

The eukaryotic translation initiation factor eIF4E wears a “cap” for many occasions

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ABSTRACT

The eukaryotic translation initiation factor eIF4E plays important roles in controlling the composition of the proteome. Indeed, dysregulation of eIF4E is associated with poor prognosis cancers. The traditional view has been that eIF4E acts solely in translation. However, over the last ~25 years, eIF4E was found in the nucleus where it acts in mRNA export and in the last ~10 years, eIF4E was found in cytoplasmic processing bodies (P-bodies) where it functions in mRNA sequestration and stability. The common biochemical thread for these activities is the ability of eIF4E to bind the 7-methylguanosine cap on the 5' end of mRNAs. Recently, the possibility that eIF4E directly binds some mRNA elements independently of the cap has also been raised. Importantly, the effects of eIF4E are not genome-wide with a subset of transcripts targeted depending on the presence of specific mRNA elements and context-dependent regulatory factors. Indeed, eIF4E governs RNA regulons through co-regulating the expression of groups of transcripts acting in the same biochemical pathways. In addition, studies over the past ~15 years indicate that there are multiple strategies that regulatory factors employ to modulate eIF4E activities in context-dependent manners. This perspective focuses on these new findings and incorporates them into a broader model for eIF4E function.

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Overview

The eukaryotic translation initiation factor eIF4E is a potent oncoprotein.¹ Forty years ago, Filipowicz and Ochoa isolated eIF4E as the first 7-methylguanosine (m⁷G) cap-binding protein, originally naming it the cap-binding protein (not to be confused with today's nuclear cap binding proteins CBP20 and CBP80).² Two years later, the eIF4E protein was purified through a cap cross-linking strategy by Sonenberg and Shatkin.³ Many groups demonstrated that eIF4E is a central part of the translation machinery where it binds the m⁷G cap of mRNAs to recruit these to the ribosome in order to increase translational efficiency.⁴ Importantly, not all mRNAs are equally affected by eIF4E where only a subset of mRNAs typically characterized by highly structured 5' UTRs are sensitive to eIF4E at the polysomal loading level.⁴ For example, eIF4E does not increase translation efficiency of GAPDH mRNAs but does increase efficiency for transcripts that encode proteins involved in proliferation, survival and invasion e.g.

vascular endothelial growth factor (VEGF).^{4,5} Consistently, modulation of eIF4E does not affect the global proteome.⁵⁻⁸ For many years, the story of eIF4E started and ended with mRNA translation. However, eIF4E has proven to be a much more diverse player in the world of post-transcriptional regulation. To fully appreciate its biochemical and cellular effects, it is necessary to integrate these new insights into a more holistic view of eIF4E function.

The same hat for many occasions: Eif4E as a multi-functional cap chaperone

There is substantial evidence to support the notion that eIF4E acts in multiple biochemical processes united by the need to bind transcripts through the m⁷G cap. Indeed, eIF4E may escort or chaperone specific mRNAs through multiple stages of the mRNA life cycle including, but not limited to, translation. One of the main lines of evidence for eIF4E to function beyond translation is the observation that eIF4E

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localization is not restricted to sites containing ribosomes. For instance, eIF4E is found in heterogeneous cytoplasmic ribonucleoparticle (RNP) granules known as processing bodies (P-bodies) as well as in multiple locations within the nucleus.^{5,9-12} In these locations eIF4E is not associated with active ribosomes.^{5,10,13} In P-bodies, eIF4E functions in the balance between sequestration and decay of specific transcripts.^{10,11} Here, eIF4E is thought to protect specific mRNAs from decapping-dependent degradation through binding the m⁷G cap on the transcript and thus, preventing association with the decapping enzymes. The eIF4E-binding protein known as the eIF4E transporter (4E-T) is required for formation of P-bodies as well as eIF4E's localization there.¹⁴ However, it is difficult to decouple the role of 4E-T in eIF4E trafficking to P-bodies from its central role in forming these structures. 4E-T binding precludes association of eIF4G with eIF4E, again indicating P-bodies are not sites of translation.¹⁵ The overall heterogeneity of P-bodies suggests that functions of individual bodies could be dependent on many contextual factors which would underpin selection of mRNAs targeted to these bodies and determine the signals that initiate mRNA entry into bodies, exit from bodies, or decay in or near bodies.^{10,11,13} Other cytoplasmic granules also contain eIF4E such as stress granules (SG).^{10,11,13} Unlike P-bodies, SGs contain other components of the translation machinery and thus could be sites of active translation for at least some mRNAs.¹¹ Interestingly, specific mRNAs and other factors can shuttle between P-bodies and SGs but importantly 4E-T is not an SG component.¹¹ Fluorescence recovery after photobleaching (FRAP) experiments indicate this exchange is rapid.¹¹ Association with these bodies is not permanent, as traffic of transcripts between P-bodies and ribosomes suggest that at least some mRNAs can be returned to active translation.¹¹ Thus in the cytoplasm, eIF4E acts in ribosome-dependent and independent activities both of which require its cap-binding functionality.

