

The expanding TOR signaling network Dietmar E Martin and Michael N Hall

Cell growth (increase in cell mass or size) is tightly coupled to nutrient availability, growth factors and the energy status of the cell. The target of rapamycin (TOR) integrates all three inputs to control cell growth. The discovery of upstream regulators of TOR (AMPK, the TSC1-TSC2 complex and Rheb) has provided new insights into the mechanism by which TOR integrates its various inputs. A recent finding in flies reveals that TOR controls not only growth of the cell in which it resides (cell-autonomous growth) but also the growth of distant cells, thereby determining organ and organism size in addition to the size of isolated cells. In yeast and mammals, the identification of two structurally and functionally distinct multiprotein TOR complexes (TORC1 and TORC2) has provided a molecular basis for the complexity of TOR signaling. Furthermore, TOR has emerged as a regulator of growthrelated processes such as development, aging and the response to hypoxia. Thus, TOR is part of an intra- and inter-cellular signaling network with a remarkably broad role in eukaryotic biology.

Addresses

Division of Biochemistry, Biozentrum, University of Basel, Klingelbergstrasse 70, CH-4056 Basel, Switzerland

Corresponding author: Hall, Michael N (M.Hall@unibas.ch)

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Introduction

TOR (target of rapamycin), an atypical serine/threonine kinase, was originally identified genetically in the unicellular budding yeast *Saccharomyces cerevisiae* [1]. *S. cer-evisiae* and other yeasts harbor two homologous TOR genes, *TOR1* and *TOR2*, whereas all other eukaryotes appear to contain only one *TOR* gene. The two TOR proteins in yeast have a redundant (shared) function in 'temporal' control of cell growth by regulating translation, transcription, ribosome biogenesis, nutrient transport and autophagy in response to nutrient availability. In addition, TOR2 uniquely has a function in 'spatial' control of cell growth by regulating the cell-cycle-dependent polarization of the actin cytoskeleton (for review see [2,3]). As

discussed below, these two distinct functions of the TOR proteins are performed by distinct TOR complexes, TORC1 and TORC2, signaling via different effector pathways [4]. Two similar TOR complexes have also been identified in mammalian cells, suggesting that the broader TOR signaling network is structurally conserved [4–7,8°,9°]. TORC1 in mammalian cells (mTORC1) phosphorylates the two well-characterized mTOR effectors S6K (p70 S6 kinase) and 4E-BP1 (eukaryotic initiation factor 4E binding protein 1), thereby promoting translation upon favorable conditions (for review see [10]). mTORC2 in mammalian cells, like TORC2 in yeast, controls the actin cytoskeleton [8°,9°]. These findings suggest that the two TOR complexes are conserved in both structure and function. However, the mechanisms by which the two mTOR complexes are regulated in response to nutrient cues, growth factors and cellular energy status remain elusive, although recent findings on mTOR and dTOR (mammalian and Drosophila TOR, respectively) upstream regulators provide new insights.

Extending TOR's known role in cell size determination, recent studies in *Drosophila* have demonstrated that TOR has a profound effect on organ and organism size. Importantly, these studies identified TOR- and nutrient-dependent humoral signaling that controls growth globally. An outstanding task now is to unravel the precise molecular mechanisms by which the two TOR complexes are controlled and in turn control their multitude of readouts in yeast and flies and in different tissues. This will be necessary to understand the fundamentally important process of cell growth and to understand the various diseases caused by defects in the TOR signaling network.

Upstream regulators of TOR

The nutrient-sensitive TOR pathway and the insulin/ IGF-PI3K (growth factor) signaling pathway are functionally connected (see Figure 1). Important new insights on the crosstalk between these two pathways came from studies on the TSC1-TSC2 heterodimer (tuberous sclerosis complex) and the small GTPase Rheb (Rashomolog enriched in brain) in Drosophila and mammalian cells. TSC1 and TSC2, also known as hamartin and tuberin, form a complex that has tumor suppressor activity (for review see [11–13]). Mutations in either TSC1 or TSC2 lead to the formation of benign tumors known as hamartomas, and cause the syndrome termed tuberous sclerosis complex. However, despite its known role in tumor suppression (for review see [14]), the molecular function of the TSC1-TSC2 heterodimer in cell growth remained elusive. Several independent studies have now



