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Skeletal Muscle and Regulation of Tissue Size

The amazing developmental path from a single cell to a complete human body involves multiple cytokine-coordinated events that affect not only the eventual size and shape of the body, but also its capacity to move and respond to challenges. The physical and mechanical characteristics of the body are influenced to a large extent by the musculoskeletal system, which consists of 206 bones connected via tendons to more than 400 muscles. Each of these skeletal muscles is voluntary and can be consciously controlled to exert specific forces or movements. Moreover, with the appropriate amount of training multiple skeletal muscles attached to various parts of the skeleton can be controlled simultaneously in order to accomplish complex tasks such as the perfect golf swing.

The human body contains an estimated 85 trillion cells, of which only a tiny fraction (about 0.0003%) is found in skeletal muscles. Yet, skeletal muscle tissue can comprise up to 50% of body mass. It is a highly organized tissue consisting of extremely large cells surrounded by uniquely structured vascularized connective tissues. Muscle cells, called fibers, are highly specialized to respond rapidly to nerve signals by generating pulling power, a function that demands intensive cell maintenance, which is performed by the large number of peripheral nuclei (up to several hundreds per cell). The plasma membrane of the cell, called sarcolemma, is specialized to respond to and conduct neuromuscular signals. The interior of the cell is packed with hundreds to thousands of slender myofibrils that run in parallel from one end of the cell to the other. They are made of the principal contractile proteins of the cell, myosin and actin, which are associated with cytoskeletal proteins. Each myofibril is composed of a large number of sarcomeres linked end to end by Z-discs. The sacromere, which is the basic functional unit of muscle contraction, consists of a centrally located array of thick filaments that interdigitate with thin filaments anchored to Z-discs (Figure 1). Thick filaments are made of myosin aggregates symmetrically organized around a single core molecule of the elastic protein titin. Titin, a muscle-specific giant protein of 3000 kDa, stabilizes the thick filament within the center of the sacromere by connecting the ends of the filament with the Z-discs (Figure 1). The thin filaments are predominantly composed of actin polymers arranged along the inflexible protein nebulin (700-900 kDa), which renders the filaments inelastic. The force-generating capacity of muscle fiber is proportional to the number of its contractile sarcomeres. The external expression of the force is accomplished by a unique cytoskeleton that acts as a force-transmitting apparatus. Longitudinal and transverse elements of the cytoskeleton are arranged in precise geometry with respect to individual sarcomeres, creating an elaborate scaffold for the contractile proteins.

**Figure 1-** Schematic Illustration of a Sarcomere
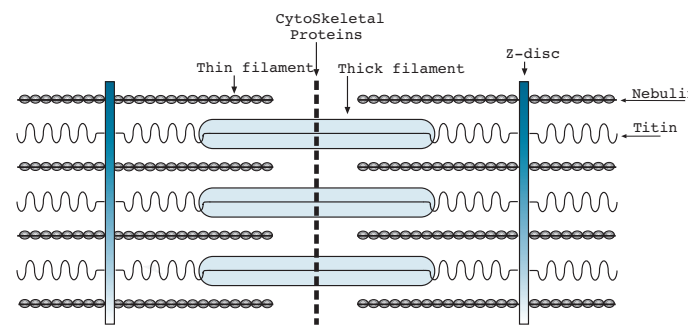
Muscle contraction occurs as a consequence of specific Ca^{2+}- dependent, ATP-driven interactions between myosin and actin, which lead to reciprocating sarcomere shortening/relaxation cycles. These myosin-actin interactions, termed cross-bridge interactions represent the principal muscular process in which chemical energy, stored in ATP molecules, is converted into mechanical energy. Unlike actin, which is expressed in many cell types, myosin is uniquely expressed in muscle cells and accounts for about 65% of the total cell protein. It is a 500 kDa protein, which by hydrolyzing ATP provides the energy necessary for muscle contraction. The protein exists in two distinct isoforms, called slow myosin heavy chains (MyHC) and fast MyHC. The two isoforms share a similar subunit structure, composed of two heavy chains and two pairs of light chains, but differ in the rate of their ATPase activity. In general, muscle fibers express either slow MyHC or fast MyHC, and are accordingly called slow or fast fibers. Slow and

