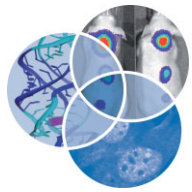
Advanced Review



Cap and cap-binding proteins in the control of gene expression

Ivan Topisirovic,[1] Yuri V. Svitkin,[1] Nahum Sonenberg[1]* and Aaron J. Shatkin[2]*

The 5′ mRNA cap structure is essential for efficient gene expression from yeast to human. It plays a critical role in all aspects of the life cycle of an mRNA molecule. Capping occurs co-transcriptionally on the nascent pre-mRNA as it emerges from the RNA exit channel of RNA polymerase II. The cap structure protects mRNAs from degradation by exonucleases and promotes transcription, polyadenylation, splicing, and nuclear export of mRNA and U-rich, capped snRNAs. In addition, the cap structure is required for the optimal translation of the vast majority of cellular mRNAs, and it also plays a prominent role in the expression of eukaryotic, viral, and parasite mRNAs. Cap-binding proteins specifically bind to the cap structure and mediate its functions in the cell. Two major cellular cap-binding proteins have been described to date: eukaryotic translation initiation factor 4E (eIF4E) in the cytoplasm and nuclear cap binding complex (nCBC), a nuclear complex consisting of a cap-binding subunit cap-binding protein 20 (CBP 20) and an auxiliary protein cap-binding protein 80 (CBP 80). nCBC plays an important role in various aspects of nuclear mRNA metabolism such as pre-mRNA splicing and nuclear export, whereas eIF4E acts primarily as a facilitator of mRNA translation. In this review, we highlight recent findings on the role of the cap structure and cap-binding proteins in the regulation of gene expression. We also describe emerging regulatory pathways that control mRNA capping and cap-binding proteins in the cell. *WIREs RNA* 2011 2 277–298 DOI: 10.1002/wrna.52

INTRODUCTION

The 5′ cap structure (m^7GpppN, where m^7G is 7-methylguanosine and N is the first nucleotide of mRNA) plays a critical role in the life cycle of eukaryotic mRNA and is necessary for efficient gene expression and cell viability from yeast to human. The cap structure is required for the optimal translation of the vast majority of cellular mRNAs and is also a prominent functional feature of most eukaryotic viral and parasite mRNAs.[1–3] In addition, the cap structure stabilizes mRNAs by protecting them against exonucleases and promotes transcription, polyadenylation, splicing, and nuclear export of mRNAs.[4,5] The cap is added co-transcriptionally to the 5′ end of nascent pre-mRNA molecules when they reach a length of 22–25 nucleotides and emerge from the RNA exit channel of RNA polymerase II (PolII).[6,7] The 5′ N^7-methyl guanosine is linked by an inverted 5′-5′ triphosphate bridge to the first nucleotide of the nascent transcript.[1,6] Capping occurs in a series of three enzymatic steps. The first reaction is the removal of the 5′-γ-phosphate group from the first transcribed nucleotide of pre-mRNA by an RNA triphosphatase (RT), which is followed by a guanylyltransferase (GT)-catalyzed transfer of guanine monophosphate (GMP) nucleotide to the RNA 5′-diphosphate end. This results in the formation of a guanosine cap (GpppN), which is subsequently methylated by RNA (guanine-N7-) methyltransferase (RNMT) to produce the 7-methylguanosine cap (m^7GpppN). These three catalytic activities, collectively referred to as 'capping enzymes', are encoded by separate genes in yeast, whereas in metazoans the first two steps of capping are catalyzed by a single enzyme consisting of two

*Correspondence to: nahum.sonenberg@mcgill.ca; shatkin@cabm.rutgers.edu

[1]Department of Biochemistry and Goodman Cancer Centre, McGill University, Montréal, QC, Canada

[2]Center for Advanced Biotechnology and Medicine, Rutgers University and UMDNJ Robert Wood Johnson Medical School, Piscataway, NJ, USA

DOI: 10.1002/wrna.52

functional domains, the N-terminal RT and the C-terminal GT.[8–13] During transcription, capping enzymes are recruited to the 5′ end of nascent transcripts, through an interaction with the C-terminal domain (CTD) of the large subunit of PolII, as they emerge from the transcribing complex.[13–15] This interaction is stimulated by the phosphorylation of Ser 5 in the CTD YSPTSPS heptad repeats by the core transcription factor II H (TFIIH) subunit cyclin-dependent kinase 7 (CDK7).[16–21] Capping can also be stimulated in vitro by transcription factor hSPT5 (21). m^7GpppN (referred to as cap 0) can be further modified by cap-specific 2′-O RNA methytransferases in the nucleus and cytoplasm that add a methyl group to ribose 2′-hydroxyl positions of the first and second nucleotides, giving rise to m^7GpppNm (cap 1) and m^7GpppNmNm (cap 2) structures, respectively.

The cap is recognized by cap-binding proteins that mediate its effects in the cell. In this review, we highlight recent findings pertinent to the role of the cap structure and cellular cap binding proteins in the regulation of gene expression. In depth description of the molecular mechanisms underlying capping and decapping, the role of the cap in regulating mRNA stability and strategies developed by viruses to modify cellular cap-dependent processes can be found elsewhere in this issue.

CAP-BINDING PROTEINS: MOLECULAR ASPECTS OF THE RECOGNITION OF mRNA CAP STRUCTURES

Cap-binding proteins mediate the effects of cap on gene expression. Two major cellular cap-binding proteins have been described to date: eukaryotic translation initiation factor 4E (eIF4E) in the cytoplasm and nuclear cap binding complex (nCBC), a nuclear complex consisting of a cap-binding subunit CBP 20 and an auxiliary protein CBP 80.[2,5] Although these proteins have strikingly different overall structures, the molecular architecture of their cap-binding pockets is remarkably similar. In both cases, this pocket consists of two aromatic amino acids (Trp 56 and Trp 102 in mammalian eIF4E and Tyr 20 and Tyr 43 in CBP 20), surrounded by basic and acidic areas which accommodate the negatively charged 5′-5′ triphosphate bridge and the positively charged π-ring system of the m^7G, respectively[22–27] (Figure 1) . The purine ring of m^7G is sandwiched between the indole and phenyl rings of the Trp and Tyr residues on eIF4E and CBP 20 resulting in a nearly perfect alignment and the optimal interplanar distance between the stacked aromatic

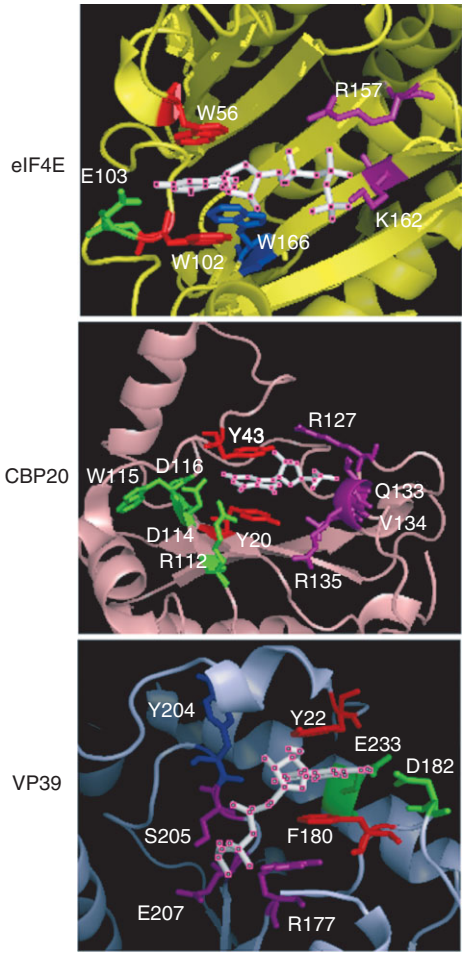


FIGURE 1 | Diagrams representing the similarities in molecular architecture of the cap-binding pockets of eIF4E, CBC20, and VP39. In each protein, aromatic residues that intercalate the cap are shown in red, residues binding the functional groups of the guanine base are shown in green, residues interacting with the 5′-5′ triphosphate bridge are shown in purple, and residues that stabilize the 7-methyl group are shown in blue. Cap analogs are shown in white. Diagrams were generated using PyMOL software (http://www.pymol.org). eIF4E-PDB accession number 1L8B; CBP 20-PDB accession number 1H2T; VP39-PDB accession number 1AV6.

rings[22–25,27,28] (Figure 1). Delocalized positive charge arising from the methyl group of the m^7G base further strengthens the interactions between the π-electrons of the stacked aromatic rings, explaining the low affinity of eIF4E and nCBC for the nonmethylated cap analogs (>100-fold difference in affinity compared to the N7-methylated cap).[28,29] The affinity of cap-binding proteins for the cap as well as their ability to discriminate between methylated and nonmethylated cap analogs are abolished when the aromatic residues in the cap-binding site are mutated to either Leu or Ala residues.[24,25,30] The interaction of cap-binding proteins with the cap is further stabilized by hydrogen

bonds and salt bridges formed between N1 and N2 of the m^7G base and the carboxyl groups of Glu 103 or Asp 116 of eIF4E and CBP 20, respectively[22–27] (Figure 1). Similarly to mutation of aromatic cap-binding residues, mutations of these amino acids to Ala decreases the affinity as well as specificity of the cap-binding proteins.[24,25,30] The 5′-5′ triphosphate bridge of the cap structure is located in a cavity composed of the residues which form salt bridges with oxygen atoms of phosphate groups (Arg 157 and Lys 162 of eIF4E and Arg 127, Glu 133, Val 134 and Arg135 of CBP 20)[25,27,31,32] (Figure 1). The interactions between the first base of the transcript with Tyr 138 in CBP 20 and Tyr 205, Lys 206 and Ser 207 in eIF4E also contribute to binding.[22–28,32] The only slight discordance between the molecular underpinnings of CBP 20 and eIF4E binding to the cap is that in the latter case the methyl group of m^7G is additionally stabilized by van der Waals interactions with Trp 166.[22,23,27,28,31]

The cap-binding pocket of the vaccinia virus cap-binding protein VP39 and the molecular mechanism which it utilizes to bind to the cap are strikingly similar to that of the cellular cap-binding proteins,[33–37] suggesting that the cellular and viral binding mechanisms evolved convergently.[31] Moreover, this specific binding to purine residues is common to the family of nucleotidyltransferases (e.g., ligases, capping enzymes) that also recognize the purine residues of adenosine 5′-triphosphate (ATP) or guanosine 5′-triphosphate (GTP) by formation of a hydrophobic sandwich between a conserved aromatic and a conserved aliphatic residue.[38]

MECHANISMS OF CAP BINDING

Structural studies have provided insights on the molecular architecture of the cap-binding pockets of eIF4E, CBP 20 and VP39 and determined the interactions between the cap and the residues present in the cap-binding pocket of each of these proteins. However, the kinetics and thermodynamics of cap binding are less well understood. eIF4E, nCBC, and VP39 have different affinities for m^7GTP, with nCBC having the strongest (~10 nM),[39] eIF4E intermediate (260–280 nM),[28] and VP39 the lowest affinity (in the μM range).[34] Although these differences correlate with the number of hydrogen bonds formed (nCBC has the highest number and VP39 the lowest number of hydrogen bonds in complexes with m^7GTP), these findings also suggest that cap-binding proteins might utilize modified mechanisms to bind the cap. This is further corroborated by studies involving different cap analogs. For instance, eIF4E and VP39 bound to m^7GpppG and m^7GTP with a similar affinity.[28,34,40] In stark contrast, nCBC had a 100-fold higher affinity for m^7GpppG than for m^7GTP,[29] which is thought to be due to stacking of the second G with Tyr 138 of CBP 20.[31] Increasing the length of the oligonucleotide (>20) dramatically increased the affinity of VP39 (~100-fold)[41] but had only a modest effect on the cap binding of eIF4E and CBP 20 (~6-fold).[25,42] Moreover, experiments using m^7GTP, m^7 guanosine 5′-diphosphate (GDP), m^7 guanosine 5′-monophospate (GMP), and m^7 guanosine (G) revealed that the triphosphate bridge stimulates and contributes to the binding of eIF4E to the cap, but not to VP39.[28,34] These data are in accordance with the initially proposed two-step binding mechanism for formation of the m^7GTP:eIF4E complex, where the binding of the 5′-5′ triphosphate moiety serves as a primary, rate-limiting step enabling the subsequent specific interactions with the m^7G base.[28,43,44] In contrast, a simple one-step binding mechanism was proposed for VP39 where it was suggested that the interactions of m^7G with the two aromatic residues is not preceded by binding of the 5′-5′ triphosphate bridge.[36] Based on stopped flow kinetics measurements, Slepenkov et al. recently challenged the 'two-step' binding model by showing that the association between eIF4E and cap analogs or capped oligoribonucleotides is a simple one-step process.[45] According to this model, the fast phase of change in fluorescence is due to m^7GpppG binding to monomeric eIF4E, while the slow phase is caused by the dissociation of eIF4E oligomers yielding reactive monomers, thereby enabling them to react with the cap analog.[45] Accordingly, in this study, the slower second phase was observed only at high eIF4E concentrations, where concentration-dependent protein self-association would be expected.[45]

NUCLEAR CAP-BINDING COMPLEX: RELATING CAP TO mRNA SPLICING, EXPORT MACHINERY AND NONSENSE MEDIATED DECAY

As explained in molecular detail in the previous section, the cap-binding subunit (CBP 20) of the heterodimeric nCBC utilizes its ribonucleoprotein (RNP) domain to bind the cap.[24,25] The resulting complex is stabilized by the interactions of the CBP 20 N-terminal tail with the CBP 80 subunit.[24,25] In addition, CBP 80 interacts with PolII CTD[46] and the REF (RNA and export factor binding protein)/Aly protein,[47,48] a component of Transcription/Export complex (TREX).[49] nCBC joins the mRNP simultaneously with capping of the transcript and facilitates pre-mRNA splicing and

nuclear export of mRNAs and PolII synthesized small, nuclear capped RNAs.[50,51] It also has an important role in pre-mRNA 3′ end processing[52] and nonsense mediated decay (NMD).[53]

