1 **Plant satellite viruses (*Albetovirus*, *Aumaivirus*, *Papanivirus*, *Virtovirus*)**

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10 **Abstract**

11 Satellite viruses are a polyphyletic group of viruses encoding structural components of their virions,
12 but incapable of completing the infection cycle without the assistance of a helper virus. Satellite
13 viruses have been described in animals, protists and plants. Satellite viruses replicating in plants
14 have small icosahedral virions and encapsidate positive-sense RNA genomes carrying a single gene
15 for the capsid protein. Thus, for genome replication these viruses necessarily depend on helper
16 viruses which can belong to different families. Plant satellite viruses are classified into genera
17 *Albetovirus*, *Aumaivirus*, *Papanivirus*, and *Virtovirus*. This chapter describes the diversity and
18 properties of these viruses.

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21 **Keywords:** satellite virus, helper virus, capsid protein, plant viruses, *Albetovirus*, *Aumaivirus*,
22 *Papanivirus*, *Virtovirus*, virus taxonomy

Introduction

Viruses span an entire range of morphological, genomic, and functional complexity. Some viruses are exceedingly complex, surpassing many unicellular organisms in terms of physical dimensions and genome sizes. For instance, members of the family *Mimiviridae* carry megabase-sized genomes encoding over 1000 proteins, including many of the molecular machineries required for their multiplication. On the other side of the complexity spectrum are viruses carrying just one or two genes. However, no matter how big or how small they are, replication of all viruses depends on certain functions provided by the host cell. Notably, some viruses depend not only on the host cells but also on other viruses for reproduction.

The phenomenon whereby one virus depends for its propagation on another virus has been first described in a plant virus system in the early 1960s. Certain preparations of tobacco necrosis viruses (TNV; genera *Alphanecrovirus* and *Betanecrosisvirus*, family *Tombusviridae*) contained two types of spherical particles that differed in size and antigenic properties; whereas the larger, TNV, particles could propagate autonomously, the smaller ones were unable to replicate in the absence of the larger ones. The smaller particles became known as particles of satellite tobacco necrosis virus (STNV), whereas TNV is referred to as STNV's helper virus. Subsequently, several other plant viruses having features similar to those of STNV have been discovered. Furthermore, viruses which depend on another virus for reproduction have been described in protists and animals. Overall, satellite viruses vary considerably in terms of complexity and evolutionary origins. Also, the functions supplemented by the helper viruses vary from one virus system to another. For instance, satellite viruses of plants rely on helper viruses for genome replication, whereas members of the *Lavidaviridae* depend on giant mimiviruses for transcription.

The tripartite relationship between satellite viruses, helper viruses and host cells is an example of hyperparasitism, which is not uncommon in complex natural ecosystems. Notably, there are other genetic elements which parasitize viruses, including satellite nucleic acids. The key difference between satellite nucleic acids and satellite viruses is that the latter necessarily encode components of their virions, whereas the former do not. Instead, satellite nucleic acids commonly encode proteins responsible for the replication of the cognate nucleic acid, whereas the components of the virion for horizontal transfer are being provided by the helper viruses.

Due to their dependency on other, quasi-autonomous viruses for reproduction, most satellite viruses, with a notable exception of members of the genera *Dependoparvovirus* and *Deltavirus*, have been for a long time considered as sub-viral agents and not officially classified by the International Committee on Taxonomy of Viruses (ICTV). However, in 2016, the arbitrary distinction between satellite viruses and other viruses has been abandoned and satellite viruses were incorporated into the ICTV classification scheme, resulting in establishment of several genera and families. Plant satellite viruses have been classified into genera *Albetovirus*, *Aumavirus*, *Papanivirus*, and *Virtovirus*; family *Sarothroviridae* has been created for classification of positive-sense (+)RNA satellite viruses that infect arthropods, and *Lavidaviridae* includes double-stranded DNA viruses replicating in protist cells and parasitizing giant viruses of the family *Mimiviridae*.

This chapter describes the diversity and properties of plant satellite viruses belonging to the genera *Albetovirus*, *Aumavirus*, *Papanivirus* and *Virtovirus*.