Figure 1

Model of intracellular TOR signaling network in mammalian cells. The mTOR pathway integrates the three inputs of nutrients (amino acids), growth factors (insulin/IGF) and cellular energy status to control cell growth. Amino acids may inhibit the TSC1–TSC2 complex, or may activate Rheb or mTOR (not shown). The growth factor signal is transduced to TSC2 via the insulin signaling pathway. Cellular energy status (low energy) is signaled to TSC2 via AMPK. mTOR, like TOR in yeast, is found in two structurally and functionally distinct multiprotein complexes, mTORC1 and mTORC2, that control many growth-related readouts of which only two, protein synthesis and actin organization, are shown. The upstream regulators of mTORC2 are unknown, but may include the TSCs and Rheb. Only mTORC1 is rapamycin-sensitive.

demonstrated that the TSC1–TSC2 complex inhibits cell growth and proliferation in *Drosophila* and mammalian cells by inhibiting TOR signaling. In *Drosophila*, TSC1 was uncovered in several genetic screens aimed at identifying regulators of cell size [15–18]. Using the fly eye as a model system, mutations in *TSC1* were found to cause large ommatidia and therefore large eyes as a consequence of activated cell growth. In contrast, simultaneous overexpression of TSC1 and TSC2 reduced overall organ size by causing a decrease in cell size and cell number. Thus, the TSC1–TSC2 complex inhibits cell and organ growth. Detailed epistasis analysis revealed that TSC1– TSC2 acts downstream of PI3K and Akt/PKB (protein kinase B) but upstream of S6K [15–18]. Interestingly, the large cell size phenotype of *tsc1* or *tsc2* mutants could be suppressed by inhibition of either TOR or S6K, suggesting that TOR acts downstream and/or in parallel to TSC1–TSC2 [18,19]. Other studies confirmed that TSC1–TSC2 acts upstream of the known TOR effectors S6K and 4E-BP1 in both *Drosophila* and mammalian cells [20–23], but the direct target of TSC1–TSC2 in cell growth still remained unknown. Once again, *Drosophila* genetics filled the gap. In the same genetic screen as described above, two laboratories independently identified Rheb as a positive regulator of cell and organ growth. Epistasis analyses suggested that Rheb acts downstream of PI3K, Akt/PKB and TSC1–TSC2, and upstream of TOR and S6K [24°,25°]. The functional link between Rheb and the TSC1–TSC2 complex was determined shortly thereafter with the finding that TSC2 acts as a GAP (GTPase activating protein) against Rheb in *Drosophila* and mammalian cells [23,26,27,28°,29]. The TSC complex was found to catalyze the conversion of GTP-loaded Rheb to its GDP-loaded form, thereby inactivating the small GTPase and causing the inactivation of TOR by an as-yet-unknown mechanism.

Until recently, the nature of the molecular links between the insulin/IGF signaling pathway and the nutrientsensitive TOR signaling pathway were unknown. Both signaling pathways play an important role in determining cell and body size. TOR mutant flies show a small cell size phenotype and reduced body size [30,31]. This phenotype is similar to loss-of-function mutations in positive regulators of the insulin/IGF pathway. In contrast, loss of a negative regulator in this pathway, PTEN (phosphatase and tensin homolog on chromosome ten), leads to an increase in cell and overall body size. Furthermore, the mTOR effectors S6K and 4E-BP1 respond to growth factors. Taken together, these results suggested that the mTOR (dTOR in flies) and insulin/IGF pathways crosstalk to coordinate overall cell and organismal growth. How does growth factor signaling impinge on the TOR pathway? Several findings in Drosophila and mammalian cells now collectively suggest that Akt/PKB phosphorylates TSC2 and thereby inhibits the TSC1-TSC2 complex [17,20,32,33] (see Figure 1). However, the consequence of TSC1-TSC2 phosphorylation by Akt/PKB remains unclear. Some suggest that the primary effect of TSC2 phosphorylation is to destabilize the TSC2 protein, while others suggest that the primary effect is to disrupt the TSC1-TSC2 complex. Additional controversy regarding Akt/PKB phosphorylation of TSC2 has been provided by the observation that mutation of the Akt/ PKB target sites in TSC2 has no effect on Drosophila growth and development [34]. The regulation of TSC1– TSC2 may be complex since the AMP-activated protein kinase (AMPK; see below), PKC and MEK signaling pathways have also been implicated in TSC2 phosphorvlation and regulation, suggesting that PI3K signaling is not the only pathway controlling TSC1-TSC2 [35].

