Length adaptation of airway smooth muscle: a stochastic model of cytoskeletal dynamics

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The model

A rectangular area represents the smooth muscle cell and contains a regular grid of nodes connected by links (Fig. 1A). These nodes are taken to correspond to focal adhesions or dense plaques, and links are taken to correspond to stress fibers or contractile filaments. This is a minimal model comprising changes in actin filament length may play a key regulatory role. Gunst and colleagues (24–26, 44, 64) have shown evidence to suggest that it is alteration of the connection of the actin filament to the focal adhesion at the cell boundary and accompanying cytoskeleton reorganization that are influenced by the histories of activation and mechanical loading. Clearly, these possibilities are not mutually exclusive.

Still another notion is that secondary but important molecules stabilize the cytoskeleton and thus modulate malleability of the cytoskeletal domain (16, 24, 27). When exposed to contractile agonists, the airway smooth muscle cell reorganizes cytoskeletal polymers, especially actin (35). Activated cells become stiffer; although cell stiffening is attributable largely to activation of the contractile machinery, an intact actin lattice has been shown to be necessary but not sufficient to account for the stiffening response (1). In that connection a role for the Rho-A pathway has been suggested (45, 70), and some evidence now suggests that the p38 MAP kinase pathway may be involved (42). In particular, heat shock protein 27, a downstream target of Rho, has been implicated as an essential element in the motility and remodeling of the airway smooth muscle cell (20, 29–31, 71). The regulation of actin polymerization in airway smooth muscle seems to be mediated through the activation of neuronal Wiskott-Aldrich syndrome protein (N-WASP) (72). The activation of N-Wasp is regulated by the small GTPase cdc42, which is in turn regulated by paxillin phosphorylation (65, 66). These findings are consistent with the hypothesis that stress response pathways may somehow stabilize the airway smooth muscle cell.

To capture the essential features of length adaptation, here we propose a highly simplified model of cytoskeletal dynamics. The cytoskeleton is represented as an evolving network of links (contractile filaments or stress fibers) that connect nodes (dense plaques or focal adhesions). The network evolves in continuous turnover with probabilities of link formation and dissolution. The probability of link formation increases with the available fraction of contractile units, increases with the degree of network activation, and decreases with increasing distance between nodes, d, as 1/d^s, where s controls the distribution of link lengths. The probability of link dissolution decreases with time to mimic progressive cytoskeletal stabilization. We computed network force (F) as the vector summation of link forces exerted at all nodes, unloaded shortening velocity (V) as being proportional to the average link length, and network compliance (C) as the change in network length per change in elastic force. Imposed deformation caused F to decrease transiently and then recover dynamically; recovery ability decreased with increasing time after activation, mimicking observed biological behavior. Isometric contractions showed small sensitivity of F to network length, thus maintaining high force over a wide range of lengths; V and C increased with increasing length. In these behaviors, link length regulation, as described by the parameter s, was found to be crucial. Concerning length adaptation, all phenomena reported thus far in the literature were captured by this extremely simple network model.

Acknowledgments

Many authors have suggested that the airway smooth muscle cell disassembles its contractile apparatus and supporting cytoskeletal scaffolding when relaxed and reassembles that apparatus when activated and accommodated at a fixed length (3, 6, 19, 24, 44, 46, 51, 53, 56, 68). In doing so, the same high active force can be maintained over an extraordinary range of muscle lengths (14, 24, 39, 41, 47, 50–52, 55–57, 68). This malleability of the cell has been called by various authors mechanical plasticity, remodeling, accommodation, or length adaptation (2). The mechanisms by which these changes come about and the factors that control the rate of adaptation are unknown.

To account for these adaptations, Ford, Seow and colleagues (14, 32, 38, 39, 49, 51, 52, 55) have suggested that the architecture of the myosin filaments themselves may change, whereas Solway and coworkers (8, 60) have suggested that

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only three processes: link formation, link dissolution, and link deformation.

