mTOR-Dependent Cell Survival Mechanisms

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The mechanistic target of rapamycin (mTOR) kinase is a conserved regulator of cell growth, proliferation, and survival. In cells, mTOR is the catalytic subunit of two complexes called mTORC1 and mTORC2, which have distinct upstream regulatory signals and downstream substrates. mTORC1 directly senses cellular nutrient availability while indirectly sensing circulating nutrients through growth factor signaling pathways. Cellular stresses that restrict growth also impinge on mTORC1 activity. mTORC2 is less well understood and appears only to sense growth factors. As an integrator of diverse growth regulatory signals, mTOR evolved to be a central signaling hub for controlling cellular metabolism and energy homoeostasis, and defects in mTOR signaling are important in the pathologies of cancer, diabetes, and aging. Here we discuss mechanisms by which each mTOR complex might regulate cell survival in response to metabolic and other stresses.

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The mechanistic target of rapamycin (mTOR) is a highly conserved serine/threonine protein kinase belonging to the phosphatidylinositol kinase-related kinase (PIKK) family and in mammalian cells is a central regulator of cell growth, proliferation, and survival (for review, see Sengupta et al. 2010; Zoncu et al. 2010). As its name implies, mTOR is the target of the naturally occurring compound rapamycin, which in association with the FK506-binding protein (FKBP12) is an allosteric inhibitor of mTOR. Although rapamycin is now known to only partially inhibit mTOR activity, derivatives of the drug have important clinical applications in oncology, in preventing restenosis after angioplasty, and as an immunosuppressant following organ transplants.

THE mTOR COMPLEXES AND THEIR REGULATION

In cells, mTOR exists in two distinct complexes called mTOR complex 1 (mTORC1) and mTORC2 that have both shared and unique subunits (Fig. 1). In addition to mTOR, both complexes contain mammalian Lethal with SEC13 protein 8 (mLST8 also known as GβL) and DEP domain-containing mTOR-interacting protein (DEPTOR). Regulatory-associated protein of mTOR (RAPTOR) and 40-kDa pro-rich Akt-substrate (PRAS40) are unique to mTORC1, whereas rapamycin-insensitive companion of mTOR (RICTOR), mammalian stressed-activated map-kinase interacting protein 1 (mSIN1), and protein observed with

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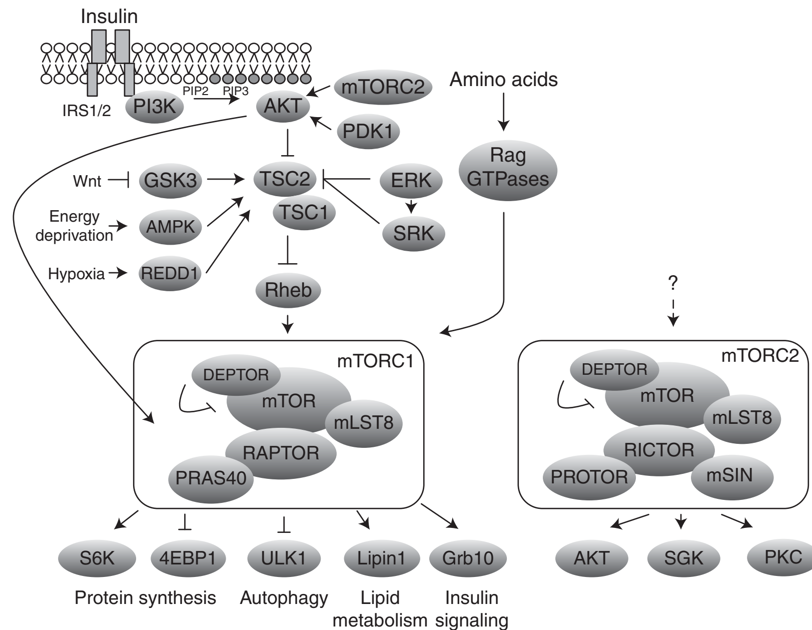

Figure 1. The mTOR pathway. The mTOR kinase exists in two distinct complexes called mTOR complex 1 (mTORC1) and mTORC2. In addition to mTOR, both complexes also contain mLST8 and DEPTOR. RAPTOR and PRAS40 are unique to mTORC1, whereas RICTOR, mSIN1, and PROTOR are specific to mTORC2. mTORC1 is well known to control cell growth. Although the downstream mechanisms by which mTORC1 controls growth are still being elucidated, several direct mTORC1 substrates have now been validated. For example, mTORC1 can regulate growth by directly phosphorylating S6K1 and 4EBP1, two regulators of protein translation; by regulating autophagy through the direct phosphorylation of ULK1; by regulating lipid metabolism at least in part by phosphorylating Lipin1; and by modulating insulin signaling through Grb10. In cells, mTORC1 activity is controlled by nutrient availability, particularly that of amino acids, through a novel pathway requiring the Rag GTPases. In the presence of amino acids, the Rags deliver mTORC1 to the lysosome, which is thought to be the signaling center for mTORC1-driven growth control (not shown but described in text). Growth factor signaling through PI3K–AKT, as well as numerous stresses, such as energy deprivation and hypoxia, can also impinge on mTORC1 activity through the TSC1–TSC2 complex. TSC2 negatively regulates a small GTPase called Rheb that directly activates mTORC1 by an unknown biochemical mechanism. AKT can also activate mTORC1 by directly phosphorylating PRAS40, which relieves an inhibitory function of this subunit. mTORC2 was discovered more recently, and although it is known to be activated in response to growth factors, the mechanisms are unknown. The best described substrates of mTORC2 are AKT and SGK, which require mTORC2-dependent phosphorylation for full biochemical activity. PKC is also regulated by mTORC2.