How does the TOR pathway sense nutrients? Several studies have suggested that abundance of branched amino acids, especially leucine, regulates TOR activity. A recent study demonstrated that overexpression of Rheb or a deficiency of TSC2 can prevent dephosphorylation of S6K induced by amino-acid starvation [22,27]. However, it is not yet known if amino acids are sensed at the level of

TOR, Rheb or the TSC1–TSC2 complex, and all have been suggested to function as nutrient sensors (for review see [13]) (see Figure 1). The fact that TOR in *S. cerevisiae* responds to nutrients despite TSCs not being present in this organism may suggest that nutrients are not sensed via the TSCs. The elucidation of the potential roles of the TSCs, Rheb and TOR proteins in nutrient sensing is of great interest.

In addition to its control by nutrients and growth factors, recent findings also suggest that the TSC complex is targeted by the energy-sensitive AMP-activated protein kinase (AMPK) (see Figure 1; see also review in this issue by Graham Hardie). AMPK is activated upon energy deprivation, in other words when the ratio of AMP to ATP is high. AMPK phosphorylates TSC2 directly, thereby enhancing the stability of the TSC1-TSC2 complex [28[•]]. Moreover, in the absence of TSC2, ATP depletion no longer leads to dephosphorylation of the two TOR effectors S6K and 4E-BP1. Consistent with these findings, the AMPK activator AICAR (5-aminoamidazole-1-β-4 carboxamide ribofuranoside) inhibits S6K in mammalian cells [36]. Taken together, these findings suggest that a low cellular energy status is transmitted by AMPK to TSC2 which, in turn, inhibits Rheb and ultimately TOR activity (for review see [37]).

Recent studies have also demonstrated an interesting link between two signaling pathways responsible for the formation of the hamartomatous syndromes tuberous sclerosis complex (TSC) and Peutz-Jeghers (PJS) [38–40] (See Figure 1). Both diseases are characterized by multiple benign hamartomas. PJS is caused by mutations in the LKB1 tumor suppressor gene. Interestingly, LKB1 controls AMPK by direct phosphorylation upon ATP depletion. Loss of LKB1 results in decreased activation of AMPK and down-regulation of TSC1-TSC2, and thereby stimulation of TOR activity. These findings further suggest that hamartomas develop as a consequence of constitutively high TOR activity. It is likely that the elucidation of the exact mode of mTOR activation by its upstream signals will contribute to the design of novel diagnostic and therapeutic tools against the tuberous sclerosis complex and other diseases [41].

Systemic growth control by TOR

Leopold and coworkers $[42^{\bullet\bullet}]$ recently made the interesting observation in *Drosophila* that TOR controls systemic growth in addition to cell autonomous growth. This study showed that dTOR in the *Drosophila* fat body controls the production of a secreted humoral factor that stimulates growth in peripheral tissues. The fat body in *Drosophila* (which is equivalent to the liver and adipocytes in vertebrates) acts as a nutrient sensor that controls growth of other tissues. Inhibition of a cationic amino acid transporter (called *slimfast*) specifically in the fat body leads to a systemic growth defect in a developing larva. In other words, inhibition of dTOR specifically in the fat body, as a consequence of a reduced intracellular concentration of amino acids resulting from slimfast inactivation, caused a pronounced growth inhibition in peripheral tissues. How does dTOR in the fat body communicate with other organs to coordinate overall growth of a fly? Under conditions of nutrient sufficiency, the expression of *Drosophila* insulin-like peptides (Dilps) in the larval brain is enhanced. Secreted Dilps have an endocrine function and activate insulin/IGF1 receptor signaling in all tissues. High amino acid concentrations in the fat body activate dTOR and induce the expression of dALS (the Drosophila ortholog of mammalian glycoprotein acid-labile subunit). dALS possibly binds and stabilizes circulating Dilps, thereby enhancing insulin signaling and growth in peripheral tissues. Conversely, down-regulation of dTOR upon nutrient deprivation may reduce secretion of dALS from the fat body. This would result in down-regulation of insulin/IGF signaling in peripheral tissues and therefore reduced overall growth. These findings suggest that TOR is a central controller of both cell and organism growth. Interestingly, they also suggest that TOR is upstream, in addition to downstream, of the insulin signaling pathway.

