Identification of a Novel Internal Ribosome Entry Site in Giardiavirus That Extends to Both Sides of the Initiation Codon*

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Giardiavirus (GLV) is a double-stranded RNA virus of the Totiviridae family, which specifically infects trophozoites of the most primitive protozoan parasite Giardia lamblia (1). Its double-stranded RNA genome of 6277 bp encodes two polypeptides, a major 100-kDa capsid protein (Gag) and a minor 190-kDa fusion protein (Gag-Pol) via a 1-ribosomal frameshift (2, 3). The coding region in GLV (+)-strand RNA is flanked by a 367-nucleotide (nt) 5′-untranslated region (UTR) and a 2022-nt 3′-untranslated region (3′-UTR). The ability of purified GLV to infect and the capability of its (+)-stranded RNA to transfect G. lamblia trophozoites, resulting in intracellular proliferation of infectious GLV particles, are the major distinguishing features of this virus among the totiviruses (1) and have enabled us to develop it into an effective transfecting vector of Giardia, which has turned out to be particularly useful for studying the mechanisms of translation in this primitive eukaryote (4, 5).

The viral transcript is not capped (6). Chimeric mRNA containing a full-length firefly luciferase transcript flanked by the 367-nt GLV 5′-UTR and a 2022-nt 3′ terminus of GLV (+)-strand RNA was introduced into GLV-infected Giardia trophozoites by electroporation (4). The chimeric mRNA thus introduced underwent vigorous replication and transcription, but only a basal level of translation ensued, resulting in an expressed luciferase activity barely above the background level (4, 7). This relatively poor translation efficiency was, however, enhanced by 5000-fold when the initial 264 nts of the capsid-coding region in GLV mRNA were fused in-frame with, and upstream from, the luciferase mRNA (7).

Extensive functional and structural analysis of the 264-nt capsid-coding region revealed several structural and sequence-specific elements that are essential for efficient translation of the chimeric transcript (6). A 13-nt downstream box (DB) sequence at position 66–78 that complements a 15-nt sequence could function as an element of the internal ribosomal scanning from the 5′-end of mRNA, and thus the 264-nt sequence revealed also that stem loops I (nts 11–35), II (nts 144–164), III (nts 166–182), and IVA (nts 193–215) all play an indispensable function in translation initiation (5). Furthermore, a pseudoknot 143 nts downstream from the initiation codon formed between a pentanucleotide sequence downstream of stem loop IVA and the loop sequence of stem loop II turned out also essential (8). This is a novel structure as it incorporates two additional stems in the loop region between stem II and the pseudoknot stem. Increasing or decreasing the distance between the pseudoknot and initiation codon results in complete loss of translation, suggesting that the position of AUG upstream of the secondary structure cannot be changed and still maintain optimal translation initiation (8). The observation suggested that initiation of translation of GLV mRNA is by a mechanism of internal ribosomal entry without ribosome scanning from the 5′-end of mRNA, and thus the 264-nt sequence could function as an element of the internal ribosomal entry site (IRES).

The presence of IRES elements in the 5′-UTRs of viral transcripts has been well documented in the past (9–11). It was initially identified exclusively in the 5′-UTRs of transcripts of picornaviruses (12, 13) and subsequently in hepatitis C virus (14, 15), classical swine fever virus (16, 17), and several other viruses (for review, see Ref. 11). Recently, IRES elements have also been identified in several mRNAs from animal cells (for review, see Ref. 18). The long and uncapped 5′-UTRs containing IRES elements have usually complex secondary and tertiary structural features that facilitate recruitment of translation machinery by internal entry. These complex structures

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‡ The abbreviations used are: GLV, giardiavirus; nt, nucleotide; UTR, untranslated region; DB, downstream box; IRES, internal ribosomal entry site; UTRCod, GLV 5′-UTR plus the 264-nt capsid-coding region; RLU, relative light unit; HCV, hepatitis C virus.
impair cap-mediated ribosome recruitment as they present significant obstacles to a scanning ribosome (9, 11). Insertion of picornaviral 5′-UTR between the two cistrons of a dicistronic construct led to the definitive demonstration that this region was able to confer internal initiation of translation of the downstream cistron, independent of the 5′-end sequence (12, 13). There is, however, no requirement of the downstream picornaviral coding region for IRES function, and the 5′-UTR alone is sufficient to drive the translation of the downstream cistron (19, 20).

