The role of contractile unit reorganization in force generation in airway smooth muscle

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Airway smooth muscle (ASM) cells undergo remodelling and reside in a tissue structure that is subject to heterogenous stress distributions that change dynamically during the breathing cycle. In this paper, we develop a structural model of an ASM cell that consists of contractile units (actin and myosin filaments) in series and parallel, anchored to a nonlinearly elastic cytoskeleton. We mimic a typical experimental protocol that involves isometric force generation through triggering of the contractile machinery, followed by oscillatory length fluctuation of the cell. We use the model to predict the effect of a single instance of rearrangement of the contractile machinery, combined with strain-stiffening of the cytoskeleton, on the force generated by the sarcomeres, and the total force generated by the cell. By linking intra-cellular events to whole-cell behaviour, the model reveals mechanistic relationships between structural properties and cell-level force–length loops. We show how contractile force, shortening velocity and sarcomere operating lengths vary as the internal cell architecture is altered. Additionally, we show how interactions between the internal contractile machinery and cytoskeletal structure play a role in the regulation of force generation and hysteresis of the cell.

Keywords: airway smooth muscle, contractile unit, plasticity, serial-to-parallel transitions.

1. Introduction

In chronic asthma, airway remodelling as a result of increased airway smooth muscle (ASM) is a well-known phenomenon (Brightling et al., 2012). The elastic stress distributions in a remodelled (and therefore thickened, relative to the lumen) airway wall are predicted to be highly heterogenous, with different regions experiencing either tensile or compressive hoop and radial stresses (Brook et al., 2010). This suggests that ASM cells within the wall are likely to be in different stress micro-environments depending on their position in the airway wall. The ability of ASM to adapt to changes in length and maintain maximal force generation over a large length range (Gunst et al., 1995; Pratusevich et al., 1995) appears to require plastic changes in sub-cellular structures that involve rearrangement of contractile units within the contractile apparatus and reshaping of the apparatus itself. Smooth muscle cell plasticity has also been demonstrated via force-fluctuation-induced re-lengthening of ASM cells (Dowell et al., 2005, 2010). In order to understand the effect of different stress environments (particularly the dynamic changes during tidal breathing) on the contractility and plasticity of the ASM and
hence on the airway, a bottom-up approach seems necessary to account for the multitude of mechanisms operating at the cell- and sub-cellular levels.

A number of mathematical models have been proposed that address different aspects of smooth muscle plasticity, such as the stochastic percolation model of Silveira et al. (2005) and the overlap one-dimensional (1D) model developed by Lambert (2004). However, these models do not incorporate the molecular mechanisms of force generation. Fredberg et al. (1999) and Mijailovich et al. (2000) based their models of active force generation on the cross-bridge mechanisms that occur in the smooth muscle cell, combining the original sliding-filament model of Huxley (1957) (which accounts for actin-myosin interactions) and the Hai–Murphy four-state model (Hai & Murphy, 1988a,b). These have subsequently been further developed and used by Wang et al. (2008), Politi et al. (2010) and Lauzon et al. (2012) in larger multi-scale models coupling molecular interactions to organ-level mechanics. In addition, there is a growing consensus that while acto-myosin cross-bridge dynamics are key (Fredberg et al., 1999; Mijailovich et al., 2000), they are not sufficient to explain all the observed behaviour of the ASM cell. Emerging data support the view of the actin cytoskeleton of smooth muscle cells as a dynamic structure (evolving through actin (de-)polymerization) which, in concert with active force-generation mechanisms, could play an integral role in regulating the development of mechanical tension and the material properties of smooth muscle tissues (Gunst & Fredberg, 2003; Gunst & Zhang, 2008; Mehta & Gunst, 1999; Zhang & Gunst, 2008). None of the models described above, however, account for force-generation, cytoskeletal dynamics and plasticity in a comprehensive quantitative model of the ASM cell as a self-contained structure that forms part of a composite tissue.

