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.

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 protein and, in muscle, 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 vivo 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 by elimination of synergistic muscles (55). Muscle-specific IGF1-AKT signaling and the control of protein turnover is dominant (9). Results could be completely different if the same pathway is acutely perturbed in adult muscle age when the role of protein turnover is minor (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 growth-promoting factors, local production of distinct IGF1 splicing products has recently raised considerable interest. A specific IGF1 splice variant 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 is more elusive, and recent results, in combination with the potential of IGF1 to promote muscle growth, the duality of IGF1 in muscle growth and atrophy, the large variety of IGF1 splice variants, and the existence of IGF1 receptors on microglia, have led to the realization that IGF1 has a complex role in the brain. Moreover, local IGF1 in activity-dependent muscle plasticity, that is, in muscle hypertrophy, has been found to be 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,
In mammals, there are three Akt genes, Akt1, Akt2, and Akt3, 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.

Exercise in vivo is associated with activation of Akt1 but not Akt2 and Akt3 kinases in contracting muscles (98). Akt activity is 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 defined.

**mTOR-S6K and the control of protein synthesis**

Two major downstream branches of the Akt pathway, which are relevant to muscle hypertrophy, are the mTOR pathway, which is activated by Akt, and glycogen synthase kinase 3β (GSK3β), which is blocked by Akt, both of them control protein synthesis. A third downstream target of Akt is FoxO pathway, which controls protein degradation and will be discussed below.

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).
protein Rheb that in turn activates mTOR in complex with the raptor adapter protein (mTOR-raptor or TORC1). Transgenic mice overexpressing TSC1 specifically in skeletal muscle show a defect in muscle growth (103).

**S6K1.** mTOR is part of two multiprotein complexes: mTORC1, which contains raptor and is rapamycin sensitive, is required for signaling to S6K1 and 4E-BP1, whereas mTORC2, which contains rictor, is required for signaling to Akt-FoxO (FIGURE 2). The effect of mTOR on the translation machinery and protein synthesis is mediated by TORC1-dependent phosphorylation of the ribosomal protein S6 kinases (S6K1 and 2) and of 4E-BP1, a repressor of the cap-binding protein eIF4E. S6K1 appears to be an important effector of the Akt pathway, since muscle fibers are smaller in S6K1-null mice, and their hypertrophic response to IGF1 and to activated Akt is blunted (71). However S6K1 knockout mice show no impairment in polysome formation, in protein synthesis, and in protein degradation (60). TORC1 complex also negatively regulates the IGF1 pathway via S6K1 (4, 100). Thus the two mTOR complexes, mTORC1 and mTORC2, may have opposite effects on Akt activity: TORC1 negatively regulates IGF1 signaling, whereas TORC2 enhances Akt activity (FIGURE 2). An additional factor that complicates the pathway is that long-term rapamycin treatment in vitro can inhibit not only TORC1 but also the mTORC2 complex and thus potentially affect, in some cell types, the Akt-FoxO signaling. This raises the possibility that, under certain circumstances, rapamycin may induce transcriptional regulation not through a direct effect, e.g., by blocking mTOR phosphorylation of downstream targets, but through an indirect transcriptional effect by inhibiting Akt and thus derepressing FoxO. It is therefore suggested that FoxO activity/localization should always be monitored in studies involving rapamycin treatment (44).

**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 transgenic mice (108) were crossed with a myostatin transgenic mouse (12), the specific force generation of skeletal muscle was increased and inactivated myostatin antibody reversed this increase (109).

