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Feng Qu
University of Nebraska-Lincoln, qu.28@osu.edu

Thomas Jack Morris
University of Nebraska-Lincoln, jmorris1@unl.edu

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Feng Qu and T. Jack Morris
School of Biological Sciences, University of Nebraska–Lincoln, Lincoln, Nebraska, USA

Taxonomy, Classification and Evolutionary Relationships

Carmovirus is one of two officially recognized genera of *Tombusviridae.* Members of *Tombusviridae* all have icosahedral virions of about 30 nm in diameter with $T = 3$ symmetry that consists of 180 coat protein (CP) subunits of about 38-43 kDa and a single-stranded (ss) RNA genome ranging in size from 4.0 to 4.7 kb. Carmoviruses share recognizable sequence similarity in both the polymerase and structural genes with members of the other genus, *Tombusvirus.* Sequence comparisons and phylogenetic analysis of these genes support the view that the genera are distinct but closely related. In addition, the genome organization of the carmoviruses is characteristically different from that of the tombusviruses.

Carmoviruses contain a single-component positive-sense genome of about 4.0 kb. The genome, as exemplified by turnip crinkle virus (TCV) in Figure 1, consists of five open reading frames (ORFs) which encode proteins of about 28, 88, 8, 9 and 38 kDa from the 5' to the 3' end, respectively. The virions are icosahedral and consist of 180 CP subunits of approximately 38 kDa. The genus name is derived from the first member of the genus to be sequenced, carnation mottle virus (CarMV). Much more detailed knowledge about virus structure and genome function is, however, known for TCV because its crystal structure has been determined and it was the first carmovirus for which infectious transcripts were produced from a cDNA clone of the genome. To date, the nucleotide sequences of six definitive carmoviruses have been determined (Table 1). These sequenced members share similar morphological and physicochemical properties with about 20 other viruses listed in Table 1 that are recognized as species or tentative species, depending on the characterized molecular detail of the viruses. Unlike the majority of tombusviruses, carmoviruses are sufficiently distant from each other to prevent them cross reacting in standard RNA hybridization or serological tests.

Carmoviruses and tombusviruses share properties with numerous other small spherical RNA viruses. Structural similarities with members of the *Dianthovirus* genus (e.g. red clover necrotic mosaic virus, RCNMV) are significant and include sequence similarity of CP as well as similarity in subunit topology and interactions within the virion. There also exists a marked sequence similarity in the RNA-dependent RNA polymerase (RDRP) genes of carmoviruses and viruses of the following genera: *Dianthovirus, Necrovirus* (e.g. tobacco necrosis virus, TNV), *Machlomovirus* (e.g. maize chlorotic mottle virus, MCMV) and *Luteovirus* (e.g. barley yellow dwarf viruses of the PAV type). Several newly sequenced viruses have been proposed for consideration as new genera of *Tombusviridae,* based primarily on similarities of nucleotide sequences and genome organization (e.g. pathos latent virus, oat chlorotic stunt virus and panicum mosaic virus). In a broader context, phylogenetic comparisons of viral RNA polymerase genes have identified the *Tombusviridae* as a representative plant virus cluster for one of three RNA virus supergroups with relatedness to animal viruses of *Flaviviridae* and the family of small RNA phage *(Leviviridae).*

Distribution, Host Range, Transmission and Economic Significance

Carmoviruses occur worldwide and are generally reported to cause mild asymptomatic infections on relatively restricted natural host ranges. Most accumulate to high concentrations in infected tissues and are mechanically transmitted with facility. Beetle transmission has been reported for a number of members, as has transmission in association with soil and/or irrigation water, and in some cases in association with fungal zoospores. Most of the viruses have been reported to cause diseases in a limited number of plants or plant types.

