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BIOLOGY AND MOLECULAR BIOLOGY OF VIRUSES IN THE GENUS *TENUIVIRUS*

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ABSTRACT

Viruses in the genus *Tenuivirus* (*Tenuiviruses*) cause a number of important diseases in economically important crop plants including rice and maize. *Tenuiviruses* are transmitted from plant to plant by specific planthopper vectors, and their transmission relationship is circulative-propagative. Thus, *Tenuiviruses* have host ranges including plants and animals (planthoppers). Four or five characteristic, circular ribonucleoprotein particles (RNPs), each containing a single *Tenuivirus* genomic RNA, can be isolated from *Tenuivirus*-infected plants. The genomic RNAs range in size from ca 9.0 kb to 1.3 kb and together give a total genome size of ca 18–19 kb. The genomic RNAs are either negative-sense or ambisense, and expression of the ambisense RNAs utilizes cap-snatching during mRNA transcription. The combination of characteristics exhibited by *Tenuiviruses* are quite different than those found for most plant viruses and are more similar to vertebrate-infecting viruses in the genus *Phlebovirus* of the *Bunyaviridae*.

INTRODUCTION

In 1977, Koganezawa reported that rice stripe virus (RSV)–infected rice (*Oryza sativa* L.) plants contained unusual fine-stranded particles, sometimes appearing as circular or branched filaments, distinctly different from particles associated with other plant viruses (73). In 1981, Gingery and co-workers suggested that maize stripe virus (MSpV) and the serologically related RSV were

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representative of a newly recognized group of plant viruses (45). They suggested that, based on a number of physical properties including the presence of unique, fine-stranded, filamentous particles and a number of nucleoprotein components that could be resolved by rate-zonal sucrose centrifugation, MSpV and the serologically related RSV were distinctly different from other plant viruses recognized at that time (45, 46). Shortly thereafter, other plant viruses including rice grassy stunt virus (RGSV) and rice hoja blanca virus (RHBV) were also recognized as having biological and molecular properties similar to MSpV and RSV, and all of these viruses were placed in the rice stripe virus group (44). Since 1995, they have been grouped together by the International Committee on Taxonomy of Viruses (ICTV) as members of the genus *Tenuivirus*, and some lesser known viruses [*Echinochloa* hoja blanca virus (EHBV)] and European wheat striate mosaic virus (EWSMV, Table 1) are tentative members of this genus (116). A few other plant viruses show similarities to those in the genus *Tenuivirus*, but they are not yet as well understood as are those mentioned here. Undoubtedly, as more viruses are discovered and characterized, more will be placed in this genus.

Previous reviews have documented the characteristics and molecular biology of the viruses in the genus *Tenuivirus* (29, 44, 99, 117). In this review, we discuss some of the original and the more recent work on these viruses. We also discuss some of the different interpretations concerning these viruses and aspects of *Tenuivirus* host interactions and vector biology. As a group, the viruses in the genus *Tenuivirus* are the only viruses whose vector range is restricted to the delphacid planthoppers. Furthermore, based on phylogenetic evidence and their genome organization and expression strategies, it appears that viruses in the genus *Tenuivirus* are more closely related to the vertebrate-infecting viruses in the genus *Phlebovirus* of the family *Bunyaviridae* than they are to other plant viruses.

TENUIVIRUS INCIDENCE AND ECONOMIC IMPORTANCE Viruses in the genus *Tenuivirus* (hereafter referred to as *Tenuiviruses*, tenui from tenuis meaning weak or slender, 116) have plant host ranges limited to the family Poaceae. *Tenuiviruses* cause serious diseases in several important food crops, including rice (*Oryza sativa* L.; rice stripe, rice hoja blanca, and rice grassy stunt) and maize [*Zea mays* L.; maize (corn) stripe, and see Table 1]. In the 1960s, RSV was estimated to affect 13–19% of the total rice acreage in Japan (117). Yield losses of 25–50% have been reported in Latin American rice due to RHBV (66), and MSpV-induced losses in maize of 80% were reported from Mauritius (103). In Florida, a scenario involved MSpV-infection of maize grown for hybrid seed production. Heavy losses occurred in the fall-grown crop, resulting in severe economic losses and the inability to economically produce winter-grown maize

Table 1 *Tenuiviruses*, their Delphacid planthopper vectors, their transmission relationships, and their known inoculated host plants

Virus	Vector species	Transmission characteristics: Minimum AAP (hours)	Transmission characteristics: Incubation period (days)	Transmission characteristics: Retention	Transmission characteristics: Transovarial rate and passage	Crop host plants	References
RSV	*Laodelphax striatellus* (Fallen); *Tethron albovittatus* (Mats.); *Unkanodes albifascia* (Mats).; *U. sapporona* (Mats).	0.25	5–10	Life	60–100%; 23–40 generations 60% 90%	*Oryza sativa*, L.; *Triticum aestivum* L.; *Hordeum vulgare* L.; *Avena sativa* L.; *Setaria italica* L.; *Sorghum bicolor*.	38, 44, 45, 53, 81, 91, 108, 113
RGSV	*Nilaparvata lugens* (Stal); *N*. spp.	0.5	6–8	Life	No	*O. sativa*.	14, 52, 53, 55, 64, 102, 116, 117
MSpV	*Peregrinus maidis* (Ashmead)	4	4–5	Life	21–58%	*S. bicolor*; *Zea mays* L.; *H. vulgare*; *A. sativa*; *T. aestivum*; *Secale cereale* L.	5, 44, 45, 48, 77, 126
RHBV	*Tagosodes* (Sogatodes) *orizicola* (Muir); *T. cubanus* (Crawford)	0.25	5–31	Life	60–100%; 10 generations	*O. sativa*; *T. aestivum*; *H. vulgare*; *A. sativa*;. *S. cereale*.	28, 40, 41, 53, 67, 82, 83
EWSMV	*Javesella pellucida* F.; *J. dubia* (Kirsch); *J. obscurella* (Boch.)	1	8–21	Life	88%	*T. aestivum*; *H. vulgare*; *A. sativa*; *S. cereale*.	3, 4, 11, 16, 44, 78
EHBV	*T. cubanus*	—	—	—	—	—	31, 42, 82, 83

Acronyms for given *Tenuiviruses* are rice stripe virus, RSV; maize stripe virus, MSpV; rice hoja blanca virus, RHBV; rice grassy stunt virus, RGSV; European wheat striate mosaic virus, EWSMV; and EHBV, *Echinochloa* hoja blanca virus (116). EWSMV and EHBV are tentative *Tenuiviruses* (116). AAP, acquisition access period.

seed (121, 125, 126) (Figure 1*C*). Most *Tenuiviruses* induce similar symptoms on their infected host plants. Typical symptom development on MSpV-infected maize plants includes fine chlorotic stippling between the veins on leaf surfaces that later develop into continuous stripes of varying width and intensity, often with a "brush-out" appearance (48) toward the tip of stripes (121) (Figure 1*B*). Young *Tenuivirus*-infected plants often exhibit complete chlorosis on the emerging whorl leaf. A syndrome so called white leaf or hoja blanca was described for diseases induced by RHBV, EHBV, and MSpV. The symptom severity and subsequent yield losses vary with age of the plant at infection (103).

The *Tenuiviruses* are somewhat limited in their geographic incidence. RSV and RGSV are limited to the Far East, and RHBV and EHBV are limited to tropical and subtropical regions of the Western Hemisphere (28, 44, 53, 83, 113, 116). However, MSpV has the most widespread geographic distribution and occurs around the world but only in subtropical and tropical regions (13, 45, 77, 104, 116, 117).

TENUIVIRUS PLANTHOPPER VECTORS

Although experimental mechanical transmission of *Tenuiviruses* has been reported, it has not been widely used (77, 79). *Tenuiviruses* are transmitted from plant to plant by planthopper vectors, and vector specificity is high (see Table 1, and Figure 1*A*). Taxonomically, *Tenuivirus* planthopper vectors are placed within the homopteran family Delphacidae (129). Although over 130 species of leafhoppers have been reported as vectors of various plant disease agents, including phytoplasmas, viruses, and bacteria-like organisms (49, 62, 89, 90, 112, 122), only 17 species of Delphacidae are known to be the vectors of only 12 plant pathogens (16, 81, 87, 129, 130). Much of our knowledge of planthoppers comes from studies on the economically important species, but planthoppers have received much less attention than have such homopteran vectors as leafhoppers and aphids.

Knowledge of planthopper biology, their environmental requirements, breeding and feeding host plants, and techniques for mass rearing are of fundamental importance to understand the potential of planthoppers as *Tenuivirus* vectors. Most planthopper vectors of *Tenuiviruses* colonize gramineous plants and are generally well adapted to members of the Poaceae (130). However, the host range of a given *Tenuivirus* does not necessarily coincide completely with the host range of its planthopper vector; the host ranges of *Tenuiviruses* are generally wider as many of the reported hosts are experimental (Table 1) (33, 48). Similarly, many rearing host plants for delphacid vectors may not be susceptible to a respective *Tenuivirus* (Table 1) (123). These latter host plants then offer the advantage of providing a means to rear nonviruliferous planthoppers.

