Contractile filament architecture and force transmission in swine airway smooth muscle

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Summary

It is well known that the cyclic interaction of myosin cross bridges with actin filaments is responsible for force and shortening generation in smooth muscle. The intracellular organization of contractile filaments, however, is still poorly understood. Here, we show electron microscopic and functional evidence that contractile filaments in airway smooth muscle lie parallel to the longitudinal axis of the cell bundle, in contrast to the obliquely arranged filaments depicted in conventional models. The parallel arrangement of contractile filaments is maintained despite the fact that individual cells are spindle-shaped. This is accomplished through filament attachment to membrane-associated dense plaques that are in turn connected to similar structures on neighboring cells. Intracellularly, the parallel arrangement is maintained despite the centrally located nucleus. This is accomplished by attachment of actin filaments to the nuclear envelope and making the nucleus a force transmitting structure. The results suggest that smooth muscle cells in tissue form a mechanical syncytium and are able to function properly only as a group.

Key words: Ultrastructure, Contractile filaments, Nuclear envelope elasticity, Electron microscopy

Introduction

Force generation in smooth muscle is achieved through cyclic interaction of myosin cross bridges with actin filaments, similar to the contraction mechanism found in striated muscle (Guilford et al., 1998). How the generated force is transmitted within and between smooth muscle cells is, however, not entirely clear. The lack of precise knowledge about the contractile filament architecture within intact smooth muscle cells in tissue has hindered our efforts to understand the mechanisms and structures responsible for force transmission in situ. It has been recognized for some time that most smooth muscle cells in situ function electrically as a syncytium in a so-called 'effector muscle bundle' (Burnstock and Prosser, 1960; Burnstock, 1970). Whether a mechanical syncytium exists in the same effector bundle of smooth muscle is not known. Many models of contractile filament organization in smooth muscle are based on observations made from isolated cells (Fay and Delise, 1973; Fisher and Bagby, 1977; Small, 1977) whose mechanical couplings with adjacent cells have been severed during the process of isolation. The models are mostly that of a single cell with obliquely orientated contractile filaments relative to the longitudinal axis of the cell (for review, see Bagby, 1983). When an isolated cell contracts, corkscrew-like shortening of the cell is often observed (Fisher and Bagby, 1977; Warshaw et al., 1987), with the exception of an observation by Small (Small, 1985) in which antivinculin staining of isolated guinea pig vas deferens cells showed filament attachment sites parallel to the longitudinal axis of the cell, even in the shortened state. A contentious point still being debated is whether the corkscrew-like shortening observed in isolated cells could happen in an intact muscle bundle or is a result of decoupling of mechanical connections of the cell with its neighboring cells and extracellular matrix. If smooth muscle cells do not work as individuals but as a group in an effector muscle bundle, the architectural design of the contractile filaments might be optimized for the group but not for individual cells. In other words, through intercellular mechanical couplings (presumably intermediate junctions) the contractile filaments might be organized into a transcellular mechanical syncytium in which force can be generated and transmitted in contractile filaments that lie parallel to the longitudinal axis of the muscle bundle and the coincidental axis of force transmission, even at the tapered ends of the cells. In that regard, force transmission might be uniform in the tissue but, within the constituent cells, it might not be uniform at all cell cross sections, and, when isolated from its neighbors, an individual cell might be dysfunctional simply because of the nonuniformity of force generation along the cell length. Studies of filament organization in smooth muscle cells might therefore be more appropriately carried out in a bundle of cells in which intercellular connections are intact.

In the present study, small bundles of swine tracheal smooth muscle cells were used in the assessment of mechanical properties and fixation for electron microscopy. Cells in these tissues had their longitudinal axes aligned to the axis of force transmission during fixation. This allowed us to examine the orientation of contractile filaments with respect to the direction of force transmission. The multicellular preparations also allowed us to examine the mechanical connections between the cells and how these connections influenced intracellular organization of contractile filaments.

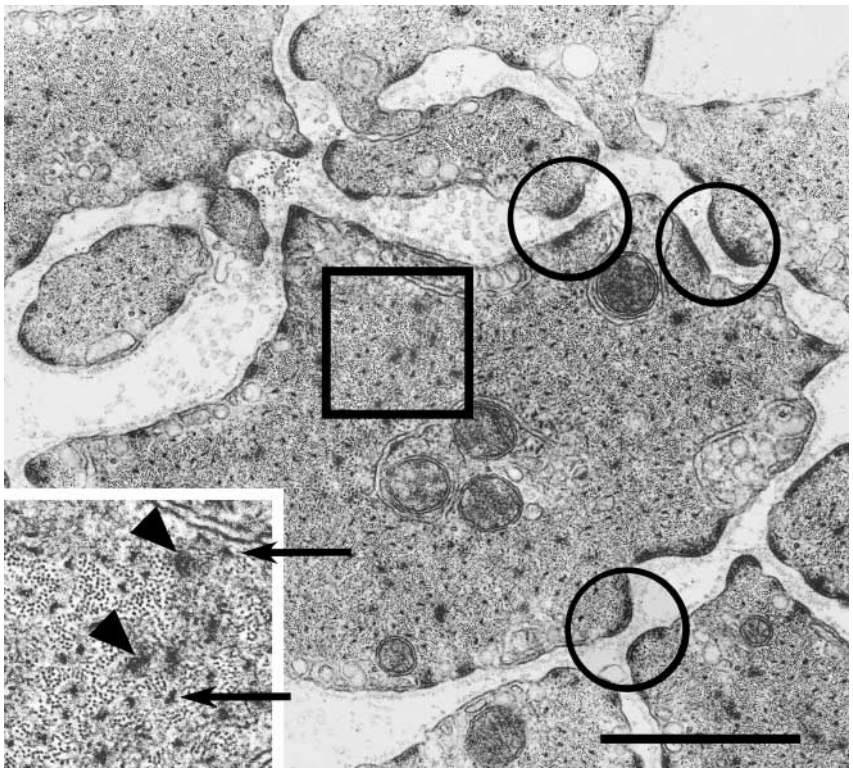

Fig. 1. Electron micrograph of a transverse section of trachealis cells. Examples of intermediate junctions are circled. Cross-sectional profiles of myosin and actin filaments are visible in the magnified area (square). Arrows point to myosin filaments surrounded by actin filaments. Arrowheads point to dense bodies. Scale bar, 1 μm.

