# TOR SIGNALLING: FROM BENCH TO BEDSIDE

*Michael N. Hall*

## *Introduction*

Cell growth is highly regulated. Cells respond to nutrients or other appropriate growth stimuli by upregulating macromolecular synthesis, and thereby increasing in size. Conversely, cells respond to nutrient limitation or other types of stress by downregulating macromolecular synthesis and enhancing turnover of excess mass. Thus, the control of cell growth involves balancing positive regulation of anabolic processes with negative regulation of catabolic processes. Growth is also controlled relative to cell division. In proliferating cells, growth is linked to the cell cycle such that most cells precisely double their mass before dividing. In other physiological contexts, such as load-induced muscle hypertrophy or growth factor-induced neuronal growth, cell growth is controlled independently of the cell cycle. Furthermore, in addition to the temporal control of cell growth described above, cell growth can be subject to spatial constraints. For example, budding yeast and neurons grow in a polarized manner as a result of new mass being laid down only at one end of the cell. Finally, in multicellular organisms, growth of individual cells is controlled relative to overall body growth such that the organs and tissues constituting the organism are properly proportioned.

What are the mechanisms that mediate and integrate the many parameters of cell growth? In other words, what determines that a cell grows only at the right time and at the right place? Remarkably, the study of these mechanisms has been largely neglected, despite their clinical relevance and despite cell growth being, along with cell division and cell death, one of the most fundamental (and obvious!) aspects of cell behavior. Also remarkable is the recent finding that cell growth control, regardless of the eukaryotic organism or the physiological context,

seems always to involve the same protein – the target of rapamycin TOR protein – and its namesake signalling pathway (Jacinto and Hall, 2003). The TOR signalling pathway controls cell growth by activating an array of anabolic processes including protein synthesis, transcription, and ribosome biogenesis, and by inhibiting catabolic processes such as bulk protein turnover (autophagy) and mRNA degradation, all in response to nutrients (Schmelzle and Hall, 2000). Dysfunction of signalling pathways controlling cell growth results in cells of altered size and, in turn, causes developmental errors and a wide variety of pathological conditions. Thus, an understanding of the TOR signalling pathway may lead to novel drugs for the treatment of, for example, cancer, diabetes, inflammation, muscle atrophy, learning disabilities, depression, obesity, and ageing. Here I review the TOR signalling pathway and its control of cell growth, with particular emphasis on studies from our laboratory with the model organism *Saccharomyces cerevisiae,* also known as budding yeast.

## *Rapamycin*

The story of the TOR signalling pathway begins with an exceptional drug, rapamycin (Abraham and Widerrecht, 1996). Rapamycin is a lipophilic macrolide produced by *Streptomyces hygroscopicus,* a bacterium isolated from a soil sample collected in Rapa-Nui (Easter Island), hence the name rapamycin. Rapamycin was originally purified in the early 1970's as an antifungal agent, but was discarded because of its then undesirable immunosuppressive side effects. Only years later was it 'rediscovered' as an immunosuppressant. It received clinical approval in 1999 for use in the prevention of organ rejection in kidney transplant patients, and additional applications in immune and inflammatory diseases are envisioned. Furthermore, prelinical studies have shown that rapamycin and it derivatives, RAD001 (Novartis) and CCI-779 (Wyeth-Ayerst), strongly inhibit the proliferation of tumor cells in culture, and the two derivatives are now in advanced clinical trials as anti-cancer agents. The candidate anti-cancer drugs are performing particularly well against tumors deficient in the tumor suppressor TSC (tuberous sclerosis complex) (Kwiatkowski, 2003), PTEN (Neshat et al., 2001;

Podsypanina et al., 2001) or p53 (Huang and Houghton, 2003; Huang et al., 2003). Rapamycin is effective against tumors because it blocks the growth of tumor cells directly and because of the indirect effect of preventing the growth of new blood vessels (angiogenesis) that supply oxygen and nutrients to the tumor cells (Guba et al., 2002). Finally, recent trials have shown that rapamycin-eluting stents prevent restenosis after angioplasty. Thus, rapamycin has clinical potential in three major therapeutic areas: organ transplantation, cancer, and coronary artery disease.

What do the seemingly very different conditions of transplant rejection, cancer, and restenosis have in common in their underlying biology such that all three can be treated with the same drug? All three conditions are due to ectopic or otherwise undesirable cell growth, suggesting that the molecular target of rapamycin is a central controller of cell growth. The target of rapamycin is indeed dedicated to controlling cell growth, but what is this target and how does it control cell growth?

