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Was the initiation of translation in early eukaryotes IRES-driven?

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The initiation of translation in eukaryotes generally involves the recognition of a 'cap' structure at the 5′ end of the mRNA. However, for some viral and cellular mRNAs, a cap-independent mechanism occurs through an mRNA structure known as the internal ribosome entry site (IRES). Here, I postulate that the first eukaryotic mRNAs were translated in a cap-independent, IRES-driven manner that was then superseded in evolution by the cap-dependent mechanism, rather than vice versa. This hypothesis is supported by the following observations: (i) IRES-dependent, but not cap-dependent, translation can take place in the absence of not only a cap, but also many initiation factors; (ii) eukaryotic initiation factor 4E (eIF4E) and eIF4G, molecules absolutely required for cap-dependent translation, are among the most recently evolved translation factors; and (iii) functional similarities suggest the evolution of IRESs from spliceosomal introns. Thus, the contemporary cellular IRESs might be relics of the past.

Initiating translation – a new start with an old beginning?

Translation is a fundamental process for the expression of genetic material. Initiation of translation in eukaryotes involves the recruitment of the 5′-untranslated region (5′-UTR) of the mRNA to the 40S ribosomal subunit. For most mRNAs, initiation of translation requires the recognition of the cap structure by the cap-binding protein eukaryotic initiation factor 4E (eIF4E). eIF4G is a scaffold protein that coordinates this process by interacting with eIF4E, the ATPase/RNA helicase eIF4A, the poly-A-binding protein (PABP) and the 40S ribosomal-subunit-associated eIF3. eIF4B stimulates the activity of eIF4A, which unwinds secondary RNA structures in the 5′-UTR, thus enabling the 40S ribosomal subunit to scan along the mRNA to reach the authenticable start codon [1] (Box 1). For some viral and cellular mRNAs, 5′-UTR recognition by the 40S ribosomal subunit occurs without involvement of eIF4E and is, instead, driven by an RNA structure located within the message itself. This structure is termed an internal ribosome entry site (IRES), and it is located in the proximity of the start codon [2–8].

In contemporary eukaryotic cells, most mRNAs are translated in a cap-dependent manner. However, I propose here that, in the past few years, evidence has accumulated suggesting that the mRNAs of the early eukaryotes were probably translated in a cap-independent IRES-driven manner. It was only after IRES-dependent initiation was already established that capped mRNAs and the molecules for the synthesis of the cap (namely RNA 5′ triphosphatase, guanylyl-transferase and guanine-N7-methyltransferase), eIF4E and eIF4G appeared, and this cap-independent initiation was later superseded in evolution by the cap-dependent mechanism of initiation of translation. Thus, the relatively recently discovered process of IRES-mediated initiation of translation could, in fact, be a relic of the past.

What is an IRES?

It has long been known that some picornaviruses (e.g. poliovirus) selectively inhibit the translation of their host mRNAs while translating their own mRNAs. The mechanism for this was, however, a mystery because the 5′-UTRs of picornavirus mRNAs are rather long (between ~600 and 1200 nucleotides compared with a few to tens of nucleotides in a canonical transcript), form complex secondary structures, contain several AUG triplets and are uncapped. Rather than promoting translation, these characteristics would typically be expected to inhibit cap-dependent translation, and it was thought that picornaviral mRNAs must be translated by a 5′ cap-independent mechanism. In the late 1980s, two independent groups [9,10] discovered that there is an internal sequence in the 5′-UTR of picornaviral mRNAs that allows the 40S ribosomal subunit to land directly on the mRNA in a cap-independent manner. This sequence is now known as an IRES [9,10]. Three years later, the first cellular IRES was discovered in the mRNA of the immunoglobulin heavy-chain binding protein (BiP) [11], an mRNA known to be translated upon poliovirus infection. Since that time, an increasing number of viral and cellular mRNAs have been observed to contain IRES elements [2,3,5–8].

Viral IRESs are found in mRNAs from several RNA virus families, as well as a DNA herpesvirus. IRESs from mRNAs within the same viral group can share complex secondary structures, but IRESs from different viral groups differ significantly in primary and secondary structure, size, requirements for translation initiation factors and the mechanism used to recruit the 40S ribosomal subunit. These differences suggest that viral IRESs arose independently several times in eukaryotic evolution, rather that directly from the same ancestral IRESs. The

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Box 1. Cap-dependent initiation of translation

The mechanism of eukaryotic cap-dependent initiation of translation (Figure I) accounts for the translation of most eukaryotic mRNAs and is mediated by the eukaryotic initiation factors (eIFs). It begins with the dissociation of the ribosomal subunits 60S (blue) and 40S (yellow). Free 40S ribosomal subunit, which is stabilized by eIF3 (pink), eIF1 and eIF1A (grey), binds to a ternary complex – consisting of eIF2 (dark green) bound to an initiator Met-tRNA$_i^{Met}$ (blue clover) and GTP – to form a 43S pre-initiation complex. eIF5 (orange) interacts with eIF2 and eIF3 and is probably recruited to the 40S ribosomal subunit with these factors. In addition, and most probably simultaneously, the cap structure (m^{7}GpppN, where N is the nucleotide located at the very 5′ end of the pre-mRNA) is recognized by the cap-binding protein eIF4E (green crescent) in complex with the scaffold eIF4G (red). Next, the recruitment of the 5′-untranslated region (5′-UTR) of the mRNA (blue line) by the 43S pre-initiation complex occurs, a process that is coordinated by eIF4G via its interaction with eIF4E, the ATPase and RNA helicase eIF4A (light green), the poly-A-binding protein (PABP, brown) and the 40S ribosomal-subunit-associated eIF3 (because of these interactions, the mRNA at this step is most probably circular). This complex scans in a 5′ to 3′ direction along the 5′-UTR to reach the start codon, usually an AUG. During the scanning (a process that requires ATP), eIF4B (dark blue) stimulates the activity of eIF4A that unwinds secondary RNA structures in mRNA. eIF1, eIF1A, and eIF5 assist in the positioning of the 40S ribosomal subunit at the correct start codon so that eIF2 can deliver the anti-codon of the initiator Met-tRNA$_i^{Met}$ as the cognate partner for the start codon directly to the peptidyl site of the 40S ribosomal subunit. Once the ribosomal subunit is placed on the correct start codon, a 48S pre-initiation complex is formed. Next eIF5 promotes GTP hydrolysis by eIF2 and the release of the initiation factors. eIF2B (light blue) and GTP recycle the dissociated eIF2–GDP complex so that it can associate with a new Met-tRNA$_i^{Met}$ and take part in a new round of initiation. Finally, the GTPase eIF5B (white) is required to join the 60S ribosomal subunit to the 40S subunit to form an 80S initiation complex. Thereafter, the polypeptide elongation begins [1].

