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REVIEW ARTICLE

Growth control via TOR kinase signaling, an intracellular sensor of amino acid and energy availability, with crosstalk potential to proline metabolism

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Abstract The TOR (Target of Rapamycin) protein kinase pathway plays a central role in sensing and responding to nutrients, stress, and intracellular energy state. TOR complex 1 (TORC1) is comprised of TOR, Raptor, and Lst8 and its activity is sensitive to inhibition by the macrolide antibiotic rapamycin. TORC1 regulates protein synthesis, ribosome biogenesis, autophagy, and ultimately cell growth through the phosphorylation of S6 K, 4E-BP, and other substrates. As TORC1 activity is positively or negatively modulated in response to upstream regulators, cellular growth rate is, respectively, enhanced or suppressed. A separate multiprotein TOR complex, TORC2, is insensitive to direct inhibition by rapamycin and does not regulate growth patterns directly; TORC2 can, however, impact certain aspects of TORC1 signaling and cell survival. TOR signaling is an ancient pathway, conserved among the yeasts, *Dictyostelium*, *C. elegans*, *Drosophila*, mammals, and *Arabidopsis*. This review will focus on the regulation of TORC1 in mammalian cells in the context of amino acid sensing/regulation and intracellular ATP homeostasis, but will also include comparisons among other organisms.

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Introduction

Exponential cellular growth requires continued accessibility to resources that sustain intracellular energy and nutrient levels. These energy and precursor sources do not function simply to support essential macromolecular synthetic events; they also participate as upstream signaling regulators within a complex intracellular network that serves to modulate cell size and cellular growth rate (Schmelzle and Hall 2000; Hay and Sonenberg 2004; Avruch et al. 2006; Wullschleger et al. 2006). Central to eukaryotic nutrient sensing is the serine/threonine protein kinase TOR, a member of the phosphatidylinositol kinase-related kinase, PIKK, family (Schmelzle and Hall 2000). The various inputs in the signaling network stimulate or repress the relative activity of TOR and consequently balance cellular growth rate with nutrient and energy availability (Fig. 1).

TOR—the target of rapamycin

The TOR kinase is the catalytic component of TOR Complex 1 (Fig. 1), TORC1 (Hara et al. 2002; Kim et al. 2002, 2003; Loewith et al. 2002). In addition to TOR (~250 kDa), TORC1 is composed of Raptor (regulatory associated protein of mTOR) and Lst8 (synthetically lethal in combination with mutations in the secretory pathway gene *SEC13*). Due to sequence similarity of Lst8 with the heterotrimeric Gβ protein, some mammalian workers have alternatively referred to Lst8 as Gβ-Like or GβL (Kim

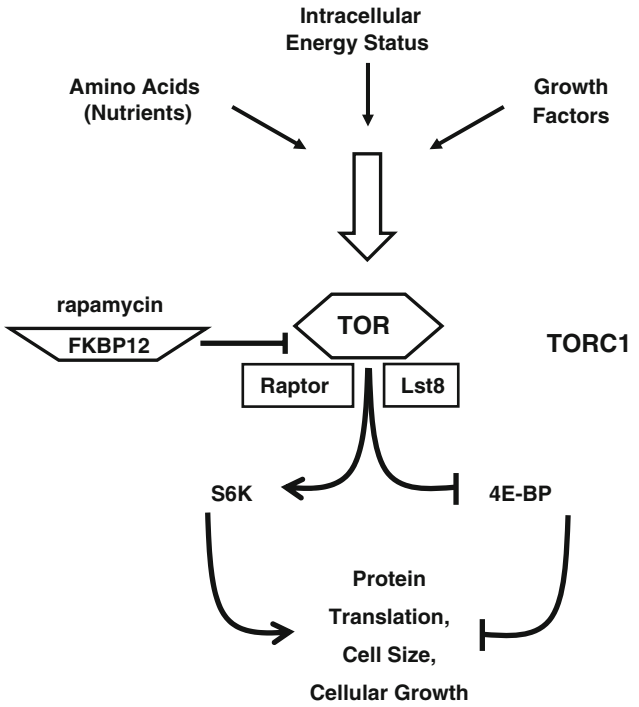

Fig. 1 TOR Complex 1, an intracellular sensor of amino acid and energy availability. TOR Complex 1 (TORC1) is comprised of TOR kinase, Raptor, and Lst8. Activated TORC1 can phosphorylate substrates S6 K and 4E-BP, altering their activities toward the regulation of protein translation and growth. TOR is the target of rapamycin. Rapamycin, in complex with FKBP12, will bind TOR, disrupt interaction with Raptor, and inhibit TORC1 function. The TORC1 signaling network integrates various upstream stimuli to control relative growth rates

