Review

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Focus: Metabolism

Nutrient sensing and TOR signaling in yeast and mammals

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Abstract

Coordinating cell growth with nutrient availability is critical for cell survival. The evolutionarily conserved TOR (target of rapamycin) controls cell growth in response to nutrients, in particular amino acids. As a central controller of cell growth, mTOR (mammalian TOR) is implicated in several disorders, including cancer, obesity, and diabetes. Here, we review how nutrient availability is sensed and transduced to TOR in budding yeast and mammals. A better understanding of how nutrient availability is transduced to TOR may allow novel strategies in the treatment for mTOR-related diseases.

Keywords amino acids; glucose; mammals; nutrients; RAG; TORC1; yeast
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Introduction

Nutrients provide energy and building blocks for organismal growth. An effective response to changes in nutrient availability is crucial for organismal viability. In response to nutrients, the target of rapamycin (TOR) signaling pathway stimulates anabolic processes such as protein, lipid, and nucleotide synthesis, and represses catabolic processes such as autophagy, to ultimately promote cell growth (for review, see Wullschleger *et al*, 2006; Loewith & Hall, 2011; Howell *et al*, 2013; Laplante & Sabatini, 2013; Shimobayashi & Hall, 2014). TOR was discovered in the budding yeast *Saccharomyces cerevisiae*, by mutations that confer resistance to the growth inhibitory effect of rapamycin (Heitman *et al*, 1991; Kunz *et al*, 1993). Shortly thereafter, it was identified in mammalian cells (Brown *et al*, 1994; Chiu *et al*, 1994; Sabatini *et al*, 1994; Sabers *et al*, 1995). TOR forms two structurally and functionally different conserved complexes termed TOR complex 1 (TORC1) and TORC2, of which only TORC1 is sensitive to rapamycin (Loewith *et al*, 2002). The essential components of budding yeast TORC1 are TOR1 or TOR2, Kog1, and Lst8; the mammalian orthologs are mTOR (mammalian TOR), RAPTOR (regulatory-associated protein of TOR), and mLST8 (mammalian lethal with SEC13 protein 8), respectively (Hara *et al*, 2002; Kim *et al*, 2002; Loewith *et al*, 2002). Nutrients, growth factors, and cellular energy regulate TORC1 activity. Nutrients are particularly important TORC1 activators as they alone are sufficient to activate TORC1 in unicellular organisms. Growth factor signaling evolved and was grafted onto the TORC1 signaling pathway in multicellular organisms. Here, we review amino acid and glucose sensing mechanisms and how nutrient availability is transduced to TORC1 in yeast and mammals.

RAG GTPases and their upstream regulators

Amino acid sufficiency regulates TORC1 via different mechanisms that largely involve the conserved RAG family of small GTPases (for review, see Jewell *et al*, 2013; Bar-Peled & Sabatini, 2014; Shimobayashi & Hall, 2015; Hatakeyama & De Virgilio, 2016; Powis & De Virgilio, 2016; Fig 1). There are four RAGs in mammals (RAGA, RAGB, RAGC, and RAGD) and two in *S. cerevisiae* (Gtr1 and Gtr2) (Schürmann *et al*, 1995; Hirose *et al*, 1998; Sekiguchi *et al*, 2001). Mammalian RAGs localize to the lysosome irrespective of amino acid availability, by interacting with the lysosomal pentameric complex RAGULATOR (Sancak *et al*, 2010; Bar-Peled *et al*, 2012). In yeast, the EGO (Ego1–Ego2–Ego3) ternary complex, the ortholog of RAGULATOR, tethers Gtr1/2 to the vacuole (the yeast equivalent of the lysosome) (Kogan *et al*, 2010; Zhang *et al*, 2012; Levine *et al*, 2013; Powis *et al*, 2015). RAGs function as heterodimers in which RAGA or RAGB dimerizes with RAGC or RAGD, and Gtr1 dimerizes with Gtr2 (Nakashima *et al*, 1999; Sekiguchi *et al*, 2001). Amino acid sufficiency promotes the active conformation of the RAG heterodimer in which RAGA/B or Gtr1 is loaded with GTP, and RAGC/D or Gtr2 is loaded with GDP (Kim *et al*, 2008; Sancak *et al*, 2008; Binda *et al*, 2009; Fig 1). In mammals, the active RAG heterodimer binds RAPTOR and thereby recruits mTORC1 to the lysosome (Sancak *et al*, 2008). Once on the lysosome, the growth factor-stimulated GTP-loaded form of the small GTPase RHEB (RAS homolog enriched in brain) binds and activates mTORC1 (Long *et al*, 2005). Growth factors stimulate lysosomal RHEB through the PI3K-PDK1-AKT pathway (reviewed in Pearce *et al*, 2010; Dibble & Cantley, 2015). AKT phosphorylates and inactivates TSC2 (tuberous sclerosis complex 2) by inducing its release from the lysosome (Inoki *et al*, 2002; Manning *et al*, 2002; Menon *et al*, 2014). TSC2 otherwise associates with TSC1 and TBC1D7 to form the TSC complex that functions as GAP (GTPase-activating protein) toward