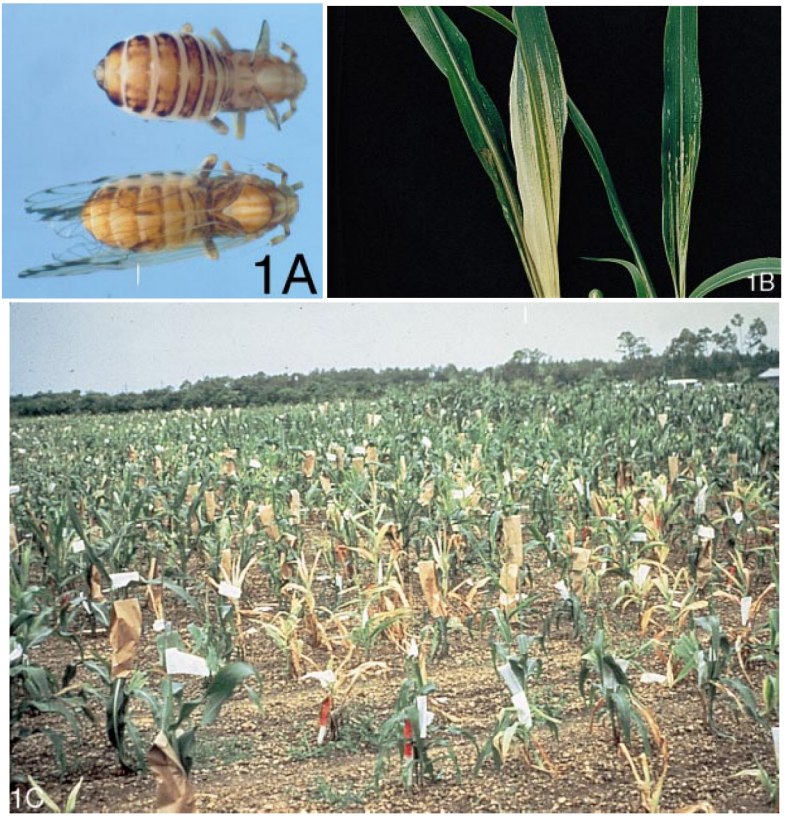

Figure 1 MSpV host plants, symptoms, and planthopper vectors. *A* shows the short-wing and long-wing forms of *Peregrinus maidis*, the MSpV planthopper vector. *B* shows two maize (*Zea mays* L.) plants exhibiting initial and advanced symptoms of infection by MSpV. Note that in advanced infections, the complete leaf lamina can become white. *C* shows a field of *Z. mays* plants affected by MSpV. Affected plants are stunted and exhibit an overall white appearance.

Most planthopper vectors feed in the phloem (36, 37, 129). This has been demonstrated by sectioning feeding tracts in plant tissue (17, 96, 124). Phloem feeders have the unique capability to inoculate pathogens into phloem tissues without destroying plant cells, thereby promoting successful infections. The feeding behavior coupled with their biological habits and geographical distribution make delphacid planthoppers ideal for transmitting the *Tenuiviruses*.

Planthopper Biology

Delphacid females are generally prolific; adult *Peregrinus maidis* females can lay 300–900 eggs, with an average of 600 eggs per female per life (125). Females oviposit on plants on which the newly hatched nymphs will feed. Eggs are usually inserted in groups into the leaf or leaf sheath of the host plant. Maturing eggs can be recognized by the appearance of red eye spots in the embryo and they can easily be removed by peeling the epidermal layer of the plant leaf. Young nymphs aggregate and feed for long periods on a single plant and often are tended by female adults. Metamorphosis is paurometabolous, with five nymphal stages that require 3–4 weeks to complete. Both nymphs and adults secrete copious amount of honeydew on feeding sites, and they are often tended by a number of ant species. Parthenogenesis has not been observed in delphacids.

Wing dimorphic species are usually found in the Delphacidae. The long-winged forms (macropters, see Figure 1*A*) play a major role in the long-distance dispersal and migration and are responsible for long-distance spread of *Tenuiviruses*. The more common short-winged forms (brachypters) are flightless and are mostly involved in *Tenuivirus* intrafield spread. The propensity for flight varies with age, season, crowding, habitat, and host plant physiology. The production of macropters in *Nilaparvata lugens*, *Laodelphax striatellus*, and *Javasella pellucida* has been attributed to the crowded conditions during nymphal development and decreased nutritional level of the host plants (71, 80). Migration of *J. pellucida* has been attributed more to planthopper physiology (97); however, the specific factors that induce migration are not yet known.

Dispersal and migration are generally facilitated by wind current. Annually *N. lugens* migrates great distances during the monsoon season from endemic areas in the tropics over the ocean northward to China, Korea, and Japan (15, 53, 127). Water current can also aid the spread of delphacid nymphs and adults that drop from the plants when disturbed (28). Delphacids that have the ability to disperse and migrate provide the opportunity for a population to spread and colonize new sites, thereby exploiting new food sources. At the same time, these actions permit exchange of genetic material between insect populations. In temperate areas, planthopper vectors are assumed to overwinter as eggs. However, species such as *L. striatellus* and *J. pellucida* overwinter in second and fourth nymphal stages (11), and *L. striatellus* can overwinter as

adults in a state of productive diapause (70). In subtropical and tropical areas, reproduction continues as long as breeding hosts are available.

Tenuivirus-*Vector Transmission Interactions*

Vector transmission of *Tenuiviruses* can be defined in three sequential phases: acquisition, incubation, and inoculation. *Tenuiviruses* can be acquired from infected plants by their planthopper vectors in times ranging between 15 min to 4 h (Table 1). After acquisition, the incubation period in the vector varies from 4 days to as many as 31 days. The inoculation threshold is generally similar to the acquisition threshold, but it can be as short as 30 sec (44). Although transmission usually continues throughout the life of the vector, inoculativity often declines with vector age and is dependent on a number of factors. Nymphs are usually more efficient *Tenuivirus* vectors than are adults (85, 126), and females are more efficient than males for RSV (44). Different populations or biotypes of the same delphacid species have shown variability in *Tenuivirus* transmission efficiency (3, 4, 72), and transmission efficiency can be increased up to 100% by continuous selective interbreeding of active vectors (39, 51, 131), or can be greatly reduced by selection and breeding inactive vectors (64). With the exception of RGSV, *Tenuiviruses* are transmitted transovarially by viruliferous females to their progeny. The transovarial transmission rate ranges from about 20% to 100% and can be for as many as 40 generations (Table 1) (3, 4, 16, 28, 40, 44, 45, 53, 55, 78, 113, 116, 126). Vertical transmission appears to be more prevalent for *Tenuiviruses* than for leafhopper-borne viruses and can complicate the ability to establish a virus-free planthopper colony in the laboratory. So far RHBV is the only known paternally transmitted *Tenuivirus* (131).

All of the *Tenuiviruses* thus far studied are transmitted by their respective vectors in a circulative-propagative manner (49, 87). Proof for *Tenuivirus* multiplication in delphacid vectors has been obtained by repeated *Tenuivirus* passage through eggs for 10 generations in *Tagosodes orizicola* (for RHBV; 39) and 40 generations in *L. striatellus* for RSV (107), such that the final inoculum dilution greatly exceeds the dilution endpoint. Additional proof of MSpV multiplication in *P. maidis* has come from serological analysis of individual *P. maidis* over time after MSpV acquisition. The percentage of *P. maidis* that were ELISA-positive for the MSpV-N (nucleoprotein) protein increased over time, corresponding with their ability to transmit MSpV to plants (35). The titer of MSpV-N protein increased in specific organs, including the salivary glands (88), and some evidence suggests that N protein titer in *P. maidis* is correlated with transmission efficiency for some MSpV isolates (5).

The effects of *Tenuivirus* infections on their vectors can vary from mild to lethal. *Tenuiviruses* can infect various planthopper organs including the brain,

digestive and respiratory tracts, salivary glands, Malpighian tubules, leg muscles, fat bodies, and reproductive tracts of both sexes (53, 88). These infections can impair the fecundity and longevity of viruliferous females (3, 11, 55, 67, 86, 89), and in extreme situations, RSV can be lethal to eggs and early instar nymphs (38). Taken together, the above data show that unlike most plant viruses but similar to plant viruses in the Rhabdoviridae, Reoviridae, and in the genera *Marafivirus* and *Tospovirus*, *Tenuiviruses* have host ranges including plants and animals, the latter being their planthopper vectors!

TENUIVIRUS CHARACTERISTICS

Tenuivirus *Ribonucleoprotein Particles*

After initial studies showed unusual ribonucleoprotein particles (RNPs) associated with *Tenuivirus*-infected plants (45, 73), several efforts were made to characterize them. Biochemical analyses showed that the RNPs were composed mostly of a ca 32- to 35-kd protein and contained RNA (45, 73, 83, 112). An additional low abundance protein of greater than 200 kd was found to be associated with some *Tenuivirus* RNPs and is discussed below (7, 114, 115). The RNPs sediment in rate-zonal sucrose density gradients as several components (Figure 2*A*). Sedimentation coefficient analysis showed that four components ranging from ca 50–190 S could be resolved for MSpV (34) (Figure 2), and three to five components with similar sedimentation coefficients have been reported for RSV, RHBV, and RGSV (18, 45, 46, 54, 63). A conflicting report suggests that the sedimentation coefficients for the RSV RNPs are much greater (685, 805, and 985 for M, B, and nB, respectively; 117); however, simultaneous comparisons of the MSpV and RSV RNPs showed similar sedimentation patterns (46), and most studies support the former estimates. Despite the fact that multiple RNPs could be separated in sucrose gradients, studies showed that *Tenuivirus* RNPs band together at a relatively low density of only ca 1.28 g/cc when analyzed by equilibrium density gradient centrifugation in cesium sulfate (34, 45, 73, 83). Furthermore, the CsSO4-purified RNPs for several *Tenuiviruses* have been shown to be infectious when injected into their respective planthopper vectors. Greater than 50% of *L. striatellus* and 80–100% of *P. maidis* planthoppers were able to transmit RSV and MSpV, respectively, to plants (34, 73).