RICTOR (PROTOR) are specific to mTORC2. Recent work is beginning to reveal insight into the structure of mTORC1 (Yip et al. 2010). A cryo-EM structure of mTORC1 at 26 Å indicates that the complex is a dimer with interlocking mTOR–RAPTOR interactions and where PRAS40 acts as a competitive inhibitor for the binding of mTORC1 substrates to RAPTOR (Wang et al. 2008; Yip et al. 2010). mLST8 associates with the mTOR kinase domain, located in the carboxyl terminus (Kim et al. 2003). In the presence of rapamycin, the mTOR–mLST8 interaction is stable, whereas the drug weakens the mTOR–RAPTOR interaction (Kim and Sabatini 2004; Yip et al. 2010). The structure of mTORC2 remains a mystery.

mTORC1 is the best understood mTOR complex and is well known to control cell autonomous growth by integrating at least four growth regulatory inputs: nutrient availability, growth factor signaling, cellular energy status,

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and cellular stress levels (for review, see Sengupta et al. 2010; Zoncu et al. 2010). The best described mechanism by which mTORC1 controls growth is by directly phosphorylating two regulators of protein translation, p70-S6 kinase 1 (S6K1) and 4E binding protein 1 (4E-BP1). mTORC1-dependent phosphorylation of S6K at T389 activates its kinase activity toward several substrates involved in mRNA maturation and protein translation. In contrast to S6K1, 4E-BP1 represses translation, and its multisite phosphorylation by mTORC1 decreases its affinity to the translation initiation factor eIF4E, thus activating cap-dependent translation (for review, see Ma and Blenis 2009). Recently, several new substrates for mTORC1 have been identified and characterized. For example, mTORC1 also controls growth by negatively regulating autophagy through the direct phosphorylation of Unc-51-like kinase (ULK1; discussed below) (Chan 2009; Ganley et al. 2009; Hosokawa et al. 2009; Jung et al. 2009). In addition, mTORC1 directly phosphorylates the phosphatidate phosphatase Lipin1 (which regulates lipid metabolism through SREBP1) and the growth factor receptor-bound protein 10 (Grb10; part of a negative feedback loop targeting insulin receptor signaling) (Hsu et al. 2011; Peterson et al. 2011; Yu et al. 2011). In fact, two recent proteomic studies identifying Grb10 as an mTORC1 substrate further suggest that the insulin-stimulated phosphorylation cascade is largely mTOR-dependent (Hsu et al. 2011; Yu et al. 2011). A role for mTORC1 in regulating PGC1α activity and mitochondrial function has also been described (Cunningham et al. 2007; Ramanathan and Schreiber 2009).

How do upstream signals regulate mTORC1? In multicellular organisms, one mechanism by which growth factor signaling regulates mTORC1 is through the PI3K–AKT pathway (for review, see Manning and Cantley 2007). Following phosphoinositide 3-kinase (PI3K) activation, AKT (also known as PKB) is recruited to the membrane, which triggers its phosphorylation and activation. Among its many substrates, AKT directly phosphorylates and inactivates TSC2, which together in a complex with TSC1 negatively regulates mTORC1 activity (Inoki et al. 2002). The TSC1/TSC2 complex inhibits mTORC1 by suppressing the activity of its activator, a small GTPase called Ras homolog enriched in brain (Rheb) (for review, see Manning and Cantley 2003). AKT can also activate mTORC1 by directly phosphorylating PRAS40 and relieving its inhibitory effect on the complex (Sancak et al. 2007; Vander Haar et al. 2007). In addition to being inactivated by PI3K–AKT signaling, the TSC1/TSC2 complex can also be inhibited by other growth-promoting signals including mitogens or cytokines through the action of the mitogen-activated protein kinase (MAPK) ERK1/2 and the p90 ribosomal S6 kinase (RSK) (for review, see Huang and Manning 2008). In contrast, growth inhibitory signals such as energy deprivation and hypoxia can activate TSC1/TSC2 through the AMP-activated protein kinase (AMPK) and regulated in development and DNA damage responses 1 (REDD1), respectively, to suppress mTORC1 activity. In addition, Wnt-regulated glycogen synthase kinase 3β (GSK3β) coordinates with AMPK to inhibit mTOR signaling such that when Wnt signaling is active, GSK3β/AMPK-dependent activation of TSC2 is inhibited to allow mTORC1 activation (Inoki et al. 2006). Thus, the TSC1/TSC2 complex integrates numerous positive and negative growth signals and adjusts mTORC1 activity accordingly.

In contrast to growth factors, nutrient sensing by mTORC1—particularly of amino acids—is an ancient function of the complex conserved from yeast to humans. However, the mechanism by which amino acids regulate mTORC1 is just beginning to be elucidated. Amino acids (in particular, leucine) promote mTORC1 activity independently of TSC2 through a pathway that requires Rheb-dependent activation of the complex (Smith et al. 2005; Sancak and Sabatini 2009). mTORC1 activation by L-leucine is also dependent on glutamine transport into the cell, although glutamine alone has no effect on mTORC1 (DeBerardinis et al. 2007; Nicklin et al. 2009). Although the amino acid-sensing mechanism is just beginning to be revealed, it appears to require a second family of GTPases: the Rag proteins, which bind to mTORC1 and translocate it from an undefined location in the

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cytoplasm to the lysosome upon amino acid stimulation (Kim et al. 2008; Sancak et al. 2008, 2010). Mammals have four Rag proteins that form heterodimers: RagA or RagB (which are more closely related to each other) with RagC or RagD (which likewise are more closely related). When RagA/B is bound to GTP, RagC/D is bound to GDP and vice versa. In the presence of amino acids, for unknown reasons, RagA/B is GTP bound, and this somehow promotes the interaction and lysosomal recruitment of mTORC1. This interaction may be facilitated by the p62/sequestosome 1, which colocalizes with the Rags at the lysosome and is required for mTORC1 recruitment there (Duran et al. 2011). The Rags are tethered to the lysosome by a complex of proteins also essential for mTORC1 function called the Ragulator (formed by p18, p14, and MP1 proteins) (Sancak et al. 2010). The Ragulator forms the mTORC1 lysosomal docking site. Because a fraction of Rheb also resides at the lysosome, the current model is that the Rags/Ragulator, by an amino-acid-dependent mechanism, bring mTORC1 into close proximity to its activator Rheb-GTP at the lysosomal surface.

