Amino Acids: Regulation of Global and Specific mRNA Translation

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Abstract

Maintenance of optimal rates of protein synthesis in both liver and skeletal muscle requires a continuous supply of essential amino acids is a prerequisite for. Deprivation of even a single essential amino acid causes a decrease in the synthesis of essentially all cellular proteins through an inhibition of the initiation phase of mRNA translation. However, the synthesis of all proteins is not repressed equally. Fore example, in contrast to most proteins, the synthesis of specific proteins, such as the transcription factor ATF4, is enhanced. Moreover, the synthesis of specific subsets of proteins, in particular those encoded by mRNAs containing a 5'-terminal oligopyrimidine (TOP) motif, is repressed to a much greater extent compared to most proteins. The specific decrease in TOP mRNA translation is a result of an inhibition of the ribosomal protein S6 kinase, S6K1, and a concomitant decline in S6 phosphorylation. Interestingly, many TOP mRNAs encode proteins involved in mRNA translation, such as the ribosomal proteins and elongation factors eEF1A and eEF2. Thus, deprivation of essential amino acids not only directly and rapidly represses global mRNA translation, but also potentially results in a reduction in the capacity to synthesize protein.

Key words: Translation initiation, Eukaryotic initiation factor eIF2, Serine/threonine protein kinase mTOR

Introduction

A universal response of metazoans to a short-term, overnight fast is a decrease in the rate of protein synthesis in various tissues and organs, including, but not limited to, skeletal muscle and liver. A similar response is likewise observed in cells in culture, where starvation for essential amino acids leads to a repression of protein synthesis. Feeding a protein-containing meal to a fasted animal, or return of the deprived amino acid to cells in culture, results in rapid restoration of rates of protein synthesis to control values. Metabolic labeling studies suggest that during starvation and refeeding the synthesis of most proteins is affected, which has led to the generalization that the effect is global in nature. However, recent studies have provided evidence that the synthesis of some proteins is affected to a greater extent compared to the majority. The purpose of this review is to summary our understanding of how starvation and refeeding regulate protein synthesis in mammals, and mechanisms for regulating protein synthesis at a global as well as a specific level will be considered. Finally, the rapid changes in protein synthesis occurring in response to feeding a protein-containing meal are mediated by an enhancement in the initiation phase of mRNA translation. Therefore, a description of mechanisms involved in the regulation of translation initiation will be presented.

Regulation of Translation Initiation

The process of translating mRNA into protein is generally divided into three functional phases: initiation, elongation, and termination. During translation initiation, initiator methionyl-$tRNA_i$ (met-$tRNA_i$) and mRNA bind to the 40S ribosomal subunit followed by localization of the 40S ribosomal subunit at the initiator AUG start codon on the mRNA (reviewed in Pestova et al., 2001). The 60S ribosomal subunit then joins and the completed initiation complex is ready to proceed to the next phase, elongation. During translation elongation, the ribosome polymerizes amino acids into a growing peptide chain based on the information encoded by the mRNA. Finally, during translation termination, the peptidyl–tRNA bond is hydrolyzed and the completed protein is released from the ribosome. Although instances of regulation at each of these steps has been reported, by far, the majority of examples of translational regulation occur through modulation at the initiation phase. Therefore, the remainder of this section will focus on mechanisms involving the regulation of translation initiation.

The process of translation initiation is mediated by over a dozen proteins referred to as eukaryotic initiation factors (abbreviated eIF). In the first step in translation initiation, a heterotrimeric protein referred to as eIF2 binds to met-$tRNA_i$ and GTP to form a ternary complex

that subsequently binds to the 40S ribosomal subunit. During a later step, the GTP bound to eIF2 is hydrolyzed to GDP through the action of eIF5, and the eIF2–GDP complex is released from the ribosome. Before eIF2 can bind to met-tRNA$_i$, the GDP bound to it must be exchanged for GTP. This GDP–GTP exchange reaction is mediated by the guanine nucleotide exchange protein, eIF2B. The best characterized mechanism for regulating eIF2B activity involves phosphorylation of the α-subunit of eIF2 where phosphorylation of eIF2α on Ser51 converts eIF2 from a substrate into a competitive inhibitor of eIF2B. Other mechanisms, for example phosphorylation of the ε-subunit of eIF2B, have also been reported to modulate eIF2B activity *in vitro*. Whether or not such mechanisms function *in vivo* is unknown.

