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REVIEW | *Translational Control of Muscle Mass*

Role of mTORC1 in mechanically induced increases in translation and skeletal muscle mass

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Goodman CA. Role of mTORC1 in mechanically induced increases in translation and skeletal muscle mass. *J Appl Physiol* 127: 581–590, 2019. First published January 24, 2019; doi:10.1152/japplphysiol.01011.2018.—Skeletal muscle mass is, in part, regulated by the rate of mRNA translation (i.e., protein synthesis). The conserved serine/threonine kinase, mTOR (the mammalian/mechanistic target of rapamycin), found in the multiprotein complex, mTOR complex 1 (mTORC1), is a major positive regulator of protein synthesis. The purpose of this review is to describe some of the critical steps in translation initiation, mTORC1 and its potential direct and indirect roles in regulating translation, and evidence that mTORC1 regulates protein synthesis and muscle mass, with a particular focus on basal conditions and the response to mechanical stimuli. Current evidence suggests that for acute contraction models of mechanical stimuli, there is an emerging pattern suggesting that there is an early increase in protein synthesis governed by a rapamycin-sensitive mTORC1-dependent mechanism, while at later poststimulation time points, the mechanism may change to a rapamycin-insensitive mTORC1-dependent or even an mTORC1-independent mechanism. Furthermore, evidence suggests that mTORC1 appears to be absolutely necessary for muscle fiber hypertrophy induced by chronic mechanical loading but may only play a partial role in the hypertrophy induced by more intermittent types of acute resistance exercise, with the possibility of mTORC1-independent mechanisms also playing a role. Despite the progress that has been made, many questions about the activation of mTORC1, and its downstream targets, remain to be answered. Further research will hopefully provide novel insights into the regulation of skeletal muscle mTORC1 that may eventually be translated into novel exercise programing and/or targeted pharmacological therapies aimed at preventing muscle wasting and/or increasing muscle mass.

mTORC1; muscle atrophy; muscle hypertrophy; protein synthesis; translation

REGULATION OF SKELETAL MUSCLE MASS

Skeletal muscle mass is largely determined by the net difference between the rates of mRNA translation (i.e., protein synthesis) and protein degradation (28). Therefore, under conditions that lead to reduced muscle fiber size (i.e., atrophy), the rate of protein degradation would exceed the rate of protein synthesis. Conversely, under conditions that stimulate muscle fiber growth (i.e., hypertrophy), the rate of protein synthesis would exceed that of protein degradation. In skeletal muscle, the rate of protein synthesis is responsive to changes in a variety of stimuli, including nutrients, neural activity, cytokines, growth factors, hormones, and mechanical stimuli (23, 77). Furthermore, changes in the rate of protein synthesis can occur relatively quickly [minutes to hours; e.g., (31, 61)], suggesting the presence of regulatory mechanisms that can rapidly increase the rate of mRNA translation in response to the appropriate stimulation. One major regulator of translation is the protein kinase known as mTOR (the mammalian/mechanistic target of rapamycin) found in the multiprotein complex, mTOR complex 1 (mTORC1), and a substantial body of evidence has accumulated showing that mTORC1 plays a critical role in stimulating increases in protein synthesis rates and skeletal muscle mass.

This review will briefly describe some of the critical steps in translation initiation, mTORC1, and its potential direct and indirect roles in regulating translation, and evidence that mTORC1 regulates protein synthesis and muscle mass, with a particular focus on basal conditions and in response to increased mechanical stimuli. Because of space limitations, this review will not cover the role of mTORC1 in regulating ribosomal biogenesis, micro-RNA, and long noncoding RNAs, all of which have the potential to regulate either overall

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translation rates, or the translation of specific mRNAs, nor will it discus mTORC1's role in regulating autophagy. It is also important to highlight that mTORC1 is not the only regulator of protein synthesis and muscle mass, and that many other signaling mechanisms can potentially regulate muscle mass by modulating rates of protein synthesis and/or protein degradation (for recent reviews, see Refs. 3, 32, 80, 81, 89, and 94).

mTOR/mTORC1

mTOR is a relatively large (289 kDa) serine (S)/threonine (T) kinase (75) that exists in two functionally and structurally distinct multiprotein complexes, mTORC1 and mTORC2. mTORC1 is made up of several different protein species (including mLST8, PRAS40, and deptor); however, the main defining feature of mTORC1 is the presence of the protein known as raptor (regulatory associated protein of mTOR) (36, 49), with mTORC1 being an obligate dimer composed of two mTOR and two raptor molecules (47, 98). Another unique feature of mTORC1 is that the macrolide antibiotic, rapamycin, in complex with specific members of the FKBP immunophilin family (e.g., FKBP12), binds to mTOR's FRB domain and inhibits many (but not all) of mTORC1's downstream effects by allosterically blocking the access of substrates to mTOR's kinase domain and/or by disrupting the dimeric architecture of mTORC1 (98). In contrast to mTORC1, the defining feature of mTORC2 is the presence of the mTOR-binding protein, rictor (rapamycin-insensitive companion of mTOR), which, in conjunction with another mTORC2-specific protein, mSin1, sterically inhibits the rapamycin/FKBP12 complex from binding to mTOR's FRB domain, rendering mTORC2 resistant to the acute inhibitory effect of rapamycin (13, 19, 45); however, with more prolonged treatment, rapamycin has been shown to eventually disrupt the mTORC2 complex and inhibit mTORC2 signaling (53, 78). mTORC2 also has different downstream targets to mTORC1, including members of the AGC kinase family, PKC (34), PKB (also known as Akt) (79), and serum- and gluccocorticoid-induced protein kinase 1 (SGK1) (25). Interestingly, Akt and SGK1 are both known regulators of protein synthesis and protein degradation, suggesting that mTORC2 may also have a role the regulation of skeletal muscle mass (1, 23). Regarding mTORC1's downstream targets, the two most studied are the eukaryotic initiation factor 4E-binding protein 1 (4E-BP1) and the 70-kDa ribosomal protein S6 kinase 1 ($p70^{S6k1}$), both of which play important roles in mRNA translation initiation (for review, see Ref. 69).