Why is the lysosome the site of mTORC1 activation in response to amino acid sufficiency and not the plasma membrane or some other cellular location? Although still a major question, the mystery is beginning to unravel. A complex of lysosomal proteins collectively called the v-ATPase is required for mTORC1 activity (Zoncu et al. 2011). Amino-acid-stimulated recruitment of mTORC1 to the lysosome and its ability to phosphorylate downstream substrates requires the v-ATPase, which interacts directly with the Ragulator. Interestingly, amino acids appear to accumulate inside the lysosomal lumen and signal through the v-ATPase to the Rags and Ragulator to activate mTORC1 by what has been dubbed an "inside-out" mechanism. Exactly how this happens is still under investigation but appears to require ATP hydrolysis and an associated rotation of the v-ATPase stalk. Interestingly, mTORC1 also negatively regulates lysosome biogenesis (Settembre et al. 2012), indicating that mTORC1 might also play an important role in controlling the number of lysosomes in the cell. Importantly, much of the understanding of the nutrient-sensing mechanism has been solved in vitro, and it will be important in future studies to determine the extent to which the amino acid-sensing pathway functions in various tissues and tumor cells.

Compared with mTORC1, mTORC2 was discovered more recently, and we know considerably less about its regulation and function. Growth factors activate mTORC2 at least in part through PI3K signaling, but the mechanism is unknown. Few substrates of mTORC2 have been described (Sparks and Guertin 2010). However, the discovery that mTORC2 directly phosphorylates AKT revealed a key role for mTORC2 in mediating downstream PI3K signaling in response to growth factor activation (Sarbassov et al. 2005). There are three mammalian AKT isoforms (AKT1, AKT2, and AKT3) that have both overlapping and distinct functions. In general, AKT1 is believed to have a more critical role in cell survival (Chen et al. 2001; Cho et al. 2001b), whereas AKT2 regulates glucose homeostasis (Cho et al. 2001a; Garofalo et al. 2003). AKT3 is implicated in brain development (Tschopp et al. 2005). As mentioned above, full AKT activation requires dual phosphorylation by both PDK1 (which phosphorylates AKT1 at T308 in the kinase motif) and by mTORC2 (which phosphorylates AKT1 at S473 in a carboxy-terminal hydrophobic motif). In addition to AKT, another AGC kinase family protein, Serum and glucocorticoid-induced kinase (SGK), is directly phosphorylated by mTORC2 (Garcia-Martinez and Alessi 2008).

Most signals upstream of mTOR appear to target either mTORC1 or mTORC2. However, a mechanism of regulation shared by both complexes may occur through DEPTOR, which is a common subunit of both mTOR complexes (for review, see Zoncu et al. 2010). Although the regulation of DEPTOR is complicated, it appears to be a natural inhibitor of both mTOR complexes because in its absence, S6K, AKT, and SGK activity increases (Peterson et al. 2009). DEPTOR levels are controlled though the ubiquitin-dependent degradation pathway by a mechanism requiring direct phosphorylation of DEPTOR by mTOR in an apparent positive feedback

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loop (Peterson et al. 2009; Duan et al. 2011; Gao et al. 2011; Zhao et al. 2011). Interestingly, DEPTOR levels are low in many cancers, which may promote mTOR-dependent cell growth, proliferation, and survival (Peterson et al. 2009). However, in a subset of multiple myelomas, DEPTOR is highly expressed. In these cells, DEPTOR overexpression inhibits mTORC1, but this relieves strong negative feedback loops to PI3K that may override its inhibitory effect on mTORC2 and consequently promote AKT-mediated cell survival (discussed below).

TSC2 PROTECTS AGAINST METABOLIC STRESS-INDUCED APOPTOSIS

As mentioned above, the TSC1/TSC2 complex integrates many growth regulatory signals to control mTORC1 activity. Many of these signals convey information regarding the metabolic state of the cell such that when nutrients are limiting, the cell will restrict mTORC1-dependent growth pathways. One of the best examples is that of AMPK-dependent phosphorylation and activation of TSC2, which is required for cell survival under glucose deprivation conditions (Inoki et al. 2003). AMPK is a major sensor of cellular energy status that is activated under conditions of metabolic stress (e.g., glucose deprivation) that decrease ATP production (for review, see Hardie 2007). AMPK is activated by increases in cellular AMP levels to promote catabolic pathways necessary to restore a critical level of ATP required for cell survival. It was initially found that AMPK activation inhibits S6K1 phosphorylation, suggesting a link between energy-sensing pathways and mTORC1 signaling (Kimura et al. 2003). It was subsequently shown that at least one mechanism by which AMPK inhibits mTORC1 is by directly phosphorylating and activating TSC2 (Inoki et al. 2003). Later work found that AMPK also directly phosphorylates RAPTOR, which can also suppress mTORC1 activity (Gwinn et al. 2008). By activating TSC2 and inhibiting mTORC1, AMPK shuts down mTORC1-dependent growth pathways from consuming cellular energy. In the absence of TSC2 function (e.g., in *TSC2*$^{-/-}$ cells), glucose deprivation results in cell death (Inoki et al. 2003). Consistent with death being driven by mTORC1 pathways, rapamycin treatment prevents the death of *TSC2*$^{-/-}$ cells when glucose is unavailable in the culture medium (Inoki et al. 2003; Choo et al. 2010).

A number of mechanisms might explain why *TSC2*-deficient cells are sensitive to apoptosis when deprived of glucose. One possibility is that uncontrolled mTORC1 activity in starving cells continues to drive protein translation through 4E-BP1 and S6K1, and because translation is a major consumer of cellular energy, this exhausts the ATP reserves. However, rapamycin is only a partial mTORC1 inhibitor and relatively inefficient at suppressing translation in mammalian cells, suggesting that other rapamycin-sensitive mTORC1 pathways might also be important (Feldman et al. 2009; Thoreen et al. 2009; Choo et al. 2010).