Cap plays a pivotal role in pre-mRNA splicing. The presence of the m^7G cap structure on pre-mRNA stimulates splicing reactions *in vitro*,[54,55] and pre-mRNAs containing m^7GpppN caps are more efficiently spliced than *in vitro* transcripts containing an ApppN cap when injected into *Xenopus* oocytes.[56] Experiments in which splicing was abolished by immunodepletion of nCBC from HeLa cell extracts[57] and microinjection of *Xenopus* oocytes with antibodies raised against CBP 20, which block binding of CBP 20 to the cap,[58,59] demonstrated that nCBC is essential for mediating the effects of the cap on the splicing reaction. nCBC stimulates binding of the core components of the small nuclear RNPs (snRNPs) to nascent transcripts, thereby promoting the assembly of the splicing-commitment complex on the cap-proximal intron of the pre-mRNA.[57,60] Concomitant with transcription and splicing, nascent transcripts become coated by proteins that ensure RNA integrity and participate in mRNA export and downstream cytoplasmic events, such as polyadenylation.[51] One of the protein complexes recruited to mRNA during splicing is the THO/TREX complex.[61–63] The complex is tetrameric in budding yeast and composed of Tho2, Hpr1, Mft1, and Thp2[64] or pentameric in human and consisting of hTho2, hHpr1, fSAP79, fSAP24, and fSAP35.[61] In *Drosophila*, the THO complex contains HPR1, THO2, THOC5, THOC6, and THOC7[65] and functions in transcription elongation and transcript-dependent recombination as well as REF/Aly RNA recognition motif (RRM)-containing RNA binding protein (Yra1 in yeast) and UAP56 RNA-dependent ATPase (Sub2 in yeast).[61–63] The nCBP 80 subunit of nCBC recruits THO/TREX complex to a region proximal to the 5′ end of mRNA through a direct interaction with REF/Aly.[49] THO/TREX complex in turn bridges the interaction of mRNA with nuclear export receptor NXF1/(TAP) which results in the cytoplasmic export of bulk cellular mRNA.[49] Accordingly, nucleo-cytoplasmic export of bulk mRNA is strongly facilitated by the deposition of the THO/TREX complex, which requires the cap and is dependent on splicing.[49] These findings are further corroborated by studies which show that the export of spliced but not intronless mRNA depends on the cap and the presence of nCBC.[66] Interaction of nCBC with Ref/Aly also promotes the positioning of the THO/TREX complex at the 5′-end of spliced mRNAs which appears to impart $5' \rightarrow 3'$ directionality to mRNA export, enabling efficient engagement of the translation apparatus.[49] Indeed, this directionality of nuclear export was observed by electron microscopy for giant Balbiani ring mRNPs of *Chironomus tentans*.[67] In addition to the THO/TREX complex, the shuttling serine/arginine-rich (SR) and SR-like proteins (i.e., ASF/SF2, SRp20, and 9G8) have also been suggested to act as mRNA export adaptors through binding of the general export receptor NXF1/TAP.[68] Finally, nucleo-cytoplasmic transport of a subset of mRNAs (e.g., transcripts exported from the nucleus in HuR- or eIF4E-dependent manner), appears to be mediated by the karyopherin chromosome region maintenance 1 (CRM1), but not by NXF1/TAP.[69–71]

In addition to mRNA, the nCBC-bound monomethylated cap represents a common export signal for PolII transcribed snRNAs.[58,72] In metazoans functional snRNPs including major spliceosomal U snRNAs are assembled in the cytoplasm where the snRNA 5′ cap structure is dimethylated on the 2-amino group and where they associate with Sm proteins.[73] In the cytoplasm, 2,2,7-trimethylguanosine cap binds snurportin1 and mature snRNPs are then re-imported into the nucleus where they function in pre-mRNA processing.[74] During the export of snRNAs, nCBC interacts with the phosphorylated adaptor for RNA export protein (PHAX) that mediates the association of CBC/snRNA complex with the CRM1 exporter.[75] In the nucleus, the phosphorylation of PHAX promotes assembly of the export complex.[75] Once snRNAs are exported to the cytoplasm, PHAX is dephosphorylated which causes the disassembly of the export complex, whereas the interaction of nCBC with importin α releases snRNAs from the complex.[75] This is followed by PHAX and nCBC being reimported to the nucleus for a subsequent round of snRNA export.[75,76]

In addition to mRNA export and splicing, nCBC plays a major role in NMD (Figure 2). NMD is a translation-dependent mRNA degradation process that targets nCBC-bound mRNAs containing a premature termination codon (PTC) (i.e., an in-frame stop codon).[53,77] NMD provides an important quality control mechanism to eliminate any errors introduced into individual mRNA molecules during transcription or pre-mRNA processing. NMD prevents production of truncated proteins whose expression might be deleterious for the cell.[53] In addition, NMD can modulate the expression of wild type genes. For instance, several pre-mRNA splicing factors appear to control their own levels via targeting their mRNAs for NMD.[78] This is achieved through the induction of alternative splicing to generate transcripts with an

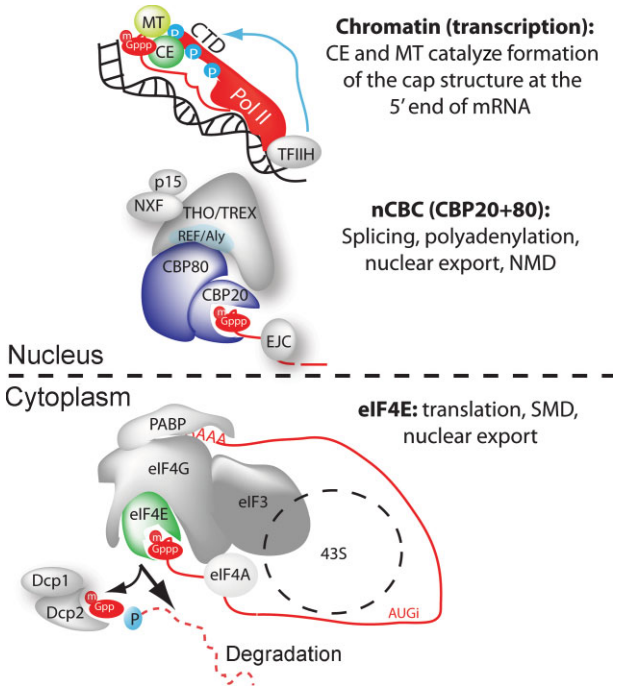


FIGURE 2 | Schematic representation of cap-dependent processes governed by cap-binding proteins. Capping occurs co-transcriptionally where capping enzyme (CE) consisting of the RNA triphosphatase and guanylyltransferase and RNA (guanine-N7-) methyltransferase (MT) are recruited to nascent transcripts through interaction with the TFIIH-phosphorylated C-terminal domain (CTD) of polymerase II (Pol II). After the cap formation, nuclear cap binding complex (nCBC) consisting of cap-binding proteins CBP 20 and 80 binds the mRNA and with other protein complexes mediates its effects on the subsequent steps of mRNA metabolism. After mRNA is exported from the nucleus, eIF4E binds the cap and recruits it to the small ribosomal subunit. In addition, eIF4E was suggested to export a subset of mRNAs from the nucleus and to play a role in Staufen-mediated decay (SMD). Finally, cap is removed by decapping enzyme (Dcp1 and 2), after which mRNA is rapidly degraded. Abbreviations: TFIIH, transcription factor II H; NXF1, nuclear export factor 1; REF, RNA and export factor binding protein; TREX, transcription/export complex; EJC, exon junction complex; PABP, poly(A) binding protein; eIF, eukaryotic translation initiation factor; 43S, 43S pre-initiation complex; Dcp, decapping protein complex.

exon junction complex (EJC) downstream of the stop codon and rendering them substrates for degradation by NMD machinery.[78] Similarly, the expression of mRNAs containing constitutively spliced introns in their 3′ untranslated region (UTR) (e.g., Arc), is also regulated in an NMD-dependent manner.[79] The efficiency of NMD is significantly increased if an EJC is located 50 or more nucleotides downstream of a PTC.[80,81] EJC is deposited on the mRNA during splicing, but unlike THO/TREX complex which is found only proximal to the 5′ end of the mRNA, EJC is found 20–24 nt upstream of every exon–exon junction.[53] In metazoans, EJC consists of Magoh, RNPS1, Y14, SRm160, REF1/Aly,[82] and the DEAD-box RNA-dependent ATPase eIF4AIII (DDX48).[83] EJC is anchored to the mRNA through eIF4AIII. DEAD-box family members exhibit helicase activity and play a well-established role in mRNP remodeling.[84] However, within the EJC core, eIF4AIII ATPase activity is inhibited by Y14 and Magoh, whereas MLN51 increases its RNA binding affinity.[85] This locks eIF4AIII into a stable RNA binding configuration.

EJCs which are deposited downstream of the stop codon stimulate NMD by facilitating the association of the NMD-specific factors UPF1 and UPF2 with mRNA. UPF1 is a RNA helicase, which together with SMG1 kinase, eRF1 and eRF3 forms a surveillance complex (SURF).[86] Binding of the SURF complex to UPF2 bound to an EJC activates SMG1, which in turn phosphorylates UPF1.[86,87] Phosphorylated UPF1 inhibits further rounds of translation most likely through an interaction with eIF3 and promotes recruitment of the RNA decay machinery by yet unknown mechanism(s), thus targeting the bound RNA for rapid exonucleolytic degradation.[87] Alternatively, degradation of the transcripts targeted to NMD can be initiated via SMG6-mediated endonucleolytic cleavage in the vicinity of the premature stop codon.[88,89]

In addition to NMD, EJC-independent, Staufen-mediated decay (SMD) targeting both eIF4E and CBC bound mRNAs has been described.[90]

CAP-DEPENDENT TRANSLATION: eIF4E RECRUITS mRNAs TO THE RIBOSOME VIA THE CAP

In eukaryotes, the vast majority of mRNAs are translated in a cap-dependent manner, with the exception of a subset of mRNAs translated through an internal ribosome entry site (IRES)-dependent mechanism. The first cap-dependent step of translation initiation is the assembly of the eIF4F complex on the cap structure[2] (Figure 2). eIF4F complex consists of the cap-binding protein eIF4E, which recruits other components of the complex to the cap.[2] These are the DEAD-box RNA helicase eIF4A and the large scaffolding protein eIF4G.[2,91–93] In addition to eIF4E and eIF4A, eIF4G binds poly(A)-binding protein (PABP) and eIF3.[2,91] eIF3 recruits the 43S pre-initiation complex consisting of a 40S subunit, eIF2-GTP-Met-tRNAi, eIF1, eIF1A, and eIF5 to the mRNA, resulting in the formation of 48S ribosomal initiation complex.[2,91,94] Cooperative binding of the eIF4E/eIF4G complex and PABP to the mRNA engenders a more effective association with the cap and effectively counteracts the inhibitory effects

of general RNA-binding proteins on translation.[95,96] In addition, PABP-eIF4G association is predicted to circularize mRNA. This 'closed-loop' mRNA configuration is believed to stimulate translation by enhancing recycling of the ribosomes on the mRNA.[2,91,94,95] The 48S initiation complex scans the 5′ untranslated region (5′ UTR) in the 5′ → 3′ direction to the initiation codon.[2,91,94,97] During the scanning, the eIF4A subunit of the eIF4F complex unwinds secondary structure in the 5′ UTR, a process stimulated by two auxiliary proteins eIF4B and eIF4H.[98–102] Because a great number of eIF4A mutants exhibit a dominant-negative effect on translation, it is generally believed that eIF4A participates in RNA unwinding as a subunit of the eIF4F complex, rather than as a singular polypeptide.[99] Binding of eIF4G induces conformational changes which increase the processivity of eIF4A by aligning its DEAD-box motifs, whereas eIF4H and eIF4B prevent re-annealing of mRNA structures, thereby promoting unidirectional 5′ → 3′ movement of eIF4A toward the initiation codon.[103,104] It is thought that eIF4A periodically dissociates from mRNA. However, its association with the 5′ end of the transcript via the eIF4E–cap interaction enables it to resume subsequent cycles of unwinding.[104] After reaching the initiation codon, eIF5 and eIF5B promote the hydrolysis of eIF2-bound GTP,[105–107] which results in the displacement of eIFs and the joining of a 60S subunit.[108,109] This is followed by a codon–anticodon base pairing accompanied by the displacement of eIF1.[108,110–112] The latter event switches the ribosome from open 'latch' to a 'closed' conformation that is locked onto the mRNA, which marks the end of translation initiation and the start of elongation.[91,94]

eIF4E: NOT ALL mRNAs ARE TREATED EQUALLY

eIF4E is a general translation initiation factor necessary for efficient cap-dependent translation of all cellular mRNAs.[2] Nonetheless, its overexpression affects global protein synthesis only modestly, while strongly increasing translation of a subset of mRNAs referred to as 'eIF4E-sensitive'.[113–116] These mRNAs are generally characterized by long, highly structured 5′ UTRs that render these transcripts strongly dependent on the unwinding activity of the eIF4A subunit of eIF4F.[117,118] mRNAs bearing short, unstructured 5′ UTRs, such as mRNAs encoding housekeeping proteins [e.g., glyceraldehyde 3-phosphate dehydrogenase (GAPDH), actin] do not strongly depend on the unwinding activity of eIF4A and are thus only marginally sensitive to the alterations in cellular eIF4E levels.[113–115] 'eIF4E-sensitive' mRNAs generally encode proto-oncogenic, proliferation, and survival promoting proteins such as cyclins, c-Myc, vascular endothelial growth factor (VEGF), and Bcl-xl.[113,115,116] Accordingly, overexpression of eIF4E results in the neoplastic transformation of rodent and human immortalized cells and leads to tumorigenesis in mice.[119,120] Notably, in both cases the cap-binding activity of eIF4E is essential, as an eIF4E mutant in which one of the cap-stacking Trp residues (Trp56) is mutated to alanine did not exhibit proto-oncogenic properties.[121,122] Consistent with the data obtained in cell culture and animal models, upregulation of eIF4E expression and/or activity has been documented in a plethora of human malignancies including colon, breast, bladder, lung, prostate, gastrointestinal, head and neck squamous cell carcinoma, lymphomas, leukemias, and neuroblastomas, where high eIF4E levels correlate with disease progression and poor clinical outcome.[113,115,116,123,124]

REGULATION OF eIF4E ACTIVITY

As eIF4E plays a pivotal role in regulating the expression of the proliferation and survival-promoting proteins, its activity in the cell is tightly regulated (Figure 3). In the last decade, several cellular mechanisms that control levels and/or activity have been described. These include the mammalian target of rapamycin (mTOR) signaling toward eIF4E-binding proteins (4E-BPs), phosphorylation of eIF4E by Mnk and the activation of transcription of the eIF4E gene by Myc.

mTOR AND 4E-BPs

The best-characterized regulators of eIF4E activity to date are the eIF4E-binding proteins (4E-BPs).[2] In mammals 4E-BP1, 2 and 3 comprise a family of low molecular weight proteins, which act as repressors of cap-dependent translation. 4E-BPs suppress eIF4F complex assembly by blocking eIF4G binding to eIF4E.[125] Namely, 4E-BP and eIF4G utilize the eIF4E binding motif [$Tyr(X)_4Leu\Phi$, where Φ is hydrophobic, and X any amino acid] to bind to the dorsal surface of eIF4E.[126] In the case of mammalian eIF4E, this interaction occurs primarily through hydrophobic interactions between Val 69 and Trp 73 residues on the dorsal surface of eIF4E and Tyr and a hydrophobic residue in the eIF4E- binding motif.[28] eIF4G enhances eIF4E's affinity for the cap.[23,127,128] It has been proposed that binding of eIF4G to the dorsal

