EJB Electronic Journal of Biotechnology ISSN: 0717-3458

Vol.1 No.2, Issue of August 15, 1998.
Invited review paper

REVIEW ARTICLE

Plant virus gene expression strategies

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Plant viruses can cause serious losses to most, if not all, major crops upon which depend for food. Many viruses are endemic, causing moderate losses each year. Others, such as those causing rice tungro, give periodic severe epidemics. There are no fully collated figures for world-wide losses due to viruses but some examples has been listed, i.e., rice tungro in SE ASIA and african cassava mosaic in Africa with 1,500 and 2,000 millions dollars per year in losses respectively.

However, in recent years the understanding of the genome organisation of plant viruses has increased in parallel with development of molecular biological techniques. The ability to obtain nucleotide sequences of complete viral genomes has also permitted the elucidation and understanding of expression strategies used by many different plant viruses. This review is aimed to summarise some aspects of the main strategies used by plant viruses to express their genomes.

To date the Virus Identification Data Exchange (VIDE) database (plant virus database operated at the Australian National University in Canberra, Australia) contains 569 characters for more than 890 plant virus species in 55 genera, according Gibbs (1994), and cited by Murphy et al. (1995). The VIDE database is accessible through the Internet from the BioWeb server http://biology.anu.edu.au/Groups/MES/vide/ (Brunt et al., 1996). Plant viruses can cause serious losses to most, if not all, major crops upon which we depend for food. Many viruses are endemic, causing moderate losses each year. Others, such as those causing rice tungro, give periodic severe epidemics. There are no fully collated figures for world-wide losses due to viruses but some examples has been listed by Hull (1994), i.e. rice tungro in SE Asia, african casava mosaic in Africa and potato viruses in UK with 1,500, 2,000 and 30-50 millions dollars per year in losses respectively.

In recent years the understanding of the genome organisation of plant viruses has increased rapidly in parallel with the development of molecular biological techniques. The ability to obtain nucleotide sequences of complete viral genomes has also permitted the elucidation and understanding of expression strategies used for many different plant viruses.

For many years, the only nucleic acid found in plant viruses was RNA, but it is now clear that viruses infecting plants may contain any one of the four types of genetic material: single-stranded RNA (ssRNA, about 75% of plant viruses), double-stranded RNA (dsRNA, reoviruses), single-stranded DNA (ssDNA, geminiviruses) or double-stranded DNA (dsDNA, caulimo- and badnaviruses). Of those for which the genome is known or can be extrapolated by being in the same group as a known virus, the vast majority have ssRNA of the (+) or messenger polarity (termed (+) RNA). These (+) strand plant viruses are classified into more than 25 distinct taxonomic groups (Murphy et al., 1995) and show a wide variation in capsid morphology ranging from the rod shaped tobravirus, the filamentous potyvirus, to the icosahedral viruses (e.g. *bromovirus*, *sobemovirus*, *comovirus*, *tombusvirus*, *nepovirus*, *tymovirus*).

There are also some economically important viruses with minus-strand and ambisense genomic RNA species (*rhabdoviruses*, *tospoviruses and tenuiviruses*). Tospovirus is the only genus of plant viruses in the *Bunyaviridae* family (German et al., 1992). However, Toriyama (1995) has also proposed to include the Tenuivirus group as a new genus of plant-related viruses of the *Bunyaviridae* family.

This review is aimed to summarise some aspects of the main strategies used by plant viruses to express their genomes.

Genome organisation

Although the majority of known plant viruses have RNA genomes, it is the smaller division of plant DNA viruses which are better known. In the following sections, mainly information related to plant RNA viruses will be discussed and for information about plant DNA viruses or some viroids, there are several reviews that cover in depth these aspects (Symons, 1991;Lazarowitz, 1992,Timmermans et al., 1994,Rothnie et al., 1994).

Plant RNA viruses show a wide variation in their genome structure and organisation and may have different terminal structures such as cap structures or genome-linked proteins (VPg) at the 5' end, and a poly(A)-tail or tRNA-like structure at the 3' end of their RNA (reviewed by Goldbach et al., 1991). For some viruses the genome needed for infection is divided between two or more segments which may be encapsidated in the same particle or in separate particles (multicomponent) and even like the *tobacco necrosis virus* (TNV), have associated satellite RNAs (Hull, 1990; Hull and Davies, 1992, Matthews, 1991). Most, if not all, plant virus genomes encode four or more proteins with functions that operate at various stages in the infection cycle.

Information on the genome organisation and sequence similarities of the non-structural proteins, in particular of their RNA-dependent RNA polymerases (RdRps) and helicases, show that most plant RNA viruses are genetically related and appear to have possible evolutionary links with some animal RNA viruses (Ishihama and Barbier, 1994,Strauss et al., 1996).

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Table 1. Characteristic of RNA virus superfamilies

Group	Lineage	Virus groups	Helicase type	Common features	Morphology	Hosts and vectors
Super 1 (POL 1)	Picorna-like Poty-like Sobemo-like Arteri-like	Picornaviridae Comovirus Nepovirus Calicivirus Potyvirus Bymovirus Sobemovirus Luteovirus Nodavirus Corona virus Arterivirus Torovirus	III II None	5'-VPG 3'-poli (A) No subgenomic RNAs Polyprotein processing No overlapping ORFs 5'-cap 3'-poly (A) Nested set of mRNAs Enveloped	Icosahedral Separate encapsidation Rod-shaped Isometric	Mammals Plants Mammals Plants Plants Insect Mammals
Super 2 (POL 2)	Phage Flavi-like Pesti-like Carmo-like	RNA coliphages Flavivirus Pestivirus Carmovirus Tombusvirus	None II II None	One ORF No 3'-poly (A)	Enveloped Icosahedral	Bacteria Humans Mammals Plants
Super 3 (POL 3)	Tymo-like Rubi-like Tobamo-like	Tymovirus Carlavirus Potexvirus Capillovirus Rubella Hepatitis E Alphaviruses Tobamovirus Tricornavirus Hordeivirus Tobravirus Closterovirus	I I I	5'-caps Subgenomics mRNAs No Overlapping ORFs Readthrough (most)	Icosahedral Filamentous Enveloped Rod-shaped	Plants
Minus strand RNA Paramyxoviridae Rhabdoviridae Orthomyxoviridae Arenaviridae Filoviridae				Enveloped Self-complementary termini Helical capsid Overlapping ORFs	Some segmented genomes Some with M protein Pleiomorphic Enveloped rod	Mammals Birds Fish Insects Plants
Double-strand RNA Reoviridae Bimaviridae				Segmented genome 5'-cap 3'-OH ss RNA intermediates		Vertebrates Plants Arthropods Mollusks

Vpg, genome-linked protein; ORF, open reading frame
Adapted from Straus et al., 1996

The analogous modular arrangement of these coding sequences also suggests that these viruses may employ similar RNA replication strategies (Dolja and Carrington, 1992;Koonin and Dolja, 1993). This has led to the proposal (Goldbach, 1986;Koonin, 1991a;Koonin et al., 1991;Dolja and Carrington, 1992;Koonin and Dolja, 1993), based on the three different types of sequence motifs in the RdRps, of the division of the positive-strand RNA viruses into three 'Supergroups'.