In our previous investigation, the potential involvement of the 5′-UTR of GLV mRNA in translation initiation was not clear, as it initiated little translation of the downstream reporter by itself (7). It remains thus possible that a portion of the 5′-UTR or the entire 5′-UTR is also a part of the GLV IRES. In this study we expressed the in vitro transcriptions of dicistronic cDNA constructs in *Giardia* and demonstrated further inclusion of a 253-nt downstream portion of the 5′-UTR in the IRES resulting in an overall 517-nt IRES extending to both sides of the initiation codon at an approximately equal distance. To our knowledge, this is the first reported case of a viral IRES that extends to both sides of the initiation codon.

**EXPERIMENTAL PROCEDURES**

**Construction of Dicistronic Viral Vectors**—In the monocistronic viral cDNA vector the reporter gene was flanked by the 367-nt GLV 5′-UTR plus the 264-nt capsid-coding region at the 5′-end and the 2022-nt 3′-terminus of GLV cDNA at the 3′-end (8). In the dicistronic construct a second reporter is placed downstream of the first reporter gene separated by a DNA fragment encoding the potential IRES. The wild-type monocistronic plasmid pC631-luc, containing the firefly luciferase gene (*Fluc*) (7), was replaced by the *Renilla* luciferase gene (*Rluc*) excised from pNull-Rluc (Promega) as a HindIII/XhoI fragment with an in-frame fusion to the 264-nt capsid-coding region of pC631 to generate pC631Rluc. Full-length Fluc cDNA carrying varying lengths of the upstream extension from its 5′-end (the GLV 5′-UTR plus the 264-nt capsid-coding region; UTRCod) was amplified by PCR using pC631-luc as template. These amplified fragments, initially cloned in pGEM-T-easy vectors and excised as XhoI/EcoRI fragments, were each cloned into pC631Rluc downstream from the Rluc gene to generate the dicistronic plasmids. Each plasmid construct was verified by DNA sequencing. For specific mutations in the IRES, PCR amplification of the UTR-Cod-Fluc region from previously constructed mutant monocistronic plasmids was performed (5, 8). The fragments thus amplified, initially cloned into pGEM-T-easy vectors and excised as XhoI/EcoRI fragments, were each cloned into the XhoI/EcoRI site of the monocistronic pC631Rluc plasmid to generate the corresponding dicistronic plasmids. For mutations in the IRES driving the first cistron, the Fluc gene in various mutant pC631-luc monocistronic plasmids was replaced by the Rluc gene to generate the corresponding mutant monocistronic pC631Rluc plasmids. The XhoI/EcoRI UTRCod-Fluc fragment excised from the dicistronic pC631Rluc-UTRCod-Fluc was cloned into the XhoI/EcoRI site in each of the mutant pC631Rluc plasmids to generate the corresponding dicistronic plasmid.

**Site-directed Mutagenesis**—Site-directed mutagenesis of the 5′-UTR sequence was carried out essentially as described previously (5) using a QuikChange site-directed mutagenesis kit and following the manufacturer’s instructions (Stratagene). Individual mutations were each verified by DNA sequencing. A stem U2 disruption, mutation U2, was made by altering GG at position 101/102 to CC. This mutation in a monocistronic construct resulted in an increase of luciferase activity to 148% of the wild-type level. Another disruptive S55 mutation in stem U5, made by substituting GC at position 317/318 with GG and AU at position 321/322 with CC, was found to decrease the luciferase activity to 0.2% of the wild-type level in a monocistronic construct.

