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invited review

Form follows function: how muscle shape is regulated by work

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Russell, Brenda, Delara Motlagh, and William W. Ashley. Form follows function: how muscle shape is regulated by work. *J. Appl. Physiol.* 88: 1127–1132, 2000.—What determines the shape, size, and force output of cardiac and skeletal muscle? Chicago architect Louis Sullivan (1856–1924), father of the skyscraper, observed that "form follows function." This is as true for the structural elements of a striated muscle cell as it is for the architectural features of a building. Function is a critical evolutionary determinant, not form. To survive, the animal has evolved muscles with the capacity for dynamic responses to altered functional demand. For example, work against an increased load leads to increased mass and cross-sectional area (hypertrophy), which is directly proportional to an increased potential for force production. Thus a cell has the capacity to alter its shape as well as its volume in response to a need for altered force production. Muscle function relies primarily on an organized assembly of contractile and other sarcomeric proteins. From analysis of homogenized cells and molecular and biochemical assays, we have learned about transcription, translation, and posttranslational processes that underlie protein synthesis but still have done little in addressing the important questions of shape or regional cell growth. Skeletal muscles only grow in length as the bones grow; therefore, most studies of adult hypertrophy really only involve increased cross-sectional area. The heart chamber, however, can extend in both longitudinal and transverse directions, and cardiac cells can grow in length and width. We know little about the regulation of these directional processes that appear as a cell gets larger with hypertrophy or smaller with atrophy. This review gives a brief overview of the regulation of cell shape and the composition and aggregation of contractile proteins into filaments, the sarcomere, and myofibrils. We examine how mechanical activity regulates the turnover and exchange of contraction proteins. Finally, we suggest what kinds of experiments are needed to answer these fundamental questions about the regulation of muscle cell shape.

sarcomere; myofibril; assembly; hypertrophy; cardiac and skeletal muscle

REGULATION OF CELL SHAPE

We know that trees add new rings of growth under the bark each year as the trunk grows bigger and the tips of the branches extend as it grows taller. What does a muscle cell do as it grows? Surprisingly, we still do not have the answer to this fundamental question of how muscle grows. Autoradiography and ultrastructural studies suggest that increased cell width is accomplished by incorporation of new material throughout the entire cross-sectional area of the cell (33). Lengthwise growth of a muscle cell occurs by addition at the tips (18), presumably because the crystalline architecture would make it hard to splice a new unit of length (the sarcomere) into the middle of the existing contractile mass. However, we do not know what mechanisms regulate growth in either the circumferential or longitudinal direction.

Muscle growth and adaptation is a complex and integrative process. The cell has an arsenal of regulatory steps that can be used in response to growth

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signals. Gene transcription is followed by the processes of translation and assembly of proteins into the contractile architecture such that the function is optimized for the task at hand. The upregulation of transcription and translation of contractile genes is regulated by work; therefore, the cell size can easily be doubled (2, 12). Moreover, this doubling is accomplished while the stoichiometry of all proteins is preserved and the correct appearance of every element of the sarcomere is maintained. Remember, a muscle cell is three dimensional; therefore, a cell is able to double its volume either in width or in length (Fig. 1). Note that the addition of sarcomeres end-to-end in series makes the cell longer, whereas the addition of sarcomeres side-by-side in parallel makes the cell wider. The direction of growth is not controlled by transcription so it must be a posttranscriptional process, such as translation or assembly.