Supergroup I, which includes, the Picorna-like, Poty-like, Sobemo-like and Arteri-like. They have common features as, a VPg protein covalently linked to the 5' end of the RNA, 3'-poly (A), no subgenomic RNAs, polyprotein processing, no overlapping ORFs (see Table 1 for more detail).
Supergroup II, which includes the coliphages, Flavi-like, Pesti-like and Carmo-like viruses. They shares such features as, enveloped virions and no 3' -poly (A) (see Table 1).

Supergroup III, which includes the Tymo-like, Rubi-like and Tobamo-like viruses. They have common features as, 5' caps, subgenomics mRNAs, no overlapping ORFs and read-through expression strategy (most of them) (see Table 1).

The grouping is based on sequence homology of three similarly organised non-structural proteins of Sindbis virus, including the RNA capping enzyme, RNA helicase and RdRp (Koonin, 1991a;Koonin, 1991b). Subgrouping can also be based on conserved sequence motifs of helicases (helicase superfamilies 1, 2 and 3), proteases and the presence of capping enzymes (reviewed in Koonin and Dolja, 1993). RdRp however is the only domain of positive-strand RNA viruses allowing an all-inclusive phylogenetic analysis.

Replication of plant RNA viruses

Most viruses encode proteins that are involved in viral nucleic acid replication. The discovery of the RdRps marked a major breakthrough in understanding the replication of progeny RNA from genomic viral RNA (reviewed in David et al., 1992; Ishihama and Barbier, 1994).

Potential RdRps have been described for many plant RNA viruses including *brome mosaic virus* (BMV) (Hardy et al., 1979), *cowpea chlorotic mosaic virus* (CCMV) (Miller and Hall, 1984), *turnip yellow mosaic virus* (TYMV) (Mouches et al., 1984), *alfalfa mosaic virus* (AlMV) (Houwing and Jaspers, 1986), *cucumber mosaic virus* (CMV) (Hayes and Buck, 1990), TMV (Young et al.., 1987), *turnip crinkle virus* (TCV) (Song and Simon, 1994), *red clover necrotic mosaic dianthovirus* (RCNMV) (Bates et al.., 1995), *tomato spotted wilt virus* (TSWV) (Adkins et al.., 1995). While it is accepted that the role of RdRp in replication of RNA viruses is essential, the mechanism of its function is unclear and may differ for different virus groups. In *in vitro* studies on BMV, CCMV, AlMV, and TYMV the enzyme has only been shown to synthesise minus-strand RNA while complete replication of both minus-strand and new progeny plus-strand RNA has been demonstrated for CMV (Hayes and Buck, l990).

In addition, host factors have also been implicated in the replication complexes of TYMV (Mouches et al.., 1984), TMV (Meshi et al.., 1988), *cowpea mosaic virus* (CPMV) (Derssers et al.., 1984), BMV (Quadt and Jaspars, 1990;Quadt et al., 1993), CMV (Hayes and Buck, l990). The requirement for host-factors goes some way in explaining the inability of some extracted viral RdRps to fully complete a replication cycle. Proposed mechanisms for the precise mode of action of several viral RdRps as well as their structure and organisation have been reviewed extensively (for comprehensive reviews see Marsh et al., 1989,David et al., 1992,Ishihama and Barbier, 1994).

In general however, the viral RdRps are complex moieties, acting as RNA replicases or transcriptases, synthesising both (-) and (+) strands. Moreover, RdRps not only catalyse RNA polymerisation but, in many viruses, also effect RNA modifications (e.g. RNA methyltransferase activity).

Replication of plus-strand RNA viruses

Replication of plant positive-strand RNA viruses takes place in the cytoplasm of infected cells. RNA polymerases appear to be membrane-bound, and some proteins implicated in replication have membrane-binding domains, e.g. P58 encoded by RNA1 of CPMV. However the precise sites where RNA replication takes place have not been clearly defined and probably differ for different viruses. Granular inclusion bodies have been invoked as the sites for TMV-RNA replication (Saito et al., 1987; Okamoto et al., 1988).

Replication of (+) strand RNA viruses can be separated into four overlapping steps:(i) The uncoating of the virus, which exposes the nucleic acid to the replication processes. (ii) Translation, during which the viral RNA serves as a messenger RNA and produces structural and non-structural proteins. This process is further divided into the primary or early translation of proteins required for replication, e.g. the RdRp, and secondary or late translation of proteins with late functions, e.g. the coat protein. (iii) Replication of the genome which yields progeny RNA molecules takes place in two stages, both catalysed by an RdRp:(1) Synthesis of a full-length complementary (negative) RNA strand using the genomic (positive) RNA strand as a template. (2) Synthesis of progeny genomic RNA and subgenomics RNAs using the negative-strand RNA as a template. And finally (iv) the progeny genomic strands are encapsidated.

The virus-encoded proteins required for RNA replication have been deduced from the composition of purified polymerases capable of copying genomic RNA to produce a negative strand, from the use of mutants, for divided genome viruses from the minimum number of RNA segments needed to infect protoplasts and from the presence of conserved sequence motifs found in polymerases in other systems (Quadt and Jaspars, 1990).

Initiation of the synthesis of a negative-strand on a positive-strand RNA template requires binding of the polymerase to a recognition site at the 3' end of the template. The 3' end of the RNA of many viruses can be folded into a characteristic secondary or tertiary structure which includes the RNA polymerase binding site. Sequences at the 5' end of the genomic RNA are also required for RNA infectivity (French and Ahlquist, 1987) and presumably reflect the requirement for binding of the polymerase at the 3' end of negative-strand RNA.

One system currently being used to study positive-strand RNA virus replication is the plant bromovirus group (Ahlquist, 1992). The bromoviruses are icosahedral, positive-strand, tripartite RNA viruses in the alphavirus-like superfamily. The two bromovirus proteins required for RNA replication, 1a and 2a, are translated from genomic RNA1 and RNA2, respectively, while proteins required for infection spread are translated from genomic RNA3 and a subgenomic mRNA, RNA4, transcribed from negative-strand RNA3. Protein 1a (109 K) contains an N-terminal m^7G methyltransferase-like domain thought to be involved in capping viral RNA (Rozanov et al., 1992) and a C-terminal helicase-like domain (Gorbalenya et al., 1988). Protein 2a (94 K) contains a central polymerase-like domain (Kamer and Argos, 1984). Site-specific mutagenesis studies showed that all three conserved domains in 1a and 2a are required for RNA synthesis (Kroner et al., 1990;Traynor et al., 1991).