### *TOR and the control of cell growth in yeast*

Studies to identify the cellular target of rapamycin and to elucidate the drug's mode of action were initiated in the late 1980's by several groups. These early studies included our efforts, which took advantage of the antifungal activity of rapamcyin. At that time, rapamycin was known to inhibit the vertebrate immune system by blocking a signalling pathway in T cells that mediates cell cycle (G1) progression in response to the lymphokine IL-2. However, the molecular mode of action of the drug was not known other than it possibly involved binding to the proline isomerase (immunophilin) FKBP12 (Schreiber, 1991). We found that rapamycin similarly inhibited cell cycle progression in yeast, suggesting that the molecular target was the same in yeast and vertebrate cells, and that yeast cells could thus be exploited to identify the target of rapamycin (Heitman et al., 1991a). Although we were interested in understanding the mechanism of rapamycin action, our main interest was to use rapamycin as a probe to identify novel growth-controlling signalling pathways (Kunz and Hall, 1993). To study drug

action, we first focused on identifying a yeast FKBP (Heitman et al., 1991b). FKBP12 had previously been identified in mammalian cells as an *in vitro* receptor for rapamycin. Yeast FKBP was purified to homogeneity and partially sequenced. The protein sequence information was then used to isolate the FKBP gene. The predicted amino acid sequence of yeast FKBP was 54% identical to that of the concurrently cloned human FKBP12, providing further support that the mode of action of rapamycin was conserved from yeast to human. Curiously, disruption of the FKBP gene in yeast *(FPR1)* revealed that FKBP is not essential for growth (Heitman et al., 1991b). Additional FKBPs and cyclophilins (also an immunophilin and proline isomerase) were subsequently discovered and cloned, and again single and multiple disruptions were constructed without consequential loss of viability (Heitman et al., 1991b; Davis et al., 1992; Kunz and Hall, 1993; Heitman et al., 1992; Dolinski et al., 1997). The finding that FKBP disruptions are not lethal was puzzling because FKBP was believed to be the *in vivo* binding protein/target for the toxic effect of rapamycin. However, our subsequent finding that an *FPR1* disruption confers rapamycin resistance (Heitman et al., 1991a; 1991b), combined with the observation by others that some drug analogs are not immunosuppressive despite still being able to bind and inhibit FKBP12 (Schreiber, 1991), led to the currently accepted model of immunosuppressive drug action: an immunophilin-drug complex (e.g., FKBP-rapamycin) gains a new toxic activity that acts on another target molecule (Heitman et al., 1992). This mode of drug action also applies to the well known immunosuppressants cyclosporin A and FK506 (form cyclophilin-cyclosporin A and FKBP-FK506 complexes), and is conserved from yeast to human. These early studies in yeast were the first of many that have since contributed to an understanding of rapamycin action and TOR signalling in mammalian cells (Crespo and Hall, 2002), indicating that studies with model systems are valuable.

To identify the target of the FKBP-rapamycin complex, rapamycin resistant yeast mutants were selected (Heitman et al., 1991a). As expected, *fpr1* mutants defective in FKBP were recovered, but also mutants altered in two novel genes, *TOR1* and *TOR2* (target of rapamycin), were obtained. The *TOR1* and *TOR2* genes were cloned, based on a dominant

rapamycin-resistance phenotype of the mutant alleles, and sequenced (Kunz et al., 1993; Helliwell et al., 1994). The TOR1 and TOR2 proteins are both 282 kDa in size and 67% identical. TOR1 and TOR2 were also the founding members of the PI kinase-related protein kinase (PIKK) family of ser/thr-specific kinases (other members include ATM, DNA-PK and MEC1). Disruption of *TOR1* and *TOR2* in combination caused a growth arrest similar to that caused by rapamycin treatment, suggesting that TOR1 and TOR2 are indeed the targets of FKBP-rapamycin and that the FKBP-rapamycin complex inhibits TOR activity (Kunz et al., 1993). It was subsequently demonstrated that the FKBP-rapamycin complex binds directly to TOR1 and TOR2 (Stan et al., 1994; Zheng et al., 1995), and that TOR is widely conserved both structurally and as the target of FKBP-rapamycin (Schmelzle and Hall, 2000).