Initial electron microscopic analyses suggested that the MSpV, RHBV, and RSV RNPs were very thin and not well defined in their morphology. The RNPs for the different *Tenuiviruses* were reported to be branched, filamentous, spiral, or circular (44, 45, 73, 77, 82, 110). However, Hibino et al (54) showed that the RSV and RGSV RNPs were mostly circular. These circular RNPs were difficult to discriminate by electron microscopy, and purified, carefully stained

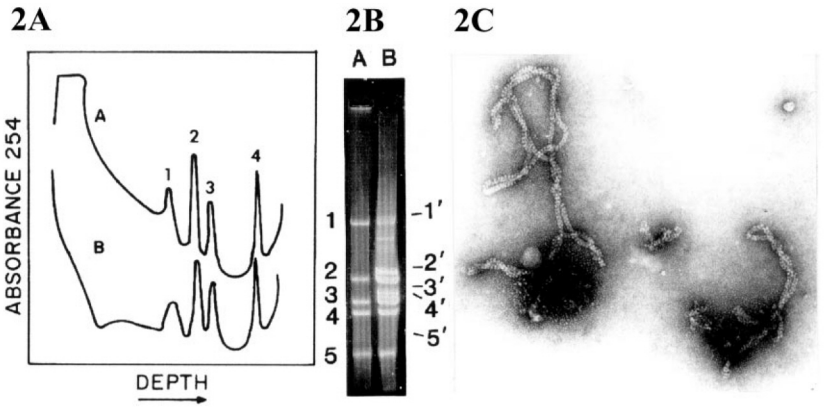

Figure 2 Characteristics of *Tenuivirus* ribonucleoprotein particles (RNPs). *A* shows a scanning profile (A260) of sucrose-density gradient purified MSpV RNPs. The profile in *A* shows RNPs purified by differential centrifugation followed by sucrose density gradient analysis. The profile in *B* shows RNPs first purified by CsSO4 equilibrium centrifugation and then fractionation in sucrose gradients. Numbers indicate the individual RNP components; component 1 is the slowest and 4 is the fastest sedimenting component. 2*B* shows MSpV RNP RNAs fractionated by nondenaturing agarose gel electrophoresis. *Lane A* shows RNAs analyzed immediately after extraction, while *lane B* shows RNAs after ethanol precipitation and resuspending RNAs. Numbers show positions of MSpV RNP ssRNAs, 1 being the largest and 5 being the smallest (see Table 2). Numbers 1′–5′ show positions of dsRNAs. 2*C* shows typical rice hoja blanca virus circular RNPs (photo courtesy of Dr A Espinoza).

preparations were required. The circular RNPs were of different circumference, and further work showed that size corresponded with the sedimentation in rate-zonal gradients [i.e. those of greater circumference had greater sedimentation coefficients (63)]. Subsequent analyses showed that for other *Tenuiviruses*, including RHBV and MSpV, the RNPs also were circular (Figure 2*C*) (13, 25). Taken together, these data suggest that multiple-sized virus-specific RNPs are present in plants infected by *Tenuiviruses*, that they are infectious, and that the circular morphology appears to be a consistent characteristic.

Genomic RNA Characteristics

Gel electrophoresis analysis showed that different-sized RNAs could be isolated from the different RNP components of *Tenuiviruses* (34, 63, 112). When the MSpV RNP RNAs were analyzed by denaturing agarose gel electrophoresis, the fastest sedimenting component (component 4, 190 S) was found to contain a large ssRNA of ca 8.5 kb (RNA1) (34). The slower sedimenting RNPs contained correspondingly smaller RNAs. For example, component 1, the slowest sedimenting RNP, contained an RNA of ca 1.4 kb (RNA5); two RNAs of ca 2.2

Table 2 Sizes of *Tenuivirus* genomic RNAs and their encoded proteins

	RSV	MSpV	RHBV	EHBV	References
RNA1, encoded proteins	8970 pc1 336,860	>8 kb	>9 kb	—	34, 101, 119
RNA2, encoded proteins	3514 p2 22,762 pc2 94,092	3337 p2 23,500 pc2 93,900	3620 p2 23,000 pc2 94,000	—	23, 27, 110
RNA3, encoded proteins	2504 p3 35,134 pc3 23,874	2357 p3 22,741 pc3 34,574	2229 p3 23,000 pc3 35,000	2336 p3 22,900 pc3 34,900	19, 20, 58, 132
RNA4, encoded proteins	2517 p4 20,541 pc4 32,474	2227 p4 19,815 pc4 31,900	1991 p4 20,076 pc4 32,469	1874 p4 19,900 pc4 32,400	22, 57, 59, 100, 133
RNA5, encoded proteins	ND	1317 pc5 44,237	ND	1334 pc5 44,000	21, 60

Acronyms used for the *Tenuiviruses* are defined in Table 1. Top number indicates the size, in nucleotides, of the given genomic segment. The encoded proteins (p or pc) and their corresponding sizes (M_r) also are indicated. For RSV, sizes are for RSV isolate T. Known and predicted functions for some of the encoded proteins include the following: RNA1-encoded pc1 is a putative RNA-dependent RNA polymerase; RNA2-encoded pc2 is a putative polyprotein that exhibits significant similarity to *Phlebovirus* virion membrane glycoproteins; RNA3-encoded pc3 is the N (nucleocapsid) protein; RNA4-encoded p4 is the major noncapsid protein, NCP. ND indicates not detected, and — indicates not characterized.

and 2.3 kb (RNAs 4 and 3, respectively) were obtained from the RNP2, and a ca 3.5 kb (RNA2) was detected from RNP3, thus suggesting five genomic RNAs for MSpV (34; and see Figure 2*B*). Likewise, five RNP RNAs have been isolated for EHBV, and some RSV isolates have five RNAs (21, 63). Only four RNAs have been identified so far for other *Tenuiviruses* (Table 2) (117, 120). Hybridization analyses have shown that for a given *Tenuivirus*, each of the RNP RNAs is mostly distinct from the others, and when combined with more recent nucleotide sequence analyses (see below), these data clearly demonstrate that the genomes of *Tenuiviruses* are multipartite and composed of four or five segments.

A complicating characteristic exhibited by the *Tenuiviruses* is that when the RNP RNAs were analyzed by nondenaturing gel electrophoresis, both single- (ss) and double-stranded (ds) RNAs were observed (Figure 2*B*). This was first seen for MSpV (34) and subsequently found to be common for other *Tenuiviruses* (18, 63, 101, 120). However, rapid extraction of RNAs followed immediately by nondenaturing gel electrophoresis showed mostly ssRNAs, but if the same RNA preparation was analyzed after repeated ethanol precipitations or storage, dsRNAs were apparent (Figure 2*B*). Thus, it appears unlikely that

both ss- and dsRNAs are encapsidated within RNPs, but that dsRNAs might result from annealing in vitro of opposite polarity ssRNAs (34). Subsequent analysis using strand-specific riboprobes complementary to the MSpV genomic RNAs showed that for each of the RNP RNAs, molecules of both polarities were encapsidated (30). However, they were not encapsidated in equimolar amounts, and for each RNA segment, there was an excess of one polarity (30).

Because each of the RNP RNAs is not represented by molecules of only one polarity, and because of the somewhat complicated coding arrangements on the different RNAs (see below), common terminology such as positive-sense or negative-sense is not easily used when referring to the *Tenuivirus* genomic RNAs. Therefore, terminology that is commonly used for many negative- and ambisense vertebrate-infecting viruses is more appropriate and is used herein to refer to the specific RNAs. Thus, vRNA (viral RNA) refers to the polarity of most abundance (i.e. vRNA1 for the more abundant RNA1), and vcRNA (viral complementary RNA) refers to the less abundant complementary RNA, respectively.

Genome Organization

In recent years, efforts have concentrated on understanding the genomic RNA structure, organization, and expression strategies used by the *Tenuiviruses*. Complementary DNA clones corresponding to several genomic RNAs have been obtained, and nucleotide sequence data are so far available for many of the genomic components of RSV, RHBV, RGSV, EHBV, and MSpV. So far, only for RSV (isolate T) has the complete nucleotide sequence been determined for each of the genomic RNAs (Table 2). Although there are some conflicting data in regards to *Tenuivirus* genome composition (four vs five genomic RNAs), current data definitively show that *Tenuivirus* genomes are complex. Genomic RNAs encode 8 or 9 proteins, and the genomes are among the largest (ca 18–19 kb) of those currently recognized for plant viruses.

Tenuivirus genomic RNAs are not capped at their 5′ termini, and the 3′ termini are not polyadenylated (111). Initial nucleotide sequence analyses confirmed previous hybridization analyses showing that the RNP RNAs were distinct genome segments; however, it also showed that conserved nucleotide sequences are present on each of the RNP RNAs. The eight nucleotides at the 5′ termini are identical for each of the *Tenuivirus* genomic RNA segments, as are the eight nucleotides at the 3′ termini. Furthermore, the nucleotide sequences of the 5′ and 3′ termini of each segment are complementary (29, 99, 111, 116, 117) (Figure 3).

Only for RSV-T has RNA1 been completely sequenced (Table 2) (119). A single, large open reading frame (ORF) is contained within vcRNA1, and thus RNA1 is referred to as a negative-sense RNA. The vcRNA1 ORF can potentially encode a protein of 336,860 M_r, and amino acid sequence analysis

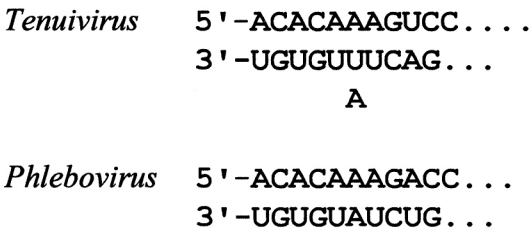

Figure 3 Comparison of the *Tenuivirus* and *Phlebovirus* genomic RNA 5′ and 3′ termini. The complementary 5′ and 3′ terminal nucleotide sequences are shown. Complementary sequences extend as far as 20 nucleotides, but the sequences shown above are conserved among all *Tenuivirus* genomic RNAs. The U is more common at the sixth position from the 3′ terminus, but some *Tenuivirus* genomic RNAs contain an A at this position (111). The 5′ and 3′ termini of the genomic RNAs of viruses in the genus *Phlebovirus* are shown for comparison (24).

suggests it is most likely an RNA-dependent RNA polymerase (119). The RSV vcRNA1-encoded protein is significantly similar to the L proteins of several viruses in the *Phlebovirus* genus of the *Bunyaviridae* and contains four conserved domains found in *Phleobovirus* L proteins (119).

Nucleotide sequence analyses of RNAs 2, 3, and 4 showed another unusual characteristic. Each of these genomic segments contains two ORFs. However, one ORF is located near the 5′ terminus of the vRNA, and a second ORF is located near the 5′ terminus of the vcRNA (Table 2, Figure 4). This coding arrangement is referred to as ambisense and was first discovered for the S RNA segments of viruses in the genus *Phlebovirus* of the *Bunyaviridae* (61). The sequence between the ORFs on the ambisense RNA segments is noncoding and ranges between ca 300 and 700 nucleotides (20, 27, 58, 59, 68, 69, 100, 110, 132, 133). Within the intergenic regions are sequences ranging from ca 30 to 90 nucleotides that appear to be conserved among *Tenuivirus* ambisense RNAs (20). Computer-assisted sequence analysis of the intergenic region has shown that it may be highly structured and could possibly form base-paired stem-loop structures (132, 133).

