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Alternative Translation Strategies in Plant Viruses

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ABSTRACT

Plant viruses have evolved several unconventional translational strategies that allow efficient expression of more than one protein from their compact, multifunctional RNAs, as well as regulation of polycistronic translation in the infected plant cell. Here, we review recent advances in our understanding of these unconventional mechanisms, which include leaky scanning, ribosome shunting, internal initiation, reinitiation, stop codon suppression and frameshifting, and compare their characteristics with related phenomena in other systems.

Keywords: cap-dependent translation initiation, closed-loop RNA model, frameshifting, internal initiation, read-through, reinitiation of translation, ribosomal shunt, termination of translation, translation initiation factors (eIFs)
Abbreviations: eEF, eukaryotic translation elongation factor; **eIF**, eukaryotic translation initiation factor; **eRF**, eukaryotic release factor; **IRES**, internal ribosome entry site; **ORF**, open reading frame; **PABP**, poly(A)-binding protein; **TLS**, tRNA-like structure

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INTRODUCTION

General translation initiation pathway in eukaryotes

Before discussing the non-canonical pathways of protein biosynthesis used by some plant viruses, we will briefly review the canonical model of translation of most cellular RNAs. Most eukaryotic cellular mRNAs have a m^7GpppN cap structure (where N is any nucleotide) at the 5′-end, a not-very-long unstructured sequence preceding the translation start codon (5′-leader), and a poly(A) tail at the 3′-terminus. These structural features are required for recruitment of the protein synthesis machinery during general transla-

Received: 15 March, 2007. Accepted: 2 May, 2007.

Special Feature

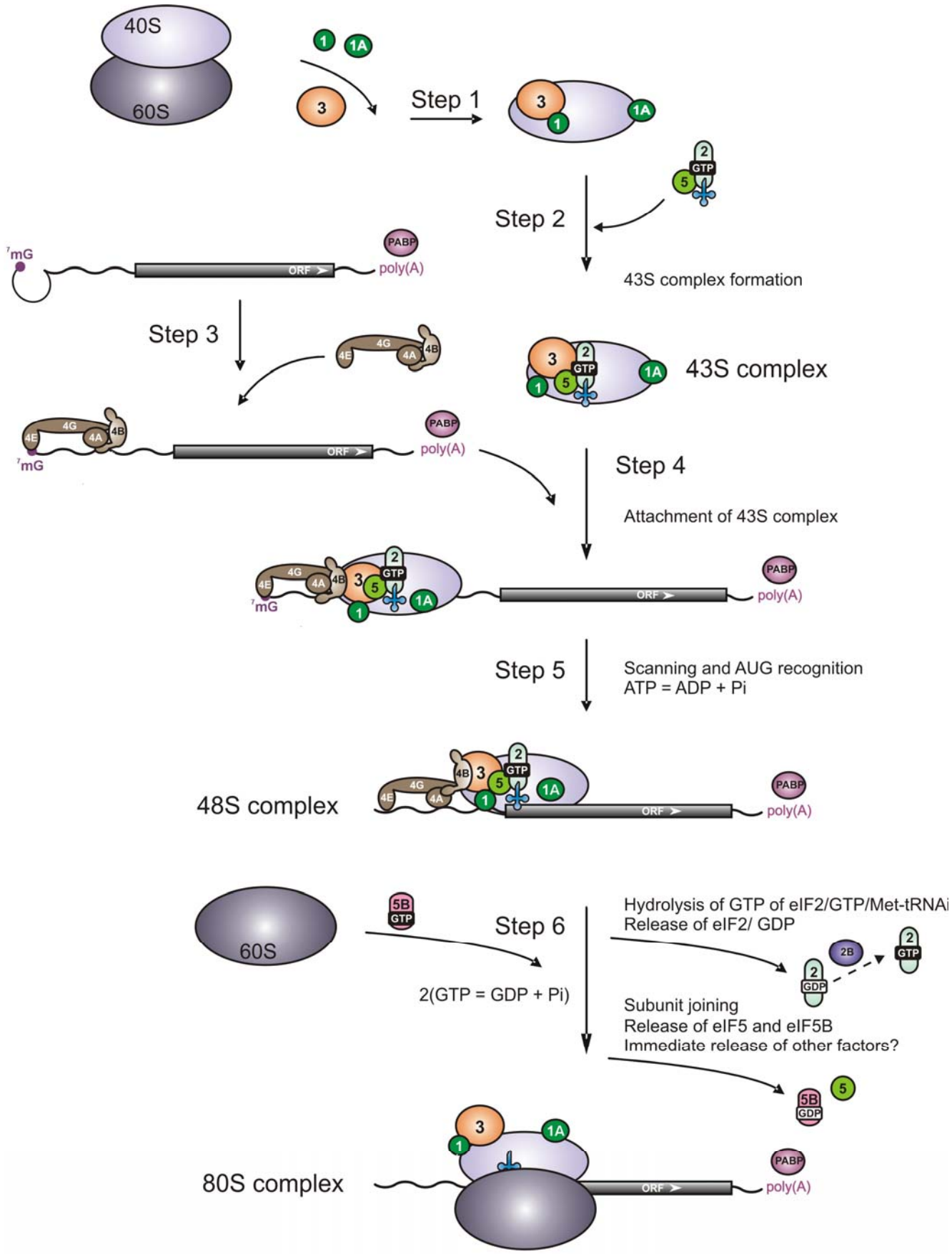

Fig. 1 The eukaryotic cap-dependent translation initiation pathway. See text for detailed description of Steps 1-6.

tion initiation *via* the cap-dependent pathway, where the translation start site is chosen by strictly linear scanning of the 40S ribosomal subunit along the 5′-leader starting from the capped 5′-end. This cap- and linear ribosome scanning-dependent mode of initiation is the main translation initiation pathway in eukaryotes, involving numerous initiation factors (eIFs) and the interplay of a succession of protein-protein and protein-RNA complexes (Hershey and Merrick 2000). The current six-step model of this pathway is presented in **Fig. 1**:

Step 1. *Separation of 80S ribosomes into 40S and 60S ribosomal subunits*. The pool of small ribosomal subunits is

then activated by binding of eIF1A, eIF1 and the largest eIF, eIF3 (Peterson *et al*. 1979; Phan *et al*. 1998; Chaudhuri *et al*. 1999; Majumdar *et al*. 2003). Importantly, eIF3 can support dissociation of 80S in the presence of mRNA or the ternary complex (TC, Met-tRNAi^{Met}/eIF2/GTP) and eIF1 in mammals (Unbehaun *et al*. 2004; Kolupaeva *et al*. 2005).

Step 2. *Binding of TC to 40S subunit*. The 40S ribosomal subunit, together with eIF3, eIF1, eIF1A, eIF5 and the TC, forms a 43S pre-initiation complex. Although eIF3, eIF1 and eIF1A can directly bind 40S, thereby stimulating the formation of the 43S complex, in yeast TC is associated with eIF3, eIF1, and eIF5 in a pre-existing multifactor complex that can interact with the 40S (Asano *et al*. 2000). eIF2 interacts with eIF3 directly via the eIF3a subunit and indirectly via eIF5 bridging the two factors.

Step 3. *Priming of the mRNA 5′-end cap structure by eIF4F, eIF4A and eIF4B*. eIF4F is comprised of the cap-binding factor eIF4E, the ATP-dependent RNA helicase eIF4A and a scaffold protein eIF4G, which contains binding domains for eIF4E, eIF4A and poly(A)-binding protein (PABP; Sachs 2000; Gross *et al*. 2003). eIF4A, the DEAD box helicase, participates in ATP-dependent unwinding of the mRNA secondary structure; its RNA melting activity is stimulated by eIF4G and eIF4B (Rogers *et al*. 2002). eIF4G can recruit other factors, including eIF3 and PABP through direct protein–protein interactions (for a review of these factors in plants, see Gallie 2002). It is thought that eIF4B promotes the RNA-dependent ATP hydrolysis activity and ATP-dependent RNA helicase activity of eIF4A in mammals (Jaramillo *et al*. 1990) and plants (Metz *et al*. 1999) and mediates binding of mRNA to ribosomes (Trachsel *et al*. 1977; Benne and Hershey 1978; Morino *et al*. 2000). eIF4B can physically interact with eIF3 in yeast and plants (via eIF3g; Vornlocher *et al*. 1999; Park *et al*. 2004) and in mammals (via eIF3a; Méthot *et al*. 1996).

PABP binds to the poly(A) tail present at the 3′-end of most cellular mRNAs, and the interaction between PABP and eIF4G brings both termini of an mRNA into close spatial proximity, effectively resulting in mRNA circularization (Wells *et al*. 1998a; Sachs 2000; for review, see Gallie 2002).

Step 4. *Binding of mRNA to the 43S complex*. eIF4G and, apparently, eIF4B potentially serve as organizing centres for loading of the 43S preinitiation complex onto the 5′-end of the mRNA, mainly via interactions between PABP, eIF4G, eIF4B, eIF3, eIF2 and mRNA (Gingras *et al*. 1999; Sachs 2000; Jivotovskaya *et al*. 2006).

Step 5. *Scanning of the mRNA leader and start codon recognition*. The 43S complex loaded at the capped 5′-end of the mRNA scans the downstream leader sequence until it encounters the first start codon in an optimal initiation context [(**A/G**)CCAUG(**G**); Kozak 1987a, 1991]. The scanning process of the 43S preinitiation complex requires ATP hydrolysis and is dependent on two eIFs, eIF1 and eIF1A, which are required for the ribosomal complex to locate the initiation codon (Pestova *et al*. 1998). Start site selection then requires cooperation between the scanning ribosome and eIF1, eIF2 and eIF5, which form the 48S preinitiation complex at the optimal start codon (for a review, see Donahue 2000; Pestova *et al*. 2002). As a result, Met-tRNAi^{Met} will be located at the ribosomal P-site (peptidyl-tRNA binding site on the ribosome), where the anticodon of Met-tRNAi^{Met} and AUG codon are base paired.

Step 6. *60S subunit joining*. As soon as the 48S complex is formed, eIF5 – a GTPase-activating protein – stimulates hydrolysis of eIF2-bound GTP, and eIF2-bound GDP is released from the 48S preinitiation complex (Merrick 1992). Joining of the 60S subunit also requires an additional factor, termed eIF5B, which has a ribosome-dependent GTPase activity (Pestova *et al*. 2000). eIF5B catalyses ribosomal subunit joining, and all other translation initiation factors are supposedly released (Unbehaun *et al*. 2004). The resulting 80S complex is ready to enter the elongation phase of translation. Recycling of eIF2-bound GDP to eIF2-bound GTP is stimulated by eIF2B.

The translational machinery of plants, despite having some unique plant-specific factors, closely resembles that of mammals. Although most eIFs are generally similar in all eukaryotes, there are a few striking differences between mammalian and plant translation initiation factors (Browning 2004). For example, higher plants possess an isozyme form of eIF4F, termed eIF(iso)4F, containing eIF(iso)4E and eIF(iso)4G, which shows preferences for initiation at unstructured non-coding regions (Gallie and Browning 2001). In the case of eIF4B, there is essentially no conservation at the primary amino acid sequence level between yeast, mammals and plants (Metz *et al*. 1999). The plant eIF4B contains three RNA binding domains, two binding domains for PABP and eIF4A, and one binding site for eIF(iso)4G (the plant isoform of eIF4G) (Cheng and Gallie 2006). Some conservation between plant and mammalian factors, in regions required for the recruitment of eIF4A and PABP, have, however, been suggested (Cheng and Gallie 2006).

Translation elongation

The working elongation cycle of the eukaryotic ribosome is basically similar to that of prokaryotes and consists of three main steps: codon-dependent binding of aminoacyl-tRNA (step 1), transpeptidation (step 2), and translocation (step 3; for a detailed description, see Merrick and Nyborg 2000). The binding sites of aminoacyl-tRNA and peptidyl-tRNA on the ribosome have been designated as the A and P sites, respectively.

Step 1. *Binding of the aminoacyl-tRNA to the A-site*. At this point the peptidyl-tRNA occupies the P site. The aminoacyl-tRNA, complexed with eEF1 and GTP, enters the ribosome and binds to the mRNA codon located in the A-site of the 80S ribosome. This binding is accompanied by the hydrolysis of a GTP molecule and the release of the eEF1/GDP complex. eEF1 consists of the eEF1A subunit, which binds GTP and elongator tRNA, and eIF1B, a three-subunit complex that is a guanine nucleotide exchange factor for eEF1A. The eEF1 holofactor containing all four subunits is known as eEF1H.

Step 2. *Transpeptidation* is catalyzed by the ribosome itself and occurs between the aminoacyl-tRNA in the A-site and the peptidyl-tRNA in the P-site, with the peptide C-terminus being transferred to the aminoacyl-tRNA. As a result, the elongated peptidyl-tRNA now occupies the A site while the deacylated tRNA formed in the reaction is relocated to the P site.

