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Alexandre Soulard[1], Adiel Cohen[1] and Michael N Hall

The Target of Rapamycin (TOR), a protein kinase, is the central node of a highly conserved signaling network that regulates cell growth in response to nutrients, hormones, and stresses. TOR is found in two functionally distinct complexes, TORC1 and TORC2. In this review we address the most recent advances in TOR signaling in invertebrate model organisms, including yeasts, plants, worms, and insects.

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Introduction

Invertebrate model organisms are valuable in biomedical research. Rapamycin was identified in the early 1970s as an antifungal agent against the pathogenic yeast *Candida albicans* [1,2] but was later developed as an immunosuppressive for use in the prevention of allograft rejection. In the early 1990s, the Target of Rapamycin (TOR) was discovered in *Saccharomyces cerevisiae* [3]. Soon thereafter, TOR was identified in many other organisms ranging from yeasts to mammals. More recently, TOR was shown to play a prominent role in many human disorders in addition to allograft rejection, including cancer, diabetes, and cardiovascular disease. TOR is a member of the PIKK family, a family of atypical serine/threonine kinases that also includes DNA-PK, ATM, and TEL1. It is found in two conserved complexes termed TOR complex 1 (TORC1) and TOR complex 2 (TORC2) [4]. TORC1 and TORC2 are essential regulators of cell growth in response to nutrients, hormones, or stresses. TORC1 mediates temporal control of cell growth by activating anabolic processes such as ribosome biogenesis, protein synthesis, transcription, and nutrient uptake and by inhibiting catabolic processes such as autophagy and ubiquitin-dependent proteolysis [4]. In contrast, TORC2 mediates spatial control of cell growth mainly by regulating actin cytoskeleton organization. Invertebrates also played a major role in elucidating these generally conserved features of the TOR signaling network. Here we review the major findings of the last two years on TOR signaling and cell growth regulation in invertebrates.

TOR complexes: composition, structure, and localization

The TOR complexes were originally described in *S. cerevisiae*, but have now been identified in a wide variety of organisms ranging from yeast to mammals (see Table 1). In *S. cerevisiae*, the core components of TORC1 are TOR (TOR1 or TOR2), KOG1, and LST8. The core components of budding yeast TORC2 are TOR2, AVO1, AVO3, and LST8. The corresponding components in other species are listed in Table 1. In addition to the core components, both complexes contain species-specific subunits (Table 1) [4]. During the last two years, TORC1 and TORC2 complexes have been identified biochemically or genetically in the fission yeast *Schizosaccharomyces pombe* [5,6••], in the pathogenic yeast *C. albicans* [7], in the unicellular green alga *Chlamydomonas reinhardtii* [8] and in the protozoan *Trypanosoma brucei* [9•], which emphasizes the high degree of conservation of these complexes in eukaryotes. As in *S. cerevisiae*, fission yeast contains two TOR genes, *TOR1* and *TOR2*. However, because of the naming of the fission yeast TORs based on order of discovery rather than function, *S. pombe* SpTOR1 corresponds to budding yeast TOR2 and vice versa. SpTOR2 is found mainly in TORC1, associated with WAT1/LST8, MIP1/KOG1, TCO89, and the uncharacterized protein TOC1 [5,6••], whereas SpTOR1 is found mainly in TORC2 associated with WAT1/LST8, SIN1/AVO1, STE20/AVO3, and BIT61 [5,6••]. Interestingly, all the so far identified, conserved TORC subunits are highly phosphorylated. Furthermore, both SpTOR1 and SpTOR2 associate with the casein kinase II ORB5, the PIKK regulator TEL2 and the uncharacterized protein TTI1 [6••]. TEL2 appears to interact with all PIKKs and, at least in mammals, it maintains the stability of the TOR protein, thereby indirectly influencing TOR downstream signaling [6••,10,11••].

In early studies, TORC1 was characterized by its sensitivity to rapamycin, while TORC2 is insensitive to rapamycin. This was later shown to be because of the ability of rapamycin (in complex with FKBP) to bind directly to TORC1 but not to TORC2. In *Arabidopsis thaliana*, TORC1 is insensitive to rapamycin due to the absence of a functional FKBP12 [12–14]. In *T. brucei*, TORC1 is insensitive to rapamycin-FKBP12 inhibition while TORC2 is sensitive. In this case, the rapamycin-FKBP12 complex can bind only to free TOR2 and not to a fully

Table 1

Listed are the protein components of *S. cerevisiae* TORC1 and TORC2 and known homologs in other invertebrates (and mammals) in which TOR has been at least partly characterized. PRR5 has been suggested to be a weak homolog of BIT61 [6••]. TOR has also been characterized in *Apis mellifera* and *Zea mays L* but no other TORC components have been studied so far in these organisms. – = no homolog found or the homologs are not known to be part of the complex

