Ring Bands in Fish Skeletal Muscle: Reorienting the Myofibrils and Microtubule Cytoskeleton Within a Single Cell


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ABSTRACT Skeletal muscle cells (fibers) contract by shortening their parallel subunits, the myofibrils. Here we show a novel pattern of myofibril orientation in white muscle fibers of large black sea bass, Centropristis striata. Up to 48% of the white fibers in fish >1168 g had peripheral myofibrils undergoing an ~90° shift in orientation. The resultant ring band wrapped the middle of the muscle fibers and was easily detected with polarized light microscopy. Transmission electron microscopy showed that the reoriented myofibrils shared the cytoplasm with the central longitudinal myofibrils. A microtubule network seen throughout the fibers surrounded nuclei but was mostly parallel to the long-axis of the myofibrils. In the ring band portion of the fibers the microtubule cytoskeleton also shifted orientation. Sarcolemmal staining with anti-synapsin was the same in fibers with or without ring bands, suggesting that fibers with ring bands have normal innervation and contractile function. The ring bands appear to be related to body-mass or age, not fiber size, and also vary along the body, being more frequent at the midpoint of the anteroposterior axis. Disturbances of the orientation of myofibrils are rare but have been described in a variety of species (Bataillon, 1891; Morris, 1959; Bethlem and Wijngaarden, 1963; Jansen et al., 1963; Korneliussen and Nicolaysen, 1973; Mühlenbey and Ali, 1978). However, they have not been reported in teleost fishes.

INTRODUCTION

Fish skeletal muscle is composed of red and white muscle arranged in a highly organized fashion. In most fishes the red muscle is located laterally, in a thin strip just under the skin along the length of the body and parallel to the lateral line (Stoiber et al., 1999). The majority of the muscle mass in fishes is white muscle and it is partitioned into myotomes flanked on either side by layers of connective tissue referred to as myosepta (Gemballa and Vogel, 2002). The organization of the white muscle fibers is complex with the fibers attached to myosepta at each end forming a continuous helical array relative to the body’s axial skeleton (Rome et al., 1988). Gemballa and Vogel (2002) refer to this arrangement as arch-like helical muscle fiber arrangements that span the length of several myotomes and are therefore intersected by multiple myosepta. In each muscle fiber the myofibrils are oriented longitudinally, connecting one myosepta to the next. Disturbances of the orientation of myofibrils are rare but have been described in a variety of species (Bataillon, 1891; Morris, 1959; Bethlem and Wijngaarden, 1963; Jansen et al., 1963; Korneliussen and Nicolaysen, 1973; Mühlenbey and Ali, 1978). However, they have not been reported in teleost fishes.

In fishes, red muscle predominantly grows by hyperplasia (increase in fiber number), whereas white muscle undergoes hyperplasia in early postlarval stages followed by hypertrophy (increase in fiber size). The muscle fibers increase in size by increasing in both length and diameter (Lampilla, 1990; Kießling et al., 1991; Johnston et al., 2011). Black sea bass undergo an extreme increase in body mass during postmetamorphic growth, where white muscle fiber diameter is positively correlated with body mass, leading to very large fibers in adults (Nyack et al., 2007; Priester et al., 2011). Furthermore, once white fibers become >120 μm...

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in diameter, a redistribution of mitochondria and nuclei is observed, presumably due to increasing diffusion constraints (Kinsey et al., 2007, 2011; Locke and Kinsey, 2008). Nuclei have been found to be strictly subsarcolemmal (SS) in fibers <120 µm but become distributed throughout the sarcoplasm (intermyofibrillar or IM) in fibers >120 µm, which is presumably a response to avoid increased diffusion distances for nuclear products such as mRNA and ribosomes (Hardy et al., 2009, 2010; Kinsey et al., 2011; Priester et al., 2011).