The acute elevation of muscle force (see below) appears to be mediated by long-term activation of mTORC1 and mTORC2. In addition, hyperphosphorylation of IGF1 receptor and downstream targets in muscle fibers is induced by IGF1 (48). mTOR is part of two multiprotein complexes: mTORC1, which contains raptor, is rapamycin sensitive, and mTORC2, which contains rictor, is required for signaling to Akt-FoxO (FIGURE 2). The effect of mTOR on the translation machinery and protein synthesis is mediated by TORC1-dependent phosphorylation of the ribosomal protein S6 kinases (S6K1 and 2) and of 4E-BP1, a repressor of the cap-binding protein eIF4E. S6K1 appears to be an important effector of the Akt pathway, since muscle fibers are smaller in S6K1-null mice, and their hypertrophic response to IGF1 and to activated Akt is blunted (71). However S6K1 knockout mice show no impairment in polysome formation, in protein synthesis, and in protein degradation (60). TORC1 complex also negatively regulates the IGF1 pathway via S6K1 (4, 100). Thus the two mTOR complexes, mTORC1 and mTORC2, may have opposite effects on Akt activity: TORC1 negatively regulates IGF1 signaling, whereas TORC2 enhances Akt activity (FIGURE 2). An additional factor that complicates the pathway is that long-term rapamycin treatment in vitro can inhibit not only TORC1 but also the mTORC2 complex and thus potentially affect, in some cell types, the Akt-FoxO signaling. This raises the possibility that, under certain circumstances, rapamycin may induce transcriptional regulation not through a direct effect, e.g., by blocking mTOR phosphorylation of downstream targets, but through an indirect transcriptional effect by inhibiting Akt and thus derepressing FoxO. It is therefore suggested that FoxO activity/localization should always be monitored in studies involving rapamycin treatment (44).

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**Beta adrenergic signaling**

Among the hormones that control skeletal muscle mass in vivo are the norepinephrine and epinephrine that are released from the adrenal medulla and that reach skeletal muscle by the bloodstream. These hormones act through beta-adrenergic receptors (β-ARs) to increase muscle force. The beta-adrenergic receptors, particularly β2, are coupled to the Gαs protein and activate the adenylate cyclase-cAMP signaling pathway. cAMP activates PKA, which in turn activates protein kinase A (PKA), where PKA activates S6K1 and 4E-BP1, which enhances cap-dependent protein synthesis.}

![FIGURE 2. Scheme illustrating the major pathways that control fiber size](http://physiologyonline.physiology.org/)

Dotted lines depict pathways whose molecular mechanisms and role in adult skeletal muscle have yet to be completely defined.
muscle growth.

It is expressed 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, nrl1 and p62, to a member of the muscle-specificRING-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 transcription factor SRF, 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).

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

Beta-agonists such as clenbuterol, acting through β2 adrenoreceptors, are known to cause muscle hypertrophy and a slow-to-fast fiber-type switch. 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, nrl1 and p62, to a member of the muscle-specificRING-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 transcription factor SRF, 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).

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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 atrogens (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, atrogin-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 atrogin-1/MAFbx or MuRF1 are partially resistant to denervation atrophy (9).

It is important to note that all the atrogens are expressed in skeletal muscle and appear to mediate the effect of inflammatory cytokines, in particular TNF-α, on muscle wasting and catabolism (10). These findings 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 (10). These findings are important to elaborate the various contributions of the different signaling pathways during muscle atrophy. The upregulation of atrogin-1/MAFbx and MuRF1 is normally blocked by Akt functioning through negative regulation of the FoxO family of transcription factors (49, 82, 93). The FoxOs family in skeletal muscle is comprised of three isoforms: FoxO1, FoxO3, and FoxO4. Akt phosphorylates and inactivates FoxOs from the nucleus to the cytoplasm. As predicted, the reduction in the activity of the Akt pathway observed in different models of muscle atrophy results in decreased levels of phosphorylated FoxO in the cytoplasm and a marked increase of nuclear FoxO protein. The translocation and activity of FoxOs members is required for the upregulation of atrogin-1/MAFbx and MuRF1, and FoxO3 was found to be sufficient to promote atrogin-1/MAFbx expression and muscle atrophy when transfected in skeletal muscles in vivo (82).

