Targeting the translation machinery in cancer

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Abstract | Dysregulation of mRNA translation is a frequent feature of neoplasia. Many oncogenes and tumour suppressors affect the translation machinery, making aberrant translation a widespread characteristic of tumour cells, independent of the genetic make-up of the cancer. Therefore, therapeutic agents that target components of the protein synthesis apparatus hold promise as novel anticancer drugs that can overcome intra-tumour heterogeneity. In this Review, we discuss the role of translation in cancer, with a particular focus on the eIF4F (eukaryotic translation initiation factor 4F) complex, and provide an overview of recent efforts aiming to 'translate' these results to the clinic.

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Protein synthesis, or mRNA translation, is the most energy-consuming process in the cell, and it has a major role in the regulation of gene expression[1–3]. Translation, which involves the orchestrated interaction of tRNAs, ribosomes, auxiliary factors and mRNA (FIG. 1), is tightly controlled[4,5]. Dysregulation of this process can be considered a hallmark of cancer and is linked to aberrant proliferation, survival, angiogenesis, alterations in immune response and cancer energetics[6–15].

Translational control provides quicker adaptive responses to environmental cues than any upstream steps in the gene expression pathway, as the onset time of regulatory events depends on the decay of all the downstream macromolecules — a concept particularly relevant in immunity[16]. Translational control is also harnessed to generate morphogen gradients[17] and localized protein synthesis[18] — events necessary for pattern development and synaptic plasticity, respectively. Several recent reports demonstrating that steady-state mRNA levels show low concordance with the cellular proteome highlight the major role the regulation of translation has in controlling gene expression[19–22].

The first evidence that mRNA translation is dysregulated in cancer dates back to 1896, when it was observed that hypertrophic nucleoli were a prominent feature of malignant cells[23]. Perhaps the most direct evidence of the importance of translation in human cancers comes from the existence of ribosomopathies, such as Diamond–Blackfan anaemia, chromosome 5q deletion syndrome and Shwachman–Diamond syndrome[24–26]. These hereditary disorders are caused by genetic alterations in ribosomal subunits or their regulatory factors, and are associated with a heightened incidence of haematological malignancies[27]. Although these particular diseases are rare, dysregulated translation is omnipresent in human cancers. Indeed, many translation initiation factors are frequently amplified or dysregulated in tumours[26] (TABLE 1). Moreover, the most common cancer-related mutations are found in pathways feeding into the translation machinery[26] (FIG. 2). Thus, several oncogenes (for example, *MYC*, *RAS* and *PIK3CA* (phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit-α) and tumour suppressors (for example, *PTEN* (phosphatase and tensin homologue) and *TP53*) affect the translation machinery[26]. Considering its position at the convergence point of such a wide range of common oncogenic lesions, understanding the molecular underpinnings of protein synthesis in cancer is crucial for the development of effective anticancer therapies.

The genetic make-up of cancer cells in any given primary tumour or metastasis can be dramatically different[28]. This intra-tumour heterogeneity is thought to be one of the major obstacles in applying targeted therapies in the clinic, as although a given drug may efficiently eliminate cells that harbour specific genetic lesions, those that are driven by alternative oncogenic pathways survive the treatment. As the components of the translation machinery integrate almost all oncogenic signals (FIG. 2), targeting the components of this machinery holds promise for overcoming a major hurdle associated with intra-tumour heterogeneity. Moreover, because malignant cells exhibit augmented activity of most of the components of the translation machinery, it is thought that they become 'addicted' to elevated protein synthesis[26,29]. This potentially provides a therapeutic window to selectively target cancer cells.

Here, we discuss progress in strategies to target components of the translation machinery for the treatment of cancer. We focus on the eukaryotic translation initiation factor 4F complex (eIF4F complex), and particularly its subunit eIF4E, as this is the best-understood and most intensely pursued target in this area. We review the evidence for the involvement of eIF4F in cancer and discuss the regulatory mechanisms controlling its assembly and function, as well as strategies to target it either directly or through its upstream regulators. We also provide a brief overview of other emerging targets among the translation machinery.

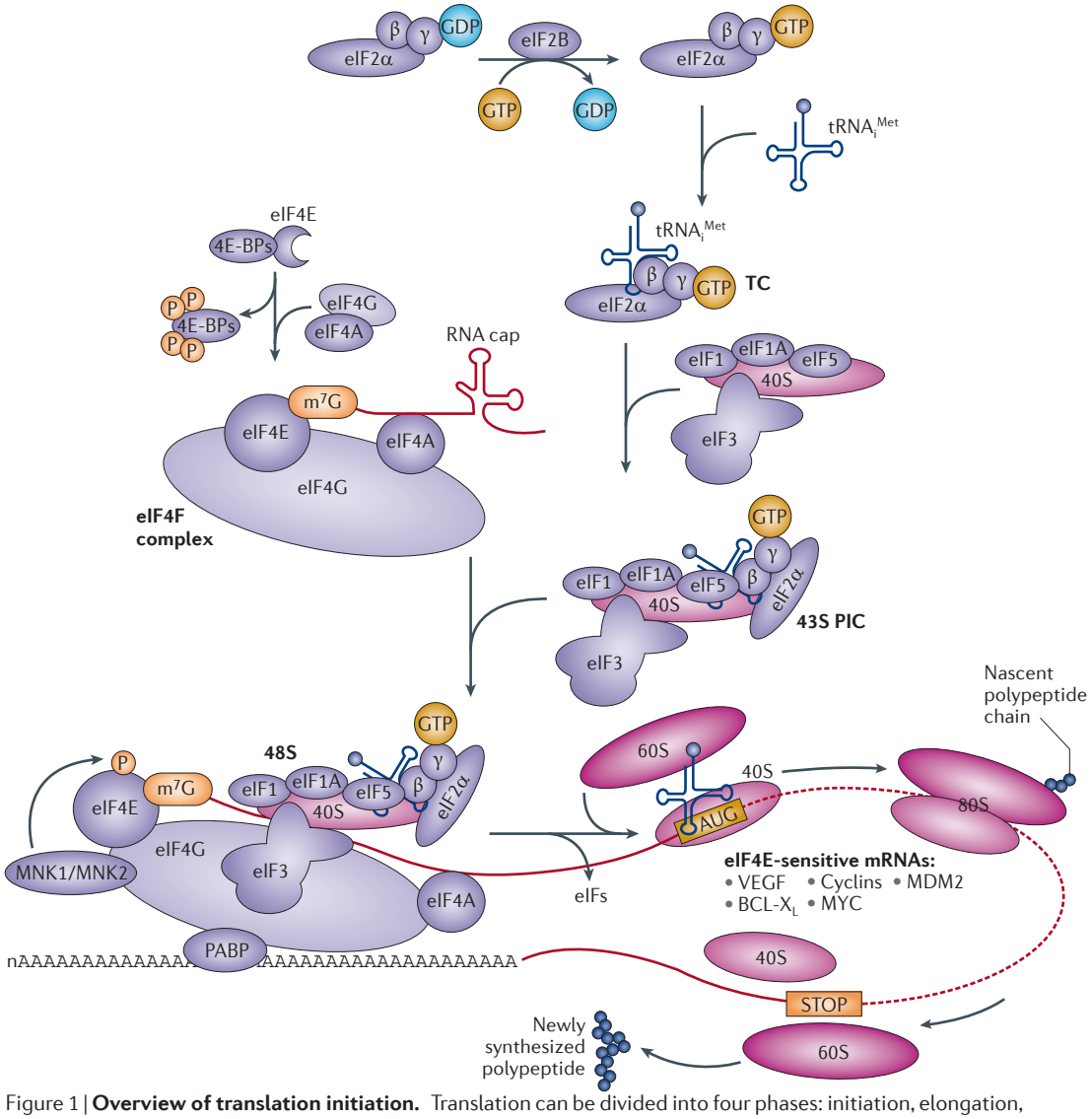


Figure 1 | **Overview of translation initiation.** Translation can be divided into four phases: initiation, elongation, termination and ribosome recycling. The two best-characterized and most prominent mechanisms that regulate translation take place at the rate-limiting phase of initiation and involve controlling the assembly of a functional 40S subunit with its associated factors (43S pre-initiation complex (43S PIC)) or altering the access of PICs to the mRNA template. The 43S PIC is a large multifactorial complex formed by the association of the 40S ribosomal subunit with eukaryotic translation initiation factors (eIFs) eIF1, eIF1A, eIF3, eIF5 and the ternary complex (TC). The TC consists of a trimeric complex involving eIF2 (containing α-, β- and γ-subunits), initiator methionyl tRNA (tRNA$_i^{Met}$) and GTP. The recruitment of the 43S PIC to the mRNA template is facilitated by eIF4F, a complex consisting of the mRNA 5′-cap-binding subunit (eIF4E), a large scaffolding protein (eIF4G) and the DEAD box RNA helicase (eIF4A), leading to 48S PIC assembly. eIF4F recruits ribosomes to mRNA through eIF4E–mRNA cap and eIF4G–eIF3 interactions, resulting in the formation of a 48S initiation complex. eIF4G also interacts with the poly(A)-binding protein (PABP), which associates with the mRNA 3′ poly(A) tail, to cause mRNA circularization to stabilize mRNAs and bolster translation. The eIF4A helicase participates in the initial interactions of eIF4F with the mRNA 5′ end and may also facilitate scanning of the 40S ribosomal subunit towards the initiation codon by resolving the secondary structure in the 5′ untranslated region (UTR). Recognition of the initiation codon by the 43S PIC leads to the release of eIFs and joining of the 60S ribosomal subunit. The formation of a translation-competent 80S ribosome marks the end of initiation and the beginning of elongation. Detailed descriptions of the molecular underpinnings of translation initiation are reviewed in REF. 235. BCL-X$_L$, B-cell lymphoma extra large; 4E-BP, 4E-binding protein; m^{7}G, 7-methylguanosine 5′-cap; MNK, MAPK-interacting kinase; VEGF, vascular endothelial growth factor.

mRNA translation
A process whereby proteins are synthesized from mRNAs by the orchestrated action of ribosomes, tRNAs and auxiliary proteins. Translation occurs in four distinct phases: initiation (positioning of the translation-competent ribosome on the initiator codon of mRNA), elongation (incorporation of amino acids into a growing polypeptide chain), termination (release of synthesized polypeptides from the ribosome) and ribosome recycling (dissociation of ribosomes and auxiliary factors to release free ribosomal subunits).

