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Where is mTOR and what is it doing there?

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Target of rapamycin (TOR) forms two conserved, structurally distinct kinase complexes termed TOR complex 1 (TORC1) and TORC2. Each complex phosphorylates a different set of substrates to regulate cell growth. In mammals, mTOR is stimulated by nutrients and growth factors and inhibited by stress to ensure that cells grow only during favorable conditions. Studies in different organisms have reported localization of TOR to several distinct subcellular compartments. Notably, the finding that mTORC1 is localized to the lysosome has significantly enhanced our understanding of mTORC1 regulation. Subcellular localization may be a general principle used by TOR to enact precise spatial and temporal control of cell growth.

Introduction

Rapamycin, an antifungal, anticancer, and immunosuppressive compound produced by a soil bacterium from Rapa Nui (better known as Easter Island), was discovered in 1975 (Vézina et al., 1975; Benjamin et al., 2011). The isolation of yeast mutants resistant to the growth-inhibitory properties of rapamycin led to the discovery of TOR (target of rapamycin; Heitman et al., 1991; Kunz et al., 1993). It was subsequently found that TOR is a highly conserved controller of cell growth and that mammalian TOR (mTOR) is implicated in human disease (Menon and Manning, 2008; Dazert and Hall, 2011; Laplante and Sabatini, 2012).

The protein kinase TOR functions in two structurally and functionally distinct multiprotein complexes termed TOR complex 1 (TORC1 in yeast, mTORC1 in mammals) and TOR complex 2 (TORC2 in yeast, mTORC2 in mammals; Wullschleger et al., 2006; Loewith and Hall, 2011; Laplante and Sabatini, 2012). mTORC1 is composed of mTOR, raptor, and mLST8 (*Saccharomyces cerevisiae* orthologues are TOR1, Kog1, and LST8, respectively). mTORC1 regulates cell growth (accumulation of cell mass) through coordination of protein anabolism (Averous and Proud, 2006; Ma and Blenis, 2009), nucleotide biosynthesis (Ben-Sahra et al., 2013; Robitaille et al., 2013), lipogenesis, glycolysis (Laplante and Sabatini, 2009; Peterson et al., 2011), and autophagy (Ganley et al., 2009; Hosokawa et al., 2009). mTORC2 is composed of mTOR, rictor, SIN1, and mLST8 (*S. cerevisiae* orthologues are TOR2, Avo3, Avo1, and LST8, respectively). mTORC2 controls growth by regulating lipogenesis, glucose metabolism (García-Martínez and Alessi, 2008; Hagiwara et al., 2012; Yuan et al., 2012), the actin cytoskeleton (Cybulski and Hall, 2009; Oh and Jacinto, 2011), and apoptosis (Datta et al., 1997).

TOR has been found at several cellular locations (Tables 1 and 2; Malik et al., 2013), which has brought cell biology to the forefront of the TOR signaling field. In this review, we discuss the subcellular localization of the TOR complexes vis-à-vis their function and regulation. However, before starting our discussion, it is important to note several caveats in determining the subcellular location of a protein or complex. Antibodies can be nonspecific, overexpressed or tagged proteins can exhibit aberrant localization, different fixation or lysis methods can influence localization, and isolated organelles can be contaminated with other organelles. Additionally, detection of one component of a TOR complex does not necessarily reflect localization of an entire complex. Furthermore, especially when dealing with highly regulated pathways, it is essential to take nutrient, stress, and cell cycle status into account and to consider that commonly used cell lines present mutations that might affect subcellular localization. Thus, when evaluating localization of TOR, or any other protein, it is advisable to consider several complementary approaches because no single technique is without weakness.

Localization of mTORC1

mTORC1 at the lysosome. mTORC1 is activated directly by GTP-bound Rheb on the surface of the lysosome (Fig. 1, Table 1). Two conditions need to be fulfilled for mTORC1 to be activated. One is that mTORC1 needs to translocate to the lysosome, a process stimulated by nutrients and the Rags (see next paragraph), where it encounters Rheb. The other is that Rheb needs to be activated, i.e., converted from a GDP- to a GTP-bound form, in response to growth factors. Rheb is a farnesylated GTPase that is anchored to the surface of the lysosome (Saito et al., 2005; Sancak et al., 2008). Rheb is inhibited by its GAP, a heterotrimer of TSC1 (tuberous sclerosis complex 1), TSC2, and TBC1D7 that is also on the lysosome. Growth factor–stimulated Akt phosphorylates and inhibits the TSC complex to activate Rheb, but it is not

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Abbreviations used in this paper: MAM, mitochondria-associated ER membrane; mTOR, mammalian TOR; TOR, target of rapamycin; TORC, TOR complex; TSC, tuberous sclerosis complex.

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Table 1. **Lysosome localization of TORC1**

Pathway	Mechanism of localization	Cell type	Technique	Reference
mTORC1	Rheb localizes to the late endosome/lysosome	MDCK; HeLa; 1321N1	Confocal live-cell imaging; expression of GFP-Rheb	Saito et al., 2005
mTORC1	The TSC complex is at the lysosome	HeLa	Wide-field imaging; PFA fixation; endogenous TSC2	Dibble et al., 2012
mTORC1	Amino acids stimulate mTOR translocation from the cytoplasm to the lysosome in a Rag- and Ragulator-dependent manner; Rheb, Ragulator, and Rags are at the lysosome independently of amino acids	HEK293T	Confocal imaging; PFA fixation; endogenous mTOR, raptor, RagA/B, RagC; validation of antibody expression of GFP-Rheb, GFP-RagB/C/D, GFP-LAMTOR1, HA-raptor, myc-mTOR, FLAG-raptor, FLAG-LAMTOR4, FLAG-LAMTOR5	Sancak et al., 2008, 2010; Bar-Peled et al., 2012
mTORC1	Glutaminolysis stimulates mTOR translocation from the cytoplasm to the lysosome	U2OS	Wide-field imaging; PFA fixation; endogenous mTOR	Durán et al., 2012b
Yeast TORC1	Tco89 (component of TORC1) is at the plasma membrane and the vacuole/lysosome	*S. cerevisiae*	Immuno-EM; PFA fixation; genomically tagged Tco89-Myc	Reinke et al., 2004
Yeast TORC1	TORC1 (Tco89; Kog1; Sch9; TOR1) is mainly at the vacuole/lysosome	*S. cerevisiae*	Live-cell, wide-field, and confocal imaging; genomically tagged Tco89-GFP, Kog1-GFP, Sch9-GFP, TOR1-GFP	Huh et al., 2003; Urban et al., 2007; Sturgill et al., 2008
Yeast TORC1	TORC1 (Tco89; Ego1; Sch9; TOR1) is mainly at the vacuole/lysosome, and this localization is independent of amino acid (Leu) availability	*S. cerevisiae*	Live-cell imaging; genomically tagged Tco89-GFP, Ego1-GFP, Sch9-GFP, TOR1-GFP	Binda et al., 2009

This table groups the most important reports that link TORC1 and mTORC1 to the lysosome. By using a range of different techniques, distinct components of TORC1 have consistently been identified at the lysosome in different cell types. PFA, paraformaldehyde; TSC, tuberous sclerosis complex.

known where Akt meets the TSC complex. Akt is generally assumed to be activated at the plasma membrane by PDK1 after growth factor stimulation (Hemmings and Restuccia, 2012).

mTORC1 translocation to the lysosome is controlled by the Rag GTPase and the so-called Ragulator, in response to amino acids (Kim et al., 2008; Sancak et al., 2008, 2010; Jewell et al., 2013). Rag is a heterodimeric GTPase consisting of RagA or B in complex with RagC or D. Ragulator, the GEF and lysosomal anchor for Rag, is a pentameric complex consisting of p14, p18, MP1, HBXIP, and C7orf59 (also known as LAMTOR1–5; Bar-Peled et al., 2012). Amino acids stimulate guanine nucleotide exchange in Rag. How does Rag sense amino acids to activate mTORC1? There may be more than one mechanism. Zoncu et al. (2011) have proposed that amino acids are sensed in the lumen of the lysosome via the v-ATPase, which then activates Rag via Ragulator and possibly the recently described Rag GAPs (Bar-Peled et al., 2013; Panchaud et al., 2013; Petit et al., 2013; Tsun et al., 2013). Activation of Rag requires ATP hydrolysis by the v-ATPase, but the function of the v-ATPase in establishing a lysosomal proton gradient appears to be dispensable. Ögmundsdóttir et al. (2012) have reported that the lysosomal proton-assisted amino acid transporter PAT1, which pumps amino acids out of the lysosomal lumen, interacts directly with Rag to activate mTORC1. However, as part of their argument that amino acids are sensed in the lysosomal lumen, Zoncu et al. (2011) reported that PAT1 overexpression inactivates mTORC1. The mechanism by which the v-ATPase actually senses amino acids to activate Ragulator–Rag remains to be determined. Durán et al. (2012b) have proposed that leucine (and glutamine) is sensed via glutaminolysis. Leucine is a particularly effective activator of Rag. Leucine is also an allosteric activator of glutamate dehydrogenase (GDH) that deaminates glutamate to produce α-ketoglutarate. α-Ketoglutarate is a cofactor for prolyl hydroxylases which may in turn, by an unknown mechanism, activate Rag (Durán et al., 2012a). Interestingly, under prolonged amino acid starvation, mTORC1 is reactivated by amino acids derived from autophagosomal lysis, thus preventing further autophagy (Yu et al., 2010).

Active Rag recruits mTORC1 to Rheb on the surface of the lysosome. Rag was originally suggested to deliver mTORC1 from the cytoplasm to the lysosome, with the implication that Rag itself shuttles on and off the lysosome (Sancak et al., 2008; Zinzalla and Hall, 2008). However, a more recent study that directly examined the cellular location of Rag showed that it appears to be fixed to the lysosome, from where it recruits mTORC1 to the lysosomal surface (Sancak et al., 2010). Most recently, it has again been suggested that Rag captures mTORC1 in the cytoplasm and then shuttles it back to the lysosomal surface (Bar-Peled et al., 2012). Furthermore, the E3 ubiquitin ligase TRAF6 and the signaling adaptor p62 appear to be involved in the recruitment of mTORC1 to the lysosome in response to amino acids (Linares et al., 2013). Thus, the mechanism by which Rag mediates lysosomal localization of mTORC1 remains to be determined. Once on the lysosome, mTORC1 forms a four-component super complex with v-ATPase, Ragulator, and Rag, as suggested by coimmunoprecipitation of mTORC1 with at least Ragulator and Rag (Sancak et al., 2010).

mTORC1 signaling in cells lacking TSC is resistant to growth factor withdrawal but still responsive to amino acids (Smith et al., 2005; Roccio et al., 2006). This indicates that to

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Table 2. **Localization of TORC1 to other organelles**

Pathway	Mechanism of localization	Cell type	Technique	Reference
mTORC1	Rheb is at the ER and the Golgi	HEK293	Confocal imaging; expression of EGFP-Rheb	Buerger et al., 2006
mTORC1	Rheb and the TSC complex are at the peroxisome	FAO; HepG2; MEF; HeLa	Confocal imaging and subcellular fractionation; quantification of colocalization; endogenous TSC1, TSC2, and Rheb	Zhang et al., 2013
mTORC1	The TSC complex is mainly cytoplasmic	COS; HeLa	Wide-field imaging and subcellular fractionation; PFA fixation; expression of TSC1 and TSC2	van Slegtenhorst et al., 1998; Nellist et al., 1999
mTORC1	The TSC complex is cytoplasmic and nuclear; Akt stimulates TSC translocation from the cytoplasm to the nucleus	NIH3T3; HeLa; HEK293; Rat-1	Subcellular fractionation	Rosner and Hengstschläger, 2007; Rosner et al., 2007,
mTORC1	mTOR is at ER, Golgi, and in the nucleus; Rheb is cytoplasmic and nuclear; mTORC1 (mTOR, raptor) is in punctate structures upon amino acid starvation	HEK293; CHO; HeLa	Single photon FRET-FLIM on live cells; expression of mTOR-EGFP, Ds-Red-raptor, and Rheb-EGFP	Yadav et al., 2013
mTORC1	mTOR and raptor are highly abundant in the nucleus but mTORC1 integrity is higher in the cytoplasm	HEK293; NIH3T3; IMR-90; MRC-5; WI-38	Subcellular fractionation	Rosner and Hengstschläger, 2008
mTORC1	mTORC1 (mTOR, raptor) is in the nucleus and the cytoplasm; mTORC1 phosphorylates NFACTc4 in the nucleus; mTOR binds to rDNA, rDNA, and tRNA genes in a rapamycin-sensitive manner; serum stimulates mTOR binding to hnRNPs	COS; HEK293; HeLa	Subcellular fractionation; ChIP	Yang et al., 2008; Goh et al., 2010; Kantidakis et al., 2010; Shor et al., 2010; Tsang et al., 2010
mTORC1	During hypoxia, PML inhibits mTORC1 (mTOR) by sequestering it in the nucleus away from cytoplasmic Rheb	MEF; HEK293	Subcellular fractionation; confocal imaging; PFA fixation	Bernardi et al., 2006
mTORC1/2	mTOR shuttles from the cytoplasm to the nucleus	HEK293; CV-1	Wide-field imaging and subcellular fractionation; PFA fixation; expression of mTOR-FLAG	Kim and Chen, 2000
mTORC1/2	mTOR is predominantly nuclear except in HEK293, where it is excluded from the nucleus	HEK293; Rh30; Rh41; IMR90; HCT8; HCT29; HCT116	Confocal imaging and subcellular fractionation; PFA fixation; endogenous mTOR	Zhang et al., 2002
mTORC1	mTOR and raptor are associated with mitochondria; mTOR is associated with the mitochondrial channel VDAC	Jurkat; HEK293	Subcellular fractionation	Schieke et al., 2006; Ramanathan and Schreiber, 2009
Yeast TORC1	TOR1 is mainly nuclear, and nutrient starvation or rapamycin treatment induce translocation to the cytoplasm	*S. cerevisiae*	Wide-field imaging and subcellular fractionation; endogenous TOR1	Li et al., 2006

