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Regulation of the mTOR Complex 1 Pathway by Nutrients, Growth Factors, and Stress

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The large serine/threonine protein kinase mTOR regulates cellular and organismal homeostasis by coordinating anabolic and catabolic processes with nutrient, energy, and oxygen availability and growth factor signaling. Cells and organisms experience a wide variety of insults that perturb the homeostatic systems governed by mTOR and therefore require appropriate stress responses to allow cells to continue to function. Stress can manifest from an excess or lack of upstream signals or as a result of genetic perturbations in upstream effectors of the pathway. mTOR nucleates two large protein complexes that are important nodes in the pathways that help buffer cells from stresses, and are implicated in the progression of stress-associated phenotypes and diseases, such as aging, tumorigenesis, and diabetes. This review focuses on the key components of the mTOR complex 1 pathway and on how various stresses impinge upon them.

Introduction

mTOR belongs to the family of phosphoinositide-3-kinase (PI3K)-related kinases (PIKKs), along with ATM, ATR, DNA-PK, and hSMG1. All of these proteins have C-terminal protein kinase domains with similarity to the lipid kinase PI3K, thus giving the family its name. While all members of the family respond to genotoxic stresses, mTOR also responds to numerous other stresses, including those related to nutrient, energy, and oxygen levels. In addition to its kinase domain, mTOR has numerous heat repeats in its N-terminal half, as well as a FRAP-ATM-TTRAP (FAT) domain of poorly understood function.

mTOR exists in two distinct complexes called complex 1 (mTORC1) and complex 2 (mTORC2) (Guertin and Sabatini, 2007). mTORC1 is a homodimer (Takahara et al., 2006; Wang et al., 2006; Yip et al., 2010; Zhang et al., 2006), and has four components in addition to mTOR: 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). The numerous heat domains in the mTOR N-terminal half are thought to mediate its interaction with binding partners. Raptor has many functions, and it regulates mTORC1 assembly and recruits kinase substrates, such as 4E-BP1 (Hara et al., 2002; Kim et al., 2002). Recent work reveals that Raptor also plays an essential role in determining the subcellular localization of mTORC1 and in sensing amino acids (Sancak et al., 2008). Despite being a core component of mTORC1 (Kim et al., 2003; Loewith et al., 2002), the role of mLST8 remains unclear, because its deletion has no effect on mTORC1 activity in vivo or in vitro (Guertin et al., 2006). PRAS40 and Deptor are components as well as substrates of mTORC1, and in the dephosphorylated state, they appear to repress mTORC1 activity. When activated, the mTOR component of mTORC1 phosphorylates both PRAS40 and Deptor, which weakens their association with the remainder of mTORC1 and promotes the

kinase activity of the complex (Oshiro et al., 2007; Peterson et al., 2009; Sancak et al., 2007; Thedieck et al., 2007; Vander Haar et al., 2007; Wang et al., 2008).

The defining component of mTORC2 is Rictor (RPTOR-independent companion of mTOR), a protein that is mutually exclusive with raptor for binding to mTOR (Jacinto et al., 2004; Sarbassov et al., 2004). mTORC2 shares several proteins with mTORC1, such as mLST8 and Deptor (Jacinto et al., 2004; Peterson et al., 2009; Sarbassov et al., 2004), and, like mTORC1, it also appears to be a multimer (Wullschlegler et al., 2005). However, mTORC2 also contains several unique components in addition to Rictor, including mammalian stress-activated protein kinase interacting protein (mSIN1) and protein observed with Rictor-1 (Protor-1, also known as PRR5). Rictor is absolutely required for mTORC2 catalytic activity (Sarbassov et al., 2004) and may serve to recruit substrates to mTORC2, much like Raptor does with mTORC1. Rictor and mSIN1 help stabilize each other and thus may provide structural integrity to mTORC2 (Frias et al., 2006; Jacinto et al., 2006). Interestingly, mSIN1 contains regions of homology to Ras-binding domains and a pleckstrin homology (PH)-like domain, suggesting that mSIN1 may have additional roles beyond providing structural support for mTORC2 (Schroder et al., 2007). Protor-1 binds Rictor, but it is not required for mTORC2 catalytic function (Thedieck et al., 2007; Woo et al., 2007). In contrast to the situation with mTORC1, mLST8 is required for mTORC2 function in vivo and its kinase activity in vitro (Guertin et al., 2006). As with mTORC1, Deptor binds to the FAT domain of mTOR, and it also inhibits mTORC2 (Peterson et al., 2009).

Compared to mTORC1, our understanding of the upstream inputs to mTORC2 is very limited, and, other than the kinases Akt and SGK, few substrates of mTORC2 are known (Sparks and Guertin, 2010). The development of new small-molecule inhibitors of mTOR and genetic loss- and gain-of-function alleles of mTORC2 components will undoubtedly lead to an expansion

in our knowledge of mTORC2 function in the next few years. This review will focus almost solely on the role mTORC1 plays in cellular stress responses, as little is known about the potential roles of mTORC2.

Downstream Actions of mTORC1

In response to a wide range of upstream inputs, mTORC1 regulates growth by maintaining the appropriate balance between anabolic processes, such as macromolecular synthesis and nutrient storage, and catabolic processes, like autophagy and the utilization of energy stores. Many of the functions ascribed to mTORC1 were first revealed through the use of the small-molecule rapamycin, an allosteric inhibitor of mTOR. Upon entering the cell, rapamycin interacts with high affinity to the peptidyl-proline isomerase FKBP12. The resulting drug-receptor complex then binds to the FKBP12-rapamycin-binding (FRB) domain of mTOR, which is located between the FAT and kinase domains (reviewed in [Sarbasov et al., 2005](#)). Interestingly, for unknown reasons, rapamycin-FKBP12 inhibits mTORC1 activity only toward a subset of mTORC1 substrates ([Gingras et al., 2001](#); [Jiang et al., 2001](#); [Kudchodkar et al., 2004](#); [Thoreen et al., 2009](#)). New approaches to inhibiting mTORC1, including loss-of-function alleles of the mTORC1 component raptor as well as ATP-competitive inhibitors of the mTOR kinase domain, are beginning to sort mTORC1 functions into rapamycin-sensitive and -resistant categories ([Feldman et al., 2009](#); [Nowak et al., 2009](#); [Thoreen et al., 2009](#); [Yu et al., 2009](#)). Below, we summarize the cellular programs controlled by mTORC1, with an emphasis on the functions of its best-known substrates: the AGC-family S6 kinases S6K1 and S6K2 and the 4E-BP1 and 4E-BP2 proteins.

