Regulation of protein synthesis by branched-chain amino acids in vivo

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Abstract

Recent advances in the understanding of mRNA translation have facilitated molecular studies on the regulation of protein synthesis by nutrients and the interplay between nutrients and hormonal signals. Numerous reports have established that, in skeletal muscle, the branched-chain amino acids (BCAAs) have the unique ability to initiate signal transduction pathways that modulate translation initiation. Of the BCAAs, leucine is the most potent. Oral administration of leucine to food-deprived rats enhances muscle protein synthesis, in part, through activation of the mRNA binding step of translation initiation. Interestingly, leucine signaling in skeletal muscle differs from that in liver, suggesting that the responses may be tissue specific. The purpose of this paper was to briefly review the current knowledge of how BCAAs act as regulators of protein synthesis in physiologically important tissues, with particular focus on the mechanisms by which BCAAs regulate translation initiation.

Although not widely appreciated, nutritional signals play an important role in controlling gene expression in mammals. The impact of nutrients on gene expression in mammals has become an important area of research. It has been shown that major (carbohydrates, fatty acids, and sterols) and minor (minerals and vitamins) dietary constituents participate in the regulation of gene expression [1–4]. Regulation of gene expression occurs at multiple levels, including transcription, mRNA processing and stability, translation, and protein modification and turnover. In recent years, it has become clear that mRNA translation, in addition to transcription, represents an important control point in gene expression. In contrast to prokaryotes, transcription and translation are spatiotemporally distinct in eukaryotes. For this reason, control of translation is more important in the regulation of gene expression in eukaryotes than in prokaryotes. Control at the level of protein synthesis allows cells to respond rapidly to changes in physiological conditions because activation or repression of mRNAs can occur essentially instantaneously. Regulation at the level of transcription, on the other hand, involves a considerable time lag because precursor RNAs must be processed and mRNA must accumulate or decay in the cytoplasm.

Translational regulation in eukaryotic cells plays a critical role in cell growth, proliferation, and development. Nutrients, such as glucose and amino acids, have a profound impact on translation [5]. Besides their role as substrates for protein synthesis, amino acids also have important regulatory roles in the control of mRNA translation. Of all amino acids, the branched-chain amino acids (BCAAs) have a unique role in this process. The purpose of this paper was to review our current understanding of how BCAAs act as regulators of protein synthesis, with a particular focus on known mechanisms by which BCAAs regulate gene expression at the level of mRNA translation.

Overview of translation initiation

Messenger RNA translation is divided into three stages, including initiation, elongation, and termination, and each of these stages is mediated by protein factors, termed initiation, elongation or release factors, respectively. Initiation appears to be the primary stage for the

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regulation of translation, although mechanisms have also been identified for the control of elongation and termination. Acute changes in nutritional status, such as those occurring during the postprandial state, have not been shown to alter tissue ribosomal content but, instead, increase the efficiency of translation [6–10]. The stimulation of protein synthesis in response to food intake is associated with reaggregation of polysomes, and, when the rate of protein synthesis per polyribosomal ribosome is calculated, it is found to increase slightly [7]. These data are interpreted as indicating that the initiation, rather than the elongation, phase of translation is the main point through which food intake stimulates protein synthesis. In this review, I have focused on recent studies on the effects of BCAA's intake on protein synthesis in fasted animals. Discussion of the control of elongation and termination is outside the scope of this report.

The initiation of mRNA translation is a complex multistep process requiring more than a dozen proteins, referred to as eukaryotic initiation factors (eIFs) [11,12]. At least two steps in the initiation pathway are subjected to regulation in vivo. In the first step of translation initiation, initiator methionyl-tRNA (met-tRNA$_i$) binds to the 40S ribosomal subunit as a ternary complex with eIF2 and GTP [13]. During a subsequent step, the GTP bound to eIF2 is hydrolyzed to GDP and eIF2 is released from the 40S ribosomal subunit as an eIF2–GDP binary complex. For eIF2 to participate in another round of initiation, it must exchange GDP for GTP prior to formation of a new ternary complex. This guanine nucleotide exchange reaction is catalyzed by a second initiation factor, eIF2B. Thus, the rate at which met-tRNA$_i$ can be bound to 40S ribosomal subunits is limited by the activity of eIF2B. The best characterized mechanism for regulating eIF2B activity is through phosphorylation of the α-subunit of eIF2. Phosphorylation of eIF2α converts it from a substrate into a competitive inhibitor of eIF2B, effectively sequestering eIF2B into an inactive complex. Because translation of essentially all mRNAs begins with met-tRNAi, phosphorylation of eIF2α results in a decline in the synthesis of almost all proteins.

