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Sequences within a Small Yeast RNA Required for Inhibition of Internal Initiation of Translation: Interaction with La and Other Cellular Proteins Influences Its Inhibitory Activity

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We recently reported purification, determination of the nucleotide sequence, and cloning of a 60-nucleotide RNA (I-RNA) from the yeast *Saccharomyces cerevisiae* which preferentially blocked cap-independent, internal ribosome entry site (IRES)-mediated translation programmed by the poliovirus (PV) 5′ untranslated region (UTR). The I-RNA appeared to inhibit IRES-mediated translation by virtue of its ability to bind a 52-kDa polypeptide which interacts with the 5′ UTR of viral RNA. We demonstrate here that the HeLa 52-kDa I-RNA-binding protein is immunologically identical to human La autoantigen. Moreover, I-RNA-mediated inhibition of PV 5′ UTR-dependent translation in cell extracts can be reversed by exogenous addition of purified La protein. By using I-RNAs with defined deletions, we have identified sequences of I-RNA required for inhibition of internal initiation of translation. Two smaller fragments of I-RNA (16 and 25 nucleotides) inhibited PV UTR-mediated translation from both monocistronic and bicistronic RNAs. When transfected into HeLa cells, these derivatives of I-RNA inhibited translation of PV RNA. A comparison of protein binding by active and inactive I-RNA mutants demonstrates that in addition to the La protein, three other polypeptides with apparent molecular masses of 80, 70, and 37 kDa may influence the translation-inhibitory activity of I-RNA.

The single-stranded, positive-sense RNA genome of poliovirus (PV), a member of the *Picornaviridae* family, translates in a unique cap-independent manner in the cytoplasm of infected cells. The translation of the viral RNA genome produces a polyprotein (~250 kDa) which is cleaved by virus-encoded proteases into structural and nonstructural proteins (28). Interestingly, PV RNA translates inside infected cells in an environment where cap-dependent translation of the majority of cellular capped mRNAs is impaired, and thus the viral RNA must translate by an alternative mechanism (49, 55). The viral RNA is naturally uncapped but instead has a small virus-encoded protein, Vpg, covalently linked to its 5′ end. The unusually long (~750 nucleotides [nt]) 5′ untranslated region (UTR) of viral RNA is highly structured and contains multiple AUG triplets (1, 40, 48). In sharp contrast to the scanning mechanism of the cap-dependent translation of eukaryotic mRNAs, the 5′ UTR of PV RNA can mediate the binding of ribosome internally to a sequence between nt 130 and 630. This sequence element has been termed ribosome landing pad or the internal ribosome entry site (IRES) (41, 52). Many other picornaviruses, such as human rhinovirus, encephalomyocarditis virus, foot-and-mouth disease virus (FMDV), coxsackievirus, Theiler's murine encephalomyelitis virus, and hepatitis A virus, have been shown to utilize this novel mechanism (2, 3, 6, 7, 17, 24, 25, 27, 32). In addition to picornaviruses, many other viruses, such as hepatitis C virus, murine leukemia virus, infectious bronchitis virus, duck hepatitis B virus, pestivirus, and plant potyviruses, are believed to utilize internal initiation of translation for synthesis of one or more gene products (4, 10, 15, 29, 30, 44, 46, 53). Cellular mRNAs encoding immunoglobulin heavy-chain-binding protein, *Drosophila* antennapedia protein, and the mouse androgen receptor also use IRES-mediated initiation of protein synthesis (21, 33, 39).

The IRES sequences of different picornaviruses are conserved and can be exchanged (24, 27). When the IRES sequence is placed upstream of a reporter gene or as an intercistronic spacer in a bicistronic construct, it can direct cap-independent translation both in vitro and in vivo (3, 25). The presence of a polypyrimidine tract near the 3′ border of the IRES and an appropriately spaced downstream AUG at nt 586 is common to all picornaviruses and is believed to be important for proper functioning of the IRES element (24, 26, 43). The precise mechanism of internal initiation of translation is unclear. Studies from several laboratories suggest the importance of specific interactions of cellular proteins with various elements containing secondary structures within the 5′ UTR. One of the two extensively studied proteins is a 52-kDa polypeptide (p52) which binds to stem loop G (nt 559 to 624) of the 5′ UTR of the poliovirus RNA (35). p52 has recently been identified as the La autoantigen (36), which causes an autoimmune response in patients with systemic lupus erythematosus and Sjögren's syndrome (51). The human La autoantigen binds to the 3′-terminal sequences of almost all nascent polymerase III transcripts. Although not absolutely necessary, La has been reported to stimulate transcription by RNA polymerase III in vitro (18, 34). Cell extracts immunodepleted of La antibodies cannot promote cap-independent translation, and exogenous addition of purified La protein can correct aberrant translation products of poliovirus RNA in a reticulocyte lysate in which p52 is limiting (36, 50). The other cellular protein, p57, has been shown to interact with three distinct domains within the 5′ UTR of PV (22, 31, 54). The p57 appears to be identical to the polypyrimidine tract-binding protein (PTB). Immunodepletion of PTB from HeLa cell extracts prevents translation of PV RNA, although exogenous addition of purified PTB

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failed to restore translation (22). Interestingly, recent studies using mutants having deletions of different stem-loop domains of the PV 5′ UTR demonstrated that interaction neither of p57 with stem-loop F-G nor of p52 with stem-loop G is required for internal ribosome binding (19, 20, 42). These studies, however, do not rule out the possibility that these polypeptides interact with other regions of the viral UTR. Thus, the functional significance of La and PTB in IRES-mediated translation is not clearly established, and possible involvement of other RNA-protein and/or protein-protein interactions in IRES-mediated translation is being explored by many laboratories. In a recent report, RNA-protein interaction with stem-loop E (nt 234 to 440) has been shown to be important for PV translation (5). A 40-kDa protein interacts directly with PV RNA, whereas two other proteins with molecular masses of 39 and 42 kDa joins the complex through protein-protein interaction (5). Additionally, it has been demonstrated that stem-loop G also harbors a binding site for a 39-kDa protein whereas a longer RNA comprising stem-loops F and G interacts with a 36-kDa protein (20). A number of other cellular proteins, such as p50, p54, and p48, have been demonstrated to interact specifically with defined regions of the 5′ UTR of the PV RNA, but the functional significance of these interactions is unknown (16, 38). Very recently, it has been demonstrated that one of the major initiation factors, eIF4B, interacts directly with the IRES element of FMDV (37).

Recently we reported purification, determination of the nucleotide sequence, and cloning of a small (60-nt) RNA (I-RNA) molecule from the yeast *Saccharomyces cerevisiae* that was isolated on the basis of its ability to selectively block translation of PV RNA both in vivo and in vitro (13). I-RNA derived from the clone inhibited PV IRES-mediated translation, did not inhibit cap-dependent translation from capped monocistronic RNAs, and selectively blocked internal initiation of translation from a bicistronic mRNA containing IRES sequences of PV RNA (13). UV cross-linking studies demonstrated that I-RNA specifically bound cellular proteins believed to be required for IRES-mediated translation. Specifically purified I-RNA competed with viral RNA elements within the 5′ UTR which bind a cellular protein with an approximate molecular mass of 52 kDa. Finally, when transfected into HeLa cells, I-RNA efficiently inhibited replication of PV RNA by inhibiting translation of input viral RNA.

In this report, we demonstrate that the HeLa 52-kDa I-RNA-binding protein is immunologically identical to the La autoantigen. Moreover, I-RNA-mediated inhibition of translation programmed by the PV 5′ UTR in cell extracts can be rescued by exogenous addition of purified La protein. By using various deleted I-RNAs, we have identified smaller segments of I-RNA which efficiently inhibit internal initiation of translation from both monocistronic and bicistronic RNA constructs. Finally, when transfected into HeLa cells, these truncated derivatives of I-RNA inhibited translation of PV RNA. A comparison of cellular protein binding by truncated I-RNAs which inhibit IRES-mediated translation with those which do not suggests a possible mode of action of I-RNA-mediated inhibition of viral translation.

MATERIALS AND METHODS

Cells and viruses. HeLa cells were grown in suspension culture in S minimum essential medium (GIBCO Laboratories) supplemented with 6% newborn calf serum. HeLa monolayers were grown in tissue culture flask in minimal essential medium supplemented with 5% fetal bovine serum. PV RNA (type 1 Mahoney) was prepared as described previously (14).

