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Signal integration by mTORC1 coordinates nutrient input with biosynthetic output

Christian C. Dibble¹ and Brendan D. Manning^{2,*}

¹Division of Signal Transduction, Beth Israel Deaconess Medical Center; Systems Biology Department, Harvard Medical School, Boston, MA 02115

²Department of Genetics and Complex Diseases, Harvard School of Public Health, Boston, MA 02115

Abstract

Flux through metabolic pathways is inherently sensitive to the levels of specific substrates and products, but cellular metabolism is also managed by integrated control mechanisms that sense the nutrient and energy status of a cell or organism. The mechanistic target of rapamycin (mTOR) complex 1 (mTORC1), a protein kinase complex ubiquitous to eukaryotic cells, has emerged as a critical signaling node that links nutrient sensing to the coordinated regulation of cellular metabolism. Here, the role of mTORC1 as a conduit between cellular growth conditions and the anabolic processes that promote cell growth is discussed. The emerging network of signaling pathways by which mTORC1 integrates systemic signals, in the form of secreted growth factors, with local signals, in the form of cellular nutrients (amino acids, glucose, and oxygen) and energy (ATP) is detailed. Our expanding understanding of the exquisite regulatory network upstream of mTORC1 provides molecular insights into the integrated sensing mechanisms by which diverse cellular signals converge to control cell physiology.

Introduction

All cells and organisms must coordinate their metabolic activity with changes in their nutrient environment. This is achieved via signaling networks that integrate the sensing of local and systemic nutrient and energy sources and relay this information to metabolic regulators and enzymes to control cellular anabolic and catabolic processes. One of the master regulators of metabolism and growth is the serine/threonine protein kinase mechanistic target of rapamycin (mTOR; formerly known as mammalian TOR), which as part of mTOR complex 1 (mTORC1) functions at the convergence point of a vast signaling network that senses fluctuations in extracellular and intracellular nutrients. The critical importance of intimately linking nutrient signals to metabolic control in human health is highlighted by the fact that aberrant regulation of mTORC1 signaling has been implicated in the pathophysiology of a diverse set of common human diseases, including cancer, metabolic diseases, neurological disorders, and inflammatory and autoimmune diseases^{1–4}

mTORC1, comprised of three essential and evolutionarily conserved core subunits (mTOR, Raptor, and mLST8)^{3, 5} is responsive to both organismal and cellular nutritional status and controls downstream metabolic processes accordingly. Systemic changes in the metabolism of the organism are generally sensed by mTORC1 through pathways activated by secreted

*Correspondence to: bmanning@hsph.harvard.edu, Phone: (617) 432-5614, Fax: (617) 432-5236.

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growth factors, cytokines, and hormones. Activation of mTORC1 is additionally dependent on sufficient levels of essential intracellular nutrients, including amino acids, glucose, and oxygen. Nutrients appear to be the more ancient input for mTORC1, as its activation in yeast depends strictly on nutrient availability⁵. In higher eukaryotes, cell culture experiments suggest that intracellular nutrients only basally activate mTORC1 but are essential for its robust stimulation by extracellular growth factors^{6, 7}. Here, we focus on mTORC1 as a key link between nutritional status and metabolic control, with an emphasis on recent advances in understanding the mechanisms of nutrient sensing and signal integration by this protein kinase complex.

Promotion of anabolic metabolism downstream of mTORC1

To understand the physiological importance of the network of signaling inputs upstream of mTORC1, we must first consider the downstream processes regulated by mTORC1. Under nutrient and energy-replete conditions, mTORC1 is activated to stimulate anabolic processes that convert nutrients and energy into macromolecules, including protein, lipid, and nucleic acids. The control of cellular and systemic metabolism by mTORC1 signaling has been the subject of several recent review articles^{2, 3, 8–10} and we briefly summarize some of the major mechanisms of metabolic regulation here (Fig. 1).

Best known for its role in promoting protein synthesis, mTORC1 activation leads to both an acute increase in the translation of specific mRNAs and a broader increase in the protein synthetic capacity of the cell. mTORC1 regulates 5'-cap-dependent mRNA translation through two sets of direct downstream targets, the eukaryotic initiation factor 4E (eIF4E)-binding proteins (4E-BP1 and 2) and the ribosomal S6 kinases (S6K1 and 2)¹¹. 4E-BP appears to have the most profound effect on mRNA translation downstream of mTORC1, binding to eIF4E at the 5'-cap of mRNAs and blocking assembly of the translation initiation complex. The phosphorylation of 4E-BP1 and 2 by mTORC1 stimulates its release from eIF4E, allowing translation initiation to proceed. This mechanism is particularly important for initiating the translation of mRNAs with 5'-terminal oligopyrimidine (5'-TOP) or 5'-TOP-like motifs, rendering this class of mRNAs especially sensitive to mTORC1 activation and inhibition^{12–14}. Importantly, 5'-TOP mRNAs are enriched for those encoding ribosomal proteins and translation factors. Therefore, the acute translational control over this class of mRNAs, allows mTORC1 signaling to globally enhance cellular protein synthesis. Additional mTORC1-dependent mechanisms are also believed to contribute to its role in increasing ribosome biogenesis to promote protein synthesis¹⁵.