In order to link the multitude of intra-cellular events to behaviour at the level of the whole cell and thus eventually to ASM tissue, we develop here a new model of an individual ASM cell that incorporates molecular cross-bridge interactions and cytoskeletal properties and the effects of contractile unit re-arrangement. We assess the ability of such a model to explain biomechanical observations from experimental protocols that are commonly used to investigate contractile and mechanical properties of ASM tissue. Typically a strip of tissue is taken from an airway and the length of the strip is held fixed, while a known amount of agonist is added. The agonist triggers contraction which evolves until a maximal isometric force is generated. The strip is then stretched sinusoidally. Contractile force is measured and plotted against the length of the strip. Typical force–length loops (taken from Bates et al., 2009) are shown in Fig. 1. In their paper, Bates et al. used a one-dimensional model for the tissue in which a nonlinear viscoelastic Kelvin–Voigt element is in series with a single active force generator, all in parallel with a second nonlinear Kelvin–Voigt body, to account for the passive and active properties of the strip. They were able to show that by selecting appropriate nonlinearity for the elastic and viscoelastic elements, the observed ‘banana-shape’ force–length loops could be reproduced to some extent. While this phenomenological tissue-scale model is useful, we believe that modelling individual cells, and their intra-cellular structure, will more clearly reveal the mechanistic basis for the nonlinear expressions used by Bates et al.

We begin this task by considering a simplified model of an individual ASM cell, accounting for nuclear, contractile unit and cytoskeletal mechanical properties. The complexity of the numerous interactions necessitates simplification of some of the mechanisms in order to understand thoroughly the effect of different intra-cellular structural elements on overall cell behaviour. At this initial stage our model allows us to explore the effect of changes in the cell’s internal architecture arising from externally imposed strain and reveals the contributions of different elements to cell-level force–length loops.
Fig. 1. Force–time and force–length traces obtained in a strip of rat trachealis muscle. The grey curves show data obtained with 2 Hz length oscillations having amplitudes of ±1, ±2, and ±4% \(L_{\text{ref}}\) as indicated, where \(L_{\text{ref}}\) is the unstressed, resting length of the tissue strip. Isometric force–time curves are shown in black (at left; solid lines obtained before collecting oscillation data, dashed lines obtained afterwards). The black curves (at right) show the first (solid line) and last (dashed line) force–length loops obtained during length oscillation. Reproduced with permission from Bates et al. (2009).

2. Methods

2.1 1D cell model

On the basis of the conceptual model of ASM cell structure proposed by Kuo & Seow (2004) (see Fig. 2), we propose a 1D model of a smooth muscle cell as depicted in Fig. 3. We assume that contractile units consisting of a myosin and two actin filaments connect to other contractile units via dense bodies in series as depicted in Fig. 4. (We will refer to these contractile units as sarcomeres although they are different in structure from the sarcomeres found in striated muscle.) The sarcomeres anchor via focal adhesions to the cell cytoskeleton directly or in series with the cell nucleus and cytoskeleton (Kuo & Seow, 2004). We thus assume for simplicity that the sarcomeres form two types of rows (termed A and B in our model) as depicted in Fig. 3. Histological sections of muscle cells (Kuo & Seow, 2004) provide information about the proportion of sarcomeres present in the cell that connect to the nucleus via actin filaments and dense bodies (type A), and the proportion that connect directly to the cytoskeleton (type B). In the present study, we model a single instance of the rearrangement of these contractile units because of an imposed length change of the whole cell.

In the model presented below, starred quantities are dimensional and bars denote quantities at maximally activated steady-state isometric conditions. At any time \(t^*\) the length of an individual cell \(L^*(t)\)
and the lengths of the individual sarcomeres $Y_A^*(t^*)$ and $Y_B^*(t^*)$ (see Fig. 3) satisfy

$$L^* = N_A^* Y_A^* + Z^* = N_B^* Y_B^*, \quad (2.1a)$$

where $N_A^*$ and $N_B^*$ are the number of sarcomeres connected in rows A and B, respectively. We assume that all the sarcomeres in each row are identical. The total number of sarcomeres is $N_S^* = N_A^* + N_B^*$. We assume that either $N_A^*$ and $N_B^*$ are constants for all time, or piecewise constant, with instantaneous change in their value from their initial state $\overline{N}_A^*, \overline{N}_B^*$, to some new value, while ensuring that $N_S^*$ is
Fig. 4. (a) Schematic of a row of ASM contractile units in series. The row depicted here is row B, the contractile units connect to the cytoskeleton via membrane adhesion complexes. (b) Reduction in the number of available cross-bridges as a result of stretch and the reduction in the overlap length between actin and myosin filaments. (c) Reduction in the overlap length via compression and hence a reduction in the number of available cross-bridges.

conserved, to mimic contractile apparatus rearrangement at a particular time. Typical parameter values for these quantities and others that follow below are given in Table 1.