A second regulated step in translation initiation involves the binding of mRNA to the 40S ribosomal subunit, a reaction mediated by a triad of initiation factors collectively referred to as eIF4F [14]. The eIF4F initiation factors include: eIF4E, a protein that binds to the m^{7}GTP cap structure at the 5′ end of mammalian cytoplasmic mRNA; eIF4A, a RNA helicase; and eIF4G, a scaffolding protein that in addition to binding to eIF4A and eIF4E binds to the poly(A) binding protein (PABP) and eIF3. Thus, mRNA binds to the 40S ribosomal subunit through the association of the eIF4F–mRNA complex with the eIF3–40S ribosomal subunit complex. Assembly of the eIF4F complex is

regulated, in part, by the association of eIF4E with the eIF4E binding proteins (4E-BP) 4E-BP1, 4E-BP2, and 4E-BP3. Of these proteins, only 4E-BP1 is well characterized. The binding site on eIF4E for 4E-BP1 overlaps with the eIF4G binding site, such that eIF4E can bind to either 4E-BP1 or eIF4G, but not both at the same time [15]. Thus, binding of eIF4E to 4E-BP1 precludes binding of the eIF4E–mRNA complex to the 40S ribosomal subunit. The interaction between eIF4E and 4E-BP1 is regulated by phosphorylation of 4E-BP1 in that hyperphosphorylation prevents and hypophosphorylation is permissive for binding. Increased eIF4F formation may stimulate global rates of protein synthesis but can preferentially enhance the translation of mRNAs with 5′-leader sequences predicted to form extensive secondary structure.

Phosphorylation of ribosomal protein S6 (rpS6) is another mechanism for the regulation of mRNA binding to 40S ribosomal subunits. rpS6 is located near the mRNA/tRNA binding site on the 40S ribosomal subunit [16] and, therefore, may be optimally positioned for a potential role in selecting mRNA to be translated. Phosphorylation of rpS6 occurs on multiple sites and is mediated by a 70-kDa protein kinase termed S6K1 [16]. Activation of S6K1 is associated with the preferential translation of mRNAs containing a terminal oligopyrimidine tract (TOP), which is an oligopyrimidine tract adjacent to the m^{7}GTP cap. Proteins encoded by such mRNA include the ribosomal proteins, eIF4G, PABP, and eukaryotic elongation factor-2; i.e., proteins that are involved in mRNA translation. Thus, activation of S6K1 results in an overall increase in the capacity to synthesize protein.

Regulation of translation initiation by BCAAs

Earlier studies established an important role for the BCAAs in regulating protein synthesis in skeletal muscle [17–19]. More recent analyses show that leucine is the most potent of the BCAAs in stimulating muscle protein synthesis, while isoleucine and valine are much less effective [20,21]. Administration of BCAAs in vivo enhances muscle protein synthesis through activation of the mRNA binding step in translation initiation, and there does not appear to be an effect on the met-tRNA$_i$ binding step. Administration of leucine to rats that have been fasted for 18 h does not cause significant alterations in either the activity of eIF2B or the phosphorylation of eIF2α [21]. Rather, 4E-BP1 is hyperphosphorylated, resulting in the release of eIF4E from the inactive 4E-BP1–eIF4E complex [21]. The freed eIF4E then associates with eIF4G to form the active eIF4F complex. Leucine administration also stimulates the phosphorylation and, thus, the activation of S6K1. Oral administration of isoleucine is less effective than leucine in

activating translation initiation [20]. In rats that have been administered isoleucine, the level of 4E-BP1 and S6K1 phosphorylation is intermediate between fasted and leucine-treated animals, and global rates of protein synthesis are not elevated. Likewise, the binding of eIF4G to eIF4E is enhanced by isoleucine but to a significantly lower level than in leucine-treated rats. Finally, valine administration has little or no effect on protein synthesis or phosphorylation of 4E-BP1 or S6K1 in skeletal muscle of fasted rats.

