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Signals mediating skeletal muscle remodeling by resistance exercise: PI3-kinase independent activation of mTORC1

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- For over 10 years, we have known that the activation of the mammalian target of rapamycin complex 1 (mTORC1) has correlated with the increase in skeletal muscle size and strength that occurs following resistance exercise. Initial cell culture and rodent models of muscle growth demonstrated that the activation of mTORC1 is common to hypertrophy induced by growth factors and increased loading. The further observation that high loads increased the local production of growth factors led to the paradigm that resistance exercise stimulates the autocrine production of factors that act on membrane receptors to activate mTORC1, and this results in skeletal muscle hypertrophy. Over the last few years, there has been a paradigm shift. From both human and rodent studies, it has become clear that the phenotypic and molecular responses to resistance exercise occur in a growth factor-independent manner. Although the mechanism of load-induced mTORC1 activation remains to be determined, it is clear that it does not require classical growth factor signaling.

mammalian target of rapamycin complex 1

Since the elegant experiments by Wong and Booth (83–85) in the late 1980s, we have known that the load across a muscle is the primary determinant of skeletal muscle hypertrophy (for review, see Ref. 3). However, how the load across a muscle is transduced into an increase in the rate of protein synthesis and the accretion of contractile protein has yet to be determined. The first molecular marker of the increase in muscle mass and strength following resistance exercise was the phosphorylation and activation of the 70-kDa ribosomal S6 protein kinase (S6K1; Refs. 4, 51, 73). Subsequent work demonstrated that S6K1 was under the control of the mammalian target of rapamycin (mTOR) complex 1 (mTORC1) and that the specific mTORC1 inhibitor, rapamycin, could directly block muscle growth (11). These initial studies have since been supported by transgenic models of mTORC1 inactivation (59) and dissociation (9) and global knockout of S6K1 (66). Collectively, these studies implicate mTORC1 and S6K1 as principal mediators of muscle growth in skeletal muscle.

The original observation that S6K1 correlated with muscle growth was striking since S6K1 had formally been identified as a mitogen (or growth factor) activated protein kinase (42, 55, 58) whose activity was correlated with the phosphorylation of ribosomal proteins and the mitogen-induced increase in protein synthesis (74). Since increased load across a muscle increased the rate of protein synthesis (16, 50, 83, 84) and growth factors like insulin-like growth factor (IGF)-1 were known to induce muscle hypertrophy (18), the natural hypothesis was that muscle hypertrophy in the adult was the result of an increase in mitogen signaling following resistance exercise. In support of this hypothesis, treating myotubes with IGF-1 in vitro resulted in the activation of S6K1 and an increase in the size of the myotubes (60); following loading there was an increase in the expression of IGF-1 mRNA in muscle that occurred even in the absence of growth hormone (23); and stretching myotubes in vitro resulted in the release of a factor into the media that was able to activate S6K1 (5). So the growth factor paradigm was set:

1) resistance exercise increased the autocrine release of IGF-1;
2) IGF-1 bound to the IGF-1 receptor on the cell surface and activated S6K1 via the canonical growth factor pathway;
3) activation of S6K1 led to an increase in protein synthesis; and
4) when repeated at a sufficient frequency, this autocrine signaling response led to an increase in muscle mass and strength (Fig. 1).

Deconstructing the paradigm: growth factor-independent activation of S6K1

Over the last few years, pharmacological, genetic, and transgenic approaches have been employed by several groups to systematically manipulate the IGF1-PI-3 kinase-PKB pathway...
and determine its importance in load-induced muscle growth. The first suggestion that the increase in S6K1 activation following loading could occur independent of canonical growth factor signaling came in 2004 when Hornberger et al. (40) showed that ex vivo stretch of isolated extensor digitorum longus muscles in the presence of the PI3-kinase inhibitor wortmannin did not prevent the stretch-induced activation in S6K1. In these experiments, insulin activation of S6K1 was prevented by both wortmannin (PI-3 kinase inhibitor) and rapamycin (an inhibitor of mTORC1), indicating that insulin activated S6K1 by the canonical growth factor PI-3 kinase/mTORC1/S6K1 pathway. However, stretch activation of S6K1 occurred in a rapamycin-sensitive, wortmannin-independent mechanism, demonstrating for the first time that PI-3 kinase was not required for stretch-induced activation of mTORC1. In support of this hypothesis, protein kinase B/Akt, the molecular link between PI-3 kinase and mTORC1, was not required for S6K1 activation by stretch (40). However, it should be noted that both wortmannin and rapamycin blocked the stretch-activated increase in protein synthesis, suggesting that PI-3 kinase was, in the least, permissive for the actions of stretch on protein synthesis.

