

REGULATION OF MUSCLE MASS BY MYOSTATIN

Se-Jin Lee

Johns Hopkins University School of Medicine, Department of Molecular Biology and Genetics, Baltimore, Maryland 21205; email: sjlee@jhmi.edu

Key Words chalone, TGF- β family, satellite cell, myoblast, latency

■ **Abstract** Myostatin is a secreted protein that acts as a negative regulator of skeletal muscle mass. During embryogenesis, myostatin is expressed by cells in the myotome and in developing skeletal muscle and acts to regulate the final number of muscle fibers that are formed. During adult life, myostatin protein is produced by skeletal muscle, circulates in the blood, and acts to limit muscle fiber growth. The existence of circulating tissue-specific growth inhibitors of this type was hypothesized over 40 years ago to explain how sizes of individual tissues are controlled. Skeletal muscle appears to be the first example of a tissue whose size is controlled by this type of regulatory mechanism, and myostatin appears to be the first example of the long-sought chalone.

CONTENTS

INTRODUCTION	61
Identification of Myostatin and Phenotype of Myostatin-Deficient	
Animals	62
Biosynthesis of Myostatin and Regulation of Myostatin Latency	65
Myostatin Signaling Pathway	71
Cellular and Tissue Responses to Myostatin Signaling	74
Clinical Applications	76
CONCLUSIONS	78

INTRODUCTION

Despite the rapid advances in our understanding of the molecules and mechanisms involved in regulating cell differentiation and tissue formation in vertebrates, relatively little is known about the control of tissue size. Over 40 years ago, Bullough (1962, 1965) proposed that tissue size is controlled by the activities of negative growth regulators that he dubbed chalones. According to this hypothesis, individual tissues secrete distinct chalones, which circulate throughout the body and act to inhibit the growth of the tissue producing the specific chalone. Despite intensive efforts in the ensuing years to provide experimen-

tal support for this theory, no molecules having the essential properties of a chalone for any tissue were identified, and this theory to explain the control of tissue mass was essentially abandoned. Recent work suggests that at least one tissue may, in fact, utilize this general type of regulatory mechanism to control tissue mass. This tissue is skeletal muscle, and the key mediator appears to be myostatin.

Identification of Myostatin and Phenotype of Myostatin-Deficient Animals

Myostatin (previously called GDF-8) was originally identified in a screen for novel mammalian members of the transforming growth factor- β (TGF- β) superfamily of growth and differentiation factors (McPherron et al. 1997). The predicted myostatin protein sequence has all the hallmarks present in other family members, including an N-terminal signal sequence, a dibasic proteolytic processing site, and a C-terminal domain following the processing site, which contains nine cysteine residues with their characteristic spacing. In this C-terminal region, the myostatin sequence shows significant homology to other family members and, together with the highly related protein GDF-11 (McPherron et al. 1997, Gamer et al. 1999, Nakashima et al. 1999), defines a distinct subgroup within the larger superfamily (Figure 1).

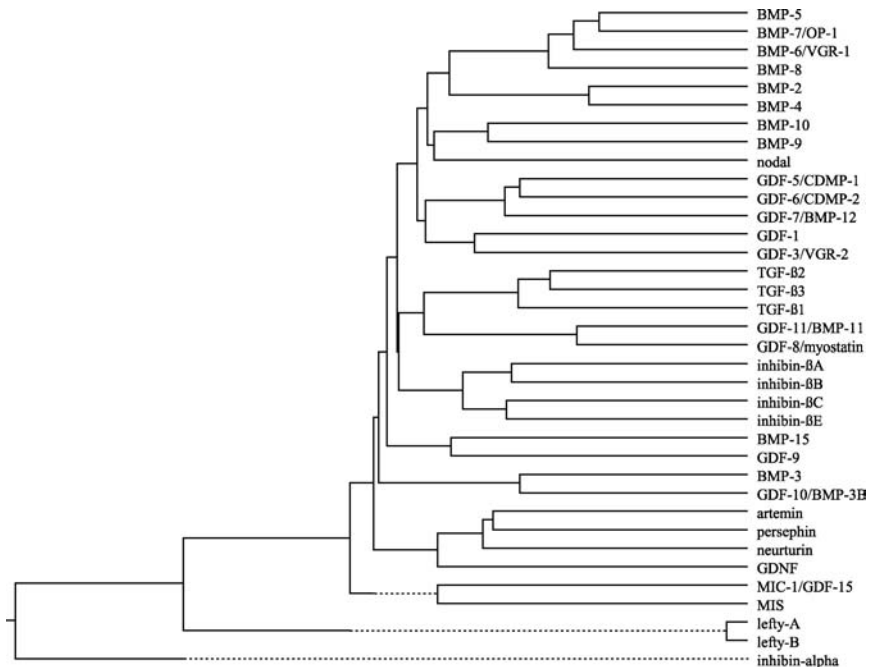


Figure 1 Sequence comparisons of members of the TGF- β superfamily.

The expression pattern of *myostatin* suggested that it might play a role in regulating muscle development or function (McPherron et al. 1997). In mice, *myostatin* is expressed in the myotome compartment of developing somites beginning at embryonic day 9.5 and continues to be expressed in developing skeletal muscles throughout embryogenesis. In adult tissues, *myostatin* is expressed almost exclusively in skeletal muscle, although clearly detectable levels of *myostatin* RNA are also present in adipose tissue. All skeletal muscles examined to date express at least some *myostatin* RNA, although the expression levels vary from muscle to muscle. The function of myostatin was elucidated by gene targeting studies in mice (McPherron et al. 1997). Mice carrying a deletion of the portion of the gene encoding the C-terminal domain of myostatin were shown to have dramatic and widespread increases in skeletal muscle mass, with individual muscles weighing about twice as much as those of wild-type mice (Figure 2a). Analysis of sections prepared from muscles of homozygous mutant mice showed that these increases in muscle mass result from a combination of increased number of muscle fibers (hyperplasia) and increased fiber size (hypertrophy). Every skeletal muscle examined appears to be affected by the mutation, and both males and females are

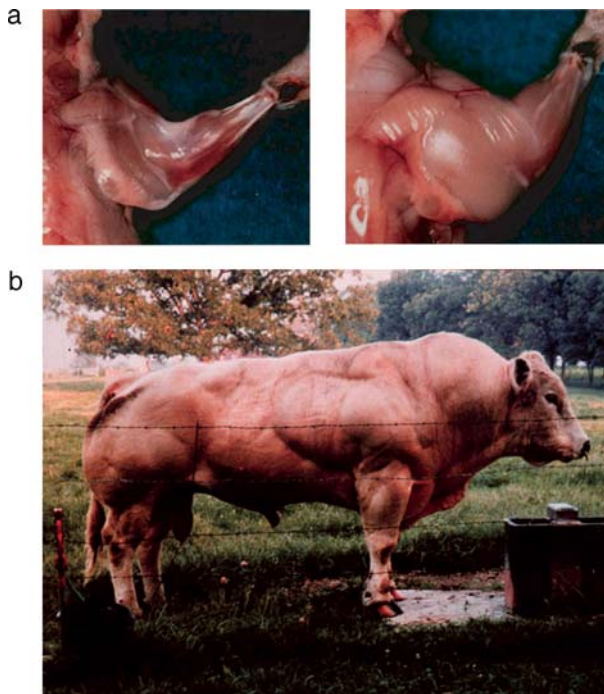


Figure 2 (a) Increased muscling in forelimbs of a myostatin knockout (*right panel*) compared with wild-type mouse (*left panel*) (reprinted from McPherron et al. 1997). (b) Belgian Blue bull showing the double-muscling phenotype (reprinted from McPherron & Lee 1997).

affected proportionately. The increases in muscle mass in *myostatin* null mice are observed at an early age and continue to be maintained for the life of the animal (McPherron & Lee 2002). Significantly, heterozygous mice are also affected, albeit to a lesser degree; muscles of heterozygous mice weigh approximately 25% more than those of wild-type mice, suggesting that the effect of myostatin is dose dependent.

The phenotype of mice lacking myostatin suggested that myostatin normally functions as a negative regulator of muscle growth, and it was on this basis that myostatin was given its name (McPherron et al. 1997). These findings raised the possibility that blocking myostatin activity might have important applications for both human therapeutics and agriculture. As a result, the myostatin gene has been analyzed in a large number of different species and has been found to be extraordinarily well conserved (McPherron & Lee 1997). The predicted myostatin protein sequences in the C-terminal domain are identical among humans, mice, rats, pigs, chickens, and turkeys. Myostatin-related sequences have also been identified in a number of different fish species (McPherron & Lee 1997, Maccatrozzo et al. 2001, Ostbye et al. 2001, Rescan et al. 2001, Roberts & Goetz 2001, Rodgers & Weber 2001, Rodgers et al. 2001, Kocabas et al. 2002). However, the predicted protein sequences in fish are significantly diverged from those of other vertebrates and are actually almost as closely related to GDF-11 as they are to myostatin. These sequence comparisons, taken together with the observation that these genes are expressed in a much wider range of tissues in fish, have led to the suggestion that the divergence of myostatin and GDF-11 occurred after the origin of fish and that the functions of myostatin and GDF-11 in fish are carried out by genes representing the original ancestral precursor (Ostbye et al. 2001). Although no other putative fish GDF-11 orthologs have been published to date, the deposition into the public databases by Iliev, Roberts, and Goetz of a partial second zebrafish sequence (accession number AF411599) much more closely related to GDF-11 than to myostatin suggests that the evolution of myostatin sequences and functions in fish may be more complicated.

Conservation of the myostatin sequence across species suggests conservation of function, and, in fact, mutations in the myostatin gene have been shown to be responsible for the double muscling phenotype in cattle (Figure 2b) (Grobet et al. 1997, 1998; Kambadur et al. 1997; McPherron & Lee 1997). For almost 200 years, certain breeds of cattle have been recognized by breeders as being significantly more muscled than standard breeds (Menissier 1982). Genetic analysis of one of these so-called double-muscled breeds, Belgian Blue, revealed that this trait segregated as a single locus on bovine chromosome 2 with a semidominant mode of inheritance (Hanset & Michaux 1985, Charlier et al. 1995). The increased muscle mass observed in *myostatin* null mice (McPherron et al. 1997), taken together with mapping studies showing that the human myostatin gene is located in a region of human chromosome 2 syntenic to the portion of bovine chromosome 2 containing the double muscling locus (Grobet et al. 1997, McPherron & Lee 1997), made the myostatin gene an obvious candidate for the double muscling locus in cattle. Indeed, analysis of the myostatin gene in a large number of double-muscled breeds

has identified at least seven different mutations, including premature stop codons, frameshift mutations, and point mutations at highly conserved amino acid residues (Grobet et al. 1997, 1998; Kambadur et al. 1997; McPherron & Lee 1997; Capucio et al. 1998; Marchitelli et al. 2003). Collectively, these mutations account for the vast majority of double-musled breeds that have been characterized, suggesting that the myostatin gene is the predominant, if not sole, locus for double muscling in cattle.

