mTOR: from growth signal integration to cancer, diabetes and ageing

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Abstract | In all eukaryotes, the target of rapamycin (TOR) signalling pathway couples energy and nutrient abundance to the execution of cell growth and division, owing to the ability of TOR protein kinase to simultaneously sense energy, nutrients and stress and, in metazoans, growth factors. Mammalian TOR complex 1 (mTORC1) and mTORC2 exert their actions by regulating other important kinases, such as S6 kinase (S6K) and Akt. In the past few years, a significant advance in our understanding of the regulation and functions of mTOR has revealed the crucial involvement of this signalling pathway in the onset and progression of diabetes, cancer and ageing.

Macrolide
A naturally occurring drug, generally an antibiotic, that is composed of a large lactone carbon ring.

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'Growth' indicates the set of biochemical processes — intimately linked to the availability of nutrients and energy — by which organisms increase their size and cell number through the synthesis of new cellular components, including proteins, nucleic acids and lipids. Cells also rely on a complex set of programmes to cope with nutrient starvation and low energy. To avoid energy imbalance and death, cells quickly suppress biosynthetic programmes during fasting, increase the recycling of 'aged' proteins and organelles to provide an internal source of metabolites, and slow or halt proliferation.

A signalling pathway that is centred on the kinase target of rapamycin (TOR) is at the interface between growth and starvation. The appearance of TOR in early eukaryotes enabled these unicellular organisms to sense the availability of nutrients and to promote growth in favourable environmental conditions. With the emergence of multicellularity, TOR acquired additional roles as a central controller of organism growth and homeostasis. As such, mammalian TOR (mTOR) is implicated in disease states where growth is deregulated and homeostasis is compromised, namely cancer, metabolic diseases and ageing. Dysregulated mTOR signalling fuels the destructive growth of cancers. Over-stimulation of the mTOR pathway by excess food consumption may be a crucial factor underlying the diabetes epidemics. Finally, recent findings suggest that mTOR signalling controls the rate at which cells and tissues age, and that inhibiting mTOR may represent a promising avenue to increase longevity.

In this Review, we begin by summarizing our current understanding of the regulatory inputs and the cellular actions of mTOR. We then discuss how mTOR controls key aspects of body metabolism and how deregulated mTOR signalling can promote metabolic diseases, cancer and ageing. Finally, we speculate how the availability of new chemical agents that control mTOR activity could pave the way towards therapeutic approaches for ageing, cancer and metabolic diseases. For the remainder of this Review, we will use the term mTOR when discussing TOR in mammalian organisms and TOR without a prefix when discussing non-mammalian organisms. In these cases, the organism (*Caenorhabditis elegans*, *Drosophila melanogaster*, *Dictyostelium discoideum* or *Saccharomyces cerevisiae*) will be specified. It should be noted that although mTOR originally stood for 'mammalian TOR', it is now also used officially as an abbreviation for 'mechanistic TOR'.

Organization and actions of mTOR complexes

Genetic and biochemical approaches in yeast and mammals led to the discovery of TOR as the target of the immunosuppressant rapamycin, a macrolide that is produced by a soil bacterium that is found on Easter Island[1–3]. TOR belongs to the phosphoinositide 3-kinase (PI3K)-related protein kinases (PIKK) family, which comprises large proteins that enable organisms to cope with metabolic, environmental and genetic stresses.

Molecular composition of mTORC1 and mTORC2. mTOR is the catalytic subunit of two distinct complexes called mTOR complex 1 (mTORC1) and mTORC2 (FIG. 1). Unique accessory proteins distinguish these complexes: regulatory-associated protein of mTOR (RAPTOR) and rapamycin-insensitive companion of mTOR (RICTOR) define mTORC1 and mTORC2, respectively[4–6]. These companions function as scaffolds for assembling the complexes and for binding substrates and regulators[4,5,7–12]. Further unique components of mTORC1

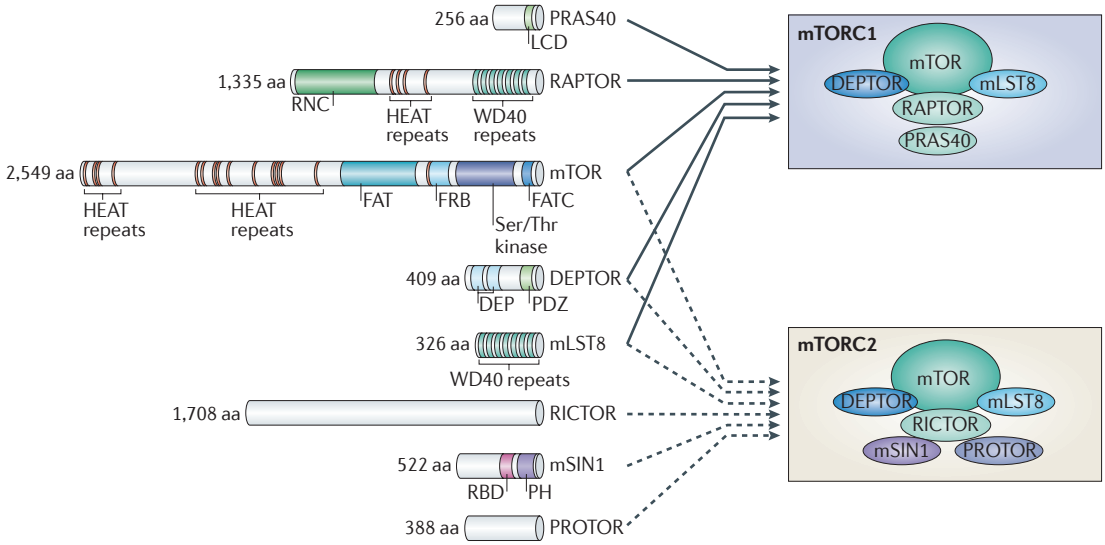


Figure 1 | **Domain organization of mTOR and mTORC proteins.** Mammalian target of rapamycin (mTOR) complex 1 (mTORC1) and mTORC2 have shared and unique components. The complexes both contain mTOR, mammalian lethal with SEC13 protein 8 (mLST8; also known as GβL) and DEP domain-containing mTOR-interacting protein (DEPTOR). Regulatory-associated protein of mTOR (RAPTOR) and 40 kDa Pro-rich Akt substrate (PRAS40; also known as AKT1S1) are unique to mTORC1; rapamycin-insensitive companion of mTOR (RICTOR), mammalian stress-activated map kinase-interacting protein 1 (mSIN1; also known as MAPKAP1) and protein observed with RICTOR (PROTOR) are specific to mTORC2. The domain organization of mTOR resembles that of other PI3K-related protein kinases (PIKK) family members. At the amino terminus, there is a cluster of HEAT (huntingtin, elongation factor 3, a subunit of protein phosphatase 2A and TOR1) repeats, which mediate protein–protein interactions. These are followed by a FRAP, ATM and TRRAP (FAT) domain; the FKBP12-rapamycin binding (FRB) domain, which mediates the inhibitory action of rapamycin on RAPTOR-bound mTOR; the Ser/Thr kinase catalytic domain; and the carboxy-terminal FATC domain. PRAS40 has a conserved Leu charged domain (LCD), at which phosphorylation by Akt occurs. The scaffolding function of RAPTOR is reflected by its composition of protein-binding domains; it consists of several HEAT repeats, followed by seven WD40 domains, which are probably arranged in a β-propeller. DEPTOR consists of tandem DEP domains (Dishevelled, EGL-10 and pleckstrin domains) that are followed by a single PDZ domain (postsynaptic density of 95 kDa, Discs large and zonula occludens 1 domain). mLST8 is highly conserved; its seven WD40 domains form a β-propeller that mediates protein–protein interactions. RICTOR and PROTOR have no clearly identifiable domains or motifs. mSIN1 contains a Ras binding domain (RBD), and a pleckstrin homology (PH) domain that is likely to interact with phospholipids. RNC, RAPTOR N-terminal conserved.

include a negative regulator, 40 kDa Pro-rich Akt substrate (PRAS40; also known as AKT1S1) [11,13], whereas mTORC2 contains protein observed with RICTOR 1 (PROTOR1) and PROTOR2, which are likely to help complex assembly, and mammalian stress-activated map kinase-interacting protein 1 (mSIN1; also known as MAPKAP1), which may target mTORC2 to membranes[12,14,15]. mTORC1 and mTORC2 share mammalian lethal with SEC13 protein 8 (mLST8; also known as GβL) and the recently identified DEP domain-containing mTOR-interacting protein (DEPTOR), which function as positive and negative regulators, respectively[16,17] (FIG. 1). Biochemical and structural evidence suggests that both mTORC1 and mTORC2 may exist as dimers[9,18].

In yeast and mammals, rapamycin inhibits the ability of mTORC1, but not mTORC2, to phosphorylate its substrates[6,16,19]. Rapamycin binds the small protein 12 kDa FK506-binding protein (FKBP12; also known as PPIase FKBP1A) and, in turn, rapamycin–FKBP12 binds and inhibits RAPTOR-bound, but not RICTOR-bound, mTOR[6,16,19]. Rapamycin might inhibit mTORC1 by dissociating RAPTOR from mTOR, thus preventing the access of mTOR to some substrates[5,18]. Complicating this picture, prolonged treatment with rapamycin can inhibit mTORC2 in a subset of tissues and cell lines[20]. This effect may involve a progressive sequestration of the cellular pool of mTOR in a complex with rapamycin–FKBP12, thus making it unavailable for assembly into mTORC2.

