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Review

Mammalian target of rapamycin and the kidney. I. The signaling pathway

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Lieberthal W, Levine JS. Mammalian target of rapamycin and the kidney. I. The signaling pathway. *Am J Physiol Renal Physiol* 303: F1–F10, 2012. First published March 14, 2012; doi:10.1152/ajprenal.00014.2012.—The mammalian target of rapamycin (mTOR) is a serine/threonine kinase that plays a fundamental role in regulating cellular homeostasis and metabolism. In a two-part review, we examine the complex molecular events involved in the regulation and downstream effects of mTOR, as well as the pivotal role played by this kinase in many renal diseases, particularly acute kidney injury, diabetic nephropathy, and polycystic kidney diseases. Here, in the first part of the review, we provide an overview of the complex signaling events and pathways governing mTOR activity and action. mTOR is a key component of two multiprotein complexes, known as mTOR complex 1 (mTORC1) and 2 (mTORC2). Some proteins are found in both mTORC1 and mTORC2, while others are unique to one or the other complex. Activation of mTORC1 promotes cell growth (increased cellular mass or size) and cell proliferation (increased cell number). mTORC1 acts as a metabolic "sensor," ensuring that conditions are optimal for both cell growth and proliferation. Its activity is tightly regulated by the availability of amino acids, growth factors, energy stores, and oxygen. The effects of mTORC2 activation are distinct from those of mTORC1. Cellular processes modulated by mTORC2 include cell survival, cell polarity, cytoskeletal organization, and activity of the aldosterone-sensitive sodium channel. Upstream events controlling mTORC2 activity are less well understood than those controlling mTORC1, although growth factors appear to stimulate both complexes. Rapamycin and its analogs inhibit the activity of mTORC1 only, and not that of mTORC2, while the newer "catalytic" mTOR inhibitors affect both complexes.

mTORC1; mTORC2; cell mass, proliferation; survival; cytoskeleton

THE "TARGET OF RAPAMYCIN" (TOR) is a serine/threonine kinase initially cloned in yeast (53). A few years later, its mammalian ortholog, the "mammalian target of rapamycin" (mTOR), was cloned (7, 17, 101). The identification of TOR and mTOR was the direct result of the discovery of rapamycin, a highly specific inhibitor of both TOR and mTOR produced by the soil bacterium *Streptomyces hygroscopicus* (53, 73). Rapamycin was essential not only for the identification of mTOR, but also for elucidating mTOR-dependent signaling events and their role in metabolism and disease. The mTOR kinase forms a part of two distinct protein complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2), that have separate downstream targets and functions. Upon entering cells, rapamycin binds to an intracellular receptor, the FK506-binding protein (FKBP12) (18). The resulting complex then binds to and inhibits mTOR kinase activity (18). Sensitivity to rapamycin varies widely among mammalian cells. The relatively high susceptibility of lymphocytes to rapamycin has led to its clinical use as an immunosuppressive therapy for recipients of organ transplantation (5, 40).

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mTOR lies at the center of an intricate signaling network with profound implications for a large number of diverse and important biological events, including embryonic development, metabolism, regenerative processes, and ageing (132). Moreover, an inappropriately high level of mTOR activity plays a central role in the progression of several highly prevalent diseases including metabolic syndrome, diabetes, and cancer (35, 51, 132). It is not surprising, therefore, that elucidation of the pathways governing the activity and effects of mTOR is currently a rapidly expanding area of research.

It has been clear for many years that mTOR has a fundamental role in the regulation of two cardinal cellular events: cell proliferation and cell growth. Throughout this review, we will use "proliferation" to refer to an increase in cell number and "growth" to refer to an increase in cell size or mass. Since mTOR promotes an increase in both cell number and cell size, it is also a major determinant of organ and total body mass (132). The role of mTOR is to act as a metabolic "sensor." mTOR monitors the cell and its immediate environment for adequacy of the conditions and ingredients necessary for proliferation and growth. Factors that stimulate cell growth and proliferation include growth factors, nutrients, cellular energy stores, and oxygen availability (4, 28, 35, 76, 112, 132). On the other hand, conditions unfavorable to proliferation and growth,

for example, a deficiency of cellular energy stores or DNA damage, suppress mTOR activity and slow both processes.

In this two-part review, we will focus on four major areas. First, we will provide an overview of the signaling events and pathways governing mTOR activity and action. Second, we will describe the critical regulatory role of mTOR in the pathophysiology of a wide range of renal diseases including acute kidney injury (AKI), diabetic nephropathy (DN), nondiabetic forms of chronic glomerular disease, and polycystic kidney disease (PKD). Third, we will discuss the potential use of rapamycin, or its analogs, in the treatment of mTOR-related renal diseases in humans. Finally, we will consider the properties and utility of a new generation of mTOR inhibitors, which have potential advantages over rapamycin (19, 31, 38, 118, 130).

Structure of the mTOR Kinase and Components of mTORC1 and mTORC2 Complexes

The mTOR kinase. mTOR is a large (~289-kDa) multidomain protein that belongs to the phosphatidylinositol 3-kinase (PI3K)-related family of kinases (66) (Fig. 1*A*). In general, members of this family of kinases are proteins that facilitate the ability of the organism to cope with metabolic, environmental, and genetic forms of stress. mTOR has a C-terminal PI3K-like catalytic domain, flanked on its N- and C-terminal sides by FRAP, ATM, and TRRAP (FAT) and FAT C-terminal (FATC) domains, respectively (Fig. 1*A*). The FAT domain is believed to mediate interactions with other proteins, while the FATC domain senses cytosolic redox potential and probably influences the rate of degradation of mTOR (24). The N terminus of mTOR contains tandem repeats of Huntington, elongation factor 3, regulatory A subunit of protein phosphatase 2A, and TOR (HEAT) domains. The HEAT domain is an α-helical structure that mediates protein-protein interactions (2) and membrane localization (74). HEAT domains also contribute to the formation of the protein complexes, mTORC1 and mTORC2, and appear necessary for mTOR function (117). A domain found exclusively in the mTOR molecule is the FKBP12-rapamycin binding (FRB) domain, to which the ligand-receptor complex between rapamycin and FKBP12 binds (5, 15, 40) (Fig. 1*A*). Phosphatidic acid (PA), a phospholipid metabolite produced by phospholipase D (PLD) that activates mTOR, also binds to the FRB domain (116, 119, 127). The opposing effects of rapamycin and PA on mTOR activity may

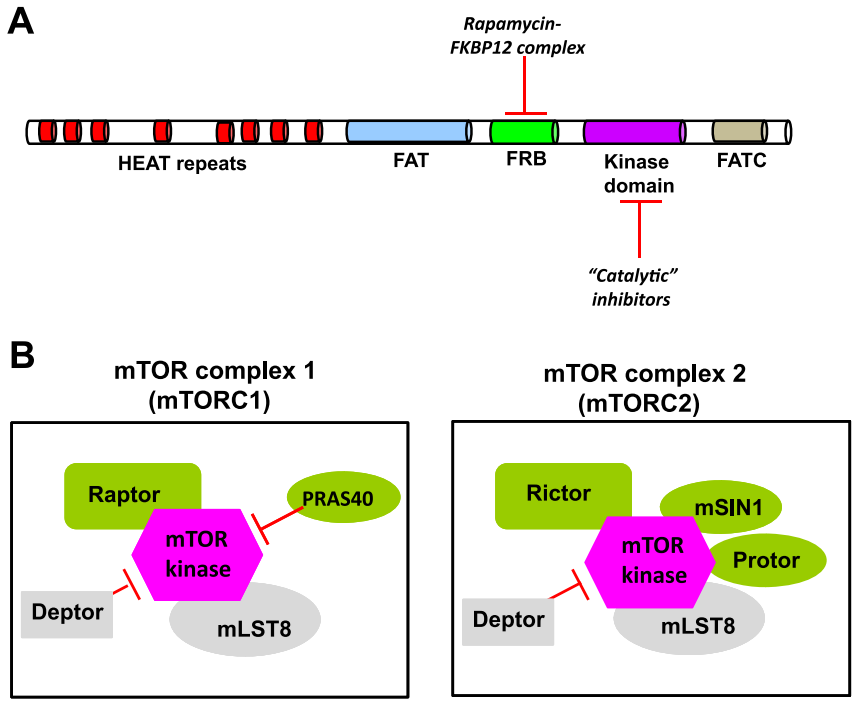


Fig. 1. Structural features of mammalian target of rapamycin (mTOR) kinase, mTOR complex 1 (mTORC1), and mTOR complex 2 (mTORC2). *A*: mTOR kinase is a large protein with a C-terminal catalytic domain, flanked on its N- terminal side by a FRAP, ATM, and TRRAP (FAT) domain, which mediates interactions with other proteins, and on its C-terminal side by a FAT C-terminal (FATC) domain, which senses cytosolic redox potential and influences degradation of mTOR. The N terminus of mTOR contains tandem repeats of Huntington, elongation factor 3, regulatory A subunit of protein phosphatase 2A, and TOR (HEAT) domains, which mediate protein-protein interactions and determine membrane localization. The FKBP12-rapamycin complex binds to the FRB (FKBP12-rapamycin binding) domain. *B*: mTOR kinase nucleates 2 distinct protein complexes, mTORC1 and mTORC2. Some proteins are common to both complexes, while others are unique. Proteins unique to mTORC1 are regulatory-associated protein of mTOR (Raptor) and proline-rich Akt substrate of 40 kDa (PRAS40). Raptor is necessary for assembly of mTORC1 and for interaction of mTORC1 with upstream regulators and downstream substrates. Inhibition by rapamycin on mTORC1 depends, in part, on preventing the association between mTOR and Raptor, thereby impairing interaction of mTORC1 with its downstream targets. PRAS40, when unphosphorylated, binds to and inhibits mTORC1. Proteins unique to mTORC2 are rapamycin-insensitive companion of mTOR (Rictor), mammalian stress-activated protein kinase interacting protein 1 (mSIN1), and protein observed with Rictor (Protor). Rictor, like Raptor within mTORC1, acts as a scaffold for assembly of mTORC2 and for interaction of mTORC2 with its substrates and regulators. mSIN1, in addition to a role in mTORC2 assembly, contributes to membrane targeting of mTORC2 and, ultimately, its phosphorylation of Akt, a major downstream substrate. The function of Protor is not yet clearly defined. Two proteins are found in both complexes. Mammalian lethal with SEC13 protein 8 (mLST8) stabilizes the interaction between Raptor and mTOR in mTORC1, and between Rictor and mTOR in mTORC2. DEPTOR, named for its tandem Dishevelled, EGL-10, and pleckstrin (DEP) domains and specific interaction with mTOR is a negative regulator of both mTORC1 and mTORC2.

arise through competition for binding to the FRB domain on mTOR.

