Control of the translational machinery by amino acids1–4

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ABSTRACT

Amino acids are the precursors for the synthesis of proteins. In humans, approximately half of the 20 different amino acids are essential, ie, must be obtained from the diet. Cells must therefore take account of amino acid availability to achieve sustainable rates of protein synthesis. One of the major mechanisms involved in this is signaling through a complex of proteins termed mammalian target of rapamycin complex (mTORC) 1, which is activated by amino acids. In turn, mTORC1 regulates the production of ribosomes, the molecular machines that make proteins, and the activity of other cellular components required for protein synthesis. mTORC1 signaling promotes the transcription of the genes for ribosomal RNAs and many other components involved in ribosome production. It also positively regulates the translation of the messenger RNAs (mRNAs) for ribosomal proteins. Indeed, recent studies have shown that mammalian target of rapamycin signaling drives the translation of mRNAs for many anabolic enzymes and other proteins involved in diverse cellular functions. The translational machinery is also regulated by the absence of amino acids through the protein kinase GCN2 (general control nonrepressed 2), which phosphorylates and in end-effect inhibits the translation initiation factor eIF2 (eukaryotic initiation factor 2). This process shuts down general protein synthesis to conserve amino acids. Am J Clin Nutr 2014;99(suppl):231S–6S.

INTRODUCTION

Protein synthesis is a key process in all living cells. Its proper control is important for normal cell physiology. First, protein synthesis is crucial in gene expression, because the majority of genes contain information for making proteins, which, in turn, perform the vast majority of cellular functions, eg, as enzymes. Second, recent data show that the major factor determining the cellular concentrations of specific proteins is the rate at which their messenger RNAs (mRNAs) are translated into protein (1). These rates vary by at least 3 orders of magnitude, implying that different mRNAs are translated with widely different efficiencies by the protein synthesis machinery of the cell. Third, protein synthesis is a demanding process for the cell; for example, it consumes ~30% of cellular energy in dividing mammalian cells (2).

Because they are the building blocks for making proteins, protein synthesis also requires an adequate supply of amino acids. Approximately half of the 20 different amino acids are essential components of the diet because mammals cannot synthesize them. Nonessential amino acids can be generated from other compounds. The lack of essential amino acids (EAAs) clearly poses a problem for mammalian cells; in this situation, they need to decrease rates of protein production.

The rate of protein synthesis is determined by the combination of the cell’s translational efficiency and its translational capacity. The latter refers to the cellular concentrations of the components required for protein synthesis, which include ribosomes, the molecular machines that assemble proteins, and translation factors. These are the proteins that are required to enable the ribosome to perform its function but are not intrinsic components of ribosomes. In the long term (hours, days), the cellular concentrations of ribosomes and translation factors are regulated by signaling through the mammalian target of rapamycin complex (mTORC) 1, which is described below.

Translational efficiency refers to the activity of the components of the translational machinery. As detailed below, the activity of translation factors in particular is subject to rapid regulation through changes in their states of phosphorylation. This allows cells to adjust their rate of protein synthesis to the prevailing conditions, eg, the availability of amino acids and energy (in the form of ATP), and in response to hormones and growth factors, which activate protein synthesis.

mTORC1 is a multiprotein complex that includes mammalian target of rapamycin (mTOR), a protein kinase, and raptor, a scaffolding protein that targets mTOR toward a subset of proteins as substrates (3). mTORC1 also interacts with Ras homolog enriched

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4 Address correspondence to CG Proud, Centre for Biological Sciences, University of Southampton, Southampton, SO17 1BJ, United Kingdom. E-mail: c.g.proud@soton.ac.uk.
5 Abbreviations used: EAA, essential amino acid; eEF2K, eukaryotic elongation factor 2 kinase; eIF, eukaryotic initiation factor; GATOR, guanosine 5′-triphosphatase–activating protein activity toward Rag-related guanosine 5′-triphosphatase; GCN2, general control nonrepressed 2 kinase; GEF, guanine nucleotide-exchange factor; GTPase; mTOR, mammalian target of rapamycin; mTORC, mammalian target of rapamycin complex; mTORC1, mammalian target of rapamycin complex 1; mTORC-KL, mammalian target of rapamycin kinase inhibitor; Pol, polymerase; Rag, Rag-related guanosine 5′-triphosphatase; Rheb, Ras homolog enriched in brain; RP, ribosomal protein; rRNA, ribosomal RNA; S6K, ribosomal protein S6 kinase; tRNA, transfer RNA; TSC, tuberous sclerosis complex; 4E-BP, eIF4E-binding protein; 5′-TOP, 5′-terminal oligopyrimidines.

