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TOR Signaling in Growth and Metabolism

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The target of rapamycin (TOR) is a conserved Ser/Thr kinase that regulates cell growth and metabolism in response to environmental cues. Here, highlighting contributions from studies in model organisms, we review mammalian TOR complexes and the signaling branches they mediate. TOR is part of two distinct multiprotein complexes, TOR complex 1 (TORC1), which is sensitive to rapamycin, and TORC2, which is not. The physiological consequences of mammalian TORC1 dysregulation suggest that inhibitors of mammalian TOR may be useful in the treatment of cancer, cardiovascular disease, autoimmunity, and metabolic disorders.

Introduction

In the 1970s, a soil sample from Easter Island (known as Rapa Nui in the local language) was found to contain a bacterial strain, *Streptomyces hygroscopicus*, that produced a potent antifungal metabolite. This metabolite was purified and found to be a macrocyclic lactone, which was named rapamycin after its place of discovery. Later, rapamycin was found to inhibit proliferation of mammalian cells and to possess immunosuppressive properties. These intriguing observations prompted further investigation into the mode of action of rapamycin.

The target of rapamycin (TOR) was originally identified by mutations, *TOR1-1* and *TOR2-1*, that confer resistance to the growth inhibitory properties of rapamycin, in the budding yeast *Saccharomyces cerevisiae* (Heitman et al., 1991). This study also demonstrated that rapamycin requires an intracellular cofactor, the peptidyl-prolyl *cis/trans* isomerase FKBP12, for toxicity. Rapamycin forms a complex with FKBP12, and this complex then binds to and inhibits TOR. Subsequent biochemical studies in mammalian cells led to the identification and cloning of the mammalian target of rapamycin, mTOR (also known as FRAP, RAFT, RAPT, or SEP; reviewed in Fingar and Blenis, 2004; Hay and Sonenberg, 2004). To date, every eukaryote genome examined (including yeasts, algae, slime mold, plants, worms, flies, and mammals) contains a *TOR* gene. Unlike yeast, which in some cases possess two *TOR* genes, higher eukaryotes possess only a single *TOR* gene (Crespo and Hall, 2002; Lee et al., 2005; Crespo et al., 2005).

Eukaryote TORs are large proteins (~ 280 kDa) that share 40%–60% identity in their primary sequence and belong to a group of kinases known as the phosphatidylinositol kinase-related kinase (PIKK) family. PIKK family members contain a carboxy-terminal serine/threonine protein kinase domain that resembles the catalytic domain of phosphatidylinositol 3-kinases (PI3Ks) and PI4Ks. Amino-terminal to the kinase domain in TOR is the FKBP12-rapamycin binding domain (FRB; Figure 1). Single amino acid substitutions in

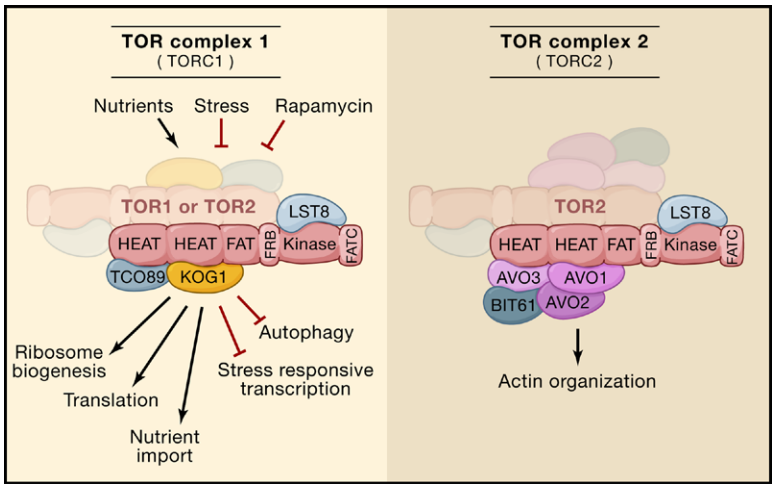

Figure 1. TOR Complex 1 (TORC1) and TOR Complex 2 (TORC2) of *S. cerevisiae*
Depicted are TOR-associated proteins (KOG1, TCO89, LST8, AVO1–3, and BIT61) and the domains found in TOR (HEAT, FAT, FRB, Kinase, and FATC). Both TORC1 and TORC2 are multimers, likely dimers. TORC1 mediates the rapamycin-sensitive signaling branch that couples growth cues to the accumulation of mass. Stimuli that positively regulate TORC1 and TORC1 outputs that promote the accumulation of mass are depicted with black arrows. Inputs that negatively regulate TORC1 and the stress- and starvation-induced processes that TORC1 regulates negatively are depicted with red bars. TORC2 signaling is rapamycin insensitive and is required for the organization of the actin cytoskeleton. Upstream regulators of TORC2 are not known.

this domain yield TOR proteins (such as TOR1-1 and TOR2-1) that are no longer bound and inhibited by the FKBP12-rapamycin complex. Located amino-terminal to the FRB domain and carboxy-terminal to the kinase domain are the FAT (FRAP, ATM, and TTRAP) and FATC domains, respectively. These domains are found in all PIKKs and always together, suggesting that they may interact. The amino-terminal half of TOR contains tandem HEAT repeats that may form an extended superhelical array with large interfaces for protein-protein interactions.

Physiological Roles for TOR

TOR Is a Central Controller of Cell Growth

Cell growth (accumulation of mass) is an extensively coordinated process that is regulated in both time and space. When nutrients and other appropriate growth stimuli are present, cells upregulate macromolecular synthesis and thereby increase in size and mass. Conversely, cells respond to nutrient limitation or other types of stress by restraining macromolecular synthesis and enhancing turnover of excess mass. Cell growth can also be subject to spatial constraints, as evidenced by the fact that many cells do not grow isotropically but rather deposit new mass at discrete loci. Studies in yeast demonstrated that TOR performs two essential functions in this organism; one regulates when a cell grows and the second regulates where a cell grows (reviewed in Loewith and Hall, 2004).

When growth conditions are favorable, TOR is active and yeast cells maintain a robust rate of ribosome biogenesis, translation initiation, and nutrient import. However, rapidly growing yeast cells treated with rapamycin, starved for nitrogen, or depleted of both TOR1 and TOR2 dramatically downregulate general protein synthesis, upregulate macroautophagy (the random sequestration and delivery of cytoplasm to the lysosome/vacuole), and activate several stress-responsive transcription factors. Thus, when growth conditions permit, rapamycin-sensitive TOR signaling promotes anabolic processes and antagonizes catabolic processes. Many of these rapamycin-sensitive readouts of TOR are conserved in mammals.

TOR2, but not TOR1, functions further to regulate spatial aspects of yeast cell growth (Loewith and Hall, 2004). In budding yeast, the majority of growth occurs in the bud (the daughter cell). To accomplish this, yeast cells polarize their actin cables and patches toward the bud. This polarization of the actin cytoskeleton facilitates trafficking of macromolecules from the mother cell to the bud for deposition. TOR2 activity is important for this cell cycle-dependent polarization of the actin cytoskeleton. Curiously, the ability of TOR2 to regulate these spatial aspects of yeast cell growth is insensitive to rapamycin. The rapamycin-insensitive ability of TOR to regulate organization of the actin cytoskeleton is also conserved in mammals.

