Review

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Regulation of mTORC1 by amino acids

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The mechanistic target of rapamycin complex I (mTORC1) is a central regulator of cellular and organismal growth, and hyperactivation of this pathway is implicated in the pathogenesis of many human diseases including cancer and diabetes. mTORC1 promotes growth in response to the availability of nutrients, such as amino acids, which drive mTORC1 to the lysosomal surface, its site of activation. How amino acid levels are communicated to mTORC1 is only recently coming to light by the discovery of a lysosome-based signaling system composed of Rags (Ras-related GTPases) and Ragulator v-ATPase, GATOR (GAP activity towards Rags), and folliculin (FLCN) complexes. Increased understanding of this pathway will not only provide insight into growth control but also into the human pathologies triggered by its deregulation.

Overview of mTORC1 signaling

Growth is a fundamental biological process that is highly influenced by an organism's environment. For multicellular eukaryotes, including mammals, nutrient availability within the local environment is a major determinant of growth and is sensed through central signaling pathways that engage anabolic programs necessary to increase cell and body size. By coupling nutrient-sensing to long-range growth factor and hormonal signaling networks, animals are able to readily adjust their growth and development programs to an ever-changing environment. One central nutrient-sensing pathway is the mechanistic target of rapamycin mTOR pathway which has emerged over the past 20 years as a master regulator of cellular, organ, and organismal growth [1].

mTOR is an atypical serine/threonine kinase [2,3] that nucleates two distinct multiprotein complexes commonly known as mTORC1 and mTORC2. Although mTORC2 promotes cell proliferation and survival [1], it is mTORC1 that that is generally associated with cell growth [1]. mTORC1 is a 1 MDa [4] homodimer composed of the scaffolding subunit raptor (regulatory associated protein of mTOR) [5,6]; two endogenous kinase inhibitors referred to as DEPTOR (DEP domain containing MTOR-interacting protein) [7] and PRAS40 (proline-rich Akt substrate of 40 kDa, also known as AKT1S1) [8]; and mLST8 (MTOR associated protein, LST8 homolog) [9] whose function remains cryptic. To stimulate cell growth, mTORC1 relies on its downstream effectors to promote coordinately anabolic programs such as mRNA translation [10] and repress catabolic programs such as autophagy [11], thereby avoiding a futile cycle of uncoordinated synthesis and degradation.

mTORC1 is regulated by the small GTPase Rheb (Ras homolog enriched in brain) [12–14] which resides at the lysosomal surface [15] where it functions as a potent stimulator of the mTORC1 kinase activity [8]. Rheb in turn is negatively controlled by the trimeric tuberous sclerosis complex (TSC) whose TSC2 component harbors GTPase-activating protein (GAP) activity towards Rheb [14,16] – converting it from the active GTP-bound state to the inactive GDP-bound state. The TSC complex, whose loss underlies a hamartomatous syndrome of the same name [17,18], serves as a central hub for numerous extracellular and intracellular inputs including mitogen and growth factor signaling [19–23], energy levels [24], oxygen availability [25,26], and genotoxic stress [27], which collectively exert their effects on the mTORC1 pathway by modulating the activity of the TSC complex.

In addition to these inputs, it has long been appreciated that amino acid levels are also crucial for mTORC1 activation and represent one of the most conserved growth signals to this pathway. Despite progress in deciphering the TSC complex–Rheb axis, we have only begun to scratch the surface in uncovering how amino acids regulate mTORC1. We focus here on the rapidly evolving field of amino acid sensing and review how deregulation of this pathway contributes to human disease.

Amino acid signaling and mTORC1 localization

Early investigations revealed that amino acids are necessary to stimulate protein synthesis in rat skeletal muscles [28], a process now known to be under the control of mTORC1. Subsequent studies in cultured mammalian cells confirmed that a mixture of all 20 amino acids activated mTORC1 and that the combination of amino acid

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and growth factor signaling was necessary for the phosphorylation of canonical mTORC1 substrates [29,30]. Whether all amino acids, one particular amino acid, or an amino acid byproduct is being sensed remains unknown. Leucine and arginine are crucial for mTORC1 activation but are insufficient for its activation in cells deprived of the remaining 18 amino acids [29]. Dissecting the amino acid signal is further complicated by the fact that some plasma-membrane amino acid transporters require additional amino acids to activate their cotransport mechanism [31], blurring the line between cellular transport and sensing.