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in brain (Rheb), a small GTPase, which, in its GTP-bound state, activates mTORC1. In the context of this article, it is relevant that signaling through mTORC1 is promoted by amino acids, especially by the branched-chain amino acid leucine, an EAA (4). mTORC1 signaling is also activated by insulin and other hormones and growth factors (Figure 1).

Hormones and growth factors such as insulin bring about the inactivation of the GTPase-activator protein for Rheb, the tuberous sclerosis complex (TSC) 1/2TSC2, thereby promoting the accumulation of Rheb-GTP and the activation of mTORC1 (3, 4). However, the control of mTORC1 by amino acids does not require TSC1/TSC2 (5). Instead, it involves Rag-related GTPases (Rags). Mammals have 4 Rag proteins, Rags A–D, which form heterodimers of Rag A or B plus either Rag C or D; activation of mTORC1 requires that Rags A/B are in their GTP-bound states, which is promoted by amino acids (reviewed in references 4 and 6). A protein complex termed “Ragulator” appears to act as a guanine nucleotide-exchange factor (GEF) for Rags A/B, thereby mediating their association with GTP. Rags localize to the cytoplasmic face of the lysosomal membrane, where Rheb is also found. When bound to GTP, Rags A/B interact with Raptor. In the current model, this mediates the recruitment of mTORC1 to where Rheb-GTP is located, facilitating the activation of mTORC1 (Figure 1). A remaining, and crucial, gap in our understanding of the control of mTORC1 by amino acids is the nature of the sensor that detects amino acid concentrations. Recent data suggest that mTORC1 is activated by amino acids within the lysosome (rather than the cytoplasm)—so-called inside-out signaling. Several other proteins, including protein and lipid kinases, the lysosomal (vacuolar) v-ATPase, and amino acid transporters, have also been implicated in the control of mTORC1 by amino acids (4). The v-ATPase is proposed to detect amino acids within the lumen of the lysosome and stimulate the GEF activity of Ragulator (Figure 1). Recent studies have identified a different multisubunit complex termed GTPase-activating protein activity toward Rags (GATOR) as interacting with the Rags (7). The GATOR subcomplex acts as a GTPase-activator protein for Rags A/B, thereby opposing the function of Ragulator.

Two recent studies have suggested roles here for the leucyl-transfer RNA (tRNA) synthetase and its equivalent in mammals or yeast by amino acids. However, the mechanisms by which this enzyme is proposed to regulate the Rags is radically different; in mammals, it is suggested to promote GTP hydrolysis by Rag D, whereas in yeast it is thought to block GTP hydrolysis by GTP-binding protein resemblance 1, the homolog of Rags A/B (reviewed in reference 4). Further work is clearly needed to resolve these discrepancies and to understand the contribution of these different components to the control of mTORC1 by amino acids. Perhaps distinct mechanisms account for its regulation by leucine and by other amino acids.

Rapamycin inhibits some, but not all, of the functions of mTORC1 (see, eg, references 8 and 9). Recent studies indicate that its differential effects may reflect the ability of rapamycin to impede phosphorylation of sites that are weak substrates for mTORC1 but not those that it phosphorylates efficiently (10). It does so by binding, together with FK506-binding protein of 12 kDa, to a region adjacent to the protein kinase domain of mTOR, but it does not directly inhibit its catalytic activity. This likely explains why rapamycin inhibits only the phosphorylation of some substrates of mTORC1, although a detailed explanation for this is lacking. The recently published crystal structure of mTOR provides some much-needed insights into this finding (11). Several compounds that do directly inhibit mTOR kinase activity are now available; these mTOR kinase inhibitors interfere with all known functions of mTORC1, as well as the other type of mTOR complex, mTORC2, which contains rictor, instead of raptor, has different substrates, and is not generally thought to be regulated by amino acids.