**In Vitro Transcription**—The monocistronic and dicistronic plasmids were each linearized with NruI at the 3′-end of GLV genomic cDNA and used as template for in vitro synthesis of transcripts using a MegaScript T7 transcription kit (Ambion).

**Transfection of *Giardia* Trophozoites**—The in vitro transcriptions of various GLV monocistronic and dicistronic chimeric cDNAs were each introduced into the GLV-infected WB strain of *Giardia* trophozoites by electroporation as described previously (4, 5). Approximately 4 × 10⁶ trophozoites were transfected with 100 µg of the in vitro transcript. Mutant and wild-type transcripts were each used in triplicates for the transfection study in duplicate experiments.

**Luciferase Assay**—The transfected *Giardia* trophozoites were incubated in culture medium at 37°C for 18 h, lyed, and assayed for luciferase activity as described previously for monocistronic transcript transfectants (4). For the dicistronic transcript transfectants, *Renilla* luciferase (Rluc) and firefly luciferase (Fluc) activities were assayed using the Dual-Luciferase® reporter assay system (Promega) according to the manufacturer’s instructions. The two enzyme activities from each transfectant in triplicates from two to three independent transfection experiments were examined and compared with the respective control transfectant. Luciferase activity was calculated in relative light units (RLU/µg of crude lysate protein determined by the Bradford method (21).

**Northern Blot Analysis**—Total RNA was extracted from the transfected *G. lamblia* GLV-infected WB trophozoites 18 h post-transfection as described previously (22) and used for Northern blots by standard procedures (23). A HindIII/XhoI fragment from pC631-Fluc containing the Fluc gene sequence (7) was labeled using RediPrime II random prime labeling system (Amersham Biosciences) in the presence of [α-32P]dCTP and used as the probe. Hybridization was carried out at 42°C for 12 h, and the blots were washed under high stringency followed by autoradiography with an exposure time from 12 to 72 h (23).

**RESULTS**

**Expression of the in Vitro Transcripts of Dicistronic Plasmids in Transfected *Giardia***—Dicistronic plasmids containing the coding regions for *Renilla* and firefly luciferases as the first and second cistrons, respectively, were constructed, and their in vitro transcriptions were expressed in the transfected *Giardia*. Among the constructs presented in Fig. 1, the 5′-end upstream from the coding region of Rluc contained the GLV 5′-UTR and the 264-nt capsid-coding region. The control construct pC631Rluc-Fluc had the two tandem reporters separated by only 10 nts between the stop codon of the first and the start codon of the second, which placed them also in two different reading frames. Only a background Fluc activity (75.5 ± 2.2 RLU/µg of protein) was detected in the lysate of *Giardia* trophozoites transfected with the transcript of this construct, whereas the Rluc activity amounted to 18,487 ± 1210 RLU/µg of protein in the same lysate. A ratio between the two activities (Fluc/Rluc) of 4 ± 0.3 × 10⁻³ is thus taken as the negative control value indicating no internal initiation of translation of the Fluc-coding region in the transcript (Fig. 1). When either the GLV 5′-UTR (pC631Rluc-UTR-Fluc) or the 264-nt capsid-coding region (pC631Rluc-FlucCod-Fluc) was introduced between the two cistrons, lysates from the transcript-transfected cells showed Fluc/Rluc ratios of 0.4 ± 0.1 × 10⁻³ and 1.0 ± 0.2 × 10⁻³, respectively (Fig. 1). Apparently, neither the 5′-UTR nor the 264-nt capsid-coding region alone can fulfill the function of an IRES. When both the 5′-UTR and the coding region were placed between the two open reading frames (pC631Rluc-UTR-Cod-Fluc), however, the Fluc activity in the lysate of transfected cells was increased to 17,489 ± 666 RLU/µg of protein, 240-fold of the background (as compared with pC631Rluc-Fluc negative control), resulting in a Fluc/Rluc ratio of 946 ± 36 × 10⁻³ (Fig. 1). The GLV IRES thus most likely includes the 264-nt coding region and a portion of or the entire 5′-UTR as well. To exclude the possibility that the enhanced translation of the second cistron in pC631Rluc-UTRCod-Fluc transcript could be due to a cleavage of the dicistronic transcript, Northern blot analysis was performed on levels and sizes of the transcripts in transfected cells at the time of luciferase assays (18 h after transfection). There were no shortened transcripts containing the second Fluc cistron detectable even after a prolonged exposure of the Northern blots up to 72 h (Fig. 2, A and B, lanes 2 and 3) of the Northern blots up to 72 h (Fig. 2, A and B, lanes 2 and 3) of the Northern blots up to 72 h (Fig. 2, A and B, lanes 2 and 3).
RNA bands in corresponding lanes used as sampling controls.