Translation initiation
The first and generally rate-limiting step of translation, during which initiation factors facilitate positioning of the translation-competent ribosome at the initiation codon of the mRNA. Most translational control occurs at this step.

Intra-tumour heterogeneity
The existence of multiple genetically heterogeneous clones of cancer cells in the same tumour bed that probably evolved through branched evolution. Intra-tumour heterogeneity hampers therapies that are tailored to target specific 'driver mutations' as cancer cells in the same tumour can be driven by several different oncogenic pathways.

Eukaryotic translation initiation factor 4F complex
(eIF4F complex).
A heterotrimeric complex composed of a 5′ mRNA cap-binding subunit eIF4E, the large scaffolding protein eIF4G, and the ATP-dependent RNA helicase eIF4A. The eIF4F complex recruits the mRNA to the ribosome and facilitates its scanning of the 5′ untranslated region (5′UTR) in search of an initiation codon.

43S pre-initiation complex
(43S PIC). A large multifactorial complex formed by association of the 40S ribosomal subunit with eukaryotic translation initiation factors (eIFs) eIF1, eIF1A, eIF3, eIF5 and the ternary complex. The 43S PIC is recruited to the mRNA by the eIF4F complex, which leads to formation of the 48S initiation complex.

The eIF4F complex and its regulation

eIF4E is the cap-binding subunit of eIF4F. Although eIF4E is required for cap-dependent translation of all nuclear-encoded mRNAs, changes in its levels disproportionally affect the translation of a subset of mRNAs encoding proliferation, survival and tumour-promoting proteins. These mRNAs include cyclins[30], ornithine decarboxylase (*ODC*)[31], vascular endothelial growth factor (*VEGF*)[11], *MYC*[32] and phosphoribosyl-pyrophosphate synthetase 2 (*PRPS2*)[33], which are called eIF4E-sensitive mRNAs. However, changes in eIF4E levels have only a small effect on the expression of house-keeping mRNAs (for example, glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) and β-actin)[34]. Unlike house-keeping mRNAs that are mostly characterized by short, low complexity 5′ untranslated regions (UTRs), most eIF4E-sensitive mRNAs have long and highly structured 5′UTRs[29,34,35] and are more dependent on the unwinding activity of eIF4A[36]. eIF4A, a bidirectional ATP-dependent DEAD box RNA helicase, is loaded onto mRNA templates as a subunit of eIF4F, thus imparting 5′ to 3′ directionality. The helicase activity of eIF4A is ~20-fold higher when it is part of the eIF4F complex than when it is free[37]. As eIF4E is the least abundant translation initiation factor, and therefore limiting for eIF4F complex assembly, its upregulation increases levels of eIF4F and stimulates unwinding by eIF4A[37–40]. Importantly, eIF4E also stimulates the RNA helicase activity of eIF4A independently of its cap-binding function[40]. The precise mechanisms underlying the 'eIF4E-sensitivity' of mRNAs, and the spectrum of mRNAs that are regulated by eIF4E, remain to be determined. Similar to eIF4E, eIF4G and eIF4A control the translation of a subset of mRNAs that partially overlaps with eIF4E[41,42], thus indicating that all three components of the eIF4F complex have major roles in the regulation of gene expression by altering the translation of specific mRNAs.

Formation and activity of the eIF4F complex is tightly regulated to maintain cellular homeostasis and is controlled by major signal transduction pathways, as described below and in FIG. 2.

The mechanistic/mammalian target of rapamycin pathway. Mammalian target of rapamycin (mTOR) is a serine/threonine kinase that exists as two functionally and structurally divergent complexes: mTOR complex 1 (mTORC1) and mTORC2 (REF. 43). mTOR has recently been renamed the 'mechanistic target of rapamycin', but the reasons behind this change in nomenclature are complex[44]. Whereas mTORC1 affects cellular proliferation, growth, protein synthesis, metabolic programmes and autophagy through several substrates[43,45], mTORC2 activates several members of the AGC family of kinases, including serum/glucocorticoid regulated kinase 1 and AKT (also known as protein kinase B), that are major regulators of cell survival[46]. Moreover, mTORC2 has been implicated in the regulation of cytoskeletal organization and degradation of newly synthesized polypeptides, as well as glucose and lipid metabolism[46]. mTORC1 links many extracellular and intracellular growth cues to the translation process, mainly by regulating the assembly of the eIF4F complex, but also on a longer timescale, by promoting the transcription of genes encoding rRNAs and tRNAs, as described below[47] (FIG. 2).

The eIF4G binding site on eIF4E also interacts with small translational suppressors, the 4E-binding proteins (4E-BPs); in mammals there are three known 4E-BPs — 4E-BP1, 4E-BP2 and 4E-BP3. By competing with eIF4G, the 4E-BPs interfere with eIF4F complex assembly[48]. Activation of the mTOR pathway leads to phosphorylation of 4E-BPs at multiple sites and causes their dissociation from eIF4E, allowing eIF4E–eIF4G association and eIF4F complex assembly[47–56]. mTORC1 also affects translation through phosphorylation and activation of S6 kinase 1 (S6K1) and S6K2 (REFS 53,54,57,58). S6Ks phosphorylate various substrates, including ribosomal protein S6 (REF. 59), eIF4B[60] and programmed cell death 4 (PDCD4)[61]. The phosphorylation of PDCD4 by S6Ks leads to its degradation by the proteasome, mediated by β-transducin repeat-containing protein 1 (β-TrCP1), which is an E3 ubiquitin ligase[61]. As PDCD4 inhibits translation initiation by binding to eIF4A, its phosphorylation and degradation lead to release of eIF4A from inhibitory PDCD4–eIF4A complexes[62]. mTORC1 also phosphorylates eIF4G[63] and stimulates the activity of eukaryotic elongation factor 2 (eEF2) by phosphorylation and inactivation of the eEF2 kinase by S6Ks[64]. Finally, mTORC1 is also thought to enhance protein synthesis through activation of the RNA polymerase I transcription initiation factor TIF1A[65], as well as through the phosphorylation and suppression of the RNA polymerase III inhibitor MAF1 (REF. 66), leading to increased ribosome biogenesis and tRNA synthesis, respectively.

It has long been known that mTOR stimulates the translation of mRNAs with 5′-terminal oligopyrimidine tracts (TOPs), which encode components of the translation machinery, including ribosomal proteins, eEF2 and poly(A)-binding protein (PABP)[67]. The 5′ TOP sequence consists of a C nucleotide at the +1 position of the mRNA (the penultimate nucleotide) followed by a stretch of 4–14 pyrimidines[67]. Recent studies using ribosome profiling[68] showed that mTOR stimulates the translation of mRNAs with TOPs and TOP-like pyrimidine-rich translational element (PRTE) motifs through phosphorylation and inactivation of 4E-BPs[69,70]. Surprisingly, in these studies the mTOR–4E-BP pathway seemed to not have an important role in regulating synthesis of proteins encoded by mRNAs for which translation was previously shown to be 'eIF4E-sensitive' and regulated by the mTOR–4E-BP pathway. Another study, using mRNA profiling across polysomes[71], showed that in addition to TOP mRNAs, mTOR promoted translation of mRNAs that encode proteins essential for mitochondrial function and biogenesis (for example, complex I and V components), as well as known 'eIF4E-sensitive' mRNAs (for example, cyclins and *ODC*)[72,73]. The variation within the different studies can be partially explained by the conditions under which the experiments were performed. However, hypoxia, nutrient deprivation and other types of stress suppress the translation of TOP mRNAs

eIF4E-sensitive mRNAs
The subset of mRNAs for which translation is disproportionately affected by changes in eIF4E levels. Most of these mRNAs encode proteins that promote proliferation, survival, invasion and metastasis. A selective increase in the expression of tumorigenic factors encoded by eIF4E-sensitive mRNAs underpins the oncogenic activity of eIF4E.

Mammalian target of rapamycin
(mTOR). A serine/threonine kinase that integrates extracellular signals and intracellular cues to regulate proliferation, growth, protein synthesis, metabolic programmes and autophagy via a multitude of substrates. mTOR exists as two functionally and structurally divergent complexes, mTOR complex 1 (mTORC1) and mTORC2. mTORC1 stimulates translation by phosphorylating 4E-binding proteins and S6 kinases.

4E-binding proteins
(4E-BPs). Small translational suppressors that impede eukaryotic translation initiation factor 4F (eIF4F) assembly by competing with eIF4G for binding to eIF4E. Mammalian target of rapamycin complex 1 (mTORC1) phosphorylates 4E-BPs at multiple sites, which leads to their dissociation from eIF4E, facilitating assembly of the eIF4F complex. 4E-BP1, 4E-BP2 and 4E-BP3 are present in mammals.

