Resistance Exercise Increases Muscle Protein Synthesis and Translation of Eukaryotic Initiation Factor 2Bε mRNA in a Mammalian Target of Rapamycin-dependent Manner*

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The contribution of mammalian target of rapamycin (mTOR) signaling to the resistance exercise-induced stimulation of skeletal muscle protein synthesis was assessed by administering rapamycin to Sprague-Dawley rats 2 h prior to a bout of resistance exercise. Animals were sacrificed 16 h postexercise, and gastrocnemius protein synthesis, mTOR signaling, and biomarkers of translation initiation were assessed. Exercise stimulated the rate of protein synthesis; however, this effect was prevented by pretreatment with rapamycin. The stimulation of protein synthesis was mediated by an increase in translation initiation, since exercise caused an increase in polysome aggregation that was abrogated by rapamycin administration. Taken together, the data suggest that the effect of rapamycin was not mediated by reduced phosphorylation of eukaryotic initiation factor 4E (eIF4E) binding protein 1 (BP1), because exercise did not cause a significant change in 4E-BP1(Thr-70) phosphorylation, 4E-BP1-eIF4E association, or eIF4F complex assembly concomitant with increased protein synthetic rates. Alternatively, there was a rapamycin-sensitive decrease in relative eIF2Bε(Ser-535) phosphorylation that was explained by a significant increase in the expression of eIF2Bε protein. The proportion of eIF2Bε mRNA in polysomes was increased following exercise, an effect that was prevented by rapamycin treatment, suggesting that the increase in eIF2Bε protein expression was mediated by an mTOR-dependent increase in translation of the mRNA encoding the protein. The increase in eIF2Bε mRNA translation and protein abundance occurred independent of similar changes in other eIF2B subunits. These data suggest a novel link between mTOR signaling and eIF2Bε mRNA translation that could contribute to the stimulation of protein synthesis following acute resistance exercise.

Understanding the molecular basis of muscle hypertrophy is critical to the development of targets for exercise, nutritional, and pharmaceutical intervention in muscle wasting conditions such as sarcopenia, cachexia, diabetes, exposure to microgravity, and extended bed rest. Regulation of muscle hypertrophy has recently been the subject of intense investigation (1). In particular, there has been a great deal of attention focused on the role of signaling through the mammalian target of rapamycin (mTOR)1 protein kinase in the hypertrophic response following load-bearing contractile activity. Several models of resistance exercise, muscle contraction, and/or muscle loading suggest that mTOR signaling is activated in the recovery period following these perturbations (2–5). At this time, the upstream regulators of mTOR following resistance exercise are not clearly defined; however, there is convincing evidence that protein kinase B (PKB/Akt) signaling to mTOR has an effect on muscle hypertrophy. Overexpression of a constitutively active form of PKB in vivo is sufficient to stimulate muscle hypertrophy and rescue muscle atrophy induced by denervation (6). The effect on muscle hypertrophy has recently been confirmed in transgenic mice in which constitutively active PKB was inductively expressed (7). The effect of PKB on muscle hypertrophy has been suggested to signal through mTOR, since muscle growth induced by synergistic ablation can be completely prevented by chronic administration of the mTOR inhibitor rapamycin (6). Furthermore, hypertrophy in response to chronic sciatic nerve stimulation is highly correlated to phosphorylation of the 70-kDa ribosomal protein S6 kinase, S6K1 (8), a well defined downstream target of mTOR signaling.

The role of PKB/mTOR signaling in the regulation of acute increases in translation initiation and protein synthesis is less clear. It has been widely assumed that activation of mTOR signaling leads to increased protein synthesis via 1) activation of S6K1, subsequent phosphorylation of ribosomal protein S6, enhanced translation of mRNAs containing a 5’-terminal oligopyrimidine tract (5’-TOP) (encoding elongation factors and ribosomal proteins), and ultimately an increase in translation capacity (9) and 2) phosphorylation of 4E-BP1, increased eIF4E availability, and eIF4F complex assembly, leading to increased rates of translation initiation (10–13). There are a number of shortcomings to these teleologically attractive yet unproven hypotheses in regard to protein synthetic regulation following acute load bearing. First, whereas 5’-TOP mRNAs have been shown to shift into actively translating polysomes following high frequency electrical stimulation of skeletal muscle (14), recent evidence has called into question the role of S6 phosphorylation in 5’-TOP mRNA translation (15, 16). Furthermore, numerous studies have shown that an acute bout of resistance

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The abbreviations used are: mTOR, mammalian target of rapamycin; PKB, protein kinase B; ERK, extracellular signal-regulated kinase.