TRANSLATION INITIATION

The process of mRNA translation is divided into four main stages, translation initiation, translation elongation, translation termination, and ribosome recycling, with the translation initiation stage being the most highly regulated, followed by the translation elongation stage (for review, see Ref. 37). Briefly, translation initiation involves the interaction of individual mRNAs with a multiprotein structure, known as the eukaryotic initiation factor 4F (eIF4F) complex, which is composed of three individual initiation factors: eIF4E, eIF4G, and eIF4A. More specifically, under stimulatory conditions, eIF4E binds to the 7-methylguanosine "cap" (m^7G cap), located on the 5′-end of all nuclear encoded mRNAs, and to the large scaffold protein, eIF4G. The helicase eIF4A is recruited to unwind 5′ secondary mRNA structures to facilitate the recruitment of the 43S preinitiation complex (PIC), which is composed of the small 40S ribosomal subunit and the eIF2/GTP/initiator tRNA ($Met\text{-}tRNA_i$) ternary complex and other initiation factors, including the large eIF3 complex. Subsequently, the now larger 48S PIC scans the mRNA in the 3′ direction for the AUG translation start codon which, when found, is followed by the recruitment of the larger 60S ribosomal subunit to create the 80S ribosome and begin translation elongation and peptide synthesis (for recent, more comprehensive reviews, see Refs. 38 and 56). As mentioned above, mTORC1 is known to directly and indirectly regulate a range of proteins involved in this process of cap-dependent translation, leading to an increase in the overall rate of protein synthesis (for recent reviews see Refs. 69 and 73).

mTORC1 SUBSTRATES INVOLVED IN TRANSLATION

4E-BP1. Under nonstimulated conditions, the binding of eIF4E to the m^7G mRNA cap, and to eIF4G, is largely inhibited by the binding of eIF4E to eIF4E-binding protein 1 (4E-BP1), thus inhibiting cap-dependent translation initiation (69). This ability of 4E-BPs to bind and inhibit eIF4E is, in part, regulated by mTORC1-mediated phosphorylation, such that upon appropriate stimulation (e.g., nutrient/growth factor/mechanical stimulation), activated mTORC1 directly phosphorylates 4E-BP1 at several different amino acid residues in a sequential manner (T37/46 first followed by S65/T70), leading to 4E-BP1 dissociation from eIF4E and the activation of translation initiation (27). Interestingly, not all of these mTORC1-mediated 4E-BP1 phosphorylation events are inhibited by rapamycin, with T37/46 phosphorylation being largely rapamycin-resistant, while S65 phosphorylation is rapamycin-sensitive (29, 87, 102). Recently, it has been shown that, depending on the amino acid residues surrounding a specific mTORC1 phosphorylation site, mTORC1 kinase activity has a higher affinity for some phosphorylation sites than others, with higher affinity sites displaying greater resistance to the inhibitory effect of rapamycin (48). This has led to different mTORC1 phosphorylation sites being termed as "good" (e.g., 4E-BP1 T37/46) and others as "poor" (e.g., 4E-BP1 S65) (48).

$p70^{S6k1}$ and its downstream translation-related targets. Another direct mTORC1 translation-related target is $p70^{S6k1}$. Similar to 4E-BP1, $p70^{S6k1}$ also has multiple mTORC1-mediated phosphorylation sites; however, it appears that rapamycin-sensitive T389 phosphorylation [classified as a poor, rapamycin-sensitive, phosphorylation site (48)] is most closely associated with $p70^{S6K1}$ activity (15, 65). Once activated by mTORC1, $p70^{S6K1}$ phosphorylates a range of translation-related substrates, including eIF4B (S422), which leads to an increased interaction with the eIF3/PIC and assists eIF4A unwind secondary mRNA structure (40, 70, 82); eukaryotic elongation factor 2 kinase (eEF2 kinase; S366), leading to its inhibition and the relief of its repression of eEF2 and the elongation process (93); and PDCD4 (S67), which leads to PDCD4's degradation by the ubiquitin proteasome system and relieves its inhibition of eIF4A and translation initiation (18). Another $p70^{S6K1}$ target is SKAR (S6K1 Aly/REF-like target; S383 and S385), which binds and recruits $p70^{S6K1}$ to newly spliced mRNAs and enhances their translation (54, 71). In addition to its potential role in stimulating ribosome biogene-

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sis, and, thus, increasing translational capacity (12), $p70^{S6K1}$ also supports translation by promoting the de novo synthesis of pyrimidine nucleotides (i.e., cytosine, uracil) by the phosphorylation of CAD [carbamoyl-phosphate synthetase 2, aspartate transcarbamoylase, dihydroorotase; S1859 (4, 72)] [Note: purine synthesis may also be regulated by mTORC1 but in a $p70^{S6K1}$-independent manner (5)].

