Life Sciences 212 (2018) 138–144

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Review article

Alternative mechanisms of translation initiation: An emerging dynamic regulator of the proteome in health and disease

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ARTICLE INFO

Keywords:
Translation
IRES
Ribosome
mRNA
ITAF

ABSTRACT

Eukaryotic mRNAs were historically thought to rely exclusively on recognition and binding of their 5′ cap by initiation factors to effect protein translation. While internal ribosome entry sites (IRESs) are well accepted as necessary for the cap-independent translation of many viral genomes, there is now recognition that eukaryotic mRNAs also undergo non-canonical modes of translation initiation. Recently, high-throughput assays have identified thousands of mammalian transcripts with translation initiation occurring at non-canonical start codons, upstream of and within protein coding regions. In addition to IRES-mediated events, regulatory mechanisms of translation initiation have been described involving alternate 5′ cap recognition, mRNA sequence elements, and ribosome selection. These mechanisms ensure translation of specific mRNAs under conditions where cap-dependent translation is shut down and contribute to pathological states including cardiac hypertrophy and cancer. Such global and gene-specific dynamic regulation of translation presents us with an increasing number of novel therapeutic targets. While these newly discovered modes of translation initiation have been largely studied in isolation, it is likely that several act on the same mRNA and exquisite coordination is necessary to maintain 'normal' translation. In this short review, we summarize the current state of knowledge of these alternative mechanisms of eukaryotic protein translation, their contribution to normal and pathological cell biology, and the potential of targeting translation initiation therapeutically in human disease.

1. Introduction

Despite the fundamental necessity for protein translation to organismal life, historically research has focused on transcription as the central governor of the proteome. An appreciation for co- and post-transcriptional gene regulation has since developed with the discovery of alternative splicing, RNA modifications, and RNA localization as critical regulators of cellular physiology. One of many post-transcriptional mechanisms capable of altering the cellular proteome is translational regulation of mRNAs, fine-tuning protein expression and rapidly altering the proteome during stress [1,2]. Alterations in translation initiation are a key feature of cancer, viral infection, and cardiac hypertrophy [3–5].

Modifying the translational program allows a rapid response to extracellular signals, effecting changes in protein expression from translation of existing mRNAs. Translation initiation is a point where many mechanisms of regulation converge to determine if an mRNA will be translated, and a canonical model of translation initiation central to our understanding of this process has arisen [6,7]. In this model, recruitment of the ribosome is dependent upon recognition of the mRNA's 5′ cap by eukaryotic initiation factor (eIF) 4E. Alternative mechanisms of translation initiation are characterized by distinct modes of initiation complex recruitment to the mRNA to facilitate translation initiation. These include 5′ cap-dependent and independent models with diverse molecular mechanisms such as alternate cap recognition and mRNA methylation capable of allowing the formation of elongation competent ribosomes. There is also growing evidence to support a critical role of the ribosome itself in regulating translation. This brief review will discuss several models of alternative translation initiation and the evidence for their occurrence in eukaryotic cells, and provide examples of mRNAs for which alternative translation is necessary to maintain normal cellular function. Yeast have served as a powerful tool to gain mechanistic insight in studies of translation initiation, however for this review we focus on studies conducted in mammalian model systems.

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https://doi.org/10.1016/j.lfs.2018.09.054
Received 4 June 2018; Received in revised form 18 September 2018; Accepted 27 September 2018
Available online 02 October 2018
0024-3205/

Translation of upstream open reading frames (uORFs), repeat associated non-AUG (RAN), and other models of alternative translation activated during pathological states have been the subjects of recent reviews, to which we refer readers [8–11].

2. Mechanisms of eukaryotic translation initiation

2.1. eIF4E cap-dependent translation

The canonical mode of eukaryotic translation initiation begins with formation of a ternary complex composed of eukaryotic initiation factor (eIF) 2, a methionine charged transfer RNA, and GTP (reviewed in [12]). Binding of the ternary complex with the 40S ribosomal subunit is promoted by eIF1, eIF1A, and eIF3, forming the pre-initiation complex (PIC). Eukaryotic mRNAs bear a 5′ cap of 7-methylguanosine (m^7G), necessary for this mode of translation initiation and maintaining mRNA stability. The cap recognition protein eIF4E binds the 5′ cap, and together with eIFs 4A, 4G, and 4B recruits the PIC to the 5′ mRNA end. Interactions of the eIF4G scaffolding protein and poly(A) binding protein (PABP) circularize the mRNA and the complex scans in a 5′ to 3′ manner until arriving at a start codon in a favorable context. Upon start codon recognition, GTP is hydrolyzed, binding of the 60S ribosomal subunit forms the 80S complex and translation is initiated (Fig. 1A).

The necessity for eIF4E in canonical cap-dependent translation make it a critical regulatory factor; eIF4E expression is tightly regulated and eIF4E activity is governed by a family of eIF4E binding proteins (4E-BPs) which sequester and inactivate eIF4E [3,13,14]. 4E-BPs are primarily regulated by the mechanistic target of rapamycin (mTOR) pathway, where phosphorylation of 4E-BPs by mTOR releases eIF4E to promote 5′ cap and eIF4E-dependent translation initiation [15,16]. Degradation or sequestration of components of the eIF4F complex inhibits this mechanism and is targeted by many viruses to induce a ‘global’ shutdown of cellular mRNA translation [17–19]. The finding that a large number of cellular mRNAs are still translated under conditions in which eIF4E cap-dependent translation is inhibited was an early clue that alternative mechanisms of translation initiation exist [20].

