Corkscrew-Like Shortening in Single Smooth Muscle Cells

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The slower and more economical contraction of smooth muscle as compared to that of skeletal muscle may relate to the arrangement of its contractile apparatus. Because the arrangement of the contractile apparatus determines the manner in which a single smooth muscle cell shortens, shortening of a contracting cell was examined by tracking of marker bead movements on the cell surface by means of digital video microscopy. Smooth muscle cells were observed to freely shorten in a unique corkscrew-like fashion with a pitch of 1.4 cell lengths (that is, the length change required for one complete rotation of cell) at a rate of 27 degrees per second. Corkscrew-like shortening was interpreted in terms of a structural model in which the contractile apparatus or cytoskeleton (or both) are helically oriented within the cell. Such an arrangement of these cytoarchitectural elements may help to explain in part the contractile capabilities of smooth muscle.

THE ABILITY OF SMOOTH MUSCLE TO actively shorten its length results from the cyclic interaction of myosin cross-bridges with actin, an interaction that generates sufficient force to slide neighboring actin filaments past the myosin filament. However, the arrangement of these filaments into specific contractile units and the orientation within smooth muscle cells of the contractile units are not well defined. Because smooth muscle contraction is characterized by a slower velocity of shortening and the ability to generate comparable or greater force per cross-sectional area of muscle with far less myosin than is found in skeletal muscle (*1*), knowledge of contractile unit arrangement and its interaction with the cytoskeleton in smooth muscle may help to explain these contractile property differences in the two muscle types.

Structural models have been proposed for the arrangement of contractile units within smooth muscle cells (*2*). Most of these models have a common feature, as first described by Rosenbluth (*3*), in that contractile units attach to the cell membrane and traverse the cell interior at small angles to the long axis of the cell. More recently, Small (*4*) and Fisher and Bagby (*5*) have proposed that contractile units are attached helically along the cell membrane. If their proposal is correct, one would predict that an isolated single cell that was fixed at one end and allowed to shorten freely would do so in a corkscrew-like manner. To test this hypothesis, we have enzymatically isolated single smooth muscle cells (*6*) from the stomach muscularis of the toad *Bufo marinus* and visually monitored the structural changes that occur during free shortening.

To characterize the shortening motion of isolated smooth muscle cells, we mixed a low calcium (0.18 m*M*) physiological salt solution (*6*) containing 10^5 cells per milliliter with a similar cell-free solution containing positively charged anionic-exchange resin beads 1 μm in diameter (9×10^6 per milliliter). The charged beads adhered strongly to the cell membrane and thus provided a means of decorating the cell surface with numerous visual markers (Fig. 1). Under a microscope and with the use of a micromanipulator, a single smooth muscle cell then was attached electrostatically at one end to a larger, 20-μm anionic-exchange resin bead that was glued to the end of a micropipette (*6*) (Fig. 1). To make certain that the entire cell length was in focus, a second micropipette, without a resin bead, was attached to a micromanipulator and used to lift the cell into the focal plane. The cell was then stimulated electrically to initiate contraction and induce cell shortening (*7*). As the cell shortened, its image was recorded on video tape and then replayed through a laboratory computer (IBM PC-XT), so that single frames were digitized (Coreco, Oculus 200) every 400 msec. The depth of field of the microscope objective (8.0 μm) allowed the bead images to be distinguished on all surfaces of the cell.

Our results demonstrate that, as predicted by the Small (*4*) and Fisher and Bagby models (*5*), single smooth muscle cells shorten in a corkscrew-like manner. The cell shortened by 30% of its initial length during the first 4.8 seconds after electrical stimulation at an average rate of 0.11 cell length per second (Fig. 1), a rate similar to that previously reported (*8*). However, when we

