Cellular mechanisms regulating protein synthesis and skeletal muscle hypertrophy in animals

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Submitted 10 October 2008; accepted in final form 21 November 2008

Miyazaki M, Esser KA. Cellular mechanisms regulating protein synthesis and skeletal muscle hypertrophy in animals. J Appl Physiol 106: 1367–1373, 2009; doi:10.1152/japplphysiol.91355.2008.—Growth and maintenance of skeletal muscle mass is critical for long-term health and quality of life. Skeletal muscle is a highly adaptable tissue with well-known sensitivities to environmental cues such as growth factors, cytokines, nutrients, and mechanical loading. All of these factors act at the level of the cell and signal through pathways that lead to changes in phenotype through multiple mechanisms. In this review, we discuss the animal and cell culture models used and the signaling mechanisms identified in understanding regulation of protein synthesis in response to mechanical loading/resistance exercise. Particular emphasis has been placed on 1) alterations in mechanical loading and regulation of protein synthesis in both in vivo animal studies and in vitro cell culture studies and 2) upstream mediators regulating mammalian target of rapamycin signaling and protein synthesis during skeletal muscle hypertrophy.

IT IS WIDELY ACCEPTED that repeated bouts of resistance exercise/high-force contractions produce compensatory growth of skeletal muscle (35, 42, 47, 95). The increase in skeletal muscle mass results from rates of protein synthesis increased more than changes in protein degradation with the net result being an accumulation of protein and increased fiber area (66, 96). While the effect of resistance exercise/contraction on muscle mass has long been recognized, the mechanisms underlying the link between high resistance contractions and muscle growth are, to date, not fully understood.

One of the important variables contributing to the growth response in skeletal muscle is the application of mechanical loading. For this review we use mechanical loading very generally as the application of force on the muscle or muscle cell. This force on the muscle can occur via active cross-bridge interactions (such as during concentric, isometric, or eccentric contractions) in a gravity-based environment or via external application of force such as passive stretching. The most established animal model for studying skeletal muscle hypertrophy in response to mechanical loading was described by Dr. A. L. Goldberg (38) and was referred to as work-induced muscle growth. This model is now commonly known as the synergist ablation/mechanical overload model and is a surgical model that involves cutting of the tendon and removal of the gastrocnemius muscle, resulting in loading and compensatory growth of the remaining plantar flexors, the plantaris, and soleus muscles. These muscles are involved in maintenance of posture and walking so are loaded by the body weight of the animal. Muscle mass changes are fairly rapid and the growth is robust, ranging from 40 to 200% depending on the muscle and species studied (7, 37, 38, 53, 56). Many labs use this model in their research programs to study molecular and cellular mechanisms associated with hypertrophy and while the extent of the surgery varies among labs, the fundamental concept of overload remains the same (3, 6, 44).

The early papers in skeletal muscle compensatory hypertrophy established that rates of protein synthesis are enhanced by 8 h after surgery and remain elevated for over a week during the growth response (37, 75). Consistent with studies of muscle growth in humans, these studies in rodents illustrate that a robust and prolonged increase in protein synthesis is associated with the hypertrophic response to increased mechanical loading (35, 37, 85). Because of the known association between tissue protein synthesis and hormones such as growth hormone and insulin, Goldberg’s group (38, 40) performed studies of muscle growth in hypophysectomized or diabetic rats. He found that skeletal muscle hypertrophy occurs to the same extent in the absence of circulating pituitary hormones or insulin. In 1975, Goldberg et al. (39) proposed that increased tension development, either passive or active, was the critical event in initiating compensatory growth in mammals. Consistent with the role that mechanical tension plays in regulating skeletal muscle mass in vivo, stretch models have been used to demonstrate that mechanical tension also regulates protein synthesis in vitro (97–99). Using a cell culture model, Vandenberg and Kaufman (97, 98) demonstrated that intermittent stretch produces a large increase in protein synthesis and a smaller decrease in protein degradation in avian
myotubes. These data suggest that in cell culture, like models in vivo, the hypertrophic response to increased mechanical tension is due, in part, to an increased rate of protein synthesis.

