

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800

Open access books available

122,000

International authors and editors

135M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



# The Target of Rapamycin: Structure and Functions

Chang-Chih Wu, Po-Chien Chou and Estela Jacinto\*

*Department of Physiology and Biophysics,  
UMDNJ-Robert Wood Johnson Medical School, Piscataway, Piscataway, NJ  
USA*

## 1. Introduction

The target of rapamycin (TOR, also called the mechanistic or mammalian target of rapamycin, mTOR) is an atypical protein kinase that is highly conserved in eukaryotes (Sarbasov et al. 2005; Wullschleger et al. 2006; Jacinto & Lorberg 2008). It modulates cell growth, metabolism, and cell survival in response to diverse extracellular and intracellular signals, such as growth factors, energy levels, and nutrient status (Reiling & Sabatini 2006; Wullschleger et al. 2006; Jacinto 2008). Inhibition of mTOR activity using rapamycin and more recently via mTOR active site inhibitors and disruption of mTOR complexes, has revealed important insights on how mTOR functions under physiological and pathological conditions (Sarbasov et al. 2005; Proud 2011; Zoncu et al. 2011).

TOR was first identified as the target of rapamycin, a potent antifungal macrolide originally purified from *Streptomyces hygroscopicus* in an Easter Island soil sample in 1975 (Sehgal et al. 1975; Vezina et al. 1975). This natural compound was later found to possess immunosuppressive and growth inhibitory properties on mammalian cells (Hall 1996; Thomas & Hall 1997; Young & Nickerson-Nutter 2005). A genetic screen in the budding yeast, *Saccharomyces cerevisiae*, identified three genes that conferred rapamycin resistance upon mutation. These genes include *TOR1*, *TOR2*, and *FPR1* (Heitman et al. 1991). Whereas *TOR1* and *TOR2* are relatively large proteins (around 300 kDa) and display homology to lipid kinases, *FPR1* (also called FKBP12) is a small protein (about 12 kDa) that has cis-trans prolyl isomerase activity (Helliwell et al. 1994; Kunz et al. 2000). The activity of TOR/mTOR becomes inhibited by the complex formed by rapamycin and FKBP12. TOR orthologues were also discovered in mammalian cells (mTOR) and other higher eukaryotes (eg *C. elegans* TOR, CeTOR; *Drosophila* TOR, dTOR; *Arabidopsis thaliana* TOR, At TOR) (Brown et al. 1994; Oldham et al. 2000; Long et al. 2002; Menand et al. 2002). In this chapter, we will review the conserved structures of TOR, the regulation of the mTOR pathway, and summarize its conserved cellular functions. We also discuss the value of targeting mTOR function in therapeutic strategies.

## 2. Structure, conserved versus divergent sequences in TOR

TOR/mTOR is encoded by a single gene in most organisms although in some yeasts there are two *TOR* genes. The encoded proteins share about 40~60% identity in amino acid

sequence among different species (Wullschleger et al. 2006). TOR belongs to the phosphatidylinositol-3 kinase-related kinase (PIKK) family, a subgroup of atypical protein kinases (Hanks & Hunter 1995; Manning et al. 2002; Miranda-Saavedra & Barton 2007). PIKKs are conserved from yeasts to mammals and have numerous functions in stress responses including DNA repair, transcription, and mRNA decay (Keith & Schreiber 1995). The PIKKs share some homology in the catalytic domain with lipid kinases including phosphatidylinositol-3 kinases (PI3Ks) (Keith & Schreiber 1995; Manning et al. 2002), but they possess serine/threonine kinase activity (Figure 1). The large size of PIKK family members (from 280 to 470 kDa) has been a major obstacle in studying the structure of these molecules (Knutson 2010). In general, these kinases are roughly defined by an  $\alpha$ -helical N-terminal region and a catalytic C-terminal region (Choi et al. 1996; Lempiainen & Halazonetis 2009). From the structural prediction of amino acid sequences, PIKK family members contain HEAT (Huntingtin, elongation factor 3, alpha-regulatory subunit of protein phosphatase 2A and TOR1) repeats at the amino-terminus, FAT (FRAP-ATM-TRRAP) domain, kinase domain (KD), the PIKK-regulatory domain (PRD) and the FAT-C-

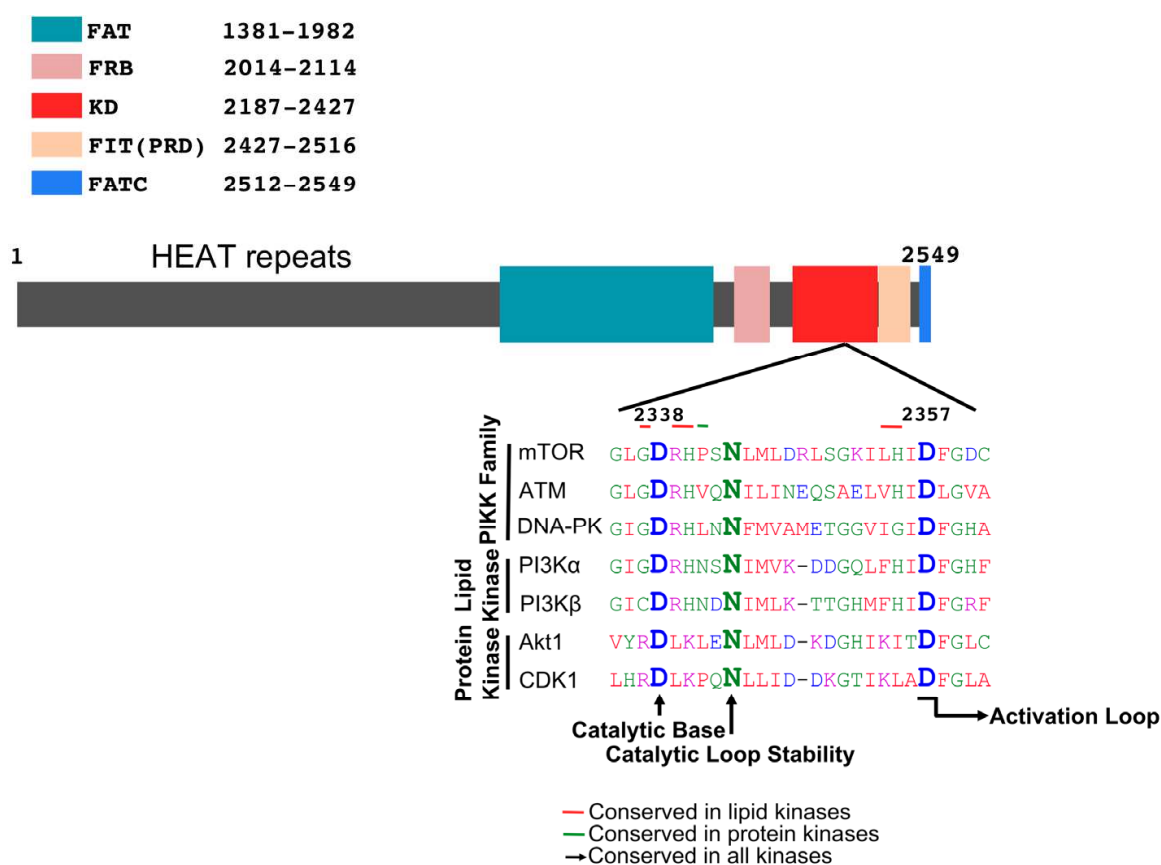


Fig. 1. Structural domains of mTOR and conserved amino acid sequences between lipid and protein kinases. Numbers indicate the residues in mTOR. FAT, FRAP-ATM-TRRAP domain; FRB, FKBP12-rapamycin-binding domain; KD, kinase domain; FIT, found in TOR domain; PRD, PIKK-regulatory domain; FATC, FAT-C-terminal domain; HEAT repeat, the protein domain found in Huntingtin, Elongation factor 3, protein phosphatase 2A, and TOR1; mTOR, mammalian target of rapamycin; ATM, Ataxia telangiectasia mutated; DNA-PK, DNA-dependent protein kinase; PI3K, Phosphatidylinositol 3-kinases; Akt, protein kinase B; CDK1, Cyclin-dependent kinase 1.

terminal (FATC) domain, at the carboxyl-terminus (Bosotti et al. 2000; Jacinto 2008; Hardt et al. 2011). The configuration of these protein motifs contributes to the catalytic activity and function of PIKKs, including TORs (Bosotti et al. 2000; Adami et al. 2007; Yip et al. 2010). Understanding the regulation of these motifs is key to unravelling the cellular function of TOR/mTOR and will provide insights on how we can manipulate the activity of this protein.

### 2.1 HEAT repeats

The amino-terminus of mTOR is characterized by HEAT repeats. This structural motif contains varied numbers of two anti-parallel  $\alpha$ -helix repeats that are linked by inter-unit loops allowing flexibility in this structure (Perry & Kleckner 2003). A recent sequence analysis strategy revealed that there are 30 to 32 tandem HEAT repeats predicted in human, fly, plant, and yeast TORs (Knutson 2010). HEAT domains confer a curved-tubular shape, facilitating multiple protein-protein interactions in the N-terminal half of mTOR (Groves & Barford 1999; Adami et al. 2007). Accordingly, this region is shown to provide a platform for protein-protein interaction, where mTOR can bind with protein regulators or substrates. In the budding yeast, the TORC components, KOG1 (Kontroller Of Growth 1), AVO1 (Adheres Voraciously 1), and AVO3 (Adheres Voraciously 3), associate with TOR at the HEAT region of the N-terminus (Wullschleger et al. 2005; Adami et al. 2007; Yip et al. 2010). Similarly, raptor, the orthologue of KOG1 in mammalian cells, also interacts with the N-terminal region of mTOR and that the intact structure of the HEAT domain in mTOR is essential for this interaction (Kim et al. 2002; Adami et al. 2007; Yip et al. 2010).

### 2.2 FAT domain and FRB domain

FAT domain is a hallmark of the PIKK family (Bosotti et al. 2000). In mTOR and other PIKK members, the FAT domain is adjacent to the N-terminal portion of the catalytic region (Lempiainen & Halazonetis 2009; Hardt et al. 2011). Although the overall structure of FAT domains in mTOR is still unclear, it is suggested that this domain is composed entirely of  $\alpha$ -helices according to sequence analysis and can be viewed as an extension of HEAT repeats. This would suggest that it serves as a platform for protein interaction as well (Perry & Kleckner 2003; Adami et al. 2007). Furthermore, the FAT domain may associate with another domain (FATC) to wedge the KD into a proper configuration and ensure the catalytic activity of mTOR (Bosotti et al. 2000).

The C-terminus of the FAT domain is where rapamycin, in complex with FKBP12, binds mTOR (Stan et al. 1994; Chen et al. 1995). This small structural motif, consisting of around 100 amino acids, has been termed as the FKBP12-rapamycin-binding (FRB) (Veverka et al. 2008). Structural and biochemical analyses have revealed that this motif can also bind to phosphatidic acid (PA), a lipid secondary messenger (Fang et al. 2001; Veverka et al. 2008). Upon mitogen stimulation, the level of PA is enhanced due to the activation of phospholipase D (English 1996). Although it is unclear whether PA can activate mTOR directly, it is suggested that PA may direct the membrane localization of mTOR (Veverka et al. 2008).

### 2.3 FATC domain

FATC, a protein motif containing around 30 amino acids, is conserved with high sequence similarity among the members of the PIKK family (Bosotti et al. 2000; Dames et al. 2005). In

these kinases, the FATC domain is at the end of the C-terminal tail and exists in combination with the FAT domain to flank the kinase domain (Bosotti et al. 2000; Jacinto & Lorberg 2008) (Figure 1). Low-resolution structure of this domain in yeast TOR1, visualized by electron microscopy, suggests that this motif protrudes from the catalytic core domain (Adami et al. 2007; Lempiainen & Halazonetis 2009). However, it is also predicted that the attachment of FATC to the KD is required for the proper conformation and activation of the latter. Structural studies utilizing NMR spectroscopy uncover that the FATC domain contains an  $\alpha$ -helix followed by a sharp turn, which is stabilized by a disulfide bond between two cysteine residues (Dames et al. 2005). The substitution of cysteine with serine increases the flexibility of FATC and leads to a lower expression level of TOR2 in budding yeast (Dames et al. 2005). Moreover, other mutagenesis assays have implied that the substitution and deletion of the hydrophobic residues in this domain abolish the autophosphorylation of mTOR and the mTOR-dependent phosphorylation of eukaryotic initiation factor 4E-binding protein (4E-BP) and p70 S6 kinase (S6K) (Peterson et al. 2000). Together, these studies indicate that the FATC domain modulates both kinase activity and stability of TOR.

#### 2.4 PRD (FIT)

The PRD domain is a newly identified motif situated between the kinase and FATC domains in PIKK family members (Mordes et al. 2008). Unlike other C-terminal domains, this domain is not conserved in all PIKKs and its length varies between 16 to 82 amino acids (Mordes et al. 2008). In TOR, this region is also named the Found in TOR (FIT) domain (Sturgill & Hall 2009; Hardt et al. 2011). Its N-terminal half, which shows almost no sequence homology with other PIKKs, is defined as a suppressor of TOR activity. The deletion of residues 2430 to 2450 of rat mTOR enhances kinase activity of both mTOR and its downstream targets, *in vitro* and *in vivo* respectively (Brunn et al. 1997; Sekulic et al. 2000). The mitogen- and nutrient-induced post-translational phosphorylation on several residues in the FIT domain, *eg* Thr 2446, Ser 2448, and Ser 2481 is used as a marker of mTOR activation (Chiang & Abraham 2005; Holz & Blenis 2005; Copp et al. 2009). Thus, the phosphorylation in this region could relieve the suppressive action conferred by this domain.

#### 2.5 Catalytic domain and kinase activity of mTOR

In the classification of eukaryotic protein kinases, mTOR belongs to the atypical group, a subset of protein kinases lacking sequence similarity to conventional protein kinases (Hanks & Hunter 1995). In fact, the catalytic sequence of mTOR shares high homology with PI3K family, a lipid kinase family, but mTOR has been experimentally demonstrated to possess Serine/Threonine protein kinase activity (Alarcon et al. 1999). The segment from residues Lys<sup>2187</sup> to Phe<sup>2421</sup> comprises the catalytic region of mTOR (Hardt et al. 2011). Within this domain, two conserved structures are proposed to contribute to the kinase activity of mTOR: the catalytic loop, which contains the predicted catalytic base (Asp<sup>2338</sup>) in the triplet DRH residues (Hardt et al. 2011), and the activation loop (also called T loop), consisting of twenty to thirty amino acids that connects the N- and C-lobes of the kinase domain (Lochhead 2009). By general definition, activation loop begins with the DFG sequence and ends with the PE sequence (Figure 1). In conventional protein kinases, there is one phosphorylatable Ser, Thr, or Tyr residue existing within the region and the

phosphorylation at this residue is required for kinase activation. In most cases, either the kinase itself (autophosphorylation) or an upstream kinase mediates the phosphorylation of this site. However, in atypical protein kinases, such as mTOR, this phosphorylatable residue is substituted by an Asp or Glu, which mimics the phosphorylated state of the T loop. Hence, instead of the conventional phosphorylation-activation mechanism in the T loop, other cis- and trans-acting mechanisms modulate mTOR activity. As discussed above, the phosphorylation at the PRD could promote mTOR activity. mTOR could also be potentially regulated via FATC domain stability. The formation of disulfide bonds may stabilize the FATC structure, or even the whole mTOR protein (Sarbasov & Sabatini 2005). Due to this unique structural feature, it has been proposed that the FATC domain could act as a redox-sensor to regulate mTOR activity (Dames et al. 2005). In this model, the presence of nutrients could enhance mitochondria metabolism that alters the intracellular redox environment. Although it remains to be examined, this redox change could be sensed by the FATC domain and confer a conformation switch in mTOR thereby altering its activity in response to intracellular stimuli (Dames et al. 2005). Other regulatory mechanisms such as association with regulatory partners and subcellular localization are discussed in Section 4.

### 3. mTOR protein complexes

Forming protein complexes is a common and efficient way to acquire different functional modules in a spatial and temporal manner (Hartwell et al. 1999; Pereira-Leal et al. 2006). As described in the previous section, the multiple motif conformation and superhelical structure of mTOR enables this protein to associate with diverse cofactors. Early studies using gel filtration chromatography suggested that TOR could be part of multi-protein complexes (Yang & Guan 2007). This was supported by findings that TOR has a rapamycin-insensitive function in yeast, implying that two complexes could perform distinct functions (Zheng, 1995; Schmidt, 1996; Zheng, 1997). Indeed, in a number of organisms with perhaps the exception of plants and algae, there are two structurally and functionally distinct TOR complexes (Loewith et al. 2002; Wedaman et al. 2003; Matsuo et al. 2007; Diaz-Troya et al. 2008). In this section, both conserved and non-conserved components of TOR complexes will be discussed.

#### 3.1 mTORC1

##### 3.1.1 Raptor (KOG1)

KOG1 was first identified to co-purify with TOR1 in budding yeast (Loewith et al. 2002). In mammals, the KOG1 orthologue, raptor, was found to associate with mTOR (Hara et al. 2002; Kim et al. 2002). It is predicted that the C-terminal half of KOG1 and raptor consists of four HEAT repeats and seven WD40 repeats (Loewith et al. 2002; Wedaman et al. 2003; Adami et al. 2007; Yip et al. 2010). Due to their motif configuration, it is speculated that KOG1 and raptor function as scaffold proteins to facilitate the association between TORC1 (mTORC1) and downstream substrates (Loewith et al. 2002; Adami et al. 2007). Deletion of *kog1* in budding yeast and knockout of *raptor* in mice both led to lethality, implying that these genes are essential for normal development and cellular functions (Loewith et al. 2002; Murakami et al. 2004; Guertin et al. 2006).

In mammalian cells, raptor binds to mTOR in a rapamycin-sensitive or nutrient-responsive manner (Kim et al. 2002; Oshiro et al. 2004). This association promotes mTORC1 activity

towards its substrates such as S6K and 4E-BP1 in response to insulin and nutrients (Kim et al. 2002). Presence of rapamycin/FKBP12 complex diminishes the association between raptor and mTOR, which could explain how rapamycin can inhibit mTORC1 function (Kim et al. 2002; Kim et al. 2003; Oshiro et al. 2004).

### 3.1.2 mLST8 (LST8)

LST8 is a 34 kDa protein composed of seven WD40 repeats (Chen & Kaiser 2003). Originally, LST8 was identified because of its function in the translocation of amino acid permease GAP1 from Golgi to cell surface (Roberg et al. 1997; Liu et al. 2001). Later, LST8 (Wat1 in fission yeast) was found in both TORC1 and TORC2 complexes (Chen & Kaiser 2003; Alvarez & Moreno 2006; Matsuo et al. 2007). Specifically, LST8 binds to the catalytic domain in the C-terminal region of TOR2 and regulates its kinase activity. In addition, it has been shown that LST8 is required for TORC2 complex integrity (Wullschleger et al. 2005). Similarly, mammalian LST8 (mLST8, also known as G $\beta$ L) was first reported to interact with raptor and mTOR in a nutrient- and rapamycin- sensitive manner (Kim et al. 2003). mLST8 is only required for mTORC2 functions in the early development of mice (Guertin et al. 2006). Knockout of mLST8 in mice revealed that it is required for mTORC2 function but not for the mTORC1 function in S6K phosphorylation (Guertin et al. 2006).

