AMPK and mTOR in Cellular Energy Homeostasis and Drug Targets

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Abstract

The mammalian target of rapamycin (mTOR) is a central controller of cell growth and proliferation. mTOR forms two distinct complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). mTORC1 is regulated by multiple signals such as growth factors, amino acids, and cellular energy and regulates numerous essential cellular processes including translation, transcription, and autophagy. The AMP-activated protein kinase (AMPK) is a cellular energy sensor and signal transducer that is regulated by a wide array of metabolic stresses. These two pathways serve as a signaling nexus for regulating cellular metabolism, energy homeostasis, and cell growth, and dysregulation of each pathway may contribute to the development of metabolic disorders such as obesity, type 2 diabetes, and cancer. This review focuses on our current understanding of the relationship between AMPK and mTORC1 signaling and discusses their roles in cellular and organismal energy homeostasis.

AMP-ACTIVATED PROTEIN KINASE

As a key physiological energy sensor, AMP-activated protein kinase (AMPK) is a major regulator of cellular and organismal energy homeostasis that coordinates multiple metabolic pathways to balance energy supply and demand and ultimately modulate cellular and organ growth (1). AMPK is an evolutionarily conserved serine/threonine protein kinase and a member of the AMPK-related kinase family that consists of 13 kinases in the human genome. The activation of AMPK-related kinases except for MELK requires phosphorylation of their activation loops by upstream kinases such as LKB1, which was initially identified as a tumor suppressor (2, 3). LKB1 requires two adaptor proteins, STRAD and MO25, to be a functional kinase in phosphorylation of AMPK (4, 5).

The AMPK holoenzyme is a trimer consisting of one α subunit, one β subunit, and one γ subunit; α is the catalytic subunit and β and γ are regulatory subunits (6). In mammals, each subunit has multiple subtypes and expresses differentially in different tissues. For instance, there are two isoforms of the catalytic α subunit (α1 and α2), whereas β and γ subunits have two (β1 and β2) and three (γ1, γ2, and γ3) isoforms, respectively (1). AMPK phosphorylates many substrates regulating the balance of intracellular energy levels. The activation loop of AMPK in the α subunit can be phosphorylated by upstream kinases, including LKB1, calcium/calmodulin-dependent protein kinase-β (CaMKK β), and TAK1 (7–9). The β subunit functions as a hinge, bringing both α and γ subunits together. It also plays an important role in the cellular localization of the complex via its myristoylation motif and carbohydrate binding domain (10, 11). A recent study revealed that branched oligosaccharides and glycogen inhibit AMPK activity (12). Thus, the β subunit may be an important regulatory unit sensing availability of cellular energy by recognizing the levels and/or the formation of glycogen. The γ subunit contains four repeats of the CBS domain, a motif originally recognized as the Bateman domain (13, 14). Two CBS domains form a pocket and create binding sites for two adenosine molecules including AMP, ADP, and ATP. Structural analyses have revealed that two of the four binding sites appear to bind AMP, ADP, or ATP in an exchangeable manner. The third site prefers to permanently bind AMP but not other forms of adenosine. The fourth site seems unable to function as a binding site even in the presence of high concentrations of AMP (15, 16).

The activity of AMPK is allosterically enhanced by AMP binding to the γ subunit. The binding of ATP or ADP to the γ subunit does not induce allosteric activation of AMPK. However, the major effect of AMP binding is to affect the activation loop phosphorylation of AMPK, which plays the most prominent role in AMPK activation. Therefore, antibodies that recognize the activation loop phosphorylation have been widely used as an indirect measurement for AMPK activity. Binding of AMP to the γ subunit protects the activation loop from dephosphorylation by the phosphatases such as PP2C, therefore leading to AMPK activation. Recent studies have demonstrated that ADP may also play a regulatory role in AMPK activation (17). Cellular concentrations of AMP or ADP are much lower than those of ATP (18, 19). Therefore, a small decrease of ATP will result in a relatively large increase of ADP and AMP. Given the above mechanisms, AMPK is able to sense small changes in cellular energy charge by monitoring AMP and ADP. Thus, AMPK is able to maintain cellular energy homeostasis at a very constant level.

AMPK is activated by a variety of cellular stresses that decrease ATP generation including metabolic poisons as well as pathologic cues such as nutrient starvation, ischemia, and hypoxia. Under these conditions, the activated AMPK phosphorylates many substrates that turn on alternative catabolic pathways to generate more ATP. It also phosphorylates some substrates that switch off anabolic biosynthetic pathways to prevent further ATP consumption.

The role of AMPK in the metabolic regulation is not the focus of this article. Excellent reviews on this topic can be found elsewhere (20–22). Nevertheless, it is important to note that AMPK

phosphorylates acetyl CoA carboxylases (ACC1 and ACC2) (23), CERB-regulated transcriptional coactivator-2 (CRTC2) (24), TBC1D1/AS160 (25, 26), PPARγ coactivator-1α (PGC1α) (27), and histone deacetylase 5 (HDAC5) (28). ACC1 and ACC2 are key enzymes for fatty acid synthesis and oxidation (29). AMPK-dependent phosphorylation of ACC inhibits its enzyme activity to suppress malonyl-CoA synthesis, thereby relieving inhibition of fatty acid uptake into mitochondria and enhancing fatty acid oxidation. Thus, AMPK allows cells to utilize an alternative source of energy such as lipids when the cells are not able to obtain energy from carbohydrates, the preferred energy source. In addition to this metabolic switch, AMPK stimulates gene expression of glucose transporter 4 (GLUT4) by inhibiting HDAC5 activity as well as glucose uptake by inducing GLUT4 translocation through inhibition of TBC1D1 and AS160, two Rab-GAP (GTPase-activating protein) proteins (28). AMPK phosphorylates and inhibits AS160, leading to Rab activation and increased plasma membrane localization of GLUT4 and glucose uptake. Furthermore, it has been postulated that AMPK-dependent phosphorylation of PGC1α stimulates mitochondrial biogenesis in muscle, whereas the phosphorylation of CRTC2 (also known as TORC2, not to be confused with the mTOR complex 2, mTORC2) by AMPK inhibits gluconeogenic gene expression in the liver (24, 27). Activation of the AMPK system in response to acute metabolic stresses is important for the survival of cells as well as whole organisms during energy crises. In addition, chronic inactivation of the AMPK system not only may have deteriorative effects on cell or organism survival, but also can contribute to the development of metabolic disorders such as type 2 diabetes and obesity.

