Intracellular Signals for Skeletal Muscle Adaptation

Leucine and mTORC1: a complex relationship

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Dodd KM, Tee AR. Leucine and mTORC1: a complex relationship. Am J Physiol Endocrinol Metab 302: E1329–E1342, 2012. First published February 21, 2012; doi:10.1152/ajpendo.00525.2011.—Amino acid availability is a rate-limiting factor in the regulation of protein synthesis. When amino acid supplies become restricted, mammalian cells employ homeostatic mechanisms to rapidly inhibit processes such as protein synthesis, which demands high levels of amino acids. Muscle cells in particular are subject to high protein turnover rates to maintain amino acid homeostasis. Mammalian target of rapamycin complex 1 (mTORC1) is an evolutionarily conserved multiprotein complex that coordinates a network of signaling cascades and functions as a key mediator of protein translation, gene transcription, and autophagy. Signal transduction through mTORC1, which is centrally involved in muscle growth through enhanced protein translation, is governed by intracellular amino acid supply. The branched-chain amino acid leucine is critical for muscle growth and acts in part through activation of mTORC1. Recent research has revealed that mTORC1 signaling is coordinated primarily at the lysosomal membranes. This discovery has sparked a wealth of research in this field, revealing several different signaling molecules involved in transducing the amino acid signal to mTORC1, including the Rag GTPases, MAP4K3, and Vps34/ULK1. This review evaluates the current knowledge regarding cellular mechanisms that control and sense the intracellular amino acid pool. We discuss the role of leucine and mTORC1 in the regulation of amino acid transport via the system L and system A transporters such as LAT1 and SNAT2, as well as protein degradation via autophagic and proteasomal pathways. We also describe the complexities of energy homeostasis via AMPK and cell receptor-mediated growth signals that also converge on mTORC1. Leucine is a particularly potent regulator of protein turnover, to the extent where leucine stimulation alone is sufficient to stimulate mTORC1 signal transduction. The significance of leucine in this context is not yet known; however, recent advancements in this area will also be covered within this review.

mammalian target of rapamycin complex 1; system A transporter 2; system L transporter 1; muscle; Rag guanosine triphosphatase; autophagy

Muscle is the primary reservoir of proteins within the body and it is therefore subject to high protein turnover rates. Muscle cells are continuously striving to balance protein synthesis and protein degradation. When the balance favors protein synthesis, muscle growth ensues, whereas when the balance favors proteolysis, the muscle atrophies. Muscle fibers dynamically modulate these processes in order to adapt to differential levels of physical activity (for review see Ref. 43).

Resistance exercise with sufficient energy and protein supplements can augment muscle protein synthesis and suppress proteolysis, resulting in an increase in muscle mass (80). This effect can be further propagated by amino acid supplementation following resistance exercise (105). Leucine in particular is both a potent activator of protein synthesis and an inhibitor of proteolysis, forcing muscle fibers to favor the anabolic phenotype. Leucine plays a significant role in the determination of muscle mass and should be considered a pharmacological entity in its own right.

It is now known that leucine and other amino acids exert these effects primarily through upregulation of an evolutionarily conserved master of protein synthesis referred to as mammalian target of rapamycin complex 1 (mTORC1, now also being referred to as mechanistic target of rapamycin complex 1 (14); see below for details). Leucine is thought to account for ∼20% of our dietary protein intake, whereas the branched-chain amino acids account for approximately one-third of muscle protein (60). Therefore, it is perhaps inevitable that leucine should play a more prominent role in the regulation of protein turnover. There is now strong evidence that leucine represses proteasomal degradation as well as enhancing mTORC1 signaling to promote growth (15, 70). Despite a wealth of evidence describing growth factor-mediated mTORC1 activity, the cellular mechanisms underpinning leucine-induced...
mTORC1 activity have remained undefined. It is still unclear how cells sense amino acids and how this signal is relayed to mTORC1. Further light may be shed on the specific potency of leucine as we begin to understand the role of amino acids in mTORC1 signaling.

mTORC1 is a Ser/Thr kinase signaling complex. The core components consist of mTOR (Ser/Thr kinase), regulatory associated protein of mTOR (Raptor), and mammalian lethal with sec13 protein 8 (mLST8, also known as GβL) (18). In order to increase muscle mass, protein synthesis is promoted by mTORC1 through regulation of translation initiation and elongation (for review see Ref. 108). mTORC1 also enhances the cellular capacity to drive translation by increasing ribosomal biogenesis and, consequently, ribosome numbers. Given that protein synthesis is an energy-costly process that requires a plentiful supply of amino acids, cells rapidly switch off mTORC1 signaling during conditions of limited nutrient or energy supply.

mTORC1 signaling also enhances muscle mass, in part by blocking one arm of protein breakdown, autophagy. mTORC1 represses autophagy, a catabolic process that breaks down the protein reservoir if amino acid supply is not maintained via amino acid transport (74). Protein degradation via the proteasome is also activated when leucine levels become low (71). Lack of mTORC1 signaling can lead to atrophy or muscle wasting, which has been linked to the aging process (28). The regulation of mTORC1 by nutrients through external and internal sources, i.e., by amino acid transporters and protein breakdown, respectively, is tightly controlled by multiple feedback mechanisms and are discussed in this review.

By functioning as a nutrient sensor within the cell, mTORC1 ascertains an appropriate level of protein synthesis, as dictated by the availability of amino acids, to promote cellular growth. mTORC1 governs protein synthesis primarily via the phosphorylation of downstream substrates eukaryotic initiation factor 4E-binding proteins (4E-BPs) and ribosomal protein (rp)S6 kinase-1 (S6K1) (108).

Dephosphorylated 4E-BPs inhibit translation initiation by binding directly to and inhibiting a critical member of the translation initiation complex eukaryotic initiation factor 4E (eIF4E). Direct phosphorylation of 4E-BPs by mTORC1 causes the release of eIF4E, allowing cap-dependent translation to commence efficiently (82).

S6K1 is directly activated by mTORC1 and promotes protein synthesis via phosphorylation of multiple downstream substrates associated with ribosomes, e.g., rpS6, which interacts directly with the 40s ribosomal subunit. S6K1 also regulates translation initiation factors such as eIF3 and eIF4B (78), elongation factors such as eukaryotic elongation factor 2 kinase (eEF2k) (107), and mRNA processing factors including the cap-binding protein-80 (CBP-80) (101). S6K1-mediated phosphorylation of SKAR also facilitates mRNA splicing through interactions with the exon junction complex, also leading to increased translation efficiency of mRNAs (65). S6K1 has also been postulated to regulate ribosomal biogenesis, which would indirectly function to enhance overall rates of protein synthesis (67).