The total force, $T^*$, that the cell exerts (which may be tensile or compressive) on its neighbours is given by the sum of the forces in each of the parallel contributions. Balancing forces, it follows that

$$T^* = F_A^* + F_B^* + E_C^*(L^* - L_0^*) + \mu B^* \dot{L}^*, \quad (2.1b)$$

where

$$F_A^* = E_A^*(Z^* - Z_0^*), \quad (2.1c)$$

the dot over $L$ in (2.1b) denotes a time derivative. $L_0^*$ and $Z_0^*$ are the unstressed cell and nucleus length, respectively. The stiffness of the cytoskeleton, $E_C^*$, represented by the spring in Fig. 3, can vary by several orders of magnitude and is thought to depend on the strain amplitude and rate (Chen et al., 2010; Krishnan et al., 2009). We, however, note that these reported measures are related to the cell stiffness measured immediately after the cell has been transiently stretched and then returned to its reference state. The hypothesis is that this observed ‘fluidization’ is a result of disrupted links between actin and myosin filaments as well as cross-linkers within the cytoskeleton and is a time-dependent effect. Here, we account for an instantaneous amplitude-dependence and apply an empirical nonlinear stress–strain relationship $E_C^* = a^* \exp(bL^*/L_0^*)$, where $a^*$ and $b$ are constants. We refer to this as strain-stiffening, consistent with the solid mechanics terminology (Holzapfel, 2000); this is distinct from, and not to be
Table 1 Physiological dimensional parameter values. All chemical kinetic rate constants are taken from Mijailovich et al. (2000)

| Quantity | Parameter name and value |
|----------|--------------------------|
| Average length of individual sarcomeres at maximum overlap | $Y_A^* \sim Y_B^* \approx 2 \mu m$ (Kuo & Seow, 2004) |
| Average length of an ASM cell at maximum activation | $L^* \approx 100 \mu m$ (Stephens et al., 1998) |
| Undeformed length of nucleus | $Z_0^* \approx 10 \mu m$ (Kuo & Seow, 2004) |
| Stiffness of the cell nucleus | $E_A^* \approx 0.7 \text{ Nm}^{-1}$ (Kuo & Seow, 2004) |
| Extensional viscosity of the cell | $\mu_D^* \approx 3 \text{ Ns m}^{-1}$ (see below) |
| Stiffness of the cytoskeleton varies with frequency | $E_C^* \approx 0.1 - 10 \text{ Nm}^{-1}$ (Trepat et al., 2007) |
| Number of available cross-bridges at max overlap in each sarcomere | $\bar{\rho}_i^* \approx 6 \times 10^7 \text{ m}^{-1}$ (see below) |
| Stiffness of each cross-bridge | $K^* = 1.8 \times 10^{-3} \text{ Nm}^{-1}$ (Warshaw & Fay, 1983) |
| Power-stroke length | $h^* = 15.6 \text{ nm}$ (Mijailovich et al., 2000) |
| Frequency of oscillation | $\omega^* = 2 \text{ Hz}$ (Bates et al., 2009) |
| Myosin light chain kinase rate | $k_1^* = k_6^* = 0.06 \text{ s}^{-1}$ |
| Myosin light chain phosphorylation rate | $k_2^* = k_5^* = 0.1 \text{ s}^{-1}$ |
| Cycling cross-bridge attachment rate constants | $f_{p1}^* = 0.88 \text{ s}^{-1}$ |
| Cycling cross-bridge detachment rate constants | $g_{p1}^* = 0.22 \text{ s}^{-1}$, $g_{p2}^* = 4(f_{p1}^* + g_{p1}^*)$, $g_{p3}^* = 3g_{p1}^*$ |
| Latch bridge detachment rate constants | $g_1^* = 0.1 \text{ s}^{-1}$, $g_2^* = 20g_1^*$, $g_3^* = 3g_1^*$ |

2.2 The Huxley–Hai–Murphy model of force generation

The Hai–Murphy four-state model (Hai & Murphy, 1988a,b) is based on observations that myosin exists in four distinct states: (i) free unattached myosin ($M^*$), (ii) phosphorylated unattached myosin ($M_p^* *$), (iii) phosphorylated myosin attached to actin ($AM_p^*$) (the so-called rapidly cycling cross-bridges) and (iv) de-phosphorylated myosin attached to actin ($AM^*$), which give rise to very slowly cycling or non-cycling cross-bridges (also called latch-bridges). The transitions from one state to another are shown in Fig. 5.

