Interaction between the AMP-Activated Protein Kinase and mTOR Signaling Pathways

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ABSTRACT

KIMBALL, S. R. Interaction between the AMP-Activated Protein Kinase and mTOR Signaling Pathways. *Med. Sci. Sports Exerc.*, Vol. 38, No. 11, pp. 1958–1964, 2006. The AMP-activated protein kinase (AMPK) has been referred to as an "energy sensor" because it binds to and is regulated by both AMP and ATP. The binding of AMP to AMPK allows it to be phosphorylated by upstream kinases, resulting in its activation. In contrast, the binding of ATP prevents its activation. AMPK regulates a multitude of metabolic processes that cumulatively function to maintain cellular energy homeostasis through repression of a number of energy-consuming processes with simultaneous enhancement of energy-producing processes. One downstream AMPK target that has been recently identified is the mammalian target of rapamycin (mTOR), a positive effector of cell growth and division. The focus of the present review is to briefly summarize current knowledge concerning the regulation of mTOR signaling by AMPK. **Key Words:** LKB1, RAPTOR, RHEB, 4E-BP1, S6K1

Both in animals *in vivo* and cells in culture, protein synthesis is regulated in part through a signaling pathway involving a protein kinase referred to as the mammalian target of rapamycin (mTOR) (1,12). In this regard, activation of mTOR stimulates both the initiation and elongation phases of mRNA translation. In addition, mTOR activation is associated with increased cell-cycle progression, selective enhancements in gene transcription, and increased ribosome biogenesis. Thus, signaling through mTOR upregulates many energy-consuming processes.

In contrast to mTOR signaling, activation of AMP-activated protein kinase (AMPK) results in a repression of energy-consuming processes (e.g., fatty acid synthesis) while simultaneously enhancing energy-producing processes (e.g., glycolysis) (6,16,25). Because mRNA translation is one of the most energy-intensive processes in a cell, it might be expected that activation of AMPK would repress protein synthesis. However, that possibility was unexplored until recently. The purpose of this review is to summarize recent findings defining the mechanisms through which AMPK represses signaling through mTOR, with a focus on the regulation of mRNA translation.

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REGULATION OF mRNA TRANSLATION BY mTOR

The process of mRNA translation is functionally divided into three phases: initiation, during which the 40S and 60S ribosomal subunits bind to mRNA and locate the AUG start codon; elongation, during which the ribosome moves along the mRNA, translating stored information into a growing peptide chain; and termination, a process resulting in release of the completed protein from the ribosome (26). Because the majority of the examples of translational regulation occur at the initiation phase, this article will focus on the regulation of initiation by mTOR and AMPK.

The initiation phase of mRNA translation is a complicated process that results in the binding of mRNA to the 40S ribosomal subunit (26). One of the first steps in this process is the association of a protein referred to as eukaryotic initiation factor (eIF)4E with the m^7GTP cap structure at the 5′-end of the mRNA. The eIF4E–mRNA complex then binds to a binary complex consisting of two other initiation factors, eIF4A and eIF4G, and the resulting eIF4F–mRNA complex associates with the 40S ribosomal subunit. The mRNA binding step in initiation is regulated through the reversible association of eIF4E with one of three eIF4E binding proteins (4E-BP). Of the 4E-BP, 4E-BP1 is the best characterized. Both 4E-BP1 and eIF4G bind to the same site on eIF4E. Consequently, binding of 4E-BP1 to eIF4E prevents its association with eIF4G and thereby decreases the binding of mRNA to the 40S ribosomal subunit. The binding of 4E-BP1 to eIF4E is regulated through phosphorylation of 4E-BP1 on multiple residues, whereby unphosphorylated and hypophosphorylated forms of the protein bind to eIF4E, but hyperphosphorylated forms do not. Phosphorylation of 4E-BP1 is controlled in large part through activation of the mTOR signaling pathway (Fig. 1). mTOR phosphorylates 4E-BP1 on at least two residues, threonine 37 and threonine 46. Phosphorylation

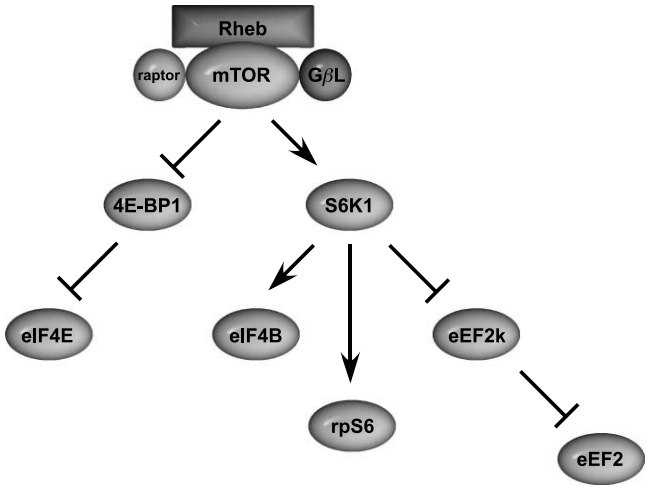

FIGURE 1—mTOR signals to multiple downstream targets impinging on mRNA translation. mTOR, in a complex with raptor, Rheb, and GβL, phosphorylates 4E-BP1 and S6K1, resulting in modulation of both translation initiation and elongation.

on those residues permits subsequent phosphorylation events by other, unidentified protein kinases, ultimately resulting in release of the protein from eIF4E.