Substrates and actions of mTORC1. The subunit composition of each mTORC dictates its substrate specificity. The mTORC1 substrates S6 kinase 1 (S6K1) and eIF4E-binding protein 1 (4E-BP1) associate with mRNAs and regulate mRNA translation initiation and progression, thus controlling the rate of protein synthesis (reviewed in REF. 21) (FIG. 2a). Unphosphorylated 4E-BP1 suppresses mRNA translation; however, when phosphorylated by mTORC1, 4E-BP1 dissociates from eukaryotic translation initiation factor 4E (eIF4E), allowing eIF4E to recruit the translation initiation factor eIF4G to the 5′ end of most mRNAs[22,23]. When phosphorylated by mTORC1, S6K1 promotes mRNA translation by phosphorylating or binding multiple proteins, including eukaryotic elongation factor 2 kinase (eEF2K)[24], S6K1 Aly/REF-like target (SKAR; also known as POLDIP3)[25], 80 kDa nuclear cap-binding protein (CBP80; also known as NCBP1) [26] and eIF4B[27], which collectively affect translation initiation and elongation.

WD40 domain
A protein domain that comprises a 40-amino-acid-long protein motif that contains a Trp–Asp (W–D) dipeptide at its carboxyl terminus. Several WD40 repeats are often arranged in a β-propeller configuration, forming a protein–protein interaction surface.

DEP domain
(Dishevelled, EGL-10 and pleckstrin domain). A domain of unknown function that is present in signalling proteins.

PDZ domain
(Postsynaptic density of 95 kDa, Discs large and zona occludens 1 domain). A protein-interaction domain that often occurs in scaffolding proteins and is named after the founding members of this protein family.

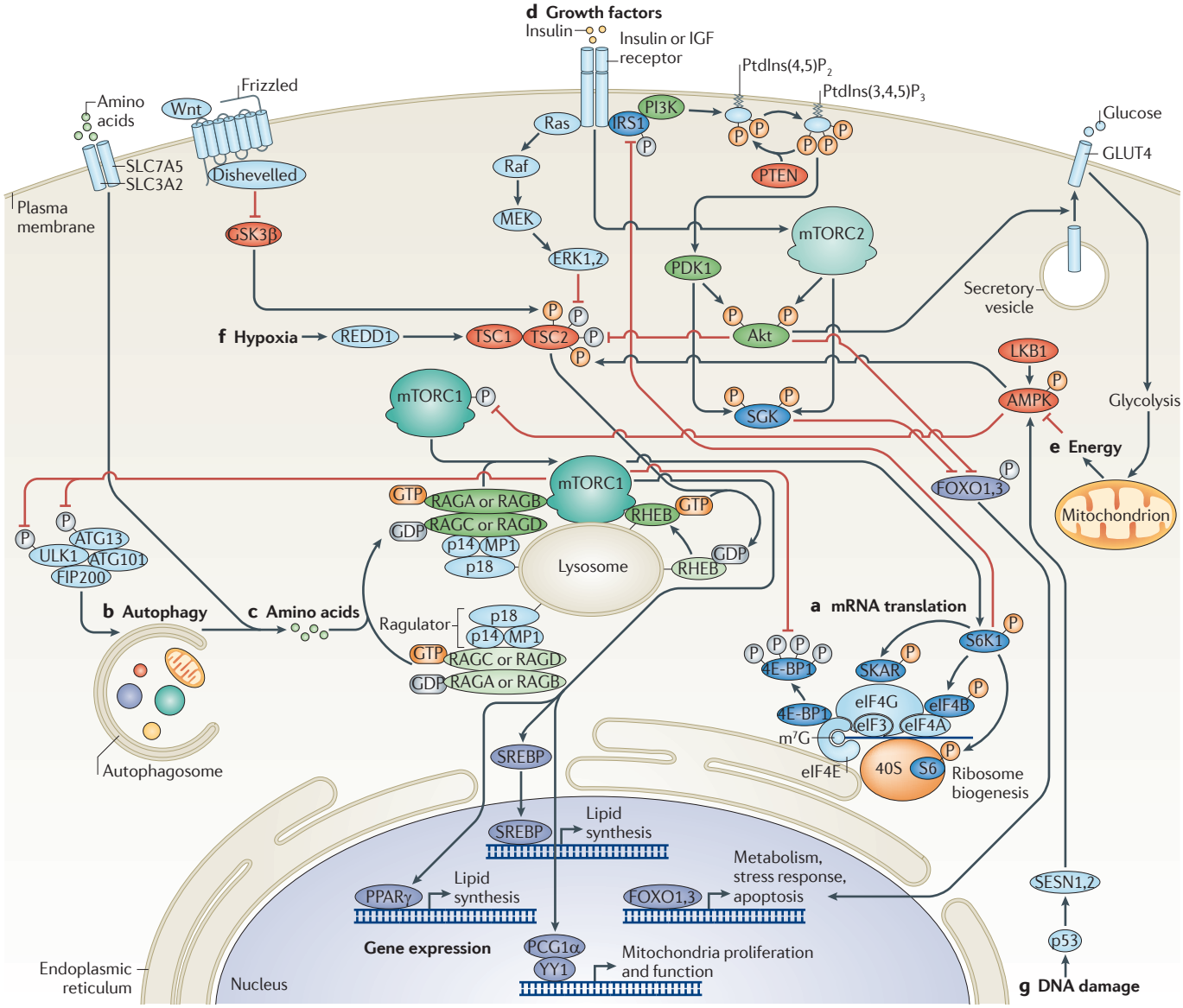


Figure 2 | **The mTOR signalling pathway.** Mammalian target of rapamycin complex 1 (mTORC1) promotes mRNA translation (**a**) and inhibits autophagy (**b**), by integrating nutrient signals that are generated by amino acids (**c**), growth factors such as insulin and insulin-like growth factors (IGFs) (**d**), energy signals that act through AMP-activated kinase (AMPK) (**e**) and various stressors including hypoxia (**f**) and DNA damage (**g**). Signal integration occurs at the level of the tuberous sclerosis 1 (TSC1; also known as hamartin)–TSC2 (also known as tuberin) complex. Akt and extracellular regulated kinase 1 (ERK1) and ERK2 phosphorylate TSC2, thus inhibiting the GTPase activating protein (GAP) activity of TSC1–TSC2 towards Ras homologue enriched in brain (RHEB). By contrast, phosphorylation of TSC2 by AMPK and glycogen synthase kinase 3β (GSK3β) results in the activation of TSC1–TSC2. The hypoxic factor protein regulated in development and DNA damage response 1 (REDD1; also known as DDIT4) promotes the assembly and activation of TSC1–TSC2. A second level of integration occurs at the lysosome: the Rag GTPases (which are held in place by the Ragulator, which consists of p18, p14 and MAPK scaffold protein 1 (MP1)) recruit mTORC1 to the lysosomal surface in response to amino acids (**c**); in turn, lysosomal recruitment enables mTORC1 to interact with GTP-bound RHEB, the end point of growth factor (**d**), energy (**e**) and stress (**f**,**g**) inputs. Growth factor receptors activate mTORC2 near the plasma membrane (**d**), where mTORC2 may be recruited through binding of mammalian stress-activated map kinase-interacting protein 1 (mSIN1; also known as MAPKAP1) to phospholipids. Because of its role in phosphorylating and activating Akt, mTORC2 forms a core component of the phosphoinositide 3-kinase (PI3K) pathway. Activating and inhibitory phosphates are orange and grey, respectively. 4E-BP1, eIF4E-binding protein 1; ATG, autophagy-related; CBP80, 80 kDa nuclear cap-binding protein; eEF2K, eukaryotic elongation factor 2 kinase; eIF, eukaryotic translation initiation factor; FIP200, 200 kDa FAK family kinase-interacting protein; FOXO, forkhead box protein O; IRS1, insulin receptor substrate 1; MEK, MAPK/ERK kinase; PDK1, 3-phosphoinositide-dependent protein kinase 1; PGC1α, PPARγ coactivator 1α; PKC, protein kinase C; PPARγ, peroxisome proliferator-activated receptor-γ; PTEN, phosphatase and tensin homologue; S6K1, S6 kinase 1; SESN, sestrin; SGK, serum- and glucocorticoid-regulated kinase; SREBP, sterol regulatory element-binding protein; SKAR, S6K1 Aly/REF-like target (also known as POLDIP3).

Recent work from the Blenis laboratory and others has added important mechanistic understanding to our knowledge of the regulation of mRNA translation by mTORC1 and S6K1. S6K1 phosphorylates and activates eIF4B; in turn, eIF4B enhances the activity of eIF4A, an RNA helicase that unwinds the structured 5′ untranslated regions (UTRs) of many mRNAs[28]. S6K1 also aids eIF4A by promoting the phosphorylation-dependent degradation of programmed cell death 4 (PDCD4), which usually blocks the association of eIF4A with the translation pre-initiation complex[29]. S6K1 is targeted to the exon junction complex, where it enhances the translation of newly generated mRNAs through its association with SKAR[25]. Finally, eIF3 binds mTORC1 and recruits it to untranslated mRNAs so that it is optimally placed to phosphorylate S6K1 and 4E-BP1 (REF. 27).

Ribosome biogenesis is highly energy-intensive and, as such, is tightly coupled to the energetic status of the cell. The synthesis of ribosomal RNAs and ribosomal proteins is positively regulated by mTORC1 (FIG. 2a). mTORC1 upregulates the transcriptional activity of the rRNA polymerase RNA polymerase I (RNAPI) through S6K1 kinase[30]. In *S. cerevisiae*, the transcription factors Rrn3 (also known as TIF1A in mammals), Fhl1 and Sfp1 mediate the transcription of ribosomal RNAs and proteins downstream of TORC1 (REFS 30–33).

