



Universiteit
Leiden
The Netherlands

Structural aspects of encapsidation signals in RNA viruses

Chen, S.C.

Citation

Chen, S. C. (2010, April 28). *Structural aspects of encapsidation signals in RNA viruses*. Retrieved from <https://hdl.handle.net/1887/15338>

Version: Corrected Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/15338>

Note: To cite this publication please use the final published version (if applicable).

Chapter II

Encapsidation signals in positive-strand RNA viruses - a literature review

Introduction

In the life cycle of a positive-strand RNA virus, genomic RNA is translated for expression of viral proteins, replicated for propagation of the virus, and encapsidated for assembly of virions. Assembly of an RNA virus is initiated with the binding of nucleocapsid proteins or core protein complexes to the encapsidation signal(s) located in the viral genomic RNA(s). The presence of an encapsidation signal in the genomic RNA promotes the oligomerization of nucleocapsid proteins and/or the interactions with other viral proteins, so that the genomic RNA is specifically packaged thereby forming virus particles. The encapsidation signals in RNA viruses are usually *cis*-acting elements exhibiting specific sequence and/or particular secondary structures that interact with (nucleo-)capsid proteins. To date, many positive-strand RNA viruses have been studied for their encapsidation signals. In this review, the structural aspects of these encapsidation signals in the positive-strand RNA viruses are highlighted.

The packaging origin of tobacco mosaic virus

In tobacco mosaic virus (TMV), the type member of the genus *Tobamovirus*, a particular fragment of genomic RNA had been originally found tightly associated with the capsid protein disks and resistant to nuclease digestion (Zimmern & Butler, 1977). The fragment is a 500-nt region located in the 3'-half of the TMV RNA (Guilley *et al.*, 1979; Ohno *et al.*, 1977; Zimmern & Wilson, 1976). The fragment containing the packaging origin was further narrowed down to the sequence between the position of nt 5420 and 5546 in TMV genomic RNA, which contains the core sequence of packaging signal (PS), 5'-AAGAAGUC(U)G-3' (Goellet *et al.*, 1982; Jonard *et al.*, 1977; Zimmern, 1977). The

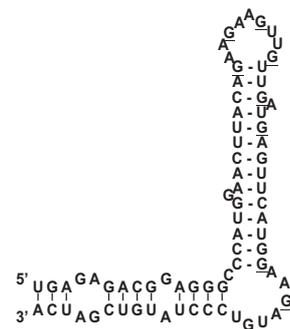


Figure 1. The packaging origin of the Tobacco mosaic virus. The critical G residues for capsid protein binding are underlined.

secondary structure for the packaging region was then proposed (Fig. 1) (Zimmern, 1983).

Structure probing of the main stem of TMV packaging origin was in agreement with the stem-loop feature, and alterations of the secondary structure were reported to interfere with the initiation of packaging (Turner *et al.*, 1988). The core sequence located in the loop region was found critical for efficient packaging; sequence deletions or substitutions resulted in reduced packaging efficiency (Turner & Butler, 1986), while heterogeneous RNA containing the packaging origin could be encapsidated into virions (Sleat *et al.*, 1986; Sleat *et al.*, 1988a, b).

Sequence comparisons further suggested that packaging origins in all known tobamoviruses exhibit stable hairpin structures with the looped-out common target sequence, GANGUUG (Okada, 1986). It was then suggested that the G residues which appear with 3-nt intervals in the origin-of-assembly sequence initiate RNA encapsidation bi-directionally (Wilson & McNicol, 1995). Thus, the (GNN)_n motifs were suggested to be specifically recognized by the disks made of TMV capsid proteins *in vitro*. On the other hand, since the genomic RNA of tobamoviruses has a statistically significant bias for G at the first position every third (or 3n) nucleotides in the region, the high frequency of GNN codons results in high Val, Ala, Gly, Asp, or Glu content in the corresponding proteins (Okada, 1999). However, the functional relevance of this observation has yet to be determined.

Although the mechanism of TMV assembly has been studied intensively *in vitro*, the nature of the *in vivo* elongation process remained unclear. It is still not known how TMV is packaged in plant cells, though self-assembled pseudovirus particles which contain heterogeneous RNAs have been reported in *E. coli* (Hwang *et al.*, 1994).

The encapsidation signal in turnip yellow mosaic virus

The knowledge of encapsidation signals in the genus *Tymovirus* was mostly obtained from studies on turnip yellow mosaic virus (TYMV). Virions of TYMV were found unstable and leading to decapsidation at high pH *in vitro* (Kaper, 1971). It had been further shown that TYMV RNA specifically binds to empty protein capsid at low pH though the RNA might not be fully encapsidated (Briand *et al.*, 1975).

Regions for initiating coat protein (CP) binding are located in the 5'-UTR of TYMV genomic RNA, which folds into two stem-loop structures, assigned as hairpin 1 (HP1) and HP2, with characteristic C:C and

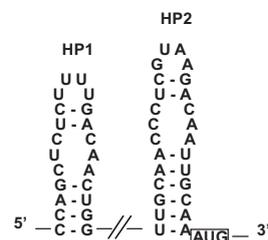


Figure 2. Turnip yellow mosaic virus packaging signal. The two mismatch containing hairpins, HP1 and 2, are shown with the boxed AUG start codon of the TYMV *gp1* gene.

C:A mismatches (Hellendoorn *et al.*, 1996) (Fig. 2). These mismatches were shown protonatable at low pH *in vitro* facilitating infectious virion assembly (Bink *et al.*, 2002, 2003; Hellendoorn *et al.*, 1996, 1997; van Roon *et al.*, 2004).

