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The impact of RNA structure on picornavirus IRES activity

Encarnación Martínez-Salas

Centro de Biología Molecular Severo Ochoa, Consejo Superior de Investigaciones Científicas, Universidad Autónoma de Madrid, Cantoblanco 28049 Madrid, Spain

Internal ribosome entry site (IRES) elements consist of *cis*-acting regions that recruit the translation machinery to an internal position in the mRNA. The biological relevance of RNA structure-mediated mechanisms involved in internal ribosome recruitment is now emerging from the structural and functional analysis of viral IRES elements. However, because IRES elements found in genetically distant mRNAs seem to be organized in different RNA structures, the definition of the structural requirements for IRES activity is challenging and demands multidisciplinary approaches. This review discusses the latest reports that establish a relationship between RNA structure and IRES function in picornavirus genomes, the first RNAs described to contain these specialized regulatory elements.

Internal initiation of protein synthesis in picornavirus

Picornaviruses belong to an important group of animal pathogens that have evolved a specialized mechanism to promote translation initiation of their mRNAs internally [1]. This mechanism of translation initiation enables the preferential translation of their genome while avoiding the modification of translation initiation factors (eIFs) and other host factors that occur in infected cells [2–4] and induce the inactivation of host protein synthesis. The process of internal initiation of translation in eukaryotic mRNAs is dependent on a *cis*-acting element known as the internal ribosome entry site (IRES) that uses an internal codon in the mRNA to start protein synthesis. IRES elements were originally found in the genomes of picornaviruses, which are a family of positive strand RNA viruses [5,6]. IRES elements have also been found in a growing number of genetically distant viral RNAs, including flaviviruses, retroviruses, coronaviruses, dicistroviruses and plant RNA viruses, in addition to several cellular mRNAs that are translated under conditions when cap-dependent initiation is inhibited [7–14].

Most eukaryotic mRNAs use a cap structure (m^7Gppp) (see Glossary) at the 5' end to initiate translation via the translation initiation factor eIF4F [15]. The process of protein synthesis is initiated by the interaction of the 40S ribosomal subunit (bound to eIF3–eIF2–methionyl-tRNA) with the eIF4F complex (eIF4G–eIF4A–eIF4E), which in turn is bound to the 5' cap of the mRNA. This macromolecular complex then scans the 5' untranslated region (UTR) to reach an AUG triplet in an appropriate

context [16]. By contrast, IRES-dependent translation initiation recruits the translational machinery to an internal position in the mRNA, regardless of the presence of upstream AUG codons, stable RNA structure and protein-binding sites [1,17].

The picornavirus genome consists of a positive sense RNA of ~7400–8500 nucleotides, in which 5' and 3' UTRs flank the single open reading frame (Figure 1). The 5' end of the viral RNA is covalently linked to viral protein g (VPg). All members of the different genera belonging to the picornavirus family initiate translation via an IRES element [17]. The success of the picornavirus replication cycle, and thus the effectiveness of infection, is dependent on the correct function of the IRES; the IRES region is therefore a target for antiviral drugs aiming to inactivate the IRES [18], and is a determinant of viral pathogenesis and virulence [19–21].

Picornavirus IRES-driven translation initiation depends on the recognition of IRES by specific cellular proteins [22]. Translation initiation factors such as eIF4G are essential for picornavirus IRES activity [23–25]. In addition, RNA-binding proteins such as the polypyrimidine tract-binding protein (PTB), the poly(rC)-binding protein (PCBP), the SRp20 splicing factor, the proliferation-associated IRES transacting factor (ITAF)₄₅ or the upstream-of-Nras protein (Unr) interact with IRES elements [26–31] presumably facilitating their structural organization by acting as RNA chaperones.

Glossary

Cap structure: m^7Gppp residue present in the 5' end of eukaryotic mRNAs that is recognized by the initiation factor eIF4E.

Dimethyl sulfate (DMS): A reagent that reacts with unpaired bases in the RNA structure, used to measure accessibility to C and A bases.

GNRA: A motif in the RNA structure that consist of 4 bases, G, N (any nucleotide), R (purine) and A.

