Translational control of mRNAs by 3’-Untranslated region binding proteins

Akio Yamashita1,* & Osamu Takeuchi2,*

1Department of Molecular Biology, Yokohama City University School of Medicine, Yokohama 236-0004, 2Laboratory of Infection and Prevention, Institute for Frontier Life and Medical Sciences, Kyoto University, Kyoto 606-8507, Japan

Eukaryotic gene expression is precisely regulated at all points between transcription and translation. In this review, we focus on translational control mediated by the 3’-untranslated regions (UTRs) of mRNAs. mRNA 3’-UTRs contain cis-acting elements that function in the regulation of protein translation or mRNA decay. Each RNA binding protein that binds to these cis-acting elements regulates mRNA translation via various mechanisms targeting the mRNA cap structure, the eukaryotic initiation factor 4E (eIF4E)-eIF4G complex, ribosomes, and the poly (A) tail. We also discuss translation-mediated regulation of mRNA fate. [BMB Reports 2017; 50(4): 194-200]

INTRODUCTION

Translational regulation of mRNA is an immediate and precise mechanism to control gene expression in various biological processes, including development, differentiation, and responses to extracellular stress. Global quantification analysis indicates that the cellular abundance of proteins in mammals is predominantly controlled at the level of translation (1). In vivo, mRNAs do not exist as bare mRNA molecules but as mRNA-protein complexes with RNA-binding proteins (RBPs) (2-5). More than one thousand RBPs have been identified, and they bind to specific cis-acting elements, consisting of sequence elements, stem-loop structures and/or modified nucleotides (6-8). For many genes, alternative poly (A) addition and alternative splicing give rise to 3’-UTR variants (Fig. 1A and B). These variants are controlled by specific post-transcriptional regulation (9, 10).

The cap-dependent mRNA translational process is divided into three major steps: initiation, elongation, and termination. Each step is elaborately regulated by multiple mRNA 3’-UTR binding proteins in a cell type- and species-specific manner (11, 12). In this review, we present examples of RBP-mediated regulation of translation and we discuss their biological roles.

MOLECULAR MECHANISM OF CAP-DEPENDENT mRNA TRANSLATION BY 3’-UTR BINDING PROTEINS

In eukaryotes, most protein coding mRNAs have a 5’-terminal cap structure and a 3’-terminal poly-adenine. Histone mRNAs, however, are an exception, having a specific stem-loop structure in the 3’-terminal region (13). The cap structure acts as an anchor and is critical for translation initiation by the eukaryotic initiation complex (14). In eukaryotes, this cap-dependent translation initiation is implemented by two macromolecular complexes, namely, the eukaryotic initiation factor-4F (eIF4F) complex, consisting of eIF4E, eIF4G and eIF4A, and the 43S initiation complex, consisting of a 40S ribosome, eIF3, elf1A, elf2 and methionyl-tRNA (11, 12). The eIF4F complex connects the 5’- and 3’-termini of the mRNA via interactions with poly(A) binding protein (PABP) or histone stem-loop binding protein (SLBP) (13). This mRNA circularization plays a significant role in efficient translation, probably by accelerating ribosome recycling (Fig. 2A) (11, 12). In addition, eukaryotic release factor 3 (eRF3) directly binds to the PABP/eIF4G complex and stimulates translation (15). Hence, mRNA translation can be modulated by the cap-binding protein complex. The circularization of mRNA is also a significant molecular feature in the regulation of cap-dependent mRNA translation by 3’-UTR RBPs (11, 12).

REGULATION OF TRANSLATION THROUGH THE CAP STRUCTURE (Fig. 2B)

The cap-binding protein, elf4E, is the foundation of a translation initiation complex at the 5’-terminal cap structure (11, 12). In addition, the elf4E-related molecule, 4E homologous protein (4EHP), competitively and directly binds the cap structure. However, 4EHP represses translation because of its weak affinity to elf4G (16). 4EHP can be recruited by RBPs such as Bicoid (which binds to a specific cis-element in the
3'-UTR of caudal mRNA) and the Pumilio/Nanos/Brat complex (which binds to a specific cis-element in the 3'-UTR of hunchback mRNA) during Drosophila melanogaster development (17, 18). Mammalian 4EHP also has the ability to suppress translation via the same mechanisms (19, 20). On the other hand, 4EHP can augment translation during hypoxia in human U87MG glioblastoma cells (21). The transcription factor, hypoxia-inducible Factor 2α (HIF-2α), can bind both DNA and RNA and forms a complex with RBM4 on the 3'-UTRs of a subset of mRNAs including FGFR mRNA. The HIF-2α/RBM4 complex then recruits 4EHP to stimulate translation. eIF4A, but not eIF4G, is present in this complex (21).

