Cap-Dependent Translation Initiation Factor eIF4E: An Emerging Anticancer Drug Target

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Abstract: Cancer cells tend to be more highly dependent on cap-dependent translation than normal tissues. Thus, proteins involved in the initiation of cap-dependent translation have emerged as potential anti-cancer drug targets. Cap-dependent translation is initiated by the binding of the factor eIF4E to the cap domain of mRNA. Detailed x-ray crystal and NMR structures are available for eIF4E in association with cap-analogs, as well as domains of other initiation factors. This review will summarize efforts to design potential antagonist of eIF4E that could be used as new pharmacological tools and anti-cancer agents and. Insights drawn from these studies should aid in the design of future inhibitors of eIF4E dependent translation initiation. © 2012 Wiley Periodicals, Inc. Med. Res. Rev., 32, No. 4, 786–814, 2012

Key words: eIF4E; translation; anticancer

1. INTRODUCTION: EUKARYOTIC TRANSLATION AND CANCER

Ribosomal recruitment to the mRNA is a crucial step in protein synthesis and is a known target for translational control.[1] In eukaryotic cells, there are two main mechanisms of ribosomal recruitment: (i) cap-dependent translation, which most cellular mRNAs use as the standard mode of translation and involves the assembly of the preinitiation translational complex at the m^7GpppX 5′ cap structure (Me^7GTP) of mRNA, where X is any nucleotide; and (ii) cap-independent translation, which is a process that is initiated through direct interaction of the translational machinery with an internal ribosomal entry site (IRES) element within RNA. This mechanism is used by plus-stranded viral RNAs and by several dozen capped eukaryotic mRNAs that possess IRES in their 5′ untranslated region (UTR).[2–4] The first step in eukaryotic translation involves the preparation of a group of small ribosomal subunits (Fig. 1A). The two subunits of the ribosome tend to remain associated under normal physiological conditions,

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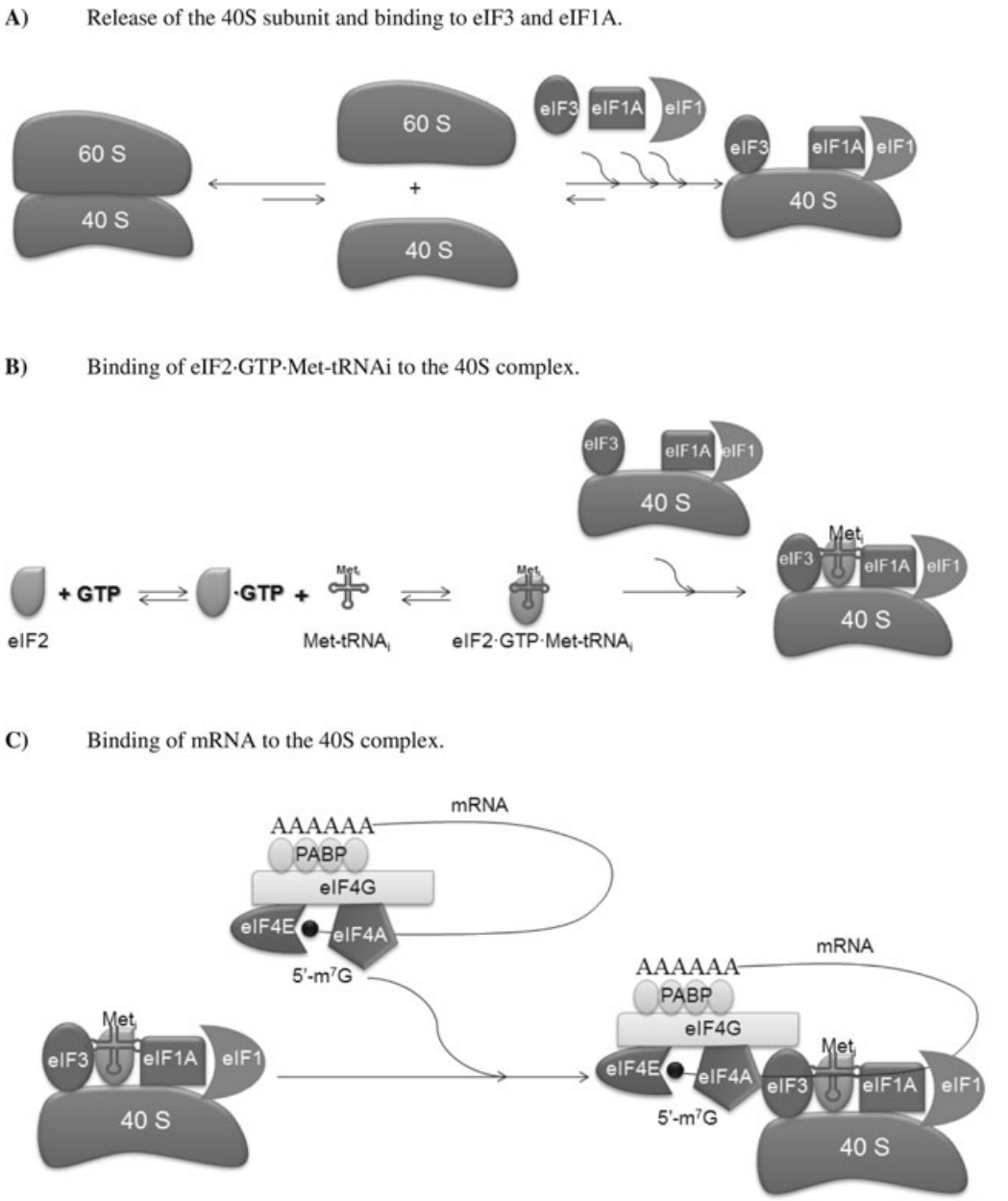


Figure 1. The mechanism of cap-dependent translation.

though some studies indicate the presence of a heterogeneous population of particles.[5] Binding of initiation factors eIF3 and eIF1A to the 40S subunit, however, leads to disassembly and formation of a pool of small ribosomal subunits.[6] Next, the 40S•eIF3•eIF1A complex binds to the initiator tRNA (Met-tRNAi) associated with the eIF2·GTP complex, which has specific sites for binding to both the 40S subunit and ribosomal-bound eIF3[6] (Fig. 1B). Recruitment of the small ribosome subunit to the 5′ end of mRNA is rate limiting, and highly regulated in the initiation of eukaryotic protein synthesis. This process includes formation of the heterotrimeric

translation initiation complex eIF4F and its association with the Me^7GTP cap structure of mRNA.[7–9] The eIF4F complex consists of eIF4E (4E), the mRNA cap-binding protein; eIF4A (4A), an RNA-dependent ATPase and helicase that unwinds the secondary structure of mRNA and forces the release of bound proteins from the mRNA 5′ end;[6] and eIF4G (4G), a scaffolding protein that serves a docking function in the assembly of the eIF4F complex and associates with the 40S ribosomal subunit through contact with the adapter complex eIF3.[9,10] (Fig. 1C). After eIF4As—the activity of ATPase and helicase is stimulated by eIF4B,[11] a small RNA-binding protein that is involved in ribosomal recruitment to mRNA, eIF4F executes the bridging function between mRNA and the ribosome via cap-eIF4E, eIF4G, and eIF3. Although a consensus has not been reached with regard to the sequence of events leading to the assembly of the eIF4F complex on the 5′-mRNA cap, most experimental results favor a model in which the interaction with the cap structure takes place after the 4F complex has been assembled.[2,6] The 40S ribosome along with its associated initiation factors scans the 5′ UTR until it encounters an initiation codon (AUG or a cognate) (Fig. 1C). Thereafter, the 60S ribosome joins to form the active 80S ribosome.[2,6]

1. Release of the 40S subunit and binding to eIF3 and eIF1A.
2. Binding of eIF2·GTP·Met-tRNAi to the 40S complex.
3. Binding of mRNA to the 40S complex.

eIF4F complex assembly is limited by the abundance of eIF4E, the cytoplasmic cap-binding protein, which specifically recognizes the N^7-methylated guanosine-cap at the 5′-end of mRNA. eIF4E, a 25 kDa polypeptide, is an evolutionarily conserved protein.[12] A growing body of experimental evidence has placed deregulated translation on the causal pathway to cancer.[13] First, cap-dependent translation is constitutively up-regulated in the majority and possibly all human malignancies.[14,15] Overexpression of eIF4E is one of the early events in breast tumorigenesis.[16] Second, mouse tumor models confirm that overexpression of eIF4E is sufficient to transform cells.[17–19] Third, genetic intervention reverses the transformed phenotype in cancer cells by normalizing deregulated translation and is associated with sustained regression of xenograft tumors.[20–23] Together, these findings suggest that the regulation of initiation step of protein synthesis contributes significantly to cancer genesis and that a wide range of malignancies become highly dependent on hyper-activated translation. Thus, each of the proteins involved in the regulation of cap-dependent translation could be considered as an anticancer target. This review will focus on the biological role and biochemical and structural characteristics of one of these proteins, eIF4E. In addition, we will survey major advances, challenges, and future directions of the design and development of antagonists of eIF4E as a potential anticancer target.

2. eIF4E: ANTICANCER TARGET

According to one formulation, cellular mRNAs can be categorized into two groups: strong mRNA, which have relatively short, unstructured 5′ UTRs; and weak mRNA, which have lengthy, highly structured 5′ UTRs.[24–26] The significant difference between the two groups of mRNAs is that weak mRNAs are much more sensitive to eIF4E availability. Weak mRNAs usually encode growth and survival factors whose level of expression are good indicators of eIF4E-relavent experimental cancer models[27] when activated, eIF4E disproportionally and dramatically stimulates translation of a limited and defined set of mRNAs encoding cancer-related proteins that control cell proliferation and viability.[28,29] (Fig. 2). eIF4E functions, therefore, as an oncogene when overexpressed in target cells.

