

Cellular and molecular events controlling skeletal muscle mass in response to altered use

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Abstract Gain or loss of skeletal muscle mass occurs in situations of altered use such as strength training, aging, denervation, or immobilization. This review examines our current understanding of the cellular and molecular events involved in the control of muscle mass under conditions of muscle use and disuse, with particular attention to the effects of resistance exercise/training. The DNA content, which is a critical determinant of protein synthesis by providing the amount of DNA necessary to sustain gene transcription, can be either increased (activation of satellite cells) or decreased (apoptosis) depending on muscle activity and ongoing physiological processes. In addition, several transcription factors are sensitive to functional demand and may control muscle-specific protein expression to promote or repress myofiber enlargement. The control of skeletal muscle mass is also markedly mediated by the regulation of transduction pathways that promote the synthesis and/or the degradation of proteins. Insulin-like growth factor-I plays a key role in this balance by activating the Akt/tuberous sclerosis complex 2/mammalian target of rapamycin pathway. Stimulation of this pathway leads to the concomitant activation of initiation and elongation factors resulting in the elevation of protein translation and the downregulation of ubiquitin proteasome components through Forkhead-box O transcription factors.

Keywords Ageing · Gene expression · Hypertrophy · IGF · Protein metabolism

Introduction

Skeletal muscle is capable of remarkable adaptations in response to altered activity. These adjustments to mechanical and metabolic demands elicit marked modifications of gene expression that could lead to gain (hypertrophy) or loss (atrophy) of muscle mass. Whereas endurance training leads to minor changes in skeletal muscle mass, strength training induces marked hypertrophy of exercising muscles. Histochemical analyses clearly show a 10 to 30% increase in muscle fiber cross-sectional area after 10–12 weeks of resistance training in sedentary subjects [146]; this rise reaching about 80% in weightlifting athletes [78]. On the other hand, muscle disuse (i.e., reduced muscle activity) resulting either from experimental designs (plaster cast immobilization, hindlimb suspension, denervation, bed rest, and tenotomy) or ongoing physiological (aging) and pathological processes (cancer, neuromuscular disorders, respiratory insufficiency, and sepsis) can lead to severe muscle mass loss [35, 98]. For instance, 2 to 5 weeks of immobilization reduces fiber cross-sectional area by about 50% ranging from 10 to 70% depending on muscle fiber type and duration of immobilization (reviewed in [152]). In some situations associated with catabolic states, amyotrophy cannot be primarily attributable to muscle disuse. Loss of muscle mass associated with these pathologies is beyond the scope of the current review and will not be discussed.

Assuming that myofibrillar proteins represent about 85% of the fiber volume [70], any situation altering the synthesis/degradation balance of myofibrillar proteins may thus contribute to muscle hypertrophy or atrophy. Consistently, the increase in soleus and plantaris muscle weights to overload is directly proportional to the increase in amino acid incorporation [53]. Similarly, animal and human studies showed significant increases in protein synthesis

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from 4.5 up to 48 h after an acute bout of resistance exercise (RE) or after 2 weeks of resistance training [166]. Elevated rates of myofibrillar or mixed muscle protein synthesis have been recorded in both fast- and slow-type muscles with eccentric, concentric, or isometric contractions [87, 97, 107, 118]. However, a single bout of RE also increases protein degradation, but to a lesser extent than protein synthesis, so that the net protein balance is increased [118]. One week of passive stretch, which is known to promote muscle growth, induces a similar pattern, i.e., a concomitant increase in both protein synthesis and degradation with the rise in synthesis being more pronounced than the one in degradation [55]. Nevertheless, a decrease in protein degradation may transiently occur at the early stages of hypertrophy process [55, 155]. Inactivity has opposite effects. Protein synthesis in skeletal muscle is reduced after 6 h of plaster-induced immobilization [23], after 14 days of bed rest [43], or in elderly [166]. Hindlimb suspension also decreases protein synthesis in gastrocnemius and soleus muscles and elevates protein degradation leading to protein loss [56, 152]. Similarly, muscle breakdown occurs after sciatic nerve section in rat soleus and EDL muscles [54]. In summary, conditions associated with skeletal muscle hypertrophy are characterized by a strong increase in protein synthesis leading to positive nitrogen balance, whereas muscle disuse is accompanied by a rapid and transient decrease in protein synthesis together with a marked increase in protein degradation.

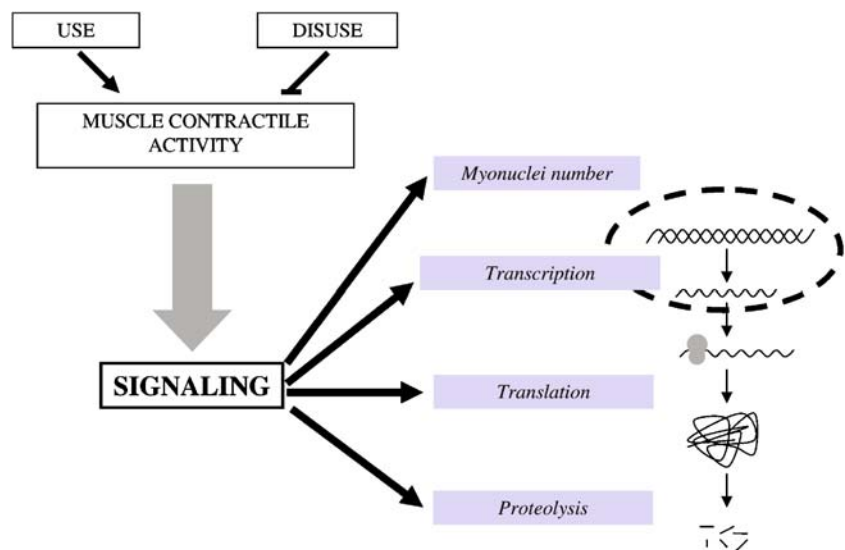
While these adaptations are now well described, the molecular and cellular mechanisms responsible for skeletal muscle mass gain or loss in response to use and disuse begin to be understood. Theoretically, skeletal muscle gene expression can be controlled by regulating the number of myonuclei (DNA content), muscle gene transcription,

translation, and/or muscle protein degradation (Fig. 1), each one of these events being susceptible to be the target of regulatory influences triggered by altered use. The purpose of the present review is to depict our recent knowledge about the mechanisms regulating muscle mass in situations of altered use. Particular interest will be accorded to the influence of RE because of its potential role for compensating disuse-related atrophy of skeletal muscle [4, 41, 63].