Metazoan TOR Is Essential for Growth during Early Development

Deletion of *CeTOR* in the nematode worm *Caenorhabditis elegans* or of *dTOR* in the fruit fly *Drosophila melanogaster* causes developmental arrests that share some features with the arrests observed in starved larvae (Long et al., 2002; Oldham et al., 2000; Zhang et al., 2000). Recent studies have shown that dTOR affects the timing of neuronal cell differentiation. Hyperactivation of dTOR signaling leads to accelerated differentiation, whereas inhibition of dTOR signaling retards differentiation (Bateman and McNeill, 2004). Thus, similar to yeast TOR, dTOR also regulates temporal aspects of cell growth. Interestingly, ablation of dTOR in the fat body (the fly equivalent of liver and adipose tissue) results in a systemic defect in larval growth (Colombani et al., 2003). Thus, in addition to controlling growth of the cell in which it resides, dTOR can also influence growth of distant cells and organs during development via a humoral mechanism.

TOR is also required for development in mice. Homozygous *mTOR*$^{-/-}$ embryos resemble embryos starved of amino acids ex vivo and die shortly after implantation due to impaired cell proliferation in both embryonic and extraembryonic compartments (Gangloff et al., 2004; Martin and Sutherland, 2001; Murakami et al., 2004). Exposure of early mouse embryos to rapamycin also arrests cell proliferation (Martin and Sutherland, 2001), indicating that rapamycin-sensitive mTOR function is essential during this stage of development. These results suggest that metazoan TOR coordinates growth and development in response to nutritional cues.

TOR Influences Memory and Aging in Adults

Rapamycin-sensitive mTOR function appears to be less important in adults because systemic administration of rapamycin to adult humans results in relatively mild side effects. However, rapamycin treatment has been found to antagonize long-term consolidation of a cerebral cortex-dependent form of memory in gerbils (Tischmeyer et al., 2003) and synapse-specific long-term facilitation in *Aplysia californica* (Casadio et al., 1999). Also, partial inhibition of TOR function in yeast, worms, and flies results in a significant increase in the life span of these organisms, possibly by mimicking calorie restriction (reviewed in Martin and Hall, 2005, and Kaeberlein et al., 2005). Although links between mTOR signaling and mammalian longevity have not yet been reported, it appears that TOR signaling plays different roles in development and adulthood in metazoans. During development, TOR primarily controls growth, whereas in the adult, where there is relatively little growth, TOR controls aging and other aspects of nutrient-related physiology.

Two TOR Complexes

TORC1 Is Sensitive to Rapamycin and Regulates Temporal Aspects of Cell Growth

As described above, genetic studies in yeast demonstrated that rapamycin-sensitive TOR signaling regulates when a cell grows, and rapamycin-insensitive TOR signaling regulates where a cell grows. Biochemical purification of TOR1 and TOR2 from yeast led to the identification of two distinct TOR protein complexes, TORC1 and TORC2, which account for the differential sensitivity of TOR signaling to rapamycin (Loewith et al., 2002). TORC1 (Figure 1; Table 1) contains KOG1, TCO89, LST8, and either TOR1 or

Table 1. Components of TORC1 and TORC2 in Various Eukaryotes

S. cerevisiae	*S. pombe*	*Dictyostelium*	*A. thaliana*	*C. elegans*	*Drosophila*	Mammals
TORC1						
TOR1 or TOR2	**Tor1p or Tor2p**	Dd-TOR	AtTOR	CeTOR	dTOR	**mTOR**
KOG1	**Mip1p**	JC1V2_0_00963[a]	AtRaptor1A AtRaptor1B	Daf-15	dRaptor	**raptor**
LST8	Wat1p/Pop3p	Dd-LST8	At3g18140[d] At2g22040[d]	C10H11.8[b]	CG3004[c]	**mLST8**
TCO89	—	—	—	—	—	—
TORC2						
TOR2	Tor1p or Tor2p?	**Dd-TOR**	AtTOR	CeTOR	dTOR	**mTOR**
AVO1	Sin1p?	**Rip3**	—	—	—	hSIN1?
AVO2	—	—	—	—	—	—
AVO3	Ste20p	**Pia**	—	—	dRictor	**rictor**
LST8	Wat1p/Pop3p	**Dd-LST8**	At3g18140[d] At2g22040[d]	C10H11.8[b]	CG3004[c]	**mLST8**
BIT61	—	—	—	—	—	—

Listed are the protein components of *S. cerevisiae* TORC1 and TORC2 and known homologs in other species in which TOR has been at least partly characterized. Proteins indicated in bold have been shown to associate in a TORC1- or TORC2-like complex. The finding that Tor1p and Tor2p interact with Mip1p in *S. pombe* is unpublished (M. Yamamoto, personal communication). It is not known whether the Tor in *S. pombe* TORC2 is Tor1p or Tor2p. It is questionable whether Sin1p (*S. pombe*) and hSIN1 (mammals) are components of TORC2 as they are only weakly homologous to AVO1. —, no homolog found.
[a]dictyBase gene name.
[b]WormBase sequence name.
[c]FlyBase gene name.
[d]TIGR locus name.

TOR2 (Loewith et al., 2002; Reinke et al., 2004). FKBP12-rapamycin binds TORC1, and TORC1 disruption mimics rapamycin treatment, suggesting that TORC1 mediates the rapamycin-sensitive temporal control of cell growth (Loewith et al., 2002).

Like TOR, KOG1 and LST8 (but not TCO89) have obvious mammalian sequence homologs, raptor and mLST8 (also known as GβL), respectively. mTOR, raptor, and mLST8 associate in a complex, mTORC1 (Figure 2; Table 1; Hara et al., 2002; Kim et al., 2002, 2003; Loewith et al., 2002). As discussed below, mTORC1 also appears to regulate temporal aspects of cell growth. mTORC1 is bound by FKBP12-rapamycin, and mTORC1 kinase activity is abrogated by FKBP12-rapamycin both in vivo and in vitro (Hara et al., 2002; Jacinto et al., 2004; Kim et al., 2002; Sarbassov et al., 2004).

The individual functions of mTORC1 components are poorly understood. Raptor is a large protein (150 kDa) containing a highly conserved, amino-terminal domain followed by several HEAT repeats and seven carboxy-terminal WD40 repeats (Hara et al., 2002; Kim et al., 2002). The domains in raptor and mTOR that interact have been difficult to map, suggesting multiple contact sites between these two proteins. Knockdown of the mammalian, worm, and fly versions of KOG1/raptor phenocopies rapamycin treatment and/or depletion of TOR in these organisms, indicating that raptor functions positively in mTOR signaling (Hara et al., 2002; Kim et al., 2002; Sarbassov et al., 2004). A number of groups have proposed that raptor acts as an adaptor to recruit substrates to mTOR (Choi et al., 2003; Hara et al., 2002; Nojima et al., 2003; Schalm et al., 2003), whereas Kim et al. (2002) have suggested that upstream signals regulate the raptor-mTOR interaction and thereby the activity of mTORC1. The mechanism by which FKBP12-rapamycin binding inhibits mTORC1 is not known and may indeed involve more than one mechanism. Under some experimental conditions, FKBP12-rapamycin dissociates raptor-mTOR (Kim et al., 2002), suggesting that FKBP12-rapamycin blocks access to substrates. This may be a common mechanism of mTORC1 inhibition because farnesylthiosalicyclic acid also dissociates raptor-mTOR and inhibits mTORC1 activity in vivo (McMahon et al., 2005). Jacinto et al. (2004) have observed that FKBP12-rapamycin inhibits mTORC1 autophosphorylation, suggesting that FKBP12-rapamycin inhibits intrinsic mTORC1 kinase activity rather than, or in addition to, access to an extrinsic substrate.

mLST8 is a 36 kDa protein that has seven WD40 repeats. mLST8 binds to the kinase domain of mTOR, and knockdown studies in mammals and yeast suggest that mLST8 also functions positively in mTORC1 signaling (Chen and Kaiser, 2003; Kim et al., 2003; Loewith et al., 2002). However, the precise role of mLST8 is not known. Kim et al. (2003) have suggested that mLST8 functions to receive upstream signals that impinge on mTORC1, whereas Chen and Kaiser (2003) have proposed that LST8 in yeast regulates signal outputs from TORC1. LST8 is necessary for full catalytic activity of TOR (Wullschleger et al., 2005), and overexpression of mLST8 stimulates mTOR kinase activity (Kim et al., 2003).