REGULATION OF TRANSLATION THROUGH THE eIF4E-eIF4G STRUCTURE (Fig. 2C)

The interaction between eIF4E and eIF4G is required for mRNA circularization and the initiation of cap-dependent translation (11, 12). D. melanogaster Cup, Xenopus laevis Maskin, mammalian Neuroguidin (NGD) and mammalian cytoplasmic fragile X mental retardation protein (FMRP) interacting protein 1 (CYFIP1) bind eIF4E competitively with eIF4G and repress translation. These translational repressors are recruited to mRNAs through specific 3'-UTR binding proteins, namely, Cup/Bruno, Cup/Smaug, Maskin/cytoplasmic polyadenylation element binding protein (CPEB), NGD/CPEB, and CYFIP1/FMRP (22-25). microRNAs (miRNAs) are small RNA molecules consisting of 21-24 nucleotides that form microRNA-induced silencing complexes (miRISCs) with Argonaute (Ago) proteins and repress translation. Ago proteins have isoform-specific mechanisms for repression of translation. In D. melanogaster, after miRISC binds to a miRNA target site in an mRNA 3'-UTR, Ago2 represses translation by competing for eIF4E binding, which is similar to Cup (26).
REGULATION OF TRANSLATION THROUGH THE 43S TRANSLATION INITIATION COMPLEX (Fig. 2D)

In contrast to D. melanogaster Ago2, D. melanogaster Ago1 and vertebrate Ago2 repress translation by interfering with the assembly of a functional elf4F complex. This occurs through the displacement of elf4A (in vertebrate elf4A1 and elf4A2) from the mRNA, leading to the functional suppression of the 43S initiation complex (27, 28). A conflicting model of vertebrate Ago2 action has been proposed. In this model, the vertebrate Ago-associated CCR4-NOT complex recruits elf4A2. The elf4A2 would then inhibit translation initiation by preventing the recruitment of active elf4A1 (29). Further investigations are necessary to verify these models (30, 31). In addition, these Ago isoforms are expected to affect mRNA circularization through complex formation with trinucleotide repeat-containing protein 6 (TNRC6), which has the ability to repress translation (30, 31).

The gamma interferon-activated inhibitor of translation (GAIIT) complex consists of a ribosomal protein L13a (rpL13a),
glyceraldehyde-3-phosphate dehydrogenase (GAPDH), glutamyl-prolyl-tRNA synthetase (EPRS), and NS1-associated protein-1 (NSAP1), and represses translation initiation by inhibiting recruitment of the 43S translation initiation complex. The GAIT complex recognizes specific stem-loop structure elements and, in response to interferon-γ, forms on a subset of 3'-UTR elements of mRNAs, including those of ceruloplasmin and vascular endothelial growth factor (VEGF). mRNA circularization is maintained during GAIT-mediated repression of translation and is expected to contribute to the action of the 3'-UTR binding protein complex on 5'-terminal cap-dependent translation initiation (32).

During female embryonic development in D. melanogaster, translation of mls2 mRNA is prevented by the Sex-lethal (SXL)/upstream of N-ras (UNR) complex formed on a specific cis-acting element in the 3'-UTR of mls2 mRNA. The SXL/UNR complex inhibits the recruitment of the 43S translation initiation complex by maintaining mRNA circularization (33). In addition, SXL binds to the 5'-UTR of mls2 mRNA and represses initiation codon scanning by the 43S initiation complex in an upstream open reading frame (uORF)-dependent manner (34).

Emerging evidence indicates that base modifications, including inosine, N6-methyladenosine (m^6A), N1-methyladenosine, 5-methylcytosine, 5-hydroxymethylcytidine, and pseudouridine, can modulate the fate of mRNA (35). Among these modifications, m^6A in 3'-UTRs promotes translation when recognized by YTH domain-containing family protein 1 (YTHDF1) (36). Similarly, methyltransferase like 3 (METTL3), a recognized by YTH domain-containing family protein 1 (YTHDF1), represses translation initiation by inhibiting recruitment of the 43S translation initiation complex. The GAIT complex recognizes specific stem-loop structure elements and, in response to interferon-γ, forms on a subset of 3'-UTR elements of mRNAs, including those of ceruloplasmin and vascular endothelial growth factor (VEGF). mRNA circularization is maintained during GAIT-mediated repression of translation and is expected to contribute to the action of the 3'-UTR binding protein complex on 5'-terminal cap-dependent translation initiation (32).

