Internal Ribosome Entry Site-mediated Translation of a Mammalian mRNA Is Regulated by Amino Acid Availability*

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James Fernandez‡, Ibrahim Yaman‡, Rangnath Mishra‡, William C. Merrick§, Martin D. Snider§, Wouter H. Lamers§, and Maria Hatzoglou‡‡

From the Departments of ‡Nutrition and §Biochemistry, Case Western Reserve University School of Medicine, Cleveland, Ohio, 44106 and the ‡Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands

The cationic amino acid transporter, Cat-1, facilitates the uptake of the essential amino acids arginine and lysine. Amino acid starvation causes accumulation and increased translation of cat-1 mRNA, resulting in a 58-fold increase in protein levels and increased arginine uptake. A bicistronic mRNA expression system was used to demonstrate the presence of an internal ribosomal entry sequence (IRES) within the 5'-untranslated region of the cat-1 mRNA. This study shows that IRES-mediated translation of the cat-1 mRNA is regulated by amino acid availability. This IRES causes an increase in translation under conditions of amino acid starvation. In contrast, cap-dependent protein synthesis is inhibited during amino acid starvation, which is well correlated with decreased phosphorylation of the cap-binding protein, eIF4E. These findings reveal a new aspect of mammalian gene expression and regulation that provides a cellular stress response; when the nutrient supply is limited, the activation of IRES-mediated translation of mammalian mRNAs results in the synthesis of proteins essential for cell survival.

Amino acids are essential nutrients for cell growth and maintenance. Mammalian cells have developed an adaptive response to changes in amino acid availability (1). When the amino acid supply is limited, protein synthesis decreases, and there are increases in catabolism of cellular proteins, amino acid biosynthesis, and amino acid transport across the plasma membrane. Together these responses provide cells with amino acids needed for survival. A significant part of this adaptive response is the increased expression of the cat-1 gene, which encodes the transporter for the essential cationic amino acids, lysine and arginine (2). We have shown that the level of the Cat-1 protein and the transport of cationic amino acids increase in amino acid-depleted cells (3). Because amino acid starvation inhibits cap-dependent initiation of protein synthesis (4), we hypothesized that the cat-1 mRNA is translated in amino acid-depleted cells through a cap-independent mechanism. This translation would involve an internal ribosomal entry sequence (IRES) within the 5'-untranslated region (5'-UTR). IRES sequences have been implicated in the translation of viral mRNAs in infected cells, where cap-dependent translation of cellular mRNAs is inhibited (5). These sequences are also important in the translation of several mammalian mRNAs encoding regulatory proteins involved in growth and differentiation (6–11). Furthermore, a role of IRES sequences in cell cycle-dependent translation of mammalian mRNAs has been demonstrated recently (6, 12–14).

This study provides support for our hypothesis by demonstrating that the 5'-UTR of the cat-1 mRNA contains an IRES sequence. Moreover, translation from this IRES is stimulated in amino acid-starved cells, when cap-dependent translation is decreased. These findings suggest a mechanism for synthesis of proteins required for amino acid accumulation in starved cells when total protein synthesis is inhibited.

EXPERIMENTAL PROCEDURES

Cloning of the cat-1 cDNA 5'-UTR—The 5'-end of the cat-1 mRNA was cloned using the RACE technique (15). RACE was performed using polyadenylated RNA from FTO2D rat hepatoma cells. Five cDNA clones were isolated, with the largest containing an insert of 224 nucleotides of 5'-UTR (cat1–224). The cat1–224 cDNA was amplified by polymerase chain reaction using the primers in Fig. 1A and cloned into the SalI/NcoI site of the pSVCAT/BiP/LUC plasmid (16) by replacing the IRES from the BiP mRNA. The resulting vector was named pSVCAT/cat1–224/LUC. The plasmid pSVhpcAT/cat1–224/LUC was constructed by replacing the BiP IRES within the pSVhpcAT/BiP/LUC (16) vector at the NcoI/SalI sites. The pSVCAT/ICS/LUC vector contains 400 nucleotides of antisense antenapedia cDNA of D. Melanogaster (16) in the ICS region and was used as a negative control for IRES-mediated translation. The pUHD10–3-cat1–224/LUC, pUHD10–3-cat1–224mut/LUC, and pUHD10–3con/LUC expression vectors were generated by cloning the chimeric LUC mRNAs into the pUHD–3 expression vector cleaved at the XbaI site 3' to the promoter region and the EcoRI site 5' to the polyadenylation signal (3). This vector contains a minimal cytomegalovirus promoter with very low promoter activity and the SV40 polyadenylation signal. The cat1–224/LUC mRNA contained 224 nucleotides upstream of the LUC ORF. The cat1–224mut/LUC contained the same sequence as cat1–224/LUC, but the 49-amino acid ORF was eliminated by mutating the initiating ATG to TTG using polymerase chain reaction-based mutagenesis. The cat1 mutants contained 25 nucleotides of linker sequence upstream of the LUC ORF. The mRNAs transcribed from these expression vectors would contain 5'-UTR sequences of 249 bases for cat1–224 and cat1–224mut and 50 bases for the con.

