*Annual Review of Biochemistry*

# The Organizing Principles of Eukaryotic Ribosome Recruitment

Jerry Pelletier[1,2,3] and Nahum Sonenberg[1,2]

[1]Department of Biochemistry, McGill University, Montreal, Quebec H3G 1Y6, Canada; email: jerry.pelletier@mcgill.ca, nahum.sonenberg@mcgill.ca

[2]Rosalind and Morris Goodman Cancer Research Center, McGill University, Montreal, Quebec H3A 1A3, Canada

[3]Department of Oncology, McGill University, Montreal, Quebec H4A 3T2, Canada





Annu. Rev. Biochem. 2019. 88:307–35

The *Annual Review of Biochemistry* is online at biochem.annualreviews.org

https://doi.org/10.1146/annurev-biochem-013118-111042



## Keywords

translation, ribosome recruitment, eukaryotic initiation factors, eIF4F, eIF4E, eIF4A, eIF4G, eIF4B, eIF4H

## Abstract

The stage at which ribosomes are recruited to messenger RNAs (mRNAs) is an elaborate and highly regulated phase of protein synthesis. Upon completion of this step, a ribosome is positioned at an appropriate initiation codon and primed to synthesize the encoded polypeptide product. In most circumstances, this step commits the ribosome to translate the mRNA. We summarize the knowledge regarding the initiation factors implicated in this activity as well as review different mechanisms by which this process is conducted.

## Contents



## INTRODUCTION

Translation of messenger RNA (mRNA) is an energetically demanding and complex process that is subject to sophisticated regulation. It is divided into four phases: initiation, elongation, termination, and ribosome recycling. In eukaryotes, translation initiation is the most intricate of all phases and is generally rate limiting. It serves to achieve three major objectives: (*a*) render the 40S ribosome subunit competent for mRNA binding, (*b*) recruit the 40S ribosome to the mRNA, and (*c*) relocate the 40S ribosome to the initiation codon, followed by joining of the 60S ribosome subunit. In most models, initiation starts with the recruitment of a ternary complex (TC) [consisting of eukaryotic initiation factor 2 (eIF2), GTP, and a methionyl-initiator transfer RNA (Met-tRNA$_i$)], eIF1, eIF1A, eIF3, and eIF5 to the 40S subunit to form a 43S preinitiation complex (PIC). The 43S PIC is then recruited to the 5′ cap structure by the eIF4F complex, and GTP is hydrolyzed upon or after 43S PIC binding, but the products (GDP and $P_i$) remain associated with eIF2. The mRNA-bound PIC scans along the 5′ leader region [also referred to as the untranslated region (UTR)] until an initiation codon is encountered, whereupon eIF1, $P_i$, and the eIF2–GDP–eIF5 complex are released. This allows eIF5B–GTP to bind the 40S ribosome to facilitate 60S joining. Next, GTP is hydrolyzed, eIF5B and eIF1A are discharged, and the newly assembled 80S ribosome is now poised for elongation. This route of initiation is referred to as "cap dependent" or "eIF4E dependent." An alternative mechanism involving recruitment of a ribosome directly to a site within the mRNA 5′ leader is known as internal ribosome entry site (IRES)–mediated initiation.

Translation initiation is controlled via three major checkpoints: the phosphoinositide 3-kinase (PI3K)/mammalian (or mechanistic) target of rapamycin (mTOR) pathway, the mitogen-activated protein kinase (MAPK) signaling cascade, and the integrated stress response (ISR). PI3K/mTOR integrates extra- and intracellular cues to effect translation by regulating the association of eIF4E with its inhibitory partners, 4E-binding proteins (4E-BPs). Activation of the MAPK pathway leads to phosphorylation of eIF4E, thereby stimulating translation of select mRNAs. The ISR engenders eIF2$\alpha$ subunit phosphorylation, reduces TC levels, and causes a depression of general translation, while paradoxically stimulating the translation of selective mRNAs [e.g., general control non-depressible 4 (GCN4) in yeast, activating transcription factor 4 (ATF4) and ATF5 in vertebrates]. In this review, we focus on the commitment step of eukaryotic protein synthesis, in particular the role that the eIF4 class of initiation factors play in the recruitment of the 43S PIC to the mRNA.



## THE eIF4 TRANSLATION INITIATION FACTORS

The principal function of eIF4F is to act as a molecular broker in recruiting ribosomes to mRNAs. eIF4F consists of three subunits: eIF4E (a cap-binding protein), eIF4A (a DEAD-box, RNA-dependent ATPase and RNA helicase), and eIF4G (a molecular platform with multiple docking sites). The RNA chaperones, eIF4B and eIF4H, stimulate eIF4A and eIF4F activity by promoting the coupling of ATP hydrolysis to RNA unwinding.

The $m^7$GpppN (where N is any nucleotide, and m is a methyl group) cap structure (**Figure 1*a***), present on all nuclear-transcribed cytoplasmic eukaryotic mRNAs, is critical for splicing, polyadenylation, mRNA stability, and translation. In most eukaryotes (notable exceptions being fungi and plants), the 2′ hydroxyl residue of the first (found in Cap 1) and second (found in Cap 2) downstream ribose is also methylated.

### The eIF4E Family

The role of the cap structure in translation is multifaceted. It is recognized by different cap-binding proteins that act to impart disparate functional outcomes on gene expression, ranging from stimulating ribosome recruitment to selective inhibition of mRNA translation and miRNA-mediated suppression of translation.

**eIF4E.** eIF4E (eIF4E1) (**Figure 1*b***), the best-characterized cap-binding protein, was initially identified by chemical cross-linking to the reovirus mRNA cap structure and was purified using cap-immobilized affinity resins (1). *EIF4E* is an essential gene (2, 3) conserved across Eukaryota kingdoms. The mammalian gene can fully substitute for yeast *eIF4E* (4). eIF4E protein levels are generally rate limiting for initiation, with levels in exponentially growing cells estimated to be at $\sim 4 \times 10^5$ molecules/cell ($\sim$0.1 molecules/ribosome) (5). Expression of eIF4E must be tightly controlled, as even a modest increase in levels (2.5-fold) is sufficient to drive tumorigenesis and drug resistance (6–8). The human *eIF4E* gene (there are five eIF4E pseudogenes) is located on chromosome 4q23. It is amplified in several cancers and can trigger resistance to PI3K/mTOR–targeted therapy (9, 10). Its transcription is stimulated by the c-MYC (MYC) proto-oncogene product (11, 12), and *MYC* translation is eIF4E responsive, establishing an oncogenic self-sustaining feed-forward loop (13). Elevated eIF4E levels are associated with autistic-like behaviors in mice (14). A heterozygous base change in the eIF4E promoter region that leads to a 2-fold increase in protein levels was reported in two unrelated families with autistic siblings (15). Reductions in eIF4E activity have recently been linked to depression-like behavior in mice due to translational effects

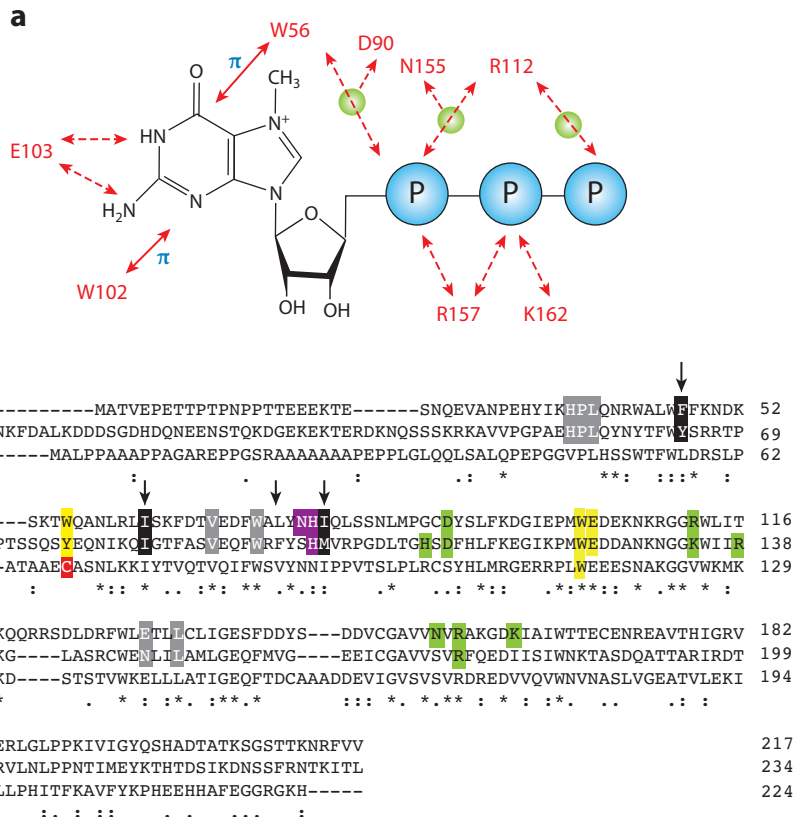


**Figure 1**

(*a*) Schematic diagram depicting cap–eIF4E interactions. Π electrons are indicated in blue. Water-mediated interactions are indicated with small green circles. Information is taken from PDB 1EJ1, PDB 1L8B, and PDB 1IPC. (*b*) CLUSTAL 2.1 multiple sequence alignment of human eIF4E (NP_001959), 4EHP (NP_001263265), and eIF4E3 (NP_001128123). Highlighted amino acids indicate $m^7G$-stacking and hydrogen-bonding amino acids (*yellow*) and amino acids interacting with the phosphates (*green*). (Note that E116 in eIF4E3 is not highlighted, as there is no information implicating it in cap binding.) In red is the C69 residue of eIF4E3 that substitutes for the canonical aromatic amino acid involved in $m^7G$ stacking. Grey and black highlights denote the amino acids involved in interacting with the 4E-BP1–conserved 4E-BM ($YX_4L\Phi$) motif and nonconserved domain, respectively (23, 55, 57, 223). Purple highlights identify amino acids interacting with the elbow loop in 4E-BP1 and eIF4G, which link 4E-BM to the noncanonical domain. The nonconserved domain of eIF4G1 interacts with the eIF4E amino acids, denoted by downward arrows. Abbreviations: 4E-BM, eIF4E-binding motif; 4E-BP, 4E-binding proteins; eIF, eukaryotic initiation factor; PDB, Protein Data Bank.

on mRNAs that are sensitive to the eIF4E phosphorylation state (16). These examples illustrate the link between perturbed eIF4E regulation and disease.

The structural basis for cap binding by eIF4E is explained by sandwiching of the $m^7G$ base between the indole side chains of tryptophans W56 and W102 (**Figure 1*a*,*b***). The N7-methyl residue ensures that the cap is in the *anti*-conformation, rather than the *syn*-conformation, thus rendering the cap more accessible for interaction with cap-binding proteins. The delocalized N7 positive charge significantly contributes to the binding energy via cation–π interactions (17, 18). Cap binding is further stabilized by E103, through interactions with the N1 and N2 hydrogens of the N7-methylguanine moiety and W102. A number of additional contacts are made between

charged side chains and the phosphates directly, or via water, to further stabilize the eIF4E–cap interaction (**Figure 1*a***). This explains why cap analogs (e.g., $m^7$GpppG, $m^7$GDP, $m^7$GTP) are more potent inhibitors of translation than $m^7$GMP or $m^7$G (19). The cap-binding cavity of eIF4E contains a pocket above the N7-methyl moiety capable of accommodating larger groups, thus explaining why mRNAs with artificial, bulky N7-ethyl or N7-benzyl caps are efficiently translated (20, 21).

In mammals, three related repressor proteins regulate the availability of eIF4E for the assembly of the eIF4F complex: 4E-BP1, 4E-BP2, and 4E-BP3 (4E-BP1 is the best characterized) (22). When hypophosphorylated, 4E-BPs compete with eIF4G for eIF4E and repress cap-dependent translation. The core eIF4E-binding motif (4E-BM; $YX_4L\Phi$, where $\Phi$ denotes a hydrophobic residue) is found in all three 4E-BPs (**Figure 2*a***). A second noncanonical eIF4E-interacting

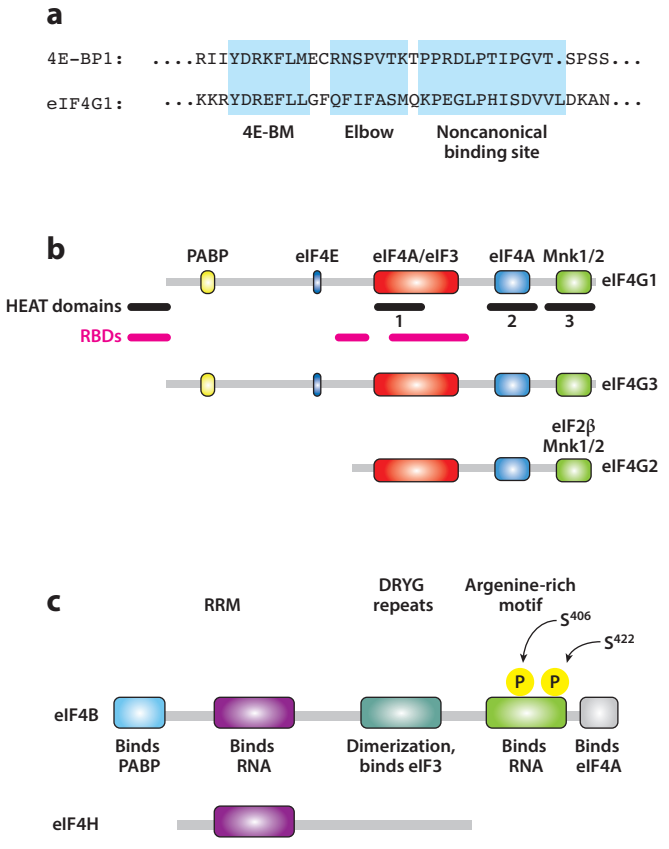


**Figure 2**

(*a*) Sequence alignment of eIF4E-interacting regions of human 4E-BP1 (NP_004086) and eIF4G1 (AAI40897) highlighting the canonical 4E-BM, the elbow region, and the noncanonical binding site. (*b*) Comparison between eIF4G1, eIF4G3, and eIF4G2 domain/motif organization. Colored blocks identify homologous regions. The HEAT domain and RBDs for eIF4G1 are shown. (*c*) Comparison between eIF4B and eIF4H domain/motif organization. The two phosphorylation sites at S406 and S422 and the function of each domain are indicated. Abbreviations: 4E-BM, eIF4E-binding motif; 4E-BP, 4E-binding protein; DRYG, aspartic acid, arginine, tyrosine, and glycine; eIF, eukaryotic initiation factor; HEAT, Huntingtin, elongation factor 3, protein phosphatase 2A, and TOR1; Mnk, MAP kinase-interacting kinase; PABP, poly(A)-binding protein; RBD, RNA-binding domain; RRM, RNA recognition motif.

domain is present downstream of the canonical 4E-BM (23–25) (**Figure 2*a***). Binding to the noncanonical region increases the affinity of 4E-BP1 for eIF4E by three orders of magnitude and enables 4E-BP1 to outcompete eIF4G for eIF4E binding (23). Binding of 4E-BP1 to eIF4E does not significantly affect the overall tertiary structure of eIF4E, including its cap-binding scaffold. In HeLa cells, the aggregate amount of 4E-BP1 and 4E-BP2 is similar to eIF4E, whereas the levels of eIF4G are in excess of eIF4E levels, indicating that sufficient 4E-BPs are available to compete with eIF4G for binding to eIF4E (5, 26).