The next major blow to the growth factor paradigm came in 2008 from the laboratory of Espen Spangenburg (67). Using a mouse with skeletal muscle that harbored an inactivating knockin mutation in the IGF receptor (MKR) that prevented receptor activation by insulin or IGF-1, Spangenburg and colleagues (67) showed that, in the absence of IGF signaling, load-induced muscle hypertrophy was identical to that of wild-type mice, even though muscles from the MKR mice did not grow as large developmentally. At 8 wk of age, the muscles of the MKR mice were ∼30% smaller than wild-type mice, and neither insulin nor IGF-1 could activate PKB/Akt, showing that IGF-1 and PI-3 kinase signaling is required for developmental muscle growth. However, following 7 or 35 days of mechanical overload, both the wild-type and the MKR mice showed an increase in muscle mass of ∼50 and 90%, respectively. Furthermore, the phosphorylation of both PKB and S6K1 was completely normal in the MKR mice in response to overload.

However, two points should be made regarding the MKR mouse model. First, it should be noted that a follow-up study by the same group (82) showed that, in response to an acute bout of resistance exercise, the MKR mice did show a delayed and diminished phosphorylation of S6K1 in response to resistance exercise, suggesting that input from PI-3 kinase maybe required for full activation of mTORC1. Second, Heron-Milhavet and colleagues (38) recently reported that the MKR mice demonstrate impaired muscle regeneration and myoblast differentiation following injury. Furthermore, the authors observed an increase in progenitor cell number in the MKR mice and speculated that the normal response to overload hypertrophy reported by Spangenburg and colleagues may be through compensatory hyperplasia (increased activation and proliferation of progenitor cells), as has previously been reported (27). However, it is not clear how this would occur if myoblast differentiation were impaired. In fact, the inability to regenerate and a decrease in myoblast differentiation suggests a decrease in satellite cell activation and fusion in the MKR mice, bringing into question the need for these cells for hypertrophy in the mouse.

Using another genetic model (electroporation of DNA into adult muscle), Goodman et al. (33) have shown that activation of PI-3 kinase is not required for mTORC1-induced skeletal...
muscle hypertrophy. In these experiments, the small G-protein activator of mTORC1, Rheb (ras homologous protein enriched in brain), was overexpressed either in vitro or in vivo to increase mTORC1 activity in a PI-3 kinase-independent fashion. The authors were then able to demonstrate that augmenting Rheb levels increased cap-dependent translation and resulted in a 64% increase in the cross-sectional area of muscle fibers. Even though Goodman et al. (32) did not demonstrate Rheb association with mTORC1 following load-induced hypertrophy, their data can be interpreted to show that PI-3 kinase-independent skeletal muscle hypertrophy is genetically possible in vivo.

It is one thing to demonstrate that experimental models can be genetically created where adult muscle growth can occur in a growth factor-independent manner; it is another thing entirely to show this in humans. West et al. (80) have done just that by taking advantage of the fact that resistance exercise using a large muscle mass increases circulating levels of anabolic hormones (1). Using heavy resistance exercise with the legs, West and colleagues were able to create a high hormone state. The high hormone state was characterized by a 5-fold increase in total testosterone, a 3-fold increase in free testosterone, and a 10-fold increase in both growth hormone and IGF-1. The authors then used a unilateral resistance exercise program in the arms where one arm performed resistance exercise immediately before heavy lifting with the legs (therefore in a high hormone state), whereas the other arm was exercised on a separate day without concomitant exercise of the legs (a low hormone state). Despite the huge differences in circulating hormone levels subsequent to each bout of exercise, there was no difference in either the increase in muscle fiber size or overall strength in the arms following 15 wk of training. Consistent with the absence of a difference in muscle strength or fiber size, they have also demonstrated that mTORC1 signaling was not different between the high and low hormone states (81). This work confirms earlier animal studies that showed that neither hypothalamic nor pancreatic hormones were required for load-induced skeletal muscle hypertrophy (30, 31). However, the fact that circulating hormones are not required for muscle hypertrophy does not preclude the possibility that local production of IGF-1 or another growth factor may play an important role in adult skeletal muscle hypertrophy.