Although it was clear for over a decade that amino acids were vital for mTORC1 activation, precisely how this signal functioned remained a mystery [32,33]. Careful cell biological analysis of this question revealed that amino acids regulate the intracellular localization of mTORC1 [34,35]. When cells are deprived of amino acids, mTORC1 is diffuse throughout the cytoplasm. However, upon addition of amino acids, mTORC1 rapidly translocates to the lysosomal surface where it is presumed to interact with the small GTPase Rheb [34]. The localization of mTORC1 to the lysosome is mediated by the raptor component of mTORC1 (see below). Attachment of a lysosomal targeting sequence to raptor constitutively places mTORC1 on this surface [35], eliminating the need for the amino acid input to activate the pathway. Thus, it appears that the main purpose of the amino acid signal is to colocalize mTORC1 with its activator, Rheb [32,36].

In budding yeast, TORC1 is localized to the vacuole, the equivalent of the mammalian lysosome [37]. Although TORC1 kinase activity is responsive to amino acids in this system, it does not appear to shuttle in response to them [37]. How amino acids actually activate TORC1 in yeast remains an open question that will certainly be addressed in the years to come.

The lysosome: key site of amino acid sensing

Extracellular amino acids must cross the plasma membrane to reactivate mTORC1 after their depletion from cell culture media [31]. Nevertheless, treating cells with cycloheximide, a protein synthesis inhibitor, preserves sufficient intracellular pools of amino acids to rescue mTORC1 signaling even in the absence of extracellular amino acids. This finding argues that the sensing mechanism must occur within the cell and not at its periphery [34]. The use of a cell-free reconstitution assay suggested that the amino acid signal initiates from within the lysosomal lumen [38]. Depleting lysosomal amino acid stores by disrupting the lysosomal membrane with detergents or ionophores inhibits amino acid-dependent recruitment of mTORC1 to purified lysosomes. Amino acids accumulate in the lysosome after their extracellular addition [38], further supporting luminal sensing in cells. Furthermore, overexpression of PAT1 (proton-assisted amino acid transporter), a lysosomal amino acid exporter, drains the lysosomal lumen of amino acids [39] and turns off mTORC1 signaling even in the presence of amino acids. Intuitively, it makes sense for mTORC1 signaling to occur at the lysosome because this organelle is the endpoint of many catabolic pathways, including autophagy, thus offering mTORC1 a window into the metabolic state of the cell.

The Rag GTPases mediate the amino acid signal to mTORC1

For a long time it was believed that the amino acid signal impinged on the TSC complex–Rheb axis; however, the development of $TSC2^{-/-}$ mice suggested otherwise. mTORC1 signaling remained sensitive to a change in amino acid levels in mouse embryonic fibroblasts (MEFs) obtained from these animals [40,41], implicating an alternative route for sensing. This alternative pathway, identified by biochemical and genetic screens [34,42], centers around the Rag GTPases which lay the molecular foundation for amino acid signaling to mTORC1.

Loss of function studies in mammalian, fly, and yeast cells indicate the requirement for Rag GTPases in communicating amino acid availability to mTORC1 [34,37,42]. Rag GTPases lie downstream of amino acids, and in their absence mTORC1 cannot translocate to the lysosome. The Rag subfamily is unique among all small GTPase subfamilies because they function as obligate heterodimers [34,42–45]. Mammalian systems contain four members of the Rag subfamily: RagA and RagB (RagA/B) are functionally redundant and bind to the highly similar RagC and RagD (RagC/D) [43–45], suggesting the existence of four possible independent heterodimeric pairs. In yeast, only two Rag orthologs exist: GTR1 (GTP binding related 1) is the equivalent of RagA/B [43] and binds to GTR2, the ortholog of RagC/D [44,46]. Interestingly, the Rags also localize to the lysosomal surface where they recruit raptor in an amino acid-dependent manner [35], substantiating their role as a docking site for mTORC1 at this compartment [34]. Linking amino acids to mTORC1 recruitment is dependent on the nucleotide-bound state of the Rags; RagA/B binds GDP during amino acid starvation and is quickly exchanged for GTP after restimulation [34]. The importance of GTP-bound RagA/B was made clear in cells or animals expressing a GTP-locked RagA/B mutant, where mTORC1 was found constitutively localized to the lysosome regardless of amino acid levels [34,36,42].

