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Functional interactions in internal translation initiation directed by viral and cellular IRES elements

EncarnaciÃ³n MartÃ-nez-Salas, Ricardo Ramos, Esther Lafuente and Sonia LÃ³pez de Quinto

Centro de BiologÃ-a Molecular 'Severo Ochoa', Consejo Superior de Investigaciones CientÃ-ficasâ€“Universidad AutÃ³noma de Madrid, Cantoblanco, 28049 Madrid, Spain

Introduction

Initiation of protein synthesis involves a series of tightly regulated events that allow the recruitment of ribosomal subunits to the mRNA. Two distinct mechanisms have evolved in eukaryotic cells to initiate translation. In one of them, the methyl-7-G(5Â´)pppN structure present at the 5Â´ end of the mRNA, known as cap, is recognized by the initiation factor eIF4F, which is composed of eIF4E, eIF4G and eIF4A. Additionally, preinitiation complex formation requires, among others, the concerted action of initiation factor eIF2, responsible for binding to the initiator tRNA-Met_i, and eIF3, which interacts with the 40S ribosomal subunit (reviewed in Hershey & Merrick, 2000).

In the alternative mechanism, translation initiation occurs internally and is mediated by a *cis*-acting element, known as the internal ribosome entry site (IRES), that recruits the translational machinery to an internal initiation codon in the mRNA with the help of *trans*-acting factors (reviewed in Jackson, 2000). During many virus infections, as well as in other cellular stress conditions, changes in the phosphorylation state of eIF2, which lower the levels of the ternary complex eIF2â€“GTPâ€“tRNA-Met_i, result in overall inhibition of protein synthesis. Conversely, specific shut-off of cap-dependent initiation depends upon modification of eIF4F functionality (reviewed in Thompson & Sarnow, 2000).

IRES elements bypass cap-dependent translation inhibition. Hence, IRES-driven translation initiation prevails during picornavirus infection (Macejak & Sarnow, 1991; Johannes & Sarnow, 1998; Johannes *et al.*, 1999). Under these circumstances, cap-dependent initiation is severely compromised, due to the presence of small amounts of functional eIF4F. This is caused by cleavage or loss of solubility of eIF4G (Gradi *et al.*, 1998; Cuesta *et al.*, 2000), 4E-BP dephosphorylation (Gingras *et al.*, 1996) or poly(A)-binding protein (PABP) cleavage (Joachims *et al.*, 1999; Kerekatte *et al.*, 1999).

Here, we discuss recent reports on the functional interactions between the key players in ribosome recruitment to internal sites in the mRNA: (i) the RNA sequences that constitute the *cis*-acting element, (ii) the eIFs required to link the RNA to the small ribosomal subunit and (iii) other *trans*-acting proteins that act as RNA chaperones and/or modulate IRES activity in response to physiological stimuli.

Occurrence of IRES elements in eukaryotic mRNAs

The development of bicistronic expression vectors containing the picornavirus 5Â´ untranslated region (UTR) located in the intercistronic region led to the definitive demonstration that this region was able to confer internal initiation of translation on an mRNA, independent of the 5Â´ end (Jang *et al.*, 1988; Pelletier & Sonenberg, 1988). Since the discovery of IRES elements in picornavirus RNAs, functional IRES elements have been identified in an increasing number of mRNAs from animal cells and their pathogens (Table 1; reviewed in Carter *et al.*, 2000). IRES-containing mRNAs encode a variety of proteins such as translation initiation factors, transcription factors, oncogenes, growth factors, homeotic genes and survival proteins, that are functional under acute cellular stress.

Table 1. Examples of IRES elements

Virus/gene type	Virus/gene	Reference
Viral RNAs		
Picornaviruses	Poliovirus (PV)	Pelletier & Sonenberg (1988)
	Encephalomyocarditis virus (EMCV)	Jang *et al.* (1988)
	Foot-and-mouth disease virus (FMDV)	KÃ¼hn *et al.* (1990)
Flavivirus	Hepatitis C virus (HCV)	Reynolds *et al.* (1995)
Pestivirus	Classical swine fever virus (CSFV)	Pestova *et al.* (1998)
Retrovirus	Murine leukaemia virus (MLV)	Berlioz & Darlix (1995)
Lentivirus	Simian immunodeficiency virus (SIV)	Ohlmann *et al.* (2000)
Insect RNA virus	Cricket paralysis virus (CrPV)	Wilson *et al.* (2000 *a*)
Cellular mRNAs		
Translation initiation factors	eIF4G	Johannes & Sarnow (1998)
	DAP5	Henis-Korenblit *et al.* (2000)
Transcription factors	c-Myc	Stoneley *et al.* (2000 *a*)
	NF-κB-repressing factor (NRF)	Oumard *et al.* (2000)
Growth factors	Vascular endothelial growth factor (VEGF)	Huez *et al.* (1998)
	Fibroblast growth factor 2 (FGF-2)	Creancier *et al.* (2000)
	Platelet-derived growth factor B (PDGF B)	Bernstein *et al.* (1997)
Homeotic genes	*Antennapedia*	Oh *et al.* (1992)
Survival proteins	X-Linked inhibitor of apoptosis (XIAP)	Holcik & Korneluk (2000)
	Apaf-1	Coldwell *et al.* (2000)
Miscellaneous	BiP	Macejak & Sarnow (1991)

The presence of IRES elements in the mRNAs of plant cells and their viruses is still under study. Several plant viral mRNAs are naturally uncapped, hence their translation initiation is cap-independent (Niepel & Gallie, 1999; Skulachev *et al.*, 1999; Wu & White, 1999). Interestingly, plant viral mRNAs often contain translational enhancers in their 3Â´ UTRs, a strategy that also allows stimulation of translation initiation independent of the 5Â´ cap (Wang *et al.*, 1997,

1999 *b*; Allen *et al.*, 1999). It is likely that this strategy has been exploited in general by plant pathogens as an alternative to IRES elements.

The presence of IRES elements in yeast and fungal mRNAs is still unclear. Although yeast extracts support both IRES- and cap-dependent translation (Iizuka *et al.*, 1994), none of the animal virus IRES elements seems to be active in yeast cells. An inhibitory activity exerted by a short inhibitor RNA (iRNA) has been reported to be responsible for the inactivation of several IRES elements when tested in yeast (Venkatesan *et al.*, 1999).

Comparison of the IRES elements of picornaviruses indicates a lack of conservation of primary sequences, with the exception of a short polypyrimidine tract (Pestova *et al.*, 1991). Moreover, when this comparison is extended to distantly related viral and cellular IRES elements, it is clear that not only the sequence but also the length varies among different IRES elements. IRES elements of around 200 nt are present in insect RNA viruses (Sasaki & Nakashima, 1999; Wilson *et al.*, 2000 *b*), whereas picornavirus RNAs have IRES elements of about 450 nt (Jang & Wimmer, 1990; KÃ¼hn *et al.*, 1990; Nicholson *et al.*, 1991). This large variation is indicative of differences in (i) structural organization and (ii) the strategies used to interact with the translational machinery. These aspects of the IRES biology are discussed below.

Structureâ€“function relationships in IRES elements

The phylogenetic conservation of IRES secondary structure is one of the strongest arguments in support of the structural requirements that determine IRES activity. Studies on the genetic variability within the IRES regions of infectious, highly variable RNAs, such as foot-and-mouth disease virus (FMDV) and hepatitis C virus (HCV), reveal that most of the substitutions cause compensatory changes in the secondary structure of the IRES. Moreover, IRES variability selected in nature is usually accompanied by a change in activity within a narrow range (MartÃ-nez-Salas *et al.*, 1993; Kamoshita *et al.*, 1997; Collier *et al.*, 1998; SÃ¡iz *et al.*, 1999; Lerat *et al.*, 2000), suggesting a restriction imposed by natural selection on the translation efficiency of viable viruses.

Nevertheless, the structural requirements for initiation of translation seem to be different in IRES elements from distant picornaviruses, defining two main groups of IRES structures (entero- and rhinoviruses versus cardio- and aphthoviruses), this difference being more remarkable when the comparison is extended to the HCV or classical swine fever virus (CSFV) IRES elements (Jackson, 2000). Hence, the same function can be accomplished by a different structural organization.

Identification of regions essential for IRES activity

On the assumption that conserved motifs often correspond to essential parts of the molecule, mutational analysis has been carried out on many IRES elements to define the precise sequences required for activity. In addition to studies performed with infectious picornavirus clones (Haller & Semler, 1992; Pilipenko *et al.*, 1992), an exhaustive mutational analysis has taken advantage of the IRES property of directing internal entry in bicistronic expression vectors (reviewed in Jackson, 2000). The latter approach offers the possibility of testing the IRES in the absence of virus infection, thus eliminating interference with other functions of the virus cycle where the IRES may also be involved, such as replication or encapsidation (Borman *et al.*, 1994; Ishii *et al.*, 1999; Johansen & Morrow, 2000).