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exercise is insufficient to cause a significant increase in ribosomal capacity (17–23), making this an unlikely contributor to the increase in protein synthesis observed after an untrained exercise bout. Additionally, 4E-BP1 phosphorylation and eIF4F complex assembly have been shown to be unchanged concomitant with elevations in protein synthesis following an in vivo model of resistance exercise (24), calling into question the role of this rate-controlling step of translation initiation. Alternatively, it has been established that eIF2B activity, the other well established rate-controlling step in translation initiation, is elevated concomitant with the stimulation of protein synthesis in the recovery period following resistance exercise (21, 25).

eIF2B is a complex, five-subunit guanine nucleotide exchange factor that exchanges GTP for GDP bound to eIF2, thus allowing eIF2 to deliver Met-tRNAi to the 40 S ribosomal subunit during each round of translation initiation (26–28). Previous work has shown that the mRNA abundance of a number of eIF2B subunits is increased subsequent to the observed stimulation of protein synthesis following an acute bout of resistance exercise, suggesting a role for transcriptional regulation on eIF2B availability and/or activity with chronic exercise training (29). Importantly, that study also reported a rapid increase in eIF2Be proteinc expression observed prior (3 h postexercise) to the stimulation of protein synthesis in the recovery period following exercise. Theoretically, this post-transcriptional increase in the catalytic e-subunit of eIF2B could participate in acute protein synthetic regulation. Interestingly, previous work in the model of resistance exercise employed in those studies has established a rapid but transient increase in mTOR signaling within the first 1 h into the recovery period (4). Since phosphorylation of ribosomal protein S6 and increased eIF4E availability (functional consequences of mTOR signaling) are both purported to be associated with translation of specific messages, it is possible that this early signaling through mTOR could lead to an increase in translation of progrowth mRNAs, such as eIF2B, that could ultimately play a role in the protein synthetic response observed later in the recovery time course.

The e-subunit of eIF2B is known to be phosphorylated on numerous residues by several kinases (e.g., GSK-3, CK1, CKII, and Dyrk). Among these kinases, GSK-3 is the only signaling protein with a well established effect on eIF2B activity. GSK-3 phosphorylates eIF2Be on Ser-535, thus reducing the activity of this positive regulator of protein translation initiation (30). Activated GSK-3 inhibits myotube hypertrophy in vitro (31) and cardiac hypertrophy both in vitro (32) and in vivo (33). Furthermore, GSK-3 is known to be inactivated by endurance exercise (34). Interestingly, GSK-3 is phosphorylated both directly by PKB (35–38) and downstream of mTOR via S6K1 (35, 39), thus inhibiting GSK-3 activity and theoretically depressurizing eIF2B activity.

Despite the clear link between eIF2B activity and initiation of translation, the role of this protein as a mediator of both skeletal muscle protein synthesis and muscle hypertrophy downstream of the PKB/mTOR pathway has been understudied. Importantly, the mechanism by which eIF2B activity is regulated following resistance exercise remains unknown, and the phosphorylation status of the e-subunit following load-bearing exercise has not been reported. The study described herein was designed to assess the contribution of PKB/mTOR signaling to the stimulation of gastrocnemius protein synthesis following an acute bout of resistance exercise. In particular, the roles of the well described mTOR targets S6K1 and 4E-BP1 in regulation of skeletal muscle protein synthesis following exercise were reexamined, and novel connections between PKB/mTOR signaling and eIF2B subunit abundance and/or phosphorylation status were explored.

EXPERIMENTAL PROCEDURES

Animal Care—All experimental procedures were approved by the Institutional Animal Care and Use Committee of the Pennsylvania State University College of Medicine. Male Sprague-Dawley rats (∼250–300 g) were housed in temperature- and humidity-controlled holding facilities on a 12:12 h light-dark cycle. They were fed a standard rodent diet (Harlan-Teklad Rodent Chow, Madison, WI), and both food and water were provided ad libitum. Animals were randomly assigned to treatment groups and fasted for 5 h prior to tissue procurement.

