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Abstract

The mTOR pathway integrates a diverse set of environmental cues, such as growth factor signals and nutritional status, to direct eukaryotic cell growth. Over the past two and a half decades, mapping of the mTOR signalling landscape has revealed that mTOR controls biomass accumulation and metabolism by modulating key cellular processes, including protein synthesis and autophagy. Given the pathway's central role in maintaining cellular and physiological homeostasis, dysregulation of mTOR signalling has been implicated in metabolic disorders, neurodegeneration, cancer and ageing. In this Review, we highlight recent advances in our understanding of the complex regulation of the mTOR pathway and discuss its function in the context of physiology, human disease and pharmacological intervention.

Introduction

In 1964, a team of pharmaceutical prospectors from Ayerst Research Laboratories struck microbial gold in a soil sample from the island of Rapa Nui (Easter Island). From a *Streptomyces hygroscopicus* soil bacterium, Sehgal and colleagues isolated a novel macrolide with potent antifungal activity, which they named 'rapamycin' in deference to its place of origin[1]. Subsequent studies of rapamycin elaborated on its immunosuppressive, antitumour and neuroprotective properties, generating significant clinical excitement[2,3,4]. Nonetheless, its mechanism of action remained elusive for more than 20 years until a series of breakthroughs in the early 1990s cracked open both the mystery of rapamycin and one of the most important signalling networks in biology.

In 1990, Schreiber and colleagues demonstrated that rapamycin acts in part by binding the prolyl-isomerase FKBP12 to form a gain-of-function complex that broadly inhibits cell growth and proliferation[5,6]. However, the full mechanism of action of rapamycin was only elucidated in 1994, when three groups used biochemical affinity purification of the FKBP12–rapamycin complex to identify a large kinase as the mechanistic (originally 'mammalian') target of rapamycin (mTOR) in mammals[7,8,9]. This discovery also revealed

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homology between mTOR and the yeast TOR/DRR proteins, which had previously emerged as rapamycin targets in genetic screens for rapamycin resistance[10,11,12,13].

As intimated by the profound effects of rapamycin treatment, we now know that the mTOR protein kinase lies at the nexus of many major signalling pathways and plays a key part in organizing the cellular and organismal physiology of all eukaryotes. In the two and a half decades since its discovery, mTOR has emerged as the central node in a network that controls cell growth. As such, it integrates information about the availability of energy and nutrients to coordinate the synthesis or breakdown of new cellular components. Dysregulation of this fundamental signalling pathway disrupts cellular homeostasis and may fuel the overgrowth of cancers and the pathologies associated with ageing and metabolic disease.

In this Review, we analyse the signalling landscape of the mTOR pathway, from the inputs that regulate mTOR activation to the downstream effectors that enact its pro-growth programmes. In particular, we highlight how the intimate association between mTOR and the lysosome can facilitate rapid mobilization of nutrients upon stress or starvation. We then discuss how the mTOR pathway responds to metabolic signals in diverse organisms, cell types and tissues. Finally, drawing on recent advances in our understanding of mTOR pathway structure and function, we examine pharmacological approaches that target the pathway and evaluate their therapeutic potential in the treatment of metabolic disease, neurodegeneration, cancer and ageing.

Architecture of mTORC1 and mTORC2

mTOR is a 289-kDa serine/threonine protein kinase in the PI3K-related protein kinases (PIKK) family[14]. In mammals, it constitutes the catalytic subunit of two distinct complexes known as mTOR complex 1 (mTORC1) and mTORC2. These complexes are distinguished by their accessory proteins and their differential sensitivity to rapamycin, as well as by their unique substrates and functions (Fig. 1a).

mTORC1 is nucleated by three core components: mTOR, mammalian lethal with SEC13 protein 8 (mLST8, also known as GβL)[15] and its unique defining subunit, the scaffold protein regulatory-associated protein of mTOR (RAPTOR)[16,17] (Fig. 1b). While structural data suggest that mLST8 may stabilize the kinase domain of mTOR[18], ablation of this protein does not affect phosphorylation of known mTORC1 substrates in vivo[19]. Meanwhile, RAPTOR is essential for proper subcellular localization of mTORC1 and can recruit substrates of mTORC1 by binding the TOR signalling motifs that are present on several canonical mTOR substrates[20,21]. In addition, RAPTOR forms a scaffold for the mTORC1 accessory factor proline-rich AKT substrate 40 kDa (PRAS40)[22,23], which acts as an endogenous inhibitor of mTORC1 activity alongside DEP-domain-containing mTOR-interacting protein (DEPTOR)[24].

In the past decade, structural studies have shed new light on the assembly and catalysis of mTORC1. Cryo-electron microscopy and crystallographic analyses have revealed that mTORC1 dimerizes to form a megaDalton 'lozenge', with dimerization occurring along the

mTOR HEAT repeats and the mTOR–RAPTOR interface[25,26]. In isolation, this complex is relatively inactive; a recent structure suggests that key residues in the kinase domain of mTOR may only shift into a catalytic position after the complex binds its essential activator, the small GTPase Rheb[27]. Similar co-crystallization approaches have also established the basis of mTORC1 inhibition by FKBP12–rapamycin and PRAS40, both of which bind the FKBP12–rapamycin binding domain of mTOR to partially occlude substrate entry into the kinase active site[18,27]. Further structural analysis of mTORC1 in the presence of its substrates and regulators may offer additional insights into the mTORC1 mechanism and function.

In contrast to mTORC1, mTORC2 retains the ability to phosphorylate its substrates upon acute rapamycin treatment. As with mTORC1, the core of mTORC2 is formed by mTOR and mLST8, the latter of which is required for mTORC2 stability and function[19,28] (Fig. 1c). In lieu of RAPTOR, however, mTORC2 is defined by the unrelated scaffolding protein RICTOR[29,30], which binds MAPK-interacting protein 1 (mSIN1)[31,32,33], DEPTOR (as in mTORC1)[24] and protein associated with rictor 1 or 2 (PROTOR1/2) to form the complex[34,35]. Of note, mSIN1 has a phospholipid-binding pleckstrin homology domain, which may help mTORC2 assemble on the plasma membrane[36]. Recent cryo-electron microscopy reconstructions of mTOR bound to mLST8, RICTOR and mSin1 show that mTORC2 also dimerizes to adopt a 'lozenge' shape[37,38]. These structures further suggest that RICTOR blocks the FKBP12–rapamycin complex binding site on mTOR, thereby rendering mTORC2 insensitive to acute inhibition by rapamycin. Nonetheless, prolonged rapamycin treatment can inhibit mTORC2 signalling by sequestering the cellular pool of mTOR into rapamycin-bound complexes that cannot nucleate new mTORC2 (refs[39,40]).

Functions of the mTOR signalling pathway

Activation of mTOR marks cellular entry into a 'growth' regime characterized by increases in both cell size and cell number. To keep pace with metabolic demand in these growing cells, mTORC1 and mTORC2 initiate biosynthetic cascades to support anabolism and cell proliferation.

Roles of mTORC1

mTORC1 phosphorylates substrates that increase the production of proteins, lipids, nucleotides and ATP while limiting autophagic breakdown of cellular components. Here, we review the major substrates and effectors downstream of mTORC1 (Fig. 2a). Many of these effectors were first identified through phosphoproteomic analyses in rapamycin-treated mammalian cell lines. However, this approach is far from comprehensive: mTORC1 function is exquisitely sensitive to the physiological and pharmacological context, and certain mTORC1 substrates are resistant to inhibition by rapamycin[41,42,43]. We posit that future studies using novel and specific mTORC1 inhibitors may uncover additional substrates and mTORC1-dependent processes.

Activation of protein synthesis—Protein synthesis is the most energy-intensive and resource-intensive process in growing cells[44]. It is therefore tightly regulated by mTORC1, which promotes protein synthesis by phosphorylating the eukaryotic initiation factor 4E-

binding proteins (4E-BPs) and p70 S6 kinase 1 (S6K1) (Fig. 2a). In its unphosphorylated state, 4E-BP1 suppresses translation by binding and sequestering eukaryotic translation initiation factor 4E (eIF4E), an essential component of the eIF4F cap-binding complex. Upon phosphorylation by mTORC1, 4E-BP1 releases eIF4E and enhances 5′ cap-dependent translation of mRNAs[45,46,47].

In concert with PDK, which phosphorylates the activation loop (T229), mTORC1 phosphorylates S6K1 on its hydrophobic motif (T389) to stimulate kinase activity[48,49] (Fig. 2a). S6K1 subsequently phosphorylates its namesake target, ribosomal protein S6, a component of the 40S subunit. The function of S6 phosphorylation remains ambiguous: ablation of all five phosphorylation-target serine residues on S6 does not impair organismal viability or translation efficiency[50], although some evidence suggests that S6 phosphorylation may promote transcription of genes involved in ribosomal biogenesis[51]. More directly, S6K1 and mTORC1 upregulate transcription of rRNA, the dominant component of newly-assembled ribosomes, by enhancing the activity of RNA polymerase I and RNA polymerase III through phosphorylation of the regulatory factors upstream binding factor (UBF)[52], transcription initiation factor 1A (TIF-1A)[53] and MAF1 (refs[54,55]). S6K1 also enhances protein synthesis by activating eIF4B (ref.[56]), a positive regulator of cap-dependent translation, and by degrading the eIF4A inhibitor programmed cell death 4 (PDCD4)[57]. In addition, S6K1 associates with SKAR at exon junction complexes to boost the rate of translation elongation in spliced transcripts[58] (Fig. 2a).

Although 4E-BP1 and S6K1 both contribute to the regulation of global translation, recent evidence indicates that 4E-BP1 has a more prominent role. Deletion of S6K1 in mouse liver and muscle cells does not reduce global translation[59,60]; likewise, rapamycin treatment, which preferentially inhibits S6K1 over 4E-BP1, produces only a weak effect on global translation. By contrast, transcriptome-scale ribosome profiling reveals that mTOR inhibition dramatically suppresses translation of mRNAs carrying 5′ terminal oligopyrimidine motifs in a 4E-BP-dependent manner[61,62]. These terminal oligopyrimidine transcripts encode much of the translation machinery, including ribosomal proteins, suggesting yet another route by which mTORC1 may modulate protein synthesis.

Biomass accumulation: lipid and nucleotide synthesis and energetic homeostasis—As cells increase in size, they must generate lipids to sustain biogenesis of new membranes. Accordingly, mTORC1 drives lipid synthesis through two axes centred on the transcription factors sterol regulatory element binding protein 1/2 (SREBP1/2) and peroxisome proliferator-activated receptor-γ (PPARγ) (Fig. 2a). When sterol levels are low, the SREBPs translocate from the endoplasmic reticulum membrane to the nucleus, where they upregulate genes for de novo lipid and cholesterol synthesis[63]. Activated mTORC1 promotes this SREBP transcriptional programme by phosphorylating the SREBP inhibitor lipin 1 to exclude it from the nucleus[64]. Although the mechanism remains unclear, mTORC1 may also enhance the nuclear translocation and processing of the SREBPs in an S6K1-dependent manner[65,66] (Fig. 2b). In addition, inhibition of mTORC1 has been shown to impair the expression of lipid homeostasis genes controlled by the nuclear receptor PPARγ[67].

To maintain DNA replication and rRNA synthesis in proliferating cells, mTORC1 regulates the supply of one-carbon units for nucleotide biosynthesis. Recent work has shown that mTORC1 activates the transcription factor ATF4 and its downstream target, mitochondrial tetrahydrofolate cycle enzyme methylenetetrahydrofolate dehydrogenase 2 (MTHFD2), to drive de novo purine synthesis[68]. Through its effector S6K1, mTORC1 also promotes phosphorylation and activation of carbamoyl-phosphate synthetase 2, aspartate transcarbamoylase, dihydroorotase (CAD), the rate-limiting enzyme in pyrimidine biosynthesis[69,70]. This mTORC1-dependent tuning of the nucleotide pool is crucial for anabolic balance and homeostasis. Indeed, in cells in which mTORC1 is hyperactive, uncoupling nucleotide biogenesis from nucleotide demand with a guanylate synthesis inhibitor leads to DNA damage, as limiting nucleotides are preferentially funnelled into rRNA to sustain high rates of ribosomal biogenesis and protein synthesis[71]. Because mTORC1 dysregulation is a signature of many cancers, inhibition of nucleotide synthesis may allow us to selectively target a metabolic vulnerability in transformed cells.