The mTORC1 and mTORC2 complexes. Each of the two mTOR complexes, mTORC1 (27, 28, 34, 112) and mTORC2 (23, 63, 81), contains multiple proteins. Some proteins are found in only one of the two complexes, while others are common to both (Fig. 1*B*).

Two proteins are unique to mTORC1 (Fig. 1*B*). These are regulatory-associated protein of mTOR (Raptor) (34, 44, 67, 93, 110) and proline-rich Akt substrate of 40 kDa (PRAS40) (56, 104, 120, 121). Activation of mTORC1 is initiated by phosphorylation of Raptor, a scaffold protein necessary for the assembly of mTORC1 as well as for interaction of mTORC1 with its various substrates and regulators (34, 44, 67). Binding of rapamycin-FKBP12 to the FRB domain of mTOR does not directly affect mTOR's kinase activity. Although the molecular mechanisms underlying rapamycin's effect remain poorly understood, inhibition appears to depend, in part, on interference with the association between mTOR and Raptor, thereby preventing mTORC1 from interacting with and phosphorylating its downstream targets (34, 67, 96). PRAS40, when unphosphorylated, binds to and inhibits mTORC1. Phosphorylation of PRAS40 by Akt causes its release from mTORC1, thus relieving mTORC1 from inhibition by PRAS40 (56, 104, 120).

Proteins unique to mTORC2 include rapamycin-insensitive companion of mTOR (Rictor) (55, 105, 126), mammalian stress-activated protein kinase interacting protein 1 (mSIN1), and protein observed with Rictor (Protor) (97) (Fig. 1*B*). Like Raptor within mTORC1, Rictor acts as a scaffold protein and facilitates both the assembly of mTORC2 and the interaction of mTORC2 with its substrates and regulators. mSIN1, in addition to a role in the assembly of mTORC2, contributes to the membrane targeting of mTORC2, and ultimately the phosphorylation of Akt, a downstream substrate of mTORC2 (36, 128) (Fig. 1*B*). The function of Protor has not yet been clearly defined.

Two proteins are found in both mTORC1 and mTORC2 (Fig. 1*B*). The first is mammalian lethal with SEC13 protein 8 (mLST8), which stabilizes the interaction between Raptor and mTOR in mTORC1 (68) and between Rictor and mTOR in mTORC2 (41). The second is DEPTOR, named for its tandem Dishevelled, EGL-10, and pleckstrin (DEP) domains and specific interaction with mTOR. DEPTOR is a negative regulator of the activity of both mTORC1 and mTORC2 (98).

Copp and his colleagues (21) have recently shown that mTOR is phosphorylated differently when part of mTORC1 vs. part of mTORC2. Phosphorylation of mTOR at Ser^{2448} is seen predominantly, although not exclusively, when mTOR is part of mTORC1, whereas phosphorylation of mTOR at Ser^{2481} occurs when it is part of mTORC2. Thus phosphorylation at residues Ser^{2448} and Ser^{2481} can be used as a biomarker to distinguish activation of mTORC1 and mTORC2, respectively (21).

Besides having distinct downstream targets, mTORC1 and mTORC2 respond differently to rapamycin. While rapamycin inhibits the catalytic activity of mTORC1 by interfering with the association between mTOR and Raptor, the drug has no direct effect on the catalytic activity of mTORC2 (81). Despite rapamycin's lack of direct effect on mTORC2, prolonged exposure to rapamycin in some cell types can diminish mTORC2 activity. Inhibition is indirect and occurs through interference with the assembly of new mTORC2 complexes (106).

The mTORC1 Signaling Pathway

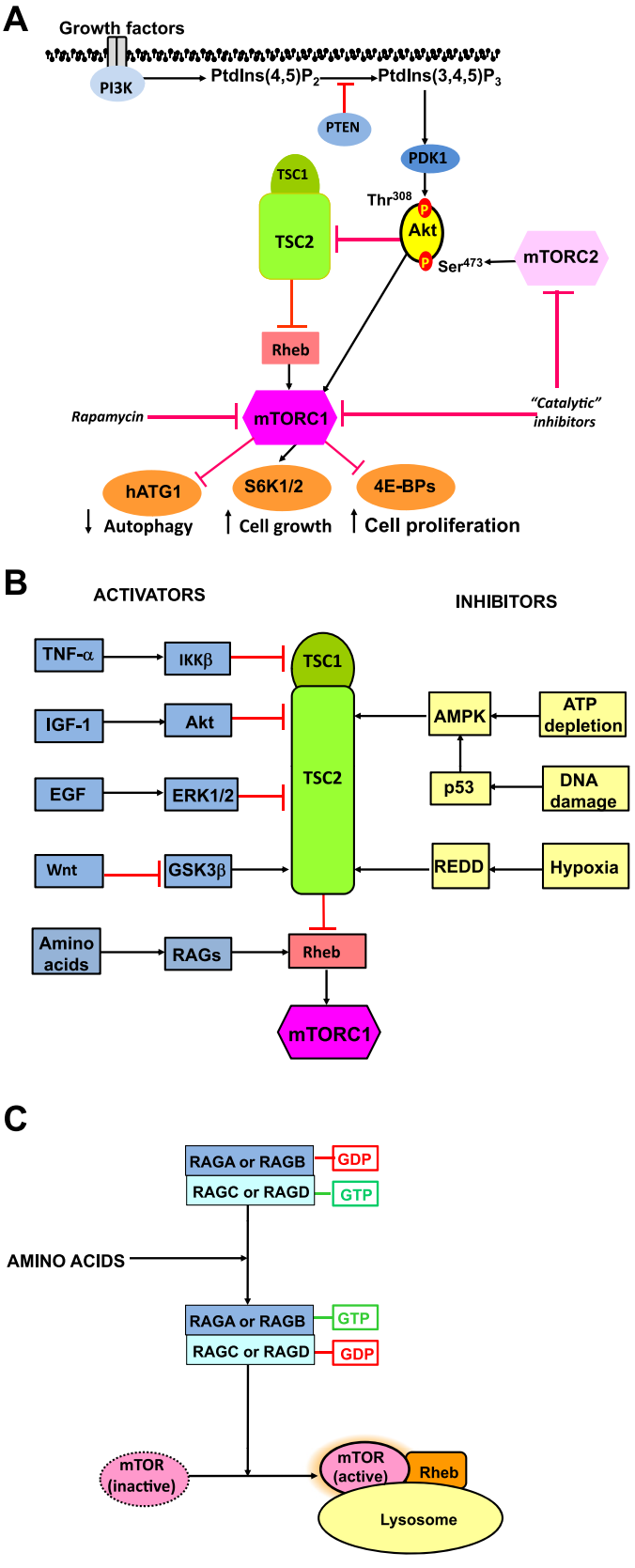
Downstream effects of mTORC1. CELL GROWTH, CELL PROLIFERATION, AND PROTEIN SYNTHESIS. mTORC1 stimulates both cell growth (increase in cell size or mass) and cell proliferation (49). In terms of the cell cycle, the major effect of mTORC1 is to promote progression through the G_0/G_1 phase of the cell cycle (51). The best-characterized targets of mTORC1 are the ribosomal S6 kinases S6K1 and S6K2 [also known as p70S6K1 (phosphoprotein 70 S6K1/2)] and the 4E-binding proteins (4E-BPs), including 4E-BP1, 4E-BP2, and 4E-BP3, which bind to and regulate the activity of the eukaryotic translation initiation factor (eIF) eIF-4E (9, 39, 51, 84) (Fig. 2*A*). Raptor and another eIF, eIF-3, play critical roles in mTOR-mediated phosphorylation of S6K1/2 and the 4E-BPs (84, 93, 110). eIF-3 acts as a scaffold, recruiting mTORC1 to the ribosomal translation initiation complex (54). Raptor then binds to conserved TOR signaling (TOS) motifs in both S6K1/2 and 4E-BPs, thereby bringing these substrates within range of mTOR (84, 93, 110). Once phosphorylated, S6K1/2 and 4E-BPs augment protein synthesis by increasing the initiation and progression of mRNA translation (84). In general, S6K1/2 is responsible for stimulating protein synthesis and cell growth, while the 4E-BPs play a predominant role in cell proliferation (26).