One hypothesis for controlling the site at which new sarcomeres are assembled is based on the potential for delivering the messenger RNA (mRNA) to specific cellular locations. For example, if the message could be delivered to the ends of the myocyte and translated there, then a cell would preferentially elongate. The myosin heavy chain (MHC) is 200 kDa and, like other large intracellular proteins, cannot diffuse quickly from its site of synthesis for incorporation into sarcomeres. Indeed, differential localization of sarcomeric and cytoskeletal mRNAs is seen in muscle during development and periods of rapid growth (reviewed in Refs. 25 and 26). We have studied this in skeletal and cardiac cells. Stretching a rabbit's leg makes mRNA accumulate at the tips of the elongating fibers as they grow longer. Furthermore, transport of mRNA via microtubules to its respective subcellular destination in the periphery of a cardiac cell only occurs when there is active contraction and ongoing translation (22). We found that cardiac muscle cells can rapidly assemble sarcomeres throughout the cell even when the mRNA is centrally located and microtubules are gone, suggesting that diffusional rates for the message or the protein is adequate. However, similar experiments with microtubular removal have not been done in vivo. It is possible that, in the whole muscle, the myofibrillar architecture is sufficiently dense so that microtubular transport is essential to overcome restricted diffusion. Therefore, it remains to be seen if local translation is a major regulatory mechanism for adjustment of cell shape.

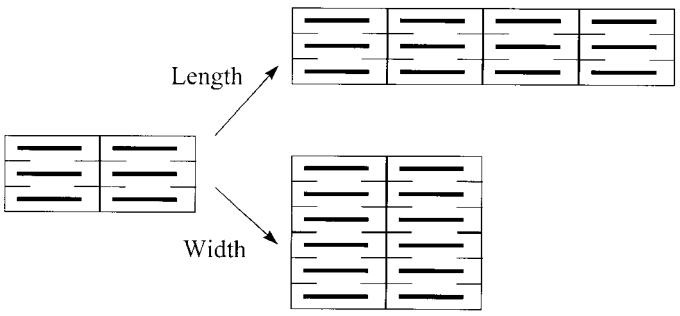

Fig. 1. A muscle cell can double its volume by increasing in either width or length. This diagram shows a cell beginning with 2 sarcomeric units of several thick and thin filaments. This cell can double in length by the addition of sarcomeres end-to-end in series, making the cell longer, or it can double in width by the addition of sarcomeres side-by-side in parallel. Direction of growth is not controlled by transcription; therefore, it must be a posttranscriptional process such as translation or assembly.

THE SARCOMERE

Cardiac and skeletal muscles are both composed of longitudinal arrays of thick and thin filaments in a repeating unit called the sarcomere. For a complete discussion of muscle structure, we refer the reader to *Gray's Anatomy* (14). According to the sliding filament theory, the thick filament protein myosin attaches to actin, a component of the thin filament, and force is developed as a result of ATP-dependent movement of the two filaments past one another. There are numerous other proteins in the sarcomere whose roles are for modulation of contraction, for maintenance of the structure, or for both. Many of these proteins do not appear in text books so we have diagrammed their location (not to scale) in Fig. 2. To complicate matters further, many of these proteins come in slightly different amino acid sequences known as isoforms. Functional variations can be achieved by combinations and amounts of various isoforms and can be readily switched by alteration of activity patterns or hormone stimulation (9, 23). We do not go into these isoform characteristics in detail here because they do not determine cell shape.

Cardiac muscle and skeletal muscle are similar in that they are both striated muscle; however, there are many important differences between the two (Table 1). Many of these differences in form arise from the significant differences in the functional requirements of the two types of muscle. The fundamental difference is that skeletal muscle is designed to do intermittent, unidirectional work against load or gravity with the force being transmitted through tendonous attachments. Cardiac muscle, however, works continuously and is designed to squeeze blood out of a chamber without the use of tendons. Furthermore, the term myofibril refers to myofilaments bundled during development or by the sarcoplasmic reticulum in the adult skeletal muscles. It is not often realized that this kind of cylindrical bundling does not occur in adult cardiac muscle, where the myofilaments are grouped into huge irregular fields named felderstrucktur by German anatomists of the nineteenth century. Interdigitating thick and thin filaments are the working units in the sarcomere; therefore, to keep the function clear, we usually refer to the term sarcomere instead of the term fibril or myofibril. Even more confusing are the terms concentric and eccentric. Concentric work is defined as the production of active tension while the muscle is shortening. Eccentric work in skeletal muscle is defined as production of active tension while the muscle is lengthening. In skeletal muscle, concentric work occurs when a weight is lifted against gravity and eccentric work occurs when a weight is lowered in a controlled fashion. The term eccentric in the cardiac literature