Protein Synthesis

Protein synthesis is energetically costly, requiring copious amounts of ATP and GTP and the production of abundant numbers of ribosomes. mTORC1 regulates the activity of the translational machinery as a whole and also specifically controls the translation of a subset of messenger RNAs (mRNAs) that are thought to promote cell growth and proliferation. The mTORC1 substrates, the S6Ks and 4E-BPs, regulate several aspects of mRNA translation in a phosphorylation-dependent fashion. The 4E-BPs, when dephosphorylated, interfere with translation initiation by binding and sequestering the eIF-4E mRNA cap-binding protein and preventing its assembly into the eIF-4F cap-binding complex, thus blocking cap-dependent mRNA translation ([Pause et al., 1994](#)). Phosphorylation of 4E-BP1 on four sites by mTORC1, two of which are rapamycin sensitive, blocks its inhibitory ability ([Beretta et al., 1996](#); [Dowling et al., 2010](#); [Feldman et al., 2009](#); [Thoreen et al., 2009](#)). The features of mRNAs, such as those for cyclin D3 and VEGF, that are particularly sensitive to 4E-BP function are not completely clear, but they probably include extensive secondary structures in the 5' untranslated regions (UTRs) that require unwinding by the eIF4F complex and/or the lack of an internal ribosome entry site (reviewed in [Hay and Sonenberg, 2004](#); [Ma and Blenis, 2009](#)). mTORC1 is also a critical regulator of the translation of an abundant class of mRNAs called 5' TOP mRNAs, which have relatively short 5' UTRs that contain stretches of pyrimidines ([Hornstein et al., 2001](#)) and tend to encode for components

of the translational machinery. Although these are the best-known class of mRNA specifically controlled by mTORC1, the mechanism through which mTORC1 controls their translation is unknown. For more on the regulation of translation by nutrient levels see [Spriggs et al. \(2010\)](#) in this issue.

The activation of S6Ks in response to mTORC1-mediated phosphorylation promotes mRNA translation through several substrates, such as S6K1 aly/REF-like target (SKAR), programmed cell death 4 (PDCD4), eukaryotic elongation factor 2 kinase (eEF-2K), eukaryotic initiation factor 4B (eIF4B), and ribosomal protein S6 ([Ma and Blenis, 2009](#)). While S6K1 and S6K2 have overlapping functions, deletion studies in mice and *in vitro* analyses have revealed separate cellular localizations and distinct functions, which are likely to be mediated through unique substrates ([Lee-Fruman et al., 1999](#); [Pende et al., 2004](#); [Shima et al., 1998](#)). However, with the exception of SKAR, which has been shown to be a specific S6K1 substrate ([Richardson et al., 2004](#)), it is unclear whether the substrates mentioned earlier are acted upon by S6K1 or S6K2 or both.

S6K-mediated phosphorylation of S6, a component of the 40S ribosomal subunit, was first characterized in regenerating livers, and it correlates with high rates of protein synthesis under growth-promoting conditions ([Gressner and Wool, 1974](#)). However, cells expressing only a nonphosphorylatable S6 have increased rather than decreased rates of protein synthesis and cell division compared to controls ([Ruvinsky et al., 2005](#)). Thus, despite being the first S6K substrate identified, the role of S6 in promoting translation remains unclear.

Better understood is the role of activated S6K1 in promoting translation through elongation factor 2 kinase (eEF-2K). This kinase normally negatively regulates translation elongation by phosphorylating and suppressing eukaryotic elongation factor 2 (eE-F2), and S6K1 relieves this inhibition by directly phosphorylating eEF-2K ([Wang et al., 2001](#)). The S6K1 substrate, PDCD4, inhibits translational initiation under serum withdrawal and is lost in aggressive carcinomas ([Carayol et al., 2008](#); [Dorrello et al., 2006](#)). PDCD4 inhibits eIF-4A, a RNA helicase needed to unwind secondary structures in the 5' UTR ([Yang et al., 2003](#)). Phosphorylation by S6K1 allows degradation of PDCD4 by promoting the binding of an ubiquitin ligase, thus relieving its repression of eIF-4A activity ([Dorrello et al., 2006](#)). S6K also activates the helicase activity of eIF-4A through direct phosphorylation of eIF4B ([Raught et al., 2004](#)). eIF4B potentiates the helicase activity of eIF-4A by binding to the eIF4F complex (which contains the helicase eIF-4A) via eIF3 ([Vornlocher et al., 1999](#)). Phosphorylation of eIF4B by S6K promotes its association with eIF3 and thus promotes translation initiation of mRNAs with secondary structure within the 5' UTR ([Shahbazian et al., 2006](#)).

S6K1 also promotes the translational efficiency of newly spliced mRNAs. Upon splicing, mRNAs recruit unique 5' cap-binding proteins and the exon-junction complex (EJC) to regions ~20 nucleotides upstream of exon-exon junctions. The EJC remains bound to mRNAs during nuclear exit and through the first round of ribosome passage, which is referred to as the pioneering round of translation. Subsequent rounds do not require EJC binding and involve different cap-binding proteins. The EJC performs quality control on mRNAs by eliminating those with premature termination codons (PTCs) (reviewed in [Lejeune](#)

and Maquat, 2005) and also enhances protein synthesis of normal mRNAs (Gudikote et al., 2005; Nott et al., 2004; Wiegand et al., 2003). In response to phosphorylation by mTORC1, S6K1 is recruited to newly spliced mRNAs by its substrate and binding partner SKAR, which also binds the EJC. Recruitment of S6K1 to the EJC leads to phosphorylation of numerous mRNA-binding proteins and correlates with increased translational efficiency of spliced mRNAs (Ma et al., 2008).

