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IS GENOME PACKAGING IN SMALL PLANT VIRUSES ENERGY INDEPENDENT?

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ABSTRACT

Genome packaging is a critical step during the viral maturation process. Viruses employ a distinct approach to package their genetic materials inside capsid: ranging from very simple strategy of nucleation of capsid proteins onto genome to complex segro-packasome machinery. The majority of small plant viruses, which falls under type I passive system, package their genome into stable virions in the cytoplasmic compartment, where chances of co-packaging of host RNA is very high, indicates viruses evolved the mechanism of selective and stringent packaging of their genomes. Recent discoveries of the unique ATPase fold in the capsid proteins of smaller plant viruses and their direct or indirect role during genome packaging have changed the perception about genome packaging in type I system. We feel that viruses of type I system have acquired unique and independent innovations for genome packaging over the course of evolution. The molecular interactions, intriguingly, cross-talk between capsid proteins and conserved signal sequence situated at the end of genome, plays an important role while viral genome packaging and translocation. Strategy utilized by smaller plant viruses for enhancement of selective genome packaging does not depend simply on nucleation of capsid proteins over genome but interestingly, configuration of viral genome, replicase, tRNA, viral encoded movement proteins are the other important key players that regulate genome packaging. The main aim of this review is to discuss and revisit the mechanism of genome packaging among viruses of agronomic importance. This study will be also useful for understanding the origin and evolution of viral genome packaging apparatuses and their roles in eukaryogenesis.

Keywords: Plant viruses, Viral genome packaging, Energy independent packaging system, Capsid protein, ATPase fold
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INTRODUCTION

Packaging of the genome into the capsid is the crucial step during the assembly of virion particles. Almost all viruses basically employ three unique mechanisms to translocate their genome inside the capsid coat. In the type I system, capsid proteins (CP) recognize and condense with the genome and form a shell (Chelikani *et al.* 2014a), employ in small plant and animal viruses with genome size lesser than 20kb (most of the plant viruses like TMV) (Burroughs *et al.,* 2007; Kumar *et al*., 2022; Ranjan *et al*., 2021). The genome condensation and capsid assembly in these viruses are coupled by coating the genetic material with viral capsid proteins without utilizing ATP leading to virus assembly (Kutluay *et al.,* 2010; Ranjan *et al*., 2021). On the basis of configuration, the genomes of viruses infecting plant can be either non-segmented or segmented and are encapsidated into more than one or a single virion, respectively. In some of the plant pathogenic viruses like Bomovirus, multipartite genomes are encapsidated in three different virions of identical size and morphology. This stringency towards selection and packaging of viral RNA is achieved by specific interaction between capsid protein and nucleic acids *viz.*, (1) protein-protein interactions for capsid assembly, (2) DNA/RNA-protein interaction for nucleation of capsid proteins on the genome, (3) sequence independent and dependent DNA/RNA-protein interactions for stabilization of the encapsidated genome and to avoid encapsidation of non-viral genome respectively within the mature virion particles (Vriend *et al.,*1986; Catalano, 2005). The unique packaging signal or OAS (origin of assembly sequence) such as stem-loop like structures, 3'-tRNA-like sequence (TLS) and 5'-untranslated region (UTR), at the genome termini are recognized by capsid protein (CP) distinguishes the viral genome from cellular genetic material (Kumar *et al*., 2022; Ranjan *et al*., 2021). For example, in case of HIV, 5'UTR act as a nucleation target for capsid assembly (Catalano, 2005; Choi and Rao, 2003). Apart from these unique packaging sites, some other factors *viz.* host encoded tRNA, viral replicase and movement proteins, etc. increase the efficiency of genome packaging (Choi and Rao, 2003; Rixon, 1993). In majority of plant viruses, RNA is extraordinarily compact with high degree of folded structure after their packaging inside capsid (Larson *et al.,* 2005; Tang *et al.,* 2001). Initially, the partly folded RNA might serve as a platform for CP assembly and later lead to the energetically favorable double stranded helices, stem-loops and folded tertiary structure of RNA (Schneemann,

2006). The CP basically serves as chaperone for genome folding and provides optimal interactions required to retain the folded RNA/DNA inside the capsid (Chen *et al.,* 1989; Fisher *et al.,* 1993; Ranjan *et al*., 2021).

The discovery of CP with a unique ATP binding and hydrolysis motifs such as repeated Walker A, Walker B, sensor and arginine motif in several plant viruses has considerably changed the opinion about energy independent type I packaging system (Kumar *et al*., 2022; Rakitinaa *et al.,* 2005; Ranjan *et al*., 2021). Interestingly, mutation and knockdown of the ATPase domain resulted in production of noninfectious with nucleic acid-deficit virions indicating its direct role in the genome packaging (Kumar *et al*., 2022). Surprisingly, apart from CP, recruitment of other accessory ATPases (for example P4 ATPase, VP3 ATPase, Hsp-70, Rep andP10 ATPase etc.) during process of virus assembly made type I packaging system very interesting (Alzhanova *et al.,* 2001; Ranjan *et al*., 2021; Wang *et al.,* 2005). However, the molecular machinery that carries out the complex operation of genome encapsidation in plant viruses with remarkable fidelity remains to be discovered and understood.

Both type II and III system are ATP dependent active packaging apparatus (Chelikani *et al.,* 2014a; Ranjan *et al.,* 2021) and operate in viruses with large genome size (>20kb) (Burroughs *et al.,* 2007). The well-known type II packaging system also known as the phage terminal-portal system, makes use of terminase protein to dock the genome onto the portal situated at a vertex of preformed empty prohead and translocate inside (for ex T4, T7, N4 and lambda phages) (Kondabagil *et al.,* 2006; Yang *et al.,* 2003). The recently discovered type III system includes giant viruses (Mimivirus, Pandoravirus etc) with genome size up to 2.5Mbp employs a unique prokaryotic segro-packasome like machinery to encapsidate their genome inside pre-assembled prohead (Chelikani *et al.,* 2014b; Iyer *et al.,* 2004).