Besides its direct effects on biosynthetic enzymes, mTORC1 also potentiates growth by dictating large-scale changes in the metabolic fates of glucose. To generate energy and carbon units, mTORC1 upregulates the transcription factor hypoxia inducible factor 1α (HIF1α), which increases expression of glycolytic enzymes and favours glycolysis over oxidative phosphorylation[66,72] (Fig. 2a,b). mTORC1-dependent activation of the SREBPs also increases flux through the pentose phosphate pathway, providing NADPH and carbon-rich precursors for lipid and nucleotide synthesis[66]. Finally, because biomass accumulation demands vast reserves of energetic currency, mTORC1 enhances translation of nuclear-encoded mitochondrial transcripts through 4E-BP1 to expand the ATP production capacity of the cell[73]. mTORC1 may additionally stimulate mitochondrial biogenesis by driving formation of the yin–yang 1 (YY1)–PPARγ coactivator 1α (PGC1α) transcriptional complex[74].

Repression of catabolism and autophagy—In order to prevent a futile cycle in which newly synthesized cellular building blocks are prematurely broken down again, mTORC1 suppresses catabolic autophagy (Fig. 2a). To that end, mTORC1 applies inhibitory phosphorylation marks to unc-51-like autophagy-activating kinase 1 (ULK1) and ATG13, two key early effectors in the induction of autophagy[75,76,77]. In complex with 200-kDa FAK family kinase-interacting protein (FIP200) and ATG101, ULK1 and ATG13 drive formation of the autophagosome[78]. mTORC1 phosphorylation of ULK1 and ATG13 blocks this process, allowing proteins and organelles — including some that may be redundant or damaged — to accumulate in the cell rather than be degraded and recycled. Under nutrient-replete conditions, mTORC1 also phosphorylates UVRAG, which normally associates with the HOPS complex to assist in trafficking and fusion, as well as Rab7 activation. By disrupting this interaction, mTORC1 inhibits autophagosome maturation and the conversion of endosomes into lysosomes, thereby acting as a check on both the early and late stages of autophagy[79].

Inhibition of mTORC1 by nutrient deprivation or rapamycin treatment flips the cell into a 'starvation' regime, shunting resources away from biosynthesis and towards autophagy. In interphase cells, turning off the mTORC1 molecular switch restores autophagosome

initiation and permits nuclear translocation of both the transcription factor EB (TFEB) and the related transcription factor E3 (TFE3), which activate genes for lysosomal biogenesis in a coordinated fashion[80,81,82] (Fig. 2a,b). Newly formed lysosomes then break down proteins and release constituent monomers back to the cytoplasm to regenerate the pool of cellular amino acids, enabling reactivation of the mTORC1 pathway after prolonged starvation[83]. Importantly, this coupling between nutrient status and autophagy is disrupted during mitosis, when CDK1 inhibits both mTORC1 and autophagosome formation to protect the genome from degradation after dissolution of the nuclear envelope[84].

Recent studies demonstrate that the feedback loop between the lysosome and mTORC1 is crucial for cell survival in nutritionally sparse environments. For example, pancreatic cancer cell lines that rely on macropinocytosis for nutrients stop proliferating when ablation of the transporter SLC38A9 traps essential amino acids inside the lysosome, impairing autophagic reactivation of mTORC1 (ref.[85]). Strikingly, a similar fitness defect is observed in nutrient-deprived cells that lack the autophagy receptor nuclear fragile X mental retardation-interacting protein 1 (NUFIP1), which recruits ribosomes to the autophagosome upon mTORC1 inhibition[86]. Defects in ribosome degradation appear to block reactivation of the mTORC1 pathway, while supplementation of exogenous nucleotides can restore growth[87]. These data suggest that ribosomes may serve as a major storage depot for amino acids and ribonucleotides and thus imply that mTORC1 may trigger selective 'ribophagy' to maintain cell viability under nutritional stress[88]. How mTORC1 balances bulk versus selective autophagy[89], how it exerts control over the kinetics of its own reactivation in starved cells and the functional importance of this reactivation are not fully understood. As lysosome–mTORC1 communication is essential in certain conditions in several tumour models, addressing these questions may shed light on the lysosome as a signalling organelle and guide new approaches for the treatment of cancer and metabolic disease.

Roles of mTORC2

The first direct substrate of mTORC2 was discovered serendipitously. While immunoblotting for T389 phosphorylation of the mTORC1 target S6K1 in RICTOR-depleted cells, researchers observed that mTORC2 knockdown did not affect S6K1 phosphorylation; instead, it suppressed a cross-reacting background band, which they identified as a homologous phosphorylation site on protein kinase Cα (PKCα)[29] (Fig. 2c). A member of the AGC (PKA/PKG/PKC) family of protein kinases, PKCα is thought to act as a cytoskeletal regulator, although the mechanistic basis of this process remains unclear[90]. Accordingly, knockdown of RICTOR, mTOR or mLST8, but not RAPTOR, impairs the reorganization of the actin cytoskeleton network and inhibits chemotaxis and migration[30,91]; this phenotype, in turn, may account in part for the well-documented role of mTORC2 in the mobility and metastasis of cancer cells[92,93].

Subsequent studies have revealed that mTORC2 also collaborates with PDK1 to activate other AGC family kinases, including several classes of PKCs[94,95], the ion transport regulator serum- and glucocorticoid-induced protein kinase 1 (SGK1)[96] and the oncogene Akt[97]. Akt is a central early effector in the PI3K pathway, where it mediates the cellular response to insulin and promotes proliferation. In that capacity, Akt rewires metabolism to resist

stressors through the forkhead-box FOXO1/3a transcription factors[98] and NAD kinase[99] (Fig. 2b,c). As one of the most frequently mutated signalling nodes in cancer cells, Akt also governs the activity of glycogen synthase kinase 3b (GSK3b) to suppress apoptosis and modulate glucose homeostasis. In addition, Akt may mediate crosstalk between the mTORC1 and mTORC2 complexes by inactivating tuberous sclerosis complex 2 (TSC2), a strong inhibitor of mTORC1 activity[100], and phosphorylating mSin1, an obligate component of mTORC2 (ref.[101]).

As yet, the relationship between mTORC2 and Akt is incompletely understood. Emerging evidence suggests that mTORC2 and Akt engage in mutually reinforcing layers of feedback phosphorylation that regulate localization and activity, although the effect of these marks — individually and cumulatively — is still unclear[102]. Moreover, unlike SGK1, Akt may not require mTORC2 for basal activation. Although mTORC2 kinase activity is necessary for phosphorylation of certain Akt substrates, such as FOXO1/3a, it is dispensable for others, including TSC2 and GSK3b (ref.[19]). Given that the FOXO proteins are regulated by both SGK1 and Akt, it is possible that SGK is, in this context, the more important mTORC2 effector, while Akt plays a subtler modulatory role.

Regulation of mTOR function

To mediate between cellular behaviour and the cellular environment, mTORC1 and mTORC2 integrate upstream signals, including nutrient levels, growth factor availability, energy and stress, to gate their own activation (Fig. 3). While the inputs and modes of regulation differ for each complex, we now recognize that mTORC1 and mTORC2 engage in substantial crosstalk — giving rise to signalling feedback loops with important consequences for health and disease.

Regulators of mTORC1

Cells must toggle mTORC1 activity in response to nutrient oscillations and other environmental changes stimulated by feeding or fasting. Because mTORC1 initiates a resource-intensive anabolic programme, it should only turn 'on' when energy, growth factors and macromolecular building blocks are all plentiful. To monitor and integrate these inputs, the mTORC1 pathway collects upstream signals at two sets of small G proteins, termed the Rheb and Rag GTPases (Fig. 3). Biochemical studies over the past decade have led to a model in which the nucleotide-loading state of the Rheb and Rag GTPases modulate, respectively, mTOR kinase activity[103,104] and intracellular localization[105,106] to promote cell growth. When the cellular environment is rich in cytokines, endocrine signals and ATP, Rheb maintains its active GTP-bound state on the surface of the lysosome and is competent to stimulate mTORC1 kinase activity[107]. However, mTORC1 can only co-localize with this population of GTP-Rheb when amino acids, glucose and other nutrients are readily available to activate the Rag heterodimer, which recruits mTORC1 from the cytoplasm to the lysosome. By funnelling all major environmental cues through this spatial 'AND gate', cells ensure that mTORC1 potentiates anabolism only when intracellular and extracellular conditions can support sustained growth.

Growth factors—mTORC1 acts as a downstream effector for growth factors and other mitogens, which often serve as proxies for broader paracrine and endocrine status. To regulate the mTORC1 pathway, these signals converge upon the tuberous sclerosis complex (TSC), a heterotrimeric signalling node upstream of Rheb that is composed of TSC1, TSC2 and TBC1D7 (ref.[108]). TSC acts as a GTPase-activating protein (GAP) for lysosomal Rheb, catalysing the conversion from the active Rheb-GTP state to the inactive GDP-bound state[103,109]. As a key 'molecular brake' for mTORC1 activation[110], TSC is subject to many levels of regulation. Upon exposure to insulin, insulin/insulin-like growth factor 1 (IGF-1) activates Akt, which phosphorylates TSC2 at multiple sites to dissociate TSC from the lysosomal surface and relieve inhibition of Rheb and mTORC1 (ref.[111,112,113,114]). To tune the extent and duration of mTORC1 activation and restore TSC regulation after this stimulus, the mTORC1 substrate S6K1 then directly phosphorylates insulin receptor substrate 1 (IRS-1) as part of a negative feedback loop, blocking further insulin-mediated activation of the PI3K–Akt pathway[115,116]. Wnt and tumour necrosis factor (TNF) signalling also repress TSC activity, although the precise mechanism of this regulation is unclear[117,118]. In addition, TSC is subject to inhibitory phosphorylation from ERK[119] and p90 ribosomal S6 kinase (RSK)[120], two downstream substrates of the Ras receptor tyrosine kinase signalling pathway. Because mutations that activate the Ras and PI3K–Akt pathways occur in many cancers, TSC regulation of mTORC1 is often lost in oncogenic contexts, resulting in constitutive mTORC1 activity even in the absence of appropriate growth factor signals.

Independently of TSC and Rheb, growth factors can also modulate mTORC1 activity through PRAS40, an endogenous inhibitor of the mTORC1 complex. A substrate and component of mTORC1, PRAS40 associates with Raptor to abolish Rheb-driven mTORC1 activation in vitro[22,23]. However, in the presence of insulin, Akt phosphorylates PRAS40, leading to its sequestration by a cellular 14–3–3 scaffold protein and restoring mTORC1 kinase activity. How growth factor signals are coordinated through PRAS40 and Rheb, and the relative importance of each branch in different cellular contexts, remains an area of active study.

Energy and oxygen availability, and other cellular stresses—Under conditions of energy or oxygen scarcity, several factors work together to activate the TSC axis and suppress mTORC1 signalling. Periods of intense metabolic exertion or glucose withdrawal can deplete cellular stores of ATP, triggering the AMP-activated protein kinase (AMPK) complex, a master regulator of cellular energy charge. As an antagonist of most major ATP-consumptive processes, AMPK inhibits mTORC1 directly, by phosphorylating Raptor, and indirectly, by activating TSC2 (refs[121,122,123]). At the same time, by reprogramming metabolism away from anabolic pathways, AMPK relieves the pressure on mitochondrial respiration and reduces the chances of cellular damage from the generation of reactive oxygen species[124].

Independently of AMPK, oxidative stress can also inhibit mTORC1 by upregulating REDD1, a small protein that activates TSC[125,126]. Other signs of cellular stress — ranging from organelle dysfunction to DNA replication stress — can further oppose mTORC1 activation[127]. For example, the endoplasmic reticulum unfolded protein response can inhibit

mTORC1 by increasing transcription of the Sestrin proteins, key negative regulators that will be discussed in greater detail below[128]. Likewise, DNA damage induces various p53 target genes, including an AMPK subunit (AMPKβ), PTEN and TSC2, all of which can dampen mTORC1 activity to slow proliferation and protect genome integrity[129].

Amino acids and other nutrients—Besides spurring growth factor release, feeding also replenishes the pool of intracellular nutrients. These nutrients, which constitute the basic molecular substrates for biology, include amino acids, nucleotides and vitamins, all of which may be partially or wholly derived from the diet. Among the major nutrients, amino acids play a dominant role in regulating the mTORC1 pathway[130]; indeed, Avruch and colleagues observed as early as 1998 that the amino acids leucine and arginine, in particular, are absolutely required for mTORC1 activation in mammalian cells[131]. How these amino acids communicate their availability to mTORC1, however, remained a complete mystery until 2008, when two groups independently reported the discovery of the Rag-GTPases as essential components of the nutrient sensing machinery[105,106].