Intergenic region (IGR): The region in the genome of dicistroviruses that separates two open reading frames and enables internal initiation of translation of the second cistron.

Internal ribosome entry site (IRES): A region in eukaryotic mRNA that enables translation initiation independently of the 5' end.

ITAFs: *Trans*-acting factors that interact with IRES regions.

Picornaviruses: A group of unsegmented and non-enveloped positive-strand RNA viruses, including enteroviruses, rhinoviruses, cardiociruses, aphthoviruses, hepatoviruses, erboviruses, teschoviruses, parechoviruses and kobuviruses.

Toe-print: A technique that determines the position of assembled 48S initiation complexes by measuring the inhibition of reverse transcriptase elongation of antisense primers.

Untranslated regions (UTR): The sequences on either end of the mRNA, flanking the coding region.

Corresponding author: Martínez-Salas, E. (emartinez@cbm.uam.es).

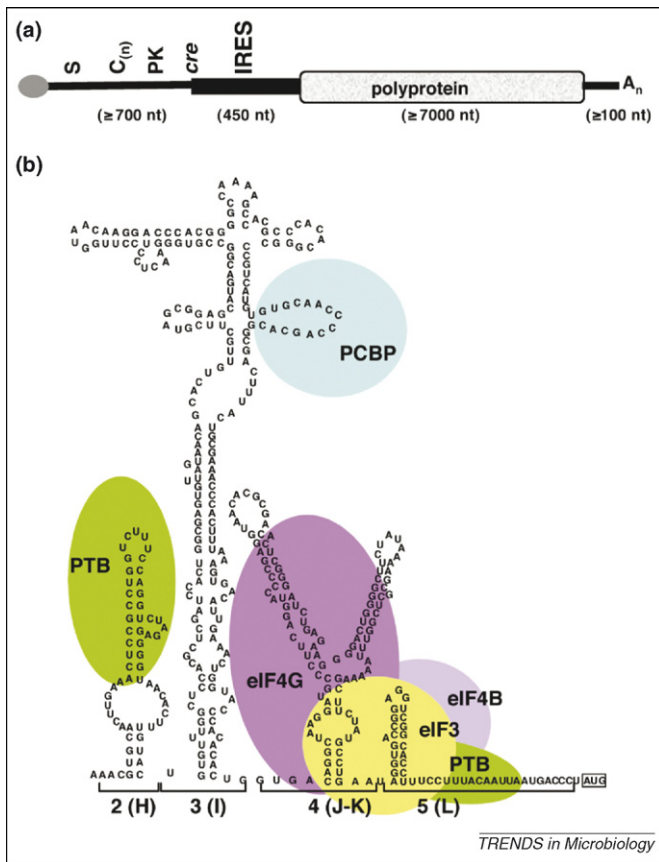


Figure 1. Diagram of protein interactions with a picornavirus IRES. (a) Upstream of the IRES element in the 5'UTR of the aphthovirus genome (top), several structural elements are arranged consecutively: the S region, the poly(C) tract, the pseudoknotted region (PK), the *cis*-replicative element (*cre*). The poly(A) tail is located at the 3'UTR. The grey circle at the 5' end depicts the viral protein VPg. Approximate nucleotide content of each region of the viral genome is indicated. The IRES region is enlarged from the aphthovirus genome (b). Position of IRES domains [2(H) to 5 (L)] is indicated at the bottom of the corresponding stem-loops. Interaction of specific sequences within domain 4 with initiation factor eIF4G is essential for IRES activity. eIF3, which is also required for IRES activity, shows preferential binding in domain 5. eIF4B-binding is restricted to the hairpin of domain 5 and moderately enhances IRES activity. The auxiliary protein PTB, which contributes to enhanced IRES activity, recognizes two separate polypyrimidine tracts, one in domain 2 and another in domain 5. The host factor PCBP interacts with the C-rich bulge in the central domain; its interaction is needed for entero- and rhinovirus IRES, but not for cardio- and aphthovirus IRES activity.