Because nuclei are long lived organelles, this reported shift in distribution raises the question of what controls the spacing and location of nuclei within skeletal muscle cells. Nuclei have been reported to be anchored in a nonrandom fashion to the sarcolemma through varying complexes of anchoring proteins and cytoskeletal elements, including actin and microtubules (MTs; Apel et al., 2000; Starr and Han, 2003; Grady et al., 2005; Bruusgaard et al., 2006; Starr, 2007, Star, 2009). Myofibrils are also organized and aligned by anchoring proteins. Dystrophin and dystrophin-associated proteins form a complex that links the myofibrils and cytoskeleton to the extracellular matrix (Campbell, 1995; Starr and Han, 2003; Starr, 2007; Han et al., 2009). A lack of dystrophin leads to a derangement of the MT network in muscle cells (Prins et al., 2009) and the appearance of IM nuclei in several species, including fish (Berger et al., 2010). Disturbances or absence of anchoring proteins have also been shown to lead to muscular dystrophies (Weller et al., 1990; Banks et al., 2008; Priester et al., 2011). Myofibrils had a separate pattern of innervation from the outer band of reoriented myofibrils had a separate pattern of innervation from the core of the fiber because the presence of separately innervated functional myofibrils with opposing orientations might act as a muscular hydrostat such as those described in Kier and Smith (1985).

MATERIALS AND METHODS

*C. striata* (0.4 g to 4840 g; n = 18) were obtained from the University of North Carolina Wilmington (UNCW) Aquaculture Facility (Wrightsville Beach, NC) and wild fish were provided by Dr. F. S. Scharf (UNCW). Fish were maintained and processed according to the UNCW Institutional Animal Care and Use Committee standards.

Small, rectangular pieces of epaxial white muscle were excised parallel to the fibers' long axis orientation at a site immediately posterior to the operculum. In large fish (>2 Kg) six different regions (cranial to caudal) were sampled at equal intervals, each ~17% (1/6th) of the distance from the operculum to the caudal peduncle. All tissue was fixed for 24 hr in 4% paraformaldehyde in Sörensen’s PBS pH 7.4 or Bouin’s fixative (Presnell and Schreibman, 1997) and subsequently processed respectively for cryomicrotomy or paraffin histology. For transmission electron microscopy (TEM) teased muscle fibers were stained with 2.5% glutaraldehyde and 1.5% paraformaldehyde in PBS and processed using the protocol described in Nyack et al. (2007). Cryosections (30 µm) were stained with 4',6-diamidino-2-phenylindole (DAPI) and acridine orange (AO) and examined with an Olympus FV1000 laser scanning confocal microscope. Stacks of ~30 fluorescence and DIC images were collected at 1 µm intervals. Companion sections were stained overnight at 4°C with a primary antibody against β-tubulin (E-7; Developmental Studies Hybridoma Bank; diluted 1:400) or for 4 hours at room temperature with a primary antibody against synapsin (SV2; Developmental Studies Hybridoma Bank, diluted 1:500). These were followed by incubation for one hour at room temperature with a 1:200 dilution of a 2 mg/mL secondary goat anti-mouse antibody conjugated with Alexa 594 fluorochrome (A-11032; Invitrogen). Negative controls included incubation with secondary antibody only for cross reactivity. Control for autofluorescence was done using sections exposed to blocking serum only, with no primary or secondary antibody. None of the controls showed any fluorescence at 594 nm.

Serial sections (10 µm) of paraffin embedded tissue were collected and every 10th section was stained with hematoxylin and eosin (H & E; Presnell and Schreibman, 1997) and examined with both bright field and polarized light microscopy. The
Fig. 1. Ring band pattern observed with laser confocal microscopy (A and D) polarized light microscopy (B and C) and in serial histological sections (E and F). (A) DAPI-stained nuclei (arrows) arranged in a circular array inside some of the acridine orange-stained white muscle fibers in a large (>1168 g) fish. (B and C) Polarized light microscopy showing fibers with the ring band of reoriented myofibrils (arrow in B) and revealing striations on these reoriented myofibrils (arrow in C). (D) Laser confocal and DIC examination of DAPI-stained whole mounts of fibers showing the ring band myofibrils (rb) wrapped around the core myofibrils (c). The arrowhead in D points to a nucleus aligned with the long axis of the fiber within the core myofibrils. The arrow in D points to a nucleus lined up with the spiraling myofibrils. (E) Section from the middle of the fiber with a visible ring band (rb) around the core of the fiber (c). (F) Section from near the end of the fiber where only the core of the fiber is present (c).
frequency of cells with the circular pattern was determined using polarized light microscopy, which allowed identification of fibers with and without the circular pattern at low magnification with a large field of view.

