

Myostatin and the control of skeletal muscle mass

Commentary

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The mechanisms by which tissue size is controlled are poorly understood. Over 30 years ago, Bullough proposed the existence of chalone, which act as tissue-specific negative growth regulators. The recent discovery of myostatin suggests that negative regulation of tissue growth may be an important mechanism for controlling skeletal muscle mass and raises the possibility that growth inhibitors may also be involved in regulating the size of other tissues.

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Abbreviations

Gdf11 growth/differentiation factor 11

TGF- β transforming growth factor- β

Introduction

In recent years, significant progress has been made in terms of identifying many of the molecules and mechanisms involved in specifying the development of individual organs and tissues. Despite this progress, however, one aspect of development and tissue homeostasis about which very little is understood is how individual tissues reach and then maintain their appropriate size. It is clear that specific regulatory mechanisms must exist for specifying tissue size because not only is the size of a given organ or tissue remarkably consistent among individual animals but tissue size is also generally maintained relative to the overall size of the animal.

The best evidence for the existence of specific regulatory mechanisms comes from studies of tissues or organs that are capable of regenerating after tissue loss or tissue injury. In mammals, the tissue that has been the best characterized in this respect and the tissue that has been shown to have one of the greatest capacities to regenerate is the liver. In fact, the remarkable ability of the liver to regenerate was described as early as ancient Greek mythology in the story of Prometheus. As the story goes, Prometheus stole fire from the Gods, and, as punishment, Zeus ordered him to be chained to a rock where each morning an eagle would descend and devour a portion of his liver. According to the story, each night, his liver grew back to replenish the portion that had been devoured.

In modern times, the ability of the liver to regenerate following tissue loss has been most extensively studied in the partial hepatectomy model in rodents. Following removal of any portion of the liver, the cells in the liver remnant

re-enter the cell cycle and continue to restore liver mass until the original liver size has been reached, at which point the hepatocytes stop proliferating [1]. How this process is controlled has been the subject of extensive study for many years. Perhaps the most fundamental question that relates to the control of tissue size is how the animal senses at any given time how much liver mass it has. That is, immediately following the surgical procedure, how does the animal 'know' that it is missing a portion of its liver mass and that it is time to start the regeneration process? And how does the animal 'know' when its liver mass has been restored in order to stop this process?

The chalone hypothesis

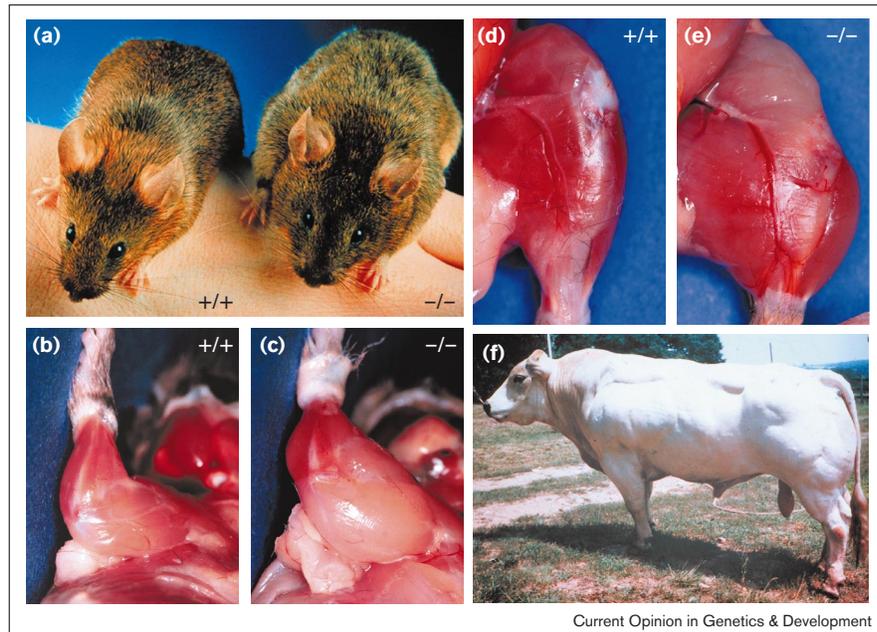
A variety of hypotheses have been put forth in an attempt to explain this phenomenon, many of which include a role for negative growth regulators [2]. Over 30 years ago, Bullough [3,4] proposed the existence of molecules that he called 'chalones', a term coined by Schäfer [5] for secreted molecules that inhibit cellular functions. Bullough suggested that the size of individual tissues is controlled independently by the activities of specific chalones that are produced by a given tissue and that act to inhibit its growth. The general idea was that the local and/or circulating concentrations of a specific chalone would be a direct reflection of the total mass of the tissue in which it is produced.

In the case of the liver, for example, the hypothesis was that liver cells produce an inhibitory molecule that circulates systemically and that acts to block liver cell proliferation. When an animal loses part of its liver, the regeneration process begins as a result of a drop in the circulating levels of the liver chalone below that required to maintain the hepatocytes in growth arrest. As regeneration proceeds, the chalone levels continue to increase until liver mass has been fully restored. At that point, the chalone concentration has reached its original inhibitory level, and further liver growth is blocked.