Acute Resistance Exercise Protocol—Details of the exercise protocol have been described previously (22). Briefly, male Sprague-Dawley rats were operantly conditioned to stand on their hind limbs and touch an illuminated bar located high on the wall of a plexiglass cage. This movement was reinforced with the use of a mild foot shock (<2 mA, 60 Hz) over the course of four familiarization sessions. Once this learning process was completed, weighted Velcro vests were strapped over the scapulae and the animals were required to touch the overhead bar 50 times during a given exercise session. The “acute” resistance exercise protocol consisted of four separate sessions with 1 day of rest between sessions. The rats performed 50 repetitions in each session with 0.2-g (day 1), 0.4-g (days 2 and 3), and 0.6-g (day 4) weighted vest/g of body weight, respectively. On training days, sedentary control animals were placed in the cage and given five mild shocks to simulate the stress experienced by the exercised animals. Sixteen hours following the last acute resistance exercise session, all animals were anesthetized by inhalation with a 75% N2O, 25% O2 gas mixture via a nose cone connected to an isoflurane vaporizer (3.0–4.0% isoflurane). Animals were deemed deeply anesthetized only if they did not respond to numerous tactile stimuli (e.g. tail pinch response and eye reflex response). These assessments were made frequently during the surgical protocol, and animals remained in a deeply anesthetized state during all described procedures. Once the appropriate anesthetic state was achieved, both gastrocnemius muscles were excised for subsequent analyses (see below). Animals were then killed by guillotine while still deeply anesthetized with isoflurane.

Administration of Rapamycin—Rapamycin (NCI, National Institutes of Health, Rockville, MD) was dissolved in 100% ethanol (1 mg/20 μl) and then added to 980 μl of sterile saline. Animals in the rapamycin treatment groups received a tail vein injection containing 0.75 mg/kg of body weight. This dosage has been previously used in rodents and demonstrated to inhibit signaling downstream of mTOR (40). Based on the timing of the injections, exercised animals received rapamycin 2 h prior to the final resistance exercise bout. Untreated animals received an injection containing an equal volume of ethanol/saline vehicle.

Assessment of Protein Synthesis—The rate of global protein synthesis was assessed in the gastrocnemius muscle by the flooding dose method previously reported (41) with the use of a previously described model (22). Sprague-Dawley rats were anesthetized as described, and the left carotid artery and right jugular vein were catheterized. A flooding dose of L-[2,3,4,5,6-3H]phenylalanine (1 μCi/rat; Amershams Biosciences) was delivered in unlabeled phenylalanine (150 μm; 1 μl/100 g of body weight) via injection into the venous catheter over a 5-s period. Arterial blood was obtained 10 min after infusion of the flooding dose and subsequently centrifuged at 1800 × g for 10 min at 4 °C to obtain serum. The specific radioactivity of [3H]phenylalanine in the serum was assessed by measuring the amount of radioactivity in the serum via liquid scintillation spectrometry (10 μl in 10 ml of 989 scintillation fluid; Packard) and the concentration of phenylalanine via high pressure liquid chromatography following amino acid extraction from the serum.

Immediately following the blood collection, the left gastrocnemius was excised and frozen between aluminum blocks cooled to the temperature of liquid nitrogen. The muscle was then powdered, and 0.5 g was homogenized in 7 volumes of homogenization buffer (20 mM HEPES (pH 7.4), 100 mM potassium chloride, 0.2 mM EDTA, 2 mM EGTA, 50 mM sodium fluoride, 50 mM β-glycerophosphate, 0.1 mM phenylmethylsulfonyl fluoride, 1 mM benzamidine, 1 mM dithiothreitol, and 0.5 mM sodium vanadate). A 500-μl aliquot of homogenate was then added to 2.5 ml of 1 N perchloric acid and boiled for 15 min. Following boiling, the samples were centrifuged at 3200 × g for 10 min at 4 °C. The resulting supernatant was aspirated, and the pellet was subjected to the following wash protocol: twice in 0.5 N perchloric acid, twice in chloroform/ethanol/ether (1:2:1), and once in ether. The pellet was then dried overnight in a fume hood, resuspended in 3 ml of 0.1 N NaOH and boiled for 15 min with occasional vortexing. The final sample was counted via...
liquid scintillation in duplicate (1-ml sample in 10 ml of 989 scintillation fluid), and 5 μl were assayed for total protein concentration using the Bio-Rad protein assay. Rates of protein synthesis (nmol of Phe/mg of protein/h) were then calculated as described previously (43).

