Chapter 1

Protein Phosphorylation as a Key Mechanism of mTORC1/2 Signaling Pathways

Elena Tchevkina and Andrey Komelkov

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/48274

1. Introduction

The mammalian target of rapamycin (mTOR) has attracted growing attention during the past decade due to the increase realization of it's extraordinarily significance in cellular life-sustaining activity on the one hand, and because of its crucial role in a variety of diseases, (including cancer, hamartoma syndromes, cardiac hypertrophy, diabetes and obesity) on the other. mTOR is an atypical serine/threonine protein kinase, belonging to the phosphatidylinositol kinase-related kinase (PIKK) family. Cumulative evidence indicates that mTOR acts as a 'master switch' of cellular energy-intensive anabolic processes and energy-producing catabolic activities. It coordinates the rate of cell growth, proliferation and survival in response to extracellular mitogen, energy, nutrient and stress signals [1, 2]. mTOR functions within two distinct multiprotein complexes, mTORC1 and mTORC2, responsible for the different physiological functions. Thus, mTORC1 is considered mostly involved in the regulation of the translation initiation machinery influencing cell growth, proliferation, and survival, while mTORC2 participates in actin cytoskeleton rearrangements and cell survival. mTORC1 and mTORC2 were initially identified in yeast on the basis of their differential sensitivity to the inhibitory effects of rapamycin, mTORC1 being originally considered as rapamycin-sensitive and mTORC2 as rapamycin-insensitive [3-5].

The history of TOR began in the early 1970s when a bacterial strain, *Streptomyces hygroscopicus*, was first isolated from Rapa Nui island during a discovery program for anti-microbial agents. These bacteria secrete a potent anti-fungal macrolide that was named rapamycin after the location of its discovery [6-9]. Later rapamycin was proven to have anti-proliferative and immunosuppressive properties. In the beginning of 1990s, two rapamycin target genes titled TOR1 (the target of rapamycin 1) and TOR2 were discovered through the yeast genetic screens for mutations that counteract the growth inhibitory properties of rapamycin [10, 11]. Further studies revealed that rapamycin forms the complex with its

intracellular receptor, FK506-binding protein 12 kDa (FKBP12), This complex binds a region in the C-terminus of TOR kinase named FRB (FKB12-rapamycin binding) domain, what leads to the inhibition of TOR functions [12-14].

At present, it becomes clear that mTORC1 and mTORC2 activities are mediated through diverse signaling pathways depending on the type of extracellular signal. Thus, signaling from growth factors is mediated predominantly through PI3K-Akt-TSC1/2 pathway and upregulates mTORC1 to stimulate translation initiation, while energy or nutrient depletion and stresses suppress mTORC1 via LKB1–AMPK cascade to trigger off the process of autophagy. In contrast, mTORC2 is insensitive to nutrients or energy conditions. mTORC2 phosphorylates Akt and some other protein kinases regulating actin cytoskeleton and cell survival in response to growth factors and hormones. The physiological functions of mTOR continue to expand. It should be stressed, that the signaling throughout the complicated mTOR network, including branched pathways and feedback loops, is regulated predominantly by phosphorylation and includes myriads of phosphorylation events. Moreover, the complexity of mTOR regulation is amplified by the crosstalk with other signaling pathways, such as MAP kinase- or TNFα-dependent cascades, which activity is also determined by vast number of phosphorylations. The complication of mTOR signaling additionally increases due to the hierarchical character of multiple site-specific phosphorylations of the main mTOR targets. Up to date there are no full clarity, concerning which kinase is responsible for each site phosphorylation as well as functional role and precise mechanisms of each phosphorylation event. The better understanding of underlying molecular mechanisms is now especially essential since inhibitors of mTOR signaling are widely used as drugs in the therapy of cancer and neurodegenerative diseases.

2. mTOR kinase structural organization

Although mTOR has limited sequence similarities in eukaryotes, it demonstrates a high level of conservation in its key cellular functions. mTOR, also known as FRAP (FKBP12-rapamycin-associated protein), RAFT1 (rapamycin and FKBP12 target), RAPT 1 (rapamycin target 1), or SEP (sirolimus effector protein), is a large 289 kDa atypical serine/threonine (S/T) kinase [15-18] and is considered a member of the phosphatidylinositol 3-kinase (PI3K)-kinase-related kinase (PIKK) superfamily since its C-terminus shares strong homology to the catalytic domain of PI3K [19, 20]. mTOR and yeast TOR proteins share > 65% identity in carboxy-terminal catalytic domains and about 40% identity in overall sequence [21]. At the amino-acid level, human, mouse and rat TOR proteins share a 95% identity [22, 23]. The knockout of mTOR in mice is embryonic lethal, indicating its physiological importance [24, 25].

Structurally, mTOR contains 2549 amino acids and the region of first 1200 N-terminal amino acids contains up to 20 tandem repeated **HEAT** (a protein-protein interaction structure of two tandem anti-parallel α-helices found in **H**untingtin, **E**longation factor 3 (EF3), PR65/**A** subunit of protein phosphatase 2A (PP2A), and **T**OR) motifs [26]. Tandem HEAT repeats are present in many proteins and may form an extended superhelical structure responsible for protein-protein interactions. HEAT repeats region is followed by a **FAT** (**F**RAP, **A**TM, and

TRRAP (PIKK family members)) domain and **FRB** (**F**KPB12-**r**apamycin **b**inding domain), which serves as a docking site for the rapamycin -FKBP12 complex formation. Downstream lies a catalytic kinase domain and a **FATC (FAT C**arboxyterminal) domain, located at the C-terminus of the protein (**Figure 1A).** The FAT and FATC domains are always found in combination, so it has been hypothesized that the interactions between FAT and FATC might contribute to the catalytic kinase activity of mTOR via unknown mechanisms [26, 27].

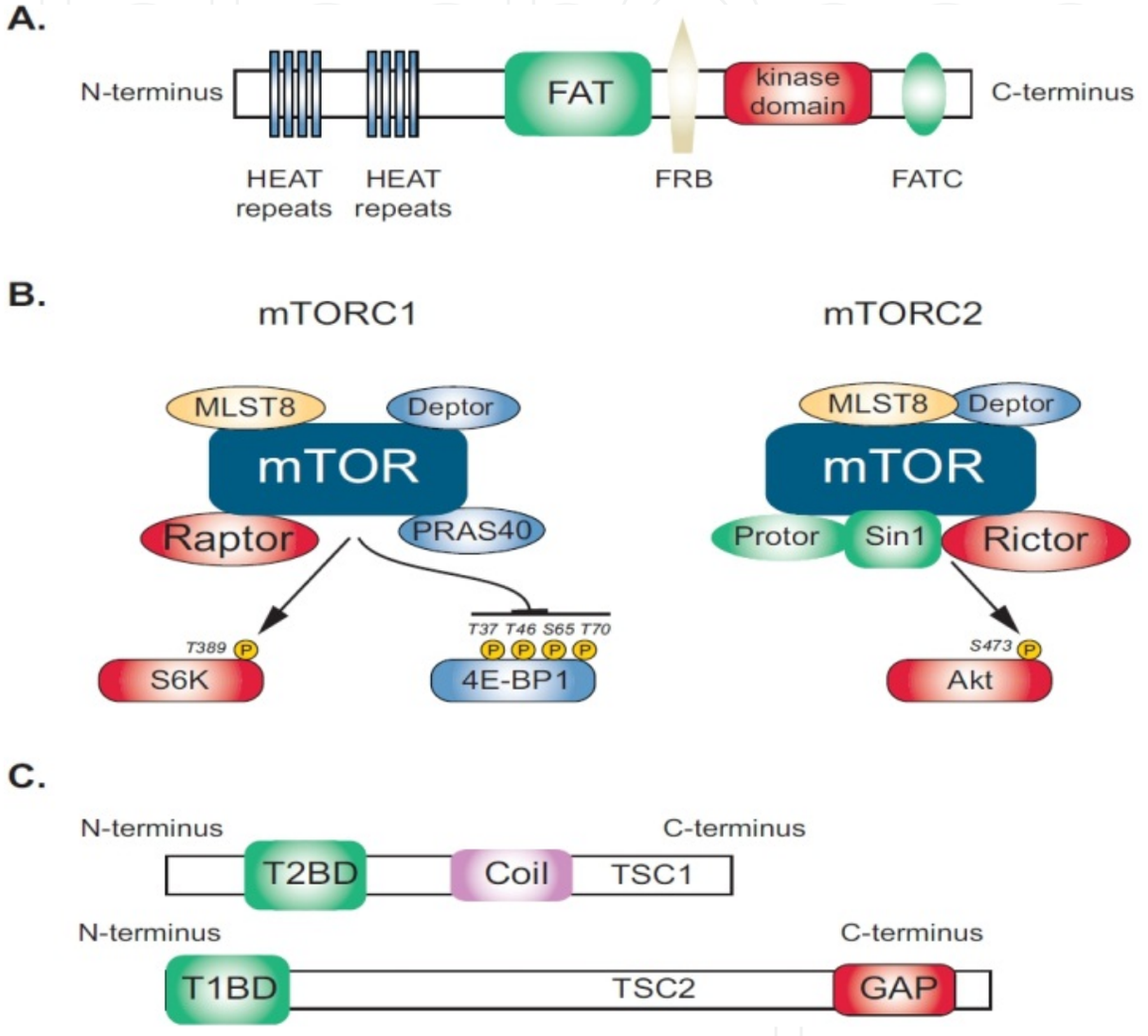

Figure 1. A. The domain structure of mTOR. mTOR contains tandem HEAT repeats, central FAT domain, FRB domain, a catalytic kinase domain and the FATC domain. Rapamycin associates with its intracellular receptor, FKBP12, and the resulting complex interacts with the FRB domain of mTOR. Binding of rapamycin–FKBP12 to the FRB domain disrupts the association of mTOR with the mTORC1 specific component Raptor and thus uncouples mTORC1 from its substrates, thereby blocking mTORC1 signaling. **B.** Composition of mTORC1 and mTORC2. mTORC1 consists of mTOR, Raptor, PRAS40, mLST8 and Deptor. mLST8 binds to the mTOR kinase domain in both complexes, where it seems to be crucial for their assembly. Deptor acts as an inhibitor of both complexes. Other protein partners differ between the two complexes. mTORC2 contains Rictor, mSIN1, and Protor1. **C.** Schematic of the TSC1 and TSC2 proteins. The functional domains (including GAP) on TSC1 and TSC2 are represented schematically. T2BD/T1BD — TSC2 and TSC1 binding domains respectively.

Up to date quite a few phosphorylation sites in mTOR have been reported, namely T2446, S2448, S2481 and S1261 and this list will be probably appended. S2481 is considered to be a site of autophosphorylation [28]. S2481 is the only site the phosphorylation of which is well established for regulating mTOR intrinsic activity [29, 30]; the significance of other phosphorylation sites for mTOR activity are not entirely clear. Recently, S1261 has been reported as a novel mTOR phosphorylation site in mammalian cells and the first evidences of this phosphorylation in regulating mTORC1 autokinase activity has been provided [31]. Although phosphorylation at T2446/S2448 was shown to be PI3K/Akt-dependent, mTORC1 downstream kinase S6K has been also reported to phosphorylate these two sites [32]. The significance of this potential feedback loop is unknown, as it is not yet clear whether and how these phosphorylations influence mTOR activity

Binding of rapamycin–FKBP12 to the FRB domain of mTOR disrupts the association of mTOR with mTORC1-specific component Raptor and thus divide mTORC1 from its targets, blocking mTORC1 signaling. However, whether rapamycin directly inhibits mTOR's intrinsic kinase activity is still not entirely clear [3, 33, 34].

The TOR complexes mTORC1 and mTORC2

The mammalian mTORC1 and mTORC2 complexes perform non-overlapping functions within the cell. Thus, the best-known function of TORC1 signaling is the promotion of translation. Other mTORC1 functions include autophagy inhibition, promotion of the ribosome biogenesis and of the tRNA production. The main known mTORC2 activity is the phosphorylation and activation of AKT and of the related kinases — serum/glucocorticoid regulated kinase (SGK) and protein kinase C (PKC) [35]. It is also involved in cytoskeletal organization. Although both mTOR complexes exist predominantly in the cytoplasm, some data indicate that they could function in different compartments. Thus, upon nutrients and energy availability mTORC1 is recruited to lysosomes where it could be fully activated [36] and where it functions to suppress autophagy. Unlike mTORC1, mTORC2 according to the most recent data localizes predominantly in ER compartment where it could directly associate with ribosomes [37, 38]. Additionally, some data evidence that mTOR may actually be a cytoplasmic-nuclear shuttling protein. The nuclear shuttling could facilitate the phosphorylation of mTORC1 substrates under the mitogenic stimulation [39]. The unique compositions of mTORC1 and mTORC2 determine the selectivity of their binding partners. Up to date we know more about mTORC1 rather then mTORC2 probably due to the lack of available and wide-spreaded inhibitors of mTORC2 activity.

TORC1 composition. Within the mammalian cells, TORC1 functions as a homodimer. Each monomer consists of mTOR, **r**egulatory **a**ssociated **p**rotein of m**TOR** (Raptor), **p**roline-**r**ich **A**KT **s**ubstrate **40** kDa (PRAS40), **DEP** domain **TOR**-binding protein (Deptor) and **m**ammalian **l***ethal* with **S**ec-13 protein **8** (mLST8, also known as GbL) [40, 41]**(Figure 1B).**

Raptor is a 150 kDa presumably non-enzymatic subunit of mTORC1 that is essential for the kinase mTORC1 activity *in vitro* and *in vivo* in response to insulin, nutrient and energy level. [42, 43]. It includes a highly conserved N-terminal region followed by 3 HEAT repeats and 7

WD40 (about **40** amino acids with conserved **W** and **D** forming four anti-parallel beta strands) repeats. The Raptor-mTOR interaction is very dynamic, and is thought to require the HEAT repeats of mTOR. It is established that Raptor is indispensable for mTOR to phosphorylate its main effectors p70S6 kinase (S6K1) and **e**ukaryotic **i**nitiation **f**actor **4E** (eIF4E) **b**inding **p**rotein 1 (4E-BP1), but whether Raptor positively or negatively regulates mTOR itself remains controversial [43]. Raptor is essential for mTORC1 complex formation and for the dimerization of TORC1 complexes as it provides direct interaction between TOR proteins from each monomer. Thus it can be considered to be a scaffolding protein that recruits substrates for mTOR thereby demonstrating a stimulating effect on mTOR activity [43]. Alternatively, other study has demonstrated that Raptor negatively regulates mTOR being tightly bound to the kinase [42]. There are also a hypothesis according to which at least two types of interaction exist between Raptor and mTOR depending on nutrients availability. One mTOR-Raptor complex that forms in the absence of nutrients is stable and leads to a repression of the mTOR catalytic activity. In contrast, the other complex that forms under nutrients-rich conditions is unstable, but it is important for *in vivo* mTOR function [42] (reviewed in [44]). Recent studies suggested that the phosphorylation status of Raptor could influence mTORC1 activity [45]. Phosphorylation on S722/792 is mediated by AMPK (AMP-activated protein kinase) and is required for the inhibition of mTORC1 activity induced by energy stress [45], whereas phosphorylation of Raptor on S719/721/722 is mediated by the p90 ribosomal S6 kinases (RSKs) and contributes to the activation of mTORC1 by mitogen stimulation [45, 46]. Most recently, S863 in Raptor was identified as mTOR-mediated phosphorylation site responsible for the insulin-dependent activation of mTORC1 [47].

PRAS40, another subunit of mTORC1, has been defined as a direct negative regulator of mTORC1 function [48]. Initially, PRAS40 was identified as a novel substrate of Akt being directly phosphorylated at T246 near its C-terminus [49]. This phosphorylation releases inhibition of mTORC1 by PRAS40. Subsequent studies showed that PRAS40 associates with mTORC1 via Raptor and inhibits mTORC1 activity [48]. A putative TOR signaling motif, FVMDE, has been identified in PRAS40 and shown to be required for interaction with Raptor. Upon binding to Raptor, PRAS40 is phosphorylated on S183 by mTORC1 both *in vivo* and *in vitro* [50] Thus, PRAS40 has been implicated as a physiological substrate of mTORC1. Most recently, two novel sites in PRAS40 phosphorylated by mTORC1, S212 and S221, have been identified [51]. Rapamycin treatment reduced the phosphorylation of S183 and S221 but not S212, indicating that besides mTORC1, other kinases may also regulate the phosphorylation of S212 *in vivo* [51].

mLST8 has been identified after Raptor as a stable component of both mTOR complexes [52]. It consists almost entirely of seven "sticky" WD40 repeats, and has been initially shown to bind to the kinase domain of mTOR, leading to the hypothesis that mLST8 positively regulates mTOR kinase activity. It was proposed that mLST8 is essential for a nutrient- and rapamycin-sensitive interaction between Raptor and mTOR [52]. However, there is no substantial evidence to support this idea. It has been speculated, that mLST8 may participate in the amino acids mediated activation of TORC1 being insignificant for other

mechanisms of TORC1 activation [52]. Alternatively, recent studies demonstrated functional importance of mLST8 for the Rictor-mTOR interaction, evidencing that mLST8 is involved in mTORC2 rather than in mTORC1 activity.

Deptor binds to mTOR at the FAT domain thus originally proposed to be a part of TORC1. Recently it has been identified as mTOR inhibitor that acts on both TORC1 and TORC2. The upstream regulators of Deptor still remain unknown [41].

mTORC2 composition and distinctions from mTORC1

In 2004, mTORC2, containing mTOR, mLST8 and Rictor was identified [3, 4]. Since mTORC2 complex was discovered later than mTORC1, its functions and regulatory mechanisms are less understood [3]. TORC2 and TORC1 contain common subunits, as is mTOR itself, mLST8 and Deptor, but instead of Raptor, mTORC2 includes two different subunits, Rictor (**r**apamycin-**i**nsensitive **c**ompanion of m**TOR)** and mSin1 (**m**ammalian **s**tress-activated protein kinase (SAPK)-**in**teracting protein **1**) [3, 4, 53]. In addition, Protor (**pr**otein **o**bserved with Rictor) was also considered a component of mTORC2 (**Figure 1B**) [54, 55]. mTORC2 was originally thought to be rapamycin-insensitive [3], however, further studies demonstrated that prolonged rapamycin treatment inhibits the assembly of mTORC2 as well as its activity towards Akt phosphorylation in certain cell lines [56].

Rictor is the first identified TORC2 specific component [3, 4]. It represents a large protein with a predicted molecular weight of about 200 kDa. Although Rictor contains no apparent catalytic domain motifs [4], knockdown of Rictor results in the loss of actin polymerization and cell spreading, the main known mTORC2 functions [4]. It was shown that the Rictor-mTOR complex does not affect the mTORC1 effectors S6K1 and 4E-BP1, but influence the activities of several proteins known as mTORC2 downstream targets, including phosphorylation of Akt, PKC and the focal adhesion proteins.

mSin1 was recently identified as a novel component of mTORC2, which is important for both the complex assembly and function [57-59]. *Sin1* is conserved among all eukaryotic species especially in the middle part of the sequence [60]. A Ras-binding domain and a C-terminal PH domain have been identified recently [61]. The several experimental techniques showed the importance of Sin1 for mTORC2 function [62]. The interaction *in vivo* between Sin1 and Rictor is more stable than their interactions with mTOR probably due to the ability of Sin1 and Rictor to stabilize each other [59]. Thus knockdown of Sin1 decreases the interaction between mTOR and Rictor, suggesting that Sin1 is important for mTORC2 assembly. Knockdown of Sin1 by RNAi in *Drosophila and* mammals crucially diminishes the Akt phosphorylation on S473 *in vitro*. The same effect was observed in Sin1−/− cells [58].

Protor-1 and Protor-2 (also known as Proline rich protein 5 (PRR5) [54, 55] and PRR5-like (PRR5L) [63] are two newly identified mTORC2 interactors which have been identified as Rictor-binding or SIN1 binding proteins [54]. Up to date their functions remain unclear. It is currently accepted that they are dispensable for mTORC2 assembly as well as for its catalytic activity [54], although Protor stability is dependent on the production of other TORC2 components. It is possible that Rictor and Sin1 act as scaffold proteins for various complexes involving different kinases.

mLST8 and Deptor, as was mentioned above, are the components of both mTORC1 and mTORC2 complexes.

3. Upstream regulation of mTOR signaling

3.1. PI3K-AKT-TSC1/2 -"Classical" pathway of mTOR regulation

Although this pathway is still considered to be the main way exerting multi-faceted control over mTORC1 activity which sense insulin and growth factors signals to regulate cell growth, at present it becomes clear that at least some of its components also function to mediate responses on other stimulus, such as energy, stress or nutrients which are provided by discrepant signaling pathways, described below.