Cells and Cell Culture—Plasmid DNA was transfected into C6 rat glioma cells using the calcium phosphate technique (2). Stable mammalian cell lines were generated by cotransfecting an expression vector containing the neo gene and selecting the transfecants in 0.1% G418. All cells were maintained in Dulbecco's modified Eagle's medium/F12 medium supplemented with 10% fetal bovine serum. Fed cells were incubated in Dulbecco's modified Eagle's medium/F12 supplemented with 5% FCS, 5'-untranslated region; CAT, chloramphenicol acetyltransferase; KRB, Krebs-Ringer bicarbonate buffer; LUC, firefly luciferase; ORF, open reading frame; RACE, rapid amplification of cDNA ends; ICS, intercistronic spacer; mut, mutant; con, control.
with fetal bovine serum dialyzed against phosphate-buffered saline. Starved cells were incubated in KRB supplemented with dialyzed fetal bovine serum (2, 3). No difference in the regulation of the cat-1 gene by amino acid starvation was observed when KRB containing all amino acids was used in place of Dulbecco’s modified Eagle’s medium/F12 medium (2). Treatments were performed by culturing cells (5 × 10⁵ cells/35-mm dish) for 48 h in growth medium followed by culture under fed or starved conditions for the appropriate times.

Enzyme and Transport Assays—Cell extracts were prepared and analyzed for LUC and CAT activities as described previously (17). The activities were normalized to the protein content of the cell extracts, which was measured using the Bio-Rad assay. To measure IRES-mediated translation, cells were plated at 2 × 10⁵ cells/18-mm plate for 48 h and were then treated with pamamicin. This compound partially inhibits cap-dependent translation by promoting the dephosphorylation and activation of 4E-BP1, a repressor of the cap-binding protein 4E (21).

RESULTS

The previously cloned cat-1 cDNA contained only 80 nucleotides of 5′-UTR sequence (19). To test our hypothesis that the cap-independent mechanism, the 5′-UTR of the cat-1 mRNA was isolated using RACE (15). The longest of the clones contained 224 base pairs of the cat-1 5′-UTR. This sequence contains an open reading frame of 49 amino acids, beginning 223 bases upstream from the cap-1 ORF (Fig. 1A).

The ability of the cat-1 5′-UTR sequence to mediate internal translation initiation was tested using vectors developed by the Sarnow laboratory that encode a bicistronic mRNA (16). The CAT enzyme is translated from the first cistron by a cap-dependent scanning mechanism. The second cistron, encoding the firefly LUC enzyme, is translated only if it is preceded by an IRES in the ICS region (Fig. 1B). The following three vectors were tested for IRES-mediated translation of the LUC gene: CAT/ICS/LUC as a negative control, CAT/BiP/LUC containing the BiP 5′-UTR as a positive control (16), and CAT/cat1–224/LUC containing the cat-1 5′-UTR sequence instead of the BiP sequence.

The plasmids were transfected into C6 rat glialoma cells, and 48 h later cell extracts were prepared and assayed for LUC and CAT activities. The data are expressed as the ratio of LUC and CAT activities to normalize for differences in transfection efficiency (Fig. 2). The normalized LUC activity for CAT/cat1–224/LUC was 35 times higher than the CAT/ICS/LUC negative control and similar to CAT/BiP/LUC (Fig. 2), suggesting that the 5′-UTR of the cat-1 mRNA contains an IRES element. Northern blot analysis of transfected cells demonstrated a single transcript hybridizing to both LUC and CAT hybridization probes (Fig. 2, inset), supporting the idea that the LUC and CAT activities derive from translation of the bicistronic mRNAs.

