Translational control by 3′-UTR-binding proteins

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Abstract

The regulation of mRNA translation is a major checkpoint in the flux of information from the transcriptome to the proteome. Critical for translational control are the *trans*-acting factors, RNA-binding proteins (RBPs) and small RNAs that bind to the mRNA and modify its translatability. This review summarizes the mechanisms by which RBPs regulate mRNA translation, with special focus on those binding to the 3′-untranslated region. It also discusses how recent high-throughput technologies are revealing exquisite layers of complexity and are helping to untangle translational regulation at a genome-wide scale.

Keywords: *RNA-binding protein; translation; UTR; RNP; CLIP; ribosome profiling*

INTRODUCTION

Translational control is a prevalent mode of gene expression regulation that chiefly contributes to the poor correlation often observed between the transcriptome and the proteome ([1, 2] and references therein). This mode of control allows rapid changes in gene expression and is used in a variety of biological situations, acquiring special relevance in those where transcription from the nucleus is diminished or shut down, such as cellular stress or early development [3, 4].

The substrate of translational control is not just naked messenger ribonucleic acid (mRNA), but mRNA covered with RNA-binding proteins (RBPs) forming RNA ribonucleoprotein particles or RNPs. RBPs dictate the life of the mRNA, from its birth to its death (reviewed in [5, 6]). Sequence-specific RBPs play important roles in translational regulation, sometimes in combination with microRNAs (miRNAs) [7–9]. RBPs bind to specific *cis*-acting elements that can be found across the whole message but are more usually located in the 5′- or 3′-untranslated regions (UTRs) [9]. Although RBPs could in principle activate or repress translation, most known examples depict proteins that repress translation by binding to the 3′-UTR. Indeed, the 3′-UTR is a repository of regulatory elements for mRNA stability, intracellular localization and translation, perhaps because it has evolved free of the constraints associated to ribosome recognition. In this review, we discuss the diverse modes of translational regulation by 3′-UTR-binding proteins and the experimental and conceptual advances provided by current high-throughput technologies.

DIVERSE MODES OF REGULATION BY 3′-UTR-BINDING PROTEINS

Translation consists of initiation, elongation and termination steps but it is the initiation phase that is often targeted for regulation. In eukaryotes, initiation is considered the rate-limiting step of translation and requires the assistance of about 12 initiation factors (eIFs), many of them composed of several polypeptides (reviewed in [10, 11]). A number of eIFs exist in several isoforms that are expressed in a tissue-specific fashion, or that function in particular cellular conditions [12, 13]. The number and nature of eIFs, some functioning as RNA recognition factors or as scaffolding proteins, and some with defined enzymatic properties, provide ample opportunities for regulation of translation initiation [14].

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Most mRNAs initiate translation in a fashion that depends on the universal m^7GpppN cap structure at the 5′-end of the mRNA. During cap-dependent initiation, the small ribosomal subunit together with some initiation factors, including eIF3, forms a 43S particle that is recruited to the mRNA in a cap-proximal position. Ribosome recruitment requires the cap-binding complex eIF4F, which is composed of three polypeptides: the cap-binding protein eIF4E, the scaffolding protein eIF4G and the RNA helicase eIF4A (Figure 1). Interactions between 43S-bound eIF3 and eIF4G contribute to ribosome recruitment in higher eukaryotes. After recruitment, the 43S complex scans the 5′-UTR until an initiator AUG codon in an appropriate sequence context (the Kozak context) is encountered. At this position, the small ribosomal subunit adopts a closed conformation and accepts the incoming large ribosomal subunit, leading to the formation of an 80S ribosome ready to initiate translation.

eIF4G also interacts with poly(A)-binding protein (PABP), which is in turn bound to the poly(A) tail resulting in mRNA pseudo-circularization [15] (Figure 1). This conformation of the mRNA is thought to promote recycling of terminating ribosomes for a new round of translation at the 5′-end and, thus, support efficient translation. The mRNA closed-loop provides a physical framework for the action of 3′-UTR effectors on translation initiation at the 5′-end.

Many RBPs interfere with closed-loop formation and ribosome recruitment. *Drosophila* Bicoid, for instance, binds to the 3′-UTR of *caudal* mRNA and recruits the eIF4E isoform 4EHP (4E homologous protein, also known in mammals as eIF4E2), which binds to the cap structure and inhibits translation because of its low affinity for eIF4G [16] (Figure 1, arrow 1). Mammalian 4EHP also seems to repress translation [17] although, surprisingly, it behaves as an activator under hypoxia [13]. The molecular mechanisms that allow 4EHP to function as a repressor or an activator in different biological contexts are unknown, but this example illustrates the complexities of translational control.