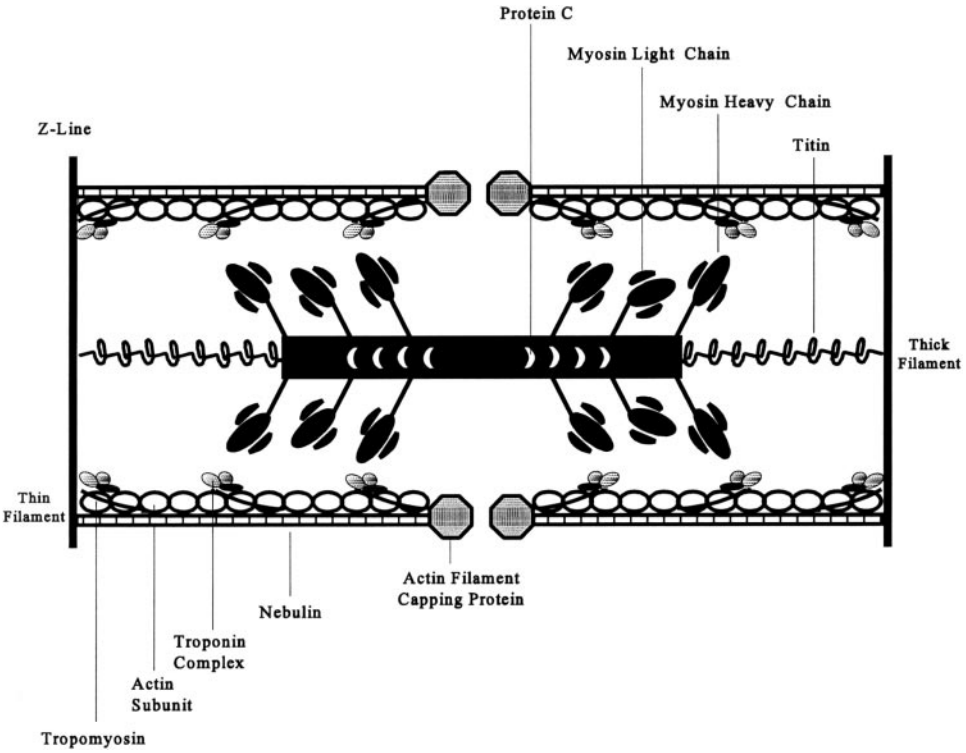

Fig. 2. Arrangement of proteins in the sarcomere. Backbone of the thin filament is actin with decoration by tropomysoin and the troponin complex. Its length is set by the long nebulin molecule (or nebulette for the heart). Turnover and growth of a thin filament may be regulated by its attachment to α-actinin at the Z band, by a capping protein at its free end, or by mechanical activity coincident with the modulation of the troponin complex (see text). Thick filament is made mainly of myosin heavy chain with its associated light chains. Its turnover and growth length may be regulated by the binding of C protein and also by mechanical activity. Central location of the thick filament in the sarcomere is determined by the long titin molecules that span from the thick filament to the Z band (see text).

arose from the anatomic position in the chest that occurs when the volume of blood returning to the heart (preload) is greater than the ejected fraction. Under these conditions, cardiac muscle must contract while being stretched by an increased volume of blood. Concentric refers to the conditions that occur when the heart must contract against a greater afterload (i.e., blood pressure). Eccentric work and concentric work are the same in both cardiac and skeletal muscle, but the results are quite different. In skeletal muscle, eccentric exercise is the most potent stimulus for functional hypertrophy, leading to bulkier and stronger muscles. In cardiac muscle, eccentric hypertrophy leads to long, thin, weak muscle cells. The anatomy of the skeletal muscle allows it to accommodate the stretching that occurs during eccentric work while maintaining functional cross bridges.

