PHYSIOLOGY 23: 160–170, 2008; doi:10.1152/physiol.00041.2007

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 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 embryo. 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 turnover 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 synergistic 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,

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direct evidence for such a role through loss-of-function approaches, such as knockout or knockdown experiments, has not yet been reported.

AKT. 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 SHIP2. Phosphatidylinositol-3,4,5-triphosphates recruit Akt to the plasma membrane by binding to its NH_2-terminal pleckstrin homology domain. At the membrane, Akt is phosphorylated on separate residues by at least two distinct kinases, PDK1 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 (99). 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 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 in the atrophy section. GSK3β is inhibited by Akt and in turn blocks the eukaryotic initiation factor 2B (eIF2B), which is involved in protein synthesis. Expression of a dominant negative kinase inactive form of GSK3β induces a dramatic hypertrophy in skeletal myotubes (75). However, it remains to be proven in vivo whether inhibiting the negative action of GSK3β on eIF2B is sufficient to promote muscle growth.

mTOR. The kinase mTOR (mammalian target of rapamycin) has recently emerged as a key regulator of cell growth that integrates signals from growth factors, nutrients, and energy status to control protein synthesis and other cell functions (32, 97). As the name implies, mTOR is selectively inhibited by rapamycin, a drug used as an immunosuppressant in organ transplantation: rapamycin binds to members of the FK binding protein (FKBP) family and the complex rapamycin/FKBP binds to mTOR and blocks its activity. The role of mTOR in muscle growth was demonstrated by in vivo studies showing that rapamycin blocked overload hypertrophy and regenerating muscle growth (10, 72). Indeed in tetracycline-inducible Akt transgenic mice, rapamycin completely blunts Akt effects on muscle growth (37). The activation of mTOR by Akt is indirect and involves the phosphorylation and inhibition by Akt of tuberous sclerosis 2 (TSC2). TSC2 is a GTPase activating protein (GAP) that functions together with TSC1 to inactivate the small G

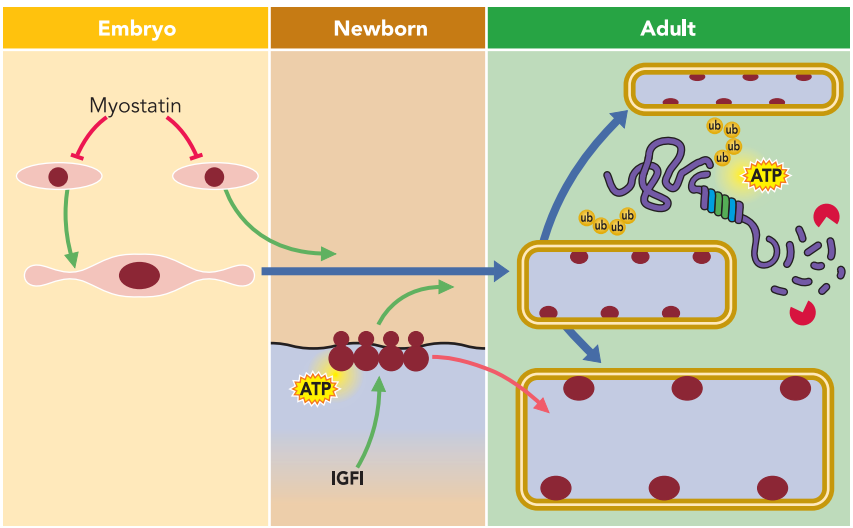

FIGURE 1. **Schematic description of spectrum of developmental stages and the different contribution of cellular turnover and protein turnover to muscle growth**
The principal pathways controlling cell and protein turnover are schematically depicted.

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 S6K and 4EBP1, 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