mTORC1 signaling can also promote lipid and nucleic acid synthesis and stimulate glucose uptake, glycolysis, and NADPH production to support these anabolic processes. This is currently understood to occur largely through the regulation of transcription factors by mTORC1¹⁶, but post-translational mechanisms have also recently been uncovered^{17, 18}. mTORC1 signaling increases the translation of Hypoxia-inducible factor 1 α (HIF1 α), which induces expression of glucose transporters and glycolytic enzymes and promotes a switch from mitochondrial oxidative metabolism to glycolysis^{19–24}. This switch to aerobic glycolysis, referred to as the Warburg effect in cancer cells, is observed in most proliferating cells, and is believed to promote metabolic flux from intermediates of glycolysis into biosynthetic branches²⁵. The sterol regulatory element-binding proteins (SREBP1 and 2) globally induce the expression of enzymes involved in *de novo* fatty acid and sterol biosynthesis²⁶, and mTORC1 signaling has been found to promote lipid synthesis through the activation of these transcription factors^{19, 27, 28}. mTORC1 signaling also promotes the expression of pentose phosphate pathway (PPP) genes and metabolic flux specific to the oxidative, NADPH-producing branch of this pathway¹⁹. The mTORC1-induced expression of the rate-limiting enzyme in the oxidative PPP, glucose-6-phosphate

dehydrogenase (G6PD), was found to be dependent on SREBP1. Given that lipid synthesis is one of the most NADPH-demanding metabolic pathways, its coregulation with the oxidative PPP by mTORC1 and SREBP is likely to help satisfy this requirement. Of equal importance, the mTORC1-mediated control of the PPP also results in increased production of ribose required for nucleotide synthesis¹⁹. In parallel to this transcriptional mechanism, mTORC1 has also been shown to acutely stimulate metabolic flux through *de novo* pyrimidine synthesis via the S6K1-mediated phosphorylation of the enzyme CAD (carbamoyl-phosphate synthetase 2, aspartate transcarbamoylase, dihydroorotatase), thereby increasing the pool of nucleotides available for RNA and DNA synthesis^{17, 18}. Therefore, mTORC1 stimulates the synthesis of proteins, lipids, and nucleic acids, as well as reducing equivalents (NADPH) required to drive those pathways.

While promoting the synthesis of macromolecules, mTORC1 at the same time potently inhibits autophagy (or macroautophagy), a cellular recycling mechanism. Autophagy is a multi-stage process in which membranous structures called autophagosomes engulf cytosolic organelles and macromolecules and, through fusion with lysosomes, target their constituents for degradation into nutrient building blocks²⁹. It is likely that mTORC1 inhibits this process at multiple steps, but the best characterized involves the direct control of ULK1 (or ATG1), a protein kinase that regulates the initiation of autophagosome formation^{30–33}. mTORC1 also directly phosphorylates the transcription factor EB (TFEB), a master regulator of lysosomal and autophagy genes, thereby exerting an inhibitory input that is likely to attenuate autophagy^{34, 35}. The major signals that stimulate the induction of autophagy include cellular nutrient and energy depletion, which lead to a decrease in mTORC1 signaling and relief of its inhibition of autophagy.

The general effect of mTORC1 activation is to promote an increase in biomass for cell growth and proliferation. However, mTORC1 signaling plays specialized roles in terminally differentiated tissues, such as promoting localized mRNA translation in neurons, which is critical for the control of synaptic plasticity³⁶, and suppression of ketogenesis in the liver upon feeding³⁷. Regardless of the setting, nutrient sensing by mTORC1 serves as a critical decision point between anabolic and catabolic metabolism within cells. The broad control that mTORC1 exerts over metabolism provides a rationale for why it must be exquisitely responsive to the local and systemic availability of metabolic raw materials.

Systemic nutrient sensing through secreted growth factors

Systemic integration of signals reflecting the physiological state of the organism, including nutritional status, is critical to maintaining homeostasis. These signals are communicated between tissues and cell types through secreted ligands classified as growth factors, hormones, and cytokines (collectively referred to as growth factors here). The archetypal systemic nutrient signal is insulin, which is produced by pancreatic beta-cells in response to increased blood glucose levels and stimulates adaptive signaling events in liver, fat, and muscle. mTORC1 is activated by insulin and most other growth factors through either receptor tyrosine kinases (RTKs) or G-protein coupled receptors (GPCRs) at the cell surface. Downstream of these receptors, two major signaling pathways are involved in mTORC1 activation, the phosphoinositide 3-kinase (PI3K)-Akt and Ras-Erk pathways (Fig. 2). These pathways are differentially activated downstream of specific receptors, with the PI3K-Akt pathway dominating downstream of the insulin and insulin-like growth factor (IGF) receptors. Importantly, many components of these signaling pathways are oncogenes or tumor suppressors, resulting in growth factor-independent activation of mTORC1 in up to 80% of human cancers, across nearly all lineages¹ (Fig. 2).