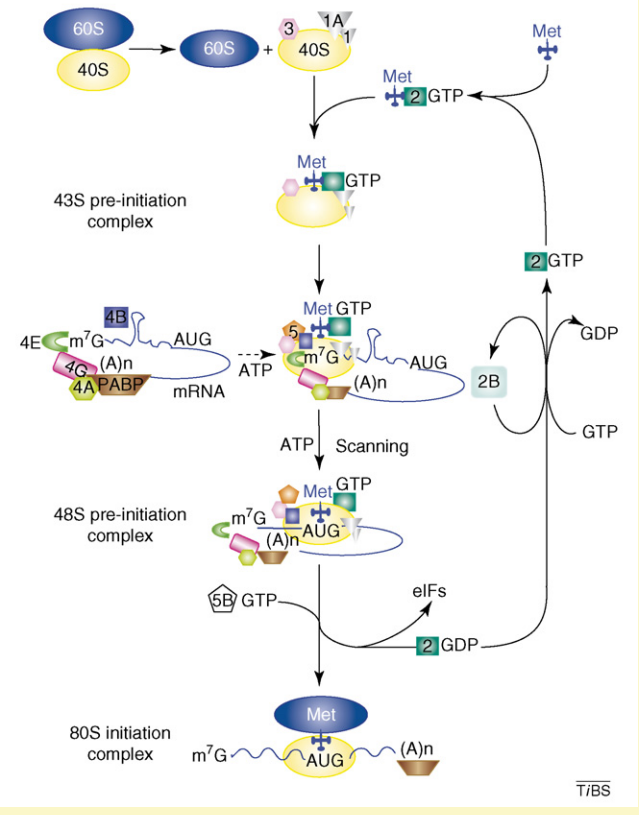

Figure I. The mechanism of eukaryotic cap-dependent initiation of translation.

archetypes of viral IRESs representing these differences are, from the simplest to the most complex, those from dicistrovirus, hepatitis C virus (HCV, a flavivirus) and picornavirus (Figure 1).

In contrast to viral IRESs, cellular IRESs share no similarity in their primary or secondary structure [7,8]. Although the requirement for the canonical translation initiation factors for most cellular IRESs is not yet known, according to the secondary structure there are apparently two types of cellular IRES (Figure 1). IRESs of the first type are adenine-rich and completely unstructured, and they are represented by the recently discovered IRESs of the mRNAs of *Drosophila melanogaster* proapoptotic genes *reaper*, *hid*, *grim* and *hsp70* [12,13], and those of the *Saccharomyces cerevisiae* genes required for starvation-induced differentiation *YMR181c*, *FLO8*, *BO11* as well as *PABP* [14]. *Drosophila* IRESs are functional in the absence of eIF4E, and there is a positive correlation between the adenine content and the efficiency of translation they confer [12]. In the case of yeast IRESs, PABP binds to the poly(A) tract preceding the AUG codon to drive translation initiation, functionally substituting for a cap and eIF4E in recruiting eIF4G [14]. Cellular IRESs of the second type (e.g. those from FGF-1, FGF-2, c-myc, L-myc, Apaf-1, and Cat-1 mRNAs) have a complex secondary structure that, for some of them, has been shown to be important for IRES functioning [5,7]. It appears that, rather than being defined structures, cellular IRESs might constitute the intrinsic ability of certain 5′-UTRs to direct the passive recruitment of ribosomes without eIF4E. This finding might explain the lack of sequence similarity among cellular IRESs.

Looking at the past of the initiation of translation

As with all evolutionary studies, we can discover the ancient nature of any current biological process by studying its present-day features. I consider that the following features of the initiation of translation in contemporary eukaryotic cells shed light on the evolutionary past of this process.

The IRES-dependent initiation of translation can be less complex than the cap-dependent one

Two basic mechanisms for the proper placement of the ribosome on the mRNA have been defined [6]. From the more complex to the simplest, they are:

(i) Initiation of translation in which the 40S ribosomal subunit is dependent on the eIF4 group of factors (particularly eIF4G) to be delivered to the mRNA. Variations on this mechanism include: (a) the cap- and scanning-dependent initiation of capped mRNAs, which requires all translation initiation factors, including eIF4E and eIF4G. This mechanism accounts for the translation of most cellular mRNAs (Box 1); (b) scanning-dependent initiation of uncapped versions of normal mRNAs [15–18] or initiation driven by picornaviral IRESs [19,20], which still require most factors, including the central domain of eIF4G, but do not require eIF4E [20].

(ii) A recently discovered prokaryotic-like mechanism in which direct binding of the 40S ribosomal subunit to