fast fibers generate the energy necessary for ATP synthesis from oxidation of either free fatty acids or glucose, respectively. Slow fibers contract slowly, generate sustainable low force, and are mainly used for posture maintenance. In contrast, fast fibers contract rapidly, generate stronger force, but tend to fatigue faster. Individual myosin molecules are rod-shaped and consist of a 1500 Å tail attached flexibly to two identical globular heads. The head regions of myosin extend outward from the thick filaments, and are called cross-bridges. Each cross-bridge contains a binding site for actin, and a site capable of binding and hydrolyzing ATP (Figure 2A). Initially, the products of this hydrolysis, ADP and inorganic phosphate (Pi), remain attached to the site, and the released energy is harnessed to induce a conformational change in the cross-bridge. In this constellation the minimal free-energy state is achieved through a relaxed cross-bridge attachment to actin. Actin is a 43 kDa globular protein which polymerizes in the cytoplasm to form twisted two-stranded thin filaments. Associated with actin filaments are two additional proteins, troponin and tropomyosin, which in the absence of Ca^{2+}, act as a complex to block access of actin to its myosin binding site. Binding of calcium ions to troponin alters the position of the complex on the thin filament, allowing myosin to interact with actin. Once this blocking is removed the myosin cross-bridge binds to the adjacent thin filament in a 90° orientation (Figure 2B). As ADP and Pi dissociate from the cross-bridge (the rate limiting step of the process), the cross-bridge moves to a 45° orientation with respect to the thin filament (Figure 2C). This movement generates a force on the thin filament, which pulls the Z-disc and causes the sarcomere to shorten. At the end of its movement the cross-bridge binds to a new ATP molecule and detaches from the thin filament (Figure 2A), ready for a new cycle of attachment. Cross-bridge activation continues for as long as the blockage of the troponin-tropomyosin is inhibited by calcium ions. When the Ca^{2+} level decreases, the troponin-tropomyosin complex returns to its blocking position and the thin filament slides back to the resting state. The Ca^{2+} level around the contractile apparatus is controlled primarily by the sarcoplasmic reticulum (SR), an intracellular extension of the sarcolemma, which forms a network of tubules and sacks that surrounds each myofibril. The SR acts both as a pump and a

Figure 2: Schematic Illustration of Cross-Bridge Interactions

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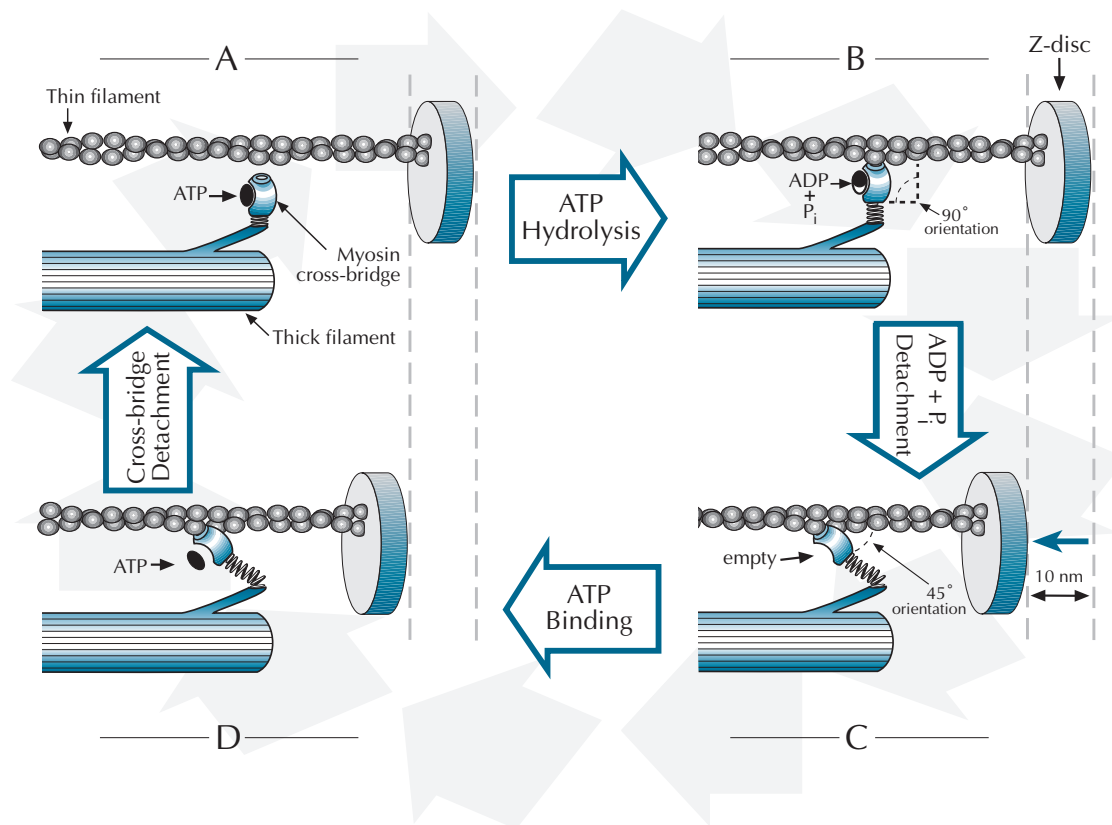
storage container for calcium ions. It responds to electrical signals by releasing calcium ions, and pumps the ions back when the signal ceases. The active, ATP-dependent pumping of calcium ions by the SR leads to dissociation of Ca^{2+} from troponin and resumption of the troponin-tropomyosin inhibition of cross-bridge interactions.

