Signaling in Muscle Atrophy and Hypertrophy

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Muscle performance is influenced by turnover of contractile proteins. Production of new myofibrils and degradation of existing proteins is a delicate balance, which, depending on the condition, can promote muscle growth or loss. Protein synthesis and protein degradation are coordinately regulated by pathways that are influenced by mechanical stress, physical activity, availability of nutrients, and growth factors. Understanding the signaling that regulates muscle mass may provide potential therapeutic targets for the prevention and treatment of muscle wasting in metabolic and neuromuscular diseases.

Cell size is determined by a balance between new protein accumulation and degradation of existing proteins. Genetic studies in both drosophila and mammals have shown that pathways controlling protein synthesis and protein breakdown have an important role to determine cell size. The two processes are tightly regulated and interrelated. The first level of connection occurs during protein synthesis when the quality control of the cell degrades proteins that are not correctly folded. At a further level, protein degradation systems determine the half-life of proteins and, in muscles, are required to replace sarcomeric proteins as a consequence of changes in muscle activity. Both systems need ATP, and muscle energy level is one of the cellular check points that decide either to promote growth and hypertrophy or activate protein breakdown and atrophy. Importantly, the proteolytic systems can produce alternative energy substrates that are used by the cell to maintain internal homeostasis in conditions of energy stress. Recent findings provide a new view, which considers the growth-promoting pathways and the proteolytic systems coordinately regulated. The following short review will focus mainly on in vitro studies and will be schematically divided into two parts: the first part will describe pathways controlling muscle hypertrophy, and the second part will be focused on signaling involved in muscle atrophy.

Muscle Hypertrophy

The growth of skeletal muscle mass, like the mass of any other tissue, depends on protein turnover and cell turnover (83). Cellular turnover plays a major role during muscle development in embryos. Moreover, satellite cell incorporation into the growing fibers takes place during postnatal muscle growth (65) concomitantly with increased protein synthesis. The activation of satellite cells is important for maintaining a constant size of each nuclear domain (quantity of cytoplasm/number of nuclei within that cytoplasm). Unlike young muscle, the contribution of cellular turnover to homeostasis of adult fibers is minor, and its role in hypertrophy has even been recently debated (56, 73). In adult muscle, the physiological conditions promoting muscle growth, therefore, do so mainly by increasing protein synthesis and decreasing protein degradation. However, satellite cells are activated in compensatory hypertrophy (65, 85), and addition of new nuclei to the growing fiber seems to be required for extreme hypertrophy. The pathways controlling cellular and protein turnover are different, and their contribution to muscle hypertrophy has to be considered during the interpretation of data resulting from studies with transgenic animals. Loss- and gain-of-function studies in which the transgene is perturbed early during postnatal growth might affect cellular growth and hypertrophy significantly more than protein synthesis. Results could be completely different if the same pathway is acutely perturbed in adult muscle age when the role of protein turnover is dominant (Figure 1).

IGF1-AKT signaling and the control of muscle growth

IGF1. IGF1 is among the best characterized muscle growth-promoting factors. In addition to circulating IGF1, mainly synthesized by the liver under GH control, local production by skeletal muscle of distinct IGF1 splicing products has recently raised considerable interest. A specific IGF1 splicing product is important for load- and stretch-induced adaptations in skeletal muscle (25). Increased IGF1 gene expression has been demonstrated following functional overload induced by elimination of sympathetic muscles (55). Muscle-specific overexpression in transgenic mice of an IGF1 isoform locally expressed in skeletal muscle results in muscle hypertrophy (68) and, importantly, the growth of muscle mass matches with a physiological increase of muscle strength. Moreover even acute ectopic expression of IGF1 in adult muscles by electroporation is sufficient to promote muscle hypertrophy (6). Although these results suggest an autocrine/paracrine role for local IGF1 in activity-dependent muscle plasticity, direct evidence, approaches, remains, has no