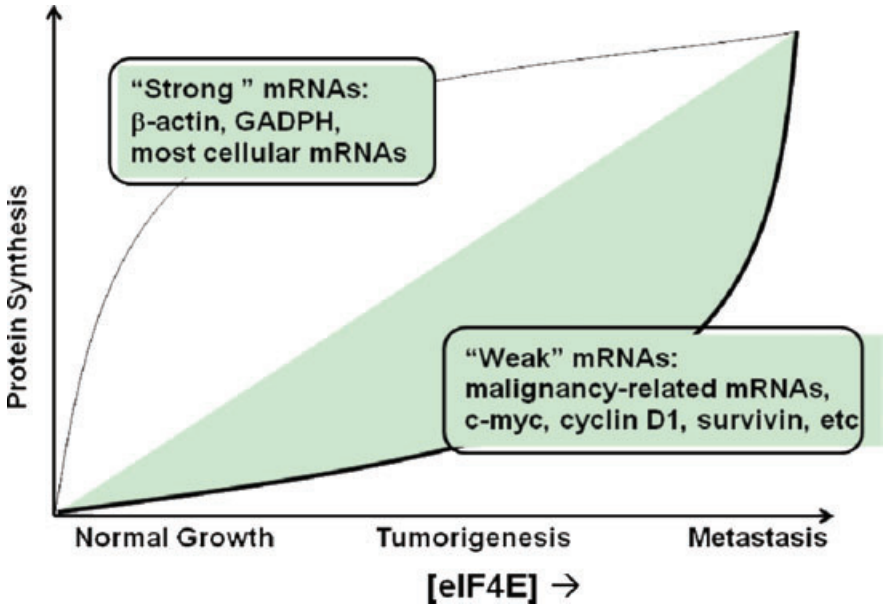


Figure 2. Reducing translation initiation factor eIF4E suppresses malignancy.[27]

Under physiological conditions, the availability of eIF4E is tightly regulated.[25,30,31] eIF4E is negatively regulated by the 4E-binding proteins (4E-BPs). Under normal physiological conditions, eIF4E is bound by 4E-BPs, a family of inhibitory proteins that sequester eIF4E by occupying the same binding site as eIF4G[32–34] (Fig. 3A). Upon trophic stimulation, 4E-BPs are phosphorylated at multiple sites through the RAS-extracellular signal-regulated kinase (RAS-ERK) and PI3 kinase/ATK signaling pathways.[35,36] Phosphorylation releases 4E-BPs from eIF4E and thereby enables the assembly of eIF4F complex followed by the delivery of mRNAs to the eIF4F complex.[15,37,38] Support for this conclusion has been demonstrated by the treatment of cancer cells with rapamycin and inhibitor of mTOR, which results in 4E-BP dephosphorylation and cancer cell growth inhibition.[39] (Fig. 3B). The hypophosphorylated 4E-BPs bind tightly to eIF4E—which cannot form the eIF4F trimer—thus inhibiting cap-dependent translation initiation.[39] Taken together, these results demonstrate that antagonism of eIF4F formation is a potential anticancer approach. Moreover, antagonists of eIF4F formation will not inhibit the translation of all mRNA equally; rather this antagonism will have a disproportionate effect on the translation of those mRNA that are most dependent on the activity of eIF4F. In general, most "oncogenic" mRNAs fall into this category and display differential sensitivity to eIF4F antagonism.[40] In addition, the inhibition of cap-dependent translation should have minimal impact on internal ribosomal entry site (IRES)-mediated translation, conferring further selectivity. Although some reports suggest that inhibition of apoptosis and translation during hypoxia in cancer cells involves IRES-mediated translation,[41–43] this topic is beyond the scope of this review.

In addition to 4EBP phosphorylation, eIF4E has also been found to be phosphorylated in cancer cells.[44] Nevertheless, the importance of eIF4E's phosphorylation status in the context of cancer remains an active area of investigation. Phosphorylation of eIF4E (usually at Ser 209) is mediated by the MAP kinase-interacting protein kinase-1 (Mnk-1). Mnk-1 in turn is activated by the RAS-regulated MAPK/ERK and p38/Jnk kinase-signaling pathways.[45] This process is facilitated by eIF4E binding to eIF4G—an event that brings eIF4E into proximity to

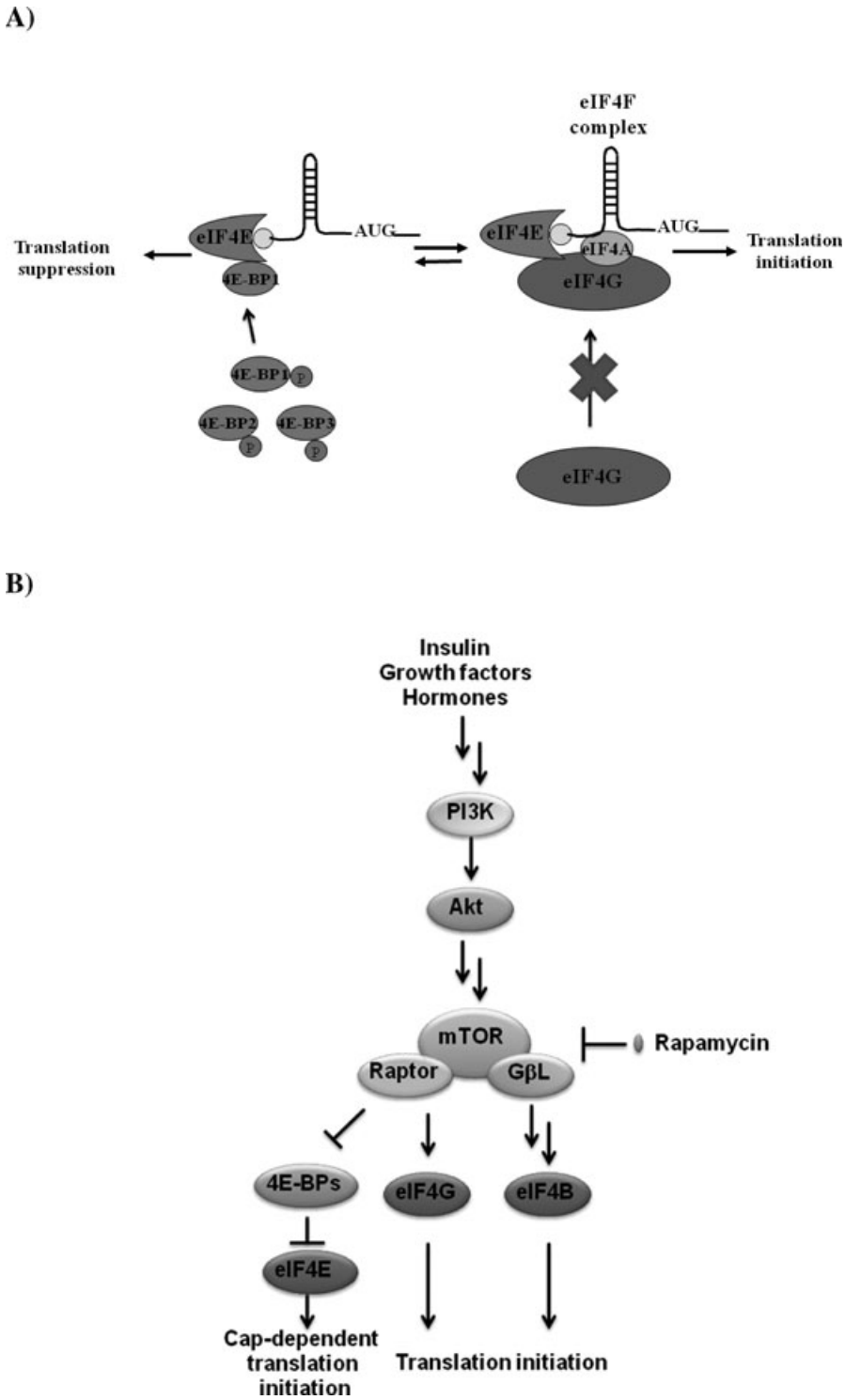


Figure 3. mTOR signaling to translation initiation. **(A)** 4E-BPs antagonism of eIF4F assembly. **(B)** mTOR signaling pathway:[37] The raptor • GbL • mTOR complex mediates the phosphorylation of 4E-BPs and S6K. Rapamycin would bind to mTOR, which dephosphorylates 4E-BPs. This hypophosphorylated form of 4E-BPs binds tightly to eIF4E and thus inhibits cap-dependent translation initiation.

the Mnk-1 kinase.[36] Phosphorylation of eIF4E is associated with the concomitant activation of cap-dependent translation and expression of the transformed phenotype.[46,47] However, the mechanism by which phosphorylation of eIF4E leads to increased translation and the role of phosphorylated eIF4E in cancer initiation and maintenance remains controversial. To promote tumorigenesis, eIF4E must be phosphorylated at Ser 209,[48,49] indicating that the mechanism of translational activation by eIF4E phosphorylation is not directly associated with the cap-binding function of eIF4E. Increased levels of phosphorylated eIF4E have been detected in about 60% of cancers[51] and in a broad spectrum of cancer cell lines.[46] Active eIF4E phosphorylation is required for overexpressed eIF4E to cooperate with deregulated Myc to promote mouse lymphomagenesis.[52] Together, these data support the speculation that increased phosphorylation of eIF4E may be on the cancer pathway. However, neither tumor progression nor aggressiveness correlates with the level of eIF4E phosphorylation.[46] These findings indicate that the role of eIF4E phosphorylation in oncogenesis requires further investigation.

