MINIREVIEW

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THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 285, NO. 38, pp. 29027–29032, September 17, 2010

Control of Translation Initiation through Integration of Signals Generated by Hormones, Nutrients, and Exercise*

Published, JBC Papers in Press, June 24, 2010, DOI 10.1074/jbc.R110.137208

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Control of translation initiation in a tissue of an intact mammalian organism is a highly complex process requiring the continuous integration of multiple positive and negative stimuli. For a tissue such as skeletal muscle, which has the capacity to undergo dramatic changes in size and protein content, translation initiation contributes importantly to the regulation of global rates of protein synthesis and is controlled by numerous stimuli, including those arising from nutrients and hormones in the circulating blood, as well as from contraction-induced signaling within the tissue. Many of the pathways conveying signals generated by these stimuli converge on mTORC1, a serine-threonine protein kinase that has been termed the nutrient and energy sensor of the cell and that plays a prominent role in the regulation of cell growth. Control of translation initiation by mTORC1 is mediated through phosphorylation of downstream targets that modulate the binding of mRNA to the 43 S preinitiation complex. Control of translation initiation is also mediated through modulation of binding of initiator methionyl-tRNA to the 40 S ribosomal subunit. Together, modulation of these two regulatory steps in translation initiation accounts in large part for changes in protein synthesis in skeletal muscle produced by the integration of inputs from hormones, nutrients, and exercise.

Previous articles in this Thematic Minireview Series on Protein Synthesis have laid the groundwork for understanding the molecular mechanisms involved in translation initiation, particularly with regard to eukaryotic organisms. Our task in this minireview is to describe the role of translation initiation in the control of protein synthesis in an intact mammalian system wherein the maintenance of homeostasis is of utmost importance. We have chosen skeletal muscle as our focus because of its unique plasticity with regard to a capacity to increase (hypertrophy) or decrease (atrophy) in size and thus protein content in response to a variety of hormonal, nutritional, and mechanical stimuli. Skeletal muscle hypertrophy and atrophy result from a homeostatic shift favoring either protein synthesis or protein degradation, respectively, and modulation of translation initiation contributes importantly to the changes in protein synthesis associated with both conditions. Moreover, skeletal muscle is a principal contributor to whole body substrate metabolism. Representing the major "reservoir" of protein in the body, it becomes a crucial source of amino acids that serve as substrates for gluconeogenesis in the liver, thus allowing for the maintenance of blood glucose concentrations during times of food deprivation.

One approach that has been used to assess changes in translation initiation in muscle is analysis of the distribution of ribosomal subunits in polysomes compared with those that are free (*i.e.* nonpolysomal), in combination with a measurement of the global rate of protein synthesis. An increase in protein synthesis in association with a shift of ribosomal subunits into polysomes indicates a stimulation of translation initiation. Conversely, loss of polysomes and accumulation of ribosomal subunits in association with a decrease in protein synthesis indicate an impairment of translation initiation. These analyses are often performed over a relatively short time frame and thus reflect acute changes in translation initiation rather than long-term ones. Analysis of acute responses has the advantage of revealing rapid changes in translation initiation factor function, *e.g.* through covalent modification of initiation factors by phosphorylation and/or protein-protein interaction profiles of the relevant initiation factors and regulatory proteins, in contrast to alterations in their expression. The results of these analyses allow for localization of changes in initiation to the two generally accepted regulatory processes (see the minireview by Merrick (68) in this thematic series), *i.e.* assembly of the 43 S preinitiation complex through binding of the Met-tRNA$_i^{Met}$·eIF2·GTP ternary complex to the 40 S ribosomal subunit and assembly of the 48 S preinitiation complex through binding of the mRNA to the 43 S preinitiation complex.

A prominent signaling pathway that controls the regulatory process wherein Met-tRNA$_i^{Met}$ joins the 40 S ribosomal subunit is represented by four separate stress-activated protein kinases that mediate phosphorylation of serine 51 on the α-subunit of eIF2 (Fig. 1). A variety of stresses, including nutrient deprivation, oxidative stress, heme deficiency, and double-stranded RNA, lead to activation of one or more of these eIF2α kinases. Phosphorylation of eIF2α converts it from a substrate into a competitive inhibitor of eIF2B, resulting in an accumulation of the eIF2·GDP binary complex that is inactive in assembly of the ternary complex (Fig. 1, *step 10*).

