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TOR, a Central Controller of Cell Growth

Review

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Summary
Cell growth (increase in cell mass) and cell proliferation (increase in cell number) are distinct yet coupled processes that go hand-in-hand to give rise to an organ, organism, or tumor. Cyclin-dependent kinase(s) is the central regulator of cell proliferation. Is there an equivalent regulator for cell growth? Recent findings reveal that the target of rapamycin TOR controls an unusually abundant and diverse set of readouts all of which are important for cell growth, suggesting that this conserved kinase is such a central regulator.

Background
In the late 1960s, Lee Hartwell isolated approximately 400 temperature-sensitive yeast mutants which he then characterized for macromolecular synthesis (protein and RNA synthesis), cell division, and cell morphology (Hartwell, 1967). He subsequently chose to focus on the mutants that displayed defects in cell division, the now famous *cdc* mutants. Importantly, Hartwell made a distinction between cell proliferation and cell growth. Proliferation is cell division which leads to an increase in cell number, whereas growth is macromolecular synthesis which leads to an increase in cell mass or size. A fortunate consequence of Hartwell's decision to focus on cell division was that we now have a relatively sophisticated understanding of the mechanisms that control the cell cycle. An inadvertent and unfortunate fallout from this decision, however, was that the study of cell growth, and in particular the mechanisms that control it, have been largely neglected. To make matters worse and confusing, the distinction between growth and proliferation has also been lost; it is common to find in the literature the terms "growth" and "proliferation" incorrectly used interchangeably. Cell growth and cell proliferation are indeed separable and thus distinct processes, as revealed by Hartwell's mutants and others' studies (Hartwell, 1967; Mitchison, 1971; Thomas and Hall, 1997; Neufeld and Edgar, 1998; Conlon and Raff, 1999).

Growth is not simply an accumulation of mass. It is a carefully orchestrated accumulation of mass, occurring only at specific times and places. In the case of Hartwell's unicellular yeast, growth occurs only when nutrients are available and only at a discrete site on the cell surface (hence the name budding yeast). In neurons, growth in response to synaptic activity occurs specifically at the synapse. In metazoans, the problem of growth is compounded by the need to adhere to an overall body plan during development. For example, the different organs of the body need to grow to a specific size to maintain body proportions. To achieve an appropriate cell, organ, or organism size, cell growth must be coordinately controlled with cell proliferation and, in the case of metazoans, cell death. How is cell growth controlled, and how is this control integrated with that of cell proliferation and death?

In recent years, interest in cell growth has been rekindled as it has become apparent that elaborate mechanisms actively control cell growth in response to favorable conditions. Cell growth is not passively controlled simply by the availability of nutrients (building blocks), as was widely thought 30 years ago when Hartwell decided to focus on his cell division mutants, but by signaling pathways that impinge on general cell physiology to elicit balanced macromolecular synthesis. There is growing evidence that the TOR kinase plays a central role in controlling these pathways and thus the various readouts that determine cell growth.

The TOR Protein
TOR (*t*arget *o*f *r*apamycin) was originally identified genetically by mutations in yeast, *TOR1–1* and *TOR2–1*, that confer resistance to the growth-inhibitory properties of the immunophilin–immunosuppressant complex FKBP (FK506 binding protein)-rapamycin (Heitman et al., 1991). The *TOR1* and *TOR2* genes encode the two large (molecular weight ~280 kDa) and highly homologous (70% identical) TOR1 and TOR2 proteins (Kunz et al., 1993; Helliwell et al., 1994). The structurally and functionally conserved mammalian counterpart mTOR (also known as FRAP, RAFT, or RAPT) was subsequently discovered biochemically based on its FKBP-rapamycin binding properties (Brown et al., 1994; Chiu et al., 1994; Sabatini et al., 1994; Sabers et al., 1995). More recently, single TOR homologs have been found encoded in the fly and worm genomes.

The TORs contain a C-terminal region with strong homology to the catalytic domain of phosphatidylinositol 3-kinase (PI3K) and phosphatidylinositol 4-kinase (Kunz et al., 1993; Keith and Schreiber, 1995) (Figure 1). Studies in yeast, flies, and mammals have revealed a TOR-related family of proteins which includes MEC1, TEL1, RAD3, MEI-41, DNA-PK, ATM, ATR, and TRRAP. All these proteins contain a characteristic C-terminal phosphatidylinositol (PI) kinase homology domain and have thus been termed PI kinase (PIK)-related kinases. The different PIK-related kinases are involved in diverse cellular functions, such as control of cell growth, cell cycle and DNA damage checkpoints, recombination and maintenance of telomere length. Accordingly, dysfunction of the PIK-related kinases results in a wide spectrum of severe diseases, ranging from cancer to immunodeficiency (Keith and Schreiber, 1995). Despite the homology to lipid kinases, none of the PIK-related kinases has been demonstrated to have lipid kinase activity, and both yeast and mammalian TOR, if not all the PIK-related

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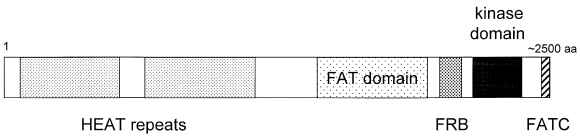

Figure 1. Architecture of a Generic TOR Protein

The domain structure is based on yeast TOR1 and TOR2 and mammalian TOR (mTOR).

kinases, are Ser/Thr protein kinases. A target phosphorylation site for yeast TOR remains to be determined. mTOR has been shown to phosphorylate a (Ser/Thr)-Pro motif (Brunn et al., 1997) or a threonine flanked by bulky hydrophobic residues (Burnett et al., 1998; Isotani et al., 1999).

