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mRNA-specific translational regulation in yeast

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Abstract: The expression of a gene is governed at various levels, from transcriptional to translational level. The translational control is widely used to regulate gene expression, especially when a rapid, local, and selective control over protein synthesis is required. The present review describes instructive examples of translational regulation in yeast, together with regulatory elements within mRNAs. The review also outlines the important contributions of mRNA-binding proteins that act in harmony with several translational elements to generate appropriate translational signals and responses.

Protein Synthesis in Yeast

The coordinated and regulated functioning of various biological processes is mediated through selective expression of genes in a chronological, spatial, and cell type-specific manner. Single-celled organisms such as *Saccharomyces cerevisiae* have evolved to adapt their intracellular environment, including their proteome, to respond to varying environmental conditions through keeping a check on the expression of specific genes. The elicitation of such an orchestrated and organized response requires an overall control of protein synthesis at all levels within a cell.

Early mRNA processing events

Gene expression can be controlled at both DNA and RNA levels. Transcription of mRNAs is a highly regulated process that works in accordance with several downstream post-transcriptional mRNA editing processes. This additional level of regulation of mRNAs results in tremendous variations in the manner in which proteins are expressed from a particular mRNA (Lackner *et al.*, 2007).

All eukaryotic mRNAs, except those coding for histones, carry a methylated guanosine residue at their 5' ends as a cap and a long poly-adenosine tail at their 3' ends. These co-transcriptionally added motifs significantly contribute to mRNA stability, as well as assist in its translation (Coppola *et al.*, 1983; Preiss and Hentze, 1998). Following transcription, introns or the non-coding regions in mRNA are spliced out to produce a functional, mature mRNA that can be translated into a specific protein. Only after a pre-mRNA has successfully undergone these events, the mature mRNA is exported to the cytoplasm for translation and further regulation (Saguez *et al.*, 2005).

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Translation

The fate of the processed mature mRNA upon its translocation from the nucleus to the cytoplasm is determined by a number of mRNA degradation and translation pathways (Palayoor *et al.*, 1981; Schroder *et al.*, 1987; Eckner *et al.*, 1991). Translation can be divided into four stages: initiation, elongation, termination, and recycling (Sonenberg *et al.*, 1978; Sonenberg *et al.*, 1979; Altmann *et al.*, 1985; Altmann *et al.*, 1989). During initiation (Fig. 1), the ribosome is assembled at the initiation codon of the mRNA along with a methionyl initiator tRNA (Met-tRNA$_i^{Met}$) bound to the peptidyl (P) site of the ribosome (Edery *et al.*, 1983; Grifo *et al.*, 1983; Pestova *et al.*, 1996). Numerous eukaryotic initiation factors (eIFs) are involved in the initiation step to prepare the mRNA for binding to the 40S ribosomal subunit, assist in locating the initiation codon, and promote binding of the 60S subunit of the ribosome (Tarun and Sachs, 1996; Tarun *et al.*, 1997; Kessler and Sachs, 1998). Prior to its recruitment to an mRNA, the 40S ribosomal subunit must acquire competency for initiation (Ladhoff *et al.*, 1981; Grifo *et al.*, 1983; Wells *et al.*, 1998). This is largely achieved through the formation of a ternary complex consisting of eIF2, Met-tRNA$_i^{Met}$, and GTP that after assembly at the initiation codon, assists in the identification of the initiation codon and delivering the initiator tRNA to the P site of the ribosome (Hoerz and McCarty, 1969; Both *et al.*, 1975; Muthukrishnan *et al.*, 1975; Nasrin *et al.*, 1986; Jivotovskaya *et al.*, 2006). eIF2 is composed of three subunits, namely α, β, and γ; the γ subunit shares considerable similarity to other GTP-binding proteins. Another initiation factor termed eIF3 also binds to the 40S ribosomal subunit (Anderson and Shafritz, 1971; Levin *et al.*, 1973). Together with the ternary complex, binding of eIF3 and eIF1A to the 40S subunit forms the 43S complex (Kozak and Shatkin, 1978; Kozak, 1980a; Kozak, 1980b).

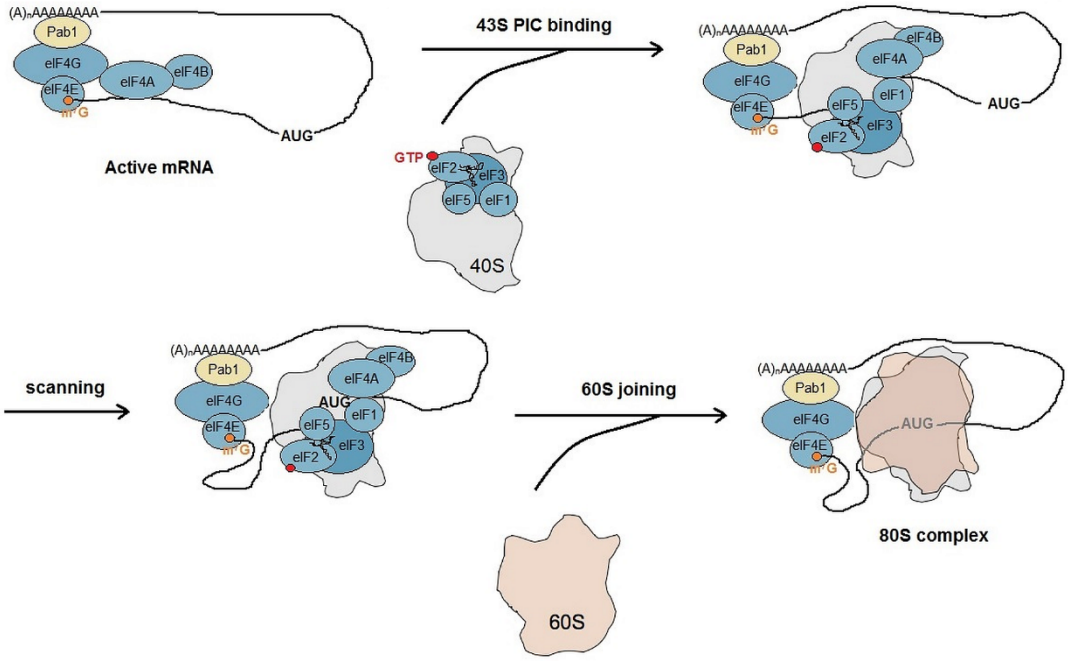

FIGURE 1. Overview of the major steps in the cap-mediated translation initiation pathway in yeast.