HeLa S10 extract and RSW preparation. HeLa cell extracts were prepared as previously described (12, 13, 47). The ribosomal salt wash (RSW) from HeLa cells was prepared as described by Brown and Ehrenfeld (8), with some modifications. Cultures of HeLa cells (4×10^5 cells per ml) were harvested by centrifugation, washed three times with cold isotonic buffer (35 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid [HEPES; pH 7.5], 146 mM NaCl, 11 mM glucose), resuspended in a 2× packed-cell volume of lysis buffer (10 mM KCl, 1.5 mM magnesium acetate, 20 mM HEPES [pH 7.4], 1 mM dithiothreitol [DTT]), and then incubated on ice for 10 min for swelling. Cells were disrupted at 0°C with 50 strokes in a type B Dounce homogenizer. After disruption, extracts were centrifuged for 15 min at 10,000 rpm at 4°C in a Sorvall SS34 rotor to remove nuclei and mitochondrial fractions. The supernatant (S10 extract) was centrifuged at 50,000 rpm for 2 h at 4°C in a Beckman Ti60 rotor. The ribosome pellet was resuspended at a concentration of approximately 250 A_{260}/ml in lysis buffer with gentle shaking on an ice bath. The KCl concentration was then adjusted to 500 mM, and the solution was stirred for 30 min on an ice bath. The resulting solution was centrifuged for 2 h at 50,000 rpm at 4°C. The supernatant (salt wash) was then subjected to 0 to 70% ammonium sulfate precipitation. The pellet containing initiation factors was dissolved in a low volume of dialysis buffer (without glycerol) and then subjected to overnight dialysis at 4°C against dialysis buffer (5 mM Tris [pH 7.5], 100 mM KCl, 0.05 mM EDTA, 1 mM DTT, 5% glycerol). The dialysate was then centrifuged at 10,000 rpm for 10 min at 4°C, and the supernatant was aliquoted in small volumes into several prechilled tubes and stored at −70°C.

In vitro transcription. Different mRNAs were transcribed in vitro by using the T7 or SP6 promoter from linear plasmids with T7 or SP6 RNA polymerase, respectively. The pSDIR clone (13) was linearized with restriction enzyme *Hin*dIII; the linear fragment was gel purified and then transcribed with T7 RNA polymerase to generate I-RNA (sense transcript). Plasmids p2CAT and pG3CAT (12, 13) were linearized with *Bam*HI and transcribed with SP6 RNA polymerase. The PV type 2 IRES containing bicistronic construct pGem CAT/P2-5′/luc (generous gift from N. Sonenberg) was linearized with *Xho*I, and the runoff capped transcript was generated with T7 RNA polymerase. The nonspecific RNA was synthesized from pGem 3Z vector DNA (Promega), linearized with *Hin*dIII, and transcribed by T7 RNA polymerase.

Construction of I-RNA deletion mutants. I-RNA deletion mutants were generated by in vitro transcription with T7 RNA polymerase from oligonucleotide templates. Different lengths of oligonucleotides were synthesized (Biosynthesis Inc.); each began with a T7 promoter adapter sequence followed by various lengths from different regions of I-RNA sequences. Oligodeoxyribonucleotide templates were mixed with equimolar amounts of the 17-mer T7 primer oligonucleotide in 0.1 M NaCl and annealed by heating at 100°C for 5 min followed by slow cooling to room temperature. The nucleotide positions of the different I-RNA deletion mutants are shown in Fig. 3. The nucleotide sequence for the yeast I-RNA has been published elsewhere (reference 13 and EMBL database library accession number X76506).

In vitro translation. In vitro translation in HeLa cell extracts was performed essentially as described elsewhere (13, 47). Approximately 2 mg of each mRNA was used with 80 μg of HeLa cell extract in the presence of 25 μCi of [^{35}S]methionine (800 Ci/mmol; Amersham) and 40 U of RNasin (Promega) in a 25-μl reaction volume. To study the effects of I-RNA-induced inhibition and La protein-induced reversal of inhibition of in vitro translation of the chloramphenicol acetyltransferase (CAT) gene from p2CAT mRNA, PV-infected HeLa extracts were preincubated on ice for 30 min with I-RNA alone (1.5 μg) and together with either purified La (1.5 μg) or bovine serum albumin (BSA; 1.5 μg) before addition to the reaction mixture.

Mobility shift electrophoresis. Fifty micrograms of HeLa S10 extract or 10 μg of HeLa RSW was preincubated at 30°C for 10 min with 4 μg of poly(dI-dC) (Pharmacia) in a 15-μl reaction mixture containing 5 mM HEPES (pH 7.6), 25 mM KCl, 2 mM $MgCl_2$, 2 mM DTT, 0.1 mM EDTA, 1.5 mM ATP, 2 mM GTP, and 3.8% glycerol. For competition experiments, 100-fold molar excesses of unlabeled competitor RNAs were added to the reaction mixtures, which were then incubated for 10 min at 30°C. Finally, 5 to 10 fmol of labeled RNA probe was added to the reaction mixtures, and incubation was continued for another 20 min at 30°C. Three microliters of gel loading dye was added to each reaction mixture to a final concentration of 5% glycerol and 0.02% each bromophenol blue and xylene cyanol. For supershift assays, the S10 extract was preincubated with either 2.5 μl of nonimmune human serum or 2.5 μl of immune human serum against La protein on ice for 10 min, the appropriate ^{32}P-labeled RNA probe was then added to the reaction mixture, and incubation was continued for another 20 min on ice. The RNA protein complexes were then analyzed on a 4% polyacrylamide gel (39:1 ratio of acrylamide to bisacrylamide) containing 5% glycerol in 0.5× Tris-borate-EDTA.

UV-induced cross-linking and immunoprecipitation. ^{32}P-labeled RNA-protein complexes generated as described above were irradiated with a UV lamp (multiband UV; 254/366 nm; model UGL; 25 UVP Inc.) at a distance of 2 to 3 cm for 15 min in a microtiter plate. Unbound RNAs were then digested with a mixture of 20 μg of RNase A and 20 U of RNase T_1 at 37°C for 15 min. For the immunoprecipitation of labeled complexes, 2 to 5 μl of either nonimmune human serum or immune human serum from a patient with lupus disease (La antibody [9]) was added, and the mixture was kept on ice for 2 h in the presence of 200 μl of 1× radioimmunoprecipitation assay buffer (5 mM Tris [pH 7.9], 150 mM NaCl, 1% Triton X-100, 0.1% sodium dodecyl sulfate [SDS], 1% sodium deoxycholate). Five milligrams of protein A-Sepharose was then added to each