DNA content

From a theoretical point of view, the number of myonuclei is a critical determinant of protein synthesis capacity by providing the amount of DNA necessary to sustain gene transcription. The relation between myofiber size and myonuclei number gave rise to the concept of myonuclear domain, which is the amount of cytoplasm supported by a single myonucleus. Although the myonuclear domain should normally be expressed as a volume ($\mu\text{m}^3/\text{myonucleus}$, [24]), most of studies present the fiber cross-sectional area per nucleus (myonuclear domain area). Particularly, Kadi et al. [80] and Petrella et al. [117] have suggested in human that there may be a ceiling size of the myonuclear domain area of about $2,000 \mu\text{m}^2$ beyond which a fiber will not be able to hypertrophy unless it can add more myonuclei. Although caricatural, this ceiling size may explain why some studies reported an increase in myonuclear number (pre-training myonuclear domain $\approx 2,000 \mu\text{m}^2$) [80] in response to strength training while others did not (pre-training myonuclear domain $< 2,000 \mu\text{m}^2$) [79, 117]. During unloading-induced muscle atrophy, a reduction in the myonuclear domain area has been reported [4, 5]. This may result from a greater (or faster) decrease in fiber cross-sectional area than in

Fig. 1 How muscle use and disuse may control skeletal muscle mass? Muscle contractions can cause the release of factors from non-muscular origin (hormones) and muscular origin (autocrine/paracrine and intracellular factors). These factors may in turn modulate muscle mass by altering the number of myonuclei, transcriptional and translational capacity, and the rate of protein degradation



myonuclei number (Fig. 2a) as observed during denervation [136]. Aging-related atrophy seems to involve a different sequence of events. Brack et al. proposed that the decrease in myonuclei number with aging does not follow fiber size reduction, but rather drives it [24]. In this situation, myonuclear death will result in a transient increase in the myonuclear domain, leading to a cytoplasmic loss and ultimately to the restoration of myonuclear domain size (Fig. 2a).

Even if terminally differentiated myotubes have been shown to dedifferentiate and proliferate when stimulated with *msx1* expression [112], these events are rare and myonuclei of mature myofibers are generally considered to be post-mitotic. In this context, supplemental genetic material can be only brought by satellite cells. Satellite cells can be activated in response to traumatic lesions requiring muscle regeneration [37]. Once activated, satellite cells proliferate and fuse together and/or with preexisting fibers to regenerate muscle tissue. Satellite cells can be also activated when the load placed upon the muscle increases. Indeed, some markers of satellite cell activation (cyclin D1) and differentiation (p21) are increased after acute RE [19, 84] or strength training in humans [80]. In accordance with these findings, studies on strength-trained athletes or on

subjects performing resistance training evidenced significant increase in satellite cell number [79, 80, 130]. What is the physiological significance of such an activation after RE? Are the satellite cells activated to repair muscle damage after strength exercise, and/or the incorporation of additional nuclei is required to enhance the synthesis capacity of the fiber and promote hypertrophy? Irradiation experiments (aimed at inhibiting satellite cell activation) strongly suggest that supplemental nuclei addition from the satellite cell pool is necessary for marked hypertrophy. Indeed, irradiated fibers, which do not hypertrophy after surgical overloading, do not exhibit increase in DNA content nor in myofibrillar proteins [1]. Nevertheless, synergistic ablation is an extreme model of muscle overloading and may likely induce damage and subsequent satellite cell activation [75]. This raises the question of whether satellite cells can be involved in skeletal muscle hypertrophy independently of any ongoing reparation/regeneration processes. Although provocative, the idea that one may need to break muscle to activate satellite cell and build more muscle should be reasonably questioned. The development of new methods to directly assess the contribution of satellite cells in skeletal muscle hypertrophy would thus be helpful. In addition, the contribution of satellite cells in muscle hypertrophy needs to be assessed in a more physiological condition such as resistance training in human.

The number of myonuclei in single muscle fiber decreases in response to reduced load such as spaceflight [5, 34], hindlimb suspension [4, 91], immobilization [143], or denervation [136]. Aging may be also associated with a decline in the number of satellite cells and nuclei per fiber [24, 79]. The loss of myonuclei with disuse results from an increase in myonuclear death by apoptosis. Consistently, elevated levels of DNA fragmentation and expression of pro-apoptotic gene such as Bax or caspase-3, caspase-6, caspase-9, or caspase-12 have been recorded after unloading [18, 91, 139, 142] or with aging [142].