Materials and Methods

Muscle preparations

Tracheal smooth muscle from adult pigs was used for the study. The swine tissues were obtained from a local abattoir. Small bundles of trachealis (~0.3×2×6 mm) were dissected from tracheas; aluminum clips were put on each end of the muscle strip for mounting onto the myograph. To ensure that we were dealing with viable muscle cells, the preparations were first 'equilibrated' at their in situ length in 37°C physiological saline (118 mM NaCl, 22.5 mM $NaHCO_3$, 5 mM KCl, 1.2 mM NaH_2PO_4, 2 mM $MgSO_4$, 2 mM $CaCl_2$ and 2 g l^{-1} glucose) bubbled with 95% O_2 / 5% CO_2 mixture. During the equilibration period, brief tetani (12 seconds) were induced by electrical stimulation once every 5 minutes. The muscle was considered viable and equilibrated when the isometric force produced by the muscle reached a steady maximal value within the normally expected range (120-250 kPa).

Fixing solutions and tissue fixation for electron microscopy

For electron microscopy, the fixing solution contained 1.5% glutaraldehyde, 1.5% paraformaldehyde and 2% tannic acid in 0.1 M sodium cacodylate buffer that was pre-warmed to the same temperature as the bathing physiological saline solution (37°C).

Tissue samples were prepared for electron microscopy as previously described (Kuo et al., 2001; Herrera et al., 2002). Briefly, muscle preparations were fixed for 15 minutes while they were still attached to the experiment apparatus. Care was taken not to disrupt the muscle physically during fixation. After the initial fixation, the strip was removed from the apparatus and cut into small blocks, approximately 1×0.5×0.3 mm, and put in the fixing solution for 2 hours at 4°C on a shaker. The blocks were then washed three times in 0.1 M sodium cacodylate (10 minutes each). In the process of secondary fixation, the blocks were put in 1% OsO_4, 0.1 M sodium cacodylate buffer for 2 hours followed by three washes with distilled water (10 minutes each). The blocks were then further treated with 1% uranyl acetate for 1 hour (en bloc staining) followed by washes with distilled water. Increasing concentrations of ethanol (50%, 70%, 80%, 90% and 95%) were used (10 minutes each) in the process of dehydration. 100% ethanol and propylene oxide were used (three 10-minute washes each) for the final process of dehydration. The blocks were left overnight in the resin (TAAB 812 mix, medium hardness) and then embedded in molds and place in an oven at 60°C for 8-10 hours. The embedded blocks were sectioned on microtome using a diamond knife and place on 400-mesh cooper grids. The section thickness was ~100 nm. The sections were then stained with 1% uranyl acetate (4 minutes) and Reynolds lead citrate (3 minutes). Images of the cross-sections of cells were made with a Phillips 300 electron microscope.

Immunoelectron microscopic localization of vinculin

The trachealis strips were quickly plunged into propane precooled by liquid nitrogen. The frozen tissues were then transferred into absolute ethanol (precooled by liquid nitrogen) and kept at –80°C for 7 days. After the 7-day freeze-substitution, the muscle strips were slowly transferred from –80°C to –20°C, –4°C and room temperature. The muscle strips were cut into small blocks (~1×1×0.3 mm) and embedded within LR White. After polymerization at 50°C for 6 hours, the blocks were sliced at a thickness of 100 nm and collected on 400-mesh grids. Sections were pretreated with a solution containing 4% normal goat serum and 0.1% Triton X-100, rinsed several times, and then incubated in primary antibody (anti-vinculin) at a dilution of 1 μg ml^{-1} for 12 hours at 4°C. The sections were then rinsed and incubated in secondary antibody conjugated with 10 nm gold particles at a dilution of 1:100 for 2 hours at room temperature. Before examination on an electron microscope operated at 60 kV, sections were stained with 1% aqueous uranyl acetate.

Results

General orientation of the contractile filaments with respect to cell axis and cell-cell connection

Smooth muscle cells normally aggregate into small bundles within which the cells lie parallel (with respect to their longitudinal axes) to one another (Bagby, 1983); so do trachealis cells. Mechanical connections among the cells are provided by intermediate junctions that contain two opposed dense plaques from adjacent cells. Fig. 1 shows a transverse section of trachealis cells within a muscle bundle. Mechanical couplings between cells are evident (examples of intermediate junctions are circled). Contractile filaments (actin and myosin filaments) seen in the section are perpendicular to the cell cross-section (inset). To see the filament orientation with

