

Chapter 6

Communication Is Key: 5'–3' Interactions that Regulate mRNA Translation and Turnover



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Abstract Most eukaryotic mRNAs maintain a 5' cap structure and 3' poly(A) tail, cis-acting elements that are often separated by thousands of nucleotides. Nevertheless, multiple paradigms exist where mRNA 5' and 3' termini interact with each other in order to regulate mRNA translation and turnover. mRNAs recruit translation initiation factors to their termini, which in turn physically interact with each other. This physical bridging of the mRNA termini is known as the “closed loop” model, with years of genetic and biochemical evidence supporting the functional synergy between the 5' cap and 3' poly (A) tail to enhance mRNA translation initiation. However, a number of examples exist of “non-canonical” 5'–3' communication for cellular and viral RNAs that lack 5' cap structures and/or poly(A) tails. Moreover, in several contexts, mRNA 5'–3' communication can function to repress translation. Overall, we detail how various mRNA 5'–3' interactions play important roles in posttranscriptional regulation, wherein depending on the protein factors involved can result in translational stimulation or repression.

Keywords mRNA translation · mRNA decay · RNA-binding proteins · Posttranscriptional control · Protein–protein interactions

6.1 The Genesis of the Closed-Loop Model

The idea that the termini of eukaryotic mRNAs functionally interact in order to regulate protein synthesis is not a new hypothesis. Primarily based on electron micrograph images of polysome-bound mRNAs, it was proposed in the mid

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twentieth century that eukaryotic mRNAs translate as circular complexes rather than as linear molecules (Mathias et al. 1964; Philipps 1965; Baglioni et al. 1969). This was posited to allow for terminating ribosomes to be “recycled” rather than falling off the mRNA, thus enhancing mRNA translation. While enticing, this model was nevertheless proposed without any genetic or biochemical evidence to support it. Studies in the following decades identified the mRNA 5' cap structure and 3' poly(A) tail, as well as the translation factors that bind these cis-acting elements and stimulate translation, including the 5' cap-bound eIF4F complex and the poly(A)-binding protein (PABP) (Tarun et al. 1997; Imataka et al. 1998). Importantly, it was shown that eIF4F physically binds PABP to stimulate protein synthesis and lent credence to a model where this interaction helps to bridge the mRNA termini. This model became commonly referred to as the “closed loop” model for translational control (Gallie 1991; Amrani et al. 2008). Moreover, there now exist multiple examples where alternative 5'–3' interactions between protein and RNA elements at the mRNA termini are utilized to stimulate the translation of select cellular and viral mRNAs that lack either a 5' cap and/or poly(A) tail. Finally, just as 5'–3' mRNA interactions promote translation, a number of examples exist where the remodeling of mRNA circularization plays an important role in repressing the translation of specific mRNAs. In this chapter, we provide a broad overview of the different modes of communication between mRNA termini and how they stimulate or inhibit mRNA translation and decay.

6.2 5' Cap- and 3' Poly(A) Tail-Dependent Translation

The majority of eukaryotic mRNAs maintain a 5' cap structure (m^7GpppN) and a 3' poly(A) tail. Early experiments in cells and cell-free systems established that the cap and poly(A) tail elements stabilize mRNAs in an additive manner, but synergistically stimulate mRNA translation (Gallie 1991; Iizuka et al. 1994; Tarun et al. 1997; Preiss and Hentze 1998). This interdependency between the cap and poly(A) tail led to the hypothesis that these elements must be directly communicating to engender optimal mRNA translation (Gallie 1991). Data that supported a physical interaction between these terminal elements came with the discovery of translation initiation factors that bind the cap and poly(A) tail structures. The 5' cap is bound by the eIF4F complex, which consists of eIF4E, eIF4A, and eIF4G (Merrick and Pavitt 2018). eIF4E physically contacts the 5' cap and binds to eIF4G, a scaffold protein that also interacts with a number of translation factors including eIF4A, an ATP-dependent RNA helicase, and eIF3. Ultimately, these translation initiation factors function to recruit the 40S ribosomal subunit as part of the 43S pre-initiation complex. Following scanning of the 5' UTR and the identification of a proper start codon, the 60S ribosomal subunit joins the 40S subunit to form a functional 80S ribosome that can initiate translation. Regardless of being at the opposite end of the mRNA, the 3' poly(A) tail stimulates translation by recruiting the poly(A)-binding protein (PABP), which serves as a bona fide translation initiation factor (Kahvejian et al. 2005).

