The Centenary Award

Signalling to eIF4E in cancer

Nadeem Siddiqui* and Nahum Sonenberg*[1]
*Department of Biochemistry and Goodman Cancer Research Centre, McGill University, 1160 Pine Avenue West, Montreal, Quebec, Canada H3A 1A3

Abstract

Translational control plays a critical role in the regulation of gene expression in eukaryotes and affects many essential cellular processes, including proliferation, apoptosis and differentiation. Under most circumstances, translational control occurs at the initiation step at which the ribosome is recruited to the mRNA. The eukaryotic translation initiation factor 4E (eIF4E), as part of the eIF4F complex, interacts first with the mRNA and facilitates the recruitment of the 40S ribosomal subunit. The activity of eIF4E is regulated at many levels, most profoundly by two major signalling pathways: PI3K (phosphoinositide 3-kinase)/Akt (also known and Protein Kinase B, PKB)/mTOR (mechanistic/mammalian target of rapamycin) and Ras (rat sarcoma)/MAPK (mitogen-activated protein kinase)/Mnk (MAPK-interacting kinases). mTOR directly phosphorylates the 4E-BPs (eIF4E-binding proteins), which are inhibitors of eIF4E, to relieve translational suppression, whereas Mnk phosphorylates eIF4E to stimulate translation. Hyperactivation of these pathways occurs in the majority of cancers, which results in increased eIF4E activity. Thus, translational control via eIF4E acts as a convergence point for hyperactive signalling pathways to promote tumorigenesis. Consequently, recent works have aimed to target these pathways and ultimately the translational machinery for cancer therapy.

Introduction

mRNA translation is divided into three steps: initiation, elongation and termination [1–3]. Initiation is the rate-limiting step of translation and is subject to extensive regulation. A large body of evidence shows that translational control occurs predominately at the initiation step [4,5]. All nuclear transcribed mRNAs contain the cap structure or '5′-cap', m7GpppN (where m is a methyl group and N is any nucleotide), which is added very early at the transcription elongation step [6]. Translation initiation in eukaryotes commences with the binding of the eukaryotic translation initiation factor 4F (eIF4F) complex to the 5′-cap (Figure 1) [6]. eIF4F consists of the cap-binding subunit, eIF4E, the RNA helicase eIF4A and the scaffolding protein eIF4G [4,5,7]. It is thought that eIF4A unwinds the secondary structure present in the 5′-UTR of the mRNA to promote the binding of the ribosome and its scanning of the 5′-UTR [4,5]. Several other helicases, such as Ded1 and DHX29 (DEAH box polypeptide 29), are also involved in this process [8]. eIF4G interacts directly with eIF4E, eIF4A, eIF3 and the poly (A)-binding protein (PABP) [4,5]. The interaction of eIF4G with the multi-component initiation factor eIF3 is required in mammals for the recruitment of the 43S pre-initiation complex (which consists of the 40S ribosomal subunit and associated initiation factors), via the direct binding of eIF3 to the 40S subunit [4,5]. Following assembly at the cap structure, the 43S pre-initiation complex traverses the mRNA 5′UTR in a 5′ to 3′ direction, until it encounters the initiation codon (usually AUG, but in rare cases a near-cognate AUG), where it stops and the 60S large ribosomal subunit joins to form the 80S ribosomal complex, which is followed by the translation elongation step [9]. The eIF4G interaction with PABP brings about the circularization of the mRNA,

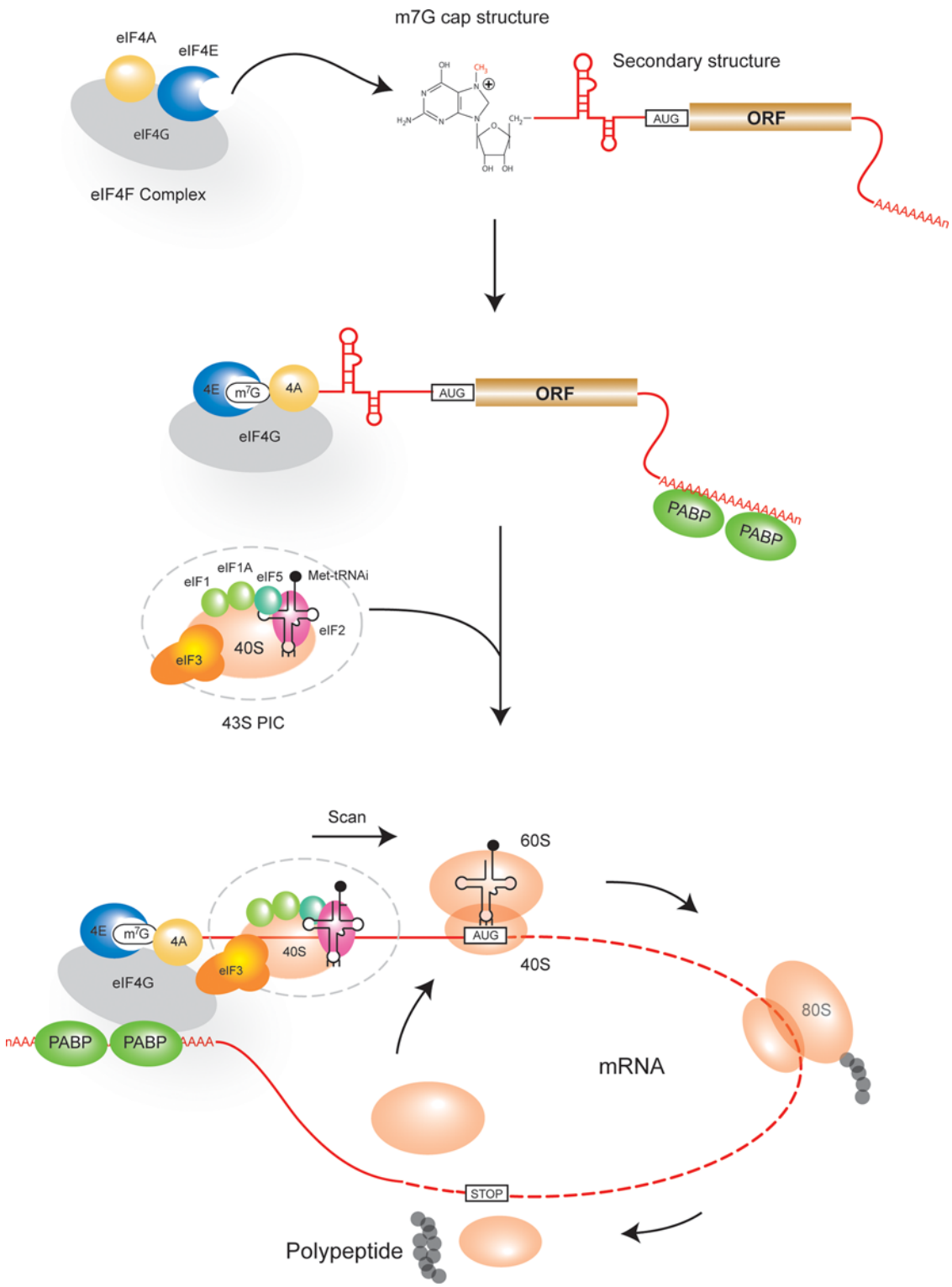
Key words: cancer therapy, eukaryotic translation initiation factor 4E (eIF4E), eukaryotic translation initiation factor 4E-binding proteins (4E-BPs), mitogen-activated protein kinase-interacting kinase 1/2 (Mnk1/2), mechanistic/mammalian target of rapamycin (mTOR), phospho-4E.

Abbreviations: ASO, anti-sense oligonucleotide; asTORi, active-site TOR inhibitor; eIF, eukaryotic translation initiation factor; 4E-BPs, eIF4E-binding proteins; EGFR, epidermal growth factor receptor; EMT, epithelial-to-mesenchymal transition; ERK, extracellular signal regulated kinase; FXS, fragile-X syndrome; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; MAPK, activated protein kinase; MMP-3, matrix metalloproteinase-3; Mnk, MAPK-interacting kinases; mTOR, mechanistic/mammalian target of rapamycin; mTORC1/2, mTOR complex 1/2; PABP, poly (A)-binding protein; PH, pleckstrin homology; PI3K, phosphoinositide 3-kinase; PIN, prostate intraepithelial neoplasia; PIP_2, phosphatidyl inositol 3,4; PIP_3, phosphatidyl inositol 3,4,5; PTEN, phosphatase and tensin homologue on chromosome 10; TGFβ, tumour growth factor β; TOP, terminal oligo pyrimidine; TSC2, tuberous sclerosis complex 2; VEGF, vascular endothelial growth factor.