Other RBPs recruit factors that bind to eIF4E and block its interaction with eIF4G (Figure 1, arrow 2). This is the case of *Drosophila* Bruno binding to the 3′-UTR of *oskar* mRNA, or of *Xenopus* CPEB binding to the 3′-UTR of *cyclin B1* mRNA. Bruno and CPEB recruit the 4E-binding proteins (4E-BP) Cup and Maskin, respectively, to repress the translation of the mRNAs to which they bind [18, 19]. The 4E-BPs block eIF4F complex formation and inhibit ribosome recruitment. Similar to 4EHP, CPEB does not always function as a repressor. During *Xenopus* oocyte maturation, phosphorylation of CPEB promotes its interaction with the cleavage and polyadenylation specificity factor (CPSF) and the poly(A) polymerase GLD-2, which elongates the poly(A) tail leading to increased translation [20, 21]. Thus, both 4EHP and CPEB are examples in which the same factor can promote opposite outcomes in translation depending on the biological circumstance.

Translation initiation can also be repressed without disrupting the closed-loop. During inflammation, the γ-interferon-activated inhibitor of translation (GAIT) complex binds to a structured element in the 3′-UTR of *ceruloplasmin* mRNA and, through one of its subunits, the ribosomal protein L13a, interferes with the interaction of eIF4G with eIF3, leading to inhibition of ribosome recruitment [22] (Figure 1, arrow 3). It has been argued that closed-loop formation is actually necessary for this translational control event, as it would place the GAIT complex close to its regulatory target [23]. Similarly, the closed-loop is not disrupted upon inhibition of *Drosophila mls2* mRNA translation by SXL. This protein binds to the 5′- and 3′-UTRs of *msl2* mRNA and inhibits both 43S complex recruitment and scanning without affecting the binding of eIF4E, eIF4G or PABP to the mRNA [24, 25]. Although the mechanism by which SXL inhibits ribosome recruitment is unclear, inhibition of scanning is achieved in part by promoting the use of an upstream open reading frame (uORF) in the 5′-UTR of *msl2* [26]. Cooperation between complexes nucleated by SXL at both UTRs of the message is necessary for synergistic repression, suggesting that UTR-to-UTR communication is important for coordinated translational control in this case. Finally, inhibition of *lipoxygenase* (*lox*) mRNA translation by hnRNPs K and E1 during erythroid differentiation is another example where regulation takes place after closed-loop formation. HnRNPs K/E1 bind to the 3′-UTR of *lox* mRNA and inhibit the joining of the large ribosomal subunit to the 43S complex positioned at the AUG [27] (Figure 1, arrow 4).

Although most RBPs target the initiation step of translation, some regulators have been recently reported to target the elongation step (Figure 1, arrow

