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The dawn of the age of amino acid sensors for the mTORC1 pathway

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Summary

The mechanistic target of rapamycin complex 1 (mTORC1) is a master regulator of cell growth that responds to a diverse set of environmental inputs, including amino acids. Over the past ten years, a number of proteins have been identified that help transmit amino acid availability to mTORC1. However, amino acid sensors for this pathway have only recently been discovered. Here, we review these recent advances and highlight the variety of unexplored questions that emerge from the identification of these sensors.

Introduction

The mechanistic target of rapamycin complex 1 (mTORC1) is a protein kinase that acts as a central controller of cell growth. mTORC1 was originally identified as the target of rapamycin, a macrolide antibiotic discovered in the soil on Easter Island (Abraham and Wiederrecht, 1996). Rapamycin is a potent antiproliferative agent (Eng et al., 1984), due to its inhibition of mTORC1, and is currently used in the clinic in a number of contexts, including to block organ transplant rejection and as a cancer therapy (Chan, 2004; Groth et al., 1999).

mTORC1 acts as a regulator of cell growth through the phosphorylation of substrates that potentiate anabolic processes and inhibit catabolic ones, such as mRNA translation and autophagy, respectively. A variety of environmental signals impinge on mTORC1 to control

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its activity, including growth factors, cellular stresses, and energy and amino acid levels (Saxton and Sabatini, 2017). The Rheb and Rag GTPases reside on the lysosomal surface and coordinate mTORC1 activity in response to environmental conditions (Buerger et al., 2006; Kim et al., 2008; Saito et al., 2005; Sancak et al., 2008). Many of these inputs, including growth factors, cellular stresses, and energy levels impinge on the TSC complex, comprised of TSC1, TSC2, and TBC1D7, which is a GTPase activating protein (GAP) for Rheb (Dibble et al., 2012; Inoki et al., 2003a; Manning et al., 2002; Potter et al., 2002). Growth factors regulate the TSC complex by controlling its localization to the lysosomal surface (Menon et al., 2014). Multiple other regulators, such as hypoxia and energy stress, impact the mTORC1 signaling pathway upstream of the TSC complex (Brugarolas et al., 2004; Inoki et al., 2003b), although whether this is by affecting its localization is unclear. Thus, in the presence of growth factors and the absence of stresses Rheb is GTP loaded and, in this state, can act as a kinase activator of mTORC1, but only if mTORC1 is on the lysosomal surface.

The Rag GTPases control mTORC1 localization to the lysosomal surface in response to nutrient levels, specifically amino acids and glucose (Efeyan et al., 2013; Sancak et al., 2008). The Rags consist of a constitutive heterodimer of RagA or B bound to RagC or D (Hirose et al., 1998; Schürmann et al., 1995; Sekiguchi et al., 2001). In the presence of nutrients, RagA/B is GTP-loaded and RagC/D is GDP-loaded. This active conformation of the Rags promotes the translocation of mTORC1 to the lysosomal surface where it can come into contact with its kinase activator, Rheb (Buerger et al., 2006; Saito et al., 2005; Sancak et al., 2008). In this way, the Rag and Rheb GTPases comprise a “coincidence detector,” such that mTORC1 is active only in the presence of both growth factors and nutrients.

Since the discovery of the Rags as components of the mTORC1 pathway, a number of other proteins have been identified as playing a role in nutrient sensing upstream of mTORC1. Ragulator, a pentameric complex composed of p18, p14, HBXIP, C7orf59, and MP1, controls the lysosomal localization and nucleotide loading state of the Rag GTPases (Bar-Peled et al., 2012; Sancak et al., 2010). Ragulator interacts with the vacuolar H^+-adenosine triphosphatase ATPase (v-ATPase), which acts as a positive regulator of the pathway through an unknown mechanism (Zoncu et al., 2011). Folliculin and its binding partners, FNIP1/2, have GAP activity towards RagC/D (Tsun et al., 2013), while GATOR1 (GAP activity towards the Rags 1) is a GAP for RagA/B (Bar-Peled et al., 2013). GATOR1 is a trimeric complex composed on DEPDC5, Nprl2, and Nprl3, which interacts with GATOR2, a pentameric complex of unknown function (Bar-Peled et al., 2013). More recently, the KICSTOR complex (which stands for KPTN, ITFG2, C12orf66, and SZT2-containing regulator of mTORC1) was identified as a scaffold for GATOR1 on the lysosomal surface (Wolfson et al., 2017; Peng et al, 2017) (Figure 1). Until recently most members of the nutrient sensing pathway that had been characterized function to directly modulate the nucleotide loading state of the Rags or localization of other components of the pathway; the amino acid sensors, however, remained elusive.