MAMMALIAN TARGET OF RAPAMYCIN

Mammalian target of rapamycin (mTOR) is an evolutionarily conserved serine/threonine protein kinase that regulates multiple cellular processes such as cell growth, cell cycle, cell survival, and autophagy. mTOR forms two functional complexes, mTORC1 and mTORC2 (30). mTORC1 exists as a multiprotein complex containing mTOR, Raptor, mLST8 (GβL), and PRAS40 (31–35), whereas mTORC2 consists of mTOR, Rictor, mSin1 (MAPKAP1), Protor (PRR5), and mLST8 (36–41). The configuration of each complex is conserved from yeast to mammals (30). mTORC1 is directly regulated by cellular energy and nutrient status, whereas mTORC2 is not. mTORC1 plays essential roles in the regulation of translation and autophagy and is sensitive to inhibition by rapamycin. Raptor, a component of mTORC1, functions as a scaffolding protein to recruit substrates such as S6 kinase (S6K) and eIF4E binding proteins (4EBPs) for phosphorylation by mTORC1 (42–44). Interestingly, recent studies have shown that Raptor also plays a significant role in intracellular localization of mTORC1 in response to amino acid availability, which is an essential cellular cue for mTORC1 activation (45).

DOWNSTREAM OF MTORC1

The most well-characterized substrates for mTORC1 are ribosomal protein (RP) S6 kinase (S6K) and eukaryotic initiation factor 4E binding protein 1 (4EBP1) (46–48) (**Figure 1**). mTORC1 primarily phosphorylates Thr389 of S6K1. Thr389 phosphorylation on S6K1 recruits phosphoinositide-dependent kinase-1 (PDK1) and enhances PDK1-dependent Thr229 phosphorylation in the activation loop of S6K1, a process essential for S6K1 activation. S6K1 phosphorylates many substrates such as eIF4B, PDCD4, Skar, and S6, thereby regulating translation initiation, mRNA processing, and cell growth (49–52). Ablation of *Drosophila* S6K1 results in a reduction in cell size and body size (53). Furthermore, disruption of the *S6K1* gene in mice displays a phenotype with small body size (54).

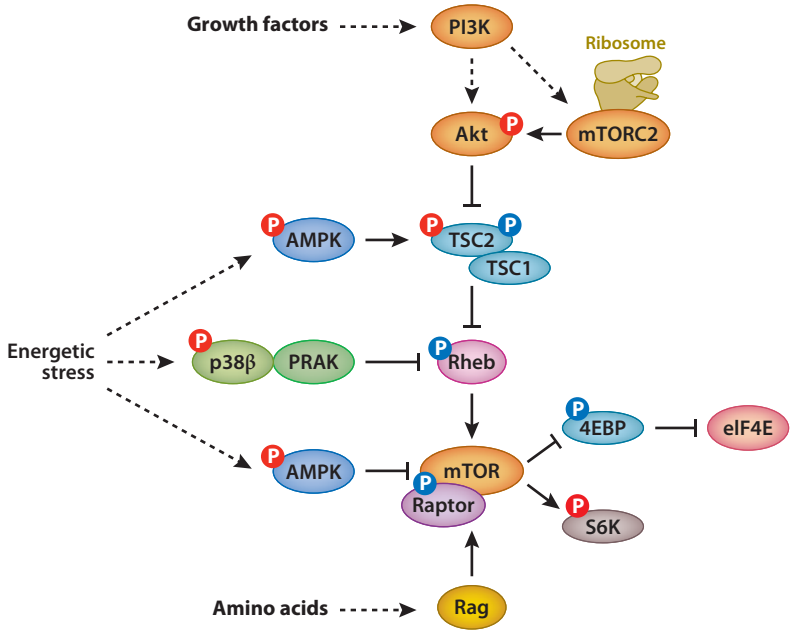

Figure 1

AMP-activated protein kinase (AMPK) inhibits the pathway of the mammalian target of rapamycin complex 1 (mTORC1) in multiple fashions. Under energetic stress conditions, AMPK phosphorylates TSC2 and Raptor to inhibit the mTORC1 pathway.

4EBP1 is a member of the 4EBP family (4EBP1, 2, and 3) that functions as a repressor of translation initiation (55). 4EBPs have a eukaryotic translation initiation factor 4E (eIF4E) binding domain, which is shared by eIF4G, an essential scaffolding protein that forms the eIF4F complex. Hypo-phosphorylated 4EBP1 strongly interacts with eIF4E, thereby interfering with the binding between eIF4E and eIF4G. Upon mTORC1-dependent 4EBP1 phosphorylation, the 4EBP1 dissociates from eIF4E, thereby relieving the inhibitory effect of 4EBP1 on eIF4E-dependent translation initiation. Recent studies have shown that 4EBPs are crucial elements of the mTORC1 pathway that regulate cell number and proliferation. mTORC1 also phosphorylates the unc-51-like kinase 1 (ULK1), a mammalian homolog of ATG1, which is the serine/threonine protein kinase that triggers autophagy initiation (56). Phosphorylation of ULK1 by mTORC1 represses the kinase activity of ULK1, thereby suppressing the initiation of autophagy (57, 58) (see discussion below).