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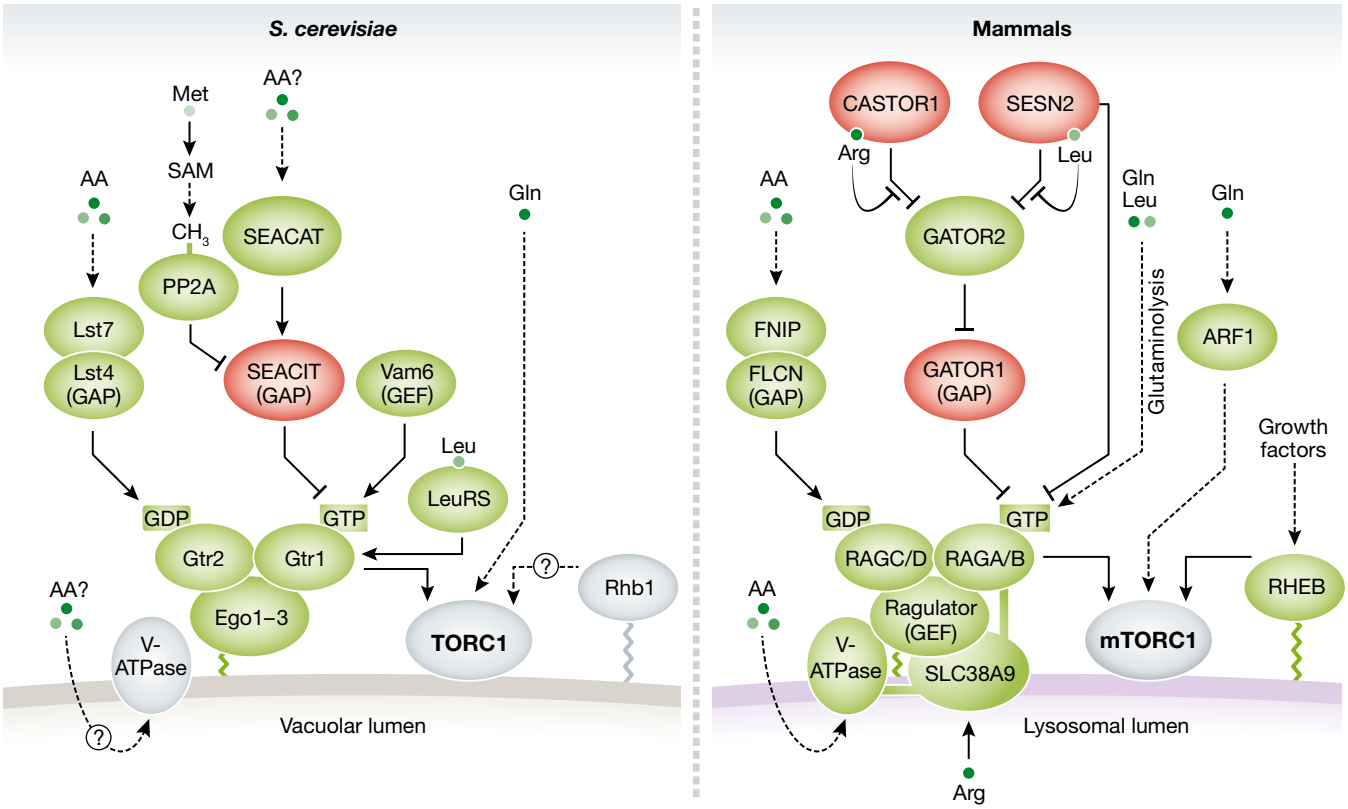

Figure 1. Regulation of TORC1 by amino acids in yeast (*Saccharomyces cerevisiae*) and mammals.
Proteins shown in green promote TORC1 activation. Proteins in red inhibit TORC1. GAP and GEF between parentheses indicate that the proteins act as GTPase-activating proteins or guanine exchange factors, respectively. Dashed lines indicate indirect interactions. There is no evidence that the yeast RHEB-related protein Rhb1 plays a role in TORC1 regulation. See main text for details.

lysosomal RHEB (Gao *et al*, 2002; Kenerson *et al*, 2002; Kwiatkowski *et al*, 2002; Onda *et al*, 2002; Tee *et al*, 2002; Garami *et al*, 2003; Inoki *et al*, 2003a; Dibble *et al*, 2012). Thus, full activation of mTORC1 requires input from amino acids and growth factors. In budding yeast, the active $Gtr1^{GTP}$–$Gtr2^{GDP}$ heterodimer similarly binds Kog1 to stimulate TORC1, but via a mechanism that possibly differs from that of mammals since (i) yeast TORC1 is constitutively localized to the limiting membrane of the vacuole or to discrete perivacuolar sites irrespective of the presence or absence of leucine (Binda *et al*, 2009) or a nitrogen source (Kira *et al*, 2014, 2015; Hughes Hallett *et al*, 2015), and (ii) budding yeast does not express TSC or RHEB orthologs. We note that yeast contains a protein, termed Rhb1 (Urano *et al*, 2000), that resembles RHEB, but is not a functional RHEB homolog.

mTORC1 inactivation is an active process that requires translocation of TSC2 to the lysosome to inhibit RHEB upon growth factor deprivation (Menon *et al*, 2014; Fawal *et al*, 2015; Demetriades *et al*, 2016), amino acid deprivation (Demetriades *et al*, 2014; Deng *et al*, 2015), or other stress conditions (e.g., hypoxia or osmotic stress) (Plescher *et al*, 2015; Demetriades *et al*, 2016). It has been proposed that the "inactive" $RAGA/B^{GDP}$–$RAGC/D^{GTP}$ heterodimer recruits TSC2 to the lysosome in amino acid-starved cells (Demetriades *et al*, 2014). However, two studies have concluded that amino acids do not regulate lysosomal localization of TSC2 (Menon *et al*, 2014; Fawal *et al*, 2015). This discrepancy is likely due to differences in cell types and experimental conditions (Demetriades *et al*, 2016). The inactive GDP-loaded version of Gtr1 has been reported to inhibit TORC1 activity and growth via the non-essential TORC1 component Tco89 (Binda *et al*, 2009).