In contrast to the observations in skeletal muscle, oral administration of leucine to fasted rats has no effect on global rates of protein synthesis in the liver [22]. However, leucine administration does promote phosphorylation of 4E-BP1, S6K1, and rpS6. Similar to the findings in skeletal muscle, valine is the least effective of the BCAAs in stimulating phosphorylation of 4E-BP1 or S6K1, while isoleucine is intermediate between leucine and valine in effectiveness. Although oral administration of leucine does not have an apparent effect on the synthesis of most hepatic proteins, it causes enhanced incorporation of ribosomal protein-encoding mRNAs into polysomes [22], indicating preferential synthesis of ribosomal proteins. Unlike the majority of mRNAs, ribosomal protein mRNAs contain an uninterrupted stretch of pyrimidine residues adjacent to the 5' cap structure, referred to as a terminal oligopyrimidine sequence. The translation of messages containing a terminal oligopyrimidine sequence is enhanced under conditions that activate S6K1, suggesting that phosphorylation of rpS6 leads to their preferential recruitment to ribosomes [23]. Thus, the induction of both S6K1 and rpS6 phosphorylation by oral administration of leucine [22] may account for the increased translation of ribosomal protein mRNAs. Overall, in liver, BCAAs enhance the translation of a particular set of mRNAs typified by those encoding the ribosomal proteins, suggesting that BCAAs increase the capacity of the tissue to synthesize protein.

The relative contribution of insulin to leucine-induced protein synthesis

Leucine has been previously shown to stimulate insulin secretion [24]. In addition to increasing plasma leucine concentrations, oral administration of leucine increases the concentration of circulating insulin. A variety of studies have attempted to prevent or attenuate these changes to help define the role of insulin in leucine-enhanced protein synthesis. Anthony et al. [25] showed that oral administration of leucine to food-deprived rats caused a transient rise in serum insulin from 15 to 45 min after administration. Serum insulin returned to food-deprived control values within 1 h even though serum and intramuscular leucine concentrations

remained elevated for at least 2 h. There was an increase in protein synthesis in gastrocnemius muscle, but it was not observed until 30 min after administration. In contrast to the changes in serum insulin concentration, alterations in protein synthesis were maintained for at least 1 h but returned to baseline by the 2 h time point. Thus, protein synthesis returned to control values even though serum and intramuscular leucine concentrations remained elevated, suggesting that both increased leucine and insulin are required for sustained stimulation of protein synthesis. Somatostatin, an inhibitor of pancreatic hormone release, prevented the leucine-induced changes in serum insulin and protein synthesis. Moreover, somatostatin attenuated the leucine-induced changes in 4E-BP1 and S6K1 phosphorylation and completely blocked the change in rpS6 phosphorylation, but it had no effect on eIF4G–eIF4E assembly. Overall, the results suggest that the transient increase in serum insulin facilitates the leucine-induced enhancement of protein synthesis and phosphorylation of 4E-BP1 and S6K1. In contrast, assembly of the eIF4G–eIF4E complex occurs independently of increases in insulin and, by itself, is insufficient to stimulate the rates of protein synthesis in skeletal muscle after leucine administration.

In a study using alloxan-induced diabetic rats, Anthony et al. [26] showed that the fasting serum insulin concentration was reduced to 25% of the value observed in food-deprived nondiabetic control animals and did not change after oral leucine administration. Interestingly, protein synthesis in skeletal muscle was stimulated by oral administration of leucine in both the control and the diabetic rats. The leucine-induced stimulation of protein synthesis in control skeletal muscle was associated with increased phosphorylation of 4E-BP1, S6K1, and rpS6, reduced binding of 4E-BP1 to eIF4E, and increased assembly of the eIF4G–eIF4E complex. Leucine administration also elevated the circulating concentration of insulin. This increase in insulin enhances the phosphorylation of 4E-BP1 and S6K1 and contributes to the leucine-dependent regulation of protein synthesis. However, a portion of the protein synthetic response must occur through an insulin-independent pathway because the rates of protein synthesis in diabetic rats were higher when they were fed leucine than when they remained food-deprived. The stimulatory effect of leucine on muscle protein synthesis in diabetic rats occurred in the absence of changes in 4E-BP1 and S6K1 phosphorylation, implying a unique and insulin-independent mechanism for the regulation of protein synthesis by leucine.