It has been shown that the cytoskeleton seems to be interconnected between cells through dense plaques, and thus it has been suggested that the smooth muscle tissue behaves as a mechanical syncytium (33, 40). Thus, although this model, defined below, is based on cellular phenomena, it can be equally taken as representing a section of the cytoskeleton, the cytoskeleton of a whole cell, or the whole muscle.

**Link formation.** Link formation depends on three factors: the pool of contractile units that is available, the degree of network activation, and the distance between the nodes that may be connected. Accordingly, each node $i$ may extend a link and connect any other node $j$ at a given time step with a probability $P_{ij}$ given by

$$P_{ij} = \frac{L_i - L_n}{L_i} (u + w) p \frac{1}{d_{ij}}$$

$L_i$ represents the total number of contractile units and $L_n$ is the number of contractile units that has been sequestered into the network by formed links. As such, the term $(L_i - L_n)/L_i$ is the fraction of contractile units still available for formation of newer links. Here we use “contractile units” as surrogate for all constitutive elements of the contractile apparatus, including actin, myosin, and regulatory proteins. The parameter $u$ represents a basal tendency to form links, whereas $w$ represents the degree of contractile activation, with $w = 0$ representing complete relaxation and $w = 1$ corresponding to full activation; $p$ is a scale factor, and $d_{ij}$ represents the distance between nodes $i$ and $j$. For $s = 0$, the probability to make a link is independent of the distance between nodes; conversely, for greater values of $s$ this probability decreases faster with increasing distance.

**Link dissolution.** The probability that any given link between two nodes ($i$ and $j$) may disconnect from one of the nodes and dissolve is given by

$$B_{ij} = b_0 + b_1 \exp(-rt)$$

where $b_0$ represents a “basal” probability and $b_1$ represents an additional probability that changes with time ($t$), thus the probability to link dissolution may be related to link dynamics.

Cytoskeleton matrix dynamics involves many proteins, their mutual interactions, and their connections to the contractile apparatus and to the dense plaques. We have recently proposed that such dynamics may fit within the framework of molecular trapping in deep energy wells and molecular hopping out of those wells driven by molecular jostling of nonthermal origin (4, 10, 11, 23, 54). This nonthermal jostling can be caused by many processes, including protein conformational changes fuelled by hydrolysis of ATP, and can be thought of as an effective temperature of the cytoskeleton matrix. This effective temperature, denoted by $x$, is experimentally accessible (Fig. 2) and is related directly to muscle hysteresivity ($\eta$) by $x = \arctan(2\eta/\pi)$ (10–12, 15, 23). It has been shown that $x$ is closely associated with the rate of matrix turnover (10–12), internal cell friction, shortening velocity, cross-bridge cycling rate (18, 23), and ATP utilization (23). There is also temporal coincidence between cytoskeleton progressive stabilization (16, 24, 27) and decreasing $x$. Accordingly, we represent the probability to dissolve a link by the function

$$B_{ij} = b_0 + b_1 \exp(-rt)$$

where $b_0$ and $b_1$ are constant values, $t$ is the time from onset of contractile activation or deactivation, and $r$ is a rate. As such, $B_{ij}$ peaks at $b_0 + b_1$ when $t = 0$ and decays afterward to the basal probability $b_0$, thus mimicking the variations of $x$ with time and cytoskeleton stabilization.

**Link deformation: the length-force relationship of each link.** The force-length relationship for a single smooth muscle contractile unit is unknown; however, the interaction between myosin and actin seems to be similar to that observed for skeletal muscles, but with the important differences that myosin is bipolar in skeletal muscle but side-polar in smooth muscle.
muscle and that their regulations differ (22). Thus contractile force is regarded as being proportional to the overlap between actin and myosin filaments. In the present model, maximum overlap is assumed for each link at the moment of its formation, which defines its optimal length. At that length the force exerted for any individual link is equal to 1 (all units in this model are arbitrary), independent of link length. If a link is deformed after its formation, three possibilities for the force-length relationship can be assessed. First, when the link length changes, its force becomes less than 1 and is given by

