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In metazoans, TOR is an essential protein that functions as a master regulator of cellular growth and proliferation. Over the past decade, there has been an explosion of information about this critical master kinase, ranging from the composition of the TOR protein complex to its ability to act as an integrator of numerous extracellular signals. Unfortunately, this plethora of information has also raised numerous questions regarding TOR function. Currently, the prevailing view is that mammalian TOR (mTOR) exists in at least two molecular complexes, mTORC1 and mTORC2, which are largely defined by the presence of either RAPTOR or RICTOR. However, additional co-factors have been identified for each complex, and their importance in mediating mTOR signals has been incompletely elucidated. Similarly, there are differences in mTOR function that reflect the tissue of origin. In this review, we present an alternative view to mTOR complex formation and function, which envisions mTOR regulation and signal propagation as a reflection of cell type- and basal state-dependent conditions. The re-interpretation of mTOR biology in this framework may facilitate the design of therapies most likely to effectively inhibit this central regulator of cell behavior.

Key words: RAPTOR, RICTOR, neurofibromatosis, glioma, tuberous sclerosis complex

Abbreviations: See page 244 for abbreviations used in the text.

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Introduction

The mammalian target of rapamycin (mTOR) is one of the most studied signaling complexes, whose deregulated function leads to a variety of human disorders, ranging from cancer and immune dysfunction to autism and epilepsy.[1-5] The function of this signaling complex is particularly relevant to nervous system biology. As a regulator of protein translation in nerve cells (neurons), mTOR signaling controls synaptic plasticity by modulating long-term potentiation (LTP) and long-term depression (LTD), important for memory and learning.[6-9] Additionally, in the hypothalamus, mTOR regulates food intake and controls hormone production relevant to the onset of puberty.[10,11] In neurons, mTOR regulates axonal regeneration in response to nerve injury,[12-14] while in glial cells, it functions to limit astrocytic scar formation in the brain and spinal cord.[15,16] Similarly, mTOR signaling is affected in a number of neurodegenerative conditions, including Alzheimer disease, Parkinson disease, stroke and Huntington disease, such that pharmacologic inhibition of mTOR activity can reduce cognitive defects associated with these conditions in mice.[17-21] In other conditions, abnormal mTOR signaling has been implicated in the genesis of recurrent seizures, where it controls mossy fiber sprouting in the hippocampus.[22-24] Finally, recent studies have suggested that deregulated mTOR activation might underlie autistic-like behaviors in rodents.[25] Together, these observations highlight the essential role of mTOR in maintaining nervous system homeostasis both in health and in disease.

The critical importance of mTOR to nervous system function derives from studies of inherited cancer predisposition syndromes in which mutations in upstream regulators of mTOR result in mTOR hyperactivation (**Fig. 1**). These conditions include neurofibromatosis type 1 (NF1) and tuberous sclerosis complex (TSC). NF1 is a common autosomal dominant disorder in which affected children and adults are prone to the

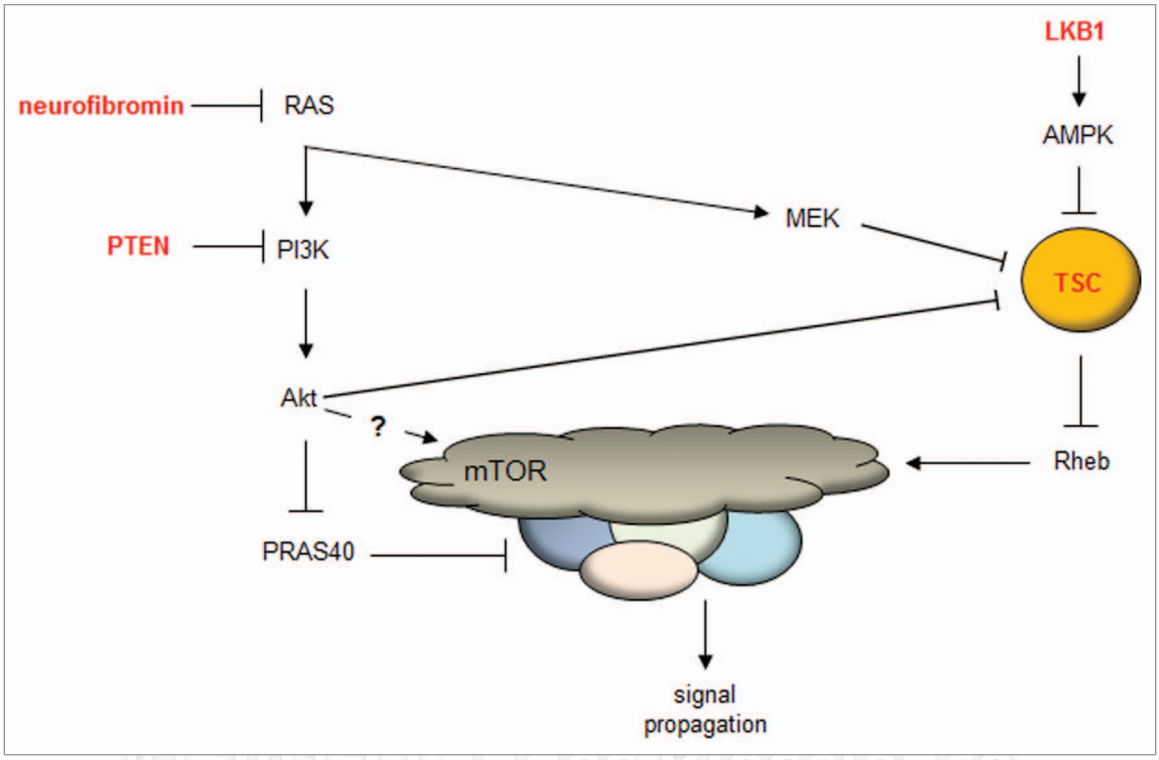

Figure 1. Current model of mTOR regulation. The mTOR complex is regulated by numerous upstream kinase molecules. Neurofibromin (*Nf1* gene product) loss leads to increased RAS activity, leading to AKT-mediated phosphorylation of PRAS40 and release of PRAS40-mediated mTOR inhibition. In addition, the increased RAS activity in *Nf1*-deficient cells leads to MEK activation, which phosphorylates tuberin (TSC complex), and increased Rheb-mediated mTOR activation. Lastly, LKB1 activates AMPK, which inhibits TSC complex function, such that loss of function mutations in LKB1 lead to increased Rheb-mediated mTOR activation.