Images of muscle fibers and ring bands were outlined with an Intuos 3 tablet (Wacom, Vancouver, WA) in Adobe Photoshop (version 7.0; Adobe Systems, San Jose, CA) and resulting polygons were analyzed with ImagePro Plus (v. 6.1.0.346; Media Cybernetics, Bethesda, MD) to determine their cross sectional area (CSA), perimeter and mean diameter. The mean diameter was determined by taking the mean of the distances across the cell through the centroid in 2° increments around the perimeter of the cell. For ring band cross sectional areas, the core area was subtracted from the total area. For sarcomere and I-band lengths 100 measurements were taken from each fiber region (ring band and core). Measurements were made on digitized TEM images with ImagePro Plus (v. 6.1.0.346; Media Cybernetics, Bethesda, MD).

RESULTS

Ring Band Pattern of Peripheral Myofibrils

The use of differential interference contrast (DIC) and especially polarized light microscopy revealed that in cross sections of those fibers that contained IM nuclei arranged in a circular pattern (Fig. 1A) the myofibrils were oriented in a ring-like pattern at the periphery. The peripheral band of myofibrils had an altered orientation, spiraling around the central core of the fiber (Fig. 1B–D). This peripheral ring band of myofibrils was close to, but not perfectly perpendicular to the core myofibrils, which was indicated by regions of extinction at ~90° intervals in the peripheral, reoriented myofibrils (Fig. 1B). Furthermore, examination of DIC images at higher magnification revealed striations on these reoriented myofibrils corresponding to the highly organized sarcomeres of skeletal muscle (Fig. 1C). Laser scanning confocal microscope examination of DAPI-stained whole fibers from similar sized fish showed that the outer myofibrils were wrapped around the center core of myofibrils in a spiral or ring band pattern (Fig. 1D). The DAPI-stained nuclei were elongate and appeared to be aligned with the long axis of the fiber within the core myofibrils whereas in the spiraling myofibrils the nuclei appeared to change their orientation so as to line up with the ring myofibrils (Fig. 1D). The ring band of reoriented myofibrils was not seen throughout the length of the fibers, rather it was usually found in the center of the fibers, as revealed by observations of both whole isolated fibers (Fig. 1D) and serial histological sections (Fig. 1E,F).

Ring Band Frequency as a Function of Body Mass and Cell Diameter

The smallest fish to display this ring band pattern weighed 1168 g (Fig. 2A,B). The smallest fiber displaying the ring band pattern was ~100 μm in diameter and was found in a fish weighing 2258 g. Fish with body mass less than 1168 g did not have cells with the ring band pattern, even though they had fibers with a mean diameter >100 μm. In fish with a body mass greater than 1168 g, 0.6–36% of the white fibers had the peripheral ring band pattern. Muscle sampled from six different sites along the length of the large fish (N = 4 fish) indicated that this ring band pattern was more prevalent in the midpoint of the anteroposterior axis, where up to 48% of the fibers had this pattern of reoriented myofibrils (Fig. 2C).

Ultrastructure, Cytoskeleton, and Innervation of the Ring Band

Ultrastructural observations verified that the peripheral myofibrils were truly reoriented, with sarcomeres being almost perpendicular to those of the central myofibrils and with no sarcolemma at their interface, indicating that both were within the same fiber (Fig. 3A,B) and thus shared the same sarcoplasm. The ring band sarcomeres appeared to be complete, suggesting that the reoriented myofibrils were functional.

When white muscle fibers of C. striata were stained with antibodies against β-tubulin, MTs were seen in all white muscle cells, including those with the peripheral ring band pattern (Fig. 4A), and these MTs were present throughout the fiber, in both the SS and IM regions. In fibers without the ring band the MTs were predominately oriented with the long axis of the cell, parallel to the myofibrils, with numerous branches projecting radially from those MT bundles (Fig. 4B). The MTs also appeared to wrap the nuclei. In the fibers containing the ring band, the MTs in the core of the fiber were also mostly longitudinally oriented. However, at the periphery the MTs were reoriented in the same manner as the myofibrils. That is, they had changed orientation and were roughly perpendicular to the MTs and myofibrils present at the core of the fiber (Fig. 4A).

After confirming that the myofibrils and MTs of the ring band shared cytoplasm with the core of the fiber we wished to determine if the altered fibers also had a similar pattern of innervation. Staining for synapsin revealed that the cells with the ring band pattern were innervated at the periphery of the fiber, in the same manner (i.e. relegated to the sarcolemma and having a stippled appearance) as the cells without the pattern (Fig. 4C–F).

