Review

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TOR complex 2: a signaling pathway of its own

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Research on TOR has grown exponentially during the last decade, generating a complex model of the TOR signaling network. Rapamycin treatment provides a simple and straightforward method to inhibit the TOR signaling pathway and to study the influence of TOR on multiple cellular processes. The discovery of two distinct TOR complexes, TORC1 and TORC2, showed that studies using rapamycin targeted only one TOR signaling branch. TORC1 is directly inhibited by rapamycin, whereas TORC2 is not. There is no known TORC2-specific inhibitor, so genetic manipulation is required to study its biological function(s). Many studies in genetically tractable model organisms have expanded our understanding of TORC2 signaling. Here we focus on the TORC2 signaling pathway as revealed by these (mostly recent) studies.

TOR signaling pathways: an overview

Target of rapamycin (TOR) is a central controller of the growth and metabolism of cells in response to nutrients, growth factors and cellular energy status (reviewed in [1,2]). This serine/threonine kinase is conserved from yeast to mammals and is found in two functionally distinct multi-protein complexes named TOR complex 1 (TORC1) and TORC2. The two complexes contain shared components, including TOR (mTOR in mammals) and Lst8 (mLST8 in mammals), and complex-specific components such as Kog1 (raptor in mammals; found in TORC1) or Avo3 (RICTOR in mammals; found in TORC2) [3–7]. In complex with binding protein 12 (FKBP12), rapamycin binds and inhibits TOR exclusively in TORC1, whereas FKBP12–rapamycin cannot bind TOR in TORC2, making this complex insensitive to direct inhibition by rapamycin. In mammals, however, long-term treatment with rapamycin can prevent TORC2 assembly and thereby indirectly inhibit TORC2 activity in a few cell types [8]. TORC1 regulates diverse cellular processes, including protein synthesis, ribosome biogenesis, transcription and autophagy, some of which are regulated through the direct substrates S6 kinase (S6K) in mammalian cells [9–11] and Sch9 in yeast [12,13], both members of the AGC kinase family. TORC2 function is less well understood; to date, the main function associated with TORC2 is the regulation of actin cytoskeleton dynamics. More recently, TORC2 has also been shown to phosphorylate several AGC kinases in different organisms, suggesting that TOR in both complexes might be the main kinase phosphorylating AGC kinases at their hydrophobic motif site (reviewed in [14]). TORC1 function has been extensively discussed in other reviews, so this review highlights the major findings on TORC2, in yeast, slime mold, flies, worms and mammals.

TORC2 in budding yeast

TOR was initially identified in the budding yeast *Saccharomyces cerevisiae* in a genetic selection for spontaneous rapamycin-resistant mutants [15]. In contrast to other eukaryotes, yeast (budding and fission yeast) contain two TOR genes, *TOR1* and *TOR2*. The existence of two TORs in yeast facilitated the study of TOR signaling because it initially helped to identify two separate TOR signaling branches. Biochemical studies subsequently demonstrated the existence of two functionally distinct TOR complexes that correspond to, and thereby confirm, the two previously identified branches. Whereas rapamycin-sensitive TORC1 contains TOR1 or TOR2, rapamycin-insensitive TORC2 contains only TOR2. TORC2 also contains Avo1, Avo2, Avo3, Lst8 and Bit61 [6,16] (Figure 1a). Avo1, Avo3 and Lst8 are essential conserved proteins required for kinase activity. By contrast, Avo2 and Bit61 are not essential, and no clear homologous

Glossary

AGC kinases: members of the conserved AGC (protein kinase A, protein kinase G, protein kinase C) protein kinase family. These kinases share a common mechanism of activation: they are fully activated upon phosphorylation within the activation loop (also known as 'T-loop'), the hydrophobic motif, and the turn motif. AGC kinases that are regulated by PI3 kinase are phosphorylated at the activation loop by PDK1.

Cell wall integrity pathway: signaling pathway in yeast which regulates cellular responses to cell wall and membrane stress. Activation of the pathway results in up-regulation of cell-wall biosynthetic enzymes, heat-shock proteins, and cell-wall components.