Because each of the ambisense RNAs encodes two proteins, but on different polarity RNAs, the terminology used to refer to them includes whether or not they are encoded by the vRNA or vcRNA. For example, p2 is the protein encoded by vRNA2, and pc2 is the protein encoded by vcRNA2. The number (i.e. 2) indicates which RNA segment encodes the specific protein and c is used to refer to a protein encoded by the vcRNA. Nucleotide and deduced amino acid sequence analyses have allowed identification of specific *Tenuivirus*-encoded proteins, and mapping them to specific ORFs. The nucleocapsid (N) protein is pc3 and p4 is the major noncapsid protein (NCP) (19, 20, 22, 57, 58, 59, 68, 69, 100, 132, 133; and see below). While other proteins have

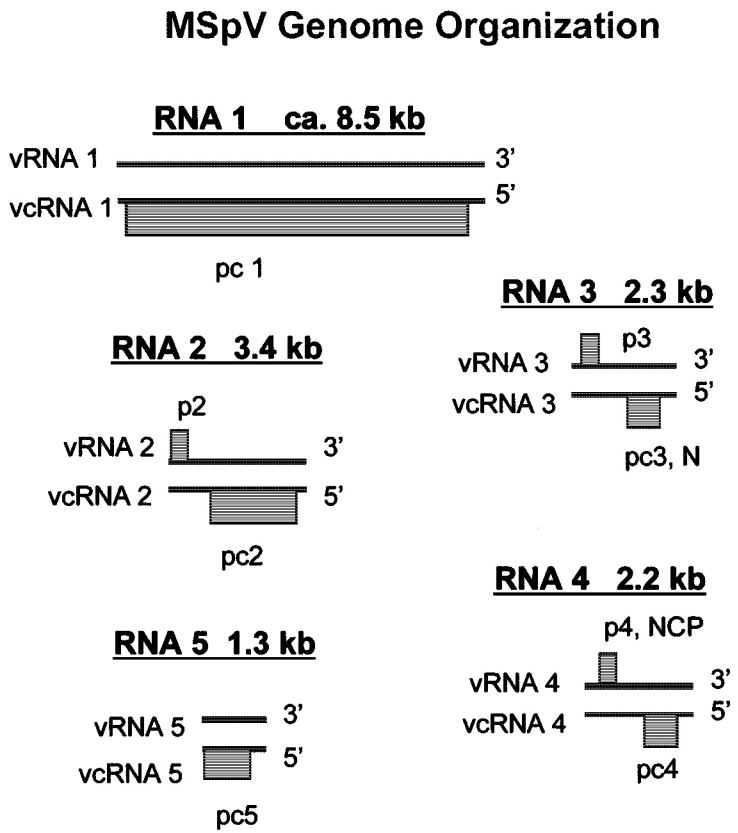

Figure 4 MSpV genome map. The 5 MSpV genomic RNAs and their open reading frames (ORFs, indicated as *hatched boxes*) are shown. Proteins (i.e. p2 or pc2) encoded by specific ORFs are indicated. Approximate sizes (in nucleotides) of each RNA are indicated. vRNA and vcRNA indicate the primary polarity of the given RNP RNA and the complementary polarity, respectively. For sizes of respective proteins, see Table 2.

been mapped to specific ORFs, the functions are as yet unknown. In some cases, potential properties can be predicted from computer analyses of amino acid sequences. For example, p2 is predicted to be a small hydrophobic protein that may be membrane-associated, and pc2 is likely a polyprotein that may be proteolytically processed to yield two glycoproteins (23, 27).

As suggested above, a complicating aspect for defining the genome composition of *Tenuiviruses* is that while four, somewhat similar genomic segments have been identified for all *Tenuiviruses*, a fifth genomic RNA has been identified for MSpV, RSV, and EHBV (21, 34, 60, 63). The MSpV and EHBV RNA5

components have been sequenced and shown to contain a single ORF in the vcRNA5. This ORF encodes a highly basic protein, pc5, for which no similar proteins were identified in database searches (21, 60).

Gene Expression

Because each of the *Tenuivirus* genomic RNAs contains an ORF positioned near the 5′ terminus of the vRNA and/or vcRNA, it seems possible that full-length genomic RNAs could function as mRNAs. In fact, the RNP RNAs can be translated in vitro, but not all of the predicted proteins are detected. When MSpV and RHBV RNP RNAs were translated in vitro, only a few proteins including the NCP (p4) and N protein (pc3) were positively identified (35, 101). However, in addition to the genomic RNAs, less than full-length RNAs corresponding to RNAs 2, 3, and 4 for several of the *Tenuiviruses* have been identified in virus-infected plants and/or planthoppers (27, 30, 58, 59). In some cases, these smaller RNAs have been shown to be associated with polyribosomes (27), and northern hybridization analysis using strand-specific probes has shown that they correspond in size and polarity to ORFs on the ambisense RNAs (27, 58, 59). Thus, current evidence suggests that they are subgenomic or transcript mRNAs.

Characterization of the mRNAs has revealed that their 5′ termini differ distinctly from the 5′ termini of the genomic RNAs (Figure 5*A*). The uncapped genomic RNAs can be labeled at their 5′ termini in vitro using polynucleotide kinase and γ-UTP, suggesting that the 5′ termini have a 5′ phosphate (58, 111). In contrast, the 5′ terminus of the RHBV NCP mRNA is capped (98). Furthermore, the NCP mRNAs for MSpV, RHBV, and RSV, and the RSV N protein mRNA contain additional nucleotides 5′ of the *Tenuivirus* conserved 5′ terminal nucleotide sequence (56, 98, 107). Nucleotide sequence analyses showed that the 5′ termini of the MSpV and RSV NCP mRNAs, and the RSV N protein mRNA varied in size and nucleotide sequence (56, 107). Most contained the 5′ conserved nucleotide sequence of 5′-ACACAAA-3′, but immediately 5′ of this sequence were heterogeneous nucleotide sequences of generally 10–15 nucleotides, although as many as 23 nucleotides were found (56, 107). No definitive, consistent characteristics of the nonviral sequences were identified (56, 107).

The origin of these heterogeneous leader sequences is believed to be via cap-snatching (56, 98, 107). Cap-snatching was first discovered for the *Orthomyxovirus*, influenza virus (9, 10, 76, 94), and subsequently was shown to be a feature of mRNA transcription for other vertebrate-infecting viruses including those in the *Arenaviridae* and *Bunyaviridae* (8, 12). A virus-encoded cap-specific endonuclease cleaves host mRNAs a short distance downstream from the 5′-cap (93, 95). The resulting 5′-capped short ribonucleotide leader then serves as a primer for mRNA synthesis. The capped primer may hybridize to

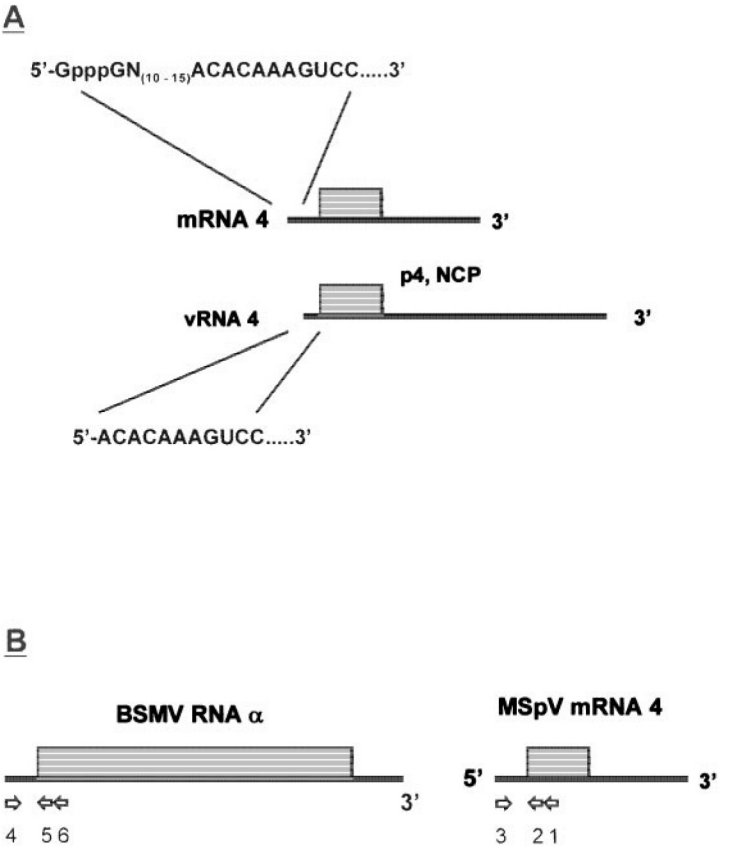

Figure 5 Characteristics of MSpV mRNAs and detection of chimeric mRNAs. *A* shows a comparison of the 5′ terminal nucleotide sequences of the MSpV vRNA4 and the mRNA4. The mRNA4 is capped and contains a heterogeneous nucleotide sequence of 10–15 nucleotides ($N_{10–15}$). *B* shows the barley stripe hordeivirus (BSMV), α genomic RNA, and the MSpV mRNA4. *Arrows* indicate approximate positions of oligonucleotide primers used in the RT-PCR assay to detect chimeric RNAs. *Arrows 1* and *6* indicated oligonucleotide primers used for reverse transcription (RT) for MSpV and BSMV RNAs, respectively. Then oligonucleotides 4 + 5, or 2 + 3 were used for PCR detection of BSMV or MSpV, respectively. RT-PCR to detect chimeric RNAs utilized oligonucleotide 1 for the RT reaction, and oligonucleotide primers 2 + 4.

a few nucleotides of the viral template RNA, and transcription ensues. Thus, the capped primer is incorporated into the mRNA transcript, and by this mechanism viral mRNAs gain a 5′ cap and a leader sequence donated from host cellular mRNAs. The resulting capped viral mRNAs presumably serve as efficient mRNAs, whereas the uncapped genome RNAs do not.