Albetoviruses: Viruses related to satellite tobacco necrosis virus

STNV is one of the most extensively studied satellite viruses. Over the years, many properties of this virus, including its genome sequence, virion structure and assembly, as well as interaction with the helper virus, have been elucidated. The linear STNV genome consists of 1,239 nucleotides and

encodes a single capsid protein (CP) of 195 amino acids, which is necessary and sufficient for virion formation. Assembly of icosahedral virions proceeds cooperatively via interactions between the packaging signals, degenerated stem-loop structures distributed throughout the genome, and multiple CP copies.

In the natural habitat, STNV is transmitted through the soil the same way as its helper virus, i.e., by zoospores of a plant-pathogenic fungus *Olpidium brassicae*, suggesting that both viruses share certain determinants. For both TNV and STNV, the 5' ends of the genomes are phosphorylated and lack a 7-methylguanylate cap or a genome-linked protein, whereas the 3' termini lack a polyadenylation sequence. Several *cis*-acting elements located within the 5' and 3' untranslated regions (UTRs) are responsible for efficient translation and replication of the STNV genome. The 3' and 5' UTRs of STNV and TNV can be exchanged without abolishing RNA accumulation, although the translation elements at the 3'-terminal regions appear to be unrelated in the two viruses.

Three serotypes of STNV have been described, including STNV-1 (or STNV), STNV-2, and STNV-C. Different STNV strains are activated by different viruses. The replication of STNV-1 and STNV-2 is supported by isolates of tobacco necrosis virus A (TNV-A is the sole member of the type species of the genus *Alphanecrovirus*, *Tobacco necrosis virus A*), whereas TNV-D, the sole member of the type species of the genus *Betanecrovirus*, *Tobacco necrosis virus D*, supports the replication of STNV-C. Genome sequences for the three STNV strains have been determined (Table 1). The organization of the genomes of the three viruses is overall similar. CPs are ~50-63% identical in sequence (Figure 1B). Whereas the 5' UTRs of STNV-1 and STNV-2 are 30 nt in length and are nearly identical, the 5' UTR of STNV-C is significantly longer (101 nt). Similarly, the 3' UTR of STNV-C RNA is significantly different compared to those of STNV-1 and STNV-2 (40 and 38% similarity, respectively), which are approximately 64% similar to each other.

STNV-like viruses STNV-1, STNV-2, and STNV-C are classified into three species, *Tobacco albetovirus 1*, *2*, and *3*, respectively, within a genus *Albetovirus* (sigil: *Al-* for *alphanecrovirus* [helper virus], *be-* for *betanecrovirus* [helper virus], *to-* for *tobacco*).

Aumaivirus: Satellite maize white line mosaic virus

The fourth, more divergent member of the STNV-like virus group is satellite maize white line mosaic virus (SMWLMV). SMWLMV depends on maize white line mosaic virus (MWLMV; species *Maize white line mosaic virus*, genus *Aureusvirus*, family *Tombusviridae*) for multiplication. MWLMV can infect maize in the absence of SMWLMV, whereas the SMWLMV particle can infect maize only when co-inoculated with MWLMV. The ssRNA genome of SMWLMV is 1,168 nucleotides in length and encodes one capsid protein. Similar to STNV-like viruses, the SMWLMV virion is 17 nm in diameter. SMWLMV CP displays 32% identity ($E=1e-14$) to the corresponding protein of STNV-1, indicating that SMWLMV and the STNV-like viruses have diverged from a common ancestor. However, due to limited sequence similarity between the CPs of STNV-like viruses and SMWLMV, the latter virus has been classified into a species, *Maize aumaivirus 1*, within a separate genus *Aumaivirus* (sigil: *Au-* for *aureusvirus* [helper virus], *mai-* for *maize*).