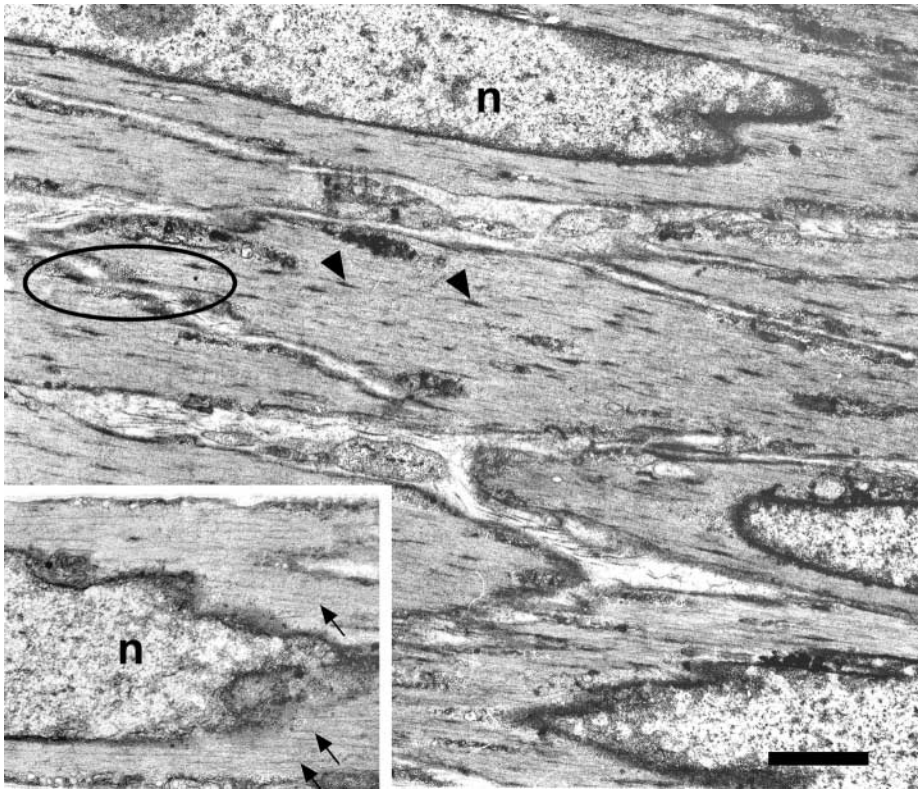

Fig. 2. Electron micrograph of a longitudinal section of trachealis cells. The general orientation of contractile filaments and dense bodies (arrowheads) aligns with the longitudinal axes of the cells. An example of intercellular coupling of dense plaques is highlighted (oval). The inset shows myosin filaments (arrows) near a nucleus (n). Scale bar, 1 μm.

respect to the cell's long axis, longitudinal sections were obtained; an example is shown in Fig. 2, for which a section was obtained by cutting the muscle bundle at a 15° angle to the longitudinal axis. Nearly 100 longitudinal sections were obtained with cutting angles (with respect to the longitudinal cell axis) ranging from zero and 30°. As shown in Fig. 2, the dense bodies and filaments are typically lined up with the axis of force transmission, which is also the longitudinal axis of the muscle bundle. The filaments lie parallel to one another; the parallelism is not interrupted by cell boundaries or nuclei. When filaments run into cell membrane, they attach to dense plaques; the plaques are located all over the cell surface and not just in the region of tapered ends (Figs 1, 2). Continuity of force transmission across cell boundary is accomplished through couplings provided by pairs of opposed adjacent dense plaques located on neighboring cells; such an example is shown in Fig. 2 (oval), in which contractile filaments in both cells attach to the inner aspects of the dense plaques that, in turn, are connected extracellularly by amorphous electron-dense material. The prevailing filament orientation is not altered by the intervening cell boundary. When filaments run into a nucleus, there is no evidence of circumvention (Fig. 2, inset). There is evidence that contractile filaments attach directly to nuclei, as shown below. There is evidence that thick filaments circumvent organelles such as mitochondria and sarcoplasmic reticulum, especially near the nuclear poles. All the muscle bundles were fixed near their in situ length. In all the longitudinal sections of trachealis examined, parallel arrangement of contractile filaments with the cells' long axes was observed. Similar observations were made in intact bundles of guinea-pig taenia coli (Gabella, 1977) and vas deferens (McGuffee et al., 1979).

Thick-filament distribution within a cell and thin-filament attachment to nuclei

Fig. 3 shows three transverse sections (out of a series of 11 consecutive slices with an average thickness of 100 nm per slice) of a trachealis cell. We counted the number of myosin thick filaments in the whole cross section for each of the 11 serial sections and correlated the filament number to the fraction of area occupied by the nucleus. We found an inverse relationship between the number of myosin filaments and the nuclear area (Fig. 4), suggesting that the number of myosin filaments in cross sections of different segments of a cell is not a constant and that part of the contractile filament arrays ended where the nucleus started.

A high-magnification electron micrograph of a region near the nuclear pole of an actively shortened trachealis cell shows direct thin filament attachment to the nuclear envelope (Fig. 5). The attachment sites on the envelope are patchy; pulling by the actin filaments appears to be responsible for the 'folds' seen on the nuclear membrane. Antivinculin immunogold labeling reveals possible actin filament attachment sites on the nuclear envelope (Fig. 6).

Near the two poles (ends) of a nucleus, clusters of mitochondria and other membranous organelles are often found. Although myosin filaments are not seen within the clusters, actin filaments are often observed to go through the clusters and are likely to attach to the nuclear envelope (Fig. 7).

Elasticity of the nuclear envelope

To determine whether the force generated by the contractile filaments acted on the nucleus, we examined the longitudinal lengths of nuclei in two groups of trachealis preparations: relaxed and isometrically contracted (i.e. activation of muscle at a constant length). Each pair of the muscle strips was obtained by bisecting a rectangular piece of trachealis to ensure that both strips had the same length. One strip was fixed for electron microscopy in the relaxed state, whereas the other was fixed in an isometrically (constant length) contracted state. The final muscle contraction was induced by addition to the muscle bath 0.1 mM of acetylcholine to ensure continued activation during fixation. Muscle strips were fixed at the plateau of contraction (120 seconds after stimulation) while they were