ABSTRACT. A specific IGF1 splicing product is important for load- and stretch-induced adaptations in skeletal muscle (25). Increased IGF1 gene expression has been demonstrated following functional overload induced by elimination of sympathetic muscles (55). Muscle-specific overexpression in transgenic mice of an IGF1 isoform locally expressed in skeletal muscle results in muscle hypertrophy (68) and, importantly, the growth of muscle mass matches with a physiological increase of muscle strength. Moreover even acute ectopic expression of IGF1 in adult muscles by electroporation is sufficient to promote muscle hypertrophy (6). Although these results suggest an autocrine/paracrine role for local IGF1 in activity-dependent muscle plasticity, direct evidence approaches, remains, has no
Akt activation is induced by IGF1 and insulin through the generation of phosphatidylinositol-3,4,5-triphosphates produced by PI3K, which is opposed by the activity of the phosphatase PTEN and SHP2. Phosphatidylinositol-3,4,5-triphosphates recruit Akt to the plasma membrane by binding to its NH2-terminal pleckstrin homology domain. At the membrane, Akt is phosphorylated on separate residues by at least two distinct kinases, PKB1 and the mTOR-Rictor complex. The role of Akt in muscle growth was first suggested by the finding that an active Ras double mutant (RasV12C40) that selectively activates the Akt pathway through the phosphatidylinositol 3 kinase (PI3K) promotes muscle growth, thus opening new perspectives in the signaling of fiber size (67) (FIGURE 2). This observation was subsequently confirmed by overexpressing a constitutively active form of Akt in adult skeletal muscle (10, 72). Similar results were obtained by the generation of conditional transgenic mice in which Akt is expressed in adult skeletal muscles only after tamoxifen (41) or tetracycline (37) treatment.

In mammals, there are three Akt genes, Akt1 (PKBα), Akt2 (PKBβ), and Akt3 (PKBγ), which appear to have distinct functions. In skeletal muscle, Akt1 and Akt2 are expressed at higher levels compared with Akt3, which is mainly expressed in the brain. Targeted deletion experiments have shown that Akt1-null mice display growth retardation and muscle atrophy, whereas Akt2-null mice suffer from a Type 2 diabetes-like syndrome, and Akt3-null mice have impaired brain development (111). Exercise in vivo is associated with activation of Akt1 but not Akt2 and Akt3 kinases in contracting muscles (98). Akt activity was increased in the rat plantaris after functional overload induced by elimination of synergistic muscles (10). Subsequent studies both in rats and humans confirmed that Akt activity is increased in response to muscle contractile activity (69, 78-80). Surprisingly, this effect was observed only in the fast EDL but not in the slow soleus muscle (78, 79). The finding that passive stretch of the fast rat EDL muscle can also induce Akt activation has suggested that mechanical tension may be a part of the mechanism by which contraction activates Akt in fast-twitch muscles (79). However, it remains to be established how mechanical stress is converted to Akt activation. Akt activity is also increased in response to hormonal and growth factor stimulation, in particular insulin is known to activate Akt2, whereas IGF1 activates primarily Akt1. Taken together with other observations, these results suggest that Akt1 is a major mediator of skeletal muscle hypertrophy. Although it has been established that Akt plays a crucial role in muscle growth, the downstream targets involved in muscle hypertrophy remain to be identified.
Myostatin and the cellular turnover

Myostatin, a member of the TGF-β family, is expressed and secreted predominantly by skeletal muscle and functions as a negative regulator of muscle growth. Mutations of the myostatin gene lead to a hypertrophic phenotype in mice, sheep, and cattle, and a loss-of-function mutation in the human myostatin gene was also found to induce increased muscle mass (14, 47, 59, 87). The increase in muscle mass is a consequence of hyperplasia, which is an increase in cell number, and hypertrophy, which is an increase in cell size. The hyperplasia suggests an activation of muscle stem cells, and, in fact, the myostatin pathway influences Pax 7, MyoD, and myogenin expression inhibiting satellite cell activation and differentiation (36, 57, 58). Only a few studies explore the effect of myostatin inhibition in adult muscle. Treating 24-wk-old mice with an anti-myostatin antibody for 5 wk induces a 12% increase in muscle mass (109). Furthermore, when tamoxifen-inducible Cre recombinase expressing transgenic mice are crossed with mice expressing tamoxifen-inducible Cre, this results in the generation of numerous myostatin-null mice, next 3 mo (108). The specific force generated in these two sets of mice is increased due to an increased mass in animals treated with myostatin antisense oligonucleotides or transgenic mice expressing a dominant-negative myostatin transgene. In these transgenic mice, the specific force generated is increased in a dose-dependent manner. This evidence suggests a role for myostatin in muscle growth and function.

