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An Independently Folding RNA G-Quadruplex Domain Directly Recruits the 40S Ribosomal Subunit

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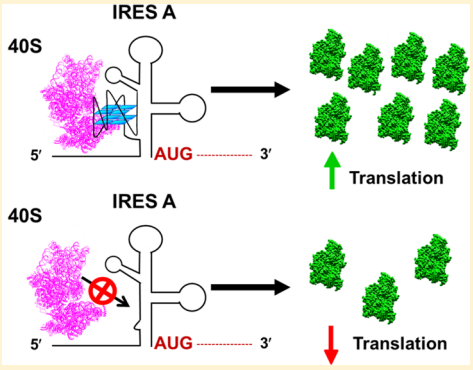
Supporting Information

ABSTRACT: In this study, we report that a 17-nucleotide independently folding RNA G-quadruplex (GQ) domain within the 294-nucleotide human VEGF IRES A interacts with the 40S ribosomal subunit. Footprinting and structure mapping analyses indicate that the RNA GQ forms independently and interacts directly with the 40S ribosomal subunit in the absence of other protein factors. Moreover, a filter binding assay in conjunction with enzymatic footprinting clearly established that the GQ-forming domain singularly dictates the binding affinity and also the function of internal ribosomal entry site (IRES) A. The deletion of the GQ domain abrogates the binding of the 40S ribosomal subunit to the IRES, which impairs cap-independent translation initiation. The findings provide a unique and defined role for a noncanonical RNA structure in cap-independent translation initiation by cellular IRESs. The GQ structure when present in an IRES acts as an essential element in contrast to their generally accepted inhibitory role in translation. The results of this study explain the hitherto unknown mechanistic necessity of the GQ structure in IRES function.



RNA G-quadruplexes (GQs) are secondary structures that when present in the 5′-untranslated (UTR) region of an mRNA can act as both necessary elements and repressors of translation.[1−5] The role of GQ structures in translational modulation depends on the context in which the GQ structure is present.[6] However, it is well-established that in a majority of the cases GQ structures inhibit translation as was observed in several clinically significant mRNAs such as *NRAS*, *ZIC1*, *BCL-2*, *TRf2*, and *MT3-MMP*.[2,3,7−9] A comprehensive analyses of six different GQ-forming sequences in the 5′-UTR region in mRNA, among many potential GQ-forming sequences in the transcriptome, suggested that in general the GQ structures act as translation repressors.[10] In fact, rational introduction of GQ structures specifically downregulates the expression of targeted genes.[11,12] Alternatively, when present in the context of an IRES, for example, in the cases of *FGF* and *VEGF*, the GQ structures act as essential elements for translation initiation.[1,5] Nevertheless, the mechanism by which the GQ structures play a context-dependent regulatory role is unknown.

The IRES-mediated translation initiation, although initially observed in viral mRNAs, has also been identified in many cellular mRNAs.[13−15] For example, the 5′-UTR of human vascular endothelial growth factor (*hVEGF*) encompasses two IRES elements.[16] The human VEGF is a key physiological and pathological angiogenic growth factor. An increase in the VEGF level is linked to not only normal physiological conditions, such as embryonic development, wound repair, and adaptation to hypoxia, but also pathological conditions, such as proliferative retinopathies, arthritis, psoriasis, and tumor angiogenesis.[17−19] The 5′-UTR of *hVEGF* contains two independently functioning IRESs (A and B) of which IRES A is a 294-nucleotide fragment (745−1038 from the 5′-end of the mRNA) immediately upstream of the canonical AUG translation start site.[16,20,21] The VEGF IRESs have been shown to play a key role in the upregulation of VEGF under hypoxic stress.[21,22] The presence of a tunable GQ structure in IRES A was determined to be necessary for optimal cap-independent translation initiation.[5] Translation initiation by IRESs involves a cap-independent mechanism wherein the 40S subunit and other IRES trans-acting factors (ITAFs) are recruited directly onto the mRNA thereby rendering the 5′-cap and some of the initiation factors nonessential.

Our investigations of structure mapping of *hVEGF* IRES A revealed that it forms a very stable and well-defined secondary structure at physiologically relevant salt concentrations. Considering the essential role of the GQ domain in IRES A function, we hypothesize that it plays a key role in IRES A-mediated translation initiation by interacting with the 40S subunit. Here we report that the direct interaction of an independently folding GQ domain in cellular *hVEGF* IRES A with the 40S ribosomal subunit (40S subunit) is critical for its cap-independent translation initiation.

