Amino Acid Regulation of Gene Expression

Leonard S. Jefferson and Scot R. Kimball

Department of Cellular and Molecular Physiology, The Pennsylvania State University College of Medicine, Hershey, PA 17033

ABSTRACT Regulation of gene expression by amino acids is mediated through a number of mechanisms affecting both the transcription of DNA and the translation of mRNA. This report reviews recent findings demonstrating a role for amino acids in regulating the initiation phase of mRNA translation. The report focuses on key regulatory events in translation initiation and discusses some of the signaling pathways through which amino acid sufficiency or the lack thereof is communicated within the cell. It concludes with a consideration of some of the important unanswered questions in this rapidly advancing area of research. J. Nutr. 131: 2460S–2466S, 2001.

KEY WORDS: leucine translation initiation signal transduction

Recent advances in biomedical research reveal a key role for amino acids as nutritional signals in the regulation of a number of cellular processes. Studies employing a variety of cell types and different tissues demonstrate that one such process affected is the regulation of gene expression through modulation of the translation of messenger RNA (mRNA). The studies show that cells recognize alterations in amino acid sufficiency and respond by either upregulating or downregulating translation initiation, i.e., the process during which the ribosome binds to mRNAs. The mRNA is translated in the absence of amino acids, and the translation process is subject to regulation by the phosphorylation status of eIF4F, eIF4E, and eIF4G, a scaffolding protein that binds eIF4E, eIF4A, a signal transduction eIF3 and the poly(A) binding protein, PABP. This step is regulated by the phosphorylation status of eIF4E, eIF4B and eIF4G, as well as by the binding of eIF4E to a family of binding proteins (4E-BP1, -2 and -3) that prevents its association with eIF4G. The response of translation initiation to a change in amino acid availability can be general, i.e., affecting the translation of most if not all mRNAs, and/or specific, i.e., affecting the translation of a single class or subset of mRNAs. The general response is mediated through regulation of both the met-tRNAi and mRNA binding steps, whereas the specific response involves the mRNA binding step and an additional regulatory site, i.e., the phosphorylation of S6, one of the proteins comprising the 40S ribosomal subunit. Sufficient availability of amino acids for optimal rates of protein synthesis is characterized by hyperphosphorylation of eIF2α allowing for unimpeded eIF2α activity, hyperphosphorylation of 4E-BP1, resulting in its dissociation from eIF4E and thus allowing association of eIF4E with eIF4G to form the active eIF4F complex, and hyperphosphorylation of S6. The increased availability of eIF4E caused by 4E-BP1 phosphorylation results in preferential translation of mRNAs containing highly structured 5′-untranslated regions. Similarly, the hyperphosphorylation status of S6 favors translation of mRNAs containing a 5′-terminal oligopyrimidine (TOP) tract. The Ser residue of the α-subunit of eIF2 is a substrate for four different protein kinases; however, the identity of the one involved in mediating the amino acid response is presently not established, and the mode through which the cell recognizes amino acid sufficiency is presently unknown.

Both 4E-BP1 and the protein kinase that phosphorylates S6, i.e., S6K1, are downstream in a signal transduction pathway involving a protein kinase referred to as the mammalian target of rapamycin (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. Learning how the cell recognizes a sufficiency of amino acids is presently the object of intense research. Present evidence, however, suggests multiple recog...

0222-3166/01 $3.00 © 2001 American Society for Nutritional Sciences.
nition sites and multiple signaling pathways. In this review, we describe mechanisms by which alterations in amino acid sufficiency mediate control of translation initiation in mammalian cells. We also provide examples of different modes of amino acid–induced regulation and discuss potential signaling pathways through which each mode of regulation is mediated.

