mTOR signaling at a glance

Mathieu Laplante[1,2] and David M. Sabatini[1,2,3,*]

[1]Whitehead Institute for Biomedical Research, Nine Cambridge Center, Cambridge, MA 02142, USA
[2]Howard Hughes Medical Institute, Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
[3]Koch Center for Integrative Cancer Research at MIT, 77 Massachusetts Avenue, Cambridge, MA 02139, USA

*Author for correspondence (sabatini@wi.mit.edu)

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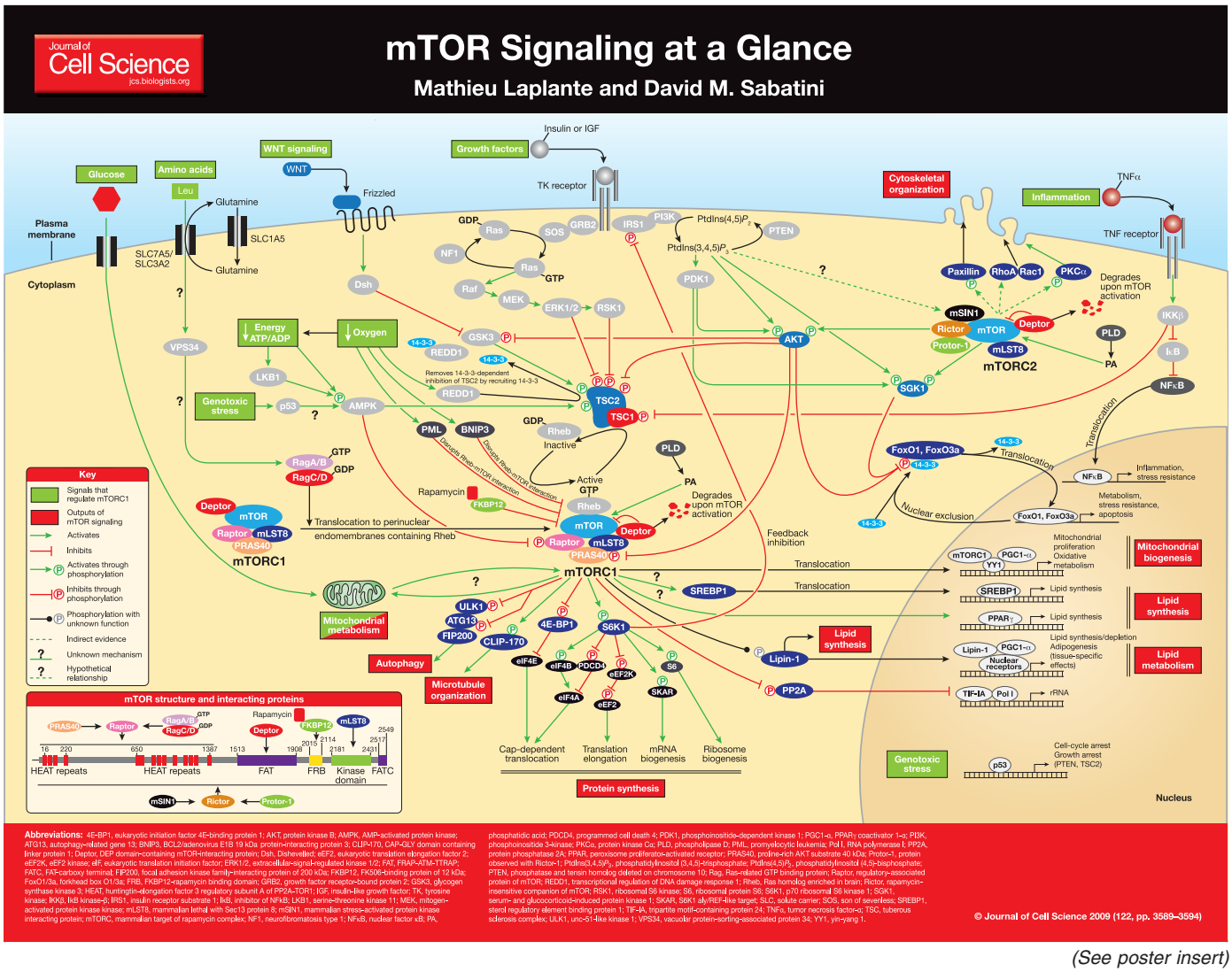
The mammalian target of rapamycin (mTOR) signaling pathway integrates both intracellular and extracellular signals and serves as a central regulator of cell metabolism, growth, proliferation and survival. Discoveries that have been made over the last decade show that the mTOR pathway is activated during various cellular processes (e.g. tumor formation and angiogenesis, insulin resistance, adipogenesis and T-lymphocyte activation) and is deregulated in human diseases such as cancer and type 2 diabetes. These observations have attracted broad scientific and clinical interest in mTOR. This is highlighted by the growing use of mTOR inhibitors [rapamycin and its analogues (rapalogues)] in pathological settings, including the treatment of solid tumors, organ transplantation, coronary restenosis and rheumatoid arthritis. Here, we highlight and summarize the current understanding of how mTOR nucleates distinct multi-protein complexes, how intra- and extracellular signals are processed by the mTOR complexes, and how such signals affect cell metabolism, growth, proliferation and survival.

