Tobamovirus

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Glossary

Origin of assembly Stem–loop structure that is the site of initiation of virion assembly.
Pseudoknot An RNA structure with base pairing between a loop and other regions of the RNA.

Introduction

Early research in the late 1800s on the causal agent of the mosaic disease of tobacco led to the discovery of viruses as new infectious agents. Thus tobacco mosaic virus (TMV), the type species of the genus *Tobamovirus*, became the first virus to be discovered, and subsequently has had a significant role in many fundamental discoveries in virology. The first quantitative biological assay for plant viruses was the use of *Nicotiana glutinosa* plants, which produce necrotic local lesions when inoculated with TMV and many tobamoviruses. The resistance gene *N* that confers this hypersensitive response-type resistance to TMV was the first resistance gene against a plant virus to be cloned and characterized. TMV was the first virus to be purified and crystallized, which led to the discovery of the nucleoprotein nature of viruses and determination of the atomic structure of the coat protein and the virion. TMV was the first virus to be visualized in the electron microscope, confirming the predicted rigid rod-shaped virions. The genetic material of TMV was shown to be RNA, a property previously thought to be restricted to DNA. The first viral protein for which an amino acid sequence was determined was the coat protein of TMV. TMV was the first virus to be mutagenized and the subsequent determination of coat protein sequences from a number of strains and mutants helped to establish the universality of the genetic code. Methods of infecting plant protoplasts with viruses were developed with the tobacco–TMV system, creating a synchronous system to study events in the infection cycle. The TMV 30 kDa protein was the first viral protein shown to be required for virus movement.

Taxonomy and Classification

The genus *Tobamovirus* has not been assigned to a family. Currently, there are 23 recognized species within the genus *Tobamovirus* (Table 1). Several recently sequenced viruses are tentative members of new species (Table 1). Although tobamoviruses comprise one of the more intensively studied plant virus genera, taxonomy has often been confusing. Historically, plant viruses with rigid virions of approximately $18 \times 300\,nm^2$ and causing various diseases were all designated strains of TMV. Thus, many viruses originally referred to as TMV strains are now recognized as belonging to separate species. For example, the tobamovirus that was referred to as the tomato strain of TMV, and is approximately 80% identical to TMV at the nucleotide sequence level, is actually tomato mosaic virus. One criterion for distinguishing the members of separate tobamovirus species is a nucleotide sequence difference of at least 10%.

Virus Structure and Composition

Tobamovirus virions are straight tubes of approximately $18 \times 300\,nm^2$ with a central hollow core 4 nm in diameter. Virion composition is approximately 95% protein and 5% RNA. For TMV, approximately 2100 subunits of a single coat protein are arranged in a right-handed helix around a single genomic RNA molecule, with each subunit associated with three adjacent nucleotides. Protein–protein associations are the essential first event of virion assembly. Coat protein subunits assemble into several types of aggregates. Coat protein monomers and small heterogeneous aggregates of a few subunits are collectively referred to as 'A-protein'. The equilibrium between A-protein and larger aggregates is primarily dependent upon pH and ionic strength. Purified coat protein and viral RNA can assemble into infectious particles *in vitro*. Larger aggregates are disks composed of two individual stacked rings of coat protein subunits, and protohelices. Protohelices contain approximately 40 coat protein subunits arranged in a spiral around a central hollow core, similar to the arrangement within the virion. A sequence-specific stem–loop structure in the RNA, the origin of assembly (OAS), initiates encapsidation and prevents defective packaging that could result from multiple independent initiation events on a single RNA molecule. Virion assembly initiates as the primary loop of the OAS is threaded through a coat protein disk or protohelix with both ends of the RNA trailing from one side. The conformation of the coat protein protohelix changes as the RNA becomes embedded within the groove between the two layers of subunits. Elongation is