In summary, our observations suggest that in fibers with the ring band pattern all of the fiber’s ultrastructural elements seem to change orientation forming a ring around the core of the muscle fiber. Myofilaments, nuclei and MTs all become spirally wound around the core of the fiber, which remains unchanged.
Cross Sectional Areas and Sarcomere Lengths

These measurements indicated that while ring bands were only seen in specimens greater than 1168 g, there was no correlation ($r^2 = 0.003$) between fiber diameter and the percentage of each fiber's cross-sectional area devoted to the ring band (Fig. 5A), and all specimens with body masses greater than 2258 g varied widely in the extent of the fiber area represented by the ring band pattern (Fig. 5B). Sarcomere and I-band lengths were also examined in the core and ring band regions of fibers from multiple sections from two fish. All fibers were processed for TEM in the same manner and mean sarcomere lengths (Z-line to Z-line) from myofibrils forming the ring band were significantly longer than the mean sarcomere length of the core myofibrils (ANOVA: DF = 1, $F = 144.8$, $P < 0.0001$; Fig. 5C). The mean length for ring band sarcomeres was $1.74 \pm 0.0152 \mu m$ (N = 2), whereas the mean length for the core sarcomeres was $1.50 \pm 0.0131 \mu m$ (N = 2). Mean I-band length was significantly shorter for myofibrils forming the ring band. Mean I-band length was $0.31 \pm 4.823e^{-3} \mu m$ in the ring band region (N = 2), and $0.54 \pm 0.0180 \mu m$ in the core region of the fibers (N = 2; ANOVA: DF = 1, $F = 167.4$, $P < 0.0001$; Fig. 5D).

DISCUSSION

The ring band pattern observed in white fibers of large black sea bass in this study is virtually identical to previously described microscopic structures variously called “ringbinden,” “striated annuliets,” “rings bands,” or “ring fibers.” These ring bands were observed as early as 1891 and have been seen in a variety of muscles from a variety of different organisms, including hagfish, *Xenopus* larva, rabbit, mouse and humans (Bataillon, 1891;...
Morris, 1959; Bethlem and Wijngaarden, 1963; Jansen et al., 1963; Korneliussen and Nicolaysen, 1973; Mühlendyck and Ali, 1978). However, the origin and function of ring bands has remained unclear.

In hagfish, the ring bands were seen in normal swimming muscle composed of white fibers (Jansen et al., 1963; Korneliussen and Nicolaysen, 1973). Ring bands in humans have been observed in older, but healthy individuals and particularly in muscles that do not have an attachment to bone such as the extra-ocular motor muscles, diaphragm, tongue, vocalis, uvulla, and others (Bethlem and Wijngaarden, 1963; Mühlendyck and Ali, 1978). Additionally, ring bands have been experimentally induced in skeletal muscle of healthy rats and rabbits by severing their attachment to a rigid structure (e.g., bone) by either cutting the tendon or the muscle itself (Bethlem and Wijngaarden, 1963).

The ring band pattern has also been observed in association with certain pathologies, especially different types of muscular dystrophy (Bethlem and Wijngaarden, 1963; Banks et al., 2008). Dystrophic muscle has altered or missing proteins in its costameres and/or dystroglycan complexes. It has been hypothesized that individuals suffering from muscular dystrophy have muscles with ‘weaker’ attachments between the sarcolemma and the myofibrils, cytoskeleton, and the basal lamina, which makes these linkages more prone to tearing and damage as the muscle fiber contracts (Goldstein and McNally, 2010; García-Pelagio et al., 2011). Han et al. (2009) have shown that a mouse model for secondary dystroglycanopathy (Large<sub>myd</sub>) has weaker links between the sarcolemma and basal lamina due to a lack of laminin globular domain-binding motif. Additionally, lengthening (eccentric) contraction has been shown to induce more damage to the sarcolemma and its attachments to the basal lamina and intracellular structures than shortening (concentric) contractions because they increase membrane tension, leading to small tears in the sarcolemma, as suggested by Weller et al. (1990) and Han et al. (2009).