eIF4E is also found in the nucleus.^{9,16} To date, the best-defined activity there is its role in the export of specific transcripts.^{5,12,17,18} Its export activity is independent of ongoing protein synthesis.¹⁸ eIF4E and target mRNAs associate with specific co-factors such as CRM1 which is the export receptor for this pathway.¹⁷⁻¹⁹ Indeed, this mRNA export pathway does not require the bulk mRNA export receptor TAP/NXF1.¹⁸

For instance, knockdown of TAP/NXF1 does not affect export of these transcripts whereas CRM1 inhibitor leptomycin B does so.^{18,19} eIF4E only associates with mRNAs after splicing and in the absence of the nuclear cap-binding complex (CBC) comprised of CBP20 and CBP80.¹⁹ No interaction between CBC and eIF4E has been detected. Presumably the hand-off of capped mRNAs between these is too rapid to readily detect.

Interactions with mRNAs in the nucleus are specific i.e. not all capped mRNAs associate with eIF4E there.^{7,17,18,20} For instance, nuclear eIF4E associates with approximately 3500 capped transcripts⁷ which typically contain a ~50 nucleotide element known as an eIF4E sensitivity element (4ESE) in their 3' UTR. The extent to which there are different subtypes of 4ESE elements is not yet known. These 4ESE-containing mRNAs encode networks of proteins that act in related pathways particularly involved in proliferation, survival, and invasion.^{17,18,20} For instance, eIF4E promotes the export of Bcl6 mRNA as well as transcripts encoding other co-factors involved in Bcl6 signaling such as BCOR, NCOR, and SMRT.⁷ Importantly, eIF4E is found in multiple locations within the nucleus including promyelocytic leukemia protein (PML) nuclear bodies, nuclear bodies containing mRNA (but not PML) and diffusely throughout the nucleoplasm.^{17,18} These multiple locations suggest that eIF4E may be involved in other nuclear cap-dependent mRNA processing events that have yet to be elucidated.

The unifying feature between these 3 eIF4E functions (translation, mRNA export and stability/sequestration) is the requirement for the m⁷G cap, to date the defining biochemical activity of eIF4E. Collectively, these observations suggest that eIF4E escorts specific, capped mRNAs through various mRNA processing events. In this way, eIF4E could be considered a cap chaperone, at least for a subset of mRNAs. In such a model, all functions of eIF4E should be considered in combination to truly understand its ultimate impact on the proteome.

eIF4E governs oncogenic RNA regulons

The ability of eIF4E to affect the expression of groups of cancer related mRNAs at multiple levels (mRNA export, translation, stability) suggests that eIF4E governs oncogenic RNA regulons.^{7,21-23} In such a model,

mRNAs that contain the right combination of Untranslated Sequence Elements for Regulation (USER) codes, such as 4ESE for mRNA export or complex 5' UTRs for translation, can be affected by eIF4E at the appropriate level.^{18,20,24} These USER codes relay this specificity through recruitment of cellular factors which engage in these processes. USER codes can be transferable to reporters such as lacZ and sensitized to translation, export or both depending on the combination of USER codes added. Whether or not there are USER codes for P-body entry or exit is not yet known, but seems likely. By targeting networks of transcripts with the same USER codes,^{7,17,18,20} eIF4E has a broad range of effects on entire pathways not simply on single proteins.^{7,17,18,20} In terms of regulation, competition between stimulatory and inhibitory factors that bind the USER codes will impact the fate of a given transcript or group of transcripts. This combinatorial affect of eIF4E on transcripts likely underpins its potent, yet specific, effects on the proteome.