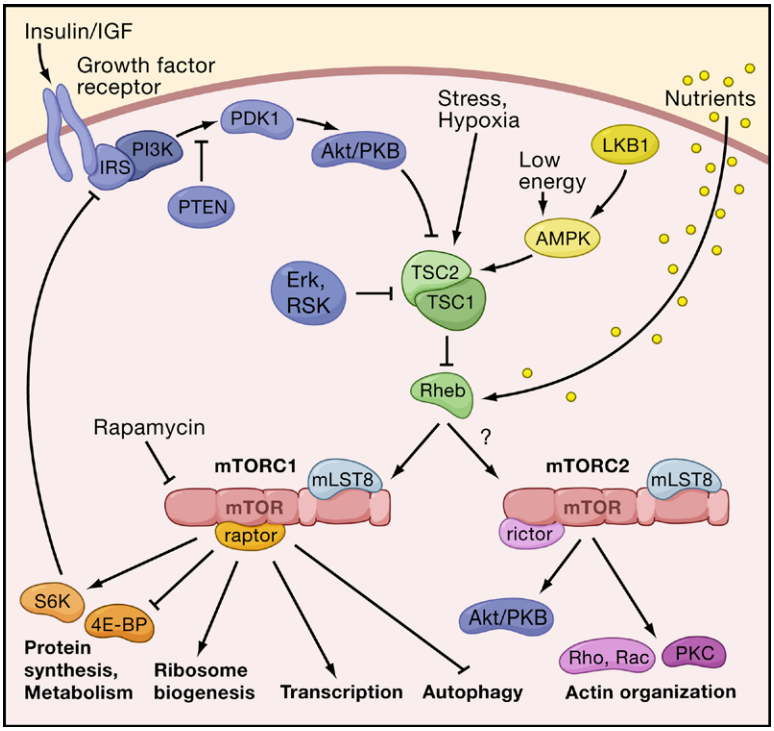

Figure 2. Model of the mTOR Signaling Network in Mammalian Cells

The mTOR signaling network consists of two major branches, each mediated by a specific mTOR complex (mTORC). Rapamycin-sensitive mTORC1 controls several pathways that collectively determine the mass (size) of the cell. Rapamycin-insensitive mTORC2 controls the actin cytoskeleton and thereby determines the shape of the cell. mTORC1 and possibly mTORC2 respond to growth factors (insulin/IGF), energy status of the cell, nutrients (amino acids), and stress. mTORC1 (and likely mTORC2) are multimeric, although are drawn as monomers. Arrows represent activation, whereas bars represent inhibition.

TORC2 Is Insensitive to Rapamycin and Regulates Spatial Aspects of Cell Growth

Yeast TORC2 contains AVO1, AVO2, AVO3, BIT61, LST8, and TOR2, but not TOR1 (Figure 1; Table 1; Loewith et al., 2002; Reinke et al., 2004). FKBP12-rapamycin does not bind to TORC2, and TORC2 disruption mimics TOR2 depletion, suggesting that TORC2 mediates the rapamycin-insensitive spatial control of cell growth (Loewith et al., 2002).AVO1 and AVO3 bind cooperatively to the amino-terminal HEAT repeats in TOR2 and are required for TORC2 integrity (Figure 1). AVO2 is a nonessential peripheral protein associated with AVO1 and AVO3 and may serve to recruit substrates to TOR2 (Audhya et al., 2004; Wullschleger et al., 2005). AVO1 and AVO3 are phosphorylated on many sites in vivo and are autophosphorylated by TORC2 in vitro (Wullschleger et al., 2005). However, the significance of these phosphorylation events is not known. TORC2 kinase activity is not required for complex stability.

TORC2 is a multimeric supercomplex that is likely a TORC2-TORC2 dimer assembled via a TOR2-TOR2 interaction (Figure 1; Wullschleger et al., 2005). Multimerization may be a general property of TOR complexes. Yeast and mammalian TORC1 are also multimeric (Wullschleger et al., 2005; T. Maeda, personal communication), as is *Drosophila* TOR (Zhang et al., 2005). The elegant genetic study of Zhang et al. (2005) demonstrated that dTOR is multimeric, but did not determine whether the multimerization corresponded to dTORC1 or dTORC2. Multimerization may play a role in the regulation of TORC kinase activity because multimeric TORC2 appears to be a more active kinase than monomeric TORC2 (Wullschleger et al., 2005).

Like TORC1, TORC2 is conserved. *Dictyostelium discoideum* TORC2 contains Dd-TOR, Dd-LST8, RIP3, and Pianissimo, orthologs of the yeast TORC2 components TOR2, LST8, AVO1, and AVO3, respectively (Lee et al., 2005; Table 1). Mutations in *Dd-LST8*, *RIP3*, and *PIA* exhibit a common set of phenotypes including reduced cell polarity and a loss of chemotaxis speed and directionality, suggesting that regulation of the actin cytoskeleton and cell polarity is a conserved function of TORC2.

Mammalian TORC2 (mTORC2) contains mTOR, rictor, and mLST8, but not raptor (Table 1; Figure 2; Jacinto et al., 2004; Sarbassov et al., 2004). Searches of mammalian genome databases indicated that there is a mammalian protein, hSIN, which has limited homology with AVO1. hSIN1 shares a similar tissue expression pattern with mTOR and mLST8, but efforts to demonstrate a stable interaction between mTOR and hSIN1 have been unsuccessful (Loewith et al., 2002). Thus, a role for hSIN1 in mTOR signaling remains uncertain. AVO2 and BIT61 appear to be unique to yeast.

Rictor (also known as mAVO3) is a large protein (~ 200 kDa) but contains no obvious catalytic motifs. Knockdown of mTOR or rictor (but not raptor) results in loss of both actin polymerization and cell spreading (Jacinto et al., 2004; Sarbassov et al., 2004), which is consistent with results from studies in yeast and *Dictyostelium*. Also consistent with studies in model organisms is the observation that mTORC2 is neither bound by FKBP12-rapamycin nor does FKBP12-rapamycin affect mTORC2 in vitro kinase activity (Jacinto et al., 2004; Sarbassov et al., 2004).

Upstream Regulators of the TOR Signaling Network

TOR integrates various signals to regulate cell growth. Four major inputs have been implicated in TOR signaling: growth factors, nutrients, energy, and stress.

Growth Factors

The mTOR pathway responds to growth factors via the PI3K pathway (Figure 2). Binding of insulin or insulin-like growth factors (IGFs) to their receptors leads to recruit-

ment and phosphorylation of the insulin receptor substrate (IRS), and subsequent recruitment of PI3K. PI3K bound to IRS converts phosphatidylinositol-4,5-phosphate (PIP2) in the cell membrane to phosphatidylinositol-3,4,5-phosphate (PIP3). PIP3 accumulation is antagonized by the lipid phosphatase PTEN. PIP3 corecruits PDK1 and Akt to the membrane, resulting in the phosphorylation and activation of Akt by PDK1. mTOR is wired to the PI3K pathway through the tuberous sclerosis proteins TSC1 (hamartin) and TSC2 (tuberin). TSC1 and TSC2 act as a heterodimer that negatively regulates mTOR signaling. TSC2 is phosphorylated and functionally inactivated by Akt in response to insulin (reviewed in Manning, 2004). However, the significance of TSC2 phosphorylation by Akt may vary depending on physiological context. In *Drosophila*, for example, a TSC2 variant that cannot be phosphorylated by Akt rescues the lethality of a *TSC2* mutant (Dong and Pan, 2004), indicating that Akt phosphorylation of TSC2 is not essential at least during fly development. As discussed below, TSC2 is also phosphorylated and regulated by other kinases.