Unlike all other known small GTPases, the Rags are obligate heterodimers, configured such that RagA or RagB is bound to RagC or RagD. Anchored to the lysosome by the pentameric Ragulator complex (comprising p18, p14, MP1, C7orf59 and HBXIP, otherwise known as LAMTOR1–LAMTOR5)[132,133,134], the Rags can be found in one of two stable conformations: an 'on' state, in which RagA/B is bound to GTP and RagC/D to GDP; and an 'off' state, in which the reverse is true. These stable nucleotide-loading states are maintained by intersubunit crosstalk between the Rags[135], but they can be modulated by the amino acid and nutrient status through a series of upstream factors with GAP or GTP exchange factor activity towards the Rags. Emerging structural evidence shows that, under amino acid-replete conditions, Raptor grasps the 'on-state' Rags via a protruding 'claw'[136]. This interaction recruits mTORC1 from the cytosol to the lysosome, allowing lysosomal Rheb to stimulate mTORC1 kinase activity. Thus, the Rags and Rheb define the two independent arms that converge to license the mTORC1 pathway (Fig. 3).

Drawing on work by several groups over the past decade, we now recognize that mTORC1 senses cytosolic and lysosomal amino acid concentrations through distinct mechanisms. Of the 'nutrient sensing complexes' that transmit cytosolic amino acid signals to the Rags, the most direct regulator of Rag status is the GAP activity towards the Rags 1 (GATOR1) complex[137]. GATOR1 is composed of three subunits — DEP domain-containing 5 (DEPDC5), nitrogen permease related-like 2 (NPRL2) and NPRL3 — with GAP activity residing in the NPRL2 subunit[138]. When cytosolic amino acid levels fall, GATOR1 experiences a poorly understood regulatory event that enables it to hydrolyse the GTP bound to RagA/B and inhibit the mTORC1 pathway[139]. In turn, GATOR1 is itself regulated by other upstream factors. The large KICSTOR complex, consisting of the proteins KPTN, ITFG2, C12orf66 and SZT2, tethers GATOR1 to the lysosome and is required for cellular sensitivity to amino acid deprivation[140,141]. Meanwhile, GATOR1 also physically interacts with GATOR2, a pentameric complex of WDR59, WDR24, MIOS, SEH1L and SEC13 (ref. [137]). Through unknown molecular mechanisms, the GATOR2 complex antagonizes GATOR1 function and acts as a potent positive regulator of mTORC1. Elucidating the link

between GATOR2 and GATOR1 activity remains one of the most intriguing challenges in basic mTOR biology.

Recently, the question of GATOR2 function has attracted special attention because of the identification of two novel 'amino acid sensors', which relay the cytosolic availability of leucine and arginine to the mTORC1 pathway through interactions with GATOR2. Upon acute leucine starvation, the cytosolic leucine sensor Sestrin2 binds and inhibits GATOR2, preventing lysosomal recruitment of mTORC1 (ref.[142]). Refeeding restores leucine levels and allows the amino acid to bind a pocket on Sestrin2, dissociating the protein from GATOR2 to relieve mTORC1 inhibition[142,143]. Although the leucine-binding affinity of Sestrin2 dictates mTORC1 sensitivity to leucine deprivation in cell culture, Sestrin2 and its relatives Sestrin1 and Sestrin3 may also be effectors of leucine-independent stress pathways. In support of this hypothesis, the Sestrins are transcriptionally upregulated by ATF4 and the endoplasmic reticulum unfolded protein response[128,144], and Sestrin overexpression alone is sufficient to suppress mTORC1 signalling in vitro[145,146]. By contrast, cellular arginine sensor for mTORC1 (CASTOR1) appears to be exquisitely sensitive to cytosolic arginine alone[147,148]. A protein that can exist either as a homodimer or as a heterodimer with CASTOR2, CASTOR1 also inhibits GATOR2 in the absence of arginine and dissociates from the complex when arginine is bound.

A second arginine sensor, SLC38A9, monitors amino acid levels inside the lysosomal lumen and defines the lysosomal branch of the nutrient sensing machinery[149,150]. SLC38A9 resides on the lysosomal membrane and transports neutral amino acids out of the organelle in an arginine-gated fashion[85]. This efflux activity may enable the products of autophagic protein degradation to reactivate the mTORC1 pathway after prolonged starvation. Synthesizing structural and biochemical evidence, we posit that the binding of lysosomal arginine to the first transmembrane helix of SLC38A9 frees the N terminus of the protein from the central pore[151]. This domain can then collaborate with Ragulator to push the Rags into the active state by promoting GTP loading of RagA/B[152]. Through a separate mechanism, the lysosomal v-ATPase, which maintains the pH gradient of the lysosome, has also been reported to interact with the Rag–Ragulator complex to influence the nucleotide-loading state of the Rags[153]. Finally, the folliculin (FLCN)–FNIP2 complex acts as a GAP for RagC/D to sustain mTORC1 activation in the presence of amino acids[154,155]. By modulating the status of RagC/D, FLCN–FNIP2 may also recruit and enhance phosphorylation of the transcription factors TFEB/TFE3, although it is unclear whether this process is mTORC1 independent[156,157]. If FLCN–RagC/D–TFEB/TFE3 does indeed constitute a distinct axis, loss of FLCN could amplify the TFEB/TFE3 transcriptional programme, an oncogenic signature in some cancers[158,159], allowing us to reconcile FLCN's status as a tumour suppressor in vivo with its activating role in the mTORC1 pathway.

The recent discovery of an S-adenosylmethionine (SAM) sensor, named SAMTOR, has shown that mTORC1 responds not only to amino acids (for example, leucine and arginine) but also to their metabolic by-products — in this case, a key methyl donor derived from methionine. Unlike Sestrin2 and CASTOR1, which oppose GATOR2 signalling when their cognate amino acids are absent, SAMTOR negatively regulates mTORC1 by binding

GATOR1 and KICSTOR under methionine or SAM deprivation[160]. Restoration of SAM levels dissociates SAMTOR from these complexes and stimulates mTORC1 activity.

At present, we do not know how other amino acids impact mTORC1 activation, nor do we understand what role, if any, the general amino acid sensors GCN2 and ATF4 play in acute mTORC1 signalling cascades. While longer-term amino acid deprivation is thought to feed from GCN2 back to mTORC1 through transcriptional upregulation of ATF4 and the Sestrins, it is not clear whether GCN2 and ATF4 regulate mTORC1 in transiently starved cells. Moreover, we still lack mechanistic explanations for how several known metabolic inputs impinge on the pathway. For example, although acute withdrawal of glucose inhibits mTORC1 at least partially through activation of AMPK, a study in AMPK-null cells has demonstrated that glucose deprivation also signals through the Rag-GTPases[161], reinforcing earlier evidence that glucose can signal independently of both AMPK and TSC[162]. Similarly, depletion of purine nucleotides inhibits mTORC1, perhaps as an indicator of replication stress, but it is not clear whether this inhibition is driven by TSC or by degradation of Rheb[163,164]. One recent study suggests that phosphatidic acid may activate mTOR signalling as a proxy for fatty acid availability[165], while another implicates glutamine in Rag-independent reactivation of mTORC1 (ref.[166]). Cholesterol has also been shown to activate mTORC1 through a complex composed of SLC38A9 and the Niemann–Pick C1 protein[281]. Whether mTORC1 senses other metabolites essential for cell growth, such as vitamins or inorganic ions, remains an open question; equally unclear is how these nutritional requirements might diverge in cell types or organisms with different dietary and metabolic needs (Box 1).

Regulators of mTORC2

In part because it has been difficult to tease apart the regulation of mTORC1 and mTORC2 with pharmacological agents, the activators of mTORC2 are still poorly defined. Even so, it is clear that mTORC2 is primarily regulated by growth factors through the PI3K pathway, with the unique mTORC2 component mSin1 acting as a key signal integrator (Fig. 3). Like other PI3K effectors, mSin1 possesses a pleckstrin homology domain, which autoinhibits mTORC2 kinase activity in the absence of insulin[36]. This inhibition is relieved by the binding of phosphatidylinositol (3,4,5)-trisphosphate (PIP3), a product of insulin-induced or serum-induced PI3K activation[36,167]. PIP3 may recruit mTORC2 and Akt to the plasma membrane, where reciprocal phosphorylations between the two kinases modulate their localization and activation[102]. In several model systems, including *Dictyostelium discoideum*, this localization and activation is also regulated by the small GTPases Rac1, Rap1 and Ras, which bind to mTORC2 to direct chemotaxis and growth[168,169,170]. A recent study extends this paradigm to human cells by showing that mSin1 can recruit oncogenic Ras to directly catalyse mTORC2 kinase activity at the plasma membrane[171]. This finding connects mTORC2 to a major cancer pathway and reinforces its role in driving survival and proliferation.

Because mTORC1 downregulates insulin–PI3K–Akt signalling through feedback inhibition, it also engages in negative crosstalk with mTORC2 (ref.[172]). As previously described, mTORC1 can disrupt PI3K–Akt signalling through S6K1-dependent degradation of IRS1

(refs[115,116]); alternatively, mTORC1 can activate Grb10, a negative regulator of the insulin/ IGF-1 receptor[173,174]. Both of these mechanisms have downstream implications for mTORC2 activity and may account for some of the paradoxical metabolic phenotypes associated with chronic rapamycin treatment (Fig. 4). Unexpectedly, mTORC2 is also activated by AMPK under energetic stress, suggesting that it may mediate cellular adaptation to oxygen-poor or nutrient-poor tumour environments in vivo[175].

mTOR in physiology and pathophysiology

Characterization of mTOR signalling nodes is a work in progress at the cellular level, but the functional regulation of the pathway becomes exponentially more complex at the organismal level, as mTOR must coordinate the storage and mobilization of nutrients and energy across different tissues. Unlike cells in culture, which are bathed in growth factors and nutrients and consequently maintain high mTOR activity, cells in vivo tend to display lower baseline activity and experience sharper fluctuations in mTOR activity upon fasting or feeding. Coordinating physiological responses with nutrient status requires the mTOR pathway to sense conditions within specialized niches and to enact tissue-specific anabolic or catabolic cascades. Appropriate regulation of mTOR is crucial for homeostasis and organismal health; conversely, imbalances in mTOR activity in various tissues can lead to metabolic dysregulation and disease.

mTOR in metabolic syndrome

As a critical regulator of glucose metabolism and lipogenesis across various tissues, the mTOR pathway is readily hyperactivated by overfeeding and underwrites many diseases of constitutive growth, including obesity and type 2 diabetes.

Insulin sensitivity and glucose homeostasis—To prevent the accumulation of nutrients in the blood, animals have evolved mechanisms to sequester macromolecules and energy after feeding. These processes are coordinated across different tissues by the release of insulin from the pancreas, which co-activates mTORC1 and mTORC2 to promote hypertrophy and growth (Fig. 4a). In skeletal muscle, insulin induces the uptake of glucose and enhances its storage as glycogen by stimulating the mTORC2–Akt axis[176]; at the same time, circulating amino acids are incorporated into new muscle biomass in an mTORC1-dependent manner.

By contrast, low levels of insulin following fasting induce autophagy in 'dispensable tissues' (that is, muscle and liver, as opposed to the brain), which break down protein stores to fuel gluconeogenesis in the liver. This catabolic programme has profound effects on metabolic organs: one study found that livers from mice fasted for 24 h decreased in weight by nearly 25%, with the difference arising not from changes in cell number but from reductions in cell size[177]. Strikingly, this fasting-induced shrinkage was abolished in mice with liver-specific knockouts of TSC1, Raptor or the autophagy gene *Atg7*, suggesting that the switch from anabolism to catabolism is primarily regulated by mTORC1 (refs[177,178]).

Substantial evidence now points to mTORC1 as a central mediator of organismal survival during nutrient restriction. For mice that cannot tune mTORC1 signalling, prolonged fasting

— like the postnatal fast caused by disruption of the placental nutrient stream — can pose an insurmountable challenge. Unlike wild-type neonates, which rapidly inhibit mTORC1 after an initial drop in circulating glucose, mice expressing a constitutively active allele of RagA (RagA-GTP) are unable to suppress mTORC1 signalling during the perinatal fasting period[161]. Because these mutant mice fail to restrict their energy expenditure or trigger autophagy to supply free amino acids for gluconeogenesis, their plasma glucose levels plummet, leading to fatal hypoglycaemia within 1 day of birth. A similar perinatal lethality occurs in mice lacking the Sestrin proteins (upstream negative regulators of mTORC1)[179] and in mice with defects in the autophagy machinery (downstream targets of mTORC1)[180], demonstrating that mTORC1 activity must be tightly coupled to diet to maintain glucose homeostasis in vivo.