Regulation of guanine nucleotide exchange activity of eIF2B

In both lower eukaryotes and in mammalian cells in culture, deprivation of single essential amino acids causes an inhibition of the guanine nucleotide exchange activity of eIF2B. The mechanism by which eIF2B activity is repressed is indirect and occurs through phosphorylation of the α-subunit of eIF2 (eIF2α). Phosphorylation of eIF2α on Ser51 converts the initiation factor from a substrate into a competitive inhibitor of eIF2B (Fig. 2). Four eIF2α kinases have been identified in mammalian cells, i.e., the heme-regulated translational inhibitor (HRI), the double-stranded RNA-dependent protein kinase (PKR), the pancreatic eIF2α kinase/PKR-like endoplasmic reticulum (ER) kinase (PEK/PERK) and the mammalian homolog of yeast GCN2 (mGCN2). HRI is activated by heme insufficiency, which occurs in reticulocytes during iron-deficiency anemia [reviewed in Chen (2000)]; PKR activity is induced by double-stranded RNA produced, for example, during viral infection [reviewed in Kaufman (2000)]; PEK/PERK is activated by the accumulation of misfolded proteins in the lumen of the endoplasmic reticulum [reviewed in Ron and Harding (2000)]; and GCN2 is regulated by nutrient availability, where deprivation of amino acids or purine nucleotides results in activation of the kinase [reviewed in Hinnebusch (2000)]. Whether mGCN2 is activated by nutrient insufficiency as is its yeast counterpart is presently not known. However, on the basis of structural homology, it is anticipated that such regulation is likely to occur with the mammalian protein. Activation of GCN2 that occurs in yeast deprived of amino acids is mediated by accumulation of uncharged tRNA, which binds to a regulatory domain in the protein homologous to histidyl-tRNA synthetases [reviewed in Hinnebusch (1997)]. Because the mammalian protein also contains a domain homologous to histidyl-tRNA synthetase (Sood et al. 2000), it is likely that mGCN2 is similarly activated by amino acid deprivation of essential amino acids causes an inhibition of the guanine nucleotide exchange activity of eIF2B. The mechanism by which eIF2B activity is repressed is indirect and occurs through phosphorylation of the α-subunit of eIF2 (eIF2α). Phosphorylation of eIF2α on Ser51 converts the initiation factor from a substrate into a competitive inhibitor of eIF2B (Fig. 2). Four eIF2α kinases have been identified in mammalian cells, i.e., the heme-regulated translational inhibitor (HRI), the double-stranded RNA-dependent protein kinase (PKR), the pancreatic eIF2α kinase/PKR-like endoplasmic reticulum (ER) kinase (PEK/PERK) and the mammalian homolog of yeast GCN2 (mGCN2). HRI is activated by heme insufficiency, which occurs in reticulocytes during iron-deficiency anemia [reviewed in Chen (2000)]; PKR activity is induced by double-stranded RNA produced, for example, during viral infection [reviewed in Kaufman (2000)]; PEK/PERK is activated by the accumulation of misfolded proteins in the lumen of the endoplasmic reticulum [reviewed in Ron and Harding (2000)]; and GCN2 is regulated by nutrient availability, where deprivation of amino acids or purine nucleotides results in activation of the kinase [reviewed in Hinnebusch (2000)]. Whether mGCN2 is activated by nutrient insufficiency as is its yeast counterpart is presently not known. However, on the basis of structural homology, it is anticipated that such regulation is likely to occur with the mammalian protein. Activation of GCN2 that occurs in yeast deprived of amino acids is mediated by accumulation of uncharged tRNA, which binds to a regulatory domain in the protein homologous to histidyl-tRNA synthetases [reviewed in Hinnebusch (1997)]. Because the mammalian protein also contains a domain homologous to histidyl-tRNA synthetase (Sood et al. 2000), it is likely that mGCN2 is similarly activated by amino acid insufficiency, which occurs in reticulocytes during iron-deficiency anemia [reviewed in Chen (2000)]; PKR activity is induced by double-stranded RNA produced, for example, during viral infection [reviewed in Kaufman (2000)]; PEK/PERK is activated by the accumulation of misfolded proteins in the lumen of the endoplasmic reticulum [reviewed in Ron and Harding (2000)]; and GCN2 is regulated by nutrient availability, where deprivation of amino acids or purine nucleotides results in activation of the kinase [reviewed in Hinnebusch (2000)]. Whether mGCN2 is activated by nutrient insufficiency as is its yeast counterpart is presently not known. However, on the basis of structural homology, it is anticipated that such regulation is likely to occur with the mammalian protein. Activation of GCN2 that occurs in yeast deprived of amino acids is mediated by accumulation