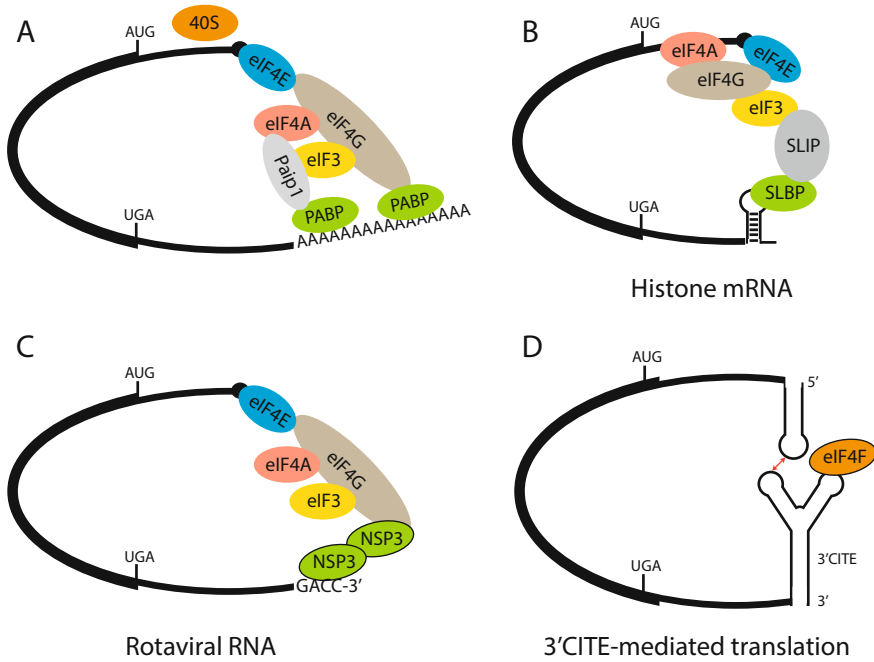


Fig. 6.1 Schematic diagram of mRNA translation initiation mechanisms. (a) Canonical cap- and poly(A) tail-dependent translation. eIF4E interacts with the 5' cap structure and forms the eIF4F complex by binding eIF4A and eIF4G. eIF4G mediates mRNA circularization by simultaneously binding to both eIF4E and PABP on the 3' poly(A) tail. Paip1 may also assist in mRNA circularization by simultaneously interacting with eIF4A, eIF3, and PABP. (b) Histone mRNA translation. Histone mRNAs maintain a 3' stemloop (SL) that recruits the SL-binding protein (SLBP). SLBP in turn interacts with the SLBP-interacting protein (SLIP) that binds eIF3 in order to promote translation initiation. (c) Rotaviral RNA translation. Rotaviral mRNAs maintain a 3' terminal sequence (GACC-3') that recruits NSP3, which dimerizes and interacts with eIF4G to promote mRNA translation. (d) 3' cap-independent translational enhancer (3' CITE)-mediated translation. The 3' CITE is located in the 3' UTR of the viral RNA, where it physically interacts with eIF4F. eIF4F then is brought into proximity with the 5' end of the RNA by a long-distance RNA-RNA interaction between the 3' CITE and an RNA structure in the 5' UTR. Base pairing between the 3' CITE and the 5' UTR is denoted by a red arrow

PABP stimulates translation at least in part by physically contacting eIF4G, an interaction that is conserved from yeast to humans as well as in plants (Tarun and Sachs 1995; Le et al. 1997; Gray et al. 2000; Wakiyama et al. 2000; Kahvejian et al. 2005). As PABP and eIF4G are bound at the 5' and 3' termini of mRNAs, it is postulated that their interaction helps to form a “closed loop” by bridging the two ends of the mRNA (Fig. 6.1a). This model was further reinforced by mRNAs forming closed-loop structures, as observed by atomic force microscopy, in the presence of yeast eIF4G, eIF4E and PABP *in vitro* (Wells et al. 1998). What exactly is the biochemical mechanism by which PABP-eIF4G contact stimulates translation initiation? Several lines of evidence, both in yeast and in cell-free *in vitro* translation

systems, indicate that PABP and the PABP-eIF4G interaction stimulate translation initiation by promoting 40S ribosomal subunit recruitment, 60S ribosomal subunit joining, as well as the interaction between eIF4E and the 5' cap (Ptushkina et al. 1998; Wei et al. 1998; Borman et al. 2000; von Der Haar et al. 2000; Kahvejian et al. 2005). Furthermore, experiments with recombinant mammalian PABP and a fragment of eIF4G that binds PABP have demonstrated that eIF4G binding to PABP increases its affinity to poly(A) RNAs (Safaei et al. 2012).