This table groups the most important reports that link TORC1 and mTORC1 to sites apart from the lysosome. By using comparable techniques to those that revealed lysosomal localization of mTORC1 (Table 1), distinct components of TORC1 have also been identified at other sites including the nucleus and mitochondria. ChIP, chromatin immunoprecipitation; PFA, paraformaldehyde.

activate mTORC1, Rheb needs amino acid–induced targeting of mTORC1 to the lysosome. On the other hand, forced localization of mTORC1 to the lysosome renders cells insensitive to amino acids and independent of Rag, but still dependent on Rheb and growth factors (Sancak et al., 2010). Overexpression of Rheb can activate mTORC1 even under amino acid starvation, possibly by enabling interaction between mislocalized Rheb and mTORC1 at a site(s) away from the lysosome (Inoki et al., 2003; Smith et al., 2005). Interestingly, amino acid starvation changes the location of the entire lysosome within the cell (Korolchuk et al., 2011), but the effect of this altered lysosomal localization on mTORC1 activity is unknown. Inhibition of mTORC1 activity with rapamycin does not affect mTORC1 localization to the lysosome (Sancak et al., 2008), and kinase-dead mTORC1 still localizes to lysosomes (Tabatabaian et al., 2010). Finally, energy stress prevents assembly and lysosomal localization of mTORC1, through the Tel2-Tti1-Tti2 (TTT)–RUVBL1/2 complex (Kim et al., 2013). Tel2 and Tti1 are critical factors for the assembly and stability of mTORC1/2 and other PIKK family members (Takai et al., 2007; Kaizuka et al., 2010).

What does mTORC1 do once it is activated on the surface of the lysosome? Does it remain on the lysosome to phosphorylate

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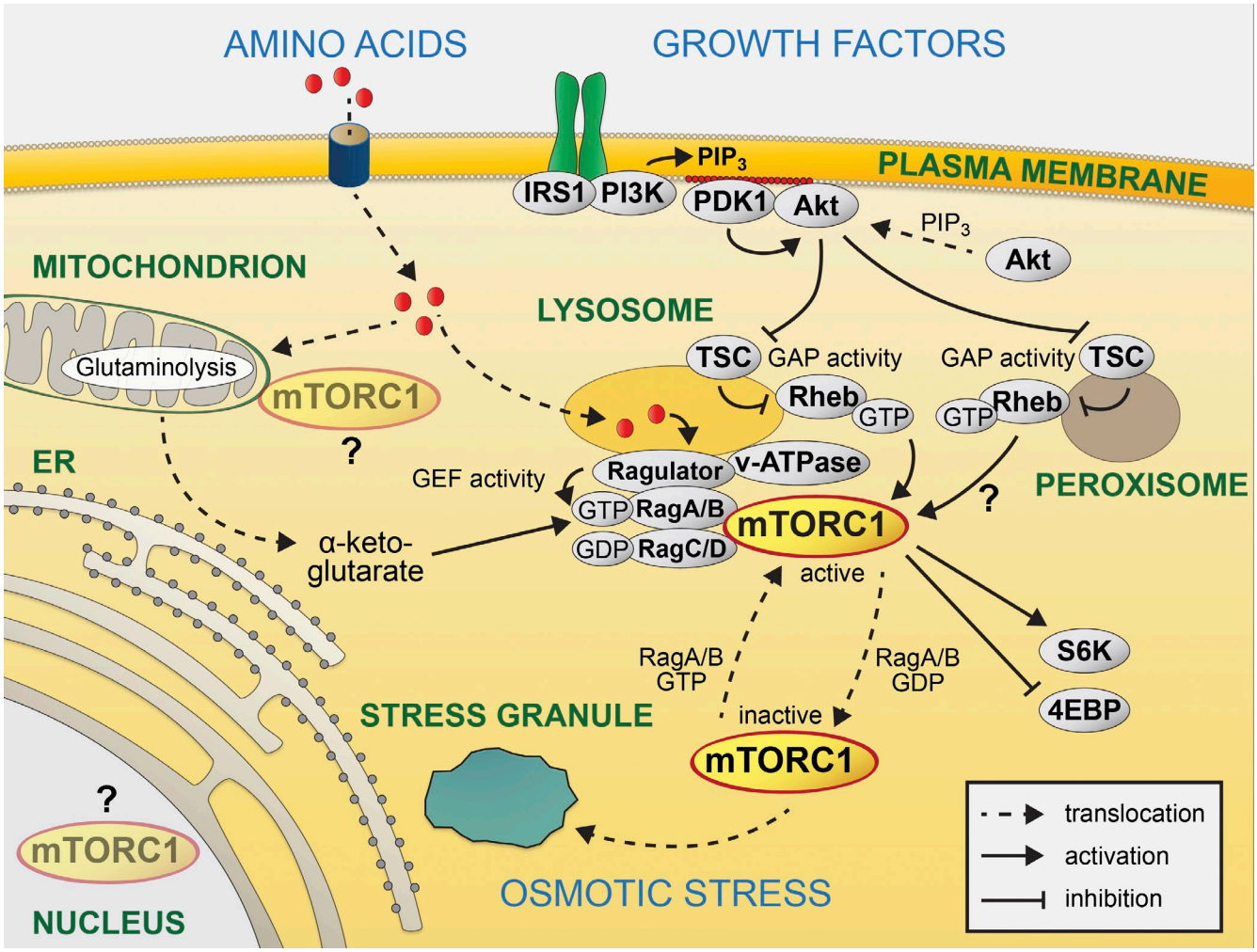

Figure 1. **Localization of mTORC1 signaling.** mTORC1 is in the cytoplasm when amino acids levels are low. Addition of amino acids stimulates the recruitment of mTORC1 in a Rag-dependent manner to the lysosomal surface. Upon growth factor stimulation, PI3K produces PIP_3 in the plasma membrane, which in turn activates PDK1 and Akt. After phosphorylation by PDK1, Akt phosphorylates and thereby inhibits the TSC complex, possibly at the lysosome and the peroxisome. Reduced TSC complex GAP activity leads to an increase in GTP-bound Rheb. Rheb-GTP at the lysosomal surface directly binds to and activates mTORC1. Osmotic stress induces sequestration of mTORC1 in stress granules. Pools of mTORC1 have also been reported at other sites, including mitochondria and the nucleus.

substrates locally? mTORC1 phosphorylates TFEB, a transcription factor involved in autophagy, at the lysosome (Martina et al., 2012; Martina and Puertollano, 2013). However, presumably active mTORC1 is also found at other cellular locations (see next subheadings), yet Rheb and Rag are required for activation of mTORC1 toward all examined substrates. It is unclear whether mTORC1 active in the cytoplasm (or in any other nonlysosomal location) remains bound to Rheb or, alternatively, it retains a "memory" of Rheb. We note that Rheb or TSC have also been identified on endomembranes other than the lysosome (Table 2; Henry et al., 1998; Plank et al., 1998; van Slegtenhorst et al., 1998; Nellist et al., 1999; Noonan et al., 2002; Murthy et al., 2004; Teleman et al., 2005; Buerger et al., 2006; Dibble et al., 2012; Yadav et al., 2013; Zhang et al., 2013). Of particular interest is the recent finding that both Rheb and TSC are on the peroxisome (Zhang et al., 2013). mTORC1 itself has so far not been found on the peroxisome but is inhibited by radical oxygen species generated in the peroxisome. The significance of TSC and Rheb on the peroxisome and whether Rag (or another recruitment factor) is also on the peroxisome should be investigated further. Future studies may reveal that mTORC1 is independently activated at different locations, possibly in response to different cues, to phosphorylate physically separate substrates. To determine what mTORC1 is doing where will require defining where it phosphorylates particular substrates.

mTORC1 localization to lysosomes is usually monitored by immunofluorescence (IF) using an antibody against mTOR (Table 1). In these experiments, mTOR is dispersed (cytoplasmic) under amino acid–starved conditions, and punctate/lysosomal upon re-addition of amino acids. An mTOR antibody should theoretically detect both mTORC1 and mTORC2, but mTORC2 does not localize to the lysosome and is unresponsive to amino acids. Thus, the mTOR antibody that is commonly used in these studies recognizes mTOR only in mTORC1, possibly because the epitope is masked in mTORC2. Another possibility would be that mTORC1 is much more abundant than mTORC2; however, immunoprecipitation experiments suggest this is not the case (Rosner and Hengstschläger, 2008). Other

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antibodies that detect mTOR at mitochondria and the ER might detect an epitope exposed only in mTORC2. Finally, immunofluorescence experiments with antibodies against mTOR or raptor have generally detected only cytoplasmic/lysosomal mTORC1, yet other approaches have revealed mTORC1 at other sites (discussed later). This may be due to mTORC1 being mainly cytoplasmic/lysosomal. Alternatively, localization to other sites may require specific conditions such as stress.

mTORC1 in the nucleus. Components of the mTORC1 signaling pathway, including mTOR, raptor, and S6K, have been detected in the nucleoplasm (Table 2; Kim and Kahn, 1997; Kim and Chen, 2000; Zhang et al., 2002; Kikani et al., 2005; Lian and Di Cristofano, 2005; Rosner et al., 2007; Yadav et al., 2013). However, although mTOR and raptor are detectable inside the nucleus where they may have a role in transcription regulation (Bernardi et al., 2006; Cunningham et al., 2007; Yang et al., 2008; Goh et al., 2010; Kantidakis et al., 2010; Shor et al., 2010; Tsang et al., 2010), these nuclear versions appear not to form an intact mTORC1 (Fig. 1; Rosner and Hengstschläger, 2008).

mTORC1 in mitochondria. mTOR has been found at mitochondria (Fig. 1), and rapamycin treatment affects mitochondrial function in Jurkat cells (Paglin et al., 2005; Schieke et al., 2006; Ramanathan and Schreiber, 2009). However, it is unclear whether mTORC1 localizes to mitochondria because mTOR, instead of complex-specific rictor or raptor, was probed to investigate mTORC1 localization. Although rapamycin acutely inhibits only mTORC1, prolonged rapamycin treatment can also inhibit mTORC2 in certain cells (including Jurkat cells; Sarbassov et al., 2006). We speculate that at least some studies might have observed mTORC2 rather than mTORC1 at mitochondria (discussed later).

mTORC1 in stress granules. mTORC1 is sequestered in stress granules in response to stress in an astrin-dependent manner (Fig. 1; Thedieck et al., 2013). This localization is mediated by physical interaction with the kinase DYRK3 but is independent of DYRK3 kinase activity (Wippich et al., 2013). Yeast TORC1 is similarly targeted to stress granules in response to heat stress (Takahara and Maeda, 2012). Localization of mTORC1 to stress granules is a mechanism to sequester mTORC1 and thus to arrest growth in unfavorable conditions.

mTORC1 at the plasma membrane. A recent report suggests that the WD40 domain in raptor binds the lipid $PI(3,5)P_2$, thereby targeting mTORC1 to the plasma membrane (Bridges et al., 2012). The authors observed that this lipid is generated at the plasma membrane and the lysosome, depending on the cell type, after insulin or amino acid stimulation, and speculate that $PI(3,5)P_2$ binding could also contribute to lysosomal targeting. Interestingly, both $PI(3,5)P_2$ and TORC1 are enriched at discrete sites on the yeast lysosome (known as the vacuole in yeast; Han and Emr, 2011), but it has yet to be determined whether TORC1 colocalizes with $PI(3,5)P_2$.

mTORC1 in the cytoplasm. mTORC1, in particular under amino acid starvation conditions, exhibits a diffuse, cytoplasmic distribution. However, it may also have a function in the cytoplasm when active. mTORC1 associates with the general translation initiation complex eIF3 and phosphorylates the translation inhibitor 4E-BP upon stimulation by growth factors and nutrients to promote translation initiation (Holz et al., 2005; Harris et al., 2006; Proud, 2009; Sonenberg and Hinnebusch, 2009). These events presumably take place in the cytoplasm.

