mRNA features that guide translational control

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Features encoded in the mRNA contribute substantially to the translation efficiency of a transcript. After processing of the 5' and 3' end and export of the mRNA to the cytoplasm, translation is initiated via a cap-dependent or cap-independent mechanism. We describe how inhibition of global translation is controlled by limiting translation initiation factors and how specific mRNAs benefit from this to increase their expression during these conditions. We also discuss how the mRNA codes for structural and regulatory elements which can be recognised by trans acting factors to promote or inhibit translation. Finally, we discuss how relatively new concepts as the epitranscriptome, codon optimality and even the sequence of the nascent peptide influence translation rates.

Introduction

Gene expression is controlled by two fundamental processes: transcription and translation, also referred to as the "central dogma" comprising the flow of information from DNA to RNA to protein. Both processes are highly regulated and go hand in hand with mRNA decay to fine tune levels of protein synthesis in a context specific manner. The observation that there is only a low correlation between mRNA levels and protein abundance suggests that transcriptional regulation alone is not sufficient to account for the dynamics of gene expression and research even suggests that translation is the predominant mechanism for the control of gene expression [1]. Since the development of ribosome profiling (Ribo-Seq) [2] that allows measurement of translation with subcodon resolution, the RNA translation field has revolutionized and measuring **translation efficiency** (TE) is now even possible at the single cell level [3]. Indeed it has been shown that TE shows a higher correlation with protein abundance [2].

Transcribed pre-mRNAs are bound by RNA binding proteins (RBPs) to form a ribonucleoprotein (RNP) complex that facilitate processing prior to export to the cytoplasm, which includes capping of the 5'end, splicing and cleavage of the mRNA followed by poly(A) synthesis (**Fig. 1**). The matured mRNA comprises a protein coding sequence (CDS) flanked by a capped 5'- and a polyadenylated 3'- untranslated region (UTR)(**Fig. 1**). Once processing of the pre-mRNA is finished, export factors will bind to the nascent transcript and is exported from the nucleus to the cytoplasm. Release in the cytoplasm makes the transcript accessible to the translational machinery to be translated into a protein but the TE can differ a 100-fold between transcripts as shown in yeast [2]. Additionally, it has been shown in mouse macrophages that the **ribosome loading time** (RLT) can differ between transcripts such as housekeeping genes which have an average RLT of 46 minutes in contrast to mRNAs encoding cyclins, histones or ribosomal proteins which have a RLT of 1−6 minutes [4].

How these differences arise and in particular which features of the mRNA contribute to these differences is the topic of this review (**Fig. 2**). First, we explain the general cap- dependent mode of translation and, whereas interference with cap-mediated initiation results in global shutdown of translation, certain regulatory elements can enhance translation of specific mRNAs. We then discuss how the mRNA codes for *cis*-acting structural elements and regulatory RNA elements that can be recognized by *trans*-acting RNA binding proteins (RBPs) or miRNAs and influence the fate of the mRNA each in its own way. Next, we discuss how chemical modifications, the "epitranscriptome" affect translation followed by how the primary sequence of the mRNA itself also determines how efficient a mRNA is translated, a concept that is known as codon optimality. Finally, we explain how
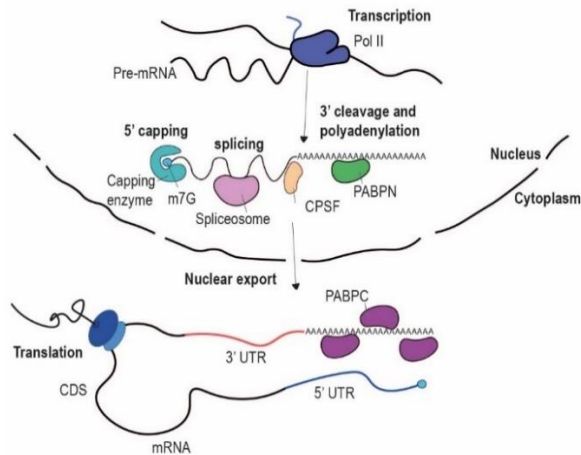
Translation efficiency
The ratio of RFP and RNA abundance determined by ribosome profiling and RNA-seq

Ribosome loading time
The time until an mRNA molecule has obtained its full load of ribosomes

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even the nascent peptide sequence contributes to TE.

Fig. 1. Maturation of the mRNA. Pre-mRNAs transcribed by RNA polymerase II (Pol II) are co transcriptionally processed. A 7-methylguanosine cap (m⁷G-cap) is added at the 5' end of the transcript, introns are removed by the spliceosome and the 3' end is cleaved followed by addition of a poly(A) tail. When processing in complete, the mature mRNA comprising a protein coding sequence (CDS) flanked by a capped 5' and a polyadenylated 3' untranslated region (UTR) is exported to the cytoplasm. The poly(A)tail is bound and protected by PABPC and translation can be initiated. Figure adapted an modified from [5].