A number of carmoviruses have been identified in association with ornamental hosts and have been widely distributed in such hosts by vegetative propagation. CarMY is the most noteworthy, being widespread in cultivated carnations, and recognized as one of the more important components of viral disease complexes in this crop worldwide. It accumulates to high concentrations without producing severe symptoms and spreads primarily by contact transmission and vegetative propagation. It has a broad experimental host range

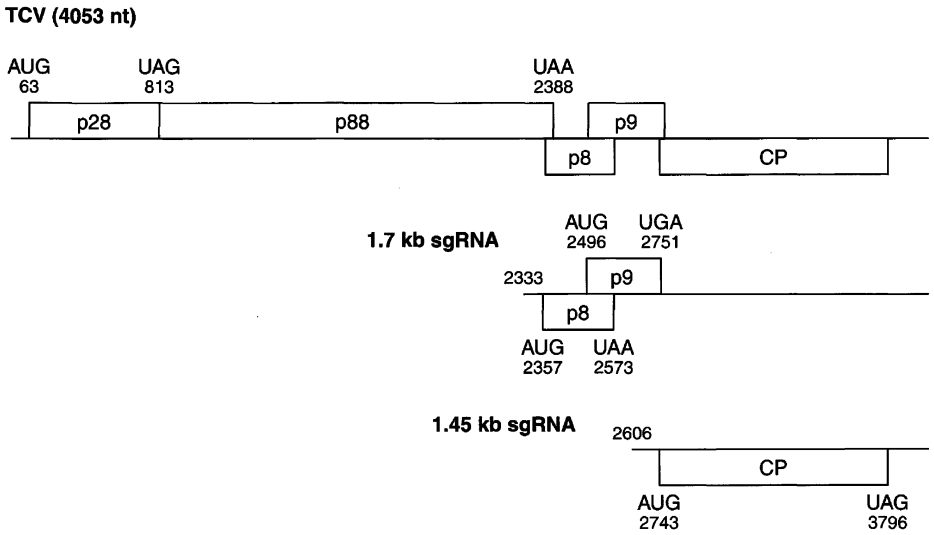

Figure 1. Genome organization of a typical carmovirus as represented by turnip crinkle virus. The genomic and subgenomic RNAs are depicted as solid lines and the sizes of the RNAs are noted. The ORFs are indicated by the boxes, with the numbers in the boxes identifying the polypeptide encoded by the ORF. The numbers above and below the diagrams give the transcriptional start sites for the sgRNAs and the nucleotide locations of the codons defining each ORF.

that includes over 30 species in 15 plant families. PFBV is widespread in vegetatively propagated *Pelargonium* species, causing disease in association with other viruses. The incidence of NTNV in *Narcissus* cultivars and HCRSV in *Hibiscus* primarily reflects distribution of infected nursery stock.

Numerous small RNA viruses have been reported to naturally infect cucurbits, causing significant disease problems. Several of these viruses are recognized tombusviruses, while others—such as MNSV, CLSV, and CSBV—have been identified as carmoviruses based on sequence and genome organization properties. MNSV occurs worldwide in greenhouse cucurbits and is both soil and seed transmitted, while CSBV has been primarily restricted to infrequent outbreaks around the Mediterranean. All three viruses have been reported to be transmitted in association with the fungus *Olpidium bornovanus*.

Several carmoviruses have been discovered in natural leguminous hosts, with GMoV being potentially the most important, causing serious disease losses in legumes in Africa. BMMV has been reported to be a latent virus widely distributed in bean cultivars in El Salvador, and BMoV has been found in *Vigna* species in Asia. Beetle vectors have been identified for these viruses, but seed transmission may also be an important factor in their distribution.

TCV is neither common nor widespread in nature in spite of the fact that it is reportedly beetle transmitted. It has a relatively wide experimental host range in some 20 plant families, including experimentally useful *Arabidopsis* and *Brassica* species, in which it accumulates to extremely high concentrations, often approaching a level equivalent to 0.5% of the fresh weight of the plant tissue. CCFV was first discovered in the Mount Kosiusko alpine region of Australia in *Cardamine lilacina,* a wild perennial *Brassica.* It has also been shown to infect *Arabidopsis* and other *Brassica* species.