In addition to 4E-BP1, mTOR also phosphorylates the 70-kDa ribosomal protein (rp)S6 kinase, S6K1 (27). Phosphorylation of S6K1 on threonine 389 by mTOR creates a docking site for another protein kinase, the phosphoinositide-dependent protein kinase (PDK)1, which phosphorylates S6K1 on serine 229 and activates the kinase (3,13). S6K1 phosphorylates multiple downstream targets relevant to mRNA translation including eIF4B (19,41), eukaryotic elongation factor (eEF)2 protein kinase (42), and rpS6 (11). Phosphorylation of eIF4B enhances its association with the eIF4F complex, resulting in increased translation of mRNAs with long, highly structured 5′-untranslated regions (41). Phosphorylation of eEF2 kinase by S6K1 inhibits it, resulting in dephosphorylation and, consequently, activation of eEF2 (55). Early studies suggested that phosphorylation of rpS6 by S6K1 promoted a preferential increase in the translation of mRNAs bearing a terminal oligopyrimidine (TOP) tract at the 5′-end of the mRNA (36). However, recent studies have shown that TOP mRNA translation is regulated normally, both in cells lacking both isoforms of S6K1 and also in cells in which the wild-type rpS6 was replaced by a variant lacking the S6K1 phosphorylation sites, suggesting that phosphorylation of rpS6 is not required for modulation of TOP mRNA translation (39,45). Thus, the effect of rpS6 phosphorylation on mRNA translation, if any, remains to be established.

REGULATION OF mTOR

mTOR forms at least two complexes in mammalian cells that are referred to as mTOR complexes 1 and 2 (mTORC1 and mTORC2, respectively). The mTORC1 complex is distinguished from mTORC2 in part by its subunit composition. Thus, in addition to mTOR, mTORC1 contains a protein referred to as the regulatory associated protein of mTOR (raptor), whereas mTORC2 contains the rapamycin-insensitive companion of mTOR (rictor) (24,47). The two complexes are also unique in their downstream targets. For example, mTORC1 phosphorylates 4E-BP1 and S6K1 (4,38,49), whereas mTORC2 phosphorylates protein kinase Cα (47) and protein kinase B (PKB, also known as Akt) (48). Because only mTORC1 has been shown to regulate mRNA translation, only it will be further considered here. In addition to mTOR and raptor, at least two other proteins comprise the mTORC1 complex, including G-protein β-subunit-like protein (GβL) and the ras homolog enriched in brain (Rheb) (Fig. 1). Raptor binds to a domain present on both 4E-BP1 and S6K1 referred to as an mTOR signaling (TOS) motif and thereby recruits them to the mTORC1 complex, permitting their phosphorylation by mTOR (38,49). Thus, raptor serves as a molecular bridge linking mTOR with its substrates. The association of raptor with mTOR is regulated by hormones such as insulin and IGF-1 and by nutrients such as leucine through an unknown mechanism. However, it is clear that raptor is required for optimal phosphorylation of 4E-BP1 and S6K1 because reducing raptor expression severely attenuates hormone- and amino acid–induced phosphorylation of both proteins (27,28). Like raptor, GβL also enhances signaling through mTOR (28). However, the mechanism through which it functions is unclear. Rheb is a GTPase that binds both GDP and GTP (14,21,33). The GTP-bound form of Rheb activates mTOR, whereas Rheb–GDP inhibits it (Fig. 2). The GTPase activity of Rheb is stimulated by the GTPase-activator protein (GAP) tuberin (also known as TSC2) functioning in a complex with a second protein, hamartin (also known as TSC1) (31,32). Tuberin GAP activity is modulated in response to phosphorylation by several protein kinases including AMPK (discussed further in the

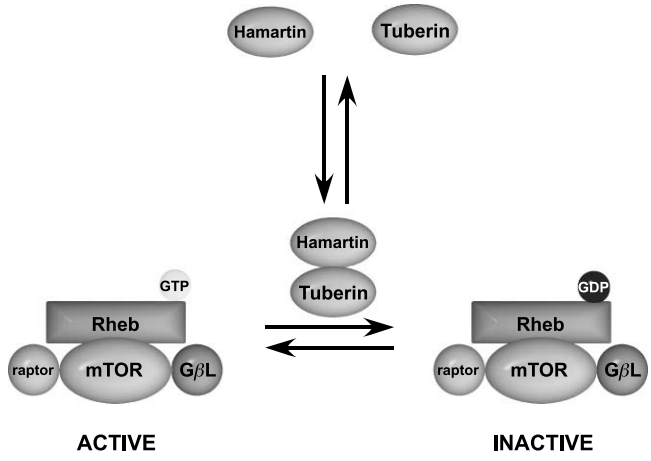

FIGURE 2—The tuberin–hamartin complex modulates mTOR signaling through activation of Rheb GTPase activity. Tuberin is a GTPase-activator protein for Rheb. Activation of Rheb GTPase activity results in hydrolysis of Rheb-bound GTP to GDP, resulting in attenuation of mTOR signaling. In part, tuberin function is regulated by its interaction with hamartin, where the tuberin–hamartin complex acts on Rheb.