An *in vivo* evolutionary assay also suggested that mutants with Watson-Crick base-pairs tend to revert to having C:C and/or C:A mismatches in HP2 (Bink *et al.*, 2003; Hellendoorn *et al.*, 1997). Specific interactions between the two 5' proximal hairpins and CPs were further proposed to regulate the pH-dependent translation and encapsidation of TYMV RNAs (Bink *et al.*, 2003).

More recently, virion assemblies have been observed *in vivo* when TYMV RNA and CP were transiently expressed in agro-infiltrated non-host plants, *N. benthamiana* leaves, and both empty capsids and virions containing (sub-)genomic RNAs were found (Cho & Dreher, 2006). However, applying a similar method to study the TYMV encapsidation in Chinese cabbage, it was found that mutations in HP1 and HP2 seriously interfere with viral replication but not encapsidation (Shin *et al.*, 2008; 2009). Moreover, it was also demonstrated that TYMV can efficiently replicate in plants held in the dark showing sufficient encapsidation of the propagating RNAs (Rohozinski & Hancock, 1996). These observations could be contradictory to the previous model for TYMV encapsidation, which stated that the packaging occurs at low pH generated by proton gradients produced by photosynthetic activity in the light (Shin *et al.*, 2009). Thus, how the protonation of TYMV HP1 and HP2 hairpins is achieved remains unclear.

The PS in brome mosaic virus and alfalfa mosaic viruses

The tRNA-like structure (TLS) at the 3' terminus of genomic RNAs has been suggested to function as a PS in brome mosaic virus (BMV), the type member of the genus *Bromovirus*. The presence of TLSs, both *in cis* and *in trans*, has been shown to initiate BMV encapsidation *in vitro*, while disruption of the TLS resulted in no packaging (Choi *et al.*, 2002). However, the necessity of 3' TLS for BMV encapsidation was uncertain. The encapsidation of mutant RNA1 and RNA2 lacking 3' TLS was proposed to be promoted by host tRNA (Rao, 2006). It was further reported that only RNA3, but not RNA1 or RNA2, requires the 3' TLS *in cis* for BMV encapsidation *in vivo* (Annamalai & Rao, 2007), suggesting that the packaging of TLS-less RNAs *in vivo* can be promoted by *trans* complementation of cellular tRNAs or that the packaging is independent to TLS *in vivo* (Annamalai & Rao, 2007).

UV cross-linking and band-shift assays demonstrated that BMV CP binds to the BMV TLS (Choi *et al.*, 2002; Damayanti *et al.*, 2002). Nevertheless, CP binding affinity of certain RNA elements does not necessarily imply their functioning as PS. For instance, the interaction between the CP and the BMV 3' and 5' UTR was reported for regulating the translation inhibition and the

packaging BMV genomic RNAs, and possibly that of other bromoviruses as well, is dependent on coupling the replication-dependent transcription and the translation of CP (Annamalai & Rao, 2006), which may complicate the identification of bromovirus encapsidation signals *in vivo*.

The PS in turnip crinkle virus

The *in vitro* dissociation and reassembly of turnip crinkle virus (TCV), the type member of the genus *Carmovirus*, have shown that neutral pH promotes formation of ribonucleoprotein (RNP) complexes which consist of viral RNA tightly bound to CP subunits (Sorger *et al.*, 1986; Golden & Harrison, 1982). It was reported that the presence of RNA is required for specific virion assembly initiated by TCV CP. The heterogeneous TMV and the closely related tomato bushy stunt virus (TBSV) genomic RNAs are shown to be packaged less efficiently than the TCV RNAs, although the 18S non-viral rRNA could also be packaged with high efficiency by TCV CP (Sorger *et al.*, 1986).

The specificity and the high efficiency of TCV encapsidation were hypothesized to be initiated by formation of RNP complexes at a specific site in the TCV genome (Sorger *et al.*, 1986; Skuzeski & Morris, 1995). The CP interacting RNA sequences have been identified using nuclease protection assays. Five protected RNA fragments which are clustered into two regions of the TCV genome were discovered (Wei *et al.*, 1990). These two clusters of sequences were subsequently reported to direct encapsidation by independent researches.

One of the two clusters, consisting of fragments a, f, and c, is situated within a region of approximately 400 nts that includes the leaky stop codon in the TCV polymerase gene. The other cluster consists of fragment d and e and is located within the CP coding region near the 3'-end of the genome. Notably, these two fragments are also present in the subgenomic RNAs which are corresponding to the 3'-terminal 1700 and 1500 nts of the TCV genomic RNA (Carrington *et al.*, 1987; 1989).

The TCV CP was suggested to contain both specific and non-specific RNA binding activities, and the specific interacting sites of the TCV RNA were first proposed to be located in the 386-nt sequences corresponding to fragments a, f, and c. (Wei & Morris, 1991). However, wobble mutations applied to the 386-nt sequence, which altered the secondary structure of the region while keeping the amino acid sequence of encoded polymerase protein identical, disabled the replication of TCV (Wei *et al.*, 1992). On the other hand, binding affinity between TCV CP and the 386-nt transcripts was shown no better than

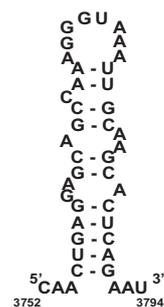


Figure 4. The packaging signal of Turnip crinkle virus. The secondary structure of the TCV PS is a stem-loop with a bulge and internal loops.

that between TCV CP and heterogeneous RNA (Skuzeski & Morris, 1995). Thus, studies of the 386-nt in fragments a, f, and c as PS was abandoned because of the difficulties in constructing viable mutants and studying the specificity of CP binding (Wei & Morris, 1991; Wei *et al.*, 1992; Skuzeski & Morris, 1995).