The FKBP-rapamycin complex, which inhibits TOR function, interacts with the FRB (FKBP-rapamycin binding) domain in TOR, adjacent to the catalytic kinase domain (Figure 1). Both original point mutations (*TOR1–1* and *TOR2–1*) that enable yeast cells to grow on rapamycin-containing medium reside within the FRB domain. The resulting amino acid substitutions (including the corresponding substitution in mTOR) prevent the binding of the FKBP-rapamycin complex to TOR, explaining the ability of the mutations to confer complete resistance to rapamycin (Stan et al., 1994; Chen et al., 1995; Choi et al., 1996).

In addition to their catalytic and FRB domains, TOR proteins also contain up to 20 tandemly repeated HEAT motifs at their N terminus, roughly grouped into two blocks (Andrade and Bork, 1995) (Figure 1). Each HEAT motif comprises approximately 40 amino acids that form a pair of antiparallel α helices (Groves et al., 1999). HEAT motifs have been proposed to mediate protein–protein interactions in multiprotein complexes (Andrade and Bork, 1995). More recently, a so-called FAT domain, spanning approximately 500 amino acids N-terminal to the FRB and catalytic domains in TOR, has been proposed (Bosotti et al., 2000) (Figure 1). The function of the FAT domain, found only in members of the PIK-related kinase family, remains to be elucidated. According to current speculation, the domain could serve as a scaffold or as a protein–protein interaction domain, similar to the HEAT repeats. Indeed, the large size of TOR suggests that it may have several interaction partners which contribute to its regulation or signaling. Finally, the PIK-related kinases contain a short ($\sim$35 amino acids) extreme C-terminal sequence that has been termed a FATC domain (Keith and Schreiber, 1995; Bosotti et al., 2000) (Figure 1). The FATC domain occurs only in combination with the FAT domain and may be important for catalytic activity of PIK-related kinases.

TOR Readouts

The immunosuppressant and antibiotic rapamycin potently inhibits growth in several evolutionarily diverse cells, suggesting that TOR has a conserved role in controlling cell growth. What are the growth-related readouts controlled by TOR? Early findings, as reviewed below, indicated that TOR is dedicated to activating translation initiation in response to nutrients. Within the

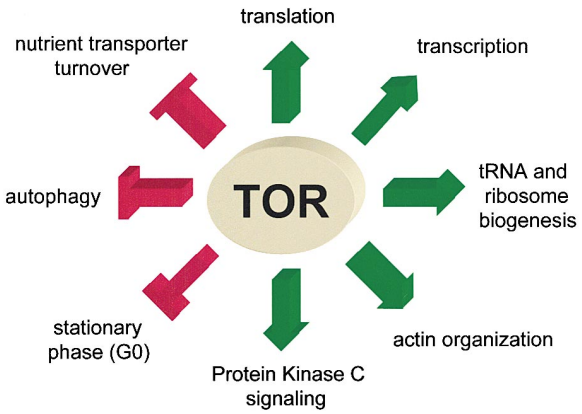

Figure 2. TOR Controls a Large and Diverse Set of Growth-Related Readouts

Green arrows indicate activation; red bars indicate repression. Shown is a composite of yeast and mammalian readouts.

last few years, yeast and mammalian TOR has been demonstrated to control several additional readouts, all of which are related to cell growth. These readouts include organization of the actin cytoskeleton, membrane traffic and protein degradation, PKC signaling, ribosome biogenesis, transcription, and, although more a consequence than a readout, cancer (Figure 2). The mechanisms by which TOR regulates such diverse readouts in response to nutrients are not known in all cases, but for some readouts, effectors or signaling mechanisms have been elucidated and will be described further below.

The studies that have described the various growth-related TOR readouts have relied extensively on the use of rapamycin to inhibit TOR. In this context, it is important to note that rapamycin is highly TOR specific. *TOR* mutations that prevent FKBP-rapamycin binding confer complete resistance to the growth-inhibitory properties of rapamycin. Furthermore, in all cases examined, these *TOR* mutations have reversed the inhibitory effects of rapamycin on the TOR readouts.