Step 3. *Translocation*. The ribosome interacts with eEF2, a single subunit protein, and GTP, and this catalyzes the displacement of the peptidyl-tRNA (its tRNA residue) along with the template codon from the A site to the P site, as well as the release of the deacylated tRNA from the P site. During these events, GTP undergoes hydrolysis and eEF2/GDP is released from the ribosome. At the end of each cycle the peptidyl-tRNA is located in the P site while the next template codon is located in the A site; thus the A site is ready to accept the next aminoacyl-tRNA molecule.

Translation of the mRNA and corresponding polypeptide elongation on the ribosome are achieved by repetition of this cycle.

Translation termination

Eukaryotic translation termination is triggered by peptide release factors eRF1 and eRF3. eRF1 recognizes all three termination codons, UAA, UAG, and UGA, at the ribosomal A-site and induces hydrolysis of peptidyl tRNA at the P site (Konecki *et al*. 1977; Frolova *et al*. 1994). As a result, the polypeptide is released from the ribosome. The function of the second termination factor – eRF3 – is not well understood (see Kisselev and Buckingham 2000), although it is known to interact with GTP and show GTPase activity in the presence of ribosomes. There is evidence that eRF3 together with GTP can form a complex with eRF1. Thus, it is the complex eRF1/eRF3/GTP that may be the functional

unit required for termination on the eukaryotic ribosome in a GTP-dependent manner. Termination of translation is described in detail in Welch *et al*. (2000).

MISSING CANONICAL RNA TERMINI

Studies of plant viruses have revealed a variety of *cis*-acting elements that influence or regulate the expression of specific mRNA via alternative initiation mechanisms. These mechanisms usually involve skipping one or more steps of the general cap-dependent translation initiation pathway (**Fig. 1**), and can proceed without some of the standard eIFs. It is thought that viruses have developed such alternative mechanisms to ensure that viral mRNAs are preferentially translated in a host cell under conditions of viral infection. Investigations of these abnormal or deviant initiation mechanisms have greatly increased our understanding of the "standard" translation pathway.

The cap structure and poly(A) tail are important determinants of the cap-dependent translation initiation pathway. Circularization of mRNA in plants is mediated by a PABP bound to the poly(A) tail and eIF4F or eIF(iso)4F bound to the cap structure (Gallie 2002). Studies in wheat germ extract indicate that these proteins interact with the termini of RNA synergistically, mutually increasing their RNA-binding affinities (Le *et al*. 1997; Wei *et al*. 1998). PABP also increases the ATPase and RNA helicase activities of translation initiation factors eIF4A, eIF4B, and eIF-iso4F (Bi and Goss 2000). It is proposed that mRNA circularization increases translational initiation efficiency by promoting recycling of terminating ribosomes on the same RNA (Wells *et al*. 1998a). In yeast during mRNA turnover PABP may protect the 5′-cap from attack of Dcp1p, the yeast decapping enzyme (Beelman *et al*. 1996).

A relatively small proportion of positive-strand RNA viruses contain both a cap and a poly(A) tail. Several groups of plant viruses contain RNAs that are neither capped nor polyadenylated. However, circularization of these viral RNAs is still possible via alternative RNA elements or via viral/ host proteins substituting for molecular bridging contacts (see **Fig. 2**, and two excellent, recently published reviews on positive-strand plant RNA viruses: Miller and White 2006; Dreher and Miller 2006).

Cap-independent internal initiation

Some (+) ssRNA plant viruses, including those of the large families *Potyviridae* and *Comoviridae*, have replaced the cap by a small protein, VPg (Viral Protein genome-linked), which is covalently attached to the first nucleotide of the mRNA. Potyviruses and comoviruses closely resemble animal picornaviruses and caliciviruses in genome structure and in having a VPg. However, all these viruses possess a conventional poly(A) tail. Lacking a cap, they seem to rely on an internal initiation pathway of translation. The internal ribosome entry site (IRES) is an RNA sequence that promotes direct binding of the 40S ribosomal subunit to internal regions of mRNA, usually upstream of the major ORF start site, thus skipping the requirement for the cap-binding protein, eIF4E (Hellen and Sarnow 2001).

The IRESes of animal picornaviruses were the first to be discovered and remain the most extensively studied RNA elements that drive internal initiation. The RNA genomes of picornaviruses contain a very long structured leader with an IRES that promotes entry of the 40S ribosomal subunit near the AUG start codon for the major polyprotein (for a review, see Jackson 2005). Direct binding of eIF4G to the encephalomyocarditis virus (EMCV) IRES is required for 40S ribosomal subunit entry via IRES/eIF4G/eIF3/40S interactions (Pestova *et al*. 1996a, 1996b).

A similar mechanism appears to operate in plants for at least two representatives of picorna-like potyviruses: tobacco etch virus (TEV, Carrington and Freed 1990) and turnip mosaic virus (TuMV, Basso *et al*. 1994). The 5′-UTRs of TuMV and TEV differ from those of animal picornaviruses by their smaller length and less complex secondary structure. Zeenko and Gallie (2005) demonstrated that a pseudoknot element (PK1) of the TEV leader is a core structure of the IRES and is sufficient to promote cap-independent translation. However, under these conditions translation still required eIF4G (Gallie 2001). Binding of the 40S ribosome to the IRES is apparently mediated by eIF4F and eIFiso4F, since direct binding of eIF4G and eIFiso4G, as well as their complexes (eIF4F and eIFiso4F), to the TEV 143-nt 5′-leader and PK1 was recently demonstrated, whereby eIF4G (or eIF4F) possesses stronger affinity to the TEV leader than does eIFiso4G (Ray *et al*. 2006). The IRES preferentially recruits eIFiso4F to form the 48S preinitiation complex.

At the same time, it is puzzling that the 5′-end of TEV genomic RNA can recruit eIFiso4E via VPg protein and that this interaction is strong enough to compete with cap binding in *Arabidopsis* (Miyoshi *et al*. 2006) despite the fact that VPg and cap bind to different sites of eIF4E (Léonard *et al*. 2000). Corresponding interactions between eIF4G, eIF4E and VPg have recently been demonstrated for lettuce mosaic virus (LMV; Michon *et al*. 2006) and TuMV (Miyoshi *et al*. 2006). Although the role of eIF4G/eIF4E/VPg complexes in regulation of translation initiation is not yet clear, their participation in IRES-mediated translation initiation has been proposed for TuMV (Khan *et al*. 2006). Indeed, in mammalian positive-stranded RNA caliciviruses, translation initiation is strictly dependent on an interaction between VPg and eIF4E, where VPg seems to substitute for the cap (Goodfellow *et al*. 2005). Whether VPg can substitute for cap in plants still needs to be clarified. The fact that PABP was found to be required for full IRES function *in vitro* (Gallie 2001) suggested that TEV genomic RNA can be circularized via eIF4G, eIF4iso4G and PABP. The RNA polymerase (RdRp) of Zucchini yellow mosaic virus (ZYMV; Wang *et al* 2000) and VPg-Pro of TuMV (Léonard *et al*. 2004) interact with PABP and can achieve circularization between 5′- and 3′- termini to facilitate replication. Although shutdown of the host translation machinery by plant viruses was not demonstrated, increasing concentrations of RdRp might interfere with the translation process.

VPg can recruit eIF4E (or eIFiso4E) for the benefit of the virus in different host plants: eIFiso4E recruitment by TEV VPg is required to infect *Arabidopsis* plants, while eIF4E recruitment is required for infection of solanaceous species (for a review, see Robaglia and Caranta 2006). The respective resistance genes of naturally occurring potyvirus-resistant plant varieties have been shown to encode defective eIF(iso)4E or eIF4E (Lellis *et al*. 2002; Ruffel *et al*. 2002). Interestingly, involvement of a VPg/eIFiso4E complex in cell-to-cell movement or viral transport has also been suggested (Dunoyer *et al*. 2004; Gao *et al*. 2004).

Putative, unstructured IRESes of tobamoviruses

IRESes identified in plant tobamoviruses are unusual in that they are very short and unstructured. The RNA genome of tobamoviruses, of which tobacco mosaic virus (TMV) strain U1 is the type member, is a polycistronic capped RNA containing four large ORFs. The first ORF and its read-through ORF, which encode the two components of viral replicase, are translated directly from the genomic RNA. The virus produces two subgenomic (sg)RNAs: the dicistronic I_2 RNA is used for translation of movement protein (MP), and a monocistronic RNA codes for CP (Palukaitis and Zaitlin 1986). Another member of the tobamoviruses, a crucifer-infecting virus (crTMV; Dorokhov *et al*. 1994), has a similar organization but its MP and CP ORFs overlap. crTMV harbours two unusual IRESes, the 148 nt $IRES_{CP}$ and the 75 nt $IRES_{MP}$, which are thought to drive translation of the CP (Ivanov *et al*. 1997) and MP (Skulachev *et al*. 1999) ORFs, respectively. Both contain a purine-rich tract upstream of the AUG start codon. Interestingly, $IRES_{CP}$ can function in plants, yeast and HeLa cells (Dorokhov *et al*. 2002). However, the functional significance of these IRESes is obscure since both MP and CP can

