Review Letter

Plant RNA viruses: strategies of expression and regulation of viral genes

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The genome of most plant viruses is composed of RNA of '+' polarity and can serve directly as messenger for protein synthesis. This paper deals with those viral RNAs whose in vitro and in vivo translation products can be compared; it reviews the strategies used for the modulation of the synthesis of the various virus-coded proteins and examines the postulated functions of these proteins.

Plant RNA virus *In vitro translation* *In vivo translation* *Protein synthesis regulation*

1. INTRODUCTION

The genome of plant viruses is composed of one or several single-stranded (ss) or double-stranded (ds) RNA or DNA molecules. Among DNA viruses the caulimoviruses, of which cauliflower mosaic virus is the main representative, possess a ds DNA genome with ss interruptions, and geminiviruses with mono- or bipartite morphology possess a circular ss DNA genome. The vast majority of plant viruses possess a ss RNA genome of + polarity since their RNAs are used directly as messenger RNA. There exist only few plant viruses whose genome is composed of ds RNA (plant reoviruses, e.g., wound tumor virus) or of ss RNA of – polarity (complementary to mRNA; plant rhabdoviruses, e.g. tomato spotted wilt virus); in these cases, the RNA is first transcribed and only then translated.

Among plant pathogens [1] there exist also viroids (free-living small circular ss RNAs that induce their own replication and are not translated), satellite particles (whose small linear RNA requires a helper virus for replication, modifies the symptoms induced by the associated virus and is often not translated: the helper virus is not dependent on the satellite) and virusoids (containing viroid-like RNA in addition to virus-like, linear RNA; the viroid-like RNA possesses properties in common with viroids and satellite RNAs, and in some cases it seems to be required for virus infectivity).

Plant viruses with a ss RNA genome of + polarity are not only the majority but are also the best studied from all points of view: structure, replication, translation, encapsidation and function of the virus-coded proteins. The viral RNA must possess information for its replication, translation, encapsidation and propagation. The viral genome is composed of one, two or three distinct RNA molecules. The RNAs of bi- and tripartite genome viruses are numbered 1 to 3 in order of their decreasing molecular mass. There also exist subgenomic RNAs which are usually 3′ co-terminal with one of the genomic RNAs and are packaged when they possess the encapsidation site. The viral RNAs are encapsidated in the same and/or distinct virus particles, the capsid being generally made up of a single protein species.

The multiplication cycle of RNA viruses with a '+' genome is believed to occur as follows (for a review see [2]. The parental virus particle trans-

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mitted mechanically, by the seeds, insects, etc., enters the cell probably by endocytosis and is rapidly uncoated. The viral RNA(s) is (are) translated giving rise to high molecular mass proteins; these are involved either directly or indirectly in virus RNA replication. Other proteins involved in virus replication are synthesized by the host during the first few hours following infection. An RNA-dependent RNA polymerase generates negative sense RNA strands from the genomic RNAs of + polarity. The progeny virus RNA strands are then synthesized on replicative intermediates yielding genomic and subgenomic RNAs that are translated. Concomitantly to coat protein synthesis, the viral RNAs are encapsidated. Short and long distance virus migration then follows and infection is established.

Recent review articles have described the structure [3–5], replication [6] and translation [4,5] of plant viral RNAs. This article briefly summarizes our present knowledge of the structures at the 5′ and 3′-ends of viral RNAs, and then deals with plant viral RNAs whose in vitro and in vivo translation can be compared, the strategies used for the synthesis of more than one polypeptide per cistron, the regulation of the synthesis of the different virus-coded proteins and the postulated functions of these proteins.

A list of plant RNA viruses (main representatives only), the group to which they belong, the type and structure of their genome and the major strategies used during translation of their RNAs are presented in table 1.

2. STRUCTURE OF VIRAL RNAs

The complete nucleotide sequence of the TMV [8], AMV [9–11], CPMV [12,13] and BMV genomic RNAs [14,15], the TSV genomic RNA 3 [16], the CMV genomic RNA 3 [17] and the TYMV sub-

Table 1

Terminal structure and major translation strategies used by the RNA genomes of + polarity of plant viruses (references cited in [3–7])