Intriguingly, YTHDF2, another m^6A decoder, competitively associates with a specific nuclear ribonucleoprotein K (hnRNPK)/hnRNPE1 complex (36). Similarly, methyltransferase like 3 (METTL3), a recognized by YTH domain-containing family protein 1 (YTHDF1), represses translation initiation by inhibiting recruitment of the 43S translation initiation complex. The GAIT complex recognizes specific stem-loop structure elements and, in response to interferon-γ, forms on a subset of 3'-UTR elements of mRNAs, including those of ceruloplasmin and vascular endothelial growth factor (VEGF). mRNA circularization is maintained during GAIT-mediated repression of translation and is expected to contribute to the action of the 3'-UTR binding protein complex on 5'-terminal cap-dependent translation initiation (32).

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of mRNA and to disrupt mRNA circularization, thereby repressing translation. However, recent studies demonstrated that median poly(A) lengths are about 60-100 nucleotides (nt), which is shorter than the 150-200 nt thought to be typical of mammalian poly(A) tails. Poly(A) lengths of > 20 nt are not correlated with translational efficiency in somatic cells (47, 48). When the poly (A) tail length is < 20 nt, translation is repressed in most genes in somatic cells (48). Because PABP binds to poly(A)s of 20 nt lengths, one PABP molecule might be sufficient to support mRNA circularization and poly(A)-dependent efficient translation.

In contrast to somatic cells, poly(A) tail length and translational efficiency are coupled in embryonic cells (47, 49). In X. laevis oocytes, cytoplasmic polyadenylation element binding protein (CPEB) binds specific cis-acting elements in the 3'-UTRs of mRNAs encoding cell cycle-related proteins, such as cyclin B. CPEB recruits PARN deadenylase and short poly(A) mRNAs are stabilized before oocyte maturation (50, 51). The stimulation of oocyte maturation induces CPEB poly(A)-dependent efficient translation.

REGULATION OF mRNA DECAY THROUGH TRANSLATION

As mentioned above, the 3'-UTR plays a key role in translational control. However, reciprocally, translation also regulates 3'-UTR-mediated mRNA decay. For instance, when exon-junction complexes are bound to the 3'-UTR, premature translation termination is recognized, and the mRNA is degraded by the nonsense-mediated mRNA decay pathway which acts as an mRNA quality control system (52). Normal translation termination codon recognition also stimulates mRNA degradation of inflammation-related mRNAs containing a specific stem-loop in the 3'-UTR. In this case, the stem-loop is recognized by Regnase-1, an endonuclease (53). Similarly, translational termination induces the degradation of replication-dependent histone mRNAs and Staufen1-mediated mRNA decay (54, 55). An RNA helicase called Up frame shift 1 (UPF1) is required for the translation-dependent mRNA decay systems described above. Taken together, 3'-UTRs can act bi-directionally in translational regulation and mRNA decay – mechanisms that are closely involved with each other.

PERSPECTIVES

In the present review, we briefly introduced the mechanism of translational control by mRNA 3'-UTR-binding proteins. Translational control is recognized as an essential regulatory mechanism of gene expression in various biological processes. For example, interferon-γ production from T-cells is regulated by translational control coupled with glycolysis. GAPDH, an enzyme essential for glycolysis, also acts as an RBP that directly binds to a cis-acting element in the 3'-UTR of the interferon-γ mRNA and acts as a translational repressor in inactive T cells. T cell activation drastically alters the metabolic status of T cells, with aerobic glycolysis promoted over oxidative phosphorylation, and GAPDH can dissociate from the interferon-γ mRNA to function as an aerobic glycolysis enzyme. In this situation, GAPDH no longer represses the translation of interferon-γ mRNA, leading to an increase in interferon-γ production (56).

Many RBPs involved in translational control and/or mRNA degradation have additional roles in alternative pre-mRNA splicing, alternative poly(A) addition and other mRNA processing events (57). In addition, analysis of translational control using plasmid vector-based reporter assays must consider the possibility of unexpected/undesired transcription from all regions of the circular plasmid DNA and unexpected processing of mRNA. Hence, in vitro reconstituted translation experiments should also be performed in addition to cell and/or animal-based analysis.

CONFLICTS OF INTEREST

The authors have no conflicting financial interests.

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