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# REVIEW OF MYOSTATIN HISTORY, PHYSIOLOGY AND APPLICATIONS

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## SUMMARY

*Myostatin, a negative regulator of muscle cell growth, is highly conserved across species. The loss of functional myostatin is known to cause the "double-muscled" phenotype in several cattle breeds, and similar phenotypes in other species. For nearly 200 years, double-muscled animals have captured the attention of livestock breeders and researchers, boasting enlarged musculature but beset by production difficulties. With the advent of transgenic technology, researchers have created a "knockout" mouse model with which to efficiently explore the biochemical pathways and influences of myostatin. Research involving this model has both agricultural and biomedical applications, and involves several cell growth and regulation mechanisms. Analysis of growth and development patterns in myostatin-null mice is necessary to link these findings with past research.*

## INTRODUCTION

For nearly two hundred years, the phenomenon of the "double-muscled" animal, a spectacular model of muscular hypertrophy, has represented a never-ending source of inspiration and argument for both livestock producers and scientists. Though livestock other than cattle produce double-muscled individuals of more or less desirability, recent characterization of the protein myostatin, a specific inhibitor of muscle cell growth, has rekindled interest in the condition (McPherron et al., 1997). Myostatin "knockouts" are the genetic and biochemical basis for one form of muscular hypertrophy in the bovine (McPherron and Lee, 1997), and advances in the field of biotechnology have shown new means to exploit the gene that is involved. Far from burying the old controversy, contemporary understanding of double-muscling in cattle has reawakened and added new issues to the old debate.

The stunning muscularity of such animals embodies the extreme in meat-producing efficiency, each animal yielding a large proportion of retail cuts that are low in fat and characteristically tender (Westhusin, 1997). At a glance, double-muscled breeds of cattle such as the Belgian Blue and Piedmontese would seem to represent a model of the modern agricultural philosophy - lean, commercially desirable meat produced in comparable quantities by fewer animals. They are economically important breeds, both at home and abroad. The Belgian Blue (Blanc Bleu Belge or BBB) is currently the premier cattle breed in Belgium, comprising 45% of the total population of cattle in that country (Fiems et al., 1995). Piedmontese and Belgian Blue bulls have both been researched as possible terminal sires, in the hopes of conferring some of their production advantage to heterozygous offspring (Kieffer and Cartwright, 1980; Baker and Lunt, 1990).

## HISTORY AND ORIGINS OF DOUBLE-MUSCLED BREEDS

The phenomenon of the double-muscled animal presents an interesting puzzle for the dedicated genetic explorer. The history of the mutation can be traced back to the middle of the eighteenth century, and was first documented in the livestock almanac of a British farmer named George Culley. However, considering the diverse breeds in which the mutation has been observed, a true chronicle of the condition would likely span several million years of history, perhaps even beyond the Pleistocene period that saw the fragmentation of the bos genus into the earliest progenitors of our modern breeds. Over two million years ago, an ancient ancestor of modern cattle gave rise to two distinct species, the aurochs, or *Bos*

*primigenius*, progenitor of modern Asian and European cattle, and *Bos namadicus*, a forebear of *Bos indicus*, the humped zebu cattle of India (Friend, 1978). Double-muscled breeds are found among descendants of both of these groups. However, any speculation about the origin of the myostatin mutation among such diverse groups as Belgian Blue and Piedmontese cattle must take into account the polymorphic nature of the double-muscling condition observed between breeds (Grobet et al., 1997; Grobet et al., 1998) and the most likely path by which the breeds developed. As discussed later, the mutation causing double-muscling in the Belgian Blue is different from that causing the condition in Piedmontese cattle (Swatland and Kieffer, 1974; Hanset, 1991; Grobet et al., 1997; McPherron and Lee, 1997; Grobet et al., 1998).

