Review

Nutrient control of TORC1, a cell-cycle regulator

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It is well established that the target of rapamycin (TOR) protein kinase has pivotal roles in controlling cell functions (including protein synthesis, cell growth and cell proliferation) and is implicated in numerous human diseases. Mammalian TOR complex 1 (mTORC1) signalling is activated by hormones and growth factors, and is also stimulated by intracellular amino acids. Recent research has provided important new insight into the poorly understood mechanism by which amino acids activate mTORC1 signalling, showing that the protein kinase MAP4K3 and Rag GTPases have important roles in this. mTORC1 is known to control the G1/S transition of the cell cycle: new data show that (m)TORC1 also controls G2/M progression in yeast and mammals, albeit in contrasting ways.

mTOR and its relatives

The mammalian target of rapamycin (mTOR) and its orthologues are multidomain proteins of approximately 300 kDa (Figure 1). Near their C termini lies a protein kinase domain related to phosphoinositide 3-kinases. The N-terminal and central domains of mTOR contain numerous HEAT repeats (originally identified in Huntingtin, elongation factor 3 [EF3], protein phosphatase 2A [PP2A] and TOR). These features usually mediate protein:-protein interactions. Indeed, mTOR and its orthologs bind partner proteins to form two types of complex, mammalian TOR complex (mTORC)1 and mTORC2 (Figure 1). mTORC1 signalling requires a supply of amino acids, although it was not known how they activate mTORC1. Recent work in this fast-moving field has provided important new insight into how amino acids control (m)TORC1 and the role of (m)TOR complexes in controlling the cell cycle. These discoveries form the main theme of this article.

Both mTORC1 and mTORC2 contain the protein GβL (also termed mLST8), the function of which in TOR signalling remains to be clarified. mTORC1 also contains raptor, a large protein that interacts with substrates for mTORC1. The small G-protein Rheb also binds mTORC1 (Figure 1). Rheb-GTP stimulates the kinase activity of mTORC1 [1], although the underlying mechanism remains elusive [2]. Instead of raptor, mTORC2 contains rictor and other protein components and performs distinct functions [3]. In the short term, rapamycin only affects functions of mTORC1 and this article focuses primarily on this complex because, recently, important new discoveries have been made about its functions and its regulation. Although mTORC2 contains mTOR, it does not bind the immunophilin FKBP12, with which rapamycin interacts, and its functions are not therefore affected by this drug. So far, there is little information on the control of mTORC2.

Rapamycin is a macrolide antibiotic, made by *Streptomyces hygroscopicus* and first found on Easter Island (local name 'Rapa Nui'). Rapamycin binds to (m)TOR (as part of mTORC1) adjacent to its kinase domain as a complex with the immunophilin FKBP12. This region is therefore called the FBKP12–rapamycin-binding domain (FRB; Figure 1). Rapamycin analogues are now in clinical use to treat certain cancers and to prevent graft rejection (Box 1).

mTORC1 signalling and protein synthesis

Signalling through mTORC1 is activated by anabolic hormones, growth factors and mitogens. Agents that stimulate mTORC1 activity within cells (e.g. insulin) induce the inactivation of the GTPase-activator protein (GAP) that hydrolyses Rheb-GTP to GDP (Box 2). This GAP function is provided by the proteins encoded by the TSC1–TSC2 genes (Figure 2). Inactivation of TSC1–TSC2 in response to insulin (and other factors) enables Rheb-GTP to accumulate, thereby stimulating mTORC1 [4]. Interestingly, Rheb undergoes farnesylation (addition of a lipid moiety) at its C terminus, which is required for its ability to activate mTORC1 signalling [5,6].

The links between mTORC1 and protein synthesis are better understood than links to other processes, such as cell proliferation and autophagy. Indeed, mTORC1 directly phosphorylates two types of protein that modulate components of the translational machinery. The S6 kinases (S6Ks) [7] phosphorylate ribosomal protein S6. The function of this modification is not known: it is not required for cell-cycle progression or overall protein synthesis because mouse cells in which the S6 phosphorylation sites have been mutated to alanines actually show faster rates of division and protein synthesis than control cells [8]. S6Ks also phosphorylate several other proteins involved in protein synthesis, including a kinase that regulates the translation elongation process [9] (see later). Because protein synthesis is a major driver of cell growth, it is of considerable interest that knocking out S6K in *Drosophila* decreases organism and cell size [10]. Knocking out the S6K1 gene in mice also results in restricted growth [11]; however, the mechanisms underlying these effects remain to be established.