Table 1 Definitive and tentative members of the genus *Tobamovirus*

Cucumber fruit mottle mosaic virus	CFMMV
Cucumber green mottle mosaic virus	CGMMV
Frangipani mosaic virus	FrMV
Hibiscus latent Fort Pierce virus	HLFPV
Hibiscus latent Singapore virus	HLSV
Kyuri green mottle mosaic virus	KGMMV
Maracuja mosaic virus	MaMV
Obuda pepper virus	ObPV
Odontoglossum ringspot virus	ORSV
Paprika mild mottle virus	PaMMV
Pepper mild mottle virus	PMMoV
Ribgrass mosaic virus	RMV
Sunn-hemp mosaic virus	SHMV
Sammons' Opuntia virus	SOV
Tobacco latent virus	TLV
Tobacco mild green mosaic virus	TMGMV
Tobacco mosaic virus	TMV
Tomato mosaic virus	ToMV
Turnip vein-clearing virus	TVCV
Ullucus mild mottle virus	UMMV
Wasabi mottle virus	WMoV
Youcai mosaic virus	YoMV
Zucchini green mottle virus	ZGMMV
Tentative members	
Brugmansia mild mottle virus	
Cucumber mottle virus	
Cactus mild mottle virus	
Streptocarpus flower break virus	
Tropical soda apple mosaic virus	

bidirectional, proceeding rapidly toward the 5′ end of the RNA as the RNA loop is extruded through the elongating virion and additional coat protein disks are added. There is disagreement about the mechanism of elongation toward the 3′ terminus of the RNA, but it appears that this slower process involves the addition of smaller protein aggregates.

Subgenomic mRNAs containing the OAS are encapsidated into shorter virions that are not required for infectivity. The OAS is located within the open reading frame (ORF) for the movement protein of most tobamoviruses. The level of accumulation of a particular subgenomic mRNA containing the OAS determines the relative proportion of that particular virion species. Thus, all tobamovirus virion populations contain a small percentage of movement protein subgenomic mRNAs. In some tobamoviruses, including cucumber green mottle mosaic virus, hibiscus latent Singapore virus (HLSV), kyuri green mottle mosaic virus, maracuja mosaic virus, sunn-hemp mosaic virus, zucchini green mottle mosaic virus, and cactus mild mottle virus, the OAS is located within the coat protein ORF. Thus, these so-called subgroup 2 tobamoviruses produce a significant proportion of small virions that contain coat protein subgenomic mRNA. Hybrid nonviral RNAs containing an OAS will also assemble with coat protein into virus-like particles of length proportional to that of the RNA.

Genome Organization

The genome of tobamoviruses consists of one single-stranded positive-sense RNA of approximately 6300–6800 nt (**Figure 1(a)**). There is a methylguanosine cap at the 5′ terminus, followed by an AU-rich leader 55–75 nt in length. The 3′ nontranslated end of the RNA consists of sequences that can be folded into a series of pseudoknot structures, followed by a tRNA-like terminus. The hibiscus-infecting tobamoviruses HLSV and hibiscus latent Fort Pierce virus contain a polyA stretch between the 3′ end of the coat protein ORF and the tRNA-like structure. The tRNA-like terminus can be aminoacylated *in vitro*, and in most cases specifically accepts histidine. The exception is the 3′ terminus of SHMV, which accepts valine and appears to have arisen by a recombination event between a tobamovirus and a tymovirus.

Four ORFs that are contained within all tobamovirus genomes (**Figure 1(a)**) correspond to the proteins found in infected tissue. Two overlapping ORFs begin at the 5′ proximal start codon. Termination at the first in-frame stop codon produces a 125–130 kDa protein. A 180–190 kDa protein is produced by readthrough of this leaky termination codon approximately 5–10% of the time. The remaining proteins are expressed from individual 3′ co-terminal subgenomic mRNAs, from which only the 5′ proximal ORF is expressed (**Figures 1(c) and 2(a)**). The next ORF encodes the 28–34 kDa movement protein, which has RNA-binding activity and is required for cell-to-cell movement. The 3′ proximal ORF encodes a 17–18 kDa coat protein. A subgenomic mRNA containing an ORF for a 54 kDa protein that encompasses the readthrough domain of the 180–190 kDa ORF has been isolated from infected tissue, although no protein has been detected.

Within the protein-coding regions of the genome, there are nucleotide sequences that also function as *cis*-acting elements for subgenomic mRNA synthesis, virion assembly, and replication. Gene expression from subgenomic mRNAs is regulated both temporally and quantitatively. The movement protein is produced early and accumulates to low levels, whereas the coat protein is produced late and accumulates to high levels. The regulatory elements for subgenomic mRNA synthesis are located on the genome-length complementary RNA overlapping the upstream ORF (**Figure 1(a)**). There is limited (40%) sequence identity between the TMV movement protein and coat protein subgenomic promoters. The TMV movement protein subgenomic promoter is located upstream of the movement protein ORF, flanking the transcription initiation site. Unlike the movement protein subgenomic promoter, full activity of the coat protein subgenomic promoter requires sequences within the coat protein ORF.