Eisosomes: immobile protein assemblies of uniform size at the plasma membrane of yeast. Eisosomes mark sites of endocytosis (from the Greek '*eis*' = into or portal, and '*some*' = body) and are found within plasma membrane microdomains that contain a distinct protein and lipid composition. The main components of these protein complexes are the two peripheral proteins Pil1 and Lsp1, and the transmembrane protein Sur7. Eisosome disruption results in aberrant plasma membrane invaginations and impaired endocytosis. Eisosome assembly and turnover are regulated by the sphingolipid–Pkh1–Ypk1 signaling pathway.

N-myc downstream regulated 1 (NDRG1): this protein is phosphorylated by SGK1 and GSK3β at several sites. NDRG1 expression is induced in response to various stress signals.

Negative feedback loop: the negative feedback loop from mTORC1 and its effector S6K to IRS blocks upstream insulin signaling. Rapamycin inhibits this negative feedback loop.

Syndecans: a family of transmembrane proteoglycans. The extracellular domain carries several heparan and chondroitin sulfate domains that allow interaction with extracellular matrix proteins, growth factors and cytokines. Syndecan-4 regulates cell adhesion; its cytoplasmic domain interacts with phosphatidylinositol-4,5-bisphosphate (PIP2).

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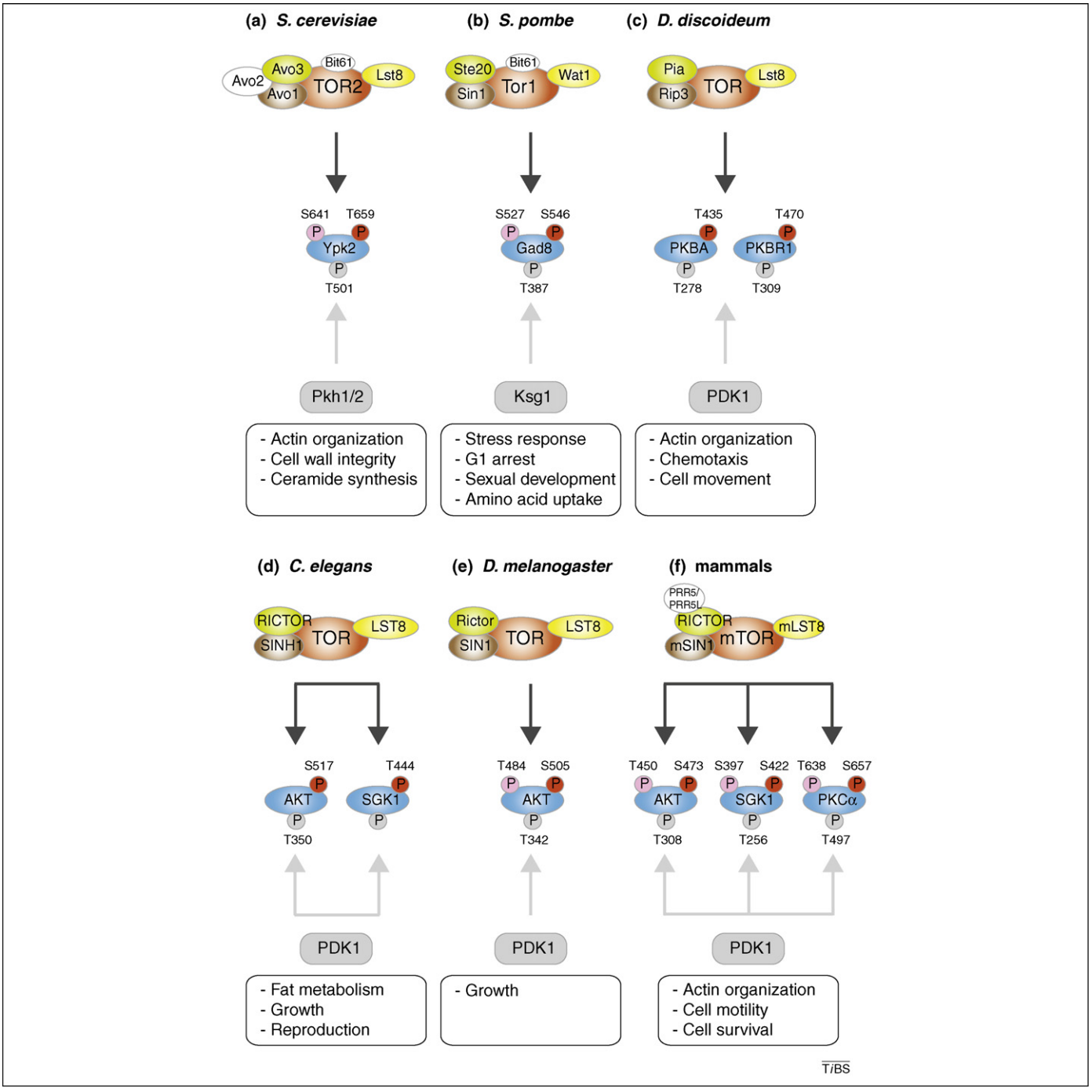