How does TSC1-TSC2 regulate mTORC1? TSC2 acts as a GAP (GTPase-activating protein) for the small GTPase Rheb (reviewed in Li et al., 2004). Rheb binds directly to the kinase domain in mTOR and activates mTOR in a GTP-dependent manner (Long et al., 2005a). However, Rheb binding to mTOR is independent of the guanyl nucleotide, and nucleotide-free Rheb inhibits mTOR activity (Long et al., 2005a; Smith et al., 2005). Long et al. (2005a) suggest that GTP loading of Rheb, rather than mediating mTORC1 recruitment, enables Rheb to induce a conformational change in mTORC1 leading to mTORC1 activation and phosphorylation of downstream targets. In *S. pombe*, Rheb binds to TOR in a GTP-dependent manner, suggesting that, in this case, GTP loading mediates effector recruitment (Urano et al., 2005). Although there is agreement that Rheb binds TOR, consensus is lacking on whether GTP loading of Rheb is required for mTOR binding or for a subsequent mTOR-activation step.

Nutrients

Nutrients, especially amino acids, regulate mTORC1 signaling. Amino acid starvation, in particular the absence of leucine, results in a rapid dephosphorylation of the mTORC1 effectors S6K1 and 4E-BP1, whereas readdition of amino acids restores S6K1 and 4E-BP1 phosphorylation in an mTORC1-dependent manner (Hay and Sonenberg, 2004). Amino acids have been proposed to activate mTORC1 via inhibition of TSC1-TSC2 or, alternatively, via stimulation of Rheb. Gao et al. (2002) have demonstrated that inactivation of TSC2 renders cells resistant to amino acid withdrawal, suggesting that the amino acids signal via TSC1-TSC2. Other studies have proposed a model in which amino acids signal to mTORC1 independently of TSC2. Amino acid withdrawal still downregulates mTORC1 signaling in TSC2-deficient cells (Smith et al., 2005), and overexpression of Rheb in *Drosophila* and mammalian cells allows TORC1 signaling in the absence of amino acids (Saucedo et al., 2003; Garami et al., 2003). Furthermore, the binding of Rheb to mTOR is regulated by amino acid sufficiency, whereas GTP charging of Rheb is independent of amino acids (Long et al., 2005b). These studies suggest that the amino acid signal impinges on Rheb. However, others failed to detect amino acid-regulated binding of Rheb to mTOR (Smith et al., 2005). Recent studies suggest that hVPS34, a class III PI3K (converts phosphatidylinositol to phosphatidylinositol-3-phosphate), signals amino acid availability to mTORC1 independently of the TSC1-TSC2/Rheb axis (Byfield et al., 2005; Nobukuni et al., 2005). TORC1 in *S. cerevisiae* responds to nutrients despite the absence of functional Rheb and TSC orthologs in this organism, and withdrawal of amino acids in mammalian cells alters the binding of raptor to mTOR (Kim et al., 2002). These latter observations may suggest that amino acids are sensed by mTORC1 directly. Clearly, the mechanism(s) by which nutrient status is communicated to mTORC1 requires further study.

Energy

Cell growth (the accumulation of cell mass) depends on a high rate of protein synthesis and consequently requires a high level of cellular energy. mTORC1 senses the energy status of a cell through AMP-activated protein kinase (AMPK). AMPK is activated in response to low cellular energy (high AMP/ATP ratio). Activated AMPK downregulates energetically demanding processes like protein synthesis and stimulates ATP-generating processes such as fatty acid oxidation. Activation of AMPK by AICAR, an AMP analog, inhibits mTORC1-dependent phosphorylation of S6K1 and 4E-BP1. Activated AMPK directly phosphorylates TSC2 and thereby enhances its GAP activity, leading to the inhibition of mTORC1 signaling (Inoki et al., 2003). The tumor suppressor LKB1 has been identified as an upstream kinase for AMPK, suggesting that LKB1 is linked to the TSC-mTORC1 signaling pathway. Indeed, *LKB1* mutant cells exhibit hyperactive mTORC1 signaling (Corradetti et al., 2004; Shaw et al., 2004). Thus, upon energy deprivation LKB1 in conjunction with AMP activates AMPK, which in turn phosphorylates and activates TSC2, resulting in the inhibition of mTORC1.

Regulation of the cellular energy supply may also be a mechanism to activate mTORC1. Hahn-Windgassen et al. (2005) have suggested that Akt activates mTORC1 not only by direct phosphorylation of TSC2, but also by regulation of cellular energy. According to this model, Akt maintains a high ATP level that causes a decrease in the AMP/ATP ratio that in turn inhibits AMPK-mediated phosphorylation and activation of TSC2. The means by which Akt maintains a high energy level involves, at least in part, the maintenance of nutrient uptake (Edinger and Thompson, 2002).

Stress

Cells respond to environmental stress, such as hypoxia, or low energy by downregulating energy-demanding processes and arresting growth. TOR has been demonstrated to play a role in the response to stress. Upon hypoxia, TOR signaling is inhibited and protein synthesis is thereby downregulated. Insights into the regulation of mTORC1 upon hypoxia have been provided by studies in *Drosophila* and mammalian cells (Brugarolas et al., 2004; Reiling and

Hafen, 2004). Hypoxia is transduced to mTORC1 via the two homologous proteins REDD1 and REDD2 (Scylla and Charybdis in *Drosophila*). The expression of REDD is upregulated upon hypoxia by the transcription factor HIF1. REDD acts downstream of Akt and upstream of TSC1-TSC2 to inhibit mTORC1 signaling. Furthermore, REDD acts independently of the LKB1-AMPK signaling branch to downregulate mTORC1. However, hypoxia and the LKB1-AMPK pathway are likely interrelated, as prolonged hypoxia would eventually lead to ATP depletion and activation of AMPK.

Other stress signals that downregulate mTOR signaling include DNA damage and reducing conditions. p53 activated upon DNA damage inhibits mTOR activity via the AMPK-TSC2 signaling pathway (Feng et al., 2005). A reducing environment inhibits mTORC1 possibly via a redox sensor in the FATC domain of mTOR (Dames et al., 2005; Sarbassov and Sabatini, 2005).

TSC2 is also phosphorylated by kinases other than Akt and AMPK. The Ras/MAPK pathway targets TSC2, as evidenced by the observation that expression of an activated allele of *ras* induces phosphorylation of TSC2 (Roux et al., 2004). Furthermore, loss-of-function mutations in the *NF1*-encoded tumor suppressor neurofibromin, a Ras-GAP, deregulate TSC2 and ultimately cause constitutive mTORC1 signaling (Johannessen et al., 2005). Ras activates the Raf-MEK1/2-Erk1/2 cascade. Activated Erk1/2 directly phosphorylates TSC2 at sites that differ from the Akt target sites, thereby causing functional inactivation of the TSC1-TSC2 complex (Ma et al., 2005a). The MAPK-activated kinase RSK1 also phosphorylates TSC2 at a unique site (Roux et al., 2004). RSK1-mediated phosphorylation of TSC2 inhibits the TSC1-TSC2 complex and thereby increases mTORC1 signaling toward S6K1. In addition, a PKC-dependent TSC2 regulatory pathway has been proposed (Ballif et al., 2005). Thus, the PI3K/Akt and Ras/Erk signaling pathways converge on TSC1-TSC2, resulting in TSC2 inactivation and ultimately mTORC1 activation. This model implies that both PI3K/Akt and Ras/Erk regulate translation via mTORC1 signaling.