next section) (23), PKB (9,22,35,40), the extracellularly regulated protein kinases (ERK) 1 and 2 (34), and $p90^{rsk}$ (44). Phosphorylation by either PKB, ERK1/ERK2, or $p90^{rsk}$ inhibits tuberin GAP activity, resulting in an increase in the proportion of Rheb associated with GTP, and increased mTOR signaling. In part, phosphorylation of tuberin may repress its function by promoting dissociation of the tuberin–hamartin complex (2).

REGULATION OF AMPK

AMPK is a heterotrimeric complex composed of a catalytic subunit (AMPKα) and two regulatory subunits (AMPKβ and AMPKγ) (6,16,25). The γ-subunit binds two AMP molecules with positive cooperativity. Thus, the binding of one molecule of AMP enhances the binding of a second, thereby increasing the sensitivity of the kinase to small changes in AMP concentration. Binding of AMP to the kinase is thought to alter its conformation, permitting subsequent phosphorylation of the α-subunit on threonine 172 by upstream protein kinases (Fig. 3). The combination of AMP binding to the γ-subunit and phosphorylation of the α-subunit results in activation of the kinase. In contrast, binding of ATP prevents phosphorylation of the α-subunit and therefore attenuates its activation. Thus, AMPK responds not only to changes in AMP concentration but also to changes in the ratio of AMP to ATP.

The upstream kinase(s) that phosphorylates AMPKα on threonine 172 was unknown until a seminal study performed in the laboratories of Drs. Dario R. Alessi and D. Grahame Hardie identified the tumor suppressor LKB1 as an AMPK kinase (17). In that study, LKB1 in a complex with two other proteins, STRAD and MO25, was shown to be required for AMPKα phosphorylation in cells treated with the AMP mimetic 5-aminoimidazole-4-carboxamide

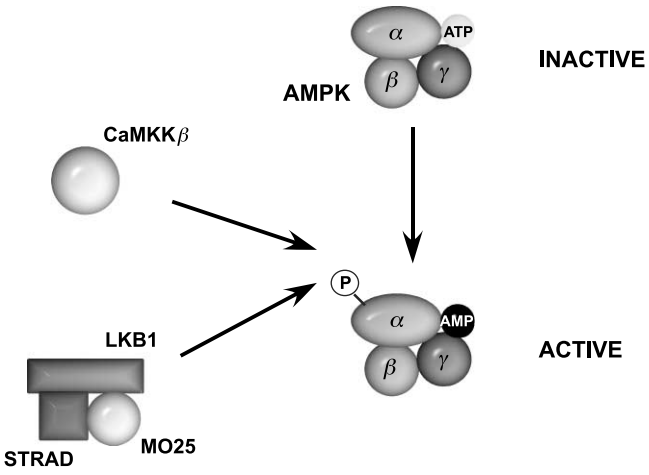

FIGURE 3—AMPK is regulated both by the AMP:ATP ratio and phosphorylation by LKB1 or CaMKKβ. Binding of ATP to the γ-subunit of AMPK restricts phosphorylation of the α-subunit. In contrast, binding of AMP is permissive for subsequent phosphorylation by CaMKKβ or the LKB1–STRAD–MO25 complex. Phosphorylation of the catalytic α-subunit of AMPK by either kinase results in its activation.

1-β-D-ribonucleoside (AICAR). AICAR does not stimulate LKB1 kinase activity; instead, it binds to AMPK and permits its phosphorylation. In addition, a complex of the three proteins was sufficient for phosphorylation of AMPKα on threonine 172 in *in vitro* studies. A subsequent study (51) confirmed the results of the initial report and extended them to show that LKB1 is required for AMPK phosphorylation in response to a variety of cellular stressors that promote energy depletion. Two more recent studies show that, under a variety of conditions, LKB1 is the primary AMPK kinase in skeletal muscle (46) and liver (52). In both studies, a portion of the wild-type LKB1 gene was replaced with a construct consisting of the wild-type sequence flanked by loxP Cre-excision sequences. In the study by Sakamoto et al. (46), expression of Cre recombinase specifically in muscle resulted in a reduction in LKB1 mRNA and protein to undetectable levels. In such mice, both activation of AMPKα2 activity and AMPK phosphorylation on threonine 172 are dramatically repressed in muscles either treated with AICAR or stimulated to contract, suggesting that LKB1 is the principal AMPK kinase activated under these conditions. Similarly, liver-specific reduction in LKB1 to less than 5% of the wild-type level dramatically attenuates AMPK phosphorylation on threonine 172 (52). Importantly, in that study, both basal mTOR signaling and the feeding-induced increase in mTOR signaling were significantly enhanced in LKB1-deficient liver.