Table 1 | **Dysregulation of translation initiation factors and regulators in human cancers**

Factor	Dysregulation	Clinical correlates in cancers
eIF4E	Overexpression	• Decreased survival in breast, head and neck, liver, prostate, bladder and stomach cancers • Correlates with disease progression and aggressive subtypes in many cancers, and with resistance to chemotherapy
eIF4E	Phosphorylation	• Elevated in early stages of development of breast, colon, gastric and lung cancers • Increased in prostate cancer and correlates with androgen independence • Poor-prognosis marker in non-small-cell lung cancer
4E-BP1	Overexpression	• Inversely correlates with tumour grade • Correlates with better survival in lung and prostate cancers • Correlates with absence of lymph node and distant metastases in gastric cancer
4E-BP1	Loss	Possibly responsible for loss of translational control in 50% of pancreatic tumours
4E-BP1	Phosphorylation	Correlates with tumour grade and poor prognosis in breast, lung, ovarian and prostate cancers
eIF4G	Increased expression	• Amplification correlates with aggressive stages in lung cancer • Overexpressed in inflammatory breast cancer and cervical cancer • Correlates with poor prognosis in nasopharyngeal carcinoma
eIF4A	Increased expression	Overexpressed in lung and cervical cancer; lowered expression after radiation predicts better survival in cervical cancer
PDCD4	Decreased expression	• Associated with poor prognosis in breast, lung, colon and ovarian cancers and gliomas • Inversely correlated with advanced tumour stage in renal cell carcinoma
eIF2α	Increased expression	Correlates with aggressive lymphoma subtypes
eIF5A	Increased expression and hypusination	Correlates with poor prognosis in early-onset colorectal cancer. Overexpression of eIF5A2 correlates with local invasion in non-small-cell lung cancer and hepatocellular carcinoma
eIF6	Altered expression and function	• Regulates ribosome biogenesis and 40S–60S joining. Promotes transformation and lymphomagenesis • Elevated in colorectal cancer, head and neck carcinomas and ovarian serous carcinoma; low expression correlates with reduced disease-free survival in ovarian serous carcinoma; mediates lymphomagenesis in Shwachman–Diamond syndrome
eIF3a	Increased expression	Associated with breast, cervical, oesophagal, lung and stomach cancers
eIF3b	Increased expression	Associated with bladder, breast and prostate cancers
eIF3c	Increased expression	Associated with meningioma and testicular seminoma
eIF3h	Increased expression	Associated with breast, colon, liver and prostate cancers
eIF3i	Increased expression	Associated with breast, head and neck, and liver cancers, as well as melanoma and neuroblastoma
eIF3m	Increased expression	Associated with colon cancer
eIF3e	Decreased expression	Associated with breast, lung and prostate cancers
eIF3f	Decreased expression	Associated with breast, colon, small intestine, ovarian, pancreatic and vulval cancers and melanoma

References provided in Supplementary information S1 (table). 4E-BP, 4E-binding protein; eIF, eukaryotic translation initiation factor; PDCD4, programmed cell death 4.

MAPK-interacting kinases (MNKs). Kinases that regulate eukaryotic translation initiation factor 4E (eIF4E) by phosphorylating it at a single site (Ser209 in mammals). MNKs are activated downstream of the MEK–ERK (MAPK/ERK kinase–extracellular signal-regulated kinase) and p38 MAPK (mitogen-activated protein kinase) pathways in response to mitogenic signals or stress. Phosphorylation of eIF4E stimulates translation of a subset of mRNAs encoding invasion-promoting and metastasis-promoting proteins and cytokines.

independently of 4E-BPs, indicating that factors other than 4E-BPs may mediate the effects of mTORC1 on translation of TOP mRNAs[74]. Indeed, a recent study showed that LARP1 modulates TOP mRNA translation downstream of mTORC1 (REFS 75,76), although control of mRNA stability may also have a role in this context[77].

The MAPK-interacting kinases. In mammals, eIF4E is also regulated by phosphorylation on Ser209 by the MAPK-interacting kinases (MNKs)[78]. The MNKs are MAPK-activated protein kinases (MAPKAPKs) (reviewed in REF. 79) that are activated downstream of the MEK–ERK (MAPK/ERK kinase–extracellular signal-regulated

kinase) and p38 MAPK (mitogen-activated protein kinase) pathways in response to mitogenic signals and stress, respectively[80–82]. There are two MNK genes in the human genome — *MKNK1* and *MKNK2*, each producing two isoforms encoding MNK1a, MNK1b, MNK2a and MNK2b proteins[80]. MNK isoforms differ in their carboxy-terminal MAPK-interacting domains, which are phosphorylated by upstream kinases[80]. MNK1a is activated by ERK and p38 MAPK, but exhibits low basal activity; by contrast, MNK2a has high basal activity, and is only modestly phosphorylated by upstream ERK signalling (MNK2a is not activated by p38 MAPK)[83]. MNK1b and MNK2b do not possess MAPK-binding sites and are therefore constitutively active[80]. To phosphorylate eIF4E, the MNKs bind to eIF4G[84], which is facilitated by the eIF3 complex, perhaps through eIF3e[85]. Although phosphorylation of eIF4E does not have a major effect on global translation, it stimulates the translation of a subset of mRNAs encoding survival (for example, myeloid cell leukaemia 1 (*MCL1*))[86] and invasion-promoting proteins (for example, matrix metalloproteinase 3 (*MMP3*))[87] and cytokines[88].

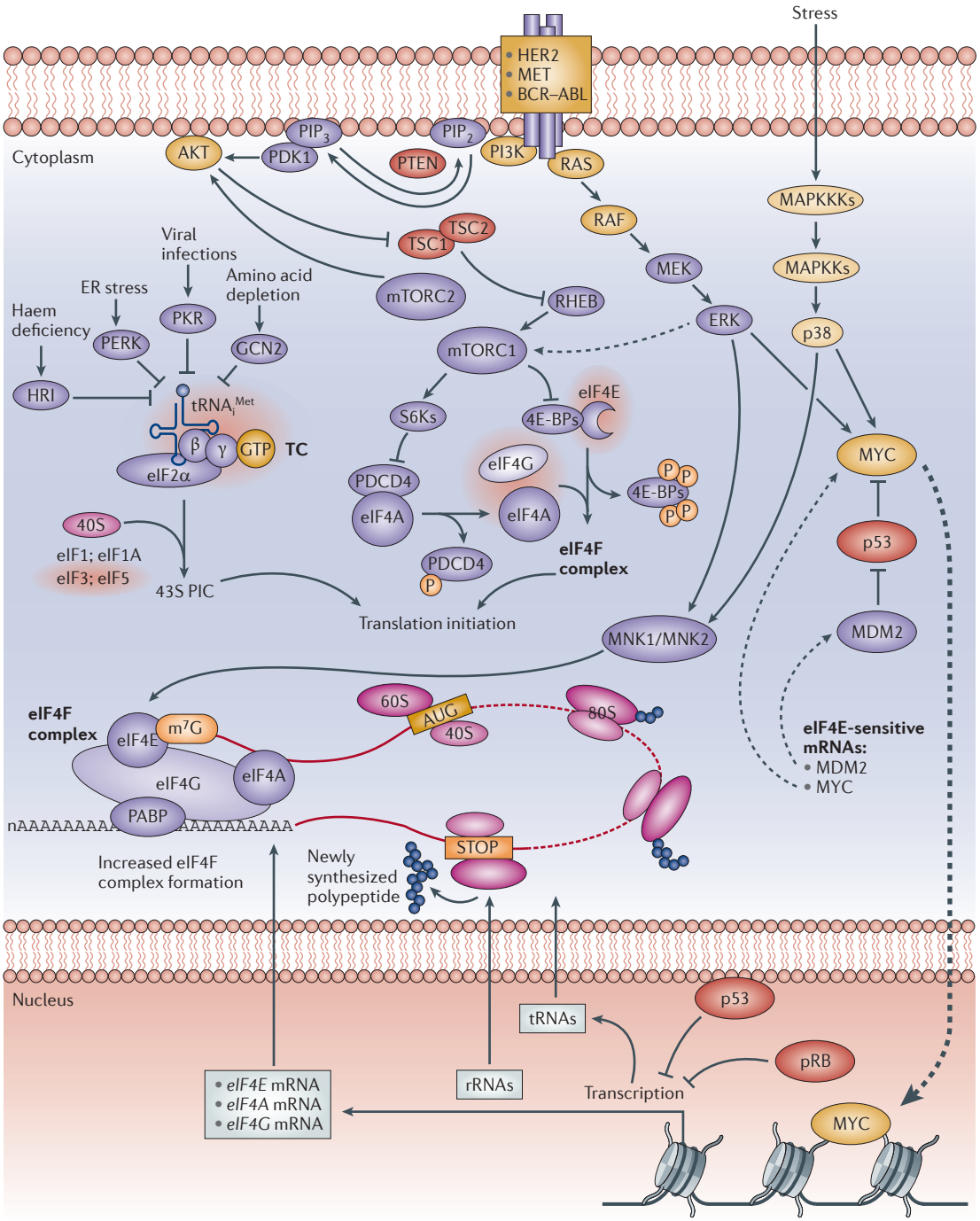
Transcriptional and post-transcriptional regulation of eIF4F complex formation. In addition to regulation by signalling pathways, eIF4F complex formation is controlled by transcriptional and post-transcriptional mechanisms. Several transcription factors including MYC[89], the p30 isoform of CCAAT/enhancer-binding protein-α (C/EBPα)[90], nuclear factor-κB (NF-κB)[91] and hypoxia-inducible factor 1α (HIF1α)[92], as well as post-transcriptional regulators such as HuR and AU-rich binding factor 1 (AUF1)[93], control eIF4E expression. The best-characterized factor that regulates eIF4E expression is the MYC oncoprotein, which promotes eIF4E transcription by binding to two conserved E-box motifs in its promoter[94]. Interestingly, MYC also promotes the transcription of eIF4A and eIF4G[95], and ribosome and tRNA biogenesis[96] (FIG. 2). As the MYC mRNA is itself a translational target of the eIF4F complex, these findings highlight a regulatory feedforward loop by which MYC increases eIF4F levels, which in turn promotes the translation of MYC mRNA[95].

The role of eIF4F in cancer. The dysregulation of many translation initiation factors has been described in various cancers (TABLE 1), although the role of the eIF4F complex, and in particular its subunit eIF4E, in neoplasia is the best-established example[29]. More than two decades ago, it was shown that eIF4E exhibits oncogenic properties *in vitro*; that is, its overexpression resulted in neoplastic transformation of mouse fibroblasts[97]. In turn, depletion of eIF4E was sufficient to abolish RAS-mediated tumorigenesis[98]. These findings were corroborated in human cells[99], as well as by *in vivo* experiments, in which overexpression of eIF4E led to multiple tumour types, reminiscent of human cancers displaying high eIF4E levels, and dramatically accelerated MYC-driven tumorigenesis[100,101]. These pro-neoplastic properties of eIF4E are correlated with selective upregulation of translation of mRNAs encoding tumour-promoting factors that stimulate proliferation (for example, cyclins, *MYC*, *ODC*, insulin-like growth factor (*IGF*)), survival (for example, B cell lymphoma extra large (*BCLXL*), *HDM2*, *MCL1*) and neo-angiogenesis (for example, *VEGF* and fibroblast growth factor 2 (*FGF2*))[34,102].

eIF4E levels and/or availability for eIF4F assembly are increased in neoplasia. Overexpression of eIF4E results from gene amplification, as documented in head and neck squamous cell carcinomas, or as a consequence of MYC-mediated transcriptional activation[103,104]. As eIF4E assembly into the eIF4F complex is controlled by 4E-BPs[48], expression of a non-phosphorylatable 4E-BP1 mutant that constitutively binds to eIF4E is sufficient to suppress cellular proliferation and neoplastic growth[105–107], whereas loss of 4E-BP expression accelerates tumorigenesis driven by p53 loss[108,109]. These findings demonstrate that the 4E-BPs function as tumour suppressors[109,110]. Therefore, eIF4E is expected to have a central role in cancers harbouring mutations that lead to mTORC1 hyperactivation — for example, mutations in *PIK3CA*, *PTEN*, *LKB1* (liver kinase B1), tuberous sclerosis complex 1 (TSC1), *TSC2*, *RAS* and others[111].