Mitogenic signaling upstream of mTORC1 is complex in higher eukaryotes, with many signaling pathways converging at the level of the TSC1/2 tumor suppressor complex. TSC1 and TSC2, as a heterodimer, function as a GTPase-activating protein toward the mTORC1 activator and small G protein Rheb. In response to growth factors or insulin stimulation, signaling cascades are triggered, resulting in TSC2 phosphorylation and the dissociation of the TSC1/2 complex (100). Loss of the TSC1/2 heterodimer allows the GTP loading of Rheb. Rheb-GTP acts as a potent activator of mTORC1 signal transduction (Fig. 1) (10, 41, 90, 102). Rheb-GTP is also thought to enhance substrate recognition of mTORC1 substrates such as 4E-BP1 and S6K1 to mTOR for their optimal phosphorylation (89).

The mechanisms leading to activation of mTORC1 by insulin and growth factors through cell membrane receptors are well defined for growth factors (shown in Fig. 1) but poorly defined for mechanical stimuli like exercise. Amino acid sufficiency is absolutely necessary for growth factor-mediated activation of mTORC1. The dominant effect of amino acid supply is a critical aspect of mTORC1 activation and also functions to directly activate mTORC1 signaling in the absence of growth factor stimuli. There is growing evidence to suggest that mechanical activation of mTORC1 also occurs independently of growth factors (34, 95), extending the complexity of signaling upstream of mTORC1.

Evidence of several key components involved in amino acid signaling to mTORC1 have emerged recently and will be described. This review evaluates the current knowledge regarding amino acid signaling to mTORC1, with particular emphasis on the role of leucine as a regulator of protein synthesis.

### Regulation of Intracellular Amino Acid Levels

Intracellular amino acid levels are determined by the equilibrium among dietary intake, cellular uptake, cellular recycling, and cellular utilization. To replenish intracellular amino acid supplies, mTORC1-dependent and -independent mechanisms are instigated to suppress protein synthesis.

Recovery of intracellular amino acids can be demonstrated on a cellular level by utilizing agents that inhibit protein synthesis. For instance, by chemically blocking translation elongation with cycloheximide (a bacterially produced toxin that reversibly interferes with ribosomal translocation), the intracellular amino acid stores quickly replenish as they are not being used to generate de novo proteins. The accumulation of amino acids during cycloheximide treatment causes a potent activation of mTORC1 despite the absence of external amino acid supply or growth factor stimuli (81). It is notable that cycloheximide treatment also suppresses the expression of the mTORC1 repressor REDD1, contributing to the activation of mTORC1 in this setting (51).

### Cellular Uptake: Amino Acid Transporters

This section of the review summarizes some of the ways in which cells regulate the intracellular amino acid pool, specifically in the context of mTORC1 signal transduction.

Muscle cells require a plentiful supply of amino acids to maximize muscular growth. Intracellular amino acid homeostasis is maintained through external amino acid supply (i.e., nutrient ingestion) and the rate of amino acid uptake from the blood. Amino acid uptake is typically coupled with countertransport of ions such as Na⁺ (also K⁺ or Cl⁻). Na⁺ and K⁺ transporters are referred to as being energy dependent, as the plasma membrane Na⁺/K⁺-ATPase maintains the ion gradi-
There are many different transport systems that regulate amino acid supply. The main systems that influence mTORC1 signaling are the system L and the system A transporters, which are described below.

The system L type transporters are amino acid exchangers that function primarily to import the branched-chain amino acids in exchange for other intracellular amino acids. The system L type exporter (LAT1) couples with the glycoprotein CD98 as well as the Na$^+/H^+$-linked system A transporter (SNAT2), all of which show increased expression in correlation with mTORC1 activity (16, 64). Despite the presence of several different glutamine transporters, SNAT2 regulates glutamine levels predominantly in skeletal muscle (6, 24, 40), where it is found in abundance (69). SNAT2 is thought to mediate the concentrative uptake of small neutral amino acids, specifically glutamine. Baird et al. describes how SNAT2 couples with LAT1. LAT1 functions to export glutamine in exchange for leucine import. Therefore, SNAT2 maintains intracellular glutamine levels to drive the LAT1/CD98 amino acid exchanger and promote leucine uptake (5). This system is referred to as tertiary active transport and is demonstrated in Fig. 3. Silencing of SNAT2 gene expression in L6 muscle cells caused a drop in both intracellular leucine and glutamine levels, indicating the dependency of glutamine import by SNAT2 (25). As a consequence, SNAT2 knockdown was also followed by a strong repression of mTORC1 and activate autophagy through reduced glutamine uptake.

As well as cellular amino acid transport, recent evidence indicates that some transporters might also be involved in signal transduction by acting as cell membrane receptors, coining the term “transceptor”. SNAT2 may have additional roles as a transceptor, given that SNAT2 knockdown causes repression of insulin signaling by PI 3-kinase. To further analyze SNAT2 signal transduction, Evans et al. (25) employed a synthetic SNAT2 substrate, methylaminoisobutyrate (MeAIB), to saturate SNAT2. Treatment with MeAIB in-
increased PI 3-kinase/Akt activity despite a drop in intracellular amino acid transport. This indicates that active SNAT2 can induce PI 3-kinase signal transduction independently of its ability to transport amino acids, as well as modulating mTORC1 through alterations in the intracellular amino acid pool. This extends the role of SNAT2 beyond that of an amino acid transceptor, suggesting a more complex role in relaying the amino acid input downstream to mTORC1.

It has been demonstrated in several different cell types that growth factor stimulation increases expression of system A and system L type transporters in an mTORC1-dependent manner (79, 85). This suggests a reciprocal relationship between amino acid transport and mTORC1 signal transduction. Conversely, a recent study examining this effect in cultured human myotubes demonstrated no change in the expression of LAT1 or SNAT2 in response to either chronic or acute insulin stimulation (31). They did, however, observe an insulin-induced increase in CD98 expression, which is required for LAT1 function. This suggests cell type-specific differences in the amino acid response. Interestingly, a link between CD98 and mTORC1 has been previously published. Edinger et al. (20) demonstrated that mTORC1 regulated the intracellular trafficking of CD98 within cells expressing an active Akt mutant, in a rapamycin-sensitive fashion. A similar mechanism may be in place in skeletal muscle to aid the uptake of leucine.