development of peripheral nerve sheath tumors (neurofibromas), brain tumors (optic gliomas), learning disabilities and attention deficit.[26] The *NF1* gene is a classic tumor suppressor gene, such that bi-allelic *NF1* gene inactivation is required for tumorigenesis. The *NF1* protein, neurofibromin, functions in part as a negative regulator of the RAS proto-oncogene and serves to accelerate the conversion of active GTP-bound RAS to inactive GDP-bound RAS by virtue of the GTPase-activating protein (GAP) domain.[27] Loss of neurofibromin expression in astrocytes or neural stem cells in the brain results in increased RAS activation, and leads to high levels of activated AKT and MEK signaling, which converge on the mTOR complex to regulate cell growth.[27-29] In this respect, inhibition of mTOR hyperactivation using the macrolide rapamycin inhibits the growth of malignant peripheral nerve sheath tumors and optic gliomas in genetically engineered mice.[30-32] These exciting preclinical observations have leveraged the design and execution of new clinical trials for nerve sheath tumors and gliomas in individuals with NF1.

Individuals with TSC are prone to the development of brain tumors (subependymal giant cell astrocytomas, SEGAs), intractable epilepsy, autism and mental retardation.[33,34] In addition, affected individuals develop renal, heart and lung tumors, especially pulmonary lymphangioleiomyomatosis (LAM).[35] In contrast to NF1, mutations in one of two genetic loci (*TSC1* or *TSC2*) underlie the pathogenesis of TSC.[36,37] However, individuals with TSC harbor mutations in either the *TSC1* or *TSC2* gene, not both. The *TSC1* gene encodes hamartin, which forms a regulatory complex with the *TSC2* protein tuberin to negatively regulate the activity of a related RAS protein, termed RAS homolog expressed in brain (RHEB).[38-43] Bi-allelic inactivation of the *TSC1* or *TSC2* gene leads to loss of function of the tuberin-hamartin complex and results in increased RHEB activation and mTOR hyperactivation (see below). Similar to NF1, treatment of *Tsc* genetically engineered mice with rapamycin to inhibit mTOR activity leads to reduced seizure frequency and severity.[43] Moreover, clinical trials using rapamycin analogs have demonstrated efficacy in the treatment of SEGAs in individuals with TSC.[44-46]

Other inherited syndromes that do not affect the nervous system have also provided insights into the role of mTOR signaling in normal growth control. Germline mutations in the *PTEN* tumor suppressor gene result in a variety of clinical conditions, including Cowden syndrome, Proteus syndrome, autism spectrum disorder (ASD) and Lhermitte-Duclos disease.[47-51] In each of these syndromes, mutational inactivation of the *PTEN* gene leads to deregulated AKT signaling and increased mTOR activation. In genetically engineered mice, *Pten* inactivation in neuroglial cells results in progressive seizures, which can be dramatically attenuated with rapamycin analog treatment.[52-54] Moreover, these *Pten* conditional knockout mice exhibit abnormal social

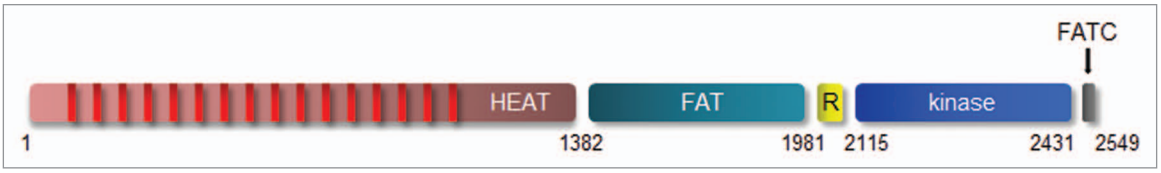

Figure 2. Anatomy of the mTOR protein. The mTOR protein is composed of several domains, including a HEAT (Huntington, EF3A, ATM, TOR), FAT (FRAP, ATM, TTRAP), R (TOR), FATC (FRAP, ATM, TRRAP C-terminal) and kinase domain. The amino acid residues are shown along the bottom.

interactions and exaggerated responses to sensory stimuli, which are also suppressed by pharmacologic mTOR inhibition.[25] In addition, mutations in the *LKB1* gene are responsible for Peutz-Jeghers syndrome, a disorder of intestinal polyp formation.[55] LKB1 is a serine/threonine kinase that activates 5'AMP-activated protein kinase (AMPK) by phosphorylation to inhibit tuberin function.[56-58] Mutational inactivation of the *LKB1* gene results in mTOR hyperactivation under low energy conditions and underlies the propensity for tumor formation in conditional knockout mouse strains.[59,60]

In this Extra View, we will review the current understanding of mTOR regulation and signaling, discuss emerging and unresolved controversies in mTOR biology and propose a new conceptual model for mTOR function.

Current Concept of mTOR and its Complexes

Although cell growth, an increase in cell mass and size through macromolecular synthesis, is distinct from cell cycle progression and division, these two processes are generally tightly regulated. In eukaryotic systems, mTOR is a key regulator of cell mass and size, identified through the elucidation of the target of an antifungal agent, rapamycin.[61] Using genetic screens, the intracellular rapamycin receptor FKBP12 and TOR were discovered.[62-65] Eukaryotic TORs are large proteins (~280 kDa) that exhibit 40–60% sequence homology at the amino acid level and are members of the phosphatidylinositol-3-kinase-related protein kinase (PIKK) family (**Fig. 2**).[66-68] Unlike PI3K, which possesses lipid kinase activity, TOR functions solely as a protein kinase.[69,70] In *Saccharomyces cerevisiae*, mutations in *Fpr1* (gene encoding FKBP12) or *Tor1* allow for growth in the presence of rapamycin, firmly establishing the products of these genes as the bona fide rapamycin targets.[62] Whereas deletion of *Tor1* in yeast results in decreased cell proliferation but not lethality, *TOR* deletion in metazoans is lethal. Genetic studies in *Drosophila melanogaster* first revealed the role of dTOR in regulating cell size; *dTor*-deficient flies exhibit a cell autonomous decrease in both cell size and proliferation.[71,72] Mammalian TOR (mTOR) is ubiquitously expressed in tissues, with highest levels of expression in the brain and skeletal muscle.[73] Consistent with this pattern of expression, conditional deletion of mTOR in muscle results in decreased muscle mass, muscle dystrophy and decreased oxidative capacity.[74]