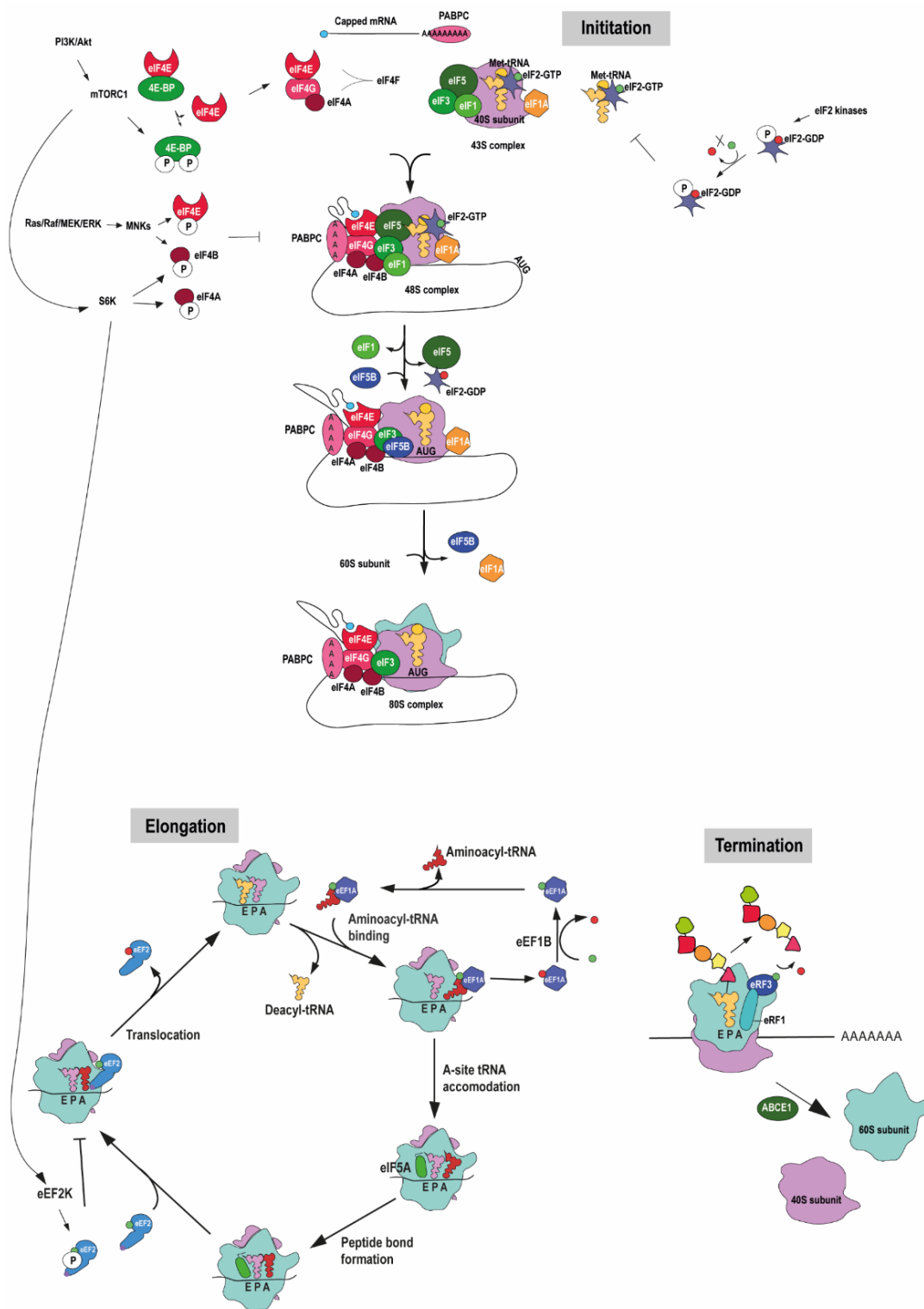## Cap-dependent translation initiation

Translation initiation is a complex process and many eukaryotic initiation factors (eIFs) are involved. The 5' cap plays an essential role in translation initiation and is recognized by cap binding protein eIF4E. Together with eIF4A and eIF4G, eIF4E forms the cap binding complex eIF4F and cooperates with eIF4B to unwind secondary structures in the 5'UTR to allow attachment of the 43S preinitiation complex (PIC), together forming the 48S complex (**Fig. 3**). eIF4G functions as a scaffold protein that binds eIF4E and the poly(A) binding protein (PABPC), circularizing the mRNA, a model that is defined as the "closed-loop" model [6]. Once the PIC, consisting of the 40S subunit, eIF1, eIF1A, eIF3, eIF5 and the initiator Met-tRNA$_i^{Met}$ in a ternary complex (TC) with GTP bound eIF2, is loaded onto the mRNA it starts scanning the 5'UTR until it reaches the start codon (**Fig. 3**). Multiple AUG codons can be present and can influence the efficiency of translation of the protein coding sequence which is discussed later. Recognition of the start codon triggers a "closed" conformational state of the ribosome that locks the initiator tRNA in the P-site of the ribosome base-paired with AUG in the mRNA. Following

this, several factors (eIF1, eIF1A, eIF3, residual eIF2–GDP and eIF5B) dissociate from the complex during the 60S subunit joining process to eventually form the elongation competent 80S ribosome (**Fig. 3**). [7-9]. In this conformation the second codon is positioned in the A-site of the ribosome. During elongation, activated GTP-bound eukaryotic elongation factor 1A (eEF1A) binds an aminoacyl-tRNA and together this TC binds in the empty A-site of the ribosome. Codon anti-codon base-pairing triggers GTP hydrolysis and GDP-bound eEF1A is released from the A-site. Following release of eEF1A, eIF5A binds in the E-site and interacts with the acceptor arm of the peptidyl-tRNA in the P-site to promote peptide bond formation between the amino acids in the peptidyl-transferase centre (PTC) (**Fig. 3**). Finally, following peptide bond formation GTP-bound eEF2 binds in the A-site and promotes translocation of the tRNAs into the canonical P- and E-sites making the ribosome ready for the next cycle of elongation [10]. The presence of all three possible stop codons in the A-site is recognised by eRF1 and GTP-bound eRF3 and GTP hydrolysis induces the release of the nascent polypeptide chain (**Fig. 3**). After termination, the 60S subunit is released from the complex by ATP-binding cassette protein ABCE1 and the deacylated transfer RNA (tRNA) and messenger RNA (mRNA) bound by the 40S subunit are further disassembled by eRF1, eIF1A, eIF3 and eIF3j and recycled enabling them to engage in another round of translation (**Fig. 3**) [11].

Global translational control

Translation initiation is considered as the rate limiting step of translation and is an important target for global translational control. One of the mechanisms to inhibit translation initiation involves the eIF4E-binding proteins (4E-BPs) that bind eIF4E and prevents interaction with eIF4G [12]. The interaction between 4E-BPs and eIF4E is mediated through their phosphorylation status. mTOR, and in particular mTORC1 (mammalian target of rapamycin complex 1) is a key nutrient sensor and modulator of protein synthesis acting downstream of the PI3K/Akt pathway and is a critical kinase that phosphorylates 4E-BPs (**Fig. 3**). In normal conditions, eIF4E is free to bind to eIF4G which allows assembly of the initiation complex to initiate translation. In various

Fig. 2. mRNA features that guide translational control. Sequence motif, such as TOP- or AU- rich motifs can affect translation by attracting RNA binding proteins. Short motifs can also act as RBP binding site for specific RBPs. miRNA binding sites can be bound by miRNAs which inhibit translation of its target mRNA. uORFs and dORFs can inhibit or promote translation. RNA modifications affect translation by several mechanisms. Structural elements such as hairpins and G4s can inhibit translation. An IRES can guide the recruitment of the 40S subunit to or near the start codon. Codon optimality determines translation efficiency of a mRNA.

stress conditions, inhibition of mTORC1 will result in unphosphorylated 4E-BPs. This makes them able to bind to eIF4E and inhibit the interaction with eIF4G resulting in inhibition of cap-dependent translation initiation [13]. Additionally, stress responsive eIF2 kinases regulate translation initiation by the phosphorylation of eIF2 subunit eIF2α and thereby reducing the level of the eIF2–GTP–tRNA$_i$^{Met} TC resulting in reduction of translation initiation (**Fig. 3**). [14]. Furthermore, eIF4E and eIF4B activity is regulated by other signaling pathways such as the Ras/Raf/MEK/ERK signaling cascades that act via the mitogen activated protein kinase (MAPK)-interacting serine/threonine kinases (MNKs)) [15, 16]. Another effector of mTORC1, S6k (ribosomal protein S6 kinase) is involved in the direct or indirect phosphorylation a number of proteins involved in translation such as ribosomal protein S6, eIF4B, eIF4A and eEF2K which has the most significant impact [17]. eEF2K phosphorylates eEF2 on a site that inhibits it from binding to the ribosome where it promotes elongation (**Fig. 3**). [18]. Al together, in response to extra- and intracellular cues these pathways converge to control global translation.

Cap-independent translation initiation
Although cap-dependent translation initiation is the primary mode of initiation in eukaryotes, certain mRNAs can initiate translation without involvement of the 5' cap structure in physiological conditions such as such as mitosis, apoptosis and hypoxia but also in stress conditions when cap-dependent translation is supressed. This alternative cap-independent mechanism of translation initiation is mediated by an internal ribosome entry site (IRES), a highly structured *cis*-regulatory element in the 5' UTR of the mRNA that guides the recruitment of the 40S subunit to or near the start codon. IRESs were originally identified in viruses whose mRNA do not contain a 5' cap structure [19, 20] but have now been found to be the major translation initiation pathway for several families of viruses, including viruses that produce cap-containing mRNAs. IRESs are classified in four groups based on their structure, the requirement of IRES-transacting factors (ITAFs) or initiation factors to promote translation and the location of the start codon relative to the IRES (reviewed in [21]). Soon after discovery in viruses, it became clear that also cellular mRNAs could be translated in a cap-independent manner mediated by an internal ribosome binding mechanism. While the mechanism of viral IRES-mediated translation is well understood, cellular IRESs lack sequential and structural similarity between transcripts [22] and their mechanism of ribosome recruitment and translation initiation is still relatively unclear but numerous ITAFs are identified [23, 24].

It is now becoming clear that IRES-mediated translation initiation even takes place when global cap-dependent translation initiation is not supressed, for example to regulate timed expression of Hox genes during development of the mammalian body [25]. Additionally, a recent screening identified that ~10% of human 5'UTRs contain cap-independent translation promoting sequences. These genes were involved in various biological processes, such as translation, transcription, signal transduction and other processes indicating that cap-independent translation is a global used mechanism [26].

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Fig. 3 . Cap-dependent translation initiation, elongation, termination and its regulation by signaling pathways. The first step in initiation is binding of eIF4F (eIF4E, eIF4G, eIF4A) to the cap. PABPC binds to the poly(A) tail. eIF4E also binds to PABPC, circularizing the mRNA. The 43S PIC binds to the mRNA mediated by eIF4F and eIF4B. The scanning competent 48S complex scans along the mRNA until it encounters a start codon which triggers base pairing of the initiator tRNA with the AUG codon. During 60S subunit joining several factors dissociate forming the elongation competent 80S ribosome. During elongation, activated GTP-bound eukaryotic elongation factor 1A (eEF1A) binds an aminoacyl-tRNA and together this TC binds in the empty A-site of the ribosome. Codon anti-codon base-pairing triggers GTP hydrolysis and GDP-bound eEF1A is released from the A-site. eIF5A mediates peptide bond formation and eEF2 promotes translocation of the tRNAs into the P-and E-site and the elongation cycle is complete. A stop codon in the A-site triggers termination. eRF1 and eRF3 bind in the empty A-site and eRF3 mediates release of the nascent polypeptide. Active mTORC1 phosphorylates eIF4E dissociating eIF4E from 4E-BPs which allows initiation. Inhibition of mTORC1 results in unphosphorylated eIF4E which can now be bound by 4E-BPs inhibiting translation initiation. eIF4E and eIF4B activity is regulated by other signaling pathways such as the Ras/Raf/MEK/ERK signaling cascades that act via the mitogen activated protein kinase (MAPK)-interacting serine/threonine kinases (MNKs). S6k is involved in the direct or indirect phosphorylation a number of proteins involved in translation such as ribosomal protein S6, eIF4B, eIF4A and eEF2K. Figures adapted and modified from [8, 10, 27].

