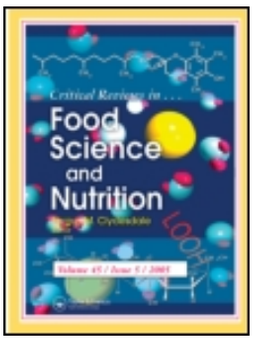

Critical Reviews in Food Science and Nutrition

ISSN: 1040-8398 (Print) 1549-7852 (Online) Journal homepage: https://www.tandfonline.com/loi/bfsn20

Cross-Talk between AMPK and mTOR in Regulating Energy Balance

Jia Xu , Jian Ji & Xiang-Hua Yan

To cite this article: Jia Xu , Jian Ji & Xiang-Hua Yan (2012) Cross-Talk between AMPK and mTOR in Regulating Energy Balance, Critical Reviews in Food Science and Nutrition, 52:5, 373-381, DOI: 10.1080/10408398.2010.500245

To link to this article: https://doi.org/10.1080/10408398.2010.500245

Accepted author version posted online: 17 Jun 2011.
Published online: 27 Feb 2012.

Critical Reviews in Food Science and Nutrition, 52:373–381 (2012)

ISSN: 1040-8398 / 1549-7852 online
DOI: 10.1080/10408398.2010.500245

Taylor & Francis
Taylor & Francis Group

Cross-Talk between AMPK and mTOR in Regulating Energy Balance

JIA XU,[1] JIAN JI,[2] and XIANG-HUA YAN[1]

[1]College of Animal Sciences and Technology, Huazhong Agricultural University, Wuhan 430070, Hubei, P.R.China
[2]College of Animal Sciences, Key Laboratory of Animal Molecular Nutrition of Education Ministry, Zhejiang University, Hangzhou 310029, Zhejiang, P.R.China

Energy balance is maintained by a complex homeostatic system involving some signaling pathways and "nutrient sensors" in multiple tissues and organs. Any defect associated with the pathways can lead to metabolic disorders including obesity, type 2 diabetes, and the metabolic syndrome. The 5'-adenosine monophosphate-activated protein kinase (AMPK) and mammalian target of rapamycin (mTOR) appear to play a significant role in the intermediary metabolism of these diseases. AMPK is involved in the fundamental regulation of energy balance at the whole body level by responding to hormonal and nutrient signals in the central nervous system and peripheral tissues that modulate food intake and energy expenditure. Mammalian target of rapamycin (mTOR),is one of the downstream targets of AMPK functions as an intracellular nutrient sensor to control protein synthesis, cell growth, and metabolism. Recent research demonstrated the possible interplay between mTOR and AMPK signaling pathways. In this review, we will present current knowledge of AMPK and mTOR pathways in regulating energy balance and demonstrate the convergence between these two pathways.

Keywords 5'-adenosine monophosphate-activated protein kinase, mammalian target of rapamycin, energy balance, signaling pathways

INTRODUCTION

Food intake, energy expenditure, and body weight are critically regulated by hypothalamic neural circuits. Complex metabolic neuronal pathways in the central nervous system have evolved such that they allow mammalian cells to receive and integrate signals that convey information about the status of energy fluxes and stores. These signals then are linked to the control of fuel metabolism and energy homeostasis. Such pathways are necessary for ensuring that vital organs meet basic metabolic needs and that various tissues receive a supply of nutrients to perform specialized functions essential for the survival and benefit of the organism. Cell growth depends on a high rate of protein synthesis and requires a high level of cellular energy; consequently cells suppress protein synthesis when there is insufficient energy or amino acid substrate. A particularly important pathway with regard to regulating energy balance is the mTOR signaling pathway. mTOR pathway plays a role in the brain mechanisms that respond to nutritional and hormonal signals (Cota et al., 2006). In peripheral cells, the mTOR signaling pathway integrates signals to control growth and development (Sabatini et al., 1994; Schmelzle and Hall, 2000; Wullschleger et al., 2006). Another fuel-sensitive signaling pathway in the hypothalamic control of energy balance is the AMPK pathway (Andersson et al., 2004; Kim et al., 2004; Minokoshi et al., 2004; Han et al., 2005; Kola et al., 2005). AMPK is regulated by intracellular AMP/ ATP ratio. When growth conditions are favorable, mTOR is active; however, unlike mTOR, AMPK activity is increased during fuel deficiency. The activation of AMPK inhibits mTOR activity. Here we provide a functional and regulatory overview of these two signaling pathways.

Address correspondence to Xiang-Hua Yan, Laboratory of Animal Molecular Nutrition, College of Animal Sciences and Technology, Huazhong Agricultural University, Wuhan 430070, P.R.China. Tel: 0086-27-8728 1033; Fax: 0086-27-8728 0408. E-mail: xhyan@mail.hzau.edu.cn

BIOCHEMICAL LINKAGE OF THE AMPK AND mTOR PATHWAYS

The AMPK exists as a heterotrimetric complex comprising a catalytic α subunit and regulatory β and γ subunits. Under conditions of hypoxia and exercise, AMPK is allosterically activated (Hardie et al., 1998; Kemp et al., 1999). AMPK when regulated responds to changes in the ratio of ATP/AMP. Activated AMPK turns on catabolic pathways to augment ATP production while turning off synthetic pathways that consume ATP. In mammalians, AMP promotes activation of AMPK by

stimulating phosphorylation of the catalytic α subunit and an upstream kinase whose identity will be discussed later. mTOR is a highly conserved serine-threonine kinase present in organisms from yeast to mammals, which controls cellular functions in response to nutrient and growth factors. In vitro, cellular levels of ATP increase mTOR signaling, and mTOR itself is thought to serve as an ATP sensor (Dennis et al., 2001). mTOR respond to nutrient availability, energy status, and in turn determines the rate of cell growth and proliferation (Colombani et al., 2003; Jacinto and Hall, 2003). The interplay of mTOR and AMPK provide a more exact mechanism for mammals to coordinate with the environmental conditions. The earliest evidence that the mTOR and AMPK pathways are linked comes from the study of eukaryotic initiation factor 4E binding protein-1(4EBP1) and S6 kinase (S6K), which are downstream targets of mTOR. The phosphorylation and activity of S6K and 4EBP1 are widely used as readouts for signaling through the mTOR pathway. AMPK regulate the activities both of S6K and 4EBP1, indicating a convergence of AMPK signaling and mTOR signaling pathways.

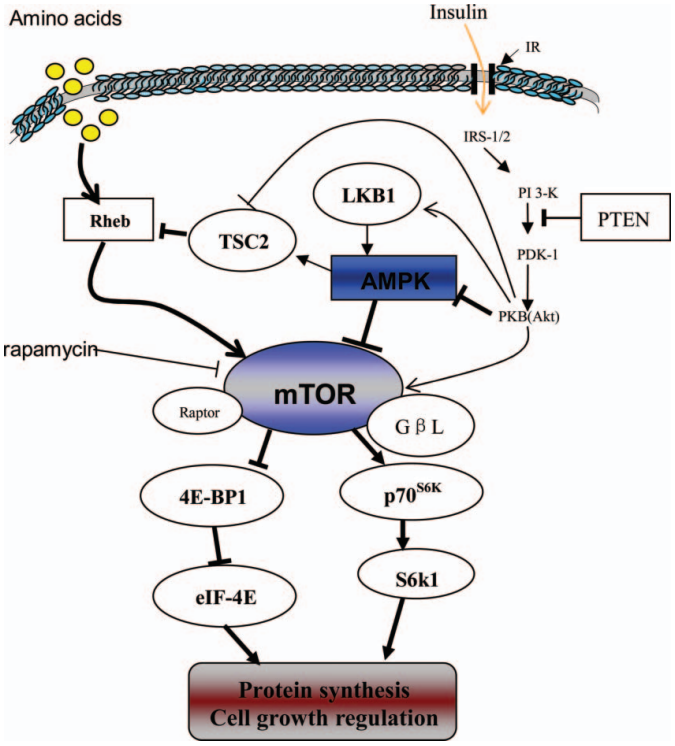

Figure 1 Model of the AMPK and mTOR signaling network in mammalian cells. AMPK inhibits mTOR activation of the mTOR pathway by the PI3K pathway occurs via the phosphorylation and inactivation of TSC2 by Akt. Arrows indicate activation, bars indicate inhibition. (color figure available online.)