Table 1. List of species in the genus *Carmovirus*

Sequenced species	
Carnation mottle	(CarMV)
Cardamine chlorotic fleck	(CCFV)
Cowpea mottle	(CPMoV)
Melon necrotic spot	(MNSV)
Saguaro cactus	(SCV)
Turnip crinkle	(TCV)
Classified species	
Ahlum water-borne	(AWBV)
Bean mild mosaic	(BMMV)
Cucumber leaf spot	(CLSV)
Cucumber soil-borne	(CSBV)
Galinsoga mosaic	(GaMV)
Hibiscus chlorotic ringspot	(HCRSV)
Pelargonium flower break	(PFBV)
Weddel water-borne	(WWBV)
Tentative species	
Blackgram mottle	(BMoV)
Elderberry latent	(ELV)
Glycine mottle	(GMoV)
Narcissus tip necrosis	(NTNV)
Plaintain 6	(PIV-6)
Squash necrosis	(SqNV)
Tephrosia symptomless	(TeSV)

Other carmoviruses have been isolated worldwide from natural hosts with little apparent disease and are presumably of little agricultural concern. These include TeSV from legumes in Kenya, GMoV from glycine in Australia, SCV from saguaro cactus in Arizona, GaMV from potato weed in Australia and PIV-6 from plantain weed in England. The infrequent isolation of these genetically similar viruses in remote locations around the world has prompted the speculation that ancestor carmoviruses may have been introduced into their natural hosts well before the last Ice Age and have since coevolved in isolation in their diverse host plants.

Virion Structure and Assembly

TCV is the only carmovirus for which refined structural studies, including high resolution X-ray crystallography, have been performed. The detailed information about CP structure and subunit–subunit interactions established that TCV and tomato bushy stunt tombusvirus (TBSV) share marked structural conservation. In this regard, the common structural features shared by other members of *Tombusviridae* have been primarily deduced from alignment of the amino acid sequence of the coat proteins of TBSV and TCV. TCV consists of a T = 3 icosahedral capsid of 180 subunits of the 38 kDa CP. The individual CP subunit folds into three distinct domains typical of the CP subunit of the tombusviruses. The relatively basic N-terminal R domain extends into the interior of the virus particle and presumably interacts with viral RNA. The R domain is connected by an arm to the S domain which constitutes the virion shell. The S domain is attached through a hinge to the P domain which projects outward from the virion surface. The protein subunits are believed to form dimers in solution and during assembly.

TCV is also the only carmovirus on which detailed *in vitro* assembly studies have been performed. The virion has been shown to dissociate at elevated pH and ionic strength to produce a stable RNA-CP complex (rp-complex) and free CP subunits. Re-assembly under physiological conditions in solution could be demonstrated using the isolated rp-complex and the soluble CP subunits. This rp-complex, consisting of six CP subunits tightly attached to viral RNA, could be generated *in vitro* and was shown to be important in selective assembly of TCV RNA. A model for assembly was proposed in which three sets of dimeric CP interact with a unique site on the viral RNA to form an initiation complex to which additional subunit dimers could rapidly bind. Preliminary characterization of the "origin of assembly" for this virus identified two possible sites based on the identification of RNA fragments in the rp-complex protected from RNase digestion by CP. Further *in vivo* studies narrowed the assembly origin site to a bulged hairpin loop of 28 nt within a 180 nt region at the 3' end of the CP gene.

