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Role of amino acids in the translational control of protein synthesis in mammals

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Abstract

Amino acids, long considered simply substrates for protein synthesis, have been recently shown to act as modulators of intracellular signal transduction pathways typically associated with growth-promoting hormones such as insulin and insulin-like growth factor-1. Many of the endpoints of the signaling pathways regulated by amino acids are proteins involved in mRNA translation. Thus, particular amino acids not only serve as substrates for protein synthesis but are also modulators of the process. The focus of this article is to review recent studies that have used intact animals as experimental models to examine the role of amino acids as modulators of signal transduction pathways.

Keywords: mRNA translation; mTOR; mGCN2; eIF2; eIF4F

1. Introduction

The role of amino acids as precursors for protein synthesis has been recognized for several decades. In contrast, the role of amino acids to function in the regulation of intracellular signal transduction pathways that control mRNA translation is a relatively recent, but highly important, concept. In this regard, essential amino acids (i.e. amino acids that the organism cannot synthesize and must be obtained from the diet) have been found to regulate signaling through at least two pathways that modulate rate-controlling steps in mRNA translation, i.e. the binding of the initiator form of methionyl-tRNA (met-tRNA$_i$) to the 40S ribosomal subunit to form the 43S preinitiation complex and the binding of mRNA to the 43S preinitiation complex. The focus of this review will be on mechanisms through which essential amino acids modulate signaling to the two regulatory steps in mRNA translation in mammalian cells, with an emphasis on studies performed in intact animals as experimental models.

2. Regulation of the met-tRNA$_i$ binding step in translation initiation by amino acids

In cells in culture, deprivation of single essential amino acids results in a decrease in global rates of protein synthesis [1–3]. It might be expected that in cells deprived of one or more essential amino acids, the decline in protein synthesis rates would be a result of an inhibition of translation elongation due to a decrease in availability of the immediate precursor for protein synthesis, i.e. aminoacyl-tRNA. This expectation is supported by the observations that protein synthesis is inhibited if the charging of tRNA is repressed through inhibition of aminoacyl-tRNA synthetases using amino acid analogues [4–6] or in cells containing a temperature-sensitive variant of the leucyl-tRNA synthetase incubated at the non-permissive temperature [7–9]. However, in mammalian cells deprived of essential amino acids, translation initiation is decreased relative to elongation (reviewed in [10,11]).

A variety of studies (reviewed in [10,11]) have shown that the step in translation initiation that is affected by amino acid deprivation is that involving the binding of met-tRNA$_i$ to

Abbreviations: eEF, eukaryotic elongation factor; eIF, eukaryotic initiation factor; GCN2, general control nonderepressible; His-RS, histidyl-tRNA synthetase; MES, mouse embryonic stem; met-tRNA$_i$, initiator methionyl-tRNA; mTOR, mammalian target of rapamycin; PI 3-kinase, phosphatidylinositol 3-kinase; rp, ribosomal protein; ser, serine; thr, threonine

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the 40S ribosomal subunit (Fig. 1). This step is mediated by a complex consisting of eukaryotic initiation factor (eIF)2, GTP, and met-tRNA$_i$ (reviewed in [12]). In a later step in initiation, the GTP bound to eIF2 is hydrolyzed to GDP, and the eIF2•GDP complex is released from the 40S ribosomal subunit leaving behind the met-tRNA$_i$. The GDP bound to eIF2 is then exchanged for GTP, permitting reformation of the eIF2•GTP•met-tRNA$_i$ complex, by the guanine nucleotide exchange factor, eIF2B. The best characterized mechanism for regulating the activity of eIF2B is through phosphorylation of the α-subunit of eIF2 (eIF2α), whereby phosphorylation converts eIF2 from a substrate into a competitive inhibitor of eIF2B. In both cells in culture (e.g. [13,14]) and in rat livers perfused in situ [15], deprivation of essential amino acids promotes phosphorylation of eIF2α and inhibition of eIF2B activity, suggesting that the inhibition of protein synthesis caused by amino acid deprivation is a result of eIF2α phosphorylation.

The kinase that phosphorylates eIF2α in response to amino acid deprivation was first identified in yeast as the product of the *GCN2* gene, Gcn2p [16]. Gcn2p has a novel domain structure, with a domain homologous to histidyl-tRNA synthetase (His-RS domain) as well as a protein kinase domain (reviewed in [17]). Current models suggest an activation mechanism whereby the binding of uncharged tRNA to the His-RS domain results in a conformational change in the structure of Gcn2p, resulting in its activation. Thus, amino acid deprivation severe enough to cause an increase in the level of uncharged (deacylated) tRNA activates Gcn2p, resulting in phosphorylation eIF2α, inhibition of eIF2B activity, and ultimately repression of protein synthesis.