In addition to LKB1, two recent studies have shown that AMPKα is also phosphorylated on threonine 172 by calcium/calmodulin-dependent protein kinase kinase-β (CaMKKβ) (18,56). Thus, in LKB1-deficient cells, activation of CaMKKβ promotes phosphorylation of AMPK independently of changes in adenine nucleotide levels (18). Moreover, recombinant CaMKKβ expressed in *E. coli* phosphorylates and activates AMPK in *in vitro* reactions (56). Interestingly, calcium ionophores promote AMPK phosphorylation and activation in cells lacking LKB1, and this effect is blocked by CaMKK inhibitors or knockdown of the protein by RNAi (18). Overall, the results of the two studies strongly suggest that AMPK activity is regulated by changes in intracellular calcium concentrations through changes in CaMKKβ activity. Future studies will hopefully address the question of whether CaMKKβ might mediate the small residual increase in contraction-stimulated AMPK activity observed in LKB1-deficient skeletal muscle (46).

REPRESSION OF mTOR SIGNALING THROUGH ACTIVATION OF AMPK

The first study linking activation of AMPK with a repression of signaling through mTOR showed that in skeletal muscle of rats treated with AICAR, AMPK phosphorylation and activity were increased concomitantly with dephosphorylation of 4E-BP1 and S6K1 (5). In particular, phosphorylation of 4E-BP1 on threonine 37 and S6K1 on threonine 389 were significantly decreased 1 h after AICAR administration. Because both phosphorylation

sites are thought to be directly phosphorylated by mTOR, the results suggest that activation of AMPK promotes inactivation of mTOR. A caveat to this finding is that because AICAR affects many metabolic pathways, its administration *in vivo* can alter blood concentrations of a number of metabolites and hormones. Thus, the observed changes in mTOR signaling could partly have been a result of changes in circulating insulin concentration. More direct evidence linking activation of AMPK with reduced mTOR signaling was presented in two studies that immediately followed publication of the initial report. In those studies (10,30), activation of AMPK using AICAR or agents that decrease intracellular ATP levels (e.g., fructose, glycerol, or oligomycin) in cells in culture was associated with decreased phosphorylation of S6K1, indicating that mTOR signaling was repressed. Moreover, in both studies, activation of AMPK by AICAR overcame the amino acid–induced activation of mTOR, providing the first evidence that negative regulation of mTOR caused by activation of AMPK is dominant to the positive input from amino acids. In contrast to the results of the three studies described above supporting a link between AMPK and mTOR, a fourth study published in the same year showed that activation of AMPK was associated with decreased rates of protein synthesis concomitant with increased phosphorylation of eEF2, but no change in phosphorylation of 4E-BP1 (20). The authors of that study conclude that the inhibition of protein synthesis caused by activation of AMPK occurred independently of changes in mTOR signaling. However, in that study, there was a noticeable shift in 4E-BP1 distribution from the hyperphosphorylated γ-form to the lesser phosphorylated β-form, a result that is consistent with reduced mTOR signaling. The finding that S6K1 phosphorylation, as assessed by changes in migration during SDS-polyacrylamide gel electrophoresis, remains elevated in cells treated with 2-deoxyglucose, may be a consequence of other signaling pathways impinging on S6K1. Alternatively, because phosphorylation of S6K1 on threonine 389 by mTOR is one of a series of phosphorylation events leading to activation of the kinase, changes in phosphorylation of a single residue may not have been detectable by the gel-shift analysis used in that study. Thus, in a variety of cell lines, activation of AMPK results in decreased S6K1 activity as assessed by an *in vitro* kinase assay (29).

Activation of AMPK has been reported to repress mTOR signaling through at least two mechanisms. In the first case, AMPK has been shown to directly phosphorylate tuberin on multiple residues (23). In that study, eight potential AMPK phosphorylation sites were identified on tuberin, each was subsequently mutated to an unphosphorylatable alanine, and the variant protein was expressed in cells. All of the individual mutants except two (where threonine 1227 and serine 1345 were changed to alanine) exhibited a wild-type pattern of phosphorylation in response to activation of AMPK, suggesting that AMPK phosphorylates tuberin on threonine 1227 and serine 1345 (Fig. 4). The finding that AMPK directly phosphorylates

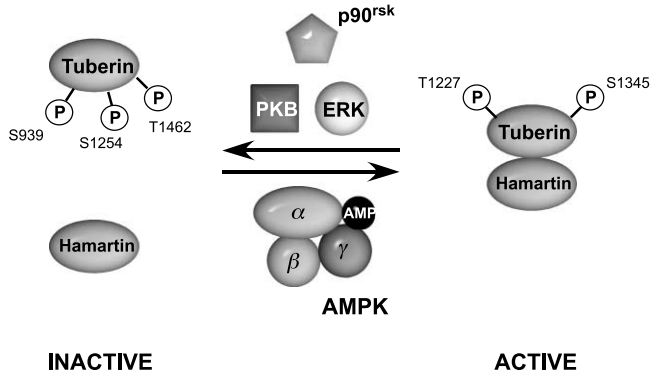

FIGURE 4—Regulation of tuberin GAP activity is a balance between negative inputs from PKB, ERK, and p90^{rsk} and positive input from AMPK. As described further in the text, phosphorylation of tuberin by PKB, ERK, or p90^{rsk} is associated with enhanced tuberin GAP activity. In contrast, phosphorylation by AMPK results in its inactivation. Activation of tuberin by AMPK is depicted in the figure as enhanced association of tuberin with hamartin because recent results from the authors' laboratory has shown an increase in association in response to activation of AMPK in cells in culture treated with AICAR and in skeletal muscle of mice subjected to treadmill running.