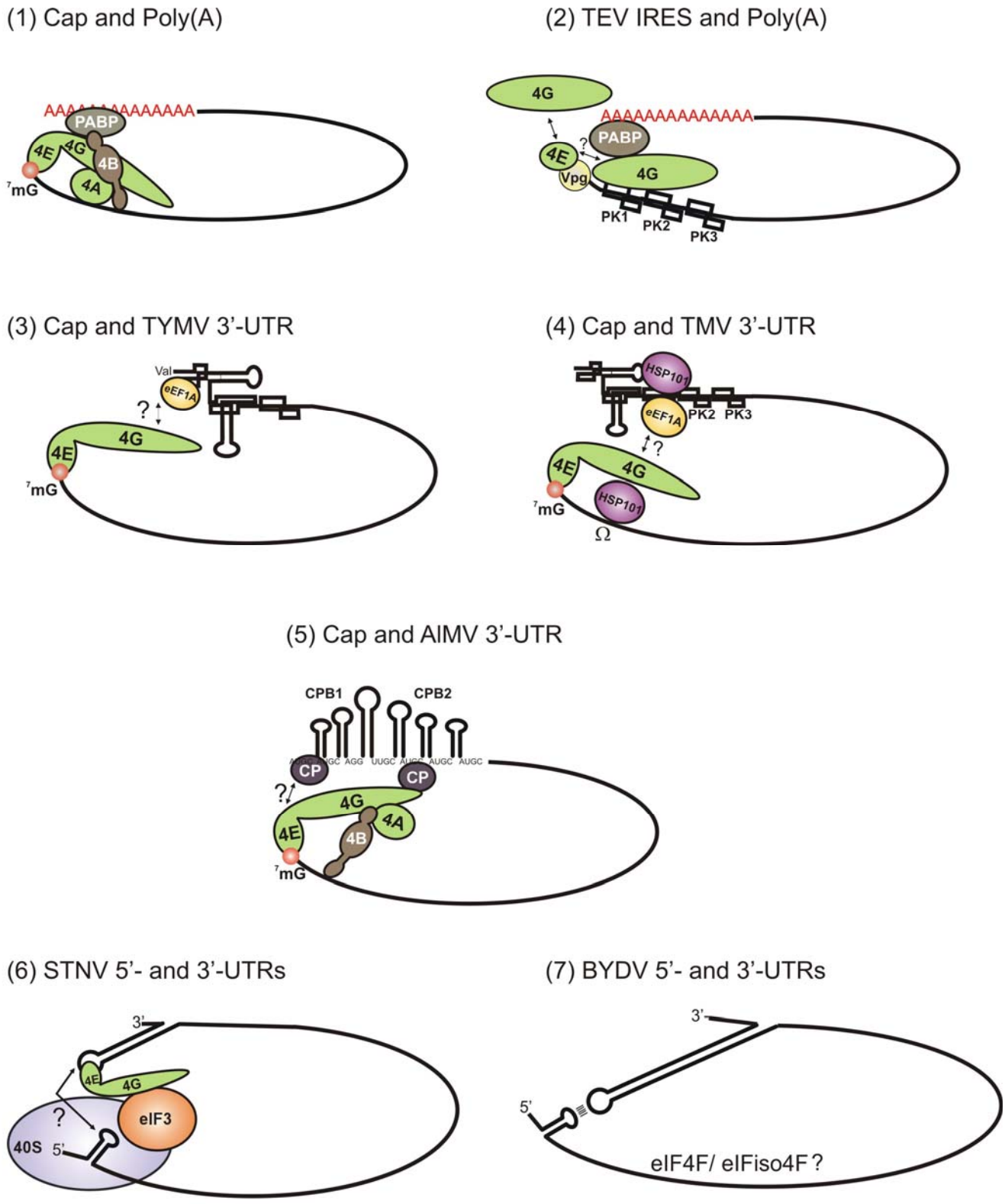

Fig. 2 5′-3′ closed-loop model in plant viruses. Proposed interactions between mRNA termini implicated in enhancement of translation in plant RNA (+) viruses. (**1**) Protein-protein interactions for the conventional 5′-3′ interactions in plants between eIF4E/eIF(iso)4E-eIF4G/eIF(iso)4G, eIF4G/eIF(iso)4G-PABP and PABP-eIF4B; (**2**) TEV RNA has a VPg protein that replaces the cap structure. eIF4G/eIF(iso)4G binds simultaneously to the PK1 pseudoknot of the TEV IRES element and poly(A)-bound PABP. The role of eIF4E/eIF(iso)4E attached to VPg in translation is unclear. TMV (**3**), TYMV (**4**) and AMV RNAs (**5**) represent capped viral RNAs without a poly(A) tail. For TYMV RNA, the interaction of eEF1A with the valine aminoacylated 3′-terminal tRNA-like structure (TLS) may be involved in formation of a closed-loop structure. This is not a case for TMV RNA, where HSP101 and/or eEF1A bound to the upstream pseudoknot (PK1) may help to circularize TMV RNA. The role of HSP101, interaction of which with the **Ω** sequence is required for the **Ω**-enhancing effect in translation, in closed-loop formation is not clear. In AMV, simultaneous binding of coat protein (CP) molecules to CPB2 and eIF4G/eIF(iso)4G bound to the cap structure via eIF4E/eIF(iso)4E is required for efficient translation and is involved in closed-loop formation. (**6**) RNA-RNA interactions in STNV RNA are mediated by eIF4E/eIF(iso)4E-eIF4G/eIF(iso)4G, where eIF4E/eIF(iso)4E binds to TED within the 3′-UTR. The 5′-UTR stem loop involves direct base-pairing to 18S ribosomal RNA; (**7**) Direct RNA-RNA interactions that would circularize BYDV genomic RNA. *Cis*-acting RNA elements such as the cap structure (7mG) and poly(A) tail (AAAAAAAAAAAA) are indicated. Question marks indicate unknown details.

be also translated from sgRNAs by a ribosome scanning mechanism (Ivanov *et al*. 1997; Skulachev *et al*. 1999). Although the $IRES_{CP}$ region is highly conserved between crucifer-infecting tobamoviruses, it differs from other tobamoviruses (Ivanov *et al*. 1997). Another very short purine-rich IRES element was identified upstream of the MP ORF of TMV U1 (Skulachev *et al*. 1999), which could be preferentially used for TMV U1 I_2 RNA due to the possible lack of a 5′ cap in this RNA. The short purine-rich stretch was suggested as an IRES module that can mediate internal initiation in plants, mammals and yeast (Dorokhov *et al*. 2002).

Poly(A)-independent initiation

The positive-strand RNA genomes of *Bromoviridae* and *Tobamoviridae* have a 5′-cap and a non-polyadenylated 3′-end. These viruses use a strategy similar to that of cap- and poly(A)-containing RNA viruses but their 3′-UTRs can functionally substitute for a poly(A)-tail and work as translational enhancers in concert with the cap structure to ensure preferential translation of the viral genome. These 3′-UTRs contain a tRNA-like structure (TLS), either combined with a series of stem-loops [alfalfa mosaic virus (AMV)], or fused to a series of pseudoknots [(brome mosaic virus (BMV), turnip yellow mosaic virus (TYMV) and TMV]. These elements provide functions similar to that of the poly(A) tail in translation initiation by using different protein partners (AMV – Krab *et al*. 2005; BMV – Barends *et al*. 2004, TMV – Gallie 1991; TYMV – Matsuda and Dreher 2004).

The TMV and TYMV 3′-UTRs enhance translation synergistically together with a 5′-cap structure (Gallie and Walbot 1990; Zeyenko *et al*. 1994; Matsuda and Dreher 2004), which suggests a 5′–3′ molecular bridge between their RNA termini. All genomic and subgenomic TMV RNAs are co-linear and contain the same 3′-UTR, composed of a three RNA pseudoknot domain followed by a TLS, which can be specifically aminoacylated and can interact with eEF1A/GTP (Mans *et al*. 1991). Using a protoplast system, Leathers *et al*. (1993) revealed the critical role of the region upstream of the tRNA-like structure pseudoknot domain in enhancement of TMV RNA translation, where it acts synergistically with the 5'-cap. The heat shock protein, HSP101, binds specifically to this upstream pseudoknot domain and might mediate translation enhancement (Tanguay and Gallie 1996). The same pseudoknot cross-links specifically to eEF1A in an aminoacylation-independent manner (Zeenko *et al*. 2002). However, additional experiments are required to confirm the possible involvement of eEF1A or HSP101 in the synergy between TMV 5′- and 3′-UTRs.

Although the 3′-UTR of TYMV, which comprises a TLS and a single upstream pseudoknot, acts synergistically with 5′-cap to enhance translation in a manner similar to that found in TMV, the TLS structure itself is required for translational enhancement (Matsuda and Dreher 2004). Moreover, only aminoacylation-competent TLS, which is able to tightly bind eEF1A-GTP, is active in translation activation (Dreher *et al*. 1999), while the pseudoknot seems to provide optimal spacing to present the TLS for aminoacylation (Matsuda and Dreher 2004). In addition, eEF1A binding to the valylated TLS represses TYMV RNA replication by RNA-dependent RNA polymerase (Matsuda *et al*. 2004b). Thus, although the tRNA-like mimicry of TYMV RNA is clearly required for synergy in translational enhancement, the requirement for eEF1A in building a bridge between the 3′- and 5′-UTRs remains to be directly demonstrated.

Direct 3′- and 5′-UTR interactions have been proposed in Alfalfa mosaic virus (AMV, family Bromoviridae), where they are mediated by coat protein (CP; Guogas 2004; Krab *et al*. 2005). The AMV RNA 3′-UTR adopts two alternative structures, of which only the one with several AUGC repeats separated by hairpins forms a strong interaction with at least two molecules of CP (Neeleman *et al*. 2004; Krab *et al*. 2005). CP enhances translation of AMV RNA *in vivo* 50- to 100-fold in the presence of the cap structure (Neeleman *et al*. 2004) and interacts with the eIF4G and eIFiso4G involved in formation of eIF4F and eIFiso4F, respectively (Krab *et al*. 2005). The complex between the AMV 3′-UTR and CP seems to mimic the PAPB/ poly(A) complex in that both can recruit eIF4F, thus converting the viral RNA into a closed loop structure.

Some 5′-UTRs of capped viral RNAs can enhance translation independently of the cap structure, using other host factors to recruit eIF4F or eIFiso4F or an internal initiation mechanism. The 68 nt TMV 5′-leader, the well-known and extensively studied Ω sequence, acts as a translation enhancer in both plant and animal species, and is now widely used in biotechnological applications. The poly (CAA) region of Ω has been shown to mediate elevated translation via recruitment of the heat shock protein HSP101 (Wells *et al*. 1998b). The complex between Ω and HSP101 might mimic eIF4E/5′-cap or PAPB/poly(A) interactions in that it efficiently recruits eIF4F in order to increase translation (Gallie 2002). Genetic analysis has demonstrated that Ω-based enhancement also requires eIF3, which can be recruited via eIF4F (Wells *et al*. 1998b).

The sequences of some plant viral leaders that can accomplish translation enhancement independently of the 3′-UTR show at least partial complementarity to the central region of 18S rRNA. These leaders bind to the 43S preinitiation complex in a cap- and eIF-independent manner (Akbergenov *et al*. 2004).

Cap- and poly(A)-independent initiation

Several groups of positive-strand RNA viruses contain RNAs that are neither capped nor polyadenylated. These viruses have evolved alternative strategies for translation that use 3′-UTR enhancer elements to recruit eIF4F or eIFiso4F and/or to base pair with their 5′-UTRs for loading the 43S preinitiation complex at the 5′- start site.

Satellite tobacco necrosis virus (STNV – a positive-strand RNA necrovirus) RNA contains within its 3′-UTR (just after the termination codon of the coat protein coding region) a translational enhancer domain (TED) that promotes efficient cap-independent translation when combined with the STNV 5′-UTR (for STNV-1 strain see Timmer *et al*. 1993; for STNV-2 strain - Danthinne *et al*. 1993; Meulewaeter *et al*. 1998). Although TED can functionally substitute for a 5′-cap structure, its function *in vitro* is dependent on the presence of eIF4F and eIFiso4F (Timmer *et al*. 1993). Indeed TED specifically binds eIF4E and eIF4iso4E *in vitro* (van Lipzig *et al*. 2002; Gazo *et al*. 2004), while the 5′-UTR of STNV-1 has the potential to base pair with TED and the 3′-end of 18S rRNA (Timmer *et al*. 1993). Thus to promote cap-independent translation initiation, TED recruits the 43S preinitiation complex by binding canonical cap-binding factors at the 3′-UTR, while potential base pairing between the viral 5′- and 3′-UTRs would be required for transfer of this 43S complex to the 5′-UTR to locate the initiation codon.

The existence of the 3′- to 5′-UTR pathway to recruit the translational machinery is probably not unique to STNV TED, but is likely to apply to other enhancer elements present within the 3′-UTRs of other non-capped, non-polyadenylated virus RNAs. Barley yellow dwarf virus (BYDV – a luteovirus) RNA also lacks a 5′-cap structure and poly (A) tail, but it harbours a cap-independent BYDV translational element (BTE) functionally similar to TED within the 3′-UTR (Wang *et al*. 1997; Guo *et al*. 2000). A BYDV-like BTE is present in all Luteoviruses (for a review, see Miller and White 2006), as well as in Dianthovirus [Red clover necrotic mosaic virus (RCNMV), Mizumoto *et al*. 2003] and Necrovirus [tobacco necrosis virus (TNV), Meulewaeter *et al*. 2004; Shen and Miller 2004], and contains the conserved sequence CGGAUCCUGGGAAACAGG, which also functions when placed in the 5′-UTR. In its natural location this sequence has the potential to base pair

to the 5′-UTR (Wang *et al*. 1997; Guo *et al*. 2000, 2001). BTE can recruit the translation machinery to the 3′-end and deliver it to the 5′-UTR by a 3′–5′ RNA interaction (Wang *et al*. 1997). The delivery of the translational machinery to the 5′-end can occur due to long-distance kissing-loop interactions between RNA hairpins in BTE and the 5′-UTR (Guo *et al*. 2001). Thus, TED and BTE behave in a similar way to strongly stimulate cap-independent translation, without exhibiting any conservation of sequence or secondary structure. Whether BTE or TED require participation of canonical translation initiation factors for their action remains to be investigated.

Another distinct enhancer element identified in Tombusvirus, Tomato bushy stunt virus (TBSV), has been termed the 3′-cap-independent translational enhancer (3′ CITE; Wu and White 1999). The TBSV 5′-UTR folds into a complex RNA structure, which was recently demonstrated to physically interact with the 3′CITE *in vitro* (Fabian and White 2004). Formation of 5′–3′ RNA interactions correlates well with efficient translation *in vivo* and might support the transfer of the translational machinery from the 3′ to the 5′-end of the RNA as suggested for BTE and TED. A similar element recently was identified in Maize necrotic streak virus (MNeSV, Scheets and Redinbaugh 2006).

RIBOSOME SHUNTING

Ribosome shunt is a special mechanism of eukaryotic translation initiation that combines features of both 5′-end dependent scanning and internal initiation (Ryabova *et al*. 2002, 2006). It was discovered in plants, first for *Cauliflower mosaic virus* (CaMV) (Fütterer *et al*. 1990, 1993) and then for *Rice tungro bacilliform virus* (RTBV) (Fütterer *et al*. 1996). Similar phenomena have also been described for several animal viruses including Sendai paramyxovirus (Latorre *et al*. 1998; de Breyne *et al*. 2003), human type C adenovirus (Yueh and Schneider 1996, 2000; Xi *et al*. 2004), human papillomavirus (Remm *et al*. 1999), and duck hepatitis B pararetrovirus (Sen *et al*. 2004), and for cellular mRNAs (Yueh and Schneider 2000; Rogers *et al*. 2004; Chappel *et al*. 2006).

In CaMV and RTBV, ribosomal shunting occurs on the leader of the capped and polyadenylated viral pregenomic (pg) RNAs. The CaMV and RTBV leaders are very long, and contain several sORFs as well as an extended stem-loop structure. In CaMV, the leader secondary structure has been determined *in vitro* by chemical and enzymatic probing (Hemmings-Mieszczak *et al*. 1997). Both the secondary structure and the multiple sORFs constitute a major barrier to linear scanning ribosomes (Pooggin *et al*. 2000; Ryabova *et al*. 2000). Nonetheless, translation initiation downstream of the CaMV pgRNA leader is cap-dependent (Schmidt-Puchta *et al*. 1997).