In addition to binding to eIF4G, PABP also interacts with the PABP-interacting protein 1 (Paip1) which functions to stimulate mRNA translation (Craig et al. 1998). Paip1 shares similarity with the middle domain of eIF4G, which interacts with both eIF3 and eIF4A. In keeping with this, Paip1 also interacts with both eIF4A and eIF3. Specifically, the binding of Paip1 to eIF3 has been reported to stimulate mRNA translation, which was suggested to be due to the stabilization of the PABP-eIF4G interaction (Craig et al. 1998; Martineau et al. 2008). Based on these data, it has been proposed that Paip1 may assist in generating circular mRNAs to promote their translation (Fig. 6.1a).

6.3 Poly(A) Tail-Independent mRNA Translation

As mentioned above, most eukaryotic mRNAs maintain both a 5' cap and 3' poly (A) tail, elements that stimulate their translation. However, several examples exist of cellular and viral RNAs that do not possess a poly(A) tail. Nevertheless, these mRNAs have adopted alternative PABP-independent mechanisms to stimulate their translation that still rely upon contact between their 5' and 3' termini. Two key examples of this are mRNAs that code for replication-dependent histone proteins and rotaviral mRNAs.

Histone mRNA Translation Histones are evolutionarily conserved amongst eukaryotes and maintain pivotal roles in packaging genetic material into chromatin and regulating transcription. There are two types of histones: replication-independent and -dependent histones. Replication-independent histones are expressed throughout the cell cycle and act to modulate chromatin state in a locus-specific manner (Talbert and Henikoff 2017). Replication-dependent histones (referred from hereon as histones) include core histones (H2A, H2B, H3 and H4) that form nucleosomes, the structural unit of chromatin, and H1 linker histones that are found between nucleosomes (Marzluff et al. 2008). Transcription of core histones-encoding genes increases at the beginning of S-phase to accommodate DNA replication, but these transcripts are rapidly degraded at the end of S-phase given that an imbalance between histone and DNA abundance is detrimental, whereby it has been shown to cause chromosome loss and genomic instability (Singh et al. 2010).

Like all eukaryotic mRNAs, histone-encoding mRNAs possess a 5' cap structure. However, histone-encoding mRNAs are unique in that they are the only cellular mRNA species to lack a 3' poly(A) tail. Nonetheless, despite the lack of a poly

(A) tail and the consequential absence of PABP association, the 5′ and 3′ termini of these histone transcripts interface in order to efficiently recruit the pre-initiation complex (Fig. 6.1b). Instead of a poly(A) tail, histone mRNAs possess a conserved 25–26 nucleotide 3′ terminal stem loop (SL). This terminal structure functions to stimulate histone mRNA translation by interacting with the histone stem-loop-binding protein (SLBP), a protein that plays a key role in regulating histone mRNA maturation, degradation, and translation (Wang et al. 1996; Tan et al. 2013; Marzluff and Koreski 2017). Just as PABP stimulates mRNA translation by interacting with eIF4G to circularize canonical mRNAs, SLBP also interacts with the 5′ cap-associated translation machinery on histone mRNAs. However, unlike PABP, SLBP does not directly bind to eIF4G. Instead, SLBP recruits the SLBP-interacting protein (SLIP1), a middle domain of initiation factor 4G (MIF4G)-like protein, which simultaneously interacts with both SLBP and eIF3 to circularize the transcript and promote efficient translation (Neusiedler et al. 2012; von Moeller et al. 2013) (Fig. 6.1b). In keeping with the cell-cycle dependent regulation of histone mRNAs, SLBP levels increase during the G1/S phase to stimulate the production of histones, and is rapidly degraded by the proteasome by the end of the S-phase (Marzluff and Koreski 2017).