The use of rapamycin has been a valuable tool for identifying molecular targets downstream of mTOR. While many proteins have been elucidated using this strategy, S6K-1 and 4EBP1 are the best-characterized molecules. S6K-1 phosphorylates the ribosomal protein S6 (rpS6).[75,76] Rapamycin treatment inhibits S6K-1 activity and, thus, reduces rpS6 phosphorylation in multiple cell types under varying stimuli.[77,78] In flies, overexpression of dS6K rescues the growth phenotype of dTOR deficiency, indicating that S6K is a critical downstream effector of dTOR in cell size determination.[79] Moreover, *S6k1*-knockout mice are considerably smaller than their wild-type littermates, further stressing the importance of S6K activity in mediating cell growth and size.[80]

4EBP1 is a potent translational repressor, binding tightly to the eIF4E mRNA cap-binding protein. Through its interaction with eIF4E, 4EBP1 effectively competes with eIF4G, preventing its access to eIF4E. Stimulation of mTOR signaling results in 4EBP1 phosphorylation and eIF4E release, allowing eIF4E to bind to eIF4G and initiate cap-dependent mRNA translation.[81,82] Treatment with rapamycin prevents the dissociation of 4EBP1 from eIF4E, resulting in continued translational repression and securing 4EBP1 as a downstream substrate of mTOR.[83]

In recent years, a distinction has been made between mTOR complexes based on their sensitivity to rapamycin treatment (**Fig. 3**). mTOR complex 1 (mTORC1) is sensitive to rapamycin and consists of RAPTOR, PRAS40, mLST8 and DEPTOR proteins.[73,84] Rapamycin binds to FKBP12, such that the rapamycin-FKBP12 complex inhibits RAPTOR-bound mTOR. mTOR complex 2 (mTORC2) is relatively insensitive to rapamycin and consists of RICTOR, PROTOR, DEPTOR, mLST8 and mSIN1.[85] However, prolonged treatment with rapamycin can inhibit mTORC2, most likely due to the progressive sequestration of pools of mTOR by rapamycin-FKBP12. This model predicts that overproduction of mTOR would restore mTORC2 assembly and activation in the prolonged presence of rapamycin, although this has not formally been tested.

While the individual proteins of the mTOR complexes and their precise functions are hotly debated, several key players have been identified through loss-of-function studies. Depletion of RAPTOR in mammalian cells results in decreased cell size and lower levels of phosphorylated S6K-1, demonstrating a crucial role for RAPTOR in mTOR signaling.[73] Moreover, non-ionic detergent treatment of mTORC1 complexes release RAPTOR from mTOR, resulting in markedly attenuated mTOR activity and a lack of 4EBP1 phosphorylation.[84] Similarly, depletion of mLST8 results in decreased cell size and lower S6K-1 phosphorylation, establishing mLST8 as an integral component of mTORC1.[86] Of note, mLST8 binds to the kinase domain of mTOR and facilitates enhanced binding of RAPTOR to mTOR. These findings further support an integral function of mLST8 in mTOR

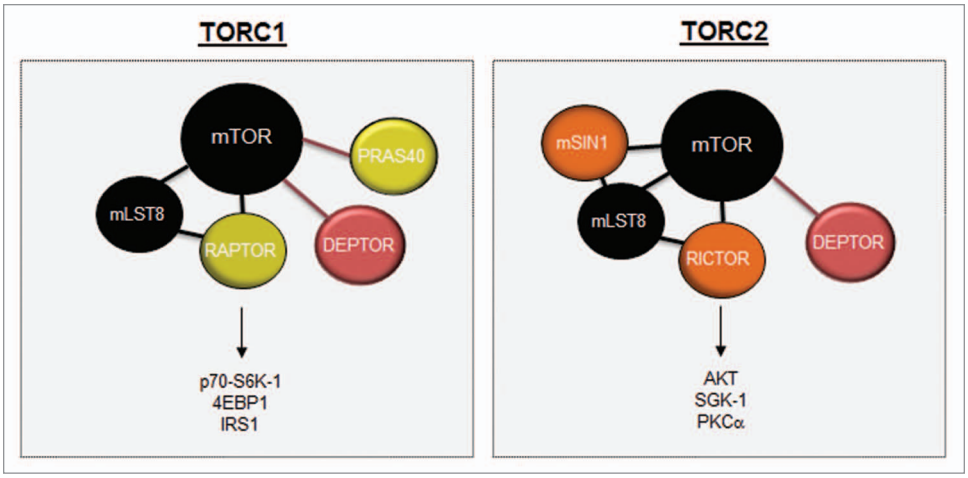

Figure 3. mTOR complexes. mTOR is composed of two distinct complexes, mTORC1 and mTORC2. TORC1 is composed of mLST8, RAPTOR, DEPTOR and PRAS40, while the mTORC2 complex is composed of mSIN1, mLST8, RICTOR and DEPTOR. mTORC1 signals to S6K1, 4EBP1 and IRS1, whereas mTORC2 activates AKT, SGK1 and PKCα.

activation through its ability to bind to mTOR.

RICTOR was discovered in mTOR immunoprecipitates and shares significant regions of homology with the Avo3p rapamycin-insensitive protein from *S. cerevisiae*. RICTOR is found in distinct mTOR complexes devoid of RAPTOR and is unable to bind FKBP12-rapamycin.[85] RICTOR-bound mTOR contains mLST8, but these complexes are unable to phosphorylate S6K-1. Instead, RICTOR-mTOR-mLST8 complexes modulate the phosphorylation of PCKα and AKT.[87] Further studies of mTORC2 identified mSIN1 as a necessary protein in the assembly of mTORC2. mSIN1 exists as multiple isoforms, with at least three of these isoforms establishing unique mTORC2 complexes, each being regulated by different upstream stimuli.[88] Moreover, mSIN1 mediates the interaction of SGK-1 with mTORC2, suggesting that it might serve as a scaffold for downstream mTOR substrates. PROTOR (1 and 2) was recently added to the list of mTORC2-specific-interacting proteins. However, knockdown of PROTOR-1 does not affect mTORC2 complex formation or activity, suggesting that PROTOR-1 is not an essential mTORC2 component.[89]

mTOR complexes are controlled by a set of negative regulators, including PRAS40 and DEPTOR. PRAS40 was first identified as an mTOR-interacting protein by mass spectrometry of mTOR complexes.[90] PRAS40 binds to the kinase domain of mTOR, although its interaction does not release mLST8. Additionally, PRAS40 is phosphorylated by activated AKT, and this phosphorylation results in the either the full release or lessened affinity of PRAS40 for mTOR through the formation of PRAS40 and 14-3-3 protein complexes.[90,91] DEPTOR was discovered following immunoprecipitation of RAPTOR and RICTOR proteins. Knockdown of DEPTOR results in the activation of both mTORC1 and 2, implying that it is a negative regulator of both complexes.[92] However, overexpression of DEPTOR only inhibits mTORC1; mTORC2 activity is elevated upon DEPTOR overexpression. While it is unclear how this asymmetrical signaling is achieved, it is clear that mTORC1 and 2 both phosphorylate DEPTOR, signaling for its destruction by the SCF (βTrCP) E3 ligase.[93]