The nucleotide binding status of the mammalian RAGs and yeast Gtr1/2 is tightly regulated by conserved GAPs and GEFs (guanine exchange factors) (for review, see Shimobayashi & Hall, 2015; Powis & De Virgilio, 2016; Fig 1). RAGULATOR, besides serving as a scaffold for the RAGs, has GEF activity toward RAGA/B (Bar-Peled *et al*, 2012). In yeast, rather than the EGO complex, the vacuolar protein Vam6 has been proposed to be the GEF for Gtr1 (Binda *et al*, 2009). The heterotrimeric protein complexes GATOR1 (GAP activity toward RAGs 1) and SEACIT (Seh1-associated subcomplex inhibiting TORC1) function as GAPs for RAGA/B and Gtr1, respectively. GATOR1 is composed of DEPDC5 (DEP domain-containing protein 5), NPRL2 (nitrogen permease regulator 2-like protein), and NPRL3 where DEPDC5 is thought to possess the GAP activity toward RAGA/B (Bar-Peled *et al*, 2013; Panchaud *et al*, 2013a). SEACIT is composed of Npr2, Npr3, and the catalytic subunit Iml1 (Panchaud *et al*, 2013a). The mammalian pentameric complex GATOR2, consisting of SEC13 (protein SEC13 homolog), SEH1L (nucleoporin SEH1), WDR24 (WD repeat-containing protein 24), WDR59, and MIOS (WD repeat-containing protein MIO), and the yeast SEACAT (Seh1-associated complex subcomplex activating TORC1), consisting of Sec13, Seh1, Sea2, Sea3, and Sea4, bind and negatively

regulate GATOR1 and SEACIT, respectively, via an undefined mechanism (Bar-Peled *et al*, 2013; Panchaud *et al*, 2013b; Dokudovskaya & Rout, 2015). Mammalian FLCN (folliculin) and its binding partners FNIP1 and 2 (folliculin-interacting proteins 1 and 2) as well as their yeast orthologs Lst4 and Lst7 are the GAPs for RAGC/D (Petit *et al*, 2013; Tsun *et al*, 2013) and Gtr2 (Péli-Gulli *et al*, 2015), respectively. The identity of the GEF for RAGC/D and Gtr2 remains unknown. Two independent studies recently demonstrated that amino acids regulate RAGA activity via ubiquitination (Deng *et al*, 2015; Jin *et al*, 2015).

Amino acid sensing and signaling to TORC1

Amino acids modulate the guanine nucleotide binding status of RAG/Gtr and eventually TORC1 activity. How amino acid sufficiency is sensed and signaled to RAGs are long-standing questions. Several mechanisms have been proposed, including amino acids being sensed in the cytosol, lysosome, and mitochondria. How many different amino acids are actually sensed remains unknown. mTORC1 activity is particularly sensitive to leucine and arginine levels (Hara *et al*, 1998), whereas yeast TORC1 responds best to the amino acid and nitrogen source glutamine (Godard *et al*, 2007; Stracka *et al,* 2014).

Leucine and glutamine sensing mechanisms

SESTRIN1 through 3 are stress-responsive proteins that mediate metabolic homeostasis in metazoans (for a review, see Lee *et al*, 2013). SESTRINS have been proposed to repress mTORC1 through at least three different mechanisms: (i) by activating AMPK (AMP-activated protein kinase) and the TSC complex (Budanov & Karin, 2008), (ii) by acting as a GDI (guanosine dissociation inhibitor) to prevent GDP dissociation from RAGA/B (Peng *et al*, 2014), and (iii) by binding and inhibiting GATOR2 to prevent mTORC1 lysosomal localization in response to amino acids (Chantranupong *et al*, 2014; Parmigiani *et al*, 2014; Kim *et al*, 2015b). Recently, Wolfson *et al* (2016) demonstrated that the cytoplasmic protein SESTRIN2 directly binds leucine *in vitro*. Leucine fails to stimulate mTORC1 in cells expressing a leucine binding-deficient mutant of SESTRIN2. Leucine (also isoleucine, methionine, and less potently, valine) disrupts the interaction between SESTRIN2 and GATOR2 *in vitro* and in cells. In cells starved for leucine, SESTRIN2 binds and inhibits GATOR2. Leucine deprivation fails to inhibit mTORC1 in SESTRIN-depleted cells expressing a GATOR2 binding-deficient mutant of SESTRIN2, indicating that SESTRIN2 controls mTORC1 via GATOR2. Upon leucine binding, SESTRIN2 dissociates from GATOR2, which results in mTORC1 translocation to the lysosome (Wolfson *et al*, 2016). Thus, Wolfson *et al* proposed that SESTRIN2 is almost certainly a cytosolic leucine sensor that acts upstream GATOR2 (Wolfson *et al*, 2016) (Fig 1). However, the role of SESTRINS as leucine sensors has been questioned, as SESTRINS can inhibit mTORC1 in cells growing in medium containing leucine (see Lee *et al*, 2016 and references therein). Recently, Saxton *et al* (2016c) resolved the structure of SESTRIN2 bound to leucine, and identified the leucine binding pocket and the GATOR2 binding site. They suggest that leucine promotes a conformational change in SESTRIN2 that alters the GATOR2 binding site, thereby causing dissociation of SESTRIN2 from GATOR2 (Saxton *et al*, 2016c). Kim *et al* recently reported a crystal structure of SESTRIN2 obtained without the addition of exogenous leucine (Kim *et al*, 2015a). This structure is largely identical to the one generated by Saxton *et al* in the presence of leucine, suggesting that leucine binding does not induce a significant conformational change in SESTRIN2 (Lee *et al*, 2016). However, the apo-SESTRIN2 crystal structure presented by Kim *et al* possibly contains leucine (Saxton *et al*, 2016b). Thus, more studies are required to elucidate how leucine binding affects the conformation of SESTRIN2 to induce its dissociation from GATOR2 and how the SESTRIN2–GATOR2 interaction affects GATOR1 and RAGs. Furthermore, it remains unknown whether additional factors regulate the dissociation of leucine from SESTRIN2 upon leucine starvation.