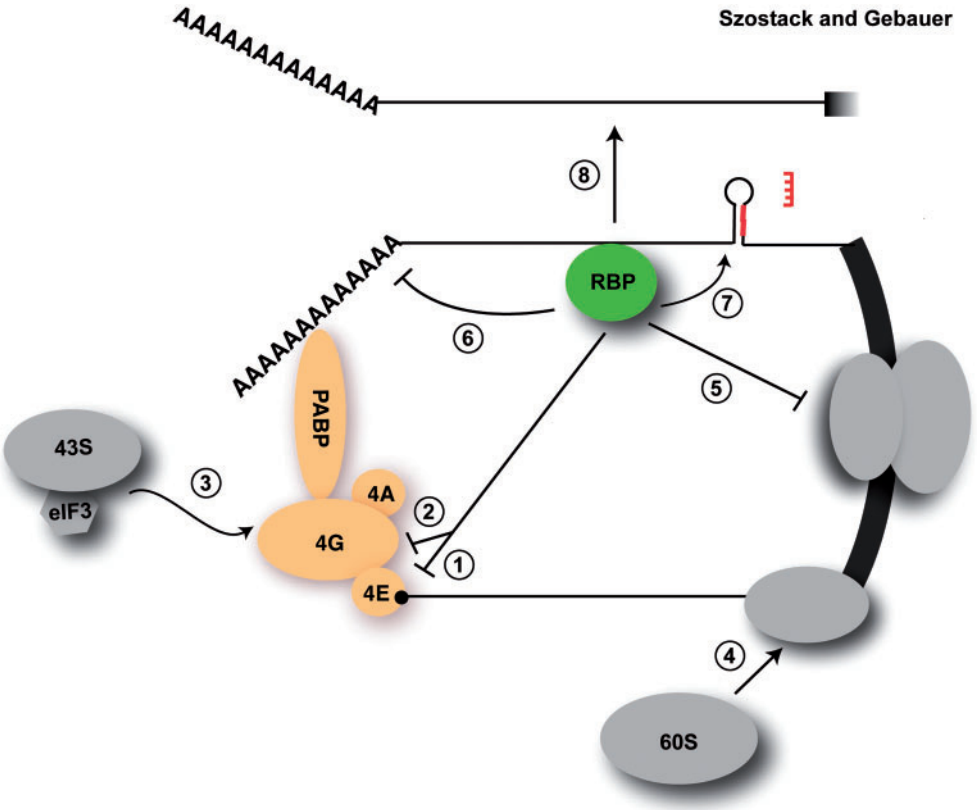

Figure 1: Mechanisms of translational regulation by 3′-UTR-binding proteins. Numbers indicate the different modes that RBPs use to modulate translation (see text for details). Large ovals depict the small (43S) and large (60S) ribosomal complexes. The initiation factors that participate in closed-loop formation are indicated. A miRNA, as well as its binding site on the target mRNA, is also highlighted.

5). Proteins of the PUF (Pumilio and FBF) family can be found in complexes containing Argonaute (Ago) and the translation elongation factor eEF1A [28]. eEF1A is a GTPase required during elongation to release aminoacyl-tRNAs upon delivery to the ribosome. The PUF/Ago complex bound to the 3′-UTR inhibits eEF1A GTPase activity, leading to attenuation of translation elongation. hnRNP E1 also interacts with eEF1A to inhibit elongation by blocking eEF1A dissociation from the ribosome [29]. Therefore, hnRNP E1 can interfere with different steps of translation depending on the associated factors.

The examples mentioned above show the variety of mechanisms by which 3′-UTR-binding proteins function to inhibit translation at the 5′-end/UTR or ORF of the mRNA, but 3′-UTR-binding regulators can also act at the 3′-end. One mechanism frequently modulated by RBPs is deadenylation (Figure 1, arrow 6). The poly(A) tail serves as an 'anti-nuclease' shield for the transcript, as the main mRNA degradation pathway starts by mRNA deadenylation, followed by decapping and 5′-to-3′ degradation. Deadenylation promotes destabilization of the mRNA, and reduces the translational efficiency by disrupting the closed-loop. An increasing number of RBPs associate to multifunctional deadenylase complexes, leading to poly(A) tail shortening of target mRNAs (reviewed in [30]). PUF proteins, for example, recruit the POP2/CCR4/NOT deadenylase complex to promote mRNA repression [31, 32]. *Drosophila* Pumilio, the founding member of the PUF family, binds to the 3′-UTR of *hunchback* (*hb*) mRNA together with Nanos (Nos) and Brain tumor (Brat) to promote *hb* deadenylation and silencing at the posterior pole of the embryo, an event necessary for correct antero-posterior axis formation. The Pum/Nos/Brat complex also recruits 4EHP to inhibit translation at the 5′-end [33]. Similarly, the protein Smaug recruits the POP2/CCR4/NOT complex to deadenylate *nanos* mRNA and Cup to promote other forms of repression [34, 35]. Indeed, deadenylation is often coupled with other mechanisms of regulation designed to 'inactivate' both ends

of the mRNA at once. Thus, rather than isolated effectors, RBPs must be viewed as centers of nucleation of more complex RNPs that can target translation by multiple mechanisms.

In addition to direct the assembly of complex RNPs with a function of their own, RBPs can modulate regulation mediated by other molecules, such as miRNAs (Figure 1, arrow 7). RBPs have been shown to either potentiate or antagonize miRNA-mediated silencing. Pumilio binding to the 3′-UTR of *p27* mRNA alters the local secondary structure and increases the accessibility of miR-221 and miR-222 to their target sites promoting miRNA-mediated repression [36]. The protein Dead end 1 (Dnd1), on the contrary, blocks miRNA-mediated silencing by competitive binding to overlapping sites [37]. HuR is another RBP that antagonizes miRNA function, most likely by a mechanism that involves HuR oligomerization along the 3′-UTR and miRNA dissociation [38].