Bromovirus RNA synthesis can be divided into three distinct steps:negative-strand synthesis, positive-strand synthesis, and subgenomic mRNA transcription. Each of these steps is differentially regulated. For example, negative-strand RNA accumulation plateaus by 8 h post-inoculation, while positive-strand genomic RNA and subgenomic mRNA continue to accumulate until or beyond 20 h post-inoculation (Kroner et al., 1990). French and Ahlquist (1987) described that BMV-directed replication of RNA3 *in vivo* depends on cis-acting sequences in three regions of RNA3:the 3' and 5' noncoding regions and the intercistronic noncoding region. Later, Janda and Ahlquist (1993) demonstrated that BMV RNA3 derivates can be replicated and direct subgenomic mRNA transcription in yeast expressing BMV proteins 1a and 2a from DNA plasmids.

Recently, it has been shown that yeast expressing 1a and 2a and replicating RNA3 derivatives can be extracted to yield BMV-specific template-dependent RdRp activity (Quadt et al., 1995). Moreover, even though RdRp activity was asssayed on *in vitro*-supplied BMV-RNA templates, it was found that RdRp can only be isolated from cells expressing certain BMV RNA template sequences as well as 1a and 2a. Strong correlation between extracted RdRp activity and BMV (-)-strand RNA accumulation *in vivo* was found for all RNA3 derivatives tested. Thus, extractable *in vitro* RdRp activity paralleled formation of a complex capable of viral RNA synthesis *in vivo*. These results suggest that assembly of active RdRp requires not only viral proteins but also viral RNA, either to direct contribute some nontemplate function or to recruit essential host factors in the RdRp complex (Quadt et al., 1995)

Zaccomer et al. (1995) have reviewed recently other elements believed to be involved in virus replication:

1) tRNA-like structures. It has long been known that the RNA genomes of certain positive-strand plant viruses have tRNA-related properties (reviewed in Mans et al., 1992). These 3' tRNA-like structures have been shown to be involved in minus-strand synthesis in the case of TMV (Dawson et al., 1988), BMV (Miller et al., 1985) and TYMV (Tsai and Dreher, 1991).

2) Pseudoknots. In addition to the pseudoknots in the tRNA-like structures, a few viruses have pseudoknots upstream of these structures which appear to participate in RNA replication. In TMV the most downstream of the six double-helical structures that compose the three pseudoknots already mentioned located just upstream of the tRNA-like structure, is required for replication (Takamatsu et al., 1990). Also Leathers et al. (1993) have reported that this region probably is involved in translation. However, pseudoknots present either in BMV-RNA3 (Lahser et al., 1993) or TYMV (Tsai and Dreher, 1992) are only involved in RNA replication.

3) Poly(A) structures. In CPMV, both M-RNA and B-RNA contain the sequence UUUUAUU immediately followed by the poly(A) tail. This heptanucleotide sequence together with the first four A residues immediately downstream can adopt a hairpin structure. A similar structure can also be formed by the M-RNA of RCMCV (Shanks et al., 1986). In CPMV B-RNA, deletions from the 3' end of the RNA can prevent formation of the hairpin and dramatically interfere with RNA replication (Eggen et al., 1989).

4) Internal control region (ICR)-like sequences. Similarities exist between viral RNA sequences (bromoviruses, cucumoviruses, tobamoviruses, tobraviruses and tymoviruses) and the ICR2 of the RNA polymerase III promoter of eukaryotes (Marsh et al., 1989). A role for these sequences in replication has been demonstrated for BMV RNA (Pogue et al., 1992) and is also proposed for the RNA of CMV (Boccard and Baulcombe, 1993) and AlMV (van der Vossen et al., 1993). The presence of ICR-like sequences suggests that a host RNA polymerase III subunit and/or one of its cofactors could participate in viral RNA replication.

Replication of minus-strand RNA viruses

Negative-strand RNA viruses are a large and diverse group of enveloped viruses. They are found in hosts from the plant and animal kingdoms, and have a wide range of morphologies, biological properties and genome organisations (Conzelmann, 1996). A major distinction is made between viruses whose genome consists of a single RNA molecule (order Mononegavirales), including the families *Rhabdoviridae*, *Paramyxoviridae* and *Filoviridae*, and those possesing multipartite (segmented) genomes, comprising the families Orthomyxoviridae (six to nine segments), *Bunyaviridae* (three segments) and *Arenaviridae* (two segments).

Characteristically, the genetic information of negative-strand RNA viruses is exclusively found in the form of a ribonucleoprotein complex (RNP) in which the genomic or antigenomic ssRNA is tightly encapsidated in a nucleoprotein (N or NP) and associated with the virus RdRp. In the case of non-segmented viruses, the latter consists of a catalytic subunit (L) and a non-catalytic cofactor, a phosphoprotein (P). After infection of a cell, the RNP serves as a template for two distinct RNA synthesis functions, transcription of subgenomic, usually non-overlapping mRNAs and the replication of full-length RNAs (for detailed reviews see Galinski, 1991). The RNP genomes appear to posses only one promoter, at the 3' end of the RNA where the virus RdRp enters for both mRNA transcription and genome replication (Conzelman, 1996).

For viruses in the family *Bunyaviridae*, the polymerase protein, either acting alone or in concert with undefined viral or cellular factors, must first functions as a cap-dependent endonuclease to generate a primer for transcriptions of a non-encapsidated transcript of subgenomic length. At some point, the polymerase must switch to a process of independently initiating transcription at the precise-3' end of the template and producing an encapsidated, full length transcript (Schmaljohn, 1996). Presumably, some viral or host factor is required to signal a suppression of the transcription termination signal responsible for generation of truncated mRNA and also to prevent the addition of the capped and methylated structures to the 5' termini of the cRNAs (Schmaljohn, 1996). For the rhabdovirus vesicular stomatitis virus, the switch to antigenome synthesis appears to be controlled by the N protein (Banerjee, 1987).

In animal viruses such Influenza A (*Orthomyxoviridae*), which has a genome consisting of eight ssRNA segments of negative polarity, the replication and transcription of the virus genome are catalysed by a virus-encoded RdRp (Kobashagi et al., 1992, Huang et al., 1990). The RdRp is composed of three subunits, PB1, PB2 and PA, which are tightly associated at the double-stranded stem region of the panhandle formed by the 5' and 3' termini of each RNA segment (Huang et al., 1990, Hsu et al., 1987). RdRp plays an essential role in both replication and transcription but little is known about the molecular mechanism of replication. However, some evidence suggests that PB1, PA and the nucleoprotein can support the replication of the influenza virus genome as well as the transcription to yield uncapped poly (A)+RNA but PB2 is specifically required for the synthesis of capped RNA (Nakagawa et al., 1995).