SNAT2 was also recently found to be a downstream target of STAT3 (signal transduction and activator of transcription 3) in response to IL-6 in human primary trophoblast cells (44). Silencing of STAT3 reduced SNAT2 mRNA levels and abolished IL-6-induced System A transporter activity. Given that STAT3 is thought to be a direct mTORC1 substrate (112), one of the ways in which mTORC1 may influence amino acid transport is through transcriptional upregulation of STAT3.

Recently, details of a third class of amino acid transporters have been affiliated with mTORC1 signal transduction, proton-assisted amino acid transporters (PATs). Genetic screens in Drosophila melanogaster revealed that PATs are required for optimal dTOR activation (30). A reciprocal study of the mammalian transporters PAT1 and PAT4 using shRNA-mediated knockdown revealed them to be indispensable in amino acid-induced mTORC1 signaling in both an MCF-7 breast cancer cell line and human embryonic kidney cells (HEK293s) (37). Interestingly, this requirement was not dependent on PATs’ ability to shuttle amino acids. PAT1 was also found to localize to endosomal membrane compartments (where mTORC1 is also localized) upon amino acid stimulation (see Rag GTPases below for details) (37), PAT1 is perhaps more likely to be involved in relaying the amino acid signal as opposed to transporting amino acids in this context. PATs may therefore play a more fundamental role in modulating mTORC1, as opposed to solely being involved in amino acid transport. Alternatively, they may facilitate amino acid transport across endomembranes where mTORC1 is situated, to directly promote signal transduction. It remains unclear whether it is the intracellular amino acid concentration that somehow signals to mTORC1 or whether PATs are able to relay the signal to mTORC1 upon amino acid binding, as was demonstrated with the SNAT2 transceptor.

In fission yeast, Rheb has also been implicated in basic amino acid uptake through proper membrane localization of amino acid permeases such as the cationic amino acid transporter (CAT) via endosomal/Golgi trafficking (3). Interestingly, the core component of both mTORC1 and mTORC2, Lst8, which colocalizes to endosomes and the Golgi apparatus, is also implicated as a negative regulator of permease sorting in yeast. Loss of Lst8 function increases the intracellular pools of glutamate and glutamine through reduced membrane delivery of the general amino acid permease Gap1p (11). Although it is unlikely that these specific signaling mechanisms are conserved from yeast to humans, the relationship between Tor and amino acid transport appears to be comparable.

Multiple links have been reported between amino acid transporters and mTORC1 signal transduction in a variety of settings. Although active mTORC1 is able to upregulate the activity/expression of amino acid transporters in muscle to increase amino acid uptake for protein synthesis, a general consensus has yet to be formed regarding how this occurs. Further research is required to determine how mTORC1 may facilitate amino acid transport under growth factor stimulation.

The mechanisms governing leucine signaling downstream to mTORC1 have also remained elusive. A drop in intracellular leucine levels is sufficient to suppress mTORC1 signaling (35). However, it is unclear how leucine is sensed within the cell and how exactly it signals to mTORC1, although it is likely to at least partially involve amino acid transceptors such as SNAT2. Several potential signaling components have been identified that could play a role in transducing the amino acid signal to mTORC1; these are discussed in detail later.

Fig. 3. Amino acid induction of mTORC1 via Rag GTPases. Under amino acid insufficiency, Rags exist as inactive GDP-bound complexes but translocate to the lysosome as governed by the Ragulator complex. Amino acids trigger GTP loading of Rags A/B. This active Rag complex then binds directly to Raptor and mediates the translocation of mTORC1 to the outer lysosomal membranes. Rags bind to the Ragulator complex or p62 (see text for details). mTORC1 is activated by proximal Rheb-GTP to suppress autophagic breakdown through ULK1/2 and AMPK and increase protein synthesis through S6K1 and 4E-BP1.
Cellular Recycling: Proteolysis

The ubiquitin system plays a major role in muscle physiology. When in a catabolic state, the ubiquitin-proteasome protein degradation pathway and its component enzymes are activated. This leads to proteasomal degradation of myosin heavy-chain and light-chain proteins as well as myosin binding proteins (36).

As indicated above, leucine is a potent activator of protein synthesis, and dietary leucine supplements are particularly effective at building muscle mass. Interestingly, studies have indicated that this is not only through increased protein synthesis but also through suppression of proteolysis. Leucine appears to inhibit muscle atrophy, in part, through downregulation of proteolysis (8, 12, 71) and has been shown to suppress expression of key components of the ubiquitin/proteasome pathway (71). Evidence for this has been demonstrated in many different experimental settings, although there is disagreement in the literature regarding the dose required to inhibit proteolysis. These discrepancies are likely to be a result of differences in intracellular leucine concentrations. As highlighted above, the cell can selectively increase intracellular leucine without increasing the total intracellular amino acid levels. Leucine’s ability to stimulate protein synthesis as well as to suppress proteolysis makes it crucial in the regulation of muscle protein turnover. It remains unclear exactly how leucine is able to suppress degradation at the proteasome. It has previously been considered to be an mTORC1-independent process. However, as indicated above, silencing of the SNAT2 amino acid transporter compromises insulin signaling through PI 3-kinase; interestingly, this coincided with an increase in proteolysis that was shown to be mTORC1 dependent. This study highlights a previously unreported association among SNAT2, PI 3-kinase, mTORC1, and proteolysis (25). Further work is required to determine whether mTORC1 has a more direct inhibitory role over proteolysis. It is possible that alterations in mTORC1 activity are impacting proteolysis indirectly by affecting the intracellular amino acid pool; this, however, remains to be seen.

Cellular Recycling: Autophagy

mTORC1 is tightly coupled to cellular energy and amino acid stores through the AMP-dependent protein kinase (AMPK) and ULK1 (also known as ATG1), respectively. To maintain energy and amino acid homeostasis, cells employ a series of negative feedback mechanisms to rapidly switch off mTORC1 and induce autophagy when energy or nutrients become limiting. The complex interplay among these three protein kinases (mTORC1, AMPK, and ULK1) has had much attention over the past few years.

