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Cellular internal ribosome entry segments: structures, *trans*-acting factors and regulation of gene expression

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Initiation of translation in eukaryotic cells can occur by two distinct mechanisms, cap-dependent scanning and internal ribosome entry. The latter mechanism requires the formation of a complex RNA structural element termed an internal ribosome entry segment (IRES). IRESs are located in the 5′ untranslated region of the message, and in the presence of *trans*-acting factors allow the ribosome to be recruited to a site that is a considerable distance from the cap structure. Many cellular mRNAs have now been shown to contain IRESs and it is likely that up to 10% of all mRNAs have the capability to initiate translation by this mechanism. The majority of IRESs that have been identified thus far are found in mRNAs whose protein products are associated with the control of cell growth and cell death, including many growth factors, proto-oncogenes and proteins required for apoptosis. In this review, we discuss the cellular situations when IRESs are required, the *trans*-acting factors that are necessary for IRES function and deregulation of IRES-mediated translation in tumorigenesis.
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Introduction

Initiation of eukaryotic protein synthesis is an intricate process culminating in the assembly of an 80S ribosome-initiation complex at a start codon within the mRNA. For the majority of cellular mRNAs, the ribosome associates with the mRNA by virtue of the cap-structure, a 7-methyl-guanylic acid residue at the 5′ terminus. A cap-binding complex, known as eukaryotic initiation factor 4F (eIF4F), interacts with the cap-structure and through its association with eIF3 recruits the 40S ribosomal subunit to the mRNA (Gingras *et al*., 1999; Hershey and Merrick, 2000) (Figure 1). However some years ago, it emerged that an alternative mechanism of translation initiation exists in eukaryotic cells. A *cis*-element was identified in the 5′ leader sequence of the poliovirus and encephalomyocarditis virus (EMCV) RNA genomes that enables ribosomes to be recruited to the RNA without the aid of a cap-structure (Jang *et al*., 1988; Pelletier and Sonenberg, 1988; Jang *et al*., 1989). Internal ribosome entry segments or sites (IRESs), as they are now called, were subsequently found in the genomes of all known picornaviruses and in some other viruses. In this mechanism of translation initiation, known as internal initiation, the ribosome is recruited to a site within the IRES that is some considerable distance downstream of 5′ terminus of the RNA (Figure 1b) (Jackson, 2000).

Soon after the discovery of internal initiation, an IRES was identified in the 5′ untranslated region (5′ UTR) of a cellular mRNA (Macejak and Sarnow, 1991). This finding initiated the search for further cellular mRNAs that are translated by internal initiation and as a result there are now numerous examples of cellular IRESs in the literature (Table 1) (http:/www.rangueil.inserm.fr/iresdatabase/). It appears that genes involved in a diverse range of cellular activities, including proliferation, growth and apoptosis employ this alternative mechanism. Moreover, a recent study has shown that 3–5% of mRNAs remain associated with polyribosomes when cap-dependent translation initiation is inhibited in poliovirus-infected cells (Johannes *et al*., 1999). It is likely that many, although by no means all, of these mRNAs are translated by internal initiation. Hence, it has become clear that internal initiation is an important cellular mechanism and not just a specialized viral strategy. Furthermore, internal initiation has been implicated in the regulation of gene expression. Here, we discuss our current understanding of cellular IRESs.

Structure–function relationships in cellular IRESs

IRESs found in the genomes of viruses have a complex RNA secondary structure. Moreover, the structure assumed by these IRESs is phylogenetically conserved in viruses of the same species and subtype. The importance of secondary structure to IRES function is underscored by studies of the genetic drift in highly infectious viruses such as FMDV and HCV. It has been shown that sequence substitutions within the IRES are accompanied by compensatory mutations that act to maintain the RNA secondary structure. Furthermore,

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mutational analysis has identified structural domains and short sequence motifs located in apical loops and internal bulges that are vital to IRES function (Martinez-Salas *et al*., 2001). Details of how these IRES structures bring about ribosome recruitment are beginning to emerge. It seems that the IRES is a complex RNA scaffold that contains multiple sites for interaction with components of the translational apparatus (Figure 1b). In the case of the EMCV and FMDV IRESs, structural domains have been identified that interact with the initiation factors eIF4G and eIF4B (Kolupaeva *et al*., 1998; Lopez de Quinto and Martinez-Salas, 2000). Using a different strategy, the HCV and CSFV IRESs interact directly with the 40S ribosome subunit at multiple sites. A domain that interacts with eIF3 has also been identified in these two IRESs (Sizova *et al*.,1998; Kieft *et al*.,2002). The CrPV IRES can also interact directly with the 40S ribosomal subunit and in addition can assemble an 80S ribosome at its initiation codon without the aid of any initiation factors or an initiator tRNA (Wilson *et al*., 2000).