In budding yeast, leucine activates TORC1 via Gtr1 (Binda *et al*, 2009), although it is unknown whether leucine signals to Gtr1 through SEACAT. Yeast lacks SESTRIN orthologs, suggesting that functional counterparts of SESTRINS exist or that yeast and mammalian cells sense leucine differently. Two studies demonstrated that yeast and mammalian leucyl-tRNA synthetases (LeuRS) act as cytoplasmic leucine sensors to activate TORC1/mTORC1, although via different mechanisms (Bonfils *et al*, 2012; Han *et al*, 2012; Fig 1). Bonfils *et al* demonstrated that yeast leucine-bound LeuRS binds Gtr1, and suggested that this interaction is necessary and sufficient to mediate leucine signaling to TORC1. Han *et al* (2012) reported that mammalian LeuRS senses leucine to induce lysosomal localization and activity of mTORC1. This study also suggested that LeuRS has GAP activity toward RAGD. The role of LeuRS as a GAP, however, has been questioned (Tsun *et al*, 2013). Yoon *et al* (2016) recently showed that LeuRS is part of a RAG-independent mechanism by which amino acid sufficiency activates mTORC1. This mechanism involves the class III PI-3-kinase VPS34 and PLD1 (phospholipase D1; Yoon *et al*, 2011). Further studies are required to reconcile the RAG-dependent and RAG-independent roles of LeuRS as an mTORC1 regulator.

Consistent with leucine sensing regulating mTORC1 activity, plasma membrane leucine (SLC7A5–SLC3A2) and glutamine (SLC1A5) transporters affect mTORC1 signaling (reviewed in Taylor, 2014). Cytosolic glutamine is used as an anti-solute to import leucine via the SLC7A5–SLC3A2 heterodimeric antiporter. Decreased leucine import due to the loss of SLC1A5 or SLC7A5–SLC3A2 impairs mTORC1 activity, indicating that glutamine acts upstream of leucine as an efflux solute to increase cytosolic leucine levels and activate mTORC1 (Nicklin *et al*, 2009). A recent study demonstrated that overexpression of LAPTM4b (lysosomal protein transmembrane 4 beta) recruits SLC7A5–SLC3A2 to the lysosome, thereby increasing leucine accumulation in the lysosome. Knockdown of *LAPTM4b* reduces mTORC1 activity in cells stimulated with leucine (Milkereit *et al*, 2015), indicating that leucine sensing occurs at lysosomes. Pharmacological inhibition of SLC1A5 also reduces mTORC1 activity in triple-negative basal-like breast cancer cells (Van Geldermalsen *et al*, 2016).

Glutaminolysis, the double deamination of glutamine to produce α-ketoglutarate, provides a mechanism for leucine and glutamine sensing in mitochondria (Durán *et al*, 2012). GLS (glutaminase) catalyzes the deamination of glutamine to yield glutamate. GDH (glutamate dehydrogenase), which requires leucine as a cofactor, then converts glutamate to α-ketoglutarate. α-Ketoglutarate activates RAG-mTORC1 through PHD (prolyl hydroxylase) (Durán *et al*, 2012, 2013). Thus, in mammalian cells, leucine and glutamine activate mTORC1 via glutaminolysis and α-ketoglutarate production upstream

of RAG (Fig 1). PHDs are conserved from yeast to mammals. It would be of interest to determine whether the budding yeast putative prolyl-4-hydroxylase Tpa1 (Henri *et al*, 2010) regulates TORC1.

Glutamine activates TORC1 also independently of the RAGs/Gtr1/2, in yeast and mammals. Stracka *et al* (2014) demonstrated that glutamine activates TORC1 in yeast cells lacking Gtr1 or Vam6. Although the Gtr1-independent mechanism of TORC1 activation remains elusive, genetic experiments suggest that it could involve the vacuolar membrane-associated phosphatidylinositol 3-phosphate binding protein Pib2 (Kim & Cunningham, 2015). Consistent with the observations reported in yeast, glutamine stimulates lysosomal translocation and activation of mTORC1 in a RAGA/B and RAGULATOR-independent manner via the small GTPase ARF1 (ADP-ribosylation factor 1) and v-ATPase (vacuolar ATPase; Jewell *et al*, 2015; Fig 1). How ARF1 senses glutamine and regulates mTORC1 is unclear.

Arginine sensing mechanisms

The lysosomal amino acid transporter SLC38A9 has been proposed as an arginine sensor upstream of mTORC1. SLC38A9 binds RAGULATOR and RAGs, and knockdown of *SLC38A9* impairs arginine-induced activation of mTORC1 (Jung *et al*, 2015; Rebsamen *et al*, 2015; Wang *et al*, 2015; Fig 1). The yeast vacuolar amino acid transporters Avt1-7 (Russnak *et al*, 2001) are the transporters most closely related to SLC38A9. Whether Avt proteins regulate TORC1 activity requires further investigation.

Recently, Chantranupong *et al* (2016) identified the GATOR2-interacting protein CASTOR1 (cellular arginine sensor for mTORC1) as a cytoplasmic arginine sensor upstream of mTORC1. CASTOR1 forms homodimers or, with the highly related protein CASTOR2, heterodimers. CASTOR1 homodimers or CASTOR1–CASTOR2 heterodimers directly bind arginine *in vitro*. Arginine binding disrupts the interaction of CASTOR dimers with GATOR2, presumably allowing free GATOR2 to inhibit GATOR1 and thereby activate mTORC1. Arginine fails to stimulate mTORC1 activity in cells expressing an arginine binding-deficient mutant of CASTOR1. Thus, binding of arginine to CASTOR1 enables GATOR2 to enhance mTORC1 activity (Fig 1). The crystal structure of CASTOR1 in complex with arginine, reported by two independent groups, illustrates in detail the arginine binding pocket of CASTOR1 (Saxton *et al*, 2016a; Xia *et al*, 2016). Furthermore, Saxton *et al* (2016a) identified several residues in CASTOR1 required for interaction with GATOR2, and speculated that arginine binding transmits an allosteric signal to trigger dissociation of CASTOR dimers from GATOR2. The structure of apo-CASTOR1 or the CASTOR1–GATOR2 complex would contribute to understanding this mechanism. In addition, it would be of interest to investigate if and how CASTOR1 and SESTRIN2 bind GATOR2 simultaneously in cells starved for arginine and leucine. CASTOR homologs are present in vertebrates, but are absent in worms, flies, and yeast. How arginine is sensed in non-vertebrates remains to be clarified.