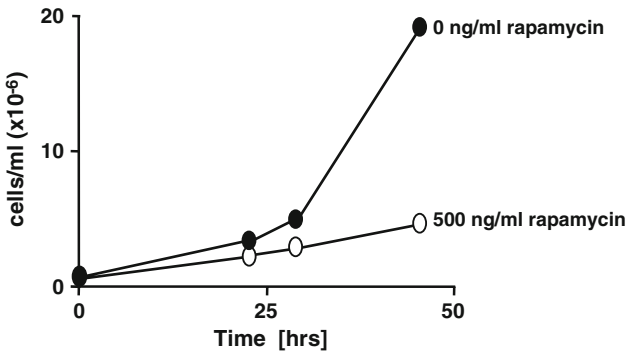

Fig. 2 Rapamycin inhibition of *Dictyostelium* growth. *Dictyostelium* were grown in nutrient-rich media to $\sim 2 \times 10^5$ cells/ml. The culture was then split, with one aliquot treated with 500 ng/ml rapamycin and one aliquot left untreated. Samples were removed from each culture at the indicated times, and cell numbers/ml determined using a hemocytometer

et al. 2003). This underscores aspects of nomenclature confusion in the TOR signaling pathway, where the TOR designation is sometimes preceded by a "species-type" modifier. Thus, while terms such as mTOR (mouse/mammalian) or dTOR (*Drosophila*) may be encountered, and perhaps thought to convey unique properties, TOR itself is largely conserved throughout the eukarya, including the yeasts, *Dictyostelium*, *C. elegans*, *Drosophila*, and mammals, including of course humans; likewise are Raptor and Lst8. Still, while the fundamental roles of TORC1 are similar in all of these diverse organisms, the positive and negative regulatory inputs and feedback responses are not fully conserved in all systems or even in all cell-types within an organism.

TOR is an acronym for Target of Rapamycin (Heitman et al. 1991). Rapamycin or Sirolimus is a natural product of *Streptomyces hygroscopicus* initially identified in the soil of Rapa Nui, i.e. Easter Island (Vezina et al. 1975). Rapamycin was first classified as a suppressor of fungal growth, but is now also recognized to be a very effective immunosuppressant and anti-neoplastic agent. Rapamycin acts to inhibit eukaryotic cellular growth (Fig. 2) by restricting the function of TORC1 kinase activity (Heitman et al. 1991; Sabatini et al. 1994; Kim et al. 2002; Loewith et al. 2002; Oshiro et al. 2004). Rapamycin binds to the endogenous protein FKBP12, a cis-trans prolyl isomerase, and the resulting FKBP12-rapamycin complex interacts with TOR near the Raptor binding domain, disrupting the structural integrity of TORC1 (Fig. 1). Cells lacking FKBP12 are resistant to rapamycin.

TORC1 promotes growth by targeting the phosphorylation of proteins that regulate protein translation, gene expression, and autophagy (Wullschleger et al. 2006); among the most studied substrates of TORC1 are the translational regulatory proteins eIF4E (eukaryotic initiation factor 4E) binding protein, 4E-BP, and ribosomal protein S6 kinase, S6 K (Hay and Sonenberg 2004). These substrates act at distinct foci within the protein translational machinery and are functionally opposed and reciprocally regulated (Fig. 1). S6 K, a positive effector of translation, is activated upon phosphorylation, whereas 4E-BP is a translational inhibitor that is de-activated by TORC1 phosphorylation.

The 4E-BP target eIF4E and its associated proteins eIF4A helicase and eIF4G define the eIF4F complex that unwinds the 5′-end of the mRNA to assist interaction with the 40S small ribosomal subunit and promote translation. The primary role of the ubiquitous 4E-BPs is to inhibit protein translation by binding to eIF4E to suppress formation of the eIF4F complex (Pause et al. 1994). TORC1-mediated phosphorylation of 4E-BP decreases its association with eIF4E and, thus, relieves inhibition (Gingras et al. 1999, 2001; Marcotrigiano et al. 1999; Tee and Proud 2002; Wang et al. 2003). As a functional inhibitor, 4E-BP may play only a minimal role during nutrient replete growth. However, under conditions of nutritional stress, when protein translation is normally limited, cells

lacking 4E-BP exhibit poorer survival in comparison to WT controls (Teleman et al. 2005).

The downstream targets of S6 K include rps6 (the S6 protein of the small, 40S ribosomal subunit), eukaryotic initiation factors eIF4B and eIF3, elongation factor 2 (eEF2) kinase, and PDCP4 (programmed cell death protein 4), among others (Browne and Proud 2004; Holz et al. 2005; Dorrello et al. 2006). Phosphorylation of these targets enhances protein synthesis by facilitating the directed assembly of the protein translational machineries. While cells expressing S6 K have selective growth advantages in comparison to S6 K-depleted counterparts, S6 K may have cell-type specific functions that more impact size regulation than absolute cell survival (Montagne et al. 1999; Fingar et al. 2002; Pende et al. 2004).

The individual effects of 4E-BP and S6 K are themselves limited and are not sufficient to explain TOR-mediated translational control. Ultimately, the TORC1-dependent actions on growth represent the collective inputs that positively regulate aspects of protein translation, transcription, and ribosome biogenesis, and that suppress autophagy and other growth-restrictive events (Schmelzle and Hall 2000; Hay and Sonenberg 2004; Avruch et al. 2006; Wullschleger et al. 2006).

TORC1 component activities

Purified, uncomplexed TOR possesses an intrinsic protein kinase activity, but lacks the ability to recognize 4E-BP as a substrate in vitro. Kinase activity toward such a heterologous substrate was first achieved with the purification of TOR in a complex with molar equivalent ratios of Raptor and Lst8. Equivalent and functionally active TOR complexes, now termed TORC1, have been purified from systems as diverse as yeast (Loewith et al. 2002), mammals (Hara et al. 2002; Kim et al. 2003), and *Dictyostelium* (Liao and Kimmel, in preparation).