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Increasing evidence indicates that other mechanisms than IRES-mediated translation exist which involves a m6A-induced ribosome engagement site (MIRES) [28]. The first study on MIRESs showed that whereas a capped unmethylated reporter did not result in translation activity without eIF4E, the presence of a single m6A residue in the 5'UTR was sufficient to promote translation in cell-free extracts [28]. Additionally, using ribo-seq data, it was shown that mRNAs with m6A residues in their 5'UTR were translated in conditions where cap-dependent translation was suppressed. Moreover, they identify eIF3 as a m6A reader and they propose a model where binding of eIF3 to m6A residues in the 5' UTR can stimulate translation initiation by directly recruiting the 43S preinitiation complex [28], although the exact nature of this process remains unclear. More recently, other m6A readers such as YTHDF3, METTL3 and ABCF1 are identified that facilitate cap-independent translation [29] even on fully capped mRNAs indicating that several initiation modes can act on the same mRNA. Although the literature on m6A-induced translation initiation of mRNAs is limited, it is a well described process in the translation of circular RNAs (circRNAs) which do not have 5' end cap and a 3' end poly (A) tail necessary for cap-dependent translation initiation [30]. Yang et al. reported that recognition of the MIRES is mediated by YTHDF3 and the translation initiation factors eIF4G2 and eIF3A which together initiate translation of circRNAs [30] and the role of YTHDF3 in facilitating cap-independent translation is consistent between several studies.

Translational control by uORFs & dORFs

Ribo-seq has identified translated regions beyond the canonical open reading frame (ORF) that were previously thought to be non-coding. These ORFs located in the 5'- and 3' UTR, called up- and downstream ORFs respectively are typically short sequences with an average length of 60 nucleotides and can code for a small peptide. The presence of the majority of the uORFs and dORFs is conserved between human and other species but not the amino acid sequence they encode

Intercistronic region
The region between the termination codon of one ORF and the initiation codon of the next ORF

for [31-33]. Although they share many features, they appear to have opposing effects on translation. Whereas uORFs typically correlate with a repressive effect, dORFs enhance translation of their canonical ORF. In both cases is observed that an increase in the number of uORFs/dORFs per transcript correlates with an increase in the repressive or enhancing effect they have. The effect is independent of their sequence suggesting a role in translation through their activity independent of their encoded peptide [31, 32]. Additionally, an uORF that is overlapping with the CDS is associated with an even stronger translational repression of the canonical ORF however, Johnstone et al, also showed that the initiation sequence composition of these overlapping ORFs is less favourable than by change indicating that these conditions are under selective evolutionary pressure to prevent otherwise constitutive repression [32].

The efficiency of the recognition of an ORF is determined by how favourable the context sequence of the start codon is. The most optimal context, the "Kozak consensus" (5′ (A/G)CCAUGG 3′), allows for direct recognition by the ribosome [34]. When this specific context is absent the ribosome can bypass the uORF by the so called "leaky scanning" mechanism. Several other features are important for leaky scanning to occur such as its proximity to the 5' cap, the total length of the 5' UTR, length of the **intercistronic region** and secondary structures. When multiple uORFs are present, the ribosome can continue to translate downstream uORFs without being first recycled by a mechanism called translation reinitiation where the 40S subunit remains attached to the mRNA and recruits a new Met-tRNA$_i$^Met TC to resume scanning although the exact mechanism is unclear. The reinitiation process is highly dependent on eIF2 activity and the corresponding levels of the Met-tRNA$_i$^Met TC. Whereas high concentrations allow for fast recruitment to the 40S subunit and subsequent recognition of the next uORF, low levels delay the recruitment and the process can be to slow to initiate translation of the next uORF and the ribosome can only initiate translation at the one thereafter (**Fig. 4**). In this way, translation of uORF is sensitive to stress conditions when translation is inhibited by phosphorylation of eIF2. Not surprisingly,

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transcripts involved in cell growth and differentiation as well as oncogenes which are highly sensitive to environmental stress are enriched in uORFs revealing a regulatory mechanism of translation of these mRNAs. In conclusion, in general the mechanism of translation inhibition by uORFs is by sequestering the ribosomes from the canonical ORF, which can be overcome in certain conditions.

A notable example of how uORFs can regulate translation is the transcriptional regulator activating transcription factor, ATF4 which has a central role in the integrated stress response and contains two open reading frames in its 5' UTR (**Fig. 4**) [35]. In normal unstressed conditions translation of uORF1 allows immediate reinitiation at the next coding region, uORF2 whereafter it dissociates from the mRNA (**Fig. 4**) [35]. However, in stress conditions the integrated stress response helps the cell to adapt to cellular stress by phosphorylation of eIF2a limiting the availability of Met-tRNA$_i^{Met}$ TC to inhibit translation. This leads to a delay in recruitment of the TC to the scanning ribosome and can therefore bypass the second open reading frame and continue to translate the CDS of ATF4 (**Fig. 4**) [35].

The exact mechanism by which dORFs are translated is unclear at the moment but several lines of evidence argue against a **readthrough model** as footprints of the intercistronic regions are absent in ribo-seq data, they occur in all three possible reading frames after the stop codon of the canonical ORF and lastly, insertion of a stem loop only affects the subsequent ORF and not the following [31]. It might be that initiation of dORF occurs via a mechanism similar to IRES-mediated translation initiation where a specific sequence and conformation can recruit the ribosome directly as a nucleotide bias close to the start codon of the dORFs was identified. However, direct evidence for this mechanism is currently lacking [31]. How the presence of dORFs facilitates the upregulated expression of the CDS in unclear, but one theory could be that, with the closed loop model in mind, it increases the pool of recycled ribosomal subunits and translation factors near the 5' end that can engage in a new round of translation. Whether dORFs only functions to enhance translation of their canonical ORF or that they have another function is not clear. At the moment, there is

Readthrough model
A model where the ribosome continues to translate after encountering a stop codon

a lot of interest in the small peptides that the uORFs and dORFs encode for that were previously undetectable. It might be that dORF do encode for functional peptides and the increase in the concentration of recycled ribosomes and initiation factors is just an additional effect that enhances the translation of the CDS [36].
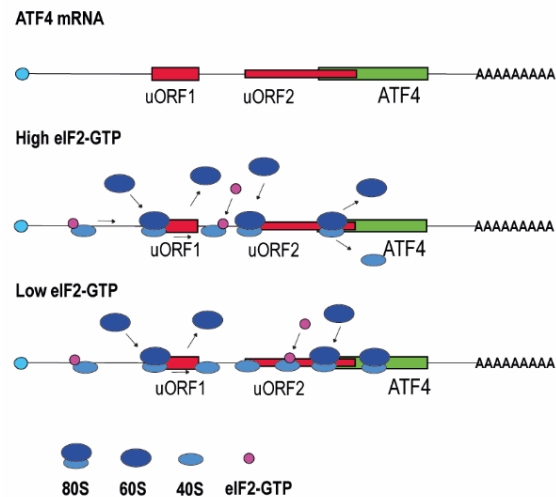
Fig. 4. Stress mediated control of ATF4 translation. ATF 4 contains 2 uORFs in its transcript. uORF1 which is always translated and uORF2, that overlaps with the CDS of ATF4, which is translated in unstressed conditions when concentrations of eIF2-GTP are high and fast reinitiation can occur after translation of uORF1. In stressed conditions, low concentrations of eIF2-GTP delay initiation and translation of uORF2 is skipped. Reinitiation can only occur at the CDS of ATF4. Adapted and modified from [37].