## 3.2 mTORC2

### 3.2.1 Rictor (AVO3)

AVO3, a 164-kDa protein, is a conserved subunit of TORC2 in budding yeast (Loewith et al. 2002). It was first identified as a suppressor of sphingolipid biosynthesis mutants in a genetic screen (Dunn et al. 1998). The presence of AVO3 is required for the integrity of rapamycin-insensitive TOR complex but it is dispensable for the *in vitro* kinase activity of TOR2 (Wullschleger et al. 2005). Therefore, AVO3 is suggested to play a role in recruiting TORC2 substrates (Ho et al. 2008).

Rictor (rapamycin-insensitive companion of mTOR) is the mammalian orthologue of yeast AVO3 and is part of mTORC2 (Jacinto et al. 2004; Sarbassov et al. 2004). It lacks common or known structural motifs but its C-terminus is conserved among vertebrates. Knockdown or ablation of rictor in mammalian cells led to defective phosphorylation of several members of the AGC (protein kinase A, G, and C) kinase family, including Akt, SGK1 and PKC, decreased cell survival upon stress induction, and impaired reorganization of actin cytoskeleton (Jacinto et al. 2004; Sarbassov et al. 2004; Guertin et al. 2006; Shiota et al. 2006; Garcia-Martinez & Alessi 2008). In mouse models, rictor knockout is embryonic lethal and the rictor<sup>-/-</sup> MEFs (mouse embryonic fibroblasts) isolated from rictor null embryos display slower growth rate compared to wild type MEFs (Guertin et al. 2006; Shiota et al. 2006). Substitution of Gly<sup>934</sup> in rictor prevented formation of rictor/SIN1 heterodimer and reduced mTORC2 activity (Aimbetov et al. 2011). Thus, the interaction between rictor and SIN1 is required to form an integral and active mTORC2.

### 3.2.2 SIN1 (AVO1)

AVO1 is another TORC2 component in budding yeast and binds to the N-terminus of TOR2. The depletion of AVO1 mimics the defective actin polarization phenotype observed in the

*tor2* mutant strain (Loewith et al. 2002). Sin1, the orthologue of AVO1 in fission yeast, was first identified as a stress-responsive protein that interacts with Sty1/Spc1 mitogen-activated protein (MAP) kinase, a member of yeast stress-activated MAP kinase (SAPK) family (Wilkinson et al. 1999; Yang et al. 2006). Mammalian SIN1 is also implicated in the JNK (c-Jun N-terminal kinase) and MAPK (mitogen-activated protein kinase)/ERK (extracellular-regulated-protein kinase) pathways (Cheng et al. 2005; Schroder et al. 2005). It was later identified as a critical subunit of mTORC2 (Frias et al. 2006; Jacinto et al. 2006; Yang et al. 2006). To date, more than five alternatively spliced isoforms of mammalian SIN1 have been discovered (Schroder et al. 2004; Cheng et al. 2005). Three of these isoforms form distinct rapamycin-insensitive mTOR complexes with rictor and mTOR (Frias et al. 2006). SIN1 disruption affects both mTORC2 assembly and function. Loss of SIN1 is embryonic lethal, indicating an important role for this protein in development (Jacinto et al. 2006; Yang et al. 2006).

### 3.3 Other interactors

In addition to the main components of TOR complexes discussed above, there are many non-conserved proteins that associate with TOR/mTOR. Some of these mTORC interactors can affect mTOR activity. These mTORC-interacting molecules could also mediate crosstalk between the mTOR pathway and other signaling pathways (Woo et al. 2007).

In budding yeast, TCO89 (TOR complex one 89 kDa subunit) has been shown to associate with TORC1 (Reinke et al. 2004). Deletion of TOR1 and TCO89 results in rapamycin hypersensitivity and defective cell-wall integrity, respectively. AVO2 and BIT61 also associate with TORC2 but their roles in regulating TORC2 functions remain to be elucidated (Loewith et al. 2002; Reinke et al. 2004). BIT61 can associate with SLM1 and SLM2, which are also TORC2-associated proteins mediating actin cytoskeleton organization (Fadri et al. 2005).

PRAS40 (proline-rich Akt substrate of 40kDa) is a negative regulator of mTORC1 (Sancak et al. 2007; Wang et al. 2007). PRAS40 and mTORC1 substrates, such as 4E-BP-1 and S6K, share a similar raptor-binding motif, the TOR signaling (TOS) motif (Wang et al. 2007). Therefore, it is speculated that PRAS40 can directly bind to raptor and interfere with the ability of mTORC1 to interact with its substrates (Wang et al. 2007). This negative regulation of mTORC1 by PRAS40 is inhibited by the insulin signaling pathway, since activated Akt can phosphorylate PRAS40 and prevent its binding to raptor (Sancak et al. 2007).

PRR5 (proline-rich protein 5), also named Protor (protein observed with rictor), and PRR5L (PRR5-like) bind to rictor and non-essential subunits of mTORC2 (Pearce et al. 2007; Thedieck et al. 2007; Woo et al. 2007). Knockdown of these two proteins did not cause significant disruption of both complex integrity and kinase activity of mTORC2. Protor1 is required for the phosphorylation of SGK1, but not of Akt and PKC $\alpha$ , specifically in mouse kidney (Pearce et al. 2011). These findings suggest that this non-conserved interactor might regulate mTORC2 function in a tissue- and target- specific manner (Pearce et al. 2011). DEPTOR (DEPDC6, DEP domain-containing protein 6), is a negative regulator of mTOR that associates with both mTORC1 and mTORC2 (Peterson et al. 2009; Proud 2009). Loss of DEPTOR activates S6K1 and Akt, downstream substrates of mTORC1 and mTORC2, respectively. In most cancer cell lines DEPTOR expression is low, except for a subset of



multiple myelomas harboring cyclin D1/D3 or c-MAF/MAFB translocations. The high DEPTOR levels in these cells are required for the activation of PI3K/Akt pathway and may suppress apoptosis (Peterson et al. 2009). Several studies have characterized how DEPTOR levels can be controlled (Duan et al. 2011; Gao et al. 2011; Zhao et al. 2011). DEPTOR is recognized and ubiquitinated by an F box protein, SCF ( $\beta$ TrCP) and degraded through the 26S-proteasome pathway (Zhao et al. 2011). Either expressing the dominant-negative mutant of  $\beta$ TrCP or interfering with the interaction between DEPTOR and  $\beta$ TrCP via mutagenesis causes the accumulation of DEPTOR and downregulation of mTOR activity (Zhao et al. 2011). Furthermore, mTORC1 and mTORC2 could directly phosphorylate DEPTOR (Gao et al. 2011). CK1 $\alpha$  (casein kinase 1 $\alpha$ ) can generate a phosphodegron on the phosphorylated DEPTOR, which is bound by  $\beta$ TrCP to induce the degradation of DEPTOR (Duan et al. 2011; Gao et al. 2011). The degron mutant and  $\beta$ TrCP deletion can inhibit DEPTOR degradation and decrease mTOR activities (Gao et al. 2011). Together, these studies suggest that DEPTOR can regulate mTORC activity via a positive feedback loop involving mTOR itself and CK1.

#### 4. Mode of regulation of mTOR complexes

mTOR serves to relay signals from growth cues to downstream events to consequently control cell growth and metabolism (Wullschleger et al. 2006; Zhou & Huang 2010). Below, we discuss how these growth cues alter mTOR activity via regulation of mTOR complex component modification, subcellular localization, and association with other regulatory molecules.

##### 4.1 Phosphorylation

mTOR itself is regulated via phosphorylation. Ser<sup>1261</sup> phosphorylation of mTOR is induced by insulin stimulation and is required for mTORC1 activity and mTOR autophosphorylation (Acosta-Jaquez et al. 2009). Furthermore, mTOR in the context of intact mTORC1 is predominantly phosphorylated at the Ser<sup>2448</sup> residue (Copp et al. 2009). This site is phosphorylated by S6K in a mitogen- and nutrient-inducible manner (Chiang & Abraham 2005). The autophosphorylation site at Ser<sup>2481</sup> is also growth-signal dependent (Peterson et al. 2000). A later report proposed that the Ser<sup>2481</sup> phosphorylation event is an indicator of functional mTORC2 (Copp et al. 2009). Prolonged but not acute rapamycin treatment, which disrupts mTORC2 (Sarbasov et al. 2006), can abolish mTOR phosphorylation at this site (Copp et al. 2009). However, mTOR from raptor immunoprecipitates is also phosphorylated at Ser<sup>2481</sup>. Furthermore, inhibition of mTORC1 by acute rapamycin treatment can reduce Ser<sup>2481</sup> phosphorylation of mTOR that is associated with raptor (Soliman et al. 2010), implying that the phosphorylation of Ser<sup>2481</sup> residue may also be involved in the regulation of mTORC1 functions. Thus, how Ser<sup>2481</sup> phosphorylation affects the specific activity of mTORC1 vs mTORC2 would need to be clarified.

mTOR complex components are also phosphorylated at numerous sites. Phosphorylation of raptor at different residues may affect the kinase activity of mTOR. For example, AMPK mediates phosphorylation of raptor at Ser<sup>722/792</sup> upon nutrient depletion and inhibits mTORC1 function (Gwinn et al. 2008). In contrast, upon mitogen stimulation, p90 ribosomal S6 kinase (RSK) and mTORC1 mediate raptor phosphorylation at Ser<sup>719/721/722</sup> and Ser<sup>863</sup>,

respectively, which is essential for mTORC1 activation (Carriere et al. 2008). Rictor is predicted to be phosphorylated in at least 37 phosphorylation sites according to MS/MS analysis and phospho-proteome database (Dibble et al. 2009; Julien et al. 2010). These putative phosphorylation sites mainly localize in the C-terminal region of rictor, which is conserved only in vertebrates (Dibble et al. 2009; Julien et al. 2010). Thus, rictor could have acquired more diverse functions and complex regulation during evolution. Several studies have examined the function of rictor phosphorylation at Thr<sup>1135</sup> residue located in the C-terminal region. This phosphorylation is mediated by S6K1 in an amino acid- and growth factor- dependent manner and is suggested to act as a feedback regulation of mTORC2 from mTORC1 signals (Dibble et al. 2009; Julien et al. 2010; Treins et al. 2010). Its effect on the mTORC2-mediated Akt activation is very minimal if any (Boulbes et al. 2010; Treins et al. 2010). Moreover, in SIN1<sup>-/-</sup> MEFs, in which mTORC2 complex integrity is disrupted, this phosphorylation is still detectable, suggesting that it might be involved in mTORC2-independent functions (Boulbes et al. 2010). Rictor is also phosphorylated at Ser<sup>1235</sup> by GSK3 $\beta$  under ER stress conditions (McDonald et al. 2008). This phosphorylation event reduces the binding between mTORC2 and its substrate, Akt, hence negatively regulating mTORC2.

SIN1 can also be phosphorylated at multiple sites although the relevant sites remain to be identified. Hypophosphorylation of SIN1 interferes with its association with mTOR (Yang et al. 2006), but not with rictor (Rosner & Hengstschlager 2008). mTOR can phosphorylate SIN1 *in vitro*, which may prevent SIN1 degradation from lysosomal pathway *in vivo* (Chen & Sarbassov 2011). Other kinases that can phosphorylate SIN1 to regulate mTORC2 activity would need to be investigated.

#### 4.2 Component stability and complex formation

The activity and specificity of mTOR can be modulated through complex assembly. Disruption of mTOR complexes via gene ablation or knockdown of a specific mTORC component has revealed the importance of an intact mTORC for phosphorylation of its downstream substrates. For instance, in the adipose-specific *raptor* knockout mice, which carry disrupted mTORC1, S6K phosphorylation in white adipose tissue was diminished (Boulbes et al. 2010). Similarly, the deletion of either SIN1 or rictor in MEFs and HeLa cells inhibited mTORC2 assembly and abolished Akt HM and TM phosphorylation (Guertin et al. 2006; Jacinto et al. 2006; Yang et al. 2006).

In mammalian cells, raptor binds to mTOR in a nutrient- responsive manner (Kim et al. 2002; Oshiro et al. 2004). Upon nutrient deprivation, raptor and mTOR form a stable interaction, which can inhibit mTORC1 activity (Kim et al. 2002). Under growth favorable conditions, the association between raptor and mTOR is less tight and presumably can promote mTORC1 activity towards its substrates such as S6K and 4E-BP (Kim et al. 2002). Instead of affecting the intrinsic kinase activity of mTOR, rapamycin/FKBP12 complex is proposed to attenuate the association between mTOR and raptor, thereby inhibiting mTORC1 (Chen et al. 1995; Choi et al. 1996; Kim et al. 2002). Supporting this model, mTOR purified from rapamycin-treated cells showed no defect of its autophosphorylation ability *in vivo* or kinase activity toward substrates *in vitro* (Peterson et al. 2000). In addition, recent studies revealed that not all mTORC1 functions can be inhibited by rapamycin, suggesting that it may only affect access to some substrates (Choo et al. 2008; Dowling et al. 2010).

While the FKBP12/rapamycin complex binds and inhibits mTORC1, it does not affect mTORC2 activity acutely perhaps because it does not bind to mTORC2 (Jacinto et al. 2004). However, chronic exposure to rapamycin could disrupt mTORC2 function in some cell lines presumably by blocking assembly of newly synthesized mTORC2 subunits (Sarbasov et al. 2006).

The integrity of mTORC2 is dependent on stability of the rictor/SIN1 heterodimer. These mTORC2 components interact tightly and deficiency in either one leads to destabilization of the other, suggesting they require each other for stability (Guertin et al. 2006; Jacinto et al. 2006; Yang et al. 2006). Other proteins associating with mTORC components have been identified that could affect mTORC activity or assembly. The folding chaperone Hsp70 interacts with rictor and its knockdown reduces rictor level as well as mTOR-rictor interaction, resulting in impaired mTORC2 formation and activity (Martin et al. 2008; Martin et al. 2008). The maturation and assembly of mTORCs was also shown to be dependent on Tel2 and Tti (Takai et al. 2007; Kaizuka et al. 2010 a; Kaizuka et al. 2010 b). Hsp90 was shown to mediate the formation of both TORCs, as well as other PIKKs (Horejsi et al. 2010; Takai et al. 2010). Whether mTORC signaling can be modulated by these interactors remains to be examined.

mTORC2 components have been found to associate with other proteins independently of mTOR. Rictor can form an E3 ligase complex with Cullin-1 and Rbx1 to promote the ubiquitination of SGK1 in an mTOR-independent manner (Gao et al. 2010). The interaction between rictor, Cullin-1, and Rbx1 is disrupted when rictor is phosphorylated at Thr<sup>1135</sup> residue by multiple AGC kinases (Gao et al. 2010). Whether the phosphorylated rictor released from the E3 ligase complexes can affect mTORC2 assembly remains to be elucidated. SIN1 also interacts with other proteins independently of mTOR and rictor. The function of SIN1 when associated with these proteins remains unclear but these proteins are involved in stress responses including ras, MEKK2, JNK, p38, ATF2 and the stress-related cytokine receptors IFNAR2, TNFR1/2 (Schroder et al. 2005; Makino et al. 2006; Schroder et al. 2007; Ghosh et al. 2008). Unlike mTORC2 components, so far, the mTORC1 subunit raptor has not been found to associate with other proteins in an mTORC1-independent manner.

### 4.3 Localization of mTOR complexes

Compartmental localization enables a protein kinase to gain access to its regulators or effectors, thereby regulating its function. Supporting this concept, yeast TOR undergoes nuclear localization and binds to the 35S rDNA promoter to enhance 35S rRNA synthesis in a nutrient-sensitive fashion (Li et al. 2006). The HTH (helix-turn-helix) motif, a region in the HEAT domain of TOR, has been demonstrated to be essential for this association since *HTH* deletion interrupts the binding of TOR1 to 35S rDNA (Li et al. 2006). TOR1 and TOR2 also localize to membrane compartments that contain actin cytoskeleton and endocytosis regulators via their HEAT domain (Kunz et al. 2000; Aronova et al. 2007). Two endoplasmic reticulum (ER) and Golgi localization sequences were characterized in the HEAT domain, supporting that the TOR complexes may localize at the membrane periphery of these organelles (Liu & Zheng 2007). The best example for how mTORC1 can be regulated via localization is the recent finding that amino acid stimulation induces shuttling of mTORC1 to late endosomes and lysosomes (LELs) by interaction with Rag GTPases (Sancak et al.

2008). How the presence of amino acids would be sensed by mTORC1 interactors in a particular organelle such as the endosomes remain to be further elucidated.

Less is known on how mTORC2 can be compartmentalized and activated in response to growth signals. It co-localizes predominantly in the ER periphery (Boulbes et al. 2011). mTORC2 associates with actively translating ribosomes and specifically interacts with the proteins from the 80S large ribosomal subunit (Oh et al. 2010; Zinzalla et al. 2011). mTORC2 components can stably interact with ribosomal proteins that line the tunnel exit of the 80S and could function in this site by modifying emerging nascent polypeptides such as Akt (Oh et al. 2010). mTORC2 becomes activated upon association with ribosomes although the precise mechanism is currently unclear (Zinzalla et al. 2011). Since the protein synthesis machinery can physically associate with cell surface receptors (Tcherkezian et al. 2010), it can be speculated that mTORC2 could be activated upon nucleation of translation machinery with a signaling receptor in the membrane periphery.

## 5. Upstream regulators of mTOR complexes

### 5.1 Activation of mTORC1

mTORC1 integrates signals from extracellular and intracellular sources of nutrients with other growth, energy and mitogenic cues (Figure 2). mTORC1 activity is sensitive to intracellular amino acid concentration, particularly leucine depletion (Hay & Sonenberg 2004). Previously, intracellular amino acid levels were proposed to modulate mTORC1 activity via TSC1/2 (tuberous sclerosis complex 1/2) (Gao et al. 2002). However, in TSC2<sup>-/-</sup> cells, attenuated mTORC1 activity upon amino acid withdrawal was still detected (Smith et al. 2005), indicating the presence of additional pathways regulating mTORC1 activity in response to amino acid levels. Furthermore, hVPS34, a class III PI3K, can signal amino acid availability to mTORC1 bypassing the TSC1/2-Rheb axis (Byfield et al. 2005; Nobukuni et al. 2005). Recently, in both yeast and mammals, TORC1/mTORC1 activity has been shown to be regulated by Rag GTPases in response to amino acids. In yeast, Rag orthologues Gtr1 and Gtr2, as part of the EGO complex (consisting of EGO1, EGO3, Gtr1 and Gtr2) localize to the vacuolar/lysosomal membrane and could mediate amino acid signals to TORC1 (Dubouloz et al. 2005; Binda et al. 2009). Mammalian Rags function as heterodimers, wherein Rag A/B associates with Rag C/D (Sekiguchi et al. 2001). A protein complex termed the Ragulator consisting of MAPK scaffold protein 1, p14, p18 localizes Rag to these membrane compartments. In nutrient replete conditions, Rag complexes are fully activated as Rag A/B in GTP form and Rag C/D in GDP form (Sekiguchi et al. 2001). Activation of Rag heterodimers recruits mTORC1 to the membrane compartment where Rheb (Ras homolog enriched in the brain) is enriched, thus promoting mTORC1 activity (Rubio-Texeira & Kaiser 2006; Meijer & Codogno 2008; Sancak et al. 2008). p62, an adaptor protein that associates with mTORC1 and Rag complex was shown to be required for mTORC1 activation (Duran et al. 2011). It was proposed that p62 can promote the localization of mTORC1 and Rag complex to the lysosomes, which is a critical step of mTORC1 activation, in an amino-acid dependent manner (Duran et al. 2011). Rheb is a small GTPase belonging to the Ras family (Garami et al. 2003; Manning & Cantley 2003; Long et al. 2005). Upon binding to mTOR, GTP-loaded Rheb induces a conformational change in the KD of mTOR to promote its activation (Long et al. 2005). In the lysosomes, the amino acid transporter PAT1 (proton-assisted amino acid transporter 1; SLC36A1) is abundant and has been described as a

lysosomal amino acid transporter (Rusznak et al. 2001). This transporter could play a role in mTORC1 activation in an amino acid-dependent manner (Heublein et al. 2010). Knockdown of *PAT1* in MCF-7 cells led to decreased S6K and 4E-BP-1 phosphorylation (Heublein et al. 2010). Two membrane transporters, *SLC7A5* and *SLC3A2*, were also shown to be required for mTORC1 activation (Nicklin et al. 2009). These two transporters function in the cellular uptake and the subsequent efflux of glutamine in the presence of essential amino acids (Nicklin et al. 2009). This process can increase intracellular concentration of leucine and can enhance mTORC1 activation via Rag complexes (Sancak et al. 2008). Thus, several regulatory molecules in the endosome/lysosomes and membrane compartments could regulate mTORC1 function in response to the presence of amino acids.