A second link between mTORC1 and protein synthesis is that mTORC1 phosphorylates the eukaryotic initiation factor (eIF) 4E-binding protein (4E-BPs), which controls

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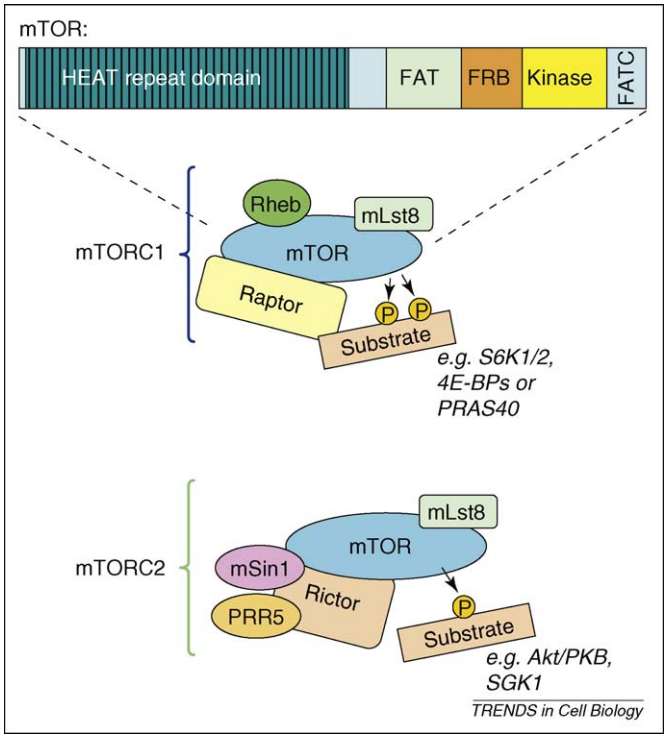

Figure 1. Composition of mTOR complexes. The domain structure of mTOR is shown schematically. Rapamycin binds to the FRB domain as a complex with the immunophilin FBKP12 and thereby inhibits functions of mTORC1. Directly below is shown the composition of mTORC1. In addition to mTOR, mTORC1 contains the proteins mLst8 and Raptor. Raptor binds substrates for mTORC1, recruiting them for phosphorylation by mTOR. The small GTPase Rheb can also interact with mTORC1, although this seems to be independent of its guanine-nucleotide-binding status. The bottom section shows the composition of mTORC2 for comparison. Substrates for mTORC2 are shown: it is not yet clear if rictor has an analogous role to raptor in mTORC1 (i.e. by interacting with substrates for mTORC2). Known interactions are depicted by overlapping shapes, otherwise the illustration is schematic. Abbreviations: ATM, TRRAP domain; FAT, FKBP12-rapamycin-associated protein (FRAP); FATC, FAT C-terminal domain; FRB, FKBP12/rapamycin binding domain; PRR5, proline-rich repeat protein 5, also known as Protor.

Box 1. Clinical applications of rapamycin (and 'rapalogs')

The earliest major medical application for rapamycin was as an immunosuppressant (e.g. in preventing kidney graft rejection); rapamycin blocks the activation of T cells by interfering with the ability of interleukin-2 to drive progression of cells through the G1/S phase of the cell cycle (for a review, see Ref. [66]). It continues to be widely used to prevent graft rejection, especially for kidney transplantation. Restenosis after angioplasty is a complication of a certain type of cardiac intervention to deal with narrowing or blockage of a blood vessel, especially the coronary artery. A balloon is first inserted to open up the coronary artery. A metal stent can be inserted to try to keep the vessel open. However, the vascular smooth muscle can regrow, causing the blood vessel to become constricted once more. The smooth muscle cell proliferation is inhibited by rapamycin, which is administered by using stents that gradually release the drug. Tuberous sclerosis involves constitutive activation of mTORC1 signalling in certain cells owing to loss of functional TSC1 or TSC2. This leads to formation of usually benign tumours that contain very large cells. mTOR inhibitors including rapamycin (Sirolimus) and its derivatives, such as Everolimus, are being assessed as potential therapeutic agents for TSC and the related lung disease sporadic lymphangioleiomyomatosis (LAM) that are caused by mutations of the TSC1 and TSC2 genes. Early clinical trials and case series have shown that TSC-associated kidney and brain tumours shrink in response to these agents and have also indicated reversible improvement in lung function in patients with LAM. Pre-clinical trials in mouse models of TSC suggest that mTOR inhibitors can also have beneficial effects on the seizures and cognitive defects associated with TSC. It is well established that rapamycin inhibits the proliferation of many cancer cell lines *in vitro*, and extensive clinical trials have been conducted to study its efficacy against tumours. As a result of this, the rapalog CCI-779 (also called temsirolimus or Torisel) received FDA approval in 2007 for use in patients with advanced renal cell carcinoma.

the activity of eIF4E. eIF4E is the translation factor that binds to the 5′-cap structure of eukaryotic mRNAs, thereby facilitating ribosome recruitment. Hypophosphorylated 4E-BPs bind to eIF4E, thereby preventing it from interacting with the scaffold protein eIF4G and blocking formation of productive initiation factor complexes. mTORC1-mediated phosphorylation of 4E-BP1 releases it from eIF4E, promoting initiation complex formation. The 4E-BPs and other known mTORC1 substrates interact with raptor via short 'TOR- signalling' (TOS) motifs. This facilitates their phosphorylation by mTORC1.