MTORC1 AND RIBOSOME BIOGENESIS

In addition to its role in translation initiation, mTORC1 is also involved in ribosomal biogenesis. Studies in yeast and mammalian cells have demonstrated that TOR activity couples nutrient availability to regulate ribosome biogenesis. Interestingly, acute inhibition of TORC1 activity with rapamycin suppresses translation, although this occurs more slowly than does transcription of ribosomal gene expression (59, 60). Ribosome biogenesis requires the coordinated activities of all three RNA polymerases (Pol I, II, and III) and consumes a large amount of total cellular energy. Pol I accounts for the transcription of rRNA, a noncoding RNA that is an essential component of ribosomes. Upon amino acid starvation, the activity of Pol I transcription is rapidly decreased

(61). Similar results are seen with rapamycin treatment, indicating that mTORC1 plays a role in nutrient-dependent regulation of Pol I transcription (62). Pol I forms a transcription initiation complex with TIF-IA, TIF-IB/SL1, and UBF (63, 64). Among these components, TIF-IA and UBF are the targets of the mTORC1 pathway (62, 65). In recent studies, rapamycin-dependent Pol I transcriptional repression was restored by exogenous TIF-IA, mTOR, or S6K1. Upon rapamycin treatment, TIF-IA, the mammalian homolog of *Saccharomyces cerevisiae* Rrn3p, translocates from the nucleus into the cytoplasm, and the association of TIF-IA with both Pol I and TIF-IB/SL1 is diminished. Furthermore, rapamycin decreases TIF-IA activity by decreasing phosphorylation at Ser44 and increasing phosphorylation at Ser199. These results suggest that mTOR- or S6K1-dependent kinase and phosphatase regulate TIF-IA in multiple manners, thereby controlling rRNA expression. Notably, a recent study demonstrated that AMPK directly phosphorylates Ser635 of TIF-IA and inhibits rRNA synthesis, highlighting an integration of AMPK and mTOR signaling at the transcriptional level (66).

RP gene expression is mediated by Pol II. Despite a considerable body of evidence about the relationship between TORC1 and RP gene expression in yeast, there is limited information regarding the impact of mTORC1 signaling on Pol II in mammalian systems. In yeast, TORC1 upregulates RP gene expression by regulating the shuttling of a transcription factor as well as corepressors that are coupled to Pol II function in RP gene expression. SFP1, a positive regulator for RP gene transcription, binds to RP gene promoters and enhances their expression in a TOR-dependent manner. In the presence of rapamycin as well as nutritional stresses, SFP1 is restricted to the cytoplasm, thereby reducing RP gene expression (67, 68). TORC1 also controls the expression of RP genes via the forkhead transcriptional factor (FHL1) and its coactivator IFH1 and corepressor CRF1 in *S. cerevisiae* (69). Under growth conditions, FHL1 binds to RP gene promoters with IFH1 and upregulates RP transcription, whereas CRF1 is excluded from the nucleus through a TORC1-dependent protein kinase A activity (70). Upon nutrient deprivation, CRF1 translocates into the nucleus and competes with IFH1 to interact with FHL1, leading to suppression of RP gene transcription. These observations clearly indicate that TORC1-mediated RP gene expression is pivotal for ribosomal biogenesis in yeast.

In mammals, the evidence that mTORC1 is actively involved in Pol II–dependent RP gene transcription is limited, although ample studies show that mTORC1-dependent RP gene expression is largely regulated at the level of translation. All functional RP genes contain a polypyrimidine tract (5′ TOP, terminal oligopyrimidine) sequence at the 5′ end of their mRNA (71). It has been postulated that mTORC1/S6K1-dependent phosphorylation of S6 may play a critical role in the translation of 5′ TOP mRNA (72). However, two genetic studies using the phospho-defective S6 knock-in mice and S6K1/2 double-knockout mice have revealed that mTORC1-dependent 5′ TOP mRNA translation requires neither S6K nor phosphorylated S6 (73, 74). Therefore, the mechanism by which mTORC1 controls the translation of 5′ TOP mRNAs including RP mRNA remains to be elucidated (75).

Pol III synthesizes 5S rRNA and tRNA. Recent studies have demonstrated that mTORC1 interacts with TFIIIC, a Pol III–specific transcription factor, and directly phosphorylates MAF1, a repressor of Pol III in the nucleus (76, 77). mTORC1-dependent MAF1 phosphorylation relieves its inhibitory effect on transcription, thereby inducing Pol III–dependent transcriptional activity. These data indicate that mTORC1 plays an important role in the regulation of 5S rRNA and tRNA gene expression.

Given the essential roles of mTORC1 function in ribosome synthesis and its overall function, dysregulation of the mTORC1 pathway may be linked to ribosome-associated human diseases such as neurodegenerative disease and cancer (78, 79). Hyperactivation of mTORC1 and overexpression of RPs have frequently been shown to be associated with tumor development (80). Strikingly, a

recent study from Hall's group demonstrated that mTORC2 associates with and is activated by ribosomes in response to PI3K signaling (81) (**Figure 1**). Notably, the translational activity driven by the ribosomes is not required for mTORC2 activation, suggesting that ribosome functions as a platform for the kinase reactions of mTORC2 to phosphorylate its substrates such as Akt (82). Thus, both the function and the amount of cellular ribosomes are key factors in the regulation of cell growth and survival.

REGULATION OF MTORC1 BY THE GROWTH FACTOR–TSC-RHEB PATHWAY

mTORC1 activity is modulated by multiple upstream factors including growth factors and nutrients such as glucose and amino acids (**Figure 1**). The molecular mechanisms by which these upstream signals regulate mTORC1 have been extensively investigated. The results of these studies have indicated that the small GTPase Rheb is the most proximal molecule with a key role in the regulation of mTORC1 activity (83). Previous studies demonstrated that Rheb directly interacts with mTOR (84). As is the case of other small GTPases, the GTP-bound form of Rheb is active, whereas the GDP-bound form is inactive (85). Importantly, the purified active Rheb is able to activate mTORC1 kinase activity in vitro (34, 86). Although intermediates between active Rheb and mTOR could exist, compelling evidence indicates that Rheb is a direct activator of mTORC1.