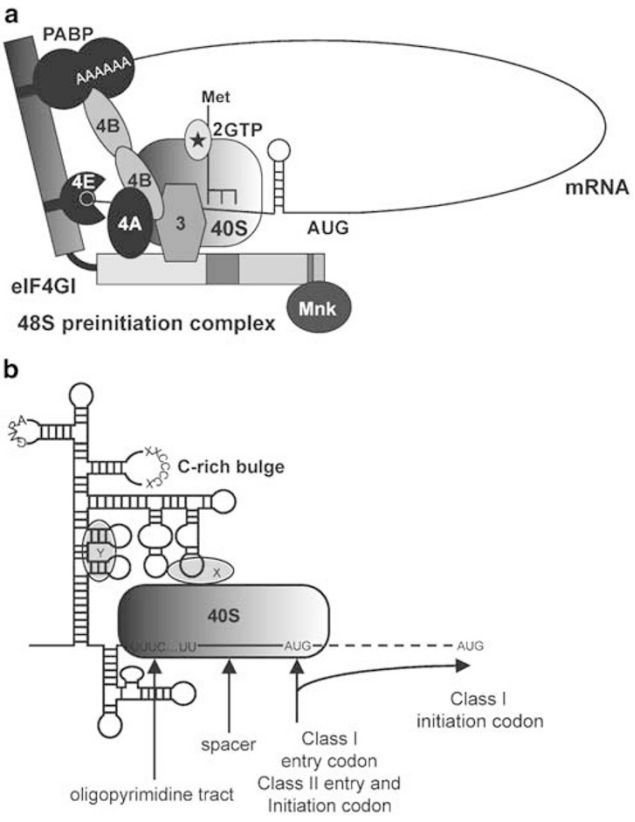

Figure 1 (**a**) Assembly of the 43S preinitiation complex onto the mRNA. EIF4G acts as a scaffold and binds to eIF4E (cap-binding protein), eIF4A (dead box helicase which is required to unwind secondary structure, the activity of this protein is stimulated by its interaction with eIF4B), eIF3 which in turn interacts with the ribosome and Mnk (Map-integrating kinase which phosphorylates eIF4E when bound in this complex). The interaction of poly A-binding protein (PABP) with eIF4G allow circularisation of the mRNA. (**b**) Schematic representation of a picornaviral IRES. Picornaviral messages are uncapped and recruit ribosomes directly to the initiation codon via an IRES. This binding is facilitated by primary sequences such as the polypyrimidine tract and secondary structural elements. Host encoded IRES-*trans*-acting factors (X and Y) may also be required to stabilize the structures or facilitate ribosome binding (adapted from Belsham and Jackson, 2000)

If there is a similar structure–function relationship for cellular IRESs? Many of the IRESs identified in cellular genes are GC-rich and therefore most likely have complex RNA secondary structures. However, thus far there is no obvious conservation of secondary structure between these IRESs. Currently structural models have been determined using chemical and enzymatic probing for only a handful of cellular IRESs (LeQuesne *et al*., 2001; Bonnal *et al*., 2003; Jopling *et al*., 2004; Mitchell *et al*., 2003; Yaman *et al*., 2003). To date no common structural features have been found, even when the IRESs of the closely related genes c-*myc* and L-*myc* are compared (Figure 2) (LeQuesne *et al*., 2001; Jopling *et al*., 2004).