Based on genetic experiments, it has been suggested that CASTOR1 and SLC38A9 regulate mTORC1 activation by arginine via parallel mechanisms (Chantranupong *et al*, 2016). However, it appears that CASTOR1 is the more important regulator of the two since mTORC1 is essentially fully active in arginine-starved, *CASTOR1*-knockout cells. Furthermore, additional regulators may exist since arginine slightly activates mTORC1 in *SLC38A9*-knockout *CASTOR1*-knockdown cells. Curiously, Carroll *et al* (2016) recently reported that arginine cooperates with growth factors to prevent the interaction between TSC2 and RHEB at the lysosome, and thereby to activate mTORC1.

Methionine sensing mechanism

It has been proposed that in yeast cells utilizing lactate as carbon source, methionine signals to Gtr1/2 through synthesis of the methyl donor SAM (S-adenosylmethionine). SAM promotes Ppm1-mediated methylation of the catalytic subunit of the type 2A protein phosphatase (PP2A). Methylated PP2A dephosphorylates the SEACIT complex component Npr2 to prevent assembly of the complex and eventually to activate TORC1 (Sutter *et al*, 2013; Fig 1).

Amino acid sensing in the lysosome

It has also been suggested that amino acid levels are sensed in the lysosome. Zoncu *et al* (2011) proposed that mTORC1 senses amino acids in the lumen of the lysosome through an "inside-out" mechanism that requires the v-ATPase. According to this model, amino acids in the lumen of the lysosome signal to the RAGs via v-ATPase and RAGULATOR. Whether the yeast v-ATPase mediates amino acid signaling toward Gtr1/2 is unknown (Fig 1).

SLC15A4 is a lysosomal proton-coupled histidine transporter, which exports histidine from the lysosome to the cytoplasm. SLC15A4 is preferentially expressed in immune cells, including dendritic and B cells. SLC15A4-depleted B cells accumulate histidine in the lysosome and display increased lysosomal pH, impaired v-ATPase function, and reduced mTORC1 activity (Kobayashi *et al*, 2014). SLC15A4 may affect mTORC1 activity through v-ATPase although the mechanism remains elusive.

The proton and amino acid symporter PAT1/SLC36A1 is required for mTORC1 activation by amino acids (Heublein *et al*, 2010). PAT1/SLC36A1 is located mainly in endosomal compartments and can potentially export amino acids to the cytoplasm. PAT1/SLC36A1 physically interacts with RAGC/D. Knockdown of *PAT1* reduces the amino acid-stimulated translocation of mTORC1 to the lysosome (Ögmundsdóttir *et al*, 2012).

Amino acid sensing in the Golgi

Thomas *et al* (2014) reported that mTORC1 on the Golgi can be activated by amino acids in a RAG-independent manner. Mechanistically, amino acids promote GTP loading of the small GTPase RAB1A (Ras-related protein RAB-1A) which in turn stimulates mTORC1 interaction with Golgi-resident RHEB (Thomas *et al*, 2014). The proton and amino acid symporter PAT4/SLC36A4 is required for mTORC1 activation by amino acids (Heublein *et al*, 2010). PAT4/SLC36A4 is mainly localized to the Golgi where it physically interacts with mTOR, RAPTOR, and RAB1A (Fan *et al*, 2016). Ypt7, the yeast RAB1A ortholog, is also required for amino acids to activate TORC1 (Thomas *et al*, 2014), indicating that amino acid sensing could occur at the Golgi in both mammals and yeast.

Extracellular amino acid sensing

The G protein-coupled receptor T1R1/T1R3 is an amino acid receptor originally discovered in gustatory neurons as a detector of the umami (glutamate) flavor (Matsunami *et al*, 2000; Nelson *et al*, 2002). Knockdown of *T1R1/T1R3* impairs amino acid-induced

mTORC1 lysosomal translocation and activation without significantly affecting intracellular amino acid levels (Wauson *et al*, 2012). This study suggests that extracellular amino acid availability could be sufficient to modulate mTORC1 activity.

The GAAC signaling pathway

The conserved GAAC (general amino acid control) signaling pathway coordinates amino acid availability with translation initiation to allow cells to adapt to amino acid starvation (reviewed in Hinnebusch, 2005). The GAAC signaling pathway senses the absence of amino acids via uncharged tRNAs that accumulate when free amino acid levels are low. In amino acid-starved cells, uncharged tRNAs bind and activate the protein kinase GCN2 (general control nonderepressible 2; Wek *et al*, 1989, 1995; Diallinas & Thireos, 1994; Dong *et al*, 2000; Narasimhan *et al*, 2004). Active GCN2 phosphorylates the alpha subunit of eIF2 (eukaryotic initiation factor 2α), thereby inhibiting eIF2 and ultimately leading to a general repression of mRNA translation (Dever *et al*, 1992). Paradoxically, this favors selective translation of mRNA with a unique 5′UTR structure containing short uORFs (upstream open reading frames). The uORF containing mRNA encodes a basic leucine zipper transcription factor termed ATF4 (activating transcription factor 4) in mammals (Harding *et al*, 2000; Vattem & Wek, 2004) and Gcn4 in yeast (Hinnebusch, 1984). ATF4/Gcn4 induces the expression of amino acid transporters, enzymes involved in amino acid metabolism (Hinnebusch & Natarajan, 2002; Siu *et al*, 2002; Averous *et al*, 2004; Hinnebusch, 2005; Kilberg *et al*, 2009; Staschke *et al*, 2010), and factors involved in autophagy (B'chir *et al*, 2013; Fig 2), thereby allowing adaptation to amino acid starvation.