The molecular mechanism of CaMV shunt has been extensively studied using shunt-competent plant protoplasts and *in vitro* translation systems (Fütterer *et al*. 1990, 1993; Dominguez *et al*. 1998; Hemmings-Mieszczak *et al*. 1998; Pooggin *et al*. 1998; Hemmings-Mieszczak *et al*. 1999; Pooggin *et al*. 2000; Ryabova and Hohn 2000; Ryabova *et al*. 2000; Pooggin *et al*. 2001). According to our current model (**Fig. 3**), shunt-mediated translation initiation on the CaMV pgRNA comprises the following steps: (1) a 40S ribosomal subunit binds the capped 5′-end of the pgRNA and scans along the leader sequence until the first AUG, the start codon of short ORF A (sORF A), is encountered; a complete 80S ribosome is assembled and initiates translation of sORF A; (2) during this short translation event, eIF3, eIF1 and eIF1A might remain ribosome-associated while RNA helicase activities are released; (3) the 80S subunit is disassembled at the sORF A stop codon (the shunt take-off site) located six nucleotides upstream of the base of the stem-loop structure; (4) the released 40S subunit (shunting ribosome) has lost initiation factor(s) capable of melting the stable secondary structure and linear scanning is thus blocked; (5) the 40S subunit shunts over (bypasses) a 480 nt structured region; (6) the shunting ribosome resumes scanning just downstream of the stem-loop structure at a shunt landing site and reaches the AUG start codon of the first large viral ORF (ORF VII), where translation is re-initiated. eIFs 1, 1A and 3, which are potentially still bound to the shunting ribosomes, assist in initiation and ribosomes are capable of reinitiating immediately downstream of the shunt landing site even at a non-AUG start site (Ryabova and Hohn 2000). Consistent with the shunt model involving a reinitiation step, the CaMV transactivator/viroplasmin (TAV) protein, which serves as a reinitiation factor to promote translation of several consecutive large ORFs on the polycistronic 35S RNA (Bonneville *et al*. 1989; Park *et al*. 2001; see below), can also increase the efficiency of shunting (Fütterer *et al*. 1993; Pooggin *et al*. 2000, 2001).

The mechanism of ribosome shunting in RTBV resembles that in CaMV. Our recent study (Pooggin *et al*. 2006) using *O. sativa* (dicot plant) and *O. violaceus* (monocot plant) protoplasts and wheat germ extract has shown that translation of the 5′-proximal sORF, which terminates a few nucleotides upstream of the base of a stem-loop structure in the RTBV leader, is absolutely required for efficient shunting, bringing ribosomes to the landing site located just downstream of the structure. The structural configuration of an sORF followed by a secondary structure element occurs in the pgRNA leader sequences of most (but not all) plant pararetroviruses sequenced so far (Pooggin *et al*. 1999; Geering *et al*. 2005), and we therefore predict that the sORF-mediated shunting mechanism is conserved in this viral family. Swapping of shunt elements, individually and in combination, between CaMV and RTBV revealed that these elements are functionally equivalent in dicot plant cells, even though their primary nucleotide sequences differ considerably (Pooggin *et al*. 2006). However, in monocot systems (rice protoplasts and wheat germ extract), the shunting mechanism shows some preference for certain *cis*-acting features. In fact, the landing site sequence of the RTBV leader failed to function in wheat germ extract, while it operated well in the two protoplast systems. In rice protoplasts, CaMV shunt elements, either complete or individual elements in various combinations with complementing RTBV elements, did not support efficient translation. In general, the RTBV shunt directs higher basal translation than the CaMV shunt and is less responsive to the CaMV reinitiation factor TAV (Pooggin *et al*. 2006). This correlates with differences in the strategies used to effect polycistronic translation from viral pgRNA, which rely on leaky scanning in RTBV and on TAV-mediated reinitiation in CaMV (see below). It is worth mentioning that differences in shunting efficiency may account for the tissue-specificity of these two viruses; CaMV infects most cell types whereas RTBV is phloem-limited (Sta Cruz *et al*. 1993).

In CaMV, point mutations of the start and the stop codon of sORF A, but not its coding sequence, abolish shunting and drastically reduce viral viability in turnip plants, leading to appearance of first and second site reversions restoring a sORF (Pooggin *et al*. 1998, 2001). This suggests that sORF-mediated shunting is essential for viral viability. Recently we tested the importance of other *cis*-elements required for shunting *in planta* (M. Pooggin and T. Hohn, in preparation). Our results indicate that the two distant regions of the CaMV leader that form the conserved, spatially proximal, shunt configuration – the sORF and the lower part of the stem structure, and the shunt landing sequence downstream of the stem – can be replaced with the corresponding regions from RTBV without any dramatic effect on shunt-mediated polycistronic translation of pgRNA and viral infectivity. The CaMV-RTBV chimeric virus was largely stable over six passages in turnip plants: a few point mutations and short deletions that did eventually accumulate within the sORF and adjacent to the RTBV sequences were indicative of fine tuning of the chimeric sequence during adaptation to a new host. Taken together, the evi-

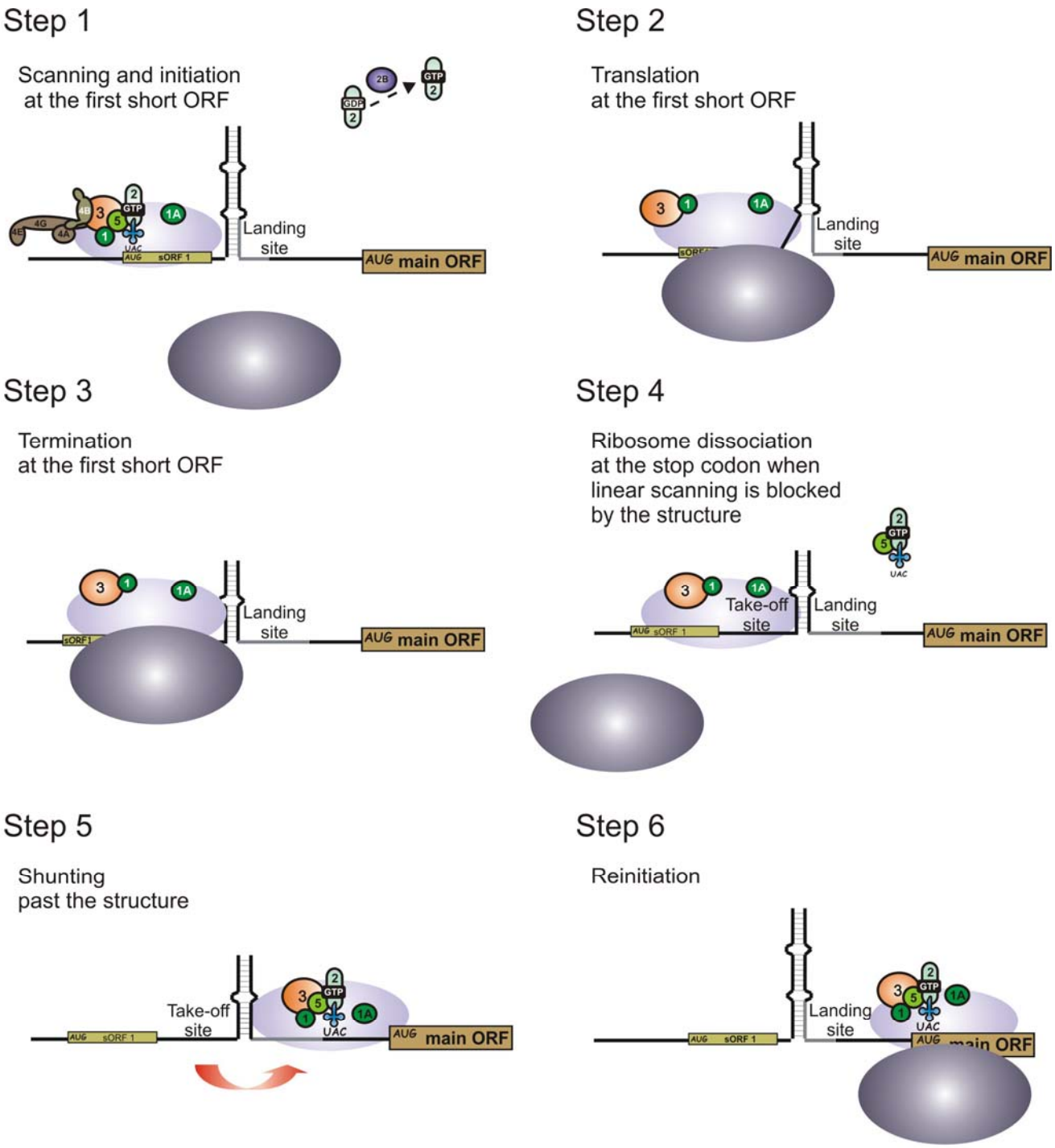

Fig. 3 Model for ribosomal shunt in CaMV and RTBV. Step 1. 5′-cap dependent scanning, where the downstream stem structure retards movement of the 40S ribosomal subunit and thereby ensures recognition of the AUG codon of the first sORF, sORF1; eIF2-GDP leaves and undergoes GDP exchange for GTP by eIF2B. **Step 2.** Conventional assembly of the 80S preinitiation complex at the sORF1 AUG codon; upon joining of 60S, eIF3 remains attached to the solvent side of 60S, while eIFs 4A and 4B are released; Short translational event where eIF3 remains unstably attached to 60S; **Step 3.** Termination at the sORF1 termination codon is an obligatory step, where the bottom helix of the stem is being melted by translating ribosomes; (**4**) On disassembly of 80S, linear scanning of 40S is blocked by the structure as the upper part of the stem is not melted by the ribosome (eIF4A and eIF4B are missing); (**5**) ribosome shunt over the structure; (**6**) Resumption of scanning downstream of the structure and reloading of the ternary complex on the 40S ribosome.

dence suggests that the molecular mechanism of ribosome shunting is evolutionarily conserved in plant pararetroviruses. Interestingly, it has been proposed that the ascending and descending arms of the CaMV leader structure building the shunt configuration may have evolved through head-to-head incorporation of long terminal repeats of an ancient retrotransposon found in the yeast genome (Shabadi *et al*. 2006).

It is worth mentioning that the pgRNA of pararetroviruses is terminally redundant and thus the 5′-part of its leader sequence, including the first sORF and half of the ascending arm of the stem-loop structure, is present as a direct repeat in the 3′-UTR preceding the poly(A) tail. Thus, the 3′-UTR can base-pair with the descending arm sequence of the leader, which, in addition to a cap-poly(A) bridge, would ensure circularization of pgRNA. Whether or not such a circularization of pgRNA is required for efficient translation initiation as described above for some plant RNA viruses remains to be investigated. An interesting possibility is that 3′-UTR-based shunt donor elements, the sORF and the assending arm sequence, might participate in recycling of ribosomes that have completed translation to the 5′UTR of the same pgRNA or a different pgRNA molecule. Indeed, a CaMV shunt can be re-constituted in *trans*