Currently, the mechanisms proposed in regulation of resistance exercise/contraction-induced compensatory growth include, but are not limited to, the involvement of local growth factors and the signaling events induced by the mechanical stimulus (39, 66, 96, 99). Previous studies have shown that mechanical stimulation of muscle tissue/cells results in local release of growth factors such as fibroblast growth factor (FGF) and insulin-like growth factor-1 (IGF-1; 1, 2, 16, 41, 101). It has also been established that mechanical loading in skeletal muscle can induce increased expression of IGF-1 at both the mRNA and protein levels (3). In addition, many other studies have demonstrated that the addition of exogenous/recombinant IGF-1 leads to an increase in protein synthesis and skeletal muscle growth/hypertrophy both in vitro and in vivo (4, 77, 86, 103). Transgenic mice in which IGF-1 was overexpressed only in muscle also led to increases in fiber cross-sectional area and skeletal muscle mass (17, 72). The results of these studies have led to a model in which mechanical loading of skeletal muscle leads to production and release of IGF-1 that functions in an autocrine/paracrine mechanism to increase protein synthesis and growth (3, 23). However, this model has been challenged recently by Spangenburg et al. (90) who suggest that IGF-1 signaling is not necessary for mechanical overload-induced muscle growth. The investigators used a transgenic mouse overexpressing a dominant negative form of the IGF-1 receptor in skeletal muscle. This receptor could bind IGF-1 but is kinase inactive so does not lead to downstream signaling (90). They demonstrated that IGF-1 and insulin signaling through Akt/protein kinase B (PKB) was impaired but they found that skeletal muscle hypertrophy was not altered in response to mechanical overload. There were no differences in mechanical overload-induced growth responses in skeletal muscle of wild-type and transgenic mice. Furthermore, they also demonstrated that mammalian target of rapamycin (mTOR) signaling pathway, which was previously shown to be necessary for skeletal muscle growth (11, 86), was activated similarly by the overload stimulus in the muscle of both wild-type and transgenic mice. Results of this study indicate that activation of the IGF-1 receptor is not necessary for the induction of muscle growth in response to increased mechanical loading in vivo.

To complement the in vivo studies, several labs have employed both in vitro cell models and ex vivo tissue models to evaluate the contribution of mechanical loading on protein synthesis. These models are valuable as they allow for more defined hormonal and mechanical environments. As noted earlier, Dr. H. H. Vandenberg (97) performed the seminal work in which they stretched avian myotubes in vitro and found an increased rate of amino acid uptake, protein synthesis, and protein accumulation. Their models included both static stretch and stretch oscillations and clearly established biochemical links between mechanical stretch and regulation of protein synthesis (97–100). More recently, Hornberger et al. (54) used an ex vivo system to test the contribution of mechanical strain oscillations on the regulation of protein synthesis in mammalian muscle. Mouse extensor digitorum longus (EDL) muscles were incubated in an organ bath and passively stretched 15% of resting length for up to 90 min. The results of these experiments found that passive stretch was sufficient to induce increases in protein synthesis. Conditioned media experiments from these ex vivo stretch studies also suggested that the increase in protein synthesis seen with mechanical stretch oscillations was not due to release of growth factors from the stretched muscle (52, 54). Taken together, the results of the in vitro/ex vivo studies support the findings in vivo and demonstrate that mechanical stretch/loading is sufficient to increase rates of protein synthesis. Additionally, the results of these studies are suggestive that mechanical strain can activate protein synthesis independent of growth factor involvement.