The communication between the nervous system and skeletal muscles is mediated by specialized nerve cells, called motor neurons, which divide in the muscle into fine terminal branches to innervate individual muscle fibers. A group of muscle fibers innervated by the same neuron, defined as a motor unit, represents a distinct functional unit of contraction, because all its fibers contract synchronously when the motor neuron fires. A motor unit may contain less than 10 fibers, as in extraocular muscles of the eye, or as many as 500 to 1500 muscle fibers as in the large muscles of the leg. At the neuromuscular junction, also called a synapse, the action potential (an electric signal) transmitted by the motor neuron induces presynaptic vesicles to release the neurotransmitter acetylcholine (ACh), which diffuses across the tiny gap between the nerve ending and the postsynaptic sarcolemma. At this end, ACh binds to AcCh receptors, thereby triggering an action potential that spreads over the cell, and induces release of calcium ions from the SR. The duration of ACh action is brief due to its quick inactivation by the enzyme ACh esterase. A single contraction of one muscle fiber in response to one nerve impulse is called a twitch, the force of which increases by adding more myofibrils to the cell (hypertrophy). In contrast, lengthening existing myofibrils, by adding more sarcomeres in series, increases the shortening capacity of the fiber and the velocity of the twitch. The output of whole-muscle contraction is a function of both the type of its fibers and their orientation in respect to the axis of the tendons. In general, parallel orientation maximizes the velocity, whereas an angled orientation increases the force of the contraction.

The formation of muscle fibers, or myogenesis, begins in the embryo during early muscle development and continues throughout life during muscle regeneration and repair. Embryonic myogenesis originates from pluripotent mesenchymal stem cells, whereas postnatally it is believed to be initiated by activation of unique fiber-associated cells termed satellite cells. However, recent studies have demonstrated that satellite cells are not the sole source of myogenic precursors, and that muscle-derived stem cells (1),

and bone marrow-derived cells (2), can significantly contribute to regenerating muscle. Independent of the cell type that initiates myogenesis, the continuation of the process appears to proceed along a common pathway, which includes the following cascade of events: (I) migration of myogenic precursor cells into their developing site, (II) commitment of the cells to the myogenic lineage to become myoblasts, through the expression of specific myogenic determining factors, (III) extensive myoblast cell-proliferation (IV) terminal differentiation of myoblasts into either slow MyHC or fast MyHC expressing cells, (V) fusion of postmitotic myoblasts into multinucleated myotubes, and finally, (VI) maturation of myotubes into muscle fibers. The progression of the process is coordinated by muscle-specific basic helix-loop-helix transcription factors, collectively referred to as myogenic regulatory factors (MRFs). The MRFs include MyoD and Myf5, required for the conversion of myogenic precursors to myoblasts, and myogenin and Mrf4, necessary for myoblast differentiation. In addition to determining the myogenic lineage, MyoD and Myf5 play an essential role in myoblast differentiation by inducing the expression of myogenin and other muscle-specific factors. Of particular interest is a downstream target gene of MyoD, called myostatin or growth and differentiation factor 8 (GDF-8).