The identification of myostatin as a negative regulator of muscle growth has raised many important questions about the control of muscle growth. Understanding the mechanism by which myostatin regulates muscle mass will be critical not only for understanding the control of tissue size in general but also for developing new strategies for increasing muscle growth both for human therapeutic applications and for livestock production. In the remainder of this review, I describe the current state of knowledge of the regulatory mechanisms by which myostatin activity is regulated, the components of the myostatin signaling pathway, the cellular and tissue responses to myostatin signaling, and the potential efficacy of blocking myostatin activity for clinical applications.

Biosynthesis of Myostatin and Regulation of Myostatin Latency

Similar to other TGF- β family members, myostatin is synthesized as a precursor protein that undergoes two proteolytic processing events in order to generate the biologically active molecule (Figure 3*a*) (McPherron et al. 1997). The first cleavage

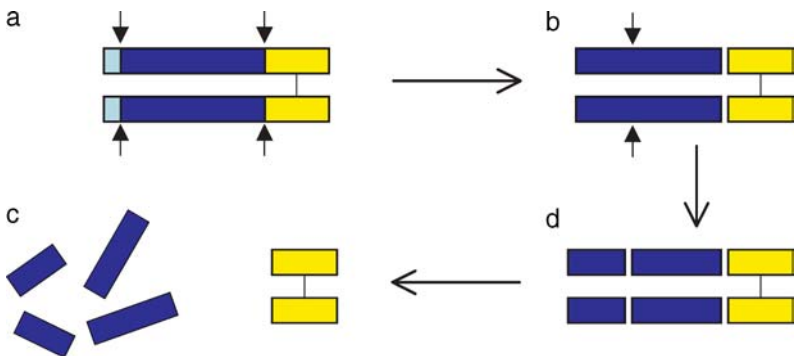


Figure 3 Processing of myostatin protein. Myostatin is synthesized in a precursor form that undergoes two proteolytic processing events; one removes the N-terminal signal sequence, a second generates the C-terminal fragment, which possesses receptor-binding activity (*a*). Following proteolytic processing, the propeptide (*blue*) and the disulfide-linked C-terminal dimer (*yellow*) remain bound noncovalently in a latent complex (*b*). Activation of latent myostatin can occur by proteolytic cleavage of the propeptide (*b*, *c*) by members of the BMP-1/tolloid family of metalloproteinases, which causes dissociation of the latent complex (*d*).

event removes the 24-amino acid signal peptide necessary for targeting the protein to the secretory pathway. The second cleavage event occurs at an RSRR (Arg-Ser-Arg-Arg) site at amino acids 240–243 (numbered from the first amino acid following the signal sequence), generating N-terminal and C-terminal fragments with predicted nonglycosylated molecular masses of 27,680 and 12,400, respectively. The precise proteinase responsible for generating this second cleavage *in vivo* is not known. The dibasic nature of the amino acids preceding the cleavage site suggests that this cleavage is carried out by paired dibasic amino acid-cleaving enzyme (PACE, also called furin), which is a member of the family of subtilisin-like serine proteinases (Wise et al. 1990); in fact, a soluble form of PACE (Rehemtulla & Kaufman 1992) is capable of fully cleaving the myostatin precursor protein at the RSRR site when expressed in Chinese hamster ovary (CHO) cells (Lee & McPherron 2001, Thies et al. 2001). Studies with C2C12 cells have also suggested a possible role for metalloproteinases in processing of myostatin, as treatment of C2C12 cells with small molecule inhibitors of metalloproteinases has been shown to result in production of an increased proportion of unprocessed myostatin as well as hypertrophy of myofibers, presumably as a result of reduced levels of mature myostatin protein being produced by these cells (Huet et al. 2001). However, no metalloproteinase has been identified to date that is capable of directly cleaving the myostatin precursor protein at the dibasic site.

The C-terminal fragment following proteolytic processing is the biologically active species, and except when explicitly stated otherwise, all subsequent references to myostatin in this review should be taken to mean the C-terminal dimer. Although the three-dimensional structure of the C-terminal fragment has not yet been determined, the high degree of sequence similarity between myostatin and other TGF- β family members suggests that the active form of myostatin is most likely a disulfide-linked dimer of C-terminal fragments folded into a cystine knot structure, as has been described for other family members (Daopin et al. 1992, Schlunegger & Grutter 1992, Griffith et al. 1996, Hinck et al. 1996, Mittl et al. 1996, Eigenbrot & Gerber 1997, Scheufler et al. 1999, Kirsch et al. 2000, Thompson et al. 2003). Indeed, myostatin produced by CHO cells can readily form homodimers, and as discussed below, the purified homodimer is active in a variety of *in vitro* assays, including receptor binding and activation of reporter genes (Lee & McPherron 2001, Thies et al. 2001, Rebbapragada et al. 2003). Whether myostatin can form heterodimers with other TGF- β family members is not known.

The N-terminal fragment following proteolytic processing has been most commonly referred to as the propeptide. On the basis of what is known about other TGF- β family members, the propeptide is presumed to play an important role in the proper folding of the C-terminal domain into a cystine knot structure. Various reports have described the purification of biologically active myostatin protein produced as an isolated C-terminal fragment in bacteria (for example, see Thomas et al. 2000, Taylor et al. 2001); however, the concentrations of bacterially produced myostatin protein required to demonstrate biological effects in these studies

were quite high (100–500 μM), suggesting that only a fraction of the purified preparation represented properly folded C-terminal dimers.

In addition to its role in proper folding of the precursor protein, the propeptide also plays an important role in regulating the activity of the C-terminal dimer following proteolytic processing. This regulatory role of the propeptide was first elucidated from the analysis of CHO cells engineered to overproduce myostatin. These cells were shown to secrete myostatin protein as a noncovalent complex of the propeptide with the C-terminal dimer (Lee & McPherron 2001) (Figure 3*b*). In this complex, the propeptide maintains the C-terminal dimer in an inactive, latent state (Lee & McPherron 2001, Thies et al. 2001, Wolfman et al. 2003) similar to that described for TGF- β (Miyazono et al. 1988), and, as in the case of TGF- β , this latent complex can be activated artificially *in vitro* by treatment, for example, with heat (Wolfman et al. 2003). The ability of the propeptide to inhibit the biological activity of the myostatin C-terminal dimer has been documented extensively *in vitro* and *in vivo*. Specifically, the purified propeptide can block the activity of the purified myostatin C-terminal dimer in both receptor binding and reporter gene assays (Lee & McPherron 2001, Thies et al. 2001), and transgenic mice overexpressing the propeptide in muscle phenocopy myostatin knockout mice in terms of increased muscle mass (Lee & McPherron 2001, Yang et al. 2001).

Although the existence of the latent complex was originally elucidated by studies with CHO cells engineered to overproduce myostatin, it is clear that this latent complex also exists *in vivo*. In particular, myostatin has been shown to circulate in the blood in a latent form that can be activated by acid treatment (Zimmers et al. 2002). The precise nature of this latent form is unknown, but biochemical analyses of proteins bound to myostatin have shown that at least one protein normally complexed to myostatin in the blood is the propeptide (Hill et al. 2002), consistent with a normal role for the propeptide in regulating the activity of myostatin *in vivo*. Therefore, understanding how the propeptide/myostatin latent complex is activated should provide important insights into the mechanisms by which myostatin activity may be modulated in different physiological states.

In this respect, one mechanism for activating myostatin latency appears to be proteolytic cleavage of the propeptide (Wolfman et al. 2003). Members of the bone morphogenetic protein (BMP)-1/tolloid family of metalloproteinases are capable of cleaving the propeptide immediately N terminal to aspartate 76 (Figure 3*b,c*). All four proteinases in this family can cleave not only the purified propeptide but also the propeptide bound to the C-terminal dimer, and cleavage of the propeptide in this complex results in activation of the C-terminal dimer. The importance of these proteinases in regulating myostatin latency *in vivo* is supported by studies using a mutant form of the propeptide in which aspartate 76 was mutated to alanine. This mutation renders the propeptide resistant to proteolysis by the BMP-1/tolloid proteinases *in vitro*, and weekly injection of the mutant propeptide into mice over a span of four weeks resulted in increases in muscle growth of approximately 25%; in contrast, no effects on muscle mass were observed in similar experiments using wild-type propeptide. The simplest interpretation of these results is that the

mutant propeptide is capable of forming latent complexes with myostatin that are resistant to activation by proteinases in the BMP-1/tolloid family. This group of proteinases has previously been demonstrated to play an analogous role in regulating the activities of certain other TGF- β family members. In particular, members of the BMP-1/tolloid family are responsible for cleaving chordin, which normally binds to and inhibits the activity of BMPs (Blader et al. 1997, Piccolo et al. 1997, Marques et al. 1997, Scott et al. 1999, Pappano et al. 2003). It is also interesting to note that one member of the BMP-1/tolloid family, TLL-2, has been shown to be expressed specifically in skeletal muscle during embryogenesis (Scott et al. 1999), raising the possibility that TLL-2 may be the proteinase that activates myostatin during muscle development.

In addition to the propeptide, several other proteins have also been shown to be capable of binding and inhibiting the activity of the myostatin C-terminal dimer. One of these is follistatin, which can bind to a number of other TGF- β family members as well (Nakamura et al. 1990, Yamashita et al. 1995, de Winter et al. 1996, Fainsod et al. 1997, Iemura et al. 1998). Several studies suggest that follistatin can function as a potent myostatin antagonist and plays an important role in modulating myostatin activity in vivo. First, follistatin is capable of blocking myostatin activity in both receptor binding and reporter gene assays (Lee & McPherron 2001, Zimmers et al. 2002), as well as in nude mice implanted with myostatin-expressing cells (Zimmers et al. 2002). Second, studies with chick embryos have shown that follistatin is expressed in myotomal cells and developing muscle at stages when myostatin is also expressed (Amthor et al. 1996, 2002a,b) and that implantation of beads soaked with follistatin into developing wing buds can cause an up-regulation of Pax-3 expression and delay muscle cell differentiation (Amthor et al. 2002a). Although these latter studies were interpreted in the context of BMP signaling, these effects would also be consistent with follistatin antagonism of myostatin activity. Finally, genetic studies in mice have shown that overexpression of follistatin in muscle can cause dramatic increases in muscle growth (Lee & McPherron 2001) and, conversely, that loss of follistatin results in reduced muscle mass at birth (Matzuk et al. 1995), which is what one might expect for excess myostatin signaling during embryonic development.