Figure 1. The conserved TOR complex 2 pathway.
The structure and function of TORC2 are conserved in **(a)** budding yeast (*S. cerevisiae*), **(b)** fission yeast (*S. pombe*), **(c)** slime mold (*D. discoideum*), **(d)** worms (*C. elegans*), **(e)** flies (*D. melanogaster*) and **(f)** mammals. In all organisms, TORC2 is composed of the TOR kinase (orange), Lst8 (yellow), Avo3 or its homolog (green), and Avo1 or its homolog (brown). In *S. cerevisiae, S. pombe* and mammals, additional TORC2 components have been identified (white). All shown TORC2 substrates (blue) are members of the AGC kinase family. The AGC kinases share a conserved mode of regulation involving phosphorylation of their hydrophobic motif (red), turn motif (pink), and activation loop (gray). TORC2 phosphorylates the hydrophobic motif in all shown kinases. TORC2 has so far been shown to phosphorylate the turn motif, directly or indirectly, in Ypk2, Gad8, and mammalian AKT and PKCα. PDK1 (gray), which is also conserved in all organisms shown, phosphorylates the activation loop.

counterparts have been identified so far in higher eukaryotes; however, Bit61 and the mammalian TORC2 (mTORC2) component proline-rich protein 5 (PRR5) share low sequence similarity [17,18]. Studies investigating the molecular organization of TORC2 in yeast revealed that TORC2 is oligomeric (probably a TORC2–TORC2 dimer) [19].

Even before the two TOR complexes were identified, TOR2 (but not TOR1) was known to regulate the cell cycle-dependent polarization of the actin cytoskeleton [20], thereby implicating TOR2 (and hence later TORC2) in the spatial control of yeast cell growth. Further studies showed that the aberrant depolarization of the actin cytoskeleton in TORC2-temperature sensitive mutants could be suppressed by hyperactivation of the cell wall integrity pathway, which involves protein kinase C1 (Pkc1). Several genetic studies revealed that Pkc1, via the Rho-like GTPases Rho1 and Rho2 and their GDP/GTP

exchange factor Rom2, mediates TORC2 signaling to actin organization [6,21–24]. However, further molecular details on the activation of this pathway required the identification of TORC2 substrates. In 2005, Kamada *et al*. identified the first yeast TORC2 substrate, yeast protein kinase 2 (Ypk2) [25]. Ypk2 is an AGC kinase and is closely related to mammalian serum and glucocorticoid-induced protein kinase-1 (SGK1). TORC2 activates Ypk2 by directly phosphorylating Ser641 and Thr659 in the turn and hydophobic motifs, respectively (Figure 1a). Recent results indicate that TORC2 activity is also required for the phosphorylation of the turn motif in Pck1 [26], although it is unclear if TORC2 phosphorylates Pkc1 directly. In addition to Ypk2, Slm proteins, which bind phosphatidylinositol-4,5-bisphosphate (PIP2), have been characterized as direct TORC2 substrates. Slm1 and Slm2 can regulate actin organization independently of Ypk2 [27]. However, a constitutively active mutant of Ypk2 suppresses the lethality provoked by the complete loss of TORC2 [25], suggesting that Ypk2 is the main TORC2 effector. Most TORC2-mediated functions, including actin remodeling, are now believed to be mediated via Ypk2.