of uncharged tRNA, which binds to a regulatory domain in the protein homologous to histidyl-tRNA synthetases [reviewed in Hinnebusch (1997)]. Because the mammalian protein also contains a domain homologous to histidyl-tRNA synthetase (Sood et al. 2000), it is likely that mGCN2 is similarly activated by amino acid insufficiency, which occurs in reticulocytes during iron-deficiency anemia [reviewed in Chen (2000)]; PKR activity is induced by double-stranded RNA produced, for example, during viral infection [reviewed in Kaufman (2000)]; PEK/PERK is activated by the accumulation of misfolded proteins in the lumen of the endoplasmic reticulum [reviewed in Ron and Harding (2000)]; and GCN2 is regulated by nutrient availability, where deprivation of amino acids or purine nucleotides results in activation of the kinase [reviewed in Hinnebusch (2000)]. Whether mGCN2 is activated by nutrient insufficiency as is its yeast counterpart is presently not known. However, on the basis of structural homology, it is anticipated that such regulation is likely to occur with the mammalian protein. Activation of GCN2 that occurs in yeast deprived of amino acids is mediated by accumulation of uncharged tRNA, which binds to a regulatory domain in the protein homologous to histidyl-tRNA synthetases [reviewed in Hinnebusch (1997)]. Because the mammalian protein also contains a domain homologous to histidyl-tRNA synthetase (Sood et al. 2000), it is likely that mGCN2 is similarly activated by amino acid insufficiency, which occurs in reticulocytes during iron-deficiency anemia [reviewed in Chen (2000)]; PKR activity is induced by double-stranded RNA produced, for example, during viral infection [reviewed in Kaufman (2000)]; PEK/PERK is activated by the accumulation of misfolded proteins in the lumen of the endoplasmic reticulum [reviewed in Ron and Harding (2000)]; and GCN2 is regulated by nutrient availability, where deprivation of amino acids or purine nucleotides results in activation of the kinase [reviewed in Hinnebusch (2000)]. Whether mGCN2 is activated by nutrient insufficiency as is its yeast counterpart is presently not known. However, on the basis of structural homology, it is anticipated that such regulation is likely to occur with the mammalian protein. Activation of GCN2 that occurs in yeast deprived of amino acids is mediated by accumulation of uncharged tRNA, which binds to a regulatory domain in the protein homologous to histidyl-tRNA synthetases [reviewed in Hinnebusch (1997)]. Because the mammalian protein also contains a domain homologous to histidyl-tRNA synthetase (Sood et al. 2000), it is likely that mGCN2 is similarly activated by amino acid insufficiency, which occurs in reticulocytes during iron-deficiency anemia [reviewed in Chen (2000)]; PKR activity is induced by double-stranded RNA produced, for example, during viral infection [reviewed in Kaufman (2000)]; PEK/PERK is activated by the accumulation of misfolded proteins in the lumen of the endoplasmic reticulum [reviewed in Ron and Harding (2000)]; and GCN2 is regulated by nutrient availability, where deprivation of amino acids or purine nucleotides results in activation of the kinase [reviewed in Hinnebusch (2000)]. Whether mGCN2 is activated by nutrient insufficiency as is its yeast counterpart is presently not known. However, on the basis of structural homology, it is anticipated that such regulation is likely to occur with the mammalian protein. Activation of GCN2 that occurs in yeast deprived of amino acids is mediated by accumulation of uncharged tRNA, which binds to a regulatory domain in the protein homologous to histidyl-tRNA synthetases [reviewed in Hinnebusch (1997)]. Because the mammalian protein also contains a domain homologous to histidyl-tRNA synthetase (Sood et al. 2000), it is likely that mGCN2 is similarly activated by amino acid
Correlation of eIF2α phosphorylation with alterations in eIF2 activity in mammalian cells was first observed in studies using perfused rat liver deprived of essential amino acids (Kimball and Jefferson 1991). The results of these studies demonstrated that deprivation of the essential amino acid, histidine, results in an increase in the proportion of eIF2α in the phosphorylated state. Addition of histidinol, a competitive inhibitor of histidyl-tRNA synthetase, to the perfusate causes a further increase in eIF2α phosphorylation. Importantly, the guanine nucleotide exchange activity of eIF2B is inversely correlated with alterations in eIF2α phosphorylation. Moreover, the reduction in eIF2B activity is directly proportional to the fall in protein synthesis, implying that eIF2B activity controls global rates of protein synthesis in response to deficiencies of essential amino acids.