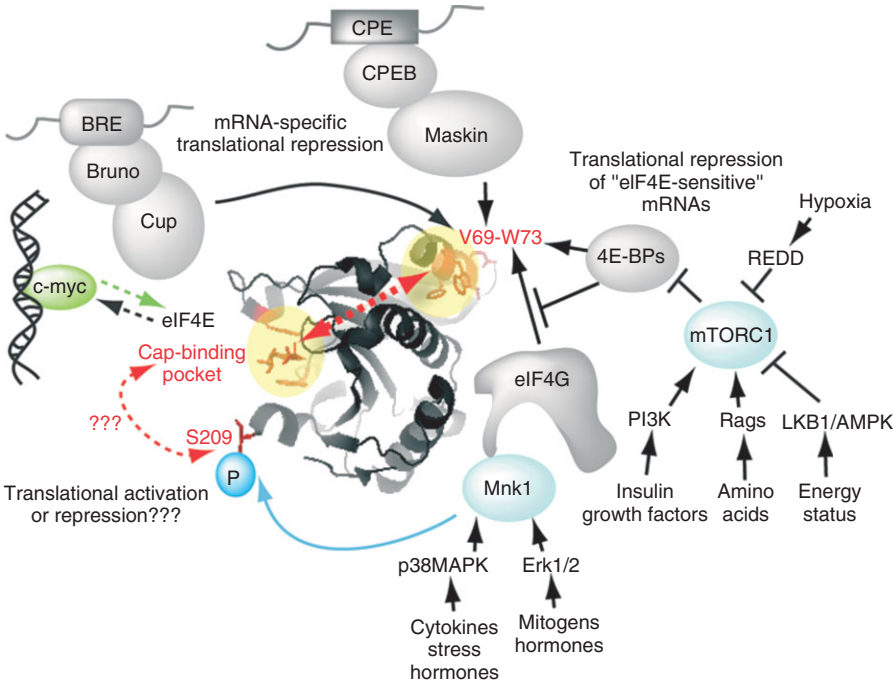


FIGURE 3 | The regulation of eIF4E activity. The activity of eIF4E is regulated by proteins that compete for binding to its dorsal surface (residues V69-W73 in mammalian eIF4E). There is an allosteric communication between dorsal surface and cap binding pocket which are on opposite sides of the eIF4E molecule (red arrow). Proteins that bind to dorsal surface either stimulate (eIF4G) or inhibit [eIF4E-binding proteins (4E-BPs), Cup and Maskin] translation. Although 4E-BPs inhibit translation of bulk cellular mRNAs, they preferentially affect 'eIF4E-sensitive' mRNAs which harbor long and structured 5′ UTRs. Activity of 4E-BPs in the cell is regulated by the mammalian target of rapamycin complex 1 (mTORC1) pathway. In contrast, Maskin and Cup inhibit translation of a limited number of specific mRNAs, where specificity is determined by the presence of the cytoplasmic polyadenylation element (CPE) or bruno response element (BRE) in the mRNA 3′ UTR, respectively. In addition, several homeobox proteins have been shown to interact with the dorsal surface of eIF4E family memebrs. eIF4E activity is also regulated via phosphorylation at residue S209 by the MAP kinase integrating kinases 1 and 2 (Mnk1 and 2). The effects of eIF4E phosphorylation on its translational activity and cap binding are still unclear. eIF4E is a transcriptional target of c-myc, whereas c-myc is regulated by eIF4E at the level of translation. Abbreviations: CPEB, cytoplasmic polyadenylation element binding protein; p38MAPK, mitogen-activated protein kinase; Erk1/2, extracellular signal-regulated kinase 1 and 2; AMPK, adenine monophosphate-activated protein kinase; LKB1, serine/threonine kinase 11; REDD1, DNA-damage-inducible transcript 4; PI3K, Phosphoinositide 3-kinase; Rags, ras-related GTP-binding proteins; eIF, eukaryotic translation initiation factor.

surface of eIF4E induces structural changes in the loops surrounding the cap-binding site, therein locking Trp56 and Trp102 residues in the optimal orientation for cap binding.[129,130] It was also proposed that eIF4G binding to the mRNA stabilizes the eIF4E interaction with the cap.[131]

Binding of 4E-BPs to eIF4E is regulated by phosphorylation via the mTOR pathway.[132–135] Stimuli, such as amino acids, insulin, growth factors and nutrients activate mTOR signaling, leading to phosphorylation of Thr 37 and 46 on 4E-BP1.[136,137] They act as priming sites for the phosphorylation of residues Thr 70 and Ser 65, leading to dissociation of 4E-BP1 from eIF4E.[136,137] Intriguingly, expression of a nonphosphorylatable mutant of 4E-BP1, which constitutively binds eIF4E or deletion of genes encoding 4E-BP1 and 2 only modestly affects global translation levels.[138] This is consistent with the experiments in which eIF4E overexpression increased translation of only a subset of 'eIF4E-sensitive' mRNAs.[118] Indeed, selected mRNAs [e.g. interferon regulatory factor 7 (IRF-7), growth arrest-specific protein 2 (Gas2)] are translated more efficiently in 4E-BP double knockout (DKO) mouse embryonic fibroblasts (MEFs) as compared to wild type MEFs.[139,140]

Similar to eIF4E expression, mTORC1 signaling is frequently dysregulated in cancer.[133] This is caused by mutations inactivating tumor suppressors [e.g. LKB1, phosphatase and tensin homolog (PTEN) and tuberous sclerosis complex (TSC)1/2] that antagonize PI3K-dependent activation of mTORC1.[133] Aberrant mTOR signaling in cancer results in increased phosphorylation of 4E-BPs, diminishing their inhibitory effect on eIF4E and contributing to dysregulation of eIF4E activity in cancer.[133,141] Indeed, several studies suggested that increased phosphorylation of 4E-BPs in tumors correlates with poor clinical outcome.[142,143]

Mnks

In mammals, eIF4E is phosphorylated at Ser 209 by the mitogen-activated protein kinase (MAPK) signal-integrating kinases 1 and 2 (Mnk1 and Mnk2).[144,145] Mnk1 is activated by growth factors and phorbol esters via an extracellular signal-regulated kinase (Erk) or by cytokines and stress via a p38 MAPK pathway.[146,147] In contrast, Mnk2 is constitutively active, and its activity is not further increased by the aforementioned stimuli.[147] Mnks are recruited to eIF4E through an interaction with the C-terminal part of eIF4G.[148] Phosphorylation of eIF4E seems to be restricted to metazoans as yeast lack Mnk homologs and a Mnk interaction domain in eIF4G.[149] Mnk 1/2 knock out mice develop normally and do not exhibit any major phenotypic changes, indicating that eIF4E phosphorylation is not essential for translation.[150] Nonetheless, the phosphorylation of eIF4E contributes to its oncogenic potential as the ability of the nonphosphorylatable mutant of eIF4E to induce transformation in cell culture and lymphomagenesis in mice is severely reduced.[122,151] Similarly, transgenic flies that express a nonphosphorylatable form of eIF4E are smaller, have morphological defects, and are less viable then wild type flies.[152]

Effects of eIF4E phosphorylation on its cap binding activity are still being debated. Ser 209 is located at the entrance to the cap-binding pocket.[22,23] Initially, based on eIF4E structure, a 'clamping' model was proposed according to which a salt bridge is formed between the negatively charged phosphate group of Ser 209 and positively charged Lys 159, forming a 'clamp' which stabilizes cap in the cap binding slot.[22,28] Molecular dynamics simulations suggested a similar model in which hydrogen-bonded clusters of water molecules could form around the phosphorylated Ser 209, thereby blocking the release of the cap from the cap binding pocket.[23] Both of these models envision that eIF4E phosphorylation leads to a closure of the cap-binding pocket. Accordingly, the phosphorylation of eIF4E would be expected to decrease the dissociation rate of eIF4E:cap complex if cap-bound eIF4E is phosphorylated, or decrease the eIF4E:cap association rate if apo-eIF4E is phosphorylated. Scheper et al., who deployed surface plasma resonance (SPR) to reveal that the phosphorylation of eIF4E accelerates dissociation of eIF4E from the cap, challenged these models.[153] They speculated that phosphorylation occurs after translation is initiated, which leads to the dissociation of eIF4E from the cap and enables it to engage in a subsequent round of translation initiation.[153] Using stopped-flow kinetic studies, Slepenkov et al., obtained an opposite result where the phosphorylation of eIF4E decreased the association rate but did not affect the dissociation rate of the eIF4E:cap complex.[45] These authors proposed that the phosphorylation of Ser-209 decreases the association of cap with eIF4E due to charge repulsion.[45] The latter model is further corroborated by the finding that the effect of eIF4E phosphorylation is diminished in high-salt buffer under conditions that shield the negative charge of phospho-Ser 209.[45]

The effects of the eIF4E phosphorylation on cap-dependent translation are also largely elusive. It has been reported that increased eIF4E phosphorylation results in increased rates of protein synthesis in studies where Mnk inhibitors[154,155] and growth factor stimulation were used in cell cultures.[156,157] In contrast, studies in which cells were recovering from hypotonic shock[158,159] or where Mnk levels were modulated[160] argued that eIF4E phosphorylation reduces or does not affect rates of global protein synthesis, respectively. Alternatively, it is plausible that the phosphorylation of eIF4E modulates the translation of only a subset of the 'eIF4E-sensitive' mRNAs. This possibility is corroborated by recent findings identifying anti-apoptotic protein Mcl-1 as a translational target of phosphorylated eIF4E.[122]

c-Myc

c-Myc is a proto-oncogene and a member of the Myc/Max/Mad family of transcription factors.[161] Myc is upregulated in response to growth factors and is essential for the proliferative response.[162] In addition to stimulating proliferation, overexpression of c-Myc results in apoptosis.[163–165] The first evidence that eIF4E and c-Myc act in the same biological pathway came from experiments where the apoptotic effect of c-Myc on cell survival was abolished when eIF4E and c-Myc were simultaneously overexpressed in mouse fibroblasts.[166] This study was followed by several reports which demonstrated that eIF4E is a bona fide transcriptional target of c-Myc.[167,168] c-Myc has also been shown to stimulate transcription of two other components of the eIF4F complex, eIF4G and eIF4A.[168] Thus, the effects of Myc on cell proliferation could at least in part be mediated via increased levels of the eIF4F complex. Indeed, high eIF4F levels are detected in a lymphoma model characterized by increased c-Myc activity.[168] Taken together, these data demonstrate that the levels of eIF4F are regulated by c-Myc. On the other hand, c-Myc mRNA is one of the first identified 'eIF4E-sensitve' mRNAs.[113,169] As mentioned before, translation of these mRNAs critically depends on the availability of the eIF4F complex.[2] Thus, increased levels of c-Myc elevate

levels of the eIF4F complex, which in turn upregulates translation of c-Myc mRNA. How do cells control this potentially oncogenic feed-forward loop? One possibility is that the stimulatory effect of Myc on the eIF4F complex activity is antagonized by cellular factors which impair its assembly and/or function, such as 4E-BPs[125] and eIF4A-inhibitory programmed cell death 4 protein (PDCD4).[170] Notably, both of these factors are regulated via mTORC1 pathway,[171] suggesting that switching off mTORC1 signaling could be a mechanism by which cells control the proto-oncogenic c-Myc-eIF4E feed-forward loop.[172]

ADDITIONAL MECHANISMS PROPOSED TO REGULATE THE ACTIVITY OF eIF4E

Several other eIF4E binding proteins were recently identified. These proteins include Maskin,[173] Cup,[174] and several homeobox proteins[175–177] which all contain an eIF4E-binding motif (notably, Maskin has a motif in which Tyr is substituted for Thr). 4E-BPs, Maskin and Cup prevent association of eIF4E with eIF4G.[178] However, in contrast to 4E-BPs, Maskin and Cup repress translation of specific mRNAs which is achieved through binding to the Maskin and Cup adapter proteins associated with specific elements present in the 3′ UTR of their target mRNAs.[178] In *Xenopus*, Maskin distinguishes its targets through an association with a cytoplasmic polyadenylation element (CPE)-binding protein that interacts with a CPE present in mRNAs such as cyclin B1.[173] In Drosphila, Cup inhibits translation of Bruno response element (BRE)-containing oskar mRNA via association with Bruno.[174,179] Similarly, in *Drosophila* it has been demonstrated that caudal mRNA is translationally repressed by a homeobox protein, Bicoid, which in this case associates with the cap-bound 4EHP.[180] 4E-HP is an eIF4E family member that does not bind to eIF4G and thus cannot engage the translational machinery.[181,182] In contrast to Maskin and Cup, Bicoid directly associates with caudal mRNA via Bicoid-binding region (BBR) present in its 3′ UTR.[183] Recently, it has been reported that Prep1 homeobox protein represses translation of 4EHP-bound HoxB4 mRNA, suggesting that a similar mechanism exists in mammalian cells.[184]

In addition to the aforementioned mechanisms of control of eIF4E, several other factors have been proposed to control transcription of the eIF4E gene (e.g., p53,[185] hnRNP K[186]). It has also been speculated that the post-translational modifications, other than phosphorylation (e.g., ubiquitination,[187] sumoylation[188]), are implicated in control of eIF4E's activity.