Numerous cellular inputs that convey growth or stress conditions are coupled to mTORC1 via the TSC1/2 tumor suppressor complex (Castro et al. 2003; Garami et al. 2003; Tee et al. 2003; Hay & Sonenberg 2004; Zoncu et al. 2011). Loss of *TSC1* or *TSC2* genes, encoding hamartin and tuberlin respectively, is observed in tuberous sclerosis, a human genetic disorder characterized by benign tumors (Green et al. 1994; Onda et al. 1999). Through phosphorylation at different residues, the activity of the TSC 1/2 complex can be regulated by different upstream signaling pathways (Huang & Houghton 2003). In the presence of growth factors, such as insulin, the insulin receptor is activated and recruits the insulin receptor substrate (IRS). IRS serves to couple signals to downstream molecules including PI3K, which converts phosphatidylinositol-4,5-phosphate (PIP2) into phosphatidylinositol-3,4,5-phosphate (PIP3) in the plasma membrane. PIP3 can recruit both PDK1 and Akt to the membrane, where Akt is phosphorylated and activated by PDK1. The activated Akt can directly phosphorylate TSC2 and block the activity of TSC1/2 complex (Huang & Houghton 2003), which consequently results in the activation of mTORC1 and the phosphorylation of S6K and 4E-BP1, the two best-characterized effectors of mTORC1 (Valentinis & Baserga 2001). The regulation of mTORC1 activity via Akt can be counteracted by GADD34 (growth arrest and DNA damage protein 34) (Minami et al. 2007; Watanabe et al. 2007). By interacting with TSC1/2 complexes, GADD34 inhibits phosphorylation of TSC2 at the Akt phosphorylation site Thr<sup>1462</sup>, thus negatively regulating mTORC1 (Minami et al. 2007; Watanabe et al. 2007).

The mitogen activated protein kinase (MAPK) pathway can also modulate mTORC1 functions via negative regulation of TSC1/2 in response to growth cues (Ma et al. 2005). In the presence of growth factors, receptor tyrosine kinases activate Ras-Erk1/2 signaling (Pearson et al. 2001). Activated Erk can phosphorylate TSC2 at Ser<sup>664</sup> and induce dissociation of TSC1/2 complex, which suppresses TSC1/2 functions toward cell proliferation and transformation (Ma et al. 2005). Active Ras can also inactivate TSC via Rsk-mediated phosphorylation of TSC2 at Ser<sup>1798</sup>, resulting in increased mTORC1 signaling (Roux et al. 2004).

AMP-activated protein kinase (AMPK) pathway is another well-established regulatory input of mTORC1 in response to energy conditions. Under low cellular energy level (high AMP/ATP ratio), LKB1, a tumor suppressor, activates AMPK to directly phosphorylate TSC2 at Thr<sup>1227</sup> and Ser<sup>1345</sup> residues (Inoki et al. 2003; Corradetti et al. 2004; Shaw et al. 2004). Unlike Akt and Erk, AMPK-mediated phosphorylation enhances the GAP activity of TSC1/2 complex and inhibits mTORC1 function. This would provide a mechanism for downregulating energy-consuming cellular processes under low ATP levels.

## 5.2 Activation of mTORC2

Early studies in yeast have elucidated how TORC2 can control actin cytoskeleton polarization but the signals that control TORC2 activity has been elusive (Schmidt et al. 1996; Schmidt et al. 1997; Loewith et al. 2002). Since this function of TORC2 is viewed to control growth spatially, it is reasonable to speculate that TORC2 is activated by growth signals. In mammals, mTORC2 activity is promoted by growth factors in a PI3K-dependent manner. After insulin stimulation, the phosphorylation of Akt at its hydrophobic motif (HM; Ser<sup>473</sup>) mediated by mTORC2 is significantly increased *in vivo* (Hresko & Mueckler 2005; Sarbassov et al. 2005). Similar results were also observed in *in vitro* kinase assays (Sarbassov et al. 2005). Since mTORC2 also phosphorylates sites in Akt and PKC in a constitutive manner (Facchinetti et al. 2008; Ikenoue et al. 2008), its responsiveness to growth factors has been puzzling. However, further studies revealed that mTORC2 could phosphorylate the constitutive site in Akt during translation (Oh et al. 2010). This would suggest that conditions that enhance translation, such as the presence of growth factors, promote mTORC2 activity. Supporting this notion, mTORC2 was found to associate with translating ribosomes and this association is enhanced in cells with increased PI3K signaling (Oh et al. 2010; Zinzalla et al. 2011). Since nutrients are essential for promoting protein synthesis, this would suggest that mTORC2 would also be regulated by nutrients. However, it remains to be determined if this is the case and how mTORC2 becomes activated upon association with ribosomes.

## 6. mTOR functions

A number of diverse functions have been ascribed to mTOR but it is now emerging that it is a central hub to regulate growth and metabolism (Figure 2). In the whole organism, mTOR is required during early development. Rapamycin treatment inhibited amino acid stimulation of embryo outgrowth in mice at blastocyst stage (Martin & Sutherland 2001). Similarly, the embryonic development of *mTOR*<sup>-/-</sup> mice is aberrant and arrested at E5.5 (Gangloff et al. 2004). Conditional knockout strategies have shed light on tissue-specific mTOR functions. The inactivation of mTOR in T cells caused defects in differentiation of peripheral T lymphocytes (Delgoffe et al. 2009). The muscle-specific mTOR knockout mice displayed reduced levels of dystrophin and severe myopathy, which led to premature death (Risson et al. 2009). While there was no significant phenotype in mice with specific deletion of *mTOR* in the prostate, tumor initiation and progression in PTEN<sup>-/-</sup> mice, which possess hyperactivated mTORC1, was suppressed (Nardella et al. 2009). These findings support a critical role for mTOR itself in organism and organ development as well as disease progression.

The cellular functions of mTOR have surfaced from numerous studies over the years. The most well characterized function of mTOR is the rapamycin-sensitive control of protein synthesis by mTORC1. More recent studies have revealed that mTOR has rapamycin-insensitive functions both as part of mTORC1 and mTORC2. Most of the mTOR functions that have been elucidated are mediated by the AGC kinases S6K, Akt, SGK, and PKC. Another well characterized mTOR substrate is 4E-BP1, a translation regulator. With the recent phospho-proteomic studies that identified hundreds of direct and indirect targets of mTOR, we can begin to elucidate how the mTOR complexes could perform its wide array of cellular functions (Hsu et al. 2011; Yu et al. 2011).

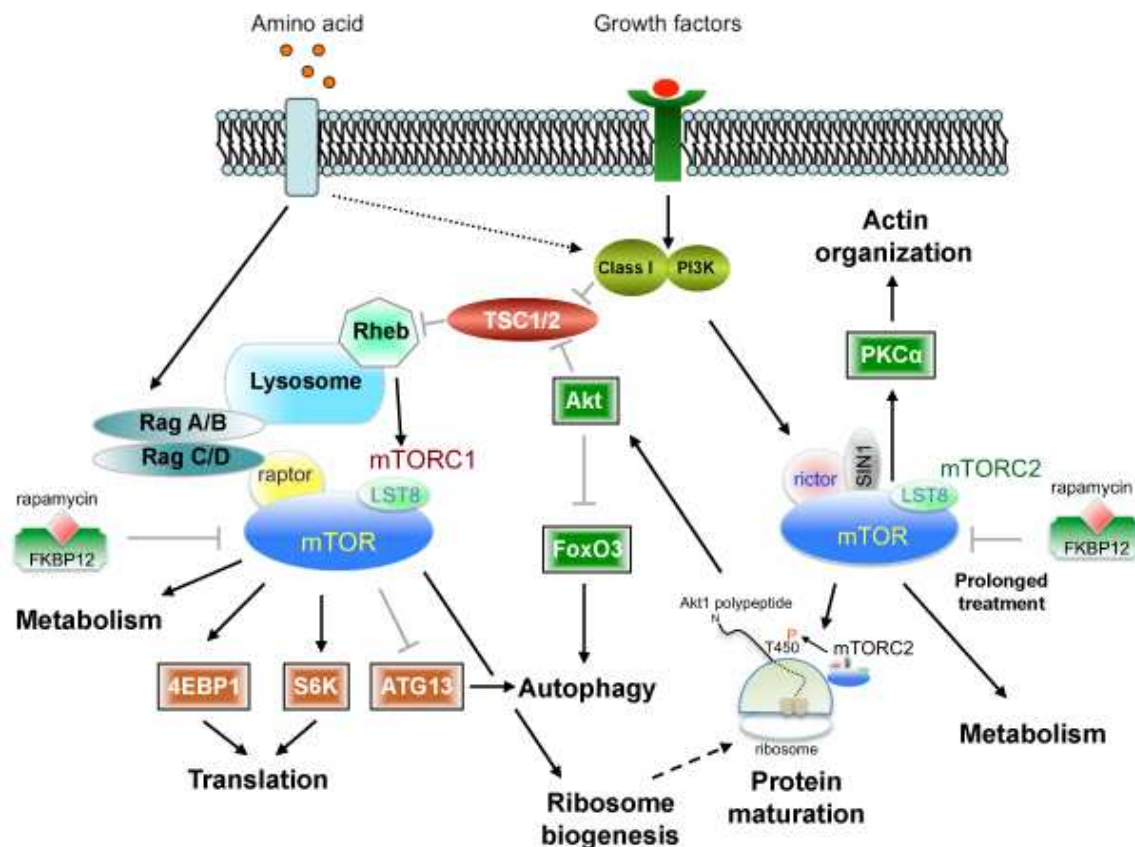


Fig. 2. Overview of mTOR complex signaling and functions. Black arrows and gray lines represent activating and inhibitory connections, respectively. Dotted lines indicate possible links.

### 6.1 Protein synthesis and maturation

Protein synthesis, ie translation, utilizes huge amounts of energy and machinery (ie. ribosomes), and consequently, the entire process is under tight control (Acker & Lorsch 2008; Malys & McCarthy 2011). mTOR, primarily mTORC1, is involved in different aspects of protein synthesis (Ma & Blenis 2009; Sonenberg & Hinnebusch 2009). Its most well characterized function is the phosphorylation of proteins involved in translation initiation, namely S6K1 and 4E-BP. 4E-BP is a negative regulator of translation initiation (Pause et al. 1994). When unphosphorylated, it interacts with eIF-4E (eukaryotic initiation factor 4E) cap-binding protein, preventing cap-dependent translation. mTORC1-mediated phosphorylation dissociates 4E-BP1 from eIF-4E and releases the inhibition of translation (Ma & Blenis 2009; Sonenberg & Hinnebusch 2009). However, there are four phosphorylation sites on 4E-BP1 that are regulated by mTORC1 but only two of them are sensitive to acute rapamycin treatment (Gingras et al. 1999; Gingras et al. 2001; Wang et al. 2003). In the presence of growth signals, translation of proteins specifically involved in cell proliferation is upregulated in a 4E-BP-dependent fashion (Dowling et al. 2010). This would suggest that mTORC1 can specifically regulate the translation of a subset of mRNA under a particular growth condition.

mTORC1 can directly phosphorylate and activate S6K1, which promotes mRNA translation by modulating multiple substrates involved in different stages of translation, from mRNA

surveillance, initiation to translation elongation (Ma & Blenis 2009). Phosphorylated S6K1 binds to newly spliced mRNAs through its binding partner, SKAR (S6K1 aly/REF-like target) and potentially facilitate translation initiation and/or elongation (Ma et al. 2008). This protein complex interacts with EJC (exon junction complex), which monitors the quality of newly spliced mRNA (Ma et al. 2008). Active S6K1 also phosphorylates the 40S ribosomal protein S6 (rpS6) at several sites. Phosphorylated rpS6 is widely used as a readout of mTORC1 activity. Its phosphorylation appears to play a role in the control of cell size but is dispensable for translation of mRNA with 5' terminal oligopyrimidine tract (TOP mRNAs) (Ruvinsky et al. 2005). S6K also phosphorylates PDCD4 (programmed cell death 4), an inhibitor of RNA helicase eIF4A. PDCD4 levels become downregulated upon phosphorylation (Dorrello et al. 2006). The degradation of PDCD4 greatly enhances eIF4A helicase activity and facilitates 40S ribosomal subunit scanning to the initiation codon. Moreover, S6K can also augment eIF-4A activity through increasing the levels of eIF-4A, eIF-4B, and eIF3 complex. The S6K-mediated phosphorylation of eIF4B can enhance its binding to eIF3 (Vornlocher et al. 1999). Thus, S6K can modulate a number of proteins involved in translation initiation. It could also regulate the elongation phase of translation. Phosphorylation of eEF2K (eukaryotic elongation factor 2 kinase) by S6K inhibits its activity towards eEF2 and consequently enhances elongation (Wang et al. 2001).

In addition to the regulation of the translation process, mTORC1 directly affects the biosynthesis of the translational machinery as well. The assembly of functional ribosomes is an energy-demanding process requiring a series of building components and assembly factors (Mayer & Grummt 2006). mTORC1 has been found to regulate ribosome biogenesis at different levels, including the production of ribosomal proteins (Cardenas et al. 1999; Hardwick et al. 1999), pre-rRNA processing (Powers & Walter 1999), and the rRNA synthesis (Hardwick et al. 1999; Hannan et al. 2003). In response to extracellular conditions, mTORC1 can coordinate all three nuclear RNA polymerases, Pol I, Pol II and Pol III, to control ribosome synthesis (Beck & Hall 1999; Miller et al. 2001; Yuan et al. 2002; Martin et al. 2004; White 2005). mTORC1 regulates the nuclear translocation of TIF1A, a transcription factor that is essential for Pol I-associated transcription initiation (Mayer et al. 2004). Furthermore, mTORC1 activity can enhance the tRNA levels via regulation of the transcription of Pol III (Shor et al. 2010). Maf1, a Pol III suppressor, directly associates with and inhibits Pol III apparatus (Reina et al. 2006). Under growth-favorable conditions, mTORC1 phosphorylates Maf1 to dissociate it from Pol III and promotes its cytoplasmic translocation (Shor et al. 2010). mTORC1 also interacts with rDNA (ribosomal DNA) promoters to promote Pol I and Pol III transcription in a growth factor-dependent and rapamycin-sensitive manner (Tsang et al. 2010).

mTORC2 is also emerging to play a role in translation. A more pronounced defect in protein synthesis and polysome assembly occurs upon mTOR inhibition with active site inhibitors, in contrast to rapamycin treatment (Yu et al. 2009; Carayol et al. 2010; Oh et al. 2010; Evangelisti et al. 2011). Although it can be argued that the exacerbated defects could be due to inhibition of rapamycin-insensitive mTORC1 functions, there is some evidence that mTORC2 inhibition could contribute to these defects. First, polysome recovery is somewhat defective in mTORC2-disrupted cells (Oh et al. 2010; Wu and Jacinto, unpublished results). Second, phosphorylation of eEF2 is aberrant in these cells. Most importantly, mTORC2 associates with actively translating ribosomes and SIN1 deficiency disengages mTOR or rictor from the ribosomes (Oh



et al. 2010; Zinzalla et al. 2011). mTORC2 components can stably associate with ribosomal proteins that are present at the tunnel exit. This would be consistent with the finding that mTORC2 can cotranslationally phosphorylate the emerging nascent Akt polypeptide (Oh et al. 2010). Thus, mTORC2 could function in cotranslational maturation of newly synthesized proteins by phosphorylating relevant sites. The maturation of conventional PKC is also mediated by mTORC2 via phosphorylation (Facchinetti et al. 2008; Ikenoue et al. 2008). It would be interesting to see if mTORC2 could also cotranslationally phosphorylate PKC and whether it has additional cotranslational targets other than Akt.

## 6.2 Autophagy

Autophagy is a catabolic process that recycles intracellular components in the lysosomes to salvage substrates for energy production when nutrients become limiting (Noda, 1998; Janku, 2011). The control of this process by TOR was first discovered in yeast. In yeast, active TORC1 correlates with hyperphosphorylation of Atg13, a regulatory component of the Atg1 complex. Under this condition, assembly of the Atg1-Atg13 complex is inhibited thereby preventing autophagy (Yorimitsu et al. 2009; Kamada et al. 2010; Kijanska et al. 2010; Yeh et al. 2010). Increased autophagy is observed upon rapamycin treatment, supporting the role of TORC1 in regulating this cellular process (Kamada et al. 2000).

Under nutrient-replete conditions, mTOR negatively regulates autophagy by interacting with a protein complex composed of ULK1 (UNC51 like kinase), Atg13, and FIP200 (Ganley et al. 2009; Hosokawa et al. 2009; Jung et al. 2009). This complex is involved in the formation of the autophagosomes. The phosphorylation of ULK1 and Atg13 is inhibited by rapamycin treatment and leucine deprivation, implying the link between mTORC1 and autophagy (Hosokawa et al. 2009; Jung et al. 2009).

mTOR is also involved in regulating expression of genes that are involved in autophagosome or lysosome biogenesis. mTORC1 could mediate phosphorylation of the transcription factor EB (TFEB), thereby controlling its nuclear shuttling and activity. TFEB binds E box related DNA sequences and controls lysosomal gene transcription (Pena-Llopis et al. 2011; Settembre et al. 2011). mTORC2 could also be involved in the control of autophagy via Akt-FoxO signaling (Mammucari et al. 2007). Disruption of rictor enhanced autophagosome formation in skeletal muscle and that this effect was abrogated upon expression of constitutively active Akt in the absence or presence of rapamycin, indicating that in this cell type, autophagy is dependent on mTORC2 function, not mTORC1 (Mammucari et al. 2007). Indeed, attenuated Akt activity caused by downregulation of mTORC2 leads to decreased phosphorylation and increased nuclear translocation of FoxO3 (Shiojima & Walsh 2006). FoxO3 induces the expression of many autophagy-related genes, such as *Atg12l* and *Ulk2*, and increases autophagosome formation in isolated adult mouse muscle fibers (Zhao et al. 2007). Together, these results indicate that by directly interacting with autophagic proteins or modulating the transcription of autophagy-related genes, mTOR complexes regulate autophagy.