In the mid-'90s, we focused on elucidating the cellular roles of TOR1 and TOR2. We found that TOR1 and TOR2 play a central role in controlling cell growth as part of two separate signalling branches (Figure 1). Although structurally similar, TOR1 and TOR2 are not functionally identical (Kunz et al., 1993; Helliwell et al., 1994). Combined disruption of *TOR1* and *TOR2,* or rapamycin treatment, mimics nutrient deprivation and causes a G0 growth arrest within one generation (Barbet et al., 1996). Disruption of *TOR1* alone has little or no effect, and disruption of *TOR2* alone causes cells to arrest growth within a few generations as small-budded cells in the G2/M phase of the cell cycle and with a randomized actin cytoskeleton (Schmidt et al., 1996; Helliwell et al., 1998a). These and other findings led to the model that TOR2 has two essential functions; one function is redundant with TOR1 (TOR-shared), and the other function is unique to TOR2 (TOR2-unique). These two TOR2 functions are two separate signalling branches that control cell growth in different ways (Barbet et al., 1996; Schmidt et al., 1997; Helliwell et al. 1998a; Bickle et al., 1998; Schmidt et al., 1998). The above findings also led to the discovery that TOR controls cell growth in response to nutrients (Barbet et al., 1996). Subsequent studies demonstrated that TOR in higher eukaryotes also controls cell growth in response to nutrients, i.e., TOR is conserved in structure and function (Thomas and Hall, 1997; Schmelzle and Hall, 2000).

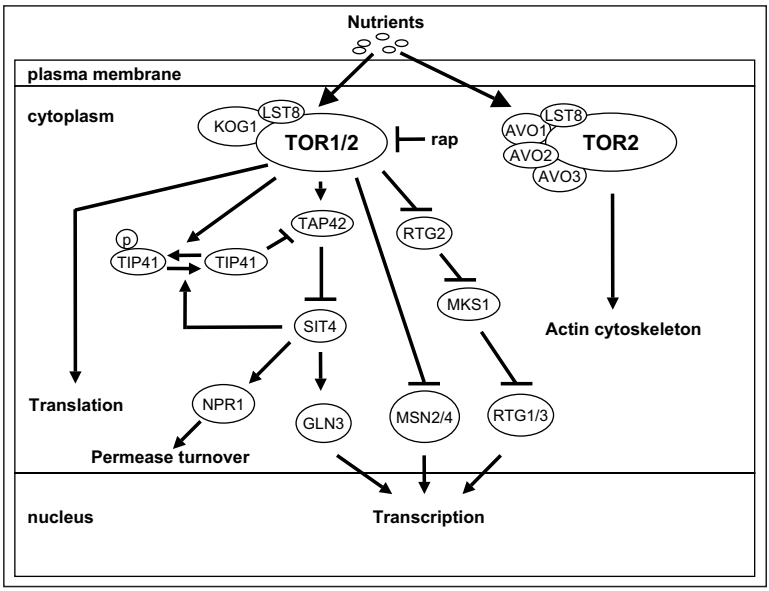


*Figure 1: TOR signalling and control of cell growth in yeast. TOR complex 1 (TORC1) and TOR complex 2 (TORC2) control cell growth via two major signalling branches. The TORC1 (TOR1 or TOR2, LST8, KOG1) branch mediates temporal control of cell growth (Translation, Transcription, etc.). The TORC2 (TOR2, LST8, AVO1-3) branch mediates spatial control of cell growth (Actin cytoskeleton). Only the TORC1 signalling branch is sensitive to rapamycin (rap) as FKBP-rapamycin binds only to TORC1 (Loewith et al., 2002).*

Since the late '90s, we have been characterizing the two separate TOR2 signalling branches. We have found that the TOR-shared signalling branch is composed of various effector pathways that control a wide variety of readouts which collectively determine the mass of the cell (Figure 1). The readouts controlled by this branch include protein synthesis and degradation, mRNA synthesis and degradation, ribosome biogenesis, nutrient transport, and autophagy (Schmelzle and Hall, 2000). We view this branch as mediating the temporal control of cell growth. Some of the effector pathways that make up this branch are known, but there are some (many?) which are not yet known, such as the pathways via which TOR controls ribosome biogenesis or mRNA

degradation. The TOR2-unique branch so far contains a single effector pathway that controls the polarized organization of the actin cytoskeleton. We view this branch as mediating the spatial control of cell growth.