Virion-associated RdRp polymerase activity has been also found in plant rhabdoviruses. In the case of wheat rosette stunt virus, both detergent-treated virions and isolated nucleocapsids exhibit RNA polymerase activity. Like animal rhabdoviruses, the enzyme activity can be regained upon mixing of L and NS proteins and using N-associated RNA template. Products synthesised *in vitro* by the virion-associated RNA polymerase of plant rhabdoviruses contain genome-length and single-strand virus complementary RNA (vcRNA) indicating that the RdRp acts not only as transcriptase but also as replicase.

Replication of ambisense viruses

RdRp activity has been detected in detergent-disrupted virions of animal-infecting members of the *Bunyaviridae* (Vialat and Bouloy, 1992) and has been directly linked to the L-protein of bunyamwera virus, the type member of the family (Jin and Elliot, 1991). An RdRp activity has been found associated with virions of TSWV, a plant- and insect-infecting member of the family *Bunyaviridae*. Radiolabelled nucleoside triphosphate was incorporated into trichloroacetic acid-precipitable products by detergent-disrupted, purified TSWV virions. The predominantly double-stranded RNA products were RNase-resistant at high but not low salt concentrations. Discrete products of approximately 3.0 kb were synthesised that hybridised to purified TSWV RNA and transcripts of cDNA clones encompassing parts of each of the three genomic RNAs. The predominant products were viral sense although significant amounts of viral complementary sense S RNA products were also synthesised (Adkins et al., 1995).

Barbier et al. (1992) working with the *Tenuivirus rice stripe virus* (RSV) a virus with some genome organisation features in common with TSWV, isolated an RNA polymerase activity by CsCl centrifugation from purified RSV ribonucleoproteins (RNPs). The active fraction contained two viral structural proteins, a 30 K nucleocapsid (N) protein and a 230 K putative polymerase protein. An *in vitro* RNA synthesis system was reconstituted using this RNA-free protein fraction and short model templates carrying the conserved 5' and 3' terminal sequences. This showed that, as in the case of influenza virus, a minimum promoter function resides in the panhandle secondary structure formed by the complementary termini or in the 3' terminal sequence of 11-14 nucleotides in length.

Modes of gene expression

Another major problem facing RNA viruses with limited genome size is their obvious dependence on the host eukaryotic protein-synthesising system. These small genomes are also expected to encode a range of virus proteins. The strategies of expression that have emerged from recent studies suggest that the viral genomes appear to have evolved to overcome the obvious constraints of the plant host system.

The eukaryotic 80S ribosome is usually able only to translate the first ORF in the 5' region of an mRNA, according the "scanning ribosome model" proposed by Kozak (1991). The model states that the 40S ribosomal subunit (carrying Met-tRNA^{imet} and various initiation factors) binds initially at the 5' end of mRNA. The ubiquitous m7G cap and the associated cap-binding protein(s) explain the predilection of eukaryotic ribosomes to engage mRNA at the 5'-end. Then the migrating 40S ribosomal subunit stalls at the first AUG codon, which is recognised in large part by base pairing with the anticodon in Met-tRNA^{imet}. However, the stop-scanning step and hence selection of the initiator codon, is susceptible to modulation, by context, at least in vertebrates and selection of more distal AUG is permitted under certain defined circumstances (Kozak, 1991).

The possibility that might be cases of internal translation initiation has been shown. Pelletier and Sonenberg (1988) have proposed that there is efficient internal initiation on poliovirus RNA. The evidence comes from experiments exploiting the fact that translation of a dicistronic mRNA with two non-overlapping ORFs (A and B) generally gives a low yield of B protein (located downstream) compared with A.

Pelletier and Sonenberg (1988) used a construct in which the entire 5' untranslated region (736 nt) of type 2 poliovirus was placed in the intercistronic region of a capped dicistronic mRNA. When the cells expressing the dicistronic mRNA are infected with the poliovirus, the synthesis of protein A (upstream) was inhibited and protein B enhanced, demonstrating that downstream cistron translation is independent of upstream. In addition, cell-free extracts from poliovirus-infected cells translated cistron B but not A. Similar results have been published also by Jang et al. (1988) for *encephalomyocarditis virus* RNA and more recently for *bovine viral diarrhea virus* by Poole et al. (1995).

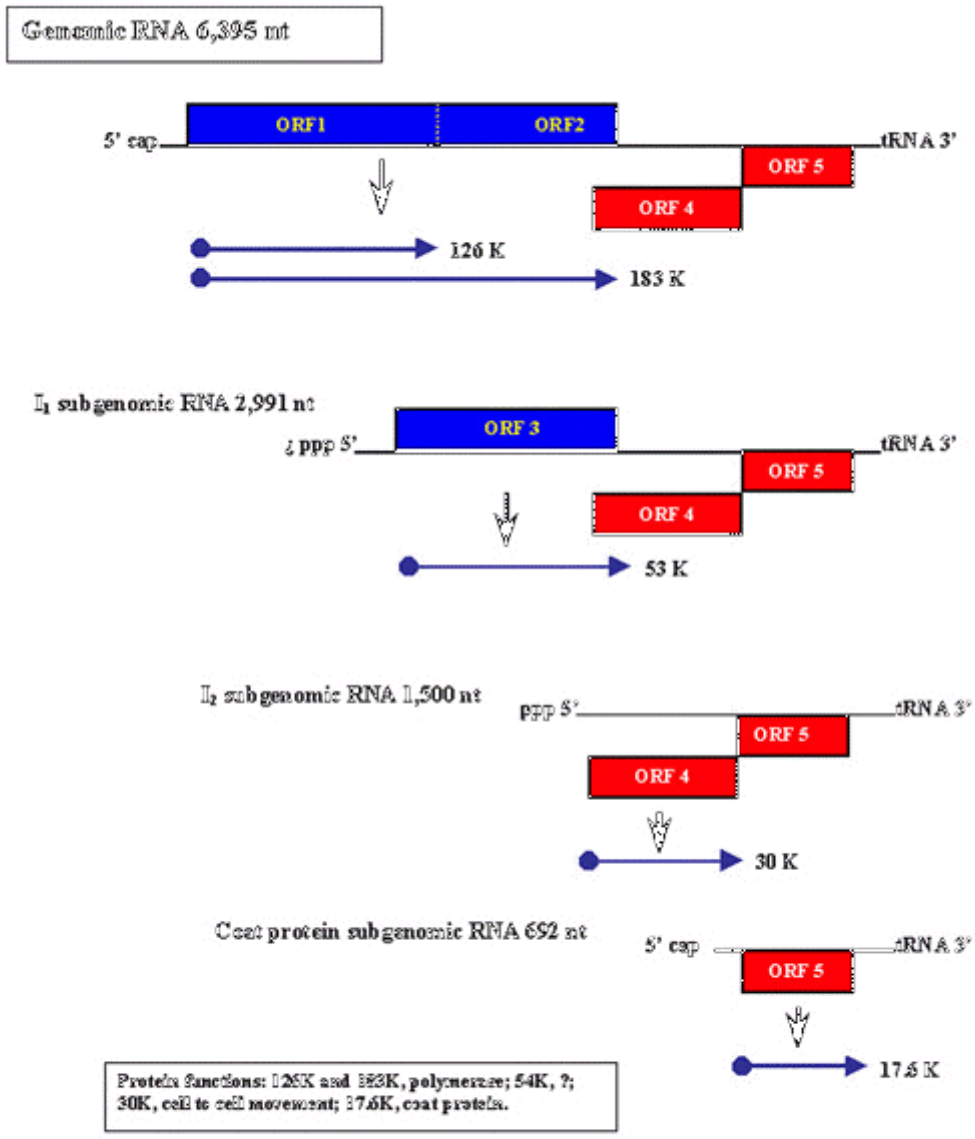
The main strategies used by plant viruses to allow protein synthesis in a eukaryotic system from positive sense RNA genome containing more than one gene are discussed below (see Figure 1 for some illustrations).

