

Plant Virus RNA Replication

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Advanced article

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Plant RNA (ribonucleic acid) viruses are obligate intracellular parasites with single-stranded (ss) or double-stranded RNA genome(s) generally encapsidated but rarely enveloped. For viruses with ssRNA genomes, the polarity of the infectious RNA (positive or negative) and the presence of one or more genomic RNA segments are the features that mostly determine the molecular mechanisms governing the replication process. RNA viruses cannot penetrate plant cell walls unaided and must enter the cellular cytoplasm through mechanically induced wounds or assisted by a biological vector. After desencapsidation, their genome remains in the cytoplasm where it is translated, replicated and encapsidated in a coupled manner. Replication occurs in large viral replication complexes (VRCs), tethered to modified membranes of cellular organelles and composed by the viral RNA templates and by viral and host proteins. Cis-acting elements located in viral RNA templates regulate the recognition by the virus-encoded RNA-dependent RNA polymerase and possibly contribute to VRC assembly and/or activation.

Introduction

Plant viruses are obligate intracellular parasites with deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) genomes that require the host's molecular machinery to complete their life cycle. The viral genome is generally encapsidated by one or more viral coat proteins (CPs) to form a virion that may have distinct architectures and that sometimes can be enveloped. RNA viruses can be divided into three classes on the basis of whether their virions package the genome as mRNA sense [positive (+) strand] single-stranded (ss) RNA, antisense [negative (–) strand] ssRNA or double-stranded (ds) RNA. In all three classes, the viral genome can be formed by either a single molecule (non-segmented genome) or by two or more molecules (segmented genome), each of which is named as genomic RNA (gRNA). Some of these features are listed for the type member of each plant RNA virus genus in **Table 1**. See also: [Capsid-Less RNA Viruses](#); [Multi-Component Plant Viruses](#); [RNA Virus Genomes](#); [Viral Classification and Nomenclature](#); [Viruses](#); [Virus Structure](#)

Plant viruses cannot penetrate plant cell walls unaided; thus, most of them are introduced into the cell by a vector organism (usually insects, nematodes or fungi) that feeds on the plant or through mechanically induced wounds. Multiplication of most RNA viruses occurs in the host cell's cytoplasm and follows a series of highly regulated and coordinated events that ultimately leads to production of newly formed virions that may then spread to nearby cells and be transmitted to other plants. The replication of the viral genome is a central step in the virus multiplication cycle and, in the case of RNA viruses, proceeds solely through RNA intermediates and takes place in large viral replication complexes (VRCs) – containing the viral RNA, viral replication proteins and proteins from the host – anchored to modified membranes of diverse cellular organelles. Distinct types of cis-acting elements located in viral RNAs dictate/regulate template selection, initiation of the synthesis of complementary RNA strands and VRC assembly and activation. See also: [Replication of Viruses Infecting Eukaryotes](#); [RNA Plant and Animal Virus Replication](#); [Virus Replication](#)

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Table 1 Plant RNA virus families and genera and important properties

Genome	Family	Genus	Type species ^a	Number of segments	Virion morphology	
ssRNA(-)	<i>Bunyaviridae</i> ^a	<i>Tospovirus</i>	TSWV	3	Spherical, enveloped	
		<i>Ophiovirus</i>	CSV	3–4	Filamentous	
	<i>Rhabdoviridae</i> ^b	<i>Cytorhabdovirus</i>	LNyV	1	Bacilliform, enveloped	
		<i>Nucleorhabdovirus</i>	PYDV	1	Bacilliform, enveloped	
	Unassigned	<i>Emaravirus</i>	EMAAV	4	Spherical, enveloped	
<i>Tenuivirus</i>		RSV	4	Filamentous		
<i>Varicosavirus</i>		LBVaV	2	Rodlike		
ssRNA(+)	<i>Alphaflexiviridae</i> ^c	<i>Allexivirus</i>	ShVX	1	Filamentous	
		<i>Lolavirus</i>	LoLV	1	Filamentous	
		<i>Mandarivirus</i>	ICRSV	1	Filamentous	
		<i>Potexvirus</i>	PVX	1	Filamentous	
	<i>Benyviridae</i>	<i>Benyvirus</i>	BNYVV	5	Rodlike	
	<i>Betaflexiviridae</i> ^c	<i>Capillovirus</i>	ASGV	1	Filamentous	
		<i>Carlavirus</i>	CLV	1	Filamentous	
		<i>Citriovirus</i>	CLBV	1	Filamentous	
		<i>Foveavirus</i>	ASPV	1	Filamentous	
		<i>Tepovirus</i>	PVT	1	Filamentous	
		<i>Trichovirus</i>	ACLSV	1	Filamentous	
		<i>Vitivirus</i>	GVA	1	Filamentous	
		<i>Bromoviridae</i>	<i>Alfamovirus</i>	AMV	3	Bacilliform
			<i>Anulavirus</i>	PZSV	3	Isometric
			<i>Bromovirus</i>	BMV	3	Isometric
	<i>Cucumovirus</i>		CMV	3	Isometric	
	<i>Ilarvirus</i>	<i>Ilarvirus</i>	TSV	3	Isometric	
		<i>Oleavirus</i>	OLV-2	3	Isometric	
		<i>Closteroviridae</i>	<i>Ampelovirus</i>	GLRaV-3	1	Filamentous
			<i>Closterovirus</i>	BYV	1	Filamentous
			<i>Crinivirus</i>	LIYV	2	Filamentous
		<i>Comovirinae</i> ^d	<i>Velarivirus</i>	GLRaV-7	1	Filamentous
			<i>Comovirus</i>	CPMV	2	Isometric
	<i>Fabavirus</i>		BBWV-1	2	Isometric	
	<i>Nepovirus</i>		TRSV	2	Isometric	
	<i>Luteoviridae</i>	<i>Enamovirus</i>	PEMV-1	1	Isometric	
		<i>Luteovirus</i>	BYDV-PAV	1	Isometric	
		<i>Polerovirus</i>	PLRV	1	Isometric	
	<i>Marnaviridae</i> ^d	<i>Marnavirus</i>	HaRNAV	1	Isometric	
	<i>Potyviridae</i>	<i>Brambyvirus</i>	BVY	1	Filamentous	
		<i>Bymovirus</i>	BaYMV	1	Filamentous	
		<i>Ipomovirus</i>	SPMMV	1–2	Filamentous	
		<i>Macluravirus</i>	MacMV	1–2	Filamentous	
<i>Poacevirus</i>		TriMV	1	Filamentous		
<i>Potyvirus</i>		PVY	1	Filamentous		
<i>Rymovirus</i>		RGMV	1–2	Filamentous		
<i>Tritimovirus</i>		WSMV	1–2	Filamentous		
<i>Secoviridae</i> ^d		<i>Cheravirus</i>	CRLV	2	Isometric	
		<i>Sadwavirus</i>	SDV	2	Isometric	
	<i>Sequivirus</i>	PYFV	1	Isometric		
	<i>Torradovirus</i>	ToTV	2	Isometric		
	<i>Waikavirus</i>	RTSV	1	Isometric		
	<i>Tombusviridae</i>	<i>Alphanecrovirus</i>	TNV-A	1	Isometric	
<i>Aureusvirus</i>		PoLV	1	Isometric		
<i>Avenavirus</i>		OCSV	1	Isometric		