\[ f(D) = \frac{-D^2 + 2D + (m-1)^2 - 1}{(m-1)^2}. \]

\[ 1 - (m-1) \leq D \leq 1 + (m-1) \]

where \( D = l' / l \) is the link distortion, \( l' \) is the current link length, \( l \) is the formation length, and \( m > 1 \) represents a parameter controlling the shape of the force-length curve (Fig. 3A). This parabolic function approximates the classical force-length relationship derived from Huxley’s theory of muscle contraction (9, 21, 36, 67); when distortion is imposed on the link, its force, \( f(D) \), decreases with decreasing overlap between myosin and actin, which implies actin and myosin filaments of comparable lengths extending between associated dense bodies. Such a hypothesis has been supported by both measurements of the force-length curve in isolated cells (28) and recently obtained measurements of the force-length curve and ultrastructural images of intact tissue (34). That is to say, for its lifetime, each contractile link is assumed to follow a classical force-length relationship with a well-defined optimal force and a well-defined optimal length.

Two other possibilities are shown in Fig. 3. Figure 3B shows \( f(D) \) decreasing only when the link is elongated, which is conceivable if myosin is much shorter than actin; the absence of an ascending limb of the length-tension curve could be the case because smooth muscles have elliptical dense bodies instead of Z-lines as in skeletal muscles, and the basis of changes in filament overlap are still to be determined (22). Figure 3C shows \( f(D) \) decreasing only when the link is shortened, which is conceivable if actin extends way beyond associated dense bodies and is much longer than myosin; however, there is no evidence of such behavior, and it is also not supported by the current knowledge of the molecular basis of the actin-myosin molecular motors (22). Nevertheless, the behavior of these three possibilities is assessed below.

**Kinematics.** The density of links in the network depends on network activation. By fully activating the network (changing the value of \( w \) from 0 to 1, Eq. 1), the rate of link formation increases, links accumulate, and a denser network is assembled. Links exert tension on nodes, which are fixed to an immobile substrate. As such, in this model all contractions simulate isometric contractions.

Deformations can be imposed on the network, however. The area containing the network is kept constant. As such, when the network is shortened the distance between nodes decreases in the vertical direction, \( z \), and increases in the horizontal direction, and vice versa. When deformation is imposed after the formation of links, each link follows the node displacement by changing both its orientation and length; with length change, a link will contribute with \( f(D) \), as indicated by its force-length relationship (Fig. 3).

**Total force.** The axial network force exerted by the system as a whole, \( F \), is computed by the summation of vector projections of force (tension) exerted by all links. The presumption is that each node, representing a focal adhesion or a dense plaque, transfers any unbalanced force to its substrate (in the case of cells in culture) or to surrounding cells and connective tissues (in the case of intact tissues). \( F \) is computed as

\[ F = \sum_{a=1}^{n} f(D_a) \sin \theta_a \]

where \( n \) is the total number of links of the system, \( f(D_a) \) is the force exerted by each link (Fig. 3), and \( \theta_a \) is the crossing angle of each link with a transection (horizontal) plane (Fig. 1C). As such, \( f(D_a) \sin \theta_a \) is the force exerted in the \( z \) direction by each link, \( f(D) \).

In addition, we also computed force by transection, \( F_x \), as the average force (also projected to the \( z \)-axis) exerted by links crossing horizontal planes evenly distributed along the network. Although \( F \) and \( F_x \) emphasize somewhat different aspects of network force, results are largely the same with either computational approach.
Unloaded shortening velocity. Although we model only isometric contractions, we can compute the shortening velocity that an assembled network would have if it were allowed to shorten without load. As do several authors (14, 43, 51, 57, 62, 63, 69), we assume that each link is composed of a chain of contractile units in series (Fig. 4). The unloaded shortening velocity of each contractile unit is taken for simplicity to be 1. Because units in series add their velocities, the unloaded shortening velocity of a link is proportional to its formation length, \( l \). We thus take the unloaded shortening velocity for a network formed by many such links (taken in the direction of the \( z \)-axis) as the projected average length of such links, given by

\[
V = \frac{\sum_{a=1}^{n} l_a \sin \theta_a}{n} \sin \theta_a > 0
\]

where \( n \) is the total number of links of the system (excluding horizontal links, which have null projections to \( z \) and do not contribute to velocity), and \( l_a \sin \theta_a \) is the length of each considered link projected in the \( z \)-axis (Fig. 1B).