A second possibility is that mTORC1 activation during nutrient stress promotes p53 synthesis and accumulation (Lee et al. 2007). AMPK has also been shown to stabilize p53 by direct phosphorylation (Jones et al. 2005). Thus, glucose deprivation in normal cells causes AMPK to inhibit mTORC1 and stabilize p53, stalling cell growth and division; but when *TSC2*$^{-/-}$ cells are glucose deprived, AMPK stabilizes p53 whereas unrestricted mTORC1 signaling drives p53 synthesis. This synergistically results in greatly elevated levels of p53 and subsequently apoptosis. Notably, the connections between p53, mTORC1 activity, and survival are complex (Feng et al. 2005). For instance, p53 also induces the transcription of *PTEN*, *TSC2*, and *REDD1*, which negatively regulate mTORC1 (Stambolic et al. 2001; Ellisen et al. 2002; Feng et al. 2005). In addition, p53 also activates AMPK as well as Sestrin 1 and Sestrin 2, which are also negative regulators of mTORC1 signaling (Feng et al. 2007; Budanov and Karin 2008; Feng 2010).

A third possibility is that in the glucose-deprived state, losing TSC2 function promotes mTORC1-dependent negative feedback loops that suppress PI3K–AKT signaling, squelching critical survival signals. The best described mTORC1 negative feedback loops function

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through S6K1 and Grb10 (Zoncu et al. 2010; Hsu et al. 2011; Yu et al. 2011). Another mechanism of negative feedback occurs through the unfolded protein response (UPR) (Ozcan et al. 2008). The UPR senses unfolded proteins in the ER lumen and transmits that information to the cell nucleus, where it drives a transcriptional program to reestablish homeostasis (Kozutsumi et al. 1988). In this model, mTOR hyperactivation during glucose deprivation induces ER stress (presumably through increased client load) and therefore, the UPR. It was found that the UPR promotes feedback inhibition of PI3K–AKT signaling, possibly through the JNK kinase (Ozcan et al. 2008). The UPR might also directly activate apoptotic pathways in response to the overwhelming demand on the ER to faithfully regulate protein folding.

Another more recent report finds that the hypersensitivity of *TSC2*-deficient cells to glucose deprivation is not linked to blocking apoptosis, to p53 levels, or to activating autophagy, but rather to rapamycin's ability to decrease metabolic consumption, maintain ATP levels, and suppress AMPK, thus preventing energetic stress (Choo et al. 2010). These investigators also find that *TSC2*$^{-/-}$ cells deprived of glucose shift to using glutamine as a carbon source and that rapamycin fails to suppress cell death in the absence of glutamine. Therefore, in this model, rapamycin's protective effect is the result of decreasing the bioenergetic demand in order to balance cellular metabolism with the supply of nutrients and to support the shift to a glutamine-based metabolism. This response is at least partially dependent on S6K1, but not on eIF4E, consistent with rapamycin's relative ineffectiveness at blocking 4E-BP1 phosphorylation (Feldman et al. 2009; Thoreen et al. 2009; Choo et al. 2010).

mTORC1 DIRECTLY REGULATES AUTOPHAGY

Although the mTORC1 pathways responsible for triggering apoptosis in cultured *TSC2*$^{-/-}$ cells deprived of glucose are complex, one clear and conserved connection between mTORC1 and a pathway critical for cell survival upon nutrient deprivation is the discovery that mTORC1 directly regulates autophagy. Normally cells activate autophagy (or macroautophagy) in times of nutrient deprivation to salvage critical nutrients essential for cell survival. By mechanisms still being worked out, autophagy targets proteins and organelles (such as the mitochondria) to the autophagosome, which then delivers the cargo to the lysosome for degradation and recycling of macromolecules (for review, see Chen and Klionsky 2010; Yang and Klionsky 2010; Das et al. 2012). More than 30 different autophagy genes (ATGs) have been identified that regulate autophagy induction, cargo selection, vesicle formation, autophagosome fusion, cargo degradation, and release (for review, see He and Klionsky 2009). In cells, autophagy is a critical survival mechanism under nutrient deprivation conditions, and when inhibited either genetically or pharmacologically, nutrient deprivation can result in apoptosis (Boya et al. 2005). Autophagy is also essential for the survival of newborn mice, which require autophagy to mobilize nutrient stores during a brief starvation period immediately after birth (Kuma et al. 2004). Defective autophagy is also implicated in neuronal degeneration, cancer, and aging-associated pathologies (for review, see Yang and Klionsky 2009).

Although understanding mammalian autophagy regulation is an emerging and intense area of research, it is generally accepted that mTORC1 is a major negative regulator of autophagosome formation. Studies in yeast suggested early on that TORC1 inhibits autophagy. For example, rapamycin treatment activates autophagy in yeast even when they are growing in nutrient-rich conditions (Noda and Ohsumi 1998). Yeast TORC1 directly prevents assembly of an ATG1 kinase-containing complex required for autophagy induction (Kamada et al. 2000). Rapamycin inhibits TORC1's ability to disrupt ATG1 complex assembly, thus activating ATG1 kinase activity. It was thought that mTORC1 also controlled autophagy in mammalian cells, but until recently, the mechanism was vague. In general, rapamycin is less effective at activating autophagy in mammalian cells, although in some cases, rapamycin treatment causes accumulation of insoluble protein aggregates characteristic of

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those typically associated with failed autophagy (Spilman et al. 2010). In contrast to rapamycin, catalytic (ATP-competitive) inhibitors of mTOR are much more potent activators of autophagy in mammalian cells, indicating the role of mTOR in autophagy regulation is clearly conserved (Thoreen et al. 2009).

A series of reports suggests that the mechanism by which mTORC1 regulates autophagy is at least in part through direct phosphorylation of Unc-51-like kinase (ULK1), the homolog of yeast ATG1 (Chan 2009; Ganley et al. 2009; Hosokawa et al. 2009; Jung et al. 2009). In mammalian cells, ULK1 is the catalytic subunit of a complex containing mAtg13, Focal adhesion kinase-interacting protein of 200 kD (FIP200), and Atg101, all of which are essential for starvation-induced autophagy (Fig. 2). mAtg13 binds ULK1 and mediates the interaction between ULK1 and FIP200, but both FIP200 and mAtg13 appear to regulate ULK1 localization and stability. In nutrient-rich conditions, mTORC1 associates with the ULK1–mAtg13–FIP200 complex through a direct interaction between RAPTOR and ULK1, and this facilitates phosphorylation of both mAtg13 and ULK1 by mTORC1. The function of mTORC1-dependent ULK1 phosphorylation is not entirely clear, but it appears to diminish ULK1 kinase activity, thus reducing autophagic vesicle formation (Kim et al. 2011; Shang et al. 2011).