Meanwhile, the 5'-cap structure binds to eIF4E, the small subunit of eIF4F. The large subunit of eIF4F, eIF4G serves to recruit several additional initiation factors, including eIF4A, eIF4B, and eIF3. The initiation factors, viz., eIF4F, eIF4A, and eIF4B, use ATP-derived energy to unwind any secondary structure present within the 5' leader sequence of mRNA that would otherwise inhibit 40S ribosome-mediated scanning of mRNA during its search for the initiation codon (Clark *et al*., 1968; Marcus *et al*., 1970; Sprinzl *et al*., 1976; Lake, 1977; Wurmbach and Nierhaus, 1979). Next, poly(A) binding protein (Pab1) combines with the poly(A) tail to come in proximity to protein eIF4G. The resultant circular mRNA becomes translationally active; moreover, the circularization protects the 5' and 3' ends of the mRNA from degradation. In a step requiring eIF4G and eIF3, the 43S complex binds at or close to the 5' terminus of an mRNA and scans its 5' leader to locate the initiation codon (Lockwood *et al*., 1972; Merrick, 1979; Peterson *et al*., 1979a; Peterson *et al*., 1979b; Haghighat and Sonenberg, 1997; Ptushkina *et al*., 1998; Kahvejian *et al*., 2005). Once the 40S subunit is positioned at the initiation codon, the 60S subunit joins to form the translationally competent 80S ribosome (Kappen *et al*., 1973; Siekierka *et al*., 1983).

Translation is initiated repeatedly for a single well-translated mRNA, leading to ample production of specific proteins (Goodman and Rich, 1963; Penman *et al*., 1963; Gross *et al*., 2003). On the other hand, initiation in poorly translated mRNAs is often aborted, leading to a reduction in ribosome occupancy, with a concomitant decrease in the level of the protein product (Gualerzi *et al*., 1977; Bergmann and Lodish, 1979). In response to several environmental stimuli, under stress conditions, and during cellular differentiation and progression during cell-cycle, the levels of certain proteins must be tightly regulated to fulfill the needs of the cell as per the situation. To accomplish this, cells have devised a process whereby subsets of or specific mRNAs are switched on and off by transitioning from highly translated condition to an untranslatable state. Following sections describe the mechanisms of translational control.

The interplay between translation and mRNA decay

All mRNAs within a cell are eventually degraded; however, these differ in their respective half-lives that vary in several orders of magnitude (Herrick *et al*., 1990; Raghavan *et al*., 2002; Sharova *et al*., 2009). The decay of the mRNAs generally begins with the removal of the poly(A) tail by deadenylase enzymes. Shortening of the poly(A) tail causes subsequent removal of the 5' cap through initiating the formation of a complex between mRNA-decapping enzyme and their activators (Brewer and Ross, 1988; Shyu *et al*., 1991; Decker and Parker, 1993). After removal of the mRNA cap, Xrn1 rapidly destroys the body of the mRNA (Jinek *et al*., 2011). Alternatively, mRNAs can be degraded by a complex called exosome (Uchida *et al*., 2004).

The enzymes responsible for degrading mRNAs generally do not have ready access to unprocessed mRNAs. They need to first compete with proteins involved in mRNA translation to get access to the target mRNA (Muhlrad *et al*., 1994; Chowdhury *et al*., 2007; Chowdhury and Tharun, 2009). The circularization of mRNA during translation efficiently protects it from the decay pathway; this configuration does not allow decapping and deadenylase machinery to interact with their respective substrates (Green *et al*., 1983; Stevens *et al*., 1991; Larimer *et al*., 1992; Schwer *et al*., 1998). The activities of protein factors associated with mRNA decay and translation are fundamentally opposite and tightly linked (Beelman *et al*., 1996). The activation of the mRNA decay machinery involves a transition to a state where translation- and stability-promoting proteins are removed from the mRNA. This is followed by the deposition of the mRNA decay machinery onto the target mRNA in a coordinated fashion, leading to its

destruction (Anderson and Parker, 1998; Lykke-Andersen *et al*., 2009; Lykke-Andersen *et al*., 2011).

Several mRNAs, along with some translation factors, are known to accumulate in the cytoplasm as P-bodies and stress granules after exposure to various stress conditions (Buchan and Parker, 2009; Buchan *et al*., 2011; Grousl *et al*., 2009; Hoyle *et al*., 2007). P-bodies are also reported to deposit various mRNA decay factors and thus act as sites of mRNA storage and/or decay. On the other hand, stress granules are probably storage sites for inactive mRNAs and some translation factors that can be reactivated into the translating pool of mRNA after the removal of stress. However, under prolonged exposure to stress, mRNAs deposited in both compartments are degraded by the mRNA-decay machinery (Buchan *et al*., 2013).

Recent advances and state-of-the-art methods in translation research

The complexity of the translation machinery and its rapid response to environmental and physiological changes have been the main challenges of the experimental tools of the translation research. Nevertheless, technical advances in recent years have brought breakthroughs in the field, and its continuous development allows us to study the features of translation in comprehensive approaches.

For each aspect of translation, specific methods have been developed. The mRNA provides the blueprint for protein synthesis. Investigating the translating mRNA has been the subject of recent studies. Due to the non-covalent association of ribosomes with mRNA and the fragile nature of the ribosome nascent-chain (RNC) complex, methods for translating mRNAs have been challenging. Several important methods were developed: full-length translating mRNA profiling (RNC-seq) (Wang *et al*., 2013), polysome profiling (Heyer *et al*., 2016), ribosome profiling (Ribo-seq) (Ingolia *et al*., 2009; 2016), and translating ribosome affinity purification (TRAP-seq) (Inada *et al*., 2002). The basic principles of these approaches are illustrated in Fig. 2. These methods allowed researchers to obtain the translational profiles during the initiation, elongation and termination stages; and to study the untranslated regions, the important players of the translational regulation, in depth.