As expected from the RNA regulon model, eIF4E activity can be regulated in context and process-specific manners. Accordingly, some regulators are found in only the nucleus or cytoplasm such as the nuclear protein PML, which is a potent suppressor of eIF4E dependent mRNA export. Here, PML directly interacts with eIF4E and reduces its affinity for the cap.^{12,25,26} Consistently, PML potently suppresses the oncogenic activity of eIF4E in vitro.^{12,20} Another nuclear inhibitor of eIF4E, the proline-rich homeodomain PRH (also known as hematopoietically-expressed homeodomain Hex), represses eIF4E dependent mRNA export in the limited number of tissues in which it is expressed. Interestingly, PRH overexpression leads to nuclear depletion of eIF4E, repression of its mRNA export activity, reduction in subsequent protein target levels and finally, reduced its oncogenic activity in vitro.²⁷ Conversely, HoxA9 simultaneously stimulates mRNA export and translation of specific transcripts in the tissues that express this protein.²⁸ Other homeodomains can also modulate eIF4E activity (see below).²⁹⁻³¹ The most studied eIF4E inhibitor, 4E-BP1 is also found in the nucleus,³² suggesting it could inhibit mRNA export. In the cytoplasm, 4E-BP1 blocks eIF4G binding and thus association with the ribosome.⁴ 4E-BP1 could similarly prevent association with relevant nuclear export factors. This remains to be tested. Some studies indicate

that 4E-BP1 is absent from P-bodies suggesting alternative control mechanisms are in play¹⁴ while other work finds 4E-BP1 present in these granules.¹¹ Thus, it is not clear if there is a universal regulator of eIF4E i.e., one present at most subcellular locations and tissues.

Considering its multiple functions, trafficking eIF4E between its different sites of activity is an important means to modulate its effects on the proteome. One example provided above is the role of 4E-T in trafficking eIF4E to P-bodies.¹⁴ In terms of nuclear trafficking, eIF4E is imported into the nucleus of at least some cells through its interaction with Importin 8.³³ Import only occurs when eIF4E is not bound to capped mRNAs.³³ This is because Importin 8 interacts with the cap-binding site of eIF4E, directly competing for the cap.³³ Thus, neither actively translating eIF4E nor newly exported eIF4E-mRNA export complexes are import cargoes. Furthermore, eIF4E is found in multiple locations within the nucleus and the factors that traffic eIF4E to these different sites are not well understood. To date, it is known that PML and the export co-factor LRPPRC compete for nuclear eIF4E where LRPPRC increases the fraction of eIF4E in active mRNA export complexes whereas, PML sequesters eIF4E in an RNA free-state in PML nuclear bodies.^{12,18,19,25} This provides an example of how the relative levels of factors can affect trafficking and thus activity of eIF4E. Finally, eIF4E exits the nucleus via the CRM1 pathway.^{18,19,34} The mechanisms determining the decision points in terms of eIF4E acting in translation or returning to the nucleus for further export rounds are not known.

How do these functions impact on the oncogenic activity of eIF4E?

eIF4E is elevated in a wide variety of malignancies.¹ eIF4E overexpression causes tumors and increased invasion in xenograft mouse models and leads to a wide array of tumors in eIF4E overexpressing mice.^{1,35-37} Interestingly, mice with a ~50% reduction in eIF4E protein levels develop normally but are more tumor resistant.³⁸ This observation questions the view that eIF4E levels are rate-limiting for translation (or other eIF4E activities), at least in most cell types. However, this observation is consistent with the idea from earlier studies that eIF4E-high cancers could be targeted systemically because the tumors had

developed an oncogene addiction to eIF4E relative to normal cells.^{6,39,40} This prediction has been borne out clinically.^{41,42} Importantly, knockout of eIF4E is lethal in mice and yeast.^{38,43} However some cell lines are still viable after CRISPR knockout of eIF4E e.g., K562, KBM7, Raji, and Jivoye human cell lines.⁴⁴ These observations suggest other systems are available to substitute for eIF4E at least in some contexts.

There is evidence that eIF4E activities can be differentially affected in different malignancies. For instance, in subtypes of acute myeloid leukemia (AML) and some lymphomas, eIF4E accumulates in the nucleus where it promotes the export of transcripts encoding oncoproteins such as c-myc and Mcl1.^{27,45} Increased cytoplasmic localization is observed in other lymphomas, supporting increased translation there.⁴⁵ Further, the level of regulation for a given transcript can also be context dependent. For instance, Bcl6 mRNA is only an mRNA export target in U2OS osteosarcoma cells but is both an mRNA export and translation target in Diffuse large B-cell lymphoma cell lines.⁴⁵ A combination of factors such as variations in USER code binding-proteins as well as differences in the transcripts themselves (e.g. via tissue specific processing) likely underlie these context-specific effects.