In addition to 4E-BPs, eIF4E binds to other proteins through canonical and noncanonical sites to influence eIF4E function (27). Examples of these include (*a*) cup, a protein required for translational repression of oskar, nanos, and gurken mRNAs during *Drosophila* development (28), and (*b*) CYFIP1, which blocks translation of FMRP-targeted mRNAs (29). In other situations, protein interactions with eIF4E exert a more global effect on translation (e.g., Gemin5) (30). Other proteins, like 4E-T, a nucleocytoplasmic shuttling protein for eIF4E (31) and a component of the mRNA decay machinery (32), and Angel1, a predominantly endoplasmic reticulum– and Golgi–restricted factor (33), alter the subcellular localization of eIF4E. Further characterization of these interacting partners and their effects on eIF4E function should advance our understanding of how the eIF4E regulome is molded.

**eIF4E homologous protein.** eIF4E homologous protein (4EHP, eIF4E2) shares 30% identity with eIF4E, is 5–10 times less abundant than eIF4E, binds to cap analogs with 30- to 200-fold lower affinity than eIF4E, and does not interact with eIF4G (34–36). Similar to eIF4E, 4EHP binds to the cap via stacking interactions mediated by two aromatic residues (Y78 and W124) and through hydrogen bonding to the guanine base by the E125 side chain and the W124 backbone amide (35). The α- and β-phosphates directly contact the side chains of H100 and R174, respectively (35) (**Figure 1*b***).

*Drosophila* 4EHP interacts with both the cap structure and proteins bound to specific mRNA 3′ UTRs to inhibit translation (37). The interaction between 4EHP and its partners is mediated through an extended 4E-BM motif ($YXYX_4L\Phi$, where the requirement of the first Y varies among different interacting partners) as well as a noncanonical site. Selectivity for recruiting 4EHP, instead of eIF4E, to binding partners is imparted by the noncanonical site. In mammals, this is illustrated by Grb10-interacting GYF proteins (GIGYF) 1 and 2, which bind 4EHP but not eIF4E—an interaction that results in the suppression of translation of a subset of mRNAs during embryonic development (38). 4EHP is also an integral component of the microRNA (miRNA)–mediated silencing machinery (39). The translational repressive activity of the miRNA-induced silencing complex (RISC) is mediated by CNOT1—the scaffolding subunit of the CCR4–CNOT complex that recruits DDX6 to bind to 4E-T (see above). Interaction between 4E-T and 4EHP leads to a 4-fold increase in the affinity of 4EHP for the cap structure (39).

**eIF4E3.** eIF4E3 shares 29% identity with eIF4E1 and has not been well studied. Its mRNA expression is low and restricted to the heart, lung, and skeletal muscle (34). Binding of eIF4E3 to $m^7$GDP and $m^7$GTP is 10- to 40-fold lower as compared with eIF4E (40). eIF4E3 interacts with an eIF4G1 fragment containing the canonical $YX_4L\Phi$ binding site but does not bind any of the 4E-BPs (34). eIF4E3 is atypical in that it has only one aromatic residue (W115) that stacks with the guanine base of the cap (**Figure 1*b***). Instead of a second aromatic residue that participates in stacking in all other known cap-binding proteins, C69 is the residue that contacts the guanine base and the N7-methyl group (40). Mutagenesis of W115 or C69 to alanine severely impairs cap recognition. Given the tissue-restricted expression and low mRNA levels of eIF4E3, as

well as eIF4E3's lower affinity for the cap structure, its physiological relevance needs to be better delineated.

## The eIF4G Family

The members of the eIF4G family of proteins serve as scaffolds that link mRNA recognition to ribosome binding. They recruit the 43S PIC, either to the 5′ end of the mRNA by hosting the eIF4E–cap interaction or internally on some IRESes by directly interacting with specific RNA structural features. Our understanding of the mechanisms whereby these events are regulated is quite rudimentary owing to a lack of complete structural information, difficulties in working with these large proteins, and redundancies in function between family members.

**eIF4G1 and eIF4G3.** Mammals have three related eIF4G proteins: eIF4G1 (widely referred to in the literature as eIF4GI), eIF4G3 (referred to as eIF4GII), and eIF4G2 [referred to as DAP5 (death associated protein)/p97/NAT1 (novel APOBEC-1 target no. 1)] (**Figure 2*b***). Multiple isoforms of the proteins arise owing to differential promoter usage, alternative splicing, and the use of multiple translation initiation codons (41–43). eIF4G1 and eIF4G3 possess binding sites for poly(A)-binding protein (PABP), eIF4E, eIF4A, eIF3, and MAP kinase-interacting kinase (MNK), although some of the eIF4G isoforms produced from 5′ distal initiation codons lack the N-terminal PABP binding site. A homozygous germline missense mutation within the C-terminal domain (CTD) of eIF4G3 (mapping to the MNK binding site) in mice leads to infertility due to defective spermatogenesis (44).

***Poly(A)-binding protein interaction.*** eIF4G1 and eIF4G3 interact with PABP through a shared motif located near the N-terminal domain (NTD) (**Figure 2*b***). PABPC1, the major cytoplasmic PABP, has four nonidentical RNA recognition motifs (RRMs) and a globular CTD, binds poly(A) tracks, and is considered a critical regulator of mRNA translation and decay (45). By simultaneously interacting with the poly(A) tail and eIF4G1/3 via a stretch of 29 amino acids, PABPC1 engenders the formation of an mRNA closed loop, which is considered the basis for the observed synergy between the cap and the poly(A) tail in translation (46). Recent single-molecule-resolution fluorescent in situ hybridization (smFISH) experiments are at apparent variance with this notion (47, 48). By monitoring the proximity of mRNA 5′ and 3′ ends, the bulk of translating test mRNAs rarely exhibit colocalized 5′ and 3′ ends, unlike nontranslating mRNAs or mRNAs in stress granules where the ends are in close proximity (47, 48). Whether the eIF4G–PABPC1 interaction is transient in cells, is more important for translation of mRNAs that have just emerged from the nucleus, or occurs on mRNAs with exceedingly long poly(A) tails (which would not have been sampled in the smFISH analysis) remains to be established. Clearly, these results highlight the need to study translational mechanisms in cells.

The mechanistic models of enhancement of translation initiation by eIF4G–PABPC1 interaction are multifaceted: The interaction (*a*) stabilizes the association of eIF4F with the mRNA cap to stimulate the recruitment of 43S PICs, (*b*) stimulates 60S ribosomal subunit joining, and (*c*) increases the concentration of terminating ribosomes in the vicinity of the cap, which is aided by an interaction between eukaryotic release factor 3 (eRF3) and PABPC1 to loop out the 3′ UTR (45). Perturbing mRNA circularization impairs translation initiation. This can be achieved, for example, through binding of PABP-interacting protein 2 (Paip2) to PABPC1 RRMs 2 and 3, resulting in decreased affinity of PABPC1 for poly(A) RNA and prevention of PABPC1–eIF4G interaction (49). In HeLa cells, Paip2 levels are ∼10-fold lower than PABPC1 and PABPC3 levels (5), suggesting that this regulatory mechanism may be cell-type specific or locally restricted in the cell.

The findings that some mRNAs are circularized even in the absence of a cap or poly(A) tail and that circularization is used by some viruses during replication suggest this to be an important gene regulation feature. For example, picornavirus mRNAs are circularized even though they do not possess a cap (50, 51). Histone mRNAs, which lack a poly(A) tail, possess a stem-loop structure in their 3′ UTR responsible for recruiting SLBP (stem-loop-binding protein) and SLIP1 (SLBP-interacting protein 1) (52). SLIP1 binds eIF3g and eIF4G at a region located N-terminal to the PABP binding site (52, 53), thus forming a closed loop. Rotavirus mRNAs lack poly(A) tails but are rendered circular by the nonstructural protein 3 (NSP3) viral protein, which binds to a sequence motif in the 3′ UTR and to eIF4G (54). Because the NSP3 binding site on eIF4G overlaps with that of PABP, NSP3 also enhances rotavirus mRNA translation by blocking eIF4G–PABP interaction on cellular mRNAs, thus dampening competing host mRNA translation (54). A robust assay that could distinguish between de novo ribosome recruitment and the recycling of termination ribosomes is needed to advance our understanding of this latter step of translation initiation.

***eIF4E binding and its regulation.*** The 4E-BM ($YX_4L\Phi$) in eIF4G is conserved across species (55). In yeast, sequences adjacent to the 4E-BM also contribute to eIF4E binding by forming a bracelet-like structure around the eIF4E amino terminus (56). In *Drosophila*, plants, and humans, a bracelet structure is not formed; instead, eIF4G utilizes a second noncanonical binding site consisting mainly of hydrophobic interactions and differing from the site used by 4E-BP1 described above (57, 58) (**Figure 2*a***).

4E-BPs are recruited to mTORC1 by its raptor subunit via a 4E-BP C-terminal motif [F(E, D, Q or S)(M, I, L or V)(E or D)(M, I, L or V)] known as TOS (TOR signaling) (59). The 4E-BPs are phosphorylated on multiple threonine and serine residues in a hierarchical manner in response to mTORC1 activation, thus reducing their affinity for eIF4E (60–62). Phosphorylation of the accessible loop region between the canonical and noncanonical 4E-BP binding sites is sufficient to disengage 4E-BP from eIF4E (63). Additionally, phosphomimetic mutations engineered in the linker region of the *Drosophila* 4E-BP ortholog, Thor, confer resistance to translational repression (23). By coupling the phosphorylation status of 4E-BPs to mTOR activity, cells propagate many extra- and intracellular inputs to the translation initiation machinery.

Free, hypophosphorylated 4E-BP1, but not its phosphorylated counterpart, is targeted by the KLHL25-CUL3 ubiquitin ligase for degradation, thus conferring a shortened half-life on the former (64). Upon eIF4E knockdown the levels of free, hypophosphorylated 4E-BP1 are rapidly reduced as an adaptive mechanism to maintain physiological levels of eIF4F (64). This homeostatic control explains how, in spite of acute reductions in eIF4E, cells are able to sustain cap-dependent translation and viability (3, 64).

***eIF4A binding.*** Human eIF4G contains three HEAT domains, which consist of tandem repeats of stacked α-helices linked by short loops and named after the proteins in which the motif was first discovered (Huntingtin, elongation factor 3, protein phosphatase 2A, and TOR1). HEAT-1 is located within the middle of the protein (eIF4Gm), whereas HEAT-2 and HEAT-3 are positioned at the C-terminal region (eIF4Gc) (**Figure 2*b***). HEAT-1 and HEAT-2 bind both the NTD and CTD of eIF4A (65, 66). Yeast eIF4G lacks the HEAT-2 and HEAT-3 domains and therefore has only one eIF4A binding site, possibly explaining why eIF4A is weakly associated with yeast eIF4F (67, 68). Binding of eIF4A to HEAT-1 stimulates ATP hydrolysis and RNA binding (69). The ability to associate with two different HEAT domains may allow eIF4A to remain associated with eIF4G during the conformational changes that occur when eIF4A binds ATP and RNA (70).

***eIF3-binding and RNA-binding domains.*** eIF4G contains a domain that binds to eIF3 ($K_d$ = 132 nM) (71) (**Figure 2*b***). The association between eIF4G and eIF3 is mediated by the eIF3c, d, and e subunits, stabilized by mTORC1, unaffected by eIF4A, and thought to be the underlying mechanism by which the 43S PIC is recruited to the metazoan mRNA (71–73). Human eIF4G contains two RNA-binding domains (RBDs): one positioned amino-terminal to HEAT-1 and the other overlapping HEAT-1 (74, 75). The interaction between eIF4E and the cap is dramatically enhanced by these RBDs (75). The N-terminal RBD in yeast eIF4G promotes eIF4F–PABP association on mRNAs (76).

***MAP-kinase interacting kinase and eIF4E phosphorylation.*** In mammals, there are two *MNK* genes (*MNK1* and *MNK2*), each producing two proteins having different C termini through alternative splicing. MNK1 binds to the eIF4G CTD HEAT domain that facilitates the phosphorylation of eIF4E on S209 (77, 78). A comparison of wild-type and $eIF4E^{S209A/S209A}$ murine embryonic fibroblasts identified a small number of mRNAs whose translation is diminished in $eIF4E^{S209A/S209A}$ cells (79). Phosphorylation of eIF4E S209 does not affect eIF4E binding to 4E-BP1 but does decrease eIF4E's affinity for the cap (80, 81). MNK-mediated eIF4E phosphorylation may be required to facilitate faster rates of eIF4E/F release from the cap following a round of initiation. Clearly, better insight is required to elucidate the mechanism by which phosphorylation affects the eIF4E translatome.

Loss of *MNK1* and *MNK2* as well as knock-in of a nonphosphorylatable mutant of eIF4E (S209A) are well tolerated in mice; however, eIF4E phosphorylation is critical to its oncogenic activity (79, 82–84). Recently, the inability to phosphorylate eIF4E at S209 in the tumor microenvironment was found to reduce metastatic burden, in part owing to decreased survival of prometastatic neutrophils (85). These results highlight the potential antineoplastic activity that MNK1/2 inhibitors hold.