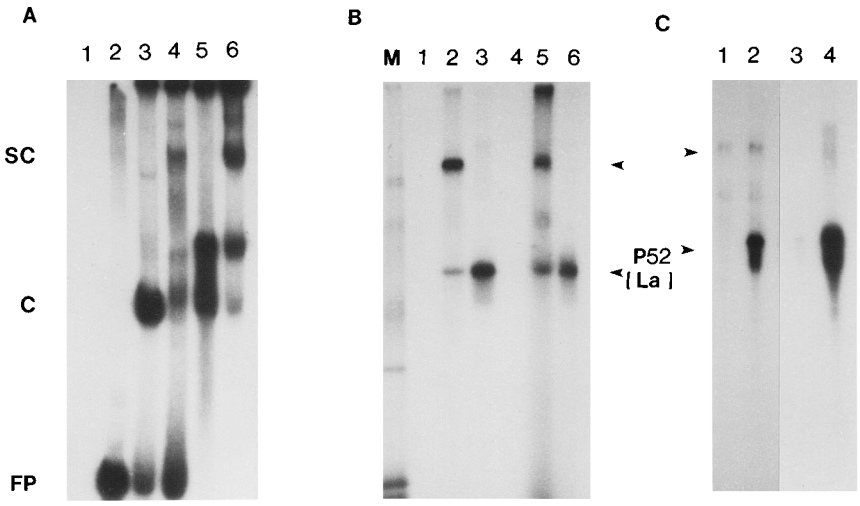

FIG. 1. The HeLa 52-kDa I-RNA-binding protein is identical to La autoantigen. (A) Gel shift. ^{32}P-labeled I-RNA probe was incubated with 50 μg of HeLa S10 extract (lanes 3 and 4) or with 0.3 μg of purified La protein expressed from the recombinant La clone (lanes 5 and 6) in the presence (lanes 4 and 6) or absence (lanes 3 and 5) of antibody to La autoantigen. I-RNA–protein complexes (C) formed with HeLa S10 or purified La protein were supershifted (SC) with anti-La antibody. Lanes 1 and 2 are minus-probe and minus-extract controls, respectively. (B) ^{32}P-labeled I-RNA (lanes 1 to 3) and ^{32}P-labeled PV 5′ UTR (nt 559 to 624) RNA (lanes 4 to 6) were UV cross-linked to HeLa S10 proteins (lanes 2 and 5) or purified La protein (lanes 3 and 6). After RNase digestion, protein-nucleotidyl complexes were immunoprecipitated with an anti-La antibody and were analyzed on an SDS-polyacrylamide gel. The immunoprecipitated bands are indicated with arrowheads. Lane M shows the migration of ^{14}C-labeled protein markers (Gibco BRL) having approximate molecular masses of 14, 18, 29, 43, 68, and 97 kDa (from bottom to top). (C) ^{32}P-labeled I-RNA was UV cross-linked to HeLa S10 proteins (lanes 1 and 2) or purified La protein (lanes 3 and 4) and then immunoprecipitated with either nonimmune human serum (lanes 1 and 3) or the La antibody isolated from the patient serum (lanes 2 and 4). The immune serum-specific ^{32}P-labeled proteins were then analyzed by SDS-polyacrylamide gel electrophoresis followed by autoradiography.

reaction tube; the tubes were rocked in a cold room for 1 h and then centrifuged at 12,000 rpm for 5 min at 4°C. Beads were washed with 1× radioimmunoprecipitation assay buffer three times to reduce nonspecific binding. Finally, resuspended beads in 1× SDS-gel loading dye (50 mM Tris [pH 6.8], 100 mM DTT, 2% SDS, 0.1% bromophenol blue, 10% glycerol) were heated at 100°C for 5 min and analyzed on an SDS–14% polyacrylamide gel.

RNA transfection. PV RNA (type 1 Mahoney) was isolated from infected HeLa cells as described elsewhere (14). Lipofectin-mediated RNA transfection of HeLa monolayer cells was performed essentially as described previously (13).

In vivo labeling and immunoprecipitation. In vivo labeling of proteins after RNA transfection and immunoprecipitation of the PV capsid proteins were done as described previously (13, 45).

RESULTS

I-RNA interacts with human La autoantigen. Previous studies from our laboratory have shown that I-RNA specifically interacts with a 52-kDa HeLa cell protein and that this interaction may play an important role in translation inhibition of IRES-mediated translation by I-RNA (13). Because a 52-kDa protein has previously been shown to interact with a specific region of PV 5′ UTR (nt 559 to 624) and this protein has been identified as the human La autoantigen (36), we determined whether the 52-kDa I-RNA-binding protein is similar to La protein. As shown in Fig. 1A, a gel-retarded complex (complex C) containing labeled I-RNA and HeLa cell proteins was supershifted by an antibody to the human La protein (Fig. 1A, lanes 3 and 4). A similar complex formed with labeled I-RNA and purified recombinant La protein (lane 5, complex C) can also be supershifted with the anti-La antibody to the same relative position as found with the HeLa cell extract. A second, more slowly migrating complex was also observed with purified La protein (lane 5), the majority of which could not be supershifted with the anti-La antibody (lane 6). This more slowly migrating complex was previously detected by Meerovitch et al. when purified La preparation was used for gel shift analysis using PV 5′ UTR nt 559 to 624 as the probe (36). The nature of this complex is not known. These results suggest that complex C formed by incubating labeled I-RNA and HeLa cell extract contains La autoantigen. To confirm that complex C indeed contains La protein, UV cross-linking studies were performed with ^{32}P-labeled I-RNA and 5′ UTR (nt 559 to 624) probes, using HeLa cell extract or purified La protein. UV cross-linked complexes were then immunoprecipitated with anti-La or nonimmune serum and analyzed by SDS-polyacrylamide gel electrophoresis. A 52-kDa UV cross-linked protein was specifically immunoprecipitated by the anti-La antibody when complexes were formed with the HeLa cell extract, using either a labeled I-RNA or UTR probe (Fig. 1B, lanes 2 and 5). This 52-kDa band comigrated with a UV cross-linked, anti-La-immunoprecipitated complex formed by incubating purified La protein with ^{32}P-labeled I-RNA (lane 3) or ^{32}P-labeled 5′ UTR (lane 6). A prominent ~120-kDa complex seen in lanes 2 and 5 was not specific to La antibody, as it could also be detected in lanes containing nonimmune serum (Fig. 1C, lanes 1 and 2). The intensity of the 120-kDa complex varied greatly between the two experiments (Fig. 1B and C), presumably because different HeLa extracts were used in these experiments. These results demonstrate that I-RNA interacts with the human La autoantigen.

Inhibition of IRES-mediated translation by I-RNA is reversed by La antigen. PV inhibits cap-dependent translation of host cell mRNAs by proteolytically cleaving the p220 component of the cap-binding protein complex. Therefore, extracts derived from virus-infected cells are active in cap-independent IRES-mediated translation but not in cap-dependent translation (55). To determine whether I-RNA-induced inhibition of IRES-mediated translation can be specifically rescued by exogenous addition of purified La protein, translation of p2CAT RNA (5′ UTR-CAT) was performed in virus-infected cell extracts. Translation of p2CAT RNA in PV-infected HeLa cell extract in the presence of I-RNA was inhibited to 20% of that of the control (Fig. 2, lanes 1 and 2). Significant stimulation (1.5-fold) of viral 5′ UTR-mediated translation was observed when purified La protein was added to the infected cell extract

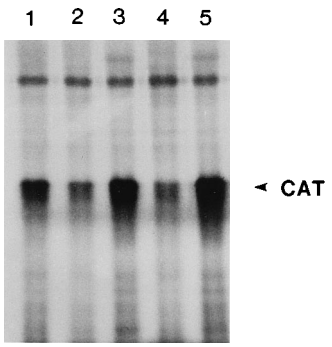

FIG. 2. Purified La protein can rescue I-RNA-mediated inhibition of translation programmed by the PV 5′ UTR. p2CAT RNA (2 μg) was translated in vitro in PV-infected HeLa cell lysate as described in Materials and Methods. Thirty micrograms of HeLa lysate was preincubated on ice for 30 min either alone (lane 1) or in the presence of 1 μg of I-RNA (lane 2) or 1 μg of I-RNA together with 1.5 μg of La (lane 3) or 1 μg of I-RNA mixed with 1.5 μg of BSA (lane 4) or only with 1.5 μg of purified La protein (lane 5). The translation products were analyzed by SDS–14% polyacrylamide gel electrophoresis. The position of the CAT gene product is indicated on the right.

(Fig. 2; compare lane 5 with lane 1). This effect is probably due to a limiting amount of La protein in virus-infected cells. Inhibition of translation mediated by I-RNA (lane 2) can be reversed by addition of purified La protein (lane 3) to approximately 90% of the value for the control containing La protein alone (lane 5). In the presence of a constant amount of I-RNA, reversal of IRES-mediated translation by exogenously added La was concentration dependent (data not shown). In contrast, addition of an equivalent amount of BSA failed to restore IRES-mediated translation (lane 4). A similar result was observed when mock-infected extracts were used instead of virus-infected cell extracts (data not shown). The higher-molecular-weight band seen in translation reactions was not a translation product, as it can also be detected in reactions lacking mRNA (data not shown). Cap-dependent translation of CAT was not stimulated by additions of La (data not shown).