UPSTREAM KINASE OF AMPK RELATED TO mTOR

LKB1

Over the past decade, the phosphorylation of the AMPK α catalytic subunit at Thr172 is essential for the activation of AMPK (Hawley et al., 1996). A breakthrough in the search for upstream AMPK kinase (AMPKK) comes from the identification of three closely related yeast protein kinases—Elm1, Pak1, and Tos3. These kinases act upstream of the yeast homologue of AMPK (Hong et al., 2003; Sutherland et al., 2003). The most closely related mammalian homologues to the kinases are members of the Ca2+–calmodulin-dependent protein kinase (CAMKK) family and the tumor suppressor LKB1 (also called serine/threonine kinase 11). LKB1 activate AMPK by phosphorylating the catalytically critical Thr172 residue and increases AMPK kinase activity (Woods et al., 2003; Hawley et al., 2003). Deficiency of LKB1 in skeletal muscle and heart of transgenic mice prevents contraction-stimulated AMPK activation (Sakamoto et al., 2005; 2006). In addition, deletion of LKB1 in liver results in a nearly complete loss of AMPK activity (Shaw et al., 2005). These findings suggest that LKB1 is a physiologically important upstream kinase for AMPK.

The LKB1 tumor suppressor is mutationally inactivated in the autosomal dominant Peutz-Jeghers syndrome (Boudeau et al., 2003), which is characterized by multiple hamartomas mainly in the intestine. Interestingly, the hamartomas in Peutz-Jeghers syndrome are similar to the benign tumors seen in tuberous sclerosis complex (TSC), an upstream negative regulator of mTOR, as will be discussed in more detail below (Yoo et al., 2002). LKB1 inhibits cell growth by decreasing the phosphorylation of S6K and 4EBP1 and positively regulates TSC2 function (Corradetti et al., 2004). Under low energy conditions, apoptosis caused by the absence of LKB1 is mTOR-dependent. The molecular mechanism LKB1 tumor suppressor may activate the TSC2 tumor suppressor via the AMPK (Inoki et al., 2003).

Akt

The serine/threonine protein kinase Akt, also known as protein kinase B, is an upstream inducer of mTOR. Akt is a positive regulator of mTOR that mediates the activation of mTOR by growth factors. Akt inactivates TSC2 by phosphorylating it on four residues, thereby activating mTOR (Manning et al., 2002; Inoki et al., 2002, Potter et al., 2002). The Akt/mTOR/p70S6K pathway is considered a central regulator of protein synthesis (see Fig. 1). Akt is also a key regulator of energy metabolism that inhibits AMPK (Hahn-Windgassen et al., 2005). The effect of Akt on ATP level causes a concomitant reduction in the AMP/ATP ratio and therefore reduces AMPK activity. Historically, the activation of mTOR via Akt-mediated phosphorylation of TSC2 and via inhibition of AMPK are viewed as two separate pathways leading to the activation of mTOR, recent studies proved Akt lies upstream of both pathways and induces full inhibition of TSC2 and activation of mTOR both through direct phosphorylation and by inhibition of AMPK-mediated phosphorylation of TSC2 (Hahn-Windgassen et al., 2005). But whether Akt affect AMPK activity via phosphorylation of AMPK upstream regulator LKB is still

unknown. Currently, another study in lung carcinoma showed the treatment with fibronectin, a matrix glycoprotein highly expressed in tobacco-related lung disease and one that stimulates non-small cell lung carcinoma (NSCLC) cell growth and survival, inhibits the mRNA and protein expression of LKB1 as well as the phosphorylation of AMPK. These observations suggest that fibronectin-induced stimulation of NSCLC cell proliferation requires activation of the Akt/mTOR/p70S6K pathway and is associated with inhibition of LKB1/AMPK signaling (Huypens, 2006).

DOWNSTREAM TARGETS OF AMPK LIE WITHIN THE mTOR SIGNALING PATHWAY AND UPSTREAM TARGET OF mTOR COORDINATE WITH AMPK

At least two downstream targets of AMPK lie within the mTOR signaling pathway, TSC and mTOR. These targets play a key role in restoring ATP levels by slowing the energy-consuming process of protein synthesis and cell growth. TSC play an important role in coordination between the AMPK and mTOR signaling pathway.

TSC

Important new insights on the crosstalk between the mTOR and AMPK pathway has come from the studies on the tuberous sclerosis complex (TSC) and the small GTPase Rheb. TSC comprises two interacting proteins that form a stable heterodimeric complex, TSC1 (hamartin) and TSC2 (tuberin). The autosomal dominant disorder tuberous sclerosis is characterized by benign tumors in the brain, eyes, skin, and kidney, and by mental retardation, seizures, and autism, and is caused by mutations in either of the TSC1 or TSC2 genes (Montague et al., 2001). Genetic studies and biochemical analyses initiated in Drosophila and confirmed in the mammalian tissue culture cells show that cells with disease-causing mutations in TSC1 or TSC2, or with RNAi-mediated reduction or genetic knockout of TSC1 or TSC2, show increased levels of S6K1 and 4E-BP1 phosphorylation (Goncharova et al., 2002; Gao et al., 2002; Inoki et al., 2002; Tee et al., 2002; Onda et al., 2002; Radimerski et al., 2002). In contrast, overexpression of TSC1 and TSC2 inhibits the phosphorylation of S6K and 4E-BP1 in response to nutrients or mitogens (Inoki et al., 2002; Tee et al., 2002). Thus, TSC1 and TSC2 act as a heterodimer that negatively regulates mTOR signaling.

How does TSC1-TSC2 regulate mTOR? The immediate downstream target of TSC is Rheb. TSC2 functions as a specific guanosine triphosphatase (GTPase) activating GAP that inhibits Ras homolog enriched in brain (Rheb), whereas TSC1 has no apparent catalytic activity. Rheb binds directly to the kinase domain in mTOR and activates mTOR in a GTP dependent manner. However, Rheb binding to mTOR is independent of the guanyl nucleotide, and nucleotide-free Rheb inhibits mTOR activity (Long et al., 2005). Long et al. (2005) suggest that GTP loading of Rheb, rather than mediating mTORC1 recruitment, enables Rheb to induce a conformational change in mTORC1 leading to mTORC1 activation and phosphorylation of downstream targets.