3. eIF4E STRUCTURE AND CAP BINDING

X-ray and Nuclear Magnetic Resonance, NMR (structures of eIF4E with cap-derived analogs, such as guanosine diphosphate) m^7 (GDP),[53,54] m^7GTP,[55] m^7GpppA,[56,57] m^7GpppG[57] have been determined. All reveal that capped nucleotides reside on a concave surface of the protein eIF4E (Fig. 4). Specificity for the 7-methyl guanosine cap structure is achieved by intercalating the nucleobase between two tryptophan residues (W56 and W102) via a cation–π interaction[53,57,58] and hydrogen bonds from the backbone of W102 to the O6 position and two H-bonds from the N2 position to the –NH and carbonyl groups of E103. In the positively charged region of the cap-binding pocket, the α, β, and γ phosphate units interact with the nearby R157 and L152 residues via coulombic interactions, which contribute 3.0, 1.9, and 0.9 kcal/mol, respectively, toward the total binding energy.[59]

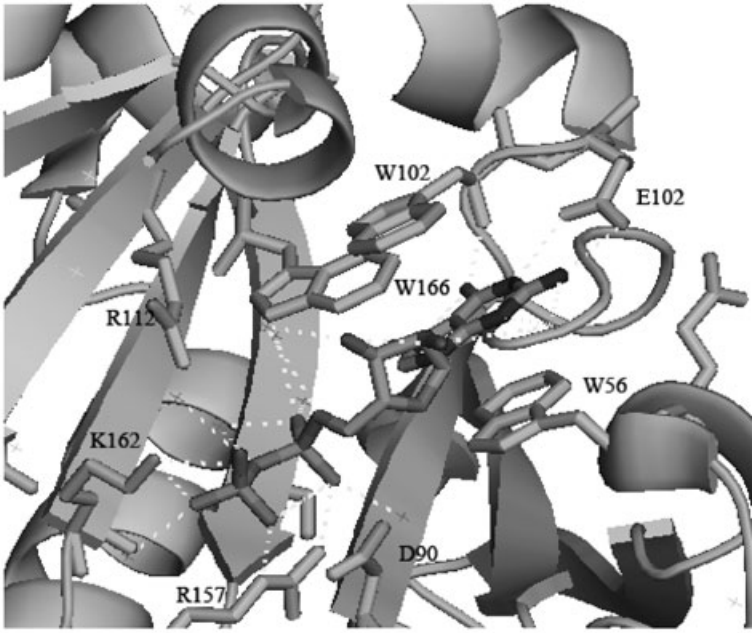
Wagner and co-workers solved the solution structure of yeast eIF4E complexed with m^7GDP by NMR[54] at almost the same time as the crystal structure of mouse eIF4E was solved.[53] The two structures are similar, but not identical. The cap analog m^7GDP in both structures was found to be sandwiched between the side chains of two tryptophan residues, Trp58 and Trp104, in a narrow hydrophobic cleft in both eIF4Es (Fig. 4). Differences include the following: (1) two helices of yeast eIF4E are not reported for the crystal structure of mouse eIF4E, (2) the β strands are slightly longer in mouse eIF4E, and (3) in the cap-binding site, the orientation of the two tryptophan side chains is entirely opposite in yeast eIF4E in comparison to those in mouse eIF4E. Solving the structures of eIF4E in different species and elucidating eIF4E interaction with the cap could be important for understanding the mechanism of translation initiation.

Besides eIF4E, there are several other proteins that recognize the mRNA cap mainly through π–cation interactions. The usually termed eIF4E protein is in fact eIF4E1, the most well characterized and studied member of eIF4E protein family. The eIF4E protein family also includes eIF4E1b, eIF4E2 (also known as 4EHP and 4E-LP), and eIF4E3.[60] These three eIF4E proteins exhibit as much as 200-fold weaker affinities with the cap.[61,62]

In addition, the vaccinia virus cap-modifying enzyme VP39 and the scavenger mRNA-decapping enzyme DcpS also undergo cation–π interactions with the cap. Structural biology studies of other proteins that adopt binding modes similar to that of eIF4E to caps might facilitate the development of drug-like inhibitors of eIF4E.

The interaction of eIF4E with cap analogs has been studied extensively by both equilibrium and kinetic studies, most of which rely on the rationale that the intrinsic fluorescence of eIF4E

A)



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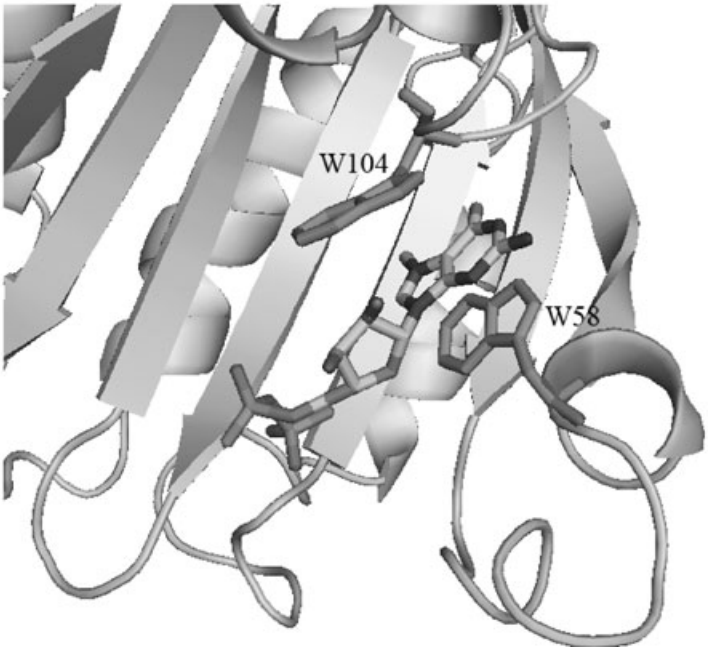


Figure 4. Structure of eIF4E complexed with m^7GDP, **(A)** mouse eIF4E (PDB: 1EJ1), **(B)** yeast eIFE (PDB: 1AP8). (Images generated by PyMOL.)

A) A two-step binding mechanism:

$$\text{eIF4E} + \text{m}^7\text{GpppG} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{eIF4E}\cdot\text{m}^7\text{GpppG}^* \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \text{eIF4E}\cdot\text{m}^7\text{GpppG}^{**}$$

B) A simple one-step binding mechanism:

$$\text{eIF4E} + \text{m}^7\text{GpppG} \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} \text{eIF4E}\cdot\text{m}^7\text{GpppG}^*$$

C) A full one-step binding mechanism:

$$(\text{eIF4E})_n \rightleftharpoons \text{eIF4E} + \text{m}^7\text{GpppG} \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} \text{eIF4E}\cdot\text{m}^7\text{GpppG}^*$$

Scheme 1. Possible mechanisms of m^7GpppG binding to eIF4E.

originating from tryptophans decreases upon cap binding. Other methods of evaluating eIF4E antagonist binding have also been developed, including surface plasmon resonance (SPR),[50] isothermal titration calorimetry[63,64], and NMR.[54] Previous studies have led to a proposed two-step binding mechanism in which an eIF4E·m^7GpppG* complex is formed in the first step accompanied by reduced eIF4E fluorescence, followed by a presumably complex reorganized second step with even lower fluorescence **(Scheme 1)**.[65] The first step is dominated by ligand binding, via the triphosphate moiety, to basic residues (Arg157 and Lys162). In the second step, binding of the m^7G base to the hydrophobic tryptophan-binding pocket is thought to result in a further decrease in fluorescence. Nevertheless, kinetic studies of cap analogs binding to eIF4E have not yielded consistent mechanistic conclusions. Reported rate constants of association, k_{on}, and the rate constants of dissociation, k_{off}, have varied by as much as two to three orders of magnitude.[50,66,67] To better understand the eIF4E–cap interaction mechanism, Rhoads and co-workers[65,68] have carried out a systematic study of cap analog binding to eIF4E, which, surprisingly, supports a one-step binding mechanism.

Stopped flow experiments were carried out under pseudo first-order conditions and varying ionic strengths. Rhoads and co-workers found that the k_{on} value—but not the k_{off} value—for m^7GpppG binding to eIF4E was ionic strength dependent. The values for k_{on} were in the range of 33–292 $\times$ 10^6 $M^{-1}s^{-1}$ (350–50 mM KCl), and those obtained for k_{off} were in the range of 70–87 s^{-1} **(Scheme 1A)**.[65]

Relative high k_{off} values are consistent with a rapid equilibrium process. K_d values calculated from the kinetic constants ranged from 0.24 to 2.48 μM depending on the ionic strength;

such K_ds are three to fivefold higher than those obtained by equilibrium methods.[69] A rationale for this discrepancy has not been proposed beyond noting the differences in protein preparation and methodology. Rhoads and co-workers have suggested that the apparent kinetic mechanism of cap binding to eIF4E is dependent on the concentration of eIF4E.[65] When the concentration of eIF4E is limiting, stopped flow study data were found to fit well to a one exponential function, which is consistent with a simple one-step association cap-binding process **(Scheme 1B)**.[65] However, when the concentration of m^7GpppG is limiting and the concentration of eIF4E varied from 0.5 to 5 μM, stopped flow data were better fit by a two-step binding **(Scheme 1C)**.[65] A fast phase and a slow phase were observed with the amplitude of the slow phase exhibiting a dependence on concentration of eIF4E. Rhoads and co-workers hypothesized that a preexisting equilibrium step was likely responsible for the observed slow phase. They hypothesized that as the concentration of eIF4E increased, the presence of inactive dimmers and/or oligomers may account for the observed slow phase.