Raptor (~150 kDa) appears essential to recruit substrates, e.g. S6 K and 4E-BP, for phosphorylation by TORC1. Raptor may serve as an adaptor, bridging TOR and these substrates to facilitate their phosphorylation. A conserved TOR signaling (TOS) motif that is shared among TORC1 substrates has a suggested recognition function (Nojima et al. 2003). Certainly, Raptor can simultaneously bind to TOR as well as S6 K or 4E-BP. As FKBP12-rapamycin complexes bind TOR in the context of TORC1 and consequently alter Raptor-TOR interactions (Kim et al. 2002), rapamycin treatment induces a rapid decrease in phosphorylation levels of S6 K and 4E-BP, presumably by reducing TOR-substrate interactions. The rapidity of de-phosphorylation upon suppression of TORC1 activity emphasizes the role of phosphatases (i.e. PP2A) in transmitting the TOR signal. Depletion of Raptor protein also reduces S6 K phosphorylation and parallels TOR deficiency in several systems (Long et al. 2002). The dependency of TOR action on Raptor is emphasized by their essential cellular functions; genetic inactivation of either causes lethality.

While these data collectively suggest that Raptor is an essential positive regulator of TOR signaling, other experiments suggest a more complicated relationship. The interactions of Raptor and TOR seem augmented in cells that have less active TORC1 (Kim et al. 2002). TORC1 isolated from cells cultured in nutrient-rich media, where S6 K and 4E-BP phosphorylation levels are high (see below), is more sensitive to rapamycin disruption, than is TORC1 in cells from nutrient-depleted media that have less active TORC1 (Kim et al. 2002). In addition, overexpression of Raptor may suppress the phosphorylation of targeted substrates. It may be argued that Raptor can act both as a pathway activator and inhibitor. However, these contrasting actions remain to be elucidated mechanistically and further generalized.

Lst8 is a small protein (~35 kDa) comprised of WD repeats. Lst8 interacts within the catalytic domain of TOR (Kim et al. 2003), but, unlike TOR and Raptor it is not essential for TORC1 activity. In accord, deletions of Lst8 do not generally cause cell lethality. Overexpression of Lst8 can increase precipitable TORC1 kinase activity, but does not induce an in vivo increase in the phosphorylation levels of TORC1 substrates S6 K and 4E-BP. In addition, Lst8 association with TOR is not altered by nutrient state or rapamycin treatment. Lst8 does not seem to be required for TORC1 kinase activity per se, but may augment the actions of TORC1.

Amino acid, growth factor, and energy-state regulation of TORC1

The composite activity of TORC1 stems from its responses to a variety of activating (e.g. insulin, amino acids) and inhibiting (e.g. AMP) signals, which, respectively, promote or suppress cellular growth (Fig. 3). The activating signals may act cooperatively (Avruch et al. 2006). While insulin stimulation promotes phosphorylation of 4E-BP and S6 K (see below), such activity requires amino acid sufficiency (Hara et al. 1998). In contrast, elevated levels of amino acids are able to fully activate TORC1 independently of stimulation by insulin or other growth factors. Data suggest that the small Ras-like GTPase Rheb (Ras homolog enriched in brain) mediates this hierarchical pathway (Fig. 3).

Rheb can bind TORC1 regardless of nucleotide-charging state (Long et al. 2005a, b). GTP-bound Rheb binds and activates TORC1, and while Rheb-GDP also binds TORC1, it does not activate, and so functions as an

