Molecular mechanisms of mTOR-mediated translational control

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Abstract | The process of translation requires substantial cellular resources. Cells have therefore evolved complex mechanisms to control overall protein synthesis as well as the translation of specific mRNAs that are crucial for cell growth and proliferation. At the heart of this process is the mammalian target of rapamycin (mTOR) signalling pathway, which senses and responds to nutrient availability, energy sufficiency, stress, hormones and mitogens to modulate protein synthesis. Here, we highlight recent findings on the regulators and effectors of mTOR and discuss specific cases that serve as paradigms for the different modes of mTOR regulation and its control of translation.

RNA helicase
An enzyme that resolves RNA base pairing through ATP hydrolysis, which leads to unfolding of structured RNAs.

PIKK family
(Phosphoinositide 3-kinase-related kinase). This family comprises high-molecular-weight signalling proteins, including mammalian target of rapamycin (mTOR), DNA protein kinase (DNA-PK), ataxia telangiectasia (A-T) mutated (ATM), ATR (A-T and RAD5-related) and SMG1. These kinases have central roles in the control of cell growth, gene expression, and genome surveillance and repair in eukaryotic cells.

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Cells promptly respond to environmental changes by efficiently and accurately altering gene expression. Proteins are produced as a consequence of new mRNA synthesis through transcription. However, translation of mRNA into protein is also regulated and often has a defining role in forming the proteome. Cells use translational control to modulate gene expression throughout life — during embryonic development, memory formation and the maintenance of normal physiology[1–4] — and its misregulation can contribute to numerous human disease states, including cancer[5–7].

As an integral biosynthetic process, translation consumes a substantial amount of cellular material and energy[8]. To grow and proliferate, cells must ensure that sufficient resources are available to drive protein production. When energy or amino acids become limiting, protein production needs to be downregulated so that cells can use their limited resources to survive. Therefore, mammalian cells have evolved elaborate mechanisms for translational control, most of which are sensitive to nutrient availability, cellular energy, stress, hormones and growth factor stimuli.

Rate-limiting steps of a biosynthetic process are often effective targets for a regulatory mechanism to control the process. The limiting step of protein synthesis is translation initiation, during which the small ribosome subunit is recruited to the 5′ end of mRNA and scans towards the start codon, where the complete ribosome is subsequently assembled to begin polypeptide formation[9–11]. Indeed, translational control mechanisms often target translation initiation factors to modulate translation[12]. For instance, the recruitment of the small ribosomal subunit to mRNA requires the assembly of the eukaryotic translation initiation factor 4F (eIF4F) complex on the 5′ cap structure of mRNA. The eIF4F complex contains three initiation factors — eIF4E, eIF4G and eIF4A[9]. To assemble the eIF4F complex, eIF4E binds to the 5′ cap and recruits eIF4G and eIF4A. The inhibitory 4E-binding protein 1 (4E-BP1; also known as eIF4EBP1) inhibits eIF4G binding to eIF4E (see below). Signal transduction-mediated phosphorylation of 4E-BP1 leads to its dissociation from eIF4E, allowing the recruitment of eIF4G and eIF4A[12] (FIG. 1a).

Some mRNA species, many of which encode proteins that are involved in promoting cell growth and proliferation, also contain inhibitory secondary structures in the 5′ untranslated region (UTR)[13–15]. The structured 5′ UTR prevents efficient scanning of the small ribosome subunit to the start codon. A group of initiation factors, such as eIF4A, are RNA helicases that can 'unwind' mRNA secondary structures and have a crucial role in translating these mRNAs[9,16]. Helicase activity can also be regulated in a signal transduction-dependent manner. For example, eIF4A helicase activity can be significantly enhanced when associated with its regulatory factor eIF4B (see below). Growth factor-mediated phosphorylation of eIF4B can dramatically increase its association with eIF4A[12,17,18] (FIG. 1b). Thus, it is important to understand how signal transduction pathways regulate protein synthesis through these complex mechanisms.

A key pathway that integrates and responds to environmental cues involves target of rapamycin (TOR), a member of the PIKK family of protein kinases that is conserved from yeast to humans[19,20]. Mammalian TOR complex 1 (mTORC1), which contains mTOR (also known as FRAP1) in complex with raptor (regulatory

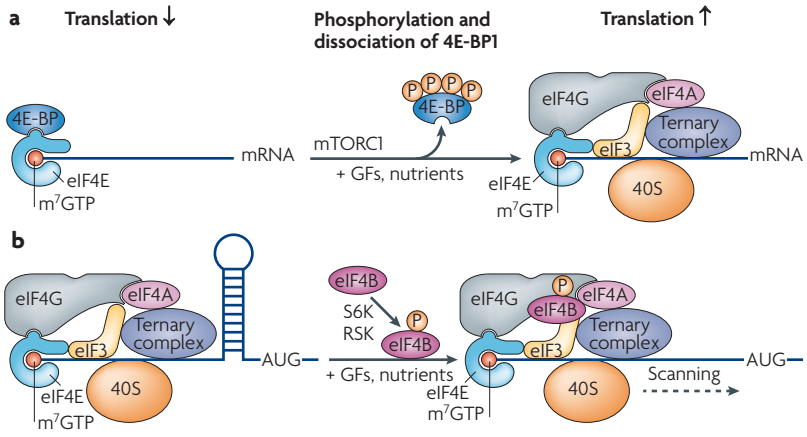


Figure 1 | **Regulating cap-dependent translation initiation. a** | The recruitment of the 40S ribosomal subunit to the 5′ end of mRNA is a crucial and rate-limiting step during cap-dependent translation. A number of translation initiation factors, including the 5′ cap-binding protein eukaryotic translation initiation factor 4E (eIF4E), have essential roles in this process. Signal transduction-mediated phosphorylation events regulate the function of eIF4E. For example, hypophosphorylated 4E-binding proteins (4E-BPs) bind tightly to eIF4E, thereby preventing its interaction with eIF4G and thus inhibiting translation. Mammalian target of rapamycin complex 1 (mTORC1)-mediated phosphorylation of 4E-BPs releases the 4E-BP from eIF4E, resulting in the recruitment of eIF4G to the 5′ cap, and thereby allowing translation initiation to proceed. **b** | Another well-studied initiation factor that is targeted by signal transduction pathways is eIF4B. Following 40S ribosomal protein S6 kinase (S6K)- or ribosomal S6 kinase (RSK)-mediated phosphorylation, eIF4B is recruited to the translation pre-initiation complex and enhances the RNA helicase activity of eIF4A. This is particularly important for translating mRNAs that contain long and structured 5′ untranslated region sequences, because the unwinding of these RNA structures is required for efficient 40S ribosomal subunit scanning towards the initiation codon. GF, growth factor.

associated protein of mTOR) and LST8 (also known as GβL), directly regulates protein synthesis in mammals[21]. A second kinase complex, mTORC2, elicits distinct biological functions to mTORC1 (BOX 1). Raptor, a defining component of mTORC1, determines the specificity of mTORC1, in part by interacting with substrates that contain a TOR signalling motif[22,23].

The phosphotransferase activity of mTORC1 is stimulated by the GTP-bound form of the small G protein RHEB (Ras homologue enriched in brain). In turn, RHEB is regulated by a tumour suppressor heterodimer that is composed of tuberous sclerosis 1 (TSC1) and TSC2 (also known as hamartin and tuberin, respectively), which are conserved in *Drosophila melanogaster* and mammals. TSC2 exhibits GTPase-activating protein (GAP) activity towards RHEB, converting it to the inactive GDP-bound form[24,25]. The main downstream targets of mTORC1 seem to be components of the translation machinery[20] (FIG. 2a), including 4E-BPs and 40S ribosomal protein S6 kinases (S6Ks), both of which are important in the physiological control of translation initiation[26]. mTORC1 signalling is potently inhibited by the naturally occurring antifungal macrolide rapamycin. As the regulation of protein synthesis has a central role in maintaining proper cell growth, misregulation of mTORC1 signalling can lead to an array of human disorders, including metabolic diseases and cancer[27–29] (BOX 2).