Translation Initiation

mTOR, in response to amino acids and growth factors, controls the mammalian translation machinery via activation of the $p70^{s6k}$ protein kinase and via inhibition of the eIF4E inhibitor 4E-BP1 (also known as PHAS-I) (see Thomas and Hall, 1997; Hara et al., 1998) (Figure 3). Activation of $p70^{s6k}$ and resulting phosphorylation of the 40S ribosomal protein S6 ultimately drives translation of 5′ TOP (terminal oligopyrimidine tract) mRNAs (Jefferies et al., 1997). These mRNAs constitute a small family of abundant transcripts (up to 20% of cellular mRNA) that encode primarily ribosomal proteins and components of the translational apparatus (Meyuhas et al., 1996). Thus, by controlling 5′ TOP mRNA translation, mTOR upregulates the translational machinery under favorable growth conditions. Early studies demonstrated that activation of $p70^{s6k}$ is potently inhibited by rapamycin, suggesting that mTOR is an upstream regulator of $p70^{s6k}$ (see Pullen and Thomas, 1997). However, it has become clear that regulation of $p70^{s6k}$ activity is very

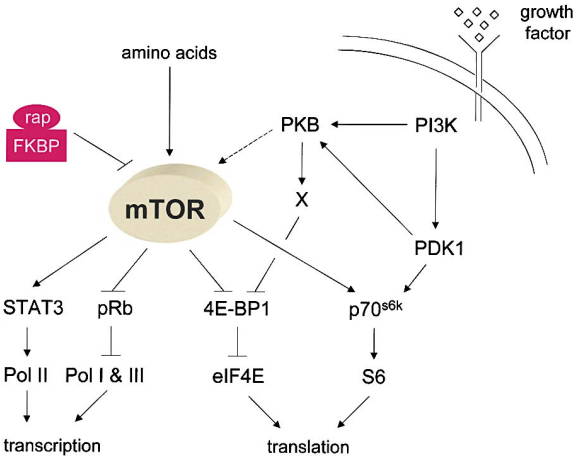

Figure 3. Model of mTOR Effectors and Signaling Pathways in Mammalian Cells

Arrows indicate activation; bars indicate repression. The dashed arrow between PKB and mTOR reflects the uncertainty as to whether PKB controls mTOR (see text). rap is rapamycin; X refers to an unidentified kinase.

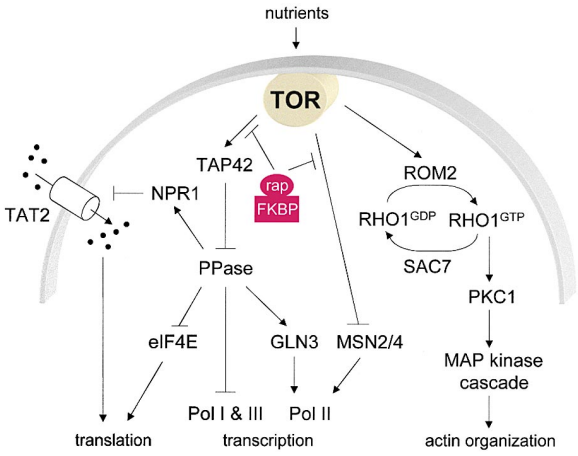

Figure 4. Model of TOR Effectors and Signaling Pathways in Yeast

Arrows indicate activation; bars indicate repression. rap is rapamycin; PPase refers to protein phosphatase (type 2A or type 2A-related). SAC7 is a GTPase-activating protein (GAP) for RHO1.

complex, involving a hierarchical phosphorylation of several sites in p70^{s6k} by different kinases (Pullen and Thomas, 1997; Alessi et al., 1998; Pullen et al., 1998). Rapamycin principally affects only one of these sites (Thr389 or Thr412, depending on the numbering system), which is one of the early sites in the phosphorylation hierarchy (reviewed in Pullen and Thomas, 1997; Weng et al., 1998). Recently, mTOR has been shown in vitro to phosphorylate p70^{s6k} directly, but it remains to be determined whether this phosphorylation is physiologically relevant in vivo (Burnett et al., 1998; Isotani et al., 1999). 4E-BP1 is a translation inhibitor that is phosphorylated and inactivated in response to a growth signal. Phosphorylated 4E-BP1 dissociates from eIF4E, the translation initiation factor that binds the cap structure (m7G(5′)ppp(5′)N) present at the 5′ termini of mRNAs, thereby allowing cap-dependent translation. Rapamycin blocks both 4E-BP1 phosphorylation and cap-dependent translation (Beretta et al., 1996), and mTOR has been demonstrated to phosphorylate 4E-BP1 directly (discussed in further detail below) (Brunn et al., 1997). Thus, mTOR also controls eIF4E function, which is particularly important in the translation of mRNAs containing a highly structured 5′ untranslated region (UTR), such as the transcripts encoding cyclin D1 and the c-*myc* oncogene (Sonenberg and Gingras, 1998).

p70^{s6k} and 4E-BP1 phosphorylation are dependent on the availability of amino acids, in addition to a growth factor, in the medium. A truncation mutation in p70^{s6k} confers resistance to rapamycin and resistance to inhibition by amino acid withdrawal, indicating that amino acid availability, like rapamycin, may impinge on p70^{s6k} and 4E-BP1 via mTOR (Hara et al., 1998) (Figure 3). Thus, like yeast TOR (see below), mTOR signals to the translation machinery in response to nutrients.