In the nutrient-deprived state, mTORC1 dissociates from the ULK1 complex, resulting in ULK1 dephosphorylation (Kim et al. 2011; Shang et al. 2011). However, this alone does not result in autophagy activation. For this to occur, ULK1 also requires direct activating phosphorylation by AMPK, emphasizing again the interplay between nutrient and energy-sensing pathways (Egan et al. 2011; Kim and Guan 2011; Kim et al. 2011; Zhao and Klionsky 2011). In starved cells, AMPK tightly binds ULK1, and this interaction is enhanced by rapamycin and disrupted by Rheb overexpression (Behrends et al. 2010; Kim and Guan 2011). In addition, AMPK can also inhibit mTORC1 by phosphorylating and activating TSC2, and directly by phosphorylating the RAPTOR subunit, which inhibits mTORC1 activity (Krause et al. 2002; Inoki et al. 2003; Gwinn et al. 2008). Interestingly, mTORC1 can be reactivated after prolonged starvation by the autolysosomal products generated by autophagy, indicating that a minimal level of mTORC1 activity is required for survival (Liang et al. 2007; Matsui et al. 2007; Herrero-Martin et al. 2009). This latter requirement for mTORC1 might be important for recycling lysosomes (Yu et al. 2010).

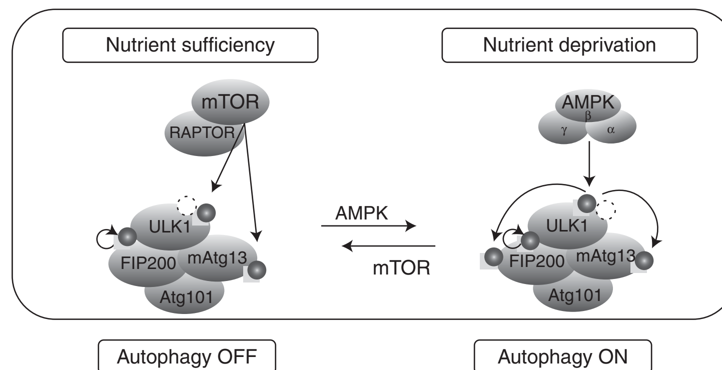

Figure 2. Regulation of the ULK complex by mTORC1 and AMPK. The ULK complex contains ULK1, mAtg13, FIP200, and Atg101. In nutrient-rich conditions, mTORC1 associates with the complex through a direct interaction between RAPTOR and ULK1 and phosphorylates both mAtg13 and ULK1. mTORC1-dependent ULK1 phosphorylation diminishes ULK1 kinase activity, preventing autophagy induction. In the nutrient-deprived state, mTORC1 dissociates from the ULK1 complex, resulting in ULK1 dephosphorylation at the mTORC1-dependent sites and phosphorylation at distinct sites by AMPK. Under these conditions, AMPK also tightly interacts with ULK1 to promote autophagy induction.

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The widely held view is that autophagy is downstream from mTORC1 such that when mTORC1 is "OFF" autophagy is "ON." However, the final destination of autophagic cargo including proteins/amino acids is the lysosome, and the amino acid signal that activates mTORC1 was recently shown to emanate from within the lysosome (discussed above). Thus, autophagy should activate mTORC1, which, in fact, has been observed (Liang et al. 2007; Matsui et al. 2007; Herrero-Martin et al. 2009). How can this be reconciled? An alternative view of the relationship between mTORC1 and autophagy might explain this. In this alternative view, autophagy is actually upstream of mTORC1, and AMPK is the main regulator of autophagy that directly activates ULK1 and suppresses mTORC1. For example, nutrient and energy deprivation reduces mTORC1 activity and activates AMPK, and active AMPK promotes autophagy as described above. But without mTORC1 activity, cells would die. Therefore, by delivering amino acids to the lysosome, autophagy actually maintains mTORC1 in a minimally active state. In turn, mTORC1 inhibits autophagy as part of a negative feedback loop to prevent cells from eating themselves to death. Thus, autophagy may actually regulate mTORC1.

How long can cells survive nutrient deprivation? It would seem that prolonged starvation would, in fact, lead cells to consume themselves to death or initiate apoptosis. In fact, under certain conditions, autophagy can kill cells through a process known as autophagic cell death or cell death type II (for review, see Eskelinen 2005). However, feedback mechanisms may exist to prevent cell death caused by prolonged activation of autophagy, and one appears to require mTORC1-dependent phosphorylation of DAP1 (death-associated protein 1) (Koren et al. 2010). Under amino acid starvation (i.e., mTORC1 inhibited), DAP1 is rapidly dephosphorylated, and by an unclear mechanism, this restricts excessive autophagy. Protein phosphatases likely also work together with mTORC1 and AMPK to control autophagy. In fact, mice lacking the protein phosphatase 1 (PP1) regulatory subunit Gadd34 (growth arrest and DNA damaged protein) cannot turn off autophagy because of sustained phosphorylation of the AMPK target site in TSC2 (Uddin et al. 2011). Thus, although the regulation of autophagy is complex, by linking autophagy induction to both mTORC1 inhibition and AMPK activation, cells can tightly regulate cellular energy homeostasis and survive under conditions of metabolic stress.

mTORC2-DEPENDENT CELL SURVIVAL PATHWAYS

In addition to its role in promoting cell growth through the TSC–mTORC1 pathway, AKT has long been thought to promote cell survival directly through several mechanisms including (1) directly phosphorylating and inhibiting pro-apoptotic proteins such as BAD; (2) directly phosphorylating and inhibiting the Forkhead box O (FoxO) transcription factors, which regulate pro-apoptotic genes such as BIM and Fas ligand (also known as CD95L); (3) promoting p53 degradation by activating the murine double minute 2 (MDM2); (4) blocking the glycogen synthase kinase 3 (GSK3)-mediated inhibitory signals to the pro-survival protein Mcl-1; and (5) activating the NF-κB survival pathway via phosphorylating IκB kinase α (IKKα) (for review, see Manning and Cantley 2007). The widespread role of AKT in regulating cell survival pathways predicts that mTORC2 might control some or all of these processes. Although this seems likely, the exact role of mTORC2-mediated AKT-S473 phosphorylation in regulating cell survival is still under investigation.