Cardiac muscle cannot accommodate significant stretch as effectively as skeletal muscle and maintain functional cross bridges. This discussion points to an important distinction. In skeletal muscle, eccentric hypertrophy is generally a physiological adaptation that leads to beneficial changes in function. In the heart, eccentric hypertrophy is a pathological change that occurs as the heart enters irreversible failure. The direction in which the heart cell grows has major clinical consequences for the mechanical output from the whole heart. In pressure overload, the heart wall thickens, and cells develop a large cross-sectional area (corresponding to concentric hypertrophy), whereas, in response to a volume overload, the heart wall becomes thin with elongated cells (corresponding to eccentric hypertrophy) (17). Thus form still follows function, but in one case the changes in form are adaptive and increase the functionality of muscle. In the latter case, however, the form changes are maladaptive and correlate with disease.

ASSEMBLY OF THE SARCOMERE AND THE MYOFIBRIL

In striated muscle, assembly of the sarcomeric proteins into highly organized sarcomeres is an ordered and complex process sometimes called sarcomerogenesis. Formation of the first fibril (myofibrillogenesis) is the process for bundling the thick and thin filaments together (1, 10). Assembly of myofilaments, in vivo, requires a complex array of structural and associated proteins. In culture, a striated muscle cell initially looks more like a fibroblast or smooth muscle cell with actin stress cables anchored at the membrane and interspersed with dense Z bodies containing α-actinin (6). This observation has been confirmed in living cultures by the use of green fluorescent protein conjugated to α-actinin (4, 24). The first short thin filaments composed of actin, tropomyosin, and the troponin complex extend in both directions away from the Z bodies, making I-Z-I brushes linked to each other by the long titin molecule (28). In cultured cells, myosin binding C protein clamps the rod region nonmuscle myosin IIB to form the initial thick filaments in the cytoplasm nearby (4, 24, 29). The NH_2 terminus of titin binds to the Z line, and the COOH terminus binds to the center of the thick filament, thereby linking the loose, nonstriated arrangements of I-Z-I complexes and capturing the isolated thick filaments to form the sarcomere. Alignment of the sarcomeres in the transverse plane is achieved by the arrival of myomesin and M protein between the thick filaments and cytosketetal proteins to form the Z bands

Table 1. *Comparison of function and composition of cardiac and skeletal muscles*

	Cardiac	Skeletal
Rest	Cycles 24 h, over 60 beats/min	Intermittent (maximum for respiratory muscles over 10 beats/min)
Load increase	Pressure overload from high peripheral resistance	Weight lifting
Length increase	Volume overload by over-filling chambers	Bone growth or stretch
Eccentric	Contraction in over-filled chamber: long, thin cells	Contraction during extension: very wide cells
Concentric	Contraction in normal or small chamber: short wide cells	Contraction during shortening: wide cells
Cell size	Diameter of 10–15 μm; length of >100 μm	Diameter of 10–100 μm; length varies up to many cm
Nuclei	One (or two) central nuclei	Multiple peripherally located nuclei
Stem cells	No	Yes, satellite cells
Apoptosis	Yes, heart failure	Yes, disuse or atrophy
Fibrils	No, irregular field like bundles; felderstruktur	Yes, cylindrical bundles; fibrillenstruktur
Myosin heavy chain isoforms	α-MHC β-MHC	Slow/type I: β-MHC; fast/type II: 2X, 2A, 2B
Myosin LC1	MLC1a; MLC1v	MLC1v; $MLC1/3_{fast}$
Myosin LC2	MLC2a; MLC2v	MLC2v; $MLC2_{fast}$
Myosin binding protein-C	$MBP\text{-}C_{cardiac}$	$MBP\text{-}C_{slow}$ $MBP\text{-}C_{fast}$
Actin	$\alpha\text{-actin}_{cardiac}$	$\alpha\text{-actin}_{skeletal}$
Tropomyosin	$\alpha\text{-Tm}_{cardiac}$	$\alpha\text{-Tm}_{slow}$ $\alpha\text{-Tm}_{fast}$ β-Tm
TnC	$TnC_{cardiac}$	$TnC_{cardiac}$ TnC_{fast}
TnI	$TnI_{cardiac}$	TnI_{slow} TnI_{fast}
TnT	$TnT_{cardiac}$	$TnT_{skeletal}$