A prominent signaling pathway that controls the regulatory process wherein the mRNA binds with the 43 S preinitiation complex involves mTORC1 (mammalian target of rapamycin complex 1), which phosphorylates the eIF4E-binding protein, 4E-BP1 (Fig. 2, *step 15*), preventing its association with eIF4E and thereby permitting eIF4E to associate with eIF4G to form the active mRNA cap-binding complex, eIF4F (Fig. 1, *step 9*). In addition, mTORC1 phosphorylates and activates the protein kinase S6K1 (Fig. 2, *step 11*). S6K1 subsequently activates the mRNA cap binding step by phosphorylating eIF4B (Fig. 2, *step 12*) and PCDC4 (*step 13*), which, in its unphosphorylated state, binds to eIF4A and

* This work was supported, in whole or in part, by National Institutes of Health Grant DK-15658. This is the fourth article in the Thematic Minireview Series on Protein Synthesis. This minireview will be reprinted in the 2010 Minireview Compendium, which will be available in January, 2011.

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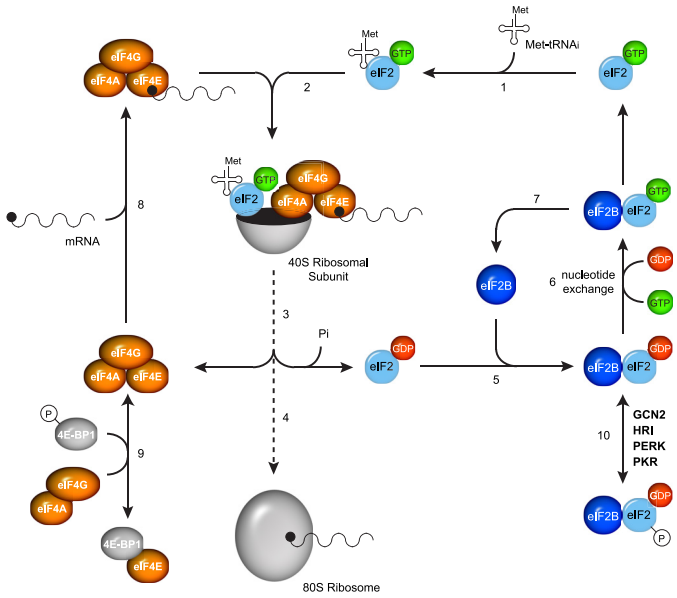

FIGURE 1. **Met-tRNA$_i^{Met}$ binding step in translation initiation.** The eIF2·GTP complex binds to Met-tRNA$_i^{Met}$ to form a ternary complex (*step 1*) that associates with the 40 S ribosomal subunit (*step 2*). During a late step in initiation, the GTP bound to eIF2 is hydrolyzed, and eIF2·GDP is released (*step 3*). A guanine nucleotide exchange factor, eIF2B, mediates exchange of GDP bound to eIF2 for GTP (*steps 5–7*), permitting reassembly of the ternary complex. Phosphorylation of serine 51 on the α-subunit of eIF2 by any of four known kinases leads to sequestration of eIF2B into an inactive complex (*step 10*), repressing the translation of most mRNAs but stimulating the translation of a selected group of mRNAs, such as the one encoding the transcription factor ATF4. GCN2 is activated when it binds to deacylated tRNA; HRI is activated both by heme deficiency and under conditions of heat shock and oxidative stress; PERK is activated in response to the accumulation of misfolded proteins in the lumen of the endoplasmic reticulum; and PKR is activated by double-stranded RNA. The binding of mRNA to the 40 S ribosomal subunit is mediated by the eIF4F complex, consisting of eIF4A, eIF4E, and eIF4G (*step 8*). Assembly of the eIF4F complex is regulated in part through the binding of eIF4E to 4E-BP1 (*step 9*).

eIF4G (*step 14*), thereby inhibiting the mRNA binding step. Thus, mTORC1 stimulates mRNA joining to the 43 S preinitiation complex through multiple downstream effectors.

Here, we summarize the evidence in support of roles for the Met-tRNA$_i^{Met}$ and mRNA binding steps in mediating the regulatory effects of amino acids (particularly leucine), insulin, glucocorticoids, and resistance exercise on translation initiation in skeletal muscle.