In its unphosphorylated state, 4E-BP1 binds to and inhibits eIF-4E (43, 45). Upon phosphorylation by mTORC1, 4E-BP1 dissociates from eIF-4E. This allows eIF-4E to interact with the translation initiation factor eIF-4G and recruit eIF-4G to the 5′-ends of a subset of mRNA transcripts known as "eIF-4E-sensitive mRNAs" (43, 45). Two other proteins, eIF-4A and eIF-4B, also play key roles in translation initiation. eIF-4B modulates the activity of eIF-4A, an RNA helicase that facilitates translation by unwinding the 5′ untranslated regions of many RNAs (100). S6K1/2, when phosphorylated by mTORC1, also promote mRNA translation by phosphorylating multiple key proteins including eIF-4B, eukaryotic elongation factor-2 kinase (eEF-2K) (122), S6K1 Aly/REF-like target (SKAR) (85), and 80-kDa nuclear cap-binding protein (CBP80), all of which are necessary for translation initiation and/or elongation (54).

AUTOPHAGY AND OTHER EFFECTS. Although regulation of protein synthesis is the best understood role of mTORC1, the complex also contributes to the regulation of other critical cellular functions. These include autophagy, ribosomal biogenesis, mitochondrial metabolism, lipid synthesis, and activity of the transcription factor hypoxia-inducible factor-1α (HIF-1α).

Autophagy is a ubiquitous process that mediates the bulk removal of abnormal or aged macromolecules and cell organelles from the cytosol (71, 80). Autophagy also provides an essential source of energy during periods of nutrient deficiency. Autophagy is mediated by the formation of autophagosomes, double-membraned structures that envelop intracellular components destined for degradation and then fuse with lysosomes to form autolysosomes, which degrade their contents (89, 123, 129).

mTORC1 is an important upstream inhibitor of autophagy (80, 132) (Fig. 2A). Many factors, including nutrient depriva-



tion, ATP depletion, hypoxia, and rapamycin, stimulate autophagy by inhibiting mTORC1. mTORC1 phosphorylates and inhibits the kinases Unc-51-like kinase-1 and -2 (ULK1/2), catalytic subunits of the multisubunit protein kinase complex that initiates the autophagic cascade. ULK1/2 is also known as human autophagy-1 (hATG1) (48, 52).

While cell growth and autophagic degradation are generally regarded as oppositely regulated, exceptions may occur. Narita et. al. (90) have recently described a mechanism by which the cell's catabolic and anabolic machineries can be coupled. In senescent cells, autolysosomes and mTOR were shown to accumulate within a distinct cellular compartment located at the *trans*-side of the Golgi apparatus. The authors named this structure the TOR-autophagy spatial coupling compartment (TASCC). Colocalization of autolysosomes and mTOR within

Fig. 2. Regulators and effectors of mTORC1. *A*: the immediate upstream regulator of mTORC1 is Rheb, a cytoplasmic G protein that acts as a molecular switch. In its "on" state (bound to GTP), Rheb activates mTOR. Rheb is inactive when bound to GDP. Rheb activity is regulated by the tuberous sclerosis complex (TSC), which comprises 2 proteins, TSC1 (hamartin) and TSC2 (tuberin). TSC2 contains a GTPase-activating protein (GAP) domain that, in its active state, converts Rheb to its inactive GDP-bound state. TSC1 is essential for functional activity of TSC2. TSC therefore acts as a negative regulator of both Rheb and mTORC1. Activation of mTORC1 by growth factors begins with activation of the lipid kinase phosphatidylinositol-3-kinase (PI3K). PI3K, in turn, phosphorylates the membrane-associated lipid phosphatidylinositol-4,5-bisphosphate [PtdIns(4,5)P_2] to yield PtdIns(3,4,5)P_3. Activity of PI3K is opposed by the phosphatase and tensin homolog on chromosome 10 (PTEN), which dephosphorylates PtdIns(3,4,5)P_3 back to PtdIns(4,5)P_2. PtdIns(3,4,5)P_3 activates 3-phosphoinositide-dependent protein kinase 1 (PDK1), which in turn activates Akt by phosphorylating it at Thr^{308}. Once activated by PDK1, Akt phosphorylates and inhibits the activity of TSC. Akt also phosphorylates and inhibits PRAS40. Akt therefore activates mTORC1 in two ways, via release of inhibition from both TSC and PRAS40. Akt is also phosphorylated on Ser^{473} by mTORC2, which promotes further activities by Akt (see Fig. 3). In addition to rapamycin, which predominantly inhibits mTORC1, there are inhibitors of the catalytic domain of mTOR, which inhibit both mTORC1 and mTORC2. Once activated, mTORC1 increases cell growth (size) and proliferation and inhibits autophagy by altering the phosphorylation and activity of ribosomal S6 kinases (S6K1/2), 4EBP, and hATG1 respectively. *B*: many factors influence the activity of mTORC1. These can be divided broadly into 2 groups: those that activate mTORC1 and those that inhibit mTORC1. Most factors activate or inhibit mTORC1 by inhibiting or activating TSC, respectively. Two exceptions are amino acids and phosphatidic acid (PA). Amino acids bypass TSC and activate Rheb (*C*), while PA binds to and activates mTOR directly. Activators of mTORC1 include growth factors such as insulin-like growth factor-1 (IGF-1), which acts via Akt, and epidermal growth factor (EGF), which acts via extracellular signal regulated kinases 1 and 2 (ERK1/2). The Wnt ligand activates mTORC1 though inhibition of glycogen synthase kinase 3β (GSK3β), thereby removing the stimulatory effect of GSK3β on TSC. Tumor necrosis factor-α (TNF-α), acting via IκB kinase (IKK), is the only known factor that activates mTORC1 via TSC1 rather than TSC2. All known inhibitors of mTORC1 act through activation of TSC2. These include AMP-activated protein kinase (AMPK), which is activated by a fall in cell energy stores. DNA damage inhibits mTORC1, in part, via p53-mediated activation of AMPK. Hypoxia inhibits mTORC1 by inducing expression of the protein regulated in development and DNA damage responses (REDD1), which activates TSC2. *C*: amino acids are absolutely required for mTORC1 signaling. Activation of mTORC1 by amino acids is largely mediated by the RAG family of G proteins, which are heterodimers of RAGA or RAGB together with RAGC or RAGD. In the absence of amino acids, RAG heterodimers are in an inactive state, in which RAGA/RAGB is bound to GDP and RAGC/RAGD to GTP. Amino acids convert RAG heterodimers to an active conformation in which RAGA/RAGB is bound to GTP and RAGC/RAGD to GDP. Active RAG heterodimers bind to Raptor and recruit mTORC1 from an unknown locus within the cytoplasm to the membranes of late endosomes and lysosomes, where Rheb is located, thereby allowing Rheb to activate mTORC1.

the TASCC allows amino acids generated by autolysosomes to activate mTORC1 in a RAG GTPase-dependent manner (see below) and thereby enhance the synthesis of proteins targeted for secretion. Interestingly, the TASCC has been shown to increase protein secretion in a number of cell types, including glomerular podocytes (90).

In addition to protein synthesis, mTORC1 also stimulates lipid synthesis (99). These effects are mediated in part through activation of lipogenic transcription factors such as sterol responsive element binding protein (SREBP1) (99) and peroxisome proliferator-activated receptor-γ (PPAR-γ) (70).

As a further means to increase protein synthesis, mTORC1 also stimulates ribosomal biogenesis, increasing the number of ribosomes within each cell (88). mTORC1 has also been shown to modulate mitochondrial function. Inhibition of mTORC1 by rapamycin leads to decreases in several mitochondrial parameters, including transmembrane potential, oxygen consumption, and ATP synthetic capacity (111). Finally, mTORC1 plays an important role in determining the balance between mitochondrial and nonmitochondrial sources of ATP generation (111).

Upstream regulation of mTORC1. mTORC1 acts as a sensor of the adequacy of factors and conditions necessary for cell growth and proliferation. Information is derived by continuous monitoring of parameters within four major areas: the availability of growth factors such as insulin, insulin-like growth factor-1 (IGF-1), and epidermal growth factor (EGF); the adequacy of nutrients such as glucose, lipids, and amino acids; the sufficiency of cellular energy stores; and the presence and extent of cell damage (Fig. 2, *A* and *B*). Input from these multiple sources can either raise or lower the overall activity of mTORC1, thereby enabling each cell to adjust its mTORC1 activity to a level appropriate for the prevailing environment (4, 27, 29, 35, 76, 84, 132). This "fine-tuning" is achieved through a complex network of signaling events and pathways that for the most part converge on two upstream regulators of mTORC1. These are Ras homolog enriched in brain (Rheb) and the tuberous sclerosis complex (TSC) (4, 28, 35, 76, 112, 132) (Fig. 2, *A* and *B*).

RHEB. The immediate upstream regulator of mTORC1 is Rheb, a cytoplasmic G protein with intrinsic GTPase activity (Fig. 2, *A* and *B*). Rheb acts as a molecular switch, depending upon whether it is bound to GTP ("on" position) or GDP ("off" position). In its on position, Rheb stimulates the kinase activity of mTORC1 (109, 115). While the precise mechanism by which Rheb-GTP controls mTORC1 remains poorly defined, part of Rheb's effect is to facilitate binding of mTORC1 to its substrates, such as S6K1/2 and the 4E-BPs (108).

TSC. TSC consists of two proteins, TSC1 (hamartin) and TSC2 (tuberin) (58, 59, 95) (Fig. 2*A*). TSC is a tumor suppressor that negatively regulates Rheb. TSC2 contains a GTPase-activating protein (GAP) domain that catalyzes the conversion of Rheb from an active GTP-bound state to an inactive GDP-bound state (35, 108). TSC1 is necessary for the function of TSC2's GAP domain.

TSC-mediated regulation of Rheb comprises the final common pathway for all known upstream regulators of mTORC1 (Fig. 2, *A* and *B*), with several exceptions. The two most important are PA, which binds to and activates mTOR directly, and amino acids, which bypass TSC and activate Rheb (Fig. 2*C*). Since TSC is a negative regulator of Rheb, activators of mTORC1, such as growth factors, do so by inhibiting TSC. In contrast, inhibitors of mTORC1, such as energy depletion, hypoxia, and DNA damage, do so by activating TSC (4, 35, 76).