Adipocyte formation and lipid synthesis—Postprandial mTOR activation also promotes longer-term energy storage by increasing the synthesis and deposition of triglycerides in white adipose tissue (WAT). As the largest repository of energy in the body, WAT serves as a metabolic hub, tailoring its biosynthetic activity to fluctuations in mTOR signalling. In these cells, the mTORC1–S6K1–SREBP axis drives de novo lipogenesis[64,65,66], while mTORC1 activation of PPARγ helps pre-adipocytes differentiate into mature tissue[67,181] (Fig. 4a). S6K1 may also increase fatty acid import into adipocytes through a complex mechanism involving the glutamyl-prolyl-tRNA synthetase (EPRS)[182]. Consistent with the importance of mTORC1 in WAT, adipocyte-specific deletion of Raptor reduces WAT tissue mass and enhances lipolysis in mouse models[183]. Tantalizingly, Adi-Raptor KO mice are also resistant to diet-induced obesity[184]. Unfortunately, these defects in adipocyte expansion can drive fat deposits to accumulate in the liver instead, leading ultimately to hepatic steatosis and insulin resistance[183].

While regulation of adipose tissue exerts second-order effects on other organs, mTOR also directly modulates lipid metabolism in the liver. Several groups have found that hepatic lipogenesis is impaired in both Raptor and Rictor-depleted mice, with mTORC2-dependent effects at least partially rescuable by constitutive activation of Akt[64,185,186]. In addition, mice with liver-specific hyperactivation of mTORC1 fail to fully stimulate the production of ketone bodies, which are synthesized from fatty acids to supply peripheral tissues with alternative energy packets during fasting[177]. Although the relationship between mTORC1 and ketogenesis is not entirely clear, insulin withdrawal likely inhibits mTORC1 phosphorylation of S6 kinase 2 (S6K2), which then enhances expression of ketogenic factors by freeing the transcription factor PPARα from its corepressor, nuclear receptor corepressor 1 (NCoR1). Similar ketogenic defects are also observed in aged mice, suggesting that long-term decline in liver function may stem from mTOR-driven dysregulation of lipid metabolism[177].

Pharmacological interventions for metabolic disease—Many diseases of overfeeding, among them obesity and type 2 diabetes, produce a major and detrimental energy imbalance in the body. By generating a constant surplus of hormones, cytokines and nutrients, these diseases collapse the metabolic cycles that underwrite tissue homeostasis, forcing mTORC1 to remain in a persistent 'on' state. Constitutive mTORC1 signalling

activates S6K1 and Grb10 to decouple the insulin/IGF-1 receptor from downstream PI3K pathway effectors, dampening the physiological response to insulin[115,116,173,174] (Fig. 4b). Moreover, PI3K inhibition suppresses mTORC2–Akt to block glucose uptake and promote gluconeogenesis[185,187], thereby further elevating the glycaemic load and exacerbating the ectopic fat deposition and glucose intolerance that constitute the hallmarks of metabolic syndrome.

Given that mTORC1 sits at the centre of a web of dysregulated metabolic signalling, it is tempting to imagine that inhibition of this node might reverse both the symptoms and underlying causes of obesity and diabetes. Lending support to these hopes, metformin, a first-line treatment for type 2 diabetes, has been shown to potently suppress mTORC1 by activating AMPK and TSC[188]; likewise, ablation of the mTORC1 effector S6K1 can protect against diet-induced obesity and enhance insulin sensitivity[172]. Unfortunately, direct pharmacological inhibition of mTORC1 yields more complex outcomes. Patients administered rapamycin experience more severe insulin resistance, perhaps because chronic rapamycin treatment disrupts not only mTORC1 but also the integrity of the mTORC2 complex, blunting the Akt-dependent insulin response[40] (Fig. 4b). To bypass these adverse effects, it will be necessary to develop new, truly specific mTORC1 inhibitors, as well as tissue-specific modulators of mTORC1 function.

mTOR regulation of brain physiology and function

Within the brain, the mTOR pathway orchestrates a wide array of neuronal functions, temporally spanning every stage of development[189,190]. From framing basic cortical architecture to remodelling neuronal circuitry in response to experience, mTOR and its molecular accomplices shape both the signalling and the physical terrain of the brain (Fig. 5a,b). Not surprisingly, loss of mTOR regulation — through either genetic or chemical perturbations — has severe repercussions for neuronal function (Fig. 5a). Brain-specific knockouts of Raptor and Rictor display remarkably similar phenotypes, typified by microcephaly — via reductions in neuron size and number — and improper differentiation[191,192]. In addition, Raptor deletion in the brain also triggers early postnatal death[191], while Rictor deletion leads to aberrant brain foliation and impaired dendrite extension[192].

mTOR in neurodevelopmental disorders—Hyperactive mTOR signalling, as observed in neurodevelopmental mTORopathies, is associated with characteristic defects (Fig. 5a). As a class, mTORopathies are caused by loss-of-function mutations in negative regulators of mTORC1, usually manifesting with some subset of the following symptoms: focal malformations in the brain, epileptic seizures, macrocephaly, autism spectrum disorder and benign tumours or cystic growths[193]. Perhaps the best-studied such disease is TSC, which arises when loss of either TSC1 or TSC2 induces constitutive mTORC1 activity. Patients with TSC often grow lesions that disrupt the laminar organization of the cortex, nucleating epileptogenic foci; these patients may also have enlarged neurons and 'giant' astrocytes[193]. Similar phenotypes are found in patients with inactivating mutations in the negative PI3K regulator PTEN[194], the AMPK activator STRADα[195] and the negative regulatory complexes GATOR1 and KICSTOR[196,197,198,199,200], as well as those with activating mutations in

Rheb or mTOR[201,202,203]. Given that mTORC1 has many roles in defining the morphology of the developing brain, the epilepsy that clinically distinguishes these disorders is likely seeded by prenatal neuronal mis-wiring[204]. However, acute rapamycin treatment can nonetheless suppress seizures caused by TSC1 loss in adult mice[205], suggesting that mTOR hyperactivity can further stimulate 'seizing' in established neural circuits. Consistent with a model in which mTORC1 participates in multiple stages of epileptogenesis, recent speculation contends that the ketogenic diet, a validated therapy for treatment-refractory epilepsy, may work by depriving the mTORC1 pathway of activating nutrients[206]. Other, more direct mTOR inhibitors are currently in clinical trials as anti-epileptic agents[207,208].

mTOR control of brain function via protein translation and autophagy regulation—Surprisingly little is known about the regulation of mTOR signalling in normal brain function and homeostasis. Unlike cell culture systems, the postnatal brain is mostly postmitotic, such that environmental inputs are consolidated not to stimulate growth or proliferation but rather to enact changes in neuronal morphology and connectivity. Although it is not clear which inputs are actually relevant in vivo, given that the brain is 'nutritionally protected' from acute fasting (that is, brain biomass and function are generally left intact for as long as possible under starvation, with the brain having first use of available glucose and ketone bodies), brain-derived neurotrophic factor (BDNF) has emerged as a major tissue-specific agonist of the neuronal mTOR pathway. As a PI3K activator, BDNF increases mTORC1 signalling near injured axons to encourage wound healing and repair[209,210]; in turn, BDNF release may itself be regulated by a feed-forward loop downstream of S6K1 (ref.[211]).

In collaboration with BDNF, mTOR regulates learning and memory by promoting translation at synapses through S6K1 and 4E-BP2 (ref.[212]) in a manner that is dependent on neuronal activity (Fig. 5c). This localized translation is rapamycin-sensitive and is crucial for the remodelling of dendritic spines that accompanies long-term potentiation[213]. Strikingly, animal models lacking TSC or 4E-BP2 recapitulate some of the social and cognitive abnormalities associated with autism spectrum disorder, suggesting that dysregulation of synaptic translation may affect higher-order brain functions[214,215]. In accordance with this paradigm, synaptic translation has also been linked to depression and psychiatric mood disorders. The NMDA receptor antagonist ketamine, a fast-acting antidepressant, has been shown to boost mTORC1 activity at the synapse, with psychiatric relief coinciding with an increase in synaptic protein, dendritic spine density and synaptic function[211,216]. In animal models, the Sestrin inhibitor NV-5138 appears to mediate similar improvements by directly activating mTORC1, independent of other upstream signals[217]. However, while these lines of evidence implicate mRNA translation in diverse aspects of synaptic plasticity and brain health, we still do not know which neuronal mRNAs are regulated by the mTORC1 pathway in response to specific stimuli, nor do we understand how mTORC1 and its substrates localize protein synthesis within individual neurons.

In recent years, it has become increasingly clear that translation is not the only mTORC1 output required for plasticity. In order to adjust the strength of a neuronal circuit, mTORC1 must simultaneously promote the building of new proteins at some synapses and the degradation of excess synaptic machinery at others. The latter process calls for local

inhibition of the mTOR pathway, which triggers macroautophagy[218] (Fig. 5c). Consistent with the apparent importance of autophagy in cognitive function (see Box 2), constitutive mTOR hyperactivity has been shown to compromise synaptic pruning and contribute to autism spectrum disorder-like social deficits in TSC-deficient mice[219]. Rapamycin treatment was sufficient to rescue these defects, but only when the autophagy pathway remained intact. While these findings are quite preliminary, taken in sum with the apparent efficacy of mTORC1 activators, like ketamine, they suggest that modulation of the mTORC1 pathway in the brain may hold promise as a therapeutic strategy to improve cognitive performance and memory in certain disease states.

mTOR in cancer

Although the mTOR kinase itself is rarely mutated in cancer, it is readily hijacked by upstream oncogenic nodes, including those in the PI3K–Akt pathway and the Ras-driven MAPK pathway. As a result, mTOR signalling is hyperactive in up to 80% of human cancers[220], in which context it plays a pivotal role in sustaining cancer cell growth and survival (Fig. 6a). Because tumour microenvironments are poorly vascularized and subject to severe nutritional restrictions, loss of the mTORC1 nutrient sensing machinery may help cancer cells evade metabolic checks on anabolism and proliferation. Thus, mutations in all three components of the GATOR1 complex have been implicated in glioblastomas[137], while RagC and FLCN mutations have been found in follicular lymphoma and Birt–Hogg–Dubé syndrome, respectively[221,222]. Meanwhile, hyperactivation of mTORC2 can aggravate negative cancer prognoses by activating Akt and by supporting the cytoskeletal transformations that underlie metastasis[92,93].

To date, mTOR inhibitors have met with limited success as chemotherapeutic agents. The first generation of clinical rapamycin derivatives, known as 'rapalogs', were approved for advanced renal cell carcinomas in the late 2000s. Outside certain exceptional contexts[223], these rapalogs have proved more cytostatic than cytotoxic, perhaps because they only partially block 4E-BP-dependent translation and fail to inhibit the pro-survival pathways regulated by mTORC2–Akt[61,224]. Inhibition of mTORC1 also drives autophagy, which has been shown to nourish cells in nutrient-poor tumour microenvironments[225]. A second generation of catalytic mTOR inhibitors (for example, Torin1, PP242, Ku-0063794) competes with ATP to occupy the kinase active site and sidesteps many of these issues by inhibiting all known substrates of mTORC1 and mTORC2 (refs[41,226,227]). Despite some concerns about tissue toxicity because of their broad effects, early clinical data suggest that catalytic mTOR inhibitors can be tolerated at effective doses[228]. However, prolonged treatment with these inhibitors can lead to a metabolic retrenchment that allows cancer cells to reactivate Akt without positive input from mTORC2, highlighting resistance as a key problem that must be tackled by next-generation therapies[229,230,231].

mTOR in ageing

In line with a growing body of genetic and pharmacological evidence, mTOR activity is now recognized as a major driver of ageing — a process defined here as a progressive decline in physiological function that increases vulnerability to disease and death (Fig. 6b). Genetic

inhibition of the mTORC1 pathway through depletion of mTOR or Raptor has been shown to extend lifespan in organisms as diverse as yeast[232], nematodes[233,234], flies[235] and mammals[236]; in a similar vein, rapamycin treatment also promotes longevity across a wide swathe of the evolutionary tree[237,238,239,240]. Tantalizingly, rapamycin appears to prolong not just lifespan but also healthspan — the length of time that an organism enjoys efficient biological performance, free of disease or disability — suggesting that mTORC1 inhibition may slow ageing by reversing molecular changes associated with cellular deterioration[241].