Transfected cells.

Similar level of various dicistronic transcripts in the transfectants. The Rluc activity remained relatively constant at 18,487 ± 2,010 RLU/μg of protein (Fig. 2, A and B, lanes 3–5), suggesting a similar stability among them inside the transfected Giardia cells.

Deletion Analysis of the 5′-UTR—With the apparent involvement of 5′-UTR in IRES, we analyzed the secondary structure of 5′-UTR with MFOLD prediction (24). A predicted structure with estimated ΔG of −138.63 kcal/mol contains 8 stem loops designated as stem loops U1 (nts 1–27), U1a (nts 28–65), U2 (nts 99–119), U3 (nts 134–164), U4a (nts 204–219), U4b (nts 221–261), U4c (nts 263–292), and U5 (nts 314–344), respectively (Fig. 3). To provide a preliminary view on whether any of the structural elements is required for the IRES function, we made a series of deletions in the 5′-UTR by the positions of these predicted stem loops in plasmid pC631Rluc-UTRCod-Fluc (see Fig. 1). Thus, pC631Rluc-82-367Cod-Fluc and pC631Rluc-92–367Cod-Fluc both have stem loops U1 and U1a deleted, but the transcripts from these constructs retained the translation efficiency in Giardia (Fig. 4). These two stem loops are thus nonessential for the viral IRES. Plasmid pC631Rluc-102–367Cod-Fluc has the stem loop U2 shortened by 4 bp, and pC631Rluc-114–367Cod-Fluc has the stem loop U2 structure destroyed. However, the transcripts from the two constructs were expressed at the wild-type level in transfected Giardia (Fig. 4), suggesting that stem loop U2 is also not required by the IRES. Nonetheless, upon a further deletion of 12 more nucleotides (pC631Rluc-126–367Cod-Fluc), which retains still U3, U4a, U4b, U4c, and U5 intact, translation of the transcript in Giardia was reduced to 15% of the wild type (Fig. 4). The upstream boundary of the IRES thus lies apparently somewhere between nts 114 and 126 in the 5′-UTR. Without further deletion analysis, these data enabled us to conclude...
that the 5'-UTR portion of the IRES contains a stretch of 253 nucleotides from nt 114 to 367, which, upon inclusion of the downstream 264-nucleotide capsid-coding region of the IRES, constitutes an IRES of 517 nucleotides with the initiation codon located at the center.

To verify that the estimated upper boundary of IRES was indeed correct and that the U3, U4a, U4b, U4c, and U5 stem loop structures are all essential for the IRES function, pC631Rluc-176–367Cod-Fluc, which has U1, U1a, U2, and U3 missing, and pC631Rluc-278–367Cod-Fluc, which has only U5 remaining, were also constructed (see Fig. 3). Their transcripts were translated in *Giardia* to 4 and 0.4% of the wild-type level, respectively (Fig. 4). To further confirm that the U2 stem loop structure is indeed not required by the IRES, a construct of pC631Rluc-U2M-CodFluc was prepared from pC631Rluc-82–367-Cod-Fluc, in which 101GG102 in stem U2 was converted to 101CC102 to destroy the stem structure (see Fig. 3). Translation of its transcript in *Giardia* reached a Fluc/Rluc ratio of 899/1036/1030, which is close to the wild-type ratio of 946/1036/1030 (Fig. 4). There is thus little doubt that the U2 stem loop structure is not required by the IRES.