In the second step in translation initiation, mRNA binds to the 40S ribosomal subunit through the action of another heterotrimeric protein referred to as eIF4F, which consists of eIF4A, an RNA helicase, eIF4E, the mRNA cap binding protein, and eIF4G, a protein that acts as a scaffold and contains binding sites for eIF4A, eIF4E, and the poly(A) binding protein, PABP (reviewed in Raught et al., 2000). eIF4G also contains a domain that binds to eIF3, which in turn binds to the 40S ribosomal subunit. Thus, the eIF4F–mRNA complex binds to the 40S ribosomal subunit through the interaction between eIF4G and eIF3. Two mechanisms for regulating mRNA binding to the 40S ribosomal subunit have been studied in detail. The first mechanism involves phosphorylation of eIF4E at Ser111. A variety of studies using cells in culture have shown that enhanced rates of protein synthesis are associated with increased incorporation of $^{32}P_i$ into eIF4E. The stimulation of protein synthesis caused by eIF4E phosphorylation reportedly is a result of an increase in the affinity of eIF4E for the m^{7}GTP cap structure present at the 5'-end of the mRNA when the protein is phosphorylated (Minich et al., 1994). However, results from studies using animals *in vivo* do not agree with those from studies performed using cells in culture. For example, in both gastrocnemius muscle and liver of overnight fasted rats, feeding a protein-containing meal causes an increase in rates of protein synthesis but a decrease in the proportion of eIF4E in the phosphorylated form (Yoshizawa et al., 1998). A proposal by Rhoads and co-workers (Rinker-Schaeffer et al., 1992; Rychlik et al., 1990) suggesting that protein synthesis may be regulated by the rate of turnover of phosphate on eIF4E rather than the net phosphorylation state may explain the apparent discrepancy between animal and cell culture studies. A second mechanism for regulating mRNA binding to the 40S ribosomal subunit involves the binding of eIF4E to a family of eIF4E binding proteins referred to as 4E-BP1, -2, and -3 (reviewed in Raught and Gingras, 1999). The domain in eIF4E to which 4E-BPs bind overlaps with the domain for eIF4G binding. Thus, the binding of 4E-BP1 and eIF4G to eIF4E is mutually exclusive and the 4E-BPs act as translational repressors when associated with eIF4E by preventing the binding of eIF4G to eIF4E. The association of 4E-BP1 with eIF4E is regulated by phosphorylation of 4E-BP1 on multiple Ser and Thr residues, where

the hyperphosphorylated protein does not bind to eIF4E, but the hypophosphorylated protein does.

Regulation of the met-tRNA$_i$ binding step by essential amino acids

Total mRNA translation

Studies dating back several decades demonstrated that in cells in culture deprived of essential amino acids, translation initiation is impaired (Vaughan and Hansen, 1973; Warrington et al., 1977). More recent studies using primary cultures of hepatocytes (Everson et al., 1989; Kimball et al., 1989) or Ehrlich ascites cells (Scorsone et al., 1987) implicated eIF2 and eIF2B in the inhibition of protein synthesis caused by amino acid deprivation. Moreover, a study using rat livers perfused *in situ* with media lacking histidine showed that eIF2α phosphorylation is enhanced and eIF2B activity is repressed by histidine deprivation (Kimball and Jefferson, 1991). The effects on eIF2α phosphorylation and eIF2B activity were magnified when an inhibitor of the histidinyl-tRNA synthetase, histidinol, was included in the media, suggesting that inhibition of tRNA charging may play a role in regulating eIF2α phosphorylation. This idea is supported by results from studies using Chinese hamster ovary cells containing a temperature-sensitive mutation in the leucyl-tRNA synthetase where incubation at the non-permissive temperature resulted in rapid inhibition of translation initiation (Austin et al., 1986; Clemens et al., 1987; Pollard et al., 1989). The repression of translation initiation associated with inhibition of the synthetase is caused by increased eIF2 phosphorylation and a reduction in eIF2B activity. Thus, using *in vitro* systems, a clear link exists between deprivation of essential amino acids and increased phosphorylation of eIF2α and inhibition of eIF2B activity.

The mechanism by which deprivation of essential amino acids enhances eIF2α phosphorylation in cells in culture involves activation of a protein kinase referred to as mGCN2 (Harding et al., 2000). Thus, in wildtype mouse embryonic stem (MES) cells, leucine deprivation results in disaggregation of polysomes and enhanced eIF2α phosphorylation. The increase in eIF2α phosphorylation is associated with enhanced phosphorylation of the kinase itself. In contrast, in MES cells containing a chromosomal disruption in the mGCN2 gene, leucine deprivation has no effect on either polysome aggregation or eIF2α phosphorylation. A similar effect has been described in yeast where amino acid deprivation causes autophosphorylation, and thereby activation, of Gcn2p which in turn results in increased phosphorylation of eIF2α (reviewed in Hinnebusch, 2000).