Finally, perhaps the most well-known target of $p70^{S6K1}$ is ribosomal protein S6 (rpS6), which is phosphorylated in response to serum stimulation on several residues in a sequential manner (90). While rpS6 was initially thought to play a positive role in the regulation of protein synthesis, other studies have shown that rpS6 phosphorylation is not sufficient to stimulate protein synthesis (51, 58, 84, 88). Furthermore, studies using cells that express an rpS6 mutant that cannot be phosphorylated ($rpS6^{P-/-}$) have reported increased or decreased rates of protein synthesis compared with wild-type cells (43, 74), while cells expressing constitutively active (ca)-Akt, combined with $rpS6^{P-/-}$, displayed a greater increase in protein synthesis than cells expressing ca-Akt alone (43). Thus, while rpS6 phosphorylation is commonly used as an indirect marker of mTORC1 activation, it remains unclear as to whether rpS6 phosphorylation plays a positive or negative role in the process of translation or in the regulation of cell size in tissues, including skeletal muscle.

eEF2 kinase. In addition to $p70^{S6K1}$-mediated phosphorylation and inhibition, eEF2 kinase is also a direct target of mTORC1, which phosphorylates S78 in a rapamycin-sensitive manner. In turn, this leads to an inhibition of eEF2 kinase activity, the relief of eEF2 kinase-mediated repression of eEF2, and the enhancement of translation elongation (10). There is also evidence that mTORC1-mediated phosphorylation of eEF2 kinase at S359 and S396 may also play an inhibitory role (10, 69).

PRAS40. PRAS40 (proline-rich Akt-substrate of 40 kDa), a component of mTORC1, binds to raptor and inhibits mTORC1 kinase activity, possibly by inhibiting substrate binding to raptor (76). While it is known that the activation of Akt leads to the phosphorylation of PRAS40 (T246), and the subsequent dissociation of PRAS40 from, and activation of, mTORC1 (97), mTORC1 can also directly phosphorylate PRAS40 (S183 and S221), thus, further relieving PRAS40-mediated inhibition of mTORC1 signaling (63, 92).

4E-BP1, Larp1, and mTORC1-mediated translation of specific subsets of mRNAs. Because of having specific features in their 5′-untranslated regions (5′-UTR), there are subsets of mRNAs that are translated with significantly reduced efficiency under basal conditions and are dependent on mTORC1 activation for increased translation. One such class of mRNAs contains a tract of pyrimidines (TOP) in their 5′-UTR (i.e., 5′-TOP mRNAs) that consists of a cytosine immediately downstream of the m^7G cap followed by a sequence of 4–15 pyrimidine nucleotides (57). Importantly, 5′-TOP mRNAs typically encode for translation initiation and elongation factors and ribosomal proteins (57, 86), and their translation is disproportionately upregulated, in part, by a rapamycin-sensitive mechanism that involves the inhibition of 4E-BP1 (86). It should be pointed out that although this suggests a role for mTORC1 in promoting 5′-TOP mRNA translation, it has also been shown that the translation of these mRNAs can occur in the absence of raptor, suggesting the possibility of a rapamycin-sensitive but mTORC1-independent mechanism (64). Another link between mTORC1 and the translation of 5′-TOP mRNAs is the mTORC1-mediated phosphorylation of Larp1 (La-related protein 1). Larp1 has been found to be associated with the mRNA 5′-cap and with mTORC1, with evidence suggesting that Larp1 represses 5′-TOP mRNA translation (22, 66). However, mTORC1-mediated phosphorylation of Larp1 leads to its dissociation from the 5′-UTR of TOP mRNAs and to their enhanced translation (41). More recently, mRNAs with 5′-UTR sequences that are similar to 5′-TOP mRNAs, but not strictly adhering to the definition above, have been termed "TOP-like" mRNAs and have also been shown to be translated by a mTORC1/4E-BP1/eIF4E-dependent mechanism (86). Other mRNAs containing a pyrimidine-rich translational sequence (PRTE) within their 5′-UTR have also been shown to be translated via a mTORC1/4E-BP1/eIF4E-dependent mechanism (44). It remains to be determined whether TOP-like and PRTE mRNAs are also regulated by mTORC1-mediated phosphorylation of Larp1.