Aronova *et al*. [28] recently described a new function for TORC2. They showed that TORC2, via Ypk2, controls the sphingolipid biosynthetic pathway and hence mediates *de novo* ceramide biosynthesis. Other studies have shown that Ypk2 and its homolog Ypk1 are also involved in regulating eisosome assembly and turnover in a sphingolipid-dependent manner [29]. Eisosomes are protein complexes near the plasma membrane involved in the early steps of endocytosis. Whether TORC2 is also directly involved in the regulation of endocytosis remains to be confirmed. Interestingly, GFP-tagged TOR2 localizes to punctate structures in the proximity of the plasma membrane, which resemble eisosomes [30]. Thus, the downstream effectors and functions of TORC2 in *S. cerevisiae* are coming into focus, but the upstream regulators of TORC2 remain completely unknown.

TORC2 in fission yeast

Schizosaccharomyces pombe, like *S. cerevisiae*, has two TOR homologs, Tor1 and Tor2. However, and this easily leads to confusion, fission and budding yeast TOR proteins are numbered in the opposite way because the *S. pombe* proteins were named based on order of discovery rather than on function. Budding yeast Tor2 is the sole TOR protein found in TORC2, whereas in fission yeast Tor1 is the main determinant of TORC2 [31], suggesting that *S. pombe* Tor1 is functionally equivalent to *S. cerevisiae* Tor2. *S. pombe* TORC2 is composed of Tor1, Sin1, Ste20, Wat1 (also known as Pop3) and Bit61 (Figure 1b). *S. pombe* Tor1 is not essential for normal growth, but is required for survival under stress conditions, proper G1 arrest, and sexual development [32,33]. Surprisingly, in contrast to other organisms, rapamycin has no effect on normal growth in *S. pombe*. Initially, rapamycin was found to affect sexual development; later experiments showed that Tor1-mediated amino-acid uptake in fission yeast is also rapamycin sensitive [34–36]. Rapamycin blocks Tor1-mediated functions, so it was believed that rapmycin in fission yeast inhibits TORC2 function. However, Petersen and Nurse recently showed that rapamycin can also inhibit Tor2 (and hence TORC1) but in a nutrient-dependent manner [37]. Currently, it remains unclear why rapamycin does not arrest fission yeast growth as in other eukaryotes. Furthermore, the molecular details of how rapamycin inhibits TORC2, and possibly TORC1, are not well understood.

To identify potential substrates of fission yeast Tor1, Matsuo *et al.* carried out a high-copy suppressor screen of a *Tor1* sterile mutant and isolated Gad8 as a potential candidate [38]. Gad8 is a Ser/Thr kinase belonging to the AGC kinase family and is the fission yeast homolog of Ypk2 in budding yeast and SGK1 in other organisms. They demonstrated that the activity and phosphorylation status of Gad8 depend on Tor1 activity. On a molecular level, they showed that the critical Tor1 phosphorylation sites in Gad8 are Ser527 and Ser546, the turn and hydrophobic motif sites, respectively (Figure 1b). These findings contributed to the idea that TOR complexes regulate many AGC kinases.

TORC2 in slime mold

Dictyostelium discoideum is a powerful model organism to study the mechanisms of cell movement and chemotaxis. This slime mold is a unicellular eukaryotic organism that, upon starvation, forms multicellular aggregates. This developmental program depends on chemotaxis toward a high extracellular level of cyclic AMP secreted by neighboring cells. Lee *et al.* showed that the underlying mechanism of cell movement and aggregate formation depends on TORC2 [39]. TORC2 in *D. discoideum* comprises TOR, Lst8, Rip3 (Avo1 in *S. cerevisiae*) and Pia (Avo3 in *S. cerevisiae*) (Figure 1c). Cells deficient in any of these components lose speed, cell polarity and directionality, i.e. they display an overall defect in chemotaxis. Furthermore, Lee *et al.* demonstrated that cells lacking any TORC2 component exhibit reduced PKBA and PKBR1 activity, two AKT (also known as protein kinase B) homologs and AGC kinase family members that are required to fully activate the chemotactic response.