Activation of autophagy is known to inhibit S6K1 and is reportedly dependent on ULK1, implying a possible feedback mechanism to mTORC1 (62). Recent work uncovered that phosphorylation of Raptor by ULK1 potently inhibited mTORC1 signaling (17, 47). Dunlop et al. (17) revealed that multisite phosphorylation of Raptor by ULK1 completely blocked substrate recognition and phosphorylation of downstream targets of mTORC1, such as elf4E-binding protein-1 (4E-BP1) and S6K1. AMPK can also phosphorylate Raptor to inhibit mTORC1 (33) as well as phosphorylating TSC2 to activate the TSC1/2 tumor suppressor complex, (see Fig. 1) (42), which indirectly inhibits mTORC1 via accumulation of inactive Rheb-GDP. To add another level of complexity to these feedback mechanisms, AMPK and ULK1 also interact with one another where AMPK drives autophagy through phosphorylation and further activation of ULK1 (21, 61). This convergence of AMPK and ULK1 signal transduction is central to understanding how cells regulate both amino acid and energy homeostasis. For instance, AMPK and ULK1 can synergize to rapidly repress the energy- and amino acid-consuming mTORC1 pathway during conditions when both energy and nutrients become limiting. By enhancing autophagy, cells quickly restore energy and amino acid stores through catabolism of intracellular lipid and protein components.

Highlighting the dynamic interplay among mTORC1, AMPK, and ULK1, mTORC1 is also known to inhibit autophagy through direct phosphorylation of ULK1 (38, 46, 58, 113). Recent evidence revealed that phosphorylation of ULK1 by mTORC1 prevents AMPK interaction with ULK1 and thus prevents autophagy (22, 92). Indeed, phosphorylation of ULK1 at Ser378 in a nutrient- and mTORC1-dependent manner blocks interaction of AMPK with ULK1 (91).

Considering that AMPK and mTORC1 signaling is acutely involved in exercise and muscle growth, it is likely that ULK1 could also be involved. Such cell signaling dynamics involving ULK1 have not been analyzed in muscle cells; however, autophagy has been implicated in muscle physiology. A recent study demonstrated that the protein levels of light-chain 3B (LC3, also referred to as ATG8II) isoform was significantly reduced upon nutrient ingestion in skeletal muscle from human subjects (91). Given that the level of LC3-II protein directly correlates with autophagosomal abundance in cells. This finding suggests that the regulation of autophagy in skeletal muscle must be involved in muscle protein breakdown and growth. Clearly, further work is required to determine whether the complex signaling interplay among mTORC1, AMPK, and ULK1 is involved in muscle physiology.

Leucine Supplementation In Vivo

Although there is a wealth of evidence demonstrating leucine induction of protein synthesis via mTORC1 in cell culture systems, there have been some conflicting reports in the equivalent in vivo studies. Tipton et al. (104) observed no difference in muscle protein in subjects given a whey protein supplement compared with a whey protein plus leucine supplement prior to resistance exercise. A similar study in the elderly showed that additional leucine supplements caused no additive effect on muscle protein synthesis compared with carbohydrate and protein supplements (54). Previously, in rat studies, muscles were known to become less responsive to leucine stimulation with age, which may partially explain these results (13). It has been suggested that leucine does not exert an additive effect on muscle protein synthesis if ample protein has been consumed. Conversely, there are a multitude of studies that report positive results on muscle protein levels through leucine supplementation (1, 55, 83). It is likely that these discrepancies arise from differing experimental conditions, for instance the intensity and duration of the exercise; as well, the age, fitness, and muscle content of the participants could impact the results. Similarly, the time and strength of the leucine supplement may...
produce varying responses across different studies. These are factors that cannot be accounted for in in vitro studies; therefore, the data should be treated with caution when extrapolating to human tissues.

**Cellular Utilization: Amino Acid Signaling to mTORC1**

The relationship between amino acid and growth factor-induced mTORC1 activity has been debated over the years. A permissive amino acid input is required for growth factor-induced mTORC1 activity (see Rag GTPases below) (35). Furthermore, amino acid withdrawal rapidly suppresses mTORC1 signaling. This is evident in TSC2-null mouse embryonic fibroblasts (MEFs) as well as in wild-type cell lines, so it was initially thought that the amino acid signal to mTORC1 was independent of the TSC1/2 complex (93). There is, however, conflicting evidence that suggests that at least a portion of leucine-induced mTORC1 activity is a result of activation of the PI3-kinase/Akt/TSC axis (see Fig. 1). Leucine supplementation causes a small, transient increase in serum insulin levels that may be contributing to the activation of mTORC1 (2). Experiments using somatostatin, which prevents secretion of a number of growth factors, including insulin, suppressed leucine-induced phosphorylation of S6K1 and 4E-BP1, suggesting that the response is partially modulated by growth factors (2). Furthermore, in diabetic rats, muscle protein synthesis is thought to be reduced by ~65% compared with starved non-diabetic controls. Leucine supplementation caused partial recovery of this; however, muscle protein synthesis was still abnormally low, supporting the notion that insulin is required for a maximal response to leucine (2). As discussed earlier, SNAT2 silencing was shown to impair insulin signaling independently of its role as an amino acid transporter. It may therefore be the case that SNAT2 is also required for the full amino acid induced insulin response.

Although we are beginning to understand how cells balance intracellular amino acid supply and demand, it is less clear how mTORC1 “senses” amino acids. After recent advancements in the field, we are now aware of multiple signaling mechanisms that either directly or indirectly influence mTORC1 signaling via amino acid supply.

Several potential upstream signaling components, including the lipid kinase Vsp34 (vacular protein sorting 34), protein kinase MAP4K3 (mitogen-activating protein kinase kinase kinase-3), as well as the small G protein Rag (Ras-related GTPases) heterodimers, have recently been linked to amino acid signaling to mTORC1. Further amino acid sensory proteins that also regulate mTORC1 have been uncovered including the unbranched-chain amino acid receptors (UBR) as well as the nitrogen permease-regulatory (NPR) proteins. It is evident that amino acid sensing involves multiple mechanisms, which are summarized below.

**Amino Acid Signaling to mTORC1: the Rag GTPases**

The Rag GTPases are a family of four Ras-related small guanosine triphosphatases, which have recently been identified as key mediators of the amino acid response to mTORC1. The Rag proteins function as heterodimers and are unique in that an active complex consists of GTP-bound Rag A or B complexed with GDP-bound Rag C or D (88). Amino acids trigger the GTP loading of Rag A/B proteins (88); however, the mechanisms behind this are currently unknown. Once this occurs, active Rag complexes bind directly to mTORC1 (specifically through the substrate recognition component of the complex, Raptor) and trigger relocation of mTORC1 to membrane surfaces where farnesylated Rheb is also situated (see Fig. 3). This translocation occurs independently of mTORC1 kinase activity and is therefore insensitive to rapamycin inhibition. It is thought that, for activation of mTORC1 by insulin or growth factors, this amino acid induced localization of mTORC1 must first occur (49, 88).