A 186-nt region consisting of the fragments d and e at the 3' end of the TCV CP-coding region was subsequently shown to be crucial for specific encapsidation in protoplasts (Qu & Morris, 1997). Furthermore, it was shown that the size of the viral RNA to be packaged is another critical factor for stable virion assembly. Eventually, it was shown that a 28-nt bulged hairpin within the 186-nt region (Fig. 4) is indispensable for packaging and directs encapsidation (Qu & Morris, 1997).

The PS in Sindbis virus

Large amounts of genomic and subgenomic RNAs are replicated and/or transcribed in alphaviruses infected cells, but usually only the genomic RNAs are packaged into virions. This implies the existence of an encapsidation signal which is responsible for the selective packaging of genomic RNA (Frolova *et al.*, 1997). In Sindbis virus, the type member of the genus *Alphavirus*, a 600-nt fragment from the position of nts 721 to 1306 in the viral methyltransferase nsP1 gene was reported to contain the specific signal for CP binding, which directs encapsidation of defective interfering (DI) RNAs (Geigenmüller-Gnirke *et al.*, 1993; Weiss *et al.*, 1989). Sindbis virus DI RNA which has one copy of the CP binding site was shown to strongly interact with CP *in vitro* while the DI RNA lacking the CP binding site does not bind to CP (Weiss *et al.*, 1989). Such difference in CP binding on the other hand resulted in efficient packaging of signal containing DI RNA and no packaging of signal-less DI RNA, respectively (Weiss *et al.*, 1989). The CP binding site in the 600-nt fragment was further specified to the 132-nt span from the position of nts 945 to 1076 in the Sindbis virus genomic RNA (Fig. 5), which specifically binds to CP or the 68-amino-acid peptide derivative (Weiss *et al.*, 1994). Insertion of the PS in the subgenomic RNA of the Sindbis virus replicon, particularly to the 5'-end, led to significant incorporation of the subgenomic RNA into extracellular particles (Bredenbeek *et al.*, 1993; Frolova *et al.*, 1997).

In contrast to what has been reported for Sindbis virus, the PS of the related Ross River virus (RRV) was

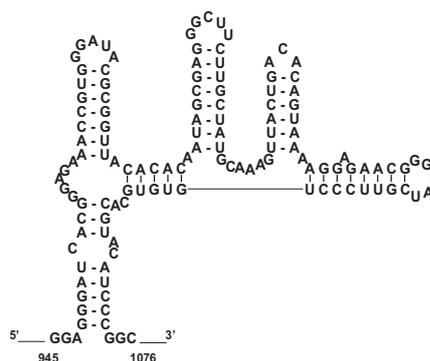


Figure 5. The packaging signal of Sindbis virus. The secondary structure of the Sindbis virus PS is shown to consist of several stem-loop structures.

found at multiple positions in the genomic RNA. Another difference considering between the encapsidation signals of RRV and Sindbis virus is that the Sindbis virus RNA could not be recognized by RRV CP, neither *vice versa* (Frolova *et al.*, 1997).

Among all the potential encapsidation signals identified in Sindbis virus, the most promising one lies between the positions of nts 2761 and 3062 in the nsP2 gene, of which the corresponding sequence was proposed to contain homologues of the Semliki Forest virus (SFV) PS, another alphavirus (Frolova *et al.*, 1997). It has been found that not only the homology of the PS but also that of the CPs is high between RRV and SFV (Frolova *et al.*, 1997). It was further suggested that the location(s) of the encapsidation signals may be correlated to the conservation of the CPs in alphaviruses (Frolova *et al.*, 1997; Strauss & Strauss, 1994). The specificity of encapsidation is determined by the interaction between the PS and the binding CP. Nevertheless, it was reported that a mutant lacking residues 97 to 106 in the CP resulted in non-specific encapsidation of Sindbis virus subgenomic RNA (Owen & Kuhn, 1996). This suggested that the specificity of encapsidation *in vivo* may not only be determined by the presence of the PS in the genomic RNA but also by the properties of the CP in Sindbis virus.

More recently, a model of the 132-nt RNA PS which can promote the dimerization of CPs was described for Sindbis virus (Linger *et al.*, 2004). Mobility shift assays showed that a 32-amino acid peptide is capable of recognizing the Sindbis virus encapsidation signal, and that the bound RNA molecule undergoes a conformational change. The multiple purine-rich stem-loops (Fig. 5) within the encapsidation signal was shown to be crucial for efficient packaging, which directs early events of the nucleocapsid assembly (Linger *et al.*, 2004).

