Signaling in Muscle Atrophy and Hypertrophy

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 (63). 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 (63) 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 (63, 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 provide additional information by which cell size is controlled. The results could be completely different if the same way is acute 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 synthetic muscles (55). Muscle-specific overexpression in transgenic mice of an IGF1 isoform locally expressed in skeletal muscle results in muscle hypertrophy (68) and, importantly, the growth of muscle mass matches with a physiological increase of muscle strength. Moreover even acute ectopic expression of IGF1 in adult muscles by electroporation is sufficient to promote muscle hypertrophy (6). Although these results suggest an autocrine/paracrine role for local IGF1 in activity-dependent muscle plasticity, direct evidence approaches, segments, has no AKT. AKT activity through the growth-promoting factors, the activity of the same pathway, the local pleiotropic action of AKT is both a distinct pathway and different. The role of AKT in different tissues, the signaling in the regulation of muscle mass, and the observation with the RASV12C40 transgenic system, which Akt is overactive after tamoxifen administration. In mammals, AKT1, AKT2, and AKT3 have distinct functions and display growth-promoting roles, whereas Akt2, like syndromic disorders, regulates muscle growth in brain development.

Exercise in vivo promotes muscle growth (54, 99). Akt activity, a functional overexpression, is also observed in experimental studies, and humans, is associated with response to training (42). Surprisingly, the growth-promoting function of Akt in EDL but not in vastus lateralis, finding that physical exercise can also induce muscle hypertrophy, by which conditions Akt1, Akt2, and Akt3, which are expressed in skeletal muscle (79). A specific role for Akt3, which is highly expressed in skeletal muscle, is a distinct feature of skeletal muscle, and in mice, Akt3 is required for muscle growth, indicating that Akt3 is involved in the control of muscle hypertrophy (86). Similarly, Akt1 plays a role in the regulation of muscle growth, and Akt2 has been shown to promote muscle hypertrophy (6). Although these results suggest an autocrine/paracrine role for local IGF1 in activity-dependent muscle plasticity, direct evidence approaches, segments, has no AKT. AKT activity through the growth-promoting factors, the activity of the same pathway, the local pleiotropic action of AKT is both a distinct pathway and different. The role of AKT in different tissues, the signaling in the regulation of muscle mass, and the observation with the RASV12C40 transgenic system, which Akt is overactive after tamoxifen administration. In mammals, AKT1, AKT2, and AKT3 have distinct functions and display growth-promoting roles, whereas Akt2, like syndromic disorders, regulates muscle growth in brain development.

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The kinase mTOR (mammalian target of rapamycin) is a critical regulator of cell growth and division that integrates signals from growth factors, nutrients, and energy status to control protein synthesis, translation, 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 (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 protein synthase kinase 3 (GSK3), 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 blocks 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 protein synthase kinase 3 (GSK3), 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.

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 S6K2) 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 deactivating 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 mice were crossed with myostatin-null mice, the increase in muscle mass in adult mice was even more pronounced (108).

**Beta adrenergic receptors**

Among the most interesting is the role of the acute elevations in the levels of the neurotransmitter epinephrine and norepinephrine through β-adrenergic receptors. Interestingly, the β-adrenergic signaling is mediated by IGF1 signaling in mouse muscle, and activating the β-adrenergic receptors mTOR pathway is blunted in the β-adrenergic receptor knockout mice (102). IGF1 stimulates the Akt signaling, which results in decreased Mstn expression, and this signaling is blocked in the β-adrenergic receptor knockout mice (102). The increased muscle mass in the β-adrenergic receptor knockout mice may be due to a decrease in the IGF1 expression, which is a negative regulator of the myostatin pathway, and to decreased Mstn expression in muscle. The results suggest that the β-adrenergic receptor signaling has a protective role in muscle hypertrophy and that the β-adrenergic receptor signaling may be a potential therapeutic target for muscle wasting diseases.
Muscle growth. 

In mice, the transcriptional repressor FoxO. It is expressed in muscle and is known to regulate muscle growth. Beta-agonists such as clenbuterol, acting through beta-2-adrenergic receptors, 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 trim, 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 trim 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 trim kinase domain (TK) is linked through two zinc-finger scaffolding proteins, nrf1 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 known to regulate muscle gene expression, and conditional deletion of the SRF gene causes severe skeletal muscle hypoplasia during the perinatal period (51). SRF regulates muscle gene expression by binding 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 genes promoters (101). The trim 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 the contribution of certain pathways to muscle loss. As mentioned above, the mass of a tissue is controlled by cellular turnover and protein turnover (FIGURES 1 AND 2). Genetic modifications that interfere with embryonic and postnatal growth result in smaller muscles in adults. This reduction in muscle size is caused by failure/inhibition of growth and not by a real atrophy process. Keeping in mind the above definition, another important concept is that protein turnover is dominant over cellular turnover during acute phases of muscle wasting when sarcomeric proteins are rapidly lost such as during fasting, disease, and denervation. Evidence that blocking satellite cells is sufficient to trigger muscle atrophy in adult muscle has never been determined. Furthermore, myonuclei are normally reduced during muscle atrophy to keep rather constant the size of the nuclear domain (16, 22). Thus the current understanding suggests that inhibiting cellular turnover would not influence protein...
breakdown and muscle weakness and would not affect nuclear domain maintenance, but it might be important for the replacement of damaged myofilaments 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).