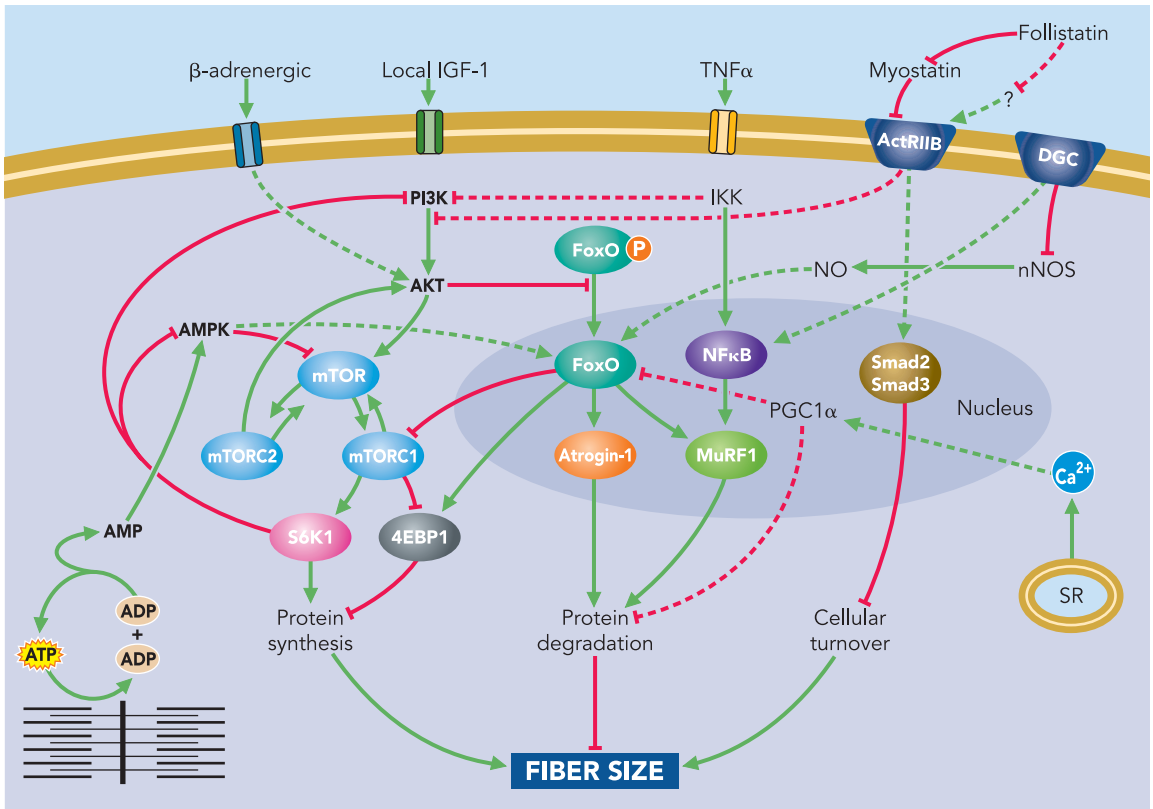

FIGURE 2. **Scheme illustrating the major pathways that control fiber size**
Dotted lines depict pathways whose molecular mechanisms and role in adult skeletal muscle have yet to be completely defined.

recombinase removes the floxed myostatin gene in 4-mo-old mice, muscle mass increases 25% during the next 3 mo (108). However, despite the effect on muscle mass, the specific muscle force has not been measured in these two studies. Overexpression of follistatin, an inhibitor of myostatin (47), promotes a great increase in muscle size. Interestingly, mice resulting from follistatin transgenic and myostatin knockout mice show tremendous increases in muscle mass supporting the concept that other myostatin-like molecules are present and relevant for muscle growth (46). There are few studies and conflicting results on the effect of exercise on myostatin gene expression in skeletal muscle (15). Furthermore, the increase of muscle mass of myostatin-null mice does not correlate with an increase in muscle force (7). Ultrastructural observations reveal an accumulation of tubular aggregates in type IIB fibers. This evidence is also supported by the decrease in specific force generation of hypertrophic ski transgenic mice (12). Ski negatively regulates Smad phosphorylation, thereby inhibiting signaling of TGF-β-like factors, such as myostatin. These findings contrast with muscle-specific overexpression of insulin-like growth factor 1, where fiber hypertrophy is accompanied by increased maximum force generation and maintained specific force levels. Myostatin binds to activin receptor IIB (48), a type II TGF-β receptor, and muscle hypertrophy is induced in transgenic mice expressing a truncated and inactive activin receptor (ActRIIB) (47). The downstream targets of the myostatin pathway and their role in protein synthesis as well as protein degradation (see below) are still to be determined. However, myostatin inhibition is benefical for maintaining muscle mass in animal models of Duchenne muscular dystrophy in which the contribution of satellite cells to rounds of regeneration is important (61, 102).

