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End-to-end communication in the modulation of translation by mammalian RNA viruses

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Abstract

A 5′–3′ end interaction leading to stimulation of translation has been described for many cellular and viral mRNAs. Enhancement of viral translational efficiency mediated by 5′ and 3′ untranslated regions (UTRs) has been shown to occur via RNA–RNA interactions or novel RNA–protein interactions. Mammalian RNA viruses make use of end-to-end communication in conjunction with both viral and cellular factors to regulate multiple processes including translation initiation and the switch between translation and RNA synthesis during the viral lifecycle.
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1. Mechanisms of translation of positive strand viruses

Due to the complexity of protein synthesis, viruses cannot encode all the components necessary for translation; therefore, they are dependent upon the availability and activity of cellular translation factors. During eukaryotic cap-dependent translation, initiation factors (eIF4F, the cap-binding complex), mediated by the cap-binding protein, eIF4E, recognize an m^{7}GpppN-cap structure at the 5′ end of mRNAs (Gingras et al., 1999). The eIF4F cap-binding complex consists of eIF4E, an adaptor protein (eIF4G), and a helicase (eIF4A) that functions in complex with the co-factor eIF4B (Fig. 1A). In the cell, the 40S ribosome associates with the initiator methionyl-tRNA/eIF2-GTP ternary complex, eIF3, and eIF1A to form the 43S pre-initiation complex (Pestova et al., 2001). Only when bound to the RNA cap structure can the eIF4F complex, mediated by eIF3, recruit the 43S ribosomal complex to the mRNA (Gingras et al., 1999). This forms the 48S complex, which scans the RNA until the AUG initiation codon is encountered, at which point GTP is hydrolyzed, the initiation factors are released, the 60S ribosomal subunit binds the pre-initiation complex to form the 80S ribosome, and translation elongation begins (Pestova et al., 2001).

Two general mechanisms exist by which viruses initiate translation: cap-dependent and cap-independent. For the purpose of

this review, the focus will be on mammalian positive-sense RNA viruses, with a particular emphasis on flaviviruses. Genomes of members of the viral families Coronaviridae, Flaviviridae, Reoviridae, and Togaviridae contain an m^{7}GpppN-cap structure at the 5′ end of mRNA and are presumed to initiate translation in a cap-dependent manner. In contrast, RNAs from a diverse group of viruses, which include members of the Caliciviridae, Flaviviridae, and Picornaviridae families, are able to bypass dependency upon an m^{7}G cap structure for translation initiation via various mechanisms. Initiation of protein synthesis through the use of an internal ribosome entry site (IRES) is one such mechanism. The IRES element directs translation in the absence or with a reduced number of cellular translation factors, thus avoiding competition for scarce initiation factors, especially eIF4E, whose availability is among the most highly regulated within the cell (Gingras et al., 1999). Once cellular translation factors have bound to IRES RNA secondary and tertiary structure, they are used to direct ribosomal subunits to the translational start site in the absence of scanning.

While there appears to be no universal mechanism of viral internal ribosome entry, the best characterized viral IRES elements are those of the Picornaviridae family and the hepacivirus (Hepatitis C virus, HCV) and pestivirus genera of the Flaviviridae family. These functionally disparate viral IRES elements associate with different groups of translation factors. Most of the picornavirus IRES elements require the same translation initiation factors as capped mRNAs except for eIF4E, poly(A)-binding protein (PABP), and the N-terminal fragment of eIF4G