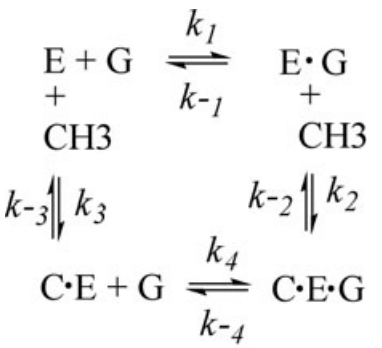
Since it has been reported that phosphorylated eIF4E enhances protein synthesis, Rhoads and co-workers[65] also determined the rate constants of cap binding to phosphorylated eIF4E (eIF4E(P)) by transient state kinetics. Results suggest that the phosphorylation of eIF4E decreased k_{on} by 2.1- to 2.3-fold at 50 to 150 mM KCl but had no effect at 350 mM KCl. Similar to nonphosphorylated eIF4E, the data were best fit to a single-exponential instead of a double-exponential function over all cap analog concentrations. The values of k_{on} for eIF4E(P) were in the range of 34–138 $\times$ 10^6 $M^{-1}s^{-1}$, while the values of k_{off} were in the range of 72–85 s^{-1}. When the K_d values were calculated from the kinetics data, the cap analog was shown to have a twofold higher affinity for eIF4E over eIF4E(P) with the exception of values determined at the highest KCl concentration (350 mM).[65] In addition, similar to eIF4E, the kinetic value derived K_d values were found to be approximately fivefold greater than the values determined by equilibrium methods.[69]

Since most studies of cap binding to eIF4E have been performed with mono- or dinucleotide cap analogs, Rhoads and co-workers[65] also carried out studies with a more physiologically relevant ligand, a capped oligoribonucleotide. This 12-mer oligonucleotide contained the antireverse cap analog (ARCA) $m_2^{7,3'\text{-O}}$GpppG[70] to ensure that all caps were in the correct orientation while all previous studies of eIF4E interaction with capped oligonucleotides[50,71,72] have used a mixture of normally capped and reverse-capped oligoribonucleotides that do not bind eIF4E. The binding affinities of m^7GpppG and $m_2^{7,3'\text{-O}}$GpppG to eIF4E were found to be nearly identical as determined by fluorescence-quenching experiments.[73] The binding affinities of capped oligonucleotides were found to be higher than that for ARCA oligonucleotides at high ionic strength, largely due to lower k_{off} values. At lower ionic strength, for instance, only modest differences in the affinities of the two oligos with either eIF4E or eIF4E(P) were observed. The results of these eIf4E stopped-flow kinetic-binding studies with capped oligonucleotide again suggested a one-step binding process, with decreased binding affinity at high ionic strength (350 mM KCl) for both eIF4E and eIF4E(P) to cap analog ARCA (Fig. 5).[65]

The authors hypothesized that the pronounced increase in affinity of the capped oligonucleotide may result from additional stabilizing interactions formed at low ionic strength with the larger ligand. Since there are several basic amino acid residues located on the surface of eIF4E both near the cap-binding side and on the lateral surface of a groove where the polynucleotide chain of mRNA conceivably could be found, it is possible that additional interactions occur between the polynucleotide chain and the basic side chain(s).

It is well established that eIF4E binds not only to capped mRNA but also to eIF4G during eIF4F assembly. Several studies have reported that the cap binding to eIF4E is modulated by its interaction with eIF4G. The basis for this assumption has been supported by several lines of evidence: (1) Ultraviolet radiation (UV) cross-linking of recombinant murine eIF4E to chloramphenicol acetyltransferase mRNA is markedly enhanced in the presence of human eIF4G (182–

A)



B)

Reaction	$k_{on} \times 10^{-6}$ ($M^{-1}s^{-1}$)	k_{off} (s^{-1})	K (nM)
1	2.6 ± 0.9	0.012 ± 0.005	4.6 ± 1.7
2	179 ± 8	79 ± 12	440 ± 90
3	184 ± 10	83 ± 23	450 ± 130
4	3.2 ± 1.5	0.014 ± 0.004	4.4 ± 1.3

Figure 5. Thermodynamic cycle and kinetic constants for assembly of the ternary complex cap analog·eIF4E·eIF4G.[68] **(A)** A scheme of the thermodynamic cycle. E: eIF4E, G: eIF4G(557– 646), C: cap analog (m^7GpppG). **(B)** Kinetic and thermodynamic constants obtained by stopped flow fluorescence quenching and SPR for reactions represented in A.

1600),[72,74] (2) the affinity of full-length recombinant human eIF4E to a fluorescently labeled cap analog (Ant-m^7GTP) is enhanced twofold in the presence of a 20-amino acid peptide bearing the consensus-binding sequence of human eIF4G,[75] and (3) m^7GTP-Sepharose pull-down assays suggest that the affinity of full-length recombinant yeast eIF4E to m^7GTP or a short-capped RNA is increased in the presence of a full-length recombinant yeast eIF4G-1 or eIF4G (393–490).[76–78] In contrast, other studies have suggested that eIF4G has minimal impact on eIF4E cap binding. Goss and co-workers found little difference in the binding affinity of tissue-

derived human eIF4E and rabbit eIF4F to rabbit globin mRNA.[72] Moreover, the presence of a 12 amino acid peptide containing the eIF4E-recognition motif of mammalian eIF4G-1 had little influence on the binding affinity of recombinant murine eIF4E to cap analogs.[57] In addition, the crystal structure of murine eIF4E in complex with the 12 amino acid eIF4G peptide was found to be isomorphous with the eIF4E·cap complex.[36]

To better understand the effect of eIF4G on eIF4E binding to the cap, Rhoads and co-workers carried out a series of transient state kinetics studies of the human eIF4E–m^7GpppG interaction with a human eIF4G (557–646)-eIF4E binding peptide utilizing stopped flow fluorescence-quenching method as well as SPR.[68] Their results suggested that eIF4G does not affect the rate constants for association or dissociation of m^7GpppG to eIF4E. The binding of m^7GpppG to the eIF4G(557–646)·eIF4E complex yielded a k_{on} value of $179 \pm 8 \times 10^6$ $M^{-1}s^{-1}$ and a k_{off} value of 79 ± 12 s^{-1} (Fig. 5, reaction 2).[68] These values, within experimental errors, do not differ significantly from the values (k_{on} of $184 \pm 10 \times 10^6$ $M^{-1}s^{-1}$ and k_{off} of 83 ± 23 s^1) determined in the absence of eIF4G(557–646) (Fig. 5, reaction 3).[68] Rhoades and co-workers concluded that while eIF4G has higher affinity for eIF4E than capped RNA, the formation of the eIF4E·m^7GpppG·eIF4G complex proceeds through a sequential, random kinetic mechanism. Thus, there is no preferential pathway for the formation of the complex.

4. APPROACHES TO EIF4E-SPECIFIC ANTICANCER THERAPY

With evidence implicating the eIF4E complex in tumorigenesis, the development of eIF4E-specific anticancer therapies has begun to attract attention. These efforts have largely focused on disrupting (1) the binding of eIF4F to capped mRNA, (2) eIF4F integrity, and (3) eIF4F enzyme activity.

A. Targeting the Interaction of eIF4E and Me^{7}G-Capped mRNA

Over the years, several nucleoside and nucleotide analogs derived from m^7GTP have been synthesized and evaluated as tools capable of modulating translation initiation in general and inhibiting eIF4E binding to capped mRNA specifically. Darzynkiewicz and co-workers have designed and synthesized various cap analogs including modifications at the N7 and N2 positions of the guanine moiety, the 5′-phosphate moiety, and the ribose ring (Fig. 6).[59,79,80] Since aryl substitution at N7 has shown an increased binding affinity, studies have therefore been focused on the utility of the synthetic nucleotide derivative 7-benzyl guanosine monophosphate (Bn^7GMP) to block the binding of eIF4E to the mRNA cap.[22,79]

Recent crystal structures comparing the eIF4E complexed with Bn^7GMP (Fig. 7A), Bn^7GMP (Fig. 7B), and *p*-F Bn^7GMP (Fig. 7C) have shown the cap-binding site to be inherently flexible and capable of conformational changes upon binding to the N^7-benzylated mononucleotide cap analogs.[55] The indole side chain of W102 flips 180Ó, allowing the bulkier benzyl group to pack into the hydrophobic cavity between W102 and W56, while still maintaining the ring stacking and hydrogen bonds necessary for efficient interactions between the analogs and residues in the cap-binding site. Gains in binding affinity due to placement of the N^7-benzyl substituent in the hydrophobic pocket dorsal to the tryptophan "clamp"[55] offers a possible explanation for a 3.5-fold increase in the strength of interaction in going from m^7GMP ($K_i = 382$ μM) to Bn^7GMP ($K_i = 113$ μM)[59] when assayed as inhibitors of in vitro translation. Likewise, K_ds determined using fluorescence titration experiments between purified eIF4E and cap analogs showed fivefold better binding affinity between m^7GMP ($K_d = 7.50 \pm 0.40$ μM and Bn^7GMP ($K_d = 1.32 \pm 0.06$ μM) upon replacement of N^7-methyl substituent by a benzyl group.[82]