Rotaviral PABP-Independent Translation Rotaviral mRNAs maintain a 5′ cap but lack a poly(A) tail and instead terminate with a 3′ GACC sequence (Vende et al. 2000) (Fig. 6.1c). However, rotaviral mRNAs are efficiently translated due to the recruitment of the viral nonstructural protein 3 (NSP3) to this 3′ terminal element. NSP3 enhances viral translation by simultaneously interacting with the 3′ GACC viral element and with eIF4G in a manner similar to PABP (Vende et al. 2000; Groft and Burley 2002; Gratia et al. 2015). Interestingly, NSP3 has been reported to interact with eIF4G as a dimer, which binds eIF4G with a tenfold higher affinity as compared to PABP (Deo et al. 2002). In addition to directly enhancing rotaviral mRNA translation initiation, NSP3 binding to eIF4G is proposed to assist rotavirus infection by displacing PABP from eIF4G and leading to PABP nuclear localization, thereby shutting down host protein synthesis (Harb et al. 2008). Thus, NSP3 provides an alternative mode of circularizing viral mRNAs and selectively enhancing viral translation in the absence of a 3′ poly(A) tail (Fig. 6.1c).

6.4 Long-Distance RNA-RNA Interactions that Support Cap- and Poly(A) Tail-Independent Translation

A number of positive-strand plant RNA viruses lack both cap and poly(A) tail structures but have adopted unique modes of mRNA circularization in order to stimulate the production of viral proteins (Miller and White 2006; Nicholson and White 2011, 2014). These include viruses from the *Tombusviridae* and *Luteoviridae* families, which maintain highly structured RNA elements in their 3′ UTRs that are termed cap-independent translational enhancer (CITE) elements (Simon and Miller

2013). In general, 3' CITEs function by physically recruiting the eIF4F translation initiation complex to the 3' UTR of viral RNAs (Gazo et al. 2004; Treder et al. 2008; Wang et al. 2009; Nicholson et al. 2010, 2013). However, some viral 3' CITE elements directly bind ribosomal subunits independently of eIF4F to stimulate viral translation (Stupina et al. 2008; Gao et al. 2012). While 3' CITEs are necessary to promote viral translation, they must communicate with the viral 5' UTR. This is mediated by long-distance RNA–RNA interactions between RNA stem loop structures in the 5' UTR and the 3' CITE, thus generating RNA–RNA-based closed-loop interactions (Fig. 6.1d). Site-directed mutagenesis experiments have demonstrated the functional significance of these long-distance RNA–RNA interactions in stimulating viral translation. Viral translation was inhibited when 5' UTR/3' CITE base pairing was disrupted, however, compensatory mutations that reestablished these long-distance interactions efficiently rescued translation (Guo et al. 2001; Fabian and White 2004, 2006; Nicholson and White 2008; Nicholson et al. 2010, 2013). Thus, it has been proposed that 3' CITE elements recruit translation factors or ribosomal subunits to viral 3' UTRs, which are then brought into proximity with the 5' UTR in order to facilitate translation initiation.

6.5 5'–3' Interactions that Repress mRNA Translation

Just as 5'–3' interactions are critical for stimulating eukaryotic translation, a number of repressive mechanisms exist that rely upon contact between the mRNA termini in order to inhibit mRNA translation. An overarching theme for these regulatory mechanisms is the tethering of translational repressor proteins to specific cis-acting elements in the 3' UTRs of select mRNAs. These, in turn, interface with the mRNA 5' terminus and shut down protein synthesis. In general, these translational repressors fall into two classes: 5' cap-binding proteins, such as the eIF4E homolog protein 4EHP, or eIF4E-binding proteins, such as 4E-T, CUP, Maskin and Neuroguidin.

4EHP and 4E-T The eIF4E homolog protein (4EHP) represents a translational repressor that is similar to eIF4E, in that it binds to the mRNA 5' cap structure (Rom et al. 1998). However, unlike eIF4E, 4EHP does not interact with eIF4G and therefore acts to repress mRNA translation initiation. In flies, *Drosophila* 4EHP (d4EHP) targets select mRNAs during embryogenesis, including the caudal and hunchback encoding mRNAs (Cho et al. 2005, 2006; Lasko 2011). d4EHP is recruited to the caudal mRNA via Bicoid, an RNA-binding protein that interacts with the Bicoid-binding region (BBR) in the caudal mRNA 3' UTR (Cho et al. 2005). d4EHP then interacts with the caudal mRNA 5' cap structure, thus circularizing the mRNA, displacing eIF4E and shutting down caudal protein synthesis (Fig. 6.2a). d4EHP uses a similar mechanism to inhibit hunchback mRNA translation by simultaneously interacting with the 5' cap and a 3' UTR-bound RNA protein complex. However, instead of binding Bicoid, d4EHP interacts with a complex of three proteins, Nanos (NOS), Pumilio (PUM), and brain tumor protein (BRAT),

