Nutrient Signaling Components Controlling Protein Synthesis in Striated Muscle[1,2]

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Abstract

Accretion of muscle mass is dependent upon faster rates of protein synthesis than degradation. When an animal is deprived of dietary protein, loss of body weight and negative nitrogen balance ensue. Likewise, refeeding accelerates protein synthesis and results in resumption of positive nitrogen balance. Amino acids and anabolic hormones both interact to maximally enhance rates of protein synthesis acutely during refeeding through an acceleration of the messenger RNA (mRNA) translation initiation. The review will illuminate the molecular mechanisms responsible for increasing mRNA translation initiation in striated muscle. The hastening of mRNA translation initiation most likely results from a stimulation of mammalian target of rapamycin (mTOR) acting through its downstream effector proteins eukaryotic initiation factors (eIF)4E binding protein1 and possibly eIF4G to enhance assembly of eIF4G with eIF4E and 70-kDa ribosomal S6 kinase1. Amino acids and leucine in particular are as effective as a complete meal in stimulating mRNA translation initiation by targeting these specific signal transduction systems. The physiologic importance lies in the potential ability of amino acids as specific nutrients designed to counteract the accelerated host protein wasting associated with a number of disease entities, including cancer, HIV infection, sepsis, and diabetes, and to improve nutrition to maintain muscle mass in aging populations and ensure muscle growth in neonatal populations. J. Nutr. 137: 1835–1843, 2007.

Introduction

Consumption of a protein-containing meal enhances the accretion of total mixed proteins in muscle. Elevations in circulating insulin concentrations and amino acid supply are the 2 critical components of the meal affecting protein turnover in a variety of tissues following refeeding. The signals that influence the stimulation of protein synthesis following feeding have been the subject of investigation for >30 y, with an elevation of amino acids and/or anabolic hormones being responsible for the accretion of muscle protein. Protein accrual occurs whenever the balance between rates of protein synthesis and protein turnover favors net synthesis. This occurs in part through an acceleration of protein synthesis. Protein synthesis in skeletal and cardiac muscle is accelerated after oral intake of nutrients (1,2). Although remarkable progress has been made in identifying the cellular mechanisms potentially responsible for stimulation of protein synthesis by insulin, the signaling pathways responding to increased amino acid availability are only beginning to be elucidated. Furthermore, a number of investigations reported that the branched chain amino acid, leucine, essentially mimics the effect of a mixture of amino acids on protein metabolism. The purpose of this review is to examine the advancements in our understanding of the mechanisms by which refeeding and amino acids in

particular function to maintain protein accretion in striated muscle. The physiologic importance lies in the potential ability of amino acids as specific nutrients designed to counteract the high host protein wasting associated with a number of disease entities, including cancer, HIV infection, sepsis, and diabetes, thereby preventing potential complications and limiting debilitating morbidities.

Indeed, parenteral administration of BCAA-enriched [45% ratio; 140 kcal/g N (586 kJ/g N)] solutions in septic patients improves nitrogen balance (3,4) and results in a faster recovery of muscle protein (5). Ultimately, mortality in septic patients is reduced (3). Likewise, BCAA-enriched oral supplementation improves morbidity and quality of life in patients undergoing major liver resection and chemo-embolization for hepatic carcinoma (6).

Overview of synthesis of proteins in mammalian systems

Synthesis of proteins in mammalian cells is a complex process (7,8) (**Fig. 1**). A series of discrete reactions that involve the association of the 40S and 60S ribosomal subunits, messenger RNA (mRNA)[3], initiator methionyl-tRNA (met-tRNA$_i^{met}$), other amino acyl-tRNAs, cofactors (i.e. GTP and ATP), and