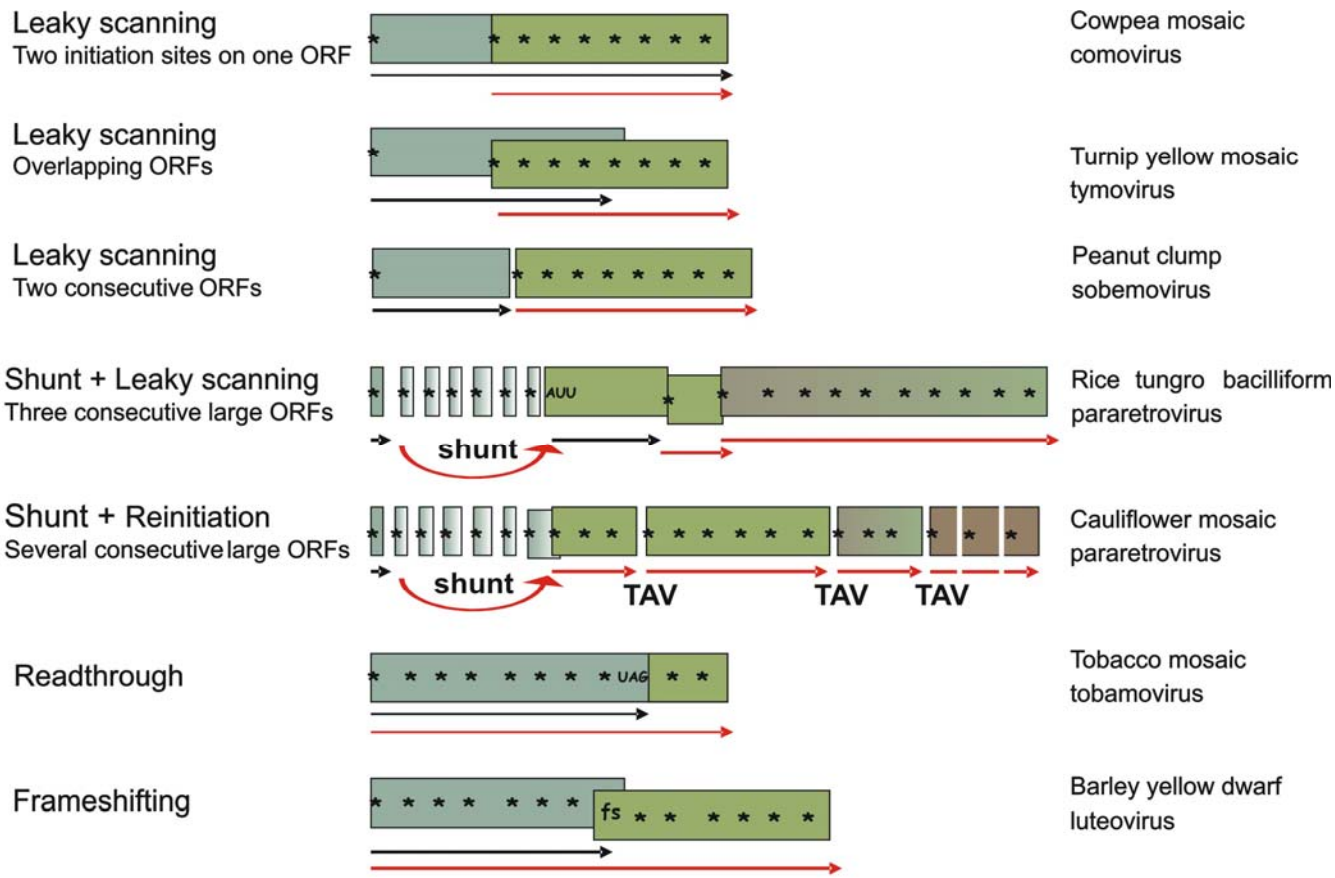

Fig. 4 Viral strategies to express polycistronic mRNAs. ORFs are shown as open boxes, where *asterisks* indicate AUG codons. The horizontal arrows show the movement of translating ribosomes, and curved arrows indicate shunting ribosomes. Examples of viruses using the strategies illustrated are given on the right.

using two separate RNA molecules, one containing the shunt donor region and another the shunt acceptor region followed by a reporter ORF (Fütterer *et al*. 1993).

Ribosome shunting that depends on a 5′-proximal sORF in the leader is used by at least one representative of plant RNA viruses: Rice tungro spherical virus (RTSV; M. Pooggin, unpublished) and one representative of animal retroviruses: Prototype foamy virus (PFV; L. Ryabova, unpublished). Interestingly, RTSV and RTBV coexist in nature and synergistically cause a severe tungro disease of rice. We hypothesise that these two viruses might have co-evolved a shunting strategy by exchanging genetic information. PFV belongs to a separate genus within Retrovirinae that falls between retroviruses and pararetroviruses. Other cases of ribosome shunting for which mechanisms have been investigated in sufficient detail to assess (Yueh and Schneider 2000; de Breyne *et al*. 2003; Chappell *et al*. 2006), differ from the CaMV case in that they do not involve translation of an sORF. However, a common theme seems to be pausing of shunting ribosomes at a take-off site. In CaMV, such a pausing might be caused by the strong secondary structure downstream of the first sORF and a lack of certain initiation factors required for efficient unwinding of the structure following the translation event at this sORF. In adenovirus late RNAs and cellular hsp70 and Gtx mRNAs, pausing might be caused by interaction of scanning ribosomes with leader regions complementary to 18S ribosomal RNA, which are required for efficient shunting in those cases (Yueh and Schneider 2000; Chappell *et al*. 2006). In the Sendai virus, pausing might occur during initiation at an upstream non-AUG start codon of the C′ gene (de Breyne *et al*. 2003).

PRODUCTION OF MULTIPLE PROTEINS FROM A SINGLE RNA

Plant viruses use various strategies to express multiple proteins from their compact genomes. Often, several viral proteins are translated from a monocistronic RNA as a large polyprotein precursor that is processed into individual components by proteolytic cleavage. In many DNA and RNA viruses, RNAs are functionally polycistronic and two or more proteins are translated separately from a single RNA molecule. In the following sections, we describe the best characterised examples of various mechanisms of viral polycistronic translation, including leaky scanning, reinitiation and recoding (see **Fig. 4**).

Leaky scanning

The mechanism most frequently used by viruses for polycistronic translation is leaky scanning, in which a fraction of scanning ribosomes bypass the first start codon and initiate translation at downstream start codons. Leaky scanning occurs when the first start codon resides in a suboptimal context, lacking both a purine at position −3 and a guanosine at position +4, or when it is of a non-AUG type (i.e. a triplet differing from AUG at one position) (for review, see Kozak 1991). Leaky scanning can result in production of two or more proteins with a common C-terminal region if translated from one open reading phase, or in totally different proteins, if the start codons are located in different phases (see **Fig. 4**).

Cowpea mosaic virus M-RNA provides an example of leaky scanning leading to translation of at least two co-C-terminal polyproteins from one large ORF (Wellink *et al*. 1993). In this case, two upstream AUGs in suboptimal context (UGC**AUG**A at position 151 and ACA**AUG**U at 161) appear to be bypassed by scanning ribosomes, which then initiate translation at the third start codon in an optimal context (GAA**AUG**G at 512). Initiation events in one reading phase at the second and the third AUGs give rise to two distinct polypeptides essential for replication and cell-to-cell movement, respectively (Wellink *et al*. 1993). It

should be noted that cowpea mosaic virus belongs to the group of picorna-like plant RNA viruses that lack a cap structure and very often use an IRES-dependent initiation mechanism (see above). Interestingly, besides leaky scanning, IRES-mediated initiation has also been suggested to contribute to translation from the third AUG of cowpea mosaic virus M-RNA (Verver *et al*. 1991).

In TYMV, the movement protein p69 and the replication polyprotein p206 are expressed from overlapping ORFs on genomic RNA by a leaky scanning mechanism: the downstream p206 start codon (GUA**AUG**G) is reached by ribosomes scanning from the 5′-end through the upstream p69 AUG, which is in a suboptimal context (CAA **AUG**A) (Matsuda *et al*. 2004a; Matsuda and Dreher 2007). Interestingly, a tRNA-like structure (TLS) located at the 3′-end of TYMV RNA was proposed to drive an alternative, "Trojan horse" mechanism for p206 translation, in which the TLS activates internal initiation at the p206 AUG without affecting cap-dependent initiation at the upstream AUG (Barents *et al*. 2003). However, the Trojan horse model has recently been ruled out in favour of a variation of canonical leaky scanning (Matsuda and Dreher 2006, 2007).

Peanut clump virus RNA2 contains two non-overlapping ORFs expressed by leaky scanning (Herzog *et al*. 1995). The start codon of the first ORF is in an unfavorable initiation context (CUU**AUG**U), therefore allowing about one-third of scanning ribosomes to initiate at the second ORF. Consistent with the leaky scanning model, there is no internal AUG between the start codons of the two ORFs.

A remarkable case of leaky scanning has been described for the pararetrovirus RTBV (Fütterer *et al*. 1997). In RTBV, ORFs I, II and III are expressed from pgRNA, which serves as a true polycistronic mRNA (Fütterer *et al*. 1997), while ORF IV is translated from a spliced, monocistronic derivative of the pgRNA (Fütterer *et al*. 1994). The first long viral ORF (ORF I) starts with a non-AUG codon (AUU) and is preceded by the long, structured, leader sequence with multiple sORFs. It has been demonstrated that around 10% of ribosomes, delivered to the 3′-end of the leader by the shunting mechanism (see above), recognize this AUU to initiate translation of ORF I, while 90% of the shunting ribosomes continue scanning towards ORFs II and III (Fütterer *et al*. 1996, 1997). The suboptimal context of the ORF II AUG codon (UAC**AUG**A) allows a significant fraction of ribosomes to scan past this AUG and reach the further downstream AUG of ORF III, which has a moderately efficient start codon (AGC**AUG**A). Notably, ORFs I and II, spanning about 1 kb, do not contain any other AUG codons (Fütterer *et al*. 1997). The lack of internal AUGs is also a feature of ORFs I and II in related badnaviruses, suggesting that the leaky scanning mechanism is conserved in the two distinct genera of bacilliform pararetroviruses (Pooggin *et al*. 1999).

Reinitiation mechanisms

In eukaryotes, ribosomes having terminated translation of a short ORF (sORF) can give rise to 40S subunits capable of resuming scanning and reinitiating at a downstream AUG. Depending on the length of the upstream ORF (uORF), translation reinitiation strategies can be divided into two types: conventional reinitiation after a short ORF, and alternative mechanisms that allow reinitiation after translation of long ORFs.

Reinitiation after a short ORF

Short ORFs of less than 30 codons located upstream of a long ORF usually have regulatory functions in controlling the rate of translation at a following downstream long ORF (for review, see Morris and Geballe 2000; Meijer and Thomas 2002). Such sORFs are often used to down-modulate production of critical effector proteins. The efficiency of reinitiation after the short translation event is constrained by structural features of the mRNA, such as the size of the uORF, and by the availability of canonical initiation factors (Hinnebusch 1997; Kozak 2001). Since factors necessary for reinitiation dissociate from the ribosome as it translates longer ORFs, the time required for the ribosome to be recharged with critical initiation factors including, at a bare minimum, the ternary complex (Met-tRNA$_i$-eIF2-GTP), is thus extended (Kozak 1987b; Hinnebusch 2005). This has been established for the GCN4 system in yeast, in which lengthening of the intercistronic region favors reinitiation efficiency (Hinnebusch 1997). Any canonical initiation factors involved in promoting primary initiation at the sORF initiation codon that are not removed during the short translation event should favour the new reinitiation event. Based on results obtained using a set of different IRESes with known protein requirements for their function, Pöyry *et al*. (2004) have suggested that, in the mammalian *in vitro* system, eIF4F and/or eIF4A might participate in reinitiation following the short elongation event. It is probable that interactions between eIF4G/eIF4A, eIF3 and the 40S subunit are weakly maintained for a short time during the elongation step. If the sORF is short enough, these interactions are not disrupted and the ribosome, still equipped with all necessary eIFs, will resume scanning and reacquire a new ternary complex. Many plant viruses contain one or more sORFs within their leader regions and may therefore use sORF-mediated reinitiation to regulate downstream translation. As described above for CaMV and RTBV, the short translational event at the 5′-proximal sORF that promotes efficient translation of the downstream ORFs via ribosome shunting can be thought of as a special case of reinitiation.

Reinitiation after a long ORF

In eukaryotes, reinitiation after translation of a long ORF is a very rare event; in mammals there are only a few well-documented examples of such reinitiation (Horvath *et al*. 1990; Ahmadian *et al*. 2000; Meyers 2003; Alisch *et al*. 2006; Luttermann and Meyers 2007). This would suggest that canonical eIFs that would be instrumental in promoting a reinitiation event are not available after their detachment during a long translation event.

Recently, a so called termination-reinitiation mechanism has been proposed for an animal calicivirus (Feline calicivirus; FCV), where close proximity of the stop codon of a first ORF to the initiation codon of a second ORF can result in efficient translation of the second ORF. In this case, both a stop codon within a certain distance from the authentic AUG codon and a cis-acting element preceding the stop codon (proposed to interact with 18S rRNA) are required to locate the terminating 40S ribosome at the reinitiation start site; moreover, the latter AUG is not required to be in optimal context and even non-AUG codons can support a significant level of ORF 2 translation (Alisch *et al*. 2006; Luttermann and Meyers 2007).

TAV-mediated reinitiation in plant pararetroviruses

According to the general translation initiation pathway (**Fig. 1**) one eIF that has to be acquired *de novo* by the reinitiating 40S ribosomal subunit is eIF2 in the form of TC (Hinnebusch 1997), since Met-tRNA loaded via eIF2 to the scanning 40S ribosome will have been used to accomplish the primary initiation even. A second required component will be the 60S ribosomal subunit, if this was lost during the first termination event. Thus a potential reinitiation factor either has to keep eIFs bound to the translating ribosome and/ or help reacquire them *de novo* during or after termination of translation of the first ORF.