Beta adrenoreceptors

Among the hormones that mediate the acute elevations in intracellular cAMP (see below) are the beta-adrenoreceptors, which are heterotrimeric G protein-coupled receptors. Activation of the beta-adrenergic receptor (β-AR) by the sympathetic nervous system and/or exogenously administered catecholamines leads to the activation of the heterotrimeric G protein complex, which in turn activates adenyl cyclase, ultimately leading to the production of cAMP. Cyclic AMP acts as a second messenger to activate downstream effector cascades, including protein kinase A (PKA), which subsequently activates downstream targets, including protein synthesis and muscle hypertrophy.

FIGURE 2. Scheme illustrating the major pathways that control fiber size

Dotted lines depict pathways whose molecular mechanisms and role in adult skeletal muscle have yet to be completely defined.
muscle growth. The muscle mass in animal models of Duchenne muscular dystrophy is decreased by 10.220.32.246 on April 20, 2017 http://physiologyonline.physiology.org/ Downloaded from

Among the hormonal responses increased by exercise, the acute elevations in catecholamines are especially interesting with respect to changes in muscle phenotype. Beta-agonists such as clenbuterol, acting through beta2-adrenoreceptors, are known to cause muscle hypertrophy and a slow-to-fast fiber-type switch. Interestingly, some effects of catecholamines could be mediated by local production of IGF-I and IGF-II by skeletal muscle (6, 96). Indeed the growth effect of beta-agonists is, at least partially, mediated by AKT/mTOR pathway since rapamycin almost completely blunts the hypertrophic effects of clenbuterol (40). Activation of beta receptors is known to also increase intracellular cAMP levels and activates protein kinase A (PKA), which may also activate the AKT pathway, and the transcription factor CREB; however, this pathway has not been explored in contracting muscle. An attractive emerging concept in muscle biology is that signals dependent on muscle activity, and specifically on mechanical load, may arise in the sarcomere, the basic unit of the contractile machinery of striated muscles, and from there transmitted to the nucleus to affect gene expression (43). The giant elastic protein titin, which spans half the sarcomere extending from the Z disk to the M band and interacts with a large number of muscle proteins, provides an exciting example of a sarcomeric activity-dependent signaling complex (signalosome) (42). A unique property of titin is the presence in the M-band region of a serine/threonine kinase domain that can be induced to acquire an open active conformation by stretch and contraction (28). In active muscle cells, the titin kinase domain (TK) is linked through two zinc-finger scaffold proteins, nhr1 and p62, to a member of the muscle-specific RING-finger proteins, MURF2 (43). In the absence of mechanical activity, the signalosome is dissociated, and MURF2 translocates to the nucleus where it can interact with the serum response factor, leading to nuclear export of SRF and loss of SRF-dependent gene expression. This pathway may thus control muscle growth because SRF is known to regulate muscle gene expression, and conditional deletion of the SRF gene causes severe skeletal muscle hypoplasia during the perinatal period (51). SRF regulates muscle gene expression by binding to muscle gene promoters (SRE) in target genes and seems to integrate different growth promoting pathways: for example, SRF is a target of Akt signaling in Hela cells (107) and can recruit the androgen receptor to muscle gene promoters (109). The titin kinase-SRF pathway described above is probably just one of several links between the sarcomere and the nucleus that are only now beginning to emerge (43).