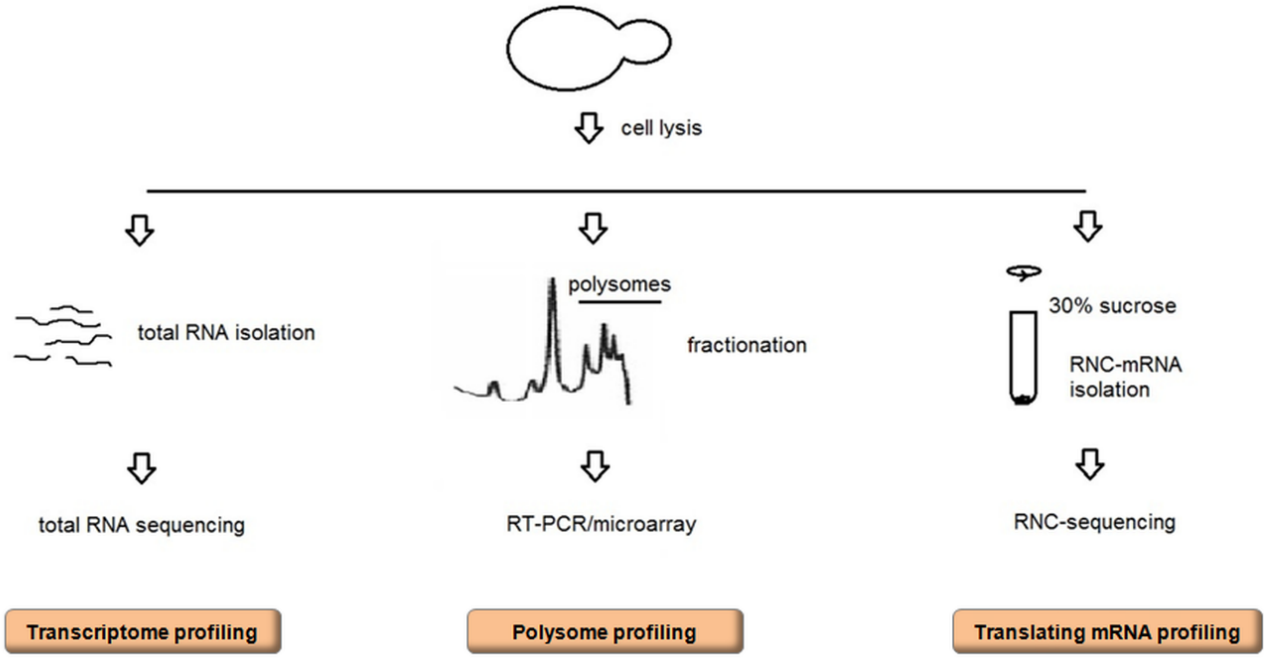

FIGURE 2. Overview of the major methods to investigate translating mRNA.

tRNAs, as essential components of translation, recognize codons on mRNA and transport corresponding amino acids for protein synthesis. Since the types and amounts of tRNAs highly influence the speed of protein synthesis, they have been the focus on many recent studies (Zhong *et al*., 2015; Lian *et al*., 2016; Chen *et al*., 2014). Various kinds of tRNA molecules are highly homologous in nucleotide sequences, and all tRNA species share similar and thermodynamically stable secondary and tertiary structures. Their nucleotides are highly modified compared to other RNA species. All these properties make separation and quantification of individual tRNA species extremely difficult. Nevertheless, recent advances in isolation and quantification of tRNAs in prokaryotes by 2-dimensional electrophoresis coupled with mass spectrometry were reported (Dong *et al*., 1996; Kanaya *et al*., 1999).

Resolution of the sequence of nascent polypeptide chain and its conformation have been the challenge for recent studies. A general and convenient method for detecting the structure of nascent polypeptide chains is the limited protease digestion (Fig. 3). The intact ribosome-nascent chain complex is treated by a non-specific protease such as protease K at low temperature. During the protease treatment, the flexible parts of the protein are easily digested, while the tightly folded elements are less accessible to the protease and thus remain uncleaved. The cleavage products can be analyzed by gel electrophoresis or autoradiography to reveal the folded regions of the nascent chains (Zhang *et al*., 2009; Chen *et al*., 2014).

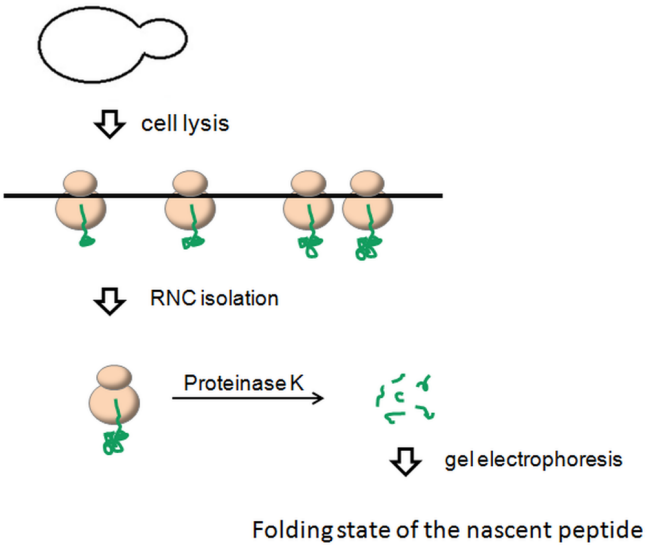

FIGURE 3. Overview of the major methods to investigate folding state of nascent polypeptide.

As discussed above, mRNA decay is an important process in controlling mRNA abundance and mRNA decay can be coupled with translation. In the recently described method called 5Pseq, 50-monophosphorylated (5P) ends of decapped transcripts and mRNAs from the same sample were treated with a phosphatase to block the 5P end of decapped transcripts and the capped mRNAs were captured and sequenced in parallel. Comparison of the sequences of the capped and decapped samples, reveals the location of mRNA degradation intermediates. 5Pseq can also reveal ribosome dynamics such as ribosome pausing and termination (Pelechano *et al*., 2017).

In order to decipher the steps of translation in live cells, single molecule fluorescence resonance energy transfer (FRET) can be employed. The reaction rates within the elongation cycles (Chen *et al*., 2013) and mRNAs undergoing translation were studied by FRET (Stevens *et al*., 2012). Nascent chain tracking (NCT) was also possible, where multi-epitope tags and antibody-based fluorescent probes were employed to monitor the protein synthesis dynamics at the single mRNA level (Morisaki *et al*., 2016).

Proteins execute all kinds of biological functions in life; thus, they are under delicate balance and control. These recent advances revealed that translational control is faster and more sensitive than transcriptional control and that the translation regulation accounts for more than half of all regulatory components. Therefore, translational regulation is the most important regulatory element in organisms.

Regulation of Translation

The regulation of translation is necessary for modulating the expression of several genes when a more rapid and spatial regulation is required. Under various stress conditions, translation is used to fine-tune the levels of several proteins in both time and space (Kuersten and Goodwin, 2003; Wickens *et al*., 2000). Two general modes of control can be described. The first one is global control, where the translation of most or all mRNAs in the cell is regulated; and the other is mRNA-specific control, involving modulation of translation of a subset of mRNAs, without affecting the general protein synthesis or the cellular proteome profile. The global regulation mainly occurs via interference with the translation initiation through the modification of translation-initiation factors. On the other hand, the mRNA-specific regulation is controlled by protein complexes that recognize and bind to certain elements present in the target mRNAs.

Global control of translation

Initiation is considered the rate-limiting step of translation. This is evident from the fact that global control of protein synthesis is mostly achieved through changes in the phosphorylation states of initiation factors or their regulators (Spriggs *et al*., 2010). For example, eIF2 delivers the Met-tRNAi to the P site of the ribosome as a part of the ternary complex with bound GTP (Simpson and Ashe, 2012). This GTP is hydrolyzed when the initiator AUG is recognized during translation initiation (Dever *et al*., 2016), producing GDP-bound eIF2. The exchange of GDP for GTP on eIF2, catalyzed by eIF2B (Jennings *et al*., 2013), is required to create a functional ternary complex for a new round of translation initiation (Hinnebusch, 2000). eIF2 consists of three subunits, namely α, β, and γ. Phosphorylation of the α subunit at residue Ser 51 converts eIF2 from a substrate to a competitive inhibitor of eIF2B (Hinnebusch, 2005; Dey *et al*., 2005), consequently leading to no GDP-GTP exchange and inhibition of global mRNA translation (Jennings *et al*., 2017; Krishnamoorthy *et al*., 2001) (Fig. 4). While the relative amounts of eIF2 and eIF2B vary among different tissues and organisms, eIF2 is always present in excess of eIF2B. Accordingly, phosphorylation of even a fraction of it inside the cell is sufficient to quantitatively inhibit eIF2B and block protein synthesis (Dever *et al*., 2016).