What are these mTORC1-sensitive molecular changes that affect ageing? One clue may come from dietary restriction, the only other intervention that produces a comparable and conserved increase in lifespan. Dietary restriction reduces nutrient intake without incurring malnutrition, pushing mTORC1 towards a catabolic regime. Indeed, dietary restriction is thought to counter ageing by acting through the mTORC1 pathway, as dietary restriction on top of chemical or genetic inhibition of mTORC1 fails to confer any additional longevity benefit in flies, worms and yeast[232,235,242]. Intriguingly, dietary restriction of a single amino acid, methionine, is sufficient to increase lifespan in flies[243], implying that restrictions on protein synthesis may have a particularly important anti-ageing effect. Consistent with this observation, loss of the translation effector S6K1 extends lifespan in worms and mice, perhaps by halting the production of misfolded or aggregated proteins[242,244]. By reducing the energetic burden of translation, mTOR inhibition also relieves oxidative stress and prevents the accumulation of harmful metabolic by-products, leading to broad improvements in cellular function[73].

In parallel with its downregulation of translation, mTOR inhibition restores autophagic capacity, which undergoes an age-related decline in many tissues[245]. Autophagy degrades obsolete or damaged cellular components and salvages them for 'spare macromolecular parts'. Through this process, aged cells refresh their molecular equipment and clear damaged proteins and organelles, which have been implicated in age-related diseases from cardiomyopathy to neurodegeneration. Underscoring the importance of autophagy in healthy ageing, direct activation of autophagic flux can significantly increase lifespan and healthspan in mice[246]. Conversely, mTOR inhibition fails to extend lifespan in ATG-deficient worms, indicating that mTOR modulates longevity at least partly through autophagy-dependent mechanisms[247,248].

mTORC1 has also been implicated in ageing at the tissue level. Several groups have shown that persistent mTORC1 signalling contributes to the exhaustion of stem cell pools, hindering tissue self-renewal in aged organisms[249,250]. mTORC1 hyperactivity is also a distinctive feature of senescent cells, which permanently arrest in the G0 phase of the cell cycle and undergo morphological alterations that eliminate sensitivity to amino acid and growth factor deprivation[251]. Exploiting translational programmes downstream of mTORC1, senescent cells synthesize and secrete pro-inflammatory cytokines to exacerbate ageing-related declines in fitness and tissue function[252,253]. Rapamycin treatment attenuates this inflammatory phenotype, although it is unclear whether mTORC1 inhibition can rescue cell cycle arrest or aid in the clearance of senescent cells.

Even though mTOR inhibitors are well validated as geroprotective agents in animal models, the potential side effects of chronic dosing (particularly insulin resistance and immunosuppression) have thus far precluded their use in healthy elderly humans. Recent studies, however, suggest that these concerns are not insurmountable. Because side-effect profiles for mTOR inhibitors have largely been inferred from patients undergoing cancer therapy or organ transplantation, they tend to reflect intense, high-dose regimens. Far lower doses are needed for anti-ageing benefits. Taking advantage of this distinction, one group found that intermittent dosing of rapamycin in mice could extend their lifespan without inciting glucose intolerance[254,255]. Another reported that low doses of mTOR inhibitors could actually improve immune function in elderly patients[256]. Efforts to harness mTOR inhibition as an anti-ageing strategy will have to build on these studies to define safe and effective doses in human cohorts.

Conclusions and perspectives

Perched at the interface between organisms and their environments, the mTOR pathway toggles the balance of anabolism and catabolism in response to contextual signals and guides nearly every aspect of metabolic function. Recent work has clarified the logical structure of the pathway and drawn the lysosome into renewed focus; structural advances have also allowed us to see, mechanistically, how key mTOR signalling nodes transduce nutritional information into molecular action. Building on careful in vivo studies, we have made remarkable progress in cataloguing the inputs and effectors of the mTOR pathway across various tissues and metabolic states, enhancing our understanding of mTOR signalling in health and disease.

Nonetheless, certain open questions remain stubbornly unresolved. Given that mTORC1 activation occurs at the lysosomal surface, how does the complex capture and phosphorylate its downstream substrates, which, with the exception of TFEB/TFE3, do not maintain lysosomal subpopulations? It is possible that lysosomal interactions with the endoplasmic reticulum, the Golgi and the plasma membrane may help bring mTORC1 into contact with some of its substrates[257,258,259]. Alternatively, while efforts to visualize the dynamics of the substrate search have not been conclusive[260,261], we speculate that activated mTORC1 could exit the lysosome to phosphorylate targets at other loci in the cell, perhaps carrying Rheb in tow. Moving forward, we also seek an integrated understanding of mTOR signalling in specific tissues. Although many components of the mTOR pathway have been identified, it is not clear which regulatory inputs are dominant in any particular physiological milieu. In order to develop new therapeutics that evade some of the metabolic side effects of existing mTOR inhibitors, we hope to identify complex-specific or tissue-specific modulators of mTOR activity and establish them as targets for rational drug development.

One emerging theme from the study of mTOR dysregulation in human disease is that these pathologies are not just linked by a common aetiological basis — they also intersect with each other in mutually reinforcing ways. Just as excessive mTOR activity can lead to metabolic syndrome, obesity accelerates molecular ageing, which in turn amplifies the risk of neurodegenerative disease and cancer. Thus, even though the complexity and breadth of the mTOR signalling network increases the risk of toxicity, the unique spectrum of mTOR-

dependent processes is also one of its most powerful advantages as a therapeutic target. More so than other strategies to delay ageing or counter disease, mTOR inhibition disrupts a wide variety of degenerative processes with a single intervention. Further insights into this fundamental pathway may ultimately lead to new treatments for currently intractable diseases and transform our ability to regulate health and homeostasis.

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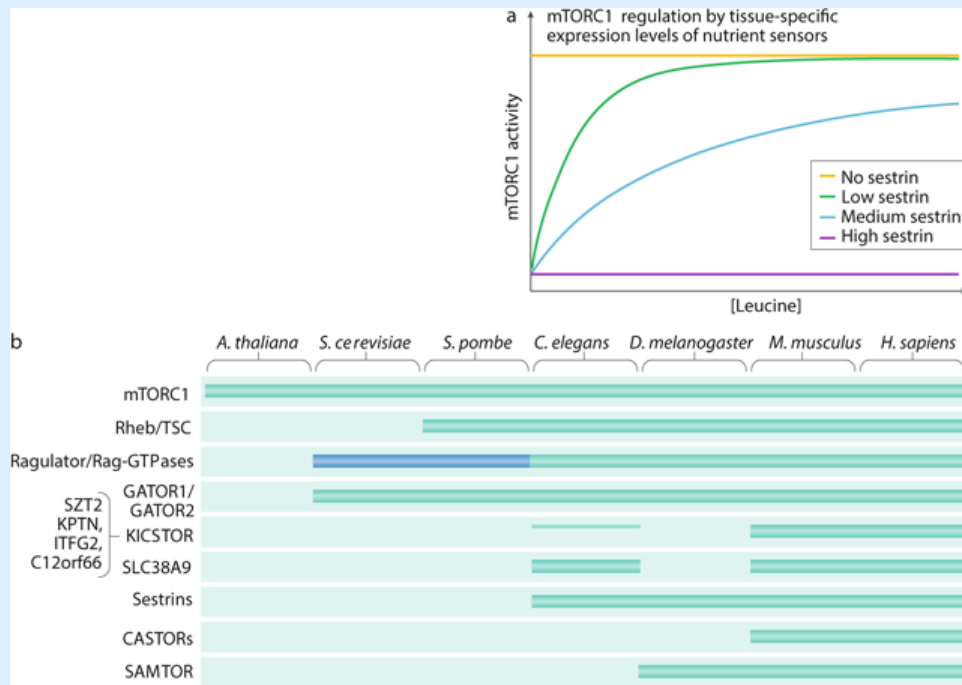
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Box 1:

Cell-specific and organism-specific regulatory mechanisms across evolution in mTORC1 signalling

In order to align mTOR complex 1 (mTORC1) activity with tissue function, some specialized human cells may respond to unique inputs, adjust the weighting of upstream signals[262] or regulate mTORC1 through non-canonical mechanisms. In muscle cells, mechanical stimuli have been shown to activate mTORC1 (refs[263,264,265]), whereas in primary osteoclasts, amino acid deprivation can abrogate mTORC1 signalling without dissociating the complex from the lysosome[266]. We postulate that specialized cells can also adapt to their niches by tuning expression of nutrient sensors. For example, in tissues where physiologically relevant leucine concentrations are relatively high, cells might selectively increase expression of Sestrin2 to raise the leucine threshold for mTORC1 activation. Conversely, cells that are protected from leucine fluctuations might abolish Sestrin2 expression altogether to render mTORC1 insensitive to leucine deprivation. Thus, differential expression of Sestrin could modulate mTORC1 sensitivity to leucine levels in a tissue-specific manner (see the figure, part **a**).

Although the core components of the nutrient sensing machinery — the Rag GTPases, Ragulator and the GAP activity towards the Rags (GATORs) — are conserved in metazoans (see note below), some of the direct amino acid sensors are absent in non-vertebrate lineages (conservation of mTORC1 pathway components in common model organisms is shown in the figure, part **b**). Based on sequence homology, *Drosophila melanogaster* retains Sestrin and the *S*-adenosylmethionine sensor SAMTOR but lacks both the lysosomal and cytosolic arginine sensors; meanwhile, *Caenorhabditis elegans* possesses SLC38A9 and Sestrin homologues but does not have a clear SAMTOR

equivalent. The irregular pattern of conservation of the sensors may be linked to the distinct nutritional needs of each organism. In support of this idea, computational searches indicate that *Saccharomyces cerevisiae*, a model organism capable of synthesizing all 20 amino acids de novo, does not have any amino acid sensors and, consequently, does not require any individual amino acid for TORC1 activation. Instead, the *S. cerevisiae* TORC1 pathway may respond to the general availability of nitrogen and carbon sources[267]. Puzzlingly, *S. cerevisiae* also does not seem to require a Rheb homologue to activate TORC1 (ref.[268]), suggesting that its molecular circuitry may diverge sharply from that of other model organisms, including *Schizosaccharomyces pombe*[269]. The blue box (see the figure, part **b**) indicates that the EGO complex in yeast shares little sequence homology with Ragulator, although it appears to serve an analogous function.

Even nutrient sensors with recognizable homology may display functional differences in divergent species. Binding assays with radioactive leucine reveal that the *D. melanogaster* homologue of Sestrin (dSesn) has 5-fold lower affinity for leucine than the human protein[142,270]. We speculate that this molecular difference may allow dSesn to sense physiological leucine fluctuations in the *D. melanogaster* haemolymph, which has about a 5-fold to 10-fold higher amino acid concentration than human plasma[270,271]. Taken together with conservation patterns, these data also suggest an attractive hypothesis: perhaps organisms evolved or retained specific nutrient sensors to enable the TORC1 pathway to respond to limiting nutrients in their metabolic niches. However, because no unique sensors have yet been identified in non-human systems and the evolutionary lineage of the sensors is not well understood, it is difficult to draw correlations between evolutionary pressures and the functional architecture of the TORC1 nutrient sensing pathway. The discovery of novel nutrient sensors outside higher eukaryotes would clarify the evolutionary logic of the nutrient sensing axis and define new inputs into the TORC1 pathway. Moreover, sensors initially characterized in other species could be conserved in human cell types with specialized metabolic environments.

Notes. For reasons that remain unclear, the KICSTOR complex is the sole exception to this generalization. KPTN, ITFG2 and C12orf66 seem to drop out of the evolutionary tree in organisms more distal than mammals; SZT2, the largest component of the complex, may have a putative homologue in *C. elegans* but is not retained in flies or yeast. If KICSTOR serves as a molecular glue that holds human GATOR1 and GATOR2 together in a supercomplex, as one study has argued[140], it is possible that it is dispensable in lower organisms where GATOR1 and GATOR2 are more constitutively bound to each other. Consistent with this hypothesis, the *S. cerevisiae* homologues for the GATORs, the SEACIT and SEACAT complexes, are indeed more tightly associated than their human counterparts and have been reported to form a supercomplex without any mediating proteins[272].

A. thaliana, *Arabidopsis thaliana*; CASTOR, cellular arginine sensor for mTORC1; *H. sapiens*, *Homo sapiens*; *M. Musculus*, *Mus musculus*; TSC, tuberous sclerosis complex.