Autophagy is the controlled self-degradation of damaged, redundant, or even dangerous cellular components, ranging from individual proteins (microautophagy) to entire organelles (macroautophagy). Autophagy is key in providing substrates for energy production during periods of low extracellular nutrients. mTORC1 actively suppresses autophagy and, conversely, inhibition of mTORC1 (by small molecules or by amino acid withdrawal) strongly induces autophagy[34,35]. In *S. cerevisiae*, TOR-dependent phosphorylation of autophagy-related 13 (Atg13) disrupts the Atg1–Atg13–Atg17 complex that triggers the formation of the autophagosome[36]. The mammalian homologues of yeast Atg13 and Atg1, ATG13 and ULK1, associate with 200 kDa FAK family kinase-interacting protein (FIP200; a putative orthologue of Atg17) and the mammalian-specific component ATG101 (FIG. 2b). By phosphorylating ATG13 and ULK1, mTORC1 blocks autophagosome initiation. However, unlike the similar complex in yeast, the formation of the ULK1–ATG13–FIP200–ATG101 complex is not regulated by nutrients[37,38].

mTORC1 also controls the activity of several transcription factors that are implicated in lipid synthesis and mitochondrial metabolism (see below).

Substrates and actions of mTORC2. *S. cerevisiae* TORC2 was identified as a mediator of actin cytoskeletal organization and cell polarization[6,16,19]. It controls a number of cytoskeletal regulators, including Rho1 GDP–GTP exchange protein 2 (Rom2) (a guanine nucleotide exchange factor (GEF) for Rho1 and Rho2) and the AGC kinase Ypk2 (REFS 39,40). The role of TORC2 in controlling cytoskeletal polarity has been confirmed in *D. discoideum* and mammalian cells.

Recent findings have revealed novel roles for mTORC2 in the phosphorylation of AGC kinase family members. mTORC2 phosphorylates and activates Akt, serum- and glucocorticoid-regulated kinase (SGK), and protein kinase C (PKC), which regulate cell survival, cell cycle progression and anabolism[41–44]. Among AGC kinases, Akt is especially important because of its role in the pathogenesis of cancer and diabetes. Using RNA interference (RNAi) in *D. melanogaster* S2 cells, TORC2 was found to mediate the phosphorylation of Akt at Ser505, which is located in the hydrophobic motif and is homologous to Ser473 in mammals[41,43,44]. This is a key finding because phosphorylation at Ser473 primes Akt for further phosphorylation at Thr308, in the catalytic domain, by 3-phosphoinositide-dependent protein kinase 1 (PDK1). Together, these two phosphorylation events cause full activation of Akt. Loss of RICTOR in worm, fly, mouse and human cells results in complete loss of Akt phosphorylation at Ser473 but, interestingly, this affects only some Akt substrates[15,44–46]. Specifically, Akt-mediated phosphorylation of the forkhead box protein O1 (FOXO1) and FOXO3 transcription factors was suppressed, but that of tuberous sclerosis 2 (TSC2; also known as tuberin), a substrate of Akt that acts upstream of mTORC1, was not[45]. Phosphorylation of FOXO1 and FOXO3 by Akt effectively prevents them from translocating to the nucleus and activating gene expression programmes that promote apoptosis; thus, mTORC2 may favour cell survival through Akt-mediated inhibition of FOXO1 and FOXO3.

Collectively, these findings place mTORC2 upstream of key cellular processes such as cell-cycle progression, anabolism and cell survival.

Upstream regulators of mTOR complexes

mTORC1 acts as a signal integrator for four major regulatory inputs: nutrients, growth factors, energy and stress (FIG. 2c–f). These inputs can cooperate with or antagonize each other, enabling the cell to fine-tune mTORC1 activity. The regulation of mTORC2 is only beginning to be discovered, but the available evidence seems to suggest that, in contrast to mTORC1 control, only growth factors directly regulate this complex.

Nutrients regulate mTORC1. Amino acids are the building blocks of proteins and are also used in the synthesis of DNA, glucose and ATP. Early studies showed that amino acids are absolutely required for mTORC1 signalling in cultured cells, and cannot be compensated for by other activating stimuli[10,47,48]. In vertebrates, amino acids may be sensed intracellularly rather than at the plasma membrane[49]; accordingly, a system of amino acid transporters plays an important part in mTORC1 signalling. This system imports Leu, which is a key amino acid for mTORC1 activation, into the cell[50]. Although the identity and mode of action of the primary amino acid sensor is unknown, candidate mediators acting downstream of amino acids have emerged, including the sterile 20 (STE20) family kinase mitogen-activated protein kinase kinase kinase kinase 3 (MAP4K3)[51,52] and PI3K catalytic subunit type 3 (also known as VPS34)[53,54].

Autophagosome
A transient membrane vesicle that engulfs and digests cellular components.

Guanine nucleotide exchange factor
(GEF). A protein that promotes the loading of GTP onto G proteins, resulting in their activation.

Anabolism
A set of chemical reactions that build complex molecules from simpler units, consuming energy in the process.

Box 1 | **mTOR and membranes**

Increasing evidence suggests that membrane trafficking might regulate mammalian target of rapamycin (mTOR) signalling. Amino acid signalling through the Rag GTPases causes mTOR complex 1 (mTORC1) to shuttle to lysosomes, enabling it to respond to growth factors (FIG. 2). The lysosomal surface hosts a molecular machinery for mTORC1 activation that includes the Rag GTPases, the trimeric Ragulator complex (which consists of p18, p14 and MAPK scaffold protein 1 (MP1)), and possibly GTPase activating proteins (GAPs) and guanine nucleotide exchange factors (GEFs) for the Rag GTPases[10,56]. Vacuolar protein sorting-associated protein 39 (Vps39; also known as Vam6), a GEF for the small GTPase Rab7, has been implicated in regulating the nucleotide loading of the GTP-binding protein Gtr1, the RAGA and RAGB orthologue in yeast[59]. Moreover, blocking early–late endosomal conversion by overexpressing Rab and Arf family GTPases strongly inhibited mTORC1 activation by amino acids[173,174].

The presence of mTORC1 at the lysosome also suggests a physical basis for the regulation of autophagy: because autophagic membranes fuse with lysosomes to promote the degradation of their contents, mTORC1 may be optimally placed to phosphorylate and inhibit key autophagy-promoting proteins. Of note, the yeast GTPases Gtr1 and Gtr2 are part of the EGO (exit from rapamycin-induced growth arrest) complex, which is involved in the regulation of microautophagy[60]. It will be interesting to assess whether mammalian Rag GTPases have a similar role; in fact, recent evidence suggests that mTORC1 might regulate the reformation of primary lysosomes following autophagy[92]. In addition, nutrient storage and release by the lysosome may allow the rapid activation of nutrient signals upstream of mTORC1.

In contrast to mTORC1, the subcellular localization of mTORC2 is unclear. However, recent studies suggest that yeast TORC2 localizes to discrete, spot-like domains on the plasma membrane[175]. Notably, membrane localization of *S. cerevisiae* TORC2 relies on the protein adheres voraciously to TOR2 1 (Avo1), the orthologue of mammalian stress-activated map kinase-interacting protein 1 (mSIN1; also known as MAPKAP1), which has a phospholipid-binding pleckstrin homology domain that may mediate the association of mTORC2 with membranes[175]. The localization of mTORC2 on peripheral membranes would be consistent with its role in phosphorylating Akt downstream of growth factor receptors and phosphoinositide 3-kinase (PI3K).

The strongest link between amino acids and mTORC1 is, arguably, the Rag family of small GTPases[10,55] (FIG. 2c). Rag GTPases are heterodimers of either RAGA or RAGB with either RAGC or RAGD; the two members of the heterodimer have opposite nucleotide loading states. In the absence of amino acids, the Rag GTPases are found in an inactive conformation, in which RAGA or RAGB is loaded with GDP and RAGC or RAGD contains GTP. Crucially, amino acids cause Rag GTPases to switch to the active conformation, in which RAGA or RAGB is loaded with GTP and RAGC or RAGD is loaded with GDP. The active Rag heterodimer physically interacts with RAPTOR, causing mTORC1 to cluster onto the surface of late endosomes and lysosomes, where the Rag GTPases reside[10,55,56]. This relocalization may enable mTORC1 to interact with the small GTPase Ras homologue enriched in brain (RHEB) (in its GTP-bound state), an essential activator of mTORC1 that is controlled by growth factor inputs[10,57,58]. This model has been largely confirmed in *S. cerevisiae*, in which the Rag GTPase orthologues GTP-binding proteins Gtr1 and Gtr2 interact physically and genetically with TORC1 (REFS 59–61). However, a RHEB homologue in *S. cerevisiae* is not essential for life and does not appear to function upstream of TORC1 (REF. 62). Thus, some aspects of *S. cerevisiae* TORC1 regulation may differ from metazoans, but this awaits clarification.

GTPase activating protein (GAP). A protein that promotes hydrolysis of GTP to GDP by G proteins, resulting in their inactivation.

Rag proteins do not show any obvious membrane targeting signals. However, three small proteins — p14, MAPK scaffold protein 1 (MP1) and p18, collectively known as the Ragulator — scaffold the Rag GTPases to the lysosomal surface. When the Ragulator is genetically inactivated, the Rag GTPases become cytoplasmic, recruitment of mTORC1 to the lysosome fails, and amino acid signalling to mTORC1 is blocked[56].

Collectively, these findings add to growing evidence that endomembranes have a key role in controlling the activity of mTOR (BOX 1); moreover, the insights suggest that the subcellular targeting of mTORC1 underlies its ability to integrate nutrient and growth factor signals (BOX 2).