(continued overleaf)

Table 1 (continued)

Genome	Family	Genus	Type species ^a	Number of segments	Virion morphology
		<i>Betanecrovirus</i>	TNV-D	1	Isometric
		<i>Carmovirus</i>	CarMV	1	Isometric
		<i>Dianthovirus</i>	CRSV	2	Isometric
		<i>Gallantivirus</i>	GMV	1	Isometric
		<i>Macanavirus</i>	FNSV	1	Isometric
		<i>Machlomovirus</i>	MDMV	1	Isometric
		<i>Panicovirus</i>	PMV	1	Isometric
		<i>Tombusvirus</i>	TBSV	1	Isometric
		<i>Umbravirus</i>	CMoV ^e	1	Isometric
		<i>Zeavirus</i>	MNeSV	1	Isometric
	<i>Tymoviridae</i> ^c	<i>Maculavirus</i>	GFkV	1	Isometric
		<i>Marafivirus</i>	MRFV	1	Isometric
		<i>Tymovirus</i>	TYMV	1	Isometric
	Unassigned	<i>Cilevirus</i>	CiLV-C	2	Bacilliform
		<i>Higrevirus</i>	HGSV-2	3	Bacilliform
		<i>Idaeovirus</i>	RBDV	3	Isometric
		<i>Ourmiavirus</i>	OuMV	3	Isometric
		<i>Polemovirus</i>	PnLV	1	Isometric
		<i>Sobemovirus</i>	SBMV	1	Isometric
	<i>Virgaviridae</i>	<i>Furovirus</i>	SBWMV	2	Rodlike
		<i>Hordeivirus</i>	BSMV	3–4	Rodlike
		<i>Pecluvirus</i>	PCV	2	Rodlike
		<i>Pomovirus</i>	PMTV	3	Rodlike
		<i>Tobamovirus</i>	TMV	1	Rodlike
		<i>Tobravirus</i>	TRV	2	Rodlike
dsRNA	<i>Amalgaviridae</i>	<i>Amalgavirus</i>	STV	1	Unknown
	<i>Endornaviridae</i>	<i>Endornavirus</i>	VFV	1	Unknown
	<i>Partitiviridae</i>	<i>Alphapartitivirus</i>	WCCV-1	2	Isometric
		<i>Betapartitivirus</i>	WCCV-2	2	Isometric
		<i>Deltapartitivirus</i>	PCV-1	2	Isometric
	<i>Reoviridae</i>	<i>Fijivirus</i>	FDV	10	Isometric
		<i>Oryzavirus</i>	RRSV	10	Isometric
		<i>Phytoreovirus</i>	WTV	12	Isometric

^aAbbreviations: ACLSV, Apple chlorotic leaf spot virus; AMV, Alfalfa mosaic virus; ASGV, Apple stem grooving virus; ASPV, Apple stem pitting virus; BaYMV, Barley yellow mosaic virus; BBWV-1, Broad bean wilt virus 1; BMV, Brome mosaic virus; BNYVV, Beet necrotic yellow vein virus; BSMV, Barley stripe mosaic virus; BVY, Blackberry virus Y; BYDV-PAV, Barley yellow dwarf virus-PAV; BYV, Beet yellow virus; CarMV, Carnation mottle virus; CiLV-C, Citrus leprosis virus C; CLB, Citrus leaf blotch virus; CLV, Carnation latent virus; CMoV, Carrot mottle virus; CMV, Cucumber mosaic virus; CPMV, Cowpea mosaic virus; CRLV, Cherry rasp leaf virus; CRSV, Carnation ringspot virus; CSV, Citrus psorosis virus; EMAAV, European mountain ash ringspot-associated virus; FDV, Fiji disease virus; FNSV, *Furcraea necrotic streak virus*; GFkV, Grapevine fleck virus; GLRaV-3/7, Grapevine leafroll-associated virus 3/7; GMV, Galinsoga mosaic virus; GVA, Grapevine virus A; HaRNAV, *Heterosigma akashiwo RNA virus*; HGSV-2, *Hibiscus green spot virus 2*; ICRSV, *Indian citrus ringspot virus*; LBVaV, *Lettuce big-vein virus*; LIYV, *Lettuce infectious yellows virus*; LoLV, *Lolium latent virus*; LNYV, *Lettuce necrotic yellows virus*; MacMV, *Maclura mosaic virus*; MCMV, *Maize chlorotic mottle virus*; MNeSV, *Maize necrotic streak virus*; MRFV, *Maize rayado fino virus*; OCSV, *Oat chlorotic stunt virus*; OLV-2, *Olive latent virus*; OuMV, *Ourmia melon virus*; PCV, *Peanut clump virus*; PCV-1, *Pepper cryptic virus 1*; PEMV-1, *Pea enation mosaic virus-1*; PLRV, *Potato leafroll virus*; PMTV, *Potato mop-top virus*; PMV, *Panicum mosaic virus*; PnLV, *Poinsettia latent virus*; PoLV, *Pothos latent virus*; PVT/X/Y, *Potato virus T/X/Y*; PYDV, *Potato yellow dwarf virus*; PYFV, *Parsnip yellow fleck virus*; PZSV, *Pelargonium zonate spot virus*; RBDV, *Raspberry bushy dwarf virus*; RGMV, *Ryegrass mosaic virus*; RRSV, *Rice ragged stunt virus*; RSV, *Rice stripe virus*; RTSV, *Rice tungro spherical virus*; SBMV, *Southern bean mosaic virus*; SBWMV, *Soil-borne wheat mosaic virus*; SDV, *Satsuma dwarf virus*; ShVX, *Shallot virus X*; SPMMV, *Sweet potato mild mottle virus*; STV, *Southern tomato virus*; TBSV, *Tomato bushy stunt virus*; Tobacco mosaic virus; TNV-A/D, *Tobacco necrosis virus A/D*; ToTV, *Tomato torrado virus*; TriMV, *Triticum mosaic virus*; TRSV, *Tobacco ringspot virus*; TRV, *Tobacco rattle virus*; TSV, *Tobacco streak virus*; TSWV, *Tomato spotted wilt virus*; TYMV, *Turnip yellow mosaic virus*; VFV, *Vicia faba endornavirus*; WCCV-1/2, *White clover cryptic virus 1/2*; WSMV, *Wheat streak mosaic virus*; WTV, *Wound tumor virus*.

^bOrder *Mononegavirales*.

^cOrder *Tymovirales*.

^dOrder *Picornavirales*.

^eSatellite virus.