Box 2:

mTOR, autophagy and neurodegeneration
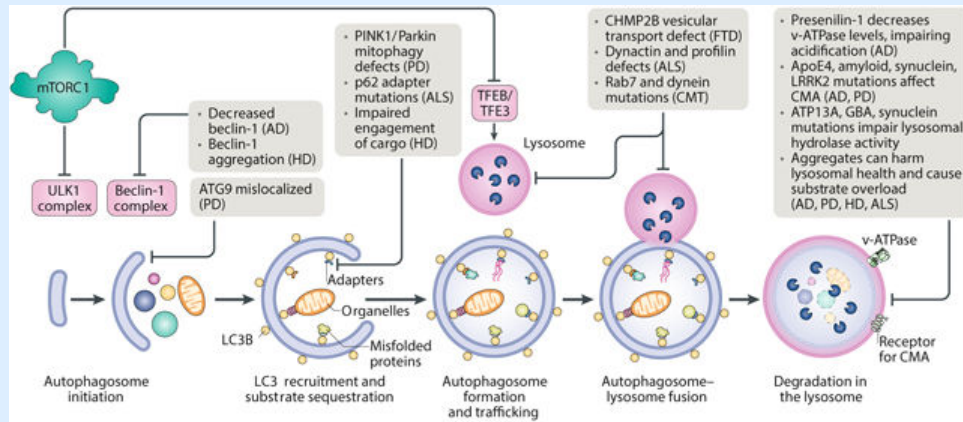
Genetic evidence implicates autophagy — and its major regulator, mTOR complex 1 (mTORC1) — in several devastating neurodegenerative disorders[273] (see the figure). These disorders, which include Alzheimer disease (AD), Parkinson disease (PD), Huntington disease (HD), amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), lead to the progressive, permanent destruction of neurons, wreaking havoc on cognition and motor control. Although most cases of neurodegeneration arise sporadically, increasing in frequency with age, certain heritable mutations can boost disease incidence and severity within families, with many such mutations mapping to genes associated with proteostasis and lysosomal function. Indeed, failures in autophagic clearance have emerged as a key hallmark of neurotoxic cell death. In AD, as in several of its pathological cousins, misfolded, ubiquitylated proteins appear to clog autophagic vacuoles, which subsequently accumulate in dystrophic neurites[274,275]. Because neuronal cells cannot divide to dilute unwanted macromolecules or organelles and must rely on autophagy, any jam in autolysosome clearance propagates through the entire endocytic machinery and may compound metabolic and immunological traumas that lie far afield from the initial amyloid stressor. Multiple groups have confirmed that deletion of essential autophagy genes in the brain is sufficient to induce neurodegeneration even in the absence of disease proteins[276,277], supporting a model that puts autophagy — and not amyloids — at the core of neurodegenerative disease.

The recent failure of drugs for AD targeting amyloid-β and tau in clinical trials has demonstrated that reduction of protein aggregates alone has little effect on cognitive function. Given the massive financial and societal costs of neurodegeneration, there is an urgent need for new therapies that delay or reverse disease progression through alternative mechanisms. Based on preclinical evidence, rapamycin may be a promising lead. Induction of autophagy through rapamycin treatment has been shown to eliminate aggregates and improve memory and behaviour in six different mouse models of AD, as well as several models of PD[278,279,280]. Moreover, as we will discuss below, rapamycin-mediated inhibition of mTOR may also reverse some of the cellular effects of ageing, the most important risk factor for neurodegeneration[237]. It should be noted, however, that

rapamycin does not penetrate the blood–brain barrier with ease and only partially blocks mTORC1 phosphorylation of autophagy regulator unc-51-like autophagy-activating kinase 1 (ULK1)[42]; in addition, chronic application of rapamycin for neuroprotection would likely disrupt major pathways inside and outside the brain. These caveats suggest that future therapeutic strategies may need to establish a precise balance of neuronal mTOR activity to maintain homeostasis — a goal that will require us to develop a more nuanced understanding of when, why, where and how mTOR acts in the brain.

CMA, chaperone-mediated autophagy; CMT, Charcot–Marie–Tooth disease; TFE3, transcription factor E3; TFEB, transcription factor EB.
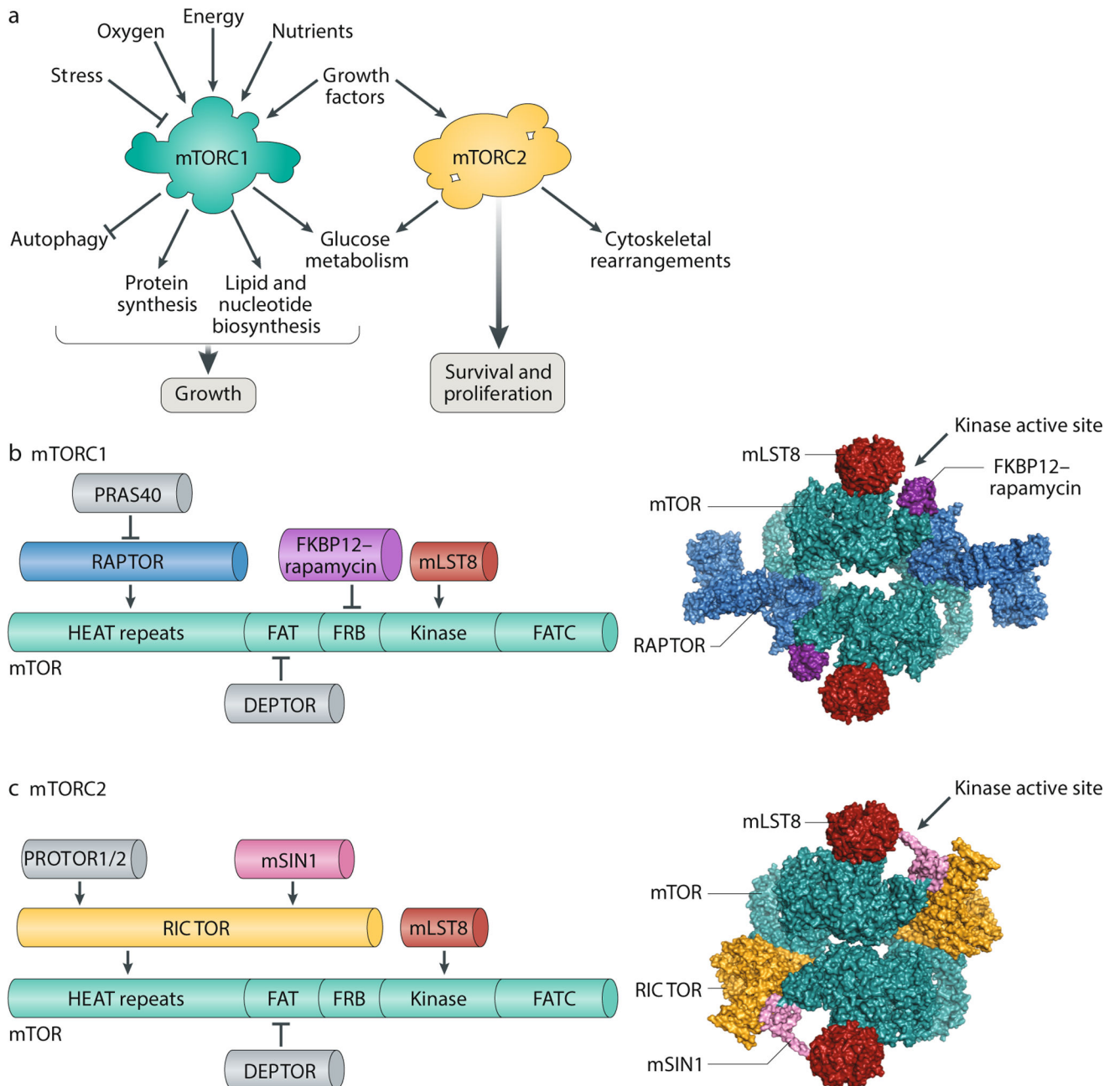
Fig. 1: Structure and function of mTORC1 and mTORC2.

A. mTOR complex 1 (mTORC1) and mTORC2 have distinct signalling roles in the cell. mTORC1 integrates information about nutritional abundance and environmental status to tune the balance of anabolism and catabolism in the cell, while mTORC2 governs cytoskeletal behaviour and activates several pro-survival pathways. Unlike mTORC1, which is acutely inhibited by rapamycin, mTORC2 responds only to chronic rapamycin treatment. **B**. Components of mTORC1 (left). The domain structure of the mTOR kinase (green) is annotated with binding sites for the other mTORC1 subunits. The N-terminus of mTOR contains clusters of huntingtin, elongation factor 3, a subunit of protein phosphatase 2A and TOR1 (HEAT) repeats, followed by a FRAP, ATM and TRRAP (FAT) domain; the

FKBP12–rapamycin binding (FRB) domain; the catalytic kinase domain; and the C-terminal FATC domain. mTOR binds mammalian lethal with SEC13 protein 8 (mLST8), a core component of the complex, and DEP-domain-containing mTOR-interacting protein (DEPTOR), an endogenous inhibitor of mTORC1 activity. Regulatory-associated protein of mTOR (Raptor), the defining subunit of mTORC1, binds mTOR with its own HEAT repeats and is required for lysosomal localization of the complex. Raptor also recruits proline-rich AKT substrate 40 kDa (PRAS40), an insulin-regulated inhibitor of mTORC1 activity. A 5.9-Å reconstruction of mTORC1 (without PRAS40 and DEPTOR) complexed with FKBP12–rapamycin is shown as a surface representation (Protein Database (PDB) ID: 5FLC) (right).
C. Components of mTORC2 (left). The mTOR kinase (green) is annotated with the binding sites for the other constituent subunits of mTORC2. These subunits include mLST8, DEPTOR and RICTOR, the defining component of mTORC2. As a scaffolding protein, RICTOR recruits protein associated with rictor 1 or 2 (PROTOR1/2) to the complex, along with MAPK-interacting protein (mSIN1), which contains a pleckstrin homology domain. A 4.9-Å reconstruction of mTORC2 (without DEPTOR and PROTOR) is shown as a surface representation (PDB: 5ZCS) (right).
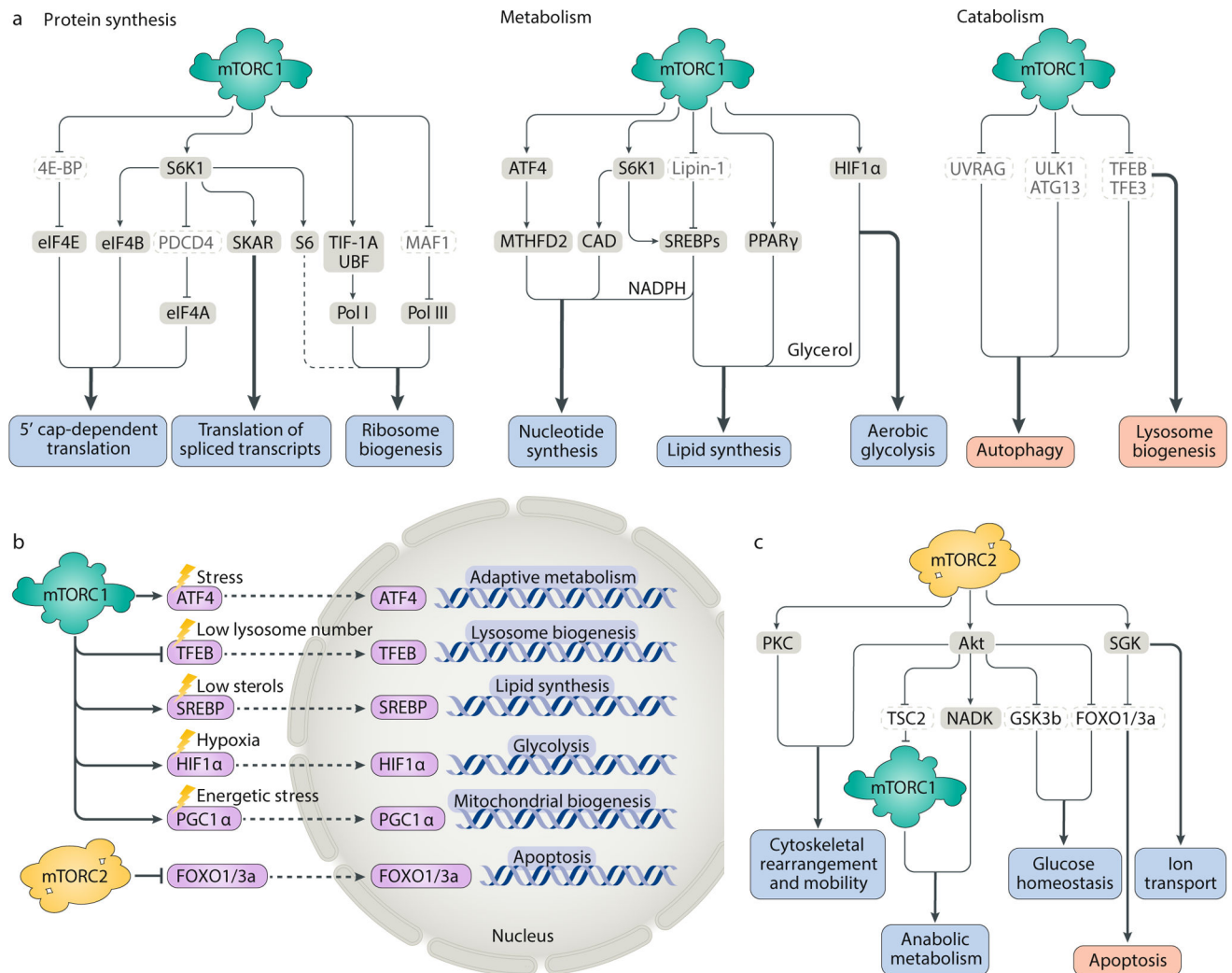
Fig. 2: Targets of mTORC1 and mTORC2 signalling.