The TOR-shared signalling branch mediates the temporal control of cell growth by positively regulating, among other readouts, translation initiation and transcription while negatively regulating a program specific to starved (G0) cells. An early observation that led to defining this branch was the finding that loss of TOR function (TOR depletion or rapamycin treatment) causes a downregulation of translation initiation, an early G1 arrest, and all other known characteristics of the G0 program (Barbet et al., 1996). A G1 cyclin mRNA whose translational control is altered by substitution of the *UBI4* 5' leader region (the polyubiquitin UBI4 is normally transcribed and translated in G0) suppressed the rapamycin-induced G1 arrest and conferred starvation sensitivity. This suggested that a TOR-shared signalling pathway is dedicated to translational control and that the cell cycle arrest is a secondary consequence of a translation (growth) defect. Cells need to grow to divide. We have proposed that TOR controls translation via the cap-binding translation initiation factor eIF4E (Barbet et al., 1996, Cosentino et al., 2000), although there are also other targets through which TOR controls translation (Berset et al., 1998; Cherkasova et al., 2003). How TOR may control eIF4E in yeast is unknown. TOR in mammalian cells controls eIF4E and translation initiation via the eIF4E inhibitor 4E-BP (Beretta et al., 1996).

TOR positively controls transcription of Pol I, II and III-dependent genes involved in ribosome biogenesis, among other genes (Zaragoza et al., 1998; Powers and Walter, 1999; Hardwick et al., 1999; Cardenas et al., 1999). How TOR controls these genes is unknown. However, TOR also negatively controls many genes whose products are needed to alter cellular metabolism in response to nutrient starvation. TOR negatively controls nuclear localization of the nutrient-responsive GATA transcription factors GLN3 and GAT1 and of the general stress-responsive transcription factors MSN2 and MSN4 (Beck and Hall, 1999; Crespo et al., 2001). The demonstration that TOR controls these transcription factors indicated that TOR signals into the nucleus, and does

not control only cytoplasmic events as previously thought. TOR inhibits nuclear localization of the GLN3 and RTG1/3 transcription factors in response to glutamine (Crespo et al., 2002), the preferred nitrogen source for yeast cells. TOR appears to regulate different transcription factors in response to the quality and quantity of different nutrients, including the nitrogen and carbon sources. How TOR senses and discriminates between different nutrients to elicit a response appropriate to a given condition is unknown. Schreiber and colleagues have referred to TOR as a multichannel processor (Shamji et al, 2000).

The TOR-shared signalling branch also controls the stability of high affinity nutrient permeases. In particular, TOR maintains the tryptophan, glucose and, most likely, other of nutrient permeases in the plasma membrane (Schmidt et al., 1998; Beck et al., 1999; Schmelzle et al., 2003). Upon TOR inactivation, by rapamycin treatment or nutrient starvation, these permeases are ubiquitinated, transported to the vacuole, and degraded. Thus, TOR ensures intracellular nutrient availability as required for growth. Furthermore, by preventing the turnover of amino acid permeases, TOR is ensuring the availability of amino acids and thereby yet again contributing to protein synthesis.

How does TOR signal to the readouts described above? TOR signals to GLN3 and the tryptophan permease via TAP42 and the type 2A phosphatase SIT4 (Di Como and Arndt, 1996; Beck et al., 1999; Schmidt et al., 1998; Jacinto et al. 2001; Jiang and Broach, 1999). TOR promotes the association of TAP42 with SIT4 and thereby maintains the phosphatase inactive. Upon TOR inactivation, SIT4 dissociates from TAP42, becomes active, and dephosphorylates GLN3 and the kinase NPR1. Dephosphorylated GLN3 is released from its cytoplasmic anchor URE2 and moves into the nucleus to activate its target genes. Dephosphorylated and activated NPR1 triggers the turnover of at least the tryptophan permease TAT2, possibly by phosphorylating TAT2 directly.

How does TOR control the association of TAP42 with the phosphatase SIT4? TOR controls the TAP42-SIT4 association via the conserved, TAP42-interacting protein TIP41 (Jacinto et al., 2001). Deletion of the *TIP41* gene confers rapamycin resistance, suppresses a *tap42* mutation,

and prevents dissociation of SIT4 from TAP42. Furthermore, a *TIP41* deletion prevents SIT4-dependent events such as dephosphorylation of the kinase NPR1 and nuclear translocation of the transcription factor GLN3. Thus, TIP41 negatively regulates the TOR pathway by binding and inhibiting TAP42. The binding of TIP41 to TAP42 is stimulated upon rapamycin treatment, via SIT4-dependent dephosphorylation of TIP41, suggesting that TIP41 is part of a feedback loop that rapidly amplifies SIT4 phosphatase activity under TOR-inactivating conditions. Interestingly, TOR in mammalian cells also appears to control the phosphorylation state of its downstream effector S6K by inhibiting a type 2A phosphatase (Peterson et al., 1999). Thus, inhibition of phosphatase activity may be a general mechanism of TOR signalling.

