Transforming growth factor-β and myostatin signaling in skeletal muscle

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Kollias HD, McDermott JC. Transforming growth factor-β and myostatin signaling in skeletal muscle. J Appl Physiol 104: 579–587, 2008; doi:10.1152/japplphysiol.01091.2007.—The superfamily of transforming growth factor-β (TGF-β) cytokines has been shown to have profound effects on cellular proliferation, differentiation, and growth. Recently, there have been major advances in our understanding of the signaling pathway(s) conveying TGF-β signals to the nucleus to ultimately control gene expression. One tissue that is potently influenced by TGF-β superfamily signaling is skeletal muscle. Skeletal muscle ontogeny and postnatal physiology have proven to be exquisitely sensitive to the TGF-β superfamily cytokine milieu in various animal systems from mice to humans. Recently, major strides have been made in understanding the role of TGF-β and its closely related family member, myostatin, in these processes. In this overview, we will review recent advances in our understanding of the TGF-β and myostatin signaling pathways and, in particular, focus on the implications of this signaling pathway for skeletal muscle development, physiology, and pathology.

myogenesis; Smad; MyoD

SKELETAL MUSCLE has a remarkable morphogenic ability to regenerate and adapt to environmental stimuli. Fundamentally, skeletal muscle regeneration is the recapitulation of skeletal muscle development in a postnatal context. Thus an understanding of the regulation of development and regeneration are mutually reinforcing. Both regeneration and development of skeletal muscle are profoundly sensitive to the extracellular milieu. In particular, signal transduction pathways initiated by growth factors such as insulin-like growth factor (IGF-1), fibroblast growth factor (FGF), and transforming growth factor-β (TGF-β) exert a potent level of control over muscle gene expression. The role of the TGF-β superfamily of growth factors is of considerable interest since many facets of muscle ontogeny and physiology are regulated by some of the ligands belonging to this cytokine superfamily. In particular, myostatin is of growing interest since it has been shown to exert profound effects on muscularity in mice, cattle, and humans (see Fig. 1; Refs. 52, 54, 68), as well as other animals. TGF-β superfamily signaling relies on a relatively parsimonious canonical pathway that interdigitates with particular aspects of the host cell machinery through cell-specific protein:protein interactions that allow precise cell context-dependent responses to the ligand. This review will focus on salient aspects of TGF-β superfamily signaling that pertain specifically to skeletal muscle. Information from a variety of studies offering insights into the molecular events of TGF-β signal transduction, the function of TGF-β in cultured muscle cells, and the effects of TGF-β in postnatal skeletal muscle physiology, will provide the foundation of this review.

AN OVERVIEW OF TGF-β SIGNALING

TGF-β1 ligand is recognized as the prototype of a class of multifunctional growth factors that regulate key events of metazoan development, disease, and repair (44, 45). In 1981, TGF-β effects were first reported by Roberts and colleagues (65), who found that TGF-β induced rat kidney fibroblasts to proliferate. Since then, TGF-β has been found to have a plethora of effects, such as regulation of cell growth, proliferation, differentiation, adhesion, migration, and apoptosis (reviewed in Refs. 44, 46, 48). These effects are highly complex and sometimes difficult to reconcile; however, one unifying feature of TGF-β signaling is that its effects are profoundly dependent on the cell context. For example, TGF-β causes increased proliferation in fibroblasts (65) but inhibits proliferation in epithelial cells, causes differentiation of neuronal cells, but blocks differentiation in mesenchymal cells (2, 18, 19, 66, 73). In skeletal muscle, TGF-β superfamily members have been shown to have potent effects on both muscle development and postnatal skeletal muscle mass.

The TGF-β superfamily consists of over 50 structurally related ligands, many of which fall into three major subfamilies: TGF-β, bone morphogenic protein (BMP), and activin (reviewed in Ref. 17). Exhaustive work on the molecular characterization of the TGF-β signal transduction pathway has led to the delineation of a canonical signaling pathway for all TGF-β superfamily members consisting of three main components (74): 1) the ligand; 2) the receptors (serine/threonine kinases); and 3) the intracellular mediators (Smads; see Fig. 2). For the TGF-β, Activin, and BMP subfamilies signal transduction begins with the ligand binding to its type II receptor. The type II receptor associates with its corresponding type I receptor, forming an activated heterotetrameric receptor complex, which transphosphorylates the type I receptor activating the latent kinase activity of the receptor complex. The activated
receptor complex then phosphorylates a receptor-regulated Smad protein that oligomerizes with a common Smad or co-Smad termed Smad4. The Smad oligomer translocates into the nucleus where it interacts with Smad binding partners to regulate transcription (27). These Smad binding partners regulate, in a cell type-specific manner, subsets of genes that are target genes of TGF-β signaling. Another group of Smads, inhibitor Smads (Smad6 and Smad7) serve to abrogate TGF-β signaling by establishing an autoinhibitory feedback loop.