Growth factor-dependent pathways stimulate mTORC1 signaling by regulating a small G protein switch directly upstream of mTORC1. Ras homolog enriched in brain (Rheb) is a ubiquitous small GTPase of the Ras superfamily, which in its GTP bound form (Rheb^{GTP}) is a direct, potent and essential activator of mTORC1^{38–44}. The Akt and Erk protein kinases promote the accumulation of Rheb^{GTP} by phosphorylating and inhibiting the tuberous sclerosis complex 2 (TSC2) protein, a GTPase-activating protein (GAP) specific for Rheb. TSC2 functions within a complex (referred to here as the TSC complex) containing two other proteins, tuberous sclerosis complex (TSC1) and Tre2-Bub2-Cdc16 (TBC) 1 domain family member 7 (TBC1D7), which are required for the stability and full GAP activity of TSC2^{40, 45}. In the absence of growth factor signaling, the TSC complex maintains Rheb in its GDP-bound state (Rheb^{GDP}), thereby blocking the activation of mTORC1. While the molecular mechanism is not well understood, phosphorylation of specific residues on TSC2 by Akt and Erk, as well as Rsk downstream of Erk (Fig. 2, inset), in response to growth factors inhibits the ability of the TSC complex to regulate Rheb, allowing Rheb^{GTP} to accumulate and activate mTORC1^{46–51}. Parallel mechanisms can also contribute to mTORC1 regulation by growth factors, including the Akt-mediated phosphorylation of PRAS40, a non-essential component of mTORC1^{44, 52}, and the ERK and RSK-dependent phosphorylation of Raptor (Fig. 2)^{53–55}. However, the TSC complex is absolutely essential for inhibition of mTORC1 signaling in the absence of growth factors. It is now recognized that many, but not all, upstream inputs into mTORC1 signaling impinge on control of the TSC complex and Rheb⁵⁶ (see below). While growth factor signaling pathways stimulate an acute and robust increase in mTORC1 activity, they can also contribute to mTORC1 activation more indirectly, through the stimulated uptake of nutrients, which are essential for the basal activation of mTORC1, as discussed below.

Amino acid sensing through the Rag GTPases

Amino acids are essential for mTORC1 activation⁷. Cell culture experiments suggest that mTORC1 is particularly sensitive to decreases in leucine, arginine, or glutamine^{7, 57, 58}. However, it is unclear whether mTORC1 truly senses individual amino acids or the total intracellular pool of amino acids, which can be differentially affected by removal of specific amino acids. It is believed that an intracellular sensor exists that likely interacts directly with amino acids, or their derivatives, and initiates a signaling mechanism to basally activate mTORC1 and allow its further stimulation by growth factors. While, the molecular nature of the upstream amino acid sensor is currently unknown, important progress has been made on the mTORC1 proximal signaling mechanism by which the amino acid sensor ultimately communicates to mTORC1.

Rheb is essential for amino acids to activate mTORC1, but the primary amino acid sensing pathway appears to function in parallel to Rheb^{59, 60} and involve a second class of small G proteins, the Rag GTPases^{61–63}. The Rag proteins belong to a highly conserved family of GTPases that consist of two subtypes, which associate to form heterodimers essential for their stability and function. In mammals, RagA or RagB, which are orthologs of budding yeast Gtr1, form a heterodimer with RagC or RagD, which are orthologs of yeast Gtr2⁶⁴. RagA/B-RagC/D heterodimers bind directly to Raptor within mTORC1⁶³. This association is highly dependent on the nucleotide-binding state of the heterodimer, with mTORC1 binding predominantly to heterodimers consisting of a GTP-bound RagA/B (RagA/B^{GTP}) and a GDP-bound RagC/D (RagC/D^{GDP}). Importantly, this nucleotide-binding state is influenced by amino acids, with starvation promoting the accumulation of RagA/B^{GDP}-RagC/D^{GTP} heterodimers, which cannot associate with mTORC1, and refeeding stimulating a change to the mTORC1-binding RagA/B^{GTP}-RagC/D^{GDP} state⁶³. In both mammalian and *Drosophila* cells, expression of constitutively GTP-bound mutants of RagA or B renders mTORC1 signaling resistant to amino acid starvation, but still under the influence of growth

factor signaling^{62, 63}. The critical nature of the nucleotide-loading state of the RagA/B subunit in amino acid sensing by mTORC1 was further confirmed with a mouse knock-in allele of RagA that is constitutively bound to GTP, which in the homozygous state renders mTORC1 signaling in cells and tissues resistant to nutrient withdrawal⁶⁵. However, unlike Rheb^{GTP}, the RagA/B^{GTP}-RagC/D^{GDP} heterodimer does not appear to directly activate mTORC1, but rather spatially regulates mTORC1 in a manner that permits its ultimate activation by Rheb (Fig. 3).