Muscle Atrophy

Atrophy is a decrease in cell size mainly caused by loss of organelles, cytoplasm, and proteins. This concept is important when a genetic approach is used to dissect the contribution of certain pathways to muscle loss. As mentioned above, the mass of a tissue is controlled by cellular turnover and protein turnover (FIGURES 1 AND 2). Genetic modifications that interfere with embryonic and postnatal growth result in smaller muscles in adults. This reduction in muscle size is caused by failure/inhibition of growth and not by a real atrophy process. Keeping in mind the above definition, another important concept is that protein turnover is dominant over cellular turnover during acute phases of muscle wasting when sarcomeric proteins are rapidly lost such as during fasting, disease, and denervation. Evidence that blocking satellite cells is sufficient to trigger muscle atrophy in adult muscle has never been determined. Furthermore, myonuclei are normally reduced during muscle atrophy to keep rather constant the size of the nuclear domain (16, 22). Thus the current understanding suggests that inhibiting cellular turnover would not influence protein...
breakdown and muscle weakness and would not affect nuclear domain maintenance, but it might be important for the replacement of damaged myofibers or myonuclei. The importance of cellular turnover for long-term muscle atrophy, e.g., long-term denervation, has yet to be addressed.

**Atrophy: an active process that requires transcriptional regulation**

A major contribution in understanding muscle atrophy comes from the pioneering studies on gene expression profiling performed independently by groups of Goldberg and Glass (9, 26). The idea to compare gene expression in different models of muscle atrophy leads to the identification of a subset of genes that are commonly up- or downregulated in atrophying muscle. Since all the diseases used for the experiments of microarray (i.e., diabetes, cancer cachexia, chronic renal failure, fasting, and denervation) have muscle atrophy in common, the commonly up or down genes are believed to regulate the loss of muscle components and are called atrophy-related genes or atrogenes (76). Together, these findings indicate that muscle atrophy is an active process controlled by specific signaling pathways and transcriptional programs. Furthermore, the two most induced genes are two novel muscle-specific ubiquitin ligases, atrogen-1/MAFbx and MuRF1, that are upregulated in different models of muscle atrophy and are responsible for the increased protein degradation through the ubiquitin-proteasome system (9, 26). In fact, knockout mice for either atrogen-1/MAFbx or MuRF1 are partially resistant to denervation atrophy (9). Importantly, recent findings suggest that myostatin heavy chains are ubiquitinated and degraded by MuRF1 (13, 23). Thus, up to now, these two genes are actually the best markers for muscle atrophy and could be considered as master genes for muscle wasting. However, several other genes among the atrophy-related genes are of potential interest, including genes coding for lysosomal proteases, transcription factors, regulators of protein synthesis, and enzymes of metabolic pathways, but their particular role in muscle wasting has to be defined in the next future.

**IGF1-AKT-FoxO signaling**

A subsequent crucial step was the identification of the two critical ubiquitin ligases. Further data support the role of this pathway in regulating muscle atrophy come from experiments of Akt transfection in adult mice. Electroporation of constitutively active Akt in adult myofibers completely blocked muscle atrophy induced by denervation (14). These findings are important to elaborate the various contributions of the different signaling pathways during muscle atrophy. The upregulation of atrogen-1/MAFbx and MuRF1 is normally blocked by Akt functioning through negative regulation of the FoxO family of transcription factors (49, 82, 93). The FoxO family in skeletal muscle is comprised of three isoforms: FoxO1, FoxO3, and FoxO4. The Akt phosphorylation of FoxO3 in the cytoplasm and a marked increase of nuclear FoxO protein. The translocation and activity of FoxO3 members is required for the upregulation of atrogen-1/MAFbx and MuRF1, and FoxO3 was found to be sufficient to promote atrogen-1/MAFbx expression and muscle atrophy when transfected in skeletal muscles in vivo (82). Accordingly, FoxO1 transgenic mice showed markedly reduced muscle mass and fiber atrophy, further supporting the notion that FoxO is sufficient to promote muscle loss (39, 92) (FIGURE 2). On the other hand, the knockdown of FoxO expression by RNAi is able to block the upregulation of atrogen-1/MAFbx expression during atrophy and muscle loss (52, 82). Cross talk between protein breakdown and protein synthesis is not limited only to Akt but also involves FoxO. Activation of FoxO upregulates 4EBP1 and downregulates both RAPTOR and mTOR (92). Thus, when AKT in active protein breakdown is suppressed but when FoxO is induced, protein synthesis is further suppressed. This is not trivial since FoxO activity is regulated by different posttranslational modifications, which include phosphorylation, acetylation, and mono- and poly-ubiquitination (34) (FIGURE 3). Most of these regulatory mechanisms are AKT independent and may play a role in muscle atrophy induced by oxidative or energy stress (see below).