Follistatin also appears to play a role in regulating the activity of GDF-11, which is highly related to myostatin (McPherron et al. 1997, Gamer et al. 1999, Nakashima et al. 1999). As in the case of myostatin, follistatin can act as a GDF-11 antagonist in vitro, because follistatin is able to block GDF-11 activity in the *Xenopus* animal cap assay (Gamer et al. 1999). Moreover, in addition to having reduced muscle mass at birth, *follistatin* knockout mice have posteriorly directed homeotic transformations of the axial skeleton (Matzuk et al. 1995). This aspect of the *follistatin* knockout phenotype is noteworthy because *Gdf11* knockout mice have extensive anteriorly directed homeotic transformations throughout the axial skeleton (McPherron et al. 1999). The contrasting phenotypes seen in *Gdf11* and *follistatin* knockout mice are consistent with the hypothesis that follistatin may normally be involved in antagonizing GDF-11 signaling in vivo.

In addition to propeptide and follistatin, two other proteins, FLRG and GASP-1, also appear to be involved in regulating the activity of the myostatin C-terminal dimer extracellularly (Hill et al. 2002, 2003). Both FLRG and GASP-1 are complexed to myostatin in the blood of mice and humans, and studies with recombinant proteins have shown that both proteins can bind with high affinity to the myostatin C-terminal dimer and inhibit its activity, as assessed by reporter gene assays. Interestingly, GASP-1 can also bind directly to the propeptide in the absence of the C-terminal dimer. *FLRG* was originally identified as a follistatin-related gene present at a chromosomal translocation breakpoint in a patient with B-cell chronic lymphocytic leukemia and was shown to be capable of blocking the activity of the TGF- β family members activin and BMP-2 in *in vitro* assays (Hayette et al. 1998, Tsuchida et al. 2000). GASP-1 is a novel protein that contains a follistatin-related domain but, in addition, contains multiple domains found in proteinase inhibitors. To date, there are no genetic data addressing the biological functions of these molecules *in vivo*.

The existence of multiple proteins capable of binding myostatin and inhibiting its activity (Figure 4) raises many questions as to the specific role that each protein plays in regulating myostatin activity. It seems likely that the propeptide becomes bound to the myostatin C-terminal dimer during the biosynthesis of the mature protein and remains bound following release of the protein into the circulation. Activation of the C-terminal dimer from this latent complex presumably requires dissociation of the propeptide, and proteolytic cleavage of the propeptide by BMP-1/tolloid proteinases may be one mechanism for achieving activation. What roles FLRG and GASP-1 play in regulating myostatin are less clear. One possibility is that these proteins, together with the propeptide, may form ternary or higher order complexes with the myostatin C-terminal dimer and that each of these proteins has a unique regulatory role in this complex in maintaining myostatin latency. In this

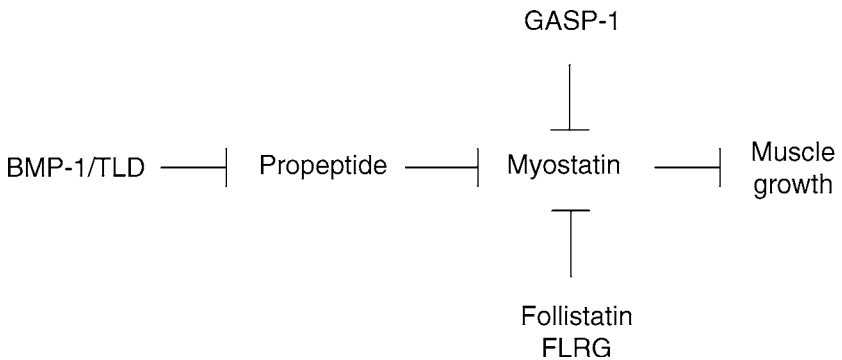


Figure 4 Myostatin activity is inhibited by extracellular binding proteins, including its propeptide, follistatin, FLRG, and GASP-1. The propeptide can be inactivated by proteolytic cleavage by BMP-1/tolloid metalloproteinases.

respect, GASP-1 can interact directly with both the propeptide and the C-terminal dimer. The possibility that GASP-1 may be able to form a ternary complex with the propeptide and C-terminal dimer, taken together with the observation that GASP-1 contains multiple proteinase inhibitory domains, has led to the suggestion that GASP-1 might be involved in regulating the activity of a proteinase that cleaves the propeptide to activate latency (Hill et al. 2003). An alternative possibility raised by these authors is that the role of GASP-1 is to regulate the activity of the proteinase that is involved in processing the myostatin precursor protein at the RSRR site; however, this role would be relevant only in terms of regulating the latent complex if cleavage of the precursor protein can normally occur either in the blood or at the myostatin target site.

A second possibility regarding the specific roles played by the various myostatin binding proteins is that the binding of the propeptide, FLRG, and GASP-1 to the myostatin C-terminal dimer may be mutually exclusive, and, as a result, the circulating form of myostatin may actually be a heterogenous mixture of these various complexes. Assuming that the initial complex formed is between the C-terminal dimer and the propeptide immediately following proteolytic processing of the precursor protein, the presumption is that a prerequisite for binding of FLRG or GASP-1 to the C-terminal dimer in this scenario would be dissociation of the propeptide from the C-terminal dimer. If multiple distinct latent complexes do exist *in vivo*, an appealing notion is that the mechanisms involved in activating each of these complexes are distinct and, therefore, that each complex potentially is responsible for responding to different physiological stimuli to regulate the overall level of active myostatin protein.

A third possibility, as suggested by Hill et al. (2002) in the context of FLRG, is that FLRG and/or GASP-1 may be involved in terminating myostatin signaling after myostatin has already bound to and activated its receptors. According to this hypothesis, these proteins could bind the myostatin/receptor complex, and given that FLRG and GASP-1 are bound to myostatin in the blood, it is possible that these proteins can dissociate myostatin from its receptor and remain bound to myostatin following dissociation. Because the complexes of myostatin with FLRG or GASP-1 could represent end products of the signaling pathway, a reasonable prediction in this scenario is that these complexes are incapable of being activated.

Clearly, identifying the true roles of each of these proteins requires not only additional biochemical approaches to further characterize the latent complexes but also genetic experiments in which the functions of FLRG and GASP-1 are eliminated. Whatever the specific roles of each of these regulatory proteins, the fact that myostatin normally exists in latent complexes provides another level of regulatory possibilities beyond simply regulating expression of the myostatin mRNA and protein. For this reason, levels of myostatin mRNA and protein under various physiological conditions or in response to various stimuli may not accurately reflect the actual level of myostatin signaling. Understanding how myostatin is activated from this latent state will be critical not only for knowing how myostatin activity is regulated but also for identifying new strategies for the development of therapeutic agents for clinical applications.

Myostatin Signaling Pathway

Upon activation from its latent state, the myostatin C-terminal dimer is capable of binding to receptors and activating a signal transduction cascade in the target cell. There is now considerable evidence that myostatin signals by a mechanism similar to that of other TGF- β -related ligands. Most members of the TGF- β superfamily are believed to signal through heteromeric complexes of type I and type II serine/threonine kinase receptors (for review, see Massagué 1998). Most ligands bind first to a type II receptor, and this ligand/type II receptor complex then recruits a type I receptor. The type II receptor kinase then phosphorylates and activates the type I receptor kinase, which in turn phosphorylates the Smad proteins. The activated Smad proteins function as the key intracellular mediators of signaling by entering the nucleus to regulate expression of downstream genes.

Cross-linking studies using purified recombinant myostatin protein have demonstrated that myostatin is capable of binding the activin type II receptors, ActRIIA and ActRIIB, in vitro (Lee & McPherron 2001, Rebbapragada et al. 2003). Qualitatively, myostatin appears to bind more strongly to ActRIIB than to ActRIIA; however, actual affinity determinations have been reported only for myostatin binding to ActRIIB (Lee & McPherron 2001). The role of the activin type II receptors in mediating myostatin signaling in vivo is supported by transgenic mouse studies in which a dominant-negative form of ActRIIB, which retains the membrane spanning domain but lacks the kinase domain, was expressed in muscle using a myosin light chain promoter and enhancer (Lee & McPherron 2001). These transgenic mice were shown to have dramatic increases in muscle mass comparable to those seen in *myostatin* knockout mice, and, as in the case of *myostatin* knockout mice, the increase in muscle mass in these transgenic mice was shown to result from a combination of muscle fiber hyperplasia and hypertrophy. Although the phenotype of these mice is certainly consistent with a role for activin type II receptors in the myostatin signaling pathway, one caveat to the interpretation of these studies is that the precise mechanism by which the dominant-negative ActRIIB enhanced muscle growth in these mice is unclear. It is possible, for example, that rather than blocking actual myostatin signaling in the target cell, the truncated receptors may have acted competitively to bind free myostatin ligand and thereby prevent myostatin from binding to its true receptors. More definitive genetic support for a role for ActRIIA and ActRIIB in mediating myostatin signaling will await the phenotypic analysis of the muscles of mice lacking one or both of these genes.