Given the data from the Spangenburg and Hornberger laboratories using transgenic approaches, growth factor-independent hypertrophy is possible. However, whether this was due to compensatory changes in the muscle and whether autocrine signaling through tyrosine phosphorylation of the IGF-1 receptor and the insulin receptor substrates (IRS) to PI-3 kinase occurred normally remained an open question. We have recently directly tested this hypothesis in rat and mouse skeletal muscle in response to high-frequency electrical stimulation (37). In support of IGF-1-independent activation of mTORC1, at no time following resistance exercise (from immediately after to 48 h later) was there an increase in tyrosine phosphorylation of the IGF-1 receptor. Furthermore, we observed a decrease in signaling through IRS1/2 to PI-3 kinase. In sharp contrast to insulin, p85 associated with either IRS1 or IRS2 decreased in the first 3 h after the resistance exercise before returning to control levels (37). To determine whether this was a measurement error or possibly that PI-3 kinase was activated in an IRS1/2-independent manner, we generated mice with muscles devoid of the phosphoinositol phosphatase PTEN. We hypothesized that if PI-3 kinase was important in the activation of mTORC1 following loading, the PTEN knockout mice would show greater accumulation of PI(3,4,5)P3 and therefore greater PKB/mTORC1 activity and muscle hypertrophy. In contrast to this hypothesis, neither the phosphorylation of S6K1 following resistance exercise nor the increase in muscle mass following overload was different between the wild-type and PTEN knockout mice.

The activation of mTORC1 in a PI-3 kinase-independent manner is consistent with both the time course of PKB and S6K1 activation and the variability observed in PKB activation following resistance exercise (Fig. 2). We and others (54) have shown that following resistance exercise the activation of S6K1 precedes or occurs concomitantly with that of PKB and, in humans, the activation of PKB following resistance exercise is more dependent on the feeding state rather than the intensity of the exercise session (22). Since most animal studies occur in the fed state, whereas the feeding state is far more controlled in human studies, these findings are completely consistent with PKB being activated by an increase in nutrients resulting from the increase in blood flow during, or immediately following, exercise and not the exercise bout or the autocrine release of growth factors.