Chemoattractant signaling triggers several cellular responses. One well-characterized response is the production of phosphatidylinositol 3,4,5-triphosphate (PIP3) at the leading edge of motile cells. It is well established that PIP3 production is an important step in regulating chemotaxis; however, chemotaxis still occurs in the absence of PIP3 [40,41], and a recent study provided insight on the molecular pathway regulating PIP3-independent chemotaxis. Kamimura *et al.* [42] showed that TORC2 is activated in a PIP3-independent manner by a heterotrimeric G protein and by cytosolic Ras GTPases. This signaling leads to activation of the PKBs, mainly PKBR1, which in turn phosphorylate several downstream targets to ultimately regulate directed cell movement. These findings suggest a possibly direct regulation of TORC2 by Ras. This idea is further supported by the presence of a Ras-binding domain in Rip3 (Ras-interacting protein-3), the mSIN1 homolog in *D. discoideum*, which, when mutated, causes an impaired chemotactic response [39]. Kamimura *et al.* also confirmed that TORC2 activity is required to phosphorylate the hydrophobic motif in PKBA and PKBR1 and thus to

activate these AGC kinases [39]. Overall, these studies in *D. discoideum* underscore the importance of TORC2 in actin remodeling and cell movement, and could provide insight on the role of TORC2 in actin organization in other organisms. *D. discoideum* might also be a particularly valuable system for unraveling the upstream regulation of TORC2. Very little is known about the upstream regulation of TORC2 in other organisms, so it would be of interest to know if Ras-mediated activation of TORC2 is conserved in other organisms.

TORC2 in worms and flies

Recently, two independent studies demonstrated that TORC2 is also present in the nematode *Caenorhabditis elegans* [43,44]. Both studies identified *Rictor* in a screen for mutants with altered lipid storage and showed that loss-of-function mutants in *Rictor* are viable, but developmentally delayed with a reduced overall body size (Figure 1d). Increased fat storage in the mutant worms suggests an important role for TORC2 in regulating fat metabolism. Interestingly, an *sgk1* null mutant, but not a mutant with impaired AKT signaling, phenocopies a *CeRictor* mutant, and a constitutively active SGK1 suppresses a *Rictor* mutation. Thus, TORC2 in worms appears to signal mainly through SGK1. This, combined with findings in yeast suggesting that the SGK1 homolog Ypk2 is the main TORC2 effector, lends doubt to whether AKT is the primary effector of TORC2 in other eukaryotes; indeed, loss of mTORC2 in mammals has a minimal effect on AKT activity (discussed below).

Similar to TORC2 mutants in worms, *rictor* and *sin1* mutants of the fly *Drosophila melanogaster* are viable, but reduced in body size [45,46]. Also in flies, TORC2 is the main kinase to phosphorylate AKT at the hydrophobic motif (Figure 1e). Although loss of TORC2 normally causes only a mild growth defect in flies, Hietakangas and Cohen [45] made the interesting observation that loss of TORC2 strongly inhibits hyperplasia caused by elevated phosphatidylinositol-3-kinase (PI3K) signaling, suggesting that TORC2-regulated AKT activity might be more important in conditions of elevated AKT signaling.

TORC2 in mammals

Mammalian TORC2 was identified in 2004. At that time, TORC2 was known to consist of mTOR, mLST8 and the TORC2-specific component RICTOR [3,7]. More recently, two additional complex-specific components were identified—the Avo1 homolog mSIN1 [47–49], and the Bit61 family members PRR5 and PRR5L (also known as Protor1 and Protor2) [18,50,51]. Except for PRR5 and PRR5L, all mTORC2 components are essential and knockout of any one of them in mice results in developmentally delayed embryos that die in mid-gestation around embryonic day E10.5 [48,49,52,53].

Similar to TORC2 in *S. cerevisiae*, mTORC2 cannot be directly inhibited by rapamycin. However, in a few cell lines, prolonged treatment with rapamycin can indirectly inhibit mTORC2 activity [54]. By example, Sarbassov *et al.* showed that long-term treatment with rapamycin prevents *de novo* mTORC2 assembly and thereby inhibits mTORC2 activity, but only in a few of the many cell lines examined. The effect of rapamyin on mTORC2 assembly appears to be due to rapamycin binding free mTOR and to an indirect consequence of rapamcyin's inhibition of protein synthesis and thus the synthesis of new mTOR.

A recent study by Copp *et al.* [55] showed that mTOR is phosphorylated in an mTORC-specific manner. mTOR phosphorylation at Ser2448 is predominantly (but not exclusively) associated with mTORC1, whereas mTOR in mTORC2 is specifically phosphorylated at Ser2481. Copp *et al.* suggest that the mTORC2-specific phosphorylation at Ser2481 could be used as a biomarker for mTORC2 sensitivity to rapamycin. However, to date, the functional importance of these phosphorylation sites is completely unknown, and it is important to point out that these sites should always be used along with other complex-specific readouts to definitively specify complex activation.