**Inflammatory cytokines and NF-κB signaling**

The NF-κB transcription factors, which play a major role as mediators of immunity and inflammation, are also expressed in skeletal muscle and appear to mediate the effect of inflammatory cytokines, in particular TNF-α, on muscle wasting and cachexia. In the inactive state, NF-κB is sequestered in the cytoplasm by a family of inhibitory proteins called IκB. In response to TNF-α, the IκB kinase (IKK) complex phosphorylates IκB, resulting in its ubiquitination and proteasomal degradation; this leads to nuclear translocation of NF-κB and activation of NF-κB-mediated gene transcription. Muscle-specific overexpression of IKKα in transgenic mice leads to severe muscle wasting mediated, at least in part, by the ubiquitin-ligase MuRF1, but not by MuRF2 (16). On the other hand, NF-κB is also able to activate transcription of inflammatory cytokines (38). Muscle atrophy, like cancer cachexia, is characterized by the release of proinflammatory cytokines and suppression of muscle protein synthesis. Therefore, Akt could be considered an active process controlled by specific signaling pathways and transcriptional programs. Furthermore, the 10.220.32.246 on April 20, 2017 http://physiologyonline.physiology.org/ Downloaded from
muscle atrophy

Different studies have shown that cathepsin-L, a lysosomal protease, is upregulated in different models of muscle wasting (18, 45). The role of cathepsin-L in muscle wasting has been reviewed by Jefcoate et al. (9).

FIGURE 3. Scheme illustrating the regulation of FoxO transcription factors and their potential role in the atrophy program. AKT is activated by growth factors and by physical activity and phosphorylates FoxO, inducing its translocation from the nucleus to the cytoplasm. Conversely, AMPK phosphorylates FoxO, increasing its transcriptional activity. Various other activators of FoxO are also indicated, including oxidative stress, which induces acetylation of peculiar lysine residues, and monoubiquitination. Polyubiquitination occurs when FoxO is relocalized in the cytoplasm triggering its proteasomal degradation.
induction is still unclear, but recent evidence suggests that the autophagy-lysosome system is activated during atrophy. Mizushima et al. (62) generated transgenic mice expressing LC3 fused with GFP. LC3 is the mammalian homolog of Atg8 gene and is critical for membrane commitment and growth to engulf organelles, cytoplasm, glycogen, and protein aggregates. Morphological analyses documented the activation of the autophagy system during fasting in skeletal muscle (62). Indeed, muscle cell culture confirmed that the autophagy-lysosome system is the major proteolytic pathway implicated in nutrient-dependent proteolysis (63). Further experiments lend insight into the signaling pathways involved and identified an autophagy-lysosome system as the major protein breakdown system during fasting in skeletal muscle (62). Indeed, muscle cell culture confirmed that the autophagy-lysosome system is activated also in denervation atrophy (24, 86). However, denervation-induced atrophy shows a slower pace of autophagy when compared with fasted-mediating atrophy. This effect is mediated by Runx1, which is upregulated during denervation and is required to preserve muscle mass. Lack of Runx1 resulted in myofibrillar disorganization and excessive autophagy in denervated muscles (105). Runx1 knockout muscles show double- or triple-membrane vacuoles, which enclose mitochondria and membranes. This finding indicates that excessive autophagy is promoting severe wasting during denervation and needs to be reduced by Runx1. We have recently shown that the autophagy-lysosome and ubiquitin-proteasome systems are coordinately regulated during muscle wasting (53, 112). In fact, some critical autophagy-related genes are among the atrogene and are under FoxO3 control. Expression of FoxO3 is sufficient and required to activate lysosomal-dependent protein breakdown in cell culture and in vivo. Interestingly, the role of mTOR signaling for the regulation of autophagy is irrelevant in skeletal muscles. However, the contribution of mTOR is crucial for the lysosome and ubiquitin-proteasome systems to organelle remodeling, protein breakdown, and, finally, muscle atrophy remains to be investigated. Mitochondrial homeostasis and energy balance: the metabolic control of cell size

Several metabolic adaptations occur in atrophying muscles. In many forms of muscle wasting, expression of a variety of genes for enzymes important in glycolysis and oxidative phosphorylation are suppressed coordinately (45). Indeed, we have recently shown that PGC-1α, the master regulatory gene for mitochondrial biogenesis, is downregulated in different models of muscle wasting. Furthermore, when the levels of PGC-1α are maintained, either by use of transgenic mice or by transfecting adult muscle fibers, excessive autophagy remains to be investigated.