[3] Abbreviations used: 4E-BP1, eIF4E binding protein1; BCAT, branched-chain amino transferase; eIF, eukaryotic initiation factor; met-tRNA$_i^{met}$, methionyl-tRNA; mRNA, messenger RNA; mTOR, mammalian target of rapamycin; PKB, protein kinase B; RAPTOR, regulatory-associated protein of mTOR; Rheb, RAS homolog enriched in brain; rpS6, ribosomal protein S6; S6K1, 70-kDa ribosomal protein S6 kinase1; 5′TOP, 5′ tract of oligopyrimidines; TSC, tumor suppressor complex.
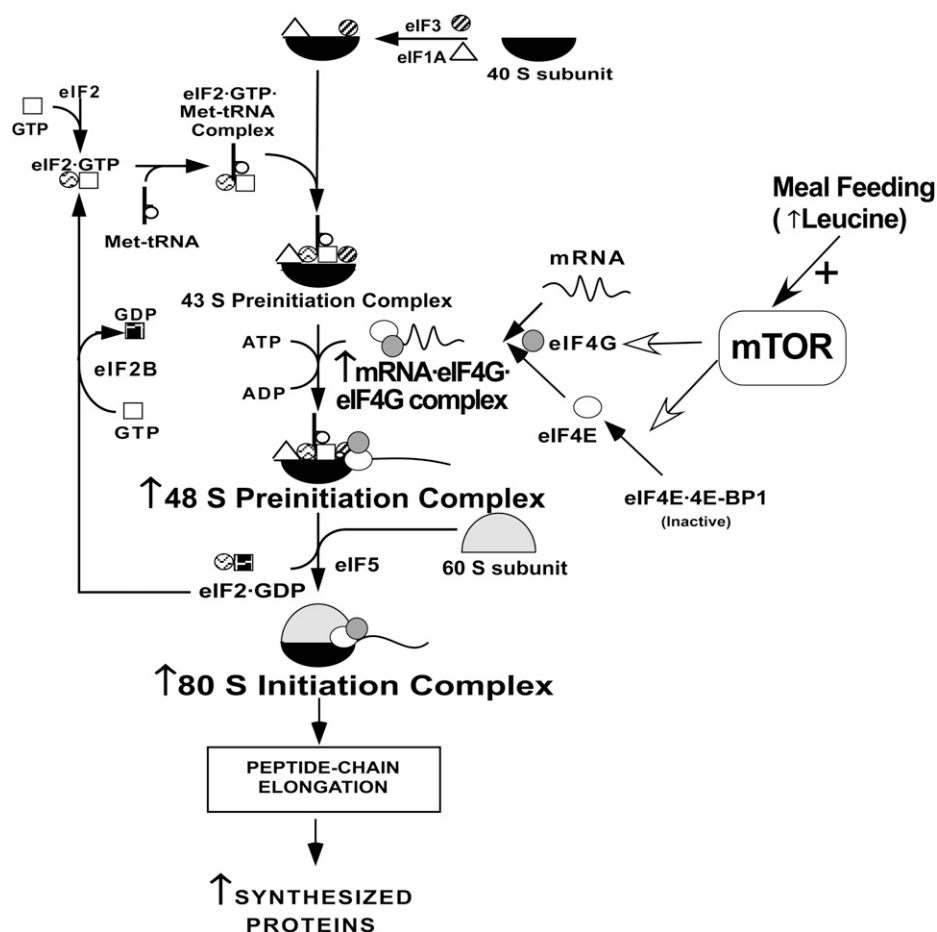
FIGURE 1 Pathway of protein synthesis in striated muscle (adapted from 7).

protein factors, known as eukaryotic initiation factors (eIF), elongation factors, and releasing factors lead to translation of mRNA into proteins. Translation of mRNA is comprised of 3 phases: *1)* initiation, whereby met-tRNA$_i^{met}$ and mRNA bind to 40S ribosomal subunits, and the binding of the 40S ribosomal subunit to the 60S subunit to form a ribosome complex capable of translation; *2)* elongation, by which tRNA-bound amino acids are incorporated into growing polypeptide chains according to the mRNA template; and *3)* termination, where the completed protein is released from the ribosome. The accelerated rates of protein synthesis appear independent of hyperacidemia. Hence, the nutrient-induced increases most likely involve processes other than amino acid availability, i.e. not a substrate effect (9). Indeed, nutrients appear to stimulate protein synthesis largely by accelerating mRNA translation initiation faster than elongation.

Two major steps in mRNA translation initiation control protein synthesis. The first limiting step is the binding of met-tRNA$_i^{met}$ to the 40S ribosomal subunit to form the 43S pre-initiation complex, mediated by eIF2 and regulated by the activity of eIF2B. Oral feeding does not enhance the formation of 43S preinitiation complex (10,11). The next regulatory step involves the recognition, unwinding, and binding of mRNA to the 43S preinitiation complex, catalyzed by a multi-subunit complex of eukaryotic factors referred to as eIF4F (12–14). Acute provision of nutrients enhances this step, the assembly of active eIF4F complex (2,15).

eIF4F is composed of: *1)* eIF4A, an RNA helicase that unwinds secondary structure in 5′-untranslated region of mRNA; *2)* eIF4E, a protein that binds directly to the 7 methyl GTP cap structure present at the 5′ end of most eukaryotic mRNAs; and *3)* eIF4G, a protein that functions as a scaffold for eIF4E, eIF4A, the mRNA, and the ribosome. The availability of eIF4E activity plays a critical role in determining global rates of mRNA translation, because, with the exception of histone mRNAs, essentially every mammalian mRNA contains the 7-methyl GTP cap structure at its 5′ end. eIF4G appears to be the nucleus around which the initiation complex forms, because it has binding sites not only for eIF4E but also for eIF4A and eIF3 (16). eIF4G recruits the 40S ribosomal subunit to the 5′ end of mRNA, coordinates the circularization of mRNA through eIF4E and poly(A)-binding protein interactions, and assists in Mnk1 and eIF4E association (17–19) (**Fig. 2**). Binding of eIF4G with eIF4E is important in accelerating mRNA translation initiation (Fig. 2). To our knowledge, we were the first to report a positive linear relationship between rates of protein synthesis and amount of eIF4G associated with eIF4E in vivo (20), consistent with the proposed role of eIF4G-eIF4E complex in the overall regulation of protein synthesis.