Early experiments seemed to provide at least some experimental support for the existence of chalone-like molecules [2–4]. For example, it was reported that the regenerative response following partial hepatectomy could be inhibited by the administration of extracts prepared from liver but not from other tissues, such as kidney. Furthermore, it was reported that regeneration could be inhibited by the administration of plasma taken from normal animals but not from animals that themselves had undergone partial hepatectomy. These data were consistent with the presence of a circulating, liver-derived inhibitory factor. Many investigators performing the same types of experiments, however, found contradictory results. Despite intensive effort in the ensuing years to isolate these inhibitory factors, the

Figure 1

Increased muscle mass in myostatin mutant animals. **(a)** Adult mice showing the altered body shape of myostatin mutant (right) as compared to wild-type (left) animals. **(b–e)** Increase in muscle size of forelimbs (b,c) and hindlimbs (d,e) of myostatin mutant (c,e) as compared to wild-type (b,d) mice. **(f)** A Belgian Blue bull homozygous for a myostatin mutation (courtesy of C Pennington, D Garrels).



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identification of molecules having all of the properties predicted for a chalone for any tissue remained elusive, and the chalone theory fell out of favor.

Myostatin

We have recently identified a negative regulator of skeletal muscle growth [6], myostatin, that we believe may provoke a new consideration of the chalone hypothesis. Myostatin is a member of the transforming growth factor- β (TGF- β) superfamily of secreted growth and differentiation factors. During mouse embryogenesis, myostatin is initially expressed specifically in the myotome layer of developing somites which gives rise to skeletal muscle. At later stages of development and in adult animals, myostatin is expressed at varying levels in all skeletal muscles throughout the body but at undetectable levels in nearly all other tissues. Adult mice carrying a targeted disruption of the myostatin gene are 25–30% heavier than their wild-type littermates and have pronounced shoulders and hips (Figure 1a). This increase in body weight is caused by a dramatic increase specifically in skeletal muscle mass throughout the animal which is evident upon removal of the skin from adult knockout animals (Figure 1b–e). Individual muscles of mutant mice weigh 2–3 times that of wild-type mice resulting from a combination of an increase in muscle-fiber number and an increase in fiber diameter.

Remarkably, for a gene that is not essential for viability or reproduction, the myostatin gene has been highly conserved through evolution; in fact, the predicted human, rat, mouse, porcine, chicken, and turkey proteins are identical in the biologically active carboxy-terminal region [7]. Myostatin is also expressed in developing and adult skeletal muscle in these and other species [7–10], and, on the

basis of the analysis of double-muscling cattle breeds that have significantly larger skeletal muscles than conventional meat breeds, it appears that myostatin has the same function in cattle as in mice. Five different mutations in the bovine myostatin gene that are predicted to either delete or nearly eliminate myostatin function have been found in these double-muscling cattle breeds, probably accounting for all true double-muscling in cattle (Figure 1f) [7–9,11]. Whether myostatin regulates muscle mass in humans is not known but myostatin immunoreactive material has been detected in human serum, and increased levels appear to correlate with the presence of cachexia in HIV-infected men [12].

Should myostatin be considered a chalone? As would be predicted for a muscle chalone, myostatin is a secreted protein that is synthesized specifically by skeletal muscle cells and loss of myostatin function leads to a dramatic and specific increase in skeletal muscle mass. However, in order for myostatin to be labelled a chalone as envisioned by Bullough, a number of key questions regarding its mechanism of action need to be answered.

First, is myostatin responsible for inhibiting the growth of skeletal-muscle in fully developed animals? It is possible that the increased muscle mass seen in the knock-out mice results entirely from the lack of myostatin activity during embryonic development. It will be essential to demonstrate that loss of myostatin activity during adult life can cause growth of muscle tissue.

Second, is the effect of myostatin dose-dependent? A key prediction of Bullough's model is that because chalone levels are a direct reflection of tissue mass, artificially

changing chalone levels should lead to changes in tissue mass in a dose-dependent manner. Assuming that myostatin protein levels are lower in heterozygous mice than in wild-type mice, one would predict that muscle weights in heterozygous mice are intermediate between those of wild-type and homozygous mice. Conversely, it will be important to show that overexpression of myostatin or exogenous administration of myostatin protein can cause loss of muscle mass in a dose-dependent manner.

Third, does myostatin act directly on muscle tissue? As envisioned by Bullough, chalone levels are sensed by the same tissue from which they are secreted, implying that if myostatin is a muscle chalone, receptors for myostatin should be present in muscle cells. Although the hormone leptin is made specifically by adipose cells and ultimately regulates adipose tissue mass, leptin would not be considered a chalone as Bullough envisioned because leptin acts indirectly on adipose tissue through its action in the central nervous system to regulate energy expenditure and food intake [13].