Genome	Virus group	Virus	Structure[a] 5′	3′	Translational strategies[b]
Monopartite	Tobamo	tobacco mosaic virus (TMV)	cap	$tRNA^{His}$	sg; RT
	Tobamo	sun hemp mosaic virus (SHMV)	cap	$tRNA^{Val}$	sg; RT
	Tymo	turnip yellow mosaic virus (TYMV)	cap	$tRNA^{Val}$	sg; pre term; RT; cleav
	Potex	potato virus X (PVX)	cap	pXOH	sg?; RT
	Poty	tomato etch virus (TEV)	VPg	poly(A)	sg?; RT?; cleav?
	unclass.	carnation mottle virus (CarMV)	?	pXOH	polycistronic
Bipartite	Tobra	tobacco rattle virus (TRV)	cap	pXOH	sg; RT
	Como	cowpea mosaic virus (CPMV)	VPg	poly(A)	cleav; int init
	Nepo	tobacco black ring virus (TBRV)	VPg	poly(A)	cleav
Bi- and tripartite	Hordei	barley stripe mosaic virus (BSMV)	cap	$tRNA^{Tyr}$	sg
Tripartite	Bromo	brome mosaic virus (BMV)	cap	$tRNA^{Tyr}$	sg
	Bromo	cowpea chlorotic mottle virus (CCMV)	cap	$tRNA^{Tyr}$	sg
	Cucumo	cucumber mosaic virus (CMV)	cap	$tRNA^{Tyr}$	sg
	Ilar	alfalfa mosaic virus (AMV)	cap	pXOH	sg; pre term; frame?
	Ilar	tobacco streak virus (TSV)	cap	pXOH	sg

[a] The viral RNAs possess at their 5′-terminus a cap ($m^7G_{5'ppp5'}Xp$-) or a VPg (virus-coded protein) linked covalently to the genome and at their 3′-terminus a poly(A) stretch, a tRNA-like region or a heteropolymeric sequence with no particular structure (-pXOH)

[b] These include the use of (i) internal initiation (int init), premature termination (pre term), readthrough (RT) and frameshift (frame) during translation, (ii) post-translational cleavage (cleav) of a polyprotein, (iii) polycistronic RNA and (iv) subgenomic (sg) RNA. ? = undefined

genomic coat protein mRNA [18] has been established. However, only partial information can be drawn from these sequences about the presence and location of the various signals involved in virus multiplication. For several other plant viral RNAs, only the nucleotide sequence in the region of the 5'- and 3'-ends is known (reviews, [3–5]).

In nearly all instances the viral RNAs carry at their 5'-terminus either a cap ($m^7G_{5'ppp5'}Xp$-) or a virus-coded genome-linked protein (VPg); at their 3'-end they possess either a tRNA-like structure, a poly(A) stretch or a heteropolymeric sequence without particular features (see table 1).

The cap structure facilitates translation of plant viral RNAs [19,20] as it does for most eukaryotic mRNAs. The presence of the VPg does not influence translation of plant viral RNAs in wheat germ extracts or reticulocyte lysates, and it is still unclear whether it is necessary for infection; by analogy with the poliovirus, one could expect the VPg to be involved in replication (review, [21]).

The tRNA-like structure formed by the 3'-region of several plant viral RNAs is recognized in vitro and in vivo by many tRNA-specific enzymes (reviews, [22,23]). The folding of the tRNA-like region of the bromo-, cucumo- and hordeiviruses is highly conserved [24–26]. It has been shown that the purified 'replicase' is able to replicate viral RNAs without prior aminoacylation ([27–29]; review, [6]). It follows that the aminoacylation of viral RNAs can not be required for viral RNA replication. It has been suggested that aminoacylation might not be required at early stages of BMV multiplication [30]. The 3'-regions of these viral RNAs must play an important role in viral RNA replication, but the exact function of viral RNA aminoacylation is not understood.

The length of the poly(A) stretch located at the 3'-end of a number of plant viral RNAs is heterogenous for the RNAs of a given virus. The role of the poly(A) stretch is unclear although it has been reported that poly(A) stabilizes the RNAs [31]. Whether the poly(A) stretch is synthesized during or after replication remains to be elucidated because the 5'-terminal sequence of the – strand of these viruses as well as the replication signal for initiation are unknown. Certain plant viral RNAs contain an internal poly(A) sequence of 19–40 residues; in BMV RNA 3 the poly(A) stretch is present in the intercistronic region [14], and in BSMV RNA between the 3'-terminal cistron and the tRNA-like region [32]. It has been suggested [32] that the formation of the poly(A) sequence could occur by 'slippage' during polymerization or by reiterative copying of the corresponding poly(U) in the – RNA strand.

Finally, some plant viral RNAs contain a heteropolymeric region without particular sequence or structure at the 3'-end. This is the case of the AMV and TSV RNAs; interestingly, coat protein interaction with the 3'-region is required for replication of these viral RNAs [33].

3. IN VITRO AND IN VIVO TRANSLATION OF PLANT VIRAL RNAs

Several systems have been used to examine the in vitro (wheat germ, reticulocyte and ascites extracts) and in vivo (leaf fragments, protoplasts and *Xenopus laevis* oocytes) translation of plant viral RNAs. The genetic map of a few plant viral RNAs is well established. Those whose in vitro and in vivo translation products are well characterized and can be compared are presented in fig.1 and are discussed below.