Several of the best-characterized mTORC1 substrates are involved in the control of the protein synthesis machinery. Ribosomal protein S6 kinases (S6Ks) 1 and 2 are activated by phosphorylation by mTORC1, and this is completely blocked by rapamycin. The S6Ks were initially discovered for their ability to phosphorylate S6, a component of the small, 40S, ribosomal subunit; however, the physiologic significance of its phosphorylation remains unclear. Nevertheless, it is now well established that S6Ks phosphorylate and regulate a number of other components of the translational machinery. These include eukaryotic initiation factor (eIF) 4B and programmed cell death protein 4, which regulate a helicase, eIF4A (12, 13). eIF4A is thought to help unwind inhibitory regions of secondary structure, which is found in the 5′-untranslated regions of some mRNAs, thereby facilitating their translation. S6Ks also phosphorylate and switch off eukaryotic elongation factor 2 kinase (eEF2K) (14). Because eEF2K phosphorylates and inactivates eukaryotic elongation factor 2, the protein that helps move the ribosome along the mRNA during translation, this link provides one way in which mTORC1 signaling can promote translation elongation and thus faster protein synthesis. It should be noted that mTORC1 signaling also promotes the phosphorylation of other sites in eEF2K (eg, Ser78 and Ser359), which inhibit eEF2K activity. However, mTORC1 has not been shown to directly

**FIGURE 1.** Control of mTORC1 by amino acids. Amino acids, probably from within the lysosome, and apparently via the v-ATPase, promote formation of Rags A/B–GTP, in which condition they can bind to raptor and recruit mTORC1 to the lysosomal surface where Rheb is located. In its GTP-bound state, Rheb can activate mTORC1. The Ragulator complex activates Rags A/B (by acting as their GEF), whereas GATOR opposes this by promoting GTP hydrolysis on Rags A/B. Signaling events that are stimulated by insulin and growth factors switch off the protein complex that hydrolyzes Rheb-bound GTP; GATOR, guanosine 5′-triphosphatase–activating protein activity toward Rag-related guanosine 5′-triphosphatase; GEF, guanine nucleotide-exchange factor; mTOR, mammalian target of rapamycin; mTORC1, mammalian target of rapamycin complex 1; Rheb, Ras homolog enriched in brain; S6K, ribosomal protein S6 kinase; TSC, tuberous sclerosis complex; v-ATPase, vacuolar ATPase; 4E-BP, eukaryotic initiation factor 4E–binding protein.
phosphorylate eEF2K (15) and the kinases that act on these sites remain to be identified.

The second small family of proteins that are substrates for mTORC1 and that regulate the translational machinery are the eIF4E-binding proteins (4E-BPs) (15), of which the best understood is 4E-BP1. eIF4E binds to the 5′-end of the mRNA (which includes 7-methylguanosine cap structure; Figure 2). By interacting with eIF4E, 4E-BPs prevent eIF4E from binding to the multidomain scaffold protein eIF4G. eIF4G itself has a number of protein partners, which include the following: eIF4A, the helicase mentioned above; the poly(A)-binding protein, which binds the other, 3′-end of the mRNA; and eIF3, a multisubunit protein complex (Figure 2). These interactions facilitate the translation of the mRNA by 1) circularizing the mRNA; 2) recruiting the 40S ribosomal subunits and associated proteins to the 5′-end of the mRNA, where it can start to move along the mRNA to seek the start codon (a process called “scanning”); and 3) recruiting eIF4A and other proteins that unwind secondary structure, as described above.