The activity of Rheb is tightly regulated by the tuberous sclerosis gene products TSC1 and TSC2 (87–90). Tuberous sclerosis complex (TSC) is an autosomal dominant disease caused by mutations of either the *TSC1* or *TSC2* gene (91). TSC is characterized by the formation of multiple hamartomas in a wide array of organs (92). *TSC1* and *TSC2* gene products (hamartin and tuberin, respectively) form a physical and functional complex in vivo and function as tumor suppressors (93). TSC1 stabilizes TSC2 and may play a role in the cellular localization of the complex (93). TSC2 has a catalytic domain in its carboxyl terminus, which is a GAP for the Rheb small GTPase. The active Rheb (GTP-bound form) is converted to the GDP form of Rheb, as the late form is unable to stimulate mTORC1 activity. Thus, the TSC1/TSC2 complex is a negative regulator of the mTORC1 pathway, thereby functioning as a tumor suppressor (**Figure 1**).

Identification of the TSC as a negative regulator for the mTORC1 pathway significantly advanced our understanding as to how upstream signals such as growth factor, glucose, and many stresses regulate mTORC1. Previous studies had demonstrated that multiple growth-related kinases such as AKT, ERK, and RSK phosphorylate TSC2 and inhibit the function of the TSC, thereby activating the Rheb-mTORC1 pathway (94–99). Consistently, mTORC1 activity is constitutively active, and deprivation of growth factors fails to efficiently inhibit mTORC1 activity in TSC-deficient cells (100).

AMPK INHIBITS MTORC1 VIA MULTIPLE MECHANISMS

Extensive studies have also revealed that mTORC1 activity is modulated by intracellular energy levels via multiple mechanisms. In 2001, the first evidence demonstrating the relationship between cellular ATP levels and mTORC1 activity was reported (101). In 2002, a reciprocal relationship between the activation of AMPK and mTORC1 was shown (102). In 2003, direct evidence that AMPK inhibits mTORC1 activity was demonstrated (103). Afterward, several groups found that AMPK directly phosphorylates multiple components in the mTORC1 pathway (**Figure 1**). AMPK phosphorylates TSC2 and activates the TSC, thereby attenuating the TORC1 pathway (104). Consistent with this model, inhibition of mTORC1 activity by aminoimidazole carboxamide ribonucleotide (AICAR), an AMPK activator, or by glucose

deprivation is largely compromised in TSC1- or TSC2-deficient cells (105, 106). In AMPK α1 α2 double-knockout MEFs (mouse embryonic fibroblasts), AICAR fails to inhibit mTORC1 activity (107). Furthermore, glucose starvation inhibits cell growth in wild-type cells but not in TSC1-deficient cells (104). Concomitantly, the TSC1-deficient cells, but not wild-type cells, undergo massive cell death under glucose-deprivation conditions, and rapamycin treatment prevents the cell death in TSC-deficient cells under glucose starvation (108, 109).

Several mechanisms by which TSC-deficient cells undergo cell death upon glucose starvation have been proposed. They include accumulation of p53 tumor suppressor, enhanced endoplasmic reticulum (ER) stress, and reduction of survival kinases (108, 110–112). Therefore, multiple factors likely contribute to the hypersensitivity of TSC mutant cells to energy starvation. AMPK is able to stabilize p53, a major proapoptotic protein, via its Ser15 phosphorylation, and the constitutive activation of mTORC1 enhances p53 translation (108, 113). In combination, these two effects cause a massive accumulation of p53 in TSC-deficient cells with glucose starvation, thereby increasing susceptibility to cell death. In addition, hyperactive mTORC1 signaling has been reported to induce ER stress and activate the unfolded protein response. The continuous ER stress induced by mTORC1 activation causes a reduction in the insulin sensitive pathway as well as the survival pathway (110). Furthermore, active S6K also leads to the downregulation of insulin receptor substrate and generates the negative-feedback inhibition for the PI3K-Akt pathway (111, 114, 115). These observations indicate that survival signaling pathways are attenuated in TSC-deficient cells. More recently, Blenis and colleagues (109) demonstrated that mTORC1 inhibition in TSC-deficient cells with rapamycin allows cells to maintain ATP levels and attenuates AMPK activation under glucose-starvation conditions. These data also suggest that the TSC-mTORC1 pathway functions upstream of the AMPK. Under energetic stress conditions, inhibition of mTORC1 is critical to conserve energy for cell survival. For example, glutamate dehydrogenase–dependent glutamine metabolism via the TCA cycle becomes the main pathway to generate energy for cell survival in rapamycin-treated TSC-deficient cells under glucose-starvation conditions (109). Collectively, these observations indicate that the response of the intact AMPK-TSC signaling is essential to suppress the mTORC1 pathway to regulate cell survival and growth under energetic stress conditions.

Although the above mechanism has been highlighted as a linear signaling pathway for the regulation of mTORC1 under stress conditions, cells can also inhibit mTORC1 through AMPK-dependent direct regulation of mTORC1 involving Raptor (116) (**Figure 1**). The inverse regulation of TOR and AMPK by glucose levels is also conserved in *Caenorhabditis elegans* and *S. cerevisiae*, both of which lack a TSC2 ortholog, thereby raising the possibility that an alternative regulatory mechanism exists in the AMPK-dependent mTORC1 mediation in these organisms. Shaw and his colleagues (116) have found that AMPK also phosphorylates Ser722 and Ser792 on Raptor. Their study demonstrated that cells expressing a phospho-defective Raptor mutant with alanine substitutions at both Ser722 and Ser792 were resistant to AICAR-induced mTORC1 inhibition, indicating that AMPK-induced Raptor phosphorylation negatively regulates mTORC1 activity. However, a more recent study (106) has shown that AICAR fails to inhibit mTORC1 activity in TSC-deficient fibroblasts although Raptor is fully phosphorylated by AMPK. Interestingly, however, AICAR inhibits mTORC1 activity in TSC-deficient hepatocytes with an increase in Raptor phosphorylation. This study (106) further suggests that the involvement of the TSC in AMPK-induced mTORC1 regulation depends on the cell types and tissues studied.