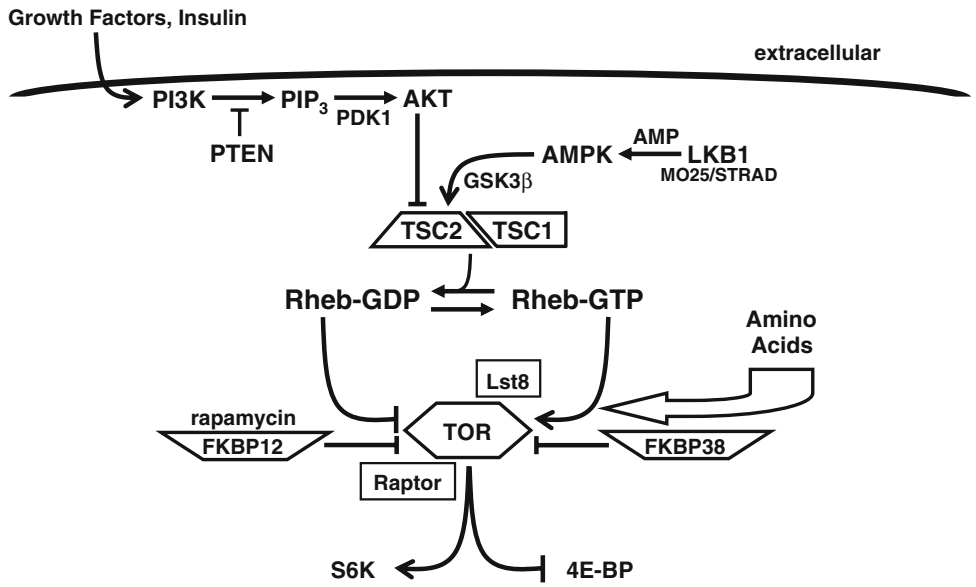

Fig. 3 Activating and inhibiting pathways for TORC1 regulation. Rheb-GTP activates TORC1, while Rheb-GDP is inhibitory. FKBP38 binds and inhibits TORC1 under conditions of depleted amino acids. Rheb-GTP and amino acid sufficiency antagonize FKBP38 binding to TOR, and relieves inhibition. TSC1/TSC2 is a Rheb GAP, subject to both negative and positive regulation by the upstream kinases AKT and AMPK, respectively. AKT activation requires phosphorylation within the kinase activation loop by PDK1. Insulin and other growth factors activate PI3 K which generates PIP3 from PIP2. AKT and PDK1 are recruited to the membrane via PIP3-binding, facilitating AKT phosphorylation and activation by PDK1. PTEN inhibits the pathway by de-phosphorylating PIP3. Activated AKT can phosphorylate and inhibit TSC2 GAP, which promotes TORC1 activation and cell growth. Under conditions of low intracellular energy levels AMPK is activated, mediated through phosphorylation by the upstream kinase LKB1, and requiring co-factors MO25 and STRAD. AMPK phosphorylation of TSC2 acts as a priming recognition site for multiple phosphorylations by GSKβ. These collective phosphorylations lead to TSC2 GAP activation, promoting the accumulation of Rheb-GDP that suppresses TORC1 activity and cell growth

effective inhibitor. Rheb binding to TOR is enhanced under amino acid replete conditions, independently of nucleotide charging (Long et al. 2005b). In contrast, insulin signaling does not influence Rheb binding to TOR.

Rheb-GTP de-activation to Rheb-GDP involves the activation of its intrinsic GTPase by the GTPase activating protein (GAP) complex TSC (Tuberous Sclerosis Complex) comprised of TSC1 (tuberin) and TSC2 (hamartin), which contains the Rheb GAP domain within its C-terminal region (Inoki et al. 2003a; Zhang et al. 2003). *Drosophila* TCTP (translationally controlled tumor protein) is suggested to function as a Rheb guanine nucleotide exchange factor (GEF) that would specifically direct Rheb-GTP loading and function antagonistically to TSC (Hsu et al. 2007). However, this activity has not been confirmed in other systems and Rheb may have an inherent binding preference for GTP. Regardless, Rheb-GTP/Rheb-GDP levels are balanced by the input of both negative and positive signals upstream of TSC that regulate GAP activity (Fig. 3).

Phosphorylation of TSC2 by AKT inhibits GAP activity, thus, promoting Rheb-GTP accumulation, TORC1 activation, and cellular growth pathways (Inoki et al. 2002; Potter et al. 2002). AKT activity requires phosphorylation within its kinase activation loop by phosphoinositide-dependent kinase 1 (PDK1), a response pathway mediated by PI3 K activation through growth factor (e.g. insulin) tyrosine kinase signaling. AKT possesses a PI(3,4,5)P3-binding plextrin homology (PH) domain. Upon PI3 K (phosphoinositide 3-kinase) activation and the resulting accumulation of PIP3, AKT becomes membrane localized where it is accessible for phospho-activation by PDK1.

In contrast to the inhibitory input of AKT on TSC2, Rheb-GAP activity is positively regulated upon depletion of intracellular energy stores (Fig. 3). As intracellular AMP/ATP levels increase, the AMP-dependent protein kinase (AMPK) becomes activated. AMPK activation is complex, requiring the two associated regulatory subunits AMPKβ and AMPKγ, as well as the upstream kinase LKB1, and its two associated regulatory proteins STRAD and MO25. AMPK phosphorylates TSC2 at sites distinct from those of AKT and stimulates GAP activity (Inoki et al. 2003b). However complete activation of TSC2 by AMPK signaling requires additional phosphorylations catalyzed by GSK3β (Inoki et al. 2006). Phosphorylation of TSC2 by AMPK serves as an essential priming site for these proximal phosphorylations by GSK3β. As available intracellular energy stores become diminished, TSC2 is activated and Rheb-GDP levels are increased, leading to suppression of TORC1 activity and growth.

Although TSC2 is clearly able to integrate positive and negative nutrient-derived inputs to regulate TORC1

activity, TSC2 does not function as a primary amino acid sensor (Roccio et al. 2006). *TSC2*$^{-/-}$ cells have intrinsically elevated levels of Rheb-GTP that are not altered upon depletion of amino acids from growth media. However, since Rheb-GTP association with TORC1 requires amino acid sufficiency, *TSC2*$^{-/-}$ cells grown in the absence of amino acids still have significantly attenuated TORC1 kinase activity (Roccio et al. 2006). Thus, amino acid regulation of TORC1 functions independently of TSC. Recent data have identified a new inhibitor of TORC1, FKBP38 (FKBP8), that is antagonized by Rheb-GTP and that mediates amino acid sensing (Bai et al. 2007).

FKBP38 is related to FKBP12 and may interact with TOR at the same site as the FKBP12-rapamycin complex. Overexpression of FKBP38 suppresses phosphorylation of S6 K and 4E-BP, but phosphorylation can be restored by overexpression of Rheb or by amino acid sufficiency (Fig. 3). Further data indicate that FKBP38 interaction with TOR is inhibited by amino acids and by Rheb-GTP, but not by Rheb-GDP (Bai et al. 2007). Under conditions of amino acid availability, there is an increase in FKBP38 interaction with Rheb-GTP and, in parallel, a decrease in FKBP38 interaction with TOR. These data follow observations that despite its role as a TORC1 activator, Rheb-GTP has a weaker association with TOR than does Rheb-GDP. Perhaps, not all aspects of amino acid control are mediated via FKBP38. While depletion of FKBP38 can increase cellular levels of p4E-PB and pS6 K during growth in nutrient-rich media, their de-phosphorylations are still induced by amino acid starvation. The precise sensor of amino acid sufficiency is still elusive, but may involve vps34 (vacuolar protein sorting 34), a class III-type PI3 K (Nobukuni et al. 2007).