Numerous growth factors are known to regulate satellite cell activity, among which insulin-like growth factor (IGF)-I [6] and myostatin [100] are of particular interest. Messenger RNA (mRNA) and protein content of IGF-I correlate with and preceded the increase in whole muscle DNA content in response to muscle overload [2]. Mechano-growth factor (MGF; a variant of IGF-I, see below) and hepatocyte growth factor also promote satellite activation [7, 164]. As mechanical stress induces HGF release and increases MGF mRNA, these factors may contribute to the regulation of myonuclei accretion when muscle load is increased. In addition, administration of testosterone, a strong anabolic agent whose serum concentration is increased after strength training [86], results in an increase in both satellite cell and myonuclei numbers [78, 141]. On the contrary, satellite cell activation

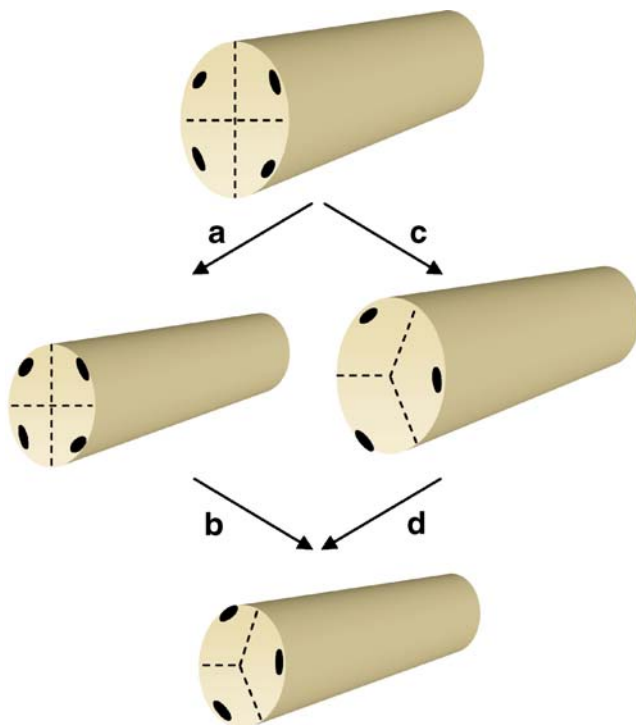


Fig. 2 Hypothetical mechanisms of myonuclear loss associated with muscle fiber atrophy. *a* Reduction in protein content without myonuclear loss (decrease in myonuclear domain area) and *b* subsequent loss of myonuclei. *c* Loss of myonuclei with constant protein content (increase in myonuclear domain area) and *d* subsequent decrease in protein content. Note that cross-sectional area within each of the triangles on *a* and *d* is similar

and self renewal is repressed by myostatin, a master negative regulator of skeletal muscle mass [100]. Other growth factors are also well known to regulate satellite cell activation and proliferation both in vitro and in vivo [67]. The physiological relevance of these factors during muscle atrophy and hypertrophy is potentially valuable but is still poorly investigated.

Transcription

The expression of muscle-specific proteins can be enhanced or repressed by numerous transcription factors. They may thus be critical in coordinating the response of skeletal muscle to altered use. Myogenic regulatory factors (MRFs), including MyoD, myogenin, Myf5, and MRF4, have been originally described to play major role in myogenesis [113]. Although sometimes controversial, increased muscle load appears to up-regulate myoD, myogenin, and MRF4 expression (Table 1) with differences between muscle fiber types, experimental design, and protocol duration. It remains to determine whether this is directly linked to changes in muscle-specific gene expression. Data regarding MRF expression during disuse are less consensual since some studies report a decrease [94] or no change [69] in MRF expression, while others show an increase [64, 147]. The significance of such variations is not clearly defined but may be related to the shift in fiber phenotype occurring

with reduced activity. Novel advances have been brought by the use of transgenic models. For example, soleus muscle weight did not differ after 2 weeks of unloading between wild-type and MyoD $-/-$ mice [140]. However, myosin heavy chain IIB expression was reduced in the KO animals, suggesting that MyoD rather drives fiber phenotype in this situation. Taken together, these data indicate that the role of MRFs in controlling muscle mass would be more effective under conditions of increased muscle load. Another level of regulation is provided by the inhibitors of differentiation (Id), a family of proteins that prevent MRFs binding to DNA, whose expression is enhanced during denervation, unloading, and aging [8, 59]. A number of molecular partners have also been demonstrated to interact with and modulate the activity of MRFs, such as pCAF, p300 [120], and Sirt1 [46]. Most of these studies have been done during in vitro myogenesis and the relevance of these observations in adult skeletal muscle during altered use needs to be established.

Several transcription factors involved in skeletal muscle remodeling are controlled in a Ca^{2+} -dependent manner, notably by the Ca^{2+} -sensitive phosphatase, calcineurin. Nuclear factor of activated T cells has been well implicated in skeletal muscle-specific gene expression and cardiomyocyte growth [106]. However, its influence on skeletal muscle hypertrophy is controversial. Indeed, some studies reported that calcineurin inhibition by cyclosporin A prevented muscle growth/hypertrophy both in vitro [109, 137] and in vivo

Table 1 Myogenic regulatory factors mRNA level in response to increased muscle use

Model	Muscle	Species	Myf5	MyoD	Myogenin	MRF4	Reference
Acute							
HFES	VL	Human		↑	↑		[19]
HFES	MG	Rat			↑		[62]
RE	VL	Human	ns	↑	↑	↑	[165]
RE	VL	Human	ns	↑	ns	↑	[122]
RE	VL	Human		ns			[66]
Chronic							
RT (16 weeks)	VL	Human		↑	↑		[14]
RT (10 weeks)	VL	Human	ns	ns	↑	ns	[69] ^a
RT (8 weeks) rest	VL	Human		ns	ns		[88]
post-ex				ns	↑		
Compensatory overloading (3 months)	Pla	Rat			↑ 1 st to 3 rd day		[1]
Stretch overload (6–72 h)	ALD	Quail	↑	↑		↑	[95]
	Pat			↑		↑	
Compensatory overloading (3 days)	Sol	Rat		↑ (1 st day)			[114]
	Pla						
Stretch (2–3 weeks)	ALD	Chicken		ns	↑		[28]
Stretch (2 days)	Sol	Rat			ns	↑	[94]
	Pla				↑	ns	

HFES High frequency electrical stimulations, RE resistance exercise, RT resistance training, VL vastus lateralis, MG medial gastrocnemius, Pla plantaris, ALD anterior latissimus dorsi, Pat patagialis, Sol soleus, ns no significant change

^a Protein level

[38], whereas others did not prevent muscle hypertrophy in response to pharmacological blockage of calcineurin [22, 41, 138]. Discrepancies between studies may depend both on muscle phenotype and stage of myofiber growth, as well as the degree of calcineurin inhibition by cyclosporin A [105]. Finally, studies from transgenic mice support the idea that calcineurin has a limited influence on skeletal muscle hypertrophy but major role in fiber phenotype regulation [39, 110, 115].