In yeast, rapamycin treatment or inactivation of both *TOR* genes results in a severe decrease in translation initiation and an arrest in the early G1 phase of the cell cycle (Kunz et al., 1993; Barbet et al., 1996). The yeast TOR1 and TOR2 proteins activate translation initiation most likely via the initiation factor eIF4E (Barbet et al., 1996; Cosentino et al., 2000) (Figure 4). The G1 cell cycle arrest observed upon TOR inactivation is a consequence of a translation defect, emphasizing that growth-induced synthesis of cell cycle machinery components, such as the yeast G1 cyclin CLN3, plays an important role in activating and allowing progression of the cell cycle (Barbet et al., 1996). These findings also support the notion that cell growth and cell proliferation are indeed distinct, yet coupled processes.

Rapamycin treatment or TOR depletion results in several physiological changes characteristic of starved (G0) cells, in addition to a downregulation of translation and a G1 arrest, including an accumulation of storage carbohydrates (e.g., glycogen) and an altered transcription pattern (Barbet et al., 1996) (Figure 2). The striking similarities between starved and rapamycin-treated cells established the concept that the TOR signaling pathway controls cell growth in response to nutrients (Barbet et al., 1996; Thomas and Hall, 1997).

What is upstream of TOR? In yeast, the mechanisms and molecules regulating TOR in response to nutrients are unknown. In mammalian cells, the regulation of TOR, in response to amino acids and growth factors, is controversial. One of the main signaling pathways activated in response to a growth factor involves the insulin receptor, insulin receptor substrates (IRS), and PI3K. Downstream of PI3K, the growth-controlling pathway includes 3′-phosphoinositide-dependent protein kinase 1 (PDK1), Protein Kinase B (PKB, also known as Akt), and p70^{s6k} (Figure 3). PDK1 phosphorylates a threonine in the activation loops of PKB and p70^{s6k}. The PDK1-mediated phosphorylation of PKB activates PKB in concert with 3′-phosphoinositide binding to the PKB pleckstrin homology domain (reviewed in Vanhaesebroeck and Alessi, 2000). Studies with the PI3K inhibitor wortmannin and with rapamycin have suggested a model in which PI3K and PKB signal to p70^{s6k} and 4E-BP1 via mTOR (see Thomas and Hall, 1997). According to this model,

mTOR would be activated by PKB. Indeed, recent studies have presented evidence that insulin stimulates phosphorylation and activity of mTOR via PKB (Scott et al., 1998), and that mTOR is a direct substrate of PKB (Nave et al., 1999). However, other studies have failed to detect significant alteration in mTOR autokinase activity or mTOR-associated 4E-BP1 kinase activity in response to amino acids or insulin (Hara et al., 1997, 1998). Gingras et al. (1999) have proposed a model in which mTOR kinase activity is largely constitutive. According to this model, mTOR-mediated phosphorylation of 4E-BP1 is a prerequisite for growth factor-induced PKB-mediated full phosphorylation of 4E-BP1 (Gingras et al., 1999) (Figure 3). mTOR may be regulated at the level of localization or substrate availability, in addition to or rather than at the level of intrinsic kinase activity. mTOR interacts with gephyrin, a protein necessary for the clustering of glycine receptors at the cell membrane in neurons. Coexpression of mTOR and gephyrin in HeLa cells causes mTOR, normally evenly distributed, to aggregate in large cytoplasmic clumps (Sabatini et al., 1999). Peterson et al. (2000) have suggested that rapamycin inhibits mTOR by altering mTOR localization or by disrupting the association between mTOR and a substrate. Thus, the mechanism(s) by which TOR is regulated remains to be determined.

Actin Organization

In yeast, TOR2 (but not TOR1) additionally controls cell cycle–dependent polarization of the actin cytoskeleton. This TOR2-unique function is rapamycin-insensitive and mediates the polarized growth characteristic of budding yeast cells (Zheng et al., 1995; Schmidt et al., 1996). Genetic and biochemical evidence has shown that TOR2, via the exchange factor ROM2, activates the RHO1 GTPase (Schmidt et al., 1997). RHO1, in turn, signals to the actin cytoskeleton via its direct effector PKC1 and a PKC1-activated MAP kinase cascade (Helliwell et al., 1998a, 1998b) (Figure 4). A similar function of TOR kinases has not been identified in any other organism and may reflect the so far unique situation of two *TOR* genes in yeast. The finding that yeast TOR controls both translation and organization of the actin cytoskeleton indicates that TOR integrates temporal and spatial control of cell growth. Temporal control is via protein synthesis whereas spatial control is via organization of the actin cytoskeleton. Coordination of these two parameters is essential to ensure that cell growth occurs only at the appropriate time and location in the cell.