The guanine nucleotide exchange activity of eIF2B is also regulated indirectly through allosteric mechanisms. Examples of such regulation include the inhibition of activity by ATP (Kimball and Jefferson 1995) and oxidized pyridine dinucleotides (Dholakia et al. 1986), and its reversal by reduced pyridine dinucleotides (Dholakia et al. 1986, Kimball and Jefferson 1995). Sugar phosphates, such as fructose-1,6-bisphosphate, and polyamines also allosterically activate eIF2B (Gross et al. 1991 and 1988, Singh and Wahba 1995). Although allosteric regulation of eIF2B activity has been demonstrated in vitro, it is important to note that such mechanisms have not been shown to be involved in mediating effects induced by alterations in amino acid sufficiency.

In addition to the indirect mechanisms described above, the guanine nucleotide exchange activity of eIF2B may also be regulated directly through phosphorylation of the e-subunit of the protein. The e-subunit is phosphorylated by at least three different protein kinases, i.e., casein kinases (CK)-I and -II, and glycogen synthase kinase (GSK)-3. Phosphorylation by either CK-I (Singh et al. 1996) or CK-II (Dholakia and Wahba 1988, Singh et al. 1994) stimulates the guanine nucleotide exchange activity of eIF2B. In contrast, phosphorylation by GSK-3 is inhibitory (Welsh et al. 1998). A precedent for this mode of regulation is suggested by studies in CHO.T cells in which phosphorylation of eIF2B by GSK-3 is concluded to be an important mechanism for mediating the action of insulin on the guanine nucleotide exchange activity of eIF2B (Welsh et al. 1997 and 1998). In CHO.T cells, insulin regulates GSK-3 through a PI 3-kinase–dependent signaling pathway. In contrast, this pathway does not appear to be modulated by amino acid sufficiency in FAO (Patti et al. 1998) or Jurkat T-lymphoblastoid (Iboshii et al. 1999) cells. Moreover, in L6 myoblasts, deprivation of single essential amino acids results in a decrease in the activity of an characterized eIF2B kinase but has no effect on GSK-3 activity (Kimball et al. 1998). The direction of change in the eIF2B kinase activity observed in L6 myoblasts deprived of single essential amino acids is opposite to what would be expected if the eIF2B kinase were GSK-3. Thus, because phosphorylation by GSK-3 is inhibitory, an increase in eIF2B activity should be associated with an increase in GSK-3 activity if GSK-3 is causative in the effect. Thus, it is unlikely that GSK-3 mediates regulation of eIF2B activity by amino acid sufficiency. However, because the activity of an as yet to be characterized eIF2B kinase decreases in response to deprivation of single essential amino acids, a role for other kinases in the regulation of eIF2B must be considered.

In vivo, feeding a diet lacking a single essential amino acid results in disaggregation of hepatic polyribosomes and repression of protein synthesis (Sidransky et al. 1967, Wunner et al. 1966). The reduction in protein synthesis is associated with a decrease in the guanine nucleotide exchange activity of eIF2B, with only minor changes in eIF2α phosphorylation (Anthony et al. 2001), suggesting that eIF2B is regulated through mechanisms distinct from eIF2α phosphorylation under these conditions. An important distinction between in vitro studies using cells in culture and in vivo studies in which diets lacking single essential amino acids are fed to animals is in the amount of the deprived amino acid that is available to the cells or tissue. In in vitro studies, the concentration of the deprived amino acid in cell culture medium is essentially zero. In contrast, in food-deprived rats fed a diet lacking a single essential amino acid, plasma concentrations of the missing amino acid fall only slightly below the value observed in a food-deprived animal. Thus, the continued availability in the plasma of the amino acid missing from the diet, albeit at fasting rather than fed concentrations, likely accounts for the lack of effect on eIF2α phosphorylation. Furthermore, in response to provision of an imbalanced mixture of amino acids, eIF2B is regulated through mechanisms other than phosphorylation of the α-subunit of eIF2.

Regulation involving signaling through mTOR to modulate the phosphorylation status of 4E-BP1

In addition to the changes in eIF2B activity noted above, in rats fed a diet lacking a single essential amino acid, eIF4F assembly and S6 phosphorylation, i.e., the steps involved in regulating the binding of mRNA to the 4OS ribosomal subunit, are repressed (Anthony et al. 2001). Assembly of the active eIF4F complex is regulated in part through reversible association of eIF4E with a family of translation repressors that currently consists of three members, eIF4E binding protein (4E-BP)1, 4E-BP2 and 4E-BP3 (reviewed in Raught and Grishin 1999). Both eIF4G and the eIF4E binding proteins contain a common structural domain that is involved in recognition of eIF4E; consequently, binding of eIF4G and the binding proteins to eIF4E is mutually exclusive. Thus, association of eIF4E with an eIF4E binding protein prevents its interaction with eIF4G and precludes assembly of the active eIF4F complex. The dynamics of eIF4E interaction with the eIF4E binding proteins is modulated by phosphorylation of the binding proteins, whereby phosphorylation causes dissociation of eIF4E from the inactive 4E-BP·eIF4E complex.