Beta adrenergic and mechanical sensors

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 β_2-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 (8, 90). Indeed the growth effect of beta-agonists is, at least partially, mediated by AKT-mTOR pathway since rapamycin almost completely blunted 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 scaffolding proteins, nbr1 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 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 know 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 serum response elements (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 (101). 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, disuse, 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, 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). Importantly, recent findings suggest that myosin 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 protease, 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 signaling pathways that regulate the expression of the two muscle-specific ubiquitin ligases. Previous studies have shown that IGF1/insulin signaling, while promoting muscle growth, is able to suppress protein breakdown (77). Furthermore, IGF1 transgenic mice are resistant to muscle atrophy induced either by angiotensin treatment or in a mouse model of cardiac cachexia (88, 91), and local IGF1 injection is sufficient to block disuse atrophy (93). In these models of muscle loss, IGF1 completely suppressed the induction of the two critical ubiquitin-ligases. Further data supporting 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 FoxO family in skeletal muscle is comprised of three isoforms: FoxO1, FoxO3, and FoxO4. Akt phosphorylates FoxOs, promoting the export of 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 FoxO 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). 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 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). 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 IKKβ in transgenic mice leads to severe muscle wasting mediated, at least in part, by the ubiquitin-ligase

MuRF1, but not by atrogin-1/MAFbx (11) (FIGURE 2). On the other hand, although muscle-specific inhibition of NF-κB by transgenic expression of a constitutively active IκB mutant leads to no overt phenotype, denervation atrophy is substantially reduced (38). Muscle atrophy induced by hindlimb unloading is likewise reduced in mice with a knockout of the p105/p50 NF-κB1 gene (35). However, TNF-α and proinflammatory cytokines also cause insulin resistance and 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).

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. 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 muscles 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 is sufficient to trigger atrogenes expression and protein breakdown in vivo. However, in muscle cell culture, myostatin is reported to upregulate the critical atrophy-related ubiquitin ligases, and interestingly the mechanism of such regulation is FoxO dependent (58). In fact, myostatin treatment blocks the IGF1-PI3K-AKT pathway and activates FoxO1, allowing the increased expression of atrogin-1. This 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 abrogates 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 unassisted binding to DNA. In addition, if their affinity for the simple sequence were higher, Smads would decorate the entire chromosome. Therefore, activated Smad proteins 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 (FoxO1, FoxO3, and FoxO4) play such a role (27). To further confuse the role of Samds during muscle wasting is the presence of TGIF, an inhibitor of Smads, among the upregulated atrophy-related genes (76). Thus the mystery of why an inhibitor of Smads is activated early, before muscle loss becomes evident, must be addressed. Dissecting the roles of Smads and identifying the co-factors that mediate Smad transcriptional activity in adult muscle are intriguing questions that require attention in the coming years.

Rediscovering lysosomes under a new light: autophagy-mediated protein breakdown

Lysosomes are the cellular system charged with the removal of organelles and protein aggregates. Indeed, autophagy is constitutively active in skeletal muscle, as shown by the accumulation of autophagosomes seen in human myopathies caused by a genetic deficiency of lysosomal proteins, e.g., Pompe's and Danon's disease, or by pharmacological inhibition of lysosomal function, as in chloroquine myopathy (89). 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

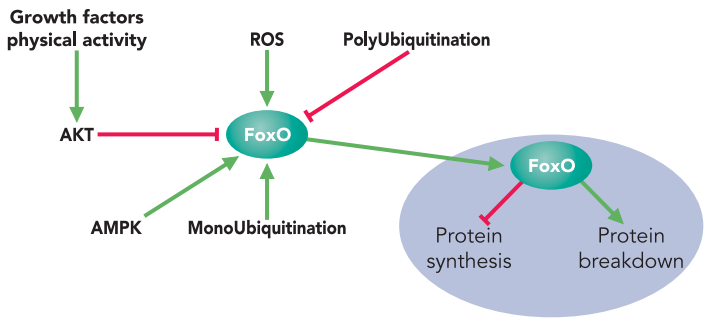

FIGURE 3. **Scheme illustrating the regulation of FoxO transcription factors and their potential role in the atrophy program**
AKT is activated by growth factors and by physical activity and phosphorylates FoxO, inducing its translocation from the nucleus to the cytoplasm. Conversely, AMPK phosphorylates FoxO, increasing its transcriptional activity. Various other activators of FoxO are also indicated, including oxdative 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 mTOR-independent but $PI3K_{III}$-beclin-dependent 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 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 mice show double- or multimembrane 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 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 muscles. However, the contribution of the autophagy-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 mitochondria 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,