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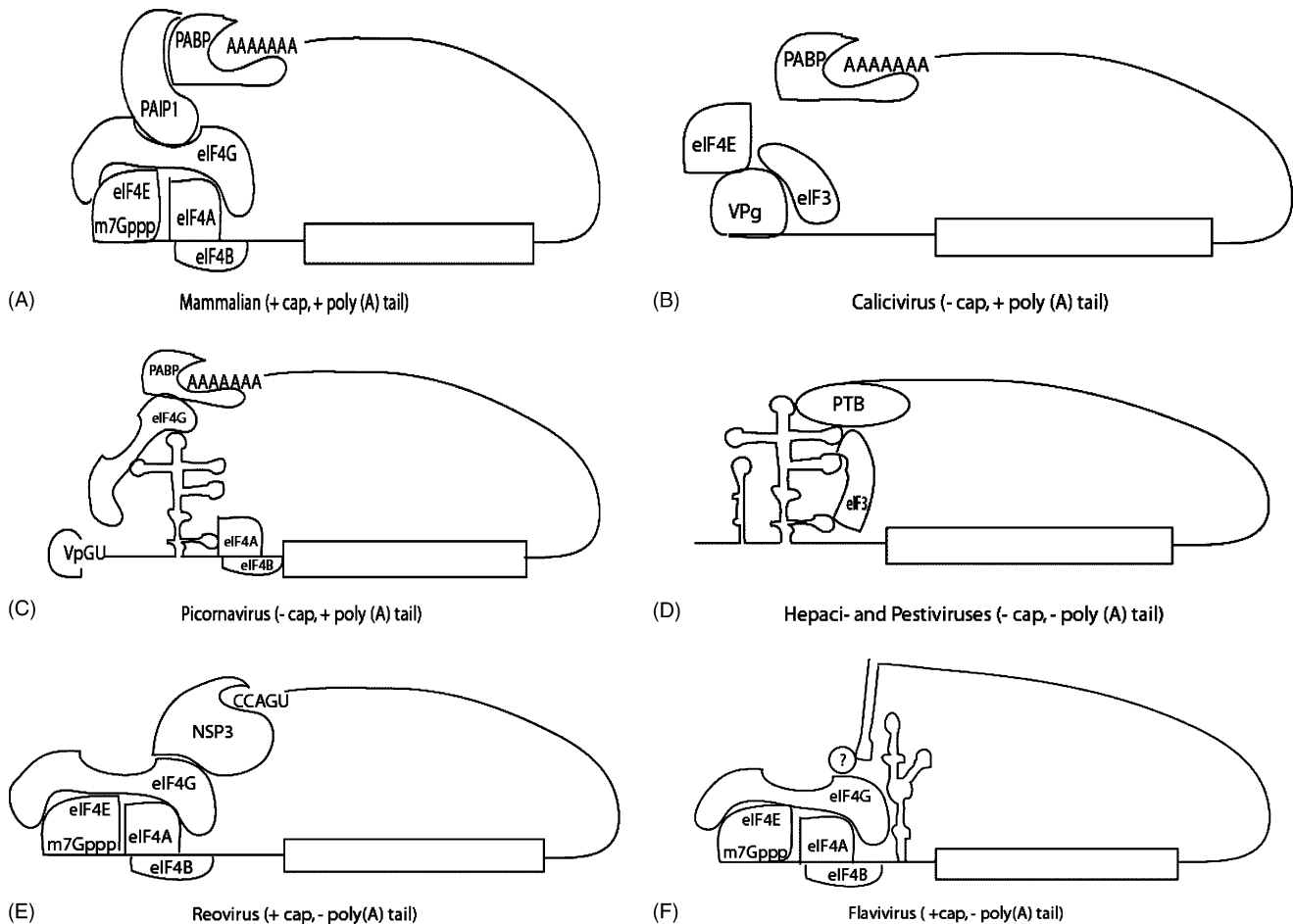*E-mail address:* eharris@berkeley.edu (E. Harris).

Fig. 1. Mechanisms of translation initiation. (A) Mammalian mRNA, (B) calicivirus RNA, (C) picornavirus RNA. Shown is the VPg moiety that is covalently bound to the 5′ end of the genomic RNA, (D) hepaci- and pestivirus RNA, (E) reovirus RNA, and (F) flavivirus RNA.

(Lomakin et al., 2000; Ohlmann et al., 2002; Pestova et al., 1996). The HCV and pestivirus IRESes, on the other hand, can bind and position the 40S subunit specifically and stably in the absence of any eIF, such that the ribosomal P site is placed immediately upstream of the initiator AUG (Pestova et al., 1998). They do, however, require eIF3 for efficient translation initiation (Kieft et al., 2001; Sizova et al., 1998).

Finally, members of the family Caliciviridae undergo cap-independent translation initiation through an entirely different mechanism. The naturally uncapped genomes of caliciviruses are instead covalently linked at their 5′ ends to the viral protein VPg (Fig. 1B) (Herbert et al., 1997). VPg has been found to interact directly with the translation initiation factors eIF4E and eIF3, promoting translation initiation from VPg-linked viral RNA while inhibiting the translation of capped mRNAs (Daughenbaugh et al., 2003; Goodfellow et al., 2005).

2. Communication between the 5′ and 3′ ends of mRNA

2.1. Enhancement of translation

In addition to the 5′ m^{7}GpppN cap structure, nearly all cellular mRNAs contain a region of polyadenylation (poly(A) tail) at their 3′ ends. Translation of mRNAs is most efficient when there

is an interaction between the 5′ cap structure and the 3′ poly(A) tail (Sachs et al., 1997; Tarun and Sachs, 1995). It has been shown that both cap-dependent and cap-independent translation are stimulated by factors that bind the poly(A) tail, presumably circularizing the mRNA (Bergamini et al., 2000; Michel et al., 2001). For many mRNAs, this 5′–3′ linkage is mediated by the interaction of the 5′-bound eIF4G and the 3′-bound poly(A)-binding protein (PABP), either directly (Otero et al., 1999; Tarun and Sachs, 1996) or via the bridging protein, PABP-interacting protein (PAIP-1) (Fig. 1A) (Craig et al., 1998). PABP may also interact specifically with ribosomal subunits through the use of RNA recognition motifs (RRM) within PABP, which allow it to bind directly to ribosomal RNA (Imataka et al., 1998).