The Belgian Blue breed has existed in one form or another since the mid-1800's, and the breed has long been scrutinized by researchers as a model for the double-muscled condition. The breed originally resulted from crosses of Dutch Friesians and English shorthorns with native cattle circa 1850 (Friend, 1978). The herd book of the Belgian blue was begun in 1919. Belgian breeders, driven by consumer demand for lean, tender meat, likely selected individuals with the characteristic conformation of hypermuscularity, purposefully or accidentally fostering the persistence of culard (double-muscled) individuals in Belgian Blue herds (Hanset, 1991).

Double-muscled Piedmontese cattle (referred to as the "Albese" variety) are believed to have resulted from the fusion of Zebu and Aurochs cattle followed by 25,000 years of evolution. Their herd book began in 1887, and the breed society was established in 1934 (Friend, 1978). Like the Belgian Blue, the Piedmontese has also been considered as a possible sire for terminal crosses, and has been used successfully in such programs for over 60 years (Swatland and Kieffer, 1974).

## PHYSIOLOGICAL CHARACTERISTICS OF DOUBLE-MUSCLED ANIMALS

One of the properties that sustained interest in the mutation causing double-muscling and ultimately led researchers to link dysfunctional myostatin to the double-muscled condition is the easily recognizable extreme phenotype of the myostatin-null individual. Myostatin knockout mice are characterized by bulging muscular development visible all over their bodies, with the most extreme hypertrophy apparent in the shoulders and hindquarters. This impression of extreme muscularity is enhanced by the lack of visible fat anywhere on the body.

Double-muscled cattle are even more easily discernable than their murine counterparts. On Belgian Blue bulls, every intramuscular groove is readily visible, due to an almost complete lack of subcutaneous fat. Instead of the "boxy" build of typical cattle, double-muscled animals have tight, "greyhound" bellies and a muscular roundness to their quarters that probably prompted the original "carthorse" description (Culley, 1804; Menissier, 1982). Indeed, they have a muscular conformation most often reserved for draft horses and bodybuilders - and such a blatant advertisement of retail product that the industry interest is obvious.

Overall body composition of double-muscled cattle varies by individual, sex, and breed. In general, however, the double-muscled phenotype is characterized by lower proportions of bone, much higher proportions of muscle, and much lower proportions of fat than conventional cattle of comparable background (Dumont, 1982; Hanset, 1986, 1991). Although the exact values of these differences vary by age and breed, independent researchers have consistently supported the general trends. Research supports these general trends not only across breeds but across species lines as well (McPherron et al., 1997).

The bones of double-muscled cattle, while significantly hypotrophied, are not affected as

drastically as other tissues in the body. Hanset (1991) reported percent losses in bone mass of double-muscled bulls compared to conventional bulls that ranged from -4.8% (tibia) to -9.1% (femur). These losses were greater in a comparison of double-muscled and normal females. In mice, the size and shape of the femoral bone was unaltered by a myostatin gene knockout (Hamrick et al., 2000). This is likely due to the fact that stress on the bones of myostatin-knockout mice is virtually identical to that of control mice in similar environmental situations, as their exercise level within the cage is nearly the same (Turner, 2000). Individual muscle weights were also found to vary in normal versus myostatin knockout mice, although the increase was much more drastic, ranging from 200-300% larger in knockout animals (McPherron et al., 1997).

In spite of the name, a double-muscled animal has the same number of muscles as a conventional animal. Rather, hypertrophy of the muscular tissue and the extreme scarcity of fat cover sharply define every muscle in the animal's body. There are, however, some striking differences in muscle structure between the types. Double-muscled cattle develop more muscle fibers than do cattle of normal conformation, and it is this hyperplastic growth that leads to the gross muscular hypertrophy. This cellular hyperplasia is pronounced in the fetal stage of growth in double-muscled cattle, with cell number increasing at a rate nearly three times that of normal cattle (Swatland and Kieffer, 1974). This hyperplastic growth is not normally accompanied by cellular hypertrophy (Swatland and Kieffer, 1974; Gerrard and Judge, 1993).