Another recent study in Drosophila demonstrated that the TSC-TOR pathway controls the timing of neuronal differentiation [43[•]]. This study showed that inappropriate activation of dTOR, as a consequence of a tsc1 mutation or activation of the upstream insulin pathway, leads to precocious cell differentiation, while inhibition of dTOR delays differentiation. Interestingly, this effect is due to alterations of the timing at which different neuronal markers appear with respect to the position of the differentiation front (the morphogenetic furrow). The role of dTOR in neuronal differentiation, like that of dTOR in the fat body, seems to be systemic, as a secreted neuronal factor may mediate the appropriate timing and location of differentiation. How might TOR control such a neuronal factor? It has been speculated that TOR and the insulin signaling pathway may regulate the translation of pre-existing mRNAs of such a neuronal factor, thereby allowing tight temporal and spatial control of differentiation of individual cells. However, clear experimental data are still missing to prove this hypothesis. Further work in fly and mammalian systems may reveal a mechanism by which TOR and the insulin/IGF signaling pathway cooperate to control differentiation processes via humoral signals.

Two TOR complexes drive cell growth

In *S. cerevisiae*, the two TOR kinases TOR1 and TOR2 control a variety of processes related to cell growth in response to nutrients. These processes can be formally divided into two major control functions: 'temporal' and 'spatial' control of cell growth. 'Temporal' control refers to TOR-regulated translation, transcription, ribosome

biogenesis, nutrient import and autophagy, readouts that collectively determine cell mass accumulation in response to changing nutrient conditions. 'Spatial' control refers to the cell-cycle-dependent regulation of the actin cytoskeleton, a prerequisite for establishing cell polarity (for review see [2]). These two major functions of the yeast TOR proteins are reflected in two structurally distinct multiprotein complexes termed TORC1 (TOR complex 1) and TORC2 (TOR complex 2) [4,44]. TORC1 mediates the temporal control of cell growth by regulating the various signaling pathways that determine mass accumulation. TORC2 mediates the spatial control of cell growth by regulating a RHO GTPase signaling pathway that ultimately impinges on the actin cytoskeleton. TORC1 consists of KOG1, LST8 and either TOR1 or TOR2. TORC2 consists of AVO1, AVO2, AVO3, LST8 and TOR2. The mammalian structural equivalent of TORC1 (mTORC1), discoverd at the same time as yeast TORC1, consists of raptor ('regulatory associated protein of mTOR', an ortholog of yeast KOG1), mLST8 (GβL) and mTOR [4-7]. mTORC1 is also the functional equivalent of yeast TORC1 as it controls similar readouts, including translation via S6K and 4E-BP1, and is the cellular target of rapamycin. The mammalian equivalent of TORC2 (mTORC2) was discovered only very recently [8[•],9[•]]. mTORC2 consists of mAVO3 (Rictor), mLST8 (GBL) and mTOR. Like yeast TORC2, mTORC2 controls the actin cytoskeleton and is rapamycin-insensitive. Individual siRNA knockdown of all TORC2 components (mTOR, mLST8 or mAVO3), but not of raptor, leads to a defect in cell spreading due to a decrease in F-actin assembly, suggesting that TORC2 controls the actin cytoskeleton. These effects are more pronounced in starved cells, suggesting that nutrient and growth factor cues regulate mTORC2 activity. As shown previously for yeast TORC2, mTORC2 may signal to the actin cytoskeleton through a small Rho-type GTPase and PKC. Activated forms of Rho and Rac restore F-actin assembly in cells in which the TORC2 component mTOR, mLST8 or mAVO3 is knocked down [8[•]]. Furthermore, mTORC2 controls the formation of activated, GTP-bound Rac1 in a growth-factor-dependent fashion. mTORC2 also controls the phosphorylation and activation of PKCa [9[•]]. In yeast, TORC2 activates PKC1 via RHO1 [2,3], but the relationship between Rho and PKC α in mTORC2 signaling remains to be determined. As stated above, mTORC2 is rapamycininsensitive. Since almost all mammalian studies to date have relied exclusively on the use of rapamycin to analyze mTOR function, it is likely that rapamycin-insensitive functions of TOR have been overlooked. Indeed, the recently described mTOR knockout in mice causes early embryonic lethality and a more severe phenotype than that observed with rapamycin-treated embryos [45,46]. It is likely that the TOR signaling network will expand further in the upcoming years. It will also be of interest to determine if mTORC2, like mTORC1, is