A recent study using streptozotocin-induced diabetic rats indicates that leucine retains the ability to induce the phosphorylation of 4E-BP1 and S6K1 in the skeletal muscle of diabetic rats [27]. However, the phosphorylation levels of 4E-BP1 and S6K1 in leucine-fed diabetic rats were significantly lower than those in leucine-fed

nondiabetic rats. Furthermore, the serum insulin concentration in diabetic rats was very low and undetectable by enzyme immunoassay. These results suggest that insulin plays a permissive role in leucine-induced protein synthesis. Specifically, insulin facilitates, but is not required for, leucine stimulation of 4E-BP1 and S6K1 phosphorylation. Moreover, this study also showed that, in contrast to skeletal muscle, leucine administration stimulated the phosphorylation of 4E-BP1 and S6K1 in the liver of nondiabetic but not diabetic rats. This suggests that in the liver, insulin is essential for mediating the leucine-induced 4E-BP1 and S6K1 phosphorylation.

Signal transduction pathways required for the stimulation of translation initiation by leucine

The signal transduction pathway that mediates leucine-induced hyperphosphorylation of 4E-BP1 and S6K1 has not been completely defined. Both 4E-BP1 and S6K1 are downstream in a signal transduction pathway involving a protein kinase referred to as the mammalian target of rapamycin protein kinase (mTOR), which appears to be a point of convergence of signals generated by the action of hormones such as insulin and those generated by the cell's recognition of a sufficiency of amino acids. Recent studies have examined the contribution of mTOR to leucine-induced stimulation of protein synthesis and translation initiation in vivo [20]. Administration of rapamycin, an inhibitor of mTOR, prior to oral leucine eliminated the increases in 4E-BP1 and S6K1 phosphorylation. There is no direct evidence, however, that leucine activates the protein kinase activity of mTOR. One mechanism through which the activity of mTOR can be modulated is through interactions with other proteins. For example, the newly discovered protein Raptor regulates both the activity of mTOR and its sensitivity to rapamycin [28,29]. It is possible that amino acids may interact with and modulate Raptor, which interacts with mTOR and plays a key role in signaling from mTOR to S6K1 and 4E-BP1.

In contrast to growth-promoting hormones such as insulin and insulin-like growth factor I, amino acids do not activate protein kinases upstream of mTOR [30]. The stimulation of mTOR by insulin or insulin-like growth factor I is mediated in part by phosphorylation of protein kinase B (PKB) on Ser^{473}, which results in its activation [31]. PKB subsequently phosphorylates a residue (Ser^{2448}) on mTOR within a domain that normally acts to repress mTOR protein kinase activity [32]. Although oral administration of leucine does not enhance the phosphorylation of PKB on Ser^{473} in skeletal muscle, leucine administration does promote phosphorylation of Ser^{2448} on mTOR (Yoshizawa, unpub-
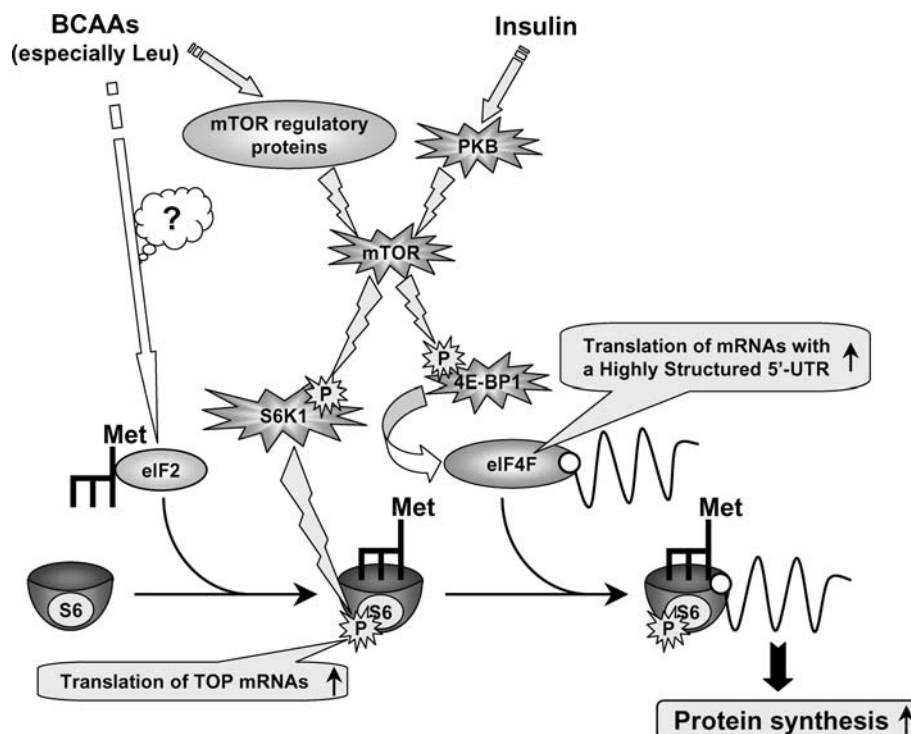