Several additional proteins have been demonstrated to interact with the TSC proteins and to modulate the activity of TSC1-TSC2. In *Drosophila*, the protein Melted interacts with TSC1 and thereby recruits TSC1 to the cell membrane. Association with Melted brings TSC1 in proximity to membrane-associated upstream signaling components such as Akt (Teleman et al., 2005). Melted also mediates binding of the transcription factor FOXO to the membrane in an insulin-dependent manner. Like TSC2, FOXO transcription factors are phosphorylated and inhibited by Akt. Consistent with the above findings, *melted* mutant flies exhibit reduced TOR activity and increased FOXO activity and resemble nutrient-starved animals. Another TSC1-TSC2-interacting protein is FIP200 (Gan et al., 2005). FIP200 interacts with TSC1 and thereby inhibits the function of TSC1-TSC2. The role of FIP200 in controlling TSC1-TSC2 function is restricted to nutrient stimulation. FIP200 is also involved in regulation of FAK (focal adhesion kinase), suggesting that FIP200 may link cell adhesion to the regulation of cell growth.

In summary, several upstream signaling cues, growth factors, energy, stress, and possibly amino acids converge on TSC1-TSC2 to regulate mTORC1 signaling. It is important to note that the effect of these upstream signals on mTOR signaling has been studied only in the context of mTORC1. The recent identification of mTORC2 raises the question of whether mTORC2 is similarly regulated. mTORC2 is involved in the organization of the actin cytoskeleton (Jacinto et al., 2004; Sarbassov et al., 2004), and TSC1-TSC2 regulates cell adhesion and migration (Astrinidis et al., 2002), suggesting that mTORC2 might also act downstream of TSC1-TSC2. Whether TSC1-TSC2 signals through mTORC2 to the actin cytoskeleton or through a different pathway remains to be elucidated. Despite the recent advances in mammals and *Drosophila*, upstream regulators of TOR in yeast remain elusive. *S. cerevisiae* does not contain TSC1 and TSC2 orthologs, indicating that upstream signaling cues may be sensed differently in budding yeast.

Downstream Targets of the TOR Signaling Network

TOR signaling plays a role in various growth-related processes in yeast and in higher eukaryotes. The best studied targets of TOR in mammalian cells are the translation regulators S6K1 and 4E-BP1. However, it is important to emphasize that, like TOR in yeast, mTOR controls several additional growth-related processes.

Translation

mTORC1 regulates translation via S6K1 and 4E-BP (Hay and Sonenberg, 2004; Tee and Blenis, 2005). S6K1 belongs to the AGC family of protein kinases and requires phosphorylation at two sites for its full activation, a site in a C-terminal hydrophobic motif and a site in the T loop of the kinase domain. mTORC1 mediates phosphorylation of Thr389 within the hydrophobic motif, whereas PDK1 is responsible for phosphorylation of the T loop. Activated S6K1 phosphorylates the 40S ribosomal protein S6, and this was commonly thought to lead to increased translation of a subset of mRNAs that contain a 5′ tract of oligopyrimidine (TOP). The 5′TOP mRNAs encode components of the translation apparatus, such as ribosomal proteins and elongation factors, and are predicted to account for 15%–20% of total cellular mRNA. Thus, via increased translation of 5′TOP mRNAs, S6K1 would upregulate general translation capacity. However, this model has now lost favor due to the recent findings that translation of 5′TOP mRNAs does not depend on S6K (S6K1 and S6K2) activity nor on S6 phosphorylation (Pende et al., 2004; Ruvinsky et al., 2005). It remains to be determined how mTORC1 controls 5′TOP mRNAs. It also remains to be determined if and how S6K controls translation other than via regulation of elongation factor 2 kinase (eEF2K; Wang et al., 2001). Interestingly, Holz et al. (2005) have shown that the eukaryotic translation initiation factor eIF3 acts as a scaffold for mTORC1 and S6K1. mTORC1 promotes cap-dependent translation initiation through phosphorylation of eIF4E binding proteins (4E-BPs). Phos-

phorylated 4E-BP1 releases eIF4E, which is then free to associate with eIF4G to stimulate translation initiation.

mTORC1 associates with S6K1 and 4E-BP1 via an interaction between raptor and a TOR signaling (TOS) motif in S6K and 4E-BP (Choi et al., 2003; Nojima et al., 2003; Schalm et al., 2003). The TOS motif is a conserved five amino acid segment found in the N terminus of S6K1 (Phe-Asp-Ile-Asp-Leu) and in the C terminus of 4E-BP1 (Phe-Glu-Met-Asp-Ile) and is necessary for the phosphorylation of these proteins by mTORC1 in vivo (Schalm and Blenis, 2002).

Ribosome Biogenesis

Ribosome biogenesis accounts for a large segment of total energy consumption by the cell. A cell must therefore have a mechanism to tightly control ribosomal biogenesis in response to the availability of energy and building blocks. Studies in both yeast and mammalian cells have demonstrated that rapamycin inhibits ribosome biogenesis. Rapamycin blocks the biosynthesis of ribosomes by inhibiting transcription of RNA polymerase I (Pol I)-dependent rRNA genes, Pol II-dependent ribosomal protein genes (RP genes), and Pol III-dependent tRNA genes and also by inhibiting the processing of 35S rRNA (Martin and Hall, 2005). In yeast and mammals, TOR controls Pol I via the transcription factor RRN3/TIF1A (Claypool et al., 2004; Mayer et al., 2004). TIF1A is an essential Pol I-associated initiation factor. Rapamycin treatment leads to TIF1A inactivation and thus impairs formation of the transcription initiation complex. Furthermore, TIF1A translocates from the nucleus to the cytoplasm upon mTOR inactivation by rapamycin (Mayer et al., 2004). Recent studies in yeast have identified the forkhead-like transcription factor FHL1 as a critical regulator of Pol II-dependent RP gene expression (Martin et al., 2004; Schawalder et al., 2004; Wade et al., 2004). FHL1 is constitutively bound to RP gene promoters and is regulated by the two cofactors IFH1 (coactivator) and CRF1 (corepressor). TORC1 maintains the corepressor CRF1 in the cytoplasm. Upon TORC1 inactivation, CRF1 translocates into the nucleus where it competes with the coactivator IFH1 for binding to FHL1, leading to inhibition of RP gene transcription (Martin et al., 2004). TOR controls RP genes also via the Zn finger transcription factor SFP1 that binds and regulates RP gene promoters in a TOR-dependent manner (Jorgensen et al., 2004). Additionally, histone modifying factors affect RP gene expression and have been implicated as TOR effectors (Humphrey et al., 2004). Thus, TOR links nutrient availability to the biosynthesis of ribosomes. The mechanism by which TOR controls the activity of all three RNA polymerases in a coordinated manner is not understood.

Macroautophagy

Starved cells degrade cytoplasmic contents, including organelles, and thereby recycle macromolecules to ensure survival under nutrient-impoverished conditions. This catabolic process, known as macroautophagy, involves the enclosure of cytoplasm by a double-membrane structure (autophagosome) and its subsequent delivery to the vacuole. TOR controls macroautophagy in yeast and higher eukaryotes (Lum et al., 2005). In yeast, TOR negatively controls macroautophagy via inhibition of the protein kinase ATG1 that mediates an early activation step in the autophagic process (Kamada et al., 2000). In *Drosophila*, *dTOR* mutant larvae display constitutive macroautophagy in the fat body, and starvation-induced macroautophagy is suppressed by constitutively active dTOR (Scott et al., 2004). The protective role of macroautophagy during starvation is underscored by the finding that inhibition of macroautophagy enhances the severity of the *dTOR* loss-of-function mutant phenotype (Scott et al., 2004). dTOR also regulates macroautophagy in response to hormonal factors during *Drosophila* development (Rusten et al., 2004). Similar to yeast TOR, *Drosophila* dTOR suppresses macroautophagy by a mechanism that involves ATG1.