mTOR structure and organization into multi-protein complexes

The mTOR protein is a 289-kDa serine-threonine kinase that belongs to the phosphoinositide 3-kinase (PI3K)-related kinase family and is conserved throughout evolution. The poster depicts an overview of mTOR structural domains. mTOR nucleates at least two distinct multi-protein complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) (reviewed by Guertin and Sabatini, 2007).

mTORC1

mTORC1 has five components: mTOR, which is the catalytic subunit of the complex; regulatory-associated protein of mTOR (Raptor); mammalian lethal with Sec13 protein 8 (mLST8, also known as GβL); proline-rich AKT substrate 40 kDa (PRAS40); and DEP-domain-containing mTOR-interacting protein (Deptor) (Peterson et al., 2009). The



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exact function of most of the mTOR-interacting proteins in mTORC1 still remains elusive. It has been proposed that Raptor might affect mTORC1 activity by regulating assembly of the complex and by recruiting substrates for mTOR (Hara et al., 2002; Kim et al., 2002). The role of mLST8 in mTORC1 function is also unclear, as deletion of this protein does not affect mTORC1 activity in vivo (Guertin et al., 2006). PRAS40 and Deptor have been characterized as distinct negative regulators of mTORC1 (Peterson et al., 2009; Sancak et al., 2007; Vander Haar et al., 2007). When the activity of mTORC1 is reduced, PRAS40 and Deptor are recruited to the complex, where they promote the inhibition of mTORC1. It was proposed that PRAS40 regulates mTORC1 kinase activity by functioning as a direct inhibitor of substrate binding (Wang et al., 2007). Upon activation, mTORC1 directly phosphorylates PRAS40 and Deptor, which reduces their physical interaction with mTORC1 and further activates mTORC1 signaling (Peterson et al., 2009; Wang et al., 2007).

mTORC2

mTORC2 comprises six different proteins, several of which are common to mTORC1 and mTORC2: mTOR; rapamycin-insensitive companion of mTOR (Rictor); mammalian stress-activated protein kinase interacting protein (mSIN1); protein observed with Rictor-1 (Protor-1); mLST8; and Deptor. There is some evidence that Rictor and mSIN1 stabilize each other, establishing the structural foundation of mTORC2 (Frias et al., 2006; Jacinto et al., 2006). Rictor also interacts with Protor-1, but the physiological function of this interaction is not clear (Thedieck et al., 2007; Woo et al., 2007). Similar to its role in mTORC1, Deptor negatively regulates mTORC2 activity (Peterson et al., 2009); so far, Deptor is the only characterized endogenous inhibitor of mTORC2. Finally, mLST8 is essential for mTORC2 function, as knockout of this protein severely reduces the stability and the activity of this complex (Guertin et al., 2006).

Now that many mTOR-interacting proteins have been identified, additional biochemical studies will be needed to clarify the functions of these proteins in mTOR signaling and their potential implications in health and disease. Below, we discuss current understanding of the functions of mTORC1 and mTORC2.

mTORC1: a master regulator of cell growth and metabolism

mTORC1 positively regulates cell growth and proliferation by promoting many anabolic processes, including biosynthesis of proteins, lipids and organelles, and by limiting catabolic processes such as autophagy. Much of the knowledge about mTORC1 function comes from the use of the bacterial macrolide rapamycin. Upon entering the cell, rapamycin binds to FK506-binding protein of 12 kDa (FKBP12) and interacts with the FKBP12-rapamycin binding domain (FRB) of mTOR, thus inhibiting mTORC1 functions (reviewed by Guertin and Sabatini, 2007). In contrast to its effect on mTORC1, FKBP12-rapamycin cannot physically interact with or acutely inhibit mTORC2 (Jacinto et al., 2004; Sarbassov et al., 2004). On the basis of these observations, mTORC1 and mTORC2 have been respectively characterized as the rapamycin-sensitive and rapamycin-insensitive complexes. However, this paradigm might not be entirely accurate, as chronic rapamycin treatment can, in some cases, inhibit mTORC2 activity by blocking its assembly (Sarbassov et al., 2006). In addition, recent reports suggest that important mTORC1 functions are resistant to inhibition by rapamycin (Choo et al., 2008; Feldman et al., 2009; Garcia-Martinez et al., 2009; Thoreen et al., 2009).