SLC38A9

The fact that this important growth regulatory pathway centers on the surface of the lysosome led to the prediction that nutrients, specifically amino acids, within the lysosome could activate the pathway (Zoncu et al., 2011). The vacuole, an acidic organelle that is the yeast equivalent of the lysosome, is considered a storage site for metabolites, including basic and neutral amino acids (Li and Kane, 2009). Mammalian lysosomes, while distinct from vacuoles, have amino acid transporters on their surface and are involved in protein degradation, particularly as part of the process of autophagy, and thus may also function as a storage site for amino acids. Indeed, isolated lysosomes can be loaded with amino acids and this is sufficient for recruitment of mTORC1 *in vitro*, indicating that the mTORC1 pathway can respond to intra-lysosomal amino acid levels (Zoncu et al., 2011). Further evidence for this model of intra-lysosomal amino acid sensing came with the identification of SLC38A9, a lysosomal transmembrane protein with homology to amino acid transporters, as a positive regulator of the mTORC1 pathway (Jung et al, 2015; Rebsamen et al., 2015; Wang et al., 2015).

SLC38A9 was identified through immunoprecipitation followed by mass spectrometry (IP/MS) analyses of the Rags and Ragulator. Unlike other proteins in the SLC38-family of transporters, SLC38A9 is unique in that it has a 119 amino acid long extension at its N terminus, through which it binds Ragulator. Surprisingly, overexpression of full-length SLC38A9 or simply this 119 amino acid N terminal extension is sufficient to render cells insensitive to amino acid deprivation, establishing SLC38A9 as a positive regulator of the pathway. Cells lacking SLC38A9 have a defect in mTORC1 activation in response to amino acids, particularly arginine. *In vitro* amino acid transport assays established that SLC38A9 has very low affinity transport activity for arginine (Wang et al., 2015). Its low affinity may reflect that for technical reasons the transport assays measure influx by SLC38A9, and that the affinity for arginine efflux, which has not been measured, could be higher, perhaps better correlating with intracellular arginine levels. Additionally, SLC38A9 may bind amino acids other than arginine with higher affinity, but extensive evaluation of its substrate preference has not yet been performed. Further, reliable methods for detecting amino acid levels within subcellular compartments, such as the lysosome, do not exist, and thus how its affinity correlates with intra-lysosomal levels of amino acids is unknown.

The identification of an arginine-binding transporter as a positive regulator of the mTORC1 pathway established SLC38A9 as a putative arginine sensor– and the first likely amino acid sensor for the pathway. However, for SLC38A9 to be validated as a bona fide amino acid sensor, it will be necessary to connect its arginine binding capacity to arginine sensing by the mTORC1 pathway. While this has not yet been accomplished for SLC38A9, bona fide amino acid sensors for relaying the levels of cytosolic amino acids to mTORC1 have been discovered.

The Sestrins

The first validated amino acid sensor for the pathway came with the discovery of Sestrin1 and Sestrin2 as leucine sensors. Sestrin1 and 2 were originally identified as proteins that are

induced under various stress conditions and named PA26 (p53-regulated protein 26) and Hi95 (hypoxia induced gene 95), respectively (Buckbinder et al., 1994; Budanov et al., 2002; Peeters et al., 2003). At first, the only hint as to their function was their weak homology to a bacterial alkyl-hydroperoxide reductase, AhpD (Budanov et al., 2004). Early studies found that Sestrin2 retained some antioxidant capacity (Budanov et al., 2004), but this has been largely refuted, both based on additional antioxidant studies as well on the location of the putatively key residues for this activity in its crystal structure (Saxton et al., 2016c; Woo et al., 2009; Kim et al, 2015). However, Sestrin2 does retain structural homology to this class of bacterial enzymes (Saxton et al., 2016c; Kim et al, 2015).

The Sestrins are a group of three homologues (Sestrin1–3) that have long been recognized to be negative regulators of the mTORC1 pathway (Budanov and Karin, 2008). Early reports argue that the Sestrins inhibit the mTORC1 pathway upstream of TSC and AMPK (Budanov and Karin, 2008), members of the growth factor sensing branch upstream of mTOR, but recent results are not compatible with this mechanism (Chantranupong et al., 2014; Peng et al., 2014). Instead, it has become clear that the Sestrins negatively regulate mTORC1 upstream of GATOR2, a positive regulator of the nutrient sensing branch of the pathway (Chantranupong et al., 2014; Parmigiani et al., 2014). Under leucine, but not arginine, deprivation conditions, Sestrin2 binds to GATOR2 and likely inhibits it although the molecular function of GATOR2 remains unknown (Wolfson et al., 2016). Leucine stimulation causes Sestrin2 to dissociate from GATOR2, likely relieving its inhibition of this positive regulator (Wolfson et al., 2016). A variety of evidence exists indicating that Sestrin2 is a leucine sensor for the mTORC1 pathway. First, Sestrin2 binds leucine with an affinity of ~20 uM, which correlates with leucine concentrations in the media that are sensed by the pathway (Wolfson et al., 2016). Sestrin2 purified from bacteria binds leucine both in an *in vitro* amino acid binding assay (Wolfson et al., 2016), as well as when crystalized in the presence of leucine, which identified residues important for the leucine binding capacity of the protein (Saxton et al., 2016c). Finally, mutants of Sestrin2 that no longer bind leucine or have reduced leucine binding capacity render cells unable to sense the presence of leucine or alter the concentration of leucine sensed by the mTORC1 pathway, establishing Sestrin2 (and by analogy Sestrin1) as leucine sensors for the mTORC1 pathway (Saxton et al., 2016c; Wolfson et al., 2016).