Many mammalian RNA viruses (e.g. togaviruses, picornaviruses, and coronaviruses) contain genomes that terminate in poly(A) tails, which are similar in length to cellular poly(A) tails and are thought to function in translation like their cellular counterparts. Despite the absence of a cap structure at the 5′-end of the picornavirus genome, the presence of PABP has been shown to enhance initiation of translation from picornavirus IRES elements (Fig. 1C) (De Benedetti et al., 1991; Michel et al., 2001; Paulous et al., 2003). However, poly(A)-mediated stimulation of picornaviral IRES activity depends on the integrity of eIF4G and the interaction of intact eIF4G with PABP (Michel et al., 2001).

Thus, since eIF4G is cleaved over the course of picornaviral infection, it is likely that this interaction is important only early in infection. Despite recent advances in understanding the role of the viral 3′UTR in the regulation of translation, the function of mRNA circularization for IRES-dependent translation is not yet fully characterized.

The 3′UTRs of IRES-containing viral genomes are also involved in the regulation of viral protein expression through the binding of cell-specific proteins required for IRES activity. For example, sequences upstream of the poly(A) tail in the picornavirus 3′UTR seem to be involved in the replication of the virus specifically in neuronal cells (Brown et al., 2004; Dobrikova et al., 2003). In addition to stimulating translation via a poly(A)/PABP interaction, sequences in the picornavirus 3′UTR upstream of the poly(A) tail are able to enhance IRES-driven translation in the absence of the poly(A) (Lopez de Quinto et al., 2002). Various cellular proteins may play a role in this process. For example, the cellular proteins eukaryotic elongation factor 1A (eEFIA), La autoantigen (La), murine proliferation-associated protein-1 (Mppl), poly-r(C)-binding protein (PCBP), and polypyrimidine tract binding protein (PTB) have been shown to bind the 5′UTR of picornaviruses and to enhance translation from picornavirus IRES elements (Blyn et al., 1996, 1997; Borman et al., 1993; Florez et al., 2005; Hellen et al., 1993; Kolupaeva et al., 1996; Meerovitch et al., 1993; Pilipenko et al., 2000; Svitkin et al., 1994), and some of these cellular proteins have also been observed to bind to the 3′UTR of the Norwalk calicivirus (La, PTB, and PABP) (Gutierrez-Escolano et al., 2003) and the HCV genomic RNA (PTB) (Fig. 1D) (Ito and Lai, 1999). Taken together, these observations suggest a potential means of communication between the viral UTRs wherein these cellular proteins interact with the viral RNA to functionally replace certain canonical translation factors.

Several viral genomes lack the poly(A) tail as a translational enhancer, including rotavirus (Reoviridae) and members of the Flaviviridae family. However, enhancement of translational efficiency via interaction of the 5′ and 3′ ends has been demonstrated for these viruses by a range of mechanisms (Chiu et al., 2005; Holden and Harris, 2004; Piron et al., 1998). For example, circularization and translational enhancement of the m^{7}G-capped rotavirus genome are mediated by the virally encoded protein NSP3, which binds to a conserved sequence in the viral 3′UTR (UGUGACC) that functionally substitutes for the poly(A) tail. The 5′ and 3′ ends of each segment of the rotavirus genome (six RNAs in total) are brought together through the direct interaction of the NSP3 protein and eIF4G (Fig. 1E) (Piron et al., 1998; Vende et al., 2000). An additional translation enhancer located only in the 3′UTR of the rotavirus gene 6 mRNA has been shown to augment expression of the major capsid protein VP6 (Yang et al., 2004).

The HCV genome is also not polyadenylated at its 3′ end. Although the HCV RNA does not require eIF4G for translation (Pestova et al., 1998), the HCV IRES has been shown to interact in vitro with recombinant cellular factors such as PTB (Ali and Siddiqui, 1995) and La (Ali and Siddiqui, 1997). PTB can bind to both the IRES (Ali and Siddiqui, 1995) and the X region at the 3′ end of the RNA (Tsuchihara et al., 1997). The multimerized PTB

(Perez et al., 1997) may then circularize the HCV genome by binding to both ends of the RNA (Fig. 1D). While this interaction may not be critical for IRES-driven translation in transfected cell lines (Kong and Sarnow, 2002), its importance in the viral lifecycle in vivo remains to be tested.