***Virus targeting of eIF4G.*** A number of viral proteins earmark eIF4G during infection. The proteins interact with eIF4G either to alter its functional properties or to cleave it. For example, at the commencement of the late phase of adenovirus infection, the adenovirus 100-kDa protein binds to the eIF4G1 CTD, which in turn blocks MNK interaction and reduces eIF4E S209 phosphorylation (86). The Epstein–Barr virus 2 (EB2) protein associates with eIF4F through interactions with PABP and eIF4G and stimulates translation in vitro (87). The influenza NS1 protein specifically binds to viral mRNAs and redirects the translational apparatus to viral mRNAs by co-opting eIF4G and PABP (88). These few selected examples underscore the untapped opportunities for developing antiviral drugs that would act by blocking eIF4G–virus protein engagement to curtail viral replication.

eIF4G is a prime target for proteolytic cleavage by viral and cellular proteases. Infection of cells by several picornaviruses, such as poliovirus, results in cleavage of eIF4G1 and eIF4G3, leading to the separation of the NTD PABP and eIF4E binding sites from the eIF4A/eIF3 binding domains. HIV (as well as several other retroviruses) cleaves eIF4G1 to generate three fragments (eIF4G3 is not efficiently cleaved by the HIV protease) (89). Cellular caspase-3 cleaves eIF4G1 and eIF4G3 at two sites, one that resides between the PABP and eIF4E binding sites and the other downstream of the eIF3 interaction site, which correlates with shutoff of translation during apoptosis (89). These examples emphasize the essential role that eIF4G plays in linking cap binding to ribosome recruitment.

**eIF4G2.** eIF4G2 lacks the PABP and eIF4E binding sites present in eIF4G1 and eIF4G3 (90–92) (**Figure 2*b***). eIF4G2 does not support cap-dependent translation, as it cannot interact with

eIF4E, but instead has been implicated in IRES-driven translation via a mechanism that recruits eIF4A1, eIF3, and eIF2β (93, 94). eIF4G2 is essential, as its knockout in mice results in death at early gastrulation (95). The role of eIF4G2 in cap-independent translation is critical for stem cell fate decisions, especially when stem cells transit from pluripotency to the differentiated state (96, 97). The absence of eIF4G2 in embryonic stem cells results in a failure of eIF4E-independent translation of, among others, the mRNAs encoding MAP3K3 and SOS1—two proteins that act upstream of ERK1/2. This deficiency causes a block to the differentiation program (97).

## The eIF4A Family

Mammals possess two highly related eIF4A paralogs: eIF4A1 (DDX2A) and eIF4A2 (DDX2B), which in humans are 90% identical at the amino acid level. eIF4A1 is more abundant and better studied than eIF4A2 (98, 99). In vitro, the two isoforms exhibit similar biochemical activities (100). In vivo, however, their roles may not be equivalent. Notable differences include the following. (*a*) eIF4A1 is predominantly synthesized during active cell growth, whereas eIF4A2 is produced during growth arrest (101). This is consistent with *EIF4A1*, but not *EIF4A2*, being transcriptionally activated by MYC (11). (*b*) Following FMDV (foot-and-mouth disease virus) infection, eIF4A1, but not eIF4A2, is cleaved, which coincides with a decline in protein synthesis (102). (*c*) Suppression of eIF4A1 leads to increased eIF4A2 levels, but this change is insufficient to compensate for the ensuing cell death associated with eIF4A1 loss (98). (*d*) eIF4A1 is essential for cell survival, but eIF4A2 is not (103).

eIF4A is the most abundant translation initiation factor in mammals (~2.4 copies/ribosome in HeLa cells), implying that only a small fraction (~5%) of the total population is incorporated into the eIF4F complex (5). eIF4A, which is the founding member of the DEAD-box RNA helicase family, contains two RecA-like domains that assume a dumbbell-shaped structure (67, 104–108). Although in vitro eIF4A shows bidirectional helicase activity, its delivery to the mRNA by eIF4F confers 5′ to 3′ directionality (109), which in yeast is imparted by the eIF4G RBD (110, 111). Unwinding by eIF4A proceeds by transitioning through reiterative cycles of open and closed conformations, as upon ATP and RNA binding, eIF4A adopts a closed conformation (112–114). Upon ATP hydrolysis and phosphate release, eIF4A assumes an open conformation and disengages from the RNA. Binding of free eIF4A to ATP or RNA is not ordered but is coupled (115). The preferred substrate for eIF4A is a single-stranded polypurine RNA (116). RNA fragments as short as 4 nucleotides can stimulate the ATPase activity of free eIF4A, but fragments of 15–20 nucleotides are optimal (117). On its own, eIF4A is a weak, nonprocessive helicase that unwinds ~11 base pairs per hydrolyzed ATP molecule at a very slow rate ($k_{cat}$) of ATP hydrolysis (~20 s) (111). In the presence of eIF4B, eIF4H, and eIF4G, eIF4A acts as a processive helicase (see below). The binding of eIF4E to eIF4G promotes RNA restructuring by stimulating eIF4A helicase activity, a property that is independent of eIF4E cap binding (118).

**eIF4A and cap dependency.** Cap dependency of mRNA translation increases with the stability of steric barriers within the 5′ leader region, consistent with eIF4A acting at the core of eIF4F's ability to discriminate among different mRNAs (119, 120). At one extreme, initiation in vitro on simple, unstructured model mRNAs containing no secondary structure (e.g., 5′ leaders harboring $[CAA]_n$ repeats) does not require eIF4A (nor eIF4F, ATP, or a cap structure), demonstrating that eIF4A (and eIF4F) is not absolutely required for initiation (121). In fact, a class of cellular mRNAs whose translation is eIF4E dependent but eIF4A independent has been identified. These mRNAs harbor a unique transcription/translation regulatory feature, termed a TISU (translation initiator of short 5′-UTR) element, consisting of very short 5′ leaders (median distance of

AUG from the cap is 12 nt) present in ~3% of human genes (122). Introduction of secondary structure at the 5′ end of a $[CAA]_n$ leader region or within a TISU element is sufficient to confer eIF4A dependency (121, 123). These are exceptional cases, however, because for the majority of mRNAs, cap-proximal steric barriers (secondary structure or protein complexes) interfere with efficient eIF4F–mRNA interaction (124–126), and this correlates with reduced translational efficiency (119, 120, 127, 128). In yeast, eIF4A is required for ribosome recruitment by all mRNAs regardless of the 5′ leader structure, but a second DEAD-box RNA helicase, Ded1, is dedicated to overcoming structural barriers in the 5′ leader to drive ribosome scanning (129–131). Under conditions of limiting Ded1 activity, the 43S PIC exhibits reduced scanning potential and initiates translation at near-cognate initiation codons proximal to structural barriers within mRNA 5′ leader regions (131). Some evidence does suggest that the mammalian Ded1 homolog, DDX3X, may participate in initiation (132), but a clear mechanistic role is difficult to obtain from genetic approaches given that DDX3X has also been implicated in nuclear processes (transcription, pre-mRNA splicing and export, and miRNA biogenesis) (133).

Recently, a role for eIF4A in initiation that is distinct from its function as a subunit of the eIF4F complex has been proposed. Earlier experiments had shown that eIF3j binds to the 43S PIC at the mRNA entry channel and A site (134–136). To allow full accommodation of the mRNA in the mRNA entry channel, eIF3j affinity for the 43S PIC is reduced in an eIF4A-dependent manner (and eIF4G- and eIF4B-dependent manners) (137). The closed ATP-bound conformation of eIF4A, but not ATP hydrolysis, is required for this activity (137).

**Modulating eIF4A availability.** eIF4A1 and eIF4A2 can be sequestered from eIF4F via their association with the programmed cell death 4 (PDCD4) tumor suppressor gene product (138–140), which binds eIF4A, blocks formation of the closed conformation, and masks the eIF4A RBD (105, 141, 142). Ectopic expression of PDCD4 preferentially represses cap-dependent translation of mRNAs containing structured 5′ leaders (143). The PDCD4–eIF4A interaction is under S6 kinase 1 (S6K1) and S6K2 control, in which phosphorylation of PDCD4 by S6K leads to PDCD4 ubiquitination and degradation (144, 145). In HeLa cells, the amount of PDCD4 is insufficient to sequester the majority of free eIF4A (5), so this regulatory mechanism is likely cell specific.

## eIF4B and eIF4H

eIF4B is the least conserved initiation factor across species, is not absolutely required for ribosome recruitment, and is dispensable in yeast (albeit the null mutant exhibits a slow-growth and cold-sensitive phenotype) (146, 147). In yeast, loss of eIF4B preferentially causes a reduction of translation of mRNAs with elevated secondary structure in their 5′ leader region (146). Mammalian eIF4B has two RBDs: an RRM within the NTD region capable of binding 18S rRNA and an arginine/serine–rich basic region at the CTD (148, 149) (**Figure 2*c***). A DRYG (aspartic acid, arginine, tyrosine, and glycine) region mediates homodimerization and interaction with eIF3 (150). Cryogenic electron microscopy (cryo-EM) studies have tentatively positioned eIF4B at the ribosomal mRNA channel entrance (151), in agreement with biochemical findings from yeast eIF4B–ribosome interaction studies (152) and consistent with its role in promoting 43S PIC recruitment. Yeast eIF4B shares only the RRM domain with mammalian eIF4B.

eIF4H is related to eIF4B (39% identity) and has one RRM but lacks the other eIF4B domains. *EIF4H* mRNA is alternatively spliced (at exon 5) to produce two isoforms encoding proteins of 25.2 and 27.3 kDa. The *EIF4H* gene resides on chromosome 7q11, in a region known as the Williams-Beuren syndrome (WBS) critical region. WBS is a multisystem developmental disorder that includes neurodevelopmental and cardiovascular abnormalities, infantile hypercalcemia,

and dysmorphic facial features. It is associated with heterozygous interstitial deletions spanning ~1.5 Mb (OMIM 603431). *Eif4h*$^{-/-}$ null mice display growth retardation, have altered brain morphology, show a reduction in the number and complexity of neurons, and exhibit impaired learning and memory formation (153).

eIF4B and eIF4H are expressed in all tissues, and in HeLa cells their levels are similar (5). Both proteins form complexes with eIF4A1 at overlapping binding sites, rendering their binding to eIF4A1 mutually exclusive (154). eIF4B and eIF4H stimulate ATP binding to eIF4A, RNA-dependent ATPase activity, ATP-dependent RNA binding, and the helicase activity of eIF4A (116, 155, 156). The stimulation of eIF4A helicase activity by eIF4B is ~10-fold, but together with eIF4G (which on its own modestly stimulates eIF4A unwinding <2-fold), a 100-fold stimulation is obtained (157). The unwinding activity observed by combining eIF4A, eIF4B, and eIF4G is impressive, as revealed by single-molecule experiments, and when combined, they can melt a 70-bp hairpin (>100 kcal/mol) (111). eIF4B and eIF4H also stabilize partially unwound substrates and/or prevent mRNA reannealing, activities that further facilitate RNA restructuring during initiation (158).

The activity of eIF4B is controlled through phosphorylation of S406 and S422 (159, 160). Phosphorylation of eIF4B on S422 increases the affinity of eIF4B for eIF3, consistent with the stimulation of translation observed with phosphomimetic mutants of eIF4B (159, 160). Translation of MYC is particularly sensitive to the phosphorylation status of eIF4B, an effect that is mediated in part by S6K1-dependent phosphorylation of eIF4B (161).

## eIF4E-DEPENDENT RIBOSOME RECRUITMENT

Despite a rich knowledge of the activity of the individual eIF4 factors, a clear understanding of the temporal order of the steps spanning cap recognition to 43S PIC recruitment is lacking. However, several plausible scenarios can be proposed, which may serve to guide future experiments (**Figure 3**).

1. The first step is eIF4E-mediated cap recognition, which does not require ATP (162). It is unlikely to involve recognition of the penultimate mRNA nucleotide (163), as surface plasmon resonance (SPR) analysis of $m^7$GTP and $m^7$GpppA binding to eIF4E detected no notable differences in affinities (164). Secondary structure positioned immediately adjacent to the cap impairs eIF4E's ability to interact with the cap (165) but not when positioned 6 nt downstream of the cap (126).
2. eIF4G likely then clamps down onto RNA via its RBDs in a process that stabilizes eIF4E–cap association (75). A role for eIF4A or other helicases in this activity cannot be ruled out. For mRNAs possessing a TISU element, eIF4A is not required for 43S PIC recruitment.
3. The next step is more of a puzzle. eIF4G-bound eIF4A may hydrolyze ATP followed by eIF4B (or eIF4H) binding to eIF4A, as suggested by experiments whereby eIF4B (and eIF4H) can be cross-linked to the cap in the presence of $Mg^{++}$/ATP (162, 166). The functional consequence of this step may be to convert eIF4A from a nonprocessive to a processive helicase, leading to unwinding of proximal secondary structure or displacement of protein–RNA complexes (111, 157, 158, 167). Alternatively, eIF4A (and eIF4B) could clamp on the mRNA, similar to the action of eIF4AIII in the EJC (exon junction complex) (W.C. Merrick, personal communication).
4. DHX29, a 40S-bound DExH-box helicase, stimulates binding of the 43S PIC to the mRNA, although the details are unclear (168).