Last, while all examples described above illustrate intramolecular connections, RBPs can also promote intermolecular interactions (Figure 1, arrow 8). Bruno binding to the 3′-UTR of *oskar* mRNA promotes mRNA oligomerization and the formation of densely packed RNPs that are inaccessible to the translational machinery [39]. Formation of higher order silenced RNP particles may facilitate the transport and localization of transcripts, and their subsequent translational activation at different cellular locations [40].

RBPS GO 'OMICS'

The last 10 years have witnessed an explosion of high-throughput technologies that are changing the way we study translation, are revealing unexpected layers of complexity and are providing a new dimension to the translational control field.

The first technique to study RNPs at large scale consisted of the immunoprecipitation of RBPs and the subsequent identification of co-immunoprecipitated mRNAs using microarrays, a technology referred to as RIP-Chip [41]. The detection power of this technology was later improved by applying sequencing methods yielding RIP-Seq. RIP-Chip and RIP-Seq identified hundreds of mRNAs associated to single RBPs. Subsets of these mRNAs encode functionally related proteins and are potentially co-regulated, constituting 'RNA regulons'. For example, the GAIT complex inhibits the translation of several mRNAs that contain GAIT hairpins in their 3′-UTRs and encode factors involved in inflammation [42]. Co-regulation of these mRNAs by the GAIT complex may lead to a coordinated resolution of the inflammatory response. RNA regulons have been described in diverse biological contexts and in organisms from yeast to mammals (reviewed in [43]).

More refined technologies have allowed the identification of RBP targets in living cells by the introduction of a UV cross-linking step. RNA–protein interactions are 'frozen' by UV cross-linking, a step that also permits harsh immunoprecipitation and washing conditions coupled to partial RNA digestion. Cross-linking and immunoprecipitation (CLIP) followed by sequencing or microarray analysis, and its derivatives photoactivatable ribonucleoside enhanced CLIP (PAR-CLIP) and individual nucleotide resolution CLIP (iCLIP) have allowed the identification of sequence motifs for RBPs and their locations within the transcript *in vivo*, as well as the definition of true- as opposed to predicted-miRNA-binding sites ([44–46], reviewed in [47]). These techniques have revealed a significant overlap between transcripts bound by different RBPs, establishing RBP-directed networks of post-transcriptional regulation and uncovering an unprecedented potential for combinatorial control.

Sequence motifs can be further refined using SEQRS, a method that integrates *in vitro* selection, high-throughput sequencing of RNA and SSL (sequence-specificity landscapes) to study the specificity of RBPs and the influence of partner proteins and co-factors on RBP-binding specificity [48].

A lesson derived from these studies is that a transcript will generally be bound and regulated by multiple RBPs, the combination of which will determine the final regulatory outcome. Establishing the 'RNP code' of a transcript is thus a prerequisite to predict its behavior. Some progress has been made in this direction. A highly predictive combinatorial code for CPE-mediated translational control has been proposed, based on the number and distance separating three regulatory *cis*-acting elements in the 3′-UTR [20, 49, 50].

The ribonomics techniques combined with large scale transcriptome analysis have great potential for novel discovery. RIP-Seq of *Drosophila* UNR in male and female flies identified a pronounced sex-specific binding bias for this protein; follow-up RNA-Seq experiments correlated UNR binding

with the sex-specific alternative processing of UTRs in target messages [51]. Processing of UTRs, and specially of the 3′-UTR, has been proposed to importantly contribute to global gene expression regulation. Proliferating and cancer cells express mRNAs, which on average, contain shorter 3′-UTRs compared with those in non-transformed cells. These shorter 3′-UTRs arise by alternative cleavage and polyadenylation, and are refractory to regulation by RBPs and miRNAs because of the loss of 3′-UTR repressive elements [52, 53].

RNA structure is also an important factor to consider. Many RBPs bind to structured elements, and binding is actually based on structural fidelity rather than primary sequence recognition. Structured *cis*-acting elements involved in translational control are, for example, the TGF-beta activated translation element present in the 3′-UTR of transcripts involved in epithelial-to-mesenchymal transition, and the GAIT element present in the 3′-UTR of transcripts involved in inflammation [54, 55]. Recently developed genome-wide methods for RNA secondary structure determination, such as PARS and SHAPE-Seq hold the promise of new research avenues [56, 57].