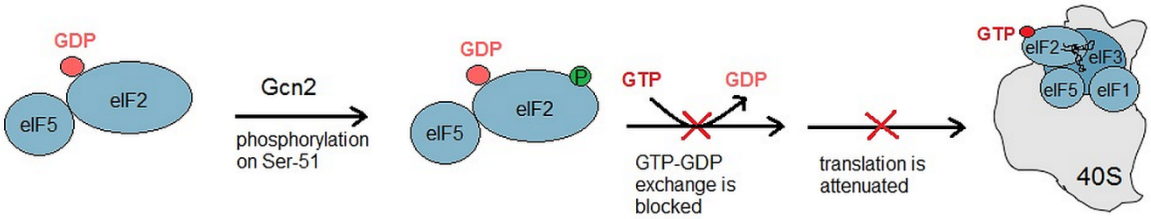

FIGURE 4. Global control of protein synthesis via the phosphorylation of eIF2α.

Four kinases have been identified that specifically phosphorylate eIF2α on Ser 51: HRI (vertebrates), PKR (mammals), PERK (metazoans), and GCN2 (all eukaryotes). All four kinases share a conserved kinase domain, although each of them has a unique regulatory domain.

Although first identified as a stimulator of *GCN4* mRNA translation in yeast (see Section 3.1), Gcn2 was found to specifically phosphorylate eIF2α on Ser 51 (Hinnebusch, 2005; Dey *et al.*, 2005). Moreover, hyperactive alleles of Gcn2 are known to inhibit total cellular protein synthesis. The opposing effects of Gcn2 on *GCN4* and global translation demonstrate that eIF2α phosphorylation can regulate both gene-specific and general translation. In addition to amino acid starvation, yeast Gcn2 can be activated by purine nucleotide starvation and glucose starvation, osmotic stress, or by treating cells with the alkylating agent MMS.

Selection of a subset of mRNAs

The integrity of the eIF4F cap-binding complex and consequently of translation is regulated via an interaction between eIF4E and eIF4G. A negative regulator that interacts with eIF4E to mediate this process, first identified in mammalian cells, 4E-BPs (for eIF4E-binding proteins) contain a distinctive amino acid motif: a tyrosine, three non-specific amino acids, followed by a leucine and a hydrophobic residue, (YXXXLφ). The sequence is similar to that present in eIF4G that binds to the conserved complement on the eIF4E protein. Consequently, 4E-BPs act as competitive inhibitors of eIF4G and compete for binding to eIF4E, thereby inhibiting translation. Several different 4E-BP proteins have been identified in mammalian cells, raising the question if these have undergone functional specialization during evolution (Altmann *et al.*, 1997; Mader *et al.*, 1995; Gingras *et al.*, 1999). The binding of 4E-BP to eIF4E in mammalian cells is regulated through phosphorylation: unphosphorylated 4E-BP binds tightly to eIF4E, whereas its phosphorylation causes it to disassociate from eIF4E (Haghighat and Sonenberg, 1995). The release of 4E-BP permits eIF4E to bind eIF4G, resulting in the phosphorylation of eIF4E, which correlates with increased translation. eIF4E is phosphorylated by Mnk1, a MAP kinase that binds to the C-terminal region of mammalian eIF4G.

Various extracellular stimuli, including hormones, growth factors, and mitogens, are capable of inducing 4E-BP phosphorylation. On the contrary, stress factors such as nutrient deprivation and certain other stress conditions reduce its phosphorylation. For example, rapamycin, an inhibitor of the kinase FRAP/mTOR, inhibits cap-dependent protein synthesis and impairs phosphorylation of several sites on 4E-BP. However, no information on kinase(s) directly phosphorylating 4E-BP *in vivo* is available.

Although 4E-BP orthologs are significantly different from each other, except for the 4E-binding motif, this motif is preserved in most eukaryotes. eIF4E is the central target for controlling eukaryotic gene expression; however, not all 4E-BPs function in the same manner. Some 4E-BP orthologs include, for example, C-terminal extensions, capable of modulating the activity of a particular 4E-BP with alternative binding partners, such as RNA-binding proteins or mRNA degradation enzymes.

In yeast, two proteins, namely Eap1 and Caf20, encoded by two non-essential genes, bind to eIF4E and block the binding of eIF4G, similar to that observed with mammalian 4E-BP. The protein Caf20 was initially identified as p20, a 20 kDa protein that was routinely found to be associated with cap-bound eIF4E (Altmann *et al.*, 1989). Moreover, *in vivo* and *in vitro* competition binding assays demonstrated that Caf20 outcompetes eIF4G for binding to eIF4E. Binding of Caf20 to eIF4E causes eIF4G displacement. *In vitro* translation assays using yeast extract suggested that Caf20 inhibited the cap-dependent translation in the same manner as by mammalian 4E-BP1 and 4E-BP2. The protein Eap1 was identified as a 4E-BP functional ortholog in a far-western assay aimed to probe 4E-interacting yeast proteins (Cosentino *et al.*, 2000). Eap1 contains a large C-terminal extension with no identifiable conserved sequence motifs apart from the eIF-4E-binding motif. Affinity experiments using an m7G cap affinity column monitored the association of eIF4E with eIF4G in the presence or absence of Eap1; it was observed that Eap1 effectively displaced eIF4G from cap-bound eIF4E.

Eap1 and Caf20 are known to inhibit the initiation of translation in response to stress conditions, such as cadmium- and diamide-mediated toxicity in the growth medium or due to the occurrence of membrane stress. The evidence for their involvement in the translational regulation of a subset of mRNAs is derived from the reports that mention these to be independently required for the induction of pseudohyphal growth during nitrogen starvation. Furthermore, mutational studies report that Eap1 mutants exhibit altered responses to oxidative and lipid stress. Yeast lacks a homolog of Mnk1, whereas yeast and plant eIF4G lack the Mnk1 interaction domain that is present in mammalian eIF4G. Therefore, the exact mechanism of activation of Eap1 and Caf20 under the aforementioned stress conditions is not completely understood. Various possibilities exist such as phosphorylation (as is the case of higher eukaryotic 4E-BPs).

However, the molecular details of their actions as an inhibitor of translation have largely remained unknown.