VIRUSES AND THE CAP-DEPENDENT REGULATION OF GENE EXPRESSION

Expression of viral genes requires the components of the translational machinery of the host. Viruses deploy different mechanisms to efficiently outcompete cellular mRNAs for the available translational machinery to enhance translation of their own mRNAs. A subset of viruses (e.g., picornaviruses) evolved a strategy to translate uncapped viral mRNAs via an internal ribosome entry mechanism.[189,190] The internal ribosome entry mechanism circumvents requirement for the cap structure, eIF4E and, depending on the virus type, additional initiation factors for translation initiation.[2,91] This is achieved via direct recruitment of ribosomal subunits through cis-acting internal ribosome entry site (IRES) elements present in the 5′ UTRs of viral RNAs.[189] Some viruses developed cap-independent mechanisms to translate their own RNAs by covalently linking a translational regulator protein to the 5′-end of their RNA. In caliciviruses and potiviruses a virus-encoded Viral Protein, genome-linked, (VPg) is covalently attached to the first nucleotide of the RNA and simultaneously interacts with eIF4E, therein ensuring efficient translation of viral mRNAs.[191,192]

In contrast to the latter cap-independent mechanisms, the cap structure is essential for the life cycle of a significant number of viruses. Thus, viruses had to develop mechanisms to adjust cap-dependent regulation of gene expression to their specific needs. Some viruses (e.g., retroviruses), utilize the capping enzymes of the host cell,[193] but the majority of viruses evolved their own, virus-specific, capping mechanism which acts independently of the cellular capping enzymes. For example, capping by Semliki Forest virus involves methylation of GTP followed by transfer of m^7GMP to viral mRNA 5′ termini.[194] Vaccinia virus utilizes cap-dependent 2′-O-ribose methyltransferases to distinguish between viral and host transcripts[41,195] (Figure 4(a)). Late gene of vaccinia virus, viral protein 39 (VP39) binds to the 5′ end of the viral mRNAs and catalyzes the transfer of a methyl group to the 2′ position of the ribose moiety of the first nucleotide of the transcript to form cap 1 ends.[41] Vesicular stomatitis virus (VSV) RNA-dependent RNA polymerase (viral L protein) possesses polyribonucleotidyltransferase activity and mediates capping of VSV transcripts[196] (Figure 4(b)). Intriguingly, in the VSV case, 2′-O-methylation apparently precedes guanine N7 methylation, which

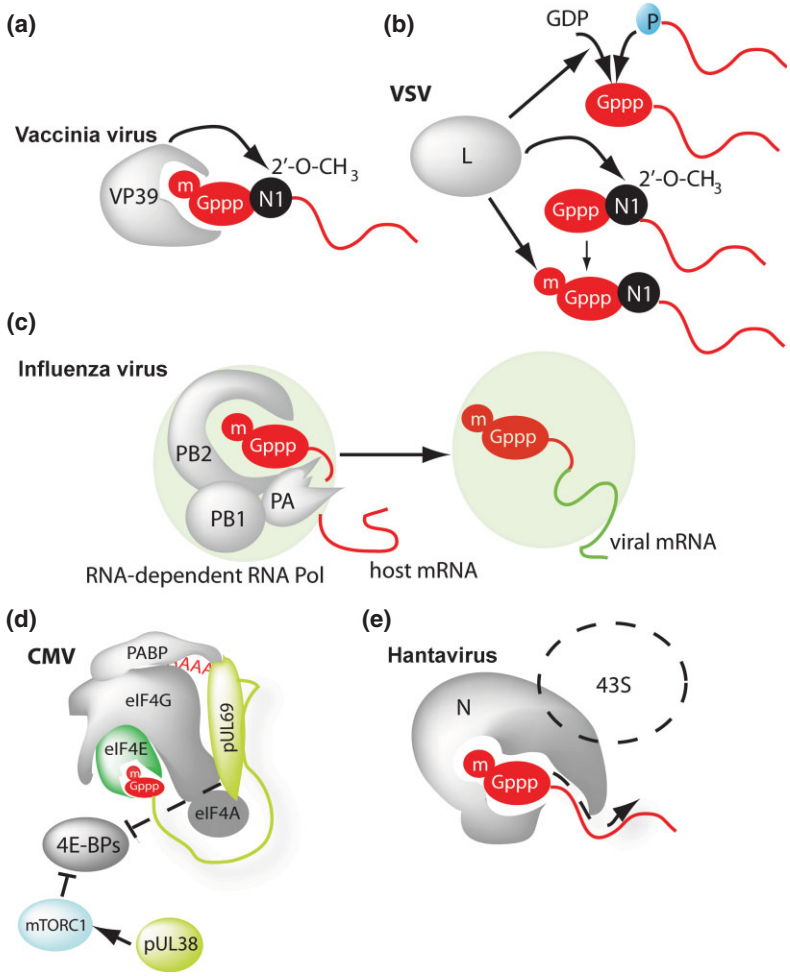


FIGURE 4 | Viruses developed mechanisms to utilize cap structure to ensure efficient expression of their genes. (a) Vaccinia virus viral protein 39 (VP39) binds the cap and functions as 2′-O-ribose methyltransferase, catalyzing the transfer of a methyl group to the first transcribed nucleotide (N1) of mRNA and converting cap 0 to cap 1. (b) RNA-dependent RNA polymerase of vesicular stomatitis virus (VSV), viral protein L, forms the cap by a mechanism distinct from cellular capping enzymes and VP39, adding GDP to monophosphate ends before 2′-O and then m7G methylation. (c) Flu virus RNA polymerase utilizes a 'cap-snatching' mechanism to prime synthesis of flu mRNA with host capped mRNA fragments. (d) Cytomegalovirus (CMV) developed strategies to overtake the host's translational machinery by stabilizing the eIF4F complex (via pUL69) and by activating the mTORC1 pathway (via pUL38). (e) N protein of hantavirus appears to act as a substitute for eIF4F activity in the cell. Abbreviations: eIF4E-binding proteins (4E-BPs); mammalian target of rapamycin complex 1 (mTORC1); PABP, poly(A) binding protein; eIF, eukaryotic translation initiation factor.

is opposite to capping reactions catalyzed by cellular enzymes and VP39, where N7 of guanine is methylated first[197,198] (Figure 4(b)). Also, L transfers GDP onto a 5′-monophosphate RNA, in contrast to the cellular capping enzymes which transfer GMP to a 5′-diphosphate RNA[197,198] (Figure 4(b)). Finally, negative-strand RNA viruses including influenza virus which do not possess their own capping enzymes, 'snatch' the cap structure from host cell mRNAs[199] (Figure 4(c)). Cap 'snatching' is mediated by the multifunctional RNA-dependent RNA polymerase binding to cellular capped pre-mRNAs, cleaving the capped oligonucleotides and subsequently utilizing them as primers to initiate viral transcription.[199] The flu RNA polymerase is composed of three subunits, PB1, PB2, and PA.[200] The PB2 subunit binds to the cap structure,[201] whereas the PA subunit mediates endonucleolytic cleavage[202] (Figure 4(c)). Albeit structurally distinct, PB2 subunit shares common features with other cap-binding proteins where similarly to eIF4E, CBP 20, and VP39, the m^7G base is stacked in a 'cation–π-sandwich'.[201] A slight difference in cap-stacking between PB2 and other cap-binding proteins (where the planes of cap intercalating aromatic residues are parallel to the plane of m^7G base) is that in type A influenza PB2, the m^7G base is stacked between Phe 404 which is tilted ~30° relative to the plane of the m^7G base and the side chain of His 357 serves as the second stacking group.[201]

In addition to developing unique capping strategies, viruses evolved a mechanism to regulate cap-dependent translation via modulation of eIF4F complex activity. Inhibition of host-cell protein synthesis by polioviruses, rhinoviruses and aphthoviruses is accompanied by the cleavage of eIF4G that effectively uncouples cap-binding and RNA helicase activities of eIF4F.[203] In contrast, in encephalomyocarditis virus (EMCV) infected cells eIF4G is not cleaved. In this case, the shut-off of host translation is believed to be triggered by the dephosphorylation and subsequent activation of 4E-BP1.[204] Under both scenarios the translation of the picornaviral mRNAs is not inhibited because it occurs via the cap-independent mechanism. That

eIF4F-mediated translational control plays an important role in the antiviral response is further underscored by the finding that 4E-BPs inhibit type-I interferon (IFN) production through suppression of IRF-7 mRNA translation.[139] Recently, it has also been revealed that cytomegalovirus pUL69 protein stimulates translation of viral mRNAs via direct interaction with the eIF4A component of the eIF4F complex and PABPC1[205] (Figure 4(d)). pUL69 appears to stabilize eIF4F complexes possibly by abrogating 4E-BP1 binding to eIF4E.[205] In addition to pUL69, another cytomegalovirus (CMV) protein, pUL38, activates mTORC1, leading to the phosphorylation and inactivation of 4E-BPs and further diminishing the ability to interfere with eIF4F complex assembly[206] (Figure 4(d)). In addition to eIF4A and PABC1 binding, pUL69 interacts with viral mRNAs,[207] suggesting that it could remodel the eIF4F complex to accommodate optimal translation of viral transcripts (Figure 4(d)). Importantly, ICP27, the herpes simplex virus (HSV) homolog of pUL69[208] that like pUL69 stimulates nuclear export and translation of mRNA,[209] has been shown to interact with PABPC1 and eIF4G.[210] This raises the possibility that this mechanism is not limited to CMV and may be shared by other herpes viruses. Nucleocapsid protein (N) of hantavirus plays an essential role in the viral genome replication and encapsidation[211] and has also been shown to act as a substitute for the cellular eIF4F complex[212] (Figure 4(e)). Namely, N binds to the cap structure and 43S pre-initiation complex and alleviates the requirement for unwinding of secondary structure present in RNA 5′ UTR, thereby mimicking the activity of eIF4E, eIF4G, and eIF4A, respectively.[212] The molecular underpinnings of this phenomenon are not completely clear, but it appears that N associates with capped mRNA primer immediately after it is 'snatched' from cellular mRNA and annealed to the 3′ end of viral RNA.[212] Binding to the cap induces conformational changes in N which stimulates its binding to a conserved 3′ stretch of nine nucleotides of viral RNA.[212] In addition to its role in translation of viral RNA, N stimulates bound capped primer annealing at the 3′ terminus of viral RNA and in conjunction with viral RNA dependent RNA polymerase ensures efficient transcription and replication of the viral genome.[213]

EMERGING MECHANISMS CONTROLLING GENE EXPRESSION VIA CAP STRUCTURE

In contrast to the enzymology of capping, structural aspects of cap binding by cap-binding proteins and the role of the cap in the post-transcriptional regulation of gene expression, little is currently known about the cellular mechanisms that control capping and whether cellular factors could have an impact on the fates of specific mRNA molecules. Thus, it is not clear whether capping plays a 'passive' role in regulating gene expression by preventing degradation of mRNAs and enabling cap-binding proteins to recruit cellular factors necessary for cap-dependent steps of mRNA metabolism, or if capping can be modulated to 'actively' regulate the fate of bulk or a selected subset of mRNAs. Recent studies provide pioneer evidence for the latter scenario as they identify the cellular factors that regulate cap methylation and suggest the presence of cellular cytoplasmic capping mechanisms.

REGULATION OF CAP METHYLATION

As mentioned above, methylation of the cap structure on N7 of the guanine base is essential for its recognition by cap-binding proteins and thus its function in the cell.[7] Emerging data suggest that cap methylation, which is catalyzed by RNMT, is actively regulated (Figure 5(a)). For instance, in yeast ubiquitin-conjugase cdc34 was demonstrated to interact with yeast RNMT, Abd1, and stimulate its activity. S-adenosylmethionine (SAM) serves as the methyl donor in the capping reaction.[214] Yeast SAM synthetase was shown to be a critical activator of Abd1.[214] Accordingly, in *Xenopus*, *Xenopus* S-adenosyl homocysteine hydrolase (xSAHH), the enzyme that hydrolyzes S-adenosyl homocysteine (SAH), was shown to bolster mRNA cap methylation.[215] SAH is a major by-product of cellular methylation reactions and potently inhibits methyltransferases.[216] Mammalian importin α was identified as an RNMT interacting partner in a yeast-two hybrid screen.[217] Moreover, importin α increases RNMT activity in vitro, which is inhibited by importin β.[217] It is well established that importin α/β complexes form in the cytoplasm and rapidly dissociate once they are imported to nucleus and exposed to high concentration of RanGTP.[218] Thus, importin α is positioned to selectively stimulate RNMT activity in the nucleus upon dissociation from importin β.

The latter findings shed light on the cellular mechanisms which could explain how cap methylation of bulk pre-mRNA is regulated. In contrast, a recent study suggested an intriguing link between Myc and E2F1 transcription factors and cap methylation wherein Myc and E2F1 were found to stimulate cap methylation mainly on their own transcriptional targets.[219] Surprisingly, at least in the case of c-Myc, this stimulatory effect on mRNA cap methylation

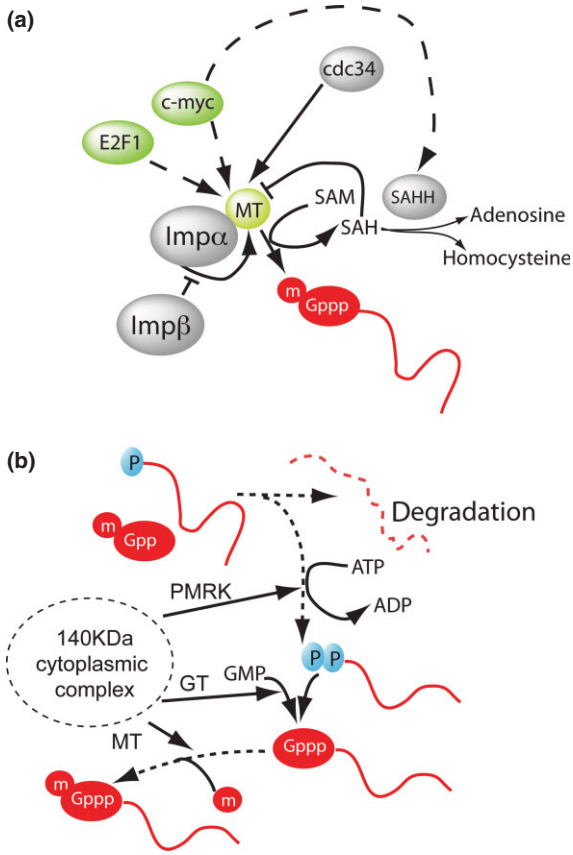


FIGURE 5 | Emerging mechanisms of the regulation of capping. (a) Efficiency of cap methylation is increased by stimulation of RNA (guanine-N7-) methyltransferase (MT) activity by ubiquitin-conjugase Cdc34 in yeast, *Xenopus* S-adenosyl homocysteine hydrolase (xSAHH) which eliminates the inhibitory by-product of cap methylation, S-adenosyl homocysteine (SAH), by interaction with importin α (Impα), which is inhibited by importin β (Impβ), and by c-myc and E2F1 transcription factors. It was suggested that c-myc stimulates transcription of SAHH. Abbreviations: SAM, S-adenosyl methionine. (b) Upon decapping, most mRNAs are rapidly degraded by 5′ → 3′ exonucleases. Several transcripts, including β globin mRNA from the erythroid cells of β thalassemia patients has been shown to produce stable degradation products. It is thought that these fragments are capped by 140 KDa cytoplasmic capping complex that exhibits polynucleotide 5′-monophosphate RNA kinase (PMRK), guanylyltransferase (GT) and RNA (guanine-N7-) methyltransferase (MT) activities.

appeared to be independent of its transcriptional activity.[220] However, the exact mechanism of how Myc and E2F1 stimulate cap methylation is yet to be discovered. Intriguingly, S-adenosyl homocysteine hydrolase (SAHH) was identified as a Myc target gene in mammals as the gene whose expression was upregulated by increased transcription and cap methylation.[221] In turn, the increased SAHH expression appeared to be necessary to permit Myc-induced cap methylation. These findings suggest the presence of a positive feedback loop, consisting of c-Myc and SAHH, which stimulates mRNA cap methylation.