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MATERIALS AND METHODS

Plasmid Construction. The transcribeable plasmid pVFIRESA and bicistronic plasmids hVEGFbicis containing the wild-type IRES A sequence were constructed as previously described.[5] The primers for deletion mutation were purchased from Integrated DNA Technologies, Inc. The deletion of the specific domains was performed with a QuikChange Lightning Site-Directed Mutagenesis Kit (Agilent Tech) following the manufacturer's protocol. Plasmids were purified by the Pure Yield Plasmid Miniprep System (Promega), and successful deletion was confirmed by sequencing at The Plant Microbe Genomic Facility (OSU).

***In Vitro* Transcription and Radiolabeling of IRES A and Mutant RNAs.** The 294-nucleotide IRES A and the mutants of the *hVEGF* mRNA were transcribed using plasmid pVFIRESA and its mutant variants.[5] The transcribed RNA was purified by 6% denaturing polyacrylamide gel electrophoresis (PAGE). The RNA bands were harvested by tumbling the gel slices at 4 °C in elution buffer [300 mM NaCl, 10 mM Tris-HCl (pH 7.4), and 0.1 mM EDTA]. The eluent was phase concentrated using 2-butanol and subsequently precipitated from the aqueous phase by ethanol.

The 5′-phosphates of the *in vitro*-transcribed RNA were enzymatically removed by calf intestinal alkaline phosphatase (CIP, NEB). The RNA was extracted with phenol and chloroform and precipitated with 70% ethanol. The CIP-treated RNA was 5′-end radiolabeled by T4 polynucleotide kinase (PNK, NEB) and [γ-^{32}P]ATP (PerkinElmer) and incubated for 1 h at 37 °C. The commercially obtained DNA sequences were directly radiolabeled with T4 PNK as the 5′-phosphate is absent. The reaction was stopped by the addition of an equal volume of stop buffer [7 M urea, 10 mM Tris-HCl (pH 7.5), and 0.1 mM EDTA]. The radiolabeled full length RNA was isolated by 6% denaturing PAGE. The radiolabeled RNA was then extracted from the gel via the crush and soak method as described previously.

Structure Mapping Analyses. The 5′-end radiolabeled RNA (60000 cpm) was dissolved in 10 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, and 2.5 mM $MgCl_2$ in the presence of 100 mM KCl or 100 mM LiCl, heated to 70 °C for 10 min, and slowly cooled to room temperature. Once reaction mixtures had reached the appropriate temperature, the RNA was digested with 0.1 unit of Ambion RNase T1 (Life Technologies), 0.005 μg of Ambion RNase A for 5 min at 37 °C, and 1.4×10^{-4} unit of RNase One (Promega) for 5 min at 37 °C. The reactions were terminated by using an equal volume of stop buffer [7 M urea, 10 mM Tris-HCl (pH 7.5), and 0.1 mM EDTA]. Treated RNA was electrophoresed on a 6% denaturing PAGE, dried on Whatman paper, and exposed to a phosphorimager screen. The gel images were then visualized by scanning the screen on a Typhoon Phosphorimager FLA 9500 (GE Healthcare, Life Sciences).

Filter Binding Assay. The filter binding assay for assessing the apparent binding constants of the interaction of the 40S ribosomal subunit was performed using a previously published protocol.[27] The salt-washed 40S ribosomal subunits isolated from rabbit reticulocyte lysate (RRL) were a kind gift from W. C. Merrick (Case Western Reserve University, Cleveland, OH), and 30S ribosomal subunits (bacterial) were kindly provided by S. A. Strobel (Yale University, New Haven, CT). The radiolabeled RNAs were folded in the presence of binding buffer (20 mM Tris-HCl, 100 mM CH_3COOK, 200 mM KCl, 2.5 mM $MgCl_2$, and 1 mM DTT). The RNA was then incubated with increasing concentrations of the 40S subunit at 37 °C for 20 min. The filter binding assay was performed using a Supor Membrane (0.45 μm) on the top of a nitrocellulose filter that in turn was placed on top of a *Hybond-N+* filter supported by filter paper. The Supor membrane was added to remove any aggregate; however, none was observed. The filters were presoaked in binding buffer assembled in a Minifold-I Dot-Blot System. The reaction mixtures were added to the wells of the Dot Blot apparatus under vacuum. The membranes were air-dried and exposed to a phosphorimager screen. The blot images were then visualized by scanning the screen on a Typhoon Phosphorimager FLA 9500 (GE Healthcare, Life Sciences). The spots were quantified using Quantity One 1-D Analysis Software. To determine the apparent K_d, the data were fit to a Langmuir isotherm described by the equation $\theta = [X]/([X] + K_d)$, where θ is the fraction of RNA bound to 40S as measured from the fraction retained on the nitrocellulose membrane and [X] is the 40S subunit concentration.