Recent studies have demonstrated that the TSC2 is a direct target of AMPK and a critical mediator of AMPK-dependent inhibition of mTOR signaling. TSC2 was shown to govern the energy sensitivity of mTOR (Inoki et al., 2003). AMPK directly phosphorylates TSC2 on T1227 and S1345, thereby enhancing the stability of the TSC1-TSC2 complex (Inoki et al., 2003). Cells expressing TSC2 with the AMPK phosphorylation sites mutated do not undergo the normal cell size reduction associated with ATP depletion. Furthermore, in the absence of TSC2, ATP depletion no longer leads to dephosphorylation of the two TOR effectors S6K and 4E-BP1. Consistent with these findings, the AMPK activator AICAR (5-aminoamidazole-1-b-4 carboxamide ribofuranoside) inhibits S6K in mammalian cells (Kimura et al., 2003). Thus, these findings suggest that a low cellular energy status is transmitted by AMPK to TSC2 which, in turn, inhibits Rheb and ultimately mTOR activity. More recently, studies discovered that phosphorylation of TSC2 by AMPK provides the priming phosphorylation for subsequent phosphorylation by GSK3 (Inoki et al., 2006). AMPK and GSK3 coordinately phosphorylate TSC2 and depend on each other to repress mTOR signaling. When GSK3 is inhibited, the AMPK inhibition on S6K phosphorylation is significantly reduced. Reciprocally, when AMPK activity is affected, the effect of GSK3 on S6K phosphorylation also changes accordingly.

mTOR

Two distinct multi-protein complexes mTORC1 and mTORC2 were found to exist in the mTOR. The first complex mTORC1, which mediates the rapamycin-sensitive temporal control of cell growth, contains mTOR, regulator-associated protein of mTOR (Raptor), and G protein β subunit–like protein (GβL). The second complex mTORC2, which mediates the rapamycin-insensitive spatial control of cell growth, contains mTOR, rapamycin-insensitive companion of mTOR (Rictor), mammalian stress–activated protein kinase–interacting protein 1 (mSin1), and GβL. mTORC1 specifically phosphorylates S6 kinase (S6K) and 4E-BP whereas mTORC2 phosphorylates Akt at Ser473, promoting Akt activation.

mTOR is one of the downstream targets of AMPK (Bolster et al., 2002; Kimura et al., 2003; Cheng et al., 2004; Reiter et al., 2005). Previous studies have identified Ser2448 as a nutrient-regulated phosphorylation site located in the mTOR catalytic domain; AMPK-induced phosphorylation is reduced when Ser2448 is phosphorylated. Upon depletion of ATP, activation of AMPK results in inhibition of mTOR signalling, thereby suppressing protein synthesis, which is an important pathway by which AMPK conserves cellular energy during low energy states (Tokunaga et al. 2004; Deldicque et al. 2005).

hVps34

The oldest member of the PI3K (phosphoinositide 3-kinase) family, hVps34 (human vacuolar protein sorting 34), was first identified as a regulator of vacuolar hydrolase sorting in yeast. Initially it was presumed that amino acids affected mTOR C1/S6K1 by acting through the generic class 1 PI3K branch of this pathway. Recently, hVp34 has shown to mediate the mTORC1/S6K1 signaling pathway. Previous finding suggested that inhibition of hVps34 blocks insulin-stimulated DNA synthesis (Siddhanta et al., 1998). Moreover, hVps34 appears to lie upstream of mTOR, as small interfering RNA knock-down of hVps34 inhibits the phosphorylation of 4EBP1. Overexpression of hVps34 or the associated hVps15 kinase activates S6K1, and insulin stimulation of S6K1 is blocked by microinjection of inhibitory anti-hVps34 antibodies. hVps34 is not regulated by insulin, nor does it affect insulin-stimulated phosphorylation of Akt or TSC2. Additional evidence indicating hVps34 as the mediator of amino acid input to S6K1 came from experiments in which S6K1 activation was attenuated by ectopic expression of a cDNA containing two FYVE domains, which bind to PI3P, preventing binding of proteins containing either FYVE or PX domains.

However, hVps34 is inhibited by amino acid or glucose starvation and by activation of AMPK. hVps34 activity was significantly inhibited in AICAR treated cells, suggesting that hVps34 is negatively regulated by the AMPK-mediated response to nutrient deprivation. Consistent with this, the Vps15 kinase domain is in fact similar to the kinase domain of AMPK (52% similar over residues 24–168 of the kinase domain by NCBI BLAST), and hVps34 has a number of potential phosphorylation sites that fit the AMPK consensus sequence (Hardie et al., 1998; Michell et al., 1996; Byfield et al., 2005). Therefore, it is possible that hVps34 act as a substrate of AMPK (Hardie, 2008).

Together, these findings suggest a novel role for hVps34 in nutrient sensing, and in the integration of signaling from amino acids and glucose to mTOR and S6K1. Interestingly, energy deprivation does not have an immediate impact on intracellular amino acid levels, nor does amino acid deprivation affect ATP levels (Dennis et al., 2001). Thus, the mechanism that hVps34 functions as a mediator between AMPK and mTOR plays an important role in regulating 4E-BP and S6K1 activity.

AMPK AND mTOR SIGNALING PATHWAYS IN CONTROL OF ENERGY HOMEOSTASIS

Inhibition of translation is a major physiological response under energy starvation conditions to maintain homeostasis. mTOR controls many aspects of cellular metabolism including amino acid biosynthesis, glucose homeostasis, and others (Thomas et al., 2004) The mTOR pathway integrates various metabolic signals (both nutrients and hormones), thereby regulating energy homeostasis and body weight, both the peripheral and central. AMPK, functions as a "fuel gauge" to monitor cellular energy status. The AMPK pathway in the hypothalamus and peripheral tissues coordinately integrates inputs from multiple hormones, peptides, and nutrients to maintain energy homeostasis.

Hypothalamic AMPK and mTOR Activity Regulates Food Intake and Body Weight

The hypothalamus is a key regulator of food intake and energy balance, coordinating body adiposity and nutritional state in response to peripheral hormones. Fat-derived hormone leptin acts directly on a subset of neurons; a deficiency of leptin is interpreted by the brain as starvation. The ancient mTOR sensing pathway integrates nutritional and hormonal signals in the neuronal to regulate the energy balance (Cota et al., 2006). Central administration of leucine activates mTOR and S6K and decreases food intake and consequently body weight. Furthermore, inhibiting mTOR signaling with the drug rapamycin prevented both leucine-mediated mTOR and S6 kinase signaling and blocked the suppression of food intake. Finally, leptin also activated hypothalamic mTOR, and rapamycin substantially blocked leptin's ability to suppress food intake and body weight gain. Thus, mTOR is a cellular fuel sensor whose hypothalamic activity is directly tied to the regulation of energy intake.