Amino Acids and Control of mRNA Translation

Early studies in rodents (1–3) and humans (4) showed that, in the fasted state, refeeding stimulates protein synthesis in skeletal muscle. Subsequent studies using isolated muscle preparations and perfused hind limb preparations demonstrated that, in large part, the feeding-induced stimulation of protein synthesis is mimicked by provision of amino acids and, in particular, by the branched-chain amino acid leucine (5, 6), which acts to stimulate translation initiation. Later studies using muscle cells in culture showed that deprivation of either leucine or histidine leads to repression of the Met-tRNA$_i^{Met}$ binding step through an increase in eIF2α phosphorylation and inhibition of eIF2B activity (Fig. 1, *step 10*) (7). However, only leucine deprivation results in repression of mTORC1, leading to impairment in the mRNA binding step (7). Thus, deprivation of any essential amino acid leads to eIF2α phosphorylation and inhibition of eIF2B activity, but mTORC1 signaling and the mRNA binding step are specifically repressed by leucine deprivation. Addition of insulin to leucine-deprived muscle cells has no effect on global rates of protein synthesis, eIF2α phosphorylation, or eIF2B activity but restores mTORC1 signaling to the level observed in cells maintained in complete medium. These results suggest that, under conditions in which the Met-tRNA$_i^{Met}$ binding step is inhibited, stimulation of the mRNA binding step through activation of mTORC1 has no global effect on protein synthesis. Instead, when eIF2B activity is rate-limiting, mTORC1 activation may promote translation of a subset of mRNAs (8).

It is curious that, in perfused rat hind limb preparations, the stimulatory effect of amino acids on mTORC1 signaling in muscle is absent. Thus, increasing amino acids from the concentration that is observed in a fasted animal (referred to hereafter as 1×) to 10 times (10×) that amount leads to increased rates of protein synthesis but has no effect on mTORC1 signaling, as assessed by 4E-BP1 or S6K1 phosphorylation, or on the association of 4E-BP1 with eIF4E (9). Similarly, in the presence of 1× amino acid concentrations, increasing leucine alone by 10-fold stimulates protein synthesis but has no effect on mTORC1 signaling (10). However, assembly of the eIF4E·eIF4G complex is increased in muscle perfused either with a complete mixture of amino acids at 10× concentrations or with a 1× amino acid mixture containing 10× leucine, and the effect is associated with increased phosphorylation of eIF4G. Together, the results of these studies demonstrate that, *in vitro*, increasing amino acids above fasting levels does not activate mTORC1 in skeletal muscle, but amino acids instead act in an mTORC1-independent manner to promote eIF4F complex assembly (possibly through a mechanism involving phosphorylation of eIF4G) and to increase protein synthesis.

Why do amino acids fail to activate mTORC1 in perfused muscle preparations? A likely explanation is that amino acid signaling to mTORC1 requires co-stimulation with insulin. For example, the leucine-induced stimulation of protein synthesis and mTORC1 signaling are blunted in skeletal muscle of severely diabetic rats (11). However, the concentration of insulin required for leucine to activate mTORC1 in muscle is likely to be low because oral administration of leucine in rats treated with somatostatin to maintain insulin concentrations at fasting levels leads to activation of mTORC1 (12). Thus, even fasting concentrations of insulin are sufficient for leucine-stimulated mTORC1 activation in muscle. Although the mechanism involved in the permissive effect of insulin on amino acid-induced mTORC1 activation is incompletely defined, it may be explained by insulin acting through the GTPase activator protein TSC1/2 to increase Rheb GTP loading (Fig. 2, *step 7*), a critical step in mTORC1 activation, whereas amino acids act downstream of TSC1/2, possibly through the RagA/B·RagC/D complex (Fig. 2, *step 9*) (13), with both TSC1/2-dependent and TSC1/2-independent inputs being required for optimal mTORC1 signaling.