The assembly of the complex containing eIF4E, eIF4G, and their partners is thus considered a key element of the process of recruitment of ribosomes to the mRNA, which is termed translation initiation; importantly, it is promoted by mTORC1 in the following way. As mentioned, 4E-BPs prevent eIF4E from binding to eIF4G. 4E-BPs are phosphorylated on several serine or threonine residues by mTORC1, highly phosphorylated 4E-BP1 cannot bind to eIF4E, thus freeing eIF4E from inhibition and allowing it to bind to eIF4G (Figure 2). Although rapamycin does interfere with the phosphorylation of some of these sites in some types of cells, mTOR kinase inhibitors (mTOR-KIs) generally have a much stronger effect. Thus, mTOR-KIs generally affect the regulation of eIF4E (i.e., inhibit its availability) much more profoundly than does rapamycin. mTORC1 signaling can therefore promote both the initiation and elongation stages of mRNA translation. The impact of mTORC1 signaling on protein synthesis will be discussed further below.

**RIBOSOME BIOGENESIS**

The number of ribosomes in the cell clearly imposes an upper limit on the achievable rate of protein synthesis. Ribosomes consist of proteins (~80 in mammals) and RNA molecules (4 in mammals); it follows that the regulation of ribosome synthesis (“ribosomal biogenesis”) requires the coordinated production of all of these components, because incomplete ribosomes are not functional. In mammalian cells, all of the ribosomal proteins (RPs) are encoded by mRNAs that contain a 5′-terminal oligopyrimidines (5′-TOP), which confers on them control through mTORC1. Thus, the translation of 5′-TOP mRNAs is promoted by signaling downstream of mTORC1 (15), and therefore by amino acids. [Although amino acids may regulate 5′-TOP mRNA translation through additional mechanisms (16).] Although S6Ks had been implicated in linking mTORC1 signaling to the control of 5′-TOP mRNA translation, it is now clear that S6Ks are not required for the control of their translation, and nor is the phosphorylation of RP S6 (17, 18).

In addition to RPs, all translation elongation and several initiation factors are also encoded by 5′-TOP mRNAs (19). Thus, amino acids, and activation of mTORC1, will drive the concerted synthesis of many proteins required for the process of protein synthesis.

Ribosome biogenesis also requires the production of the 4 ribosomal RNAs (rRNAs; Figure 3). The 3 larger rRNAs (5.8S, 18S, and 28S in mammals) are made by RNA polymerase (Pol) I in the nucleolus as a single precursor that must be processed to create the mature rRNAs. Both Pol I–mediated transcription (20) and the processing of the pre-rRNAs (21) are positively controlled by mTORC1. In the former case, the S6Ks have been implicated in regulating Pol I, as has phosphorylation of the Pol I–associated transcription factor UBF (upstream binding factor) (20). Another Pol I transcription factor, TIF-IA, may also be controlled by TOR signaling. However, further work is needed to achieve a detailed understanding of the mechanisms by which Pol I and the processing of its product, the 47S rRNA precursor, are controlled by mTORC1 signaling. Recent studies have

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**FIGURE 2.** mTORC1 controls the formation of translation initiation complexes involving the mRNA cap-binding protein eIF4E by phosphorylating 4E-BP1 and by promoting its release from eIF4E (see text for further information). The mRNA is shown only schematically; the dotted gray lines indicate that the mRNA continues beyond this point. For simplicity, some components mentioned in the text are omitted. AAA, represents the 3′-polyadenyl tail of the mRNA; eIF, eukaryotic initiation factor; mRNA, messenger RNA; mTORC1, mammalian target of rapamycin complex 1; P, phosphate group; PABP, poly(A)-binding protein; 4E-BP, eIF4E-binding protein.

**FIGURE 3.** mTORC1 coordinates ribosome biogenesis. It does so by promoting the translation of 5′-TOP mRNAs (encoding RPs), the synthesis of rRNAs by Pol I and III, and the processing of pre-rRNA. Italic type indicates these mTORC1-activated processes. mRNA, messenger RNA; mTORC1, mammalian target of rapamycin complex 1; Pol, polymerase; r-protein, ribosomal protein; RNA, ribosomal RNA; 5′-TOP, 5′-terminal oligopyrimidines.
shown that S6Ks control the transcription of genes for many proteins involved in ribosome biogenesis (22).

mTORC1 also activates transcription of the fourth rRNA, the 5S, which is made by RNA Pol III (20) (Figure 3). This likely involves the phosphorylation of Maf1, a protein that inhibits Pol III (23, 24); its phosphorylation relieves the inhibition of Pol III, leading to increased production of 5S rRNA, and tRNAs, which are also made by Pol III. The 4 rRNAs and the 80 RPs are assembled into ribosomal particles in the nucleolus (Figure 3) in a complex process that requires numerous proteins and RNA molecules. Ribosomal particles must finally be exported into the cytoplasm to participate in translation.