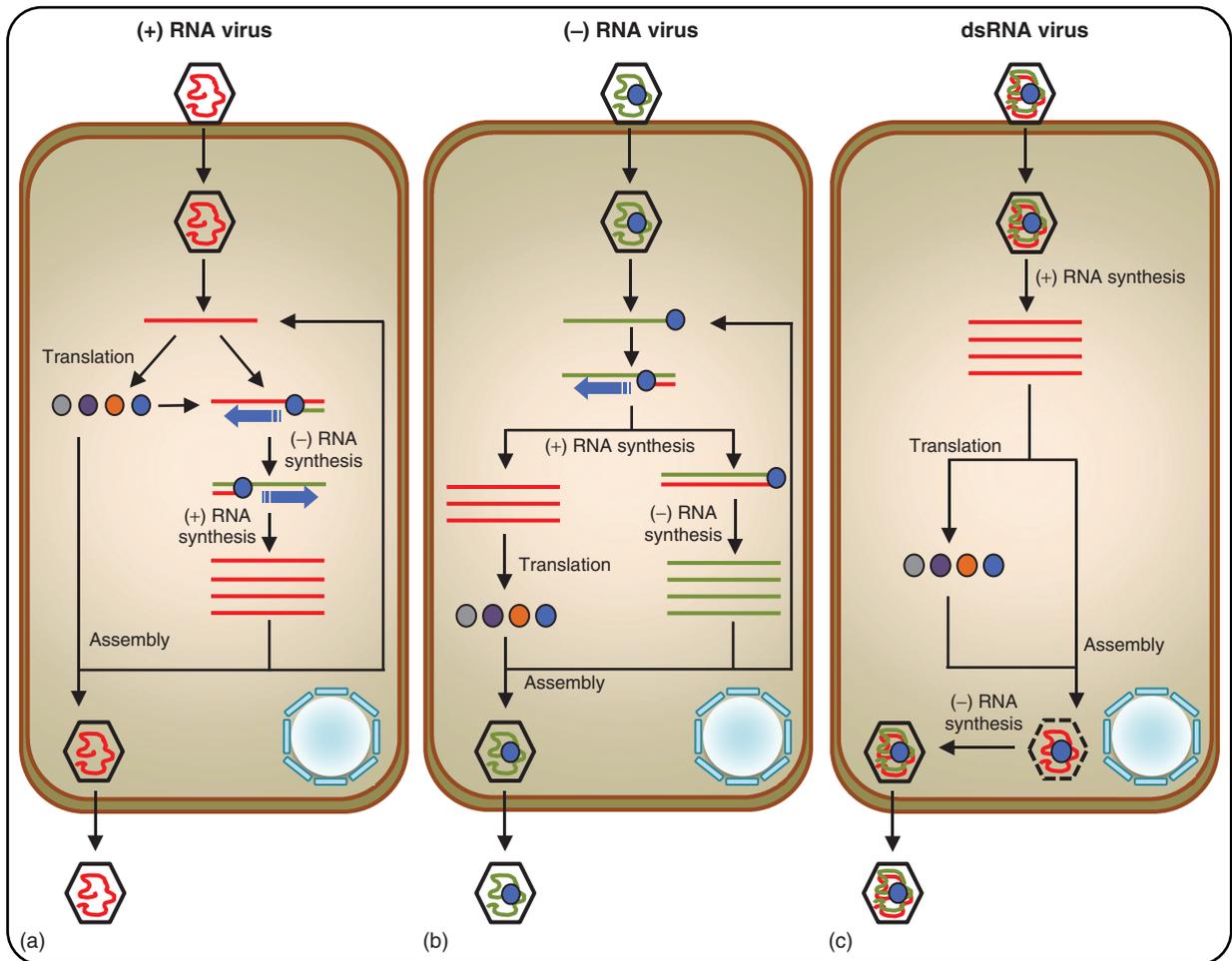


Figure 1 Cellular life cycles of plant RNA viruses. For each class of RNA virus, a simplified and representative life cycle is shown. All three virus classes replicate exclusively through RNA intermediates in the cytoplasm. Nuclear trafficking of macromolecules during viral replication could not be ruled out. Virions are shown as black hexagons containing positive (+) and/or negative (-) RNA strands in red and green, respectively, and viral-RNA-dependent RNA polymerase (RdRp, blue circle). Other viral proteins are shown as filled circles of distinct colours. (a) Life cycle of (+) RNA viruses. The genomic RNA (gRNA) from virions is released into the cytoplasm. This (+) RNA, mRNA sense, is translated to produce several viral proteins including the viral RdRp. This polymerase copies the (+) strands into (-) strands that serve as templates for the synthesis of (+) strands that can be translated, replicated or assembled in virion particles. (b) Life cycle of the majority of (-) RNA viruses. The gRNA is used as a template by the viral RdRp to generate (+) strand mRNAs that are released into the cytoplasm to be translated into viral proteins. The gRNA is also copied into full-length (+) strands that, in turn, serve as templates for (-) RNA synthesis. For members of the genus *Nucleorhabdovirus*, replication occurs in the nucleus. (c) Life cycle of double-stranded RNA (dsRNA) viruses. Viral RdRp synthesises and releases into the cytoplasm (+) strands that are first translated and then packaged to form immature virions. Virions mature by synthesising (-) RNA and by the addition of other viral proteins.

Replication Cycles and Sites of Replication of Plant RNA Viruses

Once a plant RNA virus enters a cell, desencapsidation occurs and the virus genome is released to the cytoplasm where, with the only exception of members of genus *Nucleorhabdovirus* (see below), all steps of the replication cycle are completed. The molecular events underlying replication depend on the type of RNA genome. For (+) RNA viruses, the most abundant class of plant RNA viruses, the gRNA is first translated into the different viral proteins, including the RNA-dependent RNA polymerase

(RdRp) that is responsible for the copy of (+) strands into (-) strands. The latter ones serve as templates for generating multiple (+) strands that are translated, replicated or assembled into virions that may spread throughout the plant and/or be transmitted to new hosts (Figure 1). In the case of (-) RNA viruses, the viral RdRp is packaged along the gRNA within the virion so that it can initiate synthesis of (+) strands complementary to the infectious (-) gRNA. The (+) strands are then translated into viral proteins or used as templates for the generation of new (-) strands that can either serve as templates for additional replication rounds or be assembled into progeny virions (Figure 1). Regarding dsRNA viruses, the viral RdRp is also incorporated into the