Phosphorylation of eIF4E is also implicated in cancer development and progression[86,87,112,113]. Overexpression of a non-phosphorylatable eIF4E^{S209A} mutant fails to promote neoplastic transformation in NIH 3T3 cells and in the Eμ-Myc lymphoma mouse model[86,112]. In addition, mice with the eIF4E^{S209A} mutant allele are resistant to the development of prostate and breast tumours[87,113]. Importantly, eIF4E phosphorylation promotes epithelial-to-mesenchymal transition, invasion and metastasis[113]. The pro-tumorigenic and pro-metastatic effects of eIF4E phosphorylation are dependent on translational upregulation of a subset of mRNAs, which includes mRNAs encoding proteins involved in survival (for example, *MCL1*) and metastasis (for example, snail family zinc finger 1 (*SNAI1*) and *MMP3*)[86,87,113]. Thus, similar to how mutations in the phosphoinositide 3-kinase (PI3K)–mTOR pathway funnel through eIF4E to promote neoplastic transformation, mutations activating the MAPK pathway are likely to work at least in part through increasing eIF4E phosphorylation. Taken together, these findings demonstrate that dysregulation of eIF4E can occur through several mechanisms to induce changes in gene expression that drive oncogenesis and tumour progression.

eIF4E is overexpressed by ~3–10-fold in many human cancers, including those of head and neck, bladder, colon, breast, prostate, lung and blood[29,34]. Notably, eIF4E expression in pre-malignant lesions (for example, adenomatous polyps) is lower than in neoplastic lesions (for example, adenocarcinomas)[103,114]. In most studies, eIF4E overexpression is associated with a poor prognosis; that is, increased cancer recurrence and decreased patient survival[34] (TABLE 1). Histological correlates of eIF4E overexpression include tumour angiogenesis and invasiveness[115,116]. For head and neck squamous cell carcinoma, eIF4E overexpression in the tumour margins predicts recurrence and is inversely correlated with survival[117]. In addition, mTORC1 and MAPK signalling are upregulated in most cancers[118,119]. Consistently, increased 4E-BP1 and eIF4E phosphorylation predict elevated rates of



progression in various malignancies, including prostate, breast and colon cancer[87,120,121] (TABLE 1). Overexpression of eIF4G and eIF4A is also detected in several malignancies. Increased eIF4G expression is associated with aggressive stages in lung cancer and inflammatory breast cancer[122,123]. Moreover, eIF4G is overexpressed in cervical cancer[124], and its overexpression correlates with poor prognosis in nasopharyngeal carcinoma[125]. eIF4A is overexpressed in lung and cervical cancer, in which its lower expression after radiation predicts better survival[122,124]. Furthermore, decreased expression of the eIF4A inhibitor PDCD4 has been observed in multiple cancers[126–131].

The accumulated knowledge on signalling pathways feeding into the translation machinery has provided important insights into the potential of therapeutically targeting translation initiation in cancer (FIG. 3). Thus, as described below, inhibitors of the PI3K–mTOR and MAPK pathways have in some cases had considerable influence in the treatment of various cancers (TABLE 2). However, their use has been limited by the development of resistance. Such acquired resistance to treatment is common in cancer and can be attributed in part to intra-tumour heterogeneity[28]. Interestingly, recent studies have highlighted substantial intra-tumour heterogeneity

Figure 2 | **The translation apparatus has a pivotal role in mediating the effects of commonly dysregulated oncogenic signalling pathways in cancer.** The signalling cascades by which hyperactive phosphoinositide 3-kinase (PI3K)–mammalian target of rapamycin (mTOR) and mitogen-activated protein kinase (MAPK) pathways induce formation of the eukaryotic translation initiation factor 4F (eIF4F) complex are indicated. Proteins encoded by commonly mutated oncogenes and tumour suppressors in these pathways are shown in yellow and red, respectively. On the left are the stresses leading to eIF2α phosphorylation, which inhibits translation by stabilizing the interaction between eIF2B and GDP–eIF2 (REF. 235). Also shown is the transcriptional regulation of the translation machinery by the oncoprotein MYC that activates eIF4E, eIF4A and eIF4G and bolsters tRNAs and rRNA synthesis[286], whereas inactivation of the p53 and pRB tumour suppressors releases their repression of tRNA and rRNA synthesis[287]. The net outcome of these alterations differs among tumour cells but is invariably linked to a disproportionate increase in the translation of eIF4E-sensitive transcripts such as *MYC*. Bar-headed lines indicate inhibition, and arrows indicate activation. The thick dashed arrow indicates nuclear translocation of MYC. Components of the translation machinery leading to neoplastic transformation when ectopically overexpressed are circled with red shading. 4E-BP, 4E-binding protein; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; GCN2, general control non-derepressible 2; HER2, human epidermal growth factor receptor 2; HRI, haem-regulated inhibitor; m′G, 7-methylguanosine 5′-cap; MAPKKK, mitogen-activated protein kinase kinase kinase; MEK, MAPK/ERK kinase (also known as MAPKK); MET, mesenchymal epithelial transition factor; MNK, MAPK-interacting kinase; mTORC, mTOR complex; PABP, poly(A)-binding protein; PDCD4, programmed cell death 4; PDK1, 3-phosphoinositide-dependent protein kinase 1; PERK, PKR-like ER kinase; PIC, pre-initiation complex; PIP_2, phosphatidylinositol-4,5-bisphosphate; PIP_3, phosphatidylinositol-3,4,5-trisphosphate; PKR, double-stranded RNA-dependent protein kinase; RHEB, RAS homologue enriched in brain; PTEN, phosphatase and tensin homologue; S6K, ribosomal S6 kinase; $tRNA_i^{Met}$, initiator methionyl tRNA; TSC, tuberous sclerosis.

in the amounts and/or activity of the components of major oncogenic pathways (for example, human epidermal growth factor receptor 2 (HER2), phospho-AKT, phospho-mTOR and phospho-MEK)[132,133]. By contrast, upregulation of eIF4E and increased levels of eIF4E phosphorylation are typically uniformly observed throughout the tumour[132]. Moreover, it has been demonstrated that aberrant eIF4F complex assembly makes cancer cells resistant to inhibition of MAPK or PI3K signalling, which are two major pathways dysregulated in cancer[134–136]. This suggests that cancers are 'addicted' to hyperactive eIF4F complexes irrespective of oncogenic pathways that drive their neoplastic growth; therefore, inhibiting the activity of the eIF4F complex may represent a vulnerability that is common to different cancers and that could be clinically exploited to overcome chemoresistance and intra-tumour heterogeneity. This is an argument for the development of inhibitors of translation initiation (see below and TABLE 2; FIGS 4–6).

Targeting the eIF4F complex

Several strategies that target the eIF4F complex in neoplasia have been devised and can be broadly separated in two categories: those targeting upstream regulators of mRNA translation that also affect other cellular processes (FIG. 4) (for example, PI3K–mTOR inhibitors; reviewed in REF. 137) and those directly targeting the eIF4F complex (FIG. 5). The category targeting eIF4F includes the downregulation of eIF4E with antisense oligonucleotides (ASOs), disrupting eIF4F complex formation, impeding eIF4E–cap interaction and targeting eIF4A[138].

Inhibitors of eIF4F upstream regulators

mTOR inhibitors — Rapamycin and rapalogues. Rapamycin (also known as sirolimus) is a naturally occurring macrolide produced by *Streptomyces hygroscopicus*, which was first described as an antifungal agent in 1975 (REF. 139). Yeast TOR kinases, and subsequently mTOR, were identified as the sole targets of rapamycin[140,141]. Rapamycin binds to the FKBP- and rapamycin-binding (FRB) domain of mTOR in a complex with the immunophilin FKBP12 (FK506-binding protein)[141]. This is thought to induce conformational changes that weaken the interaction between regulatory-associated protein of mTOR (RAPTOR) and mTOR[142,143], thereby inhibiting mTORC1 (REFS 144–147). In addition, rapamycin–FKBP12 seems to induce steric changes in the FRB domain that restrict substrate access to the catalytic site of mTOR[144,147]. The effects of rapamycin also seem to depend on the nature of the residues surrounding substrate phospho-acceptor sites, which partly explains why this drug inhibits phosphorylation of some, but not all, mTORC1 phosphorylation sites[148,149]. Whereas mTORC2 is insensitive to acute rapamycin treatment[145,146], prolonged rapamycin treatment suppresses mTORC2 levels in a subset of cell lines and in the liver, possibly as a consequence of newly synthesized mTOR molecules being sequestered by rapamycin–FKBP12 complexes[150,151].

Several rapamycin analogues (rapalogues; for example, everolimus, temsirolimus and ridaforolimus (FIG. 4a)) were generated to improve its pharmacodynamic properties, which exert anti-neoplastic activity in cancer cell lines and mouse models[152]. All are either in use in the clinic or in clinical trials[137]. However, the efficacy of rapalogues in the treatment of human cancers has been lower than expected[153]. This has been attributed in part to incomplete inhibition of the 4E-BPs as well as to activation of AKT through disruption of mTORC1–S6K–PI3K and mTORC1–growth factor receptor-bound protein 10 (GRB10)–PI3K negative feedback loops[76,154–156]. In addition, rapalogues activate MAPKs in a PI3K-dependent manner[157]. Thus, there are several mechanisms of resistance to this class of mTOR inhibitors.