One unresolved issue is whether mTORC2-dependent AKT phosphorylation is required for all AKT functions, or for only a subset of its targets. Typically, many protein kinases of the protein kinase A/protein kinase G/protein kinase C (AGC) family, such as AKT, serum, and glucocorticoid-induced protein kinase (SGK), and S6K, require prior phosphorylation in their hydrophobic motif (HM), which creates a docking site for PDK1 (Biondi et al. 2002; Frodin et al. 2002). For example, mTORC1-mediated phosphorylation on the HM site of S6K enhances the affinity for PDK1 binding and promotes full S6K activation (for review, see Jacinto

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and Lorberg 2008). Accordingly, acute in vitro knockdown experiments suggest that mTORC2 is required for both AKT Thr308 and Ser473 phosphorylation (Sarbassov et al. 2005). However, genetic studies indicate that Thr308 is still phosphorylated even in the complete absence of mTORC2 activity (by deleting the *rictor*, *mlst8*, or *sin1* genes), arguing that in other contexts, these events might occur independently (Guertin et al. 2006; Jacinto et al. 2006; Shiota et al. 2006). The reason for this discrepancy remains unclear but may reflect a difference between acute knockdown and chronic knockout experiments, or the existence of a compensatory mechanism. Although phosphorylation at both T308 and S473 is required for maximal AKT activity in vitro, it appears that T308 phosphorylation alone empowers AKT with enough activity to phosphorylate many of its substrates in cultured cells or in tissues (Alessi et al. 1996; Guertin et al. 2006; Jacinto et al. 2006; Yang et al. 2006; Bentzinger et al. 2008; Kumar et al. 2008, 2010; Cybulski et al. 2009; Gu et al. 2011).

To date, only a select few of the predicted AKT substrates have been examined for signaling defects caused by loss of mTORC2 activity. One AKT substrate important in cell survival that appears to require mTORC2 activity is the FoxO1/3a transcription factors (Guertin et al. 2006, 2009; Jacinto et al. 2006; Yang et al. 2006). For example, FoxO1 (T24) and FoxO3a (T32) phosphorylation is decreased upon mTORC2 inactivation in both knockdown and genetic knockout studies. In the absence of AKT-mediated phosphorylation, FoxOs accumulate in the nucleus and activate metabolic and cell survival genes (Biggs et al. 1999; Nakae et al. 1999; Rena et al. 1999; Tang et al. 1999). Interestingly, phosphorylation of other AKT targets such as BAD, TSC2, and GSK3β show little to no effect upon genetic ablation of mTORC2 (Guertin et al. 2006, 2009; Shiota et al. 2006; Yang et al. 2006). Other key survival proteins downstream from AKT such as MDM2, Caspase-9 and IKKα have not yet been investigated. Because FoxOs have critical roles in cell survival, mTORC2 may regulate cell viability through Akt–FoxO pathways.

Because AKT is predicted to activate mTORC1, it is not unreasonable to predict that losing mTORC2-dependent AKT phosphorylation might also decrease mTORC1 activity and induce autophagy. However, despite most models placing mTORC2 upstream of the AKT–TSC2–mTORC1 signaling axis, evidence that this connection is important in vivo is lacking. In fact, it appears that losing mTORC2 activity has minimal effects on mTORC1 signaling in many cell types (Guertin et al. 2006; Jacinto et al. 2006; Shiota et al. 2006; Bentzinger et al. 2008; Kumar et al. 2008, 2010; Cybulski et al. 2009; Gu et al. 2011). Interestingly, mTORC2 may regulate autophagy independently of mT ORC1 via the AKT–FoxO3a axis (Mammucari et al. 2007). In fasting skeletal muscle, FoxO3a positively controls transcription of several autophagy-related genes, including LC3 and Bnip3. Tamoxifen-induced activation of recombinant AKT (AKT fused with estrogen receptor) blocks FoxO3a activation and autophagy induction. Conversely, knockdown of RICTOR promotes FoxO3 nuclear retention and autophagosome formation. Another report indicates that insulin signaling also inhibits autophagy in the liver through a FoxO1-mediated mechanism (Liu et al. 2009). Thus, mTORC2 may regulate autophagic survival through AKT-dependent, mTORC1-independent mechanisms.

In vivo studies of conventional *rictor*, *sin1*, and *mlst8* knockout embryos (all of which result in selective mTORC2 ablation) indicate that mTORC2 is essential for progression through mid-embryonic development (Guertin et al. 2006). Although the exact cause of lethality is unknown, increased cell death was not readily apparent in the knockout embryos. To gain further insight into the tissue-specific functions of mTORC2, conditional knockout models of *rictor* have been developed, including skeletal muscle, white adipose tissue, and pancreatic β cells (Bentzinger et al. 2008; Kumar et al. 2008, 2010; Cybulski et al. 2009; Gu et al. 2011). Although some metabolic defects are reported, there is no indication from these studies that mTORC2 loss—at least under otherwise normal physiological conditions—results in increased apoptosis. This may reflect the fact that in all three of these tissues, AKT Thr308 phosphorylation is largely preserved despite

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decreased mTORC2-dependent Ser473 phosphorylation. Thus, more studies are needed to determine exactly if and how mTORC2 might regulate cell survival.

DOES mTORC2 REGULATE CANCER CELL SURVIVAL?

Although it is not clear exactly which AKT pathways require mTORC2 under normal conditions, several lines of evidence suggest that mTORC2 may be more essential for AKT signaling in cells with oncogenic activation of PI3K activity. For example, in a *PTEN*-deletion-driven mouse model of prostate cancer deleting *RICTOR* blocks tumor formation but has no effect on normal prostate growth or function (Guertin et al. 2009). Interestingly, ablating mTORC2 activity in the *PTEN*-null tumor cells reduces both AKT Ser473 and Thr308 phosphorylation, perhaps suggesting a differential requirement for mTORC2 in prostate cancer cells compared with MEFs. The experiments in the prostate cancer model are reminiscent of genetic studies in *Drosophila*, in which *dRICTOR* is less essential for fly development but is required for phenotypes induced by *PTEN* deletion or PI3K activation (Hietakangas and Cohen 2007). Importantly, it is unclear in these models whether the *rictor*/mTORC2-deficient cells have survival defects.