Unlike other small GTPases, Rags do not contain lipid modifications commonly used for intracellular protein targeting. They rely instead on a pentameric complex referred to as Ragulator to function as its lysosomal tether [35,47]. Ragulator was identified as a Rag-interacting complex and its basic architecture consists of the central Lamtor1 (late endosomal/lysosomal adaptor, MAPK and MTOR activator 1) component that functions as a scaffold for two obligate heterodimers composed of Lamtor2–Lamtor3 and Lamtor4–Lamtor5. Myristoylation and palmitoylation on the N terminus of Lamtor1 [48] promote the localization of Ragulator and Rag GTPases to lipid rafts on lysosomal surfaces. In cells lacking or depleted of Ragulator components, Rag GTPases no longer attach to lysosomes, preventing mTORC1 shuttling to this surface and resulting in pathway inactivation [35,47]. The functional ortholog of Ragulator in yeast is likely the heterodimeric EGO1–3 complex that sits at the vacuolar surface, analogously localizing GTRs and TORC1 to this membrane [37,49,50]. Although EGO1–3 and Ragulator members

Box 1. Structural studies of amino acid sensing machinery

Detailed structural studies of amino acid sensing components have provided a wealth of mechanistic insights. Perhaps the most surprising result has been the prevalence of the Roadblock domain in this pathway, found in four of five Ragulator proteins and all four Rag GTPases [47,74–76]. In its most basic form, the Roadblock domain adopts a profilin-like fold after homo- or heterodimerization of two Roadblock-containing proteins. Although the function of this domain is still poorly understood, it is often associated with regulation of GTPases [77], as made evident by its presence on Ragulator and the bacterial GAP MglB [78].

The crystal structure of the yeast GTRs has also offered clues into a potentially new area of study, intra-Rag regulation. The GTRs are stitched together by their C-terminal domains containing the aforementioned Roadblock domain, with the N terminus occupied by rather dynamic nucleotide-binding domains [74,79]. When both GTRs are bound to GTP the G domains face away from each other; however, when GTR2 becomes GDP-loaded a dramatic rearrangement occurs, with the G domain of GTR2 swinging 28° to face the G domain of GTR1 [79]. The significance of this structural rearrangement remains to be determined but, given that heterodimeric GTPases such as the SRP–SRP receptor complex are known to control the nucleotide state of each other [80], this large movement raises the possibility that the Rag GTPases also partake in this form of self-regulation.

share no primary sequence identity, EGO3 adopts a nearly identical fold as Lamtor2/3 and Lamtor4/5 [51,52] (Box 1).

Regulation of the Rag GTPases

The Rags are critical for proper amino acid sensing because their tight coordination with amino acid levels prevents deregulation of mTORC1 signaling. This coordination depends on Rag GTPase activators and inhibitors which modulate their nucleotide-bound state. The recent identification of some of these regulators highlights a complex signaling network upstream of the Rag GTPases (Figure 1).

Ragulator is a guanine nucleotide exchange factor (GEF) for RagA and RagB

Dominant active mutations in the RagA/B proteins have led to the conclusion that a crucial step in the amino acid sensing pathway is their conversion from the inactive GDP-bound state to the active GTP-bound state. In cells, GDP dissociation and GTP binding is mediated by GEFs [53]. *In vitro* experiments with the Rag GTPases suggested that their rates of GDP dissociation were not physiologically relevant, indicating the need for a GEF. Early experiments with Ragulator hinted at the auxiliary roles of this complex: Ragulator preferentially bound to Rags in their inactive state, an interaction that was driven by the nucleotide state of RagA/B [47]. Clarifying the molecular nature of this observation, *in vitro* and *in vivo* data demonstrated a strong preference for Ragulator binding to Rags devoid of nucleotide, a characteristic of GEF–GTPase interactions. Using a system that allowed preferential loading of one Rag GTPase with guanine nucleotide in the context of the Rag heterodimer, it was revealed that Ragulator indeed functions as a GEF for RagA/B; however, it did not display any activity towards RagC or an unrelated GTPase [47]. Moreover, the GEF activity of Ragulator appears to be shared across multiple surfaces of the pentameric complex, evoking comparisons to the TRAPP1 (transport protein particle 1) complex, which also requires multiple subunits for its GEF activity towards YPT1 (yeast protein transport 1) [54,55].