Hypothalamic AMPK activity also regulates food intake and body weight, which is altered by various factors and mediates their feeding effects. Hypothalamic AMPK is affected by nutritional availability in hypothalamic neurons. Administration of the glucose anti-metabolite 2-deoxyglucose (2-DG) increases hypothalamic AMPK activity, whereas co-administration of an AMPK inhibitor, compound C, inhibited the 2-DG induced glucoprivic feeding (Kim et al., 2004). Conversely, ICV administration of glucose or restoration of food intake decreases hypothalamic AMPK activity (Minokoshi et al., 2004). Furthermore, hypothalamic AMPK is regulated depending on the feeding states. Fasting results in increased AMPK activity in multiple hypothalamic regions, whereas refeeding inhibits it (Minokoshi et al., 2004). AMPK activity seems to be decreased by feeding-inhibiting factors and increased by feeding-stimulating factors. Fasting and refeeding are accompanied by oscillations of hormones and nutrients in both the periphery and the central nervous system which may bring about the alterations in AMPK activity. Injection of leptin and insulin (Andersson et al., 2004; Minokoshi et al., 2004) into rodents inhibits AMPK activity in the hypothalamus. Leptin's effects on AMPK activity are localized to the arcuate and paraventricular (PVN) nuclei (Minokoshi et al. 2004) whereas high glucose or insulin inhibits AMPK in other hypothalamic regions as well; conversely, administration of orexigenic peptides including ghrelin and cannabinoids increases AMPK activity (Minokoshi et al., 2004; Kola et al. 2005). Cannabinoids potently stimulate food intake acting via the presynaptic cannabinoid type 1 receptor (CB1) in the ventromedial hypothalamus (Jamshidi and Taylor, 2001). Ghrelin

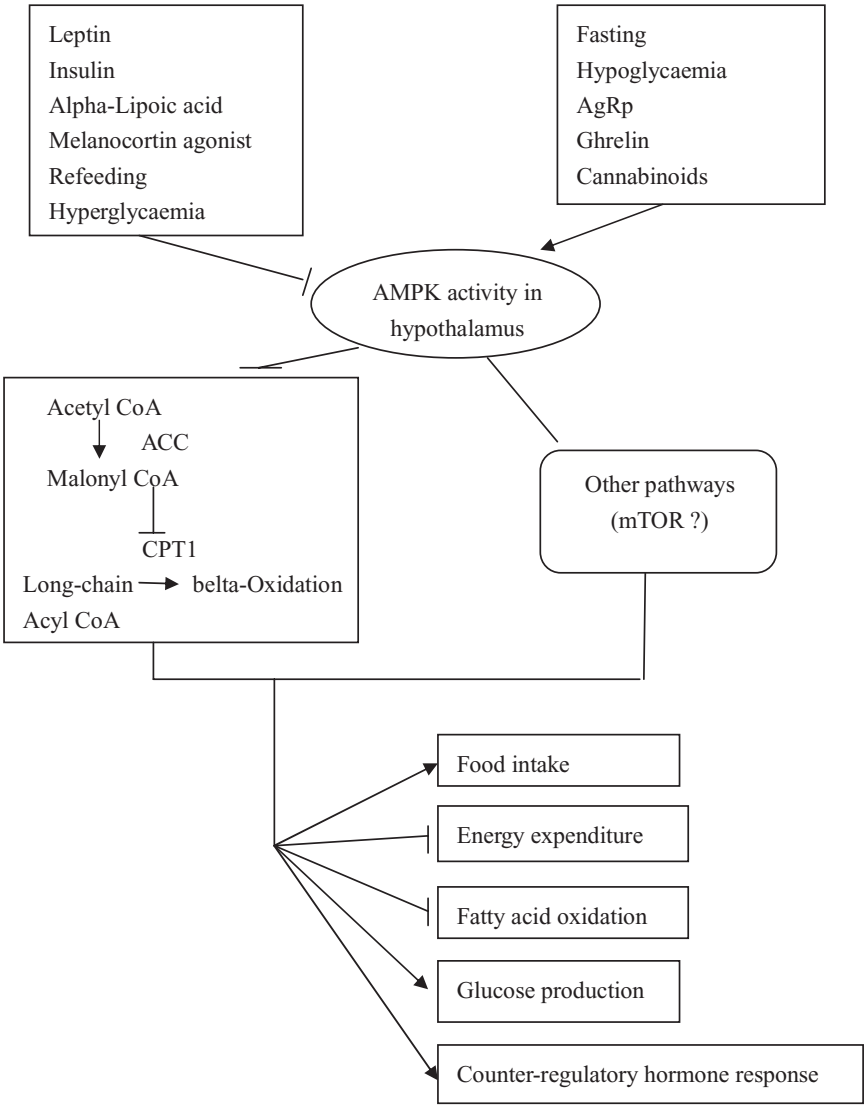

Figure 2 Interaction between hypothalamic mTOR and AMPK. Changes in AMPK activity may act through the acetyl CoA carboxylase (ACC)–malonyl CoA–CPT1 pathway to regulate food intake and other metabolic processes. Other pathways downstream of AMPK, including possibly mTOR (Cota et al., 2006), also mediate the effects of AMPK on energy balance and glucose homeostasis. An arrow represents stimulation, whereas a crossbar means inhibition.

is predominantly expressed in the gastric mucosa and acts on receptors in the arcuate and ventromedial hypothalamus (see Fig.2) (Xue and Khan, 2006; Zigman et al. 2006).

Interestingly, the expression of both neuropeptide Y (NPY) and agouti-related peptide (AgRP) in the orexigenic neurons was significantly raised while no such rise in the anorexigenic neurons took place during the activation of mTOR which is regulated by leptin to regulate energy balance. In the hypothalamic PVN nucleus, AMPK controls feeding behavior, at least in part, through the regulation of orexigenic NPY and AgRP expression. Over-expression of DN-AMPK in the medial hypothalamus decreases NPY and AGRP mRNA expression in ad libitum-fed rats, whereas over-expression of constitutively active AMPK augments the fast-induced increase in NPY and AGRP expression (Minokoshi et al. 2004). In addition, leptin activating hypothalamic mTOR activity indicates a mTOR-dependent pathway for leptin's effect on food intake (Cota et al., 2006). Conversely, it suppresses AMPK activity in the hypothalamus, and this suppression appears to be necessary for leptin's ability to reduce food intake (Minokoshi et al., 2004). The finding that leptin action is inhibited both by preventing a fall in hypothalamic AMPK activity and by preventing a rise in mTOR activity suggests the existence of such a linear pathway.

AMPK and mTOR Signaling Pathways Regulate Energy Balance Through Peripheral Tissues

Skeletal Muscle

AMPK is viewed as an energy sensor that acts to modulate glucose uptake and fatty acid oxidation in skeletal muscle. Many

experiments were designed to investigate whether AMPK activation modulates the translational control of protein synthesis in skeletal muscle. The activity of alpha1 AMPK remained unchanged in gastrocnemius muscle from AICAR-treated animals compared with controls, whereas alpha2 AMPK activity was significantly increased. Injection of AICAR resulted in a reduction in protein synthesis which was associated with decreased activation of mTOR signal transduction pathway as evidenced by reduced phosphorylation of protein kinase B on Ser(473), mTOR on Ser(2448), ribosomal protein S6 kinase on Thr(389), and the eukaryotic initiation factor eIF4E-binding protein on Thr(37). Therefore, in response to AMPK activation, changes will be seen in translation initiation and skeletal muscle protein synthesis.

The notion that during exercise muscle protein synthesis is inhibited suggests a cellular mechanism for the inhibition of muscle protein synthesis which would be associated with an activation of AMPK and an inhibition of downstream components of the mTOR pathway (Deshmukh et al., 2008). Immunoprecipitation and immunoblotting methods were utilized to measure muscle AMPK-alpha-2 activity, and mTOR-associated upstream and downstream signaling proteins find that AMPK activation and a reduced phosphorylation of 4E-BP1 may contribute to the inhibition of muscle protein synthesis during resistance exercise (Deldicque et al., 2005).