To demonstrate that expression of the LUC gene does not result from readthrough of ribosomes from the CAT cistron, we constructed a vector encoding a bicistronic CAT/cat1–224/LUC mRNA with a stable RNA hairpin upstream of the CAT ORF (see Fig. 1B and Ref. 16). RNA hairpins in the 5′-UTR of mRNAs have been shown to inhibit translation initiation at a downstream AUG (20). hpCAT/cat1–224/LUC gave normalized LUC activity that was 15-fold higher than CAT/cat1–224/LUC (Fig. 2A). This increase was due to decreased CAT and unchanged LUC activities in hpCAT/cat1–224/LUC compared with CAT/cat1–224/LUC (Fig. 2, B and C). These results are consistent with the cap-dependent initiation of CAT and the cap-independent initiation of LUC translation from an IRES in the cat-1 5′-UTR, as previously shown (16), the relative LUC activity from hpCAT/BiP/LUC was 5-fold higher than the activity from CAT/BiP/LUC (Fig. 2A).

To further demonstrate that the cat-1 5′-UTR supports IRES-mediated translation under conditions where the cap-dependent translation of cellular mRNAs is inhibited, the normalized LUC activity was measured in cells treated with rapamycin. This compound partially inhibits cap-dependent translation by promoting the dephosphorylation and activation of 4E-BP1, a repressor of the cap-binding protein 4E (21).
The regulation of CAT/cat1–224/LUC mRNA translation by amino acid starvation was studied next. Rat C6 glioma cells were transfected either with CAT/cat1–224/LUC or CAT/BiP/LUC, along with a vector expressing the neo gene, and two stable mass-culture lines, C6/pSVcat1–224 and C6/pSVBiP, were generated, each containing ~70 clones. These cell lines were cultured either in amino acid-containing or amino acid-free media, and the effects of starvation on CAT and LUC expression were examined. As expected, the cells expressed a single mRNA transcript containing both the CAT and LUC cistrons (not shown). Amino acid starvation of C6/pSVcat1–224 cells increased the normalized LUC activity after 6 h, reaching a 7-fold increase by 12 h (Fig. 3A, left panel). This increase was due to increased LUC and decreased CAT activities (Fig. 3, B and C, left panels). No further increase in LUC activity was observed when amino acid starvation exceeded 12 h (not shown). These data support the conclusion that cat1–224-IRES-mediated translation of the LUC cistron increased during amino acid starvation. Is this translational regulation specific for the cat1–224 IRES? This was tested by examining the effect of amino acid starvation on C6/pSVBiP cells. In contrast to C6/pSVcat1–224 cells, both CAT and LUC activities decreased in amino acid-starved C6/pSVBiP cells (Fig. 3, B and C, right panel). The normalized LUC activity increased by only 1.7-fold (Fig. 3A), suggesting that the BiP/IRES-mediated translation of the LUC cistron is more efficient than the cap-dependent translation of the CAT cistron, but four times less efficient than the cat1–224 IRES, under conditions of amino acid starvation.

A striking result in these studies was that following 12 h of amino acid starvation, cap-dependent translation of the CAT cistron decreased by 70% (Fig. 3), whereas IRES-mediated translation of the LUC cistron increased by 3.5-fold. What caused the dramatic decrease of CAP-dependent translation? It is well known that heat shock, serum deprivation, and viral infection inhibit cap-dependent translation by regulating the phosphorylation state of the cap-binding protein, eIF4E (22). Reduced phosphorylation of eIF4E correlates with inhibition of cap-dependent protein synthesis (23). We therefore compared the phosphorylation state of eIF4E in amino acid-fed and -starved cells. As a negative control, we analyzed rapamycin-treated cells, because this drug does not change eIF4E phosphorylation (23). Amino acid starvation induced dephosphorylation of eIF4E (75% decrease), whereas total eIF4E levels remained the same (Fig. 4). As expected, rapamycin had no effect (Fig. 4). The 75% decrease in eIF4E phosphorylation paralleled the 75% decrease in cap-dependent CAT expression from the CAT/cat1–224/LUC bicistronic mRNA following 12 h

| Control  | 167 ± 6 | 509 ± 25 | 3.0 ± 0.1 |
|----------|---------|----------|-----------|
| Rapamycin| 34.6 ± 0.5 | 537 ± 33 | 15.4 ± 0.8 |

### Table I

Rapamycin does not inhibit translation from the cat-1 IRES

C6 cells were transfected with the pSVCAT/cat1–224/LUC plasmid and 36 h after transfection were incubated without (control) or with 50 ng/ml rapamycin for 18 h. Cell extracts were analysed for LUC and CAT activities; the LUC and CAT activities (arbitrary units) and the LUC/CAT ratio are shown; values represent the mean ± S.E. of three independent experiments.