Others transcription factors may play a role in the regulation of muscle mass in situation of altered use. Serum response factor (SRF) is a transcription factor that activates the expression of muscle-specific genes [29, 49]. It has been proposed that a pathway involving integrin β 1, RhoA, focal adhesion kinase (FAK) and SRF may play a central role in transducing mechanical signals from cell membranes to skeletal muscle fiber nuclei [30, 44, 57, 133]. Works from Booth et al. evidenced that SRF is up-regulated in hypertrophied muscles [29, 44, 57]. In contrast, this pathway appears to be down-regulated in unloaded [57] or in merosin-deficient dy muscles [133]. Yin Yang 1 (YY1), which is known to repress myofibrillar gene expression in myoblasts [158], is increased with heart failure and decreased with exercise-induced left ventricular hypertrophy [149]. Whether YY1 is implicated in the regulation of muscle-specific expression in mature skeletal muscle is, however, not established. Finally, interesting observations have been reported about the runt-related transcription factor Runx1 (also known as AML1). Runx1 limits the extent of muscle atrophy and preserves the structural integrity of myofibrils after 2 weeks of denervation [159]. Whether the preventing effect of Runx1 occurs directly through the transcriptional control of muscle specific gene remains to be determined. Other transcriptional factors are involved in the regulation of muscle-specific gene expression during myogenesis (e.g., rev-erbA α , pax3, mist1, and SOX15), but there is lack of studies about their role in adult skeletal muscle.

Protein synthesis

Regulation of protein translation: the regulatory function of the Akt/TSC2/mTOR pathway

The process of protein synthesis or mRNA translation is conventionally divided into three steps. Each stage requires translation factors (eukaryotic initiation, elongation and release factors, eIFs, eEFs, and eRFs, respectively) that transiently associate with ribosomes to regulate the progress and fidelity of the overall reaction. Akt/tuberous sclerosis complex (TSC) 2/mammalian target of rapamycin (mTOR) pathway has been characterized as a crucial regulator of

skeletal muscle hypertrophy by regulating eIFs and eEFs (Fig. 3), leading to an increase in protein synthesis [72, 87]. Rommel et al. observed that expression of a kinase-inactive Akt, which blocks endogenous Akt activity, resulted in thinner myotubes, whereas expression of a constitutively active form of Akt stimulated hypertrophy of myotubes [128]. In vivo, this pathway is activated in skeletal muscle after an acute RE or strength training as illustrated by the increase in phosphatidylinositol-3-kinase (PI3K) activity and in the phosphorylated state of Akt, mTOR, p70S6K, the ribosomal protein S6, 4E-BP1, or GSK3 β [13, 87, 92] and a reduction in the phospho-to-total eIF2 β E ratio [87].

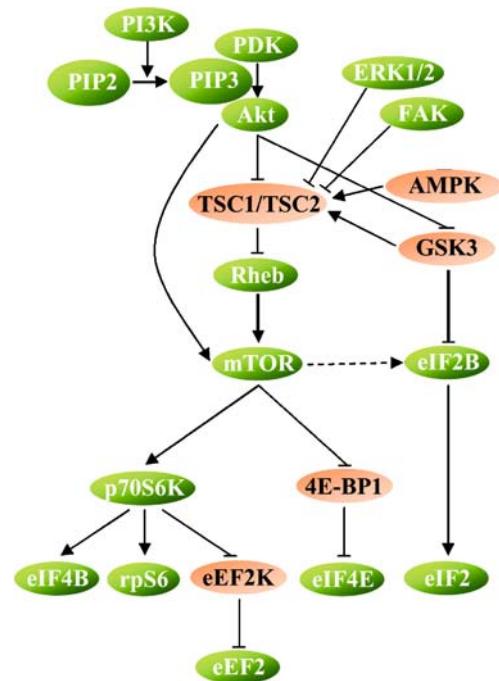


Fig. 3 Akt/TSC2/mTOR pathway. *Arrow* Activating steps; *hatched line* inhibitory steps; *broken arrow* incompletely-defined links. Activation of phosphatidylinositol-3-kinase (PI3K) results in the phosphorylation of phosphatidylinositol-biphosphate (PIP2) into phosphatidylinositol-triphosphate (PIP3), which is a membrane-binding site for two kinases: the serine/threonine kinase Akt (or protein kinase B) and phosphoinositide-dependent protein kinase (PDK). Akt is phosphorylated by PDK and thereby activated upon translocation to the membrane. Once activated, Akt phosphorylates the tuberous sclerosis complex (TSC) 2, which assembles with TSC1, leading to the release of the inhibition on Ras homologue enriched in brain (Rheb). In turn, Rheb activates the mammalian target of rapamycin (mTOR) that stimulates ribosome biogenesis, initiation, and elongation of mRNA translation via phosphorylation of the 70-kDa ribosomal protein S6 kinase (p70S6K) and by preventing the binding of 4E-BP1 to eukaryotic initiation factor (eIF) 4E. In addition, Akt promotes protein translation via the inhibition of glycogen synthase kinase (GSK) 3, which controls the activity of eIF2B. It is to note that the TSC1/2 complex represents a key step of the pathway, as it can be regulated by several kinases. Particularly, TSC2 is activated by GSK3 and the AMP kinase (AMPK) and is inhibited by Akt, the extracellular signal-regulated kinase (ERK) 1/2, and focal adhesion kinase (FAK)

Similar phosphorylation patterns have been evidenced in response to functional overload [22, 125], high frequency electrical stimulation (HFES) [11] or passive stretch [72, 132]. Although less documented, an increase in TSC2 phosphorylation (Ser1462) in skeletal muscle has also been reported after HFES [11]. Finally, mTOR inhibition with rapamycin leads to a 95% blockage of overloading-induced hypertrophy [22], clearly illustrating the functional relevance of this pathway during muscle hypertrophy.