Liver

The convergence of mTOR and AMPK in the liver comes from the study of protein synthesis, which is an energy-consuming biosynthetic process. The actions of glucagon and insulin on glucose metabolism within the liver are opposing, which are essential mechanisms for maintaining plasma glucose concentrations within narrow limits. Earlier studies showing that protein kinase A (PKA) phosphorylates LKB1 (Collins et al., 2000; Sapkota et al., 2001) provides a possible mechanism through which glucagon may repress signaling through mTOR. Administration of AICAR to rats in vivo results in a repression of signaling through mTOR as assessed by the decreased phosphorylation of mTOR on Ser-2448 and the diminished phosphorylation of 4E-BP1 and S6K1 (Bolster et al., 2002). Recently, using the perfused rat liver the effect of glucagon on amino acid-induced signaling through the mTOR was examined, and the results show that amino acids enhance signaling through mTOR resulting in phosphorylation of eukaryotic initiation factor 4E-BP1 and S6K1 (Kimball et al., 2004; Le Bacquer et al., 2007). In contrast, glucagon repressed both basal and amino acid-induced signaling through mTOR, as assessed by changes in the phosphorylation of 4E-BP1 and S6K1. The repression was associated with the activation of PKA and enhanced phosphorylation of LKB1 and the AMPK. Thus, glucagon represses phosphorylation of 4E-BP1 and S6K1 through the activation of a PKA-LKB-AMPK-mTOR signaling pathway. More recently, the effects of AICAR observed in vivo were compared with those obtained in an in situ perfused liver preparation to investigate activation of AMPK in the absence of accompanying changes in hormones and nutrients. Phosphorylation of AMPK either in vivo or in situ was associated with a repression of protein synthesis as well as decreased phosphorylation of a number of targets of mTOR signaling including S6K1, eukaryotic initiation factor (eIF)4G and 4E-BP1. Overall, AICAR directly represses protein synthesis and mTOR signaling in the liver through an AMPK-dependent mechanism.

Pancreatic Islet β Cells

The β-cell plays an important role in animals since its primary function to synthesize and secrete insulin is tightly coupled to its metabolic rate. Glucose and amino acids leucine and glutamine stimulate phosphorylation of the mTOR target proteins p70S6K and 4E-BP1 in various pancreatic β-cell lines and rodent islets (Gomez et al., 2004; Xu et al., 2001; 1998). Additionally, in the β-cell, the exposure to glucose can lead to activation of the insulin signaling pathway by an autocrine mechanism and it may also affect the regulation of mTOR by nutrients. Thus, glucose can lead to mTOR activation through stimulation of insulin secretion and activation of the insulin receptor or directly through an unknown mechanism.

A high concentration of glucose in pancreatic β-cells decreases AMPK activity (da Silva Xavier et al., 2000). Conversely, AMPK activation suppresses glucose-induced increases in glycolysis, mitochondrial oxidative metabolism, Ca^{2+} influx, and insulin secretion (da Silva Xavier et al., 2003). AMPK activation also inhibits the effect of glucose on the promoter activities of L-PK and preproinsulin (PPI) (da Silva Xavier et al., 2000) Thus, AMPK seems to antagonize the effect of glucose on insulin secretion and gene expression in β-cells.

Recent studies show that inhibition of AMPK in β-cells by high glucose inversely correlates with the mTOR pathway (Gleason et al., 2007). Forced activation of AMPK by AICAR, phenformin, or oligomycin significantly blocked phosphorylation of S6K in response to the combination of glucose and amino acids. In β-cells, glucose and the amino acids leucine and glutamine activate mTOR whereas they suppress the activity of AMPK (Leclerc et al., 2004; Gleason et al., 2007). Thus, mTOR activation is inversely related to AMPK activity and AMPK activation inhibits the mTOR signaling pathway and it may suggest that one component of the mechanism whereby glucose and other nutrients stimulate protein synthesis in the β-cells is through the inhibition of AMPK.

CROSS-TALK BETWEEN AMPK AND mTOR IN METABOLIC DISEASE

Obesity is rapidly increasing worldwide, and closely related to the accelerating rates of type 2 diabetes and cardiovascular disease. Both type 2 diabetes and obesity, among other disorders, are associated with an inability to respond to insulin. Insulin and its signaling systems are implicated in both central

and peripheral mechanisms governing the ingestion, distribution, metabolism, and storage of nutrients in organisms ranging from worms to humans. Pharmacological studies demonstrated that when insulin was injected into the hypothalamic ventromedial and paraventricular nuclei, the body temperature and energy expenditure were increased and the food intake was also reduced (McGowan et al., 1992; Menendez et al., 1991). Hypothalamic AMPK and mTOR activity is an important determinant of food intake, and alteration in hypothalamic AMPK and mTOR activity may cause dysregulation of feeding in metabolic disorders. The peripheral mTORC1 pathway is involved in the pathogenesis of obesity and obesity-associated metabolic disorders (Um et al., 2004; Khamzina et al., 2005; Lam et al., 2005). Previous studies have demonstrated that genetic deletion of S6K1 results in profound metabolic defects in peripheral tissues (Pende et al., 2000; Shima et al., 1998). mTORC1 activity is significantly elevated in the liver, muscle, and adipose tissue of both genetically-induced and diet-induced obese animals (Um et al., 2004; Khamzina et al., 2005).

Obesity is also associated with leptin resistance (El-Haschimi et al., 2000). Leptin is an adipocyte-derived hormone that primarily acts in the hypothalamus and plays a key role in diminishing appetite, reducing food intake, and long-term control of body weight. Leptin has direct peripheral effects on several tissues, and it may be independently involved in insulin secretion and action besides its effects on body weight regulation. Recent research found that hypothalamic AMPK activity is enhanced in rats with uncontrolled diabetes (Lee et al., 2005). Moreover, the inhibition of hypothalamic AMPK activity suppresses enhanced feeding in type 2 diabetes rats, suggesting that hypothalamic AMPK activation is attributed to the development of type 2 diabetes. Leptin and insulin deficiencies in diabetic animals may cause increased hypothalamic AMPK activity, based on the observations that plasma leptin and insulin concentrations are profoundly lower in diabetic animals and that ICV leptin and insulin reversed diabetes-induced increases in food intake and hypothalamic AMPK activity.

Given the importance of AMPK and mTOR in mediating the physiological effects of insulin and leptin, it is plausible to hypothesize that dysregulation of AMPK and mTOR may contribute to leptin resistance in diet-induced obesity. Leptin-resistant states, which include diet-induced obesity, pharmacological activation of AMPK, and suppression of mTOR in peripheral tissues, may be a strategy to bypass leptin resistance and help protect individuals from developing insulin resistance and type 2 diabetes. Genetic deletion of 4E-BP1 protects against high-fat diet-induced obesity and insulin resistance because of increased energy expenditure (Noland et al., 2007; Tsukiyama-Kohara et al., 2001).

PERSPECTIVES

AMPK and mTOR represent diverse, yet versatile energy sensor and metabolic regulator that exerts a regulatory effect in the hypothalamus and multiple peripheral tissues. An increasing number of hormones and nutrients are being shown to regulate AMPK and mTOR signaling. Novel molecules and pathways should be identified as targets which link these two pathways. A further understanding of the relationship between mTOR and AMPK in coordinating amino acid- and energy-sensing pathways would provide new insights into the regulation of whole body energy homeostasis. Understanding how AMPK and mTOR regulate energy balance is rapidly gaining clinical importance. Interplay of both the peripheral and central nervous system (CNS) fuel-sensing pathways may be associated with the development of obesity and diabetes. Novel therapies for metabolic disease will emerge from pharmacological or nutritional exploitation of these insights.