### FIG. 2

The 5’-UTR of the cat-1 mRNA confers IRES-mediated translation. C6 cells were independently transfected with pSVcat1–224/LUC (ICS), pSVcat1–224/LUC (cat1–224), pSVpcat1–224/LUC (hpBiP), and pSVpcat1–224/LUC (hpBiP). The data are presented as the ratio (A), CAT (B), and LUC (C) activities measured in cell extracts 48 h after transfection. The bars represent the average ± S.E. of three independent experiments. The inset shows a Northern blot of total RNA (25 μg) from cells transfected with either pSVcat1–224/LUC (BiP) or pSVcat1–224/LUC (cat1–224) expression vectors and hybridized to a LUC cDNA probe. A single mRNA transcript, hybridizing to both LUC (shown) or CAT (not shown) hybridization probes was expressed from both vectors.

Rapamycin treatment caused an 80% decrease of CAT expression from CAT/cat1–224/LUC, but LUC activity did not change (Table I). The normalized LUC activity increased 5.1-fold (Table I), supporting internal translation initiation of LUC from the cat-1 5’-UTR. As expected (16), a 5.6-fold increase in the normalized LUC activity was caused by rapamycin treatment of cells transfected with CAT/BiP/LUC as a positive control (not shown). These data support the conclusion that the 5’-UTR of the cat-1 mRNA contains an IRES element that has high activity when cellular cap-dependent translation is inhibited.
of amino acid starvation (compare Figs. 3 and 4). This is consistent with the idea that phosphorylated eIF4E is a key part of the eIF4F cap-binding complex that is required for the cap-dependent initiation of translation (22).

Amino acid starvation is also known to cause increased phosphorylation of the α subunit of the initiation factor eIF2 (4). This phosphorylation results in decreased formation of ternary complexes (initiator Met-tRNA, eIF-2, and GTP). We therefore tested the phosphorylation state of eIF2α during a time course of amino acid starvation, because it has been reported that eIF2α is phosphorylated transiently in response to different stimuli by specific kinases (24). An increase in phospho-eIF2α levels occurred during the first hour of amino acid starvation (3.2-fold) followed by a gradual decrease thereafter (Fig. 4B).

The increased phospho-eIF2α levels can be due to either increased total eIF2α or increased phosphorylation. To determine the degree of eIF2α phosphorylation, we determined the ratio of phospho-eIF2α/total eIF2α during the time course of amino acid starvation (Fig. 4B). The ratio increased by 2-fold during the first hour of amino acid starvation and then decreased in a pattern similar to the changes in phospho-eIF2α levels (Fig. 4B). We therefore conclude that eIF2α phosphorylation transiently increased in amino acid-depleted cells. Because eIF2α phosphorylation rises and then declines before any increase in LUC activity is seen, we propose that phosphorylation of eIF2α does not directly regulate cat-1/IRES activity.

The IRES-mediated translation of cat-1 mRNA during amino acid starvation could enable cells to increase Cat-1 protein levels under conditions where global protein synthesis is inhibited. Asparagine synthase protein levels, analyzed as a positive control, were increased by amino acid starvation (Fig. 5A). Cat-1 protein expressed from the endogenous gene increased by 58-fold at 12 h of amino acid starvation (Fig. 5A), whereas mRNA levels increased by only 16-fold (not shown; see Ref. 3). The fact that the Cat-1 protein was induced to a greater extent than the mRNA supports the translational regulation of cat-1 gene expression. Cat-1 protein expressed from the endogenous gene increased by 58-fold at 12 h of amino acid starvation (Fig. 5A), whereas mRNA levels increased by only 16-fold (not shown; see Ref. 3).

Furthermore, a 4.5-fold increase in high affinity arginine transport was observed after 12 h of amino acid starvation (Fig. 5B), demonstrating that cells depleted of amino acids induce Cat-1 protein synthesis to support cationic amino acid transport once amino acids become available.