Compliance. Each link is imagined as being a chain of compliant components such as the myosin S2 subfragments as well as myosin S1 backbones and actin filaments (48). To compute network compliance, dynamics are temporarily frozen and each link is represented as an ideal spring whose compliance is proportional to its formation length and follows the classical Hooke’s law. A small length change, \( \Delta L \), is then imposed on the network, while the variation in elastic network force, \( \Delta S \) (the vector summation of forces exerted by all springs), is computed. Network compliance \( C \) is taken as \( \Delta L/\Delta S \).

RESULTS

Setting the constants. We tested a variety of parameters, changed the number, and modified the relative position of the nodes (including random assignments of node positions), but we found no substantive differences in network behavior. A set of parameters shown in Table 1 was chosen to simulate the pattern of force development for contraction and relaxation depicted in Fig. 2.

Activation and deactivation of the network. In the deactivated state link appearance and dissolution are in balance and, therefore, network force is initially low and constant (Fig. 5A). When the network is activated \((w = 1, \text{Eq. 1})\), a new balance is reached when network force increases to a higher plateau (Fig. 5A). When the network is again deactivated \((w = 0)\), network force returns to the basal level (Fig. 5A). Activation does not affect the average link length (Fig. 5B), whereas compliance decreases with activation, corresponding to a stiffer network (Fig. 5C).

A more detailed view of the network changes from the deactivated to the activated status is shown in Fig. 6; all scales are arbitrary. Figure 6A is a close up of some network nodes and links, showing that activation greatly increased the density

<table>
<thead>
<tr>
<th>Table 1. Parameters chosen for the reference network</th>
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<tbody>
<tr>
<td>Cell</td>
</tr>
<tr>
<td>------</td>
</tr>
<tr>
<td>121:253 ((L_{ref}))</td>
</tr>
<tr>
<td>Nodes: 242 ((11:22))</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Status</th>
<th>( w )</th>
<th>( b_0 )</th>
<th>( b_1 )</th>
<th>( r )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deactivated</td>
<td>0</td>
<td>0.00015</td>
<td>0.034</td>
<td>0.04</td>
</tr>
<tr>
<td>Activated</td>
<td>1</td>
<td>0.00010</td>
<td>0.068</td>
<td>0.05</td>
</tr>
</tbody>
</table>

\( L_{ref} \), network reference length. The other parameters are defined in Eqs. 1, 2, and 3.

Fig. 4. Relationship between length \((l)\), force \((f)\), unloaded shortening velocity \((v)\), and compliance \((c)\) of a single link. Because each link is devised as a chain of contractile units, \( f \) does not depend on link length, but longer links show higher \( v \) and \( c \) because contractile units in series add their velocities and compliances.

Fig. 5. Typical evolution of network force \((F; A)\), average link length projected in \( z \)-axis \((l_z)\), which is proportional to unloaded shortening velocity, \( v \); \((B)\), and compliance \((C)\); average (thick lines) of 10 simulations (thin lines). When the network was activated, \( F \) attained a higher plateau whereas \( C \) decreased (representing a stiffer cell); \( l_z \), however, was not affected by activation.
of links. Figure 6, B and C, respectively, shows the increase of network force and the transient increase of the effective rate of link dissolution, $B$, after network activation. With activation, the total number of links increased with time, but the distribution of link lengths projected to the long axis, $l_z$, did not change qualitatively; there was always a greater number of shorter than longer links ($s = 1$, Eq. 1). E: distribution of links per node, $k$, changed with activation: most nodes had few connections when the network was deactivated ($t = 0$) but an intermediate number of connections when the network was activated. Ten simulations performed under identical conditions (thin lines) and average (thick lines) are shown.