In fact, the knockdown of FoxO expression by RNAi is able to block the upregulation of atrogin-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). Moreover, 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 catabolism. In the inactive state, NF-κB is sequestered in the cytoplasm by a family of inhibitory proteins called IκB. Upon binding of TNF-α, the IκB kinase (IκK) 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 IκKα in transgenic mice leads to severe muscle wasting and atrophy, at least in part, by the ubiquitin-ligase MuRF1, but not by IκB kinase. The degradation of IκBα is mediated by the ubiquitin ligase MAFbx, which is upregulated during muscle atrophy (11). The ablation of IκBα results in severe muscle wasting and cachexia, whereas the ablation of MAFbx abrogates the phenotype. Moreover, the ablation of any of these genes decreases the atrophy rate caused by Akt knockdown. The interaction between these two pathways and their role in muscle atrophy is not fully elucidated. The identification of the mechanisms underlying the interaction between these two pathways is required to understand the molecular basis of muscle wasting and cachexia.
Muscle atrophy and muscle loss of atrogin-1 proteasomal protein synthesis is inhibited by Akt pathway (92). Akt pathway also regulates 4EBP1 and its role in triggering muscle atrophy is not obvious. The first report that sustains this notion described a severe atrophy when CHO cells, engineered to express myostatin, were injected in skeletal muscles (113). The atrophy in these mice was so severe that some animals died. However, these findings were not confirmed by transgenic mice. Expressing myostatin specifically in skeletal muscle leads to only 20% of atrophy in males and no phenotype in females (74). Electroporation experiments show that myostatin expression in adult muscle induces a degree of atrophy comparable to that observed in transgenic mice (21). These findings suggest that CHO cells substantially contribute to muscle atrophy by secreting some cachectic factors and that the myostatin pathway is synergistic with other pathways. No report clearly shows whether myostatin expression is controlled by FoxO1, suppression of the IGF1 pathway (17, 19, 33). Therefore, Akt phosphorylation should always be considered and explored when the NF-κB pathway is perturbed, since Akt inhibition can substantially contribute to muscle atrophy. This concept is supported by results obtained with conditional knockout mice for IKKβ, which are resistant to muscle atrophy but show hyperphosphorylation of Akt (66). Thus the amount by which muscle atrophy is reduced by IKKβ ablation and the degree to which it is Akt dependent is unclear. The cross talk between the two pathways is nevertheless important, and future studies should establish the contribution of IKK-NF-κB pathways versus Akt-FoxO pathways in relation to muscle atrophy. Interestingly, modulation of IKK-NF-κB pathway seems to affect cellular turnover in mdx mice (2).

Muscle atrophy is reduced by IKKβ down is sufficient to trigger atrogenes expression and muscle loss breakdown and Akt but also regulates EFP1 and mTOR (92). Akt down regulation of atrogenes is insufficient to trigger muscle atrophy, further supporting the notion that Akt is insufficient to trigger muscle atrophy (2).

**Myostatin and the enigma of the downstream targets**

Despite the hypertrophic effect of myostatin inhibition, the opposite situation of myostatin activation and its role in triggering muscle atrophy is not obvious. Myostatin is a member of the TGF-β superfamily and is produced by extracellular matrix cells and bone. It is produced by extracellular matrix cells and bone. It is a secreted cytokine that inhibits muscle growth and is expressed in mice, rats, and humans. The role of myostatin in skeletal muscle cells is unclear. The cross talk between the two pathways does not require NF-κB, whose inhibition does not prevent upregulation of atrogin-1 (58). Similarly, in cardiac cells, myostatin regulates phenylephrine hypertrophic effects through inhibition of Akt (64). A further level of complexity is that myostatin expression is controlled by FoxO1, supporting the notion that the myostatin pathway is synergistic to Akt-FoxO signaling (5). However, despite the evidence of cross talk between Akt and TGF-β pathways, it is still unclear whether Smads, the transcription factors downstream of TGF-β signaling, are mediating some of the myostatin effects in muscle. Furthermore, Smads can recognize the DNA sequence CAGAC, but their affinity seems too low to support unsustained binding to DNA. In addition, if their affinity for the simple sequence were higher, Smads would be considered to be members of the Smad superfamily. Therefore, activated Smads must associate with different DNA-binding cofactors for the recognition and regulation of specific target genes (54).}

Interesting members of the forkhead box O family associate with different DNA-binding cofactors for the recognition and regulation of specific target genes (54). Myostatin inhibition is increased expression of atrogin-1. This cross talk between the two pathways is synergistic with other pathways. No report clearly shows whether myostatin expression is controlled by FoxO1, suppression of the IGF1 pathway (17, 19, 33). Therefore, Akt phosphorylation should always be considered and explored when the NF-κB pathway is perturbed, since Akt inhibition can substantially contribute to muscle atrophy. This concept is supported by results obtained with conditional knockout mice for IKKβ, which are resistant to muscle atrophy but show hyperphosphorylation of Akt (66). Thus the amount by which muscle atrophy is reduced by IKKβ ablation and the degree to which it is Akt dependent is unclear. The cross talk between the two pathways is nevertheless important, and future studies should establish the contribution of IKK-NF-κB pathways versus Akt-FoxO pathways in relation to muscle atrophy. Interestingly, modulation of IKK-NF-κB pathway seems to affect cellular turnover in mdx mice (2).