Protein synthesis

mTORC1 positively controls protein synthesis, which is required for cell growth, through various downstream effectors. mTORC1 promotes protein synthesis by phosphorylating the eukaryotic initiation factor 4E (eIF4E)-binding protein 1 (4E-BP1) and the p70 ribosomal S6 kinase 1 (S6K1). The phosphorylation of 4E-BP1 prevents its binding to eIF4E, enabling eIF4E to promote cap-dependent translation (reviewed by Richter and Sonenberg, 2005). The stimulation of S6K1 activity by mTORC1 leads to increases in mRNA biogenesis, cap-dependent translation and elongation, and the translation of ribosomal proteins through regulation of the activity of many proteins, such as S6K1 aly/REF-like target (SKAR), programmed cell death 4 (PDCD4), eukaryotic elongation factor 2 kinase (eEF2K) and ribosomal protein S6 (reviewed by Ma and Blenis, 2009). The activation of mTORC1 has also been shown to promote ribosome biogenesis by stimulating the transcription of ribosomal RNA through a process involving the protein phosphatase 2A (PP2A) and the transcription initiation factor IA (TIF-IA) (Mayer et al., 2004).

Autophagy

Autophagy – that is, the sequestration of intracellular components within autophagosomes and their degradation by lysosomes – is a catabolic process that is important in organelle degradation and protein turnover. When nutrient availability is limited, the degradation of organelles and protein complexes through autophagy provides biological material to sustain anabolic processes such as protein synthesis and energy production. Studies have shown that mTORC1 inhibition increases autophagy, whereas stimulation of mTORC1 reduces this process (reviewed by Codogno and Meier, 2005). We have observed that mTORC1 controls autophagy through an unknown mechanism that is essentially insensitive to inhibition by rapamycin (Thoreen et al., 2009). It was recently shown by three independent groups that mTORC1 controls autophagy through the regulation of a protein complex composed of unc-51-like kinase 1 (ULK1), autophagy-related gene 13 (ATG13) and focal adhesion kinase family-interacting protein of 200 kD (FIP200) (Ganley et al., 2009; Hosokawa et al., 2009; Jung et al., 2009). These studies have revealed that mTORC1 represses autophagy by phosphorylating and thereby repressing ULK1 and ATG13.

Lipid synthesis

The role of mTORC1 in regulating lipid synthesis, which is required for cell growth and proliferation, is beginning to be appreciated. It has been demonstrated that mTORC1 positively regulates the activity of sterol regulatory element binding protein 1 (SREBP1) (Porstmann et al., 2008) and of peroxisome proliferator-activated receptor-γ (PPARγ) (Kim and Chen, 2004), two transcription factors that control the expression of genes encoding proteins involved in lipid and cholesterol homeostasis. Blocking mTOR with rapamycin reduces the expression and the transactivation activity of PPARγ (Kim and Chen, 2004). The molecular mechanism of SREBP1 activation by mTORC1 is unknown. Additionally, rapamycin reduces the phosphorylation of lipin-1 (Huffman et al., 2002), a phosphatidic acid (PA) phosphatase that is involved in glycerolipid synthesis and in the coactivation of many transcription factors linked to lipid metabolism, including PPARγ, PPARα and PGC1-α. The precise impact of lipin-1 phosphorylation on lipid synthesis remains to be established.

Mitochondrial metabolism and biogenesis

Mitochondrial metabolism and biogenesis are both regulated by mTORC1. Inhibition of mTORC1 by rapamycin lowers mitochondrial membrane potential, oxygen consumption and cellular ATP levels, and profoundly alters the mitochondrial phosphoproteome (Schieke et al., 2006). Recently, it has been observed that mitochondrial DNA copy number, as well as the expression of many genes encoding proteins involved in oxidative metabolism, are reduced by rapamycin and increased by mutations that

activate mTORC1 signaling (Chen et al., 2008; Cunningham et al., 2007). Additionally, conditional deletion of Raptor in mouse skeletal muscle reduces the expression of genes involved in mitochondrial biogenesis (Bentzinger et al., 2008). Cunningham and colleagues have discovered that mTORC1 controls the transcriptional activity of PPARγ coactivator 1 (PGC1-α), a nuclear cofactor that plays a key role in mitochondrial biogenesis and oxidative metabolism, by directly altering its physical interaction with another transcription factor, namely yin-yang 1 (YY1) (Cunningham et al., 2007).