## 6.3 Metabolism

Early studies showing reduced fungal amino acid, nucleic acid, and lipid metabolism after rapamycin treatment have linked TOR to metabolic functions (Singh et al. 1979). Later

transcription profiling screening of lymphoma cells treated with rapamycin revealed a tendency towards catabolism and that the levels of many mRNA involved in lipid, nucleotide, and protein synthesis were downregulated (Peng et al. 2002). In the whole organism, the function of mTOR in metabolism is underscored by findings on the effect of rapamycin treatment on insulin-responsive tissues. Inhibition of mTORC1 in mice via feeding with rapamycin induced diabetes due to smaller pancreatic islets and abolished insulin secretion (Bussiere et al. 2006). This effect could be mediated via S6K and S6 since these two mTORC1 pathway effectors are also required for the normal morphology and function of pancreatic islet cells and that removal of these two proteins led to a diabetic phenotype (Ruvinsky et al. 2005). Corollary to this, hyperactivation of mTORC1 occurring in *TSC* knockout resulted in larger islet size and higher number of  $\beta$ -cell (Rachdi et al. 2008). Together, these findings support a role for mTORC1 in maintaining metabolic homeostasis.

Recent studies, discussed below, that have employed mTORC component gene ablation or knockdown further demonstrate the central role of mTOR in cellular and systemic metabolism. The critical role of mTORC1 in cellular metabolism is illustrated by its involvement in the biogenesis of mitochondria. In muscle-specific *raptor* knockout mice, there are reduced levels of PGC1 $\alpha$  (PPAR $\gamma$  coactivator 1), which is required for mitochondrial gene expression (Cunningham et al. 2007). In these mice, the skeletal muscle has lower mitochondria number, reduced oxidative capacity, and elevated glycogen storage, which led to muscle dystrophy (Bentzinger et al. 2008). Moreover, by genomic analysis, YY1 (yin-yang 1), a transcription factor that regulates mitochondrial gene expression and oxygen consumption, was identified as a downstream effector of mTORC1 (Cunningham et al. 2007). Both mTOR and raptor can bind to YY1 while rapamycin treatment inhibited YY1 activity by preventing its interaction with the coactivator, PGC1 $\alpha$  (Cunningham et al. 2007). Rapamycin treatment or knockdown of mTOR or raptor in muscle cells also reduced mitochondrial gene transcription and respiratory metabolism (Cunningham et al. 2007). Interestingly, this function of mTORC1 appears to be S6K1-independent. In line with these findings, enhanced muscular levels of PGC1 $\alpha$  and mitochondria were found in *S6K1* knockout mice (Um et al. 2004). Thus, mTORC1, via YY1 and PGC1 $\alpha$ , could regulate mitochondrial gene expression and thereby enhance mitochondrial oxidative functions.

In addition to skeletal muscle, raptor knockout in other insulin-responsive tissues further illustrate the role of mTORC1 in metabolism. In adipose-specific *raptor* knockout mice, adipose tissue was reduced and these mice were protected against diet-induced obesity and hypercholesterolemia. There was elevated expression of genes involved in mitochondrial respiration in white adipose tissue and the leanness of these mice could be explained by enhanced energy expenditure due to mitochondrial uncoupling (Polak et al. 2008). These mice also display higher glucose tolerance and insulin sensitivity. This could be due to defective S6K feedback regulation of IRS-1 activity, causing hyperactivated insulin receptor signaling (Polak et al. 2008). These findings underscore the role of adipose mTORC1 in whole body energy homeostasis.

In liver, inhibition of mTORC1 promotes hepatic ketogenesis in response to fasting (Sengupta et al. 2010). Active mTORC1 negatively regulates PPAR $\alpha$  (peroxisome proliferator activated receptor  $\alpha$ ), the master transcriptional activator of ketogenic gene expression, through control of its corepressor, NCoR1 (nuclear receptor corepressor 1) (Sengupta et al. 2010).

Thus, mice with hyperactivation of mTORC1 upon loss of *TSC1* in the liver manifest defects in ketone body production and enlarged liver size during fasting (Sengupta et al. 2010).

Other effectors of mTORC1 in the control of metabolic processes have emerged in recent reports. mTORC1 can modulate sterol and lipid biosynthesis through SREBP-1 (sterol regulatory element binding protein-1), a transcription factor that controls lipo- and sterolgenic gene transcription (Porstmann et al. 2008; Duvel et al. 2010). The mTORC1 target S6K1 can partially promote the activity of SREBP-1 via posttranslational modification. More recently, one critical regulator of mTORC1-SREBP-1 pathway has been identified. Lipin 1, which is directly phosphorylated and sequestered in the cytoplasm by mTORC1, induces the translocation of SREBP-1 into cytoplasm and negatively regulates its activity as a transcription factor (Peterson et al. 2011). Under high-fat and -cholesterol diet, mTORC1 activity is required for SREBP-1 function to promote fat accumulation and hypercholesterolemia in mice (Peterson et al. 2011). mTORC1 can also modulate the expression level of Hif1 $\alpha$  (hypoxia-inducible factor  $\alpha$ ), which activates numerous hypoxia-induced genes involved in cellular metabolic processes including those involved in glycolysis and glucose uptake (Goldberg et al. 1988; Brugarolas et al. 2003; Duvel et al. 2010).

There is also accumulating evidence that mTORC2 is required in metabolic processes. Knockdown of *riCTOR* in MEFs diminished metabolic activity (Shiota et al. 2006). Furthermore, deficiency of *riCTOR* in Jurkat cells, a leukemic T cell line, increased oxygen consumption (Schieke et al. 2006). However, mTORC2 could play a more complex function in mitochondrial metabolism since a PTEN-deficient cell line that is mTORC2 addicted/IL3-independent was shown to require a number of genes involved in mitochondrial functions (Colombi et al. 2011). Adipose-specific knockout of *riCTOR* in mice revealed that mTORC2 can function to control whole body growth (Cybulski et al. 2009). In these mice, there was increased size of non-adipose organs, such as heart, kidney, spleen, and pancreas (Cybulski et al. 2009). In addition, these mice also displayed hyperinsulinemia and elevated levels of IGF (insulin-like growth factor) and IGFBP3 (IGF binding protein 3) (Cybulski et al. 2009). Conversely, the deletion of *riCTOR* in pancreatic  $\beta$ -cells decreased their proliferation and mass, which led to reduced insulin secretion, hyperglycemia, and glucose intolerance in mice (Gu et al. 2011). Specific effectors of mTORC2 function in metabolism remain to be characterized. Future investigation should reveal how mTORC1 and mTORC2 signaling pathways impinge on metabolic pathways. This would be important in light of understanding how defects in cellular metabolism that occurs in cancer and other pathological conditions are linked to aberrant mTOR signaling.

#### 6.4 Actin cytoskeleton reorganization

The regulation of actin cytoskeleton reorganization is a conserved function of mTORC2. In *S. cerevisiae*, *tor2* mutations or depletion of TORC2 components depolarizes the actin cytoskeleton (Schmidt et al. 1996; Loewith et al. 2002). Normal polarization of actin towards the growing bud controls spatial growth in yeast. In mammals, mTORC2 can control actin polymerization and cell spreading via Rho and Rac, members of the Rho family of GTPases that regulates F-actin assembly (Jacinto et al. 2004). Rex1, a Rac GEF, links mTOR signaling to Rac activation and regulates cell migration (Hernandez-Negrete et al. 2007). PKC $\alpha$ , which is phosphorylated by mTORC2, is also linked to actin cytoskeleton reorganization in mammalian cells (Sarbasov et al. 2004). However, it remains unclear how the mTORC2-

dependent phosphorylation of PKC $\alpha$  can promote the actin reorganization function of this AGC kinase.

Studies using rapamycin have also linked actin cytoskeleton reorganization to the mTORC1 pathway. Rapamycin inhibits the reorganization of F-actin and the phosphorylation of focal adhesion proteins through S6K1 (Berven et al. 2004; Liu et al. 2008). S6K1 is localized to actin stress fibers in fibroblasts (Crouch 1997) and S6K1, Akt, PDK1, and PI3K colocalized with the actin arc, a caveolin-enriched cytoskeletal structure located at the leading edge of migrating Swiss 3T3 cells (Berven et al. 2004). The mTORC1 pathway is also linked to cell motility and migration. Rapamycin inhibits cell motility in several cell types, such as neutrophils (Gomez-Cambronero 2003), vascular smooth muscle cells (Poon et al. 1996), and T-lymphocytes (Finlay & Cantrell 2010). The mTORC1 target, S6K, mediates cell migration via its regulation of focal adhesion formation (Liu et al. 2008), reorganization of F-actin (Berven et al. 2004; Liu et al. 2008), as well as the upregulation of the matrix metalloproteinase 9 (MM9) (Zhou & Wong 2006), and the activity and expression of RhoA (Liu et al. 2010). How mTORC1 or mTORC2 can more directly regulate its effectors in actin cytoskeleton reorganization remains to be examined. Future studies on how the mTORC-mediated function in actin cytoskeleton reorganization is coupled to the other growth-regulatory functions of mTORCs would need to be addressed.

## 7. mTOR inhibitors and therapeutic significance

Due to the central role of mTOR in cell survival, growth and proliferation, deregulation of the mTOR signaling pathway is implicated in many human diseases including benign and malignant tumors, neurological and metabolic disorders and cardiovascular diseases (Pei & Hugon 2008; Krymskaya & Goncharova 2009; Hwang & Kim 2011; Ibraghimov-Beskrovnaya & Natoli 2011). Moreover, its role in organismal aging is highlighted by findings that inhibition of the TOR/mTOR pathway can prolong lifespan in several organisms (Vellai et al. 2003; Kapahi et al. 2004; Medvedik et al. 2007; Harrison et al. 2009). Drawing lessons from rapamycin, numerous mTOR inhibitors have been developed and are currently being refined to achieve more specific inhibition. We discuss some recent findings on the use of these inhibitors at the bench and in the clinic.

Rapamycin and its analogs (rapalogs) are allosteric mTOR inhibitors and form a complex with FKBP12 and mTOR. By binding at the FRB domain of mTOR, the interaction between mTOR and raptor is diminished and can uncouple mTOR from its substrates (Oshiro et al. 2004). Rapamycin and derivatives such as CCI-779 (temsirolimus, Torisel), RAD001 (everolimus), and AP23573 (ridaforolimus) act as cytostatic agents that slow down or arrest the growth of cells derived from several cancer types such as rhabdomyosarcoma (Hosoi et al. 1999), prostate cancer (van der Poel et al. 2003), breast cancer (Pang & Faber 2001), and B-cell lymphoma (Muthukkumar et al. 1995). Early results from clinical trials reveal that they have antiproliferative activity in a subset of cancer, such as endometrial cancer (Oza et al. 2011), pancreatic neuroendocrine tumors (Goldstein & Meyer 2011), gastric cancer (Doi et al. 2010), and malignant glioma (Reardon et al. 2011). However, only a subset of mTORC1 functions is sensitive to rapamycin treatment, hence the antiproliferative properties of this drug can be limited (Wang et al. 2005; Choo et al. 2008; Choo & Blenis 2009). In a number of cell types, the inhibition of mTORC1 results in the upregulation of the PI3K/Akt pathway. Normally, mTORC1 activates S6K1 and the active S6K1 negatively regulates the insulin

receptor substrate-1 (IRS-1) by phosphorylation at serine residues (Zhande et al. 2002; Shah & Hunter 2006; Tzatsos & Kandrор 2006). The inhibition of mTORC1 by rapalogs disrupts this feedback loop and results in increased IRS1 signaling and Akt activity that may compromise the anti-tumor activity of mTOR inhibitors (Harrington et al. 2004; Shah et al. 2004; Sun et al. 2005; O'Reilly et al. 2006). Since mTORC2 is a positive regulator of Akt, several new mTOR inhibitors that can block both mTORC1 and mTORC2 have been developed to more effectively inhibit mTOR signaling and cell proliferation.

The pyrazolopyrimidine analogs PP242 and PP30 are ATP-competitive inhibitors of mTOR that bind to its ATP-binding site and as a result, block the kinase activities of both mTORC1 and mTORC2 (Feldman et al. 2009). PP242 and PP30 both inhibit the mTORC2-induced phosphorylation of Akt at Ser473, indicating that these inhibitors can indeed interfere with mTORC2 functions. Furthermore, these two mTOR kinase domain inhibitors attenuate protein synthesis and proliferation of mouse embryonic fibroblasts (MEFs) (Feldman et al. 2009). PP242 has been shown to induce cyto reduction and apoptosis in multiple myeloma cells (Hoang et al. 2010) and cause death of mouse and human leukemia cells and delay leukemia onset *in vivo* (Janes et al. 2010), highlighting the potential therapeutic application of this compound. Another ATP-competitive mTOR inhibitor, Torin1, induces cell cycle arrest and inhibits cell growth and proliferation more efficiently than rapamycin (Thoreen et al. 2009). Preclinical studies substantiate the therapeutic value of Torin1. For example, Torin1 treatment prevented the anti-inflammatory potency of glucocorticoids both in human monocytes and myeloid dendritic cells (Weichhart et al. 2011). Moreover, Torin1 significantly inhibited the translation of viral proteins during human cytomegalovirus infection (Clippinger et al. 2011). Recently, Torin2, a novel mTOR inhibitor with improved pharmacokinetic properties and synthetic route, has been described (Liu et al. 2011). Torin2 inhibits mTOR complexes with IC<sub>50</sub> of 0.25 nM, compared to Torin1 at IC<sub>50</sub> of 2 to 10 nM. Therefore, Torin2 is suggested to be a more potent and stable mTORC inhibitor than Torin1.

Since mTORC1 inhibition leads to upregulation of IRS1 and subsequently PI3K, which in turn activates Akt, simultaneously blocking the activities of mTOR complexes and PI3K may inhibit cell proliferation and growth more effectively. As a result, several mTOR/PI3K dual inhibitors have been developed. NVP-BEZ235 inhibits PI3K and mTOR kinase activity by interacting with their ATP-binding domains (Maira et al. 2008). It has been implicated in the treatment of non-small cell lung cancer (Konstantinidou et al. 2009), melanoma (Marone et al. 2009), pancreatic cancer (Cao et al. 2009), and acute myeloid leukemia (Chapuis et al. 2010). Given the compensatory mechanisms that cells employ to adapt to growth-inhibitory conditions, it would be important to identify signaling pathways that impinge on the mTOR/PI3K pathway in order to develop combinatorial therapy for preventing malignancy.

## 8. Conclusion

Two decades after the discovery of TOR/mTOR, the function of this protein in orchestrating cellular processes in response to growth signals particularly nutrients has been established. Some key discoveries in the last few years that allowed more extensive analysis of mTOR function and regulation include identification of the mTOR complexes and regulatory proteins that link the mTOR pathway to nutrient and energy responses and the development of mTOR inhibitors. Studies to dissect the systemic function of mTOR

complexes are also gaining momentum with the use of tissue-specific mTORC component knockout mice. There are still numerous outstanding questions that need to be addressed such as the precise regulation of mTOR complexes by nutrients, how it links signals from nutrients to cellular metabolism and other processes, and distinct functions and regulation of mTORCs in different cellular compartments. The recent identification of the myriad possible direct and indirect targets of the mTORCs should provide clues on the mechanisms involved in mTOR functions. Animal models would also provide insights on the role of mTOR in physiological and pathological conditions. Finally, development of specific mTORC1 and mTORC2 inhibitors would not only be useful to determine the distinct functions of these complexes but also would have numerous clinical applications.

## 9. Acknowledgements

We thank Won Jun Oh for comments on this manuscript and acknowledge support from the NIH (GM079176), American Cancer Society (RSG0721601TBE), Cancer Research Institute, and SU2C-AACR-IRG0311 (E.J.).

## 10. References

- Acker, M. G. & Lorsch, J. R. (2008). "Mechanism of ribosomal subunit joining during eukaryotic translation initiation." *Biochemical Society transactions* 36(Pt 4): 653-657.
- Acosta-Jaquez, H. A.; Keller, J. A.; Foster, K. G.; Ekim, B.; Soliman, G. A.; Feener, E. P.; Ballif, B. A. & Fingar, D. C. (2009). "Site-specific mTOR phosphorylation promotes mTORC1-mediated signaling and cell growth." *Molecular and cellular biology* 29(15): 4308-4324.
- Adami, A.; Garcia-Alvarez, B.; Arias-Palomo, E.; Barford, D. & Llorca, O. (2007). "Structure of TOR and its complex with KOG1." *Molecular cell* 27(3): 509-516.
- Aimbetov, R.; Chen, C. H.; Bulgakova, O.; Abetov, D.; Bissenbaev, A. K.; Bersimbaev, R. I. & Sarbassov, D. D. (2011). "Integrity of mTORC2 is dependent on the rictor Gly-934 site." *Oncogene*.
- Alarcon, C. M.; Heitman, J. & Cardenas, M. E. (1999). "Protein kinase activity and identification of a toxic effector domain of the target of rapamycin TOR proteins in yeast." *Molecular biology of the cell* 10(8): 2531-2546.
- Alvarez, B. & Moreno, S. (2006). "Fission yeast Tor2 promotes cell growth and represses cell differentiation." *Journal of cell science* 119(Pt 21): 4475-4485.
- Aronova, S.; Wedaman, K.; Anderson, S.; Yates, J., 3rd & Powers, T. (2007). "Probing the membrane environment of the TOR kinases reveals functional interactions between TORC1, actin, and membrane trafficking in *Saccharomyces cerevisiae*." *Molecular biology of the cell* 18(8): 2779-2794.
- Beck, T. & Hall, M. N. (1999). "The TOR signalling pathway controls nuclear localization of nutrient-regulated transcription factors." *Nature* 402(6762): 689-692.
- Bentzinger, C. F.; Romanino, K.; Cloetta, D.; Lin, S.; Mascarenhas, J. B.; Oliveri, F.; Xia, J.; Casanova, E.; Costa, C. F.; Brink, M.; Zorzato, F.; Hall, M. N. & Rugg, M. A. (2008). "Skeletal muscle-specific ablation of raptor, but not of rictor, causes metabolic changes and results in muscle dystrophy." *Cell metabolism* 8(5): 411-424.
- Berven, L. A.; Willard, F. S. & Crouch, M. F. (2004). "Role of the p70(S6K) pathway in regulating the actin cytoskeleton and cell migration." *Exp Cell Res* 296(2): 183-195.