S. cerevisiae	*S. pombe*	*C. albicans*	*D. discoideum*	*C. reinhardtii*	*T. brucei*	*A. thaliana*	*C. elegans*	*D. melanogaster*	Mammals
TORC1									
TOR1 or TOR2	TOR1 or TOR2	TOR1	TOR	TOR	TOR1	TOR	TOR	TOR	mTOR
KOG1	MIP1	–	–	–	RAPTOR	Raptor1A Raptor1B	DAF15	RAPTOR	RAPTOR
LST8	WAT1	–	–	LST8	–	–	LST8	LST8	mLST8
TCO89	TCO89	TCO89	–	–	–	–	–	–	
–	TOC1	–	–	–	–	–	–	–	
–	–	–	–	–	–	–	–	–	PRAS40
TORC2									
TOR2	TOR1 or TOR2	TOR1	TOR	TOR	TOR2	TOR	TOR	TOR	mTOR
AVO1	SIN1	–	PiaA	–	–	–	SINH1	SIN1	mSIN1
AVO2	–	–	–	–	–	–	–	–	
AVO3	STE20	–	RIP3	–	AVO3	–	RICT1	RICTOR	RICTOR
LST8	WAT1	–	LST8	LST8	–	–	LST8	LST8	mLST8
BIT61	BIT61	–	–	–	–	–	–	–	PRR5/PRR5L

assembled TORC2 complex, suggesting that rapamycin in *T. brucei* affects the assembly and not the stability of the complex [9•], as shown previously in mammalian cells [15]. Finally, in fission yeast both TORC1 and TORC2 seem to be sensitive to rapamycin, although not under all conditions or for all readouts [5,6••,16–18].

While the composition of TORC1 and TORC2 are extensively described, their three-dimensional structures are far less well known. In 2007, the Llorca group reconstructed a low-resolution 3D structure of yeast TOR1 and the TOR1–KOG1 complex, by electron microscopy. Although the structure is only partial, it suggests that the RNC (Raptor N-terminal Conserved) domain of KOG1, which is known to interact with TORC1 substrates, is in close proximity to the kinase domain of TOR1 [19]. This finding begins to provide an explanation of how KOG1 might present substrates to the catalytic region of TOR1 within TORC1. Currently there is no structure of TORC2 and its resolution would be of great interest to better understand the regulation of TORC2 activity.

The intracellular localization of TOR and the TORCs has been debated for several years. In *S. cerevisiae*, early studies localized TORC1 at the plasma membrane, endosomes, the vacuolar membrane, or in the nucleus [20,21]. During the last couple of years, the issue of TOR localization was revisited. In *S. cerevisiae*, both TORC1 and TORC2 were shown to co-fractionate biochemically with endosomal membranes [22]. However, visualization of GFP-tagged versions of TOR1, TOR2 and their partners showed that TORC1 is localized to the vacuolar membrane [23,24•,25••,26•] while TORC2 is localized to the cytoplasm and discrete sites at the plasma membrane [24•,26•]. In *S. pombe*, GFP-tagged SpTOR2 exhibits uneven cytoplasmic localization which changes to undefined membranous perinuclear structures upon entry into G0 [6••]. In the green algae *C. reinhardtii*, the TOR and LST8 homologs were found by biochemical methods to be associated with ER membranes, and by indirect immunofluorescence to be localized at 'dot' structures near the plasma membrane and the basal body which is enriched in ER membranes [8]. In trypanosomes, TORC1 localizes mainly to the nucleus, a localization already observed in yeast and mammalian cells, and TORC2 was found to be associated with the ER and mitochondria [9•,21,27,28]. Altogether, the existence of TORC1 and TORC2 at diverse subcellular locations provides a molecular basis for the broad and distinct functions of the two TORCs.

TORC1 signaling and cell growth

Upstream regulators of TORC1

TORC1 is a key hub mediating temporal control of cell growth, by sensing a variety of extra and intracellular growth cues such as nutrients, especially nitrogen sources and amino acids availability, growth hormones (insulin/IGF), cellular energy (ATP), oxygen levels, and noxious stress [4]. In multicellular organisms, the best characterized upstream signaling cascade regulating TORC1 is the insulin–PI3K–TSC–Rheb pathway [4]. In this pathway, insulin–PI3K signaling inhibits the GTPase activating protein (GAP) activity of the TSC complex (a heterodimeric GAP consisting of the two proteins TSC1 and TSC2), allowing the small GTPase Rheb to activate TORC1. While TSC–Rheb–TORC1 signaling is not

found in *S. cerevisiae* (no TSC homologs), this pathway is present in the fission yeast *S. pombe* and in the filamentous fungal pathogen *C. albicans* [5,6••,16,29–32], indicating that the pathway appeared early in evolution. However, TSC1/2 is absent or not characterized in several organisms where TOR has been identified, including plants, worms, slime mold, and *T. brucei* (some of which evolved later than yeasts).

The translationally controlled tumor protein (TCTP), recently identified in *Drosophila* as a Guanine Exchange Factor (GEF) for Rheb, positively regulates Rheb and downstream TORC1 signaling [33]. While the role of TCTP in Rheb–mTORC1 signaling is not conserved in mammals, RNAi-mediated knockdown of TCTP in *A. thaliana* leads to growth phenotypes similar to loss of AtTOR, suggesting that TCTP is involved in TOR signaling in plants [34–36]. Furthermore, TORC1 in *Drosophila* seems to be regulated indirectly through proteasomal degradation [37]. Indeed, TSC2 can be degraded in an ubiquitin-dependent and E3 ubiquitin ligase complex (FBW5–DDB1–CUL4 complex)-dependent manner. This underscores the complexity of TORC1 regulation by the TSC–Rheb axis.