GTPase-activating protein (GAP). A protein that stimulates the intrinsic ability of a GTPase to hydrolyse GTP to GDP. Therefore, GAPs negatively regulate GTPases by converting them from active (GTP bound) to inactive (GDP bound) forms.

By highlighting some recent discoveries in this fast-developing field, we summarize both the upstream signals that modulate mTORC1 activity and the downstream components that affect protein synthesis.

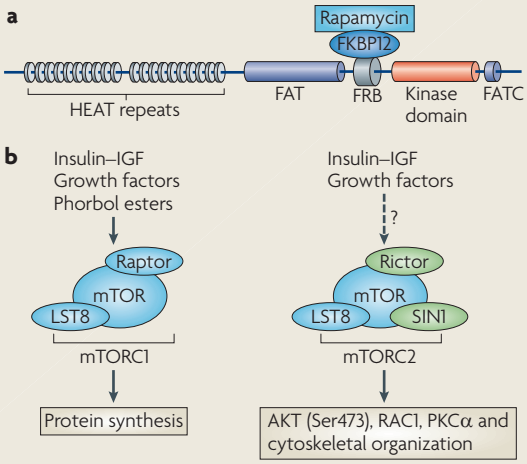
mTORC1 activation by growth factors

An increase in protein synthesis is essential for cell growth and proliferation[30]. To retain a constant cell size during proliferation, cells must approximately double in size before division, a process that is largely regulated by protein synthesis. In addition, the production of specific factors that are crucial for cell cycle progression must be carefully controlled, in part through intrinsic characteristics — such as structured 5′ UTRs — of the mRNAs that encode these proteins. Activated mTORC1 signalling promotes translation and thus cell growth and proliferation in response to growth factor stimuli[31]. Growth factors or related hormones activate receptor Tyr kinases and G protein-coupled receptors, which, in turn, activate several key signal transduction pathways. In particular, the phosphoinositide 3-kinase (PI3K)–AKT pathway and the Ras–ERK (extracellular signal-regulated kinase) pathway[32] stimulate mTORC1 signalling by inhibiting the tumour suppressor complex TSC1–TSC2, which is a negative regulator of mTORC1 (REF. 26) (FIG. 2a). Inhibition of TSC1–TSC2 is mediated mainly through the phosphorylation of TSC2 by several upstream kinases, including AKT, ERK and ribosomal S6 kinase (RSK).

PI3K–AKT and mTORC1. AKT, a downstream effector kinase of PI3K, directly phosphorylates TSC2 on a number of residues, including Ser939, Ser981 and Thr1462 (FIG. 2b). A TSC2 mutant in which AKT phosphorylation sites are substituted can be a dominant inhibitor of mTORC1 by blunting its activation in response to growth factor stimuli[33–35]. Moreover, a non-phosphorylatable mutant form of TSC2 inhibits AKT-mediated stimulation of growth in the *D. melanogaster* eye[34]. These AKT-mediated phosphorylation sites in TSC2 are conserved from *D. melanogaster* to mammals, but a mechanistic link between the requirements of these sites and the activation of mTORC1 by AKT is missing. Recent studies have suggested that AKT-mediated phosphorylation of TSC2 at Ser939 and Ser981 creates a binding site for a cytosolic anchor protein, 14-3-3. Binding of 14-3-3 to TSC2 seems to disrupt binding to TSC1 and RHEB, which are associated with endomembranes[36]. As 14-3-3 is also involved in TSC1–TSC2-independent activation of mTORC1 (see below), further investigation will be required to determine the dominant function of 14-3-3 in mTORC1 regulation.

Importantly, TSC2 is not an essential target of AKT during normal *D. melanogaster* development[37], suggesting the presence of additional targets for the AKT-mediated regulation of mTORC1. Several recent studies described PRAS40 (40 kDa Pro-rich AKT substrate; also known as AKT1S1), a novel mTORC1-binding partner that mediates AKT signals to mTORC1 (REFS 38–40) (FIG. 2a). Activated AKT phosphorylates

Box 1 | **mTORC1 and mTORC2**



Part **a** of the box figure shows the domain structure of mammalian target of rapamycin (mTOR; also known as FRAP1). The amino-terminal half of mTOR is composed of tandem HEAT repeats (protein interaction domains of two tandem antiparallel α-helices that are found in huntingtin, elongation factor 3, PR65/A and TOR). The carboxy-terminal half of the protein contains the central FAT domain (a domain shared by FRAP, ataxia telangiectasia mutated and TRRAP, all of which are PIKK family members), followed by the FRB (FKBP12–rapamycin-binding) domain, a kinase domain and the FATC (FAT C-terminus) domain.

mTOR is an unusual protein kinase that is related to lipid kinases and is an essential component of two distinct multiprotein complexes — mTOR complex 1 (mTORC1) and mTORC2 (REFS 109–114). mTORC1, the rapamycin-sensitive complex, consists of mTOR, raptor (regulatory-associated protein of mTOR) and LST8 (also known as GβL)[80] (see the figure, part **b**). Rapamycin binds to the immunophilin FKBP12 to generate a highly potent and specific inhibitor of mTORC1-dependent signalling through direct binding to the FRB domain. The mTORC1 complex signals to 4E-binding protein 1 (4E-BP1; also known as EIF4EBP1) and 40S ribosomal protein S6 kinase (S6K), which mediate efficient cap-dependent translation initiation (see the main text).

The mTORC2 complex contains mTOR, LST8, rictor (rapamycin-independent companion of mTOR) and SIN1 (stress-activated protein kinase-interacting protein 1) (see the figure, part **b**). The activity of mTORC2 responds to growth factors, but how mTORC2 is regulated is unclear. mTORC2 can be considered to be upstream of mTORC1 because mTORC2 phosphorylates AKT on Ser473 in its C-terminal hydrophobic motif[115]. This, together with phosphoinositide-dependent kinase 1 (PDK1)-mediated phosphorylation of the activation loop, is required for full AKT activation. mTORC2 also mediates the phosphorylation of RAC1 and protein kinase (PKCα) and is involved in the regulation of cytoskeletal organization[28,80]. Rapamycin–FKBP12 does not bind directly to mTORC2, but long-term rapamycin treatment disrupts mTORC2 assembly in ~20% of cancer cell lines through an unknown mechanism[116]. The reduction in mTORC2 leads to a strong inhibition of AKT signalling. Although it is likely that rapamycin–FKBP12 binding to mTOR might block the subsequent binding of rictor and SIN1 (REF. 116), it remains to be determined why rapamycin-mediated inhibition of mTORC2 assembly only occurs in certain cell types. IGF, insulin-like growth factor.

PRAS40, resulting in the dissociation of PRAS40 from mTORC1. This was proposed to be mediated through 14-3-3 binding of the phosphorylated PRAS40 (REF. 38). Thus, bypassing TSC2, AKT phosphorylates PRAS40 and prevents its ability to suppress mTORC1 signalling to S6K and 4E-BP1 (see above). More recent data indicate that PRAS40 is also a substrate of mTORC1, and that mTORC1-mediated phosphorylation of PRAS40 facilitates the removal of its inhibition on downstream signalling of mTORC1 (REFS 41–43), suggesting a positive-feedback mechanism for AKT-induced mTORC1 signalling events. PRAS40 also contains a TOR signalling motif, which has been proposed to negatively regulate mTORC1 signalling by competing with 4E-BP1 and S6K for interaction with raptor[40]. PRAS40 is a direct inhibitor of mTORC1 and antagonizes the activation of the mTORC1 by RHEB•GTP. However, constitutive mTORC1 signalling in TSC2-null mouse embryonic fibroblasts, in which AKT signalling is largely inhibited owing to a negative-feedback mechanism (see below), indicates that hyperactive RHEB can overcome PRAS40-mediated inhibition of mTORC1 (REF. 39).