Although a number of studies have shown changes in phosphorylation of 4E-BP1 in response to amino acid sufficiency (Fox et al. 1998, Harag et al. 1998, Patti et al. 1998, Wang et al. 1998, Xu et al. 1998), few have examined its consequences on the interaction of 4E-BP1 or eIF4E with eIF4E. An exception is a recent study showing that in L6 myoblasts in culture, replacement of the essential amino acid leucine to leucine-deprived myoblasts resulted in a concomitant decrease in 4E-BP1 binding to eIF4E and an increase in eIF4G binding to eIF4E (Kimball et al. 1998). A similar effect was observed in skeletal muscle of food-deprived rats in which oral administration of leucine both promoted binding of eIF4G to eIF4E and repressed 4E-BP1 association with eIF4E (Anthony et al. 2000). In each of these studies, alterations in
4E-BP1 binding to eIF4E were associated with hyperphosphorylation of the binding protein.

The kinase(s) that phosphorylates 4E-BP1 in vivo remains elusive. For example, inhibition of the extracellular-signal regulated kinase (ERK) protein kinase, MEK, by PD98059 prevents 4E-BP1 phosphorylation induced by physiologic, but not supraphysiologic concentrations of insulin in CHO or 3T3-L1 cells (Scott and Lawrence 1997), suggesting that ERK phosphorylates 4E-BP1 in vivo. Similarly, PD98059 attenuates prostaglandin F₂α-induced phosphorylation of 4E-BP1 in rat vascular smooth muscle cells (Rao et al. 1999). However, inhibition of the mammalian homolog of the yeast target of rapamycin protein kinase (mTOR), a protein kinase on a separate intracellular signal transduction pathway, also prevents the insulin- or prostaglandin F₂α-induced stimulation of 4E-BP1 phosphorylation. Inhibition of mTOR also represses 4E-BP1 phosphorylation induced by amino acids or by hormones, such as insulin-like growth factor-I (Graves et al. 1995). Thus, it may be that multiple protein kinases mediate phosphorylation of 4E-BP1. In this regard, six 4E-BP1 phosphorylation sites have been identified in cells treated with insulin or serum (Fadden et al. 1997, Gingras et al. 1999, Heesom et al. 1998, Mothe-Satney et al. 2000). Two sites, Thr³⁶ and Thr⁴⁵, reside N-terminal to the eIF4E binding domain and the remaining sites, Ser⁶⁴, Thr⁶⁹, Ser⁷² and Ser¹¹¹, are C-terminal to the binding domain. In vitro studies, both ERK2 and mTOR phosphorylate each of the sites with the exception of Ser¹¹¹, although not all sites are phosphorylated by the two kinases with equal efficiency (Brunn et al. 1997, Gingras et al. 1999, Haystead et al. 1994). In addition, dephosphorylation of Thr³⁶ and Thr⁴⁵ is refractory to inhibition of mTOR by rapamycin (Mothe-Satney et al. 2000). In contrast, phosphorylation of Ser⁶⁴ and Thr⁶⁹ in quiescent cells is acutely blocked by inhibitors or mTOR (Mothe-Satney et al. 2000). Thus, it may be that mTOR, or an mTOR-regulated protein kinase, phosphorylates Ser⁶⁴ and Thr⁶⁹, whereas a different kinase phosphorylates Thr³⁶ and Thr⁴⁵.

Of the five sites that are phosphorylated by ERK2 and mTOR, phosphorylation at Thr⁴⁵ and Ser⁶⁴, i.e., those sites that flank the eIF4E binding domain, appear to be dominant in attenuating binding to eIF4E (Mothe-Satney et al. 2000). Thus, phosphorylation of Thr⁴⁵ and Ser⁶⁴ abolishes eIF4E binding in vitro. If phosphorylation at only one or two sites can prevent eIF4E binding, then what is the function of the remaining sites? One possibility is that phosphorylation at certain sites acts to "prime" 4E-BP1 for phosphorylation at other sites. In this regard, it has been suggested that 4E-BP1 phosphorylation occurs in an ordered process, with phosphorylation at Thr³⁶ and Thr⁴⁵ occurring before, and being required for phosphorylation at Ser⁶⁴ and Thr⁶⁹ [reviewed in Raught et al. (2000b)]. This suggestion is consistent with results from a study showing that mutation of Thr³⁶ and Thr⁴⁵ to nonphosphorylatable Ala residues reduces serum-stimulated phosphorylation of the remaining sites (Gingras et al. 1999).