Viral Proteins

The tobamovirus 125–130/180–190 kDa proteins are involved in viral replication, gene expression, and movement. Both are contained in crude replicase preparations, and temperature-sensitive replication-deficient mutants map to these ORFs. The 125–130/180–190 kDa proteins contain two functional domains common to replicase proteins of many positive-stranded RNA plant and animal viruses (Figure 1(b)). The N-terminal domain has methyltransferase and guanylyltransferase activities associated with capping of viral RNA. The second common domain is a proposed helicase, based upon conserved sequence motifs. The readthrough domain of the 180–190 kDa protein has sequence motifs characteristic of RNA-dependent RNA polymerases. Both proteins are necessary for efficient replication, although the TMV 126 kDa protein is dispensable for replication and gene expression in protoplasts. The 125–130 kDa protein (or sequences within this region) of the 180–190 kDa protein are required for cell-to-cell movement. Additionally, these multifunctional proteins are symptom determinants, as mutations in mild strains map to these ORFs.

The 28–34 kDa movement protein has a plasmodesmatal binding function associated with its C-terminus and a single-stranded nucleic acid-binding domain associated with the N-terminus. The movement protein–host interaction determines whether the virus can systemically infect some plant species. Although principally a structural protein, the coat protein is also involved in other host interactions. Coat protein is required for efficient long-distance movement of the virus. Coat protein is also a symptom determinant in some susceptible plant species and an elicitor of plant defense mechanisms in other plant species.

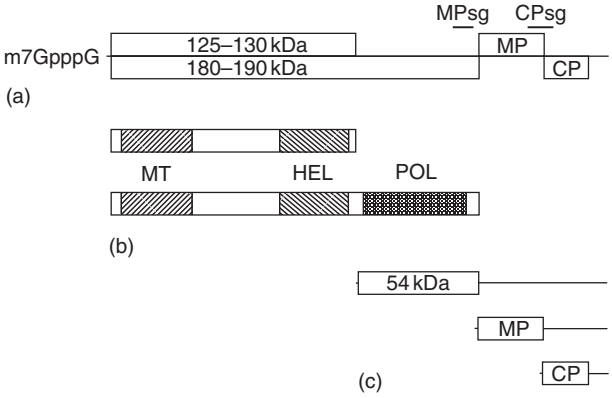

Figure 1 Tobamovirus genome organization and gene expression strategy. (a) Tobamovirus genome organization. ORFs designated as open boxes. Nontranslated sequences designated as lines; positions of subgenomic promoters are marked. (b) Nonstructural proteins involved in tobamovirus replication. Functional domains shared with other viruses within the 'alphavirus supergroup' are designated as hatched boxes. (c) Subgenomic mRNAs with 5′ proximal ORF labeled. MP, movement protein; CP, coat protein; MT, methyltransferase; HEL, helicase; POL, polymerase; MPsg, MP subgenomic mRNA; CPsg, CP subgenomic mRNA.

Interactions between Viral and Host Proteins

Available evidence suggests that the interactions of viral proteins with host factors are important determinants of viral movement and host ranges. Amino acid substitutions in the movement protein and 125–130/180–190 kDa

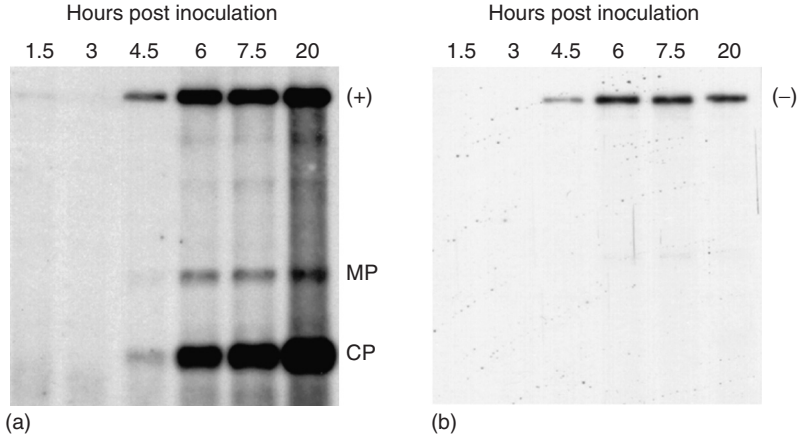

Figure 2 Time course of accumulation of TMV positive-stranded (a) and negative-stranded (b) RNAs in tobacco protoplasts. Total RNA was extracted from tobacco suspension cell protoplasts transfected with TMV *in vitro* transcripts at the time points indicated and analyzed by Northern blot hybridization. (+), TMV genomic RNA; MP, movement protein subgenomic RNA; CP, coat protein subgenomic RNA; (–), negative-stranded complement to the genomic RNA.