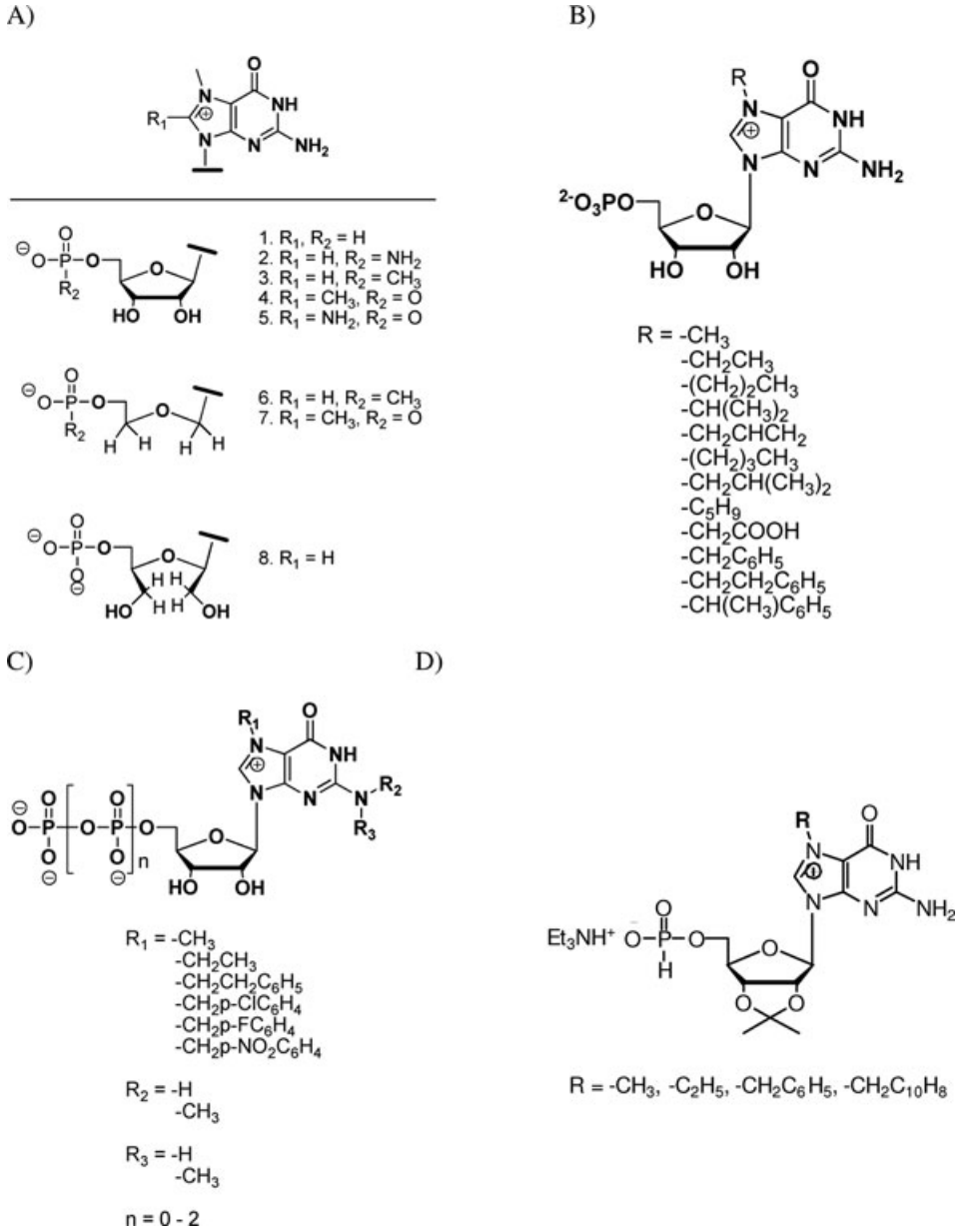


Figure 6. Structures of mRNA cap analogs. **(A)** substitutions at 5′-phosphate moieties and/or the ribose ring,[80] **(B)** aryl and alkyl substitutions at N7 of guanosine monophosphate,[79] **(C)** substitutions at N7, N2, and phosphate,[59] **(D)** constraining 2′-, 3′-hydroxy groups.[81]

The 2′- and 3′-hydroxyl groups of cap analogs do not interact with eIF4E and remain solvent exposed. Examination of the role of the ribose ring conformation by constraining it with an isopropylidine group at 2′- and 3′-positions (Fig. 6D) has been shown to enhance the potency of a N-7-benzylated cap derivative over twofold compared to the one with 2′- and 3′-hydroxyls free and the ribose ring unconstrained.[81]

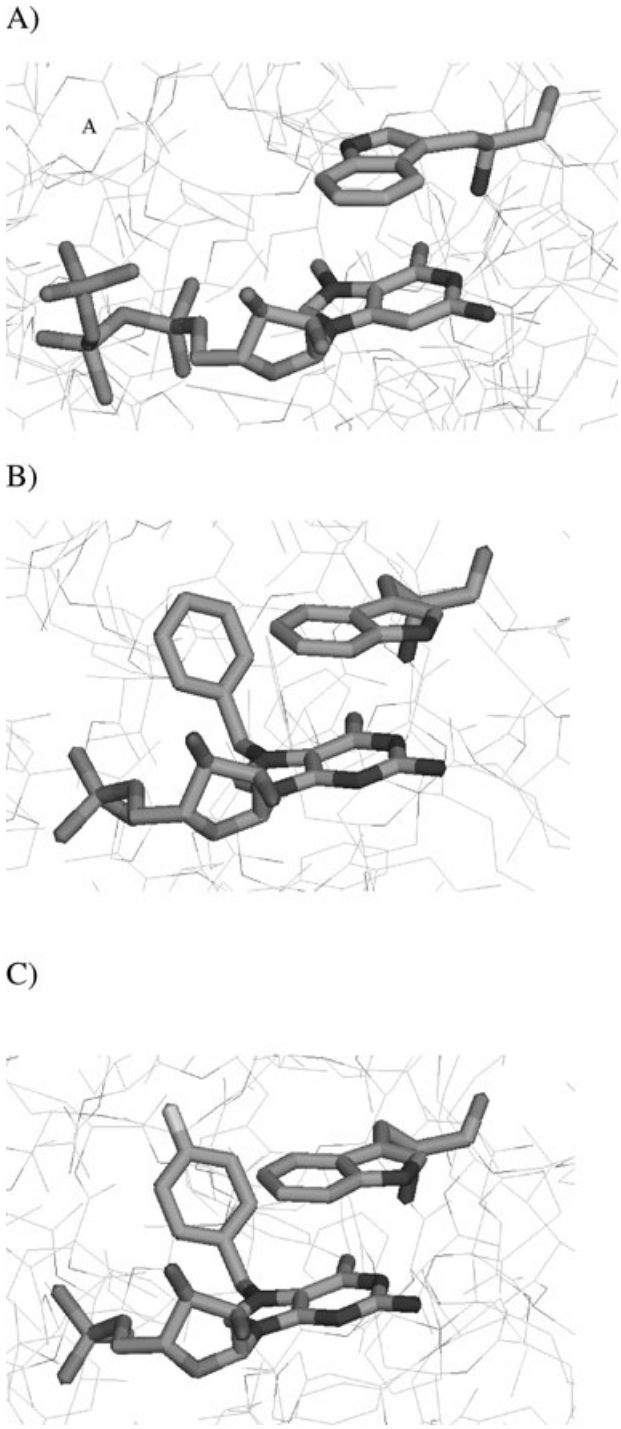


Figure 7. Comparison of eIF4E ligand structures—**(A)** m^7GTP (PDB: 2V8W), **(B)** Bn^7GMP (PDB: 2V8X), **(C)** p-F Bn7GMP (PDB: 2V8Y.[55] (Generated by PyMOL.)

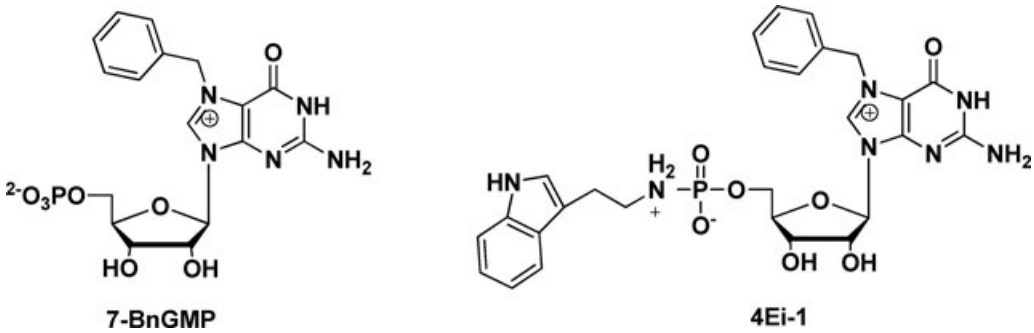


Figure 8. Structures of Bn^7GMP and 4Ei-1.