It is important to note that not all outputs from mTORC1 are inhibited by rapamycin, so that insensitivity to rapamycin does not rule out a role for mTORC1. For example, mTORC1 readily phosphorylates 4E-BP1 *in vitro* but the phosphorylation of Thr37 and Thr46 (in human 4E-BP1) is resistant to rapamycin [12]. More importantly, their phosphorylation is hardly affected by rapamycin in several cell types [13–15], although it is impaired by siRNA-mediated knock-down of raptor [16], starving cells of leucine (which inhibits mTORC1 signalling) or an ATP-competitive mTOR kinase inhibitor [15]. Similarly, only some of the mTORC1 sites in PRAS40 are affected by rapamycin [17].

mTORC1 signalling also regulates translation elongation (the process whereby the ribosome traverses the coding region of the mRNA and assembles the new polypeptide chain). The movement of the ribosome along the mRNA requires eukaryotic elongation factor eEF2. Its activity is inhibited by phosphorylation at Thr56 and this is catalysed by a very specific kinase, eEF2 kinase [18]. Treating cells with agents that activate protein synthesis rapidly inactivates eEF2 kinase and thus induces dephosphorylation of eEF2, speeding up elongation [19]. These effects are blocked by rapamycin, showing that they require signalling through mTORC1. A simple explanation would be that mTORC1 directly phosphorylates eEF2 kinase, thus inactivating it. However, eEF2 kinase neither contains a TOS motif nor binds to raptor, and is not phosphorylated by mTORC1 [20]. Other components must therefore link mTORC1 to the control of eEF2 kinase. mTORC1 regulates the phosphorylation of three sites in eEF2 kinase, each of which inactivates eEF2 kinase [18]. One site (Ser366), as noted earlier, is phosphorylated by S6Ks [9]; another, Ser359, profoundly inhibits the activity of eEF2 kinase [21]. Work to identify the Ser359 kinase recently led to the identification of a new link between mTORC1 and the cell cycle (see section on control of mitosis on TORCs). These and other recent data show that mitotic entry is regulated by amino acids.

Regulation of mTORC1 by amino acids

Starving cells for amino acids quickly results in dephosphorylation of substrates for mTORC1 (for a review, see

Box 2. Rheb and the Rags are unusual G-proteins

The Rag GTPases belong to a large class of (usually monomeric) proteins that bind guanine nucleotides (i.e. GDP or GTP). The conformation and biological activity of G-proteins usually change markedly depending on which nucleotide they are bound to (Figure I). In almost every case, it is the GTP-bound form that is biologically active and that binds to the immediate downstream targets ('effectors') of the G-protein. By contrast, the GDP-bound form cannot bind the target proteins and is therefore inactive. The irreversible hydrolysis of GTP to GDP acts as a switch, normally turning off the G-protein. This step is frequently catalysed by a separate GTPase-activator protein (GAP). The TSC1/2 complex provides this function for Rheb (see main text for details). Regeneration of the active GTP-bound form of the G-protein is frequently a slow process that requires a guanine-nucleotide-exchange factor (GEF). The control of the Rag GTPases might involve unknown GAPs or GEFs, which might be regulated by amino acids. Particularly unusual features of the Rag GTPases are, first, that they occur as heterodimers (although the GTPases associated with the signal recognition particle also heterodimerize [67]). Second, the activation of mTORC1 apparently requires one Rag partner to be bound to GTP and the other to GDP. Rheb is also unusual in that it apparently binds its presumed effector, mTOR, irrespective of its guanine-nucleotide-binding status [1]. The figure shows the general properties of G-proteins, not specifically those of Rheb or the Rag proteins.

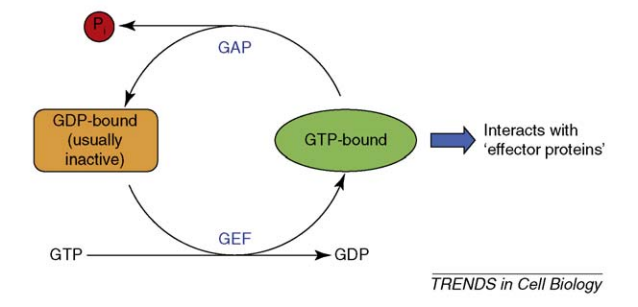

Figure I. G-proteins are usually active in their GTP-bound state, which can interact with target proteins. Interconversion between the GDP- and GTP-bound states is usually mediated by GAPs and GEFs, as described in more detail in the box text.

Ref. [22]), whereas replenishment rapidly reverses this effect. The branched-chain amino acid leucine exerts the greatest effect on mTORC1 signalling, although starving some cell types for other single amino acids can also inhibit it [23]. Several lines of evidence show it is intracellular, not extracellular, amino acids that regulate TORC1 signalling in vertebrate cells [24,25]. For example, intracellular glutamine stimulates mTORC1 signalling by promoting the uptake of leucine into cells. Bidirectional transport of amino acids regulates mTOR and autophagy [26].

This helps explain why compounds that inhibit protein synthesis (such as cycloheximide) stimulate mTORC1 signalling [26]; by inhibiting their consumption by protein synthesis, such compounds increase intracellular amino acid levels (e.g. of the branched-chain amino acids including leucine [24]) and activate mTORC1 signalling (e.g. see Refs [24,27]). It should also be noted here that the protein regulated in development and DNA damage responses 1 (REDD1), a negative regulator of mTORC1, turns over very rapidly: the decreased levels of REDD1 caused by inhibiting protein synthesis also seem to contribute to activation of mTORC1 signalling observed under such conditions [28]. The requirement for proteasomal function to maintain protein synthesis in nutrient-starved cells [29] might partly reflect the activation of mTORC1 by amino acids derived from protein breakdown under these conditions.