3.1.1. TSC1/TSC2 complex and Rheb protein

The TSC1/TSC2 complex (tuberous sclerosis complex 1/2, TSC1/2) has been established as the major upstream inhibitory regulator of mTORC1 [64, 65]. This complex mediates signals from a large number of distinct signaling pathways to modulate mTORC1 activity predominantly via different phosphorylations of TSC2. Functioning as a molecular switch, TSC1/2 suppresses mTOR's activity to restrict cell growth during the stress, and releases its inhibition under the favorable conditions. The *TSC1* and *TSC2* genes were identified in 1997 and 1993 respectively as the tumor-suppressor genes mutated in the tumor syndrome TSC 1(tuberous sclerosis complex) [66-68]. TSC is a multisystem disorder characterized by the development of numerous benign tumors (e.g. hamartomas) most commonly detected at the brain, kidneys, skin, heart and lungs. Genetic studies of *TSC1* and *TSC2* in humans, mice, *Drosophila* and yeast strongly suggest that these proteins act mainly as a complex. The 140 kDa TSC1 (also known as hamartin) and 200 kDa TSC2 (also known as tuberin) proteins share no homology with each other and very little with other proteins **(Figure 1C)** TSC1 and TSC2 associate through certain regions [69] giving a heterodimeric complex. The only known functional domain throughout these two proteins is a region of homology at the C-terminus of TSC2 to the GAP domain of small G-protein Rap1. Searches for a GTPase target regulated by the TSC2 GAP (GTPase-activating protein) domain revealed the small G-protein Rheb. Mammalian TSC2 was shown to accelerate the rate of GTP hydrolysis of Rheb, converting Rheb from the active GTP-bound to the inactive GDP-bound state [69, 70]. This evidences that Rheb is a direct target of TSC2 GAP activity, and TSC2 suppress Rheb function. While the GAP activity of TSC2 is necessary for the complex functionality, TSC1 is required to stabilize TSC2 and prevent its ubiquitin-mediated degradation [71, 72]. Under growth conditions, the TSC1/2 complex is inactive, thereby allowing Rheb-GTP to activate TORC1.

Rheb is a member of the Ras superfamily that appears to be conserved in all eukaryotes and, despite the term 'brain' in its name, is in fact ubiquitously expressed in mammals. Whether a GEF protein (guanine-nucleotide exchange factor responsible for reverse process, i.e. change GDP-bound to GTP-bound state) for Rheb exists remains unknown. Several evidences demonstrate that Rheb positively regulates mTORC1. In particular, Rheb

overexpression stimulates S6K1 and 4EBP1 phosphorylation, which are indicators of mTORC1 activity. This effect can be reversed by mTOR inactivation or by rapamycin treatment, suggesting that Rheb primarily functions through TORC1 [59]. Although genetic and biochemical studies strongly suggest that GTP-bound Rheb potently activates mTORC1, the molecular mechanism is still unclear. Overexpressed Rheb was shown to bind to mTOR [73, 74]. Associations between endogenous Rheb and mTORC1 components have not been reported. In general, Ras-related small G-proteins bind to their downstream effectors mostly in the GTP-bound state. Surprisingly, Rheb has been found to bind stronger to mTOR in its GDP-bound or nucleotide-free states [74]. At the same time it has been shown that GTP-bound Rheb rather than the GDP-bound stimulates mTOR kinase activity *in vitro* [74]. Although the mechanism by which Rheb-GTP activates mTORC1 has not been fully understood, it needs Rheb farnesylation and can be blocked by farnesyl transferase (FT) inhibitors. Recently, it was found that Rheb can directly interact with the FKBP12 homologue FKBP38 (named also FKBP8), and this binding seems to be tighter with Rheb-GTP [75]. That study suggests that Rheb-GTP binds to FKBP38 and triggers its release from mTORC1, stimulating mTORC1 activity **(Figure 2).** In support of this model, an independent study carried out that decreasing FKBP38 expression with antisense oligonucleotides blocked the growth inhibitory effects of TSC1–TSC2 overexpression [76]. Although more studies are needed, these findings suggest that FKBP38 might be a Rheb effector that regulates mTORC1 and, perhaps, unknown targets downstream of the TSC1/TSC2 complex and Rheb.

3.1.2. The PI3K-AKT pathway joins TSC-mTORC1 regulation

The responsiveness of mTORC1 signaling to growth factors and insulin is provided through activation of PI3K (phosphatidylinositol-3-kinase) and Akt kinase, but the precise mechanism is still not clear. Through PI3K signaling, Akt also termed PKB (serine/threonine protein kinase B) is activated by most growth factors to phosphorylate several downstream substrates [77].

PI3K is a heterodimeric protein containing an 85-kDa regulatory and a 110-kDa catalytic subunits (*PIK3CA*) [78, 79]. PI3K acts to phosphorylate a number of membrane phospholipids to form the lipid second messengers phosphatidylinositol 3,4-bisphosphate (PtdIns(3,4)P2 or PIP2) and phosphatidylinositol 3,4,5-trisphosphate (PtdIns(3,4,5)P3 or PIP3). In response to the upstream inputs, PI3K at the cell membrane is activated through the association of a ligand with its receptor, stimulating p85 to bind phosphorylated tyrosine residues of Src-homology 2 (SH2) domain on the receptor. This association promotes the p110 catalytic subunit to transfer phosphate groups to the membrane phospholipids [78, 80]. Consequently these lipids, particularly PtdIns(3,4,5)P3, attract several kinases to the plasmalemma initiating the signaling cascade [78, 80]. PIP3 accumulation is antagonized by the well-known tumor suppressor, lipid phosphatase PTEN (**p**hosphatase and **t**ensin homolog deleted on chromosome 10), which converts PIP3 to PIP2. One important function of PIP3 is to recruit Akt as well as PDK1 (or PDPK1, 3-phosphoinositide-dependent protein kinase-1) [81] via their PH (**p**leckstrin **h**omology) domains to the plasma membrane (**Figure 2).**

Akt, known as one of the major survival kinases, belongs to the AGC (PKA/PKG/PKC) protein kinase family and is involved in regulating a vast number of cellular processes, including transcription, proliferation, migration, growth, apoptosis and various metabolic processes [3, 82]. Being translocated to the plasma membrane, Akt undergoes partial activation through the phosphorylation of T308 residue within the activation loop by PDK1 and following full activation through the additional phosphorylation at the hydrophobic motif site S473 by PDK2 [83]. After activation Akt quits the cell membrane to phosphorylate intracellular substrates. Particularly, Akt can translocate to the nucleus [80] where it influences the activity of transcriptional factors, including CREB *(cAMP response element-binding)*, E2F (eukaryotic transcription factor 2), NF-κB (nuclear factor kappa from B cells) through Iκ-K (inhibitor kappa B protein kinase), the forkhead transcription factors, in particular, FOXO1 and FOXO3 and murine double minute 2 (MDM2) which regulates p53 activity [84, 85]. In addition, Akt is able to target some other molecules to influence cell survival including GSK-3β (glycogen-synthase kinase-3β), which regulates β-catenin protein stability, and BAD (the pro-apoptotic molecule Bcl-2-associated death promoter).

Akt was the first kinase demonstrated to phosphorylate directly the TSC1/TSC2 complex in response to growth factors. Human TSC2 contains five predicted Akt sites (S939, S981, S1130, S1132 and T1462 on full-length human TSC2), all of which have been suggested to be subjects of phosphorylation by Akt (**Figure 2**). Importantly, the two sites were shown definitively to be targeted by Akt in mammalian cells, S939 and T1462 [86]. There is also evidence that either S1130 or S1132 is phosphorylated by Akt *in vivo* [87]. Finally, Akt can phosphorylate a peptide corresponding to the sequence surrounding S981 *in vitro* [88]. This residue has been identified as an *in vivo* phosphorylation site on TSC2 by tandem-MS analyses [89]. However, whether Akt phosphorylates S981 on full-length TSC2 within cells has not been conclusively demonstrated.

The majority of studies postulated that activated AKT promotes TORC1 signaling by phosphorylating multiple sites on TSC2, thereby relieving inhibition of Rheb and activating TORC1 [86, 87, 90, 91]. The data obtained using phosphorylation-site mutants of TSC2 demonstrate that Akt mediated phosphorylation of these sites inhibits the function of the TSC1–TSC2 complex in cells, however the molecular mechanism of this inhibition has been the subject of much debate (reviewed in [92])**.** One proposed mechanism involves disruption of the TSC1–TSC2 complex. However, this does not occur rapidly and, although it might contribute to the long-term effects of Akt on mTORC1 signaling, it cannot explain the immediate effects of Akt activation on mTORC1, which are blocked by Akt phosphorylation-site mutants of TSC2. Another proposed mechanism is based on the possibility that phosphorylation of TSC2 alters its subcellular localization, such that it can no longer act as a GAP for Rheb. One study supporting this mechanism found that growth factor stimulation led to increase of the TSC2 levels within the cytosolic fraction [93]. This effect was PI3K-dependent, stimulated by activated Akt and required both S939 and S981 on TSC2. In that study, both TSC1 and Rheb were found exclusively in the membrane fraction, and unlike TSC2, did not show an increase in the cytosolic fraction following growth-factor stimulation. From these findings it was concluded that Akt-mediated phosphorylation of

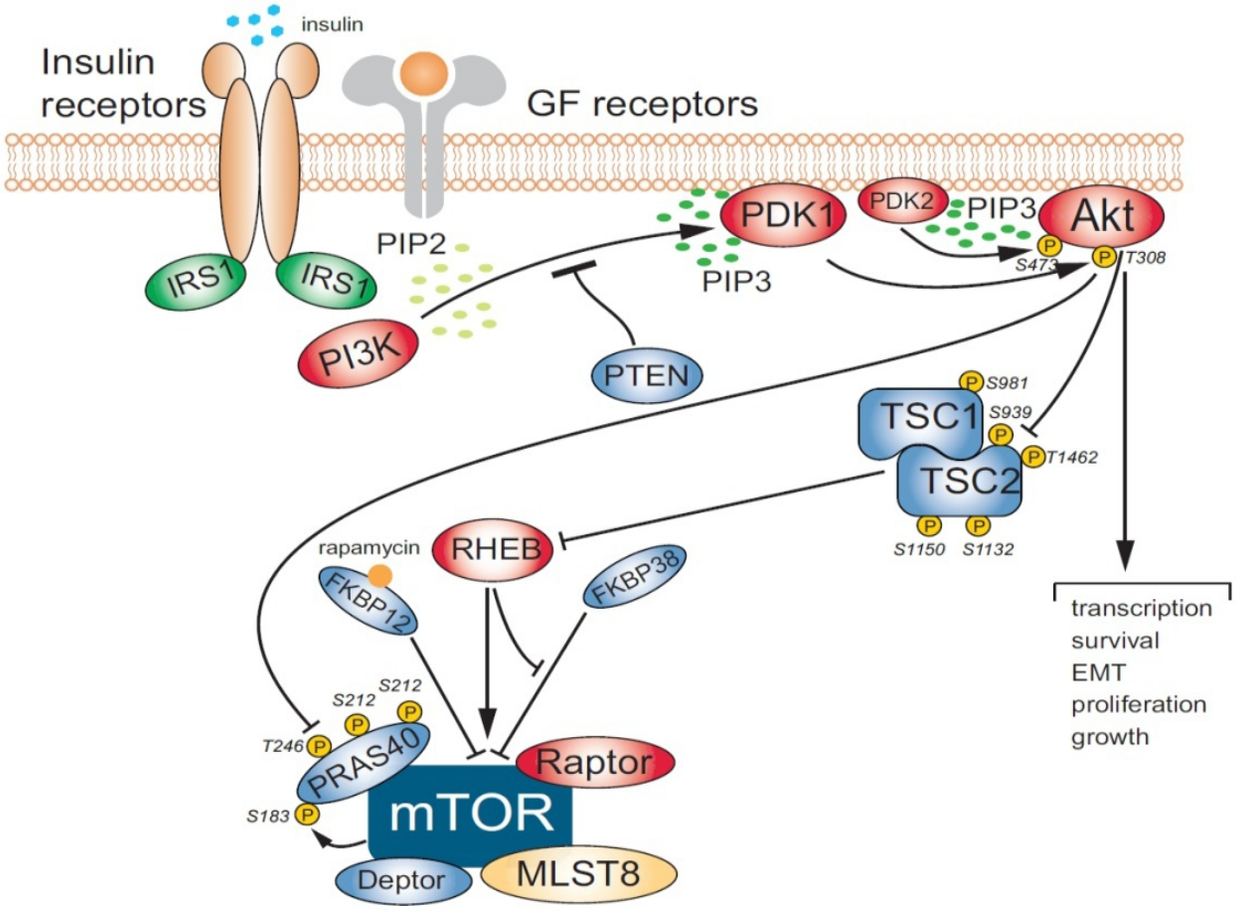

Figure 2. Growth factors and insulin regulation of mTORC signaling. mTORC1 activity is modulated by a number of positive (shown in red) and negative (shown in blue) regulators. Growth factors activate mTORC1 indirectly by suppressing the function of its negative regulator TSC1/TSC2 complex. TSC2 contains a GAP domain that converts Rheb to its inactive, GDP-bound form. PI3K-AKT dependent phosphorylation inhibits the TSC1/2 complex, thereby relieving the TSC1/2-mediated repression of Rheb and allowing activation of TORC1. AKT also activates mTORC1 through negative phosphorylation of mTORC1 suppressor, PRAS40. FKBP38 appears to associate through the FRB domain of mTOR and trigger its release from mTORC1, thereby stimulating mTORC1 activation.

TSC2 on S939 or S981 inhibits the TSC1/TSC2 complex by triggering release of TSC2 from TSC1 at an intracellular membrane also occupied by Rheb. This model points on the significant and rapid dissociation of TSC2 from TSC1 upon phosphorylation – something that has not been detected in the majority of studies to date. Recent studies have suggested that AKT mediated phosphorylation of TSC2 at S939 and S981 creates a binding site for a cytosolic anchor protein, 14-3-3 (tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, theta polypeptide also known as YWHAQ, 1C5; HS1), a mechanism of regulation shared by several other Akt substrates [77]. Examining interactions between endogenous 14-3-3 proteins and TSC2, another study found that S939 and T1462 were both required for 14-3-3 binding to TSC2 downstream of PI3K signaling. It seems likely that 14-3-3 binding to TSC2 (provided by some combination of phosphorylated S939, S981 and T1462) contributes to Akt-mediated inhibition of TSC2. Binding of 14-3-3 to TSC2 can disrupt binding TSC2 to TSC1

and RHEB, which are associated with endomembranes [93]. However, in 14-3-3 pull-down experiments, both TSC1 and TSC2 were found to bind, and 14-3-3 did not affect the association between TSC1 and TSC2 [94, 95]. It also remains unclear whether TSC2 binding to14-3-3 hindered its GAP activity towards Rheb. Importantly, TSC2 is not an essential target of AKT during normal *D. melanogaster* development [96], suggesting the presence of possible additional targets for the AKT mediated regulation of mTORC1.

Growth factors control mTORC1 independently of the TSC complex

As was mentioned above, the PRAS40 binds Raptor and thereby inactivates mTORC1 [48, 50, 57, 63]. In response to growth factors, Akt phosphorylates PRAS40 at T246. This phosphorylation leads to the dissociation of PRAS40 from mTORC1 resulting in a reduced ability of PRAS40 to inhibit TORC1 [48, 49, 57]. This was proposed to be mediated through 14-3-3 binding of the phosphorylated PRAS40 [57]. Thus, bypassing TSC2, AKT phosphorylates PRAS40 and prevents its ability to suppress mTORC1 downstream effectors. The inhibition of PRAS40 by AKT is conserved; in *Drosophila*, the PRAS40 ortholog Lobe regulates TORC1 signaling [97]. PRAS40 is in turn a substrate of mTORC1, and mTORC1 mediated phosphorylation of PRAS40 S183, [50, 63] has been proposed to negatively regulate mTORC1 signaling by competing with 4EBP1 and S6K for interaction with Raptor. PRAS40 is a direct inhibitor of mTORC1 and antagonizes the activation of the mTORC1 by Rheb•GTP. However, constitutive mTORC1 signaling in TSC2 null mouse embryonic fibroblasts, in which AKT signaling is largely inhibited owing to a negative feedback mechanism (see below), indicates that hyperactive Rheb can overcome PRAS40 mediated inhibition of mTORC1 [48]. Thus, the AKT pathway might stimulate mTORC1 through two interconnected mechanisms: by activating Rheb and/or by inhibiting PRAS40.

3.2. mTORC1 activation by nutrients

3.2.1. hVps34 PI-3-P kinase and Rag GTPases

It has long been known that mTORC1 signaling is strongly inhibited in cells under the conditions of nutrient deficiency and that the re-addition of amino acids to starved cells can strongly stimulate mTORC1 activity [22, 98]. However, the mechanisms by which amino acids convey signals to mTORC1 remain largely unknown. Earlier studies demonstrated that silencing expression of TSC1/2 confers resistance to amino acid deprivation, indicating that TSC1/2 is involved in the regulation of mTOR function by amino acids [90]. It has been suggested that branched-chain amino acids, (such as leucine), activate mTORC1 by inhibiting TSC1/TSC2 or stimulating Rheb [62]. Consequently, inhibition of Rheb binding to mTOR is critical for the inhibitory effect of amino acid withdrawal on mTOR signaling [99]. However, other studies do not support this idea. Thus, in TSC-null cells (that lack either TSC1 or TSC2), the mTORC1 activity remains sensitive to amino acid deprivation, suggesting that other than TSC2, additional mechanisms may also be involved in the regulation of mTOR by amino acids [100]. Although Rheb is required for the amino acid stimulation of mTORC1, starving of amino acids has no effect on GTP loading [99-102].

Therefore, while there is a requirement for GTP-bound Rheb to induct of mTORC1 by amino acids, amino acids probably do not affect Rheb activity – indicating that regulation of Rheb does not stimulate mTORC1 in response to amino acids.

Recently, Ste20-related kinase MAP4K3 (mitogen activated protein kinase kinase kinase kinase 3) and the class III PI3K hVps34 (human vacuolar protein sorting 34) were proposed to be activated by amino acid and be involved in the transduction of signals from amino acids to mTORC1 [103-107]. While the mechanism by which MAP4K3 regulates mTORC1 remains unknown, a mechanism for hVPS34 was recently proposed **(Figure 3**). According to this proposed mechanism, amino acids induce an extracellular calcium influx that activates calmodulin, which in turn binds and activates hVps34 [108]. hVps34 then generates PI-3-phosphate (PI-3-P) instead of the PI-3,4,5-tris-phosphate generated by type I PI3Ks [109], that somehow activates mTORC1. The mechanism also involves the formation of a calmodulin-hVps34-mTORC1 supercomplex. However, the regulation of mTORC1 by hVps34 is thought to be specific to mammalian cells because in flies Vps34 does not regulate TORC1 [106]. This is unexpected because regulation of TORC1 by amino acids is known as very conserved. Furthermore, in certain mammalian cells, amino acids appear to inhibit rather than activate mVps34 [110]. However, additional studies are needed to clarify the roles of these proteins in TORC1 activation**.**

Most recent studies identified Rag GTPases as activators of mTORC1 by sensing amino acid signals [111, 112]. Rag-mediated activation of TORC1 still requires Rheb, indicating that, during amino acid signaling, Rag complexes act upstream of Rheb. Rag family members (Rag A-D) belong to the Ras superfamily of GTPases. They are unique in their ability to dimerize through long C-terminal extensions. In the presence of amino acids, the dimeric Rag complex, which consists of a Rag A/B monomer and a Rag C/D monomer, binds Raptor and transport mTORC1 to lysosomes, the same intracellular compartment that contains Rheb [36, 111, 112]. Rag complexes are recruited to the lysosomal membrane by the trimeric Ragulator complex [36], which contains the proteins MP1 (MEK partner 1), p14 and p18. The GTP-loading of Rag A/B appears to be regulated by amino acids, and binding to TORC1 is observed most robustly under nutrient-rich conditions – when Rag A/B is in the GTP-bound state and Rag C/D is in the GDP-bound state [111, 112]. This model answers the question why mTORC1 activity cannot be stimulated by growth factors in the absence of amino acids. It also explains why Rag GTPases are not able to activate mTORC1 activity *in vitro* [111]. mTORC1 can be fully activated only under the conditions of amino acids availability, Rab-dependent mTORC1 translocation to a Rheb-containing compartment, and Rheb activated by growth factors. However, there are many key aspects that remain to be discovered, such as how branched amino acids are detected by Rag GTPases and the identification of the Rag guanine exchange factor (GEF).

3.2.2. PLD joins to amino acids dependent mTORC1 regulation

Several data evidence that phosphatidic acid (PA) is essential for mTORC1 activation. The main mechanism for generating PA is the hydrolysis of phosphatidylcholine (PC) by

phospholipase D (PLD). In mammals PLD exists as two isoforms (PLD1 and PLD2) possessing different mechanisms of regulation and subcellular distribution [113]. PLD1 is predominantly localized under steady-state conditions at the Golgi complex, endosomes, lysosomes and secretory granules, and is regulated by two major signaling categories: growth factors/mitogens like EGF, PDGF, insulin and serum that implicate tyrosine kinases, and the small GTPase proteins from Arf, Ral and Rho families. PLD2 is largely associated with lipid rafts on the membrane surface. [113]. Both PLD1 and PLD2 have a strong requirement for PIP2 as a co-factor [113]. It has been shown that PLD1 activation stimulates PLD2 by increasing levels of PIP2 (product of PA metabolic modifications) [114]. This makes more clear the involvement of both PLD1 and PLD2 in the mTORC1 activation. The generation of PA by PLD can be suppressed by primary alcohols (such as 1-butanol) through the transphosphatidylation reaction whereby inert phosphatidyl-alcohol is generated instead of PA. This reaction has been widely used to examine PLD significance, and several studies have demonstrated that the activation of mTOR was sensitive to primary alcohols. Thus, 1-butanol was able to block almost completely the serum-stimulated phosphorylation of mTOR downstream targets, S6K1 and 4E-BP1 [115]. From these findings, it can be asserted that PLD production of PA plays an essential role in the mTOR signaling pathway). In skeletal muscle, PA stimulated S6 kinase phosphorylation, and 1-butanol suppressed S6 kinase phosphorylation [116]. Nutrient-dependent multimerization of mTOR was also suppressed by 1-butanol [117]. Therefore, primary alcohols-dependent suppression of PLD activity has been shown to suppress mTORC1 signaling in several cell models [114].