Highly predictive and consistent three dimensional quantitative and structure-activity relationship (3D-QSAR) models have been derived from a combinatorial library of Bn^7GMP analogs which pinpointed several key interactions involved in the cap binding to eIF4E.[82] While effective in cell-free systems, the efficacy of Bn^7GMP in cells is poor due to its low intracellular uptake. One approach to improve its in vivo activity is to develop a stable, cell-permeable prodrug which can be, enzymatically or chemically, converted into an active species.[83]

Phosphoramidates are of special interest for this purpose, considering their generally high water solubility, low to nonexistent toxicity, and high stability. In fact, there have been successful examples of their applications for antiviral and anticancer purposes.[84–88]

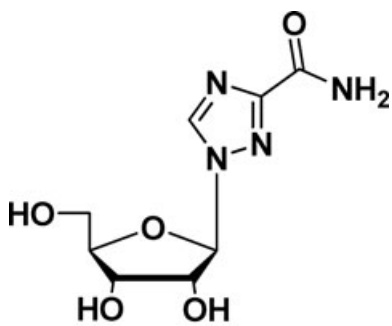
Recent work by Wagner and co-workers has led to the development of a novel class of Histidine Triad Nucleotide Binding Protein (Hint)-dependent pronucleotides that interfere the epithelial-to-mesenchymal transition (EMT), a key translationally regulated step in the development of epithelial cancers as well as pathological tissue fibrosis, by negatively regulating the association of the cap with eIF4E.[89] The compound 4Ei-1 (Fig. 8), a Bn^7GMP-derived phosphoramidate, potently inhibited in a dose-dependent manner cap-dependent translation in zebrafish embryos without causing developmental abnormalities. In addition, it inhibited up to 30% of cap-dependent translation without causing disturbanceon cell division or development.[89] More significantly, 4Ei-1, not toxic by itself, prevented eIF4E from triggering EMT in zebrafish ectoderm explants. Metabolism studies with whole zebra fish lysate confirmed that 4Ei-1 was converted to the active component Bn^7GMP rapidly presumably by zebra fish Hint. The presence of Hint activity as early as the eight-cell stage has recently been established, thus demonstrating its possible role in the early stages of embryogenesis.[90] 4Ei-1 is the first nontoxic small molecule able to inhibit EMT and represents the successful utilization of a prodrug strategy for the intracellular release of Bn^7GMP resulting inhibition of cap-dependent translation.

Ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-caboxamide) (Fig. 9) has been proposed as an eIF4E antagonist because of the potential for the base in the protonated state to be positively charged and thus, similar to Me^7GTP, capable of forming π–cation interactions with the cap-binding site, "tryptopan clamp."[91,92] Clinically, ribavirin has been used for the treatment of viral infections, such as Lassa fever virus, respiratory syncytial virus, hepatitis C virus, and severe acute respiratory syndrome coronavirus.[93–95] Ribavirin is phosphorylated by cellular kinases in vivo, with ribavirin triphosphate (RTP) being the major intracellular metabolite.[96,97] It is still not clear how to account for ribavirin's antiviral activity, though different mechanisms have been suggested, such as inhibition of inosine monophosphate dehydrogenase,[98] incorporation as an RNA virus mutagen (templates incorporation of cytidine and uridine),[99–101] and inhibition of viral RNA capping.[102,103] Studies also suggest that ribavirin can strongly inhibit the proliferation of cells which were not infected with DNA or RNA viruses.[104] Considering

the possibility that these identified mechanisms of ribavirin are not mutually exclusive and they may indeed cooperate to result in its reported efficacy, it is reasonable to posit that the mechanism of ribavirin involves modulating translation. Along these lines, Kentsis and co-workers reported the direct binding of ribavirin to eIF4E with an apparent K_d of 8.4 μM in vitro using a fluorescence-quenching assay, while the ribavirin analog 1-β-D-ribofuranosyl-1,2,3-triazole-4-carboxamide (Rib4C), which contains an uncharged 1,2,3-triazole moiety, failed to bind eIF4E.[105] More significantly, they demonstrated that RTP and m^7GTP bound to eIF4E with nearly equal affinities ($K_d \sim 0.1$ μM). By using m^7G-Sepharose affinity chromatography, they determined that RTP competed with m^7G binding to eIF4E with an apparent inhibition constant K_i of 0.3 μM, which is similar to that from m^7GTP.[105] Furthermore, ribavirin was found to disrupt the translation of mRNAs regulated by eIF4E posttranscriptionally. Ribavirin treatment caused a reduced level of cyclin D1 with an apparent EC_{50} (which is the effective concentration needed to reduce the level of cyclin D1 by 50% relative to a control) of 0.1–1 μM. They also showed that ribavirin potently suppressed eIF4E-mediated oncogenic transformation in NIH 3T3 cells with an EC_{50} of 0.1–1 μM. After treatment with ribavirin, significant suppression of tumor growth in a mouse model of human squamous cell carcinoma was recently demonstrated.[105]

In contrast, Yan and co-workers reported that in a chemical cross-linking assay, RTP did not impair the ability of eI4E to interact with capped mRNA.[106] This result was further confirmed with independent cap-affinity chromatography experiments demonstrating that RTP was unable to block eIF4E binding to Me^7GTP. Since effects on eIF4E activity by cap analogs can be evaluated as inhibition of cap-dependent translation,[59,79,80] the authors also used translation extracts to determine whether RTP could inhibit cap-dependent protein synthesis. Neither GDP nor RTP affected the synthesis of either *Renilla* luciferase (cap-dependent), or *Firefly* luciferase (cap-independent), while the synthesis of *Firefly* luciferase was inhibited by m^7GDP in a dose-dependent manner.

Kentsis and co-workers have rebutted Yan and co-workers by suggesting that the binding of cap to eIF4E is highly dependent on solution conditions.[107] They argued that any change in ionic strength, pH, or temperature could result in a variation of several orders of magnitude for cap-binding affinity to eIF4E.[57,108,109] Kentsis and co-workers repeated the affinity chromatography experiments provided using their published conditions and compared them to those used by Yan and co-workers. They found once again that micromolar concentrations of RTP competed with the binding of m^7GTP·eIF4E.[107] In contrast, RTP failed to compete with m^7GTP binding when the protocol of Yan and co-workers was used. Thus, they concluded that the reported



Ribavirin

Figure 9. Structure of ribavirin.

failure of ribavirin binding to eIF4E in vitro by Yan and co-workers was probably the result of different experimental solution conditions. With regard to the in vitro translation experiments by Yan and co-workers, Kentsis and co-workers argue that cell extracts may not properly reflect conditions in living cells. For the same reason, Kentsis and co-workers emphasized the importance of assessing ribavirin like compounds' functionality in vivo in order to determine the physiological relevance of those interactions. Kentsis and co-workers also provided a direct observation of the specific binding of ribavirin to eIF4E using mass spectrometry confirming specific binding.[107]

Independently, Westman and co-workers[110] have corroborated the findings of Yan and co-workers. Intrigued by Kentsis's work, Westman and co-workers tested ribavirin, RTP, and the dinucleotide RpppG for their ability to inhibit translation in vitro and explored their possible intrinsic relation with eIF4E; a design intended to determine whether these ribavirin-containing analogs could be substitutes for natural caps once incorporated into mRNA. Surprisingly, their in vitro translation assay suggested that these ribavirin-containing compounds did not inhibit translation at concentrations at which conventional cap analogs could effectively block cap-dependent translation, though inhibition was observed at high concentrations (millimolar). However, their work suggested that this inhibition effect at very high concentrations was inconsistent with an action through blocking eIF4E. Experimentally, they also excluded other possibilities that could lead to the poor translation activity, such as metabolic instability of these compounds in the translation system and failure to cap in the incorrect orientation. Furthermore, their independent fluorescence titration experiments suggested very low binding affinity of ribavirin and its derivatives to recombinant murine eIF4E and human recombinant nuclear cap-binding complex, two to four orders of magnitude lower than the values for true cap analogs. Therefore, they hypothesized that it was the guanine, rather than the triazole moiety, that penetrated the binding surface of eIF4E in the case of RpppG. Taking all the evidence together, they conclude that ribavirin is not a structural or functional mimic of the 7-methyl guanosine moiety for translation in vitro, even though ribavirin has confirmed translational inhibition effect in vivo. From our point of view, many—if not all—of these discrepancies are largely due to differing experimental methods and conditions. The field would be greatly advanced if investigators would agree to establish a systematic, standardized set of assays.

A proof-of-principle clinical trial with ribavirin has recently been reported on acute myelogenous leukemia patients. The clinical responses to ribavirin correlated with both reduced eIF4E expression and the relocation of eIF4E to the cytoplasma.[111] These findings seem to suggest that ribavirin is indeed a promising anticancer drug candidate functioning, at least in part, as an eIF4E inhibitor, but possibly not as a direct inhibitor.

B. Targeting eIF4E and eIF4G Interaction

eIF4E/eIF4G complex assembly plays a pivital role in the regulation of gene expression at the stage of translation initiation, an interruption of which by small molecules would establish a possible new strategy for cancer therapy. The eIF4E/eIF4G complex is regulated by the 4E-BPs, which binds to the same binding site of eIF4E as eIF4G. Results have suggested that increased level of 4E-BPs has tumor-suppressor activity.[112] Protein–protein interfaces typically contain "hot spots," which are compact regions of conserved residues significant to protein–protein binding and thus provide a target for the development of small molecule inhibitors of protein–protein interaction. High throughput screening of compound libraries has been applied to identify small molecule modulators of translation initiation.[21,113,114]

A high throughput fluorescence polarization-binding assay has been established to identify small molecules, which specifically disrupt the interaction of eIF4E and eIF4G.[22] A peptide containing the sequence KYTYDELFQLP ($K_d = 3\ \mu$M) was synthesized and labeled with a