The potential crosstalk between GAAC and mTORC1 has not been studied in detail, although inhibition of hepatic mTORC1 in mice fed a leucine-free diet or in cells starved for leucine requires GCN2 (Anthony *et al*, 2004; Xiao *et al*, 2011). Recently, two independent studies confirmed that mTORC1 inhibition in response to amino acid deprivation requires GCN2 (Ye *et al*, 2015; Averous *et al*, 2016). Averous *et al* (2016) proposed that, upon short-term (0.5 to 1 h) deprivation of leucine or arginine, GCN2 inhibits mTORC1 via an uncharacterized ATF4-independent mechanism (Fig 2). Short-term

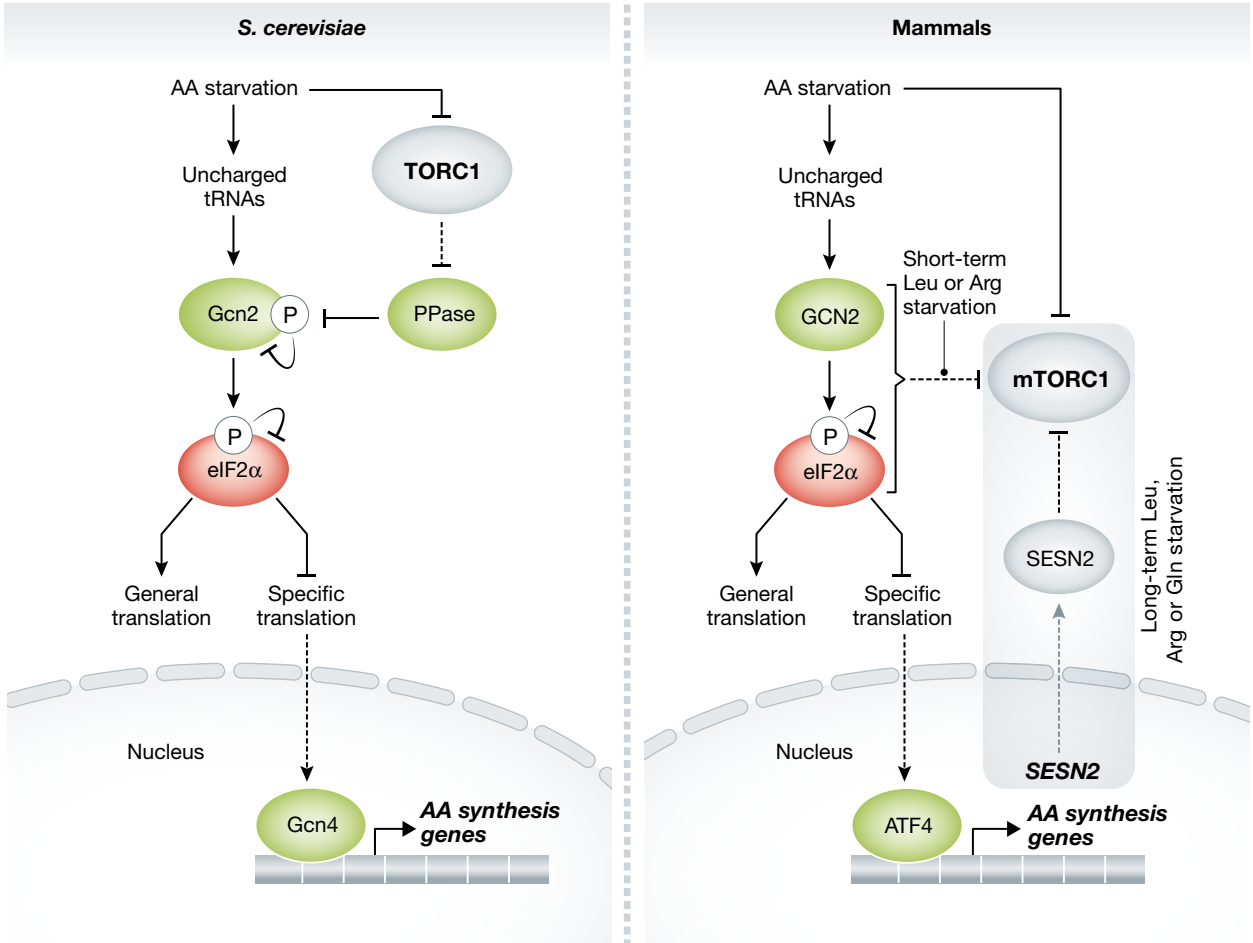

Figure 2. Crosstalk between TORC1 and GAAC signaling pathways in yeast and mammals.
Proteins shown in green promote Gcn4/ATF4-dependent transcription. Proteins in red inhibit Gcn4/ATF4-dependent transcription. PPase, protein phosphatase. See main text for details.

deprivation of leucine also requires phosphorylated eIF2α to inhibit mTORC1. Ye *et al* reported that, upon long-term (24 h) deprivation of leucine, arginine, or glutamine, GCN2 inhibits mTORC1 through ATF4-mediated induction of *SESTRIN2* expression. SESTRIN2 in turn inhibits mTORC1 in a RAGA/B-dependent manner (Ye *et al*, 2015) (Fig 2). The findings by Ye *et al* imply that SESTRIN2 inhibits mTORC1 even in the presence of leucine. It would be of interest to determine whether SESTRIN2-mediated inhibition of mTORC1 requires GATOR2 and whether leucine-binding ability of SESTRIN2 is required to inhibit mTORC1 under these conditions.