Autophagy

Autophagy is a recycling process through which cells liberate intracellular stores of nutrients by degrading cytoplasmic proteins and organelles in lysosomes. In mammalian cells the primary form of autophagy is macroautophagy (referred to from now on as autophagy) and requires the formation of double-membrane autophagosomes that sequester cytoplasmic components and then fuse with lysosomes (Klionsky, 2007; Kroemer et al., 2010, this issue). A major regulator of autophagy is mTORC1, which in the presence of nutrients and growth factors strongly inhibits the initiation of autophagy (Noda and Ohsumi, 1998). Autophagy is upregulated during periods of starvation or growth factor withdrawal, as well as in response to oxidative stress, infection, or the accumulation of protein aggregates. While mTORC1 inhibition triggers autophagy, the release of amino acids from autophagic protein degradation eventually leads to the reactivation of mTORC1, which in turn restores the cellular lysosomal population (Yu et al., 2010). Directly downstream of mTORC1 are numerous proteins that are required for the execution of the autophagic program, including the serine/threonine kinase Atg1/ULK, which plays a key role in the formation of the preautophagosome (Kamada et al., 2000). ULK1 forms a complex with Atg13 and FIP200, which promote ULK1 kinase activity and localization to the preautophagosome (Ganley et al., 2009). mTORC1 phosphorylates ULK1 and Atg13, moderately reducing ULK1 kinase activity but not affecting its association with Atg13 and FIP200. Reports conflict about whether mTORC1 binds to the complex under nutrient-replete conditions, and more evidence is needed to determine the role mTORC1 phosphorylation of ULK1 plays in its subcellular localization and interaction with other autophagy proteins. As a result, it is too early to know whether these phosphorylation events fully explain the control of autophagy by mTORC1 (Ganley et al., 2009; Hosokawa et al., 2009; Jung et al., 2009). Interfering with the ability of cells to undergo autophagy within an intact animal produces a range of phenotypes that underscore the importance of autophagy not only as an adaptive response to nutrient stress, but also in general cell and tissue housekeeping. For example, mice lacking *Atg5*, which is required for autophagosome formation, are born at mendelian ratios, but die within 1 day of delivery because they are unable to mobilize the energy and nutrient stores they require to survive the pre-suckling period (Kuma et al., 2004). Mice depleted of *Atg5* in just neural cells exhibit a progressive decline in motor activity that correlates with the buildup of protein aggregates in neurons, indicating that autophagy is essential for the basal clearance of these aggregates and to maintain proper neuronal function in adult animals (Hara et al., 2006). Tissue-specific deletions of additional genes required for autophagy have uncovered roles for autophagy in cardiac contractility, immune

cell function, and the liver detoxification of drugs (Levine and Kroemer, 2008).

Metabolism

As a sensor of nutrient and growth factor levels, it is not surprising that mTORC1 also controls numerous metabolic pathways. Although this area of mTOR research is still in its infancy, it appears that mTORC1 can regulate a variety of metabolic pathways in several tissue types and at the transcriptional, translational, and posttranslational levels (reviewed in Polak and Hall, 2009). In lymphoma cells, inhibition of mTORC1 by rapamycin changes the levels of many mRNAs encoding metabolic enzymes, including those involved in glycolysis, as well as in amino acid, sterol, lipid, and nucleotide metabolism (Peng et al., 2002). Recent work combining metabolite profiling and gene expression analyses of cultured cells also found that mTORC1 regulates glycolysis and sterol and lipid biosynthesis but in addition identified the pentose phosphate pathway as under the control of mTORC1 (Düvel et al., 2010). Many reports show that mTORC1 activates the SREBP-1 transcription factor (Düvel et al., 2010; Peng et al., 2002; Porstmann et al., 2008), which belongs to the family of sterol regulatory element binding proteins (SREBPs) that mediate the effects of sterols on the expression of enzymes involved in lipid and cholesterol homeostasis (Yokoyama et al., 1993). Intact SREBP-1 is found in an inactive state in the endoplasmic reticulum (ER). In response to stimuli such as decreased sterol levels, insulin, or saturated fatty acids, SREBP-1 is escorted from the ER to the Golgi, where it is processed into an active form. Active SREBP-1 then translocates to the nucleus, where it transactivates promoters containing sterol regulatory elements and E-box sequences (Brown and Goldstein, 1997). mTORC1 increases SREBP-1 activity at least in part by promoting its posttranslational processing via S6K1, which in turn leads to the increased transcription of genes involved in sterol and lipid biosynthesis and the oxidative arm of the pentose-phosphate pathway (Düvel et al., 2010). mTORC1-mediated upregulation of SREBP-1 activity is necessary for lipogenesis induced by Akt (Porstmann et al., 2008).

mTORC1 also promotes the expression of the hypoxia-inducible factor (HIF) (see Majmundar et al., 2010), mostly by regulating the translation, in a 4E-BP-dependent manner, of the alpha subunit of HIF (Brugarolas et al., 2003; Düvel et al., 2010; Hudson et al., 2002; Zhong et al., 2000). HIF activates the transcription of 100–200 genes involved in cellular metabolism and the adaptation of cells to hypoxic conditions (Goldberg et al., 1988; Shweiki et al., 1992), and mTORC1-dependent activation of Hif-1 α is sufficient to upregulate these genes (Düvel et al., 2010). The emerging theme is that the regulation of HIF and SREBP-1 by mTORC1 is an important mechanism through which cells coordinate carbohydrate and lipid metabolism with the availability of nutrients.

mTORC1 also appears to be an important regulator of mitochondrial number and function. 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). Conversely, hyperactivation of mTORC1 increases mitochondrial DNA copy number, as well as the expression of many genes encoding proteins involved in oxidative metabolism

(Cunningham et al., 2007). In murine skeletal muscle, the lack of raptor reduces the expression of genes involved in mitochondrial biogenesis and leads to a concomitant loss of oxidative capacity (Bentzinger et al., 2008). Cunningham and colleagues found that mTORC1 promotes the transcriptional activity of PPAR γ 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 the yin-yang 1 (YY1) transcription factor (Cunningham et al., 2007). The direct interaction between mTORC1, PGC1 α , and YY1 is somewhat at odds with fractionation and imaging studies that show little to no endogenous mTORC1 in the nucleus (Rosner and Hengstschl-äger, 2008; Sancak et al., 2008). In general, how mTORC1 signaling impinges upon transcriptional networks remains a poorly understood process. As evidence mounts demonstrating that mTORC1 regulates the function of important transcription factors such as SREBP1, PGC1 α , PPAR γ , and p53, much more work is needed to fully understand how mTORC1 and its substrates signal to transcription factors.