SKELETAL MUSCLE DEVELOPMENT AND REGENERATION

Briefly, skeletal muscle ontogeny is comprised of three developmental stages: 1) determination, 2) differentiation, and 3) maturation (Fig. 3). Determination begins with the aggregation of mesodermal precursors in the paraxial region of the embryo, which form somites. The somites are composed of pluripotent cells, of which some will become muscle progenitor cells (33). These myogenic progenitor cells give rise to proliferating cells that are committed to the myogenic lineage termed myoblasts and are derived from the myotome compartment of the somite (33). Proliferating myoblasts subsequently undergo differentiation, a process that includes withdrawal from the cell cycle, expression of muscle-specific genes, and fusion resulting in the formation of multinucleated myotubes. Myotubes eventually give rise to the vast array of muscle fibers that are used to construct the complex skeletal muscle architecture of the animal. There are two types of myoblasts, embryonic and fetal. Most embryonic myoblasts differentiate
and fuse to form primary muscle fibers, but some continue to proliferate and become fetal myoblasts. These fetal myoblasts form secondary fibers, smaller than the primary fibers, which surround the primary fibers (13). Fetal myogenesis is more similar to postnatal muscle regeneration than embryonic myogenesis. Postnatally, myoblasts mature into muscle fibers. In the adult, skeletal muscle regeneration begins with quiescent satellite cells, located between the basal lamina and the sarcolemma of the muscle fiber, that become activated by trauma, be it mechanical or biological (e.g., disease). Recent work has classified satellite cells as a heterogeneous population of cells comprised of stem cells and committed progenitors based on the combined Pax7 and Myf5 status of the cells (32). Once activated, satellite cells proliferate, undergo differentiation, and then fuse to an existing muscle fiber or alternately create fibers de novo (see review, Refs. 9, 26).

Two families of transcriptional regulatory proteins are critical in regulating developmental myogenesis: the muscle regulatory factors (MRFs) and the myocyte enhancer factor 2 (MEF2) proteins. MRF members are considered the prototypic master regulatory genes in skeletal muscle based on their capacity to convert some nonmyogenic fibroblast cells into a myogenic cell phenotype (61). MEF2 family members function as necessary coactivators for the MRFs in an evolutionarily conserved ancient code to specify skeletal muscle ontogeny (7). Both MRFs and MEF2 members have functional cis-elements within many skeletal muscle-specific promoters and enhancers (7).

**ROLE OF TGF-β AND MYOSTATIN IN SKELETAL MUSCLE DEVELOPMENT**

Since it would be untenable to consider all TGF-β ligands, we will focus on the prototypical TGF-β1 and myostatin ligands because of their potent role in skeletal muscle (35, 37, 38, 47). TGF-β1 and myostatin signaling in skeletal muscle involve two strikingly similar pathways, which mediate some overlapping and also mutually exclusive effects. TGF-β1 plays an important role in skeletal muscle development but also influences different tissues producing a plethora of outcomes. TGF-β1 activity is very specific to the cellular context, that is, its signaling outcome is determined by the intracellular milieu of the cell. This duplicistic nature of TGF-β1 proves to be necessary for its in vivo function since it regulates many tissues and processes. Conversely, myostatin plays a much more restricted role in skeletal muscle development, muscle mass regulation, and adipose tissue. The elucidation of the role of TGF-β1 in skeletal muscle began in experiments using cultured muscle cells in vitro, while the understanding of the role of myostatin began in vivo through gene targeting experiments in mice. These two different starting points led to different insights into the role of these interrelated pathways. TGF-β1 is the archetypal cytokine of this superfamily and much of the understanding of the components of the TGF-β signaling pathway have been delineated by the study of TGF-β1. Conversely, because of the pervasive hypermuscular phenotype of myostatin null mice, myostatin research has focused more on clinical applications in skeletal muscle and less on the detailed biochemical dissection of its cognate signaling pathway.