[1] **To whom correspondence should be addressed (email nahum.sonenberg@mcgill.ca).**
Award lecture presented at the Biochemical Society Centenary Celebration. Held at the Royal Society, London, U.K., 15–16 December 2011.

Figure 1 | Model of cap dependent translation initiation

eIF4F binds to the m^{7}G cap structure via the cap-binding protein eIF4E. eIF4G is a scaffold protein which also binds to the RNA helicase eIF4A and eIF3, which in turn recruits the 43S pre-initiation complex (PIC). The PIC consists of the 40S ribosomal subunit, eIF2-GTP-Met-tRNAi and several other initiation factors, which are indicated. eIF4A melts the secondary structure in the 5′-UTR and the PIC scans the mRNA until it encounters the AUG start codon where the 60S subunit joins, followed by peptide chain synthesis. eIF4G also binds to PABP which brings about the circularization of the mRNA allowing for efficient ribosome recycling.



which is thought to facilitate the shunting of ribosomes from the termination codon to the mRNA 5′-cap to stimulate translation [10,11].

With the exception of mRNAs that translate via an internal ribosome entry site (IRES), the vast majority of eukaryotic mRNAs are translated in a cap-dependent manner [4,5]. Although eIF4E is necessary for cap-dependent translation, its requirement varies dramatically among mRNAs, as it preferentially stimulates the translation of a subset of mRNAs which are referred to as 'eIF4E-sensitive' [12]. These mRNAs mostly include those encoding proliferation and survival-promoting proteins such as cyclin D1 and D3, c-Myc, MDM2 (mouse double minute 2), VEGF (vascular endothelial growth factor), survivin and Bcl-2 (B-cell lymphoma 2) [13]. Considerable research efforts have been dedicated to the understanding of the mechanism that explains the preference for eIF4E-sensitive mRNAs. In general, mRNAs containing long G/C-rich 5′-UTRs, with the potential of forming stable secondary structures are feebly translated [14]. Reducing secondary structure by denaturing the mRNA enhances translation [15], whereas inserting sequences in the mRNA 5′-UTR that can form secondary structure impairs translation [16–20]. Strikingly, mRNAs with increased secondary structure are highly dependent on the cap structure for efficient translation [16]. Consistent with these studies, mRNAs with extensive secondary-structure in their 5′-UTRs are exceedingly dependent on eIF4E and eIF4A activity [21,22]. These observations can be mechanistically explained by recent findings showing that eIF4E exhibits a novel activity [23]. The eIF4E-binding region within eIF4G can inhibit eIF4A helicase activity when not bound to eIF4E. This inhibition is alleviated upon eIF4E binding to eIF4G. Thus, in addition to its canonical function as the 5'-cap-binding protein, eIF4E indirectly stimulates eIF4A helicase activity [23]. Recent reports have identified other motifs in mammalian mRNA 5′-UTRs that confer stimulation via eIF4E. These include terminal oligo pyrimidine (TOP) sequences at the mRNA extreme 5′-end and TOP-like mRNAs, which contain pyrimidine-rich sequences distal to the mRNA 5′end [24,25]; see other reports with additional conclusions [26,27]. The mechanisms by which eIF4E preferentially up-regulates the translation of TOP mRNAs are unknown.

eIF4E amounts and activity are regulated by multiple mechanisms. These include: (1) amplification of the gene encoding eIF4E; (2) transcriptional activation, particularly by c-Myc; (3) control of eIF4E activity by eIF4E-binding proteins (4E-BPs) and (4) post-translational modifications such as phosphorylation [12]. There are three 4E-BP homologues in mammals: 4E-BP1, 2 and 3 [7]. 4E-BPs and eIF4G share a common binding site for eIF4E [28]; thus, the interaction of 4E-BPs with eIF4E precludes the binding of eIF4E to eIF4G, causing impaired assembly of the eIF4F complex [29,30] (Figure 2). 4E-BPs are phosphoproteins, whose binding to eIF4E is determined by their phosphorylation status. The dephosphorylated forms of 4E-BPs bind avidly to eIF4E and therefore act as translational suppressors.

The mTOR pathway regulates eIF4E

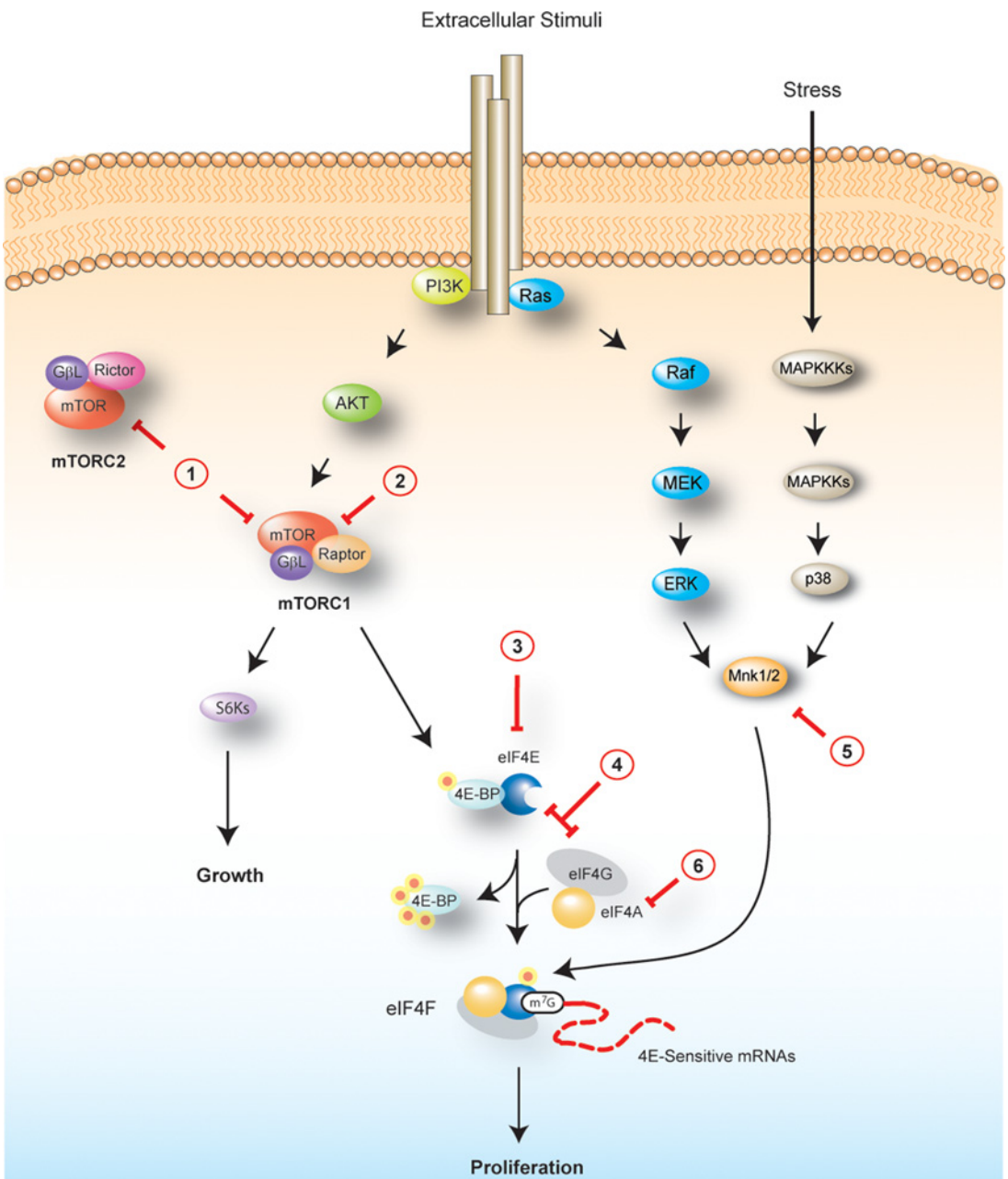
The mechanistic/mammalian target of rapamycin (mTOR) is a highly evolutionarily conserved serine/threonine kinase that controls a staggering number of key cellular processes including protein synthesis, cell growth and proliferation, lipid metabolism, mitochondrial function, autophagy and cytoskeleton organization [31–33]. mTOR forms two separate complexes: mTOR complex 1 and 2 (mTORC1 and mTORC2) [32] [34] (Figure 2). Both mTOR complexes share the protein subunit LST8 (lethal with sec13 protein 8)/GβL (G-protein beta subunit like) [34,35]. mTORC1 contains the subunit Raptor that functions as an adaptor for the substrates of mTORC1, such as 4E-BPs, S6 kinases, PRAS40 (proline rich Akt substrate of 40 kDa) and Deptor [32,36]. mTORC2 contains the subunits Rictor, Sin1 (stress activated protein-kinase interacting protein) and Protor [32]. mTORC1 integrates a large number of inputs including extracellular stimuli signals such as growth factors, hormones and stress as well as intracellular cues, such as energy status, amino acid amounts and oxygen levels [37]. mTORC1 is activated via ordered serine/threonine phosphorylation and GTPase events, which constitute the PI3K (phosphoinositide 3-kinase)/Akt/mTOR signalling pathway. PI3K is a lipid kinase that phosphorylates phosphatidyl inositol 3,4 (PIP_2) to generate phosphatidyl inositol 3,4,5 (PIP_3), which activates the kinase Akt (also known as protein kinase B) [31,38]. The action of PI3K is antagonized by PTEN (phosphatase and tensin homologue on chromosome 10) a PIP_3 phosphatase [39,40]. PIP_3 binds to the pleckstrin homology (PH) domain of Akt to promote the translocation of Akt to the plasma membrane [41–43], where it is phosphorylated and activated by several kinases including mTORC2 [44]. Among its numerous targets, phosphorylation of tuberous sclerosis complex 2 (TSC2; a subunit of the tuberous sclerosis complex) by Akt stimulates mTORC1 activity [45–47]. Arguably, the best-studied and understood function of mTORC1 is its role in translation control. mTORC1 phosphorylates (inactivates) the 4E-BPs, leading to their dissociation from eIF4E which allows eIF4F formation and translation progression [48,49] (Figure 2). In our laboratory, we investigated the role of mTOR in promoting the translation of the 'eIF4E-sensitive' subset of mRNAs via the eIF4F complex [50,51].