Consistent with the changes in gene expression that occur in cultured cells upon mTORC1 manipulation, transgenic mice with mutations in *S6K1* or the *4E-BPs* exhibit profound metabolic alterations. *S6K1*-null mice are hypoinsulinemic as a result of decreased β cell mass (Pende et al., 2000) but are also hypersensitive to insulin signaling. The combination of the decrease in mTORC1 signaling and insulin levels with the hypersensitivity to insulin makes *S6K1*-null mice resistant to diet- and age-induced obesity (Um et al., 2004). Despite not affecting food intake, the loss of *S6K1* prevents fat accumulation by increasing the lipolysis of triglycerides from adipose stores and the oxidation of fatty acids in fat and muscle. Mice deficient for both *4E-BP1* and *4E-BP2* have the opposite phenotype. While these mice are viable and appear to develop normally, they have a propensity to become obese and gain ~30% more weight than controls when fed a normal diet over the course of 16 weeks (Le Bacquer et al., 2007). These mice also have an increase in adipose tissue and plasma insulin and cholesterol levels, thus establishing them as a model for the development of obesity.

Regulation of mTORC1 by Upstream Signals

A variety of upstream signals regulate mTORC1 activity, including growth factors and amino acids, glucose, and oxygen levels. Regulation of mTORC1 by most signals occurs primarily through two types of mechanisms: the direct modification of mTORC1 components or the regulation of Rheb, a small GTPase that, when bound to GTP, directly interacts with and activates mTORC1 (Long et al., 2005; Sancak et al., 2007). Amino acid signaling to mTORC1 occurs through an interesting pathway that requires Rheb, but also the Rag GTPases, and is discussed below in its own section. While flies have only one Rheb, mammals have Rheb1 and Rheb2, of which only Rheb1 has been extensively studied. The intracellular location of Rheb is poorly defined, but it appears to be present on the endomembrane system of cells with at least some on lysosomal membranes, where, in the presence of amino acids, it is joined by mTORC1. Below, we describe how distinct upstream signals affect mTORC1 through Rheb and the Rag GTPases and by modifying mTORC1 components.

Growth Factor Sensing

The blood levels of growth factors such as insulin and IGF-1 reflect the fed status of the organism. When food is plentiful, levels of these growth factors are sustained and promote anabolic cell processes such as translation, lipid biosynthesis, and nutrient storage via mTORC1 (Figure 1). The binding of insulin to its cognate tyrosine kinase receptor recruits insulin receptor substrate 1 (IRS1) to the receptor and activates phosphoinositide 3-kinase (PI3K), which through the production of phosphatidylinositol (3,4,5)-triphosphate [PtdIns(3,4,5)P $_3$] recruits Akt to the plasma membrane, where it becomes activated by direct phosphorylation by PDK1 and mTORC2. Akt, along with other kinases downstream of growth factor signaling, such as MAPK and p90 RSK1, phosphorylates TSC2 (Ballif et al., 2005; Li et al., 2003; Potter et al., 2002; Roux et al., 2004; Tee et al., 2003a). TSC2 (also known as tuberlin) is the GTPase-activating protein (GAP) for Rheb, and together with its partner TSC1 (also known as hamartin) forms the heterodimeric tuberous sclerosis complex (TSC). *Tsc1* and *Tsc2*, when lost, lead to the development of tuberous sclerosis complex, a tumor

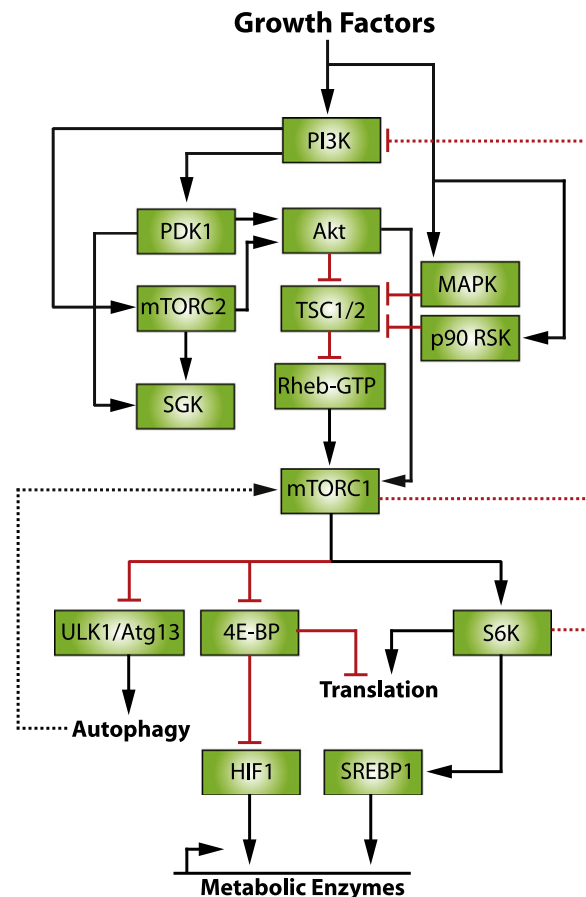


Figure 1. Growth Factors Activate mTORC1 through Multiple Pathways

Black lines signify activating connections, whereas red lines signify inhibitory inputs between proteins. Dotted lines indicate connections between proteins that are not known to be direct.