Subgenomic RNAs

The expression of internal genes such coat protein (CP) of the positive RNA viruses is frequently mediated via subgenomic RNAs, considered in this study as mRNAs (see Figure 1a). These mRNAs are encapsidated in some viruses, but not in others. Among plant RNA viruses, the mechanism of synthesis of the subgenomic RNA encoding the CP has been examined in several viruses, i.e., TMV (Palukaitis et al., 1983), CMV (Jaspars et al., 1985). From these studies, two mechanisms have been proposed to explain the synthesis of subgenomic RNA species:(1) During (-) RNA strand synthesis by the RdRp, premature termination could lead to the formation of (-) RNA strands of subgenomic length that could serve as template to generate the subgenomic (+) RNA; alternatively (2) the subgenomic (+) RNA could be synthesised via internal initiation on (-) RNA strands of genomic length.

The evidence from *in vivo* and *in vitro* experiments with various RNA viruses clearly tends to favour the second mechanism. Since subgenomic RNAs contain at their 3' end the elements required for the production of complementary subgenomic RNA chains, various explanations have been put forward to account for the lack of autonomous replication of subgenomic RNA. These are that (1) the sequence contained within the subgenomic RNA is insufficient for replication of the subgenomic RNA; (2) the subgenomic RNA, which is frequently a highly efficient mRNA, may not be available for replication; and (3) the subgenomic RNA would be produced late in infection or at time when negative-strand synthesis has ceased. From different experiments, the first explanation is certainly the most likely.

Miller et al. (1985), studying the mechanism of BMV subgenomic RNA4 formation from genomic RNA3 by using the *in vitro* RdRp system provided, the first unequivocal evidence that the subgenomic RNA of a positive-strand RNA virus is synthesised (at least *in vitro*) by internal initiation of positive-strand RNA synthesis on a negative-strand template.



Organisation and expression of a *Tobamovirus* genome (Read-through and subgenomic RNAs strategies. TMV Vulgare strain).

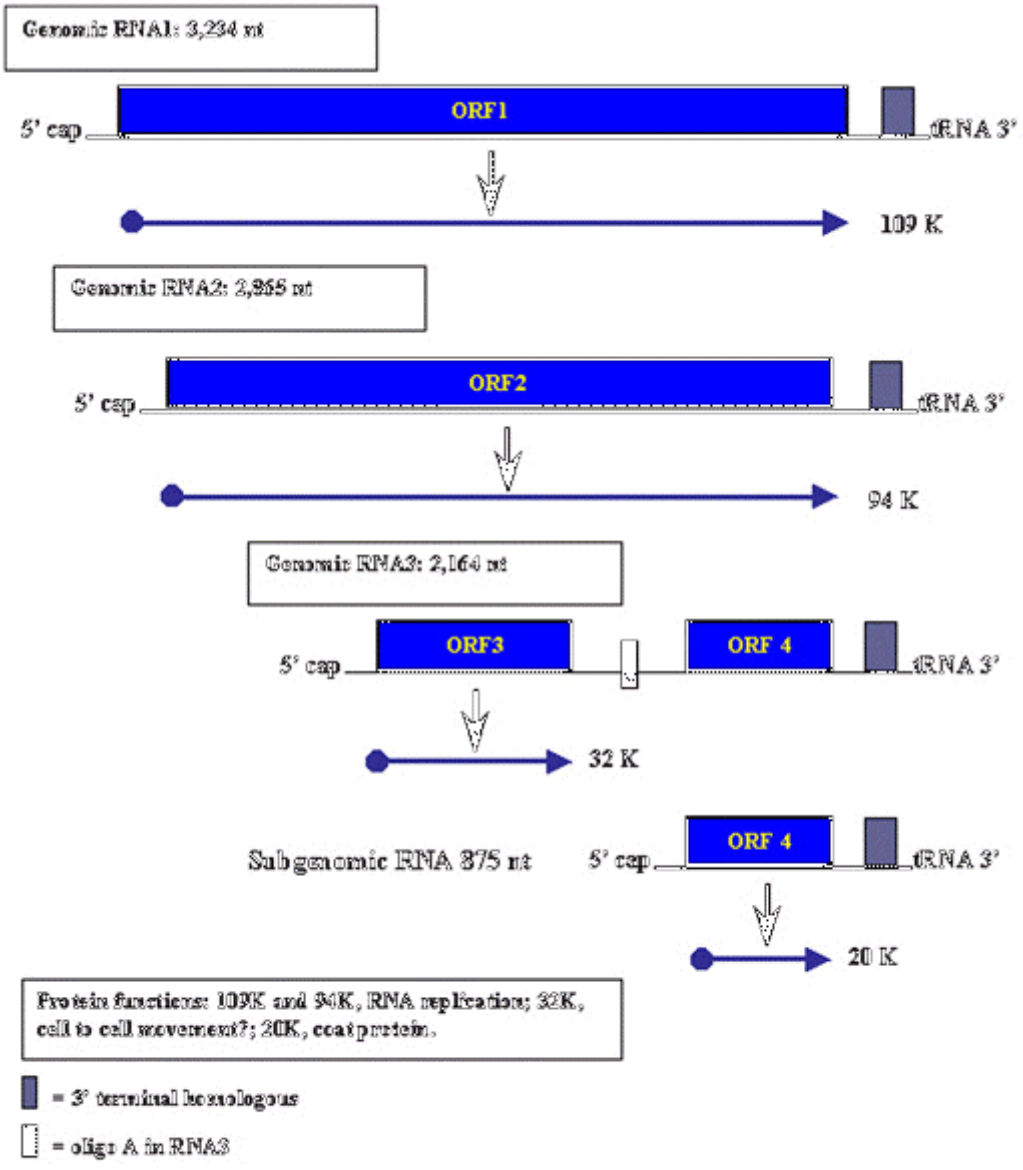
Figure 1a.