Loss-of-function mutations in genes of the TSC/Rheb axis define several human syndromes that are characterized by hamartomas, severe cellular overgrowth in multiple organ systems, and, thus, appear linked to defects in TORC1 regulation (see Fig. 3). Tuberous Sclerosis is associated with mutations in the genes for either TSC1 or TSC2 that reduce TSC-GAP activity and, thus, limit TORC1 inhibition. Peutz-Jegher's syndrome results from loss-of-function mutations in the gene for the AMPK-activating kinase LKB1 and also has apparent deficiencies in TORC1 inhibition. Finally, Cowden disease is an autosomal, dominantly inherited syndrome associated with mutations in the gene for PTEN (phosphatase and tensin homolog), leading to the hyperactivation of AKT, a TORC1 activator.

Additional modes of AKT regulation

Data from several laboratories have revealed an activating mode for AKT regulation of TORC1 that functions independently of the TSC/Rheb network (Oshiro et al. 2007; Sancak et al. 2007; Vander Haar et al. 2007; Wang et al. 2007). PRAS40 is a proline-rich, AKT substrate of ~40 kDa; non-phosphorylated PRAS40 binds to TORC1 and inhibits TORC1 activity (Fig. 4). Overexpression of PRAS40 can reduce the elevated levels of S6 K phosphorylation in cells lacking TSC2, while depletion of PRAS40 will rescue S6 K phosphorylation that has been suppressed in cells ablated for Rheb. Phosphorylation of PRAS40 by AKT antagonizes its inhibitory action on TORC1 and, consequently, increases TORC1 activity (Fig. 4).

The PRAS40 pathway may partially resolve certain data that are not fully compatible with AKT functioning solely to regulate TORC1 via TSC2/Rheb. Although *Drosophila* that lack TSC2 exhibit embryonic lethality, re-expression of non-phosphorylatable forms of TSC2 in these mutant *Drosophila* restores normal growth properties (Dong and Pan 2004). Direct inhibition of TSC2 by AKT may not be essential provided there are additional AKT-dependent mechanisms for TORC1 activation, e.g. via suppression of Lobe, a potential PRAS40 ortholog in *Drosophila*. Still, this pathway may not be conserved among all eukarya. Functionally equivalent proteins have not been identified in yeast or *Dictyostelium*.

The interactions among the various kinases upstream of TSC2 indicate additional complexities. AKT can suppress TSC2 function independently of direct phosphorylation and inhibition (Fig. 5). AKT may decrease intracellular AMP/ATP ratios and, thus, deactivate AMPK (Hahn-Windgassen et al. 2005); GSK3β activity is inhibited by AKT phosphorylation. Mechanistically, these

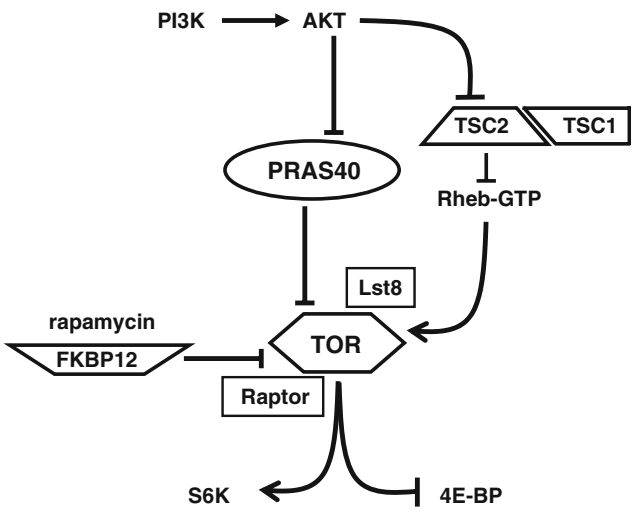

Fig. 4 PRAS40, an alternative, AKT-sensitive inhibitory pathway of TORC1. PRAS40 is a proline-rich, AKT substrate of ~40 kDa. Unphosphorylated PRAS40 can inhibit TORC1; upon phosphorylation by AKT, inhibition is relieved. PRAS40 is a mammalian protein. An equivalent protein may function in *Drosophila,* but is yet to be defined in evolutionarily more distant eukaryotes