In a recent effort to address which mRNAs interact with the pre-initiation complex and how these are translationally regulated, Costello and coworkers determined and analyzed the yeast *S. cerevisiae* mRNA-binding profiles (Costello *et al*., 2015; Costello *et al*., 2017). Their study revealed an inverse relation between the Pab1 mRNA binding profile and that of the yeast 4E-BPs, Caf20, and Eap1. Moreover, consistent with the hypothesis that yeast 4E-BPs are not global regulators of translation initiation but function to regulate in an mRNA-specific manner (Cridge *et al*., 2010; Sezen *et al*., 2009; Ibrahimo *et al*., 2006), each of the yeast 4E-BPs was found to bind to approximately 1,500 mRNAs under normal growth conditions. Of these, 1,000 mRNAs have been found to be common to both (Costello *et al*., 2015; Castelli *et al*., 2015). It is also reported that 4E-BPs targeting mRNAs typically have longer ORFs and are poorly expressed than the average mRNA, a finding consistent with the notion that these mRNAs are not critical and important during unlimited, exponential growth. Hence, their translation is repressed by the action of 4E-BPs.

The work by Costello and coworkers also revealed a large group of proteins that strongly interact with both eIF4F and repressive 4E-BPs (Costello *et al*., 2015). These proteins display a broad range of functions, including transcription, protein phosphorylation, and cell cycle regulation. Furthermore, this group comprises 79 of the 127 protein kinase-encoding mRNAs, whereas no other group contains any protein kinase mRNA. The authors infer that the mRNAs in this group encode for proteins involved in processes that are tightly regulated within the cell, including signaling pathways that get activated in response to external stimuli. This observation unleashes the central role of 4E-BPs in translational control of a subset of mRNAs via maintaining a delicate balance in the pre-initiation complex relative to 4E-BPs to fine-tune the expression of specific proteins.

An observation supporting the fundamental role of yeast eIF4E binding proteins in regulation of the translation of particular mRNA targets was reported during the yeast-to-hyphal-form transition (Park *et al*., 2006). mRNA transcripts of *STE12*, *GPA2*, and *CLN1* were found to be preferentially recruited to polyribosomes and consistently, the levels of Ste12, Gpa2, and Cln1 proteins also increased during filamentation. The up-regulation of *STE12* mRNA translation in this process was found to be dependent on *CAF20,* but the up-regulation of *CLN1* mRNA translation was independent of *CAF20*, indicating that different mechanisms regulate *CLN1* and *STE12* expression.

mRNA Specific Translational Control

mRNA selection via mRNA regulatory elements

Translation in eukaryotes initiates at the 5' end of the mRNA. The untranslated region (UTR) present at this end acts as the entry point for the mRNA into the ribosomes. Contrary to this, mammalian mitochondrial mRNAs completely lack the 5' UTR. Some mRNAs with a very short 5' UTR undergo scanning-free initiation (Haimov *et al*., 2015). Several other eukaryotic 5' UTRs are highly structured and block the entry of the associated mRNA into the ribosome. Yeast 5' UTRs are typically unstructured; however, some contain stable secondary structures that can block the assembly of the 43S pre-initiation complex onto 5' UTR (Kertesz *et al*., 2010). In this regard, the DEAD-box RNA helicase eIF4A, is crucial for unwinding such structures, thus allowing for an efficient ribosome scanning. This is evident from the immediate inhibition of translation upon glucose starvation (occurs before the onset of eIF2 phosphorylation and therefore believed to be independent of eIF2 phosphorylation), owing to the dissociation of RNA helicase eIF4A from the 43S pre-initiation complex (Kozak, 1986). In addition to eIF4A (Parsyan *et al*., 2011), another helicase, Ded1 is required to scan through long, structured 5' UTRs (Sen *et al*., 2015). Ded1 may function independently or in concert with eIF4A and eIF4G (Gao *et al*., 2016). It is speculated that Ded1 may have overlapping functions with eIF4A. Additionally, after eIF4A is released upon recognition of the start site, the DEAD-box helicase Dhh1 takes over and specifically enhances translation of mRNAs having long and highly structured coding regions (Castelli *et al*., 2011; Sen *et al*., 2016; Jungfleisch *et al*., 2017).

An alternative to the cap-dependent protein synthesis is the cap-independent manner of initiating protein synthesis in eukaryotic cells using internal ribosome entry sites (IRESs), in lieu of the cap structure to recruit the 40S ribosomal subunit (Jackson, 2013). The IRES-dependent mechanism is usually less efficient than the cap-dependent translation; however, it is a competitive form of initiation that plays a role in stress conditions when cap-dependent translation initiation is non-functional (Mitchell *et al*., 2005; Sarnow, 1989). For example, yeast Ure2 is a transcriptional regulator involved in nitrogen assimilation and has two forms: one shorter and a longer form (Komar *et al*., 2003). The balance of these two forms affects its response to nitrogen-limiting conditions. Under nitrogen-limiting conditions, the 5' UTR of *URE2*, containing an IRES element, initiates cap-independent translation from this element to produce the shorter functional form of the protein, which is repressed by the initiation factor eIF2A (Reineke and Merrick, 2009).

Another unique strategy to regulate translation exists for yeast *HAC1*. The Hac1 transcription factor in yeast up-regulates a group of genes, responsible for maintaining protein homeostasis. The *HAC1* intron is not spliced in the nucleus by the spliceosome, and the unspliced mRNA is exported to the cytoplasm. Base-pairing interactions between the sequences in the intron and the 5' UTR of the *HAC1* mRNA represses translation of the unspliced HAC1 mRNA (Sathe *et al*., 2015). However, under endoplasmic reticulum stress conditions, Ire1 kinase-endonuclease-mediated-cytoplasmic splicing of the intron leads to Hac1 synthesis. It has been reported that insertion of an in-frame AUG start codon upstream of the interaction site releases the translational block, whereas the overexpression of translation initiation factor eIF4A enhances production of Hac1 from this upstream AUG start codon. These results suggest translation is primarily blocked at the initiation stage. Thus, inhibition of translation of unspliced *HAC1* mRNA demonstrates a unique 5' UTR-intron interaction that represses its own translation at the initiation step.

Only 13% of yeast mRNAs contain upstream open reading frames (uORFs) (Lawless *et al*., 2009), which are structures implicated to have important effects on the translation of their associated main ORFs. For instance, translation of yeast *CLN3* mRNA, which is poorly translated due to the presence of a short uORF, is enhanced in cells overexpressing eIF4E. This finding suggests that increased availability of the general translation initiation factor eIF4E to the initiation sites leads to significant elevation of translation of these mRNAs.

GCN4 and *CPA1* exemplify two distinct mechanisms by which uORFs regulate translation. As discussed in Section 2.1, amino acid deprivation reduces global protein synthesis via phosphorylation of eIF2α by the kinase GCN2, whereas it enhances translation of *GCN4* mRNA (Dever, 2002). The *GCN4* mRNA encodes a transcriptional activator for genes that regulate amino acid biosynthesis. It contains four short uORFs encoding di- or tripeptides, which are scanned by ribosomes before reaching the main *GCN4* initiation codon (Hinnebusch, 1984). The translation of the first uORF promotes an efficient translation of *GCN4*, indicating that a ribosome that has already translated this first uORF resumes translation of the downstream ORF *GCN4* (Fig. 5).