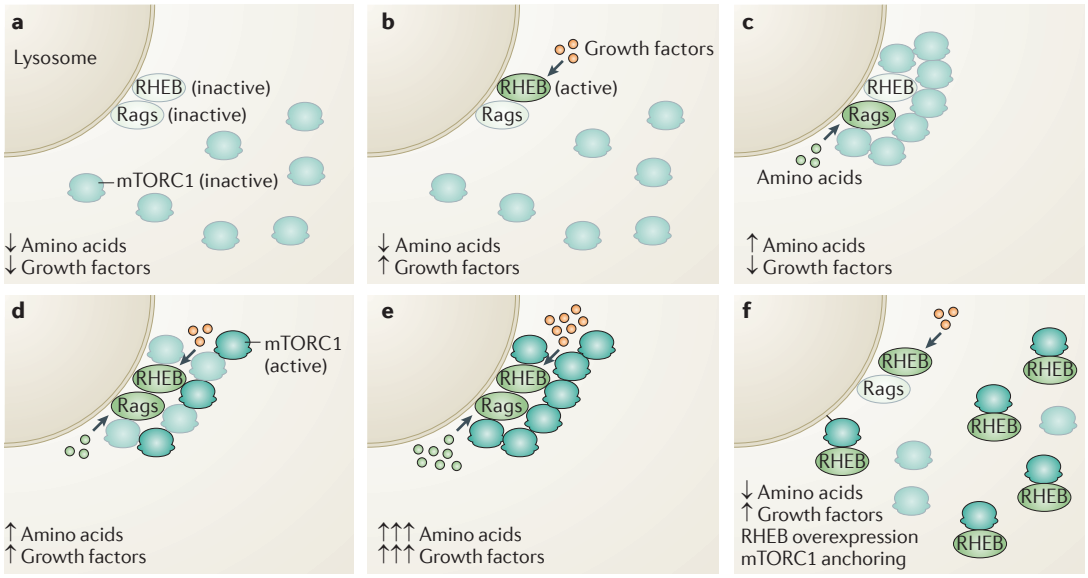
Growth factors regulate mTORC1. Multicellular organisms rely on long-range communication to coordinate the distribution of nutrients and the growth of cell populations throughout the body. With the emergence of metazoans, the mTOR pathway became wired to signalling pathways initiated by growth factors, such as insulin, which relay information on the nutritional state of the organism as a whole. One key end point of growth factor signalling to mTORC1 is the small GTPase RHEB that, when loaded with GTP, stimulates the kinase activity of mTORC1 (REFS 57,58). In metazoans, RHEB has an essential role, as its loss abolishes the activation of mTORC1 by both growth factors and nutrients. Conversely, overexpression of RHEB can maintain mTORC1 activity even when nutrients and growth factors have been withdrawn (BOX 2).

How do growth factors regulate the GTP-loading of RHEB? Binding of insulin (or insulin-like growth factor (IGF)) to its receptor activates the PI3K pathway, which leads to the phosphorylation and activation of Akt. In turn, Akt phosphorylates TSC2, a large protein that, together with TSC1 (also known as hamartin), forms the TSC1–TSC2 complex[63–65] (FIG. 2d). TSC1–TSC2 acts as a GTPase activating protein (GAP) for RHEB[66–69]; because GDP-loaded RHEB is unable to activate mTORC1, TSC1–TSC2 effectively shuts off mTORC1 signalling. Akt-mediated TSC2 phosphorylation[63–65] is likely to inhibit its GAP activity for RHEB, thus promoting mTORC1 activation. Akt also phosphorylates PRAS40 (REF. 70), which causes it to bind to 14-3-3 proteins and prevents it from inhibiting mTORC1 (REFS 11,13).

Growth factors can signal to mTORC1 through alternative pathways to the PI3K–Akt axis. For example, extracellular signal-regulated kinase (ERK) phosphorylates TSC2, downstream of the Ras–Raf–MAPK/ERK kinase (MEK)–ERK axis, to inhibit it[71]. Moreover, the Wnt pathway has been implicated in mTORC1 signalling[72,73]. Glycogen synthase kinase 3β (GSK3β) acts as a negative regulator of mTORC1 by phosphorylating TSC2; by inhibiting GSK3β, Wnt activates mTORC1.

The convergence of multiple growth factor-initiated pathways on mTORC1 is likely to allow it to participate in many developmental and physiological processes. This is supported by the absolute requirement for mTORC1 in early embryonic development[45,74,75].

Box 2 | **mTORC1 as a signal integrator**



How are mammalian target of rapamycin complex 1 (mTORC1) inputs integrated to generate a coherent signalling response? Signal integration by mTORC1 is likely to be based on the convergent regulation of its physical interactions and localization. Growth factors induce the GTP loading of Ras homologue enriched in brain (RHEB), enabling it to physically interact with mTORC1, whereas amino acids cause the Rag GTPase-mediated shuttling of mTORC1 to the endomembrane system, where RHEB resides. This explains why both inputs are required for mTORC1 activation. In the absence of growth factors and amino acids, the Rag and RHEB GTPases are inactive and mTORC1 is physically removed from RHEB (see the figure, part **a**). Growth factors activate RHEB; however, amino acids are required to bring mTORC1 into contact with RHEB and, if they are not present, mTORC1 remains inactive (part **b**). Amino acids activate the Rag GTPases (Rags), which recruit mTORC1 and allow it to bind to RHEB. However, in the absence of growth factors, RHEB is inactive and so is mTORC1 (part **c**). When both amino acids and growth factors are present, mTORC1 is recruited to active RHEB and activated (part **d**).

Importantly, nutrients and energy should not be regarded as an on–off switch: their concentrations vary smoothly in time as a function of the feeding cycle. Thus, mTORC1 probably senses fine variations in these parameters, continuously adjusting the rate of biosynthetic processes accordingly. Once mTORC1 is active, increasing concentrations of amino acids and growth factors can activate it further (part **e**). Experimental overexpression of RHEB or forced localization of mTORC1 to the lysosomal surface (by fusing a lysosomal localization signal to RAPTOR) bypass the requirement for Rag-mediated recruitment of mTORC1 and allow its activation in the absence of amino acids (part **f**), supporting the signal integration model. Other regulatory inputs, such as energy-sensing AMP-activated protein kinase (AMPK) and feedback mechanisms can modulate or entirely suppress this signal integration mechanism, ensuring that nutrients, growth factors and energy do not generate conflicting signals. Thus, the regulation of mTORC1 is a multi-step decision process that takes into account multiple indicators of the energy status of the cell before making a commitment to grow and proliferate.

Energy and stress regulate mTORC1. Chemical inhibitors of glycolysis and mitochondrial function suppress mTORC1 activity, indicating that mTORC1 senses cellular energy[5,76]. This is crucial, because mTORC1-driven growth processes consume a large fraction of cellular energy, and thus could be deleterious to starving cells.

Glycolysis and mitochondrial respiration convert nutrients into energy, which is stored in the form of ATP. Upon nutrient deprivation, cellular ATP levels quickly drop. The mTORC1 pathway indirectly senses low ATP by a mechanism that is centred on the AMP-activated protein kinase (AMPK) (reviewed in REF. 77) (FIG. 2e). Both AMP and ATP are allosteric regulators of AMPK: when the AMP:ATP ratio increases, AMPK phosphorylates TSC2 (REFS 73,78), possibly stimulating the GAP activity of TSC1–TSC2 towards RHEB to inhibit mTORC1 signalling. Moreover, AMPK phosphorylates RAPTOR, causing it to bind 14-3-3 proteins, which leads to the inhibition of mTORC1 through allosteric mechanisms[79].

Numerous stressors affect ATP levels and thus may regulate mTOR through the AMP–AMPK axis. For example, during hypoxia, mitochondrial respiration is impaired, leading to low ATP levels and activation of AMPK. Hypoxia also affects mTORC1 in AMPK-independent ways by inducing the expression of regulated in development and DNA damage response 1 (*REDD1*; also known as *DDIT4*), the protein products of which then suppress mTORC1 by promoting the assembly of TSC1–TSC2 (REFS 80–82) (FIG. 2f). Conversely, other stressors that do not primarily impinge on cellular energy signal through AMPK. DNA damage results in the inhibition of mTORC1 activity through the p53-dependent upregulation of AMPK[83,84] (FIG. 2g). Sestrin 1 and sestrin 2 are two transcriptional targets of p53 that are implicated in the DNA damage response, and it was recently shown

that sestrins potently activate AMPK, thus mediating the p53-dependent suppression of mTOR activity upon DNA damage[85].

Upstream regulation of mTORC2. Surprisingly little is known about the upstream activators of mTORC2. Given their role in regulating Akt, SGK and PKC, it is generally thought that growth factors control mTORC2, directly or indirectly. In fact, insulin stimulation of cultured cells promotes Ser473 phosphorylation of Akt by mTORC2 (REF. 44).

Because Akt, SGK and PKC respond to different growth factors, the range of upstream regulators of mTORC2 may be quite wide. Thus, how is signalling specificity achieved? One potential solution may come from the existence of multiple isoforms of mSIN1. Three out of the five known splice variants of mSIN1 can be part of mTORC2, effectively defining three distinct complexes; of these, only two are regulated by insulin[14]. Thus, mSIN1 may function as an adaptor between mTORC2 and specific growth factor receptors. The mSIN1 orthologue in *D. discoideum*, Ras-interacting protein 3 (Rip3), mediates the enhanced migratory behaviour of this organism, which is driven by activated Ras; thus, Ras may provide a link between growth factors and mTORC2 (REFS 86,87).

mTOR in the regulation of metabolism

Owing to the intermittency of food intake and the necessity to keep nutrient levels in the bloodstream within a narrow physiological range, multicellular organisms have acquired mechanisms to store energy after feeding and to mobilize this energy during periods of shortage. These mechanisms largely impinge on mTOR: when nutrients are available, mTOR is activated and drives anabolism as well as energy storage and consumption. Conversely, during fasting, mTOR must be suppressed to avoid the insurgence of conflicting metabolic signals. Finally, chronic overfeeding can lead to an excess of mTOR activation and metabolic derangements (FIG. 3).

mTOR in fasting and starvation. Having evolved in conditions of limited nutrient availability, mammals (including humans) have developed a striking ability to maximize the available resources of energy in anticipation of periods of shortage. Early during fasting, glucose and amino acid blood levels decrease, causing a drop in circulating insulin. In addition, the imbalance between energy expenditure and food intake leads to an increased cellular AMP:ATP ratio. These factors converge as inhibitory inputs on mTORC1, placing a brake on energy-intensive biosynthetic processes and upregulating macroautophagy[88]. Mitophagy (the autophagic degradation of mitochondria) provides an immediate source of energy at the expense of nutrient-intensive, long-term ATP production (reviewed in REF. 89). The pro-survival role of autophagy was elegantly demonstrated *in vivo* in ATG5-null mice: immediately after birth, interruption of the placental nutrient supply causes an energetic shortage that is compensated for by upregulation of autophagy in several organs. ATG5-deficient mice are unable to overcome this energetic challenge and die within one day after birth[90].