Mechanical signals

The dystrophic mouse model of human Duchenne muscular dystrophy (DMD) is the most widely used and characterized animal model of human muscular dystrophies. The dystrophin-deficient dystrophic mouse model of human muscular dystrophy (DMD) is the most widely used and characterized animal model of human muscular dystrophies. Duchenne muscular dystrophy (DMD) is a degenerative muscle disease caused by mutations in the dystrophin gene, which is located on the X chromosome. The dystrophin gene encodes a large protein, dystrophin, which is involved in maintaining muscle integrity by linking the extracellular matrix to the cytoskeleton. Duchenne muscular dystrophy is characterized by progressive muscle weakness and wasting, leading to respiratory and cardiac failure. Duchenne muscular dystrophy is the most common form of muscular dystrophy and affects boys and young men. The dystrophin protein is absent or severely reduced in patients with Duchenne muscular dystrophy, leading to the loss of muscle function. Duchenne muscular dystrophy is inherited as an X-linked recessive trait, meaning that it is caused by a mutation on the X chromosome. The absence of dystrophin leads to the loss of muscle function, leading to muscle weakness, fatigue, and difficulty with movement. Duchenne muscular dystrophy is a progressive disease, and patients typically require ventilator support and other medical interventions to manage their symptoms. Duchenne muscular dystrophy is a life-limiting disease, and the average lifespan of a child with Duchenne muscular dystrophy is around 20 years. Despite advances in medical care, Duchenne muscular dystrophy remains a significant medical challenge, and there is currently no cure for this disease.
muscles are protected from the atrophy induced by denervation, fasting, or expression of FoxO3 (81). Treatment with statins induces FoxO3-mediated atrogin-1 activation and related muscle alterations. In cell culture and in zebrafish, statins cause muscle atrophy, disorganization of myofibrils, and mitochondrial dys-function (31). These changes are completely aborted by either the deletion of atrogin-1 or by PGC-1α over-expression. These results may explain why oxidative fibers tend to be resistant to atrophy compared with glycolytic fibers and suggest that metabolic changes are crucial for muscle atrophy (50). Among the differ-ent energy-dependent pathways, AMPK acts as the cellular sensor of energy balance. Indeed, stressing energy in muscle cell culture either by oligomycin treatment or by replacing glucose with 2-deoxyglucose triggers myotube atrophy (4). Interestingly, increased levels of AMP, activation of AMPK, lipid consumption, and smaller muscles have all been observed in S6K1 and S6K2 knockout mice. Importantly, AMPK inhibi-tion rescues skeletal muscle growth in these mice, confirming that S6K1/2 deletion alters energy balance (4) and suggesting that AMPK activity is a key factor for myotube size. Interestingly, these mice show normal methionine incorporation and polysomal profiles as well as autophagy and upregulation of atrogin-1/MAFbx and MuRF1 (60). Altogether, these findings describe a connection between AMPK and FoxO3. AMPK phys-ophorylates several AKT-independent sites of FoxO3, stimulating its transcriptional activity on target genes (29, 30). Indeed, treating muscle cell culture with AICAR, an activator of AMPK, causes an increase of protein breakdown and atrogin-1/MAFbx expression via the FoxO3 family (70). The physiological relevance of such important control over protein breakdown and muscle atrophy in vivo remains to be explored.

Within the myotube, mitochondria are crucial in regulating metabolism and might be a potential source of catalytic signals. Insulin resistance and dia-betes have been reported to trigger caspase-3-mediated actin cleavage, which would release myofibrils from the sarcomere for subsequent degradation via the pros-tasomes (20). Caspase-3 activation is mediated by Bax, which disrupts the mitochondrial external membrane, causing cytochrome-c release and apoptosis for-mation (49). Furthermore, inhibition of caspases by XIAP overexpression protects adult skeletal muscle from atrophy in an animal model of diabetes (106). However, the contribution of caspases to muscle atrophy should be further investigated using loss of function approaches.