TOR controls not only bulk protein degradation by macroautophagy, but also the ubiquitination, internalization, and turnover of specific nutrient transporters. In yeast, TORC1 prevents the turnover of amino acid and glucose transporters (Schmelzle et al., 2004 and references therein). Similarly, mTORC1 controls trafficking of nutrient transporters and thereby promotes uptake of nutrients such as glucose, amino acids, lipoprotein, and iron (Edinger and Thompson, 2002). In support of these findings, mTOR was identified in a kinome-wide screen for regulators of membrane trafficking in mammalian cells (Pelkmans et al., 2005).

Transcription

The nuclear localization and the activity of several nutrient- and stress-responsive transcription factors in yeast are regulated by TORC1-mediated phosphorylation (Loewith and Hall, 2004). mTORC1 signaling also controls transcription of many genes, particularly genes involved in metabolic and biosynthetic pathways, as demonstrated by microarray experiments on rapamycin-treated mammalian cells (Peng et al., 2002). TOR-dependent transcription programs may be regulated through URI (unconventional prefoldin RPB5 interactor; Gstaiger et al., 2003). URI is involved in the regulation of nutrient-sensitive, TORC1-controlled transcription pathways in yeast and mammals. It interacts with all three RNA polymerases through a shared subunit and is phosphorylated in a TOR-dependent manner. In addition, ribosome biogenesis is controlled at the level of *rDNA* transcription by mTOR through the transcription factors UBF and TIF1A (Hannan et al., 2003; Mayer et al., 2004). Thus, mTOR also regulates nutrient-responsive transcription programs. Furthermore, mTOR has been demonstrated to phosphorylate STAT1 and STAT3 (signal transducer and activator of transcription; Kristof et al., 2003 and references therein) and to activate the nuclear receptor PPARγ (see below), in a rapamycin-sensitive manner. The role of mTOR-controlled transcription in mediating cell growth remains poorly understood.

Actin Organization

Early studies established a role for TOR in the organization of the actin cytoskeleton (Loewith and Hall, 2004). In yeast, rapamycin-insensitive TORC2 controls the cell cycle-dependent polarization of the actin cytoskeleton. TORC2 signals to the actin cytoskeleton by activating a Rho1 GTPase switch.

Upon activation, Rho1 interacts with and activates PKC1, which in turn signals to the actin cytoskeleton via a MAP kinase pathway. How TORC2 regulates the Rho1 GTPase switch is not completely understood but might involve SLM1/2 and YPK2, two recently identified substrates of TORC2 (Audhya et al., 2004; Fadri et al., 2005; Kamada et al., 2005), in addition to the Rho1 exchange factor ROM2 (Loewith and Hall, 2004). The PH domain-containing proteins SLM1 and SLM2 act downstream of TORC2 and the phosphatidylinositol-4-phosphate 5-kinase MSS4 to regulate actin organization. YPK2, the yeast homolog of SGK1 (serum- and glucocorticoid-activated kinase), belongs to the AGC family of protein kinases. YPK2 requires phosphorylation in a hydrophobic motif and in the T loop of the catalytic domain for its activation. Phosphorylation of the hydrophobic motif is mediated by TORC2, whereas phosphorylation of the T loop is mediated by PKH2 (yeast homolog of PDK1), which acts downstream of sphingolipids in yeast. Thus, YPK2 integrates TORC2 and PKH-mediated sphingolipid signals to control actin polarization. It is currently unknown whether SLM1/2 or YPK2 signal through Rho1 or through a parallel pathway to the actin cytoskeleton.

mTORC2 also signals to the actin cytoskeleton (Jacinto et al., 2004; Sarbassov et al., 2004). Although the direct targets of mTORC2 that mediate signaling to the actin cytoskeleton are unknown, mTORC2 signaling to the actin cytoskeleton may involve PKCα and the small GTPases Rho and Rac. The recent finding that TORC2 exists in *Dictyostelium* where it is also required for cell polarity further supports the notion that TORC2 signaling is functionally conserved (Lee et al., 2005).

Metabolism

TOR controls many aspects of cellular metabolism including amino acid biosynthesis, glucose homeostasis, and others (see above; Thomas et al., 2004). Recent studies suggest that the TOR signaling network also controls fat metabolism. In particular, mTORC1 appears to play an important role in adipogenesis as rapamycin treatment prevents adipocyte differentiation and, thus, lipid accumulation (Kim and Chen, 2004 and references therein). The mechanism by which mTOR controls adipogenesis is poorly understood but might involve the nuclear receptor PPARγ, as rapamycin treatment inhibits PPARγ activity (Kim and Chen, 2004). PPARγ plays a critical role in adipogenesis and lipid accumulation and is therefore referred to as one of the so-called "thrifty genes" that are responsible for efficient storage of energy (Lazar, 2005 and references therein). The regulation of fat metabolism by mTORC1 also involves signaling via S6K1. S6K1 mutant mice display reduced adipose tissue and a decrease in fat accumulation due to enhanced β oxidation (Um et al., 2004). Furthermore, *melted* mutant flies, which are defective in TOR signaling, are lean due to loss of fat (Teleman et al., 2005). Thus, loss of TOR activity correlates with a decrease in fat accumulation, suggesting that the TOR pathway may be a "thrifty pathway" normally required for fat accumulation. Interestingly, in addition to being a passive fat-storage depot, adipose tissue is also an active organ that secretes hormones that regulate appetite and other aspects of animal physiology, suggesting that there might be broader implications to the control of adipogenesis by mTOR. Thus, mTOR may play a role in the development of metabolic disorders such as obesity and type 2 diabetes.

Regulatory Loops: Intricacies of the TOR Network

Negative Regulation of IRS by S6K1

Recent studies have highlighted the existence of a negative feedback loop from the nutrient-sensitive TSC-mTOR-S6K1 pathway to the upstream, insulin-responsive IRS-PI3K-PDK1-Akt pathway (Figure 2; Harrington et al., 2004; Shah et al., 2004; Um et al., 2004). Earlier studies showed that increased amino acid availability can inhibit signaling through the insulin pathway (Tremblay et al., 2005). The rapamycin sensitivity of this effect suggested that the inhibition is mediated by a negative feedback loop from mTOR to a component of the insulin pathway. This model was supported and extended by more recent studies showing that loss of TSC1 or TSC2, in mouse embryonic fibroblasts (MEFs) or *Drosophila*, leads to a strong inhibition of insulin-mediated PI3K signaling (Manning, 2004). TSC-mTOR-S6K1 signaling attenuates PI3K by phosphorylation and inactivation of IRS proteins. In particular, S6K1 regulates IRS1 both at the transcriptional level and through direct phosphorylation, thereby impairing IRS1 adaptor function (Um et al., 2004). Thus, constitutive activation of mTOR-S6K1 signaling induces a negative feedback loop to attenuate PI3K via inhibition of IRS.

S6K1 knockout mice, due to loss of IRS inhibition, are hypersensitive to insulin yet do not become hypoglycemic (Um et al., 2004). The absence of hypoglycemia is most likely due to these mice also having a low mass of β cells and thus a low level of insulin. Phosphorylation-deficient S6 knockin mice exhibit a nearly identical β cell phenotype, suggesting that the β cell defect is due, at least in part, to a failure of S6K1 to phosphorylate ribosomal protein S6 (Ruvinsky et al., 2005). Curiously, phosphorylation-deficient S6 mice are also hypersensitive to insulin, suggesting that S6 may somehow be involved in the negative feedback loop to IRS.