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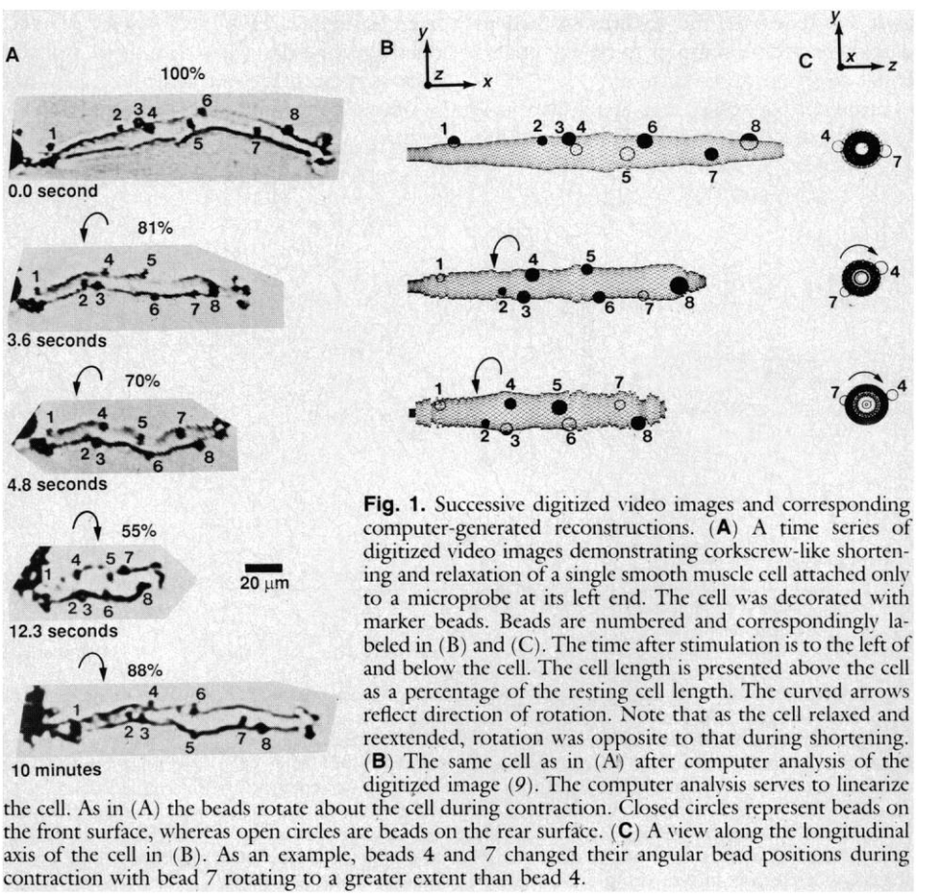

Fig. 1. Successive digitized video images and corresponding computer-generated reconstructions. (**A**) A time series of digitized video images demonstrating corkscrew-like shortening and relaxation of a single smooth muscle cell attached only to a microprobe at its left end. The cell was decorated with marker beads. Beads are numbered and correspondingly labeled in (B) and (C). The time after stimulation is to the left of and below the cell. The cell length is presented above the cell as a percentage of the resting cell length. The curved arrows reflect direction of rotation. Note that as the cell relaxed and reextended, rotation was opposite to that during shortening. (**B**) The same cell as in (A) after computer analysis of the digitized image (*9*). The computer analysis serves to linearize the cell. As in (A) the beads rotate about the cell during contraction. Closed circles represent beads on the front surface, whereas open circles are beads on the rear surface. (**C**) A view along the longitudinal axis of the cell in (B). As an example, beads 4 and 7 changed their angular bead positions during contraction with bead 7 rotating to a greater extent than bead 4.

tracked marker bead images, it was apparent that their position on the cell surface changed during cell shortening (Fig. 1). To more accurately define single-cell shortening by monitoring changes in bead position, we developed a computer-based analysis of successive digitized images to determine (i) cell length, (ii) relative position along the cell length at which a bead was located, (iii) cell diameter at a bead location, and (iv) angular bead position on the cell circumference (*9*).