Another subset of mRNAs are characterized by relatively long, highly structured, guanine/cytosine-rich 5′-UTRs, which reduce translation efficiency, in part, by impeding ribosomal scanning and start codon recognition (14). These mRNAs have been described as "weak" when compared with "strong" mRNAs, which have relatively short and uncomplicated 5′-UTR structures, such as mRNAs encoding for constitutively expressed "housekeeping" proteins (for review, see Ref. 14). Weak mRNAs include several that encode for cell growth-related proteins, such as IGF-II, c-myc, cyclin D1, and ornithine decarboxylase, and prosurvival proteins, such as Bcl-2 and BCL-xL. Importantly, these "weak" mRNAs are sensitive to changes in the availability of eIF4E (14). Therefore, mTORC1-mediated phosphorylation of 4E-BP1, and its subsequent dissociation from eIF4E, combined with the recruitment of the eIF4A helicase, as part of the eIF4F complex, and $p70^{S6k1}$-mediated phosphorylation of eIF4B have the potential to facilitate an increased translation of weak mRNAs to promote growth (14). Interestingly, there is another group of mRNAs that encode for a small subset of mitochondrial proteins that are also regulated in an mTORC1/eIF4E-dependent manner; however, these mRNAs have very short 5′-UTRs and, as such, are not dependent on eIF4A (24).

Overall, it is clear that the activation of mTORC1 signaling has the potential to, directly or indirectly, affect a range of downstream targets that lead to an overall increase in cap-dependent translation. This is, in part, facilitated by mTORC1's ability to selectively increase the efficiency of translation of specific groups of mRNAs that encode for proteins directly involved in translation and ribosomal biogenesis, cell growth and survival, and energy metabolism.

ROLE OF mTORC1 IN REGULATING TRANSLATION AND MUSCLE MASS

Is mTORC1 necessary for maintaining basal rates of translation? Given mTORC1's range of translation-related targets, it would not be unreasonable to expect that mTORC1 would play a fundamental role in regulation of basal/resting rates of protein synthesis in skeletal muscle. Interestingly, some initial studies showed that acute treatment with mTORC1 inhibitor, rapamycin, had no effect on basal protein synthesis in

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mouse muscle ex vivo (150 nM) and in rat muscle in vivo (0.75 mg/kg) (42, 52), and little-to-no effect on polysome abundance in vivo (52). More recently, however, acute in vivo rapamycin treatment (1.5 mg/kg) reduced basal protein synthesis in rat skeletal muscle by ~30–40% (61), suggesting that a higher dose of rapamycin may be required to inhibit basal rates of protein synthesis. In this context, it is interesting to note one study reported that a very high dose of rapamycin (8.0 mg/kg) led to only a ~16% reduction in active muscle ribosomes in mice; however, actual protein synthesis rates were not measured (26). Another recent rat study showed that acute in vivo treatment with either the active site mTOR kinase inhibitor AZD8055 (which would inhibit mTORC1 and mTORC2) or rapamycin (1.5 mg/kg) reduced basal protein synthesis by a similar magnitude (~40–50%) compared with vehicle controls (62). This is an interesting result because, although AZD8055 inhibited $p70^{S6K1}$ T389 phosphorylation to the same extent as rapamycin, it almost completely inhibited 4E-BP1 T37/46, S65, and S70 phosphorylation, while rapamycin had essentially no effect on 4E-BP1. These data may suggest that mTORC2 plays only a minor role, if any, in regulating basal rates of protein synthesis, and that mTORC1-mediated $p70^{S6K1}$, but not 4E-BP1, phosphorylation may play a more dominant role in regulating basal rates of protein synthesis under these conditions. Finally, using a new inducible muscle-specific raptor knockout mouse model, it was found that 21 days after the initiation of raptor knockout in adult mice, in vivo basal rates of protein synthesis were reduced by ~40% (101). When combined, the more recent rodent data suggest that mTORC1 may account for 30–50% of basal rates of protein synthesis in skeletal muscle, a value that is similar to that reported using active site mTOR kinase inhibitors (35–65%) in cultured nonmuscle cells (44, 59, 86).

To date, there have only been four human studies that have used an acute dose of rapamycin (12–16 mg) in an attempt to either specifically inhibit basal postabsorptive (16), resistance exercise-induced (20, 35) or essential amino acid-induced (17) mTORC1 signaling and protein synthesis. In all cases, rapamycin had no effect on basal rates of protein synthesis and had a limited effect on markers of mTORC1 signaling (i.e., $p70^{S6K1}$ or 4E-BP1 phosphorylation). This is likely due to the significantly lower dose used in the human studies (equivalent to 0.17–0.23 mg/kg for a 70-kg person) compared with the rodent studies. Interestingly, while these relatively low doses of rapamycin did not inhibit basal protein synthesis, they were, however, sufficient to inhibit the amino acid-induced (17), and resistance exercise-induced [(20, 35) and see below], increases in protein synthesis. The reason for rapamycin's differential effect of basal versus stimulated rates of protein synthesis under these conditions remains to be determined.

Overall, recent rodent data obtained using relatively high doses of rapamycin, mTOR kinase inhibitors, and an inducible raptor KO model indicate that mTORC1 is necessary for a significant portion (~30–50%) of basal rates of protein synthesis. The few human studies showing no effect of rapamycin on protein synthesis may suffer from the dose of rapamycin being too low to effectively inhibit basal mTORC1 activity. Importantly, these data also highlight that a significant proportion of basal protein synthesis in skeletal muscle is regulated by mTORC1-independent mechanisms.