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induction is still unclear, but recent evidence suggests that the autophagy-lysosome system is activated dur-
ing atrophy. Mizushima et al. (62) generated trans-
genic 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 aggre-
gates. Morphological analyses documented the activa-
tion of the autophagy system during fasting in skeletal
muscle (62). Indeed, muscle cell culture confirmed
that the autophagy-lysosome system is the major pro-
teolytic pathway implicated in nutrient-dependent
proteolysis (63). Further experiments lend insight into
the signaling pathways involved and identified an
autophagic control of the autophagic system in myotubes (96).
Furthermore, electron microscopic and biochemical
studies have shown that autophagy is activated also in
denervation atrophy (24, 86). However, denervation-
induced atrophy shows a slower pace of autophagy
when compared with fasting-mediated atrophy. This
effect is mediated by Runx1, which is upregulated dur-
ings denervation and is required to preserve muscle
mass. Lack of Runx1 resulted in myofibrillar disorgan-
ization and excessive autophagy in denervated
muscles (105). Runx1 knockout mice show double- or
multimembrane vacuoles, which enclose mitochon-
dria and membranes. This finding indicates that
excessive autophagy is promoting severe wasting dur-
ing 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
atrogenes 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 mus-
cles. However, the contribution of mTOR-lysosome
and ubiquitin-proteasome systems to organelle
remodelling, 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 glycoly-
sis and oxidative phosphorylation are suppressed coordinately (45). Indeed, we have recently shown that PGC-1α, the master regulatory gene for mito-
ochondria 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,
mitochondrial homeostasis and energy produc-
tion rescues atrophy (4). Induction of PGC-1, well as auton-
omaic activation of AMPK, increases the levels of PGC-1α
and smaller muscle mass. It is also possible that the
connection between PGC-1α and ATP production could
be due to a cross-talk with the mitogen-activated
protein kinase (MAPK) pathway (29, 30). Indeed,
AMPK activating mutant decreases the levels of PGC-1α
confirming that AMPK is necessary for maintaining
mitochondrial biosynthesis and oxidative phosphorylation.
Myostatin, a cytokine that regulates muscle growth,
has been shown to downregulate PGC-1α in muscle
cells. Therefore, the relationship between PGC-1α and
myostatin needs to be defined in future studies.

Mechanical signals and muscle atrophy
The dystrophic mouse model is a valuable tool to
study the molecular mechanisms underlining the
muscles are prone to rapid atrophy. Treatment with
myostatin-1 activates atrophy in muscle cell culture and in
vivo. Similarly, denervation is still unclear, but recent evidence suggests that
the dystrophin gene is associated with the development of muscle
atrophy. However, the role of dystrophin in muscle atrophy is yet to be
fully understood.

Mechanical stress is a major factor that regulates
muscle atrophy. Mechanical signals, such as stretch and
contraction, can activate various pathways that lead to
muscle atrophy. In particular, mechanical loading has
been shown to activate the unfolded protein response
(UPR), which inhibits protein synthesis and leads to
muscle atrophy. Additionally, mechanical signals can
activate the AMPK pathway, which is involved in
energy metabolism and regulates the expression of
proteolytic enzymes.

Several studies have shown that mechanical stress
increases the expression of atrogenes, such as ubiquitin
proteasome and lysosome, which are crucial for
muscle atrophy. For example, in vitro studies have
shown that mechanical loading increases the expression
of ubiquitin ligases, such as MAFbx and MuRF1, which
are key regulators of skeletal muscle atrophy.

These catabolic pathways are regulated by changes in
trophic factors, such as TNF-α, IL-6, and growth factors like IGF-1, which
inhibit muscle atrophy. For instance, IGF-1 activates Akt,
a downstream effector of PI3K, which inhibits the
expression of atrogenes. Additionally, IGF-1 activates the
mitogen-activated protein kinase (MAPK) pathway, which
inhibits muscle atrophy.

Several studies have shown that mechanical stress
activates the mechanical stretch receptor (MSR) in
muscle cells, which leads to the activation of the
mitogen-activated protein kinase (MAPK) pathway and the
expression of atrogenes.

Mechanical stretch has also been shown to activate
the autophagy-lysosome system, which is a major
pathway involved in the regulation of muscle
atrophy. In particular, mechanical stretch activates
the autophagy-lysosome system in muscle cells,
resulting in the degradation of protein aggregates and
the recycling of organelles.