A NEW PARADIGM: MECHANOSENSING DRIVES LOAD-INDUCED SKELETAL MUSCLE HYPERTROPHY

Taken together, the above data support a new paradigm where mTORC1 is activated in a PI-3 kinase/PKB-independent manner. This shift in paradigm parallels research into the activation of mTORC1 by amino acids. Like resistance exercise, amino acids activate mTORC1 in a PI-3 kinase/PKB-independent manner. Instead of working through the PI-3 kinase/PKB pathway, amino acids activate mTORC1 by altering its subcellular location (45, 46, 61, 62). In the absence of amino acids, mTORC1 is diffuse throughout the cell. When amino acids are added back, mTORC1 is shuttled to the lysosomal membrane through its interaction with the Rag family of small G-proteins and the Ragulator, a protein scaffold found on the lysosomal membrane (45, 61, 62). Once at the lysosome, mTORC1 can interact with Rheb. The interaction with Rheb increases the basal level of mTORC1 activity and permits the activation of mTORC1 by growth factors and other stimuli (61). This model fits very well with what we know about skeletal muscle hypertrophy: 1) the basal level of mTORC1 activity (as determined by S6K1 phosphorylation) is extremely low; 2) the addition of amino acids increases mTORC1 activity and protein synthesis (7, 12, 13, 20); 3) the ability of amino acids and loading to increase protein synthesis is dependent on a permissive level of hormonal activity (8, 26, 40); and 4) growth factors and resistance exercise can activate mTORC1 and increase protein synthesis more efficiently in the presence of amino acids (57, 72, 75, 76). Interestingly, we have shown that resistance exercise increases the activity of the vesicular trafficking protein Vps34 (51). This might improve the trafficking of mTORC1 to the lysosome and permit the prolonged activation of mTORC1 following resistance exercise. However, whether
A number of protein complexes serve this purpose in muscle including the dystrophin-associated glycoprotein complex, integrin-associated costameric complex, and cell-cell adherins junctions. The role of some of these protein complexes in mechanosensing has been studied in the heart with some interesting results. In the heart, the costameres appear to be the primary mechanical sensor. β-Integrins (44, 65), the integrin-linked kinase (ILK) (6), melusin (14, 21), the muscle LIM domain protein (47), and PKB all are found in costameres and all play an important role in mechanosensing and cardiac hypertrophy. Of specific interest is ILK. ILK is primarily a scaffolding protein but may still harbor some kinase activity. The primary role of ILK in hypertrophy is due to its ability to interact and bring together β-integrins, PKB, and the rapamycin-insensitive companion of mTOR (rictor). Rictor coordinates the phosphorylation of PKB at Thr473 as part of the other known mTOR complex (mTORC2). The activation of PKB can lead to muscle hypertrophy in both the heart and skeletal muscle (11), suggesting that ILK may coordinate the conversion of the mechanical stimulus from the β-integrins into the chemical signal of PKB phosphorylation. However, as discussed above, PKBo/Akt1 is not required for the activation of mTORC1 in response to stretch. This means that either other isoforms can compensate for the loss of PKBo/Akt1 in muscle or the β-integrin/ILK/PKB/mTORC1 pathway is not the mechanosensor in skeletal muscle.

Another member of costameres and adherins junctions is focal adhesion kinase (FAK) (15). Work by Fluck et al. (28) demonstrated that the amount and activity of FAK increase within 24–36 h of the onset of stretch in the chicken ALD and in the rat soleus after overload. Furthermore, the degree of FAK expression and activity in muscles is related to their loading (34). For instance, the postural soleus muscle has greater expression and tyrosine phosphorylation of FAK than the gastrocnemius or plantaris (34). Moreover, unloading reduces the phosphorylation of FAK in the soleus and the concentration of FAK in the gastrocnemius and plantaris (34). This suggests that load may be sensed through FAK. However, unlike ILK, it is unclear what the downstream targets of FAK may be involved in this process; additionally, it has yet to be determined whether FAK is activated by acute resistance exercise with a time course consistent with it being proximal to mTORC1.

Calcium has also been proposed as a mechanosensor. Guharay and Sachs (35) first identified stretch-activated ion channels that could be blocked by the antibiotic streptomycin and the cation gadolinium. Spangenburg and McBride (68) have shown that treating animals with streptomycin or gadolinium before a bout of resistance exercise can decrease S6K1 activation without impacting muscle function, suggesting that these channels may be important in mechanosensing. Further support for this theory comes from the fact that increasing intracellular calcium in muscle cells using the calcium ionophore A23187 enhances both protein synthesis and degradation rates (43) much like resistance exercise. However, stretch in combination with A23187 enhances protein synthesis further than treatment with A23187 alone (43). This means that, although calcium influx may play a role in mechanosensing, it is not the only stretch sensor in muscle.

Goldberg and Goodman (32) were the first to observe that amino acid transport into loaded muscles was increased in both
normal and hyposectomized rats. An increase in amino acid uptake, specifically the branched chain amino acids, is also seen 3 h after resistance exercise in humans (10) and 90 min after resistance exercise in rats (51). As discussed above, amino acids, specifically leucine, are known to activate mTORC1 via the RagGTPases (45, 61, 62) and increase muscle protein synthesis, suggesting that amino acid influx may function as a mechanosensor in skeletal muscle. In fact, when muscle cells are stretched in vitro, the increase in amino acid uptake is required for stretch-induced activation of protein synthesis (77). Since the permeability of muscle to amino acids increases following resistance exercise and amino acids increase mTORC1 activity and protein synthesis, it is not surprising that protein supplementation in association with resistance exercise has a synergistic effect on protein synthesis rates and muscle hypertrophy (25, 48, 75). However, the influx of amino acids is not seen immediately after resistance exercise and rapidly returns to normal (3 h in the rat), even though mTORC1 activity remains high for at least another 15 h (51).