Membrane Traffic and Protein Degradation

Delivery of nutrient transporters to the cell surface and uptake of nutrients are essential for cell growth and viability. These events are adapted to environmental conditions to optimize nutrient flow and cell growth. Coordination between the import of building blocks for a cellular process and the process itself, e.g., amino acid import and translation, is critical to adjust the growth rate of the cell to the nutritional conditions.

As part of the above mentioned starvation response occurring upon TOR inactivation, rapamycin also causes a severe decrease in amino acid import in yeast (Schmidt et al., 1998; Beck et al., 1999). This drop in amino acid uptake is a consequence of the vacuolar degradation of high affinity amino acid permeases such as the yeast tryptophan transporter TAT2. The turnover of plasma membrane TAT2 requires ubiquitination, endocytosis, and finally vacuolar proteases. Interestingly, upon rapamycin treatment or starvation, an intracellular pool of TAT2 is routed to the vacuole independently of the plasma membrane, indicating that TOR controls not only stability of nutrient permeases which already reside at the plasma membrane, but also the intracellular sorting of nutrient transporters to their final destination. Furthermore, TOR regulation of TAT2 stability and sorting is inverse to that of GAP1. GAP1 is a general amino acid permease that is upregulated by TOR inactivation or nutrient deprivation and sorted to the plasma membrane (Beck et al., 1999). Control of the inverse regulation of specific (e.g., TAT2) and broad-range (e.g., GAP1) permeases by TOR selectively coordinates amino acid import and growth. For other nutrients, such as ammonium or glucose, the situation may be analogous, with TOR controlling stability and sorting of the corresponding permeases according to the prevailing nutrient conditions.

Autophagy is the process by which cytoplasmic components are delivered in bulk to the lysosome/vacuole for degradation. Autophagy is induced upon nutrient starvation to downregulate ribosomes and other cytoplasmic components, but also occurs at a basal level in yeast during vegetative growth to eliminate old proteins and organelles. This bulk protein degradation process involves the formation of autophagosomes, vesicular membrane structures that deliver cytoplasmic material to the lysosome/vacuole. Inactivation of TOR in yeast or mammalian cells leads to induction of autophagy, even in nutrient-rich medium, indicating that TOR negatively controls starvation-induced autophagy (Blommaart et al., 1995; Noda and Ohsumi, 1998). In yeast, TOR prevents autophagy probably by inhibiting one or more of the autophagy-mediating *APG* gene products. The APG proteins form a novel protein-degradation pathway, similar to the ubiquitin-conjugation system (Mizushima et al., 1998).

Under favorable growth conditions, TOR promotes protein synthesis and inhibits protein degradation (nutrient transporter turnover and autophagy). Upon unfavorable conditions, TOR is inactive, leading to a reduction in protein synthesis and an upregulation of protein degradation. Thus, TOR maintains a balance between protein synthesis and degradation such that the cell can rapidly adjust mass accumulation to a level appropriate to the nutrient supply (reviewed in Dennis et al., 1999).

Protein Kinase C Signaling

Protein Kinase C (PKC) family members play central regulatory roles in a multitude of cellular processes, including cell growth and proliferation, apoptosis and survival, and cytoskeletal remodeling. Much attention has focused on the regulation of mammalian PKC activity, and especially on the phosphorylation of PKC (reviewed in Parekh et al., 2000). Phosphorylation of a critical residue in the activation loop of AGC kinase family members, including Protein Kinase A (PKA), PKB, PKC, and $p70^{s6k}$, is crucial for the activation of these kinases.

Phosphorylation of other sites, for instance a C-terminal hydrophobic site, also contributes to the full activation of PKC isozymes. Recently, the phosphorylation of the conserved C-terminal hydrophobic site in classical PKCα and in novel PKCδ has been shown to be positively controlled by TOR. Similar to Thr389 in $p70^{s6k}$, the phosphorylation of the hydrophobic site in PKC is sensitive to rapamycin. The kinase responsible for the rapamycin-sensitive phosphorylation of classical and novel PKC isozymes was identified as atypical PKC complex, indicating that TOR controls either this atypical PKC or an antagonizing phosphatase (Ziegler et al., 1999). Importantly, and again in agreement with previous findings for $p70^{s6k}$, amino acid availability impinges on the rapamycin-sensitive hydrophobic site in at least novel PKC, further implicating mTOR in the response to nutrients (Parekh et al., 1999).

Recent evidence has involved novel PKCδ in the mTOR-4E-BP1 signaling pathway, i.e., in the control of cap-dependent translation initiation (Kumar et al., 2000). PKCδ interacts with mTOR, is required for the phosphorylation (and inhibition) of 4E-BP1, and promotes cap-dependent translation initiation. However, unlike mTOR, PKCδ does not directly phosphorylate 4E-BP1. The role of PKCδ in the mTOR-4E-BP1 pathway remains to be further elucidated.