The forces generated by the sarcomeres are triggered by application of agonist and evolve with time. The Huxley–Hai–Murphy (HHM) model (Mijailovich et al., 2000), based on the chemical model of Hai & Murphy (1988a) and the sliding cross-bridge model of Huxley (1957), is used to determine the magnitude of the force and shortening velocity. The equations that relate force generation by the
For their unstressed position, was assumed to be constant throughout the simulation. In the model presented here, we assume that as the attachment of myosin heads to actin binding sites is more likely when the cross-bridge is within a powerstroke length, unit length of sarcomere, and the sum in (2.2b) is over the four components of . 5. Transitions between the four states accounted for in the Hai–Murphy regulation scheme.

\[
\frac{\partial \mathbf{n}_i^*}{\partial t} - V^*_i \frac{\partial \mathbf{n}_i^*}{\partial x^*} = \mathbf{S}_i^* \mathbf{n}_i^*, \quad (2.2a)
\]

\[
\sum \mathbf{n}_i^* = \rho_i^*, \quad (2.2b)
\]

for \( i = \text{A, B} \), where \( x^* \) is an internal variable representing the displacement of the cross-bridges from their unstressed position, \( \mathbf{n}_i^*(x^*, t^*) = (M_i^*(x^*, t^*), M_{pi}^*(x^*, t^*), AM_i^*(x^*, t^*), AM_{pi}^*(x^*, t^*)) \) is the number density (in \( x^* \) space) of cross-bridges in each pool, \( \rho_i^* \) is the total number of available cross-bridges per unit length of sarcomere, and the sum in (2.2b) is over the four components of \( \mathbf{n}_i^* \). \( V^*_i \) is the shortening velocity of the sarcomere and is positive for contraction, so that \( V^*_i = -\dot{Y}_i^* \).

In previous studies that implement the HHM model ([Mijailovich et al., 2000; Wang et al., 2008]), \( \rho_i^* \) was assumed to be constant throughout the simulation. In the model presented here, we assume that \( \rho_i^* \) for \( i = \text{A, B} \) are either constant and maximal (i.e. \( \rho_i^* = \overline{\rho}_i^* \)) throughout, or piecewise constant, changing from \( \overline{\rho}_i^* \) to \( \overline{\rho}_i^*/2 \) at a particular point in time. The reason for this choice is discussed further below.

The matrix \( \mathbf{S}_i^*(x^*, t^*) \) in (2.2a) describes the probabilities of transitions between the four states, and how those probabilities depend on \( x^* \). Phosphorylation transitions between \( M^* \) and \( M_p^* \), and between \( AM^* \) and \( AM_p^* \) are independent of \( x^* \). Phosphorylation of myosin facilitates interaction with actin. Transitions between \( M_p^* \) and \( AM_p^* \) and \( M^* \) and \( AM^* \) depend on \( x^* \) as the attachment of myosin heads to actin binding sites is more likely when the cross-bridge is within a powerstroke length, \( h^* \), of the nearest binding site. Likewise, detachment is more likely outside this powerstroke length. \( \mathbf{S}_i^*(x^*, t^*) \) is given in [Fredberg et al. (1999)] as

\[
\mathbf{S}_i^*(x^*, t^*) = \begin{pmatrix}
-k_1^* & k_2^* & 0 & g^*(x^*) \\
-k_1^* - f_p^*(x^*) & g_p^*(x^*) & 0 & 0 \\
0 & f_p^*(x^*) & -k_5^* - g_p^*(x^*) & k_6^* \\
0 & 0 & k_5^* & -k_6^* - g^*(x^*)
\end{pmatrix}, \quad (2.2c)
\]

where

\[
(f_p^*(x^*), g_p^*(x^*), g^*(x^*)) = \begin{cases}
(0, g_{p2}^*, g_2^*), & x^* < 0, \\
(f_{p1}^* x^*/h^*, g_{p1}^* x^*/h^*, g_1^* x^*/h^*), & 0 \leq x^* \leq h^*, \\
(0, (g_{p3}^* + g_1^* x^*/h^*)/h^*, (g_{p1}^* + g_3^*) x^*/h^*), & h^* < x^*.
\end{cases} \quad (2.2d)
\]
Strictly, the conservation law (2.2b) is valid only during isometric contraction (Fredberg et al., 1999; Mijailovich et al., 2000). Implicit in the use of this conservation law during non-isometric situations is the assumption that the distance between actin binding sites is much larger than $h^*$, so that there is no interference between neighbouring binding sites.