For GDF-11, both biochemical and compelling genetic evidence support a role for activin type II receptors in mediating signaling. Co-immunoprecipitation studies carried out with *Xenopus* embryos have shown that similar to myostatin, GDF-11 is capable of binding both ActRIIB and ActRIIA, and, as in the case of myostatin, GDF-11 appears to bind more strongly to ActRIIB than to ActRIIA (Oh et al. 2002). Moreover, the phenotype of *Gdf11* knockout mice (McPherron et al. 1999) is remarkably similar to that of mice deficient in activin type II receptor signaling (Oh & Li 1997, Oh et al. 2002). As described above, *Gdf11* knockout mice have extensive anteriorly directed transformations of the axial

skeleton, with the most dramatic manifestations being the presence of five to six additional thoracic segments and two to three additional lumbar segments (McPherron et al. 1999). Likewise, genetic studies in mice have shown that both *ActRIIA* and *ActRIIB* also play important roles in regulating axial patterning. Specifically, *ActRIIB* knockout mice have anteriorly directed transformations, including the presence of three additional thoracic segments (Oh & Li 1997), and although axial patterning appears normal in *ActRIIA*^{-/-} mice, animals lacking both copies of *ActRIIB* and one copy of *ActRIIA* have a more severe phenotype than *ActRIIB*^{-/-} mice, with most *ActRIIB*^{-/-} *ActRIIA*^{+/-} mice having four additional thoracic segments and one additional lumbar segment (Oh et al. 2002). The presumption is that mice lacking both copies of both *ActRIIA* and *ActRIIB* would have an even more severe patterning defect, although tissue-specific or conditional knockouts will be required to address this point, as the complete double knockout of these genes has been shown to result in early embryonic lethality (Song et al. 1999). This same general pattern has also been observed with respect to kidney development. *Gdf11* knockout mice have severe kidney defects with most mutant mice having complete bilateral renal agenesis (McPherron et al. 1999, Esquela & Lee 2003). About 25% of *ActRIIB* mutant mice also have kidney defects (Oh & Li 1997), and this percentage is increased to about 98% in mice that also lack one copy of *ActRIIA* (Oh et al. 2002). Taken together, these data provide strong support for the model that both *ActRIIA* and *ActRIIB* are responsible for transducing the GDF-11 signal in vivo, with *ActRIIB* perhaps playing a more dominant role. On the basis of the high degree of sequence relatedness between myostatin and GDF-11 and the ability of myostatin to bind activin type II receptors in vitro, it seems likely that *ActRIIA* and *ActRIIB* are also the relevant type II receptors for myostatin in vivo.

Myostatin and GDF-11 are the newest members of a growing list of ligands that appear to be capable of signaling through activin type II receptors. Given that these receptors as well as many of these ligands have relatively widespread expression patterns and given that some of these ligands, including myostatin itself, circulate systemically, a key question is how specificity of signaling is achieved. For myostatin, one possible mechanism for achieving specificity might be selective activation of the latent complex at the target site (see above). An attractive hypothesis, for example, is that the BMP-1/tolloid proteinases responsible for cleaving the myostatin propeptide are activated only at sites and times at which suppression of muscle growth is desired. Whether this type of regulatory mechanism operates in vivo will require a much more detailed understanding of how activation of latent myostatin is regulated under various physiological conditions.

An alternative mechanism for achieving specificity is selective utilization of coreceptors. Certain members of the TGF- β family require one of several unrelated coreceptors in order to engage the type II receptor, and it is noteworthy that in nearly every case in which this regulatory mechanism has been described to date, it is the interaction of a ligand specifically with the activin type II receptors that is modulated by the presence of these coreceptors. In particular, although the activins and BMPs are capable of binding activin type II receptors in the absence

of coreceptors, binding of inhibin requires the presence of betaglycan (Lewis et al. 2000), and binding of nodal and GDF-1 requires the presence of the EGF-CFC proteins, cripto and cryptic (Schier & Shen 2000, Cheng et al. 2003). Whether a coreceptor is required for binding of myostatin to ActRIIA and ActRIIB is not known. However, the receptor binding affinity of myostatin that has been determined for COS-1 cells transfected with ActRIIB is somewhat lower than typical affinities reported for other TGF- β family members and their cognate type II receptors (Lee & McPherron 2001), which raises the possibility that a receptor component for myostatin, such as a coreceptor, may have been limiting in COS-1 cells.

A third possible mechanism for achieving specificity of signaling is restricted expression of the appropriate type I receptors; that is, only those cells expressing both the activin type II receptors and the appropriate type I receptors would be capable of transducing the myostatin signal. In this regard, evidence for certain members of the TGF- β superfamily indicates that the type I receptor can enhance the affinity of the ligand for the type II receptor (Attisano et al. 1993). Cross-linking studies with cells cotransfected with ActRIIB and individual type I receptors have shown that myostatin can bind two type I receptors, ALK-4 and ALK-5 (Rebbapragada et al. 2003). Similarly, although a systematic survey of type I receptors has not yet been carried out for GDF-11, studies using *Xenopus* embryos have shown that GDF-11 also can bind ALK-4 (Oh et al. 2002). The interaction of ALK-4 with activin type II receptors had been documented extensively in prior studies with other ligands (for review, see Massagué 1998). However, ALK-5 had not previously been shown to be capable of interacting with type II receptors other than TGF- β R2. Hence, if ALK-5 is involved in mediating myostatin and/or GDF-11 signaling, the unique combination of ALK-5 with activin type II receptors certainly provides one possible mechanism for achieving specificity of signaling by these ligands. In contrast to the activin type II receptors, however, there are no genetic data as yet supporting a role for either ALK-4 or ALK-5 in mediating myostatin or GDF-11 signaling in vivo.

Whether ALK-4 and/or ALK-5 are the signaling type I receptors for myostatin in vivo, a variety of data suggests that myostatin signaling leads to activation of Smad proteins. Treatment of cells in culture with purified myostatin protein has been shown to cause increased levels of both phospho-Smad2 and phospho-Smad3, as well as activation of Smad2/Smad3-responsive reporter genes (Thies et al. 2001, Langley et al. 2002, Rebbapragada et al. 2003). Although less is known about the GDF-11 signaling pathway, injection of *Xenopus* embryos with GDF-11 RNA also leads to increased levels of phospho-Smad2. Finally, there are considerable genetic data showing that the nuclear protein c-ski, which is capable of interacting with and blocking the activity of Smad 2, 3, and 4 (Luo et al. 1999; Stroschein et al. 1999; Sun et al. 1999a,b; Akiyoshi et al. 1999), is a potent regulator of muscle growth. Mice lacking c-ski have a severe reduction in skeletal muscle mass (Berk et al. 1997), and transgenic mice overexpressing c-ski in muscle have dramatic

muscle hypertrophy (Sutrave et al. 1990). The simplest interpretation of these data is that c-ski normally functions to block myostatin signaling in vivo by blocking activities of Smad proteins normally activated by myostatin.

Cellular and Tissue Responses to Myostatin Signaling

Although relatively little is known about the downstream genes that are regulated by the activated Smad proteins, significant progress has been made in terms of identifying at least some of the cellular responses to myostatin signaling. As discussed above, mice carrying a deletion of *myostatin* have increases both in the number of muscle fibers and in fiber sizes (McPherron et al. 1997). These findings suggest that myostatin may play two distinct regulatory roles: one to regulate the final number of muscle fibers during development and a second to regulate muscle fiber growth postnatally. The hypothesis that myostatin may have this dual role has been borne out by a variety of experiments showing that the development of hyperplasia versus hypertrophy depends on the method used to block myostatin activity. For example, similar to *myostatin* knockout mice, transgenic mice expressing various myostatin inhibitors, such as the propeptide, follistatin, or a dominant-negative form of ActRIIB, under the control of a myosin light chain promoter/enhancer have increases in both fiber numbers and fiber sizes (Lee & McPherron 2001). In contrast, hyperplasia without hypertrophy has been reported in transgenic mice in which a chicken β -actin promoter coupled to a CMV enhancer was used to drive expression of a mutant form of myostatin in which an invariant cysteine residue in the C-terminal domain was changed to tyrosine (Nishi et al. 2002). Conversely, transgenic mice in which a muscle creatine kinase promoter was used to express a mutant form of myostatin with an altered furin cleavage site have been shown to have hypertrophy without hyperplasia (Zhu et al. 2000). Similarly, neutralizing antibodies to myostatin are capable of causing significant increases in muscle growth when administered to adult mice, and this increase in muscle growth appears to result entirely from an increase in muscle fiber sizes (Bogdanovich et al. 2002, Whittimore et al. 2003). Taken together, these results demonstrate that the effects of myostatin on fiber numbers can be dissociated from its effects on fiber sizes and that the development of hyperplasia versus hypertrophy is likely to be dependent on the time during embryonic development or postnatal life at which myostatin activity is lost.

The regulation of fiber numbers by myostatin most likely results from direct effects of myostatin on proliferation and/or differentiation of myoblasts during development. A number of studies have examined the effects of myostatin on C2C12 myoblasts either by treating cells with purified recombinant myostatin protein or by transfecting cells with sense or antisense myostatin expression constructs. These studies have demonstrated that myostatin is capable of blocking both proliferation (Thomas et al. 2000, Ríos et al. 2001, Taylor et al. 2001, Joulia et al. 2003) and differentiation (Langley et al. 2002, Ríos et al. 2002, Joulia et al. 2003) of C2C12 myoblasts. The effects of myostatin on cell proliferation appear

to be mediated by its ability to increase levels of p21, decrease levels of Cdk2, and decrease phosphorylation of Rb (Thomas et al. 2000, Ríos et al. 2001). Myostatin appears to inhibit myogenic differentiation by down-regulating expression of the myogenic regulators, MyoD, myogenin, and Myf-5 (Langley et al. 2002, Ríos et al. 2002, Joulia et al. 2003). The effects of myostatin on myoblast proliferation and differentiation do not appear to be restricted to C2C12 myoblasts, as effects on cell proliferation have also been demonstrated in primary bovine fetal myoblasts (Thomas et al. 2000), and implantation of myostatin-coated beads into developing chick limb buds has been shown to cause down-regulation of Pax-3, Myf-5, and MyoD expression and a consequent decrease in the amount of limb muscle formed (Amthor et al. 2002b). Taken together, these studies suggest that myostatin normally regulates the number of muscle fibers that are ultimately formed by regulating both proliferation and differentiation of myoblasts during development.

The ability of myostatin to regulate fiber size suggests that a major target for myostatin signaling in adult animals is likely to be the satellite cell. Satellite cells, which are mononucleated cells located between the sarcolemma and the basement membrane of individual muscle fibers, function as a stem cell population in muscle (for reviews, see Campion 1984, Bischoff 1994, Schultz & McCormick 1994). During muscle fiber growth, the normally quiescent satellite cells re-enter the cell cycle, proliferate, and fuse with existing muscle fibers. The fact that loss of myostatin can cause increased fiber sizes suggests that myostatin may normally act to suppress satellite cell proliferation and/or differentiation. This role for myostatin has been substantiated both by *in vitro* studies examining the effects of myostatin on satellite cells in culture and by direct analysis of satellite cells in *myostatin* mutant mice (McCroskery et al. 2003). Studies with isolated satellite cells have demonstrated that myostatin is capable of up-regulating p21 expression, down-regulating Cdk2 expression, and inhibiting cell proliferation. Consistent with this activity of myostatin on satellite cells *in vitro*, muscles of *myostatin* mutant mice have been shown to have an increased number of satellite cells per unit length and a higher proportion of activated satellite cells than muscles of wild-type mice. Moreover, satellite cells isolated from *myostatin* null mice appear to have a higher rate of proliferation in culture than satellite cells isolated from wild-type mice. An appealing model is that the normal function of myostatin in adult muscle is to maintain satellite cells in a quiescent state and that in circumstances in which muscle growth or regeneration is required, this tonic myostatin activity is inhibited, thereby releasing satellite cells from growth arrest.