As described by Kier and Smith (1985), muscles have a constant volume and as a muscle contracts, it shortens in length and therefore it must expand radially, increasing its circumference. An increase in circumference leads to more stress on the sarcolemma. In a muscle that is attached to bone, the extent of contraction is limited by the attachment to a rigid structure, whereas in muscles that are not attached to bone, such as the diaphragm and extra-ocular motor muscles, the extent of contraction is less limited and can result in hypercontraction and subsequent stress and strain between the sarcolemma and cytoskeleton. The same is true for muscles that have had their attachment to bone severed. As muscle is allowed to hypercontract, there appears to be a threshold fiber length at which the extreme shortening of the fiber causes the circumference to stretch to the point of breaking the links between the sarcolemma, the myofibrils, cytoskeleton, and basal lamina (Mühlendyck and Ali, 1978; Han et al., 2009). By extension, in a dystrophic muscle fiber one would expect a significantly lower stress to result in damage to the bridge between the sarcolemma and the myofibrils and basal lamina, even in muscle fibers that are

![Ultrastructure of fibers with the ring band pattern](image-url)
Fig. 4. Cytoskeletal network and innervation pattern in muscle fibers with and without the ring band. (A and B) Laser confocal z-stack of an oblique section stained for microtubules (anti-β-tubulin seen in green) and nuclei (DAPI seen in red for better contrast). (A) Fiber with ring band (rb). Arrow points to MTs spiralling around the core of the fiber (c). (B) Fiber without the ring band, with MTs running the length of the fiber (arrow) with some branching perpendicular to the longitudinal axis (arrowhead). A nucleus is seen in association with the network of MTs (circle). (C–F) Paired images showing the pattern of innervation in white muscle fibers without (C and D) and with (E and F) the ring band as revealed by an antibody against synapsin (arrows pointing to green fluorescence in D and F). DAPI-stained nuclei are seen as red. (C and E) are 1 μm optical sections of fluorescence plus DIC. (D and F) are z-stacks of 30 1 μm confocal fluorescence images without DIC. The ring band is indicated by “rb” in (E) and the core of the fiber by “c” in (C) and (E).
attached to bone (Banks et al., 2008; Han et al., 2009). Therefore, it appears that the common characteristic seen in the muscles that display this reoriented ring band of myofibrils is the disruption of the sarcolemmal attachments to intra- and extracellular structures. The disruptions can be caused by various events, including hypercontraction, lengthening contractions, and pathological conditions such as muscular dystrophies.

Once the sarcolemma is disrupted, the breaking of its linkages leads to a disconnect between intracellular structures (cytoskeleton, myofibrils and nuclei) and the sarcolemma and basal lamina. These intracellular components may then become free to reorient (or disorient) and form new connections with the sarcolemma. What dictates the orientation of the myofibrils, cytoskeleton and nuclei at this roughly 90° angle to the core of the fiber is not known. It does seem that the ring band consists of newly formed myofibrils because the fibers are continuing to grow hypertrophically. In fact, in some fibers the area composed of the ring band was very large, reaching over 75% of the CSA (Fig. 5A,B), indicating that these fibers were still undergoing hypertrophy by adding myofibrils in this reoriented, ring band pattern.

Staining for synapsin revealed no obvious differences in innervation between fibers with and without the ring band, which suggests that if the fiber is stimulated at the neuromuscular junction, all of the myofibrils would contract, both the core and ring band myofibrils. Contraction of the myofibrils of the ring band around the middle point of the fiber would potentially restrict the expansion of the fiber’s circumference. By doing this, the reoriented ring band myofibrils would be reinforcing the fiber’s middle portion, thereby preventing fur-
ther expansion of the sarcolemma and subsequent damage to the sarcolemmal linkages.

It has been previously suggested that chronic stretch induces an increase in sarcomere lengths (Houlihan and Breckenridge, 1981). This might explain why sarcomeres of the ring band are significantly longer than the sarcomeres at the core of the fiber even though their I-bands are shorter, indicating they are more contracted. Alternatively, because they are reoriented, the Z-lines of the sarcomeres in the ring band might be attaching to costameres on the sarcolemma that have a different spacing, i.e. that are further apart and therefore could lead to formation of longer sarcomeres. However, it is not known whether costameres dictate sarcomere length or whether sarcomere length dictates where Z-lines are anchored at the sarcolemma.

While we have shown that the occurrence of ring band fibers is widespread, it is somewhat rare, raising the question of why they appear in the white muscle of the black sea bass. The white fibers of black sea bass in our study grew hypertrophically to very large sizes, both in diameter and length. Furthermore, they are not directly attached to bone, but rather to a contiguous series of connective tissue layers (myosepta). The fibers are aligned end-to-end, in a row from myosepta to myosepta, in such a way that the fibers at the extremities attach to either the skin or the axial skeleton of the fish. When the fibers in a row contract, they pull on the myosepta between them and, therefore, also pull on each other. The fibers located near the center of this row of fibers would have more leeway and may hypercontract more than the fibers that are attached closer to the skin or axial-skeleton. Furthermore, because the ends of the muscle fibers are anchored to myosepta, the portions of the sarcolemma that are close to the myosepta would likely not be disrupted during hypercontractions, whereas the portions of the sarcolemma in the middle of the fiber, furthest from myosepta may be disrupted and damaged. This may explain why we observe: 1) the ring band pattern in the middle of each fiber rather than along the entire length and 2) more fibers with the ring band pattern towards the middle of the fish's anteroposterior axis, which is the position most distant from attachment sites on bone or skin.