Small deletions and point mutations in viral IRESs can have a profound effect on internal initiation. It is likely that the integrity of the intricate RNA scaffold is essential for the assembly of a competent 80S initiation complex. In contrast, deletions within many cellular IRESs rarely completely disable the element. These observations imply that the structure–function relationship is not as rigid in these cellular IRESs as in the viral IRESs. Furthermore in many instances, individual sections of cellular IRESs are able to promote internal initiation, albeit not as efficiently as the entire IRES (Yang and Sarnow, 1997; Huez *et al*., 1998; Stoneley *et al*., 1998; Coldwell *et al*., 2000; Chappell *et al*., 2000a; Miskimmins *et al*., 2001; Kullmann *et al*., 2002; Jopling *et al*., 2004). Collectively, this has led to the hypothesis

Table 1 Representative list of cellular IRESes identified to date and their cellular functions

Gene type	*Name*
Transcription factors	*Antennapedia*, *Ultrabithorax*, c-*myc*, N-*myc*, MYT2, AMLI/RUNXI, Gtx, c-Jun, Mnt, Nkx6.1, NRF, YAP1, Smad5, HIP-1alpha, Hairless
Stress response factors	XIAP, APC, Apaf-1, Bag-1, Bip/GRP78
Growth factors and growth receptors	FGF2, PDGF2/c-sis, VEGF-A, IGF-II, estrogen receptor α, IGF-1 receptor, Notch2
Translation and RNA processing factors	La, eIF4GI, TIF4631, DAP5/p97/NAT1
Cytoskeletal proteins	ARC, MAP2
Kinases and related	Pim1, p58/PITSLRE, α Cam kinase II, CDK inhibitor p27, PKCδ
Channels/transporters	KV-14, βF1-ATPase, Cat-1
Other	Bip, Connexin-43, Connexin-32, Cyr61, ODC, Dendrin, Neurogranin/RC3, NBS1, FMR1, Rbm3, NDST

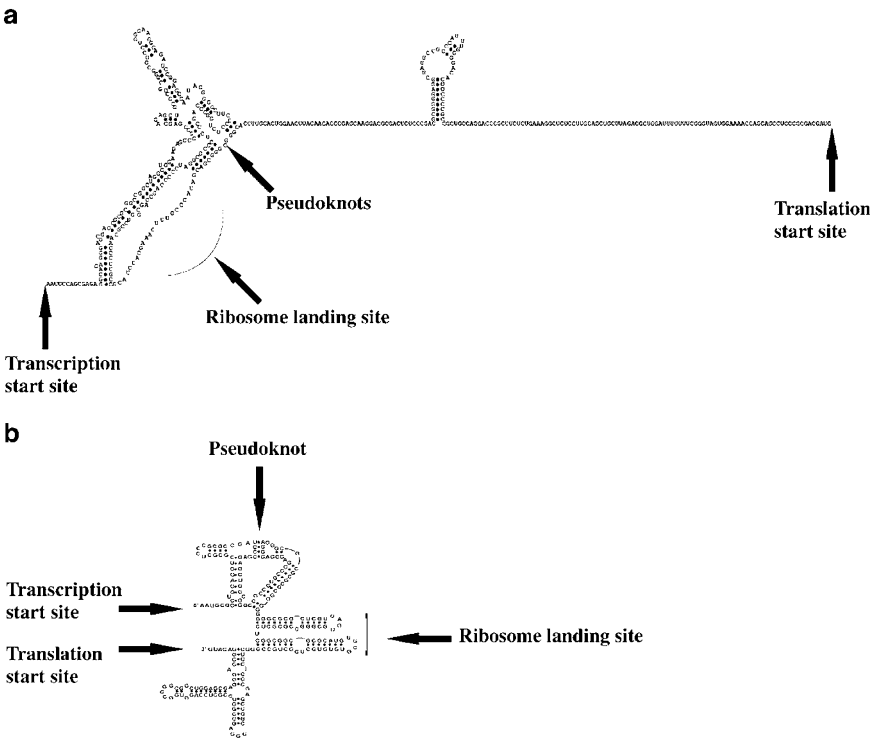

Figure 2 Secondary structural models of c-*myc* (**a**) and L-*myc* IRESs (**b**). c-*myc* or L-*myc* RNA was treated with chemicals (CMCT, kethoxal, DMS) to detect single-stranded regions or with RNase VI to detect double-stranded regions. This information was then used to add constraints to the m-fold algorithm to obtain the structural model shown. The c-*myc* IRES has a more open and flexible structure than the L-*myc* IRES. However, interestingly both IRES contain potential pseudoknots close to the region where the ribosome lands

that many cellular IRESs are composed of multiple short modules and it is the combined effect of these modules that promotes internal initiation.