Assuming that the attached cross-bridges are linear elastic springs in parallel, and that both bound species exert equal force, the total force generated by each sarcomere is calculated by

$$F_i^* = K^* \int_{-\infty}^{\infty} x^* q \cdot n_i^* \, dx^*, \quad (2.2e)$$

where $i = A, B$, $K^*$ denotes the spring stiffness of the cross-bridges and $q \equiv (0 \ 0 \ 1 \ 1)$.

### 2.3 Initial and boundary conditions

Following the experimental protocol described in Section 1, we consider two phases. First, in the period $0 \leq t^* \leq \bar{t}^*$, we simulate isometric force generation in the presence of agonist by holding $L^* = L^* = L^*_0$ and increasing the concentration of agonist from zero to some constant (simulated via a step change in the constants $k_1^*$ and $k_6^*$ from zero to the values in Table 1). We assume that initially all the cross-bridges are dephosphorylated and unbound so that

$$F_i^*(0) = 0 \quad \text{and} \quad n_i^*(0) = (\rho_i^*, 0, 0, 0). \quad (2.3)$$

In the period $0 \leq t^* \leq \bar{t}^*$, we assume that the internal architecture of the cell has a particular configuration, i.e. $N_A^* = N_B^* = N_S^*/2$ (Kuo & Seow, 2004), and that there is maximal overlap between actin and myosin filaments as depicted in Fig. 4, so that $\rho_i^* = \bar{\rho}_i^*$.

In the second phase, $t > \bar{t}^*$, length-oscillations are imposed on the tissue strip. We thus prescribe a time-dependent cell length so that for the entire protocol, we write

$$L^* = \begin{cases} 
L^*, & 0 \leq t^* \leq \bar{t}^*, \\
L^* + \bar{L}_e^* \sin(\omega^*(t^* - \bar{t}^*)), & t^* > \bar{t}^*,
\end{cases} \quad (2.4)$$

where $\bar{L}_e^*$ is the amplitude of length oscillation, and $\omega^*$ its frequency.

We wish to investigate the effect of modified or remodelled cell architecture (both internal and cytoskeletal) on force generation. We therefore assume the following possibilities for the internal architecture at $t^* = \bar{t}^*$:

(i) The internal architecture retains its initial configuration, i.e. $N_i^* = \bar{N}_i^* = 0.5N_S^*$ and $\rho_i^* = \bar{\rho}_i^*$ for $i = A, B$. Current models (Politi et al., 2010; Wang et al., 2008) that calculate force generation in ASM via the HHM cross-bridge model assume implicitly that this is the case.

(ii) Alternatively, at $t^* = \bar{t}^*$, there is an abrupt change in internal architecture. This is conceived in response to observations of Lavoie et al. (2009) who propose that length fluctuations may completely disrupt actin–myosin connectivity. A mechanism that would lead to this is as follows. Imposed strain causes $\rho_i^*$ to decrease with both increase and decrease in overlap length (as depicted in Figs 4b and c, as proposed by Herrera et al., 2005) and therefore to vary continuously as a function of overlap length and hence time. At some threshold strain, $\rho_i^*$ becomes too small for myosin to hold on to actin filaments and the contractile unit disassembles and reassembles somewhere else in the cell (i.e. a different row). The present model assumes that
the strain imposed prior to the onset of oscillations causes this disassembly and reassembly to occur instantaneously, resulting in changes in the number of row A and row B sarcomeres along with sudden reduction of $\rho^*_i$. We model the subsequent rearrangement as a drop in the number of sarcomeres in one row and an increase in the other row, ensuring conservation of $N^*_S$. Given the observation that the ratio of actin to myosin filaments is $\sim 20:1$ (Kuo et al., 2003), this assumption makes sense physiologically. Thus, at $t^* = t^*_1$, we assume that $N^*_A$ drops to $\frac{1}{4}N^*_S$ so that $N^*_B = \frac{3}{4}N^*_S$. The fact that there are fewer (more) contractile units in row A (B) compared with row B (A) also suggests that there will be a decrease in overlap length between actin and myosin filaments which will result in a concurrent drop in $\rho^*_A$ ($\rho^*_B$) (Herrera et al., 2005) as depicted in Fig. 4b and c. We arbitrarily assume here that $\rho^*_i$ drops to $\rho^*_i / 2$. Once this architecture is established, we assume that it remains in this configuration in $t^* \geq t^*_1$. (A more realistic mechanism would allow for these changes in $\rho^*_i$ and $N^*_i$ to occur dynamically and as continuous variables of overlap length which is related to our definition of sarcomere length—the distance between two dense bodies on different actin filaments (Fig. 3b and c). In this case, we would require $\rho^*_i = \rho^*_i(Y^*_i)$ and $N^*_i = N^*_i(Y^*_i)$, but this complicates the model significantly, introducing nonlinearity in the expression for the shortening velocity (see Appendix); this will further be explored in future work. We apply instantaneous changes here to ensure that this first model remains relatively simple.) The architecture description is thus given by