Factors determining selection of host mRNAs for cap-snatching are as yet unknown. Attempts to identify common features of these *Tenuivirus* heterogeneous leader sequences by computer analyses did not reveal any clues (56, 107). Recently, we utilized an alternative approach to assess whether specific donor RNAs could serve as primers for cap-snatching in vivo. We created mixed infections of MSpV and the *Hordeivirus*, barley stripe mosaic virus (BSMV), in barley (*Hordeum vulgare* L.) plants, and determined whether the 5′ termini of the BSMV genomic RNAs could serve as donors for cap-snatching. The three BSMV genomic RNAs, α, β, and γ, differ in their nucleotide sequences and each has a 5′ cap (65). The reverse transcription-polymerase chain reaction (RT-PCR) was used in attempts to detect chimeric molecules composed of BSMV 5′-termini and 3′-terminal MSpV RNA4 sequences (see Figure 5*B*). By using primers for MSpV RNA4 or the BSMV genomic RNAs, we produced the predicted-sized DNAs when RNAs from plants singly infected by MSpV and/or BSMV were used for analyses. When we mixed RNAs from plants singly infected with BSMV or MSpV for RT-PCR, we also produced only DNAs corresponding to BSMV or MSpV. However, when RNAs from doubly infected plants were analyzed, we detected RT-PCR products composed of BSMV 5′-terminal nucleotides and MSpV3′ terminal nucleotides. These were obtained only from doubly infected plants and never when RNAs from singly infected plants were mixed in vitro (EM Estabrook, JH Tsai, BW Falk, unpublished).

When these RT-PCR products were cloned and their nucleotide sequences determined, the resulting data demonstrated that RNAs from doubly infected plants contained nucleotide sequences of both co-infecting viruses. The 5′ region corresponded to BSMV α, β, or γ RNAs, and the BSMV sequence ranged from 10 to 16 nucleotides. The downstream nucleotide sequence corresponded to that of MSpV vRNA4, demonstrating that these represented chimeric RNAs containing BSMV and MSpV sequences. These chimeric RNAs were detected only from co-infection of barley plants by both BSMV and MSpV. Based on what is known and surmised in regards to *Tenuivirus* mRNA transcription, the most likely explanation is that chimeric RNAs resulted from cap-snatching, and the BSMV α, β, and γ RNAs were able to serve as primers for MSpV vRNA4 mRNA transcription.

If cap-snatching is the mechanism leading to generation of the BSMV-MSpV chimeric RNAs, this process appears to occur within the cytoplasm of cells in the doubly infected barley plants because BSMV replicates solely within the

cytoplasm (65), and it is believed that MSpV replication also is restricted to the cytoplasm (6, 26). In contrast, for *Influenza* virus cap-snatching occurs within the nucleus of the infected host cell (76).

When barley plants were inoculated with both of these two unrelated viruses, both MSpV and BSMV must co-infect at least some cells. Thus, as for many other mixed virus infections in plants, cells infected by one virus do not exclude other unrelated viruses from infecting the same cell (32, 128). Additionally, within the doubly infected cells the RNAs of each virus are not partitioned so as to prevent RNAs of one virus (i.e. BSMV) from encountering the replication/transcription complex of the other (i.e. MSpV).

Infections of Plant and Insect Hosts

Because the *Tenuivirus*-planthopper transmission relationship is circulative-propagative, by definition the *Tenuiviruses* infect and replicate in their plant hosts and in their planthopper vectors. Another interesting aspect of *Tenuivirus* biology and molecular biology has been to compare *Tenuivirus* infections in plants and their planthopper vectors. When one considers that the *Tenuiviruses* alternate between plant and animal hosts as part of their natural life cycles, several questions come to mind. For example, do *Tenuiviruses* cause pathogenic effects within the planthoppers as they do in plants? Are the same genes and gene products utilized in both hosts? Are the ultrastructural effects similar? A few of these questions have been at least partly answered and are discussed here.

One of the diagnostic features of *Tenuivirus* infections in plants is the abundant production of the major noncapsid protein (NCP) (6, 13, 18, 25, 26, 31, 33, 35, 45, 73) (see Figure 6). Within the cells of *Tenuivirus*-infected plants, the NCP is the most abundant protein, even more abundant than is rubisco (35). It can be readily detected by light and/or electron microscopic analysis, serological assays, and gel electrophoresis (25, 26, 31, 33). Large amorphous inclusion bodies are seen by electron microscopy and these appear to almost fill the entire cell cytoplasm (Figure 6) (6, 25, 26). In contrast, efforts to detect the NCP in MSpV-infected planthoppers (*P. maidis*) have so far proven unsuccessful (35). Similarly, the mRNA corresponding to the NCP-coding region of vRNA4 is highly abundant in MSpV-infected plants but was not detectable in MSpV-infected *P. maidis* (59). Analyses for other MSpV-encoded proteins and mRNAs have demonstrated that at least some of them are readily detectable in both host types. The MSpV N protein is easily detected in MSpV-infected plants and planthoppers and accumulates in both host types (5, 35, 88). Similarly, the MSpV mRNAs for each of the RNA2 ORFs are readily detected in both MSpV-infected plants and planthoppers (27). What leads to this differential accumulation of the NCP and/or its mRNA is presently unknown, but the obvious question is whether the NCP and/or its mRNA are differentially expressed in MSpV-infected plants vs planthoppers. The function of the NCP is as yet unknown, and whether

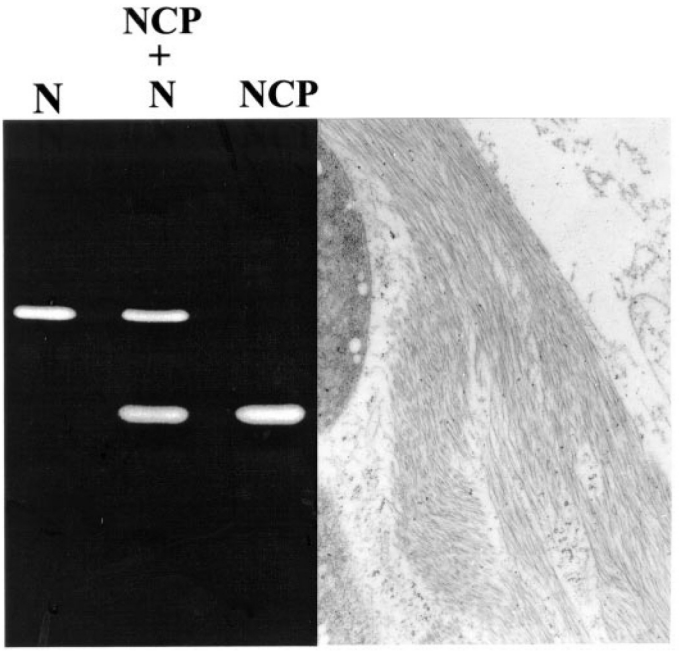

Figure 6 Characteristics of the MSpV major noncapsid protein (NCP). 6*A* shows the MSpV N protein (N) and major noncapsid protein (NCP) separated by SDS-polyacrylamide gel electrophoresis. *Panel at right* shows a portion of a MSpV-infected *Z. mays* cell. *C* indicates a portion of a chloroplast, NCP indicates the massive fibrillar NCP inclusion body.

it is necessary in infections of plants but not planthoppers is an intriguing question. Interestingly, Shikata & Galvez (106) reported accumulation of RHBV-specific structures in the intestinal cells, but not the salivary glands of *T. orizicola*. Immunogold labeling was not developed at that time, and the exact composition of these structures was not determined. However, examination and comparison with more recent electron micrographs suggest, at least to us, that these structures may be NCP-like inclusions. If so, why are they limited to the intestine when for other *Tenuiviruses* the N protein has been readily detected in a variety of organs, including salivary glands, fat bodies, and reproductive organs (88)?

Phylogenetic Relationships

When the *Tenuivirus* genome organization and gene expression strategies are compared to those of other plant viruses, the only similar plant viruses are those in the genus *Tospovirus* of the *Bunyaviridae*. Viruses in the genus *Tospovirus* have tripartite genomes and contain one negative-sense and two ambisense

segments (47, 74). They also utilize cap-snatching during mRNA transcription (75) and thus share two of the characteristic features also seen for the *Tenuiviruses*. Furthermore, the *Tospovirus* RNPs are morphologically similar to those of the *Tenuiviruses*; they are circular, containing a single genome, or antigenome segment (47). However, in contrast to the *Tenuiviruses*, viruses in the *Tospovirus* genus package their RNPs within characteristic membrane-bound virions typical of those for viruses in the *Bunyaviridae* (47). In addition, when *Tenuivirus* and *Tospovirus* nucleotide sequences and amino acid sequences of their encoded proteins are compared, they are not highly similar. Thus, *Tenuiviruses* share some characteristics with viruses in the genus *Tospovirus*, but they do not appear to be closely related.

More striking are the features shared between *Tenuiviruses* and the vertebrate-infecting viruses in the *Phlebovirus* of the *Bunyaviridae*. Like viruses in the genus *Tospovirus*, those in the genus *Phlebovirus* have three genomic segments (two negative-sense and one ambisense) packaged within spherical, membrane-bound virions, and they utilize cap-snatching during mRNA transcription (12, 24, 43, 105). However, *Tenuiviruses* and viruses in the genus *Phlebovirus* share an absolute conservation of identical nucleotides at the genomic RNA termini. The sequence 5′-ACACAAAU is present at the 5′ terminus of each *Tenuivirus* and *Phlebovirus* genomic RNA segment, and the complementary sequence AUUUGUGU-3′ is found on the 3′ termini (see Figure 3). Furthermore, comparisons of the amino acid sequences of several encoded proteins also show regions of significant similarity. The RSV RNA1-encoded protein shows significant similarity to the L proteins of viruses in the genus *Phlebovirus* (119). The *Tenuivirus* pc2 putative polyproteins show a high degree of similarity with corresponding *Phlebovirus* RNA2-encoded membrane glycoproteins, including a similar putative proteolytic processing site (23, 27), and the *Tenuivirus* N proteins are significantly similar to the corresponding *Phlebovirus* N proteins (19, 20, 58, 69, 132). Taken together, these data suggest that *Tenuiviruses* and viruses in the genus *Phlebovirus* of the *Bunyaviridae* have likely evolved from a common ancestor and retained a number of common molecular characteristics. Furthermore, at the biological level, these viruses exhibit similar strategies. Viruses in the genus *Phlebovirus* alternate between their invertebrate hosts (vectors, Phlebotomid flies) and vertebrate hosts (including humans), whereas *Tenuiviruses* alternate between invertebrate hosts (planthoppers) and plant hosts. Thus, viruses in both genera infect their invertebrate vectors, but their primary hosts differ significantly.