A link between TORC1 and GAAC has been demonstrated in *S. cerevisiae*. TORC1 prevents dephosphorylation of Ser577 in Gcn2 by inhibiting one or more phosphatases. Phosphorylation of Gcn2 at Ser577 inhibits Gcn2 by decreasing its uncharged tRNA binding ability (Cherkasova & Hinnebusch, 2003; Kubota *et al*, 2003). Thus, in budding yeast, Gcn2 activation upon amino acid starvation is a consequence of an increase in uncharged tRNAs and the release of an inhibitory effect of TORC1 (Fig 2). Despite the conserved role of Gcn2 in translation, it is unknown whether mTORC1 regulates GCN2. Interestingly, a recent report showed that mTORC1 stimulates purine synthesis through ATF4 activation independent of eIF2α phosphorylation (Ben-Sahra *et al*, 2016). Further studies are required to better understand how GAAC and TORC1 signaling pathways coordinate to allow cells to adapt to changes in nutrient availability.

The yeast SPS amino acid sensing pathway

The SPS pathway senses amino acid availability and regulates amino acid uptake (reviewed in Ljungdahl, 2009; Ljungdahl & Daignan-Fornier, 2012). The SPS pathway is present only in fungi (Martínez & Ljungdahl, 2005). In contrast to the GAAC pathway, the SPS pathway is activated by amino acids. The primary amino acid sensor is a plasma membrane-localized complex composed of Ssy1, Ptr3, and Ssy5 (named as SPS sensor) (Forsberg & Ljungdahl, 2001). Ssy1 is a multi-spanning transmembrane sensor structurally related to amino acid permeases but lacking transporting capacity (Didion *et al*, 1998; Iraqui *et al*, 1999; Klasson *et al*, 1999). Ssy1 possesses an exclusively cytoplasmic N-terminal domain, which binds the scaffold protein Ptr3, and the endoprotease Ssy5. Ssy5 is expressed as a zymogen composed of a catalytic domain attached to an inhibitory domain (Abdel-Sater *et al*, 2004a; Andréasson *et al*, 2006; Poulsen *et al*, 2006). Binding of extracellular amino acids to exposed Ssy1 induces a conformational change that stimulates the phosphorylation and ubiquitin-mediated degradation of the inhibitory domain of Ssy5 (Pfirrmann *et al*, 2010; Omnus *et al*, 2011). Active Ssy5 cleaves the N-terminal cytoplasmic retention motif of the transcription factors Stp1 and 2 (Andréasson & Ljungdahl, 2002). Processed Stp1/2 translocates into the nucleus to induce the expression of genes encoding amino acid permeases (Abdel-Sater *et al*, 2004b; Boban & Ljungdahl, 2007). The SPS pathway and