The first function ascribed to mTORC2, based on the previously known function of TORC2 in yeast, was the regulation of the actin cytoskeleton. Knockdown of mTORC2-specific components in cultured cells results in alteration of the actin cytoskeleton. Furthermore, also as in yeast, it was suggested that mTORC2 signals to the actin cytoskeleton via RhoGTPases and PKC [3,7]. However, although two research groups have independently observed an altered actin cytoskeleton upon knockdown of mTORC2-specific components, opposite phenotypes were observed. Jacinto *et al.* [3] observed that mTORC2 is required for cell spreading and assembly of actin fibers. By contrast, the findings by Sarbassov *et al.* [7] suggest that loss of mTORC2 promotes actin fiber assembly. More recently, a role for mTORC2 in regulating the actin cytoskeleton was questioned when obvious alterations in the actin cytoskeleton were not observed in embryonic fibroblasts derived from *Rictor* knockout mice [52,53]. The apparent discrepancies in mTORC2-mediated actin regulation could possibly be related to the different systems studied. The early knockdown studies looked at actin changes immediately after loss of mTORC2, but used different cell lines, whereas subsequent studies looked at knockout cells permanently deficient for mTORC2. Cells constitutively lacking mTORC2 might adapt by using other mechanisms to regulate actin cytoskeletal organization. Several other studies using different approaches have supported a role for mTORC2 in actin-regulated processes. Misregulated mTORC2 activity results in altered cell motility in various cell types, including different cancer cells where migration plays an important part in metastasis [56–58]. Overall, the molecular mechanism by which mTORC2 regulates the actin cytoskeleton remains unclear.

In 2005, the first direct substrate of mTORC2, AKT, was identified. mTORC2 was found to be a long sought-after kinase phosphorylating Ser473 in the hydrophobic motif of AKT (Figure 1f) [8,59]. Although earlier knockdown studies of RICTOR also showed reduced phosphorylation of Thr308 in the activation loop, further studies in knockout mice suggested that phosphorylation of Thr308, by phosphoinositide-dependent kinase 1 (PDK1), does not depend on prior Ser473 phosphorylation [47,48,52,53]. The independent phosphorylation of Thr308 and Ser473 contrasts with the hierarchical phosphorylation of Thr229 and

Thr389 observed in S6K; phosphorylation by mTORC1 (Thr389) is required for subsequent phosphorylation by PDK1 (Thr229) (reviewed in [60]). Furthermore, rather than being inactive, AKT without Ser473 phosphorylation appears to remain largely active as determined by the phosphorylation state of the AKT substrates glycogen synthase kinase 3 (GSK3), tuberous sclerosis complex protein 2 (TSC2), BAD and the forkhead class O transcription factors 1/3a (FOXO1/3a). Only FOXO1/3a, and possibly BAD, show decreased phosphorylation upon loss of Ser473 phosphorylation [48,49,52]. Thus, mTORC2-mediated AKT phosphorylation does not seem to determine absolute activity, but instead appears to determine substrate specificity. It is also possible that under conditions of low AKT activity, some AKT substrates can be phosphorylated by another kinase. For example, under conditions of insulin resistance when AKT is no longer active, GSK3 is phosphorylated by S6K [61]. Furthermore, FOXO can also be phosphorylated by SGK1 [62], another mTORC2 substrate, providing a possible explanation for why cells with reduced mTORC2 activity show reduced FOXO phosphorylation, but not reduced GSK3 phosphorylation.

After the identification of AKT as an mTORC2 substrate, other AGC kinases were identified as additional substrates. In particular, many research groups focused on PKC phosphorylation. Sarbassov *et al.* showed that PKCα phosphorylation (at the hydrophobic motif) and activity depend on mTORC2 [7]. However, this study suggested that the control of PKCα by mTORC2 is indirect. Ikenoue *et al.* [63] and Facchinetti *et al.* [26] later showed that mTORC2 is required for phosphorylation of all conventional PKCs and the novel PKCε at their hydrophobic motif and, in addition, at their turn motif, thereby controlling activity and stability of PKC (Figure 1f). Loss of mTORC2 activity results in a reduction in total protein levels of PKC. Ikenoue *et al.* and Facchinetti *et al.* also showed that mTORC2 directly phosphorylates the turn motif in AKT. Interestingly, only phosphorylation of the hydrophobic motif (but not the turn motif) of PKC and AKT occurs in a growth factor-dependent manner. Overall, whether mTORC2 is the direct kinase of PKC remains an open question because no study to date has been able to demonstrate direct *in vitro* phosphorylation of either site on any PKC isoform by mTORC2. Furthermore, it remains unclear how strongly mTORC2 activity influences PKC-mediated signaling events.

As discussed above, Ypk2 and Gad8 were identified early on as TORC2 substrates, in budding and fission yeast, respectively. Both AGC kinases have close homology to the mammalian SGK kinase family. However, SGK1 was identified in mammals as an mTORC2 substrate only very recently [64]. mTORC2 phosphorylates SGK1 at its hydrophobic motif site (Figure 1f) and thereby regulates the activity of SGK1 toward its physiological substrate n-myc downstream regulated 1 (NDRG1). Given the very modest reduction in AKT activity upon loss of mTORC2, as discussed above, is AKT a major mTORC2 effector? Studies in yeast and worms suggest that SGK is the main TORC2 effector in these organisms. Whether SGK1 is also the most important physiological substrate of TORC2 in mammals is not clear. *Sgk1 Sgk3* double knockout (DKO) mice display only mild phenotypes, including a defect in renal function that does not affect embryonic development [65]. By contrast, *Akt1 Akt2* DKO mice and *Akt1 Akt3* DKO mice are impaired in development, and the latter display a phenotype similar to that of *Rictor* knockout mice [52,53,66,67]. Furthermore, loss of *Rictor* [68] or *Akt1* [69] suppresses the development of prostate neoplasia in *Pten* (phosphatase and tensin homolog)-deficient mice. These findings are similar to those in *D. melanogaster* where tissue hyperplasia and increased AKT activity induced by *Pten* loss are reduced upon loss of *Rictor*. Curiously, loss of *Rictor* in the *Pten* prostate cancer mouse model reduces AKT phosphorylation at Thr308 and Ser473. Overall, AKT seems to be an important mTORC2 effector, at least upon enhanced signaling through the PI3K pathway.

Although the processes downstream of TORC2 are coming into focus, knowledge about TORC2 upstream regulators is almost completely lacking. In yeast, absolutely nothing is known about extracellular or intracellular signals controlling TORC2. In mammalian cells, mTORC2 phosphorylates AKT upon serum stimulation, in particular growth factors such as insulin and insulin-like growth factor 1 (IGF1), suggesting that mTORC2 is regulated by the PI3K pathway [47–49,63]. This observation alone does not indicate that intrinsic mTORC2 kinase activity is stimulated by the PI3K pathway. PI3K activation leads to the production of PIP3 and recruitment of AKT to the plasma membrane where it is phosphorylated by PDK1 and a possibly constitutively active, membrane-bound mTORC2. Thus, mTORC2 could be constitutively active, with its regulated phosphorylation of AKT being controlled at the level of AKT localization. However, arguing against this possibility and in favor of a model in which the PI3K pathway stimulates intrinsic mTORC2 kinase activity are the observations that mTORC2-dependent mTOR autophosphorylation at Ser2481 [55] and *in vitro* mTORC2 activity are stimulated by growth factors [47,49]. Furthermore, mTORC2 appears to phosphorylate SGK1 in response to growth factors even though SGK1 lacks a pleckstrin homology (PH) domain and is activated independently of membrane recruitment. Taken together, these latter findings suggest that growth factors, via the PI3K pathway, stimulate intrinsic mTORC2 kinase activity.

How might growth factors activate mTORC2 kinase activity? Despite several indications that growth factors stimulate mTORC2 activity, it remains a mystery how the growth factor signal is relayed within the cell to activate mTORC2. By contrast, mTORC1 upstream signaling pathways are better defined [1]. A recent report suggests that growth factors could signal to mTORC2 via the TSC1–TSC2 complex. Huang *et al.* [71] proposed that the TSC1–TSC2 complex, a GTPase-activating protein (GAP) that lies upstream of and negatively regulates mTORC1, also regulates mTORC2 function by directly binding mTORC2. In contrast to the negative regulation of mTORC1 by TSC1–TSC2, TSC1–TSC2 is thought to positively regulate mTORC2 activity in a GAP-independent manner. The GTPase RHEB, which lies directly downstream of TSC1–TSC2 and activates mTORC1 [72], does not appear to lie upstream of mTORC2. The observation that TSC1–TSC2 GAP activity is not required for mTORC2 activation suggests that activation does not occur via