A third mechanism by which glucose deprivation and AMPK activators such as AICAR and 2-deoxy-glucose (2DG) inhibit mTORC1 signaling has recently been demonstrated. Han and colleagues (105) have implicated the p38β-PRAK pathway in the downregulation of Rheb activity through a phosphorylation-dependent mechanism (**Figure 1**). Upon glucose starvation, AICAR,

or 2DG treatment, the p38β-PRAK pathway is activated in a manner independent of AMPK, and the activated PRAK directly phosphorylates Ser130 on Rheb. The phosphorylation of Rheb causes not only an impairment of its GTP binding, but also a dissociation of bound GTP from Rheb. Although it remains unclear whether Ser130 phosphorylation of Rheb induces the intrinsic Rheb GTPase activity or stimulates TSC2-dependent Rheb GTP hydrolysis, the study suggests that there is a reduction of guanine nucleotide binding, including GDP. Because p38β-PRAK-dependent Rheb phosphorylation occurs later than the initial inhibition of mTORC1 activity in AICAR-treated cells, TSC2- or Raptor-mediated mTORC1 inhibition may be responsible for the acute phase of mTORC1 suppression during energetic stress, whereas the p38β-PRAK-dependent regulation is responsible for the sustained mTORC1 inhibition.

Many studies have used different methods and chemicals to activate AMPK. For instance, glucose depletion or hypoxia is often used as a means to activate AMPK. Moreover, a variety of chemicals including AICAR, biguanides such as metformin and phenformin, glycolysis inhibitors (2DG), and metabolic poisons such as oligomycin and FCCP have been used to study the role of AMPK in the regulation of the mTORC1 pathway. As described above, the involvement of new players such as p38β-PRAK compels us to re-evaluate our view of AMPK-dependent mTORC1 regulation (105). Furthermore, Thomas and colleagues (107) have recently made the unexpected observation that metformin-induced mTORC1 inhibition depends on neither AMPK nor TSC. This work has demonstrated that phenformin inhibition of mTORC1 activity depends on Rag small GTPases, but the precise molecular mechanism by which phenformin modulates the Rag-mTORC1 pathway remains unclear.

REGULATION OF MTORC1 BY AMINO ACID–RAG PATHWAY

The mammalian Rag subfamily of GTPase is a Ras-related GTPase and consists of RagA, RagB, RagC, and RagD (117). RagA and RagB (RagA/B) are homologous to yeast Gtr1p, whereas the yeast Gtr2p is a homolog of RagC and RagD (RagC/D). A unique feature of this Rag complex is that RagA/B form a stable dimer with RagC/D. Moreover, in the Rag GTPase dimer, RagA/B must be in GTP form, whereas RagC/D must be in GDP form for the RAG complex to activate mTORC1 (45, 118). Recently Sabatini's group and our group (45, 118) found that Rag plays a crucial role in amino acid–sensitive mTORC1 regulation (**Figure 2**). Sancak et al. (45) demonstrated that the Rag heterodimer interacts with Raptor. Interestingly, the interaction between Raptor and Rag is dependent on the GTP binding state of RagA or RagB in the heterodimer. In contrast, the nucleotide binding status of RagC/D does not have a direct effect on the interaction between the Rag GTPase dimer and Raptor. Furthermore, the level of GTP-bound RagB is increased by amino acid stimulation. Knockdown of RagA/B or dominant negative RagA/B expression efficiently inhibits the mTORC1 pathway. In contrast, constitutively active RagA/B is able to activate mTORC1 activity during amino acid starvation, indicating that Rag functions downstream of amino acids to activate mTORC1 (45, 118). In *Drosophila*, loss of function of dRagA decreases cell size, wing, and fat bodies, whereas overexpression of dRagA increases cell size. Importantly, constitutively active dRagA suppresses starvation-induced autophagy in fat bodies (118). These observations indicate that Rag small GTPases relay signals from amino acids to activate the mTORC1 pathway.

The mechanism by which Rag activates mTORC1 has been further defined, revealing that Rag plays an essential role in the spatial regulation of mTORC1 in the cytoplasm. Rag small GTPases are constitutively expressed at the lysosomal membrane anchored by the MP1/p18/p14 complex (Ragulator) (119) (**Figure 2**). Unlike Rheb, active Rag has no capability to activate the kinase activity of mTORC1 in vitro, and instead it recruits mTORC1 to the lysosomal membrane

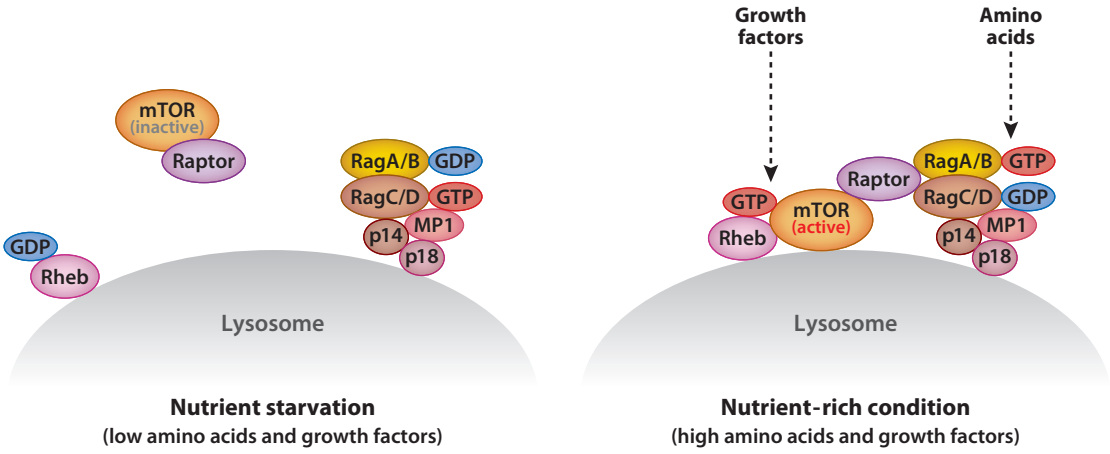

Figure 2

Spatial regulation of mammalian target of rapamycin complex 1 (mTORC1) on the lysosomal membrane. Upon amino acid stimulation, the Rag heterodimer anchored at the lysosomal membrane by the MP1/p14/p18 complex (Ragulator) recruits mTORC1 to the lysosome. Concomitantly, growth factors stimulate Rheb on the lysosomal membrane, thereby facilitating Rheb-induced mTORC1 activation.

in vivo (45). These data suggest that mTORC1 may translocate to the lysosome in a manner dependent on Rag, whose activity is regulated by amino acid availability. Because exogenous Rheb can be expressed on the lysosomes, amino acid–induced Rag activation helps to link mTORC1 and Rheb on the lysosomes, thereby enhancing mTORC1 activity (**Figure 2**). This spatial regulation of mTORC1 via Rag and Rheb explains how the signals from amino acids and growth factors are integrated and then activate the mTORC1 pathway.

