Myosin filament assembly in an ever-changing myofilament lattice of smooth muscle

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Seow, Chun Y. Myosin filament assembly in an ever-changing myofilament lattice of smooth muscle. Am J Physiol Cell Physiol 289: C1363–C1368, 2005; doi:10.1152/ajpcell.00329.2005.—A major development in smooth muscle research in recent years is the recognition that the myofilament lattice of the muscle is malleable. The malleability appears to stem from plastic rearrangement of contractile and cytoskeletal filaments in response to stress and strain exerted on the muscle cell, and it allows the muscle to adapt to a wide range of cell lengths and maintain optimal contractility. Although much is still poorly understood, we have begun to comprehend some of the basic mechanisms underlying the assembly and disassembly of contractile and cytoskeletal filaments in smooth muscle during the process of adaptation to large changes in cell geometry. One factor that likely facilitates the plastic length adaptation is the ability of myosin filaments to form and dissolve at the right place and the right time within the myofilament lattice. It is proposed herein that formation of myosin filaments in vivo is aided by the various filament-stabilizing proteins, such as caldesmon, and that the thick filament length is determined by the dimension of the actin filament lattice. It is still an open question as to how the dimension of the dynamic filament lattice is regulated. In light of the new perspective of malleable myofilament lattice in smooth muscle, the roles of many smooth muscle proteins could be assigned or reassigned in the context of plastic reorganization of the contractile apparatus and cytoskeleton.

contraction mechanism; length adaptation; thick filament; plasticity; contractile unit

THE PRIMARY FUNCTION of smooth muscle in our bodies is to control and regulate the physical dimension and mechanical function of hollow organs. The large volume change in some organs requires that smooth muscle cells lining the organ wall have a large working length range. The length range over which a striated muscle can generate maximal or near maximal force is quite limited (19), between 10% and 20% of the resting muscle length. If smooth muscle were to have the same working length range, the corresponding volume change of the smooth muscle organ would be ~30–70%, which is not adequate for organ functions such as emptying a urinary bladder or pushing a fetus through the birth canal.

A broad plateau in the length-force relationship can be observed in many types of smooth muscle. It has been recognized recently that the plateau can become even broader if the muscle is allowed to adapt at each of the lengths at which force measurements are made (48, 69). Different extents of length adaptation in smooth muscle can be induced, at a fixed length, by a single contraction (21, 54), a series of brief activations (48, 55), or a continuous submaximal activation (42) over a period of tens of minutes. Adaptation can also occur in a relaxed muscle set at a fixed length over a period of hours (41, 69) or days (2, 44, 74). In isolated single cells from toad stomach, the length-force relationship can be shifted simply by changing the length of the relaxed cells (22). Although the acute and long-term length adaptation may have different mechanisms, results obtained with the various protocols appear to be the same: regaining of (at least partial) ability of the muscle to generate maximal force at the new length and a shift of the peak force of the bell-shaped “conventional” (i.e., nonadapted) length-force relationship toward the new length. This mechanical plasticity extends the working length range of smooth muscle and allows hollow organs to function properly. Under abnormal conditions, it is conceivable that length adaptation could lead to abnormal organ dimensions, such as excessively narrowed airways or blood vessels.

What is the underlying mechanism for mechanical plasticity in smooth muscle? Evidence suggests that for short-term length adaptation, the structural malleability of the network of contractile and cytoskeletal filaments is a key factor that gives the muscle the ability to adapt and function over a large length range (3, 11, 13, 20, 24, 25, 35, 36, 48, 54, 68, 69). For long-term length adaptation, it appears that rearrangement of cell-cell connection (41) and cell hypertrophy/hyperplasia (74) are also key factors. Quantitative electron microscopy revealed changes in the myosin filament content in some smooth muscles during contractile activation (17, 23, 71) and in adaptation to different cell lengths (24, 35). These findings were corroborated by studies using birefringence (18, 57) and X-ray diffraction (70). Note that in some smooth muscles, no evidence for myosin evanescence was found (17, 27, 71). (Possible reasons for the discrepancy are discussed below). Actin filaments in intact airway smooth muscle have been found to be evanescent also, with the filament content increased during contractile activation (24, 43). Therefore, it appears that the content of contractile filaments in smooth muscle can be changed rapidly. This has led to several proposals of models of smooth muscle contractile apparatus that are structurally malleable and able to accommodate all available functional and structural data (3, 20, 25, 35, 37). A key feature in some of these models is that myosin filament content is variable. The thick filament content in intact smooth muscle can be assessed by quantitative electron microscopy (35, 71) or birefringence (18, 57); validity of these models therefore can be verified not only through functional tests but also with ultrastructural quantification.