An unanswered question is whether leucine, or a product of its metabolism, is involved in mTORC1 activation. Studies in primary cultures of adipocytes have shown that α-ketoisocap-

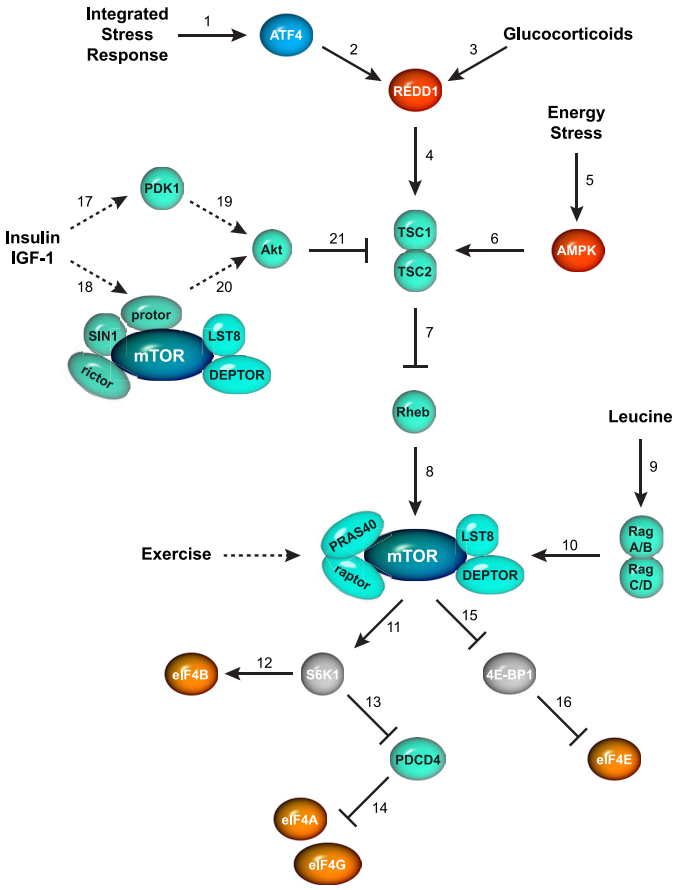

FIGURE 2. **Various signaling pathways converge on mTORC1 to mediate control of the mRNA binding step in translation initiation in skeletal muscle.** mTORC1 acts as a nexus for numerous signaling pathways, including both positive inputs, *e.g.* amino acids, exercise, and insulin/insulin-like growth factor-1 (*IGF-1*), and negative inputs, *e.g.* glucocorticoids, energy stress, and the integrated stress response. Activation of mTORC1 impinges on at least three proteins involved in the mRNA binding step, eIF4A (*steps 13* and *14*), eIF4B (*step 12*), and eIF4E (*step 16*), although future studies may reveal as yet unknown targets involved in mTORC1-mediated control of translation initiation and/or elongation.

roate (KIC),[2] generated by the branched-chain amino acid aminotransferase BCAT2 during the initial step in leucine metabolism, is as potent as leucine in stimulating mTORC1 (*e.g.* Ref. 14). However, the reaction catalyzed by BCAT2 is reversible, and therefore, conversion of KIC to leucine may account for increased mTORC1 signaling after KIC treatment. To circumvent this possibility, a recent study (15) assessed mTORC1 signaling in mice lacking BCAT2. In such mice, the increase in 4E-BP1 and S6K1 phosphorylation associated with refeeding fasted animals is magnified in skeletal muscle of wild-type compared with knock-out animals, suggesting that leucine, not KIC, promotes mTORC1 signaling. Interestingly, in the fasted state, phosphorylation of 4E-BP1 and S6K1 is the same in skeletal muscle of wild-type and knock-out animals, even though plasma leucine concentrations are elevated by 14-fold in knock-out compared with wild-type mice. In part, the lack of leucine-induced mTORC1 signaling in muscle of fasted knock-out animals may be a consequence of the plasma insulin concentration being only 35% of that in fasted wild-type mice. Even though plasma insulin concentrations are exceptionally low, the basal rates of protein synthesis are significantly increased in muscle of knock-out compared with wild-type animals. Whether or not the increase is a consequence of leucine-induced phosphorylation of eIF4G and increased assembly of the eIF4E·eIF4G complex is unknown.

Assuming that leucine specifically mediates activation of mTORC1, it might be expected that the molecule possesses a unique structural feature(s) that allows it to interact with mTORC1 or an upstream regulatory protein. Thus, the side chain of the amino acid (and in particular, the branch point of the side chain) might play an important role in mediating its signaling function. However, norleucine, an amino acid with a side chain containing the same number of carbons as leucine but in linear format, is as potent as leucine in activating mTORC1 in skeletal muscle (14, 16). Other studies examining the structural requirements for leucine in mediating mTORC1 activation have yielded inconsistent results (17, 18).