Is mTORC1 necessary for maintaining basal skeletal muscle mass? The current evidence that mTORC1 mediates a significant portion of basal rates of protein synthesis would suggest that mTORC1 is also necessary for maintaining basal levels of skeletal muscle mass. Despite this assumption, short-term (7–14 days) inhibition of mTORC1 by daily rapamycin injections (0.6–6.0 mg/kg) has no effect on resting muscle fiber size (30, 33, 83), while longer-term studies (4–6 mo) using diet-based rapamycin ingestion have also found no effect on muscle mass (21, 60, 103). Although these rapamycin-based studies might suggest that mTORC1 is not necessary to maintain basal muscle mass, they may be complicated by the dose of rapamycin being too low and/or that not all of mTORC1's functions are inhibited by rapamycin. Perhaps a better model, which negates the need for the use of rapamycin, is the developmental muscle-specific raptor KO mouse model (7). Indeed, muscles from these mice display a 4–19% reduction in their relative mass (and a dystrophic phenotype) at 3 mo of age (7), suggesting that mTORC1 may be a minor-to-moderate regulator of basal levels of skeletal muscle mass; however, because this is a developmental KO model, any phenotype developed may not accurately reflect whether mTORC1 is necessary in mature muscle per se, but instead indicate that mTORC1 may also play critical roles during embryonic, or early postnatal, development. More recently, the relatively short-term (21 days) induced knockout of raptor in mature mouse muscle did not result in a decrease in muscle mass (or a dystrophic phenotype) despite ablating $p70^{S6K1}$ T389 phosphorylation and partially inhibiting 4E-BP1 T37/46 phosphorylation (101).

In summary, because of the limitations inherent in the models used to date, the answer to whether mTORC1 is required for maintaining basal levels of skeletal muscle mass remains to be definitively determined. It is possible that mTORC1-independent mechanisms alone are sufficient to maintain basal muscle mass, at least over the short term. Further insights may be gained from following the inducible muscle-specific raptor KO mice for a longer period of time.

Is mTORC1 activation sufficient to increase rates of translation and muscle mass? Although it is unclear as to whether mTORC1 is necessary for maintaining basal skeletal muscle mass, the picture is significantly clearer regarding whether the relatively acute activation of mTORC1 is sufficient to increase rates of protein synthesis and muscle mass. Interestingly, just having an elevated expression of mTOR protein (up to ~4-fold) does not confer a hypertrophic phenotype in mice (30); although it is unclear how much of the extra mTOR would be incorporated into mTORC1 and mTORC2 or remained uncomplexed. Nevertheless, this suggests that it is the extent of mTORC1 activation that is more important for increasing muscle mass than the amount of mTOR. Indeed, evidence that mTORC1 activation is sufficient to increase skeletal muscle mass comes from studies showing that the overexpression of mTORC1's direct upstream activator, the small GTPase Rheb, is sufficient to activate mTORC1 signaling and induce muscle fiber hypertrophy in a mTOR kinase-dependent, and rapamycin-sensitive, manner over a 2- to 7-day period (31, 33). Furthermore, this Rheb-induced hypertrophy is accompanied by a significant increase in muscle fiber rates of protein synthesis (31). The direct activation of mTORC1 is also sufficient to enhance protein synthesis and to rescue immobilization-induced muscle fiber atrophy (99). In addition to

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the direct activation of mTORC1, further upstream activation of the phosphatidylinositol 3-kinase (PI3K)/Akt pathway by IGF-1 is also sufficient to activate rapamycin-sensitive mTORC1 signaling, increase protein synthesis, and induce skeletal muscle hypertrophy (28, 81).

While relatively short-term activation of mTORC1 (days) is sufficient to increase rates of protein synthesis and muscle mass, it is important to point out that long-term continuous activation of mTORC1 (months), such as that which occurs in mice with a developmental knockout of the upstream mTORC1 inhibitor, TSC1, results in a reduction in the mass of fast-twitch muscles (and body mass) at 3 and 9 mo of age (6), with signs of myopathy at 9 mo (11). These are accompanied by dysregulated autophagy and elevated markers of ubiquitin proteasome activation (6, 11). This mouse model highlights that although mTORC1 plays an important role in positively regulating protein synthesis and cell size, long-term continuous activation of mTORC1 can be deleterious to the skeletal muscle.

Overall, the current data clearly show that, at least in rodents, the relatively acute direct activation of mTORC1 is sufficient to drive an increase in rates of protein synthesis and skeletal muscle mass. As such, it is not surprising that the activation of skeletal muscle mTORC1 signaling by various stimuli, including increased mechanical loading, has been implicated in the increased rates of protein synthesis and muscle hypertrophy.