**mTOR SIGNALING AND PROTEIN SYNTHESIS**

mTOR is a serine/threonine kinase of the phosphatidylinositol kinase-related kinase family and is highly conserved from yeast to mammals (61). mTOR signaling pathway has a wide range of functions including regulation of protein synthesis, cell proliferation, apoptosis, and autophagy. In mammalian cells, mTOR exists in two distinct multi-protein complexes, mTOR complex 1 (TORC1) and mTOR complex 2 (TORC2). TORC1 is a complex comprised of mTOR, regulatory associated protein of mTOR (Raptor), and G protein β-subunit-like (Gβλ/also known as mLST8). The TORC2 complex is comprised of mTOR, rapamycin-insensitive companion of mTOR (Rictor), stress-activated-protein-kinase-interacting protein 1 (SIN1), and Gβλ. For the purpose of this review, we will focus on mTOR/TORC1 signaling and regulation of protein synthesis during hypertrophy in skeletal muscle.

To date, numerous studies have shown that mTOR plays a critical role in regulating protein synthesis and cell growth/hypertrophy in skeletal muscle (11, 36, 54, 74, 86). Consistent with its role in regulation of protein synthesis, signaling through mTOR occurs in muscle after high force contractions/mechanical loading and is maintained for many hours (18–36 h) after a single bout of loading (10). In contrast, mTOR signaling is only transiently (<6 h) upregulated after low force contractions that do not elicit growth of skeletal muscle (10, 73). Also considered in this review is that mTOR receives and integrates many different upstream signals that can function as either activators or inhibitors of activity and many of these upstream regulators are known to be active in muscle following mechanical loading. For example, activators of mTOR include growth factors, amino acid availability, and mechanical strain, while factors that can inhibit mTOR function include energy stress (via AMPK-dependent regulation) and other stress response molecules (induction of REDD1/REDD2). In the following sections we provide a brief summary of known components of the mTOR signaling pathway and these are depicted in Fig. 1. More detailed reviews of mTOR regulation can be found elsewhere (80, 84, 108).

**GROWTH FACTOR/INSULIN SIGNALING AND REGULATION OF mTOR ACTIVITY**

The most well-defined pathway regulating mTOR activity in skeletal muscle is the IGF-1/insulin pathway. Stimulation of muscle with growth factors (e.g., insulin and/or IGF-1) leads to activation of phosphoinositide 3-kinase (PI3K), triggering multiple downstream signaling events associated with the control...
of cell cycle progression, differentiation, survival, and cell growth [see Frost and Lang (31) for a more comprehensive review on this topic]. Serine/threonine kinase Akt/PKB is a well-established target of PI3K, and Akt/PKB phosphorylation of glycogen synthase kinase 3 (GSK-3) leads to its inhibition and an increase in global protein synthesis through increase in the activity of eukaryotic initiation factor 2B (eIF2B; Ref. 106). Additionally Akt/PKB is a known upstream regulator of mTOR signaling through regulation of the tuberous sclerosis complex (TSC; Refs. 20, 58, 70, 79). The TSC protein complex is a heterodimer of TSC1 and TSC2 and functions as a negative regulator of mTOR activity. Cells null for TSC1 or TSC2, cells depleted of TSC1 or TSC2 by RNA interference, and human and mouse tissues deficient in TSC1 or TSC2, all have high mTOR activity (33, 43, 58, 79). Together with its partner TSC1, TSC2 functions as a guanosine triphosphate (GTP)ase activating protein (GAP) for a small G protein named Ras homolog enriched in brain (Rheb). It has been reported that Akt/PKB directly phosphorylates TSC2 on multiple residues (at least two sites, Ser939 and Thr1462; Refs. 55, 58, 70). This phosphorylation of TSC2 by Akt/PKB is thought to inhibit its GAP activity, allowing Rheb to accumulate in its active GTP-bound form. GTP-bound Rheb strongly stimulates mTOR activity, thus TSC2 functions as a negative regulator of mTOR through increasing the intrinsic rate of GTP hydrolysis to GDP on Rheb (34, 57, 60, 92). This leads to the well-established growth factor associated signaling cascade through PI3K-Akt/PKB-TSC1/TSC2-Rheb-mTOR seen in muscle as well as other cell types.