A key question here is how amino acids activate mTORC1. Amino acid starvation still inactivates mTORC1 signalling in cells lacking TSC2, demonstrating that TSC2 is dispensable for this effect [30,31]. Consistent with this, starving cells for amino acids has little, if any, effect on the proportion of Rheb-GTP [30–32]. Amino acid starvation impairs binding of Rheb to mTORC1 [32], although the underlying mechanism remains to be identified.

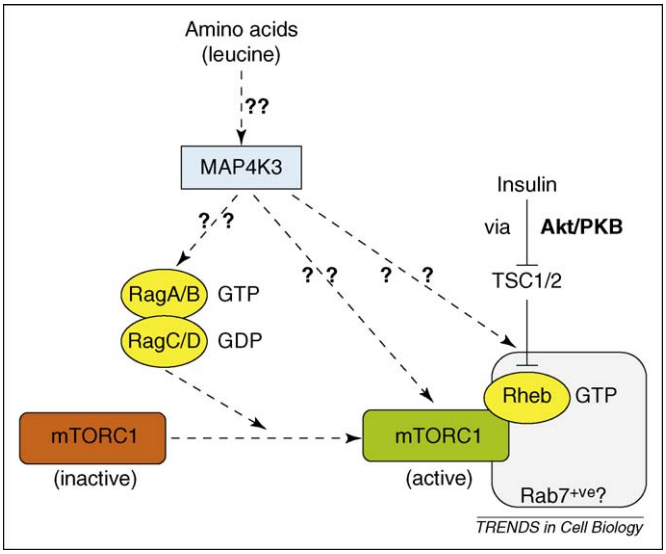

Figure 2. Upstream control of mTORC1 by amino acids. This diagram summarizes recent data by showing roles for the Rag GTPases and MAP4K3. Dotted lines and question marks denote potential signalling connections that remain to be clarified. Dashed lines indicate the proposed mechanism by which Rag GTPases activate mTORC1 (please also see the main text). Bottom right shows a Rab7-containing compartment to which the Rag GTPases may recruit mTORC1 for activation by Rheb, which in turn is activated (e.g. by insulin) through the inhibition of its GAP, the TSC1/2 heterodimer (Box 3).

Earlier data suggested a role for the phosphatidylinositide 3-kinase hVps34, a PtdIns 3-kinase, in the control of mTORC1 [33]. For example, adding amino acids back to starved cells increases the production of PtdIns 3-phosphate and the activity of hVps34. Knocking down hVps34 expression by siRNA impairs the ability of insulin to promote S6K1 phosphorylation in amino-acid-fed cells, without affecting the ability of insulin to activate protein kinase B (PKB; also known as Akt). However, overexpressing hVps34 does not stimulate mTORC1 signalling in amino-acid-starved cells. Moreover, recent work suggests that Vps34 actually lies downstream, not upstream, of TORC1 (in *Drosophila*, at least) [34]. Because hVps34 positively regulates autophagy [34,35] (a degradative processes that generates amino acids), it is possible that hVps34 seems to act upstream of mTORC1 by modulating the supply of intracellular amino acids, rather than by relaying the signal from amino acids to mTORC1 (Figure 3). Evidence has been provided that amino acids trigger a rise in intracellular Ca^{2+} ion levels,which could activate hVps34 and mTORC1 [36] (Figure 3). Because Vps34 probably lies downstream of mTORC1, this interpretation needs to be re-evaluated, but it remains possible that Ca^{2+} ions have a role in amino acid signalling.

Screens for components involved in regulating TORC1 activity in *Drosophila* cells revealed a role for a protein kinase of which the closest human orthologue is MAP4K3, a MAP kinase kinase [37]. siRNA-mediated knockdown of MAP4K3 impaired the activation of mTORC1 signalling by amino acids in human cells, whereas overexpressing it stimulated phosphorylation of S6K1 and 4E-BP1 and delayed the impairment of mTORC1 signalling owing to amino acid withdrawal. Rapamycin blocks these effects, indicating that MAP4K3 acts upstream of mTORC1 (Figure 2). MAP4K3 activity declines upon amino acid withdrawal and rises quickly when they are resupplied. These data suggest that MAP4K3 is involved in mediating the effects of amino acids on mTORC1 signalling, but its precise role remains unclear (Box 3).

Most recently, the Rag GTPases have emerged as components involved in the control of mTORC1 by amino acids (Figure 2). Sancak *et al.* [38] sought novel proteins that interact with mTORC1 and identified RagC as copurifying with raptor. Raptor was found to bind specific pairs of Rag GTPases (e.g. RagA/C and RagB/C). Almost simultaneously, Kim *et al.* [39] conducted an siRNA screen for GTPases involved in controlling TORC1 signalling in *Drosophila* S2 cells. They found that knocking down the closest relatives of the Rag proteins (CG11768 and CG8707) interfered with the phosphorylation of S6K in this system.