In the late 1980s, several groups made three key observations that enabled the identification of an important in vivo role for TGF-β in skeletal muscle development. First, TGF-β is present in large amounts in the limb bud, is produced in the ectoderm, and affects the adjacent mesenchymal cells. Second, TGF-β inhibits differentiation of fetal, but not embryonic, myoblasts (80). Last, the treatment of limb bud organ cultures with TGF-β neutralizing antibodies results in the premature appearance of large myotubes (secondary; Ref. 14). These observations suggest that TGF-β prevents premature differentiation in migrating myoblasts, allowing for proper muscle formation in the developing embryonic limb.

Myostatin was discovered during a screen for novel mammalian members of the TGF-β superfamily (54). Expression of myostatin mRNA is first seen at embryonic day 9.5 post conception in the myotome of developing somites and continues throughout muscle development. The myostatin gene is expressed in adult skeletal muscle, heart, and adipose tissue (69). In addition to its restricted pattern of expression, mice homozygous null for the myostatin gene are two to three times larger in mass than the wild type due to an enhanced mass of the musculature (Fig. 1, A and B). The increase in skeletal muscle mass is attributed to both hyperplasia (increase in muscle fiber number) and hypertrophy (increase in muscle fiber size). Myostatin homozygous null mice also have reduced stores of adipose tissue (53, 54). Furthermore, heterozygous (+/−) myostatin mice have a 25% increase in body weight, which is a lesser effect than that seen in the homozygous (−/−) mice, suggesting a dose-dependent effect of myostatin protein levels on skeletal muscle mass. Naturally occurring
myostatin mutations in cattle (Belgian Blue, Piedmontese, and Marchigiana) and in humans have subsequently been shown also to lead to pronounced hypermuscularity (28, 42, 54, 68; Fig. 1, C-E). The consistency of the hypermuscular phenotype corresponding to myostatin null mutation across species is consistent with the evolutionary conservation of the myostatin gene and protein product between humans, mice, rats, pigs, cows, chickens, and turkeys.

Since both TGF-β1 and myostatin have been shown to regulate myogenesis and postnatal muscle physiology, a comparative analysis of the two may provide useful insights into the common and divergent aspects of the intracellular signaling of these pathways. TGF-β1 signal transduction begins with the active ligand binding to the TGF-β type II receptor (TβRII) and either ALK-1 (activin like kinase-1) or TβRI/ALK-5 (TGF-β type I receptor) receptors. Myostatin signaling begins with the active myostatin ligand binding to either activin receptor type IIA (ActR-IIA) or ActR-IIIB and either TβRI/ALK-5 or ALK-4, type I receptors (63). The myostatin ligand has a higher affinity for ActR-IIIB in vitro (36, 63). Both pathways converge in the activation of Smad2 and Smad3 followed by oligomerization with Smad4. Next, the Smad complex translocates into the nucleus, where it regulates transcription of genes such as MyoD (39, 64). Additional non-Smad-dependent signaling has also been implicated in TGF-β1 effects, although this is so far less well characterized (51). To counteract TGF-β/myostatin signaling, repression of the signal can be achieved by the I-Smads, Smad6 and Smad7, or a ubiquitin-mediated proteasomal degradation pathway mediated by the Smad-ubiquitin regulatory factors (SMURFs; Ref. 5). Smad7 has been previously shown to inhibit both TGF-β1 and myostatin signaling (31, 82). Furthermore, Smad7 may have additional pro-myogenic functions. A recent study from our group (31) has shown a dramatic enhancement of skeletal muscle differentiation and growth by the myostatin signaling inhibitor Smad7, which interacts with and potentiates MyoD transcriptional activity. However, although Smad7 is able to inhibit TGF-β1 signaling, as assessed by a TGF-β responsive promoter (3TP-lux), it was not able to rescue TGF-β1-mediated inhibition of myogenesis. Therefore, consideration of how the TGF-β/myostatin downstream effectors integrate with skeletal muscle-specific transcriptional regulators to control gene expression will be paramount in understanding how these growth factors modulate the muscle phenotype during development and regeneration.