Adaptation to different lengths. Pratusevich et al. (51) and Kuo et al. (39) showed that smooth muscle preparations can adapt so as to produce roughly the same amount of force when maximally activated over a large range of muscle lengths (Fig. 7A, circles). They also showed that unloaded shortening velocity (Fig. 7B, thick line) and compliance (Fig. 7C, thick line) increase with muscle length.

We simulated those observations by assembling networks over a wide range of lengths. Network force exhibited a maximum near the reference length but decreased only slightly at smaller or greater lengths (Fig. 7A, thick line). Unloaded shortening velocity (Fig. 7B, thick line) and compliance (Fig. 7C, thick line) increased with network length in an almost linear fashion. These results compared favorably to the reported observations. Corresponding predictions from the model of Lambert et al. (43) are also shown as dashed lines.

Further aspects of length adaptation. Isolated tracheal muscle preparations exhibit a classical force-length response when passively shortened or lengthened and then quickly stimulated, showing maximum force at a given length, the so-called optimal length (Fig. 8A, middle curve). However, it was demonstrated by Pratusevich et al. (51) and Wang et al. (69) that in any given muscle this optimal length is labile; if given sufficient time, the muscle adapts to the length at which it is maintained. Such adaptation shifts the force-length curve to the left or to the right in such a way that the optimal length...
coincides with the length at which the preparation was previously adapted (Fig. 8A, left and right curves).

We simulated those observations assessing the three possibilities for link force-length relationships shown in Fig. 3. Under the assumption shown in Fig. 3A, the network was activated at its reference length and then deformed (shortened or lengthened) in quick steps, so as not to allow the time necessary for the network to readapt; network force decreased in both directions (Fig. 8B, middle curve). The procedure was then repeated at other starting lengths, showing that network force shifted to the left and to the right (Fig. 8B). These three curves located their optimal lengths at the length the networks were respectively formed. Their peaks also corresponded to the network forces depicted in Fig. 7A (represented as diamonds in Fig. 8B).

By using Fig. 3A, the results matched biological observations extremely well (Fig. 8A). We then repeated these simulations using the force-length relationship shown in Fig. 3B and found that the results roughly matched biological data (Fig. 8C). By using the force-length relationship shown in Fig. 3C, however, results did not match biological data (Fig. 8D). Therefore, the force-length relationship shown in Fig. 3A was adopted for the following simulations.

In a second experiment, Wang et al. (69) observed that length adaptation is reversible. Preparations were allowed to

Fig. 8. A: length-tension curves of rabbit trachealis; preparations adapted at the reference, shorter, or longer muscle lengths exhibit maximum force at the length at which they are adapted [modified from Wang et al. (69)]. B: simulations assuming the force-length relationship shown in Fig. 3A reproduced biological patterns best; the network was assembled at 0.6, 1.0 and 1.5 \( L_{ref} \) and then quickly deformed, showing maximum force at the length at which the networks were assembled (gray diamonds correspond to the network forces depicted in Fig. 7A). C: simulations with the force-length relationship shown in Fig. 3B roughly matched biological data, but the pattern diverged at the ascending limb with longer networks. D: simulations with the force-length relationship shown in Fig. 3C, which is unlikely according to known biomolecular basis, did not match biological data.
adapt to shorter lengths and then were stretched to their reference lengths and periodically stimulated. The fraction of force recovery (in comparison with control preparations) was measured, showing that readaptation took ~45 min (Fig. 9A); without periodic stimulation, muscle adaptation can take several hours.

Simulation of this observation showed sequential plateaus of force recovery established between each two successive peaks of $B_i$, which represented the periodic stimulation of the network (Fig. 9B, thick line). Fraction of force recovery (Fig. 9C) in relation to the network at the reference length (Fig. 9B, thin line) matched experimental data. Without periodic stimulation, force recovery became very slow (Fig. 9B, dashed line).