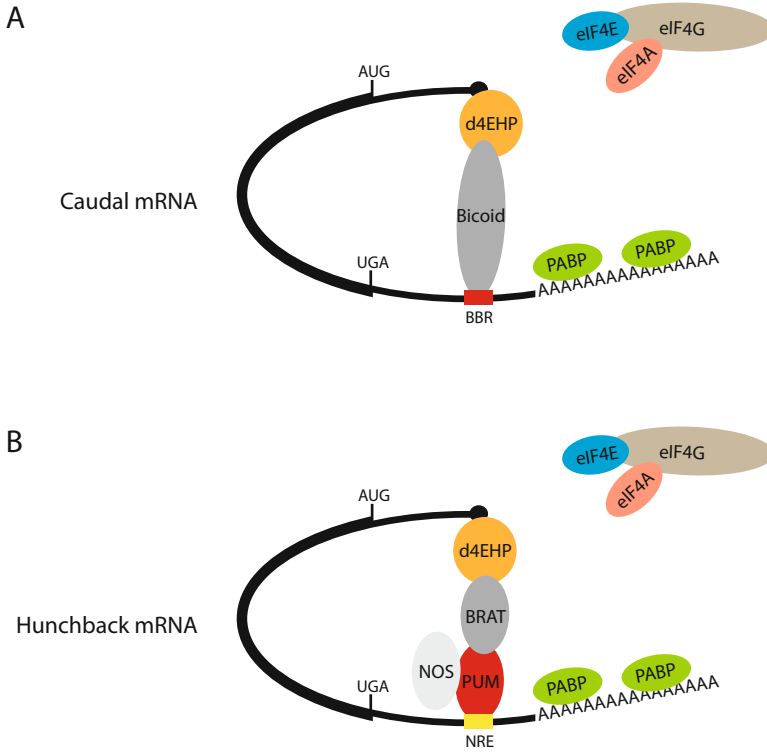


Fig. 6.2 Schematic diagram of drosophila 4EHP (d4EHP)-mediated translational repression. (a) Caudal mRNA is translationally repressed by the d4EHP/Bicoid complex, which simultaneously binds to the Bicoid-binding region (BBR) in the caudal mRNA 3' UTR and the 5' cap structure. (b) Hunchback mRNA is translationally repressed Pumilio (PUM), which binds to the Nanos response element (NRE). There, it interacts with Nanos (NOS) and Brain Tumor (BRAT). BRAT binds d4EHP, which interacts with the 5' cap structure to inhibit mRNA translation

which are recruited to the Nanos-responsive element (NRE) in the hunchback 3' UTR (Cho et al. 2006) (Fig. 6.2b). d4EHP therefore plays an important role in the development of the *Drosophila* embryo by making sure that Caudal and Hunchback proteins are produced in the proper locations within the embryo.

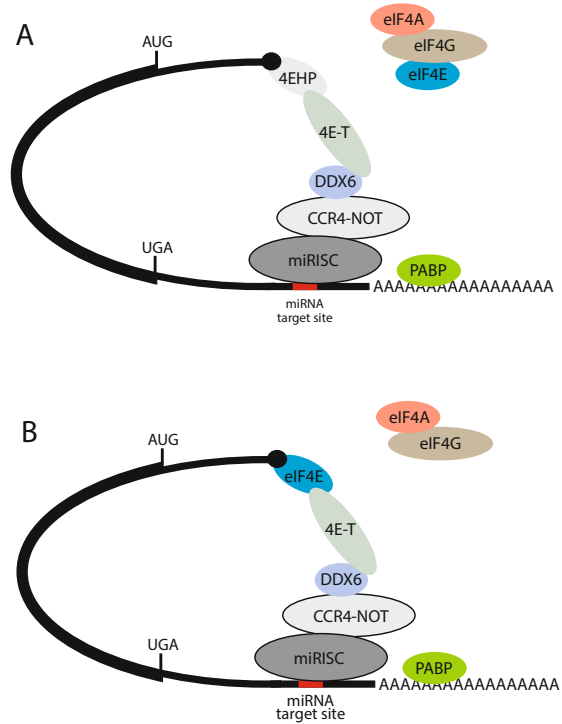
Proteomic and structural analyses of mammalian 4EHP have determined that it has two major binding partners: the RNA-binding protein GIGYF2 and the eIF4E-binding protein 4E-T (Morita et al. 2012; Chapat et al. 2017; Peter et al. 2017; Amaya Ramirez et al. 2018). Although less is currently known regarding the function of the GIGYF2/4EHP complex, several groups have implicated 4E-T in the translational repression and turnover of microRNA-targeted mRNAs (Kamenska et al. 2014a, 2016; Nishimura et al. 2015; Ozgur et al. 2015; Chapat et al. 2017; Duchaine and Fabian 2019). Like other 4E-BPs, 4E-T competes with eIF4G for binding to eIF4E (Dostie et al. 2000). However, in contrast to eIF4E-binding proteins such as eIF4G, 4E-T also has the ability to bind to 4EHP (Kubacka et al. 2013;