There are also variations in the ratios of different muscle cell types. Microscopic analysis of the semitendinosis muscle of Belgian Blue cattle has shown that double-muscled cattle develop approximately twice as many cells as normal cattle, and that this aggregate cell count contains far more of the smaller type IIB cells than found in normal counterparts (Wegner et al., 2000). The hypertrophy is specific to muscle tissue, and is observed in skeletal muscles throughout the entire body (Swatland and Kieffer, 1974; Lee and McPherron, 1999). The muscle of double-muscled cattle also contains less connective tissue, which also contributes to its tenderness (Dumont and Schmitt, 1973; Bailey et al., 1982; Hanset, 1986).

As previously mentioned, the amount of fat in the carcasses of double-muscled cattle is significantly less than that observed in the carcasses of conventional cattle. Amounts of intramuscular fat, or marbling, are particularly affected by the double-muscled condition (Hanset, 1982; Thiessen and Rollins, 1982; Hanset, 1991; Webb et al., 1998; Hocquette et al., 1999; De Smet et al., 2000). This lack of marbling contributes to the lower quality grade that often classifies double-muscled carcasses as inferior (Thiessen and Rollins, 1982). Adipocytes comprising the subcutaneous and internal fatty tissues of double-muscled cattle seem to be smaller than in conventional cattle, although adipocyte size within the intramuscular fat appeared to be similar between both types (Hocquette et al., 1999).

The significance for producers and consumers is that double-muscled animals produce a higher proportion of lean meat than conventional cattle types. The meat of double-muscled animals is generally much lower in fat, and what fat does remain is higher in the polyunsaturated varieties, both of which more closely conform to current nutritional guidelines (Webb et al., 1998).

## GENETIC BASIS OF THE DOUBLE-MUSCLED PHENOTYPE

The genetic origin of double-muscling in cattle was never truly in doubt. By the end of the 1980's, the most favored theory was a single, autosomal recessive pattern. This idea was supported by long-standing observations of heterozygous "carrier" animals, which often displayed growth characteristics between those of the normal and knockout homozygotes (Kieffer and Cartwright, 1980; Baker and Lunt, 1990).

In 1995, the research of Charlier et al. (1995) localized the mh locus to bovine chromosome 2, yielding strong evidence to support the idea that the mh locus described the single, autosomal, major gene underlying the double-muscled phenotype. This claim of simple monofactorial Mendellian segregation was verified by an F1 backcross of presumed hemizygous cattle to known homozygous knockouts (mh+/- x mh-/-), which yielded a 1:1 ratio of double-muscled to normal offspring. Although upon first evaluation the researchers found no evidence to support linking the mh locus to any particular autosome, they constructed a marker map of bovine chromosome 2, and analyzed the relative rates of recombination between markers. The results of this experiment indicated that the marker that had the lowest incidence of recombination with mh--and therefore the shortest physical distance--was indeed on chromosome 2, at the centromeric end of the BTA2 linkage group.

The year 1997 marked the world's first formal introduction to the debutante growth and development factor named "myostatin", by Johns Hopkins researchers Se Jin Lee and Alexandra McPherron. While looking for possible cousins of the well-known Transforming Growth Factor-alpha (TGF-alpha) superfamily of growth factors, the team discovered a novel gene that closely resembled previously investigated members of the family and crossed species barriers with a high degree of fidelity (McPherron et al., 1997; McPherron and Lee, 1997). Subsequent targeted mutation of the gene in mice resulted in an animal that showed runaway muscle development: dramatic, muscle-specific, and altogether like the condition observed for nearly two centuries in double-muscled cattle.