Thus, the AKT pathway might stimulate mTORC1 through two interconnected mechanisms: by inhibiting PRAS40 and/or by activating RHEB. As the TSC1–TSC2 complex is not found in some lower eukaryotes (for example, budding yeast), it is likely that the TSC1–TSC2-independent effect of AKT might be more ancient and conserved, and that the TSC1–TSC2–RHEB module evolved later in higher eukaryotes to further fine-tune mTORC1 activity in response to more complex environmental signals. Consistently, changes in the relative amount of PRAS40 or RHEB•GTP can affect mTORC1 activity both *in vitro* and in cells[38,39]. This strongly suggests that cell type-dependent variations in the activity and abundance of the two proteins might dictate different levels of the mTORC1 activity despite similar upstream signalling events.

As described above, the TSC1–TSC2–RHEB–mTORC1 module is downstream of AKT; however, activated mTORC1 signalling triggers a negative-feedback loop that inhibits the insulin–PI3K–AKT pathway (FIG. 2a). Early work indicated that the activated mTORC1 pathway represses insulin–PI3K–AKT signalling through inhibition of insulin receptor substrate 1 (IRS1), a crucial component in insulin signalling[44,45]. But it is now known that S6K1, the downstream effector kinase of mTORC1, phosphorylates and inhibits IRS1 (REFS 46,47). It is becoming increasingly appreciated that this negative-feedback loop has an important role in insulin-resistant diabetes and in cancer[48,49].

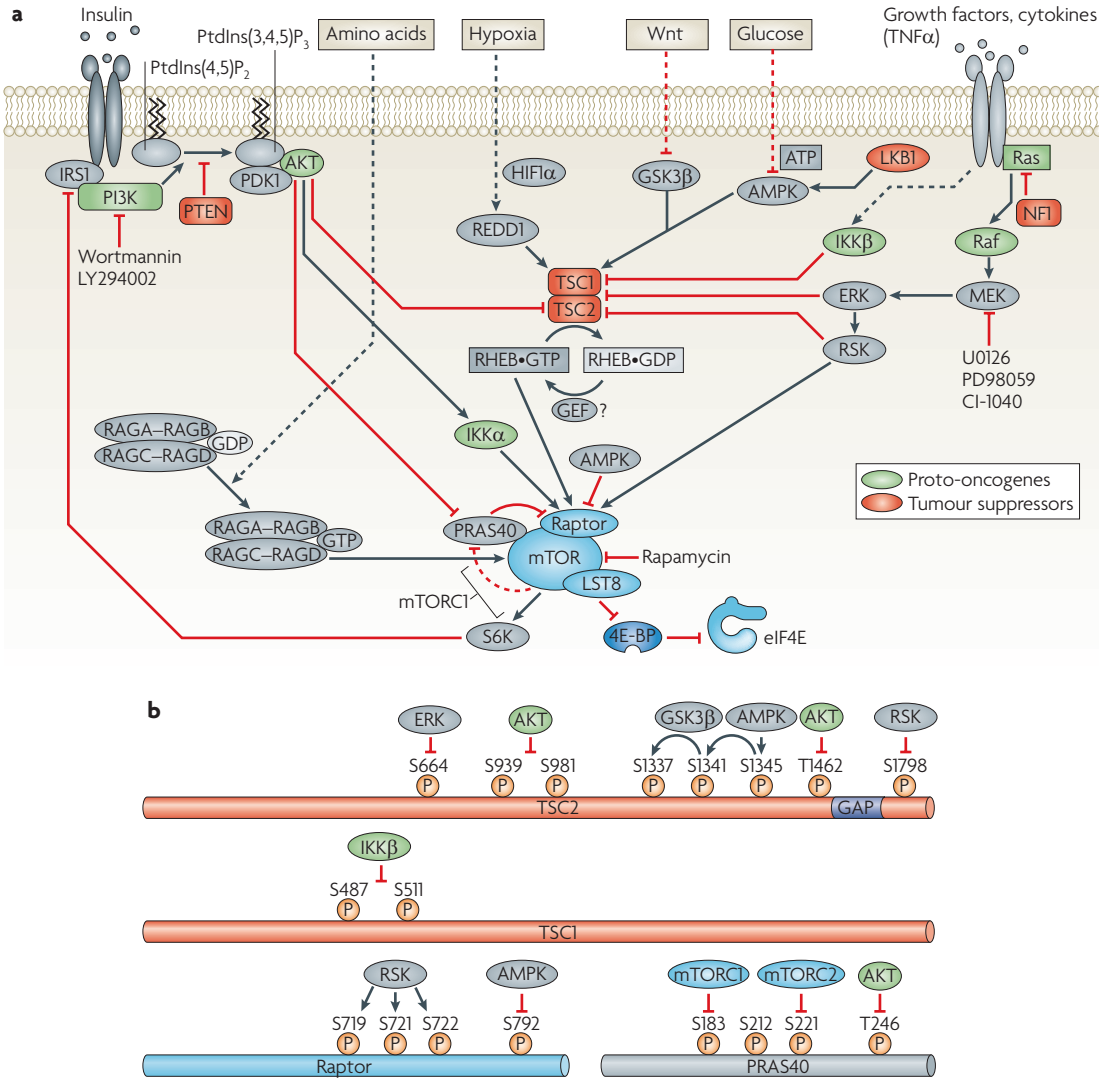


Figure 2 | **The mTORC1 signalling regulatory network. a** | Mammalian target of rapamycin complex 1 (mTORC1) is stimulated by the active, GTP-bound form of RHEB; immediately upstream of RHEB is the tuberous sclerosis 1 (TSC1)–TSC2 tumour suppressor complex. TSC2 contains a GTPase-activating protein (GAP) domain that converts RHEB to its inactive, GDP-bound form. Multiple upstream signalling inputs from PI3K–AKT, Ras–ERK–RSK, TNFα–IKKβ, AMPK–GSK3β, LKB1–AMPK and Wnt–GSK3β pathways either positively or negatively regulate mTORC1 signalling (AMPK is AMP-activated protein kinase (also known as PRKAB1), ERK is extracellular signal-regulated kinase, GSK3β is glycogen synthase kinase 3β, IKK is inhibitor of nuclear factor-κB kinase, PI3K is phosphoinositide 3-kinase, RSK is ribosomal S6 kinase and TNFα is tumour necrosis factor-α). TSC2 is phosphorylated by several different kinases, including AKT, ERK and RSK, which results in the inhibition of the GAP function of TSC2 towards RHEB. Conversely, AMPK- and GSK3-mediated phosphorylation events positively regulate the GAP activity of TSC2 towards RHEB. Furthermore, some of these kinases can modulate mTORC1 activity in a TSC2-independent manner. For example, AKT-mediated phosphorylation of the mTORC1 inhibitory factor PRAS40 (40 kDa Pro-rich AKT substrate; also known as AKT1S1) and RSK-mediated phosphorylation of raptor contribute to mTORC1 activation, whereas AMPK-mediated phosphorylation of raptor results in the inhibition of mTORC1 signalling. In addition, Rag proteins respond to amino acid sufficiency and serve to control the subcellular localization of mTORC1, thereby mediating the nutrient-sensing function of mTORC1. The mTORC1 kinase is a master modulator of protein synthesis by integrating signals from growth factors and cellular conditions. In addition to its direct phosphorylation of 4E-binding proteins (4E-BPs), activated mTORC1 promotes the activation of S6Ks, which in turn phosphorylate many translation initiation factors, including eukaryotic translation initiation factor 4B (eIF4B). mTORC1 is also thought to regulate the duration of phosphorylation of its downstream targets by inhibiting the phosphatase activity of protein phosphatase 2A (PP2A). U0126, PD98059 and CI-1040 are mitogen-activated protein kinase/ERK kinase (MEK) inhibitors, and wortmannin and LY294002 are PI3K inhibitors. **b** | Phosphorylation sites of TSC2, TSC1, PRAS40 and raptor are mediated by multiple upstream signalling molecules. Note that AMPK-mediated phosphorylation of TSC2 at Ser1345 is required for subsequent phosphorylation of TSC2 by GSK3β. GEF, guanine nucleotide-exchange factor; HIF1α, hypoxia-inducible factor 1α; IRS1, insulin receptor substrate 1; NF1, neurofibromin 1; PDK1, phosphoinositide-dependent kinase 1; PtdIns, phosphatidylinositol; PTEN, phosphatase and tensin homologue; REDD1, protein regulated in development and DNA damage response 1 (also known as DDIT4).