The PS in Mouse hepatitis virus

The PS in mouse hepatitis virus, the type member of the genus *Coronavirus*, was identified near the 3' end of the open reading frame (ORF) 1b in its genomic RNA (Makino *et al.*, 1990; van der Most *et al.*, 1991). Sequence deletion analysis suggested that a 190-nt region corresponding to 20.2-20.4kb of the genomic RNA was necessary for packaging of MHV DI RNA. A 69-nt core sequence within the region was further proposed as the PS (Fig. 6A) which was computationally predicted to consist of a stem-loop structure (Fosmire *et al.*, 1992) and found to direct packaging of heterogeneous RNA (Lin & Lai, 1993; Woo *et al.*, 1997). However, the 69-nt element showed only one-fifth efficiency in directing genome packaging compared to what has been shown for the 190-nt sequence (Fosmire *et al.*, 1992), while the 69-nt PS was on the other hand shown to bind nucleocapsid (N) protein much weaker than the 190-nt

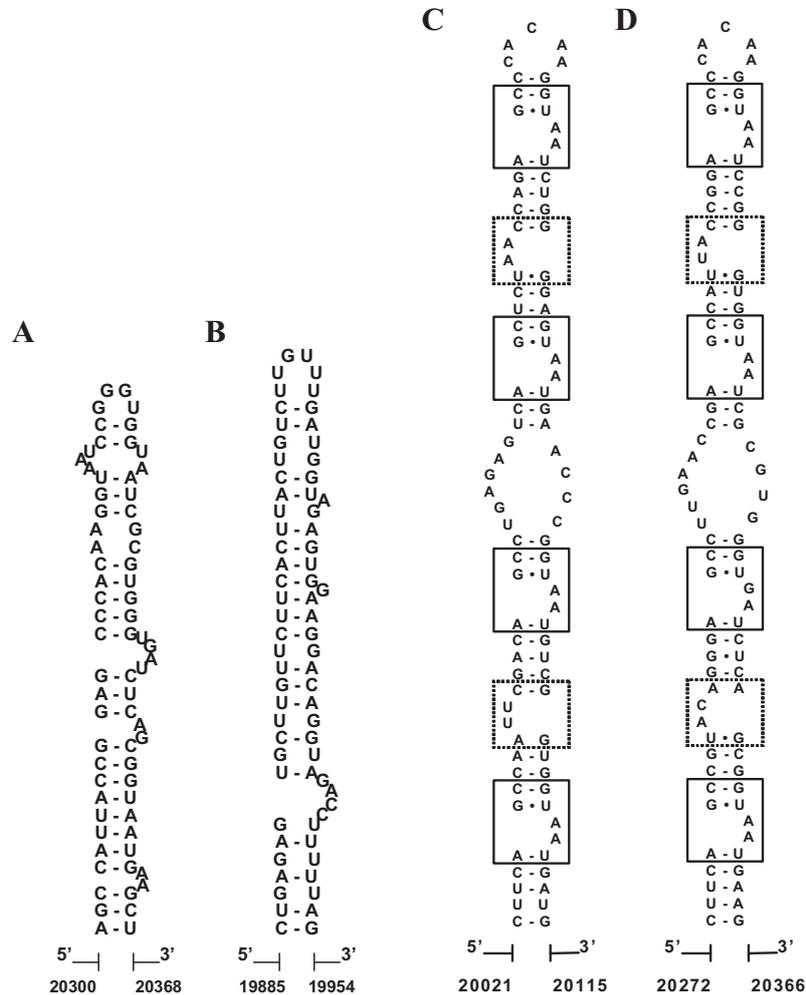


Figure 6. The packaging signals of coronaviruses. (A) The 69-nt MHV packaging signal proposed by Fosmire *et al.*, (1992, J Virol. 66:3522-30) and (B) the homologue in SARS-CoV proposed by Hsieh *et al.*, (2005, J Virol. 79:13848-55). (C, D) The 96-nt packaging signal of the group IIa coronaviruses BCoV and MHV, respectively (Chen *et al.*, 2007, J Virol. 81:6771-4).

sequence does (Molenkamp & Spaan, 1997). It has led to the assumption that the flanking sequences are necessary for promoting the 69-nt PS to fold into specific secondary structure which is selectively recognized by N protein (Molenkamp & Spaan, 1997; Narayanan & Makino, 2001). Nevertheless, mutagenesis on the 69-nt element based on the originally proposed secondary structure led to obscure results (Fosmire *et al.*, 1992). In addition, secondary structure of the MHV 69-nt PS unexpectedly exhibited poor homology to the predicted secondary structure of the closely related bovine coronavirus (BCoV) PS discovered later on (Cologna & Hogue, 2000). The structural homology between MHV and BCoV PS was not discovered until a phylogenetic analysis

was done on sequences corresponding to PS in all group IIa CoVs and an intensive structure probing study was applied to the MHV PS (Chen *et al.*, 2007).

A new secondary structure model for MHV and other group IIa CoVs PS is a 96-nt element with two stem-bulge structures separated by internal loops, which consist of repeating 2-nt (preferably AA) bulges (Fig. 7A). The model fits the mutagenesis data originally obtained by Fosmire *et al.* (1992) much more evidently, and the 96-nt PS clearly explained why the flanking sequences of the 69-nt element is required for N protein binding and how the 190-nt element directs packaging much more efficiently (Lin & Lai, 1993; Molenkamp & Spaan, 1997; Narayanan & Makino, 2001; Woo *et al.*, 1997), i.e. the 190-nt region includes the entire 96-nt MHV PS while the 69-nt element possesses only a truncated 96-nt PS and is possibly unable to fold into specific secondary structures required for encapsidation (Chen *et al.*, 2007).

The putative PS in other coronaviruses has also been located. In another group II CoV, the severe acute respiratory syndrome (SARS) CoV, homologues of the MHV 69-nt PS were proposed in the corresponding region of SARS-CoV ORF1ab (Hsieh *et al.*, 2005) (Fig. 7B). The sequence is able to incorporate non-viral RNA into virus-like particles. In transmissible gastroenteritis virus (TGEV), a group I coronavirus, the PS was located in the 5' 649 nts, though the specific sequence and the (secondary) structure of the signal have not yet been investigated (Escors *et al.*, 2003).