Assembly of an active eIF4G-eIF4E complex is dependent both on the availability of eIF4E and on activity of eIF4G. The availability of eIF4E for binding to eIF4G is regulated in part through the association of eIF4E with translational repressor 4E binding proteins (4E-BPs) (21–24). 4E-BP1 is a small protein that, in the hypophosphorylated state, tightly binds eIF4E and blocks the ability of eIF4E to bind to eIF4G (Fig. 2). When eIF4E is bound to 4E-BP1, eIF4E cannot bind to eIF4G (Fig. 2). Consequently, the mRNA cannot bind to the ribosome (25), thereby inhibiting cap-dependent translation of mRNA by physically sequestering eIF4E into an inactive 4E-BP1-eIF4E complex.
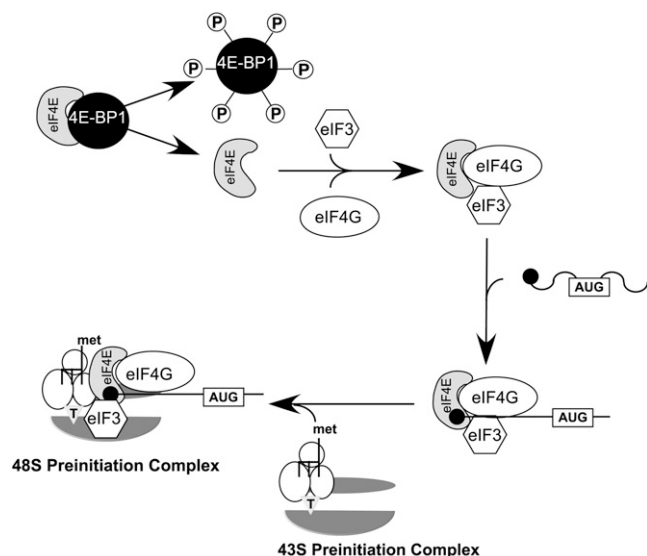
FIGURE 2 Assembly of active eIF4G-eIF4E complex. Inhibition by sequestration of eIF4E with 4E-BP1.

eIF4E binding to 4E-BP1 is reduced following phosphorylation of 4E-BP1. Phosphorylation of 4E-BP1 releases eIF4E from 4E-BP1-eIF4E complex and allows the eIF4E-mRNA complex to bind to eIF4G and then to the 40S ribosome (Fig. 2) (21). Mammalian target of rapamycin (mTOR) is thought to be an upstream kinase that phosphorylates 4E-BP1. Likewise, the 70-kDa ribosomal protein S6 (rpS6) kinase1 (S6K1) has been implicated in augmenting the translation of a subset of mRNAs that possess a 5′ tract of oligopyrimidines (5′TOP) including elongation factors. S6K1's multi-step activation involves mTOR and PDK1-dependent Ser/Thr phosphorylation (**Fig. 3**). Augmenting mTOR activity enhances phosphorylation of S6K1 (26) and 4E-BP1 (Fig. 3) (1,27–29).

In addition to availability of eIF4E, assembly of eIF4G-eIF4E complex also appears dependent upon eIF4G. eIF4G is a phosphoprotein possessing 3 phosphorylation sites located in the C-terminal one-third of the protein corresponding to Ser

residues 1108, 1148, and 1192 (30). Increased phosphorylation of eIF4G on Ser[1108] fully activates eIF4 and is associated with enhanced formation of active eIF4G-eIF4E complex in cells in culture (30,31). Increased phosphorylation of eIF4G correlates with accelerated rates of protein synthesis in cell extracts (32) and in striated muscle in vivo (33–35).

Meal feeding enhances formation of eIF4F in skeletal muscle by increased eIF4E availability and eIF4G phosphorylation