Box 2 | **mTOR, rapamycin and cancer**

It is becoming increasingly clear that deregulated protein synthesis can lead to tumorigenesis[7,117]. Therefore, inhibition of translation through silencing of the mammalian target of rapamycin (mTOR; also known as FRAP1) signalling pathway is emerging as a promising therapeutic option. In preclinical cancer models, rapamycin and its analogues exhibit antitumour properties, such as inhibiting cell proliferation and cell survival, and anti-angiogenesis. However, accumulating evidence suggests that the antitumour properties of these molecules vary significantly among different cell lines. For example, rapamycin can be pro-apoptotic[118–120] or pro-survival[121], depending on the cancer cell line tested. The pro-survival or cytostatic effects of rapamycin can be explained by the feedback loop that is shown in FIG. 2a. When mTORC1 is activated, the resulting activation of 40S ribosomal protein S6 kinase 1 (S6K1) results in the phosphorylation and inhibition of insulin receptor substrate 1 (IRS1)-dependent activation of phosphoinositide 3-kinase (PI3K), thus leading to inhibition of AKT. Therefore, mTOR complex 1 (mTORC1) inhibition by rapamycin suppresses this negative-feedback loop and leads to the activation of AKT, a well-known pro-survival signalling enzyme. The pro-apoptotic effect of rapamycin remains largely unclear, however, although it can be correlated with its effects on mTORC2 and AKT (see BOX 1). In certain cancer cells, in which prolonged rapamycin treatment inhibits mTORC2 assembly and AKT activation, the drug might promote apoptosis[116]. Finally, the molecular basis for acquisition of rapamycin resistance in some cancer cells needs to be defined, as this might also lead to predictions of whether rapamycin will be useful in treating certain patients with cancer[93]. As pro-apoptotic effects are desirable for antitumour therapeutics, it will also be necessary to develop appropriate diagnostic biomarkers to predict the combinations of molecular lesions in a tumour that will result in pro-apoptotic responses to rapamycin treatment. Finally, the potential for combinatorial therapeutic approaches involving rapamycin must also be more actively pursued.

Ras–ERK and mTORC1. The Ras–ERK pathway has an established role in regulating transcription[50,51], but a connection between this pathway and translational regulation is less clear. The ERK-activated protein kinases MNK1 and MNK2 (also known as MKNK1 and MKNK2, respectively) directly phosphorylate eIF4E[52–54]. However, Mnk-dependent phosphorylation of eIF4E is not essential for translational control, as mice that are deficient for both MNK1 and MNK2 develop normally and rates of translation seem to be normal in the cells of mutant mice[55]. However, this does not exclude a crucial regulatory role for these kinases, as compensatory events could arise as a consequence of generating these mutant mice.

Over the past few years, mitogen-activated Ras–ERK signalling has also been shown to trigger the activation of mTORC1 signalling. This is mediated by ERK- and RSK-dependent inhibitory phosphorylation of TSC2 at Ser664 and Ser1798, respectively[56–59] (FIG. 2a,b). RSK-mediated phosphorylation of TSC2 is additive to AKT-mediated inhibitory modifications of TSC2 (REF. 56), but how these phosphorylation events lead to TSC2 inhibition remains unclear. Intriguingly, it was recently shown that RSK also directly targets the mTORC1 complex by phosphorylating raptor, and thereby promotes mTORC1 kinase activity[60]. Although the underlying molecular mechanism of this was not fully defined, this study provided new insights into Ras–ERK signals to mTORC1. As tumour-promoting phorbol esters and some growth factors activate mTORC1 signalling independently of AKT, phosphorylation of raptor by RSK might provide a mechanism to overcome the inhibitory effects of PRAS40. In addition, ERK-dependent phosphorylation of TSC2 has been shown to promote TSC1–TSC2 dissociation, which in turn leads to mTORC1 activation[58]. Together, these findings suggest that the mitogen-activated Ras–ERK–RSK signalling module, in parallel with the PI3K–AKT pathway, contains several inputs to stimulate mTORC1 signalling.

TNFα–IKKα or TNFα–IKKβ, and mTORC1. Although it is well established that insulin and growth factors can stimulate mTORC1 signalling, accumulating evidence suggests that cytokines, such as tumour necrosis factor-α (TNFα), can also induce mTORC1 activity. TNFα is a pro-inflammatory cytokine and is involved in many human diseases, including cancer[61,62]. Early studies implicated the TNFα pathway in mTORC1 activation[63,64]. Recently, it has been shown that IKKβ (inhibitor of nuclear factor-κB (NF-κB) kinase-β; also known as IKBKB), a major downstream kinase in the TNFα signalling pathway, phosphorylates TSC1 at Ser487 and Ser511, resulting in the inhibition of TSC1–TSC2 and, therefore, the activation of mTORC1 (REF. 65) (FIG. 2b). However, the mechanistic link between IKKβ-mediated phosphorylation of TSC1 and TSC1–TSC2 inhibition remains to be determined. In certain human cancers, TNFα promotes vascular endothelial growth factor (VEGF) expression and angiogenesis through activated mTORC1 signalling as a result of IKKβ-mediated suppression of TSC1 (REF. 65). This has provided a plausible mechanism that could link inflammation to cancer pathogenesis.

Moreover, TNFα also signals to AKT[61]. Activated AKT in turn induces IKKα (also known as CHUK), another major downstream kinase in the TNFα signalling pathway[62]. It has been shown that IKKα associates with mTORC1 in an AKT-dependent manner[66]. Importantly, IKKα is required for efficient induction of mTORC1 activity by AKT in certain cancer cells[66,67]. It remains unclear, however, how the association of IKKα with mTORC1 can result in the activation of mTORC1.

Environmental cues and mTORC1 activity

Protein synthesis consumes amino acids and ATP, which becomes particularly essential when cells are committed to growth and proliferation. Recent studies have begun to provide exciting new insights into the mechanisms by which the mTORC1 pathway integrates environmental cues to regulate protein synthesis and cell growth.

Cellular energy status and mTORC1. Because energy depletion severely decreases mTORC1 activity[68], and because translation is a major consumer of cellular energy, it follows that mTORC1 might sense an inadequate supply of cellular energy and suppress protein synthesis when necessary. Indeed, recent studies reveal that the AMP-activated protein kinase (AMPK; also known as PRKAB1) serves as the 'energy sensor' for mTORC1.

AMPK can be activated under various conditions of cellular stress, particularly those that increase the level of AMP or the ratio of AMP to ATP. In response, AMPK turns on ATP-generating pathways while inhibiting ATP-consuming functions of the cell[69]. TSC2 is regulated by cellular energy levels and has an essential role in the cellular energy response pathway[70]. The activated AMPK phosphorylates TSC2 on Ser1345 and enhances its GAP activity towards RHEB•GTP, resulting in the inhibition of mTORC1 (REF. 70) (FIG. 2b). However, in TSC2-null cells, mTORC1 activity can still be inhibited by cellular energy depletion[71,72], suggesting that additional components in this pathway can signal energy stress to mTORC1. A recent study proposes a TSC2-independent mechanism by which AMPK can signal to mTORC1 (REF. 72). They show that AMPK directly phosphorylates the mTOR-binding partner raptor on two well-conserved Ser residues. This phosphorylation induces 14-3-3 binding to raptor, resulting in the inhibition of mTORC1 activity. Together with the RSK-mediated regulation of raptor (see above), these new findings suggest that, similar to TSC2, raptor is a major signal integrator that interprets cell growth cues as well as energy sufficiency.