Northern blotting analysis of the transcripts extracted from transfected cells indicated that there were no significant differences in the levels of transcripts among the control (pC631Rluc-UTRCod-Fluc) and the truncated mutant transcripts (see Supplementary Fig. 1). The absence of any visible shortened transcripts upon prolonged exposure of the blots indicates also that these transcripts were of similar stability and integrity inside the transfected *Giardia*. The decreased expression of luciferase activities with increasing deletions in the 5'-UTR is thus most likely attributed to a reduced translation.

Specific Mutations either in the 5'-UTR or in the Capsid-coding Region That Abolish IRES Activity—To further verify that translation of the second cistron depends on the intercistronic IRES sequence, we introduced to it the previously characterized mutations in the 264-nt capsid-coding region known to disrupt translation initiation (5). In this region of the IRES, we introduced mutations G16C, which destabilizes stem loop I, DB2, which disrupts the base pairing between the DB and the anti-DB sequences, and T160C, which destabilizes stem loop II in the pseudoknot structure (5). In the 5'-UTR of the IRES, we made an St5 mutation that destabilizes stem loop U5 (Fig. 5). In each case, translation of the downstream Fluc was significantly decreased as was observed in the previous study of the monocistronic expression (5, 8). However, the translation efficiency of Rluc (the first cistron) remained unaltered. These results demonstrate that translation of the second cistron in a dicistronic transcript depends solely on the function of intercistronic IRES, whereas that of the first cistron is not affected by the IRES at all. Northern blotting analysis of the transcripts presented in Fig. 5 indicated once again little difference in their levels and integrity and confirmed the immediate consequences from individual mutations on translation initiation (see Supplementary Fig. II).

**IRES Activity Is Independent of Translation of the First Cistron**—To demonstrate that the IRES function of the intercistronic UTRCod sequence is not attributed to or affected by translation initiation from the first cistron, we introduced the same type of mutations to the UTRCod sequence upstream from the first cistron while the intercistronic UTRCod sequence remained unchanged. Results from the transcript-
transfected cells demonstrated the anticipated disruptive effects from these mutations on the expression of the first cistron (\textit{Rluc}) (Fig. 6). But the levels of expression of the second cistron (\textit{Fluc}) remained relatively unchanged in all cases, indicating once again that translation of the second cistron is independent of the first cistron. Again, Northern analysis of the transcripts extracted from the transfected cells was performed with an overexposure as described for Fig. 2 and Supplementary Figs. I and II. The results showed similar levels and no visible sign of breakdown among the transcripts (data not shown).

The Distance between Stem Loop U5 and the Initiation Codon Cannot Be Altered without Inhibiting Translation—Our previous study indicated that the position of the initiation codon with respect to the functionally essential stem loop I in the downstream capsid-coding region could not be changed at all without disrupting translation initiation (8). Disruption of stem loop U5 in the 5’-UTR, located 21 nts upstream of the initiation codon, also drastically reduced translation of the transcript from the monocistronic construct pC631-Fluc.\textsuperscript{5} It is likely that being flanked by two functionally indispensable stem loops, U5 and I, the precise location of the initiation codon is of critical importance to accommodate the recruited ribosome small subunit for efficient translation initiation. To further verify this working hypothesis, site-directed mutageneses were carried out to either decrease or increase the distance between stem loop U5 and the initiation codon in the monocistronic luciferase transcript for potential effects on translation initiation (Fig. 7). Results from the transcript-transfected cells indicated that by decreasing the distance by 1, 2, 3, or 5 nucleotides there was a drastic loss of translation efficiency to 1.9, 3.4, 0.1, and 0.2% of the wild-type level, respectively (Fig. 7). Similarly, an increase of the distance by 3 or 5 nucleotides reduced the translation efficiency to 13 and 0.1% of the wild-type activity (Fig. 7). These results indicate that like the distance between

\textsuperscript{5} S. Garlapati and C. C. Wang, unpublished results.
stem loop I and the initiation codon (8), the distance between the stem loop U5 and the initiation codon is also highly precise and inflexible.