To date, the evidence for two distinct mTOR complexes is largely based on the identification of independent RAPTOR- and RICTOR-containing immunoprecipitates with mTOR. Clearly, RAPTOR and RICTOR are not found in immunoprecipitates of the other protein, and RICTOR appears to interact with proteins unique to its complex (mSIN1 and PROTOR). However, the finding of multiple mSIN1 isoforms pertaining to multiple mTORC2s suggests that a simple view of two complexes may warrant further scrutiny. Many of these studies were performed in fibroblasts or established cell lines, where components may not be uniformly expressed or functional. Recent studies suggest that mTOR regulation of brain cell function may not follow these rules (see below). These new findings suggest that some of the existing dogma may need to be reassessed.

Upstream Regulation of mTOR Activation

Nutrients, energy, stress and growth factors are the major upstream signaling inputs for the mTORC1 complex (**Fig. 4**). While the varying flux of signals from each of these pathways cooperates to activate mTOR, we will discuss each component separately. Amino acids are the building blocks of proteins and can be used in the synthesis of nucleic acids and ATP. The RAG family of small GTPases serves as the link between intracellular amino acids and mTORC1.[94,95] RAG proteins form heterodimers of RAGA or RAGB with either RAGC or RAGD. In the absence of amino acids, RAG complexes are inactive, and RAGA and RAGB are bound to GDP. The presence of amino acids activates RAGA and RAGB and switches them to GTP-bound proteins that interact with RAPTOR. This interaction causes mTORC1 to re-localize to the surface of endosomes and lysosomes, enabling mTORC1 to interact

with another small GTPase, Ras homologous enriched in brain (RHEB).[94,96] GDP-loaded RHEB is unable to interact with mTORC1. However, upon growth factor stimulation, AKT phosphorylates and inactivates tuberin (*TSC2* protein),[97-99] which, together with its binding partner hamartin (*TSC1* protein), acts as a GTPase-activating protein (GAP) for RHEB.[38,39,41,43] In this manner, TSC1-TSC2 complexes are potent inhibitors of mTORC1 activity. Release of hamartin/tuberin complex negative regulation of RHEB results in increased RHEB GTP-loading, which, in turn, stimulates mTOR kinase activity. Overexpression of RHEB maintains mTORC1 activity even in the absence of nutrients and growth factors,[38,39,41,100] demonstrating its central role in activating mTORC1.

Previous studies using established cell lines support a model in which the primary mode of mTOR activation involves TSC-RHEB signaling. In these studies, several signaling intermediates (AMPK, MEK and AKT) regulate mTOR activity by phosphorylating tuberin on a number of residues to result in RHEB-mediated mTOR activation.[39,98,101] For example, mTOR hyperactivation and increased tumor cell line growth was inhibited when an interfering tuberin phospho-mutant protein was introduced.[97,98] One possible exception to this route of mTOR activation involves PRAS40-mediated mTOR regulation. PRAS40 was initially shown to operate upstream of mTOR, such that AKT phosphorylation of PRAS40 relieved TORC1 suppression.[90,91] However, more recent studies suggest that PRAS40 may also be a downstream target of mTORC1 activity.[102,103] Knockdown of PRAS40 results in a reduction in RAPTOR bound to mTOR and attenuated mTORC1 activity, suggesting that PRAS40 might positively regulate the assembly or stabilization of mTORC1.[102] This would be consistent with dual roles for PRAS40 in negatively regulating the short-term activity of mTORC1 while enhancing the long-term stability of mTORC1. Analysis of the AKT phosphorylation site (Thr-246) and one of the three mTOR phosphorylation sites (Ser-221) revealed their concerted importance in promoting 14-3-3 binding for PRAS40, which helps to remove PRAS40 from the complex, thus establishing that upstream (by AKT) and downstream (by mTORC1) phosphorylation of PRAS40 are fully activated mTORC1.[104]

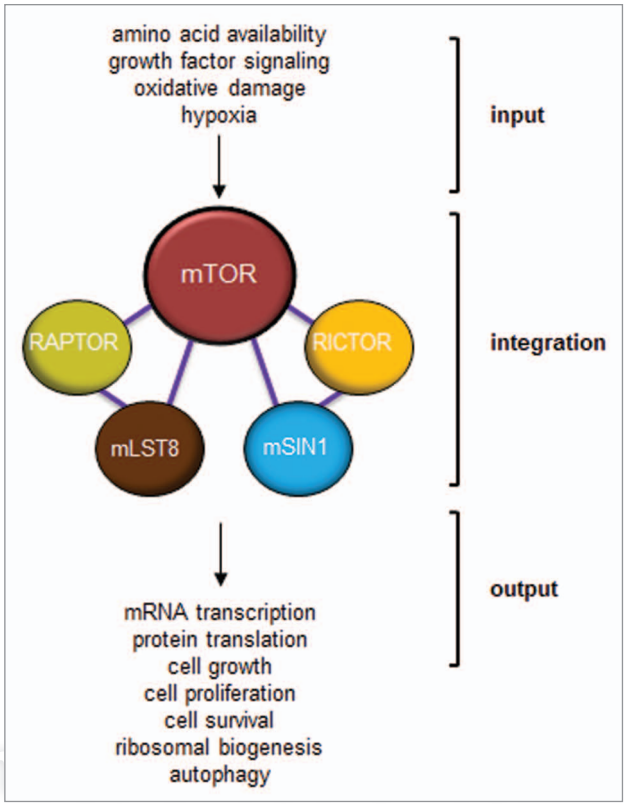

Figure 4. mTOR functions as a molecular integrator. Various inputs to mTOR provide information about amino acid availability, growth factor mitogenic signaling, oxidative damage and oxygen levels. This information must be integrated by mTOR through the use of its binding partners to provide meaningful outputs that dictate mRNA transcription, protein translation, cell growth, cell survival, ribosomal biogenesis and autophagy.