Despite the aforementioned differences between viral and cellular IRES, mutational analysis of two cellular IRESs has provided evidence that structural modules are involved in cellular internal initiation. In contrast to viral IRESs, mutations or deletions introduced into a single structural motif have relatively little impact on c-*myc* IRES function. However, the IRES can be disabled by two mutations or deletions that disrupt separate structural regions (Le Quesne *et al*., 2001). Thus, we speculate that for this IRES a combination of structural modules act in concert to promote internal initiation. Furthermore, since in addition to internal initiation, c-*myc* protein synthesis can occur by cap-dependent initiation, the structure of the IRES may well be dynamic to accommodate the scanning ribosome (West *et al*., 1998; Stoneley *et al*., 2000b). RNA structure has also been implicated in the function of the cat-1 IRES. Disruption of RNA–RNA interactions between the 5′ and 3′ end of the IRES prevents inducible internal initiation, whereas the restoration of this structural feature re-establishes the activity of the inducible IRES (Yaman *et al*., 2003). Finally, RNA structure can also have a negative impact on cellular internal initiation. For both the c-*myc* and cat-1 IRESs, evidence has been presented that a pseudoknot can inhibit the activity of the IRES, and moreover in the cat-1 IRES this structural feature has been implicated in the regulation of cat-1 internal initiation (Le Quesne *et al*., 2001; Yaman *et al*., 2003).

Primary sequence elements that are implicated in internal initiation have been identified in the IRESs of *Gtx*, *Rbm3* and *fgf2* (Chappell *et al*., 2000a; Bonnal *et al*., 2003; Chappell and Mauro, 2003). Short-sequence modules found in the *Gtx* and *Rbm3* IRESs can promote internal initiation and have been shown to bind to the 40S ribosome in cell-free assays. Such interactions may stimulate the recruitment of the 40S subunit to the IRES. It has been proposed that pairing interactions may occur between these regions and the 18S rRNA. Importantly, the putative complementary regions of the 18S rRNA are known to be accessible. Although this is an interesting proposition it must be noted that there is no evidence that such interactions can occur when these modules are present in the context of the entire IRES (Chappell *et al*., 2000a; Chappell and Mauro, 2003). Clearly these short-sequence elements can contribute to internal initiation but precisely how they do so warrants further investigation. The situation is further complicated by the observation that the 9 nt *Gtx* element can function as a translation repressor when present in the 5′ UTR of a monocistronic mRNA (Hu *et al*., 1999).

Furthermore, an identical sequence also appears in the *fgf2* IRES and in this case appears to have no effect on internal initiation (Bonnal *et al*., 2003).

Thus the evidence to date suggests that cellular IRESs differ to some extent from their viral counterparts. Nevertheless, both structural features and short-sequence elements appear to be involved in ribosome recruitment in cellular IRESs. Exactly how these motifs combine to promote internal initiation remains to be determined.

Internal initiation *trans*-acting factors (ITAFs) and cellular IRESs

In addition to their requirements for eukaryotic initiation factors, the efficiency of some viral IRESs is augmented by noncanonical initiation factors known as ITAFs (Table 2) (Martinez-Salas *et al*., 2001). Although the mechanism of action of cellular IRESs is currently not understood, it has become clear that some of these elements require similar auxiliary factors to function. Specific functional RNA–protein interactions between cellular IRESs and known ITAFs have been identified (Holcik and Korneluk 2000b; Mitchell *et al*., 2001; Evans *et al*., 2003; Holcik *et al*., 2003; Pickering *et al*., 2003) and recently progress has been made towards unravelling the roles of some of these ITAFs.

