Base-Pairing between Untranslated Regions Facilitates Translation of Uncapped, Nonpolyadenylated Viral RNA

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Summary

Translationally competent mRNAs form a closed loop via interaction of initiation factors with the 5′ cap and poly(A) tail. However, many viral mRNAs lack a cap and/or a poly(A) tail. We show that an uncapped, nonpolyadenylated plant viral mRNA forms a closed loop by direct base-pairing (kissing) of a stem loop in the 3′ untranslated region (UTR) with a stem loop in the 5′ UTR. This allows a sequence in the 3′ UTR to confer translation initiation at the 5′-proximal AUG. This base-pairing is also required for replication. Unlike other cap-independent translation mechanisms, the ribosome enters at the 5′ end of the mRNA. This remarkably long-distance base-pairing reveals a novel mechanism of cap-independent translation and means by which mRNA UTRs can communicate.

Introduction

The 5′ m7GpppN cap and poly(A) tail on eukaryotic mRNAs stabilize the message and interact synergistically to facilitate efficient translation initiation (Gallie, 1991; Preiss and Hentze, 1998; Tarun and Sachs, 1995). Eukaryotic initiation factor (eIF) 4E binding protein binds the poly(A) tail, and both of these proteins bind eIF4G to form a closed loop mRNA (Wells et al., 1998). This loop is required for efficient recruitment of the 43S ribosomal subunit complex to the 5′ untranslated region (UTR) of the mRNA (Hentze, 1997; Sachs, 2000). The 3′-5′ UTR interaction explains how translation of many mRNAs is controlled by signals in the 3′ UTR (Gunkel et al., 1998; Wicksens et al., 2000). The exact mechanism(s) by which these regulatory 3′ ends interact with the 5′ UTR and how closed loop mRNA facilitates translation initiation remain to be elucidated.

Many efficiently translated viral mRNAs lack a 5′ cap and/or a poly(A) tail. The uncapped RNAs of picornaviruses contain a long, structured, internal ribosome entry site (IRES) in the 5′ UTR that recruits ribosomes directly (Jackson, 2000). In most cases, eIF4E is unnecessary for their translation. The capped, nonpolyadenylated RNAs of rotaviruses (Piron et al., 1998) and tobornaviruses (Gallie and Walbot, 1990) harbor sequences in the 3′ UTRs that functionally mimic the poly(A) tail. Rotavirus protein NSP3A binds the poly(A)-mimic sequence and communicates with the 5′ end via eIF4G (Michel et al., 2000). RNAs of some plant, yeast, and animal (hepatitis C virus, Ito et al., 1998; Jackson, 2000) viruses are translated independently of both a cap and a poly(A) tail. How they act as efficient messages, and whether they form a closed loop, is unknown.

Members of genus Luteovirus (Wang et al., 1997) and the large Tombusviridae family (Meulewaeter et al., 1998; Qu and Morris, 2000; Wu and White, 1999) contain sequences in the 3′ UTR that promote cap-independent translation initiation at the 5′-proximal AUG on viral RNAs. A particularly efficient sequence is the 100 nt cap-independent translation element (TE) in the 3′ UTR of barley yellow dwarf luteovirus (BYDV) RNA (Wang et al., 1997). The 5′ UTRs of either BYDV genomic RNA (gRNA) or subgenomic RNA 1 (sgRNA1) are also necessary for the TE to function from the 3′ UTR, but these 5′ UTRs are not necessary in mRNAs that harbor the TE in the 5′ UTR (Wang et al., 1999). Thus, the viral 5′ UTRs are needed only for communication with the 3′ TE and do not serve other essential roles in ribosome recruitment. Here, we demonstrate that the 3′ and 5′ UTRs interact by direct Watson-Crick base-pairing, to bring about efficient translation of uncapped RNA.

Results

Sequences Required for 5′ UTR-3′ TE Interaction

To understand how the 5′ UTR interacts with the 3′ TE, we determined its secondary structure and domains needed for cap-independent translation. It consists of four stem loops (Figures 1A and 1B). Stem loop IV (SL-IV) was necessary and sufficient for significant cap-independent translation of luciferase (LUC) mRNAs containing the 3′ TE (Figure 1C), with sequence upstream of SL-IV possibly enhancing translation in vivo. Interestingly, a pyrimidine-rich tract was unnecessary. Such tracts function in many IRESes (Ito et al., 1998; Jackson, 2000). Additional viral sequence downstream of the 3′ TE was included in constructs tested in vivo, as shown previously to be necessary (Wang et al., 1999). This may be necessitated by the more competitive translation conditions that require the presence of a poly(A) tail (or a viral substitute) on mRNAs in vivo, but not in noncompetitive in vitro conditions (Michel et al., 2000; Preiss and Hentze, 1998). The additional viral sequence may also contribute to RNA stability in vivo.