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EARLY OBSERVATIONS OF MYOSIN FILAMENT EVANESCE

In the 1960s, studies (30, 31, 45) using electron microscopes reported that there were few myosin thick filaments in the relaxed smooth muscle. More thick filaments were observed in the contracted state, and this has led to the hypothesis that thick filaments form during contraction (45, 50). Some of the observations might not be accurate because of poor fixation of tissue samples (30), but the consensus was that thick filaments in smooth muscle were much less robust compared with their counterparts in striated muscle. With improved preparative techniques, later studies (4, 15, 16, 59) were able to show that there was a significant number of thick filaments in the relaxed smooth muscle. The notion that thick filaments form and dissolve during the contraction-relaxation cycle was therefore abandoned and lay dormant for more than a decade until a series of studies published in the late 1980s and 1990s showed that at least in some smooth muscle, thick filament number increased as a result of contractile activation (17, 18, 70, 71). The physiological significance of this finding was recognized in our proposals regarding possible mechanisms governing mechanical plasticity in smooth muscle (14, 53).

RAPID THICK FILAMENT ASSEMBLY AND DISASSEMBLY COULD FACILITATE PLASTIC ADAPTATION OF SMOOTH MUSCLE TO LARGE LENGTH CHANGE

Measurement of length adaptation in airway smooth muscle has revealed that over a large length range, the maximal isometric force is independent of muscle length (after adaptation) and that shortening velocity, on the other hand, is highly length dependent (48). A simple model that explains these observations consists of contractile units arranged in series and in parallel; adaptation of the muscle to different lengths involves adding or subtracting the units in series. That is, smooth muscle cells adapted at longer lengths will have more units in series and vice versa. Because myosin filaments are likely central components of contractile units, any change in the number of contractile units inside a cell likely accompanies a change in the number of myosin filaments, possibly through polymerization and depolymerization. Furthermore, the change has to occur with sufficient rapidity to account for the observed swift time course of length adaptation (48).

In airway smooth muscle, it has been recently shown that the content of myosin filaments dramatically increased upon stimulation (23, 24, 57). The rapidity of the change (within seconds) (57) confers feasibility to the proposed models based on myosin evanescence (25, 35) and suggests that filament formation and dissolution in smooth muscle cells is likely governed by equilibrium between nonfilamentous and filamentous myosins. A corollary is that in a relaxed smooth muscle cell, there exists a relatively large pool of myosin monomers compared with that in an activated cell. Similarly, a cell adapted at a short length should have a larger pool of monomeric myosin than that in a cell adapted at a long length, because in a long cell, more myosin monomers are incorporated into filaments. This presumes that the total content of monomeric myosins is constant inside a cell, at least over a short period of time during which protein synthesis is minimal. In tracheal cells adapted to a stretched length, we have observed a substantial increase in myosin filament content (35). The increase is possibly due to additional contractile units being incorporated into the contractile apparatus during the process of length adaptation. The observed increase in muscle power output and rate of ATP consumption also agrees with this explanation (35), and so does the observed change in the length-force relationship adapted to different lengths (25). Taken together, available evidence suggests that rapid myosin polymerization and depolymerization play a crucial role in facilitating adaptation of airway smooth muscle to length change. In vitro studies have provided valuable insights regarding molecular mechanisms of thick filament formation and dissolution. Applicability of these in vitro results to intact smooth muscle, however, has yet to be established.