In general, for a given virus at least 3 proteins are synthesized. The RNAs of viruses with a monopartite genome are usually polygenic, and those of viruses with a bi- or tripartite genome are mono- and/or polygenic. In nearly all cases, the viral RNAs are functionally monocistronic: only the 5'-proximal cistron is translated. Subgenomic RNAs, on which the internal genes carried by the genomic RNA become 5'-proximal are generally responsible for the translation of these genes.

In the case of TMV, the monopartite genomic RNA codes in phase for two high-molecular-mass proteins, and the two subgenomic RNAs code for a 30-kDa protein and for the coat protein, respectively (fig.1; [20,34–41]). Interestingly, a similar pattern of protein synthesis is found with TRV whose genome is segmented: the molecular masses of the proteins encoded by its RNAs are about the same [42] as those of TMV RNA. The two high-molecular-mass proteins are synthesized on genomic RNA 1, whilst the coat protein is exceptionally encoded by the 5'-proximal cistron of genomic RNA 2 and the 30-kDa protein by a subgenomic RNA deriving from the 3'-region of RNA 2 [43].

Como- and nepoviruses possess a bipartite

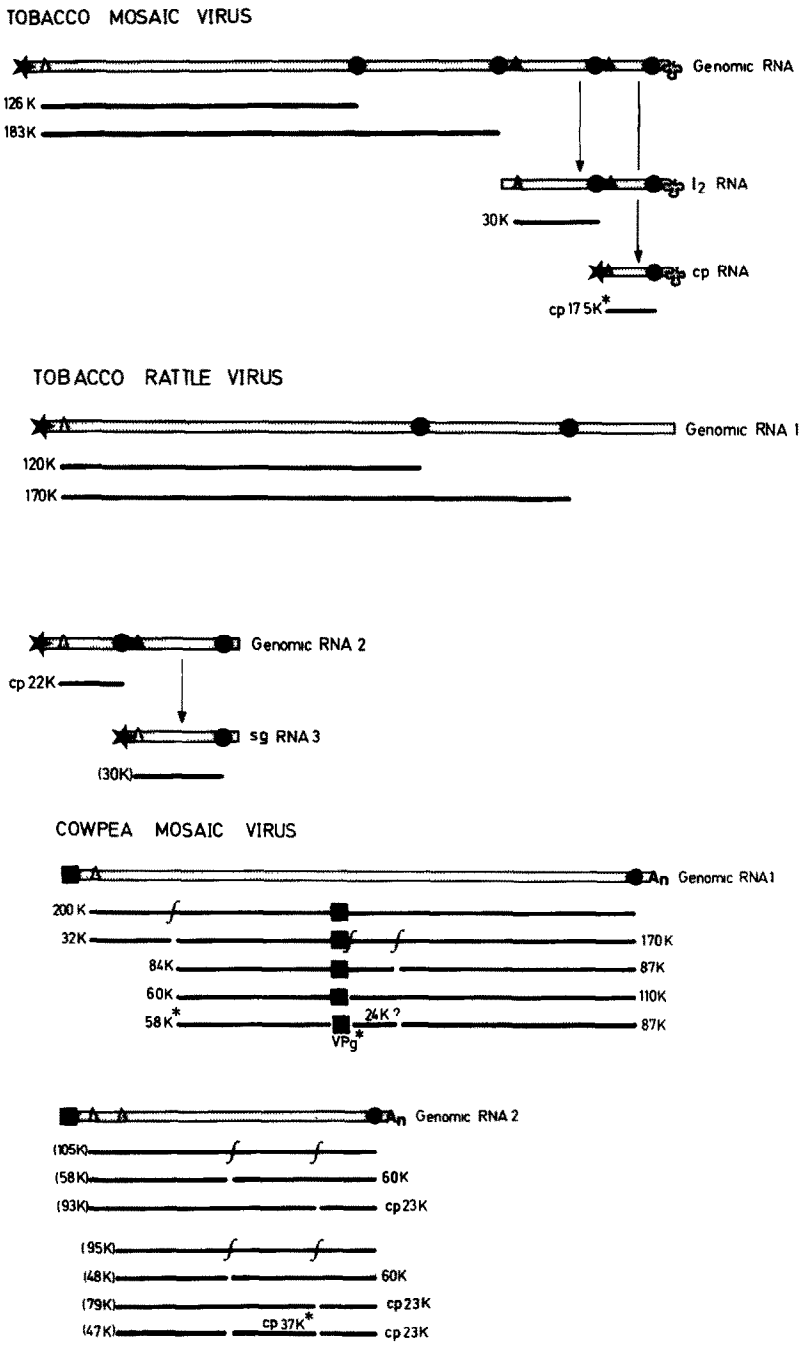
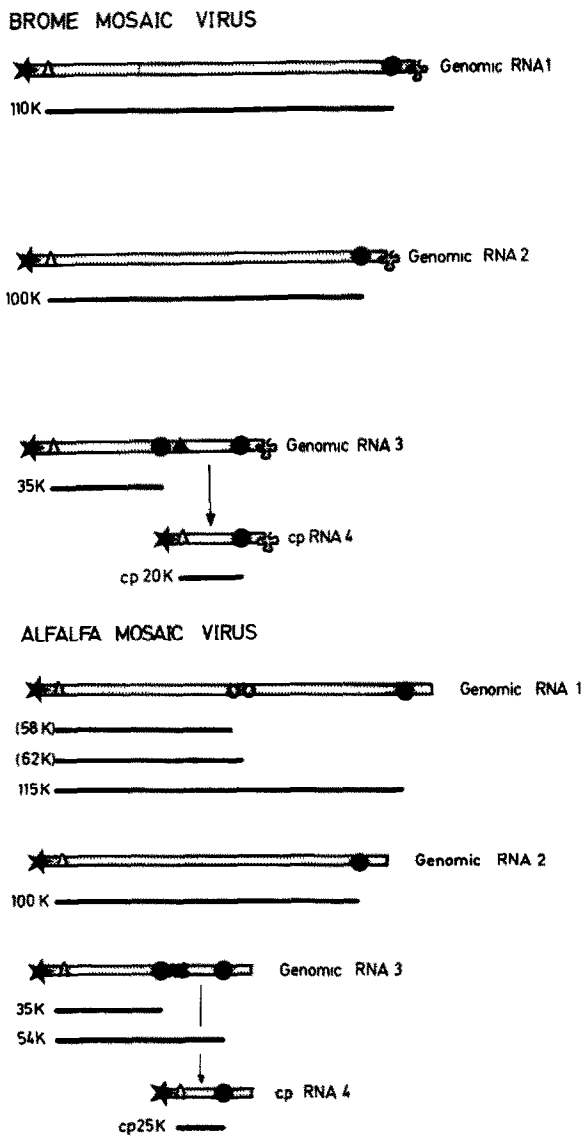