In this review, we discuss structural aspects of picornavirus IRES elements that are crucial for internal initiation activity. We suggest that the acquisition of specific structural organization by the IRES has a dual function; it is necessary for recognition by RNA-binding proteins and for RNA-mediated regulation of internal translation initiation.

Functional link between the 5' and 3' ends of the viral RNA

Polyadenylation is a conserved feature in picornavirus RNAs and cellular mRNAs. The poly(A) tail present in the majority of eukaryotic mRNAs improves the efficiency of translation initiation through recruitment of poly(A)-binding protein (PABP), enabling its interaction with eIF4F located at the mRNA 5' end. In picornavirus RNAs the poly(A) tail together with the sequence of the 3' UTR, which is specific for each genus, constitute essential determinants of the virus multiplication cycle [32–34]. The

efficiency of viral RNA replication is enhanced by protein bridges that mediate RNA circularization by interacting with both ends of the viral RNA. In poliovirus RNAs the cloverleaf (CL) structure and the adjacent C-rich spacer interact with the host protein PCBP2 and the viral protein 3CD [35] generating a ternary complex that is essential for viral RNA replication, and that possibly regulates the switch from translation to replication [36]. Viral proteins 3A and 3C also contribute to the formation of a functional complex with the IRES and 3' UTR, which is important for viral propagation [37].

Poliovirus IRES activity in neuronal cells was stimulated by the presence of specific 3' UTR structural elements [38] suggesting a functional role for the 3' end–IRES interaction in viral pathogenesis. Similarly, a functional link between the aphthovirus IRES and the 3' end of the viral RNA was suggested by the specific stimulation of IRES activity by the foot-and-mouth disease virus (FMDV) 3' UTR [39]. In agreement with data reported in other RNA viruses [40], the 3' end of the FMDV genome establishes strand-specific long range RNA–RNA interactions, but in this case there are two different interactions, one with the IRES element and another with the S region at the 5' end [41] (Figure 1). Different RNA motifs seem to be involved in these interactions because a high-order structure adopted by the entire IRES and the 3' UTR was essential for RNA interaction, whereas the S region interacted with each of the stem-loops at the genome 3' end. The 3D structure of the FMDV 3' UTR is unknown; however in enteroviruses the 3' UTR is organized as two stem-loops that adopt a quasi globular organization [42].

Thus, bridging of 5' and 3' ends in the picornavirus genome involves direct RNA–RNA contacts and RNA–protein interactions. It is noteworthy that the host factor PCBP2, which is required for translation initiation and viral RNA replication, is cleaved during poliovirus infection [2] resulting in a truncated protein that is unable to function in translation but that maintains its activity in viral RNA replication. This event might promote the switch from viral translation to RNA replication, a key step in the picornavirus infection cycle that needs to be tightly regulated.

Relevance of RNA structure in IRES function

Understanding IRES biology is crucial for increasing our knowledge of translational control in viral RNAs. Several studies have shown a strong relationship between RNA structure and IRES function, but the basic mechanisms through which IRES elements recruit the ribosome have only begun to emerge recently. This is due, at least in part, to the lack of a conserved core either in the primary sequence or in the predicted RNA structure of genetically distant IRES elements.

From a functional and structural point of view, the picornavirus IRES together with IRES elements present in the viral RNA of hepatitis C virus (HCV) [7] and the dicistrovirus intergenic region (IGR) [14] provide good model systems for addressing the relevance of RNA structure in IRES function. The IRES elements present in these three positive strand RNA viruses belonging to different families possibly encounter the largest variation in RNA

organization and eIF requirements. The dicistrovirus IGR is organized in a pseudoknotted RNA structure and it does not require eIFs for assembly of 48S initiation complexes [43,44]. The HCV IRES adopts a different folded RNA structure (distributed in three domains, II, III and IV, with IV including a pseudoknot) and requires eIF3 and eIF2, but no eIF4G, to assemble initiation complexes [45–47]. In this scale of growing complexity, picornavirus IRES represent the most complex group in terms of sequence length and factor requirements [23,25].