Taken together, these mechanisms allow the amino acid–activated mTORC1 signaling pathway to promote the synthesis of RPs, rRNAs, and tRNAs, allowing the coordinated upregulation of the cellular components required for protein synthesis (Figure 3). Accelerated ribosome production (ribosome biogenesis) is likely important in conditions such as cancer (faster cell proliferation) and tissue hypertrophy (cell growth). Indeed, alterations in nucleolar morphology in cancer tissue were noted more than a century ago (25). It is important to note that mTORC1 signaling is activated in many cancer cells (eg, due to dysregulation of the pathways upstream of TSC1/2) and is implicated in cardiac hypertrophy, a potentially lethal disorder (26). Defects in ribosome production give rise to a range of disorders generally termed ribosomopathies (27).

Furthermore, because mTORC1 signaling is activated by amino acids, these mechanisms allow amino acid availability to promote the accumulation of components of the translational machinery, and thus the storage of amino acids as protein. Looked at another way, the requirement of mTORC1 signaling for amino acids provides a “check” whereby cells do not synthesize ribosomes, etc, at rates that exceed the availability of amino acids both to make ribosomes (~40% of whose mass is protein) and to use them to synthesize other proteins.

**REGULATION OF eIF2 BY AMINO ACIDS**
eIF2 mediates the recruitment of the initiator methionyl-tRNA to the 40S ribosomal subunit. This tRNA recognizes the initiator (start) codon in the mRNA and needs to be bound to the 40S ribosomal subunit to allow it to locate the start codon during the scanning process described above. eIF2 is active only when it is bound to GTP; the GTP is hydrolyzed during the initiation of translation, and thus eIF2 leaves the ribosome as the inactive eIF2-GDP species (Figure 4). The “recycling” of eIF2-GDP back to active eIF2-GTP involves the exchange of GDP for GTP, a slow process that is accelerated by another translation factor, eIF2B, which acts as a GEF. eIF2B is made up of 5 different subunits, of which the largest, eIF2Be, contains the catalytic domain (28). The recycling of eIF2 by eIF2B is critical for the initiation of translation and for its control.

Amino acids regulate eIF2/eIF2B in 2 ways, both of which operate such that insufficiency of amino acids leads to inhibition of their activities, causing protein synthesis to slow down, thereby conserving amino acids. In the first, well-characterized, mechanism, eIF2 is phosphorylated on Ser51 in its α-subunit (eIF2α) by a protein kinase termed general control nonrepressed 2 (GCN2), which is activated when amino acid concentrations are low. GCN2 contains a domain that resembles many amino acyl-tRNA synthetase enzymes, the enzymes that attach specific amino acids to the correct tRNAs, a process termed “charging.” When a specific amino acid is missing, there is an accumulation of “uncharged” tRNAs for that amino acid. These are thought to be recognized by GCN2, via the amino acyl-tRNA synthetase domain, activating it; the resulting phosphorylation of eIF2 and the inhibition of eIF2B result in the inhibition of protein synthesis (29). This is clearly a logical response to inadequate amino acid concentrations. One would expect it to be triggered by a deficiency of any EAA. It is interesting to note that GCN2 is needed for mice to distinguish between diets with a balanced or imbalanced amino acid content (30).

The activity of eIF2B can also be regulated more directly by amino acids. The removal of amino acids from the culture medium leads to the phosphorylation of eIF2Be at a site (Ser525) adjacent to its catalytic domain (31). This inhibits eIF2B’s activity. It remains unclear how amino acids regulate the phosphorylation of Ser525, eg, which kinase is involved; however, this does not involve known amino acid signaling mechanisms that impinge on protein synthesis, ie, mTORC1 and GCN2.