Importantly, functional studies indicate that both translation and mRNA export can contribute to the oncogenic activity of eIF4E.^{1,45} Although the contribution of P-body or SG activity to eIF4E's oncogenic activity has not been directly tested, there is some evidence these will also play a role.⁴⁶ For instance, P-bodies may function in the response to hypoxia and there is substantial evidence that SGs play roles in resistance to various cancer therapies.⁴⁶

Mutational studies have been a useful means to assess the effects of different eIF4E activities on its physiological activities. Importantly, mutations which disrupt cap-binding (e.g., W56A) impair mRNA export, translation and oncogenic transformation highlighting the importance of cap-binding to all of its activities.^{12,18,25} Interestingly, mutation of serine 53 to alanine impairs the ability of eIF4E to transform cells.^{37,45} This phenotype was originally attributed to a loss of phosphorylation at this site, but it was later determined that eIF4E was phosphorylated on S209 not S53.⁴⁷ Interestingly, the S53A mutant is inactive not only in transformation but also in the formation of nuclear mRNA export complexes and in the

promotion of mRNA export.⁴⁵ However, the S53A mutant rescues yeast null in wild-type eIF4E and enhances translation of eIF4E sensitive transcripts such as VEGF in mammalian cell lines.^{8,43,45} Further the mutant is folded and active for cap binding.⁴⁵ In addition to the effects on mRNA export, it is also possible that S53 is required for translation of a specific subset of transcripts that have not been identified yet and/or also modulates eIF4E functions relating to P-bodies or SGs. Interestingly, a mutant active in mRNA export but which does not enhance eIF4E specific translation (W73A), transforms cells as readily as wildtype eIF4E in NIH3T3, MEFs and U2OS cells.^{12,17,45} Consistently, the addition of a classical nuclear localization signal to wild-type eIF4E increases its mRNA export and *in vitro* transformation activities relative to wildtype eIF4E.^{19,33} These findings support the view that non-translation activities of eIF4E also contribute to its oncogenic potential. The phosphorylation status of eIF4E is important for its ability to transform cells,^{48,49} but it affects both mRNA export and translation, with unknown effects on P-body or SG activity.

Sites of control on the eIF4E protein

There are a wide variety of protein motifs used to bind multiple surfaces of eIF4E underlying specific regulation of its activities. For the point of this discussion, eIF4E can be considered to have 2 major structural features, the cap-binding site and the dorsal surface (Fig. 2). The m⁷G cap is intercalated between 2 tryptophan residues (W56 and W102) in the cap-binding site of eIF4E. This site is ~35 Å away from the dorsal surface where many regulatory proteins bind^{50,51} (Fig. 2). The traditional view is that eIF4E-interacting proteins almost exclusively use a conserved consensus-binding motif to interact with the dorsal surface of eIF4E. This motif was initially identified in eIF4G and the 4E-BPs and is comprised of YXXXXLΦ where X is any amino acid and Φ is any hydrophobic.⁵² More recent studies indicate that 4E-BPs have additional binding sites which increase affinity for eIF4E⁵³ (Fig. 2). It seems likely that this second site will be used by other proteins in conjunction with the consensus-binding motif. Although far apart in space, interactions on the dorsal surface affect the cap-binding site (and vice versa) via allostery.⁵⁴⁻⁵⁷ Indeed, 4E-BP1 not only blocks eIF4G binding to the dorsal surface but also modestly