ACTIVATION OF MTORC1 BY GROWTH FACTORS. Growth factors, like insulin and IGF-1, activate mTORC1 predominantly via the kinase Akt (Fig. 2*A*). Activation of mTORC1 begins with activation of the lipid kinase PI3K. PI3K phosphorylates the membrane-associated lipid, phosphatidylinositol-4,5-bisphosphate [PtdIns(4,5)P_2] to yield PtdIns(3,4,5)P_3 (87). PtdIns(3,4,5)P_3 then activates 3-phosphoinositide-dependent protein kinase 1 (PDK1), which in turn activates Akt by phosphorylating it at Thr^{308}.

Akt activates mTORC1 through at least two mechanisms. Both involve release of inhibition through phosphorylation of a negative regulator of mTORC1. The first target of Akt is TSC2 (25, 60). Interestingly, phosphorylation of TSC2 by Akt does not alter its intrinsic GAP activity. Rather, phosphorylation results in translocation of TSC2 from the cell membrane to the cytosol, where it is sequestered by 14-3-3 proteins, thereby separating TSC2 from both its partner (TSC1) and its target (Rheb) (11). The second target of Akt is PRAS40, a negative regulator within the mTORC1 complex. Phosphorylation of PRAS40 by Akt causes its dissociation from mTORC1 (104, 120). Relief of inhibition from these two sources, the first upstream of mTORC1 and the second within the mTORC1 complex itself, leads to activation of mTOR (Fig. 2*A*).

The activity of PI3K is opposed by the tumor suppressor phosphatase and tensin homolog on chromosome 10 (PTEN), which dephosphorylates PtdIns(3,4,5)P_3 back to PtdIns(4,5)P_2 (50, 65) (Fig. 2*A*). Since PtdIns(3,4,5)P_3 is the major activator of Akt, the predominant antiapoptotic kinase in cells (8, 9), loss of PTEN function leads to excessive Akt activity and conditions conducive to tumorigenesis (72).

Growth factors also modulate mTORC1 activity via pathways independent of Akt. For example, EGF activates mTORC1 via extracellular signal-regulated kinase-1 and -2 (ERK1/2), which phosphorylates and inhibits TSC2 (83) (Fig. 2*B*). The Wnt pathway also activates mTORC1 through inhibition of TSC2, but in this case inhibition involves decreased phosphorylation of TSC2. Activation of the Wnt pathway leads to inhibition of glycogen synthase kinase-3β (GSK3β), which in its uninhibited state phosphorylates and activates TSC2 at a site distinct from that of Akt and ERK1/2 (13, 61). Lack of phosphorylation by GSK3β leads to decreased TSC2 activity and therefore activation of mTORC1 (Fig. 2*B*).

ACTIVATION OF MTORC1 BY AMINO ACIDS. Amino acids are absolutely required for mTORC1 activation, and a deficiency of amino acids cannot be overcome by other activating stimuli (132). The availability of amino acids, especially the essential amino acids leucine and arginine, was the earliest identified activator of mTORC1 (3, 46). Notably, as opposed to other known upstream regulators of mTORC1, amino acids act directly on Rheb and independently of TSC (113) (Fig. 2*C*). Recently, the RAG family of small cytoplasmic G proteins has been identified as important mediators of amino acid-induced activation of mTORC1 (69, 103). Sabatini and coworkers (69, 102, 103) have proposed a model in which amino acids convert inactive GDP-loaded RAG heterodimers to their active GTP-loaded form. A protein complex which has been termed the

"Ragulator," interacts with the RAG GTPases, recruits them to lysosomes, and is essential for mTORC1 activation (102).

Amino acids signal their availability from within lysosomes through their interaction with the luminal aspect of vacuolar H^+-adenosine triphosphatase (v-ATPase). This interaction leads to dissociation of v-ATPase from the "Ragulator" (102), which then activates the RAG heterodimers (131). Active RAG heterodimers bind Raptor and recruit mTORC1 to the membrane of lysosomes where Rheb is situated (Fig. 2*C*).

The role of other putative mediators of amino acid-induced activation of mTORC1, such as the class III PI3K hVPS34 (10, 92), mitogen-activated protein kinase kinase kinase kinase-3 (MAP4K3) (33), and the cytoplasmic G protein RalA (86), remains to be elucidated. Intracellular availability of leucine and arginine, the most important amino acid regulators of mTORC1, is determined by an interconnected system of amino acid transporters, in which glutamine also plays a key role (20, 91).

INHIBITION OF MTORC1 BY DEPLETION OF CELL ENERGY STORES. The adequacy of cellular energy stores represents a second major determinant of mTORC1 activity (Fig. 2*B*). Decreases in cellular energy stores inhibit mTORC1. The ability of cells to "sense" energy status is dependent largely on the activity of AMP-activated protein kinase (AMPK) (47, 49). ADP and AMP both activate AMPK, whereas ATP inhibits AMPK. ADP and AMP activate AMPK through an allosteric effect on AMPK that facilitates its phosphorylation at Thr^{172} by liver kinase B1 (LKB1), a constitutively active kinase. (124). ADP and AMP also maintain AMPK in an active state through blocking its dephosphorylation by phosphatases (49, 94). When ATP stores are replete, dephosphorylation of AMPK is uninhibited and AMPK remains inactive (49, 94). The net effect of these regulatory events is that AMPK activity is related inversely to a cell's energy charge, as reflected by the ratio of ATP to either AMP or ADP (49, 94).

When active, AMPK inhibits mTORC1 in at least two ways. First, like GSK3β, AMPK phosphorylates and activates TSC2 (22, 61) (Fig. 2, *A* and *B*). Second, AMPK phosphorylates Raptor. Phosphorylation of Raptor by AMPK leads to Raptor's sequestration by 14-3-3 proteins, rendering Raptor incapable of fulfilling its role as a scaffolding molecule for mTORC1 and its downstream substrates (42). Thus, through coupling of AMPK activity to a cell's energy status, AMPK ensures that cell growth and proliferation cannot proceed unless the energy required to fuel these processes are in adequate supply.

In addition to inhibiting cell growth and proliferation, AMPK also promotes autophagy. AMPK does this in both an mTORC1-dependent and an mTORC1-independent manner. Via phosphorylation and inhibition of mTORC1, AMPK promotes autophagy by releasing ULK1/2 from mTORC1-mediated inhibition. AMPK also more directly promotes autophagy by phosphorylating and activating ULK1/2 (30) Thus AMPK delivers two independent signals, both leading to activation of the autophagic program (30, 48).

OTHER UPSTREAM MODULATORS OF MTORC1. Hypoxia, if severe enough to reduce cellular ATP levels, will inhibit mTORC1 via activation of AMPK. Hypoxia can also inhibit mTORC1 independently of AMPK. Regulated in development and DNA damage responses (REDD1), an HIF-1α-inducible protein, suppresses mTORC1 by activating TSC2 (8) (Fig. 2*B*). PA, a metabolite of PLD, has also been implicated in the regulation of mTORC1. PA activates mTORC1 by direct interaction with mTOR and may compete with rapamycin for binding to mTOR's FRB domain. DNA damage inhibits mTORC1 through activation of p53, which, in turn, activates AMPK (32) (Fig. 2*B*) and also stimulates increased transcription of PTEN (114) (Fig. 2*A*). Finally, tumor necrosis factor-α, a mediator of inflammation, can activate mTORC1 through IκB kinase-β (IKKβ), which phosphorylates and inhibits TSC1 (78) (Fig. 2*B*).

The mTORC2 Signaling Pathway

While substantial progress has been made in our understanding of the regulation and function of mTORC1, far less is known about mTORC2. Progress in this area has been slowed by two main factors: lack of a specific inhibitor of mTORC2, and the early embryologic lethality that follows deletion of components of this complex (4, 34, 35, 76).

Downstream effects of mTORC2. Among the downstream targets of mTORC2 are Akt, protein kinase Cα (PKCα), and serum and glucocorticoid-induced protein kinase 1 (SGK1)

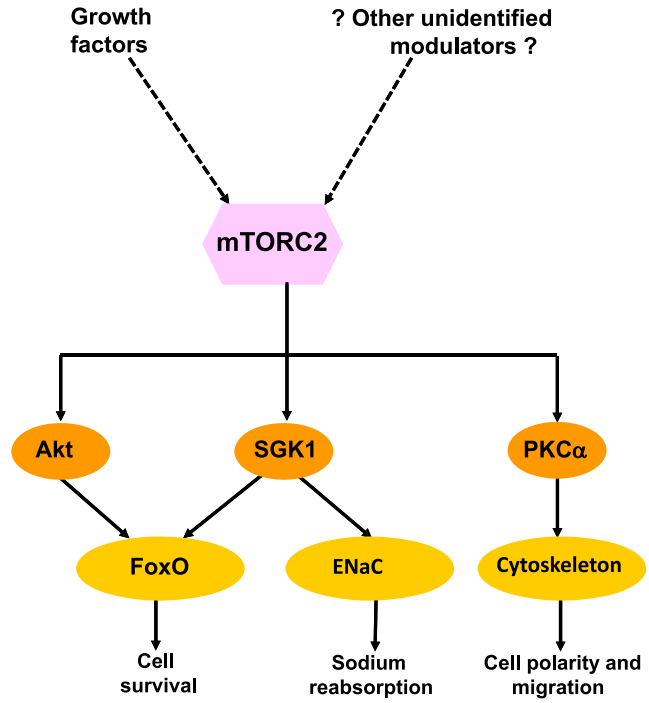


Fig. 3. Regulators and effectors of mTORC2. Relatively little is known about the upstream regulators of mTORC2 activity. Growth factors probably activate mTORC2 as well as mTORC1. Downstream targets of mTORC2 include Akt, protein kinase Cα (PKCα), and serum- and glucocorticoid-induced protein kinase 1 (SGK1). These kinases are involved in regulation of diverse cell functions, including cell survival, cytoskeletal organization, and aldosterone-mediated sodium reabsorption. mTORC2 activates Akt by phosphorylating it at Ser^{437}, an event essential for phosphorylation and activation of a distinct subset of Akt substrates, such as the FoxO family of Forkhead transcription factors. FoxO factors induce transcription of a number of genes that encode proteins with important antiapoptotic effects. mTORC2 also phosphorylates and activates SGK1, which, like Akt also has prosurvival effects mediated by the FoxO family of transcription factors. In addition, mTORC2-mediated activation of SGK1 increases the activity of the epithelial sodium (Na^+) channel (ENAC)-and increases aldosterone-regulated sodium reabsorption by the distal nephron. Finally, activation of PKCα by mTORC2 also accounts for some of the effects of mTORC2 on the cytoskeleton, including changes in polarity and migration.