Meal feeding in skeletal muscle or heart enhanced the assembly of the active eIF4G-eIF4E complex, which returned to basal levels within 3 h of removal of food (33). The increased assembly of the active eIF4G-eIF4E complex was associated with a marked rise in the level of phosphorylation of eIF4G(Ser[1108]) (either eIF4G in the eIF4G-eIF4E complex or tissue homogenates) and a decreased assembly of inactive 4E-BP1-eIF4E complex (Fig. 4). Meal feeding augmented the phosphorylation of 4E-BP1 and S6K1(Thr[389]), both of which are associated with activation of these modulators of translation initiation. The upstream kinase responsible for phosphorylating both S6K1 and 4E-BP1 appears to be mTOR, a proline-directed Ser/Thr kinase, which also undergoes activation via phosphorylation and nonphosphorylation mechanisms. The extent of mTOR phosphorylation on Ser[2448] or Ser[2481] was increased by meal feeding. The level of phosphorylation of protein kinase B (PKB)(Thr[308] or Ser[473]) is associated with enhanced PKB activity. Phosphorylation PKB, an upstream kinase responsible for phosphorylating mTOR, on Thr[308] was only elevated after one-half hour of meal feeding, whereas phosphorylation Ser[473] was significantly elevated up to 1 h after initiation of feeding. Hence, meal feeding stimulates 2 signal pathways in skeletal muscle, elevating eIF4G-eIF4E complex through increased phosphorylation of eIF4G and decreased association of 4E-BP1 with eIF4E.

In cardiac muscle, a slightly different response to meal feeding was observed (36). Meal feeding raised the extent of phosphorylation of eIF4G(Ser[1108]) in the eIF4G-eIF4E complex, which returned to basal levels within 3 h of removal of food. Likewise, meal feeding was associated with an increase in phosphorylation of 4E-BP1 in the γ-form during feeding. Phosphorylation of mTOR
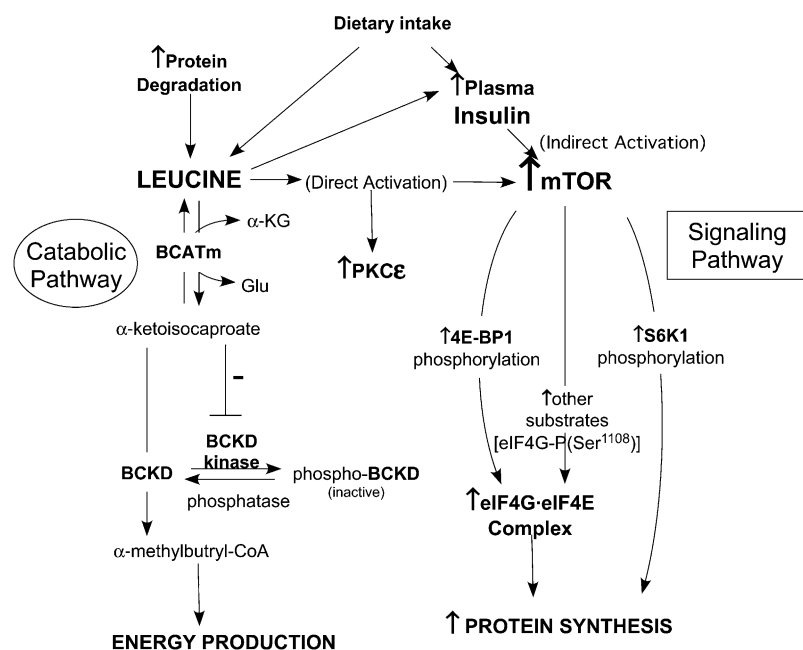

FIGURE 3 Dual function of leucine as oxidative fuel and activator of signaling pathways by direct and indirect processes (adapted from 49).

on Ser[2448] or Ser[2481] was not affected by meal feeding. The phosphorylation of S6K1 on Thr[389], an mTOR downstream effector molecule, was also unchanged. Phosphorylation of PKB(Thr[308]) and PKCε but not PKCδ was elevated at all time points following initiation of meal feeding. Hence, meal feeding stimulates at least 2 signal pathways in cardiac muscle that raises phosphorylation of eIF4G and 4E-BP1 during meal feeding and results in sustained increases in phosphorylation of PKB and PKCε.

Leucine can substitute for a complete protein meal

Of the nutrients provided by a complete meal, postprandial increases in the plasma concentration of amino acids after a mainly protein-containing meal may provide a signal for accelerating protein synthesis (37,38). Infusing amino acids in vivo enhances muscle protein synthesis independent of any stimulatory effect induced by changes in plasma anabolic hormone concentrations (1,39–44). Indeed, an oral gavage with leucine resulted in a stimulation of protein synthesis that was independent of changes of plasma insulin concentrations, whereas a gavage containing carbohydrates (glucose plus sucrose) that raised insulin concentrations over 2.5 times the fasting glucose concentration did not affect protein synthesis (10). This does not mean that anabolic hormones are not required for the effects of amino acids on mRNA translation initiation. Indeed, administration of anti-insulin antibodies blunts the effects of refeeding to stimulate protein synthesis (45,46). Moreover, when insulin secretion is retarded by somatostatin infusions (47), oral leucine gavage was ineffective in augmenting rates of protein synthesis in skeletal muscle (48).