The human genome encodes several hundred predicted RBPs. Two recent studies have identified the mRNA-bound proteome or 'mRNA interactome' of Hela and embryonic kidney cells, and have increased this number by about 30% [58, 59]. These studies have revealed novel RBPs that bind to RNA through yet-unknown RNA-binding domains, RNA-binding kinases and a catalog of protein–mRNA contact sites. Interestingly, these studies have also uncovered RNA-binding enzymes of the intermediary metabolism, suggesting a direct connection between metabolism and RNA regulation (reviewed in [60]). Curiously, one of the first RNA-binding proteins shown to regulate translation was an enzyme, the iron responsive protein 1 (IRP1) [61]. In its iron-free form, IRP1 binds to the iron responsive element in the UTRs of mRNAs involved in iron metabolism and regulates their translation or stability, whereas in its iron-bound form, IRP behaves as the cytoplasmic aconitase. Another enzyme that binds to RNA is the glutamyl-prolyl tRNA synthetase (EPRS), a component of the GAIT complex [62]. In both cases, IRP1 and EPRS bind to mRNA dynamically, depending on the availability of small molecule metabolites or on protein phosphorylation, respectively. This suggests, perhaps not surprisingly, that the mRNA interactome of a cell changes according to metabolic conditions.

Several techniques allowing high-throughput estimation of translation have been developed in the last few years, including polysome profiling and pulsed SILAC [63, 64]. However, these technologies suffer from limited resolution. More recently, a new high resolution technique to systematically monitor mRNA translation has been reported [65]. Ribosome profiling is based on deep sequencing of ribosome-protected mRNA fragments, and allows the analysis of translation at nucleotide resolution. The positional and quantitative precision provided by this technology makes it an extremely powerful tool for the study of translational regulation. Ribosome profiling has already provided a number of surprising observations. Pervasive translation initiation at GUG and CUG codons in the 5′-UTR of transcripts was detected using ribosome profiling in yeast and mammals [65–67]. Indeed, initiation at CUG was later shown to contribute to the generation of short peptides that are presented by the major histocompatibility complex during immune surveillance [68]. Translation of short ORFs in stable transcripts that were previously classified as long noncoding RNAs was also reported using this technique, as well as the existence of short polycistronic ribosome-associated RNAs in metazoans [66, 67]. The ribosome profiling technology has estimated a 10- to 100-fold range in the translational efficiencies of mRNAs, and agrees with previous studies indicating that translation contributes substantially to the dynamic range of gene expression.

In summary, new genome-wide technologies have opened the Pandora box to translational regulation. The full catalog of RBPs, combined with estimations of the RBP-bound transcriptome and the global translational effects caused by disruption of RBP function should allow a comprehensive understanding of the transcriptome-to-proteome transition.

PERSPECTIVE

The dimension of translational control has changed in the last 10 years. From single regulators to complex mRNPs to genome-wide studies, research on RBP-mediated regulation has evolved in highly complementary, parallel directions that promise to converge to allow accurate predictions of systems

behavior. Recent findings have uncovered a layer of complexity that is probably just 'the tip of the iceberg' in translational regulation. RBPs function in concert with other factors to target translation by several mechanisms at once. The reasons for this are unclear, but one can imagine that redundant translational control mechanisms ensure the precise and fail-safe regulation of mRNA expression. A variety of sequence or structure-based *cis*-acting elements direct the formation of complex RNPs that cooperate in translation regulation. The number of transcripts bound by given RBPs together with the diversity of RBPs that can bind a given transcript provides an enormous capacity for combinatorial control. Precise positional and quantitative information provided by high-throughput technologies such as CLIP or ribosome profiling are dramatically expanding the types of questions that can be asked and are pushing forward the translation field in a manner that rivals transcriptional regulation. Systematic studies of dynamic biological scenarios will allow us to fully appreciate the potential of translational control, and to manipulate translation to alleviate diseases in the future.

Key Points

- RBPs nucleate the formation of RNP complexes that generally target translation by more than one mechanism.
- One factor can behave as a repressor or an activator of translation depending on the interacting co-factors or on post-translational modifications.
- Genome-wide techniques such as ribosome profiling, CLIP or transcriptome capture, yield highly precise quantitative and positional information that will illuminate the field of translational control with unprecedented detail.

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