Several laboratories have shown that mTORC1 is activated in response to exogenously supplied PA. For example, exogenously provided PA stimulated the activation of S6 kinase and phosphorylation of 4E-BP1 in cancer HEK293 cells. The effect of PA was sensitive to rapamycin [115, 118] and was dependent on the presence of amino acids [115]. Coexpression of TSC1/2 was shown to inhibit PA-dependent stimulation of S6K. This indicates that PA-induced S6K activity is mediated through TSC1/2-mTOR signaling. PA was also shown to activate mTOR in macrophages in an Akt-dependent manner [119].

In addition, several studies have explored the influence of PLD1 and PLD2 expression on mTORC1 activation. Particularly it was reported that PLD2 overexpression increases S6K phosphorylation in MCF7 cells [120]. Overexpression of PLD1 also stimulated S6K phosphorylation in rat fibroblasts [121]. siRNA-mediated knockdown of PLD1 blocked S6K phosphorylation in B16 melanoma cells, and suppression of either PLD1 or mTOR led to melanoma cells differentiation [122].

At the same time, up to date the precise mechanism of PA-dependent stimulation of mTOR signaling remains unclear. One possibility is that PA binds to mTOR at the FRB domain, the region where the rapamycin-FKBP12 molecule binds mTOR as well. This binding was specific for PA as other phospholipids were unable to bind the FRB with such specificity. It was hypothesized that the competition between the rapamycin-FKBP12 complex and PA for the FRB site may be one of the regulating factors in mTOR activation [115]. According to the

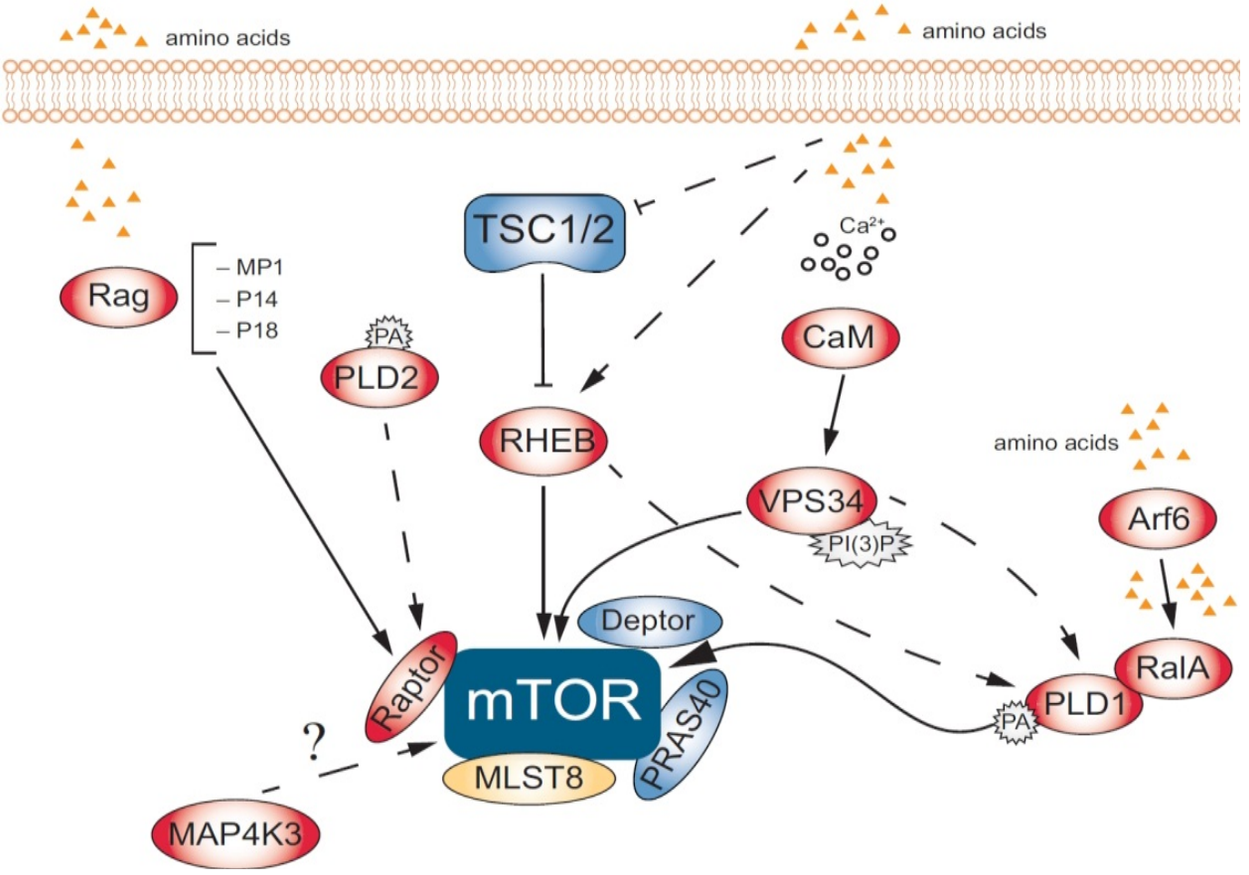

Figure 3. Nutrients regulation of mTORC signaling. mTORC1 could be activated by amino acids through few proposed molecular mechanisms. In the response to amino acid sufficiency Rag complex is recruited to the lysosomal membrane by the trimeric Ragulator complex which consists of MP1, p14 and p18 thereby allowing Rheb to activate mTORC1. Amino acids also induce an extracellular calcium influx that activates calmodulin, which in turn binds and activates hVps34 that generates PI-3-P, what leads somehow to the mTORC1 activation. One model puts PLD downstream of hVps34 suggesting hVps34(PI-3-P)-PLD-mTORC1 pathway mediating response to amino acids. According to this model nutrient activation of PLD requires interaction with small G proteins RalA and Arf6. In addition, several studies evidence that PLD probably via generation of PA contributes to the mTORC1 activation in response to the nutrient stimulation. Particularly, PA could compete with rapamycin-FKBP12 complex for the mTOR FRB domain binding or reduce the pH around mTOR. PLD2 has also been reported to form a functional complex with mTOR and Raptor through a TOS (TOR signaling) motif. It has also been proposed that branched-chain amino acids could activate mTORC1 by inhibiting TSC1/TSC2 or stimulating Rheb.

other hypothesis the pH locally around mTOR is reduced by PA-generated PLD, which eventually promotes its kinase activity, or allows for interaction with yet unknown promoter substrates [114]. It was shown that PLD1 is an effector of the small GTPase Rheb (see above) within the mTORC1 signaling pathway [123, 124] **(Figure 3)**. It was also reported that PLD2 forms a functional complex with Raptor and mTOR via a TOS (TOR signaling) motif in PLD2, and this interaction was essential for mitogen stimulation of mTORC1 [125]. More recently, dominant negative mutants of both PLD1 and PLD2 were able to suppress

the activation of mTORC1 [126]. Therefore, besides PA ability to activate mTORC1, there are several data indicating requirement of PLD itself for the activation of mTORC1. Very recent study provided additional evidence that nutrient stimulation of mTORC1 is dependent on PLD activity which in turn is activated by small GTPases RalA and Arf6 [127]. According to this study, amino acids dependent activation of PLD is mediated trough generated by Vps34 PI-3-phosphate [127], that could interact with PX domains of PLD1 and PLD2 which are known to be critical for PLD activity [128]. This activation also requires PLD interaction with both RalA and Arf6. Interestingly, these small GTPases have been earlier shown to be implicated in both responding to nutrients and the stimulation of PLD activity. RalA is constitutively associated with PLD1, but does not activate PLD1 by itself. RalA contributes to the activation of PLD1 by recruiting ARF6, which does activate PLD1 activity, into RalA/ARF6/PLD1 complex. While it is still not clear how the presence of nutrients activates RalA and ARF6, the data provided in this study indicate that PLD is a key target of RalA and ARF6 for the stimulation of mTORC1. In concordance with these findings data from our lab evidence that expression of constitutively active Arf6 stimulates PLD activity which leads to the mTORC1 dependent phosphorylation of downstream targets 4E-BP1, S6K1 kinase and its effector **r**ibosomal **p**rotein **S6** (rpS6). We also show that mTORC1 signaling stimulation contributes to the Arf6 promitogenic activity [129].

3.3. Control of mTOR signaling in response to energy stress

AMPK (the AMP-activated protein kinase, also known as PRKAB1) is activated under the low level of intracellular ATP and found in all eukaryotes. It was initially identified as a serine/threonine kinase that negatively regulates several key enzymes of the lipid anabolism [130]. At present, AMPK is considered to be the major energy-sensing kinase that activates a whole variety of catabolic processes in multicellular organisms such as glucose uptake and metabolism, while simultaneously inhibiting several anabolic pathways, such as lipid, protein, and carbohydrate biosynthesis (reviewed in [130]). AMPK is upregulated under energy stress conditions in response to nutrient deprivation or hypoxia when intracellular ATP level decreases and AMP increases [131]. In response, AMPK turns on ATP generating pathways while inhibiting ATP consuming functions of the cell [131]. AMPK functions as heterotrimeric kinase complex, which consists of a catalytic (α) subunit and two regulatory (β and γ) subunits. Upon energy stress, AMP directly binds to tandem repeats of crystathionine-β-synthase (CBS) domains in the AMPK γ subunit [132]. Since the ratio of AMP to ATP represents the most accurate way to precisely measure the intracellular energy level, both AMP and ATP are able to oppositely regulate the activity of AMPK. While AMP binding to the γ–subunit allosterically enhances AMPK kinase activity and prevents the dephosphorylation of T172 [133], ATP is known to counteract the activating properties of AMP [130]. Although ADP does not allosterically activate AMPK, it also binds to AMPK and enhances phosphorylation at T172 [134, 135]. The phosphorylation of the activation loop T172 is absolutely necessary for AMPK activation. At present, several AMPK-phosphorylating kinases have been identified. In addition to the ubiquitously expressed and constitutively active kinase LKB1, Ca^{2+}-activated Ca^{2+}/calmodulin-dependent kinase kinase β

(CaMKKβ) [136] and transforming growth factor β-activated kinase-1 (TAK1) are both known as AMPK activators. Genetic and biochemical studies in worms, flies, and mice have identified the serine/threonine liver kinase B1 (LKB1) as major kinase phosphorylating the AMPK activation loop at T172 residue, under conditions of energy stress [130]. Within the TOR signaling pathway, LKB1 dependent activation of AMPK inhibit mTORC1 activity by two ways **(Figure 4).** Firstly, AMPK directly phosphorylates the TSC2 on S1387 and T1227 [2, 64, 70, 87, 137, 138]. AMPK phosphorylation of TSC2 has also been reported to act as a primer for the phosphorylation and enhancement of TSC2 function by glycogen synthase 3β (GSK3β). GSK3β dependent phosphorylation of TSC2 on S1341 and S1337 stimulates its GAP activity towards Rheb, leading to the inhibition of mTORC1 [138]. It is possible that GSK3β cooperates with AMPK to fully activate the GAP activity of TSC2. The second, TSC2 independent mechanism by which AMPK can signal to mTORC1, [45] is a direct phosphorylation of Raptor at two highly conserved residues — S722 and S792. These phosphorylation events induce Raptor direct binding to 14-3-3 protein, which leads to a suppression of mTORC1 kinase downstream activity [45]. Therefore, mTORC1 itself serves as an AMPK substrate for inhibiting phosphorylation.

3.4. mTOR signaling regulating by hypoxia

mTOR signaling pathway is strictly regulated by hypoxia [139, 140], since the sufficiency of oxygen is also essential for cellular metabolism. Hypoxia inhibits mTORC1 signaling via multiple signal pathways, two of them being mediated through activation of the TSC1/TSC2 complex **(Figure 4**). First, activation of AMPK by hypoxia can enhance TSC complex function. Particularly, it was shown, that brief hypoxia exposure prevents insulin-mediated stimulation of mTORC1 and phosphorylation of its targets p70S6K and 4E-BP1 [139]. Under these conditions mTOR suppression is mediated through a HIF1α (hypoxia-inducible factor 1α)-independent pathway involving AMPK-dependent activation of TSC1/TSC2 [2, 87, 141]. Second way includes the upregulation of TSC1/TSC2 through transcriptional regulation of stress-induced protein REDD1 (Regulated in Development and DNA damage responses, also known as DDIT4 or RTP801) [142, 143]. This response is mediated in part through a mechanism that involves HIF1α, a transcription factor that is stabilized under hypoxic conditions and drives the expression of several genes, including *REDD1.* Induction of REDD1 can activate the TSC1/2 complex by competing with TSC2 for 14-3-3 proteins binding [142, 144]. Thus, increased REDD1 levels that occur following exposure to hypoxia prevent the inhibitory binding of 14-3-3 to TSC2 [144], which eventually leads to the inhibition of mTORC1 signaling**.** Therefore inhibitory effect of REDD1 on mTOR signaling seemed to be dependent on the presence of the TSC1/2 complex, but independent on the LKB1-AMPK signaling [142, 145, 146]. However, most recent studies proposed that hypoxia and the LKB1-AMPK signaling are highly interrelated at least in some type of cells [140]. In response to prolonged hypoxia, REDD1 expression was enhanced by AMPK activation, leading to the inhibition of mTOR pathway. Indeed, it was demonstrated that prolonged hypoxia induced ATP depletion and eventually activate AMPK [140]. Taken together, under

hypoxic stress, the inhibition of mTOR activity by REDD1 activation may be mediated either through AMPK-independent or -dependent mechanisms.

Hypoxia may also downregulate mTORC1 through proteins that hinder the the Rheb–mTOR interaction. The PMl (promyelocytic leukaemia tumour suppressor) has been found to bind mTOR during hypoxia and inactivate it via sequestration in nuclear bodies [147]. Likewise, the hypoxia-inducible proapoptotic protein BNIP3 (BCl2/adenovirus E1B 19 kDa protein-interacting protein 3) was found to regulate mTOR by direct association with Rheb [148]. (Reviewed in [149]).

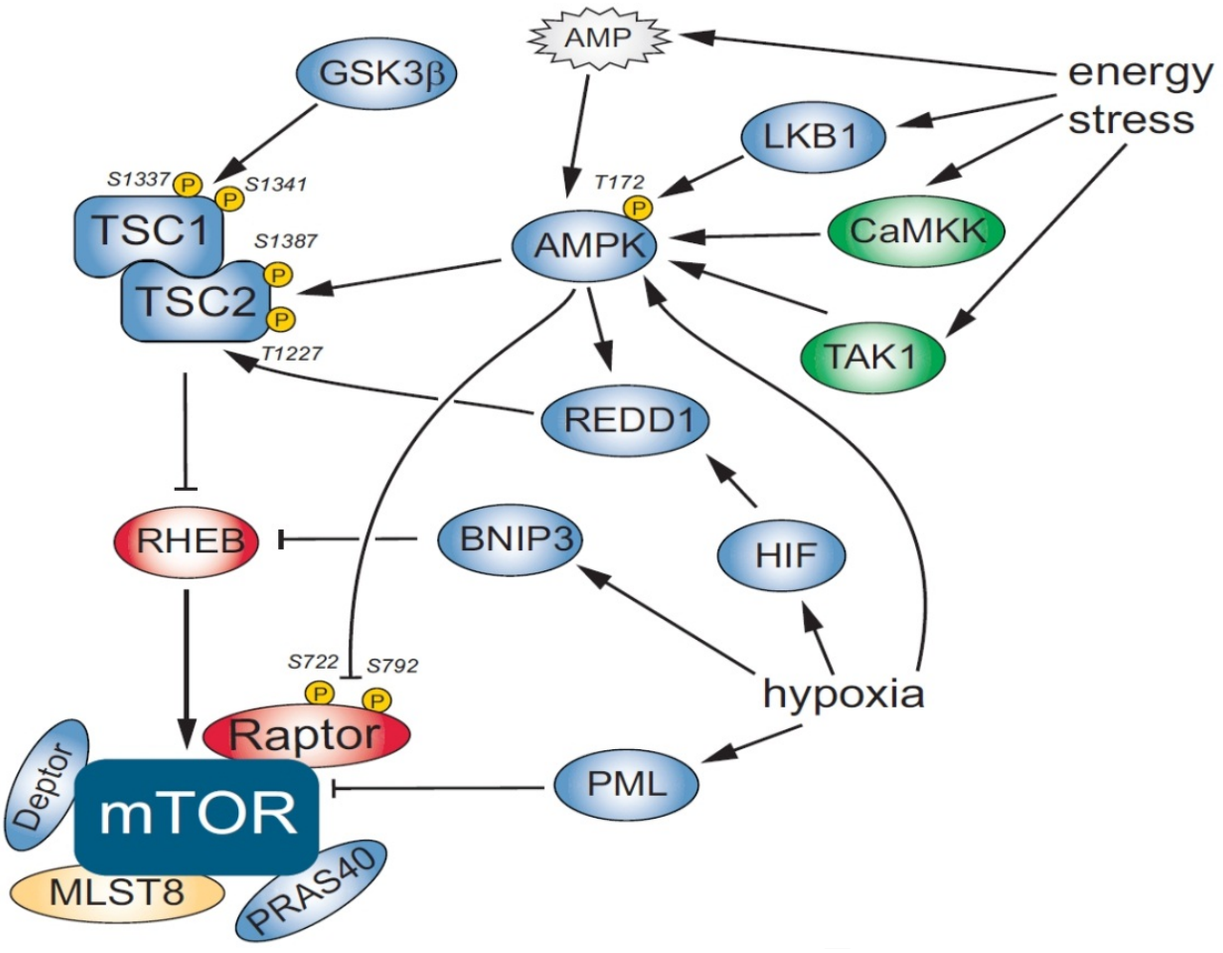

Figure 4. mTORC1 regulation in response to energy deprivation and hypoxia. Low cellular energy levels (conveyed by AMP) and hypoxia activate AMPK, which represses mTORC1 both through direct negative phosphorylation of TSC2 and through Raptor inhibition. LKB1, CaMKK and TAK1 are known as AMPK activators. AMPK- and GSK3β-mediated phosphorylation of the TSC1/2 complex positively regulates the GAP activity of TSC2 towards Rheb, abrogating its stimulative activity towards mTORC1. Under hypoxic stress, the inhibition of mTOR activity could be mediated by REDD1 either through AMPK-independent or -dependent mechanisms. Hypoxia-inducible proapoptotic protein BNIP3 is reported to regulate mTOR by direct binding to Rheb, while PML can binds mTOR and inactivate it through sequestration in nuclear bodies.

4. Signaling downstream of mTOR

4.1. TORC1 regulates translation machinery

The protein synthesis stimulation and the inhibition of autophagy are two mostly known biological outputs controlled by this pathway under the favorable conditions, such as nutrient and oxygen availability. By sensing the presence of growth factors and the sufficiency of nutrients, activated mTORC1 mediates the signals to various components of the translation initiation machinery through direct or indirect phosphorylation events [22]. Several data also evidence that mTOR regulates the synthesis of many classes of lipids (such as phosphatidylcholine, phosphatidylglycerol, and sphingolipids, unsaturated and saturated fatty acids) that are required for membrane biosynthesis and energy storage (For the detailed review see [150]. Since the best characterized effectors of mTOR signaling are proteins controlling the translational initiation machinery it is important to understand how mTORC1 signal transduction pathways contribute to protein synthesis regulation (reviewed in [151]).

The earliest identified and best-studied mTORC1 targets are S6K kinases (p70 ribosomal protein S6 kinase 1 and 2) and 4EBP1 (eIF4E binding protein 1); both proteins involved in the translation initiation process [152] **(Figure 5A**). Protein synthesis is one of the most energy consuming processes within the cell and translation rates are strictly regulated mostly through modification of the eukaryotic initiation factors (eIFs). In eukaryotes, several mRNAs are translated in a cap-dependent manner. The cap structure, m7GpppN (where N is any nucleotide), is present at the 5′ terminus of the majority cellular eukaryotic mRNAs (except those in organelles) [153]. The cap structure is bound by the eIF4F (eukaryotic initiation factor 4F) complex, which contains three initiation factors — the mRNA 5′ cap-binding protein eIF4E, an ATP-dependent RNA helicase eIF4A and a large scaffolding protein eIF4G, which provides docking sites for the other proteins. Briefly, to assemble the eIF4F complex, eIF4E binds the 5′ cap and recruits eIF4G and eIF4A. eIF4A along with eIF4B acts to unwind the mRNA 5′ secondary structure to facilitate ribosome binding [153]. It is especially essential, since stable secondary structures are often found in the 5′ UTR of specific mRNA species, many of which encode proteins that are involved in promoting cell growth and proliferation, and significantly suppress their translation efficiency [154]. As the translation preinitiation complex is recruited near the 5′ end of mRNA, this requires the structured UTR to be 'linearized' — not only for the initial binding of the 40S ribosome but also for subsequent searching for the downstream initiation codon. Although eIF4A alone exhibits low levels of RNA helicase activity the last one is substantially stimulated by its regulatory cofactor, eIF4B. Thus, eIF4B enhances the affinity of eIF4A binding to ATP, which, in turn, increases the processivity of the eIF4A helicase function [155]. eIF4G recruits the small ribosomal subunit to the mRNA (and the poly(A)-binding protein, PABP) through the ribosome associated large multisubunit factor eIF3. As a result the assembly of the 48S translation preinitiation complex takes place, allowing for the ribosome scanning and translation initiation [22, 26]. The translation initiation factors and cofactors that are regulated by mTORC1 signaling include eIF4G, eIF3, eIF4B, eIF4E and 4EBP1, of which 4EBP1 is considered to be the most well-known mTORC1 direct effector protein.

eIF4G serving as a modular scaffold for the translation preinitiation complex formation, is phosphorylated in response to growth factor stimuli at multiple sites, some of which are dependent on mTORC1. These sites are clustered in a hinge region of eIF4G that joins two structural domains, and it has thus been predicted that the modification might induce conformational changes in the protein that affect its activity [22]. Nevertheless, the precise molecular mechanism by which eIF4G phosphorylation regulates its function remains to be determined. Regulation of the mRNA cap binding protein eIF4E is mediated mainly in two ways, firstly, through phosphorylation at S209 in its C-terminus by MAP kinase signaling integration kinases 1 and 2 (Mnk1/2) [156] and, secondly, through the sequestration by small, heat stable phosphoproteins termed 4E-binding proteins, 4E-BPs [153] belonging to the 4E-BPs translation repressors family. One of these proteins, 4E-BP1 is a direct mTORC1 phosphorylation target. In quiescent cells, hypophosphorylated 4EBP1 binds tightly to eIF4E. As 4EBP1 and eIF4G share the same eIF4E-binding motif 4EBP1 competes with eIF4G for an overlapping binding site on eIF4E, and prevents eIF4G from interacting with eIF4E. On mTORC1 activation, hyperphosphorylated 4EBP1 dissociates from eIF4E, allowing for the recruitment of eIF4G and eIF4A to the 5′ end of an mRNA. Thus, the effects of 4E-BP1 on protein translation are not limited to switching 'off ' or 'on' protein synthesis; they can also alter the range of nascent proteins by mediating a switch between cap-dependent and cap-independent translation. Indeed, during specific stress conditions, such as nutrient depletion, hypoxia or metabolic stress, the cell can reduce the activity of mTORC1, resulting in the cessation of cap-dependent translation and the concomitant promotion of cap-independent translation of essential pro-survival factors. Rapamycin inhibits mTORC1-dependent 4E-BP1 phosphorylation, stimulating the interaction between eIF4E and 4E-BP1, what leads to cap-dependent translation inhibition [157].