It has long been appreciated that Sestrin2 levels increase in response to various stresses (Buckbinder et al., 1994), and recent evidence indicates that the ATF4 transcription factor, which is activated by long-term amino acid deprivation, can control Sestrin2 expression (Ye et al., 2015). These data lead to the model that Sestrin2 likely has a bimodal regulation. Sestrin2, leucine, and GATOR2 exist in an equilibrium such that in the absence of any cellular stresses, and the presence of leucine, Sestrin2 binds leucine (and not GATOR2), thus allowing the mTORC1 pathway to be active (Figure 2A). This equilibrium can be pushed by one of two levers – the concentration of leucine or that of Sestrin2. With short-term leucine deprivation, Sestrin2 levels are unchanged, favoring free Sestrin2 binding to GATOR2 and inhibiting the pathway. Under conditions of cellular stress, including long-term amino acid deprivation or DNA damage, Sestrin2 levels increase, pushing both equilibriums forward, such that the amount of Sestrin2 bound to leucine increases (if leucine is available) and the level of Sestrin2 bound to GATOR2 increases, inhibiting the mTORC1 pathway even in the

presence of leucine. In this manner, the mTORC1 pathway is sensitive to both leucine as well as Sestrin2 levels.

For example, at constant leucine and GATOR2 levels, an increase in Sestrin2 expression will inhibit mTORC1 signaling. At lower levels of Sestrin2 this inhibition will be weak, as there is plenty of leucine to bind to Sestrin2 and prevent its interaction with GATOR2 (Figure 2B). However, upon the higher expression of Sestrin2 the cellular levels of leucine will not saturate Sestrin2, given the relatively low affinity of Sestrin2 for leucine, and more Sestrin2 will bind to GATOR2, until the positive regulator becomes saturated and the pathway is fully inhibited, regardless of leucine concentrations (Figure 2B). In a similar manner, one can assess how changing both the levels of Sestrin2 and leucine will modulate mTORC1 activity. For example, in the absence of Sestrin2 (and its homologues Sestrin1 and 3) mTORC1 activity will be completely insensitive to leucine concentrations (Figure 2C). At low Sestrin2 levels, the mTORC1 pathway will be responsive to leucine fluctuations, such that under leucine deprivation the pathway is inhibited and increasing leucine levels increases pathway activity. With higher Sestrin2 expression, more leucine is needed for the same degree of mTORC1 activity, as additional leucine is needed to compete the increased amount of Sestrin2 off of GATOR2. Finally, extremely high levels of Sestrin2 will render the pathway constitutively inhibited, regardless of leucine concentrations (Figure 2C). In this manner, Sestrin2 can act as a leucine sensor at basal conditions, and as an inhibitor of mTORC1 in response to a variety of stresses that increase its expression.

One major unsolved question in the Sestrin field revolves around how the Sestrins inhibit GATOR2. The solution awaits the understanding of the molecular function of GATOR2, but some insight into this problem could come from identifying structural differences between Sestrin2 that is leucine-bound, free, and GATOR2-bound. At this point, however, only the structure of leucine-bound Sestrin2 has been solved (Saxton et al., 2016c). Although claims have been made that apo-Sestrin2 was crystalized (Kim et al., 2015; Lee et al., 2016), further analyses revealed that those crystals also contained leucine (Saxton et al., 2016b), and thus understanding the exact conformation change that Sestrin2 undergoes on binding to leucine or GATOR2, and how this could impact its activity, awaits further investigation. Thermal shift assays revealed that leucine shifts the melting temperature of Sestrin2 by up to 8.5°C (Wolfson et al., 2016), indicating that even though the structure of apo-Sestrin2 remains elusive a conformational change in Sestrin2 most likely occurs on leucine binding.

CASTOR1

Following the discovery of Sestrin1/2 as cytosolic leucine sensors that bind to GATOR2, a protein-protein interaction database generated using IP/MS analyses from 2,594 proteins stably expressed in HEK-293T cells revealed the proteins encoded by the GATSL3 and GATSL2 (GATS protein-like 3/2) genes as putative GATOR2 interactors (Chantranupong et al., 2016). The proteins were renamed CASTOR1/2 (for cellular arginine sensor for mTORC1) after their identification as cytosolic arginine sensors for the pathway, through a mechanism analogous to that of the Sestrins. The CASTOR proteins, specifically either a homodimer of CASTOR1 or heterodimer of CASTOR1 and 2, bind to GATOR2 only under arginine deprivation conditions, thereby likely inhibiting it. Both an *in vitro* binding assay

and crystal structure found that these proteins directly bind arginine, and do so with an affinity of ~30 uM, which correlates with concentrations of arginine in the media that lead to half maximal mTORC1 signaling (Chantranupong et al., 2016; Saxton et al., 2016a). Finally, CASTOR1 mutants that are unable to bind arginine render the mTORC1 pathway insensitive to arginine stimulation when added back to cells.