The TOR2-unique signalling branch controls the cell cycle-dependent organization of the actin cytoskeleton and thereby mediates spatial control of cell growth (Figure 1). The first indication that TOR2 is linked to the actin cytoskeleton came from the isolation of *TCP20,* encoding an actin-specific chaperone, as a dosage suppressor of a dominant-negative TOR2 "kinase-dead" mutation (Schmidt et al., 1996). This, in turn, led to the discovery that *tor2* mutants display an actin organization defect (Schmidt et al., 1996). The subsequent isolation of *sac7,* encoding a Rho-GAP (GTPase activating protein), as a second site suppressor of a *tor2*-ts mutation, suggested that TOR2 was linked to the actin cytoskeleton via a signalling pathway containing a RHO GTPase. It was later demonstrated that SAC7 is indeed a GAP for RHO1 and that TOR2 activates the RHO1 switch via the RHO1-GEF (guanine nucleotide exchange factor) ROM2 (Schmidt et al., 1997; Bickle et al., 1998). ROM2 GEF activity is reduced in extracts from a *tor2*-ts mutant (Schmidt et al., 1997). The finding that overexpression of ROM2 suppresses a *tor2*-ts mutation whereas overexpression of catalytically active ROM2 lacking its lipid-binding PH domain does not suppress suggested that TOR2 signals to ROM2 via the PH domain. We next examined through which RHO1 effector (FKS, PKC1, BNI1 or SKN7) TOR2 signals to the actin cytoskeleton. TOR2 signals to the actin cytoskeleton mainly, if not exclusively, via the RHO1 effector PKC1 (protein kinase C) and the PKC1-controlled cell integrity MAP kinase cascade (Helliwell et al., 1998b).

How does TOR2 signal to ROM2 to activate the RHO1 GTPase switch? The PH domain in ROM2 suggests that the mechanism of ROM2 activation may involve a lipid intermediate. This possibility is supported by the observation that overexpression of the PI-4-P 5-kinase MSS4 suppresses a *tor2*-ts mutation (Helliwell et al., 1998a; Desriviéres et al., 1998). However, this model has yet to be confirmed nor has a mechanism by which TOR2 activates ROM2 been elucidated, despite extensive investigation of lipid signalling pathways (Inagaki et al., 1999; Friant et al., 2001; Desriviéres et al., 2002) and of other RHO1-related regulatory proteins (Schmelzle et al., 2002; Schmidt et al., 2002).

The elucidation of two major TOR signalling branches that integrate temporal and spatial control of cell growth was a major step toward understanding TOR signalling and cell growth control. However, the two signalling branches have also raised some new and interesting questions. For example, what are the molecular determinants of the specificity and diversity of TOR signalling? Why can TOR2 signal in both branches whereas TOR1 is restricted to only one branch? Furthermore, how does TOR sense and discriminate nutrients? To answer these questions, we recently purified TOR1 and TOR2 in the hope of identifying co-purifying accessory proteins that mediate TOR function. Two functionally and structurally distinct, membrane-bound TOR complexes were identified (Kunz, et al., 2000; Loewith et al., 2002) (Figure 1). TOR complex 1 (TORC1) contains TOR1 or TOR2, LST8 and the uncharacterized, essential protein KOG1. TOR complex 2 (TORC2) contains TOR2, LST8 and the uncharacterized proteins AVO1, AVO2 and AVO3. As suggested by the fact that TORC1 contains either TOR1 or TOR2 whereas TORC2 contains only TOR2, TORC1 mediates the TOR-shared signalling branch and TORC2 mediates the TOR2-unique signalling branch. We also showed that TORC1 is conserved in mammalian cells (Loewith et al., 2002). mTOR forms a complex with raptor (also known as mKOG1) and mLST8, the mammalian orthologs of KOG1 and LST8, respectively. In parallel with our studies, Hara et al. (2002) and Kim et al. (2002) discovered mKOG1 as an mTOR interactor and named it raptor. It remains to be determined whether TORC2 is conserved. The molecular functions of the individual TOR partner proteins are not known, but most of the partners are essential proteins (only

AVO2 is not essential) and likely play an important role in determining the specificity and diversity of TOR sensing or signalling. Thus, the identification of TORC1 and TORC2 has been a significant step in the ongoing characterization of TOR signalling. Our current and future work focuses largely on further characterizing the two TOR complexes and on elucidating the roles of the TOR partner proteins. We hope thereby to determine the molecular mechanism by which TOR senses and signals nutrient availability.