proteins can alter the movement function in different hosts. Some viruses, including tobamoviruses, can assist movement of other viruses that are incapable of movement in a particular plant species. These interactions suggest that there are more precise associations of viral proteins with host factor(s) than with viral RNA. Additionally, precise coat protein–plant interactions are required for movement to distal positions within the plant. The helicase domain of the 130–190 kDa proteins elicits the *N* gene-mediated resistance in *N. glutinosa.*

Virus Replication

Virions or free viral RNA will infect plants or protoplasts. Because tobamoviruses have a genome consisting of messenger-sense RNA that is infectious, one of the first events is translation of the 5′-proximal ORFs to produce the proteins required for replication of the genomic RNA and transcription of subgenomic mRNAs. When virions are the infecting agent, the first event is thought to be co-translational disassembly, in which the coat protein subunits at the end of the virion surrounding the 5′ end of the RNA loosen, making the RNA available for translation. Ribosomes then associate with the RNA, and translation of the 126/183 kDa ORFs is thought to displace coat protein subunits from the viral RNA. After the formation of an active replicase complex, a complementary negative-strand RNA is synthesized from the genomic positive-strand RNA template. Negative-strand RNA serves as template for both genomic and subgenomic mRNAs. Negative-strand RNA synthesis ceases early in infection, while positive-strand RNA synthesis continues. This results in an asymmetric positive- to negative-strand RNA ratio. Early in infection, genomic RNA functions as template for negative-strand RNA synthesis and as mRNA for production of the 126/183 kDa proteins. Later in the infection cycle, most of the newly synthesized genomic RNA is encapsidated into virions. Subgenomic mRNAs transcribed during infection function as mRNA for the 3′ ORFs. Within cells of an infected leaf, replication proceeds rapidly between approximately 16 and 96 h post infection within a cell, then ceases. Even though the infected cells become packed with virions, these cells remain metabolically active for long periods. During the early stages of infection of an individual cell, the infection spreads through plasmodesmatal connections to adjacent cells. This event requires the viral movement protein that modifies plasmodesmata to accommodate larger molecules and the 126/183 kDa proteins. Movement through plasmodesmata does not require the coat protein. A second function of the movement protein appears to be binding to the viral RNA to assist its movement through the small plasmodesmatal openings. The movement protein also appears to associate with the cytoskeleton. As the virus spreads from cell to cell throughout a leaf, it enters the phloem for rapid long-distance movement to other leaves and organs of the plant, a complex process that requires the coat protein.

cis-Acting Sequences

The 5′ nontranslated region contains sequences that are required for replication. This region is an efficient translational leader. The 3′ nontranslated region contains *cis*-acting sequences that are involved in replication. Certain deletions within the pseudoknots are not lethal, but result in reduced levels of replication. Exchange of 3′ nontranslated elements between cloned tobamovirus species has resulted in some lethal and nonlethal hybrids, suggesting a requirement for sequence specificity and/or secondary structure. The 3′ nontranslated region appears to be a translational enhancer, both in the viral genome and when fused to heterologous reporter mRNAs. Sequences encoding the internal ORFs for the movement and coat proteins are dispensable for replication. Duplication of the subgenomic promoters results in transcription of an additional new subgenomic mRNA. Heterologous tobamovirus subgenomic promoters inserted into the viral genome are recognized by the replicase complex and transcribed. Foreign sequences inserted behind tobamovirus subgenomic mRNA promoters have been expressed to high levels in plants and protoplasts.