threonine 1227 and serine 1345 in an *in vitro* kinase assay provides further support for this conclusion. Importantly, expression of a tuberin variant with the two AMPK phosphorylation sites changed to alanine attenuates the decrease in 4E-BP1 and S6K1 phosphorylation caused by 2-deoxyglucose-mediated activation of AMPK. A more recent study (53) provides further evidence for a role for tuberin in AMPK signaling to mTOR. In that study, phosphorylation of 4E-BP1 was dramatically elevated in cells lacking tuberin compared with wild-type cells. Activation of AMPK with AICAR attenuated 4E-BP1 phosphorylation in wild-type cells but not in cells lacking tuberin. Overall, the results suggest that one mechanism through which AMPK represses mTOR signaling is through phosphorylation and activation of tuberin.

A second mechanism through which AMPK may regulate mTOR signaling involves phosphorylation of mTOR on threonine 2446 by AMPK. In a study by Cheng et al. (7), activation of AMPK using either AICAR or dinitrophenol was associated with enhanced phosphorylation of mTOR on threonine 2446. A 111–amino acid peptide containing the sequence surrounding threonine 2446 is phosphorylated by AMPK in an *in vitro* kinase assay, which further suggests that AMPK directly phosphorylates mTOR on that residue. It is not known whether phosphorylation of mTOR on threonine 2446 by AMPK alters it catalytic activity or its interaction with mTOR regulatory proteins such as raptor or Rheb. However, it is interesting that phosphorylation of mTOR on threonine 2446 is inversely correlated with phosphorylation of an adjacent residue, serine 2448 (7). Thus, phosphorylation of threonine 2446 is attenuated when serine 2448 is already phosphorylated, and PKB-mediated phosphorylation of serine 2448 is repressed when threonine 2446 is mutated to an acidic residue to mimic phosphorylation. Serine 2446

on mTOR has been reported to be phosphorylated by either PKB (37,50) or S6K1 (8). Phosphorylation of serine 2448 typically correlates with enhanced mTOR signaling (43). Therefore, phosphorylation of threonine 2446 may interfere with mTOR function by preventing phosphorylation of serine 2448. However, this possibility must be viewed with caution because mutation of serine 2448 to alanine has no detectable effect on mTOR signaling to 4E-BP1 and S6K1 (50).

In addition to phosphorylating tuberin and mTOR, AMPK also phosphorylates Raf on serine 621 and enhances phosphorylation of ERK1 and ERK2 on threonine 202 and tyrosine 204 (54). Recent studies have shown that tuberin is phosphorylated both by ERK1/ERK2 (34) and by a downstream target of ERK1/ERK2 signaling, $p90^{rsk}$ (44). In contrast to the activation of tuberin GAP activity caused by phosphorylation by AMPK, phosphorylation of the protein by ERK1/ERK2 or $p90^{rsk}$ is associated with decreased tuberin GAP activity and enhanced mTOR signaling (34,44). Thus, by activating ERK1/ERK2 and $p90^{rsk}$, AMPK would be expected to activate rather than repress mTOR signaling. However, it has been observed that in C2C12 myotubes, activation of AMPK by treatment with AICAR is associated with enhanced phosphorylation of ERK1/ERK2 and decreased phosphorylation of 4E-BP1 and S6K1, suggesting that the repressive action of AMPK on mTOR signaling is dominant to the stimulatory action of ERK1/ERK2 (57). In that study, the decrease in mTOR signaling was associated with enhanced association of tuberin with hamartin, suggesting that AMPK may increase tuberin GAP activity by promoting assembly of the tuberin–hamartin complex. Similarly, a recent study (15) reports that activation of AMPK attenuates insulin-induced PKB activation and represses mTOR signaling. In skeletal muscle of mice run on a treadmill, both AMPK and ERK1/ERK2 phosphorylation increase in conjunction with decreased phosphorylation of 4E-BP1 and S6K1 (58). Therefore, a possible mechanism through which AMPK might repress mTOR signaling despite the potential opposing actions of ERK1/ERK2 and/or $p90^{rsk}$ would involve enhanced binding of tuberin to hamartin (Fig. 4).