Ribosome Biogenesis and tRNA Synthesis

Ribosome synthesis, a major consumer of the cell's resources, is the classic growth-related readout. Upregulation of ribosome synthesis occurs in response to favorable conditions and allows a cell to grow faster—but only if all components of the ribosome and other essential machineries are coordinately available. Thus, ribosome synthesis reflects many regulatory decisions a cell must make when determining whether to grow and, if so, at what rate (Warner, 1999).

In mammalian cells, the mTOR-$p70^{s6k}$ signaling pathway induces synthesis of ribosomal proteins by stimulating translation of 5′ TOP mRNAs, as discussed above. Earlier studies have indicated that rRNA synthesis in mammalian cells is rapamycin-sensitive and thus also controlled by mTOR (Mahajan, 1994; Leicht et al., 1996). mTOR positively controls RNA Polymerase I (PolI, large rRNA transcription) and RNA Polymerase III (PolIII, 5S rRNA, and tRNA transcription) activity, possibly via inactivation of the retinoblastoma protein (pRb). pRb inhibits PolI and PolIII transcription (reviewed in White, 1997), and pRb phosphorylation and inactivation are blocked upon rapamycin treatment (Hashemolhosseini et al., 1998) (Figure 3).

TOR also regulates ribosome biogenesis in yeast. Rapamycin treatment rapidly and severely reduces RNA Polymerase II (PolII)-mediated transcription of genes encoding ribosomal proteins (Cardenas et al., 1999; Hardwick et al., 1999; Powers and Walter, 1999). Yeast TOR also controls rRNA and tRNA synthesis, via PolI and PolIII, and processing of at least the 35S precursor rRNA (Zaragoza et al., 1998; Powers and Walter, 1999) (Figure 4). Thus, combining the evidence obtained in mammalian systems and the latest results from yeast, the TOR signaling pathway plays an essential role in controlling ribosome biogenesis at many levels: transcription (PolI, II, and III), rRNA processing, and translation. The finding that TOR positively controls both the biosynthesis and activity of the translation machinery underscores the central role of TOR in mediating cell growth.

Transcription

Upon nutrient limitation, yeast cells enter a quiescent (stationary) phase characterized not only by a reduction in protein synthesis and many other distinctive phenotypes, but also by an altered transcription pattern. Most genes transcribed by RNA PolI, II and III are downregulated in G0. However, a relatively few PolII-transcribed genes, important for survival under nutrient-limiting conditions, are induced. TOR, in addition to positively controlling PolII-mediated transcription of ribosomal protein genes, also negatively controls PolII-mediated transcription specific to starved cells (Barbet et al., 1996; Beck and Hall, 1999; Cardenas et al., 1999; Hardwick et al., 1999). The general mechanism by which TOR negatively controls starvation-specific PolII transcription is by preventing nuclear localization of nutrient-responsive transcriptional activators (see below for further details) (Beck and Hall, 1999). Thus, TOR also controls growth via transcriptional regulation of nutrient metabolism.

In mammalian cells, mTOR controls the transcriptional activator STAT3 (Figure 3). Maximal activation of STAT3, in response to the cytokine CNTF (ciliary neurotrophic factor), requires tyrosine and serine phosphorylation. Tyrosine phosphorylation is mediated by members of the JAK/Tyk family of kinases. Serine phosphorylation of STAT3, and STAT3-dependent transcription, is rapamycin-sensitive. The activation of STAT3 by mTOR may be direct, as mTOR efficiently phosphorylates the relevant serine (Ser727) in a STAT3 peptide (Yokogami et al., 2000).

Cancer

Several lines of evidence have implicated mTOR in cancer. First, although an oncogenic version of mTOR has yet to be found, many of the proteins linked to TOR signaling, such as PI3K, PKB, and eIF4E, have been identified in oncogenic versions or demonstrated to have transforming potential (see Thomas and Hall, 1997). Second, mTOR controls translation of c-Myc, a transcription factor often deregulated in tumors. c-Myc target genes govern several growth-related processes including rRNA transcription and processing, ribosomal protein transcription, and translation (reviewed in Schmidt, 1999)—processes remarkably similar to the growth-related readouts of TOR. mTOR mediates translational induction of c-*myc*, in response to serum, via phosphorylation and inhibition of 4E-BP1 (West et al., 1998). Third, STAT3, whose activation is controlled by mTOR (see above), is persistently activated in many human cancers and causes cellular transformation (Bromberg et al., 1999). Fourth, malignant transformation by the nonreceptor tyrosine kinase v-Src is mediated via the Ras-MAPK pathway and the PI3K-mTOR pathway (Penuel and Martin, 1999). Fifth, a constitutively active mTOR-$p70^{s6k}$ signaling pathway has been shown to be responsible for the transformed state of pancreatic cancer cells

(Grewe et al., 1999). Sixth, mitogen-stimulated downregulation of the cyclin-dependent kinase inhibitor $p27^{Kip1}$ is prevented by rapamycin (Nourse et al., 1994; Luo et al., 1996). Finally, some neuroblastoma and glioblastoma cells are particularly sensitive to rapamycin (Hosoi et al., 1998). The above findings also suggest that rapamycin may provide a novel therapeutic approach in cancer treatment. The involvement of mTOR signaling in cancer further illustrates the link between cell growth and proliferation.