This process termed as "reinitiation" is a relatively rare event, where the 60S ribosomal subunit dissociates at the stop codon during translation termination of the uORF, and the 40S subunit remains associated with the mRNA and resumes scanning. According to this model, the 40S subunit acquires a ternary complex and recruits other initiation factors after the uORF during scanning so as to initiate translation at the downstream *GCN4* ORF. The probability with which the 40S subunit acquires a ternary complex increases as the distance between the uORF and the main ORF increases. Consequently, and in contrast to uORF1, the translation of uORF4 strongly inhibits the translation of *GCN4* ORF. The GC-rich sequence that surrounds the uORF4 stop codon promotes ribosome dissociation and release and contributes to this phenomenon (Grant and Hinnebusch, 1994; Grant *et al*., 1995; Gunišová *et al*., 2016; Munzarová *et al*., 2011; Szamecz *et al*., 2008).

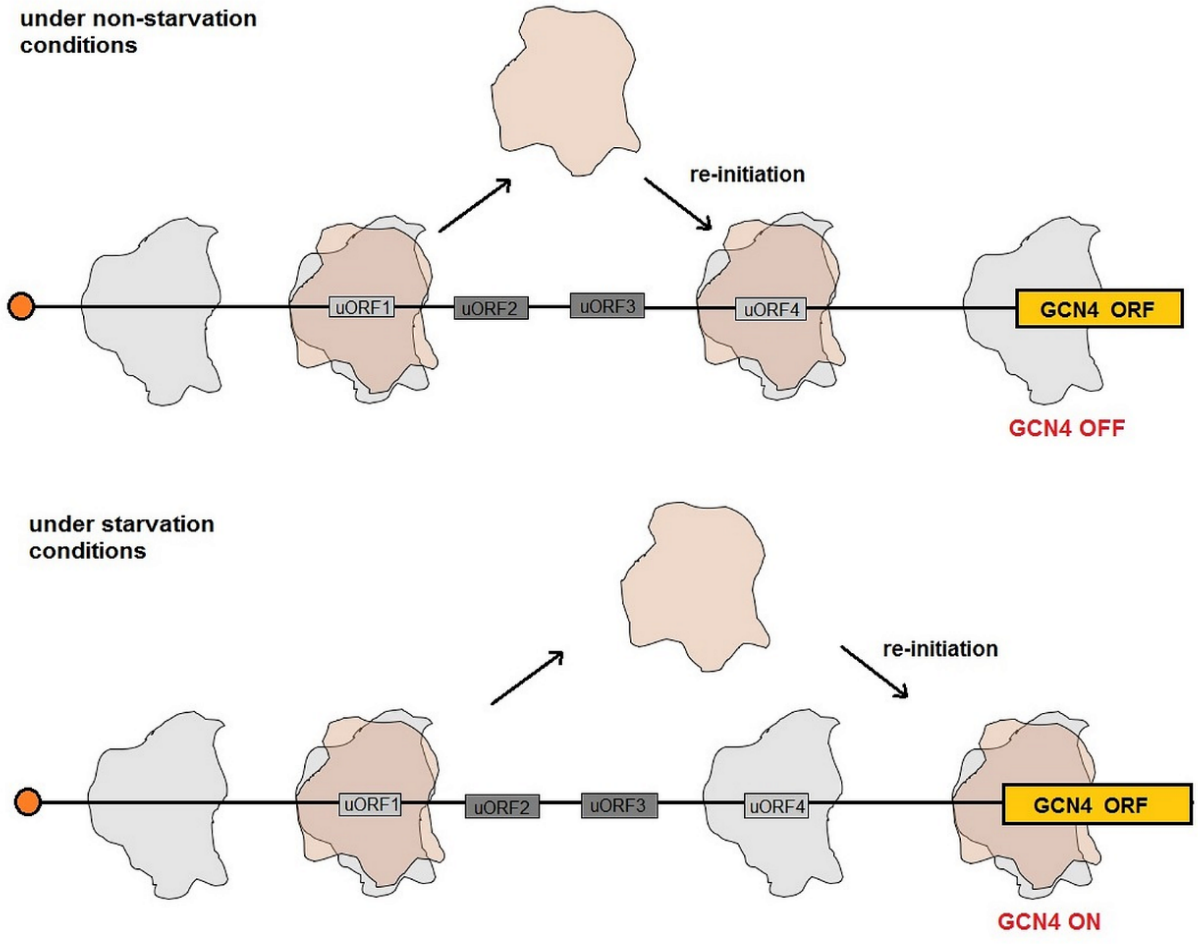

FIGURE 5. Mechanism of regulation of *GCN4* mRNA translation.

When sufficient amino acids are available, the small ribosomal subunit more readily recruits an active ternary complex following the translation of uORF1; translation resumes before *GCN4* ORF and at uORF3 and uORF4. For this reason, few recharged 40S subunits reach the *GCN4* initiation codon, and only basal levels of GCN4 are produced. However, under conditions of amino acid deprivation, the kinase GCN2 phosphorylates eIF2α, thereby reducing the number of active ternary complexes in the cell (Abastado *et al*., 1991). This decreases the recharging efficiency of small ribosomal subunits and increases the number of active 40S subunits that get recruited to the initiation codon of *GCN4*. This explains the paradoxical increase in *GCN4* translation when eIF2α is phosphorylated. Gcn4 activates transcription of amino acid biosynthetic enzymes. Its low expression during normal conditions and specific induction during starvation ensures a strict control of cellular resources.

Yeast *CPA1*, on the other hand, contains a single uORF that encodes for the arginine attenuator peptide (AAP) (Werner *et al*., 1987). Cpa1 catalyzes a step in the synthesis of citrulline, an arginine precursor, and its synthesis is repressed by the translation of this uORF under high arginine levels. Under elevated levels of arginine, it specifically interacts with the uORF-encoded AAP within the ribosome exit channel, causing ribosomes to stall, thereby decreasing the number of ribosomes reaching the downstream initiation codon. When arginine is in low levels and its biosynthesis is required, a leaky scanning of the *AAP* uORF allows for the migration of ribosomes until the *CPA1* ORF. Thus, efficient synthesis of Cpa1 attenuates arginine biosynthesis pathway (Gaba *et al*., 2001; Wang *et al*., 1999). *In vitro* studies have demonstrated that while more efficient reinitiation of translation of the main ORF followed by the *GCN4* uORF1 is possible as compared to the *AAP* uORF, Arg-regulated leaky scanning is observed for *AAP* uORF and *CPA1*. Thus, *GCN4* and *CPA1* uORFs demonstrate uORF-dependent translation control in different ways.

mRNA Selection via mRNA-Binding Proteins

The mRNA-specific translational repression of 4E-BPs is achieved via specialized mRNA-binding proteins that bind to specific sequence motifs or secondary structural elements in mRNA. One of the first examples to be described includes regulation of translation of maternal mRNA during *Xenopus* oocyte maturation and early development by cytoplasmic-polyadenylation-element-binding protein (CPEB). This protein binds to a uridine-rich sequence, i.e., the cytoplasmic polyadenylation element (CPE) that is located in the 3' UTR of target mRNAs. Its binding to mRNA promotes both silencing of the mRNA before oocyte maturation and subsequent cytoplasmic polyadenylation and translational activation. To repress translation, CPEB binds a protein known as Maskin that contains an eIF4E-binding domain, which resembles the one in eIF4G. During early oogenesis, when Maskin is absent, a different mechanism operates to silence the translation of maternal mRNAs in which CPEB binds to eIF4E through 4E-T (4E-transporter). A similar gene-specific inhibition of *oskar* mRNA has been reported in *Drosophila*. Cup, an eIF4E-binding protein, is recruited to the *oskar* mRNA by Bruno; it inhibits the recruitment of the mRNA to the ribosome by competing with eIF4G for eIF4E binding.