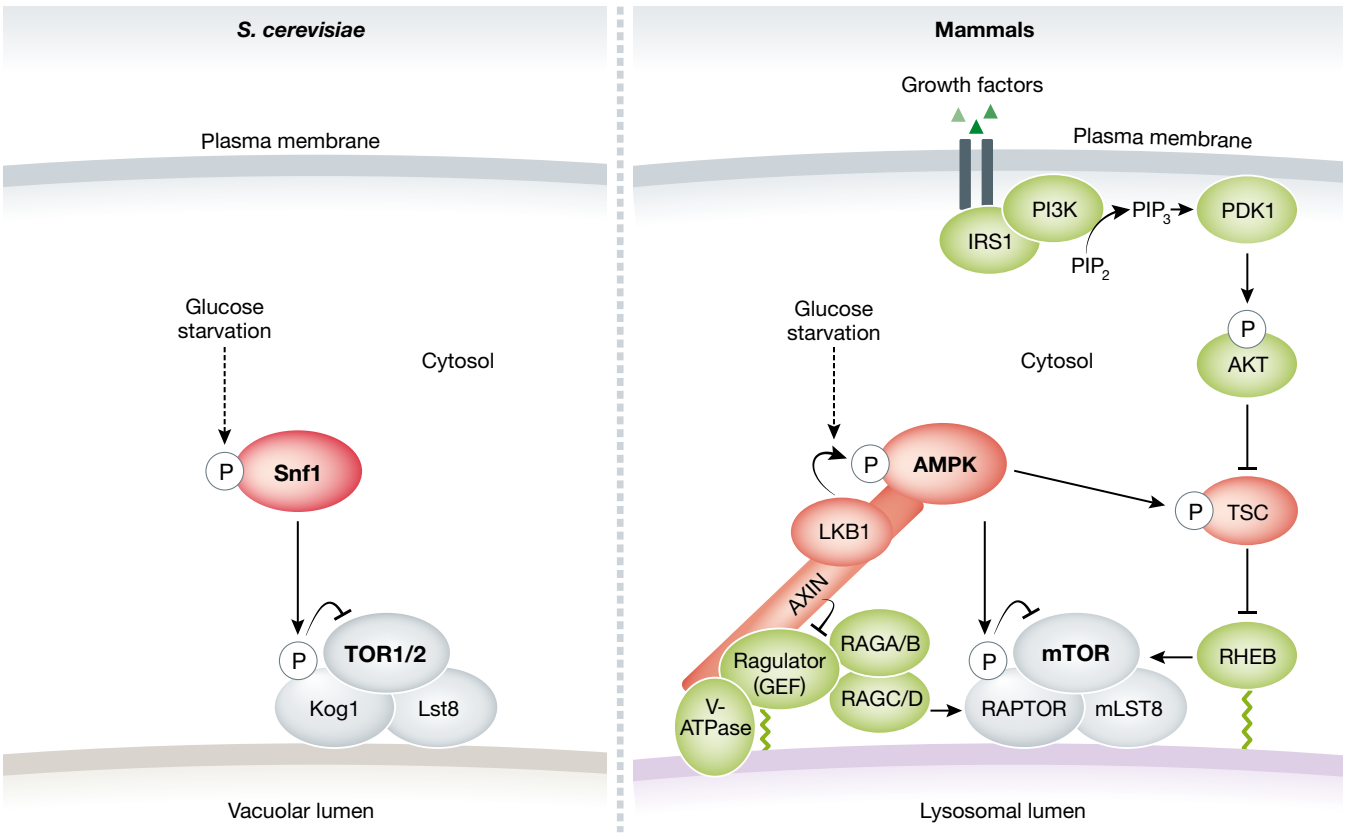

Figure 3. Crosstalk between TORC1 and AMPK signaling pathways in yeast and mammals.
Proteins shown in green promote TORC1 activation. Proteins in red inhibit TORC1. IRS1, insulin receptor substrate 1. See main text for details.

TORC1 are interconnected. TORC1, via the PP2A-like phosphatase Sit4, promotes the stability of nuclear Stp1 and thus amino acid uptake (Shin *et al*, 2009).

Glucose sensing and signaling to TORC1

AMPK is a conserved sensor of cellular energy status. It is activated by metabolic stress, such as glucose deprivation, that increases cellular ADP/ATP and AMP/ATP ratios (reviewed in Hardie, 2007; Hardie *et al*, 2012). AMPK promotes catabolic processes such as autophagy and inhibits anabolic processes such as protein synthesis, in part by negatively regulating TORC1 signaling (for review, see Hardie, 2014; Hindupur *et al*, 2015). In mammals, AMPK inhibits mTORC1 via at least two different mechanisms (Fig 3): (i) AMPK phosphorylates and activates TSC2, thereby inactivating RHEB (Inoki *et al*, 2003b), and (ii) AMPK phosphorylates RAPTOR on Ser722 and Ser792 to inhibit mTORC1 (Gwinn *et al*, 2008). Although budding yeast does not express TSC2, the AMPK Snf1 is required for TORC1 inactivation in glucose-starved cells (Hughes Hallett *et al*, 2014). Active Snf1 phosphorylates Kog1 at Ser491 and Ser494. Curiously, phosphorylated Kog1 dissociates from TORC1 and translocates to discrete perivacuolar sites, leading to a reduction in TORC1 activity (Hughes Hallett *et al*, 2015). The Snf1 phosphorylation sites in Kog1 are located in a glutamine-rich, prion-like motif. This motif and a similar motif, separated by 300 residues, are essential for Kog1 translocation to perivacuolar sites upon glucose deprivation (Fig 3). Interestingly, organisms that express Kog1/RAPTOR proteins containing prion-like motifs (e.g., *S. cerevisiae* and *C. elegans*) lack TSC orthologs, whereas species lacking such motifs in Kog1/RAPTOR (e.g., fission yeast, flies, and mammals) express TSC proteins. Thus, mechanisms by which AMPK inhibits TORC1 may have diverged during evolution.

Glucose deprivation inhibits TORC1 in yeast cells expressing constitutively active versions of Gtr1 and Gtr2 ($Gtr1^{GTP}$–$Gtr2^{GDP}$) (Hughes Hallett *et al*, 2015), suggesting that TORC1 inhibition upon glucose starvation does not require Gtr1/2. In contrast, Efeyan *et al* (2014) reported that glucose deprivation fails to inhibit mTORC1 in primary MEFs expressing a constitutively active form of RAGA ($RAGA^{GTP}$), indicating that RAGs may signal glucose sufficiency to mTORC1. In this regard, Zhang *et al* reported that AXIN (axis inhibition protein 1), originally discovered as an inhibitor of WNT signaling (Zeng *et al*, 1997), is required for AMPK activation by its upstream kinase LKB1 (liver kinase B1) at the lysosomal surface (Zhang *et al*, 2013). A subsequent study demonstrated that, upon glucose starvation, AXIN/LKB1 promotes AMPK phosphorylation and activation at the lysosomal surface via v-ATPase-RAGULATOR. Concurrently, AXIN inhibits GEF activity of RAGULATOR toward RAGA/B, thereby inactivating mTORC1 (Zhang *et al*, 2014) (Fig 3). These observations provide an explanation for how glucose availability is transduced to RAGs and suggest that the lysosomal surface may represent a key platform where nutrients are sensed in a reciprocal manner by mTORC1 and AMPK. A better understanding of the interplay between TORC1 and AMPK in coordinating nutrient-sensing pathways in yeast and mammals will provide new insights into the regulation of cellular metabolism.

Concluding remarks and future directions

Although it has long been known that TORC1 promotes cell growth in response to nutrients (Barbet *et al*, 1996) and that amino acids activate mTORC1 (Hara *et al*, 1998), the identity of amino acid sensors upstream of TORC1 has started to emerge only recently. Amino acid sufficiency regulates TORC1 via different RAG/Gtr-dependent and RAG/Gtr-independent mechanisms. The RAGs, as well as their upstream regulators, are largely conserved from yeast to mammals. Intriguingly, the recently identified mammalian cytosolic leucine and arginine sensors seem to lack yeast counterparts although they impinge on GATOR2 that does have a counterpart in yeast. The reason for this is unclear and follow-up studies are required to elucidate if and how amino acids regulate the yeast GATOR2 ortholog SEACAT.

Finally, mutations affecting mammalian amino acid sensing components are linked to immunodeficiency, epilepsy, and cancer (Shimobayashi & Hall, 2015), and mTOR is often deregulated in metabolic disorders such as obesity, diabetes, and cancer (Efeyan *et al*, 2012; Liko & Hall, 2015). A better understanding of how nutrient availability is transduced to TOR may allow novel therapies against mTOR-related diseases.

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Conflict of interest
The authors declare that they have no conflict of interest.

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