The mammalian ortholog of Gcn2p, mGCN2, was first cloned by de Haro and co-workers [18] and its role in amino acid-regulated protein synthesis subsequently characterized by two different research groups [14,19]. In those studies, it was shown that eIF2α phosphorylation is enhanced in wildtype mouse embryonic stem (MES) cells deprived of leucine, but not in MES cells lacking mGCN2. Moreover, Zhang et al. [19] reported that livers of wildtype (mGCN2$^{+/+}$) mice exhibit increased phosphorylation of eIF2α and a concomitant reduction in eIF2B activity when perfused in situ with medium lacking histidine and containing the histidyl-tRNA synthetase inhibitor, histidinol, whereas livers from mice containing a chromosomal disruption in the mGCN2 gene (mGCN2$^{-/-}$ mice) are insensitive to such treatment. Thus, mGCN2 in mammalian cells deprived of amino acids in vitro appears to be regulated through similar mechanisms as reported previously in yeast.

In animals in vivo, protein synthesis is repressed in a variety of tissues in response to a short-term fast including liver and skeletal muscle, and rapidly returns to control values after feeding a single meal (e.g. [20,21–24]). The restoration of protein synthesis in response to feeding is only observed in animals fed a meal containing amino acids, and is not observed in animals fed an amino acid-free meal [25], demonstrating the important role amino acids play in promoting protein synthesis in the intact animal. However, eIF2α phosphorylation and eIF2B activity are unaffected by short-term fasting or refeeding in either liver or skeletal muscle [26], suggesting that the overall reduction in plasma amino acid levels associated with a short-term fast is insufficient to activate mGCN2. This idea is supported by the observation that the charging state of aminoacyl-tRNAs is maintained during fasting (unpublished observation).

The finding that mGCN2 is not activated during food deprivation raises the question as what the physiological role of mGCN2 might be. A clue that might provide an answer to that question comes from a study examining the effect of feeding a meal lacking certain essential amino acids to fasted rats [27]. In that study, rats fasted for 18 h were fed either a complete meal, a complete meal lacking the nonessential amino acid glycine, or complete meals lacking either of two essential amino acids, leucine or tryptophan. Feeding the glycine-deficient diet had the same effect on liver protein synthesis as the complete meal and eIF2 phosphorylation was low in animals fed either diet. In contrast, in animals fed either of the diets lacking a single essential amino acid, protein synthesis and eIF2B activity were lower and eIF2α phosphorylation was enhanced compared to animals fed the complete meal or the glycine-deficient diet. The results of that study suggest that an imbalance in plasma amino acid concentrations may activate mGCN2.

A more definitive role for mGCN2 in recognizing an imbalance in amino acid availability in vivo is provided by a study using wildtype (mGCN2$^{+/+}$) or mGCN2$^{-/-}$ mice [17]. Similar to the findings in rats [27], feeding fasted mGCN2$^{+/+}$ mice a complete meal or a complete meal lacking glycine had no effect on eIF2α phosphorylation or eIF2B activity in liver. In contrast, feeding mGCN2$^{+/+}$ mice a meal lacking leucine both enhanced eIF2α phosphorylation and decreased eIF2B activity. However, in mGCN2$^{-/-}$ mice, feeding the meal lacking leucine had no effect on either eIF2α phosphorylation or eIF2B activity, demonstrating that mGCN2 is required for the effect. A subsequent study confirmed the initial observation and extended it to show that eIF2α phosphorylation was not elevated in mGCN2$^{-/-}$ mice, even after feeding the leucine-deficient diet for six days [28].

The results of studies using mGCN2$^{-/-}$ mice suggest that mGCN2 is activated in liver in vivo when the plasma concentration of an essential amino acid is imbalanced relative to other amino acids, but do not provide a mechanism for activation of the kinase. It might be postulated that because plasma leucine concentrations fall below the normal fasting level in mice fed a leucine-free diet, i.e. to about 50% of the value previously reported for fasted animals [29], uncharged leucyl-tRNA might accumulate and activate mGCN2. However, because the residual plasma leucine concentration (approximately 60 μM [28]) is still well above the K_m of the aminoacyl-tRNA synthetases (typically about 1 μM), it seems likely that leucyl-tRNA would be completely charged even in animals fed a leucine-free diet. Thus, other