**Role of mTOR in Muscle Protein Synthesis following Exercise**

**RESULTS**

**Effect of Resistance Exercise and Rapamycin Treatment on mTOR-dependent Signaling**—Previous work has established that mTOR signaling is rapidly but transiently increased within 1 h following the model of acute resistance exercise employed in this study (4). The results presented herein suggest that there is also a small but significant increase in the hyperphosphorylation status of the mTOR targets S6K1 (+19%; Fig. 1A) and 4E-BP1 (+30%; Fig. 1B) 16 h into the recovery period following acute resistance exercise compared with sedentary control values. In both cases, hyperphosphorylation status was assessed by dividing the combined densitometry of the slower migrating phosphorylated β and γ bands by the total densitometry signal from all of the bands. Previously published results established that tail vein administration of rapamycin (0.75 mg/kg) is sufficient to severely blunt phosphorylation of the aforementioned mTOR targets 3 h following injection (40) even following an oral gavage of leucine (a known stimulator of mTOR signaling) 1 h prior to sacrifice. In the present study, this dose of rapamycin significantly decreased hyperphosphorylation of both S6K1 (Fig. 1A) and 4E-BP1 (Fig. 1B) in both sedentary control and exercised animals, even 18 h postinjection, thus confirming the efficacy of the inhibitor. Whereas muscle contractions are known to transiently increase phosphorylation of members of the mitogen-activated protein kinase pathway, phosphorylation of ERK1/2 (Thr-202/Tyr-204) (Fig. 1C) and its downstream target eIF4E (Ser-209) (Fig. 1D) were unchanged 16 h following resistance exercise. Importantly, rapamycin administration had no nonspecific effects on the phosphorylation of these proteins.

**Assessment of Protein Synthesis following Resistance Exercise with and without Pretreatment with Rapamycin**—It has been well established that the model of resistance exercise employed in this study leads to an increase in the rate of gastrocnemius protein synthesis 16 h into the recovery period following load bearing (21, 22, 47), as assessed by incorporation of [3H]phenylalanine into muscle protein using the flooding dose technique (41). As expected, there was a significant increase in the rate of protein synthesis in the gastrocnemius 16 h following resistance exercise compared with sedentary control animals in the present investigation (+43%; Fig. 2). Administration of rapamycin had no effect on the basal rate of protein synthesis; however, rapamycin administration 2 h before exercise completely prevented the normally observed increase in protein synthesis 16 h into the recovery period.

In order to examine the role of translation initiation in the exercise-induced increase in protein synthesis and delineate the effect of rapamycin treatment on abrogating this increase, muscle homogenates were analyzed by sucrose density gradient centrifugation (Fig. 3). To identify the fractions containing the 40 and 60 S ribosomal subunits, 80 S monosomes, and polysomes, six fractions were collected across the gradient (Fig. 3A), and total RNA was extracted using TRIzol reagent. The RNA samples were analyzed using an Agilent 2100 Bioanalyzer (Santa Cruz, CA). Antibodies raised against the GSK-3β (Santa Cruz, CA). Antibodies raised against the GSK-3β (number 9102), phospho-GSK-3β (Ser-21) (number 9255), phospho-GSK-3β (Thr-27) (number 9455), and phospho-GSK-3β (Ser-9) (number 9336) antibodies were obtained from Cell Signaling Technologies (Beverly, MA). GSK-3β antibody (number 361528) was also available from Cell Signaling Technologies. The antibodies raised against the β, δ, and ε-subunits of eIF2β (44), eIF4E and eIF4G (45), and eIF2α (46) used in the current study were developed in our laboratory and have been described previously. Total actin was assessed using an antibody (A-5806) from Sigma.