Although there is considerable evidence that myoblasts and satellite cells are targets for myostatin signaling *in vivo*, it seems likely that other cells are also capable of responding to myostatin. In particular, overexpression of myostatin in adult mice has been shown to induce a dramatic systemic wasting syndrome (cachexia) characterized by extensive loss of muscle and adipose tissue mass (Zimmers et al. 2002). The rate and extent of muscle loss in these animals cannot

be explained as an effect of myostatin solely on satellite cell function and raises the possibility that this effect may be mediated by myostatin signaling directly on myotubes; consistent with this possibility is the observation that myostatin is capable of inhibiting protein synthesis in myotubes derived from differentiation of C2C12 cells in culture (Taylor et al. 2001). In addition, the rapid depletion of fat stores seen upon overexpression of myostatin in mice raises the possibility that myostatin may also have direct effects on adipocytes; in this regard, myostatin is capable of blocking adipogenic differentiation of both 3T3-L1 and C3H 10T1/2 cells in culture (Kim et al. 2001, Zimmers et al. 2002, Rebbapragada et al. 2003), although effects of myostatin on fully differentiated adipocytes have not yet been reported. Alternatively, it is possible that the effects on muscle mass and/or fat stores seen upon overexpression of myostatin may reflect the activity of some other mediator of cachexia whose production or activity is induced by myostatin. If the effect of myostatin is indirect, the direct mediator is unlikely to be TNF- α or IL-6, which are known to induce cachexia in mice (Oliff et al. 1987, Black et al. 1991), as serum levels of these proteins do not appear to be elevated in the myostatin-overexpressing mice (Zimmers et al. 2002).

Although this depletion of fat stores was observed in mice in which the serum concentrations of myostatin were artificially elevated, it is clear that myostatin can influence fat metabolism and adipocyte function even under normal conditions. Mice lacking myostatin have a reduction in total body fat, which is particularly pronounced in older animals (Lin et al. 2002, McPherron & Lee 2002). The reduction in fat stores is contrary to what one might predict based on the effects of overexpressing myostatin in mice and on the effects of myostatin on adipogenesis *in vitro*. One possible explanation to reconcile these findings might be that the rapid depletion of fat stores seen upon overexpression of myostatin results from inappropriate direct signaling of myostatin on adipocytes, whereas the reduction in fat accumulation in *myostatin* mutant mice might represent an indirect effect of the profound metabolic changes resulting from loss of myostatin signaling in muscle.

Clearly, determining whether myostatin plays a direct or indirect role on adipocyte development or function and whether myostatin can act directly on myotubes to regulate fiber growth independently of its effect on satellite cells will be essential for understanding the full range of myostatin activities under both normal and pathologic conditions. Definitive experiments to identify the cells that are direct targets for myostatin will almost certainly require conditional transgenic and gene knockout approaches to block myostatin signaling in a cell type-specific manner *in vivo*.

Clinical Applications

The biological functions of myostatin have raised the possibility that targeting the myostatin pathway may be an effective strategy for increasing muscle growth for a variety of clinical applications. Given that satellite cells may be targets for

myostatin signaling in adult animals, there has been considerable interest in the possibility that inhibition of myostatin may have beneficial effects in terms of maintaining muscle mass or promoting muscle growth in settings of chronic muscle degeneration, such as in sarcopenia, which is the progressive muscle loss that occurs in the elderly (for review, see Hepple 2003), or in patients with muscle degenerative diseases, such as muscular dystrophy. In this regard, two studies (Wagner et al. 2002, Bogdanovich et al. 2002) have investigated the effect of loss of myostatin activity in *mdx* mice, which carry a mutation in the *dystrophin* gene (Sicinski et al. 1989) and have therefore been used as a mouse model for Duchenne muscular dystrophy. Wagner et al. (2002) analyzed the effect of the *myostatin* null mutation in *mdx* mice, and Bogdanovich et al. (2002) analyzed the effect of administering myostatin-neutralizing monoclonal antibodies to *mdx* mice. Perhaps not surprisingly, both studies showed that loss of myostatin activity led to an increase in muscle mass and muscle strength in *mdx* mice, as in wild-type mice. However, the beneficial effects of loss of myostatin activity seemed to extend beyond simply enhancing muscle mass. In particular, although the muscles of these mice did exhibit some of the dystrophic changes typical of *mdx* mice, loss of myostatin led to a significant histological improvement of the muscle, including decreased fibrosis, suggesting that inhibition of myostatin activity may actually enhance the regenerative capacity of muscle in the setting of repeated cycles of degeneration. In this respect, one concern is that blocking myostatin activity might rapidly deplete the satellite cell pool and thereby prematurely exhaust the ability of the muscle to regenerate in disease states characterized by chronic degeneration. An encouraging finding, however, was that the increased muscle mass and decreased fibrosis were seen even in 9-month-old *myostatin* null, *mdx* mice, suggesting that the beneficial effects of loss of myostatin could be maintained for at least this time period (Wagner et al. 2002).

In addition to these settings of chronic muscle degeneration, targeting myostatin may also have beneficial effects in disease states in which muscle loss is more acute. One example is the muscle loss that occurs in cachexia, which is a wasting syndrome often seen in patients with chronic diseases such as cancer, AIDS, and sepsis and which is a major contributor to both morbidity and mortality (for review, see Tisdale 1999). Although the effects of inhibiting myostatin activity in models of cachexia have not yet been reported, it seems plausible that blocking this pathway might be an effective method for enhancing muscle growth or regeneration to offset the wasting that occurs in this syndrome. Moreover, as discussed above, overexpression of myostatin in mice can induce a wasting syndrome that has many of the hallmarks of human cachexia, including fat and muscle loss in the setting of normal caloric intake (Zimmers et al. 2002). This finding raises the intriguing possibility that either myostatin itself or some other mediator capable of activating this same signaling pathway may play a critical role in inducing cachexia in humans. If so, targeting this pathway may directly counteract the molecular signals responsible for inducing cachexia in various disease states. In this respect, circulating levels of myostatin have been reported to be elevated in HIV-infected

patients with muscle wasting (Gonzalez-Cadavid et al. 1998), although one caveat to the interpretation of these studies is the uncertainty regarding the specificity of the antibodies used to measure myostatin levels.

Finally, targeting the myostatin pathway may have applications not only for muscle degenerative and wasting conditions but also for metabolic diseases, such as obesity and type II diabetes. As discussed above, *myostatin* mutant mice fail to accumulate fat as a function of age (Lin et al. 2002, McPherron & Lee 2002). This observation suggested the possibility that loss of myostatin might have beneficial effects in settings of abnormal fat accumulation. Indeed, introduction of the *myostatin* null mutation into two different obese strains, *agouti lethal yellow* and *ob/ob*, resulted in a significant reduction in fat accumulation and suppression of the development of insulin resistance (McPherron & Lee 2002). Although the precise mechanisms by which myostatin regulates fat and glucose metabolism *in vivo* are not completely understood, these findings raise the possibility that inhibition of the myostatin pathway may be a new strategy for the prevention or treatment of obesity or type II diabetes.

Clearly, much more work will be required to determine whether targeting the myostatin pathway will have beneficial effects in settings of human diseases. A major unanswered question is whether myostatin plays a role in regulating muscle mass in humans as it does in mice and cattle, although the high degree of sequence conservation and expression of myostatin in human muscle are certainly consistent with a similar role for myostatin in humans. If targeting this pathway does turn out to be an effective strategy for treating human diseases, a number of considerations make myostatin a particularly attractive target for drug development. First, the effects of loss of myostatin are highly specific, as significant effects on tissues other than muscle and adipose tissue have not yet been reported. Second, myostatin normally functions extracellularly and is therefore accessible to a variety of potential pharmacologic agents, including proteins capable of binding myostatin; similarly, the mechanisms involved in regulating myostatin latency also operate extracellularly, making them readily accessible as well. Third, the effect of myostatin is dose dependent in that partial inhibition of myostatin activity results in a partial increase in muscle growth; hence, it may not be necessary to completely block myostatin signaling in order to produce a therapeutically beneficial effect.

CONCLUSIONS

Although many details regarding the mechanism of action of myostatin and the mechanisms by which myostatin activity is regulated *in vivo* remain to be elucidated, it is now firmly established that myostatin is synthesized by skeletal muscle, circulates in the blood, and acts in a concentration-dependent manner as a negative regulator of muscle growth. These properties of myostatin are precisely those hypothesized by Bullough (1962, 1965) for molecules that act to regulate tissue

size, and to be consistent with the terminology that he proposed, it seems clear that myostatin should be considered to be a muscle chalone.

Of all the tissues that could potentially utilize this type of mechanism to regulate tissue size, it is perhaps a bit unexpected that the one tissue for which this type of regulatory mechanism has been established is skeletal muscle. After all, growth of individual muscles is known to be controlled locally in response to a variety of different environmental stimuli and stresses, including exercise and injury. How, then, does one reconcile this type of local control with the fact that a key regulator like myostatin circulates systemically in the blood? Almost certainly, a critical aspect of the biology of myostatin is that myostatin circulates in the blood in a complex with inhibitory proteins and is therefore biologically inactive. It seems reasonable to suppose that levels of myostatin activity are regulated primarily at the target site by local control of the mechanisms involved in activating myostatin latency.