The nucleotide sequence of the 5’-UTR of the cat-1 mRNA revealed the presence of a 49-amino acid ORF (Fig. 1A). Because ORFs within the 5’-UTR of mRNAs are almost always associated with translational control of the mRNA (25), we studied the role of the ORF in the translation of the cat-1 mRNA. We studied two monocistronic mRNAs. One (cat1–224/LUC) had the cat-1 5’-UTR upstream of the LUC coding sequence. The other (cat1–224mut/LUC) had the initiating AUG of the upstream ORF mutated to UUG. Both mRNAs were expressed from the minimal cytomegalovirus promoter (3). C6
cells were transiently transfected with one of the expression vectors along with a vector expressing the β-galactosidase gene to normalize for transfection efficiency. Cell extracts were prepared 48 h after transfection, and the LUC and β-galactosidase activities were measured and expressed as the LUC/β-galactosidase ratio. In amino acid-fed cells, the cat1–224mut/LUC mRNA increased in amino acid-depleted cells. The 2-fold difference in the LUC activity between the wild-type and mutant 5′-UTRs suggests that the upstream ORF does contribute to the increased translation of the cat-1 mRNA during amino acid starvation. The smaller increase in translation of cat1–224mut/LUC mRNA may be due to the higher level of translation in fed cells (Fig. 6, A and C).

Finally, we compared the translation of the mRNAs containing the cat-1 5′-UTR to the control mRNA (con/LUC). Amino acid starvation of the C6/con/LUC cells caused a 40% decrease in LUC activity, consistent with translation of this mRNA via a cap-dependent scanning mechanism. LUC expression from this mRNA was at least 50-fold higher than from the mRNAs with the cat-1 5′-UTR. This is consistent with inefficient initiation of translation caused by the secondary structure of the cat-1 5′-UTR mRNA. This low level of expression was seen with both the cat1–224/LUC and cat1–224mut/LUC mRNAs. Consequently, the inefficient translation initiation from these mRNAs cannot be due solely to the presence of the ORF in the 5′-UTR. This is in agreement with the inhibitory effects of IRESs within the 5′-UTR of mRNAs on cap-dependent translation initiation (26).

**DISCUSSION**

Our studies have shown that the 5′-UTR of the cat-1 mRNA contains an IRES sequence. Moreover, we have shown that translation from this IRES is induced by amino acid starvation. To our knowledge, this is the first report of an IRES that shows this regulation. We propose that some mammalian mRNAs use IRES-mediated translation to provide cells with proteins essential for survival when the nutrient supply is limited. Among these proteins is the Cat-1 arginine/lysine transporter, which also provides cells with the substrate for NO synthesis (27). Expression of the cat-1 gene has been shown to be regulated at the transcriptional and post-transcriptional level by hormones (28, 29), nutrients (2, 3) and growth factors (30, 31). The translational regulation reported in this study further extends the complex regulatory mechanisms that control the cell’s supply of arginine and lysine.

We have shown in this study that amino acid starvation caused a 50-fold increase in Cat-1 protein levels and only a 4-fold increase in y+ amino acid transport. This difference may be due to the fact that this transporter is stimulated by intracellular cationic amino acids, a property called trans-stimulation (32, 33). Arginine transport in this study was performed in cells starved of all amino acids but lysine. We speculate that the difference between cat-1 protein levels and y+ transport in starved cells is due to low cytoplasmic cationic amino acids that cause inadequate trans-stimulation. However, we cannot exclude the possibility that Cat-1 protein synthesis during amino acid starvation is not functional, because it is not properly folded or is not transported to the plasma membrane.

Translational regulation by amino acid availability has also been demonstrated for the yeast transcription factor GCN4 (1). However, the mechanism of this regulation is different from the one we observed for cat-1 mRNA. Amino acid starvation increases translation of GCN4 mRNA by 8-fold by a mechanism involving cap-dependent initiation and reinitiation at the GCN4 ORF, following ribosome scanning of four small ORFs in
the 5'-UTR (1). Removal of the ORFs increased basal expression of the GCN4 mRNA by 80-fold and abolished translational control by amino acid starvation (34). Thus the upstream ORFs inhibit basal translation and participate in the starvation-induced stimulation. Another example of translation by induced amino acid starvation was recently reported for the branched-chain a-ketoacid dehydrogenase kinase, but the mechanism of translational control was not studied (35).

This is the first report that nutrient supply can regulate expression of a mammalian mRNA via an IRES-mediated mechanism. What features of the cat-1 IRES enable it to recruit ribosomes and regulate translation? Computer analysis suggests that the cat-1 IRES is very structured (not shown), a general feature of other cellular and viral IRESs (26). However, studies of other IRESs have not revealed a consensus sequence (36) nor a specific RNA conformation important for activity (37). In fact, segments as short as 9 nucleotides may have independent IRES activity or affect the activity of the IRES in which they occur (37). The fact that amino acid depletion induced translation mediated by the cat1–224/IRES four times more than the BiP/IRES suggests that the cat1/IRES has special features that support enhancement of translation during starvation. The residues within the cat-1 IRES that are important in translational regulation will be the focus of future studies.