Fig.1. Genetic map of plant virus RNAs of + polarity based on in vitro and in vivo experiments. The following genetic maps are shown: TMV RNA [8,20,34–41], TRV RNA campinas strain [42,43], CPMV RNA ([13,44–46] review, [21]), BMV RNA [47–49] and AMV RNA [50–55]. The genetic map of CCMV RNA [56,57] is very similar to that of BMV RNA. The genetic map of BSMV RNA has recently been reported [58,59] but is not presented here since it is based essentially on in vitro data, and since a relationship between all the in vitro and



virus-coded or virus-induced in vivo translation products has not yet been established. (★) cap sructure; (A_n) poly(A) stretch; (♆) tRNA-like structure; (■) VPg; (▭) RNA; (▬) protein; (△, ▲) open and closed initiation sites for translation, respectively; (●) termination codon; (○) premature termination due to a 'stop' codon different from termination codons; (♈) frameshift; (∫) post-translational cleavage; (sg) subgenomic; (cp) coat protein; (K) kilodalton; (♈) proteins detected only upon in vitro translation of the packaged RNAs; (*) proteins detected only in vivo; (?) protein detected neither in vivo nor in vitro. All other proteins have been detected both in vivo and in vitro.

genome; their RNAs direct the synthesis of 3 high-molecular-mass proteins. One of them is encoded by RNA 1 (also referred to as B RNA) and gives rise after post-translational cleavage to the replicase or to a subunit thereof, and to VPg [44]. The two others are synthesized by in-phase reading on RNA 2 (also referred to as M RNA) and both are post-translationally cleaved to generate the coat proteins ([45,46]; review, [21]).

In bromo-, cucumo- and ilarviruses with a tripartite genome, RNAs 1 and 2 direct the synthesis of two high-molecular-mass proteins, and RNA 3 of a 35-kDa protein. In addition, a subgenomic RNA 4 pertaining to the 3'-region of RNA 3 directs the synthesis of the coat protein [47–57]. Based on RNA sequence data, it has been demonstrated that two regions of the protein encoded by BMV RNA 1 and AMV RNA 1 are strikingly similar; moreover, the central portions of the proteins encoded by BMV RNA 2 and AMV RNA 2 also contain considerable sequence homology, suggesting common functions for these two pairs of high-molecular-mass proteins [60,61].