However, even if local signals do dictate the final level of myostatin signaling in muscle, it is possible that circulating levels of myostatin are regulated to limit the overall capacity for muscle growth. A speculative model is that circulating levels of myostatin change under different environmental conditions, such as temperature or food availability, or under different physiological states, such as physical activity, illness, pregnancy, or age, and that these changing levels then alter the metabolic homeostatic balance between fat storage and muscle growth. Under certain conditions, it may be advantageous to shift this balance toward fat storage, and under different conditions, it may be advantageous to shift this balance toward muscle growth. Numerous studies have examined the regulation of myostatin expression in response to a variety of stimuli and under different physiological conditions, and up-regulation or down-regulation of myostatin expression was detected in many of these studies (Gonzalez-Cadavid et al. 1998; Ji et al. 1998; Carlson et al. 1999; Sharma et al. 1999; Lalani et al. 2000; Mendler et al. 2000; Sakuma et al. 2000; Wehling et al. 2000; Kawada et al. 2001; Lang et al. 2001; Ma et al. 2001, 2003; Marcell et al. 2001; Schulte & Yarasheski 2001; Brill et al. 2002; Kocamis et al. 2002; Tseng et al. 2002; Welle et al. 2002; Yarasheski et al. 2002; Armand et al. 2003; Chauvigné et al. 2003; Jeanplong et al. 2003; Liu et al. 2003; Pampusch et al. 2003; Peters et al. 2003; Rodgers et al. 2003; Roth et al. 2003; Vianello et al. 2003; White et al. 2003); however, additional experiments will be required to determine whether the magnitude of these observed changes is large enough to cause significant physiological effects. Although we still have much to understand regarding the biology of myostatin, it is clear that myostatin is a major regulator of the metabolic state of the animal, and it is the profound metabolic consequences resulting from altered myostatin activity that likely explain the extraordinary degree to which myostatin has been conserved through evolution.

It remains to be determined whether myostatin is unique as a negative regulator of muscle growth or whether the activity of myostatin is at least partially redundant with those of other signaling molecules, such as the highly related protein,

GDF-11. It will also be important to determine whether this general type of regulatory mechanism may be utilized by tissues other than muscle to regulate tissue size. Because activation of the TGF- β signaling pathway results in growth inhibition in a variety of cell types and because the TGF- β superfamily contains an enormously large group of ligands with a wide range of expression patterns, this family of signaling molecules may be a reasonable starting point in the search for other chalone-like modulators of tissue size.

ACKNOWLEDGMENTS

I thank Christine Moss for assistance with preparation of this manuscript. I apologize to those colleagues whose work I did not cite as a result of either space limitations or oversight. Work in my laboratory on myostatin is supported by National Institutes of Health grants R01HD35887 and R01CA88866. Myostatin was licensed by the Johns Hopkins University to MetaMorphix, Inc., and sublicensed to Wyeth. I am entitled to a share of sales royalty received by the University from sales of this factor. The University and I also own MetaMorphix stock, which is subject to certain restrictions under University policy. I am a paid consultant to MetaMorphix. The terms of these agreements are being managed by the University in accordance with its conflict of interest policies.

**The Annual Review of Cell and Developmental Biology is online at
<http://cellbio.annualreviews.org>**

LITERATURE CITED

- Akiyoshi S, Inoue H, Hanai J-i, Kusanagi K, Nemoto N, et al. 1999. c-Ski acts as a transcriptional co-repressor in transforming growth factor- β signaling through interaction with smads. *J. Biol. Chem.* 274:35269–77
- Amthor H, Christ B, Rashid-Doubell F, Kemp CF, Lang E, Patel K. 2002a. Follistatin regulates bone morphogenetic protein-7 (BMP-7) activity to stimulate embryonic muscle growth. *Dev. Biol.* 243:115–27
- Amthor H, Connolly D, Patel K, Brand-Saberi B, Wilkinson DG, et al. 1996. The expression and regulation of *follistatin* and a *follistatin-like* gene during avian somite compartmentalization and myogenesis. *Dev. Biol.* 178:343–62
- Amthor H, Huang R, McKinnell I, Christ B, Kambadur R, et al. 2002b. The regulation and action of myostatin as a negative regulator of muscle development during avian embryogenesis. *Dev. Biol.* 251:241–57
- Armand A-S, Gaspera BD, Launay T, Charbonnier F, Gallien CL, Chanoine C. 2003. Expression and neural control of follistatin versus myostatin genes during regeneration of mouse soleus. *Dev. Dyn.* 227:256–65
- Attisano L, Cárcamo J, Ventura F, Weis FMB, Massagué J, Wrana JL. 1993. Identification of human activin and TGF β type I receptors that form heteromeric kinase complexes with type II receptors. *Cell* 75:671–80
- Berk M, Desai S, Heyman HC, Colmenares C. 1997. Mice lacking the *ski* proto-oncogene have defects in neurulation, craniofacial patterning, and skeletal muscle development. *Genes Dev.* 11:2029–39
- Bischoff R. 1994. The satellite cell and muscle regeneration. In *Myology: Basic and*

- Clinical*, ed. A Engel, C Franzini-Armstrong, pp. 97–118. New York: McGraw-Hill
- Black K, Garrett IR, Mundy GR. 1991. Chinese hamster ovarian cells transfected with the murine interleukin-6 gene cause hypercalcemia as well as cachexia, leukocytosis and thrombocytosis in tumor-bearing nude mice. *Endocrine* 128:2657–59
- Blader P, Rastegar S, Fischer N, Strahle U. 1997. Cleavage of the BMP-4 antagonist chordin by zebrafish tolloid. *Science* 278:1937–40
- Bogdanovich S, Krag TOB, Barton ER, Morris LD, Whittemore L-A, et al. 2002. Functional improvement of dystrophic muscle by myostatin blockade. *Nature* 420:418–21
- Brill KT, Weltman AL, Gentili A, Patrie JT, Fryburg DA, et al. 2002. Single and combined effects of growth hormone and testosterone administration on measures of body composition, physical performance, mood, sexual function, bone turnover, and muscle gene expression in healthy older men. *J. Clin. Endocrinol. Metab.* 87:5649–57
- Bullough WS. 1962. The control of mitotic activity in adult mammalian tissues. *Biol. Rev.* 37:307–42
- Bullough WS. 1965. Mitotic and functional homeostasis: a speculative review. *Cancer Res.* 25:1683–727
- Campion D. 1984. The muscle satellite cell: a review. *Intl. Rev. Cytol.* 87:225–51
- Cappuccio I, Marchitelli C, Serracchioli A, Nardone A, Filippini F, et al. 1998. A G-T transversion introduces a stop codon at the mh locus in hypertrophic Marchigiana beef subjects. *Anim. Genet.* 29:51
- Carlson CJ, Booth FW, Gordon SE. 1999. Skeletal muscle myostatin mRNA expression is fiber-type specific and increases during hindlimb unloading. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 277:R601–6
- Charlier C, Coppieters W, Farnir F, Grobet L, Leroy PL, et al. 1995. The mh gene causing double-muscling in cattle maps to bovine chromosome 2. *Mamm. Genome* 6:788–92
- Chauvigné F, Gabillard JC, Weil C, Rescan PY. 2003. Effect of refeeding on IGFI, IGFII, IGF receptors, FGF2, FGF6, and myostatin mRNA expression in rainbow trout myotomal muscle. *Gen. Comp. Endocrinol.* 132:209–15
- Cheng SK, Olale F, Bennett JT, Brivanlou AH, Schier AF. 2003. EGF-CFC proteins are essential coreceptors for the TGF- β signals Vg1 and GDF1. *Genes Dev.* 17:31–36
- Daopin S, Piez KA, Ogawa Y, Davies DR. 1992. Crystal structure of transforming growth factor- β 2: an unusual fold for the superfamily. *Science* 257:369–73
- de Winter JP, ten Dijke P, de Vries CJM, van Achterberg TAE, Sugino H, et al. 1996. Follistatins neutralize activin bioactivity by inhibition of activin binding to its type II receptors. *Mol. Cell. Endocrin.* 116:105–14
- Eigenbrot C, Gerber N. 1997. X-ray structure of glial cell-derived neurotrophic factor at 1.9 Å resolution and implications for receptor binding. *Nat. Struct. Biol.* 4:435–38
- Esquela AF, Lee S-J. 2003. Regulation of metanephric kidney development by growth/differentiation factor 11. *Dev. Biol.* 257:356–70
- Fainsod A, Deißler K, Yelin R, Marom K, Epstein M, et al. 1997. The dorsalizing and neural inducing gene *follistatin* is an antagonist of *BMP-4*. *Mech. Dev.* 63:39–50
- Gamer LW, Wolfman NM, Celeste AJ, Hattersley G, Hewick R, Rosen V. 1999. A novel BMP expressed in developing mouse limb, spinal cord, and tail bud is a potent mesoderm inducer in *Xenopus* embryos. *Dev. Biol.* 208:222–32
- Gonzalez-Cadavid NF, Taylor WE, Yarasheski K, Sinha-Hikim I, Ma K, et al. 1998. Organization of the human myostatin gene and expression in healthy men and HIV-infected men with muscle wasting. *Proc. Natl. Acad. Sci. USA* 95:14938–43
- Griffith DL, Keck PC, Sampath TK, Rueger DC, Carlson WD. 1996. Three-dimensional structure of recombinant human osteogenic protein 1: structural paradigm for the transforming growth factor β superfamily. *Proc. Natl. Acad. Sci. USA* 93:878–83