downstream of TSC1–TSC2, Rheb, AMPK and the insulin pathway.

A key aspect of TORC1-mediated growth control in yeast is the regulation of ribosome biogenesis, not only because ribosomes are directly required for growth, but also because ribosome biogenesis is a major consumer of cellular energy. To maintain robust growth in response to favorable conditions, yeast cells synthesize ~ 2000 ribosomes per minute. This requires the coordinated activity of all three RNA polymerases transcribing several hundred genes, including 35S rRNA genes by Pol I, ribosomal protein (RP) genes by Pol II, and 5S rRNA and tRNA genes by Pol III [47-49]. Thus, in a growing cell, ~95% of total transcription and a large portion of total cellular energy are dedicated to ribosome biogenesis, underscoring the need for tight regulation of ribosomal genes in response to nutrient and energy conditions. Despite the fundamental importance of this regulation, it is poorly understood. In S. cerevisiae, TOR plays a major role in the regulated transcription of ribosomal genes. RNA Pol I- and RNA Pol III-dependent transcription and 35S rRNA processing are strongly reduced upon TOR inhibition by rapamycin treatment. Moreover, rapamycin treatment leads to a rapid and pronounced down-regulation of RNA Pol II-dependent RP genes [50-52]. How does TOR control the transcription of all ribosomal (RP, rRNA, and tRNA) genes? Recently, the rapamycinsensitive transcription factor RRN3 has been shown to mediate Pol I-dependent transcription in yeast and mammalian cells [53-56]. Moreover, two histone H4 modifying factors — ESA1, a histone acetylase subunit of NuA4, and RPD3, a histone deacetylase subunit of the RPD3-SIN3 complex — have been implicated in the activation and repression of RP genes in yeast, respectively [57,58]. However, the mechanism by which RRN3, ESA1 and RPD3 are controlled either directly or indirectly by TOR is unknown. A recent report has demonstrated that rapamycin-induced down-regulation of ribosomal genes is suppressed by activation of the RAS-cAMP-PKA (protein kinase A) pathway [51]. Furthermore, this study showed that TOR controls the subcellular localization of PKA and the PKA-regulated kinase YAK1. These results suggested that TOR controls ribosomal gene expression via the RAS-cAMP-PKA pathway. However, no PKA-regulated transcription factors involved in regulation of ribosomal genes were known. In recent studies, the forkhead transcription factor FHL1 was identified as a key regulator of RP gene transcription [59–61]. FHL1 has a dual role as an activator and a repressor of RP transcription that is determined by its direct interactions with two specific cofactors, the coactivator IFH1 and the corepressor CRF1 [59]. TOR, via PKA, negatively regulates the kinase YAK1 and thereby maintains CRF1 in the cytoplasm. Upon TOR inactivation, activated YAK1 phosphorylates and activates CRF1. Phosphorylated CRF1 accumulates in the