Chapat et al. 2017). In addition to containing an N-terminal eIF4E/4EHP-binding motif, 4E-T also interacts with proteins involved in mRNA translational repression and turnover, including UNR, LSM14, PATL1, and DDX6 (Dostie et al. 2000; Nishimura et al. 2015; Kamenska et al. 2016; Brandmann et al. 2018). How is 4E-T recruited to miRNA-targeted mRNAs? Briefly, the miRNA-induced silencing complex (miRISC) recruits a number of factors to targeted mRNAs that engender translational repression and mRNA decay (Jonas and Izaurralde 2015; Duchaine and Fabian 2019). These include the CCR4-NOT deadenylase complex and the translational repressor and decapping enhancer protein DDX6, which binds to the CNOT1 subunit of the deadenylase machinery. The crystal structure of the CNOT1/DDX6/4E-T complex was recently solved and demonstrates that DDX6, when directly bound to CNOT1, forms a unique complex with 4E-T (Ozgur et al. 2015). From a functional standpoint, several studies have reported that the 4E-T/4EHP complex plays a role in miRNA-mediated translational repression (Chapat et al. 2017; Jafarnejad et al. 2018). Knocking down 4EHP in mammalian cells partially impaired miRNA-mediated translational repression (Chapat et al. 2017; Chen and Gao 2017) and 4EHP has also been reported to be important for silencing the DUSP6 mRNA by miR-145 (Jafarnejad et al. 2018). Taken together, these data lend credence to a model where the 4E-T/4EHP complex is recruited by the CCR4-NOT complex to miRNA-targeted mRNAs where it has been postulated that 4EHP competes with eIF4E for the 5' cap, thus inhibiting mRNA translation (Fig. 6.3a).

In addition to playing a role in translational repression, 4E-T has also been linked to enhancing mRNA decay of CCR4-NOT targets, including miRNA-targeted mRNAs and transcripts regulated by the AU-rich element (ARE)-binding protein tristetraprolin (TTP) (Ferraiuolo et al. 2005; Nishimura et al. 2015). Complementation experiments in HeLa cells demonstrated that a 4E-T mutant that cannot bind to eIF4E (or 4EHP) was unable to efficiently bring about the destabilization of miRNA- and TTP-targeted mRNAs. It was thus suggested that 4E-T acts to enhance mRNA decay by bringing its interaction partners, that include the decapping factors LSM14, PATL1 and DDX6, into proximity with the 5' terminus by binding to 4EHP (Fig. 6.3a) or eIF4E (Fig. 6.3b) (Nishimura et al. 2015).

CUP The *Drosophila* protein CUP is a well-characterized eIF4E-binding protein (4E-BP) that functions in the spatial and temporal regulation of specific mRNAs during oogenesis and embryogenesis. 4E-BPs [reviewed in (Kamenska et al. 2014b)] represent a class of translational regulators that compete with eIF4G bound on eIF4E to inhibit translation initiation. 4E-BPs include, but are not limited to, 4E-T, 4E-BP1, and Thor, which possess a canonical (C) (YXXXXLΦ, where X is any residue and Φ is hydrophobic) and a noncanonical (NC) eIF4E-binding site (Igreja et al. 2014). CUP was initially identified as a cytoplasmic protein present in *Drosophila* oocytes that functions in the translational repression and localization of oskar mRNA (Keyes and Spradling 1997; Wilhelm et al. 2003). Importantly, the proper localization of oskar mRNA is critical for posterior patterning of the embryo and germ line establishment (Ephrussi et al. 1991; Kimha et al. 1991). Instead of acting as a general translational repressor, CUP represses specific mRNAs (oskar and nanos)