- Binda, M.; Peli-Gulli, M. P.; Bonfils, G.; Panchaud, N.; Urban, J.; Sturgill, T. W.; Loewith, R. & De Virgilio, C. (2009). "The Vam6 GEF controls TORC1 by activating the EGO complex." *Molecular cell* 35(5): 563-573.
- Bosotti, R.; Isacchi, A. & Sonnhammer, E. L. (2000). "FAT: a novel domain in PIK-related kinases." *Trends in biochemical sciences* 25(5): 225-227.
- Boulbes, D.; Chen, C. H.; Shaikenov, T.; Agarwal, N. K.; Peterson, T. R.; Addona, T. A.; Keshishian, H.; Carr, S. A.; Magnuson, M. A.; Sabatini, D. M. & Sarbassov, D. (2010). "Rictor phosphorylation on the Thr-1135 site does not require mammalian target of rapamycin complex 2." *Molecular cancer research : MCR* 8(6): 896-906.
- Boulbes, D. R.; Shaiken, T. & Sarbassov, D. (2011). "Endoplasmic reticulum is a main localization site of mTORC2." *Biochemical and biophysical research communications* 413(1): 46-52.
- Brown, E. J.; Albers, M. W.; Shin, T. B.; Ichikawa, K.; Keith, C. T.; Lane, W. S. & Schreiber, S. L. (1994). "A mammalian protein targeted by G1-arresting rapamycin-receptor complex." *Nature* 369(6483): 756-758.
- Brugarolas, J. B.; Vazquez, F.; Reddy, A.; Sellers, W. R. & Kaelin, W. G., Jr. (2003). "TSC2 regulates VEGF through mTOR-dependent and -independent pathways." *Cancer cell* 4(2): 147-158.
- Brunn, G. J.; Fadden, P.; Haystead, T. A. & Lawrence, J. C., Jr. (1997). "The mammalian target of rapamycin phosphorylates sites having a (Ser/Thr)-Pro motif and is activated by antibodies to a region near its COOH terminus." *The Journal of biological chemistry* 272(51): 32547-32550.
- Bussiere, C. T.; Lakey, J. R.; Shapiro, A. M. & Korbitt, G. S. (2006). "The impact of the mTOR inhibitor sirolimus on the proliferation and function of pancreatic islets and ductal cells." *Diabetologia* 49(10): 2341-2349.
- Byfield, M. P.; Murray, J. T. & Backer, J. M. (2005). "hVps34 is a nutrient-regulated lipid kinase required for activation of p70 S6 kinase." *The Journal of biological chemistry* 280(38): 33076-33082.
- Cao, P.; Maira, S. M.; Garcia-Echeverria, C. & Hedley, D. W. (2009). "Activity of a novel, dual PI3-kinase/mTOR inhibitor NVP-BEZ235 against primary human pancreatic cancers grown as orthotopic xenografts." *Br J Cancer* 100(8): 1267-1276.
- Carayol, N.; Vakana, E.; Sassano, A.; Kaur, S.; Goussetis, D. J.; Glaser, H.; Druker, B. J.; Donato, N. J.; Altman, J. K.; Barr, S. & Plataniias, L. C. (2010). "Critical roles for mTORC2- and rapamycin-insensitive mTORC1-complexes in growth and survival of BCR-ABL-expressing leukemic cells." *Proceedings of the National Academy of Sciences of the United States of America* 107(28): 12469-12474.
- Cardenas, M. E.; Cutler, N. S.; Lorenz, M. C.; Di Como, C. J. & Heitman, J. (1999). "The TOR signaling cascade regulates gene expression in response to nutrients." *Genes & development* 13(24): 3271-3279.
- Carriere, A.; Cargnello, M.; Julien, L. A.; Gao, H.; Bonneil, E.; Thibault, P. & Roux, P. P. (2008). "Oncogenic MAPK signaling stimulates mTORC1 activity by promoting RSK-mediated raptor phosphorylation." *Current biology : CB* 18(17): 1269-1277.
- Castro, A. F.; Rebhun, J. F.; Clark, G. J. & Quilliam, L. A. (2003). "Rheb binds tuberous sclerosis complex 2 (TSC2) and promotes S6 kinase activation in a rapamycin- and farnesylation-dependent manner." *The Journal of biological chemistry* 278(35): 32493-32496.

- Chapuis, N.; Tamburini, J.; Green, A. S.; Vignon, C.; Bardet, V.; Neyret, A.; Pannetier, M.; Willems, L.; Park, S.; Macone, A.; Maira, S. M.; Ifrah, N.; Dreyfus, F.; Herault, O.; Lacombe, C.; Mayeux, P. & Bouscary, D. (2010). "Dual inhibition of PI3K and mTORC1/2 signaling by NVP-BEZ235 as a new therapeutic strategy for acute myeloid leukemia." *Clin Cancer Res* 16(22): 5424-5435.
- Chen, C. H. & Sarbassov, D. D. (2011). "Integrity of mTORC2 is dependent on phosphorylation of SIN1 by mTOR." *The Journal of biological chemistry*.
- Chen, E. J. & Kaiser, C. A. (2003). "LST8 negatively regulates amino acid biosynthesis as a component of the TOR pathway." *The Journal of cell biology* 161(2): 333-347.
- Chen, J.; Zheng, X. F.; Brown, E. J. & Schreiber, S. L. (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): 4947-4951.
- Cheng, J.; Zhang, D.; Kim, K.; Zhao, Y. & Su, B. (2005). "Mip1, an MEKK2-interacting protein, controls MEKK2 dimerization and activation." *Molecular and cellular biology* 25(14): 5955-5964.
- Chiang, G. G. & Abraham, R. T. (2005). "Phosphorylation of mammalian target of rapamycin (mTOR) at Ser-2448 is mediated by p70S6 kinase." *The Journal of biological chemistry* 280(27): 25485-25490.
- Choi, J.; Chen, J.; Schreiber, S. L. & Clardy, J. (1996). "Structure of the FKBP12-rapamycin complex interacting with the binding domain of human FRAP." *Science* 273(5272): 239-242.
- Choo, A. Y. & Blenis, J. (2009). "Not all substrates are treated equally: implications for mTOR, rapamycin-resistance and cancer therapy." *Cell Cycle* 8(4): 567-572.
- Choo, A. Y.; Yoon, S. O.; Kim, S. G.; Roux, P. P. & Blenis, J. (2008). "Rapamycin differentially inhibits S6Ks and 4E-BP1 to mediate cell-type-specific repression of mRNA translation." *Proceedings of the National Academy of Sciences of the United States of America* 105(45): 17414-17419.
- Clippinger, A. J.; Maguire, T. G. & Alwine, J. C. (2011). "The changing role of mTOR kinase in the maintenance of protein synthesis during human cytomegalovirus infection." *Journal of virology* 85(8): 3930-3939.
- Colombi, M.; Molle, K. D.; Benjamin, D.; Rattenbacher-Kiser, K.; Schaefer, C.; Betz, C.; Thiemeyer, A.; Regenass, U.; Hall, M. N. & Moroni, C. (2011). "Genome-wide shRNA screen reveals increased mitochondrial dependence upon mTORC2 addiction." *Oncogene* 30(13): 1551-1565.
- Copp, J.; Manning, G. & Hunter, T. (2009). "TORC-specific phosphorylation of mammalian target of rapamycin (mTOR): phospho-Ser2481 is a marker for intact mTOR signaling complex 2." *Cancer research* 69(5): 1821-1827.
- Corradetti, M. N.; Inoki, K.; Bardeesy, N.; DePinho, R. A. & Guan, K. L. (2004). "Regulation of the TSC pathway by LKB1: evidence of a molecular link between tuberous sclerosis complex and Peutz-Jeghers syndrome." *Genes & development* 18(13): 1533-1538.
- Crouch, M. F. (1997). "Regulation of thrombin-induced stress fibre formation in Swiss 3T3 cells by the 70-kDa S6 kinase." *Biochem Biophys Res Commun* 233(1): 193-199.
- Cunningham, J. T.; Rodgers, J. T.; Arlow, D. H.; Vazquez, F.; Mootha, V. K. & Puigserver, P. (2007). "mTOR controls mitochondrial oxidative function through a YY1-PGC-1 $\alpha$  transcriptional complex." *Nature* 450(7170): 736-740.



- Cybulski, N.; Polak, P.; Auwerx, J.; Ruegg, M. A. & Hall, M. N. (2009). "mTOR complex 2 in adipose tissue negatively controls whole-body growth." *Proceedings of the National Academy of Sciences of the United States of America* 106(24): 9902-9907.
- Dames, S. A.; Mulet, J. M.; Rathgeb-Szabo, K.; Hall, M. N. & Grzesiek, S. (2005). "The solution structure of the FATC domain of the protein kinase target of rapamycin suggests a role for redox-dependent structural and cellular stability." *The Journal of biological chemistry* 280(21): 20558-20564.
- Delgoffe, G. M.; Kole, T. P.; Zheng, Y.; Zarek, P. E.; Matthews, K. L.; Xiao, B.; Worley, P. F.; Kozma, S. C. & Powell, J. D. (2009). "The mTOR kinase differentially regulates effector and regulatory T cell lineage commitment." *Immunity* 30(6): 832-844.
- Diaz-Troya, S.; Perez-Perez, M. E.; Florencio, F. J. & Crespo, J. L. (2008). "The role of TOR in autophagy regulation from yeast to plants and mammals." *Autophagy* 4(7): 851-865.
- Dibble, C. C.; Asara, J. M. & Manning, B. D. (2009). "Characterization of Rictor phosphorylation sites reveals direct regulation of mTOR complex 2 by S6K1." *Molecular and cellular biology* 29(21): 5657-5670.
- Doi, T.; Muro, K.; Boku, N.; Yamada, Y.; Nishina, T.; Takiuchi, H.; Komatsu, Y.; Hamamoto, Y.; Ohno, N.; Fujita, Y.; Robson, M. & Ohtsu, A. (2010). "Multicenter phase II study of everolimus in patients with previously treated metastatic gastric cancer." *J Clin Oncol* 28(11): 1904-1910.
- Dorrello, N. V.; Peschiaroli, A.; Guardavaccaro, D.; Colburn, N. H.; Sherman, N. E. & Pagano, M. (2006). "S6K1- and betaTRCP-mediated degradation of PDCD4 promotes protein translation and cell growth." *Science* 314(5798): 467-471.
- Dowling, R. J.; Topisirovic, I.; Alain, T.; Bidinosti, M.; Fonseca, B. D.; Petroulakis, E.; Wang, X.; Larsson, O.; Selvaraj, A.; Liu, Y.; Kozma, S. C.; Thomas, G. & Sonenberg, N. (2010). "mTORC1-mediated cell proliferation, but not cell growth, controlled by the 4E-BPs." *Science* 328(5982): 1172-1176.
- Duan, S.; Skaar, J. R.; Kuchay, S.; Toschi, A.; Kanarek, N.; Ben-Neriah, Y. & Pagano, M. (2011). "mTOR Generates an Auto-Amplification Loop by Triggering the betaTrCP- and CK1alpha-Dependent Degradation of DEPTOR." *Molecular cell* 44(2): 317-324.
- Dubouloz, F.; Deloche, O.; Wanke, V.; Cameroni, E. & De Virgilio, C. (2005). "The TOR and EGO protein complexes orchestrate microautophagy in yeast." *Molecular cell* 19(1): 15-26.
- Dunn, T. M.; Haak, D.; Monaghan, E. & Beeler, T. J. (1998). "Synthesis of monohydroxylated inositolphosphorylceramide (IPC-C) in *Saccharomyces cerevisiae* requires Scs7p, a protein with both a cytochrome b5-like domain and a hydroxylase/desaturase domain." *Yeast* 14(4): 311-321.
- Duran, A.; Amanchy, R.; Linares, J. F.; Joshi, J.; Abu-Baker, S.; Porollo, A.; Hansen, M.; Moscat, J. & Diaz-Meco, M. T. (2011). "p62 Is a Key Regulator of Nutrient Sensing in the mTORC1 Pathway." *Molecular cell* 44(1): 134-146.
- Duvel, K.; Yecies, J. L.; Menon, S.; Raman, P.; Lipovsky, A. I.; Souza, A. L.; Triantafellow, E.; Ma, Q.; Gorski, R.; Cleaver, S.; Vander Heiden, M. G.; MacKeigan, J. P.; Finan, P. M.; Clish, C. B.; Murphy, L. O. & Manning, B. D. (2010). "Activation of a metabolic gene regulatory network downstream of mTOR complex 1." *Molecular cell* 39(2): 171-183.
- English, D. (1996). "Phosphatidic acid: a lipid messenger involved in intracellular and extracellular signalling." *Cellular signalling* 8(5): 341-347.

- Evangelisti, C.; Ricci, F.; Tazzari, P.; Tabellini, G.; Battistelli, M.; Falcieri, E.; Chiarini, F.; Bortul, R.; Melchionda, F.; Pagliaro, P.; Pession, A.; McCubrey, J. A. & Martelli, A. M. (2011). "Targeted inhibition of mTORC1 and mTORC2 by active-site mTOR inhibitors has cytotoxic effects in T-cell acute lymphoblastic leukemia." *Leukemia : official journal of the Leukemia Society of America, Leukemia Research Fund, U.K* 25(5): 781-791.
- Facchinetti, V.; Ouyang, W.; Wei, H.; Soto, N.; Lazorchak, A.; Gould, C.; Lowry, C.; Newton, A. C.; Mao, Y.; Miao, R. Q.; Sessa, W. C.; Qin, J.; Zhang, P.; Su, B. & Jacinto, E. (2008). "The mammalian target of rapamycin complex 2 controls folding and stability of Akt and protein kinase C." *The EMBO journal* 27(14): 1932-1943.
- Fadri, M.; Daquinag, A.; Wang, S.; Xue, T. & Kunz, J. (2005). "The pleckstrin homology domain proteins Slm1 and Slm2 are required for actin cytoskeleton organization in yeast and bind phosphatidylinositol-4,5-bisphosphate and TORC2." *Molecular biology of the cell* 16(4): 1883-1900.
- Fang, Y.; Vilella-Bach, M.; Bachmann, R.; Flanigan, A. & Chen, J. (2001). "Phosphatidic acid-mediated mitogenic activation of mTOR signaling." *Science* 294(5548): 1942-1945.
- Feldman, M. E.; Apsel, B.; Uotila, A.; Loewith, R.; Knight, Z. A.; Ruggero, D. & Shokat, K. M. (2009). "Active-site inhibitors of mTOR target rapamycin-resistant outputs of mTORC1 and mTORC2." *PLoS biology* 7(2): e38.
- Finlay, D. & Cantrell, D. (2010). "Phosphoinositide 3-kinase and the mammalian target of rapamycin pathways control T cell migration." *Ann N Y Acad Sci* 1183: 149-157.
- Frias, M. A.; Thoreen, C. C.; Jaffe, J. D.; Schroder, W.; Sculley, T.; Carr, S. A. & Sabatini, D. M. (2006). "mSin1 is necessary for Akt/PKB phosphorylation, and its isoforms define three distinct mTORC2s." *Current biology : CB* 16(18): 1865-1870.
- Gangloff, Y. G.; Mueller, M.; Dann, S. G.; Svoboda, P.; Sticker, M.; Spetz, J. F.; Um, S. H.; Brown, E. J.; Cereghini, S.; Thomas, G. & Kozma, S. C. (2004). "Disruption of the mouse mTOR gene leads to early postimplantation lethality and prohibits embryonic stem cell development." *Molecular and cellular biology* 24(21): 9508-9516.
- Ganley, I. G.; Lam du, H.; Wang, J.; Ding, X.; Chen, S. & Jiang, X. (2009). "ULK1.ATG13.FIP200 complex mediates mTOR signaling and is essential for autophagy." *The Journal of biological chemistry* 284(18): 12297-12305.
- Gao, D.; Inuzuka, H.; Tan, M. K.; Fukushima, H.; Locasale, J. W.; Liu, P.; Wan, L.; Zhai, B.; Chin, Y. R.; Shaik, S.; Lyssiotis, C. A.; Gygi, S. P.; Toker, A.; Cantley, L. C.; Asara, J. M.; Harper, J. W. & Wei, W. (2011). "mTOR Drives Its Own Activation via SCF(betaTrCP)-Dependent Degradation of the mTOR Inhibitor DEPTOR." *Molecular cell* 44(2): 290-303.
- Gao, D.; Wan, L.; Inuzuka, H.; Berg, A. H.; Tseng, A.; Zhai, B.; Shaik, S.; Bennett, E.; Tron, A. E.; Gasser, J. A.; Lau, A.; Gygi, S. P.; Harper, J. W.; DeCaprio, J. A.; Toker, A. & Wei, W. (2010). "Rictor forms a complex with Cullin-1 to promote SGK1 ubiquitination and destruction." *Molecular cell* 39(5): 797-808.
- Gao, X.; Zhang, Y.; Arrazola, P.; Hino, O.; Kobayashi, T.; Yeung, R. S.; Ru, B. & Pan, D. (2002). "Tsc tumour suppressor proteins antagonize amino-acid-TOR signalling." *Nature cell biology* 4(9): 699-704.
- Garami, A.; Zwartkruis, F. J.; Nobukuni, T.; Joaquin, M.; Rocco, M.; Stocker, H.; Kozma, S. C.; Hafen, E.; Bos, J. L. & Thomas, G. (2003). "Insulin activation of Rheb, a mediator

- of mTOR/S6K/4E-BP signaling, is inhibited by TSC1 and 2." *Molecular cell* 11(6): 1457-1466.
- Garcia-Martinez, J. M.&Alessi, D. R. (2008). "mTOR complex 2 (mTORC2) controls hydrophobic motif phosphorylation and activation of serum- and glucocorticoid-induced protein kinase 1 (SGK1)." *The Biochemical journal* 416(3): 375-385.
- Ghosh, D.; Srivastava, G. P.; Xu, D.; Schulz, L. C.&Roberts, R. M. (2008). "A link between SIN1 (MAPKAP1) and poly(rC) binding protein 2 (PCBP2) in counteracting environmental stress." *Proceedings of the National Academy of Sciences of the United States of America* 105(33): 11673-11678.
- Gingras, A. C.; Gygi, S. P.; Raught, B.; Polakiewicz, R. D.; Abraham, R. T.; Hoekstra, M. F.; Aebersold, R.&Sonenberg, N. (1999). "Regulation of 4E-BP1 phosphorylation: a novel two-step mechanism." *Genes & development* 13(11): 1422-1437.
- Gingras, A. C.; Raught, B.; Gygi, S. P.; Niedzwiecka, A.; Miron, M.; Burley, S. K.; Polakiewicz, R. D.; Wyslouch-Cieszynska, A.; Aebersold, R.&Sonenberg, N. (2001). "Hierarchical phosphorylation of the translation inhibitor 4E-BP1." *Genes & development* 15(21): 2852-2864.
- Goldberg, M. A.; Dunning, S. P.&Bunn, H. F. (1988). "Regulation of the erythropoietin gene: evidence that the oxygen sensor is a heme protein." *Science* 242(4884): 1412-1415.
- Goldstein, R.&Meyer, T. (2011). "Role of everolimus in pancreatic neuroendocrine tumors." *Expert Rev Anticancer Ther* 11(11): 1653-1665.
- Gomez-Cambronero, J. (2003). "Rapamycin inhibits GM-CSF-induced neutrophil migration." *FEBS Lett* 550(1-3): 94-100.
- Green, A. J.; Smith, M.&Yates, J. R. (1994). "Loss of heterozygosity on chromosome 16p13.3 in hamartomas from tuberous sclerosis patients." *Nature genetics* 6(2): 193-196.
- Groves, M. R.&Barford, D. (1999). "Topological characteristics of helical repeat proteins." *Current opinion in structural biology* 9(3): 383-389.
- Gu, Y.; Lindner, J.; Kumar, A.; Yuan, W.&Magnuson, M. A. (2011). "Rictor/mTORC2 is essential for maintaining a balance between beta-cell proliferation and cell size." *Diabetes* 60(3): 827-837.
- Guertin, D. A.; Stevens, D. M.; Thoreen, C. C.; Burds, A. A.; Kalaany, N. Y.; Moffat, J.; Brown, M.; Fitzgerald, K. J.&Sabatini, D. M. (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): 859-871.
- Gwinn, D. M.; Shackelford, D. B.; Egan, D. F.; Mihaylova, M. M.; Mery, A.; Vasquez, D. S.; Turk, B. E.&Shaw, R. J. (2008). "AMPK phosphorylation of raptor mediates a metabolic checkpoint." *Molecular cell* 30(2): 214-226.
- Hall, M. N. (1996). "The TOR signalling pathway and growth control in yeast." *Biochemical Society transactions* 24(1): 234-239.
- Hanks, S. K.&Hunter, T. (1995). "Protein kinases 6. The eukaryotic protein kinase superfamily: kinase (catalytic) domain structure and classification." *The FASEB journal : official publication of the Federation of American Societies for Experimental Biology* 9(8): 576-596.
- Hannan, K. M.; Brandenburger, Y.; Jenkins, A.; Sharkey, K.; Cavanaugh, A.; Rothblum, L.; Moss, T.; Poortinga, G.; McArthur, G. A.; Pearson, R. B.&Hannan, R. D. (2003). "mTOR-dependent regulation of ribosomal gene transcription requires S6K1 and is