Papaniviruses: Viruses related to satellite panicum mosaic virus

Satellite panicum mosaic virus (SPMV) is completely dependent on panicum mosaic virus (PMV), a member of the species *Panicum mosaic virus*, genus *Panicovirus*, family *Tombusviridae*, for replication as well as systemic spread in plants. Like in the case of STNV, the 5'-terminus of the SPMV genome is phosphorylated and lacks a 7-methylguanylate cap. Several secondary structure elements implicated in the replication of the SPMV genome were predicted in the 5' and 3' UTRs. The 826 nt-

long ssRNA genome of SPMV contains two open reading frames. However, only one of them (encoding CP) was found to be expressed in *in vitro* translation assays. The sequence of SPMV CP is not appreciably similar to those of STNV-like viruses (below 15% identity; Figure 1B). However, X-ray structure analysis of the SPMV particle revealed that the protein has a similar jelly-roll fold as the corresponding protein of STNV (Figure 1A). Beside its structural role, SPMV CP has several other biological functions, most notably systemic accumulation, maintenance, and movement of the cognate SPMV RNA. Interestingly, the latter activities of SPMV CP apparently extend to the helper virus RNA, assisting in its maintenance or stabilization. It is noteworthy that PMV and SPMV are involved in a peculiar tripartite association with a 350 nt-long satellite RNA (satRNA), whereby PMV provides necessary factors for satRNA replication and SPMV provides CPs for satRNA encapsidation.

Two other viruses encoding SPMV-like CPs have been reported. The first one, satellite St. Augustine decline virus (SSADV), is associated with the St. Augustine decline strain of PMV. SSADV is 95% identical to SPMV over the entire genome length (36 nt changes, 5 aa changes) and can be considered as a different strain of SPMV. The second putative satellite virus, satellite grapevine virus (SGVV), has been discovered by deep sequencing of total genomic RNA from grapevine. However, neither the viral particles nor the associated helper virus have been characterized. SGVV CP shares ~24% sequence identity with SPMV CP (Figure 1B).

SPMV and SSADV have been assigned into a genus *Papanivirus* (sigil: *Pa-* for *panicovirus* [helper virus], *pani-* for *panicum*), within a species *Panicum papanivirus 1*. By contrast, SGVV remains unclassified due to lack of information on its ability to form virions as well as on the identity of its helper virus.

Homologs of SPMV CP are encoded by certain satRNAs. Particularly, bamboo mosaic virus satellite RNA (satBaMV; 836 nt) encodes a protein, P20, which is 44% identical to the CP of SPMV (Figure 1B). P20 plays a role in the accumulation and movement of the satBaMV but does not participate in satRNA encapsidation. Instead, satBaMV is packaged into rod-shaped particles by the CP of the helper bamboo mosaic virus, a member of the family *Alphaflexiviridae*. In addition, a sequence of olive viral satellite RNA (OVsatRNA) has been deposited to GenBank (Table 1) that encodes a protein 35% identical to P20 of satBaMV (Figure 1B). Considering the conservation of the SPMV-like CPs, it appears likely that satBaMV and OVsatRNA have evolved from genuine satellite viruses, once again emphasizing the apparent ease with which transitions between different types of mobile elements (i.e., parasitic nucleic acids and viruses) occur.

Virtovirus: Satellite tobacco mosaic virus

Satellite tobacco mosaic virus (STMV) has been isolated from tree tobacco (*Nicotiana glauca*) and is naturally associated with and dependent on tobacco mild green mosaic virus (TMGMV), a member of the species *Tobacco mild green mosaic virus*, genus *Tobamovirus*, family *Virgaviridae*. However, under experimental settings, STMV can adapt and replicate in many plant hosts (e.g., tobacco, pepper, tomato) in association with other tobamoviruses, including tobacco mosaic virus (TMV). Thus far, STMV is the only known satellite virus that uses rod-shaped viruses as helpers.

The STMV genome is a linear ssRNA molecule of 1,059 nt that contains two open reading frames (ORF), both of which are functional in the *in vitro* translation assay. The first ORF encodes a protein of 58 aa which lacks similarity to proteins in the public databases and appears to be dispensable for STMV multiplication. Indeed, certain naturally occurring isolates of STMV contain a deletion within ORF1 and do not produce the corresponding product. The second ORF encodes STMV CP, which also has no identifiable homologs in sequence databases. However, structural analysis shows that the

STMV CP has a jelly-roll fold similar to those of STNV and SPMV (Figure 1A), suggesting that the three satellite viruses might be evolutionarily related.