The internal promoter involved in RNA4 synthesis was identified using an altered negative-RNA3 strand as template (Miller et al., 1985; Marsh et al., 1986). Deletions 3' from the RNA4-corresponding sequence were performed on the RNA3 negative-strand to identify the core sequence required for initiation of positive-RNA4 synthesis. They are located, respectively, 20 nucleotides downstream from the RNA4 start site and 17 nucleotides into the RNA4 sequence.

An oligo (U) region 3' from this negative-strand core promoter sequence seems to function as a spacer, ensuring accessibility of the promoter for the viral RdRp, since its removal leads to an important decrease in RNA4 synthesis (Marsh et al., 1986).

Further studies have been performed to investigate the nature and behaviour of sequences influencing RNA4 production *in vivo*. The roles of additional downstream sequences and positional effects on promoter functions have also been studied (Allison et al., 1988). Four regions can be identified as playing a role in RNA4 initiation *in vivo*.

First, as demonstrated *in vitro*, initiation of subgenomic RNA synthesis does not require more than 17 nucleotides of the RNA4 sequence (nucleotides +1 to +17). Second, sequences 3' from the start site of RNA4 can be divided into three different domains. They correspond to the 20 nucleotides upstream (nucleotide -1 to -20), an oligo(U) stretch (nucleotides -20 to -38) downstream from this region, and finally, an (A+U) rich sequence (nucleotides -38 to -95) adjacent to the oligo (U). The region containing the first two domains contributes favouring correct initiation of positive-RNA synthesis. The entire promoter sequence is 112 nucleotides long.



Organisation and expression of *Bromovirus* genome (Multipartite genome and subgenomic RNAs strategies. BMV)

Figure 1b.

For TMV, however, two separate subgenomic promoter sites have been identified in the negative-strand, which control synthesis of the mRNAs for P30 (expressed relatively early) and CP (expressed late) (Young et al.,1985; Lehto and Dawson, 1990).

For the expression of ambisense viruses several lines of evidence indicate that subgenomic mRNAs are formed from each RNA strand, and full-length vRNA and genome-size vcRNA are synthesised only during replication. For several animal-infecting members of the Bunyaviridae the process of initiation and termination of transcription has been further

studied by characterising the 5' and 3' ends of viral mRNAs (reviewed by German et al., 1992).

These studies have shown the presence of short heterogeneous non-viral sequences at the 5' ends of the mRNAs, indicating that the viral transcriptase uses RNA primers to initiate transcription (Bishop et al., 1983;Bouloy et al.,1990;Simons and Pettersson, 1991; Gro et al., 1992; Jin and Elliot, 1993). These primers are generated from capped host messenger RNA species by a process referred to as "cap-snatching", i.e. the 5' terminal sequence of a cellular mRNA is cleaved off by an endonuclease and subsequently used to initiate transcription on the viral genome (Braam et al., 1983; Ulmanen et al., 1981). Less is known about the termination of transcription, but some typical structures features, e.g., palindromic sequences or hairpin structures, have been found close to the site where termination occurs (Bouloy et al., 1990; Simons and Pettersson, 1991).

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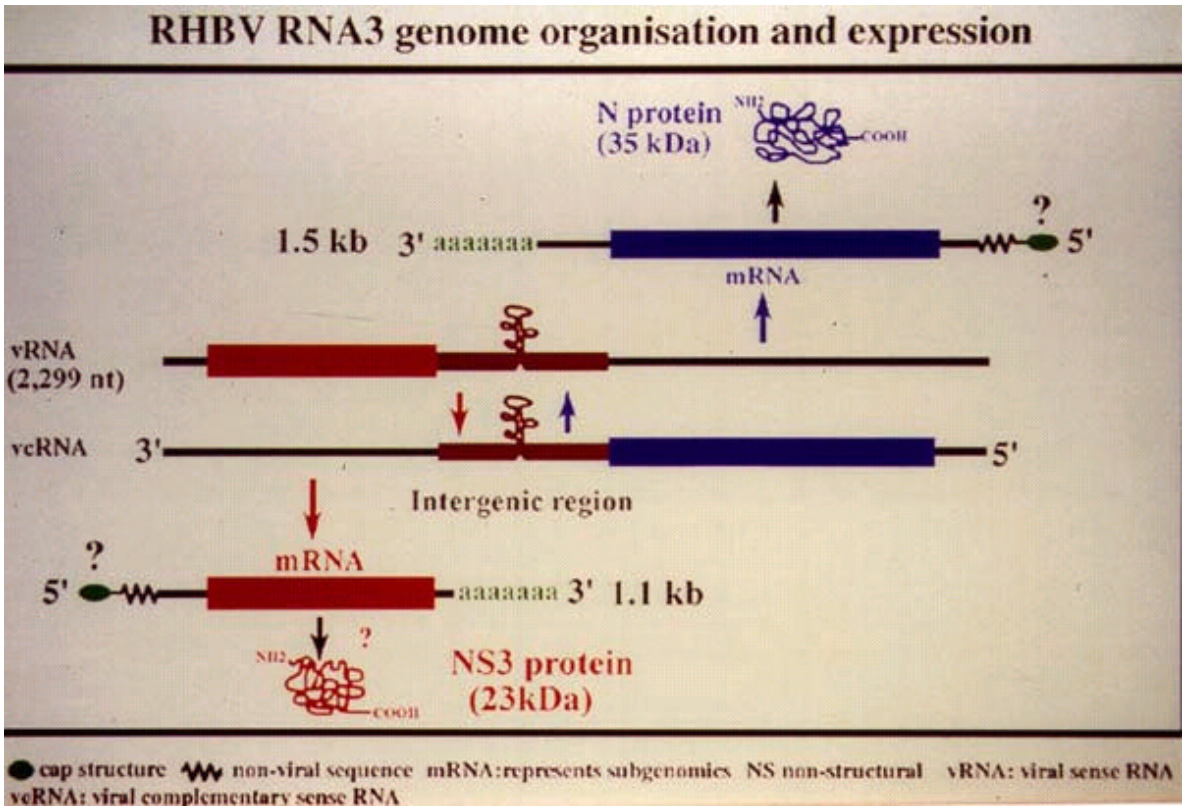

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genome is approximately 10 kb in length and encodes a single polyprotein that is processed by three viral proteinases to yield nine or more mature proteins (Riechmann et al., 1992). Two of the proteinases, P1 and helper component-proteinase (HC-Pro), each catalyse cleavage only at their respective C termini (Verchot et al., 1991). The remaining cleavage sites (at least six) are processed by the NIa proteinase, a homologue of the picornaviral 3C proteinase. This enzyme possesses a serine-type proteinase fold but contains a nucleophilic Cys residue rather than Ser at the active site (Gorbalenya et al., 1989).