Amino acids and nitrogen sources are well known regulators of TORC1 in several organisms; however, the molecular mechanism that triggers TORC1 activation specifically by amino acids was unknown until recently. Two new activators of TORC1 in response to amino acids were identified in *D. melanogaster*. The MAP kinase homolog MAP4K3 activates TORC1 leading to the phosphorylation of S6K and 4EBP in response to amino acids but not to insulin [38•]. A dimeric complex of the two small GTPases RagA and RagC also activates TORC1 specifically in response to amino acids, independently of the TSC complex and downstream or in parallel to Rheb [39••,40]. The Rag complex affects cell growth and organ size in a nutrient-dependent manner and inhibits autophagy and starvation induced cell death [39••,40]. The underlying molecular mechanism is likely conserved in *S. cerevisiae* because the vacuolar proteins GTR1 and GTR2 (homologs of RagA and RagC) are involved in microautophagy and have been proposed to mediate the amino acid signal to TORC1 [20,41]. This notion is supported by the finding that GTR1 and GTR2 are required to inhibit nitrogen catabolite-repressed (NCR) genes, which are well known target genes of TORC1 signaling, and that GTR deletion causes rapamycin hypersensitivity, an indication of a defect in TORC1 signaling [42,43].

In *S. cerevisiae*, membrane trafficking to the vacuole appears to be important to trigger the amino acid signal to TORC1. Indeed, several recent studies have shown that TORC1 co-fractionates and genetically interacts with several proteins involved in protein sorting to the vacuole, including the class C and class D VPS (Vacuolar Protein Sorting) proteins [22,44,45]. Mutants defective in these VPS proteins exhibit rapamycin hypersensitivity, reduced intracellular amino acid pools, and constitutive GLN3 nuclear accumulation [44,45] — all phenotypes suggesting a defect in TORC1 signaling. These findings, together with the localization of TORC1 at the vacuolar membrane and at the endosome, suggest a model in which intracellular protein trafficking (from Golgi to endosomes and from endosomes to vacuole) controls the level of intracellular amino acids which in turn affect TORC1 activity at the vacuolar membrane and at endosomes.

Targets and processes downstream of TORC1

TORC1 mediates temporal control of cell growth in response to nutrients and, in metazoans, growth factors. The growth-related processes controlled by TORC1 include transcription, protein synthesis, ribosome biogenesis, autophagy, stress responses, and nutrient transport. Despite the large number on TORC1-regulated processes, only a few direct TORC1 substrates have been identified. In metazoans, the best characterized substrates for TORC1 are the translational inhibitor 4E-BP and the S6 kinase S6K [4]. In *Drosophila*, the dephosphorylation of S6K upon rapamycin treatment or amino acid withdrawal depends on the activity of the type 2A phosphatase PP2A, linking two well known TORC1 functions — activation of kinases and inhibition of phosphatases [46]. TORC1–S6K signaling seems to be conserved in yeast. In *S. cerevisiae*, the AGC kinase and S6K homolog SCH9 is directly phosphorylated and activated by TORC1 [25••]. SCH9 is required for TORC1-dependent regulation of ribosome biogenesis, control of translation initiation via phosphorylation of the S6 ortholog, activation of RNA polymerase III through direct phosphorylation and inhibition of the transcriptional repressor MAF1, and inhibition of entry into G0 phase via direct phosphorylation of the kinase RIM15 [25••,47,48]. Contrary to the known inhibitory effect of noxious stresses on TORC1–SCH9 [25••,47,49], osmotic stress seems to promote the binding of SCH9 to the promoter of osmostress-responsive genes where it acts as a transcriptional activator [50]. This finding together with genome-wide expression profiling suggests that SCH9 also has TORC1-independent functions in yeast [50,51].

One of the major roles of TORC1 in the promotion of cell growth is the positive regulation of ribosome biogenesis and function [4,52]. In yeast, TORC1 regulates ribosomal protein (RP) gene and ribosomal biogenesis (RiBi) gene expression through SCH9 [25••] and through the control of at least two transcription factors, FHL1 and SFP1 [4,52]. Interestingly, it was recently shown in yeast that TORC1 binds and phosphorylates SFP1 directly, in a rapamycin sensitive manner, and that this phosphorylation by TORC1 is essential to maintain SFP1 in the

nucleus where it can activate RP and RiBi gene expression [53•]. The authors of this study propose that SFP1 is a functional homolog of the proto-oncogene c-MYC that regulates RP and Ribi gene transcription in mammals [52,53•,54]. This notion was recently strengthened by the finding that MYC in flies is downstream of TORC1 in activating cell growth, by promoting Ribi gene expression in response to nutrients [55•,56•]. MYC binds and activates several Ribi genes in a TORC1-dependent manner [56•]. Furthermore, an overgrowth phenotype due to TSC1 knockdown in flies is completely abolished in the absence of MYC, while the effect of MYC overexpression in muscle cell endoreplication is abolished by TSC overexpression [55•,56•]. However, MYC is regulated not only via the TOR signaling pathway but also by the EGFR–Ras pathway [57•]. Taken together, these data support a model in which MYC in flies is a downstream effector of TOR signaling in regulating Ribi gene expression and cell growth in response to nutrients.