Genome Structure

Complete nucleotide sequences have been determined for six carmoviruses as listed in **Table 1**: CarMV (4003 nt), TCV (4053-4054 nt), MNSV (4262 nt), CCFV (4041-4072 nt), CPMoV (4029 nt), and SCV (3879 nt). Comparative studies of the deduced ORFs revealed that all these viruses encode a similar set of genes that are closely related and in the same gene order as illustrated for TCV in **Figure 1.** The genome organization of the carmoviruses is quite compact, with most of the identified ORFs overlapping each other. Both the product of the most 5′ proximal ORF (26-28 kDa) and its readthrough product (86-89 kDa) have been shown to be essential for replication of the TCV genome. The 3′ proximal gene encodes the viral CP which varies from 37 to 42 kDa for the different viruses. All of the sequenced carmoviruses characteristically encode two small ORFs in the middle of the genome that have both been shown in TCV to be indispensable for cell-to-cell movement (movement proteins or MPs). Although the genome organizations of all sequenced carmoviruses are quite similar, there are some unique features evident in the individual carmoviruses. In CarMV, a second readthrough event could extend the translation of the polymerase gene into the p9 MP gene to produce a p98 product. This double readthrough event is not predicted for any other carmovirus. In another example, the two small central ORFs in MNSV (p7a and p7b) are connected by an in-frame amber codon that could result in the production of a 14 kDa fusion protein of the two ORFs. An unusual feature of the CPMoV sequence is the prediction of a sixth ORF (p28) nested within the 3′ proximal CP gene.

There is no direct evidence for the existence of a cap structure at the 5′ end of carmovirus genomes. Absence of a cap seems most likely given the recent identification of cap-independent translational enhancer sequences in the leaders of the genomic and subgenomic RNAs of TCV. The 5′ noncoding region varies from 34 nt in CPMoV to 88 nt in MNSV. No extensive sequence homology was observed within this region. The 3′ noncoding region of carmoviruses varies from about 200 to 300 nt in length and possesses neither a poly(A) tail nor a tRNA-like structure.

Replication and Gene Expression

Carmoviruses replicate to very high concentrations in protoplast infections, with the genomic RNA accumulating to levels approaching that of the ribosomal RNAs. Upon infection of susceptible plants, carmoviruses transcribe two 3′ coterminal subgenomic RNAs (sgRNAs) for expression of the MP and CP genes. The smaller sgRNA *(c.* 1.5 kb) is the mRNA for CP. The larger sgRNA *(c.* 1.7 kb) presumably functions as the mRNA for the two MP genes utilizing a leaky scanning mechanism. This is supported from *in vitro* translation experiments performed for TCV and SCV. Recent results involving transgenic expression of the p8 and p9 gene products of TCV in *Arabidopsis* plants have demonstrated that both of them are essential for viral cell-to-cell movement and that they function by *in trans* complementation in the same cell. It was also shown that cell-to-cell movement of TCV in *Arabidopsis* does not require the CP gene.

Viral specific double-stranded RNAs (dsRNAs), corresponding in size to the genomic RNA and sgRNAs, characteristically accumulate in infected plant tissue. The product of the 5′ proximal ORF (p28 in TCV) and its readthough product (p88 in TCV) are the only virus encoded components of the polymerase complex. Mutagenesis studies demonstrated that p28 and p88 are both essential for viral RNA replication in protoplasts. When expressed from two separate molecules, p28 and p88 complemented *in trans* to enable the genome replication. Putative host factors needed for viral RNA replication have not yet been well studied, although extracts from infected turnip plants have been shown to contain RDRP activity capable of synthesizing complementary full-length molecules from both (+)-strand and (-)-strand TCV associated RNA templates. Experiments utilizing these RDRP extracts have provided useful information about *cis*-elements important in replication and gene expression. For example, a stable stem-loop structure at the 3′ end of the TCV genome has been identified as the promoter for (-)-strand synthesis from the (+)-strand TCV genome. A similar stem-loop structure has also been identified in other carmoviruses, including CarMV and CCFV, by sequence comparisons. The essential promoter sequences for the two subgenomic RNAs have also been mapped in TCV. The promoter for (+)-strand genomic RNA synthesis has not yet been identified. Another potentially important *cis*-acting hairpin structure in TCV (the FaFf element, see **Figure** 1) has been identified in the vicinity of the UAG stop codon that punctuates the polymerase gene. A regulatory function for this element is proposed because it binds CP with high affinity, and disruption of the RNA structure, but not the coding sequence, abolishes replication.