**DISCUSSION**

In this study we expressed the chimeric transcripts instead of the cDNA constructs in *Giardia* in pursuing the structure-function analysis of GLV IRES. It enabled us to look into potential involvement of the 5′-UTR portion in the IRES without infringing on the involvement of 5′-UTR in transcription initiation (25). The results provided a clear demonstration that a downstream portion of the 5′-UTR from nt 114 to 367 plus the downstream 264-nt capsid-coding region constitute the viral IRES comprising a full sequence of 517 nts. This combination of a portion of upstream 5′-UTR and a long stretch of downstream coding region in forming an IRES has not been, to our knowledge, reported previously among other viral mRNAs. The expression of dicistronic transcripts in *Giardia* in our study helped also verifying the true identity of the IRES, which is capable of initiating translation while located at an internal position in the mRNA. Its function is unaffected by the translation or the lack of translation of the upstream cistron. It is a *bona fide*, albeit unique, IRES.

The precise upstream boundary of the IRES can be only identified within a region between nts 114 and 126 in the 5′-UTR at the present time. The sequence of this region 114CUUCUAGAUUCGC126 was not predicted to be involved in any secondary structure by MFOLD (Fig. 3), and stem U3 is still 6 nucleotides downstream from it. Apparently, the sequence between nts 114 and 126 plays an important role in the function of IRES in initiating translation in *Giardia*.

The actual presence of all the MFOLD-predicted secondary structures in 5′-UTR (Fig. 3) has been verified by chemical modification followed by primer extension in our recent studies.6 We then conducted specific site-directed mutagenesis to disrupt these individual secondary structures and restore them in changed sequences. Our data suggest that stem loops U3, U4, U4c, and U5 (but not U1, U1a, U2, or U4a) are all essential for the IRES function but that the space they occupy and their sequences are not.7

An extension of ~253 nts upstream and 264 nts downstream from the initiation codon to constitute the IRES suggests that the secondary structure of the IRES flanking the initiation codon at the center may constitute a suitable physical arrangement for efficient ribosome recruiting and translation initiation. Results from our previous investigation (8) and the current study, both showing an inflexibility of the location of the initiation codon between stem loops U5 and I, lend support to this elucidation. These observations suggest that there is little ribosome scanning prior to AUG recognition and that the structures surrounding the initiation codon, stem loops U5 and I, may play a major role in precisely positioning the ribosome.

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onto the AUG codon. The spacing (including the AUG triplet itself) between the two stem loop structures is 31 nts, which could provide a suitable structural environment for accommodating a ribosome small subunit to the initiator AUG (Fig. 8).

An experiment on *Giardia* small ribosome subunit protection of this specific RNA region will be performed to verify the point in the future.

Direct recruitment of ribosome by IRES to the initiation codon has been also reported in hepatitis C virus (HCV) (17) and cricket paralysis virus (26) despite the fact that these IRESs are confined exclusively within the 5'-UTR. Pseudoknot structures present in the IRESs of HCV (27) and cricket paralysis virus (28) were found to play essential functions for precise positioning of ribosome onto the initiation codon. In HCV IRES, domain IIIb interacts with the initiation factor eIF3 (29, 30) and plays a role in translation initiation after the 48 S complex formation (17) suggesting that the overall structure of the IRES plays an active role in internal initiation in this virus (31). The overall complex secondary structures identified in the GLV IRES (Fig. 8) play also an essential function in translation initiation (Fig. 3) (5, 8). The essential complex structural elements in the downstream region, including stem loop I, DB, stem loops II, III, and IVA, and a pseudoknot, have not yet been observed among other viruses. These elements could be involved in the downstream events after a successful binding of the ribosome to the initiation codon followed by formation of the 80 S initiation complex (32). Movement of the ribosome complex would require melting stem loop I with help from initiation factors with RNA helicase activity.8 Once the translating ribosome complex moves past the sequence involving stem loop I, the latter needs to refold into its original structure for another round of translation initiation. One could speculate that the DB sequence and the pseudoknot structure would pause the translating ribosome so as to allow sufficient time for stem loop I to refold into its original shape. Alternatively, the pseudoknot could retard the downstream movement of ribo-