MHC, myosin heavy chain; MLC, myosin light chain; MBP, myosin binding protein; Tm, tropomyosin; TnC, troponin C; TnI, troponin I; TnT, troponin T.

from the I-Z-I brushes. The final thin filament length of 1 μm is determined by the long nebulin molecule in skeletal muscle (nebulette in cardiac muscle) and by an actin capping protein (15). The other long molecule, titin, appears to be necessary for the determination of both the length of the thick filament (1.6 μm) and for bringing it to the center of the sarcomere. This is evident because titin binds to the Z line at its NH_2 terminus and to the M line at its COOH terminus.

The first sarcomeres in culture form in close proximity to the membrane and are coupled by focal adhesions to the extracellular environment. It is worth noting that a cardiac myocyte changes from a cylindrical, rod-shaped cell in vivo with a central nucleus embedded in contractile material to one that is shaped like a fried egg with the contractile proline lying near the lower surface like the white and the nucleus sitting on top like the yolk in vitro. Given that cultured cells are flattened on the surface of the dish, this is hardly surprising that this adaptation to the new environment yields many nonphysiological properties in the cell. However, when myofibrillogenesis was observed in vivo in normally situated cells in early development of the heart of the chick embryo by confocal microscopy, a difference in the sequence of the appearance of sarcomeric proteins was found. No stress fibers or premyofibrils were observed in vivo, suggesting that these findings could be an artifact supported by the artificial two-dimensional properties of the current cell culture system (7). In studies of new sarcomere formation in elongating skeletal muscle, myofibrils formed well away from lateral association with the membrane. There were actin stress fibers, Z bodies, and insertion to a focal adhesion at the end of the fiber only (5). Therefore, we cannot assume that the observation on fibril formation in the flattened cells in culture holds for the three-dimensional architecture in vivo.

MECHANICAL REGULATION OF PROTEIN TURNOVER AND EXCHANGE

All biological materials are constantly in a state of flux, with a cycle of molecules entering and leaving every structure. Therefore, a sarcomere today will not be made of the same molecules as tomorrow (21). To understand such replacement at the level of the contractile machinery, contractile proteins have been labeled and followed (8, 24, 26). These exchange processes have not been measured directly in vivo except by isoform exchange as witnessed by immunoelectron microscopy. The natural incorporation of the newly synthesized α-MHC was detected in a day or two, and, notably, the exchange rate was greater near the free ends of the thick filaments than in the center (32). Tropomyosin is also preferentially replaced at the ends of the thin filaments (20), suggesting that the ends of filaments are less tightly bound than the central regions.

Every protein has its own steady-state exchange rate that varies from seconds to weeks. The contractile proteins in vivo are among the longest to live of known proteins. For example, sarcomeric actin's half-life is ~20 days and MHC turns over with a half-life of 7–10 days, whereas the components of the troponin complex have turnover rates similar to troponin I, troponin T, and troponin C at 3.2, 3.5, and 5.3 days, respectively (19).