CaMV and related pararetroviruses have developed an unique reinitiation strategy in which a viral factor promotes reinitiation after translation of one or several long viral ORFs. This translation reinitiation mechanism was first documented for CaMV (Bonneville *et al*. 1989; Fütterer and Hohn 1991) and Figwort mosaic virus (FMV, Gowda *et al*. 1989), and has since been confirmed also for Peanut chlo-

rotic streak virus (PCSV, Maiti *et al*. 1998). The CaMV genomic DNA contains two promoters, which direct production of the polycistronic 35S pregenomic RNA and the 19S subgenomic RNA. The 35S RNA encodes all six functional viral proteins from ORF I to ORF VI, with the ORFs being mostly closely spaced or with a short overlap. The 19S subgenomic RNA contains a single ORF, ORF VI, which encodes a transactivator protein, TAV, essential for translation of the 35S RNA. TAV is the most abundant viral protein in the cytoplasm of infected cells and forms so-called inclusion bodies (viroplasms) where all viral gene products are found (Givord *et al*. 1984; Martinez-Izquierdo *et al*. 1987). TAV functions in transactivation of polycistronic translation in plant protoplasts (Fütterer *et al*. 1990; Fütterer and Hohn 1991, 1992; Kiss-László *et al*. 1995) and plants (Ziljstra and Hohn 1992). TAV-mediated transactivation is not significantly dependent on virus-specific sequences, with artificial bicistronic messages also being good substrates for the action of TAV (Fütterer and Hohn 1991, 1992). TAV-activated reinitiation is not much affected by the distance between the two consecutive ORFs: reinitiation can occur immediately after translation termination, when the two ORFs are linked by an AUGA quadruplet, and, still as efficiently, if the second ORF is located as far as 700 nt further downstream (Fütterer and Hohn 1991). Thus, unlike the reinitiation strategy used after sORF translation in the yeast GCN4 system (Hinnebusch 1997) or termination-reinitiation mechanisms (Alisch *et al*. 2007; Luttermann and Meyers 2007), TAV-activated reinitiation is not distance- or stop codon context-dependent.

To accomplish its transactivation function, TAV is proposed to interact with eIF3 and the 60S ribosomal subunit via multiple contacts mediated by at least three ribosomal proteins: L13, L18 and L24 (Leh *et al*. 2001; Park *et al*. 2001). TAV interactions with eIF3 (via its subunit g) and L24 proved critical for its activity (Park *et al*. 2001). Two domains of TAV are required for transactivation of polycistronic translation: the so-called miniTAV domain (MAV; de Tapia *et al*. 1993), and the multiple protein binding domain (MBD; Park *et al*. 2001). The MAV domain is essential for transactivation since deletion of the conserved tetrapeptide YNPG abolished transactivation activity (de Tapia *et al*. 1993). The MAV peptide, expressed in a high excess in *Nicotiana plumbaginifolia* protoplasts, can provide 20-25% of wild-type TAV-mediated transactivation activity. The presence of the MBD domain is absolutely required for transactivation in *Orychophragmus violaceus* protoplasts and increases the MAV-dependent level of transactivation by 70-75% in *N. plumbaginifolia* protoplasts (de Tapia *et al*. 1993). It has been shown that the same binding site of MAV can recruit either L13 or L18 (Leh *et al*. 2001; Bureau *et al*. 2004) or double-stranded RNA, while MBD interacts with eIF3 or, less strongly, with L24 (Park *et al*. 2001). What could be the functional meaning of TAV interactions with these components of the translational machinery?

Role of L24 in TAV-mediated reinitiation

Overexpression of L24 in plant protoplasts led to significant enhancement of TAV-mediated reinitiation (Park *et al*. 2001). L24 is located at the internal surface of the 60S ribosomal subunit in the main factor-binding site (Ban *et al*. 2000). Although L24 is not essential in yeast, its removal causes defects in the 60S subunit joining step during translation initiation (Baronas-Lowell 1990; Spahn *et al*. 2001). Thus, it was speculated that TAV functions in recruitment of the 60S ribosomal subunit for the reinitiation event. Recently, we have identified another partner of TAV of unknown function, provisionally termed TAIP (TAV-interacting protein), which can strongly interact with the 60S ribosomal subunit (OT and LR, unpublished data). This protein may be exploited by TAV for rapid recruitment of the 60S ribosomal subunit during the reinitiation event. On the other hand, binding of TAV to L24 located in the 60S active centre might inhibit translation elongation, freeing the pgRNA for reverse transcription and/or packaging as part of the CaMV replication cycle (Park *et al*. 2001).

Interestingly, L24 has been implicated in reinitiation events on plant mRNA containing upstream ORFs (Nishimura *et al*. 2004). In *Arabidopsis thaliana*, an L24-deficient mutant, *stv*, shows a gynoecium developmental phenotype similar to that observed in *ett* and *mp* mutants, where *ETT* and *MP* translation is negatively regulated by upstream ORFs and apparently requires reinitiation mechanisms. The first uORF of *ETT* is 279 nt long, and Nishimura *et al*. (2004) postulated that L24 might play a role in translation regulation by long uORFs. It will be interesting to determine whether the *stv* mutant can support CaMV infection.

Role of the eIF3-TAV interaction in reinitiation

In vitro experiments suggest that the TAV/eIF3 complex binds both 40S and 60S ribosomal subunits via eIF3, and TAV as a bridge, respectively (Park *et al*. 2001), and the complex co-sediments with the 80S ribosomal fraction in sucrose gradients (Park *et al*. 2004). eIF3 co-sediments with the 40S ribosomal fraction in healthy plants, but CaMV infection of turnip plants leads to accumulation of both TAV and eIF3 in the polyribosomal fractions of sucrose gradients (Park *et al*. 2001). Based on these data, one apparent function of TAV would be to prevent dissociation of eIF3 from the 80S translating ribosome during the preceding elongation event, to allow resumption of scanning and/or reinitiation at a downstream AUG (Ryabova *et al*. 2004, 2006). eIF3 is the largest and most complex initiation factor (Browning *et al*. 2001; see also a recent review by Hinnebusch 2006) that plays a crucial role during cap-dependent translation initiation, where it stimulates binding of TC to the 40S ribosomal subunit in a manner enhanced by eIF1 and eIF1A, and interacts with mRNA to assist loading of the 40S ribosomal subunit on the mRNA (Majumdar *et al*. 2003; **Fig. 1**, steps 1 and 2). eIF3 is required for rapid reloading of TC on the scanning 40S ribosome to re-initiate at a GCN4 ORF *in vivo* (Garcia-Barrio *et al*. 1995). Thus, the TAV/eIF3/40S complex can re-recruit TC via eIF3, resume scanning and reinitiate at downstream ORF.

Interestingly, eIF3 may play a role in the termination-reinitiation mechanism operating in FCV described above through binding to the cis-element preceding the stop codon of the upstream ORF (Jackson 2005).

Role of other factors in TAV-mediated reinitiation

Interaction of TAV with eIF3 can be precluded by eIF4B, which out-competes TAV for eIF3 binding: the central G-rich domain of Arabidopsis eIF4B interacts with the TAV-binding domain of eIF3g (Park *et al*. 2004). Recent data suggest that plant eIF4B is a scaffold protein that can form a complex with eIF4A, PABP, eIF3 and mRNA (Cheng and Gallie 2006), so that TAV apparently cannot enter the host translational machinery via interaction with eIF3 until the 60S subunit joining step, during which eIF4B and other eIFs are likely displaced. Overexpression of eIF4B in plant protoplasts negatively affects TAV-mediated transactivation (Park *et al*. 2004), probably by sequestering eIF3 from the translation machinery. The fact that TAV does not interfere with the first initiation event speaks in favor of the notion that formation of a stable complex between eIF3/40S occurs after the 48S preinitiation complex formation and eIF4B removal (Park *et al*. 2004).

What of the other canonical translation initiation factors? Two such factors, eIF4G and eIF4A, have been proposed to be retained at the 40S ribosomal subunit to accomplish reinitiation at the second ORF after a short elongation event (Pöyry *et al*. 2004). However, in the case of FCV, reinitiation was not inhibited by dominant-negative eIF4A mutants, suggesting that eIF4G and eIF4A are not required for the reinitiation–termination event (Jackson 2005). Ribo-

somal proteins L13 and L18 are apparently located somewhere close to the outer surface of the 60S ribosomal subunit near the neck region (Marion and Marion 1987; Ban *et al*. 2000), so it has been speculated that L13 and L18 might bind TAV dimers (Bureau *et al*. 2004). However, their functional role in transactivation needs to be clarified.

A model of TAV-mediated reinitiation

Our current model of TAV-mediated reinitiation is presented in **Fig. 5** (originally proposed by Park *et al*. 2004). After disruption of the eIF3-eIF4B interaction, TAV is sequestered by the eIF3/40S complex, thus strengthening this otherwise unstable complex, which remains attached to the translation machinery during the elongation process. The affinity of TAV for the 60S ribosomal subunit due to its interaction with L18 or L13 might lead to relocation of eIF3 to the solvent surface of 60S, where it would not interfere with the elongation process. The 60S-TAV-eIF3 interaction might also enable eIF3 to travel with elongating ribosomes. During translation termination, the TAV-eIF3 complex might be transferred back to the 40S subunit. The TAV/eIF3/40S complex would thus quickly reacquire the ternary complex and be ready to drive ribosome scanning or immediate reinitiation at the next AUG triplet.

Recoding

Normally, translation terminates at any one of the three nonsense (stop) codons UAG, UAA or UGA. However, genetic readout can be extended by alteration of the standard rules in a manner that is specific for individual mRNAs. Such extensions of the genetic code are termed recoding events (for a review, see Baranov *et al*. 2002). Recoding is often in competition with standard decoding (see **Fig. 4**). In general, viruses can use recoding events both to regulate gene expression during the viral life cycle and to expand the genetic information held in their relatively small genome. Two distinct classes of recoding are used by plant viruses: programmed-frameshifting and stop-codon suppression. In the case of frameshifting, the reading frame is shifted in the 5′ or 3′ direction. In stop-codon suppression (or read-through), the stop codon is read as a sense codon by normal cellular tRNAs known as natural suppressor tRNAs.