A major breakthrough in understanding the spatial regulation of mTORC1 came with the discovery of a protein complex dubbed the Ragulator, which is responsible for both the subcellular localization of the Rags and regulation of their nucleotide-binding state^{66, 67}. In yeast, Gtr1-Gtr2 heterodimers interact with Ego1 and Ego3 to form the EGO complex, which associates with the outer surface of the vacuole, the yeast equivalent of the lysosome^{5, 61}. In mammalian cells, RagA/B-RagC/D heterodimers localize to the lysosome through an interaction with the Ragulator, which is similar in architecture to the EGO complex but whose components are not orthologs of the Ego proteins⁶⁶. Amongst the five Ragulator subunits (LAMTOR1–5), LAMTOR1 (p18), anchors the complex, and hence the Rag heterodimers, to the lysosomal surface via dual N-terminal lipid modifications (myristoyl and palmitoyl moieties). LAMTOR2 (p14), LAMTOR3 (MP1), LAMTOR4 (C7 or f59), and LAMTOR5 (HBXIP) share common structural elements called Roadblock domains, which are also present in the Rag proteins but serve an unknown molecular function^{67, 68}. Like the Rag proteins, all components of the Ragulator are required for amino acid sensing to mTORC1^{66, 67}. mTORC1 acutely translocates to the Rag-Ragulator complex at the lysosome in response to amino acid refeeding, and this requires a switch to the activated RagA/B^{GTP}-RagC/D^{GDP} state^{66, 67}. This translocation to the lysosome is both necessary and sufficient for amino acid sensing by mTORC1. Importantly, the Ragulator not only serves as a lysosomal scaffold, but also stimulates the nucleotide switch in the Rag proteins in response to amino acids by acting as a guanine nucleotide exchange factor (GEF) towards RagA and RagB⁶⁷. The molecular origins of this activity within the pentameric complex are unknown, but it induces the RagA/B subunit within Rag heterodimers to release GDP, allowing subsequent loading with GTP. Through an unknown mechanism, this change coincides with a switch of the RagC/D subunit from a GTP to GDP-bound form. Therefore, by promoting the RagA/B^{GTP}-RagC/D^{GDP} state, the Ragulator GEF activity stimulates the recruitment of mTORC1 to the lysosome in response to amino acids.

How amino acids are sensed by the Ragulator at the lysosome is unknown but has been found to involve intra-lysosomal amino acids and the v-ATPase, a large protein complex spanning the lysosomal membrane that acts as proton pump to acidify the lysosome⁶⁹. The v-ATPase was found to be essential for amino acid sensing by mTORC1 in both *Drosophila* and mammalian cells. Ragulator subunits copurify with those of the v-ATPase, and the two complexes make multiple contacts that vary in the presence or absence of amino acids. Importantly, v-ATPase catalytic activity is required for an amino acid-induced conformational shift between the two complexes and stimulation of the switch to the RagA/B^{GTP}-RagC/D^{GDP} state that recruits mTORC1 to the lysosome. However, this mechanism does not appear to involve the lysosomal proton gradient. Therefore, it appears that the v-ATPase stimulates the GEF activity of the Ragulator in response to amino acids⁶⁷. Somewhat unexpectedly, Zoncu et al⁶⁹ found that signals affecting the v-ATPase-Ragulator interactions and mTORC1 activation originate from intralysosomal, rather than cytosolic, amino acids. These data provide compelling evidence that the unknown amino acid sensor lies at, and likely within, the lysosome.

Here, we briefly discuss a few of the many other factors that have been found to influence amino acid sensing by mTORC1. For any putative amino acid-sensing pathway upstream of

mTORC1 it will be critical to determine how it interfaces with the Rag proteins, as their regulation appears to represent the most proximal event to mTORC1 activation in response to amino acids. Leucyl-tRNA Synthetase (LRS) has been proposed through independent studies in yeast⁷⁰ and mammalian cells⁷¹ to function as an amino acid sensor that regulates the Rag proteins, but major mechanistic differences exist between these studies. Most notably, the yeast study suggested that LRS regulates Gtr1, the RagA/B ortholog, whereas the mammalian study suggested specific regulation of RagD, a Gtr2 ortholog. Another study has indicated that p62 (also known as sequestome 1 or SQSTM1), which targets proteins for degradation via autophagy and that, itself, is a substrate of autophagy, is involved in amino acid sensing by mTORC1 through a direct interaction with RagC/D⁷². However, unlike components of the Rag-Ragulator circuit, p62 is dispensable for insulin to stimulate mTORC1. Mitogen-activated protein kinase kinase kinase 3 (MAP4K3) is activated in response to amino acids and functions upstream of the Rag GTPases to promote mTORC1 signaling in both *Drosophila* and mammalian systems^{73–75}. Understanding the nature of the molecular connection of MAP4K3 to both amino acids upstream and the Rags downstream requires further study. Interestingly, the two deamination steps of glutaminolysis, which convert glutamine to α -ketoglutarate have been shown to influence the ability of leucine and glutamine to stimulate mTORC1 signaling⁷⁶. While the molecular basis of this link is unknown, it appears that glutaminolysis promotes GTP-loading of RagB, and the product α -ketoglutarate can stimulate mTORC1 translocation to the lysosome even in the absence of amino acids. The vertebrate-specific protein SH3BP4 has been found to directly bind to the Rags and attenuate the stimulation of mTORC1 signaling by amino acids⁷⁷. The relationship between other emerging amino acid-sensing pathways and the Rag GTPases is poorly understood. These include the class III PI3K VPS34 and phospholipase D (discussed further in Box 1)^{130–132}, the G protein-coupled taste receptors (T1R1 and T1R3)⁷⁸, and the inositol polyphosphate multikinase (IMPCK)⁸². It is clear from these and other studies that there will be many cellular pathways and processes that impinge, either directly or indirectly, on the emerging amino acid-sensing system at the lysosome to influence the basal state of mTORC1 activation.