In the genome of all picornaviruses, the IRES element is surrounded by RNA structural elements. This is particularly evident in cardiovirus and aphthovirus, which have long 5' UTRs burdened with different structural elements required for viral infectivity [41] (Figure 1). The IRES region spans approximately 450 nucleotides of the 5' viral RNA, and on average <50% of the primary nucleotide sequence is conserved between different picornaviruses. However, the secondary structure is conserved between different members of this family [48]. According to secondary structure prediction, representative members such as poliovirus (PV), coxsackievirus B3 (CVB3) or human rhinovirus (HRV) belong to group I, whereas encephalomyocarditis virus (EMCV) or FMDV belong to group II. A novel group that shares RNA structural similarities with the IRES element of HCV has been described recently [49,50].

All picornavirus IRES elements are organized in stable domains termed II to VII in enteroviruses and G to L or 2 to 5 in cardio- and aphthovirus IRES [1,17,48]. Structural analysis of type II IRES encompassing either single domains or the entire IRES have shown the presence of self-folding structural elements that acquire a similar conformation [51,52]. Consistent with the lack of effect of upstream sequences, the IRES element occupies an internal position in the viral RNA where the different 5' UTR upstream elements perform their specific roles during the replication cycle without interfering with IRES activity. Consequently, the picornavirus IRES elements promote efficient protein synthesis in polycistronic vectors [53].

Biochemical and functional analyses have shown that distal domains within the IRES structure are involved in interactions with host factors [17]. Recognition of the different picornavirus IRES elements by host transacting factors is mainly dependent on the structural organization of specific domains, as demonstrated by mutational analysis of the binding site of proteins such as eIF4G, eIF4B or PTB (Figure 1; reviewed in [54]). However, the role of the central domain of picornavirus IRES that occupies a significant portion of the entire sequence remains elusive. Few interactions with host factors have been found in this region; a conserved C-rich motif that interacts with PCBP2 is essential for IRES activity in the viral RNA of entero- and rhinovirus, but not in cardio- and aphthovirus [27].

A distinctive feature of the central domain (termed 3 or I in cardio- and aphthovirus; Figure 2) is the presence of a cruciform structure at the most apical region [51]. This region of domain 3 contains two conserved purine-rich motifs, GNRA and RAAA (N, any nucleotide, R, purine), located in distal loops that do not tolerate nucleotide

substitutions, deletions or insertions [55,56]. The proximal region of this domain is organized as a base-paired structure interrupted with bulges that includes several non-canonical base pairings and a helical structure at its proximal region that is crucial for IRES activity [57]. These results suggest that the central domain could have a regulatory role during internal initiation, in which the proximal region of the central domain contributes to IRES activity by serving as a platform that holds the apical region in a conformation appropriate for its recognition by the translation machinery.

The GNRA motifs of different picornavirus IRES (FMDV, PV, CVB3 and EMCV) adopt a tetraloop conformation at the tip of a stem-loop [51,58–60]. GNRA tetraloops are often involved in RNA folding, generating RNA tertiary contacts [61]. Sequence variability of the IRES region in FMDV field isolates shows substitutions in the GUAA sequence yielding GCAA or GCGA, always compatible with the GNRA consensus motif. Conversely, as shown by RNA probing, IRES residues involved in base pairing often show nucleotide covariation (Figure 2), strongly supporting the need for preserving IRES structure for internal initiation [54]. Site-directed nucleotide substitutions disrupting the FMDV GNRA motif led to the reorganization of the apical region of the central domain, demonstrating the significant role of this conserved motif in dictating the stability of the cruciform structure. Subsequent structural probing of GNRA mutants enabled the identification of a distant region (motif A) that became more susceptible to RNase attack [51]. Functional analysis of mutants in this region indicated a reduction in IRES activity similar to that reported for GUAG mutants. Conversely, RNA probing of the second-site substitutions showed that motif A mutants contained a reorganization that affected the mutated region and also the distant GNRA motif [62]. Based on the reciprocity of RNA structural changes this short region behaves as a receptor of the GNRA motif. Lack of genetic variability in this region in approximately one hundred FMDV sequences [63] showed a strong selective pressure for keeping the primary sequence. This feature can be explained either because of its involvement in distant contacts with other RNA motifs, or by recognition by auxiliary factors involved in translation initiation that remain to be identified.

