Internal Ribosome Entry Sites in Cellular mRNAs: Mystery of Their Existence*

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Although studies on viral gene expression were essential for the discovery of internal ribosome entry sites (IRESs), it is becoming increasingly clear that IRES activities are present in a significant number of cellular mRNAs. Remarkably, many of these IRES elements initiate translation of mRNAs encoding proteins that protect cells from stress (when the translation of the vast majority of cellular mRNAs is significantly impaired). The purpose of this review is to summarize the progress on the discovery and function of cellular IRESs. Recent findings on the structures of these IRESs and specifically regulation of their activity during nutritional stress, differentiation, and mitosis will be discussed.

Initiation of protein synthesis in eukaryotes is a complex process requiring numerous accessory proteins called initiation factors (also termed canonical initiation factors) (1). Assembly of the 80 S ribosome at a start codon within the majority of eukaryotic mRNAs involves binding of the mRNA 5′-mG cap structure to a group of proteins referred to as the cap-binding complex or eIF4F (which consists of three proteins: eIF4E, eIF4G, and eIF4A) (1–3). This is followed by recruitment of the 40 S ribosomal subunit and associated initiation factors (43 S initiation complex comprising a 40 S subunit, eIF2-GTP-Met-tRNAi, and eIF3) and movement of the 43 S initiation complex along the 5′-untranslated region (5′-UTR) in search of the initiation codon (1–3) (Fig. 1, left panel). This mechanism of translation initiation is known as “ribosome scanning” (1–3). Initiation factor eIF4G functions as a scaffolding protein. It binds eIF4E (a cap-binding protein) and eIF4A (an ATP-dependent RNA helicase, which is thought to unwind the secondary structure in the mRNA 5′-UTR) and bridges the mRNA and the ribosome via its interaction with the 40 S bound initiation factor eIF3 (1–3). eIF3 is a multiprotein complex directly associated with the small ribosomal subunit and was shown to impede the association of the 40 S and 60 S ribosomal subunits in the absence of eIF2-GTP-Met-tRNAi, ternary complex (1–3). eIF2 binds GTP and Met-tRNAi and transfers Met-tRNAi to the initiation factors of the eIF4F complex (1–3). Initiation factor eIF2α dephosphorylation (21), or the cleavage of PABP (22). Thus, three major conclusions can be drawn regarding viral IRES

Viral Ribosome Landing Pads: How the Story Began

The poliovirus and encephalomyocarditis virus (EMCV) mRNAs were the first to be described to utilize IRES elements (17, 18) originally termed ribosome landing pads by Nahum Sonenberg (18). Pelletier and Sonenberg (18) developed the dicistronic test, which became the standard for assaying IRES activity. Their dicistronic mRNAs had two open reading frames of reporter genes (also called cistrons); thymidine kinase and chloramphenicol acetyltransferase and the entire poliovirus leader sequence or parts of it inserted in between (18). The first cistron (thymidine kinase) measured cap-dependent initiation, whereas the second (chloramphenicol acetyltransferase) reflected the existence of internal initiation of translation (18). The dicistron mRNA expression system was used to show that IRES elements allow initiation to bypass many regulatory mechanisms specific for cap-dependent translation involving the eIF4F complex. Hence, it appeared that IRES-driven translation initiation prevails when cap-dependent initiation is severely compromised (6, 7, 19). During viral infection, inhibition of cellular protein synthesis can be caused by the cleavage and partial loss of activity of eIF4G (19, 20), 4-E-BP dephosphorylation (21), or the cleavage of PABP (22).

The subset of canonical initiation factors required for viral internal initiation can vary depending on the IRES type and structure (e.g. recruitment of the 40 S ribosomes to hepatitis C virus and swine fever virus mRNAs does not require any of the initiation factors of the IRES “family” (23)). Furthermore the cricket paralysis virus IRES containing mRNA directs initiation without a requirement for any of the canonical initiation factors at all (24, 25). The same holds true for the IRES element found in an RNA virus that infects penaid shrimp (26). Soon after the first reports describing the IRES activity in the picornavirus 5′-UTR, specific proteins were identified that were shown to bind and modulate IRES activity (27, 28) (reviewed in Refs. 10 and 29–31). Surprisingly, none of these proteins were translation initiation factors (10, 27–31). The functional roles of these IRES trans-acting factors (ITAFs) were generally addressed by in vitro translation assays (for references, see Refs. 27–32). However, recent studies provided evidence for their important functional role in vivo as well (for references, see Ref. 10 and supplemental Table 1).