Yeast TORC1. TORC1 in *S. cerevisiae* is on the limiting membrane of the vacuole, the major nutrient reservoir in yeast cells (Reinke et al., 2004; Urban et al., 2007; Berchtold and Walther, 2009; Binda et al., 2009). The yeast vacuole is equivalent to the lysosome in higher organisms. At the vacuole, TORC1 localizes to discrete, PI3P-enriched subdomains (Sturgill et al., 2008). Interestingly, in sharp contrast to mTORC1 at lysosomes, yeast TORC1 localization to the vacuole is independent of nutrient availability (Binda et al., 2009). TORC1 at the vacuole/lysosome in both yeast and higher eukaryotes is the best-characterized localization of TORC1. However, as in mammals, the precise function of TORC1 at the vacuole/lysosome is unknown. A small fraction of yeast TORC1 is near the plasma membrane (Wedaman et al., 2003; Reinke et al., 2004). Curiously, Li et al. (2006) have reported that TORC1 is mainly nuclear.

TORC1 in other organisms. TORC1 in the alga *Chlamydomonas* is localized to the ER and regulates Bip (Grp78) phosphorylation (Díaz-Troya et al., 2008, 2011). TORC1 in plants is cytoplasmic and nuclear and regulates development and growth (Ren et al., 2011). In *Drosophila*, TOR appears to localize to vesicular structures, possibly the lysosome (Hennig et al., 2006), and to a perinuclear structure, possibly the ER (Chang and Neufeld, 2009). Although TORC1 is highly conserved in eukaryotes, subcellular localization in organisms such as worms or fish has to our knowledge not been reported.

Localization of mTORC2

mTORC2 at mitochondria-associated ER membrane. mTORC2 interacts with the ER proteins Hsp70 and Grp58 (Martin et al., 2008; Ramírez-Rangel et al., 2011), is sensitive to ER stress (Hosoi et al., 2007; Yung et al., 2011; Chen et al., 2011; Appenzeller-Herzog and Hall, 2012), and is localized to the ER (Boulbés et al., 2011). It is not fully understood which steps in mTORC2 signaling occur at the ER and in what chronological order. Akt, a major, direct mTORC2 substrate, is also found at the ER (Hresko and Mueckler, 2005; Boulbés et al., 2011), although the first step in Akt activation—PDK1-mediated phosphorylation of the activation loop in Akt—is generally thought to occur at the plasma membrane. However, PDK1, PTEN (the PIP_3 phosphatase), and PI3K have been observed on other organelles, including the ER (Daniele et al., 1999; Lim et al., 2003; Downes et al., 2004). It is thus possible that the first step in Akt activation might also occur at the ER. Alternatively, PDK1-phosphorylated Akt may translocate to the ER to be phosphorylated by mTORC2. It is unclear whether reported cotranslational phosphorylation of Akt and IMP1 by mTORC2 (Oh et al., 2010; Dai et al., 2013) occurs at the ER.

There are longstanding indications that mTORC2 is also associated with mitochondria. Mitochondrial membrane potential, respiration, and the phosphorylation status of several mitochondrial proteins are changed upon rapamycin treatment or rictor knockdown in Jurkat cells (Desai et al., 2002; Schieke et al., 2006). As noted earlier, mTORC2 is highly sensitive to rapamycin in Jurkat cells (Sarbassov et al., 2006). Furthermore,

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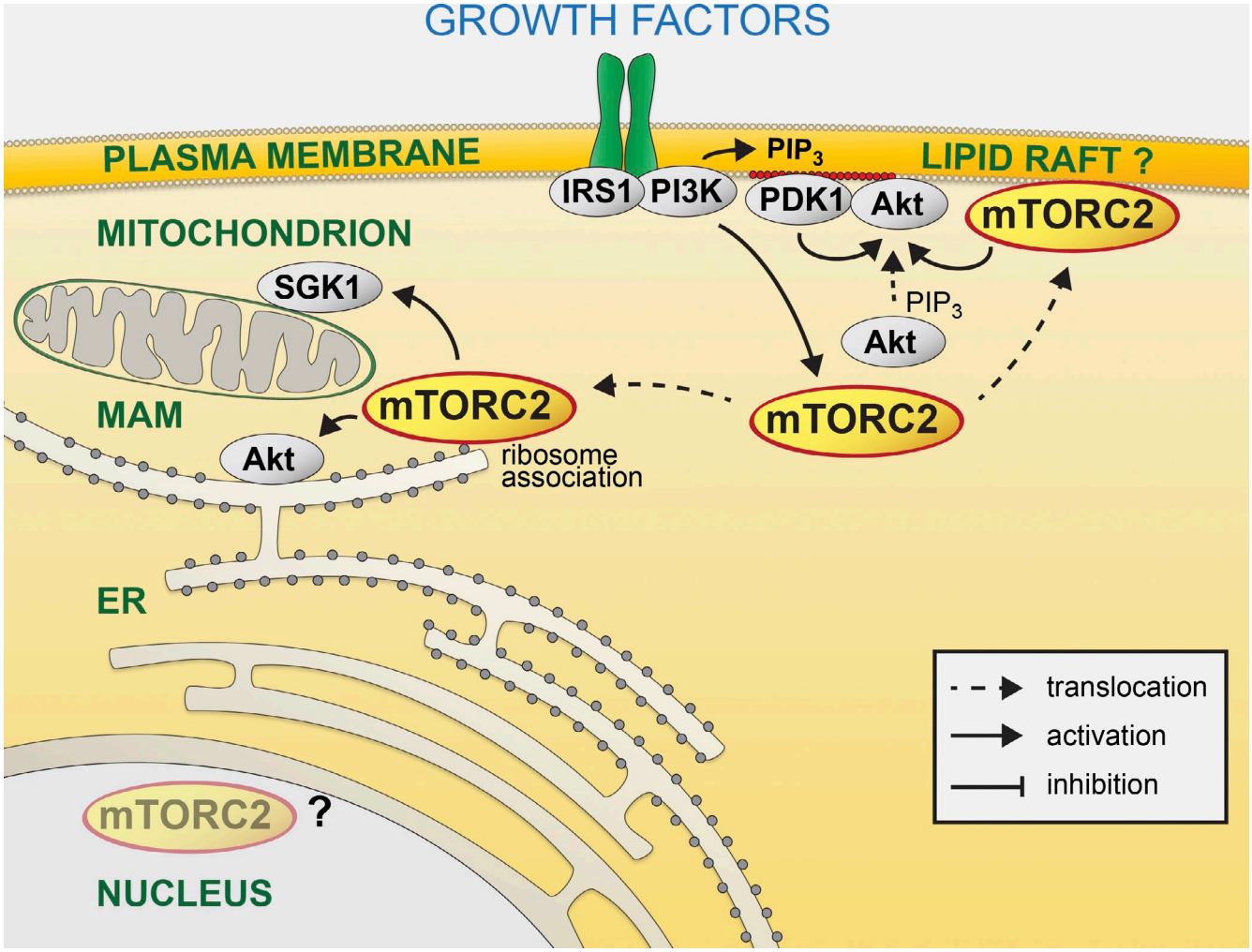

Figure 2. **Localization of mTORC2 signaling.** mTORC2 interacts with ribosomes in a PI3K-dependent manner. Upon growth factor stimulation, mTORC2 is recruited to MAMs, presumably from the cytoplasm. mTORC2 has also been observed in the nucleus and on lipid rafts at the plasma membrane.

mTOR localizes to structures in close proximity to the mitochondrial outer membrane (OMM; Desai et al., 2002) and interacts with VDAC, an OMM channel (Ramanathan and Schreiber, 2009), although these two studies did not address whether the observed mTOR localization was mTORC1 or mTORC2. We suggest that mitochondrial mTOR may correspond to mTORC2. This would be consistent with other studies linking mTORC2 to mitochondrial function (Wang et al., 2010; Colombi et al., 2011; Murata et al., 2011; Hagiwara et al., 2012; Yuan et al., 2012; Wu et al., 2013). Furthermore, Akt has been detected at mitochondria (Bijur and Jope, 2003; Miyamoto et al., 2008; Antico Arciuch et al., 2009; Su et al., 2012), and another direct substrate of mTORC2, SGK1 (serum- and glucocorticoid-inducible kinase 1), is primarily localized to mitochondria (Engelsberg et al., 2006; Cordas et al., 2007). PTEN has been observed at mitochondria (Zhu et al., 2006; Zu et al., 2011) and mitochondria-associated ER membranes (MAMs; Bononi et al., 2013), although it is unclear whether mitochondrial PTEN functions as a PIP_3 phosphatase.

Is there a link between mitochondrial mTORC2 and ER-associated mTORC2? MAM is a subdomain of the ER that is physically tethered to mitochondria (Raturi and Simmen, 2012). The main function of MAM is to facilitate the transfer of lipids and calcium between the two organelles. MAM thereby controls mitochondrial metabolism and apoptosis (Rizzuto et al., 1998; Csordás et al., 1999). Several observations have linked mTORC2 to MAM. First, mTOR and mLST8 interact with VDAC1 (Ramanathan and Schreiber, 2009) and Grp75 (Behrends et al., 2010), respectively. VDAC1 together with Grp75 and IP3R tethers mitochondria to the ER to form MAM (Szabadkai et al., 2006). Second, the MAM resident proteins IP3R, PACS2, and the VDAC-interacting protein hexokinase 2 (HK2) are Akt substrates (Simmen et al., 2005; Khan et al., 2006; Szado et al., 2008; Aslan et al., 2009; Marchi et al., 2012), and Akt itself is associated with MAM (Giorgi et al., 2010). Third, MAM appears to have a role in modulating ER stress (Simmen et al., 2010; Malhotra and Kaufman, 2011; Verfaillie et al., 2012), which in turn can inhibit mTORC2 (Appenzeller-Herzog and Hall, 2012). Fourth, liver-specific knockout of Mfn2 (Sebastián et al., 2012), a key MAM protein, confers a whole body phenotype strikingly similar to that of liver-specific rictor knockout (Hagiwara et al., 2012). Fifth, a recent proteomic study on MAM detected mTOR (Poston et al., 2013). These results suggest that the previously described mitochondrial and ER-associated mTORC2 might actually be at MAM.

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Table 3. **Mitochondria, ER, MAM, and other localization of mTORC2**

Pathway	Mechanism of localization	Cell type	Technique	Reference
mTORC2	mTORC2 (mTOR, rictor) is mainly at the ER	MDA-MB-435; A549	Confocal imaging and subcellular fractionation; PFA fixation; endogenous mTOR and rictor	Boulbés et al., 2011
mTORC1/2	mTOR is associated with mitochondria	NIH3T3	Confocal imaging, subcellular fractionation, and immuno-EM; PFA fixation; endogenous mTOR	Desai et al., 2002
mTORC2	mTORC2 (mTOR; Sin1; rictor) is at MAM	HeLa; MEF; mouse liver	Confocal imaging, subcellular fractionation, and immuno-EM; PFA fixation; endogenous mTOR, Sin1, and rictor; validation of antibody	Betz et al., 2013; Poston et al., 2013
mTORC2	A part of mTORC2 (mTOR; rictor; Sin1) is nuclear; rapamycin treatment induces translocation of mTORC2 to the cytoplasm	HEK293; IMR-90; NIH3T3	Subcellular fractionation	Rosner et al., 2007; Rosner and Hengstschläger, 2008, 2011, 2012
Yeast TORC2	TORC2 (TOR2; Bit61; Avo1-3) is at or near the plasma membrane, possibly cortical ER	*S. cerevisiae*	Subcellular fractionation, immuno-EM, live-cell imaging, and wide-field and confocal imaging; PFA fixation; TOR2-GFP, Bit61-GFP, Avo1-3-GFP	Kunz et al., 2000; Wedaman et al., 2003; Aronova et al., 2007; Sturgill et al., 2008; Berchtold and Walther, 2009

This table groups the most important reports that investigate TORC2 and mTORC2 localization. mTORC2 has consistently been identified in proximity to mitochondria, the ER, and MAM. Other studies suggest that mTORC2 is nuclear or at the plasma membrane. EM, electron microscopy; PFA, paraformaldehyde.