The PS in Aichi virus and Poliovirus

Poliovirus and Aichi virus are members of the *Picornaviridae*. Poliovirus contains a 3'-polyadenylated 7450-nt positive-sense genomic RNA which consists of three coding regions, P1, P2, and P3. It has been shown that only the positive-sense viral genomic RNA is packaged into virions, which is covalently bound to a small viral protein, the VPg, at its 5'-terminus (Lee *et al.*, 1977; Nomoto *et al.*, 1977; Novak & Kirkegaard, 1991). Although the VPg is associated with the genomic RNA, the specificity and the efficiency of the encapsidation are not likely to be dependent on the interaction between VPg and genomic RNA because the VPg-bound viral RNA was found not to be packaged in cells (Harber *et al.*, 1991) while encapsidation has been shown in mutants of which the amino acid sequence in VPg

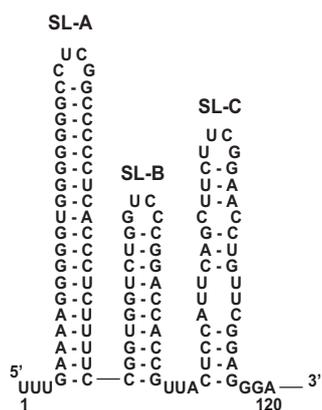


Figure 8. The packaging signals of Aichi virus. The secondary structure of the Aichi virus packaging signal is shown, exhibiting three stem-loop structures.

was seriously mutated (Kuhn *et al.*, 1988). The specificity of the encapsidation was subsequently suggested to be coupled with the replication of poliovirus (Nugent *et al.*, 1999).

Although no encapsidation signals have been identified yet in the poliovirus, studies on packaging of the poliovirus DI RNA have indicated some clues. First, the signal may not be located in the coding region of the structural proteins (P1) since that the encapsidation was observed in mutant viruses or DI RNA with this region truncated and/or substituted by non-poliovirus sequences when the P1 proteins were expressed *in trans* (Ansardi *et al.*, 1993; Barclay *et al.*, 1998; Kajigaya *et al.*, 1985; Porter *et al.*, 1995). Secondly, the encapsidation signal may not be located in the 5'UTR or in the coding region of the non-structural 2A protease because substituting the 5'UTR and the coding region of the 2A protease for the corresponding sequence of other viruses still leads to encapsidation of poliovirus genomic RNA (Lu *et al.*, 1995; Xiang *et al.*, 1995).

In another picornavirus, the Aichi virus which is associated with acute gastroenteritis in humans (Yamashita *et al.*, 1998), a potential PS has been proposed to be located in the 5'-proximal 120 nts, where three stem-loop structures (SL-A, SL-B, and SL-C) have been predicted (Fig. 8). The SL-A was shown to be required for viral RNA replication and encapsidation (Sasaki *et al.*, 2001; Sasaki & Taniguchi, 2003). However, the role of secondary structure in directing encapsidation was still unclear because sequence substitutions in the stem of SL-A seriously interfered with the encapsidation although the secondary structure was maintained (Sasaki *et al.*, 2001).

References

1. **Annamalai P & Rao AL.** 2006. Packaging of Brome Mosaic Virus Subgenomic RNA Is Functionally Coupled to Replication-Dependent Transcription and Translation of Coat Protein. *J Virol.* **80**:10096-10108.
2. **Annamalai P & Rao AL.** 2005. Dispensability of 3' tRNA-like sequence for packaging cowpea chlorotic mottle virus genomic RNAs. *Virology.* **332**:650-658.
3. **Annamalai P & Rao AL.** 2007. In vivo packaging of brome mosaic virus RNA3, but not RNAs 1 and 2, is dependent on a cis-acting 3' tRNA-like structure. *J Virol.* **81**:173-181.
4. **Ansardi DC, Porter DC, & Morrow CD.** 1993. Complementation of a poliovirus defective genome by a recombinant vaccinia virus which provides poliovirus P1 capsid precursor in trans. *J Virol.* **67**:3684-3690.
5. **Barclay W, Li Q, Hutchinson G, Moon D, Richardson A, Percy N, Almond JW, & Evans DJ.** 1998. Encapsidation studies of poliovirus subgenomic replicons. *J. Gen. Virol.* **79**:1725-1734.
6. **Bink HH & Pleij CW.** 2002. RNA-protein interactions in spherical viruses. *Arch Virol.* **147**:2261-2279.
7. **Bink HH, Schirawski J, Haenni AL, & Pleij CW.** 2003. The 5'-proximal hairpin of turnip yellow mosaic virus RNA: its role in translation and encapsidation. *J Virol.*