Control of the 4E-BPs by mTOR

Upon the stimulation (by growth factors, mitogens and hormones), human 4E-BP1 is phosporylated at 7 sites, 4 of which are involved in mTOR signaling [157, 158]. These are T37, T46 and T70, and S65. The 4E-BP1 phosphorylation is proceeded in a hierarchical manner (first T37 and T46, then T70 and last S65) [157]. S65 and T70 are located near the eIF4E-binding site. Often phosphorylation of these residues is stimulated by insulin in a rapamycin-sensitive manner. Some data evidence that phosphorylation of S65 is required for release of eIF4E from 4E-BP1, however the role of phosphorylation of this site is unclear [159]. Molecular dynamics findings [160] and earlier biophysical data suggest that phosphorylation of S65 and T70 is insufficient to bring about release from eIF4E. Phosphorylation of both S65 and T70 depends upon the prior phosphorylation of the N-terminal threonines, and modification of T46 is considered to be essential for phosphorylation of T37 [157, 161]. The phosphorylation of T70 and S65 in human 4E-BP1 depends upon a further site, S101 [162]. The phosphorylation of the N-terminal threonin residues in 4E-BP1 depends upon a certain sequence in the N-terminus, which includes the Arg-Ala-Ile-Pro ('RAIP' motif) [91, 163]. This phosphorylation is not significantly influenced by TOS motif inactivation and according to some data is rather insensitive to rapamycin [158]. This suggests that it could be mediated independently of mTORC1. However, several

data evidence that phosphorylation is mediated by mTORC1: (i) it is inhibited by starvation of cells for amino acids; (ii) it is activated by Rheb; (iii) it is suppressed by TSC1/2; (iv) it is sensitive to inhibitors of the kinase activity of mTOR and (v) it is decreased in cells in which mTOR levels have been knocked down [158]. Therefore, further study of this process needs to clarify the molecular mechanisms of mTOR downstream signaling.

Control of the S6Ks by mTOR

Another important target of mTORC1 is the S6 kinases family, including S6K1 and S6K2. Ribosomal protein S6 (rpS6) is highly phosphorylated protein containing at least five phosporylating sites in its C-terminus. There are two main classes of protein kinases which are responsible for rpS6 phosphorylation *in vitro*, namely the p70 S6 kinases (S6Ks) and the p90 ribosomal protein S6 kinases, also known as RSKs [164, 165], (reviewed in [151]). The observed sensitivity of rpS6 to rapamycin lead to the speculation that S6K are responsible for rpS6 phosphorylation as their activation is mediated by mTOR. Unlike S6Ks, the RSKs are not influenced by rapamycin since they are known to be activated through the classical MAP kinase (ERK) pathway (see below). There are two similar S6 kinase proteins, S6K1 and S6K2, in mammals [166], which show 70% of amino acid homology. Each p70S6K gene encodes two distinct proteins due to alternative splicing of the mRNAs. Several data confirm that activation of both the S6K1 and S6K2 are regulated by mTORC1 [118, 167, 168]. S6K1, which was discovered earlier than S6K2, is ubiquitously expressed and appears to be more critical for the control of cell growth. S6K1 can be activated by a wide variety of extracellular signals and is known as the major rpS6 kinase in mammalian cells and key player in the control of cell growth (cell size) and proliferation [169, 170].

Earlier it was thought that activated S6K1 regulates translation of a class of mRNA transcripts that bear a 5′-terminal oligopolypyrimidine (5′-TOP). Particularly, it was shown that S6K1 phosphorylates eIF4B on S422, which is located in the RNA binding region that is necessary for promoting the helicase activity of eIF4A [171]. Few data indicate that eIF4B phosphorylation by S6K1 is both sufficient and necessary for its recruitment to the translation preinitiation complex [172]. However, there are also some data that disprove this model. In S6K1/2–/– cells, 5′-TOP mRNA translation is intact and still rapamycin-sensitive [173]. These results are in concordance with earlier data showing that mitogenic-stimulated or amino acid dependent 5′-TOP mRNAs translation is dependent on PI3K mediated signaling, and does not require S6K1 activity and ribosomal protein S6 phosphorylation [174, 175]. Instead, a role for the S6 kinases in controlling the cell size has been suggested as deletion of S6K leads to animal size decrease [173]. Studies performed on 'knock-in' mice in which all sites phosphorylated by the S6 kinases were mutated also indicated a role for S6 phosphorylation in cell growth [176]. These knock-in cells still demonstrated faster rates of protein synthesis at the same time being decreased in size. This could be explained by elevated access of the S6K to other substrates involved in translation, such as eIF4B and eEF2 kinase (see below) [151].

Another pool of data connecting S6K1 activity and translation initiation occurs from the study of potential tumor suppressor, Programmed cell death 4 (PDCD4) protein **(Figure**

5A). PDCD4 binds to eIF4A and is thought to inhibit its helicase activity [177]. PDCD4 is also thought to prevent eIF4A from incorporating into the eIF4F complex by competing with eIF4G for eIF4A binding [178]. S6K1 phosphorylates PDCD4 on S67 in response to growth factor stimulation resulting in its subsequent degradation through the ubiquitin ligase βTrCP101. Therefore, S6K1-dependent phosphorylation of PDCD4 prevents the inhibitory effect of PDCD4 towards eIF4A helicase function within the eIF4F complex.

Recent data give new evidence on interconnection of the mTOR/S6K1 pathway and translation preinitiation complex assembly [172, 179]. Under the poor growth conditions, S6K1 but not mTORC1 binds with multisubunit initiation factor eIF3 that was identified as a dynamic scaffold for mTORC1 and S6K1 binding [172] **(Figure 5B).** Upon growth factors or nutrients availability, the mTORC1 is recruited to eIF3 and phosphorylates S6K1. Based on polysome analysis and cap-binding assays, it is thought that the mTORC1–eIF3 complex associates with the mRNA 5′ cap, bringing mTORC1 into proximity with 4EBP1. Phosphorylation of S6K1 at T389 leads to its dissociation from eIF3. T389-phosphorylated S6K1 binds to PDK1 **(Figure 5A**), which phosphorylates S6K1 at T229. The fully activated S6K1 is able to phosphorylate eIF4B and S6. Phosphorylation of eIF4B by S6K1 at S422 promotes its association with eIF3 [172, 180]. The interaction of mTOR with eIF3 also strengthens the association between eIF4G and eIF3 [181]. Described interactions cooperate to enhance the assembly of translation initiation complex and facilitate cap-dependent translation.

The S6 kinases are activated by phosphorylation at multiple sites. Several of them lie within the C-terminus, while two others lay immediately C-terminal to the catalytic domain. One of these, T389 in the shorter form of S6K1, which is located at a hydrophobic motif carboxyterminal to the kinase domain, is directly phosphorylated by mTOR as part of the mTORC1 complex [182, 183]. Phosphorylation here is required for the consequent phosphorylation of S6K1 by PDK1 at a T229 in the activation loop of the catalytic domain. Phosphorylation at T229 allows full activation of S6K1. S6K2 is likely regulated in a similar manner. Both S6K1 and 2 contain a TOS motif within their N-terminus region, which interacts with Raptor, promoting phosphorylation of S6Ks by mTORC1 [184]. The phosphorylation within the C-terminal region seems to open access to the sites T389/T229, phosphorylation of which provides the complete activation. It is not known exactly which kinase is responsible for C-terminal phosphorylation sites. Nevertheless mTOR also indirectly contributes to the phosphorylation of the C-terminal sites. A motif RSPRR exists in this region probably plays a significant role in the inhibitory effect of the C-terminal region. It has been speculated that a negative S6K1 regulator binds S6K via this motif and that mTOR could broke this binding [184]. The C-terminal region of S6K1 also determines whether S6K1 can be phosphorylated by mTORC2. Mutant S6K1 with deletion of this region is a substrate for mTORC2 [185]. Some data indicate that for S6K1 activation, mTOR can directly phosphorylate S371 *in vitro*, and this event modulates T389 phosphorylation by mTOR [186, 187].

In addition to the discussed above mTORC1 targets, S6Ks and 4E-BP1, both of which modulate translation initiation, mTOR signaling also regulates the translation elongation process through the phosphorylation of eukaryotic elongation factor 2 (eEF2). eEF2 is a GTP binding protein that mediates the translocation step of elongation [188]. eEF2 is

phosphorylated at T56 within the GTP-binding domain and this phosphorylation impedes its ability to bind the ribosome, thus inhibiting its function [188, 189]. Insulin and other stimuli induce the dephosphorylation of eEF2, and this effect is blocked by rapamycin, indicating that this effect is also mediated through mTOR [190]. The eEF2 phosphorylation function is attributed to a highly specific enzyme called eEF2 kinase (eEF2K) [190]. Phosphorylation of eEF2 at T56 by eEF2 kinase impedes the eEF2 binding of to the ribosome and the translocation step of the elongation [188]. The calcium/calmodulin-dependent protein kinase eEF2K is an atypical enzyme since the sequence of its catalytic domain differs substantially from that of other protein kinases, and it is not a member, e.g., of the main Ser-Thr-Tyr kinase superfamily [191]. The C-terminal half of the eEF2K polypeptide contains several sites of phosphorylation including the binding site for the substrate eEF2 at the C-terminus [192]. mTOR negatively regulates eEF2 kinase and consequently activates eEF2. mTOR is considered being able to phosphorylate 3 sites, as was determined by their rapamycin- and/or amino-acid starvation sensitivities [188, 193]. S366 in the C terminus of the catalytic domain has been identified as the site being phosphorylated by S6K and by p90RSK [190]. The phosphorylation at S359 has been shown to be also regulated in a rapamycin-sensitive manner in response to insulin-like growth factor 1 (IGF1) and inactivates eEF2K [194], but the kinase responsible for this phosphorylation remains to be determined. Recently, a novel phosphorylation site located immediately adjacent to the CaM-binding site in eEF2K that is regulated markedly in response to insulin in an mTOR dependent manner has been identified. This site (S78) is not known to be phosphorylated by any known protein kinase in the mTOR pathway. Phosphorylation at this third site also causes the inactivation of eEF2 kinase, in this case by inhibiting the binding of CaM, which binds immediately C-terminal to S78 [193]. eEF2K is thought to be a target of signaling from mTOR independently of other known targets of this pathway, which implies the existence of a novel (probably mTOR-controlled) protein kinase that could acts upon S78 in eEF2K. These data provide a molecular mechanism by which mTOR could regulate peptide chain elongation.

Since the protein synthesis depends on the amount of ribosomes and transfer RNAs (tRNAs) it is important to know that mTOR signaling also contributes to the regulation of tRNA production, promotion of rRNA synthesis and ribosome biogenesis. Thus mTOR signaling tightly regulates transcription of ribosomal RNAs (rRNAs) and tRNAs by RNA polymerases I and III [195]. mTOR can associate with general transcription factor III C (TFIIIC) and relieve its inhibitor Maf1, leading to increased tRNA production. mTORC1 activity also promotes association between transcription initiation factor 1A (TIF-1A) and polymerase I (PolI), thereby promoting rRNA synthesis [35]. The activity of several other transcription factors, such as signal transducer and activator of transcription-1 and -3 (Stat-1 and Stat-3) is also suggested to be regulated by mTORC1-mediated phosphorylation in a rapamycin-sensitive manner [196].

4.2. TORC1-mediated repression of autophagy

Autophagy is a lysosomal-dependent cellular degradation process that generates nutrients and energy to maintain essential cellular activities upon nutrient starvation. A term

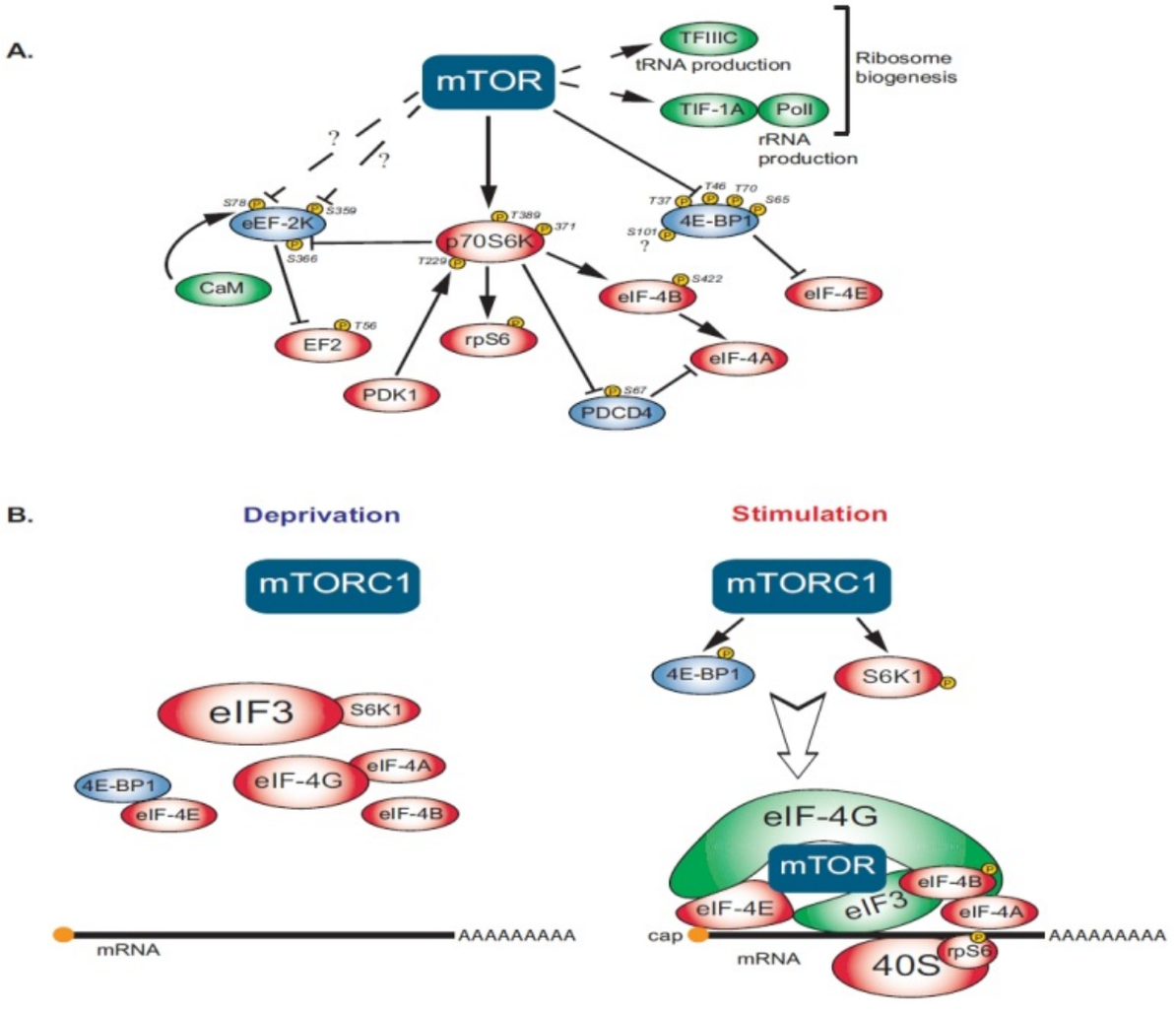

Figure 5. mTORC1 downstream signaling and translation regulation.
A. mTOR phosphorylates two major targets: 4E-BP1 and S6Ks. Hypophosphorylated 4E-BP1 binds tightly to eIF-4E, thereby preventing its interaction with eIF-4G and thus inhibiting translation. Phosphorylated 4E-BP1 is released from eIF-4E resulting in the recruitment of eIF-4G to the 5´-cap, and thereby allowing translation initiation to proceed. Phosphorylation of p70S6K stimulates its activity towards several substrates, including 40S ribosomal protein S6, translation initiation factor eIF-4B, elongation factor kinase eEF2K, and PDCD4 protein. Following S6K-mediated phosphorylation, eIF-4B is recruited to the translation pre-initiation complex and enhances the RNA helicase activity of eIF-4A. S6K1dependent phosphorylation of PDCD4 prevents its inhibitory effect towards eIF-4A helicase. mTORC1 also contributes to the translation elongation through the regulation of eEF2. mTOR negatively regulates eEF2 kinase (either directly or via p70S6K activation) and thereby activates eEF2. mTOR signaling also contributes to the regulation of tRNA production, promotion of rRNA synthesis and ribosome biogenesis activating TFIIIC and promoting the association between transcription initiation factor 1A and polymerase I respectively.
B. In the absence of extracellular stimuli, S6K1 is associated with eIF3 while 4E-BP1 binding to eIF-4E prevents its interaction with eIF-4G and thus inhibiting translation. In response to extracellular stimuli, such as growth factors or nutrients, the mTOR complex is recruited to eIF3 to phosphorylate S6K1 and 4E-BP1. Phosphorylation and activation of S6K1 leads to its dissociation from eIF3. Activated S6K1 then phosphorylates eIF4B and S6. Phosphorylation of eIF-4B 2 promotes its association with eIF3. mTOR also stimulates the association between eIF3 and eIF-4G.

autophagy appeared from Greek "auto" (self) and "phagy" (to eat), refers to an evolutionarily conserved, multi-step lysosomal degradation process in which a cell degrades long-lived proteins and damaged organelles. Three forms of autophagy have been identified, namely macroautophagy, microautophagy and chaperone-mediated autophagy that differ with respect to their modes of delivery to lysosome and physiological functions [197]. Macroautophagy (hereafter autophagy) is the major regulated catabolic mechanism that involves the delivery of cytosolic cargo sequestered inside specific intracellular double-membrane vesicles, called autophagosomes to the lysosomal compartment and subsequent fusion with lysosomes to form single-membrane-bound autophagolysosomes, in which the sequestered material is degraded by acidic lysosomal hydrolases. On one hand, autophagy is crucial for cell survival under extreme conditions through degradation of intracellular macromolecules, which provides the energy required for minimal cell functioning when nutrients are deprived or scarce. Also, autophagy-mediated elimination of altered cytosolic constituents, such as aggregated proteins or damaged organelles, preserves cells from further damages, indicating that autophagy plays a protective role in early stages of cancer [198]. On the other hand, autophagy plays a death-promoting role as type II programmed cell death (type II PCD), compared to apoptosis (type I PCD), as a bona fide tumor suppressor mechanism in cancer [199].

The ability of mTORC1 to regulate autophagy is as highly conserved as well as the process of autophagy itself. AMPK has been indicated as a main upstream regulator of mTORC1 mediated autophagy inhibition.