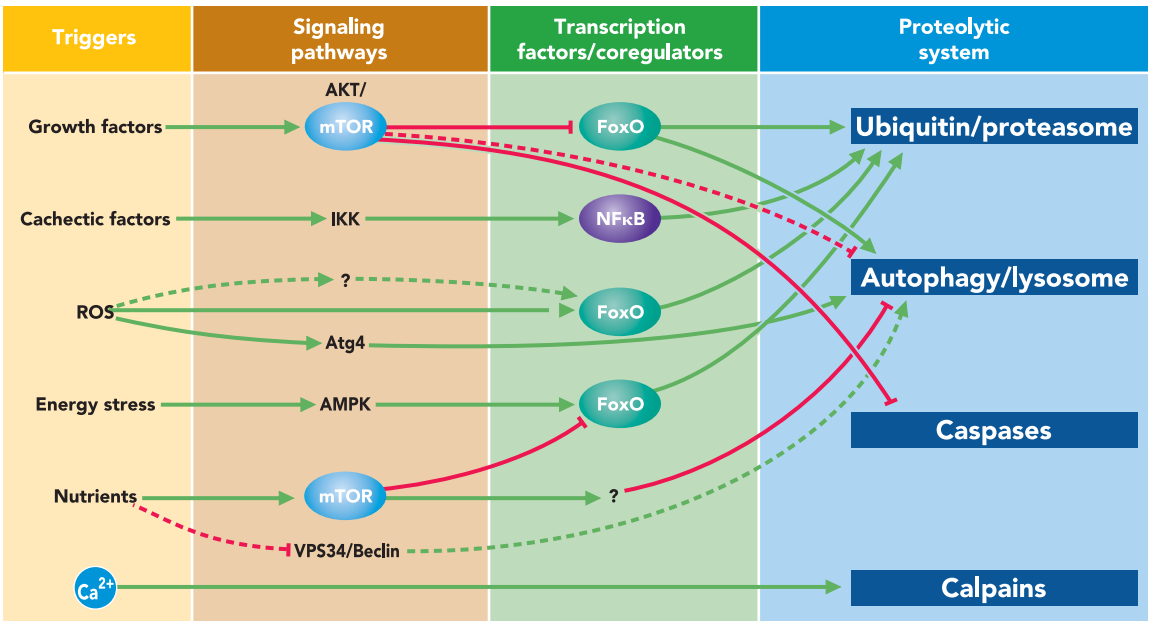

FIGURE 4. **Scheme illustrating some of the triggers that could activate proteolytic systems that lead to muscle atrophy**
These catabolic pathways are regulated by changes in *1*) growth factors, *2*) cachectic factors such as TNF-α, IL-6, and myostatin, *3*) oxidative stress mediated by ROS or NO, *4*) metabolic postcontraction events, such as the increase in ATP consumption, activation of adenylate kinase (2ADP, 3ATP + AMP) which rises intracellular AMP, and consequent activation of AMP-activated protein kinase (AMPK), *5*) nutrient availability such as aminoacids and glucose, *6*) Ca^{2+} imbalance. Few transcription factors have been identified to mediate these effects. The major proteolytic systems, the ubiquitin-proteasome and lysosome, are recruited to degrade most of the myofiber content, whereas the role of caspases and calpains is suggested by evidence, but final proofs are still missing. Dotted lines depict pathways whose molecular mechanisms and role in adult skeletal muscle have to be defined.

muscles are protected from the atrophy induced by denervation, fasting, or expression of FoxO3 (81). Treatment with statins induces FoxO-mediated atrogin-1 activation and related muscle alterations. In cell culture and in zebrafish, statins cause muscle atrophy, disorganization of myofibrils, and mitochondrial dysfunction (31). These changes are completely aborted by either the deletion of atrogin-1 or by PGC-1α overexpression. 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 different 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 inhibition 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 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 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 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 catabolic signals. Insulin resistance and diabetes have been reported to trigger caspase3-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 apoptosome 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 oxygene 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, adrenalectomy 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 p300/HAT, 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 dexamethaspone-dependent MyoD and Id1 degradation by NH_2-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 breakdown is 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 myofiber 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 new and exciting perspectives to the field and introduce a series of new stimulating questions to the community, setting the base for future studies, which should enable us to identify new therapeutic targets and drugs. ■

The critical reading of Kenneth Dyar is gratefully acknowledged. I apologize to colleagues whose studies were not cited owing to space limitations.

Our work is supported by grants from ASI (OSMA project), Telethon-Italy (TCP04009), and Compagnia San Paolo.

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