Consistent with the role of AMPK in the regulation of mTORC1, the tumour suppressor LKB1 phosphorylates and activates AMPK[69] and is required for the AMPK-dependent inhibition of mTORC1 (REF. 73). Cells from LKB1 mutant mice exhibit elevated signalling downstream of mTORC1. Moreover, the LKB1-mediated inhibition of mTORC1 signalling is dependent on AMPK and TSC2 (REF. 73), suggesting that dysregulation of the LKB1–AMPK–TSC2 signalling module might contribute to aberrant cell growth and proliferation in cancers with LKB1 mutations.

Wnt signalling also has a pivotal role in cell growth control and differentiation, and a well-established role of the Wnt pathway is to regulate transcription of a wide array of growth-promoting genes[74]. For instance, activation of the Wnt pathway inhibits glycogen synthase kinase 3β (GSK3β), which results in the stabilization of transcription factors, including β-catenin[74]. Evidence for a link between Wnt signalling and the mTORC1 pathway came from the observation that GSK3β phosphorylates TSC2 on Ser1341 and Ser1337, and that these two phosphorylation events require priming by the AMPK-mediated phosphorylation of Ser1345 (REFS 70,75) (FIG. 2a,b). GSK3β-dependent phosphorylation of TSC2 stimulates its GAP activity towards RHEB, leading to the inhibition of mTORC1 (REF. 75). Thus, activation of the Wnt signalling pathway stimulates the mTORC1 activity through GSK3β repression[75]. Importantly, expression of a *TSC2* mutant that cannot be modified by GSK3β makes the mTORC1 pathway resistant to energy deprivation[75], suggesting that GSK3β cooperates with AMPK to fully activate the GAP activity of TSC2. Curiously, both AKT and RSK, the two kinases implicated in phosphorylation and inhibition of TSC2 (see above), can also phosphorylate and inhibit GSK3β (REF. 76). It would thus be interesting to determine whether the inhibition of GSK3β by AKT or RSK is necessary for the activation of mTORC1 by growth factors or mitogens.

In addition to cellular energy levels, the sufficiency of oxygen is also essential for cellular metabolism. Long-term hypoxic stress results in energy deprivation and contributes to LKB1- or AMPK-mediated mTORC1 inhibition[73,77]. In response to short-term hypoxic stress, cells promptly limit energy expenditure by inhibiting energy-consuming processes, such as protein synthesis[77]. This rapid response is mediated in part by the inhibition of mTORC1 activity through a mechanism that involves hypoxia-inducible factor 1α (HIF1α), REDD1 (also known as DDIT4), TSC1 and TSC2, and 14-3-3 (REF. 78). HIF1α, a transcription factor that is stabilized under hypoxic conditions, drives the expression of a set of genes, including *REDD1*. As described above, AKT-mediated phosphorylation of TSC2 induces the binding of 14-3-3 to TSC2, the inhibition of the GAP function of TSC and activation of mTORC1 signalling. A recent study suggests that REDD1 competes with TSC2 for 14-3-3 binding. Thus, increased REDD1 levels that occur following exposure to hypoxia prevent the inhibitory binding of 14-3-3 to TSC2 (REF. 79), which eventually leads to the inhibition of mTORC1 signalling (FIG. 2a).

Amino acid sufficiency and mTORC1. It has long been known that mTORC1 signalling is strongly inhibited in cells cultured under low nutrient conditions, and that the re-addition of amino acids to starved cells can dramatically stimulate mTORC1 activity[21,26]. However, the mechanism by which amino acids signal to mTORC1 has remained largely unknown. Earlier studies suggested that amino acids, particularly Leu, activate mTORC1 by inhibiting TSC1–TSC2 or stimulating RHEB[80]. However, in cells that lack either TSC1 or TSC2, the hyperactivated mTORC1 activity remains sensitive to amino acid deprivation[81].

Recent studies from two groups proposed a mechanism that links amino acid sensing and the regulation of mTORC1 activity[82–84]. The Rag proteins, a family of four related small GTPases[84], interact with mTORC1 in an amino acid-sensitive manner and seem to be both necessary and sufficient for mediating amino acid signalling to mTORC1 (FIG. 2a). A constitutively active RAGB mutant, which binds exclusively to GTP, interacts strongly with mTORC1 and its expression makes the mTORC1 pathway resistant to amino acid deprivation. Furthermore, a dominant-negative form of RAGB that binds only to GDP, prevents the stimulation of mTORC1 by amino acids[82,83]. Interestingly, unlike RHEB, the Rag-mediated stimulation of mTORC1 kinase activity does not seem to be direct. In cells starved of amino acids, mTOR is distributed throughout the cytoplasm, but following a brief restimulation with amino acids, it is rapidly localized to the same intracellular compartment that contains

Wnt signalling
Wnt proteins are highly conserved secreted signalling molecules that regulate interactions between cells during embryogenesis. Wnt proteins bind to the Frizzled and low density lipoprotein receptor-related protein (LRP) families of receptors, and the signal is transduced to β-catenin, which then drives the transcription of Wnt target genes. Mutations in Wnt genes or Wnt pathway components lead to developmental defects and cancer.

Hypoxic stress
A lack of oxygen induces numerous changes in cell metabolism. Under hypoxic stress, inadequate ATP production leads to the downregulation of energy-consuming processes, such as protein synthesis. Hypoxia-inducible factor 1α (HIF1α) is the key transcription factor involved in cellular adaptation to hypoxia.

Rag proteins
In mammals, the Rag subfamily of Ras small GTPases comprises four members. They form heterodimers of RAGA or RAGB with RAGC or RAGD. Recent studies show that Rag proteins are required for amino acids to stimulate mammalian target of rapamycin complex 1 (mTORC1) signalling.

Box 3 | **Other signal-mediated translational control mechanisms**

A well-characterized mechanism of global translational control that seems to be independent of mammalian target of rapamycin complex 1 (mTORC1) is mediated through eukaryotic translation initiation factor 2 (eIF2), an essential component of the ternary complex (which comprises eIF2, Met-tRNA and GTP)[11]. eIF2–GTP is converted to eIF2–GDP during translation initiation when the start codon is recognized. Reconstitution of a functional ternary complex requires the exchange of GDP for GTP on eIF2, a reaction that is catalysed by eIF2B. eIF2 consists of three subunits (α, β and γ), and phosphorylation of eIF2α at residue Ser51 prevents the GTP-exchange reaction by inhibiting the dissociation of eIF2 from eIF2B[4,10]. As a result, the functional ternary complex becomes limited and global protein synthesis is inhibited. An array of kinases, including HRI, GCN2, PKR and PERK, which are activated in response to different cellular conditions, can phosphorylate eIF2α at Ser51 and thus inhibit global translation[11].

Another mechanism of regulating protein synthesis involves eukaryotic elongation factor 2 (eEF2). Phosphorylation of eEF2 at Thr56 by eEF2 kinase (eEF2K), a calcium/calmodulin-dependent protein kinase, interferes with the binding of eEF2 to the ribosome and the translocation step during elongation[122]. mTORC1 indirectly regulates eEF2K through S6K1-mediated inhibitory phosphorylation of eEF2K at residue Ser366 (REF. 122). However, evidence that mTORC1 indeed regulates elongation rates is lacking. Additional phosphorylation sites on eEF2K include Ser78 and Ser359, of which Ser359 is modified by stress-activated protein kinase 4 (SAPK4; also known as p38 mitogen-activated protein kinase δ)[27]. Recently, the CDC2–cyclin B complex was shown to phosphorylate eEF2K at Ser359 in a cell cycle-dependent manner[123]. Taken together, signal transduction-mediated translational control mechanisms also regulate the rates of peptide elongation through eEF2 phosphorylation.

RHEB. Importantly, the interaction between Rag and mTORC1 that is induced by amino acids is essential for the relocalization of mTOR to RHEB[82]. It is also possible that the recruitment of mTORC1 to intracellular membranes by the Rag GTPases brings it into proximity with membrane-associated translational complexes. These early results raise many important questions, such as: how do amino acids regulate the GTPase activity and cellular distribution of the Rag proteins at a molecular level? Further characterization of this pathway should prove to be highly enlightening.