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The 49 K NIa protein is actually a polyprotein consisting of a proteinase domain (27 K) near the C terminus and a VPg (21 K) near the amino terminus (Dougherty and Parks, 1991). The VPg domain is attached covalently to the 5' terminus of viral RNA via a phosphodiester linkage with Tyr-62 (Murphy et al., 1990). The N-terminal domain also contains a nuclear localisation signal that directs the majority of NIa molecules to the nucleus of infected cells (Carrington et al., 1991)

contain conserved Tyr and Gln residues at the P3 and P1 positions, respectively, the internal NIa site contains Thr and Glu (Dougherty and Parks, 1991).

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The first cistron in the genomic viral RNA may have a "leaky" termination codon (UAG or a UGA) that can be suppressed by a host transfer RNA (tRNA), thereby permitting some of the ribosomes to read through into a downstream cistron as a result, giving rise a second longer functional polypeptide (see Figure 1a, Figure 1d.).

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For a number of retroviruses, heptanucleotide signals are involved as "slippery sequences" in the frameshift. These include the sequences AAAAAAC (mouse mammary tumour virus); AAAUUUA (Rous sarcoma virus) and UUUUUUA (human immunoeficiency virus) which allow for tRNA slippage during translation (Hatfield et al., 1989). In addition to the specific sequence signal a second type of information bears relevance to frameshifting (Hatfield and Oroszlan, 1990): stem-loop structures immediately downstream of the "slippery sequences" have significant influence on the efficiency of the frameshift event.

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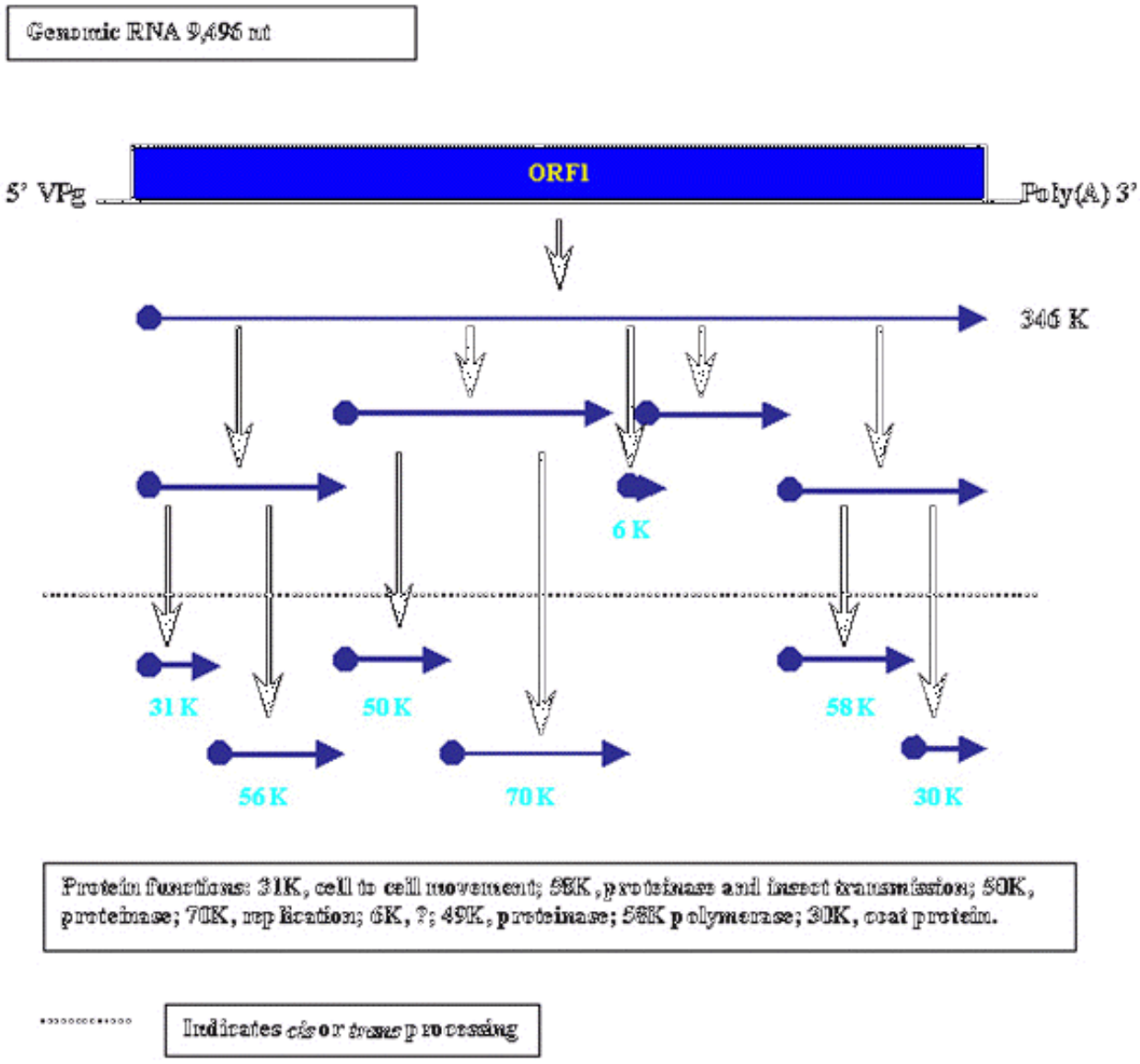
The signal responsible for efficient frameshift in PLRV is composed of the slippery sequence UUUAAAU followed by a sequence that has the potential to adopt two alternative folding patterns, either a structure involving a pseudoknot, or a simple stem-loop structure. Kujawa et al. (1993) have confirmed that in PLRV-P (Polish isolate) the -1 frameshift in the overlap region depends on the slippery site and on the downstream positioned sequence a proposed pseudoknot is required for efficient frameshifting. These results are in agreement with those reported for beet western yellows luteovirus by García et al. (1993).



Organisation and expression of a *Potyvirus* genome (Polyprotein strategy, TEV)

Figure 1c.

Frameshifting has also been postulated as an expression strategy of PEMV RNA2 (Demler et al., 1993). Moreover, in beet yellows virus, a unique case among viruses of +1 frameshift has also been proposed (Agranovsky et al., 1994).

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A mechanism whereby the 5' cistron was bypassed by ribosomes has been suggested for Agronowski (1991), cited by Hull (1992), for the expression of the beet yellows closterovirus 65-K cistron, which is downstream of a 6.4-K cistron. The latter cistron has no internal AUG codons in any of the reading frames, from where the expression of the 65-K cistron could be initiated. Recently, Grieco et al. (1995) has reported the presence of a bicistronic RNA from the olive latent virus 2. However, the RNA3 of the virus contains two clear-cut ORFs in same reading frame The encapsidation at a very low level, of an RNA species (smaller by about 350 nt

and denominated RNA 4) with a very high sequence homology with the RNA3, could explain the expression of the second ORF.