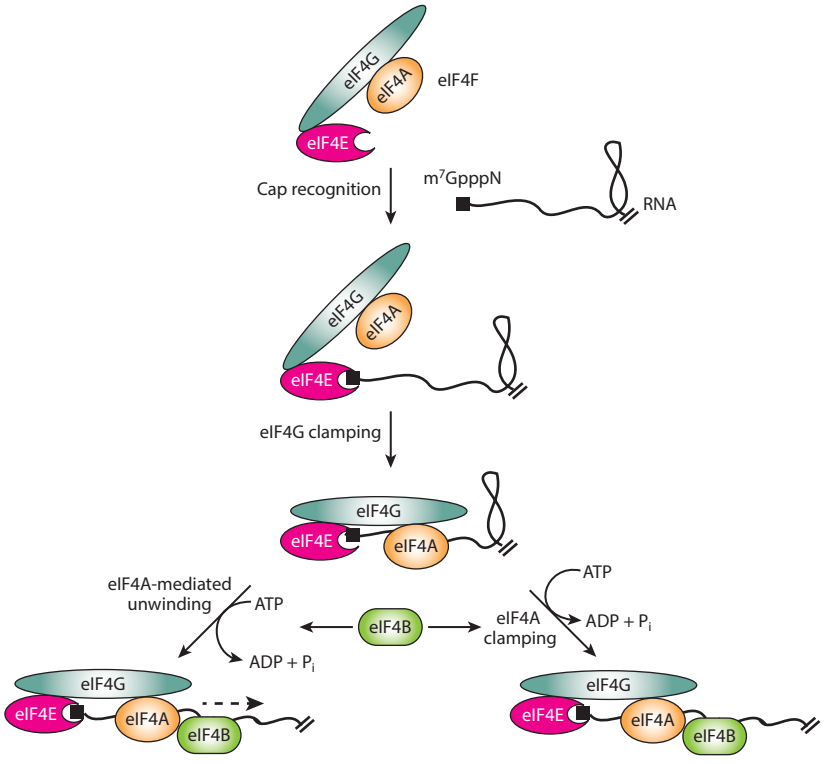


**Figure 3**

A possible mechanism for preparing the mRNA template for eIF4E-mediated, cap-dependent initiation. Although both eIF4B and eIF4H function in conjunction with eIF4A, only eIF4B interplay is shown. Cap recognition by eIF4E is ATP independent and is likely followed by RNA clamping by eIF4G to stabilize the eIF4F complex at the mRNA 5′ end. In an ATP-dependent step, eIF4A, in conjunction with eIF4B and/or eIF4H, resolves structural barriers adjacent to the cap structure, preparing the mRNA template to accommodate a 43S PIC. Abbreviations: eIF, eukaryotic initiation factor; mRNA, messenger RNA; PIC, pre-initiation complex.

5. Interactions between eIF4G and ribosome-bound eIF3 are required for recruiting the 43S PIC to the mRNA. A recent cryo-EM study placed the eIF4G-interacting eIF3 subunits (c, d, and e) near the mRNA exit channel of the 40S ribosome and eIF4B at the mRNA entrance channel (151), a finding that seems at odds with the need for eIF4F to interact with both eIF3 and eIF4B during ribosome recruitment. However, eIF4G is sufficiently large to possibly toggle between the entrance and exit channels. The cryo-EM view is also a static one and does not inform on the dynamic changes that could occur during different binding events.
6. How the mRNA is inserted into the ribosome mRNA channel is unknown. (*a*) A threading model has been proposed, whereby eIF4F binds near the entrance site and a handoff is made, transferring the 5′ mRNA cap from eIF4E to eIF3l (169). eIF3l possesses cap-binding activity, although unlike eIF4E, the N7-methyl moiety is not essential for this binding, as GTP and GpppG can compete with the eIF3l–cap interaction (169). This model is consistent with experiments showing that ribosomes do not have a blind spot when it comes to AUG recognition, with AUGs 2 nt downstream from the cap being recognized as start codons

(169). (*b*) A slotting mechanism could be used if eIF4F were to bind near the exit site, but this would be at variance with the aforementioned experiments, indicating the absence of an AUG blind spot (169). (*c*) A third mechanism could entail binding of the eIF4F–mRNA complex near the entrance channel followed by repositioning of eIF4E or eIF4F to the exit channel. During this process, eIF4E remains cap-bound and the mRNA is dragged into, and through, the 40S channel. In this model, every nucleotide could still be inspected, but a handoff of the mRNA cap would not occur. eIF4A helicase activity could serve to pull the mRNA through the channel, or if eIF4A were to act as a clamp, this could serve to prevent backward movement of the ribosome. Perhaps DHX29 and Ded1 (in yeast) could then resolve the cap-distal secondary structure to promote scanning. That IRESes can recruit ribosomes to an internal sequence implies that mRNAs must be able to slot or be dragged into the 40S channel.

Whether eIF4F disengages from the mRNA and recycles to a different mRNA for each round of ribosome recruitment or it (or eIF4E) remains mRNA bound for multiple rounds is unclear. UV cross-linking experiments on a 40S-bound RNA containing an AUG codon 28 bases downstream from the cap showed reduced interaction with eIF4E, suggesting that eIF4E–cap interaction is not maintained during scanning (169). Consistent with this finding is the absence of footprint density near the cap in translation complex profiling (TCP)-Seq, a novel approach that reports on small ribosome subunit footprints within mRNA 5′ leaders (170). The fast dissociation rate of eIF4E from the cap ($K_{off} = 79\ s^{-1}$) also supports a model by which eIF4E does not remain cap associated, although the effect that eIF4G could exert on this rate was never reported (171). Alternatively, the ability of cap analogs to inhibit de novo initiation, but not ongoing translation, would argue that eIF4E does not recycle (efficiently) during multiple rounds of initiation (172).

## INTERNAL RIBOSOME RECRUITMENT

### Viral Internal Ribosome Entry Sites

IRESes were first described in picornaviruses (173, 174) and subsequently extensively studied for a small set of viral mRNAs. Significant variation in the structural features and the set of initiation factors required for ribosome recruitment by IRESes exists, and they are stratified into four types (175). Type I IRESes occur in the *Enterovirus* genus. In the case of poliovirus, the first initiation event on the newly uncoated RNA plus strand utilizes intact eIF4G as the initiation factor. Here, bound eIF4E stimulates the rate of poliovirus IRES restructuring by eIF4A as part of the intact eIF4F complex (175). Following translation, viral encoded 2A protease cleaves eIF4G, separating the eIF4E and eIF4A binding sites. Subsequent rounds of initiation utilize 2A-cleaved eIF4G, retaining the eIF4A and eIF3 binding sites. Poly(C)-binding protein 2, one of several documented IRES trans-acting factors (ITAFs), binds to C-rich motifs within the IRES and also promotes its usage (175). Following ribosome recruitment, the ribosome scans in the 5′ to 3′ direction in search of the initiation codon.

Type II IRESes, found predominantly in *Cardiovirus and Aphthovirus* genera, have structural domains unrelated to type I IRESes with the exception of a conserved C-rich loop and GNRA tetraloop (175). Type II IRESes can function independent of eIF4E, although some require one or more ITAFs (175).

Type III IRESes, as found in hepatitis C virus (HCV), require only eIF2, 40S ribosomes, and Met-tRNAi to form initiation complexes and position the AUG codon in the P site (auxiliary factors are involved and influence the rate of initiation). Initiation on HCV is independent of eIF3,

eIF4F, eIF4A, or eIF4B (176). Cryo-EM studies revealed that the location of the HCV IRES and the eIF3 binding sites on the 40S ribosome overlap (177). The HCV IRES contains a domain upstream of the ribosome binding site that interacts with and sequesters eIF3, thus enabling the HCV IRES to bind to the 40S ribosome while simultaneously reducing eIF3-dependent 48S formation on cellular mRNAs (177).

Type IV IRESes, as exemplified by the insect cricket paralysis virus (CrPV), bind ribosomes in the absence of any translation initiation factors (178). The CrPV IRES harbors a structural element [pseudoknot I (PKI) domain], which mimics a tRNA–codon interaction and positions itself into the A site, blocking binding of aminoacyl-tRNAs (179). A pseudotranslocation step, mediated by eEF2, shifts the PKI domain into the P site and inserts the adjacent codon (encoding alanine) into the A site, with the resulting complex poised to undertake the first round of elongation (179).

## Cellular Internal Ribosome Entry Sites

The existence of cellular IRESes was proposed to rationalize the efficient translation of mRNAs harboring long, complex 5′ leaders and the persistent translation of some mRNAs under conditions of stress. The absence of conserved primary or structural features among cellular IRESes necessitated the use of functional assays—generally involving bicistronic mRNAs to identify cellular IRESes—which had shortcomings (activation of cryptic promoters or splice sites) (180). A recent genome-wide approach to identify cellular IRESes reported that as many as 10% of cellular mRNAs use IRESes for initiation (181). Cellular and biochemical validation are required to authenticate these as bona fide IRESes.

## $N^6$-Methyladenosine and Internal Ribosome Entry Site Activity

$N^6$-methyladenosine ($m^6A$) base modifications in mRNA have been reported to stimulate translation. Meyer et al. (182) reported that 48S complexes can form on $m^6A$-containing mRNAs in the absence of eIF4A, eIF4F, and eIF4B. They concluded that eIF3 is recruited to 5′ leader $m^6A$ sites and is responsible for stimulating 43S PIC loading. The $m^6A$ methylase, METTL3 (methyltransferase-like 3), has been implicated in enhancing translation by directly recruiting eIF3 to the mRNA (183). In tethering assays, in which METTL3 was artificially recruited to binding sites at the 3′ UTR of the target mRNA, an ∼2-fold stimulation in expression was reported. Additionally, Wang et al. (184) showed that the $m^6A$ reader, YTHDF1 (YT521-B homology $N^6$-methyladenosine RNA binding protein 1), promoted ribosome occupancy of $m^6A$-targeted mRNAs. Using a tethering assay in which multiple copies of YTHDF1 were recruited to a target mRNA within the 3′ UTR, they found that translation was stimulated ∼1.7-fold. It is difficult to reconcile these results with other findings concerning $m^6A$. First, $m^6A$ modifications are not restricted to one location but are heterogeneously distributed along the body of the mRNA. Second, the frequency of $m^6A$ is low (∼3 $m^6A$ residues/mRNA) (185). And last, the $m^6A$ modification can alter mRNA stability (186), which has to be accounted for in experiments assessing potential effects of $m^6A$ on translation.

## Circular RNAs

Circular RNAs (circRNAs) arise from pre-mRNA back splicing and are dependent on intronic sequences that juxtapose the donor and acceptor splice sites (187). CircRNAs can act as miRNA sponges and as docking sites for RNA-binding proteins (188). Inserting a viral IRES into a chimeric circRNA promotes translation of the downstream ORF (open reading frame) (189, 190).

Several cytoplasmic cellular circRNAs are translated, but the mechanism of ribosome recruitment is not well understood. It was postulated that translation of circRNAs may be driven by internal $m^6A$ modifications in which the YTH3 reader protein recruits eIF4G2 to promote cap-independent initiation (188). Rigorous experimental approaches are needed to ensure that translation products arise from circRNAs rather than from linear trans-spliced products.

## eIF4E-INDEPENDENT, CAP-DEPENDENT MODES OF TRANSLATION INITIATION

### Nuclear Cap-Binding Proteins nCBP20 and nCBP80

Initiation on an mRNA that emerges from the nucleus is referred to as the pioneer round of translation and appears to be distinct from subsequent rounds. The nuclear cap-binding complex (nCBC), comprised of nCBP20 and nCBP80, binds the cap synergistically through two aromatic residues in nCBP20 (191). How ribosomes are then recruited to the mRNA is unclear; some experiments suggest that eIF4G interacts with the nCBP20–nCBP80 complex, whereas others invoke an nCBC-dependent translation initiation factor (CTIF) that exhibits affinity for ribosome-bound eIF3g (53). Importins have been implicated in dissociating the nCBC from the mRNA to facilitate the transition from nCBC- to eIF4E-mediated initiation. In yeast, the nCBC is not essential, as eIF4E mediates the pioneer round of translation (192).

### eIF3d

Lee et al. (193) demonstrated that translation of the *c-Jun* mRNA is eIF4F independent but requires eIF3d cap-binding activity. They proposed that a stem-loop element within the *c-Jun* mRNA prevents recruitment of eIF4F to the 5′ end and directly recruits eIF3, whose subunit eIF3d binds to the *c-Jun* cap structure (193). Recent structural studies have positioned eIF3d near the mRNA exit channel in the 40S ribosome, where it does not contact the mRNA (151). In cross-linking experiments, eIF3d did not show cap-binding activity, which suggests that eIF3d's cap-binding activity may be mRNA specific (169).

## CAP-INDEPENDENT, 5′-END–DEPENDENT TRANSLATION INITIATION

Norovirus is responsible for acute gastroenteritis. Like several other animal and plant mRNAs, norovirus mRNA contains a covalently linked small viral peptide, VPg (viral protein genome-linked), at its 5′ end. During norovirus infection, a 20-amino-acid region at the VPg CTD interacts with eIF4G HEAT-1 and substitutes for the requirement of a 5′ cap (194). The VPg binding site on eIF4G does not overlap with the eIF4A binding site. In plants, an interaction between the potyvirus VPg and eIF(iso)4E is critical for viral infection and has been linked to increased viral mRNA expression, presumably due to redirection of the translation machinery to viral mRNAs (195, 196). These studies underscore the antiviral potential that small-molecule inhibitors could have by interdicting VPg–eIF4G and VPg–eIF4E interactions.

## SCANNING

The signal or signals that trigger the 40S ribosome to begin the search for an initiation codon following mRNA binding are unknown. ATP is required for scanning, but the question remains whether eIF4A–eIF4B or eIF4A–eIF4H complexes are sufficient for this process. Studies in yeast

showed that Ded1 plays a major role in scanning (130, 131). In yeast, mRNAs with extended 5′ leader length and greater secondary structure exhibit a higher dependency on Ded1, whereas most mRNAs display a similar dependency on eIF4A, possibly due to a more universal role for eIF4A in 43S PIC attachment. TCP-Seq revealed that small ribosome subunit footprints from relatively short and unstructured mRNAs show a uniform distribution of reads throughout the 5′ leader regions, suggesting smooth continuous scanning (170). In contrast, mRNAs with longer 5′ leaders showed evidence of read clustering, suggesting pausing during the scanning process.

Secondary structures present upstream of an initiation codon can block scanning (197). In contrast, when located downstream of, and in proximity to, an initiation codon, a secondary structure can increase utilization of the initiation codon by slowing 40S migration and allowing increased codon sampling time (198). Multiple 40S ribosomes can accumulate on a 5′ leader region (199). Whether the helicase power needed to load and support scanning of the first 43S PIC is the same as that required by subsequent 43S PICs is an open question. It is noteworthy that increasing 5′ leader length does not necessarily lead to reduced translational efficiency (200). Perhaps ribosome queuing is an effective way of keeping the mRNA 5′ leader region partially unwound to reduce the need for persistent helicase activity.



## REINITIATION

Approximately 40% of eukaryotic mRNAs harbor upstream (u)ORFs (201). Ribosome profiling experiments uncovered widespread translation at many of these uORFs (202). The ability to resume scanning following termination at uORFs is inversely correlated with the length of the uORF, negatively influenced by structural constraints in the uORF that decrease elongation, and dependent on the distance between the ORFs (203). It was suggested that a subset of initiation factors are required for reinitiation and that they remain ribosome bound for a very short period of time following the start of elongation (204). Translation of average-sized ORFs ultimately leads to the ejection of these factors and loss of reinitiation potential.