A recent study that directly investigated mTORC2 localization confirmed that mTORC2 indeed localizes to MAM (Fig. 2, Table 3; Betz et al., 2013). mTORC2 localization to MAM is stimulated by growth factors, and mTORC2 at MAM controls Akt and the Akt targets PACS2, IP3R, and HK2 to ultimately control MAM integrity, calcium release, and mitochondrial physiology (Betz et al., 2013). Thus, it appears that MAM is a hub for mTORC2-Akt signaling. MAM regulates many physiological functions including mitochondrial metabolism, the synthesis and maturation of cholesterol, phospho- and sphingolipids, autophagy, and ER stress (Fujimoto et al., 2012; Raturi and Simmen, 2012; Verfaillie et al., 2012; Hamasaki et al., 2013), and is implicated in a number of diseases including diabetes, neurodegeneration, and cancer (Bononi et al., 2012; Leem and Koh, 2012). Interestingly, mTORC2 is also linked to many of these processes and diseases, suggesting that they may be due to mTORC2 function (or dysfunction) at the MAM signaling hub.

How does mTORC2 localization to MAM relate to activation of mTORC2 by ribosome association? Electron microscopy images (Ruby et al., 1969; Csordás et al., 2006; Lebiedzinska et al., 2009) and proteomic profiling (Poston et al., 2011, 2013; Zhang et al., 2011) of MAM suggest that ribosomes are present at this ER subdomain. Calnexin, a MAM-enriched chaperone (Lynes et al., 2012), interacts with the MAM protein PACS2 and anchors ribosomes at the ER and possibly MAM (Lakkaraju et al., 2012). The role of MAM ribosomes has yet to be defined. However, certain viruses that replicate at MAM, such as CMV, actively recruit the translation machinery to MAM (Zhang et al., 2011), suggesting that ribosomes at MAM are required for translation. Interestingly, CMV infection also increases mTORC2 activity (Clippinger et al., 2011), and a MAM deficiency results in reduced mTORC2 activity (Sebastián et al., 2012). Thus, it appears that ribosomes at MAM and activation of mTORC2 are functionally linked. A recent report suggests that ATP regulates mTORC2 integrity (Chen et al., 2013). At MAM, mTORC2 is in a complex with VDAC, the exit channel for mitochondrial ATP, thus providing more evidence that MAM localization is important for mTORC2 activity. Altogether, the above suggests that MAM is a signaling platform for mTORC2 like the lysosome is for mTORC1 (Fig. 2).

mTORC2 at the plasma membrane. mTORC2 and its substrate Akt have been isolated from lipid rafts traditionally associated with the plasma membrane (Fig. 2; Hill et al., 2002; Partovian et al., 2008; Gao et al., 2011), although lipid raft domains also exist in the ER, where they form MAM (Poston et al., 2011; see previous section). Evaluation of rictor localization by immunofluorescence (Zhang et al., 2010) and subcellular fractionation (Boulbés et al., 2011) suggests that only a minor fraction of mTORC2 is at the plasma membrane.

mTORC2 in the nucleus. mTORC2 has been reported to shuttle between the cytoplasm and the nucleoplasm (Table 3, Fig. 2; Rosner et al., 2007; Rosner and Hengstschläger, 2008, 2011, 2012). A nuclear function for mTORC2 is so far unknown.

TORC2 in yeast. TORC2 in yeast is at or near the plasma membrane (Kunz et al., 2000; Wedaman et al., 2003; Aronova et al., 2007; Sturgill et al., 2008; Berchtold and Walther, 2009). TORC2 near the plasma membrane might actually be at cortical ER, as suggested by its association with distinct membranous tracks just under the plasma membrane (Wedaman et al., 2003). It is currently unknown whether TORC2 can also localize to MAM in yeast, as this structure is structurally and functionally different from mammalian MAM (Kornmann et al., 2009; Michel and Kornmann, 2012).

TORC2 in other organisms. TORC2 in *Trypanosomes* localizes to both the ER and mitochondria (Barquilla et al., 2008). Although present in *Dictyostelium*, *Drosophila*, *Caenorhabditis*, and zebrafish, TORC2 localization has not yet been investigated in these organisms.

Considerations and future directions

A major question is, why is mTOR at different cellular locations? The initial response to this question is that mTOR phosphorylates

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functionally distinct substrates at different locations. Another possibility is that mTORC1 and mTORC2 sense different inputs at different locations. For example, mTORC1 at the lysosome senses amino acids, whereas mTORC1 at the peroxisome would sense peroxisome-generated hydrogen peroxide (Benjamin and Hall, 2013). The answer to the above question will be revealed as we learn more about the cellular biology of mTOR.

Several mTOR complex components, including mTOR, rictor, raptor, and Sin1 are phosphorylated in vivo. Although the functionality of most of these modifications is unclear, post-translational modifications (PTMs), including phosphorylation, are prime suspects in regulating protein targeting. Farnesylation is a key determinant of Rheb subcellular localization to endomembranes (Buerger et al., 2006). Palmitoylation, a PTM that strongly affects protein localization, has not been reported for mTOR components; however, bioinformatic analysis (Ren et al., 2008) predicts at least one strong candidate palmitoylation site (C322) in the Sin1 isoform Sin1.5 (NP_001006619; unpublished data). It is of interest to determine the potential role of PTMs in the localization of TOR pathway components.

TORC1 localization is well characterized and consistent in mammalian and yeast cells. It has been shown by many methods that both yeast TORC1 and mTORC1 are mainly at the vacuole/lysosome. TORC2 localization is more ambiguous. mTORC2 is at MAM, and yeast TORC2 is at the plasma membrane. Although this diversity can be attributed to cell type specificity, there might also be multiple pools of TORC2 (or TORC1), each at a different subcellular location. Furthermore, there are at least three different mTORC2 pools, defined by different Sin1 isoforms (Frias et al., 2006). These alternatively spliced Sin1 isoforms differ in containing PH and RBD (Ras binding) domains, and thus present an interesting mechanism for how different mTORC2 complexes might localize to different compartments. Future studies may reveal the molecular basis of differently localized TORC1 or TORC2 subpopulations.

Another important unresolved issue is how the TORCs at different locations are regulated. For example, is all mTORC1 activated by Rheb and Rag at the lysosome and then distributed to other sites, or are there so far undetected amounts of Rheb and Rag at other locations? The following questions have also not been addressed carefully and are interesting points for future investigation. Is there a specific site inside the cell where TOR complexes are assembled? What role do lipids play in TOR localization? What forces/factors mediate TOR translocation in the cell? How does disease influence mTOR localization and vice versa? Does TOR localization play a role in developmental processes? To answer these questions, new technologies that allow sensitive detection of intact mTOR complexes in live cells, such as FRET-FLIM (Ishikawa-Ankerhold et al., 2012), may be necessary. Other methodological or technological developments such as proximity ligation (Blazek et al., 2013) and super-resolution microscopy, respectively, will also enhance our understanding of TOR localization. The ultimate goal is to obtain a comprehensive, dynamic, and spatial model of TOR signaling.

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References

Antico Arciuch, V.G., S. Galli, M.C. Franco, P.Y. Lam, E. Cadenas, M.C. Carreras, and J.J. Poderoso. 2009. Akt1 intramitochondrial cycling is a crucial step in the redox modulation of cell cycle progression. *PLoS ONE*. 4:e7523. http://dx.doi.org/10.1371/journal.pone.0007523

Appenzeller-Herzog, C., and M.N. Hall. 2012. Bidirectional crosstalk between endoplasmic reticulum stress and mTOR signaling. *Trends Cell Biol.* 22:274–282. http://dx.doi.org/10.1016/j.tcb.2012.02.006

Aronova, S., K. Wedaman, S. Anderson, J. Yates III, and T. Powers. 2007. Probing the membrane environment of the TOR kinases reveals functional interactions between TORC1, actin, and membrane trafficking in *Saccharomyces cerevisiae*. *Mol. Biol. Cell*. 18:2779–2794. http://dx.doi.org/10.1091/mbc.E07-03-0274

Aslan, J.E., H. You, D.M. Williamson, J. Endig, R.T. Youker, L. Thomas, H. Shu, Y. Du, R.L. Milewski, M.H. Brush, et al. 2009. Akt and 14-3-3 control a PACS-2 homeostatic switch that integrates membrane traffic with TRAIL-induced apoptosis. *Mol. Cell*. 34:497–509. http://dx.doi.org/10.1016/j.molcel.2009.04.011

Averous, J., and C.G. Proud. 2006. When translation meets transformation: the mTOR story. *Oncogene*. 25:6423–6435. http://dx.doi.org/10.1038/sj.onc.1209887

Bar-Peled, L., L.D. Schweitzer, R. Zoncu, and D.M. Sabatini. 2012. Ragulator is a GEF for the rag GTPases that signal amino acid levels to mTORC1. *Cell*. 150:1196–1208. http://dx.doi.org/10.1016/j.cell.2012.07.032

Bar-Peled, L., L. Chantranupong, A.D. Cherniack, W.W. Chen, K.A. Ottina, B.C. Grabiner, E.D. Spear, S.L. Carter, M. Meyerson, and D.M. Sabatini. 2013. A Tumor suppressor complex with GAP activity for the Rag GTPases that signal amino acid sufficiency to mTORC1. *Science*. 340:1100–1106. http://dx.doi.org/10.1126/science.1232044

Barquilla, A., J.L. Crespo, and M. Navarro. 2008. Rapamycin inhibits trypanosome cell growth by preventing TOR complex 2 formation. *Proc. Natl. Acad. Sci. USA*. 105:14579–14584. http://dx.doi.org/10.1073/pnas.0802668105

Behrends, C., M.E. Sowa, S.P. Gygi, and J.W. Harper. 2010. Network organization of the human autophagy system. *Nature*. 466:68–76. http://dx.doi.org/10.1038/nature09204

Ben-Sahra, I., J.J. Howell, J.M. Asara, and B.D. Manning. 2013. Stimulation of de novo pyrimidine synthesis by growth signaling through mTOR and S6K1. *Science*. 339:1323–1328. http://dx.doi.org/10.1126/science.1228792

Benjamin, D., and M.N. Hall. 2013. TSC on the peroxisome controls mTORC1. *Nat. Cell Biol.* 15:1135–1136. http://dx.doi.org/10.1038/ncb2849

Benjamin, D., M. Colombi, C. Moroni, and M.N. Hall. 2011. Rapamycin passes the torch: a new generation of mTOR inhibitors. *Nat. Rev. Drug Discov.* 10:868–880. http://dx.doi.org/10.1038/nrd3531

Berchtold, D., and T.C. Walther. 2009. TORC2 plasma membrane localization is essential for cell viability and restricted to a distinct domain. *Mol. Biol. Cell*. 20:1565–1575. http://dx.doi.org/10.1091/mbc.E08-10-1001

Bernardi, R., I. Guernah, D. Jin, S. Grisendi, A. Alimonti, J. Teruya-Feldstein, C. Cordon-Cardo, M.C. Simon, S. Rafii, and P.P. Pandolfi. 2006. PML inhibits HIF-1alpha translation and neoangiogenesis through repression of mTOR. *Nature*. 442:779–785. http://dx.doi.org/10.1038/nature05029

Betz, C., D. Stracka, C. Prescianotto-Baschong, M. Frieden, N. Demaurex, and M.N. Hall. 2013. Feature article: mTOR complex 2-Akt signaling at mitochondria-associated endoplasmic reticulum membranes (MAM) regulates mitochondrial physiology. *Proc. Natl. Acad. Sci. USA*. 110:12526–12534. http://dx.doi.org/10.1073/pnas.1302455110

Bijur, G.N., and R.S. Jope. 2003. Rapid accumulation of Akt in mitochondria following phosphatidylinositol 3-kinase activation. *J. Neurochem.* 87:1427–1435. http://dx.doi.org/10.1046/j.1471-4159.2003.02113.x

Binda, M., M.-P. Péli-Gulli, G. Bonfils, N. Panchaud, J. Urban, T.W. Sturgill, R. Loewith, and C. De Virgilio. 2009. The Vam6 GEF controls TORC1 by activating the EGO complex. *Mol. Cell*. 35:563–573. http://dx.doi.org/10.1016/j.molcel.2009.06.033

Blazek, M., C. Betz, M. Reth, M.N. Hall, R. Zengerle, and M. Meier. 2013. Proximity ligation assay for high content profiling of cell signaling pathways on a microfluidic chip. *Mol. Cell. Proteomics*. http://dx.doi.org/10.1074/mcp.M113.032821