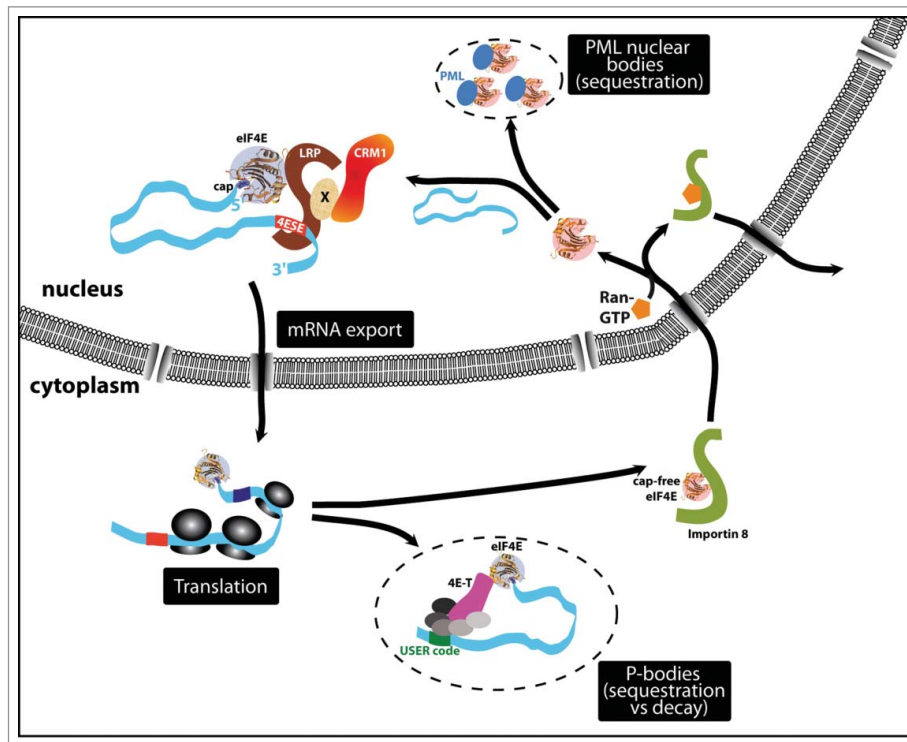


Figure 1. Model demonstrating the multiple activities of eIF4E and how they are connected in the cell. Many key partner proteins are not shown for the sake of simplicity. Cap-free eIF4E is shown in orange (pdb 2GPQ) and cap-bound eIF4E in violet (pdb 1EJ1). The blue thread is mRNA. Different USER codes are color coded. The various cellular structures are not shown to scale. The idea that 4E-T mediates interactions (directly or indirectly) with target transcripts is conjecture and the multiple gray/black ellipses indicate the possible presence of intermediary factor(s). Whether USER codes for P-bodies would be 5' or 3' (or both) is not known and is put in the 3' UTR for simplicity. In the nuclear mRNA export complex, the X indicates that it is not known whether LRPPRC directly binds CRM1 or if it uses an adaptor(s).

increases cap-affinity suggesting that it could sequester target mRNAs away from the ribosome as well.⁵⁶ While most studies focus on the 4E-BPs as the major

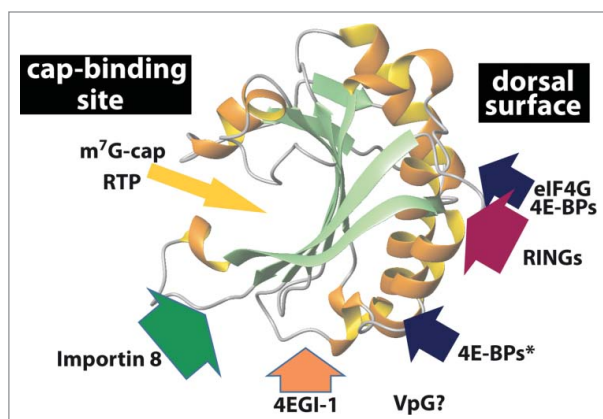


Figure 2. Diagram of the multiple surfaces that protein co-factors and small molecules use to interact with eIF4E. The human apo-eIF4E structure (pdb 2GPQ) was rendered using pymol and a subset of co-factors are shown to demonstrate the various surfaces used to interact with eIF4E. Importin 8 binds the cap site and other sites on eIF4E simultaneously, consistent with its large size (120 kDa). The binding site for VpG is unknown. 4E-BPs* indicates the secondary site surface, not the consensus binding site which is indicated by 4E-BP.

regulators of eIF4E, this family is not conserved in flies or yeast where other factors regulate eIF4E activity. Interestingly, bioinformatics analysis indicates that roughly 200 homeodomain proteins contain unconfirmed consensus-binding motifs and thus could bind eIF4E and further, could recruit specific transcripts potentially through their homeodomain motifs.^{28,58,59} Biochemical studies confirm that several of these do indeed directly bind eIF4E including Emx2, Engrailed 2, OTX2, bicoid, Hox11, PRH/Hex and HoxA9.^{28,30,58,59} Indeed, Bicoid was found to directly bind both eIF4E and caudal mRNA through its homeodomain to suppress translation.⁵⁹ Thus, many proteins potentially control eIF4E activity, some of which use the consensus-binding motif, such as 4E-T¹⁵, while others use different strategies.