In contrast to the universal findings of *in vitro* studies, early studies examining the regulation of eIF2α phosphorylation and eIF2B activity in animals *in vivo* suggested that amino acids might not modulate the met-tRNA$_i$ binding step in translation initiation in mammals. For example, Yosizawa et al. (Yoshizawa et al., 1997) reported that in rat liver and gastrocnemius muscle, neither eIF2α phosphorylation nor eIF2B activity is affected by an overnight fast or refeeding a protein-containing diet. Moreover, oral administration of leucine to fasted rats stimulates protein synthesis in skeletal muscle to values observed in fed animals, with no change in eIF2α phosphorylation or eIF2B activity (Anthony et al., 2000a). However, a more recent study using young, meal-trained rats revealed that feeding fasted animals a diet lacking a single essential amino acid results in both an increase in eIF2α phosphorylation and an inhibition of eIF2B activity (Anthony et al., in press). In contrast, feeding a diet lacking the nonessential amino acid, glycine, had no effect on either parameter. Thus, in liver *in vivo*, provision of an imbalanced mixture of amino acids, but not changes in a complete mixture, enhances eIF2α phosphorylation and represses eIF2B activity. Whether or not provision of imbalanced amino acid mixtures affects eIF2α phosphorylation in other tissues is unknown.

Translation of specific mRNAs

Under conditions that promote eIF2α phosphorylation, the translation of mRNAs encoding essentially all proteins is repressed. A notable exception to this generalization is the transcription factor ATF4. In particular, in mouse embryonic stem cells, deprivation of leucine promotes phosphorylation of eIF2α as well as disaggregation of polysomes (Harding et al., 2000). However, although the translation of most mRNAs is impaired, the synthesis of ATF4 is specifically enhanced. The mechanism by which phosphorylation of eIF2α enhances translation of ATF4 mRNA is complex and involves multiple short open reading frames present in the ATF4 mRNA that are upstream of the authentic AUG start site. The mechanisms by which upstream open reading frames regulate translation initiation have been the subject of a recent review article (Morris and Geballe, 2000), and the reader is referred there for a detailed description. Briefly, the mechanism involved in regulating ATF4 mRNA translation is thought to be similar to that described for the yeast GCN4 mRNA. Like the 5'-leader sequence of ATF4, the 5'-leader sequence of the mRNA encoding GCN4 contains multiple short upstream open reading frames (uORF) (reviewed in Hinnebusch, 1997). The available evidence suggests that uORF1 is translated constitutively, but ribosomes continue to scan down the message following termination of translation at uORF1. Under amino acid-sufficient conditions, the initiation factors required for reinitiation reassemble on the ribosomes as they scan the mRNA and the majority of the ribosomes reinitiate at uORF4. After translation of uORF4, ribosomes dissociate

from the message and never reach the start codon of GCN4, resulting in little synthesis of the protein. In contrast, under amino acid-deficient conditions, translation initiation is impaired due to eIF2α phosphorylation, and not all of the ribosomes that are scanning the message have time to reaccumulate initiation factors prior to reaching uORF4. These ribosomes bypass uORF4 and reinitiate at GCN4 resulting in increased synthesis of the protein.

Regulation of the mRNA binding step by essential amino acids

Regulation of 4E-BP1 phosphorylation and eIF4F assembly by amino acids

The eIF4E binding protein 4E-BP1 (also referred to as PHAS-I) was first identified as a target for insulin-stimulated phosphorylation in adipose tissue (Belsham and Denton, 1980). However, its function as a translational repressor wasn't delineated for another 14 years (Lin et al., 1994; Pause et al., 1994). Since that time a plethora of studies have shown that 4E-BP1 is phosphorylated both *in vitro* and *in vivo* in response to essential amino acids and growth promoting hormones such as insulin and the insulin-like growth factor IGF-1 (reviewed in Raught et al., 2000). As discussed above, hyperphosphorylation of 4E-BP1 prevents it from binding to eIF4E, allowing eIF4G to bind to eIF4E and form the active eIF4F complex.

In either pigs (Davis et al., 2000) or rats (Anthony et al., 2000a; Yoshizawa et al., 1999; Yoshizawa et al., 1997) subjected to an overnight fast, rates of protein synthesis are reduced in skeletal muscle and liver. Feeding either a complete meal, but not a meal lacking protein, rapidly reverses the inhibition, implying that provision of amino acids is crucial in restoring protein synthesis. The feeding-induced stimulation of protein synthesis in both pigs and rats is associated with enhanced phosphorylation of 4E-BP1, dissociation of eIF4E from the inactive 4E-BP1–eIF4E complex, and assembly of the active eIF4G–eIF4E complex (Anthony et al., 2000a; Davis et al., 2000; Yoshizawa et al., 1999; Yoshizawa et al., 1997).