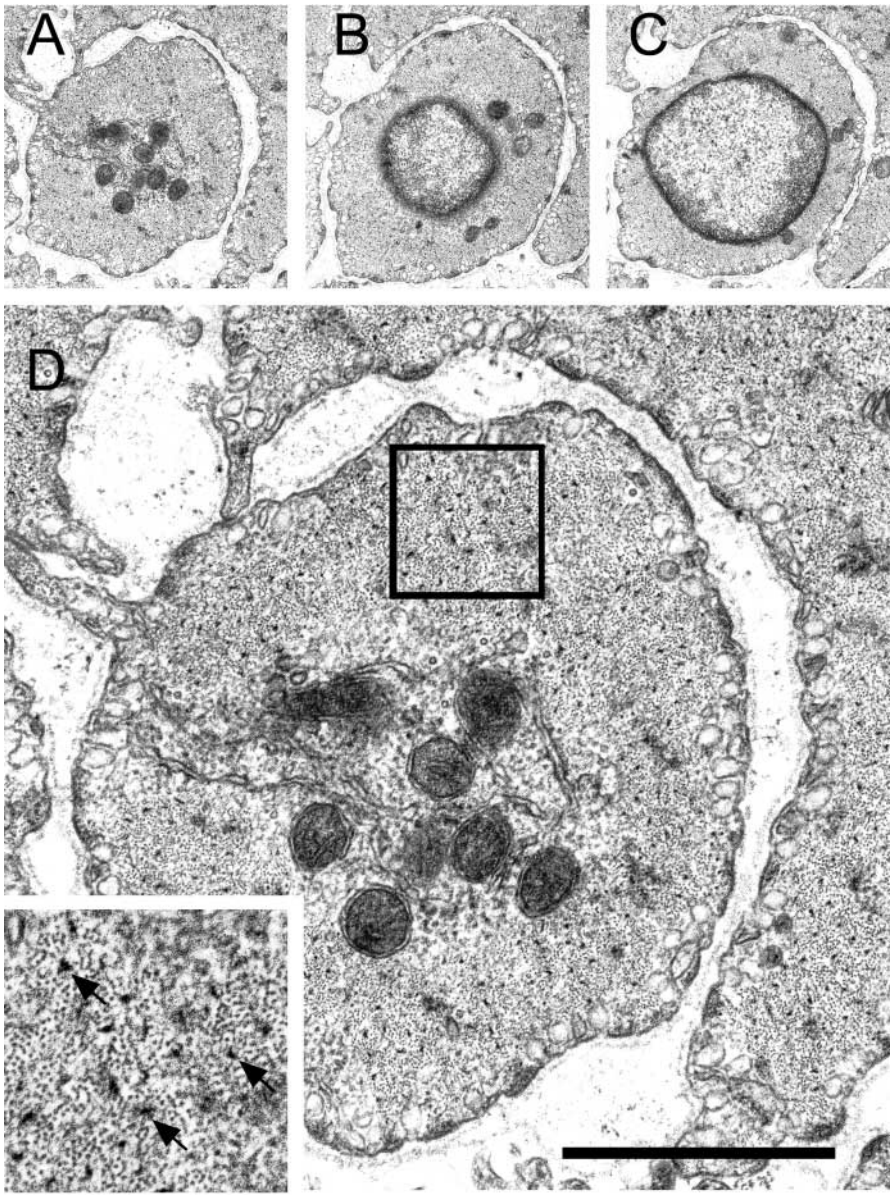

Fig. 3. Electron micrographs showing a series of transverse sections from one trachealis cell. (A) Transverse section near the nuclear pole with a cluster of centrally located mitochondria and other organelles. (B) Transverse section cutting across the tip of the cigar-shaped nucleus. (C) Transverse section showing full cross-section of the nucleus. (D) Enlarged version of (A). The inset shows myosin thick filaments (arrows) surrounded by actin thin filaments. All cross sections come from a series of 11 consecutive sections (~100 nm in thickness for each section). Scale bar, 1 μm.

still attached to the experiment apparatus (Herrera et al., 2002; Qi et al., 2002) and force generated by the muscle was monitored throughout the period of initial fixation (15 minutes). In the contracted muscles, after reaching a force peak, there was a small decline in force (<5% maximal force) during fixation. Longitudinal lengths of the nuclei from the two groups (relaxed and contracted) were measured from electron micrographs (Fig. 8). The averaged values from five pairs of muscle preparations from five tracheas were 9.22±0.12 μm and 11.36±0.12 μm for the relaxed and activated groups, respectively. The ratio of the activated over the relaxed group indicated a 23.4±1.7% (s.e.m.) increase in the longitudinal lengths of nuclei, suggesting that activated contractile filaments (located between the nucleus and either ends of the cell) were exerting tension on the nucleus. The strain of the nucleus under maximal isometric force was therefore 0.234±0.017.

To calculate the axial tensile strength of the nuclear envelope, we first determined the stress in the activated muscles by dividing the active isometric force (88.3±11.0 mN, n=5) by the cross-sectional area occupied by the muscle cells. In these trachealis preparations, muscle cells on average occupied 76±9.6% of the total cross-sectional area, the balance of the area was occupied by connective tissue. The averaged active stress in the muscle cells was calculated to be 196±10 kPa (n=5). If we assume that the tensile stress is uniform across the cell cross-section then the average stress on the nucleus is the same as that on the rest of the cell (196 kPa). The elasticity of the nucleus under isometric stress can be calculated as 196±10 kPa ÷ 0.234±0.017, which gives a value of 837.6±74.4 kPa. The stress, however, might not be evenly distributed in a cell cross section. For example, in the cell illustrated in Fig. 3, the proportion of nuclear area increased from 0% to ~60% of the total area, the total number of myosin filaments per cell cross-section decreased only by ~47%. If force is proportional to the

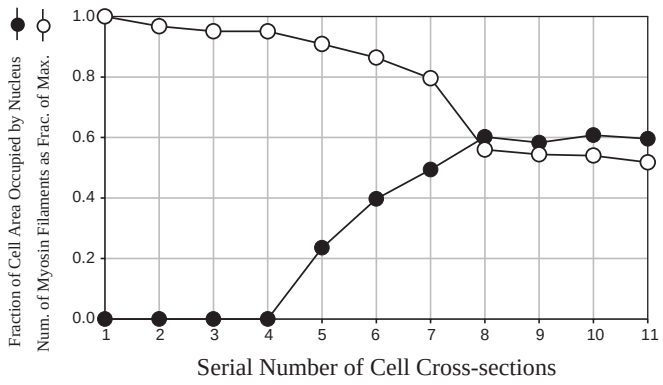

Fig. 4. A plot of cell cross-sectional area occupied by the nucleus and the number of myosin thick filaments found in the cytoplasmic area in 11 consecutive serial sections (three of which are shown in Fig. 3).