CASTOR1 contains four tandem ACT (Aspartate kinase, Chorismate mutase, TyrA) domains, which are evolutionarily ancient domains associated with binding a diverse set of small molecules, and shares structural homology to the regulatory domain of bacterial aspartate kinases (Saxton et al., 2016a). Specifically, the arginine-binding pocket has striking structural similarities to the lysine-binding pocket of an aspartate kinase in *Escherichia coli* (AKeco). While aspartate kinases are well conserved throughout bacteria and fungal species, they were lost before the emergence of metazoa, in which CASTOR1 homologues are exclusively expressed (Saxton et al., 2016a). Thus, the mTORC1 pathway may have adapted this ancient lysine-sensitive regulatory mechanism for the evolution of arginine-sensing in metazoa.

Intracellular amino acid levels

The discovery of the elusive leucine and arginine sensors for the mTORC1 pathway represents an exciting advance in the field. How the affinities of these sensors for their respective amino acids correlate with concentrations of those amino acids in cells or *in vivo*, however, is not entirely clear. Sestrin1 and 2, for example, bind leucine with affinities of ~15–20 uM (Wolfson et al., 2016), which is similar to that of another cytosolic leucine-binding protein, leucyl tRNA-synthetase (LRS), which binds leucine with an affinity of 45 uM (Chen et al., 2011) (Table 1). Furthermore, while it is clear that these sensors bind leucine and arginine with affinities that are consistent with those in the media that lead to half maximal mTORC1 pathway activity (Wolfson et al., 2016), it is not known how concentrations of amino acids in the media correlate with intracellular, specifically cytosolic, amino acid concentrations. Despite recent advances in measuring small molecules with mass spectrometry, a few challenges arise when trying to measure intracellular amino acid concentrations.

First, amino acid levels within different subcellular compartments, specifically within various organelles, could easily vary with respect to the cytosol, as organelles are enclosed by membranes and contain a distinct set of amino acid transporters than those that are on the plasma membrane. Recent advances in rapid isolation of mitochondria have allowed for the accurate measurement of metabolites within this compartment (Chen et al., 2016), but these advances need to be expanded to other organelles that may be more difficult to isolate. Indeed, the varied concentrations of amino acids within organelles means that whole cell measurements of amino acid levels may vary drastically from those that are actually available to proteins within certain subcellular spaces. Finally, when estimating concentrations of amino acids in certain compartments, the compartmental volume is necessary. While it is relatively simple to measure the volume of a certain organelle, say the mitochondria, it is difficult to measure the volume of the space that does not contain any

organelles (i.e. the cytosol), further complicating estimations of cytosolic amino acid concentrations.

In addition, concentrations of amino acids may exist in gradients across the intracellular space. For example, as mentioned previously lysosomes may serve as amino acids storage sites, perhaps containing higher concentrations of amino acids than exist in the cytosol. It is not difficult to imagine, then, that at the lysosomal surface, where the mTORC1 pathway machinery resides, amino acid concentrations are locally higher than in other parts of the cytosol. Measuring these gradients of amino acid concentrations across the cell presents a particular challenge given currently available technology.

Finally, inside the cell, amino acids exist in both free and bound pools, including being bound specifically and non-specifically to proteins, lipids, and other molecules. The pools of amino acids that are bound are not available to bind to SLC38A9, Sestrin2, or CASTOR1, but would be measured when intact cells are extracted and analyzed by mass spectrometry-based metabolomics. These pools likely exist in equilibrium with the free pools, and thus estimating what fraction of each amino acid measured is bound or free is a significant challenge to understanding the levels of these small molecules that are available to the sensors.

Amino acid levels *in vivo*

With cells in culture, researchers often completely starve cells of amino acids by removing them from the media. How amino acid levels fluctuate *in vivo*, however, would likely differ considerably from these *in vitro* manipulations, as even under starvation conditions proteolysis would keep amino acid levels from falling to 0. Amino acid concentrations in the plasma have been measured in humans and mice in the fed and fasted states. For leucine in humans, these values range from ~130 uM under the fasted state to ~300 uM under the fed state, while arginine ranges from ~100 uM to ~150 uM (Stegink et al., 1991; Stein and Moore, 1954) (Table 1). While these levels are above the binding affinities of Sestrin1/2 and CASTOR1 (Chantranupong et al., 2016; Wolfson et al., 2016), how the plasma levels of amino acids under these conditions correlate with interstitial amino acid levels in various tissues is unknown. Even once the interstitial concentrations of amino acids are appreciated, all the challenges described previously for estimating free cytosolic amino acid concentrations in cells in culture will persist in cells *in vivo*, further complicating our ability to fully understand how the *in vitro* binding affinities of the sensors correlate with the fluctuations of amino acids under fed and fasted states *in vivo*.