ACKNOWLEDGEMENTS

This work was supported by the grants from the National Natural Science Foundation of China (No. 30700580, No. 31072036, and No. 30700580), the Fundamental Research Funds for the Central Universities (No. 2010PY011) and the Open Project of State Key Laboratory of Animal Nutrition (No. 2004DA125184F0906).

REFERENCES

Andersson, U., Filipsson, K., Abbott, C. R., Woods, A., Smith, K., Bloom, S. R., Carling, D., and Small, C. J. (2004). AMP-activated protein kinase plays a role in the control of food intake. *J Biol. Chem.* **279**: 12005–12008.

Bolster, D. R., Crozier, S. J., Kimball, S.R., and Jefferson, L. S. (2002). AMPK-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.

Boudeau, J., Sapkota, G., and Alessi, D. R. (2003). LKB1: A protein kinase regulating cell proliferation and polarity. *FEBS Lett*. **546**: 159–165.

Byfield, M. P., Murray, J. T., and Backer, J. M. (2005). hVps34 Is a nutrient-regulated lipid kinase required for activation of p70 S6 kinase. *J. Biol. Chem.* **280**: 33076–33082.

Cheng, S. W. Y., Fryer, L. G. D., Carling, D., and Shepherd, P.R. (2004). Thr2446 is a novel mammalian target of rapamycin (mTOR) phosphorylation site regulated by nutrient status. *J. Biol. Chem.* **279**: 15719–15722.

Collins, S. P., Reoma, J. L., Gamm, D. M., and Uhler, M. D. (2000). LKB1, a novel serine/threonine protein kinase and potential tumour suppressor, is phosphorylated by cAMP-dependent protein kinase (PKA) and prenylated in vivo. *Biochem. J.* **345**: 673–680.

Colombani, J., Raisin, S., Pantalacci, S., Radimerski, T., Montagne, J., and Leopold, P. (2003). A nutrient sensor mechanism controls Drosophila growth. *Cell*. **114**: 739–749.

Corradetti, M. N., Inoki, K., Bardeesy, N., DePinho, R. A., and Guan, K. L. (2004). Regulation of the TSC pathway by LKB1: Evidence of a molecular link between tuberous sclerosis complex and Peutz-Jeghers syndrome. *Genes Dev.* **18**: 1533–1538.

Cota, D., Proulx, K., Smith, K. B., Kozma, S., Thomas, G., Woods, S. C., and Seeley, R.J. (2006). Hypothalamic mTOR regulates food intake. *Science*. **312**: 927–930.

da Silva Xavier, G., Leclerc, I., Salt, I. P., Doiron, B., Hardie, D. G., Kahn, A., and Rutter, G. A. (2000). Role of AMP activated protein kinase in the

regulation by glucose of islet beta cell gene expression. *Proc Natl Acad Sci USA*. **97**: 4023–4028.

da Silva Xavier, G., Leclerc, I., Varadi, A., Tsuboi, T., Moule, S. K., and Rutter, G. A. (2003). Role for AMP-activated protein kinase in glucose-stimulated insulin secretion and preproinsulin gene expression. *Biochem J.* **371**(Pt 3): 761–774.

Deldicque, L., Theisen, D., and Francaux, M. (2005). Regulation of mTOR by amino acids and resistance exercise in skeletal muscle. *Eur. J. Appl. Physiol.* **94**: 1–10.

Dennis, P. B., Jaeschke, A. J., Saito, M., Fowler, B., Kozma, S. C., and Thomas, G. (2001). Mammalian TOR: A homeostatic ATP sensor. *Science*. **294**: 1102–1105.

Deshmukh, A. S., Treebak, J. T., Long, Y. C., Viollet, B., Wojtaszewski, J. F., and Zierath, J. R. (2008). Role of adenosine 5'-monophosphate-activated protein kinase subunits in skeletal muscle mammalian target of rapamycin signaling. *Mol Endocrinol.* **22**(5): 1105–1112.

El-Haschimi, K., Pierroz, D. D., Hileman, S. M., Bjorbaek, C., and Flier, J. S. (2000). Two defects contribute to hypothalamic leptin resistance in mice with diet-induced obesity. *J Clin. Invest.* **105**: 1827–1832.

Gao, X., Zhang, Y., Arrazola, P., Hino, O., Kobayashi, T., Yeung, R. S., Ru, B., and Pan, D. (2002). Tsc tumour suppressor proteins antagonize amino-acid-TOR signaling. *Nat. Cell Biol.* **4**(9): 4699–4704.

Gleason, C. E., Lu, D., Witters, L. A., Newgard, C. B., and Birnbaum, M. J. (2007). The role of AMPK and mTOR in nutrient sensing in pancreatic beta-cells. *J Biol. Chem.* **282**(14): 10341–10351.

Gomez, E., Powell, M. L., Greenman, I. C., and Herbert, T. P. (2004). Glucose-stimulated protein synthesis in pancreatic beta-cells parallels an increase in the availability of the translational ternary complex (eIF2-GTP.Met-tRNAi) and the dephosphorylation of eIF2 alpha. *J Biol. Chem*. **279**(52): 53937–53946.

Goncharova, E. A., Goncharov, D. A., Eszterhas, A., Hunter, D. S., Glassberg, M. K., Yeung, R. S., Walker, C. L., Noonan, D., Kwiatkowski, D. J., Chou, M. M., Panettieri, R. A., Jr., and Krymskaya, V. P. (2002). Tuberin regulates p70 S6 kinase activation and ribosomal protein S6 phosphorylation. A role for the TSC2 tumor suppressor gene in pulmonary lymphangioleiomyomatosis (LAM). *J. Biol. Chem*. **277**: 30958–30967.

Hahn-Windgassen, A., Nogueira, V., Chen, C. C., Skeen, J. E., Sonenberg, N., and Hay, N. (2005). Akt activates the mammalian target of rapamycin by regulating cellular ATP level and AMPK activity. *J Biol. Chem.* **280**: 32081–32089.

Han, S. M., Namkoong C., Jang, P. G., Park, I. S., Hong, S. W., Katakami, H., Chun, S., Kim, S. W., Park, J. Y., Lee, K. U., and Kim, M. S. (2005). Hypothalamic AMP-activated protein kinase mediates counter-regulatory responses to hypoglycaemia in rats. *Diabetologia*. **48**: 2170–2178.

Hardie, D. G., Carling, D., and Carlson, M. (1998). The AMP activated/SNF1 protein kinase subfamily: Metabolic sensors of the eukaryotic cell? *Annu. Rev. Biochem.* **67**: 821–855.

Hardie, D. G. (2008). AMPK and Raptor: Matching cell growth to energy supply. *Mol. Cell.* **30**(3): 263–265.

Hawley, S. A., Davison, M., Woods, A., Davies, S. P., Beri, R. K., Carling, D., and Hardie, D. G. (1996). Characterization of the AMP-activated protein kinase kinase from rat liver and identification of threonine 172 as the major site at which it phosphorylates AMP-activated protein kinase. *J Biol. Chem.* **271**(44): 27879–27887.

Hawley, S. A., Boudeau, J., Reid, J. L., Mustard, K. J., Udd, L., Makela, T. P., Alessi, D. R., and Hardie, D. G. (2003). Complexes between the LKB1 tumor suppressor, STRAD alpha/beta and MO25 alpha/beta are upstream kinases in the AMP-activated protein kinase cascade. *J Biol.* **2**(4): 28.