- 77:7452-7458.
8. **Bredenbeek PJ, Frolov I, Rice CM, & Schlesinger S.** 1993. Sindbis virus expression vectors: packaging of RNA replicons by using defective helper RNAs. *J. Virol.* **67**:6439-6446.
 9. **Briand JP, Bouley JP, Jonard G, Witz J, & Hirth L.** 1975. Low pH RNA-protein interactions in turnip yellow mosaic virus. II. Binding of synthetic polynucleotides of TYMV capsids and RNA. *Virology.* **63**:304-311.
 10. **Carrington JC, Heaton LA, Zuidema D, Hillman BI, & Morris TJ.** 1989. The genome structure of turnip crinkle virus. *Virology.* **170**:219-226.
 11. **Carrington JC, Morris TJ, Stockley PG, & Harrison SC.** 1987. Structure and assembly of turnip crinkle virus. IV. Analysis of the coat protein gene and implications of the subunit primary structure. *J Mol Biol.* **194**:265-276.
 12. **Chen SC, van den Born E, van den Worm SH, Pleij CW, Snijder EJ, & Olsthoorn RC.** 2007. New structure model for the packaging signal in the genome of group IIa coronaviruses. *J Virol.* **81**:6771-6774.
 13. **Cho TJ & Dreher TW.** 2006. Encapsidation of genomic but not subgenomic turnip yellow mosaic virus RNA by coat protein provided in trans. *Virology.* **356**:126-135.
 14. **Choi YG & Rao AL.** 2003. Packaging of brome mosaic virus RNA3 is mediated through a bipartite signal. *J Virol.* **77**:9750-9757.
 15. **Choi YG, Dreher TW, & Rao AL.** 2002. tRNA elements mediate the assembly of an icosahedral RNA virus. *Proc Natl Acad Sci U S A.* **99**:655-660.
 16. **Cologna R & Hogue BG.** 2000. Identification of a Bovine Coronavirus Packaging Signal. *J Virol.* **74**:580-583
 17. **Damayanti TA, Nagano H, Mise K, Furusawa I, & Okuno T.** 2002. Positional effect of deletions on viability, especially on encapsidation, of Brome mosaic virus D-RNA in barley protoplasts. *Virology.* **293**:314-319.
 18. **Damayanti TA, Tsukaguchi S, Mise K, & Okuno T.** 2003. cis-acting elements required for efficient packaging of brome mosaic virus RNA3 in barley protoplasts. *J Virol.* **77**:9979-9986.
 19. **Duggal R & Hall TC.** 1993. Identification of domains in brome mosaic virus RNA-1 and coat protein necessary for specific interaction and encapsidation. *J Virol.* **67**:6406-6412.
 20. **Escors D, Izeta A, Capiscol C, & Enjuanes L.** 2003. Transmissible gastroenteritis coronavirus packaging signal is located at the 5' end of the virus genome. *J Virol.* **77**:7890-7902.
 21. **Fosmire JA, Hwang K, & Makino S.** 1992. Identification and characterization of a coronavirus packaging signal. *J Virol.* **66**:3522-3530.
 22. **Frolova E, Frolov I, & Schlesinger S.** 1997. Packaging Signals in Alphaviruses. *J Virol.* **71**:248-258
 23. **Geigenmüller-Gnirke U, Nitschko H, & Schlesinger S.** 1993. Deletion analysis of the capsid protein of Sindbis virus: identification of the RNA binding region. *J Virol.* **67**:1620-1626.
 24. **Goelet P, Lomonosoff GP, Butler PJG, Akam ME, Gait MJ, & Karn J.** 1982. Nucleotide sequence of tobacco mosaic virus RNA. *Proc. Natl Acad. Sci. USA* **79**: 5818-5822.
 25. **Golden JS & Harrison SC.** 1982. Proteolytic dissection of turnip crinkle virus subunit in solution. *Biochemistry.* **21**:3862-3866.
 26. **Gougas LM, Filman DJ, Hogle JM, & Gehrke L.** 2004. Cofolding organizes alfalfa mosaic virus RNA and coat protein for replication. *Science.* **306**:2108-11
 27. **Guilley, H, Jonard G, Kukla B, & Richards KE.** 1979. Sequence of 1000 nucleotides at the 3' end of tobacco mosaic virus RNA. *Nucleic Acids Res.* **6**:1287-1308.
 28. **Harber JJ, Bradley J, Anderson CW, & Wimmer E.** 1991. Catalysis of poliovirus