TORC1 also regulates other factors involved in RP gene expression or ribosome biogenesis. In yeast, TORC1 regulates the subcellular localization of the 40S ribosome biogenesis factor DIM2, and the function of HMO1 in the regulation of rRNA and RP genes expression [58,59]. In *Drosophila*, TORC1 controls the RNA pol I dependent transcription factor TIF-IA in the regulation of rRNA synthesis, similarly to what was observed for the regulation of TIF-IA homologs in yeast and mammals [60–62].

In response to nutrients, TORC1 inhibits autophagy, a process of bulk degradation of protein and organelles by the vacuole/lysosome [63]. Autophagy is mediated by a number of conserved ATG proteins each of which acts at a specific step in this process. Activation of the protein kinase ATG1 is a critical step in the induction and formation of the preautophagosomal structure (PAS). In yeast, TORC1 inactivation upon starvation leads to the dephosphorylation of ATG13 (see Figure 1). Dephosphorylated ATG13 then forms a complex with ATG1 and ATG17 in which the ATG1 kinase is activated [64]. At the signaling level, it appears that both SCH9 and the type 2A phosphatases, two well known TORC1 targets, are also implicated in the regulation of autophagy [65,66]. However, it should be noted that SCH9 cooperates with PKA to inhibit autophagy and that this effect seems to be partially independent of TORC1 activity [65]. The ATG1–ATG13 complex is conserved in *Drosophila* as a nutrient-sensitive regulator of autophagy. In flies, ATG1 interacts with TOR, and the ATG1–ATG13 complex is phosphorylated in a TOR-dependent and ATG1-dependent manner (see Figure 1). However, contrary to what is observed in yeast, ATG13 in flies is hyperphosphorylated even during starvation and remains associated with ATG1 during feeding conditions [67]. Interestingly, in flies (and mammalian cells), overexpression of ATG1 inhibits TORC1 signaling while ATG1 disruption increases TORC1 activity as measured by variation in TOR-dependent S6K phosphorylation [68•,69•]. Furthermore, overexpression of ATG1 or depletion of ATG13 significantly affects the cellular localization of TOR in flies [67]. These results suggest a model whereby ATG1–ATG13, by antagonizing TORC1, is involved in a self-reinforcing feedback loop [67,68•,69•] (see Figure 1).

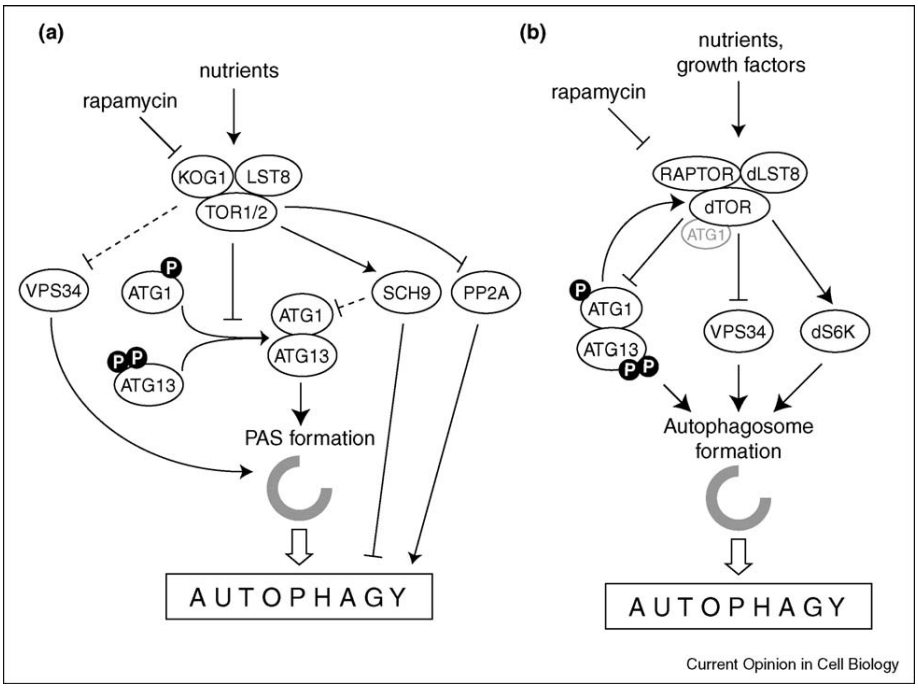
A second target of TORC1 during autophagy in *Drosophila* might be the class III PI3K VPS34 (see Figure 1). VPS34 is activated by TORC1 and ATG1 and is required for TORC1-dependent and ATG1-dependent PAS formation during starvation [70]. These findings suggest that VPS34 is a downstream target of nutrient–TORC1–ATG1 signaling, contrary to what has been observed in mammals where VPS34 seems to be upstream of mTORC1 [71]. The regulation of VPS34 by TORC1, as seen in flies, might be conserved in *S. cerevisiae*. A *VPS34* deletion in yeast is synthetic lethal with a *TOR1* deletion, and a *VPS34* deletion impairs GLN3-dependent gene expression (a well known TORC1 readout) in response to poor nitrogen source [44,45]. Finally, as discussed below, autophagy has emerged as an important process in the regulation of longevity by TORC1 in flies, worms, and yeast.