TGF-β SIGNALING IN SKELETAL MUSCLE

TGF-β represses skeletal muscle specific gene expression and has also been reported to modulate proliferation in satellite cells (3, 12, 19, 24, 47, 59). Taken together, these studies indicate that TGF-β reprograms gene expression in muscle cells resulting in an alteration of proliferative control and a potent inhibition of the program of gene expression underlying myogenic differentiation. As key orchestrators of muscle gene expression, the MRFs were shown to be targeted by TGF-β signaling. Therefore, logical targets of TGF-β1 are the myogenic transcription factors, the MRFs and the MEF2s, as the quintessential regulators of myogenesis. The MRFs are comprised of MyoD, Myf5, myogenin, and MRF4, whereas the MEF2s include MEF2A-D. To that end, Brennan and colleagues (8) showed that TGF-β inhibited the transcriptional activity of myogenin without affecting its DNA binding affinity. Furthermore, it has been shown that TGF-β1 targets the basic helix-loop-helix (bHLH) region of all MRFs, decreasing their DNA transcriptional activity without affecting their binding properties (43). Not only does TGF-β1 inhibit the transcriptional activity of the MyoD protein it also inhibits the transcription of the MyoD gene, thus reducing both levels and activity (76).

How does TGF-β1 inhibit myogenesis? What role do Smad2 and Smad3 have in myogenesis? In recent years several studies have begun to elucidate the role of Smad2 and Smad3 during TGF-β1-mediated myogenic inhibition and in “normal” differentiation (39, 62). It has been shown that Smad3 is the key mediator of TGF-β inhibition of myogenesis. Liu and colleagues (39) showed that Smad3 alone, and not Smad2, via MyoD inhibition is critical in TGF-β signaling in myoblasts. Smad3 was found to physically interact with MyoD, inhibiting the transactivation properties of MyoD. Furthermore, Smad3 was also shown to physically interact with MEF2C and decrease its transcriptional activity (40). However, the relevance of this biochemical interaction in vivo is controversial because the MEF2C gene is not expressed in myoblasts endogenously (50), although MEF2C does play a role in the late stages of differentiation and conditional deletion in skeletal muscle affects sarcomere integrity (60).

The role of Smad2 in TGF-β inhibition of myogenesis is less clear. Using myoblasts De Angelis et al. (15) reported that MEF2, normally found in the nucleus, translocates to the cytoplasm upon TGF-β treatment. Forced expression of MEF2C rescued TGF-β inhibition by maintaining a pool of MEF2C in the nucleus. Furthermore, endogenous Smad2 and MEF2 are complexed together in the nuclei of differentiating myotubes, but not in myoblasts, where Smad2 is cytoplasmic and where MEF2A and 2C are not highly expressed (62). Thus the link between MEF2 and Smad2 protein is complex and may depend on the differentiation status of the cells. Together, these data suggest that Smad2 is an important component of TGF-β-mediated repression of differentiation in myoblasts, although the precise mechanism mediating this effect still requires further characterization (60).

TGF-β in skeletal muscle: a role in vivo. Recently, an in vivo role for TGF-β1 in skeletal muscle regeneration was confirmed. Mice deficient in fibrillin-1 have increased TGF-β signaling activity that causes a failure of skeletal muscle to regenerate (11). These mice are used as a model for the Marfan syndrome (MFS) in humans, which is caused by a mutation of the FBN1 gene that encodes fibrillin-1 (an extracellular matrix protein). MFS symptoms include bone overgrowth, ocular lens dislocation, emphysema, cardiac complications, and an inability to increase skeletal muscle mass in response to exercise and other physiological signals (16). Antagonizing TGF-β signaling with a TGF-β neutralizing antibody or by losartan treatment rescues skeletal muscle regeneration in the fibrillin-1-deficient mice. Increased TGF-β signaling was also found in the skeletal muscle of dystrophin-deficient mice, a model of Duchenne muscular dystrophy, and antagonizing TGF-β signaling restored the regeneration program in these mice (11). Furthermore, the fibrillin-1-deficient mice exhibited increased satellite cell numbers when TGF-β was antagonized. Thus
inhibition of TGF-β signaling results in an improved skeletal muscle profile in several genetically invoked myopathies.