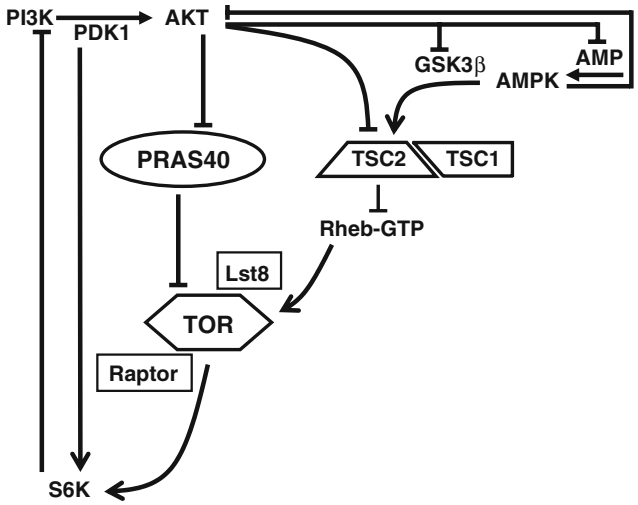

Fig. 5 Pathway cross-talk. Activated AKT can phosphorylate and inhibit GSK3β and may alter intracellular ATP levels to suppress AMP activation. Conversely, activated AMPK can feedback-inhibit AKT. Further, cells constitutively activated for TORC1 can exhibit downregulation of PI3 K/PDK1 activation of AKT, by a pathway that requires pS6 K. S6 K is activated by phosphorylations within the activation loop by PDK1 and within a hydrophobic motif (HM) by TORC1

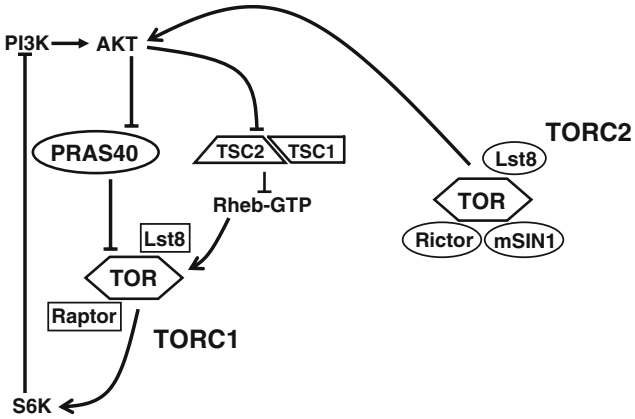

Fig. 6 Antagonistic actions of TORC1 and TORC2 toward AKT. AKT is phosphorylated within the activation loop by PDK1, and within a hydrophobic motif (HM) at the C terminus, as mediated by TOR Complex 2 (TORC2). TORC2 is comprised of TOR, Lst8, Rictor, and mSIN1. Rictor and mSIN1 are mammalian nomenclatures. In *Dictyostelium*, Rictor and mSIN1 are, respectively, called Pia and RIP3; their discoveries and functional linkages preceded that of mammalian cells

actions would limit TSC2 GAP activity even in the absence of its direct phosphorylation by AKT (Fig. 5).

Conversely, several feedback paths may cooperate to limit unregulated TORC1 action (Fig. 5). As an example, activated AMPK may suppress AKT activity. More importantly, cells lacking TSC2 are predicted to have hyperactivated S6 K, even in the presence of functional PRAS40. However, unregulated S6 K serves to limit insulin receptor signaling and, thus, PI3 K-mediated activation of AKT (Shah et al. 2004). Since S6 K is activated by phosphorylations within the activation loop by PDK1 and within a hydrophobic motif (HM) by TORC1, diminished PI3 K-signaling via PDK may additionally impact S6 K.

One supplementary input to AKT should be noted (Fig. 6). It may be deduced from the "TOR Complex 1" nomenclature that TOR can function within another multiprotein complex. TOR Complex 2 (TORC2) is comprised of TOR and Lst8, as in TORC1, but it lacks Raptor (Hara et al. 2002; Kim et al. 2002, 2003; Loewith et al. 2002; Sarbassov et al. 2004; Lee et al. 2005; Jacinto et al. 2006; Yang et al. 2006). Instead, TORC2 has two additional proteins Rictor (rapamycin-insensitive companion of mTOR), termed Pianissimo (Pia) in *Dictyostelum*, and mSIN1 (Ras-interacting protein 3 (RIP3) in *Dictyostelum*); mSIN1 derives its name from sequence similarity to the *S. pombe* protein SIN1, SAPK (stress-activated MAP kinase) interacting protein 1 (Wilkinson et al. 1999). Phenotypes resulting from the selective inactivation of TORC1 or TORC2 are largely distinct.

TORC1 and TORC2 components have separate substrate specificities. In addition to the phosphorylation of AKT by PDK1, a hydrophobic motif (HM) within the C-terminus of AKT is also phosphorylated; HM phosphorylation of AKT is mediated by TORC2, and not be TORC1 (Sarbassov et al. 2005; Guertin et al. 2006; Jacinto et al. 2006; Yang et al. 2006). The phosphorylations of AKT via PDK1 and TORC2 occur independently of each other in mammalian cells, but both are required for full enzymatic activation of AKT. Nonetheless, it is suggested that phosphorylation by PDK1 may be the more critical for control of growth (Hietakangas and Cohen 2007). Loss of HM phosphorylation by inactivation of TORC2 impairs the ability of AKT to act on only a limited subset of substrates, e.g. FoxO (forkhead box O class) transcription factors. In contrast, AKT phosphorylation of TSC2 and GSK3β is unaffected by loss of HM phosphorylation.

Perspectives: speculations on feedback connections among TORC1, amino acid metabolism, and homeostasis

Amino acid signaling is clearly a crucial upstream component for the functional activation of TORC1 to promote growth, but several responses also interpose TORC1 in the maintenance of amino acid and energy homeostasis upon nutrient stress (Arsham and Neufeld 2006). Thus, autophagy, the highly regulated process that recycles essential cellular components by directed degradation, is suppressed under nutrient-rich conditions, but is activated if amino acids and energy stores are depleted. Additional perspectives suggest

that the downregulation of TORC1 in response to nutrient stress can promote other pathways which may stimulate bioenergy recovery in mammalian cells (Phang et al. 2008).