Previously, we identified a candidate sequence in the TE for involvement in 3′–5′ communication. Changes to the loop (L-III) of stem loop III in the TE (SL-III, Figure 2A) knocked out translatibility when the TE was in the 3′ UTR, but did not affect its function in the 5′ UTR context (Guo et al., 2000). Thus, L-III may be involved primarily in communication between the 3′ and 5′ UTRs, and not directly in recruitment of translation machinery. The seven bases in L-III differ from those in loop (L-IV), of SL-IV in the 5′ UTR, only in the middle base. This difference results in complementarity between the central five bases in the two loops (Figure 2A). We propose that base-pairing (kissing) between these loops forms a translationally competent closed loop mRNA.
Figure 1. 5’ UTR Element Required for 3’ TE-Mediated Translation
(A) Alignment and structure probing of the BYDV genomic 5’ UTR. 5’ UTRs of known BYDV isolates (Genbank accession numbers: PAV-Vic, X07653; PAV-ILL, AF235167; PAV-JPN, D85783; PAV-P, D11032; PAV-129, AF218798; MAV-PS1, D11028) were aligned using CLUSTAL W (Thompson et al., 1994). Arrows and shading indicate predicted base-paired regions. Covariations that support base-pairing are underlined. Dashed box: pyrimidine-rich domain (PRD). The substrate was a 5’ end-labeled transcript consisting of the 143 nt 5’ UTR of isolate PAV-ILL followed by the first 47 nt of the LUC ORF. T1 nuclease sequencing ladder and 0.4 M imidazole cleavage reactions (cuts most single-stranded nucleotides) are shown on the horizontal gel (top of gel at right) under the aligned sequences.

(B) The MFOLD-predicted (http://bioinfo.math.rpi.edu/mfold/rna/form1.cgi) secondary structure of the 5’ UTR superimposed with imidazole cleavage sites.

(C) Translation of 3’ TE-containing reporter transcripts with viral 5’ UTR truncations or substitutions indicated at left. Thick lines: portions of 5’ UTR with positions indicated by base numbers. Thin line: vector sequence. In the map of the reporter construct (top), 3’ TE represents 101 nt 3’ UTR (nts 4818–4918) for in vitro constructs and 869 nt viral 3’ end (nts 4809–5677) for in vivo constructs. Luciferase activity is in relative light units (RLU), normalized to the construct with the complete BYDV 5’ UTR (1–143). Each RNA was translated at least in triplicate (Experimental Procedures).

Potential Base-Pairing between UTRs Is Phylogenetically Conserved
The potential kissing stem loop interaction between the 3’ and 5’ UTRs is conserved in all mRNAs known or suspected to harbor a 3’ TE. This includes all genomic and subgenomic mRNAs of all sequenced BYDV isolates (Figure 1A and [Guo et al., 2000]), the related Soybean dwarf virus (SDV), and the unrelated Tobacco necrosis virus (TNV, Tombusviridae), which also has un capped, nonpolyadenylated RNA (Figure 2A). The predicted kissing stem loop in the 5’ UTR of BYDV sgRNA1 is in a region necessary for 3’ TE-mediated cap-independent translation of the RNA (Wang et al., 1999). The putative 3’ TEs of SDV and TNV were identified by a conserved, essential (Guo et al., 2000) 17 nt tract followed by a stable stem loop resembling SL-III. In TNV, the sequences of the 5’ UTR loop and putative L-III differ from those in BYDV and SDV, supporting the notion that base-pairing between the loops, rather than their sequences, is important for function (Figure 2A).

Base-Pairing between UTRs Is Necessary for Cap-Independent Translation
To test the role of base-pairing between the loops, the potential base-pairing was disrupted by altering the central base in either L-IV or L-III, and restored in the double mutant with both base changes. As predicted, each point mutation (SL-IVA–U or 3’ TEU–A, Figure 2A) reduced LUC expression to levels similar to that obtained with vector-derived 5’ UTR (Figure 2B). Compared to the single mutants, LUC expression from the double mutant (SL-IVA–U/3’ TEU–A) was doubled in vitro and increased at least 10-fold in vivo (Figure 2B).