Initiation at near-cognate AUG codons has been documented for ∼30 years. Until recently, few attempts to study the underlying mechanism have been made, as it was generally thought not to differ from canonical AUG initiation. Recent studies have underscored the increased prevalence and importance of translation from near-cognate AUG codons: (*a*) ribosome profiling has uncovered that initiation at near-cognate start sites is widespread (202); (*b*) non-AUG initiation appears to increase during cellular stress (205); and (*c*) non-AUG initiation has been shown to drive cancer progression in a squamous cell carcinoma (SCC) model, and its suppression led to an antineoplastic effect (206). Although eIF2 is generally required for Met-tRNA$_i$ delivery to the P site, eIF2A, eIF2D (ligatin), and the multiple copies in T cell lymphoma 1–density regulated protein (MCT-1–DENR) complex can substitute for eIF2. A well-studied example of an mRNA that requires eIF2A to support initiation at a CUG codon is the phosphatase and tensin homolog (PTEN), which leads to synthesis of the PTEN$\alpha$ isoform (207). eIF2A was shown to be essential for cancer progression in an SCC model, in which it promoted translation of select cancer genes and was associated with poor prognosis (206). eIF2A is not necessary for normal development because eIF2A$^{-/-}$ mice are viable (208), indicating that it might represent a tumor-selective vulnerability.

## LONG NONCODING RNAs

There are ∼20,000 vertebrate long noncoding RNAs (lncRNAs) (RNAs >200 nt), with biological functions attributed to only a small fraction. Evidence has been found that a sizable fraction of

lncRNAs are translated (202, 209, 210), producing short peptides that originate from some of the encoded small ORFs (211, 212). Recent analysis of ribosome profiling data across eight human cell lines revealed that ribosome footprints from lncRNAs exhibit a high degree of cell-type specificity and only a small fraction (1.4% out of 4,709 sampled lncRNAs) displayed ribosome footprints across all cell lines (213). The mechanism by which ribosomes are loaded onto lncRNAs remains to be established.

## REPEAT-ASSOCIATED NON-AUG TRANSLATION

Nucleotide expansion repeats within genes are the underlying cause of a number of neurological diseases. For example, the molecular basis for Huntington's disease is an expansion of a CAG repeat sequence in the coding sequence of the *HTT* gene. Expansion of the nucleotide repeats results in repeat-associated non-AUG (RAN) translation, in which non-AUG codons are used to initiate translation in all three reading frames from within the expanded repeat, generating protein products that form toxic aggregates. RAN translation is orders of magnitude less efficient than canonical cap-dependent initiation (214). Initiation of RAN translation requires the 5′ cap, eIF4E, and eIF4A and entails scanning (214), but in a manner not well understood, it bypasses the canonical normal requirements for start codon selection.

## INHIBITING INITIATION BY CAP SEQUESTRATION

Ribosomal biogenesis in multicellular organisms is under stringent translational regulation. Transcripts encoding ribosomal proteins, many translation factors, and some RNA-binding proteins carry a unique 5′ terminal oligopyrimidine (TOP) sequence, with known exceptions being yeast and *Caenorhabditis elegans* (215, 216). The TOP motif consists of an invariant 5′ cytosine as the penultimate base, $m^7$GpppC, followed by a track of 4–14 pyrimidines. La-related protein 1 (LARP1) is a downstream target of mTORC1 and mediates the translational control of TOP-containing mRNAs (217, 218). LARP1 is a member of the LARP family of proteins (consisting of seven proteins), which are defined by the presence of an evolutionarily conserved La module comprising the La motif and an RRM, which together recognize and bind a variety of RNA sequence elements.

A tripartite cocrystal structure of the human LARP1 DM15 region in complex with a 5′ TOP motif and a cap analog ($m^7$GTP or $m^7$GpppC) has recently been published (219). The cap-binding pocket of LARP1 contains two aromatic residues that stack the guanosine of the cap as well as an acidic side chain that interfaces with the Watson-Crick face of the $m^7$G base.

LARP1 interacts with the first four bases adjacent to the cap structure, explaining why it selectively recognizes capped oligopyrimidine RNA sequences (219, 220). LARP1 is thus able to compete with eIF4F for binding to TOP mRNAs. LARP1 associates with raptor when mTORC1 is active, but upon mTORC1 inhibition, nonphosphorylated LARP1 dissociates from raptor and binds to TOP-containing mRNAs (219), leading to occlusion of eIF4F and inhibition of translation (217). Phosphorylation of LARP1 by mTORC1 and Akt/S6K1 causes it to dissociate from TOP mRNAs, relieving translational repression. LARP1 binding to TOP mRNAs also stabilizes the target mRNAs (216).

## CONCLUSIONS AND PERSPECTIVES

Much of our knowledge of the many steps of the mRNA translation pathway has been garnered thorough biochemical and genetic investigations. Computation modeling of translation rates in

yeast, based on ribosome footprinting data, predict median initiation rates of ~40 s/round, whereas elongation rates are predicted to be 9.3 aa/s with a mean distance between consecutive bound ribosomes of 60 codons (221). Single-molecule imaging in live cells has estimated elongation rates of 3.1–4.9 aa/s with initiation rates of 17–43 s/round (222). These results are consistent with the notion that initiation is a generally slow, and rate-limiting, step in vivo.

Biophysical studies aimed at deciphering the sequence of steps between eIF4F cap recognition and 43S ribosome–mRNA binding are required to further our understanding of translation initiation. These studies will go hand in hand with detailing the precise location of eIFs. In vivo imaging approaches have much to offer in visualizing these steps and documenting possible nuances. Considering the presence of two eIF4A and eIF4G genes, there are four possible eIF4F complexes (more if we consider the different isoforms encoded by each single eIF4G gene), yet we have no idea if these complexes are equivalent in activity.

Finally, enormous progress in identifying small-molecule inhibitors of the translation initiation pathway has been made (10). Consequently, there are untapped opportunities for the discovery of novel probes that could be used to help elucidate the function of key factors. The impact of deregulated translational control in human diseases makes translation a promising therapeutic target.



## DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

## ACKNOWLEDGMENTS

We wish to dedicate this review to the memory of Aaron J. Shatkin, codiscoverer of mRNA capping, admired and compassionate mentor, and valued colleague. We are grateful to Leo Shen (McGill University), Jennifer Chu (McGill University), Yuri Svitkin (McGill University), Wayne Sossin (McGill University), William Merrick (Case Western Reserve University, Cleveland, Ohio), Alan Hinnebusch (National Institutes of Health, Bethesda, Maryland), Christopher S. Fraser (University of California, Davis), Hiroaki Imataka (University of Hyogo, Japan), Franck Martin (Université de Strasbourg), and John Hershey (University of California, Davis) for their insightful comments and suggestions on this review. Work in the authors' laboratories is supported by the Canadian Institutes of Health Research.

## LITERATURE CITED

1. Sonenberg N, Morgan MA, Merrick WC, Shatkin AJ. 1978. A polypeptide in eukaryotic initiation factors that crosslinks specifically to the 5′-terminal cap in mRNA. *PNAS* 75:4843–47
2. Altmann M, Handschin C, Trachsel H. 1987. mRNA cap-binding protein: cloning of the gene encoding protein synthesis initiation factor eIF-4E from *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 7:998–1003
3. Truitt ML, Conn CS, Shi Z, Pang X, Tokuyasu T, et al. 2015. Differential requirements for eIF4E dose in normal development and cancer. *Cell* 162:59–71
4. Altmann M, Muller PP, Pelletier J, Sonenberg N, Trachsel H. 1989. A mammalian translation initiation factor can substitute for its yeast homologue in vivo. *J. Biol. Chem.* 264:12145–47
5. Kulak NA, Pichler G, Paron I, Nagaraj N, Mann M. 2014. Minimal, encapsulated proteomic-sample processing applied to copy-number estimation in eukaryotic cells. *Nat. Methods* 11:319–24
6. Lazaris-Karatzas A, Montine KS, Sonenberg N. 1990. Malignant transformation by a eukaryotic initiation factor subunit that binds to mRNA 5′ cap. *Nature* 345:544–47

7. Wendel HG, de Stanchina E, Fridman JS, Malina A, Ray S, et al. 2004. Survival signalling by Akt and eIF4E in oncogenesis and cancer therapy. *Nature* 428:332–37
8. Rousseau D, Kaspar R, Rosenwald I, Gehrke L, Sonenberg N. 1996. Translation initiation of ornithine decarboxylase and nucleocytoplasmic transport of cyclin D1 mRNA are increased in cells overexpressing eukaryotic initiation factor 4E. *PNAS* 93:1065–70
9. Ilic N, Utermark T, Widlund HR, Roberts TM. 2011. PI3K-targeted therapy can be evaded by gene amplification along the MYC-eukaryotic translation initiation factor 4E (eIF4E) axis. *PNAS* 108:E699–708
10. Bhat M, Robichaud N, Hulea L, Sonenberg N, Pelletier J, Topisirovic I. 2015. Targeting the translation machinery in cancer. *Nat. Rev. Drug Discov.* 14:261–78
11. Lin CJ, Cencic R, Mills JR, Robert F, Pelletier J. 2008. c-Myc and eIF4F are components of a feedforward loop that links transcription and translation. *Cancer Res.* 68:5326–34
12. Schmidt EV. 2004. The role of c-*myc* in regulation of translation initiation. *Oncogene* 23:3217–21
13. Lin CJ, Nasr Z, Premsrirut PK, Porco JA Jr., Hippo Y, et al. 2012. Targeting synthetic lethal interactions between Myc and the eIF4F complex impedes tumorigenesis. *Cell Rep.* 1:325–33
14. Gkogkas CG, Khoutorsky A, Ran I, Rampakakis E, Nevarko T, et al. 2013. Autism-related deficits via dysregulated eIF4E-dependent translational control. *Nature* 493:371–77
15. Neves-Pereira M, Muller B, Massie D, Williams JH, O'Brien PC, et al. 2009. Deregulation of *EIF4E*: a novel mechanism for autism. *J. Med. Genet.* 46:759–65
16. Aguilar-Valles A, Haji N, De Gregorio D, Matta-Camacho E, Eslamizade MJ, et al. 2018. Translational control of depression-like behavior via phosphorylation of eukaryotic translation initiation factor 4E. *Nat. Commun.* 9:2459
17. Marcotrigiano J, Gingras AC, Sonenberg N, Burley SK. 1997. Cocrystal structure of the messenger RNA 5′ cap-binding protein (eIF4E) bound to 7-methyl-GDP. *Cell* 89:951–61
18. Matsuo H, Li H, McGuire AM, Fletcher CM, Gingras AC, et al. 1997. Structure of translation factor eIF4E bound to m7GDP and interaction with 4E-binding protein. *Nat. Struct. Biol.* 4:717–24
19. Darzynkiewicz E, Ekiel I, Lassota P, Tahara SM. 1987. Inhibition of eukaryotic translation by analogues of messenger RNA 5′-cap: chemical and biological consequences of 5′-phosphate modifications of 7-methylguanosine 5′-monophosphate. *Biochemistry* 26:4372–80
20. Chen X, Kopecky DJ, Mihalic J, Jeffries S, Min X, et al. 2012. Structure-guided design, synthesis, and evaluation of guanine-derived inhibitors of the eIF4E mRNA-cap interaction. *J. Med. Chem.* 55:3837–51
21. Brown CJ, McNae I, Fischer PM, Walkinshaw MD. 2007. Crystallographic and mass spectrometric characterisation of eIF4E with N7-alkylated cap derivatives. *J. Mol. Biol.* 372:7–15
22. Pause A, Belsham GJ, Gingras AC, Donze O, Lin TA, et al. 1994. Insulin-dependent stimulation of protein synthesis by phosphorylation of a regulator of 5′-cap function. *Nature* 371:762–67
23. Peter D, Igreja C, Weber R, Wohlbold L, Weiler C, et al. 2015. Molecular architecture of 4E-BP translational inhibitors bound to eIF4E. *Mol. Cell* 57:1074–87
24. Igreja C, Peter D, Weiler C, Izaurralde E. 2014. 4E-BPs require non-canonical 4E-binding motifs and a lateral surface of eIF4E to repress translation. *Nat. Commun.* 5:4790
25. Lukhele S, Bah A, Lin H, Sonenberg N, Forman-Kay JD. 2013. Interaction of the eukaryotic initiation factor 4E with 4E-BP2 at a dynamic bipartite interface. *Structure* 21:2186–96
26. Merrick WC, Pavitt GD. 2018. Protein synthesis initiation in eukaryotic cells. In *Translation Mechanisms and Control*, ed. MB Mathews, N Sonenberg, JW Hershey. Cold Spring Harbor, NY: Cold Spring Harb. Lab.
27. Rhoads RE. 2009. eIF4E: new family members, new binding partners, new roles. *J. Biol. Chem.* 284:16711–15
28. Kinkelin K, Veith K, Grunwald M, Bono F. 2012. Crystal structure of a minimal eIF4E–Cup complex reveals a general mechanism of eIF4E regulation in translational repression. *RNA* 18:1624–34
29. Napoli I, Mercaldo V, Boyl PP, Eleuteri B, Zalfa F, et al. 2008. The fragile X syndrome protein represses activity-dependent translation through CYFIP1, a new 4E-BP. *Cell* 134:1042–54
30. Pacheco A, Lopez de Quinto S, Ramajo J, Fernandez N, Martinez-Salas E. 2009. A novel role for Gemin5 in mRNA translation. *Nucleic Acids Res.* 37:582–90