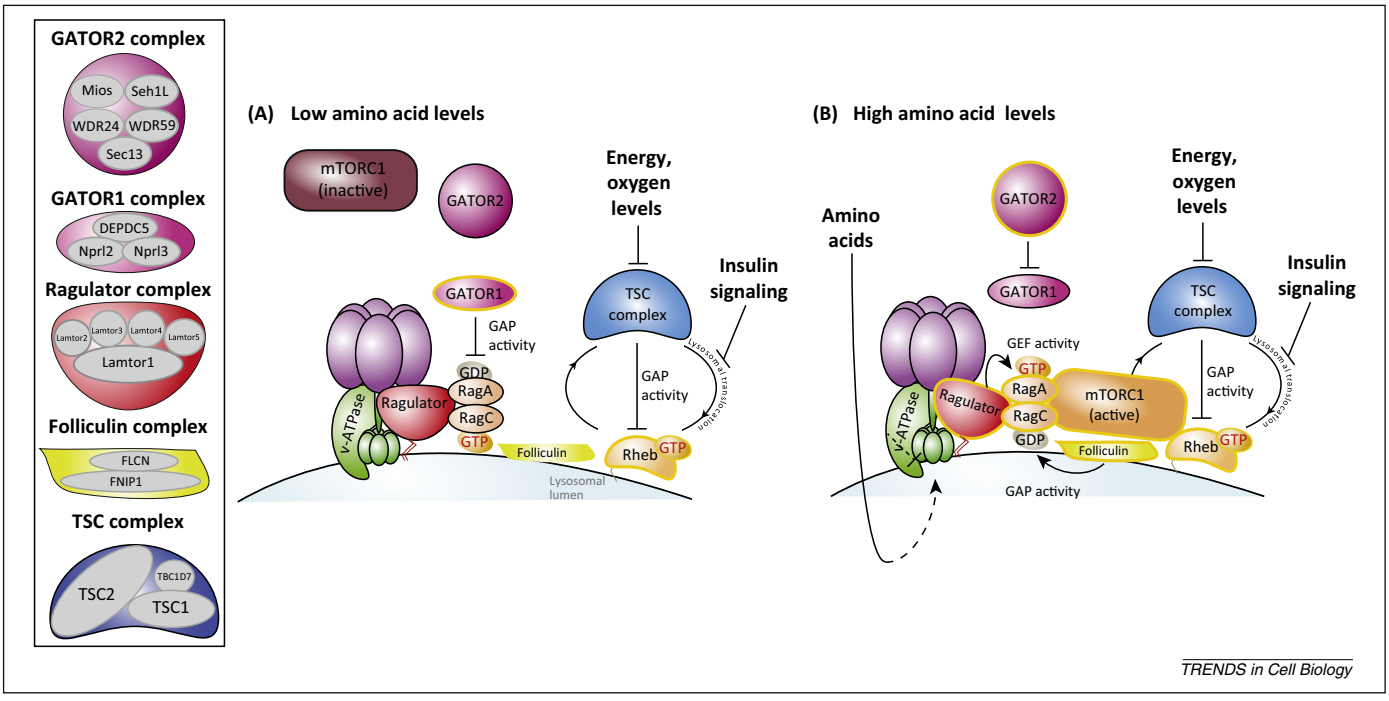

Figure 1. The mechanistic target of rapamycin complex I (mTORC1) amino acid sensing pathway. **(A)** Under low amino acid conditions Ragulator is found in an inhibitory state with the v-ATPase, and GATOR1 [GAP activity towards Rags (Ras-related GTPases)] exerts its GTPase-activating protein (GAP) activity towards RagA, keeping this GTPase in the inactive GDP-bound state that is not sufficient to recruit mTORC1. Insulin signaling inhibits tuberous sclerosis complex (TSC) translocation to the lysosomal surface where it functions as a GAP for Rheb (Ras homolog enriched in brain), inactivating this G protein. **(B)** Upon amino acid stimulation, GATOR1 may be inhibited by GATOR2, and Ragulator and v-ATPase undergo a conformational change, unleashing the guanine nucleotide exchange factor (GEF) activity of Ragulator towards RagA, while the folliculin complex promotes RagC GTP hydrolysis. The now active heterodimer, consisting of GTP-bound RagA and GDP-loaded RagC, recruits mTORC1 to the lysosomal surface where it interacts with and is activated by Rheb.