TOR Effectors and Signaling Mechanisms

The TOR effectors and signaling mechanisms mediating the growth-related readouts described above are known in some, but not all cases. mTOR signals to the translation initiation machinery via $p70^{s6k}$ and 4E-BP1, as described above. In yeast, TOR signals to the translation machinery via TAP42, an essential protein homologous to the murine α4 phosphoprotein. TAP42 associates, in a TOR- and nutrient-dependent manner, with the catalytic subunits of type 2A or type 2A-related (SIT4) protein phosphatases (PP2As), and mutations in the *TAP42* gene confer dominant rapamycin resistance (Di Como and Arndt, 1996). Similar α4-phosphatase complexes are present in mammalian cells, but there is substantial controversy regarding the rapamycin-sensitivity of these complexes (Murata et al., 1997; Chen et al., 1998; Inui et al., 1998; Nanahoshi et al., 1998). TAP42 has been demonstrated to be directly phosphorylated by TOR2 and this phosphorylation promotes formation of the TAP42-PP2A complex (Jiang and Broach, 1999) (Figure 4). The mechanism by which TAP42 signals to activate protein synthesis is unknown. TAP42 also mediates the TOR signal controlling nutrient permease stability and sorting. In the case of the tryptophan transporter TAT2, TAP42 regulates the phosphorylation state of the Ser/Thr kinase NPR1. Under favorable nutrient conditions, TOR and TAP42 maintain NPR1 in an inactive, phosphorylated state. Upon rapamycin treatment or nutrient deprivation, NPR1 is dephosphorylated and activated, resulting in the degradation of TAT2 and the stabilization of the general amino acid permease GAP1 (Schmidt et al., 1998; Beck et al., 1999) (Figure 4).

In yeast, TOR globally represses starvation-specific transcription by sequestering several nutrient-responsive transcription factors in the cytoplasm. TOR prevents transcription of genes normally induced upon nitrogen limitation by promoting association of the GATA transcription factor GLN3 with the cytoplasmic URE2 protein. The binding of GLN3 to URE2 requires TOR-dependent phosphorylation of GLN3 and/or URE2 (Beck and Hall, 1999; Cardenas et al., 1999; Hardwick et al., 1999). Phosphorylation and cytoplasmic retention of GLN3 are also dependent on the TOR effector TAP42, and are antagonized by the phosphatase SIT4 (Beck and Hall, 1999). The similarities in the regulation of GLN3 and NPR1 suggest a general model for TOR-mediated signaling. According to this model, TOR inhibits phosphatases, via formation of a TAP42-PP2A complex. Upon inactivation of TOR, the TAP42-PP2A complex dissociates, and the released phosphatase dephosphorylates and thereby activates GLN3 and NPR1 (Figure 4). In mammalian cells, mTOR has also been proposed to negatively regulate PP2A as a mechanism to maintain $p70^{s6k}$, 4E-BP1 and PKC isozymes phosphorylated (Hara et al., 1997, 1998; Parekh et al., 1999; Peterson et al., 1999, 2000).

Yeast TOR also controls starvation-specific transcription by sequestering the redundant zinc-finger transcription factors MSN2 and MSN4 in the cytoplasm. MSN2/4 activate a large number of genes in response to several types of stress, including carbon limitation. TOR sequesters the carbon-responsive factors MSN2/4 in the cytoplasm by a mechanism similar to that by which PKB and 14-3-3 proteins inactivate the growth-inhibiting or proapoptotic Forkhead transcription factors in mammalian cells (Brunet et al., 1999; Kops et al., 1999). In response to a good carbon source, TOR promotes the association of MSN2/4 with the cytoplasmic 14-3-3 protein BMH2 (Bertram et al., 1998; Beck and Hall, 1999). TOR-mediated control of MSN2/4 is independent of TAP42 and SIT4 (Beck et al., 1999) (Figure 4). TOR may control MSN2/4 in conjunction with the RAS-PKA pathway, as nuclear localization of MSN2/4 is also controlled negatively by PKA (Gorner et al., 1998). Interestingly, as TOR controls MSN2/4 and GLN3 via different effector pathways and in response to different types of starvation, TOR appears to discriminate between nitrogen and carbon starvation.

The effector pathways by which yeast and mammalian TOR controls other readouts, such as PolI/III transcription and rRNA processing, are not known. The observation that yeast PP2A mutants are defective in ribosome biogenesis suggests that TOR controls several, but not necessarily all, readouts via TAP42-PP2A (Zaragoza et al., 1998; Powers and Walter, 1999) (Figure 4). Future studies may reveal additional TOR effector pathways, or TOR cross-talk with other growth-controlling pathways.