BOX 1

Signals from endogenous and dietary lipids

Relatively little is known regarding how intracellular and dietary lipids influence mTORC1 signaling. Two lipid signaling molecules found on endomembranes, phosphatidylinositol-3-phosphate (PI3P) and phosphatidic acid (PA), and the enzymes that produce them, Vps34 and phospholipase D (PLD) respectively, have been found to promote mTORC1 signaling in some settings^{130–132}. These enzymes and phospholipids have been partially localized to late endosomes and lysosomes, and their regulation of mTORC1 signaling might be tied together. The activities of VPS34 and PLD appear to be responsive to both amino acids and glucose, and their knockdown can impair acute mTORC1 activation by these nutrients^{79,80,133,134}, though it has been pointed out that these effects can often be compensated for under steady state conditions¹³¹. Through the production of PI3P, Vps34 activity has been proposed to recruit PLD to the lysosome, where its product PA is believed to stimulate mTORC1^{131,133}. However, genetic ablation of Vps34 in *Drosophila* or in specific mouse tissues has no apparent effect on mTORC1 signaling^{135,136}, and PLD-deficient flies and both *PLD1*^{−/−} and *PLD2*^{−/−} mice are viable, suggesting a non-essential role for these enzymes in activating mTORC1^{137,138}. It is worth noting that other cellular sources of these lipids do exist, but the enzymes responsible for their production have not been specifically implicated in mTOR signaling. Further studies are required to clarify how these lipid signaling pathways fit into the emerging model of spatial integration of signals by mTORC1 at the lysosome.

In mammals, high fat diets and obesity are associated with elevated mTORC1 signaling in metabolic tissues, which is believed to contribute to the development of insulin resistance under such conditions^{139–142}. The molecular nature of this chronic mTORC1 activation and whether there are cell autonomous effects of dietary lipids on mTORC1 signaling are not well understood. Free fatty acids have been described in a few studies to acutely stimulate mTORC1 signaling^{142–146}. The upstream events that mediate this effect are unknown but could depend on either energy production through the β -oxidation of fatty acids or signaling from extracellular fatty acids engaging specific GPCRs¹⁴⁷. However, it is somewhat paradoxical that mTORC1 would be activated by free fatty acids, given that it can promote the *de novo* synthesis of fatty acids through its induction of SREBP^{19, 27}. The relationship between lipid sensing and synthesis by mTORC1 remains an important area of investigation, as aberrant activation of mTORC1 under conditions of obesity is believed to be a molecular factor underlying many of the pathological manifestations of obesity.

Integrating nutrient sensing with growth factor signaling: an emerging model

David Sabatini and colleagues have proposed that spatial regulation of mTORC1 through the Ragulator-Rag circuit, in conjunction with its requirement for the GTP-loading of Rheb, serves as a coincidence detector or a molecular “and gate”, allowing a hierarchy of signals to be integrated by mTORC1^{3, 66}. While growth factor signaling pathways that increase Rheb^{GTP} levels by impinging on the TSC complex are required for maximal mTORC1 activation, they fail to activate mTORC1 in the absence of amino acids. Rheb localizes on the surface of multiple endomembrane compartments, at least in part, through a C-terminal sequence that is farnesylated^{83–85}, a modification required for Rheb to activate mTORC1 signaling^{40, 86}. Importantly, a subpopulation of Rheb resides at the lysosome⁶⁶. Therefore, the recruitment of mTORC1 to this compartment through the Ragulator-Rag system in response to amino acids brings mTORC1 in contact with its essential upstream activator Rheb (Fig. 3B). This mechanism helps explain the dominance of amino acid signaling over growth factor signaling to mTORC1. Through the lysosomal shuttling of mTORC1, the amino acid sensing pathway facilitates the association of Rheb and mTORC1, but the ultimate activation of mTORC1 appears to be determined by the GTP/GDP-loading state of Rheb, which is controlled by the TSC complex. Interestingly, growth factor signaling pathways might also impinge on Rheb regulation directly at the lysosome, as the TSC complex also localizes to the lysosomal surface⁴⁵. This current spatial model of signal integration is enticing, but it will be important to more completely define the molecular mechanisms and temporal nature of these and other signals influencing both the activation and inhibition of mTORC1 signaling.

Multiple inputs from glucose, oxygen, and cellular energy levels

In addition to amino acids, the presence of two other essential cellular nutrients, glucose and oxygen, are sensed by mTORC1 and are required for both basal and maximal (growth factor-stimulated) activation of mTORC1. A decrease in the availability of glucose or oxygen to cells results in profound changes in cellular metabolism and can cause an acute, but often transient, drop in cellular energy levels, in the form of ATP. Cells respond to such changes by tipping the metabolic balance from anabolic processes that consume energy and carbon for macromolecular biosynthesis to catabolic pathways that produce energy. As a major promoter of anabolic processes, mTORC1 is a key target in this metabolic adaptation and is negatively regulated by decreases in glucose, oxygen, and/or energy levels^{87–89}. This

regulation is now known to occur through multiple interconnected adaptive response mechanisms lying upstream of mTORC1 (Fig. 4).