More recent work by Sancak et al. (87) identified that amino acids induced mTORC1 translocation to the lysosomal or late endosomal membrane, where mTORC1 interacted with the aptly named “Ragulator complex” consisting of MP1, p14, and p18. The Ragulator complex translocates to the lysosomal membranes when the p18 subunit becomes lipidated at NH2-terminal myristoylation and palmitoylation sites. It is thought that this is crucial for lysosomal localization of the Ragulator complex (87). The Ragulator complex then functions to recruit Rag GTPases to the lysosomal membranes, placing mTORC1 in proximity with its activator Rheb-GTP to promote signal transduction (Fig. 3). In agreement with the importance of mTOR localization, constitutive targeting of mTOR to the lysosomal surface is sufficient to render it unresponsive to amino acids (87). A similar mechanism has been described in yeast, the recently identified GSE complex, consisting of Gse1p and Gse2p, actually shares less than 5% sequence homology with the mammalian Ragulator complex. However, the crystal structure is strikingly similar, and a direct interaction between the GSE complex and the yeast Rag proteins Gtr1p and Gtr2p has been reported, suggesting that this mechanism is highly conserved (53).

Interestingly, a recent study (19) demonstrated evidence that the intracellular signaling adapter molecule p62 is also a Raptor interacting protein required for amino acid-induced activation of mTORC1. The authors demonstrate an interaction between p62 and the Rag GTPases, with enhanced p62 binding to active Rag complexes. The study shows that p62 is a key mediator of Rag signaling to mTORC1, promoting formation of active Rag complexes to enhance Rag-mTORC1 interactions and localization of mTORC1 to the lysosomal membrane (Fig. 3). Conversely, the authors failed to show interaction between p62 and the “Ragulator complex” identified by Sancak et al. (87). This may suggest that p62 provides an alternative mechanism for Rag signaling to mTORC1, although it is unclear at present what the differences are between the p62-Rag association and the Ragulator-Rag association. Although p62 appears to favor the active Rag complexes, it is unclear from the study whether p62 is itself involved in amino acid sensing. Further research is required to elucidate exactly how the amino acid signal is transmitted to the Rag proteins.

As stated earlier, Sancak et al. (88) demonstrated that amino acids promote GTP loading of the Rag A/B proteins. It is therefore possible that a guanine exchange factor (GEF) for the Rags exists that is specifically activated by amino acids. Binda et al. (7) identified Vam6 as the GEF for the yeast Rag A/B ortholog (Gtr1), which is critical in transducing the amino acid signal to Tor1. It has been speculated in the literature that the mammalian homolog of Vam6 may also be activated by amino acids and act as a GEF for the Rag GTPases. There are at least two identified mammalian homologs of Vam6, which share
~44% amino acid homology. They are Vps39 (vacuolar protein sorting-39) and TGFBRAP1 (TGFβ receptor-associated protein-1) (77). Both Vps39 and TGFBRAP1 are involved in TGFβ signal transduction and are essential for embryonic development (68). Knockdown of Vps39 in mammalian cells has also been shown to inhibit amino acid and insulin signaling to mTORC1. Conversely, knockdown of Vsp39 had no effect on mTORC1 localization (27). This suggests that GTP-Rag signaling to mTORC1 remains intact in the absence of Vps39. So, although Vps39 appears to play a role in nutrient signaling to mTORC1, it does not appear to act as a GEF for the Rags in mammalian cell systems.

Vam6 has also been identified as a GEF for the yeast homolog of Rab7. This is interesting when you consider that Sancak et al. (88) identified mTORC1 as localizing to Rab7 positive endosomes upon amino acid stimulation. It is possible that Rab7 is involved in nutrient signaling to mTORC1. During maturation from early to late endosomes, Rab5 is substituted for Rab7, as active Rab5 mutants block early to late endosomal conversion (84). Interestingly, overexpression of a constitutively active Rab5 mutant also inhibits amino acid- and insulin-induced mTORC1 activity in a similar manner to Vps39 knockdown. It is likely that mTORC1 activity is dependent on the late endosomes providing a platform for signal transduction. With this in mind, Vps39, Rab5, and Rab7 are more likely involved in the progression from early to late endosomes as opposed to playing a direct role in nutrient signaling of mTORC1.

There is a wealth of evidence connecting the endocytic pathway to nutrient mTORC1 signaling. In addition to the connections between Vps39, Rab5, and Rab7, vacuolar sorting protein-34 (Vps34) has also been implicated in nutrient signaling to mTORC1 (discussed below). Furthermore, there is evidence to suggest that Rheb is involved in the formation of late endosomes independently of its ability to activate mTORC1 (86). This suggests a reciprocal relationship between the mTORC1 and endocytic pathways. It remains unclear whether the other Vam6 homolog TGFBRAP1 is involved in GTP loading of Rag proteins, or if indeed the Rag proteins are connected to endosomal trafficking themselves. It is also unclear how these small G proteins, Rags, Rabs, and Rheb, are actually targeted to the late endosome/lysosomal membranes and the significance of this with regard to nutrient signaling to mTORC1. Rheb is COOH-terminally farnesylated, which is required for mTORC1 activation (103). Presumably, farnesylation is necessary for proper Rheb localization to late endosomes.

Much of our current knowledge of the mTORC1 signaling pathway is derived from researching diseases where mTORC1 signaling is aberrant. One particularly interesting example that may be relevant in the case of nutrient signaling to mTORC1 is type 2 diabetes (T2DM). T2DM is characterized by acquired insulin resistance. Normally, insulin is released in response to elevated glucose levels. Insulin functions to increase the synthesis of lipids and reduce further generation of glucose from catabolic processes to restore glucose homeostatic balance. It is thought that suppression of gluconeogenesis occurs downstream of Akt, whereas lipogenesis is regulated downstream of mTORC1 via SREBP-1c (sterol regulatory element binding protein-1c) (59); the insulin signal therefore bifurcates at Akt.