One example of a different motif is the really interesting new gene (RING) domains. The RING domains from PML and the arenavirus protein Z directly interact with eIF4E. Additionally, the RING domain from the human homolog of ariadne (HHARI) directly binds eIF4E2, another eIF4E family member.^{12,25,26,60}

The RINGs bind a part of the dorsal surface distinct from the consensus-binding site²⁶ (Fig. 2) thereby promoting differential allosteric effects which alter the dynamics in the cap-binding site and lead to 50–100 fold reduction in the cap affinity of eIF4E.²⁶

Interactions with regulatory proteins are not restricted to the dorsal surface of eIF4E. For instance, Importin 8 occludes the cap-binding site thereby competing with the cap for eIF4E³³ (Fig. 2). This selectivity relies on substantial charge differences for the cap-binding surface of apo- versus cap-bound eIF4E. Future studies will determine whether other proteins utilize a similar mechanism to select different forms of eIF4E.³³ These observations also explain previous studies demonstrating that m⁷G cap analogs or cap competitors such as ribavirin (see below) prevent nuclear import of eIF4E to the cytoplasm.^{12,33,34} Importantly NMR data indicate that Importin 8 also simultaneously binds other surfaces on eIF4E, thus its binding is not restricted to the cap site.³³ The viral protein genome linked VpG from potyviruses also uses a unique strategy to bind novel sites on eIF4E. Specifically, VpG binds eIF4E-cap-eIF4G ternary complexes, indicating it interacts with a novel binding surface.⁶¹ Further, VpG contains no known eIF4E binding motifs. Future structure studies will be key in elucidating the strategy used by VpG and whether other proteins bind similarly.

Interactions with small molecule inhibitors have also been characterized. One example is the anti-viral drug ribavirin and its active metabolite, ribavirin triphosphate (RTP), which act as cap competitors. Ribavirin and RTP directly bind eIF4E as observed using multiple methods e.g. NMR, mass spectrometry, fluorescence and cap-affinity chromatography.^{39,40,62} Mutation of the cap-binding site impairs ribavirin and RTP binding.^{39,62} NMR studies show that RTP binds in the cap-binding site, but likely deeper into the pocket than most cap analogs.^{39,40,62} Interestingly, under conditions where eIF4E and RTP aggregate (e.g., in HEPES), RTP no longer binds eIF4E thereby highlighting the importance of choosing optimal conditions for binding studies.^{40,63} Importantly, ribavirin interacts with eIF4E in live cells and lysates as observed by eIF4E immunoprecipitation of ³H ribavirin.^{64–66} Consistently, ribavirin impairs eIF4E activity in mRNA export and translation.^{7,39,40} Its effects on P-bodies and SGs are unknown.

Another pharmacological inhibitor of eIF4E is 4EGI-1. 4EGI-1 was designed to interact with the dorsal surface and thus interfere with eIF4G recruitment.⁶⁷ 4EGI-1 inhibits eIF4E dependent translation and reduces cell growth in many cancer lines.⁶⁷ Its effects on mRNA export and P-body or SG activity are not known. Despite NMR studies supporting 4EGI-1 binding the dorsal surface, a subsequent crystal structure of the complex showed that 4EGI-1 bound between the cap and dorsal surfaces suggesting that 4EGI-1 is working through an allosteric mechanism⁶⁸ (Fig. 2). In total, it is clear that our understanding of the structural regulation of eIF4E activity has burgeoned in the 20 years since the first eIF4E structure⁵⁰ was reported.

Binding RNAs beyond the m⁷G cap?