Amino acids probably stimulate key regulatory protein factors controlling protein synthesis rather than simply increasing substrate supply, which would be expected to enhance only peptide-chain elongation and not translation initiation (49,50). Amino acids, BCAA (leucine, isoleucine, and valine) in particular, not only provide substrate for protein synthesis but are also now recognized to stimulate a number of cell-signaling pathways important in the regulation of mRNA translation (38,51–57). Not all amino acids have the same potency in stimulating protein synthesis. Leucine seems to be the most potent of the BCAA with regard to most of these effects and therefore may be the most physiologically relevant. Oral gavage with a solution containing leucine, but not isoleucine or valine, stimulates protein synthesis with an increased assembly of eIF4G, S6K1 phosphorylation, and eIF4G phosphorylation in gastrocnemius (10,58–60) and heart (61).

Similar to meal feeding, the mTOR signaling pathway has been proposed as 1 potential intracellular signaling target for mediating the effects of amino acids on mRNA translation initiation. In adipocytes, the efficacy of amino acids in activating mTOR signaling appears to be related to their structural similarity to leucine. Thus, leucine and norleucine are posited to be agonists at a common leucine recognition site in adipocytes, LeuR$_a$ (27,62).

Leucine and its analog, norleucine, constitute a direct-acting nutrient signal (1,27,62) that communicates the presence of an ingested protein-containing meal to peripheral organs, including striated muscle (1,50,63). Leucine is ideally suited for its role as a nutrient signal for several reasons. First, plasma leucine concentrations rise rapidly after a meal and remain elevated during feeding in meal-trained rats. Second, rises in circulating plasma leucine concentration observed after a meal are within the range over which leucine stimulates mTOR activity (27,62,64). Third, leucine is the most efficacious amino acid in stimulating protein synthesis in skeletal muscle (38,65) and, more importantly,

mTOR (27,62,64). Fourth, leucine is derived from the diet and cannot be synthesized by mammals, allowing for a defined administration. Fifth, the rise in plasma leucine after a protein-containing meal is facilitated by the liver's inability to catalyze the first step in leucine metabolism (BCAT2, the enzyme that transaminates leucine to α-ketoisocaproate acid), in contrast to the liver's well-known capacity to metabolize other amino acids (64). Lastly, skeletal muscle has a mechanism to regulate and eliminate this putative nutrient signal through oxidation of leucine, although this pathway is generally repressed except when plasma leucine concentrations rise (64).

Leucine stimulates mTOR phosphorylation in skeletal muscle

Leucine alone can substitute for a meal in stimulating signal transduction pathways, leading to a stimulation of protein synthesis by accelerating mRNA translation initiation. Oral leucine gavage increased muscle protein synthesis within 20 min (10,66). Like meal feeding, the activity of the mTOR-signaling pathway in muscle is augmented following the oral leucine gavage. Leucine markedly increased the level of phosphorylation of 4E-BP1 in gastrocnemius and this response was associated with a release of eIF4E from the inactive eIF4E-4E-BP1. In addition, leucine gavage induced increases in the level of phosphorylation of eIF4G. The combination of increased eIF4E availability and phosphorylation of eIF4G enhanced the assembly of eIF4G-eIF4E complexes. Moreover, the synthesis of 5′TOP mRNAs should be augmented through increased phosphorylation of S6K1 and the rpS6. Thus, it appears that signaling through mTOR is important for mediating the effects of leucine feeding on translation in muscle. However, the precise detection mechanism or sensor protein in mediating the effects of leucine on mTOR remains unresolved.

mTOR exists in 2 structurally and functionally distinct protein complexes in mammalian systems, namely TORC1 and TORC2. TORC1 is composed of mTOR and 2 other proteins: regulatory-associated protein of mTOR (RAPTOR) and Gβl (67). The association of RAPTOR with mTOR enhances mTOR's ability to phosphorylate 4E-BP1 and S6K1 (68,69). The kinase activity of TORC1 is inhibited by rapamycin (rapamycin sensitive), whereas activity of TORC2 appears rapamycin insensitive (67,68,70). Changes in binding of RAPTOR with mTOR would be expected to decrease the phosphorylation of S6K1 and 4E-BP1, thereby limiting translation initiation and representing a potential mechanism to account for the decreased phosphorylation of 4E-BP1 and S6K1 and/or eIF4G during sepsis. mTOR fails to bind the FKBP12·rapamycin complex when contained in TORC2. The mammalian TORC2 complex is composed of mTOR, RICTOR (also known as Pianissimo), GβL, and hSIN1 (71). TORC2 is a strong candidate for transmission of rapamycin-insensitive signals. Amino acid deprivation may limit the TORC1 pathway, leading to decreased phosphorylation of S6K1 and 4E-BP1 and reduced eIF4E availability while lowering the abundance of TORC2 and thereby leading to a reduced level of eIF4G phosphorylation.