To date there have been very few studies that have incorporated analysis of TSC1/TSC2 or Rheb in the regulation of protein synthesis during skeletal muscle growth/hypertrophy. Consistent with the role of TSC complex as a negative regulator of muscle growth Wan et al. (104) reported that overexpression of human TSC1 in mouse skeletal muscle leads to 20% reduction of muscle mass and decreased fiber cross-sectional area in the tibialis anterior and EDL muscles compared with controls. In response to overexpression of TSC1, TSC2 was stabilized and this was associated with significant inhibition of mTOR signaling. Other studies in rodents have reported that TSC2 is modified by phosphorylation at several different sites (Ser1345 and Thr1462 sites) after ex vivo high-frequency electrical stimulation (8) or in vivo mechanical overload model (71). Experiments evaluating the mRNA levels of TSC1, TSC2, and Rheb were recently reported from human muscle biopsies following one bout of high-resistance contractions and nutritional supplements. Drummond et al. (27) found that mRNA levels of TSC1 and TSC2 (negative regulators of mTOR) were decreased and Rheb (positive regulator of mTOR) was increased at 6 h after single bout of resistance exercise in young subjects. While limited, these findings support the likely contribution of the TSC complex and Rheb to regulation of muscle protein synthesis and size.

**mTOR AND AMINO ACID AVAILABILITY**

In addition to growth factors, mTOR is also known to be regulated by the availability of amino acids (33, 46, 63, 68, 88). In mammalian cells, amino acid deprivation leads to decreased mTOR signaling and to decreased rates of protein synthesis, effects that are rapidly reversed by readition of amino acids (30, 46). Among the amino acids, changes in leucine levels alone are sufficient to regulate the phosphorylation state and activity of the mTOR pathway (46, 69). Several studies in rodents and human have shown that administration/ingestion of essential amino acids (specifically leucine) leads to increased/enhanced protein synthesis in skeletal muscle primarily through activation of mTOR signaling (5, 24, 25, 32, 102). Currently, it is widely accepted that amino acid availability affects mTOR signaling and protein synthesis rate; however, how amino acids regulate the mTOR signaling pathway remains poorly understood.

For instance, it has been proposed that amino acids activate...
mTOR in both a TSC1/TSC2-dependent (33) and TSC1/TSC2-independent (88) manner. Some studies suggest that Rheb plays an essential role in regulation of the mTOR activity in response to amino acids (57) and that nutrient deprivation may decrease Rheb binding to mTOR (68). However, other work implies that Rheb may not be involved in nutrient-induced mTOR signaling, but rather that nutrient poor conditions lead to alterations in the proteins associated in the mTOR complex and this results in decreased activity (63). Recently, a class III PI3K, hVps34, has been implicated in the amino acid-induced activation of mTOR signaling and this is independent of Akt/PI3K-TSC-Rheb pathway (15, 76). In addition, two research groups [Kim et al. (64) and Sancak et al. (87)] reported that a heterodimeric complex of the Rag proteins, a family of four related small GTPases, plays a fundamental role in amino acid-induced regulation of mTOR/TORC1 activity both in Drosophila and mammalian cells. It is still unclear, however, what the relationship is between hVps34 and the Rag proteins in activating mTOR/TORC1 in response to amino acids. The lack of consensus in the understanding of the mechanisms by which amino acids regulate mTOR suggests that other molecules, not yet identified, are involved in the signaling and/or the mechanisms for signaling might be varied among different cell types.