To determine the effects of mutations on mRNA stability, the functional half-lives of the mRNAs were estimated by monitoring the rate of LUC accumulation (Figure 2A). The predicted kissing stem loop in the 5’ UTR of BYDV sgRNA1 is in a region necessary for 3’ TE-mediated cap-independent translation of the RNA (Wang et al., 1999). The putative 3’ TEs of SDV and TNV were identified by a conserved, essential (Guo et al., 2000) 17 nt tract followed by a stable stem loop resembling SL-III. In TNV, the sequences of the 5’ UTR loop and putative L-III differ from those in BYDV and SDV, supporting the notion that base-pairing between the loops, rather than their sequences, is important for function (Figure 2A).

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To determine the effects of mutations on mRNA stability, the functional half-lives of the mRNAs were estimated by monitoring the rate of LUC accumulation (Meulewaeter et al., 1998). The half-lives of the mutants were not reduced enough to account for the drastic drop in LUC synthesis, nor did they correlate with total LUC expression (Figure 2C). Thus, LUC synthesis reflects translation efficiency.

It is possible that the double mutant did not restore translation fully because the wild-type 3’ –5’ sequence may play an additional role in recruiting ribosomes beyond the 3’–5’ communication function, or the mutation may have altered the TE structure in an unpredicted way. To avoid these possibilities while testing the role of 3’–5’ UTR base-pairing, additional mRNAs with the wild-type 3’ TE sequence but altered 5’ stem loop were constructed. In the 5’ UTR, SL-IV was replaced with a copy of SL-III from the 3’ TE. Thus, this mRNA harbored one copy of SL-III in the 5’ UTR and one in the 3’ TE.
Kissing UTRs Promote Cap-Independent Translation

Figure 2. Base-Pairing between the 3' TE and 5' UTR

(A) Secondary structures of portions of the viral 5' UTRs, and the 3' TE. Structures for SDV and TNV are the most stable predicted using MFOLD (Zuker et al., 1999). Bold italics: invariant 17 nt tract. Dashed lines: potential base-pairing. Shaded boxes: predicted stem loops at 5' ends of sgRNAs (Meulewaeter et al., 1992; Wang et al., 1999), with bold bases complementary to L-III. Genomic positions of bases are in italic numbers; their positions in sgRNAs are in parentheses. Circled bases: mutated in panel B.

(B) Relative luciferase activity produced by translation of LUC mRNAs containing the indicated UTRs (with longer 3' UTRs in protoplasts as in Figure 1C). RLU were normalized against those obtained with wild-type SL-IV and 3' TE flanking the LUC gene (top row). 3' TEBF contains a four base duplication (GAUC) in BamHI4837 site that eliminates cap-independent translation (Wang et al., 1997). U! Ao rA! U indicates the base change in one or both of the circled bases in panel A.

(C) Time course of LUC synthesis (RLU) in protoplasts electroporated with mRNAs containing some of the same UTR mutations as in panel B. Approximate half-lives were calculated as in Meulewaeter et al. (1998).

(D) Effect of mutations on the structure of the 5' UTR. LUC mRNAs with the indicated 5' UTRs were digested partially with nuclease T1 under translation ionic conditions, and then used as templates for primer extension (Experimental Procedures). Sequencing ladder is in the four lanes at right. Dots at left indicate reverse transcriptase termination sites present in the absence of T1 treatment. G105 is the guanosine in the SL-IV loop that is predicted to base-pair to SL-III in wild-type and double mutant structures. The ratio of radioactivity in each G 105 band to total radioactivity in its lane was normalized to the ratio obtained with wild-type UTRs (WT) and indicated below each lane.

This construct gave background levels of LUC activity presumably because SL-III cannot base pair with itself (Figure 2B). The L-IIIU!A mutation was introduced only in the 5' copy of SL-III, to allow base-pairing between it and the wild-type L-III in the 3' TE. In this case, nearly wild-type levels of LUC were obtained (Figure 2B). Thus, base-pairing is necessary and sufficient for communication between the 3' TE and the 5' UTR. Moreover, because the stem of SL-III is different from that of SL-IV, the stem structure in the 5' UTR is not important for 3'--5' communication or ribosome recruitment. This is supported by the very different sequence of the SL-IV stem in the 5' UTR of BYDV isolate PAV-129 (Figure 1A).