\[
(N^*_A, N^*_B, \rho^*_A, \rho^*_B) = \begin{cases} 
(N^*_A, N^*_B, \rho^*_A, \rho^*_B), & 0 \leq t^* < t^*_1, \\
(\frac{1}{4}N^*_S, \frac{3}{4}N^*_S, \frac{1}{2}\rho^*_A, \frac{1}{2}\rho^*_B), & t^* \geq t^*_1.
\end{cases}
\]  

(2.5)

The cytoskeleton is also thought to be highly malleable (Chen et al., 2010; Krishnan et al., 2009) with large changes in cell stiffness observed on applying transient stretch. This response, often termed ‘fluidization’, appears to be time-dependent one and is not accounted for here. On the other hand, the instantaneous elastic response to imposed strain is characterized by a nonlinear stress–strain relationship (referred to as ‘strain-stiffening’ in the solid mechanics literature). We account for such cytoskeletal changes phenomenologically via the following cases:

(A) The cytoskeletal stiffness is constant (either $E^*_C = 0.1 \text{ N m}^{-1}$ or $E^*_C = 0.001 \text{ N m}^{-1}$) .

(B) The cytoskeletal stiffness varies with strain (and hence cell length Kim et al., 2011) according to

\[
E^*_C = a^* e^{bL^*/L_0^*},
\]  

(2.6)

where $a^*$ and $b$ are constants chosen to ensure that $E^*_C$ varies from 0.001 to 10 N m$^{-1}$ over a length range of $\pm 10\%$ of the reference length.

2.4 Maximally activated steady state at fixed length

In the first phase, $0 \leq t^* \leq t^*_1$, the proportions of myosin in the different states evolve as the myosin phosphorylates (driven by the imposed change in $k^*_1$ and $k^*_6$ from 0 to 0.35 s$^{-1}$) and begin to attach to the actin filament, thus generating a time-dependent force in both rows of sarcomeres. The reactions eventually settle to an activation steady state ($\overline{\rho}_i$) at which the contractile force is maximal at the imposed length $L^*$, referred to as the maximum isometric force (which we denote as $F^*_i$) and where the
shortening velocity $\dot{Y}_i^* = 0$. In Section 2.5, we will rescale the variables with respect to this maximally activated steady-state configuration.

The length of the row B sarcomeres, in the initial isometric phase, remains constant so that
\[ N_B^* Y_B^* = L^* \quad V_B^* = 0. \] (2.7)

Equation (2.2a) reduces to a system of one-dimensional equations (parametrized by $x^*$):
\[ \frac{\partial n_B^*}{\partial t^*} = S_B(x^*) n_B^*. \] (2.8)

subject to the initial condition $n_B^*(x^*, 0) = \rho_B^*(1, 0, 0, 0)$. The time-dependent isometric solution is given by
\[ n_B^*(x^*, t^*) = \sum_{k=1}^{4} a_k v_k(x^*) e^{\lambda_k(x^*)t^*}, \quad (0 \leq t^* \leq T^*), \] (2.9)

where $a_k$ are constants; $\lambda_k(x^*)$ are the eigenvalues and $v_k(x^*)$ the corresponding eigenvectors, of $S_B(x^*)$ satisfying $S_B v_k = \lambda_k v_k$. We numerically find that all but one of the eigenvalues are negative, and the remaining one is always zero (and is true for all $x^*$). The zero eigenvalue (equilibrium) solution satisfies the simultaneous equations
\[ S_B \bar{n}_B^* = 0. \] (2.10)