The present review aims to upgrade type I genome packaging system with more emphasize on viruses infecting plant. Until now, reported plant viruses are classified in to 80 genera; out of which 53 possess icosahedral and 25 genera exhibits helical symmetry. The remaining two have unknown capsid symmetry (Catalano, 2005). In spite of differences in terms of architecture and host, the packaging of infectious virions is basically coordinated by precision interaction and nucleation of CP onto the nucleic acid (Catalano, 2005; Ni *et al.,* 2013; Ranjan *et al*., 2021; Sarah *et al.,* 2011; Zhu *et al.,* 2015). The final destination for matures plant virion particles is their long distance spreading from cell-to-cell and their procurement and dissemination to new hosts by vectors such as insect, nematode, mite and fungi (Vriend *et al.,* 1986; Benitez-Alfonso *et al.,* 2010). Thus the detailed knowledge of mechanism of assembly and genome packaging in plant viruses is essential for understanding their biology. Plant viruses are responsible for huge losses in crop production and cause 50-60% of total damage alone (Kreuze *et al*., 2020). Targeting viral maturation steps may prevent huge economical loss (Kumari *et al*., 2021; Kumar *et al*., 2022). This kind of study will not be only helpful for agricultural biologists but also would be useful for understanding the origin and evolution of packaging mechanisms and role of small viruses in eukaryogenesis.

Genome packaging in small viruses infecting plant: Plant virus assembly takes place by the nucleation of CP onto nucleic acids instead of direct translocation of genome inside the empty prohead (Rao *et al.,* 2005; Chelikani *et al.,* 2014a) (Figure 1A & Figure 2). During infection, viruses enter, disassemble and release their genetic material inside the cytoplasm (Figure 1A &B) (Rao, 2006). Early translation synthesizes many viral encoded proteins such as movement protein (MP) (Figure 1C.II) and simultaneously during the progress, a vesicular-like structure called the 'replication factory' assembles in the cytoplasm due to perturbation of inner membranes (Figure 1C.I) (Rao *et al.,* 2005). The replication factory is equipped with different proteins involve in multiplication of viral genome. After amplification of genomic copy number, the CP and MP synthesis takes place in a large amount at late translation stage (Figure 1D, E, F& 1E.I) (Francki *et al.,* 1985; Buck, 1996; Catalano, 2005). The CP has the ability to discriminate the viral nucleic acids from the pools of host cytoplasmic RNA (Francki *et al.,* 1985). It seems that viruses have evolved with efficient machinery for selective packaging of their genomes inside coat (Ranjan *et al*., 2021; Fox *et al.,* 1998). The stringency towards selective packaging of genome is accomplished by many factors *viz.,* configuration of nucleic acid, packaging signals, interaction between nucleic acid-protein, protein-protein and some additional factors for example tRNA (Rao, 2006). Finally, CP recognizes the signals at the genomic end and start co-condensing over it (with or without hydrolyzing ATP), lead to the generation of mature virus particles (Figure 1G&H, Figure 2). Our extensive bioinformatics data has found a novel ATP binding and hydrolysis motifs on the polypeptide chain of CP, suggesting their role during the viral maturation process (Kumari *et al.,* 2020; Kumar *et al.,* 2020; Kumar *et al*., 2022; Ranjan *et al.,* 2021). Apart from this, enough studies have been done by our lab which further confirms the role of ATP in genome packaging and virus assembly process (Kumar *et al*., 2022; Ranjan *et al.,* 2021). Co-condensation is usually arranged in a helical fashion throughout the RNA genome in TMV (Figure 2). Finally, MP helps in spreading of mature virions to the neighbor cells (Figure 1I). MP either transport virus particles through the enlarged pore of plasmodesmata (Figure 1I) or directly allow the genome as nucleoprotein complex to the adjoining cells (Figure 1C.III & IV) (Kumari *et al*.,

2021; Sunpapao, 2013). At last, newly assembled viral particles exit from the cell and are transmitted to another host by the help of insect and other means (Figure 1J).

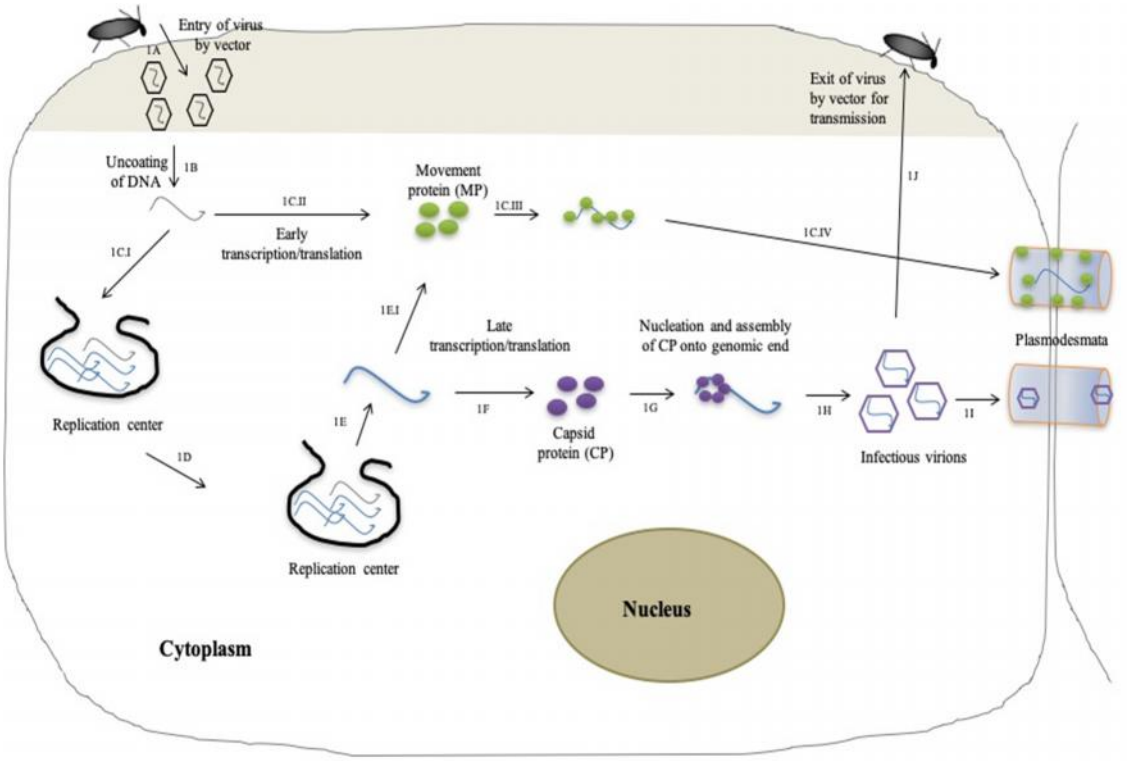

Figure 1: Schematic representation of life cycle of plant viruses. After entry inside the host with the help of vector (1A & B) viruses increase their genomic copy numer within a specialized cytoplasmic space known as 'replication centre' (1C-D). Further, after early (1E) and late translation (1F), the viral encoded capsid protein and movement protein transport the completely packaged (1G-H) and mature virus particle to the neighbour cells through plasmodesmata (1I). Movement protein can also transport the naked viral genomes into the neighbour cells, where virus completes their life cycle (1C II-IV).