Given the importance of mTORC1 in controlling ribosome biogenesis and mRNA translation, both of which require massive amounts of cellular energy, it is appropriate that mTORC1 acts as a sensor of energy and stress. In the absence of nutrients, cellular ATP levels rapidly decline due to altered mitochondrial respiration and glycolysis and trigger the AMP-activated kinase (AMPK). Under increased levels of AMP, AMPK phosphorylates TSC2 to activate its GAP activity toward RHEB and inactivate mTOR.[56,105] Additionally, AMPK phosphorylates RAPTOR, leading to RAPTOR-14-3-3 binding and allosteric inhibition of mTORC1.[106] AMPK is capable of relaying signals from both energy stress, such as hypoxia,[107] as well as other cellular stresses, such as DNA damage.[108] The latter is achieved through the p53 transcriptional induction of SESTRIN1 and SESTRIN2, which are both potent activators of AMPK.[109] Thus, AMPK is positioned as a central cellular stress relay kinase to mTORC1.

Of note, little is known about the upstream activating signals for mTORC2. While it is appreciated that mTORC2 is largely activated by growth factors,[110] its ability to signal in any specific manner seems improbable given the plethora of potential upstream signals. This is further highlighted by the divergence of AKT, SGK and PKCα regulation by mTORC2 in response to various growth factors. Thus, mTORC2 must somehow be capable of distinguishing between growth factor inputs to elicit the proper downstream kinase activation. Our earlier discussion of mSIN1 isoforms might provide some clues to this achieved diversity of mTORC2, but it seems more likely that other, as-yet-unidentified mechanisms

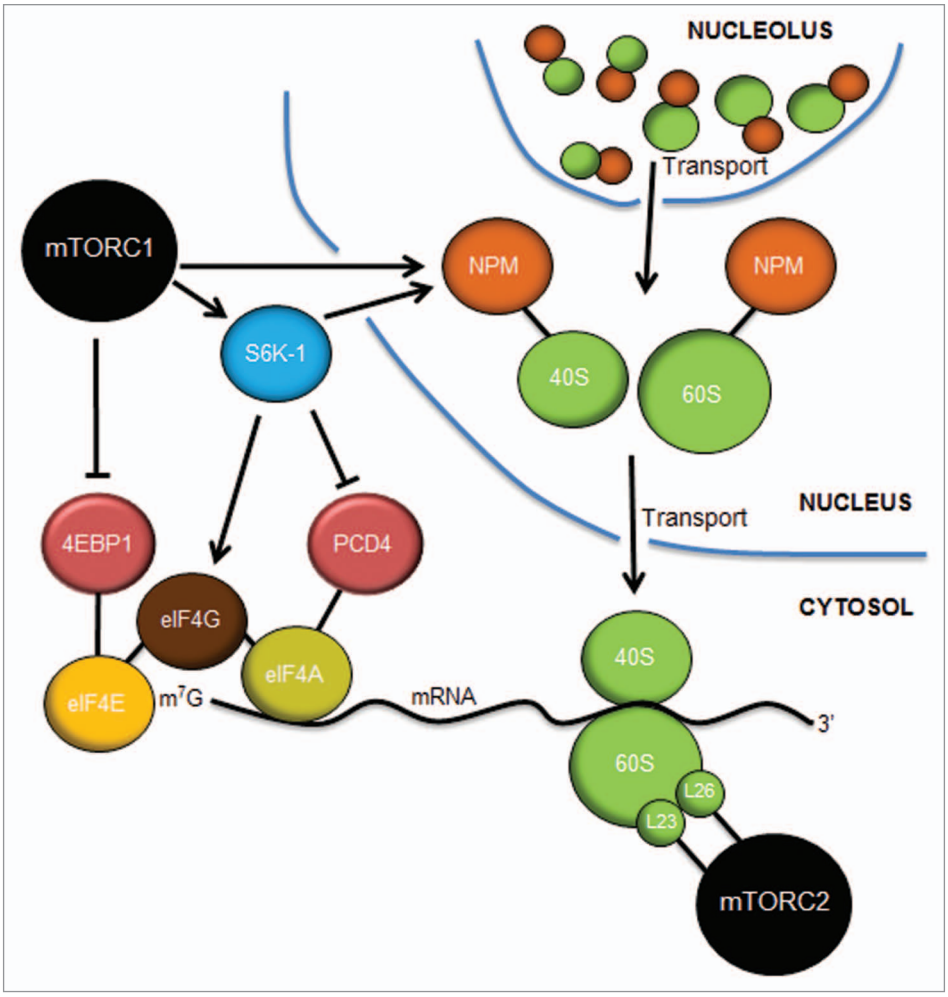

Figure 5. Control of translation by mTORC1 and mTORC2. mTORC1 controls cap-dependent translation through the phosphorylation and inactivation of 4EBP1, freeing eIF4E to bind to the 7-methylguanosine (m^7G) cap structure at the 5' end of mature mRNAs. S6K-1 phosphorylates eIF4G to initiate its interaction with eIF4G at the cap while also inactivating PCD4, the negative regulator of the eIF4A RNA helicase. mTORC1 (in astrocytes) and S6K-1 (in fibroblasts) stimulates the increased expression of nucleophosmin (NPM), which acts in a rate-limiting manner to transport mature 40S and 60S ribosome subunits from the nucleolus/nucleus into the cytosol, where they engage in mRNA translation. mTORC2 interacts with rpL23 and rpL26 of the 60S subunit on polysomes during translational elongation. Inhibition of mTORC1 or mTORC2 dramatically attenuates mRNA translation.

must be in place to provide this diversity in mTORC2 signaling.