Amino acid sensors for the mTORC1 pathway in model organisms

The discovery of SLC38A9, Sestrin1/2, and CASTOR1 as putative or bona fide amino acid sensors for the mTORC1 pathway was an important advance in the field. While several components of the pathway are conserved in many eukaryotes, amino acid sensors upstream of mTORC1 have not been discovered or characterized in most other species, and even if amino acid sensing per se is conserved throughout eukaryotes is unclear.

One putative amino acid sensor in a non-mammalian organism that has been recently studied is the *D. melanogaster* Sestrin (dSestrin). While mammals have three Sestrin homologues, *D. melanogaster* have only one, which has been previously characterized as a negative regulator of the mTORC1 pathway, as its loss leads to phenotypes in flies that are analogous to those seen with lack of autophagy, an important process downstream of TORC1 (the *D. melanogaster* homologue of mTORC1) (Lee et al., 2010). dSestrin retains leucine binding capacity, but it binds leucine more weakly than Sestrin2 (Wolfson et al., 2016), with an affinity of ~100 uM (unpublished). The relative affinities of dSestrin for leucine over similar amino acids such as methionine and isoleucine are unchanged in comparison to Sestrin2, but because the affinity of dSestrin is lower for leucine, its affinity for methionine and isoleucine are also much lower, approximately 2.5 mM and 10 mM, respectively (unpublished). Although leucine and methionine concentrations have not been studied in fly hemolymph, concentrations of six other amino acids were measured and found to be in the low millimolar range (Piyankarage et al., 2008), while most of these amino acids are in the hundreds of micromolar range in humans and mice (Cantor et al., 2017; Stegink et al., 1991; Stein and Moore, 1954). Additionally, in Schneider's media (a commonly used media for the culture of fly cells) leucine and methionine concentrations are at 1.1 mM and 5.4 mM, respectively (Schneider, 1964). While this concentration of leucine is well above the binding affinity of dSestrin for leucine, this concentration of methionine is similar to the binding affinity of dSestrin for methionine. Methionine restriction is known to lead to lifespan extension via the TORC1 pathway in *D. melanogaster* (Lee et al., 2014), and thus it is tantalizing to hypothesize that dSestrin could act, at least under certain situations, as a methionine sensor in this organism.

Conservation of the amino acid sensors across evolution

Study of the nutrient sensing pathway revealed that some of its components were conserved in many eukaryotes (Figure S1). Components of mTORC1, such as TOR itself and raptor, are conserved throughout all eukaryotes, including plants (Figure 3). Much of the rest of the core machinery of the pathway, such as the Rag GTPases, or components that control the nucleotide loading state of the Rags (like GATOR1), are conserved through fungi (Figures 3–4). Additionally, GATOR2, a complex of unknown function that acts upstream or parallel to GATOR1, has a relatively similar pattern of conservation as GATOR1 (Figure S1). Two components of Ragulator that lack homologues by sequence were recently found to have structural homologues in yeast (Kogan et al., 2010; Zhang et al., 2012). Similarly, structural homologues that were not uncovered by sequence analysis alone allowed for the identification of Lst4-Lst7 as the FLCN-FNIP complex orthologues in *S. cerevisiae* (Peli-Guilli et al, 2015). Certain amino acids, such as glutamine, have a stronger effect than others on Lst4-Lst7 localization to the lysosomal membrane, indicating that this complex may play a role in the sensing of particular amino acids (Peli-Guilli et al, 2015). As of yet, the FLCN-FNIP complex has not been indicated to have any specific amino acid sensing functions.

The discovery of the amino acid sensors, however, has revealed that not all components of the nutrient sensing pathway are as conserved beyond vertebrates (Figure S1). SLC38A9 has a homologue in *C. elegans*, but lacks a detectable one in *D. melanogaster* or in any fungal species (Wang et al., 2015). The Sestrins have a clear homologue in *D. melanogaster* and *C.*

elegans, but are not as well conserved across various parasites or fungal species. Some fungal species, such as *Tuber melanosporum* and *Arthrobotrys oligospora*, do seem to have a Sestrin homologue, but most lack one (Supplementary Figure 1). The CASTOR proteins lack homologues in *D. melanogaster* or *C. elegans*, but follow a similar pattern as the Sestrin proteins in lower organisms (Figures S1 and 3).

These different patterns of conservation across components of the mTORC1 pathway lead to many intriguing questions. Are the sensors less well conserved because other organisms evolved in distinct environments with different nutritional requirements and thus sense other molecules? There are a variety of data suggesting that nitrogen sensing occurs in fungi, but if amino acids are sensed in non-engineered non-auxotrophic fungal organisms remains under debate (Chantranupong et al., 2015). Perhaps the species that lack SLC38A9, Sestrin, and CASTOR homologues have another set of sensors that are responsible for stimulating the mTORC1 pathway in the presence of nutrients other than leucine and arginine. Until more sensors have been discovered in other organisms, it will be hard to address these questions (Figure 4).