Mechanical sensor of inactivity

The dystrophin glycoprotein complex (DGC) anchors the muscle cytoskeleton to the cell membrane via dystrophin and its binding partners. This complex is important for correct transduction of myosin move-ments on actin to the extracellular matrix. Indeed, lack of dystrophin exacerbates mechanical stress on the myotube plasma membrane, causing muscle damage and degeneration. This classical view con-siders a purely structural function of DGC proteins. However, an attractive role of dystrophin in transduc-tion of mechanical signals to the nucleus has recently been proposed. In fact, dystrophin is lost from the cell membrane under atrophic conditions, causing a loss of continuity between the cell membrane of the myotube and the extracellular matrix (1). This decreased interaction between membrane and matrix is required for muscle loss since forced dys-trophin expression in transgenic mice counteracts both cachexia-induced muscle wasting and upregu-lation of atrogin-1/MAFbx and MuRF1. Further sup-port for the DGC acting as a mechanical sensor has recently been published. Neuronal NOS (nNOS) is normally bound to the DGC, but when the DGC is disrupted, as occurs in dystrophic muscle, its localization is disturbed and it is no longer at the sar-colemma. A similar nNOS dysregulation has recently been described during disuse-induced muscle atro-phy. Free nNOS dissociates to the cytoplasm where it enhances FoxO3-mediated transcription and upreg-ulation of atrogin-1 and MuRF1 (95). Interestingly, the NF-κB pathway is not involved in nNOS-mediated muscle atrophy (95). Since nNOS generates NO, an important player in protein nitrosylation and in reactive oxygen species generation, the role of oxidative stress in muscle atrophy is an important aspect to be analyzed in future studies.

Glucocorticoids-induced muscle atrophy

Glucocorticoids are elevated in many pathological conditions associated with muscle loss. Moreover glu-coorticoid treatment induces atrogin-1 and MuRF1 expression and muscle wasting both in cell culture and in vivo (10, 13, 77, 82, 84). Importantly, adrenalectomy or treatment with a glucocorticoid receptor antagonist (RU-486) attenuates muscle loss in some diseases (84). However, the mechanisms of glucocorti-coid-mediated muscle atrophy are unclear. In fact, none of the atrophy-related genes have been found to be directly regulated by glucocorticoids, and no glu-coorticoid response elements on atrogenes promoters have been identified as critical for their expression (45). Thus most of the glucocorticoid effects are indi-rect, mainly affecting pathways previously described to be crucial for muscle growth or loss. Glucocorticoids are reported to decrease IGF1 pro-duction and increase myostatin secretion. Other effects like downregulation of the anabolic transcrip-tion factor ATF4, activation of p38α/ERK, or upregu-la-tion of REDD1, an inhibitor of mTOR signaling, are described, but no obvious mechanism can explain the...
direct involvement of corticosteroids in regulation of atrophy-related genes (3, 84, 104, 110). Even less clear is the recent description of dexamethasone-dependent
MyoD and M1 delta degradation by NIH-3T3 subcultivation pathway, especially if we consider that MyoD is described to be an atrogen-1 substrate (94, 98). How MyoD loss can contribute to weakness and muscle atrophy remains unclear. Loss and gain of function experiments on glucocorticoid receptor should be performed to address the direct role of this pathway in muscle loss.

Conclusions
Over the last few years, the mechanisms controlling muscle loss have attracted the attention of the scientific community due to their relevance in various fields of study such as ageing, prognosis of many diseases, quality of life, and sports medicine. From the recent findings, a new scenario emerges that considers the size of the myotub and muscle performance a result of not a single pathway but of a network of signaling (FIGURES 2 AND 4). Intriguingly, the different pathways cross talk and modulate one another at different levels, coordinating protein synthesis and degradation simultaneously. Findings of the last few years offer levels, coordinating protein synthesis and degradation ways cross talk and modulate one another at different

REFERENCES