2.2. Alternative 5′ cap-dependent models of translation initiation

The eIF3 complex promotes efficient canonical, cap-dependent translation initiation and is central to several alternative models of translation initiation [21–23]. Lee et al. (2015) performed photoactivatable ribonucleoside-enhanced crosslinking and immunoprecipitation (PAR-CLIP) to identify eIF3-interacting transcripts [24]. eIF3 selectively bound mRNAs involved in cell growth and proliferation, exerting translational control by binding the 5′ untranslated regions (UTRs) of these transcripts. Four subunits of eIF3 cross-linked directly to mRNAs, and deep sequencing of these eIF3 bound mRNA sequences revealed specific binding to just under 500 cellular transcripts [24]. Notably, the authors find that eIF3 can act as a positive and negative regulator of translation, dependent upon transcript *cis* elements [24]. eIF3 complex composition ranges from as few as five subunits in yeast, considered the eIF3 “core complex” due to its species conservation, to up to thirteen subunits in mammalian cells, with requirement for distinct subunits varying between initiation mechanisms [25,26]. The eIF3d subunit has been shown to bind the 5′ cap directly and allow cap-dependent translation initiation in the absence of eIF4E [27,28]. The *c-Jun* mRNA on which eIF3d cap binding activity has been described, harbors an RNA sequence element that appears inhibitory to eIF4E cap binding. In this study, alternate 5′ cap recognition promotes translation initiation, however an inhibitory role of novel cap binding proteins has also been described. In an effort to identify the mechanism by which La-related protein 1 (LARP1) represses translation of terminal oligopyrimidine (TOP) mRNAs specifically, Lahr et al. reveal 5′ cap binding by LARP1 blocks eIF4F complex assembly to negatively regulate translation [29,30]. Together, these data highlight the importance of alternate 5′ cap recognition in the regulation of eukaryotic translation initiation.

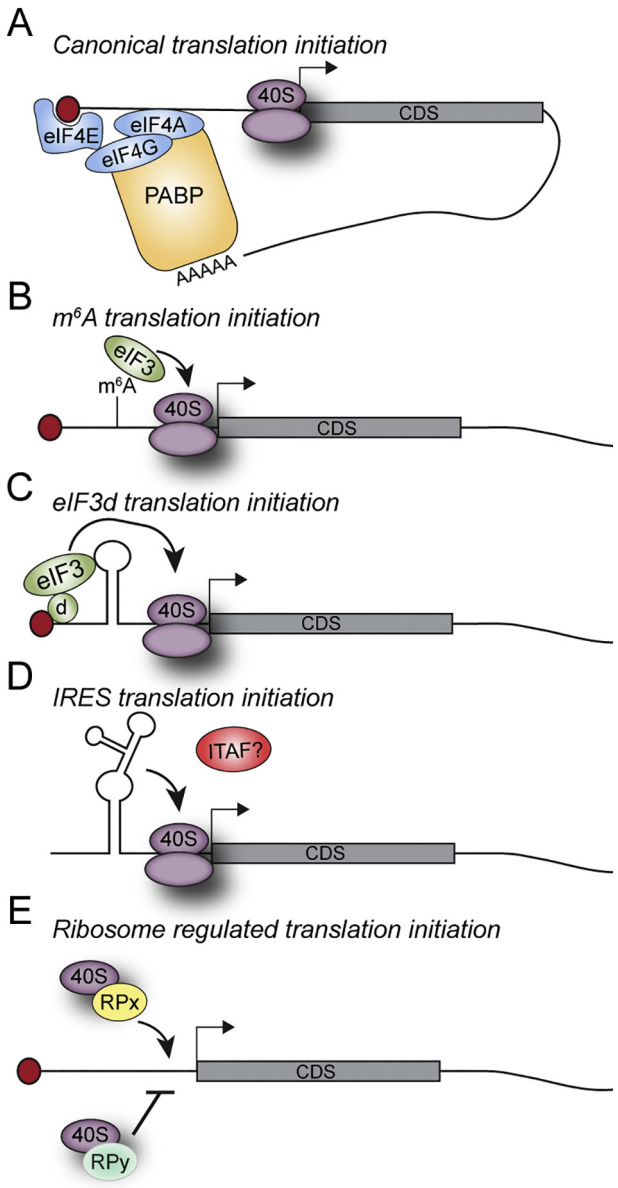

Fig. 1. Mechanisms of translation initiation currently known. A) eIF4E recognition of the 5′ mRNA cap facilitates binding of the 43S preinitiation complex in the canonical pathway of translation initiation. Upon recognition of the initiation codon the 60S subunit joins and translation is initiated. This mechanism of eIF4E and cap-dependent translation initiation is responsible for the majority of mRNA translation in the eukaryotic cell. B) *N*(6)-methyladenosine (m^6A) in the mRNA 5′ UTR can directly bind the eIF3 multiprotein complex and allow recruitment of the 43S complex to initiate translation independent of the 5′ cap. C) A subset of mRNAs are bound by the d subunit of the eIF3 complex. eIF3d cap recognition directs translation initiation allowing cap-dependent translation independent of eIF4E, the canonical cap recognition protein. D) Highly structured mRNA elements, in many cases with the assistance of IRES *trans* acting factors (ITAF) recruit ribosomes to specific start codons in viral mRNAs, allowing cap-independent translation initiation. The requirement for mRNA structure, ITAFs, and other factors in eukaryotic IRES remains a subject of research. E) Ribosomes vary in their ribosomal protein composition, and selectively translate mRNAs. Ribosomal proteins, in tandem with *cis* elements of the message, may direct ribosomes to translate specific mRNAs in changing conditions.

The role of eIF3 as a governor of the translational program during