(75) (Fig. 3). These kinases are all members of the so-called AGC family (protein kinases A, G, and C), which contribute to the regulation of diverse cell functions including cell survival, cell polarity, cytoskeletal rearrangement, and aldosterone-mediated sodium reabsorption by the distal nephron (1, 64). S6K1/2, a major downstream target of mTORC1, is also a member of the AGC family of kinases (64).

CELL SURVIVAL. mTORC2 activates Akt by phosphorylating it at Ser^{473} (41, 62, 107) (Fig. 2*A*). While phosphorylation of Akt at Thr^{308} by PDK1 is required for phosphorylation and inactivation of many proapoptotic mediators, including BAD, Bcl-xL, and GSK3β (6), phosphorylation of Akt at Ser^{473} by mTORC2 is not required for these events (6, 41, 62). Rather, phosphorylation of Akt at Ser^{473} by mTORC2 is essential for the phosphorylation and activation of a distinct subset of Akt substrates, which includes the FoxO family of Forkhead transcription factors (12) (Fig. 3). These transcription factors induce the expression of a number of genes important in ameliorating age-dependent diseases, including diabetes, cancer, and neurodegenerative processes (6, 12). SGK1, another target of mTORC2 (37), which is activated by growth factors and shares homology with Akt (75), has also been shown to phosphorylate and activate the FoxO family of transcription factors (1, 37) (Fig. 3).

CYTOSKELETAL REARRANGEMENT. Activation of mTORC2 leads to changes in the actin cytoskeleton and cell polarity (Fig. 3). Although the specific mechanism for these changes has not been clearly identified (23, 63, 105), cytoskeletal rearrangement is mediated in part by activation of PKCα, which, in turn, activates Rho and Rac1, two G proteins and major regulators of the cytoskeleton. PKCα also phosphorylates paxillin and induces its localization to focal adhesions (105).

SODIUM HOMEOSTASIS. Besides its prosurvival effects, SGK1 has been known for many years to stimulate sodium reabsorption in the distal nephron by increasing activity of the aldosterone-regulated epithelial sodium channel (ENaC) (16, 125). Activation of SGK1 requires phosphorylation within a C-terminal domain known as the hydrophobic motif. Recently, Lu and colleagues (82) have shown that mTORC2 is the kinase responsible for activation of SGK1. This suggests that mTOR, in addition to its other effects on renal function, is involved in sodium homeostasis and perhaps also regulation of blood pressure (Fig. 3).

Upstream regulation of mTORC2. It is generally believed that mTORC2, like mTORC1, is activated by growth factors. In support of this concept, stimulation with IGF-1 induces phosphorylation of Akt at Ser^{473} (107). Given that activation of Akt, SGK1, and PKCα occurs in response to a wide range of growth factors, the number of growth factors that activate mTORC2 may be quite large. In addition to activating mTORC1, PA may also activate mTORC2 (119).

Since phosphorylation of Akt by mTORC2 occurs at the cell membrane (77), membrane localization of mTORC2 may be critical to its activation. It has been suggested that translocation of mTORC2 to the cell membrane is mediated by mSIN1 via its C-terminal pleckstrin homology (PH) domain (76) (Fig. 1*B*). Additional studies, however, are needed to confirm this model. Alternatively, mSIN1 may function as an adaptor between mTORC2 and certain growth factor receptors (14, 79). Finally, an intriguing report suggests that TSC may lie upstream not only of mTORC1, but also mTORC2 (57). As opposed to its effects on mTORC1, activated TSC would promote rather than inhibit mTORC2 activity, and do so in a Rheb-independent manner.

NOTE ADDED IN PROOF

Until recently, as discussed in this review, it has been believed that rapamycin predominantly inhibits mTORC1. However, a recent study by Lamming and associates (Lamming DW, Ye L, Katajisto P, Goncalves MD, Saitoh M, Stevens DM, Davis JG, Salmon AB, Richardson A, Ahima RS, Guertin DA, Sabatini DM, Bauer JA. Rapamycin-induced insulin resistance is mediated by mTORC2 loss and uncoupled from longevity. *Science* 335: 1638–1643, 2012) shows conclusively that rapamycin inhibits both complexes. Rapamycin inhibits mTORC2 by disrupting the mTORC2 complex, rather than through interference with its assembly (as suggested by earlier work). Lamming et al. also demonstrate that the downstream effects of rapamycin on mTORC1 and mTORC2 can be dissociated. Whereas rapamycin-mediated inhibition of mTORC1 activity promotes longevity, its disruption of mTORC2 induces insulin resistance and impaired glucose homeostasis.

DISCLOSURES

No conflicts of interest, financial or otherwise. are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: W.L. prepared figures; W.L. drafted manuscript; J.S.L. edited and revised manuscript; W.L. and J.S.L. approved final version of manuscript.

REFERENCES

1. **Alessi DR, Pearce LR, Garcia-Martinez JM.** New insights into mTOR signaling: mTORC2 and beyond. *Sci Signal* 2: 27–29, 2009.
2. **Andrade MA, Bork P.** HEAT repeats in the Huntington's disease protein. *Nat Genet* 11: 115–116, 1995.
3. **Avruch J, Lin Y, Long X, Murthy S, Ortiz-Vega S.** Recent advances in the regulation of the TOR pathway by insulin and nutrients. *Curr Opin Clin Nutr Metab Care* 8: 67–72, 2005.
4. **Bai X, Jiang Y.** Key factors in mTOR regulation. *Cell Mol Life Sci* 67: 239–253, 2010.
5. **Ballou LM, Lin RZ.** Rapamycin and mTOR kinase inhibitors. *J Chem Biol* 1: 27–36, 2008.
6. **Brazil DP, Yang ZZ, Hemmings BA.** Advances in protein kinase B signalling: AKTion on multiple fronts. *Trends Biochem Sci* 29: 233–242, 2004.
7. **Brown EJ, Albers MW, Shin TB, Ichikawa K, Keith CT, Lane WS, Schreiber SL.** A mammalian protein targeted by G1-arresting rapamycin-receptor complex. *Nature* 369: 756–758, 1994.
8. **Brugarolas J, Lei K, Hurley RL, Manning BD, Reiling JH, Hafen E, Witters LA, Ellisen LW, Kaelin WG Jr.** Regulation of mTOR function in response to hypoxia by REDD1 and the TSC1/TSC2 tumor suppressor complex. *Genes Dev* 18: 2893–2904, 2004.
9. **Burnett PE, Barrow RK, Cohen NA, Snyder SH, Sabatini DM.** RAFT1 phosphorylation of the translational regulators p70 S6 kinase and 4E-BP1. *Proc Natl Acad Sci USA* 95: 1432–1437, 1998.
10. **Byfield MP, Murray JT, Backer JM.** hVps34 is a nutrient-regulated lipid kinase required for activation of p70 S6 kinase. *J Biol Chem* 280: 33076–33082, 2005.
11. **Cai SL, Tee AR, Short JD, Bergeron JM, Kim J, Shen J, Guo R, Johnson CL, Kiguchi K, Walker CL.** Activity of TSC2 is inhibited by AKT-mediated phosphorylation and membrane partitioning. *J Cell Biol* 173: 279–289, 2006.
12. **Calnan DR, Brunet A.** The FoxO code. *Oncogene* 27: 2276–2288, 2008.
13. **Castilho RM, Squarize CH, Chodosh LA, Williams BO, Gutkind JS.** mTOR mediates Wnt-induced epidermal stem cell exhaustion and aging. *Cell Stem Cell* 5: 279–289, 2009.
14. **Charest PG, Shen Z, Lakoduk A, Sasaki AT, Briggs SP, Firtel RA.** A Ras signaling complex controls the RasC-TORC2 pathway and directed cell migration. *Dev Cell* 18: 737–749, 2010.
15. **Chen J, Zheng XF, Brown EJ, Schreiber SL.** Identification of an 11-kDa FKBP12-rapamycin-binding domain within the 289-kDa FKBP12-rapamycin-associated protein and characterization of a critical serine residue. *Proc Natl Acad Sci USA* 92: 4947–4951, 1995.