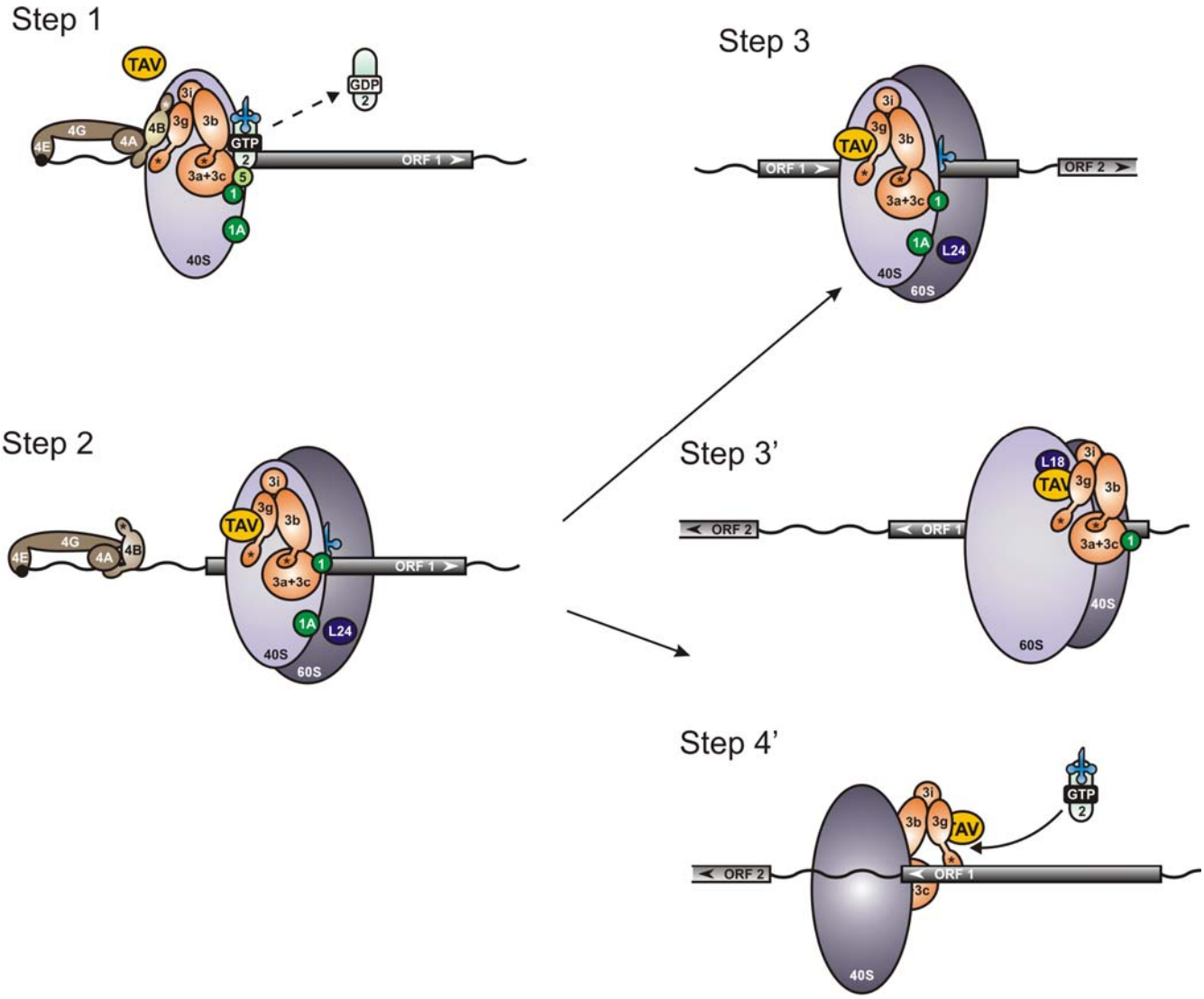

Fig. 5 Model of the role of TAV in retaining eIF3 on the translating ribosome during the elongation phase of translation of a long ORF. Note that in steps 1-3, the model is viewed from the "front", with movement of the initiation complex from left to right, and in steps 3′ and 4′ from the "back" (movement from right to left). **Step 1.** Conventional assembly of the 48S pre-initiation complex at the first suitable start codon. **Step 2.** During the 60S subunit joining step eIF4B is replaced by TAV on the eIF3 subunit g. eIF3 is still associated with the solvent side of the 40S subunit. **Step 3.** During elongation of ORF1 TAV binding can stabilize the eIF3/40S complex on the solvent surface of the 40S ribosomal subunit. **Step 3′.** In an alternative scenario the TAV/eIF3/80S complex is stabilized by transfer of TAV/eIF3 to the 60S subunit through TAV interaction with L18. In this model a fourth step (**Step 4′**) would be required, in which the TAV/eIF3 complex is relocated back to the 40S subunit during ORF 1 termination. The TAV/eIF3/40S complex scans for ORF 2 and re-acquires the ternary complex on the way. eIF3 and its subunits g (3g), i (3i), b (3b), a and c (3a+3c); eIF1 (1), eIF2 (2), eIF5 (5), tRNA and TAV are indicated. The solvent side of 40S and 60S ribosomal subunits is depicted in grey, with the internal interface side in white. *Asterisks* RNA recognition domains (RRM) within eIF4B and eIF3g.

In both cases, the ribosome is not released by the eukaryotic release factor complex and an elongated polypeptide is synthesized.

Stop codon read-through

In some RNAs, the stop codon of the 5′ gene may be "leaky", which allows a proportion of ribosomes to continue translation until the next stop codon. Such "read-through", or "stop-codon suppression", results in a C-terminally extended version of the protein. The eukaryotic release factor complex, eRF1/eRF3/GTP, normally decodes stop signals efficiently. However, depending on the context of the stop codon and the presence of the suppressor tRNA, competition between the eukaryotic release factor complex and a suppressor tRNA for the stop signal shifts in favor of the tRNA, resulting in a read-through rate of about 1-10%.

This phenomenon was first reported for TMV (Pelham 1978) and later for at least 17 plant virus genera, including the *Luteoviridae* and *Tombusviridae*. A large number of positive-sense ssRNA plant viruses use the read-through strategy to produce components of RNA-dependent RNA-polymerases (RdRp, e.g. tobamo- and tombusviruses) or elongated coat proteins (luteoviruses). The latter products are thought to be involved in transmission vector interactions (reviewed by Gray and Banerjee 1999). To date, nonsense codon suppression has not been reported for plant DNA viruses.

Biological significance of the read-through products

The best characterized example of suppression of a UAG termination codon occurs in TMV RNA. This RNA can be translated *in vitro* to yield a "normal" 126 kDa protein and a 183-kDa read-through protein (Pelham 1978). Both proteins have been detected in TMV-infected cells (Paterson and Knight 1975). The read-through mechanism results in the expression of large quantities of P126, which includes helicase and methyl transferase domains, and much smaller amounts of RdRp (P183), which harbors at its C terminal end the highly conserved GDD motif responsible for replicase activity (Maia *et al*. 1996). Both polypeptides are essential for TMV multiplication (Ishikawa *et al*. 1986).

Tobraviruses also express the catalytic domain of RdRp by read-through in plants. These viruses possess a bipartite genome that is packaged separately in rod-shaped particles. RNA-1 encodes two open reading frames separated by the UGA stop codon (Hamilton *et al*. 1987), which give two proteins of about 130 and 190 kDa. For TRV, a read-through protein of 194 kDa was detected *in vitro* (Fritsch *et al*. 1977; Hughes *et al*. 1986) and *in vivo* (Mayo 1982). Both proteins are thought to be involved in RNA replication (Hamilton *et al*. 1987).

In luteoviruses, Beet necrotic yellow vein virus (BNYVV) and the fungal-transmitted viruses of the genera *Pomovirus* and *Furovirus*, expression of the capsid protein is also regulated by read-through. In luteoviruses, the capsid is composed of two structural proteins: the major 21 kDa CP and a minor component, the 75 kDa read-through protein containing the read-through domain (RTD). CP alone is sufficient to form infectious virions but the RTD is required for aphid transmission (Brault *et al*. 1995). The N-terminal (conserved) half of the RTD may be the site of the primary vector specificity determinant for all luteoviruses (Brault *et al*. 2005). The RTD is also important for efficient accumulation of the virus in whole plants (Brault *et al*. 1995; Chay *et al*. 1996; Bruyère *et al*. 1997; see Mutterer *et al*. 1999 and references therein).

RNA-3 of Potato mop-top virus (PMTV) – the type member of the genus Pomovirus – has a single ORF encoding the 20 kDa CP and a 67 kDa protein produced by read-through of the CP amber termination codon (Kashiwazaki *et al*. 1995). The read-through protein of PMTV is thought to be involved in transmission by the vector *Spongospora subterranean* (Reavy *et al*. 1998) and has been located near one extremity of the virus particle (Cowan *et al*. 1997). For BNYVV, the read-through protein intervenes in virion assembly (Schmitt *et al*. 1992) and plays a role in virus-vector interactions (Tamada and Kusume 1991). The read-through protein is translated from RNA 2 as a fusion protein (75 kDa), with the viral coat protein (21 kDa) at its N terminus. Schmitt *et al*. (1992) demonstrated that a short deletion in the read-through domain of P75 interfered with accumulation of BNYVV virions during infection without inhibiting the ability of the virus to replicate and form local lesions on leaves. Mutation of a peptide motif (KTER) within the C-terminal half of the read-through domain did not inhibit assembly but blocked protist transmission of the virus (Tamada *et al*. 1996).

Stop codon context effects

Stop codons are all subject to read-through, and all have different efficiencies of termination (UAA>UAG>UGA), but UAA is by far the least frequently used. One major determinant known to affect the efficiency of translation termination is the local sequence context surrounding the termination codon (Skuzeski *et al*. 1991). However, Brown *et al*. (1996) have shown in BYDV that a recoding signal for read-through can also be located at some distance from the stop codon. The codon context, which has a major influence on read-through, extends to the hexanucleotide at the 3′ side of the mis-read codon. The read-through region at the 3′ side can be classified into three groups according to sequence homology (Beier and Grimm 2001). The type 1 read-through region is found in plant viral RNAs like tobamovirus. These viruses share the 6 nucleotides CAA UYA (Y=pyrimidine) at the 3′ side of the suppressed UAG or UAA codon. Any change in these nucleotides can abolish or greatly reduce UAG suppression *in vitro* (Zerfass and Beier 1992). The type II region present in tobravirus or furovirus is characterized by the sequence CGG or CUA at the 3′ side of the suppressed UGA (rarely UAA) codon. The type III region present in luteovirus is more complex: a conserved purine-rich spacer sequence consisting of 8 nt immediately downstream of the suppressor UAG resembles the octanucleotide sequence in animal retroviruses (see Beier and Grimm 2001 for a detailed description). However, for the Luteoviridae, this sequence is not sufficient for read-through *in vivo*: a cytidine-rich repeat $(CCNNNN)_{8\text{-}16}$ beginning about 20 nt downstream of the stop codon and an essential sequence located over 700 nt downstream were also required for read-through (Brown *et al*. 1996).

An alternative classification scheme for read-through stop codon contexts has been proposed by the Atkins group (Harrel *et al*. 2002). From a database of virus read-though contexts, they used the six most frequent triplets found at the +1 position of the misread codon to form six different groups. Group 1 is similar to Type 1 and corresponds to plant viruses like TMV that contain the CAA-UUA consensus sequence. Group 5 includes the luteovirus signal (CCNNNN repeat) of Type III. Group 2 (CGG) and Group 6 (CUA) also belong to Type III. Atkins and colleague also tested the importance of different nucleotides within these contexts using a dual luciferase fusion reporter. A C or G at the +1 position seems to be critical for read-through in most systems. Substitution of positions +2, +3 and +4 does not show significant effect. Only in the UGA of TRV did the +5 U to G substitution significantly increase read-through. However, alteration of the +6 position caused the most dramatic change (Harrel *et al*. 2002).

Suppressor tRNAs

Synthesis of a read-through protein depends primarily on the presence of appropriate suppressor tRNAs. *In vitro* translation of TMV RNA has been used as a convenient system for assaying 'natural' UAG suppressor tRNAs. To identify a UAG suppressor, tobacco rattle virus (TRV) was

used. To promote read-through over the leaky UAG codon of TMV RNA, the natural suppressor $tRNA^{Tyr}$ was isolated from tobacco leaves (Beier *et al*. 1984a). This tRNA was the first natural UAG suppressor characterized in plants. Only the $tRNA^{Tyr}$ with a GΨA anticodon allows effective read-through. This $tRNA^{Tyr}$(GΨA) is quite efficient, since addition of pure $tRNA^{Tyr}$ to reticulocyte lysate increases the yield of TMV UAG read-through by up to 35% (Beier *et al*. 1984a). This tRNA is rare in young wheat leaves but is abundant in older tissue, suggesting that read-through is developmentally regulated (Fütterer and Hohn 1996). The $tRNA^{Tyr}$ with a QΨA anticodon, which is abundant in wheat germ, is unable to stimulate UAG read-through (Beier *et al*. 1984b).

Grimm *et al*. (1998) identified two $tRNA^{Gln}$ isoacceptors from tobacco leaves as a second class of natural UAG suppressors. This $tRNA^{Gln}$ can promote read-through over the UAG termination codon in the TMV context in wheat germ extract depleted of endogenous tRNA. Two $tRNA^{Leu}$ have been isolated from calf liver and both are functional *in vitro* as amber suppressors of UAG codons of TMV RNA and RNA-2 of beet necrotic yellow vein virus (BNYVV) (Valle *et al*. 1987). The leaky UGA stop codon of TRV is suppressed by various suppressor tRNAs. A $tRNA^{Trp}$ with a CmCA anticodon originates from the chloroplast and was found to be more efficient than that from the cytoplasm (Zerfass and Beier 1992). TRV appears to be associated with mitochondria in infected cells and tRNAs found in chloroplasts can also be found in mitochondria. Thus, the virus may use mitochondrial tRNA as a suppressor *in vivo*. Baum and Beier (1998) have found that $tRNA^{Arg}$ U*CG is an efficient UGA supressor *in vitro* in the TRV context. Moreover, $tRNA^{Arg}$ U*CG is able to misread the UGA at the end of the coat protein cistron in RNA-1 of pea enation mosaic virus.

Frameshifting

For positive-stranded RNA viruses, ribosomal frameshifting is the prevailing recoding mechanism, having the added advantage of providing an economical means of storing and expressing viral genetic information. The efficiency of frameshift events are crucial, as it determines the stoichiometry of viral structural and enzymatic proteins that are available for virus particle assembly and replication, and therefore controls virus propagation (Dinman *et al*. 1998; Harger *et al*. 2002).

In most plant viruses known to undergo frameshifting, it is the catalytic domain of the RdRp that is expressed via frameshift, which allows the low-level synthesis of the polymerase to be controlled. The frameshift strategy is found in some genera of *Tombusviridae*, *Closteroviridae*, *Luteoviridae* and Sobemovirus and Umbravirus. All employ −1 frameshifting except the *Closteroviridae* family, which use the +1 direction.