In vivo studies have also documented the presence of TGF-β during muscle injury. Muscle injury caused by eccentric contractions (6), cardiotoxin injection (22), and muscle strain injury (72) resulted in increased TGF-β transcript and protein levels within 48 h of injury. However, increases in TGF-β1 and TGF-β2 precursors did not correspondingly lead to an increase in TGF-β activity following muscle strain, suggesting a delay in TGF-β activation after injury (72). Furthermore, TGF-β type I receptor expression is regulated by myotube excitability. TβRI was downregulated when primary rat myotubes were electrically stimulated and upregulated in adult rat muscle 72 h after denervation (75). Thus careful dissection of the role of TGF-β signaling in these in vivo contexts is warranted to further understand the role played by the endogenous cytokine. Nearly three decades have passed since the potent effects of TGF-β on myogenesis were first reported and only recently has an in vivo role for TGF-β begun to be elucidated. Future studies using tissue-specific gene targeting and the discovery of new TGF-β pathway mediators may further elucidate the role of TGF-β in skeletal muscle.

**MYOSTATIN SIGNALING IN SKELETAL MUSCLE**

While the relevance of TGF-β1 in vivo has just begun to be understood, the in vivo importance of myostatin was obvious from the early phase of its discovery. However, characterization of the intracellular signaling pathway for myostatin is still in its infancy. Much of what is known has been inferred by knowledge of the TGF-β signaling pathway but there may still be some surprises to unfold since studies are now identifying differences in TGF-β1 and myostatin signaling (31).

**Myostatin: a role in vivo.** Myostatin null mice (Mstn<sup>−/−</sup>) have proved to be an excellent model for understanding the genetic role of myostatin during both muscle development and postnatal muscle physiology (52). In addition to alterations in muscle mass, changes in fiber contractile properties, fiber type, and fiber recovery have now been reported in this genetic model (4, 55, 79).

In addition to the increased muscle mass and decreased fat in the Mstn<sup>−/−</sup> mice there are also corresponding increases in strength and changes in fiber type distribution with a shift toward the type IIB fibers. Furthermore, the contractile properties of Mstn<sup>−/−</sup> mice differed from heterozygous (Mstn<sup>+/−</sup>) and wild-type mice (55). In physiological studies of skeletal muscle derived from the mice, the extensor digitorum longus (EDL) muscle and the soleus muscle are often used as indicators of fast twitch and slow twitch muscle fibers, respectively. In vitro contractile analysis of EDL and soleus muscles from wild-type, Mstn<sup>+/−</sup>, and Mstn<sup>−/−</sup> mice revealed that the maximum tetanic force (P<sub>T</sub>) in the EDL muscle and soleus muscle was greater in the Mstn<sup>−/−</sup> than in the wild-type animals (55).

Additionally, the specific maximum tetanic force (sP<sub>T</sub>, corrected for cross sectional area, N/mm<sup>2</sup>) was reduced in the Mstn<sup>−/−</sup> compared with the wild-type EDL muscle. However, in the soleus, no difference was found in the sP<sub>T</sub> of the Mstn<sup>−/−</sup> compared with mice with the wild-type genotype. In the case of the EDL muscle, the phenomenon of decreased sP<sub>T</sub> with increased P<sub>T</sub> occurs because as the whole muscle cross sectional area increases the angle of pennation by muscle fibers increases, thus decreasing the transmission of force, a phenomenon previously described by Maxwell et al. (49). While the lack of any difference in the sP<sub>T</sub> of the soleus muscle is more difficult to explain, one possibility is that P<sub>T</sub> in the soleus muscle of the Mstn<sup>−/−</sup> compared with the wild type was not large enough to affect the sP<sub>T</sub>. Interestingly, following two lengthening contractions, the EDL muscle from the Mstn<sup>−/−</sup> mice had a greater force deficiency than the wild type. However, no difference in force production was found in the soleus muscle. Molecular analysis revealed that the levels of ActRIIB in the EDL muscle were twice as high as those in the soleus muscle suggesting that the higher levels of ActRIIB in the EDL muscle could prime it for greater sensitivity to the ligand compared with the soleus muscle.

While Mendias et al. (55) have reported an increase in strength of the EDL of Mstn<sup>−/−</sup> null mice, there is some conflict in this area since another study reported that Mstn<sup>−/−</sup> null mice do not have increased P<sub>T</sub> generation in their EDL muscle compared with wild-type mice despite their larger muscle mass (4). However, consistent with other studies, the sP<sub>T</sub>, in the myostatin-null state was reduced compared with that of their wild-type littermates. As well, the P<sub>T</sub> was slightly higher in the EDL muscle of the Mstn<sup>−/−</sup> mice compared with the wild-type mice. Further comparison of the contractile properties of the EDL muscle from Mstn<sup>−/−</sup> and wild-type mice exhibited differences in single twitch characteristics. EDL muscle from Mstn<sup>−/−</sup> mice contracted and relaxed faster during a single twitch (4).