Footprinting of the Interaction of 40S Ribosomal Subunit with IRES A. The 5′-end radiolabeled RNA (150000 cpm) was dissolved in 30 mM HEPES (pH 7.5), 2.5 mM $MgCl_2$, and 100 mM KCl, heated to 70 °C for 10 min, and slowly cooled to room temperature. The folded RNA was then incubated with 100 nM 40S ribosomal subunit at 37 °C for 20 min. The RNA was then treated with 0.1 unit of RNase T1 for 3 min at 37 °C, and the reaction was stopped by adding 1% SDS and then the mixture extracted with phenol and chloroform followed by ethanol precipitation. The precipitated RNA was then dissolved in formaldehyde loading buffer, counted by a scintillation counter and normalized for uniform loading in each well, electrophoresed on a 6% denaturing PAGE, dried on Whatman paper, and exposed to a phosphorimager screen. The gel images were then visualized by scanning the screen on a Typhoon Phosphorimager FLA 9500 (GE Healthcare, Life Sciences).

Cell Culture and Transfection of the Reporter Plasmid. HeLa cells were grown in 96-well plates in Dulbecco's modified Eagle's medium (DMEM) with low glucose supplemented with 10% fetal bovine serum and 1% antibiotics at 37 °C in 5% CO_2 in a humidified incubator. The plasmids were transfected with Lipofectamine-2000 for 6 h. After 24 h, a dual luciferase assay was performed with a Dual-Glo Luciferase Assay System as per the manufacturer's protocol, and the luminescence signals were read by the SpectraMax M4 plate reader (Molecular Devices, LLC). The ratios of the Firefly and Renilla luciferase luminescence were calculated and normalized to the ratio of the wild-type plasmid.

RESULTS

The 40S Ribosomal Subunit Interacts with the 5′-Proximal End of IRES A. IRES A forms a stable secondary structure because of its highly GC rich (~77%) sequence. The predominance of guanine (G) residues in the 294-nucleotide IRES A constituting ~45% of the total number of residues rendered RNase T1 as the enzyme of choice for structure mapping and footprinting analyses. RNase T1 cleaves single-stranded G residues in an RNA; however, the G residues involved in base pairing to form secondary structures, GQ structures, and tertiary interactions are protected from undergoing cleavage.[28] Mapping of IRES A–40S ribosomal subunit interaction by enzymatic footprinting clearly indicates a region of ~60 nucleotides protected from RNase T1 cleavage in the 5′-proximal end of 294-nucleotide IRES A (Figure 1a). Interestingly, this region harbors the 17-nucleotide GQ-forming region consisting of five stretches

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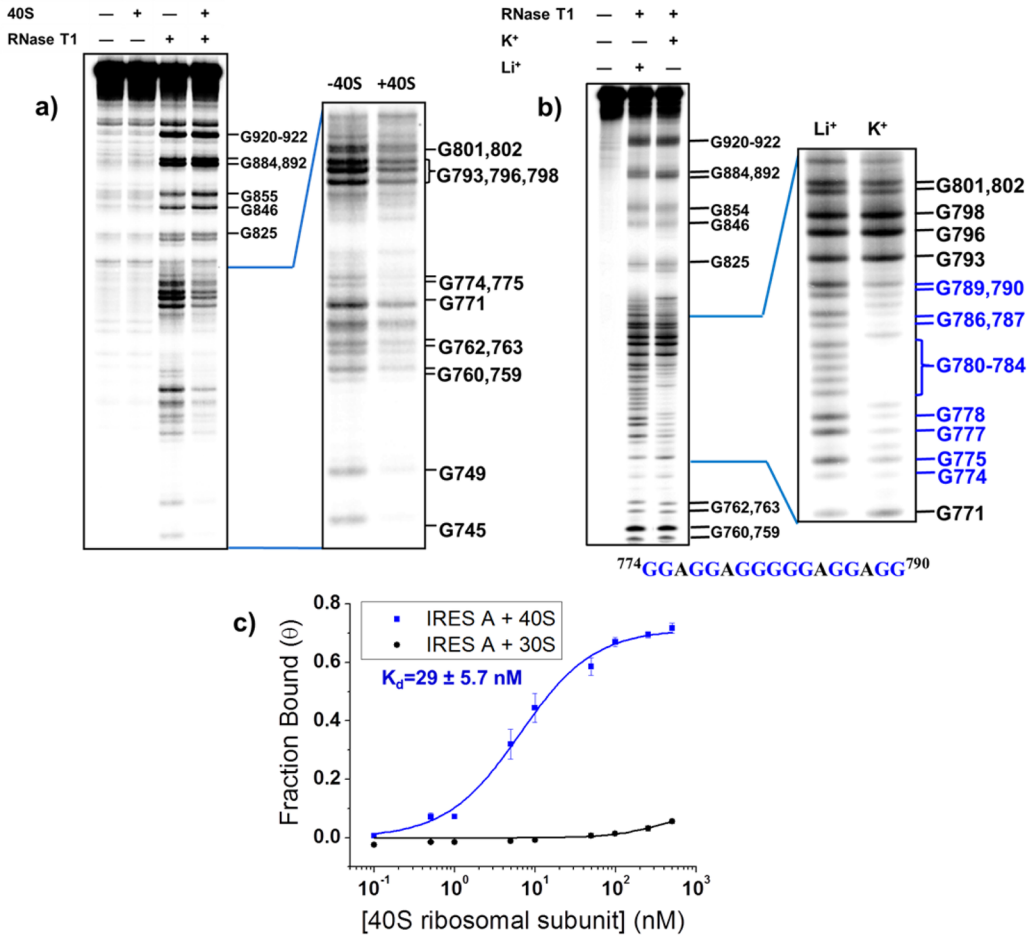