Table 2 Site of replication of different plant RNA viruses

Family	Genus	Virus	Membranes used for replication	
<i>Bromoviridae</i>	<i>Alfavirus</i>	<i>Alfalfa mosaic virus</i> (AMV)	Vacuolar membrane	
	<i>Bromovirus</i>	<i>Brome mosaic virus</i> (BMV)	Endoplasmic reticulum	
	<i>Cucumovirus</i>	<i>Cucumber mosaic virus</i> (CMV)	Vacuolar membrane	
<i>Potyviridae</i>	<i>Potyvirus</i>	<i>Tomato aspermy virus</i> (TAV)	Vacuolar membrane	
		<i>Plum pox virus</i> (PPV)	Endoplasmic reticulum	
		<i>Tobacco etch virus</i> (TEV)	Endoplasmic reticulum	
<i>Rhabdoviridae</i>	<i>Cytorhabdovirus</i>	<i>Lettuce necrotic yellows virus</i> (LNYV)	Endoplasmic reticulum	
	<i>Nucleorhabdovirus</i>	<i>Potato yellow dwarf virus</i> (PYDV)	Nuclear membrane	
		<i>Sonchus yellow net virus</i> (SYNV)	Nuclear membrane	
<i>Secoviridae</i>	<i>Comovirus</i>	<i>Cowpea mosaic virus</i> (CPMV)	Endoplasmic reticulum	
	<i>Nepovirus</i>	<i>Grapevine fanleaf virus</i> (GFLV)	Endoplasmic reticulum	
<i>Tombusviridae</i>	<i>Carmovirus</i>	<i>Melon necrotic spot virus</i> (MNSV)	Mitochondria	
		<i>Pelargonium flower break virus</i> (PFBV)	Mitochondria	
		<i>Red clover necrotic mosaic virus</i> (RCNMV)	Endoplasmic reticulum	
	<i>Dianthovirus</i>	<i>Carnation Italian ringspot virus</i> (CIRV)	Mitochondria	
		<i>Tombusvirus</i>	<i>Cucumber necrosis virus</i> (CNV)	Peroxisome
		<i>Cymbidium ringspot virus</i> (CymRSV)	Peroxisome	
		<i>Tomato bushy stunt virus</i> (TBSV)	Peroxisome	
	<i>Umbravirus</i>	<i>Lettuce speckles mottle virus</i> (LSMV)	Vacuolar membrane	
	<i>Tymoviridae</i>	<i>Tymovirus</i>	<i>Turnip yellow mosaic virus</i> (TuMV)	Chloroplast outer membrane
	Unassigned	<i>Benyvirus</i>	<i>Beet necrotic yellow vein virus</i> (BNYVV)	Mitochondria
<i>Virgaviridae</i>	<i>Pecluvirus</i>	<i>Peanut clump virus</i> (PCV)	Endoplasmic reticulum	
	<i>Tobamovirus</i>	<i>Cucumber green mottle mosaic virus</i> (CGMMV)	Mitochondria	
	<i>Tobravirus</i>	<i>Tobacco mosaic virus</i> (TMV)	Endoplasmic reticulum	
		<i>Tobacco rattle virus</i> (TRV)	Mitochondria	

virions where it synthesises (+) RNA strands that are extruded from the particle. Such (+) RNA strands are first translated to produce, among others, the RdRp and the CP and then packaged to form immature virions that develop by synthesising (–) RNA strands. In this way, while the dsRNA genome is being produced, it is protected from host antiviral systems such as RNA silencing that detect and inactivate dsRNA molecules (**Figure 1**). **See also: Replication of Viruses Infecting Eukaryotes; RNA Plant and Animal Virus Replication; RNA Virus Genomes; Viral Classification and Nomenclature; Virus Replication**

Replication occurs in membranes of cytoplasmic organelles such as the endoplasmic reticulum (ER), mitochondria, vacuoles, peroxisomes or chloroplasts, except for (–) RNA viruses of the genus *Nucleorhabdovirus* that replicate in the nucleus in association with nuclear membranes (**Table 2**). Certain plant RNA viruses can exploit alternative subcellular membranes for their replication in the absence of their primary membrane, as shown for tombusviruses *Tomato bushy stunt virus* (TBSV) and *Cymbidium ringspot virus* (CymRSV) or potyvirus *Turnip mosaic virus* (TuMV) (Xu and Nagy, 2014). In this article we describe essentially key aspects of (+) RNA virus replication, as the lack of reverse genetics approaches for (–) RNA and dsRNA viruses has limited the study of these two classes of viral agents. **See also: Cell Membrane Features; Nuclear Membranes; Plant Chloroplasts and Other Plastids; Plant Endoplasmic Reticulum; Plant Golgi Apparatus; Plant Mitochondria; Plant Peroxisomes and Glyoxysomes; Plant Vacuoles; Rhabdoviruses**

Structure and Dynamics of Viral Replication Complexes

For plant RNA viruses, the architecture of *Brome mosaic virus* (BMV) VRCs is the best known. BMV replication occurs in vesicles – known as spherules – derived from ER invaginations and with an open neck that connects its interior with the cytoplasm (Diaz and Wang, 2014). It has been proposed that these spherules may protect BMV VRCs (as do capsids in retroviruses or dsRNA viruses) from cellular defenses, and that similar structures may be involved in the replication of other ssRNA viruses (den Boon *et al.*, 2010; Nagy and Pogany, 2012). Interestingly, BMV replication can also occur in very different membranous structures. By modulating the relative levels and interactions of BMV replication factors, it is possible to produce multilayered stacks of ER-derived appressed double membranes that support RNA replication as efficiently as the spherules (Diaz and Wang, 2014).

Importantly, the membranous structures where VRCs are assembled are not static during the infection process. For instance, multiple replication sites that are distributed randomly throughout the cytoplasm in the early stages of *Cowpea mosaic virus* (CPMV) infection rapidly coalesce into a large body located in the centre of the cell, often in the perinuclear region (Carette *et al.*, 2002). Similar perinuclear structures have also been observed in infections by other RNA viruses (Heinlein, 2015). While those from secoviruses CPMV and *Grapevine*

fanleaf virus (GFLV) seem to derive from ER membranes, those from potexvirus *Potato virus X* (PVX) and potyvirus TuMV also contain elements of the Golgi apparatus, and in the case of TuMV, of chloroplasts. TuMV vesicles move along the perinuclear body and the nuclear periphery, and traffic through the actomyosin motility system from the ER to the chloroplasts, where they amalgamate and induce chloroplast membrane invaginations. Similarly, *Alfalfa mosaic virus* (AMV) VRCs are assembled in multivesicular bodies before their recruitment to the vacuolar membrane or tonoplast. Also, it has been observed that the intracellular trafficking of *Tobacco mosaic virus* (TMV) VRCs along microfilaments is required for the cell-to-cell spread of the infection (Heinlein, 2015). **See also: Secoviridae: A Family of Plant Picorna-Like Viruses with Monopartite or Bipartite Genomes**

Viral Proteins Involved in Plant Virus RNA Replication

All plant RNA viruses encode a viral RdRp that is responsible for the synthesis of new gRNA using parental gRNA as template. In the most simple plant RNA viruses, viral proteins involved in replication are restricted to such RdRp and to a second protein with variable functions depending on the virus species. For example, TMV produces a 180K protein, with an RNA polymerase domain, and a 130K protein that methylates guanosine-5'-triphosphate (GTP) and transfers the resulting m⁷GTP to the 5' end of the nascent RNA molecule to generate a cap structure (Ishibashi *et al.*, 2012). Besides its role as methyl-transferase, this protein also acts as RNA helicase hydrolysing nucleoside triphosphates (NTPs) and using the resulting energy to unwind the two strands of the dsRNA replication intermediates (Goregaoker and Culver, 2003; Nishikiori *et al.*, 2012). Moreover, the interaction of 130K with host proteins is critical for membrane targeting of the ribonucleic complexes formed by viral RNAs and replication proteins that are assembled co-translationally and that ultimately constitute the functional VRCs (Ishibashi *et al.*, 2012).