A greater proportion of type IIB fibers in the Mstn<sup>−/−</sup> mice can explain the shorter contraction and relaxation times reported. Assessed by myosin heavy chain (MHC) isoform expression, Mstn<sup>−/−</sup> mice had less IIA and IIX fibers in EDL, but had more IIB fibers than the wild-type mice (4, 21). In the soleus muscle, Mstn<sup>−/−</sup> mice had less IIA mRNA, but more IIX and IIB mRNA than the wild type, although this change at the mRNA level needs to be confirmed at the protein level. However, functional assessment using ATPase staining and succinate dehydrogenase (SDH) activity did confirm a shift toward more fast fibers and glycolytic fibers, respectively (21). The analysis of oxidative enzymes in the EDL muscle reported a decrease in mitochondrial enzymes, SDH, cytochrome oxidase (COX), and NADH reductase, in the Mstn<sup>−/−</sup> mice consistent with the shift to fast glycolytic IIB fibers. Furthermore, the number of mitochondria per unit area was reduced. Taken together these data suggest that modification of myostatin levels does alter adult muscle contractile properties.

Reduced myostatin levels have been shown to be potentially beneficial in aging muscle. Comparing older Mstn<sup>−/−</sup> mice (24 mo) to older wild-type mice it was shown that Mstn<sup>−/−</sup> mice had less IIB fibers and size losses compared with wild-type mice (70). Genetic removal of myostatin from conception exhibits profound effects on muscle mass and fiber type, but another extant question concerns whether postnatal myostatin repression can also have the same effects. Reducing myostatin in mice postweaning through the use of myostatin neutralizing antibodies, or by postnatal conditional genetic targeting, also leads to increased muscle mass (77–79). Depending on the length of treatment and the age of the mice there is a 10–30% increase in muscle mass with neutralizing antibodies (77). Whittemore and colleagues (79) reported that mice treated with neutralizing antibodies had increased skeletal muscle mass and
increased grip strength. Critically, this was the first study in postnatal animals to show that repressing myostatin signaling not only increases muscle mass but also enhances functional muscle strength. Recently, postnatal conditional genetic targeting of myostatin also showed increase muscle mass (78). Older mice that have ceased normal muscle growth also respond to myostatin repression with increased muscle mass but to a lesser degree. Additionally, short-term inhibition of myostatin in aged mice (14–16 mo) enhanced muscle regeneration and activated satellite cell activation (71). Since older mice retain the capability to respond to myostatin repression with increased muscle mass it is tantalizing to consider the possibility of treating sarcopenia, age-related loss of muscle mass and strength, by inhibiting myostatin. These findings indicate that repression of myostatin could proffer a treatment for sarcopenia. Furthermore, inhibiting the myostatin pathway using the iSmads could be another potentially therapeutic strategy for maintaining muscle mass in ageing animals.

In concert with the functional consequence of loss of myostatin signaling, when myostatin signaling is increased, mice undergo a predicted severe muscle loss. In experiments in which mice were systemically administered myostatin, pronounced skeletal muscle loss was observed (83). These mice exhibited symptoms similar to those of cachexia, which occurs in many disease states such as cancer, heart failure, AIDS, and sepsis. Thus it is now becoming clear that myostatin has the capacity to act as a specific and potent negative regulator of skeletal muscle mass during development, which is also paralleled in postnatal skeletal muscle.

Recently, several studies have implicated modulation of myostatin mRNA levels in response to altered functional demand imposed by exercise training. It is clear that resistance training represses myostatin mRNA. Long-term resistance training reduces myostatin levels in rodents and humans (20, 67). An acute bout of resistance exercise also inhibits myostatin mRNA expression in rodents and humans (25, 29). Furthermore, resistance training mitigates atrophy and increases in myostatin caused by skeletal muscle unloading in rodents (1). Interestingly, hypertrophic capability of an individual had no impact on the level of myostatin reduction caused by resistance exercise (30). Thus both long-term and acute resistance training inhibit myostatin mRNA expression. However, individuals with greater hypertrophy in response to training did not concomitantly have the lowest levels of myostatin.