31. Dostie J, Ferraiuolo M, Pause A, Adam SA, Sonenberg N. 2000. A novel shuttling protein, 4E-T, mediates the nuclear import of the mRNA 5′ cap-binding protein, eIF4E. *EMBO J.* 19:3142–56
32. Nishimura T, Padamsi Z, Fakim H, Milette S, Dunham WH, et al. 2015. The eIF4E-binding protein 4E-T is a component of the mRNA decay machinery that bridges the 5′ and 3′ termini of target mRNAs. *Cell Rep.* 11:1425–36
33. Gosselin P, Martineau Y, Morales J, Czjzek M, Glippa V, et al. 2013. Tracking a refined eIF4E-binding motif reveals Angel1 as a new partner of eIF4E. *Nucleic Acids Res.* 41:7783–92
34. Joshi B, Cameron A, Jagus R. 2004. Characterization of mammalian eIF4E-family members. *Eur. J. Biochem.* 271:2189–203
35. Rosettani P, Knapp S, Vismara MG, Rusconi L, Cameron AD. 2007. Structures of the human eIF4E homologous protein, h4EHP, in its m7GTP-bound and unliganded forms. *J. Mol. Biol.* 368:691–705
36. Zuberek J, Kubacka D, Jablonowska A, Jemielity J, Stepinski J, et al. 2007. Weak binding affinity of human 4EHP for mRNA cap analogs. *RNA* 13:691–97
37. Cho PF, Poulin F, Cho-Park YA, Cho-Park IB, Chicoine JD, et al. 2005. A new paradigm for translational control: inhibition via 5′-3′ mRNA tethering by Bicoid and the eIF4E cognate 4EHP. *Cell* 121:411–23
38. Morita M, Ler LW, Fabian MR, Siddiqui N, Mullin M, et al. 2012. A novel 4EHP-GIGYF2 translational repressor complex is essential for mammalian development. *Mol. Cell. Biol.* 32:3585–93
39. Chapat C, Jafarnejad SM, Matta-Camacho E, Hesketh GG, Gelbart IA, et al. 2017. Cap-binding protein 4EHP effects translation silencing by microRNAs. *PNAS* 114:5425–30
40. Osborne MJ, Volpon L, Kornblatt JA, Culjkovic-Kraljacic B, Baguet A, Borden KLB. 2013. eIF4E3 acts as a tumor suppressor by utilizing an atypical mode of methyl-7-guanosine cap recognition. *PNAS* 110:3877–82
41. Byrd MP, Zamora M, Lloyd RE. 2005. Translation of eukaryotic translation initiation factor 4GI (eIF4GI) proceeds from multiple mRNAs containing a novel cap-dependent internal ribosome entry site (IRES) that is active during poliovirus infection. *J. Biol. Chem.* 280:18610–22
42. Byrd MP, Zamora M, Lloyd RE. 2002. Generation of multiple isoforms of eukaryotic translation initiation factor 4GI by use of alternate translation initiation codons. *Mol. Cell. Biol.* 22:4499–511
43. Coldwell MJ, Sack U, Cowan JL, Barrett RM, Vlasak M, et al. 2012. Multiple isoforms of the translation initiation factor eIF4GII are generated via use of alternative promoters, splice sites and a non-canonical initiation codon. *Biochem. J.* 448:1–11
44. Sun F, Palmer K, Handel MA. 2010. Mutation of *Eif4g3*, encoding a eukaryotic translation initiation factor, causes male infertility and meiotic arrest of mouse spermatocytes. *Development* 137:1699–707
45. Gray NK, Hrabalkova L, Scanlon JP, Smith RW. 2015. Poly(A)-binding proteins and mRNA localization: Who rules the roost? *Biochem. Soc. Trans.* 43:1277–84
46. Gallie DR. 1991. The cap and poly(A) tail function synergistically to regulate mRNA translational efficiency. *Genes Dev.* 5:2108–16
47. Adivarahan S, Livingston N, Nicholson B, Rahman S, Wu B, et al. 2018. Spatial organization of single mRNPs at different stages of the gene expression pathway. *Mol. Cell* 72:727–38.e5
48. Khong A, Parker R. 2018. mRNP architecture in translating and stress conditions reveals an ordered pathway of mRNP compaction. *J. Cell Biol.* 217:4124–40
49. Khaleghpour K, Kahvejian A, De Crescenzo G, Roy G, Svitkin YV, et al. 2001. Dual interactions of the translational repressor Paip2 with poly(A) binding protein. *Mol. Cell. Biol.* 21:5200–13
50. Svitkin YV, Imataka H, Khaleghpour K, Kahvejian A, Liebig HD, Sonenberg N. 2001. Poly(A)-binding protein interaction with elF4G stimulates picornavirus IRES-dependent translation. *RNA* 7:1743–52
51. Michel YM, Borman AM, Paulous S, Kean KM. 2001. Eukaryotic initiation factor 4G-poly(A) binding protein interaction is required for poly(A) tail-mediated stimulation of picornavirus internal ribosome entry segment-driven translation but not for X-mediated stimulation of hepatitis C virus translation. *Mol. Cell. Biol.* 21:4097–109
52. Cakmakci NG, Lerner RS, Wagner EJ, Zheng L, Marzluff WF. 2008. SLIP1, a factor required for activation of histone mRNA translation by the stem-loop binding protein. *Mol. Cell. Biol.* 28:1182–94

53. von Moeller H, Lerner R, Ricciardi A, Basquin C, Marzluff WF, Conti E. 2013. Structural and biochemical studies of SLIP1-SLBP identify DBP5 and eIF3g as SLIP1-binding proteins. *Nucleic Acids Res.* 41:7960–71
54. Piron M, Vende P, Cohen J, Poncet D. 1998. Rotavirus RNA-binding protein NSP3 interacts with eIF4GI and evicts the poly(A) binding protein from eIF4F. *EMBO J.* 17:5811–21
55. Marcotrigiano J, Gingras AC, Sonenberg N, Burley SK. 1999. Cap-dependent translation initiation in eukaryotes is regulated by a molecular mimic of eIF4G. *Mol. Cell* 3:707–16
56. Gross JD, Moerke NJ, von der Haar T, Lugovskoy AA, Sachs AB, et al. 2003. Ribosome loading onto the mRNA cap is driven by conformational coupling between eIF4G and eIF4E. *Cell* 115:739–50
57. Gruner S, Peter D, Weber R, Wohlbold L, Chung MY, et al. 2016. The structures of eIF4E-eIF4G complexes reveal an extended interface to regulate translation initiation. *Mol. Cell* 64:467–79
58. Miras M, Truniger V, Silva C, Verdaguer N, Aranda MA, Querol-Audi J. 2017. Structure of eIF4E in complex with an eIF4G peptide supports a universal bipartite binding mode for protein translation. *Plant Physiol.* 174:1476–91
59. Schalm SS, Fingar DC, Sabatini DM, Blenis J. 2003. TOS motif-mediated raptor binding regulates 4E-BP1 multisite phosphorylation and function. *Curr. Biol.* 13:797–806
60. Brunn GJ, Fadden P, Haystead TA, Lawrence JC Jr. 1997. The mammalian target of rapamycin phosphorylates sites having a (Ser/Thr)-Pro motif and is activated by antibodies to a region near its COOH terminus. *J. Biol. Chem.* 272:32547–50
61. Gingras AC, Gygi SP, Raught B, Polakiewicz RD, Abraham RT, et al. 1999. Regulation of 4E-BP1 phosphorylation: a novel two-step mechanism. *Genes Dev.* 13:1422–37
62. Gingras AC, Raught B, Gygi SP, Niedzwiecka A, Miron M, et al. 2001. Hierarchical phosphorylation of the translation inhibitor 4E-BP1. *Genes Dev.* 15:2852–64
63. Bah A, Vernon RM, Siddiqui Z, Krzeminski M, Muhandiram R, et al. 2015. Folding of an intrinsically disordered protein by phosphorylation as a regulatory switch. *Nature* 519:106–9
64. Yanagiya A, Suyama E, Adachi H, Svitkin YV, Aza-Blanc P, et al. 2012. Translational homeostasis via the mRNA cap-binding protein, eIF4E. *Mol. Cell* 46:847–58
65. Imataka H, Sonenberg N. 1997. Human eukaryotic translation initiation factor 4G (eIF4G) possesses two separate and independent binding sites for eIF4A. *Mol. Cell. Biol.* 17:6940–47
66. Marintchev A, Edmonds KA, Marintcheva B, Hendrickson E, Oberer M, et al. 2009. Topology and regulation of the human eIF4A/4G/4H helicase complex in translation initiation. *Cell* 136:447–60
67. Schutz P, Bumann M, Oberholzer AE, Bieniossek C, Trachsel H, et al. 2008. Crystal structure of the yeast eIF4A-eIF4G complex: an RNA-helicase controlled by protein-protein interactions. *PNAS* 105:9564–69
68. Dominguez D, Altmann M, Benz J, Baumann U, Trachsel H. 1999. Interaction of translation initiation factor eIF4G with eIF4A in the yeast *Saccharomyces cerevisiae*. *J. Biol. Chem.* 274:26720–26
69. Korneeva NL, First EA, Benoit CA, Rhoads RE. 2005. Interaction between the $NH_2$-terminal domain of eIF4A and the central domain of eIF4G modulates RNA-stimulated ATPase activity. *J. Biol. Chem.* 280:1872–81
70. Hilbert M, Kebbel F, Gubaev A, Klostermeier D. 2011. eIF4G stimulates the activity of the DEAD box protein eIF4A by a conformational guidance mechanism. *Nucleic Acids Res.* 39:2260–70
71. Villa N, Do A, Hershey JW, Fraser CS. 2013. Human eukaryotic initiation factor 4G (eIF4G) protein binds to eIF3c, -d, and -e to promote mRNA recruitment to the ribosome. *J. Biol. Chem.* 288:32932–40
72. Harris TE, Chi A, Shabanowitz J, Hunt DF, Rhoads RE, Lawrence JC Jr. 2006. mTOR-dependent stimulation of the association of eIF4G and eIF3 by insulin. *EMBO J.* 25:1659–68
73. LeFebvre AK, Korneeva NL, Trutschl M, Cvek U, Duzan RD, et al. 2006. Translation initiation factor eIF4G-1 binds to eIF3 through the eIF3e subunit. *J. Biol. Chem.* 281:22917–32
74. Marcotrigiano J, Lomakin IB, Sonenberg N, Pestova TV, Hellen CU, Burley SK. 2001. A conserved HEAT domain within eIF4G directs assembly of the translation initiation machinery. *Mol. Cell* 7:193–203

75. Yanagiya A, Svitkin YV, Shibata S, Mikami S, Imataka H, Sonenberg N. 2009. Requirement of RNA binding of mammalian eukaryotic translation initiation factor 4GI (eIF4GI) for efficient interaction of eIF4E with the mRNA cap. *Mol. Cell. Biol.* 29:1661–69
76. Park EH, Walker SE, Lee JM, Rothenburg S, Lorsch JR, Hinnebusch AG. 2011. Multiple elements in the eIF4G1 N-terminus promote assembly of eIF4G1·PABP mRNPs *in vivo*. *EMBO J.* 30:302–16
77. Waskiewicz AJ, Johnson JC, Penn B, Mahalingam M, Kimball SR, Cooper JA. 1999. Phosphorylation of the cap-binding protein eukaryotic translation initiation factor 4E by protein kinase Mnk1 in vivo. *Mol. Cell. Biol.* 19:1871–80
78. Pyronnet S, Imataka H, Gingras AC, Fukunaga R, Hunter T, Sonenberg N. 1999. Human eukaryotic translation initiation factor 4G (eIF4G) recruits Mnk1 to phosphorylate eIF4E. *EMBO J.* 18:270–79
79. Furic L, Rong L, Larsson O, Koumakpayi IH, Yoshida K, et al. 2010. eIF4E phosphorylation promotes tumorigenesis and is associated with prostate cancer progression. *PNAS* 107:14134–39
80. Scheper GC, van Kollenburg B, Hu J, Luo Y, Goss DJ, Proud CG. 2002. Phosphorylation of eukaryotic initiation factor 4E markedly reduces its affinity for capped mRNA. *J. Biol. Chem.* 277:3303–9
81. Zuberek J, Jemielity J, Jablonowska A, Stepinski J, Dadlez M, et al. 2004. Influence of electric charge variation at residues 209 and 159 on the interaction of eIF4E with the mRNA 5′ terminus. *Biochemistry* 43:5370–79
82. Ueda T, Sasaki M, Elia AJ, Chio II, Hamada K, et al. 2010. Combined deficiency for MAP kinase-interacting kinase 1 and 2 (Mnk1 and Mnk2) delays tumor development. *PNAS* 107:13984–90
83. Topisirovic I, Ruiz-Gutierrez M, Borden KLB. 2004. Phosphorylation of the eukaryotic translation initiation factor eIF4E contributes to its transformation and mRNA transport activities. *Cancer Res.* 64:8639–42
84. Wendel HG, Silva RL, Malina A, Mills JR, Zhu H, et al. 2007. Dissecting eIF4E action in tumorigenesis. *Genes Dev.* 21:3232–37
85. Robichaud N, Hsu BE, Istomine R, Alvarez F, Blagih J, et al. 2018. Translational control in the tumor microenvironment promotes lung metastasis: phosphorylation of eIF4E in neutrophils. *PNAS* 115:E2202–9
86. Cuesta R, Xi Q, Schneider RJ. 2004. Structural basis for competitive inhibition of eIF4G-Mnk1 interaction by the adenovirus 100-kilodalton protein. *J. Virol.* 78:7707–16
87. Juillard F, Bazot Q, Mure F, Tafforeau L, Macri C, et al. 2012. Epstein-Barr virus protein EB2 stimulates cytoplasmic mRNA accumulation by counteracting the deleterious effects of SRp20 on viral mRNAs. *Nucleic Acids Res.* 40:6834–49
88. Burgui I, Aragon T, Ortin J, Nieto A. 2003. PABP1 and eIF4GI associate with influenza virus NS1 protein in viral mRNA translation initiation complexes. *J. Gen. Virol.* 84:3263–74
89. Castelló A, Álvarez E, Carrasco L. 2011. The multifaceted poliovirus 2A protease: regulation of gene expression by picornavirus proteases. *J. Biomed. Biotechnol.* 2011:369648
90. Levy-Strumpf N, Deiss LP, Berissi H, Kimchi A. 1997. DAP-5, a novel homolog of eukaryotic translation initiation factor 4G isolated as a putative modulator of gamma interferon-induced programmed cell death. *Mol. Cell. Biol.* 17:1615–25
91. Imataka H, Olsen HS, Sonenberg N. 1997. A new translational regulator with homology to eukaryotic translation initiation factor 4G. *EMBO J.* 16:817–25
92. Yamanaka S, Poksay KS, Arnold KS, Innerarity TL. 1997. A novel translational repressor mRNA is edited extensively in livers containing tumors caused by the transgene expression of the apoB mRNA-editing enzyme. *Genes Dev.* 11:321–33
93. Liberman N, Gandin V, Svitkin YV, David M, Virgili G, et al. 2015. DAP5 associates with eIF2β and eIF4AI to promote Internal Ribosome Entry Site driven translation. *Nucleic Acids Res.* 43:3764–75
94. Liberman N, Marash L, Kimchi A. 2009. The translation initiation factor DAP5 is a regulator of cell survival during mitosis. *Cell Cycle* 8:204–9
95. Yamanaka S, Zhang XY, Maeda M, Miura K, Wang S, et al. 2000. Essential role of NAT1/p97/DAP5 in embryonic differentiation and the retinoic acid pathway. *EMBO J.* 19:5533–41
96. Yoffe Y, David M, Kalaora R, Povodovski L, Friedlander G, et al. 2016. Cap-independent translation by DAP5 controls cell fate decisions in human embryonic stem cells. *Genes Dev.* 30:1991–2004