Minimum sequences required for I-RNA activity. To determine I-RNA sequences required for inhibition of PV IRES-mediated translation, a nested set of 15-nt-long deletions was generated (I-1, I-2, I-3, and I-4; Fig. 3). Effects of these truncated RNAs on in vitro translation programmed by p2CAT RNA containing the PV 5′ UTR were determined. As shown in Fig. 4A, both I-1 and I-2 RNAs were still active in translation inhibition, although they were not as active as intact I-RNA (compare lanes 2, 3, and 6 with lane 1). Deletion of nt 31 to 45 and 46 to 60 (I-3 and I-4) from I-RNA, however, almost totally destroyed its ability to inhibit IRES-mediated translation (lanes 4 and 5). These results suggest that the 3′-terminal half of I-RNA contains major sequences necessary for inhibition of viral IRES-mediated translation. However, it should be noted that sequences present within the first 15 (i.e., I-1) or the next 15 (I-2) nt also play a role in inhibition, since these mutants are not as active as intact I-RNA (lanes 2 and 3). Further deletion analysis showed that a 25-nt-long fragment of I-RNA (I-7 RNA, nt 26 to 50) was as active as I-RNA in viral translation inhibition (Fig. 4B, lane 5). A similar deletion mutant that contained an extra 10 nt at its 3′ end (I-6 RNA, nt 26 to 60) was active but not as active as I-7 RNA (Fig. 4B, lane 4). A fragment of I-RNA containing nt 1 to 25 (I-8 RNA), however, was totally inactive in translation inhibition (Fig. 4C, lane 2). Further deletion of I-7 RNA resulted in a smaller fragment (I-9 RNA, nt 30 to 45) capable of inhibiting IRES-mediated translation (Fig. 4C, lane 3). The ability of I-9 RNA to inhibit translation (Fig. 4C, lane 3) is fully consistent with the inability of I-3 RNA to arrest translation (Fig. 4A, lane 4), as I-3 RNA lacks nt 31 to 45 (Fig. 3). None of these mutant I-RNAs were able to inhibit cap-dependent translation of pCAT RNA (Fig.

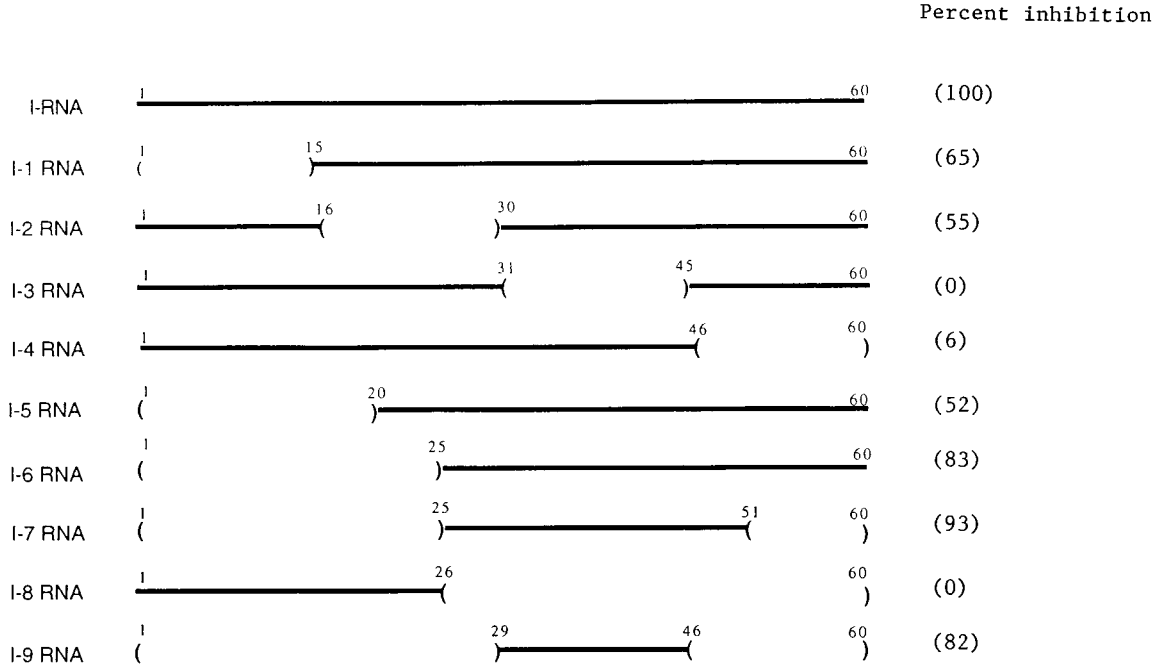

FIG. 3. I-RNA deletions. The diagram shows the I-RNA deletion mutant constructs. The nucleotide positions of the mutation sites are indicated for each mutant. The names of the mutants are given at the left. The numbers in parentheses indicate percentages of translation inhibition by mutant I-RNAs compared with wild-type I-RNA (100%), calculated by averaging results of three independent experiments, including those in Fig. 4.

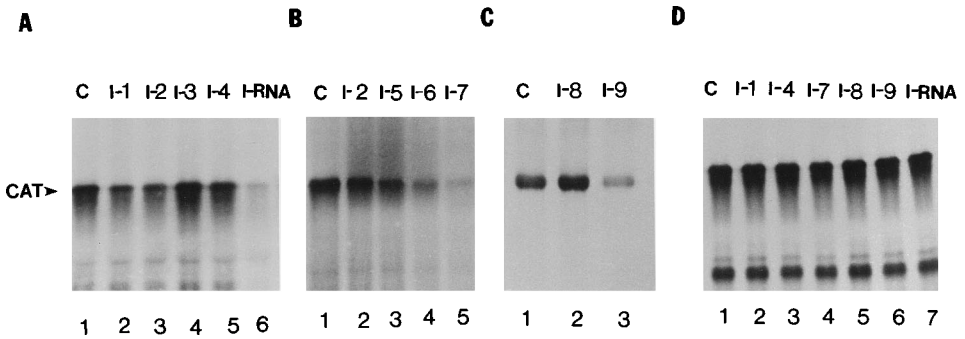

FIG. 4. Minimum I-RNA sequences necessary for inhibition of in vitro translation in HeLa lysates programmed by the 5′ UTR of PV RNA. The effects of different I-RNA deletion mutants on in vitro translation of pG3CAT and p2CAT RNAs in HeLa lysates were determined. In vitro translations were performed with approximately 2 μg of either uncapped p2CAT RNA (A to C) or capped pG3CAT RNA (D) in the absence (lanes C of all the panels) or in the presence of 2 μg of I-RNA deletion mutant as indicated. The position of the CAT protein is indicated at the left.

4D and data not shown). Quantitation of translation inhibition by various deletion mutants of I-RNA is shown in Fig. 3.

I-7 and I-9 RNAs inhibit internal initiation of translation. To determine whether the truncated I-RNAs are capable of inhibiting internal initiation of translation, their effects on translation from a bicistronic construct were determined. A bicistronic construct containing CAT and luciferase genes flanked by the PV 5′ UTR was used in this experiment. The initiation of cap-independent translation occurring internally from the PV 5′ UTR would result in the synthesis of luciferase, whereas cap-dependent translation would normally produce the CAT protein. In uninfected HeLa cell extracts, translation from the capped bicistronic message produced both CAT and luciferase proteins in separate experiments using different cell extracts (Fig. 5A, lane 1; Fig. 5B, lane 1). Addition of full-length I-RNA preferentially inhibited synthesis of luciferase but not that of CAT (Fig. 5A, lane 3). The 25-nt-long I-7 RNA almost totally (>90%) inhibited production of luciferase (lane 2). CAT synthesis, however, was stimulated approximately 3.5-fold in reactions containing I-7 RNA compared with the control (lane 2). No inhibition of luciferase synthesis was apparent with the mutant I-4 RNA (lane 4). Synthesis of CAT protein was also stimulated 3.7-fold by I-4 RNA (lane 4). Similar results were obtained with I-9 and I-8 RNAs. The 16-nt-long I-9 RNA inhibited luciferase synthesis significantly compared with the control (Fig. 5B, lanes 1 and 2). Although 20% inhibition of CAT production was observed in presence of I-9 RNA, luciferase synthesis was inhibited almost 85% over the control (lane 2). In contrast, I-8 RNA did not significantly inhibit synthesis of either luciferase or CAT (lane 3). These results suggest that I-7 and I-9 RNAs, but not I-4 and I-8 RNAs, preferentially inhibit internal initiation of translation programmed by the PV 5′ UTR. Although not shown here, all deletion mutants shown in Fig. 3 showed similar activities of translation inhibition irrespective of whether mono- or bicistronic mRNAs were used (data not shown).