The effect of submaximal intensity running exercise on myostatin mRNA levels has also been reported (41). Acute bouts of both resistance exercise (3 sets of 10 repetitions at 70% 1RM) and submaximal running (75% $\dot{V}O_2$ max for 30 min) reduced myostatin mRNA levels in young untrained participants. Postexercise time points comparing the two modes of acute exercise showed that resistance exercise has a more pronounced and longer suppression of myostatin. Resistance exercise reduced myostatin over sixfold for 23 h (1–24 h postexercise) while submaximal running decreased myostatin over threefold for 3 h (8–12 h postexercise). Myostatin expression is suppressed by both resistance exercise and running, but with the former being a much more robust suppressor. Thus these data implicate an inverse correlation between exercise training and myostatin mRNA levels in a variety of exercise regimens. Decreases in myostatin mRNA levels in response to resistance exercise are consistent with the known role of myostatin as a negative regulator of fiber size. However, the decrease in myostatin mRNA during running is more difficult to reconcile as one would expect myostatin levels to increase with this perturbation.

A possible mechanism for exercise modulation of myostatin mRNA may be through calcium-dependent signaling via calcineurin. Calcineurin is a calcium-dependent phosphatase that has been shown to play a key role in the control of muscle gene expression by its regulation of the nuclear factor of activated transcription (NFAT) transcriptional regulator (10). Myostatin mRNA was reduced in mouse plantaris muscle that was overloaded. However, when calcineurin is inhibited through the use of cyclosporin A, myostatin mRNA was maintained at normal levels, suggesting that calcineurin may play a potentially important role in the regulation of myostatin levels during muscle remodeling (56, 57). Furthermore, expression of constitutively activated calcineurin in transgenic mice resulted in higher levels of myostatin mRNA levels (56). In addition, a number of experimental manipulations that result in reduced levels of calcineurin activity, such as calmodulin-binding protein (CaMBP)-overexpressing mice, parvalbumin-overexpressing mice, and NFATc2 null mice also exhibited reduced myostatin mRNA levels (56). Collectively, these data clearly indicate a connection between calcineurin activity and myostatin mRNA levels.

The next and, probably, most important question to address is whether the alteration in myostatin levels is fundamental to the adaptation of the muscle in these contexts. The question as to whether myostatin levels are functionally linked to muscle performance is indeed intriguing. A recent study suggests that it may be, based on the observation that in a particular breed of racing dogs (whippets), performance characteristics were correlated with the mutation in the myostatin gene in that the dogs with the mutation are faster than those with the wild-type allele (58). This is the first report of myostatin gene mutation leading to enhanced athletic performance. Although increased muscle mass and strength in myostatin null animals is firmly established, there has been little evidence to date that it necessarily translates to increased athletic performance. With regard to this issue, whippets heterozygous for a mutant copy of myostatin (mh/+) are more muscular than normal whippets, but were not as fast as the homozygous dogs. Thus it is important to consider that the balance between muscle mass and muscular performance may be more optimal in the heterozygous rather than the homozygous null state.

FUTURE PERSPECTIVES

Repressing TGF-β and myostatin signaling in muscle could have potential therapeutic applications since both TGF-β and myostatin activity are elevated in a variety of clinical conditions associated with muscle loss. Increased TGF-β signaling in mice deficient in fibrillin-1 have impaired skeletal muscle regeneration (11). A correlation between enhanced myostatin levels and muscle atrophy has also been observed in AIDS (23), bed rest (81), limb unloading, and exposure to micro-
gravity (34). These observations position myostatin as a strong therapeutic target for manipulating phenotypic muscle mass in disease states and throughout the mammalian life span. Controversially, preliminary evidence in dogs indicates that myostatin inhibition may also potentially have ergogenic properties for athletic performance in events that require strength and power such as sprinting.

On the molecular mechanistic level, much work still needs to be done to fully understand the intracellular mechanisms of the TGF-β and myostatin signaling pathways in skeletal muscle. To date, very few TGF-β or myostatin target genes have been annotated and this analysis needs to be carried out on a genome wide level. Clearly there are overlapping but also distinct features of the signaling initiated by these two cytokines that may result from common and divergent modulation of gene expression programs. Also, the role of the inhibitor Smads, such as Smad7, are intriguing because of their capacity to reverse the signaling and dramatically impinge on muscle cell differentiation and growth (31).

Manipulation of TGF-β and myostatin signaling thus holds considerable promise for the modulation of skeletal muscle mass and strength. This may have important implications for skeletal muscle regeneration in disease or injury states and also for skeletal muscle maintenance in the ageing population.

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