**Dynamics after network deformation.** Gunst et al. (23, 24) studied muscle strips that were shortened immediately before (Fig. 10A), 1 min after (Fig. 10B), or 10 min after (Fig. 10C) activation. They showed that the later the imposed shortening, the slower the subsequent pace of force recovery. This behavior was captured by the network model (Fig. 10, D–F). In addition, Fig. 10, G–I, shows the average force exerted for individual links, and Fig. 10, J–L, shows the average orientation of links.

**Sensitivity analysis.** The parameter $s$ describes the influence of distance between two nodes on the probability of link formation. We tested a range of values for $s$, finding that all curves computed for force by transection crossed at, approximately, 0.7 $L_{ref}$ (Fig. 11B); it was by using the values of network force, unloaded shortening velocity, and compliance obtained at this length and $s = 1$ that we found the best agreement between experimental data and simulations shown in Fig. 7.

Figure 11, A, C, and D, shows the behavior of network force, unloaded shortening velocity, and compliance with varying $s$. When $s$ approached zero, network force decreased excessively at shorter lengths, whereas unloaded shortening velocity and compliance increased faster with network length; in addition, under this condition compliance lost its typical behavior, abruptly increasing at shorter lengths. For greater values of $s$, network force was constant or increased at shorter lengths, but unloaded shortening velocity and compliance increased more slowly than expected with increasing network length.

We also computed the distribution of link lengths (Fig. 12). As expected, when $s = 0$, links are longer and may span the entire network length. When $s = 3$, by contrast, the distribution of lengths was strongly concentrated at small link lengths, with very few spanning the entire network. When $s = 1$, the behavior was intermediate.

Another key factor was $m$, the parameter that sets the shape of the force-length curve for individual links and comes into play with deformation imposed on the network. We adopted $m = 1.2$ to reproduce biological patterns best. The $m$ value determined the amount of network force dropping after any network deformation (Figs. 9B and 10, E and F) and also the shape of the network force-length relationship, as observed in Fig. 8B. Smaller values of $m$ (close to 1.0) produced greater force dropping and narrower network force-length relationships; bigger values of $m$ had the opposite effect (not shown).

**DISCUSSION**

Here we have presented a simple stochastic model of cytoskeletal dynamics of the airway smooth muscle cell. The main finding is that simple formation and turnover of network links seemed to capture all known mechanical features of smooth muscle length adaptation; a rather minimalist model expressed a relatively rich phenomenology. The remainder of
the DISCUSSION summarizes the idealizations that are implicit in the model, contrasts this model with that of Lambert et al. (43), and highlights key mechanisms by which this model accounts for length adaptation.

Three main mechanisms have been put forward to explain length adaptation of airway smooth muscle (Fig. 13, A–C). Gunst et al. (24) have suggested that length adaptation reflects a shift in the connection of the actin filament termination among the various adhesion plaques to which it might attach (Fig. 13A). Shifting connections, however, are only conceivable if the cytoskeleton can remodel by modifications in length and number of actin filaments. In fact, it has been demonstrated that, with contractile stimulation, integrin-associated proteins in focal adhesions may work as mechanotransducers (64), being involved in downstream regulation of actin polymerization, which also affects the length sensitivity of contractile force in smooth muscle (44). In other words, the whole organization of the actin cytoskeleton and its associated binding proteins may be fluid and subject to remodeling. This notion easily reconciles with Seow, Ford and coworkers (43, 51, 57), who have suggested as a second mechanism that length adaptation reflects a parallel-to-series transition where either cross bridges are rearranged (i.e., thick filament length changes, or, as alternatively suggested by Kuo et al. (39), thick filaments of the same length are added in series (Fig. 3). Finally, Solway and coworkers (8, 60) have recently suggested that length adaptation might be attributable to systematic modulation in actin filament length; in that case, cells with shorter actin filaments may entail parallel-to-series transition with cell lengthening, but cells with longer actin filaments need not (Fig. 13C). These three possibilities are not mutually exclusive, and to some extent they must be interdependent. The key insight of the network model evaluated here is that these three main mechanisms would appear to be the by-product of a common underlying process, namely, network formation and turnover governed by very simple rules.