FINAL THOUGHTS

The *Tenuiviruses* are successful plant viruses. They survive by infecting and replicating within plant and animal hosts. They cause economically important

plant diseases in some of our most important food crops including rice and maize. Our understanding of the *Tenuivirus* genome, gene products, and infections of their plant and planthopper hosts is incomplete. Their taxonomic status is still under consideration, and inconsistency in specific properties raises questions about some tentative members of the genus *Tenuivirus* (118).

Based on what we know now about *Tenuiviruses*, a persisting question in regards to our understanding of *Tenuiviruses* is what exactly is the composition of *Tenuivirus* virions? Based on molecular and phylogenetic evidence, *Tenuiviruses* appear most similar to viruses in the genus *Phlebovirus*. Then because of the similarities, we might predict that spherical membrane-bound virions typical for the *Phleoviruses* should also be seen for *Tenuiviruses*. Despite a number of elaborate efforts, no membrane-bound virions have been identified in *Tenuivirus*-infected plants or planthoppers (6, 25, 26, 106, 109). Immunogold labeling and transmission electron microscopy have allowed localizing N protein in *Tenuivirus*-infected cells, but no typical virions have been identified (25, 109). The typical *Tenuivirus* RNPs have been purified from infected plants and are infectious. Perhaps the presence of four or five genomic segments for *Tenuiviruses* as opposed to only three for typical viruses in the *Bunyaviridae* suggests that the all *Tenuivirus* RNPs could not be packaged into spherical membrane-bound virions of only 80–100 nm diameter, the typical size of virions observed for viruses in the *Bunyaviridae*. Alternatively, *Tenuiviruses* may not need such elaborate virions. The RNPs are infectious; they contain all necessary requirements to initiate infections and even have an associated RNA-dependent RNA polymerase (34, 73, 89b, 114, 115). Many plant viruses have their genomes divided among distinct genomic segments, and at least 12 of the currently recognized genera of plant viruses encapsidate their individual genome segments into separate virions. In contrast, none of the viruses affecting bacteria, algae, fungi, protozoa, invertebrates, or vertebrates do (84). Thus, for plant viruses to be successful, all genomic segments need not be packaged together. By this reasoning, the RNPs of *Tenuiviruses* could then be considered to be analogous in function to virions; they do not need to be packaged together. Somewhat in support of this idea is the finding that some tomato spotted wilt virus (TSWV) isolates can be mechanically transmitted and cause systemic infections in plants, but they no longer package RNPs into virions (47). But why then do *Tenuiviruses* retain genome segments (i.e. RNA2) that encode for proteins that have the characteristics of virion membrane proteins? One could reason that these proteins may not be needed for virion assembly, but might perform other essential functions of the *Tenuivirus* life cycle. Many plant virus–encoded proteins are multifunctional, and thus they could be retained for other essential functions.

The *Tenuiviruses* are complex plant viruses and offer exciting advantages as well as challenges for study. Not only do they exhibit many atypical

properties compared with other plant viruses, but they also cause significant plant diseases in important food crops. In some regions of the world, controlling diseases caused by *Tenuiviruses* is presently of great importance (53). Genetically engineered resistance to RSV in rice plants has already been reported (50). However, because *Tenuiviruses* infect their planthopper vectors, can genetic engineering approaches directed at the planthopper vector offer alternative and possibly even more effective strategies to control plant virus diseases? Genetically engineered resistance to virus transmission has already been reported for the mosquito (*Aedes aegypti*)-borne Dengue-2 virus (*Flaviviridae*) (92), and additional approaches are under way for other invertebrate-borne pathogens of animals (1, 2). Therefore, a greater understanding is needed of *Tenuivirus* infections of their plant hosts and their planthopper vectors, and such information could be useful not just for plant viruses, but for other invertebrate-borne viruses that affect plant or animal hosts.

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Literature Cited

1. Aldhous P. 1993. Malaria: focus on mosquito genes. *Science* 261:546–48
2. Aldhous P. 1993. Bacteria may provide access to the Tsetse fly. *Science* 261:548
3. Ammar ED. 1975. Effect of European wheat striate mosaic, acquired by feeding on diseased plants, on the biology of its planthopper vector *Javesella pellucida*. *Ann. Appl. Biol.* 79:195–202
4. Ammar ED. 1975. Effect of European wheat striate mosaic, acquired transovarially, on the biology of its planthopper vector *Javesella pellucida*. *Ann. Appl. Biol.* 79:203–13
5. Ammar ED, Gingery RE, Madden LV. 1995. Transmission efficiency of three isolates of maize stripe tenuivirus in relation to virus titre in the planthopper vector. *Plant Pathol.* 44:239–43
6. Ammar ED, Gingery RE, Nault LR. 1985. Two types of inclusions in maize infected with maize stripe virus. *Phytopathology* 75:84–89
7. Barbier P, Takahashi M, Nakamura I, Toriyama S, Ishihama A. 1992. Solubilization and promoter analysis of RNA polymerase from rice stripe virus. *J. Virol.* 66:6171–74
8. Bishop DHL. 1991. Arenaviridae and their replication. In *Fundamental Virology*, ed. BM Fields, DM Knipe, RM Chanock, MS Hirsch, JL Melnick, et al, pp. 567–79. New York: Raven. 2nd ed.
9. Bouloy M, Morgan MA, Shatkin AJ, Krug

RM. 1979. Cap and internal nucleotides of reovirus mRNA primers are incorporated into influenza viral complementary RNA during transcription in vitro. *J. Virol.* 32:895–904
10. Bouloy M, Plotch SJ, Krug RM. 1978. Globin mRNAs are primers for the transcription of influenza viral RNA in vitro. *Proc. Natl. Acad. Sci. USA* 75:4886–90
11. Brcak J. 1979. Leafhopper and planthopper vectors of plant disease agents in central and southern Europe. See Ref. 79b, pp. 97–135
12. Calisher CH. 1996. History, classification, taxonomy of viruses in the Family *Bunyaviridae*. See Ref. 24, pp. 1–17
13. Chen CC, Chiu RJ. 1982. Three symptomatic type of rice virus diseases related to grassy stunt in Taiwan. *Plant Dis.* 66: 15–18
14. Chen CC, Tsai JH, Whiu RJ. 1993. Purification, characterization, serological analysis of maize stripe virus in Taiwan. *Plant Dis.* 77:367–72
15. Cheng SN, Chen JC, Si H, Yan LM, Chu TL. 1982. Studies on the migrations of brown planthopper, *Nilaparvata lugens* Stal. *Acta Entomol. Sin.* 22:1–21
16. Conti M. 1985. Transmission of plant viruses by leafhoppers and planthoppers. See Ref. 89a, pp. 289–307
17. Day MF, Irzykiewiez H, McKinnon A. 1952. Observations on the feeding of the virus vector *Orosius argentatus* (Evans), comparisons with certain other jassids. *Aust. J. Sci. Res. Ser. B* 5:128–42
18. de Miranda JR, Espinoza AM, Hull R. 1996. Rapid, small scale purification of rice hoja blanca and *Echinochloa* hoja blanca tenuivirus ribonucleoprotein. *J. Virol. Methods* 56:109–13
19. de Miranda J, Hernandez M, Hull R, Espinoza AM. 1994. Sequence analysis of rice hoja blanca virus RNA-3. *J. Gen. Virol.* 75:2127–32
20. de Miranda JR, Munoz M, Madriz J, Wu R, Espinoza AM. 1996. Sequence of Echinochloa hoja blanca Tenuivirus RNA-3. *Virus Genes* 13:65–68
21. de Miranda JR, Munoz M, Wu R, Espinoza AM. 1996. Sequence of Echinochloa hoja blanca Tenuivirus RNA-5. *Virus Genes* 12:131–34
22. de Miranda JR, Munoz M, Wu R, Espinoza AM. 1996. Sequence of Echinochloa hoja blanca Tenuivirus RNA-4. *Virus Genes* 13:61–64
23. de Miranda JR, Munoz M, Wu R, Hull R, Espinoza AM. 1996. Sequence of rice hoja blanca Tenuivirus RNA-2. *Virus Genes* 12:231–37
24. Elliott RM. 1996. The *Bunyaviridae*: concluding remarks and future prospects, In *The Bunyaviridae*, ed. RM Elliott, pp. 295–322. New York: Plenum. 337 pp.
25. Espinoza-Esquivel AM, Hernandez M, Pereira R, Falk B, Medina V. 1992. In situ immunogold labelling analysis of the rice hoja blanca virus nucleoprotein and major noncapsid protein. *Virology* 191:619–27
26. Espinoza AM, Pereira R, Macaya-Lizano AV, Hernandez M, Goulden M, Rivera C. 1993. Comparative light and electron microscopic analyses of Tenuivirus major noncapsid protein (NCP) inclusion bodies in infected plants, of the NCP in vitro. *Virology* 195:156–66
27. Estabrook EM, Suyenaga K, Tsai JH, Falk BW. 1996. Maize stripe virus Tenuivirus RNA2 transcripts in plant and insect hosts and analysis of pvc2, a protein similar to the *Phlebovirus* virion membrane glycoproteins. *Virus Genes* 12:239–47
28. Everett TR, Lamey HA. 1969. Hoja blanca. See Ref. 79a, pp. 361–77
29. Falk BW. 1994. Tenuiviruses. In *Encyclopedia of Virology*, ed. RG Webster, A Granoff, pp. 1410–16. London: Academic
30. Falk BW, Klaassen VA, Tsai JH. 1989. Complementary DNA cloning and hybridization analysis of maize stripe virus RNAs. *Virology* 173:338–42
31. Falk BW, Morales FJ, Tsai JH, Niessen AI. 1987. Serological and biochemical properties of the capsid and major noncapsid proteins of maize stripe virus, rice hoja blanca virus and *Echinochloa* hoja blanca virus. *Phytopathology* 77:196–201
32. Falk BW, Passmore BK, Watson MT, Chin L-S. 1995. The specificity and significance of heterologous encapsidation of virus and virus-like RNAs. In *Biotechnology and Plant Protection: Viral Pathogenesis and Disease Resistance*, ed. DD Bills S-D Kung, pp. 391–415. Singapore: World Sci.
33. Falk BW, Tsai JH. 1983. Assay for maize stripe virus-infected plants by antiserum produced to a purified noncapsid protein. *Phytopathology* 73:1259–62
34. Falk BW, Tsai JH. 1984. Identification of single- and double-stranded RNAs associated with maize stripe virus. *Phytopathology* 74:909–15
35. Falk BW, Tsai JH, Lommel SA. 1987. Differences in levels of detection for the maize stripe virus capsid and major noncapsid proteins in plant and insect hosts. *J. Gen. Virol.* 68:1801–11
36. Foster S, Goodman LJ, Duckett JG. 1983. Ultrastructure of sensory receptors on the