Phosphorylation of eIF4E and cancer

eIF4E was suggested to act as an oncogene because it malignantly transformed NIH 3T3 murine fibroblasts [52] and subsequently rat primary embryo fibroblasts, in combination with c-Myc or adenovirus E1A [53] and human mammary cells [54]. It is remarkable that a relatively modest level of overexpression (~2.5-fold) of eIF4E in NIH 3T3 was sufficient to cause transformation [55]. This is highly

Figure 2 | Signal transduction pathways converging on eIF4E

The PI3K pathway is activated in response to many extracellular stimuli resulting in the activation of the downstream serine/threonine kinase, mTOR. mTOR is a multi-domain protein which associates with several binding partners to form two different complexes mTORC1 and mTORC2. mTORC1 phosphorylates the translation repressors 4E-BPs, which then dissociate from eIF4E, allowing eIF4F formation and thus promoting translation. Mitogenic and stress signals activate components of the MAPK pathway including the ERK and p38 MAP kinase. Both converge to activate Mnk1/2, which bind to eIF4G and phosphorylate eIF4E at Ser^{209}. Both pathways are hyperactivated in the majority of human malignancies. Drugs targeting, these signalling pathways and translation initiation factors are shown in red.



1	2	3	4	5	6
asTORis	**Rapalogs**	**Inhibitors of eIF4E**	**Inhibitors of eIF4E-4G interaction**	**Mnk Inhibitors**	**eIF4A Inhibitors**
PP242 Torin1 INK128 AZD8055	Siromilus Everolimus Temsirolimus	Cap analog 4Ei-1 Anti-sense eIF4E oligo (ASO)	4EGI-1 4E1R-Cat 4E2R-Cat	CGP5738 Cercosporamide 2-anilino Pyrimidines	Silvestrol Hippuristanol Pateamine A

significant, because in human cancers the magnitude of eIF4E increase is in this range [56]. Consistent with these results, depletion of eIF4E significantly reduced Ras-mediated tumorigenesis [57]. The conclusions from the *in vitro* experiments were bolstered by subsequent *in vivo* work in mice, which showed that overexpression of eIF4E augmented Eμ-Myc-driven lymphomas [58] and engendered cancers in a multitude of organs, when expressed from the β-actin promoter [59]. eIF4E activity is also regulated via the MAPK (mitogen-activated protein kinase) pathway through direct phosphorylation by the MAPK-interacting kinases (Mnk1 and Mnk2) at a single residue, Ser^{209} [60,61]. Phosphorylation of eIF4E plays an important role in cancer development and progression [62–65]. Ectopic expression of the $eIF4E^{S209A}$ mutant protein failed to cause neoplastic transformation in NIH 3T3 cells and in the Eμ-Myc lymphoma mouse model [62,63]. Engineered knockin mice, in which the wild-type allele of eIF4E was replaced by the $eIF4E^{S209A}$ allele, were crossed with mice in which PTEN was deleted in the prostate. This deletion causes early onset of prostate intraepithelial neoplasia (PIN) and invasive carcinoma [66]. However, strikingly, the $eIF4E^{S209A}$ mutant mice were resistant to PIN and invasive carcinoma [64]. These results are highly relevant to human prostate cancer, inasmuch as eIF4E amounts and phosphorylation are gradually elevated in the progression of prostate cancer from PIN through hormone-sensitive and hormone-resistant forms [64]. In more recent studies, the mutant $eIF4E^{S209A}$ mouse was also shown to be resistant to polyoma middle-T driven mammary tumours [65]. Availability and phosphorylation of eIF4E promote metastasis in mice [67,68]. Translation of a subset of mRNAs, encoding several pro-metastatic proteins, such as MMP-3 (matrix metalloproteinase-3) and MMP-9, was reduced in the mutant $eIF4E^{S209A}$ mouse. MMPs cleave constituents of the extracellular matrix and promote migration and invasion [69]. eIF4E phosphorylation stimulated the translation of *Mmp3* and *Snail* mRNAs whose proteins promote invasion and epithelial-to-mesenchymal transition (EMT), which is required for metastasis [64]. Indeed, tumour growth factor β (TGFβ), which is an established inducer of EMT [70], promotes the phosphorylation of eIF4E via activation of ERK (extracellular signal regulated kinase) and p38 MAPK, which phosphorylate Mnk [71]. Strikingly, the phosphorylation of eIF4E by MNK1 is required for TGFβ-induced EMT [65].

Strategies for targeting eIF4E in cancer therapy

In light of the idea that eIF4E is a convergence point for the major cancer related signalling pathways [72,73] (Figure 2) and that eIF4E is activated or overexpressed in a large number of tumours, there has been considerable effort to target eIF4E directly or indirectly for cancer therapy. eIF4E activity in cancer can be targeted indirectly by inhibitors of the PI3K/Akt/mTOR pathway, which cause the dephosphorylation of 4E-BPs and inhibition of eIF4E. Some of these compounds, prominently rapamycin derivatives (rapalogues) are in use in the clinic for certain cancers, but many more are in clinical trials, particularly PI3K inhibitors and active-site mTOR inhibitors (asTORi); the latter inhibiting both mTORC1 and mTORC2 [74,75]. A highly pertinent question is whether eIF4E is a pivotal target that mediates the therapeutic activity of these inhibitors in cancer. Some affirmative answers to this question were obtained recently showing that cells in culture, which develop resistance to these drugs exhibit amplified eIF4E. Cells that became resistant to NVP-BEZ235, which is a dual PI3K/mTOR inhibitor, exhibited amplified c-Myc and eIF4E genes [76] and cells which acquired resistance to AZD8055, an asTORi, had amplified eIF4E [77]. These results support earlier findings from our laboratory that the ratio of eIF4E/4E-BP is a prime predictor of the efficacy of asTORi in reducing tumour growth in mice [78]. Moreover, asTORi inhibit cell proliferation, but not cell growth via inhibition of 4E-BP phosphorylation and subsequent suppression of translation of 'eIF4E-sensitive' mRNAs [79].