Several cytoplasmic proteins have the potential to modulate mTOR, including the tumor suppressor complex (TSC). TSC patients are characterized by mutations in either TSC1 or TSC2, whose gene products, hamartin (TSC1) and tuberin (TSC2), constitute a putative tumor suppresser complex (72) (**Fig. 4**). Both tuberin and hamartin are expressed in muscle. Lethality of the generation of TSC1 or TSC2 knockout mice has hampered dissection of the function of TSC1/2 complex in mammalian tissues in vivo. Hamartin and tuberin interact to form a stable
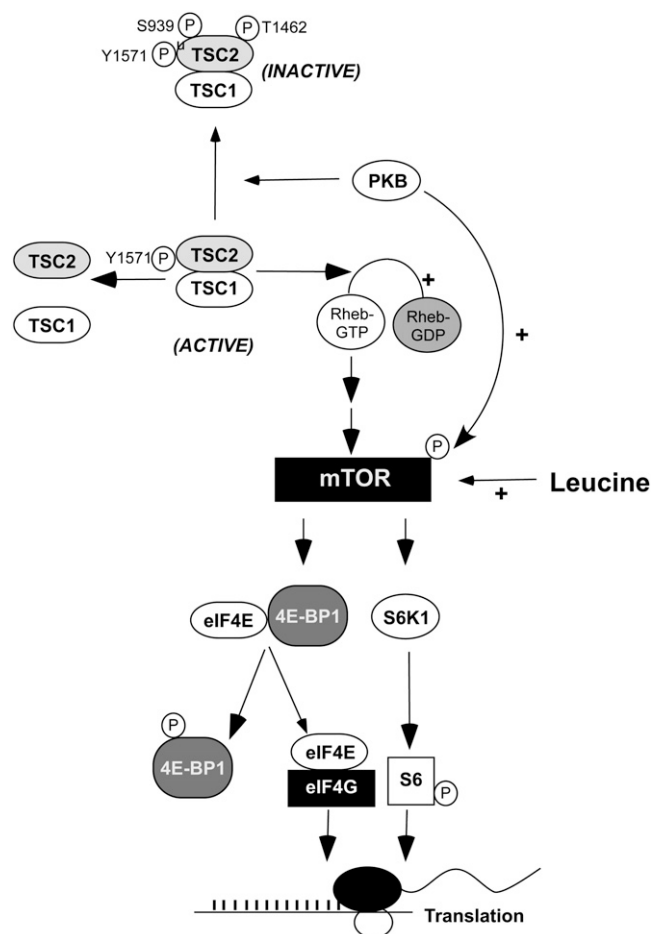
FIGURE 4 Activation of rapamycin-sensitive mTOR signaling by leucine.

complex that is responsible for its activity and function together in the complex to inhibit mTOR-mediated signaling to 4E-BP1 and S6K1 (73). TSC2 does not share any homology with protein kinases or phosphatases. Tuberin possesses a GTPase-activating protein domain that acts on the Ras family small GTPase called RAS homolog enriched in brain (Rheb) (73–76). Small G proteins are molecular switches that are in an active state when bound to GTP but become inactive when GTP is hydrolyzed to GDP (77,78). The GTPase-activating protein domain is important for tuberin's function in growth retardation (79).

Rheb increases phosphorylation of mTOR on Ser^{2448} in response to stimuli known to enhance mTOR activity (75). Tuberin-dependent stimulation of GTP hydrolysis of Rheb blocks phosphorylation of mTOR (Ser^{2448}), which antagonizes the mTOR-signaling pathway. Hence, the tuberin-hamartin complex functions as an mTOR suppressor, ultimately resulting in diminished phosphorylation of S6K1 and 4E-BP1. Indeed, RNAi inhibition of Rheb abolishes $S6K1(Thr^{389})$ phosphorylation (76). Conversely, loss of TSC2 function leads to constitutive activation of S6K1 (80).

Tuberin activity is regulated through phosphorylation by a number of protein kinases and the multiple phosphorylation events represent a point of convergence of inhibitory signaling from PKB (81), p90[RSK] (82), and ERK1/2 (83). Ser^{939} and Thr^{1462} are sites phosphorylated by PKB, whereas Ser^{1210} is directly phosphorylated by p38 mitogen-activated protein kinase-activated protein kinase 2 (84).

TSC2 appears regulated through phosphorylation (85), which serves 2 distinct functions. First, prevention of phosphorylation of Tyr^{1571} leads to loss of function of TSC1/2, curtailing the growth inhibitory effects of tuberin (86). Second, phosphorylation of Thr^{1462} causes dissociation of the tuberin-hamartin complex (81) and limits the tuberin-hamartin complex reductions of Rheb-GTPase activity, thereby relieving inhibition mTOR. This phosphorylation is dependent upon the PI3K/PKB signaling pathway whereby activated PKB phosphorylates tuberin at residue (Thr^{1462}) (85,87). The potential importance of TSC2 phosphorylation in control of TORC1 has been observed in transformed cells in culture. In contrast, the extent of phosphorylation of TSC2(Thr^{1462}) was not significantly altered during meal feeding despite increased phosphorylation of PKB in either gastrocnemius (34) or cardiac muscle (35) in vivo. Likewise, enhanced PKB phosphorylation was not associated with phosphorylation of TSC2 in cardiac muscle stimulated with insulin-like growth factor I (35). Thus, TSC2(Thr^{1462}) phosphorylation does not appear to be an important regulatory factor in controlling mTOR activity and, hence, mRNA translation initiation following meal feeding in muscle in vivo.