labium of the rice brown planthopper. *Cell Tiss. Res.* 230:353–66
37. Foster S, Goodman LJ, Duckett JG. 1983. Sensory receptors associated with the stylets and cibarum of the rice brown planthopper, *Nilaparvata lugens*. *Cell Tiss. Res.* 232:111–19
38. Fukushi T. 1969. Relationships between propagative rice viruses and their vectors. See Ref. 79a, pp. 279–301
39. Galvez GE. 1968. Transmisssion studies of the hoja blanca virus with highly active virus-free colonies of *Sogatodes oryzicola*. *Phytopathology* 58:818–21
40. Galvez GE. 1969. Transmission of hoja blanca virus of rice. In *The Virus Diseases of the Rice Plant*. Baltimore: Johns Hopkins Press. 155 pp.
41. Galvez GE, Thurston HS, Jennings PR. 1960. Transmission of hoja blanca of rice by the planthopper, *Sogata cubana*. *Plant Dis. Report.* 44:394
42. Galvez GE, Thurston HS, Jennings PR. 1961. Host range and insect transmission of hoja blanca disease of rice. *Plant Dis. Report.* 45:949–53
43. Giorgi C. 1996. Molecular biology of Phleboviruses. See Ref. 24, pp. 105–28
44. Gingery R. 1988. The rice stripe virus group. In *The Plant Viruses*, ed. RG Milne, 4:297–329. New York: Plenum
45. Gingery RE, Nault LR, Bradfute OE. 1981. Maize stripe virus: characteristics of a new virus class. *Virology* 182:99–108
46. Gingery RE, Nault LR, Yamashita S. 1983. Relationship between maize stripe virus and rice stripe virus. *J. Gen. Virol.* 64:1765–70
47. Goldbach R, Peters D. 1996. Molecular and biological aspects of Tospoviruses. See Ref. 24, pp. 129–57
48. Greber RS. 1983. Characteristics of viruses affecting maize in Australia. In *Proc. Int. Maize Virus Dis. Colloq. Workshop*, ed. DT Gordon, JK Knoke, LR Nault, RM Ritter, pp. 206–18. Wooster: Ohio State Univ, Ohio Agric. Res. Dev. Cent. 266 pp.
49. Harris KF. 1981. Arthropod and nematode vectors of plant viruses. *Annu. Rev. Phytopathol.* 19:391–426
50. Hayakawa T, Zhu Y, Itoh K, Kimura Y, Izawa T, et al. 1992. Genetically engineered rice resistant to rice stripe virus, an insect-transmitted virus. *Proc. Natl. Acad. Sci. USA* 89:9865–69
51. Hendrick RD, Everett TR, Lamey HA, Showers WB. 1965. An improved method of selecting and breeding for active vectors of hoja blanca virus. *J. Econ. Entomol.* 58:539–42
52. Hibino H. 1986. Rice grassy stunt virus—purification and serology. *Tech. Bull. Trop. Agric. Res. Cent. Jpn.* 21:34–40
53. Hibino H. 1996. Biology and epidemiology of rice viruses. *Annu. Rev. Phytopathol.* 34:249–74
54. Hibino H, Usugi T, Omura T, Tsuchizaki T, Shohara K, et al. 1985. Rice grassy stunt virus: a planthopper-borne circular filament. *Phytopathology* 75:894–99
55. Hirao J, Oya S, Inoue H. 1987. Transmission of rice grassy stunt virus (RGSV) by the brown planthopper, *Nilaparvata lugens* Stal. (Homoptera: Delphacidae). *Bull. Kyushu Natl. Agric. Exp. Stn.* 24: 307–35
56. Huiet L, Feldstein PA, Tsai JH, Falk BW. 1993. The maize stripe virus major noncapsid protein messenger RNA transcripts contain heterogeneous leader sequences at their 5′ termini. *Virology* 197: 808–12
57. Huiet L, Klaassen VA, Tsai JH, Falk BW. 1990. Identification and sequence analysis of the maize stripe virus major noncapsid protein gene. *Virology* 179:862–66
58. Huiet L, Klaassen VA, Tsai JH, Falk BW. 1991. Nucleotide sequence and RNA hybridization analyses reveal an ambisense coding strategy for maize stripe virus RNA-3. *Virology* 182:47–53
59. Huiet L, Tsai JH, Falk BW. 1992. Complete sequence of maize stripe virus RNA4 and mapping of its subgenomic RNAs. *J. Gen. Virol.* 73:1603–7
60. Huiet L, Tsai JH, Falk BW. 1993. Maize stripe virus RNA5 is of negative polarity and encodes a highly basic protein. *J. Gen. Virol.* 74:549–54
61. Ihara T, Hiroomi A, Bishop DHL. 1984. Novel coding strategy (ambisense genomic RNA) revealed by sequence analyses of Punta Toro Phlebovirus S RNA. *Virology* 136:293–306
62. Ishihara T. 1969. Families and genera of leafhopper vectors. See Ref. 79a, pp. 235–54
63. Ishikawa K, Omura T, Hibino H. 1989. Morphological characteristics of rice stripe virus. *J. Gen. Virol.* 70:3465–68
64. Iwasaki M, Nakano M, Shinkai A. 1982. Variation in the transmission rates of rice grassy stunt virus in various colonies of brown planthopper. *Proc. Assoc. Plant Prot. Kyushu* 28:1–3 (In Japanese)
65. Jackson AO, Hunter BG, Gustafson GD. 1989. Hordeivirus relationships and genome organization. *Annu. Rev. Phytopathol.* 27:95–121
66. Jennings PR. 1963. Estimating yield loss

in rice caused by hoja blanca. *Phytopathology* 53:492
67. Jennings PR, Pineda A. 1971. The effect of the hoja blanca virus on its insect vector. *Phytopathology* 61:142–43
68. Kakutani T, Hayano Y, Hayashi T, Minobe Y. 1990. Ambisense segment 4 of rice stripe virus: possible evolutionary relationship with phleboviruses and uukuviruses (Bunyaviridae). *J. Gen. Virol.* 71:1427–32
69. Kakutani T, Hayano Y, Hayashi T, Minobe Y. 1991. Ambisense segment 3 of rice stripe virus: the first case of a virus containing two ambisense segments. *J. Gen. Virol.* 72:465–68
70. Kisimoto R, Dyck VA. 1976. Climate and rice insects. *IRRI Symp. Clim. Rice*, pp. 367–91. Los Banos, Philippines
71. Kisimoto R. 1956. Factors determining the wing form of adult, with special reference to the effect of crowding during the larval period of the brown planthopper, *Nilaparvata lugens* Stal. Studies of the polymorphism in the planthopper (Homoptera: Araeopidae). I. *Oya Kontyu* 12: 105–11
72. Kisimoto R. 1967. Genetic variation in the ability of a planthopper vector, *Laodelphax striatellus* (Fallen), to acquire the rice stripe virus. *Virology* 132:144–52
73. Koganezawa H. 1977. Purification and properties of rice stripe virus. In *Symp. Virus Dis. Trop. Crops. Trop. Agric. Res. Ser. No. 10*, pp. 151–54. Trop. Agric. Res. Cent., Minist. Agric. For., Jpn.
74. Kormelink R, de Haan P, Meurs C, Peters D, Goldbach R. 1992. The nucleotide sequence of the mRNA segment of tomato spotted wilt virus, a bunyavirus with two ambisense RNA segments. *J. Gen. Virol.* 73:2795–804
75. Kormelink R, van Poelwijk F, Peters D, Goldbach R. 1992. Non-viral heterogeneous sequences at the 5′ ends of tomato spotted wilt virus mRNAs. *J. Gen. Virol.* 73:2125–28
76. Krug RM, Broni BA, Bouloy M. 1979. Are the 5′ ends of influenza viral mRNAs synthesized in vivo donated by host mRNAs? *Cell* 18:329–34
77. Lastra R, Carballo O. 1985. Mechanical transmission, purification and properties of an isolate of maize stripe virus from Venezuela. *Phytopathol. Z.* 114:168–79
78. Lindsten K. 1979. Planthopper vectors and plant disease agents in Fennoscandia. See Ref. 79b, pp. 155–79
79. Louie R. 1995. Vascular puncture of maize kernels for the mechanical transmission of maize while line mosaic virus and other viruses of maize. *Phytopathology* 85:139–43
79a. Maramorosch K, ed. 1969. *Viruses, Vectors, Vegetation.* New York: InterScience. 666 pp.
79b. Maramorosch K, Harris KF, eds. 1979. *Leafhopper Vectors and Plant Disease Agents.* New York: Academic. 654 pp.
80. Mochida O. 1973. The characters of the two wing-forms of *Javesella pellucida* (F.) (Homoptera: Delphacidae), with special reference to reproduction. *Trans. R. Entomol. Soc. London* 125:177–225
81. Mochida O, Okada T. 1971. A list of the Delphacidae (Homoptera) in Japan with special reference to host plants, transmission of plant diseases, natural enemies. *Bull. Kyushu Agric. Exp. Stn.* 15:737–843
82. Morales FJ, Niessen AI. 1983. Association of spiral filamentous viruslike particles with rice hoja blanca. *Phytopathology* 73:971–74
83. Morales FJ, Niessen AI. 1985. Rice hoja blanca virus. In *Descriptions of Plant Viruses*, No. 199, Kew, Surrey, UK: Commonw. Mycol. Inst./Assoc. Appl. Biol. 5 pp.
84. Murphy FA, Fauquet CM, Bishop DHL, Ghabrial SA, Jarvis AW, et al, eds. 1995. *Virus taxonomy. Sixth Rep. Int. Comm. Taxon. Viruses.* Vienna/New York: Springer-Verlag
85. Narayana YD, Muniyappa V. 1996. Virus-vector relationships of a planthopper (*Peregrinus maidis*)-borne sorghum stripe tenuivirus. *Int. J. Pest Manag.* 42:165–70
86. Nasu S. 1963. Studies on some leafhoppers and planthoppers which transmit diseases of rice plant in Japan. *Bull. Kyushu Natl. Agric. Exp. Stn.* 8:153–340 (In Japanese)
87. Nault LR, Ammar ED. 1989. Leafhopper and planthopper transmission of plant viruses. *Annu. Rev. Entomol.* 34:503–29
88. Nault LR, Gordon DT. 1988. Multiplication of maize stripe virus in *Peregrinus maidis*. *Phytopathology* 78:991–95
89. Nault LR, Knoke JK. 1981. Maize vectors. In *Virus and Virus-Like Diseases of Maize in the United States*, ed. DT Gordon, JK Knoke, GE Scott, pp. 77–84. *South. Crop Ser. Bull.* 247. 218 pp.
89a. Nault LR, Rodriguez JG, eds. 1985. *The Leafhoppers and Planthoppers.* New York: Wiley
89b. Nguyen M, Ramirez B-C, Goldbach R, Haenni A-L. 1997. Characterization of the in vitro activity of the RNA-dependent RNA polymerase associated with the