mTORC1 and the negative feedback loop (a hallmark of activated mTORC1 signaling). As part of the negative feedback loop the mTORC1 substrate S6K directly phosphorylates and promotes the degradation of insulin receptor substrate (IRS) [70]. As a consequence, further insulin-mediated AKT signaling is attenuated and cells are in an insulin-resistant state. Indeed, Huang *et al.* argue that TSC-mediated activation of mTORC2 does not occur via the negative feedback loop. However, the way in which TSC1–TSC2 binding regulates mTORC2 activity, as well as potential GAP-independent activities for TSC1–TSC2, remain poorly understood.

Is mTORC2 found at the plasma membrane and is mTORC2 localization regulated? In the *Pten* prostate cancer mouse model, where PI3K signaling is increased, RICTOR and Ser473-phosphorylated AKT are enriched at the plasma membrane. Furthermore, Partovian *et al.* [73] demonstrated that deficiency of syndecan-4 reduces mTORC2 localization to detergent-insoluble membrane fractions (rafts) in endothelial cells. Interestingly, syndecan-4, which is a single-pass transmembrane proteoglycan, recruits PKCα to the plasma membrane and thereby regulates PKCα activity; this, in turn, is required for appropriate mTORC2 localization to the rafts and subsequent AKT activation. However, the mechanism by which PKCα regulates mTORC2 recruitment is unknown, and discovery efforts are complicated by the fact that PKCα is known to be a downstream target of mTORC2.

Although we have some insights into how mTORC2 might be regulated, it will be a major breakthrough to identify upstream regulators of the TORC2 signaling branch, in yeast and mammals.

Concluding remarks and future perspectives

We have summarized and highlighted the major recent findings on TORC2 in various organisms. The sum of all studies clearly shows conserved functions of TORC2 across organisms (Figure 1). In plants and algae, many components of the TOR signaling pathway have been elucidated, and *Arabidopsis thaliana* TOR (AtTOR) is important in the control of plant growth [74–77]. However, no RICTOR or Sin1 homolog has been identified in *A. thaliana* or the green alga *Chlamydomonas reinhardtii*, thus drawing question to the existence of TORC2 in photosynthetic organisms. TORC2 was recently identified in the protozoan parasite *Trypanosoma brucei*, which causes sleeping sickness in humans [78]. Interestingly, and in contrast to other eukaryotes, rapamycin treatment of *T. brucei* inhibits cell growth by exclusively preventing TORC2 assembly without affecting TORC1.

TORC2, together with its sibling complex TORC1, is the main kinase that phosphorylates and thereby regulates the activity of several AGC kinases. TORC2 also regulates actin cytoskeletal organization in most systems studied. However, it remains unclear how TORC2 specifically regulates the actin cytoskeleton in the context of diverse physiological processes that involve motility such as embryogenesis, inflammation, metastasis, or wound healing.

Several studies have shown that a full-body knockout of any mTORC2 component is embryonic lethal. As a next step, determining how mTORC2 in individual organs influences whole-body growth and metabolism will be interesting. Conditional knockout studies of the mTORC2-specific component *Rictor* in skeletal muscle display minimal phenotypes [79,80], suggesting that the role of mTORC2 in muscle is less important. Loss of *Rictor* in adipose tissue, however, results in a more dramatic phenotype: adipose mTORC2 negatively controls whole-body growth and also influences glucose metabolism by influencing IGF1 and insulin levels, respectively [81].

Does mTORC2 have a role in diseases such as cancer or metabolic disorders? Guertin *et al.* [68] made the interesting observation that mTORC2 is important for the development of prostate cancer induced by *Pten* loss, but is not important for non-cancerous prostate epithelial cells. Moreover, conditional knockout studies show a role for mTORC2 in regulating glucose metabolism, so mTORC2 could play a part in the development of type-2 diabetes [80,81]. The involvement of mTORC2 in diseases is only starting to be considered. Future studies might reveal the need for drugs that specifically inhibit mTORC2, such as rapamycin for mTORC1.

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