Mammals possess four Rag-family proteins, RagA–D. Like other G-proteins (Box 2), the function of Rag proteins depends upon their GDP/GTP binding status, but in a rather unusual way, such that the $RagB^{GTP}$–C^{GDP} combination binds best to mTORC1. Evidence that Rag GTPases are involved in the regulation of mTORC1 comes from the findings that overexpressing $RagB^{GTP}$-$RagC^{GDP}$ or 'constitutively active' forms of RagA and RagB activates mTORC1 signalling and also made it resistant to withdrawal of amino acids. Conversely, expressing dominant interfering (GDP-bound) forms of RagA or RagB or an activated form of RagC inhibited the phosphorylation of S6K. This indicates that RagB normally has a role in controlling mTORC1: other data suggest that this is not a general effect on mTORC1, because $RagB^{GTP}$ cannot 'substitute' for insulin in the activation of mTORC1 or override the inactivation of mTORC1 caused by energy deprivation [38] or osmotic stress [39]. Amino acid stimulation modestly increased the proportion of RagB bound to GTP [38].

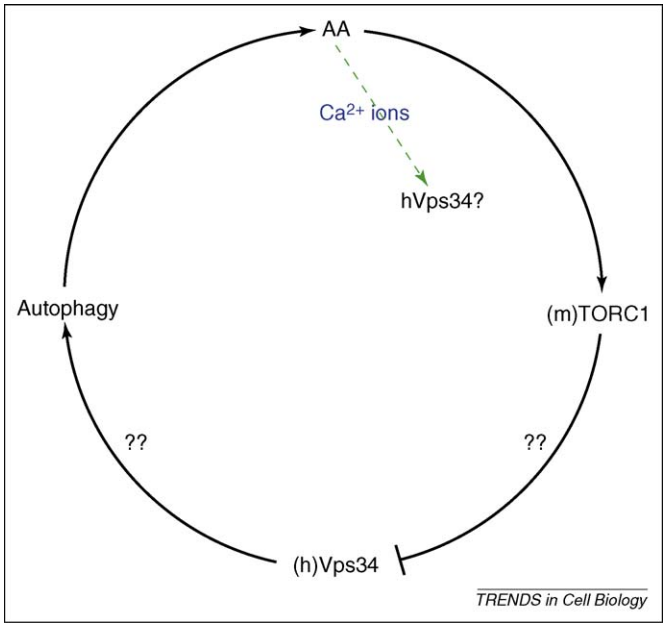

Figure 3. Links between mTORC1 and autophagy. Intracellular amino acids regulate mTORC1 signalling but the mechanism is unknown: it has been suggested that this involves the AA-induced elevation of Ca^{2+} ions which activate Vps34 and stimulate mTORC1. However, recent evidence suggests Vps34 is downstream (not upstream) of TORC1 and controls autophagy. Autophagy is one source of intracellular amino acids. There could therefore be a 'homeostatic' regulatory loop as depicted here, in which, under conditions of low amino acid availability, the resulting suppression of mTORC1 signalling leads to activation of autophagy (possibly via Vps34, uncertainty indicated by question marks), thus increasing protein breakdown; this could restore the supply of intracellular amino acids and restimulate mTORC1, turning off autophagy again. Abbreviations: AA, amino acids.

GTP-bound forms of RagA increase cell size, whereas a dominant-interfering mutant had the converse effect [39]. This is consistent with the established role of dTORC1 signalling in regulating cell size. In the larval fat body, which is involved in nutrient sensing and signalling, the growth-regulatory effects of Rag mutants were only seen in response to changes in nutrient availability, pointing to a role for these GTPases specifically in the nutrient regulation of cell size. The inhibition of mTORC1 signalling caused by dominant interfering Rag mutants is not overcome by cycloheximide, again indicating that the Rag proteins operate 'downstream' of intracellular amino acids [38]. Further data indicate that the Rag proteins and TSC1, TSC2 and Rheb operate in parallel, not series, to regulate TORC1.

It is notable that the closest yeast orthologues of the Rag GTPases, Gtr1p and Gtr2p, control the intracellular sorting of Gap1p (an amino acid permease [40]), a process that is regulated by amino acids and by TORC1. In fact, Gtr2 was identified earlier as a positive regulator of yeast microautophagy, a process that seems to be activated by TOR, and as

being required for yeast cells to exit from rapamycin-induced growth arrest [41]. Indeed, Gtr2 (and Gtr1) are components of the exit from rapamycin-induced growth arrest complex (EGOC) suggested to function upstream of TORC1, perhaps by relaying nutrient signals to TORC1 [21,41].