CONCLUSIONS

The finding that activation of AMPK results in decreased mTOR signaling and inhibition of protein synthesis perhaps isn't surprising, considering the known role of AMPK in repressing energy-consuming metabolic processes and the large energy expenditure devoted to protein synthesis in cells. Once the link between AMPK and mTOR was identified, a number of studies quickly provided evidence for at least two mechanisms through which the regulation might occur. Together, the results of these studies provide support for a model in which phosphorylation of tuberin by AMPK would enhance its association with hamartin, resulting in an increase in Rheb GTPase activity and in an increased association of Rheb with GDP. The resulting Rheb–GDP complex would inhibit mTOR activity toward downstream targets including 4E-BP1 and S6K1. In this model, phosphorylation of tuberin by AMPK would overcome positive input from other kinases such as PKB, ERK1/ERK2, and $p90^{rsk}$. However, despite the growing number of studies examining the mechanism(s) through which AMPK represses mTOR signaling, a number of questions remain unanswered. For example, what role, if any, does phosphorylation of mTOR on threonine 2446 have in the regulation of mTOR by AMPK? How does phosphorylation of tuberin by AMPK overcome positive signaling through PKB, ERK1/ERK2, and $p90^{rsk}$? What role does CaMMKβ play in the regulation of AMPK in animals *in vivo*? These and many other questions remain to be answered in future studies.

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REFERENCES

1. Abraham, R. T. TOR signaling: an odyssey from cellular stress to the cell growth machinery. *Curr. Biol.* 15:R139–R141, 2005.
2. Aicher, L. D., J. S. Campbell, and R. S. Yeung. Tuberin phosphorylation regulates its interaction with hamartin. Two proteins involved in tuberous sclerosis. *J. Biol. Chem.* 276: 21017–21021, 2001.
3. Alessi, D. R., M. T. Kozlowski, Q.-P. Weng, N. Morrice, and J. Avruch. 3-Phosphoinositide-dependent protein kinase 1 (PDK1) phosphorylates and activates the p70 S6 kinase *in vivo* and *in vitro*. *Curr. Biol.* 8:69–81, 1998.
4. Beugnet, A., X. Wang, and C. G. Proud. Target of rapamycin (TOR)-signaling and RAIP motifs play distinct roles in the mammalian TOR-dependent phosphorylation of initiation factor 4E-binding protein 1. *J. Biol. Chem.* 278:40717–40722, 2003.
5. Bolster, D. R., S. J. Crozier, S. R. Kimball, and L. S. Jefferson. AMP-activated protein kinase suppresses protein synthesis in rat skeletal muscle through down-regulated mammalian target of rapamycin (mTOR) signaling. *J. Biol. Chem.* 277:23977–23980, 2002.
6. Carling, D. AMP-activated protein kinase: balancing the scales. *Biochimie* 87:87–91, 2005.
7. Cheng, S. W. Y., L. G. D. Fryer, D. Carling, and P. R. Shepherd. Thr2446 is a novel mammalian target of rapamycin (mTOR) phosphorylation site regulated by nutrient status. *J. Biol. Chem.* 279:15719–15722, 2004.
8. Chiang, G. G., and R. T. Abraham. Phosphorylation of mTOR at Ser-2448 is mediated by p70S6 kinase. *J. Biol. Chem.* 280: 25485–25490, 2005.
9. Dan, H. C., M. Sun, L. Yang, et al. Phosphatidylinositol 3-kinase/Akt pathway regulates tuberous sclerosis tumor suppressor complex by phosphorylation of tuberin. *J. Biol. Chem.* 277: 35364–35370, 2002.
10. Dubbelhuis, P. F., and A. J. Meijer. Hepatic amino acid-dependent signaling is under the control of AMP-dependent protein kinase. *FEBS Lett.* 521:39–42, 2002.
11. Ferrari, S., H. R. Bandi, J. Hofsteenge, B. M. Bussian, and G. Thomas. Mitogen activated 70K S6 kinase. Identification of *in vitro* 40S ribosomal S6 phosphorylation sites. *J. Biol. Chem.* 266:22770–22775, 1991.