Taken together, both nutrient availability and energy supply are key elements in an environment that is supportive of protein synthesis. These requirements should be satisfied before cells become committed to growth and proliferation, as activation of AKT and/or RSK will fail to trigger mTORC1 activation if AMPK is activated owing to energy stress[71]. These observations are consistent with the existence of a dominant and crucial energy and nutrient checkpoint mechanism, which controls mTORC1 signalling and is essential for the proper control of protein synthesis, cell growth and proliferation.

mTORC1 targets in translation machinery

By sensing the presence of growth factors and the sufficiency of nutrients, activated mTORC1 signals to various components of the translation initiation machinery in a coordinated fashion through direct or indirect phosphorylation events[26,27,85]. The translation initiation factors that are regulated by mTORC1 signalling include eIF4G, eIF4B and 4E-BP1, of which 4E-BP1 regulates the function of eIF4E that binds to the 5′ mRNA cap structure.

Ternary complex
A complex that comprises eIF2, Met-tRNA and GTP. During cap-dependent translation initiation, the complex associates with 40S ribosomal subunit, eIF3 and eIF1A to form the 43S pre-initiation complex. The assembly of the ternary complex is regulated by eIF2B.

Regulation of the mRNA cap-binding protein eIF4E is mediated directly by mTORC1, which phosphorylates the eIF4E inhibitors — the 4E-BPs[26,86] (FIG. 1a). In quiescent cells, hypophosphorylated 4E-BP1 binds tightly to eIF4E. As 4E-BP1 competes with eIF4G for an overlapping binding site on eIF4E, 4E-BP1 prevents eIF4G from interacting with eIF4E. On mTORC1 activation, hyperphosphorylated 4E-BP1 dissociates from eIF4E, allowing for the recruitment of eIF4G and eIF4A to the 5′ end of an mRNA. Finally, eIF3, the small ribosomal subunit and the ternary complex (comprising eIF2, Met-tRNA and GTP) are recruited to the cap, resulting in the assembly of the 48S translation pre-initiation complex, ribosome scanning and translation initiation[26,86] (BOX 3).

Serving as a modular scaffold in the assembly of the translation pre-initiation complex, eIF4G is also phosphorylated in response to growth factor stimuli at multiple sites, some of which are dependent on mTORC1 (REF. 26). These sites are clustered in a hinge region of eIF4G that joins two structural domains, and it has thus been predicted that the modification might induce conformational changes in the protein that affect its activity[26]. However, the molecular details of how eIF4G phosphorylation regulates its function remain to be determined.

Another important target of mTORC1 are the S6Ks, including S6K1 and S6K2 (REF. 20) (FIG. 2a). Although a full and sustained S6K activation requires multiple growth factor-induced phosphorylation events, two essential phosphorylation sites have been identified, including Thr229, which is located in the catalytic activation loop, and Thr389, which is located at a hydrophobic motif that is carboxy-terminal to the kinase domain[20]. S6K activation is initiated by mTORC1-mediated phosphorylation of Thr389, resulting in the formation of a docking site for phosphoinositide-dependent kinase 1 (PDK1), which then phosphorylates Thr229 to activate S6K[20]. Although accumulating evidence suggests that S6Ks modulate the functions of translation initiation factors during protein synthesis (see below), S6Ks are also thought to coordinate the regulation of ribosome biogenesis, which in turn drives efficient translation[87].

Importantly, the results from the *S6k1* and *S6k2* mouse knockouts and from RNA interference studies reveal both redundant and isoform-specific functions for the two S6Ks[20]. In particular, S6K1, but not S6K2, seems to contribute more significantly to the ability of mTORC1 to regulate cell growth[88,89]. Prior to the generation of the *S6k1* knockout mouse, it was predicted that S6K1 stimulated cell growth by regulating the translation of a set of mRNAs that encode components of the ribosome and certain translation factors. However, the finding that translation of these mRNAs is normal in the absence of S6K1 is inconsistent with this notion[26]. Similarly, ribosomal protein S6 (RPS6), which is often used as a biomarker for S6K activity, does not seem to be the crucial target of S6Ks in regulating cell growth[90]. Consistently, RPS6 phosphorylation levels are nearly normal in S6K1-deficient mice[91]. It is important to note that the *S6k1*$^{-/-}$*S6k2*$^{-/-}$ double knockout mice are small, but only a small percentage of the mice develop owing to

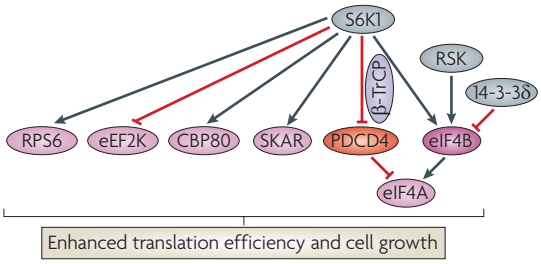


Figure 3 | **Regulation of PDCD4.** In addition to several translation factors and RNA-binding proteins — including ribosomal protein S6 (RPS6), eukaryotic translation elongation factor 2 kinase (eEF2K), cap-binding protein 80 (CBP80), SKAR and eukaryotic translation initiation factor 4B (eIF4B) — activated 40S ribosomal protein S6 kinase 1 (S6K1) also phosphorylates PDCD4 (programmed cell death 4), a tumour suppressor that binds to eIF4A. This binding is thought to prevent translation by inhibiting the helicase activity of eIF4A, by preventing eIF4A from interacting with eIF4G, or both. S6K1-mediated phosphorylation of PDCD4 results in its ubiquitylation and subsequent degradation through the E3 ubiquitin ligase β-TrCP. 14-3-3δ is required for inhibiting eIF4B activity during mitosis. In addition, ribosomal S6 kinase (RSK) and S6K1 converge on eIF4B to regulate its function in translation initiation.

perinatal lethality[91]. These results underscore the importance of these enzymes for normal growth and survival. However, the fact that some mice survive indicates that the S6Ks are not essential for life. Alternatively, compensatory mechanisms may be at work. For example, the upregulation of AKT or RSK activities, which share several targets with S6K1 or S6K2, could result in compensation for some S6K functions. The development of S6K1- or S6K2-specific inhibitors will be useful in defining the effects of acute inhibition of these enzymes. Importantly, based on the results from the knockout mice and RNA interference-based studies, the search for S6K1-specific targets that could contribute to growth control has been initiated. Recent studies have provided important new insights into the mechanism by which mTORC1 and S6K1 regulate translation initiation and cell growth (see below).

On mTORC1 inhibition with rapamycin, the dephosphorylation of its downstream targets seems to be rapid. For instance, rapamycin induces prompt dephosphorylation of S6K1, including sites that are not phosphorylated by mTORC1. This indicates that mTORC1 might also regulate the phosphorylation of its substrates, possibly by suppressing phosphatase interaction with or activity towards mTORC1 targets[26]. Interestingly, mTORC1 phosphorylates protein phosphatase 2A (PP2A) *in vitro*, suggesting a model in which phosphorylation of PP2A by mTORC1 could inhibit its phosphatase activity[92]. Thus, exactly how mTORC1 regulates the phosphorylation of its two most extensively characterized targets is still unclear. A recent study showed that 4E-BP1 regains its mTORC1-dependent phosphorylation in some but not all cells after long-term incubation with rapamycin[93]. This explains, at least in part, why cap-dependent translation can be maintained in some cells following an extended treatment with rapamycin. Importantly, in these rapamycin-treated cells, S6K1 does not regain phosphorylation activity. How mTORC1 gains resistance against the effects of rapamycin towards 4E-BP1 but not S6K1 is not clear but might reveal important mechanistic information regarding mTORC1 signalling. These results also highlight an important cautionary note regarding the use of phospho-S6 antibodies as a biomarker in animal and clinical studies using rapamycin or its analogues, to determine whether mTORC1 is being inhibited or not.