- mediated by phosphorylation of the carboxy-terminal activation domain of the nucleolar transcription factor UBF." *Molecular and cellular biology* 23(23): 8862-8877.
- Hara, K.; Maruki, Y.; Long, X.; Yoshino, K.; Oshiro, N.; Hidayat, S.; Tokunaga, C.; Avruch, J. & Yonezawa, K. (2002). "Raptor, a binding partner of target of rapamycin (TOR), mediates TOR action." *Cell* 110(2): 177-189.
- Hardt, M.; Chantaravisoot, N. & Tamanoi, F. (2011). "Activating mutations of TOR (target of rapamycin)." *Genes to cells: devoted to molecular & cellular mechanisms* 16(2): 141-151.
- Hardwick, J. S.; Kuruvilla, F. G.; Tong, J. K.; Shamji, A. F. & Schreiber, S. L. (1999). "Rapamycin-modulated transcription defines the subset of nutrient-sensitive signaling pathways directly controlled by the Tor proteins." *Proceedings of the National Academy of Sciences of the United States of America* 96(26): 14866-14870.
- Harrington, L. S.; Findlay, G. M.; Gray, A.; Tolkacheva, T.; Wigfield, S.; Rebholz, H.; Barnett, J.; Leslie, N. R.; Cheng, S.; Shepherd, P. R.; Gout, I.; Downes, C. P. & Lamb, R. F. (2004). "The TSC1-2 tumor suppressor controls insulin-PI3K signaling via regulation of IRS proteins." *The Journal of cell biology* 166(2): 213-223.
- Harrison, D. E.; Strong, R.; Sharp, Z. D.; Nelson, J. F.; Astle, C. M.; Flurkey, K.; Nadon, N. L.; Wilkinson, J. E.; Frenkel, K.; Carter, C. S.; Pahor, M.; Javors, M. A.; Fernandez, E. & Miller, R. A. (2009). "Rapamycin fed late in life extends lifespan in genetically heterogeneous mice." *Nature* 460(7253): 392-395.
- Hartwell, L. H.; Hopfield, J. J.; Leibler, S. & Murray, A. W. (1999). "From molecular to modular cell biology." *Nature* 402(6761 Suppl): C47-52.
- Hay, N. & Sonenberg, N. (2004). "Upstream and downstream of mTOR." *Genes & development* 18(16): 1926-1945.
- Heitman, J.; Movva, N. R. & Hall, M. N. (1991). "Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast." *Science* 253(5022): 905-909.
- Helliwell, S. B.; Wagner, P.; Kunz, J.; Deuter-Reinhard, M.; Henriquez, R. & Hall, M. N. (1994). "TOR1 and TOR2 are structurally and functionally similar but not identical phosphatidylinositol kinase homologues in yeast." *Molecular biology of the cell* 5(1): 105-118.
- Hernandez-Negrete, I.; Carretero-Ortega, J.; Rosenfeldt, H.; Hernandez-Garcia, R.; Calderon-Salinas, J. V.; Reyes-Cruz, G.; Gutkind, J. S. & Vazquez-Prado, J. (2007). "P-Rex1 links mammalian target of rapamycin signaling to Rac activation and cell migration." *J Biol Chem* 282(32): 23708-23715.
- Heublein, S.; Kazi, S.; Ogmundsdottir, M. H.; Attwood, E. V.; Kala, S.; Boyd, C. A.; Wilson, C. & Goberdhan, D. C. (2010). "Proton-assisted amino-acid transporters are conserved regulators of proliferation and amino-acid-dependent mTORC1 activation." *Oncogene* 29(28): 4068-4079.
- Ho, H. L.; Lee, H. Y.; Liao, H. C. & Chen, M. Y. (2008). "Involvement of *Saccharomyces cerevisiae* Avo3p/Tsc11p in maintaining TOR complex 2 integrity and coupling to downstream signaling." *Eukaryotic cell* 7(8): 1328-1343.
- Hoang, B.; Frost, P.; Shi, Y.; Belanger, E.; Benavides, A.; Pezeshkpour, G.; Cappia, S.; Guglielmelli, T.; Gera, J. & Lichtenstein, A. (2010). "Targeting TORC2 in multiple myeloma with a new mTOR kinase inhibitor." *Blood* 116(22): 4560-4568.
- Holz, M. K. & 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): 26089-26093.

- Horejsi, Z.; Takai, H.; Adelman, C. A.; Collis, S. J.; Flynn, H.; Maslen, S.; Skehel, J. M.; de Lange, T. & Boulton, S. J. (2010). "CK2 phospho-dependent binding of R2TP complex to TEL2 is essential for mTOR and SMG1 stability." *Molecular cell* 39(6): 839-850.
- Hosoi, H.; Dilling, M. B.; Shikata, T.; Liu, L. N.; Shu, L.; Ashmun, R. A.; Germain, G. S.; Abraham, R. T. & Houghton, P. J. (1999). "Rapamycin causes poorly reversible inhibition of mTOR and induces p53-independent apoptosis in human rhabdomyosarcoma cells." *Cancer Res* 59(4): 886-894.
- Hosokawa, N.; Hara, T.; Kaizuka, T.; Kishi, C.; Takamura, A.; Miura, Y.; Iemura, S.; Natsume, T.; Takehana, K.; Yamada, N.; Guan, J. L.; Oshiro, N. & Mizushima, N. (2009). "Nutrient-dependent mTORC1 association with the ULK1-Atg13-FIP200 complex required for autophagy." *Molecular biology of the cell* 20(7): 1981-1991.
- Hresko, R. C. & Mueckler, M. (2005). "mTOR.RICTOR is the Ser473 kinase for Akt/protein kinase B in 3T3-L1 adipocytes." *The Journal of biological chemistry* 280(49): 40406-40416.
- Hsu, P. P.; Kang, S. A.; Rameseder, J.; Zhang, Y.; Ottina, K. A.; Lim, D.; Peterson, T. R.; Choi, Y.; Gray, N. S.; Yaffe, M. B.; Marto, J. A. & Sabatini, D. M. (2011). "The mTOR-regulated phosphoproteome reveals a mechanism of mTORC1-mediated inhibition of growth factor signaling." *Science* 332(6035): 1317-1322.
- Huang, S. & Houghton, P. J. (2003). "Targeting mTOR signaling for cancer therapy." *Current opinion in pharmacology* 3(4): 371-377.
- Hwang, S. K. & Kim, H. H. (2011). "The functions of mTOR in ischemic diseases." *BMB Rep* 44(8): 506-511.
- Ibraghimov-Beskrovnaia, O. & Natoli, T. A. (2011). "mTOR signaling in polycystic kidney disease." *Trends Mol Med*.
- Ikenoue, T.; Inoki, K.; Yang, Q.; Zhou, X. & Guan, K. L. (2008). "Essential function of TORC2 in PKC and Akt turn motif phosphorylation, maturation and signalling." *The EMBO journal* 27(14): 1919-1931.
- Inoki, K.; Zhu, T. & Guan, K. L. (2003). "TSC2 mediates cellular energy response to control cell growth and survival." *Cell* 115(5): 577-590.
- Jacinto, E. (2008). "What controls TOR?" *IUBMB life* 60(8): 483-496.
- Jacinto, E.; Facchinetti, V.; Liu, D.; Soto, N.; Wei, S.; Jung, S. Y.; Huang, Q.; Qin, J. & Su, B. (2006). "SIN1/MIP1 maintains rictor-mTOR complex integrity and regulates Akt phosphorylation and substrate specificity." *Cell* 127(1): 125-137.
- Jacinto, E.; Loewith, R.; Schmidt, A.; Lin, S.; Ruegg, M. A.; Hall, A. & Hall, M. N. (2004). "Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive." *Nature cell biology* 6(11): 1122-1128.
- Jacinto, E. & Lorberg, A. (2008). "TOR regulation of AGC kinases in yeast and mammals." *The Biochemical journal* 410(1): 19-37.
- Janes, M. R.; Limon, J. J.; So, L.; Chen, J.; Lim, R. J.; Chavez, M. A.; Vu, C.; Lilly, M. B.; Mallya, S.; Ong, S. T.; Konopleva, M.; Martin, M. B.; Ren, P.; Liu, Y.; Rommel, C. & Fruman, D. A. (2010). "Effective and selective targeting of leukemia cells using a TORC1/2 kinase inhibitor." *Nat Med* 16(2): 205-213.
- Janku, F.; McConkey, D. J.; Hong, D. S. & Kurzrock, R. (2011). "Autophagy as a target for anticancer therapy." *Nat Rev Clin Oncol* 8(9): 528-539.

- Julien, L. A.; Carriere, A.; Moreau, J.&Roux, P. P. (2010). "mTORC1-activated S6K1 phosphorylates Rictor on threonine 1135 and regulates mTORC2 signaling." *Molecular and cellular biology* 30(4): 908-921.
- Jung, C. H.; Jun, C. B.; Ro, S. H.; Kim, Y. M.; Otto, N. M.; Cao, J.; Kundu, M.&Kim, D. H. (2009). "ULK-Atg13-FIP200 complexes mediate mTOR signaling to the autophagy machinery." *Molecular biology of the cell* 20(7): 1992-2003.
- Kaizuka, T.; Hara, T.; Oshiro, N.; Kikkawa, U.; Yonezawa, K.; Takehana, K.; Iemura, S.; Natsume, T.&Mizushima, N. (2010). "Tti1 and Tel2 are critical factors in mammalian target of rapamycin complex assembly." *J Biol Chem* 285(26): 20109-20116.
- Kaizuka, T.; Hara, T.; Oshiro, N.; Kikkawa, U.; Yonezawa, K.; Takehana, K.; Iemura, S.; Natsume, T.&Mizushima, N. (2010). "Tti1 and Tel2 are critical factors in mammalian target of rapamycin complex assembly." *The Journal of biological chemistry* 285(26): 20109-20116.
- Kamada, Y.; Funakoshi, T.; Shintani, T.; Nagano, K.; Ohsumi, M.&Ohsumi, Y. (2000). "Tor-mediated induction of autophagy via an Apg1 protein kinase complex." *The Journal of cell biology* 150(6): 1507-1513.
- Kamada, Y.; Yoshino, K.; Kondo, C.; Kawamata, T.; Oshiro, N.; Yonezawa, K.&Ohsumi, Y. (2010). "Tor directly controls the Atg1 kinase complex to regulate autophagy." *Molecular and cellular biology* 30(4): 1049-1058.
- Kapahi, P.; Zid, B. M.; Harper, T.; Koslover, D.; Sapin, V.&Benzer, S. (2004). "Regulation of lifespan in *Drosophila* by modulation of genes in the TOR signaling pathway." *Current biology : CB* 14(10): 885-890.
- Keith, C. T.&Schreiber, S. L. (1995). "PIK-related kinases: DNA repair, recombination, and cell cycle checkpoints." *Science* 270(5233): 50-51.
- Kijanska, M.; Dohnal, I.; Reiter, W.; Kaspar, S.; Stoffel, I.; Ammerer, G.; Kraft, C.&Peter, M. (2010). "Activation of Atg1 kinase in autophagy by regulated phosphorylation." *Autophagy* 6(8): 1168-1178.
- Kim, D. H.; Sarbassov, D. D.; Ali, S. M.; King, J. E.; Latek, R. R.; Erdjument-Bromage, H.; Tempst, P.&Sabatini, D. M. (2002). "mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery." *Cell* 110(2): 163-175.
- Kim, D. H.; Sarbassov, D. D.; Ali, S. M.; Latek, R. R.; Guntur, K. V.; Erdjument-Bromage, H.; Tempst, P.&Sabatini, D. M. (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.
- Knutson, B. A. (2010). "Insights into the domain and repeat architecture of target of rapamycin." *Journal of structural biology* 170(2): 354-363.
- Konstantinidou, G.; Bey, E. A.; Rabellino, A.; Schuster, K.; Maira, M. S.; Gazdar, A. F.; Amici, A.; Boothman, D. A.&Scaglioni, P. P. (2009). "Dual phosphoinositide 3-kinase/mammalian target of rapamycin blockade is an effective radiosensitizing strategy for the treatment of non-small cell lung cancer harboring K-RAS mutations." *Cancer Res* 69(19): 7644-7652.
- Krymskaya, V. P.&Goncharova, E. A. (2009). "PI3K/mTORC1 activation in hamartoma syndromes: therapeutic prospects." *Cell Cycle* 8(3): 403-413.

- Kunz, J.; Loeschmann, A.; Deuter-Reinhard, M. & Hall, M. N. (2000). "FAP1, a homologue of human transcription factor NF-X1, competes with rapamycin for binding to FKBP12 in yeast." *Molecular microbiology* 37(6): 1480-1493.
- Kunz, J.; Schneider, U.; Howald, I.; Schmidt, A. & Hall, M. N. (2000). "HEAT repeats mediate plasma membrane localization of Tor2p in yeast." *The Journal of biological chemistry* 275(47): 37011-37020.
- Lempiainen, H. & Halazonetis, T. D. (2009). "Emerging common themes in regulation of PIKKs and PI3Ks." *The EMBO journal* 28(20): 3067-3073.
- Li, H.; Tsang, C. K.; Watkins, M.; Bertram, P. G. & Zheng, X. F. (2006). "Nutrient regulates Tor1 nuclear localization and association with rDNA promoter." *Nature* 442(7106): 1058-1061.
- Liu, L.; Chen, L.; Chung, J. & Huang, S. (2008). "Rapamycin inhibits F-actin reorganization and phosphorylation of focal adhesion proteins." *Oncogene* 27(37): 4998-5010.
- Liu, L.; Luo, Y.; Chen, L.; Shen, T.; Xu, B.; Chen, W.; Zhou, H.; Han, X. & Huang, S. (2010). "Rapamycin inhibits cytoskeleton reorganization and cell motility by suppressing RhoA expression and activity." *J Biol Chem* 285(49): 38362-38373.
- Liu, Q.; Wang, J.; Kang, S. A.; Thoreen, C. C.; Hur, W.; Ahmed, T.; Sabatini, D. M. & Gray, N. S. (2011). "Discovery of 9-(6-aminopyridin-3-yl)-1-(3-(trifluoromethyl)phenyl)benzo[h][1,6]naphthyridin-2(1H)-one (Torin2) as a potent, selective, and orally available mammalian target of rapamycin (mTOR) inhibitor for treatment of cancer." *J Med Chem* 54(5): 1473-1480.
- Liu, X. & Zheng, X. F. (2007). "Endoplasmic reticulum and Golgi localization sequences for mammalian target of rapamycin." *Molecular biology of the cell* 18(3): 1073-1082.
- Liu, Z.; Sekito, T.; Epstein, C. B. & Butow, R. A. (2001). "RTG-dependent mitochondria to nucleus signaling is negatively regulated by the seven WD-repeat protein Lst8p." *The EMBO journal* 20(24): 7209-7219.
- Lochhead, P. A. (2009). "Protein kinase activation loop autophosphorylation in cis: overcoming a Catch-22 situation." *Science signaling* 2(54): pe4.
- Loewith, R.; Jacinto, E.; Wullschlegel, S.; Lorberg, A.; Crespo, J. L.; Bonenfant, D.; Oppliger, W.; Jenoe, P. & Hall, M. N. (2002). "Two TOR complexes, only one of which is rapamycin sensitive, have distinct roles in cell growth control." *Molecular cell* 10(3): 457-468.
- Long, X.; Lin, Y.; Ortiz-Vega, S.; Yonezawa, K. & Avruch, J. (2005). "Rheb binds and regulates the mTOR kinase." *Current biology : CB* 15(8): 702-713.
- Long, X.; Spycher, C.; Han, Z. S.; Rose, A. M.; Muller, F. & Avruch, J. (2002). "TOR deficiency in *C. elegans* causes developmental arrest and intestinal atrophy by inhibition of mRNA translation." *Current biology : CB* 12(17): 1448-1461.
- Ma, L.; Chen, Z.; Erdjument-Bromage, H.; Tempst, P. & Pandolfi, P. P. (2005). "Phosphorylation and functional inactivation of TSC2 by Erk implications for tuberous sclerosis and cancer pathogenesis." *Cell* 121(2): 179-193.
- Ma, X. M. & Blenis, J. (2009). "Molecular mechanisms of mTOR-mediated translational control." *Nature reviews. Molecular cell biology* 10(5): 307-318.
- Ma, X. M.; Yoon, S. O.; Richardson, C. J.; Julich, K. & Blenis, J. (2008). "SKAR links pre-mRNA splicing to mTOR/S6K1-mediated enhanced translation efficiency of spliced mRNAs." *Cell* 133(2): 303-313.