Insulin and Control of mRNA Translation

Evidence suggests that insulin regulates a number of steps in the pathway of protein synthesis (Fig. 2, *steps 17–20*), although the exact biochemical and molecular mechanisms whereby the hormone mediates this control remain to be completely defined. The first evidence of a role for insulin in the control of mRNA translation comes from studies using isolated perfused preparations of rat heart (19) and skeletal muscle (20). The conclusion drawn from these studies is that insulin acts to stimulate translation initiation. A similar conclusion comes from studies with diabetic rats in which a defect in translation initiation is observed (21). The mechanism suggested initially to explain this action of insulin is that it acts to stimulate assembly of the 43 S preinitiation complex (22). Further studies with diabetic rats suggested that insulin acts to control the activity of eIF2B (23, 24) through a mechanism not involving changes in phosphorylation of eIF2α (25).

As noted above, a prominent site in mediating the action of insulin in the control of translation initiation in skeletal muscle is mTORC1 and the subsequent assembly of the 48 S preinitiation complex. An initial study showed that insulin and diabetes cause reciprocal changes in the association of eIF4E and 4E-BP1 in rat skeletal muscle *in vivo* (26). A subsequent study using an isolated perfused preparation of rat skeletal muscle showed that insulin acts directly to enhance association of eIF4E with eIF4G in conjunction with stimulation of protein synthesis (27). A conclusion drawn from that study is that the enhanced association of eIF4E with eIF4G is mediated in part through phosphorylation of 4E-BP1, resulting in release of eIF4E from the inactive 4E-BP1·eIF4E complex (Fig. 1, *step 9*). Studies designed to identify the signaling pathway(s) through which insulin acts to control assembly of the eIF4F complex in skeletal muscle show that it stimulates phosphorylation of 4E-BP1 as well as dissociation of the 4E-BP1·eIF4E complex and that these effects are blocked by rapamycin, thus implicating mTORC1 in mediating the effects of the hormone.

In contrast to the consistent findings with animal models, a number of studies have failed to demonstrate an effect of insu-

[2] The abbreviations used are: KIC, α-ketoisocaproate; caAkt, constitutively active Akt; EGFP, enhanced green fluorescent protein.

lin on protein synthesis in human skeletal muscle. A potential explanation for the different response in humans compared with animals is the dramatic effect the hormone has on lowering plasma amino acid levels. To address this possibility, a recent study (28) compared increasing insulin alone while maintaining amino acids and glucose at fasted levels with a condition in which insulin, amino acids, and glucose were all raised to the level observed in fed individuals. In that study, raising all three effectors led to stimulation of protein synthesis, activation of mTORC1 as evidenced by increased phosphorylation of 4E-BP1 and S6K1, and decreased association of 4E-BP1 with eIF4E compared with subjects in which only insulin was increased.

Glucocorticoids and Control of mRNA Translation

Glucocorticoid hormones are known to have a general action on protein metabolism in skeletal muscle opposite that of insulin, producing a catabolic rather than an anabolic response. The overall effects of excess amounts of glucocorticoids, whether from endogenous (*e.g.* Cushing syndrome) or exogenous sources, are widely recognized. Administration of exogenous glucocorticoids has been shown to cause loss of body weight (29, 30), marked atrophy of certain skeletal muscles (29–33), and a reduction in rates of protein synthesis in skeletal muscle as measured in the intact animal (34), perfused hind limb preparations (32, 35), isolated muscle preparations (36, 37), and cultures of L6 myoblasts (38). Administration of the potent glucocorticoid analog dexamethasone produces within 4 h a maximal reduction in protein synthesis in skeletal muscle *in vivo* (39) and in L6 myoblasts (38).

From initial attempts to identify the mechanism(s) responsible for the reduction in protein synthesis came the conclusion that glucocorticoids act to repress initiation of mRNA translation (35) and that this effect involves assembly of the eIF4F complex and not a change in eIF2α phosphorylation or eIF2B activity (39). The effect on assembly of the eIF4F complex is associated with down-regulated phosphorylation of 4E-BP1 and S6K1 (Fig. 2, *steps 11* and *15*) (39, 40), which correlates temporally with the reduction in protein synthesis in skeletal muscle *in vivo* (39) and L6 myoblasts (38) following administration of dexamethasone.