What is the role of the 49-amino acid ORF in the cat-1 5'-UTR? A general feature of IRESs is the presence of ORFs (26). It is believed that the ORFs are present within the IRES to inhibit cap-dependent translation. Our studies of the wild-type and mutant mRNAs have shown that the cat-1 ORF inhibits translation. This is in agreement with the very low levels of Cat-1 protein in amino acid-fed cells (Fig. 5). Amino acid starvation increased translation of monocistronic mRNAs containing the cat-1 5'-UTR whether they contained the 49-amino acid ORF, consistent with the idea that the regulation does not depend on the presence of the ORF. However, the extent of induction was 2-fold greater in the ORF-containing mRNA than from the wild-type 5'-UTR. This could reflect a change in the regulation by amino acid starvation. Alternatively, it could be due to the fact that there is greater cap-dependent translation from the mutant than from the wild-type 5'-UTR. This translation will be inhibited by amino acid-starvation, causing a decrease in the overall induction seen with this construct. This is in agreement with the 3.5-fold induction of the LUC activity in the bicistronic mRNA, where the LUC cistron was translated exclusively by the IRES. Future studies will determine the mechanism (cap-
dependent or IRES-mediated) via which the cat-1 mRNA is translated in amino acid-fed and -starved cells.

Using the bicistronic mRNA system, we have shown that translation mediated by the ORF-containing cat-1/IRES increased 3.5-fold (Fig. 3C). Is the ORF translated within the bicistronic mRNA? Translation of the 49-amino acid ORF might occur either by reinitiation of cap-dependent translation of the first cistron or by initiation at the IRES at or before the AUG of the ORF. Using the hpCAT/cat1–224/LUC bicistronic mRNA, we have shown that the cat-1/IRES activity is independent of the cap-dependent translation of the first cistron (Fig. 2). Consequently if this 49-amino acid ORF is translated this must occur by initiation within the IRES. The structure of the cat-1 IRES and its role in the regulation of translation initiation by amino acid starvation will be the subject of future studies.

The cellular proteins that interact with the IRESs and modulate translation are not known. We have shown that during amino acid starvation, cat-1 IRES-mediated translation increased, whereas cap-dependent translation decreased. The increased phosphorylation levels of eIF2α may contribute to the IRES-mediated stimulation of cat-1 expression in starved cells. The inhibition of cap-dependent translation may also be due to changes in the activity of the cap binding complex, eIF4F. We showed that amino acid starvation decreased the amount of phosphorylated eIF4E, a component of eIF4F and a regulator of its activity (36). However, eIF4E may not play a role in the increased translation from the cat-1 IRES. We showed that rapamycin, which reduces the amount of active eIF4E, caused an 80% decrease in cap-dependent protein synthesis but did not induce IRES-mediated translation, consistent with previous findings (38). It is therefore likely that the increased activity of the cat-1 IRES in amino acid-starved cells is due to the synthesis or activation of regulatory proteins. The 3-h lag in the increase of cat1–224/IRES-mediated translation in amino acid-deficient cells may represent the time required for synthesis or modification of protein(s) that interact with the IRES and/or the recruited ribosomes. In fact, cellular IRES-binding proteins have been shown to control the functional state of viral IRESs (14). The elucidation of these regulatory mechanisms will be of great importance in our understanding of cell responses to nutritional stress.

Nuclear proteins have also been implicated in IRES-mediated translation (36). A few nuclear RNA-binding proteins, such as the polypyrimidine tract and polypyrimidine tract-binding proteins, have been shown to enhance IRES-mediated translation (36, 39). The cat-1 IRES contains three pyrimidine tracts (∼47, −111, and −183, relative to the cat-1 ORF; see Fig. 1) that may bind to pyrimidine tract-binding proteins.

IRES-mediated translation in mammals has also been described for viral mRNAs (5) in infected cells where cap-dependent translation is inhibited. It is likely that these viral mRNAs are translated using a normal cellular mechanism that is activated by infection. Interestingly, the Cat-1 protein is the receptor for the type C ecotropic retrovirus (40, 41), whose mRNAs are translated via an IRES-dependent mechanism (42). Based on the common regulatory elements in the mRNAs of the type C ecotropic retrovirus and its receptor, Cat-1, it is intriguing to speculate on their coevolution.

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