–1 frameshifting

The phenomenom of −1 frameshifting was first discovered in animal retroviruses, where two cis-acting elements in the viral mRNA are required for the frame shift to occur: a heptanucleotide sequence of the form X-XXY-YYZ (X= A/G/C/U; Y=A/U; Z=A/C/U); and an RNA secondary structure, usually a pseudoknot, beginning about 5 or 6 nt downstream (ten Dam *et al*. 1990). The length and sequence of the spacer between the two cis-acting signals also contributes to the efficiency of frameshifting. Changes to the "slippery heptamer" sequence, but not upstream or downstream of the heptamer, which interrupt its repetitive structure, can drastically reduce the frequency of frameshifting (Jacks *et al*. 1988). Jacks *et al*. (1988) suggested a simultaneous-slippage model for −1 frameshifting in RSV (Rous Sarcoma Virus), and the evidence points to a similar mechanism in plant viruses. Each of the two ribosome-bound tRNAs slip simultaneously in the 5′ direction from their initial position in the zero frame (XXY-YYZ) to the −1 frame (XXX-YYY), and translation resumes in the new (−1) reading frame. In this model, the tRNAs form at least two base pairs in the shifted frame with the viral RNA and allow a mismatch only at the wobble positions. The slippage of the peptidyl-tRNA and aminoacyl-tRNAs bound in the ribosomal P and A sites, respectively, occurs before peptide transfer. Weiss *et al*. (1989) proposed that the slip occurs after peptide transfer (reviewed in Farabaugh 1996). Frameshifting is stimulated by secondary structures such as mRNA pseudoknots (Brierley *et al*. 1989) or, more rarely, stem loops (Kim and Lommel 1998). mRNA pseudoknots consists of two nested stems, the loop of one stem forming the base-pairs of the second. The structure of the pseudoknot in BWYV RNA has been determined by X-ray crystallography (Su *et al*. 1999) and NMR (Cornish *et al*. 2005). It is thought that the pseudoknot causes elongating ribosomes to pause over the slippery site (Somogyi *et al*. 1993; Lopinski *et al*. 2000), allowing more time for anticodon: mRNA realignment to occur. However, the precise mechanism by which downstream RNA pseudoknots stimulate frameshifting remains unknown (for review, see Giedroc *et al*. 2000). The pseudoknot may interact with the elongating ribosome with the same domain required for the ribosomal helicase site. This would transiently block helicase activity, impeding the melting of the secondary structure and causing a pause in ribosome movement (Takyar *et al*. 2005).

Other elements can affect the efficiency of frameshifting. A downstream termination codon in the −1 frame enhances frameshifting (Lucchesi *et al*. 2000), probably because it represents another pausing element for the translating ribosome. Upstream and spacer sequences can influence frameshifting (Kim *et al*. 2001; Mäkeläinen and Mäkinen 2005). Interestingly, for Cocksfoot mottle virus (CfMV), the viral proteins produced via −1 frameshifting can themselves regulate −1 frameshifting. Co-expression of the frameshift products, P27 and replicase, together with a dual reporter vector containing a minimal frameshift signal revealed that only P27 reduces production of the downstream reporter (Mäkeläinen and Mäkinen 2005).

In plant viruses, the most detailed studies on −1 frameshifting have been performed on luteoviruses, particularly BYDV. Luteoviruses have a single-stranded, positive-sense RNA genome containing six ORFs. The −1 frameshift is used by luteoviruses to control the molar ratio of the P1-encoded viral protease and the P2-encoded RdRp. The efficiency of this frameshifting event varies between 2% and 15% among different viruses. For BYDV-PAV (PAV serotype), the first two ORFs, 39 kDa and 60 kDa, overlap by 13 nucleotides. The 60 kDa ORF is expressed by ribosomal frameshifting (Brault and Miller 1992). Before encountering the termination codon, a small proportion of the ribosomes slip into the −1 frame and continue to translate the 60 kDa ORF to give a low-abundance 99 kDa fusion protein. The frameshift takes place at the "shifty" site, G GGU UUU, and a large bulged stem-loop structure is predicted (Di *et al*. 1993). Barry and Miller (2002) have shown the presence of an additional sequence located 4 kb downstream of the shifty signal to be essential for frameshifting in wheat germ extract. This sequence includes a 50-base essential "core" element and an adjacent "enhancer" region, and can base-pair to the large bulged stem-loop adjacent to the frameshift site. Because the translating ribosomes and the viral replicase molecules are moving in the opposite direction on the same mRNA template, Miller and colleagues (reviewed in Miller and White 2006 and references therein) have proposed a model in which the long-distance base-pairing event allows replication and translation to occur without competition between viral replicase and ribosome.

The carlavirus PVM (potato virus M) is unique among plant viruses in that the frameshift produces a capsid protein. Translation of the 46 kDa CP/12K transframe protein requires a −1 frameshift at a particular slippage site (Gramstat *et al*. 1994). An *in vitro* assay has shown that only four adenosine nucleotides and a 3′ flanking UGA stop codon

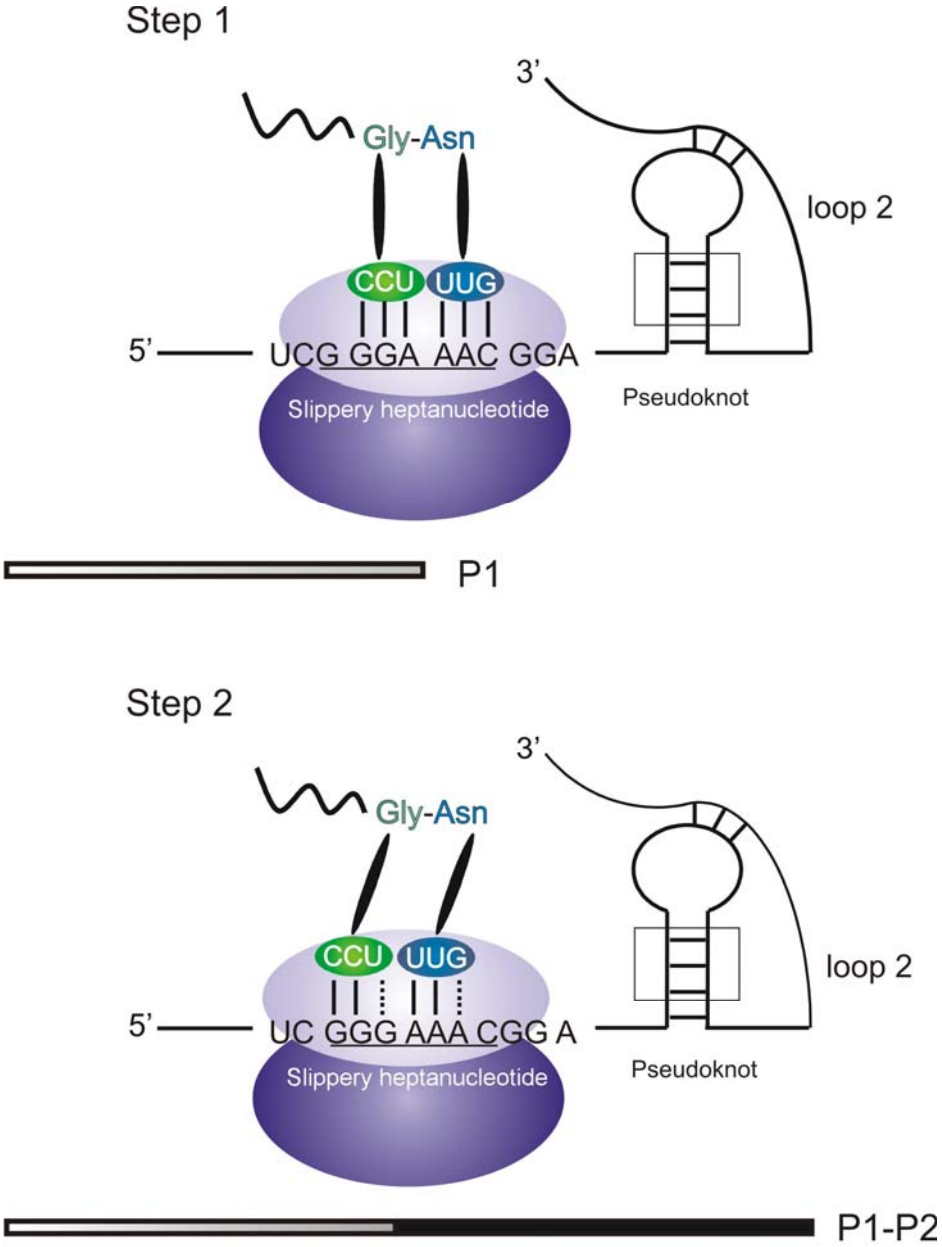

Fig. 6 –1 frame shifting strategy of BWYV. The simultaneous slippage of the aminoacyl-tRNA and the peptidyl-tRNA in the –1 direction (from step 1 to step 2). The loop 2 and stem of three G-C pairs (boxed) are conserved in luteoviruses. The P1-P2 frame-shifted protein shares 146 aa with P1.

are required for efficient frameshifting. The stop codon may cause the ribosome to pause, allowing the slippage to occur with a tRNA in only the P site (Gramstat *et al*. 1994).

+1 frameshifting

In the *Closteroviridae* family, was postulated to express their RdRp via a +1 ribosomal frameshift in the ORF 1a/1b region (Agranovsky *et al*. 1994; Karasev *et al*. 1995; Klaassen *et al*. 1995; Karasev *et al*. 1996). This is the only documented example of a eukaryotic virus group that uses +1 frameshifting. In BYV, Agranovsky *et al*. (1994) proposed a model of +1 frameshifting that is quite similar to –1 frameshift models (**Fig. 6**). It involves a GGGUUU slippery sequence immediately upstream of the UAG terminator and two hairpins, one preceding and another following the terminator. However, the differences in nucleotide sequence within the ORF 1a/1b region between BYV and CTV (the slippery sequence and the RNA secondary structure are not conserved in CTV) suggest that the mechanism mediating the +1 frameshift might differ, even though the frameshift event occurs at the same point (Karasev *et al*. 1995). Another model involving ribosome pausing at a terminator or at a rare codon similar to the models proposed for yeast retrotransposons with +1 frameshifting has been suggested (Farabaugh *et al*. 1993). Analysis of the BYSV sequence seems to favour the CTV model of a +1 frameshift (Karasev *et al*. 1996). No conservation between the amino acid sequence of the LIYV product and those of CTV, BYSV and BYV has been detected (Klaassen *et al*. 1995). Another mechanism suggested for the expression between LIYV ORF1a and ORF1b consists of slippage by $tRNA^{Lys}$ on the sequence AAAG.

CONCLUDING REMARKS

Viruses depend on the translational machinery of their hosts, but seem to know well how to manipulate the regulatory mechanisms existing in the cell and even how to activate mechanisms that are normally prohibited. Even without canonical RNA termini, plant RNA viruses can mimic RNA closed-loop structures, often using canonical translation initiation factors as a means to circularize their RNA. The interplay of eIFs and regulatory regions on the non-canonical viral 5′ and 3′ RNA termini seems to be important for controlling the efficiency and efficacy of viral translation. The competitiveness of viral mRNAs could explain why plant viruses probably do not globally shut off host gene expression. Instead of cleaving eIF4G or eIFiso4G to shut off cellular translation, as some animal viruses do, plant viruses appear rather to manipulate these factors for their benefit. IRES elements, which provide animal viruses with the ability to initiate translation despite the global shut-off of cap-dependent translation, have also been identified in some

plant viruses. However, these elements are structurally distinct from those found in animal viruses and it remains to be demonstrated whether IRES-driven translation initiation is essential for viral infectivity in plants. Translational recoding via read-through and frameshifting mechanisms endows viruses with increased coding capacity. However both mechanisms occur at low frequency, suggesting the importance of particular expression ratios. Plant pararetroviruses make use of several non-canonical mechanisms of translation initiation, including ribosome shunting, leaky scanning and reinitiation. Ribosome shunting is thought to preserve a leader-based packaging signal on the viral pgRNA from being melted by scanning ribosomes, which would interfere with binding of coat protein. Interestingly, *cis*-elements comprising the shunt configuration are evolutionary conserved. Leaky scanning and virus-mediated reinitiation are strategies allowing expression of more than one protein from a polycistronic viral RNA. Remarkably, the cellular taboo regarding ribosome reinitiation after translation of a long ORF is broken by a single pararetroviral protein, TAV, through its multiple interactions with host factors including ribosomal proteins and eIFs.

We believe that plant viruses will continue to serve as among the best tools for studying the molecular mechanisms of eukaryotic translation and for discovering novel cellular factors regulating translation.

ACKNOWLEDGEMENTS

We thank Helen Rothnie and Mario Keller for helpful comments on the manuscript. Work in our laboratory was supported by grants from Fonds National de la Science du France, ACI BCMS364 (Biologie Cellulaire, Moléculaire et Structurale), and from Agence Nationale de la Recherche de France, ANR-BLANC-2_135889, to LR and by grants Swiss National Science Foundation and Indo-Swiss Collaboration in Biotechnology to MP.

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