Three other mechanisms will be briefly mentioned here to explain the expression strategies used by caulimoflower mosaic virus (CaMV), a dsDNA virus that, to express the different cistrons, transcribes their genome into two RNA species, the polycistronic 35S and the monocistronic 19S (Rothnie et al., 1994; Kiss-Laszlo et al., 1995).

1.- "Ribosome shunt". Studies carried out to analyse the translation of the CaMV in protoplasts suggested a mechanism by which ribosomes enter at the cap site as normal and begin scanning. But at some point near the 5' end of the leader, they are somehow transferred to a region at the 3' end of the leader, without scanning linearly through the central portion of the leader. This process has been termed "ribosome shunt" and the sites between which it occurs have been defined (Futterer et al., 1993).

The shunt mechanism has been observed to operate between two separate RNA molecules, thus reinforcing the fact that the ribosomes are transferred directly from one part of the leader to another without scanning the sequence in between (Rothnie et al., 1994).

2.- "Translational trans-activation". Translation of downstream ORFs on the polycistronic RNAs of caulimoviruses depends on the presence of a virally encoded translational trans-activator (TAV), which is the product of ORF VI. TAV is a complex protein that appears to be involved in many aspects of the virus life cycle (De Tapia et al., 1993). It has been shown to be part of abundant vacuolated inclusion bodies as well as determinant of host specificity and a factor influencing symptom development in infected plants (Broglio, 1995; De Tapia et al., 1993). As mention above, another of its role is to control translation from the polycistronic CaMV 35S RNA. Translational trans-activation has been demonstrated for CaMV and figwort mosaic virus (Bonneville et al., 1989; Gowda et al., 1989) and appears to enhance expression of all the majors ORFs on the pregenomic 35S RNA (Scholthof et al., 1992). The TAV is itself translated from the 19S RNA, but can probably also trans-activated its own expression from ORF VI on the 35S RNA (Driesen et al., 1993). Like the "ribosome shunt", trans-activation seems to act on ribosomes that have begun scanning of the RNA at the 5' end. The process of trans-activation seems to allow ribosomes that have translated one ORF to remain competent to translate further downstream ORFS, or to become initiation competent once more (Rothnie et al., 1994).

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Evidence indicates that NIa catalyses internal cleavage at a rate much slower than that at other NIIa-mediate cleavage sites, most likely as a result of a sub optimal context of the cleavage site. While other NIa-mediated cleavage sites in the TEV polyprotein contain conserved Tyr and Gln residues at the P3 and P1 positions, respectively, the internal NIa site contains Thr and Glu (Dougherty and Parks, 1991).

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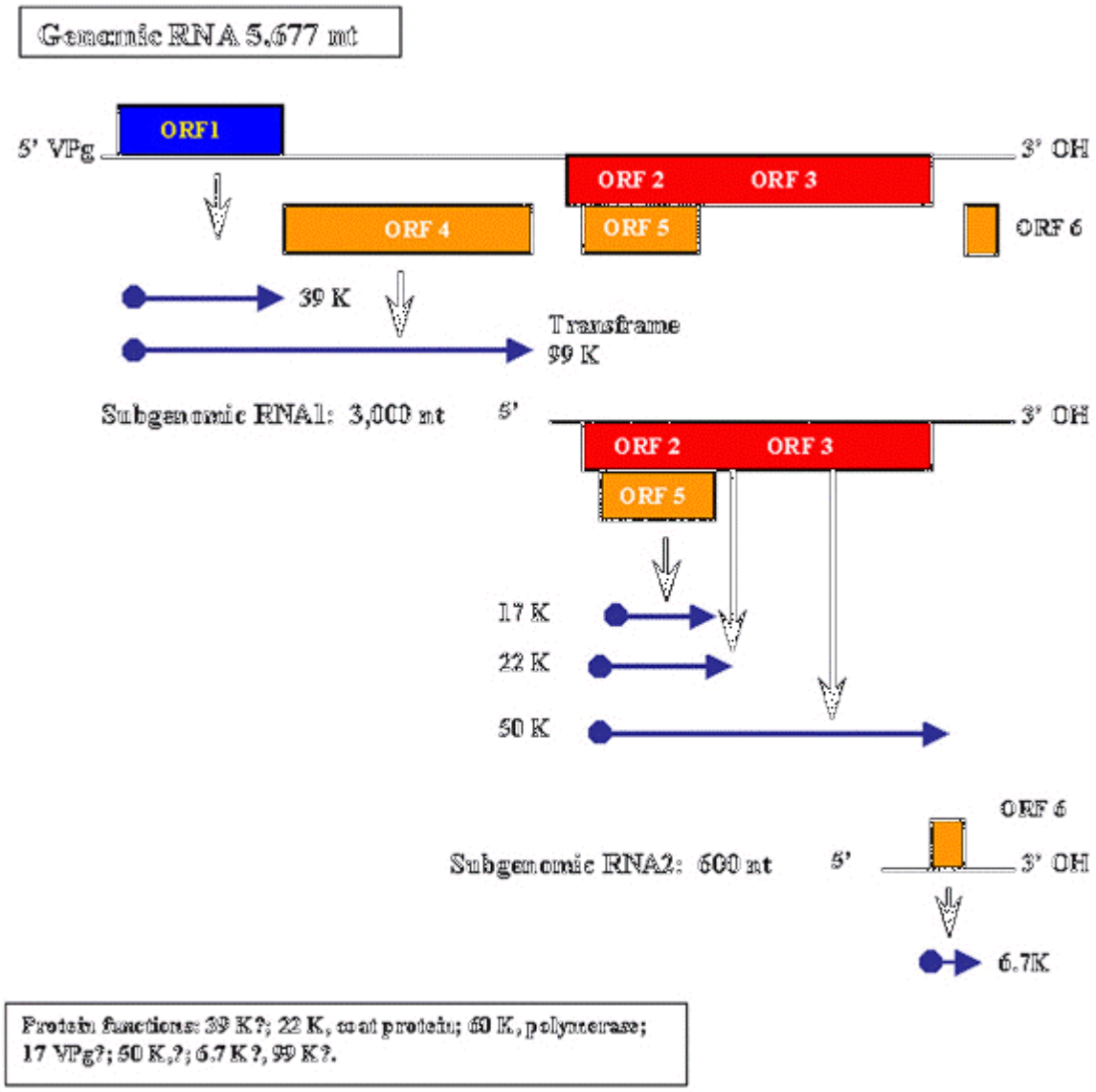

Organisation and expression of a *Luteovirus* genome (Frameshift, Read-trough and subgenomic RNAs strategies. BYDV).

Figure 1d.

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