The dexamethasone-induced reduction in 4E-BP1 and S6K1 phosphorylation is attenuated both by inhibitors of glucocorticoid receptor function and by inhibitors of DNA transcription and mRNA translation (41). Moreover, the glucocorticoid receptor is both necessary and sufficient for the dexamethasone-induced dephosphorylation of S6K1 (41). Mutational analysis of the glucocorticoid receptor reveals that the DNA binding and transcriptional activation functions, but not the transcriptional repression function, of the receptor are required for S6K1 regulation (41). Taken together, these studies demonstrate that glucocorticoids induce transcription of a gene encoding a protein that acts to reduce phosphorylation of 4E-BP1 and S6K1.

Given that 4E-BP1 and S6K1 are downstream targets of mTORC1, it had been considered likely that the protein induced by glucocorticoids would act to repress signaling through this pathway. The timely identification of two novel repressors of mTORC1 signaling, *i.e.* proteins referred to as REDD1 and REDD2 (regulated in development and DNA damage responses) (Fig. 2, *step 4*), has provided an opportunity to assess their role in mediating the effects of glucocorticoids on protein synthesis and mRNA translation (42–46). Studies show that REDD1, but not REDD2, mRNA expression is dramatically induced following acute dexamethasone treatment both in rat skeletal muscle *in vivo* and in L6 myoblasts in culture (40). In L6 myoblasts, the effect of the drug on mTORC1 signaling is efficiently blunted in the presence of REDD1 RNA interference oligonucleotides. Moreover, the dexamethasone-induced assembly of the mTORC1 regulatory complex TSC1/2 is disrupted in L6 myoblasts following siRNA-mediated repression of REDD1 expression. Finally, overexpression of Rheb (Fig. 2, *step 8*) reverses the effect of dexamethasone on phosphorylation of mTORC1 substrates. Overall, the data lead to the conclusion that REDD1 functions upstream of TSC2 and Rheb to down-regulate mTORC1 signaling in response to dexamethasone.

Subsequent studies show that changes in mTORC1 signaling are inversely proportional to alterations in expression of REDD1 and that REDD1 is rapidly degraded with a half-life estimated to be 10 min or less (47). In addition, changes in REDD1 in skeletal muscle in response to fasting and refeeding (48) and in REDD2 in skeletal muscle following resistance exercise (49) correlate with altered mTORC1 signaling. Thus, this mTORC1 repressor is likely to play an important role in the control of translation initiation in skeletal muscle under a number of physiological and pathophysiological conditions.

Resistance Exercise and Control of mRNA Translation

The preponderance of evidence shows that resistance exercise induces skeletal muscle hypertrophy by enhancing protein synthesis (50). The first evidence that translation initiation is involved in mediating the exercise-induced stimulation of muscle protein synthesis comes from the work of Baar and Esser (51), who examined the distribution of ribosomal subunits between polysomal and nonpolysomal fractions after electrical stimulation of rat hind limb muscles. They showed that, in the extensor digitorum longus muscle, but not the soleus muscle, translation initiation is enhanced under these conditions. This conclusion has been confirmed by Kubica *et al.* (52), who showed an increase in polysome aggregation in the gastrocnemius muscle of rats subjected to an acute resistance exercise protocol.

A study by Bodine *et al.* (53) was the first to provide convincing data supporting a direct role for the mTORC1 signaling pathway in controlling translation initiation and promoting skeletal muscle hypertrophy in response to exercise. The study shows that chronic overloading of the plantaris muscle (through synergistic ablation of the soleus and gastrocnemius muscles) leads to increased phosphorylation of Akt and proteins downstream of mTORC1, such as 4E-BP1 and S6K1. The central role of mTORC1 in mediating the hypertrophic response under these loading conditions is verified through *in vivo* treatment with rapamycin, which completely blocks the muscle hypertrophy associated with synergistic ablation (53, 54) and the exercise-induced increase in protein synthesis and mTORC1 signaling in humans (55). In other studies,