In the liver, induction of autophagy causes the simultaneous recycling of mitochondria, cytoplasmic proteins and stored glycogen (FIG. 3b). This effect is so dramatic that the murine liver shrinks to around one-third of its normal size in a 24-hour fasting period. Furthermore, during starvation, white adipose tissue (WAT) and liver cells mobilize lipid stores, converting them into free fatty acids that are utilized by the liver and muscle through β-oxidation (FIG. 3b). Recent evidence indicates that, during fasting, autophagosomes sequester lipid droplets and break them down into free fatty acids[91]. Autophagy also mediates massive protein breakdown in the muscle[88], releasing amino acids into the bloodstream to be converted to glucose in the liver. Thus, the flow of amino acids, glucose and other metabolites that are released by autophagy feeds back onto mTORC1, causing its partial reactivation[92] (FIG.3b).

Changes in mitochondrial function that stem from mTORC1 inhibition may also contribute to fasting responses. Activation of 4E-BP1 under limiting nutrients in *D. melanogaster* led to the selective translation of mRNAs encoding the mitochondrial respiratory chain[93], which is consistent with an attempt to increase the efficiency of ATP production. Moreover, mTORC1 has been reported to promote mitochondria biogenesis and enhance respiration by forming a ternary complex with the transcription factors PPARγ coactivator 1α (PGC1α) and YY1 (REF. 94). In agreement with this, deletion of RAPTOR in skeletal muscle leads to a defect in mitochondrial biogenesis and decreased oxidative capacity[95]. Thus, inhibition of mTORC1 during starvation acts on mitochondrial function at three different levels: by placing a brake on the synthesis of new mitochondria, by eliminating a subset of the existing mitochondria by mitophagy and by increasing the efficiency of existing mitochondria through the 4E-BP1 translational programme.

mTOR, overfeeding, and insulin sensitivity. Growth control programmes have evolved under conditions of scarce nutrients that were prevalent during mammalian evolution, but these conditions are no longer prevalent in most of the Western world. Overfeeding may be pathogenic because selection has favoured organismal responses, which are partly mediated by mTOR, that accumulate and store energy in anticipation of periods of shortage. This translates into aberrant cellular responses when food and energy are plentiful and constantly available.

One of the most efficient forms of energy storage in the body occurs in the WAT. There, lipids are stored in the form of triglycerides, which provide a higher energetic yield per unit of mass than carbohydrates. mTORC1 has a key role in lipid store formation; it not only mediates the synthesis of triglycerides but also drives the differentiation of preadipocytes into WAT (FIG. 3a). Adipocyte-specific deletion of RAPTOR in mice leads to reduced WAT tissue and enhanced fatty-acid oxidation[96]. Moreover, mTORC1 indirectly upregulates peroxisome proliferator-activated receptor-γ (PPARγ), which is a factor that is necessary and sufficient for the differentiation of preadipocytes and lipid accumulation through its ability to upregulate adipocite-specific gene

β-oxidation
The breakdown of fatty acids that occurs in the mitochondria and generates acetyl CoA, which is the entry substrate for the tricarboxylic acid cycle.

Triglyceride
A lipid that is formed by the esterification of fatty acids with glycerol. Triglycerides are the most abundant form of lipid storage.

programmes[97–100]. mTORC1 may affect PPARγ activity by increasing its translation[101] and by activating the transcription factor sterol regulatory element-binding protein 1c (SREBP1c) that, in turn, mediates the production of a PPARγ-activating ligand (the identity of which is currently unknown)[102,103]. Consistently, TSC2-deficient mouse embryonic fibroblasts (MEFs) show enhanced adipogenesis and PPARγ levels[104].

Recent evidence also implicates mTORC2 in lipid biogenesis. Akt activation by mTORC2 leads to the induction of PPARγ as a result of its ability to activate mTORC1 (REF. 105) and to directly inhibit FOXO1, which usually antagonizes PPARγ activity[106,107]. In *C. elegans*, TORC2 participates in lipid accumulation, as worms carrying inactivating mutations in RICTOR display increased body fat, which is partly due to reduced activity of both Akt and SGK[46].

In liver and muscle, the conversion of glucose to glycogen (glycogen synthesis) provides an energy source that, although not as efficient as triglycerides, can be rapidly mobilized for a sudden surge in demand. mTORC2 participates in the accumulation of glycogen by activating Akt which, by inhibiting GSK3β, drives the activation of the enzyme glycogen synthase[108] (FIG. 3a).

When glucose is abundant, insulin signalling through Akt suppresses the expression of phosphoenolpyruvate carboxykinase (PEPCK), a transcriptional target of FOXO1 that is the limiting enzyme of gluconeogenesis in the liver[109,110]. Because of its role upstream of Akt, mTORC2 may thus directly participate in the suppression of gluconeogenesis (FIG. 3a).

From a clinical perspective, chronic mTORC1 activation contributes to obesity by mediating excess fat deposition in WAT, liver and muscle; in turn, ectopic fat deposition has a role in the insurgence of insulin resistance. Furthermore, mTORC1 hyperactivation during overfeeding triggers an S6K1-dependent negative feedback loop: activated S6K1 dampens the function of insulin receptor substrate 1 (IRS1), which is an adaptor protein that recruits key downstream effectors to the insulin receptor. Thus, S6K1-mediated phosphorylation of IRS1 leads to insulin desensitization[111]. This results in dampened Akt activation, which translates into reduced glucose uptake and glycogen synthesis in liver and muscle, and increased gluconeogenesis and glucose release by the liver. Collectively, these effects lead to a worsening of the hyperglycaemia and hyperinsulinaemia that are generated by excess nutrients (FIG. 3c). Supporting the role of the S6K1–IRS1 feedback loop in the pathogenesis of type 2 diabetes, S6K1-deficient mice displayed enhanced insulin sensitivity when chronically maintained on a high fat diet[111].

Of note, mTORC1 hyperactivation in the context of insulin resistance poses a paradox: how can mTORC1 be constitutively active in an insulin-resistance state if insulin is responsible for its activation? This apparent paradox can be explained if excess nutrients keep mTORC1 hyperactivated. Chronically high blood levels of amino acids, as seen in obesity[112], will keep mTORC1 at work, driving the S6K1–IRS1 loop and, consequently, insulin resistance (FIG. 3c).

Gluconeogenesis
The chain of enzymatic reactions, mainly occurring in the liver, which leads to the *de novo* production of glucose from more simple carbon precursors and ATP.