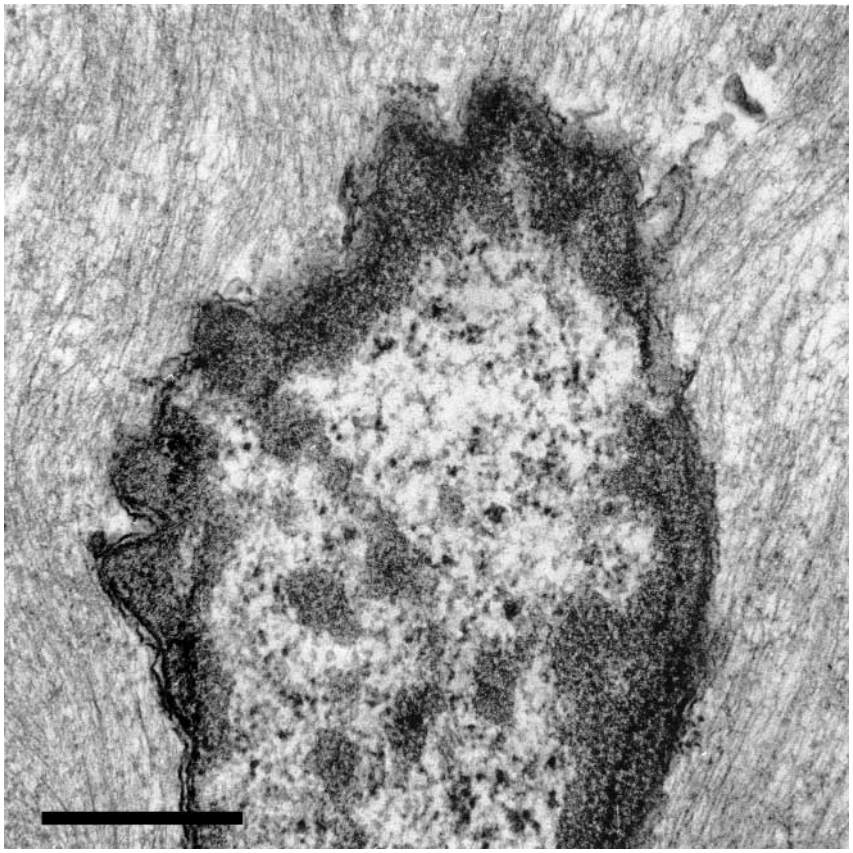

Fig. 5. Electron micrograph showing actin filaments attaching to nucleus in an isotonically shortened trachealis cell. Scale bar, 1 μm.

number of myosin filaments, as suggested by our previous observations (Kuo et al., 2001), this means that force transmitted through the cytoplasmic area surrounding the nucleus in this particular cell is 53% of the total force generated by the cell, which leaves 47% of the force going through the nucleus. The disparity between the percentage area occupied by the nucleus (60%) and the force transmitted through it (47% of total force) means that the stress on the nucleus is about 78% (i.e. 0.47/0.6 ≈ 0.78) that of the averaged stress in this particular example. We examined the fractional areas occupied by the nuclei in cell cross sections morphometrically in the five trachealis strips fixed in the contracted state. In 40 cell cross sections showing full nuclei (eight cells per strip), we found that the fraction of total cell area occupied by the nucleus was 0.549±0.026. The number of myosin filaments in these sections expressed as a fraction of the number of myosin filaments in a separate group (but from the same tracheas) of 40 cell cross-sections without nuclei, was 0.551±0.061. This means that, although the nuclear area on average is slightly greater than 50% of the total cell area, the number of myosin filaments attaching (via actin filaments) to the nucleus is on average slightly less than 50%. The stress on the nucleus is therefore less than the average stress calculated for the whole cell. Assuming that isometric force is proportional to the number of myosin filaments in a cross-section, a correction factor can be obtained: (0.449±0.061) ÷ (0.549±0.026) = 0.818±0.118. With this correction, we obtained a corrected stress on the nucleus (160.3±24.5 kPa) and a stiffness of 685±116 kPa for the nuclear envelope. This axial stiffness of the nuclei is close to the modulus of elasticity of elastin fibers [~600 kPa (Fung, 1993)].

Discussion

Morphological and functional evidence gathered in this study supports the notion that individual cells in an airway smooth muscle bundle might not be a 'self-contained' functional unit of force generator. Non-uniformity in terms of the number of myosin filaments per cell cross section in individual cells is prevalent. This raises the question of how smooth muscle as a tissue maintains mechanical equilibrium (i.e. uniform force generation and transmission) along its entire length. Another question stemming from the observations is where the obliquely oriented filaments are that are supposed to be a standard feature of filament organization in smooth muscle?