97. Sugiyama H, Takahashi K, Yamamoto T, Iwasaki M, Narita M, et al. 2017. Nat1 promotes translation of specific proteins that induce differentiation of mouse embryonic stem cells. *PNAS* 114:340–45
98. Galicia-Vázquez G, Cencic R, Robert F, Agenor AQ, Pelletier J. 2012. A cellular response linking eIF4AI activity to eIF4AII transcription. *RNA* 18:1373–84
99. Nielsen PJ, McMaster GK, Trachsel H. 1985. Cloning of eukaryotic protein synthesis initiation factor genes: isolation and characterization of cDNA clones encoding factor eIF-4A. *Nucleic Acids Res.* 13:6867–80
100. Conroy SC, Dever TE, Owens CL, Merrick WC. 1990. Characterization of the 46,000-Dalton subunit of eIF-4F. *Arch. Biochem. Biophys.* 282:363–71
101. Williams-Hill DM, Duncan RF, Nielsen PJ, Tahara SM. 1997. Differential expression of the murine eukaryotic translation initiation factor isogenes eIF4A$_I$ and eIF4A$_{II}$ is dependent upon cellular growth status. *Arch. Biochem. Biophys.* 338:111–20
102. Li W, Ross-Smith N, Proud CG, Belsham GJ. 2001. Cleavage of translation initiation factor 4AI (eIF4AI) but not eIF4AII by foot-and-mouth disease virus 3C protease: identification of the eIF4AI cleavage site. *FEBS Lett.* 507. **https://doi.org/10.1016/S0014-5793(01)02885-X**
103. Galicia-Vázquez G, Chu J, Pelletier J. 2015. eIF4AII is dispensable for miRNA-mediated gene silencing. *RNA* 21:1826–33
104. Caruthers JM, Johnson ER, McKay DB. 2000. Crystal structure of yeast initiation factor 4A, a DEAD-box RNA helicase. *PNAS* 97:13080–85
105. Chang JH, Cho YH, Sohn SY, Choi JM, Kim A, et al. 2009. Crystal structure of the eIF4A-PDCD4 complex. *PNAS* 106:3148–53
106. Loh PG, Yang HS, Walsh MA, Wang Q, Wang X, et al. 2009. Structural basis for translational inhibition by the tumour suppressor Pdcd4. *EMBO J.* 28:274–85
107. Johnson ER, McKay DB. 1999. Crystallographic structure of the amino terminal domain of yeast initiation factor 4A, a representative DEAD-box RNA helicase. *RNA* 5:1526–34
108. Oberer M, Marintchev A, Wagner G. 2005. Structural basis for the enhancement of eIF4A helicase activity by eIF4G. *Genes Dev.* 19:2212–23
109. Rozen F, Edery I, Meerovitch K, Dever TE, Merrick WC, Sonenberg N. 1990. Bidirectional RNA helicase activity of eucaryotic translation initiation factors 4A and 4F. *Mol. Cell. Biol.* 10:1134–44
110. Rajagopal V, Park EH, Hinnebusch AG, Lorsch JR. 2012. Specific domains in yeast translation initiation factor eIF4G strongly bias RNA unwinding activity of the eIF4F complex toward duplexes with 5′-overhangs. *J. Biol. Chem.* 287:20301–12
111. García-García C, Frieda KL, Feoktistova K, Fraser CS, Block SM. 2015. Factor-dependent processivity in human eIF4A DEAD-box helicase. *Science* 348:1486–88
112. Andersen CB, Ballut L, Johansen JS, Chamieh H, Nielsen KH, et al. 2006. Structure of the exon junction core complex with a trapped DEAD-box ATPase bound to RNA. *Science* 313:1968–72
113. Sengoku T, Nureki O, Nakamura A, Kobayashi S, Yokoyama S. 2006. Structural basis for RNA unwinding by the DEAD-box protein *Drosophila* Vasa. *Cell* 125:287–300
114. Bono F, Ebert J, Lorentzen E, Conti E. 2006. The crystal structure of the exon junction complex reveals how it maintains a stable grip on mRNA. *Cell* 126:713–25
115. Lorsch JR, Herschlag D. 1998. The DEAD box protein eIF4A. 1. A minimal kinetic and thermodynamic framework reveals coupled binding of RNA and nucleotide. *Biochemistry* 37:2180–93
116. Rogers GW Jr., Komar AA, Merrick WC. 2002. eIF4A: the godfather of the DEAD box helicases. *Prog. Nucleic Acid Res. Mol. Biol.* 72:307–31
117. Peck ML, Herschlag D. 1999. Effects of oligonucleotide length and atomic composition on stimulation of the ATPase activity of translation initiation factor elF4A. *RNA* 5:1210–21
118. Feoktistova K, Tuvshintogs E, Do A, Fraser CS. 2013. Human eIF4E promotes mRNA restructuring by stimulating eIF4A helicase activity. *PNAS* 110:13339–44
119. Svitkin YV, Pause A, Haghighat A, Pyronnet S, Witherell G, et al. 2001. The requirement for eukaryotic initiation factor 4A (elF4A) in translation is in direct proportion to the degree of mRNA 5′ secondary structure. *RNA* 7:382–94

120. Waldron JA, Raza F, Le Quesne J. 2018. eIF4A alleviates the translational repression mediated by classical secondary structures more than by G-quadruplexes. *Nucleic Acids Res*. 46:3075–87
121. Pestova TV, Kolupaeva VG. 2002. The roles of individual eukaryotic translation initiation factors in ribosomal scanning and initiation codon selection. *Genes Dev*. 16:2906–22
122. Elfakess R, Sinvani H, Haimov O, Svitkin Y, Sonenberg N, Dikstein R. 2011. Unique translation initiation of mRNAs-containing TISU element. *Nucleic Acids Res*. 39:7598–609
123. Gandin V, Masvidal L, Hulea L, Gravel SP, Cargnello M, et al. 2016. nanoCAGE reveals 5′ UTR features that define specific modes of translation of functionally related MTOR-sensitive mRNAs. *Genome Res*. 26:636–48
124. Lawson TG, Cladaras MH, Ray BK, Lee KA, Abramson RD, et al. 1988. Discriminatory interaction of purified eukaryotic initiation factors 4F plus 4A with the 5′ ends of reovirus messenger RNAs. *J. Biol. Chem*. 263:7266–76
125. Svitkin YV, Ovchinnikov LP, Dreyfuss G, Sonenberg N. 1996. General RNA binding proteins render translation cap dependent. *EMBO J*. 15:7147–55
126. Pelletier J, Sonenberg N. 1985. Photochemical cross-linking of cap binding proteins to eucaryotic mRNAs: effect of mRNA 5′ secondary structure. *Mol. Cell. Biol.* 5:3222–30
127. Pelletier J, Sonenberg N. 1985. Insertion mutagenesis to increase secondary structure within the 5′ noncoding region of a eukaryotic mRNA reduces translational efficiency. *Cell* 40:515–26
128. Babendure JR, Babendure JL, Ding JH, Tsien RY. 2006. Control of mammalian translation by mRNA structure near caps. *RNA* 12:851–61
129. Yourik P, Aitken CE, Zhou F, Gupta N, Hinnebusch AG, Lorsch JR. 2017. Yeast eIF4A enhances recruitment of mRNAs regardless of their structural complexity. *eLife* 6:e31476
130. Sen ND, Zhou F, Ingolia NT, Hinnebusch AG. 2015. Genome-wide analysis of translational efficiency reveals distinct but overlapping functions of yeast DEAD-box RNA helicases Ded1 and eIF4A. *Genome Res*. 25:1196–205
131. Guenther UP, Weinberg DE, Zubradt MM, Tedeschi FA, Stawicki BN, et al. 2018. The helicase Ded1p controls use of near-cognate translation initiation codons in 5′ UTRs. *Nature* 559:130–34
132. Soto-Rifo R, Rubilar PS, Limousin T, de Breyne S, Decimo D, Ohlmann T. 2012. DEAD-box protein DDX3 associates with eIF4F to promote translation of selected mRNAs. *EMBO J*. 31:3745–56
133. Sharma D, Jankowsky E. 2014. The Ded1/DDX3 subfamily of DEAD-box RNA helicases. *Crit. Rev. Biochem. Mol. Biol.* 49:343–60
134. Fraser CS, Hershey JW, Doudna JA. 2009. The pathway of hepatitis C virus mRNA recruitment to the human ribosome. *Nat. Struct. Mol. Biol.* 16:397–404
135. Sokabe M, Fraser CS. 2014. Human eukaryotic initiation factor 2 (eIF2)-GTP-Met-tRNAi ternary complex and eIF3 stabilize the 43S preinitiation complex. *J. Biol. Chem*. 289:31827–36
136. Aylett CH, Boehringer D, Erzberger JP, Schaefer T, Ban N. 2015. Structure of a yeast 40S-eIF1-eIF1A-eIF3-eIF3j initiation complex. *Nat. Struct. Mol. Biol.* 22:269–71
137. Sokabe M, Fraser CS. 2017. A helicase-independent activity of eIF4A in promoting mRNA recruitment to the human ribosome. *PNAS* 114:6304–9
138. Yang HS, Jansen AP, Komar AA, Zheng X, Merrick WC, et al. 2003. The transformation suppressor Pdcd4 is a novel eukaryotic translation initiation factor 4A binding protein that inhibits translation. *Mol. Cell. Biol.* 23:26–37
139. Yang HS, Knies JL, Stark C, Colburn NH. 2003. Pdcd4 suppresses tumor phenotype in JB6 cells by inhibiting AP-1 transactivation. *Oncogene* 22:3712–20
140. Zakowicz H, Yang HS, Stark C, Wlodawer A, Laronde-Leblanc N, Colburn NH. 2005. Mutational analysis of the DEAD-box RNA helicase eIF4AII characterizes its interaction with transformation suppressor Pdcd4 and eIF4GI. *RNA* 11:261–74
141. LaRonde-LeBlanc N, Santhanam AN, Baker AR, Wlodawer A, Colburn NH. 2007. Structural basis for inhibition of translation by the tumor suppressor Pdcd4. *Mol. Cell. Biol.* 27:147–56
142. Waters LC, Veverka V, Böhm M, Schmedt T, Choong PT, et al. 2007. Structure of the C-terminal MA-3 domain of the tumour suppressor protein Pdcd4 and characterization of its interaction with eIF4A. *Oncogene* 26:4941–50

143. Yang HS, Cho MH, Zakowicz H, Hegamyer G, Sonenberg N, Colburn NH. 2004. A novel function of the MA-3 domains in transformation and translation suppressor Pdcd4 is essential for its binding to eukaryotic translation initiation factor 4A. *Mol. Cell. Biol.* 24:3894–906
144. Dorrello NV, Peschiaroli A, Guardavaccaro D, Colburn NH, Sherman NE, Pagano M. 2006. S6K1- and βTRCP-mediated degradation of PDCD4 promotes protein translation and cell growth. *Science* 314:467–71
145. Liwak U, Thakor N, Jordan LE, Roy R, Lewis SM, et al. 2012. Tumor suppressor PCD4 repressed internal ribosome site-mediated translation of antiapoptotic proteins and is regulated by S6 kinase 2. *Mol. Cell. Biol.* 32:1818–29
146. Altmann M, Muller PP, Wittmer B, Ruchti F, Lanker S, Trachsel H. 1993. A *Saccharomyces cerevisiae* homologue of mammalian translation initiation factor 4B contributes to RNA helicase activity. *EMBO J.* 12:3997–4003
147. Coppolecchia R, Buser P, Stotz A, Linder P. 1993. A new yeast translation initiation factor suppresses a mutation in the eIF-4A RNA helicase. *EMBO J.* 12:4005–11
148. Methot N, Pause A, Hershey JW, Sonenberg N. 1994. The translation initiation factor eIF-4B contains an RNA-binding region that is distinct and independent from its ribonucleoprotein consensus sequence. *Mol. Cell. Biol.* 14:2307–16
149. Naranda T, Strong WB, Menaya J, Fabbri BJ, Hershey JW. 1994. Two structural domains of initiation factor eIF-4B are involved in binding to RNA. *J. Biol. Chem.* 269:14465–72
150. Methot N, Pickett G, Keene JD, Sonenberg N. 1996. In vitro RNA selection identifies RNA ligands that specifically bind to eukaryotic translation initiation factor 4B: the role of the RNA remotif. *RNA* 2:38–50
151. Eliseev B, Yeramala L, Leitner A, Karuppasamy M, Raimondeau E, et al. 2018. Structure of a human cap-dependent 48S translation pre-initiation complex. *Nucleic Acids Res.* 46:2678–89
152. Walker SE, Zhou F, Mitchell SF, Larson VS, Valasek L, et al. 2013. Yeast eIF4B binds to the head of the 40S ribosomal subunit and promotes mRNA recruitment through its N-terminal and internal repeat domains. *RNA* 19:191–207
153. Capossela S, Muzio L, Bertolo A, Bianchi V, Dati G, et al. 2012. Growth defects and impaired cognitive-behavioral abilities in mice with knockout for *Eif4h*, a gene located in the mouse homolog of the Williams-Beuren syndrome critical region. *Am. J. Pathol.* 180:1121–35
154. Rozovsky N, Butterworth AC, Moore MJ. 2008. Interactions between eIF4AI and its accessory factors eIF4B and eIF4H. *RNA* 14:2136–48
155. Parsyan A, Svitkin Y, Shahbazian D, Gkogkas C, Lasko P, et al. 2011. mRNA helicases: the tacticians of translational control. *Nat. Rev. Mol. Cell Biol.* 12:235–45
156. Richter-Cook NJ, Dever TE, Hensold JO, Merrick WC. 1998. Purification and characterization of a new eukaryotic protein translation factor: eukaryotic initiation factor 4H. *J. Biol. Chem.* 273:7579–87
157. Ozes AR, Feoktistova K, Avanzino BC, Fraser CS. 2011. Duplex unwinding and ATPase activities of the DEAD-box helicase eIF4A are coupled by eIF4G and eIF4B. *J. Mol. Biol.* 412:674–87
158. Rogers GW Jr., Richter NJ, Lima WF, Merrick WC. 2001. Modulation of the helicase activity of eIF4A by eIF4B, eIF4H, and eIF4F. *J. Biol. Chem.* 276:30914–22
159. van Gorp AG, van der Vos KE, Brenkman AB, Bremer A, van den Broek N, et al. 2009. AGC kinases regulate phosphorylation and activation of eukaryotic translation initiation factor 4B. *Oncogene* 28:95–106
160. Shahbazian D, Roux PP, Mieulet V, Cohen MS, Raught B, et al. 2006. The mTOR/PI3K and MAPK pathways converge on eIF4B to control its phosphorylation and activity. *EMBO J.* 25:2781–91
161. Csibi A, Lee G, Yoon SO, Tong H, Ilter D, et al. 2014. The mTORC1/S6K1 pathway regulates glutamine metabolism through the eIF4B-dependent control of c-Myc translation. *Curr. Biol.* 24:2274–80
162. Sonenberg N. 1981. ATP/$Mg^{++}$-dependent cross-linking of cap binding proteins to the 5′ end of eukaryotic mRNA. *Nucleic Acids Res.* 9:1643–56
163. Lawson TG, Ray BK, Dodds JT, Grifo JA, Abramson RD, et al. 1986. Influence of 5′ proximal secondary structure on the translational efficiency of eukaryotic mRNAs and on their interaction with initiation factors. *J. Biol. Chem.* 261:13979–89