16. **Chen SY, Bhargava A, Mastroberardino L, Meijer OC, Wang J, Buse P, Firestone GL, Verrey F, Pearce D.** Epithelial sodium channel regulated by aldosterone-induced protein sgk. *Proc Natl Acad Sci USA* 96: 2514–2519, 1999.
17. **Chiu MI, Katz H, Berlin V.** RAPT1, a mammalian homolog of yeast Tor, interacts with the FKBP12/rapamycin complex. *Proc Natl Acad Sci USA* 91: 12574–12578, 1994.
18. **Choi J, Chen J, Schreiber SL, Clardy J.** Structure of the FKBP12-rapamycin complex interacting with the binding domain of human FRAP. *Science* 273: 239–242, 1996.
19. **Chresta CM, Davies BR, Hickson I, Harding T, Cosulich S, Critchlow SE, Vincent JP, Ellston R, Jones D, Sini P, James D, Howard Z, Dudley P, Hughes G, Smith L, Maguire S, Hummersone M, Malagu K, Menear K, Jenkins R, Jacobsen M, Smith GC, Guichard S, Pass M.** AZD8055 is a potent, selective, and orally bioavailable ATP-competitive mammalian target of rapamycin kinase inhibitor with in vitro and in vivo antitumor activity. *Cancer Res* 70: 288–298, 2010.
20. **Cohen A, Hall MN.** An amino acid shuffle activates mTORC1. *Cell* 136: 399–400, 2009.
21. **Copp J, Manning G, Hunter T.** TORC-specific phosphorylation of mammalian target of rapamycin (mTOR): phospho-Ser2481 is a marker for intact mTOR signaling complex 2. *Cancer Res* 69: 1821–1827, 2009.
22. **Corradetti MN, Inoki K, Bardeesy N, DePinho RA, Guan KL.** Regulation of the TSC pathway by LKB1: evidence of a molecular link between tuberous sclerosis complex and Peutz-Jeghers syndrome. *Genes Dev* 18: 1533–1538, 2004.
23. **Cybulski N, Hall MN.** TOR complex 2: a signaling pathway of its own. *Trends Biochem Sci* 34: 620–627, 2009.
24. **Dames SA, Mulet JM, Rathgeb-Szabo K, Hall MN, Grzesiek S.** The solution structure of the FATC domain of the protein kinase target of rapamycin suggests a role for redox-dependent structural and cellular stability. *J Biol Chem* 280: 20558–20564, 2005.
25. **Dan HC, Sun M, Yang L, Feldman RI, Sui XM, Ou CC, Nellist M, Yeung RS, Halley DJ, Nicosia SV, Pledger WJ, Cheng JQ.** Phosphatidylinositol 3-kinase/Akt pathway regulates tuberous sclerosis tumor suppressor complex by phosphorylation of tuberin. *J Biol Chem* 277: 35364–35370, 2002.
26. **Dowling RJ, Topisirovic I, Alain T, Bidinosti M, Fonseca BD, Petroulakis E, Wang X, Larsson O, Selvaraj A, Liu Y, Kozma SC, Thomas G, Sonenberg N.** mTORC1-mediated cell proliferation, but not cell growth, controlled by the 4E-BPs. *Science* 328: 1172–1176, 2010.
27. **Dunlop EA, Dodd KM, Seymour LA, Tee AR.** Mammalian target of rapamycin complex 1-mediated phosphorylation of eukaryotic initiation factor 4E-binding protein 1 requires multiple protein-protein interactions for substrate recognition. *Cell Signal* 21: 1073–1084, 2009.
28. **Dunlop EA, Tee AR.** Mammalian target of rapamycin complex 1: signalling inputs, substrates and feedback mechanisms. *Cell Signal* 21: 827–835, 2009.
29. **Efeyan A, Sabatini DM.** mTOR and cancer: many loops in one pathway. *Curr Opin Cell Biol* 22: 169–176, 2010.
30. **Egan DF, Shackelford DB, Mihaylova MM, Gelino S, Kohnz RA, Mair W, Vasquez DS, Joshi A, Gwinn DM, Taylor R, Asara JM, Fitzpatrick J, Dillin A, Viollet B, Kundu M, Hansen M, Shaw RJ.** Phosphorylation of ULK1 (hATG1) by AMP-activated protein kinase connects energy sensing to mitophagy. *Science* 331: 456–461, 2011.
31. **Feldman ME, Apsel B, Uotila A, Loewith R, Knight ZA, Ruggero D, Shokat KM.** Active-site inhibitors of mTOR target rapamycin-resistant outputs of mTORC1 and mTORC2. *PLoS Biol* 7: e38, 2009.
32. **Feng Z, Zhang H, Levine AJ, Jin S.** The coordinate regulation of the p53 and mTOR pathways in cells. *Proc Natl Acad Sci USA* 102: 8204–8209, 2005.
33. **Findlay GM, Yan L, Procter J, Mieulet V, Lamb RF.** A MAP4 kinase related to Ste20 is a nutrient-sensitive regulator of mTOR signalling. *Biochem J* 403: 13–20, 2007.
34. **Foster KG, Acosta-Jaquez HA, Romeo Y, Ekim B, Soliman GA, Carriere A, Roux PP, Ballif BA, Fingar DC.** Regulation of mTOR complex 1 (mTORC1) by raptor Ser863 and multisite phosphorylation. *J Biol Chem* 285: 80–94, 2010.
35. **Foster KG, Fingar DC.** Mammalian target of rapamycin (mTOR): conducting the cellular signaling symphony. *J Biol Chem* 285: 14071–14077, 2010.
36. **Frias MA, Thoreen CC, Jaffe JD, Schroder W, Sculley T, Carr SA, Sabatini DM.** mSin1 is necessary for Akt/PKB phosphorylation, and its isoforms define three distinct mTORC2s. *Curr Biol* 16: 1865–1870, 2006.
37. **Garcia-Martinez JM, Alessi DR.** mTOR complex 2 (mTORC2) controls hydrophobic motif phosphorylation and activation of serum- and glucocorticoid-induced protein kinase 1 (SGK1). *Biochem J* 416: 375–385, 2008.
38. **Garcia-Martinez JM, Moran J, Clarke RG, Gray A, Cosulich SC, Chresta CM, Alessi DR.** Ku-0063794 is a specific inhibitor of the mammalian target of rapamycin (mTOR). *Biochem J* 421: 29–42, 2009.
39. **Goncharova EA, Goncharov DA, Eszterhas A, Hunter DS, Glassberg MK, Yeung RS, Walker CL, Noonan D, Kwiatkowski DJ, Chou MM, Panettieri RA Jr, Krymskaya VP.** Tuberin regulates p70 S6 kinase activation and ribosomal protein S6 phosphorylation A role for the TSC2 tumor suppressor gene in pulmonary lymphangioleiomyomatosis (LAM). *J Biol Chem* 277: 30958–30967, 2002.
40. **Guertin DA, Sabatini DM.** The pharmacology of mTOR inhibition. *Sci Signal* 2: pe24, 2009.
41. **Guertin DA, Stevens DM, Thoreen CC, Burds AA, Kalaany NY, Moffat J, Brown M, Fitzgerald KJ, Sabatini DM.** Ablation in mice of the mTORC components raptor, rictor, or mLST8 reveals that mTORC2 is required for signaling to Akt-FOXO and PKCalpha, but not S6K1. *Dev Cell* 11: 859–871, 2006.
42. **Gwinn DM, Shackelford DB, Egan DF, Mihaylova MM, Mery A, Vasquez DS, Turk BE, Shaw RJ.** AMPK phosphorylation of raptor mediates a metabolic checkpoint. *Mol Cell* 30: 214–226, 2008.
43. **Haghighat A, Mader S, Pause A, Sonenberg N.** Repression of cap-dependent translation by 4E-binding protein 1: competition with p220 for binding to eukaryotic initiation factor-4E. *EMBO J* 14: 5701–5709, 1995.
44. **Hara K, Maruki Y, Long X, Yoshino K, Oshiro N, Hidayat S, Tokunaga C, Avruch J, Yonezawa K.** Raptor, a binding partner of target of rapamycin (TOR), mediates TOR action. *Cell* 110: 177–189, 2002.
45. **Hara K, Yonezawa K, Kozlowski MT, Sugimoto T, Andrabi K, Weng QP, Kasuga M, Nishimoto I, Avruch J.** Regulation of eIF-4E BP1 phosphorylation by mTOR. *J Biol Chem* 272: 26457–26463, 1997.
46. **Hara K, Yonezawa K, Weng QP, Kozlowski MT, Belham C, Avruch J.** Amino acid sufficiency and mTOR regulate p70 S6 kinase and eIF-4E BP1 through a common effector mechanism. *J Biol Chem* 273: 14484–14494, 1998.
47. **Hardie DG.** AMP-activated/SNF1 protein kinases: conserved guardians of cellular energy. *Nat Rev Mol Cell Biol* 8: 774–785, 2007.
48. **Hardie DG.** Cell biology: why starving cells eat themselves. *Science* 331: 410–411, 2011.
49. **Hardie DG.** Signal transduction: wow cells sense energy. *Nature* 472: 176–177, 2011.
50. **Harrington LS, Findlay GM, Lamb RF.** Restraining PI3K: mTOR signalling goes back to the membrane. *Trends Biochem Sci* 30: 35–42, 2005.
51. **Hay N, Sonenberg N.** Upstream and downstream of mTOR. *Genes Dev* 18: 1926–1945, 2004.
52. **He C, Klionsky DJ.** Regulation mechanisms and signaling pathways of autophagy. *Annu Rev Genet* 43: 67–93, 2009.
53. **Heitman J, Movva NR, Hall MN.** Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast. *Science* 253: 905–909, 1991.
54. **Holz MK, Ballif BA, Gygi SP, Blenis J.** mTOR and S6K1 mediate assembly of the translation preinitiation complex through dynamic protein interchange and ordered phosphorylation events. *Cell* 123: 569–580, 2005.
55. **Hresko RC, Mueckler mTOR M.** RICTOR is the Ser473 kinase for Akt/protein kinase B in 3T3-L1 adipocytes. *J Biol Chem* 280: 40406–40416, 2005.
56. **Huang B, Porter G.** Expression of proline-rich Akt-substrate PRAS40 in cell survival pathway and carcinogenesis. *Acta Pharmacol Sin* 26: 1253–1258, 2005.
57. **Huang J, Dibble CC, Matsuzaki M, Manning BD.** The TSC1-TSC2 complex is required for proper activation of mTOR complex 2. *Mol Cell Biol* 28: 4104–4115, 2008.
58. **Huang J, Manning BD.** The TSC1-TSC2 complex: a molecular switchboard controlling cell growth. *Biochem J* 412: 179–190, 2008.
59. **Inoki K, Li Y, Xu T, Guan KL.** Rheb GTPase is a direct target of TSC2 GAP activity and regulates mTOR signaling. *Genes Dev* 17: 1829–1834, 2003.