S6K and the eIF4 RNA helicase complex

Roughly one-half of mRNA bases are thought to self-pair, mostly in the form of short hairpins, but longer hairpins or more stable structures are not uncommon[94,95]. Interestingly, stable secondary structures are often found in the 5′ UTR of specific mRNA species and significantly suppress their translation efficiency[13,14]. The translation pre-initiation complex is recruited near the 5′ end of mRNA, and this requires the structured UTR to be 'linearized' — not only for the initial binding of the 40S ribosome but also for subsequent scanning towards the downstream initiation codon[9,11]. The RNA helicase activity in the eIF4F complex carries out this important function. The initiation factor eIF4A contains the RNA helicase activity and unwinds structured mRNAs during translation initiation[16]. Although eIF4A alone exhibits low levels of RNA helicase activity, it has long been known that its activity is substantially stimulated by a cofactor, eIF4B[9]. Recent studies reveal new and important mechanisms by which S6K1 modulates the RNA helicase function of eIF4A (FIG. 3).

S6K1 regulates eIF4B recruitment. eIF4B enhances the affinity of eIF4A binding to ATP, which, in turn, increases the processivity of the eIF4A helicase function[16]. Interestingly, eIF4B is a phosphoprotein, and its phosphorylation can be stimulated by growth factors or tumour-promoting phorbol esters[96]. Importantly, eIF4B phosphorylation correlates with its function in promoting the translation of mRNAs with long and structured 5′ UTRs, suggesting that this modification is crucial for signalling-mediated translational control in response to growth factor stimuli[17,18]. Consistent with the role of mTORC1 signalling as a master modulator of protein synthesis, S6K1 phosphorylates eIF4B on Ser422, which is located in the RNA-binding region that is necessary for promoting the helicase activity of eIF4A[97]. An eIF4B mutant that cannot be modified by S6K1 results in a loss of its activity in an *in vitro* translation assay system[97], indicating that phosphorylation of this site has an important role in eIF4B function. eIF4B phosphorylation by S6K1 is both sufficient and necessary for its recruitment to the translation pre-initiation complex[17]. Interestingly, RSK can also phosphorylate eIF4B on residue Ser422 (REF. 18), suggesting that AKT and ERK or RSK pathways converge not only on TSC2 to regulate mTORC1 but also on eIF4B to control its function in translation (FIG. 3). This observation also provides another possible mechanism for gaining resistance to rapamycin, as RSK activation and signalling is insensitive to mTORC1 inhibition.

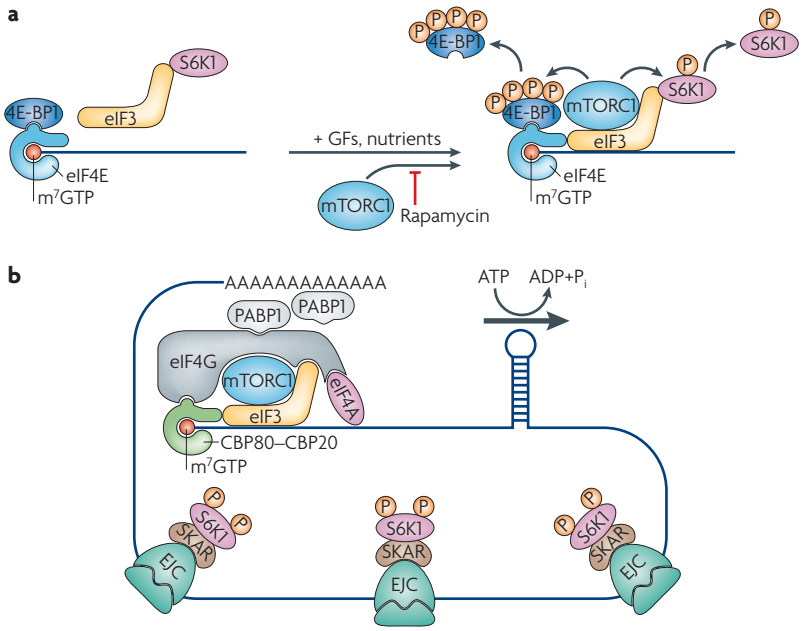


Figure 4 | **mTORC1 and S6K associate with mRNAs. a** | The multisubunit eukaryotic initiation factor complex 3 (eIF3) is a dynamic scaffold for mammalian target of rapamycin complex 1 (mTORC1)- and 40S ribosomal protein S6 kinase 1 (S6K1)-binding to mRNAs. When inactive, S6K1 associates with the eIF3 complex, whereas mTORC1 does not. On activation, mTORC1 is recruited to the eIF3 complex and phosphorylates S6K1 (REF. 17). The mTORC1-mediated phosphorylation of S6K1 at Thr389 results in its dissociation from eIF3, and subsequent phosphorylation and activation by phosphoinositide-dependent kinase 1 (PDK1; not shown). Following activation, the eIF3–mTORC1 complex is found at the 5′ cap, where it is now close to 4E-binding protein 1 (4E-BP1; also known as eIF4EBP1). Phosphorylation of 4E-BP1 leads to its dissociation from the cap complex, thus promoting assembly of the eIF4F complex (see FIG. 1). **b** | As described in the main text, an extensive connection between the regulation of transcription and translation has led us to predict that newly synthesized mRNAs are translated efficiently as their expression is required for cells to respond promptly to environmental changes. The molecular mechanisms that link new gene transcription and rapid expression of these new genes are beginning to emerge. For example, the scaffold protein SKAR is associated with the exon-junction complexes (EJCs) of newly generated mRNAs in a splicing-dependent manner[103]. The binding of SKAR to the newly synthesized mRNAs facilitates the recruitment of activated S6K1 to these new mRNAs. This, in turn, can lead to an enhanced translation efficiency of these new mRNAs. CBP, cap-binding protein; GF, growth factor; PABP1, polyadenylate-binding protein 1.

During mitosis, when protein synthesis is largely inhibited, eIF4B function is regulated through a mechanism that requires 14-3-3δ[98], a tumour suppressor. Binding of 14-3-3δ to essential regulators of cell cycle progression, such as CDC2 and cyclin B1, sequesters these proteins in the cytoplasm after DNA damage and thus inhibits cell cycle progression. In the absence of 14-3-3δ, cells exhibit abnormal proliferation after DNA damage, resulting in multinucleated cells and tumour formation. Recent studies show that 14-3-3δ binds to several proteins that are required for translation, including eIF4B[98]. When bound to 14-3-3δ, eIF4B cannot be recruited to the translation pre-initiation complex near the 5′ cap of mRNA. Consequently, cap-dependent translation is inhibited, whereas mRNAs with internal ribosome entry sites, such as the mRNA that encodes the cell cycle regulator PITSLRE (also known as p58 and CDC2L2), can still be translated in a cap-independent manner. However, it remains to be determined whether inhibiting eIF4B by 14-3-3δ is sufficient to inhibit cap-dependent translation during mitosis. If not, it will be important to identify additional factors that are implicated in this novel mechanism of translational control.

S6K1 regulates PDCD4-mediated inhibition of eIF4A. Programmed cell death 4 (PDCD4), which is a reported tumour suppressor, binds to eIF4A and is thought to inhibit the helicase activity of eIF4A[99]. Both PDCD4 and eIF4G contain a conserved motif that mediates binding to eIF4A; therefore, PDCD4 is also thought to prevent eIF4A from incorporating into the eIF4F complex by competing with eIF4G for eIF4A binding[100]. On growth factor stimulation, PDCD4 can be rapidly phosphorylated on Ser67 by S6K1 and subsequently degraded through the ubiquitin ligase β-TrCP[101]. Therefore, S6K1-dependent phosphorylation of PDCD4 prevents the inhibitory effect of PDCD4 towards eIF4A helicase function (FIG. 3).