IS DECAPPING THE END OF THE ROAD? CYTOPLASMIC RE-CAPPING

Much evidence suggests that the cap accompanies mRNA throughout its life cycle and that once the cap is removed by decapping enzymes, mRNA is rapidly degraded.[4,51] Removal of the cap is thus considered to be an irreversible early step in mRNA degradation.[222,223] However, stable transcripts lacking sequences from their 5′ ends have been described, suggesting that these mRNAs contain a cap or cap-like structure. For instance, degradation intermediates of PTC-containing β globin mRNAs isolated from the erythroid cells of β thalassemia patients are stable,[224,225] can be immunoprecipitated with an anti-m^{7}G cap antibody and exhibit sensitivity to TAP which hydrolyzes the 5′ -5′ triphosphate bridge of the cap structure.[226] Thus, the 5′ end modification of β globin mRNAs with truncated 5′ termini appears to be identical to the 5′ cap of the parental β globin transcripts and suggests that these fragments are re-capped. Interestingly, β globin mRNA fragments are generated in the cytoplasm by endonucleolytic cleavage,[227] which results in products that have a single phosphate group on the 5′ end. However, the 5′-monophosphate mRNA is not a suitable substrate for the cellular capping enzyme which transfers GMP to the 5′-diphosphate mRNA.[7] Furthermore, the capping enzyme is almost exclusively confined to the nucleus.[228] In a recent study, Schoenberg et al. set out to resolve the conundrum of how β globin mRNAs fragments are re-capped and identified a cytoplasmic activity that is identical to the nuclear-capping enzyme.[228] These authors isolated a putative 140 kDa cytoplasmic capping complex which also included a polynucleotide 5′-monophosphate RNA kinase activity which catalyzes conversion of 5′-monophosphate to 5′-diphosphate mRNA[228] (Figure 5(b)). Thus the enzymology of nuclear and cytoplasmic capping appear to differ in the first step wherein 5′-diphosphate mRNA is generated by removal of the 5′-γ-phosphate group from 5′-triphosphate RNA versus addition of 5′-β-phosphate to 5′-monophosphate RNA, respectively. Components of the cytoplasmic capping complex still await cloning and identification. Nonetheless, these findings suggest a hitherto unknown mechanism in which a subset of mRNAs could be stored in an uncapped, translationally inactive state and, when cells are stimulated, are swiftly capped by cytoplasmic capping complex and recruited

to the translational machinery. Shortening of the 5′ UTR of a given mRNA could increase its translational efficiency, which could in turn serve as a mechanism by which cells could regulate the levels of the protein encoded by such a mRNA.

CONCLUSION AND PERSPECTIVES

The m^7G cap structure and cap-binding proteins play a central role in mRNA, snRNA, and viral RNA metabolism. In this review we highlighted the known aspects of the role of the m^7G cap structure of mRNA and cap-binding proteins in the regulation of gene expression. However, there is an emerging body of data suggesting that the impact of cap-dependent mechanisms on gene expression might be much broader than what is considered today. Namely, recent studies indicated that m^7G cap could play transcript-, tissue- and/or species-specific roles and that proteins other than eIF4E family members and CBC act as cap-binding proteins (e.g., Dcp2 which is a catalytic subunit of decapping enzyme[4,229]; Snuportin1 which recognizes trimethylated caps of snRNAs[74] and poly(A)-specific ribonuclease (PARN) which deadenylates mRNA and utilizes a seemingly unique mechanism to bind the cap in which the m^7G base is not sandwiched between two aromatic residues but rather stacks on a single tryptophan[230,231]). Cap or cap-like structures were also detected on a subset of noncoding RNAs, which are emerging as major regulators of gene expression.[232] A plethora of new studies suggest that cap-binding proteins regulate multiple steps of mRNA metabolism; taken together with the finding that c-Myc regulates both N7-methylation of the cap and the levels of eIF4F complex, these data suggest that these factors could modulate gene expression through orchestration and coordination of different cap-dependent processes. Finally, it is important to stress that the overexpression of eIF4E and, as recently shown, RNMT[233] leads to the malignant transformation of the cell and metastasis and correlates with poor prognosis of cancer patients.[91,113,115,124] Targeting cap-dependent gene expression could serve as a valid strategy to curb dysregulated gene expression in cancer. Indeed, today several therapeutic approaches, including the mTOR inhibitors,[234] eIF4E antisense–oligonucleotides,[235] and ribavirin,[236] which directly or indirectly aim at cap-dependent gene expression, are in clinical use or in clinical trials.

REFERENCES

1. Furuichi Y, Shatkin AJ. Viral and cellular mRNA capping: past and prospects. *Adv Virus Res* 2000, 55:135–184.
2. Sonenberg N, Hinnebusch AG. Regulation of translation initiation in eukaryotes: mechanisms and biological targets. *Cell* 2009, 136:731–745.
3. Shuman S. What messenger RNA capping tells us about eukaryotic evolution. *Nat Rev Mol Cell Biol* 2002, 3:619–625.
4. Gu M, Lima CD. Processing the message: structural insights into capping and decapping mRNA. *Curr Opin Struct Biol* 2005, 15:99–106.
5. Lewis JD, Izaurralde E. The role of the cap structure in RNA processing and nuclear export. *Eur J Biochem* 1997, 247:461–469.
6. Shatkin AJ. Capping of eucaryotic mRNAs. *Cell* 1976, 9(suppl 4 Pt 2):645–653.
7. Shatkin AJ, Manley JL. The ends of the affair: capping and polyadenylation. *Nat Struct Biol* 2000, 7:838–842.
8. Mao X, Schwer B, Shuman S. Yeast mRNA cap methyltransferase is a 50-kilodalton protein encoded by an essential gene. *Mol Cell Biol* 1995, 15:4167–4174.
9. Shibagaki Y, Itoh N, Yamada H, Nagata S, Mizumoto K. mRNA capping enzyme. Isolation and characterization of the gene encoding mRNA guanylytransferase subunit from Saccharomyces cerevisiae. *J Biol Chem* 1992, 267:9521–9528.
10. Tsukamoto T, Shibagaki Y, Imajoh-Ohmi S, Murakoshi T, Suzuki M, Nakamura A, Gotoh H, Mizumoto K. Isolation and characterization of the yeast mRNA capping enzyme beta subunit gene encoding RNA 5′-triphosphatase, which is essential for cell viability. *Biochem Biophys Res Commun* 1997, 239:116–122.
11. Tsukamoto T, Shibagaki Y, Niikura Y, Mizumoto K. Cloning and characterization of three human cDNAs encoding mRNA (guanine-7-)-methyltransferase, an mRNA cap methylase. *Biochem Biophys Res Commun* 1998, 251:27–34.
12. Yamada-Okabe T, Doi R, Shimmi O, Arisawa M, Yamada-Okabe H. Isolation and characterization of a human cDNA for mRNA 5′-capping enzyme. *Nucleic Acids Res* 1998, 26:1700–1706.
13. Yue Z, Maldonado E, Pillutla R, Cho H, Reinberg D, Shatkin AJ. Mammalian capping enzyme complements mutant Saccharomyces cerevisiae lacking mRNA

guanylyltransferase and selectively binds the elongating form of RNA polymerase II. *Proc Natl Acad Sci USA* 1997, 94:12898–12903.

14. Ho CK, Sriskanda V, McCracken S, Bentley D, Schwer B, Shuman S. The guanylyltransferase domain of mammalian mRNA capping enzyme binds to the phosphorylated carboxyl-terminal domain of RNA polymerase II. *J Biol Chem* 1998, 273:9577–9585.
15. McCracken S, Fong N, Rosonina E, Yankulov K, Brothers G, Siderovski D, Hessel A, Foster S, Shuman S, Bentley DL. 5′-Capping enzymes are targeted to pre-mRNA by binding to the phosphorylated carboxy-terminal domain of RNA polymerase II. *Genes Dev* 1997, 11:3306–3318.
16. Ho CK, Shuman S. Distinct roles for CTD Ser-2 and Ser-5 phosphorylation in the recruitment and allosteric activation of mammalian mRNA capping enzyme. *Mol Cell* 1999, 3:405–411.
17. Phatnani HP, Greenleaf AL. Phosphorylation and functions of the RNA polymerase II CTD. *Genes Dev* 2006, 20:2922–2936.
18. Komarnitsky P, Cho EJ, Buratowski S. Different phosphorylated forms of RNA polymerase II and associated mRNA processing factors during transcription. *Genes Dev* 2000, 14:2452–2460.
19. Schroeder SC, Schwer B, Shuman S, Bentley D. Dynamic association of capping enzymes with transcribing RNA polymerase II. *Genes Dev* 2000, 14:2435–2440.
20. Serizawa H, Makela TP, Conaway JW, Conaway RC, Weinberg RA, Young RA. Association of Cdk-activating kinase subunits with transcription factor TFIIH. *Nature* 1995, 374:280–282.
21. Wen Y, Shatkin AJ. Transcription elongation factor hSPT5 stimulates mRNA capping. *Genes Dev* 1999, 13:1774–1779.
22. Marcotrigiano J, Gingras AC, Sonenberg N, Burley SK. Cocrystal structure of the messenger RNA 5′ cap-binding protein (eIF4E) bound to 7-methyl-GDP. *Cell* 1997, 89:951–961.
23. Matsuo H, Li H, McGuire AM, Fletcher CM, Gingras AC, Sonenberg N, Wagner G. Structure of translation factor eIF4E bound to m7GDP and interaction with 4E-binding protein. *Nat Struct Biol* 1997, 4:717–724.
24. Mazza C, Ohno M, Segref A, Mattaj IW, Cusack S. Crystal structure of the human nuclear cap binding complex. *Mol Cell* 2001, 8:383–396.
25. Mazza C, Segref A, Mattaj IW, Cusack S. Large-scale induced fit recognition of an m(7)GpppG cap analogue by the human nuclear cap-binding complex. *EMBO J* 2002, 21:5548–5557.
26. Calero G, Wilson KF, Ly T, Rios-Steiner JL, Clardy JC, Cerione RA. Structural basis of m7GpppG binding to the nuclear cap-binding protein complex. *Nat Struct Biol* 2002, 9:912–917.
27. Tomoo K, Shen X, Okabe K, Nozoe Y, Fukuhara S, Morino S, Sasaki M, Taniguchi T, Miyagawa H, Kitamura K, et al. Structural features of human initiation factor 4E, studied by X-ray crystal analyses and molecular dynamics simulations. *J Mol Biol* 2003, 328:365–383.
28. Niedzwiecka A, Marcotrigiano J, Stepinski J, Jankowska-Anyszka M, Wyslouch-Cieszynska A, Dadlez M, Gingras AC, Mak P, Darzynkiewicz E, Sonenberg N, et al. Biophysical studies of eIF4E cap-binding protein: recognition of mRNA 5′ cap structure and synthetic fragments of eIF4G and 4E-BP1 proteins. *J Mol Biol* 2002, 319:615–635.
29. Izaurralde E, Stepinski J, Darzynkiewicz E, Mattaj IW. A cap binding protein that may mediate nuclear export of RNA polymerase II-transcribed RNAs. *J Cell Biol* 1992, 118:1287–1295.
30. Morino S, Tomoo K, Nishi N, Okabe K, Doi M, Ishida T, Kitamura K. Crystallization and preliminary X-ray diffraction study of recombinant human eukaryotic initiation factor-4E. *J Biochem* 1996, 119:224–225.
31. Fechter P, Brownlee GG. Recognition of mRNA cap structures by viral and cellular proteins. *J Gen Virol* 2005, 86(Pt 5):1239–1249.
32. Tomoo K, Shen X, Okabe K, Nozoe Y, Fukuhara S, Morino S, Ishida T, Taniguchi T, Hasegawa H, Terashima A, et al. Crystal structures of 7-methylguanosine 5′-triphosphate (m(7)GTP)- and P(1)-7-methylguanosine-P(3)-adenosine-5′, 5′-triphosphate (m(7)GpppA)-bound human full-length eukaryotic initiation factor 4E: biological importance of the C-terminal flexible region. *Biochem J* 2002, 362(Pt 3):539–544.
33. Hodel AE, Gershon PD, Shi X, Wang SM, Quiocho FA. Specific protein recognition of an mRNA cap through its alkylated base. *Nat Struct Biol* 1997, 4:350–354.
34. Hu G, Gershon PD, Hodel AE, Quiocho FA. mRNA cap recognition: dominant role of enhanced stacking interactions between methylated bases and protein aromatic side chains. *Proc Natl Acad Sci USA* 1999, 96:7149–7154.
35. Hu G, Oguro A, Li C, Gershon PD, Quiocho FA. The "cap-binding slot" of an mRNA cap-binding protein: quantitative effects of aromatic side chain choice in the double-stacking sandwich with cap. *Biochemistry* 2002, 41:7677–7687.
36. Hu G, Tsai AL, Quiocho FA. Insertion of an N7-methylguanine mRNA cap between two coplanar aromatic residues of a cap-binding protein is fast and selective for a positively charged cap. *J Biol Chem* 2003, 278:51515–51520.
37. Quiocho FA, Hu G, Gershon PD. Structural basis of mRNA cap recognition by proteins. *Curr Opin Struct Biol* 2000, 10:78–86.