Downstream mTOR Signaling

After dealing with such a diverse array of input signals, both mTORC1 and mTORC2 must direct a signaling program that leads to the activation of critical cellular processes.[111] In this regard, it has been easier to establish consequences of mTORC signaling than to determine the underlying intricate mechanisms of how the mTOR complex performs these decisions. As such, the hallmark of mTORC1 activity is the stimulation of ribosome biogenesis and mRNA translation (**Fig. 5**). Both processes are inhibited by rapamycin and rescued by proteins downstream of mTORC1. First, active S6K-1 stimulates the translation of mRNAs containing unique 5'-terminal oligopyrimidine tracts (TOPs).[112,113] TOP-containing mRNAs encode ribosomal proteins, elongation factors and other critical components of ribosome production.[114] However, S6K-1 activity is not obligatory for this important process; cells lacking S6K-1 still actively translate TOP mRNAs in response to growth factor stimulation through a potentially redundant mechanism that does not involve the phosphorylation of rpS6.[113,115]

Second, phosphorylation of 4EBP1 by mTORC1 signals the onset of cap-dependent translation.[116-118] Without 4EBP1 phosphorylation, 40S ribosome subunits (newly produced by S6K-1 signals) would not engage the cap complex of eIF4E, eIF4G and eIF4A to enable the beginning of mRNA scanning. The eIF4G protein is also a direct target of S6K-1. Mitogen signaling induces phosphorylation of Ser-422 in a rapamycin-sensitive and S6K-1-dependent manner.[119] A Ser422Ala mutant mimicking non-phosphorylated eIF4G is unable to stimulate cap-dependent translation,[119] and RNAi knockdown of eIF4G leads to polysome reduction and translational repression of key proteins involved in cell survival and proliferation.[120] Additionally, S6K-1 phosphorylates and inactivates the programmed cell death 4 protein (PCD4). Phosphorylation

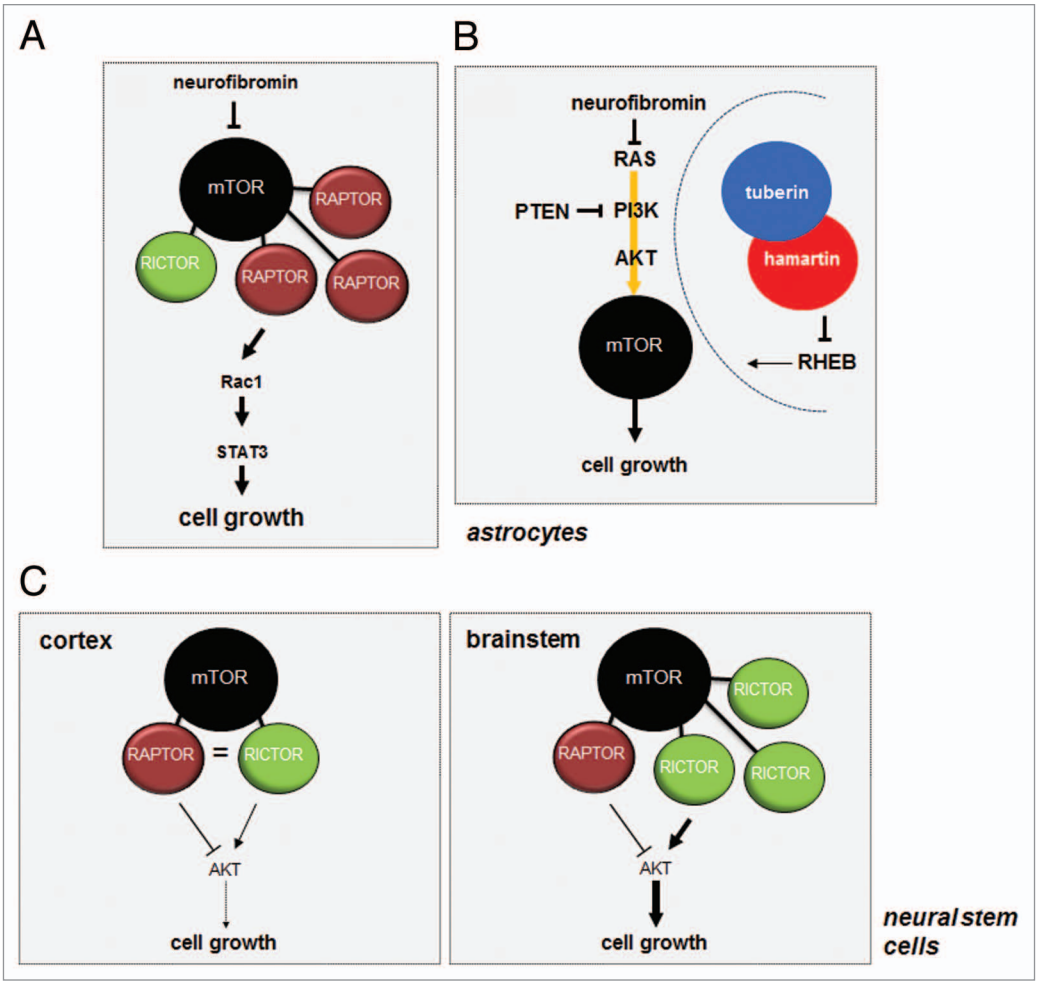

Figure 6. mTOR signaling in the brain does not follow the established canonical rules. (A) In astrocytes, neurofibromin negatively regulates mTOR through RAS/AKT-mediated signaling. Loss of neurofibromin leads to increased mTOR activation, which results in Rac1 and STAT3 activation through RAPTOR. (B) While tuberin phosphorylation results in loss of TSC complex function and increased RHEB-mediated mTOR activation, *NF1* protein (neurofibromin) and PTEN loss in astrocytes leads to AKT-dependent mTOR activation, which does not involve TSC complex regulation. Moreover, *Tsc1/Tsc2* inactivation or Rheb overexpression in vitro or in vivo does not recapitulate the effects of *Nf1* or *Pten* loss on astrocyte growth or gliomagenesis in genetically engineered mice. (C) mTOR-regulated AKT activation in neural stem cells (NSCs) following *Nf1* gene loss is controlled by the levels of RAPTOR and RICTOR. NSCs from the cortex fail to activate AKT and increase their proliferation following *Nf1* loss, whereas those from the brainstem activate AKT and exhibit increased proliferation. This region-specific difference reflects the relative abundance of RAPTOR and RICTOR in NSCs from these two brain regions.

of PCD4 on Ser67 prevents its association with eIF4A and signals the destruction of PCD4 by β-Trap.[121] Released eIF4A then interacts with active eIF4G to initiate translation.[122] This clearly places S6K-1 as a central kinase in the activation of the cap-dependent pre-initiation complex. Moreover, mTORC1 provides the key signals to both produce ribosomes and to enable those ribosomes to engage in mRNA translation in response to appropriate nutrient, energy and growth signals.