$\bar{n}_B^*(x^*)$ is non-zero only in the region $0 \leq x^* \leq h^*$, in which its components are
\[ \bar{M}_{pB}^*(x^*) = \frac{k_5^* k_1^*}{(1 + \kappa)k_1^* k_5^* + k_4^* (k_6^* + g(x^*)) \kappa + k_2^* k_5^* + g(x^*) \kappa k_5^*}, \] (2.11a)
\[ \bar{M}_{B}^*(x^*) = \left( \frac{k_5^*}{k_1^*} + \frac{g(x^*)}{k_1^* \kappa} \right) \bar{M}_{pB}^*(x^*), \] (2.11b)
\[ \bar{M}_{B}^*(x^*) = \kappa \bar{M}_{pB}^*(x^*), \] (2.11c)
\[ \bar{M}_{pB}^*(x^*) = \frac{k_5^* + g(x^*)}{k_5^*} \kappa \bar{M}_{pB}^*(x^*), \] (2.11d)

where
\[ \kappa(x^*) = \frac{f_p(x^*) k_5^*}{(k_5^* + g_p(x^*)) - k_5^* k_6^*}. \] (2.11e)

The remaining eigenmodes decay as $t^*$ increases but contain boundary layers of time-dependent thickness near $x^* = 0^+$ (based on numerical evidence). Since the equilibrium solution $\bar{n}_B^*(x^*)$ obtained directly from (2.10) does not include such boundary layers, the maximum isometric force calculated via (2.2e)
\[ \bar{F}_B^* = K^* \int_{-\infty}^{\infty} x^* \mathbf{q} \cdot \bar{n}_B^*(x^*) \, dx^*, \] (2.12)

is only the leading-order approximation.
We now consider the row A sarcomeres. These are able to shorten while the cell is held at fixed length subject to (2.1a). Thus, at maximally activated steady state, where $\dot{Y}_A^* = 0$,

$$N_A^* Y_A^* + Z^* = \mathcal{L}^*,$$

(2.13a)

and from (2.1c), we obtain

$$Z^* = \frac{E_A^*}{E_A^*} + Z_0^*.$$

(2.13b)

At equilibrium, therefore, $\mathbf{n}_A^*$ will again be the zero-eigenmode, satisfying $S_A(x^*) \mathbf{n}_A^* = 0$. Again the leading order approximation for maximum isometric force $F_A^*$ is calculated via (2.2e) and can be substituted into (2.13) to obtain the equilibrium lengths $Z^*$ and $Y_A^*$.

The steady-state lengths are thus given by (2.13b) and

$$Y_B^* = \mathcal{L}^*/N_B^*, \quad Y_A^* = \frac{1}{N_A^*} \left( \mathcal{L}^* - Z_0^* - \frac{E_A^*}{E_A^*} \right).$$

(2.14)

In the transients leading to equilibrium, since the row A sarcomeres are able to shorten, the shortening velocity is not zero and is given by eliminating $Z^*$ from (2.1) and differentiating with respect to time so that

$$\dot{Y}_A^* = -\frac{1}{N_A^*} \left( \frac{\dot{F}_A^*}{E_A^*} \right).$$

(2.15)

This is given further consideration when we consider solutions during length oscillation (see Appendix).

### 2.5 Non-dimensionalization

We non-dimensionalize lengths and stiffnesses with respect to the maximally activated steady state according to

$$L^* = L^*/\eta_A, \quad Y_i^* = Y_i^*/Y_j, \quad Z^* = Z_0^*/Z, \quad x^* = h^*/x, \quad t^* = \frac{1}{\omega^*} t,$$

$$F_i^* = \frac{E_i^*}{h^*} F_i.$$

(2.16)

for $i = A, B$, where the steady-state lengths are given by (2.14) and $\mathcal{L}^* = L_0^*$ is the unstressed cell length. Normalizing the number of sarcomeres with respect to $N_S^*$ gives sarcomere fractions $N_i = N_i^*/N_S^*$ for $i = A, B$, so that $N_A^* + N_B^* = 1$.