Moreover, a PDCD4 mutant that cannot bind the ubiquitin ligase β-TrCP inhibited translation of an mRNA with a structured 5′ UTR. Importantly, expression of mutant *PDCD4* results in reduced cell growth and proliferation[101], suggesting that S6K1-mediated phosphorylation and subsequent degradation of PDCD4 promotes efficient protein synthesis. It is important to note that there are several other Ser residues on PDCD4 that are phosphorylated in response to growth factor stimuli[101]. Given that multiple upstream signalling pathways converge on the translation initiation machinery to modulate protein synthesis, it would be interesting to determine the corresponding kinases that are responsible for these modifications and the function of these phosphorylation events in regulating the effect of PDCD4 on translation.

mTORC1 and S6K associate with mRNAs

Supporting the role of mTORC1 and S6Ks in assembly of the translation pre-initiation complex, the multisubunit initiation factor complex eIF3 was identified as a dynamic scaffold for mTORC1 and S6K1 binding[17] (FIG. 4). When inactive, S6K1 associates with the non-polysome-associated eIF3 complex, whereas mTORC1 does not. On activation, mTORC1 is recruited to the eIF3 complex and phosphorylates S6K1 (REF. 17). The mTORC1-mediated phosphorylation of S6K1 at Thr389 results in its dissociation from eIF3 (REF. 17) and subsequent phosphorylation and activation by PDK1. Moreover, a non-phosphorylatable S6K mutant (Thr389Ala) that eliminates S6K1 activity constitutively binds eIF3, whereas active S6K1 mutants (with Thr389Glu) exhibit reduced binding to eIF3 (REF. 17). Based on polysome analysis and cap-binding assays, it is thought that the mTORC1–eIF3 complex associates with the mRNA 5′ cap, bringing mTORC1 into proximity with its other major target, 4E-BP1. Together, these findings explain how mTORC1 encounters its substrates during translation initiation. However, whether and how activated S6K1 remains associated with the translation machinery such that it would be in proximity with its downstream effectors was not addressed in this study.

Internal ribosome entry site
A structure in the 5′ untranslated region or open reading frame of some mRNAs of cellular or viral origin. This site mediates translation initiation independently of the cap structure by recruiting the ribosome directly to an internal position of the mRNA.

Polysome
Two or more ribosomes attached to different points on the same strand of mRNA. Also known as a polyribosome.

Recently, SKAR, a downstream target of S6K1 (REF. 102), was identified as a scaffold protein that is used for the recruitment of activated S6K1 to newly generated mRNAs[103] (FIG. 4). SKAR was previously shown to interact specifically with hyperphosphorylated, active S6K1, but not with S6K2. Importantly, earlier studies suggested that SKAR, like S6K1, is also involved in cell growth control[102]. SKAR has now been shown to be associated with newly synthesized mRNAs in a splicing-dependent manner[103]. Mapping of the SKAR-binding site on mRNA localizes SKAR predominantly to the exon-junction complex[103], a multisubunit structure that is recruited to mRNA following pre-mRNA splicing[104,105]. Importantly, SKAR, as well as the exon-junction complex, is required for the recruitment of activated S6K1 to newly synthesized mRNAs, which in turn contributes to the increased translational yield of spliced messages[103]. Thus, activated S6K1 is placed in proximity with additional translational targets during the initial translation of spliced mRNAs. However, the identity and function of potential S6K1 downstream effectors that are unique to the newly synthesized mRNAs remain to be investigated. The 5′ cap structures of newly synthesized mRNAs are associated with cap-binding protein 80 (CBP80) and CBP20 (also known as NCBP1 and NCBP2, respectively), which form a cap-binding complex. CBP80 can be phosphorylated by S6K1 on growth factor stimulation[20]. However, the biological significance of this phosphorylation event is unclear. Importantly, we propose that the ability of S6K1 to contribute to the translation efficiency of newly processed mRNA also provides the cell with an important checkpoint mechanism used to downregulate protein production at the first round of translation in times of nutrient and energy insufficiency.

Finally, in another recent study, an additional novel mechanism was proposed that potentially links mTORC1 to splicing and translation initiation[106]. The study provided evidence that SF2/ASF, a splicing factor and mRNA-binding protein, stimulates translation initiation by directly recruiting mTORC1 to mRNA[106]. However, it remains to be determined whether the SF2/ASF-dependent recruitment of mTORC1 is regulated in response to nutrients and growth factors, as is the case for eIF3-dependent recruitment of mTORC1 (REF. 17). Intriguingly, SF2/ASF is an oncoprotein that controls the alternative splicing of a group of genes, including S6K1 (REF. 107). Fittingly, an unusual oncogenic isoform of S6K1 recapitulates the transforming activity of SF2/ASF (REF. 107). It remains to be determined, however, which of the multifaceted functions of SF2/ASF have a major role in promoting protein synthesis.

Exon-junction complex
A complex of proteins that is deposited as a consequence of pre-mRNA splicing 20–24 nucleotides upstream of splicing-generated exon–exon junctions of newly synthesized mRNA. These proteins are thought to mediate the enhanced accuracy and efficiency of gene expression of spliced mRNAs.

Conclusions and perspectives

An increasing number of oncogenic signalling pathways, including Ras–ERK and Wnt, that have long been thought to be primarily responsible for transcriptional control have recently been shown to be involved in the regulation of protein synthesis through mTORC1 signalling. One could envisage a scenario whereby crucial mRNAs that are transcribed owing to the activation of transcription factors are then efficiently translated by the concurrently activated mTORC1 pathway. The coordination of these processes makes energetic and biological sense. It follows that many other oncogenic pathways might also directly or indirectly regulate mTORC1 signalling and translation. Intriguingly, many studies have provided evidence for links between mTORC1 and signalling by notch, hedgehog, TGFβ, hormone receptors, interferons and a range of other receptor-based signalling systems. The molecular basis for these connections will likely be the subject of future studies.

It is fascinating that cells have evolved several independent mechanisms by which mTORC1 regulates translation initiation. mTORC1, through S6K1, might have a crucial role in relaying the decision of a cell of whether to invest the energy and building material required for the translation of newly synthesized and processed mRNA. The association of activated S6K1 with spliced mRNA also provides it with the potential to contribute to decisions regarding mRNA storage, stability and transport into the cytoplasm. mTORC1 contributes to overall cap-dependent translation by phosphorylating 4E-BPs, and, when combined with mTORC1-mediated activation of S6Ks, might further enhance the translation efficiency, through enhanced RNA helicase activities, of a subset of mRNAs that contain long and structured 5′ UTRs. Importantly, these 5′ UTR-containing mRNAs often encode proteins with crucial biological functions, such as MYC, HIF1α, ODC1, cyclin D1, fibroblast growth factor (FGF), VEGF and insulin-like growth factor (IGF), all of which contribute to efficient cell cycle progression. To unwind the structured 5′ UTR, S6K-mediated activation of eIF4A helicase activity is essential. Consistent with this notion, a recent study showed that unwinding a structured mRNA by ribosomal helicase activity is surprisingly time-consuming, ranging from a few seconds to 1–2 min[108]. To initiate translation of mRNAs that contain these highly structured 5′ UTRs, the elongated duration of ribosomal scanning requires sustained activity of mTORC1 and S6Ks, which in turn can only be warranted by the commitment of the environmental cues that are supportive of cell growth, including sufficient supply of nutrients and energy in addition to the presence of growth factors.

An important avenue for future studies is to understand cell-type-specific regulation of protein synthesis. For example, mTORC1 signalling in differentiated neurons is probably distinct from that in de-differentiated highly proliferative cancers. Accumulating evidence suggests that the temporal and spatial regulation of translational activity can vary dramatically despite the same environmental conditions. It is essential to delineate upstream signals and downstream effectors that are crucial for dictating the readout of mTORC1 activity in different cell types. The molecular specifics of mTORC1 signalling can then be applied to a better understanding of human diseases, such as diabetes, neurodegeneration and cancer.

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DATABASES

UniProtKB: http://www.uniprot.org
β-TrCP | 4E-BP1 | AKT | AMPK | CDC2 | eIF4A | eIF4B | eIF4E | eIF4G | IKKα | IKKβ | IRS1 | LST8 | mTOR | PDCD4 | PRAS40 | raptor | RHEB | S6K1 | S6K2 | TSC1 | TSC2 | VEGF

FURTHER INFORMATION

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