nucleus and displaces IFH1 from FHL1, which is constitutively bound to RP gene promoters. In summary, the activity of the forkhead factor FHL1 seems to be a key target of the TOR-PKA pathway to regulate RP gene expression. However, the regulation of RP genes is likely to be more complex, as RP genes respond to many stress conditions including heat, oxidative stress, high osmolarity and DNA damage (for review see [49]). SFP1, a zinc finger transcription factor, was recently discovered to integrate a wide variety of different stress conditions and to mediate regulation of RP gene expression. SFP1 nuclear localization and the ability of SFP1 to bind to RP gene promoters are rapidly lost upon various stress conditions [62,63]. Similar to the above-described finding for CRF1, SFP1 localization and RP gene-promoter binding are regulated by TOR and PKA. Taken together, these findings show that the TOR-PKA pathway may target various transcription factors to control the coordinated expression of ribosomal genes and, thereby, ribosome biogenesis. How does coordinated transcription of all ribosomal genes occur mechanistically, and does this type of regulation also occur in higher eukaryotes? Since forkhead factors like FHL1 and histone deacetylases like RPD3 are conserved among eukaryotes and have been implicated in rDNA as well as RP gene transcription in yeast, they are promising candidates for further investigation of these questions.

TOR and lifespan regulation

Recent findings indicate that TOR, in addition to the insulin pathway, controls reproductive lifespan in worms and flies. It has been known for several years that lifespan in many organisms can be enhanced by nutrient limitation (calorie restriction), suggesting that nutrient-sensing signaling pathways may play a role in ageing. In C. elegans, genetic screens identified factors involved in the formation of a long-lived, stress-resistant dauer larvae. These factors included central components of the insulin/IGF signaling pathway such as DAF-2 (an insulin/IGF-like receptor) and the forkhead (FOXO) transcription factor DAF-16, and the NAD-dependent protein deacetylase Sir2 (silent information regulator) (for review see [64,65]). Interestingly, Sir2 also mediates lifespan extension in S. *cerevisiae*, suggesting that lifespan regulation is, at least in part, conserved in eukarvotic evolution. Recent reports now establish that TOR has a role in lifespan regulation [66,67,68[•]]. In *C. elegans*, loss-of-function mutations in *daf*-15 or let-363, which encode raptor (KOG1) and TOR orthologs, respectively, significantly increase lifespan. Deficiencies in the nutrient-signaling TORC1 components DAF-15 and LET-363 most likely extend lifespan by mimicking calorie restriction. Thus, under good nutrient conditions, LET-363 and DAF-15 signal to downstream effectors leading to the expression of reproductive and metabolic genes that, in turn, lead to a shorter lifespan. Upon nutrient limitation, a reduction of TORC1 activity leads to activation of genes required for dauer

formation and longevity. These genes include autophagy genes that are known to modulate lifespan and dauer formation. Autophagy seems to have a role in lifespan determination since *bec-1* (an ortholog of the yeast and mammalian autophagy gene APG6/VSP30/beclin1) is required for lifespan extension in *daf2* mutants [69]. Interestingly, DAF-2 activates *daf-15* transcription, via negative regulation of the *daf-15* transcription inhibitor *daf-16*. This provides yet another link between the insulin pathway and TORC1 signaling [66], and may account for at least a part of the lifespan-enhancing effect of mutations in the insulin pathway.

The above findings are consistent with earlier observations that defects in the *dTOR*, *Inr* (insulin-like receptor) or chico (insulin receptor substrate) gene extend lifespan in Drosophila [70,71]. In addition to regulating reproductive and metabolic genes, as discussed above, the TOR and insulin signaling pathways likely regulate lifespan via systemic, humoral mechanisms. In support of this, a recent study showed that down-regulation of the dTOR pathway specifically in the fat body of Drosophila increases lifespan of the fly [68[•]]. Thus, systemic signaling by dTOR in the fat body appears to control organism size during development and then aging in the adult. In line with these findings, a recent study in C. elegans showed that expression of DAF-16 specifically in the intestine (the worm equivalent of the fat body) is sufficient to restore longevity in *daf-16* germline mutants, highlighting again that humoral, systemic effects control longevity in a wide variety of organisms [72]. Taken together, the above-mentioned studies show that lifespan regulation via the TOR and the insulin/IGF signaling pathways is conserved, and demonstrate once more the systemic effect of TOR signaling in multicellular organisms.