The organization of the RAAA stem-loop was also dependent on the local RNA structure determined by GNRA-dependent interactions [62]. Thus, multiple contacts within the apical region of the central domain presumably contribute to formation of a tight RNA structure that is essential for IRES activity, as also suggested by the finding of Mg²⁺-dependent RNA–RNA interactions mediated by this IRES region [64]. Along this line, RNA probing of the entire CVB3 5' NTR has shown that the previously known domains II to VII, defined by mutational and bioinformatic approaches, comprises a long-range tertiary interaction involving bases in domain II and domain V [60] that connects the 5' end (domain I, which folds in a CL structure) with the IRES element. This physical association opens new perspectives for crosstalk between the IRES element and the CL regarding translation and replication of viral RNA.

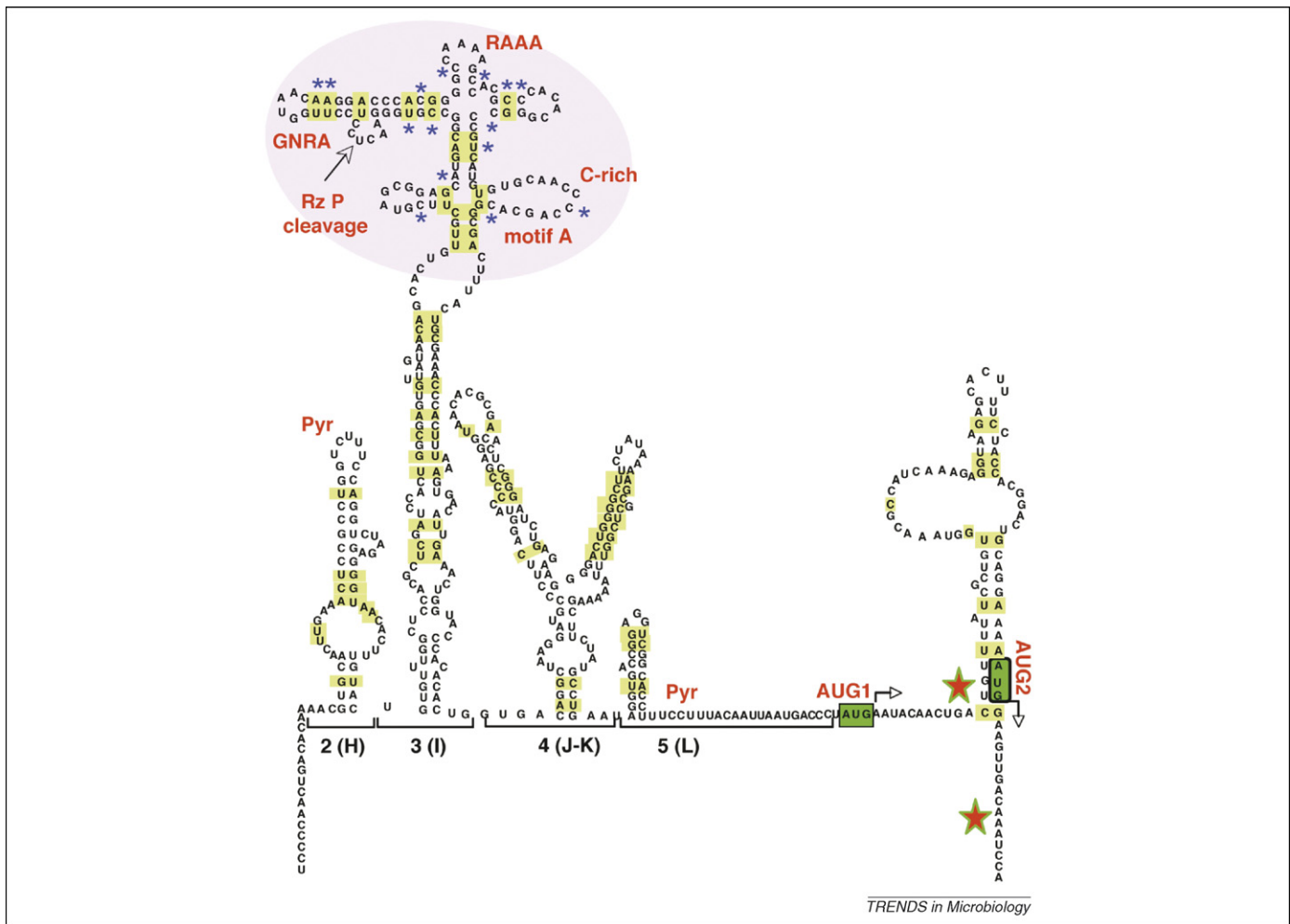


Figure 2. Schematic representation of the secondary structure of a picornavirus IRES. Nucleotide covariation is indicated by yellow rectangles; Pyr denotes the polypyrimidine tracts; GNRA, RAAA, C-rich and motif A denote picornavirus IRES conserved motifs; Rz P cleavage denotes the cleavage site of the FMDV IRES transcript *in vitro* by the cyanobacteria RNase P ribozyme. The apical region of domain 3 (I) is highlighted by a purple circle that contains the RNase P recognition motif overlapping with the GNRA stem-loop. In this region the nucleotides marked with blue asterisks showed a differential accessibility in the cellular cytoplasm. The positions of the functional AUG codons (arrows on green boxes) as well as the toe-prints (green and red stars) are depicted.

Structural studies performed in other viral IRES showed that the tertiary structure of IRES elements contributes to regulation of translation efficiency [46,43,47]. The IRES elements of cricket paralysis virus (CrPV) and HCV have different RNA structures and distinct binding sites in the ribosomal subunit, yet they induce similar conformational changes in the 40S ribosomal subunit [65]. This observation suggested that IRES elements could share the property of having unique structural elements that mediate direct interaction with the 40S subunit. Accordingly, it has been speculated that the central domain of the aphthovirus and its structurally related cardiovirus IRES could have a fundamental role in dictating the formation of a stable tertiary structure, thereby providing the correct orientation to recruit the ribosome subunits to the initiation sites [62].