Soon after, independent researchers established that the novel protein did indeed map to the mh locus (Smith et al., 1997), and showed via the first successful bovine positional cloning experiment that defects in myostatin were responsible for the double-muscled phenotype in both Belgian Blue and Asturiana de los Valles cattle (Grobet et al., 1997; Vaiman, 1999). A flurry of other research followed, yielding more evidence to support the link between myostatin and muscle hypertrophy. Casas et al. (1998) documented the "partial recessive character" of myostatin in cattle, submitting that hemizygous (mh +/-) animals in that study had a muscle mass 1.6 standard deviations higher than homozygous normal (mh +/+) animals.

DNA sequencing of the myostatin gene in other breeds known to produce a large number of double-muscled animals showed that several mutations were capable of inducing the double-muscled phenotype. In addition to the 11 base pair deletion at bovine myostatin nucleotide 821 (nt821(del11)) found in double-muscled Belgian Blue and Asturiana de los Valles (Grobet et al., 1997; McPherron and Lee, 1997) and the G to A transition at nucleotide 938, which causes a cysteine to tyrosine shift in the mature Piedmontese myostatin protein (Grobet et al., 1997; Kambadur et al., 1997; McPherron and Lee, 1997), other polymorphisms can disrupt myostatin function. Grobet et al. (1998) pointed out seven different possible mutations in the coding region of the myostatin gene, five of which were likely to have severe effects on myostatin function. These results also suggested a hypothetical model for the evolution of myostatin haplotypes among beef cattle.

Isolating the myostatin gene and protein and definitively linking mutant myostatin to the double-

muscled phenotype had a broader effect than simply characterizing a cause of muscle cell hyperplasia. It allowed researchers to put away the nearly two-century long subjective selection of subjects and the corresponding mixture of genotypes that previously made up studies of double-muscled beef cattle. Instead of the physical observation once used, it became possible to directly genotype individuals, differentiating both those individuals who carry one copy of dysfunctional myostatin, and those "homozygous" individuals that receive two different varieties of dysfunctional myostatin gene (Casas et al., 1998; Casas et al., 1999). This knowledge is of particular importance as researchers move from breeding programs that employ simple selection of the double muscled phenotype to inducing functional changes in the myostatin genes of other species. Biochemically speaking, structure is function, so identifying these differences between species, breeds, and types allows us to better understand how small changes in the molecular structure of a protein can radically alter its effects.

## STRUCTURAL CHARACTERISTICS

Members of the TGF-alpha superfamily of signaling cytokines have characteristic sequence and structural patterns that dictate their function (Piek et al., 1999). Their physiological duties run the gamut of cell growth regulation from directing the differentiation of neural tissue to inducing growth of mesenchymal cells (Piek et al., 1999), but most relevant to this discussion are those family members that act as highly specific, highly potent inhibitors of cell proliferation. Myostatin is dissimilar enough, especially in the C-terminal region, to defy classification into any of the major TGF-alpha subfamilies, such as inhibins, TGF-alpha, and bone morphogenic proteins (McPherron et al., 1997); however, it shares several characteristics in common with other members of the superfamily. The TGF-alpha superfamily sequences encode a secretion signal sequence, proteolytic processing site, and a conserved pattern of cysteine residues in the C-terminal end, and are highly conserved across species (McPherron et al., 1997).

The TGF-alpha superfamily peptides are characterized by a conserved pattern of nine cysteine residues in the carboxy-terminal end (McPherron and Lee, 1997). In other TGF-alpha family members, similar cysteine patterns in the mature proteins form intramolecular and intermolecular disulfide bridges within the biologically active pocket of the homodimeric complex, the so-called "cysteine knot" structure (Thomas et al., 2000). This highly conserved pattern is one of the factors that originally led the Johns Hopkins researchers to myostatin (McPherron and Lee, 1997). One of the nullifying mutations, C313Y, which changes the fifth of the nine cysteine residues in mature myostatin to tyrosine, causes the functional loss seen in the Piedmontese breed (Grobet et al., 1998). The striking effects of this one-residue alteration give some indication of the structural importance of this pattern in the mature protein function. The amino acid sequence that targets the mature protein for secretion consists of a core of hydrophobic amino acids close to the N-terminal end, and the Arg-Ser-Arg-Arg (RSRR) proteolytic processing signal is located close to the protein's C-terminal end (Thomas et al., 2000).