Thus, three major conclusions can be drawn regarding viral IRES function. First, viral IRES-driven translation initiation can prevail when cap-dependent initiation is severely compromised. Second, viral IRES-driven translation has a reduced requirement for the canonical translation initiation factors. Third, viral IRES-driven translation can often be enhanced by a number of trans-acting factors (ITAFs).

Cellular IRESs

Very soon after the first IRES was identified in the picornavirus 5′-UTR (17, 18), it was found by Peter Sarnow and colleagues (33)
that a cellular mRNA, encoding the immunoglobulin heavy chain binding protein (BiP), can be translated in poliovirus-infected cells at a time when cap-dependent translation is inhibited. It was concluded that the BiP mRNA can mediate internal entry of ribosomes in mammalian cells, indicating that translation initiation by an internal ribosome-binding mechanism can be used by cellular eukaryotic mRNAs (33). For a long time this example of a cellular mRNA containing an IRES element (along with the mRNA for the Drosophila melanogaster homeotic genes Antennapedia (34) and Ultrabithorax (35)) has been considered as an exception for the general cap-dependent mode of translation initiation in eukaryotes. However, it was later shown that 3–5% of the cellular mRNAs remain associated with polyribosomes in poliovirus-infected cells at a time when cap-dependent initiation is impaired (36). Indeed, in the past few years, IRES elements have been detected in an increasing number of cellular mRNAs from various species (9, 10), and the list is growing (37). So far IRES elements were mainly found in mRNAs involved in regulating gene expression during development, differentiation, cell cycle progression, cell growth, apoptosis, and stress (Refs. 9, 10, and 37 and supplemental Table I).

Canonical Factors Requirement—Despite the identification of IRESs in an increasing number of cellular mRNAs, the mechanism of internal initiation is still poorly understood. It is clear that similarly to viral IRES elements, cellular IRESs enable translation of the mRNAs under conditions when cap-dependent protein synthesis is impaired (such as a variety of stress conditions, the G1/M phase of the cell cycle, and apoptosis). Under these conditions, the activity of the cap-binding factor eIF4E is down-regulated (3). This favors the expression from the IRES elements (9, 10). Interestingly, the majority of the stress conditions (such as starvation for growth factors/nutrients, heat shock, UV light irradiation, hypoxia, endoplasmic reticulum stress, and virus infection) also lead to the down-regulation of activity of initiation factor eIF2 through phosphorylation of its α subunit (39–40). The reduction in eIF2-GTP levels leads to the inhibition of the overall rate of cap-dependent protein synthesis (38–40). However, a number of cellular mRNAs containing IRES elements such as platelet-derived growth factor-2, vascular endothelial growth factor (41), oncopogene c-myc (41, 42), and PI3K/Akt kinase (43) continue to be efficiently translated under these conditions. Interestingly, the IRES element found in the cationic amino acid transporter (Cat-1) requires prior eIF2α phosphorylation for its following activation by stress (44). A number of reports have also indicated that some viral (EMCV and hepatitis C virus) IRES elements can function under conditions of eIF2α phosphorylation (41, 45). The case of the EMCV IRES element continues to be controversial (41, 43, 45).
though two reports have indicated that EMCV IRES can function under the conditions of eIF2α phosphorylation (41, 45), the other did not support this observation (43).

Information on the requirement of other canonical initiation factors for the activity of cellular IRESs is just beginning to emerge (46). Because mammalian IRESs are generally resistant to proteolytic cleavage of eIF4G and even stimulated under these conditions (46). It should also be noted that inhibition of protein synthesis in apoptosis is accompanied by a caspase-dependent cleavage of initiation factors eIF4F, eIF4B (eIF4B stimulates the helicase activity of eIF4A), eIF2α, and the p35 subunit of eIF3 (47). Proteolytic cleavage of these proteins yields distinct, characteristic products (47, 48). However, there is strong evidence that translation of c-myc, death-associated protein 5 (DAP5), X chromosome-linked inhibitor of apoptosis protein (XIAP), inhibitor of apoptosis protein 2 (HAIP2/c-IAP1), a pro-apoptotic protein Reaper, chaperone Hsp70, anti-apoptotic proteins Bcl-2 and Survivin, protein kinase C8, and the apoptotic protease activating factor 1 (Apaf-1) mRNAs is maintained under these conditions and is driven by their IRES elements (supplemental Table I, and references therein). This indicates that these IRESs would probably hide additional cellular IRES elements for the initiation of translation eIF4F as well as eIF4B, eIF2α, and the p35 subunit of eIF3.