Finally, although flaviviruses, such as the dengue (DENV) and West Nile (WNV) viruses, are not polyadenylated, the viral 3′UTR contains conserved regions including: the terminal 100 nucleotides (nt), which form a conserved stem loop termed the 3′SL (Brinton et al., 1986); structures that are predicted to form pseudoknots (Shi et al., 1996); and a 3′ cyclization sequence (CS) that is complementary to a CS at the 5′ end of the genome (Hahn et al., 1987). The DENV 3′UTR has been shown to stimulate translation of reporter constructs (Edgil et al., 2003; Holden and Harris, 2004), and half of this stimulation can been attributed to the action of the 3′SL (Fig. 1F) (Holden and Harris, 2004). This has been confirmed by other investigators who have found that the 3′SL contributes significantly to translational enhancement by the 3′UTR in DENV reporter constructs (Chiu et al., 2005). Consistent with these results, recent studies using phosphorodiamidate morpholino oligomers (PMOs) directed to the top of the DENV 3′SL demonstrated an approximately 50% reduction in translation using DENV reporter constructs and DENV replicons containing the nonstructural protein genes in addition to a luciferase reporter (Holden et al., in press). DENV replicons containing mutations in the loops at the top of the 3′SL also reduced translation by approximately 50% compared to the wildtype replicon (K. Clyde, K.L. Holden, E. Harris, unpublished results). Furthermore, similar PMOs targeting the 3′SL of WNV have recently been shown to reduce replication of WNV replicons (Deas et al., 2005), and mutations in the 3′SL of infectious viral RNA negatively impact flavivirus RNA synthesis, host-range and viability (Markoff et al., 2002; Zeng et al., 1998). Conversely, an earlier study using WNV reporter constructs suggested that the WNV 3′SL in the absence of the rest of the viral 3′UTR may inhibit translation of reporter RNAs (Li and Brinton, 2001). While it is clear that the flavivirus 3′UTR plays a role in the modulation of translation efficiency, the mechanism of action is likely regulated by many factors, including the genomic context of the 3′SL.

One of the main functions of the 3′SL seems to be the recruitment of viral and cellular proteins. The flavivirus 3′SL has been shown to bind to the viral proteins NS5, NS3, and NS2A, which presumably form part of the replication complex (Chen et al., 1997; Khromykh et al., 2000), as well as to the cellular proteins eEFIA, La, and PTB (Blackwell and Brinton, 1995, 1997; De Nova-Ocampo et al., 2002; Garcia-Montalvo et al., 2004). While the functional consequence of the interaction of these proteins with the 3′UTR has not yet been demonstrated, it may serve to more effectively recruit and/or stabilize critical translation factors at the 5′UTR. These ribonucleoprotein (RNP) complexes may mediate the interaction of the viral UTRs as well as play a role in the switch between translation and replication of the viral genome (see below).

Conserved domains other than the 3′SL in the 3′UTR of flaviviruses have been shown to regulate flavivirus translation. For instance, when both pseudoknot domains are deleted from

DENV reporter replicons or reporter constructs, translation is reduced 40–75%, respectively (Alvarez et al., 2005a; Chiu et al., 2005) (K.L. Holden, E. Harris, unpublished results). In addition, these domains appear to play a more dramatic role in regulation of viral RNA synthesis (Alvarez et al., 2005a; Lo et al., 2003; Tilgner et al., 2005). Interestingly, when the entire 3′UTR is deleted from DENV or WNV reporter replicons, little effect on translation is observed (Alvarez et al., 2005a; Tilgner et al., 2005), implying that both positive and negative regulators of translation exist within the flavivirus 3′UTR.

Although it is clear that the circularization of cellular and viral mRNAs stimulates translation, the exact mechanism has not been defined. In the 'closed-loop' model of mRNA translation, it is thought that cyclization may serve to stabilize the mRNA and the translation complex. Furthermore, the interaction of the UTRs may ensure translation only of full-length RNAs that contain both the 5′ and 3′ ends. However, under certain conditions, stimulation of translation of cellular mRNAs can be mediated by the poly(A) in *trans* in a cell-free system, suggesting that it is not circularization of mRNAs, but the interaction of the ends with the translational machinery that prompts maximal translation efficiency (Borman et al., 2002). Alternatively, circularization may promote efficient recycling of ribosomes and rapid re-initiation of translation on the same strand of RNA (Sachs, 2000). This strategy would be particularly advantageous for viral RNAs that need to compete effectively with cellular messages for limited translation factors and ribosomes.

2.2. Alternative strategies of viral translational control

While DENV and other flaviviruses containing capped RNA genomes undergo cap-dependent translation, DENV has been shown to translate efficiently under circumstances in which cap-dependent cellular translation is suppressed through the depletion of eIF4E (Edgil et al., submitted for publication). To investigate this phenomenon, several approaches were used to inhibit cap-dependent translation. Initially, the compounds rapamycin, wortmannin and LY294002 were used to suppress cellular translation initiation through the sequestration of eIF4E. The downstream effect of the interaction of rapamycin with the mammalian target of rapamycin (mTOR) is the hypo-phosphorylation of the eIF4E-binding protein 1 (4E-BP). Similarly, wortmannin and LY294002 inhibit the phosphoinositol-3-kinase (PI3-kinase) pathway, which also results in the hypo-phosphorylation of 4E-BP (Vanhaesebroeck et al., 2001). In its hypo-phosphorylated form, 4E-BP binds to and sequesters eIF4E from the eIF4F translation initiation complex, thus inhibiting cap-dependent translation. The impact of treatment with these drugs on translation of cellular cap-dependent mRNAs was compared to their effect on DENV reporter constructs and on replication of infectious viral RNA. When cells were incubated with DENV in the presence of rapamycin, LY294002 or wortmannin, viral titers remained unchanged at 24 h post-infection (Fig. 2A). These results are consistent with recent studies that have shown both DENV and JEV infection to be resistant to treatment with LY294002 (Lee et al., 2005). Furthermore, whereas metabolic labeling of total cellular protein

in inhibitor-treated cells revealed an inhibition of cellular protein synthesis 12 h post-infection, translation of DENV proteins, represented by the DENV RNA-dependent RNA polymerase (NS5), was relatively unaffected (Fig. 2B).