4. STRATEGIES USED FOR THE EXPRESSION AND REGULATION OF VIRUS-CODED PROTEINS

Plant RNA viruses resort to several strategies to synthesize more than one polypeptide per cistron, and to regulate the synthesis of their genomic and/or subgenomic RNA-coded proteins. These strategies include: the existence of multipartite RNA genomes, the formation of (a) subgenomic RNA(s), readthrough, post-translational cleavage, the presence of multi-initiation sites on monocistronic mRNAs, premature termination, frameshift (see table 1) and differential translation efficiency. Only those strategies utilized during translation will be considered here.

4.1. *Initiation of translation*

As is the case in eukaryotic systems, in plant viral RNAs also, the first AUG triplet from the 5'-end generally serves for initiation of translation. However, in CPMV RNA 2, the first AUG triplet is silent, but both the second and the third AUGs from the 5'-terminus are efficiently used in phase for in vitro translation [12,21,45]. In at least one strain of AMV, the first AUG on RNA 1 is followed in phase by a UGA codon [9].

An interesting and exceptional situation is that of CarMV whose RNA might resemble a prokaryotic RNA insofar as all 3 cistrons of its genomic RNA seem to be translated in vitro probably by internal initiation [62]. This translation strategy needs to be confirmed.

4.2. *Elongation of translation*

Two types of regulation can occur during the elongation of peptide chains: premature termination or a shift in the reading frame. These modalities appear to take place during translation of AMV RNAs 1 and 3, respectively.

During translation of AMV RNA 1 in a reticulocyte lysate, in addition to the full-length translation product, two shorter proteins are synthesized by premature termination. The synthesis of the two low-molecular-mass proteins appears to be due to the lack of a glutamine-charged tRNA, since upon addition of either glutamine or of the corresponding tRNA an increase in the synthesis of the full-length translation product ensues [52]. Surprisingly, no particular feature in the sequence or structure of RNA 1 can be discerned in the region corresponding to the C-terminal portion of the low-molecular-mass proteins, except that a coat protein binding site is present about halfway down the RNA molecule [63].

In a wheat germ extract [50] and in vivo in AMV-infected tobacco leaves [55], AMV RNA 3 directs the synthesis of a 35-kDa polypeptide and of a higher-molecular-mass protein. Based on the sequence of AMV RNA 3, the synthesis of the high-molecular-mass protein can be explained by two +1 frameshifts or by readthrough of the UGA termination codon at the end of the 35-kDa protein gene followed by one −1 frameshift [55]. The higher-molecular-mass protein is recognized by the antibodies raised against the 35-kDa protein and against the virus. However, direct confirmation of the use of frameshift during translation will have to await sequencing of the high-molecular-mass protein.

4.3. *Termination of translation*

The 5′-proximal cistron of the genomic RNA of TMV [36] and of TYMV [64] is terminated by a UAG codon and that of TRV RNA 1 by a UGA codon (H. Beier, personal communication). The readthrough of these codons occurs to a certain extent in the presence of total (host) tRNAs and is increased by addition in vitro of the corresponding (host-coded) suppressor tRNAs. Readthrough of the TMV and TYMV UAG codons is also favored in vitro by the presence of polyamines such as spermine or spermidine; it has been suggested that this could occur via conformational changes in a tRNA [64]. A $tRNA^{Tyr}$ isolated from *Drosophila melanogaster* [65], from tobacco leaves [66] and from wheat germ or wheat leaves [67] devoid of modification in the first position of the anticodon has recently also been shown to suppress the UAG codon during translation of TMV RNA. In an in vitro system, readthrough of the UAG termination codon increases at low rather than at high TMV RNA concentration [20], suggesting that in vivo synthesis of the readthrough product could be favored early upon infection; this conclusion is strengthened by results obtained in vivo [68].

4.4. *Post-translational cleavage*

Upon in vitro and in vivo translation of the comovirus RNAs, high-molecular-mass proteins are synthesized which undergo post-translational cleavages to produce mature proteins. Two proteins deriving from the CPMV RNA 1-encoded 200-kDa polyprotein contain protease activity. The 32-kDa protein is involved in the cleavage of the primary translation products of RNA 2 [46]. The second protease (24 kDa) probably participates in the processing of the 200-kDa polyprotein itself [69]; this observation is strengthened by computer data [70]. Indeed, there exists considerable amino acid sequence homology between the C-terminal portion of the 24-kDa protein and the C-terminal region of P3-7c, the protease of poliovirus.

A similar situation probably exists among the nepoviruses [71] and the potyviruses [72–74]. Upon in vitro translation of their RNA, a polyprotein is synthesized from which functional proteins are derived by proteolytic cleavage. It is remarkable that picornaviruses (review, [75]) as well as como-, nepo- and potyviruses which all bear a VPg and a poly(A) stretch at their 5′- and 3′-terminus respectively, should have adpoted the same translational strategy.