**Statistical Analysis**—Treatment comparisons were conducted using a one-way analysis of variance (GraphPad Prism 4, San Diego, CA). If the analysis of variance reached statistical significance at a 95% confidence level, a Student-Newman-Keuls multiple comparison test was applied to assess significant differences (p < 0.05) between the various treatment groups. All data sets were assessed for potential outliers using a Grubbs test. All results are expressed as a fraction of control and presented as mean ± S.E.
Fractions 1–4, whereas progressively larger polysome peaks lie in Fractions 5 and 6 and beyond. This sucrose density gradient strategy reveals that there was an increase in polysomal aggregation in the gastrocnemius 16 h postexercise compared with basal levels of polysome aggregation in sedentary control muscles (Fig. 3, E versus C). Whereas rapamycin had little effect on polysome aggregation in control animals (Fig. 3, D versus C), pretreatment with the inhibitor prior to resistance exercise completely abrogated the exercise-induced increased in polysome aggregation (Fig. 3, F versus E). This result suggests that the exercise-induced stimulation of protein synthesis was mediated through a mTOR-dependent increase in translation initiation.

Analysis of Functional End Points Downstream of mTOR Signaling following Resistance Exercise—Next, well established functional end points downstream of mTOR signaling were examined in an attempt to understand how mTOR participates in the exercise-induced stimulation of translation ini-

Fig. 1. Analysis of downstream targets of mTOR in the gastrocnemius 16 h following a bout of acute resistance exercise with or without rapamycin pretreatment. Hyperphosphorylation status of S6K1 (A) and 4E-BP1 (B) was examined by dividing the combined densitometry of the slower migrating phosphorylated β and γ bands by the density of all three bands. For both proteins, representative Western blots and graphical summaries of mean densitometry values are shown. A, S6K1 hyperphosphorylation status in sedentary control animals (lane 1), control animals treated with rapamycin (lane 2), exercised animals (lane 3), and exercised animals pretreated with rapamycin (lane 4). The graphical results represent the mean ± S.E. (n = 20–28/group). †, p < 0.001 versus control; *, p < 0.01 versus control; #, p < 0.001 versus exercise. B, 4E-BP1 hyperphosphorylation status 16 h following a bout of acute resistance exercise (n = 12/group). †, p < 0.001 versus control; *, p < 0.001 versus exercise. Exercise and/or rapamycin treatment had no effect on ERK1/2 Thr-202/Tyr-204 phosphorylation (C) or the downstream target of ERK signaling, eIF4E(Ser-209) phosphorylation (D) 16 h following an acute bout of resistance exercise (n = 12/group).
Role of mTOR in Muscle Protein Synthesis following Exercise

**Fig. 2. Assessment of rates of protein synthesis in the gastrocnemius muscle.** Rates of protein synthesis were measured by incorporation of \(^{3}H\)-labeled phenylalanine into muscle protein of sedentary control animals (n = 16), control animals treated with rapamycin (n = 8), exercised animals (n = 14), and exercised animals pretreated with rapamycin (n = 8). *, p < 0.05 versus control; #, p < 0.01 versus exercise.

**DISCUSSION**

The present study provides the first direct evidence that mTOR signaling contributes to the acute stimulation of skeletal muscle protein synthesis following load-bearing exercise. As expected, a bout of resistance exercise caused a significant increase in the rate of protein synthesis in the gastrocnemius muscle 16 h into the recovery period (Fig. 2). The stimulation of protein synthesis was completely abolished by rapamycin treatment 2 h prior to the exercise bout. It is important to note that whereas previous theoretical arguments concerning the role of mTOR signaling in the regulation of protein synthesis have, in part, revolved around regulation of translational capacity via S6K1 activation (14), total RNA (an index of translation capacity) is unchanged at the time point examined in the exercise model employed in the present study (21).