fribonucleoproteins of rice hoja blanca Tenuivirus. *J. Virol.* 71:2621–27
90. Nielson NW. 1979. Taxonomic relationships of leafhopper vectors of plant-pathogens. See Ref. 79b, pp. 3–27
91. Okuyama S, Yora K, Asuyama H. 1968. Multiplication of the rice stripe virus in its vector, *Laodelphax striatellus* Fallen. *Ann. Phytopathol. Soc. Jpn.* 34:255–64
92. Olson KE, Higgs S, Gaines PJ, Powers AM, Davis BS, et al. 1996. Genetically engineered resistance to Dengue-2 virus transmission in mosquitoes. *Science* 272:884–86
93. Patterson JL, Holloway B, Kolakofsky D. 1984. LaCrosse virions contain a primer-stimulated RNA polymerase and a methylated cap-dependent endonuclease. *J. Virol.* 52:215–22
94. Plotch SJ, Bouloy M, Krug RM. 1979. Transfer of 5′-terminal cap of globin mRNA to influenza viral complementary RNA during transcription in vitro. *Proc. Natl. Acad. Sci. USA* 76:1618–22
95. Plotch SJ, Bouloy M, Ulmanen I, Krug RM. 1981. A unique cap (m7GpppXm)-dependent influenza virion endonuclease cleaves capped RNAs to generate the primers that initiate viral RNA transcription. *Cell* 23:847–58
96. Putnam WL. 1941. The feeding habits of certain leafhoppers. *Can. Entomol.* 73: 39–53
97. Raatikainen M. 1967. Bionomics, enemies and population dynamics of *Javesella pellucida* (F.). *Ann. Agric. Fenn.* 6:149
98. Ramirez B-C, Garcin D, Calvert LA, Kolakofsky D, Haenni A-L. 1995. Capped-nonviral sequences at the 5′ end of the mRNAs of rice hoja blanca virus RNA4. *J. Virol.* 69:1951–54
99. Ramirez B-C, Haenni A-L. 1994. The molecular biology of tenuiviruses—a remarkable group of plant viruses. *J. Gen. Virol.* 75:467–75
100. Ramirez B-C, Lozano I, Constantino L-M, Haenni A-L, Calvert LA. 1993. Complete nucleotide sequence and coding strategy of rice hoja blanca virus RNA4. *J. Gen. Virol.* 74:2463–68
101. Ramirez B-C, Macaya G, Calvert LA, Haenni A-L. 1992. Rice hoja blanca virus genome characterization and expression in vitro. *J. Gen. Virol.* 73:1457–64
102. Rivera CT, Ou SH, Iida TT. 1966. Grassy stunt disease of rice and its transmission by the planthopper, *Nilaparvata lugens* Stal. *Plant Dis. Report.* 50:453–56
103. Roca de Doyle MM, Autrey LJC. 1992. Assessment of yield losses as a result of co-infection by maize streak virus and maize stripe virus in Mauritius. *Ann. Appl. Biol.* 120:443–50
104. Roca de Doyle MM, Autrey LJC, Jones P. 1992. Purification, characterization and serological properties of two virus isolates associated with the maize stripe disease in Mauritius. *Plant Pathol.* 41: 325–34
105. Schmaljohn CS, Beaty BJ, Calisher CH, Dalrymple JM, Elliott RM, et al. 1995. *Family Bunyaviridae in virus taxonomy*. See Ref. 84, pp. 300–15
106. Shikata E, Galvez GE. 1969. Fine flexuous threadlike particles in cells of plants and insect hosts infected with rice hoja blanca virus. *Virology* 39:635–41
107. Shimizu T, Toriyama S, Takahashi M, Akutsu K, Yoneyama K. 1996. Non-viral sequences at the 5′ termini of mRNAs derived from virus-sense and virus-complementary sequences of the ambisense RNA segments of rice stripe tenuivirus. *J. Gen. Virol.* 77:541–46
108. Shinkai A. 1962. Studies on insect transmission of rice virus diseases in Japan. *Bull. Natl. Agric. Sci. Ser. C.* 14, 1
109. Suzuki Y, Fuji S, Takahashi Y, Kojima M. 1992. Immunogold localization of rice stripe virus particle antigen in thin sections of insect host cells. *Ann. Phytopathol. Soc. Jpn.* 58:480–84
110. Takahashi M, Toriyama S, Hamamatsu C, Ishihama A. 1993. Nucleotide sequence and possible ambisense coding strataegy of rice stripe virus RNA segment 2. *J. Gen. Virol.* 74:769–73
111. Takahashi M, Toriyama S, Kikuchi Y, Hayakawa T, Ishihama A. 1990. Complementarity between the 5′ and 3′ terminal sequences of rice stripe virus RNAs. *J. Gen. Virol.* 71:2817–21
112. Toriyama S. 1982. Characterization of rice stripe virus: a heavy component carrying infectivity. *J. Gen. Virol.* 61:187–95
113. Toriyama S. 1983. Rice stripe virus. *Descriptions of Plant Viruses*, No. 269. Kew, Surrey, UK: Commonw. Mycol. Inst./Assoc. Appl. Biol. 5 pp.
114. Toriyama S. 1986. An RNA-dependent RNA polymerase associated with the filamentous nucleoproteins of rice stripe virus. *J. Gen. Virol.* 67:1247–55
115. Toriyama S. 1987. Ribonucleic acid polymerase activity in filamentous nucleoproteins of rice grassy stunt virus. *J. Gen. Virol.* 68:925–29
116. Toriyama S. 1995. Genus *Tenuivirus*. See Ref. 84, pp. 316–18
117. Toriyama S. 1995. Viruses and molecular biology of *Tenuivirus*. In *Pathogene-*

sis and Host Specificity in Plant Diseases, Vol. 3, *Viruses and Viroids*, ed. RP Singh, US Singh, K Kohmoto, pp. 211–23. Oxford: Pergamon
118. Toriyama S, Kimishima T, Takahashi M. 1997. The proteins encoded by rice grassy stunt virus RNA5 and RNA6 are only distantly related to the corresponding proteins ofother members of the genus *Tenuivirus*. *J. Gen. Virol.* 78:2355–63
119. Toriyama S, Takahashi M, Sano Y, Shimizu T, Ishihama A. 1994. Nucleotide sequence of RNA 1, the largest genomic segment of rice stripe virus, the prototype of the tenuiviruses. *J. Gen. Virol.* 75:3569–79
120. Toriyama S, Watanabe Y. 1989. Characterization of single- and double-stranded RNAs in particles of rice stripe virus. *J. Gen. Virol.* 70:505–11
121. Tsai JH. 1975. Occurrence of a corn disease in Florida transmitted by *Peregrinus maidis*. *Plant Dis. Report.* 59:830–33
122. Tsai JH. 1979. Vector transmission of mycoplasmal agents of plant diseases. In *The Mycoplasmas III*, ed. RF Whitcomb, JG Tully, pp. 265–307. New York: Academic
123. Tsai JH. 1996. Development and oviposition of *Peregrinus maidis* (Homoptera: Delphacidae) on various host plants. *Fla. Entomol.* 79:19–26
124. Tsai JH, Fisher JB. 1993. Feeding sites of some leaf and planthopper insects (Homoptera: Auchenorryncha) associated with coconut palms. *Principe* 37:35–41
125. Tsai JH, Wilson SW. 1986. Biology of *Peregrinus maidis* with description of immature stages (Homoptera: Delphacidae). *Ann. Entomol. Soc. Am.* 79:395–401
126. Tsai JH, Zitter TA. 1982. Characteristics of maize stripe virus transmission by the corn delphacid. *J. Econ. Entomol.* 75:397–400
127. Tu CW. 1980. The brown planthopper and its control in China. In *Rice Improvement in China and Other Asian Countries*, ed. TR Hargrove, pp. 149–56. Los Banos, Philippines: IRRI
128. Vance VB. 1991. Replication of potato virus X RNA is altered in coinfections with potato virus Y. *Virology* 182:486–94
129. Wilson MR, Claridge MF. 1985. The leafhopper and planthopper faunas of rice fields. See Ref. 89a, pp. 381–404
130. Wilson SW, O'Brien LB. 1987. A survey of planthopper pests of economically important plants (Homoptera: Fulgoroidea). *Proc. Int. Workshop Leafhoppers Planthoppers Econ. Importance, 2nd.* pp. 343–60
131. Zeigler RS, Morales FJ. 1990. Genetic determination of replication of rice hoja blanca virus within its planthopper vector, *Sogatodes oryzicola*. *Phytopathology* 80:559–66
132. Zhu Y, Hayakawa T, Toriyama S, Takahashi M. 1991. Complete nucleotide sequence of RNA3 of rice stripe virus: an ambisense coding strategy. *J. Gen. Virol.* 72:763–67
133. Zhu Y, Hayakawa T, Toriyama S. 1992. Complete nucleotide sequence of RNA-4 ofrice stripe virus isolate T, comparison with another isolate and with maize stripe virus. *J. Gen. Virol.* 73:1309–12