In vitro studies using several human cancer cell lines further indicate that knocking down RICTOR is toxic to transformed cells with elevated AKT activity. For example, mTORC2 activity is elevated in gliomas and is required for anchorage-independent growth and proliferation in vitro and for tumor growth in a xenograft model (Masri et al. 2007). Moreover, mTORC2 promotes cell cycle progression and anchorage-independent growth of breast (MCF7) and prostate (PC3) cancer cell lines (Hietakangas and Cohen 2008; Guertin et al. 2009). However, regarding a specific role for mTORC2 in cell survival, only one report shows that stable knockdown of RICTOR specifically impairs survival of a cancer cell line in vitro (in this case, colorectal cancer cells) (Gulhati et al. 2009). Cell proliferation is also inhibited in these cells.

One possible explanation for the differential requirement for mTORC2 activity in normal cells versus cancer cells is that under normal conditions, basal AKT activity (Thr308 phosphorylation only) is sufficient for maintaining AKT's essential functions, whereas in *PTEN*-deficient or *PI3KCA* (phosphatidyl inositol 3-kinase catalytic subunit) mutant transformed cells, the demand for AKT signaling is maximal, requiring both T308 and S473 phosphorylation to achieve its full activation potential. Alternatively, the difference may reflect a compensatory signal that reactivates AKT by up-regulated T308 phosphorylation upon prolonged loss of mTORC2 activity, and this pathway might not yet be functional following acute loss of mTORC2 in transient knockdown experiments. These compensatory mechanisms may exist specifically to avoid cell death. Such protective circuits may be cell type specific or only active under specific conditions, and clearly this needs further examination. Nevertheless, studies to date suggest that cancer cells with an abnormally high level of PI3K–AKT activity may have a greater requirement for mTORC2 than otherwise normal cells, and this provides rationale for developing a therapeutic strategy that selectively targets mTORC2. Importantly, however, it remains unclear to what extent mTORC2 activity is required for cancer cell survival in more advanced, therapeutically relevant stages of cancer, and whether a selective mTORC2 inhibitor (if it existed) would have an acceptable therapeutic window.

In addition to AKT, another AGC kinase family protein, SGK, is also directly phosphorylated by mTORC2 (Garcia-Martinez and Alessi 2008). It is reported that SGK can regulate cell viability through activation of MDM2-dependent p53 degradation (Amato et al. 2009). Moreover, like AKT, SGK also exists in three isoforms (SGK1, SGK2, and SGK3) in human and mouse (Brunet et al. 2001). However, in contrast to AKT, even basal SGK activity is controlled by mTORC2 because phosphorylation in its hydrophobic motif by mTORC2 is required for phosphorylation in the kinase domain by PDK1 (Garcia-Martinez and Alessi 2008). mTORC2 regulation of SGK could also have relevance in cancer cell survival as suggested by a recent

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study that finds SGK3 signaling (but not AKT signaling) downstream from PIK3CA mutations is essential for the survival of certain cancer cells (Vasudevan et al. 2009). Thus, mTORC2 may regulate cell survival through both AKT-dependent and AKT-independent pathways, although definitive mechanisms require further investigation.

DO mTOR INHIBITORS AFFECT CANCER CELL SURVIVAL?

Aberrant PI3K–AKT–mTOR signaling is a common feature of most cancers (Shaw and Cantley 2006; Zoncu et al. 2010; Hanahan and Weinberg 2011). Consequently, there is intense interest in developing mTOR inhibitors as cancer therapeutics. First-generation mTOR inhibitors are based on the chemical structure of rapamycin, but despite the strong rationale for using rapamycin in oncology, this class of drugs has unfortunately had limited success (O'Reilly et al. 2006; Sudarsanam and Johnson 2010; for review, see Guertin and Sabatini 2009; Benjamin et al. 2011). The best albeit modest responses to rapamycin as a therapy have been reported in renal cell carcinoma, mantle cell lymphoma, neuroendocrine tumors of the pancreas, and in treating tuberous sclerosis (caused by mutations in *TSC1* or *TSC2*) (Benjamin et al. 2011). There are several reasons that could explain this including the fact that rapamycin is an allosteric inhibitor of mTOR that binds outside the kinase domain and only partially inhibits mTORC1 activity. For example, rapamycin universally inhibits mTORC1-dependent S6K1 phosphorylation but has only minor and acute inhibitory effects on other mTORC1 substrates including 4E-BP1 (Choo et al. 2008; Feldman et al. 2009; Thoreen et al. 2009). In addition,