Many roads lead to mTORC1: overview of a complex signaling network

mTORC1 integrates four major signals – growth factors, energy status, oxygen and amino acids – to regulate many processes that are involved in the promotion of cell growth. One of the most important sensors involved in the regulation of mTORC1 activity is the tuberous sclerosis complex (TSC), which is a heterodimer that comprises TSC1 (also known as hamartin) and TSC2 (also known as tuberin). TSC1/2 functions as a GTPase-activating protein (GAP) for the small Ras-related GTPase Rheb (Ras homolog enriched in brain). The active, GTP-bound form of Rheb directly interacts with mTORC1 to stimulate its activity (Long et al., 2005; Sancak et al., 2007). The exact mechanism by which Rheb activates mTORC1 remains to be determined. As a Rheb-specific GAP, TSC1/2 negatively regulates mTORC1 signaling by converting Rheb into its inactive GDP-bound state (Inoki et al., 2003; Tee et al., 2003). Consistent with a role of TSC1/2 in the negative regulation of mTORC1, inactivating mutations or loss of heterozygosity of TSC1/2 give rise to tuberous sclerosis, a disease associated with the presence of numerous benign tumors that are composed of enlarged and disorganized cells (reviewed by Crino et al., 2006).

Growth factors

Growth factors stimulate mTORC1 through the activation of the canonical insulin and Ras signaling pathways. The stimulation of these pathways increases the phosphorylation of TSC2 by protein kinase B (PKB, also known as AKT) (Inoki et al., 2002; Potter et al., 2002), by extracellular-signal-regulated kinase 1/2 (ERK1/2) (Ma et al., 2005), and by p90 ribosomal S6 kinase 1 (RSK1) (Roux et al., 2004), and leads to the inactivation of TSC1/2 and thus to the activation of mTORC1. Additionally, AKT activation by growth factors can activate mTORC1 in a TSC1/2-independent manner by promoting the phosphorylation and dissociation of PRAS40 from mTORC1 (Sancak et al., 2007; Vander Haar et al., 2007; Wang et al., 2007).

The binding of insulin to its cell-surface receptor promotes the tyrosine kinase activity of the insulin receptor, the recruitment of insulin receptor substrate 1 (IRS1), the production of phosphatidylinositol (3,4,5)-triphosphate [PtdIns(3,4,5)P_3] through the activation of PI3K, and the recruitment and activation of AKT at the plasma membrane. In many cell types, activation of mTORC1 strongly represses the PI3K-AKT axis upstream of PI3K. Activation of S6K1 by mTORC1 promotes the phosphorylation of IRS1 and reduces its stability (reviewed by Harrington et al., 2005). This auto-regulatory pathway, characterized as the S6K1-dependent negative feedback loop, has been shown to have profound implications for both metabolic diseases and tumorigenesis (reviewed by Manning, 2004). Other pathways that are independent of IRS1 are also likely to contribute to the retro-inhibition of mTORC1. For example, loss of TSC1/2 suppresses platelet-derived growth factor receptor (PDGFR) expression in a rapamycin-sensitive manner (Zhang et al., 2007). How mTOR signaling controls PDGFR expression remains to be determined.

Energy status

The energy status of the cell is signaled to mTORC1 through AMP-activated protein kinase (AMPK), a master sensor of intracellular energy status (reviewed by Hardie, 2007). In response to energy depletion (low ATP:ADP ratio), AMPK is activated and phosphorylates TSC2, which increases the GAP activity of TSC2 towards Rheb and reduces mTORC1 activation (Inoki et al., 2003). Additionally, AMPK can reduce mTORC1 activity in response to energy depletion by directly phosphorylating Raptor (Gwinn et al., 2008).