The signaling pathway through which amino acids promote 4E-BP1 phosphorylation has not yet been delineated. However, based on studies performed both *in vitro* and *in vivo*, it is clear that the Ser/Thr protein kinase referred to as the mammalian target of rapamycin (mTOR; aka FRAP or RAFT) must be active for amino acid-induced phosphorylation of 4E-BP1 to occur. For example, treating fasted rats with rapamycin, an inhibitor of mTOR, prior to oral administration of leucine, completely prevents in skeletal muscle the increased phosphorylation of 4E-BP1 and decreased association of 4E-BP1 with eIF4E caused by the amino acid in nontreated animals (Anthony et al., 2000b). Interestingly, rapamycin attenuates, but does not completely prevent the leucine-induced increase in muscle protein synthesis. A similar effect is

observed in neonatal pigs treated with rapamycin prior to feeding (Kimball et al., 2000). Thus, phosphorylation of 4E-BP1 and the resulting changes in its association with eIF4E accounts for part, but not all of the changes in global protein synthesis observed in response to fasting and feeding.

Regulation of TOP mRNA translation by amino acids

In addition to stimulating phosphorylation of 4E-BP1, amino acids promote phosphorylation and thereby activation of the ribosomal protein S6 (rpS6) kinase, S6K1. In cells in culture, phosphorylation of S6K1 enhances the translation of a specific subset of mRNAs containing a 5'-terminal oligopyrimidine (TOP) motif adjacent to the m^7GTP cap structure (reviewed in Fumagalli and Thomas, 2000; Meyuhas and Hornstein, 2000). Such mRNAs include those encoding ribosomal proteins, elongation factors eEF1A and eEF2, and poly(A)-binding protein. Thus, activation of S6K1 results in increased synthesis of many proteins involved in the process of mRNA translation.

A recent study reported that in livers of fasted rats, both rpS6 and S6K1 are hypophosphorylated (Anthony et al., 2001). Oral administration of leucine promotes phosphorylation of both proteins, whereas valine has little effect and the effect of isoleucine is intermediate between the other two branched-chain amino acids. Interestingly, the major portion of the mRNAs encoding ribosomal proteins S4, S8, and L26 is not associated with polysomes in livers of fasted rats, indicating that most of the mRNA encoding these proteins is not being translated. In contrast, two mRNAs that do not contain a TOP sequence, e.g. those encoding β-actin and albumin, are almost entirely present in polysomes and are therefore being actively translated. In response to oral administration of leucine, the mRNAs encoding the ribosomal proteins become polysome-associated, indicating that leucine enhances the translation of these mRNAs. In contrast, isoleucine has a minimal and valine has no effect on the polysomal distribution of ribosomal protein mRNAs. Thus, in rat liver, activation of S6K1 is associated with a preferential increase in translation of mRNAs containing the TOP sequence.

As discussed in the previous section for 4E-BP1, the signaling pathway through which amino acids promote S6K1 phosphorylation and activation is unclear, but seems to involve mTOR (reviewed in Fumagalli and Thomas, 2000). For example, amino acid-induced phosphorylation of S6K1 is blocked by rapamycin in both cells in culture (Fox et al., 1998; Kimball et al., 1999; Patti et al., 1998) and *in vivo* (Anthony et al., 2000b; Kimball et al., 2000). However, activation of S6K1 requires phosphorylation at multiple serine and threonine residues, few, if any, of which are phosphorylated by mTOR *in vitro*. Thus, a variety of protein kinases

have been shown to be upstream effectors involved in activation of S6K1, including PDK1, protein kinase B, protein kinase C (PKC)λ, and PKCζ.

Summary

In animals *in vivo*, deprivation of essential amino acids represses protein synthesis by inhibiting multiple steps in translation initiation. In livers of fasted rats, feeding a meal lacking individual, essential amino acids results in both phosphorylation of eIF2α and inhibition of eIF2B activity. Such changes result in a decrease in synthesis of almost all proteins, with a few currently identified exceptions such as ATF4. In addition to changes in eIF2α phosphorylation, 4E-BP1 becomes dephosphorylated, binds to eIF4E, and thereby prevents the assembly of the active eIF4F complex. Dephosphorylation of 4E-BP1 also occurs in skeletal muscle during an overnight fast and is rapidly reversed in both liver and muscle after consumption of a protein-containing meal. Reduced binding of eIF4G to eIF4E likely repressed the synthesis of most proteins. However, some proteins are translated by a cap-independent process and are minimally affected by decreased formation of the eIF4F complex. Finally, an overnight fast also results in dephosphorylation of S6K1 which leads to dephosphorylation of rpS6. Decreased S6K1 activity has little or no effect on the synthesis of most proteins, but instead preferentially represses the translation of TOP mRNAs, i.e. many of the mRNAs encoding components of the translation machinery. Thus, deprivation of essential amino acids not only directly and rapidly represses the synthesis of most proteins, but because many proteins that are involved in mRNA translation are encoded by TOP mRNAs, dephosphoryaltion of S6K1 potentially results in a reduction in the capacity to synthesize protein.

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