Second-generation mTOR inhibitors. More recent therapeutic strategies aimed at mTOR have addressed some of the shortcomings associated with rapamycin. The activation of AKT can be avoided by using dual-specificity inhibitors (FIG. 4b) that target both mTOR and PI3K, which is the main AKT-activating kinase[137,158]. The clinical applicability of these dual inhibitors in cancer is unclear, and the results of current ongoing clinical trials of PI-103, NVPBEZ235 and other PI3K–mTOR inhibitors are not available[137]. Active-site mTOR inhibitors (asTORi; also known as TORCi and TORKinibs) have also been synthesized[153] (FIG. 4c). These potently inhibit both mTORC1 and mTORC2 (REFS 159–161). As mTORC2 can activate AKT by phosphorylating it on Ser473 (REFS 162,163), asTORi suppress AKT signalling[153] and exhibit stronger anti-neoplastic effects compared to rapalogues[153]. However, loss of mTORC2 activity does not reduce the anti-proliferative activity of the asTORi PP242 or Torin1 (REFS 159,160), thus demonstrating that

Rapamycin
A macrolide that associates with mammalian target of rapamycin (mTOR) in a complex with the immunophilin FKBP12, resulting in allosteric modifications in mTOR that lead to its dissociation from the mTOR complex 1 (mTORC1)-specific component RAPTOR (regulatory associated protein of mTOR). This is thought to be the mechanism of selective inhibition of mTORC1, but not mTORC2, by acute rapamycin treatment. In several cell lines and hepatocytes *in vivo*, prolonged rapamycin treatment also inhibits mTORC2. Rapamycin analogues (rapalogues) work in a similar way.

Active-site mTOR inhibitors (asTORi)
ATP-competitive inhibitors that suppress both mammalian target of rapamycin complex 1 (mTORC1) and mTORC2 signalling by blocking the active site of mTOR.

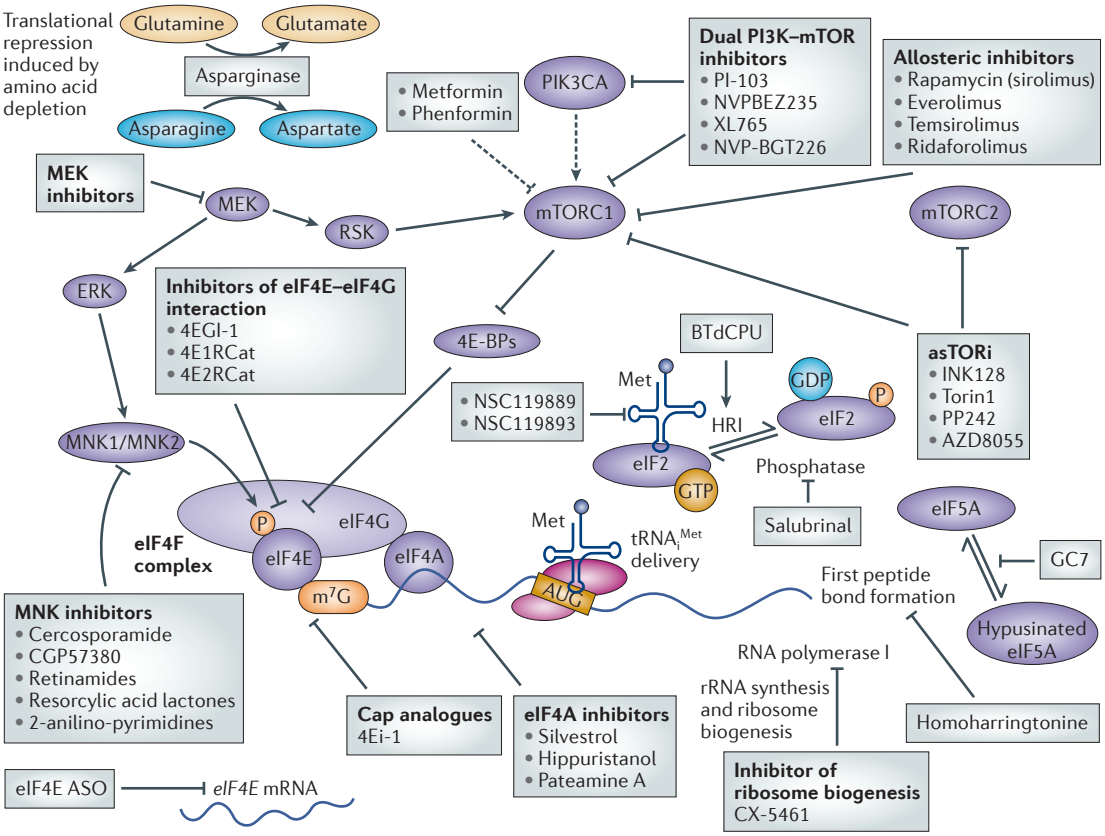


Figure 3 | **Therapeutic agents being investigated to target the translation machinery in cancer.** Important drug targets in the translation machinery and compounds that target them are shown. Arrows indicate activation, bar-headed lines indicate inhibition and dotted lines indicate indirect effects. Drugs targeting the translation machinery are in grey, translation initiation factors are in purple and the ribosome is in pink. As can be seen by comparing FIG. 3 with FIG. 2, almost every step of translation initiation can be targeted by small-molecule inhibitors, including mitogen-activated protein kinase (MAPK) signalling (left), phosphoinositide 3-kinase (PI3K)–mammalian target of rapamycin (mTOR) signalling (top right), eukaryotic translation initiation factor 2α (eIF2α) phosphorylation and ternary complex formation (middle), eIF4F complex formation, enzymatic activity and cap-binding (bottom left), as well as amino acid availability (top left), ribosome biogenesis (bottom) and first peptide bond formation by eIF5A (bottom right). 4E-BP, 4E-binding protein; 4Ei-1, N-7-benzyl guanosine monophosphate tryptamine phosphoramidate pronucleotide; ASO, antisense oligonucleotide; asTORi, active-site mTOR inhibitors; ERK, extracellular signal-regulated kinase; GC7, N1-guanyl-1,7-diaminoheptane; HRI, haem-regulated inhibitor; m^{7}G, 7-methylguanosine 5′-cap; MEK, MAPK/ERK kinase; MNK, MAPK-interacting kinase; mTORC, mTOR complex; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit-α; RSK, ribosomal S6 kinase; tRNA$_i^{Met}$, initiator methionyl tRNA.

the inhibition of mTORC1 is responsible for the anti-proliferative effects of asTORi. Considering that rapamycin strongly and sustainably inhibits phosphorylation of some (for example, S6Ks) but not all (for example, 4E-BPs) mTORC1 substrates[164], the superiority of the anti-neoplastic effects of asTORi relative to rapamycin might be a consequence of asTORi drastically inhibiting rapamycin-resistant mTORC1 outputs, including 4E-BP phosphorylation[153,159,160].

In preclinical models, asTORi have shown promising results, including bioavailability, anti-metastatic properties and increased potency compared to rapamycin *in vivo*, as well as displaying specificity for cancer cells over normal cells[70,165–167]. However, asTORi cannot completely escape the resistance mechanisms inherent to targeting of upstream signalling molecules. Several studies indicate that eIF4E and/or 4E-BP expression levels affect the efficacy of PI3K and mTOR inhibitors[134,135,168–171]. These studies demonstrate that mTOR-targeted therapies are probably ineffective in tumours that exhibit an elevated eIF4E/4E-BP ratio (reviewed in REF. 172). Mechanistically, this is explained by the inability of mTOR inhibitors to suppress eIF4F assembly, with translation of eIF4E-sensitive mRNAs proceeding unabated in these tumours[134]. These findings suggest that the eIF4E/4E-BP ratio in a tumour could be used as a stratification marker for patients receiving mTOR-targeted therapies[134], as expression of these markers varies widely — a situation that has been documented in several cancers, including prostate and breast cancers[121,173]. Additional mechanisms of resistance to mTOR inhibition include a switch from cap-dependent to cap-independent translation[174] and compensation for reduced mTOR signalling by activation of MAPKs[157].