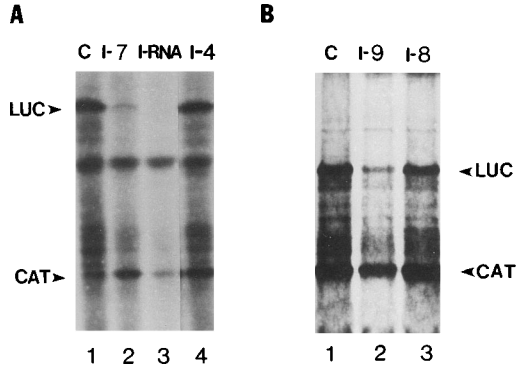

FIG. 5. Effects of I-RNA deletions on internal initiation of translation in vitro. A bicistronic construct containing CAT and luciferase genes flanked by the PV type 2 5′ UTR was translated in vitro in HeLa cell lysates in the absence (lanes C) or presence of 2 μg of either I-RNA (A, lane 3) or the I-RNA deletion indicated above each lane. Products were analyzed on an SDS–14% polyacrylamide gel. Arrowheads indicate positions of the luciferase (LUC) and CAT gene products.

I-7 and I-9 RNAs inhibit translation of PV RNA in vivo. To determine whether mutant I-RNAs inhibit translation of PV RNA in vivo, PV RNA was transfected (by using liposome) into HeLa cells singly or together with purified I-7, I-9, I-4, I-8, and I-RNAs. Proteins were labeled with [^{35}S]methionine, and the synthesis of viral proteins was monitored by immunoprecipitation of viral capsid proteins from cell extracts by anticapsid antisera. As shown in Fig. 6A, no capsid protein could be precipitated from mock-transfected cells (lane 1). Upon transfection of cells with PV RNA alone, synthesis of capsid proteins was clearly detected (Fig. 6A, lane 2; Fig. 6B, lane 1). Cotransfection of I-7 RNA (Fig. 6A, lane 3) or I-RNA (Fig. 6A, lane 4; Fig. 6B, lane 5) with PV RNA resulted in over 90% inhibition of capsid protein synthesis. Activity of I-9 RNA was approximately 50% of that observed with I-7 or I-RNA (Fig. 6B, lane 4). Higher concentrations of I-9 RNA, however, inhibited viral protein synthesis to the extent seen with I-7 RNA (data not shown). As expected, I-8 RNA and I-4 RNA were unable to inhibit translation of viral proteins (Fig. 6B, lanes 2 and 3). It should be noted that similar amounts of intracellular PV RNAs were detected in both cells transfected with PV RNA alone and those transfected with a mixture of PV RNA and I-7 or I-9 RNA at early time points, suggesting that the stability of input PV RNA is not altered significantly in cells containing I-RNA or its derivatives (data not shown).

Protein binding of I-RNA mutants examined by UV cross-linking. Results presented in Fig. 4 to 6 demonstrated differences in activities of various I-RNA mutants in inhibiting IRES-mediated translation. While I-7 and I-9 RNAs were capable of inhibiting PV IRES-mediated translation, I-4 and I-8 RNAs were almost totally inactive as translation inhibitors. To determine whether similar or different proteins were bound by these RNAs, various labeled RNA probes were incubated with HeLa proteins, and protein-RNA complexes were examined by UV cross-linking following RNase digestion.

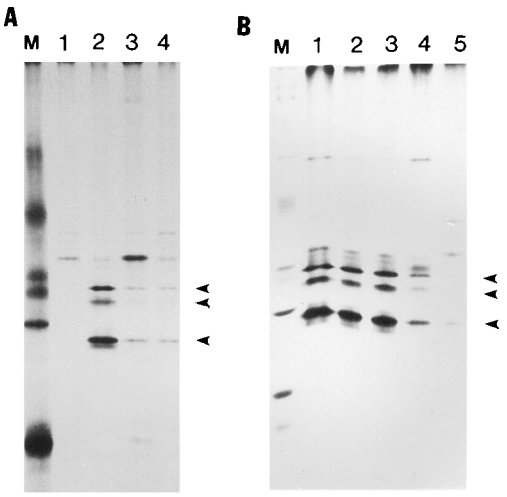

FIG. 6. Inhibition of PV translation in vivo by the I-RNA deletion mutants. HeLa monolayer cells were transfected with PV RNA alone (A, lane 2; B, lane 1) or viral RNA together with 4 μg of either intact I-RNA (A, lane 4; B, lane 5) or I-7 RNA (A, lane 3), I-4 RNA (B, lane 2), I-8 RNA (B, lane 3), or I-9 RNA (B, lane 4). After transfection, cells were labeled with [^{35}S]methionine, and in vivo-labeled proteins were immunoprecipitated with anticapsid antibody and analyzed on an SDS–14% polyacrylamide gel. Positions of the PV capsid proteins are indicated by arrowheads. In panels A and B, 1.5 and 1 μg, respectively, of PV RNAs were used for transfection. The total RNA concentration in each transfection was adjusted to 20 μg by adding carrier tRNA. Lanes M show the migration of ^{14}C-labeled protein markers (Gibco BRL) having approximate molecular masses of 14, 18, 29, 43, 68, 97 kDa (from bottom to top).

Two sources of HeLa proteins were used for these experiments, S10 (Fig. 7A, lanes 1 to 6) and RSW (lanes 7 to 12). When full-length labeled I-RNA was incubated with HeLa S10 extract, two major bands with approximate molecular masses of 52 and 110 kDa were detected in addition to minor bands at 100, 70, 48, and 46 kDa (lane 2). When RSW proteins were used, the profile of protein-nucleotidyl complexes was significantly different from that with S10 (lane 8). First, the 110-kDa band was present in very low amounts in reactions containing RSW. Second, the 52-kDa protein was present as a doublet of 54 to 52 kDa and in relatively lower amounts than in S10. Third, new bands at approximately 80 and 37 kDa were apparent in RSW-containing reactions. In contrast to full-length I-RNA, when labeled truncated I-RNAs were used in UV cross-linking experiments, the protein-nucleotidyl profiles for each RNA were remarkably similar between S10 (lanes 3 to 6) and RSW (lanes 9 to 12). While I-4 and I-8 RNAs bound mainly polypeptides of 70, 52, 48, 46, and 37 kDa, I-7 and I-9 RNAs interacted with a new band at 80 kDa (compare lanes 3 and 4 with lanes 5 and 6). The 80-kDa band was more pronounced with I-7 RNA. Additionally, the 70-kDa polypeptide bound by I-4 and I-8 RNAs was not detected with I-7 and I-9 RNAs. Another very high molecular weight polypeptide (running faster than the 220-kDa marker) was present only in reactions containing I-4 and I-8 RNAs (lanes 3 and 4). Similarly, in experiments using RSW, the 100-kDa polypeptide was preferentially bound by I-7 and I-9 RNAs (lanes 11 and 12). The autoradiogram shown in Fig. 7A was overexposed purposely to detect even minor protein-nucleotidyl complexes.

In competition experiments, it was observed that unlabeled I-7 and I-4 RNAs successfully competed with all major protein bands bound by labeled I-7 and I-4 RNA probes (Fig. 7B). For example, all three polypeptides, 80, 52, and 37 kDa, complexed to I-7 RNA (Fig. 7B, lane 2) were diminished with unlabeled I-7 and I-4 RNAs (lanes 3 and 4) but not with a nonspecific RNA (lane 5). Similarly, with labeled I-4 RNA, the 70-, 52-, and 37-kDa bands were diminished with unlabeled I-7 and I-4 RNAs (lanes 8 and 9) but not with a nonspecific competitor (lane 10). A higher molecular weight polypeptide was nonspecifically bound to I-4 RNA, as it could be totally abolished with a nonspecific competitor (compare lanes 7 and 10). These results demonstrate that while the active I-7 and inactive I-4 RNAs bind the same two polypeptides (52 and 37 kDa), these two truncated I-RNAs differ from each other in binding at least one polypeptide; while I-7 RNA binds the 80-kDa polypeptide, I-4 RNA binds a 70-kDa polypeptide.