More recently, Rubinsztein and colleagues (120) demonstrated that nutrients such as amino acids regulate lysosomal positioning that plays a critical role in the regulation of mTORC1 activity and autophagy. The study used mild amino acid starvation to examine mTORC1-lysosome localization and found that mTORC1 remains on lysosomes even when its activity is significantly reduced. Under these conditions, lysosomes translocate the perinuclear region with mTORC1. These observations suggest that dissociation of mTORC1 from lysosomes in response to amino acid starvation may not be the sole basis for the inhibition of mTORC1 activity. The study further demonstrated that KIF1Bβ and KIF2, two kinesin proteins, as well as ARL8B (small GTPase ADP-ribosylation factor-like 8B) play a positive role in the redistribution of lysosomes to the cell periphery and the activation of mTORC1 in response to amino acid stimulation. It is possible that mTORC1 remains in the late endosomal LAMP2-positive compartment and that subsequently these compartments redistribute to the cell periphery in response to increases in amino acid concentrations. Consistent with this idea, loss of function of p18, a component of the Ragulator, also disrupts lysosomal maturation and positioning (121). Therefore, Rag-Ragulator may function not only in the recruitment of mTORC1 but also in the trafficking of lysosomes, possibly via the kinesins and ARL8. Interestingly, expression of a loss of function of ARL8B increases autophagosome formation as well as autophagosome-lysosome fusion. The study illustrates the important role of the dynamics of lysosomes for both amino acid–induced mTORC1 activation and for limiting the process of autophagy.

The importance of organelle and protein trafficking for mTORC1 activation in response to amino acids is consistent with recent reports that the Rab small GTPases have a role in mTORC1

activation (122). The Rab family GTPases play a key role in intracellular vesicle trafficking. Expression of constitutively active Rab, including Rab5, Rab7, Rab11, and Rab31, selectively blocks mTORC1 activation in response to amino acids. Surprisingly, expression of dominant negative Rab GTPases also inhibits mTORC1. Although the precise mechanisms by which Rab family small GTPases regulate mTORC1 remain unclear, it is likely that the Rab family may be involved in the control of trafficking for the essential components in amino acid–sensitive mTORC1 signaling.

Besides the Rag family GTPases, both VPS34 and MAP4K3 have also been implicated in mTORC1 activation in response to amino acids (123, 124). The role of VPS34 is complicated; VPS34 likely regulates the mTORC1 pathway in an indirect manner, because it also plays a key role in the intracellular trafficking machinery (125). Moreover, VPS34 is essential for inducing autophagy (126), which may contribute to mTORC1 activation by increasing intracellular amino acids as a result of autophagy-based degradation of cellular proteins (see next section). Good evidence also supports a role for MAP4K3 in mTORC1 activation by amino acids. However, this role may be limited because MAP4K3 knockdown delays mTORC1 inactivation by amino acid withdrawal (124). With such a wide array of potential regulators in the amino acid–sensitive mTORC1 pathway, it is clear that the mechanisms governing mTORC1 activation by amino acids are much more complicated than anticipated, and our current understanding of these signaling pathways remains far from complete.

REGULATION OF AUTOPHAGY BY MTOR AND AMPK

Autophagy is a cellular degradative process that functions to maintain fundamental biological activities during cellular stresses, especially nutrient starvation (127). Once autophagy is activated, cellular components are embedded into a double-membrane vacuolar structure (autophagosome), which is further fused with lysosomes (autolysosome) as a means to degrade its contents, providing a nutrient source to maintain vital cellular activities (128, 129).

Many studies of *S. cerevisiae* have shown that TORC1 negatively regulates autophagy, and the observation that rapamycin treatment is sufficient to induce autophagy even in the presence of nutrients provided key evidence for this conclusion. Extensive genetic and biochemical studies indicate that ATG1 is an autophagy-initiating kinase and its activity is under the control of TORC1 (56, 130). In *S. cerevisiae*, the ATG1 mutant is defective in autophagy even under nutrient starvation or TORC1 inhibition, suggesting that ATG1 acts downstream of TORC1. ATG1 interacts with several autophagy proteins, including ATG13 and ATG17. The interaction of ATG13 and ATG17 with ATG1 is induced by rapamycin or nutrient starvation, and formation of this complex is important for ATG1 kinase activity (131). TORC1 appears to phosphorylate ATG13 on multiple residues to disrupt the ATG1 complex (132), thereby repressing autophagy induction. Consistently, starvation or rapamycin treatment enhances ATG1 kinase activity.

The function of TORC1 in the regulation of autophagy is conserved in eukaryotes (130). The human genome has ATG1 homologs, such as ULK1 and ULK2 (56). Several studies have revealed that mammalian ULK1 is involved in autophagy regulation (133, 134) and functions downstream of mTORC1. Moreover, recent reports show that mTORC1 interacts with ULK1-ATG13-FIP200 (a mammalian functional homolog of ATG17) (135) and directly phosphorylates ULK1 kinase and ATG13 proteins, even though the precise sites and functional impact of phosphorylation are yet to be established (57, 136–138). This may provide a mechanism for autophagy inhibition by mTORC1. As an energy sensor, not surprisingly, AMPK is also involved in autophagy (139–141). Typically, AMPK inhibits mTORC1 through phosphorylation of TSC2 (142) and Raptor (116); thus, AMPK is assumed to induce autophagy by suppressing mTORC1 in response to cellular energy cues.