Figure 1. Binding of IRES A to the 40S ribosomal subunit. (a) RNase T1 footprinting of IRES A in the presence of the 40S subunit protects a segment of IRES A from cleavage (nucleotides 745−805). The protected region is enlarged on the sides. (b) The 40S interacting region in IRES A comprises a 17-nucleotide sequence that adopts GQ structure in the presence of physiologically relevant K^+ concentrations. (c) The 40S ribosomal subunit binds to IRES A with a strong affinity (K_d = 29 ± 5.7 nM) as observed by the filter binding assay, whereas there was no binding observed with the 30S ribosomal subunit.

of G residues, which are resistant to RNase T1 cleavage in the presence of K^+ (Figure 1b).

To investigate the binding affinity of IRES A for the 40S ribosomal subunit, we used a filter binding assay to determine the apparent equilibrium dissociation constant (K_d) of the 40S subunit−IRES A complex and used the bacterial 30S ribosomal subunit as a control. IRES A was incubated with increasing concentrations (0.1−500 nM) of either 40S or 30S salt-washed ribosomal subunits. The RNA bound to the 40S or 30S ribosomal subunit stays with the nitrocellulose membrane, while the unbound RNA binds to the positively charged membrane underneath. The equilibrium dissociation constant (K_d) was calculated by plotting the fraction of bound RNA (θ) to the 40S versus the 40S concentration, and data were fit to the equation $\theta = [X]/([X] + K_d)$. The K_d of the IRES A−40S complex was determined to be ∼29 nM (Figure 1c) and is comparable to previously reported values for the EMCV and HIV-1 *gag* viral IRESs.[29] Our results precisely trace the region of interaction of a cellular IRES and 40S subunit and their apparent binding affinity in the absence of other ITAFs. The direct association of 40S with an IRES has been observed only in some types of viral IRESs, and our finding suggests that IRES A-mediated translation initiation is similar to that mechanism of initiation.[27,30] Prior to this report, potential interaction of the 40S subunit with a cellular IRES was observed only in the case of *c-Src* kinase, although its binding affinity and the precise region important for the interaction are not known.[31] Overall, cellular VEGF IRES A has the distinct ability to directly recruit the 40S ribosomal subunit with a low nanomolar binding constant.

The Independently Folding GQ Domain in hVEGF IRES A Determines 40S Ribosomal Subunit Recruitment. We determined that the GQ is a stable structure formed within the 40S subunit binding region given the strong protection detected in enzymatic structural mapping.[5] A more detailed analysis of the secondary structure of the IRES A was conducted via a series of enzymatic structure mapping (Figure S1 of the Supporting Information). The updated secondary structure of the IRES A that incorporated the constraints derived from structure mapping data was created with the help of Mfold[32] with the 40S subunit interacting region traced on the folded RNA structure (Figure S2 of the Supporting Information). Next we sought to precisely identify the RNA segment that determines the IRES A−40S subunit interaction. To accomplish that, the putative 40S interacting region in IRES A was divided into three different segments with the central one being the GQ-forming region (D2) and its 5′ flanking region (D1) and the 3′ flanking segment (D3). Deletion mutants were prepared by site-directed mutagenesis of a transcribeable plasmid pVFIRESA and used