Bononi, A., S. Missiroli, F. Poletti, J.M. Suski, C. Agnoletto, M. Bonora, E. De Marchi, C. Giorgi, S. Marchi, S. Patergnani, et al. 2012. Mitochondria-associated membranes (MAMs) as hotspot Ca(2+) signaling units. *Adv. Exp. Med. Biol.* 740:411–437. http://dx.doi.org/10.1007/978-94-007-2888-2_17

Bononi, A., M. Bonora, S. Marchi, S. Missiroli, F. Poletti, C. Giorgi, P.P. Pandolfi, and P. Pinton. 2013. Identification of PTEN at the ER and MAMs and its regulation of Ca(2+) signaling and apoptosis in a protein phosphatase-dependent manner. *Cell Death Differ.*

Boulbés, D.R., T. Shaiken, and D. Sarbassov. 2011. Endoplasmic reticulum is a main localization site of mTORC2. *Biochem. Biophys. Res. Commun.* 413:46–52. http://dx.doi.org/10.1016/j.bbrc.2011.08.034

Bridges, D., J.-T. Ma, S. Park, K. Inoki, L.S. Weisman, and A.R. Saltiel. 2012. Phosphatidylinositol 3,5-bisphosphate plays a role in the activation and subcellular localization of mechanistic target of rapamycin 1. *Mol. Biol. Cell*. 23:2955–2962. http://dx.doi.org/10.1091/mbc.E11-12-1034

Buerger, C., B. DeVries, and V. Stambolic. 2006. Localization of Rheb to the endomembrane is critical for its signaling function. *Biochem. Biophys. Res. Commun.* 344:869–880. http://dx.doi.org/10.1016/j.bbrc.2006.03.220

Chang, Y.-Y., and T.P. Neufeld. 2009. An Atg1/Atg13 complex with multiple roles in TOR-mediated autophagy regulation. *Mol. Biol. Cell*. 20:2004–2014. http://dx.doi.org/10.1091/mbc.E08-12-1250

Chen, C.-H., T. Shaikenov, T.R. Peterson, R. Aimbetov, A.K. Bissenbaev, S.-W. Lee, J. Wu, H.-K. Lin, and D. Sarbassov. 2011. ER stress inhibits mTORC2 and Akt signaling through GSK-3β-mediated phosphorylation of rictor. *Sci. Signal.* 4:ra10. http://dx.doi.org/10.1126/scisignal.2001731

Chen, C.-H., V. Kiyan, A.A. Zhylkibayev, D. Kazyken, O. Bulgakova, K.E. Page, R.I. Bersimbaev, E. Spooner, and D. dos Sarbassov. 2013. Autoregulation of the mTOR Complex 2 integrity is controlled by the ATP-dependent mechanism. *J. Biol. Chem.* 288:27019–27030. http://dx.doi.org/10.1074/jbc.M113.498055

Clippinger, A.J., T.G. Maguire, and J.C. Alwine. 2011. The changing role of mTOR kinase in the maintenance of protein synthesis during human cytomegalovirus infection. *J. Virol.* 85:3930–3939. http://dx.doi.org/10.1128/JVI.01913-10

Colombi, M., K.D. Molle, D. Benjamin, K. Rattenbacher-Kiser, C. Schaefer, C. Betz, A. Thiemeyer, U. Regenass, M.N. Hall, and C. Moroni. 2011. Genome-wide shRNA screen reveals increased mitochondrial dependence upon mTORC2 addiction. *Oncogene*. 30:1551–1565. http://dx.doi.org/10.1038/onc.2010.539

Cordas, E., A. Náray-Fejes-Tóth, and G. Fejes-Tóth. 2007. Subcellular location of serum- and glucocorticoid-induced kinase-1 in renal and mammary epithelial cells. *Am. J. Physiol. Cell Physiol.* 292:C1971–C1981. http://dx.doi.org/10.1152/ajpcell.00399.2006

Csordás, G., A.P. Thomas, and G. Hajnóczky. 1999. Quasi-synaptic calcium signal transmission between endoplasmic reticulum and mitochondria. *EMBO J.* 18:96–108. http://dx.doi.org/10.1093/emboj/18.1.96

Csordás, G., C. Renken, P. Várnai, L. Walter, D. Weaver, K.F. Buttle, T. Balla, C.A. Mannella, and G. Hajnóczky. 2006. Structural and functional features and significance of the physical linkage between ER and mitochondria. *J. Cell Biol.* 174:915–921. http://dx.doi.org/10.1083/jcb.200604016

Cunningham, J.T., J.T. Rodgers, D.H. Arlow, F. Vazquez, V.K. Mootha, and P. Puigserver. 2007. mTOR controls mitochondrial oxidative function through a YY1-PGC-1[agr] transcriptional complex: Article: Nature. *Nature*. 450:736–740. http://dx.doi.org/10.1038/nature06322

Cybulski, N., and M.N. Hall. 2009. TOR complex 2: a signaling pathway of its own. *Trends Biochem. Sci.* 34:620–627. http://dx.doi.org/10.1016/j.tibs.2009.09.004

Dai, N., J. Christiansen, F.C. Nielsen, and J. Avruch. 2013. mTOR complex 2 phosphorylates IMP1 cotranslationally to promote IGF2 production and the proliferation of mouse embryonic fibroblasts. *Genes Dev.* 27:301–312. http://dx.doi.org/10.1101/gad.209130.112

Daniele, N., F. Rajas, B. Payrastre, G. Mauco, C. Zitoun, and G. Mithieux. 1999. Phosphatidylinositol 3-kinase translocates onto liver endoplasmic reticulum and may account for the inhibition of glucose-6-phosphatase during refeeding. *J. Biol. Chem.* 274:3597–3601. http://dx.doi.org/10.1074/jbc.274.6.3597

Datta, S.R., H. Dudek, X. Tao, S. Masters, H. Fu, Y. Gotoh, and M.E. Greenberg. 1997. Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. *Cell*. 91:231–241. http://dx.doi.org/10.1016/S0092-8674(00)80405-5

Dazert, E., and M.N. Hall. 2011. mTOR signaling in disease. *Curr. Opin. Cell Biol.* 23:744–755. http://dx.doi.org/10.1016/j.ceb.2011.09.003

Desai, B.N., B.R. Myers, and S.L. Schreiber. 2002. FKBP12-rapamycin-associated protein associates with mitochondria and senses osmotic stress via mitochondrial dysfunction. *Proc. Natl. Acad. Sci. USA*. 99:4319–4324. http://dx.doi.org/10.1073/pnas.261702698

Díaz-Troya, S., F.J. Florencio, and J.L. Crespo. 2008. Target of rapamycin and LST8 proteins associate with membranes from the endoplasmic reticulum in the unicellular green alga *Chlamydomonas reinhardtii*. *Eukaryot. Cell*. 7:212–222. http://dx.doi.org/10.1128/EC.00361-07

Díaz-Troya, S., M.E. Pérez-Pérez, M. Pérez-Martín, S. Moes, P. Jenö, F.J. Florencio, and J.L. Crespo. 2011. Inhibition of protein synthesis by TOR inactivation revealed a conserved regulatory mechanism of the BiP chaperone in *Chlamydomonas*. *Plant Physiol.* 157:730–741. http://dx.doi.org/10.1104/pp.111.179861

Dibble, C.C., W. Elis, S. Menon, W. Qin, J. Klekota, J.M. Asara, P.M. Finan, D.J. Kwiatkowski, L.O. Murphy, and B.D. Manning. 2012. TBC1D7 is a third subunit of the TSC1-TSC2 complex upstream of mTORC1. *Mol. Cell*. 47:535–546. http://dx.doi.org/10.1016/j.molcel.2012.06.009

Downes, C.P., S. Walker, G. McConnachie, Y. Lindsay, I.H. Batty, and N.R. Leslie. 2004. Acute regulation of the tumour suppressor phosphatase, PTEN, by anionic lipids and reactive oxygen species. *Biochem. Soc. Trans.* 32:338–342. http://dx.doi.org/10.1042/BST0320338

Durán, R.V., E.D. Mackenzie, H. Boulahbel, C. Frezza, L. Heiserich, S. Tardito, O. Bussolati, S. Rocha, M.N. Hall, and E. Gottlieb. 2012a. HIF-independent role of prolyl hydroxylases in the cellular response to amino acids. *Oncogene*.

Durán, R.V., W. Oppliger, A.M. Robitaille, L. Heiserich, R. Skendaj, E. Gottlieb, and M.N. Hall. 2012b. Glutaminolysis activates Rag-mTORC1 signaling. *Mol. Cell*. 47:349–358. http://dx.doi.org/10.1016/j.molcel.2012.05.043

Engelsberg, A., F. Kobelt, and D. Kuhl. 2006. The N-terminus of the serum- and glucocorticoid-inducible kinase Sgk1 specifies mitochondrial localization and rapid turnover. *Biochem. J.* 399:69–76. http://dx.doi.org/10.1042/BJ20060386

Frias, M.A., C.C. Thoreen, J.D. Jaffe, W. Schroder, T. Sculley, S.A. Carr, and D.M. Sabatini. 2006. mSin1 is necessary for Akt/PKB phosphorylation, and its isoforms define three distinct mTORC2s. *Curr. Biol.* 16:1865–1870. http://dx.doi.org/10.1016/j.cub.2006.08.001

Fujimoto, M., T. Hayashi, and T.-P. Su. 2012. The role of cholesterol in the association of endoplasmic reticulum membranes with mitochondria. *Biochem. Biophys. Res. Commun.* 417:635–639. http://dx.doi.org/10.1016/j.bbrc.2011.12.022

Ganley, I.G., H. Lam, J. Wang, X. Ding, S. Chen, and X. Jiang. 2009. ULK1.ATG13.FIP200 complex mediates mTOR signaling and is essential for autophagy. *J. Biol. Chem.* 284:12297–12305. http://dx.doi.org/10.1074/jbc.M900573200

Gao, X., P.R. Lowry, X. Zhou, C. Depry, Z. Wei, G.W. Wong, and J. Zhang. 2011. PI3K/Akt signaling requires spatial compartmentalization in plasma membrane microdomains. *Proc. Natl. Acad. Sci. USA*. 108:14509–14514. http://dx.doi.org/10.1073/pnas.1019386108

García-Martínez, J.M., and D.R. Alessi. 2008. mTOR complex 2 (mTORC2) controls hydrophobic motif phosphorylation and activation of serum- and glucocorticoid-induced protein kinase 1 (SGK1). *Biochem. J.* 416:375–385. http://dx.doi.org/10.1042/BJ20081668

Giorgi, C., K. Ito, H.-K. Lin, C. Santangelo, M.R. Wieckowski, M. Lebiedzinska, A. Bononi, M. Bonora, J. Duszynski, R. Bernardi, et al. 2010. PML regulates apoptosis at endoplasmic reticulum by modulating calcium release. *Science*. 330:1247–1251. http://dx.doi.org/10.1126/science.1189157

Goh, E.T.H., O.E. Pardo, N. Michael, A. Niewiarowski, N. Totty, D. Volkova, I.R. Tsaneva, M.J. Seckl, and I. Gout. 2010. Involvement of heterogeneous ribonucleoprotein F in the regulation of cell proliferation via the mammalian target of rapamycin/S6 kinase 2 pathway. *J. Biol. Chem.* 285:17065–17076. http://dx.doi.org/10.1074/jbc.M109.078782

Hagiwara, A., M. Cornu, N. Cybulski, P. Polak, C. Betz, F. Trapani, L. Terracciano, M.H. Heim, M.A. Rüegg, and M.N. Hall. 2012. Hepatic mTORC2 activates glycolysis and lipogenesis through Akt, glucokinase, and SREBP1c. *Cell Metab.* 15:725–738. http://dx.doi.org/10.1016/j.cmet.2012.03.015

Hamasaki, M., N. Furuta, A. Matsuda, A. Nezu, A. Yamamoto, N. Fujita, H. Oomori, T. Noda, T. Haraguchi, Y. Hiraoka, et al. 2013. Autophagosomes form at ER-mitochondria contact sites. *Nature*. 495:389–393. http://dx.doi.org/10.1038/nature11910

Han, B.-K., and S.D. Emr. 2011. Phosphoinositide [PI(3,5)P2] lipid-dependent regulation of the general transcriptional regulator Tup1. *Genes Dev.* 25:984–995. http://dx.doi.org/10.1101/gad.1998611

Harris, T.E., A. Chi, J. Shabanowitz, D.F. Hunt, R.E. Rhoads, and J.C. Lawrence Jr. 2006. mTOR-dependent stimulation of the association of eIF4G and eIF3 by insulin. *EMBO J.* 25:1659–1668. http://dx.doi.org/10.1038/sj.emboj.7601047

Heitman, J., N.R. Movva, and M.N. Hall. 1991. Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast. *Science*. 253:905–909. http://dx.doi.org/10.1126/science.1715094