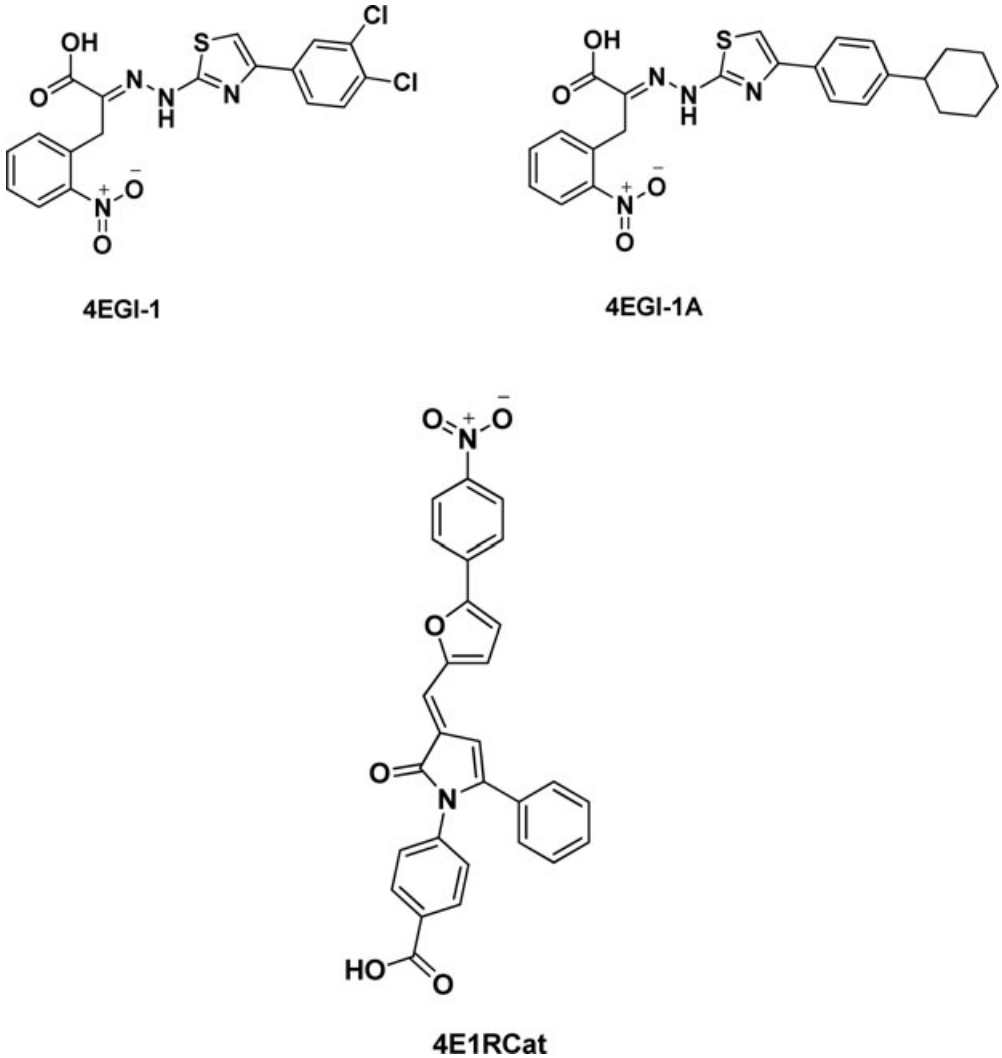


Figure 10. Structures of 4EGI-1, 4EGI-1A, and 4E1RCat.

fluorescein tag. The principle of the high throughput screening was to identify compounds that displace the fluorescein-labeled peptide from eIF4E by detecting the fluorescence polarization. A library of 16,000 compounds (Chembridge DiverSet E) was screened, and a small molecule 4EGI-1 was identified as a competitive inhibitor of eIF4G peptide binding (16 $\pm$ 6 μM). A similarly active analog, 4EGI-1A (25 $\pm$ 11 μM) was also synthesized (Fig. 10).

Both 4EGI-1 and 4EGI-1A caused displacement of eIF4G from eIF4E in a dose-dependent manner and inhibited cap-dependent translation in vitro in *Renilla luciferase* reporter assay system. Neither compound had any inhibitory effect on cap-independentIRES-driven translation. Similar effects were observed in several mammalian cancer cell lines treated with 4EGI-1; 4EGI-1 treatments caused cell death in the Jurkat cell line and inhibited the proliferation of A549 lung cancer cells. Since 4E-BP1 competes with eIF4E for binding to eIF4G, 4EGI-1 would be expected to inhibit binding of 4E-BP1 to eIF4E. However, in both in vitro and cancer cell line experiments, 4EGI-1 caused a significant apparent increase in the amount of 4E-BP1 that

is bound as shown by the results from an m^7GTP pull-down assay. One possible explanation is that 4EGI-1 displaces eIF4G from eIF4E and clears the docking site for 4E-BPs since 4E-BPs probably occupy a larger footprint though eIF4G and 4E-BPs bind eIF4E at overlapping sites.[7,8]

Though 4EGI-1 was discovered as a small molecule inhibitor of eIF4E that disrupted the interaction of eIF4E and eIF4G, a recent study by Fan et al.[51] revealed a novel biological function of 4EGI-1: sensitizing human lung cancer cells by augmenting tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-mediated apoptosis independent of cap-dependent translation. The death ligand TRAIL binds to its receptors 4 and 5 (DR4 and DR5) and therefore mediates apoptosis, preferentially in transformed or malignant cells but not in normal cells.[115] Therefore, TRAIL has attracted research interest as a cancer therapeutic target, and currently, recombinant human TRAIL is being tested in clinical trials as an anticancer agent.[116]

Nevertheless, certain cancer cells are intrinsically resistant to TRAIL/death receptor-induced apoptosis. Thus, certain sensitization agents have been used in combination with TRAIL to enhance TRAIL-based cancer therapy.[117–120] Additionally, Panner et al.[121,122] define translational control as a new mechanism in which the mTOR pathway plays an important role in control of death ligand-induced apoptosis and TRAIL sensitivity. They discovered that TRAIL resistance in glioblastoma multiforme was a consequence of overexpression of the short isoform of the c-FLICE inhibitory protein ($FLIP_s$) and that $FLIP_s$ expression is translationally upregulated by activation of mTOR pathway in which both S6 kinase and eIF4E are activated and drives the association of the $FLIP_s$ mRNA with translating polyribosomal complexes. With the understanding of the linkage between death ligand-induced apoptosis and translation control, Fan et al.[51] attempted to resolve the intrinsic TRAIL resistance in certain cancer cells by testing the efficiency of 4EGI-1 and TRAIL, alone and combined, in human lung cancer cells. Fan et al.[51] found that 4EGI-1 alone not only induced apoptosis of human lung cancer cells but also cooperated with TRAIL for enhanced apoptosis. As expected, 4EGI-1 as a single agent inhibited eIF4E/eIF4G complex formation, inhibited the cell growth, and induced apoptosis of human lung cancer cells. Moreover, the combination of 4EGI-1 and TRAIL was much more effective than either 4EGI-1 or TRAIL alone in terms of killing human lung cancer cells. The detailed mechanistic studies of Fan et al. suggested that the 4EGI-1 increased DR5 expression through a CHOP-dependent mechanism (CHOP refers to a chemotherapy regime that consists of cyclophosphamide, hydroxydaunorubicinm, oncovin, and prednisolone) and reduced c-FLIP levels through ubiquitin/proteasome-mediated degradation, both of which are often involved in drug-induced sensitization of TRAIL/death receptor-induced apoptosis. Interestingly, neither knockdown of eIF4E with eIF4E siRNA nor rapamycin increased DR5 expression, reduced c-FLIP or enhanced TRAIL-induced apoptosis in the cancer cells they tested, suggesting that the 4EGI-1 modulation of DR5 and c-FLIP and the enhancement of TRAIL-induced apoptosis are independent of inhibition of cap-dependent translation, thus suggesting that the biological activity of the compound is at least partially dependent on a significant "off target" mechanism.

Another small molecule inhibitor 4E1RCat (Fig. 10), which disrupts the eIF4E and eIF4G interaction, has been recently discovered by Pelletier and co-workers.[123] 4E1RCat was found after screening a library of 217,341 compounds using a time resolved (TR)-fluorescence resonance energy transfer (FRET) based assay. Molecular modeling of 4E1RCat binding to eIF4E suggests a potential mechanism by which 4E1RCat inhibits the interactions between eIF4E and either eIF4G or 4E-BP1 by possibly binding to four shallow pockets of eIF4E which overlap with the eIF4G- and 4E-BP1-binding sites. Western blot analysis confirmed that 4E1RCat indeed blocked formation of eIF4E:eIF4G and eIF4E:4E-BP1 complexes. 4E1RCat inhibited

	49 54 56 59 68
4EBP-WT	Gly-Thr-Arg-Ile-Ile-Tyr-Asp-Arg-Lys-Phe-Leu-Met-Glu-Gys-Arg-Asn-Ser-Pro-Val-Thr
TAT	Tyr-Gly-Arg-Lys-Lys-Arg-Gln-Arg-Arg
TAT-4EBP-WT	Tyr-Gly-Arg-Lys-Lys-Arg-Gln-Arg-Arg-Gly-Thr-Arg-Ile-Ile-Tyr-Asp-Arg-Lys-Phe-Leu-Met-Glu-Gys-Arg-Asn-Ser-Pro-Val-Thr
[DLys6]GnRH	pGlu-His-Trp-Ser-Tyr-DLys-Leu-Arg-Pro-Gly
[DLys6]GnRH-4EBP1-WT	pGlu-His-Trp-Ser-Tyr-DLys-Leu-Arg-Pro-Gly-Gly-Thr-Arg-Ile-Ile-Tyr-Asp-Arg-Lys-Phe-Leu-Met-Glu-Gys-Arg-Asn-Ser-Pro-Val-Thr

Figure 11. Peptide agonists of eIF4E binding to eIF4G derived from 4EBP-1 (conserved residues 54, 56, and 59 of 4EBP proteins are important for binding to eIF4E).[123, 125]

cap-dependent translation in a dose-dependent manner, with an IC_{50} of ~4 μM, while not affecting cap-independent translation in an in vitro translation assay. In addition, 4E1RCat inhibited protein synthesis in vivo in MDA-MB-231 and HeLa cells without greatly affecting RNA or DNA levels. These results demonstrate that while 4E1RCat enhances the amount of free eIF4E by reducing the amount of eIF4E:4E-BP1, it also reduces the concentration of translationally active eIF4E:eIF4G, thus functioning as a small molecule mimic of 4E-BP1.