The feedback inhibition of insulin-PI3K signaling is relevant to the development of metabolic disorders such as obesity and diabetes. Under conditions where mTOR signaling is inappropriately activated, due to a TSC deficiency or excess of nutrients, the feedback loop dampens the duration and strength of PI3K signaling and thus leads to insulin resistance. In addition, the benign nature of TSC-related tumors might be explained by the action of this negative feedback. Indeed, analyses of tumors in heterozygous *TSC2* mutant mice revealed that feedback inhibition of Akt correlates with limited tumor growth (Ma et al., 2005b; Manning et al., 2005).

Regulation of Akt/PKB by mTORC2

mTORC2 was discovered only recently. Thus, the upstream regulators and downstream effectors of this rapamycin-insensitive mTOR complex are largely unknown. mTORC2 phosphorylates and activates Akt/PKB, another member of the AGC protein kinase family (Sarbassov et al., 2005). The role of Akt includes regulation of cell proliferation, survival, metabolism, and transcription. Through its PH

Table 2. Summary of Protooncogenes and Tumor Suppressors that Are Functionally Linked to mTORC1 Signaling

Protooncogenes	Evidence	References
PI3K	Aberrantly high PI3K activity has been implicated in cell transformation and tumor progression and has been observed in a variety of human cancers.	Vivanco and Sawyers, 2002
PKB/Akt	Akt is amplified in a subset of human cancers.	Vivanco and Sawyers, 2002
Rheb	Rheb expression is elevated in many tumor cells, and Rheb may be the critical target by which farnesyl transferase inhibitors mediate their antitumor activity.	Basso et al., 2005
ras	*ras* mutations that result in hyperactivation of the protein are among the most frequent alterations in human cancers.	Coleman et al., 2004
eIF4E	Ectopic overexpression of eIF4E can transform cells ex vivo and in vivo. eIF4E is overexpressed in many human tumors.	Bjornsti and Houghton, 2004; Ruggero et al., 2004
S6K1	*S6K1* is amplified or overexpressed in a large fraction of breast cancers, and this correlates with poor prognosis.	Barlund et al., 2000
Tumor Suppressors		
PTEN	Loss of PTEN function has been found in a large fraction of advanced human cancers. Individuals with inherited mutations in *PTEN* develop hamartoma tumor syndromes (Cowden disease, Bannayan-Riley-Ruvalcaba syndrome, Proteus syndrome, Lhermitte-Duclos disease) and are at higher risk of developing several types of cancer.	Inoki et al., 2005; Sansal and Sellers, 2004; Vignot et al., 2005
TSC1, TSC2	Individuals with mutations in *TSC1* or *TSC2* develop tuberous sclerosis complex (TSC) characterized by the formation of hamartomas in a wide variety of organs.	Kwiatkowski, 2003
NF1	Individuals with mutations in *NF1* develop neurofibromatosis type 1, characterized by benign and malignant peripheral nerve sheath tumors.	Johannessen et al., 2005
LKB1	Individuals with mutations in *LKB1* develop Peutz-Jeghers syndrome, characterized by hamartomas in the gastrointestinal tract.	Inoki et al., 2005; Tee and Blenis, 2005
p53	p53 performs a variety of antineoplastic activities and is mutated in the majority of human tumors.	Sigal and Rotter, 2000
4E-BP1	Overexpression of activated 4E-BP1 blocks c-Myc induced cellular transformation.	Lynch et al., 2004
beclin-1	Decreased expression of beclin-1 (a protein required for autophagy) is associated with human breast carcinomas, and *beclin-1* mutant mice have a high frequency of tumor formation.	Lum et al., 2005

domain, Akt binds PIP3 generated by PI3K upon stimulation by insulin or insulin-like growth factors. Similar to other AGC kinases, membrane bound Akt is activated by phosphorylation at two sites, a site in the activation loop of the kinase (Thr308) and a site in the hydrophobic motif (Ser473). Whereas PDK1 phosphorylates Akt in the activation loop, the kinase responsible for phosphorylation of the hydrophobic site was recently determined to be mTORC2 (Sarbassov et al., 2005). Thus, mTORC2 performs a positive role in the activation of Akt. Importantly, Akt is phosphorylated by mTORC2 but not by mTORC1. The logic of mTORC2 activating Akt remains elusive. Because Akt activates mTORC1, mTORC2 could indirectly activate mTORC1. However, knockdown of mTORC2 does not affect the mTORC1 effector S6K1 (Sarbassov et al., 2004; Jacinto et al., 2004), suggesting that mTORC2 activates a pool of Akt that is not upstream of mTORC1. In support of the finding that mTORC2 is upstream of Akt, TORC2 in *Dictyostelium* signals to Akt to regulate chemotaxis and cell polarity (Lee et al., 2005).

Phosphorylation of mTOR by S6K1

mTOR is phosphorylated at Thr2446 and Ser2448. The phosphorylation at these two sites was shown to be PI3K dependent and increased in response to constitutively active Akt. These observations led to the model that Akt acts directly on mTOR, in addition to acting indirectly via TSC. Two groups have now shown that S6K1, and not Akt, is the kinase responsible for phosphorylation of these two sites (Chiang and Abraham, 2005; Holz and Blenis, 2005). The significance of this potential feedback loop is unknown as it is not yet clear whether Thr2446/Ser2448 phosphorylation is a positive, negative, or inconsequential modification. It is also unknown whether S6K1 phosphorylates mTOR in mTORC1, mTORC2, or both.

TOR and Disease

Cancer and Hamartoma Syndromes

The ongoing elucidation of the signaling components upstream and downstream of mTORC1 has suggested a causal link between aberrant mTORC1 signaling and tumor

formation. As summarized in Table 2, the signaling components upstream and downstream of mTORC1 are frequently altered in a number of human tumors. Specifically, aberrantly high mTORC1 activity appears to be an underlying cause of cancers and hamartoma syndromes (Inoki et al., 2005; Tee and Blenis, 2005). Hamartoma syndromes, which are characterized by benign tumors that contain architecturally disorganized but otherwise normally differentiated cells, affect a wide variety of tissues, including brain, skin, kidneys, heart, lung, and the gastrointestinal tract. Although benign, hamartoma syndromes can progress to malignancy. Notable among the hamartoma syndromes is tuberous sclerosis complex resulting from mutation of *TSC1* or *TSC2* (Kwiatkowski, 2003). The correlation between tumor growth and hyperactive mTORC1 signaling suggests that tumors may be sensitive to mTORC1 inhibitors. To this end, rapamycin (sirolimus, Wyeth) and its derivatives temsirolimus (CCI-779, Wyeth), everolimus (RAD-001, Novartis Pharma AG) and AP-23573 (Ariad Pharmaceuticals) are currently being evaluated in clinical trials as cancer treatments. Preclinical studies suggest that sensitivity of tumors to mTORC1 inhibition may correlate with aberrant activation of the PI3K pathway and/or with aberrant expression of cell cycle regulatory or antiapoptotic proteins. Clinical trial results show that mTOR inhibitors are generally well tolerated and may induce prolonged stable disease and even tumor regressions in a subset of patients (Dancey, 2005; Vignot et al., 2005).

Allograft Rejection and Autoimmune Disorders

Rapamycin interferes with the activation of T cells, and clinical trials in the late 1990s confirmed the efficacy of sirolimus and everolimus as potent immunosuppressive agents in renal transplantation. These drugs have also shown promise in liver transplantation (sirolimus) and cardiac transplantation (everolimus), and appear to facilitate weaning of patients off nephrotoxic calcineurin inhibitors. Sirolimus and everolimus are themselves not without side effects. Both drugs present metabolic, hematological, and dermatological effects including increases in serum cholesterol and triglycerides, anemia, skin rashes, and diarrhea. Current protocols employ a combination of complementary agents (e.g., calcineurin inhibitors, mTORC1 inhibitors, and steroids) to yield optimal immunosuppression with minimal side effects.