In the case of T2DM, partial insulin resistance develops whereby gluconeogenesis is not suppressed but lipogenesis continues. It is thought that insulin resistance develops due to S6K1-mediated degradation of insulin receptor substrate-1 (IRS-1), which is a negative feedback mechanism induced by the mTORC1/S6K1 pathway. This is in combination with mTORC1-mediated phosphorylation of Grb10, which suppresses insulin and insulin-like growth factor signaling (39, 113). These feedback mechanisms suppress insulin-induced activation of Akt and mTORC1. It has been suggested that mTORC1 but not Akt signaling is activated directly by amino acids. Therefore, overeating, which is a known risk factor for T2DM, leads to increased amino acid intake keeping mTORC1 signaling “switched on” while Akt is suppressed. In support of this notion, it has been reported that obese individuals exhibit 14% higher blood leucine levels than leaner individuals (73). This remains controversial, however, as there is evidence in the literature that leucine supplementation increases insulin sensitivity in rats (114). Furthermore, it has been shown that increased exercise is effective in reducing insulin resistance (114), yet exercise also acts a mechanical stimulus to activate mTORC1 (34, 95). However, S6K1 knockout mice do not develop insulin resistance when raised on a high-fat diet, unlike their wild-type counterparts, suggesting that, in at least some cases, aberrant mTORC1 signal transduction is likely to play a role in insulin resistance (106). mTORC1/S6K1 signaling therefore provides the connection between insulin signaling and nutrient intake. Further research is required to clarify the mechanisms leading to insulin resistance as they are evidently more complex than initially thought.

Metformin is often used therapeutically to target T2DM. Metformin is thought to alleviate the symptoms through inhibition of mTORC1 via AMPK activation (see Fig. 1). A recent study reveals that metformin sufficiently suppresses mTORC1 in AMPK-deficient cell lines. Furthermore, Kalender et al. (48) demonstrated how mTORC1 inhibition by metformin is relieved through overexpression of a constitutively active Rag mutant. This evidence implies that metformin is inhibitory to amino acid sensing through the Rag proteins and does not require AMPK activation.

Metformin may be a useful tool in future studies deciphering the mechanisms of Rag signaling to mTORC1, as well as having potential as an anticancer therapeutic in cases where mTORC1 signaling is aberrant.

Interestingly, recent research exploring the relationship between mTORC2 and amino acids has revealed differences in the way cells respond to amino acids, which is dependent on the starvation conditions used. Tato et al. (99) demonstrated that when cells are starved for 2–3 h using D-PBS (Dulbecco’s phosphate-buffered saline), amino acid stimulation caused activation of mTORC2/Akt signaling. Conversely, they demonstrated that when DMEM (-AA) was used for starvation, amino acids were able to induce mTORC1 activity independently of mTORC2/Akt activation, as expected.

This evidence suggests that there is a component within DMEM that is absent from D-PBS, which could alter the cellular response to determine whether amino acids induce the activity of mTORC1 or mTORC2. Further research investigating the differences between these two starvation methods could therefore aid our understanding of how cells respond to amino acids (99).
Inositol Polyphosphate Multikinase

Inositol polyphosphate multikinase (IPMK) has recently been implicated in amino acid signaling to mTORC1 (50). IPMK is a signaling molecule with inositol phosphate and lipid kinase activity; however, its role in mTORC1 signaling is thought to be independent of this function. Kim et al. (50) showed evidence where IPMK acts as a cofactor for mTORC1 by stabilizing the mTOR/Raptor interaction in the presence of amino acids. The authors propose that IPMK facilitates the interaction between mTORC1 and Raptor in response to amino acids, allowing binding of the Rag proteins to promote mTORC1 localization. Further research is evidently required to confirm this mechanism and also to elucidate how amino acids might signal to IPMK.

Vps34

Vps34 is considered a positive regulator of mTORC1 and an amino acid sensor. Vps34 is also involved in vesicular trafficking processes and autophagy (45). Byfield et al. (9) showed that overexpression of Vps34 causes upregulation of mTORC1 signal transduction. Furthermore, Vps34 is inhibited by amino acid/glucose withdrawal but not rapamycin, suggesting that it lies upstream of mTORC1 (9, 75). Another study utilizing C2C12 myoblasts revealed that leucine increased Vps34 activity threefold when supplied at physiological concentrations, this correlated with mTORC1 activation (101).

Vps34 binds and forms an active complex with Vps15. Vps34/Vps15 phosphorylates PtdIns (phosphoinositide) to produce PtdIns(3)P1 on endosomal/vesicular membranes (76). PtdIns(3)P1 promotes recruitment of various proteins to the early endosome and may provide a platform for cell signal transduction that also includes mTORC1 (9, 75).

Further studies revealed a regulatory Ca2+–calmodulin binding domain within the PI 3-kinase accessory domain of Vps34. Binding of Ca2+ to Vps34 increases lipid kinase activity and increases mTORC1 signaling, suggesting in fact that Vps34 senses Ca2+ rather than amino acids (32). In dispute of this argument, Yan et al. (111) indicated that Vps34 activity was unaffected by Ca2+ chelators or Ca2+-calmodulin inhibitors. Therefore, it remains unclear whether Vps34 responds specifically to amino acids or calcium. Given that amino acids induce membrane polarity changes due to Na+ and polar amino acid fluxing (see amino acid transporters above) and that this appears to stimulate Ca2+ influx, Ca2+ and amino acid signaling may be more interconnected than initially thought.

Interestingly, it has also been shown that Vps34 and Vps15 interact with Rab7 at the late endosomes (97). It is plausible that Vps34 is involved in the localization of mTORC1 to the Rab7-positive endosomes where Rheb-GTP is situated. Given that Vps34 is also an effector of Rab5 and overexpression of Rab5 suppresses mTORC1 (see Rag GTPases above), it may be more likely that Vps34, like Vps39, Rab5, and Rab7, is involved in the progression from early to late endosomes and therefore provides a platform for mTORC1 signaling.

Conversely, it is well documented that Vps34 complexes with beclin-1 and plays a positive role in regulating autophagy (for review see Ref. 29). Vps34 therefore plays conflicting roles within the cell, activating mTORC1 to suppress autophagy as well as positively regulating autophagy itself. It is unclear how or why this occurs; it may be that the positive effect on mTORC1 occurs indirectly, with Vps34 promoting autophagic breakdown, thus increasing intracellular amino acid levels to promote mTORC1 signaling. Alternatively, it may be that nutrients are able to differentially regulate distinct pools of Vps34 to either promote or inhibit autophagy in accordance with nutrient availability. Further research is required to determine whether Vps34 acts primarily as a positive mediator of autophagy or whether it has a more complex role as a nutrient sensor within the cell.