Inhibition of mTOR partially limits the effects of leucine signaling

Rapamycin is a bacterial macrolide that either abolishes or severely inhibits the ability of cells to progress through the G1 phase of the cell cycle by inhibiting the activity of mTOR (88,89). As such, it has proved a useful tool to probe the role of mTOR in modulating signaling pathways. Rapamycin treatment reduced rates of protein synthesis independently of leucine administration. Protein synthesis rates in rats treated with rapamycin and then administered leucine were only 72% of those in rats fed leucine alone and were equal to those of food-deprived control rats administered saline (66). These results suggest that the leucine-dependent stimulation of muscle protein synthesis is in part rapamycin sensitive and involves mTOR. Rapamycin administration completely blocked the leucine-dependent inhibition of 4E-BP1-eIF4E complex formation through a reduced 4E-BP1 phosphorylation. 4E-BP1 phosphorylation in drug-treated rats was <10% of that in rats fed leucine alone and significantly lower than that in food-deprived control rats. Furthermore, rapamycin treatment reversed the leucine-stimulated formation of the active eIF4G-eIF4E complex, resulting in values that were 50% less than those not receiving the mTOR inhibitor. Additionally, rapamycin also inhibited the association of eIF4E with eIF4G in rats not receiving leucine. Finally, rapamycin reversed the leucine-dependent decrease in eIF4E phosphorylation, resulting in values that were not different from those of food-deprived rats.

Pretreatment with rapamycin prevented the feeding-induced phosphorylation of mTOR, eIF4G, and S6K1 but only partially attenuated the shift in 4E-BP1 into the γ-form. In contrast, the feeding-induced increase in phosphorylation of PKCε was not reduced by rapamycin. Rapamycin also prevented the augmented association of eIF4G with eIF4E and the decreased association of eIF4E with 4E-BP1. Similar findings were observed in gastrocnemius from animals after oral administration of leucine. Collectively, these results suggest that the leucine-dependent increase in eIF4E availability for formation of the active eIF4G-eIF4E complex occurs via a rapamycin-sensitive signaling pathway (90). However, signaling through mTOR alone may not be sufficient to explain the leucine-dependent stimulation of muscle protein synthesis in postabsorptive rats.

Leucine stimulates the level of phosphorylation of eIF4G in perfused hind limb

The regulatory effects attributed to leucine on protein synthesis can be demonstrated in vitro perfused hind limb, where the contribution of hormones and substrates in the medium can be controlled (1,27,52,60,64,91–93). Even though leucine stimulates mTOR activity, the studies described above do not delineate whether mTOR is required for stimulation of protein synthesis. Protein synthesis was raised in both gastrocnemius and soleus muscles perfused with buffer containing 1.5 mmol/L leucine, indicating the effects are not limited to muscles composed only of fast-twitch fibers (60). The stimulatory effects of leucine on rates of protein synthesis were unaffected when LY 294002 inhibited PI3-kinase, indicating activation of the PI3-kinase pathway was not mandatory for the response. Moreover, signaling through mTOR, as monitored by the phosphorylation status of 4E-BP1 or S6K1 under these experimental conditions, was maintained near the fed-state value by plasma leucine concentrations and was not further enhanced by raising the leucine. In contrast, the binding of eIF4E to eIF4G to form the active eIF4F complex was enhanced. Additionally, eIF4GSer[1108] phosphorylation in the eIF4E immunoprecipitate also significantly increased (60). Leucine modulates distinct steps of translation initiation and protein synthesis directly in skeletal muscle through enhanced assembly of active eIF4G-eIF4E complex and phosphorylation of eIF4G. Collectively, these results illustrate an experimental model whereby leucine in the absence of other regulatory agents modulates distinct steps of translation initiation and protein synthesis directly in skeletal muscle through a signaling pathway independent of mTOR.

Chronic leucine administration

With chronic supplementation of leucine in the diet, protein synthesis was elevated in adipose tissue, liver, and skeletal muscle but not in kidney or heart (63). In a preceding acute administration study, leucine and norleucine had different effects on protein synthesis (1). Leucine administration stimulated protein synthesis in adipose tissue, muscle, and kidney, whereas norleucine was effective in all tissues (1). The tissue-specific differences in effects of leucine and norleucine supplementation in these 2 models suggests that there may be varied pathways by which amino acids such as leucine affect protein synthesis in body tissues and/or that the mechanisms involved in leucine's acute and chronic effects on protein synthesis occur by different mechanisms. Indeed, the acute affects of leucine are mediated by both rapamycin-sensitive and rapamycin-insensitive pathways (94,95). The rapamycin-sensitive pathway involves 4E-BP1, S6K1, and mTOR. However, little is known about the rapamycin-insensitive pathway, but it may be controlled through the distribution of mTOR between TORC1 and TORC2 described above or a yet-undetermined alternative pathway.