In the absence of external amino acids, protein degradation may play an important role in supplying amino acids for the activation of mTORC1 and protein synthesis (63, 64). Early studies in fasting humans demonstrated a direct association between protein synthesis and degradation following resistance exercise (56). However, when essential amino acids are supplied, the increase in degradation is attenuated (75), suggesting that degradation serves to increase internal amino acids, provide a source of essential amino acids for de novo synthesis, and possibly activate mTORC1.

Hornberger and his colleagues have suggested that the phospholipid cleaving enzyme phospholipase D (PLD) could serve as a mechanoreceptor. One of the products of PLD, the second messenger phosphatidic acid (PA), increases in response to 15–90 min of mechanical stretch (39) or within 2 min following the start of electrical stimulation (17). Hornberger and his group (39, 54) have used two chemically distinct inhibitors of PLD to demonstrate that blocking PLD prevents the production of PA in response to stretch and the mechanical activation of mTORC1 phosphorylates its downstream targets 4EBP and S6K1, upregulates miR-1 resulting in an increase in follistatin and myostatin inhibition, and increases amino acid transporters possibly through the myc protooncogene. S6K1 in turn phosphorylates upstream binding factor (UBF) and elongation factor (EF) 2 kinase, resulting in a decrease in eEF2 phosphorylation.
mTORC1 (Fig. 3). Furthermore, the same group has shown that the increase in PA with in vivo contractions only occurs in muscles that will undergo hypertrophy (54). These data suggest that PA is important in the activation of mTORC1 by mechanical loading. However, whether PLD activation is the mechanical sensor or simply a marker of an upstream event has yet to be determined. RNA interference experiments have revealed that the mTORC1 activator Rheb binds to, and activates, PLD in a GTP-dependent manner (70). Knockdown of PLD prevents Rheb-induced activation of mTORC1, whereas knockdown of Rheb decreases the activation of PLD in response to serum (70). In contrast, Rheb overexpression activates PLD in serum-starved cells. Together, these data suggest that activation of Rheb recruits PLD to mTORC1 and enhances the local production of phosphatidic acid, resulting in the activation of mTORC1. Therefore, the activation of PLD by mechanical loading may simply reflect the upstream activation of Rheb and not that PLD is a mechanosensor per se.

CONCLUSIONS

Even though we have focused on the role of mTORC1 and S6K1 in the development of skeletal muscle hypertrophy, this is not the only mediator of adult muscle growth. Myostatin (78, 79), notch (19, 53), and microRNAs (24, 52) also play an important role in the development of muscle hypertrophy. However, whether these are distinct pathways or part of a coordinated system has yet to be determined. For instance, mTORC1 can regulate the production of miR-1, resulting in an increase in the endogenous inhibitor of myostatin, follistatin decreasing myostatin activity (71). In turn, myostatin can inhibit the activation of mTORC1 (2, 78). Together, these effects would produce a positive feedback loop following resistance exercise where the activation of mTORC1 would decrease myostatin signaling, the decrease in myostatin would increase mTORC1 activity, and the cycle would repeat. It is likely that more than one factor is responsible for growth signaling in response to resistance exercise. It is possible that one sensor starts the cycle and the others function to maintain mTORC1 activity at various times after resistance exercise. Since the ability to increase protein synthesis and accrue muscle is an important and evolutionarily conserved process, it would not be surprising if significant redundancy existed, making it difficult to dissect out the hierarchy of these signals. It is important to make clear that for the sake of this concise review series we decided to discuss factors involved in muscle hypertrophy following mechanical loading. As noted in the recent Point-Counterpoint series in this Journal (29, 69), IGF-1 has important functions in development, the remodeling of skeletal muscle, the extracellular matrix, and the activation of progenitor cells. Certainly, in humans it is likely that many molecular signals are required to maintain or increase muscle mass. Therefore, future research, such as the innovative approach employed by West et al. (80), is needed to determine whether murine models translate completely to humans. These studies are extremely relevant because, with the growing elderly population and the anabolic resistance and muscle loss that occurs with age (49), viable clinical options to rectify this situation are desperately needed. These issues, as well as how mTORC1 is activated and what mTORC1 does, should keep us busy for years to come.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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