In contrast to Rheb-GTP, the Rag proteins do not stimulate the *in vitro* kinase activity of mTORC1 [38]. Instead, they are suggested to modulate the intracellular localization of mTORC1: in amino-acid-fed cells, mTORC1 is distributed throughout the cytoplasm. Stimulating starved cells with amino acids rapidly caused relocalization of mTORC1 to vesicular and perinuclear structures [38]. Expressing $RagB^{GTP}$ caused mTORC1 to show the latter localization even in amino-acid-starved cells (in which $RagB^{GTP}$ prevents the shutoff of mTORC1 signalling). Such localization seems similar to that of Rab7, a 'marker' for endosomal and lysosomal structures. Interestingly, GFP-tagged Rheb shows a vesicular pattern of distribution, which is dependent upon its C-terminal farnesylation motif [42]. GFP-tagged Rheb was found to colocalize with tagged Rab7 and this was not discernibly affected by amino acids [38]. Sancak *et al.* [38] therefore proposed that Rag-proteins control the localization of mTORC1 in response to amino acids, such that mTORC1 is recruited to the same locale as Rheb, which then activates it (Figure 2). However, other studies have reported different patterns of localization for Rheb. When overexpressed, Rheb has also been reported to localize to the Golgi and endoplasmic reticulum, and to the cytoplasm [42,43]. Furthermore, endogenous Rheb is reported to be cytosolic [42,44], although more was membrane-associated in cells lacking TSC2 [44]. This contrasts with the apparent colocalization of Rag GTPases and Rab7, because the latter is associated with late endosomes. Further studies are thus required to establish the mechanism by which the Rag GTPases, and indeed Rheb, regulate mTORC1 activity.

The discovery that Rag GTPases have a role in amino acid control of mTORC1 signalling is a potentially important advance. But it also raises new questions (Box 3).

mTORC1 controls the cell cycle

Starving human cells for amino acids, which inhibits mTORC1, decreases cdc2 activity. These and other data point to links between (m)TORC1 and the control of mitotic kinases in mammals and in yeast [20,45,46]. The cell cycle is conventionally divided into four stages: G1; S (where DNA synthesis occurs); G2; and M (mitosis, where the cell divides) (Figure 4). The transitions between G1 and S, and between G2 and M, are tightly controlled by sophisticated mechanisms. A fifth, quiescent state, termed G_0, corresponds to a non-dividing 'resting' condition. In yeast, inactivation of the TORC1 pathway causes cells to enter G_0 through a protein kinase called Rim15. TORC1 apparently maintains Rim15 in a phosphorylated state in which it associates with 14-3-3 proteins and consequently remains in the cytoplasm. Rim15 is a substrate for the kinase Sch9 [47], the probable S6K ortholog in yeast [48]. Inactivation of TORC1 signalling leads to dephosphorylation of Rim15 and its entry into the nucleus, where it induces the G_0 programme. Rim15 acts as a node of convergence for additional signalling pathways that sense nutrient availability including cAMP-dependent protein kinase (PKA) and Pho80 (a cyclin-dependent kinase CDK involved in phosphate sensing [49]).

In the early 1990s, rapamycin was found to block T-cell proliferation by impairing entry into S-phase (for a review, see Ref. [50]). This effect underlies the use of rapamycin as an immunosuppressant (Box 1). Its ability to block the cell cycle at the G1/S boundary is probably involved in its anti-proliferative effects on tumour cells and in the context of restenosis (Box 1).

The entry of cells in G1 into S-phase involves distinct types of cyclin-dependent kinase (CDK)/cyclin complexes, each of which is regulated by specific CDK-inhibitor proteins (CDKIs) [51], termed p21 (also called CIP1 or WAF1) and p27 (or KIP1). The expression of both p21 and p27 can be controlled at transcriptional and post-transcriptional levels. In their active states, CDKs phosphorylate the retinoblastoma gene product (Rb), relieving its inhibitory effect on the transcription factor E2F and promoting S-phase progression.

Much attention has focused on the control of p27 expression by mTORC1. p27 levels are high in quiescent cells and mitogenic stimulation leads to decreased p27 levels. Rapamycin treatment can thus lead to the accumulation of (inactive) cyclin E/Cdk2/$p27^{Kip1}$ complexes. Because S-phase entry requires active cyclin E/Cdk2 complexes, binding of $p27^{Kip1}$ blocks cell-cycle progression. Rapamycin blocks the ability of mitogens to downregulate $p27^{Kip1}$ and data from cells lacking $p27^{Kip1}$ suggest that this effect is important in its anti-proliferative effects [52], although other events are also important, as illustrated by the continued sensitivity of $p27^{-/-}$ T cells to rapamycin. Interestingly, amino acid withdrawal causes increased levels of p27 and p21 through stabilization of their mRNAs [53].

In addition to p27 and p21, mTORC1 signalling can also regulate other components involved in the G1/S transition. These include cyclins D1 [54], D3 [55], E [55] and A [56]. Rapamycin seems to decrease levels of cyclins D1 [57] and D3 [55] through repression of the translation of their mRNAs. Other work has indicated that mTORC1 signalling can regulate the expression of cyclin D1 at multiple levels, including gene transcription and the stability of both the cyclin D1 mRNA and the corresponding protein [58]. Because cyclin D1 can sequester $p27^{Kip1}$, decreased levels of cyclin D1 might inhibit cyclin E-Cdk2, and decrease Cdk4 activity, the partner for cyclin D1.

A recent study reported that mTORC1 is involved in activating the protein kinase SGK1, a close relative of PKB, and that SGK1 phosphorylates $p27^{Kip1}$, resulting in its retention in the cytoplasm (i.e. away from cyclin E-Cdk2) and thus favouring S-phase progression [59]. Although this could provide a link between mTORC1 and S-phase entry, other findings indicate that it is actually the rapamycin-insensitive mTORC2 complex that phosphorylates and activates SGK1 [60].