Satellites, Defective-Interfering RNAs and RNA Recombination

TCV is the only carmovirus in which replication of associated small subviral RNAs in infected plants has been characterized and the situation for this virus is curiously complex. For example, TCV infections have been shown to contain defective-interfering RNAs derived totally from the parent genome (e.g. RNA G of 342-346 nt), satellite RNAs of nonviral origin (e.g. RNAs D, 194 nt and F, 230 nt), and chimeric RNAs (e.g. RNA C of 356 nt) with a 5′ region derived from satellite RNA D and a 3′ region derived from the 3′ end of the TCV genome. All three types of small RNAs depend on the helper virus for their replication and encapsidation within the infected plant. The different satellite and defective-interfering RNAs have been shown to affect viral infections in different ways. Both RNA C and G intensify viral symptoms while interfering with the replication of the helper virus, whereas RNAs D and F seem to produce no detectable effects on either expression of symptoms or helper virus replication.

TCV has proved to be a good model for studies on RNA recombination. The frequency and diversity of the small RNA species that appear to have arisen spontaneously in plant infections suggests that RNA recombination occurs frequently and with facility. The *de novo* generation of defective-interfering RNAs in plants after inoculation with cDNA-derived transcripts of the genome is an obvious example. Detailed studies in Anne Simon's laboratory, utilizing the chimeric satellite RNA C as a model, have provided some detailed insight into the copy-choice mechanisms that are important in viral RNA recombination. They have shown, for example, that the majority of recombinants produced are not viable for further replication. These studies have also identified a novel 3′-end repair mechanism by which the RDRP complex synthesizes short oligoribonucleotides (up to 8 nt) complementary to the 3′ end of TCV RNA, which in turn serve as primer for RNA synthesis using a different template (e.g. satellite RNA C with 3′ end up to 6 nt deleted).

Virus-Host Interaction

Studies are beginning to emerge in the area of viral–host interactions utilizing *Arabidopsis* and TCV as a model system. In one study from L. A. Heaton's laboratory, an *Arabidopsis* encoded protein, Apt8, was identified as interacting specifically with the TCV pS protein in a yeast two-hybrid screen.

Apt8 was shown to be localized in plasma membrane and possibly linked to the cytoskeleton. Transgenic *Arabidopsis* and tobacco plants expressing antisense Apt8 mRNA were resistant to TCV infection.

Some studies suggest that the CP of carmoviruses may also interact with host plants in other ways besides assembly. For example, minor amino acid changes within TCV CP have been shown to modify symptoms of infection. In addition, the CP has been implicated as a possible viral determinant in the observed resistance of *Arabidopsis thaliana* ecotype Di-0 to TCV infection. In these studies, systemic invasion by chimeric virus occurred in the Di-0 ecotype when the TCV CP ORF was replaced by the CCFV CP ORF. It was also shown that TCV replicated normally in Di-0 protoplasts and inoculated leaves, suggesting the genetic resistance was due to restricted systemic movement.

CP has also been found to be important in satellite RNA C interactions in the host plant. Normally, the presence of RNA C results in symptom intensification in TCV infections. However, when the TCV CP ORF is either deleted or replaced by the CCFV CP ORF (see above), RNA C will then attenuate symptoms caused by the helper virus. Additional experiments in protoplasts suggested that the CP either down-regulated the replication of RNA C or enhanced its own competitiveness.

Finally, the replicase gene has also been implicated in the symptom modification by satellite RNA C. The 3′ end of the TCV genome, common in TCV RNA, RNA C, and defective-interfering RNA G, was also suggested to be a symptom determinant. Environmental conditions were also found to affect the extent of resistance of *Arabidopsis* plants to TCV.

See also: **Defective interfering viruses; Dianthoviruses *(Tombusviridae);* Luteovirus; Necroviruses *(Tombusviridae);* Tombusviruses; Virus structure: Atomic structure, Principles of virus structure**

Further Reading

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