The mechanism by which TORC1 negatively regulates the autophagic machinery has first been described in yeast. Genetic screenings for autophagy defective mutants led to the identification of more than 30 essential autophagy-related genes (Atg).These proteins can be classified into several groups depending on their function and interdependency. Most upstream is a protein complex that comprises the serine/threonine kinase Atg1, as well as two accessory proteins Atg13 and Atg17. In mammals, two homologs of Atg1, uncoordinated 51-like kinase 1 (ULK1) and ULK2 have been identified. Accumulating evidence suggests that ULK1 is a key regulator of autophagy initiation. ULK1 is directly phosphorylated by TORC1 [200-202]. Recently, it has also been shown that mTORC1-mediated phosphorylation of ULK1 impairs its activation by AMPK and results in an overall decrease in autophagy [203]. ULK1 and ULK2 are found in a stable complex with mammalian autophagy-related protein 13 (mAtg13), the scaffold protein FAK-family interacting protein of 200 kDa (FIP200) [204] [201] and Atg101, an additional binding partner of Atg13 that has no ortholog in yeast [205]. In contrast to yeast, the composition of this complex does not change with the nutrient status. Several data evidence that the phosphorylation status within the Ulk1/2-Atg13-FIP200 complex dramatically changes with the nutrient availability. Under rich growth conditions, mTORC1 associates with the Ulk1/2-Atg13-FIP200 complex, via direct interaction between Raptor and Ulk1/2 (37). The active mTOR phosphorylates Atg13 and Ulk1/2 [201], thereby downregulating Ulk1/2 kinase activity and suppressing autophagy **(Figure 6)**. In response to starvation, the mTORC1-dependent phosphorylation sites in Ulk1/2 are rapidly dephosphorylated by yet unknown phosphatases, what stimulates Ulk1/2 autophosphorylation and phosphorylation of both

Atg13 and FIP200. Several serine and threonine residues in human Ulk1 whose phosphorylation was decreased after starvation have been recently identified from which S638 and S758 have been proposed to be most probable mTORC1 negative phosphorylation sites [203, 206]. Ulk1/2 autophosphorylation and following FIP200 and Atg13 phosphorylation in turn leads to translocation of the entire complex to the pre-autophagosomal membrane and to autophagy induction [200, 201, 205]. However, the functional relevance of Ulk1/2-mediated phosphorylation of Atg13 and FIP200 for this recruitment and the relevant phosphorylation sites has not been verified yet. Interestingly, another Ulk1-dependent phosphorylation site in human Atg13 (S318) has been identified recently [207]. The authors of that study could show that the Hsp90-Cdc37 chaperone complex selectively stabilizes and activates Ulk1.

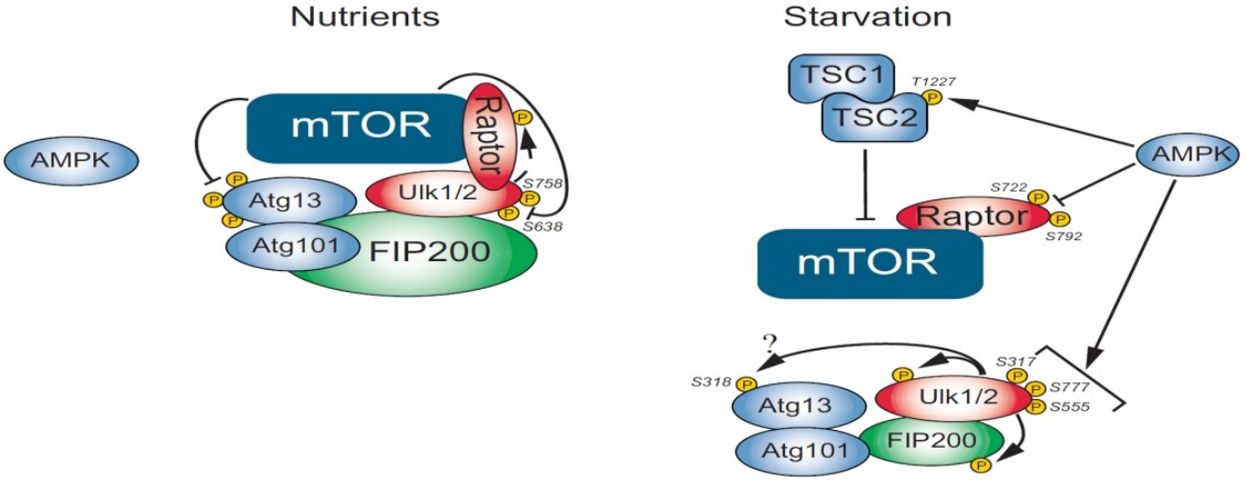

Figure 6. mTORC1 downstream signaling and autophagy regulation. Ulk1 and Ulk2 form a stable complex with Atg13, FIP200 and Atg101. Under fed conditions mTORC1 phosphorylates Ulk1/2 and Atg13, thereby inhibiting the Ulk1/2 kinase activity and complex stability. In response to starvation, the mTORC1-dependent phosphorylation sites in Ulk1/2 are rapidly dephosphorylated, and Ulk1/2 autophosphorylates and phosphorylates Atg13 and FIP200 resulting in translocation of the entire complex to the pre-autophagosomal membrane and autophagy induction. Alternatively, Ulk1/2 is phosphorylated by AMPK and thereby activated. In addition, AMPK indirectly leads to the induction of autophagy by inhibiting mTORC1 through phosphorylation of Raptor or TSC2.

In yeast, autophagosomes originate from a single preautophagosomal structure. Although an equivalent structure seems to be absent from mammalian cells, a special subdomain in the endoplasmic reticulum (ER) termed the "omegasome" has been suggested as a putative origin of autophagosomes. This structure is enriched in PI(3)P, a product of the phosphatidylinositol 3-kinase (PI3K). A hierarchical analysis of the mammalian Atg proteins could recently confirm the recruitment of Ulk1 proximal to these omegasomes [208]. The translocation of Ulk1, presumably in a complex with Atg13 and FIP200, is the initial step of autophagosome biogenesis and is completely abrogated in *FIP200–/–* cells [208]. The subsequent recruitment of the PI3K depends on Ulk1 and its kinase activity [208]. Recently, two groups found evidence for the mechanism by which Ulk1 and Ulk2 in turn negatively regulate mTORC1 signaling. Particularly, the phosphorylation of Raptor at numerous sites

was strongly enhanced after overexpression of Ulk1. Interestingly, one of these residues (T792) is the abovementioned effector site through which AMPK negatively regulates mTORC1 activity [45]. The multiple Ulk1-dependent phosphorylation of Raptor either results in direct inhibition of mTORC1 kinase activity [209], or interferes with Raptor-substrate interaction [210], thus finally leads to reduced phosphorylation of mTORC1 downstream targets.

Apart from mTOR, Ulk1/2 is phosphorylated (probably on S317 and S777 or S555 according to different studies) by AMPK under glucose starvation and thereby activated [112, 203, 211, 212]. Under nutrient sufficiency phosphorylation of ULK1 S758 by active mTORC1 disrupts ULK1 interaction with, and hence activation by, AMPK [203]. Although the data concerning the role of ULK1/2 certain sites phosphorylation is rather discrepant it is clear that in mammals, phosphorylation of ULK1 by AMPK is strongly required for ULK function in the response to nutrient deprivation. Therefore, AMPK could control ULK1 via a two-pronged mechanism, ensuring activation only under the appropriate cellular conditions – firstly, by direct phosphorylation and secondly, by suppression of mTORC1-mediated ULK1 inhibition [212]. Several studies demonstrated that Ulk1 in addition directly interferes with mTORC1 downstream signaling and negatively regulates S6K1 activity, both in *Drosophila* and mammalian cells [213]. Taken together these data evidence that mTOR subnetwork occupy the key position in autophagic pathways.

5. Signaling up and downstream of mTORC2

In contrast to mTORC1, very little is known about the upstream regulation of TORC2. Rapamycin–FKBP12 complex does not bind directly to mTORC2, but long-term rapamycin treatment disrupts mTORC2 assembly in ~20% of cancer cell lines through an unknown mechanism [56]. It remains to be determined why rapamycin-mediated inhibition of mTORC2 assembly only occurs in certain cell types. One hypothesis suggests that some mTORC2 subunits could prevent the binding of rapamycin/FKBP12 complex to the mTOR FRB domain by the competing mechanism (reviewed in [62]). However, there are no enough data to support this model.

It seems that mTORC2 is activated in response to growth factors but is insensitive to nutrients and energetic stress, [214]. Thus, like TORC1, TORC2 can be stimulated by growth factors through PI3K [3]. Consequently, treatment with PI3K inhibitors can inhibit TORC2-mediated target phosphorylation [85]. Thus it was suggested that mTORC2 lies downstream of PI3K signaling [85]. Rheb which is known as a key upstream activator of mTORC1 showed negative and indirect effect on the regulation of mTORC2 both in *Drosophila* and mammalian cells [59]. Some data pointed on TSC1-TSC2 function in mTORC2 regulation [92]. Moreover, the TSC1/TSC2 complex was found to physically associate with mTORC2, but not with mTORC1. The molecular mechanism through which the TSC1/TSC2 complex promotes mTORC2 activation is unclear. It is also currently unknown whether some pathways that regulate TSC1/TSC2 ability to inhibit mTORC1, also influence on mTORC2 activation.

The best-characterized target of mTORC2 is AKT, which is phosphorylated at S473 upon TORC2 activation [53, 58, 85]. Numerous studies attempted to identify the crucial kinase(s) (often referred to as PDK2) responsible for the phosphorylation of S473 in Akt. Several enzymes are in the candidate list, including PDK1, integrin-linked kinase (ILK), Akt itself, DNA-dependent protein kinase catalytic subunit (DNA-PKcs) and mTORC2 [215]. Since mTORC2 complex fulfills the role of the Akt S473 kinase, mTORC2 has been identified as the PDK2 [85]. Akt is a member of the AGC kinase family (see above), which also includes S6Ks, serum glucocorticoid-induced protein kinase (SGKs), RSKs, and PKCs [62, 216]. mTORC2 has been shown to phosphorylate AKT, SGK and PKCα [85]. mTORC2 seems to regulate Akt by phosphorylation of its two different sites. The mTORC2-mediated Akt hydrophobic motif phosphorylation on the regulatory S473 site is dependent on growth factor signaling, whereas a basal activity of mTORC2 maintains the constitutive phosphorylation of Akt on T450 site in its turn motif [217]. This difference indicates that phosphorylation of the T450 and S473 sites on Akt by mTORC2 are separate events and might take place at different locations. It has been proposed that translocation of Akt to the plasma membrane coupled with its phosphorylation on T308 and S473 is a critical step in activation of Akt by growth factor signaling [217]. Phosphorylation of AKT on S473 enhances the activation phosphorylation motif at T308, which is absolutely required for AKT activity.

The major functions of mTORC2 include the regulation of cytoskeletal organization and the promotion of cell survival. If the last one is mediated apparently through AKT activation, the mechanisms, which realize mTORC2 function in cytoskeletal reorganization, are not obvious. Paxillin, which functions as a docking protein, localizing to the focal adhesions of adherent cells [218] has been shown to be highly phosphorylated at Tyr118. Knockdown of mTORC2 inhibited the phosphorylation of paxillin [3]. Rho, Rac and Cdc42, three best-characterized members of the Rho family of small GTPases, were demonstrated to be involved in actin cytoskeleton assembly and disassembly [219]. It was reported that mTORC2 may function as upstream regulator of Rho GTPases to regulate the actin cytoskeleton [3].

Interestingly, the TORC2-mediated activation of AKT places TORC2 upstream of TORC1 in the TOR signaling cascade. A most recent publication has highlighted a role for ribosomes in the activation of TORC2 [38]. The authors have found that active mTORC2 was physically associated with the ribosome, and insulin-stimulated PI3K signaling promoted mTORC2-ribosome binding. Interaction of mTORC2 with NIP7 (nuclear import 7, a protein responsible for ribosome biogenesis and rRNA maturation) was shown to be required for full activation of mTORC2 by insulin. Noteworthy, inhibition of protein translation had no effect on mTORC2 activation, supporting the notion that mTORC2 is activated by the ribosome, but not translation. Ribosome associated mTORC2 displays kinase activity toward AKT *in vitro*. Inhibition of PI3K activity blocks the interaction between the ribosome and mTORC2, as well as inhibits mTORC2 activation in response to insulin, confirming that NIP7-ribosome assembly activates mTORC2 downstream from PI3K. It appears that the mTORC2 components, Rictor and/or Sin1, which are not found in mTORC1, interact with

the 60S subunit of ribosome. Interestingly, another study [220] has also recently reported the association of mTORC2 with the ribosome and proposed that the ribosomal association is important for the cotranslational phosphorylation of the AKT turn motif. These findings are coherent with very recent data that point on endoplasmic reticulum (ER), the cellular organelle highly enriched with ribosomes, as a major compartment of mTORC2 localization. Moreover, the signaling from growth factor does not change the ER localization of mTORC2 as well as its translocation to the plasma membrane. Besides it was suggested that the mTORC2-dependent phosphorylation of Akt on S473 occurs on the surface of ER [37]. These observations raise many interesting questions regarding the regulation of TORC2 and its ribosomal interactions, but it also indicates that additional levels of interplay between TORC2 and TORC1 may exist, as both complexes are linked to the process of ribosome biogenesis.

6. Crosstalk of mTORC1/2 and major cytokine signaling pathways

6.1. mTORC1/2 and Ras-MAP kinases pathways

Ras-Erk-RSKs

In addition to the PI3K–Akt pathway, activation of Ras-MAPK signaling can also stimulate mTORC1 activity. The Ras–mitogen-activated protein kinase (MAPK) pathway is a key signaling pathway that is involved in the regulation of normal cell proliferation, survival, growth and differentiation. This pathway includes the whole number of kinases, being regulated through phosphorylation in consecutive order. The Ras–MAPK signaling network has been the subject of intense research because mutations in (or overexpression of) many of the signaling components from this pathway are a hallmark of several human cancers and other human diseases [221]. The Ras–ERK (extracellular signal-regulated kinase-1 and -2) pathway has an established role in regulating transcription [222], but a connection between this pathway and translational regulation is less clear. Over the past few years, mitogen activated Ras–ERK pathway has been shown to trigger the activation of mTORC1 signaling. This is mediated by ERK and RSK dependent phosphorylations of TORC1 pathway components.

p90RSKs (also known as MAPKAP kinase 1 (mitogen-activated protein kinase-activated protein) kinase-1) are a family of Ser/Thr kinases that lies downstream of the Ras–MAPK cascade and has overlapping substrate specificity with Akt. The RSK isoforms are directly activated by ERK1/2 in response to growth factors, many polypeptide hormones, neurotransmitters, chemokines and other stimuli. RSKs phosphorylate several cytosolic and nuclear targets and they are involved in the regulation of different cellular processes, including cell survival, cell proliferation, cell growth and motility. Following the stimulation of cells with growth factors, RSKs are phosphorylated at multiple Ser and Thr residues by several kinases; these phosphorylation events are directly or indirectly initiated by the activation of the ERK/MAPK cascade [223]. Six different phosphorylation sites have been mapped in RSK1/2 (and are conserved in RSK3/4), of which four have been shown to be important for their activity

(S221, S363, S380 and T573 in human RSK1). Following mitogen stimulation, ERK1/2 phosphorylates T573 at the C-terminal domain (CTKD) activation loop of RSK, resulting in CTKD activation. ERK1/2 might also phosphorylate S363 (the turn motif) and T359 (unknown function) in the RSK linker region. The activated CTKD of RSK then autophosphorylates S380 in the hydrophobic motif, creating a docking site for PDK1. After binding, PDK1 phosphorylates the NTKD activation loop S221, leading to the complete activation of the protein and following phosphorylation of the substrates by the NTKD. The NTKD also phosphorylates S749 in the CTKD domain of RSK, differentially modulating the interaction of RSK isoforms with ERK1/2 and thereby completing a sequence of coordinated phosphorylation events and protein–protein interactions that culminate in RSK activation and downstream signaling throughout the cell [224]. Other factors that have been shown to be involved in the activation of RSK include the p38 MAPK, the ERK5 MAPK and fibroblast growth factor receptor-3 (FGFR3). RSK was found to phosphorylate TSC2 at the C-terminus S1798 [225] and, to a lesser extent, the two conserved Akt sites (S939 and T1462) and inactivates its suppressor function, thereby promoting mTOR signaling and translation (**Figure 7)**. RSK mediated phosphorylation of TSC2 is additive to AKT mediated inhibitory modifications of TSC2, but how these phosphorylation events lead to TSC2 inhibition remains unclear.

Erk1/2 kinase itself also impacts the mTORC1 regulation. Thus, a number of additional sites on TSC2 were found to be weakly induced by PMA [89], including an ERK consensus site S664. This site, and a second site on TSC2, S540, was independently found to be directly phosphorylated by ERK and to contribute to ERK-mediated activation of mTORC1 signaling [226]. Strikingly, phosphorylation of S540 and/or S664 by ERK was found to disrupt the association between TSC1 and TSC2. This effect was also detected following phosphorylation of the TSC1/TSC2 complex *in vitro,* suggesting that it is direct and does not require other proteins.

In addition, it was recently shown that RSK also directly impacts the mTORC1 complex phosphorylating Raptor, and thereby promotes mTORC1 kinase activity [46]. RSK phosphorylates at least two evolutionarily conserved Raptor serine residues that lie within a region with no homology to known functional domains. Whereas S721 lies within a classical RSK consensus sequence (RXRXXpS/T), S719 is located within a minimal phosphoacceptor sequence (RXXpS) that was found to be sufficient in other RSK substrates, such as DAPK, c-Fos, and CREB. Although the underlying molecular mechanism of this was not fully defined, this study provided new insights into Ras–ERK signals to mTORC1. As tumor promoting phorbol esters and some growth factors activate mTORC1 signaling independently of AKT, phosphorylation of Raptor by RSK might provide a mechanism to overcome the inhibitory effects of PRAS40 inhibitory phosphorylation of TSC2 at S664 and S1798, respectively [89, 226, 227]. Collectively, these data suggest that ERK signaling activates mTORC1 through multisite phosphorylation events by both ERK and its downstream target RSK.

Erk1/2-RSK pathway also contributes to the mTORC1 downstream signaling, this includes RSK dependent *in vivo* and *in vitro* phosphorylation of eukaryotic translation initiation

factor-4B (eIF4B) and rpS6 [180]. Although early studies indicated S6K1 and S6K2 as the major rpS6 kinases in somatic cells [228, 229], the role of RSK in regulating site-specific rpS6 phosphorylation and translation in somatic cells has been recently readdressed [230]. Particularly, *in vitro* and *in vivo* evidence suggests that S6Ks phosphorylate every site in rpS6, while RSK predominantly phosphorylates S235 and S236 [230]. Studies from *S6k1*/*S6k2*-knockout mice showed that there was minimal phosphorylation of rpS6 at S240/244, but there was persistent phosphorylation at S235/236 [173]. In accordance with this finding, RSK1 and RSK2 were shown to phosphorylate rpS6 on S235/236 in response to Ras–MAPK-pathway activation, using an mTOR-independent pathway [230]. The RSK mediated S235/236 phosphorylation correlated with formation of the translation pre-initiation complex and increased cap-dependent translation, pointing that RSK provides an additional mitogen- and oncogene-regulated input that links the ERK pathway to the regulation of translation initiation [230]. Translation initiation factor-4B is also phosphorylated by RSK and S6K on S422 [171, 180]. Therefore, phosphorylation and regulation of eIF4B function by RSK and S6K exemplifies the convergence of two major signaling pathways that are involved in translational control. Together, these findings suggest that the mitogen-activated Ras–ERK–RSK signaling module, in parallel with the PI3K–AKT pathway, contains several inputs to stimulate mTORC1 signaling.

6.2. mTORC1 and TNFα-IKKβ-TSC1 pathway

Although the activation of mTORC1 downstream of most cytokines including insulin and growth factors is likely to occur through the Akt and ERK signaling mechanisms described above, accumulating evidence suggests that other cytokines, such as tumor necrosis factor α (TNFα), can also induce mTORC1 activity. TNFα is a proinflammatory cytokine that is involved in many human diseases, including cancer [231, 232]. Early studies implicated the TNFα pathway in mTORC1 activation [233]. Recently, it has been shown that IKKβ (inhibitor of nuclear factor κB (NFκB) kinaseβ; also known as IKBKB), a major downstream kinase in the TNFα signaling pathway, can associate with and phosphorylates TSC1 at S487 and S511, resulting in the inhibition of TSC1–TSC2 and, therefore, the activation of mTORC1 [234]. *Tsc1*−/− mouse embryo fibroblasts expressing TSC1 mutants lacking these sites lose their responsiveness to TNFα for activation of mTORC1, whereas phosphomimetic mutation lead to a basal increase in mTORC1 signaling. Authors proposed a mechanism involving rapid dissociation of the complex and increased degradation of TSC1. However, the results suggest minimal effects on the stability of the TSC1–TSC2 complex, and the precise mechanism of acute complex inhibition by phosphorylation of these sites is not known. In certain human cancers, TNFα promotes vascular endothelial growth factor (vEGF) expression and angiogenesis through activated mTORC1 signaling as a result of IKKβ mediated suppression of TSC1 [234]. This has provided a plausible mechanism that could link inflammation to cancer pathogenesis. Moreover, TNFα also signals to AKT [231]. Activated AKT in turn induces IKKα (also known as CHUK), [232]. It has been shown that IKKα associates with mTORC1 in an AKT dependent manner [235]. Importantly, IKKα is required for efficient induction of mTORC1 activity by AKT in certain cancer cells [235, 236].

It remains unclear, however, how the association of IKKα with mTORC1 can result in the activation of mTORC1.