In addition to phosphorylating 4E-BP1, mTOR also phosphorylates S6K1 in vitro (Isotani et al. 1999). However, the sites in 4E-BP1 phosphorylated by mTOR are flanked by proline residues, whereas those sites on S6K1 (Thr³⁸⁹) have neighboring large, bulky residues. Moreover, mTOR exhibits a strong (>10-fold) preference for S6K1 over 4E-BP1 (Burnett et al. 1998). Therefore, mTOR may not act as a 4E-BP1 kinase in vivo. Instead, it may act by inhibiting the activity of a protein phosphatase that dephosphorylates 4E-BP1 (Fig. 3). Indeed, Saccharomyces cerevisiae, two TOR isoforms, Tor1p and Tor2p, control translation initiation in response to nutrient availability (Barbet et al. 1996). In this system, TOR is linked to PPH21 and PPH22, two type 2A protein phosphatase catalytic subunits (PP2Ac) (Jiang and Broach 1999), and SIT4, a homolog of the mammalian type 6 protein phosphatase (Bastians and Ponstingl 1996). Pph21p, Pph22p and Sip4p all interact with the essential protein, Tap42p (Di Como and Arndt 1996). The interaction between Tap42p and the protein phosphatase catalytic subunits is observed in growing cells, but not in cells in stationary phase. Furthermore, the association is prevented by rapamycin, and mutations in the TAP42 gene can result in rapamycin resistance, implicating Tor in the effect. Further evidence that Tor is upstream of, and regulates Tap42p interaction with protein phosphatase catalytic subunits is that at the nonpermissive temperature, a yeast strain expressing a temperature-sensitive variant of Tap42p, expressed a cold-sensitive temperature-sensitive variant of Tap42p.

![Diagram](https://example.com/diagram.png)

**FIGURE 3** Regulation of translation initiation by mammalian target of rapamycin (mTOR)-mediated alterations in phosphorylation of 4E-BP1 and S6K1. Hormones, such as insulin and insulin-like growth factor-I, cause activation of mTOR and subsequently, hyper phosphorylation of 4E-BP1 and S6K1. Although still not completely defined in mammalian cells, in yeast, mTOR functions through inhibition of a protein phosphatase rather than through activation of a protein kinase. Although hyper phosphorylation of 4E-BP1 and S6K1 in response to regulatory amino acids requires mTOR to be active, there is no evidence that amino acids actually act directly through mTOR. In the figure, the alternative possibility that amino acids regulate a protein kinase(s) distinct from mTOR is presented. In this model, mTOR activity would be required to repress the phosphatase that dephosphorylates 4E-BP1 and S6K1, whereas amino acids signal to 4E-BP1 and S6 protein kinases. Regardless of the mechanism involved in hyperphosphorylation, it is clear that phosphorylation of 4E-BP1 stimulates eukaryotic initiation factor (eIF)4F assembly, leading to enhanced translation of mRNAs having high secondary structure at their 5′-termini. Increased eIF4F assembly would also be expected to increase global rates of protein synthesis. Activation of S6K1 by hyperphosphorylation leads to a preferential increase in translation of mRNAs with a terminal oligopyrimidine (TOP) sequence adjacent to their 5′-termini.
exhibits a defect in translation initiation (Di Como and Arndt 1996) similar to wild-type cells treated with rapamycin (Barbet et al. 1996). Moreover, Tap42p is phosphorylated in growing cells, but not in cells treated with rapamycin (Jiang and Broach 1999). Finally, expression of a rapamycin-insensitive Tor variant confers rapamycin resistance to Tap42p phosphorylation. Overall, results obtained in yeast suggest that Tor phosphorylates and thereby promotes association of Tap42p with the catalytic subunit of PP2A and PP6 and that such an association is a requisite step in Tor-mediated signaling. In mammals, the α4 protein is a homolog of Tap42p (Inui et al. 1995, Kuwahara et al. 1994). As observed for Tap42p in yeast (Jiang and Broach 1999), α4 binds to PP2Ac as well as the related PP4 and PP6 protein phosphatases (Chen et al. 1998, Inui et al. 1998, Murata et al. 1997, Nanahoshi et al. 1998). As in yeast, interaction between α4 and PP2Ac in mammalian cells is regulated through an mTOR-dependent process. For example, in COS7 cells expressing α4 containing a FLAG epitope at the N-terminus of the protein, the catalytic subunit of PP2A is detected in anti-FLAG immunoprecipitates (Murata et al. 1997). Treating the cells with rapamycin prior to immunoprecipitation has no effect on recovery of α4, but drastically reduces the amount of PP2Ac in the immunoprecipitate. Similarly, in Jurkat T-cells, rapamycin reduces the amount of PP2Ac in α4 immunoprecipitates in a dose-dependent manner (Inui et al. 1998). In contrast, in rapamycin-resistant Raji B-cells, the macrolide has no effect on the recovery of PP2Ac in α4 immunoprecipitates. Thus, the results from studies in mammalian cells are also consistent with a model in which mTOR enhances the binding of α4 to PP2Ac, which decreases protein phosphatase activity toward 4E-BP1 and S6K1, resulting in a net increase in phosphorylation of the two proteins.