Furthermore, sucrose density gradient centrifugation analysis suggests that there is an increase in polysome aggregation 16 h postexercise compared with sedentary control levels (Fig. 3, E versus C) and that this increase is prevented by pretreatment with rapamycin (Fig. 3, E versus F). This evidence implies that increased efficiency of translation initiation plays a role in the observed stimulation of muscle protein synthesis. However, 4E-BP1 phosphorylation and eIF4F complex assembly have been reported to be unchanged at the time point examined, using the identical model employed in the present study (24). Despite the modest increase in 4E-BP1 hyperphosphorylation reported herein (Fig. 1B), the absence of a significant exercise effect on 4E-BP1 (Thr-70) phosphorylation (Fig. 4D), 4E-BP1-eIF4E association (Fig. 4E), and/or eIF4E-eIF4G complex assembly (Fig. 4F) argues against the involvement of mTOR signaling to E4-BP1 in the rapamycin-sensitive stimulation of protein synthesis observed following muscle loading. Therefore, an alternative explanation for the role of mTOR signaling in regulating acute changes in skeletal muscle protein synthesis following resistance exercise is likely to exist.

Previous studies have demonstrated that there is a significant increase in eIF2B activity 16 h following an acute bout of resistance exercise (21, 25). Those studies utilized an experimental model of acute resistance exercise identical to the model...
employed in the present study. Furthermore, the time of sacrifice was chosen to be concomitant with a significant increase in the rate of muscle protein synthesis. Therefore, the work in this experimental model has consistently suggested that increased eIF2B activity plays a role in acute exercise-induced stimulation of skeletal muscle protein synthesis. The results of the present study suggest that the increase in eIF2B activity could be caused in part by a significant increase in expression of the catalytic e-subunit of eIF2B (Fig. 5C). The increase in eIF2B\textsubscript{e} protein expression occurs in the absence of an increase in phosphorylation at the Ser-535 residue (Fig. 5B), theoretically leading to a larger, more catalytically active protein pool that would enhance eIF2B activity (see below). Previously published work has demonstrated that transcriptional regulation of eIF2B\textsubscript{e} gene expression is not increased during the time course considered in this study (29); therefore, the potential involvement of mRNA translation was examined. There was a significant increase in the percentage of e-subunit mRNA associated with actively translating polysomes 16 h following a bout of acute resistance exercise compared with sedentary con-

**FIG. 3. Qualitative analysis of polysomal aggregation in the gastrocnemius.** Polysome profiles were produced using sucrose density gradient ultracentrifugation. In order to ensure the identities of the various peaks on the spectrophotometer tracing generated from the gradients, six fractions (1–6) were collected (A), and total RNA was isolated from each fraction. RNA was analyzed using an Agilent 2100 Bioanalyzer (B), and ribosomal fractions were identified based on the presence and/or relative abundance of the 28 and 18 S rRNA present. Gastrocnemius tissue from animals in each treatment group (n=12/group) was analyzed using this methodology, and polysome aggregation was observed qualitatively in sedentary control animals (C), control animals treated with rapamycin (D), exercised animals (E), and exercised animals pretreated with rapamycin (F). A representative profile from each condition is presented.
control muscle (Fig. 7). Tail vein administration of rapamycin 2 h prior to the exercise bout completely abrogated this redistribution of the eIF2Be message into polysomes (Fig. 7), prevented the increase in eIF2Be protein expression (Fig. 5, B and C), and disallowed increases in skeletal muscle protein synthesis (Fig. 2) following a bout of resistance exercise.
FIG. 5. Western blot analysis of eIF2B subunit phosphorylation status and protein abundance in the gastrocnemius. A, GSK-3β (Ser-9) phosphorylation was examined in sedentary control animals (lane 1), control animals treated with rapamycin (lane 2), exercised animals (lane 3), and exercised animals pretreated with rapamycin (lane 4). The results represent the mean values ± S.E. (n = 19–27/group). B, phosphorylation of eIF2Bβ on the inhibitory Ser-535 residue 16 h following a bout of acute resistance exercise (n = 19–28). *, p < 0.001 versus control; #, p < 0.001 versus exercise. C, total eIF2Bε protein abundance was also assessed in all treatment groups (n = 19–28). A significant increase in eIF2Bε was observed 16 h following a bout of acute resistance exercise (*, p < 0.001 versus control); however, pretreatment with rapamycin prior to exercise completely prevented this increase (#, p < 0.001 versus exercise). D and E, protein expressions of both eIF2Bβ and eIF2Bδ were unchanged in all of the treatment groups examined in the current study (n = 19–20 per group).
Taken together, the results suggest a novel role for mTOR signaling to eIF2B mRNA translation and protein abundance. This potential mechanism for regulation of eIF2B activity is particularly interesting because eIF2B is found in rate-limiting quantities relative to its primary known substrate eIF2 (50). Expression of the \( \alpha \)- and \( \beta \)-subunits of eIF2 were shown to be unchanged (Fig. 6, A and B) in all of the treatments examined. It is important to point out that the increase in eIF2B protein expression occurred in the absence of a change in two other eIF2B subunits (Fig. 5, D and E). Furthermore, as predicted from the Western blots, eIF2B mRNA association with poly-somes was not altered in any of the treatment groups examined (Fig. 7). When these data are considered in the context of the previously established increase in eIF2B activity 16 h following completion of the resistance exercise protocol, they imply that eIF2B may act in isolation to promote guanine nucleotide exchange, enhance the rate of translation initiation, and thus increase muscle protein synthesis.