These results suggest that mechanical stress is a
major factor that regulates muscle atrophy through
the activation of the ubiquitin-proteasome, lysosome,
and autophagy-lysosome systems. Further studies
are needed to elucidate the molecular mechanisms
underlying the regulation of muscle atrophy by
mechanical signals.
cells are among the factors that suppress muscles, whose expression and activity are regulated by PGC-1α (95). Interestingly, increased levels of AMPK, activation of AMPK, lipid consumption, and smaller muscles have all been observed in S6K1 and S6K2 knockout mice. Importantly, AMPK inhibition rescues skeletal muscle growth in these mice, confirming that S6K1/2 depletion alters energy balance (4) and suggesting that AMPK activity is a key factor for myofiber 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 confirm that a metabolic program controls cell size, but the molecular mechanisms of such control remain to be understood. Very recent findings describe a connection between AMPK and FoxO3. AMPK phosphorylates several ACT-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 FoxO family (70). The physiological relevance of such important control over protein breakdown and muscle atrophy in vivo remains to be explored.

Within the myofiber, mitochondria are crucial in regulating metabolism and might be a potential source of catalytic signals. Insulin resistance and diabetes have been reported to trigger caspase-mediated actin cleavage, which would release myofibrils from the sarcomere for subsequent degradation via the proteasome (20). Caspase3 activation is mediated by Bax, which disrupts the mitochondrial external membrane, causing cytochrome-c release and apoptosis formation (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 movements on actin to the extracellular matrix. Indeed, lack of dystrophin exacerbates mechanical stress on the myofiber plasma membrane, causing muscle damage and degeneration. This classical view considers a purely structural function of DGC proteins. However, an attractive role of dystrophin in transduction 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 myofiber and the extracellular matrix (1). This decreased interaction between membrane and matrix is required for muscle loss since forced dystrophin expression in transgenic mice counteracts both cachexia-induced muscle wasting and upregulation of atrogin-1/MAFbx and MuRF1. Further support 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 sarcolemma. A similar nNOS dysregulation has recently been described during disuse-induced muscle atrophy. Free nNOS dislocates to the cytoplasm where it enhances FoxO3-mediated transcription and upregulation 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, glucocorticoid treatment induces atrogin-1 and MuRF1 expression and muscle wasting both in cell culture and in vivo (10, 13, 77, 82, 84). Importantly, adrenalec- tomy or treatment with a glucocorticoid receptor antagonist (RU-486) attenuates muscle loss in some diseases (84). However, the mechanisms of glucocorticoid-mediated muscle atrophy are unclear. In fact, none of the atrophy-related genes have been found to be directly regulated by glucocorticoids, and no glucocorticoid response elements on atrogenes promoters have been identified as critical for their expression (45). Thus most of the glucocorticoid effects are indirect, mainly affecting pathways previously described to be crucial for muscle growth or loss. Glucocorticoids are reported to decrease IGF1 production and increase myostatin secretion. Other effects like downregulation of the anabolic transcription factor ATF4, activation of p38/HEAT, or upregulation 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-depend- ent MyoD and M1 degragation by NEl, terminal ubiquitination pathway, especially if we consider that MyoD is described to be an atrogin-1 substrate (94, 98). How MyoD loss can contribute to weakness and muscle protein loss is a common theme in the muscle atrophy literature and cross talk and modulate one another at different pathway in muscle loss.

...function experiments on glucocorticoid receptor 98). How MyoD loss can contribute to weakness and MyoD is described to be an atrogin-1 substrate (94, 98). How MyoD loss can contribute to weakness and muscle protein loss is a common theme in the muscle atrophy literature and cross talk and modulate one another at different pathway in muscle loss.

Conclusions

Over the last few years, the mechanisms controlling muscle loss have attracted the attention of the scientif- ic community due to their relevance in various fields of study such as aging, prognosis of many diseases, quality of life, and sports medicine. From the recent findings, a new scenario emerges that considers the size of the myotroph and muscle performance a result of not a single pathway but of a network of signaling (FIGURES 2 AND 4). Strikingly, the different path- ways cross talk and modulate one another at different levels, coordinating protein synthesis and degradation simultaneously. Findings of the last few years offer new and existing perspectives to the field and introduce a new series of stimulating questions to the com- munity, setting the base for future studies, which should enable us to identify new therapeutic targets and drugs.

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References