Fig. 6.3 Schematic models for 4E-T involvement in microRNA-mediated translational repression. Of note, these models are not mutually exclusive. (a) A miRNA-targeted mRNA is bound by the miRISC, which in turn recruits the CCR4-NOT deadenylase complex. The CCR4-NOT/DDX6 complex recruits 4E-T and 4EHP, the latter functioning to displace eIF4E and associated translation factors (i.e. eIF4G and eIF4A) from the cap structure. (b) The CCR4-NOT/DDX6 complex recruits 4E-T, which binds to eIF4E, thereby displacing eIF4G. Figure modified from Duchaine and Fabian (2019) with permission from Cold Spring Harbor Laboratory Press © 2018



by tethering to their 3' UTRs. CUP is recruited to oskar and nanos mRNAs via the RNA-binding proteins Bruno and Smaug (SMG), respectively, that bind to response elements in their 3' UTRs (Wilhelm et al. 2003; Nakamura et al. 2004; Nelson et al. 2004). Thus, CUP represents a tethered translational repressor that simultaneously interacts with eIF4E and 3' UTR-bound RBPs (Bruno or Smaug) in order to bridge the mRNA termini, displace eIF4G, and inhibit the translation of oskar and nanos mRNAs (Fig. 6.4a, b).

Maskin Maskin is an eIF4E-binding protein that plays an important role in regulating gene expression in *Xenopus* oocytes. Translational repression is pivotal during vertebrate oocyte maturation as immature oocytes are arrested at prophase of meiosis I (stage IV) where they synthesize large amounts of mRNA that are silenced and will serve to drive subsequent meiotic progression that takes place in the absence of transcription (Reyes and Ross 2016). Maskin is recruited to targeted mRNAs by directly interacting with the cytoplasmic polyadenylation element-binding (CPEB) protein, which binds to mRNAs containing cytoplasmic polyadenylation elements (CPEs) in their 3' UTRs (Huang et al. 2006; Pique et al. 2008; Igea and Mendez 2010; Novoa et al. 2010). Specifically, Maskin is recruited by CPEB to maternal mRNAs with short poly(A) tails. There, Maskin simultaneously interacts with both CPEB and eIF4E, thereby preventing the association of eIF4E with eIF4G and consequently inhibiting mRNA translation (Stebbins-Boaz et al. 1999) (Fig. 6.4c).

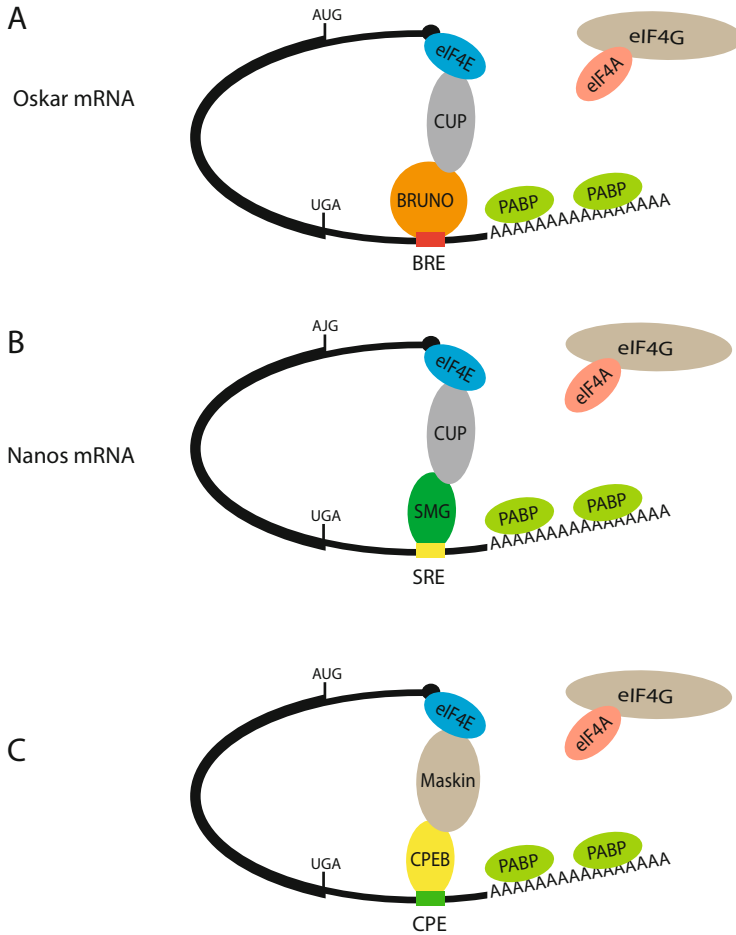


Fig. 6.4 Tethered eIF4E-binding protein-mediated translational repression. (a) Oskar mRNA is translationally repressed by Bruno, which binds to the Bruno response element (BRE). There, Bruno interacts with the eIF4E-binding protein CUP, which binds to the 5' cap-bound eIF4E in order to displace eIF4G and inhibit translation initiation. (b) Nanos mRNA is translationally repressed by Smaug (SMG), which binds to the Smaug response element (SRE). There, Smaug interacts with the eIF4E-binding protein CUP, which binds to the 5' cap-bound eIF4E in order to displace eIF4G and inhibit translation initiation. (c) CPEB is recruited to CPE-containing mRNAs in late-stage *Xenopus* oocytes to repress their translation. CPEB binds to the eIF4E-binding protein Maskin, which binds to the 5' cap-bound eIF4E in order to displace eIF4G and inhibit translation initiation