It was also demonstrated that eIF4A activity is essential and limiting for the activity of c-myc and BIP IRES (46). It is unclear whether the activity of other cellular IRESs will also be dependent on eIF4A. Information on other canonical initiation factors that might influence the translation driven by cellular IRESs is limited.

Start Site Location and Selection—It is assumed that translation of most of the cellular mRNAs that contain IRES elements proceeds predominantly through the internal entry of ribosomes. Although all cellular mRNAs are capped and in principle should be able to bind the eIF4F complex, it is generally believed that the conventional scanning from the 5′-end is not possible for the majority of IRES-containing cellular mRNAs because their 5′-UTRs are long and structured (9, 10). The vast majority of the cellular IRES elements are located within the 5′-UTRs in close proximity to the initiation codon, and thus even if cap-dependent translation from the 5′-end were possible, the translation products would be indistinguishable. The mRNA for neurogenin, a neuronal calmodulin-binding protein, is an example of a mechanism that is translationally independent of the initiation of a subset of cellular IRESs (including c-myc, Apaf-1, Cat-1, GFP-1, and others (54, 63–67)) revealed complex structures that included stem loops and pseudoknots (54, 63–67). Yet, it is unclear how these structures can promote and facilitate efficient internal entry of ribosomes. tRNA-like elements (68), initially found within the structures of viral IRES elements (69), could facilitate docking of the cellular IRESs to the e or P sites on the 40 S ribosome. Recent cryo-electron micrograph visualization of two viral (hepatitis C virus and cricket paralysis virus intergenic region CrPV) internal ribosome entry sites bound to the 40 S ribosome seems to support this suggestion (70, 71).

Interestingly, a number of cellular mRNAs containing IRES elements in their 5′-UTRs also contain small upstream open reading frames (uORFs) located within the IRES sequence (60, 65, 72–76). In most cases, the significance of these uORFs for translation is not clear. Initially it was believed that these uORFs are present in the 5′-UTRs of cellular mRNAs to inhibit cap-dependent translation (Ref. 65, and see Ref. 77 for a review). However, recent studies on Cat-1 IRES-mediated translation showed that translation of the 48-amino acid uORF is required for increased Cat-1 mRNA translation (69). This strongly supports the new concept of a “dynamic IRES” (65). Thus, we believe that cellular IRES elements are not rigid structures but can undergo transitions that can substantially influence their activity (64, 65, 68).

ITAFs—All known ITAFs are cellular RNA-binding proteins that play a variety of functions in cells (reviewed in Refs. 10 and 29–32). For example, the levels of ITAF expression were shown to correlate with pathogenic properties and tissue specificity of picornaviruses (29–32). The properties and tissue distribution of ITAFs were suggested to determine the biological properties of a variety of viruses that use the IRES-dependent translation initiation (29–32).

RNA-protein complexes containing multiple protein components have been also reported for a number of cellular IRESs (Ref. 10 and references therein; supplemental Table I), and some of these proteins have been shown to modulate the efficiency of internal ribosome entry. A striking feature of many of these ITAFs is that they belong to the group of heterogeneous nuclear ribonucleoproteins (A1, C1/C2, I, E1/E2, K, and L) known to shuttle between the nucleus and the cytoplasm. It was suggested that the relative levels of ITAFs present in the cytoplasm vary under different stress conditions and their intracellular distribution would significantly modulate the level of IRES-mediated translation (10) (supplemental Table I). One hypothesis is that ITAFs may help to recruit the 40 S ribosomal subunit to the mRNA through specific interactions with canonical translation initiation factors or ribosomal components. On the other hand ITAFs may promote or stabilize specific active conformations of the IRES. For example remodeling of the Apaf-1 structure upon interaction with UNR protein was shown to promote binding of poly pyrimidine tract-binding protein, and these events led to stimulation of Apaf-1 internal initiation (64). Therefore, it is possible that ITAFs play an important role in regulating IRES activity by causing a conformational
Thus, internal initiation represents a cellular "backup plan" for survival under conditions when cap-dependent protein synthesis is greatly reduced (such as starvation for growth factors/nutrients, heat shock, UV light irradiation, hypoxia, endoplasmic reticulum stress). The rapid inhibition of protein synthesis under these conditions is believed to function as a protective homeostatic mechanism. Thus, internal initiation represents a cellular "backup plan" for survival under conditions when cap-dependent protein synthesis is greatly reduced (such as starvation for growth factors/nutrients, heat shock, UV light irradiation, hypoxia, endoplasmic reticulum stress). The rapid inhibition of protein synthesis under these conditions is believed to function as a protective homeostatic mechanism.

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