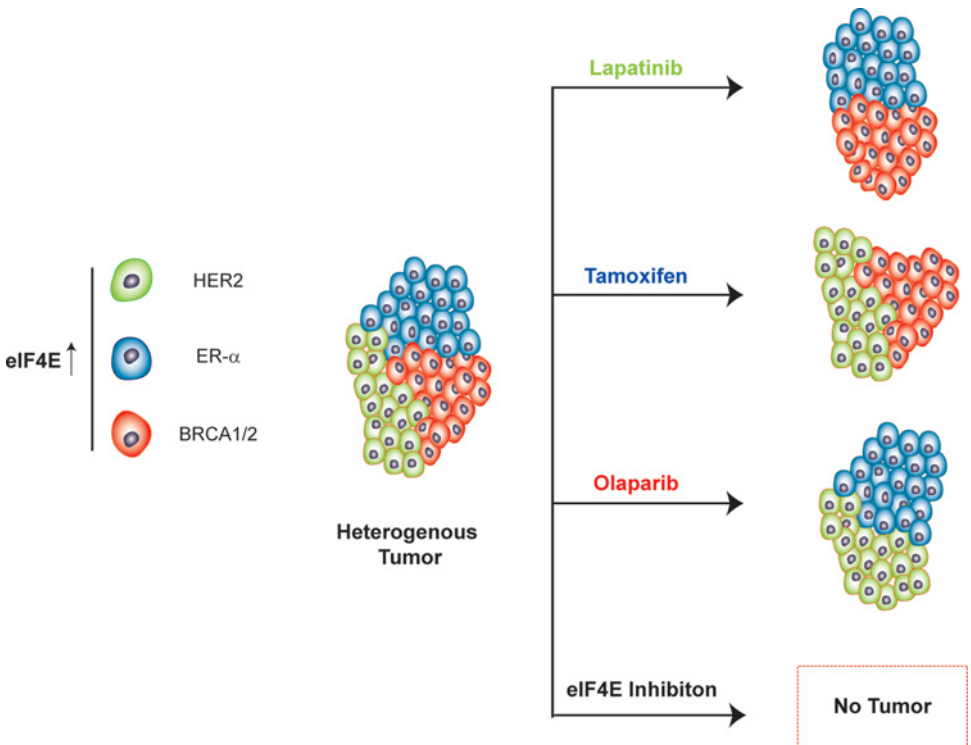
One of the first attempts to target eIF4E directly was undertaken by Graff et al. [80] by developing an anti-sense oligonucleotide (ASO) against eIF4E, which preferentially inhibited the translation of eIF4E-sensitive mRNAs encoding proteins, such as VEGF, cyclin D1, survivin, c-Myc, and Bcl-2, in cultured cells. Most striking was the observation that intravenous administration of ASO selectively reduced eIF4E expression in human tumour xenografts and dramatically suppressed tumour growth. eIF4E ASO reduced eIF4E levels in the mouse (80 % in the liver), but importantly, had no effect on body weight, organ weight or liver transaminase levels [80]. The puzzling question as to why a dramatic reduction in eIF4E did not significantly impair translation but rather caused only minimal deleterious effects in the mouse is most probably explained by results obtained from cells in culture in which shRNA was used to deplete eIF4E [81]. When eIF4E is dramatically reduced, the non-phosphorylated 4E-BPs, which no longer have a binding partner, undergo ubiquitination and subsequent degradation [81]. Thus, depletion of eIF4E also causes a reduction in the amounts of its inhibitory 4E-BPs and therefore, the potential deleterious effects of eIF4E depletion are significantly ameliorated.

As described above, phosphorylation of eIF4E plays an important role in cancer progression. Thus, another strategy to diminish eIF4E activity has been to develop drug-candidate compounds that target Mnks to prevent eIF4E phosphorylation [82]. These include CGP57380 [83], cercosporamide [84] and 5-(2-(phenylamino) pyrimidin-4-yl)thiazol-2(3H)-one derivatives [85]. As double 'knockout' Mnk1/2 mice develop normally and appear healthy [86], compounds that acutely inhibit Mnk activity are highly attractive candidates to treat cancer.

Another class of promising inhibitors comprises compounds that prevent eIF4E–eIF4G interactions [87] (Figure 2). These compounds disrupt the formation of the eIF4F complex, and therefore impair translation of eIF4E-sensitive mRNAs. The archetype of this family of compounds,

Figure 3 | Targeting eIF4E in tumour heterogeneity

A schematic diagram illustrating a simplified version of diversity within a hypothetical heterogeneous tumour. Sub-populations of cells expressing HER2 (green), ERα (blue) or presenting BRCA1/2 mutations (red) are shown. In contrast, all tumour cells contain elevated eIF4E. The tumours containing the indicated targets are treated in the clinic by the corresponding drugs listed in the figure. We hypothesize that direct eIF4E inhibitors will target all types of tumour cells, regardless of their genetic make-up.



4EGI-1, suppressed the growth and induced apoptosis of multiple myeloma and lung cancer cells *in vitro* and inhibited myeloma and breast cancer xenografts without apparent toxicity *in vivo* [88–92]. Accordingly, current efforts are focused on developing more potent analogues of 4EGI [93–95]. Mechanistically, 4EGI-1 binds to a region of eIF4E that is distant from the eIF4E-eIF4G interface and induces an allosteric inhibition of the interaction between the two-subunits [87]. In addition to preventing eIF4G–eIF4E interaction, 4EGI-1 also stabilizes the 4E-BP binding to eIF4E, which exacerbates the translation inhibition of eIF4E-sensitve mRNA [87]. This observation was initially surprising and suggested that eIF4G and 4E-BPs may have some non-overlapping interaction sites with eIF4E. Indeed, recent structural studies have shown that 4E-BP2 contains a non-canonical motif (conserved in all 4E-BPs), which contacts a region of eIF4E that is not used by eIF4G [96,97]. Further structural characterization of eIF4E complexes revealed unique binding elements in different eIF4E binding partners that could be fused into a chimeric '4E-BP-like' mimic and dampen translational activity [98]. NMR studies using the full-length 4E-BP revealed that phosphorylation of 4E-BP results in a transition from a binding-competent disordered state to a folded state that cannot bind to eIF4E [99]. The latter findings raise for the first time the intriguing possibility of targeting a regulator of eIF4E. It is proposed that small molecules could be designed to alter the stability of 4E-BPs and thus alter its affinity for eIF4E [99]. Taken together, these detailed structural studies will aid in the rational design of more potent treatments such as 4EGI-like compounds, 4E-BPs mimics or small molecules that stabilize 4E-BP-eIF4E interactions.

As eIF4E's role in tumorigenesis depends on its ability to bind the 5′-cap, a key strategy has focused on developing bioavailable 5′-cap analogues which directly target eIF4E [63,100]. For instance, numerous atomic structures of eIF4E have been studied in great detail, with and without different 5′-cap structures [87], which have aided in the design of 5′-cap-mimetics such as 4Ei-1 [101]. 4Ei-1 inhibited cap-dependent translation and exhibited anti-neoplastic activities with minimal toxic effects [101–103]. High throughput screening efforts for more effective 5′-cap mimetics are ongoing [104].

The bevy of inhibitors of eIF4E and also eIF4A (see below) [105] provides an intriguing opportunity to cope with the pernicious problem of tumour heterogeneity in

targeting cancer. It is well established that most cancers consist of heterogeneous tumour types with different genetic aberrations [106,107]. Consequently, personalized medicine/targeted monotherapies are effective only for relatively short times (less than 1 year) in the majority of cancers, as they lead to the selection of pre-existing intrinsically resistant sub-population of cells (Figure 3) [107]. For example, mammary tumours are considered clinically HER2 (human epidermal growth factor receptor 2)-positive when immunohistochemical analysis identifies as few as 30 % of HER2-expressing cells [108], whereas the cut-off for EGFR (epidermal growth factor receptor) positivity is only 1 % [109]. Therefore, only a small fraction of tumour cells are required to strain positive by IHC (immunohistochemistry) in order to be considered for clinically targeted therapy [110]. Although the treatment of cancer with the EGFR/HER2 inhibitor Lapatinib provides initial benefit, the presence of tumour cells having acquired EGFR/HER2 independence or contain other mutations (i.e. PI3K mutation, TSC2 loss, PTEN loss, eIF4E amplification, etc.) will eventually lead to resistance to treatment. One possible solution is to employ a combination of drugs directed against two or more targets which can lead to enhanced efficacy; however, this approach also generally leads to additive or even synergistic toxicity [111,112].

In general, many therapeutic cancer targets display similar heterogeneous expression patterns; however, Ramon et al. [113] obtained IHC data demonstrating a homogenous distribution of elevated eIF4E and 4E-BP and their phosphorylated forms in breast cancer. If this striking observation were to be repeated in other tumours, this would provide an intriguing opportunity to use inhibitors of eIF4F as monotherapies for cancer. Consistent with this idea, recent studies have found that an inhibitor of eIF4F suppressed B-Raf (B-Raf proto-oncogene serine/threonine-protein kinase) resistant melanoma [114]. In addition to the inhibitors of eIF4E described above, several anti-eIF4A natural compounds were discovered, which exhibit potent inhibition of proliferation and anti-tumorigenic activities [105]. These compounds include, silvestrol, hippuristanol and pateamine A. Notably, Pelletier's group showed that multiple myeloma cancer cells which are resistant to standard therapy could be eliminated by the eIF4A inhibitor silvestrol [105]. Taken together, these experiments imply that a highly promising application of single eIF4F inhibitors for cancer treatment is to combat drug resistance.