As in the case of STNV and SPMV, but different from the helper TMV virus, the genome of STMV lacks a 7-methylguanylate cap and the first 6 nucleotides of the STMV RNA are identical to those of the STNV genome. However, in contrast to STNV and SPMV, the 5' end of STMV genome is not phosphorylated. The 3' UTR is predicted to contain a series of pseudoknots followed by a tRNA-like structure which can be amino acylated with histidine. The latter features are strikingly similar to those of the genome of helper TMV and other tobamoviruses, with 40–50 nt-long regions of near identity among the STMV and TMV 3' UTRs. These secondary structure elements play critical roles in STMV genome replication, translation, and initiation of virion assembly.

STMV is currently a sole representative of the species *Tobacco virtovirus 1* within the genus *Virtovirus* (sigil: *Vir*- for *vir*gavirus [helper virus], *to*- for *to*bacco).

Effect of mixed infections on the host and the helper viruses

The parasitic lifestyle of satellite viruses is usually associated with negative effects on the fitness of the helper virus, whereas the effect on the host cell during a co-infection might differ drastically between viruses. The negative effect of STNV on the propagation of its helper virus manifests as a decrease in (i) the number of necrotic lesions formed in co-inoculated plant leaves, (ii) diameter of the lesions, and (iii) the amount of TNV produced in inoculated leaves. However, several factors influence the extent to which TNV multiplication is affected, particularly the relative concentrations of the satellite and helper viruses in the inoculum, the physiological state of the host plants before and during the infection, and the type of plant used for the assay. An excess of STNV present during co-infection reduces the yield of TNV to non-detectable levels. This reduction is associated with a selective decrease in the synthesis of TNV capsid protein, suggesting that STNV affects TNV virion production. By contrast, co-infection of PMV with SPMV exacerbates PMV disease phenotype in millet plants resulting in a severe chlorosis and stunting. Expression of the SPMV CP from a Potato virus X-based vector was shown to induce the formation of chlorotic spots on the host as well as non-host plants, confirming that SPMV CP is a pathogenicity determinant.

The effects of STMV on the multiplication of its helper virus, as well as on the helper virus-induced symptoms, are dependent on the host. In tobacco plants, STMV does not change the mild mosaic symptom caused by TMGMV, whereas in jalapeno pepper severe leaf blistering induced by TMGMV is attenuated by STMV infection. Furthermore, tobamovirus titers are greatly decreased by STMV in pepper compared to other hosts.

Common ancestry of plant satellite viruses?

Plant satellite viruses from the four groups described above all propagate in flowering plants (angiosperms) and share several genomic and structural characteristics that distinguish them from other known viruses. Namely, representatives of all four satellite virus groups form capsids with $T=1$ icosahedral symmetry and composed of 60 CP subunits (Figure 1A). Although common among ssDNA viruses (e.g., nanoviruses and circoviruses), $T=1$ capsids are not used by other known ssRNA viruses, which typically have larger $T=3$ or $T=4$ capsids. Furthermore, the CPs of all described plant satellite viruses have the same jelly-roll fold (Figure 1A), despite negligible sequence similarity. The similarities also extend to the genomic characteristics. In particular, in all plant satellite viruses, the linear ssRNA genomes lack the 5' 7-methylguanylate caps and polyadenylation sequences in their 3' UTRs. Furthermore, 5' ends of the STNV and SPMV genomes are phosphorylated, whereas the first

six nucleotides at the 5' terminus of the STMV genome are identical to those in STNV. Considering these similarities, it is conceivable that all plant satellite viruses have evolved from a common ancestor. Notably, in structural comparison of the satellite virus CPs with the jelly-roll CPs of other viruses, satellite viruses formed a monophyletic clade, indicating that they indeed might share a common ancestor, despite high sequence divergence.