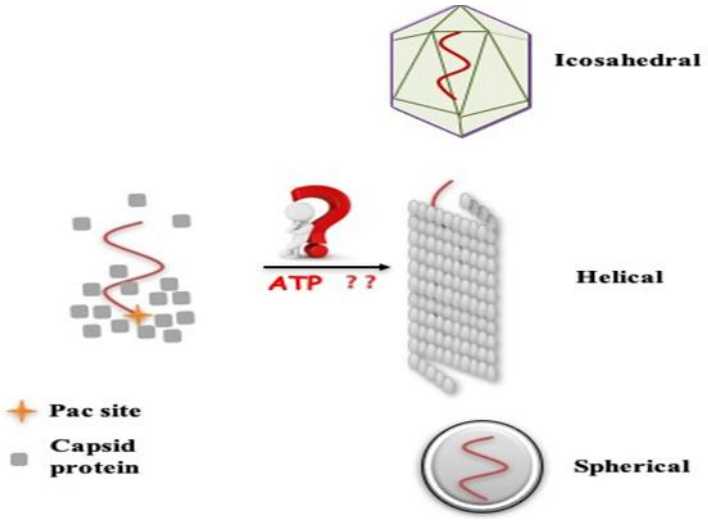

Figure 2: A generalized scheme for genome packaging and assembly in plant viruses with all three types of head configurations. Capsid protein recognises the packaging signal or site (pac site) situated at the genomic end. We propose that, the oligomerization of capsid protein onto genome and further their packaging process is fueled by energy or ATP.

Factors governing genome encapsidation in plant viruses

Genomic Organization: Genome of plant viruses exhibits different forms ranging from segmented to non-segmented. Most of the spherical plant virus possesses partite genome. Viruses (such as Tymovirus, Sobemovirus, Luteovirus, and Tombus virus) with monopartite genome (Table 1) encode an extra sub-genomic RNA (sgRNA), which is packaged into a separate particle. Bipartite viruses with two RNA copies either package or translocate their RNAs into a single virion particle (Diantbovirus) or in two separate virions particle (Comovirus and Nepovirus) (Table 1). Bromovirus, Cucumovirus and Alfamovirus are typical example of multipartite virus (Koev and Miller, 2000). These Icosahedral plant viruses encode more than two segments of RNA (Table 1). In Bromovirus and Cucumovirus, first two segments of RNA package into two separate virion particles but third RNA segment package in a separate compartment along with their sgRNA. Interestingly, in case of Alfamovirus, all the three segments and one sgRNA are package inside four distinct particles (Table 1). Study suggests that packaging of different segments into a single or separate capsid may be co-operative and in other words, packaging of one segment further helps other segments to be encapsidated (Koev and Miller, 2000). Apart from genomic RNA, many of the plant virus needs some secondary RNA such as sgRNA, satRNA during encapsidation/or genome packaging. Although these RNAs does not have direct role during infection process but their involvement during gene regulation has been reasonably well understood (Koev and Miller, 2000; Catalano, 2006). In case of plant and animal icosahedral virus (Polio virus and Hepatitis A virus), (+) ssRNA arranged in stem-loop like secondary structure and helps in nucleation of capsid protein with high affinity. Studies have shown that disruption of such kind of structure leads to the abruption of mature virions production (Frolova *et al.,* 1997). Other secondary structure like 5'-UTR with single stranded RNA dimer in case of HIV allows Gag protein (a major capsid protein) to interact with each other and oligomerize over it. And later Gag protein docks the genome to the membrane raft of host where assembly takes place by the aiding of other structural proteins (Momany *et al.,* 1996).

Table 1: Characteristic features of majority of plant viruses with their typical example.

Name of Virus	Genome	Genomic configuration	No. of genome packaged (sg/sat Genome)	Head configuration
Bromovirus	RNA	Tripartite	3(1)	Spherical
Cucumovirus	RNA	Tripartite	3(3)	Spherical
Alfamovirus	RNA	Tripartite	3(1)	Spherical
Oleavirus	RNA	Tripartite	3(1)	Spherical
Ourmiavirus	RNA	Tripartite	3(0)	Spherical
Ilavirus	RNA	Tripartite	3(1)	Spherical
Fabavirus	RNA	Bipartite	2(0)	Spherical
Nepovirus	RNA	Bapartite	2(0)	Spherical
Tymovirus	RNA	Monopartite	1(1)	Spherical
Sobemovirus	RNA	Monopartite	1(1)	Spherical
Luteovirus	RNA	Monopartite	1(2)	Spherical
Polerovirus	RNA	Monopartite	1(1)	Spherical
Sequivirus	RNA	Monopartite	1(0)	Spherical
Tombusvirus	RNA	Monopartite	1(2)	Spherical
Carmovirus	RNA	Monopartite	1(2)	Spherical
Tobacco Mosaic Virus	RNA	Monopartite	1(3)	Rod
Bromo Mosaic Virus	RNA	Tripartite	3(1)	Icosahedral
Geminivirus	DNA	segmented	1	Icosahedra

The sum of nucleic acid that can be packaged inside the spherical/icosahedral/helical virion depends upon the interior capacity or volume of protein shell and this may differ from virus to virus. For example, Cowpea mosaic virus package their genome segments I and II (3.2 & 5.8kb, respectively) in to the two different capsids with an inner capsid volume of 4.32×10^{-6} Å. The nucleic acid density in human picornavirus is higher than that of

crystalline duplex RNA (Johnson *et al.,* 1997). The extent of distribution and arrangement of hydrogen bonding inside virions can be solved by several techniques like solution X-ray scattering, Raman spectroscopy and neutron diffraction (Schmidt *et al.,* 1983; Li *et al.,* 1992; Timmins *et al.,* 1994).

Molecular Interactions: Elimination of host RNA and precise viral genome packaging inside the capsid coat is crucially governed by molecular interactions, which could be either protein-nucleic acid interaction or protein-protein interaction (Catalano, 2005). Contribution of above two molecular interactions varies from genera to genera. These interactions operate while capsid-capsid oligomerization and capsid-nucleic acid cross talk during viral assembly and morphogenesis. In the members of genera Tymovirus and Comovirus, virion assembly is predominantly stabilized by protein-protein interaction and oligomerization of capsid protein is not dependent on the presence of RNA (Schneemann, 2006; Rao, 2006). Assembly of several plant viruses is guided by mostly nucleic acid-protein interaction, where oligomerization of CP is critically initiated in the presence of genome only as seen in Alfamovirus, Bromovirus and Cucumovirus etc (Schneemann, 2006; Rao, 2006). This kind of physical interaction ensures the encapsidation of viral genome exclusively into the progeny virions. One of the structural proteins of picornavirus viz., VP3 with ATPase activity behaves like chaperone and helps in condensation of genome with the icosahedral symmetry (Zhu *et al*., 2015).