- Maira, S. M.; Stauffer, F.; Brueggen, J.; Furet, P.; Schnell, C.; Fritsch, C.; Brachmann, S.; Chene, P.; De Pover, A.; Schoemaker, K.; Fabbro, D.; Gabriel, D.; Simonen, M.; Murphy, L.; Finan, P.; Sellers, W. & Garcia-Echeverria, C. (2008). "Identification and characterization of NVP-BEZ235, a new orally available dual phosphatidylinositol 3-kinase/mammalian target of rapamycin inhibitor with potent in vivo antitumor activity." *Mol Cancer Ther* 7(7): 1851-1863.
- Makino, C.; Sano, Y.; Shinagawa, T.; Millar, J. B. & Ishii, S. (2006). "Sin1 binds to both ATF-2 and p38 and enhances ATF-2-dependent transcription in an SAPK signaling pathway." *Genes to cells : devoted to molecular & cellular mechanisms* 11(11): 1239-1251.
- Malys, N. & McCarthy, J. E. (2011). "Translation initiation: variations in the mechanism can be anticipated." *Cellular and molecular life sciences : CMLS* 68(6): 991-1003.
- Mammucari, C.; Milan, G.; Romanello, V.; Masiero, E.; Rudolf, R.; Del Piccolo, P.; Burden, S. J.; Di Lisi, R.; Sandri, C.; Zhao, J.; Goldberg, A. L.; Schiaffino, S. & Sandri, M. (2007). "FoxO3 controls autophagy in skeletal muscle in vivo." *Cell Metab* 6(6): 458-471.
- Manning, B. D. & Cantley, L. C. (2003). "Rheb fills a GAP between TSC and TOR." *Trends in biochemical sciences* 28(11): 573-576.
- Manning, G.; Whyte, D. B.; Martinez, R.; Hunter, T. & Sudarsanam, S. (2002). "The protein kinase complement of the human genome." *Science* 298(5600): 1912-1934.
- Marone, R.; Erhart, D.; Mertz, A. C.; Bohnacker, T.; Schnell, C.; Cmiljanovic, V.; Stauffer, F.; Garcia-Echeverria, C.; Giese, B.; Maira, S. M. & Wymann, M. P. (2009). "Targeting melanoma with dual phosphoinositide 3-kinase/mammalian target of rapamycin inhibitors." *Mol Cancer Res* 7(4): 601-613.
- Martin, D. E.; Soulard, A. & Hall, M. N. (2004). "TOR regulates ribosomal protein gene expression via PKA and the Forkhead transcription factor FHL1." *Cell* 119(7): 969-979.
- Martin, J.; Masri, J.; Bernath, A.; Nishimura, R. N. & Gera, J. (2008). "Hsp70 associates with Rictor and is required for mTORC2 formation and activity." *Biochem Biophys Res Commun* 372(4): 578-583.
- Martin, J.; Masri, J.; Bernath, A.; Nishimura, R. N. & Gera, J. (2008). "Hsp70 associates with Rictor and is required for mTORC2 formation and activity." *Biochemical and biophysical research communications* 372(4): 578-583.
- Martin, P. M. & Sutherland, A. E. (2001). "Exogenous amino acids regulate trophoblast differentiation in the mouse blastocyst through an mTOR-dependent pathway." *Developmental biology* 240(1): 182-193.
- Matsuo, T.; Otsubo, Y.; Urano, J.; Tamanoi, F. & Yamamoto, M. (2007). "Loss of the TOR kinase Tor2 mimics nitrogen starvation and activates the sexual development pathway in fission yeast." *Molecular and cellular biology* 27(8): 3154-3164.
- Mayer, C. & Grummt, I. (2006). "Ribosome biogenesis and cell growth: mTOR coordinates transcription by all three classes of nuclear RNA polymerases." *Oncogene* 25(48): 6384-6391.
- Mayer, C.; Zhao, J.; Yuan, X. & Grummt, I. (2004). "mTOR-dependent activation of the transcription factor TIF-IA links rRNA synthesis to nutrient availability." *Genes & development* 18(4): 423-434.
- McDonald, P. C.; Oloumi, A.; Mills, J.; Dobrev, I.; Maidan, M.; Gray, V.; Wederell, E. D.; Bally, M. B.; Foster, L. J. & Dedhar, S. (2008). "Rictor and integrin-linked kinase



- interact and regulate Akt phosphorylation and cancer cell survival." *Cancer research* 68(6): 1618-1624.
- Medvedik, O.; Lamming, D. W.; Kim, K. D.&Sinclair, D. A. (2007). "MSN2 and MSN4 link calorie restriction and TOR to sirtuin-mediated lifespan extension in *Saccharomyces cerevisiae*." *PLoS biology* 5(10): e261.
- Meijer, A. J.&Codogno, P. (2008). "Nutrient sensing: TOR's Ragtime." *Nature cell biology* 10(8): 881-883.
- Menand, B.; Desnos, T.; Nussaume, L.; Berger, F.; Bouchez, D.; Meyer, C.&Robaglia, C. (2002). "Expression and disruption of the Arabidopsis TOR (target of rapamycin) gene." *Proceedings of the National Academy of Sciences of the United States of America* 99(9): 6422-6427.
- Miller, G.; Panov, K. I.; Friedrich, J. K.; Trinkle-Mulcahy, L.; Lamond, A. I.&Zomerdijs, J. C. (2001). "hRRN3 is essential in the SL1-mediated recruitment of RNA Polymerase I to rRNA gene promoters." *The EMBO journal* 20(6): 1373-1382.
- Minami, K.; Tambe, Y.; Watanabe, R.; Isono, T.; Haneda, M.; Isobe, K.; Kobayashi, T.; Hino, O.; Okabe, H.; Chano, T.&Inoue, H. (2007). "Suppression of viral replication by stress-inducible GADD34 protein via the mammalian serine/threonine protein kinase mTOR pathway." *Journal of virology* 81(20): 11106-11115.
- Miranda-Saavedra, D.&Barton, G. J. (2007). "Classification and functional annotation of eukaryotic protein kinases." *Proteins* 68(4): 893-914.
- Mordes, D. A.; Glick, G. G.; Zhao, R.&Cortez, D. (2008). "TopBP1 activates ATR through ATRIP and a PIKK regulatory domain." *Genes & development* 22(11): 1478-1489.
- Murakami, M.; Ichisaka, T.; Maeda, M.; Oshiro, N.; Hara, K.; Edenhofer, F.; Kiyama, H.; Yonezawa, K.&Yamanaka, S. (2004). "mTOR is essential for growth and proliferation in early mouse embryos and embryonic stem cells." *Molecular and cellular biology* 24(15): 6710-6718.
- Muthukkumar, S.; Ramesh, T. M.&Bondada, S. (1995). "Rapamycin, a potent immunosuppressive drug, causes programmed cell death in B lymphoma cells." *Transplantation* 60(3): 264-270.
- Nardella, C.; Carracedo, A.; Alimonti, A.; Hobbs, R. M.; Clohessy, J. G.; Chen, Z.; Egia, A.; Fornari, A.; Fiorentino, M.; Loda, M.; Kozma, S. C.; Thomas, G.; Cordon-Cardo, C.&Pandolfi, P. P. (2009). "Differential requirement of mTOR in postmitotic tissues and tumorigenesis." *Science signaling* 2(55): ra2.
- Nicklin, P.; Bergman, P.; Zhang, B.; Triantafellow, E.; Wang, H.; Nyfeler, B.; Yang, H.; Hild, M.; Kung, C.; Wilson, C.; Myer, V. E.; MacKeigan, J. P.; Porter, J. A.; Wang, Y. K.; Cantley, L. C.; Finan, P. M.&Murphy, L. O. (2009). "Bidirectional transport of amino acids regulates mTOR and autophagy." *Cell* 136(3): 521-534.
- Nobukuni, T.; Joaquin, M.; Roccio, M.; Dann, S. G.; Kim, S. Y.; Gulati, P.; Byfield, M. P.; Backer, J. M.; Natt, F.; Bos, J. L.; Zwartkruis, F. J.&Thomas, G. (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): 14238-14243.
- Noda, T & Ohsumi, Y. (1998). "Tor, a phosphatidylinositol kinase homologue, controls autophagy in yeast." *The Journal of biological chemistry* 273(7): 3963-3966.
- O'Reilly, K. E.; Rojo, F.; She, Q. B.; Solit, D.; Mills, G. B.; Smith, D.; Lane, H.; Hofmann, F.; Hicklin, D. J.; Ludwig, D. L.; Baselga, J.&Rosen, N. (2006). "mTOR inhibition

- induces upstream receptor tyrosine kinase signaling and activates Akt." *Cancer Res* 66(3): 1500-1508.
- Oh, W. J.; Wu, C. C.; Kim, S. J.; Facchinetti, V.; Julien, L. A.; Finlan, M.; Roux, P. P.; Su, B. & Jacinto, E. (2010). "mTORC2 can associate with ribosomes to promote cotranslational phosphorylation and stability of nascent Akt polypeptide." *The EMBO journal* 29(23): 3939-3951.
- Oldham, S.; Montagne, J.; Radimerski, T.; Thomas, G. & Hafen, E. (2000). "Genetic and biochemical characterization of dTOR, the Drosophila homolog of the target of rapamycin." *Genes & development* 14(21): 2689-2694.
- Onda, H.; Lueck, A.; Marks, P. W.; Warren, H. B. & Kwiatkowski, D. J. (1999). "Tsc2(+/-) mice develop tumors in multiple sites that express gelsolin and are influenced by genetic background." *The Journal of clinical investigation* 104(6): 687-695.
- Oshiro, N.; Yoshino, K.; Hidayat, S.; Tokunaga, C.; Hara, K.; Eguchi, S.; Avruch, J. & Yonezawa, K. (2004). "Dissociation of raptor from mTOR is a mechanism of rapamycin-induced inhibition of mTOR function." *Genes to cells : devoted to molecular & cellular mechanisms* 9(4): 359-366.
- Oza, A. M.; Elit, L.; Tsao, M. S.; Kamel-Reid, S.; Biagi, J.; Provencher, D. M.; Gotlieb, W. H.; Hoskins, P. J.; Ghatage, P.; Tonkin, K. S.; Mackay, H. J.; Mazurka, J.; Sederias, J.; Ivy, P.; Dancey, J. E. & Eisenhauer, E. A. (2011). "Phase II study of temsirolimus in women with recurrent or metastatic endometrial cancer: a trial of the NCIC Clinical Trials Group." *J Clin Oncol* 29(24): 3278-3285.
- Pang, H. & Faber, L. E. (2001). "Estrogen and rapamycin effects on cell cycle progression in T47D breast cancer cells." *Breast Cancer Res Treat* 70(1): 21-26.
- Pause, A.; Methot, N.; Svitkin, Y.; Merrick, W. C. & Sonenberg, N. (1994). "Dominant negative mutants of mammalian translation initiation factor eIF-4A define a critical role for eIF-4F in cap-dependent and cap-independent initiation of translation." *The EMBO journal* 13(5): 1205-1215.
- Pearce, L. R.; Huang, X.; Boudeau, J.; Pawlowski, R.; Wullschleger, S.; Deak, M.; Ibrahim, A. F.; Gourlay, R.; Magnuson, M. A. & Alessi, D. R. (2007). "Identification of Protor as a novel Rictor-binding component of mTOR complex-2." *The Biochemical journal* 405(3): 513-522.
- Pearce, L. R.; Sommer, E. M.; Sakamoto, K.; Wullschleger, S. & Alessi, D. R. (2011). "Protor-1 is required for efficient mTORC2-mediated activation of SGK1 in the kidney." *The Biochemical journal* 436(1): 169-179.
- Pearson, G.; Robinson, F.; Beers Gibson, T.; Xu, B. E.; Karandikar, M.; Berman, K. & Cobb, M. H. (2001). "Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions." *Endocrine reviews* 22(2): 153-183.
- Pei, J. J. & Hugon, J. (2008). "mTOR-dependent signalling in Alzheimer's disease." *J Cell Mol Med* 12(6B): 2525-2532.
- Pena-Llopis, S.; Vega-Rubin-de-Celis, S.; Schwartz, J. C.; Wolff, N. C.; Tran, T. A.; Zou, L.; Xie, X. J.; Corey, D. R. & Brugarolas, J. (2011). "Regulation of TFEB and V-ATPases by mTORC1." *The EMBO journal* 30(16): 3242-3258.
- Peng, T.; Golub, T. R. & Sabatini, D. M. (2002). "The immunosuppressant rapamycin mimics a starvation-like signal distinct from amino acid and glucose deprivation." *Molecular and cellular biology* 22(15): 5575-5584.

- Pereira-Leal, J. B.; Levy, E. D. & Teichmann, S. A. (2006). "The origins and evolution of functional modules: lessons from protein complexes." *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* 361(1467): 507-517.
- Perry, J. & Kleckner, N. (2003). "The ATRs, ATMs, and TORs are giant HEAT repeat proteins." *Cell* 112(2): 151-155.
- Peterson, R. T.; Beal, P. A.; Comb, M. J. & Schreiber, S. L. (2000). "FKBP12-rapamycin-associated protein (FRAP) autophosphorylates at serine 2481 under translationally repressive conditions." *The Journal of biological chemistry* 275(10): 7416-7423.
- Peterson, T. R.; Laplante, M.; Thoreen, C. C.; Sancak, Y.; Kang, S. A.; Kuehl, W. M.; Gray, N. S. & Sabatini, D. M. (2009). "DEPTOR is an mTOR inhibitor frequently overexpressed in multiple myeloma cells and required for their survival." *Cell* 137(5): 873-886.
- Peterson, T. R.; Sengupta, S. S.; Harris, T. E.; Carmack, A. E.; Kang, S. A.; Balderas, E.; Guertin, D. A.; Madden, K. L.; Carpenter, A. E.; Finck, B. N. & Sabatini, D. M. (2011). "mTOR complex 1 regulates lipin 1 localization to control the SREBP pathway." *Cell* 146(3): 408-420.
- Polak, P.; Cybulski, N.; Feige, J. N.; Auwerx, J.; Ruegg, M. A. & Hall, M. N. (2008). "Adipose-specific knockout of raptor results in lean mice with enhanced mitochondrial respiration." *Cell metabolism* 8(5): 399-410.
- Poon, M.; Marx, S. O.; Gallo, R.; Badimon, J. J.; Taubman, M. B. & Marks, A. R. (1996). "Rapamycin inhibits vascular smooth muscle cell migration." *J Clin Invest* 98(10): 2277-2283.
- Porstmann, T.; Santos, C. R.; Griffiths, B.; Cully, M.; Wu, M.; Leever, S.; Griffiths, J. R.; Chung, Y. L. & Schulze, A. (2008). "SREBP activity is regulated by mTORC1 and contributes to Akt-dependent cell growth." *Cell metabolism* 8(3): 224-236.
- Powers, T. & Walter, P. (1999). "Regulation of ribosome biogenesis by the rapamycin-sensitive TOR-signaling pathway in *Saccharomyces cerevisiae*." *Molecular biology of the cell* 10(4): 987-1000.
- Proud, C. G. (2009). "Dynamic balancing: DEPTOR tips the scales." *Journal of molecular cell biology* 1(2): 61-63.
- Proud, C. G. (2011). "mTOR Signalling in Health and Disease." *Biochemical Society transactions* 39(2): 431-436.
- Rachdi, L.; Balcazar, N.; Osorio-Duque, F.; Elghazi, L.; Weiss, A.; Gould, A.; Chang-Chen, K. J.; Gambello, M. J. & Bernal-Mizrachi, E. (2008). "Disruption of Tsc2 in pancreatic beta cells induces beta cell mass expansion and improved glucose tolerance in a TORC1-dependent manner." *Proceedings of the National Academy of Sciences of the United States of America* 105(27): 9250-9255.
- Reardon, D. A.; Wen, P. Y.; Alfred Yung, W. K.; Berk, L.; Narasimhan, N.; Turner, C. D.; Clackson, T.; Rivera, V. M. & Vogelbaum, M. A. (2011). "Ridaforolimus for patients with progressive or recurrent malignant glioma: a perisurgical, sequential, ascending-dose trial." *Cancer Chemother Pharmacol*.
- Reiling, J. H. & Sabatini, D. M. (2006). "Stress and mTOR signaling." *Oncogene* 25(48): 6373-6383.
- Reina, J. H.; Azzouz, T. N. & Hernandez, N. (2006). "Maf1, a new player in the regulation of human RNA polymerase III transcription." *PloS one* 1: e134.

- Reinke, A.; Anderson, S.; McCaffery, J. M.; Yates, J., 3rd; Aronova, S.; Chu, S.; Fairclough, S.; Iverson, C.; Wedaman, K. P. & Powers, T. (2004). "TOR complex 1 includes a novel component, Tco89p (YPL180w), and cooperates with Ssd1p to maintain cellular integrity in *Saccharomyces cerevisiae*." *The Journal of biological chemistry* 279(15): 14752-14762.
- Risson, V.; Mazelin, L.; Roceri, M.; Sanchez, H.; Moncollin, V.; Corneloup, C.; Richard-Bulteau, H.; Vignaud, A.; Baas, D.; Defour, A.; Freyssenet, D.; Tanti, J. F.; Le-Marchand-Brustel, Y.; Ferrier, B.; Conjard-Duplany, A.; Romanino, K.; Bauche, S.; Hantai, D.; Mueller, M.; Kozma, S. C.; Thomas, G.; Ruegg, M. A.; Ferry, A.; Pende, M.; Bigard, X.; Koulmann, N.; Schaeffer, L. & Gangloff, Y. G. (2009). "Muscle inactivation of mTOR causes metabolic and dystrophin defects leading to severe myopathy." *The Journal of cell biology* 187(6): 859-874.
- Roberg, K. J.; Bickel, S.; Rowley, N. & Kaiser, C. A. (1997). "Control of amino acid permease sorting in the late secretory pathway of *Saccharomyces cerevisiae* by SEC13, LST4, LST7 and LST8." *Genetics* 147(4): 1569-1584.
- Rosner, M. & Hengstschlager, M. (2008). "Cytoplasmic and nuclear distribution of the protein complexes mTORC1 and mTORC2: rapamycin triggers dephosphorylation and delocalization of the mTORC2 components rictor and sin1." *Human molecular genetics* 17(19): 2934-2948.
- Roux, P. P.; Ballif, B. A.; Anjum, R.; Gygi, S. P. & 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): 13489-13494.
- Rubio-Teixeira, M. & Kaiser, C. A. (2006). "Amino acids regulate retrieval of the yeast general amino acid permease from the vacuolar targeting pathway." *Molecular biology of the cell* 17(7): 3031-3050.
- Russnak, R.; Konczal, D. & McIntire, S. L. (2001). "A family of yeast proteins mediating bidirectional vacuolar amino acid transport." *The Journal of biological chemistry* 276(26): 23849-23857.
- Ruvinsky, I.; Sharon, N.; Lerer, T.; Cohen, H.; Stolovitch-Rain, M.; Nir, T.; Dor, Y.; Zisman, P. & Meyuhas, O. (2005). "Ribosomal protein S6 phosphorylation is a determinant of cell size and glucose homeostasis." *Genes & development* 19(18): 2199-2211.
- Sancak, Y.; Peterson, T. R.; Shaul, Y. D.; Lindquist, R. A.; Thoreen, C. C.; Bar-Peled, L. & Sabatini, D. M. (2008). "The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1." *Science* 320(5882): 1496-1501.
- Sancak, Y.; Thoreen, C. C.; Peterson, T. R.; Lindquist, R. A.; Kang, S. A.; Spooner, E.; Carr, S. A. & Sabatini, D. M. (2007). "PRAS40 is an insulin-regulated inhibitor of the mTORC1 protein kinase." *Molecular cell* 25(6): 903-915.
- Sarbassov, D. D.; Ali, S. M.; Kim, D. H.; Guertin, D. A.; Latek, R. R.; Erdjument-Bromage, H.; Tempst, P. & Sabatini, D. M. (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): 1296-1302.
- Sarbassov, D. D.; Ali, S. M. & Sabatini, D. M. (2005). "Growing roles for the mTOR pathway." *Current opinion in cell biology* 17(6): 596-603.

- Sarbassov, D. D.; Ali, S. M.; Sengupta, S.; Sheen, J. H.; Hsu, P. P.; Bagley, A. F.; Markhard, A. L. & Sabatini, D. M. (2006). "Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB." *Molecular cell* 22(2): 159-168.
- Sarbassov, D. D.; Guertin, D. A.; Ali, S. M. & Sabatini, D. M. (2005). "Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex." *Science* 307(5712): 1098-1101.
- Sarbassov, D. D. & Sabatini, D. M. (2005). "Redox regulation of the nutrient-sensitive raptor-mTOR pathway and complex." *The Journal of biological chemistry* 280(47): 39505-39509.
- Schieke, S. M.; Phillips, D.; McCoy, J. P., Jr.; Aponte, A. M.; Shen, R. F.; Balaban, R. S. & Finkel, T. (2006). "The mammalian target of rapamycin (mTOR) pathway regulates mitochondrial oxygen consumption and oxidative capacity." *The Journal of biological chemistry* 281(37): 27643-27652.
- Schmidt, A.; Bickle, M.; Beck, T. & Hall, M. N. (1997). "The yeast phosphatidylinositol kinase homolog TOR2 activates RHO1 and RHO2 via the exchange factor ROM2." *Cell* 88(4): 531-542.
- Schmidt, A.; Kunz, J. & Hall, M. N. (1996). "TOR2 is required for organization of the actin cytoskeleton in yeast." *Proceedings of the National Academy of Sciences of the United States of America* 93(24): 13780-13785.
- Schroder, W.; Bushell, G. & Sculley, T. (2005). "The human stress-activated protein kinase-interacting 1 gene encodes JNK-binding proteins." *Cellular signalling* 17(6): 761-767.
- Schroder, W.; Cloonan, N.; Bushell, G. & Sculley, T. (2004). "Alternative polyadenylation and splicing of mRNAs transcribed from the human Sin1 gene." *Gene* 339: 17-23.
- Schroder, W. A.; Buck, M.; Cloonan, N.; Hancock, J. F.; Suhrbier, A.; Sculley, T. & Bushell, G. (2007). "Human Sin1 contains Ras-binding and pleckstrin homology domains and suppresses Ras signalling." *Cellular signalling* 19(6): 1279-1289.
- Sehgal, S. N.; Baker, H. & Vezina, C. (1975). "Rapamycin (AY-22,989), a new antifungal antibiotic. II. Fermentation, isolation and characterization." *The Journal of antibiotics* 28(10): 727-732.
- Sekiguchi, T.; Hirose, E.; Nakashima, N.; Ii, M. & Nishimoto, T. (2001). "Novel G proteins, Rag C and Rag D, interact with GTP-binding proteins, Rag A and Rag B." *The Journal of biological chemistry* 276(10): 7246-7257.
- Sekulic, A.; Hudson, C. C.; Homme, J. L.; Yin, P.; Otterness, D. M.; Karnitz, L. M. & Abraham, R. T. (2000). "A direct linkage between the phosphoinositide 3-kinase-AKT signaling pathway and the mammalian target of rapamycin in mitogen-stimulated and transformed cells." *Cancer research* 60(13): 3504-3513.
- Sengupta, S.; Peterson, T. R.; Laplante, M.; Oh, S. & Sabatini, D. M. (2010). "mTORC1 controls fasting-induced ketogenesis and its modulation by ageing." *Nature* 468(7327): 1100-1104.
- Settembre, C.; Di Malta, C.; Polito, V. A.; Garcia Arencibia, M.; Vetrini, F.; Erdin, S.; Erdin, S. U.; Huynh, T.; Medina, D.; Colella, P.; Sardiello, M.; Rubinsztein, D. C. & Ballabio, A. (2011). "TFEB links autophagy to lysosomal biogenesis." *Science* 332(6036): 1429-1433.
- Shah, O. J. & Hunter, T. (2006). "Turnover of the active fraction of IRS1 involves raptor-mTOR- and S6K1-dependent serine phosphorylation in cell culture models of tuberous sclerosis." *Molecular and cellular biology* 26(17): 6425-6434.