In contrast, hindlimb suspension or denervation reduces the phosphorylated state of Akt, mTOR, and p70S6K [22, 71, 125], as well as Akt protein content [22]. In addition, loss of muscle mass mediated by hindlimb suspension is associated with an increase in 4E-BP1/eIF4E complex formation [22]. Overexpression of human TSC1 in mice leads to skeletal muscle atrophy associated to an up-regulation of TSC2 protein level [157]. Data regarding the phosphorylation state of downstream targets (eIFs and eEFs) with muscle disuse are less consensual. For example, phosphorylation of eEF2—that leads to inhibition of protein translation—is reduced in soleus muscle after 7 days of hindlimb unloading but is increased in denervated EDL muscle [71]. Aging does not seem to alter the phosphorylation status and protein content of several molecules of the Akt/TSC2/mTOR pathway (4E-BP1 and GSK3 phosphorylation, eIF4E/4G complex formation) in tibialis anterior muscle [47], whereas the level of Akt activation is decreased in tibialis anterior [31] or medial gastrocnemius muscle of old rats [62]. Importantly, the response to a single bout of HFES is attenuated in old rats suggesting that impairment of Akt/TSC2/mTOR pathway may contribute to the limited capacity of hypertrophy in aged animals [47, 62].

Akt/TSC2/mTOR pathway can integrate multiple regulatory influences, which ultimately determine the overall response. Hornberger et al. evidenced that stretch-induced activation of mTOR signaling was not abolished in the skeletal muscle of Akt1 $-/-$ mice [72], showing that Akt is not necessary for mechanically induced signaling through mTOR. Furthermore, Akt-independent stimulation of mTOR may be positively or negatively regulated by phosphorylation of TSC2. For instance, TSC2 is inhibited by FAK in 293T cells [50] suggesting that up-regulation of FAK with increased load could stimulate protein synthesis via TSC2 inhibition. By contrast, AMP kinase activation may limit muscle hypertrophy by stimulating TSC2 phosphorylation (Ser 1387) thus accounting for the observation that endurance training is generally not associated with a gain of muscle mass [11]. All these regulatory influences may explain why a decrease in TSC2 phosphorylation by Akt (Ser 1462) after RE has been reported concomitantly with a rise in the level of phosphorylated p70S6K [32]. p70S6K activation may also result from the release of the inhibitory action of proline-rich Akt-substrate

(PRAS40), which has recently been involved in insulin-induced activation of mTOR in HEK293T cells [60]. The relevance of such a mechanism in skeletal muscle needs to be determined. Lastly, TSC2 may be inhibited by the extracellular signal-regulated kinase (ERK) 1/2 (Ser 664) [96]. This is consistent with the observation that ERK or MEK kinase inhibition blunted IGF-I-mediated hypertrophy [61] or RE-induced increase in protein synthesis [45], although others showed no effect of ERK inhibition on IGF-I-induced myotube hypertrophy in vitro [128].

IGF-I: a master trigger of muscle hypertrophy

IGF-I has been characterized as a strong anabolic agent of skeletal muscle notably by activating the Akt/TSC2/mTOR pathway and promoting protein synthesis. IGF-I is secreted by the liver and skeletal muscle where two main variants have been identified in human: IGF-IEa, the hepatic-like isoform, and IGF-IEc, also called MGF because of its susceptibility to mechanical stress [66]. While both isoforms have been shown to equally activate satellite cells [164], our current knowledge on the respective effects of IGF-IEa and MGF on protein synthesis remains to be elucidated. Furthermore, the role of MGF on skeletal muscle tissue should be interpreted with caution because the identification of its receptor is still missing and that only mRNA data are available. Therefore, when not specified, the term IGF-I will refer to both IGF-IEa and MGF.

Whereas increase in skeletal muscle IGF-I induced by infusion or genetic manipulation is sufficient to stimulate hypertrophy [3, 108], systemic administration of IGF-I and/or growth hormone (GH) does not necessarily cause significant skeletal muscle hypertrophy. For example, co-infusion of GH/IGF-I in suspended rats had no effect on muscle mass in the absence of mechanical load [4]. Furthermore, GH supplementation did not alter the hypertrophic response in elderly men subjected to resistance training [151], suggesting that the decline in the GH-IGF-I axis with aging do not impair skeletal muscle sensitivity to resistance training. Conversely, transgenic mice whose liver cells were knocked out for IGF-I gene grew normally, although IGF-I serum levels were lowered [163]. Lastly, in an early report, Goldberg [52] showed that tenotomy-induced hypertrophy of synergistic muscles was not impaired in hypophysectomised rats. Accordingly, neither a single bout of RE (sufficient to cause a transient increase in interstitial IGF-I) [36] nor 10 weeks of strength training [156] alter plasmatic concentration of IGF-I. Overall, this suggests that systemic IGF-I has only a limited influence in the hypertrophic response after overloading. This also suggests that autocrine/paracrine expression of growth factors within the muscle in response to altered workload is probably a major event controlling skeletal muscle mass.