We divide protein-RNA interactions into two subtypes, which could be either sequence specific or non-specific. A specific interaction between nucleic acid and capsid protein leads to the nucleation of other capsid proteins and ensure the exclusive encapsidation of viral genome. Non-specific interactions provide stability or compactness of packaged genome inside the envelope through interaction between N-terminal region of capsid protein (rich in basic amino acids) and RNA molecules (Vriend *et al.,* 1986; Johnson, 2003). Apart from these interactions, genome trans-encapsidation mechanism, where capsid protein of one virus package nucleic acid of other one has also been reported in viruses with similar taxonomic groups (Creamer and Falk, 1990; Hull, 2002).

Impacts of molecular interactions on selective genome packaging: The CP of plant viruses usually do not package the genomes of different viruses after co-infection of the same host. To increase the stringicity of selective packaging, the CP has capability to distinguish between viral genomes. However, studies also suggest nucleocapsid-independent viral RNA packaging in case of Coronavirus (Catalano, 2005, Masters *et al*., 2019). Genome packaging mechanism is very simple in non-segmented genomic viruses, where only one segment has to go inside a single compartment (Cuillel *et al.,* 1979; Damayanti *et al.,* 2000). In case of bipartite and tripartite viruses, the packaging mechanism is relatively complex as CP has to distinguish gRNA from sgRNA prior to their segregation into individual virions. Virus has to act smartly in distribution of segmented genome to the multiple virion particles. In multipartite virus, genome packaging seems to be co-operative, where RNA binding capability of CP facilitates RNA-RNA interaction. The binding of CP to nucleic acids exposes the packaging signal, which further allows their recruitment and oligomerization (Lazinski *et al.,* 1989; Qu *et al.,* 1997).

The CP with both sequence non-specific and specific nucleic acid binding activity, plays a vital role while selective packaging of viral genome via specific RNA-protein interaction from a large pool of cellular RNAs (Duggal and Hall, 1993). The specific RNA-protein interaction in plant and other viruses is mainly governed by 'arginine rich RNA binding motif' (ARM) of CP, which resulted in nucleation of CP onto genome while packaging (Ford *et al.,* 2013). The ARM is found in CP of several plant (such as CMV and BMV) and animal (such as HIV) viruses and first time it was discovered in lambda bacteriophage (Lazinski *et al.,* 1989; Weeks *et al.,* 1991; Wei *et al.,* 1991; Zeltins, 2018). The ARM is consisting of small stretch of basic amino acids with the α-helix, β hairpin and extended chains conformations (Dreher, 1999; Catalano, 2005). This ARM is a hallmark of CP, which lowers the chance of co-packaging of cellular RNAs. It seems that during the course of evolution, viruses have developed stringency towards selective packaging of nucleic acids and minimized the chances of encapsidation of high backgrounds of cellular RNAs (Catalano, 2005; Lazinski *et al*., 1989; Burd and Dreyfuss, 1994). The CP of sobemovirus has T=3 symmetry, made up of highly flexible basic amino acids rich R-domain and jelly-roll containing motif S-domain (Choi *et al.,* 2000; Tamm and Truve, 2000). Studied have shown that removal of R-domain leads to the capsid T=1 symmetry without RNA, indicates flexibility of N-terminal R-domain has role in protein-nucleic acid interaction and ultimately in packaging. Apart from ARM, R-domain contains another motif which has beta-annulus like structure and thought to impart a critical role in transition of capsid symmetry from T=1 to T=3 (Satheshkumar *et al.,* 2004; Basnak *et al.,* 2009; Sarah *et al.,* 2011).

It has been hypothesized that ARM specifically interacts with RNA in their alpha-helical conformation, which makes them highly flexible prior to communication between CP and RNA (Vriend *et al.,* 1986). This flexibility of N-terminal ARM has a great impact in term of degree of freedom, which increases the probability of interaction to nucleic acids. In case of majority of plant viruses and few animal viruses such as HIV, the helical configuration of ARM makes more accessible and suitable to interact with nucleic acids via

salt bridge interaction between basic amino acids (ARM region of CP) and phosphate backbone of nucleic acid (Tan and Frankel, 1995). Interestingly, alpha configuration of ARM is essentially not required for genome packaging in case of Bromovirus, where proline (a helix destabilizer) residues are prominent in CP (Catalano, 2005). Mutational study indicates the contribution of N-terminal ARM (specifically amino acids from 9 to 19) in cooperative recognition and packaging of genome in multipartite viruses such as BMV. Duggal and Hall in 1993 also suggests that amino acids between 9 to 20 residues of ARM critically required for recognition of 5' 943 nucleotides of RNA1 in case of BMV. Evidences suggest that N-ARM also contains critical amino acids which are more specific to sgRNA (Duggal and Hall, 1993). The N-terminus of CP is highly flexible which tends to be located inside the internal cavity of capsid coat and encapsidate the genome via several kind of interaction with them (Ni and Kao, 2013). Recent high resolution *de novo* atomic model indicates that not only N-terminal region of CP is solely responsible for genome encapsidation but C-terminal region also critically imparts in selective viral genome packaging. Similarly, C-terminal region is also highly basic in nature and rich with arginine amino acids particularly. Mutagenesis study by replacing arginine residues (R193, R195) with other amino acid shows complete absence of mature virion particle production (Hesketh *et al.,* 2015; Ni and Kao, 2013).

Structure of RNA: RNA can adopt several secondary and tertiary conformations such as 5' UTR, 3' tRNA-like structure, and stem-loop and these unique conformations are very crucial for assembly and nucleation of CP (Barry and Miller, 2002; Momany *et al.,* 1996; Wang *et al.,* 1999). Studies indicate that stem loop-like structure at the end of genomes act like a nucleation center for CP during packaging in BMV. Not only these higher secondary structure at the genomic end but overall structure is also important for interaction between CP and nucleic acid. For example, packaging of 300 nucleotide satRNA of CMV is efficiently regulated by the overall structure of RNA rather than any specific secondary configuration at the end (Qu and Morris, 1997; Choi *et al.,* 2000; Choi *et al.,* 2003; Catalano, 2005). Thus, a correlation between structural features of RNA and its interconnection to replication, translation and genome packaging needs to be explored in details and this could be done by the help of advancement in several techniques like x-ray and others. Small angle X-ray can offer us for direct anticipation of shape and size of RNA molecules inside envelope and their interaction with capsid protein.