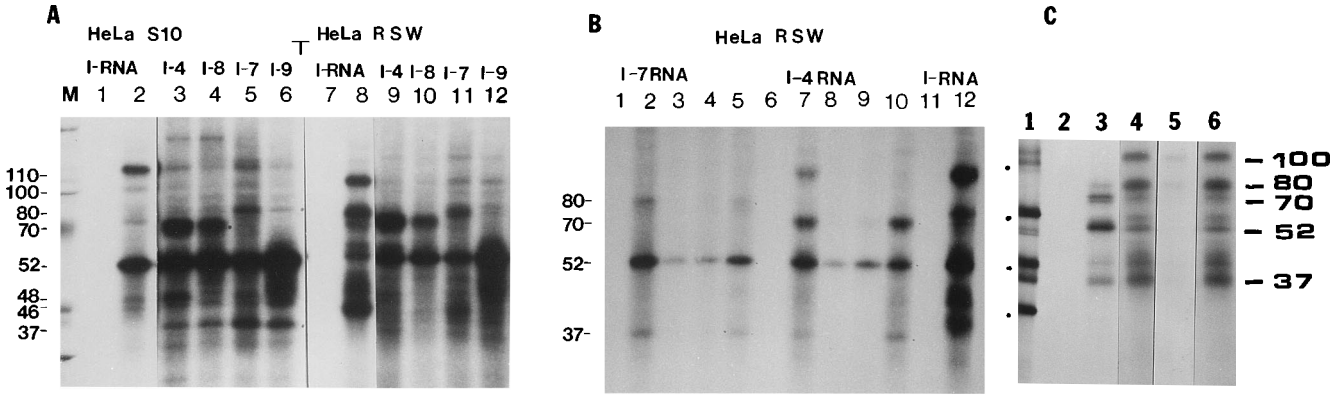

FIG. 7. Cellular proteins that interact with full-length and deleted I-RNAs. (A) UV cross-linking of ^{32}P-labeled I-RNA or truncated I-RNAs with HeLa S10 or RSW proteins was performed as described in Materials and Methods. Numbers at the left refer to the approximate molecular masses (in kilodaltons) of the proteins that cross-link to the labeled RNA probes. Lane M shows the migration of ^{14}C-labeled rainbow protein markers (Amersham) with molecular masses of 30, 46, 66, 97, and 220 kDa (from bottom to top). Lanes 1 and 7 contained the probes but no S10 and RSW, respectively. The labeled probes used for UV cross-linking are shown above the lanes. (B) Competition UV cross-linking studies with ^{32}P-labeled I-7 RNA (lanes 1 to 5), I-4 RNA (lanes 6 to 10), and I-RNA (lanes 11 and 12) probes were performed with 10 μg of HeLa RSW in the presence (lanes 3 to 5 and 8 to 10) or absence (lanes 1, 2, 6, 7, 11, and 12) of unlabeled competitor RNAs. A 100-fold molar excess of each unlabeled competitor RNA was used. Lanes 3, 4, and 5 contained unlabeled I-7 RNA, I-4 RNA, and nonspecific RNA, respectively. Lanes 8, 9, and 10 contained unlabeled I-4, I-7, and nonspecific RNA, respectively. Lanes 1, 6, and 11 contained labeled probes but no RSW proteins. (C) UV cross-linking studies were performed with ^{32}P-labeled 5′ UTR (13) (lanes 2 and 3) and ^{32}P-labeled I-RNA (lanes 4 to 6). Ten micrograms of HeLa RSW was incubated with ^{32}P-UTR (lane 3) and ^{32}P-I-RNA in the absence (lane 4) or presence of a 50-fold molar excess of unlabeled UTR (lane 5) or a nonspecific RNA (lane 6). Lane 2 had ^{32}P-UTR but no protein. Lane 1 shows the migration of protein markers. The dots at the left show migration of 94-, 67-, 43-, and 29-kDa marker proteins (from top to bottom).

To determine whether the PV 5′ UTR binds polypeptides similar to those observed with I-RNA, UV cross-linking studies were performed with HeLa RSW and labeled 5′ UTR or I-RNA. As can be seen in Fig. 7C, both UTR (lane 3) and I-RNA (lane 4) bound polypeptides with apparent molecular masses of 80, 70, 52, 43, and 37 kDa. A more slowly migrating band at approximately 100 kDa was observed when I-RNA was used as the probe (lane 4). That the polypeptides bound by UTR and I-RNA were similar was confirmed by a competition experiment using labeled I-RNA and unlabeled 5′ UTR (lanes 5 and 6). Almost all polypeptides bound to labeled I-RNA could be abolished by inclusion of unlabeled UTR during the assay (lane 5). However, a similar amount of a nonspecific RNA was totally ineffective in inhibiting cross-linking of I-RNA to these polypeptides (lane 6). These results suggest that similar polypeptides (80, 70, 52, and 37 kDa) bind to both I-RNA and the viral 5′ UTR. Although the 100-kDa polypeptide did not bind to labeled UTR (lane 3), binding of this polypeptide to labeled I-RNA was inhibited by unlabeled UTR (lane 5) for an unknown reason.

In an attempt to determine whether I-RNA deletion mutants demonstrate different La binding activities in an assay other than UV cross-linking, various labeled I-RNA deletion mutants were incubated with purified La and protein-RNA complexes were analyzed by a gel shift assay. No significant difference in La binding by I-4, I-8, I-7, and I-9 RNAs was apparent in this experiment (data not shown). Thus, there is no direct correlation between the activities of I-RNA deletion mutants and their abilities to bind La.

DISCUSSION

We have shown here that a small yeast RNA (I-RNA) which selectively blocks PV IRES-mediated translation physically interacts with the human La autoantigen. Both immunoprecipitation following UV cross-linking of the recombinant La protein to I-RNA and the ability to supershift the La–I-RNA complex with an anti-La antibody attest to this fact (Fig. 1). That binding of La to I-RNA may be relevant is indicated by the fact that purified recombinant La protein is able to restore PV IRES-mediated translation in the presence of the inhibitor RNA (Fig. 2). Our results are consistent with a previously published report which demonstrated the ability of La to correct aberrant translation of PV RNA in reticulocyte lysate, which is naturally deficient in La protein (36). Using deletion analysis, we also identified regions of I-RNA as small as 16 nt which retain the ability to inhibit PV IRES-mediated translation both in vitro and in vivo (Fig. 4 to 6). Finally, a comparison of protein binding by active and inactive truncated I-RNA mutants suggests that in addition to the La (p52) protein, three other polypeptides with apparent molecular masses of 80, 70, and 37 kDa may influence the translation-inhibitory activity of I-RNA (Fig. 7).

It is clear from the deletion analysis that the minimum sequence required to inhibit PV IRES-mediated translation appears to reside between nt 30 and 45. This notion is supported by two observations. First, a deletion mutant (I-3 RNA) which contains the entire I-RNA sequence except nt 31 to 45 is totally inactive in inhibiting viral IRES-mediated translation. Second, a truncated I-RNA (nt 30 to 45; I-9 RNA) retains a considerable amount of translation-inhibitory activity (Fig. 4C). However, a 25-nt-long truncated RNA (I-7 RNA) containing the I-9 RNA sequence appears to be more active, particularly in vivo (Fig. 6A). The shorter I-9 RNA was only 50% as active as I-RNA in vivo. It is worth mentioning in this context that both I-7 and I-9 RNAs can assume secondary structures having stem-loops (data not shown). Clearly, because of its smaller size, I-9 RNA is much less stable than I-RNA, which may affect its stability inside a cell. It will be interesting to determine whether a thio derivative of I-9 RNA is more active than the normal I-9 RNA. The structure(s) of I-RNA or its truncated derivatives may be important in IRES-mediated translation inhibition. The fact that addition of an extra 10 nt to the 3′ end of I-7 RNA (nt 26 to 50) significantly reduces the translation-inhibitory activity of the resultant construct (I-6 RNA, nt 26 to 60) may be indicative of alteration of structure of this RNA (Fig. 3 and 4B). Similarly, addition of another 5 nt to the 5′ end of I-6 RNA drastically reduces the ability of the resultant construct (I-5 RNA, nt 20 to 60) to inhibit translation (Fig. 4B).