While most fungal organisms lack Sestrin and CASTOR homologues, a very few species do appear to have homologues of these sensors (Figure S1). How these were retained (or acquired through horizontal transmission) is unclear, and if they act as amino acid sensors (or even modulators of the mTORC1 pathway) has not been investigated. For example, *Tuber melanosporum*, or the black truffle, appears to have a Sestrin homologue, while *Microbotryum violaceum*, a fungus that infects a specific plant species, may have a homologue of the CASTOR proteins. However, only a few fungal species appear to have any homologues to Sestrin or CASTOR, and these species are the exceptions. Further work will be needed to uncover if more fungal species have homologues to these sensors. It is tantalizing to imagine that the specific environment of an organism would dictate the type of sensors that it may need, and that as more sensors are discovered we can begin to dissect out these correlations.

Finally, unlike other core members of the pathway, the components of KICSTOR are much less well conserved. KPTN, ITFG2, and C12orf66 are not even conserved throughout all metazoans, as *C. elegans* and *D. melanogaster* lack homologues to these proteins (Figure 3). The final component of KICSTOR, SZT2, which is necessary for the other three components to bind GATOR1, has a homologue in *C. elegans* but lacks one in *D. melanogaster* or other lower organisms, such as fungi, with a few rare exceptions (Figure S1). This different pattern of conservation between KICSTOR and other complexes that regulate the pathway but are not amino acid sensors (such as GATOR1/2, Ragulator, and the Rags) suggests that perhaps targeting GATOR1 to the lysosome is not the only function of KICSTOR. Further work will be needed to uncover if these proteins have any direct nutrient sensing properties, or if environmental inputs that are specific to higher eukaryotes feed in to the mTORC1 pathway upstream of KICSTOR.

Perspectives

The mTORC1 pathway has emerged as a critical growth regulatory node that is deregulated in a variety of diseases, including cancer, diabetes, and epilepsy. While it has been well appreciated that amino acids are a key input to the pathway, how these molecules were sensed remained elusive until recently. The discovery of three amino acid sensors upstream of mTORC1 (SLC38A9, Sestrin1/2, and CASTOR1) has explained, at least in part, how leucine and arginine are sensed. However, if other amino acid sensors exist remains undetermined – across subcellular compartments, cell types, tissues, or species. These recent discoveries represent only the first glimpse at how amino acids are directly sensed – the dawn of the age of amino acid sensors for the mTORC1 pathway.

Methods

Evolutionary Analysis

Evolutionary analysis was performed using the PHMMER software (as described in Wolfson et al., 2017). To decrease false positives, common domains of some of the protein sequences were excluded, and thus portions of the sequences were used for the analysis (Figure S2).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- Review recent advances in understanding how amino acids are sensed upstream of mTORC1
- Clarify the role of Sestrin2 as a leucine sensor for the pathway
- Suggest future areas of investigation, including identifying intracellular amino acid levels
- Analyze patterns of conservation of components of the mTORC1 pathway

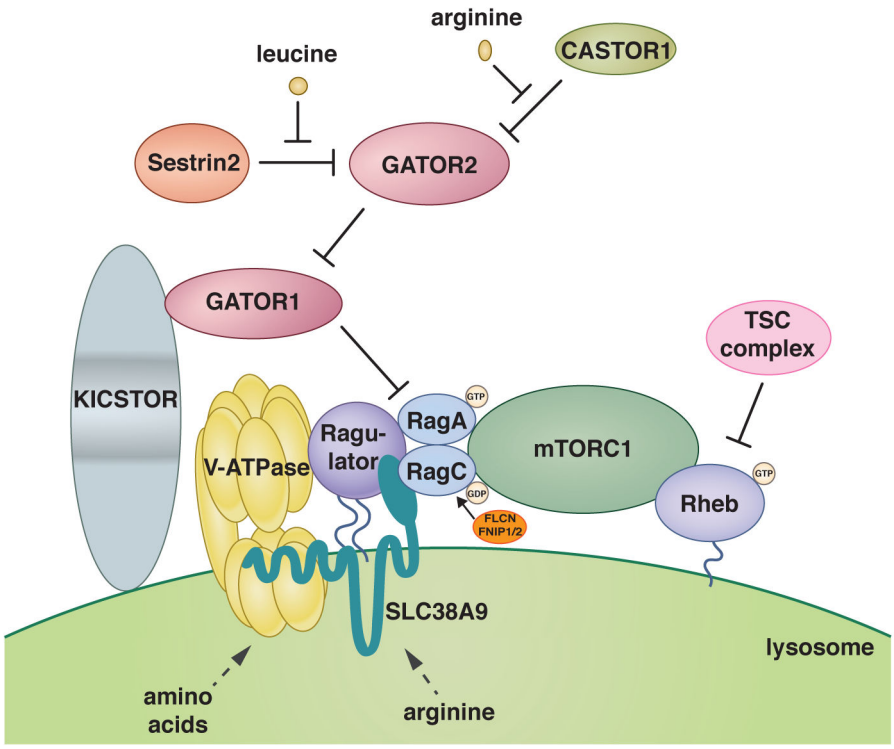

Figure 1.