Fig. 1. Regulation of protein synthesis through modulation of translation initiation by BCAAs and insulin. The diagram outlines the mechanisms through which in vivo administration of BCAAs enhances the initiation phase of mRNA translation. The details are discussed in the text. PKB, protein kinase B; mTOR, mammalian target of rapamycin protein kinase; eIF, eukaryotic initiation factor; 4E-BP1, eIF4E binding protein-1; S6K1, 70-kDa ribosomal protein S6 protein kinase; S6, ribosomal protein S6; TOP, terminal oligopyrimidine tract; and UTR, untranslated region.

lished data). Recent findings suggest that the proteins hamartin and tuberin (also termed TSC1 and TSC2, respectively) regulate mTOR function, and there is evidence that they may modulate the control of mTOR signaling by amino acids [33,34]. Although the precise mechanisms through which TSC1 and TSC2 regulate the activity of mTOR have not been determined, the available evidence suggests that the TSC1–TSC2 complex binds to mTOR and prevents PKB-mediated phosphorylation and activation of mTOR. Thus, leucine may promote dissociation of the TSC1–TSC2 complex from mTOR, allowing PKB access to Ser^{2448}. Such a mechanism would not require activation of PKB but would require release of the TSC1–TSC2 complex (see Fig. 1).

Rapamycin treatment prevents the leucine-induced hyperphosphorylation of 4E-BP1 and S6K1 as well as the release of eIF4E from the inactive 4E-BP1–eIF4E complex. However, rapamycin attenuates but does not prevent the leucine-induced stimulation of protein synthesis or eIF4F complex assembly. This suggests that mTOR signaling is essential for the leucine-induced stimulation of translation initiation but that signaling through mTOR alone is not sufficient for the stimulation of muscle protein synthesis. Indeed, there appears to be an mTOR-independent pathway for activation of eIF4F complex assembly that participates in leucine-induced protein synthesis. Thus, leucine regulates muscle protein synthesis through both an insulin- and mTOR-dependent signaling pathway, which results in 4E-BP1 and S6K1 phosphorylation, and an insulin- and mTOR-independent pathway, which results in enhanced eIF4F complex assembly.

Conclusions

The accumulating evidence strongly supports a key role for BCAAs as signaling molecules in the regulation of protein synthesis by modulating mRNA translation. However, a number of questions remain unanswered. A key question is the specificity of response. In particular, why do certain amino acids, e.g., BCAAs, stimulate mRNA translation in some tissues but not others? Another important question is how mammalian cells perceive the BCAA's supply and relay this information to signaling pathways within the cell. The signal transduction pathways through which BCAAs stimulate translation initiation are not completely defined. It is clear that the mTOR must be active in order for leucine to be effective, but signaling through mTOR alone is not sufficient to explain the increased rates of protein synthesis following leucine administration. Further studies will undoubtedly uncover novel mechanism(s) through which BCAAs modulate the translational control of protein synthesis. Elucidation of the sensing and sig-

naling events involved in mammalian amino acid regulation is fundamentally important for understanding not only mammalian cell physiology but is also of substantial clinical significance with respect to amino acid nutrition.

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