The fact that I-7 and I-9 RNAs inhibit viral IRES-mediated translation but I-4 and I-8 do not (Fig. 4 to 6) prompted us to examine protein binding to these RNAs. UV cross-linking studies using various labeled RNAs and competition experiments demonstrated that both I-7 and I-4 RNAs bound two common polypeptides, of 52 and 37 kDa. However, these two RNAs differed from each other in that I-7 RNA bound an 80-kDa polypeptide whereas I-4 RNA interacted with a 70-kDa polypeptide. Many possible explanations can be thought of regarding the difference in protein binding of I-7 and I-4 RNAs. It is possible that in addition to 52- and 37-kDa polypeptides, binding of the 80-kDa protein to the viral 5′ UTR is important for internal initiation to occur and that I-7 RNA directly competes with the 5′ UTR in binding these polypeptides. A recent study by Meyer et al. using UV cross-linking indicates the importance of a 80-kDa protein in IRES-mediated translation of FMDV (37). This 80-kDa protein has been identified as initiation factor eIF-4B. The results presented by Meyer et al. suggest that additional protein factors contribute to this interaction of eIF-4B with the FMDV IRES (37). It is possible that binding of eIF-4B to the viral IRES requires La and 37-kDa polypeptides and or other polypeptides. I-7 RNA may interfere with IRES-mediated translation by binding these polypeptides (Fig. 7). Despite their interactions with 52- and 37-kDa polypeptides, I-4 and I-8 RNAs may not efficiently inhibit translation because of their inability to interact with the 80-kDa polypeptide. It appears from our results (Fig. 7) that binding of the 70-kDa protein to I-4 and I-8 RNAs inhibit their ability to interfere with IRES-mediated translation. It is possible that binding of the 70-kDa polypeptide to I-4 and I-8 RNAs prevents these RNAs from interacting with the 80-kDa polypeptide. Alternatively, these RNAs may lack the binding site (or structure) for the 80-kDa polypeptide. Future studies will be directed toward identifying the 80- and 70-kDa polypeptides, which may lead to a better understanding of the roles of these polypeptides in IRES-mediated translation.

Despite its inability to bind the 80-kDa polypeptide, I-4 RNA, when used as a competitor, prevents binding of the 80-kDa protein to a labeled I-7 RNA probe (Fig. 7B). Because both I-7 and I-4 RNAs bind La and 37-kDa polypeptides, this result suggests that the interaction of the 80-kDa polypeptide with I-7 RNA may be primarily through La and/or 37-kDa polypeptides, although the polypeptide (80 kDa) must somehow touch the RNA because it can be cross-linked (albeit weakly) to the I-7 RNA. In contrast, interaction of the 70-kDa protein to I-4 RNA appears relatively strong, suggesting direct interaction with I-4 RNA (Fig. 7). Because I-7 and I-9 RNAs do not bind the 70-kDa polypeptide but I-8 and I-4 RNAs do, it appears that at least the 5′-terminal 25 nt of I-RNA may be important in 70-kDa protein binding in the form of either a sequence or structure or both. Consistent with this idea is the

fact that I-3 RNA also binds the 70-kDa polypeptide (data not shown).

The normal function of I-RNA in the yeast *S. cerevisiae* is not known. Very recently, Christopher and Wolin reported identification of a human La-homologous protein in *S. cerevisiae* (11). It would be interesting to determine whether the yeast La protein interacts with I-RNA. This may lead to a better understanding of the function of I-RNA in *S. cerevisiae*. Indeed, some evidence of IRES-mediated translation in *S. cerevisiae* has recently been reported (23, 56). Our future studies will be directed toward more defined mutational analysis of I-RNA function and protein binding. These studies along with determination of I-RNA structure should help us delineate the mechanism of IRES-mediated initiation of translation in eukaryotes.

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REFERENCES

1. **Agol, V. I.** 1991. The 5′ untranslated region of picornaviral genomes. Adv. Virus Res. **40:**103–180.
2. **Bandopadhyay, P. K., C. Wang, and H. Lipton.** 1992. Cap-independent translation by the 5′-untranslated region of Theiler's murine encephalomyelitis virus. J. Virol. **66:**6249–6256.
3. **Belsham, G. J., and J. K. Brangwyn.** 1990. A region of the 5′-noncoding region of foot-and-mouth disease virus RNA directs efficient internal initiation of protein synthesis within cells: involvement with the role of L protease in translational control. J. Virol. **64:**5389–5395.
4. **Berlioz, C., and J. L. Darlix.** 1995. An internal ribosomal entry mechanism promotes translation of murine leukemia virus Gag polyprotein precursors. J. Virol. **69:**2214–2222.
5. **Blyn, B. L., R. Chen, B. L. Semler, and E. Ehrenfeld.** 1995. Host cell proteins binding to domain IV of the 5′-noncoding region of poliovirus RNA. J. Virol. **69:**4381–4389.
6. **Borman, A., M. T. Howell, J. G. Patton, and R. J. Jackson.** 1993. The involvement of a spliceosome component in internal initiation of human rhinovirus RNA translation. J. Gen. Virol. **74:**1775–1788.
7. **Borman, A., and R. J. Jackson.** 1992. Initiation of translation of human rhinovirus RNA: mapping the internal ribosome entry site. Virology **188:** 685–696.
8. **Brown, B. A., and E. Ehrenfeld.** 1979. Translation of poliovirus RNA in vitro: changes in cleavage pattern and initiation sites by ribosomal salt wash. Virology **97:**396–405.
9. **Chambers, J. C., D. Kenan, B. J. Martin, and J. D. Keene.** 1988. Genomic structure and amino acid sequence domains of the human La auto antigen. J. Biol. Chem. **263:**18043–18051.
10. **Chang, L.-J., P. Prycrak, and D. Ganem.** 1989. Biosynthesis of the reverse transcriptase of hepatitis B viruses involves de novo translation initiation not ribosomal frame shifting. Nature (London) **337:**364–368.
11. **Christopher, Y. J., and S. L. Wolin.** 1994. La proteins from *Drosophila melanogaster* and *Saccharomyces cerevisiae*: a yeast homolog of the La auto antigen is dispensable for growth. Mol. Cell. Biol. **14:**5412–5424.
12. **Coward, P., and A. Dasgupta.** 1992. Yeast cells are incapable of translating RNAs containing the poliovirus 5′-untranslated region: evidence for a translational inhibitor. J. Virol. **66:**286–295.
13. **Das, S., P. Coward, and A. Dasgupta.** 1994. A small yeast RNA selectively blocks internal initiation of translation programmed by poliovirus RNA: specific interaction with cellular proteins that bind to the viral 5′-untranslated region. J. Virol. **68:**7200–7211.
14. **Dasgupta, A.** 1983. Purification of host factor required for in vitro transcription of poliovirus RNA. Virology **128:**245–251.
15. **Fukushi, S., K. Katayama, C. Kurihara, N. Ishiyama, F. B. Hoshino, T. Andor, and A. Oya.** 1994. Complete 5′-noncoding region is necessary for the efficient internal initiation of hepatitis C virus RNA. Biochem. Biophys. Res. Commun. **199:**425–432.
16. **Gebhard, J. R., and E. Ehrenfeld.** 1992. Specific interactions of HeLa cell proteins with proposed translation domains of the poliovirus 5′ noncoding region. J. Virol. **66:**3101–3109.
17. **Glass, M. J., X. Jia, and D. Summers.** 1993. Identification of the hepatitis A virus internal ribosome entry site: *in vivo* and *in vitro* analysis of bicistronic RNAs containing HAV 5′-noncoding region. Virology **193:**842–852.
18. **Gottlieb, E., and J. A. Steitz.** 1989. The RNA binding protein La influences both the accuracy and the efficiency of RNA polymerase III transcription *in vitro*. EMBO J. **8:**841–850.
19. **Haller, A. A., J. H. C. Nguyen, and B. L. Semler.** 1993. Minimum internal ribosome entry site required for poliovirus infectivity. J. Virol. **67:**7461–7471.
20. **Haller, A. A., and B. L. Semler.** 1995. Stem-loop structure synergy in binding cellular proteins to the 5′-noncoding region of poliovirus RNA. Virology **206:**923–934.
21. **He, W. W., J. K. Lindzey, J. L. Prescott, M. V. Kumar, and D. J. Tindall.** 1994. The androgen receptor in the testicular feminized (Tfm) mouse may be a product of internal translation initiation. Receptor **4(2):**121–134.
22. **Hellen, C. U. T., G. W. Witherell, M. Schmid, S. H. Shin, T. V. Pestova, A. Gil, and E. Wimmer.** 1993. A cytoplasmic 57 kd protein (p57) that is required for translation of picornavirus RNA by internal ribosome entry is identical to the nuclear polypyrimidine tract binding protein. Proc. Natl. Acad. Sci. USA **90:**7642–7646.
23. **Iizuka, N., L. Najita, A. Franzusoff, and P. Sarnow.** 1994. Cap-dependent and cap-independent translation by internal initiation of mRNAs in cell extracts prepared from *Saccharomyces cerevisiae*. Mol. Cell. Biol. **14:**7322–7330.
24. **Jackson, R., M. T. Howell, and A. Kaminski.** 1990. The novel mechanism of initiation of picornavirus RNA translation. Trends Biochem. Sci. **15:**477–483.
25. **Jang, S. K., H. G. Krausslich, M. J. H. Nicklin, G. M. Duke, A. C. Palmenberg, and E. Wimmer.** 1988. A segment of the 5′ nontranslated region of encephalomyocarditis virus RNA directs internal entry of ribosomes during in vitro translation. J. Virol. **62:**2636–2643.
26. **Jang, S. K., T. V. Pestova, C. U. T. Hellen, G. W. Witherell, and E. Wimmer.** 1990. Cap-independent translation of picornavirus RNAs: structure and function of the internal ribosomal entry site. Enzyme (Basel) **44:**292–309.
27. **Johnson, V. H., and B. L. Semler.** 1988. Defined recombinants of poliovirus and coxsackie virus: sequence-specific deletions and functional substitutions in the 5′-noncoding region of viral RNAs. Virology **162:**47–57.
28. **Kitamura, N., B. L. Semler, P. G. Rothberg, G. R. Larsen, C. J. Adler, A. J. Dorner, E. A. Emini, R. Hanecak, J. J. Lee, S. Vander Werf, C. W. Anderson, and E. Wimmer.** 1981. Primary structure, gene organization, and polypeptide expression of poliovirus RNA. Nature (London) **291:**547–553.
29. **Le, S.-Y., N. Sonenberg, and J. V. Maizel, Jr.** 1994. Distinct structural elements and internal entry of ribosomes in mRNA3 encoded by infectious bronchitis virus. Virology **198:**405–411.
30. **Liu, D. X. M., and S. C. Inglis.** 1992. Internal entry of ribosomes on a tricistronic mRNA encoded by infectious bronchitis virus. J. Virol. **66:**6143–6154.
31. **Luz, N., and E. J. Beck.** 1990. A cellular 57 kDa protein binds to two regions of the internal initiation site of foot-and-mouth disease virus. FEBS Lett. **269:**311–314.
32. **Luz, N., and E. J. Beck.** 1991. Interaction of a cellular 57-kilodalton protein with the internal translation initiation site of foot-and-mouth disease virus. J. Virol. **65:**6486–6494.
33. **Macejak, D. G., and P. Sarnow.** 1991. Internal initiation of translation mediated by the 5′-leader of a cellular mRNA. Nature (London) **353:**90–94.
34. **Malaria, R. J., D. J. Kenan, and J. D. Keene.** 1994. Eukaryotic transcription termination factor La mediates transcript release and facilitates reinitiation by RNA polymerase III. Mol. Cell. Biol. **14:**2141–2158.
35. **Meerovitch, K., J. Pelletier, and N. Sonenberg.** 1989. A cellular protein that binds to the 5′ noncoding region of poliovirus RNA: implications for internal translation initiation. Genes Dev. **3:**1026–1034.
36. **Meerovitch, K., Y. V. Svitkin, H. S. Lee, F. Lejbkowicz, D. J. Kenan, E. K. L. Chan, V. I. Agol, J. D. Keene, and N. Sonenberg.** 1993. La autoantigen enhances and corrects aberrant translation of poliovirus RNA in reticulocyte lysate. J. Virol. **67:**3798–3807.
37. **Myer, K., A. Petersen, M. Niepmann, and E. Beck.** 1995. Interaction of eukaryotic initiation factor eIF-4B with a picornavirus internal translation initiation site. J. Virol. **69:**2819–2824.
38. **Najita, L., and P. Sarnow.** 1990. Oxidation-reduction sensitive interaction of a cellular 50-kD protein with an RNA hairpin in the 5′-noncoding region of the poliovirus genome. Proc. Natl. Acad. Sci. USA **87:**5846–5850.
39. **Oh, S. K., M. P. Scott, and P. Sarnow.** 1992. Homeotic gene *Antennapedia* mRNA contains 5′-noncoding sequences that confer translational initiation by internal ribosome binding. Genes Dev. **6:**1643–1653.
40. **Pelletier, J., M. E. Flynn, G. Kaplan, V. R. Racaniello, and N. Sonenberg.** 1988. Mutational analysis of upstream AUG codons of poliovirus RNA. J. Virol. **62:**4486–4492.
41. **Pelletier, J., and N. Sonenberg.** 1988. Internal initiation of translation of eukaryotic mRNA directed by a sequence derived from poliovirus RNA. Nature (London) **334:**320–325.
42. **Percy, N., G. J. Belsham, J. K. Brangwyn, M. Sullivan, D. M. Stone, and J. W. Almond.** 1992. Intracellular modifications induced by poliovirus reduce