In addition to cancer, eIF4E has been implicated in neurodevelopmental diseases, in which translation is dysregulated, such as autism and fragile-X syndrome (FXS). Notably, drugs that are being developed for cancer, such as 4EGI, were shown to correct autistic-like deficits in mice [115] and cercosporamide reversed many of the FXS-like symptoms in mice [116]. In summary, the cap-binding protein eIF4E plays a critical role in cell homoeostasis in health and disease. It is hoped that some of the currently tested candidate drugs or their derivatives that inhibit eIF4E activity will prove effective as anti-cancer drugs. It is also clear that the accumulated exhaustive knowledge of the structure and function of eIF4E and its regulators will prove to be instrumental in designing effective drugs against cancer.

Acknowledgements

We thank Nathaniel Robichaud and Christopher Rouya for their comments on the manuscript. Nahum Sonenberg is a Howard Hughes Medical Institute Senior International Scholar.

Funding

This work was supported by the Susan G. Komen Breast Cancer Foundation [KGMS IIR12224057], the Canadian Institute of Health Research [MOP-7214], Howard Hughes Medical Institute [HHMI 55007654], and the Canadian Cancer Society Research Institute [CCSRI 702317].

References

1 Mathews, M.B., Sonenberg, N. and Hershey, J. (2007) Translation Control in Biology and Medicine. Cold Spring Harbor Monograph Series 48, Cold Spring Harbor, NY
2 Hinnebusch, A.G., Asano, K., Olsen, D.S., Phan, L., Nielsen, K.H. and Valasek, L. (2004) Study of translational control of eukaryotic gene expression using yeast. Ann. N.Y. Acad. Sci. **1038**, 60–74 CrossRef PubMed
3 Dever, T.E. and Green, R. (2012) The elongation, termination, and recycling phases of translation in eukaryotes. Cold Spring Harb. Perspect. Biol. **4**, a013706 CrossRef PubMed
4 Jackson, R.J., Hellen, C.U. and Pestova, T.V. (2010) The mechanism of eukaryotic translation initiation and principles of its regulation. N. Rev. Mol. Cell Biol. **11**, 113–127 CrossRef
5 Sonenberg, N. and Hinnebusch, A.G. (2009) Regulation of translation initiation in eukaryotes: mechanisms and biological targets. Cell **136**, 731–745 CrossRef PubMed
6 Topisirovic, I., Svitkin, Y.V., Sonenberg, N. and Shatkin, A.J. (2011) Cap and cap-binding proteins in the control of gene expression. Wiley Interdiscip. Rev. RNA **2**, 277–298 CrossRef PubMed
7 Gingras, A.C., Raught, B. and Sonenberg, N. (1999) eIF4 initiation factors: effectors of mRNA recruitment to ribosomes and regulators of translation. Annu. Rev. Biochem. **68**, 913–963 CrossRef PubMed
8 Parsyan, A., Svitkin, Y., Shahbazian, D., Gkogkas, C., Lasko, P., Merrick, W.C. and Sonenberg, N. (2011) mRNA helicases: the tacticians of translational control. Nat. Rev. Mol. Cell Biol. **12**, 235–245 CrossRef PubMed
9 Hinnebusch, A.G. (2014) The scanning mechanism of eukaryotic translation initiation. Annu. Rev. Biochem. **83**, 779–812 CrossRef PubMed
10 Tarun, Jr, S.Z. and Sachs, A.B. (1996) Association of the yeast poly(A) tail binding protein with translation initiation factor eIF-4G. EMBO J. **15**, 7168–7177 PubMed
11 Kahvejian, A., Svitkin, Y.V., Sukarieh, R., M'Boutchou, M.N. and Sonenberg, N. (2005) Mammalian poly(A)-binding protein is a eukaryotic translation initiation factor, which acts via multiple mechanisms. Genes Dev. **19**, 104–113 CrossRef PubMed
12 Bhat, M., Robichaud, N., Hulea, L., Sonenberg, N., Pelletier, J. and Topisirovic, I. (2015) Targeting the translation machinery in cancer. Nat. Rev. Drug Discov. **14**, 261–278 CrossRef PubMed
13 Graff, J.R., Konicek, B.W., Carter, J.H. and Marcusson, E.G. (2008) Targeting the eukaryotic translation initiation factor 4E for cancer therapy. Cancer Res. **68**, 631–634 CrossRef PubMed
14 Gehrke, L., Auron, P.E., Quigley, G.J., Rich, A. and Sonenberg, N. (1983) 5′-Conformation of capped alfalfa mosaic virus ribonucleic acid 4 may reflect its independence of the cap structure or of cap-binding protein for efficient translation. Biochemistry **22**, 5157–5164 CrossRef PubMed

15 Payvar, F. and Schimke, R.T. (1979) Methylmercury hydroxide enhancement of translation and transcription of ovalbumin and conalbumin mRNA's. J. Biol. Chem. **254**, 7636–7642 PubMed

16 Pelletier, J. and Sonenberg, N. (1985) Insertion mutagenesis to increase secondary structure within the 5′ noncoding region of a eukaryotic mRNA reduces translational efficiency. Cell **40**, 515–526 CrossRef PubMed

17 Goossen, B., Caughman, S.W., Harford, J.B., Klausner, R.D. and Hentze, M.W. (1990) Translational repression by a complex between the iron-responsive element of ferritin mRNA and its specific cytoplasmic binding protein is position-dependent *in vivo*. EMBO J. **9**, 4127–4133 PubMed

18 Sagliocco, F.A., Vega Laso, M.R., Zhu, D., Tuite, M.F., McCarthy, J.E. and Brown, A.J. (1993) The influence of 5′-secondary structures upon ribosome binding to mRNA during translation in yeast. J. Biol. Chem. **268**, 26522–26530 PubMed

19 Babendure, J.R., Babendure, J.L., Ding, J.H. and Tsien, R.Y. (2006) Control of mammalian translation by mRNA structure near caps. RNA **12**, 851–861 CrossRef PubMed

20 Kozak, M. (1986) Influences of mRNA secondary structure on initiation by eukaryotic ribosomes. Proc. Natl. Acad. Sci. U.S.A. **83**, 2850–2854 CrossRef PubMed

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

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

23 Feoktistova, K., Tuvshintogs, E., Do, A. and Fraser, C.S. (2013) Human eIF4E promotes mRNA restructuring by stimulating eIF4A helicase activity. Proc. Natl. Acad. Sci. U.S.A. **110**, 13339–13344 CrossRef PubMed

24 Thoreen, C.C., Chantranupong, L., Keys, H.R., Wang, T., Gray, N.S. and Sabatini, D.M. (2012) A unifying model for mTORC1-mediated regulation of mRNA translation. Nature **485**, 109–113 CrossRef PubMed

25 Hsieh, A.C., Liu, Y., Edlind, M.P., Ingolia, N.T., Janes, M.R., Sher, A., Shi, E.Y., Stumpf, C.R., Christensen, C., Bonham, M.J. et al. (2012) The translational landscape of mTOR signalling steers cancer initiation and metastasis. Nature **485**, 55–61 CrossRef PubMed

26 Miloslavski, R., Cohen, E., Avraham, A., Iluz, Y., Hayouka, Z., Kasir, J., Mudhasani, R., Jones, S.N., Cybulski, N., Ruegg, M.A. et al. (2014) Oxygen sufficiency controls TOP mRNA translation via the TSC-Rheb-mTOR pathway in a 4E-BP-independent manner. J. Mol. Cell Biol. **6**, 255–266 CrossRef PubMed

27 Meyuhas, O. and Kahan, T. (2014) The race to decipher the top secrets of TOP mRNAs. Biochim. Biophys. Acta. **1849**, 801–811 CrossRef PubMed

28 Mader, S., Lee, H., Pause, A. and Sonenberg, N. (1995) 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. **15**, 4990–4997 PubMed

29 Pause, A., Belsham, G.J., Gingras, A.C., Donze, O., Lin, T.A., Lawrence, Jr, J.C. and Sonenberg, N. (1994) Insulin-dependent stimulation of protein synthesis by phosphorylation of a regulator of 5′-cap function. Nature **371**, 762–767 CrossRef PubMed

30 Poulin, F., Gingras, A.C., Olsen, H., Chevalier, S. and Sonenberg, N. (1998) 4E-BP3, a new member of the eukaryotic initiation factor 4E-binding protein family. J. Biol. Chem. **273**, 14002–14007 CrossRef PubMed