The examples of mRNA-specific translational repression in yeast mediated via mRNA-binding proteins that belong to the Pumilio family (PUF) domain, La motif (LaM) or the K homology (KH) domain of the mRNAs are described below.

Pumilio family proteins

Proteins belonging to the Pumilio family (Puf) are among the mRNA-binding proteins that play a key role in RNA decay and translational control. The PUF domain is defined by eight repeats that are typically located within the 3' UTRs; each of these provides specificity for a single RNA nucleotide base. The high-affinity PUF site begins invariably with bases UGUA, displaying divergence. Depending on the changes observed, repeats 5 to 8, a specific subset of mRNAs that each PUF associates with was determined (Gavis, 2001). The six Puf family proteins in yeast display shared functions and bind to different sets of mRNAs (Gerber *et al*., 2004). For example, translational products of Puf4- and Puf5-associated mRNAs are mainly nucleolar (Gerber *et al*., 2004), whereas Puf3 primarily binds to mRNAs that are localized to the mitochondrial periphery (Saint-Georges *et al*., 2008). Their translation products are reported to be involved in mitochondrial biogenesis and respiration (Lapointe *et al*., 2017). The exposure of cells to hydrogen peroxide weakens the Puf3–polysome, downregulating the translation of Puf3-bound mRNAs. Glucose starvation conversely causes activation of translation of bound mRNAs following phosphorylation of N-terminal region of the Puf3 protein (Kershaw *et al*., 2015). Thus, Puf family proteins represent a class of mRNA-binding proteins that can up- or downregulate the translation of a specific set of mRNAs in response to different external stimuli (Rowe *et al*., 2014; Lee and Tu, 2015; Haramati *et al*., 2017).

Next example is of yeast *ASH1* mRNA localization, required for mating-type switching (Deng *et al*., 2008; Paquin *et al*., 2007). The *ASH1* transcripts are localized at the bud cortex during late anaphase, which restricts the Ash1 protein to the daughter cell. *ASH1* mRNA localization depends on active transport along actin bundles through the action of various proteins, such as She1/Myo4, She2, and She3. She2 is an RNA-binding protein that recognizes structural elements in the *ASH1* transcript. It recruits Myo4, a type V myosin, to the *ASH1* mRNA via the adaptor protein She3. *ASH1* expression is confined to the bud cortex through its translational repression by preventing its transport and avoiding premature protein synthesis. The *ASH1* mRNA contains stem-loops in both its ORF and 3' UTR. Its silencing before it is localized to the bud cortex in late anaphase is mediated by binding of translational repressors Puf6 and Khd1 to these secondary structures (Fig. 6). It is proposed that Puf6 interferes with the conversion of the 48S complex into the 80S complex by preventing recruitment of 60S subunit during initiation; this repression is mediated through the general translation factor eIF5B. This repression is relieved by CK2 phosphorylation in the N-terminal region of Puf6 upon localization of the mRNA to the bud tip, thereby restricting its synthesis to its own special niche.

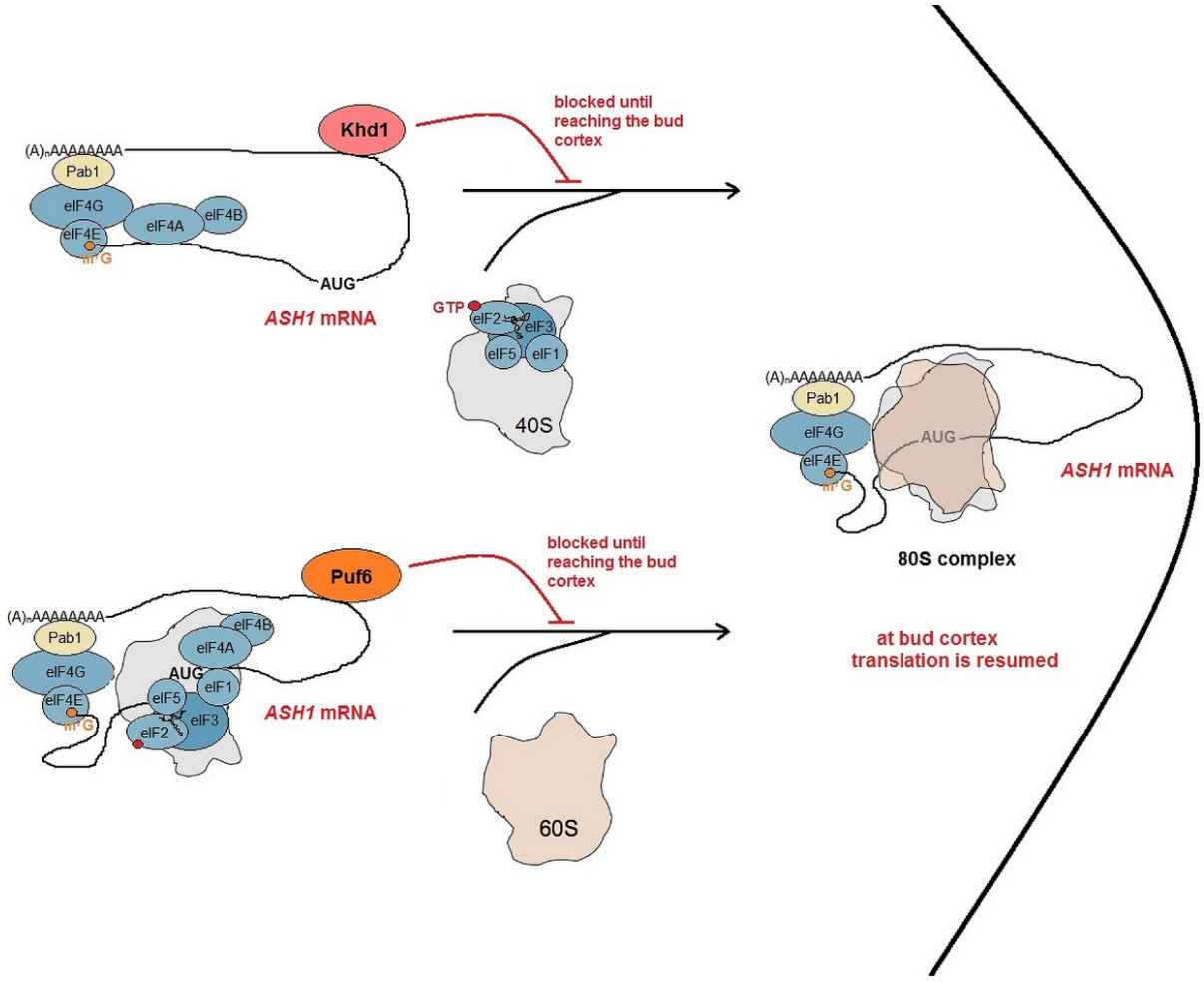

FIGURE 6. Local regulation of *ASH1* mRNA translation.