MOLECULAR MECHANISM OF THICK FILAMENT ASSEMBLY IN VITRO AND ITS IN VIVO RELEVANCE

It has been known for a long time that myosin filaments of smooth muscle are not stable and can be dissociated in solution at physiological levels of MgATP, ionic strength, and pH, and that phosphorylation of the regulatory myosin light chain stabilizes the filaments (61). It was found subsequently that dephosphorylated myosin monomers exist in a folded form, especially at low ionic strength (47, 64, 65), and phosphorylation of the light chain converts the folded myosins into extended ones, which are able to form filaments (7, 56). It appears that a folded myosin monomer is able to trap the hydrolysis products of ATP (i.e., ADP and inorganic phosphate) and therefore is unable to consume ATP continuously (8, 10). This could be an important in vivo mechanism for preventing monomeric myosins from utilizing ATP when they are not part of the thick filaments and not doing useful work.

Assembly of nonphosphorylated myosin monomers into filaments in solution has been found to be dependent on the monomer concentration; once a critical concentration is reached, self-assembly occurs (32). The required critical concentration increases dramatically when ATP is present in the solution (32) and decreases when the regulatory light chain of the myosin is phosphorylated (32, 66). A short region near the carboxy terminus (tail end) of myosin has been shown to be crucial for filament self-assembly (33, 63). Cross et al. (9) proposed that a tail tip-to-tip association of two myosin molecules is responsible for forming an anti-parallel dimer, which then serves as a nucleation site for filament growth. The filaments thus formed exist in two conformations, a closely packed, tight conformation in equilibrium with a loosely packed, “flared” conformation (9). Filament stability associated with the two conformations is not known. The side-polar structure of thick filaments observed in vitro (9) is believed to be also the in vivo structure. This predominant view is supported by evidence provided mostly by Craig and colleagues (5, 6, 28, 62, 72). In a recent study (25), we examined the possibility of smooth muscle thick filament being bipolar and possessing a central (cross-bridge free) bare zone. Over a large length range, we found no evidence of a plateau in the length-force relationship that could be attributed to the central bare zone. Functional evidence (25) therefore supports side-polar or similar structure, such as the row-polar model proposed by Hinssen et al. (26), which does not have a central bare zone. A side-polar thick filament could endow smooth muscle with
greater ability to shorten, especially if we assume that during shortening the thick filament could slide over dense bodies.

The carboxy terminal isoforms (SM1 and SM2) of smooth muscle myosin heavy chain have been found to affect stability of filament assembly (51), with the longer SM1 tailpiece conferring greater stability. Why would a less stable isoform such as SM2 be preserved in smooth muscle? A possible answer is that thick filament lability is required for the cell’s mechanical plasticity and that different degrees of plasticity may be determined by different mixes of SM1 and SM2 in the muscle cell. It has been shown that the expression of SM1 and SM2 isoforms is different in different smooth muscles and that it varies during development and hypertrophy (12, 40). The difference in expression therefore provides an opportunity for testing the hypothesis that SM2 (or other isoforms) enhances mechanical plasticity and facilitates the muscle’s adaptation to large changes in length.

Relevance of the in vitro mechanisms described above to intact smooth muscle cells is still a question. At physiological levels of MgATP, ionic strength, and pH, and in the absence of myosin regulatory light chain phosphorylation, there should be few (if any) thick filaments formed in solution, according to Craig et al. (7). Unfacilitated self-assembly of nonphosphorylated monomers into thick filaments in vivo is therefore not likely. The fact that thick filaments are found in relaxed smooth muscle cells suggests that there are other factors contributing to thick filament formation in vivo. One factor could be that myosin monomers in intact cells are distributed unevenly, with some local concentrations exceeding the critical concentration required for filament self-assembly (32). In intact smooth muscles from avian gizzard (27) and guinea pig taenia coli (17, 18, 70, 71), no evidence was found for a significant pool of monomeric myosin. On the other hand, in intact smooth muscles from rat anococcygeus (17, 18, 70, 71) and porcine (23, 24) and canine (57) airways, a large increase in thick filament content during contractile activation was found, suggesting that there is a large pool of myosin monomers. It is possible that the size of the monomer pool is dependent on specific smooth muscle cell types and also on the resting state of myosin light chain phosphorylation (71). More studies are needed to clarify this issue.