syndrome characterized by the appearance in a variety of tissues of benign tumors containing large cells (Kwiatkowski and Manning, 2005; Orlova and Crino, 2010). When active, TSC2 inhibits mTORC1 by promoting the conversion of Rheb-GTP to Rheb-GDP (Inoki et al., 2003a; Tee et al., 2003b). The phosphorylation of TSC2 in response to growth factors correlates with an increase in mTORC1 activation, but exactly how TSC2 phosphorylation leads to its repression is unclear. The initial assumption was that the phosphorylation of TSC2 by Akt would directly suppress its GAP activity toward Rheb, but in vitro assays have not confirmed this, and it is still unclear exactly how the GAP function of TSC2 is regulated. Inoki et al. observed a weakened interaction between Akt-phosphorylated TSC2 and TSC1 in mammalian cells, which lead to ubiquitin-mediated degradation of TSC2 (Inoki et al., 2002). Recent work shows that hypophosphorylated TSC2 is present within the membrane fraction of cells, where it interacts with TSC1 (Cai et al., 2006). Phosphorylation of TSC2 by Akt promotes 14-3-3 binding and the subsequent sequestration of TSC2 in the cytosolic fraction away from TSC1, which, along with Rheb, likely remain in the membrane compartment (Cai et al., 2006). This shift from a membrane to cytosolic compartment correlates with the inhibition of TSC2 function and activation of mTORC1 but has not yet been proven to be the key regulatory event. In contrast, Manning et al. immunoprecipitated TSC2 from PDGF-stimulated cells and found that, over time, Akt phosphorylation of TSC2 did not alter its interaction with TSC1 (Manning et al., 2002). Similarly, in *Drosophila* cells, expression of a TSC2 mutant having residues normally phosphorylated by Akt changed to either alanine or phospho-mimicking amino acids, interacted with TSC1 to the same extent as wild-type TSC2 (Dong and Pan, 2004). Remarkably, both mutants of TSC2 sufficiently rescued the growth defects in *Tsc2*-null mutants, indicating a key difference between flies and mammals in growth regulation by Akt via the TSC complex (Dong and Pan, 2004). Further molecular analysis of TSC1 and TSC2 is needed before a consensus can be reached regarding its repression by growth factor signaling.

Growth factors also regulate mTORC1 through mechanisms that are independent of TSC. Growth factor-activated Akt phosphorylates PRAS40, relieving its repression of mTORC1 (Oshiro et al., 2007; Sancak et al., 2007; Thedieck et al., 2007; Vander Haar et al., 2007; Wang et al., 2008; Wang et al., 2007). In TSC2-null cells, GTP-loaded Rheb can overcome the repressive actions of PRAS40, indicating an unequal balance in these two points of regulation of mTORC1 (Sancak et al., 2007). Activation of mTORC1 in response to growth factors also correlates with phospholipase-D (PLD)-dependent accumulation of phosphatidic acid (PA). RNA interference (RNAi)-mediated depletion of PLD1 moderately inhibits mTORC1, and PA has been shown to bind directly to the FRB domain of mTOR (Fang et al., 2003; Fang et al., 2001; Foster, 2009; Sun et al., 2008). Recent data demonstrate that loss of TSC2 or overexpression of Rheb-GTP can activate PLD1, indicating that PA activation of mTORC1 may not be fully independent of TSC (Sun et al., 2008). A full understanding of the importance of this mechanism requires more analysis because PA is not sufficient on its own to activate mTORC1, has no effect on its kinase activity in vitro,

and, like other upstream inputs, requires the presence of amino acids (Fang et al., 2001).

Attenuated growth factor signaling represses mTORC1 activity, which downregulates nutrient and energy use and helps cells survive periods of starvation and intermittent fasting. Indeed, many organisms mutant for growth factors or their receptors can tolerate greater levels of stress, have an increased life span, and are frequently resistant to metabolic disease. Loss-of-function mutations in the IGF-1 receptor (IGF-1R) in mice or its orthologs in flies (InR) and worms (DAF-2) promote longevity, with female *Igf-1R* heterozygous mice living 33% longer than controls (Guarente and Kenyon, 2000; Holzenberger et al., 2003). The degree that suppression of mTORC1 contributes to the increased life span of IGF-1R heterozygous mice is not known, but it is interesting to note that rapamycin treatment of mice also increased life span (Harrison et al., 2009).

Hyperactivation of growth factor signaling is associated with decreased tolerance to multiple stresses, as well as cancer, cardiac hypertrophy, and neuronal dysfunction. Hyperactivation of mTORC1 caused by loss of TSC1 or TSC2 blocks cells from initiating appropriate responses to growth factor deprivation, such as autophagy, and triggers processes such as ER stress. The induction of ER stress leads to increased reactive oxygen species (ROS) production and apoptosis in fibroblasts, kidney tumors, and neurons (Di Nardo et al., 2009; Ozcan et al., 2008). In future work, it will be important to understand how the increased stress levels of cells lacking TSC1 or TSC2 contributes to the development and progression of tumors in patients with tuberous sclerosis.

Upon being activated by growth factors, mTORC1 limits the extent of growth factor signaling via what the mTOR field calls the "negative feedback loop." However, in contrast to classic feedback loops where a threshold is reached before inhibition occurs, mTORC1 seems to suppress growth factor signaling in a more gradual and continual fashion. Accordingly, acute rapamycin treatment leads to enhanced PI3K signaling, whereas, as expected, constitutively active mTORC1 signaling continually suppresses PI3K signaling (Harrington et al., 2004). There appear to be multiple mechanisms through which the negative feedback loop is initiated. In one well-characterized mechanism, S6K1 reduces IRS1 activity and expression by phosphorylating it, which interferes with its binding to the insulin receptor, promotes its degradation, and decreases the levels of its mRNA (Harrington et al., 2004; Shah and Hunter, 2006; Tremblay et al., 2007). Activated S6K1 can also suppress the activity of other growth factor receptors that do not rely on IRS1, such as platelet-derived growth factor receptor (PDGFR), indicating that additional targets besides IRS1 must exist (Zhang et al., 2003). In another mechanism, mTORC1 directly interacts with IRS1 via Raptor and phosphorylates IRS1 at sites that interfere with its association with PI3K (Tzatsos, 2009).