12. FINGAR, D. C., and J. BLENIS. Target of rapamycin (TOR): an integrator of nutrient and growth factor signals and coordinator of cell growth and cell cycle progression. *Oncogene* 23:3151–3171, 2004.
13. FRODIN, M., T. L. ANTAI, B. A. DUMMIER, et al. A phosphoserine/threonine-binding pocket in AGC kinases and PDK1 mediates activation by hydrophobic motif phosphorylation. *EMBO J.* 21:5396–5407, 2002.
14. GARAMI, A., F. J. T. ZWARTKRUIS, T. NOBUKUNI, et al. Insulin activation of Rheb, a mediator of mTOR/S6K/4E-BP1 signaling, is inhibited by TSC1 and 2. *Molec. Cell* 11:1457–1466, 2003.
15. HAHN-WINDGASSEN, A., V. NOGUEIRA, C.-C. CHEN, J. E. SKEEN, N. SONENBERG, and N. HAY. Akt activates the mammalian target of Rapamycin by regulating cellular ATP level and AMPK activity. *J. Biol. Chem.* 280:32081–32089, 2005.
16. HARDIE, D. G. New roles for the LKB1→ AMPK pathway. *Curr. Opinion Cell Biol.* 17:167–173, 2005.
17. HAWLEY, S. A., J. BOUDEAU, J. L. REID, et al. Complexes between the LKB1 tumor suppressor, STRADa/b and MO24a/b are upstream kinases in the AMP-activated protein kinase cascade. *J. Biol.* 2:28, 2003.
18. HAWLEY, S. A., D. A. PAN, K. J. MUSTARD, et al. Calmodulin-dependent protein kinase kinase-[beta] is an alternative upstream kinase for AMP-activated protein kinase. *Cell Metab.* 2:9–19, 2005.
19. HOLZ, M. K., B. A. BALLIF, S. P. GYGI, and J. BLENIS. mTOR and S6K1 mediate assembly of the translation preinitiation complex through dynamic protein interchange and ordered phosphorylation events. *Cell* 123:569–580, 2005.
20. HORMAN, S., G. J. BROWNE, U. KRAUSE, et al. Activation of AMP-activated protein kinase leads to the phosphorylation of elongation factor 2 and an inhibition of protein synthesis. *Curr. Biol.* 12:1419–1423, 2002.
21. INOKI, K., Y. LI, T. XU, and K.-L. GUAN. Rheb GTPase is a direct target of TSC2 GAP activity and regulates mTOR signaling. *Genes Dev.* 17:1829–1834, 2003.
22. INOKI, K., Y. LI, T. ZHU, J. WU, and K.-L. GUAN. TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signaling. *Nature Cell Biol.* 4:648–657, 2002.
23. INOKI, K., T. ZHU, and K.-L. GUAN. TSC2 mediates cellular energy response to control cell growth and survival. *Cell* 115:577–590, 2003.
24. JACINTO, E., R. LOEWITH, A. SCHMIDT, et al. Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. *Nature Cell Biol.* 6:1122–1128, 2004.
25. KAHN, B. B., T. ALQUIER, D. CARLING, and D. G. HARDIE. AMP-activated protein kinase: ancient energy gauge provides clues to modern understanding of metabolism. *Cell Metab.* 1:15–25, 2005.
26. KAPP, L. D., and J. R. LORSCH. The molecular mechanics of eukaryotic translation. *Annu. Rev. Biochem.* 73:657–704, 2004.
27. KIM, D.-H., D. D. SARBASSOV, S. M. ALI, et al. mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. *Cell* 110:163–175, 2002.
28. KIM, D.-H., D. D. SARBASSOV, S. M. ALI, et al. GβL, a positive regulator of the rapamycin-sensitive pathway required for the nutrient-sensitive interaction between raptor and mTOR. *Molec. Cell* 11:895–904, 2003.
29. KIMURA, N., C. TOKUNAGA, S. DALAL, et al. A possible linkage between AMP-activated protein kinase (AMPK) and mammalian target of rapamycin (mTOR) signalling pathway. *Genes Cells* 8:65–79, 2003.
30. KRAUSE, U., L. BERTRAND, and L. HUE. Control of p70 ribosomal protein S6 kinase and acetyl-CoA carboxylase by AMP-activated protein kinase and protein phosphatases in isolated hepatocytes. *Eur. J. Biochem.* 269:3751–3759, 2002.
31. KRYMSKAYA, V. P. Tumour suppressors hamatin and tuberin: intracellular signalling. *Cell Signal.*15:729–739, 2003.
32. KWIATKOWSKI, D. J. Tuberous sclerosis: from tubers to mTOR. *Annals Hum. Genet.* 67:87–96, 2003.
33. LI, Y., K. INOKI, and K.-L. GUAN. Biochemical and functional characterization of small GTPase Rheb and TSC2 GAP activity. *Molec. Cell Biol.* 24:7965–7975, 2004.
34. MA, L., Z. CHEN, H. ERDJUMENT-BROMAGE, P. TEMPST, and P. P. PANDOLFI. Phosphorylation and functional inactivation of TSC2 by Erk: implications for tuberous sclerosis and cancer pathogenesis. *Cell* 121:179–193, 2005.
35. MANNING, B. D., A. R. TEE, M. N. LOGSDON, J. BLENIS, and L. C. CANTLEY. Identification of the tuberous sclerosis complex-2 tumor suppressor gene product tuberin as a target of the phosphoinositide 3-kinase/Akt pathway. *Molec. Cell* 10:151–162, 2002.
36. MEYUHAS, O. Synthesis of the translational apparatus is regulated at the translational level. *Eur. J. Biochem.* 267:6321–6330, 2000.
37. NAVE, B. T., D. M. OUWENS, D. J. WITHERS, D. R. ALESSI, and P. R. SHEPHERD. Mammalian target of rapamycin is a direct target for protein kinase B: identification of a convergence point for opposing effects of insulin and amino acid deficiency on protein translation. *Biochem. J.* 344:427–431, 1999.
38. NOJIMA, H., C. TOKUNAGA, S. EGUCHI, et al.The mammalian target of rapamycin (mTOR) partner, raptor, binds the mTOR substrates p70 S6 kinase and 4E-BP1 through their TOR signaling (TOS) motif. *J. Biol. Chem.* 278:15461–15464, 2003.
39. PENDE, M., S. H. UM, V. MIEULET, et al. S6K1−/−/S6K2−/− mice exhibit perinatal lethality and rapamycin-sensitive 5-terminal oligopyrimidine mRNA translation and reveal a mitogen-activated protein kinase-dependent S6 kinase pathway. *Molec. Cell Biol.* 24:3112–3124, 2004.
40. POTTER, C. J., L. G. PEDRAZA, and T. XU. Akt regulates growth by directly phosphorylating Tsc2. *Nature Cell Biol.* 4:658–665, 2002.
41. RAUGHT, B., F. PEIRETTI, A.-C. GINGRAS, et al. Phosphorylation of eucaryotic translation initiation factor 4B Ser422 is modulated by S6 kinases. *EMBO J.* 23:1761–1769, 2004.
42. REDPATH, N. T., E. J. FOULSTONE, and C. G. PROUD. Regulation of translation elongation factor-2 by insulin via a rapamycin-sensitive signalling pathway. *EMBO J.* 15:2291–2297, 1996.
43. RICHARDSON, C. J., S. S. SCHALM, and J. BLENIS. PI3-kinase and TOR: PIKTORing cell growth. *Semin. Cell Dev. Biol.* 15:147–159, 2004.
44. ROUX, P. P., B. A. BALLIF, R. ANJUM, S. P. GYGI, and J. BLENIS. Tumor-promoting phorbol esters and activated Ras inactivate the tuberous sclerosis tumor suppressor complex via p90 ribosomal S6 kinase. *Proc. Natl. Acad. Sci. U. S. A.* 101:13489–13494, 2004.
45. RUVINSKY, I., N. SHARON, T. LERER, et al. Ribosomal protein S6 phosphorylation is a determinant of cell size and glucose homeostasis. *Genes Dev.* 19:2199–2211, 2005.
46. SAKAMOTO, K., A. MCCARTHY, D. SMITH, et al. Deficiency of LKB1 in skeletal muscle prevents AMPK activation and glucose uptake during contraction. *EMBO J.* 24:1810–1820, 2005.
47. SARBASSOV, D. D., S. M. ALI, D.-H. KIM, et al. Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton. *Curr. Biol.* 14:1296–1302, 2004.
48. SARBASSOV, D. D., D. A. GUERTIN, S. M. ALI, and D. SABATINI. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science* 307:1098–1101, 2005.
49. SCHALM, S. S., D. C. FINGAR, D. M. SABATINI, and J. BLENIS. TOS motif-mediated raptor binding regulates 4E-BP1 multisite phosphorylation and function. *Curr. Biol.* 13:797–806, 2003.
50. SEKULIC, A., C. C. HUDSON, J. L. HOMME, et al. A direct linkage between the phosphoinositide 3-kinase-Akt signaling pathway and the mammalian target of rapamycin in mitogen-stimulated and transformed cells. *Cancer Res.* 60:3504–3523, 2000.
51. SHAW, R. J., M. KOSMATKA, N. BARDEESY, et al. The tumor suppressor LKB1 kinase directly activates AMP-activated kinase and regulates apoptosis in response to energy stress. *Proc. Natl. Acad. Sci. U. S. A.* 101:3329–3335, 2004.
52. SHAW, R. J., K. A. LAMIA, D. VASQUEZ, et al. The kinase LKB1