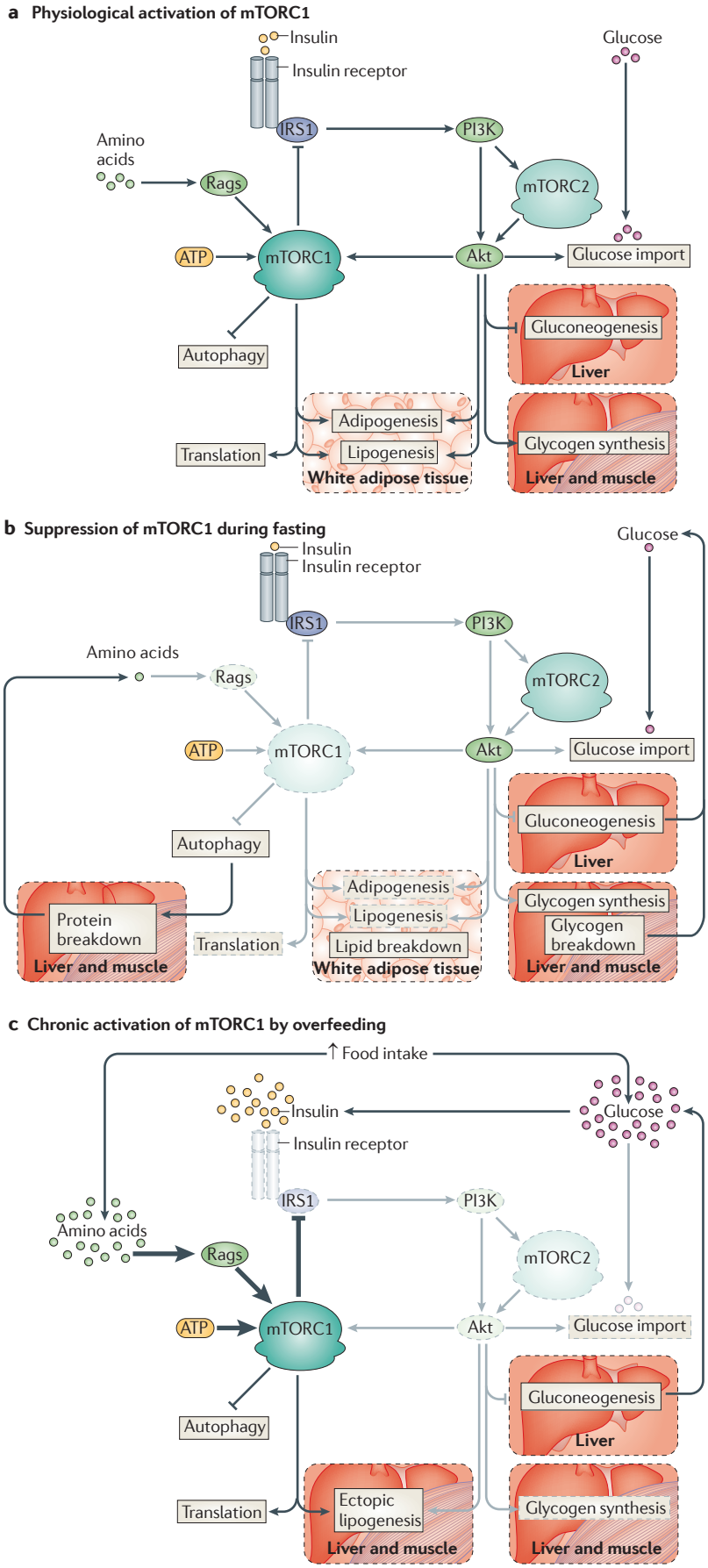
Figure 3 | **mTOR in metabolism. a** | Mammalian target of rapamycin (mTOR) links nutrient abundance with growth and the accumulation of energy stores in anticipation of future nutrient shortage. Feeding raises nutrient (amino acids and glucose) and insulin levels in the bloodstream. These converge to activate mTOR complex 1 (mTORC1) and mTORC2. mTORC1 activates translation, cell mass increase (especially in skeletal muscle) and lipogenesis (which leads to lipid accumulation) in white adipose tissue, while inhibiting autophagy. mTORC2 promotes glucose import in most tissues and promotes glycogen synthesis, but inhibits gluconeogenesis, in the liver. **b** | During fasting, blood levels of glucose, amino acids and insulin drop. This leads to decreased activation of mTORC1 by the amino acid pathway (through the Rag GTPases (Rags)) and by the insulin pathway (through Akt). Decreased mTORC1 activity leads to suppression of translation, glycogen synthesis in the liver and lipid synthesis and adipogenesis in white adipose tissue. Conversely, autophagy, gluconeogenesis and breakdown of lipids, proteins and glycogen are stimulated; collectively, these processes release metabolites into the bloodstream and stabilize cellular ATP levels. Decreased mTORC1 activity also results in the suppression of the S6 kinase 1 (S6K1)–insulin receptor substrate 1 (IRS1) feedback loop, which may help to boost insulin sensitivity. **c** | An overabundance of nutrients leads to chronic mTORC1 activation, which disrupts energetic homeostasis in different tissues. During chronic hyperinsulinaemia, as occurs in overfeeding states, mTORC1 activity towards S6K1 inhibits insulin receptor signalling at the cellular membrane, in particular in liver and muscle, contributing to the onset of the diabetic state. In an insulin-resistance state, phosphoinositide 3-kinase (PI3K) and Akt are not activated, leading to decreased cellular glucose uptake and to increased hepatic gluconeogenesis, which worsens the hyperglycaemic condition. Despite decreased insulin signalling and an absence of Akt activation, mTORC1 remains active, maintaining the negative feedback loop at work. The hyperactive amino acid input to mTORC1 may explain sustained mTORC1 activity in the context of insulin resistance and may drive the ectopic accumulation of lipids that occurs in muscle and liver. Inactivated pathways are faded out in the figure.

Strikingly, the same molecular circuitry that controls metabolism in peripheral tissues also influences food intake in the central nervous system. When locally applied to the hypothalamus, Leu induced satiety through activation of mTORC1; conversely, inhibition of mTORC1 in the hypothalamus by rapamycin injection increased food intake[113].

Thus, mTORC1 coordinates food intake with energy storage at multiple levels, from the central control of food-seeking to energy storage and expenditure in peripheral tissues. This multi-level regulation explains the profound consequences that deregulated mTOR signalling exerts on human metabolism.

mTOR in cancer aetiology and therapy

The most direct evidence that mTOR can drive tumorigenesis comes from familial cancer syndromes arising from mutations of negative mTOR regulators such as TSC1–TSC2, LKB1 (also known as STK11) and phosphatase and tensin homologue (PTEN)



(see Supplementary information S1 (figure)). Moreover, epidemiological data indicates that sporadic mutation or deregulation of PI3K, Akt and PTEN are, together with p53, among the most prevalent alterations in human cancer[114].

4E-BP1 is emerging as a key regulator of cell proliferation downstream of mTORC1, and its inactivation may directly contribute to the growth of sporadic cancers. MEFs lacking 4E-BP1 and 4E-BP2 displayed increased proliferation but not increased size, indicating that inhibition of 4E-BPs by mTORC1 drives cell cycle progression but not cell growth[115]. Mechanistically, on mTORC1-mediated inhibition of 4E-BP1, activated eIF4E preferentially drives the translation of mRNAs for pro-tumorigenic genes, including cell cycle regulators (FIG. 4A). Indeed, eIF4E promoted cell survival in *in vivo* mouse models of lymphoma by upregulating the translation of the anti-apoptotic protein myeloid leukaemia cell differentiation 1 (MCL1)[116–118]. Loss of 4E-BP1 and 4E-BP2 increased tumorigenesis caused by inactivation of p53 in mice[119]; conversely, expression of a non-phosphorylatable, constitutively active 4E-BP1 suppressed the growth of tumours driven by concomitant PI3K and KRAS mutations[120].

mTORC1 indirectly upregulates the enzyme fatty acid synthase (FAS), a transcriptional target of SREBP1 involved in lipid biogenesis that favours the rapid proliferation of cancer cells (reviewed in REF. 121) (FIG. 4A).

Increasing evidence suggests that autophagy has a role in tumour suppression. The most direct data supporting the anti-cancer roles of autophagy come from mice that are heterozygous for the autophagic protein beclin[122,123] and from ATG4C-deficient mice[124], both of which are tumour-prone. Thus, constitutive mTORC1 activation may indirectly favour tumorigenesis by suppressing autophagy (FIG. 4A). In addition, mTORC1 potently promotes angiogenesis by regulating hypoxia-inducible factor 1α (HIF1α); thus, mTORC1 signalling favours the growth of tumours by supplying them with nutrients and oxygen[125].

By activating Akt[44] and SGK[42], mTORC2 may directly drive tumorigenesis. Akt promotes proliferation, survival and nutrient uptake in cancer cells (reviewed in REF. 126). Tumours that are driven by inactivation of the tumour suppressor PTEN (an inhibitor of Akt signalling) or by oncogenic mutations in PI3K (which promotes Akt signalling) may be especially dependent on the pro-survival activities of Akt: thus, targeting mTORC2 in this context may prove especially useful. In fact, RICTOR is required for the growth of tumour cell lines and prostate tumours in PTEN-deficient mice[127–130].

Rapamycin as an mTOR-centred cancer therapy. The existence of rapamycin, a potent, naturally occurring inhibitor of mTOR, appeared to be a lucky strike for cancer therapies. However, to date, the limited success of rapamycin as an anti-cancer drug in clinical trials has generated disappointment. Here, we discuss the limitations of rapamycin and the current efforts to move beyond this drug towards an effective mTOR-centred cancer therapy.