To detect the long-distance base-pairing directly, we observed the effect of disrupting and restoring L-IV:L-III base-pairing on nuclease accessibility of L-IV. mRNAs with viral 5' UTR and 3' TE were probed with nuclease T1, which cleaves only single-stranded guanosine nucleotides, and cleavage sites were mapped by primer extension. Although several nonspecific bands that did not correspond to G residues appeared, presumably due to premature termination by reverse transcriptase, the G banding pattern was consistent with the predicted kissing stem loop structure (Figure 2D). Disruption of predicted base-pairing between UTRs resulted in 3- to 5-fold more T1 cleavage at the G in L-IV (G105) compared to wild-type RNA or the double mutant in which base-pairing would be restored (Figure 2D). Thus, a point mutation over 1.6 kb downstream clearly affects T1 nuclease sensitivity of a base (G105) in the 5' UTR (3' TEU!A mutants, Figure 2D), supporting the predicted interaction.

L-III:L-IV Base-Pairing Is Necessary for Replication

To determine the biological relevance of the above mutations in their natural context, they were introduced into full-length viral RNA, and the effects on translation in vitro and on virus replication (which requires translation) in vivo were observed. The effects of single and compensatory mutations in L-III and L-IV on translation of genomic RNA (Figure 3B) were similar to their effects on translation of the reporter gene (Figure 2B), but in the double mutants viral RNA translation was fully restored.

Replication was assessed by quantification of subgenomic RNAs that accumulated in transfected oat protoplasts (Figure 3C). Unlike genomic RNA inoculum, which can linger in the absence of replication, subgeno-
Figure 3. Base-Pairing between UTRs Is Necessary for Viral Translation and Replication
(A) BYDV genome organization. Black box: genomic 5' UTR. Gray box: 100 nt 3' TE. Hatched box: additional 3' UTR in mRNAs assayed in protoplasts.
(B) In vitro translation products of full-length wild-type (PAV6) and mutant BYDV RNAs. PAV6BF contains a GAUC insertion in the TE that knocks out cap-independent translation and replication (Allen et al., 1999). Mobilities of products of ORF 1 (39 kDa) and the fusion of ORFs 1 and 2 generated by ribosomal frameshifting (99 kDa) are indicated. Relative amount of 39 kDa product produced is shown below gel.
(C) Northern blot of total RNA 24 hr after inoculation of protoplasts with the same RNAs used in panel B. Equal loading of each lane was verified by ethidium bromide staining. Relative accumulation of sgRNAs is indicated below each lane. Residual full-length inoculum RNA is in all lanes, even in the absence of replication (Allen et al., 1999).

Ribosome Entry at the 5' End
Finally, we explored whether the ribosome must enter the mRNA from the 5' end. Translation initiates only at the 5'-proximal AUG in BYDV gRNA (Wang et al., 1997). Thus, a 5' scanning mechanism seems likely. Alternatively, the 3' TE may facilitate ribosome entry directly at L-IV. To distinguish between these mechanisms, we introduced a stable stem loop (K-SL) that blocks ribosome entry on capped mRNAs when located ≤12 nt from the 5' end, but allows ribosome binding, scanning, and translation when located more 5'-distally in the 5' UTR (Kozak, 1989). Presumably the 43S ribosome complex requires a single-stranded 5' terminus to bind the mRNA, but then can melt out distal secondary structures as it scans toward the first AUG. In the 5'-proximal construct (K-SL-5'UTR56-143), BYDV bases 1-55 were replaced by K-SL (Figure 4A). In the 5'-distal construct (5'UTR56-143-K-SL), K-SL was inserted between SL-IV and the start codon. As shown in Figure 4B, the 5'-proximal stem loop blocked translation, whereas the 5'-distal stem loop had little effect. This effect was observed with 3' TE-mediated translation of uncapped mRNAs, and, in agreement with Kozak (1989), on capped mRNAs (Figure 4B). Thus, the ribosome must scan from the very 5' end for translation of uncapped mRNAs in a manner indistinguishable from scanning on capped, polyadenylated mRNAs, and markedly different from IRES-containing mRNAs arise only if replication takes place (Allen et al., 1999). Replication of the single mutants was reduced to very low levels (undetectable in L-IIIU→A), and most importantly, the compensatory mutations restored replication to a level roughly proportional to that restored in translation in vivo (compare Figures 2B and 3C). Thus, the base-pairing is necessary for translation of replicating viral RNA. These results also indicate that the viral replication machinery tolerates variations in the sequences of L-III and L-IV.