mediates glucose homeostasis in liver and therapeutic effects of metformin. *Science* 310:1642–1646, 2005.
53. SMITH, E. M., S. G. FINN, A. R. TEE, G. J. BROWNE, and C. G. PROUD. The tuberous sclerosis protein TSC2 is not required for the regulation of the mammalian target of rapamycin by amino acids and certain cellular stresses. *J. Biol. Chem.* 280:18717–18727, 2005.
54. SPRENKLE, A. B., S. P. DAVIES, D. CARLING, D. G. HARDIE, and T. W. STURGILL. Identification of Raf-1 Ser621 kinase activity from NIH 3T3 cells as AMP-activated protein kinase. *FEBS Lett.* 403:254–258, 1997.
55. WANG, X., W. LI, M. R. WILLIAMS, N. TERADA, D. R. ALESSI, and C. G. PROUD. Regulation of elongation factor 2 kinase by p90RSK1 and p70 S6 kinase. *EMBO J.* 20:4370–4379, 2001.
56. WOODS, A., K. DICKERSON, R. HEATH, et al. Ca2+/calmodulin-dependent protein kinase kinase-[beta] acts upstream of AMP-activated protein kinase in mammalian cells. *Cell Metab.* 2:21–33, 2005.
57. WILLIAMSON, D. L., S. R. KIMBALL, and L. S. JEFFERSON. Time course changes in signaling pathways, effector mechanisms of mRNA translation, and protein synthesis in C2C12 myotubes following AMPK activation by AICAR. *Am. J. Physiol.* 291:E80–E89, 2006.
58. WILLIAMSON, D. L., N. KUBICA, S. R. KIMBALL, and L. S. JEFFERSON. Exercise-induced alterations in ERK112 and mTOR signaling to regulatory mechanisms of mRNA translation in mouse muscle. *J. Physiol.* 573:497–510, 2006.