With this experimental approach, parts of the secondary structure of IRES elements have been associated with activity. Some essential sequences were found to form part of double-stranded regions (Jang & Wimmer, 1990; Hoffman & Palmenberg, 1996; Honda *et al.*, 1996, 1999; MartÃ-nez-Salas *et al.*, 1996), whereas others, located in apical or internal loops, required a specific primary sequence to allow IRES activity (LÃ³pez de Quinto & MartÃ-nez-Salas, 1997; Psaridi *et al.*, 1999; Robertson *et al.*, 1999; Jubin *et al.*, 2000). Disruption of some of these regions is associated with the modification of essential RNAâ€“protein interactions (LÃ³pez de Quinto & MartÃ-nez-Salas, 2000). However, other motifs such as the conserved GNRA tetraloop located at a distal loop in the central domain of FMDV IRES (Fig. 1) are candidates to determine long-range RNA interactions. Taken together, there is experimental evidence that not only point substitutions but also small deletions and/or insertions inactivate the IRES, indicating that the overall RNA structure

needs to be maintained in order to retain activity.

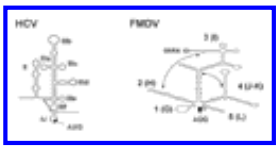

Fig. 1. IRES structural organization. Schematic representation of the HCV and FMDV RNA structure. Domain numbering of HCV is taken from Honda *et al.* (1999); IIIa, IIIb, IIIc, IIId as well as the AUG loop are exposed (Kieft *et al.*, 1999). The pseudoknot structure is depicted according to Wang *et al.* (1995). Domain numbering of FMDV (1â€“5) is taken from LÃ³pez de Quinto & MartÃ-nez-Salas (2000). The alternative numbering (Gâ€“L) used for EMCV is from Pilipenko *et al.* (2000). Double-headed arrows depict RNAâ€“RNA interactions between separated domains (Ramos & MartÃ-nez-Salas, 1999). The position of the essential GNRA motif (LÃ³pez de Quinto & MartÃ-nez-Salas, 1997) is shown by a thick line. The positions of the AUG initiator codons are highlighted by black boxes.

The structural organization of cellular IRES elements is poorly defined. Mutations found in the c-myc IRES identified a C-to-U transition within this element that modified the predicted secondary structure and led to enhanced activity in multiple myeloma cell lines (Chappell *et al.*, 2000 *b*). In the case of vascular endothelial growth factor (VEGF) mRNA, two IRES elements seem to occupy non-overlapping sequences (Huez *et al.*, 1998), each of them directing initiation at a different codon. On the other hand, a modular organization, consisting of repetitions of a short sequence, has been proposed for the Gtx IRES (Chappell *et al.*, 2000 *a*).

IRES tertiary structure interactions

Experimental evidence in support of tertiary structure generated by RNAâ€“RNA interactions in IRES elements is available for FMDV and HCV (Fig. 1). A pseudoknot structure in the HCV IRES was shown to be absolutely required for IRES activity, as mutations that destabilized the tertiary interactions between residues of loop IIIf and those complementary in domain IV were accompanied by a strong reduction in translation initiation (Wang *et al.*, 1995; Honda *et al.*, 1996). Similarly, the IRES located in the intergenic region of an insect virus RNA also contains a pseudoknot-like structure, as shown by phylogenetic and mutational studies (Wilson *et al.*, 2000 *a*; Sasaki & Nakashima, 2000).

Long-range RNAâ€“RNA interactions have been shown to occur *in vitro* between functional domains of the FMDV IRES (Ramos & MartÃ-nez-Salas, 1999). These interactions are strand-specific and depend on RNA concentration, ionic conditions and temperature, suggesting a dynamism in the tertiary structure of the IRES that may play an important role in the biology of this element. The RNAâ€“RNA interactions observed *in vitro* between separated domains of the FMDV IRES, in the absence of proteins, suggest that the IRES adopts a specific folding, depending upon environmental conditions. A similar situation has been described for the HCV IRES, which adopts different folding patterns in response to increased ion concentrations (Kieft *et al.*, 1999). Since the intracellular concentration of cations varies with time in picornavirus-infected cells (Carrasco, 1995), it is likely that changes in the tertiary structure of the IRES occur readily during infection and, as a consequence, modulate translation efficiency.

Interestingly, the central region (domain 3) of the FMDV IRES is unique in its ability to interact with all the other domains (see Fig. 1), including the whole IRES (Ramos & MartÃ-nez-Salas, 1999). This result has two implications: the first is that domain 3 acts as a scaffold structure that holds together the remaining domains of the IRES and the second is that domain 3 is necessary to determine intermolecular interactions with other IRES molecules. These interactions can account for reports of complementation between defective IRES elements (Drew & Belsham, 1994; Roberts & Belsham, 1997). In the context of a virus infection where replication occurs in precise cellular regions (Bolten *et al.*, 1998), the local RNA concentration can reach the levels required to allow intermolecular interactions.

Is the rearrangement of IRES structure relevant to biological activity? Physiological changes in the intracellular milieu affecting ionic conditions, pH gradients, temperature, free radical formation, expression of specific RNA-binding proteins

and so on are likely to induce reorganization of the IRES structure, which may have important consequences for recruiting *trans*-acting factors. Consistent with this, artificial reorganization of the spatial RNA structure has strong effects on the binding of cellular factors to the HCV IRES (Odreman-Macchioli *et al.*, 2000). Additionally, mutations in loop IIId cause structural reorganization of the HCV IRES, as measured by RNase T1 sensitivity and Fe(II) EDTA cleavage, concomitant with a reduction in IRES activity (Kieft *et al.*, 1999; Jubin *et al.*, 2000). Taken together, the experimental evidence indicates that the overall RNA structure of the IRES has an active role in internal initiation.

Interaction of viral IRES elements with the translational machinery

Soon after the first reports of IRES activity in the picornavirus 5Â´ UTR, specific RNAâ€“protein interactions were described (Jang & Wimmer, 1990; Luz & Beck, 1991; reviewed in Stewart & Semler, 1997; Andino *et al.*, 1999). Surprisingly, none of the first proteins reported was catalogued as a translation eIF. Hence, the question was: do IRES elements represent a different strategy for recruiting ribosomes and, then, do all IRES elements use the same mechanism to interact with the translational machinery?

RNAâ€“protein interaction assays have been exploited extensively, with cellular extracts as well as purified proteins, to study the mechanism of ribosome recruitment by viral IRES elements. However, binding per se does not necessarily mean a functional requirement for IRES activity. A correlation between lack of activity and absence of binding should be followed by the restoration of binding upon gain of IRES function. In recent years, it has become increasingly clear that eIFs play an essential role in IRES-dependent initiation. According to their functional requirements for RNAâ€“protein interactions (Table 2), viral IRES elements can be separated into different categories, which are discussed below.

eIF4G-dependent IRES: encephalomyocarditis virus (EMCV) and FMDV

The canonical initiation factors eIF4A, eIF4G, eIF2 and eIF3 were reported to be required for 48S complex formation in a reconstituted 40S ribosome-binding assay with the EMCV IRES (Pestova *et al.*, 1996; Kolupaeva *et al.*, 1998). This assay reveals the minimal requirements for assembly of the initiation complex *in vitro*.

A mutational analysis in conjunction with UV cross-linking, immunoprecipitation, Western blot and competition assays led to the identification of a structural region in the base of domain 4 as an essential component of the FMDV IRES involved in binding to eIF4G (LÃ³pez de Quinto & MartÃ-nez-Salas, 2000) (see Fig. 2 A). In agreement with this observation, eIF4G interacts with the related EMCV IRES in a similar position, as shown by toeprint as well as footprint analysis (Kolupaeva *et al.*, 1998). Binding of eIF4G to a transcript encoding FMDV domain 4 alone was as effective as binding to the full-length IRES, suggesting strongly that no additional sites for recognition of this protein in the FMDV IRES are required. Furthermore, the C-terminal region of the proteolytically processed form of eIF4G (eIF4G-Ct), present in cells transfected with the FMDV Lb protease, binds as efficiently to the FMDV IRES as does the unprocessed protein (LÃ³pez de Quinto & MartÃ-nez-Salas, 2000). The strong correlation found between eIF4Gâ€“RNA interaction and IRES activity in transfected cells demonstrates that eIF4G binding is an essential step in recruitment of the translational machinery *in vivo*.

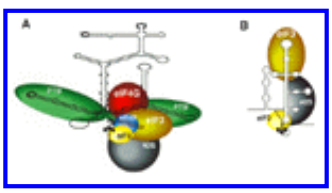

Fig. 2. Functional RNAâ€“protein interactions in the FMDV and HCV IRES elements. (A) FMDV interactions. PTB-binding sites are taken from Luz & Beck (1991) and Kolupaeva *et al.* (1996). The interaction sites of eIF4G and eIF4B are taken from LÃ³pez de Quinto & MartÃ-nez-Salas (2000). Thick lines correspond to regions essential for IRES activity (Luz & Beck, 1991; MartÃ-nez-Salas *et al.*, 1996; LÃ³pez de Quinto & MartÃ-nez-Salas, 1997, 2000). (B) HCV interactions. Binding sites of eIF3 and the 40S ribosome subunit are described in Buratti *et al.* (1998), Sizova *et al.* (1998) and Kolupaeva *et al.* (2000).

The C-terminal region of eIF4G that interacts with the IRES contains the binding sites for eIF3 and eIF4A (Gingras *et al.*, 1999). Accordingly, eIF4A stimulates binding of the central part of eIF4GI (aa 746â€“949) to the EMCV and FMDV IRES elements (Lomakin *et al.*, 2000; Pilipenko *et al.*, 2000). Although eIF4GI contains two non-canonical RNA-binding motifs in this central region (Gingras *et al.*, 1999), it is not yet known which residues in eIF4G are responsible for IRES binding. Consistent with the interaction of eIF4G-Ct with picornavirus IRES elements, internal initiation of translation promoted by these elements is efficient under conditions of eIF4G cleavage (Ohlmann *et al.*, 1996). However, the hepatitis A virus IRES requires intact eIF4G (Borman & Kean, 1997).

The initiation factor eIF4B stimulates 48S complex formation on EMCV (Pestova *et al.*, 1996) and also binds to sequences within the 3Â´ end of the FMDV IRES (Meyer *et al.*, 1995). However, in contrast to eIF4G, eIF4B binding to the FMDV IRES requires sequences within domain 5 (LÃ³pez de Quinto & MartÃ-nez-Salas, 2000), suggesting that both eIFs interact with the 3Â´ region of the IRES but do so by using different residues (Fig. 2 A). Although strong eIF4Bâ€“RNA binding has been shown, the biological significance of this interaction in IRES-dependent initiation has yet to be resolved.

Several non-canonical initiation factors have been shown to interact functionally with picornavirus IRES elements (Table 2). In contrast to Theiler's murine encephalitis virus and EMCV, the FMDV IRES requires both the polypyrimidine tract-binding protein (PTB) (Kolupaeva *et al.*, 1996; Niepmann *et al.*, 1997) and the proliferation-associated factor $ITAF_{45}$ for 48S complex formation *in vitro* (Pilipenko *et al.*, 2000). Therefore, even closely related IRES elements that share secondary structure and primary sequence in essential regions behave differently in terms of functional RNAâ€“protein associations.

Table 2. Functional RNAâ€“protein interactions in viral IRES elements

IRES	Translation initiation factors	*Trans*-acting factors
EMCV	eIF4G-Ct, eIF4A, eIF3, eIF2	PTB*
FMDV	eIF4G-Ct, eIF4A, eIF3, eIF2	PTB, $ITAF_{45}$
Poliovirus	NK	PTB, PCBP2, La, unr
Rhinovirus	NK	PTB, PCBP2, La, unr
HCV	eIF3, eIF2	PTB, PCBP2, La
CrPV	None	NK

* Conditional (Kaminski & Jackson, 1998).
NK, Not known.

The other group of IRES elements in the picornaviruses (poliovirus and rhinovirus) interacts with La, PCBP2 (hnRNP E2), unr and PTB (hnRNP I) (Table 2) (Meerovitch *et al.*, 1993; Blyn *et al.*, 1997; Gamarnik & Andino, 1997; Hunt *et al.*, 1999; Walter *et al.*, 1999). Most of these are cellular proteins with previously assigned functions. Additionally, specific stimulation of poliovirus and rhinovirus IRES activity is exerted by 2A and Lb picornavirus proteases (Ziegler *et al.*, 1995; Ohlmann *et al.*, 1996) by as yet unknown mechanisms.

Some of the factors mentioned above (PTB, $ITAF_{45}$ and PCBP2) contain several RNA-binding motifs and display multiple interactions with the IRES molecule and their capacity to dimerize has been described (Blyn *et al.*, 1997; Conte *et al.*, 2000; Gamarnik & Andino, 2000; Kim *et al.*, 2000; Pilipenko *et al.*, 2000). Hence, it is likely that

these proteins act as RNA chaperones, directing or stabilizing the tertiary folding of the RNA.

eIF4G-independent, eIF2-dependent IRES elements: HCV and CSFV

The results of toeprinting analysis indicate that 48S initiation complex formation driven by the HCV and CSFV IRES elements requires only eIF2â€“GTP/Met-tRNA$_i$, eIF3 and 40S subunits (Pestova *et al.*, 1998; see Table 2). The binding site of eIF3 has been mapped to residues in stemâ€“loop IIIb (Fig. 2 B) (Buratti *et al.*, 1998; Sizova *et al.*, 1998). The 40S ribosome subunit seems to interact at multiple sites, including sequences in loop IIId, the helix between IIIc and IIId and residues flanking the pseudoknot as well as the initiator AUG (Kolupaeva *et al.*, 2000). To date, HCV is the only IRES for which a ribosomal protein, S9, has been shown to interact with IRES sequences (Fukushi *et al.*, 1999). Recently, eIF2B and eIF2 have been identified as cofactors of HCV IRES-mediated translation (Kruger *et al.*, 2000).

In support of the biological relevance of the interactions mentioned above, mutation of the regions that constitute the binding site for these proteins is detrimental to IRES activity (Honda *et al.*, 1996; Jubin *et al.*, 2000). As in the case of the picornaviruses, the HCV IRES also binds *trans*-acting factors other than the canonical eIFs (Table 2) (Ali & Siddiqui, 1997; Kamoshita *et al.*, 1997; Hahm *et al.*, 1998; SpÃ¥ngberg & Schwartz, 1999).

HCV IRES-dependent initiation resembles the mechanism used by the Shineâ€“Dalgarno region in prokaryotic mRNAs (Pestova *et al.*, 1998; reviewed in Jackson, 2000). It was proposed long ago that the IRESâ€“ribosome interaction may take place by base-pair complementarity between a purine-rich sequence close to the 3Â´ end of the 18S rRNA and the conserved polypyrimidine tract present in the picornavirus IRES (Beck *et al.*, 1983; Pestova *et al.*, 1991). However, this possibility has never been documented experimentally.

eIF2-independent IRES: cricket paralysis virus (CrPV)

It has been shown that the intergenic region of CrPV has IRES activity when tested in bicistronic expression vectors (Wilson *et al.*, 2000 *b*). Surprisingly, toeprinting assays carried out in the presence of drugs that interfere with AUG recognition by the 40Sâ€“eIF2â€“GTP/Met-tRNA$_i$ complex demonstrated that this peculiar IRES recruits 40S ribosomes *in vitro* in the absence of eIFs (Wilson *et al.*, 2000 *a*; see Table 2). This is indicative of a molecular mimicry mechanism, by which the IRES itself substitutes functionally for the role played by the initiator Met-tRNA in the eIF2â€“GTP ternary complex (Wilson *et al.*, 2000 *a*). In agreement with this, the IRES present in the closely related *Plautia stali* intestinal virus (PSIV) is also able to direct protein synthesis initiation at a CAA codon in the absence of eIF2, Met-tRNA or GTP (Sasaki & Nakashima, 2000).

Modular organization of IRES elements: distribution of functions in structural domains

Is a core structural motif required for basal IRES activity? An IRES could be envisaged as a regulatory element having modular organization, in which the activity of a core region, able to interact with the translational machinery, is modulated, either stimulated or repressed, by other IRES subunits. This model theoretically could apply to EMCV and FMDV, where the domains located at the 3Â´ end of the IRES (Jâ€“K or 4â€“5, respectively) (Fig. 2 A) mediate the interaction with eIFs required for 48S initiation complex formation *in vitro* (Kolupaeva *et al.*, 1998; Pilipenko *et al.*, 2000) and establish RNAâ€“eIF4G interactions, which are essential for IRES activity *in vivo* (LÃ³pez de Quinto & MartÃ-nez-Salas, 2000). Assuming that this hypothetical model is true, basal IRES activity should be detected when this region alone is used to promote internal initiation. To date, there is no experimental evidence to indicate that this region is active by itself in promoting internal initiation *in vivo*.

In contrast, there is extensive evidence in support of the requirement for the whole length of the picornavirus IRES for

detecting full activity. Several point substitutions, outside domains 4â€“5, abolish IRES activity completely by altering essential motifs that are candidates to mediate RNA folding (LÃ³pez de Quinto & MartÃ-nez-Salas, 1997; Robertson *et al.*, 1999). Furthermore, precise deletions removing each of the five predicted stemâ€“loops of FMDV were shown to reduce IRES activity (Drew & Belsham, 1994). Hence, IRESâ€“eIF4G binding (achieved by domain 4 in FMDV or domains Jâ€“K in EMCV) is necessary, but not sufficient, for fully efficient internal initiation.

Thus, we favour an alternative model of RNA functional compartmentalization, which takes into account the distribution of functions among the different regions of the IRES molecule. Hence, whereas the main function of domains 4â€“5, in the 3Â´ region of the IRES, is to interact with eIFs (Fig. 2 A), the 5Â´ and central regions (domains 1â€“2 and 3) are involved in the organization of the IRES architecture (Fig. 1), directing intramolecular RNAâ€“RNA interactions (Ramos & MartÃ-nez-Salas, 1999). In agreement with the latter model, the PTB protein that seems to act as a RNA chaperone has its main binding site in the 5Â´ end of the IRES, but it also interacts with sequences in the 3Â´ end (Luz & Beck, 1991; Kolupaeva *et al.*, 1996).

Cellular IRES elements: specific stimulation

A characteristic feature of cellular mRNAs that contain IRES elements is that they are also translated in a cap-dependent manner, although quite inefficiently, often leading to the production of a different version of the protein (Huez *et al.*, 1998; Henis-Korenblit *et al.*, 2000; Cornelis *et al.*, 2000). Hence, cellular IRES elements represent a mechanism of gene expression regulation that is functional during nuclear inactivity, as in the case of homeotic genes (Oh *et al.*, 1992), and responds quickly to acute stress conditions.

It is known that several cellular IRES elements are activated by physiological stimuli (Table 3) including, among others, hypoxia, vascular lesions, serum deprivation, γ-irradiation, as well as factors that induce apoptosis, growth arrest and angiogenesis (Huez *et al.*, 1998; Stein *et al.*, 1998; Holcik *et al.*, 2000; Stoneley *et al.*, 2000 *a*). Activation of IRES elements present in the ornithine decarboxylase and p58PITSLRE protein kinase mRNAs is observed during specific phases of the cell cycle or in a proliferation-dependent manner (Cornelis *et al.*, 2000; Pyronnet *et al.*, 2000). On the other hand, tissue-specific regulation of fibroblast growth factor 2 (FGF-2) (Creancier *et al.*, 2000), c-myc (Stoneley *et al.*, 2000 *b*) or platelet-derived growth factor B (PDGF B/c-sis) (Bernstein *et al.*, 1997; Sella *et al.*, 1999) is likely related to the involvement of IRES elements in the fine-tuning of gene expression. However, the activating factors have not yet been clearly established, and it is not known whether different IRES elements share activator molecules.

Table 3. RNAâ€“protein interactions in cellular IRES elements

IRES	*Trans*-acting factors	Stimulatory conditions
DAP5	DAP5	Apoptosis
XIAP	hnRNP C, La	Apoptosis, γ-irradiation
Apaf-1	PTB, unr	Apoptosis
c-Myc	PTB, IRP, unr, DAP5	Apoptosis, Bloom syndrome
VEGF	PTB	Vascular lesions, hypoxia
PDGF2	hnRNP C	Megakaryocytic differentiation
Antennapedia	NK	Third-instar larvae

NK, Not known.

RNAâ€“protein interaction studies conducted on cellular IRES elements have identified a number of proteins (Table 3). With the exception of the apoptotic translation-initiation factor DAP5, none of the currently known cellular IRES-binding proteins is a canonical eIF and in most cases (hnRNP C, PTB, PCBP2, La) they have a role in nuclear RNA metabolism (Sella *et al.*, 1999; Holcik & Korneluk, 2000; Stoneley *et al.*, 2000 *b*). In contrast to viral IRES elements, several cellular IRES elements function only when expressed from within the nucleus (Stoneley *et al.*, 2000 *b*), suggesting that the nuclear compartment tags the RNA, although the nature of this tagging is unknown.

It remains to be elucidated whether or not cellular IRES elements require canonical eIFs to initiate translation. As the studies carried out are still very limited, it is too early to include these IRES elements in any of the categories defined above for viral IRES elements. In one example, complementarity of a 9 nt repeat in the Gtx homeodomain protein mRNA and the 18S rRNA has been suggested to direct RNAâ€“RNA interaction between the IRES and the rRNA (Chappell *et al.*, 2000 *a*).

Start-codon recognition in IRES-dependent initiation

Discrimination of the authentic initiation site is achieved by both the IRES element and functional sequences surrounding the AUG codon (LÃ³pez de Quinto & MartÃ-nez-Salas, 1998; Ohlmann & Jackson, 1999; reviewed in MartÃ-nez-Salas, 1999; Belsham & Jackson, 2000). The parameters that influence initiation site recognition in cellular IRES elements are as yet poorly characterized.

The initiation site in aphthovirus RNAs is of particular interest. In contrast to poliovirus and EMCV, which use one AUG codon to start translation (Nicholson *et al.*, 1991; Hellen *et al.*, 1994; Kaminski *et al.*, 1994), FMDV translation initiation occurs at two in-frame AUG codons separated by 84 nt, with the second being used most frequently (Cao *et al.*, 1995; LÃ³pez de Quinto & MartÃ-nez-Salas, 1999; reviewed in Belsham & Jackson, 2000). Studies conducted recently on equine rhinitis A virus, another member of the genus *Aphthovirus*, that also contains two initiator AUGs suggested that the second AUG is not reached by leaky scanning (Hinton *et al.*, 2000). Hence, the experimental evidence to explain aphthovirus start codon selection is compatible with two possibilities. One is the existence of a unique ribosome-loading site in front of the first AUG, followed by transfer to the vicinity of the second AUG. The second possibility is that two ribosome-loading sites exist in the FMDV RNA. In both of these models, ribosomes initiate translation at the second site in the vast majority of the viral RNAs (LÃ³pez de Quinto & MartÃ-nez-Salas, 1999).

Synergism between the IRES and 3Â´ mRNA regions

Cap-dependent translation initiation is strongly enhanced by poly(A) tails (Gallie, 1991; reviewed in Sachs, 2000). This synergism is due to the physical interaction of PABP with eIF4G which, in turn, respectively tether the ends of the mRNA through their specific recognition of the poly(A) tail and the capâ€“eIF4E complex (Tarun & Sachs, 1996; Preiss & Hentze, 1998).

The evidence for RNA circularization during IRES-dependent initiation is still limited. A small enhancement of IRES-driven translation by poly(A) tails *in vitro* has been reported recently (Michel *et al.*, 2000). However, the PABPâ€“eIF4Gâ€“RNA interaction is disrupted in picornavirus-infected cells by the action of 2A or Lb proteases. Although eIF4G-Ct supports IRES-dependent translation and binds efficiently to the IRES (Kolupaeva *et al.*, 1998; LÃ³pez de Quinto & MartÃ-nez-Salas, 2000), the binding motif for PABP is retained in the N-terminal fragment of the processed protein (Gingras *et al.*, 1999). PABP, eIF4G and eIF4B are also cleaved during apoptosis (reviewed in Clemens *et al.*, 2000), another circumstance in which IRES-dependent initiation is induced. Hence, the stimulation observed *in vitro*

could not be extrapolated to living cells unless the proteolytic fragments are able to interact with each other or there are other proteins involved in such an interaction.

In agreement with the notion that interactions among hnRNP E2, I, K and L, in concert with their RNA-binding preferences, could mediate interactions between the IRES and the 3Â´ end of the mRNA, proteinâ€“proteinâ€“hnRNP interactions have recently been found (Kim *et al.*, 2000). Accordingly, PTB, which binds both the HCV 3Â´ end and the IRES, has been proposed to stimulate the HCV IRES (Hahm *et al.*, 1998; Ito *et al.*, 1998). Other possibilities to close the circle could be the interactions eIF4Aâ€“PAIPâ€“PABP (Craig *et al.*, 1998), eIF4Bâ€“PABP (Le *et al.*, 2000) or PCBPâ€“PABP (Wang *et al.*, 1999 *a*), but none of these has yet been reported to stimulate IRES activity.

Concluding remarks

The RNA that constitutes the IRES plays a pivotal role in determining its activity. The IRES seems to substitute functionally for one (eIF4E) or more (eIF4F, eIF3 and eIF2) components of the initiation machinery necessary for cap-dependent initiation.

At present, there are several possibilities to account for the differences in the mechanism of IRES-driven protein synthesis. One is that it may be a fail-safe strategy that has evolved from, or co-evolved with, cap-dependent initiation that allows synthesis of proteins required under stress conditions. On the other hand, the IRES may be reminiscent of the Shineâ€“Dalgarno sequences that govern translation initiation in prokaryotic cells. In the case of the picornavirus IRES, the features shared with cap-dependent initiation in terms of requirements for eIFs favour the first possibility. With the exception of eIF4E, the rest of the eIFs are required, and non-canonical eIFs exert a regulatory role on IRES activity. However, the mechanism used by the HCV IRES favours the second explanation.

The diversity of IRES sequences, in terms of length, primary sequence and structural requirements, together with the variety of *trans*-acting proteins found in the IRES elements described so far, suggests that they have probably appeared at different points in evolution, being positively selected for by their ability to interact with factors that are essential for recruitment of ribosomal subunits. On the other hand, the differences found in the molecular mechanisms used by IRES elements to recruit the translational machinery indicate that internal initiation is not simply an alternative mechanism, rather it represents a fail-safe strategy to ensure the synthesis of certain proteins under physiological stress conditions.

In view of the rapidly increasing amount of information available on IRES structural organization and their exploitation of particular functional interactions to recruit ribosomes internally, we should look forward to an exciting future in understanding fully the biological relevance of IRES elements.

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References

Ali, N. & Siddiqui, A. (1997). The La antigen binds 5Â´ noncoding region of the hepatitis C virus RNA in the context of the initiator AUG codon and stimulates internal ribosome entry site-mediated translation. *Proceedings of the National Academy of Sciences, USA* **94**, 2249â€“2254.

Allen, E., Wang, S. & Miller, W. A. (1999). Barley yellow dwarf virus RNA requires a cap-independent translation sequence because it lacks a 5Â´ cap. *Virology* **253**, 139â€“144.

Andino, R., Boddeker, N., Silvera, D. & Gamarnik, A. V. (1999). Intracellular determinants of picornavirus replication.

Trends in Microbiology **7**, 76â€“82.

Beck, E., Forss, S., Strebel, K., Cattaneo, R. & Feil, G. (1983). Structure of the FMDV translation initiation site and of the structural proteins. *Nucleic Acids Research* **11**, 7873â€“7885.

Belsham, G. J. & Jackson, R. J. (2000). Translation initiation on picornavirus RNA. In *Translational Control of Gene Expression*, pp. 869â€“900. Edited by N. Sonenberg, J. W. B. Hershey & M. B. Mathews. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Berlioz, C. & Darlix, J. L. (1995). An internal ribosomal entry mechanism promotes translation of murine leukemia virus gag polyprotein precursors. *Journal of Virology* **69**, 2214â€“2222.

Bernstein, J., Sella, O., Le, S. Y. & Elroy-Stein, O. (1997). PDGF2/c-sis mRNA leader contains a differentiation-linked internal ribosomal entry site (D-IRES). *Journal of Biological Chemistry* **272**, 9356â€“9362.

Blyn, L. B., Towner, J. S., Semler, B. L. & Ehrenfeld, E. (1997). Requirement of poly(rC) binding protein 2 for translation of poliovirus RNA. *Journal of Virology* **71**, 6243â€“6246.

Bolten, R., Egger, D., Gosert, R., Schaub, G., Landmann, L. & Bienz, K. (1998). Intracellular localization of poliovirus plus- and minus-strand RNA visualized by strand-specific fluorescent *in situ* hybridization. *Journal of Virology* **72**, 8578â€“8585.

Borman, A. M. & Kean, K. M. (1997). Intact eukaryotic initiation factor 4G is required for hepatitis A virus internal initiation of translation. *Virology* **237**, 129â€“136.

Borman, A. M., Deliat, F. G. & Kean, K. M. (1994). Sequences within the poliovirus internal ribosome entry segment control viral RNA synthesis. *EMBO Journal* **13**, 3149â€“3157.

Buratti, E., Tisminetzky, S., Zotti, M. & Baralle, F. E. (1998). Functional analysis of the interaction between HCV 5Â´UTR and putative subunits of eukaryotic translation initiation factor eIF3. *Nucleic Acids Research* **26**, 3179â€“3187.

Cao, X., Bergman, I. E., FÃ¼llkrug, R. & Beck, E. (1995). Functional analysis of the two alternative translation initiation sites of foot-and-mouth disease virus. *Journal of Virology* **69**, 560â€“563.

Carrasco, L. (1995). Modification of membrane permeability by animal viruses. *Advances in Virus Research* **45**, 61â€“112.

Carter, M. S., Kuhn, K. M. & Sarnow, P. (2000). Cellular internal ribosome entry site elements and the use of cDNA microarrays in their investigation. In *Translational Control of Gene Expression*, pp. 615â€“636. Edited by N. Sonenberg, J. W. B. Hershey & M. B. Mathews. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Chappell, S. A., Edelman, G. M. & Mauro, V. P. (2000 *a*). A 9-nt segment of a cellular mRNA can function as an internal ribosome entry site (IRES) and when present in linked multiple copies greatly enhances IRES activity. *Proceedings of the National Academy of Sciences, USA* **97**, 1536â€“1541.

Chappell, S. A., LeQuesne, J. P., Paulin, F. E., deSchoolmeester, M. L., Stoneley, M., Soutar, R. L., Ralston, S. H., Helfrich, M. H. & Willis, A. E. (2000 *b*). A mutation in the c-myc-IRES leads to enhanced internal ribosome entry in multiple myeloma: a novel mechanism of oncogene de-regulation. *Oncogene* **19**, 4437â€“4440.

Clemens, M. J., Bushell, M., Jeffrey, I. W., Pain, V. M. & Morley, S. J. (2000). Translation initiation factor modifications and the regulation of protein synthesis in apoptotic cells. *Cell Death and Differentiation* **7**, 603â€“615.

Coldwell, M. J., Mitchell, S. A., Stoneley, M., MacFarlane, M. & Willis, A. E. (2000). Initiation of Apaf-1 translation by internal ribosome entry. *Oncogene* **19**, 899â€“905.

Collier, A. J., Tang, S. & Elliott, R. M. (1998). Translation efficiencies of the 5Â´ untranslated region from representatives of the six major genotypes of hepatitis C virus using a novel bicistronic reporter assay system. *Journal of General Virology* **79**, 2359â€“2366.

Conte, M. R., Grune, T., Ghuman, J., Kelly, G., Ladas, A., Matthews, S. & Curry, S. (2000). Structure of tandem RNA recognition motifs from polypyrimidine tract binding protein reveals novel features of the RRM fold. *EMBO Journal* **19**, 3132â€“3141.

Cornelis, S., Bruynooghe, Y., Denecker, G., Van Huffel, S., Tinton, S. & Beyaert, R. (2000). Identification and characterization of a novel cell cycle-regulated internal ribosome entry site. *Molecular Cell* **5**, 597â€“605.

Craig, A. W. B., Haghighat, A., Yu, A. T. K. & Sonenberg, N. (1998). Interaction of polyadenylate-binding protein with the eIF4G homologue PAIP enhances translation. *Nature* **392**, 520â€“523.

Creancier, L., Morello, D., Mercier, P. & Prats, A. C. (2000). Fibroblast growth factor 2 internal ribosome entry site (IRES) activity *ex vivo* and in transgenic mice reveals a stringent tissue-specific regulation. *Journal of Cell Biology* **150**, 275â€“281.

Cuesta, R., Laroia, G. & Schneider, R. J. (2000). Chaperone hsp27 inhibits translation during heat shock by binding eIF4G and facilitating dissociation of cap-initiation complexes. *Genes & Development* **14**, 1460â€“1470.

Drew, J. & Belsham, G. J. (1994). *trans* complementation by RNA of defective foot-and-mouth disease virus internal ribosome entry site elements. *Journal of Virology* **68**, 697â€“703.

Fukushi, S., Okada, M., Kageyama, T., Hoshino, F. B. & Katayama, K. (1999). Specific interaction of a 25-kilodalton cellular protein, a 40S ribosomal subunit protein, with the internal ribosome entry site of hepatitis C virus genome. *Virus Genes* **19**, 153â€“161.

Gallie, D. R. (1991). The cap and poly(A) tail function synergistically to regulate mRNA translational efficiency. *Genes & Development* **5**, 2108â€“2116.

Gamarnik, A. V. & Andino, R. (1997). Two functional complexes formed by KH domain containing proteins with the 5Â´ noncoding region of poliovirus RNA. *RNA* **3**, 882â€“892.

Gamarnik, A. V. & Andino, R. (2000). Interactions of viral protein 3CD and Poly(rC) binding protein with the 5Â´ untranslated region of the poliovirus genome. *Journal of Virology* **74**, 2219â€“2226.

Gingras, A.-C., Svitkin, Y., Belsham, G. J., Pause, A. & Sonenberg, N. (1996). Activation of the translational suppressor 4E-BP1 following infection with encephalomyocarditis virus and poliovirus. *Proceedings of the National Academy of Sciences, USA* **93**, 5578â€“5583.

Gingras, A. C., Raught, B. & Sonenberg, N. (1999). eIF4 initiation factors: effectors of mRNA recruitment to ribosomes and regulators of translation. *Annual Review of Biochemistry* **68**, 913â€“963.

Gradi, A., Svitkin, Y. V., Imataka, H. & Sonenberg, N. (1998). Proteolysis of human eukaryotic translation initiation factor eIF4GII, but not eIF4GI, coincides with the shutoff of host protein synthesis after poliovirus infection. *Proceedings of the National Academy of Sciences, USA* **95**, 11089â€“11094.

Hahm, B., Kim, Y. K., Kim, J. H., Kim, T. Y. & Jang, S. K. (1998). Heterogeneous nuclear ribonucleoprotein L interacts with the 3Â´ border of the internal ribosomal entry site of hepatitis C virus. *Journal of Virology* **72**, 8782â€“8788.

Haller, A. A. & Semler, B. L. (1992). Linker scanning mutagenesis of the internal ribosome entry site of poliovirus RNA. *Journal of Virology* **66**, 5075â€“5086.

Hellen, C. U. T., Pestova, T. V. & Wimmer, E. (1994). Effect of mutations downstream of the internal ribosome entry site on initiation of poliovirus protein synthesis. *Journal of Virology* **68**, 6312â€“6322.

Henis-Korenblit, S., Strumpf, N. L., Goldstaub, D. & Kimchi, A. (2000). A novel form of DAP5 protein accumulates in apoptotic cells as a result of caspase cleavage and internal ribosome entry site-mediated translation. *Molecular and Cellular Biology* **20**, 496â€“506.

Hershey, J. W. B. & Merrick, W. C. (2000). Pathway and mechanism of initiation of protein synthesis. In *Translational Control of Gene Expression*, pp. 33â€“88. Edited by N. Sonenberg, J. W. B. Hershey & M. B. Mathews. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Hinton, T. M., Li, F. & Crabb, B. S. (2000). Internal ribosomal entry site-mediated translation initiation in equine rhinitis A virus: similarities to and differences from that of foot-and-mouth disease virus. *Journal of Virology* **74**, 11708â€“11716.

Hoffman, M. A. & Palmenberg, A. C. (1996). Revertant analysis of Jâ€“K mutations in the encephalomyocarditis virus internal ribosome entry site detects an altered leader protein. *Journal of Virology* **70**, 6425â€“6430.

Holcik, M. & Korneluk, R. G. (2000). Functional characterization of the X-linked inhibitor of apoptosis (XIAP) internal ribosome entry site element: role of La autoantigen in XIAP translation. *Molecular and Cellular Biology* **20**, 4648â€“4657.

Holcik, M., Yeh, C., Korneluk, R. G. & Chow, T. (2000). Translational upregulation of X-linked inhibitor of apoptosis (XIAP) increases resistance to radiation induced cell death. *Oncogene* **19**, 4174â€“4177.

Honda, M., Brown, E. A. & Lemon, S. M. (1996). Stability of a stemâ€“loop involving the initiator AUG controls the efficiency of internal initiation of translation on hepatitis C virus RNA. *RNA* **2**, 955â€“968.

Honda, M., Beard, M. R., Ping, L.-H. & Lemon, S. M. (1999). A phylogenetically conserved stemâ€“loop structure at the 5Â´ border of the internal ribosome entry site of hepatitis C virus is required for cap-independent viral translation. *Journal of Virology* **73**, 1165â€“1174.

Huez, I., Creancier, L., Audigier, S., Gensac, M. C., Prats, A. C. & Prats, H. (1998). Two independent internal ribosome entry sites are involved in translation initiation of vascular endothelial growth factor mRNA. *Molecular and Cellular Biology* **18**, 6178â€“6190.

Hunt, S. L., Hsuan, J. J., Totty, N. & Jackson, R. J. (1999). unr, a cellular cytoplasmic RNA-binding protein with five cold-shock domains, is required for internal initiation of translation of human rhinovirus RNA. *Genes & Development* **13**, 437â€“448.

Iizuka, N., Najita, L., Franzusoff, A. & Sarnow, P. (1994). Cap-dependent and cap-independent translation by internal initiation of mRNAs in cell extracts prepared from *Saccharomyces cerevisiae*. *Molecular and Cellular Biology* **14**, 7322â€“7330.

Ishii, T., Shiroki, K., Iwai, A. & Nomoto, A. (1999). Identification of a new element for RNA replication within the internal ribosome entry site of poliovirus RNA. *Journal of General Virology* **80**, 917â€“920.

Ito, T., Tahara, S. M. & Lai, M. M. (1998). The 3Â´-untranslated region of hepatitis C virus RNA enhances translation from an internal ribosomal entry site. *Journal of Virology* **72**, 8789â€“8796.

Jackson, R. J. (2000). Comparative view of initiation site selection mechanisms. In *Translational Control of Gene Expression*, pp. 127â€“184. Edited by N. Sonenberg, J. W. B. Hershey & M. B. Mathews. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Jang, S. K. & Wimmer, E. (1990). Cap-independent translation of encephalomyocarditis virus RNA: structural elements of the internal ribosomal entry site and involvement of a cellular 57-kD RNA-binding protein. *Genes & Development* **4**, 1560â€“1572.

Jang, S. K., Krausslich, H. G., Nicklin, M. J., Duke, G. M., Palmenberg, A. C. & Wimmer, E. (1988). A segment of the 5Â´ nontranslated region of encephalomyocarditis virus RNA directs internal entry of ribosomes during *in vitro* translation. *Journal of Virology* **62**, 2636â€“2643.

Joachims, M., Van Breugel, P. C. & Lloyd, R. E. (1999). Cleavage of poly(A)-binding protein by enterovirus proteases concurrent with inhibition of translation *in vitro*. *Journal of Virology* **73**, 718â€“727.

Johannes, G. & Sarnow, P. (1998). Cap-independent polysomal association of natural mRNAs encoding c-myc, BiP, and eIF4G conferred by internal ribosome entry sites. *RNA* **4**, 1500â€“1513.

Johannes, G., Carter, M. S., Eisen, M. B., Brown, P. O. & Sarnow, P. (1999). Identification of eukaryotic mRNAs that are translated at reduced cap binding complex eIF4F concentrations using a cDNA microarray. *Proceedings of the National Academy of Sciences, USA* **96**, 13118â€“13123.

Johansen, L. K. & Morrow, C. D. (2000). Inherent instability of poliovirus genomes containing two internal ribosome entry site (IRES) elements supports a role for the IRES in encapsidation. *Journal of Virology* **74**, 8335â€“8342.

Jubin, R., Vantuno, N. E., Kieft, J. S., Murray, M. G., Doudna, J. A., Lau, J. Y. N. & Baroudy, B. M. (2000). Hepatitis C virus internal ribosome entry site (IRES) stem loop IIId contains a phylogenetically conserved GGG triplet essential for translation and IRES folding. *Journal of Virology* **74**, 10430â€“10437.

Kaminski, A. & Jackson, R. J. (1998). The polypyrimidine tract binding protein (PTB) requirement for internal initiation of translation of cardiovirus RNAs is conditional rather than absolute. *RNA* **4**, 626â€“638.

Kaminski, A., Belsham, G. J. & Jackson, R. J. (1994). Translation of encephalomyocarditis virus RNA: parameters influencing the selection of the internal initiation site. *EMBO Journal* **13**, 1673â€“1681.

Kamoshita, N., Tsukiyama-Kohara, K., Kohara, M. & Nomoto, A. (1997). Genetic analysis of internal ribosomal entry site on hepatitis C virus RNA: implication for involvement of the highly ordered structure and cell type-specific transacting factors. *Virology* **233**, 9â€“18.

Kerekatte, V., Keiper, B. D., Badorff, C., Cai, A., Knowlton, K. U. & Rhoads, R. E. (1999). Cleavage of poly(A)-binding protein by coxsackievirus 2A protease *in vitro* and *in vivo*: another mechanism for host protein synthesis shutoff? *Journal of Virology* **73**, 709â€“717.

Kieft, J. S., Zhou, K., Jubin, R., Murray, M. G., Lau, J. Y. & Doudna, J. A. (1999). The hepatitis C virus internal ribosome entry site adopts an ion-dependent tertiary fold. *Journal of Molecular Biology* **292**, 513â€“529.

Kim, J. H., Hahm, B., Kim, Y. K., Choi, M. & Jang, S. K. (2000). Proteinâ€“protein interaction among hnRNPs shuttling between nucleus and cytoplasm. *Journal of Molecular Biology* **298**, 395â€“405.

Kolupaeva, V. G., Hellen, C. U. T. & Shatsky, I. N. (1996). Structural analysis of the interaction of the pyrimidine tract-binding protein with the internal ribosomal entry site of encephalomyocarditis virus and foot-and-mouth disease virus RNAs. *RNA* **2**, 1199â€“1212.

Kolupaeva, V. G., Pestova, T. V., Hellen, C. U. T. & Shatsky, I. N. (1998). Translation eukaryotic initiation factor 4G recognizes a specific structural element within the internal ribosome entry site of encephalomyocarditis virus RNA. *Journal of Biological Chemistry* **273**, 18599â€“18604.

Kolupaeva, V. G., Pestova, T. V. & Hellen, C. U. T. (2000). An enzymatic footprinting analysis of the interaction of 40S ribosomal subunits with the internal ribosomal entry site of hepatitis C virus. *Journal of Virology* **74**, 6242â€“6250.

Kruger, M., Beger, C., Li, Q. X., Welch, P. J., Tritz, R., Leavitt, M., Barber, J. R. & Wong-Staal, F. (2000).

Identification of eIF2B and eIF2 as cofactors of hepatitis C virus internal ribosome entry site-mediated translation using a functional genomics approach. *Proceedings of the National Academy of Sciences, USA* **97**, 8566â€“8571.

KÃ¼hn, R., Luz, N. & Beck, E. (1990). Functional analysis of the internal translation initiation site of foot-and-mouth disease virus. *Journal of Virology* **64**, 4625â€“4631.

Le, H., Browning, K. S. & Gallie, D. R. (2000). The phosphorylation state of poly(A)-binding protein specifies its binding to poly(A) RNA and its interaction with eukaryotic initiation factor (eIF) 4F, eIFiso4F, and eIF4B. *Journal of Biological Chemistry* **275**, 17452â€“17462.

Lerat, H., Shimizu, Y. K. & Lemon, S. M. (2000). Cell type-specific enhancement of hepatitis C virus internal ribosome entry site-directed translation due to 5Â´ nontranslated region substitutions selected during passage of virus in lymphoblastoid cells. *Journal of Virology* **74**, 7024â€“7031.

Lomakin, I. B., Hellen, C. U. T. & Pestova, T. V. (2000). Physical association of eukaryotic initiation factor 4G (eIF4G) with eIF4A strongly enhances binding of eIF4G to the internal ribosomal entry site of encephalomyocarditis virus and is required for internal initiation of translation. *Molecular and Cellular Biology* **20**, 6019â€“6029.

LÃ³pez de Quinto, S. & MartÃ-nez-Salas, E. (1997). Conserved structural motifs located in distal loops of aphthovirus internal ribosome entry site domain 3 are required for internal initiation of translation. *Journal of Virology* **71**, 4171â€“4175.

LÃ³pez de Quinto, S. & MartÃ-nez-Salas, E. (1998). Parameters influencing translational efficiency in aphthovirus IRES-based bicistronic expression vectors. *Gene* **217**, 51â€“56.

LÃ³pez de Quinto, S. & MartÃ-nez-Salas, E. (1999). Involvement of the aphthovirus RNA region located between the two functional AUGs in start codon selection. *Virology* **255**, 324â€“336.

LÃ³pez de Quinto, S. & MartÃ-nez-Salas, E. (2000). Interaction of the eIF4G initiation factor with the aphthovirus IRES is essential for internal translation initiation *in vivo*. *RNA* **6**, 1380â€“1392.

Luz, N. & Beck, E. (1991). Interaction of a cellular 57-kilodalton protein with the internal translation initiation site of foot-and-mouth disease virus. *Journal of Virology* **65**, 6486â€“6494.

Macejak, D. G. & Sarnow, P. (1991). Internal initiation of translation mediated by the 5Â´ leader of a cellular mRNA. *Nature* **353**, 90â€“94.

MartÃ-nez-Salas, E. (1999). Internal ribosome entry site biology and its use in expression vectors. *Current Opinion in*

Biotechnology **10**, 458â€“464.

MartÃ-nez-Salas, E., SÃ¡iz, J. C., DÃ¡vila, M., Belsham, G. J. & Domingo, E. (1993). A single nucleotide substitution in the internal ribosome entry site of foot-and-mouth disease virus leads to enhanced cap-independent translation *in vivo*. *Journal of Virology* **67**, 3748â€“3755.

MartÃ-nez-Salas, E., Regalado, M. P. & Domingo, E. (1996). Identification of an essential region for internal initiation of translation in the aphthovirus internal ribosome entry site, and implications for viral evolution. *Journal of Virology* **70**, 992â€“998.

Meerovitch, K., Svitkin, Y. V., Lee, H. S., Lejbkowicz, F., Kenan, D. J., Chan, E. K., Agol, V. I., Keene, J. D. & Sonenberg, N. (1993). La autoantigen enhances and corrects aberrant translation of poliovirus RNA in reticulocyte lysate. *Journal of Virology* **67**, 3798â€“3807.

Meyer, K., Petersen, A., Niepmann, M. & Beck, E. (1995). Interaction of eukaryotic initiation factor eIF-4B with a picornavirus internal translation initiation site. *Journal of Virology* **69**, 2819â€“2824.

Michel, Y. M., Poncet, D., Piron, M., Kean, K. M. & Borman, A. M. (2000). Capâ€“poly(A) synergy in mammalian cell-free extracts: investigation of the requirements for poly(A)-mediated stimulation of translation initiation. *Journal of Biological Chemistry* **275**, 32268â€“32276.

Nicholson, R., Pelletier, J., Le, S. Y. & Sonenberg, N. (1991). Structural and functional analysis of the ribosome landing pad of poliovirus type 2: *in vivo* translation studies. *Journal of Virology* **65**, 5886â€“5894.

Niepel, M. & Gallie, D. R. (1999). Identification and characterization of the functional elements within the tobacco etch virus 5Â´ leader required for cap-independent translation. *Journal of Virology* **73**, 9080â€“9088.

Niepmann, M., Petersen, A., Meyer, K. & Beck, E. (1997). Functional involvement of polypyrimidine tract-binding protein in translation initiation complexes with the internal ribosome entry site of foot-and-mouth disease virus. *Journal of Virology* **71**, 8330â€“8339.

Odreman-Macchioli, F. E., Tisminetzky, S. G., Zotti, M., Baralle, F. E. & Buratti, E. (2000). Influence of correct secondary and tertiary RNA folding on the binding of cellular factors to the HCV IRES. *Nucleic Acids Research* **28**, 875â€“885.

Oh, S. K., Scott, M. P. & Sarnow, P. (1992). Homeotic gene Antennapedia mRNA contains 5Â´-noncoding sequences that confer translational initiation by internal ribosome binding. *Genes & Development* **6**, 1643â€“1653.

Ohlmann, T. & Jackson, R. J. (1999). The properties of chimeric picornavirus IRESes show that discrimination between internal translation initiation sites is influenced by the identity of the IRES and not just the context of the AUG codon. *RNA* **5**, 764â€“778.

Ohlmann, T., Rau, M., Pain, V. M. & Morley, S. J. (1996). The C-terminal domain of eukaryotic protein synthesis initiation factor (eIF) 4G is sufficient to support cap-independent translation in the absence of eIF4E. *EMBO Journal* **15**, 1371â€“1382.

Ohlmann, T., LÃ³pez-Lastra, M. & Darlix, J. L. (2000). An internal ribosome entry segment promotes translation of the simian immunodeficiency virus genomic RNA. *Journal of Biological Chemistry* **275**, 11899â€“11906.

Oumard, A., Hennecke, M., Hauser, H. & Nourbakhsh, M. (2000). Translation of NRF mRNA is mediated by highly efficient internal ribosome entry. *Molecular and Cellular Biology* **20**, 2755â€“2759.

Pelletier, J. & Sonenberg, N. (1988). Internal initiation of translation of eukaryotic mRNA directed by a sequence derived from poliovirus RNA. *Nature* **334**, 320â€“325.

Pestova, T. V., Hellen, C. U. T. & Wimmer, E. (1991). Translation of poliovirus RNA: role of an essential cis-acting oligopyrimidine element within the 5Â´ nontranslated region and involvement of a cellular 57-kilodalton protein. *Journal of Virology* **65**, 6194â€“6204.

Pestova, T. V., Hellen, C. U. T. & Shatsky, I. N. (1996). Canonical eukaryotic initiation factors determine initiation of translation by internal ribosomal entry. *Molecular and Cellular Biology* **16**, 6859â€“6869.

Pestova, T. V., Shatsky, I. N., Fletcher, S. P., Jackson, R. J. & Hellen, C. U. T. (1998). A prokaryotic-like mode of cytoplasmic eukaryotic ribosome binding to the initiation codon during internal translation initiation of hepatitis C and classical swine fever virus RNAs. *Genes & Development* **12**, 67â€“83.

Pilipenko, E. V., Gmyl, A. P., Maslova, S. V., Svitkin, Y. V., Sinyakov, A. N. & Agol, V. I. (1992). Prokaryotic-like cis elements in the cap-independent internal initiation of translation on picornavirus RNA. *Cell* **68**, 119â€“131.

Pilipenko, E. V., Pestova, T. V., Kolupaeva, V. G., Khitrina, E. V., Poperechnaya, A. N., Agol, V. I. & Hellen, C. U. T. (2000). A cell cycle-dependent protein serves as a template-specific translation initiation factor. *Genes & Development* **14**, 2028â€“2045.

Preiss, T. & Hentze, M. W. (1998). Dual function of the messenger RNA cap structure in poly(A)-tail-promoted translation in yeast. *Nature* **392**, 516â€“520.

Psaridi, L., Georgopoulou, U., Varaklioti, A. & Mavromara, P. (1999). Mutational analysis of a conserved tetraloop in the 5Â´ untranslated region of hepatitis C virus identifies a novel RNA element essential for the internal ribosome entry site function. *FEBS Letters* **453**, 49â€“53.

Pyronnet, S., Pradayrol, L. & Sonenberg, N. (2000). A cell cycle-dependent internal ribosome entry site. *Molecular Cell* **5**, 607â€“616.

Ramos, R. & MartÃ-nez-Salas, E. (1999). Long-range RNA interactions between structural domains of the aphthovirus internal ribosome entry site (IRES). *RNA* **5**, 1374â€“1383.

Reynolds, J. E., Kaminski, A., Kettinen, H. J., Grace, K., Clarke, B. E., Carroll, A. R., Rowlands, D. J. & Jackson, R. J. (1995). Unique features of internal initiation of hepatitis C virus RNA translation. *EMBO Journal* **14**, 6010â€“6020.

Roberts, L. O. & Belsham, G. J. (1997). Complementation of defective picornavirus internal ribosome entry site (IRES) elements by the coexpression of fragments of the IRES. *Virology* **227**, 53â€“62.

Robertson, M. E., Seamons, R. A. & Belsham, G. J. (1999). A selection system for functional internal ribosome entry site (IRES) elements: analysis of the requirement for a conserved GNRA tetraloop in the encephalomyocarditis virus IRES. *RNA* **5**, 1167â€“1179.

Sachs, A. (2000). Physical and functional interactions between the mRNA cap structure and the poly(A) tail. In *Translational Control of Gene Expression*, pp. 447â€“465. Edited by N. Sonenberg, J. W. B. Hershey & M. B. Mathews. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

SÃ¡iz, J. C., LÃ³pez de Quinto, S., Ibarrola, N., LÃ³pez-Labrador, F. X., SÃ¡nchez-Tapias, J. M., Rodes, J. & MartÃ-nez-Salas, E. (1999). Internal initiation of translation efficiency in different hepatitis C genotypes isolated from interferon treated patients. *Archives of Virology* **144**, 215â€“229.

Sasaki, J. & Nakashima, N. (1999). Translation initiation at the CUU codon is mediated by the internal ribosome entry site of an insect picorna-like virus *in vitro*. *Journal of Virology* **73**, 1219â€“1226.

Sasaki, J. & Nakashima, N. (2000). Methionine-independent initiation of translation in the capsid protein of an insect RNA virus. *Proceedings of the National Academy of Sciences, USA* **97**, 1512â€“1515.

Sella, O., Gerlitz, G., Le, S. Y. & Elroy-Stein, O. (1999). Differentiation-induced internal translation of c-sis mRNA: analysis of the cis elements and their differentiation-linked binding to the hnRNP C protein. *Molecular and Cellular Biology* **19**, 5429â€“5440.

Sizova, D. V., Kolupaeva, V. G., Pestova, T. V., Shatsky, I. N. & Hellen, C. U. T. (1998). Specific interaction of eukaryotic translation initiation factor 3 with the 5Â´ nontranslated regions of hepatitis C virus and classical swine fever virus RNAs. *Journal of Virology* **72**, 4775â€“4782.

Skulachev, M. V., Ivanov, P. A., Karpova, O. V., Korpela, T., Rodionova, N. P., Dorokhov, Yu. L. & Atabekov, J. G. (1999). Internal initiation of translation directed by the 5Â´-untranslated region of the tobamovirus subgenomic RNA I_2. *Virology* **263**, 139â€“154.

SpÃ¥ngberg, K. & Schwartz, S. (1999). Poly(C)-binding protein interacts with the hepatitis C virus 5Â´ untranslated region. *Journal of General Virology* **80**, 1371â€“1376.

Stein, I., Itin, A., Einat, P., Skaliter, R., Grossman, Z. & Keshet, E. (1998). Translation of vascular endothelial growth factor mRNA by internal ribosome entry: implications for translation under hypoxia. *Molecular and Cellular Biology* **18**, 3112â€“3119.

Stewart, S. R. & Semler, B. L. (1997). RNA determinants of picornavirus cap-independent translation initiation. *Seminars in Virology* **8**, 242â€“255.

Stoneley, M., Chappell, S. A., Jopling, C. L., Dickens, M., MacFarlane, M. & Willis, A. E. (2000 *a*). c-Myc protein synthesis is initiated from the internal ribosome entry segment during apoptosis. *Molecular and Cellular Biology* **20**, 1162â€“1169.

Stoneley, M., Subkhankulova, T., Le Quesne, J. P., Coldwell, M. J., Jopling, C. L., Belsham, G. J. & Willis, A. E. (2000 *b*). Analysis of the c-myc IRES; a potential role for cell-type specific trans-acting factors and the nuclear compartment. *Nucleic Acids Research* **28**, 687â€“694.

Tarun, S. Z. & Sachs, A. B. (1996). Association of the yeast poly(A) tail binding protein with translation initiation factor eIF-4G. *EMBO Journal* **15**, 7168â€“7177.

Thompson, S. R. & Sarnow, P. (2000). Regulation of host cell translation by viruses and effects on cell function. *Current Opinion in Microbiology* **3**, 366â€“370.

Venkatesan, A., Das, S. & Dasgupta, A. (1999). Structure and function of a small RNA that selectively inhibits internal ribosome entry site-mediated translation. *Nucleic Acids Research* **27**, 562â€“572.

Walter, B. L., Nguyen, J. H., Ehrenfeld, E. & Semler, B. L. (1999). Differential utilization of poly(rC) binding protein 2 in translation directed by picornavirus IRES elements. *RNA* **5**, 1570â€“1585.

Wang, C., Le, S-Y., Ali, N. & Siddiqui, A. (1995). An RNA pseudoknot is an essential structural element of the internal ribosome entry site located within the hepatitis C virus 5Â´ noncoding region. *RNA* **1**, 526â€“537.

Wang, S., Browning, K. S. & Miller, W. A. (1997). A viral sequence in the 3Â´-untranslated region mimics a 5Â´ cap in facilitating translation of uncapped mRNA. *EMBO Journal* **16**, 4107â€“4116.

Wang, Z., Day, N., Trifillis, P. & Kiledjian, M. (1999 *a*). An mRNA stability complex functions with poly(A)-binding protein to stabilize mRNA in vitro. *Molecular and Cellular Biology* **19**, 4552â€“4560.

Wang, S., Guo, L., Allen, E. & Miller, W. A. (1999 *b*). A potential mechanism for selective control of cap-independent translation by a viral RNA sequence *in cis* and *in trans*. *RNA* **5**, 728â€“738.

Wilson, J. E., Pestova, T. V., Hellen, C. U. T. & Sarnow, P. (2000 *a*). Initiation of protein synthesis from the A site of the ribosome. *Cell* **102**, 511â€“520.

Wilson, J. E., Powell, M. J., Hoover, S. E. & Sarnow, P. (2000 *b*). Naturally occurring dicistronic cricket paralysis virus RNA is regulated by two internal ribosome entry sites. *Molecular and Cellular Biology* **20**, 4990â€“4999.

Wu, B. & White, K. A. (1999). A primary determinant of cap-independent translation is located in the 3Â´-proximal region of the tomato bushy stunt virus genome. *Journal of Virology* **73**, 8982â€“8988.

Ziegler, E., Borman, A. M., Kirchweger, R., Skern, T. & Kean, K. M. (1995). Foot-and-mouth disease virus Lb proteinase can stimulate rhinovirus and enterovirus IRES-driven translation and cleave several proteins of cellular and viral origin. *Journal of Virology* **69**, 3465â€“3474.

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