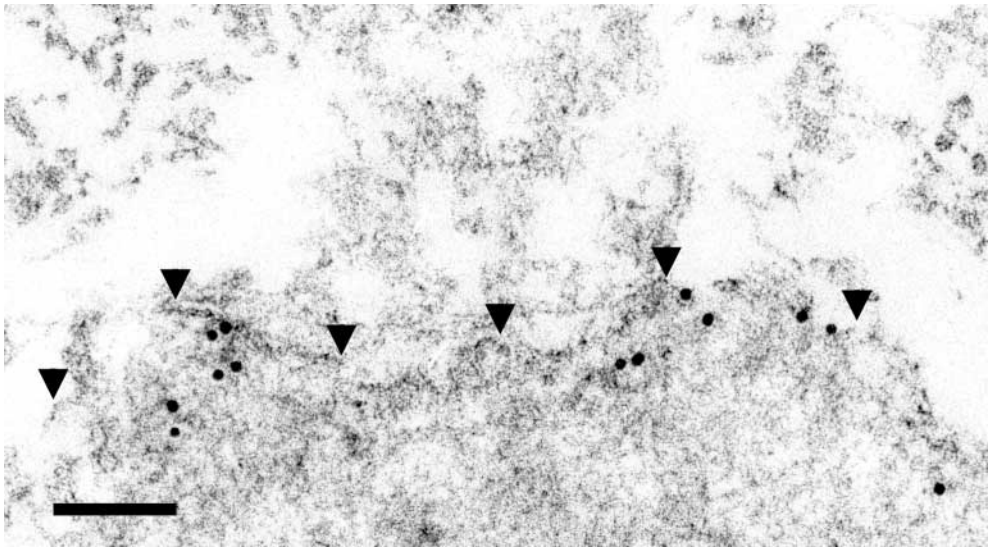

Fig. 6. Electron micrograph of a transverse section of quickly frozen trachealis cell labeled with antivinculin primary antibodies and 10-nm-gold-particle-conjugated secondary antibodies. Arrowheads indicate the nuclear envelope. Scale bar, 100 nm.

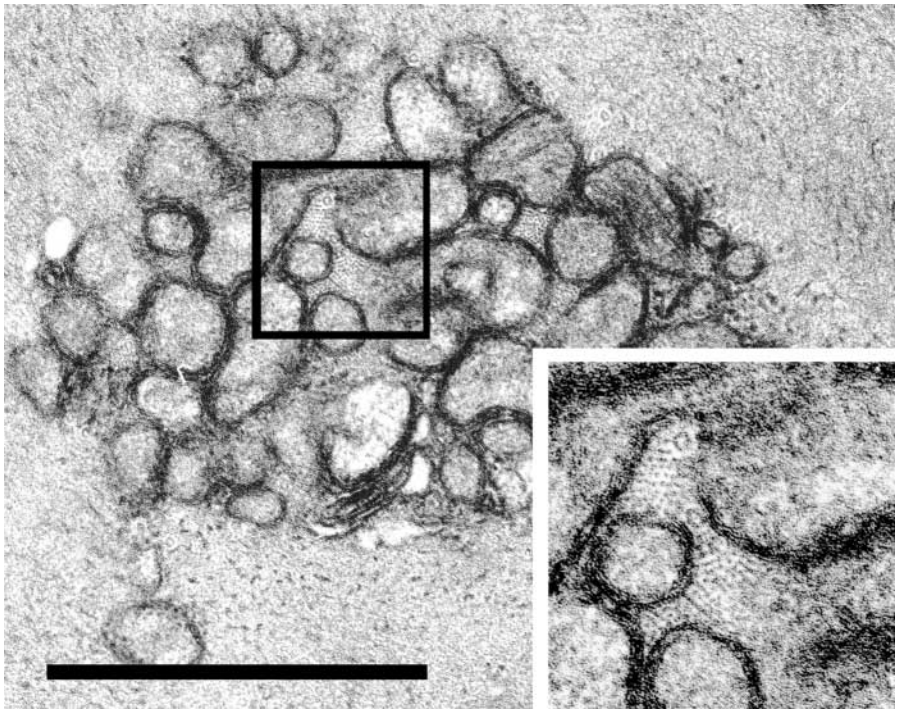

Fig. 7. Electron micrograph of a transverse section of trachealis cell showing a cluster of mitochondria and other organelles. The enlarged area shows microtubules (hollow, 25 nm diameter) and actin thin filaments (solid, 6 nm diameter) going through the cluster. Scale bar, 1 μm.

Perhaps the oblique angle is too shallow to be detected, as suggested by Small and Gimona (Small and Gimona, 1998); if this is true then the functional significance of oblique filament arrangement in intact smooth muscle cells in situ might be doubtful. A final question is whether we need to incorporate oblique filament architecture in models for intact smooth muscle cells residing in tissue, especially when there is no conclusive structural evidence for such an arrangement. It should be pointed out that the angled filament arrangement in many proposed models stemmed from functional considerations and based on observations made in isolated cells, as discussed below, and many models depicted exaggerated angles of filament orientation with respect to the longitudinal cell axis (Small and Gimona, 1998).

Early models of contractile filament organization in smooth muscle cell

A very good discussion of various models of the smooth muscle cell is provided by Bagby (Bagby, 1983), with one of the earliest models proposed by Rosenbluth (Rosenbluth, 1965). The model featured parallel contractile filaments inserted obliquely into the sides of the cell. This type of filament arrangement could explain the relatively high force and low shortening velocity produced by smooth muscle compared with those produced by striated muscle. Based on studies on isolated smooth muscle cells, Fay and Delise (Fay and Delise, 1973) proposed a model similar to that of Rosenbluth, with obliquely oriented filaments crisscrossing one another. A three-dimensional model that incorporated the main features of the models of Rosenbluth (Rosenbluth, 1965) and of Fay and Delise (Fay and Delise, 1973) was proposed by Fisher and Bagby (Fisher and Bagby, 1977) (reviewed by Bagby, 1983). Later models all had a common feature: obliquely oriented contractile filaments. The oblique arrangement offered a convenient way for contractile filaments to circumvent the relatively large and centrally located nucleus (Bagby, 1983). A criticism can be raised against all of the models mentioned above that there is considerable evidence against angled filament alignment in intact smooth muscle bundles from the present study and from other early studies (Shoenberg and Needham, 1976; Gabella, 1977; McGuffee et al., 1979). The findings of the present study also suggest that most contractile filaments do not circumvent the nucleus.