*TOR signalling in mammals*

TOR and TOR signalling are conserved from yeast to human, including worms, flies and plants. Mammalian TOR (mTOR, also known as FRAP, RAFT and RAPT) was identified biochemically via its FKBP12-rapamycin binding activity (Brown et al., 1994; Chiu et al., 1994; Sabatini et al., 1994; Sabers et al., 1995). Strong evidence that these biochemical approaches had indeed identified the *in vivo* target of FKBP12-rapamycin was that the isolated protein was homologous to the previously identified yeast TOR proteins. mTOR, in a complex with its conserved partner proteins raptor and mLST8 (mTORC1), controls protein synthesis and thereby cell growth via the two effectors S6 kinase (S6K) and 4E-BP (Gingras et al., 2001). mTOR phosphorylates and activates S6K, and phosphorylates and inactivates the eIF4E-inhibitor 4E-BP. Like TOR in yeast cells, mTOR controls cell growth in response to nutrients, amino acids in particular (Kimball and Jefferson, 2002; van Sluijters et al., 2000). Thus, the TOR signalling pathway appears to be a primordial pathway that has been conserved throughout eukaryotic evolution to control the fundamental process of cell growth.

In mammalian cells, growth is stimulated by a combination of nutrients and growth factors. Accumulating evidence indicates that mTOR mediates signalling in response to both stimuli. The mTOR pathway mediates phosphatidylinositol 3-kinase (PI3K)-dependent growth factor signalling. Studies using PTEN deficient cancer cells, in which the PI3K pathway is activated due to upregulation of the lipid second messenger phosphatidylinositol (3,4,5)$P_3$ (PtdInsP3), indicate that

inhibition of mTOR blocks signalling through the PI3K pathway (Neshat et al., 2001; Podsypanina et al., 2001). Reduction of neoplastic proliferation and tumor size by inhibition of mTOR in PTEN deficient cells is largely due to inhibition of S6K. The targets of mTOR, S6K and 4E-BP, are also components of the insulin-PI3K-PDK1 (3-phosphoinositide dependent protein kinase-1)-PKB (protein kinase B) pathway.

What is the nature of the link between mTOR and the insulin signalling pathway in the control of S6K and 4E-BP? mTOR and the insulin signalling pathway converge on S6K and 4E-BP to mediate the complex, hierarchical phosphorylation of these common downstream targets (Gingras et al., 2001; Alessi et al., 1998; Pullen at al., 1998). However, recent evidence suggests that the insulin pathway also impinges on mTOR signalling upstream of mTOR. Genetic evidence in flies and biochemical evidence in mammalian cells indicate that the TSC1–TSC2 protein complex inhibits TOR (Gao et al., 2002; Inoki et al., 2002; Tee et al., 2002). TSC1 (also known as hamartin) and TSC2 (tuberin) are products of the tumor suppressor genes *TSC1* and *TSC2,* mutations in which cause tuberous sclerosis. The TSC1–TSC2 complex is inactivated in response to insulin via PKB-mediated phosphorylation of three sites in TSC2 (Potter et al., 2002). Furthermore, mTOR seems to respond positively to phosphatidic acid (PA) and ATP, both of which are produced in response to growth factors (Dennis et al., 2001; Fang et al., 2001). Thus, mTOR signalling is controlled by growth factor inputs upstream and downstream of mTOR.

As TOR controls cell growth in yeast and plants, which lack a PI3K signalling pathway, the PI3K pathway was grafted onto the TOR pathway only late in evolution. The joint control by the mTOR and PI3K pathways meets the need of multicellular animals to coordinate growth of individual cells with overall body growth such that a properly proportioned organism is obtained. Multicellularity evolved independently in plants and animals, and plants must use another strategy to coordinate growth. Interestingly, worms have both TOR and a PI3K pathway, but, in this case, the PI3K pathway does not seem to be involved in controlling cell growth (Long et al., 2002).