TOR and hypoxia

Animals alter their metabolism to adapt to changes in oxygen tension. Upon low oxygen conditions (hypoxia), individual cells down-regulate energy-consuming anabolic processes, such as translation, to avoid an energy crisis. In addition to its effect on translation, hypoxia regulates the abundance of HIF-1, a transcription factor that activates genes required for adaptation to hypoxia. HIF-1 is thought to contribute to tumor formation by increasing expression of several growth factors, including TGF, PDGF and VEGF. In addition, HIF-1 activates genes involved in glucose uptake and metabolism, thereby stimulating cell growth. HIF-1 is a heterodimeric protein complex composed of HIF-1a and HIF-1B subunits. The HIF-1 α subunit is rapidly degraded under normoxic conditions, whereas it is stabilized and accumulates upon hypoxia.

What is the signaling pathway(s) that regulates translation and HIF-1 in response to oxygen? Several recent studies in *Drosophila* and mammalian cells have suggested a role for TOR in oxygen sensing and adaptation to hypoxia. Hypoxia, like rapamycin, causes the dephosphorylation of TOR and its effector proteins 4E-BP1, S6K and S6. This effect is independent of growth factors, Akt1, LKB, AMPK and phosphatase 2A (PP2A), suggesting that oxygen deprivation impinges on mTOR function via a novel, unidentified signaling pathway [73,74]. More recently, the TSC1–TSC2 complex has been implicated in the cellular response to hypoxia [74]. Hypoxia-induced dephosphorylation of S6K and S6 in MEFs is suppressed by inactivation of *TSC1* or *TSC2*, suggesting that hypoxia activates the TSC1–TSC2 complex which in turn inhibits TOR and translation.

Despite these interesting new findings, there is still a substantial lack of understanding concerning the mechanism by which hypoxia acts on downstream targets. In contrast to the findings described above, a recent study in PC3 prostate cancer cells shows that rapamycin prevents hypoxia-induced accumulation of HIF-1 and HIF-1dependent transcription [75]. Moreover, expression of extra copies of wild-type TOR enhances HIF-1 activation by hypoxia, while expression of a rapamycin-resistant TOR allele restores HIF-1a stabilization and HIF-1 transcriptional activity upon rapamycin treatment. These results suggest that TOR is an upstream activator of HIF-1 in hypoxic cancer cells. Thus, there appear to be conflicting reports on whether TOR has a positive or a negative role in the hypoxic response. Notwithstanding this confusion, the dysregulation of TOR and HIF1 is common in hamartomatous syndromes, and new insights into the regulation of HIF-1 by TOR will likely be important for developing new therapeutic agents against these diseases.

Is the regulation of cell growth in response to oxygen conserved in metazoans? A screen in Drosophila recently identified two homologous hypoxia-induced genes termed scylla and charybdis. scylla and charybdis were identified as suppressors of a PDK- (phosphoinositidedependent kinase) and PKB-dependent eye overgrowth phenotype [76]. Loss of scylla and charybdis caused an increase in body weight and cell size, suggesting that both gene products inhibit growth. Epistasis analyses revealed that scylla acts downstream of PKB but upstream of TSC and Rheb. scylla and charybdis are hypoxia- and starvationinduced genes, suggesting they play a role in adaptation to a wide variety of stress conditions. Down-regulation of scylla or charybdis decreased the lifespan of Drosophila under starvation conditions, underscoring again the fundamental role of cell growth regulators in lifespan regulation. Interestingly, inhibition of mTOR by hypoxia correlates with increased expression of the REDD1 gene, the conserved mammalian counterpart of scylla and charybdis. Moreover, inhibition of mTOR by hypoxia requires the Redd1 protein, suggesting that Redd1, like Scylla and charybdis in flies, acts upstream of the TSC1–TSC2 complex to negatively regulate mTOR [74].

Conclusions

Although many new insights have been obtained in recent years, several open questions remain. How do the three major growth signals (nutrients, growth factors and energy) converge to regulate TOR activity? Is mTORC2, like mTORC1, controlled by nutrients, growth factors, energy status, TSC, Rheb and the insulin pathway? How does Rheb activate TOR? How do different cell types vary in their regulation of growth, a key aspect of cell differentiation and organ development? The TOR signaling network is likely to be very complex, including crosstalk and feedback mechanisms. Indeed, the recent discovery of a feedback loop from mTOR-S6K to the insulin receptor substrate (IRS) highlights the complexity of the TOR signaling network (for review see [77]). The answers to these questions will be of fundamental and clinical importance.

Acknowledgements

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