As mentioned previously, the IRES region constitutes a structural entity within the viral genome; this model is supported by the ability of IRES to fold in essentially the same manner, regardless of the downstream viral sequences that encompass the initiator codons, and fold as a stem-loop [66] (Figure 2). In the aphthovirus genome, two in-frame AUG triplets separated by 84 nucleotides

are used as translation initiator codons of the viral polyprotein. The second functional triplet (AUG2) is used to initiate protein synthesis more frequently (80%) than AUG1 in infected cells, and also in cells transfected with chimeric RNAs [67,68]. A conserved A-rich sequence precedes AUG2 (Figure 2) and is engaged in base pair formation within a stem-loop phylogenetically conserved in FMDV field isolates. By contrast, the first functional start codon (AUG1) is located in a single-stranded region, as judged by its accessibility to chemicals and enzymes [66]. Initiation of protein synthesis from both AUGs is IRES-dependent but the mechanism operating in codon selection remains poorly understood. It has been proposed that following entry of the ribosome the initiation complex scans along this viral region until AUG2 is reached [68], or that the initiation complex is transferred to the vicinity of AUG2 [67]. Recently it was shown that translation initiation at AUG2 was barely abrogated by the presence of a modified, more stable RNA structure in the spacer sequence [66], consistent with previous observations of lack of effect of antisense molecules bound to AUG1 [67]. However, the factors required for reconstituting 48S initiation complexes at each initiation codon *in vitro* are

different. In addition to eIF3, eIF4G and eIF4A, recognition of AUG1 is dependent on eIF1A, whereas initiation at AUG2 is dependent on eIF1 [66], suggesting that the mechanisms operating for selection of the initiation codon in FMDV depend on the sequence preceding the initiation triplet. This conclusion is in agreement with the observation that non-viral sequences adopting a stable hairpin structure placed in a spacer region between the IRES and the AUG of a reporter coding region abrogated translation initiation [69], but did not do so in the viral RNA [66,67].