In agreement with this idea, isometric or dynamic contractions [62, 84], as well as passive stretch [102], induce increase in muscular IGF-IEa and MGF mRNA. Importantly, the anabolic effect of IGF-I may be potentiated by mechanical strains. Indeed, addition of IGF-I induces greater elevation of protein synthesis when myotubes were mechanically stimulated [116].

The role of IGF-I in atrophying conditions is less obvious. IGF-I mRNA has been shown to decrease after 2 days of unloading but returned to control value at day 8 [12], while IGF-I protein content was reduced after 14 days of hindlimb suspension in plantaris muscle but not in TA muscle [167]. However, neither 5 weeks of unilateral limb suspension in human [63] nor spinal cord isolation [64] did result in a decrease in IGF-I mRNA level. By contrast, aging seems to attenuate IGF-I mRNA level in human [161] and rat [31] skeletal muscle. Furthermore, there is evidence that aging is associated with an attenuation of the exercise-induced increase in MGF expression [66, 114]. Moreover, adenoviral-mediated delivery of murine IGF-IEa and MGF both promote muscle hypertrophy in young mice (without any difference on muscle gain between the two isoforms), whereas MGF was ineffective in old animals [17]. It should be noted that bioavailable IGF-IEa can be regulated by IGF-binding proteins (IGFBPs), especially IGFBP-4 and IGFBP-5. Hypertrophic conditions such as RE [19, 63], electromyostimulation [20], or overloading [1, 12] have all been accompanied by an increase in IGFBP-4 mRNA, whereas IGFBP-5 variations are less consensual [1, 12, 145, 147]. On the other hand, testosterone administration increased IGF-I mRNA but decreased IGFBP-4 mRNA levels in skeletal muscle of elderly, together with an increase in protein synthesis [154]. Although the signification of such variations is not well established, the IGFBPs may have a crucial role in modulating IGF-I activity in skeletal muscle.

MicroRNAs: novel candidates for the regulation of protein translation?

Recent findings highlighted the role of small RNA fragments termed microRNA (miRNA) in the post-transcriptional regulation of gene expression. miRNAs can down-regulate protein expression by either of two mechanisms: cleavage of target mRNAs or inhibition of the target mRNA translation into protein [16]. They may be involved in the control of muscle size as (1) the muscle-specific miR-1, miR-133, and miR-206 expression is regulated by myoD and myogenin [121], and (2) miR-1 and miR-133 are downregulated after 7 days of overload-induced hypertrophy in the plantaris muscle [99]. Data in the field are sparse but may represent an interesting way to understand the fine regulation of gene expression in skeletal muscle in response to altered use.

Proteolysis

Role of the ubiquitin proteasome system in protein degradation: the control by Foxo

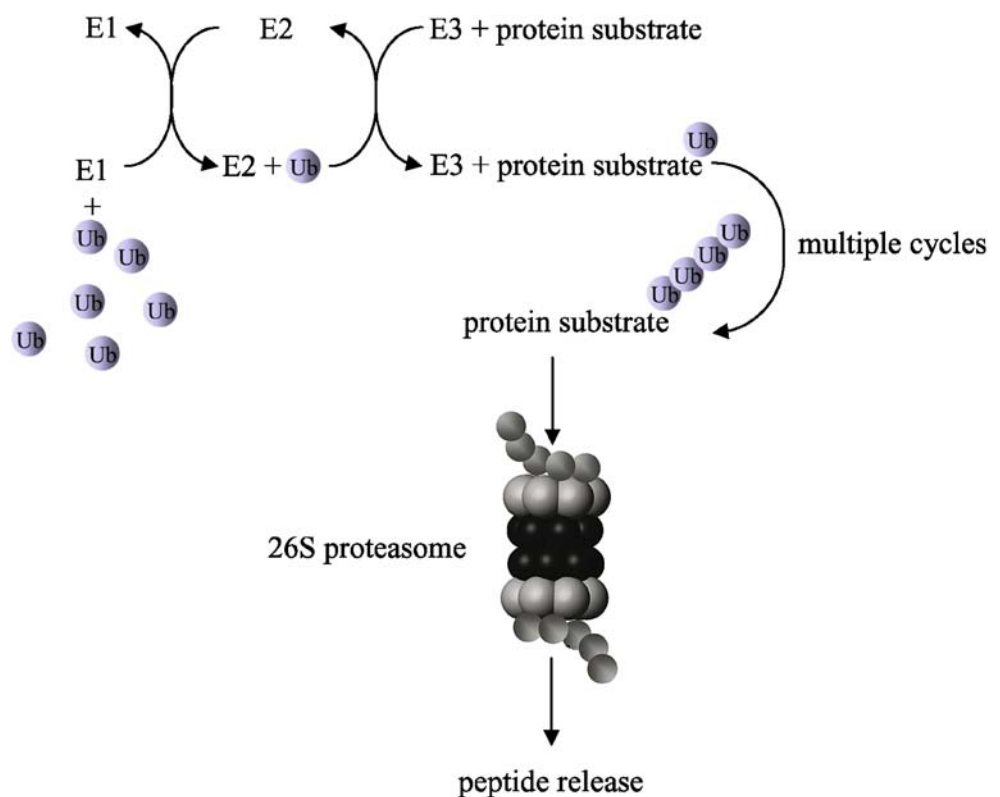
The ubiquitin proteasome system (Fig. 4) is an important component of the proteolytic machinery in eukaryotic cells [127] and is activated with reduced muscle activity [124]. mRNA level of two muscle-specific E3 ligases, atrogin-1 (or MAFbx) and MuRF1, are increased in response to unweighting, denervation, dexamethasone, and interleukin-1 administration in rats [21, 135, 159]. Expression of these atrogenes (i.e., genes involved in skeletal muscle atrophy) is also markedly increased in catabolic states such as cancer cachexia, diabetes, and uremia [90]. In addition, two other E3 ligases named neuronal precursor cell-expressed developmentally downregulated-4 (Nedd4) and X-chromosome-linked inhibitor of apoptosis (XIAP), are up-regulated in soleus muscle in response to either 1, 4, 7 or 14 days of hindlimb suspension [40, 147]. The increase in Nedd4 protein level occurs in situation of disuse such as hindlimb suspension or denervation, but not in catabolic states such as starvation or diabetes [85], suggesting that Nedd4 is specifically involved in atrophying conditions where muscular tension is removed. Curiously, RE attenuates the increase in atrogin-1 and MuRF1 mRNA in response to unloading, whereas Nedd4 and XIAP mRNA levels remain elevated [40]. If E3 ligases appear to be key molecules for muscular atrophy, knowledge about their substrate and substrate specificity is limited. It has been evidenced that MyoD and Notch1 are substrates for atrogin-1 [153] and Nedd4 [85], respectively, whereas troponin I was a target for MuRF1 in cardiomyocytes [83]. In agreement with these data, an increase in protein ubiquitination (notably myosin heavy chain) has been recorded after unloading [40, 74, 77, 167] or denervation [162]. Similarly, analysis of transcriptional changes reveals that expression of structural and regulatory components of the 26S proteasome is increased with denervation [131], unloading [40, 74, 147], or immobilization [76]. Surprisingly, the three main enzymatic activities of 20S proteasome (chymotrypsin-, trypsin-, and caspase-like activities) do not follow the same pattern in response to unloading, some studies describing an increase [74, 167] whereas others do not show any changes [40, 74]. Data regarding proteasome activity or expression with aging are more controversial, as an increase [31], no change, or even a decrease [33] in proteasome components have been reported. Interestingly, in this latter study Combaret et al. [33] observed a defective post-prandial inhibition of proteasome with aging but a decrease in proteasome activities in the postabsorptive state, illustrating time-dependent variations in proteasome activity as a function of absorptive state.