Concluding remarks

Due to their simplicity, satellite viruses have been (and continue to be) used as models for elucidating fundamental biological processes, including mechanisms of cap-independent protein translation as well as virion assembly and viral genome encapsidation. For instance, it has been recently demonstrated that assembly of the icosahedral STNV virion proceeds through

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Figure legends

Figure 1. Relationships between plant satellite viruses. A. Structural similarity between the virions (top) and jelly-roll capsid proteins (bottom) of satellite tobacco necrosis virus (STNV; PDB ID: 2BUK), satellite panicum mosaic virus (SPMV; PDB ID: 1STM) and satellite tobacco mosaic virus (STMV; PDB ID: 4OQ8). All three virions have $T=1$ icosahedral symmetry. Images of the depicted virions were downloaded from the VIPER database (viperd.b.scripps.edu/). B. Pairwise identities between capsid proteins of plant satellite viruses were calculated using SIAS (<http://imed.med.ucm.es/Tools/sias.html>). Identity values are highlighted in cyan, orange, green and red, respectively. SPMV-like capsid protein homologs encoded by satellite RNAs (OVsatRNA and satBaMV) and SGVV are shaded in light green. The boxes containing identity values among the capsid proteins of STNV-like viruses, SMWLMV, SPMV-like viruses, and STMV are coloured according to the proposed classification of the plant satellite viruses: *Albetovirus*, cyan; *Aumaivirus*, orange; *Papanivirus*, green; *Virtovirus*, red. Abbreviations: SMWLMV, satellite maize white line mosaic virus; SSADV, satellite St. Augustine decline virus; SGVV, satellite grapevine virus; OVsatRNA, olive viral satellite RNA; satBaMV, bamboo mosaic virus satellite RNA. Note that OVsatRNA and satBaMV are not satellite viruses but satellite nucleic acids because they are packed into the virions of helper viruses.

297 **Table 1.** General properties of plant satellite viruses.

Satellite virus	Species	Helper virus	Accession #	Genome, nt	Capsid Ø, nm	Comments
Albetovirus						
satellite tobacco necrosis virus (STNV-1)	<i>Tobacco albetovirus 1</i>	tobacco necrosis virus A (<i>Tombusviridae</i>)	V01468	1,239	17	STNV suppresses the replication of its helper virus and ameliorates the TNV-induced symptoms in different hosts.
satellite tobacco necrosis virus 2 (STNV-2)	<i>Tobacco albetovirus 2</i>	tobacco necrosis virus A (<i>Tombusviridae</i>)	M64479	1,245	17	STNV and STNV-2 coat protein genes share 55% nucleotide sequence identity, whereas the UTRs are more similar.
satellite tobacco necrosis virus C (STNV-C)	<i>Tobacco albetovirus 3</i>	tobacco necrosis virus D (<i>Tombusviridae</i>)	AJ000898	1,221	17	STNV and STNV-C coat proteins share 62% sequence identity, whereas the 3'UTRs are 40% identical.
Aumaivirus						
satellite maize white line mosaic virus (SMWLMV)	<i>Maize aumaivirus 1</i>	maize white line mosaic virus (<i>Tombusviridae</i>)	M55012	1,168	17	CP is 32% identical to that of STNV-1.
Papanivirus						
satellite panicum mosaic virus (SPMV)	<i>Panicum papanivirus 1</i>	panicum mosaic virus (<i>Tombusviridae</i>)	M17182	826	16	Besides virion formation, CP of SPMV has several other biological functions, including systemic accumulation, maintenance and movement of the SPMV RNA.
satellite St. Augustine decline virus (SSADV)	<i>Panicum papanivirus 1</i>	St. Augustine decline virus strain of PMV (<i>Tombusviridae</i>)	L10083	824	ND	SSADV is a strain of SPMV (36 nt substitutions; 5 aa changes).
satellite grapevine virus (SGVV)	unclassified	grapevine virus F (<i>Betaflexiviridae</i>) ?	KC149510	1,060	ND	SGVV and GVF share stem-loop structures at the 5' ends of the genomes.
Virtovirus						
satellite tobacco mosaic virus (STMV)	<i>Tobacco virtovirus 1</i>	tobacco mosaic virus (<i>Virgaviridae</i>)	M25782	1,059	17	The 3'UTR is similar to that of tobamoviruses, with a clear sequence similarity between STMV and TMV.

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