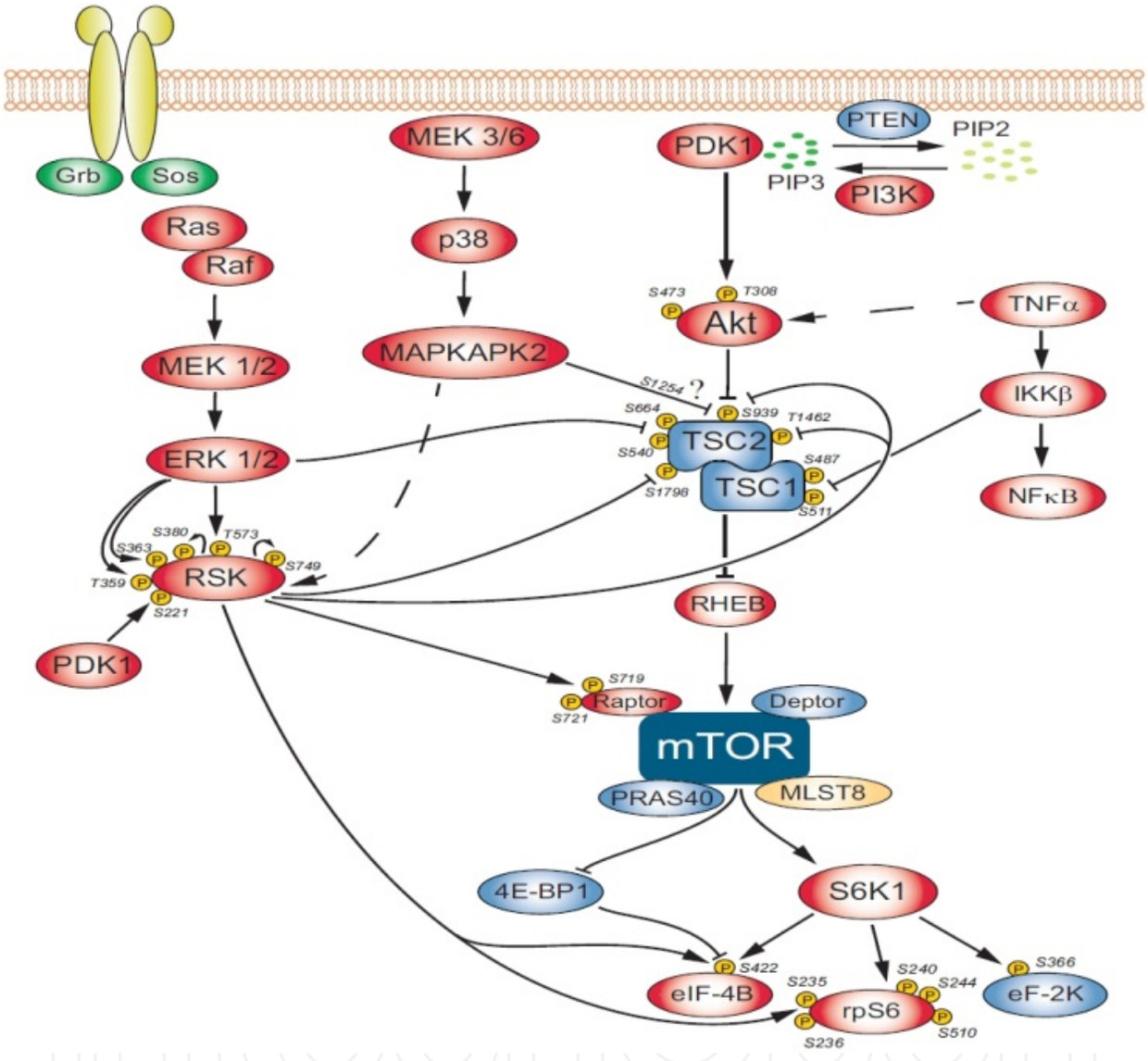

Figure 7. mTORC1 crosstalk with the major cytokine signaling pathways. MAPK pathway impinges on the mTORC1 signaling in a few ways. RSK phosphorylates TSC2 at the C-terminus and, to a lesser extent, the two conserved Akt sites thus inactivating its suppressive effect on mTORC1. RSK also directly impacts the mTORC1 complex by phosphorylation of Raptor and thereby upregulates mTORC1 kinase activity. Besides, RSK phosphorylates the rpS6 and eIF-4B to promote cap-dependent translation in response to Ras – MAPK-pathway activation. ERK1/2 kinase contributes to the activation of mTORC1 signaling through direct phosphorylation of TSC2 and probably through the disruption of the association between TSC1 and TSC2. Stress activated signaling pathway might also influence mTORC1 signaling through TSC2 phosphorylation by p38-activated MAPKAPK2 kinase. IKK β, a major downstream kinase in the TNF α signaling pathway, can associate with and phosphorylate TSC1 leading to the inhibition of TSC1/TSC2 and, therefore, the activation of mTORC1.

7. Conclusion

Maintenance of cellular energy homeostasis and life-sustaining activity requires their appropriately adaptation to the continually varying surrounding environment. This adaptation is provided by the differential expression of genes that is strictly controlled at the levels of transcription and translation. To provide the rapid response to the environmental cues cells switch vast number of intracellular signaling cascades that define the activity of key proteins responsible for transcription and translation regulation. This implies operative and directed changes in the activities of proteins mediating the signal transduction. Phosphorylation represents one of the most important intracellular regulatory molecular mechanisms since it provides the rapid and reversible activation or downregulation of protein activities. Not surprisingly, mTOR signaling network, which integrates and promotes the prompt respond to environmental changes is mainly regulated through this type of posttranslational modification. mTOR is known as a "switch master" that converts vast array of nutrient-, cytokine-, energy- and stress-sensitive signals into the alterations of cellular metabolism including protein and lipid biosynthesis and autophagy. Indeed such resources consuming processes as growth and proliferation could occur only under the conditions of nutrient and energy sufficiency. When energy or amino acids become limiting, cell growth needs to be restricted and protein production needs to be downregulated so that cells can use their limited resources to survive. mTORC1 contributes to overall cap-dependent translation including initiation and elongation steps by several different pathways. Significantly, all of these pathways use phosphorylation as common molecular mechanism of regulation. Most of the proteins from mTOR-dependent pathways (for instance TSC2, Akt, S6K, etc.) contain multiple phosphorylating sites, which mediate stimulating or negative effect on their activity. Some of mTOR partners are characterized by the hierarchical mode of phosphorylation (rpS6, Akt as the examples), whereby each previous phosphorylation opens the opportunity for the subsequent ones. Interestingly, one certain site could serve as phosphorylation target for more than one kinase, therefore implementing the competitive mechanism of regulation (for instance, eIF4B S422). The complexity of mTOR signaling increases due to the presence of positive or negative feedback loops as well as crosstalk with other pathways. The number of reported phosphorylation sites throughout mTOR pathways constantly increases although the precise molecular meaning of several already discovered phosphorylation events remains unclear. This is also true to some molecular mechanisms of mTORC1 and especially mTORC2 functioning. About mTORC1 signaling, a number of issues remain unresolved. For example, aside from S6K, 4EBP1 and ULK1, the downstream direct targets that mediate the cellular effects of TORC1 signaling are largely unaccounted for. In addition, how the specificity of TORC1 signaling is achieved and how multiple signals are integrated is not known. Concerning TORC2, the upstream regulators are poorly defined. This knowledge seems to be of great significance since mTOR is considered a central node of intracellular signaling network and deregulation of its activity strongly contributes to the wide spectrum of human diseases. Further studies will give us a better understanding of the whole picture of mTORC1/mTORC2 functioning that could be applied to the development of new approaches to the treatment of mTOR-associated diseases.

Author details

Elena Tchevkina
Corresponding Author
Oncogenes Regulation Department, N.N. Blokhin Russian Cancer Research Center, Moscow, Russia

Andrey Komelkov
Oncogenes Regulation Department, N.N. Blokhin Russian Cancer Research Center, Moscow, Russia

8. References

[1] Dennis PB, Jaeschke A, Saitoh M, Fowler B, Kozma SC, et al. (2001) Mammalian TOR: a homeostatic ATP sensor. Science.294(5544):1102-5. Epub 2001/11/03.
[2] Inoki K, Zhu T, Guan KL. (2003) TSC2 mediates cellular energy response to control cell growth and survival. Cell.115(5):577-90. Epub 2003/12/04.
[3] Jacinto E, Loewith R, Schmidt A, Lin S, Ruegg MA, et al. (2004) Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. Nature cell biology.6(11):1122-8. Epub 2004/10/07.
[4] Sarbassov DD, Ali SM, Kim DH, Guertin DA, Latek RR, et al. (2004) Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton. Current biology: CB.14(14):1296-302. Epub 2004/07/23.
[5] Loewith R, Jacinto E, Wullschleger S, Lorberg A, Crespo JL, et al. (2002) Two TOR complexes, only one of which is rapamycin sensitive, have distinct roles in cell growth control. Molecular cell.10(3):457-68. Epub 2002/11/01.
[6] Vezina C, Kudelski A, Sehgal SN. (1975) Rapamycin (AY-22,989), a new antifungal antibiotic. I. Taxonomy of the producing streptomycete and isolation of the active principle. The Journal of antibiotics.28(10):721-6. Epub 1975/10/01.
[7] Eng CP, Sehgal SN, Vezina C. (1984) Activity of rapamycin (AY-22,989) against transplanted tumors. The Journal of antibiotics.37(10):1231-7. Epub 1984/10/01.
[8] Douros J, Suffness M. (1981) New antitumor substances of natural origin. Cancer treatment reviews.8(1):63-87. Epub 1981/03/01.
[9] Sehgal SN, Baker H, Vezina C. (1975) Rapamycin (AY-22,989), a new antifungal antibiotic. II. Fermentation, isolation and characterization. The Journal of antibiotics.28(10):727-32. Epub 1975/10/01.
[10] Cafferkey R, Young PR, McLaughlin MM, Bergsma DJ, Koltin Y, et al. (1993) Dominant missense mutations in a novel yeast protein related to mammalian phosphatidylinositol 3-kinase and VPS34 abrogate rapamycin cytotoxicity. Mol Cell Biol.13(10):6012-23. Epub 1993/10/01.
[11] Heitman J, Movva NR, Hall MN. (1991) Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast. Science.253(5022):905-9. Epub 1991/08/23.
[12] Chen J, Zheng XF, Brown EJ, Schreiber SL. (1995) Identification of an 11-kDa FKBP12-rapamycin-binding domain within the 289-kDa FKBP12-rapamycin-associated protein and characterization of a critical serine residue. Proceedings of the National Academy of Sciences of the United States of America.92(11):4947-51. Epub 1995/05/23.

[13] Choi J, Chen J, Schreiber SL, Clardy J. (1996) Structure of the FKBP12-rapamycin complex interacting with the binding domain of human FRAP. Science.273(5272):239-42. Epub 1996/07/12.
[14] Sabers CJ, Martin MM, Brunn GJ, Williams JM, Dumont FJ, et al. (1995) Isolation of a protein target of the FKBP12-rapamycin complex in mammalian cells. The Journal of biological chemistry.270(2):815-22. Epub 1995/01/13.
[15] Brown EJ, Albers MW, Shin TB, Ichikawa K, Keith CT, et al. (1994) A mammalian protein targeted by G1-arresting rapamycin-receptor complex. Nature.369(6483):756-8. Epub 1994/06/30.
[16] Chiu MI, Katz H, Berlin V. (1994) RAPT1, a mammalian homolog of yeast Tor, interacts with the FKBP12/rapamycin complex. Proceedings of the National Academy of Sciences of the United States of America.91(26):12574-8. Epub 1994/12/20.
[17] Sabatini DM, Erdjument-Bromage H, Lui M, Tempst P, Snyder SH. (1994) RAFT1: a mammalian protein that binds to FKBP12 in a rapamycin-dependent fashion and is homologous to yeast TORs. Cell.78(1):35-43. Epub 1994/07/15.
[18] Chen Y, Chen H, Rhoad AE, Warner L, Caggiano TJ, et al. (1994) A putative sirolimus (rapamycin) effector protein. Biochemical and biophysical research communications.203(1):1-7. Epub 1994/08/30.
[19] Keith CT, Schreiber SL. (1995) PIK-related kinases: DNA repair, recombination, and cell cycle checkpoints. Science.270(5233):50-1. Epub 1995/10/06.
[20] Kunz J, Henriquez R, Schneider U, Deuter-Reinhard M, Movva NR, et al. (1993) Target of rapamycin in yeast, TOR2, is an essential phosphatidylinositol kinase homolog required for G1 progression. Cell.73(3):585-96. Epub 1993/05/07.
[21] Wiederrecht GJ, Sabers CJ, Brunn GJ, Martin MM, Dumont FJ, et al. (1995) Mechanism of action of rapamycin: new insights into the regulation of G1-phase progression in eukaryotic cells. Progress in cell cycle research.1:53-71. Epub 1995/01/01.
[22] Hay N, Sonenberg N. (2004) Upstream and downstream of mTOR. Genes & development.18(16):1926-45. Epub 2004/08/18.
[23] Janus A, Robak T, Smolewski P. (2005) The mammalian target of the rapamycin (mTOR) kinase pathway: its role in tumourigenesis and targeted antitumour therapy. Cellular & molecular biology letters.10(3):479-98. Epub 2005/10/12.
[24] Gangloff YG, Mueller M, Dann SG, Svoboda P, Sticker M, et al. (2004) Disruption of the mouse mTOR gene leads to early postimplantation lethality and prohibits embryonic stem cell development. Mol Cell Biol.24(21):9508-16. Epub 2004/10/16.
[25] Murakami M, Ichisaka T, Maeda M, Oshiro N, Hara K, et al. (2004) mTOR is essential for growth and proliferation in early mouse embryos and embryonic stem cells. Mol Cell Biol.24(15):6710-8. Epub 2004/07/16.
[26] Gingras AC, Raught B, Sonenberg N. (2001) Regulation of translation initiation by FRAP/mTOR. Genes & development.15(7):807-26. Epub 2001/04/12.
[27] Perry J, Kleckner N. (2003) The ATRs, ATMs, and TORs are giant HEAT repeat proteins. Cell.112(2):151-5. Epub 2003/01/30.
[28] Foster KG, Fingar DC. (2010) Mammalian target of rapamycin (mTOR): conducting the cellular signaling symphony. The Journal of biological chemistry.285(19):14071-7. Epub 2010/03/17.

[29] Soliman GA, Acosta-Jaquez HA, Dunlop EA, Ekim B, Maj NE, et al. (2010) mTOR Ser-2481 autophosphorylation monitors mTORC-specific catalytic activity and clarifies rapamycin mechanism of action. The Journal of biological chemistry.285(11):7866-79. Epub 2009/12/22.
[30] Caron E, Ghosh S, Matsuoka Y, Ashton-Beaucage D, Therrien M, et al. (2010) A comprehensive map of the mTOR signaling network. Molecular systems biology.6:453. Epub 2010/12/24.
[31] Acosta-Jaquez HA, Keller JA, Foster KG, Ekim B, Soliman GA, et al. (2009) Site-specific mTOR phosphorylation promotes mTORC1-mediated signaling and cell growth. Mol Cell Biol.29(15):4308-24. Epub 2009/06/03.
[32] Chiang GG, Abraham RT. (2005) Phosphorylation of mammalian target of rapamycin (mTOR) at Ser-2448 is mediated by p70S6 kinase. The Journal of biological chemistry.280(27):25485-90. Epub 2005/05/19.
[33] Peterson RT, Beal PA, Comb MJ, Schreiber SL. (2000) FKBP12-rapamycin-associated protein (FRAP) autophosphorylates at serine 2481 under translationally repressive conditions. The Journal of biological chemistry.275(10):7416-23. Epub 2000/03/04.
[34] Edinger AL, Linardic CM, Chiang GG, Thompson CB, Abraham RT. (2003) Differential effects of rapamycin on mammalian target of rapamycin signaling functions in mammalian cells. Cancer research.63(23):8451-60. Epub 2003/12/18.
[35] Russell RC, Fang C, Guan KL. (2011) An emerging role for TOR signaling in mammalian tissue and stem cell physiology. Development.138(16):3343-56. Epub 2011/07/28.
[36] Sancak Y, Bar-Peled L, Zoncu R, Markhard AL, Nada S, et al. (2010) Ragulator-Rag complex targets mTORC1 to the lysosomal surface and is necessary for its activation by amino acids. Cell.141(2):290-303. Epub 2010/04/13.
[37] Boulbes DR, Shaiken T, Sarbassov dos D. (2011) Endoplasmic reticulum is a main localization site of mTORC2. Biochemical and biophysical research communications.413(1):46-52. Epub 2011/08/27.
[38] Zinzalla V, Stracka D, Oppliger W, Hall MN. (2011) Activation of mTORC2 by association with the ribosome. Cell.144(5):757-68. Epub 2011/03/08.
[39] Kim JE, Chen J. (2000) Cytoplasmic-nuclear shuttling of FKBP12-rapamycin-associated protein is involved in rapamycin-sensitive signaling and translation initiation. Proceedings of the National Academy of Sciences of the United States of America.97(26):14340-5. Epub 2000/12/13.
[40] Yip CK, Murata K, Walz T, Sabatini DM, Kang SA. (2010) Structure of the human mTOR complex I and its implications for rapamycin inhibition. Molecular cell.38(5):768-74. Epub 2010/06/15.
[41] Peterson TR, Laplante M, Thoreen CC, Sancak Y, Kang SA, et al. (2009) DEPTOR is an mTOR inhibitor frequently overexpressed in multiple myeloma cells and required for their survival. Cell.137(5):873-86. Epub 2009/05/19.
[42] Kim DH, Sarbassov DD, Ali SM, King JE, Latek RR, et al. (2002) mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. Cell.110(2):163-75. Epub 2002/08/02.
[43] Hara K, Maruki Y, Long X, Yoshino K, Oshiro N, et al. (2002) Raptor, a binding partner of target of rapamycin (TOR), mediates TOR action. Cell.110(2):177-89. Epub 2002/08/02.

[44] Zhou H, Huang S. (2010) The complexes of mammalian target of rapamycin. Current protein & peptide science.11(6):409-24. Epub 2010/05/25.
[45] Gwinn DM, Shackelford DB, Egan DF, Mihaylova MM, Mery A, et al. (2008) AMPK phosphorylation of raptor mediates a metabolic checkpoint. Molecular cell.30(2):214-26. Epub 2008/04/29.
[46] Carriere A, Cargnello M, Julien LA, Gao H, Bonneil E, et al. (2008) Oncogenic MAPK signaling stimulates mTORC1 activity by promoting RSK-mediated raptor phosphorylation. Current biology : CB.18(17):1269-77. Epub 2008/08/30.
[47] Wang L, Lawrence JC, Jr., Sturgill TW, Harris TE. (2009) Mammalian target of rapamycin complex 1 (mTORC1) activity is associated with phosphorylation of raptor by mTOR. The Journal of biological chemistry.284(22):14693-7. Epub 2009/04/07.
[48] Sancak Y, Thoreen CC, Peterson TR, Lindquist RA, Kang SA, et al. (2007) PRAS40 is an insulin-regulated inhibitor of the mTORC1 protein kinase. Molecular cell.25(6):903-15. Epub 2007/03/28.
[49] Kovacina KS, Park GY, Bae SS, Guzzetta AW, Schaefer E, et al. (2003) Identification of a proline-rich Akt substrate as a 14-3-3 binding partner. The Journal of biological chemistry.278(12):10189-94. Epub 2003/01/14.
[50] Oshiro N, Takahashi R, Yoshino K, Tanimura K, Nakashima A, et al. (2007) The proline-rich Akt substrate of 40 kDa (PRAS40) is a physiological substrate of mammalian target of rapamycin complex 1. The Journal of biological chemistry.282(28):20329-39. Epub 2007/05/23.
[51] Wang L, Harris TE, Lawrence JC, Jr. (2008) Regulation of proline-rich Akt substrate of 40 kDa (PRAS40) function by mammalian target of rapamycin complex 1 (mTORC1)-mediated phosphorylation. The Journal of biological chemistry.283(23):15619-27. Epub 2008/03/29.
[52] Kim DH, Sarbassov DD, Ali SM, Latek RR, Guntur KV, et al. (2003) GbetaL, a positive regulator of the rapamycin-sensitive pathway required for the nutrient-sensitive interaction between raptor and mTOR. Molecular cell.11(4):895-904. Epub 2003/04/30.
[53] Frias MA, Thoreen CC, Jaffe JD, Schroder W, Sculley T, et al. (2006) mSin1 is necessary for Akt/PKB phosphorylation, and its isoforms define three distinct mTORC2s. Current biology : CB.16(18):1865-70. Epub 2006/08/22.
[54] Pearce LR, Huang X, Boudeau J, Pawlowski R, Wullschleger S, et al. (2007) Identification of Protor as a novel Rictor-binding component of mTOR complex-2. The Biochemical journal.405(3):513-22. Epub 2007/04/28.
[55] Woo SY, Kim DH, Jun CB, Kim YM, Haar EV, et al. (2007) PRR5, a novel component of mTOR complex 2, regulates platelet-derived growth factor receptor beta expression and signaling. The Journal of biological chemistry.282(35):25604-12. Epub 2007/06/30.
[56] Sarbassov DD, Ali SM, Sengupta S, Sheen JH, Hsu PP, et al. (2006) Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. Molecular cell.22(2):159-68. Epub 2006/04/11.
[57] Vander Haar E, Lee SI, Bandhakavi S, Griffin TJ, Kim DH. (2007) Insulin signalling to mTOR mediated by the Akt/PKB substrate PRAS40. Nature cell biology.9(3):316-23. Epub 2007/02/06.