Recent biophysical data show that eIF4E also binds the m^{2,2,7} G tri-methyl guanosine (TMG) cap characteristic of small nuclear and small nucleolar RNAs. Crystal structures of nematode eIF4E-TMG complexes indicate that TMG binds similarly to the m⁷G cap.⁷³ Nematode eIF4E readily translates TMG RNAs indicating this is a functional interaction. Consistent with the structural homology between nematode and human eIF4E, human eIF4E also binds the TMG cap albeit with a lower affinity than for the m⁷G cap.⁷³ Given the high concentrations of U snRNAs in the cell, the human eIF4E-TMG interaction may underlie some novel physiological role for eIF4E in mammals. Indeed, eIF4E has been reported to associate with U1 snRNAs in the nucleus, but given it associates with target mRNAs after splicing this must be related to some other biochemical activity.^{19,34} Further, there are reports suggesting that the TMG cap is used in translation in organisms other than nematodes, such as in mammalian cells during HIV infections.⁷⁴ Thus, it is possible that eIF4E play roles outside of m⁷G cap binding in mammals, as it does in nematodes.

There is also evidence that eIF4E binds RNA elements in addition to caps. In nematodes, the 22-nucleotide trans-spliced leader sequence increases the affinity of eIF4E for the TMG cap by inducing conformational changes to the cap-binding pocket.⁷³ Another example is the cap-independent translation element (PTE) in Pea enation mosaic virus which directly binds eIF4E.⁷⁵ Modeling studies suggest that eIF4E, through its cap-binding site, clamps a

guanosine in a pseudo-knot in the PTE. In Histone H4 mRNA, eIF4E directly binds a paired-stem loop element (structurally similar to the 4ESE but found in its coding region) to promote translation.⁷⁶ This element binds to eIF4E independently of the m⁷G cap. Interestingly, eIF4E also binds the m⁷G cap of Histone H4 mRNA independently of the 4ESE-like element.⁷⁶ Previous studies showed that the 4ESE in the 3' UTR did not effect translation,¹⁷ but its affects in the 5' UTR or coding regions were not tested prior to the above studies. Thus, eIF4E could be positioned to directly bind USER codes and thus may not always require a protein mediator for specific recognition of target mRNAs.

eIF4E can also modulate functions of other proteins to affect activity. Recent studies showed that eIF4E stimulates eIF4A helicase activity.⁷⁷ Here, eIF4E alleviates eIF4G-mediated repression of eIF4A helicase activity through inducing conformational changes in eIF4G.⁷⁷ This eIF4E activity does not require cap-binding. These findings provide a mechanism for how eIF4E preferentially stimulates translation of mRNAs with highly structured 5' UTRs. Such mechanisms are undoubtedly important for its oncogenic potential and have broader biochemical implications as well.

What have we learned from targeting eIF4E in patients?

The link between dysregulated eIF4E and cancer is widely studied.¹ eIF4E dysregulation occurs at multiple levels including (but not limited to) elevation of the eIF4E protein, increased phosphorylation and nuclear accumulation.¹ This has made eIF4E an attractive cancer target, especially given that several types of cancer cells have developed an oncogene addiction to eIF4E.^{6,39,41,42} However, only 3 clinical

trials designed to directly target eIF4E have been published.^{41,42,69} The strategies involved either ribavirin as a cap competitor or antisense oligonucleotides (ASO) to reduce eIF4E levels. Currently, only ribavirin-based therapies led to objective responses in the clinic^{41,42} (Table 1). These clinical results are described below.

The first published trial in humans assessed the clinical benefit of ribavirin monotherapy to refractory and relapsed acute myeloid leukemia (AML) patients in a phase II study.⁴¹ It is noteworthy that the average survival for the majority of newly diagnosed AML patients (age over 60) is 7.4 months.⁷⁰ In our study, we observed dramatic clinical improvements including multiple objective responses with the longest response lasting 9 months: 1 complete remission (CR), 2 partial remissions (PR) and 3 blast responses (BR) (50% or more reduction in leukemia blast count) out of 15 evaluable patients. Similar frequency of responses (5/14) was achieved in a phase I trial combining ribavirin with low-dose cytarabine, with some evidence for increased duration of responses (up to 24 months).⁴² In each case, we observed that targeting eIF4E's activity correlated with clinical response.^{41,64} Conversely, loss of eIF4E targeting correlated with relapse^{41,42,64} and was associated with chemical modification of ribavirin which impaired its interaction with eIF4E.^{39,40,67} A trial targeting this form of drug resistance is ongoing (Clinical Trials.gov NCT02073838).