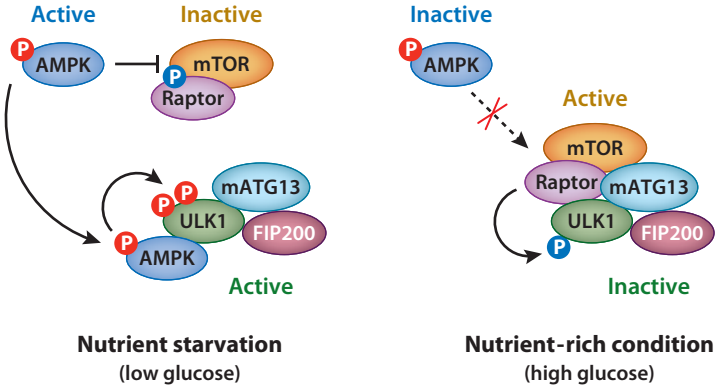

Figure 3

Regulation of ULK1 by AMP-activated protein kinase (AMPK) and mammalian target of rapamycin complex 1 (mTORC1). In the nutrient-rich condition, active mTORC1 phosphorylates ULK1, which negatively regulates the ULK1-AMPK interaction. Once the cellular energy level is decreased, AMPK phosphorylates to inhibit mTORC1 at the level of TSC2 and Raptor, relieving mTORC1-dependent ULK1 phosphorylation. It allows AMPK-ULK1 interaction, followed by ULK1 activation by AMPK-dependent phosphorylation.

In a recent paper, we provided new molecular insight into autophagy regulation by mTORC1 and AMPK (58) (**Figure 3**). We observed that ULK1 is activated by glucose starvation in a manner that depends on AMPK-mediated phosphorylation. ULK1 cannot be activated when AMPK-knockout MEFs are subjected to glucose deprivation, indicating an obligatory role of AMPK in ULK1 activation. Importantly, ULK1 can be directly activated by AMPK in vitro. Similarly, Egan et al. (143) also showed that ULK1 is a direct target of AMPK. They showed that autophagy was promoted by expression of an active AMPK in worm hypodermal cells, which was suppressed by ULK1 siRNA. These genetic data also support the ideas that AMPK lies upstream of ULK1 and that AMPK regulation of ULK1 is required for proper autophagy. Consistent with these two observations, Lee et al. (144) reported that AMPK association with ULK1 plays an important role in autophagy induction. In this study, the authors suggest that AMPK induces autophagy, at least in part, by phosphorylation of Raptor, an event that relieves the inhibitory effect of mTOR on the ULK1 autophagic complex.

In parallel, our group and others have demonstrated that AMPK directly phosphorylates multiple sites in ULK1 (S317, S467, S555, T575, S637, and S777) and promotes ULK1 function in autophagy (58, 143) (**Figure 3**). Analyses of ULK1-knockout cells reconstituted with ULK1 mutants that cannot be phosphorylated by AMPK indicate that the cells expressing the ULK1 mutants are defective in autophagy induction. These observations demonstrate the functional importance of ULK1 phosphorylation by AMPK in autophagy induction. Moreover, ULK1 can be directly activated by AMPK phosphorylation in vitro. Phosphorylation of S317 and S777 is essential for ULK1 activation by AMPK both in vitro and in vivo. These observations establish a direct role for AMPK in ULK1 activation, hence also in autophagy induction.

The mechanism of mTORC1 in ULK1 regulation and autophagy induction has also been elucidated (**Figure 3**). Our group showed that the ULK1-AMPK interaction was enhanced by rapamycin, indicating that mTORC1 may inhibit the interaction between ULK1 and AMPK. mTORC1 directly phosphorylates ULK1 on S757, which is located in the AMPK binding motif (711–828) on ULK1. Notably, phosphorylation of the AMPK sites and the mTORC1 site in ULK1 are oppositely regulated under various conditions. A recent report proposed a different

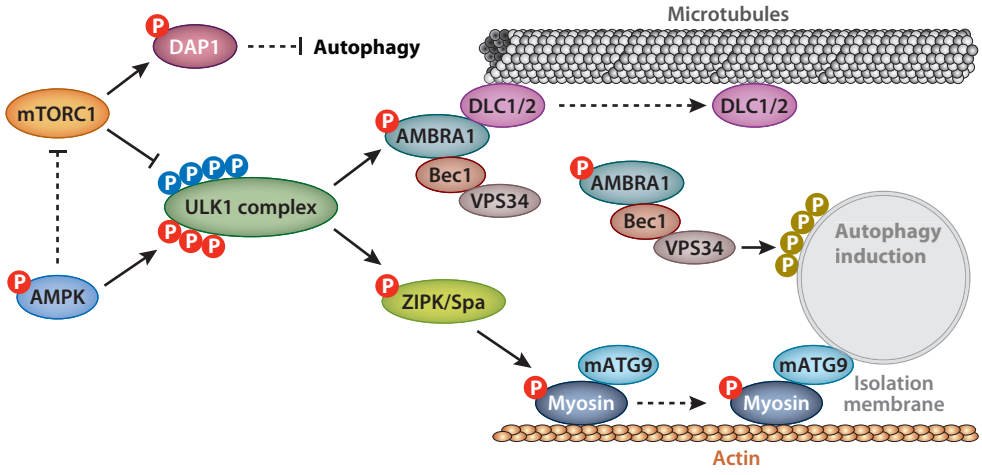

Figure 4

ULK1-dependent autophagy induction. Under autophagy-inducing conditions, such as nutrient starvation, active ULK1 phosphorylates AMBRA1, a component of the VPS34 complex. The VPS34 complex produces PI(3)P, which provides a docking platform for the autophagy protein machinery. Phosphorylation of AMBRA1 induces the release of the VPS34 complex from the dynein complex on microtubules and subsequent relocation of the autophagy core complex to the endoplasmic reticulum, which enables autophagosome nucleation. Also, actin-associated motor protein myosin II is activated by ULK1-dependent phosphorylation. The activated myosin complex delivers mammalian ATG9 (mATG9) to the isolation membrane for autophagy.