MAP4K3

MAP4K3 is an Ste20-related kinase that was identified in 2007 as a regulator of mTORC1 in response to amino acids (26). Findlay et al. (26) established that overexpression of MAP4K3 propagates mTORC1 signaling, as determined by increased phosphorylation of S6K1 and 4E-BP1. This was subject to inhibition by rapamycin but not by the PI 3-kinase inhibitor wortmannin. MAP4K3 activity was shown to be regulated by amino acids but not insulin or rapamycin. In addition knockdown of MAP4K3 prevented amino acid induced S6K1 phosphorylation. This work suggests that MAP4K3 lies upstream of mTORC1 and functions to regulate mTORC1 in response to amino acids (26).

Later work in 2010 utilized D. melanogaster MAP4K3 mutants to investigate this further. Interestingly, the mutant flies were viable, indicating that dTOR signaling could still occur. Given that the mutant flies displayed reduced dTOR signaling rather than a complete inhibition, MAP4K3 may function to only partially modulate TORC1 signaling. Furthermore, differences between mutant flies and wild-type flies diminished when the flies were raised under low-nutrient conditions. This finding implies that MAP4K3 might play a more pivotal role in dTOR signaling when nutrients are plentiful.

Yan et al. (110) observed that suppression of the Rag proteins reduced the ability of MAP4K3 to propagate mTORC1 signaling but concluded that the Rag GTPases were unlikely to be directly modulated by MAP4K3. Further work is required to determine how the two pathways are related. Yan et al. also identified Ser170 as a phosphorylation site within the kinase activation domain of MAP4K3 that is required for amino acid modulation of MAP4K3 activity and for MAP4K3 activation of mTORC1. Phosphorylation at Ser170 was eliminated by amino acid withdrawal but unaffected by insulin treatment. Interestingly, they showed that amino acid withdrawal caused an acute drop in Ser170 phosphorylation within 5 min and postulated that this rapid dephosphorylation was likely to be the action of a phosphatase. Further studies revealed that MAP4K3 dephosphorylation could be inhibited by incubation with the specific PP2A (protein phosphatase 2A) inhibitor okadaic acid (109). PP2A is a multiprotein serine/threonine phosphatase that functions to reverse the action of kinases in many major signaling pathways. PP2A consists of a structural A-subunit, a regulatory B-subunit, and a catalytic C-subunit. The regulatory B-subunit is thought to determine substrate specificity (23). Yan et al. revealed that MAP4K3 binds to the B-subunit of PP2A, PR61δ, and that ectopic expression of PR61e abolishes Ser170 phosphorylation of MAP4K3 (and, hence, mTORC1 signaling) even in the presence of amino acids. Furthermore, amino acid deprivation...
caused an increase in PP2A binding to MAP4K3 (Fig. 4) (111). This suggests a model of competitive inhibition between PP2AT61/H9280 (PP2A in complex with the B-subunit) and amino acids (more likely a factor regulated by both), but further work is required to fully elucidate how amino acids regulate MAP4K3.

RalA

RalA is a member of the Ras small G protein superfamily involved in modulation of protein transcription, membrane transport, and cell migration. It has also been implicated in cellular proliferation, with RalA participating in Ras-induced oncogenic transformation of cells (63). More recently, RalA was implicated in nutrient regulation of mTORC1. Maehema et al. (66) demonstrated that knockdown of RalA or its activator Ral-GDS (Ral guanine nucleotide dissociation stimulator) blocked amino acid signaling. It was reported that amino acids increased the levels of GTP-bound RalA but not RalB, concluding that amino acids were able to regulate RalA to activate mTORC1. Furthermore, RalA knockdown was sufficient to suppress mTORC1 signaling in cells overexpressing a hyperactive mutant of Rheb without affecting its nucleotide bound status, placing RalA downstream of Rheb. However, they were unable to show direct interaction of RalA with mTORC1 or with FKBP38 [an inhibitor of mTORC1 which was postulated to be displaced from mTORC1 by Rhet-GTP (4)]. From this, they hypothesized that nutrients may independently activate mTORC1 downstream from Ral in a Ral-dependent manner; however, the mechanism is yet to be uncovered.

UBR1 and UBR2

The branched-chain amino acids, in particular leucine, elicit a much stronger impact on mTORC1 signaling than other amino acids (35, 52). Leucine withdrawal alone is as effective as complete amino acid starvation at suppressing mTORC1 signaling. Stimulation with leucine alone is sufficient to promote mTORC1 signal transduction. It has been speculated that leucine may be more frequently utilized in protein synthesis, making mTORC1 more responsive to its depletion (35).

A recent study identified UBR1 and UBR2 as both leucine binding proteins and negative regulators of mTORC1. UBR1 and UBR2 are E3 ubiquitin ligases that specifically recognize “N-degrons”, which are destabilizing NH2-terminal basic or bulky hydrophobic residues of protein substrates. UBR1 and UBR2 function as “N-recognins” that identify N-degrons and target these specific proteins for ubiquitination and subsequent degradation at the 26S proteasome (known as the “N-end rule pathway”) (98). A study by Kume et al. demonstrated that overexpression of UBR1 and UBR2 suppressed mTORC1 signal transduction, which could be rescued with high concentrations of leucine. They demonstrated that leucine bound directly to the substrate recognition domain of UBR2, preventing degradation via the N-end rule pathway. Consequently, this promoted signaling via mTORC1 (56). It is unclear how UBR1 and UBR2 function to inhibit mTORC1 signaling. One possibility is that the UBRs promote degradation of a critical component of the mTORC1 complex or an upstream activator. Alternatively, they may have signal transduction functions outside of their roles as ubiquitin ligases. Irrespective to mTORC1’s involvement, it is likely that the UBRs link proteasome function with leucine signaling.

NPR2 and NPR3

Neklesa et al. (72) developed a flow cytometry-based genetic screen in yeast to discover regulators of Tor1. They identified a highly conserved complex consisting of nitrogen transporters NPR2 and NPR3.

NPRL1/2

NPRL1/2 may signal to mTORC1 through phosphoinositide-dependent kinase-1 (PDK1) inhibition. The yeast homologs of NPRL1/2 act as amino acid sensors in yeast to regulate mTORC1 signaling. In mammalian cells, NPRL1/2 (nitrogen permease regulators) have been shown to have tumor suppressor activity through suppression of PDK1. PDK1 plays a role in activating Akt, which suppresses mTORC1 inhibitors PRAS40 and the TSC1/2 complex.
permease regulators NPR2 and NPR3, which responded specifically to amino acid deprivation to inactivate Tor1/2. Unfortunately, they were unable to identify the mechanistic action of NPR2 and NPR3 nor whether this activity was conserved among higher eukaryotes. The human analogs NPRL2 and NPRL3 both function as tumor suppressors in mammalian cells (96). It may be possible that the NPRL2/3 human analogs perform similar functions to the yeast NPR2/3 proteins in conveying the amino acid signal to mTORC1. Interestingly, a recent study by Kuruta et al. (57) demonstrated that NPRL2 interacted with and inhibited PDK1 (phosphoinositide-dependent kinase-1). PDK1 is upstream of the Akt/mTORC1 pathway (see Fig. 1). Active NPRL2 can, therefore, function to inhibit PDK1 causing indirect inhibition of mTORC1 (see Fig. 5 for potential mechanism). Unfortunately, Kurata et al. did not investigate NPRL2 activity under the context of nutrient deprivation. Since a similar mechanism occurs in eukaryotes as in yeast, and NPRL2 can inhibit mTORC1, it is possible that NPRL2 may also be involved in amino acid regulation of mTORC1.