We investigated the method of cell attachment to the microprobe and possible nonuniformities of contraction after activation as possible explanations for the observed corkscrew-like shortening. To control for the mode of attachment, decorated cells resting on a glass slide were also observed to rotate during contraction. With regard to nonuniformities in activation, relative bead position along the cell length as a function of the percentage of cell shortening (Fig. 2A) indicated that no significant shifts in the relative bead positions occurred during shortening. Thus, for a bead to maintain a constant relative position along the cell length as the cell shortened, uniform cell shortening must have occurred. Although the relative position of the bead along the cell length remained constant during shortening, significant changes in the angular position of a bead on the cell surface were observed that indicated that the cell rotated as it shortened. In the 15 cells studied to date there does not appear to be any preferential direction of rotation.

From data on the change in angular position of a bead, it appeared that beads at different relative positions along the cell length rotated at varying speeds after activation (shown as different slopes in Fig. 2B), and that the maximum rate of angular change increased the farther the bend was from the fixed cell end (Figs. 1C and 2D). These changes in bead angular position were also used to describe the corkscrew-like shortening of the cell in terms of a helical pitch. An estimate of bead pitch was obtained from the slope of the relation between the angular change of a bead and the percentage of cell shortening after activation (Fig. 2C). For the cell in Fig. 1, a bead pitch of 1.4 ± 0.1 ($n = 7$) cell lengths was obtained during the initial 20% of cell shortening. After contraction, cells were observed to spontaneously relax and reextend to within 90% of their original length (Fig. 1A), as previously observed in this preparation (*10*). The reextension suggests that during contraction some structure within the cell may be compressed and that once active contractile force is removed the stored compressive forces reextend the cell. During this reextension, marker beads reversed their direction of angular change, suggesting an uncoiling of the cell's helical pattern of shortening. Although isolated cells provide a useful model for smooth muscle behavior, the isolation procedure could induce significant changes to the cellular structure, a change that could lead to the observed corkscrew-like shortening. However, studies of contractile properties (*6, 8*) in these isolated cells suggest that contractile function has not been compromised by the isolation procedure.

Although it may be premature to analyze our data concerning cell surface events in terms of interactions between the cytoskeleton and contractile apparatus, it is reasonable to assume that surface events are governed by the arrangement of the underlying cellular architecture. A functional link between the cell surface and contractile apparatus in smooth muscle cells may be supposed because actin filaments attach to amorphous dense plaques on the cell membrane (*11*) and, upon contraction, large membrane evaginations form as a result of inwardly directed forces exerted on the membrane at these attachment points (*10, 12, 13*). Thus surface marker movements must be coupled in part to the behavior of the contractile apparatus.

In addition to the contractile apparatus, the cytoskeleton must play an important role in the contractile function of a cell. Therefore, it is important to consider how the arrangement of the cell's cytoskeleton and contractile apparatus relate to the observed cell rotation upon shortening, reextension, and unwinding upon relaxation. One possible arrangement for these structures within the cell is in the form of a helix, as proposed by Small (*4*) and Fisher and Bagby (*5*). Although it is not discernible from the bead rotational data whether the cytoskeleton or contractile apparatus is helically oriented within the cell, the bead data can be interpreted within the framework of at least one structural model (Fig. 3) in which the observed 1.4–cell length bead pitch is the result of a cytoarchitecture that is helically attached to the cell membrane. If it is as-