Hong, S. P., Leiper, F. C., Woods, A., Carling, D., and Carlson, M. (2003). Activation of yeast Snf1 and mammalian AMP-activated protein kinase by upstream kinases. *Proc. Natl. Acad. Sci. U.S.A.* **100**: 8839–8843.

Huypens, P. R. (2006). Leptin and adiponectin regulate compensatory beta cell growth in accordance to overweight. *Med. Hypotheses.* **68**(5): 1134–1137.

Inoki, K., Li, Y., Zhu, T., Wu, J., and Guan, K. L. (2002). TSC2 is phosphorylated and inhibited by AKT and suppresses mTOR signaling. *Nat. Cell Biol.* **4**: 648–657.

Inoki, K., Zhu, T., and Guan, K. L. (2003). TSC2 mediates cellular energy response to control cell growth and survival. *Cell*. **115**: 577–590.

Inoki, K., Ouyang, H., Zhu, T., Lindvall, C., Wang, Y., Zhang, X., Yang, Q., Bennett, C., Harada, Y., Stankunas, K., Wang, C. Y., He, X., MacDougald, O. A., You, M., Williams, B. O., and Guan, K. L. (2006). TSC2 integrates wnt and energy signals via a coordinated phosphorylation by AMPK and GSK3 to regulate cell growth, *Cell*. **126**(5): 955–968.

Jacinto, E. and Hall, M. N. (2003). TOR signalling in bugs, brain and brawn. *Nat. Rev. Mol. Cell. Biol.* **4**: 117–126.

Jamshidi, N. and Taylor, D. A. (2001). Anandamide administration into the ventromedial hypothalamus stimulates appetite in rats. *Br. J. Pharmacol.* **134**: 1151–1154.

Kemp, B. E., Mitchelhill, K. I., Stapleton, D., Michell, B. J., Chen, Z. P., and Witters, L. A. (1999). Dealing with energy demand: The AMP-activated protein kinase. *Trends Biochem. Sci*. **24**: 22–25.

Khamzina, L., Veilleux, A., Bergeron, S., and Marette, A. (2005). Increased activation of the mammalian target of rapamycin pathway in liver and skeletal muscle of obese rats: Possible involvement in obesity-linked insulin resistance. *Endocrinology*. **146**: 1473–1481.

Kimball, S. R., Siegfried, B. A., and Jefferson, L. S. (2004). Glucagon represses signaling through the mammalian target of rapamycin in rat liver by activating AMP-activated protein kinase. *J. Biol. Chem.* **279**(52): 54103–54109.

Kim, M. S., Park, J. Y., Namkoong, C., Jang, P. G., Ryu, J. W., Song, H. S., Yun, J. Y., Namgoong, I. S., Ha, J., Park, I. S., Lee, I. K., Viollet, B., Youn, J. H., Lee, H. K., and Lee, K. U. (2004). Anti-obesity effects of α-lipoic acid mediated by suppression of hypothalamic AMP-activated protein kinase. *Nat. Med.* **10**: 727–733.

Kimura, N., Tokunaga, C., Dalal, S., Richardson, C., Yoshino, K., Hara, K., Kemp, B. E., Witters, L. A., Mimura, O., and Yonezawa, K. (2003). A possible linkage between AMP-activated protein kinase (AMPK) and mammalian target of rapamycin (mTOR) signalling pathway. *Genes Cells*. **8**: 65–79.

Kola, B., Hubina, E., Tucci, S. A., Kirkham, T. C., Garcia, E. A., Mitchell, S. E., Williams, L. M., Hawley, S. A., Hardie, D. G., Grossman, A. B., and Korbonits, M. (2005). Cannabinoids and ghrelin have both central and peripheral metabolic and cardiac effects via AMP-activated protein kinase. *J Biol. Chem*. **280**: 25196–25201.

Lam, T. K., Schwartz, G. J., and Rossetti, L. (2005). Hypothalamic sensing of fatty acids. *Nat. Neurosci*. **8**: 579–584.

Le Bacquer, O., Petroulakis, E., Paglialunga, S., Poulin, F., Richard, D., Cianflone, K., and Sonenberg, N. (2007). Elevated sensitivity to diet-induced obesity and insulin resistance in mice lacking 4E-BP1 and 4E-BP2. *J. Clin. Invest.* **117**: 387–396.

Leclerc, I., daSilvaXavier, G., and Rutter, G. A. (2004). AMP-activated protein kinase: a new beta-cell glucose sensor? Regulation by amino acids and calcium ions, *Diabetes*. **53**, Suppl 3: S67–S74.

Lee, K., Li, B., Xi, X., Suh, Y., and Martin, R. J. (2005). Role of neuronal energy status in the regulation of adenosine 5'-monophosphate-activated protein kinase, orexigenic neuropeptides expression, and feeding behavior. *Endocrinology*. **146**(1): 3–10.

Long, X., Lin, Y., Ortiz-Vega, S., Yonezawa, K., and Avruch, J. (2005). Rheb binds and regulates the mTOR kinase. *Curr Biol*. **15**(8):702–713.

Manning, B. D., Tee, A. R., Logsdon, M. N., Blenis, J., and Cantley, L. C. (2002). Identification of the tuberous sclerosis complex-2 tumor suppressor gene product tuberin as a target of the phosphoinositide 3-kinase/akt pathway. *Mol. Cell.* **10**: 151–162.

McGowan, M. K., Andrews, K. M., and Grossman, S. P. (1992). Chronic intrahyphothalamic infusions of insulin or insulin antibodies alter body weight and food intake in the rat. *Physol. Behav.* **51**: 753–766.

Menendez, J. A. and Atrens, D. M. (1991). Insulin and the paraventricular hypothalamus: Modulation of energy balance. *Brain Res*. **555**: 193–201.

Michell, B. J., Stapleton, D., Mitchelhill, K. I., House, C. M., Katsis, F., Witters, L. A., and Kemp, B. E. (1996). Isoform-specific purification and substrate specificity of the 5'-AMP-activated protein kinase. *J. Biol. Chem.* **271**: 28445–28450.

Minokoshi, Y., Alquier, T., Furukawa, N., Kim, Y. B., Lee, A., Xue, B. Z., Mu, J., Foufelle, F., Ferre, P., Birnbaum, M. J., Stuck, B. J., and Kahn, B.

B. (2004). AMP-kinase regulates food intake by responding to hormonal and nutrient signals in the hypothalamus. *Nature*. **428**: 569–574.

Montague, J., Radimerski, T., and Thomas, G. (2001). Insulin signaling: Lessons from the Drosophila tuberous sclerosis complex, a tumor suppressor. *Sci. STKE.* **105**: PE36.

Noland, R. C., Thyfault, J. P., Henes, S. T., Whitfield, B. R., Woodlief, T. L., Evans, J. R., Lust, J. A., Britton, S. L., Koch, L. G., Dudek, R. W., Dohm, G. L., Cortright, R. N., and Lust R. M. (2007). Elevated sensitivity to diet-induced obesity and insulin resistance in mice lacking 4E-BP1 and 4E-BP2. *J. Clin. Invest.* **117**:387–396.

Onda, H., Crino, P. B., Zhang, H., Murphey, R. D., Rastelli, L., Gould Rothberg, B. E., and Kwiatkowski, D. J. (2002). Tsc2 null murine neuroepithelial cells are a model for human tuber giant cells, and show activation of an mTOR pathway. *Mol. Cell. Neurosci.* **21**(4): 561–574.