Although there is little evidence for oblique filaments in intact smooth muscle cells in a tissue bundle (especially under tension), convincing evidence can be found in isolated cells that shows myofibrils with distinct angles to the longitudinal axis of the cell (Small, 1974; Small, 1977; Draeger et al., 1990). We have no good explanation for the discrepancy. The attachment of contractile

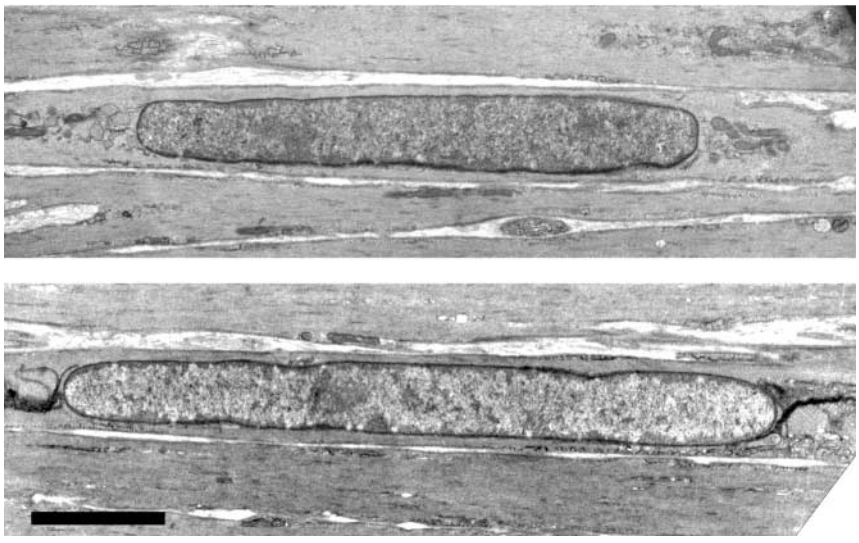

Fig. 8. Longitudinal sections of trachealis cells fixed in the relaxed (top) and contracted (bottom) state. The average lengths of the nuclei measured from 40 such pictures for each condition were 9.22±0.12 μm (s.e.m.) and 11.36±0.12 μm, respectively, for relaxed and contracted cells. Scale bar, 2 μm.

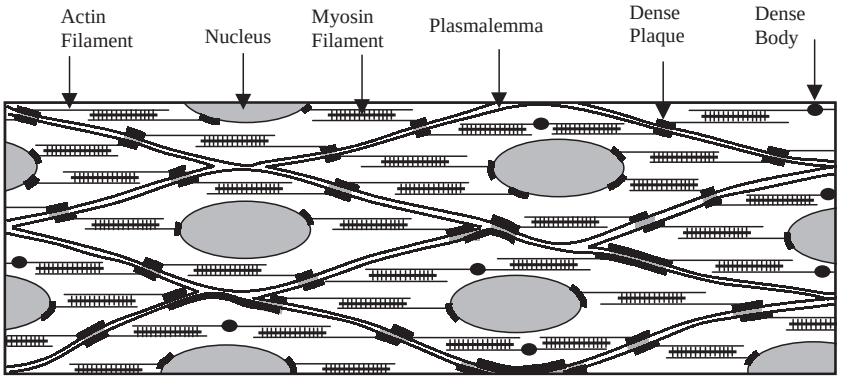

Fig. 9. Schematic representation of contractile filament architecture in a bundle of airway smooth muscle cells.

filaments to cytoskeleton has recently been shown to be dynamic (reviewed by Gunst and Fredberg, 2003). It is conceivable that, without extracellular tethers, rearrangement/reattachment of contractile filaments (with respect to the cytoskeleton) could occur in isolated cells. This rearrangement could produce angled filaments and the corkscrew-like behavior or undulation seen in isolated cells during shortening (Fisher and Bagby, 1977; Warshaw et al., 1987). In an evenly dissected trachealis bundle, we have never observed twisting of the preparation during shortening or in isometric contraction. Examination of data similar to those presented in Fig. 8 failed to yield evidence of twisted filaments. Intact cells in a bundle are mechanically coupled to one another and to extracellular matrix (Figs 1, 2); it is unlikely that individual cells will undergo corkscrew-like shortening without the whole tissue undergoing the same motion.

Proposed model of filament architecture in a bundle of trachealis – a mechanical syncytium

A possible answer to the questions brought up in the above discussion and a possible explanation for the inconsistency between models developed from isolated cell preparations and ultrastructural evidence from intact cell bundles is shown in Fig. 9. To achieve uniform force transmission along the length of a muscle bundle, the same amount of force has to be transmitted across each cross section (perpendicular to the direction of force transmission) of the bundle. In this proposed model, force is actively generated and carried by the contractile filaments and passively borne by the nuclei. This arrangement allows an aggregate of 'non-uniform' cells to form a functional unit, a syncytium, in which force can be generated and transmitted uniformly. Evidence supporting the model includes the observation that the prevailing orientation of the contractile filaments in a muscle bundle is maintained from one end of the bundle to the other, with the orientation not altered by the presence of irregular cell boundaries and the nuclei. This model excludes the need for obliquely oriented filaments; it can, however, accommodate oblique filaments as long as the resultant vector for all vectors representing the directions of all filaments is parallel to the direction of force transmission.

If smooth muscle cells within a tissue function as a mechanical syncytium, mechanical equilibrium of force transmission in individual cells is no longer required. This removes a crucial constraint on the modeling of filament architecture in a single cell (as part of a tissue bundle). For instance, force generated in the tapered region of a cell can be much less than the force generated in the mid-region. In Fig. 1, the thick and thin filament numbers in the tapered ends (small cell cross-sections on the upper left) are much less than those seen in the cross section near the mid-segment (large cell cross-section in the middle). This is consistent with the model proposed in Fig. 9 and no special filament arrangement is required to account for the apparent difference in the number of contractile filaments found in different cell cross-sections if the individual cells belong to a mechanical syncytium. An implicit assumption made here is that force transmitted through a cross section is proportional to the number of myosin filaments found in that cross section. The only supporting evidence we know is from our previous studies (Kuo et al., 2001) that showed parallel change in thick filament density and isometric force. It is possible that, under some circumstances, the thick filament density might not be a good index of force: the thick filaments might have different lengths; they might be longer and thus able to generate more force near the tapered ends of a cell. Another possibility is that active force generated by contractile filaments in one segment of a cell is transmitted by intermediate filaments (as part of the cytoskeleton) in another segment. In that case, myosin filament density in a cell cross section will not correlate with force transmitted through that section. An argument against this scenario is that the distribution of myosin thick filaments within intact cells in a muscle bundle is generally quite even (Kuo et al., 2001).