However, if a molecule leaves the protection of the intact filament, it is highly susceptible to rapid degradation, with a half-life in minutes when disassembled in both cardiac and skeletal muscles (11, 27). This leads to the question of what processes foster unraveling of the filaments so that rapid degradation follows. The simple answer is removal of the activity or load, for example, tenotomy of skeletal muscle, space flight for cardiac and skeletal muscle, or separation of tissues into cells to culture them. It might not matter whether the removal of load is externally or internally generated, since even inhibition of contractile activity (e.g., by blockade of calcium transients or inhibition of actin-

myosin cross-bridge cycling) reduces the MHC and actin content of cultured cells and leads to a time-dependent disappearance of intact sarcomeres. There is both a decrease in MHC and actin synthesis and an increase in the rate of MHC and actin degradation as sarcomeres disappear (3, 27). These effects are entirely reversible, and an increase in load or activity enhances assembly of the sarcomere. Passive stretch causes MHC and actin accumulation in contracting cells, due to both an increase in the rate of protein synthesis and a reduction in the rate of degradation (30). Surprisingly, cardiac myocytes cyclically stretched are not aligned to the force vector as they would be in any muscle in vivo. Rather, they swing to the perpendicular direction and lie transverse to the axis of strain (29, 30). We think this might be due to the abnormal attachment between the culture cells and the slippery surface of the supporting membrane. The chemistry and surface features adjacent to the cell are important to both cell shape and attachment (16, 29).

To study the mechanics of the cross bridge, methods were developed to allow exchange of the natural contractile proteins with those engineered by molecular techniques (20). New proteins can be driven into the filaments by the law of mass action if they are supplied at many times the normal concentration in vitro or overexpressed in transgenic animals. Almost nothing goes in at physiological concentrations; therefore, high concentrations are needed for mass action to work effectively. For example, the isolated, skinned muscle strips can have 80% of the troponin sites occupied with the new protein and be functioning 1 h later. We can glean other useful information from the methods sections of this series of mechanical papers and see that turnover rates may well vary in different mechanical conditions. For example, the affinity of troponin T to tropomyosin, which governs the resting exchange rate, is more rapid in rigor when the myosin head is tightly bound to actin (for a review, see Ref. 31). Perhaps stabilization of the thick filaments can be regulated in a similar manner by the phosphorylation or calcium binding through associated thick filaments proteins, such as the C proteins (34). Relative binding affinities could well link calcium regulation of contractility to favor either assembly or disassembly.

FUTURE DIRECTIONS

Gene transfer experiments in vivo and in vitro allow introduction of constructs into muscle cells to probe molecular and morphological responses to altered work. It appears that the need for timed, controlled manipulations of the functional properties are necessary. The Sanger team (4) has recently had great success with introduction of labeled contractile molecules in cultured cells, since the green fluorescent protein has the advantage of being visible in living cells. Modern molecular techniques have provided us with much needed avenues in which to explore contractile function and assembly. Immunocytochemistry has been a useful tool in visualizing various cellular components. Refinements have been made to tagged proteins to allow for detection of a larger variety of structures. For example, the FLAG peptide (DYKDDDDK) is an improved affinity tag in use for detection and purification of recombinant antibody fragments (20). Such tagged proteins can be introduced in vitro by transfection (adnenovirus or lipofectamine) and are easily detected with the use of commercially available antibodies.

Transgenic animals allow a mutated or tagged protein to be introduced into the whole animal. With newer expression systems, we can turn these new genes on at a specific time in the adult rather than have them active throughout embryological development. However, when one is trying to explore cell shape control, these do not permit much better experimental control than do the natural isoform exchange and anatomic descriptions that were done in past decades. They are also much more expensive.

The use in whole intact animals has the advantage of seeing the real response to altered functional demands in vivo and, therefore, will always be essential. Unfortunately, cardiac mechanobiological research is hampered because interventions in vivo cause the death or demise of the experimental animal, whereas studies in vitro do not yet have a life-like cell culture system. As we strive to understand the fundamental mechanisms of mechanical transduction at the cellular level, there is a strong need to create more physiologically relevant models of cells in vitro. Specifically, to understand cell shape, we must address questions of how contractile function in cells modulates addition of new contractile filaments in parallel or series. Our notion is that the ultimate shape of individual myocytes is the fundamental process by which the muscle cells grow and remodel to meet altered work demands.

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