Akt modulates the transcription of atrogin-1 and MuRF1 by regulating the nuclear translocation of the Foxo family transcription factors (Foxo1 and Foxo3). When phosphorylated by Akt, Foxo proteins are sequestered in the cytosol where they are unable to stimulate atrogenes [135, 148] (Fig. 5). On the contrary, a non-phosphorylatable active mutant of Foxo1 is almost exclusively nuclear, [148] and overexpression of Foxo1 or Foxo3a leads to muscle fiber atrophy in vivo and in vitro [81, 135]. Accordingly, strength training reduces nuclear protein content of Foxo1 concomitantly with increased Akt phosphorylation in human skeletal muscle [92]. Curiously, marked increase in atrogin-1 and MuRF1 expression was recorded, suggesting that these E3 ligases can be regulated by others pathways than Akt/Foxo. This rise in atrogin-1 and MuRF1 mRNA level could be related to an increase in protein breakdown as it might occur during RE [118]. In addition to its role on atrogin-1 expression, Foxo1 inhibits mTOR signaling by increasing 4E-BP1 expression while decreasing the phosphorylation of both mTOR and 4E-BP1 and the expression of mTOR protein [144]. A recent work pointed out the role of the peroxisome proliferator-activated receptor coactivator (PGC)-1 α in protecting skeletal muscle from denervation- or fasting-induced atrophy [134]. In this study, overexpression of PGC-1 α reduced Foxo3-dependent transcription of MuRF-1, atrogin-1, and cathepsin L (but did not increase

skeletal muscle mass in control animals). Consistent with this, muscle disuse after denervation induces concomitant fall in PGC-1 α mRNA and rise in Foxo1 mRNA [131].

Proteolysis during disuse may also be activated by the transcription factors of the nuclear factor (NF)- κ B family (i.e., p65, Rel B, c-Rel, p52, and p50; reviewed in [82]). This has been well illustrated by Cai et al. [26] who demonstrated that NF- κ B activation results in muscle loss through ubiquitin-dependent proteolysis degradation and that blockade of NF- κ B reduces denervation-mediated atrophy. p50^{-/-} mice also exhibit a lesser atrophy of soleus muscle with hindlimb suspension [73]. Similarly, transfection of a dominant negative inhibitor of NF- κ B (I κ B α) reduces fiber atrophy by 40% in suspended rodents concomitantly with a reduced increase in Foxo3a, atrogin-1, Nedd4, 4E-BP1, and cathepsin L mRNA levels [77]. Several in vitro studies suggest a role for reactive oxygen species in regulating NF- κ B [93] and Foxo [48]. Interestingly, in vivo supplementation with vitamin E reduces disuse-associated amyotrophy after immobilization [9] and hindlimb suspension [139]. In this latter study, the prevention of muscle mass loss was associated with a decrease in the up-regulation MuRF1 and atrogin-1 transcripts, suggesting a potential role for reactive oxygen species in triggering disuse-mediated atrophy through Foxo and/or NF- κ B.