The replication of members of the *Bromoviridae* family also requires only two viral proteins: 1a protein, with a methyltransferase and an NTPase/helicase domain similar to those of TMV 130K, and 2a protein, containing a central RNA polymerase domain and an N-terminal domain that interacts with the helicase domain of 1a. BMV 1a has been shown to play a critical role also in VRC assembly. In particular, BMV 1a localises in ER membranes, where it oligomerises, induces the formation of invaginations and recruits both the 2a RNA polymerase and the template RNAs to these compartments where replication takes place (Diaz and Wang, 2014). **See also: Bromoviridae and Allies**

It seems that a general characteristic of plant (+) RNA viruses is to express a protein to induce precise modifications in cellular membranes and to target there the viral factors that are required to assemble functional VRCs. Hence, p33 from tombusviruses induces the formation of spherules that are similar to those induced by BMV 1a, and directs to them the RNA template

and the p92 RNA polymerase (Stork *et al.*, 2011). Protein p33 has also RNA chaperone activity, which might assist in both the assembly and activity of VRCs. *Red clover necrotic mosaic virus* (RCNMV), a dianthovirus also from the *Tombusviridae* family, expresses two proteins, p27 and p88, involved in RNA replication. Protein p27 has a strong affinity for ER membranes, interacts with itself and with p88 as well as with the viral RNA and with several host proteins to facilitate the VRC assembly (Hyodo *et al.*, 2014). In tymovirus *Turnip yellow mosaic virus* (TYMV) infections, the 140K protein, containing methyltransferase, proteinase and NTPase/helicase domains, recruits the 66K RNA polymerase to the chloroplast membrane (Prod'homme *et al.*, 2003). **See also: Tombusviridae**

Other plant RNA viruses with more complex genomes such as closteroviruses or potyviruses devote a larger number of proteins to their replication. Genetic studies have revealed that the majority of potyviral gene products participate either directly or indirectly in replication. However, while the roles that certain potyviral proteins such as the RNA polymerase NIb or the NTPase/helicase CI play in replication are well known, those of other proteins such as P3 remain to be ascertained. An example of potyviral protein with a known role in replication is 6K₂, a small hydrophobic peptide that anchors the VRC to the membrane. Despite this protein being able to localise by itself to the ER and induce the formation of membranous vesicles, it seems that the partially processed 6K₂-VPg-Pro product is actually the skeleton upon which the VRC is built (Heinlein, 2015). Moreover, this protein contains the protease domain of NIa and the multifunctional protein VPg, which binds covalently to the 5' end of the potyviral gRNA and is uridylylated by the NIb viral RNA polymerase (Puustinen and Mäkinen, 2004; Anindya *et al.*, 2005). The resulting product is thought to be used as the initiator of viral RNA replication. **See also: Closteroviridae; Potyviridae**

Host Proteins Involved in Plant Virus RNA Replication

The relatively small size and consequently limited coding capacity of viral genomes could explain why plant RNA viruses must hijack certain cellular pathways, co-opt host factors (HFs) and manipulate cellular structures to be able to complete all the steps of their life cycle, including genome replication. The search for co-opted HFs involved in virus replication has been the subject of intense research and has been addressed through different approaches including *in vitro* replication assays, reverse and direct genetics and intracellular localisation studies. In addition, the yeast *Saccharomyces cerevisiae* has been reported to sustain replication of some plant (+) RNA viruses such as BMV or TBSV. Genome-wide screenings in this model organism have been extremely useful in identifying HFs participating in the replication of these viruses (Galao *et al.*, 2007; Nagy *et al.*, 2014). Identified HFs include RNA-binding proteins, chaperones, translation factors or proteins involved in membrane trafficking or synthesis, among others (Table 3). Generally, co-opted HFs control the biogenesis of replication organelles, the composition,

assembly or activity of the VRCs, or mediate post-translational modifications of viral replication proteins. The best studied HFs regulating these processes are described below and are listed in **Table 3**, together with other factors that are not mentioned in the text due to space limitations.

Host factors involved in the biogenesis of viral replication organelles

Increasing evidence indicates that viral proteins and co-opted HFs orchestrate the protein targeting to specific membranes as well as the remodelling of certain cellular membranes used as scaffolds for tethering the VRCs. A first group of HFs involved in the biogenesis of replication organelles includes proteins participating in lipid metabolism and membrane organisation. Indeed, membrane rearrangements are necessary for the formation of VRCs and require the modification of the lipid composition of the membrane. For example, *OLE1*, a $\Delta 9$ fatty acid desaturase, is important for BMV replication because it regulates the fluidity or plasticity of the membrane at the replication site (Diaz and Wang, 2014). In contrast, BMV replication is severely inhibited when ACB1-encoded acyl-CoA binding protein (ACBP), a critical enzyme maintaining lipid homeostasis, is mutated (Diaz and Wang, 2014). Other genetic studies in yeast have identified several host genes involved in lipid, fatty acid or sterol biosynthesis/metabolism that affect TBSV replication (Wang, 2015). **See also: Lipids; Membrane Lipid Biosynthesis**

It has also been observed that viruses use components of the cellular secretory pathway for the organisation of the membranous compartments where VRCs are assembled. For instance, DOA4 and BRO1, two components of the multivesicular body secretory pathway, are important for BMV replication because they are required for the ubiquitination and proteasome-dependent activation of transcription factors MGA2 and SPT23 regulating several lipid metabolism genes, including *OLE1* (Diaz and Wang, 2014). Another component of the cellular secretory machinery that is important for viral replication is ARF1, a small GTPase involved in COPI vesicle formation in the Golgi apparatus. Its interaction with RCNMV p27 redirects ARF1 to the ER membranes where it participates in VRC formation (Hyodo *et al.*, 2013). Similarly, ARL8 GTPase interacts with *Tomato mosaic virus* (ToMV) 130K, and, together with TOM1 and TOM3, contributes to 130K targeting to ER membranes and to activate the VRC (Ishibashi *et al.*, 2012). PEX19, required for the synthesis of peroxisome membranes, also acts as a cellular transporter for directing TBSV p33 to the peroxisomal membrane to initiate TBSV VRC assembly (Pathak *et al.*, 2008). Finally, another function of certain secretory proteins is to induce membrane bending in order to facilitate the formation of spherules where viral RNA is synthesised. This is apparently the case of ESCRT (endosomal sorting complexes required for transport) and RHP (reticulon homology proteins) factors in TBSV and BMV infections (Wang, 2015). **See also: Protein Secretory Pathways**

Host factors involved in the assembly and activity of viral replication complexes