- VP0 maturation cleavage is not mediated by serine 10 of VP2. *J Virol.* **65**:326-334.
29. **Hellendoorn K, Michiels PJ, Buitenhuis R, & Pleij CW.** 1996. Protonatable hairpins are conserved in the 5'-untranslated region of tymovirus RNAs. *Nucleic Acids Res.* **24**:4910-4917.
 30. **Hellendoorn K, Verlaan PW, & Pleij CW.** 1997. A functional role for the conserved protonatable hairpins in the 5' untranslated region of turnip yellow mosaic virus RNA. *J Virol.* **71**:8774-8779.
 31. **Hsieh PK, Chang SC, Huang CC, Lee TT, Hsiao CW, Kou YH, Chen IY, Chang CK, Huang TH, & Chang MF.** 2005. Assembly of severe acute respiratory syndrome coronavirus RNA packaging signal into virus-like particles is nucleocapsid dependent. *J Virol.* **79**:13848-13855.
 32. **Hwang DJ, Roberts IM, & Wilson TMA.** 1994. Expression of tobacco mosaic virus coat protein and assembly of pseudovirus particles in *Escherichia coli*. *Proc. Natl Acad. Sci. U S A* **91**:9067-9071.
 33. **Jonard G, Richards KE, Guilley H, & Hirth L.** 1977. Sequence from the assembly nucleation region of TMV RNA. *Cell.* **11**:483-493.
 34. **Kajigaya S, Arakawa H, Kuge S, Koi T, Imura N, & Nomoto A.** 1985. Isolation and characterization of defective-interfering particles of poliovirus Sabin 1 strain. *Virology.* **142**:307-316.
 35. **Kaper JM.** 1971. Studies on the stabilizing forces of simple RNA viruses. I. Selective interference with protein-RNA interactions in turnip yellow mosaic virus. *J Mol Biol.* **56**:259-276.
 36. **Kuhn RJ, Hong Z, & Strauss JH.** 1990. Mutagenesis of the 3' nontranslated region of Sindbis virus RNA. *J Virol.* **64**:1465-1476.
 37. **Kuhn RJ, Tada H, Ypma-Wong MF, Semler BL, & Wimmer E.** 1988. Mutational analysis of the genome-linked protein VPg of poliovirus. *J Virol.* **62**:4207-4215.
 38. **Laforest SM & Gehrke L.** 2004. Spatial determinants of the alfalfa mosaic virus coat protein binding site. *RNA.* **10**:48-58.
 39. **Lee YF, Nomoto A, Detjen BM, & Wimmer E.** 1977. A protein covalently linked to poliovirus genome RNA. *Proc Natl Acad Sci U S A.* **74**:59-63.
 40. **Lin YJ & Lai MM.** 1993. Deletion mapping of a mouse hepatitis virus defective interfering RNA reveals the requirement of an internal and discontinuous sequence for replication. *J Virol.* **67**:6110-6118.
 41. **Linger BR, Kunovska L, Kuhn RJ, & Golden BL.** 2004. Sindbis virus nucleocapsid assembly: RNA folding promotes capsid protein dimerization. *RNA.* **10**:128-138.
 42. **Lu HH, Li X, Cuconati A & Wimmer E.** 1995. Analysis of picornavirus 2A(pro) proteins: separation of proteinase from translation and replication functions. *J Virol.* **69**:7445-7452.
 43. **Makino S, Yokomori K & Lai MM.** 1990. Analysis of efficiently packaged defective interfering RNAs of murine coronavirus: localization of a possible RNA-packaging signal. *J Virol.* **64**:6045-6053.
 44. **Molenkamp R & Spaan WJ.** 1997. Identification of a specific interaction between the coronavirus mouse hepatitis virus A59 nucleocapsid protein and packaging signal. *Virology.* **239**:78-86.
 45. **Narayanan K & Makino S.** 2001. Cooperation of an RNA packaging signal and a viral envelope protein in coronavirus RNA packaging. *J Virol.* **75**:9059-9067.
 46. **Nomoto A, Kitamura N, Golini F, & Wimmer E.** 1977. The 5'-terminal structures of poliovirion RNA and poliovirus mRNA differ only in the genome-linked protein VPg. *Proc Natl Acad Sci U S A.* **74**:5345-5349.
 47. **Novak JE & Kirkegaard K.** 1991. Improved method for detecting poliovirus negative strands used to demonstrate specificity of positive-strand encapsidation and the ratio of positive to negative strands in infected cells. *J Virol.* **65**:3384-3387.
 48. **Nugent CI, Johnson KL, Sarnow P, & Kirkegaard K.** 1999. Functional coupling

- between replication and packaging of poliovirus replicon RNA. *J. Virol.* **73**:427-435.
49. **Ohno T, Sumita M, & Okada Y.** 1977. Location of the initiation site on tobacco mosaic virus RNA involved in assembly of the virus in vitro. *Virology.* **78**:407-414.
 50. **Okada Y.** 1986. Molecular assembly of tobacco mosaic virus in vitro. *Adv Biophys.* **22**:95-149.
 51. **Okada Y.** 1999. Historical overview of research on the tobacco mosaic virus genome: genome organization, infectivity and gene manipulation. *Philos Trans R Soc Lond B Biol Sci.* **354**:569-582.
 52. **Olsthoorn RC, Mertens S, Brederode FT, & Bol JF.** 1999. A conformational switch at the 3' end of a plant virus RNA regulates viral replication. *EMBO J.* **18**:4856-4864.
 53. **Owen KE & Kuhn RJ.** 1996. Identification of a region in the Sindbis virus nucleocapsid protein that is involved in specificity of RNA encapsidation. *J Virol.* **70**:2757-2763
 54. **Porter DC, Ansardi DC, & Morrow CD.** 1995. Encapsidation of poliovirus replicons encoding the complete human immunodeficiency virus type 1 gag gene by using a complementation system which provides the P1 capsid protein in trans. *J Virol.* **69**:1548-1555.
 55. **Qu F & Morris TJ.** 1997. Encapsidation of turnip crinkle virus is defined by a specific packaging signal and RNA size. *J Virol.* **71**:1428-1435.
 56. **Rao AL.** 2006. Genome packaging by spherical plant RNA viruses. *Annu Rev Phytopathol.* **44**:61-87.
 57. **Rohozinski J. & Hancock J.M.** 1996. Do light-induced pH changes within the chloroplast drive turnip yellow mosaic virus assembly? *J. Gen. Virol.* **77**:163-165.
 58. **Sasaki J & Taniguchi K.** 2003. The 5'-end sequence of the genome of Aichi virus, a picornavirus, contains an element critical for viral RNA encapsidation. *J Virol.* **77**:3542-3548.
 59. **Sasaki J, Kusuhara Y, Maeno Y, Kobayashi N, Yamashita T, Sakae K, Takeda N, & Taniguchi K.** 2001. Construction of an infectious cDNA clone of Aichi virus (a new member of the family Picornaviridae) and mutational analysis of a stem-loop structure at the 5' end of the genome. *J. Virol.* **75**:8021-8030.
 60. **Shin HI, Kim IC, & Cho TJ.** 2008. Replication and encapsidation of recombinant turnip yellow mosaic virus RNA. *BMB Rep.* **41**:739-74.
 61. **Shin HI, Tzanetakakis IE, Dreher TW, & Cho TJ.** 2009. The 5'-UTR of turnip yellow mosaic virus does not include a critical encapsidation signal. *Virology.* **387**:427-435.
 62. **Skuzeski JM & Morris TJ.** 1995. Quantitative analysis of the binding of turnip crinkle virus coat protein to RNA fails to demonstrate binding specificity but reveals a highly cooperative assembly interaction. *Virology.* **210**:82-90.
 63. **Sleat DE, Gallie DR, Watts JW, Deom CM, Turner PC, Beachy RN, & Wilson TMA.** 1988a. Selective recovery of foreign gene transcripts as virus-like particles in TMV-infected tobaccos. *Nucleic Acids Res.* **16**:3127-3140.
 64. **Sleat DE, Plaskitt KA, & Wilson TMA.** 1988b. Selective encapsidation of CAT gene transcripts in TMV-infected transgenic tobacco inhibits CAT synthesis. *Virology.* **165**:609-612.
 65. **Sleat DE, Turner PC, Finch JT, Butler PJG, & Wilson TMA.** 1986. Packaging of recombinant RNA molecules into pseudovirus particles directed by the origin-of-assembly sequence from tobacco mosaic virus RNA. *Virology.* **155**: 299-308.
 66. **Sorger PK, Stockley PG, & Harrison SC.** 1986. Structure and assembly of turnip crinkle virus. II. Mechanism of reassembly in vitro. *J Mol Biol.* **191**:639-658.
 67. **Strauss JH & Strauss EG.** 1994. The alphaviruses: gene expression, replication, and evolution. *Microbiol. Rev.* **58**:491-562.
 68. **Turner DR & Butler PJ.** 1986. Essential features of the assembly origin of tobacco mosaic virus RNA as studied by directed mutagenesis. *Nucleic Acids Res.*