Fig. 4 The ubiquitin proteasome system. Proteins targeted to degradation by the proteasome are tagged with ubiquitin via a three-step mechanism involving ubiquitin-activating (*E1*), ubiquitin-conjugating (*E2*), and ubiquitin-ligating (*E3*) enzymes



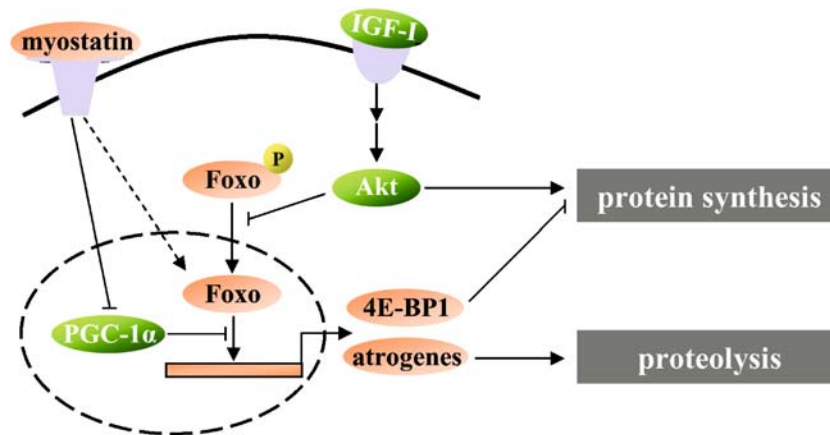


Fig. 5 Dual control of Forkhead-box O (*Foxo*) transcriptional activity by IGF-I and myostatin. *Arrow* Activating steps; *hatched line* inhibitory steps; *broken arrow* incompletely-defined links. Foxo stimulates atrogene expression (atrogin-1, MuRF1, proteasome subunits, cathepsin L) and represses mTOR signaling via stimulation of

4E-BP1 expression. IGF-I inactivates the nuclear translocation of Foxo through direct phosphorylation by Akt, whereas myostatin may enhance Foxo activity (1) by downregulating the peroxisome proliferator-activated receptor coactivator (PGC) 1 α expression and (2) possibly by increasing Foxo expression

Myostatin: the catabolic counterpart of IGF-I?

In contrast to IGF-I, myostatin is a strong negative regulator of skeletal muscle mass. Myostatin gene deletion causes hyper-muscle phenotypes [104], and its ectopic overexpression is associated with atrophy of adult skeletal muscle [42]. Several weeks of strength training reduce myostatin mRNA and protein content in human [129, 156] and rat skeletal muscles [68]. Moreover, myostatin mRNA is reduced after acute isometric or concentric exercise in adult rat and human [62, 84, 122]. As observed with IGF-I, an impairment of the myostatin response with RE has been observed with aging [62, 84]. Conversely, myostatin protein and/or mRNA contents are increased in some types of disuse-mediated muscle atrophy such as microgravity [89], unloading [160], or in patients with chronic osteoarthritis [123]. Besides, myostatin release is regulated by the titin-cap/teletonin, a 19 kDa protein that can bind to the giant sarcomeric protein titin [111]. As titin functions in helping to maintain the integrity of sarcomeres, one may speculate about the role of mechanical strains on structural proteins in regulating myostatin bioavailability.

In addition to its inhibitory action on satellite cell activation, myostatin has been shown to repress myoD and myogenin protein expression [42] and to promote proteolysis by up-regulating some components of the ubiquitin proteasome system [51] (Fig. 4). In this latter study, dexamethasone-mediated increase in atrogin-1, MuRF1, and Foxo3a mRNAs was abolished in myostatin KO mice [51]. Furthermore, myostatin treatment of C2C12 myotubes is associated with a rise in atrogin-1 and MuRF1 mRNA together with an increase in ubiquitinated proteins and a reduction in the phospho-to-total ratio of Foxo1

[101]. In agreement with these data, expression of PGC-1 α , which prevents atrogin-1 and MuRF1 expression in response to muscle disuse [134], was decreased in an in vivo model of ectopic myostatin expression [42]. Nevertheless, one should note that myostatin is dispensable for disuse-induced amyotrophy, as *mstn*^{-/-} mice show equal or greater muscle atrophy than wild-type mice in response to unloading [103].

Conclusion

The understanding of the mechanisms by which muscle grows or atrophies has made considerable progress in the recent years. Taken together, all these studies draw a complex interplay of regulatory processes involving the regulation of satellite cell activation, transcription, proteosynthesis, and/or proteolysis. Particularly, the control of the Akt/TSC2/mTOR pathway emerges as a critical step in the response of skeletal muscle to altered use, as it can potentially integrate many regulatory influences from autocrin/paracrin, mechanical (integrin/RhoA/FAK), and bioenergetic (AMPK) origins. However, numerous aspects require further investigations and clarifications. Thus far, we do not know the implication of satellite cells in RE-induced hypertrophy in human. Transcription factors that control muscle-specific gene expression have been mostly studied during in vitro myogenesis. What is really known about their role in adult skeletal muscle? The molecular mechanisms that transduce variations in mechanical strains into chemical signals are also still poorly understood. One may question about the contribution of other proteases such as calpains, caspases, and cathepsins. The role of myokines

is promising and requires further exploration. For instance, interleukin (IL)-15 expression is altered by acute RE, aging, or unloading [119, 126]. IL-15 promotes protein accretion in healthy muscles [25] and limits muscle wasting by antagonizing proteolysis in cachectic muscles [27]. Conversely, IL-6 can have deleterious effects on skeletal muscle mass as observed in HIV patients or in some cancers and as inhibition by antibodies may prevent cachexia in these patients [10]. The atrophying effect of IL-6 may be related to interference with downstream components of IGF-I signaling [65]. Musclin is another myokines whose mRNA expression is inversely regulated to changes in muscle mass [15], supporting a potential role for musclin in the control of muscle mass. In addition, the role of several molecules, such as c-ski [150], SOCS2 [58], and Runx1 [159], whose contribution to the regulation of skeletal muscle mass has been clearly illustrated in KO animals, could be examined in adult skeletal muscle independently of any developmental cues.

Skeletal muscle mass is essential for maintaining an active lifestyle, but it is also a critical determinant of whole-body metabolism. Clarifying the exact mechanisms and interplay between regulatory processes will be fundamental for a comprehensive analysis of how muscle mass is controlled.

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