[58] Jacinto E, Facchinetti V, Liu D, Soto N, Wei S, et al. (2006) SIN1/MIP1 maintains rictor-mTOR complex integrity and regulates Akt phosphorylation and substrate specificity. Cell.127(1):125-37. Epub 2006/09/12.
[59] Yang Q, Inoki K, Ikenoue T, Guan KL. (2006) Identification of Sin1 as an essential TORC2 component required for complex formation and kinase activity. Genes & development.20(20):2820-32. Epub 2006/10/18.
[60] Wilkinson MG, Pino TS, Tournier S, Buck V, Martin H, et al. (1999) Sin1: an evolutionarily conserved component of the eukaryotic SAPK pathway. The EMBO journal.18(15):4210-21. Epub 1999/08/03.
[61] Schroder WA, Buck M, Cloonan N, Hancock JF, Suhrbier A, et al. (2007) Human Sin1 contains Ras-binding and pleckstrin homology domains and suppresses Ras signalling. Cellular signalling.19(6):1279-89. Epub 2007/02/17.
[62] Yang Q, Guan KL. (2007) Expanding mTOR signaling. Cell research.17(8):666-81. Epub 2007/08/08.
[63] Thedieck K, Polak P, Kim ML, Molle KD, Cohen A, et al. (2007) PRAS40 and PRR5-like protein are new mTOR interactors that regulate apoptosis. PloS one.2(11):e1217. Epub 2007/11/22.
[64] Tee AR, Fingar DC, Manning BD, Kwiatkowski DJ, Cantley LC, et al. (2002) Tuberous sclerosis complex-1 and -2 gene products function together to inhibit mammalian target of rapamycin (mTOR)-mediated downstream signaling. Proceedings of the National Academy of Sciences of the United States of America.99(21):13571-6. Epub 2002/09/25.
[65] Manning BD, Cantley LC. (2003) United at last: the tuberous sclerosis complex gene products connect the phosphoinositide 3-kinase/Akt pathway to mammalian target of rapamycin (mTOR) signalling. Biochemical Society transactions.31(Pt 3):573-8. Epub 2003/05/30.
[66] Kandt RS, Haines JL, Smith M, Northrup H, Gardner RJ, et al. (1992) Linkage of an important gene locus for tuberous sclerosis to a chromosome 16 marker for polycystic kidney disease. Nature genetics.2(1):37-41. Epub 1992/09/01.
[67] Consortium ECTS. (1993) Identification and characterization of the tuberous sclerosis gene on chromosome 16. Cell.75(7):1305-15. Epub 1993/12/31.
[68] van Slegtenhorst M, de Hoogt R, Hermans C, Nellist M, Janssen B, et al. (1997) Identification of the tuberous sclerosis gene TSC1 on chromosome 9q34. Science.277(5327):805-8. Epub 1997/08/08.
[69] Hodges AK, Li S, Maynard J, Parry L, Braverman R, et al. (2001) Pathological mutations in TSC1 and TSC2 disrupt the interaction between hamartin and tuberin. Human molecular genetics.10(25):2899-905. Epub 2001/12/14.
[70] Garami A, Zwartkruis FJ, Nobukuni T, Joaquin M, Roccio M, et al. (2003) Insulin activation of Rheb, a mediator of mTOR/S6K/4E-BP signaling, is inhibited by TSC1 and 2. Molecular cell.11(6):1457-66. Epub 2003/06/25.
[71] Benvenuto G, Li S, Brown SJ, Braverman R, Vass WC, et al. (2000) The tuberous sclerosis-1 (TSC1) gene product hamartin suppresses cell growth and augments the expression of the TSC2 product tuberin by inhibiting its ubiquitination. Oncogene.19(54):6306-16. Epub 2001/02/15.

[72] Chong-Kopera H, Inoki K, Li Y, Zhu T, Garcia-Gonzalo FR, et al. (2006) TSC1 stabilizes TSC2 by inhibiting the interaction between TSC2 and the HERC1 ubiquitin ligase. The Journal of biological chemistry.281(13):8313-6. Epub 2006/02/09.
[73] Tee AR, Blenis J, Proud CG. (2005) Analysis of mTOR signaling by the small G-proteins, Rheb and RhebL1. FEBS letters.579(21):4763-8. Epub 2005/08/16.
[74] Long X, Lin Y, Ortiz-Vega S, Yonezawa K, Avruch J. (2005) Rheb binds and regulates the mTOR kinase. Current biology : CB.15(8):702-13. Epub 2005/04/28.
[75] Bai X, Ma D, Liu A, Shen X, Wang QJ, et al. (2007) Rheb activates mTOR by antagonizing its endogenous inhibitor, FKBP38. Science.318(5852):977-80. Epub 2007/11/10.
[76] Rosner M, Hofer K, Kubista M, Hengstschlager M. (2003) Cell size regulation by the human TSC tumor suppressor proteins depends on PI3K and FKBP38. Oncogene.22(31):4786-98. Epub 2003/08/02.
[77] Manning BD, Cantley LC. (2007) AKT/PKB signaling: navigating downstream. Cell.129(7):1261-74. Epub 2007/07/03.
[78] Zhao L, Vogt PK. (2010) Hot-spot mutations in p110alpha of phosphatidylinositol 3-kinase (pI3K): differential interactions with the regulatory subunit p85 and with RAS. Cell Cycle.9(3):596-600. Epub 2009/12/17.
[79] Franke TF, Kaplan DR, Cantley LC, Toker A. (1997) Direct regulation of the Akt proto-oncogene product by phosphatidylinositol-3,4-bisphosphate. Science.275(5300):665-8. Epub 1997/01/31.
[80] Martelli AM, Evangelisti C, Chiarini F, Grimaldi C, Cappellini A, et al. (2010) The emerging role of the phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin signaling network in normal myelopoiesis and leukemogenesis. Biochimica et biophysica acta.1803(9):991-1002. Epub 2010/04/20.
[81] McManus EJ, Collins BJ, Ashby PR, Prescott AR, Murray-Tait V, et al. (2004) The in vivo role of PtdIns(3,4,5)P3 binding to PDK1 PH domain defined by knockin mutation. The EMBO journal.23(10):2071-82. Epub 2004/04/30.
[82] Hay N. (2005) The Akt-mTOR tango and its relevance to cancer. Cancer Cell.8(3):179-83. Epub 2005/09/20.
[83] Toker A, Newton AC. (2000) Cellular signaling: pivoting around PDK-1. Cell.103(2):185-8. Epub 2000/11/01.
[84] Guertin DA, Stevens DM, Thoreen CC, Burds AA, Kalaany NY, et al. (2006) 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. Developmental cell.11(6):859-71. Epub 2006/12/05.
[85] Sarbassov DD, Guertin DA, Ali SM, Sabatini DM. (2005) Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. Science.307(5712):1098-101. Epub 2005/02/19.
[86] Potter CJ, Pedraza LG, Xu T. (2002) Akt regulates growth by directly phosphorylating Tsc2. Nature cell biology.4(9):658-65. Epub 2002/08/13.
[87] Inoki K, Li Y, Zhu T, Wu J, Guan KL. (2002) TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. Nature cell biology.4(9):648-57. Epub 2002/08/13.
[88] Dan HC, Sun M, Yang L, Feldman RI, Sui XM, et al. (2002) Phosphatidylinositol 3-kinase/Akt pathway regulates tuberous sclerosis tumor suppressor complex by

phosphorylation of tuberin. The Journal of biological chemistry.277(38):35364-70. Epub 2002/08/09.

[89] Ballif BA, Roux PP, Gerber SA, MacKeigan JP, Blenis J, et al. (2005) Quantitative phosphorylation profiling of the ERK/p90 ribosomal S6 kinase-signaling cassette and its targets, the tuberous sclerosis tumor suppressors. Proceedings of the National Academy of Sciences of the United States of America.102(3):667-72. Epub 2005/01/14.

[90] Gao X, Zhang Y, Arrazola P, Hino O, Kobayashi T, et al. (2002) Tsc tumour suppressor proteins antagonize amino-acid-TOR signalling. Nature cell biology.4(9):699-704. Epub 2002/08/13.

[91] Tee AR, Proud CG. (2002) Caspase cleavage of initiation factor 4E-binding protein 1 yields a dominant inhibitor of cap-dependent translation and reveals a novel regulatory motif. Mol Cell Biol.22(6):1674-83. Epub 2002/02/28.

[92] Huang J, Manning BD. (2008) The TSC1-TSC2 complex: a molecular switchboard controlling cell growth. The Biochemical journal.412(2):179-90. Epub 2008/05/10.

[93] Cai SL, Tee AR, Short JD, Bergeron JM, Kim J, et al. (2006) Activity of TSC2 is inhibited by AKT-mediated phosphorylation and membrane partitioning. The Journal of cell biology.173(2):279-89. Epub 2006/04/26.

[94] Nellist M, Goedbloed MA, de Winter C, Verhaaf B, Jankie A, et al. (2002) Identification and characterization of the interaction between tuberin and 14-3-3zeta. The Journal of biological chemistry.277(42):39417-24. Epub 2002/08/15.

[95] Shumway SD, Li Y, Xiong Y. (2003) 14-3-3beta binds to and negatively regulates the tuberous sclerosis complex 2 (TSC2) tumor suppressor gene product, tuberin. The Journal of biological chemistry.278(4):2089-92. Epub 2002/12/07.

[96] Dong J, Pan D. (2004) Tsc2 is not a critical target of Akt during normal Drosophila development. Genes & development.18(20):2479-84. Epub 2004/10/07.

[97] Wang YH, Huang ML. (2009) Reduction of Lobe leads to TORC1 hypoactivation that induces ectopic Jak/STAT signaling to impair Drosophila eye development. Mechanisms of development.126(10):781-90. Epub 2009/09/08.

[98] Jacinto E, Hall MN. (2003) Tor signalling in bugs, brain and brawn. Nature reviews Molecular cell biology.4(2):117-26. Epub 2003/02/04.

[99] Long X, Ortiz-Vega S, Lin Y, Avruch J. (2005) Rheb binding to mammalian target of rapamycin (mTOR) is regulated by amino acid sufficiency. The Journal of biological chemistry.280(25):23433-6. Epub 2005/05/10.

[100] Smith EM, Finn SG, Tee AR, Browne GJ, Proud CG. (2005) The tuberous sclerosis protein TSC2 is not required for the regulation of the mammalian target of rapamycin by amino acids and certain cellular stresses. The Journal of biological chemistry.280(19):18717-27. Epub 2005/03/18.

[101] Wang X, Proud CG. (2009) Nutrient control of TORC1, a cell-cycle regulator. Trends in cell biology.19(6):260-7. Epub 2009/05/08.

[102] Roccio M, Bos JL, Zwartkruis FJ. (2006) Regulation of the small GTPase Rheb by amino acids. Oncogene.25(5):657-64. Epub 2005/09/20.

[103] Byfield MP, Murray JT, Backer JM. (2005) hVps34 is a nutrient-regulated lipid kinase required for activation of p70 S6 kinase. The Journal of biological chemistry.280(38):33076-82. Epub 2005/07/29.

[104] Nobukuni T, Joaquin M, Roccio M, Dann SG, Kim SY, et al. (2005) Amino acids mediate mTOR/raptor signaling through activation of class 3 phosphatidylinositol 3OH-kinase. Proceedings of the National Academy of Sciences of the United States of America.102(40):14238-43. Epub 2005/09/24.

[105] Findlay GM, Yan L, Procter J, Mieulet V, Lamb RF. (2007) A MAP4 kinase related to Ste20 is a nutrient-sensitive regulator of mTOR signalling. The Biochemical journal.403(1):13-20. Epub 2007/01/27.

[106] Juhasz G, Hill JH, Yan Y, Sass M, Baehrecke EH, et al. (2008) The class III PI(3)K Vps34 promotes autophagy and endocytosis but not TOR signaling in Drosophila. The Journal of cell biology.181(4):655-66. Epub 2008/05/14.

[107] Yan L, Mieulet V, Burgess D, Findlay GM, Sully K, et al. (2010) PP2A T61 epsilon is an inhibitor of MAP4K3 in nutrient signaling to mTOR. Molecular cell.37(5):633-42. Epub 2010/03/17.

[108] Gulati P, Gaspers LD, Dann SG, Joaquin M, Nobukuni T, et al. (2008) Amino acids activate mTOR complex 1 via Ca2+/CaM signaling to hVps34. Cell metabolism.7(5):456-65. Epub 2008/05/08.

[109] Backer JM. (2008) The regulation and function of Class III PI3Ks: novel roles for Vps34. The Biochemical journal.410(1):1-17. Epub 2008/01/25.

[110] Tassa A, Roux MP, Attaix D, Bechet DM. (2003) Class III phosphoinositide 3-kinase--Beclin1 complex mediates the amino acid-dependent regulation of autophagy in C2C12 myotubes. The Biochemical journal.376(Pt 3):577-86. Epub 2003/09/12.

[111] Sancak Y, Peterson TR, Shaul YD, Lindquist RA, Thoreen CC, et al. (2008) The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1. Science.320(5882):1496-501. Epub 2008/05/24.

[112] Kim E, Goraksha-Hicks P, Li L, Neufeld TP, Guan KL. (2008) Regulation of TORC1 by Rag GTPases in nutrient response. Nature cell biology.10(8):935-45. Epub 2008/07/08.

[113] Exton JH. (2002) Phospholipase D-structure, regulation and function. Reviews of physiology, biochemistry and pharmacology.144:1-94. Epub 2002/05/04.

[114] Foster DA. (2007) Regulation of mTOR by phosphatidic acid? Cancer research.67(1):1-4. Epub 2007/01/11.

[115] Fang Y, Vilella-Bach M, Bachmann R, Flanigan A, Chen J. (2001) Phosphatidic acid-mediated mitogenic activation of mTOR signaling. Science.294(5548):1942-5. Epub 2001/12/01.

[116] Hornberger TA, Chu WK, Mak YW, Hsiung JW, Huang SA, et al. (2006) The role of phospholipase D and phosphatidic acid in the mechanical activation of mTOR signaling in skeletal muscle. Proceedings of the National Academy of Sciences of the United States of America.103(12):4741-6. Epub 2006/03/16.

[117] Takahara T, Hara K, Yonezawa K, Sorimachi H, Maeda T. (2006) Nutrient-dependent multimerization of the mammalian target of rapamycin through the N-terminal HEAT repeat region. The Journal of biological chemistry.281(39):28605-14. Epub 2006/07/28.

[118] Park IH, Bachmann R, Shirazi H, Chen J. (2002) Regulation of ribosomal S6 kinase 2 by mammalian target of rapamycin. The Journal of biological chemistry.277(35):31423-9. Epub 2002/06/28.

[119] Lim HK, Choi YA, Park W, Lee T, Ryu SH, et al. (2003) Phosphatidic acid regulates systemic inflammatory responses by modulating the Akt-mammalian target of

rapamycin-p70 S6 kinase 1 pathway. The Journal of biological chemistry.278(46):45117-27. Epub 2003/09/10.

[120] Chen Y, Rodrik V, Foster DA. (2005) Alternative phospholipase D/mTOR survival signal in human breast cancer cells. Oncogene.24(4):672-9. Epub 2004/12/08.

[121] Hui L, Abbas T, Pielak RM, Joseph T, Bargonetti J, et al. (2004) Phospholipase D elevates the level of MDM2 and suppresses DNA damage-induced increases in p53. Mol Cell Biol.24(13):5677-86. Epub 2004/06/17.

[122] Ohguchi K, Banno Y, Nakagawa Y, Akao Y, Nozawa Y. (2005) Negative regulation of melanogenesis by phospholipase D1 through mTOR/p70 S6 kinase 1 signaling in mouse B16 melanoma cells. Journal of cellular physiology.205(3):444-51. Epub 2005/05/17.

[123] Fukami K, Takenawa T. (1992) Phosphatidic acid that accumulates in platelet-derived growth factor-stimulated Balb/c 3T3 cells is a potential mitogenic signal. The Journal of biological chemistry.267(16):10988-93. Epub 1992/06/05.

[124] Sun Y, Fang Y, Yoon MS, Zhang C, Roccio M, et al. (2008) Phospholipase D1 is an effector of Rheb in the mTOR pathway. Proceedings of the National Academy of Sciences of the United States of America.105(24):8286-91. Epub 2008/06/14.

[125] Ha SH, Kim DH, Kim IS, Kim JH, Lee MN, et al. (2006) PLD2 forms a functional complex with mTOR/raptor to transduce mitogenic signals. Cellular signalling.18(12):2283-91. Epub 2006/07/14.

[126] Toschi A, Lee E, Xu L, Garcia A, Gadir N, et al. (2009) Regulation of mTORC1 and mTORC2 complex assembly by phosphatidic acid: competition with rapamycin. Mol Cell Biol.29(6):1411-20. Epub 2008/12/31.

[127] Xu L, Salloum D, Medlin PS, Saqcena M, Yellen P, et al. (2011) Phospholipase D mediates nutrient input to mammalian target of rapamycin complex 1 (mTORC1). The Journal of biological chemistry.286(29):25477-86. Epub 2011/05/31.

[128] Frohman MA, Morris AJ. (1999) Phospholipase D structure and regulation. Chemistry and physics of lipids.98(1-2):127-40. Epub 1999/06/08.

[129] Knizhnik AV, Kovaleva OV, Komelkov AV, Trukhanova LS, Rybko VA, et al. (2012) Arf6 promotes cell proliferation via the PLD-mTORC1 and p38MAPK pathways. Journal of cellular biochemistry.113(1):360-71. Epub 2011/09/20.

[130] Hardie DG. (2007) AMP-activated/SNF1 protein kinases: conserved guardians of cellular energy. Nature reviews Molecular cell biology.8(10):774-85. Epub 2007/08/23.

[131] Kahn BB, Alquier T, Carling D, Hardie DG. (2005) AMP-activated protein kinase: ancient energy gauge provides clues to modern understanding of metabolism. Cell metabolism.1(1):15-25. Epub 2005/08/02.

[132] Shaw RJ. (2009) LKB1 and AMP-activated protein kinase control of mTOR signalling and growth. Acta Physiol (Oxf).196(1):65-80. Epub 2009/02/28.

[133] Sanders MJ, Grondin PO, Hegarty BD, Snowden MA, Carling D. (2007) Investigating the mechanism for AMP activation of the AMP-activated protein kinase cascade. The Biochemical journal.403(1):139-48. Epub 2006/12/07.

[134] Oakhill JS, Steel R, Chen ZP, Scott JW, Ling N, et al. (2011) AMPK is a direct adenylate charge-regulated protein kinase. Science.332(6036):1433-5. Epub 2011/06/18.

[135] Xiao B, Sanders MJ, Underwood E, Heath R, Mayer FV, et al. (2011) Structure of mammalian AMPK and its regulation by ADP. Nature.472(7342):230-3. Epub 2011/03/15.

[136] Hawley SA, Pan DA, Mustard KJ, Ross L, Bain J, et al. (2005) Calmodulin-dependent protein kinase kinase-beta is an alternative upstream kinase for AMP-activated protein kinase. Cell metabolism.2(1):9-19. Epub 2005/08/02.

[137] Zhang Y, Gao X, Saucedo LJ, Ru B, Edgar BA, et al. (2003) Rheb is a direct target of the tuberous sclerosis tumour suppressor proteins. Nature cell biology.5(6):578-81. Epub 2003/05/29.

[138] Inoki K, Ouyang H, Zhu T, Lindvall C, Wang Y, et al. (2006) TSC2 integrates Wnt and energy signals via a coordinated phosphorylation by AMPK and GSK3 to regulate cell growth. Cell.126(5):955-68. Epub 2006/09/09.

[139] Arsham AM, Howell JJ, Simon MC. (2003) A novel hypoxia-inducible factor-independent hypoxic response regulating mammalian target of rapamycin and its targets. The Journal of biological chemistry.278(32):29655-60. Epub 2003/06/05.

[140] Schneider A, Younis RH, Gutkind JS. (2008) Hypoxia-induced energy stress inhibits the mTOR pathway by activating an AMPK/REDD1 signaling axis in head and neck squamous cell carcinoma. Neoplasia.10(11):1295-302. Epub 2008/10/28.

[141] Liu L, Cash TP, Jones RG, Keith B, Thompson CB, et al. (2006) Hypoxia-induced energy stress regulates mRNA translation and cell growth. Molecular cell.21(4):521-31. Epub 2006/02/18.

[142] Brugarolas J, Lei K, Hurley RL, Manning BD, Reiling JH, et al. (2004) Regulation of mTOR function in response to hypoxia by REDD1 and the TSC1/TSC2 tumor suppressor complex. Genes & development.18(23):2893-904. Epub 2004/11/17.

[143] Sofer A, Lei K, Johannessen CM, Ellisen LW. (2005) Regulation of mTOR and cell growth in response to energy stress by REDD1. Mol Cell Biol.25(14):5834-45. Epub 2005/07/01.

[144] DeYoung MP, Horak P, Sofer A, Sgroi D, Ellisen LW. (2008) Hypoxia regulates TSC1/2-mTOR signaling and tumor suppression through REDD1-mediated 14-3-3 shuttling. Genes & development.22(2):239-51. Epub 2008/01/17.

[145] Corradetti MN, Inoki K, Guan KL. (2005) The stress-inducted proteins RTP801 and RTP801L are negative regulators of the mammalian target of rapamycin pathway. The Journal of biological chemistry.280(11):9769-72. Epub 2005/01/06.

[146] Schwarzer R, Tondera D, Arnold W, Giese K, Klippel A, et al. (2005) REDD1 integrates hypoxia-mediated survival signaling downstream of phosphatidylinositol 3-kinase. Oncogene.24(7):1138-49. Epub 2004/12/14.

[147] Bernardi R, Guernah I, Jin D, Grisendi S, Alimonti A, et al. (2006) PML inhibits HIF-1alpha translation and neoangiogenesis through repression of mTOR. Nature.442(7104):779-85. Epub 2006/08/18.

[148] Li Y, Wang Y, Kim E, Beemiller P, Wang CY, et al. (2007) Bnip3 mediates the hypoxia-induced inhibition on mammalian target of rapamycin by interacting with Rheb. The Journal of biological chemistry.282(49):35803-13. Epub 2007/10/12.

[149] Wouters BG, Koritzinsky M. (2008) Hypoxia signalling through mTOR and the unfolded protein response in cancer. Nature reviews Cancer.8(11):851-64. Epub 2008/10/11.

[150] Laplante M, Sabatini DM. (2009) An emerging role of mTOR in lipid biosynthesis. Current biology : CB.19(22):R1046-52. Epub 2009/12/02.