Translational control by secondary structures

Besides elements in the primary sequence that influence translation, the RNA is able to form high-order structures which play an important role in modulating translation by acting as an element that can be recognised by RNA binding proteins or by directly interfering with the translational machinery which results in repression of translation. Altering RNA structure, even in non-coding regions may alter translational output and these regions are very conserved between species [38]. Wan et al. that showed that single nucleotide variants that disrupts the structure are evolutionarily selected against and are significantly depleted in 3' UTRs, around predicted miRNA target sites and RBP binding sites indicating the importance of secondary structures [39].

Key studies performed by Kozak already showed that RNA can fold back on itself to form a hairpin and that stable hairpin

structures of −50 kcal/mol in the 5' UTR can reduce translation per se [40] and moderately stable hairpins of -30 kcal/mol selectively inhibit translation only when occurring close to the 5' cap preventing translation initiation [41]. Agreeing with this, Babendure et al. showed in live cells that increasing the thermal stability of the hairpin reduces translation efficiency and hairpin structures close to the 5' cap inhibits translation. In addition, they also showed that increased GC content in the hairpin decreases translation efficiency dramatically [42]. Similar findings were reported in the Hoxa11 5' UTR where a GC-rich stem loop inhibits cap-dependent translation when inserted in other mRNAs [33].

Stretches of guanines exhibit a high propensity to self-assemble into RNA G-quadruplexes (G4s). G4s are identified in thousands of mammalian transcripts and are enriched in 5′ and 3′ UTRs of mRNAs pointing to a regulatory role in translation. Their high thermal stability makes it impossible for the ribosomes helicase activity to unwind this structure and therefor halts translation elongation and inhibit expression of their corresponding mRNA [43]. Resolving these structures requires specialized proteins and numerous RNA binding proteins that bind and unwind G4s to stimulate translation of individual mRNAs are identified. For example DHX36, a DEAH-box helicase binds and unwinds G4s formed in the 5'UTR of the *Gnai2* mRNA thereby regulating its translation which is essential for muscle stem-cell regenerative functions [44]. Additionally, G4s are involved in regulation of translation of ribosomal proteins as the majority of the mRNAs encoding ribosomal protein contain a folded G4 structure and mutation of the G4 led to increased protein expression [45]. Several interactors of G4s in ribosomal protein mRNAs are identified including DDX3X, DHX36 [45] and CNBP [46] indicating that there are several mechanisms in controlling ribosomal protein synthesis. The helicase eIF4A is also identified as a factor that promotes translation of many oncogenes and transcriptional regulators that also contain G4s in their 5' UTR [47]. This indicates that regulation of the helicase activity of eIF4A is an additional mechanism to control the translation of certain mRNAs. Additionally, it is shown that highly expressed transcripts often have a low structed 5'-UTR and first ~30 nucleotides of the CDS which may facilitate ribosome binding and translation initiation and is consistent between several studies [48, 49]. In contrast to this, secondary structures in the CDS positively correlate with elongation rate and protein output [48, 50] which might be due to the fact that mRNA secondary structures mediate sufficient spacing between ribosomes to prevent collisions later on or it might be that more structured mRNAs have an increased mRNA half-life and therefor can increase protein output.

Cis-regulatory elements and *trans*-acting factors

Sequence motifs act as *cis*-regulatory elements which can serve as binding sites for *trans*-acting RBPs or microRNAs (miRNAs) and regulate mRNA fate at many steps. Translation is highly interconnected with stability and therefore many RBPs regulate translation by stabilizing the mRNA or by recruiting the decay machinery fine tune protein expression (**Box 1**). Numerous RNA elements and their corresponding binding proteins are identified [51, 52] and interestingly, many RBPs recognize similar motifs however, binding specificity differs due to compositional complexity and structure of the RNA so many factors contribute to the RBP specificity [52, 53]. Also the mechanism of miRNA mediated repression was the subject of active research the last few decades (reviewed in [54]). Below we discuss several well characterized regulatory elements that control mRNA fate through interaction with RBPs or miRNAs.

Terminal oligopyrimidine (TOP) motifs

As indicated before, mTORC1 is a key modulator of translational control. mTORC1 activity has particularly an effect on mRNAs bearing a 5' terminal oligopyrimidine (5' TOP) motif comprising a 4–14 pyrimidine stretch directly downstream of the 5' cap followed by a G-rich stretch. Interestingly, many of the TOP containing mRNAs encode proteins associated with the translational machinery which enables the cell to respond quickly when protein synthesis needs to be repressed [55]. Various mechanisms by which

TOP containing mRNA are selectively more sensitive to translation inhibition are described. Kroun Damgaard et al. identified RNA-binding proteins TIA-1 and TIAR as key proteins in the repression of TOP containing mRNA (*Fig. 5a*) [56]. TIA-1 and TIAR are associated with **stress granules** and upon amino acid starvation these proteins bind to TOP mRNAs and assemble them in stress granules to inhibit their translation. Later was proposed that the inhibitory effect of mTORC1 on TOP containing transcript was mainly attributed to the phosphorylation of 4E-BPs via a mechanism that prevents the interaction between eIF4G and eIF3 that weakens the affinity of eIF4E for the mRNA cap (**Fig. 5a**)[57]. TOP mRNAs highly rely on eIF4G binding to their cap to recruit the translation initiation machinery as depletion of eIF4G selectively supressed TOP containing mRNAs [57].

The La-related protein 1 (LARP1) is also identified as a key repressor of TOP mRNAs (**Fig. 5a**)[58]. mTORC1 phosphorylates LARP1s TOP binding domain (DM15) making it unable to bind to the TOP motif and repress translation. Upon inactivation of mTORC1, LARP1 is able to bind the TOP motif resulting in repression of translation of TOP motif containing mRNAs [58]. Recently, it has been shown that that the eIF4E paralog eIF4E2 enhances the repression as it preferentially binds to TOP containing mRNAs and increases the affinity for LARP1 [59].

Lastly, AUF1 is also identified as a regulator of TOP containing mRNAs by promoting their translation although the mechanism in unclear [60].

AU- rich element (ARE)

Adenylate-uridylate (AU)-rich elements (ARE) are typically located in the 3'UTR and were originally identified as sequences that promote rapid degradation of mRNAs that have to be expressed only transiently such as, mRNAs coding for inflammatory cytokines or growth factors. However, research has now shown that their role extends far beyond this and depending on the RPB that is bound to the ARE it can also promote translation of certain mRNAs and is implicated in mRNA export and splicing. Over the years, various proteins that bind AREs are identified of which AUF1 was the first. It was first only

associated with degradation of its bound mRNA as AUF1 knock out mice are unable to degrade mRNAs of tumour necrosis factor-α (TNF-α) and interleukin-1β [61]. However, it is also appears to block degradation although this might be cell type specific [62]. On the mechanistic side, it has been shown that AUF1 can recruit the exosome to initiate degradation of the mRNA (**Fig. 5b**)[63].

Tristetraprolin (TTP) is another well characterized ARE binding protein that promotes mRNA decay of predominantly inflammatory cytokines by recruiting the CCR4-NOT complex [64] and decapping complexes (**Fig. 5b**) [65]. Furthermore, TTP can also repress translation by recruiting 4EHP which interferes with the initiation complex [66].

HuR is a member of the ELAV family of proteins and is associated with enhanced stability, translation and splicing of its target mRNAs. HuR typically resides in the nucleus and in response of stress conditions it can shuttle to the cytoplasm to increase the translation of its bound target mRNA. In human hepatocarcinoma cells it has been shown that binding of HuR to the 3' UTR of the CAT-1 mRNA mediates release form the **p-body** and reactivates it expression which was repressed by miRNA miR-122 (**Fig. 5b**) [67]. Binding of HuR to the ARE and its oligomerization on the mRNA leads to dissociation of the miRISC from the CAT-1 mRNA. Additionally, it was also shown to inhibit miRNA-mediated deadenylation of mRNA [68].