The amino acid sensing pathway upstream of mTORC1

Schematic detailing the key molecular players in the nutrient sensing branch upstream of mTORC1.

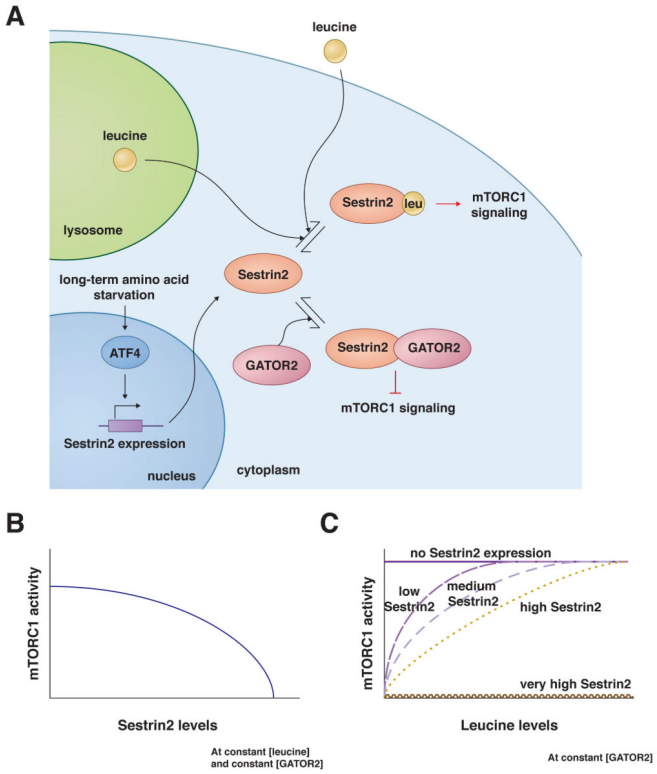

Figure 2.

Bimodal regulation of Sestrin2

A) Sestrin2 is a leucine sensor and inhibitor of mTORC1 signaling in response to stresses. Sestrin2, leucine, and GATOR2 exist in a equilibrium in the cell that dictates mTORC1 activity in response to both leucine levels and other stresses. Increasing leucine levels pushes the Sestrin2-leucine equilibrium forward such that more GATOR2 is free, leading to increased mTORC1 signaling. Increasing levels of Sestrin2, downstream of ATF4 in response to cellular stresses, pushes the Sestrin2-GATOR2 equilibrium forward, inhibiting mTORC1 signaling.

B) Schematic depicting mTORC1 activity in response to increasing Sestrin2 levels, at constant levels of GATOR2 and leucine.

C) Diagram showing how modulating leucine levels will impact mTORC1 activity at varying cellular levels of Sestrin2.