Growth Control in Metazoans

All the studies reviewed above pertain to growth control in single cells, yeast and cultured mammalian cells. However, control of cell growth is also important in determining overall organ and body size in multicellular systems. Animals attain characteristic body size and proportions via the coordinate regulation of cell growth, cell proliferation, and cell death (Conlon and Raff, 1999).

How is cell growth controlled within the context of overall body size in multicellular organisms? Recent results from the fruit fly *Drosophila* have begun to answer this question and have, albeit so far only indirectly, implicated TOR. Manipulation of members of the PI3K signaling pathway in *Drosophila* alters organ and organism size, indicating that this pathway controls cell size (growth) and/or number (proliferation and death) (reviewed in Edgar, 1999 and in Weinkove and Leevers, 2000). *Drosophila* PI3-kinase (Dp110-Dp60) and its direct regulator, the IRS1–4 homolog CHICO, control cell size and cell number (Leevers et al., 1996; Bohni et al., 1999; Weinkove et al., 1999). Interestingly, manipulation of the more distal component of the pathway $p70^{s6k}$ (DS6K) affects cell size, but not cell number (Montagne et al., 1999). Importantly, DS6K mutant flies (*dS6K*) are reduced in body size but perfectly formed, suggesting that $p70^{s6k}$ controls body size but not body proportions (Montagne et al., 1999). Perfectly proportioned "micro-flies" also develop under starvation conditions, suggesting that body size is controlled in response to

nutrients, independently of patterning, cell-fate specification, or cell differentiation (see Edgar, 1999). The role of PKB (DAkt1) in controlling cell size and/or number is unclear as it has been reported to affect only cell size (Verdu et al., 1999), or both cell size and number (H. Stocker and E. Hafen, as cited in Leevers, 1999). PI3K, CHICO, DAkt1, and DS6K control cell size in a cell-autonomous manner, meaning that these signaling molecules regulate growth of the individual cells in which they reside rather than of other cells via production of a systemic growth factor.

How can one explain the finding that PI3K and CHICO mutants are reduced in both cell size and cell number, whereas DS6K mutants are reduced only in cell size? An explanation could be that the PI3K signaling pathway bifurcates upstream of DS6K, possibly at the level of DAkt1. The DS6K branch, probably also involving *Drosophila* TOR, is dedicated to the control of cell size, whereas a so far unknown, parallel branch controls cell number. The unknown branch may ultimately determine cell number by modulating only cell proliferation as apoptosis is not enhanced in, at least, CHICO mutants (Bohni et al., 1999; Edgar, 1999; Verdu et al., 1999). Importantly, the *Drosophila* studies described above demonstrate that the coordinated regulation of cell growth and proliferation, and ultimately overall body size, is mediated at least in part by the PI3K signaling pathway.

Manipulation of other *Drosophila* signal transducers, such as the Myc transcription factor (dMyc) or the small GTPase Ras (Ras1), also alters the regulation of cell growth during development (Johnston et al., 1999; Prober and Edgar, 2000). Loss of dMyc or Ras1 results again in reduced cell size, and enhancement of either one promotes cell growth. Enhancement of dMyc and Ras1 also promotes G1 to S phase progression, but this enhancement ultimately fails to accelerate cell division, due to a compensating lengthening of the S and G2 phases. Consistent with the finding that alterations in dMyc and Ras1 cause similar phenotypes, Ras1 upregulates dMyc. dMyc, in turn, regulates growth via candidate target genes such as *pitchoune* (Zaffran et al., 1998). *pitchoune* encodes a DEAD box RNA helicase that may be involved in translation initiation or rRNA processing. Both Ras1 and dMyc also increase levels of cyclin E posttranscriptionally, explaining the effects of these regulators on cell cycle progression, and again suggesting that growth is coupled to proliferation via cyclin synthesis (Barbet et al., 1996; Polymenis and Schmidt, 1997; Johnston et al., 1999; Prober and Edgar, 2000).

Does TOR control growth in *Drosophila*? *Drosophila* TOR remains to be characterized. However, counterparts of the molecules implicated in *Drosophila* growth control (e.g., PI3K, PKB, $p70^{s6k}$, Myc) have been linked to TOR signaling and growth control in mammalian cells. It is thus reasonable to predict that *Drosophila TOR* mutations, like *dS6K* mutations, will cause a reduction in cell and, ultimately, body size. Such a result would confirm the role of TOR as a central controller of cell growth. In this context, it is also noteworthy that among the components involved in the growth-controlling PI3K signaling pathway, TOR is the only protein functionally conserved from yeast to man. TOR may be a key signaling molecule utilized by many, if not all, organisms to control growth in response to nutrients.

Concluding Remarks

The multitude and diversity of growth-related readouts controlled by TOR indicate that this conserved kinase may not be simply part of a single, linear, growth-controlling pathway. Indeed, as suggested by the findings described above, TOR may act radially on several different pathways. TOR may even be viewed primarily as a "cross-talker" that broadly integrates cell physiology to elicit balanced growth. Thus, TOR can be regarded as a central controller of cell growth.

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