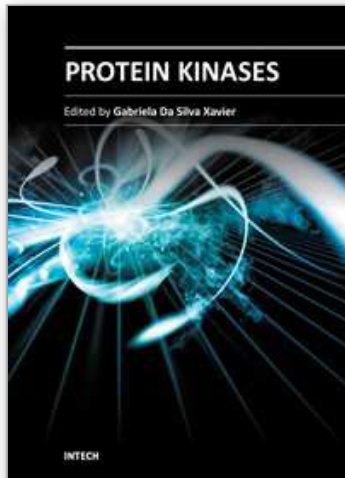
- Shah, O. J.; Wang, Z.&Hunter, T. (2004). "Inappropriate activation of the TSC/Rheb/mTOR/S6K cassette induces IRS1/2 depletion, insulin resistance, and cell survival deficiencies." *Current biology : CB* 14(18): 1650-1656.
- Shaw, R. J.; Bardeesy, N.; Manning, B. D.; Lopez, L.; Kosmatka, M.; DePinho, R. A.&Cantley, L. C. (2004). "The LKB1 tumor suppressor negatively regulates mTOR signaling." *Cancer cell* 6(1): 91-99.
- Shiojima, I.&Walsh, K. (2006). "Regulation of cardiac growth and coronary angiogenesis by the Akt/PKB signaling pathway." *Genes Dev* 20(24): 3347-3365.
- Shiota, C.; Woo, J. T.; Lindner, J.; Shelton, K. D.&Magnuson, M. A. (2006). "Multiallelic disruption of the rictor gene in mice reveals that mTOR complex 2 is essential for fetal growth and viability." *Developmental cell* 11(4): 583-589.
- Shor, B.; Wu, J.; Shakey, Q.; Toral-Barza, L.; Shi, C.; Follettie, M.&Yu, K. (2010). "Requirement of the mTOR kinase for the regulation of Maf1 phosphorylation and control of RNA polymerase III-dependent transcription in cancer cells." *The Journal of biological chemistry* 285(20): 15380-15392.
- Singh, K.; Sun, S.&Vezina, C. (1979). "Rapamycin (AY-22,989), a new antifungal antibiotic. IV. Mechanism of action." *The Journal of antibiotics* 32(6): 630-645.
- Smith, E. M.; Finn, S. G.; Tee, A. R.; Browne, G. J.&Proud, C. G. (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): 18717-18727.
- Soliman, G. A.; Acosta-Jaquez, H. A.; Dunlop, E. A.; Ekim, B.; Maj, N. E.; Tee, A. R.&Fingar, D. C. (2010). "mTOR Ser-2481 autophosphorylation monitors mTORC-specific catalytic activity and clarifies rapamycin mechanism of action." *The Journal of biological chemistry* 285(11): 7866-7879.
- Sonenberg, N.&Hinnebusch, A. G. (2009). "Regulation of translation initiation in eukaryotes: mechanisms and biological targets." *Cell* 136(4): 731-745.
- Stan, R.; McLaughlin, M. M.; Cafferkey, R.; Johnson, R. K.; Rosenberg, M.&Livi, G. P. (1994). "Interaction between FKBP12-rapamycin and TOR involves a conserved serine residue." *The Journal of biological chemistry* 269(51): 32027-32030.
- Sturgill, T. W.&Hall, M. N. (2009). "Activating mutations in TOR are in similar structures as oncogenic mutations in PI3K $\alpha$ ." *ACS chemical biology* 4(12): 999-1015.
- Sun, S. Y.; Rosenberg, L. M.; Wang, X.; Zhou, Z.; Yue, P.; Fu, H.&Khuri, F. R. (2005). "Activation of Akt and eIF4E survival pathways by rapamycin-mediated mammalian target of rapamycin inhibition." *Cancer research* 65(16): 7052-7058.
- Takai, H.; Wang, R. C.; Takai, K. K.; Yang, H.&de Lange, T. (2007). "Tel2 regulates the stability of PI3K-related protein kinases." *Cell* 131(7): 1248-1259.
- Takai, H.; Xie, Y.; de Lange, T.&Pavletich, N. P. (2010). "Tel2 structure and function in the Hsp90-dependent maturation of mTOR and ATR complexes." *Genes & development* 24(18): 2019-2030.
- Tcherkezian, J.; Brittis, P. A.; Thomas, F.; Roux, P. P.&Flanagan, J. G. (2010). "Transmembrane receptor DCC associates with protein synthesis machinery and regulates translation." *Cell* 141(4): 632-644.
- Tee, A. R.; Manning, B. D.; Roux, P. P.; Cantley, L. C.&Blenis, J. (2003). "Tuberous sclerosis complex gene products, Tuberin and Hamartin, control mTOR signaling by acting

- as a GTPase-activating protein complex toward Rheb." *Current biology : CB* 13(15): 1259-1268.
- Thedieck, K.; Polak, P.; Kim, M. L.; Molle, K. D.; Cohen, A.; Jeno, P.; Arriemerlou, C.&Hall, M. N. (2007). "PRAS40 and PRR5-like protein are new mTOR interactors that regulate apoptosis." *PloS one* 2(11): e1217.
- Thomas, G.&Hall, M. N. (1997). "TOR signalling and control of cell growth." *Current opinion in cell biology* 9(6): 782-787.
- Thoreen, C. C.; Kang, S. A.; Chang, J. W.; Liu, Q.; Zhang, J.; Gao, Y.; Reichling, L. J.; Sim, T.; Sabatini, D. M.&Gray, N. S. (2009). "An ATP-competitive mammalian target of rapamycin inhibitor reveals rapamycin-resistant functions of mTORC1." *The Journal of biological chemistry* 284(12): 8023-8032.
- Treins, C.; Warne, P. H.; Magnuson, M. A.; Pende, M.&Downward, J. (2010). "Rictor is a novel target of p70 S6 kinase-1." *Oncogene* 29(7): 1003-1016.
- Tsang, C. K.; Liu, H.&Zheng, X. F. (2010). "mTOR binds to the promoters of RNA polymerase I- and III-transcribed genes." *Cell Cycle* 9(5): 953-957.
- Tzatsos, A.&Kandror, K. V. (2006). "Nutrients suppress phosphatidylinositol 3-kinase/Akt signaling via raptor-dependent mTOR-mediated insulin receptor substrate 1 phosphorylation." *Molecular and cellular biology* 26(1): 63-76.
- Um, S. H.; Frigerio, F.; Watanabe, M.; Picard, F.; Joaquin, M.; Sticker, M.; Fumagalli, S.; Allegrini, P. R.; Kozma, S. C.; Auwerx, J.&Thomas, G. (2004). "Absence of S6K1 protects against age- and diet-induced obesity while enhancing insulin sensitivity." *Nature* 431(7005): 200-205.
- Valentinis, B.&Baserga, R. (2001). "IGF-I receptor signalling in transformation and differentiation." *Molecular pathology : MP* 54(3): 133-137.
- van der Poel, H. G.; Hanrahan, C.; Zhong, H.&Simons, J. W. (2003). "Rapamycin induces Smad activity in prostate cancer cell lines." *Urol Res* 30(6): 380-386.
- Vellai, T.; Takacs-Vellai, K.; Zhang, Y.; Kovacs, A. L.; Orosz, L.&Muller, F. (2003). "Genetics: influence of TOR kinase on lifespan in *C. elegans*." *Nature* 426(6967): 620.
- Veverka, V.; Crabbe, T.; Bird, I.; Lennie, G.; Muskett, F. W.; Taylor, R. J.&Carr, M. D. (2008). "Structural characterization of the interaction of mTOR with phosphatidic acid and a novel class of inhibitor: compelling evidence for a central role of the FRB domain in small molecule-mediated regulation of mTOR." *Oncogene* 27(5): 585-595.
- Vezina, C.; Kudelski, A.&Sehgal, S. N. (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): 721-726.
- Vornlocher, H. P.; Hanachi, P.; Ribeiro, S.&Hershey, J. W. (1999). "A 110-kilodalton subunit of translation initiation factor eIF3 and an associated 135-kilodalton protein are encoded by the *Saccharomyces cerevisiae* TIF32 and TIF31 genes." *The Journal of biological chemistry* 274(24): 16802-16812.
- Wang, L.; Harris, T. E.; Roth, R. A.&Lawrence, J. C., Jr. (2007). "PRAS40 regulates mTORC1 kinase activity by functioning as a direct inhibitor of substrate binding." *The Journal of biological chemistry* 282(27): 20036-20044.
- Wang, X.; Beugnet, A.; Murakami, M.; Yamanaka, S.&Proud, C. G. (2005). "Distinct signaling events downstream of mTOR cooperate to mediate the effects of amino acids and insulin on initiation factor 4E-binding proteins." *Molecular and cellular biology* 25(7): 2558-2572.

- Wang, X.; Li, W.; Parra, J. L.; Beugnet, A. & Proud, C. G. (2003). "The C terminus of initiation factor 4E-binding protein 1 contains multiple regulatory features that influence its function and phosphorylation." *Molecular and cellular biology* 23(5): 1546-1557.
- Wang, X.; Li, W.; Williams, M.; Terada, N.; Alessi, D. R. & Proud, C. G. (2001). "Regulation of elongation factor 2 kinase by p90(RSK1) and p70 S6 kinase." *The EMBO journal* 20(16): 4370-4379.
- Watanabe, R.; Tambe, Y.; Inoue, H.; Isono, T.; Haneda, M.; Isobe, K.; Kobayashi, T.; Hino, O.; Okabe, H. & Chano, T. (2007). "GADD34 inhibits mammalian target of rapamycin signaling via tuberous sclerosis complex and controls cell survival under bioenergetic stress." *International journal of molecular medicine* 19(3): 475-483.
- Wedaman, K. P.; Reinke, A.; Anderson, S.; Yates, J., 3rd; McCaffery, J. M. & Powers, T. (2003). "Tor kinases are in distinct membrane-associated protein complexes in *Saccharomyces cerevisiae*." *Molecular biology of the cell* 14(3): 1204-1220.
- Weichhart, T.; Haidinger, M.; Katholnig, K.; Kopecky, C.; Poglitsch, M.; Lassnig, C.; Rosner, M.; Zlabinger, G. J.; Hengstschlager, M.; Muller, M.; Horl, W. H. & Saemann, M. D. (2011). "Inhibition of mTOR blocks the anti-inflammatory effects of glucocorticoids in myeloid immune cells." *Blood* 117(16): 4273-4283.
- White, R. J. (2005). "RNA polymerases I and III, growth control and cancer." *Nature reviews. Molecular cell biology* 6(1): 69-78.
- Wilkinson, M. G.; Pino, T. S.; Tournier, S.; Buck, V.; Martin, H.; Christiansen, J.; Wilkinson, D. G. & Millar, J. B. (1999). "Sin1: an evolutionarily conserved component of the eukaryotic SAPK pathway." *The EMBO journal* 18(15): 4210-4221.
- Woo, S. Y.; Kim, D. H.; Jun, C. B.; Kim, Y. M.; Haar, E. V.; Lee, S. I.; Hegg, J. W.; Bandhakavi, S. & Griffin, T. J. (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): 25604-25612.
- Wullschleger, S.; Loewith, R. & Hall, M. N. (2006). "TOR signaling in growth and metabolism." *Cell* 124(3): 471-484.
- Wullschleger, S.; Loewith, R.; Oppliger, W. & Hall, M. N. (2005). "Molecular organization of target of rapamycin complex 2." *The Journal of biological chemistry* 280(35): 30697-30704.
- Yang, Q. & Guan, K. L. (2007). "Expanding mTOR signaling." *Cell research* 17(8): 666-681.
- Yang, Q.; Inoki, K.; Ikenoue, T. & Guan, K. L. (2006). "Identification of Sin1 as an essential TORC2 component required for complex formation and kinase activity." *Genes & development* 20(20): 2820-2832.
- Yeh, Y. Y.; Wrasman, K. & Herman, P. K. (2010). "Autophosphorylation within the Atg1 activation loop is required for both kinase activity and the induction of autophagy in *Saccharomyces cerevisiae*." *Genetics* 185(3): 871-882.
- Yip, C. K.; Murata, K.; Walz, T.; Sabatini, D. M. & Kang, S. A. (2010). "Structure of the human mTOR complex I and its implications for rapamycin inhibition." *Molecular cell* 38(5): 768-774.
- Yorimitsu, T.; He, C.; Wang, K. & Klionsky, D. J. (2009). "Tap42-associated protein phosphatase type 2A negatively regulates induction of autophagy." *Autophagy* 5(5): 616-624.
- Young, D. A. & Nickerson-Nutter, C. L. (2005). "mTOR--beyond transplantation." *Current opinion in pharmacology* 5(4): 418-423.



- Yu, K.; Toral-Barza, L.; Shi, C.; Zhang, W. G.; Lucas, J.; Shor, B.; Kim, J.; Verheijen, J.; Curran, K.; Malwitz, D. J.; Cole, D. C.; Ellingboe, J.; Ayril-Kaloustian, S.; Mansour, T. S.; Gibbons, J. J.; Abraham, R. T.; Nowak, P. & Zask, A. (2009). "Biochemical, cellular, and in vivo activity of novel ATP-competitive and selective inhibitors of the mammalian target of rapamycin." *Cancer research* 69(15): 6232-6240.
- Yu, Y.; Yoon, S. O.; Poulogiannis, G.; Yang, Q.; Ma, X. M.; Villen, J.; Kubica, N.; Hoffman, G. R.; Cantley, L. C.; Gygi, S. P. & Blenis, J. (2011). "Phosphoproteomic analysis identifies Grb10 as an mTORC1 substrate that negatively regulates insulin signaling." *Science* 332(6035): 1322-1326.
- Yuan, X.; Zhao, J.; Zentgraf, H.; Hoffmann-Rohrer, U. & Grummt, I. (2002). "Multiple interactions between RNA polymerase I, TIF-IA and TAF(I) subunits regulate preinitiation complex assembly at the ribosomal gene promoter." *EMBO reports* 3(11): 1082-1087.
- Zhande, R.; Mitchell, J. J.; Wu, J. & Sun, X. J. (2002). "Molecular mechanism of insulin-induced degradation of insulin receptor substrate 1." *Molecular and cellular biology* 22(4): 1016-1026.
- Zhao, J.; Brault, J. J.; Schild, A.; Cao, P.; Sandri, M.; Schiaffino, S.; Lecker, S. H. & Goldberg, A. L. (2007). "FoxO3 coordinately activates protein degradation by the autophagic/lysosomal and proteasomal pathways in atrophying muscle cells." *Cell Metab* 6(6): 472-483.
- Zhao, Y.; Xiong, X. & Sun, Y. (2011). "DEPTOR, an mTOR Inhibitor, Is a Physiological Substrate of SCF(betaTrCP) E3 Ubiquitin Ligase and Regulates Survival and Autophagy." *Molecular cell* 44(2): 304-316.
- Zheng, X. F.; Florentino, D.; Chen, J.; Crabtree, G. R. & Schreiber, S. L. (1995). "TOR kinase domains are required for two distinct functions, only one of which is inhibited by rapamycin." *Cell* 82(1): 121-130.
- Zheng, X. F. & Schreiber, S. L. (1997). "Target of rapamycin proteins and their kinase activities are required for meiosis." *Proceedings of the National Academy of Sciences of the United States of America* 94(7): 3070-3075.
- Zhou, H. & Huang, S. (2010). "The complexes of mammalian target of rapamycin." *Current protein & peptide science* 11(6): 409-424.
- Zhou, H. Y. & Wong, A. S. (2006). "Activation of p70S6K induces expression of matrix metalloproteinase 9 associated with hepatocyte growth factor-mediated invasion in human ovarian cancer cells." *Endocrinology* 147(5): 2557-2566.
- Zinzalla, V.; Stracka, D.; Oppliger, W. & Hall, M. N. (2011). "Activation of mTORC2 by association with the ribosome." *Cell* 144(5): 757-768.
- Zoncu, R.; Efeyan, A. & Sabatini, D. M. (2011). "mTOR: from growth signal integration to cancer, diabetes and ageing." *Nature reviews. Molecular cell biology* 12(1): 21-35.



## **Protein Kinases**

Edited by Dr. Gabriela Da Silva Xavier

ISBN 978-953-51-0640-1

Hard cover, 484 pages

**Publisher** InTech

**Published online** 05, June, 2012

**Published in print edition** June, 2012

Proteins are the work horses of the cell. As regulators of protein function, protein kinases are involved in the control of cellular functions via intricate signalling pathways, allowing for fine tuning of physiological functions. This book is a collaborative effort, with contribution from experts in their respective fields, reflecting the spirit of collaboration - across disciplines and borders - that exists in modern science. Here, we review the existing literature and, on occasions, provide novel data on the function of protein kinases in various systems. We also discuss the implications of these findings in the context of disease, treatment, and drug development.

### **How to reference**

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Chang-Chih Wu, Po-Chien Chou and Estela Jacinto (2012). The Target of Rapamycin: Structure and Functions, Protein Kinases, Dr. Gabriela Da Silva Xavier (Ed.), ISBN: 978-953-51-0640-1, InTech, Available from: <http://www.intechopen.com/books/protein-kinases/the-target-of-rapamycin-structure-and-functions>

**INTECH**  
open science | open minds

### **InTech Europe**

University Campus STeP Ri  
Slavka Krautzeka 83/A  
51000 Rijeka, Croatia  
Phone: +385 (51) 770 447  
Fax: +385 (51) 686 166  
[www.intechopen.com](http://www.intechopen.com)

### **InTech China**

Unit 405, Office Block, Hotel Equatorial Shanghai  
No.65, Yan An Road (West), Shanghai, 200040, China  
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元  
Phone: +86-21-62489820  
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen