# Regulation of TOR by small GTPases

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**TOR is a conserved serine/threonine kinase that responds to nutrients, growth factors, the bioenergetic status of the cell and cellular stress to control growth, metabolism and ageing. A diverse group of small GTPases including Rheb, Rag, Rac1, RalA and Ryh1 play a variety of roles in the regulation of TOR. For example, while Rheb binds to and activates TOR directly, Rag and Rac1 regulate its localization and RalA activates it indirectly through the production of phosphatidic acid. Here, we review recent findings on the regulation of TOR by small GTPases.**

See Glossary for abbreviations used in this article.

## Introduction

The growth-controlling TOR signalling pathway is structurally and functionally conserved from unicellular eukaryotes to humans. TOR, an atypical serine/threonine kinase, was originally discovered in *Saccharomyces cerevisiae* as the target of rapamycin (Heitman *et al*, 1991). It was later described in many other organisms including the protozoan *Trypanosoma brucei,* the yeast *Schizosaccharomyces pombe*, photosynthetic organisms such as *Arabidopsis thaliana* and *Chlamydomonas reinhardtii*, and in metazoans such as *Caenorhabditis elegans*, *Drosophila melanogaster* and mammals. TOR integrates various stimuli to control growth, metabolism and ageing (Avruch *et al*, 2009; Kim & Guan, 2011; Soulard *et al*, 2009; Wullschleger *et al*, 2006; Zoncu *et al*, 2011a). In mammals, mTOR is activated by nutrients, growth factors and cellular energy, and is inhibited by stress. Thus, the molecular regulation of TOR is complex and diverse. Among the increasing number of TOR regulators, small GTPases are currently garnering much attention. Small GTPases (20–25 kDa) are either in an inactive GDP-bound form or an active GTP-bound form (Bos *et al*, 2007). GDP–GTP exchange is regulated by GEFs, which mediate the replacement of GDP by GTP, and by GAPs, which stimulate the intrinsic GTPase activity of a cognate GTPase to convert GTP into GDP (Fig 1). Upon activation, small GTPases interact with effector proteins, thereby stimulating downstream signalling pathways. Small GTPases constitute a superfamily that comprises several subfamilies, such as the Rho, Ras, Rab, Ran and

Arf families. Rheb, Rag, RalA, Rac1 and Ryh1, all members of the small GTPase superfamily, play a role in the concerted regulation of TOR by different stimuli. This review summarizes recent advances in the understanding of TOR regulation by these small GTPases.

## The TOR complexes

TOR is found in two functionally and structurally distinct multi-protein complexes, named TORC1 and TORC2 (Avruch *et al*, 2009; Kim & Guan, 2011; Soulard *et al*, 2009; Wullschleger *et al*, 2006; Zoncu *et al*, 2011a). TORC1 regulates several cellular processes including protein synthesis, ribosome biogenesis, nutrient uptake and autophagy. TORC2, in turn, regulates actin cytoskeleton organization, cell survival, lipid synthesis and probably other processes. TORC1 and TORC2 are rapamycin-sensitive and rapamycin-insensitive, respectively, although in some organisms, for example *A. thaliana* and *T. brucei,* this rule does not apply (Barquilla *et al*, 2008; Mahfouz *et al*, 2006). Nevertheless, long-term treatment with rapamycin can also indirectly inhibit TORC2 in mammalian cell lines (Sarbassov *et al*, 2006). Furthermore, there is accumulating evidence that not all TORC1 readouts are rapamycin-sensitive (Choo & Blenis, 2009; Dowling *et al*, 2010; Peterson *et al*, 2011).

 *Saccharomyces cerevisiae* has two TORs encoded by two separate genes, *TOR1* and *TOR2*. TOR1 is part of TORC1 only, whereas TOR2 is found in both TORC1 and TORC2. The core components of TORC1 in budding yeast are TOR (TOR1 or TOR2), KOG1 and LST8. The core components of TORC2 are TOR2, AVO1, AVO2, AVO3 and LST8. The fission yeast *S. pombe* also has two TORs (SpTOR1 and SpTOR2), but their numbering is reversed compared with those in budding yeast—SpTOR1 is the orthologue of TOR2 in *S. cerevisiae,* and SpTOR2 is orthologous to budding yeast TOR1. By contrast, mammals have a single mTOR. The core components of mTORC1 are mTOR, Raptor (KOG1 orthologue) and mLST8. Other proteins, such as the regulators PRAS40 and Deptor, interact directly with TORC1 to regulate its kinase activity (Peterson *et al*, 2009; Sancak *et al*, 2007; Thedieck *et al*, 2007; Vander Haar *et al*, 2007). However, most significant among the regulating interactors are the small GTPases Rheb and Rag (Avruch *et al*, 2009; Kim *et al*, 2008; Sancak *et al*, 2008). Mammalian mTORC2 is composed of mTOR, Rictor, mSIN1 (AVO1 orthologue), mLST8 and PRR5 (also known as Protor). Similarly to mTORC1, Deptor also binds to mTORC2 (Peterson *et al*, 2009).

## Upstream of TOR

Four main inputs regulate mTORC1: nutrients, growth factors, the bioenergetic status of the cell and oxygen availability. It is well established that growth factors activate mTORC1 through the PI3K–AKT

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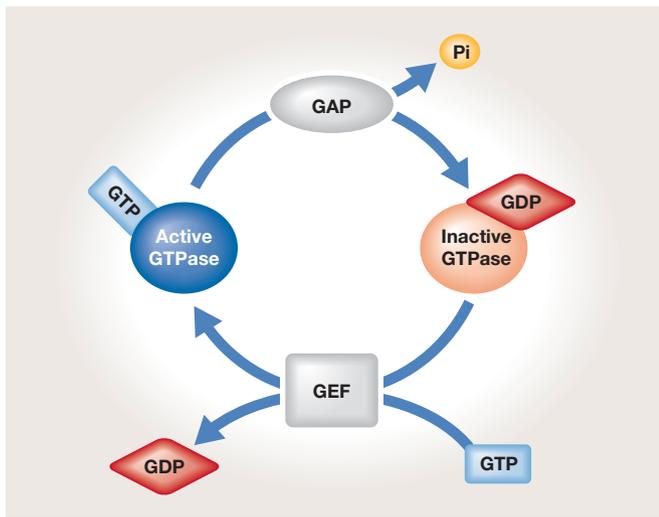E-mail: m.hall@unibas.ch

**Fig 1** | Regulation of small GTPases by GEFs and GAPs. A guanine nucleotide exchange factor (GEF) replaces GDP with GTP to activate the signalling function of the GTPase. Conversely, a GTPase-activating protein (GAP) stimulates hydrolysis of GTP into GDP to inactivate the small GTPase.

**Glossary**

| | |
|---|---|
| AMPK | AMP-activated protein kinase |
| Arf | ADP-ribosylation factor |
| AVO | adheres voraciously to TOR |
| CTD | carboxy-terminal domain |
| Deptor | DEP domain-containing mTOR-interacting protein |
| eIF4E | eukaryotic initiation factor 4E |
| ERK | extracellular signal-regulated kinase |
| GAP | GTPase-activating protein |
| GEF | guanine nucleotide exchange factor |
| HIF1 | hypoxia-inducible factor 1 |
| MAPK | mitogen-activated protein kinase |
| MEK | MAPK/ERK kinase |
| PA | phosphatidic acid |
| PI3K | phosphatidylinositol-3 kinase |
| PKC | protein kinase C |
| PLD | phospholipase D |
| PP2A | protein phosphatase 2A |
| PRAK | p38-regulated/activated kinase |
| PRAS40 | proline-rich AKT substrate 40 |
| Protor | protein observed with Rictor |
| PRR5 | proline-rich protein 5 |
| Raptor | regulatory associate protein of TOR |
| REDD1 | regulated in development and DNA damage response 1 |
| Rheb | Ras homologous enriched in brain |
| Rictor | rapamycin-insensitive companion of TOR |
| RSK | ribosomal s6 kinase |
| SGK1 | serum and gucocorticoid-induced kinase 1 |
| TCTP | translationally controlled tumour protein |
| TOR | target of rapamycin |
| TORC1/2 | TOR complex 1/2 |
| tRNA | transfer RNA |
| TSC | tuberous sclerosis complex |

pathway. Once activated, AKT phosphorylates and inhibits the heterodimeric complex TSC1–TSC2, a GAP for Rheb and thus an inhibitor of mTORC1 (Avruch *et al*, 2009). The TSC1–TSC2 heterodimer is a 'reception centre' for various stimuli that are then transduced to mTORC1, including growth factor signals transduced through the AKT and ERK pathways, hypoxia through HIF1 and REDD1, and energy status through AMPK (Wullschleger *et al*, 2006). In addition to the small GTPases Rheb and Rag (see below), PA also binds to and activates mTORC1 (Fang *et al*, 2001). Pharmacological or genetic inhibition of PA production, through the inhibition of PLD, impairs activation of mTORC1 by nutrients and growth factors (Fang *et al*, 2001). Moreover, elevated PLD activity leads to rapamycin resistance in human breast cancer cells (Chen *et al*, 2003), further supporting a role for PA as an mTORC1 regulator. As discussed below, the small GTPase RalA participates in the mechanism by which PA activates mTORC1 (Maehama *et al*, 2008; Xu *et al*, 2011).

In the case of nutrients, amino acids in particular, several elements mediate the activation of TORC1. As discussed below, the Rag GTPases are necessary to activate TORC1 in response to amino acids (Binda *et al*, 2009; Kim *et al*, 2008; Sancak *et al*, 2008). In mammals, it has also been proposed that amino acids stimulate an increase in intracellular calcium concentration, which in turn activates mTORC1 through the class III PI3K Vps34 (Gulati *et al*, 2008). However, this model has not been confirmed in other organisms (Juhasz *et al*, 2008). Furthermore, the Ste20-like kinase MAP4K3 plays a role in the activation mTORC1 in response to amino acids (Findlay *et al*, 2007; Yan *et al*, 2010). It is not yet clear how the above nutrient signalling components are functionally related—that is, whether they are in a single pathway or in parallel pathways. It has been proposed that amino acid signalling might involve both activation of kinases and inhibition of counteracting phosphatases, namely PP2A (Meijer & Dubbelhuis, 2004; Yan *et al*, 2010). Finally, tRNAs have been invoked as part of an amino acid sensing and signalling mechanism, but short-term amino acid deprivation does not affect aminoacyl-tRNA or free tRNA levels (Dennis *et al*, 2001).

By contrast, the regulation of TORC2 is poorly understood. In mammals, insulin stimulates mTORC2 through the PI3K pathway. mTORC2 interacts with the ribosome (Oh *et al*, 2010), and this direct interaction is required for the efficient activation of mTORC2 by insulin and PI3K (Zinzalla *et al*, 2011). As TORC2 appears to interact with the ribosome in both mammalian and yeast systems, this is probably a conserved mechanism of TORC2 activation. PA has also been proposed to positively regulate mTORC2, as inhibition of PLD blocks mTORC2-mediated phosphorylation of AKT-Ser 473 and PRAS40 (Toschi *et al*, 2009).

## Downstream of TOR

TORC1 regulates growth-related processes such as transcription, ribosome biogenesis, protein synthesis, nutrient transport and autophagy (Wullschleger *et al*, 2006). In mammals, the best-characterized substrates of mTORC1 are S6K and 4E-BP1, through which mTORC1 stimulates protein synthesis. mTORC1 activates S6K, which is a positive regulator of protein synthesis, and inhibits 4E-BP1, which is a negative regulator of protein synthesis. Upon phosphorylation by mTORC1, 4E-BP1 releases eIF4E. Once released from 4E-BP1, eIF4E interacts with the eIF4G subunit of the eIF4F complex, allowing initiation of translation. In mammals, 4E-BP1 participates mainly in the regulation of cell proliferation and metabolism (Dowling *et al*, 2010). In *S. cerevisiae*, the main substrate of TORC1 is the S6K orthologue Sch9 (Urban *et al*, 2007). Sch9 is required for the activation of ribosome biogenesis and

translation initiation stimulated by TORC1. Furthermore, it participates in TORC1-dependent inhibition of G0 phase entry.

In part due to the fact that TORC2 is rapamycin-insensitive, its downstream effects have been analysed to a lesser extent than those of TORC1. TORC2 controls organization of the actin cytoskeleton, and phosphorylation and activation of AGC kinase family members such as AKT, SGK1 and PKC in mammals (Cybulski & Hall, 2009) and the SGK1 orthologues YPK2 and GAD8 in *S. cerevisiae* and *S. pombe*, respectively. Phosphorylation of YPK2 by TORC2 in *S. cerevisiae* mediates sphingolipid biosynthesis, whereas phosphorylation of GAD8 by TORC2 in *S. pombe* controls entry into mitosis and G1 arrest in response to stress. In *C. elegans*, TORC2 phosphorylates SGK1 to control fat storage, body size and development (Soulard *et al*, 2009).

## Regulation of TOR by Rheb

The small GTPase Rheb was first identified in 1994 in a screen for genes induced in neurons in response to synaptic activity (Yamagata *et al*, 1994), and was first described to interact with the Raf1 kinase (Yee & Worley, 1997). A later report showed that loss of Rhb1, the Rheb orthologue in *S. pombe*, causes a starvation-like growth arrest (Mach *et al*, 2000). In 2003, several independent groups working with mammalian cells *in vitro* and *Drosophila in vivo* demonstrated that Rheb is the target of the TSC1–TSC2 GAP and a TORC1 activator (Avruch *et al*, 2009).

*Drosophila* and fission yeast have a single *Rheb* gene, whereas mammals have two, termed *RHEB1* and *RHEB2* (Patel *et al*, 2003). *RHEB1* mRNA is relatively widespread, whereas *RHEB2* is expressed mainly in the brain (Yamagata *et al*, 1994). A recent study performed in mice demonstrated that RHEB1 is dominant over RHEB2 in the regulation of mTORC1 *in vivo* (Zou *et al*, 2011). The mechanism underlying TORC1 activation by Rheb is not completely understood. Rheb binds to TOR directly (Long *et al*, 2005a), although this interaction appears to be weak and has not been detected between endogenous proteins.

Interestingly, the Rheb–mTOR interaction both *in vivo* and *in vitro* does not depend on GTP loading of Rheb. This is unusual for GTPases as GTP loading usually regulates effector binding. However, GTP loading of Rheb is crucial for the activation of mTOR kinase activity (Sancak *et al*, 2007). Conversely, mTOR becomes inactive after association with a nucleotide-deficient Rheb (Long *et al*, 2005a; Fig 2). Similar results were obtained in *S. pombe*, making use of mutations that hyperactivate Rheb by increasing its overall GTP:GDP binding ratio (Urano *et al*, 2005). In contrast to the situation in mammals, interaction of Rheb with SpTOR2 in fission yeast is detected only with a hyperactive Rheb mutant. This suggests that, in *S. pombe*, Rheb binds to SpTOR2 in a GTP-dependent manner.

The TSC1–TSC2 complex plays a crucial role in the regulation of Rheb by growth factors. As the Rheb GAP, TSC1–TSC2 promotes conversion of GTP to GDP in Rheb. Growth factors stimulate several kinases, including AKT, ERK and RSK, that phosphorylate TSC2 and thereby inhibit the TSC1–TSC2 complex (Wullschleger *et al*, 2006). The phosphorylation status of TSC2 correlates with TORC1 activation. In the active unphosphorylated state, TSC1–TSC2 localizes at the membrane where it exerts its GAP activity to inhibit Rheb and ultimately TORC1. Phosphorylation of TSC2 promotes translocation of TSC2 to the cytosol, allowing for Rheb and TORC1 activation (Cai *et al*, 2006). The TSC1/2–Rheb pathway also participates in stress-mediated activation of mTORC1. Indeed, redox stress regulates
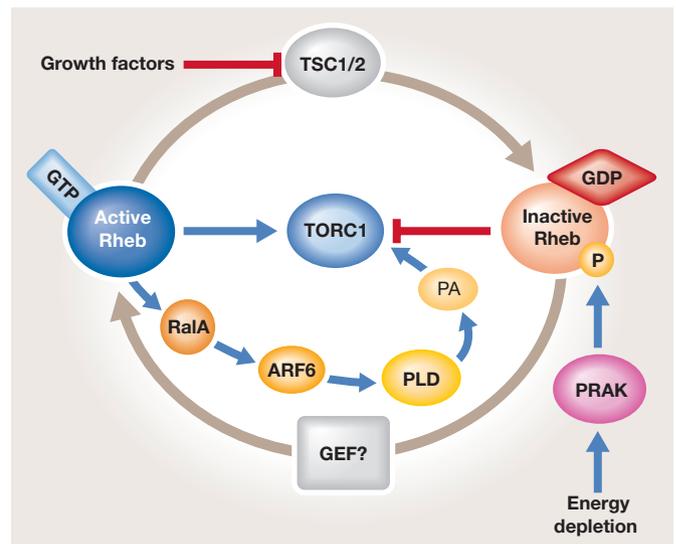


**Fig 2** | Rheb activates TORC1 both directly and indirectly. GTP-bound Rheb interacts directly with TORC1 to activate TORC1 kinase. GTP-bound Rheb also activates RalA, which activates PLD to increase production of PA. PA in turn interacts with TORC1 to stimulate TORC1 kinase activity. Rheb is inactivated by TSC1–TSC2, which acts as a GAP for Rheb. GAP, GTPase-activating protein; PA, phosphatidic acid; PLD, phospholipase D; TORC1, TOR complex 1; TSC, tuberous sclerosis complex.

mTORC1 activity independently of Rag GTPase. Instead, redox stress increases the GTP-bound state of Rheb (Yoshida *et al*, 2011). A role for TSC1–TSC2 upstream of Rheb has also been established in *S. pombe*, where disruption of *TSC2* results in partial activation of TORC1 under nitrogen depletion, similarly to the activation of TORC1 observed on hyperactivation of Rheb (Nakashima *et al*, 2010). By contrast, much less is known about the GEF regulating the conversion of Rheb-GDP to Rheb-GTP. One possible reason for this is that Rheb does not require a GEF. This is supported by the finding that Rheb mutants (S20N, D60V and D60K) with a decreased affinity for nucleotides do not act as dominant negatives (Li *et al*, 2004), although these conclusions are still under debate (Long *et al*, 2005a). Another possible reason for the lack of a viable RhebGEF candidate is genetic redundancy among several possible GEFs.

One study in *Drosophila* suggested that the TCTP protein is the GEF for Rheb (Hsu *et al*, 2007; Fig 2). However, subsequent studies in mammalian cells showed that reducing TCTP levels did not affect mTORC1 signalling in amino-acid-replete/insulin-stimulated cells. Moreover, overexpression of TCTP does not rescue mTORC1 signalling in amino-acid-starved cells (Rehmann *et al*, 2008; Wang *et al*, 2008). These findings suggest that TCTP does not regulate mTORC1 signalling, at least in mammals.

In addition to the direct interaction between mTOR and Rheb, activation of PA production by Rheb is an additional mechanism by which Rheb might regulate mTORC1. Rheb binds to and activates PLD in a GTP-dependent manner (Sun *et al*, 2008). PLD produces PA, which binds directly to and upregulates mTORC1. This finding reveals cross-talk between the TSC–Rheb and the PA pathways in the regulation of mTORC1 signalling. A recent study by Yoon and colleagues further demonstrated the role of PLD in mTORC1 regulation (Yoon *et al*, 2011). They showed that amino acids activate PLD

through translocation of PLD to the lysosomal compartment. This translocation is positively regulated by human Vps34 and is necessary for the activation of mTORC1 by amino acids. These authors propose the existence of a Vps34–PLD1 pathway that activates mTORC1 in parallel to the Rag pathway (Yoon *et al*, 2011).

Rheb is also regulated through direct phosphorylation by PRAK (Zheng *et al*, 2011). Using a mammalian cell culture approach, this report demonstrated that energy stress activates the p38β–PRAK cascade and that PRAK is essential for energy-depletion-induced inhibition of mTORC1. Furthermore, the action of PRAK on mTORC1 is independent of TSC2 and AMPK, but instead involves a direct interaction and subsequent phosphorylation of Rheb-Ser130 by PRAK. This phosphorylation impairs the guanyl-nucleotide-binding ability of Rheb and therefore inhibits Rheb-mediated mTORC1 activation (Fig 2). These results are in contrast to previously reported findings demonstrating that p38 is an important positive regulator of TORC1. Indeed, phosphorylation of TSC2 at Ser1210 by MK2, a kinase downstream from p38α, inhibits TSC2 and thereby activates Rheb and mTORC1 (Li *et al*, 2003), further inducing mTORC1 activation. Moreover, phosphorylation of Raptor by p38β participates in arsenite-induced mTORC1 activation in cultured mammalian cells (Wu *et al*, 2011). Thus, the cross-talk between MAPK and mTOR pathways is complex and operates at different levels, in both a Rheb-dependent and a Rheb-independent manner.

Although Rheb is required for the activation of mTORC1 by amino acids, Rheb itself does not participate in amino acid sensing, and GTP-loading of Rheb is not affected by amino acid depletion (Long *et al*, 2005b). Furthermore, amino acid depletion inhibits mTORC1 even in *TSC2*$^{-/-}$ fibroblasts (Roccio *et al*, 2006). Nevertheless, interaction of mTORC1 with Rheb depends on amino acid availability (Long *et al*, 2005b). As discussed below, the current model proposes that amino acids mediate translocation of mTORC1 to the lysosomal surface where mTORC1 interacts with and is activated by GTP-loaded Rheb (Sancak *et al*, 2008). The role of Rheb in development in mammals has also been investigated. Rheb is essential for murine development beyond embryonic day 12 (Goorden *et al*, 2011), and conditional knockout in the brain reduces brain size and impairs postnatal myelination (Zou *et al*, 2011). Rheb signalling also plays an important role in regulating mTORC1-dependent T-cell differentiation (Delgoffe *et al*, 2011).

## Regulation of TOR by Rag

Rag GTPases have unique features among the Ras GTPase subfamily members: they form heterodimers and lack a membrane-targeting sequence (Nakashima *et al*, 1999; Sekiguchi *et al*, 2001). Gtr1 in *S. cerevisiae* was the first member of this GTPase subfamily to be identified (Bun-Ya *et al*, 1992). The mammalian RagA and RagB GTPases were later described as Gtr1 orthologues (Hirose *et al*, 1998). Gtr2 in yeast (Nakashima *et al*, 1999) and its mammalian orthologues RagC and RagD (Sekiguchi *et al*, 2001) were subsequently discovered due to their ability to form heterodimers with Gtr1 in yeast and RagA and RagB in mammals, respectively. The crystal structure of the Gtr1–Gtr2 complex has been determined recently (Gong *et al*, 2011). Gtr1 and Gtr2 have similar structures, organized in two domains: an amino-terminal GTPase domain (designated as the G domain) and a carboxy-terminal domain. The Gtr1–Gtr2 heterodimer presents a pseudo-twofold symmetry resembling a horseshoe. The crystal structure reveals that Gtr1–Gtr2 dimerization results from extensive contacts between

the C-terminal domains of both proteins, while the G domains do not contact each other (Gong *et al*, 2011).

Gtr1 was initially implicated in phosphate uptake and the Ran/Gsp1–GTPase pathway (Bun-Ya *et al*, 1992; Hirose *et al*, 1998). The Rag family GTPases were only recently shown to have a role in TOR regulation. In yeast, Gtr1 and Gtr2 participate in the EGO complex, a vacuolar-membrane-associated protein complex that, in conjunction with TOR, positively regulates microautophagy (Dubouloz *et al*, 2005; Gao & Kaiser, 2006). Further studies demonstrated that the EGO complex functions upstream from TORC1, and that TORC1 activity is determined by the nucleotide-bound state of Gtr1 (Binda *et al*, 2009). In *Drosophila* and mammals, two independent reports published in 2008 demonstrated that the Rag proteins are necessary for the activation of TORC1 by amino acids (Kim *et al*, 2008; Sancak *et al*, 2008). In mammals, RagA or RagB interact with RagC or RagD to constitute a heterodimer. According to the current model of mTORC1 activation by amino acids through the Rag proteins (Fig 3), a Rag complex is constitutively anchored on the surface of the lysosome. The Rag heterodimer is anchored to the lysosome by the MP1–p14–p18 complex (also known as the 'Ragulator'; Sancak *et al*, 2010). However, the Ragulator does not appear to act exclusively with Rags as it was previously shown to serve as an anchoring element also for MEK1 and ERK (Nada *et al*, 2009). In addition to the MP1–p14–p18 complex, the signalling adaptor p62 also interacts with Rags, thereby favouring the formation of the Rag complex and ultimately the activation of mTORC1 by Rags and amino acids (Duran *et al*, 2011). In the absence of amino acids, RagA or RagB, the dominant member of the Rag heterodimer (Binda *et al*, 2009), is GDP loaded and unable to recruit mTORC1 to the surface of the lysosome. As a result, the mTORC1 complex is dispersed throughout the cytosol in an inactive conformation (Kim *et al*, 2008; Sancak *et al*, 2008). The presence of amino acids inside the cell induces the exchange of GDP by GTP in RagA/B. The exchange mechanism is not well understood, but it has been proposed to be mediated by the Vam6 GEF in yeast (Binda *et al*, 2009). GTP-bound RagA/B binds to and recruits mTORC1 to the surface of the lysosome in a process that also requires the vacuolar H$^+$-ATPase (Zoncu *et al*, 2011b). Once at the lysosome, mTORC1 is able to interact with active Rheb (activated by a separate growth factor input; Sancak *et al*, 2010). Structural data support the idea that Rag GTPase interacts with p18 in the MP1–p14–p18 complex. In a manner dependent on GTP-loading of RagA/B, the Rag heterodimer interacts with Raptor mainly through the G domain of RagA, although RagC/D is also required. Indeed, although the G domain of RagC/D plays a minor role in the interaction with Raptor, the nucleotide binding status of RagC/D still regulates the binding affinity of the complex (Gong *et al*, 2011). Unexpectedly, in this case it appears that RagC/D is bound to GDP and, consistent with this, expression of a GTP-bound mutant of RagB combined with a GDP-bound mutant of RagD activates mTORC1 (Sancak *et al*, 2008). Similar results were obtained in yeast (Binda *et al*, 2009).

The above model explains why a GDP-bound RagB mutant exerts a dominant negative effect on mTORC1. Such a mutant prevents mTORC1 from interacting with the lysosome. Furthermore, it explains how the interaction of Rheb with mTORC1, but not the GTP loading of Rheb, is stimulated by amino acids to activate mTORC1 (Long *et al*, 2005b). Finally, it also explains how overexpression of Rheb promotes mTORC1 activation even in the absence of amino acids. Overexpressed Rheb is mislocalized throughout the cell, and
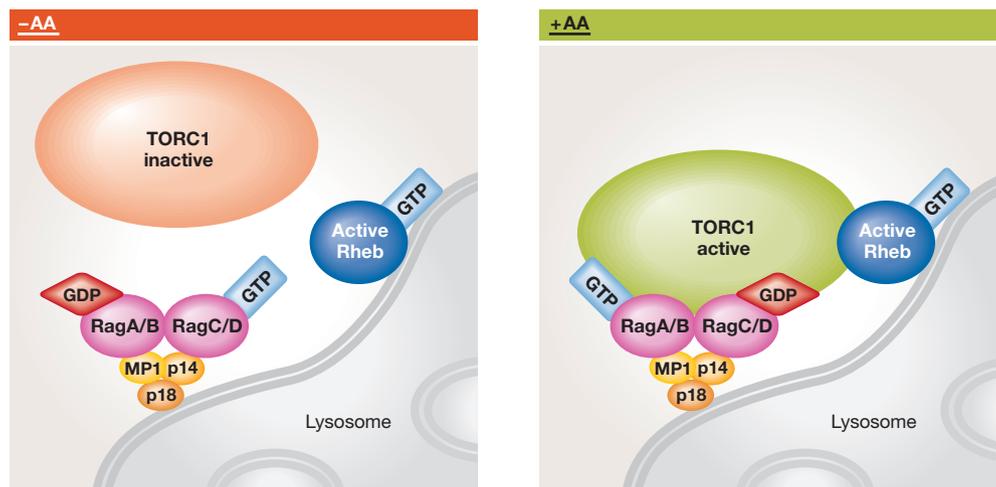
**Fig 3 |** Rag proteins mediate the activation of TORC1 in response to amino acids. The RagA/B–RagC/D heterodimer is anchored to the MP1–p14–p18 complex on the surface of the lysosome. In the absence of amino acids (left panel), RagA/B is GDP-bound whereas RagC/D is GTP-bound, and the Rag heterodimer is inactive and unable to recruit TORC1 to the lysosomal surface. In the presence of amino acids (right panel), RagA/B exchanges GDP for GTP and RagC/D converts its GTP to GDP. This Rag heterodimer acquires an active conformation that binds to and thereby recruits TORC1. Once recruited to the surface of the lysosome, TORC1 interacts with a GTP-loaded Rheb. TORC1, TOR complex 1.

therefore interaction of mTORC1 with Rheb does not require amino-acid-induced translocation of mTORC1 to the lysosome. The model is further supported by observations in *Drosophila* showing that expression of a constitutively active mutant of RagA significantly increases the size of individual cells, whereas expression of a dominant negative mutant of RagA reduces cell size (Kim *et al*, 2008). Moreover, Rag plays a role in TORC1-mediated inhibition of autophagy both in *Drosophila* (Kim *et al*, 2008) and in human cells (Narita *et al*, 2011).

Little is known about the mechanism of activation of Rag GTPases in response to an amino acid signal. In yeast, Vam6 is a GEF for Gtr1 (Binda *et al*, 2009). Vam6 was identified in a genome-wide synthetic lethal screen using a mutant of Gtr1 that preferentially binds to GDP. Loss of Vam6 and Gtr1 similarly affect TORC1. Furthermore, over-expression of Vam6 renders wild-type but not Gtr1-deficient cells resistant to rapamycin. Finally, Vam6 stimulates GDP release from Gtr1, and loss of Vam6 severely reduces the interaction between Gtr1 and Ego1 (Binda *et al*, 2009). These results suggest that Vam6 is a GEF for Gtr1 and explain the finding that class C *vps* mutants (such as *vam6*) fail to recover from rapamycin-induced growth arrest or to survive nitrogen starvation (Zurita-Martinez *et al*, 2007). Vam6 appears to be evolutionarily conserved (26% identity with human Vam6), suggesting that the regulation of Rag by the mammalian Vam6 orthologue could also be conserved.

## Regulation of TOR by RalA

PA acts upstream of both mTORC1 and mTORC2, as suggested by the fact that inhibition of PLD impairs activation of both complexes (Fang *et al*, 2001; Toschi *et al*, 2009). PA is produced by PLD-mediated hydrolysis of phosphatidylcholine. The small GTPase RalA plays a role in this process (Fig 2; Xu *et al*, 2011) through interaction with PLD and its subsequent activation (Jiang *et al*, 1995). However, RalA does not activate PLD directly, but instead promotes the association of PLD with ARF6, a member of the ARF family of GTPases, which in turn activates PLD (Xu *et al*, 2003). As PLD is a positive regulator of both mTORC1 and mTORC2, a potential connection

between RalA and the mTOR pathway has been proposed and indeed proven for mTORC1 (Maehama *et al*, 2008). The amount of GTP-bound RalA in cultured human cells increases when amino acids are added to the cells, suggesting that RalA responds to amino acid availability. Furthermore, RalA is indispensable for the Rheb-dependent activation of mTORC1 induced by extracellular nutrients. Inhibition of RalA abolishes amino-acid- and glucose-induced mTORC1 activation even after overexpression of a hyperactive Rheb mutant (Maehama *et al*, 2008). Therefore, RalA—similarly to PLD—seems to act downstream of Rheb to activate mTORC1 (Sun *et al*, 2008). In support of this model, activation of both PLD and mTORC1 in human cancer cells by nutrients is dependent on RalA and ARF6 (Xu *et al*, 2011). In conclusion, a RalA–ARF6–PLD complex appears to promote activation of mTORC1 in response to nutrients.

## Regulation of TOR by Rac1

The Rho subfamily of GTPases is present in all eukaryotic cells, from yeast to mammals, regulating both actin organization and morphogenesis. In yeast, Rho proteins mediate polarized growth, cell integrity, cytokinesis and mating (Park & Bi, 2007). In mammals, the most prominent members of the Rho family are RhoA, Rac1 and CDC42, which regulate actin cytoskeleton reorganization to promote cell growth, exert anti-apoptotic functions and regulate gene expression through the activation of different signalling pathways (Wennerberg & Der, 2004). Activation of Rac1 promotes actin polymerization and the formation of lamellipodia (Hall, 1998). P-Rex1 is the GEF for Rac1 and, interestingly, P-Rex1 interacts directly with and is subsequently activated by mTORC2 (Hernandez-Negrete *et al*, 2007). Indeed, activated Rac1 suppresses actin cytoskeleton defects owing to a loss of mTORC2 function, suggesting that mTORC2 might signal to the actin cytoskeleton through Rac1 (Jacinto *et al*, 2004). Accordingly, mTORC2 deficiency causes a 20–30% decrease in GTP-bound Rac1, further suggesting that mTORC2 regulates Rac1 activity and thus signals through Rac1 (Jacinto *et al*, 2004). Besides its function as an mTORC2 target, Rac1 has been shown recently
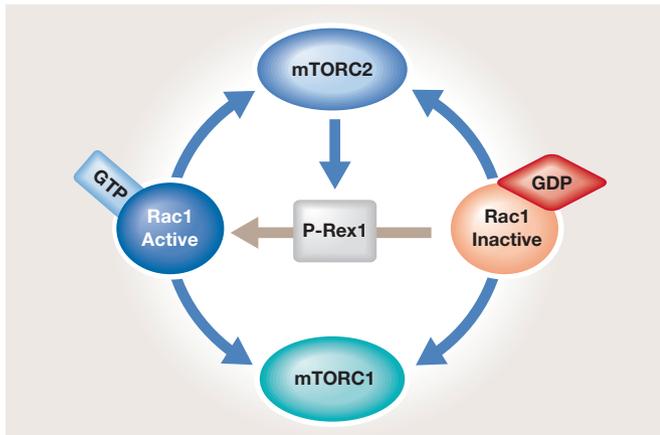
Fig 4 | In mammalian cells, Rac1 functions both upstream and downstream of mTOR. Once activated by TORC2, P-Rex1 (the GEF for Rac1) promotes the exchange of GDP for GTP in Rac1. Rac1 in turn interacts with and activates both mTORC1 and mTORC2 independently of its GTP loading. GEF, guanine nucleotide exchange factor; TOR, target of rapamycin; TORC1/2, target of rapamycin complex 1/2.

Sidebar A | In need of answers

(i)    How are amino acids sensed by the cell?
(ii)   What is the mechanism by which amino acids regulate the GTP-loading of Rag proteins? What are the GEF and GAP for the Rag proteins?
(iii)  Is there a GEF that regulates the GTP-loading of Rheb?
(iv)   What is the molecular mechanism by which Rheb activates TORC1?
(v)    How is the dual effect of Rac1 being both upstream and downstream from TOR regulated?
(vi)   How are the diverse GTPases that impinge on TOR integrated?

to act upstream of mTOR. Inhibition of Rac1 in mammalian cells blocks the activation of both mTORC1 and mTORC2 by growth factors (Saci et al, 2011). Similarly to P-Rex1, Rac1 also interacts directly with mTOR. This interaction has been proposed to be involved in mTORC1 and mTORC2 localization, as deletion of Rac1 affects the subcellular distribution of mTOR. Surprisingly, the regulation of mTORC1 and mTORC2 by Rac1 is independent of the GTP loading of Rac1 (Saci et al, 2011). Thus, Rac1 seems to have an intricate relationship with mTOR: it interacts directly with mTORC1 and mTORC2 and is both upstream and downstream of them (Fig 4).

### Regulation of TOR by Rab family members

A recent study in *S. pombe* revealed that the Rab GTPase subfamily is also involved in the regulation of the TOR pathway (Tatebe et al, 2010). TORC2 in fission yeast phosphorylates and activates Gad8, a member of the AGC kinase family orthologue of mammalian SGK1 (see above). Ablation of Gad8 sensitizes cells to osmotic stress and high temperature, causes a mating deficiency and prevents G1 arrest under nitrogen starvation, a global phenotype similar to that observed after SpTOR1 deletion. Furthermore, overexpression of Gad8 suppresses the stress sensitivity caused by SpTOR1 deletion (Matsuo et al, 2003). A genetic screen in fission yeast showed that deletion of Ryh1, a GTPase homologous to human RAB6, prevents phosphorylation of Gad8 by TORC2. Similar results were obtained after ablation of SAT1 and SAT4, the putative GEFs for Ryh1 (Tatebe et al, 2010). GTP-bound Ryh1 interacts with SpTOR1 and enhances the interaction of TORC2 with its substrate Gad8, stimulating the phosphorylation of Gad8 by mTORC2. Although Ryh1 is not implicated in the subcellular distribution of mTORC2, the SAT1/4–Ryh1–TORC2–GAD8 pathway seems to be implicated in vacuolar integrity in *S. pombe*.

As mentioned above, Ryh1 is homologous to human RAB6, which regulates transport pathways in and out of the Golgi. Several RAB6 effectors have been identified, including subunits of the dynein–dynactin complex, Rab kinesin-6/MKLP2, GAPCenA, TMF/ARA160, mint3, Rab6IP1 and Rab6IP2/ELKS, all involved in cellular compartmentalization (Fernandes et al, 2009). At present, no

experimental evidence links RAB6 with the mTOR pathway in mammals, but interestingly, human RAB6 can stimulate TORC2 when expressed in *S. pombe*, suggesting that the role of RAB6 as a positive regulator of TOR might be conserved (Tatebe et al, 2010). As Rab proteins play a role in the establishment of compartmental specificity within the eukaryotic endomembrane system (Zerial & McBride, 2001), it has been proposed that Ryh1 GTPase, as well as its GEF and GAP, confer spatial regulation on TORC2 activity (Tatebe & Shiozaki, 2010).

In addition to TORC2, Rab family members also regulate TORC1. Inhibition of Rab1, Rab5 or Rab11 reduces the phosphorylation of S6K in *Drosophila* S2 cells, suggesting an important role of Rab proteins in TORC1 activation. In addition, constitutively active mutants of Rab5, Rab7, Rab10 or Rab31 potently inhibit S6K phosphorylation in human cells (Li et al, 2010). Rab5 appears to play a particularly important role in mTORC1 activation, as a constitutively active mutant of Rab5 inhibits Rag- and amino-acid-dependent phosphorylation of S6K without interfering with activation of mTORC1 by Rheb (Li et al, 2010). However, Rab5 is not sufficient to activate mTORC1 and it does not interact with mTORC1. These results suggest that disruption of the GTP–GDP cycling of Rab proteins can disrupt normal cellular transport and inhibit TORC1 activation. Furthermore, these results suggest concerted regulation of intracellular transport and mTOR activation.

### Concluding remarks

mTOR and small GTPases are therapeutic targets in the treatment of cancer (Berndt et al, 2011; Dazert & Hall, 2011). Aberrant activation of GTPases, including Ras, Rho, Rab or Ran GTPases, promotes cell transformation and cancer (Agola et al, 2011; Ly et al, 2010; Pylayeva-Gupta et al, 2011), in some cases by acting in the mTOR pathway. Targeting GTPases by using farnesyltransferase inhibitors or geranylgeranyltransferase inhibitors affects signal transduction pathways, cell cycle progression, proliferation and cell survival. Both types of inhibitor are currently under investigation for cancer therapy, although only a small subset of patients responds to these inhibitors (Berndt et al, 2011). A better understanding of the relationship between GTPases and mTOR is essential for the design of combined therapies.

From a mechanistic point of view, research on TOR in different systems is continually adding new insight on the role of TOR in cell biology. However, what is lacking is an integration of the various proposed regulators of TOR, in particular small GTPases (see Sidebar A). Future work should clarify how the various proposed mechanisms that exert an effect on TOR are coordinately regulated and integrated. In the case of small GTPases, further understanding of how the GEF/GAP systems are regulated for each GTPase incoming signal and how TOR discerns the various inputs it receives from different GTPases is essential.

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