Much less attention has been devoted to the control of later stages in the cell cycle by (m)TOR. However, three recent reports from eukaryotes as far apart (in an evol-

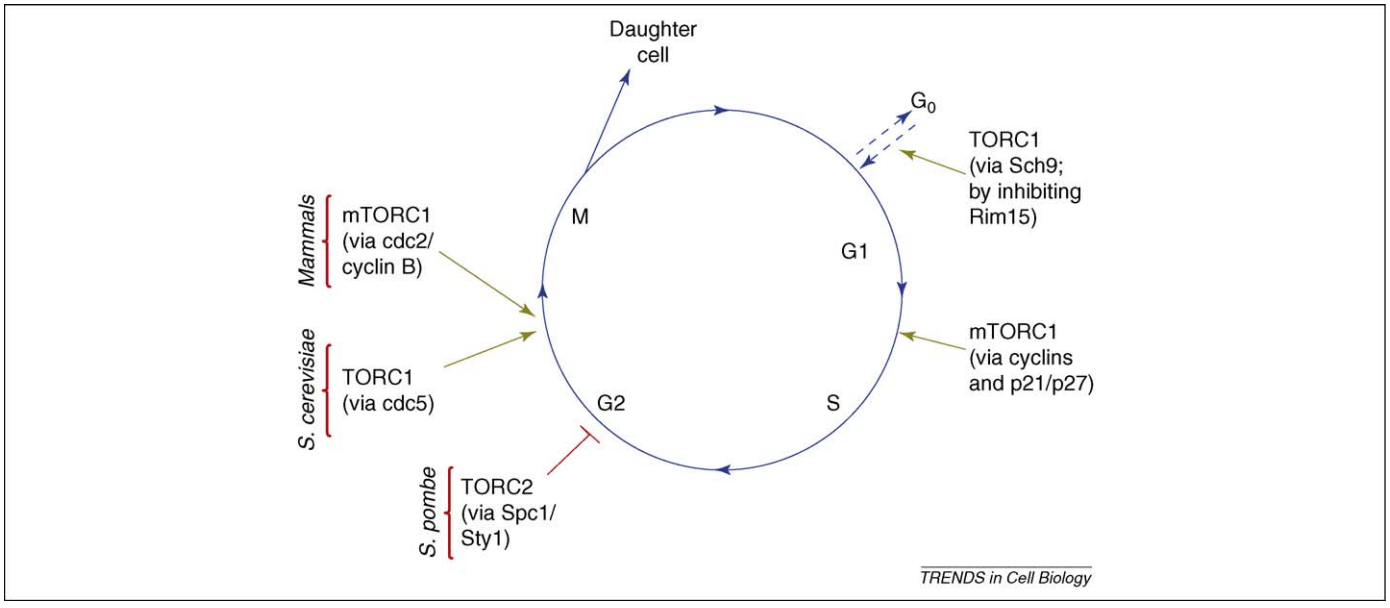

Figure 4. Schematic diagram of the cell cycle showing known inputs to the control of cell-cycle progression. Selected components that link (m)TOR complexes to the control of the cell cycle are shown. See main text for a more detailed discussion. Abbreviations: TORC1, yeast target of rapamycin complex 1.

utionary sense) as yeast and mammals indicate that (m)TOR signalling also controls events involved in the entry of cells into mitosis.

Control of mitosis by TORCs

As described earlier, eEF2K is regulated by mTORC1, but indirectly rather than through direct phosphorylation. Thus, mTORC1 signalling acts indirectly to inhibit eEF2K by promoting its phosphorylation at three sites, Ser78, Ser359 and Ser366. Ser366 was shown some time ago to be phosphorylated by the S6 kinases [9], but the kinases acting on the other two sites were not known. Recently, our own laboratory [20] used a biochemical approach to isolate the Ser359 kinase and showed it to be cdc2/cyclin B. Consistent with cdc2/cyclin B acting to inhibit eEF2K, treating cells with roscovitine, an inhibitor of cdc2, blocks the phosphorylation of Ser359 and increases the phosphorylation of eEF2. Although treating cells with rapamycin does not markedly affect the activity of cdc2, two other manipulations that affect mTORC1 signalling (amino acid starvation and ablation of TSC2) do alter cdc2 activity in a manner consistent with cdc2 being positively regulated by mTORC1.

Consistent with the ability of cdc2/cyclin B to phosphorylate and inactivate eEF2K, the phosphorylation of eEF2 is low in mitotic cells [20]. This probably serves to keep the translation elongation machinery active in mitosis, where certain proteins continue to be made [61]. It has been reported that 4E-BP1 is less phosphorylated during mitosis, leading to suppression of cap-dependent translation (and implying that mTORC1 signalling is inhibited) [61,62]. However, another study showed that 4E-BP1 phosphorylation is high in mitosis and that cdc2 phosphorylates Thr70, an important regulatory site in 4E-BP1 [63].

It remains to be established how amino acids and mTORC1 control the activity of cdc2/cyclin B against eEF2K, and whether or not this effect applies to other cdc2 substrates. The observation that starving cells of leucine delays entry of HeLa cells into mitosis [20] suggests that impaired mTORC1 signalling inhibits the mitosis-promoting function of cdc2/cyclin B. This implies that mTORC1 signalling promotes both entry into S-phase and entry into mitosis (Figure 4).