The v-ATPase controls Ragulator

Because the prominence of the lysosome for mTORC1 signaling is established, a limited RNA interference (RNAi) screen in *Drosophila* cells was undertaken [38] to determine if additional lysosomal proteins partake in amino acid sensing. This screen led to the discovery that reducing the levels of lysosomal v-ATPase components severely inhibits dTORC1 signaling. Complementing the RNAi results, the use of v-ATPase-specific chemical inhibitors in mammalian cells verified the importance of this complex in mediating the amino acid signal to mTORC1. The v-ATPase is composed of two multiprotein complexes termed V1 and V0, and is best appreciated for its role in lysosomal lumen acidification [56]. Although this acidification appears to be dispensable for mTORC1 signaling, the v-ATPase engages in extensive amino acid-dependent interactions with Ragulator [38]. Interestingly, the interactions between the two complexes during times of starvation are mimicked by pharmacological inhibition of the v-ATPase, offering a model in which the GEF activity of Ragulator is blocked during amino acid starvation but is fully reactivated after amino acids induce a conformational change between the v-ATPase and Ragulator [38,47]. This model raises the question of whether the v-ATPase is a direct amino acid sensor – an answer that will be forthcoming through the application of advanced biophysical and *in vitro* reconstitution assays.

FLCN: a tumor suppressor complex that regulates RagC and RagD

Although the key importance of RagA/B in controlling mTORC1 is established, the functional significance of RagC/D to this pathway has remained largely unanswered. Recently, studies employing new *in vitro* and *in vivo* Rag–raptor binding assays indicated that the nucleotide state of RagC but not RagA governs the raptor–Rag GTPase interaction. Specifically, when RagC is bound to GDP, the Rag heterodimer strongly interacts with raptor, whereas GTP loading of RagC abolishes this interaction [57]. These results raise the question of how GTP binding to RagA/B activates the heterodimer and in turn mTORC1 (Box 1).

Although the function of RagC has greatly expanded, the positive and negative regulators of RagC are only beginning to emerge. One such regulator is the tumor suppressor FLCN that functions as a RagC/D GAP. FLCN is not a new member of the mTORC1 pathway; truncating mutations in the protein are known to underlie a hamartoma-like syndrome referred to as Birt–Hogg–Dubé (BHD), which is characterized by aberrant mTORC1 activity [58,59]. Paradoxically, acute loss of FLCN, in human and fly cells, inactivates this pathway [57,60], suggesting that FLCN could function as either a GEF for RagA/B or a GAP for RagC/D. *In vitro* studies established that FLCN together with its binding partner FNIP1 (folliculin interacting protein 1) function as a GAP for RagC and RagD, but not as a GEF for RagA/B, thus providing another avenue of control over mTORC1 translocation [57]. In light of these new studies, the mechanism by which the loss of FLCN in BHD triggers mTORC1 pathway activation along with its control by amino acids must be revisited.

Other positive regulators: VAM6 (vacuolar morphology 6) and LRS (leucyl tRNA-synthetase)

In addition to the role of Ragulator and FLCN in regulating mTORC1 activity via RagA/B and RagC/D respectively, other novel regulators of TORC1 activity in yeast have been identified including Vam6 [37]. Although VAM6 has traditionally been recognized as part of the HOPS (homotypic fusion and vacuole protein sorting) endosome/lysosome maturation pathway, and was previously thought to be a GEF for the GTPase Ypt7 [61,62], new evidence suggests that it also has GEF activity towards GTR1 [37]. The GEF activity of VAM6 might only be conserved in lower eukaryotes because its mammalian ortholog VPS39 (vacuolar protein sorting 39 homolog) is neither a RagA GEF nor an interacting protein [47]. However, it is important to consider that deletion of VAM6 severely disrupts endosomal trafficking [37], a process known to be crucial for proper mTORC1 signaling [63], implying that TORC1 may be indirectly regulated by VAM6. Resolving the differences in how Rag GTPases become activated in these two systems will be critical for our understanding of this pathway.