Studies in yeast provide an interesting background that connects the downregulation of TORC1 to amino acid mobilization, apart from autophagy. When yeast are transferred from sources rich in nitrogen (e.g. glutamine and ammonia) to nitrogen-poor media (e.g. proline and urea), signaling cascades become activated that stimulate the import of nitrogen metabolites and their conversion to more useable precursors. A direct role for TORC1 in this process had been implied as rapamycin treatment induces the transcriptional upregulation of genes necessary for the increased uptake of such metabolites (Cardenas et al. 1999; Hardwick et al. 1999). These induced genes encode GAP1 and AGP1 (general amino acid permeases), MEP2 (an ammonia permease), PUT4 (a proline permease), and CAN1 (an arginine permease), among others. Similarly, genes involved in the regulation of the allantoin (a urea precursor) utilization pathway (e.g. *DAL1*, *DAL1*, *DAL7*, *DAL80*), the proline utilization pathway (*PUT1*, *PUT2*), and the glutamine biosynthetic pathway (*GDH2*, *GLN1*) are also upregulated (Cardenas et al. 1999; Hardwick et al. 1999).

The expression of nitrogen source utilization genes in yeast is regulated by a family of GATA-type, zinc finger transcription factors, including Gln3 (Cooper 2002). In turn, TORC1 regulates the functional state of Gln3 by directing its cytosolic/nuclear localization. In rich media, Gln3 is excluded from the nucleus through association with the phosophorylated form of Ure2, a transcriptonal repressor. When cells are transferred to nitrogen poor sources or are treated with rapamycin, Ure2 becomes dephosphorylated; Gln3 protein is released from the cytosolic Ure2-Gln3 complex, enters into nucleus, and thus activates transcription of *GAP1*, *MEP2*, etc. and, consequently, amino acid transport (Mitchell and Magasanik 1984; Daugherty et al. 1993; Blinder et al. 1996; Beck and Hall 1999; Kulkarni et al. 2001). The regulated phosphorylation/de-phosphorylation of Ure2 is inversely correlated with the relative activity of TORC1 (Cooper 2002); responses to diverse nitrogen sources are varied and, although TORC1 may play a central role, multiple additional regulators are involved (Saxena et al. 2003).

Linkage between amino acid metabolism and TORC1 suppression in mammalian cells has not been as well defined at the molecular level. However, a recent study may suggest a potential functional parallel for TORC1 regulation of cellular homeostasis in mammals (Phang et al. 2008). POX/PRODH is a proline oxidase (i.e. dehyrdogenase) that catalyzes the first step in proline degradation, utilizing proline to generate ATP (Phang et al. 2008)]. Indeed, the downregulation of TORC1 through

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Hs   MA--------LRRA--LPALRPCIPRFVQLSTAPASREQPAAGPAAVPGGGSAT------------------------    44
Dd   MIKNTVRIINKNSNTFINIRNNNNNNINSSLKSGFGTIKRFNTTLNHNSSNSNIQTPISINSTIINNNNNSNNNNSNNI    79

Hs   ------------------------------------------------------AVRPPVPAVDFGNAQEAYRSRRTWE    69
Dd   INNDLNVVKFSTISTPNSILDTLNENHSNQTNNVNNKNYNNNNNNFEKDDKFGPPNNQNNNKLDL-DTSKLYVSKSTGE   157

Hs   LARSLLVLRLCAWPALLA-----RHEQLLYVSRKLLGQRLFNKLMKMTFYGHFVAGEDQESIQPLLRHYRAF----GVS   139
Dd   LFFTFMILKVCSINFISD-----NSQKFLNLFEKLGLSKPLNFFIKYSFFKQFCAGETIRETEIFTEKLNKL----GIG   227

Hs   AILDYGVEEDLSPEEAEHKEMESCTSAAERDGSGTNKRDKQYQAHWAFGDRRNGVISARTYFYANEA-KCDSHMETFLR   217
Dd   TILDYAIEEL------------------------------------------------------AGSSEGFDSVAENICE   253

Hs   CIEASGRVSDDGF--IAIKLTALGRPQFLLQFSEVLAKWRCFFHQMAVEQGQAGLAAMDTKLEVAVLQESVAKLGIASR   294
Dd   TIRVAAKNPTNSFSCV--KFTGLVTPSVLEKMNTLVS----------------------------NVTTNVSELPIES-   300

Hs   AEIEDWFTAETLGVSGTMDLLDWSSLIDSRTKLSKHLVVPNAQTGQLEPLLSRFTEEEELQMTRMLQRMDVLAKKATEM   373
Dd   --------------------NFNSPLDFYLNQSSSLMKQGS-----EPLL---TSKDIKEIKEFFNRMDKIFQLCHQR   350

Hs   GVRLMVDAEQTYFQPAISRLTLEMQRKFNV--EKPLIFNTYQCYLKDAYDNVTLDVELARREGWCF--GAKLVRGAYLA   448
Dd   GVPILVDAEQSYYQVAIHHLTMSYSIKYN--KEKPIIYNTYQMYLVNGMNVLKQHFELSSSQKFNFKLGAKIVRGAYMV   427