The Apaf-1 IRES can form a specific RNA–protein complex with the ITAFs upstream of N-ras (UNR) and polypyrimidine tract–binding protein (PTB). In combination, these factors stimulate Apaf-1 internal initiation (Mitchell *et al*., 2001). The UNR-binding site is located in a structural domain and recognition of this site by UNR unwinds this region. In addition, a distal domain is also remodelled by UNR, which in turn promotes the interaction of PTB with the IRES. Together the conformational changes precipitated by these RNA-binding proteins aid the recruitment of the ribosome to the IRES. This is in part achieved through the disruption of RNA–RNA interactions in the vicinity of the ribosome entry window (Figure 3) (Mitchell *et al*., 2003). A similar mechanism has also been documented in the Bag-1 IRES. In this instance, the RNA chaperone activities of PTB and poly-(rC)-binding protein 1 (PCBP1) unwind a specific region of the IRES structure. As with the Apaf-1 IRES, this structural change occurs in the region to which the ribosome is recruited (Pickering *et al*., 2003; Pickering *et al*., 2004). Members of the poly-(rC)-binding protein family have also been shown to stimulate the activity of the c-*myc* IRES (Evans *et al*., 2003). Therefore, structural remodelling by the RNA chaperone activities of ITAFs such as PTB,

Table 2 IRES-*trans*-acting factors and the corresponding IRESs with which they interact

ITAF/trans-*acting factor*	*IRESes interacts with*
PTB (hnRNP1)	EMCV, FMDV, TMEV, PV1, HRV, HCV, HAV, human T-lymphotrophic virus type 1, Apaf-1, IGF-IR, BAG-1
N-PTB (neuronal PTB)	TMEV, Apaf-1
La	EMCV, HCV, PV1, human T-lymphotrophic virus type 1, HIV-1 (gag RNA), XIAP, Bip/Grp78, coxsackievirus B3
Unr	HRV, Apaf-1
ITAF45	FMDV
HnRNPE2 (PCBP2)	PV1, HRV, Coxsackievirus B3, c-*myc*
HnRNPE1 (PCBP1)	PV1, c-*myc*
HnRNPC1/C2	PDGF2/c-sis, XIAP, c-*myc*
HnRNPL	HCV
HnRNPK	c-*myc*
DAP5	DAP5, c-*myc*, Apaf-1, XIAP
GAPDH	HAV
Nucleolin	HRV, PV1
ELAV/Hu	p27
Ribosomal protein S9	HCV, CSFV
Ribosomal protein S5	HCV

Data from the IRES database at http://ifr31w3.toulouse.inserm.fr/IRESdatabase/

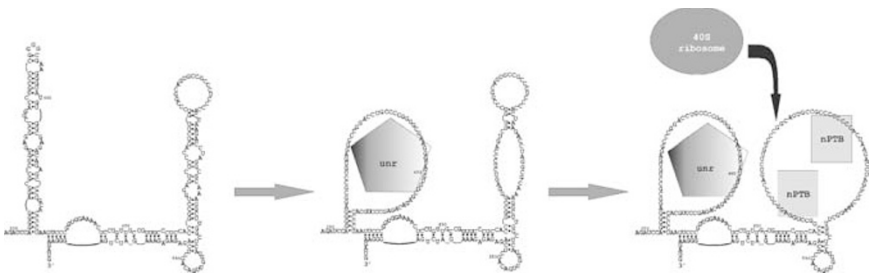

Figure 3 Model for binding of unr and nPTB to the Apaf-1 IRES. IRES *trans*-acting factors interact with IRESs to remodel the RNA into a structure that permits binding of the 40S ribosomal subunit (modified from Mitchell *et al*., 2003)

UNR and PCBP1 may prove to be a common theme in the mechanism of cellular IRESs. However, it should be noted that these factors do not affect all cellular IRESs. Moreover, PTB can function as a repressor of Bip internal initiation (Kim *et al*., 2000), suggesting that RNA chaperones may exert both positive and negative influences on IRESs. Since ITAFs such as UNR and PTB have multiple RNA recognition motifs they can interact with spatially distinct sites within an IRES, and this may explain their RNA chaperone activity.