One of the first lines of defense against energy stress, defined as the depletion of cellular ATP, is acute activation of the AMP-dependent protein kinase (AMPK), which is activated by even subtle decreases in cellular ATP levels⁹⁰. Through numerous downstream targets, AMPK initiates an adaptive program that promotes catabolic metabolism and inhibits anabolic processes. For instance, AMPK stimulates autophagy, while inhibiting lipid and protein synthesis^{33, 90–92}. Critical to this adaptive response is the inhibition of mTORC1 signaling, which occurs through the AMPK-mediated phosphorylation of at least two pathway components, TSC2 and Raptor. Through phosphorylation of TSC2 on S1387, which acts as a priming event for subsequent phosphorylation of additional sites by GSK3, AMPK promotes the inhibition of Rheb and mTORC1 by the TSC complex^{93–95}. Hence, loss of any TSC complex component renders the mTORC1 pathway at least partially resistant to energy stress-inducing conditions, including glucose starvation, inhibition of glycolysis or oxidative phosphorylation, or hypoxia^{45, 93, 95–97}. AMPK has also been found to have a direct inhibitory input into mTORC1 by phosphorylating Raptor on S792⁹⁸. The relative contributions of the AMPK-mediated activation of the TSC complex, resulting in decreased levels of Rheb^{GTP}, and this more direct inhibition of mTORC1 are unknown. However, one possibility is that Raptor phosphorylation blocks basal activation of mTORC1, whereas AMPK-mediated phosphorylation of TSC2 overrides growth factor signaling through the TSC complex that would otherwise activate TORC1. Importantly, the regulation of mTORC1 by AMPK renders mTORC1 signaling sensitive to a rapidly expanding list of chemicals, xenobiotics, and natural products that activate AMPK, including commonly prescribed drugs such as metformin and aspirin⁹⁹.

Another major regulator of mTORC1 signaling that is involved in the adaptation to hypoxia, glucose starvation, and perhaps other cellular stresses is the protein REDD1 (regulated in development and DNA damage responses 1; also known as DDIT4, Dig2, and RTP801). The *Drosophila* orthologs of REDD1 were identified in a genetic screen for regulators of cell growth, with their overexpression or loss resulting in a respective decrease or increase in cell and organ growth, similar to that observed with TSC1 and TSC2 in the fly¹⁰⁰. Importantly, hypoxia stimulates the expression of these genes, and mammalian *REDD1*, through the stabilization and activation of HIF1^{100, 101}, which in the presence of oxygen is degraded through the action of oxygen-dependent prolyl-hydroxylases and the von-Hippel Lindau (VHL) E3-ubiquitin ligase¹⁰². The HIF1-mediated induction of REDD1 appears to be required for the sustained inhibition of mTORC1 signaling under hypoxia⁹⁶ (i.e., 1% oxygen). Genetic and cell biological evidence indicate that REDD1 inhibits mTORC1 signaling through the TSC complex, although the mode of action of REDD1 has yet to be fully elucidated^{96, 100, 103, 104}. The activation of HIF1, its induction of REDD1, and the subsequent inhibition of mTORC1 signaling in response to hypoxia appears to also require upstream input from the ATM tumor suppressor, a protein kinase best known for its role in the DNA damage response¹⁰⁵. Interestingly, REDD1 has also been found to be required for the inhibitory effects of energy stress on mTORC1 signaling, including that induced by glucose starvation, 2-deoxyglucose, and metformin^{106, 107}. Under these and other stress conditions that inhibit mTORC1, it is likely that REDD1 expression is driven by the transcription factors p53 or ATF4 rather than HIF1^{107–109}. The ATF4-mediated expression of REDD1 appears to be independent of energy stress, but rather is stimulated downstream of endoplasmic reticulum (ER) stress resulting from the deleterious effects of glucose starvation, 2-deoxyglucose, or hypoxia on the maturation of proteins in the ER^{108, 110, 111}. In general, the sensitivity of protein glycosylation and disulfide bond formation to the availability of glucose and oxygen, respectively, renders the ER and the adaptive stress response originating therein, known as the unfolded protein response, a key component of

cellular nutrient sensing. It seems likely that the relative contributions of acute activation of AMPK and the transcriptional induction of REDD1 to inhibitory signals affecting mTORC1 in response to hypoxia will depend on many factors, including the duration of exposure to hypoxia, the dependence of the cell on oxidative metabolism, the secretory properties of the cell, and the tissue microenvironment^{97, 112, 113}. Similar principles are likely to apply to glucose deprivation and various other forms of nutrient and energy stress.