Even in smooth muscles that possess a large pool of myosin monomers, unaided self-assembly of nonphosphorylated myosins in vivo may not occur due to the presence of MgATP at intracellular ionic strength and pH. Besides phosphorylation of the myosin regulatory light chain, other facilitating factors inside an intact smooth muscle cell may be crucial for regulating thick filament assembly in vivo.

POSSIBLE MECHANISMS FOR THICK FILAMENT ASSEMBLY IN INTACT SMOOTH MUSCLE

If a significant fraction of myosins in intact smooth muscle is monomeric, activation-induced phosphorylation of the regulatory myosin light chain, besides serving the important role of activating the cross bridges, may also serve to promote thick filament formation in intact cells. The second role could be as important as the first. In intact airway smooth muscle, we tested the idea that phosphorylation of the regulatory myosin light chain may facilitate thick filament formation. By applying large-amplitude length oscillation to relaxed trachealis muscle strips, we observed that thick filaments could be partially disassembled (36). Reassembly of the filaments occurred when the muscle was repeatedly activated isometrically in the absence of mechanical agitation (36). However, this thick filament reassembly was prevented when phosphorylation of the regulatory myosin light chain was inhibited by 1 μM wortmannin (49). It appears, therefore, that phosphorylation of the regulatory light chain is just as important for thick filament formation in vivo as it is in vitro (7).

A critical question is why there are still so many thick filaments present in intact smooth muscle in the relaxed state when there is no sign of the light chain phosphorylation (49). A possible answer is that the actin filament network plays a crucial role in guiding and facilitating thick filament formation. Mahajan et al. (39) showed that myosin filament formation was accelerated fivefold in the presence of actin filaments and that transient binding of myosin monomers to actin filaments before incorporating into thick filaments appeared to be the key step for the accelerated assembly. Applegate and Pardee (1) also reached the same conclusion that filamentous actin facilitates myosin filament formation. Katayama et al. (29) observed that MgATP at physiological concentrations was able to disassemble dephosphorylated thick filaments but that the addition of calsdesmon caused the formation of thick filaments under the same condition. In the presence of actin filaments and under conditions that mimicked the relaxed intracellular environment, calsdesmon was able to induce the formation of long, thick filaments resembling those seen in intact muscle (29). Other thick filament-stabilizing proteins such as telokin (also known as kinase-related protein) (34, 58) and 38k protein (46) could also contribute to the maintenance of thick filaments in the relaxed smooth muscle. It appears, therefore, that many proteins in smooth muscle serve the role of thick filament preservation.

Formation of thick filaments cannot be random in terms of their location inside smooth muscle cells. For the contractile apparatus to function properly, thick filaments have to be placed into an actin filament lattice in which they can interact with thin filaments possessing the appropriate polarities to generate force. Perhaps it is for this reason that many of the thick filament-stabilizing proteins are part of the thin filament network. These proteins are likely placed in specific areas of the thin filament network, and their locations likely determine where the thick filaments will form. Calsdesmon, for example, is found interspersed in the thin filament network where myosin filaments are also found (38, 67).