Discussion
The fact that BYDV has evolved to require base-pairing between UTRs suggests that there is strong selective pressure for formation of closed loop mRNA, and that this closed loop may be necessary in all mRNAs, regardless of the terminal structures. In support of this conclusion, uncapped, polyadenylated mRNAs (Bergamini et al., 2000), and capped, nonpolyadenylated mRNAs (Michel et al., 2000) are also likely to form closed loops.

The formation of the closed loop by long-distance pairing of just five bases raises questions regarding stability and specificity of this interaction. First, it is possible that host proteins strengthen the long-distance interaction. Second, the base-pairing between the 3' TE the 5' UTR is probably more stable than predicted by Watson-Crick pairing.
Crick interactions alone, because kissing stem loops are kinetically and thermodynamically favored compared to equivalent base-pairing of linear RNAs (Zeller and Simons, 1997). However, internal sequences within the coding regions of the mRNA might be expected to compete with L-IV of the 5′ UTR for base-pairing to L-III of the TE. In fact, BYDV genomic RNA contains an internal loop capable of base-pairing to L-III. This loop is in the 5′ UTR of subgenomic RNA 1 (Figure 2A). In that context, it probably does base-pair to L-III during translation, whereas it does not inhibit translation from its internal location in the full-length genomic RNA context. Thus, the proximity to the 5′ end may be necessary for stable base-pairing. This is supported by the requirement for ribosomal scanning from the 5′ end in Figure 4.

The TE-mediated translation mechanism differs from those of other mRNAs by requiring the 3′–5′ interaction even in the poly(A) tail-insensitive wheat germ extract. This interaction, via the kissing stem loop, is required only when the TE is in the 3′ UTR (Guo et al., 2000). Thus, we propose that the closed loop is necessary to deliver either ribosomes or initiation factors to the 5′ UTR, and that mRNA can then enter the ribosome for scanning and translation only via its 5′ terminus. This model is supported by preliminary data indicating that the TE specifically binds canonical initiation factors (E. Allen, our unpublished data). The mechanism is complicated by the requirement for additional viral sequence, mostly downstream of the 100 nt 3′ TE, for cap-independent translation in vivo (Wang et al., 1999). This additional sequence can be replaced partially by a 60 nt poly(A) tail, suggesting that it may function as a poly(A) tail mimic (Guo et al., 2000). The in vivo-required sequence may bind additional factors that further promote recruitment of ribosomes, or fortify the closed loop interactions in the more competitive in vivo conditions. The additional sequence may also provide stability against exonucleases that are absent in vitro (Jacobson and Peltz, 1996).

Specific, long-range base-pairing between short sequences in loops separated by kilobases (Klovins et al., 1998; Miller and Koev, 2000; van Marle et al., 1999), or even in separate molecules (Mujeeb et al., 1998; Sit et al., 1998), have been shown to control synthesis or encapsidation of viral RNAs. Unlike the above examples, we show a long-range base-pairing interaction that controls the universal process of translation. Moreover, BYDV cap-independent translation also differs by not requiring viral proteins, because reporter constructs behave similarly to viral genomic RNA. Interaction between the 3′ UTR and the 5′ IRES may regulate cap-independent translation of HCV RNA, which is also nonpolyadenylated (Ito et al., 1998), but this interaction is protein-mediated and the effects on translation are of a lower magnitude than that seen with the TE.

Base-pairing between UTRs was proposed for cap-independent translation mediated by the 3′ UTR of Satellite tobacco necrosis virus (STNV) RNA, but covariation mutagenesis did not support this hypothesis (Meulewaeter et al., 1998). It is possible that the particular mutations caused unexpected structural changes, preventing restoration of a functional element in the double mutants. Interestingly, the STNV sequence shows no apparent similarity to the 3′ TE (Wang et al., 1997), yet the 3′ UTR of its helper virus, TNV, is clearly similar (Figure 2A).

The base-pairing between UTRs may also play a role in replication. Mounting evidence indicates that replication templates of some RNA viruses are circular. The 5′ UTR of poliovirus RNA interacts with the 3′ end (via proteins) to regulate initiation of (-) strand synthesis (Gamarnik and Andino, 1998). Substantial complementarity exists between the UTRs of flaviviral RNAs (Hahn et al., 1987), This base-pairing controls replication of Dengue virus RNA in vitro (You and Padmanabhan, 1999).