MECHANOTRANSDUCTION OF mTOR SIGNALING

Work by Bodine et al. (11) established that mechanical overload-induced signaling through mTOR was necessary for skeletal muscle hypertrophy. Since then, many studies have confirmed the association between mechanical loading and activation of mTOR signaling in mammalian muscle. However, there is still very little known about the actual biochemical events that link the mechanical loading of a muscle/muscle fiber to mTOR signaling. Ex vivo muscle stretch and in vitro muscle cell stretch models of mechanical loading have defined that 1) passive mechanical loading is sufficient to activate mTOR signaling in skeletal muscle, 2) stretch-induced activation of mTOR occurs in a PI3K-independent manner, and 3) mechanical stretch can activate mTOR in the absence of amino acids or growth factors (49, 50, 54). Our lab has found that treatment of the myotubes with cytochalasin D to disrupt the microfilament system (49) will block stretch-induced signaling to mTOR even though insulin activation of mTOR was still intact. These results suggest that there is a cytoskeletal requirement for communication of signaling pathway from mechanical stretch to mTOR. Other intracellular targets of stretch include phospholipase D1 (PLD1) and the lipid second messenger phosphatidic acid (PA) as the mediators of stretch-induced mTOR signaling in skeletal muscle (51). The PLD1 pathway has been proposed to contribute to the activation of mTOR through generating PA from the hydrolysis of phosphatidylycerol. PA binds specifically to the FRB domain of the mTOR in a manner competitive with inhibitor rapamycin/FKBP12 complex (28, 29). Recently, studies from Dr. Y. Chen’s laboratory (91) identified that PLD1 is required for Rheb activation of mTOR signaling, and their experiments place PLD1 downstream of Rheb and upstream of mTOR. Thus mechanical loading/stretch clearly acts independently of amino acids and growth factors to activate mTOR signaling in skeletal muscle. We believe that this mechanical pathway likely acts synergistically with changes in amino acid uptake and growth factor availability to contribute to the prolonged activation of mTOR signaling following high resistance contractions/mechanical overload.

CELLULAR ENERGY STATUS AND mTOR ACTIVITY

In addition to the availability of growth factors and amino acids, the rate of protein synthesis is regulated by the cellular energy status in muscle and non-muscle cells (13, 81). It has been shown that glucose deprivation, through use of a non-hydrizable glucose analog (2-deoxyglucose), results in decreased mTOR signaling (21). It is currently suggested that mTOR functions as a sensor of cellular energy state through input from the AMP-activated protein kinase (AMPK) pathway (60). AMPK is well known as a sensor of cellular energy status, which is regulated by changes in the cellular levels of AMP-to-ATP ratio (62). Inoki et al. (60) identified that, under low cellular energy conditions, activated AMPK phosphorylates TSC2 on Thr1227 and Ser1345 residues (these residues correspond to Thr1271 and Ser1387, respectively, in human TSC2) and enhances its inhibitory function leading to decreased mTOR activity. This research group has also reported that AMPK-dependent phosphorylation of TSC2 on Ser1387 primes TSC2 for further phosphorylation by GSK-3 on multiple residues. This series of phosphorylation steps leads to subsequent inhibition of mTOR activity under energy-deprived conditions (59). Additionally, a recent study by Gwinn et al. (45) reported that AMPK can directly phosphorylate the mTOR binding partner Raptor on two well-conserved serine residues (Ser722/Ser792), and Raptor phosphorylation is required to inhibit mTOR activity through cellular energy stress-induced AMPK activation. Consistent with these observations, it was demonstrated that activation of AMPK, by treatment with 5-aminoimidazole-4-carboxamide-1-β-d-ribonucleoside (AICAR), results in decreased protein synthesis and a repression of mTOR-mediated signaling in skeletal muscle in both in vitro and in vivo experimental models (12, 82, 107). Deshmukh et al. (22) reported that preincubation with AICAR completely inhibited insulin-induced activation of mTOR signaling using an ex vivo organ bath system. Thomson et al. (93) also reported that AICAR treatment inhibited mTOR activation, which is induced by high-frequency electrical stimulation of rat EDL muscle. Finally Thomson and Gordon (94) found a correlation between increased phosphorylation of AMPK and reduced muscle hypertrophy in aging rats.