A. mTOR complex 1 (mTORC1) activation initiates a downstream anabolic programme that enhances the production of proteins, lipids, nucleotides and other macromolecules while inhibiting catabolic processes, such as autophagy and lysosome biogenesis. **B**. By regulating the expression or nuclear localization of transcription factors, mTORC1 and mTORC2 control the expression of genes that promote organelle biogenesis or alter metabolic flux through biosynthetic pathways. Although these transcription factors can be independently activated by specific, acute cellular stress signals (for example, hypoxia inducible factor 1α (HIF1α) can be directly activated by hypoxia and ATF4 can be directly activated by endoplasmic reticulum stress), mTORC1 and mTORC2 toggle the activation of these factors in a coordinated manner to support growth and proliferation. Thus, activation of mTORC1 can simultaneously activate ATF4, the sterol regulatory element binding proteins (SREBPs), HIF1α and yin–yang 1 (YY1)–peroxisome proliferator-activated receptor-γ (PPARγ) coactivator 1α (PGC1α) to drive diverse processes involved in cellular growth, all while blocking lysosomal biogenesis through transcription factor EB (TFEB). **C**. mTORC2 activates the AGC family kinases protein kinase C (PKC), Akt and serum- and

glucocorticoid-induced protein kinase (SGK) to regulate the cytoskeleton, metabolism and ion transport and promote cell survival. CAD, carbamoyl-phosphate synthetase 2, apartate transcarbamoylase, dihydroorotase; 4E-BP, 4E-binding protein; eIF4, eukaryotic translation initiation factor 4; GSK3b, glycogen synthase kinase 3b; MTHFD2, methylenetetrahydrofolate dehydrogenase 2; PDCD4, programmed cell death 4; Pol I/Pol III, RNA polymerase I/RNA polymerase III; S6K1, p70 S6 kinase 1; TFE3, transcription factor E3; TIF-1A, transcription initiation factor 1A; TSC2, tuberous sclerosis complex 2; UBF, upstream binding factor; ULK1, unc-51-like autophagy-activating kinase 1.
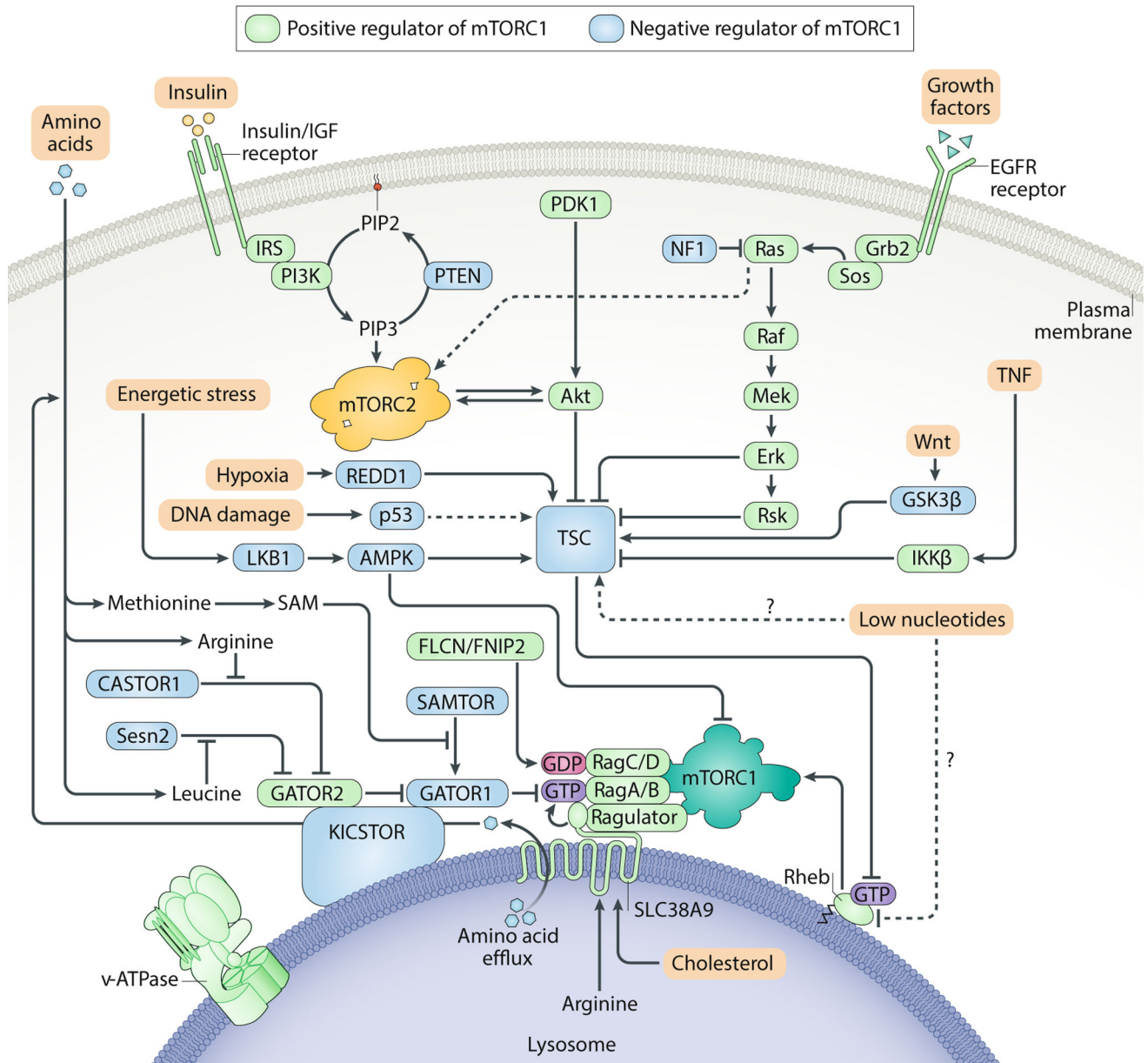
Fig. 3: Upstream regulators of the mTOR signalling pathway.
mTOR complex 1 (mTORC1) and mTORC2 integrate upstream environmental information
to gate their own activation. Because mTORC1 controls cellular entry into an anabolic state
that requires copious amounts of energy and macromolecules, activation of the complex
should only occur when amino acids, insulin/growth factors, ATP and oxygen are all readily
available. To ensure that all of these requirements are satisfied, mTORC1 must translocate to
the lysosome by anchoring onto the Rag GTPases, which are only competent to recruit
mTORC1 in the presence of amino acids. Once localized to the lysosomal surface, mTORC1
can be then be activated by the small GTPase Rheb in its GTP-bound state. Importantly,
GTP loading of Rheb is promoted by growth factors and opposed by energetic stress or
hypoxia. All of these inputs converge on tuberous sclerosis complex (TSC), which acts as a
GAP for Rheb. mTORC2 is thought to be primarily regulated by growth factors. Although it

is not clear where mTORC2 activation occurs, the pleckstrin homology domain on MAPK-interacting protein 1 (mSIN1) may recruit mTORC2 to the plasma membrane. Positive regulators of the mTORC1 pathway are shown in green, while negative regulators of mTORC1 are shown in blue. AMPK, AMP-activated protein kinase; CASTOR, cellular arginine sensor for mTORC1; EGFR, epidermal growth factor receptor; FLCN, folliculin; GATOR, GAP activity towards the Rags; Grb2, growth factor receptor-bound protein 2; GSK3, glycogen synthase kinase 3; IGF, insulin-like growth factor; IKKβ, inhibitor of nuclear factor κB kinase β; IRS, insulin receptor substrate; LKB1, liver kinase B1; Mek, MAPK/ERK kinase; NF1, neurofibromatosis type 1; PIP2, phosphatidylinositol (4,5)-bisphosphate; PIP3, phosphatidylinositol (3,4,5)-trisphosphate; PTEN, phosphatase and tensin homologue; RSK, p90 ribosomal S6 kinase; SAM, *S*-adenosylmethionine; SAMTOR, *S*-adenosylmethionine sensor; Sos, son of sevenless; TNF, tumour necrosis factor.
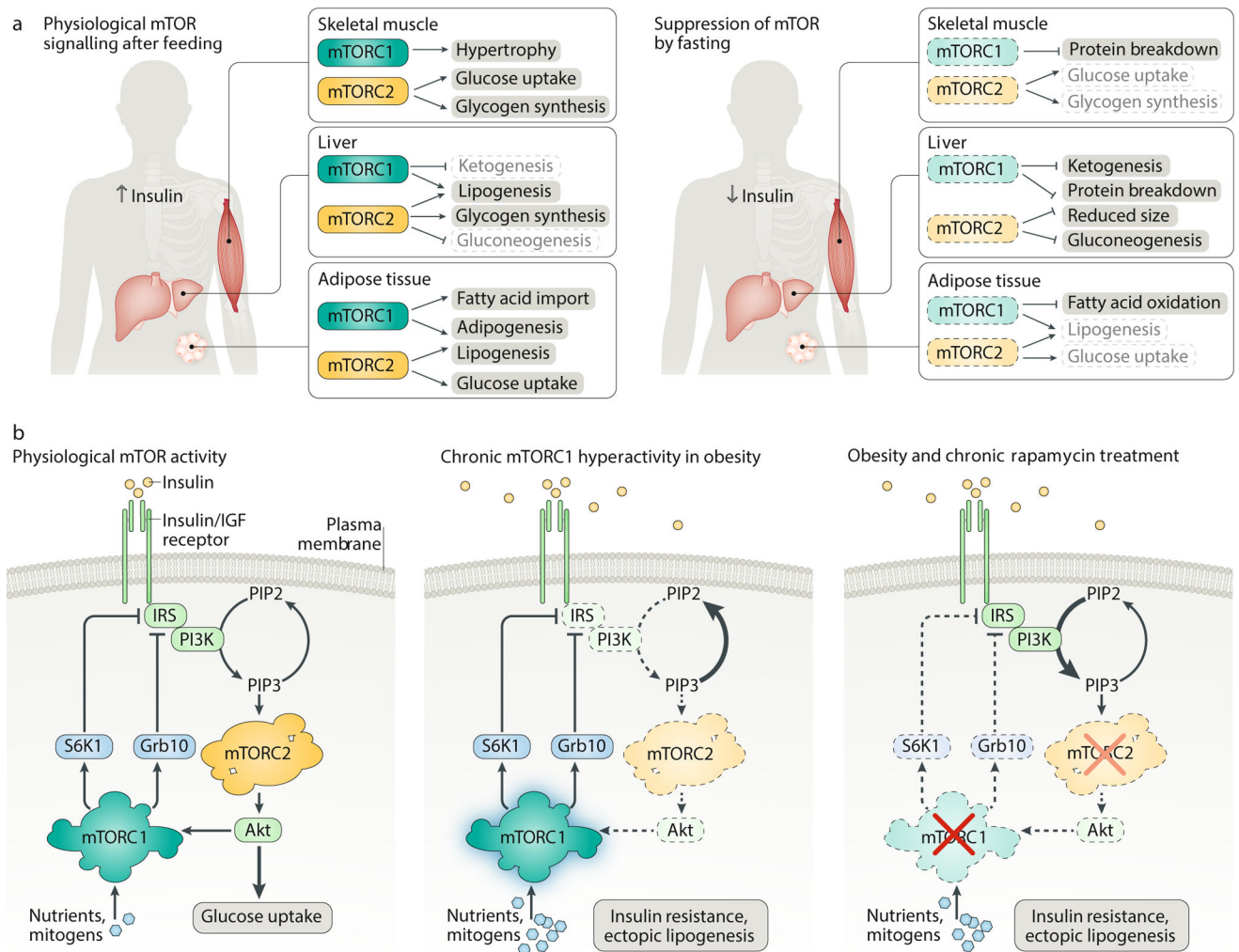
Fig. 4: mTOR signalling in metabolism.