- Grobet L, Martin LJR, Poncelet D, Pirotin D, Brouwers B, et al. 1997. A deletion in the bovine myostatin gene causes the double-muscling phenotype in cattle. *Nat. Genet.* 17: 71–74
- Grobet L, Poncelet D, Royo LJ, Brouwers B, Pirotin D, et al. 1998. Molecular definition of an allelic series of mutations disrupting the myostatin function and causing double-muscling in cattle. *Mamm. Genome* 9:210–13
- Hanset R, Michaux C. 1985. On the genetic determinism of muscular hypertrophy in the Belgian White and Blue cattle breed. I. Experimental data. *Genet. Sel. Evol.* 17:359–68
- Hayette S, Gadoux M, Martel S, Bertrand S, Tigaud I, et al. 1998. *FLRG* (follistatin-related gene), a new target of chromosomal rearrangement in malignant blood disorders. *Oncogene* 16:2949–54
- Hepple RT. 2003. Sarcopenia—a critical perspective. *Sci. Aging Knowl. Environ.* 2003: pe31–37
- Hill JJ, Davies MV, Pearson AA, Wang JH, Hewick RM, et al. 2002. The myostatin propeptide and the follistatin-related gene are inhibitory binding proteins of myostatin in normal serum. *J. Biol. Chem.* 277:40735–41
- Hill JJ, Qiu Y, Hewick RM, Wolfman NM. 2003. Regulation of myostatin in vivo by growth and differentiation factor-associated serum protein-1: a novel protein with protease inhibitor and follistatin domains. *Mol. Endocrinol.* 17:1144–54
- Hinck AP, Archer SJ, Qian SW, Roberts AB, Sporn MB, et al. 1996. Transforming growth factor β 1: three-dimensional structure in solution and comparison with the X-ray structure of transforming growth factor β 2. *Biochemistry* 35:8517–34
- Huet C, Li Z-F, Liu H-Z, Black RA, Galliano M-F, Engvall E. 2001. Skeletal muscle cell hypertrophy induced by inhibitors of metalloproteases; myostatin as a potential mediator. *Am. J. Physiol. Cell Physiol.* 281:C1624–34
- Iemura S-I, Yamamoto TS, Takagi C, Uchiyama H, Natsume T, et al. 1998. Direct binding of follistatin to a complex of bone-morphogenetic protein and its receptor inhibits ventral and epidermal cell fates in early *Xenopus* embryo. *Proc. Natl. Acad. Sci. USA* 95:9337–42
- Jeanplong F, Bass JJ, Smith HK, Kirk SP, Kambadur R, et al. 2003. Prolonged underfeeding of sheep increases myostatin and myogenic regulatory factor Myf-5 in skeletal muscle while IGF-I and myogenin are repressed. *J. Endocrinol.* 176:425–37
- Ji S, Losinski RL, Cornelius SG, Frank GR, Willis GM, et al. 1998. Myostatin expression in porcine tissues: tissue specificity and developmental and postnatal regulation. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 275:R1265–73
- Jouliia D, Bernardi H, Garandel V, Rabenoelina F, Vernus B, Cabello G. 2003. Mechanisms involved in the inhibition of myoblast proliferation and differentiation by myostatin. *Exp. Cell Res.* 286:263–75
- Kambadur R, Sharma M, Smith TPL, Bass JJ. 1997. Mutations in myostatin (GDF8) in double-muscling Belgian Blue and Piedmontese cattle. *Genome Res.* 7:910–15
- Kawada S, Tachi C, Ishii N. 2001. Content and localization of myostatin in mouse skeletal muscles during aging, mechanical unloading and reloading. *J. Muscle. Res. Cell Motil.* 22:627–33
- Kim HS, Liang L, Dean RG, Hausman DB, Hartzell DL, Baile CA. 2001. Inhibition of preadipocyte differentiation by myostatin treatment in 3T3-L1 cultures. *Biochem. Biophys. Res. Commun.* 281:902–6
- Kirsch T, Sebald W, Dreyer MK. 2000. Crystal structure of the BMP-2-BRIA ectodomain complex. *Nat. Struct. Biol.* 7:492–96
- Kocabas AM, Kucuktas H, Dunham RA, Liu Z. 2002. Molecular characterization and differential expression of the myostatin gene in channel catfish (*Ictalurus punctatus*). *Biochim. Biophys. Acta* 1575:99–107
- Kocamis H, Gahr SA, Richter J, Kirkpatrick-Keller DC, Killefer J. 2002. Myostatin and TGF- β 2 gene expression patterns in response to in ovo administration of rhIGF-I during

- chicken embryonic development. *Growth Dev. Aging* 66:3–10
- Lalani R, Bhasin S, Byhower F, Tarnuzzer R, Grant M, et al. 2000. Myostatin and insulin-like growth factor-I and -II expression in the muscle of rats exposed to the microgravity environment of the NeuroLab space shuttle flight. *J. Endocrinol.* 167:417–28
- Lang CH, Silvis C, Nystrom G, Frost RA. 2001. Regulation of myostatin by glucocorticoids after thermal injury. *FASEB J.* 15:1807–9
- Langley B, Thomas M, Bishop A, Sharma M, Gilmour S, Kambadur R. 2002. Myostatin inhibits myoblast differentiation by down-regulating MyoD expression. *J. Biol. Chem.* 277:49831–40
- Lee S-J, McPherron AC. 2001. Regulation of myostatin activity and muscle growth. *Proc. Natl. Acad. Sci. USA* 98:9306–11
- Lewis KA, Gray PC, Blount AL, MacConnell LA, Wiater E, et al. 2000. Betaglycan binds inhibin and can mediate functional antagonism of activin signalling. *Nature* 404:411–14
- Lin J, Arnold HB, Della-Fera MA, Azain MJ, Hartzell DL, Baile CA. 2002. Myostatin knockout in mice increases myogenesis and decreases adipogenesis. *Biochem. Biophys. Res. Commun.* 291:701–6
- Liu W, Thomas SG, Asa S, Gonzalez-Cadavid N, Bhasin S, Ezzat S. 2003. Myostatin is a skeletal muscle target of growth hormone anabolic action. *J. Clin. Endocrinol. Metab.* 88:5490–95
- Luo K, Stroschein SL, Wang W, Chen D, Martens E, et al. 1999. The Ski oncoprotein interacts with the Smad proteins to repress TGF β signaling. *Genes Dev.* 13:2196–206
- Ma K, Mallidis C, Artaza J, Taylor W, Gonzalez-Cadavid N, Bhasin S. 2001. Characterization of 5'-regulatory region of human myostatin gene: regulation by dexamethasone in vitro. *Am. J. Physiol. Endocrinol. Metab.* 281:E1128–36
- Ma K, Mallidis C, Bhasin S, Mahabadi V, Artaza J, et al. 2003. Glucocorticoid-induced skeletal muscle atrophy is associated with up-regulation of myostatin gene expression. *Am. J. Physiol. Endocrinol. Metab.* 285:E363–71
- Maccatrozzo L, Bargelloni L, Cardazzo B, Rizzo G, Patarnello T. 2001. A novel second myostatin gene is present in teleost fish. *FEBS Lett.* 509:36–40
- Marcell TJ, Harman SM, Urban RJ, Metz DD, Rodgers BD, Blackman MR. 2001. Comparison of GH, IGF-I, and testosterone with mRNA of receptors and myostatin in skeletal muscle in older men. *Am. J. Physiol. Endocrinol. Metab.* 281:E1159–64
- Marchitelli C, Savarese MC, Crisà A, Nardone A, Marsan PA, Valentini A. 2003. Double muscling in Marchigiana beef breed is caused by a stop codon in the third exon of myostatin gene. *Mamm. Genome* 14:392–95
- Marques G, Musacchio M, Shimell MJ, Wunnenberg-Stapleton K, Cho KW, O'Conner MB. 1997. Production of a DPP activity gradient in the early *Drosophila* embryo through the opposing actions of the SOG and TLD proteins. *Cell* 91:417–26
- Massagué J. 1998. TGF- β signal transduction. *Annu. Rev. Biochem.* 67:753–91
- Matzuk MM, Lu N, Vogel H, Sellheyer K, Roop DR, Bradley A. 1995. Multiple defects and perinatal death in mice deficient in follistatin. *Nature* 374:360–63
- McCroskery S, Thomas M, Maxwell L, Sharma M, Kambadur R. 2003. Myostatin negatively regulates satellite cell activation and self-renewal. *J. Cell Biol.* 162:1135–47
- McPherron AC, Lawler AM, Lee S-J. 1997. Regulation of skeletal muscle mass in mice by a new TGF- β superfamily member. *Nature* 387:83–90
- McPherron AC, Lawler AM, Lee S-J. 1999. Regulation of anterior/posterior patterning of the axial skeleton by growth/differentiation factor 11. *Nat. Genet.* 22:260–64
- McPherron AC, Lee S-J. 1997. Double muscling in cattle due to mutations in the myostatin gene. *Proc. Natl. Acad. Sci. USA* 94:12457–61
- McPherron AC, Lee S-J. 2002. Suppression of body fat accumulation in myostatin-deficient mice. *J. Clin. Invest.* 109:595–601

- Mendler L, Zador E, Ver Heyen M, Dux L, Wuytack F. 2000. Myostatin levels in regenerating rat muscles and in myogenic cell cultures. *J. Muscle Res. Cell Motil.* 21:551–63
- Mennisier F. 1982. Present state of knowledge about the genetic determination of muscular hypertrophy or the double muscled trait in cattle. In *Muscle Hypertrophy of Genetic Origin and Its Use to Improve Beef Production*, ed. JWB King, F Mennisier, pp. 387–428. The Hague: Nijhoff
- Mittl PR, Priestle JP, Cox DA, McMaster G, Cerletti N, Grutter MG. 1996. The crystal structure of TGF- β 3 and comparison to TGF- β 2: implications for receptor binding. *Protein Sci.* 5:1261–71
- Miyazono K, Hellman U, Wernstedt C, Heldin CH. 1988. Latent high molecular weight complex of transforming growth factor β 1. Purification from platelets and structural characterization. *J. Biol. Chem.* 263:6407–15
- Nakamura T, Takio K, Eto Y, Shibai H, Titani K, Sugino H. 1990. Activin-binding protein from rat ovary is follistatin. *Science* 247:836–38
- Nakashima M, Toyono T, Akamine A, Joyner A. 1999. Expression of growth/differentiation factor 11, a new member of the BMP/TGF- β superfamily during mouse embryogenesis. *Mech. Dev.* 80:185–89
- Nishi M, Yasue A, Nishimatu S, Nohno T, Yamaoka T, et al. 2002. A missense mutant myostatin causes hyperplasia without hypertrophy in the mouse muscle. *Biochem. Biophys. Res. Commun.* 293:247–51
- Oh SP, Li E. 1997. The signaling pathway mediated by the type IIB activin receptor controls axial patterning and lateral asymmetry in the mouse. *Genes Dev.* 11:1812–26
- Oh SP, Yeo C-Y, Lee Y, Schrewe H, Whitman M, Li E. 2002. Activin type IIA and IIB receptors mediate Gdf11 signaling in axial vertebral patterning. *Genes Dev.* 16:2749–54
- Oloff A, Defeo-Jones D, Boyer M, Martinez D, Kiefer D, et al. 1987. Tumors secreting human TNF/cachectin induce cachexia in mice. *Cell* 50:555–63
- Ostbye T-K, Galloway TF, Nielsen C, Gabestad I, Bardal T, Andersen O. 2001. The two myostatin genes of Atlantic salmon (*Salmo salar*) are expressed in a variety of tissues. *Eur. J. Biochem.* 268:5249–57
- Pampusch MS, Johnson BJ, White ME, Hathaway MR, Dunn JD, et al. 2003. Time course of changes in growth factor mRNA levels in muscle of steroid-implanted and non-implanted steers. *J. Anim. Sci.* 81:2733–40
- Pappano WN, Steiglitiz BM, Scott IC, Keene DR, Greenspan DS. 2003. Use of *Bmp1/Tll1* doubly homozygous null mice and proteomics to identify and validate in vivo substrates of BMP-1/tolloid-like metalloproteinases. *Mol. Cell. Biol.* 23:4428–38
- Peters D, Barash IA, Burdi M, Yuan PS, Mathew L, et al. 2003. Asynchronous functional, cellular and transcriptional changes after a bout of eccentric exercise in the rat. *J. Physiol.* 553:947–57
- Piccolo S, Agius E, Lu B, Goodman S, Dale L, DeRobertis EM. 1997. Cleavage of chordin by xolloid metalloprotease suggests a role for proteolytic processing in the regulation of Spemann organizer activity. *Cell* 91:407–16
- Rebbapragada A, Benchabane H, Wrana J, Celeste AJ, Attisano L. 2003. Myostatin signals through a transforming growth factor β -like signaling pathway to block adipogenesis. *Mol. Cell. Biol.* 23:7230–42
- Rehemtulla A, Kaufman RJ. 1992. Preferred sequence requirements for cleavage of pro-von Willebrand factor by propeptide-processing enzymes. *Blood* 79:2349–55
- Rescan P-Y, Jutel I, Rallièrè C. 2001. Two myostatin genes are differentially expressed in myotomal muscles of the trout (*Oncorhynchus mykiss*). *J. Exp. Biol.* 204:3523–29
- Ríos R, Carneiro I, Arce VM, Devesa J. 2001. Myostatin regulates cell survival during C2C12 myogenesis. *Biochem. Biophys. Res. Comm.* 280:561–66
- Ríos R, Carneiro I, Arce VM, Devesa J. 2002. Myostatin is an inhibitor of myogenic