Oxygen levels

Oxygen levels affect mTORC1 activity through multiple pathways (reviewed by Wouters and Koritzinsky, 2008). Under conditions of mild hypoxia, the reduction in ATP levels activates AMPK, which promotes TSC1/2 activation and inhibits mTORC1 signaling as described in the previous section (Arsham et al., 2003; Liu et al., 2006). Hypoxia can also activate TSC1/2 through transcriptional regulation of DNA damage response 1 (REDD1) (Brugarolas et al., 2004; Reiling and Hafen, 2004). REDD1 blocks mTORC1 signaling by releasing TSC2 from its growth-factor-induced association with 14-3-3 proteins (DeYoung et al., 2008). This ability of REDD1 to reduce mTORC1 signaling by disrupting the interaction of TSC2 and 14-3-3 has probably evolved to limit energy-consuming processes when oxygen, but not growth factors, is scarce. Additionally, promyelocytic leukemia (PML) tumor suppressor and BCL2/adenovirus E1B 19 kDa protein-interacting protein 3 (BNIP3) reduce mTORC1 signaling during hypoxia by disrupting the interaction between mTOR and its positive regulator Rheb (Bernardi et al., 2006; Li et al., 2007).

Amino acids

Amino acids represent a strong signal that positively regulates mTORC1 (reviewed by Guertin and Sabatini, 2007). It was recently shown that leucine, an essential amino acid required for mTORC1 activation, is transported into cells in a glutamine-dependent fashion (Nicklin et al., 2009). Glutamine, which is imported into cells through SLC1A5 [solute carrier family 1 (neutral amino acid transporter) member 5], is exchanged to import leucine via a heterodimeric system composed of SLC7A5 [antiport solute carrier family 7 (cationic amino acid transporter, y+ system, member 5] and SLC3A2 [solute carrier family 3 (activators of dibasic and neutral amino acid transport) member 2]. The mechanism by which intracellular amino acids then signal to mTORC1 remained obscure for many years. The activation of mTORC1 by amino acids is known to be independent of TSC1/2, because the mTORC1 pathway remains sensitive to amino acid deprivation in cells that lack TSC1 or TSC2 (Nobukuni et al., 2005). Some studies have implicated human vacuolar protein-sorting-associated protein 34 (VPS34) in nutrient sensing (Nobukuni et al., 2005); however, the precise role of human VPS34 in this process still remains to be established (Juhasz et al., 2008).

Recently, two independent teams, including ours, have shown that the Rag proteins, a family of four related small GTPases, interact with mTORC1 in an amino acid-sensitive manner and are necessary for the activation of the mTORC1 pathway by amino acids (Kim et al., 2008; Sancak et al., 2008). In the presence of amino acids, Rag proteins bind to Raptor and promote the relocalization of mTORC1 from discrete locations throughout the cytoplasm to a perinuclear region that contains its activator Rheb (Sancak et al., 2008). The physical dissociation of mTORC1 and Rheb with amino acid deprivation might explain why activators of Rheb, such as growth factors, cannot stimulate mTORC1 signaling in the absence of amino acids.

Other cellular conditions and signals

In addition to the key signals described above, other cellular conditions and signals, such as genotoxic stress, inflammation, Wnt ligand and PA, have all been shown to regulate mTORC1 signaling. Genotoxic stress reduces

mTORC1 activity through many mechanisms. For instance, the activation of p53 in response to DNA damage rapidly activates AMPK through an unknown process, which in turn phosphorylates and thereby activates TSC2 (Feng et al., 2005). Additionally, p53 negatively controls mTORC1 signaling by increasing the transcription of phosphatase and tensin homolog deleted on chromosome 10 (PTEN) and TSC2, two negative regulators of the pathway (Feng et al., 2005; Stambolic et al., 2001). Inflammatory mediators also signal to mTORC1 via the TSC1/2 complex. Pro-inflammatory cytokines, such as TNFα, activate IκB kinase-β (IKKβ), which physically interacts with and inactivates TSC1, leading to mTORC1 activation (Lee et al., 2007). This positive relationship between inflammation and mTORC1 activation is thought to be important in tumor angiogenesis (Lee et al., 2007) and in the development of insulin resistance (Lee et al., 2008). Wnt signaling also increases mTORC1 activity through the inactivation of TSC1/2. Stimulation of the Wnt pathway inhibits glycogen synthase kinase 3 (GSK3), a kinase that promotes TSC1/2 activity by directly phosphorylating TSC2 (Inoki et al., 2006). Finally, PA has been identified as another activator of mTORC1. Many groups have shown that exogenous PA or overexpression of PA-producing enzymes such as phospholipase D1 (PLD1) and PLD2 significantly increases mTORC1 signaling (reviewed by Foster, 2007). A recent study suggests that PA affects mTOR signaling by facilitating the assembly of mTOR complexes, or stabilizing the complexes (Toschi et al., 2009).

mTORC2 still has many secrets to reveal

In contrast to mTORC1, for which many upstream signals and cellular functions have been defined (see above), relatively little is known about mTORC2 biology. The early lethality caused by the deletion of mTORC2 components in mice, as well as the absence of mTORC2 inhibitors, have complicated the study of this protein complex. Nonetheless, many important discoveries have been made over the last few years. Using various genetic approaches, it has been demonstrated that mTORC2 plays key roles in various biological processes, including cell survival, metabolism, proliferation and cytoskeleton organization. The role of mTORC2 in these processes is discussed in more detail below.