Finally, this report provides evidence of a novel cap-independent translation mechanism that resembles translation of capped mRNAs by its requirement for ribosomal scanning from the 5′ terminus of the mRNA. Given the large number of viruses that lack caps or poly(A) tails or have potential base-pairing between UTRs (Hahn et al., 1987), and given the numerous nonviral mRNAs that undergo translational regulation via their 3′ UTRs (Gunkel et al., 1998; Wickens et al., 2000), interactions similar to those reported here may be widespread.

**Experimental Procedures**

**mRNA Construction**

RNAs were synthesized by in vitro transcription from plasmids with T7 polymerase using Megascript (for uncapped RNAs) or mMessage mMachine (for capped RNAs) kits (Ambion, Austin, TX). The in vitro and in vivo reporter plasmids were derived by PCR from p5 UTR-LUC-TE105 and p5 UTR-LUC-TE869, respectively (Guo et al., 2000). For the 5′ truncations, a set of 5′ primers with EcoRI site extensions was made starting from the deletion boundaries indicated in Figure 1C. The 3′ primer initiated at a site complementary to the 3′ end of the LUC ORF. The PCR products were cut with EcoRI (one is an internal EcoRI site in LUC) and cloned into p5 UTR-LUC-TE105 or p5 UTR-LUC-TE869. The 3′ deletions in the 5′ UTR were made similarly by a 5′ primer in the vector upstream of the 5′ UTR and a set of 3′ antisense primers at the points of deletions shown in Figure 1C. The 3′ substitution bases were introduced in the 3′ primer. The point mutations in Figures 2 and 3 were generated by PCR using two complementary primers with the designated point mutation. In one tube, the sense mutagenic primer was paired with a downstream antisense primer; in the other tube the antisense mutagenic primer was paired with an upstream sense primer. The two overlapping PCR products were mixed as templates, and amplified using the two flanking primers. The resulting mutated PCR product was cut with appropriate restriction enzymes to replace the wild-type fragment. The K-SL stem loop, containing a BstBI site for cloning, was introduced into different positions of the 5′ UTR by the above mutagenesis methods. All constructs were verified by sequencing on an ABI377 automated sequencer.

**Translation and Replication Assays**

Subsaturating levels (0.2-0.3 pmol) of transcript were translated in wheat germ S30 extract (Promega), or 2–3 pmol of transcript were electroporated in oat protoplasts, and products assayed, at least in triplicate, for luciferase activity as in Guo et al., 2000. mRNA half-life was approximated using the equation t 1/2 = P(−) • ln2/A (Meulewaeter et al., 1998), where P(−) is LUC in relative light units (RLU) at 10s min; A is the slope (RLU/min) at t0. For replication studies, oat protoplasts were inoculated with 10 pmol of full-length BYDV genomic RNA (or mutant versions) transcribed from Small-linearized pPAV6 (Allen et al., 1999). After 24 hr, RNA was extracted and Northern blots performed as in Allen et al. (1999).

**RNA Structure Probing**

The 5′ UTR structure in Figure 1 was determined on transcript from XhoI-digested p5′ UTR-LUC-TE105. Twenty pmol of [γ-32P]ATP end-labeled RNA was purified on a 6% polyacrylamide, 7 M urea denaturing gel and eluted, and T1 nuclease ladder generated all as described previously (Guo et al., 2000). The labeled RNA was probed in 0.4 M imidazole solution containing 40 mM NaCl, 1 mM EDTA, 10 mM MgCl2, for 15 hr at 25°C as in Vlasov et al. (1995). Probed products were separated by 8% polyacrylamide, 7 M urea gel electrophoresis.
trophoresis, and visualized with a STORM 840 Phosphorimager (Molecular Dynamics).

The 5' UTR structure of full-length mRNA, and mutant versions, (Figure 2D) transcribed from Smal-linearized p5'UTR-LUC-TE105, was analyzed by partial digestion with T1 nuclease (Guo et al., 2000) under the same ionic conditions used for in vitro translation, followed by reverse transcription using the 5' end-labeled primer, CATAGCC TTATGCAGTTGC (complementary to bases 72 to 90 in the LUC ORF), as described by Mervyn and Noller (1998).

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References


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