Based on specific sequence on genomic RNA, three different models were found to operate in monopartite, bipartite and tripartite viruses (Catalano, 2005). Study in monopartite spherical Turnip crinkle virus (TCV) indicates that there are three high affinity CP-binding sites in genomic RNA; one in polymerase gene about 700 nucleotides from 5' end, second in CP gene about 700 nucleotides from 3' end and third one and third one in 186 nucleotides fragment at 3' end. For the efficient and selective packaging of genomic and sgRNA inside confined space of capsid coat, contribution of these CP binding sites is very critical (Wei *et al.,* 1990; Qu and Morris, 1997). The nucleotide region starting from 1410 to 1438 has been found to attain stem-loop like secondary structure in case of Southern beam mosaic virus (SBMV), where CP interact, oligomerize and nucleate to initiate the packaging process (Bink *et al.,* 2003). Similarly, in Turnip yellow mosaic virus (TYMV), packaging is initiated on to the two hairpins like structures at 5'UTR region in genomic RNA (Hacker, 1995; Bink *et al.,* 2003).

In case of bipartite virus, CP recognizes and encapsidate segmented genomes either within the same virion or into a separate virion particle containing each segment of RNA. Experiments suggest that both of the above strategies are being operated in bipartite virus. Irradiation and heat treatment experiment suggest formation of high molecular weight genome complex (RNA heterodimer and multimers) inside capsid after packaging. Based on this observation another strategy, called hybrid model for virus assembly (hybrid of both above strategies discussed) was proposed (Basnayake *et al.,* 2005). Importantly, most these partite viruses have evolved with a mechanism of co-operativity while their genome encapsidation (Hollings and Stone, 1977; Gould *et al.,* 1981; Hamilton *et al.,* 1996). For example, in Dianthovirus, cross-talk between RNAI and II is very essential for expression of CP and genome packaging. Thus the arrangement of genome and interaction of both RNA while CP expression makes their encapsidation into two independent virion particles interlinked (Red dover necrotic mosaic virus and Sweet clover necrotic mosaic virus) (Okuno *et al.,* 1983; Sit *et al.,* 1998). Although in Red clover necrotic mosaic virus (RCNMV), both RNA I & II have their independent origin of assembly (OAS) but RNA II usually possess a minimal OAS site. Most of the viruses have evolved themselves in such a way to encapsidate particular amount of their genome for optimum packaging and slight deviation may lead to the very low packaging efficiency (Basnayake *et al.,* 2005).

Experiments suggest that gRNA I and II package independently into two different particles, but gRNA III and sgRNA IV co-package together form separate virion particles in the tripartite BMV with icosahedral symmetry (Rao, 2000). Despite the fact that gRNA I, II, III, and sgRNA IV have different sizes, all three virion particles are morphologically and physically indistinguishable. This could be the one of the reason of tight regulations during genome distribution into three independent particles. Several experimental studies

suggest a critical role of cis-acting elements/signals in assortment as well as packaging. At the end of each of the eight segments of the influenza A virus genome encodes a unique packaging signal (Rao, 2000; Catalano, 2005). The panhandle structures created by base-pairing at the ends of all 11 segments of the rotavirus genome may serve as a site for genome assortment and packing (Sarah *et al.,* 2011).

Transfer RNA: Apart from their primary involvement in protein synthesis and biomolecule formation (chlorophyll, Heme), tRNA has been linked to a variety of additional processes such as replication. In the case of retroviruses, host tRNA acts as a primer during reverse transcription. (Jahn *et al.,* 1992; Marquet *et al.,* 1995). The 3' transfer RNA like sequence (TLS) serves as the origin of replication and primitive telomeres in plant viruses such as Bromovirus, Cucumovirus, and others. According to the findings, cellular tRNA is also detected in various plant icosahedral viruses, such as the Eggplant mosaic virus (Rao *et al.,* 1989; Hema *et al.,* 2005). Till date, we do not have any clue on co-packaging and active selection of cellular tRNA inside the capsid. For the first time, Choi *et al* in 2005 has suggested the role of tRNA in genome packaging in case of BMV (Choi *et al.,* 2002).

All four RNAs in BMV have a 200-nucleotide-long 3'UTR that folds into a tRNA-like structure (TLS) by imitating themselves. According to the findings, the highly organized structure (such as TLS) enhances CP nucleation and genome packaging. The deletion of 3'UTR leads to inhibition of virus assembly and infectivity of virus can be restored by adding 200 nucleotides (Dreher, 1999; Catalano, 2005). Thus TLS mediated assembly is strictly dependent on its ordered structure and this hypothesis further can be supported by the evidence that TLS from CMV, TMV and tRNA as well as from yeast supports BMV assembly (Zlotnick *et al.,* 2000). This could explain the fact of how viruses take advantage of host tRNA in their genome replication, translation and packaging. The TLS basically act as a scaffold for oligomerization of CP which could be dimers to multimers (Choi *et al.,* 2000; Barry and Miller, 2002; Choi *et al.,* 2002).

Unlike BMV (Infect to monocotyledonous) where packaging of RNA III is dependent on bipartite signal, CCMV (infect dicotyledonous) RNA III does not depend on CP ORF, MP ORF and 3'UTR (Rao, 1997). Apart from this CCMV differ in several aspects from the BMV. For example, CCMV transfer their genetic material between cells through plasmodesmata without utilizing CP but BMV has to ensure assembly of virion particles before movement to neighbor cells (Figure 1). This might be the reason of different mode of genome packaging operating in these two viruses (Schmidt *et al.,* 1983). The genomic RNA of alfalfa virus has 3' non-coding 112 nucleotide region, which can attain either stem-loop or TLS type structure. The switching between these two secondary structures regulates the transition between translations to replication in these viruses. Like BMV, genomic RNA III of CCMV encodes TLS, which plays a trans-acting role during the packaging of genome (Olsthoorn *et al.,* 1999).

Interestingly, in 2003, Choi and Rao reported that apart from TLS, OAS and UTR, a cis-acting position dependent 180 nucleotide present in the MP ORF is critically required in genome packaging of BMV. Study also suggests that deletion of this region from MP ORF leads to the disruption of assembly but replication and translation was observed efficiently (Wang *et al.,* 1999).