- 14:9229-9242.
69. **Turner DR, Joyce LE, & Butler PJ.** 1988. The tobacco mosaic virus assembly origin RNA. Functional characteristics defined by directed mutagenesis. *J Mol Biol.* **203**:531-547
 70. **van der Most RG, Bredenbeek PJ, & Spaan WJ.** 1991. A domain at the 3' end of the polymerase gene is essential for encapsidation of coronavirus defective interfering RNAs. *J Virol.* **65**:3219-3226.
 71. **van Roon AM, Bink HH, Plaisier JR, Pleij CW, Abrahams JP, & Pannu NS.** 2004. Crystal structure of an empty capsid of turnip yellow mosaic virus. *J Mol Biol.* **341**:1205-1214.
 72. **Wei N & Morris TJ.** 1991. Interactions between viral coat protein and a specific binding region on turnip crinkle virus RNA. *J Mol Biol.* **222**:437-443.
 73. **Wei N, Hacker DL, & Morris TJ.** 1992. Characterization of an internal element in turnip crinkle virus RNA involved in both coat protein binding and replication. *Virology.* **190**:346-355.
 74. **Wei N, Heaton LA, Morris TJ, & Harrison SC.** 1990. Structure and assembly of turnip crinkle virus. VI. Identification of coat protein binding sites on the RNA. *J Mol Biol.* **214**:85-95.
 75. **Weiss B, Geigenmüller-Gnirke U, & Schlesinger S.** 1994. Interactions between Sindbis virus RNAs and a 68 amino acid derivative of the viral capsid protein further defines the capsid binding site. *Nucleic Acids Res.* **22**:780-786.
 76. **Weiss B, Nitschko H, Ghattas I, Wright R, & Schlesinger S.** 1989. Evidence for specificity in the encapsidation of Sindbis virus RNAs. *J Virol.* **63**:5310-5318.
 77. **Wilson TM & McNicol JW.** 1995. A conserved, precise RNA encapsidation pattern in Tobamovirus particles. *Arch Virol.* **140**:1677-1685.
 78. **Woo K, Joo M, Narayanan K, Kim KH, & Makino S.** 1997. Murine coronavirus packaging signal confers packaging to nonviral RNA. *J. Virol.* **71**: 824-827
 79. **Xiang W, Harris KS, Alexander L, & Wimmer E.** 1995. Interaction between the 5'-terminal cloverleaf and 3AB/3CDpro of poliovirus is essential for RNA replication. *J Virol.* **69**:3658-3667.
 80. **Yamashita T, Sakae K, Tsuzuki H, Suzuki Y, Ishikawa N, Takeda N, Miyamura T, & Yamazaki S.** 1998. Complete nucleotide sequence and genetic organization of Aichi virus, a distinct member of the Picornaviridae associated with acute gastroenteritis in humans. *J. Virol.* **72**:8408-8412.
 81. **Yi G, Letteney E, Kim CH, & Kao CC.** 2009. Brome mosaic virus capsid protein regulates accumulation of viral replication proteins by binding to the replicase assembly RNA element. *RNA.* **15**:615-626
 82. **Zhu J, Gopinath K, Murali A, Yi G, Hayward SD, Zhu H, & Kao C.** 2007. RNA-binding proteins that inhibit RNA virus infection. *Proc Natl Acad Sci U S A.* **104**:3129-3134.
 83. **Zimmern D. & Butler PJG.** 1977. The isolation of tobacco mosaic virus RNA fragments containing the origin for viral assembly. *Cell.* **11**:455-462.
 84. **Zimmern D. & Wilson TMA.** 1976. Location of the origin for viral reassembly on tobacco mosaic virus RNA and its relation to stable fragment. *FEBS Lett.* **71**: 294-298.
 85. **Zimmern D.** 1977. Nucleotide sequence at the origin for assembly on tobacco mosaic virus RNA. *Cell.* **11**:463-482.
 86. **Zimmern D.** 1983. An extended secondary structure model for the TMV assembly origin, and its correlation with protection studies and an assembly defective mutant. *EMBO J.* **2**:1901-1907.