The active TGF-alpha family member myostatin is a 26 kDa homodimeric protein expressed specifically in the myotome layer of developing somites during embryogenesis, and later in all skeletal muscles (McPherron and Lee, 1997; Lee and McPherron, 1999). Recent evidence suggests that, like TGF-alpha, myostatin exists as a large, latent complex with other proteins, including its propeptide (Lee and McPherron, 2001). In vitro, the propeptide blocked myostatin binding to activin type IIB receptors, and in vivo, increased expression of the propeptide in transgenic mice resulted in increased muscle mass, due to both increased muscle fiber number and size; thus, the propeptide acts as a myostatin inhibitor (Lee and McPherron, 2001).

The actual amino acid sequence of myostatin has a strikingly high degree of conservation across species boundaries, considering that the protein is patently not necessary for viability. According to

McPherron and Lee (1997) and Grobet et al. (1997), in the 1,128 base pair overlapping region of the murine and bovine coding sequence, there is an 89.1% incidence of matching, while the predicted protein shows a 92.5% identity between the two species.

Mutations that disrupt the bioactive carboxy-terminal region of the myostatin gene or the structure of the cysteine knot can lead to functional knockouts, and the accumulation of two copies of dysfunctional myostatin leads to the extreme double-muscled phenotype. A survey of myostatin polymorphisms carried out by Grobet et al. (1998) revealed a number of different ways to abrogate myostatin's effect in cattle by structurally reorganizing the gene.

Lee and McPherron (2001) recently demonstrated that activin type II receptors are involved in myostatin signaling. In vitro, myostatin binding to Act RIIB receptors was specific and saturable, and transgenic mice with increased muscle expression of a dominant negative form of Act RIIB had increased muscle weights. As noted above, Lee and McPherron (2001) showed that the myostatin propeptide inhibited binding of myostatin to Act RIIB receptors and blocked its inhibitory action on muscle growth in vivo. They also investigated another potential myostatin inhibitor, follistatin, which has been shown to inhibit the activity of other TGF-alpha family members (Gamer et al., 1999). Mice expressing increased levels of follistatin in muscle had dramatic increases in muscle weight, caused by both hyperplasia and hypertrophy; thus, follistatin appears to be a potent myostatin antagonist (Lee and McPherron, 2001).

## PHYSIOLOGICAL ACTIONS OF MYOSTATIN

Myostatin has been identified as a circulating factor, secreted by muscle cells and acting upon those cells to inhibit growth (McPherron et al., 1997; McPherron and Lee, 1997; Gonzalez-Cadavid et al., 1998). Previous work showed that serum from double-muscled fetuses failed to inhibit the replication of myoblasts in vitro. This was first interpreted as a case of double-muscled fetal serum stimulating growth (Gerrard and Judge, 1993). Later research, however, identified the causal agent of double-muscling as a blood-borne factor that was incapable of inhibiting muscle cell proliferation (Gerrard et al., 1995).

Based on what is known about myostatin function, it is possible to consider several different aspects of its in vivo role. First, it can be considered a limiting factor in normal muscle development. Given its pattern of expression: highly expressed in embryonic and fetal stages and expressed to a lesser degree in adult muscle tissue, it can be primarily viewed as a growth regulator in early development (McPherron et al., 1997). In spite of this variation of expression during phases of development, myostatin is expressed in postnatal muscle tissue, and has been shown to affect adult tissue as well (Thomas et al., 2000). Over-expression of myostatin has been linked to muscle wasting, such as that seen in individuals infected with HIV, limiting growth or regrowth in adult humans (Gonzalez-Cadavid et al., 1998). Upregulation of myostatin gene expression has also been associated with glucocorticoid-induced muscle atrophy (Ma et al., 2001). Myostatin expression patterns have been shown to change with physiological state, becoming upregulated in cardiomyocytes after heart damage (Sharma et al., 1999), and downregulated in regenerating muscle (Sakuma et al., 2000).