31 Hay, N. and Sonenberg, N. (2004) Upstream and downstream of mTOR. Genes Dev. **18**, 1926–1945 CrossRef PubMed

32 Laplante, M. and Sabatini, D.M. (2012) mTOR signaling in growth control and disease. Cell **149**, 274–293 CrossRef PubMed

33 Caron, E., Ghosh, S., Matsuoka, Y., Ashton-Beaucage, D., Therrien, M., Lemieux, S., Perreault, C., Roux, P.P. and Kitano, H. (2010) A comprehensive map of the mTOR signaling network. Mol. Syst. Biol. **6**, 453 CrossRef PubMed

34 Wullschleger, S., Loewith, R. and Hall, M.N. (2006) TOR signaling in growth and metabolism. Cell **124**, 471–484 CrossRef PubMed

35 Kim, D.H., Sarbassov, D.D., Ali, S.M., Latek, R.R., Guntur, K.V., Erdjument-Bromage, H., Tempst, P. and Sabatini, D.M. (2003) GbetaL, a positive regulator of the rapamycin-sensitive pathway required for the nutrient-sensitive interaction between raptor and mTOR. Mol. Cell **11**, 895–904 CrossRef PubMed

36 Ma, X.M. and Blenis, J. (2009) Molecular mechanisms of mTOR-mediated translational control. Nat. Rev. Mol. Cell. Biol. **10**, 307–318 CrossRef PubMed

37 Bar-Peled, L. and Sabatini, D.M. (2014) Regulation of mTORC1 by amino acids. Trends Cell Biol. **24**, 400–406 CrossRef PubMed

38 Cantley, L.C. (2002) The phosphoinositide 3-kinase pathway. Science **296**, 1655–1657 CrossRef PubMed

39 Yuan, T.L. and Cantley, L.C. (2008) PI3K pathway alterations in cancer: variations on a theme. Oncogene **27**, 5497–5510 CrossRef PubMed

40 Pearce, L.R., Huang, X., Boudeau, J., Pawlowski, R., Wullschleger, S., Deak, M., Ibrahim, A.F., Gourlay, R., Magnuson, M.A. and Alessi, D.R. (2007) Identification of Protor as a novel Rictor-binding component of mTOR complex-2. Biochem. J. **405**, 513–522 CrossRef PubMed

41 Kandel, E.S. and Hay, N. (1999) The regulation and activities of the multifunctional serine/threonine kinase Akt/PKB. Exp. Cell Res. **253**, 210–229 CrossRef PubMed

42 Brazil, D.P. and Hemmings, B.A. (2001) Ten years of protein kinase B signalling: a hard Akt to follow. Trends Biochem. Sci. **26**, 657–664 CrossRef PubMed

43 Scheid, M.P. and Woodgett, J.R. (2001) Phosphatidylinositol 3′ kinase signaling in mammary tumorigenesis. J. Mammary Gland Biol. Neoplasia **6**, 83–99 CrossRef PubMed

44 Sarbassov, D.D., Ali, S.M., Sengupta, S., Sheen, J.H., Hsu, P.P., Bagley, A.F., Markhard, A.L. and Sabatini, D.M. (2006) Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. Mol. Cell **22**, 159–168 CrossRef PubMed

45 Inoki, K., Li, Y., Zhu, T., Wu, J. and Guan, K.L. (2002) TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. Nat. Cell Biol. **4**, 648–657 CrossRef PubMed

46 Manning, J., Beutler, K., Knepper, M.A. and Vehaskari, V.M. (2002) Upregulation of renal BSC1 and TSC in prenatally programmed hypertension. Am. J. Physiol. Renal Physiol. **283**, F202–F206 CrossRef PubMed

47 Dibble, C.C., Elis, W., Menon, S., Qin, W., Klekota, J., Asara, J.M., Finan, P.M., Kwiatkowski, D.J., Murphy, L.O. and Manning, B.D. (2012) TBC1D7 is a third subunit of the TSC1-TSC2 complex upstream of mTORC1. Mol. Cell **47**, 535–546 CrossRef PubMed

48 Gingras, A.C., Gygi, S.P., Raught, B., Polakiewicz, R.D., Abraham, R.T., Hoekstra, M.F., Aebersold, R. and Sonenberg, N. (1999) Regulation of 4E-BP1 phosphorylation: a novel two-step mechanism. Genes Dev. **13**, 1422–1437 CrossRef PubMed

49 Gingras, A.C., Kennedy, S.G., O'Leary, M.A., Sonenberg, N. and Hay, N. (1998) 4E-BP1, a repressor of mRNA translation, is phosphorylated and inactivated by the Akt(PKB) signaling pathway. Genes Dev. **12**, 502–513 CrossRef PubMed

50 Mamane, Y., Petroulakis, E., Martineau, Y., Sato, T.A., Larsson, O., Rajasekhar, V.K. and Sonenberg, N. (2007) Epigenetic activation of a subset of mRNAs by eIF4E explains its effects on cell proliferation. PLoS One **2**, e242 CrossRef PubMed

51 Larsson, O., Sonenberg, N. and Nadon, R. (2010) Identification of differential translation in genome wide studies. Proc. Natl. Acad. Sci. U.S.A. **107**, 21487–21492 CrossRef PubMed

52 Lazaris-Karatzas, A., Montine, K.S. and Sonenberg, N. (1990) Malignant transformation by a eukaryotic initiation factor subunit that binds to mRNA 5′ cap. Nature **345**, 544–547 CrossRef PubMed

53 Lazaris-Karatzas, A. and Sonenberg, N. (1992) The mRNA 5′ cap-binding protein, eIF-4E, cooperates with v-myc or E1A in the transformation of primary rodent fibroblasts. Mol. Cell. Biol. **12**, 1234–1238 PubMed

54 Avdulov, S., Li, S., Michalek, V., Burrichter, D., Peterson, M., Perlman, D.M., Manivel, J.C., Sonenberg, N., Yee, D., Bitterman, P.B. and Polunovsky, V.A. (2004) Activation of translation complex eIF4F is essential for the genesis and maintenance of the malignant phenotype in human mammary epithelial cells. Cancer Cell **5**, 553–563 CrossRef PubMed

55 Rousseau, D., Kaspar, R., Rosenwald, I., Gehrke, L. and Sonenberg, N. (1996) Translation initiation of ornithine decarboxylase and nucleocytoplasmic transport of cyclin D1 mRNA are increased in cells overexpressing eukaryotic initiation factor 4E. Proc. Natl. Acad. Sci. U.S.A. **93**, 1065–1070 CrossRef PubMed

56 Schneider, R.J. and Sonenberg, N. (2007) Translational Control in Cancer Development and Progression. Cold Spring Harbor Monograph Archive, Cold Spring Harbor, NY

57 Rinker-Schaeffer, C.W., Graff, J.R., De Benedetti, A., Zimmer, S.G. and Rhoads, R.E. (1993) Decreasing the level of translation initiation factor 4E with antisense RNA causes reversal of ras-mediated transformation and tumorigenesis of cloned rat embryo fibroblasts. Int. J. Cancer **55**, 841–847 CrossRef PubMed

58 Wendel, H.G., De Stanchina, E., Fridman, J.S., Malina, A., Ray, S., Kogan, S., Cordon-Cardo, C., Pelletier, J. and Lowe, S.W. (2004) Survival signalling by Akt and eIF4E in oncogenesis and cancer therapy. Nature **428**, 332–337 CrossRef PubMed

59 Ruggero, D., Montanaro, L., Ma, L., Xu, W., Londei, P., Cordon-Cardo, C. and Pandolfi, P.P. (2004) The translation factor eIF-4E promotes tumor formation and cooperates with c-Myc in lymphomagenesis. Nat. Med. **10**, 484–486 CrossRef PubMed

60 Waskiewicz, A.J., Johnson, J.C., Penn, B., Mahalingam, M., Kimball, S.R. and Cooper, J.A. (1999) Phosphorylation of the cap-binding protein eukaryotic translation initiation factor 4E by protein kinase Mnk1 *in vivo*. Mol. Cell. Biol. **19**, 1871–1880 PubMed

61 Fukunaga, R. and Hunter, T. (1997) MNK1, a new MAP kinase-activated protein kinase, isolated by a novel expression screening method for identifying protein kinase substrates. EMBO J. **16**, 1921–1933 CrossRef PubMed

62 Topisirovic, I., Ruiz-Gutierrez, M. and Borden, K.L. (2004) Phosphorylation of the eukaryotic translation initiation factor eIF4E contributes to its transformation and mRNA transport activities. Cancer Res. **64**, 8639–8642 CrossRef PubMed

63 Wendel, H.G., Silva, R.L., Malina, A., Mills, J.R., Zhu, H., Ueda, T., Watanabe-Fukunaga, R., Fukunaga, R., Teruya-Feldstein, J., Pelletier, J. and Lowe, S.W. (2007) Dissecting eIF4E action in tumorigenesis. Genes Dev. **21**, 3232–3237 CrossRef PubMed

64 Furic, L., Rong, L., Larsson, O., Koumakpayi, I.H., Yoshida, K., Brueschke, A., Petroulakis, E., Robichaud, N., Pollak, M., Gaboury, L.A. et al. (2010) eIF4E phosphorylation promotes tumorigenesis and is associated with prostate cancer progression. Proc. Natl. Acad. Sci. U.S.A. **107**, 14134–14139 CrossRef PubMed

65 Robichaud, N., Del Rincon, S.V., Huor, B., Alain, T., Petruccelli, L.A., Hearnden, J., Goncalves, C., Grotegut, S., Spruck, C.H., Furic, L. et al. (2014) Phosphorylation of eIF4E promotes EMT and metastasis via translational control of SNAIL and MMP-3. Oncogene **34**, 2032–2042 CrossRef PubMed

66 Trotman, L.C., Niki, M., Dotan, Z.A., Koutcher, J.A., Cristofano, A., Xiao, A., Khoo, A.S., Roy-Burman, P., Greenberg, N.M., Dyke, T. et al. (2003) Pten dose dictates cancer progression in the prostate. PLoS Biol. **1**, CrossRef

67 Konicek, B.W., Stephens, J.R., McNulty, A.M., Robichaud, N., Peery, R.B., Dumstorf, C.A., Dowless, M.S., Iversen, P.W., Parsons, S., Ellis, K.E. et al. (2011) Therapeutic inhibition of MAP kinase interacting kinase blocks eukaryotic initiation factor 4E phosphorylation and suppresses outgrowth of experimental lung metastases. Cancer Res. **71**, 1849–1857 CrossRef PubMed

68 Nasr, Z., Robert, F., Jr, J.A., Muller, W.J. and Pelletier, J. (2013) eIF4F suppression in breast cancer affects maintenance and progression. Oncogene **32**, 861–871 CrossRef PubMed

69 Friedl, P. and Alexander, S. (2011) Cancer invasion and the microenvironment: plasticity and reciprocity. Cell **147**, 992–1009 CrossRef PubMed

70 Xu, J., Lamouille, S. and Derynck, R. (2009) TGF-beta-induced epithelial to mesenchymal transition. Cell Res. **19**, 156–172 CrossRef PubMed

71 Proud, C.G. (2014) Mnks, eIF4E phosphorylation and cancer. Biochim. Biophys. Acta 766–773

72 Armengol, G., Rojo, F., Castellvi, J., Iglesias, C., Cuatrecasas, M., Pons, B., Baselga, J. and Cajal, S. (2007) funnel factor. Cancer Res. **67**, 7551–7555 CrossRef PubMed

73 Pelletier, J., Graff, J., Ruggero, D. and Sonenberg, N. (2015) Targeting the eIF4F translation initiation complex: a critical nexus for cancer development. Cancer Res. **75**, 250–263 CrossRef PubMed

74 Liu, Q., Kang, S.A., Thoreen, C.C., Hur, W., Wang, J., Chang, J.W., Markhard, A., Zhang, J., Sim, T., Sabatini, D.M. and Gray, N.S. (2012) Development of ATP-competitive mTOR inhibitors. Methods Mol. Biol. **821**, 447–460 CrossRef PubMed

75 Feldman, M.E., Apsel, B., Uotila, A., Loewith, R., Knight, Z.A., Ruggero, D. and Shokat, K.M. (2009) Active-site inhibitors of mTOR target rapamycin-resistant outputs of mTORC1 and mTORC2. PLoS Biol. **7**, CrossRef

76 Ilic, N., Utermark, T., Widlund, H.R. and Roberts, T.M. (2011) PI3K-targeted therapy can be evaded by gene amplification along the MYC-eukaryotic translation initiation factor 4E (eIF4E) axis. Proc. Natl. Acad. Sci. U.S.A. **108**, E699–E708 CrossRef PubMed

77 Cope, C.L., Gilley, R., Balmanno, K., Sale, M.J., Howarth, K.D., Hampson, M., Smith, P.D., Guichard, S.M. and Cook, S.J. (2014) Adaptation to mTOR kinase inhibitors by amplification of eIF4E to maintain cap-dependent translation. J. Cell Sci. **127**, 788–800 CrossRef PubMed

78 Alain, T., Sonenberg, N. and Topisirovic, I. (2012) mTOR inhibitor efficacy is determined by the eIF4E/4E-BP ratio. Oncotarget **3**, 1491–1492 PubMed

79 Dowling, R.J., Topisirovic, I., Alain, T., Bidinosti, M., Fonseca, B.D., Petroulakis, E., Wang, X., Larsson, O., Selvaraj, A., Liu, Y. et al. (2010) mTORC1-mediated cell proliferation, but not cell growth, controlled by the 4E-BPs. Science **328**, 1172–1176 CrossRef PubMed

80 Graff, J.R., Konicek, B.W., Vincent, T.M., Lynch, R.L., Monteith, D., Weir, S.N., Schwier, P., Capen, A., Goode, R.L., Dowless, M.S. et al. (2007) Therapeutic suppression of translation initiation factor eIF4E expression reduces tumor growth without toxicity. J. Clin. Invest. **117**, 2638–2648 CrossRef PubMed

81 Yanagiya, A., Suyama, E., Adachi, H., Svitkin, Y.V., Aza-Blanc, P., Imataka, H., Mikami, S., Martineau, Y., Ronai, Z.A. and Sonenberg, N. (2012) Translational homeostasis via the mRNA cap-binding protein, eIF4E. Mol. Cell **46**, 847–858 CrossRef PubMed

82 Hou, J., Lam, F., Proud, C. and Wang, S. (2012) Targeting Mnks for cancer therapy. Oncotarget **3**, 118–131 PubMed

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

84 Konicek, B.W., Dumstorf, C.A. and Graff, J.R. (2008) Targeting the eIF4F translation initiation complex for cancer therapy. Cell Cycle **7**, 2466–2471 CrossRef PubMed

85 Diab, S., Teo, T., Kumarasiri, M., Li, P., Yu, M., Lam, F., Basnet, S.K., Sykes, M.J., Albrecht, H., Milne, R. and Wang, S. (2014) Discovery of 5-(2-(phenylamino)pyrimidin-4-yl)thiazol-2(3H)-one derivatives as potent Mnk2 inhibitors: synthesis, SAR analysis and biological evaluation. ChemMedChem **9**, 962–972 CrossRef PubMed

86 Ueda, T., Sasaki, M., Elia, A.J., Chio, II, Hamada, K., Fukunaga, R. and Mak, T.W. (2010) Combined deficiency for MAP kinase-interacting kinase 1 and 2 (Mnk1 and Mnk2) delays tumor development. Proc. Natl. Acad. Sci. U.S.A. **107**, 13984–13990 CrossRef PubMed

87 Papadopoulos, E., Jenni, S., Kabha, E., Takrouri, K.J., Yi, T., Salvi, N., Luna, R.E., Gavathiotis, E., Mahalingam, P., Arthanari, H. et al. (2014) Structure of the eukaryotic translation initiation factor eIF4E in complex with 4EGI-1 reveals an allosteric mechanism for dissociating eIF4G. Proc. Natl. Acad. Sci. U.S.A. **111**, E3187–E3195 CrossRef PubMed

88 Fan, S., Li, Y., Yue, P., Khuri, F.R. and Sun, S.Y. (2010) The eIF4E/eIF4G interaction inhibitor 4EGI-1 augments TRAIL-mediated apoptosis through c-FLIP down-regulation and DR5 induction independent of inhibition of cap-dependent protein translation. Neoplasia **12**, 346–356 CrossRef PubMed

89 Chen, L., Aktas, B.H., Wang, Y., He, X., Sahoo, R., Zhang, N., Denoyelle, S., Kabha, E., Yang, H., Freedman, R.Y. et al. (2012) Tumor suppression by small molecule inhibitors of translation initiation. Oncotarget **3**, 869–881 PubMed

90 Descamps, G., Gomez-Bougie, P., Tamburini, J., Green, A., Bouscary, D., Maiga, S., Moreau, P., Gouill, S., Pellat-Deceunynck, C. and Amiot, M. (2012) The cap-translation inhibitor 4EGI-1 induces apoptosis in multiple myeloma through Noxa induction. Br. J. Cancer **106**, 1660–1667 CrossRef PubMed

91 Attar-Schneider, O., Drucker, L., Zismanov, V., Tartakover-Matalon, S. and Lishner, M. (2014) Targeting eIF4GI translation initiation factor affords an attractive therapeutic strategy in multiple myeloma. Cell. Signal. **26**, 1878–1887 CrossRef PubMed

92 Yi, T., Kabha, E., Papadopoulos, E. and Wagner, G. (2014) 4EGI-1 targets breast cancer stem cells by selective inhibition of translation that persists in CSC maintenance, proliferation and metastasis. Oncotarget **5**, 6028–6037 PubMed

93 Mahalingam, P., Takrouri, K., Chen, T., Sahoo, R., Papadopoulos, E., Chen, L., Wagner, G., Aktas, B.H., Halperin, J.A. and Chorev, M. (2014) Synthesis of rigidified eIF4E/eIF4G inhibitor-1 (4EGI-1) mimetic and their *in vitro* characterization as inhibitors of protein-protein interaction. J. Med. Chem. **57**, 5094–5111 CrossRef PubMed

94 Takrouri, K., Chen, T., Papadopoulos, E., Sahoo, R., Kabha, E., Chen, H., Cantel, S., Wagner, G., Halperin, J.A., Aktas, B.H. and Chorev, M. (2014) Structure-activity relationship study of 4EGI-1, small molecule eIF4E/eIF4G protein-protein interaction inhibitors. Eur. J. Med. Chem. **77**, 361–377 CrossRef PubMed

95 Yefidoff-Freedman, R., Chen, T., Sahoo, R., Chen, L., Wagner, G., Halperin, J.A., Aktas, B.H. and Chorev, M. (2014) 3-substituted indazoles as configurationally locked 4EGI-1 mimetics and inhibitors of the eIF4E/eIF4G interaction. Chembiochem **15**, 595–611 CrossRef PubMed

96 Lukhele, S., Bah, A., Lin, H., Sonenberg, N. and Forman-Kay, J.D. (2013) Interaction of the eukaryotic initiation factor 4E with 4E-BP2 at a dynamic bipartite interface. Structure **21**, 2186–2196 CrossRef PubMed

97 Igreja, C., Peter, D., Weiler, C. and Izaurralde, E. (2014) 4E-BPs require non-canonical 4E-binding motifs and a lateral surface of eIF4E to repress translation. Nat. Commun. **5**, 4790 CrossRef PubMed

98 Peter, D., Igreja, C., Weber, R., Wohlbold, L., Weiler, C., Ebertsch, L., Weichenrieder, O. and Izaurralde, E. (2015) Molecular architecture of 4E-BP translational inhibitors bound to eIF4E. Mol. Cell **57**, 1074–1087 CrossRef PubMed

99 Bah, A., Vernon, R.M., Siddiqui, Z., Krzeminski, M., Muhandiram, R., Zhao, C., Sonenberg, N., Kay, L.E. and Forman-Kay, J.D. (2015) Folding of an intrinsically disordered protein by phosphorylation as a regulatory switch. Nature **519**, 106–109 CrossRef PubMed

100 Jia, Y., Polunovsky, V., Bitterman, P.B. and Wagner, C.R. (2012) Cap-dependent translation initiation factor eIF4E: an emerging anticancer drug target. Med. Res. Rev. **32**, 786–814 CrossRef PubMed

101 Li, S., Jia, Y., Jacobson, B., McCauley, J., Kratzke, R., Bitterman, P.B. and Wagner, C.R. (2013) Treatment of breast and lung cancer cells with a N-7 benzyl guanosine monophosphate tryptamine phosphoramidate pronucleotide (4Ei-1) results in chemosensitization to gemcitabine and induced eIF4E proteasomal degradation. Mol. Pharm. **10**, 523–531 CrossRef PubMed

102 Ghosh, B., Benyumov, A.O., Ghosh, P., Jia, Y., Avdulov, S., Dahlberg, P.S., Peterson, M., Smith, K., Polunovsky, V.A., Bitterman, P.B. and Wagner, C.R. (2009) Nontoxic chemical interdiction of the epithelial-to-mesenchymal transition by targeting cap-dependent translation. ACS Chem. Biol. **4**, 367–377 CrossRef PubMed

103 Chen, E.Z., Jacobson, B.A., Patel, M.R., Okon, A.M., Li, S., Xiong, K., Vaidya, A.J., Bitterman, P.B., Wagner, C.R. and Kratzke, R.A. (2014) Small-molecule inhibition of oncogenic eukaryotic protein translation in mesothelioma cells. Invest. New Drugs **32**, 598–603 CrossRef PubMed

104 Chen, X., Kopecky, D.J., Mihalic, J., Jeffries, S., Min, X., Heath, J., Deignan, J., Lai, S., Fu, Z., Guimaraes, C. et al. (2012) Structure-guided design, synthesis, and evaluation of guanine-derived inhibitors of the eIF4E mRNA-cap interaction. J. Med. Chem. **55**, 3837–3851 CrossRef PubMed

105 Chu, J. and Pelletier, J. (2014) Targeting the eIF4A RNA helicase as an anti-neoplastic approach. Biochim. Biophys. Acta 781–791

106 Marusyk, A., Almendro, V. and Polyak, K. (2012) Intra-tumour heterogeneity: a looking glass for cancer? Rev. Cancer **12**, 323–334 CrossRef

107 Polyak, K. (2014) Tumor heterogeneity confounds and illuminates: a case for Darwinian tumor evolution. Nat. Med. **20**, 344–346 CrossRef PubMed

108 Onsum, M.D., Geretti, E., Paragas, V., Kudla, A.J., Moulis, S.P., Luus, L., Wickham, T.J., McDonagh, C.F., Macbeath, G. and Hendriks, B.S. (2013) Single-cell quantitative HER2 measurement identifies heterogeneity and distinct subgroups within traditionally defined HER2-positive patients. Am. J. Pathol. **183**, 1446–1460 CrossRef PubMed

109 Rao, C., Shetty, J. and Prasad, K.H. (2013) Immunohistochemical profile and morphology in triple - negative breast cancers. J. Clin. Diagn. Res. **7**, 1361–1365 PubMed

110 Wolff, A.C., Hammond, M.E., Schwartz, J.N., Hagerty, K.L., Allred, D.C., Cote, R.J., Dowsett, M., Fitzgibbons, P.L., Hanna, W.M., Langer, A. et al. (2007) American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. J. Clin. Oncol. **25**, 118–145 CrossRef PubMed

111 Widakowich, C., de Castro, G., de Azambuja, E., Dinh, P. and Awada, A. (2007) Review: side effects of approved molecular targeted therapies in solid cancers. Oncologist **12**, 1443–1455 CrossRef PubMed

112 Larkin, J., Ascierto, P.A., Dreno, B., Atkinson, V., Liszkay, G., Maio, M., Mandala, M., Demidov, L., Stroyakovskiy, D., Thomas, L. et al. (2014) Combined vemurafenib and cobimetinib in BRAF-mutated melanoma. N. Engl. J. Med. **371**, 1867–1876 CrossRef PubMed

113 Ramon, Y.C.S., De Mattos-Arruda, L., Sonenberg, N., Cortes, J. and Peg, V. (2014) The intra-tumor heterogeneity of cell signaling factors in breast cancer: p4E-BP1 and peIF4E are diffusely expressed and are real potential targets. Clin. Transl. Oncol. **16**, 937–941 CrossRef PubMed

114 Boussemart, L., Malka-Mahieu, H., Girault, I., Allard, D., Hemmingsson, O., Tomasic, G., Thomas, M., Basmadjian, C., Ribeiro, N., Thuaud, F. et al. (2014) eIF4F is a nexus of resistance to anti-BRAF and anti-MEK cancer therapies. Nature **513**, 105–109 CrossRef PubMed

115 Gkogkas, C.G., Khoutorsky, A., Ran, I., Rampakakis, E., Nevarko, T., Weatherill, D.B., Vasuta, C., Yee, S., Truitt, M., Dallaire, P. et al. (2013) Autism-related deficits via dysregulated eIF4E-dependent translational control. Nature **493**, 371–377 CrossRef PubMed

116 Gkogkas, C.G., Khoutorsky, A., Cao, R., Jafarnejad, S.M., Prager-Khoutorsky, M., Giannakas, N., Kaminari, A., Fragkouli, A., Nader, K., Price, T.J. et al. (2014) Pharmacogenetic inhibition of eIF4E-dependent Mmp9 mRNA translation reverses fragile X syndrome-like phenotypes. Cell Rep. **9**, 1742–1755 CrossRef PubMed

Received 27 May 2015
doi:10.1042/BST20150126