Significantly, Pelletier and co-workers have demonstrated that 4E1RCat is capable of reversing tumor chemoresistance in a genetically engineered Eμ−Myc lymphoma mouse model.[123] Treatment of mice bearing Pten^{+}/$^{-}$Eμ−Myc with 4E1RCat and doxorubicin extended tumor-free remissions for up to 14 days over a control set treated with just doxorubicin. Examination of the tumors revealed that animals treated with 4E1RCat and doxorubicin exhibited an increased number of apoptotic cells and a decreased level of the antiapoptotic protein, Mcl-1, when compared to animals dosed with only doxorubicin. Taken together, these results demonstrate that 4E1RCat is an exciting new small pharmacophore that has the potential to be useful for the development of additional chemical genetic probes and drugs targeting the eIF4E:eIF4G interface.[124]

Another strategy developed by Naora and co-workers[123, 125] is to use 4EBP-based peptides to interfere with the interaction of eIF4E with eIF4G. Since phosphorylation of the 4EBPs by mTOR releases 4EBPs from eIF4E and then eIF4E binds to eIF4G, 4EBP-based peptides might bind eIF4E, and therefore prevent eIF4E from binding eIF4G. They synthesized a peptide containing residues 49–68 of 4EBP1, and fused the peptide to an analog of gonadotropin-releasing hormone (GnRH), [DLys6]GnRH (Fig. 11). The utilization of the fusion of a 4EBP peptide to a GNRH agonist (GnRH-4EBP1-WT) is mainly based on the following considerations: (1) GnRH-R1 is overexpressed in epithelial ovarian cancers and other endocrine cancers, which is suited for a targeted strategy, (2) GnRH agonists possess the capability to facilitate cellular uptake of the 4EBP peptide since they are efficiently internalized by receptor-mediated endocytosis, (3) the small size of GnRH agonists insures it will not interfere with the binding of the 4EBP peptide to eIF4E. They found that the peptide, [DLys6]GnRH-4EBP1-WT, bound to eIF4E and inhibited cap-dependent translation in multiple cell lines that express GnRH-R1, while the GnRH agonist alone did not bind eIF4E. In addition, a fusion peptide that contains mutations of three critical residues for 4EBP binding did not inhibit cap-dependent translation. Naora and co-workers[123, 125] found that [DLys6]GnRH-4EBP1-WT peptide inhibited growth of GnRH-positive cells in a dose-dependent manner whereas no inhibition was observed in cells that lack GnRH-R1 expression. More significantly, treatment of female nude mice bearing i.p. ovarian tumor xenografts with the fusion peptide suggested a ~30% reduced tumor burden compared to that with the [DLys6]GnRH truncated peptide alone or with saline. Although the author could not totally exclude the possibility that this 4EBP-based peptide might inhibit growth in part by mechanisms unrelated to inhibiting eIF4E activity, their approach has resulted in a potent compound that is able to target eIF4E in epithelial ovarian cancer. Intriguingly, the approach of Naora and co-workers should be generally applicable to the design

of tissue-specific agonists of eIF4E, by substitutions of the GnRN peptide agonist with other tissue-specific targeting peptides.

C. Targeting Specific RNAs—Antisense Oligonucleotides (ASOs)

Graff and co-workers have provided the first in vivo evidence that cancers may be more susceptible to eIF4E inhibition than normal cells using eIF4E-specific ASO administration.[21] ASOs have been substantially explored as pharmacological tools and therapeutics. They recognize and hybridize target mRNA by Watson–Crick base pairing, followed by RNase H-mediated RNA destruction.[126]

Several ASOs have been designed to target growth factors, kinases, Bcl-2, etc. as cancer therapeutics.[126–128] Earlier generation of ASOs, however, have innate defects for their utilization in system therapy, such as resistance to nuclease and tissue stability issues. The second generation of ASOs, by contrast, has been engineered to accommodate these issues by incorporating modifications such as 2′-O-methyl and 2′-O-methoxyethyl (MOE). Their clinical trials are currently in process. These modifications have resulted in a three- to tenfold increase in biological activity, altered pharmacokinetic properties, and increased nuclease resistance and tissue stability.[126,129–131]

Graff and co-workers designed ASOs capable of targeting both murine and human eIF4E and evaluated their in vivo effects on eIF4E reduction in both human xenograft tissues and normal mouse tissues.[21] Both mRNA levels and protein levels of eIF4E in cultured human cancer cells were suppressed by treatment with eIF4E ASO. Four ASOs were selected for the inhibition of eIF4E in cultured human and murine cells. The sequences are as follows: eIF4E-ASO1 5′-TGCTATCTTATCACCTTTAG-3′, eIF4E-ASO2 5′-GGCGAATGAGACTTCTCTTA-3′, eIF4E-ASO3 5′-TCCTGGATCCTTCACCAATG-3′, and eIF4E-ASO4 5′-TGTCATATTCCTGGATCCTT-3′ (the MOE-modified based are underlined). These ASOs reduce eIF4E RNA expression level by over 50% at a concentration of 25 nM in human tumor cells and murine endothelial cells.[21] ASOs also substantially decreased the expression of many malignancy-related proteins, including the oncogenes c-myc and cyclin D1, the antiapoptotic proteins Bcl-2, the angiogenesis factor VEGF, and the inhibitor of apoptosis protein survivin.[21] The decrease was observed 72 hr after transfection with eIF4E-ASO, along with decreased eIF4E expression. This study is consistent with the previous hypothesis that altering eIF4E levels selectively changes the expression of growth regulatory proteins.[126]

In contrast, altering eIF4E levels has limited effects on global protein synthesis.[15] ^{35}S incorporation into total protein was reduced by only 20% in MDA-MB-231 cells transfected with 75 nM ASO compared to the mock-transfected control.[21] Similarly, for Hela cells, ^{35}S incorporation into total protein was only marginally reduced after transfection with 100 nM eIF4E-ASO while eIF4E RNA expression decreased over 80%.[21] Furthermore, systemic eIF4E-ASO administration decreases eIF4E expression in xenograft tumors, and suppresses tumor growth and angiogenesis.[21] eIF4E-ASO dosing revealed a 64% reduction in eIF4E expression in the MDA-MB-231 breast cancer xenograft tumors.[21] Similarly, a 56% reduction in eIF4E expression was observed in the PC-3 human prostate cancer xenografts in mice dosed with 25 mg/kg thrice weekly for 2 months. More significantly, eIF4E levels decreased in normal mouse tissues without obvious toxicity. There was no noticeable change in liver weight (where ASOs preferentially accumulate), spleen weight, body weight, or liver transaminase levels in ASO-treated nontumor bearing mice, although the expression level of eIF4E RNA decreased by up to 80%.[21]

Tumor-specific RNAi targeting eIF4E was recently shown to downregulate eIF4E gene expression effectively and specifically, which has led to breast carcinoma cell apoptosis induction,

tumor growth suppression, and enhancement of chemosensitivity of cisplatin both in vitro and in vivo.[132] Collectively, these results suggest eIF4E is a promising therapeutic target for the human malignancy treatments.

5. CONCLUSIONS AND FUTURE DIRECTIONS

The regulation of cap-dependent translation is an important contributor to tumorgenesis. Consequently, members of the translation initiation complex, eIF4F, have been proposed as potential anticancer drug targets. The eIF4F protein, eIF4E, which initiates eIF4F complex formation to mRNA cap, is the rate-limiting factor in cap-dependent translation initiation and is necessary for the regulation of tumor cell apoptosis, proliferation, and, potentially, metastasis. Several approaches attempting to block the function of eIF4E have been tried over the years, such as small molecule inhibitors that disrupt eIF4E/eIF4G interaction and cap analogs that directly target the eIF4E cap-binding site. Although the availability of NMR and X-ray structures has greatly enhanced the possibility of structure-based inhibitor design of eIF4E antagonists, enthusiasm for these approaches is tempered by the substantial structural flexibility of eIF4E. The use of ASOs to reduce the expression level of eIF4E has proved to be useful and has advanced to clinical trials in prostate cancer patients. Nevertheless, despite the tantalizing promise of targeting cancer cap-dependent translation, our understanding of the role of eIF4E, as well as other members of the eIF4F complex on cancer cell oncogene expression is still in its infancy. The success of these studies will depend on the development and use of chemical biological tools, a subset of which may show potential as anticancer drug leads.

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Carston R. Wagner *received his B. S. degree in chemistry at the University of North Carolina-Chapel Hill in 1981 where he worked with Professor William Little on the synthesis of cobalamin analogs. He received his graduate education in chemistry under the direction of Professor Ned A. Porter at Duke University where he studied phosphatidyl choline lipid peroxidation. In 1987, he began an NIH post-doctoral fellowship at the Pennsylvania State University with Dr. Stephen J. Benkovic where he investigated the role of active site hydrophobic amino acids on the binding and catalysis of dihydrofolate reductase. In 1991, he joined the faculty of the University of Minnesota where he is currently a Professor of Medicinal Chemistry. His laboratory seeks to apply the principles of organic chemistry, enzymology analytical chemistry, molecular & cellular biology, biophysics and nanotechnology to protein design, biocatalyis and drug design and delivery. His laboratory has been particularly interested in the development of new pronucleotide approaches for cancer, viral and bacterial diseases. Recently, his group has also developed a strong interest in methods for the tissue specific delivery of therapeutic nucleic acids to cells and tissues.*