38. Shuman S, Lima CD. The polynucleotide ligase and RNA capping enzyme superfamily of covalent nucleotidyltransferases. *Curr Opin Struct Biol* 2004, 14:757–764.
39. Wilson KF, Fortes P, Singh US, Ohno M, Mattaj IW, Cerione RA. The nuclear cap-binding complex is a novel target of growth factor receptor-coupled signal transduction. *J Biol Chem* 1999, 274:4166–4173.
40. Carberry SE, Rhoads RE, Goss DJ. A spectroscopic study of the binding of m7GTP and m7GpppG to human protein synthesis initiation factor 4E. *Biochemistry* 1989, 28:8078–8083.
41. Lockless SW, Cheng HT, Hodel AE, Quiocho FA, Gershon PD. Recognition of capped RNA substrates by VP39, the vaccinia virus-encoded mRNA cap-specific 2′-O-methyltransferase. *Biochemistry* 1998, 37:8564–8574.
42. Goss DJ, Carberry SE, Dever TE, Merrick WC, Rhoads RE. Fluorescence study of the binding of m7GpppG and rabbit globin mRNA to protein synthesis initiation factors 4A, 4E, and 4F. *Biochemistry* 1990, 29:5008–5012.
43. Blachut-Okrasinska E, Bojarska E, Niedzwiecka A, Chlebicka L, Darzynkiewicz E, Stolarski R, St Pinski J, Antosiewicz JM. Stopped-flow and Brownian dynamics studies of electrostatic effects in the kinetics of binding of 7-methyl-GpppG to the protein eIF4E. *Eur Biophys J* 2000, 29:487–498.
44. Sha M, Wang Y, Xiang T, van Heerden A, Browning KS, Goss DJ. Interaction of wheat germ protein synthesis initiation factor eIF-(iso)4F and its subunits p28 and p86 with m7GTP and mRNA analogues. *J Biol Chem* 1995, 270:29904–29909.
45. Slepenkov SV, Darzynkiewicz E, Rhoads RE. Stopped-flow kinetic analysis of eIF4E and phosphorylated eIF4E binding to cap analogs and capped oligoribonucleotides: evidence for a one-step binding mechanism. *J Biol Chem* 2006, 281:14927–14938.
46. Lejeune F, Ishigaki Y, Li X, Maquat LE. The exon junction complex is detected on CBP80-bound but not eIF4E-bound mRNA in mammalian cells: dynamics of mRNP remodeling. *EMBO J* 2002, 21:3536–3545.
47. Stutz F, Bachi A, Doerks T, Braun IC, Seraphin B, Wilm M, Bork P, Izaurralde E. REF, an evolutionary conserved family of hnRNP-like proteins, interacts with TAP/Mex67p and participates in mRNA nuclear export. *RNA* 2000, 6:638–650.
48. Zhou Z, Luo MJ, Straesser K, Katahira J, Hurt E, Reed R. The protein Aly links pre-messenger-RNA splicing to nuclear export in metazoans. *Nature* 2000, 407:401–405.
49. Cheng H, Dufu K, Lee CS, Hsu JL, Dias A, Reed R. Human mRNA export machinery recruited to the 5′ end of mRNA. *Cell* 2006, 127:1389–1400.
50. Aguilera A. Cotranscriptional mRNP assembly: from the DNA to the nuclear pore. *Curr Opin Cell Biol* 2005, 17:242–250.
51. Moore MJ. From birth to death: the complex lives of eukaryotic mRNAs. *Science* 2005, 309:1514–1518.
52. Flaherty SM, Fortes P, Izaurralde E, Mattaj IW, Gilmartin GM. Participation of the nuclear cap binding complex in pre-mRNA 3′ processing. *Proc Natl Acad Sci USA* 1997, 94:11893–11898.
53. Maquat LE. Nonsense-mediated mRNA decay in mammals. *J Cell Sci* 2005, 118(Pt 9):1773–1776.
54. Edery I, Sonenberg N. Cap-dependent RNA splicing in a HeLa nuclear extract. *Proc Natl Acad Sci USA* 1985, 82:7590–7594.
55. Konarska MM, Padgett RA, Sharp PA. Recognition of cap structure in splicing in vitro of mRNA precursors. *Cell* 1984, 38:731–736.
56. Inoue K, Ohno M, Sakamoto H, Shimura Y. Effect of the cap structure on pre-mRNA splicing in *Xenopus* oocyte nuclei. *Genes Dev* 1989, 3:1472–1479.
57. Lewis JD, Izaurralde E, Jarmolowski A, McGuigan C, Mattaj IW. A nuclear cap-binding complex facilitates association of U1 snRNP with the cap-proximal 5′ splice site. *Genes Dev* 1996, 10:1683–1698.
58. Izaurralde E, Lewis J, Gamberi C, Jarmolowski A, McGuigan C, Mattaj IW. A cap-binding protein complex mediating U snRNA export. *Nature* 1995, 376:709–712.
59. Izaurralde E, Lewis J, McGuigan C, Jankowska M, Darzynkiewicz E, Mattaj IW. A nuclear cap binding protein complex involved in pre-mRNA splicing. *Cell* 1994, 78:657–668.
60. Berget SM. Exon recognition in vertebrate splicing. *J Biol Chem* 1995, 270:2411–2414.
61. Strasser K, Masuda S, Mason P, Pfannstiel J, Oppizzi M, Rodriguez-Navarro S, Rondon AG, Aguilera A, Struhl K, Reed R, et al. TREX is a conserved complex coupling transcription with messenger RNA export. *Nature* 2002, 417:304–308.
62. Masuda S, Das R, Cheng H, Hurt E, Dorman N, Reed R. Recruitment of the human TREX complex to mRNA during splicing. *Genes Dev* 2005, 19:1512–1517.
63. Reed R, Cheng H. TREX, SR proteins and export of mRNA. *Curr Opin Cell Biol* 2005, 17:269–273.
64. Chavez S, Beilharz T, Rondon AG, Erdjument-Bromage H, Tempst P, Svejstrup JQ, Lithgow T, Aguilera A. A protein complex containing Tho2, Hpr1, Mft1 and a novel protein, Thp2, connects transcription elongation with mitotic recombination in Saccharomyces cerevisiae. *EMBO J* 2000, 19:5824–5834.
65. Rehwinkel J, Herold A, Gari K, Kocher T, Rode M, Ciccarelli FL, Wilm M, Izaurralde E. Genome-wide analysis of mRNAs regulated by the THO complex in

Drosophila melanogaster. *Nat Struct Mol Biol* 2004, 11:558–566.

66. Masuyama K, Taniguchi I, Kataoka N, Ohno M. RNA length defines RNA export pathway. *Genes Dev* 2004, 18:2074–2085.
67. Daneholt B. Packing and delivery of a genetic message. *Chromosoma* 2001, 110:173–185.
68. Huang Y, Steitz JA. SRprises along a messenger's journey. *Mol Cell* 2005, 17:613–615.
69. Brennan CM, Gallouzi IE, Steitz JA. Protein ligands to HuR modulate its interaction with target mRNAs in vivo. *J Cell Biol* 2000, 151:1–14.
70. Gallouzi IE, Steitz JA. Delineation of mRNA export pathways by the use of cell-permeable peptides. *Science* 2001, 294:1895–1901.
71. Topisirovic I, Siddiqui N, Lapointe VL, Trost M, Thibault P, Bangeranye C, Pinol-Roma S, Borden KL. Molecular dissection of the eukaryotic initiation factor 4E (eIF4E) export-competent RNP. *EMBO J* 2009, 28:1087–1098.
72. Hamm J, Mattaj IW. Monomethylated cap structures facilitate RNA export from the nucleus. *Cell* 1990, 63:109–118.
73. Yong J, Wan L, Dreyfuss G. Why do cells need an assembly machine for RNA-protein complexes? *Trends Cell Biol* 2004, 14:226–232.
74. Huber J, Cronshagen U, Kadokura M, Marshallsay C, Wada T, Sekine M, Luhrmann R. Snurportin1, an m3G-cap-specific nuclear import receptor with a novel domain structure. *EMBO J* 1998, 17:4114–4126.
75. Ohno M, Segref A, Bachi A, Wilm M, Mattaj IW. PHAX, a mediator of U snRNA nuclear export whose activity is regulated by phosphorylation. *Cell* 2000, 101:187–198.
76. Gorlich D, Kraft R, Kostka S, Vogel F, Hartmann E, Laskey RA, Mattaj IW, Izaurralde E. Importin provides a link between nuclear protein import and U snRNA export. *Cell* 1996, 87:21–32.
77. Jackobson A. EI: Nonsense-mediated mRNA decay: from yeast to metazoan. In: Sonenberg N, Mathews M, Hershey J, eds. *Nonsense-mediated mRNA Decay: From Yeast to Metazoan*. 3rd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press; 2007.
78. McGlincy NJ, Smith CW. Alternative splicing resulting in nonsense-mediated mRNA decay: what is the meaning of nonsense? *Trends Biochem Sci* 2008, 33:385–393.
79. Giorgi C, Yeo GW, Stone ME, Katz DB, Burge C, Turrigiano G, Moore MJ. The EJC factor eIF4AIII modulates synaptic strength and neuronal protein expression. *Cell* 2007, 130:179–191.
80. Stalder L, Muhlemann O. The meaning of nonsense. *Trends Cell Biol* 2008, 18:315–321.
81. Nagy E, Maquat LE. A rule for termination-codon position within intron-containing genes: when nonsense affects RNA abundance. *Trends Biochem Sci* 1998, 23:198–199.
82. Reichert VL, Le Hir H, Jurica MS, Moore MJ. 5′ exon interactions within the human spliceosome establish a framework for exon junction complex structure and assembly. *Genes Dev* 2002, 16:2778–2791.
83. Shibuya T, Tange TO, Sonenberg N, Moore MJ. eIF4AIII binds spliced mRNA in the exon junction complex and is essential for nonsense-mediated decay. *Nat Struct Mol Biol* 2004, 11:346–351.
84. Jankowsky E, Fairman ME. RNA helicases—one fold for many functions. *Curr Opin Struct Biol* 2007, 17:316–324.
85. Ballut L, Marchadier B, Baguet A, Tomasetto C, Seraphin B, Le Hir H. The exon junction core complex is locked onto RNA by inhibition of eIF4AIII ATPase activity. *Nat Struct Mol Biol* 2005, 12:861–869.
86. Kashima I, Yamashita A, Izumi N, Kataoka N, Morishita R, Hoshino S, Ohno M, Dreyfuss G, Ohno S. Binding of a novel SMG-1-Upf1-eRF1-eRF3 complex (SURF) to the exon junction complex triggers Upf1 phosphorylation and nonsense-mediated mRNA decay. *Genes Dev* 2006, 20:355–367.
87. Isken O, Kim YK, Hosoda N, Mayeur GL, Hershey JW, Maquat LE. Upf1 phosphorylation triggers translational repression during nonsense-mediated mRNA decay. *Cell* 2008, 133:314–327.
88. Eberle AB, Lykke-Andersen S, Muhlemann O, Jensen TH. SMG6 promotes endonucleolytic cleavage of nonsense mRNA in human cells. *Nat Struct Mol Biol* 2009, 16:49–55.
89. Huntzinger E, Kashima I, Fauser M, Sauliere J, Izaurralde E. SMG6 is the catalytic endonuclease that cleaves mRNAs containing nonsense codons in metazoan. *RNA* 2008, 14:2609–2617.
90. Kim YK, Furic L, Desgroseillers L, Maquat LE. Mammalian Staufen1 recruits Upf1 to specific mRNA 3′UTRs so as to elicit mRNA decay. *Cell* 2005, 120:195–208.
91. Jackson RJ, Hellen CU, Pestova TV. The mechanism of eukaryotic translation initiation and principles of its regulation. *Nat Rev Mol Cell Biol* 2010, 11:113–127.
92. Grifo JA, Tahara SM, Morgan MA, Shatkin AJ, Merrick WC. New initiation factor activity required for globin mRNA translation. *J Biol Chem* 1983, 258:5804–5810.
93. Browning KS, Lax SR, Humphreys J, Ravel JM, Jobling SA, Gehrke L. Evidence that the 5′-untranslated leader of mRNA affects the requirement for wheat germ initiation factors 4A, 4F, and 4G. *J Biol Chem* 1988, 263:9630–9634.
94. Pestova TV, Lorsch JR, Hellen CUT, eds. The mechanism of translation initiation in eukaryotes. *Translational Control in Biology and Medicine*. Cold Spring

Harbor, NY: Cold Spring Harbor Laboratory Press; 2007.

95. Kahvejian A, Svitkin YV, Sukarieh R, M'Boutchou MN, Sonenberg N. Mammalian poly(A)-binding protein is a eukaryotic translation initiation factor, which acts via multiple mechanisms. *Genes Dev* 2005, 19:104–113.

96. Svitkin YV, Evdokimova VM, Brasey A, Pestova TV, Fantus D, Yanagiya A, Imataka H, Skabkin MA, Ovchinnikov LP, Merrick WC, et al. General RNA-binding proteins have a function in poly(A)-binding protein-dependent translation. *EMBO J* 2009, 28:58–68.

97. Kozak M, Shatkin AJ. Migration of 40 S ribosomal subunits on messenger RNA in the presence of edeine. *J Biol Chem* 1978, 253:6568–6577.

98. Grifo JA, Abramson RD, Satler CA, Merrick WC. RNA-stimulated ATPase activity of eukaryotic initiation factors. *J Biol Chem* 1984, 259:8648–8654.

99. Pause A, Methot N, Svitkin Y, Merrick WC, Sonenberg N. Dominant negative mutants of mammalian translation initiation factor eIF-4A define a critical role for eIF-4F in cap-dependent and cap-independent initiation of translation. *EMBO J* 1994, 13:1205–1215.

100. Richter-Cook NJ, Dever TE, Hensold JO, Merrick WC. Purification and characterization of a new eukaryotic protein translation factor. Eukaryotic initiation factor 4H. *J Biol Chem* 1998, 273:7579–7587.

101. Rogers GW Jr, Richter NJ, Lima WF, Merrick WC. Modulation of the helicase activity of eIF4A by eIF4B, eIF4H, and eIF4F. *J Biol Chem* 2001, 276:30914–30922.

102. Rozen F, Edery I, Meerovitch K, Dever TE, Merrick WC, Sonenberg N. Bidirectional RNA helicase activity of eucaryotic translation initiation factors 4A and 4F. *Mol Cell Biol* 1990, 10:1134–1144.

103. Schutz P, Bumann M, Oberholzer AE, Bieniossek C, Trachsel H, Altmann M, Baumann U. Crystal structure of the yeast eIF4A-eIF4G complex: an RNA-helicase controlled by protein-protein interactions. *Proc Natl Acad Sci USA* 2008, 105:9564–9569.

104. Marintchev A, Edmonds KA, Marintcheva B, Hendrickson E, Oberer M, Suzuki C, Herdy B, Sonenberg N, Wagner G. Topology and regulation of the human eIF4A/4G/4H helicase complex in translation initiation. *Cell* 2009, 136:447–460.

105. Shin BS, Kim JR, Acker MG, Maher KN, Lorsch JR, Dever TE. rRNA suppressor of a eukaryotic translation initiation factor 5B/initiation factor 2 mutant reveals a binding site for translational GTPases on the small ribosomal subunit. *Mol Cell Biol* 2009, 29:808–821.

106. Fringer JM, Acker MG, Fekete CA, Lorsch JR, Dever TE. Coupled release of eukaryotic translation initiation factors 5B and 1A from 80S ribosomes following subunit joining. *Mol Cell Biol* 2007, 27:2384–2397.

107. Jennings MD, Pavitt GD. eIF5 has GDI activity necessary for translational control by eIF2 phosphorylation. *Nature* 2010, 465:378–381.

108. Unbehaun A, Borukhov SI, Hellen CU, Pestova TV. Release of initiation factors from 48S complexes during ribosomal subunit joining and the link between establishment of codon-anticodon base-pairing and hydrolysis of eIF2-bound GTP. *Genes Dev* 2004, 18:3078–3093.

109. Pestova TV, Lomakin IB, Lee JH, Choi SK, Dever TE, Hellen CU. The joining of ribosomal subunits in eukaryotes requires eIF5B. *Nature* 2000, 403:332–335.

110. Lomakin IB, Kolupaeva VG, Marintchev A, Wagner G, Pestova TV. Position of eukaryotic initiation factor eIF1 on the 40S ribosomal subunit determined by directed hydroxyl radical probing. *Genes Dev* 2003, 17:2786–2797.