These studies indicate that activation of AMPK in skeletal muscle will contribute to diminished protein synthesis through inhibiting mTOR signaling. However, it is still controversial whether an AMPK-dependent regulatory mechanism plays an active role in exercise/contraction-induced skeletal muscle growth responses. To address the potential role of AMPK in muscle growth, McGee et al. (71) used the compensatory hypertrophy model with mice lacking LKB1, the primary upstream kinase for AMPK. The hypothesis for this study was that decreased phosphorylation of AMPK, via loss of LKB1, would enhance mTOR signaling and lead to a greater degree of muscle hypertrophy in response to functional overload. To their surprise, loss of LKB1 did not alter the growth of skeletal muscle after 7 or 28 days of functional overload. Normal hypertrophic responses were accompanied in skeletal muscle of LKB1−/− mice compared with the wild-type strain. How-
ever, these authors found that while α2-AMPK activity was reduced in the muscles of these mice, α1-AMPK was still activated following overload in both the LKB1+/− mice as well as the wild-type mice. Thus the conclusion was that α2-AMPK is likely not an important molecule in the regulation of mechanical load signaling to mTOR and growth.

STRESS RESPONSE GENES REDD1/REDD2 AND mTOR SIGNALING

Another negative regulator of mTOR activity are two factors that were originally identified from a genetic screen for negative regulators of the Drosophila TOR pathway (83). The mammalian orthologs are called REgulated in Development and DNA damage responses 1 (REDD1) and REDD2 (also called RTP801/DDIT4 and RTP801L/DDIT4L, respectively). REDD1 is ubiquitously expressed in all cell types while expression of REDD2 is significantly enriched in human and mouse skeletal muscle (http://symatlas.gnf.org/SymAtlas/). Cros et al. (19) identified REDD2 (also named SMHS1) as a highly upregulated gene in soleus muscle undergoing atrophy following 14 days of hindlimb unloading (78). Biochemical studies of both REDD1 and REDD2 found that they inhibit mTOR kinase activity in non-muscle and muscle cells downstream of Akt/PKB but upstream of TSC2 (14, 18, 89, 105). In skeletal muscle, REDD1 has been shown to inhibit mTOR signaling and protein synthesis in response to dexamethasone and alcohol intoxication (67, 105). In addition, Kimball et al. (65) found that rapid degradation of REDD1 in muscle was associated with enhanced mTOR signaling. Recent studies in human skeletal muscle have also suggested that expression of REDD1 and REDD2 plays a role in regulating mTOR activity and protein synthesis (26, 27). These observations suggest the possibility that REDD1 and REDD2 are stress responsive negative regulators of mTOR signaling with implications for controlling skeletal muscle size.

SUMMARY

In summary, there is a long history of research in understanding the contribution of mechanical loading/resistance exercise to regulation of protein synthesis in skeletal muscle and growth. The goal of this short review was to provide a general overview linking what is currently known using well-established animal models and cell culture models of mechanical loading and hypertrophy with regulation of mTOR, a key kinase involved in protein synthesis and growth. One of the unique features seen across all models of skeletal muscle hypertrophy in humans and rodents is that rates of protein synthesis and signaling through mTOR stay elevated for hours to days after one bout of high-resistance contractions. We propose in this review that the prolonged activation of mTOR signaling, and thus rates of protein synthesis, is the cumulative result of synergism among several independent positive signals including mechanical loading, amino acids, and growth factors. We also suggest that resistance exercise/mechanical loading can act to dampen the inhibitory contributors to mTOR signaling via downregulation of the TSC complex and REDD1/REDD2 molecules. Thus to understand the critical molecular and cellular steps involved in resistance exercise-induced regulation of protein synthesis will require carefully designed experiments that will consider balancing both the positive and negative inputs to mTOR signaling in skeletal muscle.

GRANTS

This work was supported by National Institutes of Health AR-045617 to K. A. Esser and American Heart Association Postdoctoral Fellowship 0825668D to M. Miyazaki.

REFERENCES


mTOR REGULATION IN SKELETAL MUSCLE