Hs   QERARAAEIGYEDPINPTYEATNAMYHRCLDYVLEELKHNAKA-KVMVASHNEDTVRFALRRMEELGLHPADHQVYFGQ   526
Dd   TESERSQRLSTENPVLPTIQDTHKSYNTALDFLLNQIKSDPNSIGLMIASHNEDSINLGTKLIKQYKIDPTNPNIQFGQ   506

Hs   LLGMCDQISFPLGQAGYPVYKYVPYGPVMEVLPYLSRRALENSSLMKGTHRERQLLWL--ELLRRLRTGNLFHRPA      600
Dd   LFGMADFLSFNLVDQHQRIFKYVPFGPVEEVLPYLIRRMHENKGFIGSNS-DKELFYLKKEIKRRLF              572
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Fig. 7 Sequence alignment of the proline oxidases (POX/PRODH) of *Dictyostelium discoideum* (*Dd*) and Human (*Hs*). Amino acid identities/similarities among all the proteins are in *red*. The conserved POX (proline dehyrogenase) catalytic domain is enclosed in a *box*

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Hs  MAAATGPS-------------------FWLGNETLKVPLALFALNRQRLCERL--RKNPAVQAG-SIVVLQQGGE   52
Dd  MSVHPRNQGKDDCCEKTHLDSQYSPGYYWLGNNTLKVPLVLHKENRQRLVSQILSKHKDQVKE-NSFILLEESGK   73

Hs  ETQRYCTDTGVLFLQESFFHWAFGVTEPGCYGVIDVDT-GKSTLFVPRLPASHATWMGKIHSKEHFKEKYAVDDV  126
Dd  STMQYDTDHEPLFKQERYFFWTFGSDIPDCFGIVGLDEQATSILCIPKLPAEYATWMGEIRSKEYYKSIFLVDQV  148

Hs  QYVDEIA----SVLTSQKPSVLLTLRGVNTDSGSVCREASFDGISK-FEVNNTILHPEIVESRVFKTDMELEVLR  196
Dd  LYVDEMM----DYLKSKNASTIYTILGTNTDSGSTFVEPQYPGLRETFNVNNTLLFPEIAECRVIKSPKEVEVIR  219

Hs  YTNKISSEAHREVMKAVKVGMKEYGLESLFEHYCYSRGGMRHSSYTCICGSGENSAVLHYGHAGAPNDRTIQNGD  271
Dd  YCVDASVSAHKHVMRKVKVGLKEYQCESEFLHHVYNEWGCRNVGYTCICAANKNSAVLHYGHAGEPNSATISENG  294

Hs  MCLFDMGGEYYSVASDITCSFPRNGKFTADQKAVYEAVLLSSRAVMGAMKPGDWWPDIDRLADRIHLEELAHMGI  346
Dd  FCLFDMGAEYHSYTADITCSFPATGKFSPEQRVVYQAVLDASVAVMEAMRPGVSWVDMHKLAERCILAALLKAGI  369

Hs  LSGSVDAMVQAHLGAVFMPHGLGHFLGIDVHDVGGYPEGVERIDEPGLRSLRTARHLQPGMVLTVEPGIYFIDHL  421
Dd  LVGDLQDLIANKIGSVFFPHGLGHFLGLDTHDVGGYLGDCQ----PKVHSLRTTRTLKAGMVITSEPGCYFINHL  440

Hs  LDEALADPARASFLNREVLQRFRGFGGVRIEEDVVVIDSGIELLT-CVPRTVEEIEACMAGCDKAFTPFSGPK   493
Dd  LTQALSNPETAKFFNLTELDKYRNIGGVRIEDDILVTETGCDNLSKNLPRTIDEIEAFMLK               501
```

Fig. 8 Sequence alignment of the prolidases of *Dictyostelium discoideum* (*Dd)* and Human (*Hs*). Amino acid identities/similarities among all the proteins are in *red*

either nutrient stress or rapamycin treatment activates POX/PRODH expression. Thus, increased metabolism of proline may impact intracellular energy state recovery under nutrient poor growth conditions. Further, metabolic proline sources are suggested to derive endogenously from the prolidase-catalyzed degradation of collagen-rich extracellular matrices (Phang et al. 2008) as well as other pathways (Wu and Morris 1998).

To ascertain if TORC1-linked proline metabolism were a possible universal eukaryotic parameter, we investigated if proline metabolic enzymes were encoded in the genome of *Dictyostelium discoideum*, an ancient eukaryote that grows as a unicellular organism in nutrient-rich media, but that enters a developmental cycle upon nutrient withdrawal (Kimmel and Firtel 2004). Proline is not an essential amino acid in *Dictyostelium*, and *Dictyostelium* do not secrete or grow on collagen-like matrices. Regardless, the *Dictyostelium* genome encodes proteins that share extreme sequence similarity to human POX/PRODH (Fig. 7) and prolidase (Fig. 8). It remains to be determined if these genes are under functional control by TORC1 and, if indeed, TORC1 and growth suppression can be partially relieved by enhancing intracellular ATP levels through the stimulated degradation of proline. Still, the potential for additional modes of cross-regulation between TORC1 and amino acid response throughout the eukarya is intriguing and a worthy focus for additional intense investigation (Phang et al. 2008).

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