Mechanically induced activation of mTORC1 signaling in skeletal muscle. Using an in vivo rat model of evoked maximal eccentric contractions, Baar and Esser (2) were the first to show that an acute bout of mechanical stimuli led to an increase in overall $p70^{S6K1}$ phosphorylation and that this was associated with an increase in the size of the polysome pool. Furthermore, they showed that the increase in $p70^{S6K1}$ phosphorylation was correlated with the subsequent training-induced increase in muscle mass (2). Although raptor, and hence mTORC1, had not been discovered at this time, these data provided the first evidence that acute muscle contractions are sufficient to activate mTORC1 and that this activation might play a role in the mechanically induced increase in protein synthesis and muscle mass. Because these initial observations, numerous cell, rodent, and human studies have reported that various traditional markers of mTORC1 signaling are increased in a range of acute and chronic mechanical loading models (28). Despite this, it has only recently been investigated whether signaling events traditionally thought to be mediated by mTORC1, such as the acute mechanically induced increases in $p70^{S6K1}$, 4E-BP1, and rpS6 phosphorylation, are in fact mediated by mTORC1 (101). Specifically, using the same eccentric contraction protocol as Baar and Esser (2), it was found that, as expected, rapamycin (1.0 mg.kg) completely blocked the contraction-induced increase in $p70^{S6K1}$ T389 phosphorylation in muscles from wild-type (WT) mice, but not in muscles from transgenic mice expressing a rapamycin-resistant mutant of mTOR (RR-mTOR) (101). Next, to determine whether this rapamycin-sensitive event was truly mTORC1-dependent, muscles from mice with induced raptor knockout were examined, and it was shown that, consistent with the effect of rapamycin, the contraction-induced increase in $p70^{S6K1}$ T389 phosphorylation was again ablated (101). Interestingly, the contractions in WT mice had no effect on the level of 4E-BP1 T37/46 phosphorylation. They did, however, increase the hyperphosphorylated form of the total 4E-BP1 pool (the slower migrating γ band found using Western blot analysis), indicating that another 4E-BP1 phosphorylation site (possibly S65 and/or S70), was increased by contractions. This hyperphosphorylation of 4E-BP1 was abolished in WT mice, but not in RR-mTOR mice, when treated with rapamycin, and in induced raptor knockout mice, showing that this event is also mTORC1-dependent. Finally, while rapamycin suppressed basal levels of rpS6 S235/236 and S240/244 phosphorylation in WT mice, it had no effect on the contraction-induced increase at these sites, suggesting that a possible rapamycin-insensitive mTORC1-dependent, or mTORC1-independent, mechanism may be involved. When tested in the raptor knockout mice, the increase in rpS6 phosphorylation remained intact, demonstrating that these sites are, in part, regulated by mTORC1-independent mechanisms (101). Similar results have also been obtained using ex vivo passive stretch and in vivo chronic overload models combined with rapamycin and RR-mTOR mice (29, 30, 102).

Combined, these studies show that skeletal muscle mTORC1 is, indeed, activated by acute mechanical stimulation and is responsible for some, but not all, of the signaling events traditionally thought to be mediated by mTORC1. Furthermore, some signaling events might be regulated in a rapamycin-insensitive, but mTORC1-dependent manner. These data suggest that mTORC1 does have the potential to play a vital role of mechanically induced increases in protein synthesis and muscle mass.

Role of mTORC1 in mechanically induced increases in rates of protein synthesis. It has long been known that resistance exercise in humans results in an increase in protein synthesis, which can last for up to 48 h poststimulation (for review, see Ref 55). This suggests that the mechanically induced activation of mTORC1 signaling may play a role in this increase in protein synthesis. To date, several studies have investigated this link using rodent models of passive stretch ex vivo, in vivo electrically induced contractions, and synergist ablation (SA)- or myotenectomy (MTE)-induced chronic overload, and with acute bouts of resistance exercise in humans. Regarding ex vivo passive stretch, one study has reported that rapamycin (150 nM) completely inhibited the mechanically induced increase in protein synthesis (42), while a more recent study has surprisingly reported that the induced muscle-specific knockout of raptor had no effect on the poststretch increase in protein synthesis, despite the severe inhibition of $p70^{S6K1}$ T389 phosphorylation (101). The reason for these two divergent results is unclear, but the data could suggest that a rapamycin-sensitive, but mTORC1-independent, mechanism is responsible for passive stretch-induced increases in protein synthesis. In this case, it would be of interest in future studies to combine rapamycin with passive stretch in the inducible raptor KO mouse model.

Another recent unexpected finding was that rapamycin, at a dose that completely inhibits SA-induced muscle hypertrophy in mice ($0.6\ mg \cdot kg^{-1} \cdot day^{-1}$) (30), had no effect on the associated increase in protein synthesis (101). Furthermore, the same study also reported that the induced muscle-specific knockout of raptor had no effect on the MTE-induced increases in protein synthesis, despite ablating the hypertrophic response (101). The inability of rapamycin to inhibit the SA-induced increase in protein synthesis may be related to its inability to inhibit all of mTORC1's downstream effects, such as 4E-BP1

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phosphorylation, which is known to be sustained during SA-induced overload, despite the presence of rapamycin (29). Importantly, these findings indicate that, at least under these two chronic overload conditions, the protein synthetic response was completely dissociated from the hypertrophic response, suggesting that there must have been a concomitant rapamycin-induced increase in protein degradation to account for the lack of protein accretion. In support of such a proposition, it is well known that mTORC1 is a negative regulator of autophagy and that rapamycin activates autophagy [for review, see Ref. (50)]. Furthermore, recent evidence has shown that rapamycin can upregulate ubiquitin proteasome system-mediated proteolysis (104, 105). Clearly, further studies are required to clarify the mechanism(s) at work in these chronic forms of overload with and without rapamycin or raptor.