Satellite Tobacco Mosaic Virus

Satellite tobacco mosaic virus (STMV), a tobamovirus-dependent satellite virus, was isolated from *Nicotiana glauca* plants infected with tobacco mild green mosaic virus (TMGMV). The STMV genome consists of one single-stranded positive-sense RNA of 1059 nt. The 240 3′ nucleotides share approximately 65% sequence identity with TMGMV and TMV, contain pseudoknot structures, and have a tRNA-like terminus. No sequence similarity with any tobamovirus exists over the remainder of the genome. Two overlapping ORFs that are expressed in *in vitro* translation reactions are present in the genomic RNA of most STMV isolates. The 5′-proximal ORF encodes a 6.8 kDa protein that has not been detected *in vivo* and is not present in all STMV isolates. The second ORF encodes a 17.5 kDa coat protein that is not serologically related to any tobamovirus coat protein. The 17 nm icosahedral virions are composed of a single STMV genomic RNA encapsidated within 60 STMV coat protein subunits. Replication of natural populations of STMV is supported by other tobamoviruses, but at lower levels than with the natural helper virus, TMGMV. The host range of STMV parallels that of the helper virus.

See also: Plant Virus Diseases: Economic Aspects; Satellite Nucleic Acids and Viruses; Tobacco Mosaic Virus.

Further Reading

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Tobravirus

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Taxonomy and Characteristics

The genus *Tobravirus* is comprised of three species, the type species *Tobacco rattle virus* (TRV) together with *Pea early-browning virus* (PEBV) and *Pepper ringspot virus* (PepRSV), which was previously referred to as the CAM strain of TRV. The genus has not been assigned to a virus family. Tobraviruses have a genome of two, positive-sense, single-strand RNAs that are packaged separately into rod-shaped particles. In some situations, the larger genomic RNA (RNA1) can cause a systemic infection in the absence of the second, smaller RNA (RNA2) and without the formation of virus particles. Tobraviruses are transmitted between plants by root-feeding nematodes of the genera *Trichodorus* and *Paratrichodorus*, and in some plant species are also seed transmitted.

Virus Particle Production and Structure

Tobravirus RNA1 is encapsidated into the L (long) particle with a length of 180–215 nm, depending on virus species, and RNA2 is encapsidated into S (short) particles which range in length from 46 to 115 nm, depending on virus isolate (**Figure 1**). Both L and S particles have an apparent diameter of 20–23 nm, depending on the technique used to examine them. *In vitro* translation experiments using RNA extracted from purified virus preparations, as well as studies with the TRV SYM isolate, which has an unusual genome structure, showed that some, if not all, tobravirus subgenomic (sg)RNAs are also encapsidated, in particles of various lengths. The tobraviruses encode a single coat protein (CP), molecules of which assemble in a helical arrangement around a central cavity with a diameter of 4–5 nm, and with a distance of 2.5 nm between successive turns of the helix. *In vitro* reconstitution experiments suggested that virus particle formation initiates at the 5′ end of the viral RNA, although the encapsidated sgRNAs do not carry the 5′ terminal part of the virus genomic RNAs. Peptide mapping showed that the major antigenic regions of the CP are, in descending order of strength, the C-terminal 20 amino acids (aa), 5 aa in the central region of the protein and 5 aa at the N-terminus. This and other spectroscopic analyses suggest that the N- and C-termini are exposed on the outer surface of the particle, while the central region is exposed in the central canal (where interactions of the CP with the viral RNA take place). The C-terminal domain appears to be unstructured and

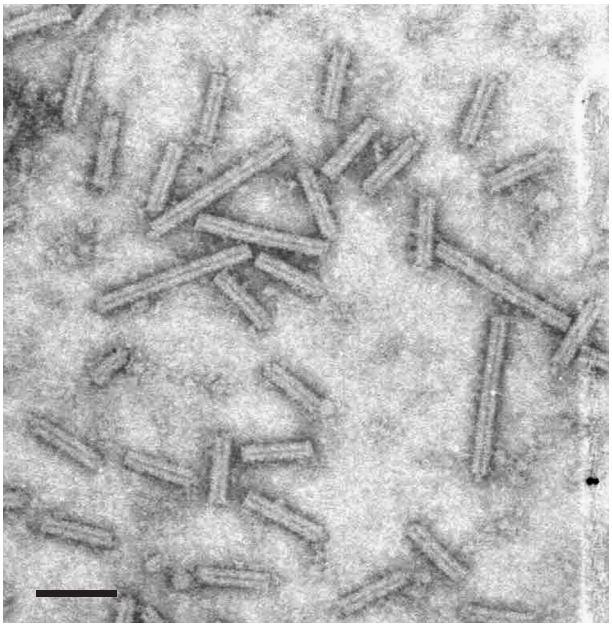

Figure 1 Electron micrograph of long and short particles of TRV isolate RH. Scale = 100 nm.