An implication of the proposed model of mechanical syncytium is that isolated single cells of smooth muscle might not be a good model for studying mechanical function, because smooth muscle cells are not designed to function as individuals. However, under certain conditions (for example, when an isolated smooth muscle cell is long and the tapered ends are not included in the mechanical measurement), reliable results can still be obtained (Warshaw and Fay, 1983).

Mechanical integrity of the nuclear envelope

The nuclear envelope of all eukaryotic cells consists of the nuclear membrane and the underlying fibrous layer called nuclear lamina (Moir et al., 1995). Cytoskeletal proteins and intermediate filaments form an extensive network that connects the nuclear envelope to the cortical cytoskeleton underlying the plasma membrane (Djabali, 1999). Unlike that in skeletal muscle, the relatively large and centrally located nucleus of smooth muscle interrupts the array of contractile filaments spanning from one end of the cell to the other. Mechanical equilibrium requires that force transmitted by the contractile filaments along the cell length be continuous and uniform. This means that the contractile filaments must either go around the nucleus or anchor onto the nuclear envelope and use the envelope as a passive structure to transmit force. Evidence from the present study suggests that the latter is probably the

case. This implies that physical integrity of the nuclear envelope is important for smooth muscle's mechanical function. Anchoring of actin filaments to nuclear envelope has been described recently (Zhen et al., 2002; Starr and Han, 2003). Our finding that vinculin (or a vinculin-like protein that cross reacts with anti-vinculin antibodies) is located near the vicinity of the nuclear envelope is consistent with the picture of actin-based mechanism for mechanically connecting the nucleus to the outside world.

Stretching a relaxed trachealis strip also caused an increase in the average length of the nuclei (results not shown). Although one can get an estimate of the stiffness of the nuclear envelope by measuring the force of stretch and the amount of nuclear elongation, there are factors that complicate the analysis. The nucleus is connected to the rest of the cell in all directions through a network of intermediate filaments and cytoskeletal proteins (Bagby, 1983); stretching a cell longitudinally will result in forces acting axially on the nuclear poles as well as a lateral compression of the nucleus, and both contribute to the elongation of the nucleus. Without knowing the exact architecture and mechanical properties of the cytoskeletal network, it is impossible to separate the contributions to nuclear elongation by the axial and lateral forces, and this renders the estimate of the axial stiffness inaccurate. The use of force generated by the contractile filaments in an isometric contraction simplifies the analysis because the muscle length is not changed and therefore no lateral force is involved.

A compliant nuclear envelope will result in substantial internal movement when the muscle is activated and lead to low mechanical efficiency. The elongation of the envelope in airway smooth muscle under isometric tension is about 2 μm. In a 100-200 μm cell, this represents a negligible 1-2% change in cell length.

The ultrastructure of nuclear 'ghosts' or nuclear envelopes without the membrane (Riley and Keller, 1978) has been examined and it appears that the structure is essentially the same in a great variety of cells derived from mammals, birds and plants. The present characterization of mechanical properties of nuclear envelope in airway smooth muscle might therefore be applicable to other cells types.

Nuclear envelope as a force-transmitting structure

Implications in disease

The finding that the nuclear envelope participates in the transmission of force in smooth muscle implies that laminopathies or nuclear envelopathies, a group of disorders linked to defects in genes encoding envelope-specific proteins such as A-type lamins (Flier, 2000; Wilson, 2000; Morris, 2001; Burke and Stewart, 2002), could involve dysfunction of smooth muscles and impairment of smooth-muscle-related regulation such as maintenance of blood pressure. Dilated cardiomyopathy has also been found in patients with laminopathy (Burke et al., 2001).

Currently, the mechanical integrity of a defective nuclear envelope can only be assessed morphologically by observing changes in the shape of the envelope (e.g. formation of blebs or herniations) (Vigouroux et al., 2001). The present study demonstrates a simple, straightforward way of accurately quantifying axial stiffness of the nuclear envelope under physiological strain. Mutations in genes that encode different envelope proteins can now be quantitatively studied to gain insights into the roles of the proteins in conferring integrity to the nuclear envelope.

Implications in signal transduction

Cyclic mechanical strains applied to cultured smooth muscle cells are known to enhance production of certain proteins by the cells (Smith et al., 2000; Lee et al., 2001). The strain-initiated signal transduction pathway in these cells has not been clearly delineated. Although 'stretch receptors' on the plasma membrane might be involved, our finding indicates that strains applied to smooth muscle cells can be transmitted directly to the nucleus, and the transmission is more effective if the muscle is activated. Cyclic isometric contraction and relaxation of smooth muscle cells can also lead to mechanical perturbation of the nuclei and might have a modulatory function on specific protein transcriptions. Although strain-initiated signal transduction targeting the nucleus could still be mediated through plasma membrane receptors, the present study suggests that this is not necessary; strain signals can be transmitted directly to the nucleus without any intervening steps that require transduction of mechanical signal into chemical or other types of signals before it reaches the nucleus.

Conclusions

In a bundle of intact airway smooth muscle cells, the orientation of contractile filaments are found to be parallel to both the common longitudinal axis of the cells and the direction of force transmission. The transcellular parallelism is maintained through the use of mechanical couplings between cells and nuclear envelopes as passive structures for force transmission. The finding indicates that the cells in a tissue bundle work as a mechanical syncytium and hence that, when separated, individual cells might not work properly. The oblique filament architecture depicted in many models of isolated smooth muscle cells might not apply to intact smooth muscle cells in their in situ environment. The finding that contractile filaments attach to nuclear envelope suggests that the physical integrity of the envelope is important for the cell's mechanical function; also, the attachment might provide a direct pathway that links the signal of mechanical strain to gene expression and other nuclear events.

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