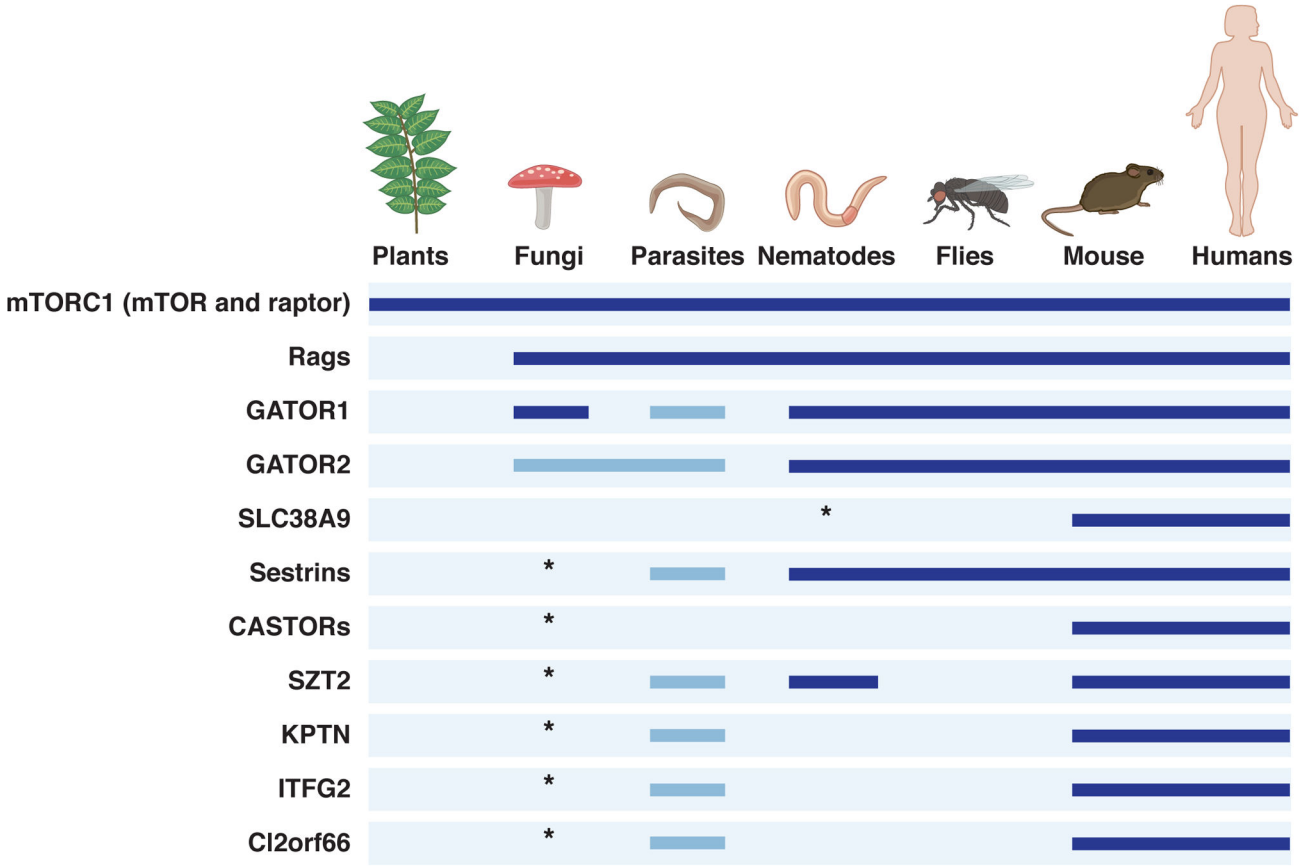

Figure 3.

Conservation of members of the nutrient sensing pathway across evolution Diagram depicting conservation of mTORC1 pathway members across evolution. Dark blue bars indicate that the protein is well conserved across the specified group or species, light blue bars indicate that the protein is conserved in many but not all organisms within the group, asterisks indicate conservation in only a few organisms within the group, and no bars indicate the protein is not conserved in the indicated group or organism.

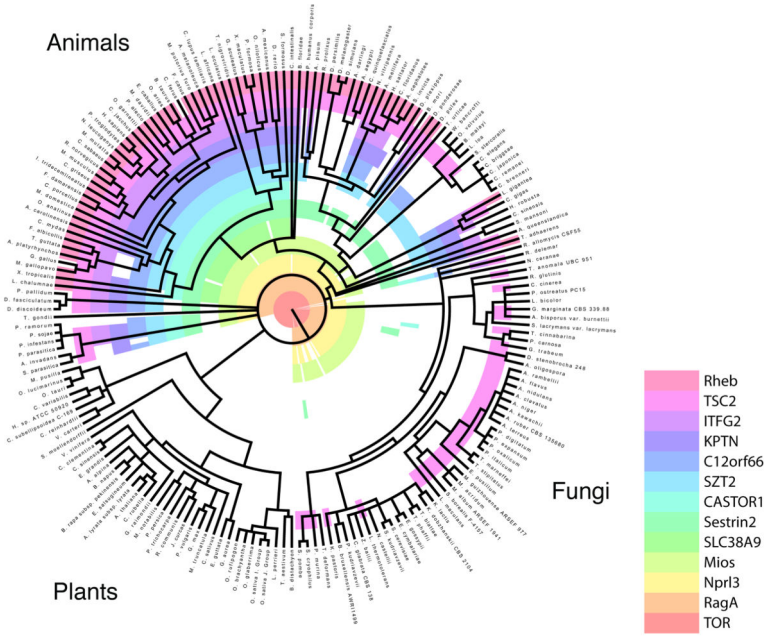

Figure 4.

mTORC1 signaling pathway conservation across evolution

Cladogram showing conservation of members of the mTORC1 signaling in various organisms across evolution. Data are a subset of the organisms shown in Figure S1. Legend on the right side indicates colored bars for each gene. Locations of the three kingdoms (plants, fungi, and animals) are listed.

Table 1

Affinities of nutrient sensors for their amino acid substrates and corresponding plasma concentrations of those amino acids (Data from Stegink et al., 1991; Stein and Moore, 1954; Piyankarage et al., 2008)

AA	AA binding protein	K_d (μM)	Plasma Concentration (μM)		Interstitial Concentration		Free Cytosolic Concentration	
			Fed	Fasted	Fed	Fasted	Fed	Fasted
Leu	Sestrin1	10–15	287	129	?	?	?	?
	Sestrin2	20	287	129	?	?	?	?
	Sestrin3	N/A	287	129	?	?	?	?
	LRS	45	287	129	?	?	?	?
	dSestrin	100	? (mm range for other AAs)		?	?	?	?
Arg	CASTOR1	30	161	97	?	?	?	?
	CASTOR2	N/A	161	97	?	?	?	?