Computed network structures and their changes subsumed all three proposed mechanisms (Fig. 13D). The first mechanism, connection shifting, emerged from length-regulated links changing their connections to relatively closer nodes (one example is indicated by a curved arrow in Fig. 13D). The second mechanism, parallel-to-series transition, appeared at two levels: at the level of each link (because longer links represent more contractile units in series), and at the level of the whole network (because these links were disposed in a more serial arrangement); this mechanism can be observed by comparison between the networks in Fig. 13D. Finally, the third mechanism, modulation of link lengths, was a necessary condition for the parallel-to-series transition.

Solway et al. (60) have suggested that smooth muscle cells in the asthmatic lung may have longer actin filaments and thereby prevent parallel-to-series transition when they are stretched (Fig. 14A). They have suggested, furthermore, that
this lack of rearrangement may explain why asthmatic subjects are refractory to the well-known bronchodilating response to a deep inspiration (13, 17, 61). This network model supports the idea that longer links may inhibit architectural transitions; when regulation favored longer link lengths (i.e., \( s = 0 \)), lengthened networks exhibited poorer rearrangement of links at the network level (Fig. 14B). This lack of rearrangement is a consequence of the distribution of longer links in a restricted area; however, we recall that longer links are also related to the increased unloaded shortening velocity and increased compliance observed in Fig. 11, C and D. Interestingly, increased shortening velocity and compliance of airway smooth muscles have been reported in association with asthma (63).

The present model addresses itself to dynamics of the cytoskeletal network in a highly idealized geometry while ignoring in all but the most primitive way underlying molecular events. Instead, the model focuses attention on forces transferred across the cell wall to focal adhesion plaques. Dense bodies, which transmit force between thin filaments within the cell, are treated implicitly (Fig. 4). Network structures that evolve (Fig. 1A) are crudely reminiscent of stress fibers seen in airway smooth muscle cells passaged in culture (5), whereas structures in intact tissues are more highly aligned (7, 33, 37, 40, 58, 59). Clearly, the specific model geometry
used here is closer to the former than the latter, but there is no reason to suppose that the basic mechanisms of adaptation might differ qualitatively.

The model presented here and that of Lambert et al. (43) are contrasted in Table 2. Both start with the assumption of a contractile filament comprised of several contractile units in series (Fig. 4). In that case the force per filament is a constant whereas compliance and shortening velocity of individual filaments increase in proportion with filament length. Beyond this common basis, these models differ with respect to network structure, connectivity, and dynamics. Primary data trends seen in Fig. 7 are captured by both models, but it is important to underscore the fact that in Lambert’s model these trends are assigned from the beginning whereas in the model presented here they are computed from underlying network dynamics. Moreover, the present network model better captures secondary features expressed by the curvilinearity of these relationships (Fig. 7) and also captures dynamic adaptive behaviors shown in Figs. 8–10; Lambert’s model, being a static one, is unable to address these dynamics.

In the present model, the network is in a state of continuous turnover. The dynamic balance between formation and dissolution of links determined the levels of force plateaus (Fig. 5A). The level was higher when the network was activated because a much greater number of links was accumulated as a consequence of the facilitated link formation. Activation did not affect the average link length, which represents unloaded shortening velocity (Fig. 5B). Compliance, however, decreased dramatically with activation (Fig. 5C) because the activated network can accumulate more links in parallel. When fully activated, although the density of links seems very high (Fig. 6C), less than 10% of all possible links were formed (for 242 nodes, less than 2,000 of 29,161 possible pairs of nodes were linked). Thus contractile units released from a dissolved link are likely to be used for the formation of links in other positions; there are plenty of network configurations that this model can dynamically adopt. In addition, activation modified the distribution of link lengths (Fig. 6D) and the distribution of connections per node (Fig. 6E). It is noticeable, however, that these distribution modifications took place almost immediately after activation, even while the matrix turnover of links was still high (Fig. 6C). It is perhaps not a coincidence that the greater part of the shortening and accompanying structural modifications in real smooth muscle seem to happen in the early phases of muscle contraction (62).