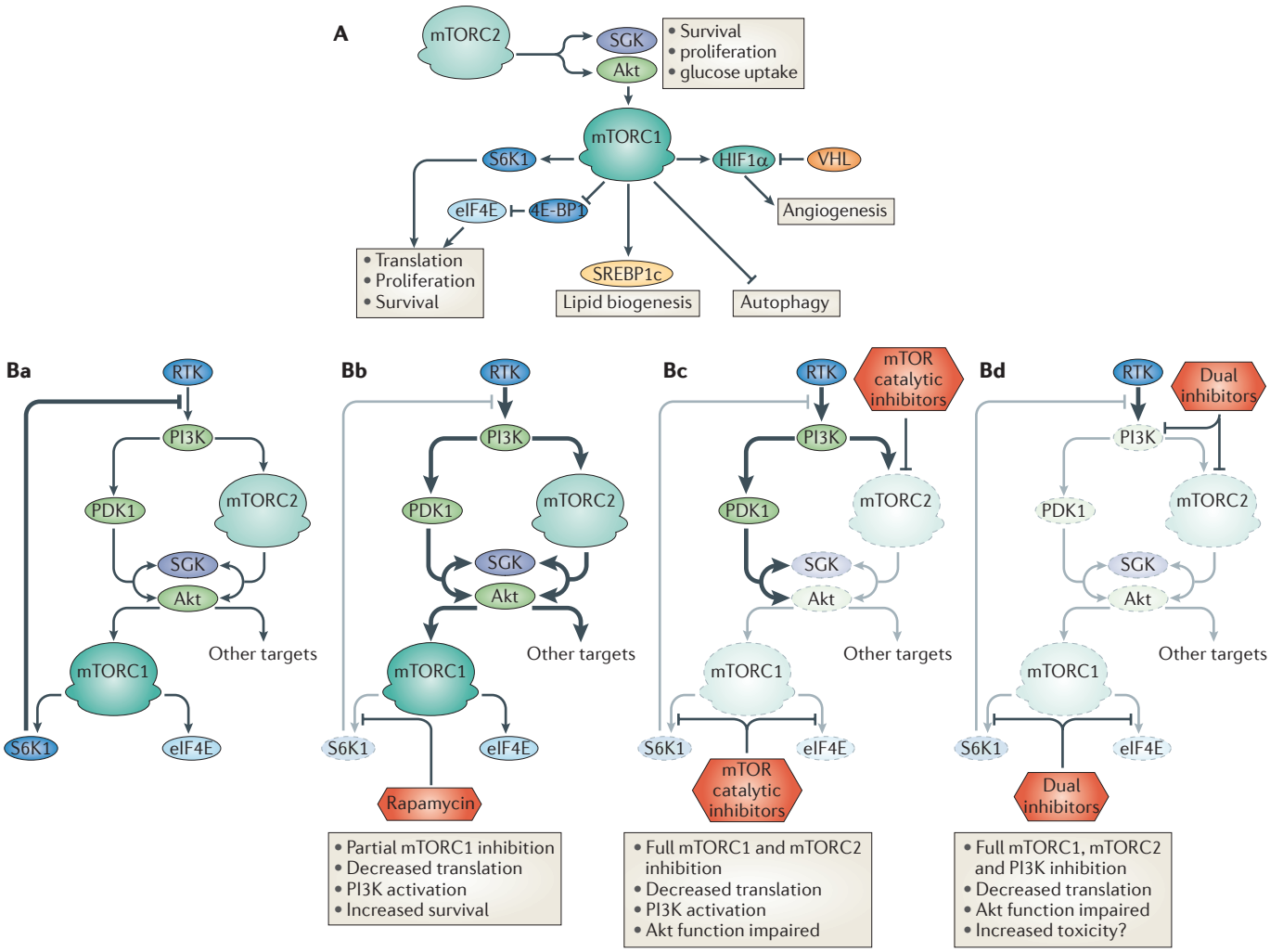


Figure 4 | **mTOR in cancer. A** | Mammalian target of rapamycin (mTOR)-regulated cellular processes that have a role in cancer. mTOR complex 1 (mTORC1) favours tumorigenesis by driving the translation of oncogenes (by relieving the eIF4E-binding protein 1 (4E-BP1)-mediated inhibition of eukaryotic translation initiation factor 4E (eIF4E)); by inhibiting autophagy; by upregulating hypoxia-inducible factor 1α (HIF1α) to increase angiogenesis; and by enhancing the accumulation of lipids by activating the transcription factor sterol regulatory element binding protein 1c (SREBP1c). mTORC2 plays a part in tumorigenesis by activating Akt and other AGC family proteins, such as serum- and glucocorticoid-regulated kinase (SGK), which promote proliferation and survival. Moreover, by promoting Akt-mediated glucose uptake, mTORC2 fuels the metabolism of cancer cells. **B** | Therapeutic inhibition of mTOR activity by rapamycin, mTOR catalytic inhibitors and dual phosphoinositide 3-kinase (PI3K)–mTOR inhibitors (dual inhibitors). The PI3K–mTOR loop is shown in the absence of inhibitors (**Ba**). Rapamycin only partially suppresses mTORC1 function, efficiently inhibiting S6 kinase 1 (S6K1) but not eIF4E (**Bb**); thus, it only partially blocks translation. Moreover, owing to the inhibition of the S6K1-dependent feedback loops, rapamycin indirectly upregulates PI3K activity to promote cell survival. By contrast, ATP-competitive mTOR catalytic inhibitors target all known functions of mTORC1 as well as mTORC2 (**Bc**); thus, they inhibit translation more potently. Although PI3K overactivation still occurs, Akt phosphorylation by mTORC2 is impaired. Dual PI3K–mTOR inhibitors block all functions of PI3K, including 3-phosphoinositide-dependent protein kinase 1 (PDK1)- and mTORC2-mediated activation of Akt (**Bd**). However, they might cause increased toxicity. RTK, receptor tyrosine kinase; VHL, Von Hippel-Lindau disease tumour suppressor. Inactivated pathways are faded out in the figure. Upregulated pathways are indicated by bold arrows.

Rapamycin-based therapeutic approaches may have encountered a stumbling block in the S6K1-mediated feedback loop (FIG. 4Ba), the inhibition of which leads to a severe upregulation of PI3K signalling and provides important pro-survival and proliferative signals through Akt and other AGC kinases[131]. Additionally, S6K1 inhibition activates the MEK–ERK signalling cascade[132], as well as transcription of platelet-derived growth factor receptor (PDGFR)[133]. These trigger feedback loops to counteract the action of rapamycin, dampening its effectiveness in cancer models and in patients[131,132] (reviewed in REF. 134) (FIG. 4Bb), and may explain why rapamycin is cytostatic but not cytotoxic in many tumours.

Although high doses of rapamycin or its prolonged delivery can block mTORC2 in some cell lines[20,135], rapamycin is largely selective for mTORC1. Given the role

of mTORC2 and especially its downstream target Akt as drivers of tumorigenesis[127], this poses a major concern. Furthermore, rapamycin does not fully inhibit all the functions of mTORC1; of note, rapamycin only affects 4E-BP1 phosphorylation transiently and partially[35,135–138]. Thus, by suppressing the S6K1–IRS1 feedback loop and hyperactivating the PI3K–Akt pathway, rapamycin may ultimately stimulate 4E-BP1 phosphorylation (and thus eIF4E activity and translation) and perhaps other tumour-promoting functions of mTORC1. These two major drawbacks have motivated the search for second-generation inhibitors of mTOR function.

mTOR and mTOR–PI3K catalytic inhibitors. Recently, independent groups generated a series of synthetic small molecules that function as ATP-competitive inhibitors and block all known mTORC1 and mTORC2 actions[35,138–141]. For example, Torin1 inhibits 4E-BP1 phosphorylation and triggers autophagy to a far greater extent than rapamycin[35]. Moreover, unlike rapamycin, Torin1 and the other catalytic inhibitors also completely block mTORC2-mediated phosphorylation of Akt[35,138–141]. Supporting the applicability of this drug in a clinical setting, pre-clinical data in genetically engineered mice argue that even full inhibition of mTORC1 and mTORC2 could be well tolerated in adult tissues[127,142]. Moreover, the mTOR-catalytic inhibitor PP242 showed a better therapeutic response than rapamycin in a mouse model of experimental leukaemia, together with a surprising milder effect on normal lymphocytes[143].

However, mTOR catalytic inhibitors are not immune to potential drawbacks. Loss of the S6K1-mediated feedback loop resulting from mTORC1 inhibition enhances PDK1-mediated phosphorylation of Akt at Thr308 (FIG. 4Bc). Consequently, when suboptimal doses of mTOR catalytic inhibitors were used, the residual mTORC2 activity towards Ser473 potently activated Akt[17]. Furthermore, although acute inhibition of mTORC2 by one such inhibitor, Ku-0063794, effectively suppressed Thr308 phosphorylation in wild-type cells, it failed to do so in cells in which mTORC2 was genetically inactivated[140]. This result suggests that under chronic inhibition of mTORC2, which may occur in a clinical setting, alternative pathways may ensure Thr308 Akt phosphorylation even in the absence of the priming Ser473 phosphorylation. In addition, as we have described earlier, suppressing mTORC2-dependent phosphorylation of Akt may specifically affect some substrates, such as FOXO1 and FOXO3, but not others, such as TSC1–TSC2 (REF. 45).

Of note, an alternative therapeutic approach could be the generation of inhibitors that are specific for mTORC2. Such molecules would block the pro-survival functions of mTORC2 without inhibiting mTORC1, thus ensuring that the S6K1–IRS1 feedback loop is not perturbed and that PI3K is not hyperactivated.

The similarity between the catalytic domains of mTOR and class I PI3K has also enabled the design of ATP-competitive drugs that simultaneously block the activity of both kinases (FIG. 4Bd). When two inhibitors of this kind, PI-103 and NVP-BEZ235, were delivered to tumour cells that were driven by PI3K, they strongly suppressed both S6K1 and Akt activation[144,145]. More importantly, this class of compounds suppressed the proliferation of cancer cells more efficiently than rapamycin or the PI3K inhibitor LY294002, and to a similar degree as a combination of the two.

Dual PI3K–mTOR inhibitors may be conceptually superior to catalytic mTOR inhibitors because they disable both inputs to Akt, namely PI3K–PDK1 and mTORC2. However, this broad inhibition may be toxic to normal cells. In fact, although the ATP-competitive mTOR inhibitor PP242 and the dual PI3K–mTOR inhibitor PI-103 showed antileukaemic effects *in vivo,* PI-103 also harmed normal lymphocytes, suggesting that the therapeutic range of dual inhibitors might be narrow[143].

It remains unclear to what extent mTOR inhibitors and mTOR–PI3K dual inhibitors are effective in inducing the death of cancer cells[139,144–146], although apoptotic responses were observed in cells from gliomas and breast and haematological tumours[143,147,148]. Nevertheless, these compounds show a consistently good effect against tumours that are driven by PI3K–Akt, whereas they were ineffective against tumours that are driven by mutations of the Ras GTPase, which is able to signal through multiple pathways, such as the MEK–ERK pathway[144]. In the case of tumours that are driven by Ras mutations, a combination therapy of dual mTOR–PI3K inhibitors together with a MEK inhibitor was required to achieve antitumoural effects.

mTOR in ageing

Ageing can be defined as a time-dependent decline of the physiological functions of cells, tissues and organs. Ageing can favour the insurgence of sporadic diseases such as cancer, or can itself lead to death through organ failure. Because of this, ageing is increasingly viewed as a disease in its own right, and one for which molecular therapies can be designed.

In recent years, the manipulation of nutrient sensing and stress response pathways has extended the lifespans of organisms from yeast to mammals. The rationale behind these results is that growth-promoting programmes may accelerate ageing by generating metabolic by-products and by directly inhibiting the clearance of these by-products. Conversely, suppression of growth programmes through chemical and genetic manipulations, or by reducing food intake, results in the activation of salvage programmes that preserve the functionality of cells and tissues for extended periods of time (reviewed in REF. 149). Owing to its role at the interface of growth and starvation, mTOR is a prime target in the genetic control of ageing, and evidence from genetic studies supports the view that mTOR may be a master determinant of lifespan and ageing in yeast[150,151], worms[152,153], flies[154,155] and mice[156].