Currently, 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, IGFBP 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 muscle 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: FoxO1, FoxO3a, and FoxO4. Inactivation of FoxO is associated with increased protein breakdown (77). Furthermore, IGF1 transgenic mice are resistant to muscle atrophy induced either 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 effects 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 atrogin-1 (39). On the other hand, a recent study from our laboratory suggests that MuRF1, but not atrogin-1, is a downstream target of NF-κB-mediated muscle atrophy (92)

Muscle atrophy in a mouse model of cardiac cachexia, chronic renal failure, and cancer cachexia (39, 92) (FIGURE 3). Interestingly, 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 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 and muscle loss (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). Evidence supporting this notion comes from the experiments of Akt transfection in skeletal muscle, which is sufficient to promote atrogin-1/MAFbx expression and muscle atrophy when transfected in skeletal muscles in vivo (82). Alternatively, FoxO1 and FoxO3a were found to be sufficient to promote atrogin-1/MAFbx expression and muscle atrophy when transfected in skeletal muscles in vivo (82).

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Muscle atrophy and muscle wasting are major negative factors that contribute to muscle atrophy. The ubiquitin-ligase atrogin-1/MAFbx and their relatives FoxO members are considered and explored when the NF-κB pathway is 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 Smad 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 in 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. FoxO is activated by growth factors and by physical activity and phosphorylates FoxO, inducing its translocation from the nucleus to the cytoplasm. Conversely, AMPK phosphorylates FoxO, increasing its transcriptional activity. Various other activators of FoxO are also indicated, including oxidative stress, which induces acetylation of peculiar lysine residues, and monoubiquitination. Polyubiquitination occurs when FoxO is retrolocalized in the cytoplasm triggering its proteasomal degradation.

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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 PI3KIII-beclin-dependent role in the regulation of autophagy during fasting in skeletal muscle (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 lysosome and ubiquitin-proteasome systems to organelle remodeling, protein breakdown, and, finally, muscle atrophy remains to be investigated.

Mitochondrial homeostasis and energy balance: the metabolic control of cell size

Several metabolic adaptations occur in atrophying muscles. In many forms of muscle wasting, expression of a variety of genes for enzymes important in glycolysis and oxidative phosphorylation are suppressed coordinately (45). Indeed, we have recently shown that PGC-1α, the master regulatory gene for mitochondrial biogenesis, is downregulated in different models of muscle wasting. Furthermore, when the levels of PGC-1α are maintained, either by use of transgenic mice or by transfecting adult muscle fibers, expression of such important genes are restored, confirming that PGC-1α is required to preserve muscle mass. Lack of Runx1 resulted in myotubular disorganization and excessive autophagy in denervated muscles (105). Runx1 knockout mice show double- or triple-membrane vacuoles, which enclose mitochondria and membranes. This finding indicates that excessive autophagy is promoting severe wasting during denervation and needs to be reduced by Runx1. We have recently shown that the autophagy-lysosome and ubiquitin-proteasome systems are coordinately regulated during muscle wasting (53, 112). In fact, 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 PI3KIII-beclin-dependent role in the regulation of autophagy during fasting in skeletal muscle (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 lysosome and ubiquitin-proteasome systems to organelle remodeling, protein breakdown, and, finally, muscle atrophy remains to be investigated.

Mechanical damage

The dystrophic heart has both a mechanical dysfunction and a metabolic dysfunction. The myocardial cell cannot contract properly under these conditions, and the cardiomyocytes are damaged by the repeated contraction and relaxation that the heart cannot control. Thus, the heart is a site of mechanical damage in the disease process. The mechanical damage is exacerbated by the metabolic dysfunction, and the metabolic dysfunction is exacerbated by the mechanical damage. Thus, the two processes are interrelated and cannot be separated.
muscles are protected from the atrophy induced by denervation, fasting, or expression of FoxO3 (81). Treatment with statins induces FoxO3-mediated atrogin-1 activation and related muscle alterations. In cell culture and in zebrafish, statins cause muscle atrophy, disorganization of myofilaments, 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 suggest a connection between AMPK and FoxO3. AMPK phosphorylates several AKT-independent sites of FoxO3, enhancing FoxO3-mediated transcription and upregulation of 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 by the FoxO3 family (70). The physiological relevance of such important control over protein breakdown and muscle atrophy in vivo remains to be explored.

Within the 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 caspase-3-mediated actin cleavage, which would release myofilaments from the sarcomere for subsequent degradation via the prosaome (20). Caspase-3 activation is mediated by Bax, which disrupts the mitochondrial external membrane, causing cytochrome-c release and apoptosis (49). Furthermore, inhibition of caspasases by XIAP overexpression protects adult skeletal muscle from atrophy in an animal model of diabetes (106). However, the contribution of caspasases 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 myoblast 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 diabolic-induced muscle atrophy. NOS-does not dislocate 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, 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 p38MAPK, 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-dependend

endMyoD and md degradation by Nlrp3/IL-1β sensitization pathway, especially if we consider that MyoD is described to be an atrog-1 substrate [94, 98]. How MyoD loss can contribute to weakness and muscle loss needs to be elucidated in the near future.

loss and gain of function experiments on glucocorticoid receptor should be performed to address the direct role of this pathway in muscle mass.

Conclusions

Over the last few years, the mechanisms controlling muscle loss have attracted the attention of the scientif-

community, setting the base for future studies, which should enable us to identify new therapeutic targets and drugs.

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