60. **Inoki K, Li Y, Zhu T, Wu J, Guan KL.** TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. *Nat Cell Biol* 4: 648–657, 2002.
61. **Inoki K, Ouyang H, Zhu T, Lindvall C, Wang Y, Zhang X, Yang Q, Bennett C, Harada Y, Stankunas K, Wang CY, He X, MacDougald OA, You M, Williams BO, Guan KL.** TSC2 integrates Wnt and energy signals via a coordinated phosphorylation by AMPK and GSK3 to regulate cell growth. *Cell* 126: 955–968, 2006.
62. **Jacinto E, Facchinetti V, Liu D, Soto N, Wei S, Jung SY, Huang Q, Qin J, Su B.** SIN1/MIP1 maintains rictor-mTOR complex integrity and regulates Akt phosphorylation and substrate specificity. *Cell* 127: 125–137, 2006.
63. **Jacinto E, Loewith R, Schmidt A, Lin S, Ruegg MA, Hall A, Hall MN.** Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. *Nat Cell Biol* 6: 1122–1128, 2004.
64. **Jacinto E, Lorberg A.** TOR regulation of AGC kinases in yeast and mammals. *Biochem J* 410: 19–37, 2008.
65. **Katso R, Okkenhaug K, Ahmadi K, White S, Timms J, Waterfield MD.** Cellular function of phosphoinositide 3-kinases: implications for development, homeostasis, and cancer. *Annu Rev Cell Dev Biol* 17: 615–675, 2001.
66. **Keith CT, Schreiber SL.** PIK-related kinases: DNA repair, recombination, and cell cycle checkpoints. *Science* 270: 50–51, 1995.
67. **Kim DH, Sarbassov DD, Ali SM, King JE, Latek RR, Erdjument-Bromage H, Tempst P, Sabatini DM.** mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. *Cell* 110: 163–175, 2002.
68. **Kim DH, Sarbassov DD, Ali SM, Latek RR, Guntur KV, Erdjument-Bromage H, Tempst P, Sabatini DM.** GbetaL, a positive regulator of the rapamycin-sensitive pathway required for the nutrient-sensitive interaction between raptor and mTOR. *Mol Cell* 11: 895–904, 2003.
69. **Kim E, Goraksha-Hicks P, Li L, Neufeld TP, Guan KL.** Regulation of TORC1 by Rag GTPases in nutrient response. *Nat Cell Biol* 10: 935–945, 2008.
70. **Kim JE, Chen J.** Regulation of peroxisome proliferator-activated receptor-gamma activity by mammalian target of rapamycin and amino acids in adipogenesis. *Diabetes* 53: 2748–2756, 2004.
71. **Klionsky DJ.** Autophage: from phenology to molecular understanding in less than a decade. *Nat Rev Mol Cell Biol* 8: 931–937, 2007.
72. **Kondo K, Yao M, Kobayashi K, Ota S, Yoshida M, Kaneko S, Baba M, Sakai N, Kishida T, Kawakami S, Uemura H, Nagashima Y, Nakatani Y, Hosaka M.** PTEN/MMAC1/TEP1 mutations in human primary renal-cell carcinomas and renal carcinoma cell lines. *Int J Cancer* 91: 219–224, 2001.
73. **Kunz J, Henriquez R, Schneider U, Deuter-Reinhard M, Movva NR, Hall MN.** Target of rapamycin in yeast, TOR2, is an essential phosphatidylinositol kinase homolog required for G1 progression. *Cell* 73: 585–596, 1993.
74. **Kunz J, Schneider U, Howald I, Schmidt A, Hall MN.** HEAT repeats mediate plasma membrane localization of Tor2p in yeast. *J Biol Chem* 275: 37011–37020, 2000.
75. **Lang F, Bohmer C, Palmada M, Seebohm G, Strutz-Seebohm N, Vallon V.** (Patho)physiological significance of the serum- and glucocorticoid-inducible kinase isoforms. *Physiol Rev* 86: 1151–1178, 2006.
76. **Laplante M, Sabatini DM.** mTOR signaling at a glance. *J Cell Sci* 122: 3589–3594, 2009.
77. **Lawlor MA, Alessi DR.** PKB/Akt: a key mediator of cell proliferation, survival and insulin responses? *J Cell Sci* 114: 2903–2910, 2001.
78. **Lee DF, Kuo HP, Chen CT, Hsu JM, Chou CK, Wei Y, Sun HL, Li LY, Ping B, Huang WC, He X, Hung JY, Lai CC, Ding Q, Su JL, Yang JY, Sahin AA, Hortobagyi GN, Tsai FJ, Tsai CH, Hung MC.** IKK beta suppression of TSC1 links inflammation and tumor angiogenesis via the mTOR pathway. *Cell* 130: 440–455, 2007.
79. **Lee S, Comer FI, Sasaki A, McLeod IX, Duong Y, Okumura K, Yates JR, 3rd Parent CA, Firtel RA.** TOR complex 2 integrates cell movement during chemotaxis and signal relay in Dictyostelium. *Mol Biol Cell* 16: 4572–4583, 2005.
80. **Lieberthal W.** Macroautophagy: a mechanism for mediating cell death or for promoting cell survival? *Kidney Int* 74: 555–557, 2008.
81. **Loewith R, Jacinto E, Wullschleger S, Lorberg A, Crespo JL, Bonenfant D, Oppliger W, Jenoe P, Hall MN.** Two TOR complexes, only one of which is rapamycin sensitive, have distinct roles in cell growth control. *Mol Cell* 10: 457–468, 2002.
82. **Lu M, Wang J, Jones KT, Ives HE, Feldman ME, Yao LJ, Shokat KM, Ashrafi K, Pearce D.** mTOR complex-2 activates ENaC by phosphorylating SGK1. *J Am Soc Nephrol* 21: 811–818, 2010.
83. **Ma L, Chen Z, Erdjument-Bromage H, Tempst P, Pandolfi PP.** Phosphorylation and functional inactivation of TSC2 by Erk implications for tuberous sclerosis and cancer pathogenesis. *Cell* 121: 179–193, 2005.
84. **Ma XM, Blenis J.** Molecular mechanisms of mTOR-mediated translational control. *Nat Rev Mol Cell Biol* 10: 307–318, 2009.
85. **Ma XM, Yoon SO, Richardson CJ, Julich K, Blenis J.** SKAR links pre-mRNA splicing to mTOR/S6K1-mediated enhanced translation efficiency of spliced mRNAs. *Cell* 133: 303–313, 2008.
86. **Maehama T, Tanaka M, Nishina H, Murakami M, Kanaho Y, Hanada K.** RalA functions as an indispensable signal mediator for the nutrient-sensing system. *J Biol Chem* 283: 35053–35059, 2008.
87. **Marone R, Cmiljanovic V, Giese B, Wymann MP.** Targeting phosphoinositide 3-kinase: moving towards therapy. *Biochim Biophys Acta* 1784: 159–185, 2008.
88. **Mayer C, Zhao J, Yuan X, Grummt I.** mTOR-dependent activation of the transcription factor TIF-IA links rRNA synthesis to nutrient availability. *Genes Dev* 18: 423–434, 2004.
89. **Mizushima N, Levine B.** Autophagy in mammalian development and differentiation. *Nat Cell Biol* 12: 823–830, 2010.
90. **Narita M, Young AR, Arakawa S, Samarajiwa SA, Nakashima T, Yoshida S, Hong S, Berry LS, Reichelt S, Ferreira M, Tavare S, Inoki K, Shimizu S.** Spatial coupling of mTOR and autophagy augments secretory phenotypes. *Science* 332: 966–970, 2011.
91. **Nicklin P, Bergman P, Zhang B, Triantafellow E, Wang H, Nyfeler B, Yang H, Hild M, Kung C, Wilson C, Myer VE, MacKeigan JP, Porter JA, Wang YK, Cantley LC, Finan PM, Murphy LO.** Bidirectional transport of amino acids regulates mTOR and autophagy. *Cell* 136: 521–534, 2009.
92. **Nobukuni T, Joaquin M, Roccio M, Dann SG, Kim SY, Gulati P, Byfield MP, Backer JM, Natt F, Bos JL, Zwartkruis FJ, Thomas G.** Amino acids mediate mTOR/raptor signaling through activation of class 3 phosphatidylinositol 3OH-kinase. *Proc Natl Acad Sci USA* 102: 14238–14243, 2005.
93. **Nojima H, Tokunaga C, Eguchi S, Oshiro N, Hidayat S, Yoshino K, Hara K, Tanaka N, Avruch J, Yonezawa K.** The mammalian target of rapamycin (mTOR) partner, raptor, binds the mTOR substrates p70 S6 kinase and 4E-BP1 through their TOR signaling (TOS) motif. *J Biol Chem* 278: 15461–15464, 2003.
94. **Oakhill JS, Steel R, Chen ZP, Scott JW, Ling N, Tam S, Kemp BE.** AMPK is a direct adenylate charge-regulated protein kinase. *Science* 332: 1433–1435, 2011.
95. **Orlova KA, Crino PB.** The tuberous sclerosis complex. *Ann NY Acad Sci* 1184: 87–105, 2010.
96. **Oshiro N, Yoshino K, Hidayat S, Tokunaga C, Hara K, Eguchi S, Avruch J, Yonezawa K.** Dissociation of raptor from mTOR is a mechanism of rapamycin-induced inhibition of mTOR function. *Genes Cells* 9: 359–366, 2004.
97. **Pearce LR, Huang X, Boudeau J, Pawlowski R, Wullschleger S, Deak M, Ibrahim AF, Gourlay R, Magnuson MA, Alessi DR.** Identification of Protor as a novel Rictor-binding component of mTOR complex-2. *Biochem J* 405: 513–522, 2007.
98. **Peterson TR, Laplante M, Thoreen CC, Sancak Y, Kang SA, Kuehl WM, Gray NS, Sabatini DM.** DEPTOR is an mTOR inhibitor frequently overexpressed in multiple myeloma cells and required for their survival. *Cell* 137: 873–886, 2009.
99. **Porstmann T, Santos CR, Griffiths B, Cully M, Wu M, Leevers S, Griffiths JR, Chung YL, Schulze A.** SREBP activity is regulated by mTORC1 and contributes to Akt-dependent cell growth. *Cell Metab* 8: 224–236, 2008.
100. **Raught B, Peiretti F, Gingras AC, Livingstone M, Shahbazian D, Mayeur GL, Polakiewicz RD, Sonenberg N, Hershey JW.** Phosphorylation of eucaryotic translation initiation factor 4B Ser422 is modulated by S6 kinases. *EMBO J* 23: 1761–1769, 2004.
101. **Sabatini DM, Erdjument-Bromage H, Lui M, Tempst P, Snyder SH.** RAFT1: a mammalian protein that binds to FKBP12 in a rapamycin-dependent fashion and is homologous to yeast TORs. *Cell* 78: 35–43, 1994.
102. **Sancak Y, Bar-Peled L, Zoncu R, Markhard AL, Nada S, Sabatini DM.** Ragulator-Rag complex targets mTORC1 to the lysosomal surface and is necessary for its activation by amino acids. *Cell* 141: 290–303, 2010.