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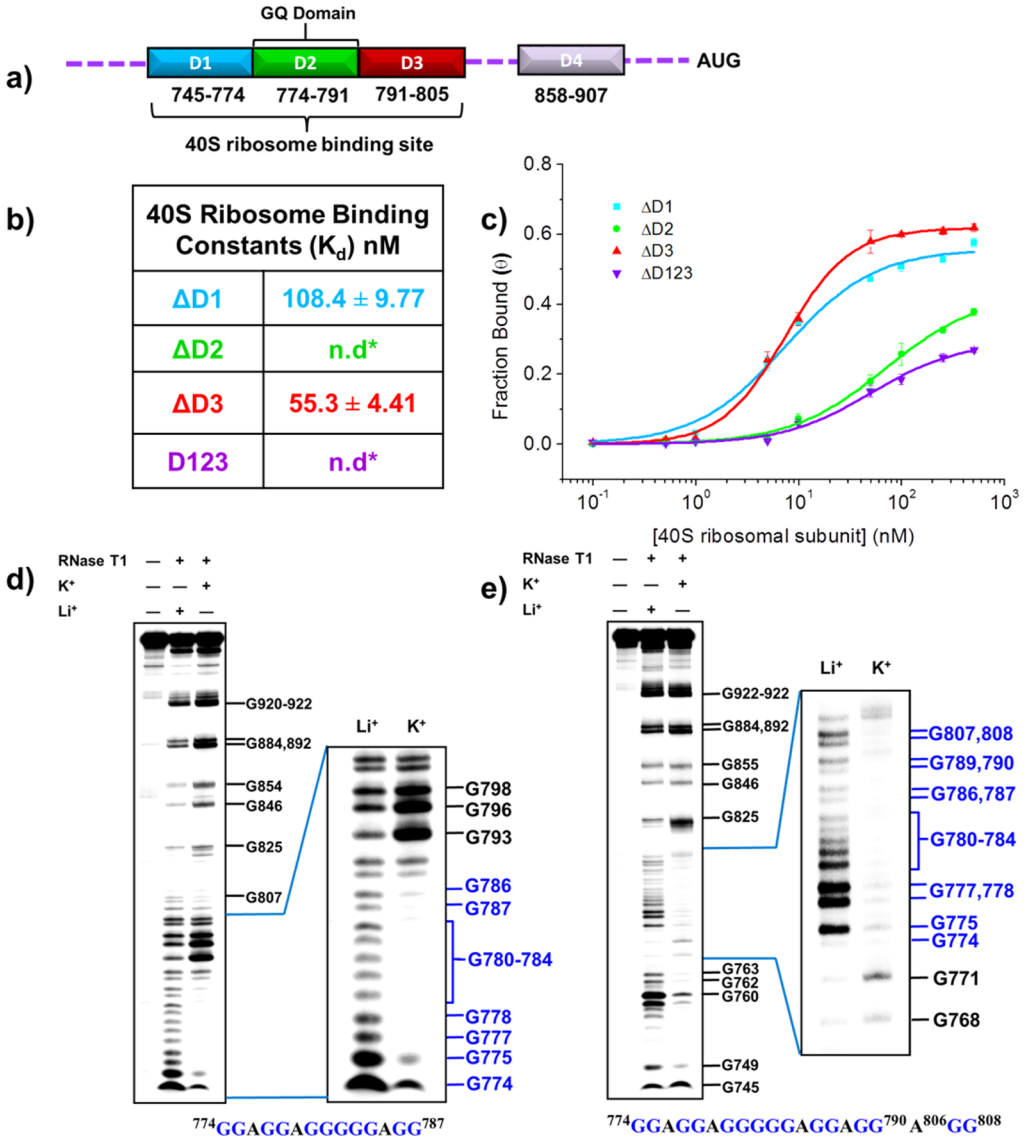

Figure 2. Independently folding D2 domain determines 40S subunit binding. (a) Schematic of IRES A indicating different sections D1−D4. (b) 40S ribosomal subunit dissociation constants (K_d) calculated from the isotherms (c) of the mutants indicate substantial loss of binding affinity in the absence of GQ, i.e., D2 domain. The dissociation constants for ΔD2 and ΔD123 could not be determined (n.d) at the highest 40S concentrations used for the assay. (d and e) The D2 domain forms a GQ structure despite the deletion of D1 and D3 domains in ΔD1 and ΔD3 mutants. *n.d indicates K_d could not be determined at a 40S ribosomal subunit concentration of 500 nM.

for *in vitro* transcription of wild-type IRES A and mutants ΔD1, ΔD2, ΔD3, and ΔD123 (Figure 2a and Figure S3 of the Supporting Information).