Fourth, does myostatin act locally or systemically to regulate muscle mass? The simplest model would be that a liver chalone, for example, functions systemically. As discussed by Bullough, however, one could also imagine that the circulation merely acts as a sink to deplete local concentrations of the chalone. If the myostatin-immunoreactive material that was detected by Gonzalez-Cadavid *et al.* in serum [12] can be shown to correspond to biologically active myostatin — or at least a form of myostatin that is capable of being activated — their data would suggest that myostatin does indeed circulate *in vivo*.

Growth/differentiation factor 11

One piece of evidence that complicates models involving the systemic action of myostatin is the existence of a highly related gene, *growth/differentiation factor 11* (*Gdf11*). GDF11 shares 90% identity with myostatin at the amino acid level in the biologically active carboxy-terminal region of the molecule and is also expressed in skeletal muscle ([14–16]; A McPherron, S-J Lee, unpublished observations). Unlike myostatin, however, *Gdf11* is widely expressed in mice during development and adulthood in tissues such as the eye, nasal epithelium, spinal cord, and various regions of the brain. The earliest expression seen is in the late primitive streak and tailbud regions in the developing embryo.

Although the function of GDF11 in skeletal muscle is unknown, it is clear from the analysis of *Gdf11* knockout mice that GDF11 is involved in the patterning of other tissues [16]. The most obvious abnormality in *Gdf11* mutant mice is the presence of extensive homeotic transformations of the axial skeleton in which individual vertebrae have morphological characteristics typical of more anterior segments. *Gdf11* mutant mice also have other abnormalities, including palate defects and extensive renal anomalies.

The fact that myostatin and GDF11 proteins are highly related in the active portion of the molecules raises the possibility that the two molecules may share a common receptor or that each of these molecules is capable of binding and activating the other ligand's receptor. Although it would seem to be difficult to reconcile the existence of a common receptor with mechanisms involving systemic actions of either molecule, it is important to keep in mind that regulation of the activities of TGF- β family members is known to occur at many levels. For example, it is known in the case of TGF- β that the mature carboxy-terminal dimer that is responsible for biological activity is normally held in an inactive form in a tight complex with the pro-region of the molecule along with at least one other protein [17]. The mechanisms by which the active molecule is released from this latent complex are not fully understood but it is known that a variety of different treatments, such as exposure to acid, chaotropic agents, or plasmin, as well as interactions with certain proteins, such as thrombospondin and integrin $\alpha v \beta 6$, can cause activation of the latent complex *in vitro* [17,18]. If myostatin also exists in a latent complex, one could postulate that specificity might be achieved by the presence of an activation mechanism that is restricted to skeletal muscle.

It is also known that the activities of TGF- β family members are regulated by other binding proteins besides the pro-peptide. The list of these binding proteins continues to expand and presently includes follistatin, noggin, chordin, and members of the DAN family [19–23]. In all of these cases, the binding protein is known to inhibit the biological activity of the TGF- β family member. In fact, it has already been demonstrated that follistatin can block the ability of GDF11 to induce mesoderm formation in the *Xenopus* animal cap assay [14]. Whether follistatin inhibits GDF11 activity *in vivo* is not known but it is intriguing to note that, like *Gdf11*, follistatin is expressed in the primitive streak region during early embryogenesis [24] and that one of the phenotypes of follistatin knockout mice is the loss of a lumbar vertebra and, in most cases, the loss of a pair of ribs [25]. This phenotype could be explained as posteriorly directed homeotic transformations of vertebral segments, which is what one might predict for overactivity of GDF11. On the basis of the high degree of homology between GDF11 and myostatin, it is certainly possible (though not yet reported) that follistatin may also inhibit myostatin. In this regard, it is also intriguing to note that follistatin is expressed in developing somites [24] and that another phenotype of follistatin knockout mice is atrophy of the intercostal muscles and diaphragm at birth [25], which is what one might predict for overactivity of myostatin. If the biological activity of myostatin is regulated by follistatin or other binding proteins *in vivo*, it is not difficult to imagine how specificity might be achieved by the absence of such inhibitory molecules in skeletal muscle. Alternatively, given that the activities of the binding proteins themselves can be regulated by specific proteases [26–28], one could postulate that follistatin or another

inhibitory binding protein is present in many tissues but that the protease that cleaves the binding protein is present only in skeletal muscle.

Hence, given the complexity of the layers of regulation that have evolved for TGF- β family members, the existence of GDF11 — even if it shares a common receptor with myostatin — does not necessarily rule out a possible systemic role for myostatin. Perhaps the most definitive studies will be the analysis of parabiosis experiments in which the circulatory systems of wild-type and myostatin knockout mice have been joined.

Conclusion

Clearly, many questions regarding the mechanism of action of myostatin remain unanswered. Nevertheless, the phenotype of myostatin-deficient animals raises the possibility that myostatin may be one of the long-sought tissue-specific growth inhibitors that were hypothesized to exist over 30 years ago. Whether or not myostatin is demonstrated to function in exactly the manner proposed by Bullough, the discovery of myostatin should lead to fundamental insights into how skeletal muscle mass is regulated and may also reinvigorate the search for negative growth regulators of other tissues.

Acknowledgements

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