To measure the impact of these inhibitors on viral translation specifically, a series of reporter constructs containing either the DENV 5′UTR or the control human beta-globin (BG) 5′UTR fused to the firefly luciferase gene (Luc) followed by either the DENV-2 3′UTR, a 60-mer poly(A) tail, or 268 nt of vector sequence was generated (Fig. 3A). Luc activity was measured from RNA transcripts that were generated from the constructs above and transfected into cells in the presence of rapamycin, wortmannin, or LY294002. It was observed that translation of only the reporter construct containing both the DENV 5′ and 3′UTRs was unaffected by the presence of drugs that inhibit translation of cap-dependent cellular messages, whereas that of the other mRNAs was reduced (Fig. 3B). Similar results were observed in cells in which cap-dependent translation was inhibited via small interfering RNA (siRNA)-mediated gene silencing of eIF4E or expression of constitutively hypo-phosphorylated 4E-BP (data not shown). Collectively, these results indicate that, despite the presence of a 5′ cap structure on the DENV genome, DENV replication and DENV translation are resistant to the effects of inhibition of cap-dependent translation.

To further examine the ability of the DENV genome to replicate in the absence of a functional cap structure under conditions in which eIF4E is limiting, RNA was transcribed from the DENV-2 infectious clone to contain either a functional 5′ m^{7}GpppA cap structure or a nonfunctional 5′ ApppA cap. Equal amounts of these RNAs were then transfected into cells treated with inhibitors of cap-dependent translation. When supernatants were analyzed by plaque assay 48 h post-transfection, cells containing the ApppA-capped viral RNA released no infectious progeny, compared with titers of ∼10^3 pfu/ml released by cells that had been transfected with the m^{7}G-capped viral RNA (Table 1, column 1). These results suggest that, in cells competent for cap-dependent translation, DENV translates exclusively via its 5′ m^{7}G-cap structure. Replication of the m^{7}G-capped viral RNAs was resistant to the presence of the inhibitors of cap-dependent translation (Table 1, row 1), similar to results obtained with the virus itself (Fig. 2A). Moreover, replication of the nonfunctionally capped viral genomes was increased to ∼10^2 pfu/ml (Table 1, row 2), indicating that the DENV viral genome can be induced to replicate independently of the cap structure under conditions of reduced eIF4E. This data is also consistent with increased levels of translation observed with ApppA-capped DENV reporter RNAs in inhibitor-treated cells relative to ApppN-capped reporter RNAs containing the beta-globin 3′UTR, the poly(A) tail, or both (data not shown).