Using a more physiological model of acute resistance exercise in rats (i.e., in vivo evoked eccentric contractions), West et al. (95) has shown that a single dose of rapamycin (1.5 mg/kg) abolished the increase in protein synthesis at 6 h postexercise, but it only partially inhibited the elevated rates at 18 h postexercise, despite the continued inhibition of $p70^{S6K1}$ T389 phosphorylation. These data indicate that the early initial increase in protein synthesis could be governed by a rapamycin-sensitive mTORC1-dependent mechanism, whose influence declines over time, while either a mTORC1-dependent, but rapamycin-insensitive, mechanism (e.g., 4E-BP1 phosphorylation), or a completely mTORC1-independent mechanism, may play a more dominant role at later time points. In a second rat study, using in vivo evoked isometric contractions, rapamycin (1.5 mg/kg) completely inhibited the increase in protein synthesis at 1 h, but not at 3 or 6 h, postcontraction (62). Interestingly, the increases in protein synthesis at 1, 3, and 6 h were inhibited by the active site mTOR kinase inhibitor, AZD8055, suggesting that either a mTORC1-dependent but rapamycin-insensitive, and/or mTORC2-dependent, mechanism(s) may play a role in the later time points in this model.

To date, only two human studies have used rapamycin to investigate the role of mTORC1 in the regulation of a resistance exercise-induced increase in protein synthesis. The first showed that rapamycin (12 mg) completely inhibited the early (1–2 h) resistance exercise-induced increase in protein synthesis (20); however, rapamycin only inhibited the early exercise-induced increase in $p70^{S6K1}$ T389 phosphorylation at 1 h, but not at 2 h, postexercise, and had no effect on 4E-BP1 T37/46 phosphorylation. In a more recent study, blood flow-restricted resistance exercise induced a biphasic increase in protein synthesis at 3 h and 24 h postexercise (35). Interestingly, while a single dose of rapamycin (16 mg) completely blocked the 3-h postexercise increases, there appears to be trend for rapamycin to only partially inhibit the increase at 24 h, despite the continued inhibition of $p70^{S6K1}$ T389 phosphorylation (35). Although this finding may support the idea that rapamycin-insensitive mTORC1-dependent, or mTORC1-independent, mechanism(s) may play greater roles at later time points, again, it cannot be ruled out that the amount/activity of intracellular rapamycin may have diminished over the >24-h period, such that it was no longer able to inhibit all of mTORC1's downstream targets.

Finally, one mouse study has examined the effect of a different type of mechanical stimuli, in the form of an acute 1-h bout of treadmill running, on myofibrillar and mitochondrial fraction protein synthesis rates, with and without rapamycin (1.5 mg/kg) (68). This study found that endurance exercise induced a large increase in protein synthesis in both fractions at 30 min, 3 h, and 6 h postexercise. Importantly, rapamycin inhibited the early (30 min) increase in myofibrillar protein synthesis, but not the later time points and had no effect at all on synthesis rates of the mitochondrial fraction (68). The lower myofibrillar synthesis rates in the presence of rapamycin at 30 min, compared with 3 and 6 h, could not be accounted for by differences in markers of mTORC1 signaling. These data suggest that the early endurance exercise-induced increase in myofibrillar protein synthesis occurred via rapamycin-sensitive, possibly mTORC1-dependent mechanism, and that the later protein synthetic response may be mediated by a rapamycin-insensitive mechanism that could be mTORC1-dependent or mTORC1-independent.

In summary, the limited number of studies that have examined the relationship between mechanical overload and mTORC1-mediated increases in protein synthesis show a range of differing results that appear to depend on the model of overload (i.e., acute passive stretch vs. chronic overload vs. acute resistance exercise vs. acute endurance exercise), the species (human vs. mice vs. rat), the method of mTORC1 inhibition (rapamycin vs. raptor KO vs. active site mTOR kinase inhibitors), and the time point poststimulation. Nonetheless, with reference to the more physiological acute resistance and endurance exercise types of overload, there may be an emerging pattern that the early increase in protein synthesis is governed by a rapamycin-sensitive mTORC1-dependent mechanism, while at later poststimulation time points, the mechanism may change to a rapamycin-insensitive mTORC1-dependent or to a mTORC1-independent mechanism. Clearly, more research is required to clarify these issues.

Role of mTORC1 in mechanically induced increases in muscle mass. In regard to the role of mTORC1 in mechanically induced increases in muscle mass, pharmacological (rapamycin) and genetic evidence has established that mTORC1 plays an absolutely necessary role in chronic SA-induced muscle hypertrophy in mice (6, 8, 30). More recently, using the milder MTE form of chronic mechanical overload, it was found that the induced knockout of raptor in mouse skeletal muscle also completely ablated the hypertrophic response (101). These data suggest that, under conditions of essentially constant mechanical overload, mTORC1 is absolutely necessary for the increase in muscle mass. Using a more physiological 4-wk model of rat resistance training, however, Ogasawara et al. (61) has shown that rapamycin (1.5 mg/kg) only inhibited ~50% of the training-induced muscle hypertrophy, suggesting that, under these conditions, there are rapamycin-sensitive and rapamycin-insensitive components responsible for the muscle growth response. This may be explained, in part, by the fact that rapamycin did not inhibit all of mTORC1's downstream effects [i.e., rapamycin fully inhibited $p70^{S6k1}$ T389 phosphorylation but had no effect on the proportion of hyperphosphorylated 4E-BP1 (61)]. Alternatively, these findings may suggest that mTORC1-independent mechanisms also play a role in promoting muscle hypertrophy in this more intermittent type of mechanical loading compared with constant mechanical overload models.