The only 'natural' method that is available to counter ageing is dietary restriction (DR), where the caloric intake is decreased by 10–50%. DR appears to act mainly through the inhibition of mTORC1, and genetic inactivation of mTORC1 pathway components in yeast and flies provides no additional benefit over DR[151,154,155]. In mice, DR causes lifespan extension and changes in gene expression profile that are similar to those resulting from loss of S6K1 (REF. 157), further supporting the view that DR acts through inhibition of mTORC1.

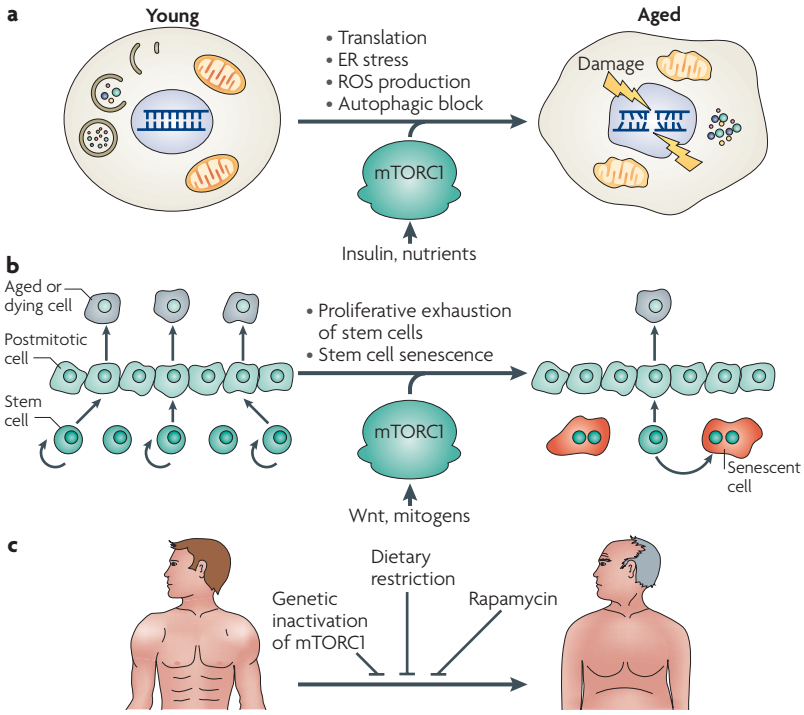


Figure 5 | **mTOR in ageing. a** | Mammalian target of rapamycin complex 1 (mTORC1)-regulated processes that may accelerate cellular ageing. mTORC1-dependent translation in ageing cells may result in the accumulation of unfolded proteins and endoplasmic reticulum (ER) stress. Stimulation of mitochondrial function by mTORC1 may increase the production of reactive oxygen species (ROS), resulting in oxidative damage to DNA, proteins and membranes. Inhibition of autophagy by mTORC1 reduces the turnover of cellular components and promotes the accumulation of their damaged forms. **b** | mTORC1 promotes stem cell exhaustion and tissue ageing. In young tissues, each stem cell divides asymmetrically to generate a new stem cell and a new postmitotic cell, which replaces one that has died (left). Continued exposure to mitogens that signal through mTORC1 causes stem cell exhaustion through hyperproliferation or senescence (right); thus, in aged tissues postmitotic cells are no longer replaced, and the overall performance of the tissue is degraded. **c** | Inhibiting mTORC1 activity by various means allows lifespan extension in multiple organisms, and may have beneficial effects on human ageing.

mTOR and cellular ageing. Cellular functions degrade over time due to the cumulative action of metabolic by-products, exogenous chemicals and ionizing radiation, as well as order-degrading stochastic processes. Inhibition of mTORC1 may counter these sources of damage and enhance repair mechanisms (FIG. 5a). Although their exact contribution is still unclear, experimental evidence supports the involvement of nearly all of the mTOR-regulated processes in ageing.

Reducing mRNA translation by genetic methods extends the lifespan of yeast, worms and flies[158–161]. Reduced translation may place smaller demands on the protein folding systems, thus decreasing the production of misfolded protein aggregates as by-products. Accordingly, prolonged rapamycin treatment in a mouse model of Huntington's disease decreased the formation of toxic huntingtin aggregates[162]. Lifespan extension can also be explained by increased autophagy following mTORC1 inhibition. Autophagy was required for lifespan extension by virtually all protocols in worms and flies[154,163,164]. Interestingly, an age-dependent drop in the expression of lysosome-associated membrane glycoprotein 2 (LAMP2A), which mediates chaperone-mediated autophagy, contributed to the ageing of hepatocytes[165]. DR and rapamycin deliver a boost to the autophagic pathway that may compensate for its age-dependent decline.

Surprisingly, inhibiting mTORC1 may lead to the increased translation of a subset of genes that exert a protective function. In a recent report, the activation of 4E-BP1 by DR in *D. melanogaster* resulted in the increased translation of several components of the mitochondrial electron transport chain[93]. This selective upregulation led to improved mitochondrial respiration. One may speculate that the resulting decrease in the production of reactive oxygen species should result in less cellular damage.

Finally, inhibition of mTORC1 may result in the activation of specific gene expression programmes that are related to the regulation of lifespan. For example, stress-response programmes that are controlled by the transcription factor Gis1 function downstream of TOR in regulating *S. cerevisiae* lifespan[166].

mTOR and tissue ageing. Tissue-specific stem cells help to maintain organ function by replacing differentiated cells that are undergoing turnover as well as those that have succumbed to damage (for a comprehensive review, see REF. 167). There is evidence that the number of stem cells, as well as their propensity to undergo novel divisions for tissue turnover and repair purposes, declines over time, leading to an irreversible degradation of organ function and, thus, ageing. For instance, the cell cycle regulators p16 (also known as INK4a) and p19 (also known as ARF) act as a brake to limit the proliferation of the stem cell pool, but their age-dependent accumulation ultimately causes stem cells to undergo senescence[168,169].

Moreover, aberrant growth signals or stress signals can accelerate stem cell senescence and tissue ageing (FIG. 5b). In a recent report[72], the persistent expression of Wnt proteins in mouse epidermis led to hyperproliferation of epithelial stem cells, ultimately causing them to undergo senescence and exhausting the stem cell niche. Importantly, these actions seemed to occur through Wnt-mediated activation of the mTOR pathway: rapamycin treatment prevented both the hyperproliferation and premature senescence of epidermal stem cells that were exposed to excess Wnt[72]. In mouse haematopoietic stem cells (HSCs), constitutive mTORC1 activation through deletion of TSC1 led to increased expression of p16, p19 and p21 (also known as CIP1), resulting in depletion of HSCs. An age-dependent increase in the activity of mTORC1 was detected and, moreover, prolonged rapamycin treatment preserved the pool of HSCs to levels that were similar to those in young animals[170].

Finally, over-activation of the PI3K pathway by deletion of PTEN also led to the hyperproliferation of HSCs followed by their depletion, probably through mTORC1. In fact, treatment with rapamycin restored the capacity of PTEN-null HSCs to reconstitute the blood lineage of irradiated mice[171].

Altogether, these findings point to mTORC1 as a key mediator of growth signals that drive the exit of tissue stem cells from quiescence. Furthermore, mTORC1

Senescence
An almost irreversible stage of permanent G0–G1 cell-cycle arrest that is linked to morphological changes, metabolic changes and changes in gene expression (for example, of the gene encoding β-galactosidase).

Quiescence
Cells in this state have exited the cell cycle and are in the G0 ('resting') phase, but can re-enter the cell cycle.

inhibition may represent a viable approach to preserve the stem cell pool and, thus, the functionality of tissues and organs over time.

Concluding remarks

The recent identification of novel regulators and their modes of action has further strengthened the idea that the basic layout of the mTOR pathway is that of a signal integrator. The TSC node computes signals from growth factors, stressors and energy to regulate RHEB. A second node is mTORC1 itself, at which RAPTOR and PRAS40 are modified by energy and growth factor inputs. Finally, the lysosomal membrane acts as a platform for the integration of nutrient inputs with the RHEB axis. Whether signal integration also occurs at the level of mTORC2 and whether mTORC1 and mTORC2 are coordinated to a greater extent than is currently known have yet to be determined. The identification of upstream regulators of mTORC2 will probably shed light on these uncertainties.

Given its many cellular actions, it is puzzling that only a few substrates of mTOR have been identified so far. This is partly due to the weak and transient nature of the mTOR–substrate interaction. The continuous improvement of mass spectrometry techniques, combined with the use of novel mTOR catalytic inhibitors, is likely to bring important advances in this area. Moreover, the emerging concept that whole classes of genes may be co-regulated by mTORC1-mediated translational control further expands the variety of its downstream effectors.

A more integrated understanding of the mTOR pathway will pave the way for novel approaches to old diseases; mTOR has evolved to accelerate growth, but it also speeds up cancer, metabolic derangement and ageing in adulthood. For these reasons, a chronic but well-tolerated inhibition of mTOR starting in mid-life could bring significant improvements to human health. However, this lifestyle improvement may come at a cost. For instance, it has been observed that lifespan extension by various manipulations comes at the expense of fertility and reproductive success, although recent findings indicate that there may be a way around this problem: in *D. melanogaster*, supply of a single amino acid, Met, allows the maintenance of reproductive potency in the context of DR-induced lifespan extension[172]. Furthermore, the many feedback loops in which mTOR participates may actually result in harmful outcomes if interrupted. Thus, the particular regimen of mTOR inhibition may have to be carefully chosen by considering the advantages of rapamycin versus catalytic inhibitors and chronic versus intermittent administration.

Finally, it remains to be seen whether limiting mTOR activity in adult humans would really enable a longer lifespan or whether it would only increase the quality of life and the way we age, without necessarily affecting how long we live.

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The authors declare no competing financial interests.

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