Similar to yeast TOR, mTOR also responds to the availability of nutrients. Branched chain amino acids, particularly leucine, activate the mTOR signalling pathway (Kimball and Jefferson, 2002; van Sluijters, 2000). A high level of ambient amino acids, even in the absence of insulin, promotes phosphorylation and activation of S6K, without activating PKB. Withdrawal of amino acids leads to rapid dephosphorylation of S6K and 4E-BP, which mimics rapamycin treatment. Furthermore, rapamycin treatment renders the phosphorylation of S6K refractory to amino acid stimulation (Hara et al., 1998). Although amino acids seem to regulate the mTOR pathway, the effect of amino acids on mTOR kinase activity is unclear. Dennis et al (2001) report that amino acids have no effect on mTOR kinase activity toward S6K, as assayed with mTOR immunopurified from amino acid stimulated cells. However, Kim et al (2002) have performed similar experiments, but with conditions that favor the inclusion of the newly identified mTOR interactor raptor. In the presence of raptor, mTOR kinase activity is increased when cells are stimulated with leucine (Kim et al., 2002). Consistent with the above observations, raptor has been proposed to function as a scaffold protein that links mTOR to S6K and 4E-BP (Hara et al., 2002). mTOR has also been proposed to respond to a mitochondrial signal, but this signal could be simply amino acids that are synthesized in the mitochondria.

How might mTOR kinase activity be regulated by amino acids? The recent observations that loss of the TSC1–TSC2 complex results in an increase in S6K activity and renders cells resistant to amino acid starvation suggest that amino acids, as well as growth factors, signal to TOR via inhibition of the TSC complex (Inoki et al., 2002). The finding that nutrient deprivation stabilizes the mTOR-raptor association and inhibits mTOR kinase activity suggests that nutrients might regulate mTOR via raptor (Kim et al., 2002), although the effect of nutrients on the mTOR-raptor association is debated (Hara et al., 2002; Loewith et al., 2002). The precise roles of raptor, TSC1/2 and possibly mLST8 in controlling mTOR activity in response to amino acids remain to be determined. However, as S6K and 4E-BP phosphorylation in response to both insulin and amino acids is rapamycin sensitive, mTOR integrates nutrient and insulin signals to control cell growth. Interestingly, as the production of

insulin as well as the response to insulin is sensitive to nutrients, mTOR as a nutrient sensor might be involved in regulating insulin production in addition to the response to insulin (McDaniel et al., 2002).

The studies reviewed above concern the role of TOR in controlling growth of proliferating cells. However, in some cells, such as muscle and neuronal cells, growth can occur in the absence of cell division. An increased workload on a muscle causes an increase in muscle mass. This increase in mass, or hypertrophy, is due to an increase in the size of individual muscle cells. A reduced workload, ageing, or myopathies lead to atrophy, a loss of skeletal muscle mass due to shrinkage of individual muscle cells. How a mechanical stimulus is propagated as a chemical signal, and the molecular mechanisms underlying skeletal muscle hypertrophy are poorly understood. However, muscle hypertrophy requires an increase in the rate of protein synthesis and signalling via the mTOR and the insulin-like growth factor (IGF-1) pathways (Rommel et al., 2001; Bodine et al., 2001; Musaro et al., 1999; Semsarian et al., 1999). Treatment with rapamycin prevents IGF-1-induced hypertrophy, whereas expression of activated PKB promotes hypertrophy (Rommel et al., 2001; Bodine et al., 2001; Pallafacchina et al., 2002). Increased phosphorylation of mTOR at Ser2448, a site that is phosphorylated by PKB *in vitro,* also occurs during muscle hypertrophy (Reynolds et al., 2002). The mTOR effector S6K shows rapamycin-sensitive increase in phosphorylation during hypertrophy (Baar and Esser, 1999). Thus, the mTOR and IGF-1 pathways seem to increase the size of skeletal muscle cells through the activation of protein synthesis.

A role of the mTOR and PKB pathways in muscle cell growth is further supported by the finding that transgenic mice that overexpress activated PKB specifically in the heart have enlarged cardiomyocytes, which results in a larger heart (Shioi et al., 2002). The PKB-induced size increase is rapamycin-sensitive, indicating that the effect is mediated by mTOR. The observed increase in myocyte size is independent of cell division, as an increase in heart size occurs when myocytes are in a postmitotic state. Taken together, the above studies on muscle cells show how both cell and organ size are controlled by mTOR, and provide an example of how mTOR can control growth in non-proliferating cells.