103. **Sancak Y, Peterson TR, Shaul YD, Lindquist RA, Thoreen CC, Bar-Peled L, Sabatini DM.** The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1. *Science* 320: 1496–1501, 2008.
104. **Sancak Y, Thoreen CC, Peterson TR, Lindquist RA, Kang SA, Spooner E, Carr SA, Sabatini DM.** PRAS40 is an insulin-regulated inhibitor of the mTORC1 protein kinase. *Mol Cell* 25: 903–915, 2007.
105. **Sarbassov DD, Ali SM, Kim DH, Guertin DA, Latek RR, Erdjument-Bromage H, Tempst P, Sabatini DM.** Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton. *Curr Biol* 14: 1296–1302, 2004.
106. **Sarbassov DD, Ali SM, Sengupta S, Sheen JH, Hsu PP, Bagley AF, Markhard AL, Sabatini DM.** Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. *Mol Cell* 22: 159–168, 2006.
107. **Sarbassov DD, Guertin DA, Ali SM, Sabatini DM.** Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science* 307: 1098–1101, 2005.
108. **Sato T, Nakashima A, Guo L, Tamanoi F.** Specific activation of mTORC1 by Rheb G-protein in vitro involves enhanced recruitment of its substrate protein. *J Biol Chem* 284: 12783–12791, 2009.
109. **Saucedo LJ, Gao X, Chiarelli DA, Li L, Pan D, Edgar BA.** Rheb promotes cell growth as a component of the insulin/TOR signalling network. *Nat Cell Biol* 5: 566–571, 2003.
110. **Schalm SS, Fingar DC, Sabatini DM, Blenis J.** TOS motif-mediated raptor binding regulates 4E-BP1 multisite phosphorylation and function. *Curr Biol* 13: 797–806, 2003.
111. **Schieke SM, Phillips D, McCoy JP Jr, Aponte AM, Shen RF, Balaban RS, Finkel T.** The mammalian target of rapamycin (mTOR) pathway regulates mitochondrial oxygen consumption and oxidative capacity. *J Biol Chem* 281: 27643–27652, 2006.
112. **Sengupta S, Peterson TR, Sabatini DM.** Regulation of the mTOR complex 1 pathway by nutrients, growth factors, and stress. *Mol Cell* 40: 310–322, 2010.
113. **Smith EM, Finn SG, Tee AR, Browne GJ, Proud CG.** The tuberous sclerosis protein TSC2 is not required for the regulation of the mammalian target of rapamycin by amino acids and certain cellular stresses. *J Biol Chem* 280: 18717–18727, 2005.
114. **Stambolic V, MacPherson D, Sas D, Lin Y, Snow B, Jang Y, Benchimol S, Mak TW.** Regulation of PTEN transcription by p53. *Mol Cell* 8: 317–325, 2001.
115. **Stocker H, Radimerski T, Schindelholz B, Wittwer F, Belawat P, Daram P, Breuer S, Thomas G, Hafen E.** Rheb is an essential regulator of S6K in controlling cell growth in Drosophila. *Nat Cell Biol* 5: 559–565, 2003.
116. **Sun Y, Chen J.** mTOR signaling: PLD takes center stage. *Cell Cycle* 7: 3118–3123, 2008.
117. **Takahara T, Hara K, Yonezawa K, Sorimachi H, Maeda T.** Nutrient-dependent multimerization of the mammalian target of rapamycin through the N-terminal HEAT repeat region. *J Biol Chem* 281: 28605–28614, 2006.
118. **Thoreen CC, Kang SA, Chang JW, Liu Q, Zhang J, Gao Y, Reichling LJ, Sim T, Sabatini DM, Gray NS.** An ATP-competitive mammalian target of rapamycin inhibitor reveals rapamycin-resistant functions of mTORC1. *J Biol Chem* 284: 8023–8032, 2009.
119. **Toschi A, Lee E, Xu L, Garcia A, Gadir N, Foster DA.** Regulation of mTORC1 and mTORC2 complex assembly by phosphatidic acid: competition with rapamycin. *Mol Cell Biol* 29: 1411–1420, 2009.
120. **Vander Haar E, Lee SI, Bandhakavi S, Griffin TJ, Kim DH.** Insulin signalling to mTOR mediated by the Akt/PKB substrate PRAS40. *Nat Cell Biol* 9: 316–323, 2007.
121. **Wang L, Harris TE, Roth RA, Lawrence JC Jr.** PRAS40 regulates mTORC1 kinase activity by functioning as a direct inhibitor of substrate binding. *J Biol Chem* 282: 20036–20044, 2007.
122. **Wang X, Li W, Williams M, Terada N, Alessi DR, Proud CG.** Regulation of elongation factor 2 kinase by p90(RSK1) and p70 S6 kinase. *EMBO J* 20: 4370–4379, 2001.
123. **Weide T, Huber TB.** Implications of autophagy for glomerular aging and disease. *Cell Tissue Res* 343: 467–473, 2011.
124. **Woods A, Johnstone SR, Dickerson K, Leiper FC, Fryer LG, Neumann D, Schlattner U, Wallimann T, Carlson M, Carling D.** LKB1 is the upstream kinase in the AMP-activated protein kinase cascade. *Curr Biol* 13: 2004–2008, 2003.
125. **Wulff P, Vallon V, Huang DY, Volkl H, Yu F, Richter K, Jansen M, Schlunz M, Klingel K, Loffing J, Kauselmann G, Bosl MR, Lang F, Kuhl D.** Impaired renal Na^+ retention in the sgk1-knockout mouse. *J Clin Invest* 110: 1263–1268, 2002.
126. **Wullschleger S, Loewith R, Oppliger W, Hall MN.** Molecular organization of target of rapamycin complex 2. *J Biol Chem* 280: 30697–30704, 2005.
127. **Xu L, Salloum D, Medlin PS, Saqcena M, Yellen P, Perrella B, Foster DA.** Phospholipase D mediates nutrient input to mTORC1. *J Biol Chem* 2011.
128. **Yang Q, Inoki K, Ikenoue T, Guan KL.** Identification of Sin1 as an essential TORC2 component required for complex formation and kinase activity. *Genes Dev* 20: 2820–2832, 2006.
129. **Yang Z, Klionsky DJ.** Eaten alive: a history of macroautophagy. *Nat Cell Biol* 12: 814–822, 2010.
130. **Yu K, Toral-Barza L, Shi C, Zhang WG, Lucas J, Shor B, Kim J, Verheijen J, Curran K, Malwitz DJ, Cole DC, Ellingboe J, Ayral-Kaloustian S, Mansour TS, Gibbons JJ, Abraham RT, Nowak P, Zask A.** Biochemical, cellular, and in vivo activity of novel ATP-competitive and selective inhibitors of the mammalian target of rapamycin. *Cancer Res* 69: 6232–6240, 2009.
131. **Zoncu R, Bar-Peled L, Efeyan A, Wang S, Sancak Y, Sabatini DM.** mTORC1 senses lysosomal amino acids through an inside-out mechanism that requires the vacuolar H-ATPase. *Science* 334: 678–683, 2011.
132. **Zoncu R, Efeyan A, Sabatini DM.** mTOR: from growth signal integration to cancer, diabetes and ageing. *Nat Rev Mol Cell Biol* 12: 21–35, 2011.