Interestingly, it has been established that overexpression of a cDNA encoding rat eIF2B alone can increase guanine nucleotide exchange activity in S9 (51) and S21 insect cells (44). Similarly, extracts from yeast cells overexpressing the GCD6 gene encoding eIF2B have been shown to exhibit higher gua-

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**FIG. 6.** Western blot analysis of total protein abundance of eIF2 subunits and members of the eIF4F complex in the gastrocnemius. A, eIF2\( \alpha \) protein expression was examined in sedentary control animals (lane 1), control animals treated with rapamycin (lane 2), exercised animals (lane 3), and exercised animals pretreated with rapamycin (lane 4). The results represent the mean values ± S.E. (\( n = 12 \) group). There was no significant difference in the total protein expression of eIF2\( \alpha \) in any of the treatment groups examined. Similarly, there was no significant difference in eIF2\( \beta \) (B), eIF4E (C), or eIF4G (D) protein abundance in any of the treatment groups examined (\( n = 12 \) group).

**FIG. 7.** Assessment of specific mRNA translation status in the gastrocnemius using sucrose density gradient ultracentrifugation followed by quantitative real time PCR analysis. Postmitochondrial supernatants were resolved over a 15–50% sucrose gradient (\( n = 12 \) per group), and subpolysomal and polysomal fractions were collected. Total RNA was isolated from these two fractions, RNA was reverse transcribed, and the relative abundance of eIF2B\( \alpha \), eIF2B\( \beta \), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was evaluated using SYBR Green qRT-PCR. The data are expressed as the percentage of the relative mRNA expression in the actively translated polysomal fraction. In each case, the data represent mean values ± S.E. (\( n = 8–11 \) group). *, \( p < 0.05 \) versus control; #, \( p < 0.05 \) versus exercise.
nine nucleotide exchange activity compared with control yeast cells (52). The homologue of eIF2B from Drosophila melanogaster was also shown to have independent catalytic activity via overexpression in HEK293 cells and Escherichia coli (53). These studies have suggested that the e-subunit of eIF2B only has 5–20% of the activity of the complete five-subunit complex. However, it is important to note that the eIF2B expressed in insect cell lines and yeast may or may not have post-translational modifications associated with full catalytic activity in mammalian cells. For example, modifications necessary for eIF2B association with other proteins critical to guanine nucleotide exchange activity may be absent in these model systems. Furthermore, previous studies did not examine eIF2Be(Ser-535) phosphorylation status to determine the possible inhibition of this overexpressed protein. Overexpression of wild-type eIF2B in cardiomyocytes (54) has been shown to induce a significant increase in phosphorylation at Ser-535 that would be expected to decrease eIF2B activity. Indeed, whereas overexpression of wild-type eIF2B causes a significant increase in cardiomyocyte cell size (+31%) and protein synthesis (~75%), overexpression of a nonphosphorylatable eIF2Be(S535A) mutant has a much more dramatic effect on these end points (+107% and −105%, respectively).