Host proteins with chaperone activity (e.g. heat-shock proteins, HSPs) or RNA-binding capacity have also been found as a part of VRCs (**Table 3**) (Nagy *et al.*, 2011; Verchot, 2012). In tombusvirus infections, HSP70 recruitment is required for the insertion of VRC components in the corresponding intracellular membranes, as well as for their correct assembly in these structures. HSP70 is also necessary for the replication of ToMV, TMV, TuMV and RCNMV (Verchot, 2012). In *Potato virus A* (PVA) infections, HSP70 requires its co-chaperone CPIP to regulate the amount of CP in VRCs in early stages of the infection (Hafren *et al.*, 2010). Another cellular chaperone involved in replication of (+) RNA viruses is HSP90, which stimulates *Bambusa mosaic virus* (BaMV) replication through its interaction with both the 3' untranslated region (UTR) of BMV gRNA and RdRp (Huang *et al.*, 2012). Indeed, both HSP70 and HSP90 participate in RCNMV replication and, despite both of them interacting with p27, they regulate different steps of VRC assembly (Mine *et al.*, 2012). Another HSP, HSP40, contributes to the folding of BMV p2a and to its assembly into active VRCs (Tomita *et al.*, 2003). In contrast to HSPs, other cellular proteins with chaperone activity can inhibit viral replication, as observed for CPR1 and CPR7 that interact with TBSV replication proteins (Mendu *et al.*, 2010; Lin *et al.*, 2012).

Other HFs have a more direct role in gRNA synthesis. For instance, eukaryotic translation elongation factor 1A (eEF1A) and/or eukaryotic translation elongation factor 1B (eEF1B) interact with the RdRp of several RNA viruses and, therefore, probably participate in regulating viral gRNA synthesis (Hwang *et al.*, 2013). In TBSV infection, eEF1A is present in the VRC and interacts with p33 and p92 as well as with a cis-acting RNA element present at the 3' end of TBSV RNA (Li *et al.*, 2009). eEF1A assists the recruitment of the viral RNA and the VRC assembly, and promotes (–) RNA synthesis (Li *et al.*, 2010). Another co-opted HF involved in virus replication is GAPDH (glyceraldehyde-3-phosphate dehydrogenase), which has opposite effects on viral replication depending on the virus. In TBSV infection, GAPDH is recruited by TBSV p92 to the VRC, binds an AU pentamer sequence near the 3' end of (–) RNA and regulates asymmetry in the synthesis of (+) and (–) viral RNAs by anchoring (–) strands inside the VCR while the (+) RNA progeny is released to the cytosol (Wang and Nagy, 2008; Huang and Nagy, 2011). In contrast, GAPDH has more affinity for BaMV (+) RNA strands and has an inhibitory effect on viral replication (Prasanth *et al.*, 2011). On the other hand, viruses with small genomes such as those of the *Tombusviridae* family do not encode helicase-like proteins. Thus, it is assumed that they must recruit host helicases to assist in their replication. In line with this view, distinct DEAD-box RNA helicases (DED1 and DBP2 from yeast, and AtRH20, AtRH2 and AtRH5 from *Arabidopsis thaliana*) have been found to be involved directly in TBSV RNA replication. These HFs seem to bind and unwind local secondary structures at the 3' end of the viral (–) RNA, thus facilitating the synthesis of (+) RNA (Kovalev and Nagy, 2014).

Table 3 Cellular factors involved in plant virus RNA replication

Host factor ^a	Cellular function	Function in viral replication	Virus ^b
ACBP	Membrane synthesis	Lipid synthesis	BMV
ARF1	Membrane trafficking	COPI vesicle formation, remodeling of ER membranes	RCNMV
ARL8, TOM1, TOM2A, TOM3	Integral membrane protein	VRC assembly, enhance VRC RNA synthesising and capping functions	ToMV
AtRH2, AtRH5, AtRH8, RH20	DEAD-box RNA Helicases	Co-localises with VRC, facilitates asymmetric RNA replication	TBSV, TuMV
BRO1, DOA4	Membrane synthesis	Lipid metabolism	BMV
CDC34	E2 Ubiquitin conjugating enzyme	Ubiquitination of a viral RNA replication protein	TBSV
CHO1, INO1, INO2, INO4, OPI3	Membrane synthesis	Phospholipid homeostasis required for activation or assembly of VRC	TBSV
CPIP	Co-chaperone	Regulation of coat protein functions	PVA
CIPK12	Phosphorylation	Phosphorylation of viral RNA polymerase to inhibit replication	CMV
CPR1, CPR7	Chaperone	Initiation of RNA replication	TBSV
DED1, DBP2, RH20	RNA-binding protein	Synthesis of RNA(+)	BMV, TBSV
eEF1A	RNA-binding protein	Synthesis of RNA (+) or (-) strand	TBSV, TMV, TSWV, TuMV, TYMV
eEF1B	RNA-binding protein	Interaction with VRC	TMV
eEF1B _γ	RNA-binding protein	Synthesis of RNA (-) strand	TBSV
eIF3	RNA-binding protein	Synthesis of RNA (-) strand	BMV
ERG25, SMO1, SMO2	Membrane synthesis	Sterol biosynthesis required for activation or assembly of VRC	Tombusvirus
ESCRT	Membrane trafficking	Remodelling membranes for activation or assembly of VRC	BMV, CIRV, TBSV
GAPDH	Cytosolic energy production	VRC assembly or inhibition of RNA replication	BaMV, CNV, RCNMV, TBSV
HSF1	Trimeric heat shock transcription factor	Modulation of RNA polymerase levels or localisation	BMV
HSP70, HSP90	Chaperone	VRC assembly or activation	BaMV, BYV, CNV, CTV, PVA, RCNMV, TBSV, TMV, ToMV, TuMV
LSm1-7/Dhh1p/Pat1p	mRNA decay	VRC assembly	BMV
OLE1	Membrane synthesis	Fatty acids synthesis	BMV
OPI1P	Membrane synthesis	Transcriptional repressor of phospholipid synthesis in yeast	Tombusvirus
ORD, VAP	Membrane synthesis	Biogenesis of replication organelles	TBSV
OSBP	Membrane synthesis	Sterol channelling to replication sites	TBSV
PABP	RNA-binding protein	VRC assembly	Potyvirus
PAP85	Seed storage protein	VRC assembly	TMV
PE	Membrane synthesis	VRC assembly	TBSV
PEX19	Cellular membrane related	VRC assembly	TBSV
PGK	Glycolysis	Synthesis of RNA (-) strand	BaMV
PKC1	Phosphorylation	Phosphorylation of viral protein to inhibit replication	TBSV
PLD	Membrane synthesis	VRC assembly	BMV, RCNMV
PRE1, RPT6	Proteasome components	Modulation of RNA polymerase levels or localisation	BMV
RHPs	Membrane trafficking	Membrane rearrangements	BMV
Rpb11	Transcription factor	Regulation of viral replication proteins accumulation	Tombusvirus

(continued overleaf)

Table 3 (continued)