Recent data also demonstrate that TOR signalling regulates commitment to mitotic entry in both fission yeast (*Schizosaccharomyces pombe* [45]) and budding yeast (*Saccharomyces cerevisiae* [46]), albeit in different and apparently contradictory ways. Nakashima and colleagues [46] show that temperature-sensitive mutants in KOG1 (which encodes raptor in *S. cerevisiae*) delay entry into mitosis, as does treatment with rapamycin, indicating that TORC1 is required for G2→M progression. Overall, this is analogous in effect to the regulation of Cdc2 by mTORC1 in human cells [20]; however, in *S. cerevisiae,* TORC1 promotes nuclear localization and activity of the polo-like kinase Cdc5.

A rather different picture has emerged from studies in *S. pombe.* When budding yeast cells are changed from a rich to a poor nitrogen source, they advance into mitosis and then continue to divide at a smaller size. (It should be noted that this treatment corresponds to poor 'nitrogen quality' rather than to starvation for nitrogen.) How does nutrient availability control commitment to mitosis in this organism? Treating cells with rapamycin also advances mitosis [45] (and thus also causes a reduction in cell size), suggesting that TORC1 controls mitotic progression here. However, rapamycin apparently interferes with the functions of TORC1 and TORC2 in *S. pombe* [45] and both complexes control mitotic entry. Tor1 (which forms TORC2 in this organism) is inhibited by poorer nitrogen quality, leading to activation of the Polo kinase, Plo1, through the Spc1/Sty1 MAP kinase and its cognate phosphatase, Pyp2. Plo1 promotes the activation of the mitotic CDK, cdc2, and, in so doing, promotes M-phase entry (Figure 4). Importantly, this implies that TORC2 is regulated by nutritional cues in budding yeast: both TORC1 and TORC2 seem to be inhibited by rapamycin in this species. Tor2 (i.e. TORC1) is

Box 3. Outstanding questions

The discoveries that Rag GTPases and MAP4K3 are involved in the control of mTORC1 by amino acids are important advances. However, they also raise several new questions, including:

- How do Rag GTPases regulate the localization of mTORC1? Amino acids increase the proportion of RagB-GTP, but it is not clear how they do this or whether they regulate other Rag GTPases. Alternatively, does the input from amino acids to mTORC1 occur via a distinct pathway that requires the Rag GTPases to activate mTORC1?
- Why is it that the highly unusual combination of one GTP-bound and one GDP-bound Rag partner activates mTORC1?
- Is there a link between MAP4K3 and the Rag GTPases?
- How do amino acids regulate the activity of MAP4K3? And how does MAP4K3 regulate mTORC1? Presumably, as a protein kinase, MAP4K3 phosphorylates a protein(s) involved in controlling mTORC1; perhaps an MAP4K3 substrate regulates the Rag GTPases?
- Above all, how are intracellular amino acids levels detected?

inhibited by loss of nitrogen, leading first to faster cell division followed by exit from the cell cycle and G1 arrest.

A further twist to this story is added by the observations that insulin treatment delays the progression of *Drosophila* cells from S-phase through G2/M, and that this effect is countered by rapamycin [64]. Indeed, in this organism, mild impairment of dTORC1 signalling can actually speed up cell division and increase cell number (thereby contrasting with the positive role of dTORC1 in G1 progression). The biochemical mechanisms underlying these very interesting effects remain to be clarified, as does the relationship to the apparent ability of mTORC1 to promote cdc2 activity [20].

It thus seems that signalling through (m)TORC1 modulates mitotic entry in organisms as diverse as fission yeast and mammalian cells. However, such signalling has converse roles in these organisms; inhibiting TORC1 signalling advances mitosis in fission yeast but delays it in mammalian cells or *S. cerevisiae*. In this context, it is relevant to point out two important differences between these systems: first, in the fission yeast experiments, what is being manipulated is the quality of the nitrogen available to an organism that can make its own amino acids. It seems appropriate here to continue to proliferate but at a smaller cell size. In mammalian cells, inhibition of mTORC1 signalling mimics the effect of starvation for one or more essential amino acids; in auxotrophic metazoan organisms, halting cell division is a logical response to this situation.

An important related point is that, although rapamycin decreases cell size in fission yeast and mammalian cells, the underlying mechanisms differ [65]; in the former, it is apparently a consequence of advanced mitosis (i.e. division at a smaller cell size); in the latter, it probably reflects inhibition of anabolic processes, such as protein synthesis, and activation of catabolic events, such as autophagy.

Concluding remarks

The discoveries described here are important advances. First, they help explain a fundamental mechanism of cell physiology (i.e. how amino acids regulate signalling through an anabolic signalling pathway, [m]TORC1). Second, they identify new links between nutrients, (m)TOR and control of cell division. There is now a pressing need to elucidate the precise role of the Rag GTPases in the control of mTORC1; for example, how are amino acid levels sensed and how is this information relayed through these proteins to mTORC1? Further studies are also needed to determine how TOR complexes control components involved in regulating mitotic progression. Such information will aid our understanding of fundamental cell physiology and will help to guide development of new therapies for diseases involving dysregulated mTOR signalling.

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