Viral replicase: Apart from multiplication of copy number, a role of viral replicase in their genome encapsidation has also been demonstrated in plant viruses (Annamalai and Rao, 2005).The role of viral replicase in genome encapsidation in many of animal viruses such as Poliovirus, Floch house virus (FHV), and Kunjin virus has already been well understood. Although the precise role of replicase in genome packing is still unknown, but investigations suggest that its knockdown may cause early disruption in genome condensing and encapsidation process inside the capsid coat. Replicase and CP work together to distinguish the viral genome from the cytoplasmic pool and package it (Traynor *et al.,* 1991; Nugent *et al.,* 1999). According to the findings, the lack of a functional replicase results in generation of genome deficit virus particle (Annamalai and Rao, 2005). Size/volume of viral capsid is determined by the interaction between identical CP subunits and conformational changes induced due to these interactions. Interaction between multiple identical subunits of CP sometimes leads to the spherical and icosahedral head conformation. In case of adenovirus and herpesvirus, scaffold protein is known to regulate the correct procapsid assembly. Evidences suggest that absence of scaffold protein leads to the generation of abnormal procapsid which is unable to package the genome (Rixon, 1973). In case of some animal viruses, host encoded histone proteins has also an impact on genome packaging and condensation inside capsid coat. For example, SV40 has minichromosome like structure wrapped with the nucleosome formed with the help of host histone proteins (Polisky *et al.,* 1975).

Conclusion: Mechanisms of genome packaging and encapsidation vary from simple coating of the genome with capsid proteins as in most-small viruses to the highly complex genome packaging mechanisms of larger DNA viruses that resemble prokaryotic chromosome segregation and pumping mechanisms. Here, we have made an attempt to revisit and understand the mechanism of genome packaging in small plant viruses (type I packaging system). Finding of a novel ATP hydrolysis motif in CP of several plant viruses has raised questions

about their place under passive packaging system. Using potexvirus and geminivirus as a model, our group is attempting to decipher the mystery of genome packaging in plant viruses. The details mechanism of viral packaging systems will help us in better understanding the viral packaging mechanisms. We believe that the molecular machinery that carries out the complex operation of genome encapsidation in plant viruses with remarkable fidelity remain to be discovered and understood in detail.

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REFERENCES

Alzhanova, D.V., Napuli, A.J., Creamer, R., Dolija, V.V. (2001) Cell-to-cell movement and assembly of a plant closterovirus: role for the capsid proteins and Hsp 70 homolog. The EMBO Journal. 20: 6997-7007.

Annamalai, P., Rao, A.L. (2005) Replication-independent expression of genome components and capsid protein of brome mosaic virus in plant: a functional role for viral replicase in RNA packaging. Virology. 338(1): 96–111.

Basnak, G., Morton, V.L., Rolfsson, O., Stonehouse, N.J., Ashcroft, A.E., Stockley, P.G. (2009) Viral genomic single-stranded RNA directs the pathway toward a $T = 3$ capsid. J. Mol. Biol. 395:924–936.

Benitez-Alfonso, Y., Faulkner, C., Ritzenthaler, C. and Maule, A.J. (2010) Plasmodesmata: Gateways to Local and Systemic Virus Infection. Molecular Plant-Microbe Interactions. 23(11):1403-1412.

Barry, J.K., Miller, W.A. (2002) A-1 ribosomal frame-shift element that requires base pairing across four kilo-bases suggests a mechanism of regulating ribosome and replicase traffic on a viral RNA. Proc. Natl. Acad. Sci. USA. 99(17):11133–38.

Basnayake, V.R., Sit, T.L., Lommel, S.A. (2005) The genomic RNA packaging scheme of red clover necrotic mosaic virus. Virology. 345(2): 532-539.

Bink, H.H., Schirawski, J., Haenni, A.L., Pleij, C.W. (2003) The 5-proximal hairpin of turnip yellow mosaic virus RNA: its role in translation and encapsidation. J. Virology. 77:7452–58.

Buck, K.W. (1996) Comparison of the replication of positive-stranded RNA viruses of plants and animals. Adv. Virus Res. 47(2):159–251.

Burroughs, A., Iyer, L., Aravind, L. (2007) Comparative genomics and evolutionary trajectories of viral ATP dependent DNA-packaging systems. Genome Dyn. 3: 48–65.

Burd, C.G., Dreyfuss, G. (1994) Conserved structures and diversity of functions of RNA binding proteins. Science. 265(5172):615–21.

Catalano, C. (2005) Viral genome packaging machines. Viral Genome Packaging Machines: Genetics, Structure, and Mechanism, 1-4.

Chelikani, V., Ranjan, T., Kondabagil, K. (2014a) Revisiting the genome packaging in viruses with lessons from the 'Giants'. Virology.466:15-26.

Chelikani, V., Ranjan, T., Zade, A., Shukla, A., Kondabagil, K. (2014b) Genome segregation and packaging machinery in *Acanthamoeba polyphaga* Mimivirus is reminiscent of bacterial apparatus. J. Virology. 88:6069–75.

Chen, Z.G., Stauffacher, C., Li, Y., Schmidt, T., Bomu, W. (1989) Protein-RNA interactions in an icosahedral virus at 3.0 A resolution. Science. 245(4914):154–59.

Choi, Y.G., Grantham, G.L., Rao, A.L. (2000) Molecular studies on bromovirus capsid protein. Virology. 270(3):377–85.

Choi, Y.G., Dreher, T.W., Rao, A.L. (2002) tRNA elements mediate the assembly of anicosahedral RNA virus. Proc. Natl. Acad. Sci. USA. 99(2):655–60.

Choi, Y.G., Rao, A.L.N. (2003) Packaging of brome mosaic virus RNA3 is mediated through a bipartite signal. J. Virology. 77(18):9750-9757.

Creamer, R., Falk, B.W. (1990) Direct detection of trans encapsidated barley yellow dwarf luteoviruses in doubly infected plants. J. Gen. Virol. 71:211–17.

Cuillel, M., Herzog, M., Hirth, L. (1979) Specificity of in vitro reconstitution of bromegrassmosaic virus. Virology. 95(1):146–53.

Damayanti, T.A., Tsukaguchi, S., Mise, K., Okuno, T. (2003) Cis-acting elements required for efficient packaging of brome mosaic virus RNA3 in barley protoplasts J. Virology. 77(18):9979–86.

Dreher, T.W. (1999) Functions of the 3-untranslated regions of positive strand RNA viral genomes. Annu. Rev. Phytopathol. 37(1):151–74.

Duggal, R., Hall, T.C. (1993) Identification of domains in brome mosaic virus RNA-1 and coat protein

necessary for specific interaction and encapsidation. J. Virology. 67(11):6406–12.

Fisher, A.J., Johnson, J.E. (1993) Ordered duplex RNA controls capsid architecture in an icosahedral animal virus. Nature. 361(6408):176–79.