Maskin-mediated translational repression is then relieved in mature oocytes upon its phosphorylation on one or more of six major sites (T58, S152, S311, S343, S453, S638) by CDK1, which causes the release of eIF4E from Maskin (Barnard et al. 2005). Importantly, Minshall et al. showed that Maskin is only expressed after stage

IV and thus proposed another mode of silencing maternal transcripts in stages I–IV (Minshall et al. 2007). Instead, they found that CPEB interacted with a number of translational repressors, including DDX6 and 4E-T, as well as an eIF4E isoform (eIF4E1b). In contrast to eIF4E, eIF4E1b shows weak binding to both eIF4G and the cap structure. Based on these data, it was proposed that the CPEB/4E-T/eIF4E1b translational repression complex plays a role in early maternal silencing in growing oocytes.

Neuroguidin In addition to being expressed in *Xenopus* oocytes, CPEB is also expressed in neural tissues where it plays a role in regulating synaptic plasticity and memory formation (Darnell and Richter 2012; Rayman and Kandel 2017). Neuroguidin represents a neural-specific CPEB-interacting eIF4E-binding protein. Neuroguidin is not detected in *Xenopus* oocytes, however, ectopic expression of Neuroguidin in oocytes bound CPEB and led to the translational repression of CPEB-containing mRNAs. In addition, knocking down Neuroguidin in the *Xenopus* embryo led to defects in neural crest migration and neural tube closure (Jung et al. 2006). Thus, Neuroguidin may act in a manner similar to Maskin to translationally repress CPEB-targeted mRNAs in neural tissues.

6.6 Conclusions and Future Perspectives

Overall, there is an abundance of biochemical and genetic evidence indicating that interactions between the mRNA 5' and 3' termini regulate mRNA translation. Notwithstanding these data, several key aspects of 5'–3' mRNA communication remain to be elucidated. mRNA circularization via PABP–eIF4G enhances mRNA translation; yet, we do not know how stable these closed-loop structures are. Are they long-lasting interactions during protein synthesis, or transient structures that briefly form and then are disrupted upon translation initiation? Moreover, as much of the data behind this model have been generated using reporter mRNAs or in the context of select mRNA species, it remains to be determined whether all mRNAs require PABP–eIF4G contact or whether specific types of mRNAs are more dependent on mRNA circularization for their efficient translation (Archer et al. 2015; Thompson and Gilbert 2017).

While many observations favor the closed-loop model for promoting translation initiation, it is still a working model that is under investigation (Thompson and Gilbert 2017). Experiments in cell-free systems have shown that the translation of capped mRNAs lacking 3' poly(A) tails can be stimulated upon the addition of free poly(A) RNA in trans (Borman et al. 2002). In addition, this stimulation was abolished upon the addition of a viral protein that disrupts the PABP–eIF4G interaction. Taken together, these data suggest that while the PABP–eIF4G interaction stimulates translation, it may not always act to circularize mRNAs. Recent investigations using cryo-electron tomography suggest that circular polysomes in cell-free systems can exist on mRNAs that lack both a 5' cap structure and a 3' poly

(A) tail (Afonina et al. 2014). Finally, mRNA closed-loop dynamics during translation have recently been investigated *in cellulo* using single-molecule resolution fluorescent *in situ* hybridization (smFISH) and super-resolution microscopy (Adivarahan 2018; Khong and Parker 2018). Both studies conclude that the 5' and 3' ends of actively translating mRNAs rarely co-localize, and that the distance between the mRNA termini increases as a function of ribosome occupancy. Thus, the mRNA closed-loop state may not be stable during translation, and the interaction between eIF4G and PABP may only occur during specific stages of the translation cycle and/or for a subset of mRNAs. In conclusion, while communication between the mRNA termini is a key aspect of translational control, it would be premature to close the book on the closed-loop model.

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