Memory formation is achieved by changes in synaptic strength or plasticity, a process which involves long-term potentiation (LTP) or, in the snail *Aplysia,* long-term facilitation (LTF). Studies in *Aplysia* have shown that, despite nuclear events, long-term changes in synaptic function and structure are confined to a stimulated synapse and require local protein synthesis from pre-existing mRNAs. Thus, proteins are synthesized and deposited specifically at a stimulated synapse, which in turn leads to the synapse-specific growth that is necessary for encoding memory (Martin et al., 1997; Casadio et al., 1999). As the control of protein synthesis is an important aspect of LTP/LTF, it is perhaps not surprising that mTOR is involved in LTP/LTF (Casadio et al., 1999; Tang et al., 2002). The induction of LTF in invertebrates and LTP in hippocampal slices by electrical stimulation or by brain-derived neurotrophic factor stimulation is inhibited by rapamycin (Tang et al., 2002; Takei et al., 2001). Post-synaptic protein synthesis and S6K activity in response to a stimulus are also inhibited by rapamycin (Khan et al., 2001; Raymond et al., 2002). Finally, components of the mTOR and insulin pathways are enriched at post-synaptic sites (Tang et al., 2002; Abbott et al., 1999). These findings show that mTOR controls synaptic protein synthesis and so also memory formation in response to a stimulus. Furthermore, this is an interesting example of mTOR controlling cell growth in a localized manner and, again, in non-proliferating cells. mTOR may also control overall neuronal growth as deletion of PTEN in the brain results in enlargement of neuronal cells (Backman et al., 2002).

Does mTOR control the size of proliferating cells? Cultured mammalian cells treated with rapamycin have a reduced proliferative rate and are smaller at all stages of the cell cycle (Fingar et al, 2002). RNAi-mediated inhibition of mTOR or the mTOR partner protein raptor also leads to decreased size in proliferating cells (Kim et al., 2002). Finally, S6K1 deficient mice have smaller pancreatic β-cells as a result of a growth defect during embryogenesis (Pende et al., 2000). Thus, mTOR also seems to control cell size in proliferating mammalian cells, which is consistent with the previous finding that *Drosophila* mutants defective in dTOR (*Drosophila* TOR) have smaller cells (Oldham et al., 2000; Zhang et al., 2000).

*Future directions*

The studies reviewed above describe the role of TOR in controlling the growth of individual cells. In other words, they describe the pathways and mechanisms by which TOR controls the growth of the cell in which it resides. Interestingly, Colombani et al. (2003) have recently found that TOR, through a humoral mechanism, also controls the growth of distant cells. Colombani et al. (2003) induced amino acid starvation specifically in the *Drosophila* fat body. The fat body is a tissue with important storage and humoral functions associated with nutrition, comparable to the vertebrate liver and adipose tissue. They found that amino acid-responsive dTOR signalling in the fat body modulates insulin signalling and growth in peripheral tissues. This is a major advance in the field because it represents a new, humoral mode of TOR-mediated growth control, and also because it provides a mechanism by which the growth of cells in different tissues is coordinated such that overall body growth is properly balanced. These findings also have interesting implications when considering other hormone- and nutrient-related processes, such as appetite regulation and ageing. Appetite and lifespan are regulated by hormones produced in response to nutrients. However, the nutrient sensor that signals the production of such hormones is not known. The findings of Colombani et al. (2003) suggest that TOR might be such a sensor. Thus, TOR may play a here-to-fore unknown and key role in the control of appetite and lifespan. Indeed, a role for TOR in the control of appetite and ageing is supported by the finding that TOR modulates insulin signalling which has already been implicated in the control of food intake and lifespan (Spiegelman and Flier, 2001; Guarente and Kenyon, 2000; Clancy et al. 2001).

TOR biology is of interest to both the research scientist and the clinician. As discussed earlier in this review, the study of TOR began with a clinically relevant drug, rapamycin, whose mode of action was unknown. Research scientists became interested in rapamycin not only to elucidate the drug's target and mode of action, but also because they viewed it as a valuable probe to identify a growth controlling signal transduction pathway. As befits a story with a happy ending, the scientists did indeed discover an interesting and fundamentally important

signalling pathway. But, this was not the true end of the story. Much basic science remains to be done on TOR signalling and the control of cell growth. The challenge also remains to translate our knowledge of TOR signalling into new and better drugs. Rapamycin or its derivatives are proving useful in the treatment of transplant rejection, cancer and restenosis. The new findings coming from the laboratory bench now suggest that TOR-targeted drugs could have many additional bedside applications, for example, the treatment of diabetes, muscle atrophy, learning disabilities, depression, obesity and even ageing.

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