**Summary**

Cells balance nutrient demand and supply, where nutrient demand is regulated in part through mTORC1 signaling to drive protein synthesis and cell growth. Nutrient supply is governed through amino acid transporters, and during times of fasting, cells scavenge amino acids via proteolysis and autophagy. In this review, we have summarized how leucine levels play a particularly important role in protein turnover, where intracellular leucine modulates mTORC1 signaling via multiple nutrient-sensing pathways as well as regulating the proteasome via a yet unknown mTORC1-independent mechanism.

This review covers a multitude of amino acid sensing mechanisms that converge on mTORC1. The most prominent amino acid sensory mechanism discussed is via the Rag small G proteins, which govern mTORC1 translocation to membrane surfaces such as lysosomes and endosomes. Evidently, there is a clear link between nutrient sensing of mTORC1 and the lysosome. This is highlighted by the array of lysosomal localized mTORC1 signaling components including the Regulator

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**Fig. 6. Amino acid input to mTORC1.** Insulin/growth factors trigger PI3K signaling, leading to GTP-loading of Rheb. Farnesylated Rheb-GTP is then localized to the endomembranes. This signaling cascade is negatively regulated by AMPK when energy levels are depleted. Intracellular amino acids trigger GTP loading of Rags, and active Rag complexes transport mTORC1 to the endomembranes (where it can be activated by Rheb-GTP) through interactions with the Ragulator complex and or p62. Inositol polyphosphate multikinase (IPMK) is thought to stabilize the mTOR/Raptor interaction in response to amino acids to facilitate Rag binding. MAP4K3 promotes mTORC1 activity and is activated by amino acids. PP2A functions to negatively regulate this activity when amino acids are depleted. RapA is also thought to be regulated by amino acids and appears to positively regulate mTORC1 signaling. mTORC1 upregulates expression of the amino acid transporter SNAT2, possibly via activation of STAT3. Na+ linked SNAT2 mediates concentrative uptake of glutamine, which can then be exported in exchange for leucine import by LAT1, the expression of which is also upregulated by mTORC1. SNAT2 is also proposed to promote PI3K signaling to mTORC1 as well as inhibiting proteolysis. Proton-assisted amino acid transporter 1 (PAT1) acts as a receptor, transducing the amino acid signal to mTORC1 at endomembranes through undefined mechanisms. Amino acid uptake triggers calcium influx, activating Vps34. Vps34 may be involved in transducing the amino acid signal to mTORC1 or may increase amino acid levels through its role in autophagy. mTORC1 negatively regulates autophagy through phosphorylation of ULK1, while AMPK functions to activate autophagy when energy levels are depleted. The intracellular amino acid pool is also replenished through proteolysis. Leucine is thought to inhibit this process either directly or via inhibition of unbranched-chain amino acid receptor (UBR) proteins (see text for details) to promote an anabolic phenotype.
complex, Vps34, and ULK1. One area that requires further clarification is the connection between Ca\textsuperscript{2+} signaling and mTORC1, which most likely involves the Ca\textsuperscript{2+}-dependent Vps34. Given that both mTORC1 and Vps34 regulate autophagy, the autophagy kinase ULK1 will undoubtedly join the forefront as a pivotal signaling component in the amino acid response. Furthermore, energy sensing via AMPK is also likely to converge at this mTORC1/Vps34/ULK1 lysosomal signaling platform via interaction with ULK1. It is interesting to speculate that nutrient and energy sensing are orchestrated in tandem by mTORC1 and AMPK. It is likely that together, mTORC1 and AMPK coordinate whether the cells grow by anabolic processes such as protein synthesis or generate energy and amino acids through catabolic processes such as autophagy.

It is evident that mTORC1 localization to membrane surfaces plays a critical point in this regulation, presumably resulting in the convergence of many of these nutrient-induced signaling inputs to endomembrane signaling platforms. Similarities can be drawn between mTORC1 structure and function and the concept of lipid rafts. Lipid rafts are considered to function as mobile signaling platforms on the surface of membranes. Although there is controversy regarding their existence, it is interesting to speculate that mTORC1 might form a signaling complex on mobile lipid raft platforms which may actively translocate between cellular membrane compartments, possibly via vesicular trafficking. While nutrient regulation of mTORC1 appears multifaceted, mitogenic regulation of mTORC1 appears relatively linear in comparison (summarized in Fig. 6). Whether lipid rafts occur or not, these multiple nutrient and mitogenic signaling inputs must coordinate their activities toward a membrane-localized mTORC1 signaling platform. It is clear that full activation of mTORC1 by mitogens involves membrane-tethered GTP-bound Rheb or RhebL1. Not all nutrient signaling pathways appear to be absolutely necessary for mTORC1 signal transduction to occur but are instead involved in maximizing the efficiency of mTORC1 activation under nutrient-fed conditions, for example, MAPK4K3 and transceptors such as PATs and SNAT2. It is interesting to speculate that the internalization of amino acid transporters, which occurs when amino acids are plentiful, functions to positively signal to mTORC1. Indeed, during amino acid stimulation, PAT1 becomes densely localized to intracellular endosomal membrane compartments where it could be involved in direct signaling to mTORC1.

The multiple signaling mechanisms discussed in this review are important for efficient growth control in mammalian cells. Skeletal muscle is no exception; where muscle growth or atrophy is critically dependent on whether there is a sufficient amino acid supply, mTORC1 is a crucial player in this regulation.

Figure 6 summarizes the complex signaling cascades initiated by growth factors, energy, and amino acids that are currently known to converge at mTORC1. However, many questions regarding how mammalian cells “sense” and respond to amino acids remain unanswered. Despite considerable progress in the field over the last decade, it is evident that the dynamic interplay between these signaling components is far more complex than first thought.

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