Ford, R.J., Barker, A.M., Bakker, S.E., Coutts, R.H., Ranson, N.A., Phillips, S.E., Pearson, A.R., Stockley, P.G. (2013) Sequence-specific, RNA-protein interactions overcome electrostatic barriers preventing assembly of satellite tobacco necrosis virus coat protein. J. Mol. Biol. 425:1050–1064.

Fox, J.M., Wang, G., Speir, J.A., Olson, N.H., Johnson, J.E. (1998) Comparison of the native CCMV virion with in vitro assembled CCMV virions by cryoelectron microscopy and image reconstruction. Virology. 244:212–18.

Francki, R.I.B., Milne, R.G., Hatta, T. (1985) *Atlas of Plant Viruses*. Boca Ranton, FL: CRC Press., 1, 2.

Frolova, E., Frolov, I., Schlesinger, S. (1997) Packaging signals in alphaviruses. J. Virology. 71:248–58.

Gould, A.R., Francki, R.I., Hatta, T., Hollings, M. (1981) The bipartite genome of red clover necrotic mosaic virus. Virology. 108(2):499–506.

Hacker, D.L. (1995) Identification of a coat protein-binding site on southern bean mosaicvirus RNA. Virology. 207(2):562–65.

Hamilton, R.I., Tremaine, J.H. (1996) Dianthoviruses: properties, molecular biology, ecologyand control. In *The Plant Viruses Polyhedral Virions and Biparetite RNA Genomes*, ed. BD Harrison, AF Murant, pp. 251–82. New York: Plenum.

Hema, M., Gopinath, K., Kao, C. (2005) Repair of the tRNA-like CCA sequence in a multipartite positive-strand RNA virus. J. Virology. 79(3):1417–27.

Hesketh, E.L., Meshcheriakova, Y., Dent, K.C., Saxena, P., Thompson, R.F., Cockburn, J.J., Lomonossoff, G.P., Ranson, N.A. (2015) Mechanisms of assembly and genome packaging in an RNA virus revealed by high-resolution cryo-EM. Nature Communications. 10:6.

Hollings, M., Stone, O.M. (1977) Red clover necrotic mosaic virus. *CMI/AAB Descr.* Plant Viruses. 181.

Hull, R. (2002) Matthews' Plant Virology. Academic press 1001 pp.

Iyer, L.M., Makarova, K.S., Koonin, E.V., Aravind, L. (2004) Comparative genomics of the FtsK-HerA superfamily of pumping ATPases: Implications for the origins of chromosome segregation, cell division and viral capsid packaging. Nucleic Acids Res. 32: 5260–5279.

Jahn, D., Verkamp, E., Soll, D. (1992) Glutamyl-transfer RNA: a precursor of heme andchlorophyll biosynthesis. Trends Biochem. Sci. 17(6):215–18.

Johnson, J.E., Rueckert, R.R. (1997) Packaging and release of the viral genome. In Structural Biology of Viruses, pp. 269–87. New York: Oxford Univ. Press.

Johnson, J.E. (2003) Virus particle dynamics. *Adv. Protein Chem.* 64, 197–218.

Kondabagil, K.R., Zhang, Z., Rao, V.B. (2006) The DNA Translocating ATPase of Bacteriophage T4 Packaging Motor. J. Mol. Biol., 363: 786–799.

Koev, G., Miller, A.W. (2000) A Positive-Strand RNA virus with Three Very Different Subgenomic RNA Promoters. J. Virology. 74(13): 5988-5996.

Kutluay, S.B., Bieniasz, P.D. (2010) Analysis of the initiating events in HIV-1 particle assembly and genome packaging. PLoS pathogens. 6(11): e1001200.

Kumar, R.R., Ansar, M., Rajani, K., Kumar, J., Ranjan, T. (2020). First report on molecular basis of potato leaf roll virus (PLRV) aggravation by combined effect of tuber and prevailing aphid. BMC Research Notes. 13(1):1-4.

Kumari, P., Kumar, J., Kumar, R.R., Ansar, M., Rajani, K., Kumar, S., Ranjan, T. (2021) Inhibition of potato leafroll virus multiplication and systemic translocation by siRNA constructs against putative ATPase fold of movement protein. Scientific reports. 10(1):1-1.

Kumar, J., Kumar, R. R., Das, D. K., Mohanty, A., Rajani, K., Kumari, N., Kumar, V., Kumar, S., Ranjan, T. (2022). Knockdown of a novel ATPase domain of capsid protein inhibits genome packaging in potato leaf roll virus. 3Biotech. 12:66.

Li, T., Chen, Z., Johnson, J.E., Thomas, G.J.J. (1992) Conformations, interactions, and thermo-stabilities of RNA and proteins in bean pod mottle virus: investigation of solution and crystal structures by laser Raman spectroscopy. Biochemistry. 31(29):6673–82.

Larson, S.B., Lucas, R.W., Greenwood, A., McPherson, A. (2005) The RNA of turnip yellow mosaic virus exhibits icosahedral order. Virology. 334(2):245–54.

Lazinski, D., Grzadzielska, E., Das, A. (1989) Sequence-specific recognition of RNA hairpinsby bacteriophage anti-terminators requires a conserved arginine-rich motif. Cell. 59(1): 207–18.

Marquet, R., Isel, C., Ehresmann, C., Ehresmann, B. (1995) tRNAs as primer of reverse transcriptases. Biochimie. 77(1): 113–24.

Masters, P.S. (2019) Coronavirus genomic RNA packaging. Virology. 537: 198-207.

Momany, C., Kovari, L.C., Prongay, A.J., Keller, W., Gitti, R.K., Lee, B.M., Gorbalenya, A.E., Tong, L., McClure, J., Ehrlich, L.S. (1996) Crystal structure of dimeric HIV-1 capsid protein. Nature Structural & Molecular Biology. 3(9):763-770.

Ni, P., Kao, C. (2013) Non-encapsidation activities of the capsid proteins of positive-strand RNA viruses. Virology. 446:123–132.

Nugent, C.I., Johnson, K.L., Sarnow, P., Kirkegaard, K. (1999) Functional coupling betweenreplication and packaging of poliovirus replicon RNA. J. Virology. 73(2):427–35.

Olsthoorn, R.C., Mertens, S., Brederode, F.T., Bol, J.F. (1999) A conformational switch at the3-end of a plant virus RNA regulates viral replication. EMBO J. 18(2):4856–64.