Together, these data support a model in which DENV is able to alternate from a canonical cap-dependent form of translation initiation to a noncanonical mechanism under conditions of reduced eIF4E (Fig. 4). It is clear that the interaction of the DENV 5′ and 3′UTRs stimulates translation; when eIF4E is present, translation initiation presumably occurs through a classical cap-dependent scanning mechanism. However, under conditions in which eIF4E or other cellular translation factors
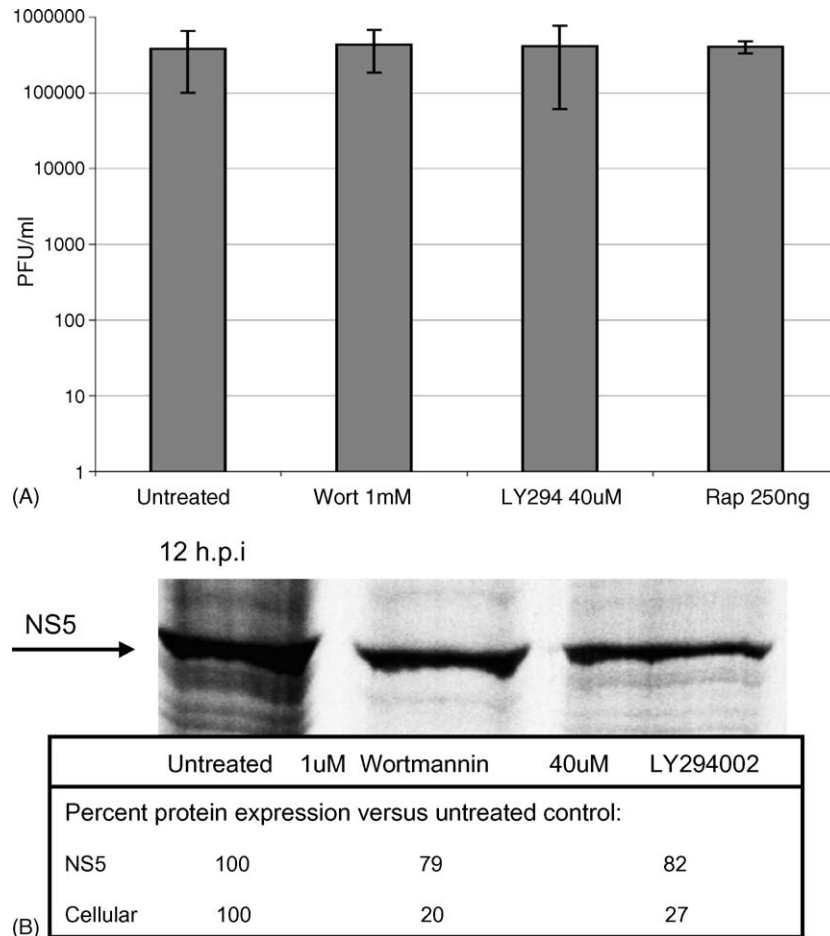
Fig. 2. DENV replication and translation are not affected by inhibitors of cap-dependent translation, (A) DENV is replicated in the presence of rapamycin, LY294002 or wortmannin. Cells were exposed to DENV for 1 h in the presence of inhibitors, washed, and incubated with rapamycin (250 ng/ml), LY294002 (40 μM) or wortmannin (1 μM) for 24 h. Supernatants were collected and titered by plaque assay (PFU/ml), (B) DENV RNA is translated in the presence of LY294002 or wortmannin. Cells were prepared as described above. At 12 h post-infection, cells were metabolically labeled and analyzed by SDS-PAGE. ImageQuant software (Molecular Dynamics) was used to quantify the DENV protein NS5 (arrow) and representative cellular proteins.

are limiting, such as in differentiated cell types with low levels of eIF4E (Grolleau et al., 1999; Krichevsky et al., 1999) or as a result of the IFN antiviral response (Gil et al., 1999), efficient viral translation requires both the DENV 5′ and 3′UTRs. This data suggests a model in which viral RNA that is translated in cellular conditions where eIF4E is limiting undergoes a reorganization of viral RNP complexes such that RNA structures or sequences in the 3′UTR interact with higher affinity with protein complexes containing eIF4G and eIF4A. This would result in effective delivery by the DENV 3′UTR of key translation initiation factors to the DENV 5′UTR, similar to the mechanism

proposed for cap-independent translation of *Luteovirus* RNAs, such as the barley yellow dwarf virus genome (Guo et al., 2001).

2.3. Switch between translation and RNA synthesis

Adding to the complexity of translational control, the 3′UTRs of cellular and viral messages often contain regulatory elements that negatively modulate expression. Whereas the poly(A) tail is thought to stimulate translation, regulatory elements upstream of the poly(A) tail in the 3′UTR may maintain viral RNA in an inactive state via binding of repressor proteins (Fu et al.,

Table 1
DENV can replicate independently of a functional 5′ cap under conditions of reduced eIF4E

| | Cell treatment | | |
	Untreated	40 μM LY294002	1 μM Wortmannin
m^{7}GpppA-capped DENV RNA (pfu/ml)	822 ± 269	1022 ± 758	2455 ± 1296
ApppA-capped DENV RNA (pfu/ml)	0	183 ± 42	378 ± 270