Myostatin is a TGF-β family member that acts as a negative regulator of skeletal muscle growth by suppressing proliferation and differentiation of myoblasts (3, 4). Initially, it is secreted as a precursor protein composed of two identical 352 amino-acid polypeptide chains, held together by disulphide bonds. The N-terminal 243 amino-acid segments of this dimer, called propeptides, render the myostatin precursor biologically inactive by preventing access of the protein to membrane receptors. Proteolytic cleavage of these segments generates the mature form of myostatin, which exhibits biological activity only after its complete detachment from the propeptides. Prior to this detachment, the complex is referred to as a 'latency-associated protein' (LAP). It is worth noting that propeptides liberated from precursor TGF-β proteins possess high binding affinities for their mature forms, and therefore could be useful as effective antagonists for the biologically active forms. Mature myostatin is a 25 kDa protein composed of two identical 109 amino acid polypeptide chains held together by a single disulfide bond. The amino-acid sequence of mature myostatin is extremely conserved across species (identical in murine, rat, chicken, turkey,

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porcine, and human) suggesting that the protein is essential for maintaining a vital biological function. Myostatin has been shown to bind to Activin type II receptors, (ActRIIA and ActRIIB) implying that its activity can be blocked by the activin-binding protein follistatin (5). Binding of myostatin to these receptors activates a signal transduction pathway which ultimately leads to an efficient suppression of myogenesis. This pathway has been shown to include phosphorylation of the transcription factor Smad-3, which then associates with and antagonizes MyoD expression and activity (4). Genetic deletion of myostatin in mice caused a large increase in skeletal muscle mass, reminiscent of the double muscling seen in certain breeds of myostatin-mutant cattle (3,6). To examine the potential therapeutic use of myostatin antagonists in treating muscle wasting syndromes, transgenic mice overexpressing myostatin propeptide, follistatin, or a dominant-negative form of ActRIIB (using a skeletal muscle-specific promoter) were generated. These mice exhibited large increases (up to 200%) in skeletal-muscle mass similar to those observed in myostatin knockout mice (5). These results clearly demonstrate that tissue-size can be highly influenced by the action of a single growth-inhibitory cytokine.

Why aren't human beings as small as mice or as big as elephants? The exact mechanisms that coordinate growth and morphogenesis of individual tissues to produce the characteristic size and shape of the species are yet to be determined. However, a number of molecular mechanisms governing growth and homeostasis of specific tissues, as well as principal signaling molecules regulating cell growth, proliferation, differentiation, and apoptosis have already been identified. Major roles in these processes are played by various members of the TGF-β superfamily, and their natural antagonists such as noggin, chordin, and follistatin. Other important signaling molecules include a large group of fibroblast growth factors (FGFs), Hedgehog proteins, Wnt proteins, and many members of the TNF-superfamily of ligands and receptors. For a single-cell embryo to develop into a specific multi-tissue animal, the proportional size of the tissues generated during this process must be coordinated. A long-standing hypothesis in developmental biology states that during tissue growth, an inhibitor that suppresses growth has to be produced, and when its concentration reaches a certain threshold value, it causes a transient or terminal cessation of the growth (7). The first experimental evidence for this kind of autoregulation of tissue size was demonstrated in skeletal muscle, where myostatin is produced by the tissue in order to suppress its own growth. This finding paved the way to the discovery that a myostatin-homologous protein, called GDF-11, exerts a similar feedback inhibitory effect on nerve-tissue growth (8). GDF-11 has been shown to suppress neurogenesis through a myostatin-like pathway, which involves arrest of progenitor cell-cycle in the G1 phase (4, 8). The striking structural similarity between myostatin and GDF-11, which are 90% identical in their amino acid sequence, suggests that the regulatory mechanisms responsible for maintaining proper tissue size during neural and muscular development might be the same. Moreover, such mechanisms of tissue-size control may also operate in invertebrates. The recent characterization of myoglianin, a Drosophila TGF-β superfamily member homologous to myostatin/GDF-11, which is expressed in muscle, glial and neuronal progenitor cells of the fruit-fly (9), points to the possibility that the regulatory mechanisms coordinating growth and morphogenesis in vertebrate and invertebrate species are evolutionarily-conserved. The elucidation of these mechanisms together with detailed characterization of their key molecular components should expedite the development of new pharmacological agents for treating indications such as muscular wasting due to aging or AIDS, and other tissue deteriorating conditions.

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