Given the role of insulin and insulin-related growth factors in metabolic disorders and cancer, the negative input from mTORC1 to the insulin pathway likely has clinical implications. For example, the relatively poor ability of rapamycin to slow tumor growth in cancer patients may be because by relieving the feedback signal the drug increases PI3K activity, a known promoter of cell proliferation and survival (O'Reilly et al., 2006).

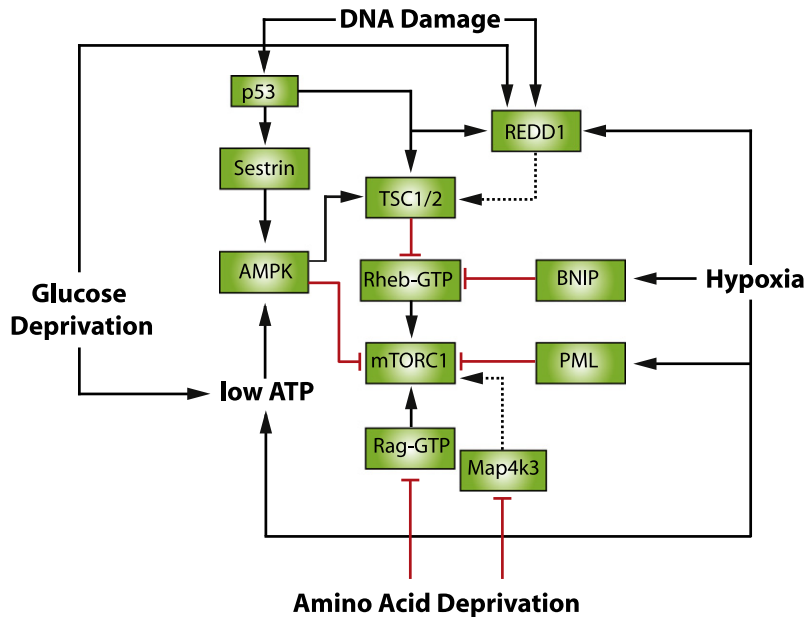


Figure 2. A Variety of Stresses Regulate mTORC1 through Multiple Pathways

Black lines signify activating connections, whereas red lines signify inhibitory inputs between proteins. Dotted lines indicate connections between proteins that are not known to be direct.

The differential effects of rapamycin on tumor growth may also reflect in part the varying effects of rapamycin on mTORC2 and its substrates Akt and SGK. Normally, acute rapamycin treatment is unable to inhibit mTORC2, likely because mTORC2 components prevent the binding of rapamycin-FKBP12 to mTOR by obscuring its FRB domain. However, in certain cell types, chronic exposure of rapamycin leads to inhibition of mTORC2, as well as of its best-known substrates, Akt and SGK. In these cells, disassociation and/or assembly of mTORC2 occurs in a manner that allows rapamycin-FKBP12 to bind free mTOR, which interferes with assembly of mTORC2 components (Sarbasov et al., 2006). Our inability to understand why certain tumors are sensitive to rapamycin precludes identification of a biomarker that would help in selecting patients who could benefit from the drug.

Multicellular organisms use growth factor signaling to communicate the fed status of the organism to their cells, but individual cells also retain systems that sense the levels of specific nutrients, such as carbohydrates and amino acids. Below, we describe the molecular underpinnings of how the mTORC1 pathway senses specific nutrients.

Glucose and Energy Sensing

Glucose deprivation leads to decreased glycolytic flux, which, by lowering ATP levels, inhibits mTORC1 (Figure 2). Depletion of cellular ATP by the hexokinase inhibitor, 2-deoxy-glucose (2DG), or the mitochondrial uncoupler, FCCP, inhibits mTORC1 in wild-type cells but not in those lacking TSC2 (Inoki et al., 2003b; Kimura et al., 2003). The signaling of low ATP levels to TSC2 requires the 5' AMP-activated protein kinase (AMPK). Decreased ATP production, and therefore an increase in the AMP/ATP ratio, activates AMPK, a heterotrimeric kinase composed of a catalytic (α) and two regulatory (β and γ) subunits (Kahn et al., 2005). AMP binds AMPK and puts the trimer in

a conformation that allows the kinase subunit to be phosphorylated by the upstream activating kinase LKB1 (Sanders et al., 2007), thus slowing cell growth and its many ATP-consuming processes (Shaw, 2009). AMPK directly phosphorylates TSC2, which through an unknown mechanism leads to its activation and thus to the suppression of mTORC1 (Corradetti et al., 2004; Inoki et al., 2003b; Shaw et al., 2004) and is vital for cell survival under glucose deprivation conditions (Inoki et al., 2003b).

Energy depletion may inhibit mTORC1 independently of TSC2 in *Drosophila*, where dTSC2 lacks the AMPK phosphorylation sites (Kalender et al., 2010), and there is evidence that TSC2-independent mechanisms also exist in mammalian cells (Gwinn et al., 2008). This mechanism

is mediated in part by the direct phosphorylation by AMPK of raptor on two serine residues that are conserved throughout evolution. These phosphorylation events induce 14-3-3 to bind raptor and inhibit, again through an unknown mechanism, the kinase activity of mTORC1 (Gwinn et al., 2008). The inhibition of mTORC1 caused by the AMPK-mediated phosphorylation of raptor is required for cell-cycle arrest in response to energy stress.