Table 2 | **Therapeutic inhibitors of translation in cancer**

Mechanism	Inhibitor*	Stage of development
Upstream signalling inhibitors		
Allosteric inhibition of mTORC1	• Rapamycin (sirolimus) • Rapalogues: Everolimus; Temsirolimus; Ridaforolimus	• Everolimus is FDA-approved for the treatment of advanced neuroendocrine tumours[272], breast cancer[273,274] and RCC, as well as subependymal giant cell astrocytomas[279] in patients who carry germline mutations in *TSC1* and *TSC2* • Sirolimus is used 'off-label' in kidney transplant recipients with Kaposi sarcoma[275] and in renal angiomyolipomas[278] • Temsirolimus is FDA-approved for the treatment of advanced RCC[271,277] • Phase III trial of Temsirolimus as single agent for relapsed or refractory mantle cell lymphoma completed and demonstrated significantly improved progression-free survival versus investigator's choice therapy (4.8 versus 1.9 months; $P = 0.0009$)[276] • Ridaforolimus in Phase III clinical trials for sarcomas[280]
Dual PI3K and mTOR inhibition	• NVP-BEZ235 • PI-103 • XL765 • NVP-BGT226	• XL765 in Phase I clinical trial for breast cancer (NCT01082068) and glioblastoma (NCT01240460) • NVP-BGT226 (NCT00600275) and NVP-BEZ235 (NCT00620594) in Phase I/II clinical trials for breast cancer • NVP-BEZ235 in clinical trials: Phase I/II for RCC (NCT01453595), Phase I for prostate cancer (NCT01634061) and Phase II for pancreatic neuroendocrine tumours (NCT01628913), among others • PI-103 did not enter clinical trials owing to issues related to rapid *in vivo* metabolism[288]
Active-site mTOR inhibition	• MLN0128 (previously known as INK128) • AZD8055 • Torin1 • PP242	• AZD8055 is effective in xenograft models[165] • PP242 is more potent than rapamycin in a mouse model of leukaemia[166] • INK128 has anti-metastatic properties[70] and selectively targets cancer cells while sparing normal bone marrow cells in animal models[167] • Results of Phase I clinical trials of MLN0128 (NCT01058707, NCT01351350 and NCT01899053), CC-223 (NCT01177397) and AZD8055 (NCT00999882 and NCT00999882) are expected
MNK inhibition	• Cercosporamide • CGP57380 • Resorcylic acid lactones • Retinamides • 2-anilino-pyrimidines	• MNK inhibitors inhibit eIF4E phosphorylation, colony formation, epithelial-to-mesenchymal transition, invasion and MCL1 expression in cancer cell lines[113,180–182,185–187] • Cercosporamide inhibits lung colony formation in a mouse experimental metastasis model[180]
Indirect mTOR inhibition	• Metformin • Phenformin	• Metformin appears to reduce cancer incidence in patients with type 2 diabetes, although this has been the subject of controversy[175,176,289] • Phenformin was taken off the market for the treatment of type 2 diabetes since late 1970s because of fatal lactic acidosis • Both inhibit tumour growth in mouse[281–283] and hamster[284] models
Direct inhibitors of the translation machinery		
Reduction of eIF4E expression	4E-antisense oligonucleotide	• Inhibits tumour growth and angiogenesis with low toxicity in mice[99,121,193] • Reduces eIF4E protein levels in human tumours[285]; Phase I/II clinical trials in combination with established chemotherapies in non-small-cell lung cancer and castration-resistant prostate cancer are in progress (NCT01234038 and NCT01234025)
Inhibition of eIF4E binding to 5′ cap	Cap analogues including the pro-drug 4Ei-1	• Delivery *in vivo* was recently made possible using virus-like particles[200] • 4Ei-1 pro-drug blocks cap-dependent translation and epithelial-to-mesenchymal transition in zebrafish[196]
Inhibition of eIF4E–eIF4G interaction	• 4EGI-1 • 4E1RCat • 4E2RCat	• 4EGI-1 induces apoptosis in several cancer cell lines[213,215] and inhibits melanoma and breast cancer xenograft growth[215] • 4E1RCat and 4E2RCat reverse drug resistance in a MYC-driven lymphoma model[214]
Inhibition of eIF4A helicase activity	• Silvestrol • Hippuristanol • Pateamine A	• Hippuristanol shows pre-clinical efficacy in a mouse model of HTLV1-infected T cell leukaemia[226] and resensitizes tumours to DNA-damaging agents in the Eμ-Myc lymphoma model[227] • Pateamine A derivatives display single-agent activity in xenograft models[224,225] • Silvestrol induces apoptosis and tumour regression in mouse models and improves the potency of doxorubicin in the Eμ-Myc lymphoma model[222,227,228]
Activation of HRI and eIF2α phosphorylation	BTdCPU	Effective in xenograft models[215]
Inhibition of eIF2α dephosphorylation	Salubrinal	Displays synthetic lethality in combination with proteasome inhibitors[252]; in Phase II clinical trial in combination with carfilzomib (NCT01775553)
Prevention of $tRNA_i^{Met}$–eIF2 interaction	• NSC119889 • NSC119893	Inhibition of global translation *in vitro*[257]

Table 2 (cont.) | **Therapeutic inhibitors of translation in cancer**

Mechanism	Inhibitor*	Stage of development
Other inhibitors		
Prevention of formation of first peptide bond	• Homoharringtonine • GC7	• Homoharringtonine is approved for the treatment of chronic myeloid leukaemia[264] • GC7 impairs tumour growth in a mouse model of melanoma[268]
Depletion of amino acid pools	Asparaginase	Approved for the treatment of acute lymphoblastic leukaemia and paediatric acute myeloid leukaemia[259,260]
RNA polymerase I inhibition	CX-5461	Effective in xenograft models[266]

4Ei-1, N-7-benzyl guanosine monophosphate tryptamine phosphoramidate; eIF, eukaryotic translation initiation factor; FDA, US Food and Drug Administration; GC7, N1-guanyl-1,7-diaminoheptane; HRI, haem-regulated inhibitor; HTLV1, human T-cell lymphotropic virus type 1; MCL1, myeloid cell leukaemia 1; MNK, MAPK-interacting kinase; mTORC, mammalian target of rapamycin complex; PI3K, phosphoinositide 3-kinase; RCC, renal cell carcinoma; $tRNA_i^{Met}$, initiator methionyl tRNA; TSC, tuberous sclerosis. *Also see FIGS 4–6.

Finally, the biguanide metformin (FIG. 4d) has been shown to reduce the risk of cancer in patients with diabetes[175–178]. Metformin suppresses mTOR[179] and represses translation of cancer-related mRNAs[72]. However, the relative contribution of the effects of metformin on translation to its anticancer properties remains unclear.

MNK inhibitors. Several small-molecule inhibitors targeting MNKs have been described (TABLE 2; FIG. 4e), including CGP57380 and cercosporamide[180–182]. These reduce eIF4E phosphorylation and repress neoplastic growth in cell culture[180]. Although CGP57380 is ineffective *in vivo*, cercosporamide was shown to drastically decrease the growth of lung colonies in an experimental metastasis assay, suggesting a possible use for MNK inhibitors in the treatment of metastatic cancers[180]. However, both compounds show notable off-target effects[180,183]. To address this problem, new compounds for targeting MNKs have been developed. Several 5-(2-(phenylamino)pyrimidin-4-yl)thiazol-2(3*H*)-one derivatives take advantage of features unique to the catalytic domain of the MNKs: the presence in the magnesium binding site of an Asp-Phe-Asp motif rather than the typical Asp-Phe-Gly motif seen in other kinases, three atypical insertions in the kinase domain, and a propensity to crystallize in the inactive conformation. This crystallization of the inactive conformation, unusual for a kinase, results in the exposure of an additional hydrophobic pocket, which can be targeted for increased specificity[184,185]. Other strategies are based on resorcylic acid lactone analogues that chemically react with cysteine residues present in the catalytic domain[186], and retinoic acid metabolism blocking agents (RAMBAs), which indirectly lead to MNK1 degradation and decreased eIF4E phosphorylation in breast cancer cell lines[187].

Although inhibiting MNKs could prevent resistance to other chemotherapeutic drugs, including rapamycin, gemcitabine and herceptin[188–190], resistance to MNK inhibitors remains unexplored; indeed, the determinants of sensitivity to anti-MNK therapy are unknown. However, the position of MNKs downstream of MAPKs indicates that at least some of the mechanisms of resistance to MEK inhibitors (reviewed in REF. 191) may be relevant to MNKs.

Direct eIF4F inhibitors

Antisense oligonucleotides. Early experiments targeting eIF4E synthesis using antisense-based strategies showed the anti-tumorigenic potential of this approach[98,99,192]. Subsequently, second-generation ASOs reduced tumour burden in breast and prostate xenograft models, but showed minimal toxicity[193] (see also TABLE 2). This was associated with a minimal effect on global protein synthesis (<20% reduction) with a dose-dependent decrease in expression of pro-survival and pro-growth proteins encoded by eIF4E-sensitive mRNAs[193]. eIF4E suppression was also accompanied by reduced angiogenesis, a consequence of blunting endothelial cell tube formation[193]. Mouse models designed to genetically mimic long-term suppression of eIF4E, using germline inducible short hairpin RNAs to inhibit eIF4E, demonstrated that low eIF4E levels are well tolerated in many tissues, affecting a subset of normal regenerating cells such as those in the gut epithelium[194]. Taken together, these results bode well for strategies aimed at suppressing eIF4E. Phase II clinical trials of an eIF4E ASO in combination with established chemotherapies are ongoing (ClinicalTrials.gov identifiers: NCT01234038 and NCT01234025).

Inhibitors of eIF4E–cap interaction. The ability of eIF4E to cause neoplastic transformation depends on its cap-binding activity, as overexpression of an eIF4E mutant defective in cap binding is not tumorigenic[86]. Cap analogues have long been used in *in vitro* studies of eIF4E function; however, they suffer from poor permeability and stability *in vivo*[195]. To circumvent this problem, pro-drugs with desirable pharmacokinetic properties have been designed. *N-7*-benzyl guanosine monophosphate tryptamine phosphoramidate pronucleotide 4Ei-1 (FIG. 5a) was reported to inhibit cap-dependent translation and the epithelial-to-mesenchymal transition in zebrafish[196] and cause chemosensitization of lung cancer cells to treatment with gemcitabine[197]. High-throughput screening of chemical libraries for effective, bioavailable cap mimetics is ongoing[198,199]. More recently, delivery of traditional cap analogues has been achieved *in vivo* using virus-like particles, opening up new possibilities for eIF4E targeting in cancer[200].

Ribavirin was reported to be an eIF4E-cap inhibitor[201], but this has been disputed[202,203]. A clinical trial reported benefits in patients with acute myeloid leukaemia[204], but