- differentiation. *Am. J. Physiol. Cell Physiol.* 282:C993–99
- Roberts SB, Goetz FW. 2001. Differential skeletal muscle expression of myostatin across teleost species, and the isolation of multiple myostatin isoforms. *FEBS Lett.* 24639:1–5
- Rodgers BD, Weber GM. 2001. Sequence conservation among fish myostatin orthologues and the characterization of two additional cDNA clones from *Morone saxatilis* and *Morone americana*. *Comp. Biochem. Phys. B* 129:597–603
- Rodgers BD, Weber GM, Kelley KM, Levine MA. 2003. Prolonged fasting and cortisol reduce myostatin mRNA levels in tilapia larvae; short-term fasting elevates. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 284:R1277–86
- Rodgers BD, Weber GM, Sullivan CV, Levine MA. 2001. Isolation and characterization of myostatin complementary deoxyribonucleic acid clones from two commercially important fish: *Oreochromis mossambicus* and *Morone chrysops*. *Endocrine* 142:1412–18
- Roth SM, Martel GF, Ferrell RE, Metter EJ, Hurley BF, Rogers MA. 2003. Myostatin gene expression is reduced in humans with heavy-resistance strength training: a brief communication. *Exp. Biol. Med. (Maywood)* 228:706–9
- Sakuma K, Watanabe K, Sano M, Uramoto I, Totsuka T. 2000. Differential adaptation of growth and differentiation factor 8/myostatin, fibroblast growth factor 6 and leukemia inhibitory factor in overloaded, regenerating and denervated rat muscles. *Biochim. Biophys. Acta* 1497:77–88
- Scheufler C, Sebald W, Hülsmeier M. 1999. Crystal structure of human bone morphogenetic protein-2 at 2.7 Å resolution. *J. Mol. Biol.* 287:103–15
- Schier AF, Shen MM. 2000. Nodal signalling in vertebrate development. *Nature* 403:385–89
- Schlunegger MP, Grütter MG. 1992. An unusual feature revealed by the crystal structure at 2.2 Å resolution of human transforming growth factor- β 2. *Nature* 358:430–34
- Schulte JN, Yarasheski KE. 2001. Effects of resistance training on the rate of muscle protein synthesis in frail elderly people. *Int. J. Sport Nutr. Exerc. Metab.* 11 (Suppl.):S111–18
- Schultz E, McCormick KM. 1994. Skeletal muscle satellite cells. *Rev. Physiol. Biochem. Pharmacol.* 123:213–57
- Scott IC, Blitz IL, Pappano WN, Imamura Y, Clark TG, et al. 1999. Mammalian BMP-1/tolloid-related metalloproteinases, including novel family member mammalian tolloid-like 2, have differential enzymatic activities and distributions of expression relevant to patterning and skeletogenesis. *Dev. Biol.* 213:283–300
- Sharma M, Kambadur R, Matthews KG, Somers WG, Devlin GP, et al. 1999. Myostatin, a transforming growth factor- β superfamily member, is expressed in heart muscle and is upregulated in cardiomyocytes after infarct. *J. Cell. Physiol.* 180:1–9
- Sicinski P, Geng Y, Ryder-Cook AS, Barnard EA, Darlison MG, Barnard PJ. 1989. The molecular basis of muscular dystrophy in the *mdx* mouse: a point mutation. *Science* 244:1578–80
- Song J, Oh SP, Schrewe H, Nomura M, Lei H, et al. 1999. The type II activin receptors are essential for egg cylinder growth, gastrulation, and rostral head development in mice. *Dev. Biol.* 213:157–69
- Stroschein S, Wang W, Zhou S, Zhou Q, Luo K. 1999. Negative feedback regulation of TGF- β signaling by the SnoN oncoprotein. *Science* 286:771–74
- Sun Y, Liu X, Ng-Eaton E, Lane WS, Lodish HF, Weinberg RA. 1999a. Interaction of the Ski oncoprotein with Smad3 regulates TGF- β signaling. *Mol. Cell* 4:499–509
- Sun Y, Liu X, Ng-Eaton E, Lodish HF, Weinberg RA. 1999b. SnoN and Ski protooncoproteins are rapidly degraded in response to transforming growth factor β signaling. *Proc. Natl. Acad. Sci. USA* 96:12442–47
- Sutrave P, Kelly AM, Hughes SH. 1990. ski can cause selective growth of skeletal muscle in transgenic mice. *Genes Dev.* 4:1462–72

- Taylor WE, Bhasin S, Artaza J, Byhower F, Azam M, et al. 2001. Myostatin inhibits cell proliferation and protein synthesis in C₂C₁₂ muscle cells. *Am. J. Physiol. Endocrinol. Metab.* 280:E221–28
- Thies RS, Chen T, Davies MV, Tomkinson KN, Pearson AA, et al. 2001. GDF-8 propeptide binds to GDF-8 and antagonizes biological activity by inhibiting GDF-8 receptor binding. *Growth Factors* 18:251–59
- Thomas M, Langley B, Berry C, Sharma M, Kirk S, et al. 2000. Myostatin, a negative regulator of muscle growth, functions by inhibiting myoblast proliferation. *J. Biol. Chem.* 275:40235–43
- Thompson TB, Woodruff TK, Jardetzky TS. 2003. Structures of an ActRIIB:activin A complex reveal a novel binding mode for TGF- β ligand:receptor interactions. *EMBO J.* 22:1555–66
- Tisdale MJ. 1999. Wasting in cancer. *J. Nutr.* 129:243S–46S
- Tseng BS, Zhao P, Pattison JS, Gordon SE, Granchelli JA, et al. 2002. Regenerated *mdx* mouse skeletal muscle shows differential mRNA expression. *J. Appl. Physiol.* 93:537–45
- Tsuchida K, Arai KY, Kuramoto Y, Yamakawa N, Hasegawa Y, Sugino H. 2000. Identification and characterization of a novel follistatin-like protein as a binding protein for the TGF- β family. *J. Biol. Chem.* 275:40788–96
- Vianello S, Brazzoduro L, Dalla Valle L, Belvedere P, Colombo L. 2003. Myostatin expression during development and chronic stress in zebrafish (*Danio rerio*). *J. Endocrinol.* 176:47–59
- Wagner KR, McPherron AC, Winik N, Lee S-J. 2002. Loss of myostatin attenuates severity of muscular dystrophy in *mdx* mice. *Ann. Neurol.* 52:832–36
- Wehling M, Cai B, Tidball JG. 2000. Modulation of myostatin expression during modified muscle use. *FASEB J.* 14:103–10
- Welle S, Bhatt K, Shah B, Thornton CA. 2002. Insulin-like growth factor-1 and myostatin mRNA expression in muscle: comparison between 62–77 and 21–31 yr old men. *Exp. Gerontol.* 37:833–39
- White ME, Johnson BJ, Hathaway MR, Dayton WR. 2003. Growth factor messenger RNA levels in muscle and liver of steroid-implanted and nonimplanted steers. *Anim. Sci.* 81:965–72
- Whittemore L-A, Song K, Li X, Aghajanian J, Davies MV, et al. 2003. Inhibition of myostatin in adult mice increases skeletal muscle mass and strength. *Biochem. Biophys. Res. Commun.* 300:965–71
- Wise RJ, Barr PJ, Wong PA, Kiefer MC, Brake AJ, Kaufman RJ. 1990. Expression of a human proprotein processing enzyme: correct cleavage of the von Willebrand factor precursor at a paired basic amino acid site. *Proc. Natl. Acad. Sci. USA* 87:9378–82
- Wolfman NM, McPherron AC, Pappano WN, Davies MV, Song K, et al. 2003. Activation of latent myostatin by the BMP-1/tolloid family of metalloproteinases. *Proc. Natl. Acad. Sci. USA* 100:15842–46
- Yamashita H, ten Dijke P, Huylebroeck D, Sampath TK, Andries M, et al. 1995. Osteogenic protein-1 binds to activin type II receptors and induces certain activin-like effects. *J. Cell Biol.* 130:217–26
- Yang J, Ratovitski T, Brady JP, Solomon MB, Wells KD, Wall RJ. 2001. Expression of myostatin pro domain results in muscular transgenic mice. *Mol. Reprod. Dev.* 60:351–61
- Yarasheski K, Bhasin S, Sinha-Hikim I, Pak-Loduca J, Gonzalez-Cadavid N. 2002. Serum myostatin-immunoreactive protein is increased in 60–92 year old women and men with muscle wasting. *J. Nutr. Health Aging* 6:343–48
- Zhu X, Hadhazy M, Wehling M, Tidball JG, McNally EM. 2000. Dominant negative myostatin produces hypertrophy without hyperplasia in muscle. *FEBS Lett.* 474:71–75
- Zimmers TA, Davies MV, Koniaris LG, Haynes P, Esquela AF, et al. 2002. Induction of cachexia in mice by systemically administered myostatin. *Science* 296:1486–88