In addition, fish swim by alternating contractions of either side of the body such that when one side approaches the end of a contraction cycle, the other side is already starting to contract. This helps maintain body stiffness during burst swimming and leads to lengthening contractions on one side of the fish during each tail beat. For example, Davies et al. (1995) have showed that during subcarangiform swimming in trout, caudal muscles are activated while still lengthening. Subcarangiform is also the swimming mode of black sea bass. Because lengthening contractions induce more damage of the sarcolemma and its attachments than isometric- or shortening-contractions (Weller et al., 1990; Han et al., 2009), this could favor the formation of ring bands in black sea bass.

In contrast, hagfish have an anguilliform mode of swimming, which involves undulation of the entire body, rather than bending in the mid-portion of the body like the subcarangiform swimming (Lindsey, 1978; Colgate and Lynch, 2004). It is interesting to note that while dissecting hagfish for a related study, we have observed that their swimming muscles are not firmly attached to the skin, and therefore are not firmly anchored. This characteristic would make their muscle fibers more likely to hypercontract, and may give an insight into why Korneliussen and Nicolaysen (1973) found ring bands in hagfish muscle. Following this logic, one would expect to find ring bands in fish with other types of swimming modes wherever there is more bending along the fish's body length. For example, fish with carangiform swimming mode (Lindsey, 1978) would be likely to possess ring bands more caudally than a fish with a subcarangiform swimming mode, and fish with a thunniform swimming mode would be expected to possess ring bands only at their caudal peduncle. However, a comparison between black sea bass and fish with thunniform swimming mode such as tuna becomes complicated due to their very different arrangement of fibers and fiber types.

The fact that this ring band pattern appears only in larger black sea bass suggests that this phenomenon is either size related or age related, however these two factors cannot be uncoupled. Muscle fibers from larger fish have greater distances from connection sites, so the hypercontraction possibility would increase with size. However, the possible effects of aging on the anchoring proteins cannot be ruled out. In either case, the appearance of this pattern seems to be a response to damage to the sarcolemma and its links to the cytoskeleton and basal lamina. Studies on the mechanisms for repairing the sarcolemma have suggested that dysferlin is one of the proteins involved in mediating the resealing of the membrane and maintaining its integrity (Han and Campbell, 2007; Han et al., 2009). Part of that repair mechanism involves activation of proteases, such as calpain, by the high levels of calcium that are allowed to enter the fiber when the membrane is damaged. The activation of proteases leads to disassembly of cortical cytoskeleton proteins in the vicinity of the damage (Han and Campbell, 2007), which could likely also release anchoring proteins and thus allow for reorientation of intracellular structures and organelles.

The ring band likely restricts the expansion of the middle of the fiber, therefore serving as reinforcement against such expansion. This would pro-
tect the sarcolemma from further stretch and damage during muscle contraction and relaxation cycles. The opposing forces of the two groups of differently oriented myofibrils make the fiber function somewhat like a muscular hydrostat (Kier and Smith, 1985), but by interactions of bundles of myofibrils in a single cell rather than groups of whole muscle cells.

Together, these observations and the previous literature give insights into what controls muscle ultrastructural organization, directionality and how all these factors can change orientation due to disturbances of the connections to the sarcolemma. We propose that disturbing the sarcolemma through stretching in the circumferential direction disrupts the links between extracellular anchoring sites (basal lamina), the dystroglycan complexes and intracellular structures, namely the myofibrils, cytoskeletal elements (MTs), and nuclei. This overstretching can result from hypercontraction or lengthening contraction of muscle fibers that are not attached to bone (normally or experimentally). Once these links are broken, the intracellular elements are released from the sarcolemma and free to reorient in a different direction. In our study, the disturbance of sarcolemma seems to also allow for the release of nuclei from the periphery of the fiber, which then become IM nuclei once new myofibrils are formed around them.

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