In addition to directly targeting the translational machinery, mTORC1 indirectly enhances ribosome biogenesis.[123] Indeed, one of the major targets of mTORC1 signaling is the nucleophosmin (NPM) proto-oncogene, a TOP-containing mRNA. Growth signals, such as those emanating from RAS, stimulate the translation of existing NPM mRNAs in a rapamycin-sensitive mechanism[124] through the binding of far upstream binding protein-1 (FUBP-1) to the 3' untranslated region (UTR) of NPM mRNA.[125] In agreement with this finding, astrocytes lacking *Nf1* express elevated NPM protein levels and exhibit enhanced protein synthesis.[126] NPM proteins engage assembling ribosomes in the nucleolus and aid in their transport into the cytosol, placing NPM as a critical nucleolar sensor of mTOR signals[127,128] (**Fig. 5**). Moreover, actin cytoskeleton rearrangement and enhanced proliferation of *Nf1*-deficient astrocytes is dependent on elevated NPM expression and its ability to properly transport ribosomes to the cytosol.[129]

Only recently have we begun to appreciate the complexity of downstream mTORC2 signaling. Nonetheless, mTORC2 activation of AKT, SGK1 and PKCα appears to place mTORC2 as the effector of numerous and diverse biological processes. AKT itself possesses pleiotropic

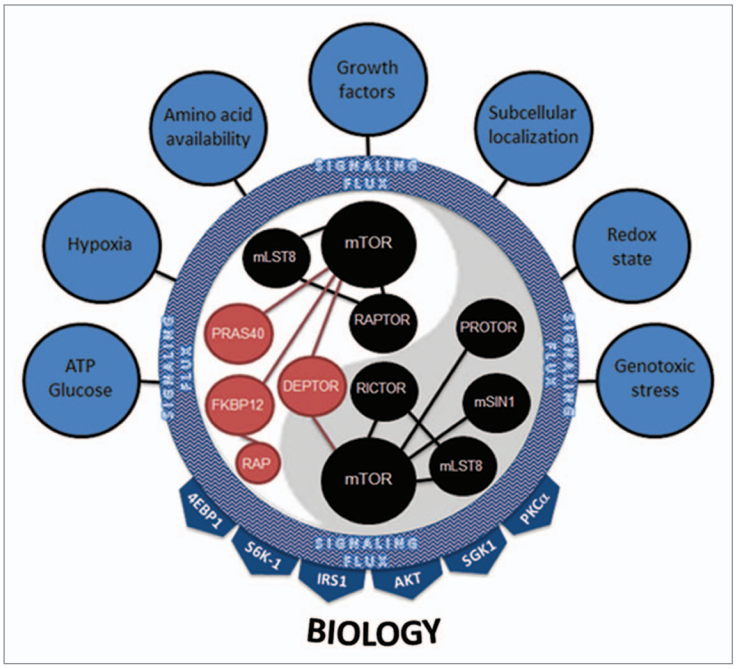

Figure 7. mTORC1 and mTORC2 integrate multiple inputs and stimulate numerous effectors. Seven major categories of inputs signal to mTOR complexes. Their combined inputs are viewed as aggregate signaling flux that is ultimately interpreted by both mTORC1 and mTORC2. The dynamics of this interpretation most likely involve changes in the protein expression level of positive (black) and negative (red) regulators of mTOR kinase activity, post-translational modification of mTORC members, binding of complexes to downstream effectors or, most likely, a combination of all of these. mTOR complexes then activate downstream signaling effectors based on their interpretation of the aggregate signaling flux flowing into mTORC1 and mTORC2. The 4EBP1, S6K-1, IRS1, AKT, SGK1 and PKCα effectors coalesce to illicit the proper cellular biology (e.g., ribosome biogenesis, translation, growth, proliferation, survival, autophagy, lipid biogenesis, cytoskeletal changes, angiogenesis).

cellular effects, regulating such events as metabolism, survival and proliferation. While many AKT substrates remain to be uncovered, GSK3β and FOXO1/3A are well-studied targets of AKT, controlling many of the processes previously ascribed to mTORC2. PROTOR-1 serves as an adaptor for the phosphorylation of SGK1 by mTORC2; cells lacking PROTOR fail to activate SGK1.[130] SGK1 is also regulated by osmotic stress, and its activation corresponds with increased epithelial sodium channel-dependent Na^+ transport.[131,132] In glioma cell lines, increased RICTOR levels and higher mTORC2 activity enhanced cellular motility and correlated with increased PKCα activity.[133] Although it is unclear how mTORC2 precisely regulates changes in the actin cytoskeleton, PKCα has been implicated in this function.[134,135] Additionally, knockdown of mTOR, mLST8 and RICTOR, but not RAPTOR, leads to severe defects in actin reorganization in the presence of serum, implicating mTORC2 in cytoskeleton regulation.[85] Moreover, RICTOR knockdown also decreased RAC1 activation, further linking mTORC2 to RHO-type GTPase control of the cytoskeleton and cellular motility.[136] Importantly, this process is not inhibited by rapamycin, squarely placing mTORC2 as a central mediator of cell migration and metastasis.[136,137]

While mTORC1 has been ascribed a primary role in regulating mRNA translation, recent evidence also points to mTORC2 as a key player in this process. mTORC2 has been isolated in polysomes, where it readily associated with individual ribosomal proteins of the 60S large subunit, including rpL23a and rpL26.[138,139] Moreover, SIN1 associated with poly(rC) binding protein 2 (PCBP2) and RNA binding protein, which controls mRNA stability and translation in response to various cellular stresses.[140] Thus, it appears that mTORC2 is as connected to mRNA translation as mTORC1 (**Fig. 5**). Indeed, consistent with this notion, cells disrupted for mTORC2 function exhibit more severely attenuated translation compared with those treated with rapamycin,[138] forcing a reassessment of what was once thought of as an mTORC1-specific function.

Heterogeneity in mTOR Function

The canonical view of mTOR signaling envisions the presence of two static complexes with a unique set of associated proteins that collectively signal to a distinct set of downstream effectors (**Fig. 6**). The notion that all cells harbor the same collection of static complexes with limited hard-wired signaling effector pathways has recently been called into question by several new observations in brain cells (astrocytes and neural stem cells). Using a high throughput chemical library screening approach, we identified STAT3 as a downstream effector of Ras/mTOR signaling in *Nf1*-deficient astrocytes.[141] In these astrocytes, STAT3 activation was inhibited by rapamycin (**Fig. 6A**). mTOR-mediated STAT3 regulation involved Rac1 activation,[129] which resides upstream of both mTORC1 and mTORC2.[142] However, astrocytes express very low levels of RICTOR, and STAT3 regulation was instead RAPTOR-dependent. These findings raise the intriguing possibility that the previously rigid signaling diagrams might not fully represent the true nature of mTOR signaling complexity in primary cells or tissues. Furthermore, it is also possible that different tissues have differing levels of mTOR components, which could determine exactly how the mTOR complex signals in any given tissue (Gutmann DH and Weber JD, unpublished observations).