phosphorylation of both 4E-BP1 and S6K1 has been shown to be enhanced in response to muscle loading and an acute bout of resistance exercise in rodents and in humans and to positively contribute to the up-regulation of select gene expression patterns necessary to elicit long-term increases in skeletal muscle accretion (55–57). Further evidence of a role for Akt in mediating these effects on mTORC1 signaling is provided by the finding that phosphorylation of Ser^{2448} on mTOR, an Akt site, is increased in skeletal muscle in response to ablation-induced overload (58–60) or an acute bout of resistance exercise (61, 62). Direct evidence linking Akt to muscle hypertrophy is provided by studies wherein a constitutively active form of the kinase (caAkt) was exogenously expressed in rat skeletal muscle. In tibialis anterior muscle expressing a hybrid protein consisting of caAkt linked to enhanced green fluorescent protein (EGFP), muscle size is increased by 1.6-fold compared with muscle expressing EGFP alone (53). Moreover, expression of caAkt-EGFP significantly reduces muscle atrophy induced by denervation (53) or injury (63).

In the acute resistance exercise model, the increases in phosphorylation of Akt, mTOR, 4E-BP1, and S6K1 in the gastrocnemius muscle are accompanied by enhanced activity of eIF2B, which correlates temporally with the stimulation of protein synthesis (64). In this model, the relative expression of the catalytic subunit of eIF2B, *i.e.* eIF2Bϵ, is increased in the gastrocnemius muscle by >2-fold 3 h after exercise, and this increase is sustained for 48 h (65). An increase in the relative abundance of the mRNA for eIF2Bϵ is not observed until 48 h post-exercise. In a subsequent study, it was shown that the increase in the relative expression of the eIF2Bϵ protein 16 h post-exercise is mediated by up-regulated translation of its mRNA (52). Moreover, this effect, as well as the exercise-induced stimulation of protein synthesis, is prevented by pretreatment of rats with rapamycin, demonstrating that mTORC1 signaling is necessary for both. Finally, using a cell culture model in which Rheb is overexpressed exogenously, it was shown that activation of mTORC1 is both necessary and sufficient to stimulate eIF2Bϵ mRNA translation and protein synthesis (66). Additional studies in support of a role for the eIF2 ternary complex binding step in skeletal muscle protein synthesis include a report by Hwee and Bodine (67) showing increased expression of eIF2Bϵ following ablation-induced hypertrophy, a response that wanes as a result of aging.

Conclusions and Future Directions

A major challenge in designing experiments and interpreting results from *in vivo* studies is the complex response of an organism to a seemingly simple physiological perturbation. For example, protein synthesis in skeletal muscle is regulated by an array of anabolic and catabolic stimuli that function in a coordinated manner to modulate translation initiation. Consequently, *in vivo*, the response to a particular perturbation represents an integration of a variety of positive (*e.g.* amino acids, insulin) and negative (*e.g.* glucocorticoids) inputs, as well as modulation by neural signaling. Notably, the plasticity exhibited by muscle is much less apparent in other tissues, and therefore, although the overall pathway depicted in Fig. 2 is likely to be similar, the upstream signals are likely to differ. Delineating the complex interactions among the various modulatory signals in the control of translation initiation not only in muscle, but in other tissues, needs to be an important goal for future studies. Such knowledge is critical for designing studies to assess, for example, the attenuated response of protein synthesis to anabolic stimulation in catabolic conditions such as sarcopenia, cachexia, sepsis, and aging. Based on the complex response elicited by many perturbations, it may seem surprising that so many studies have focused on the role of a single protein kinase, mTOR, in the control of translation initiation in skeletal muscle. However, mTOR as part of the mTORC1 complex is a key regulator of muscle hypertrophy and integrates signals from a variety of pathways to regulate the function of several proteins involved in the control of translation initiation, as well as translation elongation factor 2. The relative contribution of changes in initiation compared with elongation in modulating muscle protein synthesis is largely unexplored. Moreover, mTORC1 is not the only mechanism through which the control of translation initiation is mediated in skeletal muscle. For example, the mechanism through which amino acids act to promote eIF4G phosphorylation and assembly of the eIF4E·eIF4G complex in the absence of insulin is unknown. Further investigation of mTORC1-independent mechanisms in the control of translation initiation *in vivo* is sorely needed. Finally, the majority of *in vivo* studies have assessed changes in global protein synthesis. Few studies have examined changes in the translation of mRNAs encoding specific proteins or subsets of proteins or the mechanisms that mediate such regulation.

Acknowledgments—We thank Alexander Tuckow, Jeremiah Winter, and Drs. Michael Dennis and Rudolf Schilder for critical reading of the manuscript.

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