In this way, myostatin, like leptin, can be described as a route of continual communication between individual tissues and the organism as a whole, a "chalone" (McPherron and Lee, 1997; Slack, 1997), helping to report the status of tissue and maintain a global balance in tissue growth. Both of these approaches to understanding myostatin's function are supported by an exploration of its mechanism of action in muscle and other tissues. The inhibitory effects of myostatin have been shown to be reversible in vitro (Thomas et al., 2000), which tends to support the notion of myostatin as a reporter and regulator whose effects can be modulated with changes in muscle tissue size and cell number.

Like all tissues of the body, the specialized cells that make up muscle begin as undifferentiated precursor cells or stem cells that commit to the mesoderm. Mesodermal progenitor cells then undergo modulation by growth factors at the determination and differentiation stages, which collectively assign the final identity of the cells (Kelvin et al., 1989). Members of the TGF-alpha superfamily all act as positive and/or negative regulators of points in the pathway that sculpt a myocyte from an undifferentiated stem cell, and they operate by way of tissue specific cell-surface receptors (Kelvin et al., 1989). TGF-alpha regulates at the terminal differentiation level, blocking the differentiation of myoblasts (Kelvin et al., 1989).

Myoblasts proliferate during myogenesis, then withdraw at G1 of the cell cycle and commit to form myotubes. Progression through the cell cycle, and cell cycle arrest, are often controlled by cyclin-dependent kinase and cyclin-dependent kinase inhibitor (CDK/CKI) complexes. Myostatin is thought to control the G1 to S and G2 to M transitions of the cell cycle for myoblasts through modulation of p21cip1 and Cdk2 protein levels (Thomas et al., 2000). Myostatin upregulates expression of several genes involved in proliferation and differentiation of skeletal muscle cells, including p21cip1 (a CKI) (Thomas et al., 2000; Rios et al., 2001; Taylor et al., 2001), and downregulates expression of Cdk2, inactivating the Cyclin/CDK complex that allows progression from G1 to S (Thomas et al., 2000). Also a key factor is P21cip1 in the survival of myocytes, strongly inhibiting myocyte apoptosis (Rios et al., 2001). Another cyclin-dependent kinase inhibitor, P27kip, also plays a role in myostatin's regulation of muscle growth. P27kip knockout mice exhibit growth enhancement characterized by hyperplasia in almost every organ. Muscle tissue from these mice had decreased myostatin mRNA (Lin et al., 2001). Over-expression of normal myostatin, therefore, induces cell cycle arrest at the G1 stage and termination of proliferation, and increases the survival of differentiating myocytes. A functional myostatin knockout removes both the inhibition and the resistance to apoptosis, which may have some effect on the final structure of the muscle tissue: increased size due to hyperplastic growth of small myotubes (Kelvin et al., 1989) (Figure 1).

Myostatin specifically affects muscle cells. However, it is expressed in other tissues and may carry out cell cycle control functions in these tissues as well. For example, although myostatin is expressed only at low levels in adipocytes (McPherron et al., 1997), it has been shown to inhibit the differentiation of preadipocytes into adipocytes, probably by inhibition of transcription factors (Kim et al., 2001). Myostatin can thus be said to have a direct effect on adipogenesis, in addition to its well-described indirect effects that result from radically changing the ratio of muscle to adipose tissue.