Pende, M., Kozma, S. C., Jaquet, M., Oorschot, V., Burcelin, R., Le Marchand-Brustel, Y., Klumperman, J., Thorens, B., and Thomas G. (2000). Hypoinsulinaemia, glucose intolerance and diminished beta-cell size in S6K1-deficient mice. *Nature*. **408**: 994–997.

Potter, C. J., Pedraza, L. G., and Xu, T. (2002). Akt regulates growth by directly phosphorylating Tsc2. *Nat. Cell Biol.* **4**(9): 4658–4665.

Radimerski, T., Montagne, J., Hemmings-Mieszczak, M., and Thomas, G. (2002). Lethality of Drosophila lacking TSC tumor suppressor function rescued by reducing dS6K signaling. *Genes Dev.* **16**(20):2627–2632.

Reiter, A,K., Bolster, D. R., Crozier, S. J., Kimball, S. R., and Jefferson, L. S. (2005). Repression of protein synthesis and mTOR signaling in rat liver mediated by the AMPK activator aminoimidazole carboxamide ribonucleoside. *Am. J. Physiol. Endocrinol. Metab.* **288**: E980–E988.

Sabatini, D. M., Erdjument-Bromage, H., Lui, M., Tempst, P. S., and Snyder, H. (1994). RAFT1: A mammalian protein that binds to FKBP12 in a rapamycin-dependent fashion and is homologous to yeast TORs. *Cell*. **78**(1): 35–43.

Sakamoto, K., McCarthy, A., Smith, D., Green, K. A., Grahame Hardie, D., Ashworth, A., and Alessi, D. R. (2005). Deficiency of LKB1 in skeletal muscle prevents AMPK activation and glucose uptake during contraction. *EMBO J*. **24**(10): 1810–1820.

Sakamoto, K., Zarrinpashneh, E., Budas, G. R., Pouleur, A. C., Dutta, A., Prescott, A. R., Vanoverschelde, J. L., Ashworth, A., Jovanović, A., Alessi, D. R., and Bertrand, L. (2006). Deficiency of LKB1 in heart prevents ischemia-mediated activation of AMPKalpha2 but not AMPKalpha1., *Am. J. Physiol. Endocrinol. Metab.* **290**(5): E780–788.

Sapkota, G. P., Kieloch, A., Lizcano, J. M., Lain, S., Arthur, J. S. C., Williams, M. R., Morrice, N., Deak, M., and Alessi, D. R. (2001). Phosphorylation of the protein kinase mutated in Peutz-Jeghers cancer syndrome, LKB1/STK11, at Ser^{431} by $p90^{RSK}$ and cAMP-dependent protein kinase, but not its farnesylation at Cys^{433}, is essential for LKB1 to suppress cell growth. *J. Biol. Chem.* **276**: 19469–19482.

Schmelzle, T. and Hall, M. N. (2000). TOR, a central controller of cell growth. *Cell*. **103**: 253–262.

Shaw, R. J., Lamia, K. A., Vasquez, D., Koo, S. H., Bardeesy, N., DePinho, R. A., Montminy, M., and Cantley, L. C. (2005). The kinase LKB1 mediates glucose homeostasis in liver and therapeutic effects of metformin. *Science.* **310**: 1642–1646.

Shima, H., Pende, M., Chen, Y., Fumagalli, S., Thomas, G., and Kozma, S. C. (1998). Disruption of the p70(s6k)/p85(s6k) gene reveals a small mouse phenotype and a new functional S6 kinase. *EMBO J*. **17**: 6649–6659.

Siddhanta, U., McIlroy, J., Shah, A., Zhang, Y. T., and Backer, J. M. (1998). Distinct roles for the p110alpha and hVPS34 phosphatidylinositol 3'-kinases in vesicular trafficking, regulation of the actin cytoskeleton, and mitogenesis. *J. Cell Biol.* **143**: 1647–1659.

Sutherland, C. M., Hawley, S. A., McCartney, R. R., Leech, A., Stark, M. J., Schmidt, M. C., and Hardie, D. G. (2003). Elm1p is one of three upstream kinases for the Saccharomyces serevisiae SNF1 complex. *Curr. Biol.* **13**: 1299–1305.

Tee, A. R., Fingar, D. C., Manning, B. D., Kwiatkowski, D. J., Cantley, L. C., and Blenis, J. (2002). Tuberous sclerosis complex-1 and -2 gene products function together to inhibit mammalian target of rapamycin (mTOR)-mediated downstream signaling, *Proc. Natl. Acad. Sci. U.S.A.* **99**(21): 13571–13576.

Thomas, G., Sabatini, D. M., and Hall, M. N. (2004). TOR-Target of Rapamycin. Springer-Verlag Co., Heidelberg, 364 pages.

Tokunaga, C., Yoshino, Y. I., and Yonezawa, K. (2004). mTOR integrates amino acid- and energy-sensing pathways. *Biochem. Biophys. Res. Commun.* **313**: 443–448.

Tsukiyama-Kohara, K., Poulin, F., Kohara, M., DeMaria, C. T., Cheng, A., Wu, Z., Gingras, A. C., Katsume, A., Elchebly, M., Spiegelman, B. M., Harper, M. E., Tremblay, M. L., and Sonenberg, N. (2001). Adipose tissue reduction in mice lacking the translational inhibitor 4E-BP1., *Nat. Med.* **7**(10): 1128–1132.

Um, S. H., Frigerio, F., Watanabe, M., Picard, F., Joaquin, M., Sticker, M., Fumagalli, S., Allegrini, P. R., Kozma, S. C., Auwerx, J., and Thomas, G. (2004). Absence of S6K1 protects against age- and diet-induced obesity while enhancing insulin sensitivity. *Nature.* **431**: 200–205.

Woods, A., Johnstone, S. R., Dickerson, K., Leiper, F. C., Fryer, L. D., Neumann, D., Schlattner, U., Wallimann, T., Carlson, M., and Carling, D. (2003). LKB1 is the upstream kinase in the AMP-activated protein kinase cascade. *Curr. Biol.* **13**: 2004–2008.

Wullschleger, S., Loewith, R., and Hall, M. N. (2006). TOR signaling in growth and metabolism. *Cell*. **124**: 471–484.

Xue, B. and Khan, B. (2006). AMPK integrates nutrient and hormonal signals to regulate food intake and energy balance through effects in the hypothalamus and peripheral tissues, *J. Physiol.* **574**(pt 1): 73–83.

Xu, G., Kwon, G., Cruz, W. S., Marshall, C. A., and McDaniel, M. L. (2001). Metabolic regulation by leucine of translation initiation through the mTOR-signaling pathway by pancreatic beta-cells. *Diabetes*. **50**: 353–360.

Xu, G., Marshall, C. A., Lin, T. A., Kwon, G., Munivenkatappa, R. B., Hill, J. R., Lawrence J C, Jr., and McDaniel, M. L. (1998). Insulin mediates glucose-stimulated phosphorylation of PHAS-I by pancreatic beta cells. An insulin-receptor mechanism for autoregulation of protein synthesis by translation, *J. Biol. Chem.* **273**(43): 28178–28184.

Yoo, L. I., Chung, D. C., and Yuan, J. (2002). LKB1–a master tumour suppressor of the small intestine and beyond. *Nat. Rev. Cancer.* **2**(7): 529–535.

Zigman, J. M., Jones, J. E., Lee, C. E., Saper, C. B., and Elmquist, J. K. (2006). Expression of ghrelin receptor mRNA in the rat and the mouse brain. *J. Comp. Neurol.* **494**: 528–548.