Equal amounts of functionally (m^{7}GpppA) or nonfunctionally (ApppA) capped in vitro transcripts generated from the DENV-2 infectious clone were transfected into cells for 4 h. Cells were washed and incubated with LY294002 or wortmannin for 24 h. Supernatants were then collected and titered on BHK cells.
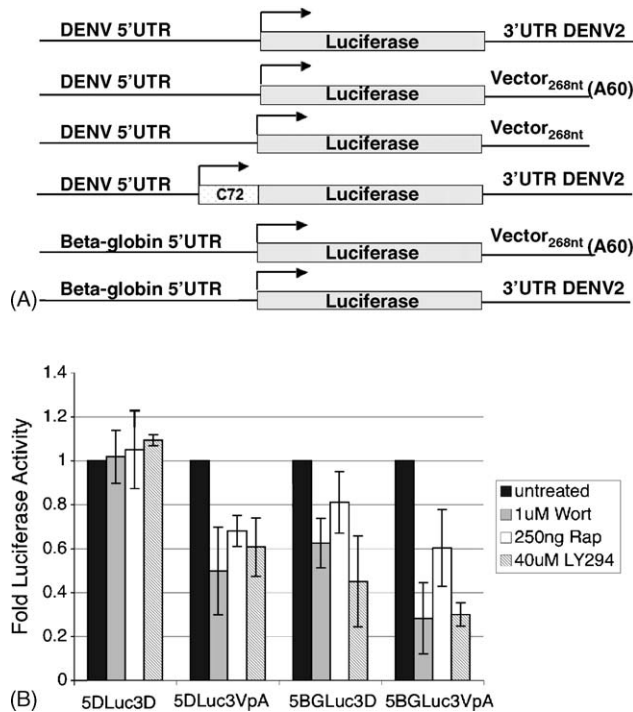
Fig. 3. Translation of DENV reporter RNA is not affected by inhibitors of cap-dependent translation (A) DENV RNA reporter constructs. RNA reporter constructs were generated containing either the DENV-2 5′UTR plus or minus the first 72 nt of the DENV coding region or the human β-globin (βg) 5′UTR fused to the firefly luciferase (Luc) gene, followed by either vector sequence, the DENV-2 3′UTR or vector sequence plus a 60-mer poly(A) tail. RNA transcribed in vitro from these constructs using T7 polymerase was transfected into cells, (B) m7G-capped DENV RNA reporter constructs are translated in BHK cells treated with rapamycin, LY294002 or wortmannin. Equal amounts of in vitro transcripts of the constructs described above were capped with m7GpppN and transfected into BHK cells. One hour post-transfection, cells were washed and 250 ng/ml rapamycin, 40 μM LY294002 or 1 μM wortmannin were added. Luc activity was assayed after 12 h by luminometry.
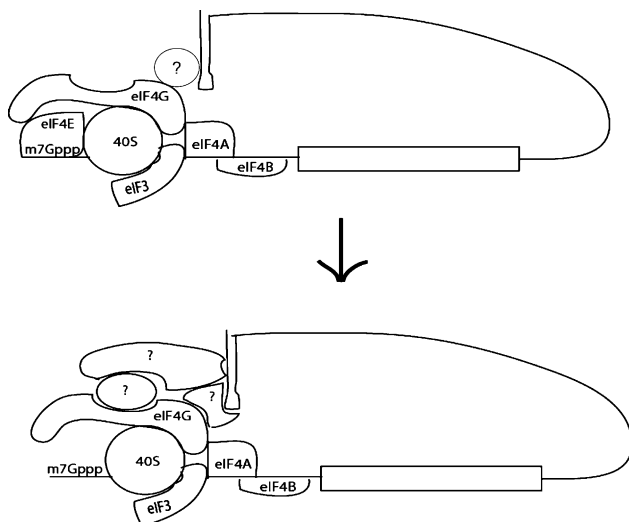
1999; Tadauchi et al., 2001). One reason for this level of control may involve the switch from translation to RNA synthesis that must occur in positive-stranded RNA viruses, which are unable to replicate RNA templates undergoing translation due to the collision of translation and replication complexes proceeding in opposite directions. For picornaviruses, the alternate binding of the cellular factor PCBP or the viral protein 3CD to certain viral RNA structures has been shown to control the function of the genomic RNA in translation versus RNA synthesis (Barton et al., 1999; Gamarnik and Andino, 1997, 1998). Additionally, both the 5′ and the 3′ termini of the pestivirus bovine viral diarrhea virus (BVDV) RNA genome have been shown to associate specifically with the 'NFAR' protein group (Isken et al., 2003). This interaction appears to be important for efficient translation termination and RNA replication and may function to regulate the switch between the two viral states (Isken et al., 2004).

Although the flavivirus DENV is not polyadenylated, the DENV 3′UTR contains conserved regions, including the cyclization sequences (CS) and tandem repeats (Hahn et al., 1987). The 5′CS, which comprises an 11-nt region ~35 nt downstream of the 5′ end of the capsid gene, and its complementary 3′CS sequence located ~100 nt from the end of the 3′UTR are the most conserved linear sequences in the flavivirus genome. Recently, additional regions of complementarity between the DENV 5′ and 3′UTRs have been identified and named UAR (for upstream AUG region); the 5′ sequence is 16 nt long and is located immediately upstream of the initiation codon while the 17 nt 3′UAR is found in the stem of the 3′SL (Alvarez et al., 2005b). Similar to the model for alphaviruses and bunyaviruses (Hsu et al., 1974; Talmon et al., 1987), it is hypothesized that base-pairing between the 5′ and 3′ ends, which circularizes the genome, ensures the synthesis of full-length genomic RNAs (Hahn et al., 1987). In fact, visualization of individual DENV viral RNA molecules by atomic force microscopy confirmed that cyclization of the viral genome is a direct result of the physical interaction of the CS and UARs (Alvarez et al., 2005b). Moreover, this circularization appears to be necessary for flavivirus replication; mutation of the CS or UAR sequences in infectious DENV RNA resulted in a lethal phenotype in mammalian cells (Alvarez et al., 2005b; Men et al., 1996). Similarly,
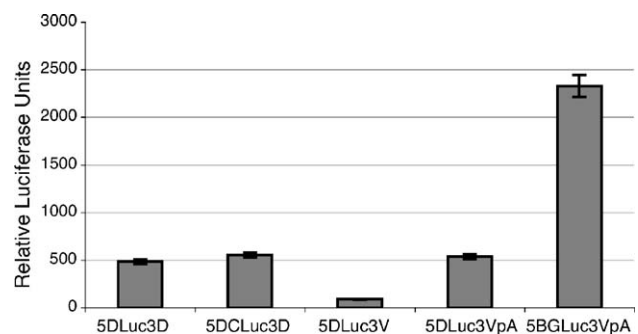

Fig. 4. Model of the reorganization of RNP complexes at the 5′ and 3′UTRs of the flavivirus genome under conditions of limiting eIF4E.