Recently, two independent studies revealed another positive regulator of mTORC1 activity, the tRNA charging enzyme LRS, which was found to mediate the leucine signal to mTORC1 [64,65]. In yeast, LRS was identified as a GTR1-interacting protein that positively regulates TORC1 by blocking its inactivation by an unknown negative regulator upon LRS binding to leucine [65]. Meanwhile, in mammalian cells, LRS may bind to RagD and function as a GAP for this GTPase in a leucine-dependent manner [65]. Although this study identifies an important region in LRS analogous to GAP domains found in Arf GAPs, and eschews the widely held belief that GAP domains are highly divergent among different GTPase families, the GAP activity of LRS towards RagD has not been reproduced in a subsequent study [57]. The alternative preferences of LRS for RagA/B or RagD in yeast and mammalian cells, respectively, is a point of contention that must also be reconciled with the function of other positive regulators of Rags.

The GATOR complex is a GAP for RagA and RagB

Although our understanding of how amino acid stimulation activates the Rags has evolved, the identity of negative regulators of these GTPases has eluded the field. Recently, an octomeric complex that interacts with the Rag GTPases and GTRs, termed GATOR [66] in humans and SEA (Seh1-associated) in yeast [67], has been identified. GATOR is composed of two distinct interacting subcomplexes known as GATOR1 and GATOR2. Although the GATOR orthologs in yeast are identifiable, they differ in their hierarchical organization because they exist in stoichiometric ratios in SEA, forming one complex as opposed to two [68]. Consistent with the localization of Rags to the lysosome, components from both GATOR subcomplexes have been found there via immunofluorescence and organellar mass-spectrometry studies [66,67,69]. However, only GATOR1 was found to interact directly with the Rags. Loss of function studies in both species revealed a surprising bifunctional role for this complex: GATOR1 negatively regulates mTORC1, conferring complete insensitivity to amino acid

Box 2. Deregulation of amino acid signaling in human pathologies

Given the ubiquity of cellular processes under the control of mTORC1, it is not surprising that deregulation of this pathway underlies many human pathologies including immunodeficiencies and various cancer types. Although diseases stemming from mutation of the TSC complex–Rheb axis are well appreciated, emerging evidence suggests mutations in components of the amino acid branch may also underlie several human diseases.

A previously unknown primary immune disorder has been linked to a reduction in the protein levels of the Ragulator component, Lamtor2 (p14). Although complete absence of Lamtor2 results in embryonic lethality in mice [81], its reduction in humans leads to a decrease in the function of neutrophils, B cells, cytotoxic T cells, and melanocytes [82]. Consistent with a positive regulation of organismal size by mTORC1, affected individuals also display significant growth defects – with growth profiles below the first percentile compared to healthy age-matched peers [82]. Moreover, in cells isolated from patients, mTORC1 activity was drastically reduced [35], making this disorder the first human disease associated with a reduction in a positive component of mTORC1.

Growing evidence suggests metabolic pathways play a large role in regulating tumor growth. The identification of GATOR1 as a novel negative regulator of mTORC1 suggested that tumor suppressors might exist in the amino acid sensing pathway. Indeed, approximately 3% of glioblastoma and 2% of ovarian cancers analyzed contain inactivating mutations in two GATOR1 components (DEPDC5 and NPRL2), and analysis of NPRL3 still remains to be completed [66]. Future large-scale sequencing endeavors are likely to uncover even more cancers with mutations in GATOR1 genes and cancers that overexpress GATOR2 components, the negative regulator of GATOR1. Intriguingly, GATOR1-null cells with hyperactive mTORC1 signaling are highly sensitive to treatment with the mTORC1 inhibitor rapamycin [66], suggesting the use of GATOR1 mutations as biomarkers to identify tumors in patients that might be sensitive to mTORC1 inhibitors.