Additional mechanisms by which glucose and energy stress impinge on mTORC1 signaling independently of the TSC complex have also emerged. Depletion of cellular energy levels can activate the stress-responsive mitogen-activated protein kinase p38 and its direct target p38-regulated/activated kinase (PRAK), albeit through an unknown mechanism^{114, 115}. In cells deficient for either p38 β or PRAK, mTORC1 signaling is largely unresponsive to 2-deoxyglucose, which blocks glycolysis and other glucose-utilizing processes, suggesting that the p38 β -PRAK pathway is required to suppress mTORC1 signaling under such conditions¹¹⁵. It appears that this pathway inhibits mTORC1 signaling through the PRAK-mediated phosphorylation of S130 on Rheb, which is proposed to disrupt its ability to bind GTP. It is worth noting, however, that independent studies have found that p38-dependent signaling stimulates, rather than inhibits, mTORC1^{116–118}. Severe conditions of energy stress, such as that caused by combined glucose and glutamine starvation in cell culture, can even impair the assembly of mTORC1, thereby overriding all other upstream regulatory events¹¹⁹. This occurs by disrupting the association between mTOR and the Tel2-Tti1-Tti2 (TTT)-RUVBL1/2 complex, which is required for the proper folding and stability of mTOR and related kinases^{120, 121}. Within the TTT-RUVBL complex, the ATPase activity of RUVBL is susceptible to cellular ATP depletion, and loss of this activity results in a defect in higher order assembly of mTORC1 into homodimers¹¹⁹, which is the functional signaling complex¹²².

Genetic evidence has emerged that the Rag GTPases are also involved in glucose sensing by mTORC1. Efeyan and colleagues⁶⁵ found that mouse embryonic fibroblasts that are homozygous for a constitutively GTP-bound mutant of RagA (*RagA^{GTP}*) are resistant to the inhibitory effects of either amino acid or glucose withdrawal on mTORC1 signaling. Furthermore, due to an inability to downregulate mTORC1 in response to a natural drop in blood glucose levels immediately after birth, *RagA^{GTP/GTP}* neonates perish prior to suckling. An independent study also provided evidence that the Rag GTPases are involved in the sensing of some forms of energy stress by mTORC1¹²³. It is predicted from these studies that the GTP/GDP-loading state of the RagA/B-RagC/D heterodimer is affected by glucose withdrawal or energy stress-inducing conditions, but like amino acids, the sensing mechanism is currently unknown. It is interesting to note that in both yeast and mammalian cells, glucose starvation has been found to induce an acute disassembly of the v-ATPase, and this is rapidly reversed by reintroduction of glucose^{124, 125}. Such a mechanism could influence the ability of the v-ATPase to stimulate Ragulator GEF activity, thereby blocking RagA/B-GTP loading and the recruitment of mTORC1 to the lysosome under glucose starvation.

The myriad of mechanisms that have been uncovered thus far by which decreases in intracellular glucose, oxygen, and/or ATP lead to inhibition of mTORC1, even in the presence of growth factors, underscore the importance of attenuating mTORC1 signaling and its downstream anabolic processes for the adaptation to such states of nutrient and energy depletion.

Conclusions and outstanding questions

The mTORC1 signaling network integrates information about the complex nutrient environment of individual cells, tissues, and organisms to mount an appropriate physiological response to that environment. While impressive progress has been made over the past decade in understanding mTORC1 signaling, critical questions remain. For instance, outside of AMPK and the prolyl hydroxylases upstream of HIF1, direct sensors of nutrients and metabolites within the upstream signaling network have not been identified. Importantly, the molecular details by which signal sensing on mTORC1 regulation have been revealed, almost exclusively, through cell culture studies under largely non-physiological conditions. The experimental comparison of two extreme conditions, such as complete removal of a specific nutrient followed by acute refeeding, has been essential to provide robust biochemical and cell biological readouts to characterize the signaling mechanisms underlying a given response. While the molecular pathways characterized in such studies are likely to be similar *in vivo*, we must define the signals that dominantly control mTORC1 in different tissues, where unlike cell culture models, mTORC1 is generally in the “off” state and only transiently activated in response to specific stimuli. Cell culture experiments suggest that nutrients only basally activate mTORC1, but the relative contribution of nutrient and growth factor signals to mTORC1 activation *in vivo* is poorly understood. Conditions referred to as “energy stress” in cell culture models, which are maintained under super-physiological levels of growth factors and nutrients, are likely to be closer to the homeostatic state *in vivo*. This is illustrated by the fact that loss of the LKB1 tumor suppressor, which is required for AMPK activation by energy stress, results in the formation of gastrointestinal polyps that exhibit high levels of mTORC1 signaling relative to the normal epithelium⁹⁵. Therefore, removal of the inhibitory signal from AMPK in this setting is sufficient to activate mTORC1. Consistent with cell culture studies, mTORC1 signaling in the liver is inhibited under fasting conditions through a pathway dependent on the TSC complex¹²⁶. However, feeding induces a robust and transient activation of mTORC1 in this tissue through a mechanism that appears to be independent of insulin signaling¹²⁷, suggesting that an unknown nutrient input might be dominating this response. Under conditions of dietary (or calorie) restriction, which has been shown to prolong the lifespan of many organisms through mechanisms believed to involve mTORC1 inhibition, the attenuation of mTORC1 signaling in different tissues is likely to reflect decreases in specific local nutrients, as well as circulating insulin and IGF1^{128,129}. The importance of defining the molecular mechanisms and hierarchy of signals that regulate mTORC1 signaling *in vivo* is underscored by the diverse disease settings in which mTORC1 is aberrantly activated, including aging-related diseases such as cancer and diabetes.