Fig. 5. DENV translation is independent of the CS. Equal amounts of m7GpppA-capped in vitro transcripts of the reporter constructs containing the DENV 5′ and 3′UTRs plus or minus the CS were transfected into cells, along with control reporter RNAs (see Fig. 2A). Cells were washed 1 h post-transfection, and Luc activity was assayed after 8 h by luminometry.
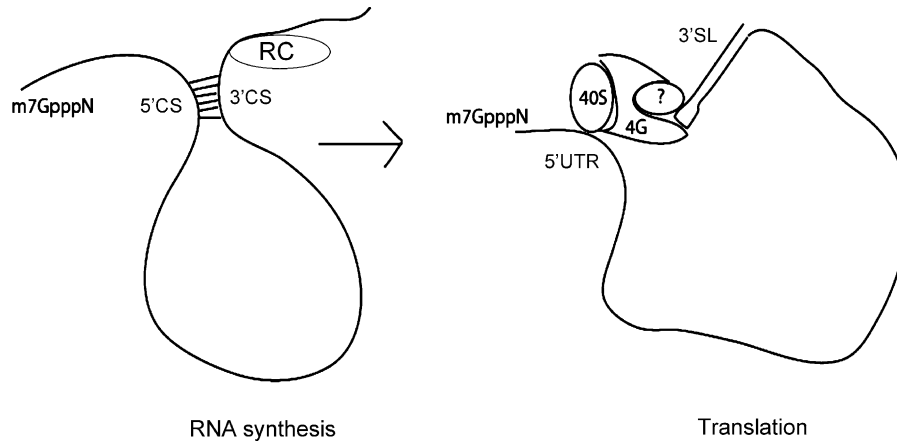
Fig. 6. Model depicting the switch between translation and replication of the flavivirus genome mediated through the architectural remodeling of viral RNA.

PMOs targeted to the 3′CS of a DENV or WNV replicon suppressed RNA replication specifically (Deas et al., 2005; Holden et al., in press). The flavivirus RNA-dependent RNA polymerase (RdRp) also appears to require CS base-pairing for RNA synthesis both in vitro and in vivo (Khromykh et al., 2001; You and Padmanabhan, 1999), whereas the precise role of the UAR sequences in RNA synthesis remains to be determined. Together, these data underscore the importance of the interaction between the 5′ and 3′UTRs in the viral lifecycle.

On the other hand, the flavivirus CS do not appear to be necessary for viral protein synthesis. To measure the influence of the CS on DENV translation, reporter constructs containing the DENV 5′ and 3′UTRs in the presence or absence of the first 72 nt of the capsid protein, which includes the 5′CS, were generated (Fig. 2A). Luc activity measured from cells transfected with either of these RNA constructs demonstrated similar translation efficiencies (Fig. 5), indicating that DENV was translated independently of the interaction of the CS. These results are consistent with recent reports demonstrating that hybridization of the 5′ and 3′CS in the context of either DENV reporter constructs or DENV replicons is not necessary for translation, and may even inhibit translation (Alvarez et al., 2005a; Chiu et al., 2005). However, the role of 5′–3′ end interaction in enhancing viral translation combined with the requirement for CS base-pairing for RNA synthesis suggests the existence of alternative conformations of the flavivirus CS and UTRs for distinct viral functions. Like the picornavirus genome, the flavivirus genomic RNA is the template for multiple stages of the viral lifecycle that cannot occur simultaneously on the same RNA. Regulation of these stages depends on the communication of the viral UTRs. During replication, base-pairing of the CS appears to be necessary for viral RNA synthesis. However, this same interaction may inhibit translation of the RNA. We speculate that the switch between translation and replication of the flavivirus genome is mediated by the architectural remodeling of portions of the genome from that of an RNA–protein bridge between the 5′ and 3′UTRs to RNA–RNA interactions between the CS and possibly the UARs at the extreme ends of the viral genome (Fig. 6). In this model, the switch between translation and replication is presumably mediated by viral and/or cellular factors that facilitate communication between the viral UTRs.

The genome of RNA viruses is, of necessity, extremely versatile. The compact size of an RNA genome demands ingenuity, in the form of overlapping open reading frames, frame-shifting, and multiple uses of the viral 5′ and 3′UTRs. To maximize the efficacy of the genome, RNA viruses regulate translation, RNA synthesis, and the switch between these two stages through the communication of the UTRs via both RNA–RNA and RNA–protein interactions.

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