Cell survival, metabolism and proliferation

Cell survival, metabolism and proliferation are all highly dependent on the activation status of AKT, which positively regulates these processes through the phosphorylation of various effectors (reviewed by Manning and Cantley, 2007). Full activation of AKT requires its phosphorylation at two sites: Ser308, by phosphoinositide-dependent kinase 1 (PDK1), and Ser473, by a kinase that remained unidentified for many years, but was demonstrated to be mTORC2 by our group in 2005 (Sarbassov et al., 2005). Other studies have subsequently observed that ablation of various mTORC2 components specifically blocks AKT phosphorylation at Ser473 and the downstream phosphorylation of some, but not all, AKT substrates (Guertin et al., 2006; Jacinto et al., 2006). Inhibition of AKT following mTORC2 depletion reduces the phosphorylation of, and therefore activates, the forkhead box protein O1 (FoxO1) and FoxO3a transcription factors, which control the expression of genes involved in stress resistance, metabolism, cell-cycle arrest and apoptosis (reviewed by Calnan and Brunet, 2008). By contrast, the phosphorylation state of TSC2 and GSK3 is not affected by mTORC2 inactivation. Recently, serum- and glucocorticoid-induced protein kinase 1 (SGK1), which shares homology with AKT, was also shown to be regulated by mTORC2 (Garcia-Martinez and Alessi, 2008). In contrast to AKT, which retains a basal activity when mTORC2 is inhibited, SGK1 activity is totally abrogated under these conditions. Because SGK1 and AKT phosphorylate FoxO1 and FoxO3a on common sites, it is possible that the lack of SGK1 activity in mTORC2-deficient cells is responsible for the inhibition of phosphorylation of FoxO1 and FoxO3a.

Cytoskeletal organization

mTORC2 regulates cytoskeletal organization. Many independent groups have observed that knocking down mTORC2 components affects actin polymerization and perturbs cell morphology (Jacinto et al., 2004; Sarbassov et al., 2004). These studies have suggested that mTORC2 controls the actin cytoskeleton by promoting protein kinase Cα (PKCα) phosphorylation, phosphorylation of paxillin and its re-localization to focal adhesions, and the GTP loading of RhoA and Rac1. The molecular mechanism by which mTORC2 regulates these processes has not been determined.

Signaling to mTORC2: the black box

The signaling pathways that lead to mTORC2 activation are not well characterized. Because growth factors increase mTORC2 kinase activity and AKT phosphorylation at Ser473, they are considered to be a plausible signal for regulating this pathway (reviewed by Guertin and Sabatini, 2007). With growth-factor stimulation, AKT is phosphorylated at the cell membrane through the binding of PtdIns(3,4,5)P_3 to its pleckstrin homology (PH) domain. Under these conditions, PDK1 is also recruited to the membrane through its PH domain and phosphorylates AKT at Ser308 (reviewed by Lawlor and Alessi, 2001). Interestingly, the mTORC2 component mSIN1 possesses a PH domain at its C-terminus, suggesting that mSIN1 can promote the translocation of mTORC2 to the membrane and the phosphorylation of AKT at Ser473. Additional work is needed to support this model and to identify other cellular signals that play a role in the regulation of mTORC2.

Perspectives

Over the last decade, knowledge of the mTOR signaling pathway has greatly progressed, enabling researchers to better understand the mechanism of diseases such as cancer and type 2 diabetes. Despite these advances, our understanding of this signaling network is far from complete and many important questions remain to be answered. For example, how is mTORC2 regulated and which biological processes does it control? How are the mTORC1 and mTORC2 signaling pathways integrated with each other? What are the functions of these complexes in adult tissues and organs and what are the implications of their dysfunction or dysregulation in health and disease? Are there additional mTOR complexes that regulate other biological processes? Finding answers to these important questions will advance our understanding of cellular biology, and will also help the development of therapeutic avenues to treat many human diseases.

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