Besides the examples given above, both the La autoantigen and the hnRNPC1/C2 proteins have been reported to functionally interact with the XIAP IRES. In fact, these ITAFs appears to be components of an RNA–protein complex consisting of at least four cytoplasmic proteins (Holcik *et al*., 2000b, 2003). However, the role of these *trans*-acting factors in cellular internal initiation is currently unknown. RNA–protein complexes containing multiple protein components have been reported for several other cellular IRESs (Yang and Sarnow, 1997; Paulin *et al*., 1998; Holcik *et al*., 2000b; Chappell and Mauro, 2003). Given the modular nature of some cellular IRESs, it is tempting to speculate that the combined effect of positive and negative ITAFs may be important in the regulation of internal initiation. Indeed the *Rbm3* IRES appears to be composed of separate *cis*-acting sequences that either inhibit or enhance internal initiation. Distinct *trans*-acting factors that bind to these regions were identified but their role in regulating the IRES remains to be confirmed (Chappell and Mauro, 2003).

One striking feature of many of these ITAFs is that they are predominantly located in the nucleus. However, since these proteins are known to shuttle between the nucleus and cytoplasm they can clearly influence internal initiation. Furthermore, the nucleocytoplasmic trafficking of ITAFs may be particularly relevant in the light of the observation that exposure to the nuclear compartment is essential for efficient internal initiation on some cellular IRESs (Stoneley *et al*., 2000b; Holcik *et al*., 2003; Shikoki *et al*., 2003).

Regulation of gene expression and cellular IRESs

Why have eukaryotic cells evolved two separate mechanisms for translation initiation? It is now well documented that cells can be exposed to various physiological conditions during which the efficiency of cap-dependent protein synthesis is greatly reduced (Table 3). There is a growing body of evidence to support the hypothesis that cellular IRESs are involved in the regulation of gene expression under such conditions.

Amino-acid starvation of mammalian cells results in a significant increase in the phosphorylation of eIF2α and a concomitant decrease in global protein synthesis. However, the translation of the cationic amino-acid transporter gene cat-1 is stimulated under these conditions. It was found that an IRES whose activity is induced by amino-acid deprivation is responsible for the increased synthesis of cat-1 (Fernandez *et al*., 2001). Yaman and co-workers have proposed that the activity of this IRES is regulated by two sequential events. Firstly, the translation of a small upstream open reading frame within the cat-1 5′ UTR causes structural remodelling of the IRES, leading to the attainment of an active IRES conformation. Secondly, induction of cat-1 IRES activity is dependent on the phosphorylation of eIF2α. A time lag between this phosphorylation event and the increased translation of cat-1 has led to speculation that an essential ITAF is synthesized during this period (Fernandez *et al*., 2002; Yaman *et al*., 2003). It must be emphasized that direct evidence for this ITAF is currently lacking. Regardless of the precise mechanistic details, this represents a clear example of regulated gene expression by internal initiation under stress conditions.

Internal initiation has also been implicated in the regulation of gene expression during the cell cycle. Both the p110^{PITSLRE} and p58^{PITSLRE} kinases are translated from a single mRNA, but the synthesis of p58^{PITSLRE} increases substantially in cells arrested in the G2/M phase. Studies revealed that an IRES is located just upstream of the p58^{PITSLRE} initiation codon (Cornelis *et al*., 2000). Importantly, the IRES can stimulate translation initiation during the G2/M phase when cap-dependent protein synthesis is inhibited (Bonneau and Sonenberg, 1987; Cornelis *et al*., 2000). Evidence has also been presented for a G2/M-regulated IRES in the ornithine decarboxylase gene (Pyronnet *et al*., 2000). In the same study, the c-*myc* IRES was found to function in all phases of the cell cycle, including when cells were arrested in the G2/M phase (Pyronnet *et al*., 2000). However in our laboratory, similar experiments revealed that the c-*myc* IRES is not active in cells progressing through G2/M (Subkhankulova and Willis, unpublished observations). Clearly further work will be required to resolve this discrepancy. Significantly, cDNA microarray studies are apparently revealing that not all mRNAs that contain an IRES are translated in the G2/M phase (Hellen and Sarnow, 2001). These data imply that individual IRESs are specifically regulated in the G2/M phase of the cell cycle.

Table 3 Cellular circumstances where cap-dependent translation is compromised but IRES-mediated translation maintains production of the proteins listed

Cellular circumstance	*IRESs regulated*
Devlopment	Antennapedia, Ultrabithorax, c-*myc*, FGF-2
Apoptosis	c-*myc*, DAP5, XIAP, PKCδ
Genotoxic stress	c-*myc*
Cell cycle	Hairless, p58/P ITSLRE, PKCδ, ODC
Hypoxia	c-*myc*, HIF-1α, VEGFA
Differentiation	AML1/RUNX1, PDGF2/c-*sis*
Heat/cold shock	Bip, Bag-1, Rbm3
Amino-acid starvation	Cat-1

Data from the IRES database at: http://ifr31w3.toulouse.inserm.fr/IRESdatabase/

During programmed cell death, there is a considerable inhibition of cap-dependent protein synthesis, which appears to correlate with the cleavage and/or modification of several eukaryotic initiation factors (Clemens *et al*., 2000). However, the synthesis of some proteins is not affected by these changes. In particular, there is a strong evidence that c-*myc* protein synthesis is maintained during apoptosis by internal initiation (Stoneley *et al*., 2000a). Other cellular IRESs that promote internal initiation in apoptotic cells include those of the DAP5, XIAP and PKCδ genes (Henis-Korenblit *et al*., 2000; Holcik *et al*., 2000a; Morrish and Rumsby, 2002). It is important to note that although the Apaf-1 gene contains an IRES and its gene product is involved in the initiation of apoptosis, in our hands the Apaf-1 IRES does not appear to function efficiently during TRAIL-induced and staurosporine-induced apoptosis (Coldwell and Willis, unpublished observations). However, it has been reported that the activity of this IRES is enhanced during etoposide-induced apoptosis (Nevins *et al*., 2003). Thus the mere presence of an IRES within a gene does not necessarily mean that the mRNA can be translated during apoptosis but IRESs may be subject to differential regulation depending on the apoptotic stimulus. Studies are currently underway using polysome profiling to identify other mRNAs that are translated efficiently during apoptosis (Bushell, Willis and Sarnow, unpublished). This analysis will help to uncover the true extent of the involvement of internal initiation in the regulation of gene expression during cell death.

Efficient translation of several other mRNAs is maintained by internal initiation in a number of other cellular conditions when cap-dependent protein synthesis is reduced. For instance, the IRESs in the vascular endothelial growth factor (VEGF) and hypoxia inducible factor-1α (HIF-1α) genes promote the translation of the corresponding mRNAs in hypoxic cells (Stein *et al*., 1998). Under conditions of genotoxic stress, the c-*myc* IRES directs the synthesis of c-*myc* polypeptides (Subkhankulova *et al*., 2001). An IRES in the bag-1 gene is essential for the synthesis of the 36 kDa BAG-1S protein during the recovery period in cells that have been stressed by heat shock (Coldwell *et al*., 2001). Finally, in cells arrested by serum starvation, the XIAP and $p27^{kip1}$ IRESs have been shown to function (Holcik *et al*., 1999; Miskimmins *et al*., 2001).

Clearly IRESs are implicated in the regulation of gene expression in diverse physiological states and they enable cells to respond to these conditions against the background of a general reduction in protein synthesis. In addition, tissue-specific regulation of internal initiation has also been reported. Several studies have demonstrated that IRES activity can vary in a cell type-specific manner in proliferating cells (Creancier *et al*., 2000; 2001; Stoneley *et al*., 2000b; Jopling and Willis, 2001; Jopling *et al*., 2004; Mitchell *et al*., 2003; Pickering *et al*., 2003a). Moreover, there is significant variation in *fgf2* and c-*myc* internal initiation in different tissues during murine embryogenesis (Creancier *et al*., 2000, 2001). Strikingly, the efficiency of the c-*myc* IRES is very low in adult tissues suggesting that repression of the IRES may play a role in the downregulation of c-*myc* expression in differentiated and quiescent cells (Creancier *et al*., 2001). In contrast, *fgf2* internal initiation is very efficient in the adult brain, a tissue in which this growth factor is known to play a significant role (Creancier *et al*., 2000). It has been suggested that the expression or activity of regulatory ITAFs could influence the efficacy of an IRES in a particular tissue. For example, there is evidence that the neuronal-specific ITAF nPTB may be responsible for enhanced Apaf-1 internal initiation in cell lines of neuronal origin (Mitchell *et al*., 2003).

Internal initiation and tumorigenesis

The presence of IRESs in so many genes that are involved in cell growth, proliferation, apoptosis and angiogenesis raises the possibility that deregulated internal initiation may contribute towards tumorigenesis. Indeed aberrant translational regulation through an IRES-mediated mechanism has been identified in the human neoplasia multiple myeloma (MM). Patients with this disorder have an expansion of a plasma cell type in the bone marrow and exhibit osteolysis. Initial studies indicated that the significantly increased level of c-*myc* proteins found in MM cell lines was due to enhanced translation initiation and moreover this aberrant regulation correlated with a single C to U transition in the c-*myc* 5′ UTR (Paulin *et al*., 1996). Subsequently, the same sequence substitution was identified in 42% of bone marrow samples collected from MM patients. This mutation enhances the activity of the c-*myc* IRES and its effect is particularly pronounced in MM cell lines (Chappell *et al*., 2000b). It is known that the C to U transition can stabilize the formation of RNA–protein complexes containing the c-*myc* IRES (Paulin *et al*., 1998). Recently, a member of the poly-(rC)-binding protein family hnRNPK has been shown to interact with the mutant IRES more strongly than the wild-type sequence. Moreover, this ITAF stimulates the activity of the mutant IRES to a greater extent. Hence, the increased affinity of hnRNPK for the mutant IRES may contribute towards enhanced c-*myc* internal initiation in MM (Evans *et al*., 2003). In addition, using our experimentally determined model for the c-*myc* IRES, we predict that this mutation could subtly modify the secondary structure in the region of domain II. Further support for this hypothesis is provided by an A to G mutation that elicits an identical structural change in this region, and like the MM mutation also stimulates the activity of the IRES (LeQuesne and Willis, unpublished).

Concluding remarks

Despite the identification of cellular IRESs in an increasing number of genes, we are currently some

way short of understanding the mechanism of internal initiation in cellular IRESs. Progress has been hampered by the lack of a suitable *in vitro* system and in some IRESs matters are complicated further by the requirement for a nuclear event. A genetic approach may yet yield vital clues as IRESs have been found in genes from both *Drosophila melanogaster* and more recently *Sacharomyces cerevisiae*. Nonetheless, since the structure-function relationship in many cellular IRESs is not clear-cut, it is apparent that these examples do not conform to the models proposed for viral IRESs. Moreover, the available data support the hypothesis that some cellular IRESs are composed of multiple modules that act in combination to regulate ribosome recruitment. In fact both structural features and sequence-specific modules have been identified and therefore there may be considerable diversity in the mode of action of cellular IRESs. As with viral IRESs, evidence is accumulating that ITAFs (Table 2) play a key role in the process of cellular internal initiation. Structural remodelling of the ribosome entry window by RNA chaperones has recently been highlighted as one function of such auxillary factors.

What is clear is that cellular IRESs enable gene expression to be regulated under conditions when cap-dependent protein synthesis is reduced, such as during cell death, in the G2/M phase of the cell cycle or under certain stress conditions (Table 3). Cellular IRESs have been shown to maintain or stimulate the synthesis of polypeptides in these physiological states. However, it is becoming increasingly apparent that not all IRESs function efficiently under the same cellular conditions. Furthermore, the activity of some cellular IRESs varies between different cell-types and tissues. Regulation of cellular internal initiation has been attributed to the activity of ITAFs, which can have both a positive and negative influence. Studies into the effect of these factors on ribosome recruitment will likely provide insight into the mechanism of internal initiation.

It is noticeable that of the various genes shown to contain an IRES, many are involved in the control of cell proliferation, cell growth, apoptosis and angiogenesis. Hence, the regulation of gene expression through internal initiation can impact on cellular processes that are involved in tumorigenesis. Indeed deregulated expression of the proto-oncogene c-*myc* can occur through internal initiation. As we expand our understanding of cellular IRESs, we will no doubt uncover further evidence of the involvement of internal initiation in the deregulation of proto-oncogene and tumour suppressor gene expression.

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