TORC1 and aging

Over the last five years, TORC1 has been shown to promote aging in invertebrates. Similar to the well known negative effect of dietary restriction (DR) on aging, inactivation of TORC1 leads to an increase in lifespan in *C. elegans*, *D. melanogaster*, and *S. cerevisiae* [72]. Furthermore, in yeast and worms, DR is not able to further increase lifespan when TORC1 is inactive, suggesting that DR and TORC1 are in the same pathway regulating aging [72].

During the last two years, important advances were made in understanding how TORC1 regulates lifespan. Several independent studies in worms and yeast have shown that translation capacity of the ribosomal pool, which is well known to be positively regulated by TORC1, is a determinant of longevity. In *C. elegans*, knockdown of genes encoding ribosomal proteins (RPs) or translation initiation factors, such as the S6K homolog RSKS-1 or the eIF4E homolog IFE-2, significantly increases lifespan [73,74•,75•,76••]. Similarly, in *S. cerevisiae*, deletion of nonessential RP genes, especially those encoding the 60S ribosome subunit, also extends lifespan [77,78••]. Contrary to what is observed in *C. elegans*, DR is unable to extend further the long lifespan phenotype of RP gene mutants in yeast, similar to a *tor1* mutant [74•,76••,78••]. This suggests that translation is an important determinant of longevity downstream of DR/TORC1 signaling. Interestingly, in *S. cerevisiae*, the nutrient-controlled transcription factor GCN4 is required for full lifespan extension in

Figure 1



Regulation of autophagy by TORC1 in **(a)** *S. cerevisiae* and **(b)** *D. melanogaster*. In response to nutrient availability, TOR complex 1 (TOR1/TOR2/dTOR, KOG1/RAPTOR, and LST8/dLST8) negatively regulates autophagy in yeast and flies. (a) In *S. cerevisiae*, TORC1 inhibits autophagy by promoting hyperphosphorylation of the kinase ATG1 and ATG13. Upon TORC1 inhibition (nutrient limitation or rapamycin treatment) ATG1 and ATG13 are dephosphorylated and associated. This leads to the formation of the preautophagosomal structure (PAS), an essential early step in autophagy. The protein kinase SCH9, a direct target of TORC1, and the TORC1 controlled type 2A phosphatase (PP2A) SIT4 inhibit and promote autophagy. The class III PI3K VPS34 is likely involved in the TORC1-dependent regulation of autophagy. (b) Contrary to yeast, in *D. melanogaster*, the ATG1–ATG13 interaction is not altered upon TORC1 inhibition. ATG1 interacts directly with dTOR and, once activated, signals back to TORC1 in a positive feedback loop reinforcing autophagy. VPS34 is required for the early step of autophagosome formation. The AGC kinase S6K is required for normal induction of autophagy.

response to either DR, to inhibition of TORC1–SCH9 signaling or to 60S ribosome subunit depletion, placing GCN4 downstream of TORC1 in regulating lifespan [78••].

Autophagy is another TORC1-dependent process that seems to be important for DR-dependent lifespan extension. Several analyses in worms have shown that DR induces autophagy, and that blocking autophagy by RNAi-mediated knockdown of *ATG* genes blocks the extension of lifespan normally observed during DR or upon TORC1 loss of function (*let63* and *daf15* RNAi) [79•,80•,81•]. In *Drosophila*, mutation or knockdown of ATG genes shortens lifespan, while induction of autophagy by the overexpression of ATG8a in the brain reduces age-associated phenotypes and extends lifespan [81•,82•,83•]. Finally, in *S. cerevisiae* deletion of *ATG1* or *ATG7* shortens lifespan of post mitotic cells, known as chronological lifespan (CLS) [84]. Interestingly, in worms the forkhead transcription factor PHA4/FOXA seems to be a downstream effector of TORC1–S6K that promotes autophagy and longevity in response to DR [79•,85••,86••]. Taken together, these findings suggest a conserved mechanism in which TORC1 promotes aging through, at least, the inhibition of autophagy.

In budding yeast, different studies have highlighted the importance of the SCH9 branch of TORC1 signaling in longevity. TORC1 promotes aging through the inhibition of mitochondrial respiration [87••] whereas deletion of *SCH9* leads to increased respiration and increased lifespan. These findings suggest that SCH9 is an important downstream target of TORC1 inhibiting mitochondrial activity during growth in the presence of glucose [88]. Furthermore, the protein kinase RIM15, which is directly phosphorylated and inhibited by SCH9, and the RIM15 target transcription factors MSN2/4 and GIS1 are required for the increase in lifespan upon TORC1 inhibition [47,89•,90]. The activation of MSN2/4 upon TORC1 inhibition promotes the expression of the pro-longevity nicotinamidase gene *PNC1*. This leads to the activation of the well-known longevity regulator SIR2, a

histone deacetylase, suggesting that TORC1 promotes aging via negative regulation of the Sirtuin SIR2 in yeast [89•]. In both flies and worms, the PNC1 homolog was shown to be involved in regulating longevity and stress resistance [91,92]. However, at least in *Drosophila*, the nicotinamidase homolog does not seem to be regulated by TORC1 or DR [92].