A. mTOR coordinates feeding and fasting with nutrient storage and mobilization. In the liver, skeletal muscle and adipose tissue, rising insulin levels after feeding activate both mTOR complex 1 (mTORC1) and mTORC2, promoting lipogenesis, glycogen synthesis and protein synthesis (left). During fasting, the nutrient, growth factor and insulin levels drop precipitously, tilting the metabolic balance in favour of gluconeogenesis, ketogenesis and lipolysis (right). **B**. Dysregulation of mTOR signalling in metabolic syndrome. Although the negative feedback loop between mTORC1 and mTORC2 is carefully balanced under physiological conditions (left), chronic hyperactivation of mTORC1 by excessive nutrients and mitogens can shut off PI3K–mTORC2 signalling, leading to insulin resistance, ectopic accumulation of lipids in the muscle and liver, and type 2 diabetes (middle). Rapamycin-based therapies have not been effective in diabetes patients with hyperactive mTORC1 signalling because prolonged rapamycin treatment also inhibits mTORC2 (right). Grb10, growth factor receptor-bound protein 10; IGF, insulin-like growth factor; IRS, insulin receptor substrate; PIP2, phosphatidylinositol (4,5)-bisphosphate; PIP3, phosphatidylinositol (3,4,5)-trisphosphate; S6K1, p70 S6 kinase 1.
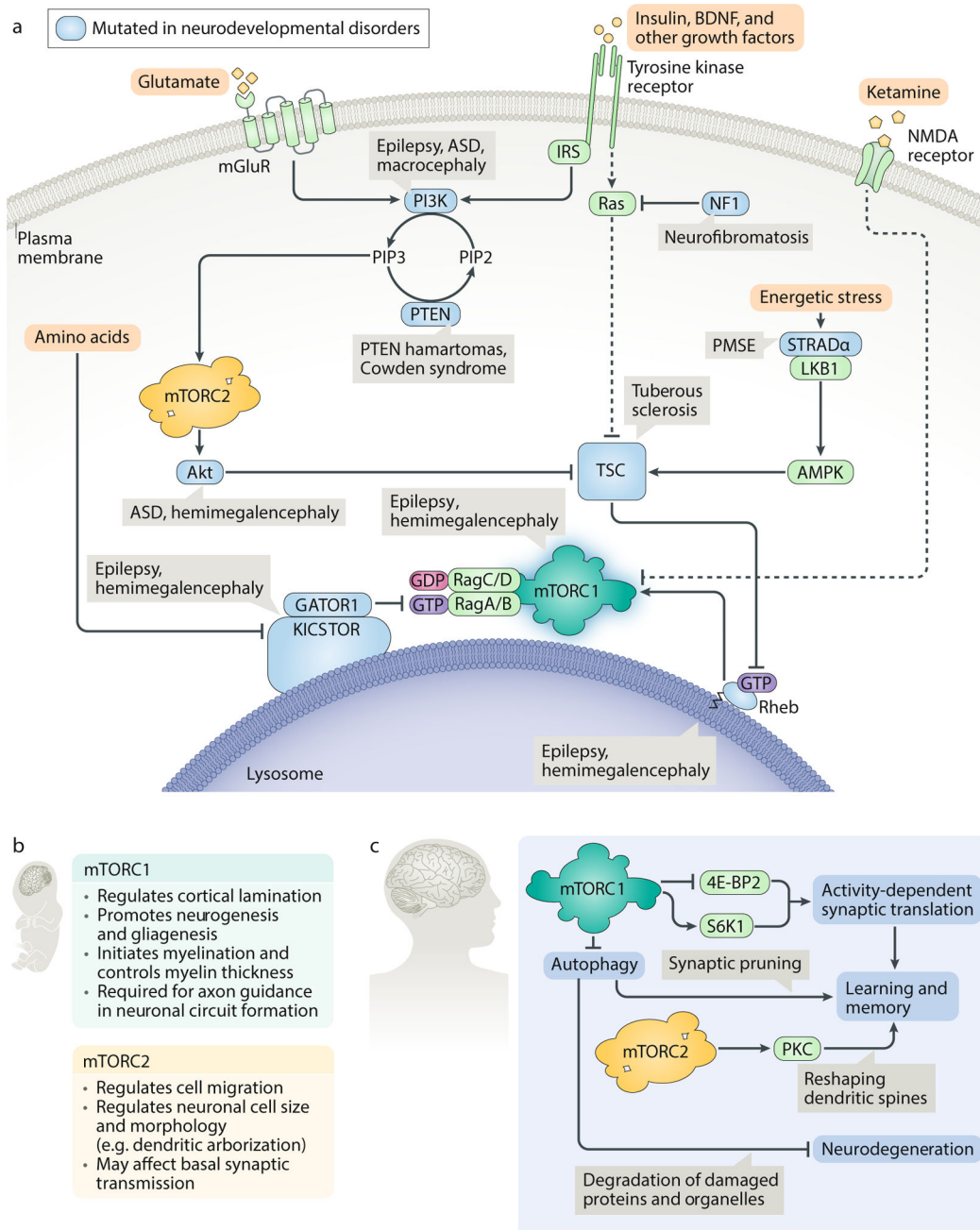
Fig. 5: mTOR signalling in the brain.

A. In the brain, mTOR complex 1 (mTORC1) signalling is activated not just by nutrients and insulin but also by several tissue-specific inputs, including the neurotransmitter glutamate and the neurotrophic growth factor brain-derived neurotrophic factor (BDNF). Dysregulation of the mTORC1 pathway is associated with a set of characteristic neurodevelopmental diseases, collectively termed 'mTORopathies'. Patients with mTORopathies suffer from severe epilepsy and may also display focal cortical dysplasia, macrocephaly or megalencephaly, cognitive and social defects, and benign tumours. Proteins from genes bearing mutations in neurodevelopmental diseases are shown in blue. **B**. Roles of

mTORC1 and mTORC2 during neuronal development. Ablation of mTORC1 or mTORC2 in the nervous system perturbs cell and organ size and disrupts the cortical architecture of the brain. mTORC1 deletion also causes early postnatal lethality. **C**. Roles of mTORC1 and mTORC2 in postnatal maintenance of synaptic plasticity and homeostasis. mTORC1 regulates activity-dependent synaptic translation through its substrates eukaryotic initiation factor 4E-binding protein 2 (4E-BP2) and p70 S6 kinase 1 (S6K1) to strengthen or weaken a given neuronal circuit; moreover, it also promotes synaptic plasticity by pruning obsolete synapses through autophagy. Autophagy may additionally play a neuroprotective role by degrading misfolded proteins and damaged organelles. mTORC2 remodels the actin cytoskeleton in response to neuronal signal transmission and helps convert transient excitatory events into long-term memory. AMPK, AMP-activated protein kinase; ASD, autism spectrum disorder; GATOR, GAP activity towards the Rags; IRS, insulin receptor substrate; LKB, liver kinase B1; NF1, neurofibromatosis type 1; PIP2, phosphatidylinositol (4,5)-bisphosphate; PIP3, phosphatidylinositol (3,4,5)-trisphosphate; PKC, protein kinase C; PMSE, polyhydramnios, megalencephaly and symptomatic epilepsy; PTEN, phosphatase and tensin homologue; STRADɑ, STE20-related kinase adapter protein-ɑ; TSC, tuberous sclerosis complex.
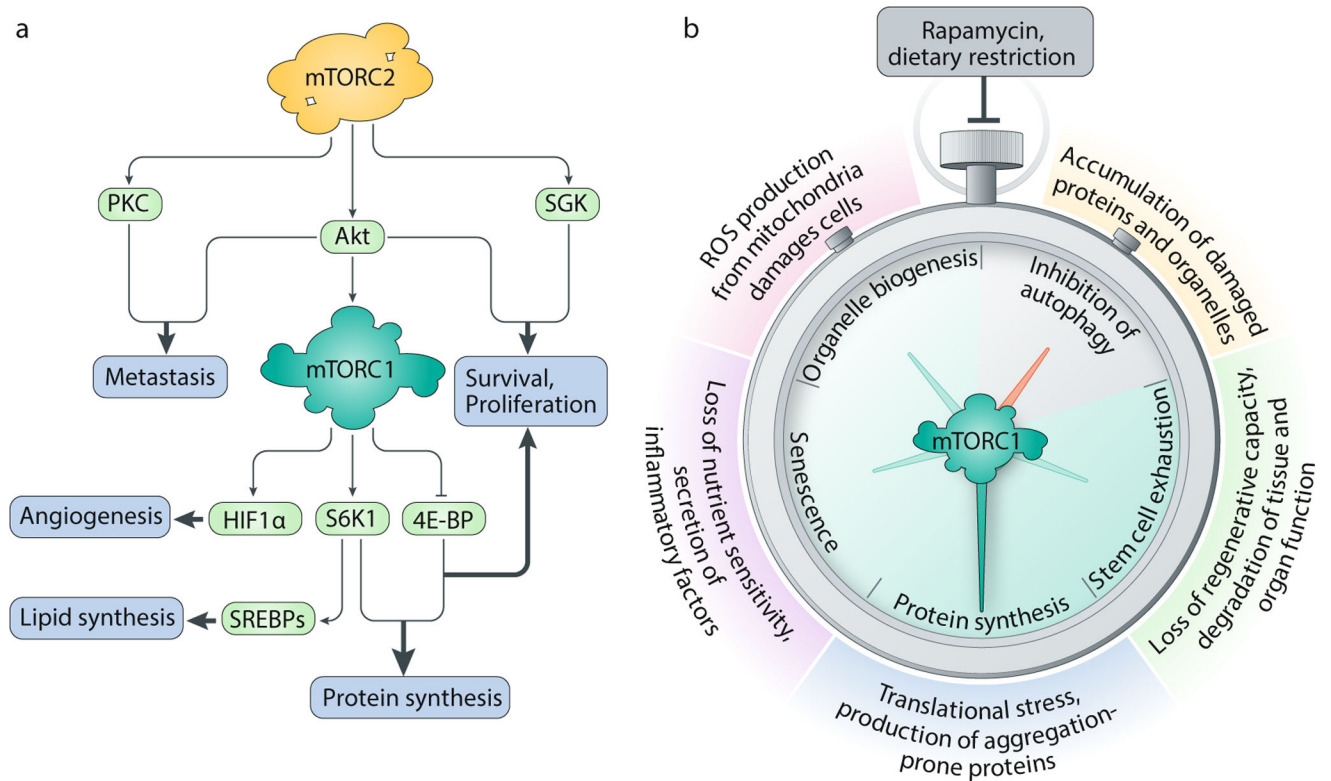
Fig. 6: mTOR in cancer and ageing.

A. mTOR complex 1 (mTORC1) and mTORC2 participate in cancer pathogenesis by underwriting biosynthetic programmes and promoting proliferation and survival. Emerging evidence also implicates mTORC2 activity in metastatic transformations. **B**. Modulation of mTORC1 signalling in ageing cells may enable us to slow the molecular clock. mTORC1 activates processes that may accelerate cellular and tissue ageing, including protein synthesis, mitochondrial energy production and entry into senescence. Chronic mTORC1 activation also blocks autophagic clearance of damaged cellular components. Inhibition of this pathway — by either rapamycin treatment, genetic inactivation of mTORC1 or dietary restriction — has been shown to extend lifespan and improve physiological performance across a range of model organisms. 4E-BP, 4E-binding protein; HIF1α, hypoxia inducible factor 1α; PKC, protein kinase C; ROS, reactive oxygen species; SGK, serum- and glucocorticoid-induced protein kinase; S6K1, p70 S6 kinase 1; SREBP, sterol regulatory element binding protein.