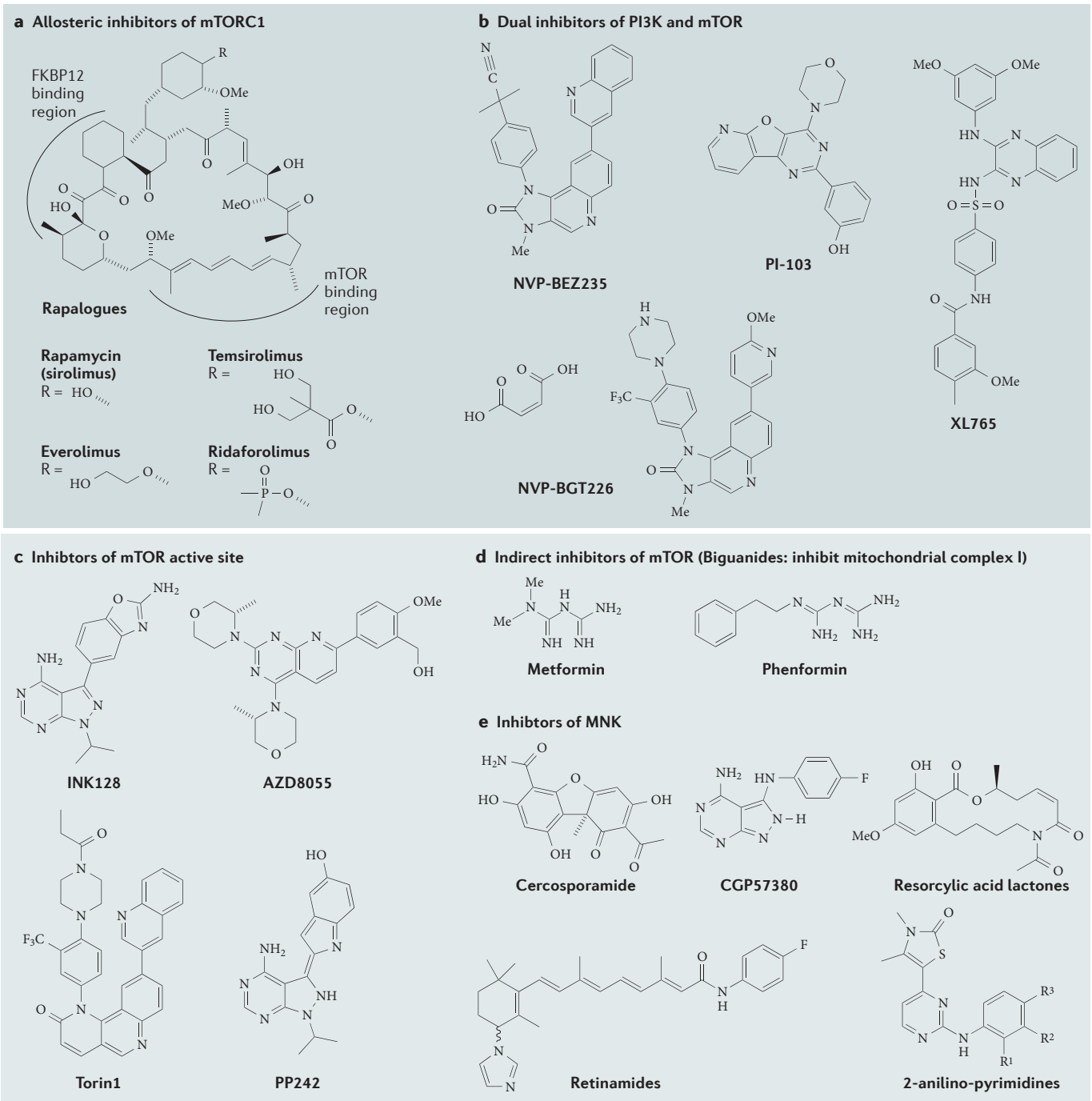


Figure 4 | **Upstream signalling inhibitors.** Structures are derived from PubChem, except for resorcylic acid lactones (example from REF. 186), retinamides (example from REF. 187) and 2-anilino-pyrimidines, in which R^1 can be NH_2, Me, NHEt or NHMe; R^2 can be NO_2, SO_2NH_2, SO_2NHMe, SO_2Me, SO_2NHEt, H, CN or NO_2; and R^3 is H or Me (example from REF. 185). MNK, MAPK-interacting kinase; mTOR, mammalian target of rapamycin; mTORC, mTOR complex; PI3K, phosphoinositide 3-kinase.

the mechanism of action is unknown and may involve translation-independent biological activities inherent to ribavirin[205].

Inhibitors of eIF4E–eIF4G interaction. A promising strategy for targeting the translation machinery is to interfere with the assembly of the eIF4F complex. eIF4G binds to the dorsal surface of eIF4E that lies opposite its cap-binding site[206,207]. This is accomplished through interaction of the eIF4E-binding motif YXXXLΦ (X is any amino acid and Φ is a hydrophobic amino acid) of eIF4G, with Val69 and Trp73 residues on the dorsal surface of eIF4E[206–208]. In addition, eIF4G binds directly to the mRNA, and this interaction stabilizes

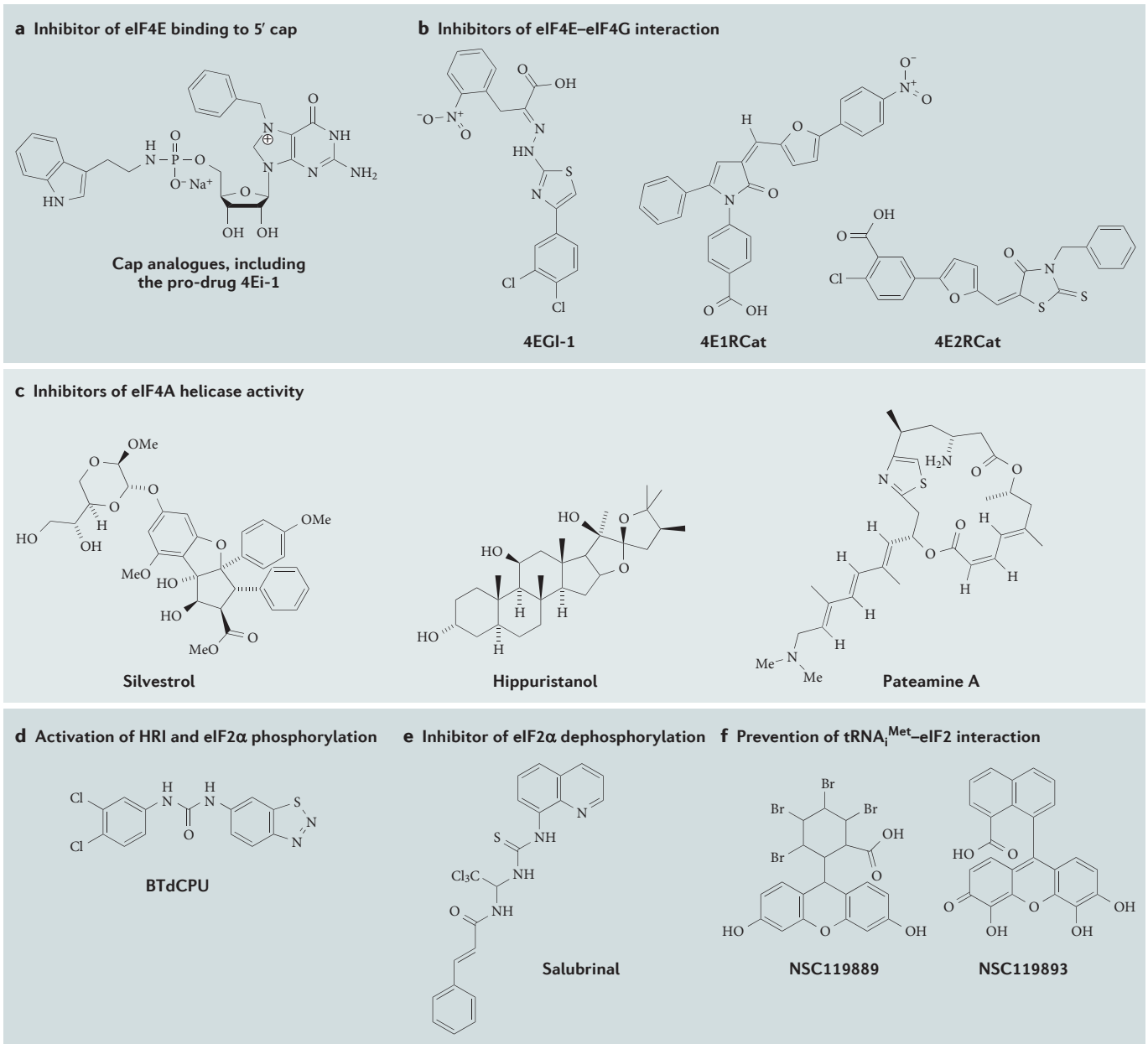


Figure 5 | **Direct inhibitors of the translation machinery.** Structures are derived from PubChem, except for pro-drug 4Ei-1 (from REF. 196). eIF, eukaryotic translation initiation factor; HRI, haem-regulated inhibitor; tRNA$_i^{Met}$, initiator methionyl tRNA.

the 5′-mRNA-cap–eIF4E association[209,210]. 4E-BPs bind to the same region as eIF4G on the dorsal surface of eIF4E, thereby blocking eIF4E–eIF4G association[211,212].

High-throughput screening of chemical libraries identified 4EGI-1, 4E1RCat and 4E2RCat (FIG. 5b) as inhibitors of the eIF4E–eIF4G interaction[213,214]. These molecules decrease the translation of eIF4E-sensitive mRNAs, and have shown promise in preclinical models[213,215] (TABLE 2). Intriguingly, although 4EGI-1 abrogates binding of eIF4E to eIF4G, it does not prevent binding of 4E-BPs to eIF4E[213]. Recently, structural studies of 4EGI-1 in complex with eIF4E showed that this compound binds to a hydrophobic pocket distal to the eIF4G-binding site, causing localized conformational changes that result in allosteric inhibition of the eIF4E–eIF4G interaction[216]. 4EGI-1 also inhibits eIF4E-independent translation, possibly owing to activation of stress response pathways[217,218]. An alternative means of disrupting the eIF4E–eIF4G interaction is through the fusion of 4E-BPs to the ligand of a cancer-specific cell surface receptor. For example, 4E-BP1 fused to an analogue of gonadotropin-releasing hormone prevented eIF4F complex formation and inhibited tumour growth in a mouse model of ovarian cancer[219]. Taken together, these results demonstrate the potential clinical value of targeting the eIF4E–eIF4G interaction directly.

Inhibitors of eIF4A. Hippuristanol, pateamine A and silvestrol are eIF4A inhibitors that suppress translation[138] (FIG. 5c). Hippuristanol belongs to a family of polyoxygenated steroids[220], which bind to the C-terminal domain of eIF4A. It allosterically prevents eIF4A from interacting with RNA and blocks its helicase activity, both in its free form and as part of eIF4F[220]. Pateamine A and silvestrol increase the ATPase, RNA-binding and helicase activities of eIF4A[221,222]. Both compounds seem to function as chemical inducers of dimerization, and they increase the RNA-binding affinity of eIF4A in a non-sequence dependent manner, resulting in its depletion from the eIF4F complex[221]. Pateamine A is an irreversible inhibitor of protein synthesis, probably as a consequence of covalent inhibition of eIF4A, and is therefore very toxic *in vivo*[221,223]; however, better-tolerated derivatives have been developed[224,225].