Leucine regulation of protein synthesis in perinatal period

Rapid growth is a hallmark of the neonatal period. High rates of protein synthesis exceeding those of degradation appear responsible for the profound accretion of skeletal muscle protein during this period (96,97). The feeding-induced stimulation of protein synthesis occurs in virtually all tissues of the neonate; however, the postprandial rise in protein synthesis is most pronounced in skeletal muscle. In skeletal muscle of neonates, feeding or the acute infusion of amino acids to raise concentrations to within the physiological fed range enhances the phosphorylation of S6K1 and 4E-BP1. The consequence of increased phosphoryla-

tion of 4E-BP1 is the release of eIF4E from the inactive 4E-BP1-eIF4E complex (31,98). The increased phosphorylation of S6K1 stimulates the synthesis of proteins containing 5′TOP sequences in their mRNA and includes ribosomal proteins and elongation factors. Phosphorylation of S6K1 and 4E-BP1 appears independent of the insulin effects to stimulate protein synthesis. Hence, leucine acts as a nutrient signal stimulating protein synthesis in muscle of neonatal pigs. Furthermore, this response to leucine is time specific and dose dependent. Prolonging leucine infusion blunts the response in muscle protein synthesis most likely because there is a concomitant decline in circulating levels of several essential amino acids, including isoleucine, valine, methionine, threonine, tryptophan, and tyrosine (99). Most likely, these essential amino acids became limiting, because they were used for protein synthesis. Hence, protein synthesis in muscle of neonatal pigs could be increased during prolonged leucine infusion provided circulating concentrations of essential amino acids were maintained at fasting levels. Similarly, leucine, but not other BCAA, was able to stimulate protein synthesis in heart (61). Leucine infusions resulted in the phosphorylation of 4E-BP1, but surprisingly, the amount of eIF4E associated with 4E-BP1 was unaffected in cardiac muscle. Moreover, phosphorylation of S6K1 or rpS6 was unaffected by infusion of leucine in heart. In contrast, infusion of leucine augmented the phosphorylation of eIF4G(Ser[1108]) with a corresponding increase in the abundance of the active eIF4G-eIF4E complex, consistent with a role for eIF4G-eIF4E in the leucine-mediated stimulation of protein synthesis in cardiac muscle (61).

Leucine regulation of protein synthesis in aging

The leucine signal is also observed in old subjects who may be less sensitive to leucine (100). Dose dependency curves involving the stimulation of muscle protein synthesis by leucine showed that response was shifted to the right, whereby leucine concentrations 1–2 times greater than in young animals were necessary to observe the same changes in adult rats. Moreover, a single leucine-supplemented meal corrected the defect of postprandial muscle protein synthesis stimulation in old rats (101). This beneficial effect of leucine supplementation on postprandial muscle protein anabolism persists at least 10 d (102). This suggested that the defect in postprandial muscle protein anabolism in old subjects was related to the alterations of the leucine signal. However, the molecular mechanism responsible for the attenuated signaling pathways for leucine remain unresolved.

Future directions

The following scenario is put forth as a potential mechanism accounting for stimulation of assembly of eIF4G-eIF4E complex following meal feeding. Meal feeding causes a rise in both insulin and certain amino acids, including leucine. The initial rise in insulin causes stimulation of PI3-kinase-PKB signal pathway-enhancing mTOR phosphorylation and the subsequent stimulation of phosphorylation of S6K1 and 4E-BP1. However, this stimulation of PKB is not maintained throughout the feeding period. Whereas PKB phosphorylation returns to baseline values, mTOR activation continues presumably because of the elevation of plasma amino acid concentrations. The BCAA are the most robust of the plasma amino acids in their ability to cause increased phosphorylation of mTOR (27,62). Meal feeding-induced activation of mTOR phosphorylation is maintained as long as the food is present. With removal of food, the assembly of eIF4G-eIF4E returns to levels prior to feeding. A fall in both plasma insulin and amino acid concentrations correlates with the reversal of the effects of meal feeding. Thus, meal feeding data are

consistent with previous reports suggesting that acute leucine-induced stimulation of protein synthesis and the phosphorylation states of 4E-BP1 and S6K1 are facilitated by the transient increases in serum insulin concentrations (15,48) and indicates assembly of an active eIF4F complex through both insulin-dependent and -independent mechanisms involved with increased phosphorylation of 4E-BP1, eIF4G, and S6K1.

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