We now consider the determinants of the three main dynamic behaviors expressed by this model: the immediate force depression associated with a length change, the relatively slow isometric force recovery, and the rate of that force recovery. After a fast change of muscle length the network force became dramatically depressed (Figs. 9B and 10, E and F) as a direct effect of the shape of the force-length curve of the individual links (Fig. 3A).

Once the network was set at a new fixed length, network force started to recover (e.g., Fig. 10E, after shortening at 1 min) as deformed links gradually disappeared and were replaced by new undeformed links. These new links tended to have a systematically different link topology, had different orientation (e.g., Fig. 10K), and, most importantly, exerted greater force than the ones they replaced because they form at their optimal length (e.g., Fig. 10H).

The rate at which force recovered was set by two main factors, namely, the rate at which deformed links disappeared and the rate at which new undeformed links formed. The rate at which deformed links disappear is set by the effective matrix temperature $x$ and its changes in time (Eq. 2 and Fig. 2). The rate at which new links form is in part by the number of contractile units available (Eq. 1), but such availability is strongly influenced by the rate at which deformed links disappear. Taken together, these effects cause the rate of force recovery to be highest when the length change is imposed early in the contractile event, and slower when the length change is imposed later in the contractile event (Fig. 10, D–F). Gunst and Fredberg (23) have suggested that the onset of contraction corresponds to an epoch of high effective temperature ($x$) of the cell matrix, whereas the present network model suggests that it is variations in $x$ together with availability of contractile units (representing actin and myosin availability in real cells) that may interact to control the rate of adaptation. Such decreasing rate can be taken as analogous to the progressive stabilization of the cytoskeleton observed in biological cells (16, 24, 27).

A particularly interesting feature of this model was the role played by the regulatory parameter $s$ (Eq. 1), which is readily appreciated in Fig. 11, together with the correspondent distributions of link lengths in Fig. 12. When $s$ was in a narrow range close to 1, link length varied little with network length and did not excessively sequester contractile units; unsequestered contractile units were then available to form more links and maintain force almost constant over a broad range of network lengths. In addition, such modulation of link shortening led to moderate increases of unloaded shortening velocity and compliance that mimicked experimental data, as discussed above. $s = 1$ gives the probability to form links decreasing inversely with increasing distance as $1/d$. Values of $s$ smaller than 1 led to longer links, with the network showing decreased force, increased unloaded shortening velocity, and increased compliance; values of $s$ larger than 1 led to numerous shorter links, with the network showing higher force (which was also too insensitive to network length), decreased unloaded shortening velocity, and decreased compliance.

In the present cytoskeleton model, each link is a highly simplified structure appearing and disappearing instantaneously and instantaneously generating force when formed. However, from assembly of these simplified links in a network there emerged adaptation phenomena; moreover, the regulation of length of these links by $s$ was by far the most critical parameter. This suggests that regulation of link lengths might
be critical for the understanding of normal airway smooth muscle length adaptation and its alteration in asthma.

In summary, the present model is a simplified representation designed to capture only the crudest essential features of network dynamics in the airway smooth muscle cell. This model is not meant to be taken as a literal description of the real structure, but rather is a representation of a connectivity map of the cytoskeleton landscape. The simple network model does not explicitly address signaling pathways, cross-bridge dynamics, or other biochemical details but nonetheless captures well all known facets of airway smooth muscle length adaptation. The model suggests that functional adaptation of smooth muscle to length change is closely connected to network structure and turnover. This model also supports length control of filamentary structures as a major regulatory factor for the cytoskeleton dynamics.

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GRANTS

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REFERENCES