Regardless of the mechanism, it is clear that essential amino acids, and in particular leucine, regulate eIF4F assembly. This effect is observed in cells in culture and in liver and skeletal muscle of food-deprived animals. Thus, in contrast to phosphorylation of eIF2α, which manifests only during severe amino acid deprivation, modulation of the steps involved in mRNA binding to the 4OS ribosomal subunit is observed under conditions of both amino acid deprivation and administration of the essential amino acid leucine.

**Regulation involving signaling through mTOR to modulate the phosphorylation status of S6K1**

The S6Ks are a family of mitogen-activated, Ser/Thr protein kinases that participate in translational and cell cycle control (reviewed in Fumagalli and Thomas (2000)). The S6K1 gene encodes cytoplasmic and nuclear proteins referred to as S6K1β/p70s6k and S6K1α/p85s6k, respectively, whereas the S6K2 gene encodes S6K2β and S6K2β. Both S6K1 and S6K2 are phosphoproteins whose enzymatic activities are regulated by phosphorylation at multiple Ser and Thr residues. With regard to S6K1, current studies suggest that activation of the enzyme involves an ordered series of phosphorylation events whereby phosphorylation of a cluster of sites located near the C-terminus permits phosphorylation of three internal sites, Thr389, Ser246 and Thr389, which collectively confer maximal kinase activity. Mitogen- and amino acid-induced phosphorylation of one of the activating sites, Thr389, is blocked by the mTOR inhibitor, rapamycin, which prevents activation of the kinase. Thus, both 4E-BP1 and S6K1 are phosphorylated in an mTOR-dependent manner. Although phosphorylation of Thr389 is inhibited by rapamycin, it is unlikely that mTOR is the relevant kinase because deletion of the C-terminal region of the protein containing the cluster of phosphorylation sites abrogates the effect, suggesting that the C-terminus confers rapamycin sensitivity.

Although mitogen-stimulated phosphorylation of S6 has been known to occur for many years, its role in regulating translation initiation has been delineated only recently. In this regard, rather than stimulating global rates of protein synthesis, activation of S6K1 results in the preferential translation of mRNAs encoding proteins that play important roles in protein synthesis [reviewed in Dufner and Thomas (1999)]. Thus, activation of S6K1 causes a preferential increase in the synthesis of proteins, such as ribosomal proteins and elongation factors eIF1A and eIF2, which are encoded by mRNAs containing 5′-terminal oligopyrimidine tracts (referred to as TOPS mRNAs). For example, in liver of food-deprived rats, oral administration of leucine promoted phosphorylation of S6K1 and ribosomal protein S6 (Anthony et al. 2001). Similar results were observed in rat livers perfused in situ with medium containing leucine at a concentration four times that found in plasma of food-deprived animals, with the remaining amino acids present at plasma concentrations (Shah et al. 1999). In both cases, unbalanced provision of leucine did not stimulate global rates of protein synthesis, but rather specifically increased translation of mRNAs containing the TOP sequence (Anthony et al. 2001). Thus, in the liver of food-deprived rats, mRNAs encoding ribosomal proteins S4, S8 and L26 are not associated predominantly with polysomes. Leucine administration results in a redistribution of ribosomal protein mRNAs, such that the bulk of these mRNAs exhibit a polysomal distribution. In contrast, mRNAs encoding albumin and β-actin are predominantly polysome associated, regardless of feeding status, indicating that imbalanced provision of leucine preferentially upregulates the translation of TOP mRNAs.