Host factor ^a	Cellular function	Function in viral replication	Virus ^b
RPN11	Proteasome component	VRC assembly	Tombusvirus
RSP5	Ubiquitin ligase	Cell-intrinsic restriction factor regulating viral replication	Tombusvirus
SAR1	Membrane trafficking	COPII vesicle formation, remodelling of ER membranes	RCNMV, WYMV
SCE1	SUMOylation	Interaction with viral RdRP to inhibit replication	TuMV
SEC39	Membrane trafficking	Recognises COPI vesicles and functions in peroxisome biogenesis	TBSV
SEE1	Methyltransferase	eEF1A methylation	TBSV
SKP1	Chaperone	Favours viral asymmetric RNA replication	Nanovirus, PVX
STI1P	Chaperone	Cell-intrinsic restriction factor regulating viral replication	CIRV
SYP71	Membrane trafficking	Traffic to chloroplasts	TuMV
TIP1, TIP2	Aquaporins	Association of VRC to the tonoplast	CMV
YDJ1	Chaperone	VRC assembly	BMV

^aACBP, acyl-CoA-binding protein; ARF1, ADP ribosylation factor 1, GTPase; ARL8 GTPase; BRO1 and DOA4, components of the multivesicular body secretory pathway; CDC34, ubiquitin conjugating enzyme; CHO1, phospholipid synthesis protein; CIPK12, calcineurin B-like calcium sensor-interacting protein kinase 12; CPIP, co-chaperone of HSP70; CPR1, CPR7: cyclophilins; DED1, DPB2 and 7/Dhh1p/Pat1p, protein complexes promoting mRNA degradation; eEF1A, translation elongation factor; eEF1B_γ, translation elongation factor; eIF3, translation initiation factor; ERG25, SMO1 and SMO2, sterol biosynthesis proteins; ESCRT, endosomal sorting complexes required for transport, transport of endosomal proteins; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HSF1, trimeric heat shock transcription factor 1; HSP70 and HSP90, heat-shock proteins; INO1 and INO2, phospholipid synthesis proteins; LSm1-7/Dhh1p/Pat1p, protein complexes promoting mRNA degradation; OLE1, unsaturated fatty acid Δ9; OPI1P, FFAT-motif (two phenylalanines with acidic tail)-containing protein; OPI3, phospholipid synthesis protein; OSBP, oxysterol binding protein; PABP, poly(A)-binding protein; PAP85, vicilin-like seed storage protein; PE, phosphatidylethanolamine; PEX19, cellular transporter; PGK, phosphoglycerate kinase; PKC1, protein kinase C1; PLD, phosphatidic acid-generating enzyme, phospholipase D; PRE1, beta 4 subunit of the 20S core of the 26S proteasome; RHPs, protein homologues to the reticulum; RPN11, 26S proteasome non-ATPase subunit; RPT6, ATPase 6 of the 19S regulatory particle of the 26S proteasome; RSP5, ubiquitin ligase; SAR1, secretion-associated RAS-related 1; SCE1, SUMO-conjugating E2 enzyme; SEC39, vesicle-mediated transport protein; SEE1, METTL10-like methyltransferase; SKP1, S-phase kinase-associated protein 1; STI1P, Hop-like stress-inducible protein 1; SYP71, plant-specific Qc-SNARE protein; TIP1 and TIP2, tonoplast intrinsic proteins 1 and 2; TOM1 and TOM3, transmembrane proteins; VAP, VAMP-associated protein; YDJ1, protein of the family of heat-shock proteins HSP40.

^bBaMV, *Bamboo mosaic virus*; BMV, *Brome mosaic virus*; BYV, *Beet yellow virus*; CMV, *Cucumber mosaic virus*; CNV, *Cucumber necrotic virus*; CIRV, *Carnation Italian ringspot virus*; CTV, *Citrus tristeza virus*; RCNMV, *Red clover necrotic mosaic virus*; ToMV, *Tomato mosaic virus*; TBSV, *Tomato bushy stunt virus*; TMV, *Tobacco mosaic virus*; TSWV, *Tomato spotted wilt virus*; TuMV, *Turnip mosaic virus*; PVA, *Potato virus A*; PVX, *Potato virus X*; TYMV, *Turnip yellow mosaic virus*; WYMV, *Wheat yellow mosaic virus*.

Host factors involved in the post-translational modification of viral proteins

Different events of the virus life cycle (e.g. translation, assembly and spread to neighbouring cells) are coupled to viral RNA replication. Therefore, it is not surprising that viral proteins undergo post-translational modifications to increase their functional diversity. Phosphorylation of certain viral proteins negatively regulates virus replication, as described for RNA polymerase 2a from CMV that is phosphorylated by CIPK12 (calcineurin B-like calcium sensor-interacting protein kinase 12) at the late infection stages in *Nicotiana tabacum* (Kim *et al.*, 2002). Another example of viral proteins undergoing phosphorylation are the replication-related proteins 66K and p33 from TYMV and *Cucumber necrosis virus* (CNV), respectively (Wang, 2015). In the case of TBSV, the overexpression of protein kinase C1 (PKC1) identified in a

genome-wide overexpression proteome screen in yeast inhibits TBSV replication (Shah Nawaz-ul-Rehman *et al.*, 2012).

Ubiquitination of viral proteins also appears to play an important role in regulating viral replication, as observed for TBSV p33 whose balanced ubiquitination by E2 ubiquitin-conjugating enzyme CDC34 is required for its interaction with HFs in yeast (Barajas and Nagy, 2010). RSP5, an E3 ubiquitin ligase, interacts with TBSV p33 and p92, and inhibits TBSV replication in yeast when overexpressed (Barajas *et al.*, 2009). Interestingly, host-dependent ubiquitination of TYMV 66K can be counteracted by viral 98K RNA polymerase that deubiquitinates 66K to regulate viral replication (Camborde *et al.*, 2010; Alcaide-Loridan and Jupin, 2012; Chenon *et al.*, 2012). More recently, other post-translational modifications of viral or host proteins affecting viral replication have been described. This is the case of the SUMOylation of TuMV NIB RNA polymerase by SUMO-conjugating E2 enzyme SCE1 (Xiong and Wang, 2013), or the methylation of host eEF1A by the mETTL10-like SEE1