111. Maag D, Algire MA, Lorsch JR. Communication between eukaryotic translation initiation factors 5 and 1A within the ribosomal pre-initiation complex plays a role in start site selection. *J Mol Biol* 2006, 356:724–737.

112. Maag D, Fekete CA, Gryczynski Z, Lorsch JR. A conformational change in the eukaryotic translation preinitiation complex and release of eIF1 signal recognition of the start codon. *Mol Cell* 2005, 17:265–275.

113. De Benedetti A, Graff JR. eIF-4E expression and its role in malignancies and metastases. *Oncogene* 2004, 23:3189–3199.

114. Sonenberg N, Gingras AC. The mRNA 5′ cap-binding protein eIF4E and control of cell growth. *Curr Opin Cell Biol* 1998, 10:268–275.

115. Graff JR, Konicek BW, Carter JH, Marcusson EG. Targeting the eukaryotic translation initiation factor 4E for cancer therapy. *Cancer Res* 2008, 68:631–634.

116. Silvera D, Formenti SC, Schneider RJ. Translational control in cancer. *Nat Rev Cancer* 2010, 10:254–266.

117. Svitkin YV, Pause A, Haghighat A, Pyronnet S, Witherell G, Belsham GJ, Sonenberg N. The requirement for eukaryotic initiation factor 4A (eIF4A) in translation is in direct proportion to the degree of mRNA 5′ secondary structure. *RNA* 2001, 7:382–394.

118. Koromilas AE, Lazaris-Karatzas A, Sonenberg N. mRNAs containing extensive secondary structure in their 5′ non-coding region translate efficiently in cells overexpressing initiation factor eIF-4E. *EMBO J* 1992, 11:4153–4158.

119. Lazaris-Karatzas A, Montine KS, Sonenberg N. Malignant transformation by a eukaryotic initiation factor

subunit that binds to mRNA 5′ cap. *Nature* 1990, 345:544–547.

120. Ruggero D, Montanaro L, Ma L, Xu W, Londei P, Cordon-Cardo C, Pandolfi PP. The translation factor eIF-4E promotes tumor formation and cooperates with c-Myc in lymphomagenesis. *Nat Med* 2004, 10:484–486.
121. Cohen N, Sharma M, Kentsis A, Perez JM, Strudwick S, Borden KL. PML RING suppresses oncogenic transformation by reducing the affinity of eIF4E for mRNA. *EMBO J* 2001, 20:4547–4559.
122. Wendel HG, Silva RL, Malina A, Mills JR, Zhu H, Ueda T, Watanabe-Fukunaga R, Fukunaga R, Teruya-Feldstein J, Pelletier J, et al. Dissecting eIF4E action in tumorigenesis. *Genes Dev* 2007, 21:3232–3237.
123. Mamane Y, Petroulakis E, Rong L, Yoshida K, Ler LW, Sonenberg N. eIF4E—from translation to transformation. *Oncogene* 2004, 23:3172–3179.
124. De Benedetti A, Harris AL. eIF4E expression in tumors: its possible role in progression of malignancies. *Int J Biochem Cell Biol* 1999, 31:59–72.
125. Pause A, Belsham GJ, Gingras AC, Donze O, Lin TA, Lawrence JC Jr, Sonenberg N. Insulin-dependent stimulation of protein synthesis by phosphorylation of a regulator of 5′-cap function. *Nature* 1994, 371:762–767.
126. Mader S, Lee H, Pause A, Sonenberg N. The translation initiation factor eIF-4E binds to a common motif shared by the translation factor eIF-4 gamma and the translational repressors 4E-binding proteins. *Mol Cell Biol* 1995, 15:4990–4997.
127. Marcotrigiano J, Gingras AC, Sonenberg N, Burley SK. Cap-dependent translation initiation in eukaryotes is regulated by a molecular mimic of eIF4G. *Mol Cell* 1999, 3:707–716.
128. Gross JD, Moerke NJ, von der Haar T, Lugovskoy AA, Sachs AB, McCarthy JE, Wagner G. Ribosome loading onto the mRNA cap is driven by conformational coupling between eIF4G and eIF4E. *Cell* 2003, 115:739–750.
129. Volpon L, Osborne MJ, Topisirovic I, Siddiqui N, Borden KL. Cap-free structure of eIF4E suggests a basis for conformational regulation by its ligands. *EMBO J* 2006, 25:5138–5149.
130. Brown CJ, Verma CS, Walkinshaw MD, Lane DP. Crystallization of eIF4E complexed with eIF4GI peptide and glycerol reveals distinct structural differences around the cap-binding site. *Cell Cycle* 2009, 8:1905–1911.
131. Yanagiya A, Svitkin YV, Shibata S, Mikami S, Imataka H, Sonenberg N. Requirement of RNA binding of mammalian eukaryotic translation initiation factor 4GI (eIF4GI) for efficient interaction of eIF4E with the mRNA cap. *Mol Cell Biol* 2009, 29:1661–1669.
132. Hay N, Sonenberg N. Upstream and downstream of mTOR. *Genes Dev* 2004, 18:1926–1945.
133. Guertin DA, Sabatini DM. Defining the role of mTOR in cancer. *Cancer Cells* 2007, 12:9–22.
134. Guertin DA, Sabatini DM. The pharmacology of mTOR inhibition. *Sci Signal* 2009, 2:pe24.
135. Wullschleger S, Loewith R, Hall MN. TOR signaling in growth and metabolism. *Cell* 2006, 124:471–484.
136. Gingras AC, Gygi SP, Raught B, Polakiewicz RD, Abraham RT, Hoekstra MF, Aebersold R, Sonenberg N. Regulation of 4E-BP1 phosphorylation: a novel two-step mechanism. *Genes Dev* 1999, 13:1422–1437.
137. Gingras AC, Raught B, Gygi SP, Niedzwiecka A, Miron M, Burley SK, Polakiewicz RD, Wyslouch-Cieszynska A, Aebersold R, Sonenberg N. Hierarchical phosphorylation of the translation inhibitor 4E-BP1. *Genes Dev* 2001, 15:2852–2864.
138. Lynch M, Fitzgerald C, Johnston KA, Wang S, Schmidt EV. Activated eIF4E-binding protein slows G1 progression and blocks transformation by c-myc without inhibiting cell growth. *J Biol Chem* 2004, 279:3327–3339.
139. Colina R, Costa-Mattioli M, Dowling RJ, Jaramillo M, Tai LH, Breitbach CJ, Martineau Y, Larsson O, Rong L, Svitkin YV, et al. Translational control of the innate immune response through IRF-7. *Nature* 2008, 452:323–328.
140. Petroulakis E, Parsyan A, Dowling RJ, LeBacquer O, Martineau Y, Bidinosti M, Larsson O, Alain T, Rong L, Mamane Y, et al. p53-dependent translational control of senescence and transformation via 4E-BPs. *Cancer Cells* 2009, 16:439–446.
141. Mamane Y, Petroulakis E, LeBacquer O, Sonenberg N. mTOR, translation initiation and cancer. *Oncogene* 2006, 25:6416–6422.
142. Rojo F, Najera L, Lirola J, Jimenez J, Guzman M, Sabadell MD, Baselga J, Ramon y Cajal S. 4E-binding protein 1, a cell signaling hallmark in breast cancer that correlates with pathologic grade and prognosis. *Clin Cancer Res* 2007, 13:81–89.
143. Petricoin EF 3rd, Espina V, Araujo RP, Midura B, Yeung C, Wan X, Eichler GS, Johann DJ Jr, Qualman S, Tsokos M, et al. Phosphoprotein pathway mapping: Akt/mammalian target of rapamycin activation is negatively associated with childhood rhabdomyosarcoma survival. *Cancer Res* 2007, 67:3431–3440.
144. Waskiewicz AJ, Flynn A, Proud CG, Cooper JA. Mitogen-activated protein kinases activate the serine/threonine kinases Mnk1 and Mnk2. *EMBO J* 1997, 16:1909–1920.
145. Fukunaga R, Hunter T. MNK1, a new MAP kinase-activated protein kinase, isolated by a novel expression screening method for identifying protein kinase substrates. *EMBO J* 1997, 16:1921–1933.

146. Wang X, Flynn A, Waskiewicz AJ, Webb BL, Vries RG, Baines IA, Cooper JA, Proud CG. The phosphorylation of eukaryotic initiation factor eIF4E in response to phorbol esters, cell stresses, and cytokines is mediated by distinct MAP kinase pathways. *J Biol Chem* 1998, 273:9373–9377.

147. Scheper GC, Morrice NA, Kleijn M, Proud CG. The mitogen-activated protein kinase signal-integrating kinase Mnk2 is a eukaryotic initiation factor 4E kinase with high levels of basal activity in mammalian cells. *Mol Cell Biol* 2001, 21:743–754.

148. Pyronnet S, Imataka H, Gingras AC, Fukunaga R, Hunter T, Sonenberg N. Human eukaryotic translation initiation factor 4G (eIF4G) recruits mnk1 to phosphorylate eIF4E. *EMBO J* 1999, 18:270–279.

149. Van Der Kelen K, Beyaert R, Inze D, De Veylder L. Translational control of eukaryotic gene expression. *Crit Rev Biochem Mol Biol* 2009, 44:143–168.

150. Ueda T, Watanabe-Fukunaga R, Fukuyama H, Nagata S, Fukunaga R. Mnk2 and Mnk1 are essential for constitutive and inducible phosphorylation of eukaryotic initiation factor 4E but not for cell growth or development. *Mol Cell Biol* 2004, 24:6539–6549.

151. Topisirovic I, Ruiz-Gutierrez M, Borden KL. Phosphorylation of the eukaryotic translation initiation factor eIF4E contributes to its transformation and mRNA transport activities. *Cancer Res* 2004, 64:8639–8642.

152. Lachance PE, Miron M, Raught B, Sonenberg N, Lasko P. Phosphorylation of eukaryotic translation initiation factor 4E is critical for growth. *Mol Cell Biol* 2002, 22:1656–1663.

153. Scheper GC, van Kollenburg B, Hu J, Luo Y, Goss DJ, Proud CG. Phosphorylation of eukaryotic initiation factor 4E markedly reduces its affinity for capped mRNA. *J Biol Chem* 2002, 277:3303–3309.

154. Walsh D, Mohr I. Phosphorylation of eIF4E by Mnk-1 enhances HSV-1 translation and replication in quiescent cells. *Genes Dev* 2004, 18:660–672.

155. Worch J, Tickenbrock L, Schwable J, Steffen B, Cauvet T, Mlody B, Buerger H, Koeffler HP, Berdel WE, Serve H, et al. The serine-threonine kinase MNK1 is post-translationally stabilized by PML-RARalpha and regulates differentiation of hematopoietic cells. *Oncogene* 2004, 23:9162–9172.

156. Kaspar RL, Rychlik W, White MW, Rhoads RE, Morris DR. Simultaneous cytoplasmic redistribution of ribosomal protein L32 mRNA and phosphorylation of eukaryotic initiation factor 4E after mitogenic stimulation of Swiss 3T3 cells. *J Biol Chem* 1990, 265:3619–3622.

157. Manzella JM, Rychlik W, Rhoads RE, Hershey JW, Blackshear PJ. Insulin induction of ornithine decarboxylase. Importance of mRNA secondary structure and phosphorylation of eucaryotic initiation factors eIF-4B and eIF-4E. *J Biol Chem* 1991, 266:2383–2389.

158. Morley SJ, Naegele S. Phosphorylation of eukaryotic initiation factor (eIF) 4E is not required for de novo protein synthesis following recovery from hypertonic stress in human kidney cells. *J Biol Chem* 2002, 277:32855–32859.

159. Naegele S, Morley SJ. Molecular cross-talk between MEK1/2 and mTOR signaling during recovery of 293 cells from hypertonic stress. *J Biol Chem* 2004, 279:46023–46034.

160. Knauf U, Tschopp C, Gram H. Negative regulation of protein translation by mitogen-activated protein kinase-interacting kinases 1 and 2. *Mol Cell Biol* 2001, 21:5500–5511.

161. Eilers M, Eisenman RN. Myc's broad reach. *Genes Dev* 2008, 22:2755–2766.

162. Grandori C, Cowley SM, James LP, Eisenman RN. The Myc/Max/Mad network and the transcriptional control of cell behavior. *Annu Rev Cell Dev Biol* 2000, 16:653–699.

163. Askew DS, Ashmun RA, Simmons BC, Cleveland JL. Constitutive c-myc expression in an IL-3-dependent myeloid cell line suppresses cell cycle arrest and accelerates apoptosis. *Oncogene* 1991, 6:1915–1922.

164. Evan GI, Wyllie AH, Gilbert CS, Littlewood TD, Land H, Brooks M, Waters CM, Penn LZ, Hancock DC. Induction of apoptosis in fibroblasts by c-myc protein. *Cell* 1992, 69:119–128.

165. Shi Y, Glynn JM, Guilbert LJ, Cotter TG, Bissonnette RP, Green DR. Role for c-myc in activation-induced apoptotic cell death in T cell hybridomas. *Science* 1992, 257:212–214.

166. Polunovsky VA, Rosenwald IB, Tan AT, White J, Chiang L, Sonenberg N, Bitterman PB. Translational control of programmed cell death: eukaryotic translation initiation factor 4E blocks apoptosis in growth-factor-restricted fibroblasts with physiologically expressed or deregulated Myc. *Mol Cell Biol* 1996, 16:6573–6581.

167. Jones RM, Branda J, Johnston KA, Polymenis M, Gadd M, Rustgi A, Callanan L, Schmidt EV. An essential E box in the promoter of the gene encoding the mRNA cap-binding protein (eukaryotic initiation factor 4E) is a target for activation by c-myc. *Mol Cell Biol* 1996, 16:4754–4764.

168. Lin CJ, Cencic R, Mills JR, Robert F, Pelletier J. c-Myc and eIF4F are components of a feedforward loop that links transcription and translation. *Cancer Res* 2008, 68:5326–5334.

169. De Benedetti AJB, Graff JR, Zimmer SG. CHO cells transformed by the translation factor eIF-4E display increased c-myc expression, but require overexpression of Max for tumorigenicity. *Mol Cell Differ* 1994, 2:347–371.

170. Yang HS, Jansen AP, Komar AA, Zheng X, Merrick WC, Costes S, Lockett SJ, Sonenberg N, Colburn NH. The transformation suppressor Pdcd4 is a novel

eukaryotic translation initiation factor 4A binding protein that inhibits translation. *Mol Cell Biol* 2003, 23:26–37.