Structural organization of picornavirus IRES elements in living cells

The basis of RNA structure-mediated mechanisms involved in ribosome recruitment during internal initiation is currently under active investigation. In the past few years, determination of the RNA structure of several viral IRES elements using *in vitro* approaches has represented a major challenge in this area of research [44,70]. IRES elements found in unrelated mRNAs do not share primary sequences and they seem to be organized in different RNA structures [7,54]. Thus, the structural requirements for IRES activity remain to be defined.

The picornavirus IRES have been found to interact specifically with some eIFs *in vitro*, using reconstituted initiation complexes [1], or in functional analysis performed in transfected cells [24,71]. Therefore, it is conceivable that in the competitive environment of the cellular cytoplasm IRES function depends on the availability of eIFs and other ITAFs and their coordinated interaction with the IRES RNA. These functional interactions might be compromised owing to their preferential use in cap-dependent initiation as well as in other processes related to RNA biology [17].

Despite multiple efforts to understand IRES biology, information on the organization of IRES elements in living cells remains limited. Recent studies have taken advantage of reagents permeable to the cellular membrane that recognize RNA molecules in a structure-dependent manner to elucidate the organization of picornavirus IRES elements in living cells [52]. Notably, specific bases of the central domain in the FMDV IRES were differentially reactive towards dimethyl sulfate (DMS) *in vivo* than *in vitro* (Figure 2), demonstrating that the pattern of modification found in the cytoplasm of living cells differed from that obtained *in vitro*. In addition, new DMS-reactive bases specifically detected *in vivo* affected nucleotides involved in the formation of G:C base pairs in the secondary structure model generated *in vitro*. This result again suggests that the central region of the IRES adopts a different local RNA conformation in the cellular cytoplasm. Decreased attack of residues in the C-rich loop *in vivo* suggested a potential RNA-protein interaction; this conserved motif is a candidate for interaction with PCBP [27]. Conversely, strong protection in the region upstream of the initiator codon suggested the formation of RNA-protein complexes, which overlap the region described to interact with translation initiation factors in tissue culture cells [24,71].

Complementary studies on the organization of FMDV IRES in the cell cytoplasm using the photoreactive reagent

amino-methyl psoralen revealed a hot-spot of crosslinked pyrimidines in the central domain [34], consistent with the formation of inter-strand crosslinks in the secondary structure. The comparison of the UV-psoralen crosslink pattern performed *in vitro* to that seen *in vivo* showed changes compatible with a local reorganization of RNA structure within the apical region of the central domain.

Overall, the results derived from different approaches are consistent with an active role of the RNA structure in this region during translation initiation. The modification of RNA accessibility detected in the central domain *in vivo* could be because of different scenarios. The interaction with host transacting factors in the cellular cytoplasm could be responsible for this reorganization, as observed *in vitro* for other IRES elements [72]. Another possibility is the acquisition of a structural conformation that might facilitate a direct involvement of the apical region of the central domain with the ribosomal components. In support of this hypothesis, a tRNA-like structural motif that serves as substrate for RNase P *in vitro* resides in this IRES region [73]. These two possibilities are not mutually exclusive, and both need further investigation because no 48S initiation complexes have been detected in the absence of eIFs and the corresponding IRES-dependent toe-prints are located downstream of the respective initiation codon [66] (Figure 2).

Functional and evolutionary implications of IRES-related structural elements in viral RNAs

At present, the question of how the IRES elements appeared during evolution is unresolved. Although the primary sequence is remarkably divergent, conservation of specific motifs in IRES structural organization could provide some hints on their evolutionary history. As an example, the conservation of the pseudoknot motif – a characteristic feature of the HCV IRES – supports the possibility that the differences in RNA structure found among picornavirus IRES might have come about by horizontal transmission between different ancestors [50]. Another conserved feature of viral IRES is the presence of one or more polypyrimidine tracts [1,48]. However, a search of IRES elements on the basis of short conserved primary sequences (e.g. the polypyrimidine tract or the C-rich motifs that provide PTB or PCBP-binding sites, respectively) does not guarantee the presence of a functional element. The data available are theoretically compatible with a high-order structure generated by a combination of several structural elements that differ between divergent IRES elements; how many different combinations can generate a functionally active element remains an unresolved question.