Drugs that trigger the cellular stress response to a high AMP/ATP ratio have long been used to treat diabetes, and recent research has focused on their potential use as anticancer agents. Drugs such as metformin and phenformin decrease ATP levels through the inhibition of mitochondrial respiration, which, through unclear mechanisms, relieves hyperglycemia in diabetics by promoting insulin-dependent glucose uptake in peripheral tissues while decreasing hepatic glucose production (Bailey and Turner, 1996). Activation of AMPK and subsequent inhibition of mTORC1 occurs in response to these drugs, but the contribution of this signaling pathway to the antidiabetic effects of the drugs remains unclear (Foretz et al., 2010; Shaw et al., 2005). A recent report indicates that, even in the absence of AMPK, these drugs can inhibit mTORC1 and increase glucose uptake in L6 myotubes, indicating that additional mechanisms may exist through which altered AMP/ATP ratios affect mTORC1 signaling (Kalender et al., 2010).

Oxygen Sensing

Low oxygen levels, or hypoxia, inhibit mTORC1 signaling through multiple mechanisms (Figure 2). Hypoxia decreases cellular ATP levels by inhibiting metabolic programs such as oxidative phosphorylation and as a result activates AMPK, which inhibits mTORC1 via TSC2 and raptor phosphorylation as described above. Hypoxia also represses mTORC1 through the actions of Redd1/RTP801/ DDIT4—first characterized in *Drosophila* as Scylla and Charybdis (Brugarolas et al., 2004;

Reiling and Hafen, 2004). These genes encode hypoxia-inducible transcripts that suppress mTORC1 in a poorly understood fashion. Redd1 is a 232 amino acid cytoplasmic protein with no recognizable domains, and genetic studies have placed its activation of TSC2 and consequent inhibition of mTORC1 in a parallel pathway to AMPK and PI3K (Sofer et al., 2005). It has been posited that Redd1 inhibits mTORC1 by controlling the release of TSC2 from its growth factor-induced association with 14-3-3, thereby stabilizing the interaction between TSC1 and TSC2 (DeYoung et al., 2008; Vega-Rubin-de-Celis et al., 2010). Many other insults besides hypoxia, including energy depletion, DNA damage, glucocorticoids, and oxidizing agents, also induce Redd1 (Ellisen et al., 2002; Wang et al., 2003), suggesting a broad role for Redd1 in conveying stress signals to mTORC1. Interestingly, cigarette smoke also induces Redd1, which may mediate smoking-induced lung injury by inhibiting mTORC1 and enhancing oxidative stress-induced cell death (Yoshida et al., 2010). In addition to AMPK and Redd1, there are reports of additional pathways through which hypoxia suppresses mTORC1 but that require confirmation and further follow up. For example, the promyelocytic leukemia tumor suppressor (PML) has been reported to bind mTOR and inactivate it through sequestration in nuclear bodies during hypoxia (Bernardi et al., 2006). Similarly, the hypoxia-inducible proapoptotic protein BNIP3 (BCI2/adenovirus E1B 19 kDa protein-interacting protein 3) may bind and inhibit Rheb1 (Li et al., 2007). The multiple pathways through which hypoxia suppresses mTORC1 indicate the importance to cells of this adaptive response.

Amino Acid Sensing

Cells respond to the stress of amino acid deprivation through the induction and suppression of multiple systems. Decreased intake of amino acids deprives the cell of substrates needed for protein synthesis as well as intermediates for feeding the TCA cycle and other metabolic processes. Low levels of amino acids inhibit TORC1 signaling in a wide range of organisms from yeast to mammals. In flies, deletion of dTOR mimics amino acid withdrawal by inhibiting larval growth and causing the accumulation of lipid vesicles in the larval fat body that reflects the breakdown and mobilization of stored nutrients (Britton and Edgar, 1998; Oldham et al., 2000; Zhang et al., 2000). In *C. elegans*, deletion of the intestinal amino acid transporter pep-2 inhibits ceTOR and insulin signaling and, by attenuating postembryonic growth, reduces body length and the number of progeny. While reducing growth, the inhibition of ceTOR also increases the ability of worms to withstand stresses such as heat and oxidative damage (Meissner et al., 2004).

In mammals, the protein composition of the diet influences the development of lean body mass, weight control, satiety, insulin sensitivity, and bone and cardiovascular health (Millward et al., 2008). The removal of individual amino acids from cells growing in culture inhibits mTORC1 by variable amounts with leucine or arginine withdrawal most closely phenocopying total amino acid deprivation (Hara et al., 1998). Moreover, amino acids sometimes affect each other in unexpected ways. For example, the transport of the leucine that is important for activating mTORC1 requires the efflux of glutamine via the bidirectional transporter SLC7A5 (Nicklin et al., 2009).

Fasting, intense exercise, and many systemic diseases exert stress upon skeletal muscle, resulting in decreased protein synthesis, increased protein degradation, and branch chain amino acid oxidation (Lecker et al., 1999; Norton and Layman, 2006). Many reports hypothesize that inhibition of mTORC1 contributes to the manifestation of these stress-related phenotypes (Khamzina et al., 2005; Kimball et al., 2000; Lang et al., 2010; McGhee et al., 2009). Modulation of mTORC1 in muscle probably occurs downstream of amino acids, in particular leucine, as well as insulin and IGF-1 signaling. Muscle-specific ablation of the mTORC1 component raptor confirmed the important role mTORC1 plays in balancing catabolic and anabolic cellular programs. *Raptor*-deficient muscles are small, become progressively more dystrophic and have altered metabolic function, including increased oxidative capacity (Bentzinger et al., 2008).