The apparent equilibrium dissociation constant of the 40S ribosomal subunits to the mutants was observed to be 108.4 ± 9.77 nM for ΔD1 and 55.3 ± 4.41 nM for the ΔD3 mutant. The GQ-forming region was determined to be the key determinant in the binding of the 40S subunit because the deletion of that domain (D2) resulted in extensive weakening of the binding that precluded us from measuring the apparent K_d for mutants ΔD2 and ΔD123, indicating D2's indispensability in 40S subunit recruitment (Figure 2b,c). The filter binding results along with the footprinting confirm the interaction of the 40S ribosomal subunit specifically to a particular region in IRES A and annul the possibility of nonspecific interaction. The evidence suggests an unprecedented role of a GQ structure. Interestingly, a previously identified 50-nucleotide region (858−907) termed D4, which was reported to be important for optimal IRES activity, was ascertained to be not involved in the 40S recruitment (Figure S4 of the Supporting Information) as the mutant ΔD4 interacts with the 40S subunit with an affinity (K_d = 33.6 ± 4.27 nM) that is similar to the K_d value of wild-type IRES A.[16] However, deletion of the D2 domain in the ΔD4 mutant to form ΔD24 practically eliminated its ability to interact with the 40S subunit. Notably, the deletion of the 50-nucleotide D4 domain did not affect the formation of the GQ structure (Figure S5 of the Supporting Information). We also verified the 40S ribosome binding affinity of a previously described mutant in which four G residues in the D2 domain were mutated to uridines to disrupt the G-stretches required for adoption of a GQ structure. The mutant that was previously established not to adopt GQ structure was unable to bind to the 40S ribosomal subunits, further confirming the requirement of the GQ structure for interaction with the 40S (Figure S4 of the Supporting Information).[5]

The formation of the GQ structure was confirmed in the mutants ΔD1 and ΔD3 by RNase T1 structure mapping in the

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presence of K^+ and Li^+. The formation of the GQ structure was observed by a protection from the RNase T1 cleavage in the presence of K^+. The D2 domain's ability to form the GQ structure in spite of the deletion of one or both of the flanking regions (Figure 2d,e) established its true independence in terms of adopting the GQ structure. The region of interaction with 40S subunit with mutants ΔD1 and ΔD3 was further confirmed by footprinting (Figures S6 and S7 of the Supporting Information). The region of protection indicated the area of interaction of the mutant IRESs with the 40S subunit and was consistent with the pattern observed for wild-type IRES A except for the deleted segments. Additionally, mutants ΔD2 and ΔD123, which lacked the D2 domain, showed no significant structural differences between the Li^+- and K^+-treated samples (Figure 3) and also

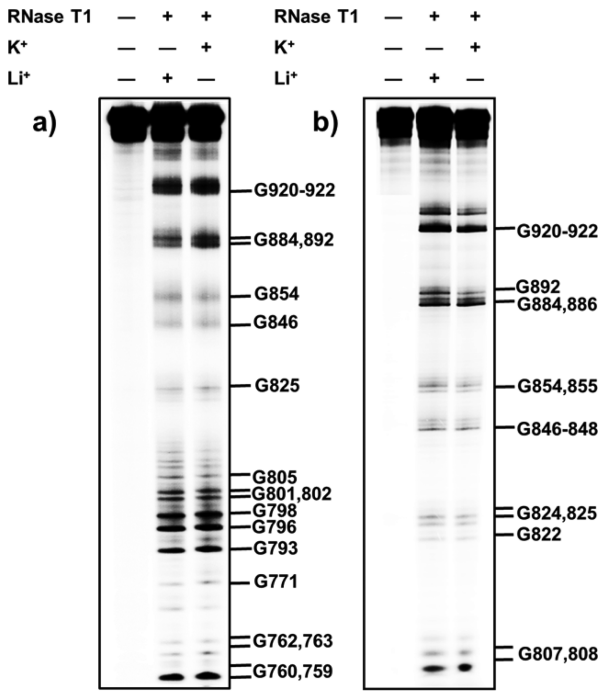

Figure 3. RNase T1 structure mapping of mutants ΔD2 and ΔD123 showed no significant difference in their overall secondary structure between the lanes containing Li^+ and K^+. The D2 domain was absent in both the mutants, which eliminated the possibility of GQ formation.

showed no interaction with the 40S subunit (Figure S8 of the Supporting Information) due to the absence of the key GQ domain. The structure mapping of the D2 domain also suggests that there was no significant change in the overall secondary or tertiary structure of IRES A based upon the relatively unchanged nature in the band pattern. Importantly, we established that the D2 segment (i) folds independently to form the GQ structure despite the deleted flanking regions, (ii) folds in a manner that is independent of the overall IRES A secondary structure, and (iii) is the key determinant of the 40S subunit interaction.

The D2 Domain Is Essential for Optimal IRES A-Mediated Translation Initiation. The next key question that needed to be addressed was the functional relevance of the GQ–40S subunit interaction and the role of different regions of IRES A in the regulation of cap-independent translation. To test that, we constructed IRES A mutants similar to the ones described above, but in the context of the dual luciferase bicistronic plasmid hVEGFbicis.[5] The plasmid was designed in a way such that one of the reporter genes (*Firefly luciferase*) was translated in a cap-independent manner by IRES A whereas the other reporter gene (*Renilla luciferase*) initiates translation in the canonical pathway serving as the control (Figure S9 of the Supporting Information).[5,12] Previously, we knocked down eIF-4E, the cap-binding protein essential for canonical translation initiation, and when bicistronic plasmid hVEGFbicis activity was measured, a sharp decrease in the *Renilla*/*Firefly* luciferase luminescence was observed. This indicates that the cap-dependent translation initiation of the *Renilla* luciferase was inhibited specifically on knocking down eIF-4E, which in turn suggests the bicistronic nature of the construct.[12]

It turned out that the lack of the GQ domain (mutants ΔD2 and ΔD123) significantly decreased IRES A activity (Figure 4;

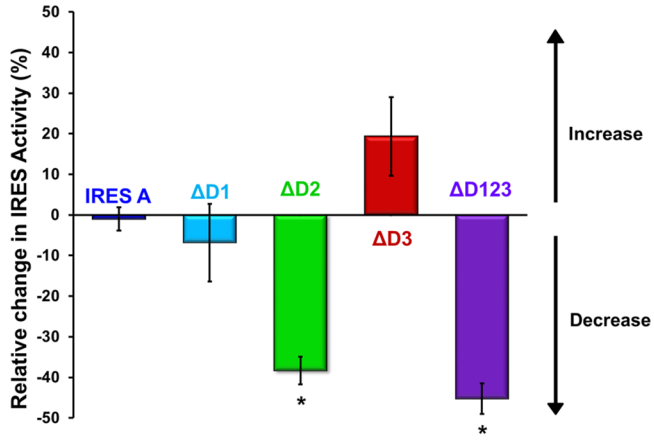

Figure 4. Presence of the D2 domain is essential for optimal IRES-mediated translation initiation in a bicistronic plasmid. HeLa cells were transfected with the bicistronic plasmid, and the reporter gene activity was measured 24 h post-transfection. IRES A activity was significantly ($p < 0.001$) decreased because of the deletion of the GQ-forming D2 domain from the sequence.

$p < 0.001$). The slight decrease in the activity of the ΔD1 mutant could be explained by its observed peripheral involvement in 40S binding. We also observed an increase in the level of cap-independent translation in the case of the ΔD3 mutant despite the deletion of the entire region responsible for 40S binding. In previous reports, deletion of functional elements has been observed to only partially disrupt the activity and in some cellular IRESs was found to upregulate the activity.[33] Our structure mapping analyses suggest that variable numbers of G-tracts are utilized to form GQ in ΔD1, WT, and ΔD3 (Figures 1b and 2d,e). These observations indicate the tunability of the GQ might also be important along with the functional elasticity of the IRES and perhaps the role played by yet to be identified regulatory elements within the hVEGF IRES. Overall, our data indicate that the independently folding GQ domain is essential for direct interaction of IRES A with the 40S subunit and for optimal IRES function.

DISCUSSION

It has been suggested that stable GQ structures in the 5′-UTR interfere with the formation of the 43S preinitiation complex or interrupt the scanning mechanism,[6] and in the coding region, GQ was shown to stall the ribosome movement along the mRNA leading to premature termination of translation and consequent inhibition of protein synthesis.[34] Thus, the GQs in mRNAs are almost exclusively translation inhibitors except when present in the context of a cellular IRES in 5-UTR, where it plays an

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essential role. However, the underlying mechanism of how the GQ structures play an essential role in the context of the cellular IRES was not understood. Our findings explain the hitherto undefined mechanistic role of a GQ structure present within a cellular IRES in cap-independent translation initiation. The presence of ∼3000 putative GQ-forming sequences in the 5′-UTR poses the question of whether this interaction is generic for all GQ structures.[2,35] Our findings when taken in conjunction with the current knowledge of cap-independent translation initiation in cellular IRESs indicate that the interactions of the 40S subunit with the GQ structure appear to be directly functionally relevant, only in the context of an IRES function. The association of 40S to GQ structures alone may not warrant canonical translation initiation because of the requirement of other factors for such a process. The discovery of GQ structures in 5′-UTR, which augment the translation of TGFβ2 that is not known to harbor any IRES, further complicates the mechanistic paradigm of the GQ structure.[4]

There has yet to be any identification of a common sequence or structural motif that can predict the presence of cellular IRESs. In several instances, the canonical stem−loop secondary structures in viral and cellular IRESs have been directly implicated for their role in 40S ribosomal subunit binding; however, a noncanonical structural domain directly playing that role has not been reported previously to the best of our knowledge. The GQ domain in hVEGF IRES A is probably the singular domain identified in any IRES that not only folds into the GQ structure in a manner independent of the surrounding native sequences and structures but also appears to "single-handedly" determine the interaction with the 40S ribosomal subunit. There are only two types of viral IRESs, type 3 (HCV-like) and type 4 (Dicistroviridae), that can directly interact with the 40S subunit.[36] The structural details of those IRESs indicate that type 4 have independently folding regions consisting of stem−loop structures and pseudoknots that determine interaction with the 40S ribosomal subunit.[14,30] Thus, hVEGF IRES A most likely belongs to either type 3 or type 4. It should be noted that although, functionally important, the pseudoknot domain alone is insufficient for recruiting the 40S subunit, unlike the GQ domain in IRES A. However, it is interesting that the G-quadruplex and pseudoknot, two unrelated noncanonical RNA structures, are crucial for cellular and viral IRES functions, respectively. Most of the viral IRESs except for type 4 IRESs involve other canonical translation initiation factors for cap-independent translation.[14] Similarly, in cellular IRESs of *c-myc* and *N-myc*, canonical translation initiation factors such as eIF-4A and eIF3 were observed to be essential for IRES-mediated translation initiation.[26] Furthermore, several ITAFs that are observed to be heterogeneous ribonucleoproteins are known to facilitate cellular IRES-mediated translation.[24] The requirement of the canonical translation initiation factors along with other ITAFs and their interaction with the GQ or other domains in IRES A requires further investigation to elucidate the mechanism of IRES A-mediated translation initiation completely.

The post-transcriptional regulation of VEGF is highly complex and consists of almost all known regulatory processes, some of which are involved in cross-talking.[19] The VEGF mRNA harbors two IRESs that are capable of initiating translation independent of each other.[16] The cellular IRESs are known to be active under physiological conditions when the canonical translation initiation is impaired such as mitosis, hypoxia, nutritional stress, and cell differentiation.[13,24] The context of the IRES-mediated translation initiation in VEGF is of paramount importance with its established role in wound healing, angiogenesis, and cancer.[17,37,38] More importantly, most of the aforementioned conditions that induce IRES-mediated translations are highly relevant to angiogenesis, which results in new blood vessels under hypoxic conditions and tumorigenesis. The identification of a GQ structure that plays a central role in the translation regulation of VEGF expression opens up the possibility of modulation of GQ structures by small molecules that can be an effective strategy for regulating VEGF expression for anticancer therapeutics.

CONCLUSION

This work reveals a novel mechanism of cap-independent translation initiation in a cellular IRES that is controlled by a GQ, a noncanonical RNA secondary structure. RNA GQ structures are known to play diverse roles in several biochemical processes such as pre-mRNA polyadenylation, splicing, mRNA targeting, and transcription termination, among several others,[39] and a GQ's direct role in interacting with the 40S subunit adds to that list of functions. Given hVEGF's role in metastasis and tumor angiogenesis, the GQ located within its 5′-UTR can potentially be a new antitumor target. Because of hVEGF's normal physiological role, potentially it can also be a pro-angiogenic target if a ligand can stabilize the GQ domain. Finally, this illustration of the recruitment of the 40S ribosomal subunit by an independently folding GQ domain in cap-independent translation initiation contributes to our nascent understanding of the structural basis of a key aspect of cellular IRES function.

ASSOCIATED CONTENT

Supporting Information

Structure mapping of IRES A; design of the mutants; filter binding and structure mapping of ΔD4, ΔD24, and 4 mut; footprinting of ΔD1 and ΔD3 in the presence of the 40S ribosomal subunit; footprinting of ΔD2 and ΔD123; and design of the hVEGFbicis plasmid. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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