The efficacy of ASO strategies to suppress eIF4E production was also examined in a phase I study of advanced solid tumors. This clinical work was based on promising results in mouse models of prostate cancer.⁶ However, the ASO treatment was not successful in humans.⁶⁹ Specifically, no patients achieved remissions with 7 stable diseases and 15 progressive diseases out of 22 patients with only 2 patients on the study for more than 3 months. The reduction in eIF4E levels

Table 1. Clinical studies designed to inhibit eIF4E.

Treatment	% OR (CR + PR + BR)	% SD	% Other	% PD	Comments	Other
Ribavirin Monotherapy n = 15	40%	40%	n/a	20%	Molecular targeting of eIF4E corresponded to clinical benefit; AML	Assouline et al., 2009, Blood & Zahreddine et al., Nature 2014
Refractory, Relapsed/unfit Ribavirin+low dose Ara-C n = 14	36%	29%	7% (SD+HI)	29%	Reporting on ribavirin plasma 20+ uM; AML	Assouline et al., 2015 Haematologica
eIF4E Antisense Oligonucleotide ISIS 183750 n = 22	0%	9%		91%	eIF4E reduction was less striking in humans than in earlier mouse studies: multiple solid tumors	Hong et al., 2011, Clinical Cancer Research

OR indicates overall response with CR (complete remission); PR (partial remission), BR (blast response), SD stable disease, HI haematological improvement and PD progressive disease. n indicates the number of patients.

was not as substantial in patients as it had been in mice suggesting that better ASO delivery into human cells is required to increase the efficacy of this strategy.

There are ongoing trials monitoring the effects of reducing eIF4E phosphorylation ClinicalTrials.gov NCT02605083. Mnk kinase inhibitors impair eIF4E-mediated transformation by impairing its phosphorylation thereby affecting both mRNA export and translation^{48,49} and perhaps P-body function. Mnk kinases are the only kinases to phosphorylate eIF4E, but importantly these phosphorylate other proteins including factors that affect mRNA processing such as hnRNPA1.^{71,72} The fact that targeting Mnk kinases will affect multiple proteins may be a great clinical strength, but careful molecular studies will be needed to determine the relative importance of targeting phosphorylation of eIF4E vs. other proteins.

eIF4E back-up systems

There are other factors that potentially substitute for eIF4E's translation (and likely other functions). For example in yeast, CBC localizes to the cytoplasm during hyperosmotic stress and actively engages polyosomes.⁷⁸ These effects are specific, with ~600 transcripts targeted for CBC-mediated translation (i.e. 10% of all transcripts). Under these conditions, general translation is inhibited and there is an increase in P-bodies.¹¹ Indeed, deletion of eIF4E during osmotic stress actually increases growth while simultaneously targeting eIF4E and CBC leads to synthetic sickness at both restrictive and permissive temperatures.⁷⁸ Aside from the CBC, eIF4E family members which normally have inhibitory functions can become active in translation during stress e.g., eIF4E2 during hypoxia and eIF4E3 during Mnk inhibition.⁷⁹⁻⁸¹ Other initiation factors (e.g. eIF3d and eIF3l) also bind the cap and can act in translation of specific transcripts suggesting other backup systems are in place as well.^{82,83} These and other back-up systems likely explain the observation that targeting eIF4E with ribavirin or ASOs does not lead to overt toxicity in patients.

Conclusions

eIF4E can chaperone or escort groups of functionally related transcripts through a variety of processing steps. This underpins the far-reaching effects of eIF4E on the proteome. Through its effects, eIF4E is even positioned to modify the epigenome by increasing

expression of DNA methyltransferases, histone deacetylation enzymes and other related factors.⁷ More studies are needed to fully understand the combinatorial effects of eIF4E on post-transcriptional regulation. For instance, how many cohorts of RNAs are co-regulated by eIF4E and how does this system adapt to cellular stress and extracellular signals? What combination of USER codes underpins these responses? Does eIF4E act in other modes of cap-dependent mRNA processing or are its activities restricted to the 3 functions described? The answers to such questions will provide the basis for a full understanding of the combinatorial effects of eIF4E. The last 40 years have taught us much about eIF4E, it will be fascinating to see what the next 40 will bring.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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Unfortunately, due to space restrictions, I was not able to cite all the outstanding papers that merited discussion. I apologize to the authors of those works. Many thanks to critical reading of the manuscript by Michael Osborne, Biljana Culjkovic-Kraljacic, Laurent Volpon, and Maggie Davis. Special thanks for production of figures by Laurent Volpon.

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