Hemmings, B.A., and D.F. Restuccia. 2012. PI3K-PKB/Akt pathway. *Cold Spring Harb. Perspect. Biol.* 4:a011189. http://dx.doi.org/10.1101/cshperspect.a011189

Hennig, K.M., J. Colombani, and T.P. Neufeld. 2006. TOR coordinates bulk and targeted endocytosis in the *Drosophila melanogaster* fat body to regulate cell growth. *J. Cell Biol.* 173:963–974. http://dx.doi.org/10.1083/jcb.200511140

Henry, K.W., X. Yuan, N.J. Koszewski, H. Onda, D.J. Kwiatkowski, and D.J. Noonan. 1998. Tuberous sclerosis gene 2 product modulates transcription

mediated by steroid hormone receptor family members. *J. Biol. Chem.* 273:20535–20539. http://dx.doi.org/10.1074/jbc.273.32.20535

Hill, M.M., J. Feng, and B.A. Hemmings. 2002. Identification of a plasma membrane Raft-associated PKB Ser473 kinase activity that is distinct from ILK and PDK1. *Curr. Biol.* 12:1251–1255. http://dx.doi.org/10.1016/S0960-9822(02)00973-9

Holz, M.K., B.A. Ballif, S.P. Gygi, and J. Blenis. 2005. mTOR and S6K1 mediate assembly of the translation preinitiation complex through dynamic protein interchange and ordered phosphorylation events. *Cell*. 123:569–580. http://dx.doi.org/10.1016/j.cell.2005.10.024

Hosoi, T., K. Hyoda, Y. Okuma, Y. Nomura, and K. Ozawa. 2007. Akt up- and down-regulation in response to endoplasmic reticulum stress. *Brain Res.* 1152:27–31. http://dx.doi.org/10.1016/j.brainres.2007.03.052

Hosokawa, N., T. Hara, T. Kaizuka, C. Kishi, A. Takamura, Y. Miura, S.-I. Iemura, T. Natsume, K. Takehana, N. Yamada, et al. 2009. Nutrient-dependent mTORC1 association with the ULK1-Atg13-FIP200 complex required for autophagy. *Mol. Biol. Cell*. 20:1981–1991. http://dx.doi.org/10.1091/mbc.E08-12-1248

Hresko, R.C., and M. Mueckler. 2005. mTOR.RICTOR is the Ser473 kinase for Akt/protein kinase B in 3T3-L1 adipocytes. *J. Biol. Chem.* 280:40406–40416. http://dx.doi.org/10.1074/jbc.M508361200

Huh, W.-K., J.V. Falvo, L.C. Gerke, A.S. Carroll, R.W. Howson, J.S. Weissman, and E.K. O'Shea. 2003. Global analysis of protein localization in budding yeast. *Nature*. 425:686–691. http://dx.doi.org/10.1038/nature02026

Inoki, K., Y. Li, T. Xu, and K.-L. Guan. 2003. Rheb GTPase is a direct target of TSC2 GAP activity and regulates mTOR signaling. *Genes Dev.* 17:1829–1834. http://dx.doi.org/10.1101/gad.1110003

Ishikawa-Ankerhold, H.C., R. Ankerhold, and G.P.C. Drummen. 2012. Advanced fluorescence microscopy techniques—FRAP, FLIP, FLAP, FRET and FLIM. *Molecules*. 17:4047–4132. http://dx.doi.org/10.3390/molecules17044047

Jewell, J.L., R.C. Russell, and K.-L. Guan. 2013. Amino acid signalling upstream of mTOR. *Nat. Rev. Mol. Cell Biol.* 14:133–139. http://dx.doi.org/10.1038/nrm3522

Kaizuka, T., T. Hara, N. Oshiro, U. Kikkawa, K. Yonezawa, K. Takehana, S.-I. Iemura, T. Natsume, and N. Mizushima. 2010. Tti1 and Tel2 are critical factors in mammalian target of rapamycin complex assembly. *J. Biol. Chem.* 285:20109–20116. http://dx.doi.org/10.1074/jbc.M110.121699

Kantidakis, T., B.A. Ramsbottom, J.L. Birch, S.N. Dowding, and R.J. White. 2010. mTOR associates with TFIIIC, is found at tRNA and 5S rRNA genes, and targets their repressor Maf1. *Proc. Natl. Acad. Sci. USA*. 107:11823–11828. http://dx.doi.org/10.1073/pnas.1005188107

Khan, M.T., L. Wagner II, D.I. Yule, C. Bhanumathy, and S.K. Joseph. 2006. Akt kinase phosphorylation of inositol 1,4,5-trisphosphate receptors. *J. Biol. Chem.* 281:3731–3737. http://dx.doi.org/10.1074/jbc.M509262200

Kikani, C.K., L.Q. Dong, and F. Liu. 2005. "New"-clear functions of PDK1: beyond a master kinase in the cytosol? *J. Cell. Biochem.* 96:1157–1162. http://dx.doi.org/10.1002/jcb.20651

Kim, E., P. Goraksha-Hicks, L. Li, T.P. Neufeld, and K.-L. Guan. 2008. Regulation of TORC1 by Rag GTPases in nutrient response. *Nat. Cell Biol.* 10:935–945. http://dx.doi.org/10.1038/ncb1753

Kim, J.E., and J. Chen. 2000. Cytoplasmic-nuclear shuttling of FKBP12-rapamycin-associated protein is involved in rapamycin-sensitive signaling and translation initiation. *Proc. Natl. Acad. Sci. USA*. 97:14340–14345. http://dx.doi.org/10.1073/pnas.011511898

Kim, S.G., G.R. Hoffman, G. Poulogiannis, G.R. Buel, Y.J. Jang, K.W. Lee, B.-Y. Kim, R.L. Erikson, L.C. Cantley, A.Y. Choo, and J. Blenis. 2013. Metabolic stress controls mTORC1 lysosomal localization and dimerization by regulating the TTT-RUVBL1/2 complex. *Mol. Cell*. 49:172–185.

Kim, S.J., and C.R. Kahn. 1997. Insulin stimulates p70 S6 kinase in the nucleus of cells. *Biochem. Biophys. Res. Commun.* 234:681–685. http://dx.doi.org/10.1006/bbrc.1997.6699

Kornmann, B., E. Currie, S.R. Collins, M. Schuldiner, J. Nunnari, J.S. Weissman, and P. Walter. 2009. An ER-mitochondria tethering complex revealed by a synthetic biology screen. *Science*. 325:477–481. http://dx.doi.org/10.1126/science.1175088

Korolchuk, V.I., S. Saiki, M. Lichtenberg, F.H. Siddiqi, E.A. Roberts, S. Imarisio, L. Jahreiss, S. Sarkar, M. Futter, F.M. Menzies, et al. 2011. Lysosomal positioning coordinates cellular nutrient responses. *Nat. Cell Biol.* 13:453–460. http://dx.doi.org/10.1038/ncb2204

Kunz, J., R. Henriquez, U. Schneider, M. Deuter-Reinhard, N.R. Movva, and M.N. Hall. 1993. Target of rapamycin in yeast, TOR2, is an essential phosphatidylinositol kinase homolog required for G1 progression. *Cell*. 73:585–596. http://dx.doi.org/10.1016/0092-8674(93)90144-F

Kunz, J., U. Schneider, I. Howald, A. Schmidt, and M.N. Hall. 2000. HEAT repeats mediate plasma membrane localization of Tor2p in yeast. *J. Biol. Chem.* 275:37011–37020. http://dx.doi.org/10.1074/jbc.M007296200

Lakkaraju, A.K., L. Abrami, T. Lemmin, S. Blaskovic, B. Kunz, A. Kihara, M. Dal Peraro, and F.G. van der Goot. 2012. Palmitoylated calnexin is a key component of the ribosome-translocon complex. *EMBO J.* 31:1823–1835. http://dx.doi.org/10.1038/emboj.2012.15

Laplante, M., and D.M. Sabatini. 2009. An emerging role of mTOR in lipid biosynthesis. *Curr. Biol.* 19:R1046–R1052. http://dx.doi.org/10.1016/j.cub.2009.09.058

Laplante, M., and D.M. Sabatini. 2012. mTOR signaling in growth control and disease. *Cell*. 149:274–293. http://dx.doi.org/10.1016/j.cell.2012.03.017

Lebiedzinska, M., G. Szabadkai, A.W.E. Jones, J. Duszynski, and M.R. Wieckowski. 2009. Interactions between the endoplasmic reticulum, mitochondria, plasma membrane and other subcellular organelles. *Int. J. Biochem. Cell Biol.* 41:1805–1816. http://dx.doi.org/10.1016/j.biocel.2009.02.017

Leem, J., and E.H. Koh. 2012. Interaction between mitochondria and the endoplasmic reticulum: implications for the pathogenesis of type 2 diabetes mellitus. *Exp. Diabetes Res.* 2012:242984. http://dx.doi.org/10.1155/2012/242984

Li, H., C.K. Tsang, M. Watkins, P.G. Bertram, and X.F.S. Zheng. 2006. Nutrient regulates Tor1 nuclear localization and association with rDNA promoter. *Nature*. 442:1058–1061. http://dx.doi.org/10.1038/nature05020

Lian, Z., and A. Di Cristofano. 2005. Class reunion: PTEN joins the nuclear crew. *Oncogene*. 24:7394–7400. http://dx.doi.org/10.1038/sj.onc.1209089

Lim, M.A., C.K. Kikani, M.J. Wick, and L.Q. Dong. 2003. Nuclear translocation of 3′-phosphoinositide-dependent protein kinase 1 (PDK-1): a potential regulatory mechanism for PDK-1 function. *Proc. Natl. Acad. Sci. USA*. 100:14006–14011. http://dx.doi.org/10.1073/pnas.2335486100

Linares, J.F., A. Duran, T. Yajima, M. Pasparakis, J. Moscat, and M.T. Diaz-Meco. 2013. K63 polyubiquitination and activation of mTOR by the p62-TRAF6 complex in nutrient-activated cells. *Mol. Cell*. 51:283–296. http://dx.doi.org/10.1016/j.molcel.2013.06.020

Loewith, R., and M.N. Hall. 2011. Target of rapamycin (TOR) in nutrient signaling and growth control. *Genetics*. 189:1177–1201. http://dx.doi.org/10.1534/genetics.111.133363

Lynes, E.M., M. Bui, M.C. Yap, M.D. Benson, B. Schneider, L. Ellgaard, L.G. Berthiaume, and T. Simmen. 2012. Palmitoylated TMX and calnexin target to the mitochondria-associated membrane. *EMBO J.* 31:457–470. http://dx.doi.org/10.1038/emboj.2011.384

Ma, X.M., and J. Blenis. 2009. Molecular mechanisms of mTOR-mediated translational control. *Nat. Rev. Mol. Cell Biol.* 10:307–318. http://dx.doi.org/10.1038/nrm2672

Malhotra, J.D., and R.J. Kaufman. 2011. ER stress and its functional link to mitochondria: role in cell survival and death. *Cold Spring Harb. Perspect. Biol.* 3:a004424. http://dx.doi.org/10.1101/cshperspect.a004424

Malik, A.R., M. Urbanska, M. Macias, A. Skalecka, and J. Jaworski. 2013. Beyond control of protein translation: what we have learned about the non-canonical regulation and function of mammalian target of rapamycin (mTOR). *Biochim. Biophys. Acta*. 1834:1434–1448. http://dx.doi.org/10.1016/j.bbapap.2012.12.010

Marchi, S., M. Marinello, A. Bononi, M. Bonora, C. Giorgi, A. Rimessi, and P. Pinton. 2012. Selective modulation of subtype III IP_3R by Akt regulates ER Ca^{2+} release and apoptosis. *Cell Death Dis.* 3:e304. http://dx.doi.org/10.1038/cddis.2012.45

Martin, J., J. Masri, A. Bernath, R.N. Nishimura, and J. Gera. 2008. Hsp70 associates with Rictor and is required for mTORC2 formation and activity. *Biochem. Biophys. Res. Commun.* 372:578–583. http://dx.doi.org/10.1016/j.bbrc.2008.05.086

Martina, J.A., and R. Puertollano. 2013. Rag GTPases mediate amino acid-dependent recruitment of TFEB and MITF to lysosomes. *J. Cell Biol.* 200:475–491. http://dx.doi.org/10.1083/jcb.201209135

Martina, J.A., Y. Chen, M. Gucek, and R. Puertollano. 2012. MTORC1 functions as a transcriptional regulator of autophagy by preventing nuclear transport of TFEB. *Autophagy*. 8:903–914. http://dx.doi.org/10.4161/auto.19653