It is striking that, in response to nutrients, TORC1 promotes aging in different organisms through a common set of downstream processes such as mitochondrial respiration, translation, autophagy, and transcription. Are these TORC1-regulated processes coordinated to regulate lifespan in response to nutrients? Recent findings seem to suggest an affirmative answer to this question. As shown in yeast, mature ribosomes are subject to selective, ubiquitin-dependent autophagy during nutrient starvation, linking autophagy, and translation capacity of the cell [93]. Furthermore, mRNA mistranslation in yeast induces *PNC1* expression which in turn activates SIR2, linking translation efficiency and SIR2 function [94].

TORC1 controls development

Several studies in invertebrates have highlighted the role of TORC1 as a regulator of development. In the filamentous pathogenic fungus *C. albicans*, nitrogen limitation induces a switch from nonfilamentous growth to filamentous growth, leading to virulence. Recently, different reports have suggested that nitrogen limitation induces this filamentation and virulence through a conserved TSC2–RHEB–TORC1–GLN3–MEP2 signaling cascade [30,95–97]. In the plant *Zea mays L* (maize), TOR expression is induced during germination. While TOR is not expressed early in germination, it appears during germination and remains at this high level in different tissues at later developmental stages and in adult plants [98].

TOR is important for development in *Drosophila*. For example, insulin–TOR–S6K signaling appears to be a critical pathway controlling neuronal growth and differentiation [99,100]. Furthermore, when fly larvae are starved, there is a delay in the transition from larva to pupa. This delay in the timing of development is mediated by TOR in the prothoracic gland (PG), a gland that regulates the production of the hormone ecdysone. It has been suggested that a nutrient-dependent signal activates TOR in the PG which in turn leads to the production of ecdysone and subsequent pupariation [101]. Similarly, in the honeybee *Apis mellifera*, change in nutrient quality during feeding of female larvae leads to dimorphic caste development. While normal nutrition leads to the development of female larvae into sterile worker bees, feeding of female larvae with a particularly nutritious 'royal jelly' results in the development of queen bees. Inhibiting TOR in female larvae fed royal jelly, by rapamycin treatment or RNAi, prevents development of queen bees and results in bees with worker phenotypes [102]. Furthermore, queen development is associated with high *TOR* gene expression [102,103]. This suggests that TORC1 is a central switch in diphenic caste development in response to nutrients, and identifies nutrient–TORC1 signaling as a social determinant.

TORC2 and growth control

Current knowledge on TORC2 is lagging behind that on TORC1, mainly because of the lack of a good pharmacological tool, such as rapamycin, for TORC2 inhibition. However, since the identification of TORC2 in yeast [104,105] and mammals [106,107], significant progresses has been made toward a better understanding of TORC2. Below we focus on the function and regulation of TORC2 in invertebrates. In general, the pattern that seems to be emerging is that TORC2 in different organisms is more varied than TORC1, in function and regulation.