La-related proteins

The superfamily of LaM-containing proteins has been divided into two distinct subfamilies, namely the genuine, or "authentic" La proteins and the La-related proteins (LARPs). The La family proteins are known to recognize terminal oligouridines (…UUU-OHs) that are present in newly synthesized RNA polymerase III transcripts (Maraia *et al*., 2017). These have been reported to function in important pathways related to noncoding RNA metabolism. The primary function of La is to assist in the folding of these transcripts, thus protecting them from exonucleolytic degradation. The genuine or "authentic" La family proteins, including yeast Lhp1, consist of adjacent RNA recognition motifs to mediate RNA binding. Lhp1 acts as a chaperone for RNA polymerase III transcripts and has a role in their 3' end maturation. On the other hand, the yeast LARPs, such as Slf1 and Sro9, consist of LaM, but lack the adjacent RNA-recognition motif. Slf1 and Sro9 are known to selectively bind to approximately 500 mRNA targets and function in RNA metabolism pathways (Sobel and Wolin, 1999; Kershaw *et al*., 2015; Schenk *et al*., 2012). Slf1 protein has been shown to play a central role in translational regulation of approximately 40% of the proteome under oxidative stress. They may also exert protective effects on cells under copper ion exposure stress and glucose starvation, owing to their hyperphosphorylation and storage in the P bodies under these conditions.

Yeast LARPs Slf1 and Sro9 have also been reported to associate with ribosomes through binding with the 40S ribosomal protein Asc1 (Opitz *et al*., 2017; Schäffler *et al*., 2010). Through this interaction, they are proposed to act as translational activators for the bound mRNAs via an unresolved mechanism.

Scp160

The yeast homolog of human vigilin, Scp160, is a highly conserved mRNA-binding protein. It contains 14 tandem repeats of heterogeneous KH domains that are implicated in RNA binding (Li *et al*., 2004). It interacts with free and membrane-bound ribosomes (Frey *et al*., 2001) and predominantly localizes to the ER in a microtubule-dependent manner. Several microarray analyses data of RNAs obtained from affinity-isolated Scp160-containing complexes revealed that Scp160 associates with a specific subset of mRNAs comprising over 1000 mRNAs. Moreover, Scp160 binds mRNAs that encode for functionally (proteins functioning in the cell wall and ER organization, and ribosome biogenesis and assembly (Hogan *et al*., 2008; Li *et al*., 2003)) and cytotopically (cell wall, plasma membrane, and ER-residing proteins) related proteins. These findings and the fact that mRNAs undergo changes in ribosome association following depletion of Scp160 (Hirschmann *et al*., 2014), raise the possibility of the existence of Scp160-based, gene-specific regulation of translation.

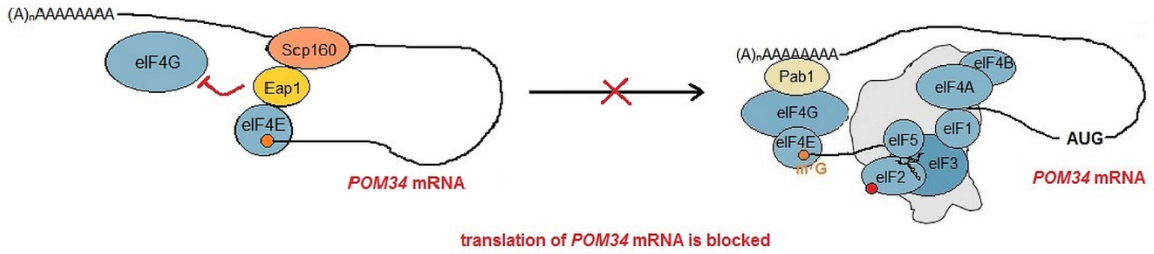

FIGURE 7. Specific translational repression of *POM34* mRNA.

The protein Scp160 interacts with several translation factors. For example, protein-protein crosslinking studies have identified an interaction between the C-terminus of Scp160 and eIF1A. Scp160 physically interacts with 40S ribosomal subunit protein Asc1 (Baum *et al*., 2004). Moreover, Scp160-containing mRNPs also contain Pab1. Similarly, synthetic lethal interactions between *scp160* and yeast 4E-BP *eap1* have been reported. Scp160 was also shown to physically associate with Eap1 in an RNA-dependent manner. Moreover, loss of *EAP1* significantly affects the complex associations and protein localization of Scp160. The loss of *SCP160* has been shown to alter the complex associations and protein localizations of Eap1.

Given these properties, Scp160 appears to function to select mRNAs and bring these into close contact with the translation machinery or the translational repressor Eap1. Thus, it may be proposed that Scp160 may function in both translational activation and repression of its target mRNAs. A recent study that identified SESA (composed of proteins *S*my2, *E*ap1, *S*cp160, and *A*sc1) system to be a part of the mechanism regulating translation of *POM34* mRNA confirms this hypothesis (Sezen *et al*., 2009) (Fig. 7). *POM34* encodes an integral membrane protein, which together with the other two nuclear pore complex (NPC) components, namely Pom152 and Ndc1, constitutes an important structure for NPC biogenesis. SESA does not affect the mRNAs of other NPC proteins (e.g., *POM152*, *NDC1*) and selectively suppresses the translation of only *POM34* mRNA (Ergüden, 2017). Another interesting finding is that activation of SESA system by an unknown mechanism upon spindle pole body (SPB) duplication defects causes *POM34* mRNA to remain in the cytoplasm. Under this condition, translation is inhibited without binding to the polysomal- rich endoplasmic reticulum. As a result, Pom34 levels decrease to one-fifth of the normal levels, thereby allowing these cells to survive when the SPB duplication is defected.

Conclusions

Both global control of protein synthesis and regulation of mRNA-specific translation represent key mechanisms of gene modulation. Although mechanisms of global translation control have been studied extensively, literature related to mRNA-specific translational regulation is scant and being uncovered recently. The mRNA-specific regulation mostly involves mRNA sequence and structural elements and multiprotein regulatory assemblies. Understanding the detailed mechanistic steps at which these assemblies control translation initiation and their interplay with the translation–initiation factors along with the contribution of recently described ribosome structural heterogeneity, are fields that need further and a thorough investigation to get a better understanding of the complex regulation of translation process.

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