Iron response element (IRE)

One of the best explored element is the iron response element (IRE), a 30-nucleotide stem-loop structure in the 5'- or 3'UTR, that can be bound by iron regulatory proteins, IRP1 and IRP2 in humans, and play a crucial role in iron homeostasis. Iron deficiency triggers binding of the IRP to the IRE, located within the 5'UTR of target mRNA, and blocks for example mRNA translation of several ferritin (a protein involved in iron storage) subunits by preventing the eIF4F guided recruitment of the 43S pre-initiation complex to the mRNA (Fig. 5c). In the presence of excess iron, metal ions decrease the stability of the interaction between the IRE and the IRPs resulting in a conformational change and

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Stress granule
Stress granules are dense aggregations in the cytosol composed of RNAs and stalled translation pre-initiation complexes that appear when the cell is under stress

P-body
P-bodies are cytoplasmic ribonucleo-protein granules which contain proteins involved in mRNA degradation, repression and their corresponding target mRNA.

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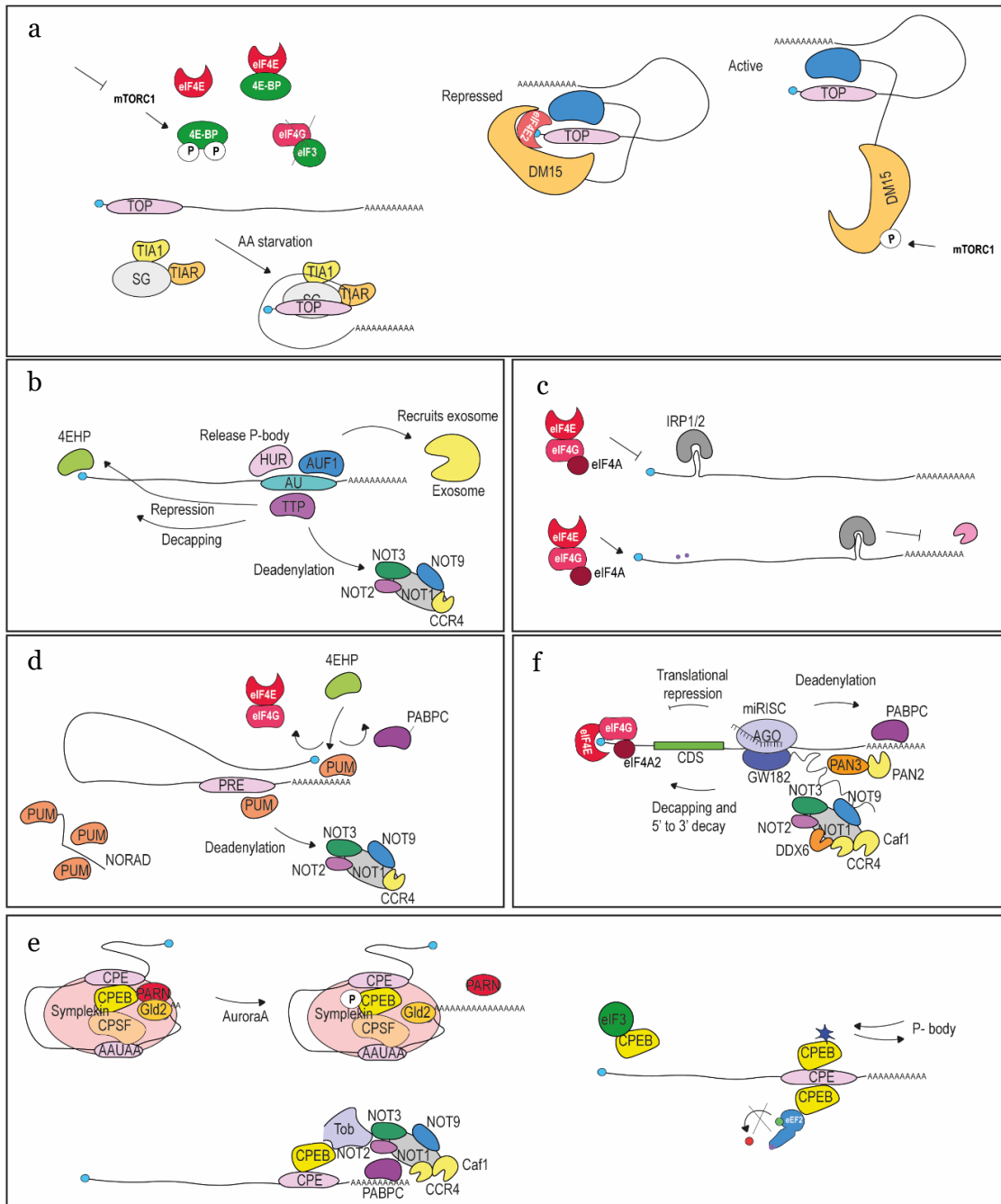
Fig. 5. Trans-acting factor mediated translational control. a. Stress granule associated proteins TIA-1 and TIAR bind TOP motifs upon amino acid deprivation thereby inhibiting their translation. The inhibitory effect of mTORC1 on TOP containing transcript is attributed to the phosphorylation of 4E-BPs via a mechanism that prevents the interaction between eIF4G and eIF3 that weakens the affinity of eIF4E for the mRNA cap. mTORC1 phosphorylates LARP1s TOP binding domain (DM15) making it unable to bind to the TOP motif and repress translation. Upon inactivation of mTORC1, LARP1 is able to bind the TOP motif resulting in repression of translation of TOP motif containing mRNAs. eIF4E paralog eIF4E2 enhances the repression. **b**, Translation of AU-rich containing transcripts is regulated by RBP via several mechanisms including, recruiting the exosome (AUF1), initiate deadenylation and decapping or recruit 4EHP that interferes with cap recognition (TTP) or release from inhibitory P-body (HUR). **c**, Iron deficiency stabilises the interaction between a hairpin structure (IRE) and IRPs which inhibits translation initiation. Excess iron results in a conformational change and release of the IRPs followed by translation initiation. IREs located in the 3'UTR protect the mRNA from rapid degradation. **d**, Translation of PRE containing transcripts is regulated by various mechanisms mediated by PUMs including recruitment of 4EHP or the deadenylation complex, antagonizing PABPC and competing with eIF4E or eIF4G. Long non coding RNA NORAD sequesters PUM from binding to target mRNAs. **e**, Translation of CPE containing transcripts is regulated by various mechanisms mediated by CPEB proteins including polyadenylation which activates translation, recruiting the deadenylation complex to stop translation, transport to p-bodies to repress translation, interaction with eEF2 to repress elongation or direct binding to eIF3 to inhibit translation initiation. **f**, as part of the miRISC, miRNAs repress translation of a multitude of mRNAs. miRNAs can inhibit translation initiation by interfering with the recognition of the 5' cap by eIF4F which involves recruitment of eIF4A2 rather than eIF4A, or by recruiting the deadenylation complex which is followed by decapping and degradation of the corresponding mRNA. Figures adapted and modified from [58, 69, 70].

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dissociation of IRP from the mRNA whereas it enhances the interaction between IRE and eIF4F promoting the recruitment of the 43S pre-initiation complex and translation of the mRNA [71]. IREs located in the 3' UTR are associated with regulation of the stability of the RNA. For example interaction of IRPs with IRE in the mRNA of the transferrin receptor protects the RNA from rapid degradation during iron depletion (**Fig. 5c**)[72].

Pumilio Recognition/Response Element (PRE)

Members of the PUF family of proteins, the human pumilio proteins, PUM1 and PUM2 are another well-known example of proteins that regulate mRNA fate of transcripts that contain a pumilio response element (PRE) in their 3' UTR. PUM1 and PUM2 share 76% sequence similarity and share many but not all target mRNAs. PUMs bind to the pumilio response element (PRE) with their highly conserved RNA-binding domain, the Pumilio Homology Domain (Pum-HD) that is identical for 91% between the two proteins. PUMs predominantly repress translation of a subset of mRNAs although translation promoting effects are also reported although this mechanism remains unclear [73]. PREs are predominantly located in the 3'UTR in a multitude of mRNAs involved in stem cell maintenance, development, fertility and neurological processes [74] and PUMs repress their translation by several mechanisms.

The best characterized mechanism is by recruiting the CCR4-NOT deadenylase complex with its N-terminal region, via direct binding to its subunits to promote poly(A) shortening and initiate the degradation of the mRNA to stop its translation (**Fig. 5d**) [75]. Furthermore, PUMs use another mechanism to repress translation that requires the poly(A) binding protein, PABPC where it antagonizes its function to promote translation resulting in decreased levels of protein expression. Although the exact mechanism of inhibition is unclear it is speculated that it involves competition with eIF4E or eIF4G and thereby disrupting translation initiation by interfering with the 'closed loop model' (**Fig. 5d**) [76, 77]. Additionally, PUMs can also inhibit translation of mRNAs lacking a poly(A) tail indicating that they can repress translation in a deadenylation independent way. Indeed, in other organisms other mechanisms of repression are identified such as recruitment of 4EHP, a cap binding protein, that binds the 5′ cap like eIF4E, but does not bind eIF4G therefore interfering with the initiation complex [78] and direct binding of PUM2 to the 5' cap to inhibit binding of eIF4E (**Fig. 5d**) [79].

The long noncoding RNA (lncRNA) NORAD is identified as a major regulator of PUM activity [80, 81]. Depending on the cell line used around 80-1000 copies of NORAD are present in the cytoplasm each containing at least 15 functional binding sites for PUM2 and to a lesser extend PUM1 indicating that it can sequester a significant fraction of the total pool from binding to its targets (**Fig. 5d**) [80, 81]. Depletion of NORAD resulted in downregulation of regulators of the cell cycle, mitosis, DNA repair, and DNA replication and subsequently to chromosomal instability indicating that NORAD is an essential regulator of PUMs in the maintenance of genomic stability [80].

Cytoplasmic polyadenylation element (CPE)

The cytoplasmic polyadenylation element binding (CPEB) proteins recognize a specific U-rich motif, the cytoplasmic polyadenylation element (CPE), in the 3' UTR of several classes of mRNAs involved many processes including oogenesis [82], the cell cycle [83], neural development, learning and memory [84]. Vertebrates contain four *CPEB* genes, *CPEB1-4*, and CPEB2-4 are more similar to each other than to CPEB1. Key studies are performed in *Xenopus* and other model organisms but the proteins and their functions is very conserved among species. CPEB1 and CPEB4 are the only members that can induce polyadenylation which was initially identified in arrested *Xenopus* oocytes that resume meiosis which requires translation of dormant mRNAs [85, 86].

Two elements in the 3' UTR are needed for polyadenylation to occur, the CPE that is bound by CPEB1 and the polyadenylation hexanucleotide AAUAAA that is recognised by cleavage and polyadenylation specificity factor (CPSF) (**Fig. 5e**). Phosphorylation of CPEB1 by the kinase Aurora A leads to increased interaction CPEB1 with CPSF. Together with the scaffold protein Symplekin, Gld2, a cytoplasmic poly(A) polymerase,

ePAB, a variant of the poly(A) binding protein and Maskin, an eIF4E binding protein they form a ribonucleoprotein (RNP) complex. PARN, a poly(A)-specific ribonuclease also associates with the machinery and competes with Gld2 to shorten the poly(A) tail. In response to developmental cues the phosphorylation of CPEB1 results in dissociation of PARN from the complex to allow Gld2-catalyzed polyadenylation, binding of ePAB to the poly(A) tail and subsequent displacement of Maskin and assembly of the initiation complex on the mRNA to initiate translation [87]. The transcripts that are polyadenylated includes the CPEB4 mRNA and when CPEB1 is degraded in later stages, CPEB4 replaces CPEB1 and recruits the factors needed for polyadenylation [86]. Their role is not restricted to meiosis only as it is shown that they also regulate mitotic entry and cell proliferation [88]. Interestingly, in cancer cells CPEB4 is reactivated and results in poly(A) tail elongation and translational activation of genes that are silenced in healthy tissue [89].

CPEB proteins are also implicated in decay and translational repression. CPEB1 mediates deadenylation on many transcripts including c-myc mRNA where Tob binds to CPEB1 and recruits the Caf1 deadenylase to PABPC to promote deadenylation in a serum-starved quiescent state while in unstressed cells Tob and Caf1 dissociate from CPEB1 probably mediated by phosphorylation of Tob to allow translation of the mRNA (**Fig. 5e**)[90, 91]. The same deadenylation mechanism is used by CPEB3 [92]. CPEB3 is also identified as a translational repressor in a decay independent manner in neuronal cells as it shown to localise to p-bodies when SUMOylated (**Fig. 5e**) [93]. Following synaptic activity, CPEB3 is de-SUMOylated allowing for its oligomerization, aggregation and translocation form the P-body to polysomes to promote translation of its target mRNA [93, 94]. CPEB2 uses a distinct mechanism to repress translation at the elongation step rather than inducing decay of the mRNA as is shown on the HIF-1α mRNA [95]. When bound to its target sequence, CPEB2 can interact with eEF2 to inhibit elongation likely mediated by steric hinderance that reduces GTP hydrolysis by eEF2 that is needed to complete a translocation step resulting in reduced speed of elongation (**Fig. 5e**). Upon oxidative stress, CPEB2 dissociates from the mRNA to allow fast elongation [95]. CPEB4 uses again a different mechanism to repress translation namely by direct binding to the eIF3 complex and interfering with the translation initiation machinery (**Fig. 5e**)[96]. Additionally, CPEB2 is shown to promote expression of PDGFRα [97] and GRASP1 [98] although the mechanism by which CPEB2 promotes translation is unclear.

microRNAs

miRNAs are conserved short non-coding RNAs of ~21-23 nucleotides that mediate repression or degradation by binding to a complementary sequence in the 3' UTR of their target RNA. They originate from a primary miRNA (pri-miRNA) which is processed to a ~60-75 nucleotide long hairpin precursor pre-miRNA and eventually to a ~21-23 nucleotides long duplex forming miRNA. When bound to an Argonaut (AGO) protein one of the strands is released. Together with AGO proteins and the protein GW182, the remaining miRNA forms a miRNA-induced silencing complex (miRISCs) and regulates more than 60% of the protein coding genes in humans [99] and therefor substantially contributes to translational control. In humans the family of AGO proteins consists of four proteins AGO1-4 and AGO2 differs from the other members as it is the only member that is catalytically active and can cleave the mRNA when a miRNA is fully complementary to the mRNA. However, in humans the vast majority of the miRNAs is only partially complementary to the mRNA [54]. Nevertheless, partial binding is sufficient to repress translation of the mRNA or to induce degradation. Multiple miRNA binding sites can be present in a transcript and multiple miRNAs can cooperate to enhance repression or degradation.

The accessibility of the miRNA binding sites appear to be very important for miRNA mediated repression. Wan et al. showed that only structural accessible predicted sites are truly bound by AGO in contrast to predicted structured sites that were not bound by AGO [39]. In line with this, RBPs and miRNAs can cooperate as is shown for more than 100 RBPs and typically they enhance miRNA mediated repression by changing the RNA structure to make the miRNA binding site

more accessible [100]. One such an example is PUM1 and miR-221 and miR-222 on the p27 mRNA [101]. The PRE and miRNA binding sites form a stem loop that prevents binding of miR-221 and miR-222 to their binding sites. Upon phosphorylation of PUM1, it is able to bind to the PRE resulting in an open conformation of the stem loop allowing miR-221 and miR-222 to bind to their binding sites [101].

Several mechanisms by which miRNAs inhibit translation or induce deadenylation and decapping and subsequent decay are identified (reviewed in [69]). Both the Pan2-Pan3 and Ccr4-Not complex can directly bind to miRISC complex member GW182 to initiate deadenylation, the latter can also interacts with DEAD-box ATPase DDX6 to promote decapping of the mRNA (**Fig. 5f**) [102, 103]. Additionally, the miRISC complex can also inhibit translation initiation by interfering with the recognition of the 5' cap by eIF4F which involves recruitment of eIF4A2 rather than eIF4A, as observed with miRNA let-7 (**Fig. 5f**) [104, 105]. miRNA target sites are also identified in the CDS where they inhibit translation independent of GW182 and seem to stall the ribosome during elongation coupled with degradation of the nascent polypeptide rather than the mRNA itself [106].

Box 1 mRNA decay

Degradation of a mRNA is initiated by deadenylation at 3' end followed by decapping of the 5' cap and subsequent exonucleolytic degradation of the mRNA. Two deadenylation complexes exist, the Ccr4-Not complex consisting of exonuclease CCR4 and several non-enzymatic proteins and the Pan2-Pan3 complex where Pan2 has exonuclease activity and Pan3 mediates Pan2 recruitment. Caf1 is another deadenylase that can associate with the Ccr4-not complex. After deadenylation, the mRNA can be degraded in 3' − 5' direction by the exosome complex and in a 5'-3' direction by exonuclease XRN1. Before degradation in 5'-3' direction can be initiated, the 5' cap structure of the mRNA has to be removed first. Cap removal is catalysed Dcp2 and its activity is enhanced by several proteins including Dcp1 and DDX6 [107].

The epitranscriptome in translation efficiency

Besides modification at the 5' end, mRNA is highly subjected to internal base modifications thereby carrying dynamic epitranscriptomic information to control mRNA fate. From the >160 chemical modifications of RNA that are characterized the majority is found on non-coding RNA and only a subset is found in mRNA [108]. As said, these modifications are dynamic and are catalysed by RNA modifying proteins that deposit and remove them, so called "writers" and "erasers" respectively and are recognised by "reader" proteins that triggers downstream reactions.

m^6A

Among all the RNA modifications that have been identified N6-methyladenosine (m^6A) is the most prevalent modification found in mRNA and can occur in the 5' UTR, the CDS and the 3'UTR but is predominantly found in the vicinity of the stop codon [109]. Most transcripts are only methylated in one of these regions, some transcripts contain methylation residues at two of the sites and only very few are methylated in all three sites [110]. The role of m^6A in mRNA is coupled to several steps of mRNA metabolism such as pre-mRNA splicing, nuclear transport, transcript stability and translation and is highly dependent on the location in the transcript [111]. The effect of m^6A is dependent on the binding of reader proteins that recognize m^6A. YTHDF1 is such a reader protein and promotes translation efficiency in an m^6A-dependent manner (**Fig. 6a**). YTHDF1 binds predominantly to m^6A in the 3'UTR of mRNA transcripts and appears to promotes ribosome loading on its target RNA thereby elevating mRNA translation efficiency. YTHDF1 interacts directly with several subunits of the translation initiation complex including several subunits of eIF3 thereby promoting the "closed loop" formation [112]. Writer protein METTL3, originally identified as a methyltransferase responsible for m^6A modification also promotes translation of a subset of m^6A-modified mRNAs independent of its catalytic activity [113]. Lin, et al have shown direct interaction between both wild-type and catalytically inactive METTL3 and the translation initiation machinery and propose

a model where METTL3 helps recruit eIF3 to promote translation initiation also independent of downstream m6A reader YTHDF1 (**Fig. 6a**) [113]. In support of this data Choe, et al also identified a direct interaction between METTL3 and the eukaryotic translation initiation factor 3 subunit h (eIF3h) and showed via electron microscopy that METTL3 is in close proximity with cap binding proteins on individual polyribosomes supporting a looping model to promote efficient translation [114]. Besides m6A in the untranslated regions, approximately 35% of m6A residues are located within the CDS and are usually located in regions with predicted stable secondary structures [110]. Using ribo-seq data ribosome pausing is observed when the A-site codon is methylated which increases in the absence of m6A indicating that methylation reduces ribosomal pausing via a mechanism in which m6A acts to unfold secondary structures that can otherwise act as a roadblock for the ribosome [110]. It has become clear that the presence of m6A can have various effects on several stages of translation but its role is more widespread than discussed here. Many more writers and readers are present and are involved in these processes and additional processes such as mRNA decay that has a large impact on translation and is comprehensively described elsewhere [115].

m^6A_m

If the first nucleotide adjacent to the 5' cap is an adenosine, which is typically already methylated on the ribose at the 2'O-hydroxyl position (Am) it can be further methylated at the N6 position to form N6,2′-O-dimethyladenosine (m^6A_m) co-transcriptionally by the methyltransferase PCIF1 [116] in a cap-dependent manner [117]. The presence of m^6A_m is associated with increased stability of a subset of mRNAs as it protects against Dcp2 mediated decapping such as during microRNA-mediated degradation [118]. However, depletion of m^6A_m does not impair the stability of mRNAs that are highly expressed suggesting that other features account for the observed stability as well [116]. Interestingly, low expressed mRNAs harbouring m^6A_m were sensitive to m^6A_m depletion indicating that m^6A_m promotes stability in a transcript specific manner [116]. Additionally, m^6A_m is

implicated in the upregulation of cap-dependent translation of mRNAs involved in mRNA transport, metabolic processes and translation [117]. Cells depleted from m^6A_m were highly susceptible to oxidative stress which might due to decreased translation efficiency of the mRNA of superoxide dismutase (SOD1), upon loss of m^6A_m [117] indicating a regulatory role of m^6A_m during the stress response.
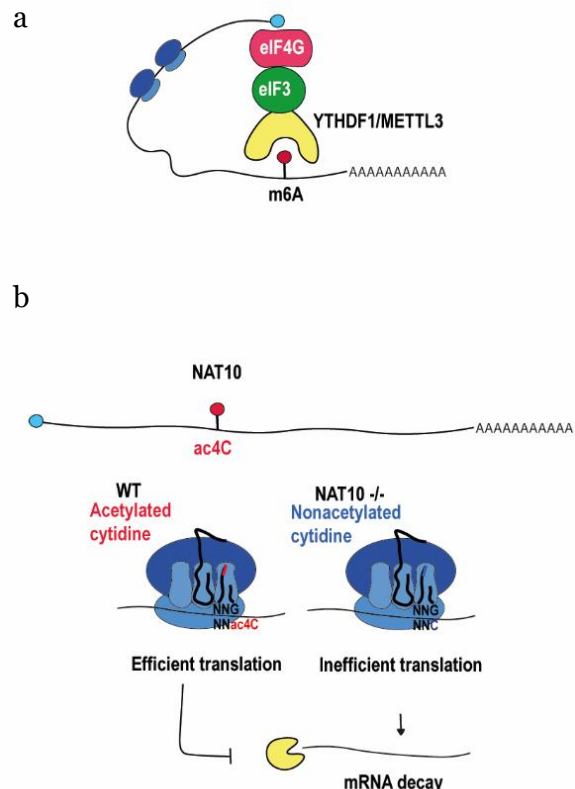
Fig. 6. Epitranscriptomic marks influence translation by multiple mechanisms. a, Several m6A readers including YTHDF1/METTL3 enhance translation by promoting a closed loop formation which stimulates translation. **b**, ac4C writer NAT10 deposits ac4C primarily at the 5' end of the transcript. Upon loss of NAT10, a decrease in expression was observed only of mRNAs normally marked with ac4C within their coding sequence through decreased stability of these mRNAs. ac4C is normally enriched in wobble positions in these mRNAs and this enhances translation of these mRNAs. Figure adapted and modified from [119].

m^1A

Decades ago, N1-methylation on RNA adenosine (m1A) was already identified on tRNA and rRNA where it has a role in structure and function of these RNAs. Later m1A was also identified in mRNA and is highly conserved between mammals [120]. m1A-seq identified that the most of the genes that contain m1A, contain only one m1A site (70%) and are enriched in classes related to

translation and RNA processing. Just like m⁶A, m¹A can occur in all segments of the transcript (5' UTR/CDS/3' UTR) however, in contrast to m⁶A, m¹A is almost absent in the 3'UTR and is enriched around translation initiation start sites [120, 121] and appears to be associated with the first splicing site of the transcript. Furthermore, m¹A correlates with a higher GC-content and high structured 5'UTR around the start codon in particular and correlates with increased translation efficiency and increased protein output when located in the 5'UTR compared to transcripts without m¹A [120, 122].

m⁵C

5-methylcytosine (m⁵C) is a widespread modification in diverse classes of RNAs including tRNAs, rRNAs, non-coding RNAs and also mRNAs [123]. In mRNAs m⁵C is non-randomly distributed predominantly close to the translation start site in the 5'UTR and AGO protein binding regions in the 3′UTR [123] although direct evidence for a role in miRNA mediated translational control is currently lacking [124]. Several studies indicate that the presence of m⁵C sites in the CDS correlates with lower translation efficiency [125, 126] but higher mRNA stability [126] as is shown in bladder cancer where reader YBX1 recognizes m⁵C present in the 3'UTR of heparin binding growth factor (HDGF) and stabilizes the mRNA by recruiting ELAVL1, a protein known to be involved in mRNA stability [127].

ac⁴C

Acetylation of cytidine was initially identified at the wobble position of the anticodon of elongator tRNA$_{met}$ in E. *coli* and later also on eukaryotic Serine and Leucine tRNAs. Arango, et al identified the presence of ac4C on mRNA, mediated by NAT10, predominantly in 5'UTR and in the CDS with enrichment around the translation start site implying a role in gene expression [119]. Upon loss of NAT10, a decrease in expression was observed only of mRNAs normally marked with ac4C within their CDS through decreased stability of these mRNAs. They found enrichment of ac4C in the wobble position and this enhances translation of these mRNAs indicating a direct role for ac4C

in translation efficiency by supporting tRNA recognition and promoting stability (**Fig. 6b**) [119].

Codon optimality

Except for Methionine, every amino acid is encoded by multiple synonymous codons. Not every synonymous codon is used to the same extend and there is a bias towards specific codon usage between species and also within groups of genes and preferred codons are more frequently used in highly expressed genes. The rate of elongation can vary between transcripts mediated by differences in codon composition of the mRNA and the efficiency of the selection of the corresponding cognate tRNA form the pool of tRNAs present in the cytoplasm, a concept known as codon optimality. Analysis of ribo-seq data in yeast showed that indeed ribosome densities inversely correlates with tRNA abundances indicating that the higher the concentration of a certain tRNA is the faster it is decoded by the ribosome [128] and recently it was shown that the same holds true in human cells [129, 130]. In line with this, Schott et al. showed that optimal codon content correlates with faster RLT [4]. Indeed, ribosomal proteins do have a high optimal codon content of ~88% [131] and were shown to have a fast RLT [4] suggesting that fast movement over the mRNA increases the pool of recycled ribosomes and increases the RLT [4]. An interesting example of how codon bias influences gene expression is the RAS family of small GTPase members KRAS and HRAS. Although the proteins share ~85% sequence similarity, their codon composition varies widely and KRAS is predominantly composed of non-optimal codons to keep its protein expression low [132]. Synonymous mutations that increase KRAS protein expression are associated with the development of cancer.

Codon content is not only associated with elongation rate and thus protein output, it is also a major determinant of mRNA stability [131]. Slow ribosomal movement over non optimal codons is directly recognised by the deadenylation complex Ccr4-not [133] which is followed by decapping of the mRNA by Dhh1p in yeast and subsequent degradation of the mRNA [134]. While non optimal codons are associated with lower stability of the mRNA they also slow down or pause elongation to allow correct co-translational

polypeptide folding of secondary structures in the ribosome exit tunnel such as α-helices, β-turns and small zinc finger domains [135].

Peptide sequence

Formation of the nascent polypeptide chain can also influence translation rates. This starts already during the peptide bond formation in the PTC. Depending on the amino acids present in the PTC, the speed of peptide bond formation may vary and thus affect the rate of elongation. In particular Proline has been shown to have a lower reactivity compared to other amino acids and slows down elongation by pausing the ribosome [136, 137]. In addition, two or more consecutive positively charged amino acids generate forces that pushes the P-site amino acid away from the A-site amino acid, which increases the time needed to incorporate the amino acid and alters the translation speed. This is in contrast to negatively charged amino acids which generate forces that bring the two amino acids closer together [138].

The nascent polypeptide leaves the ribosome through the exit tunnel which has a negative electrostatic potential. Positively charged amino acids may change the electrostatic potential when proceeding through the tunnel with pauses or arrests as a consequence allowing for correct folding of the protein which also results in slower elongation speed [139].

Concluding remarks

The state of knowledge on the mechanisms of translation regulation is rapidly growing with the development of new molecular techniques. We can conclude that there is not one feature that influences the translation efficiency of a transcript at the time, but in fact many features cooperate at the same time to allow translation in a precisely controlled manner. We need to keep in mind that there are more aspects of RNA metabolism such as stability, transport and storage that contribute to translational control but are not completely covered here. In this review, we took a mechanistic look at how certain features of the mRNA affect translation. 1) The presence of an IRES or MIRES can mediate cap-independent translation in certain physiological- and stress conditions. 2) The presence of uORFs inhibit translation of the canonical ORF and only allows translation of the canonical ORF in certain conditions, dORFs enhance translation of the canonical ORF. 3) An unstructured region near the start codon allows for efficient recognition of the start codon. Highly structured regions inhibit translation and activity of helicases that resolve them regulate translation of the mRNA. Structures also contribute to sufficient spacing between the ribosomes to prevent collisions later on. 4) Depending on the context, a RBP can have a translation promoting or repressive effect. miRNAs inhibit initiation followed by degradation of the mRNA. 5) Epitranscriptomic marks influence translation on several levels, they can enhance initiation by promoting circularization of the mRNA, increase elongation by supporting tRNA recognition or stabilize the mRNA to increase protein output. However, epitranscriptomics is a relatively new field and the exact mechanism of translation regulation of many of the marks are unclear at the moment. 6) The codon composition of a mRNA determines elongation speed and RLT. Optimal codons correlate with faster decoding and RLT. Non-optimal codons allow for pauses needed for correct folding of the nascent peptide. The balance between optimal and non-optimal codons also determine how fast a mRNA is degraded. 7) Depending on the amino acids, peptide bond formation may take longer. Positively charged amino acids reduce the speed of the nascent peptide through the ribosome exit tunnel which results in reduced elongation speed. All these factors contribute to the translation efficiency of a given transcript an together they for control correct timing and production of a functional protein. As the technology advances, the development of new techniques in the future will continue to reveal the molecular complexity of translation regulation. This is important because deregulation of mRNA translation is a hallmark of many diseases. Targeting mRNA translation has become a hot area in drug discovery the last few years. In particular in the field of epitranscriptomics, the development of small molecule inhibitors for RNA-modifying proteins has attracted a lot of attention and also inhibitors of proteins involved in translation initiation are under investigation [140]. Not only provides mRNA translation as a process a therapeutic window to treat diseases, mRNA itself has also emerged as a promising therapeutic drug as

we have seen for example with the COVID-19 vaccines but also in cancer immunotherapy it shows promising results in clinical testing [141]. Understanding the exact mechanism of how elements of the mRNA contribute to translation efficiency is therefor of importance so that protein output can be precisely controlled.

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Layman's summary

Our genetic information is stored in our DNA. The DNA encodes RNA, which can be converted into a protein by the ribosome, a process that is called: translation. Each cell can contain millions of ribosomes so in theory every free RNA can be translated into a protein. However, certain proteins are needed more or less than others while the same amount of RNA is present in the cell. By regulating how efficient a RNA is translated, the cell can control protein output per transcript. The efficiency is controlled by many mechanisms including features that are encoded by the mRNA transcript itself. In this review we discuss which features exist in the mRNA and how they contribute to the control of mRNA expression. The protein coding sequence (canonical ORF) of a mRNA is flanked on both sides by an untranslated region (UTR), the 5' and 3' UTR. Both ends are protected against rapid degradation. The 5' end with a so called 5' cap and the 3' end with a poly(A) tail. Before the ribosome can start to translate, many other proteins have to bind to the RNA. Many of these proteins are regulated during stress conditions so that translation can be stopped to save energy for the other processes in the cell. However, mRNAs that are essential during stress can still be translated because they evolved in such a way that they can initiate translation without binding of all those proteins. Some mRNAs also contain a second ORF in their 5' UTR that sequesters all the ribosomes from the canonical ORF and only in stress conditions allows translation of the canonical ORF. Depending on the sequence, the mRNA can fold into structures. We describe how several structures affect translation. The mRNA also contains many sequence motifs that can be recognised by *trans*-acting factors such as RNA binding proteins and miRNAs which control the fate of the mRNA. We discuss several of these elements and their corresponding *trans*-acting factors and how they control mRNA fate. Chemical modifications on the mRNA can also influence translation rates in several ways. From the >160 modifications that are identified at the moment, only a subset is identified on mRNA and we discuss how several of them influence translation rates. Every nucleotide triplet of the RNA forms a codon which is recognised by a tRNA with corresponding anticodon. Depending on the presence of the corresponding tRNA, a codon can be optimal (when tRNA is abundant) or non-optimal (when tRNA is rare). We explain how the optimality of the composition of the mRNA also contributes to translation efficiency. Finally, we discuss how the charge of the nascent peptide affects elongation speed.