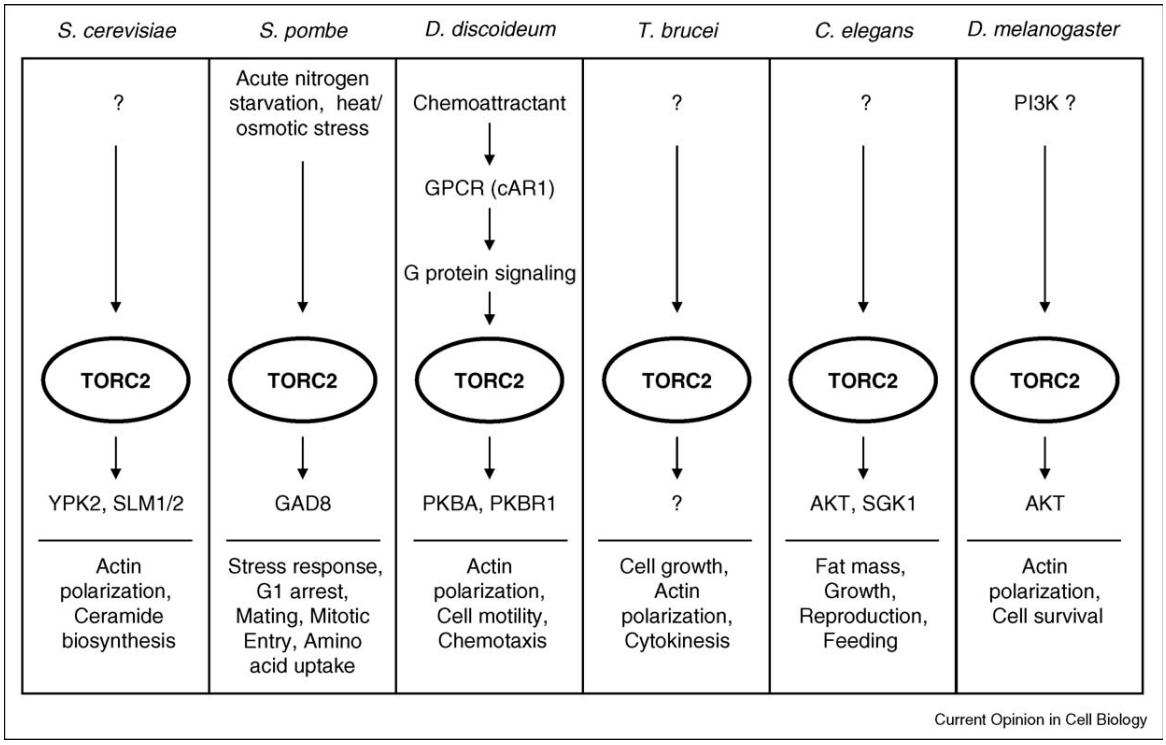
Upstream regulators of TORC2

While processes and targets downstream of TORC2 are known (actin cytoskeleton, lipid metabolism, etc.) the nature of the upstream regulators of TORC2 are poorly characterized. In *Drosophila*, like in mammals, TORC2 appears to be regulated by the insulin–PI3K pathway. However, in *Dictyostelium discoideum*, another pathway activates TORC2. In this organism, TORC2 is activated in response to a chemoattractant in a PIP3-independent manner. The activation of TORC2 by chemoattractant is mediated by heterotrimeric G proteins and by RAS-like G proteins rasC and rasG [108••]. But other intermediate G proteins are also suggested to be involved in this process. In yeast, while nitrogen source and stress are well known regulators of TORC1, their effect on TORC2 is unclear. In *S. pombe*, TOR1 is part of TORC2, but upon mild nitrogen limitation TOR1 seems also to be part of TORC1. TOR1 in TORC1 controls G2/M transition upon mild nitrogen limitation. TOR1 in TORC2 controls G1 arrest, activation of mating, and mitotic entry in response to heat and osmotic stress and upon complete nitrogen depletion [109,110]. These findings suggest that both TORC1 and TORC2 can be regulated by nitrogen at least in *S. pombe*. It is still unclear if the TSC1/2-RHEB pathway is able to regulate TORC2, in addition to TORC1, in *S. pombe*.

Targets and processes downstream of TORC2

Similar to TORC1, only few direct substrates have been identified for TORC2. The best characterized substrates of TORC2 are protein kinases of the AGC kinase family, such as YPK2 in *S. cerevisiae* and Akt/PKB in mammals, *D. melanogaster* and *C. elegans* [4,111]. TORC2 regulates these AGC kinases by phosphorylating a conserved serine or threonine in a C-terminal regulatory domain known as the hydrophobic motif. Over the last two years, this mechanism of regulation by TORC2 has shown to be conserved in other invertebrates. In *S. pombe*, TORC2

Figure 2



TORC2 signaling in different invertebrates. The upstream regulators of TORC2 are largely unknown. In *Schizosaccharomyces pombe*, TORC2 is activated by different stresses, including acute nitrogen starvation, heat, and osmotic stress. In *Dictyostelium discoideum*, TORC2 is activated by chemoattractant through the activation of G-protein-coupled receptors (GPCR-cAR1) and G protein signaling. It is unknown if PI3K is upstream of TORC2 in *Drosophila melanogaster.* Known or assumed direct phosphorylation substrates of TORC2 in different organisms are shown. YPK2 in *Saccharomyces cerevisiae*, GAD8 in *S. pombe*, and SGK1 in *C. elegans* are all SGK1 homologs. AKT in *C. elegans* and *D. melanogaster* and PKBA and PKBR1 in *D. discoideum* are all AKT/PKB homologs. Also indicated are downstream processes controlled by TORC2.

phosphorylates the hydrophobic motif in the YPK2 homolog GAD8 to control mitotic initiation and G1 arrest in response to stress [112]. Similarly, in *C. elegans*, TORC2 phosphorylates and activates the YPK2 homolog SGK1 [113••,114••]. This finding is supported by the observation that mTORC2 phosphorylates the hydrophobic motif of mammalian SGK1 in response to insulin [115••]. In *D. melanogaster*, TORC2 phosphorylates the hydrophobic motif in AKT, thereby activating AKT and mediating AKT-dependent inhibition of the forkhead transcription factor FOXO [116]. This activation of AKT is also found in *D. discoideum*, where TORC2 phosphorylates the AKT homolog PKBR1 in response to a chemoattractant.

Phosphorylation of the hydrophobic motif is not the only way that TORC2 regulates AGC kinases. TORC2 also phosphorylates another conserved AGC kinase region known as the turn motif. In *D. melanogaster* and in mammals, SIN1 knockout decreases phosphorylation of the turn motif in AKT [117]. Similar to AKT, it has been shown in *S. cerevisiae* and mammals that a TORC2 deficiency reduces phosphorylation of the turn motif in PKC [117]. This suggests conservation of a TORC2–PKC signaling pathway at least in yeast and mammals. In mammals, TORC2-dependent phosphorylation of the PKCα turn motif regulates PKC stability [117,118]. All together, these data support the notion that TORC2 is a master regulator of AGC kinases, including AKT, PKC, and YPK2/GAD8/SGK1 (Figure 2) [111].