In the case of TYMV RNA only two of the 3 high-molecular-mass proteins are cleaved in vitro [76]; the protease activity could be located in the C-terminal part of these proteins [77].

4.5. *Efficiency of translation*

In general, the coat protein mRNA is much more efficient in translation than are the genomic RNAs. This has been established by examining the in vitro translation of total (genomic and subgenomic) RNAs: at high RNA concentrations, translation of the coat protein mRNA is favored whereas that of the genomic RNA is reduced [51,78–80]. Coat protein is synthesized in vivo in much larger amounts than the genomic RNA-coded proteins and even in larger amounts than would be expected of the 5:1 ratio of subgenomic to genomic RNA usually encapsidated; consequently, the subgenomic coat protein mRNA is very efficiently translated. These RNAs all possess a 5′-terminal cap structure. They differ in their leader sequences, and apart from the fact that the length of this region in the coat protein mRNA is generally short, it is still unknown why certain mRNAs are more efficient templates than others (review, [5]).

There also exist certain subgenomic RNAs that are less efficient in translation than genomic or other subgenomic RNAs. As recently shown in TMV RNA, the subgenomic intermediate-length RNA 2 (I_2 RNA) is uncapped and is less efficient in translation than the capped genomic RNA [20].

5. POSSIBLE FUNCTIONS OF VIRUS-CODED PROTEINS

Several proteins encoded by the viral genome participate, at least in part, in RNA replication and encapsidation. The virus-coded proteins may be multifunctional and/or act in association with host proteins (reviews, [5–7]).

5.1. *Coat protein*

The most obvious function of the coat protein is to encapsidate the viral RNA and to protect it from nucleases. The coat protein–viral RNA interaction must be stable and highly specific to avoid inadvertent packaging of cellular RNAs.

Recently, it has been demonstrated that in TMV, a 26.5-kDa protein, previously referred to as the host-coded 'H' protein, shares sequences with the coat protein (17.5 kDa), and is also present in the virions to the extent of one molecule per virus particle [81,82]. The function of this protein remains unknown.

In ilarviruses, the coat protein also has a function in 'genome activation': two to six molecules of coat protein per viral RNA are required for infection ([33]; review, [7]). The coat proteins of different ilarviruses such as AMV and TSV are interchangeable although they possess no sequence similarity. Even though the sequences at the 3′-end of the TSV and AMV RNAs are different, they possess similar secondary structures. There exists a high-affinity site for the coat protein in the 3′-region of the genomic RNAs. It has been proposed that coat protein–RNA interaction could be involved in template recognition by the 'replicase'. Removal of the N-terminal region of the protein molecule [83] renders the coat protein inactive in this respect.

5.2. *'Replicase'*

At least two virus-coded proteins could be involved in TMV RNA replication [84,85], and one in TYMV RNA replication [29]. A more direct indication that virus-coded proteins must either directly or indirectly be involved in replication of the viral RNA comes from viruses with a bi- or tripartite genome in which the larger or the two largest genomic RNAs respectively, can replicate independently [21,49].

In CPMV, the RNA 1-encoded 60-kDa protein present in the membranous fractions is the direct precursor of the VPg of about 4 kDa. VPg could be involved in initiation of replication by acting as primer for the initiation of RNA synthesis, or it could discriminate between those RNA molecules to be encapsidated and those to serve as mRNA for viral protein synthesis [21]. The 110-kDa protein resulting from post-translational cleavage of the 200-kDa protein constitutes the core of the RNA replication complex [86]. In support of a role of the 110-kDa protein in RNA replication is the observation that there exists significant sequence homology between the 87-kDa domain of the 110-kDa protein of CPMV and the polymerase from two picornaviruses, foot-and-mouth disease virus and poliovirus [70].

In bromo- and cucumoviruses such as BMV [28] and CMV [27], the replicase has been purified extensively. Interestingly, there exists a 110-kDa protein in the replicating system of the BMV RNA whose peptide map is virtually identical to that of the 110-kDa protein encoded in vitro by BMV RNA 1 [28]. This demonstrates clearly that at least

one virus-coded protein is involved in viral RNA replication. It has been demonstrated that the N-terminal and the C-terminal regions of the 110-kDa protein encoded by BMV RNA 1 bear considerable sequence homology with the corresponding regions in the 115-kDa protein of AMV RNA 1 and the 126-kDa protein of TMV RNA, supporting the notion that all 3 proteins may operate equivalent functions in these viruses [60,61]. BMV RNA 2 and AMV RNA 2 are also required for viral replication; in support of this is the observation that the central portion of their proteins is strikingly similar. Since it is also homologous to the C-terminal portion of the TMV readthrough protein of 183-kDa, these 3 proteins may play a similar role [60,61].