Another study addressing the functional role of eIF2B expression was published by Balachandran and Barber (55) during the preparation of this manuscript. The authors of that study demonstrated that transformed mouse embryonic fibroblasts had increased eIF2B activity, along with a >10-fold increase in the relative expression of endogenous eIF2B and a ~2-fold increase in protein synthesis, compared with genetically equivalent parental immortalized cells. As in the present study, the increase in eIF2B protein expression occurred in the absence of an increase in the other eIF2B subunits. Furthermore, the authors overexpressed the full-length rat eIF2Be cDNA and showed that an increase in eIF2Be alone was sufficient to stimulate translation ~2-fold. Finally, transformed mouse embryonic fibroblasts were infected with vesicular stomatitis virus, and eIF2Be expression was repressed using small interfering RNA technology. Small interfering RNA treatment resulted in a 10-fold reduction in virus and nearly complete repression of viral cytolysis. Therefore, increased eIF2Be protein expression may be a common mechanism for regulating increases in translation initiation and protein synthesis in both physiological and pathological states. Future studies will focus on the role of exogenous overexpression of eIF2Be on skeletal muscle eIF2B activity, protein synthesis, and hypertrophic growth.

Another important question that remains is the identity of downstream mTOR targets that participate in the increased eIF2Be mRNA translation following exercise. In the present study, we report a significant increase in phosphorylation of the S6K1 target ribosomal protein S6 (Fig. 4, A and B) 16 h into the recovery period following resistance exercise. As expected, the increase in S6 phosphorylation was completely inhibited by rapamycin, and this reduction was concomitant with a complete prevention of the increase in eIF2Be observed following exercise alone. Whereas these data might tentatively suggest a role for S6 phosphorylation in increased translation of eIF2Be mRNA, the e-subunit mRNA does not include a TOP sequence in its 5′-untranslated region. As discussed earlier, recent work has challenged the role of S6 phosphorylation in 5′-TOP mRNA translation, and this seems at best to be a cell- and tissue-specific phenomenon. Therefore, important questions remain concerning the functional consequences of ribosomal S6 phosphorylation. It is possible that this phosphorylation event is involved in translation of specific messages (such as eIF2Be) in a TOP-independent manner. Alternatively, a newly described target of S6K1 termed SKAR (56), which is involved in growth control and related to a family of proteins involved in mRNA processing and export, may also be involved. More efficient message export from the nucleus could be another possible explanation for increased translation of eIF2Be mRNA following exercise.

The other well characterized downstream target of mTOR, as described previously, is 4E-BP1. Whereas functional effects downstream of mTOR signaling to 4E-BP1 appear not to be affected 16 h following exercise, a previous study using this model of resistance exercise has demonstrated significant, but transient, elevations in 4E-BP1 phosphorylation and eIF4E binding to eIF4G within the first 1 h into the recovery period (4). The rapid induction of these changes is reminiscent of the rapid up-regulation of the relative expression of eIF2Be protein previously observed (29). Whereas eIF4F complex assembly is associated with increased protein synthesis under certain conditions, eIF4E availability is also commonly associated with enhanced translation of mRNA species with highly structured 5′-untranslated regions (57, 58) through the physical proximity of these messages with the RNA helicase eIF4A in the eIF4F complex. Computer predictions of secondary structure in the rat eIF2Be 5′-untranslated region along with the first 150 base pairs of the coding region (mFOLD 3.1) (59) reveal a significant hairpin structure (~80.5 kcal/mol) located immediately upstream of the translational start site. Theoretically, this structure is substantial enough to prevent normal ribosomal scanning. Future studies will also address functional elements within the eIF2Be message that are required to mediate mTOR-dependent translational regulation.

In conclusion, the present study has provided the first direct evidence that the mTOR signaling pathway is necessary for acute increases in global rates of protein synthesis following resistance exercise. Based on the rapamycin-sensitive increase in polysome aggregation following exercise, the role of mTOR in acute protein synthetic regulation is mediated through an increase in translational initiation. This effect is apparently not mediated through phosphorylation of 4E-BP1 and a subsequent increase in eIF4F complex assembly 16 h following exercise. Instead, the data suggest that an mTOR-dependent increase in eIF2Be mRNA translation and the subsequent increase in e-subunit protein expression could play a role in the acute increase in protein synthesis in the recovery period following resistance exercise.

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