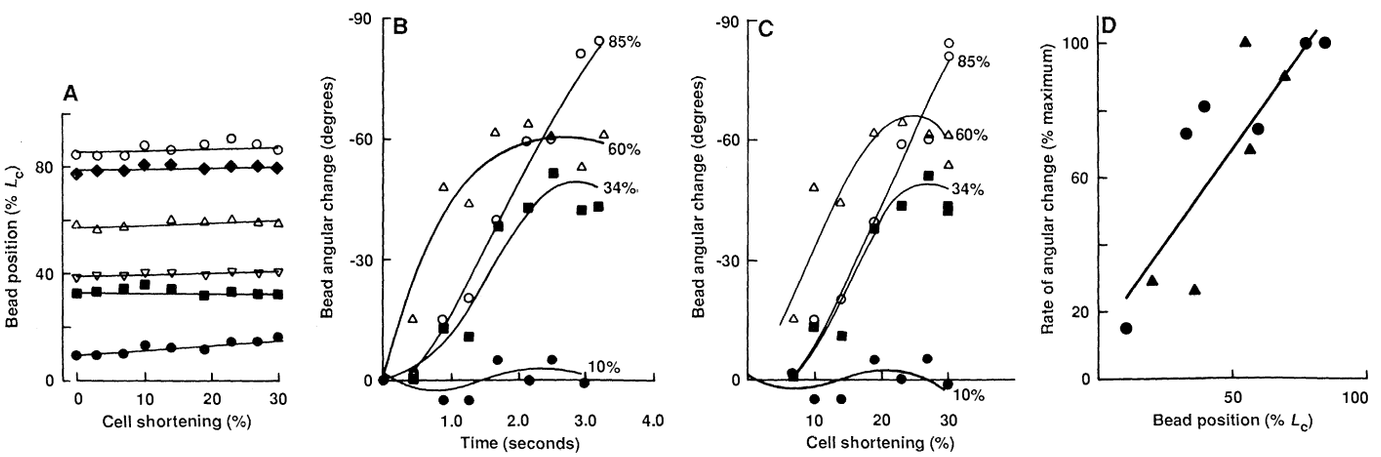

Fig. 2. Graphs representing the contraction parameters for free shortening in smooth muscle cells. (**A**) Relative position of bead along cell length (L_c) as a function of percentage of cell shortening for the cell in Fig. 1. (**B**) Change in bead angular position on cell circumference as a function of time of contraction. The symbols and relative position of the bead as a percentage of L_c correspond to the beads in (A). Curves are the best fit to a third-order polynomial. The angular change for the bead at 85% L_c was not measured until 0.8 second after contraction because of its position on the top surface of the cell where errors in estimating bead angular position are large (*9*). (**C**) Change in bead angular position as a function of the percentage of cell length. The symbols and relative position of the bead (as a percentage) correspond to the beads in (A). From these data a value for the pitch, describing the bead motion, was calculated by dividing the maximum slope determined from the polynomial fit by 360°. (**D**) The rate of normalized bead angular change as a function of the relative position of the bead beginning at the fixed end. Individual data points were obtained from the maximum slope for curves in (B) (filled circles). Triangles are data from another cell.

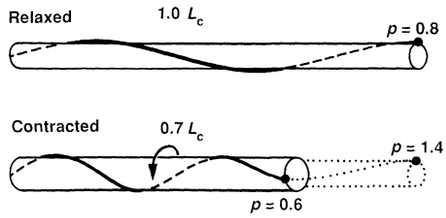

Fig. 3. Model for a helically oriented cytoarchitecture that may explain the observed pitch in bead position. A model cell with a length-to-width ratio of 15 is represented by a cylinder. The helical attachment sites for a single group of cytoarchitectural elements are depicted as a solid helix on the cell surface, with the portion behind the model cell shown as a dashed line. In the relaxed cell, the helix is described by a pitch (p) in cell lengths (L_c) having a fixed arc length. As the cell contracts isovolumetrically, it is assumed that the cytoarchitecture remains attached to the cell membrane and that the arc length of the helix remains constant. The observed cell shortening and bead rotation for the cell in Fig. 1 are predicted if the helical pitch in the contracted cell decreases. The observed bead trajectory having a pitch of 1.4 cell lengths is depicted as a dotted line in the contracted cell model. The bead trajectory pitch is greater than the internal helix in both the relaxed and contracted cell.

sumed that during contraction both the arc length of the helical attachment and cell volume (*14*) remain constant, then the helical pitch must decrease and thus could give rise to the observed bead rotation on the cell surface. Given the cell data, a helical cytostructure having a pitch of 0.8 cell length in the resting cell would have to reduce its pitch to 0.6 cell length as the cell shortens by only 30% of its length. Evidence for a decrease in the pitch of the cell's cytoarchitecture upon contraction may come from the undulating pattern of contractile and cytoskeletal elements that is apparent only during contraction in these single cells (*4, 5, 15*).