model regarding the ULK1-AMPK interaction upon nutrient starvation (145). These authors performed quantitative analysis of ULK1 phosphorylation and found that phosphorylation of S556 (for human ULK1, which is equivalent to mouse S555) was decreased more than fivefold upon starvation, whereas Egan et al. (143) showed increased S555 phosphorylation upon starvation. Further studies are needed to clarify how phosphorylation of S555 is regulated by starvation, as the phosphorylation of ULK1 S757, which is phosphorylated by mTORC1, was decreased upon starvation (58, 145). However, Shang et al. (145) argued that the ULK1-AMPK interaction was disrupted by nutrient starvation, mainly through dephosphorylation of S758 of ULK1 (equivalent to mouse S757), as evidenced by the observation that the S758A mutant impaired the ULK1-AMPK interaction. In contrast, our study (58) showed that mutation of S757 to either alanine (S757A) or aspartate (S757D) abolished AMPK binding, suggesting that the chemistry of this residue is important for ULK1-AMPK binding. More importantly, mutation of S757 to cysteine, which is structurally and chemically similar to serine but cannot be phosphorylated, retains some ULK1-AMPK binding but the binding is resistant to mTORC1, demonstrating the importance of S757 phosphorylation in regulating the association between ULK1 and AMPK in response to mTORC1 activation.

Recent studies are starting to shed light on a downstream target of ULK1. Tang et al. (146) showed that the actin-associated motor protein myosin II was activated by ATG1/ULK1-dependent phosphorylation in *Drosophila* and mammalian cells. These authors demonstrated that activation of myosin II plays important roles in the regulation of starvation-induced autophagy and mammalian ATG9 (mATG9) trafficking when cells are deprived of nutrients (**Figure 4**). Also, another recent paper reported that the VPS34 complex is a target of ULK1 (147). Di Bartolomeo et al. (147) showed that the VPS34 complex is tethered to the cytoskeleton through

an interaction between one component of the VPS34 complex, AMBRA1, and dynein light chains 1 and 2 (DLC1 and DLC2) (**Figure 4**). When autophagy is induced, ULK1 phosphorylates AMBRA1, releasing the autophagy core complex from dynein. Its subsequent relocation to the ER enables autophagosome nucleation.

In addition, DAP1 (death-associated protein 1) is a novel mTORC1 substrate with an inhibitory role in autophagy (148). Although the underlying mechanism of DAP1 in autophagy inhibition is unknown, it would be interesting to determine both if DAP1 acts upstream or downstream of ULK1 and its relative contribution in mediating the effect of mTORC1 in autophagy regulation. Surprisingly, Yu et al. (149) recently demonstrated that mTORC1 activity is also required at the late stage of autophagy to recycle lysosomes for another cycle of autophagy, even though mTORC1 has long been believed to inhibit the initiation step of this degradative process. Thus, the function of mTORC1 in autophagy is complex. It inhibits the initiation of autophagy at early stages, yet it positively contributes to the completion of autophagy at later stages.

Accumulating reports indicate that mTORC1 and AMPK serve as master switches for the process of autophagy. AMPK and mTORC1 have opposing effects on autophagy induction via coordinated phosphorylation of ULK1 (**Figure 4**). However, many questions still remain regarding how these two energy-sensing kinases, mTOR and AMPK, accomplish and coordinate their complex regulatory functions in response to the wide variety of conditions that trigger autophagy.

CONCLUSION AND PERSPECTIVE

Extensive biochemical, cell biological, genetic, and physiological studies confirm that AMPK is a key cellular energy sensor and that its activation promotes energy-producing catabolism and inhibits energy-consuming anabolism. Many of the AMPK substrates are metabolic enzymes directly involved in energy metabolism, such as glycolysis and fatty acid synthesis and oxidation. As illustrated by its effects on protein synthesis, AMPK activation also inhibits biosynthesis of macromolecules by, for example, inhibiting mTORC1 or eukaryotic elongation factor activity. Moreover, AMPK induces hydrolysis of cellular contents, such as proteins and organelles, via autophagy induction. This is accomplished in part by inhibiting mTORC1 and activating ULK1. Thus, AMPK activation modulates cellular metabolism of both small metabolites and macrobiomolecules. AMPK also plays a role in organismal energy balance by its actions in the neuroendocrine system. mTORC1 integrates cellular nutrient status, including energy levels, and plays a major role in cell growth. High mTORC1 activity promotes cell growth, whereas low mTORC1 activity inhibits growth and induces autophagy. Under nutrient starvation, decreased mTORC1 activity leads to reductions in ribosome biosynthesis and protein translation, which normally consume a large fraction of cellular energy. Therefore, mTORC1 has a key role not only in nutrient response, but also in cellular energy homeostasis. It is not surprising that mTORC1 activity is coupled to and inhibited by AMPK.

AMPK and mTORC1 are key cellular nutrient indicators and cell growth regulators. Thus, their dysregulation is associated with many human diseases. For example, uncontrolled mTORC1 activation by mutation in TSC1/TSC2 or constitutive activation of the PI3K pathway contributes to tumorigenesis. As such, mTORC1 inhibitors have been shown to have therapeutic benefits for TSC disease. Moreover, mTORC1 inhibitors, rapamycin analogs, have been approved as drugs to treat late-stage renal cancer, and many clinical trials under way use mTOR inhibitors for cancer treatment. mTORC1 can also regulate protein metabolism, targeting both synthesis and degradation (by autophagy); thus, inhibition of mTORC1 may prove useful in the development of drugs that target proteinopathies, including neurodegenerative disorders such as Alzheimer's disease. Similarly, defects in AMPK activation may be linked to tumorigenesis, given that the

upstream kinase LKB1 has long been recognized as a tumor suppressor. Moreover, metformin, the most widely used diabetic drug, has been reported to suppress cancers because patients using metformin present with a significant reduction in cancer incidence. Furthermore, the prominent role of AMPK and mTOR in energy metabolism makes them attractive drug targets for metabolic diseases, such as diabetes and obesity. Finally, inhibition of mTORC1 delays aging and extends life span in *C. elegans*, *Drosophila*, and mice. Therefore, both AMPK and mTORC1 will continue to attract wide attention from the pharmaceutical industry as prominent drug targets.

DISCLOSURE STATEMENT

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Errata

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