Amino acid signaling to mTORC1 remains poorly understood, but some of the key players are beginning to be uncovered (Kim et al., 2008; Sancak et al., 2008) (Figure 2). An essential step appears to be the amino acid-induced recruitment of mTORC1 to lysosomal membranes, where it can interact with a pool of Rheb that also appears to reside on this compartment (Sancak et al., 2010; Sancak et al., 2008). Lysosomal membranes contain a multimeric complex consisting of MP1, p14, and p18, which has been termed Ragulator (Sancak et al., 2010). This complex acts as a scaffold for a heterodimer of Rag GTPases of which there are four (RagA, RagB, RagC, and RagD). RagA and RagB are functionally redundant and interact with either RagC or RagD, which are also functionally redundant, to form a Rag heterodimer, which appears to constitutively reside on lysosomal membranes. The loading of the Rags with GTP or GDP appears to be regulated by amino acids. In cells deprived of amino acids, RagA/B is bound to GDP and RagC/D to GTP. Stimulation of cells with amino acids leads to a flipping, via an unknown mechanism, of the bound nucleotide so that RagA/B and RagC/D become GTP and GDP bound, respectively. This is the active state of the Rag heterodimer, which, through its interaction with raptor, serves as a docking site for mTORC1 on the lysosomal surface (Sancak et al., 2008). There, mTORC1 is proposed to interact with the lysosomal pool of Rheb-GTP. Because Rheb is required for the activation of mTORC1 by all upstream inputs, the amino acid-dependent recruitment of mTORC1 to the lysosomal surface should be necessary for activating mTORC1 by all signals (Sancak et al., 2010; Sancak et al., 2008). Indeed, loss of TSC1 or TSC2 does not activate mTORC1 in the absence of amino acids or Rag or Ragulator function (Kim et al., 2008; Sancak et al., 2008; Smith et al., 2005), and forced recruitment of mTORC1 to Rheb-containing lysosomes makes mTORC1 shed its requirement for amino acids, the Rag GTPases, and the Ragulator.

Genetic and RNAi studies indicate that the Rag GTPases and the Ragulator are absolutely necessary for amino acids to activate mTORC1. Conversely, overexpression of just a mutant of RagB that is constitutively bound to GTP is sufficient to activate mTORC1 in amino acid-free media and in response to metformin (Kalender et al., 2010; Sancak et al., 2010). Interestingly, metformin treatment phenocopies the effects of amino acid deprivation on the localization of mTORC1, thus suggesting

that this drug may inhibit mTORC1 via modulation of Rag signaling (Kalender et al., 2010). Further work is needed to determine whether the results found with metformin represent a novel pathway through which lowered ATP levels inhibit mTORC1 independently of AMPK.

Several additional upstream factors have been proposed to participate in amino acid signaling to mTORC1. Using double-stranded RNA (dsRNA) screens in *Drosophila* cells, the Lamb group identified a role for the Ste-20 related kinase Map4k3 in the activation of mTORC1 (Findlay et al., 2007). In cell culture models, knockdown of Map4k3 prevents the activation of mTORC1 by amino acids, whereas overexpression delays the inhibition of mTORC1 by amino acid withdrawal. It is unclear where in the pathway of amino acid activation Map4k3 lies and, in data not shown of a subsequent paper, the authors find that suppression of the Rag GTPases blunts the ability of Map4k3 to activate mTORC1, indicating that Map4k3 may act upstream of the Rag GTPases (Yan et al., 2010). Whether Map4k3 directly acts on the Rag GTPases to help recruit mTORC1 to the lysosomes is unclear. While one report showed a direct interaction between Map4k3 and RagA and RagC in *Drosophila* S2 cells (Bryk et al., 2010), another found no evidence for this interaction (Yan et al., 2010). Flies mutant for Map4k3 are viable, leaner, and show growth defects that are correlated with reduced, but not abrogated, mTORC1 signaling (Bryk et al., 2010). For more on nutrient sensing see Wellen and Thompson (2010) in this issue.

DNA Damage Sensing

Damage to DNA induces responses that enable cells to repair themselves or activate programmed cell death (Ciccia and Elledge, 2010, this issue). It is now appreciated that mTORC1 inhibition is an important part of the cellular response to DNA damage and mTORC1 hyperactivation caused by TSC1 or TSC2 deficiency sensitizes cells to the effects of DNA damage (Ghosh et al., 2006). Central to the coordination of DNA damage responses is the tumor suppressor, p53 (Riley et al., 2008; Vousden and Ryan, 2009). Multiple mechanisms have been proposed for how p53 inhibits mTORC1 in response to DNA damage. p53 induces the transcription of PTEN, TSC2, and Redd1, which all act to inhibit mTORC1 as discussed earlier (Ellisen et al., 2002; Feng et al., 2005; Stambolic et al., 2001). p53 also transactivates Sestrin1 and Sestrin 2, which can repress mTORC1 via AMPK-dependent regulation of the TSC1/TSC2 complex (Budanov and Karin, 2008; Feng et al., 2005) (Figure 2). Chronic activation of the *Drosophila* TOR homolog, dTOR, was also found to lead to Sestrin accumulation (Lee et al., 2010), suggesting a negative feedback on dTOR activation. In addition to repressing mTORC1 via transcriptional targets, there is indirect evidence that p53 causes a rapid decrease in translation initiation partly by regulating the phosphorylation of S6K and 4E-BP1 (Horton et al., 2002). These multiple mechanisms suggest that the induction of p53 upon genotoxic stress can repress mTORC1 at multiple levels (Ellisen et al., 2002; Feng et al., 2005; Stambolic et al., 2001).

Conclusion

An emerging view is that the mTOR complex 1 (mTORC1) protein kinase is a critical mediator of the cellular response to many

stresses, including DNA damage as well as drops in the levels of energy, glucose, amino acids, and oxygen. These stress responses impact a wide variety of both pathological and physiological states, such as aiding in the resistance of tumor cells to conventional therapy and modulating life span. Additional work is needed to understand how mTORC1 function and activity vary temporally during the course of a stress and the mechanisms that reset mTORC1 signaling once a stress subsides. A good example of the former comes from new insights into the role of mTORC1 during autophagy (Yu et al., 2010). Amino acid deprivation inhibits mTORC1, which then leads to the induction of autophagy. Over time, however, the autophagic process liberates amino acids, which reactivate mTORC1 and whose activity then promotes the replacement of lysosomes consumed during autophagy. mTORC1 is likely to orchestrate similarly elegant long term responses to the other stresses it senses. A better understanding of how stress responses impinge upon mTORC1 as a function of time may inform the timing and dosage of therapeutics aimed at manipulating mTORC1 activity in various disease states.

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