171. Ma XM, Blenis J. Molecular mechanisms of mTOR-mediated translational control. *Nat Rev Mol Cell Biol* 2009, 10:307–318.
172. Lin CJ, Malina A, Pelletier J. c-Myc and eIF4F constitute a feedforward loop that regulates cell growth: implications for anticancer therapy. *Cancer Res* 2009, 69:7491–7494.
173. Stebbins-Boaz B, Cao Q, de Moor CH, Mendez R, Richter JD. Maskin is a CPEB-associated factor that transiently interacts with elF-4E. *Mol Cell* 1999, 4:1017–1027.
174. Nakamura A, Sato K, Hanyu-Nakamura K. Drosophila cup is an eIF4E binding protein that associates with Bruno and regulates oskar mRNA translation in oogenesis. *Dev Cell* 2004, 6:69–78.
175. Topisirovic I, Culjkovic B, Cohen N, Perez JM, Skrabanek L, Borden KL. The proline-rich homeodomain protein, PRH, is a tissue-specific inhibitor of eIF4E-dependent cyclin D1 mRNA transport and growth. *EMBO J* 2003, 22:689–703.
176. Topisirovic I, Kentsis A, Perez JM, Guzman ML, Jordan CT, Borden KL. Eukaryotic translation initiation factor 4E activity is modulated by HOXA9 at multiple levels. *Mol Cell Biol* 2005, 25:1100–1112.
177. Nedelec S, Foucher I, Brunet I, Bouillot C, Prochiantz A, Trembleau A. Emx2 homeodomain transcription factor interacts with eukaryotic translation initiation factor 4E (eIF4E) in the axons of olfactory sensory neurons. *Proc Natl Acad Sci USA* 2004, 101:10815–10820.
178. Richter JD, Sonenberg N. Regulation of cap-dependent translation by eIF4E inhibitory proteins. *Nature* 2005, 433:477–480.
179. Chekulaeva M, Hentze MW, Ephrussi A. Bruno acts as a dual repressor of oskar translation, promoting mRNA oligomerization and formation of silencing particles. *Cell* 2006, 124:521–533.
180. Cho PF, Poulin F, Cho-Park YA, Cho-Park IB, Chicoine JD, Lasko P, Sonenberg N. A new paradigm for translational control: inhibition via 5′–3′ mRNA tethering by Bicoid and the eIF4E cognate 4EHP. *Cell* 2005, 121:411–423.
181. Rom E, Kim HC, Gingras AC, Marcotrigiano J, Favre D, Olsen H, Burley SK, Sonenberg N. Cloning and characterization of 4EHP, a novel mammalian eIF4E-related cap-binding protein. *J Biol Chem* 1998, 273:13104–13109.
182. Joshi B, Cameron A, Jagus R. Characterization of mammalian eIF4E-family members. *Eur J Biochem* 2004, 271:2189–2203.
183. Rivera-Pomar R, Niessing D, Schmidt-Ott U, Gehring WJ, Jackle H. RNA binding and translational suppression by bicoid. *Nature* 1996, 379:746–749.
184. Villaescusa JC, Buratti C, Penkov D, Mathiasen L, Planaguma J, Ferretti E, Blasi F. Cytoplasmic Prep1 interacts with 4EHP inhibiting Hoxb4 translation. *PLoS One* 2009, 4:e5213.
185. Zhu N, Gu L, Findley HW, Zhou M. Transcriptional repression of the eukaryotic initiation factor 4E gene by wild type p53. *Biochem Biophys Res Commun* 2005, 335:1272–1279.
186. Lynch M, Chen L, Ravitz MJ, Mehtani S, Korenblat K, Pazin MJ, Schmidt EV. hnRNP K binds a core polypyrimidine element in the eukaryotic translation initiation factor 4E (eIF4E) promoter, and its regulation of eIF4E contributes to neoplastic transformation. *Mol Cell Biol* 2005, 25:6436–6453.
187. Murata T, Shimotohno K. Ubiquitination and proteasome-dependent degradation of human eukaryotic translation initiation factor 4E. *J Biol Chem* 2006, 281:20788–20800.
188. Xu X, Vatsyayan J, Gao C, Bakkenist CJ, Hu J. Sumoylation of eIF4E activates mRNA translation. *EMBO Rep* 2010, 11:299–304.
189. Cullen BR. Viral RNAs: lessons from the enemy. *Cell* 2009, 136:592–597.
190. Hellen CU, Sarnow P. Internal ribosome entry sites in eukaryotic mRNA molecules. *Genes Dev* 2001, 15:1593–1612.
191. Oruetxebarria I, Guo D, Merits A, Makinen K, Saarma M, Valkonen JP. Identification of the genome-linked protein in virions of Potato virus A, with comparison to other members in genus Potyvirus. *Virus Res* 2001, 73:103–112.
192. Goodfellow I, Chaudhry Y, Gioldasi I, Gerondopoulos A, Natoni A, Labrie L, Laliberte JF, Roberts L. Calicivirus translation initiation requires an interaction between VPg and eIF 4 E. *EMBO Rep* 2005, 6:968–972.
193. Goff SP. Host factors exploited by retroviruses. *Nat Rev Microbiol* 2007, 5:253–263.
194. Ahola T, Kaariainen L. Reaction in alphavirus mRNA capping: formation of a covalent complex of nonstructural protein nsP1 with 7-methyl-GMP. *Proc Natl Acad Sci U S A* 1995, 92:507–511.
195. Condit RC, Niles EG. Regulation of viral transcription elongation and termination during vaccinia virus infection. *Biochim Biophys Acta* 2002, 1577:325–336.
196. Ogino T, Banerjee AK. Unconventional mechanism of mRNA capping by the RNA-dependent RNA polymerase of vesicular stomatitis virus. *Mol Cell* 2007, 25:85–97.
197. Ogino T, Banerjee AK. Formation of guanosine(5′) tetraphospho(5′)adenosine cap structure by an unconventional mRNA capping enzyme of vesicular stomatitis virus. *J Virol* 2008, 82:7729–7734.
198. Ogino T, Yadav SP, Banerjee AK. Histidine-mediated RNA transfer to GDP for unique mRNA capping by

vesicular stomatitis virus RNA polymerase. *Proc Natl Acad Sci USA* 2010, 107:3463–3468.

199. Plotch SJ, Bouloy M, Ulmanen I, Krug RM. A unique cap(m7GpppXm)-dependent influenza virion endonuclease cleaves capped RNAs to generate the primers that initiate viral RNA transcription. *Cell* 1981, 23:847–858.
200. Honda A, Mukaigawa J, Yokoiyama A, Kato A, Ueda S, Nagata K, Krystal M, Nayak DP, Ishihama A. Purification and molecular structure of RNA polymerase from influenza virus A/PR8. *J Biochem* 1990, 107:624–628.
201. Guilligay D, Tarendeau F, Resa-Infante P, Coloma R, Crepin T, Sehr P, Lewis J, Ruigrok RW, Ortin J, Hart DJ, et al. The structural basis for cap binding by influenza virus polymerase subunit PB2. *Nat Struct Mol Biol* 2008, 15:500–506.
202. Dias A, Bouvier D, Crepin T, McCarthy AA, Hart DJ, Baudin F, Cusack S, Ruigrok RW. The cap-snatching endonuclease of influenza virus polymerase resides in the PA subunit. *Nature* 2009, 458:914–918.
203. Belsham GJ, Sonenberg N. RNA-protein interactions in regulation of picornavirus RNA translation. *Microbiol Rev* 1996, 60:499–511.
204. Gingras AC, Svitkin Y, Belsham GJ, Pause A, Sonenberg N. Activation of the translational suppressor 4E-BP1 following infection with encephalomyocarditis virus and poliovirus. *Proc Natl Acad Sci USA* 1996, 93:5578–5583.
205. Aoyagi M, Gaspar M, Shenk TE. Human cytomegalovirus UL69 protein facilitates translation by associating with the mRNA cap-binding complex and excluding 4EBP1. *Proc Natl Acad Sci USA* 2010, 107:2640–2645.
206. Moorman NJ, Cristea IM, Terhune SS, Rout MP, Chait BT, Shenk T. Human cytomegalovirus protein UL38 inhibits host cell stress responses by antagonizing the tuberous sclerosis protein complex. *Cell Host Microbe* 2008, 3:253–262.
207. Toth Z, Lischka P, Stamminger T. RNA-binding of the human cytomegalovirus transactivator protein UL69, mediated by arginine-rich motifs, is not required for nuclear export of unspliced RNA. *Nucleic Acids Res* 2006, 34:1237–1249.
208. Winkler M, Rice SA, Stamminger T. UL69 of human cytomegalovirus, an open reading frame with homology to ICP27 of herpes simplex virus, encodes a transactivator of gene expression. *J Virol* 1994, 68:3943–3954.
209. Sandri-Goldin RM. The many roles of the regulatory protein ICP27 during herpes simplex virus infection. *Front Biosci* 2008, 13:5241–5256.
210. Fontaine-Rodriguez EC, Taylor TJ, Olesky M, Knipe DM. Proteomics of herpes simplex virus infected cell protein 27: association with translation initiation factors. *Virology* 2004, 330:487–492.
211. Osborne JC, Elliott RM. RNA binding properties of bunyamwera virus nucleocapsid protein and selective binding to an element in the 5′ terminus of the negative-sense S segment. *J Virol* 2000, 74:9946–9952.
212. Mir MA, Panganiban AT. A protein that replaces the entire cellular eIF4F complex. *EMBO J* 2008, 27:3129–3139.
213. Panganiban AT, Mir MA. Bunyavirus N: eIF4F surrogate and cap-guardian. *Cell Cycle* 2009, 8:1332–1337.
214. Schwer B, Saha N, Mao X, Chen HW, Shuman S. Structure-function analysis of yeast mRNA cap methyltransferase and high-copy suppression of conditional mutants by AdoMet synthase and the ubiquitin conjugating enzyme Cdc34p. *Genetics* 2000, 155:1561–1576.
215. Radomski N, Barreto G, Kaufmann C, Yokoska J, Mizumoto K, Dreyer C. Interaction of S-adenosylhomocysteine hydrolase of *Xenopus* laevis with mRNA(guanine-7-)methyltransferase: implication on its nuclear compartmentalisation and on cap methylation of hnRNA. *Biochim Biophys Acta* 2002, 1590:93–102.
216. Chiang PK, Gordon RK, Tal J, Zeng GC, Doctor BP, Pardhasaradhi K, McCann PP. S-Adenosylmethionine and methylation. *FASEB J* 1996, 10:471–480.
217. Wen Y, Shatkin AJ. Cap methyltransferase selective binding and methylation of GpppG-RNA are stimulated by importin-alpha. *Genes Dev* 2000, 14:2944–2949.
218. Terry LJ, Shows EB, Wente SR. Crossing the nuclear envelope: hierarchical regulation of nucleocytoplasmic transport. *Science* 2007, 318:1412–1416.
219. Cole MD, Cowling VH. Specific regulation of mRNA cap methylation by the c-Myc and E2F1 transcription factors. *Oncogene* 2009, 28:1169–1175.
220. Cowling VH, Cole MD. The Myc transactivation domain promotes global phosphorylation of the RNA polymerase II carboxy-terminal domain independently of direct DNA binding. *Mol Cell Biol* 2007, 27:2059–2073.
221. Fernandez-Sanchez ME, Gonatopoulos-Pournatzis T, Preston G, Lawlor MA, Cowling VH. S-adenosyl homocysteine hydrolase is required for Myc-induced mRNA cap methylation, protein synthesis, and cell proliferation. *Mol Cell Biol* 2009, 29:6182–6191.
222. Cougot N, van Dijk E, Babajko S, Seraphin B. Captabolism. *Trends Biochem Sci* 2004, 29:436–444.
223. Parker R, Sheth U. P bodies and the control of mRNA translation and degradation. *Mol Cell* 2007, 25:635–646.
224. Lim S, Mullins JJ, Chen CM, Gross KW, Maquat LE. Novel metabolism of several beta zero-thalassemic beta-globin mRNAs in the erythroid tissues of transgenic mice. *EMBO J* 1989, 8:2613–2619.

225. Lim SK, Sigmund CD, Gross KW, Maquat LE. Nonsense codons in human beta-globin mRNA result in the production of mRNA degradation products. *Mol Cell Biol* 1992, 12:1149–1161.

226. Lim SK, Maquat LE. Human beta-globin mRNAs that harbor a nonsense codon are degraded in murine erythroid tissues to intermediates lacking regions of exon I or exons I and II that have a cap-like structure at the 5′ termini. *EMBO J* 1992, 11:3271–3278.

227. Stevens A, Wang Y, Bremer K, Zhang J, Hoepfner R, Antoniou M, Schoenberg DR, Maquat LE. Beta-globin mRNA decay in erythroid cells: UG site-preferred endonucleolytic cleavage that is augmented by a premature termination codon. *Proc Natl Acad Sci USA* 2002, 99:12741–12746.

228. Otsuka Y, Kedersha NL, Schoenberg DR. Identification of a cytoplasmic complex that adds a cap onto 5′-monophosphate RNA. *Mol Cell Biol* 2009, 29:2155–2167.

229. She M, Decker CJ, Chen N, Tumati S, Parker R, Song H. Crystal structure and functional analysis of Dcp2p from Schizosaccharomyces pombe. *Nat Struct Mol Biol* 2006, 13:63–70.

230. Wu M, Nilsson P, Henriksson N, Niedzwiecka A, Lim MK, Cheng Z, Kokkoris K, Virtanen A, Song H. Structural basis of m(7)GpppG binding to poly(A)-specific ribonuclease. *Structure* 2009, 17:276–286.

231. Nagata T, Suzuki S, Endo R, Shirouzu M, Terada T, Inoue M, Kigawa T, Kobayashi N, Guntert P, Tanaka A, et al. The RRM domain of poly(A)-specific ribonuclease has a noncanonical binding site for mRNA cap analog recognition. *Nucleic Acids Res* 2008, 36:4754–4767.

232. Shiraki T, Kondo S, Katayama S, Waki K, Kasukawa T, Kawaji H, Kodzius R, Watahiki A, Nakamura M, Arakawa T, et al. Cap analysis gene expression for high-throughput analysis of transcriptional starting point and identification of promoter usage. *Proc Natl Acad Sci USA* 2003, 100:15776–15781.

233. Cowling VH. Enhanced mRNA cap methylation increases cyclin D1 expression and promotes cell transformation. *Oncogene* 2010, 29:930–936.

234. Dancey J. mTOR signaling and drug development in cancer. *Nat Rev Clin Oncol* 2010, 7:209–219.

235. Graff JR, Konicek BW, Vincent TM, Lynch RL, Monteith D, Weir SN, Schwier P, Capen A, Goode RL, Dowless MS, et al. Therapeutic suppression of translation initiation factor eIF4E expression reduces tumor growth without toxicity. *J Clin Invest* 2007, 117:2638–2648.

236. Assouline S, Culjkovic B, Cocolakis E, Rousseau C, Beslu N, Amri A, Caplan S, Leber B, Roy DC, Miller WH Jr, et al. Molecular targeting of the oncogene eIF4E in acute myeloid leukemia (AML): a proof-of-principle clinical trial with ribavirin. *Blood* 2009, 114:257–260.