Another conclusion based on our model is that beads located at the free end of the cell should rotate to a greater extent than beads closer to the fixed end. This in fact was observed (Fig. 2D). Finally, if we assume that the contractile apparatus is attached to this helix at any two points along the helix, then a maximum 9.5° angle is subtended by the contractile apparatus relative to the cell's long axis. This slight angle is due to the large length-to-width ratio (for example, 15) typical of these cells. Myosin filaments, a known component of the contractile apparatus, are observed in relaxed isolated or intact smooth muscle preparations at angles no larger than 10° to the cell's longitudinal axis (*2, 16*). In addition, after studying the cell surface patterns of fluorescently labeled vinculin, an actin-binding protein, Small (*17*) suggests that actin, a component of both the contractile apparatus and cytoskeleton, binds to the cell membrane along longitudinal "ribs" that are coaxial and not helical as he hypothesized earlier. However, there was no attempt to measure a pitch associated with these ribs, and, as with the myosin filaments, which appear to be longitudinally aligned in relaxed muscle, these structures may make shallow angles relative to the cell's long axis and thus be consistent with the corkscrew-like cell shortening we have seen.

At the other extreme, contractile filaments at very large angles to the long axis of the cell are characteristic of isolated cells that have freely shortened or of cells within a tissue contracting isometrically at short lengths (*2, 16*). The large angles could result from a more exaggerated reflection of the helically oriented cytoarchitecture. If we assume that the contractile apparatus is helically oriented (*4, 5*), then such an arrangement might in part explain smooth muscle's slower shortening velocity, as only a fraction of the shortening ability of the contractile unit would be realized at the cell ends. In addition, more contractile units would effectively be placed in parallel and thus increase the force per cross-sectional area in smooth muscle (*1, 3*). Thus a helically oriented contractile apparatus as well as longer contractile filaments and a cross-bridge cycle that is both slower and has a greater percentage of attached, force-generating cross-bridges (*1*) may all contribute to the slower and greater force-generating capabilities of smooth muscle.

Finally, is corkscrew-like shortening consistent with cell contraction within the intact tissue where constraints of the connective matrix are present? Data that support this view have been presented by Gabella (*13*), who studied smooth muscle structure of intact tenia coli after isotonic shortening of the tissue to 25% of its original length. Gabella observed a "twisting" of cells and suggested that torsional forces were placed on the cell during shortening. Thus data concerning the possible helical arrangement of the cytoskeleton and contractile apparatus are of great interest and may help explain the slow, economical contraction of smooth muscle cells either after isolation or in situ.

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9. Video images of cells were digitized into a 480 by 512 pixel array, with a spatial resolution of 0.7 μm horizontally and 0.6 μm vertically between pixels. Each pixel has 128 possible gray levels. Using a "mouse," the investigator traces the cell outline from the digitized video image. The digitized outline is smoothed by a least squares digital filtering routine to eliminate high-frequency noise associated with the investigator's hand motions. The midline of the cell, a line equidistant between the upper and lower cell surface outline, is determined, and its linear distance is used to estimate the cell length. In addition, the radius of the cell is determined at every point along its midline. The investigator then determines whether the bead is on the front or rear surface and locates the marker bead center and a point on the bead's circumference. With these data, the computer determines the relative position of the bead along the cell's length and angular position on the circumference of the cell. Angular position is calculated with the following equation: θ = arc sin $[h/(R+r)]$, where h is height of the bead center above the midline of the cell projected onto a vertical plane through the cell axis; R is cell radius at the bead location; and r is bead radius. The average error in estimating the angular position of the bead was 2% for beads positioned at angles between +60° and −60° off center, with 0° projected from the cell center toward the viewer. Thus measurements of bead motion at points near the upper and lower surfaces were neglected because of errors as large as 6% that arise in estimating bead angle at these locations.
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