Menon, S., and B.D. Manning. 2008. Common corruption of the mTOR signaling network in human tumors. *Oncogene*. 27(Suppl 2):S43–S51. http://dx.doi.org/10.1038/onc.2009.352

Michel, A.H., and B. Kornmann. 2012. The ERMES complex and ER-mitochondria connections. *Biochem. Soc. Trans.* 40:445–450. http://dx.doi.org/10.1042/BST20110758

Miyamoto, S., A.N. Murphy, and J.H. Brown. 2008. Akt mediates mitochondrial protection in cardiomyocytes through phosphorylation of mitochondrial hexokinase-II. *Cell Death Differ.* 15:521–529. http://dx.doi.org/10.1038/sj.cdd.4402285

Murata, H., M. Sakaguchi, Y. Jin, Y. Sakaguchi, J.-I. Futami, H. Yamada, K. Kataoka, and N.-H. Huh. 2011. A new cytosolic pathway from a Parkinson disease-associated kinase, BRPK/PINK1: activation of AKT via mTORC2. *J. Biol. Chem.* 286:7182–7189. http://dx.doi.org/10.1074/jbc.M110.179390

Murthy, V., S. Han, R.L. Beauchamp, N. Smith, L.A. Haddad, N. Ito, and V. Ramesh. 2004. Pam and its ortholog highwire interact with and may negatively regulate the TSC1.TSC2 complex. *J. Biol. Chem.* 279:1351–1358. http://dx.doi.org/10.1074/jbc.M310208200

Nellist, M., M.A. van Slegtenhorst, M. Goedbloed, A.M. van den Ouweland, D.J. Halley, and P. van der Sluijs. 1999. Characterization of the cytosolic tuberin-hamartin complex. Tuberin is a cytosolic chaperone for hamartin. *J. Biol. Chem.* 274:35647–35652. http://dx.doi.org/10.1074/jbc.274.50.35647

Noonan, D.J., D. Lou, N. Griffith, and T.C. Vanaman. 2002. A calmodulin binding site in the tuberous sclerosis 2 gene product is essential for regulation of transcription events and is altered by mutations linked to tuberous sclerosis and lymphangioleiomyomatosis. *Arch. Biochem. Biophys.* 398:132–140. http://dx.doi.org/10.1006/abbi.2001.2682

Ögmundsdóttir, M.H., S. Heublein, S. Kazi, B. Reynolds, S.M. Visvalingam, M.K. Shaw, and D.C.I. Goberdhan. 2012. Proton-assisted amino acid transporter PAT1 complexes with Rag GTPases and activates TORC1 on late endosomal and lysosomal membranes. *PLoS ONE*. 7:e36616. http://dx.doi.org/10.1371/journal.pone.0036616

Oh, W.J., and E. Jacinto. 2011. mTOR complex 2 signaling and functions. *Cell Cycle*. 10:2305–2316. http://dx.doi.org/10.4161/cc.10.14.16586

Oh, W.J., C.-C. Wu, S.J. Kim, V. Facchinetti, L.-A. Julien, M. Finlan, P.P. Roux, B. Su, and E. Jacinto. 2010. mTORC2 can associate with ribosomes to promote cotranslational phosphorylation and stability of nascent Akt polypeptide. *EMBO J.* 29:3939–3951. http://dx.doi.org/10.1038/emboj.2010.271

Paglin, S., N.-Y. Lee, C. Nakar, M. Fitzgerald, J. Plotkin, B. Deuel, N. Hackett, M. McMahill, E. Sphicas, N. Lampen, and J. Yahalom. 2005. Rapamycin-sensitive pathway regulates mitochondrial membrane potential, autophagy, and survival in irradiated MCF-7 cells. *Cancer Res.* 65:11061–11070. http://dx.doi.org/10.1158/0008-5472.CAN-05-1083

Panchaud, N., M.-P. Péli-Gulli, and C. De Virgilio. 2013. Amino acid deprivation inhibits TORC1 through a GTPase-activating protein complex for the Rag family GTPase Gtr1. *Sci. Signal.* 6:ra42. http://dx.doi.org/10.1126/scisignal.2004112

Partovian, C., R. Ju, Z.W. Zhuang, K.A. Martin, and M. Simons. 2008. Syndecan-4 regulates subcellular localization of mTOR Complex2 and Akt activation in a PKCalpha-dependent manner in endothelial cells. *Mol. Cell.* 32:140–149. http://dx.doi.org/10.1016/j.molcel.2008.09.010

Peterson, T.R., S.S. Sengupta, T.E. Harris, A.E. Carmack, S.A. Kang, E. Balderas, D.A. Guertin, K.L. Madden, A.E. Carpenter, B.N. Finck, and D.M. Sabatini. 2011. mTOR complex 1 regulates lipin 1 localization to control the SREBP pathway. *Cell*. 146:408–420. http://dx.doi.org/10.1016/j.cell.2011.06.034

Petit, C.S., A. Roczniak-Ferguson, and S.M. Ferguson. 2013. Recruitment of folliculin to lysosomes supports the amino acid-dependent activation of Rag GTPases. *J. Cell Biol.* 202:1107–1122. http://dx.doi.org/10.1083/jcb.201307084

Plank, T.L., R.S. Yeung, and E.P. Henske. 1998. Hamartin, the product of the tuberous sclerosis 1 (TSC1) gene, interacts with tuberin and appears to be localized to cytoplasmic vesicles. *Cancer Res.* 58:4766–4770.

Poston, C.N., E. Duong, Y. Cao, and C.R. Bazemore-Walker. 2011. Proteomic analysis of lipid raft-enriched membranes isolated from internal organelles. *Biochem. Biophys. Res. Commun.* 415:355–360. http://dx.doi.org/10.1016/j.bbrc.2011.10.072

Poston, C.N., S.C. Krishnan, and C.R. Bazemore-Walker. 2013. In-depth proteomic analysis of mammalian mitochondria-associated membranes (MAM). *J. Proteomics*. 79:219–230. http://dx.doi.org/10.1016/j.jprot.2012.12.018

Proud, C.G. 2009. mTORC1 signalling and mRNA translation. *Biochem. Soc. Trans.* 37:227–231. http://dx.doi.org/10.1042/BST0370227

Ramanathan, A., and S.L. Schreiber. 2009. Direct control of mitochondrial function by mTOR. *Proc. Natl. Acad. Sci. USA*. 106:22229–22232. http://dx.doi.org/10.1073/pnas.0912074106

Ramírez-Rangel, I., I. Bracho-Valdés, A. Vázquez-Macías, J. Carretero-Ortega, G. Reyes-Cruz, and J. Vázquez-Prado. 2011. Regulation of mTORC1 complex assembly and signaling by GRp58/ERp57. *Mol. Cell. Biol.* 31:1657–1671. http://dx.doi.org/10.1128/MCB.00824-10

Raturi, A., and T. Simmen. 2012. Where the endoplasmic reticulum and the mitochondrion tie the knot: The mitochondria-associated membrane (MAM). *Biochim. Biophys. Acta*.

Reinke, A., S. Anderson, J.M. McCaffery, J. Yates III, S. Aronova, S. Chu, S. Fairclough, C. Iverson, K.P. Wedaman, and T. Powers. 2004. TOR complex 1 includes a novel component, Tco89p (YPL180w), and cooperates with Ssd1p to maintain cellular integrity in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 279:14752–14762. http://dx.doi.org/10.1074/jbc.M313062200

Ren, J., L. Wen, X. Gao, C. Jin, Y. Xue, and X. Yao. 2008. CSS-Palm 2.0: an updated software for palmitoylation sites prediction. *Protein Eng. Des. Sel.* 21:639–644. http://dx.doi.org/10.1093/protein/gzn039

Ren, M., S. Qiu, P. Venglat, D. Xiang, L. Feng, G. Selvaraj, and R. Datla. 2011. Target of rapamycin regulates development and ribosomal RNA expression through kinase domain in Arabidopsis. *Plant Physiol.* 155:1367–1382. http://dx.doi.org/10.1104/pp.110.169045

Rizzuto, R., P. Pinton, W. Carrington, F.S. Fay, K.E. Fogarty, L.M. Lifshitz, R.A. Tuft, and T. Pozzan. 1998. Close contacts with the endoplasmic reticulum as determinants of mitochondrial Ca2+ responses. *Science*. 280:1763–1766. http://dx.doi.org/10.1126/science.280.5370.1763

Robitaille, A.M., S. Christen, M. Shimobayashi, M. Cornu, L.L. Fava, S. Moes, C. Prescianotto-Baschong, U. Sauer, P. Jenoe, and M.N. Hall. 2013. Quantitative phosphoproteomics reveal mTORC1 activates de novo pyrimidine synthesis. *Science*. 339:1320–1323. http://dx.doi.org/10.1126/science.1228771

Roccio, M., J.L. Bos, and F.J.T. Zwartkruis. 2006. Regulation of the small GTPase Rheb by amino acids. *Oncogene*. 25:657–664. http://dx.doi.org/10.1038/sj.onc.1209106

Rosner, M., and M. Hengstschläger. 2007. Cytoplasmic/nuclear localization of tuberin in different cell lines. *Amino Acids*. 33:575–579. http://dx.doi.org/10.1007/s00726-007-0541-0

Rosner, M., and M. Hengstschläger. 2008. Cytoplasmic and nuclear distribution of the protein complexes mTORC1 and mTORC2: rapamycin triggers dephosphorylation and delocalization of the mTORC2 components rictor and sin1. *Hum. Mol. Genet.* 17:2934–2948. http://dx.doi.org/10.1093/hmg/ddn192

Rosner, M., and M. Hengstschläger. 2011. mTOR protein localization is cell cycle-regulated. *Cell Cycle*. 10:3608–3610. http://dx.doi.org/10.4161/cc.10.20.17855

Rosner, M., and M. Hengstschläger. 2012. Detection of cytoplasmic and nuclear functions of mTOR by fractionation. *Methods Mol. Biol.* 821:105–124. http://dx.doi.org/10.1007/978-1-61779-430-8_8

Rosner, M., A. Freilinger, and M. Hengstschläger. 2007. Akt regulates nuclear/cytoplasmic localization of tuberin. *Oncogene*. 26:521–531. http://dx.doi.org/10.1038/sj.onc.1209812

Ruby, J.R., R.F. Dyer, and R.G. Skalko. 1969. Continuities between mitochondria and endoplasmic reticulum in the mammalian ovary. *Cell Tissue Res.* 97:30–37.

Saito, K., Y. Araki, K. Kontani, H. Nishina, and T. Katada. 2005. Novel role of the small GTPase Rheb: its implication in endocytic pathway independent of the activation of mammalian target of rapamycin. *J. Biochem.* 137:423–430. http://dx.doi.org/10.1093/jb/mvi046

Sancak, Y., T.R. Peterson, Y.D. Shaul, R.A. Lindquist, C.C. Thoreen, L. Bar-Peled, and D.M. Sabatini. 2008. The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1. *Science*. 320:1496–1501. http://dx.doi.org/10.1126/science.1157535

Sancak, Y., L. Bar-Peled, R. Zoncu, A.L. Markhard, S. Nada, and D.M. Sabatini. 2010. Ragulator-Rag complex targets mTORC1 to the lysosomal surface and is necessary for its activation by amino acids. *Cell*. 141:290–303. http://dx.doi.org/10.1016/j.cell.2010.02.024

Sarbassov, D.D., S.M. Ali, S. Sengupta, J.-H. Sheen, P.P. Hsu, A.F. Bagley, A.L. Markhard, and D.M. Sabatini. 2006. Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. *Mol. Cell*. 22:159–168. http://dx.doi.org/10.1016/j.molcel.2006.03.029

Schieke, S.M., D. Phillips, J.P. McCoy Jr., A.M. Aponte, R.-F. Shen, R.S. Balaban, and T. Finkel. 2006. The mammalian target of rapamycin (mTOR) pathway regulates mitochondrial oxygen consumption and oxidative capacity. *J. Biol. Chem.* 281:27643–27652. http://dx.doi.org/10.1074/jbc.M603536200

Sebastián, D., M.I. Hernández-Alvarez, J. Segalés, E. Sorianello, J.P. Muñoz, D. Sala, A. Waget, M. Liesa, J.C. Paz, P. Gopalacharyulu, et al. 2012. Mitofusin 2 (Mfn2) links mitochondrial and endoplasmic reticulum function with insulin signaling and is essential for normal glucose homeostasis. *Proc. Natl. Acad. Sci. USA*. 109:5523–5528. http://dx.doi.org/10.1073/pnas.1108220109

Shor, B., J. Wu, Q. Shakey, L. Toral-Barza, C. Shi, M. Follettie, and K. Yu. 2010. Requirement of the mTOR kinase for the regulation of Maf1 phosphorylation and control of RNA polymerase III-dependent transcription in cancer cells. *J. Biol. Chem.* 285:15380–15392. http://dx.doi.org/10.1074/jbc.M109.071639