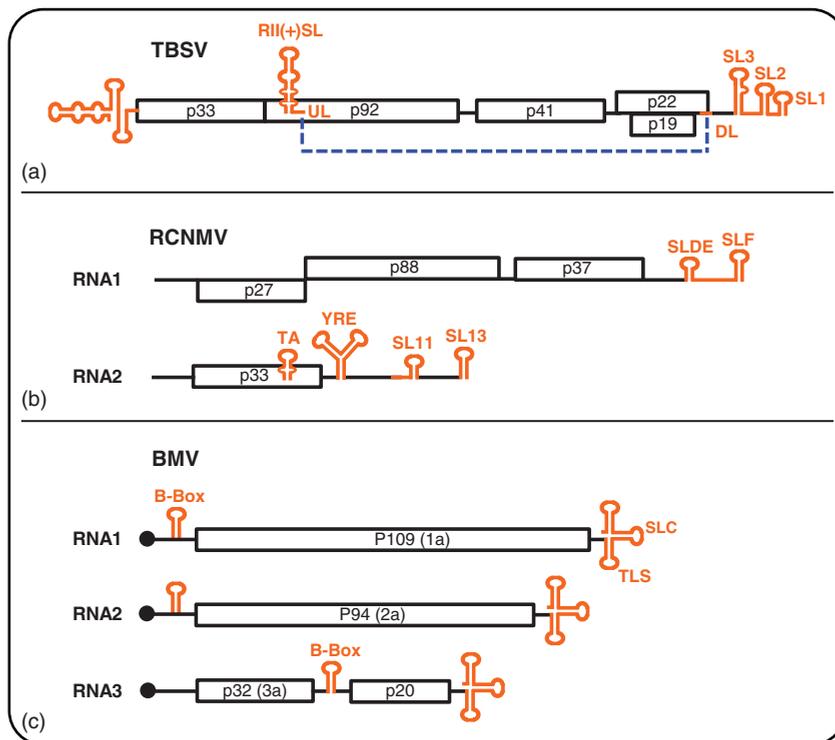


Figure 2 Cis-acting RNA elements in viral genomic RNAs (gRNAs) involved in genome replication. A diagram corresponding to the linear RNA genome is shown for each selected virus described in the main text. RNA elements in the viral RNA genome involved in replication are shown in orange, and the long-range interactions between them are shown in blue dotted lines. (a) RNA elements in *Tomato bushy stunt virus* (TBSV) gRNA. (b) RNA elements in *Red clover necrotic mosaic virus* (RCNMV) gRNAs. (c) RNA elements in *Brome mosaic virus* (BMV) gRNAs. Adapted from Newburn and White (2015) © Elsevier.

methyltransferase that inhibits TBSV replication in yeast and plants (Li *et al.*, 2014). **See also: Proteins: Postsynthetic Modification - Function and Physical Analysis; SUMOylation**

Functions of Viral RNA in Genome Replication

The viral RNA performs both template- and non-template functions during replication. The latter ones include its recruitment to the replication site and the assembly of a functional VRC. A variety of RNA elements (most of which act in cis) regulating these processes have been identified and characterised (Newburn and White, 2015). They are present either in the 3' UTR, internal regions, or the 5' UTR of viral RNAs, and include linear nucleotide sequences, RNA segments that may fold into characteristic secondary structures (e.g. stem-loops, SLs) or others adopting high-order structures that may also participate in medium- or long-range RNA:RNA interactions. Some of the best-studied examples of viral cis-acting RNA elements involved in genome replication of plant RNA viruses are described below.

The replication of tombusviruses, exemplified by TBSV, has been intensively studied in the past years. This work has led to the identification of the RNA motifs that act as core promoters

for (+) and (–) strand synthesis initiation (Pathak *et al.*, 2011). In the 3' UTR of TBSV (+) gRNA, an SL structure (SL1) and the adjacent 3'-terminal nucleotides constitute the core promoter for (–) strand synthesis, whose activity is negatively regulated by its interaction with an internal loop in a proximal SL (SL3) (Figure 2a). The presence in other tombusvirids of similar short-range interactions regulating core promoter activity suggests that this is a well-conserved mechanism for the modulation of viral replication. The core promoter for (+) strand synthesis corresponds to the 3'-terminal 11 nt of TBSV (–) strand of gRNA, and its activity is positively regulated by elements that are located on the same (–) strand molecule. The existence of elements in the (+) and (–) gRNA that act as repressors and enhancers, respectively, of the synthesis of the corresponding complementary strands most likely accounts for the strand asymmetry that characterises the replication process in (+) RNA viruses, with (+) strands being produced in a large excess over (–) strands.

Another important RNA element for TBSV replication is located in the 5' UTR of the gRNA (Figure 2a). The sequence and the secondary and tertiary structures of this element are important for the accumulation of TBSV RNA of both polarities (Ray *et al.*, 2004). In addition, another SL structure, known as RII(+)/SL, that contains a critical CC mismatch and that is located in the p92/RdRp coding region of TBSV RNA (Figure 2a), is recognised by the viral protein p33, a recognition that seems essential for recruitment of the viral template for replication.

The interactions between RII(+)-SL and the 3' terminal SLs, and between an upstream (UL) and a downstream (DL) linker, result in the formation of a bipartite RNA platform that assists the VRC assembly (**Figure 2a**) (Pathak *et al.*, 2011, 2012). In the case of the dianthovirus RCNMV, a *Tombusviridae* member with bipartite RNA genome, both gRNAs contain RNA elements important for replication (Pathak *et al.*, 2011). RNA1 contains two SLs (SLDE and SLF) that form the core promoter for (–) strand synthesis (**Figure 2b**). RNA2 also contains two SLs (SL11 and SL13) that are the core promoter for (–) strand synthesis of this genomic segment (**Figure 2b**). In addition, RNA2 includes at least two other RNA elements that are critical for (–) strand synthesis: a Y-shape RNA element (YRE) located in the 3' UTR which routes RNA2 to the ER membrane for VRC assembly and an element (TA) in the coding region of p33 that might interact with host proteins required for viral replication (Pathak *et al.*, 2011) (**Figure 2b**). **See also: Tombusviridae**

Another extensively studied virus in terms of replication is BMV. The three BMV gRNAs fold in a tRNA-like (TSL) structure at their 3'-terminal regions, with an SL (SLC) being critical for VRC assembly (**Figure 2c**) (Wang, 2015). Moreover, an SL RNA motif named B-box is present in the 5' UTR of RNA1 and RNA2 as well as in the intercistronic region of RNA3 (**Figure 2c**). In all cases, these B-box motifs are critical for the interaction with BMV 1a protein and subsequent recruitment of the viral RNAs for replication (Wang, 2015). Another SL structure at the 3' end of RNA2 (–) strand has been identified as the core promoter for (+) strand production (Sivakumaran *et al.*, 1999). **See also: Bromoviridae and Allies**

Concluding Remarks

Replication is at the core of the infection cycle of plant RNA viruses. It represents the complex process by which RNA viruses copy and package their genome to infect new cells and, hence, ensure their survival. During the past years, our knowledge of how RNA viruses replicate in plant cells has increased significantly. In particular, for (+) RNA viruses, this is explained by (1) the availability of suitable reverse genetic approaches due to the relative ease of constructing infectious cDNA clones that allow modifying virus genomes and evaluating *in planta* the effect of these modifications on virus biology, and (2) the ability of some (+) RNA viruses to replicate in yeast, an alternative experimental host with a well-annotated genome that has facilitated genome-wide searches for HFs involved in virus replication. The future development of similar approaches for studying (–) RNA and dsRNA viruses is thus critical to better understand how these two classes of virus replicate. More generally, a deeper understanding of virus replication should facilitate the development of novel strategies for inducing more effective antiviral resistance in plants.

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