Signaling in Muscle Atrophy and Hypertrophy

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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 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 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 display growth whereas Akt2-like syndromes in brain development. Exercise increases Akt1 expression but not Akt2 (99). Akt activity can also induce the functional overload in dystrophic muscles and humans with response to exercise. Surprisingly, the transgenic muscle EDL but not tibialis anterior finding that people can also induce mechanical stress by which contractions (79). How Akt functions in skeletal muscle hypertrophy activity is also known to act directly Akt1. Taken together these results support that skeletal muscle is established during growth, the direct Akt1 and hypertrophy role.
is minor, and is currently debated whether IGF1 receptor activation in skeletal muscle can also induce Akt activation has suggested that Akt is a major mediator of 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.

**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 3 (GSK3), 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. GSK3 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 GSK3 induces a dramatic hypertrophy in skeletal myotubes (75). However, it remains to be proven in vivo whether inhibiting the negative action of GSK3 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 blocks overload hypertrophy and regenerating muscle growth (10, 72). Indeed in tetracycline-inducible Akt transgenic mice, rapamycin completely blunts Akt effects on muscle growth (137). 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 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 down-stream 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 male old mice, next 3 mo (108 mass, the specific inhibitor of myostatin transgenic mice show tremendous increase in muscle size. Furthermore, statistically mice show increased muscle force (107) and increased specific force (108a). This evidence is consistent with the concept that the acute elevation of beta-adrenoreceptor activity is induced by tamoxifen (12) and may have a role in protein synthesis, which, thereby, affects the effects of tamoxifen on muscle growth in a manner similar to that in which the Akt-FoxO molecular mechanisms and role in adult skeletal muscle have yet to be completely defined.

**Beta adrenoreceptor signaling**

Among the hormones that affect muscle growth, the acute elevation of beta-adrenoreceptor activity is of particular interest. Beta-adrenoceptor signaling is mediated by a critical process that involves the recruitment of beta-adrenoceptor kinase to activated beta-adrenoceptors, resulting in the increased phosphorylation of downstream targets such as myostatin and Akt. Interestingly, activation of myostatin by beta-adrenoreceptor signaling is inhibited by rapamycin (108a), which blunts the hypertrophic response of skeletal muscle. The Akt-FoxO pathway is activated by beta-adrenoreceptor signaling. An attractive model for this pathway is that signals generated by beta-adrenoreceptors activate PKA, which increases the phosphorylation of Akt, leading to increased FoxO activity and decreased muscle growth.
muscle growth. A hypertrophic role in protein synthesis as well as protein degradation downstream targets of the myostatin pathway and their inhibition in adult muscle (14, 47, 59, 61). Only a 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 type II (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 beneficial 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 β2-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 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 scaffolding proteins, nH1 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 SRE, leading to nuclear export of SRE and loss of SRE-dependent gene expression. This pathway may thus control muscle growth because SRE is known to regulate muscle gene expression, and conditional deletion of the SRE gene causes severe skeletal muscle hypoplasia during the perinatal period (51). SRE regulates muscle gene expression by binding serum response elements (SRE) in target genes and seems to integrate different growth promoting pathways: for example, SRE 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 al 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 starvation. 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 near 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, when 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 denervation atrophy (83). 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 (14). 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: FoxD1, FoxO4, and FoxO3. Akt phosphorylation of FoxO3α and FoxO4α 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 FoxO3α in the cytoplasm and a marked increase of nuclear FoxO4 protein. The translocation and activity of FoxO members is required for the upregulation of atrogin-1/MAFbx and MuRF1, and FoxO3α 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 (82). 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 (58, 92). On the other hand, knockout mice for NF-κB subunits show marked resistance to muscle atrophy induced by denervation or severe muscle wasting. The role of NF-κB in muscle atrophy, irrespective of the type of injury, is a major issue in the pathogenesis of myopathies. The NF-κB signaling network includes cytokines, NF-κB, and its role in the proliferation of immune cells, myostatin, and inflammatory cytokines, in particular TNF-α and IL-6. Therefore, Akt phosphorylation of IκBα is perturbed, since Akt has no role in IκBα degradation and in the NF-κB signaling pathway. Interestingly, recent findings suggest that Akt is able to suppress NF-κB signaling in muscles (58). In fact, Akt signaling between the two pathways is still unclear. To date, only NF-κB-AKT interaction has been described. 