TORC2 was originally identified as a regulator of actin cytoskeleton organization in yeast and mammals and was later shown to have a similar role in *D. discoideum* [4]. This function was recently shown to be conserved in the protozoan parasite *T. brucei* where TORC2 controls actin polarization and cytokinesis [9•]. In yeast and mammals, TORC2 regulates the actin cytoskeleton via Rho-type small GTPases [4]. This mechanism is likely conserved in *D. discoideum*, as the TORC2 substrate PKBR1 regulates GACQ, a RHO-GTPase activating protein [108••]. Recently, it was shown in *S. cerevisiae* that TORC2 controls RHO1 via two independent effector pathways. One is mediated by AVO1 and the other by AVO2 and SLM1/2 [119]. Furthermore, the recent finding that TORC2 colocalizes and genetically interacts

with the endocytic pathway, which relies on the actin cytoskeleton, suggests that TORC2 may directly regulate endocytosis in *S. cerevisiae* [22].

TORC2 regulates lipid metabolism in several organisms. Sphingolipids, glycerolphospholipids, and sterols are the major lipids in eukaryotic membranes whose concentrations are tightly controlled. For a long time, there were hints suggesting the involvement of TORC2 in sphingolipid metabolism in *S. cerevisiae*, first genetic evidence [120] and later through the discovery of SLM1/2 as a TORC2 substrate [121–123]. More recently, the temperature sensitive *avo3-30* mutation, a new TORC2 mutant allele defective in AVO3, allowed the analysis of sphingolipid content in *S. cerevisiae* and revealed that TORC2 mediates *de novo* ceramide synthesis. The *avo3-30* mutant displays a strong reduction in the major yeast ceramide (phytosphingosine), because of reduced ceramide synthase activity. TORC2 controls ceramide synthase via phosphorylation of YPK2. Furthermore, previous reports suggested that calcineurin antagonizes TORC2 [121,124], and it was shown recently that inhibition of calcineurin, by deletion of its regulatory subunit CNB1, restores ceramide levels in the *avo3-30* mutant. The regulation of ceramide synthase is a novel function of TORC2 signaling, separate from TORC2-dependent actin polarization [125••]. The link between TORC2 and lipid metabolism is conserved in multicellular organisms. In *C. elegans*, mutants defective in RICTOR accumulate triacylglycerol (fat mass), and show developmental delay, small body size, smaller brood size, and abnormal feeding behavior. These observed phenotypes are due mainly to a defect in TORC2–SGK1 signaling rather than to a defect in TORC2-mediated activation of AKT or PKC. Only the fat mass phenotype of the RICTOR mutant could be attributed to a defect in AKT (and SGK1) activation. Thus, SGK1 appears to be the major downstream effector of TORC2. However, TORC2 clearly regulates more than SGK1 in *C. elegans*, since an *SGK1*-active allele cannot suppress the phenotypes of a strong RICTOR mutant [113••,114••]. TORC2 also regulates SGK1 in mammals [115••], but it remains to be determined whether mTORC2 controls lipid synthesis.

Finally, while TORC2 is essential in yeasts and mammals, flies lacking the TORC2 component RICTOR or SIN1 are viable and show only minor growth phenotypes despite a severe reduction in phosphorylation of the hydrophobic motif in AKT [126]. However, similar to what is observed in a mouse model for prostate cancer, TORC2 in flies becomes essential for hyperplasia caused by a high level of PI3K signaling [126,127]. This suggests that the importance of TORC2 in cell growth regulation differs depending on the organism and physiological context.

Future directions

Although research on TOR signaling in invertebrates continues to make important contributions, many questions remain unanswered. The observation that both TORC1 and TORC2 are at multiple locations in the cell suggests that there are functionally different subpopulations of each TORC. It would be of interest to assign location-specific functions to the TORCs. The different localizations of the TOR complexes, together with the many different biological processes regulated by TORC1 and TORC2, also suggest that there are many direct substrates remaining to be identified. The identification of these substrates either by targeted or by proteomic approaches, will likely be a major goal in the future. Furthermore, mechanisms that determine localization of the TORCs, possibly in response to growth conditions, would also be of interest. Another important, understudied aspect of TOR signaling is how and where TOR complexes are assembled and if this biogenesis is regulated.

While biological readouts of TORC1 are relatively well established, the exact mechanism by which nutrients act on TORC1 and the direct substrates of TORC1 are largely unknown. In *Drosophila* (and mammals), how do amino acids feed into the RAG proteins and MAP4K3 to regulate TORC1? What is the molecular mechanism by which signals are relayed to TORC1, particularly in *S. cerevisiae*, worms and plants where the TSC1/2-RHEB axis does not exist or is incomplete? The upstream regulators and direct substrates of TORC2 are even less well known. Thus, research on TOR signaling in invertebrates should remain an active area in the coming years.

Acknowledgements

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