**Regulation involving mTOR-independent signaling to modulate the activation state of the eIF4F complex**

Although association of eIF4E with 4E-BP1 is an important mechanism for modulating assembly of the eIF4F complex, it is not the sole means of regulating the interaction of eIF4E and eIF4G. For example, in skeletal muscle of food-deprived rats, oral administration of leucine enhanced 4E-BP1 phosphorylation concomitant with dissociation of the 4E-BP1·eIF4E complex and increased association of eIF4G with eIF4E (Anthony et al. 2000). Treatment with rapamycin before leucine administration prevented completely leucine-induced changes in 4E-BP1 phosphorylation and dissociation of the 4E-BP1·eIF4E complex. However, binding of eIF4G to eIF4E was nonetheless significantly enhanced by leucine administration in rapamycin-treated rats. A similar phenomenon was observed in neonatal pigs, in which rapamycin treatment abrogated the feeding-induced dissociation of the 4E-BP1·eIF4E complex, but only partially reduced the increase in eIF4G binding to eIF4E (Kimball et al. 2000).

A possible mechanism for TOR-independent signaling to eIF4F is suggested by a recent study showing that stimulation of eIF4G binding to eIF4E by epidermal and nerve growth factors (EGF and NGF, respectively) was blocked by the MEK inhibitor, PD98059 (Kleijn and Proud 2000). In that study, both EGF and NGF also promoted dissociation of the 4E-BP1·eIF4E complex. However, PD98059 had no effect on 4E-BP1 binding to eIF4E, indicating that signaling through the mitogen-activated protein (MAP) kinase pathway can modulate eIF4F assembly in a 4E-BP1-independent manner.

How signaling through the MAP kinase pathway promotes eIF4G binding to eIF4E remains an unanswered question. On
the basis of observations that eIF4G is a phosphoprotein, and that its phosphorylation is enhanced by mitogen- and serum-stimulation of cells in culture [reviewed in Raught et al. (2000b)], it is tempting to speculate that phosphorylation of eIF4G might stimulate eIF4F assembly. However, phosphorylation of the three serum-stimulated phosphorylation sites in eIF4G is phosphorylated in response to feeding a complete meal to food-deprived rats. However, in that study, an increase in plasma insulin concentration may have contributed to the increased phosphorylation. Further studies are required to establish what, if any role, changes in amino acid sufficiency have in regulating mTOR-independent signaling to eIF4F assembly.

CONCLUSIONS

The recent identification of amino acids as signaling molecules that regulate translation initiation has provided the incentive to gain a better understanding of the mechanisms through which they operate. A plethora of studies have demonstrated that essential amino acids, and in particular leucine, stimulate phosphorylation of 4E-BP1 and S6K1. In contrast, relatively few studies have examined their effects on eIF4F assembly or eIF2B activity. On the basis of information presently available, a number of questions remain unanswered. One key question concerns the specificity of the response. In particular, why do certain amino acids stimulate 4E-BP1 phosphorylation and/or S6K1 phosphorylation in some cells, but not others? Another important question is how amino acid sufficiency is sensed by the cell. It would appear that at least two mechanisms exist through which amino acid sufficiency is detected, although their identities remain elusive. One mechanism by which amino acid sufficiency is detected appears to involve a recognition molecule whose signaling to 4E-BP1 and S6K1 is dependent upon mTOR (Lynch et al. 2000) (Fig. 4). Although incompletely characterized, the recognition molecule preferentially responds to leucine sufficiency, although other essential amino acids appear to be recognized to lesser extents. In addition to modulating eIF4F complex assembly indirectly through phosphorylation of 4E-BP, the recognition molecule may also regulate eIF4F complex assembly directly through the MAP kinase signaling pathway. A second mechanism by which amino acid sufficiency is detected involves an eIF2α kinase, most likely mGCN2. By modulating the phosphorylation state of eIF2α, the kinase indirectly regulates the guanine nucleotide exchange activity of eIF2B. In contrast to the specificity demonstrated by the putative leucine recognition molecule, activation of the eIF2α kinase is responsive to insufficiency of any essential amino acid. The activity of eIF2B may also be regulated through modulation of an unidentified eIF2B kinase. A third unanswered question concerns the exact role of mTOR in amino acid signaling to translation initiation. It is clear that such signaling requires mTOR to be active, but whether changes in amino acid sufficiency modulate mTOR activity is unclear. Finally, a topic not discussed in this report, but of considerable interest, involves the possible cooperation between amino acids and growth-promoting hormones in the regulation of translation initiation.

LITERATURE CITED