164. Tomoo K, Matsushita Y, Fujisaki H, Abiko F, Shen X, et al. 2005. Structural basis for mRNA Cap-Binding regulation of eukaryotic initiation factor 4E by 4E-binding protein, studied by spectroscopic, X-ray crystal structural, and molecular dynamics simulation methods. *Biochim. Biophys. Acta Proteins Proteom.* 1753:191–208
165. Parkin NT, Cohen EA, Darveau A, Rosen C, Haseltine W, Sonenberg N. 1988. Mutational analysis of the 5′ non-coding region of human immunodeficiency virus type 1: effects of secondary structure on translation. *EMBO J.* 7:2831–37
166. Lindqvist L, Imataka H, Pelletier J. 2008. Cap-dependent eukaryotic initiation factor-mRNA interactions probed by cross-linking. *RNA* 14:960–69
167. Sun Y, Atas E, Lindqvist L, Sonenberg N, Pelletier J, Meller A. 2012. The eukaryotic initiation factor eIF4H facilitates loop-binding, repetitive RNA unwinding by the eIF4A DEAD-box helicase. *Nucleic Acids Res.* 40:6199–207
168. Pisareva VP, Pisarev AV, Komar AA, Hellen CUT, Pestova TV. 2008. Translation initiation on mammalian mRNAs with structured 5′UTRs requires DExH-box protein DHX29. *Cell* 135:1237–50
169. Kumar P, Hellen CU, Pestova TV. 2016. Toward the mechanism of eIF4F-mediated ribosomal attachment to mammalian capped mRNAs. *Genes Dev.* 30:1573–88
170. Archer SK, Shirokikh NE, Beilharz TH, Preiss T. 2016. Dynamics of ribosome scanning and recycling revealed by translation complex profiling. *Nature* 535:570–74
171. Slepenkov SV, Korneeva NL, Rhoads RE. 2008. Kinetic mechanism for assembly of the m7GpppG·eIF4E·eIF4G complex. *J. Biol. Chem.* 283:25227–37
172. Asselbergs FAM, Peters W, van Venrooij WJ, Bloemendal H. 1978. Diminished sensitivity of reinitiation of translation to inhibition by cap analogues in reticulocyte lysates. *Eur. J. Biochem.* 88:483–88
173. Pelletier J, Sonenberg N. 1988. Internal initiation of translation of eukaryotic mRNA directed by a sequence derived from poliovirus RNA. *Nature* 334:320–25
174. Jang SK, Kräusslich HG, Nicklin MJ, Duke GM, Palmenberg AC, Wimmer E. 1988. A segment of the 5′ nontranslated region of encephalomyocarditis virus RNA directs internal entry of ribosomes during in vitro translation. *J. Virol.* 62:2636–43
175. Mailliot J, Martin F. 2018. Viral internal ribosomal entry sites: four classes for one goal. *Wiley Interdiscip. Rev. RNA* 9:e1458
176. Pestova TV, Shatsky IN, Fletcher SP, Jackson RJ, Hellen CU. 1998. A prokaryotic-like mode of cytoplasmic eukaryotic ribosome binding to the initiation codon during internal translation initiation of hepatitis C and classical swine fever virus RNAs. *Genes Dev.* 12:67–83
177. Hashem Y, des Georges A, Dhote V, Langlois R, Liao HY, et al. 2013. Hepatitis-C-virus-like internal ribosome entry sites displace eIF3 to gain access to the 40S subunit. *Nature* 503:539–43
178. Wilson JE, Pestova TV, Hellen CU, Sarnow P. 2000. Initiation of protein synthesis from the A site of the ribosome. *Cell* 102:511–20
179. Fernández IS, Bai XC, Murshudov G, Scheres SH, Ramakrishnan V. 2014. Initiation of translation by cricket paralysis virus IRES requires its translocation in the ribosome. *Cell* 157:823–31
180. Jackson RJ. 2013. The current status of vertebrate cellular mRNA IRESs. *Cold Spring Harb. Perspect. Biol.* 5:a011569
181. Weingarten-Gabbay S, Elias-Kirma S, Nir R, Gritsenko AA, Stern-Ginossar N, et al. 2016. Comparative genetics. Systematic discovery of cap-independent translation sequences in human and viral genomes. *Science* 351:aad4939
182. Meyer KD, Patil DP, Zhou J, Zinoviev A, Skabkin MA, et al. 2015. 5′ UTR $m^6A$ promotes cap-independent translation. *Cell* 163:999–1010
183. Lin S, Choe J, Du P, Triboulet R, Gregory RI. 2016. The $m^6A$ methyltransferase METTL3 promotes translation in human cancer cells. *Mol. Cell* 62:335–45
184. Wang X, Zhao BS, Roundtree IA, Lu Z, Han D, et al. 2015. $N^6$-methyladenosine modulates messenger RNA translation efficiency. *Cell* 161:1388–99
185. Krug RM, Morgan MA, Shatkin AJ. 1976. Influenza viral mRNA contains internal $N^6$-methyladenosine and 5′-terminal 7-methylguanosine in cap structures. *J. Virol.* 20:45–53

186. Fu Y, Dominissini D, Rechavi G, He C. 2014. Gene expression regulation mediated through reversible $m^6A$ RNA methylation. *Nat. Rev. Genet.* 15:293–306
187. Salzman J. 2016. Circular RNA expression: its potential regulation and function. *Trends Genet*. 32:309–16
188. Chekulaeva M, Rajewsky N. 2018. Roles of long noncoding RNAs and circular RNAs in translation. In *Translation Mechanisms and Control*, ed. MB Mathews, N Sonenberg, JW Hershey, pp. 213–27. Cold Spring Harbor, NY: Cold Spring Harb. Lab.
189. Chen CY, Sarnow P. 1998. Internal ribosome entry sites tests with circular mRNAs. *Methods Mol. Biol.* 77:355–63
190. Wang Y, Wang Z. 2015. Efficient backsplicing produces translatable circular mRNAs. *RNA* 21:172–79
191. Mazza C, Ohno M, Segref A, Mattaj IW, Cusack S. 2001. Crystal structure of the human nuclear cap binding complex. *Mol. Cell* 8:383–96
192. Gao Q, Das B, Sherman F, Maquat LE. 2005. Cap-binding protein 1-mediated and eukaryotic translation initiation factor 4E-mediated pioneer rounds of translation in yeast. *PNAS* 102:4258–63
193. Lee AS, Kranzusch PJ, Doudna JA, Cate JH. 2016. eIF3d is an mRNA cap-binding protein that is required for specialized translation initiation. *Nature* 536:96–99
194. Leen EN, Sorgeloos F, Correia S, Chaudhry Y, Cannac F, et al. 2016. A conserved interaction between a C-terminal motif in norovirus VPg and the HEAT-1 domain of eIF4G is essential for translation initiation. *PLOS Pathog*. 12:e1005379
195. Eskelin K, Hafrén A, Rantalainen KI, Makinen K. 2011. Potyviral VPg enhances viral RNA translation and inhibits reporter mRNA translation *in planta*. *J. Virol*. 85:9210–21
196. Leonard S, Plante D, Wittmann S, Daigneault N, Fortin MG, Laliberte JF. 2000. Complex formation between potyvirus VPg and translation eukaryotic initiation factor 4E correlates with virus infectivity. *J. Virol.* 74:7730–37
197. Kozak M. 1989. Circumstances and mechanisms of inhibition of translation by secondary structure in eucaryotic mRNAs. *Mol. Cell. Biol.* 9:5134–42
198. Kozak M. 1986. Influences of mRNA secondary structure on initiation by eukaryotic ribosomes. *PNAS* 83:2850–54
199. Kozak M, Shatkin AJ. 1978. Migration of 40 S ribosomal subunits on messenger RNA in the presence of edeine. *J. Biol. Chem.* 253:6568–77
200. Kozak M. 1991. Effects of long 5′ leader sequences on initiation by eukaryotic ribosomes in vitro. *Gene Expr*. 1:117–25
201. Calvo SE, Pagliarini DJ, Mootha VK. 2009. Upstream open reading frames cause widespread reduction of protein expression and are polymorphic among humans. *PNAS* 106:7507–12
202. Ingolia NT, Lareau LF, Weissman JS. 2011. Ribosome profiling of mouse embryonic stem cells reveals the complexity and dynamics of mammalian proteomes. *Cell* 147:789–802
203. Kozak M. 2001. Constraints on reinitiation of translation in mammals. *Nucleic Acids Res*. 29:5226–32
204. Poyry TA, Kaminski A, Jackson RJ. 2004. What determines whether mammalian ribosomes resume scanning after translation of a short upstream open reading frame? *Genes Dev*. 18:62–75
205. Starck SR, Tsai JC, Chen K, Shodiya M, Wang L, et al. 2016. Translation from the 5′ untranslated region shapes the integrated stress response. *Science* 351:aad3867
206. Sendoel A, Dunn JG, Rodriguez EH, Naik S, Gomez NC, et al. 2017. Translation from unconventional 5′ start sites drives tumour initiation. *Nature* 541:494–99
207. Liang H, He S, Yang J, Jia X, Wang P, et al. 2014. PTENα, a PTEN isoform translated through alternative initiation, regulates mitochondrial function and energy metabolism. *Cell Metab*. 19:836–48
208. Golovko A, Kojukhov A, Guan BJ, Morpurgo B, Merrick WC, et al. 2016. The eIF2A knockout mouse. *Cell Cycle* 15:3115–20
209. Chew GL, Pauli A, Rinn JL, Regev A, Schier AF, Valen E. 2013. Ribosome profiling reveals resemblance between long non-coding RNAs and 5′ leaders of coding RNAs. *Development* 140:2828–34
210. Ruiz-Orera J, Messeguer X, Subirana JA, Alba MM. 2014. Long non-coding RNAs as a source of new peptides. *eLife* 3:e03523

211. Bazzini AA, Johnstone TG, Christiano R, Mackowiak SD, Obermayer B, et al. 2014. Identification of small ORFs in vertebrates using ribosome footprinting and evolutionary conservation. *EMBO J.* 33:981–93
212. Mackowiak SD, Zauber H, Bielow C, Thiel D, Kutz K, et al. 2015. Extensive identification and analysis of conserved small ORFs in animals. *Genome Biol.* 16:179
213. Wang H, Wang Y, Xie S, Liu Y, Xie Z. 2017. Global and cell-type specific properties of lincRNAs with ribosome occupancy. *Nucleic Acids Res.* 45:2786–96
214. Kearse MG, Green KM, Krans A, Rodriguez CM, Linsalata AE, et al. 2016. CGG repeat-associated non-AUG translation utilizes a cap-dependent scanning mechanism of initiation to produce toxic proteins. *Mol. Cell* 62:314–22
215. Meyuhas O, Kahan T. 2015. The race to decipher the top secrets of TOP mRNAs. *Biochim. Biophys. Acta Gene Regul. Mech.* 1849:801–11
216. Fonseca BD, Lahr RM, Damgaard CK, Alain T, Berman AJ. 2018. LARP1 on TOP of ribosome production. *Wiley Interdiscip. Rev. RNA* 9:e1480
217. Fonseca BD, Zakaria C, Jia JJ, Graber TE, Svitkin Y, et al. 2015. La-related protein 1 (LARP1) represses terminal oligopyrimidine (TOP) mRNA translation downstream of mTOR complex 1 (mTORC1). *J. Biol. Chem.* 290:15996–6020
218. Tcherkezian J, Cargnello M, Romeo Y, Huttlin EL, Lavoie G, et al. 2014. Proteomic analysis of cap-dependent translation identifies LARP1 as a key regulator of 5′TOP mRNA translation. *Genes Dev.* 28:357–71
219. Lahr RM, Fonseca BD, Ciotti GE, Al-Ashtal HA, Jia JJ, et al. 2017. La-related protein 1 (LARP1) binds the mRNA cap, blocking eIF4F assembly on TOP mRNAs. *eLife* 6:e24146
220. Philippe L, Vasseur J-J, Debart F, Thoreen CC. 2017. La-related protein 1 (LARP1) repression of TOP mRNA translation is mediated through its cap-binding domain and controlled by an adjacent regulatory region. *Nucl. Acids Res.* 46:1457–69
221. Shah P, Ding Y, Niemczyk M, Kudla G, Plotkin JB. 2013. Rate-limiting steps in yeast protein translation. *Cell* 153:1589–601
222. Yan X, Hoek TA, Vale RD, Tanenbaum ME. 2016. Dynamics of translation of single mRNA molecules in vivo. *Cell* 165:976–89
223. Peter D, Weber R, Sandmeir F, Wohlbold L, Helms S, et al. 2017. GIGYF1/2 proteins use auxiliary sequences to selectively bind to 4EHP and repress target mRNA expression. *Genes Dev.* 31:1147–61

Annual Review of Biochemistry

Volume 88, 2019

# Contents

**Errata**

An online log of corrections to *Annual Review of Biochemistry* articles may be found at http://www.annualreviews.org/errata/biochem