Taken together, it appears that the lability of myosin filament in smooth muscle, especially in the relaxed state (when the filaments are not phosphorylated), is crucial for enabling the muscle to adapt to changes in configuration of the thin filament lattice brought about by changes in cell dimension. Somehow the mechanical strain that reshapes the filament lattice also results in depolymerization of the thick filaments. As mentioned above, we were able to induce partial depolymerization of the thick filaments in airway smooth muscle by applying length oscillation to muscle in the relaxed state (36) (Fig. 1A). A substantial decrease in isometric force in the subsequent contraction was also observed. By allowing the muscle to recover in the absence of mechanical disturbance, both isometric force and thick filament density returned to their preoscillation values after a few stimulations (isometrically)
over a period of \( \sim 30 \) min (Fig. 1A). Although the mechanism of this recovery is not entirely clear, one hypothesis is provided in Fig. 1B. It is assumed that thick filaments disassemble (at least partially) when the thin filament lattice is distorted. The weak interaction between thin filament-associated caldesmon and myosin cross bridges may provide sufficient shear force to disrupt the thick filaments. If this is true, then thick filaments in a relaxed muscle must be very unstable and may exist in the loosely packed conformation observed in vitro by Cross et al. (9). Another possibility is that dissolution of thick filaments is not caused directly by mechanical perturbation, but rather through a cascade of chemical reactions initiated by the mechanical perturbation. In dictyostelium, Yumura and Fukui (73) observed that cAMP (a chemoattractant for the amoebae) caused a rapid redistribution of myosin filaments within the cell through filament dissolution at some locations and reformation at others. Whether thick filaments in smooth muscle are dissociated by mechanical perturbation or through chemical reactions, their disassembly is likely the first step of the adaptation process through which the muscle regains its optimal configuration of filament overlap and contractility. Once the thin filament lattice settles into a stable configuration (or a relatively stable configuration; see Ref. 3), repolymerization of the thick filaments starts to take place. Formation of thick filaments is not likely to occur at random locations within the muscle cell, as mentioned above. To form a functional contractile unit, thick filaments have to be located in the “right” thin filament lattice. The appropriate places for thick filament formation are probably where the thick-filament-stabilizing proteins reside (Fig. 1B). Binding of myosin monomers by the thin-filament-associated caldesmon may provide a nucleation site for thick filament formation (9, 67). Cross et al. (9) have suggested that lengthening of existing thick filaments is the predominate mode of myosin polymerization in solution. Inside an intact cell, the thin filament lattice may provide a better environment for filament nucleation, and therefore a thick filament could be formed at multiple nucleation sites and upon the joining of multiple segments. Theoretically, this can increase the overall rate of thick filament formation. Note that the myosin-caldesmon interaction occurs only under the relaxed condition, i.e., no phosphorylation of the regulatory myosin light chain and caldesmon itself (29, 34). Under the activated condition, phosphorylation of caldesmon abolishes its own affinity for myosin (56). As discussed above, binding of myosin monomers to thin filaments could also provide guidance for thick filament formation (1, 39).

The mechanism outlined in Fig. 1B indicates that there is no need for a separate mechanism regulating thick filament length: the length is determined by the dimension of the thin filament lattice. Also, in a fully adapted smooth muscle, the thick filament length likely spans the entire lattice space, from dense body to dense body (Fig. 1B). This specific feature is exactly
what is needed to explain our recent data on the length-force relationships obtained at different adapted lengths (25). We found that regardless of the initial length at which the muscle was adapted, shortening of the muscle led to an immediate decrease in the ability of the muscle to carry load, and this decrease was linearly related to the amount of shortening (25).

If thick filament length is regulated by the dimension of thin filament lattice, then any pharmacological agents that disrupt thin filament lattice would be expected to disrupt thick filament assembly as well. Not many agents are known to interfere directly with thick filament formation; there are, however, many classes of compounds that sever or cap thin filaments. These agents can serve as experimental tools for testing the hypothesis alluded to above. The question of how the dimension of the thin filament lattice is regulated is still wide open. In tackling this question, the possibility should be kept in mind that smooth muscle cytoskeleton is not a random network of filaments and that there are subdivisions within the network that provide lattice space for thick filament formation.

SMOOTH MUSCLE, A MALLEABLE AND ADAPTABLE TISSUE

To understand how smooth muscle contracts, we have to recognize the tissue’s unique properties that are different from those of striated muscles. Malleability and adaptability are qualities of smooth muscle that are not compatible with a static filament lattice seen in striated muscle, and they are probably responsible for making smooth muscle “smooth” (52). From a functional point of view, these qualities are ideal for maintaining contractility over a large length range for muscle cells lining hollow organs that undergo large volume changes. For other organs such as blood vessels and airways, these smooth muscle qualities may put the organs in a precarious state, for adaptation at excessively short lengths may underlie the pathophysiology of diseases such as hypertension and asthma.

Many areas of smooth muscle research are currently focused on the regulation of contraction and relaxation. In light of what has been discussed above, the research probably will benefit from a change in perspective that places more emphasis on the regulation of structural protein organization and reorganization that is associated with muscle length adaptation. In our effort to assign functional roles for some proteins in the regulation of contraction, we may have neglected structural roles that these proteins may actually play. There are still many proteins (some of them highly abundant) in smooth muscle that do not seem to serve any function. A change in our perspective may aid us in finding their purposes in this highly malleable and adaptable cell.

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