the requirement for structural motifs in the 5′-noncoding region of the genome involved in internal initiation of protein synthesis. J. Virol. **66:**1695–1701.

43. **Pilipenko, E. V., A. P. Gmyl, S. V. Maslova, Y. V. Svitkin, A. N. Sinyakov, and V. I. Agol.** 1992. Prokaryotic-like cis-elements in the cap-independent internal initiation of translation on picornavirus RNA. Cell **68:**119–131.
44. **Poole, T. L., C. Wang, R. A. Popp, L. N. Potgieter, A. Siddique, and M. S. Collett.** 1995. Pestivirus translation initiation occurs by internal ribosome entry. Virology **206:**750–754.
45. **Ransone, L. J., and A. Dasgupta.** 1987. Activation of double-stranded RNA-activated protein kinase in HeLa cells after poliovirus infection does not result in increased phosphorylation of eukaryotic initiation factor 2. J. Virol. **61:**1781–1787.
46. **Riechman, J. L., S. Lain, and J. A. Garcia.** 1991. Identification of the initiation codon of plum pox potyvirus genomic RNA. Virology **185:**544–552.
47. **Rose, J. K., H. Trachsel, K. Leong, and D. Baltimore.** 1978. Inhibition of translation by poliovirus: inactivation of a specific initiation factor. Proc. Natl. Acad. Sci. USA **75:**2732–2736.
48. **Skinner, M. A., V. R. Racaniello, G. Dunn, J. Cooper, P. D. Minor, and J. W. Almond.** 1989. New model for the secondary structure of the 5′-noncoding RNA of poliovirus is supported by biochemical and genetic data that show that RNA secondary structure is important in neurovirulence. J. Mol. Biol. **207:**379–392.
49. **Sonenberg, N., and K. Meerovitch.** 1990. Translation of poliovirus mRNA. Enzyme **44:**278–291.
50. **Svitkin, Y. V., K. Merovitch, H. S. Lee, J. N. Dholakia, D. J. Kenan, V. I. Agol, and N. Sonenberg.** 1994. Internal translation initiation on poliovirus RNA: further characterization of La function in poliovirus translation in vitro. J. Virol. **68:**1544–1550.
51. **Tan, E. M.** 1989. Antinuclear antibodies: diagnostic markers for autoimmune diseases and probes for cell biology. Adv. Immunol. **44:**93–151.
52. **Trono, D., R. Andino, and D. Baltimore.** 1988. An RNA sequence of hundreds of nucleotides at the 5′ end of poliovirus RNA is involved in allowing viral protein synthesis. J. Virol. **62:**2291–2299.
53. **Tsukiyama-Kohara, K. I., N. Zuka, M. Kohara, and A. Nomoto.** 1992. Internal ribosome entry site within hepatitis C virus RNA. J. Virol. **66:**1476–1483.
54. **Witherrell, G. W., A. Gil, and E. Wimmer.** 1993. Interaction of polypyrimidine tract binding protein with the encephalomyocarditis virus mRNA internal ribosome entry site. Biochemistry **32:**8268–8275.
55. **Wyekoff, E. E., J. W. B. Hershey, and E. Ehrenfeld.** 1990. eIF-3 is required for poliovirus 2A protease-induced cleavage of the p220 component of eIF-4F. Proc. Natl. Acad. Sci. USA **87:**9529–9533.
56. **Yun, D. F., and F. Shermann.** 1995. Initiation of translation can occur only in a restricted region of the *cyc1* mRNA of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **15:**1021–1033.