In AMV the translation product(s) of RNA 3 are involved in the regulation of the synthesis of the + and − strand RNA populations [87].

Nothing is known concerning the nature of the host proteins involved in replication of the viral RNAs nor what determines host specificity. Generally, in infected protoplasts, the host-coded proteins are synthesized during the first 8 h of infection [88]. Inhibition of transcription during this period results in inhibition of viral RNA replication although the viral RNAs which served for infection are translated [89].

5.3. *The 30–35-kDa protein*

A 30–35-kDa protein is synthesized in tobamo-, tobra-, bromo-, cucumo- and ilarviruses; its function is still a matter of speculation. It has been suggested that the TMV RNA-coded 30-kDa protein could be involved in cell-to-cell movement of the virus [90]. Nucleotide sequence analysis of the 30-kDa protein gene has revealed that the wild type and the cell-to-cell movement-deficient strain (Ls1) of TMV differ by only one amino acid in the 30-kDa protein [91]. A point mutation in the open reading frame of the TMV 30-kDa protein gene results in temperature-sensitive assembly and local lesion spreading of the mutant Ni 2519 [92,93]. In neither case are mutations elsewhere in the genome excluded. The maximum rate of synthesis of the 30-kDa protein in infected plants is reached earlier than that of other virus-coded proteins [20,40], suggesting that the former protein could also operate prior to replication of the viral RNA; one could speculate that early in infection the function of the 30-kDa protein is to disaggregate the polysomes containing the genomic RNA so as to allow the synthesis of − strand RNA of the length of the genomic RNA [94]. Alternatively, the 30-kDa protein might be involved in the internal initiation of transcription of subgenomic mRNA.

In comoviruses, the independent replication of RNA 1 is restricted to the initially infected cells [95]; either a protein encoded by RNA 2, or synthesis of the coat protein and packaging of the viral RNA might be required for cell-to-cell movement of the virus.

Interestingly, in TRV, RNA 1 alone can replicate and migrate from cell to cell, and the symptoms of infection are even more severe than those observed upon infection with RNAs 1 and 2 together [96].

The short and long distance movements of the virus, and the changes in host phenotype upon infection have recently been reviewed [7].

5.4. *The helper component*

Aphid transmission of potyviruses is dependent on the presence of a virus-coded helper component (HC) present in potyvirus-infected cells. Anti-HC antibodies not only precipitate the in vitro-synthesized HC protein (~56 kDa) but also a protein of ~82 kDa. This implies that proteolytic or other post-translation modifications are required to produce active HC [97].

6. DISCUSSION

6.1. *Discrimination between viral and cellular RNAs*

To discriminate between viral and cellular mRNAs during replication, translation and encapsidation of viral particles, specific signals must exist on the viral RNAs.

The last ~150 nucleotides at the 3′-end of the RNAs of multipartite genome viruses are highly conserved. In ilarviruses the coat protein binding site is located near the 3′-end of the genome and binding of the protein to the RNA is thought to trigger viral RNA replication. It seems likely that this region of the viral RNAs constitutes the recognition signal for the synthesis of − RNA strands and/or for encapsidation.

Knowledge of the structure at the 5′-end of viral RNAs will provide insight on the features required for the synthesis of + strand RNAs and those required for efficient translation. Finally, contrary

to cellular mRNAs which are capped and polyadenylated, only very few viral RNAs possess both these structures.

6.2. *Evaluation of the different strategies used by plant RNA viruses of + polarity*

6.2.1. Functionally monocistronic: use of subgenomic RNAs

Between 3 and 10 proteins are encoded by viruses of this category; the genomic RNAs are often polygenic but are functionally monocistronic, only the 5′-terminal cistron being expressed. In these viruses (see table 1) subgenomic RNAs are synthesized during infection for the expression of internal genes, and these may be more or less efficient in translation than the genomic counterparts. Generally, the coat protein cistron is 3′-terminal and the coat protein is synthesized on a subgenomic RNA. Apart from I_2 RNA (in the case of TMV) and the coat protein mRNA, the function of most other subgenomic RNAs is unknown and it is still unclear whether these RNAs are translated in vivo. The exact mechanism of the genesis of the subgenomic RNAs is not yet well defined. This strategy is highly economical for the virus since it permits the synthesis of different virus-coded proteins in different amounts. A striking analogy exists [60,61] between the amino acid sequences contained in certain non-structural proteins of viruses using this strategy (AMV, BMV and TMV) and of two alphaviruses (Sindbis virus and Middelburg virus).