Okuno, T., Hiruki, C., Rao, D.V., Figueiredo, G.C. (1983) Genetic determinants distributed in two genomic RNAs of sweet clover necrotic mosaic, red clover necrotic mosaic and clover primary leaf necrosis viruses. J. Gen. Virol. 64(9):1907–14.

Polisky, B., McCarthy, B. (1975) Location of histones on simian virus 40 DNA. Proc. Natl. Acad. Sci. USA. 72(8): 2895-2899.

Qu, F., Morris, T.J. (1997) Encapsidation of turnip crinkle virus is defined by a specific packaging signal and RNA size J. Virology.71(2):1428–35.

Rakitinaa, D.V., Kantidzea, O.L., Leshchinera, A.D., Solovyeva, A.G., Novikovb, V.K., Morozova, S.Y., Kalininaa, N.O. (2005) Coat proteins of two filamentous plant viruses display NTPase activity in vitro. FEBS Letters. 579: 4955–4960.

Ranjan, T., Pal, A.K., Prasad, B.D., Kumar, R.R., Kumar, M., Shamim, M., Jambhulkar, S. (2021) Reassessing the mechanism of genome packaging in plant viruses with lessons from ATPase fold. Australasian Plant Pathology. 1-4.

Rao, V.B., Black, L.B. (2005) Viral Genome Packaging Machines: Genetics, Structure, and Mechanism, 40. 135-144.

Rao, A.L.N. (2000) Bromoviruses. In *Encyclopedia of Plant Pathology*, ed. OC Maloy, TDMurray, pp. 155–58. New York:Wiley.

Rao, A.L. (1997) Molecular studies on bromovirus capsid protein. III. Analysis of cell-to-cell movement competence of coat protein defective variants of cowpea chlorotic mottle virus. Virology. 232(2):385–95.

Rao, A.L., Dreher, T.W., Marsh, L.E., Hall, T.C. (1989) Telomeric function of the tRNA-likestructure of brome mosaic virus RNA. Proc. Natl. Acad. Sci. USA. 86(14):5335–39.

Rixon, F.J. (1993) Structure and assembly of herpesviruses. Seminars in Virology. 4(2).

Rao, L.N. (2006) Genome packaging by spherical plants. Annu. Rev. Phytopathol. 44 (1): 61-87.

Satheshkumar, P.S., Lokesh, G.L., Sangita, V., Saravanan, V., Vijay, C.S. (2004) Role of metal ion-mediated interactions in the assembly and stability of Sesbania mosaic virus T=3 and T=1 capsids. J. Mol. Biol. 342(3):1001–14.

Sarah, M., McDonald, J., Patton, T. (2011) Assortment and packaging of the segmented rotavirus genome. Trend in Microbiology. 19(3):136-144.

Schneemann, A. (2006) The Structural and Functional Role of RNA in Icosahedral Virus Assembly. Annu. Rev. Microbiol. 60(2): 51-67.

Schmidt, T., Johnson, J.E., Phillips, W.E. (1983) The spherically averaged structures of cowpea mosaic virus components by X-ray solution scattering. Virology. 127(1): 65–73.

Sit, T.L., Vaewhongs, A.A., Lommel, S.A. (1998) RNA-mediated *trans*-activation of transcription from a viral RNA. Science. 281(2):829–32.

Sunpapao, A. (2013)Virus-Induced Symptoms in Plants: A Review of Interactions between Viral Trafficking and RNA Silencing. Philipp Agric Scientist. 96(2): 210-218.

Tan, R., Frankel, A.D. (1995) Structural variety of arginine-rich RNA-binding peptides. Proc. Natl. Acad. Sci. USA. 92(2):5282–86.

Tang, L., Johnson, K.N., Ball, L.A., Lin, T., Yeager, M., Johnson, J.E. (2001) The structure of pariacoto virus reveals a dodecahedral cage of duplex RNA. Nat. Struct. Biol. 8(1):77–83.

Tamm, T., Truve, E. (2000) Sobemoviruses. J. Virol.74:6231–41.

Timmins, P.A., Wild, D., Witz, J. (1994) The three-dimensional distribution of RNA and protein in the interior of tomato bushy stunt virus: a neutron low-resolution single-crystal diffraction study. Structure. 2(94):1191–201.

Traynor, P., Young, B.M., Ahlquist, P. (1991) Deletion analysis of brome mosaic virus 2a protein: effects on RNA replication and systemic spread. J. Virology. 65(6):2807–15.

Vriend, G., Verduin, B.J.M., Hemminga, M.A. (1986) Role of the N-terminal part of the coat protein in the assembly of cowpea chlorotic mottle virus: A 500 MHz proton nuclear magnetic resonance study and structural calculations. J. Molecular Biology. 191(3): 453-460.

Wang, J., Carpenter, C.D., Simon, A.E. (1999) Minimal sequence and structural requirements of a sub-genomic RNA promoter for turnip crinkle virus. Virology. 253(2):327–36.

Wang, X., Lee, W.M., Watanabe, T., Schwartz, M., Janda, M., Ahlquist, P. (2005) Brome Mosaic Virus 1a Nucleoside Triphosphatase/Helicase Domain Plays Crucial Roles in Recruiting RNA

Replication Templates. J. Virology. 79:13747–13758.

Weeks, K.M., Crothers, D.M. (1991) RNA recognition by Tat-derived peptides: interaction in the major groove? Cell. 66(3):577–88.

Wei, N., Heaton, L.A., Morris, T.J., Harrison, S.C. (1990) Structure and assembly of turnip crinkle virus. VI. Identification of coat protein binding sites on the RNA. J. Mol. Biol. 214(1):85–95.

Wei, N., Morris, T.J. (1991) Interactions between viral coat protein and a specific binding region on turnip crinkle virus RNA. J. Mol. Biol. 222(3):437–43.

Yang, Q., Catalano, C.E. (2003) Biochemical characterization of bacteriophage lambda genome packaging in vitro. Virology. 305(2): 276–287.

Zeltins, A. (2018) Protein complexes and virus-like particle technology. Virus protein and nucleoprotein complexes, pp.379-405.

Zhu, L., Wang, X., Ren, J., Porta, C., Wenham, H., Ekstro, J.O., Panjwani, A., Knowles, N.J., Kotecha, A., Siebert, C.A., Lindberg, A.M., Fry, E.E., Rao, Z., Tuthill, T.J., Stuart, D.I. (2015) Structure of Ljungan virus provides insight into genome packaging of this picornavirus. Nature Communication. 6(2):8316.

Zlotnick, A., Aldrich, R., Johnson, J.M., Ceres, P., Young, M.J. (2000) Mechanism of capsid assembly for an icosahedral plant virus. Virology. 277(2):450–56.