The immunosuppressive activity of rapamycin and its derivatives suggests that these drugs may also have potential in the treatment of autoimmune disorders including rheumatoid arthritis, psoriasis, multiple sclerosis, and Parkinson's disease. Indeed, a number of pharmaceutical companies are investigating the efficacy of mTORC1 inhibitors in treating these autoimmune disorders (reviewed in Young and Nickerson-Nutter, 2005).

Cardiovascular Disease

Cardiovascular disease is a leading killer in the developed world. In the year 2000, more than 1,000,000 percutaneous transluminal coronary procedures were performed in the US, and about half of these included the placement of intracoronary stents (an expandable metal coil that is inserted into the newly opened area of the artery to keep the artery from narrowing or closing again). Although stenting brings long-term benefit to a majority of patients, a substantial number of patients experience overgrowth of smooth muscle cells surrounding the stent, similar to scar tissue, and reblockage of the artery (in-stent restenosis). A number of approaches have been employed to reduce the incidence of in-stent restenosis, ranging from systemic pharmacologic treatments to modification of the composition of the stent. Drug-eluting stents have shown much promise in this regard. A drug that inhibits cell growth is attached to the metal of a stent, and an outer layer of biocompatible polymers allows the drug to elute over an ~30 day period. Paclitaxel- and sirolimus-eluting stents have been analyzed in a number of clinical trials and both have been found to improve patient outcome (Gershlick, 2005). Future clinical studies will evaluate stents that elute different drugs (including other rapamycin derivatives) at different release kinetics.

Cardiac hypertrophy is also a major risk factor for heart failure. Overgrowth of cardiomyocytes is dependent on the PI3K-mTORC1 pathway (reviewed in Inoki et al., 2005; Tee and Blenis, 2005). Furthermore, inherited mutations in the gene encoding AMPKγ2, a regulatory subunit of AMPK, result in reduced AMPK activity and cause familial hypertrophic cardiomyopathy and Wolff-Parkinson-White syndrome, presumably via hyperactivation of mTORC1. These observations suggest that mTORC1 inhibitors may be useful therapeutic agents for the treatment of lifestyle-induced and inherited cardiac hypertrophy.

Metabolic Disorders

Both type 2 diabetes and obesity, among other disorders, are associated with an inability to respond to insulin (insulin resistance). Recent studies (reviewed in Manning, 2004) have demonstrated that inhibition of IRS protein function is one means by which cells become desensitized to insulin. As described above, one mechanism by which IRS function is inhibited is via a negative feedback loop from mTOR-S6K. This suggests that mTORC1 inhibitors may be effective for the treatment of metabolic disorders involving insulin resistance.

Future Directions

Ongoing clinical trials indicate that mTORC1 inhibitors show remarkable clinical potential in a variety of disease settings. However, major questions concerning the design of these clinical trials and in particular the future of mTORC1 inhibitors as anticancer agents remain. For example, what distinguishes a cancer that is likely to respond favorably to mTORC1 inhibition from a cancer that will not respond favorably? To answer this question, we must fully understand the pathways that regulate mTORC1 and how these pathways are altered in disease. This includes a full understanding of the consequences that mTORC1 inhibition will have on Akt signaling. For example, will mTORC1 inhibition prevent the negative feedback loop from S6K to IRS (and ultimately to Akt) and thereby exacerbate some cancers? Additionally, the anticancer capabilities of mTORC1

inhibitors appear to be augmented when used in combination with other anticancer agents (Beuvink et al., 2005). What additional pathways should be targeted for optimal response? Also lacking is an understanding of how nutrient abundance/quality is sensed and signaled to mTORC1. Studies in yeast, worms, flies, and mammals have all linked nutrient status to mTORC1 activity, and yet the relevant sensing mechanisms remain obscure.

It is also important to note that mTORC1 inhibition has relied almost exclusively on rapamycin or closely related derivatives of rapamycin. However, the mechanism of action of these compounds is poorly understood, and therefore the existence of medically relevant, rapamycin-insensitive mTORC1 substrates cannot be ruled out. Conversely, the generation of novel mTORC1 inhibitors that interrupt specific mTORC1-substrate interactions may be just as efficacious as rapamycins but present fewer side effects. FKBP12-rapamycin is probably the most potent and specific kinase inhibitor known (Davies et al., 2000). A better understanding of the mode of action of FKBP12-rapamycin action could lead to the development of potent and specific inhibitors of other kinases.

It is estimated that up to one-third of proteins in a cell are phosphorylated, many at multiple sites. The existence of roughly 600 kinases in the human kinome implies that each human kinase phosphorylates on average ~ 20 substrates. Identification of mTORC substrates will be crucial to understand the mechanisms by which TORCs control growth. As noted in Table 2, pathways downstream of mTORC1 are also believed to have important roles in tumorigenesis. What are the critical substrates of mTORC1 for tumor formation? These effectors may themselves serve as potential drug targets. The molecular characterization of downstream pathways may also facilitate the development of molecular markers useful for diagnosis and prognosis and to monitor biological effects of mTORC1 inhibitors.

The specific functions of mTOR-associated proteins remain to be determined. Tissue-specific disruptions of mTORC1 and mTORC2 will be important to define when and where mTORC functions are needed. This is particularly interesting given the extreme toxicity of rapamycin in early embryos versus the relatively mild affects of rapamycin in adults. These studies may suggest additional settings where mTORC inhibitors/agonists may be of use. Possibilities include enhancement of life span or treatment of cognitive disorders. The clinical efficacies of mTORC1 inhibitors as agents for the treatment of metabolic disorders and cardiac hypertrophy also remain to be evaluated.

Lastly, the existence of rapamycin-insensitive mTORC2 has important implications in mTOR biology. Cellular processes that are unaltered by rapamycin treatment are not necessarily mTOR independent. Furthermore, it appears that growth factors may regulate mTORC2 (Jacinto et al., 2004; Sarbassov et al., 2005), but whether this involves the same signaling cascades that regulate mTORC1 remains to be determined. If mTORC2 indeed shares upstream regulators with mTORC1, does mTORC2 signaling contribute to the pathology of diseases so far attributed solely to mTORC1 hyperactivity? For example, does hyperactive mTORC2, via altered regulation of the actin cytoskeleton, cause the morphological changes observed in hamartoma cells? The recent observation that mTORC2 regulates Akt also suggests that, as is the case for mTORC1, mTORC2 dysregulation and/or chemical inhibition may be clinically relevant. Finally, how does TORC2 signaling, in mammalian and nonmammalian systems, contribute to the control of normal physiological processes that were previously ascribed solely to TORC1? For example, does the control of lifespan, memory, or fat metabolism by TOR involve TORC2 in addition to TORC1? Although our understanding of TOR signaling has come a long way since the fateful collection of soil on Easter Island three decades ago, much remains unknown.

Note on Nomenclature

mLST8 is referred to in the literature as either mLST8 or GβL, due to it having been identified as an mTOR binding protein in two independent studies (Loewith et al., 2002; Kim et al., 2003). Similarly, rictor is referred to as either rictor or mAVO3, also because it was demonstrated to bind mTOR by two independent studies (Sarbassov et al., 2004; Jacinto et al., 2004). To avoid further confusion in the literature, we propose that the field adopt mLST8 and rictor as the official names for these two proteins. These are the names used in the first of the two binding studies that were published for each protein (Loewith et al., 2002; Sarbassov et al., 2004). This proposal applies only to the mammalian orthologs. The nomenclature of the nonmammalian orthologs is unambiguous, and these proteins should continue to be referred to by their current names (see Table 1). Finally, given the diversity of names for TOR-associated proteins from different species, we urge use of TORC1 and TORC2 (mTORC1, etc.) as a standardized nomenclature for the complexes.

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