6.2.2. Functionally monocistronic: post-translational cleavage of polyproteins

The genomic RNAs of potyviruses are probably monocistronic, although evidence for the presence and possible functional significance of subgenomic RNAs has also been provided [98].

The genomic RNAs of como- and nepoviruses are monocistronic. The coat protein(s) is (are) encoded by one genomic RNA and the replicase by the other. Upon translation of these RNAs, polyproteins are synthesized that are post-translationally cleaved to liberate mature, functional proteins. Unless differential stability exists between the different mature proteins, one must assume that this strategy leads to the appearance in equimolar amounts of all the proteins present on the polyprotein, a situation that may not necessarily be required during infection. It is interesting to note that homologous sequences are encountered in the non-structural proteins of the viruses using this strategy and in picornaviruses [70].

6.2.3. Polycistronic: internal initiation

CarMV has been reported to be polycistronic and to resort to internal initiation. Since several prokaryotic polycistronic mRNAs appear to be correctly translated in eukaryotic systems, it is not surprising that this strategy could also be used by plant viral RNAs.

6.2.4. One gene – multiple proteins

Several stretegies are used during translation of plant viral RNAs at the level of initiation, elongation and termination, leading to the synthesis of proteins that have peptides in common. The ratio of these proteins varies depending on the strategy used. In the case of TYMV RNA, these proteins also behave differently during post-translational cleavage [76]. Such proteins may fulfill different functions during virus propagation.

6.2.5. Multipartite genome

The genome parts of multicomponent viruses are separately encapsidated. The ratio of the different genomic RNAs varies between 1 and 5. For infection of a cell, more than one particle is thus required, and this reduces the efficiency of mechanical transmission. However, in nature these viruses are often transmitted by seed or by invertebrate vectors allowing large numbers of virus particles to enter per cell.

Since RNA-dependent RNA polymerases lack error-correcting mechanisms, the estimated error frequency for these enzymes lies between 10^{-3} and 10^{-4}. Clearly, for a fixed error level, the longer the RNA, the greater the possible loss of information. This problem may be resolved by the presence of multipartite genomes [99]. Since during encapsidation different genomic RNAs are withdrawn randomly from the intracellular pool of RNAs, a number of combinations exists and this strategy may ensure that the optimally adopted combination of composite viruses arises at each round of replication.

With multipartite genome viruses, new pseudo-recombinants can be obtained in vitro and in vivo. This increase in variability could represent an im-

portant evolutionary advantage, especially since recombination at the RNA level takes place only at low frequency [100]. The multipartite genome strategy is also well adapted to the translational machinery of the host cell which preferentially initiates close to the 5'-terminus of mRNAs. Such a genome offers the possibility of regulating separately, in amount and time, the expression of each gene.

6.3. *Regulation of synthesis of viral proteins and RNAs*

One obvious function required by the virus during infection is replication of its genome. The amount of the different genomic/subgenomic RNAs, as well as of their translation products, is regulated during infection. The relative efficiencies of the replication signals for each genomic/subgenomic RNA may determine the amount of the different RNAs produced; likewise the 5'-terminal structure and the efficiency of the leader sequence for translation may regulate the level of synthesis of the different proteins. Various strategies may be used during translation producing different amounts of normal, readthrough and/or premature termination products.

The time after infection at which different viral RNAs are synthesized may depend on the need for a specific protein involved in replication, on the accessibility of the RNA species for replication, or in the case of subgenomic RNA species also on the strategy used for their replication. As a result, synthesis of the viral proteins may be triggered at different times during infection, depending on the availability of the corresponding RNAs and on the relative efficiencies of these RNAs in translation.

6.4. *Conclusions and perspectives*

The study of plant virus RNAs has already contributed greatly to our understanding of the different steps involved in translation as well as of the RNA features required for efficient translation.

An RNA-dependent RNA polymerase is involved in viral RNA replication and is clearly different [101,102] from the one detected in healthy plants. This latter activity is increased upon rubbing of the leaves in the absence of any virus, and also increases in leaves other than those thus treated [103]. One can therefore wonder whether plants also make use of an RNA-dependent RNA-synthesizing machinery. A few viral RNAs are also able to migrate from one cell to another [96]. It will be interesting to determine whether host mRNAs are also able to migrate from cell to cell.

The detailed knowledge of the mechanism of viral genomic and subgenomic RNA replication, translation and encapsidation as well as of the functions of the different virus- and host-coded proteins involved might allow insertion and expression of any desired gene into plant virus genomes [104]. A better understanding of virus multiplication and propagation should permit detection, control and/or prevention of plant diseases, and also development of new chromosomal vectors for plant genetic engineering.

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NOTE ADDED IN PROOF

The complete nucleotide sequence of the CMV genomic RNA 2 has been reported [105].

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