Structural motifs in HCV RNA that serve as substrate for the human RNase P have been discovered recently, one in the IRES and another in the coding region [74]. On the basis of the recognition by RNase P it was inferred that the HCV and pestivirus IRES contained a structural element that mimics the tRNA-like structure [75]. RNase P is a structure-dependent endonuclease involved in the processing of the tRNA precursor [76], which also recognizes RNA viruses containing tRNA-like structures at the 3' end of the viral genome as substrate [77]. The aphthovirus IRES

contains a structural motif that acts as substrate for the RNase P ribozyme *in vitro* (Figure 2). The core structural element recognized by the ribozyme resides in the central domain of the FMDV IRES [73], overlapping with a self-folding region that contains the conserved motif GNRA, which is essential for IRES activity. As mentioned previously, the GNRA motif mediates the local RNA structure [51], presumably with the involvement of tertiary contacts. In support of the recognition of a structural element, defective FMDV IRES mutants with modified RNA structure responded differentially to ribozyme cleavage leading to an enhanced recognition that was accompanied by cleavage sites in nearby residues [73] as it also occurs in a variant RNA molecule of HCV [78]. As the viral cycle of picornaviruses and flaviviruses occurs in the cell cytoplasm, the viral RNA has no access to RNase P, which resides in the nucleus of eukaryotic cells [76].

The ribozyme recognition motif in FMDV is located in a different position relative to the HCV or the pestivirus IRES, which have this motif at the 3' end of the IRES region [74]. From an evolutionary point of view, this observation can be interpreted in several ways. First, the differences in the relative position within the IRES and also within the genome of other distantly related viral RNAs [74,77] could be the remnants of an ancestor module without function in the viral genome. Second, because overlapping information in the small genome of RNA viruses occurs often, it is also possible that this motif is involved in a new function still to be determined. Third, regarding the possibility of an active role of this motif during IRES activity, the differences found between IRES elements could be indicative of the distinct strategies used by the genome of RNA viruses belonging to different families to interact with the translational machinery.

In this regard, structural studies performed on HCV and the IGR of dicistroviridae members have shown that these IRES elements are located in the interface of the ribosomal subunits [79,70,44]. These results suggested that specialized motifs of the IRES region mimic the initiator tRNA (tRNA_i) during initiation of protein synthesis and have recently been confirmed in domain 3 of the CrPV IGR [80]. It is proposed that the IGR structure undergoes subtle structural changes during the process of translation initiation, suggesting a direct involvement of the RNA structure in the ribosome assembly and translocation process. It can be envisaged that a tRNA-like motif within the picornavirus IRES could contribute to efficient recognition of the viral RNA by the translational machinery because it seems to occur in the dicistrovirus IGR [14,80]. However, to date there is no evidence of the formation of binary complexes mediated by the picornavirus IRES and 40S subunits in the absence of eIFs. Thus, a functional connection between RNA organization detected by structural analysis and a putative tRNA-like structure detected by ribozyme recognition in IRES elements requires further investigation.

Concluding remarks and future perspectives

Understanding how RNA structure-mediated mechanisms operate in genetically divergent IRES elements is necessary for generating a model of the internal initiation process

occurring in eukaryotic cells. Picornavirus IRES elements seem to work as a ribonucleoprotein engine that assembles a large number of factors into a specialized, appropriately folded RNA structure that can be recognized by the ribosomal subunits. Other viral IRES elements seem to work as docking RNA structures that can gain access to specific sites in the ribosomal subunit without the help of additional factors. In between these divergent types of IRES elements, large variations in the mode of action exist that extend from picornavirus-like to the poorly understood cellular IRES elements. The idea that a universal IRES motif could consist of a structural element that mediates the interaction with the ribosomal machinery has gained support from studies using different approaches. In particular, structural and functional studies of the relatively small dicistrovirus IRES models have led to the idea that the IRES element itself mimics the initiator tRNA during internal initiation, in a process in which flexibility of the RNA molecule is crucial. Evaluating this model in the more complex, but also more efficient, picornavirus IRES elements represents a major challenge for the near future.

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