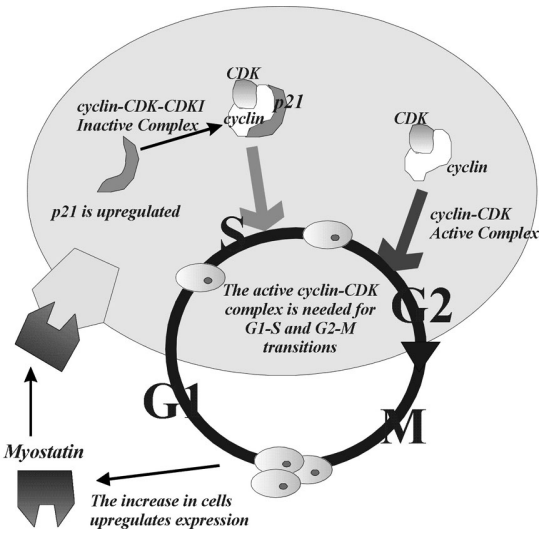

Figure 1. Proposed Myostatin Mechanism

## FUTURE DIRECTIONS

Month by month, new research has increased our understanding of the mechanism of myostatin function and its specific roles in the development of diverse species. Two hundred years' worth of breeding for the double-muscled trait in cattle is some indication of our general willingness to exploit a condition without full understanding. However, with new information about the specific effects of a myostatin knockout in model organisms such as the mouse, it becomes possible to explore this pathway in an economical and time-efficient way.

It seems logical that the same technological revolution that uncovered the basis for double-muscling in cattle should have a hand in alleviating the associated concerns and providing new ways to exploit the trait. New understanding of the role of myostatin gene expression in growth and development, along with research into the structural and functional characteristics of the myostatin protein, has offered researchers several potential methods to manipulate the pathway. Altering the time of expression, for instance, may provide a way to circumvent some of the problems associated with dystocia survival at parturition and in newborn calves. With the advent of transgenic technology, it may also be possible to induce the mutation in livestock animals other than the bovine species, such as pigs, which do not suffer the same difficulties with large birth weights. The myostatin gene is highly conserved across species, and there is evidence to suggest that its function in muscle growth regulation is similarly conserved. Changes in myostatin function induced either by targeted mutation of the gene or pharmacological or immunological targeting of the myostatin pathway have already produced comparable growth and body composition alterations in mice, chickens, and sheep.

The high conservation of myostatin function across livestock species, along with the inability to easily differentiate hemizygous and normal animals, may also mean that endogenous myostatin mutations already exist, undetected, within breeding herds today. Identification of myostatin polymorphisms that can interrupt function opens the door for widespread screening of possible carrier animals and breeding strategies that can take advantage of the useful nature of a myostatin knockout while selecting against undesirable companion traits. Plans already underway to screen large numbers of various livestock breeds and species will help producers to identify the mutation within their own herds and develop a breeding strategy to maximize its potential.

Along with the direct applications of such functional myostatin knockouts among livestock animals, the creation of myostatin knockout mice by homologous recombination has provided an invaluable model animal for the further study of the biological pathway myostatin follows. Corresponding function of the human myostatin protein suggests that murine models with altered myostatin expression may be of value in biomedical research. Identification of the human myostatin gene and analysis of its expression patterns has indicated that myostatin may play a role in certain diseases characterized by cachexia or muscle wasting, including AIDS (Gonzalez-Cadavid et al., 1998).

Increased knowledge of expression patterns and function mechanisms of myostatin have already hinted at some possible methods to circumvent the deleterious effects of a full myostatin knockout. The work of Lee and McPherron (2001) on agents that block myostatin receptor binding and action may offer a way to "turn off" myostatin function in adult animals, bypassing the problems for gestating, fetal, and neonatal cattle. This method may have a place in human medicine as well, offering a way to fight AIDS-related or cancer-related forms of cachexia.

## CONCLUSION

Increasing understanding of cell-cycle control mechanisms, along with these varied approaches to exploiting myostatin mutations and blocking myostatin action may represent a significant gain for several industries. The two century-long drive to explore myostatin has been a study in tenacity for the livestock industry in particular, embodying the best outcome of perseverance and creative problem solving. Now, new understanding and new technology have shown that this mechanism can be extremely valuable in the pursuit of muscle-specific growth control.

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