In the past several years deregulation of the mTORC1 pathway has been appreciated to be an important contributor to epilepsy [83], a notion underscored by the fact that a majority of TSC patients suffer from at least one epileptic seizure during their lifetimes [84]. Connecting amino acid sensing to epilepsy, two independent studies reported that mutations in the GATOR1 component DEPDC5 are responsible for many cases of familial focal epilepsy with variable foci, an autosomal dominant form of epilepsy [85,86]. These new studies, coupled with previous research on TSC patients, suggest that mTORC1 inhibitors may be beneficial for treating this disease.

starvation when deleted in cancer cell lines, whereas GATOR2 functions as a positive regulator. This bipartite regulation was explained through epistasis analysis that places GATOR1 downstream of GATOR2, emphasizing that the positive function of GATOR2 stems from its inhibition of GATOR1. Confirming the strong genetic evidence for its negative role in this pathway, GATOR1 was discovered to have GAP activity towards RagA/B similar to its yeast counterpart towards GTR1, converting these G proteins to an inactive state incapable of supporting mTORC1 signaling [66,67]. The exact GATOR1 subunit that confers GAP activity remains to be determined. Mutations in GATOR1 components occur in human tumors [66,70] (Box 2), suggesting that the ability to maintain mTORC1 activity in tumor microenvironments, where reduced nutrient concentrations would otherwise not support this type of signaling, may confer a selective advantage to cancer cells that have lost these negative regulators [71].

SH3BP4 is a negative modulator of RagB

In addition to GATOR1, SH3BP4 [SH3 (Src homology 3 domain)-binding protein 4] was found to interact with the Rags and reduce mTORC1 signaling by increasing both RagB GTP hydrolysis and preventing RagB GDP dissociation; in short, this protein ensures that RagB is kept inactive [72]. In contrast to all previously identified regulators, SH3BP4 is not conserved in lower eukaryotes, and its effect on mTORC1 signaling is more similar to that of a modulator. Therefore, it will be interesting to understand how SH3BP4 fits into the existing amino acid signaling pathway.

Spatial regulation of the TSC complex

A new rigorous study shows that that, like mTORC1, the TSC complex translocates to and from the lysosomal surface in response to insulin signaling but not to amino acid levels [73]. Akt-dependent phosphorylation of TSC2, presumed by many to inhibit TSC complex GAP activity, is responsible for driving the TSC complex off the lysosomal surface, allowing mTORC1 activation by removing TSC from Rheb, the target of its GAP activity [73]. Given the number of signals upstream of mTORC1 that converge on TSC complex phosphorylation, it is likely that other pathways, such as the AMP-activated protein kinase (AMPK) pathway, also affect TSC shuttling to and from the lysosome [73].

Concluding remarks

This is an exciting time to study how amino acids are sensed by mTORC1. With the discovery of so many new pathway components there remain many more questions than answers. Clearly, understanding the interplay between positive and negative regulators, and the existence of additional human pathologies associated with these factors are of high interest (Box 3). With the use of a combination of bioinformatic and systems-biology approaches together with more traditional discovery platforms, the identity of the long-sought amino acid sensor finally seems within reach.

Box 3. Outstanding questions

The complexity of how amino acids are sensed by mTORC1 raises more questions than answers. Below we list these outstanding questions that will be increasingly important to address in the years to come.

- What is the identity of the amino acid sensor? Although there is evidence suggesting that the v-ATPase may be an amino acid sensor, it remains to be determined whether this is the sole sensing mechanism or if additional sensors exist that modulate the activity of GATOR1 and GATOR2.
- How does GATOR2 regulate GATOR1? Studies in both yeast and mammalian cells have established a clear genetic and biochemical interaction between the two complexes, but at the molecular level it remains unclear how GATOR2 inactivates GATOR1, presumably doing so under conditions of amino acid sufficiency.
- Is there crosstalk between different Rag regulators? In GATOR1-null cell lines, mTORC1 is hyperactive and non-responsive to amino acid regulation. Interestingly, pharmacological inhibition of the v-ATPase does not reduce mTORC1 activity in these cell lines, formally suggesting that the v-ATPase/Ragulator arm functions either upstream or in parallel to GATOR1 [66]. How these multicomponent signaling complexes actually communicate with each other represents a ripe area for future study.

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