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Some for a sound and error-proof recognition of the initiation codon (33).

Among examples of the IRES in other viruses, hepatitis C virus requires, however, a 12–30 nt viral polyprotein-coding sequence for efficient IRES-mediated translation initiation (15, 34). Deletion of the adenosine-rich domain near the 5′-end of CAT reporter sequence or insertion of a stable hairpin structure between HCV IRES and CAT sequences substantially reduced IRES-mediated translation. Mutational analysis of the inserted protein-coding sequences demonstrated no requirement for either a specific nucleotide- or amino acid-coding sequence to restore an efficient IRES-mediated translation (35). Similar observations were also made in classical swine fever virus, wherein the inclusion of 17 or more codons of the virus requires, however, a 12–14 nt viral polyprotein-coding sequence downstream from the 5′-UTR exhibited a full IRES activity. With only 12 codons, however, the activity was ~66% of the maximum in vitro (36). In contrast, efficient IRES activity was reported with constructs containing only 3–8 nts of HCV-coding sequences placed between the IRES and a downstream sequence encoding a reporter protein (14, 37, 38).

The role of these coding sequences in translation initiation remains, however, unclear. By placing a hairpin structure (ΔG = −18 kcal/mol) in the HCV IRES downstream of the AUG codon, an inhibitory effect on translation initiation was observed (35), whereas a stable stem loop I (ΔG = −12 kcal/mol) in the capsid-coding region of the GLV IRES was found to be essential for translation initiation (5). These apparent contradictions remain unexplained.

Ironically, the presence of IRES within the coding region of eukaryotic cellular mRNA has been recently demonstrated. Two PITSLRE protein kinase isoforms, P110PITSLRE and p58PITSLRE, are translated from a single transcript. The second protein is resulted from an internal initiation of translation that is enhanced upon down-regulation of eIF4E in the G2/M phase of the cell cycle (39). In a recent publication by Komar et al. (40), the mRNA encoding Ure2p in Saccharomyces cerevisiae was found to possess an IRES that synthesizes an N-terminally truncated active form of the protein that is independent of eIF4E. The URE2 IRES, tentatively localized between nts 203 and 368, has the initiation codon contained at nts 280–282 and thus provides an example of IRES in a cellular mRNA that extends to both sides of the initiation codon very much like GLV IRES.

Our identification of a novel IRES element in GLV may yet carry an additional biological significance. The dependence of GLV on a downstream sequence for translation could reflect one of the uniquely primitive features of the translation machinery operating in its host, Giardia. The cellular mRNAs in this primitive protozoan have very short 5′-UTR in the range of 0–14 nts (41). In the absence of a substantial 5′-UTR, how mRNA molecules can recruit the 40 S ribosome subunit to the precise decoding region to initiate translation has not been clearly understood. The GLV transcript is translated by the same machinery as the cellular mRNA of Giardia, and translation of the viral mRNA is known to exert no inhibitory effect on translation of cellular mRNA (1). Insights obtained from the mechanism of translation initiation on GLV IRES may shed light on whether the cellular mRNA in Giardia could also depend on similar elements present downstream of the initiation codon for translation initiation.

Translation of GLV transcript may serve as a model system for understanding the translation initiation mechanisms in this most primitive and one of the earliest diverging and living eukaryotes known to man.

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