To date, while numerous studies have shown that increased mechanical loading in humans leads to increases in various

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markers of mTORC1 signaling (for review, see Ref. 28), there are no human studies directly demonstrating that mTORC1 signaling is required for mechanical overload-induced muscle growth. Despite this, some, but not all, studies have correlated the initial acute resistance exercise-induced increases in mTORC1 signaling with the prolonged training-induced increase in muscle mass [e.g., (85)]. More recently, resistance training at the same relative intensity for 6 wk (3 times per week) with untrained subjects, combined with deuterium oxide-derived measures of relatively long-term changes in muscle protein synthesis, showed that increases in protein synthesis and muscle hypertrophy were greatest in the first 3 wk of training and that these essentially plateaued over the second 3 wk (9). This decrease in the protein synthetic response to a bout resistance exercise after a period of training is in line with previous studies (e.g., 67). Importantly, however, the training-induced changes in long-term protein synthesis and muscle hypertrophy were paralleled by changes in markers of mTORC1 signaling. More specifically, the initial bout of resistance exercise induced a robust increase in $p70^{S6K1}$ T389, and a slight decrease in eEF2 T56, phosphorylation (but no change in 4E-BP1 T37/46 phosphorylation), and these responses were essentially absent by 3 and 6 wk (9). These data suggest a correlation between mTORC1 signaling, protein synthesis, and muscle growth in response to resistance exercise and support the hypothesis that mTORC1 signaling may be required for mechanically induced muscle hypertrophy in humans, at least in the early stages of training. Furthermore, these data suggest that the ability to activate mTORC1 may become desensitized over time, despite training at the same relative intensity. Further research is required to elucidate the mechanism behind this phenomenon.

In summary, rodent studies have shown that while muscle cell-specific mTORC1 appears to be fundamentally important for muscle fiber hypertrophy induced by chronic mechanical loading, it may only play a partial role in more intermittent types of acute resistance exercise, with mTORC1-independent mechanisms also possibly involved in the hypertrophic response to this type of exercise. While there is currently no direct evidence that mTORC1 is required for mechanical overload-induced muscle growth in humans, a limited amount of evidence exists suggesting a correlation between the activation of mTORC1 and muscle hypertrophy in human muscle but that the activation of mTORC1 by resistance exercise is diminished with training.

SOME REMAINING QUESTIONS

From the above discussion, it is clear that significant progress has been made in our understanding of the role of mTORC1 in the regulation of protein synthesis and skeletal muscle mass; however, it is also clear that many questions remain to be answered. Other questions, not specifically raised above include what is the upstream mechanosensor that is responsible for the mechanically induced activation of mTORC1? Is the sensor the same for all types of mechanical stimuli, or are there several sensors located in critical regions of the muscle cell (e.g., costameres or the sarcomeric Z-line), whose relative role may vary depending on the type of mechanical stimuli (i.e., passive stretch, active stretch/eccentric contractions, concentric and isometric contractions)? Furthermore, how does the sensor(s) transduce the signal to mTORC1? Regarding this last question, progress is being made with a recent focus on the role of phosphatidic acid, changes in TSC1 or TSC2 phosphorylation and/or cellular location, and on changes in the localization of mTOR within the cell (28, 39, 46, 91, 100); however, other mechanisms may be involved. Looking further downstream, we still only have limited knowledge of the events that are required for mTORC1-mediated increases in protein synthesis and muscle fiber growth. Indeed, of the mTORC1-related targets described at the beginning of this review, there is very little information as to whether any or all are necessary for mechanically induced protein synthesis and hypertrophy in skeletal muscle cells and, if so, to what extent. Moreover, there could be, as yet, unidentified direct or indirect mTORC1 targets that are activated by mechanical stimuli? Hopefully, future studies examining changes to the muscle proteome and phospho-proteome (96), combined with rapamycin and/or raptor knockout will begin to shed light on this issue. Also, what mTORC1-independent mechanisms could be involved in the prolonged increases in protein synthesis? Finally, why does the mechanical activation of mTORC1 and protein synthesis appear to diminish over time with resistance training, despite maintained relative exercise intensity (9). Does the mechanoreceptor become desensitized over time and, if so, can it somehow be resensitized? Importantly, answers to these questions will hopefully provide novel insights into the regulation of skeletal muscle mass that may eventually be translated into novel exercise programing and/or targeted pharmacological therapies aimed at preventing muscle atrophy/wasting and/or increasing muscle mass.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

C.A.G. drafted manuscript; C.A.G. edited and revised manuscript; C.A.G. approved final version of manuscript.

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