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Figure 1. mTORC1 signaling links cellular growth conditions with metabolic processes underlying anabolic cell growth and proliferation

Many physiological and pathological signals affect the activation status of mTORC1, including cellular nutrients and energy, growth factors, oncogenes and tumor suppressors, and a variety of intracellular pathogens (i.e., infectious agents). When activated, mTORC1 regulates a number of cellular processes, with those affecting the metabolic state of the cell emphasized in this model. Through various downstream mechanisms, mTORC1 signaling inhibits autophagy, while stimulating mRNA translation, glycolysis, lipid synthesis, the pentose phosphate pathway, and de novo pyrimidine synthesis, thereby promoting the production of energy (ATP), reducing equivalents (NADPH), and the major macromolecules required for cell growth.

Figure 2. Secreted growth factors stimulate mTORC1 activity through the PI3K-Akt and Ras-Erk pathways

Through receptor tyrosine kinases (RTKs), a variety of secreted growth factors stimulate the recruitment and activation of PI3K, via binding to RTKs or scaffolding adaptor proteins. PI3K activity generates phosphoinositide-3,4,5-trisphosphate (PIP3), which recruits Akt to the plasma membrane, where it is activated by upstream kinases (not pictured). Ras is also activated downstream of RTKs and stimulates a kinase cascade leading to the activation of ERK and RSK. In response to growth factors, the Akt, ERK, and RSK protein kinases phosphorylate specific residues on TSC2 (inset) within the TSC complex, thereby negatively regulating the ability of this complex to act as a GAP for Rheb. Consequently, GTP-bound Rheb accumulates and activates mTORC1. Parallel inputs into mTORC1 from these kinases also exist, with Akt phosphorylating PRAS40 and ERK and RSK both phosphorylating residues on Raptor. Oncogenes and tumor suppressors mutated in human cancers and tumor syndromes are indicated (*).

Figure 3. Model of the Rag-dependent recruitment of mTORC1 to the lysosome in response to amino acids and the integration with Rheb-dependent growth factor signaling

(A) Amino acid-stimulated recruitment of mTORC1 to the lysosome. Under amino acid depletion conditions, the Ragulator is not active as a RagA/B GEF and RagA/B^{GDP}-RagC/D^{GTP} heterodimers that accumulate are unable to recruit mTORC1 to the lysosome. In the presence of amino acids, which enter the lysosome, an unknown signal or sensor within the lysosomal lumen triggers a conformational change within the V-ATPase that, through direct interactions, promotes the Ragulator GEF activity. In this manner, amino acids stimulate the formation of RagA/B^{GTP}-RagC/D^{GDP} heterodimers, which bind directly to mTORC1 and recruit the inactive kinase complex to the lysosomal surface. (B) The amino acid signal allows mTORC1 to come in contact with its essential upstream activator Rheb. In the absence of growth factors, Rheb is maintained in its GDP-bound state through the GAP activity of the TSC complex, and mTORC1 remains inactive. Growth factor signaling inhibits the TSC complex, allowing formation of Rheb^{GTP}, which binds to and activates mTORC1.

Figure 4. Transcriptional and post-translational regulation of mTORC1 by glucose, oxygen, and cellular energy

Sufficient glucose and oxygen levels are required for mTORC1 activation, and many sensing mechanisms have been identified. Glucose, glutamine, and oxygen are utilized for ATP production via glycolysis, the TCA cycle, and oxidative phosphorylation. Decreased availability of these nutrients can lower ATP levels, with a subsequent rise in AMP levels, conditions that stimulate the activation of AMPK. AMPK inhibits mTORC1 through activation of the TSC complex, which inhibits Rheb, and phosphorylation of Raptor within mTORC1. Glucose or oxygen deprivation, as well as other forms of energy stress, also stimulates the transcription of REDD1 through the action of either the HIF1, ATF4, or p53 transcription factors. REDD1 somehow cooperates with the TSC complex to inhibit Rheb and mTORC1. Through their sensing of AMP and oxygen, respectively, AMPK and the PHD proteins (in yellow) represent the only known direct sensors of cellular metabolic status within this network. In addition to energy stress, glucose and oxygen can also be sensed through ER homeostasis, as they are required for proper protein glycosylation and disulfide bond formation, respectively. Disrupting these processes results in activation of PERK and inhibition of eIF2 α , which results in the selective translation of ATF4. Glucose starvation and energy stress also appear to signal to mTORC1 via the Rag GTPases, albeit through unknown mechanisms, and through a pathway involving the p38 and PRAK kinases leading to direct phosphorylation of Rheb. Severe states of ATP depletion inhibit the ability of the TTT-RUVBL1/2 complex to promote formation of functional mTORC1 dimers. Note:

mTORC1 is depicted as a single unit at the lysosome for simplicity. Compounds such as 2-deoxyglucose (2-DG), AICAR, and the biguanides metformin and phenformin also have inputs into these different mechanisms of mTORC1 inhibition. Dashed lines denote unknown molecular mechanisms.