**CONTROL OF THE SYNTHESIS OF SPECIFIC PROTEINS BY AMINO ACIDS**
The discussion so far has mainly centered on mechanisms by which the overall translational machinery can be controlled by amino acids, the main exception being that mTORC1, and thus amino acids, promote the translation of 5′-TOP mRNAs. This effect is a increased recruitment of ribosomes onto such mRNAs, ie, regulation of the initiation of their translation.

Given the importance of amino acid availability for cell and animal physiology, it is relevant to ask whether amino acids regulate the translation of other mRNAs in addition to the known 5′-TOP mRNAs [most of which, as mentioned above, encode RPs or translation factors (19)]. Three recent studies have shed light on this. These studies used 2 new, but distinct, methods for studying the translation of specific mRNAs or the synthesis of specific proteins. Two used a technique termed “ribosome profiling,” first developed by Ingolia et al (32), in which next-generation sequencing is used to identify those parts of mRNAs that are associated with ribosomes [by virtue of the fact that such
regions are protected from nuclease digestion and can therefore be recovered, sequenced, and identified (33, 34). The third study used a mass spectrometric approach whereby cells are incubated in medium containing lysine and arginine in which several carbon or nitrogen atoms have been replaced with heavy isotopes \((^{13}\text{C}, ^{15}\text{N})\) (1, 35). Thus, newly synthesized proteins are tagged with heavy lysine or arginine, and peptides derived from them can be identified and quantitated by mass spectrometric methods. If a given signaling inhibitor such as rapamycin impairs the synthesis of the parent protein from which a given peptide is derived, the magnitude of the corresponding peak in the mass spectrum is decreased; this informs on the effects of specific signaling pathways on the spectrum of proteins being made.

These studies generated large data sets, especially the ribosome-profiling experiments. Several key points emerged from these studies. First, inhibiting mTORC1 with rapamycin impairs the synthesis of many proteins, including a large number that are not encoded by known 5′-TOP mRNAs. Second, mTOR-KIs inhibit the synthesis of these proteins (or the polysomal association of the corresponding mRNAs) much more strongly than rapamycin. This could reflect a role for mTORC2, but it is notable that the effects of inhibiting mTORC1 activity on general protein synthesis are similar in wild-type and rictor-knockout cells (which do not contain mTORC2) (8). Thus, given that amino acid starvation and mTOR-KIs inhibit (as far as we know) all functions of mTORC1, the impact of mTORC1s on mRNA translation may be similar to that of amino acid depletions. Third, many of the mRNAs whose translation is strongly inhibited by mTOR-KIs (and to a lesser extent by rapamycin) appear to contain a 5′-TOP or similar sequence. Some may contain a second regulatory element (33). Fourth, there is evidence that the strong inhibition of the translation of certain mRNAs by mTOR-KIs reflects dephosphorylation of 4E-BPs, and the consequent inhibition of eIF4E. However, not all of the data are consistent with this conclusion (35), and it is not clear why the inhibition of eIF4E by 4E-BPs has such a strong effect on translation of 5′-TOP mRNAs. Fifth, and perhaps most important, many of the proteins whose synthesis is tightly controlled in this way are, like those encoded by the “classical” 5′-TOP mRNAs, involved in anabolic processes. They include enzymes involved in intermediary and anabolic metabolism, proteins involved in protein or RNA metabolism, and proteins involved in cell migration. This implies that amino acids promote the synthesis of these sets of proteins. It should be noted that the control of 5′-TOP mRNA translation by amino acids may involve other components (eg, RNA-binding proteins) in addition to mTORC1 (16).

It is clear that amino acid–activated mTORC1 signaling drives ribosome production, the synthesis of specific proteins, and the activation of translation factors. mTORC1 signaling also regulates the transcription of many genes (36, 37). Many of the mRNAs whose translation is promoted by mTORC1 are also transcriptionally regulated by this pathway. The effect of mTORC1 signaling on their translation provides a rapid way of increasing the synthesis of the corresponding proteins, before the slower-acting effects of mTORC1 on the concentrations of these mRNAs.

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