Compared with other tissues,[143-145] the biological effects of mTOR activation resulting from *Pten* loss, *Nf1* loss, *Tsc1* loss and RHEB overexpression in astrocytes are distinct[28] (**Fig. 6B**). In these experiments, *Tsc1* loss and RHEB overexpression did not increase astrocyte proliferation in vitro, whereas *Pten* and *Nf1* loss led to increased cell growth. In all four groups of genetically modified astrocytes, mTOR activation, as measured by ribosomal S6

phosphorylation, was elevated and could be ameliorated by rapamycin treatment. However, neither neurofibromin nor PTEN loss resulted in increased tuberin phosphorylation, suggesting that TSC inactivation was not involved. Consistent with a TSC-independent mechanism of mTOR activation, RHEB silencing using shRNA completely blocked mTOR activation in *Tsc1*-null astrocytes but had no effect on either mTOR activation or proliferation in *Nf1*- or *Pten*-deficient cells. Since previous studies had shown that optic glioma growth in *Nf1* genetically engineered mouse models could be inhibited by rapamycin treatment,[30] we hypothesized that RAS activation, but not *Tsc1* loss or RHEB overexpression, in glial cells in vivo would result in gliomagenesis. In agreement with a model in which mTOR activation due to *Tsc1* loss or RHEB overexpression are not equivalent to neurofibromin loss, *Nf1* genetically engineered mice with glial *Tsc1* loss or RHEB overexpression did not develop optic glioma, whereas those with glial Ras activation did. While the precise mechanism underlying the differential activation of mTOR remains to be completely elucidated, these results support a model in which mTOR function is differentially dictated by the mode of mTOR activation by its upstream regulators.

Further functional support for mTOR heterogeneity dictating cell biology was provided by studies in which neural stem cells (NSCs) from two different regions of the mouse brain were analyzed.[146] In these experiments, *Nf1*-deficient NSCs from the brainstem exhibited a cell autonomous increase in proliferation in vitro and increased gliogenesis in vivo, whereas NSCs from the neocortex did not (**Fig. 6C**). Examination of the signaling pathways deregulated following neurofibromin loss revealed that only brainstem NSCs had increased AKT activation. In contrast, no increase in AKT activity was observed in *Nf1*$^{-/-}$ cortex NSCs. Interestingly, the AKT hyperactivation and increased NSC proliferation was TORC2-dependent and could be blocked by rapamycin treatment or RICTOR genetic silencing but not by RAPTOR shRNA knockdown. The basis for this striking difference in both mTOR function and NSC biology reflected differential RICTOR expression. In brainstem NSCs, there was significantly higher RICTOR expression relative to cortex NSCs (**Fig. 6**). These observations establish differential mTOR component expression as a primary determinant of cellular heterogeneity even in nearly identical cell types.

mTOR as a Dynamic Molecular Sensor and Integrator

In light of recent experimental observations on the central role of mTOR as a molecular sensor and integrator in a plethora of biological settings, the current models of mTOR function require thoughtful re-examination. We propose a model in which the function of mTOR is dynamically regulated by spatially-, temporally- and signaling network-defined conditions. In this regard, which molecules are physically assembled into the mTOR complex is dictated by their expression levels and activity status. Not only will differing levels of mTOR component expression define the mTOR signaling complex, but also the interplay of the signal transduction pathways activated at any given time within the cell. The activation of these pathways likely do not operate in a linear fashion, such that MEK activation not only affects tuberin phosphorylation to impact on mTOR signaling, but also on the activity and subcellular localization of other proteins that define how mTOR signaling is regulated or the context in which its signals are transmitted. We view this state as the aggregate signaling flux (**Fig. s7**). In this setting, various mTORC components are individually (on a molecule by molecule basis) modified at the protein level (through expression or modification) to provide an overall landscape of mTOR-associated proteins. In effect, this provides the necessary complexity that allows mTOR to interpret a wide multitude of input signals. Based on this landscape, mTORC1 and mTORC2 act to target select downstream effectors. Many of these targets control cellular processes specific to each mTOR complex but may be maintained by overlapping mTORC1 and mTORC2 functions (e.g., mRNA translation). In any case, the key to understanding mTOR complex function resides in our ability to merge upstream signaling flux into mTOR complex control of downstream substrates and cellular processes. While this may seem a daunting task, recent advances in genetic and proteomic technologies should permit a more detailed view of mTOR complex assembly and how these complexes are dynamically regulated to perform such intricate multitasking within the cell.

Abbreviations

4EBP1, eukaryotic translation initiation factor 4E binding protein 1; AKT, protein kinase-B; AMPK, AMP-activated protein kinase; DEPTOR, DEP domain-containing mTOR-interacting protein; FKBP12, FK506 binding protein 12; FKBP38, FK506 binding protein 38; FUBP-1, far upstream binding protein-1; GAP, GTPase activating protein; MEK, mitogen-activated kinase (ERK) kinase; mLST8, mammalian lethal with SEC13 protein 8; GβL, G protein beta subunit-like; mSIN1, mammalian stress-activated protein kinase-interacting protein 1; mTOR, mammalian target of rapamycin; NF1, neurofibromatosis type 1; NPM, nucleophosmin; PCD4, programmed cell death 4; PI3K, phosphatidylinositol-3-kinase; PCBP2, poly(rC) binding protein 2; PRAS40, proline-rich AKT substrate of 40 kDa; PROTOR, protein observed with RICTOR-1; PTEN, phosphatase and tensin homolog; RAPTOR, regulatory associated protein of TOR; RHEB, Ras homolog enriched in brain; RICTOR, Rapamycin-insensitive companion of TOR; S6K, ribosomal S6 kinase; SGK1, serum- and glucocorticoid-induced protein kinase 1; STAT3, signal transducer and activator of transcription 3; TOP, terminal oligopyrimidine; TORC1, mTOR complex 1; TORC2, mTOR complex 2; TSC, tuberous sclerosis complex

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Ethical Statement

The authors have complied with all of the legal requirements governing the use of animals at the Washington University School of Medicine under active and approved Animal Studies Protocols.

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