Simmen, T., J.E. Aslan, A.D. Blagoveshchenskaya, L. Thomas, L. Wan, Y. Xiang, S.F. Feliciangeli, C.-H. Hung, C.M. Crump, and G. Thomas. 2005. PACS-2 controls endoplasmic reticulum-mitochondria communication and Bid-mediated apoptosis. *EMBO J.* 24:717–729. http://dx.doi.org/10.1038/sj.emboj.7600559

Simmen, T., E.M. Lynes, K. Gesson, and G. Thomas. 2010. Oxidative protein folding in the endoplasmic reticulum: tight links to the mitochondria-associated membrane (MAM). *Biochim. Biophys. Acta*. 1798:1465–1473. http://dx.doi.org/10.1016/j.bbamem.2010.04.009

Smith, E.M., S.G. Finn, A.R. Tee, G.J. Browne, and C.G. Proud. 2005. The tuberous sclerosis protein TSC2 is not required for the regulation of the

mammalian target of rapamycin by amino acids and certain cellular stresses. *J. Biol. Chem.* 280:18717–18727. http://dx.doi.org/10.1074/jbc.M414499200

Sonenberg, N., and A.G. Hinnebusch. 2009. Regulation of translation initiation in eukaryotes: mechanisms and biological targets. *Cell*. 136:731–745. http://dx.doi.org/10.1016/j.cell.2009.01.042

Sturgill, T.W., A. Cohen, M. Diefenbacher, M. Trautwein, D.E. Martin, and M.N. Hall. 2008. TOR1 and TOR2 have distinct locations in live cells. *Eukaryot. Cell*. 7:1819–1830. http://dx.doi.org/10.1128/EC.00088-08

Su, C.-C., J.-Y. Yang, H.-B. Leu, Y. Chen, and P.H. Wang. 2012. Mitochondrial Akt-regulated mitochondrial apoptosis signaling in cardiac muscle cells. *Am. J. Physiol. Heart Circ. Physiol.* 302:H716–H723. http://dx.doi.org/10.1152/ajpheart.00455.2011

Szabadkai, G., K. Bianchi, P. Várnai, D. De Stefani, M.R. Wieckowski, D. Cavagna, A.I. Nagy, T. Balla, and R. Rizzuto. 2006. Chaperone-mediated coupling of endoplasmic reticulum and mitochondrial Ca2+ channels. *J. Cell Biol.* 175:901–911. http://dx.doi.org/10.1083/jcb.200608073

Szado, T., V. Vanderheyden, J.B. Parys, H. De Smedt, K. Rietdorf, L. Kotelevets, E. Chastre, F. Khan, U. Landegren, O. Söderberg, et al. 2008. Phosphorylation of inositol 1,4,5-trisphosphate receptors by protein kinase B/Akt inhibits Ca2+ release and apoptosis. *Proc. Natl. Acad. Sci. USA*. 105:2427–2432. http://dx.doi.org/10.1073/pnas.0711324105

Tabatabaian, F., K. Dougherty, M. Di Fulvio, and J. Gomez-Cambronero. 2010. Mammalian target of rapamycin (mTOR) and S6 kinase down-regulate phospholipase D2 basal expression and function. *J. Biol. Chem.* 285:18991–19001. http://dx.doi.org/10.1074/jbc.M110.111542

Takahara, T., and T. Maeda. 2012. Transient sequestration of TORC1 into stress granules during heat stress. *Mol. Cell*. 47:242–252. http://dx.doi.org/10.1016/j.molcel.2012.05.019

Takai, H., R.C. Wang, K.K. Takai, H. Yang, and T. de Lange. 2007. Tel2 regulates the stability of PI3K-related protein kinases. *Cell*. 131:1248–1259. http://dx.doi.org/10.1016/j.cell.2007.10.052

Teleman, A.A., Y.-W. Chen, and S.M. Cohen. 2005. *Drosophila* Melted modulates FOXO and TOR activity. *Dev. Cell*. 9:271–281. http://dx.doi.org/10.1016/j.devcel.2005.07.004

Thedieck, K., B. Holzwarth, M.T. Prentzell, C. Boehlke, K. Kläsener, S. Ruf, A.G. Sonntag, L. Maerz, S.N. Grellscheid, E. Kremmer, et al. 2013. Inhibition of mTORC1 by astrin and stress granules prevents apoptosis in cancer cells. *Cell*. 154:859–874. http://dx.doi.org/10.1016/j.cell.2013.07.031

Tsang, C.K., H. Liu, and X.F.S. Zheng. 2010. mTOR binds to the promoters of RNA polymerase I- and III-transcribed genes. *Cell Cycle*. 9:953–957. http://dx.doi.org/10.4161/cc.9.5.10876

Tsun, Z.-Y., L. Bar-Peled, L. Chantranupong, R. Zoncu, T. Wang, C. Kim, E. Spooner, and D.M. Sabatini. 2013. The folliculin tumor suppressor is a GAP for the RagC/D GTPases that signal amino acid levels to mTORC1. *Mol. Cell*.

Urban, J., A. Soulard, A. Huber, S. Lippman, D. Mukhopadhyay, O. Deloche, V. Wanke, D. Anrather, G. Ammerer, H. Riezman, et al. 2007. Sch9 is a major target of TORC1 in *Saccharomyces cerevisiae*. *Mol. Cell*. 26:663–674. http://dx.doi.org/10.1016/j.molcel.2007.04.020

van Slegtenhorst, M., M. Nellist, B. Nagelkerken, J. Cheadle, R. Snell, A. van den Ouweland, A. Reuser, J. Sampson, D. Halley, and P. van der Sluijs. 1998. Interaction between hamartin and tuberin, the TSC1 and TSC2 gene products. *Hum. Mol. Genet.* 7:1053–1057. http://dx.doi.org/10.1093/hmg/7.6.1053

Verfaillie, T., N. Rubio, A.D. Garg, G. Bultynck, R. Rizzuto, J.-P. Decuypere, J. Piette, C. Linehan, S. Gupta, A. Samali, and P. Agostinis. 2012. PERK is required at the ER-mitochondrial contact sites to convey apoptosis after ROS-based ER stress. *Cell Death Differ.* 19:1880–1891. http://dx.doi.org/10.1038/cdd.2012.74

Vézina, C., A. Kudelski, and S.N. Sehgal. 1975. Rapamycin (AY-22,989), a new antifungal antibiotic. I. Taxonomy of the producing streptomycete and isolation of the active principle. *J. Antibiot.* 28:721–726. http://dx.doi.org/10.7164/antibiotics.28.721

Wang, Y., L.M. Weiss, and A. Orlofsky. 2010. Coordinate control of host centrosome position, organelle distribution, and migratory response by Toxoplasma gondii via host mTORC2. *J. Biol. Chem.* 285:15611–15618. http://dx.doi.org/10.1074/jbc.M109.095778

Wedaman, K.P., A. Reinke, S. Anderson, J. Yates III, J.M. McCaffery, and T. Powers. 2003. Tor kinases are in distinct membrane-associated protein complexes in *Saccharomyces cerevisiae*. *Mol. Biol. Cell*. 14:1204–1220. http://dx.doi.org/10.1091/mbc.E02-09-0609

Wippich, F., B. Bodenmiller, M.G. Trajkovska, S. Wanka, R. Aebersold, and L. Pelkmans. 2013. Dual specificity kinase DYRK3 couples stress granule condensation/dissolution to mTORC1 signaling. *Cell*. 152:791–805. http://dx.doi.org/10.1016/j.cell.2013.01.033

Wu, Z., T. Sawada, K. Shiba, S. Liu, T. Kanao, R. Takahashi, N. Hattori, Y. Imai, and B. Lu. 2013. Tricornered/NDR kinase signaling mediates PINK1-directed mitochondrial quality control and tissue maintenance. *Genes Dev.* 27:157–162. http://dx.doi.org/10.1101/gad.203406.112

Wullschleger, S., R. Loewith, and M.N. Hall. 2006. TOR signaling in growth and metabolism. *Cell*. 124:471–484. http://dx.doi.org/10.1016/j.cell.2006.01.016

Yadav, R.B., P. Burgos, A.W. Parker, V. Iadevaia, C.G. Proud, R.A. Allen, J.P. O'Connell, A. Jeshtadi, C.D. Stubbs, and S.W. Botchway. 2013. mTOR direct interactions with Rheb-GTPase and raptor: sub-cellular localization using fluorescence lifetime imaging. *BMC Cell Biol.* 14:3. http://dx.doi.org/10.1186/1471-2121-14-3

Yang, T.T.C., R.Y.L. Yu, A. Agadir, G.-J. Gao, R. Campos-Gonzalez, C. Tournier, and C.-W. Chow. 2008. Integration of protein kinases mTOR and extracellular signal-regulated kinase 5 in regulating nucleocytoplasmic localization of NFATc4. *Mol. Cell. Biol.* 28:3489–3501. http://dx.doi.org/10.1128/MCB.01847-07

Yu, L., C.K. McPhee, L. Zheng, G.A. Mardones, Y. Rong, J. Peng, N. Mi, Y. Zhao, Z. Liu, F. Wan, et al. 2010. Termination of autophagy and reformation of lysosomes regulated by mTOR. *Nature*. 465:942–946. http://dx.doi.org/10.1038/nature09076

Yuan, M., E. Pino, L. Wu, M. Kacergis, and A.A. Soukas. 2012. Identification of Akt-independent regulation of hepatic lipogenesis by mammalian target of rapamycin (mTOR) complex 2. *J. Biol. Chem.* 287:29579–29588. http://dx.doi.org/10.1074/jbc.M112.386854

Yung, H.W., D.S. Charnock-Jones, and G.J. Burton. 2011. Regulation of AKT phosphorylation at Ser473 and Thr308 by endoplasmic reticulum stress modulates substrate specificity in a severity dependent manner. *PLoS ONE*. 6:e17894. http://dx.doi.org/10.1371/journal.pone.0017894

Zhang, X., L. Shu, H. Hosoi, K.G. Murti, and P.J. Houghton. 2002. Predominant nuclear localization of mammalian target of rapamycin in normal and malignant cells in culture. *J. Biol. Chem.* 277:28127–28134. http://dx.doi.org/10.1074/jbc.M202625200

Zhang, F., X. Zhang, M. Li, P. Chen, B. Zhang, H. Guo, W. Cao, X. Wei, X. Cao, X. Hao, and N. Zhang. 2010. mTOR complex component Rictor interacts with PKCzeta and regulates cancer cell metastasis. *Cancer Res.* 70:9360–9370. http://dx.doi.org/10.1158/0008-5472.CAN-10-0207

Zhang, A., C.D. Williamson, D.S. Wong, M.D. Bullough, K.J. Brown, Y. Hathout, and A.M. Colberg-Poley. 2011. Quantitative proteomic analyses of human cytomegalovirus-induced restructuring of endoplasmic reticulum-mitochondrial contacts at late times of infection. *Mol. Cell Proteomics.* 10:M111.009936.

Zhang, J., J. Kim, A. Alexander, S. Cai, D.N. Tripathi, R. Dere, A.R. Tee, J. Tait-Mulder, A. Di Nardo, J.M. Han, et al. 2013. A tuberous sclerosis complex signalling node at the peroxisome regulates mTORC1 and autophagy in response to ROS. *Nat. Cell Biol.* 15:1186–1196. http://dx.doi.org/10.1038/ncb2822

Zhu, Y., P. Hoell, B. Ahlemeyer, and J. Krieglstein. 2006. PTEN: a crucial mediator of mitochondria-dependent apoptosis. *Apoptosis*. 11:197–207. http://dx.doi.org/10.1007/s10495-006-3714-5

Zinzalla, V., and M.N. Hall. 2008. Signal transduction: Linking nutrients to growth. *Nature*. 454:287–288. http://dx.doi.org/10.1038/454287a

Zoncu, R., L. Bar-Peled, A. Efeyan, S. Wang, Y. Sancak, and D.M. Sabatini. 2011. mTORC1 senses lysosomal amino acids through an inside-out mechanism that requires the vacuolar H(+)-ATPase. *Science*. 334:678–683. http://dx.doi.org/10.1126/science.1207056

Zu, L., X. Zheng, B. Wang, N. Parajuli, C. Steenbergen, L.C. Becker, and Z.P. Cai. 2011. Ischemic preconditioning attenuates mitochondrial localization of PTEN induced by ischemia-reperfusion. *Am. J. Physiol. Heart Circ. Physiol.* 300:H2177–H2186. http://dx.doi.org/10.1152/ajpheart.01138.2010