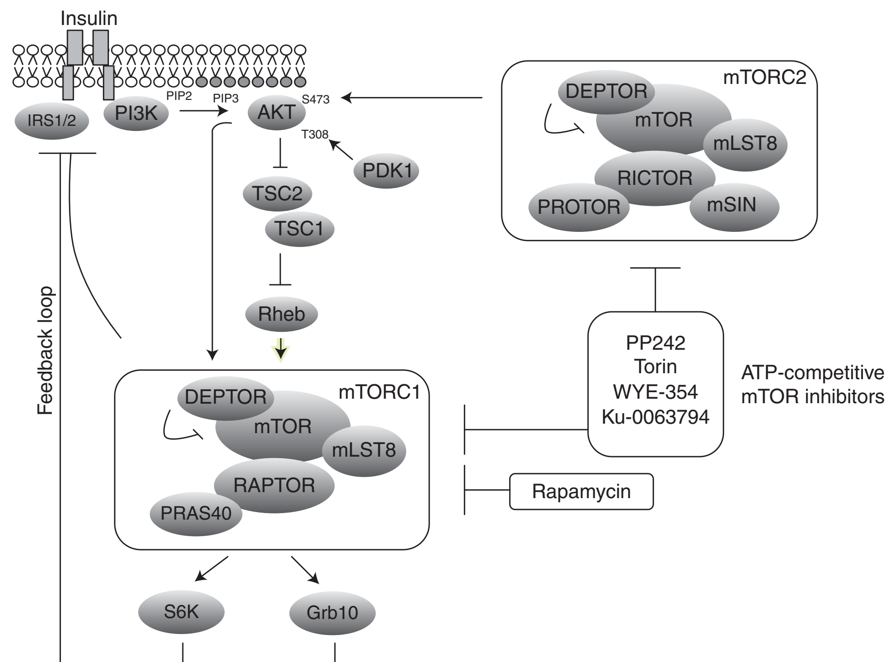

Figure 3. Feedback inhibition of insulin signaling. Several mechanisms of negative feedback inhibition of insulin signaling exist downstream from mTORC1. The best described are mediated by S6K1, which can phosphorylate and inhibit IRS1; and by Grb10, which is stabilized by mTORC1-dependent phosphorylation and suppresses signaling from the insulin and insulin-like growth factor receptors. mTOR inhibitors such as rapamycin and the new generation of ATP-competitive inhibitors can relive these negative feedback loops, resulting in PI3K–AKT activation.

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rapamycin relieves strong negative feedback loops to PI3K that exist downstream from mTORC1 (Fig. 3) (Choo and Blenis 2009). As mentioned above, these feedback loops can function through mTORC1 substrates including S6K1 and Grb10. S6K1 directly phosphorylates insulin receptor substrate 1 (IRS-1), mislocalizing it and targeting it for degradation. The recently discovered mTORC1 substrate Grb10 directly binds to and negatively regulates the insulin and insulin-like growth factor receptors (Hsu et al. 2011; Yu et al. 2011). mTORC1 phosphorylation of Grb10 promotes its stability. Interestingly, Grb10 levels are often decreased in cancer, suggesting that it could have tumor-suppressor functions. In both cases, rapamycin relieves feedback inhibition and promotes PI3K–AKT survival signaling. The clinical relevance of losing feedback inhibition is emphasized in human trials that find rapamycin increases AKT activation in many malignancies (O'Reilly et al. 2006; Tabernero et al. 2008; Sudarsanam and Johnson 2010). Rapamycin can also activate the MAPK pathway, providing another potential avenue to resistance (Carracedo et al. 2008). Thus, as a single agent, rapamycin may actually promote cancer cell survival; however, rapamycin may ultimately prove to be useful in combination with agents such as PI3K, AKT, or MAPK inhibitors.

The discovery of mTORC2, which is generally rapamycin insensitive, and the widespread ineffectiveness of rapamycin as a monotherapy led to development of inhibitors that directly target the mTOR catalytic site (for review, see Guertin and Sabatini 2009). The first to be reported include Torin1, PP242, Ku-0063794, and WYE-354 (Feldman et al. 2009; Garcia-Martinez et al. 2009; Thoreen et al. 2009; Yu et al. 2009). The ATP-competitive inhibitor class more completely inhibits mTORC1 and additionally inhibits mTORC2. Of note, prolonged exposure to rapamycin can inhibit mTORC2 in a subset of cell types, and this might explain some of the clinical successes with the drug (Sarbassov et al. 2006; Gulhati et al. 2009). The mechanism is not entirely understood but may result from rapamycin blocking the assembly of new mTORC2 complexes. The mTOR ATP-competitive inhibitors are just beginning to be tested for clinical efficacy, and it is hoped that they will outperform rapamycin in the clinic. In a few preclinical studies, the mTOR catalytic inhibitors were shown to induce cell death in combination with other inhibitors (Janes et al. 2010; Sini et al. 2010).

Although the preclinical studies with mTOR catalytic site inhibitors are exciting, several questions regarding their efficacy remain. For example, will feedback activation of PI3K–AKT signaling still promote survival even though mTORC2 is also inhibited? Evidence that this could be problematic comes from studies of the natural mTOR inhibitor DEPTOR (discussed above), which emphasize the fact that losing feedback inhibition by inhibiting mTORC1 can override mTORC2 inhibition with respect to AKT activation (Peterson et al. 2009). Another potential concern is whether mTOR catalytic inhibitors will be well tolerated, although preclinical tests in rodent models are promising (for review, see Benjamin et al. 2011). The mTOR catalytic inhibitors are also more formidable activators of autophagy compared with rapamycin (Chresta et al. 2009; Thoreen et al. 2009). Because autophagy can promote cell survival in nutrient-limiting conditions, increasing autophagic activity could also promote cancer cell survival in the nutrient-deprived tumor microenvironment. In fact, in melanoma cells, inhibiting autophagy in combination with nutrient deprivation induces apoptosis, suggesting that autophagy can protect cancer cells from nutrient-limiting conditions (Sheen et al. 2011).

CONCLUSION

In this article, we review mechanisms by which cells respond to nutrient deprivation that impinge on mTORC1 signaling. We also discuss possible mechanisms by which the less-well-understood mTORC2 might regulate cell survival. Although starvation is clearly detrimental to a cell's ability to maintain long-term homeostasis, nutrient overload also stresses cells by forcing them to elevate their metabolism, increasing damaging reactive oxygen species (ROS) and oxidative stress (for review, see Wellen and

Thompson 2010). This is the case in cancer, in which oncogenic pathways drive aberrant nutrient uptake and metabolism; and diabetes, where nutrient overload promotes obesity and insulin resistance. mTOR, by functioning as a point of convergence between a nutrient-sensing pathway and PI3K–AKT signaling (i.e., as part of mTORC1) and as a regulator of AKT itself (i.e., as part of mTORC2), is central to understanding how both normal and cancer cells survive nutrient excess and is a growing area of research. In sum, mTOR integrates growth signals from diverse mechanisms that sense nutrient availability and as part of the response regulates cell survival. Pathways deregulated in many human diseases clearly impinge on mTOR signaling; thus, defining the cell and tissue-specific mechanisms through which mTOR regulates cell survival will be critical to developing therapies to treat cancer and metabolic diseases.

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