[151] Averous J, Proud CG. (2006) When translation meets transformation: the mTOR story. Oncogene.25(48):6423-35. Epub 2006/10/17.

[152] Fingar DC, Blenis J. (2004) Target of rapamycin (TOR): an integrator of nutrient and growth factor signals and coordinator of cell growth and cell cycle progression. Oncogene.23(18):3151-71. Epub 2004/04/20.

[153] Gingras AC, Raught B, Sonenberg N. (1999) eIF4 initiation factors: effectors of mRNA recruitment to ribosomes and regulators of translation. Annual review of biochemistry.68:913-63. Epub 2000/06/29.

[154] Nielsen FC, Ostergaard L, Nielsen J, Christiansen J. (1995) Growth-dependent translation of IGF-II mRNA by a rapamycin-sensitive pathway. Nature.377(6547):358-62. Epub 1995/09/28.

[155] Rogers GW, Jr., Komar AA, Merrick WC. (2002) eIF4A: the godfather of the DEAD box helicases. Progress in nucleic acid research and molecular biology.72:307-31. Epub 2002/09/11.

[156] Joshi B, Cai AL, Keiper BD, Minich WB, Mendez R, et al. (1995) Phosphorylation of eukaryotic protein synthesis initiation factor 4E at Ser-209. The Journal of biological chemistry.270(24):14597-603. Epub 1995/06/16.

[157] Gingras AC, Raught B, Gygi SP, Niedzwiecka A, Miron M, et al. (2001) Hierarchical phosphorylation of the translation inhibitor 4E-BP1. Genes & development.15(21):2852-64. Epub 2001/11/03.

[158] Wang X, Beugnet A, Murakami M, Yamanaka S, Proud CG. (2005) Distinct signaling events downstream of mTOR cooperate to mediate the effects of amino acids and insulin on initiation factor 4E-binding proteins. Mol Cell Biol.25(7):2558-72. Epub 2005/03/16.

[159] Ferguson G, Mothe-Satney I, Lawrence JC, Jr. (2003) Ser-64 and Ser-111 in PHAS-I are dispensable for insulin-stimulated dissociation from eIF4E. The Journal of biological chemistry.278(48):47459-65. Epub 2003/09/26.

[160] Tomoo K, Matsushita Y, Fujisaki H, Abiko F, Shen X, et al. (2005) Structural basis for mRNA Cap-Binding regulation of eukaryotic initiation factor 4E by 4E-binding protein, studied by spectroscopic, X-ray crystal structural, and molecular dynamics simulation methods. Biochimica et biophysica acta.1753(2):191-208. Epub 2005/11/08.

[161] Herbert TP, Tee AR, Proud CG. (2002) The extracellular signal-regulated kinase pathway regulates the phosphorylation of 4E-BP1 at multiple sites. The Journal of biological chemistry.277(13):11591-6. Epub 2002/01/19.

[162] Wang X, Li W, Parra JL, Beugnet A, Proud CG. (2003) The C terminus of initiation factor 4E-binding protein 1 contains multiple regulatory features that influence its function and phosphorylation. Mol Cell Biol.23(5):1546-57. Epub 2003/02/18.

[163] Beugnet A, Wang X, Proud CG. (2003) Target of rapamycin (TOR)-signaling and RAIP motifs play distinct roles in the mammalian TOR-dependent phosphorylation of initiation factor 4E-binding protein 1. The Journal of biological chemistry.278(42):40717-22. Epub 2003/08/13.

[164] Avruch J, Belham C, Weng Q, Hara K, Yonezawa K. (2001) The p70 S6 kinase integrates nutrient and growth signals to control translational capacity. Progress in molecular and subcellular biology.26:115-54. Epub 2001/09/29.

[165] Roux PP, Blenis J. (2004) ERK and p38 MAPK-activated protein kinases: a family of protein kinases with diverse biological functions. Microbiology and molecular biology reviews : MMBR.68(2):320-44. Epub 2004/06/10.

[166] Reinhard C, Thomas G, Kozma SC. (1992) A single gene encodes two isoforms of the p70 S6 kinase: activation upon mitogenic stimulation. Proceedings of the National Academy of Sciences of the United States of America.89(9):4052-6. Epub 1992/05/01.

[167] Shima H, Pende M, Chen Y, Fumagalli S, Thomas G, et al. (1998) Disruption of the p70(s6k)/p85(s6k) gene reveals a small mouse phenotype and a new functional S6 kinase. The EMBO journal.17(22):6649-59. Epub 1998/11/21.

[168] Price DJ, Grove JR, Calvo V, Avruch J, Bierer BE. (1992) Rapamycin-induced inhibition of the 70-kilodalton S6 protein kinase. Science.257(5072):973-7. Epub 1992/08/14.

[169] Montagne J, Stewart MJ, Stocker H, Hafen E, Kozma SC, et al. (1999) Drosophila S6 kinase: a regulator of cell size. Science.285(5436):2126-9. Epub 1999/09/25.

[170] Radimerski T, Montagne J, Rintelen F, Stocker H, van der Kaay J, et al. (2002) dS6K-regulated cell growth is dPKB/dPI(3)K-independent, but requires dPDK1. Nature cell biology.4(3):251-5. Epub 2002/02/28.

[171] Raught B, Peiretti F, Gingras AC, Livingstone M, Shahbazian D, et al. (2004) Phosphorylation of eucaryotic translation initiation factor 4B Ser422 is modulated by S6 kinases. The EMBO journal.23(8):1761-9. Epub 2004/04/09.

[172] Holz MK, Blenis J. (2005) Identification of S6 kinase 1 as a novel mammalian target of rapamycin (mTOR)-phosphorylating kinase. The Journal of biological chemistry.280(28):26089-93. Epub 2005/05/21.

[173] Pende M, Um SH, Mieulet V, Sticker M, Goss VL, et al. (2004) S6K1(-/-)/S6K2(-/-) mice exhibit perinatal lethality and rapamycin-sensitive 5'-terminal oligopyrimidine mRNA translation and reveal a mitogen-activated protein kinase-dependent S6 kinase pathway. Mol Cell Biol.24(8):3112-24. Epub 2004/04/03.

[174] Tang H, Hornstein E, Stolovich M, Levy G, Livingstone M, et al. (2001) Amino acid-induced translation of TOP mRNAs is fully dependent on phosphatidylinositol 3-kinase-mediated signaling, is partially inhibited by rapamycin, and is independent of S6K1 and rpS6 phosphorylation. Mol Cell Biol.21(24):8671-83. Epub 2001/11/20.

[175] Stolovich M, Tang H, Hornstein E, Levy G, Cohen R, et al. (2002) Transduction of growth or mitogenic signals into translational activation of TOP mRNAs is fully reliant on the phosphatidylinositol 3-kinase-mediated pathway but requires neither S6K1 nor rpS6 phosphorylation. Mol Cell Biol.22(23):8101-13. Epub 2002/11/06.

[176] Ruvinsky I, Sharon N, Lerer T, Cohen H, Stolovich-Rain M, et al. (2005) Ribosomal protein S6 phosphorylation is a determinant of cell size and glucose homeostasis. Genes & development.19(18):2199-211. Epub 2005/09/17.

[177] Yang HS, Jansen AP, Komar AA, Zheng X, Merrick WC, et al. (2003) The transformation suppressor Pdcd4 is a novel eukaryotic translation initiation factor 4A binding protein that inhibits translation. Mol Cell Biol.23(1):26-37. Epub 2002/12/17.

[178] Yang HS, Cho MH, Zakowicz H, Hegamyer G, Sonenberg N, et al. (2004) A novel function of the MA-3 domains in transformation and translation suppressor Pdcd4 is essential for its binding to eukaryotic translation initiation factor 4A. Mol Cell Biol.24(9):3894-906. Epub 2004/04/15.

[179] Peterson TR, Sabatini DM. (2005) eIF3: a connecTOR of S6K1 to the translation preinitiation complex. Molecular cell.20(5):655-7. Epub 2005/12/13.
[180] Shahbazian D, Roux PP, Mieulet V, Cohen MS, Raught B, et al. (2006) The mTOR/PI3K and MAPK pathways converge on eIF4B to control its phosphorylation and activity. The EMBO journal.25(12):2781-91. Epub 2006/06/10.
[181] Harris TE, Chi A, Shabanowitz J, Hunt DF, Rhoads RE, et al. (2006) mTOR-dependent stimulation of the association of eIF4G and eIF3 by insulin. The EMBO journal.25(8):1659-68. Epub 2006/03/17.
[182] Alessi DR, Kozlowski MT, Weng QP, Morrice N, Avruch J. (1998) 3-Phosphoinositide-dependent protein kinase 1 (PDK1) phosphorylates and activates the p70 S6 kinase in vivo and in vitro. Current biology : CB.8(2):69-81. Epub 1998/03/21.
[183] Pullen N, Dennis PB, Andjelkovic M, Dufner A, Kozma SC, et al. (1998) Phosphorylation and activation of p70s6k by PDK1. Science.279(5351):707-10. Epub 1998/02/21.
[184] Schalm SS, Blenis J. (2002) Identification of a conserved motif required for mTOR signaling. Current biology : CB.12(8):632-9. Epub 2002/04/23.
[185] Ali SM, Sabatini DM. (2005) Structure of S6 kinase 1 determines whether raptor-mTOR or rictor-mTOR phosphorylates its hydrophobic motif site. The Journal of biological chemistry.280(20):19445-8. Epub 2005/04/06.
[186] Saitoh M, Pullen N, Brennan P, Cantrell D, Dennis PB, et al. (2002) Regulation of an activated S6 kinase 1 variant reveals a novel mammalian target of rapamycin phosphorylation site. The Journal of biological chemistry.277(22):20104-12. Epub 2002/03/27.
[187] Isotani S, Hara K, Tokunaga C, Inoue H, Avruch J, et al. (1999) Immunopurified mammalian target of rapamycin phosphorylates and activates p70 S6 kinase alpha in vitro. The Journal of biological chemistry.274(48):34493-8. Epub 1999/11/24.
[188] Browne GJ, Proud CG. (2002) Regulation of peptide-chain elongation in mammalian cells. European journal of biochemistry / FEBS.269(22):5360-8. Epub 2002/11/09.
[189] Carlberg U, Nilsson A, Nygard O. (1990) Functional properties of phosphorylated elongation factor 2. European journal of biochemistry / FEBS.191(3):639-45. Epub 1990/08/17.
[190] Wang X, Li W, Williams M, Terada N, Alessi DR, et al. (2001) Regulation of elongation factor 2 kinase by p90(RSK1) and p70 S6 kinase. The EMBO journal.20(16):4370-9. Epub 2001/08/14.
[191] Ryazanov AG, Ward MD, Mendola CE, Pavur KS, Dorovkov MV, et al. (1997) Identification of a new class of protein kinases represented by eukaryotic elongation factor-2 kinase. Proceedings of the National Academy of Sciences of the United States of America.94(10):4884-9. Epub 1997/05/13.
[192] Diggle TA, Seehra CK, Hase S, Redpath NT. (1999) Analysis of the domain structure of elongation factor-2 kinase by mutagenesis. FEBS letters.457(2):189-92. Epub 1999/09/03.
[193] Browne GJ, Proud CG. (2004) A Novel mTOR-Regulated Phosphorylation Site in Elongation Factor 2 Kinase Modulates the Activity of the Kinase and Its Binding to Calmodulin. Molecular and Cellular Biology.24(7):2986-97.

[194] Knebel A, Morrice N, Cohen P. (2001) A novel method to identify protein kinase substrates: eEF2 kinase is phosphorylated and inhibited by SAPK4/p38delta. The EMBO journal.20(16):4360-9. Epub 2001/08/14.
[195] Tsang CK, Liu H, Zheng XF. (2010) mTOR binds to the promoters of RNA polymerase I- and III-transcribed genes. Cell Cycle.9(5):953-7. Epub 2009/12/30.
[196] Dobashi Y, Watanabe Y, Miwa C, Suzuki S, Koyama S. (2011) Mammalian target of rapamycin: a central node of complex signaling cascades. International journal of clinical and experimental pathology.4(5):476-95. Epub 2011/07/09.
[197] Klionsky DJ. (2007) Autophagy: from phenomenology to molecular understanding in less than a decade. Nature reviews Molecular cell biology.8(11):931-7. Epub 2007/08/23.
[198] Levine B, Klionsky DJ. (2004) Development by self-digestion: molecular mechanisms and biological functions of autophagy. Developmental cell.6(4):463-77. Epub 2004/04/08.
[199] Liu JJ, Lin M, Yu JY, Liu B, Bao JK. (2011) Targeting apoptotic and autophagic pathways for cancer therapeutics. Cancer letters.300(2):105-14. Epub 2010/11/03.
[200] Chang YY, Neufeld TP. (2009) An Atg1/Atg13 complex with multiple roles in TOR-mediated autophagy regulation. Molecular biology of the cell.20(7):2004-14. Epub 2009/02/20.
[201] Jung CH, Jun CB, Ro SH, Kim YM, Otto NM, et al. (2009) ULK-Atg13-FIP200 complexes mediate mTOR signaling to the autophagy machinery. Molecular biology of the cell.20(7):1992-2003. Epub 2009/02/20.
[202] Kamada Y, Yoshino K, Kondo C, Kawamata T, Oshiro N, et al. (2010) Tor directly controls the Atg1 kinase complex to regulate autophagy. Mol Cell Biol.30(4):1049-58. Epub 2009/12/10.
[203] Kim J, Kundu M, Viollet B, Guan KL. (2011) AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. Nature cell biology.13(2):132-41. Epub 2011/01/25.
[204] Behrends C, Sowa ME, Gygi SP, Harper JW. (2010) Network organization of the human autophagy system. Nature.466(7302):68-76. Epub 2010/06/22.
[205] Hosokawa N, Sasaki T, Iemura S, Natsume T, Hara T, et al. (2009) Atg101, a novel mammalian autophagy protein interacting with Atg13. Autophagy.5(7):973-9. Epub 2009/07/15.
[206] Shang L, Chen S, Du F, Li S, Zhao L, et al. (2011) Nutrient starvation elicits an acute autophagic response mediated by Ulk1 dephosphorylation and its subsequent dissociation from AMPK. Proceedings of the National Academy of Sciences of the United States of America.108(12):4788-93. Epub 2011/03/09.
[207] Joo JH, Dorsey FC, Joshi A, Hennessy-Walters KM, Rose KL, et al. (2011) Hsp90-Cdc37 chaperone complex regulates Ulk1- and Atg13-mediated mitophagy. Molecular cell.43(4):572-85. Epub 2011/08/23.
[208] Itakura E, Mizushima N. (2010) Characterization of autophagosome formation site by a hierarchical analysis of mammalian Atg proteins. Autophagy.6(6):764-76. Epub 2010/07/20.
[209] Jung CH, Seo M, Otto NM, Kim DH. (2011) ULK1 inhibits the kinase activity of mTORC1 and cell proliferation. Autophagy.7(10):1212-21. Epub 2011/07/29.

[210] Dunlop EA, Hunt DK, Acosta-Jaquez HA, Fingar DC, Tee AR. (2011) ULK1 inhibits mTORC1 signaling, promotes multisite Raptor phosphorylation and hinders substrate binding. Autophagy.7(7):737-47. Epub 2011/04/05.
[211] Efeyan A, Sabatini DM. (2010) mTOR and cancer: many loops in one pathway. Current opinion in cell biology.22(2):169-76. Epub 2009/12/01.
[212] Egan DF, Shackelford DB, Mihaylova MM, Gelino S, Kohnz RA, et al. (2011) Phosphorylation of ULK1 (hATG1) by AMP-activated protein kinase connects energy sensing to mitophagy. Science.331(6016):456-61. Epub 2011/01/06.
[213] Lee SB, Kim S, Lee J, Park J, Lee G, et al. (2007) ATG1, an autophagy regulator, inhibits cell growth by negatively regulating S6 kinase. EMBO reports.8(4):360-5. Epub 2007/03/10.
[214] Lee S, Comer FI, Sasaki A, McLeod IX, Duong Y, et al. (2005) TOR complex 2 integrates cell movement during chemotaxis and signal relay in Dictyostelium. Molecular biology of the cell.16(10):4572-83. Epub 2005/08/05.
[215] Feng J, Park J, Cron P, Hess D, Hemmings BA. (2004) Identification of a PKB/Akt hydrophobic motif Ser-473 kinase as DNA-dependent protein kinase. The Journal of biological chemistry.279(39):41189-96. Epub 2004/07/21.
[216] Guertin DA, Sabatini DM. (2007) Defining the role of mTOR in cancer. Cancer Cell.12(1):9-22. Epub 2007/07/07.
[217] Pearce LR, Komander D, Alessi DR. (2010) The nuts and bolts of AGC protein kinases. Nature reviews Molecular cell biology.11(1):9-22. Epub 2009/12/23.
[218] Schaller MD. (2001) Paxillin: a focal adhesion-associated adaptor protein. Oncogene.20(44):6459-72. Epub 2001/10/19.
[219] Etienne-Manneville S, Hall A. (2002) Rho GTPases in cell biology. Nature.420(6916):629-35. Epub 2002/12/13.
[220] Oh WJ, Wu CC, Kim SJ, Facchinetti V, Julien LA, et al. (2010) mTORC2 can associate with ribosomes to promote cotranslational phosphorylation and stability of nascent Akt polypeptide. The EMBO journal.29(23):3939-51. Epub 2010/11/04.
[221] Roberts PJ, Der CJ. (2007) Targeting the Raf-MEK-ERK mitogen-activated protein kinase cascade for the treatment of cancer. Oncogene.26(22):3291-310. Epub 2007/05/15.
[222] Murphy LO, Blenis J. (2006) MAPK signal specificity: the right place at the right time. Trends in biochemical sciences.31(5):268-75. Epub 2006/04/11.
[223] Dalby KN, Morrice N, Caudwell FB, Avruch J, Cohen P. (1998) Identification of regulatory phosphorylation sites in mitogen-activated protein kinase (MAPK)-activated protein kinase-1a/p90rsk that are inducible by MAPK. The Journal of biological chemistry.273(3):1496-505. Epub 1998/01/27.
[224] Anjum R, Blenis J. (2008) The RSK family of kinases: emerging roles in cellular signalling. Nature reviews Molecular cell biology.9(10):747-58. Epub 2008/09/25.
[225] Roux PP, Ballif BA, Anjum R, Gygi SP, Blenis J. (2004) Tumor-promoting phorbol esters and activated Ras inactivate the tuberous sclerosis tumor suppressor complex via p90 ribosomal S6 kinase. Proceedings of the National Academy of Sciences of the United States of America.101(37):13489-94. Epub 2004/09/03.
[226] Ma L, Chen Z, Erdjument-Bromage H, Tempst P, Pandolfi PP. (2005) Phosphorylation and functional inactivation of TSC2 by Erk implications for tuberous sclerosis and cancer pathogenesis. Cell.121(2):179-93. Epub 2005/04/27.

[227] Ma L, Teruya-Feldstein J, Bonner P, Bernardi R, Franz DN, et al. (2007) Identification of S664 TSC2 phosphorylation as a marker for extracellular signal-regulated kinase mediated mTOR activation in tuberous sclerosis and human cancer. Cancer research.67(15):7106-12. Epub 2007/08/03.

[228] Blenis J, Chung J, Erikson E, Alcorta DA, Erikson RL. (1991) Distinct mechanisms for the activation of the RSK kinases/MAP2 kinase/pp90rsk and pp70-S6 kinase signaling systems are indicated by inhibition of protein synthesis. Cell growth & differentiation : the molecular biology journal of the American Association for Cancer Research.2(6):279-85. Epub 1991/06/01.

[229] Chung J, Kuo CJ, Crabtree GR, Blenis J. (1992) Rapamycin-FKBP specifically blocks growth-dependent activation of and signaling by the 70 kd S6 protein kinases. Cell.69(7):1227-36. Epub 1992/06/26.

[230] Roux PP, Shahbazian D, Vu H, Holz MK, Cohen MS, et al. (2007) RAS/ERK signaling promotes site-specific ribosomal protein S6 phosphorylation via RSK and stimulates cap-dependent translation. The Journal of biological chemistry.282(19):14056-64. Epub 2007/03/16.

[231] Magnusson C, Vaux DL. (1999) Signalling by CD95 and TNF receptors: not only life and death. Immunology and cell biology.77(1):41-6. Epub 1999/04/02.

[232] Karin M. (2008) The IkappaB kinase - a bridge between inflammation and cancer. Cell research.18(3):334-42. Epub 2008/02/28.

[233] Glantschnig H, Fisher JE, Wesolowski G, Rodan GA, Reszka AA. (2003) M-CSF, TNFalpha and RANK ligand promote osteoclast survival by signaling through mTOR/S6 kinase. Cell death and differentiation.10(10):1165-77. Epub 2003/09/23.

[234] Lee DF, Kuo HP, Chen CT, Hsu JM, Chou CK, et al. (2007) IKK beta suppression of TSC1 links inflammation and tumor angiogenesis via the mTOR pathway. Cell.130(3):440-55. Epub 2007/08/19.

[235] Dan HC, Adli M, Baldwin AS. (2007) Regulation of mammalian target of rapamycin activity in PTEN-inactive prostate cancer cells by I kappa B kinase alpha. Cancer research.67(13):6263-9. Epub 2007/07/10.

[236] Dan HC, Baldwin AS. (2008) Differential involvement of IkappaB kinases alpha and beta in cytokine- and insulin-induced mammalian target of rapamycin activation determined by Akt. J Immunol.180(11):7582-9. Epub 2008/05/21.