All three eIF4A inhibitors show preclinical efficacy in various cell and mouse models[222,224–228] (TABLE 2); silvestrol has the highest potency *in vivo*[228]. The fact that inhibition of eIF4A is the mechanism by which silvestrol targets translation has been demonstrated by the identification of eIF4A mutants that are resistant to silvestrol[229]. As expected for an eIF4F inhibitor, mRNAs translated with increased secondary structure in their 5′UTR are more sensitive to inhibition by silvestrol[42,228,230,231].

The demonstration that the eIF4A inhibitors can re-sensitize lymphomas to DNA-damaging agents in tumours overexpressing eIF4E suggests that directly targeting the eIF4F complex can overcome the resistance mechanisms described previously[134,136,169,232] that lead to increased eIF4E availability or expression. Interestingly, one of the barriers to the development of silvestrol as an anti-neoplastic agent is the fact that resistance can be mediated by overexpression of ATP-binding cassette sub-family B1 (ABCB1; also known as P-glycoprotein 1). Structure–activity relationship studies are underway to overcome this limitation[230,233,234].

Inhibitors of ternary complex formation

The other major regulatory pathway of translation initiation involves ternary complex (TC) formation (FIGS 1,2). Although dysregulation of this pathway in cancer is not as well understood as the role of eIF4F, new evidence for its importance and novel means of targeting the TC have emerged.

Regulation of ternary complex formation. Following start codon recognition by initiator methionyl tRNA ($tRNA_i^{Met}$), eIF2-bound GTP is hydrolysed, and the resulting eIF2–GDP complex is released from the 40S ribosome[235]. The multi-subunit factor eIF2B functions as a guanine nucleotide exchange factor (GEF) to convert eIF2–GDP to eIF2–GTP, thus facilitating TC assembly and allowing a subsequent round of initiation to occur[236,237]. Phosphorylation of the α-subunit of eIF2 (eIF2α) at Ser51 increases the affinity of eIF2–GDP for the limiting amounts of eIF2B, thereby sequestering eIF2B and limiting its availability[238]. Various stresses such as the accumulation of unfolded proteins in the endoplasmic reticulum (ER), amino acid depletion, haem deficiency and viruses activate eIF2α kinases (PKR-like ER kinase (PERK), general control non-derepressible 2 (GCN2), haem-regulated inhibitor (HRI) and double-stranded RNA-dependent protein kinase (PKR), respectively)[239]. Phosphorylation of eIF2α causes inhibition of protein synthesis, but paradoxically also stimulates the translation of a small subset of mRNAs with short, upstream ORFs (uORFs)[240]. These mRNAs encode master transcriptional regulators of the stress response, such as GCN4 in yeast[240] and ATF4 in mammals[241]. In non-stressed cells, initiation occurs at the first-encountered AUG codon of the uORF[242]. Following translation of this uORF, ribosomes must acquire a new ternary complex to become competent for re-initiation at the next downstream ORF[243]. The organization of the uORFs causes the ribosomes to bypass the AUG initiation codon of the major ORF owing to overlap and out-of-frame positioning of a preceding uORF or too short a distance between the termination codon of the uORF and the major AUG codon[242,243]. When eIF2 becomes limiting, as it does on eIF2α phosphorylation, this enables some ribosomes to bypass uORFs and initiate translation at the downstream major AUG codon, as more time (distance) is available to acquire a functional ternary complex[244].

The role and targeting of the TC in cancer. Although the biochemistry of TC formation and activity is well studied, understanding of the role of the TC in cancer biology and its potential for exploitation as an anticancer target is limited. Depending on the stimulus, intensity and duration, eIF2α phosphorylation can promote cell survival or have

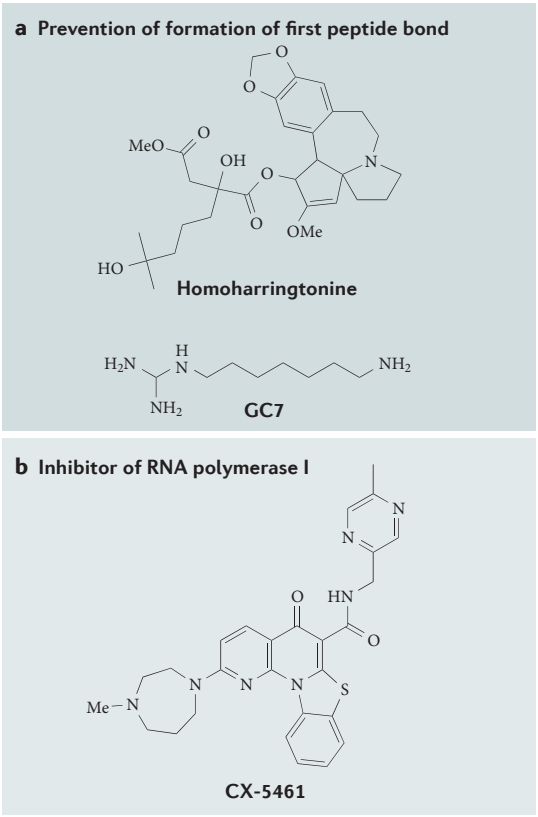


Figure 6 | **Other inhibitors.** Structures are derived from PubChem. GC7, N1-guanyl-1,7-diaminoheptane.

Ternary complex (TC). A complex of eIF2 (containing α, β and γ subunits), initiator $tRNA^{Met}$ and GTP.

a deleterious effect on cell fate[245,246]. A mutant of eIF2α that cannot be phosphorylated transforms NIH 3T3 cells[247], whereas sustained phosphorylation induces apoptosis[248]. Therefore, increasing eIF2α phosphorylation is an attractive strategy to treat cancer[239]. One way to achieve this is to activate the kinases upstream of eIF2α. BTdCPU (FIG. 5d) and related *N,N′*-diarylureas promote eIF2α phosphorylation by the HRI kinase and show promising effects *in vitro* and *in vivo*[249,250]. Another strategy is to inhibit the dephosphorylation of eIF2α using phosphatase inhibitors, such as salubrinal[251] (FIG. 5e). There are *in vitro* data suggesting a synthetic lethal relationship between salubrinal and the proteasome inhibitor bortezomib[252], and this is the basis of a clinical trial combining salubrinal with the proteasome inhibitor carfilzomib (ClinicalTrials.gov identifier NCT01775553). However, an important consideration in developing strategies aimed at increasing eIF2α phosphorylation is its pro-survival function in response to stressors, such as hypoxia and nutrient deprivation[253,254]. The dual effects of stimulators of eIF2α phosphorylation are exemplified by guanabenz, a compound that binds to protein phosphatase 1 and inhibits stress-mediated eIF2α dephosphorylation. Guanabenz was shown to promote the survival of HeLa cells under conditions of toxic ER stress[255], but it inhibited tumour growth in a breast cancer mouse model[256]. One possible way of avoiding the potentially detrimental effects of compounds like guanabenz would be to directly target TC formation, the feasibility of which has been demonstrated using brominated derivatives of fluorescein, NSC119889 and NSC119893 (FIG. 5f). These inhibitors prevent binding of $tRNA_i^{Met}$ to eIF2 *in vitro*[257]; however, the efficacy of direct TC inhibitors *in vivo* has yet to be established.

Other inhibitors of translation

Although they are not the focus of this Review, several other inhibitors of translation show promise in the treatment of cancer and deserve to be mentioned here. For example, a bacterial enzyme, asparaginase, catalyses the hydrolysis of L-asparagine and, at a lower rate, L-glutamine (5% of the asparagine rate)[258], and it is used for the treatment of acute lymphoblastic leukaemia and paediatric acute myeloid leukaemia[259,260]. Asparaginase leads to depletion of L-asparagine and L-glutamine, which is accompanied by perturbations in amino acid pools, increased eIF2α phosphorylation by GCN2 and inactivation of mTORC1 (REF. 261). Another clinically approved natural product that inhibits protein synthesis is homoharringtonine, which prevents the formation of the first peptide bond[262,263] and is approved for the treatment of chronic myeloid leukaemia[264] (TABLE 2; FIG. 6a).

Transcription of ribosomal DNA by RNA polymerase I is often increased in cancer[265]. The small molecule CX-5461 (FIG. 6b) effectively inhibits RNA polymerase I[266]. This causes accumulation of free ribosomal proteins, leading to disruption of nucleolar function and induction of p53-dependent apoptosis[267]. CX-5461 has so far demonstrated antitumour activity in mouse xenograft models[266]. Another noteworthy inhibitor is GC7 (N1-guanyl-1,7-diaminoheptane) (FIG. 6a), which blocks formation of the first peptide bond via inhibition of the hypusination of eIF5A[268,290]. This is particularly interesting because eIF5A is the only known protein to contain the amino acid hypusine, which is a modified lysine, and is highly conserved across eukaryotes[269]. GC7-mediated inhibition of hypusination was shown to lead to apoptotic cell death and impaired tumour growth in a mouse model of melanoma[268].

Conclusions

Encouragingly, there are many ways to target translation in the clinical setting, and these are increasingly designed to directly target the translation machinery. Considering the accumulating possibilities of directly targeting eIF4F by ASOs, 4EGI-1, cap analogues and so on, it seems that the use of such small-molecule inhibitors to treat cancer is imminent and holds promise to minimize the issue of acquired resistance. The transition to the clinical setting will present a series of new challenges unique to direct translation inhibitors. In particular, eIF4A inhibitors display impressive potency, and the development of analogues with improved pharmacodynamic properties is awaited impatiently. There is a rich history of fundamental research that has been germane to our deep understanding of the translation process and the identification and development of the previously mentioned compounds. This body of knowledge must continue to grow so that we can understand the physiological consequences of translation inhibitors as they are applied in various clinical settings. Indeed, a recent review of AstraZeneca's small-molecule drug projects from 2005 to 2010 has shown that key determinants of clinical success include confirming the compound-mechanism hypothesis, compound–target engagement and pharmacodynamic activity[270]. It would seem prudent to set high standards for the science accompanying translation inhibitors as they move into the clinic if the likelihood of success is to be maximized. Validation by genetic and biochemical assays that can directly quantify the proposed mechanisms of action is crucial. The early successes of therapeutics that target dysregulated translation in cancer bode well for their transition from the bench to the bedside in the not-too-distant future.

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Competing interests statement

The authors declare no competing interests.

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