**Mystatin as a downstream regulator of muscle wasting**

Despite the huge number of studies on muscle wasting and its role in the pathogenesis of cachexia, the molecular mechanisms of muscle wasting remain poorly understood. The first report that myostatin, a muscle-acting cytokine, could induce muscle wasting was published by Wood and colleagues in 1998 (3). Myostatin is a secreted member of the TGF-β superfamily and was shown to be expressed in skeletal muscles and to be downregulated in the regenerating muscle during regeneration of muscle mass in rats. Myostatin's mechanism of action is still unknown, but it seems to affect the balance between muscle cell proliferation and differentiation. Nevertheless, the reduction in the activity of the Akt pathway observed in different models of muscle atrophy results in decreased levels of phosphorylated FoxO3α in the cytoplasm and a marked increase of nuclear FoxO4 protein. The translocation and activity of FoxO members is required for the upregulation of atrogin-1/MAFbx and MuRF1, and FoxO3α 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 (82). 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).
Transfection in vivo with active Akt or muscle atrophy inhibitors and findings are blemishes of the muscle atrophy pathway. Although negative factors muscle wasting and FOXO members (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-κB gene (35). However, TNF-α and pro-inflammatory cytokines also cause insulin resistance and suppression of the IGF1 pathway (17, 19, 33). Therefore, Akt phosphorylation should always be considered and 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). Electrophysiological 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 (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 Smads during muscle wasting is the presence of TGF-β, 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 catterpil-L, a lysosomal protease, is upregulated in different models of muscle wasting (18, 45). The role of catterpil-L in muscle atrophy is still to be elucidated.

**FIGURE 2.** Schematic illustrating the regulation of FoxO transcription factors and their potential role in the atrophy program. FOXO is activated by growth factors and by physical activity and phosphorylates FoxO1, inducing its translocation from the nucleus to the cytoplasm. Conversely, AMPK phosphorylates FoxO1, 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 pro-
tection pathway implicated in nutrient-dependent proteolysis (63). Further experiments lend insight into the signaling pathways involved and identified an mTOR-independent but PI3KIII-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 disorganiza-
tion and excessive autophagy in denervated muscles (105). Runx1 knockout mice show double- or triple-stranded muscle fibers with reduced levels of PGC-1α, the master regulatory gene for mitochondria biogenesis, is downregulated in different pathological situations (45). Indeed, we have recently shown that PGC-1α is sufficient and required to activate lysosomal-related proteolytic systems (112). In fact, the role of mTOR signaling for the regulation of autophagy is irrelevant in skeletal muscles. However, the contribution of the autophagy-lysosome system is the major pro-
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tection system during fasting in skeletal muscle.

**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 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 pathological situations (45). Indeed, we have recently shown that PGC-1α is sufficient and required to activate lysosomal-related proteolytic systems (112). In fact, the role of mTOR signaling for the regulation of autophagy is irrelevant in skeletal muscles. However, the contribution of the autophagy-lysosome system is the major protection system during fasting in skeletal muscle.

**Mechanical stress**

The dystrophin protein is required for muscle cell membrane stability and is markedly reduced in Duchenne muscular dystrophy (DMD) patients. Dystrophin is a 427-kDa protein located in the sarcolemmal membrane and is essential for the prevention of sarcolemmal membrane defects. The dystrophin-deficient state is characterized by muscle fiber degeneration, spontaneous release of muscle enzymes, and a severe myopathic phenotype. The role of autophagy in DMD has not been fully elucidated. However, recent studies have shown that autophagy is activated in dystrophic muscles, and its activation appears to be crucial for maintaining muscle integrity and preventing muscle degeneration. The role of autophagy in DMD is still under investigation, but it is evident that autophagy plays a key role in the pathogenesis of muscular dystrophy.
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, promoting its transcriptional activity on target genes (49). Furthermore, inhibition of caspases by caspase-3 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 cells from atrophy in an animal model of diabetes (106). Importantly, AMPK inhibits BAX, an activator of caspases, a key factor for the muscle size (4). Interestingly, these mice show normal muscle size in skeletal muscle, and smaller muscles have 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 muscle size. Interestingly, these mice show normal methionine incorporation and polysomal profiles as well as autophagy and upregulation of autophagy-related genes (49). 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, promoting 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-3-mediated actin cleavage, which would release myofibrils from the sarcomere for subsequent degradation via the proteasome (20). Caspase-3 activation is mediated by Bax, which disrupts the mitochondrial external membrane, causing cytokrome-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 dystrophic 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 movement from 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. Furthermore, nNOS silences the cytoplasm where it enhances FoxO3-mediated transcription and upregulation of atrogin-1 and MuRF1 (95). Interestingly, the NF-kB 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, 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 p380/ERK, or upregulation of REED1, 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 deamethasone-dependent MyoD and M1 degradation by NIH-3T3 ubiquitin-ligase 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 protein loss have attracted the attention of the scientific community due to their relevance in various fields such as studies on 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 myotube 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 new opportunities for therapeutic intervention.

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