Applying (2.16) together with (2.14) to (2.1a–c) yields

$$L = \frac{1}{\eta_A} [1 - \beta - \alpha_A F_A] Y_A + \beta + \alpha_A F_A = \frac{Y_B}{\eta_B},$$

(2.17a)

$$\alpha_A F_A = \beta (Z - 1),$$

(2.17b)

$$T = \alpha_A F_A + \alpha_B F_B + E_C (L - 1) + \tau \dot{L}.$$  

(2.17c)
revealing the following dimensionless parameters:

\[
\eta_i = \frac{N_i}{\bar{N}_i} = \frac{\bar{N}^*_i}{N^*_i}, \quad \gamma_i = \frac{\bar{Y}^*_i}{h^*}, \quad \rho_i = \frac{\bar{p}^*_i}{\rho^*_i}, \quad \alpha_i = \frac{\bar{\rho}^*_i K^* h^{*2}}{E^*_A L^*}, \quad \beta = \frac{Z_0^*}{L^*}, \quad E_C = \frac{E^*_C}{E^*_A}, \quad \tau = \frac{\mu^*_D \omega^*}{E^*_A},
\]

for \(i = A, B\).

The dimensionless equations governing the HHM model of force generation are thus

\[
\frac{\partial \mathbf{n}_i}{\partial t} + \gamma_i \dot{Y}_i \frac{\partial \mathbf{n}_i}{\partial x} = \mathbf{S}_i \mathbf{n}_i, \quad (2.18a)
\]

\[
\sum \mathbf{n}_i = 1, \quad (2.18b)
\]

for \(i = A, B\), \(\mathbf{n}_i = (M_i, M_{pi}, AM_{pi}, AM_i)\) is the fraction of cross-bridges in each pool (i.e. \(M_i = M^*_i / \rho^*_i\), etc.), and \(\mathbf{S}_i\) is the matrix of dimensionless rate constants. The dimensionless \(x\)-dependent rate functions are given by

\[
(f_p(x), \gamma_p(x), \gamma(x)) = \begin{cases} 
(0, g_{p2}, g_2), & x < 0, \\
(f_{p1x}, g_{p1x}, g_{1x}), & 0 \leq x \leq 1, \\
(0, (g_{p1} + g_{p3})x, (g_1 + g_3)x), & x > 1,
\end{cases}
\]

where \(f_{p1}^* = \omega^* f_{p1}\) and so on. The dimensionless force generated by each sarcomere is then given by

\[
F_i(t) = \rho_i \int_{-\infty}^{\infty} x \mathbf{q} \cdot \mathbf{n}_i(x, t) \, dx, \quad (2.18d)
\]

for \(i = A, B\), where \(\rho_i = \rho^*_i / \bar{\rho}^*_i\).

The dimensionless boundary and initial conditions are given by

\[
\dot{Y}_i(t) = 0, \quad (2.19a)
\]

\[
L(t) = 1 + \epsilon \sin(t - \bar{t}), \quad (2.19b)
\]

for \(t \geq \bar{t}\), where \(\epsilon = L^*_e / L^*\) is the dimensionless amplitude of length oscillation. Continuity of \(\mathbf{n}_i\) gives

\[
\mathbf{n}_i(0^-, t) = \mathbf{n}_i(0^+, t) \quad \text{and} \quad \mathbf{n}_i(1^-, t) = \mathbf{n}_i(1^+, t). \quad (2.19c)
\]

Assuming that binding sites are independent of their neighbours requires

\[
AM_{pi}(x) \to 0 \quad \text{and} \quad AM_i(x) \to 0 \quad \text{as} \quad x \to \infty \quad (2.19d)
\]

with \(M_i\) and \(M_{pi}\) given by dimensionless versions of (2.10a,b) with \(\kappa = 0\).

The two internal architectures are characterized by the following dimensionless parameters:

\[
\eta_i = 1, \quad \rho_i = 1 \quad \text{for} \quad t \geq 0 \quad \text{and} \quad i = A, B; \quad (2.20a)
\]
Table 2  Dimensionless parameter values

| Parameter                                                                 | Parameter value |
|----------------------------------------------------------------------------|-----------------|
| Ratio of sarcomere numbers at non-maximally activated state to that at     | \( \eta_A = 1, 2 \) |
| maximally activated state                                                 |                 |
| Ratio of sarcomere length to powerstroke length                           | \( \gamma_i = 100 \) |
| Ratio of available cross-bridge numbers relative to that at maximally     | \( \rho_A = 1, 0.5 \) |
| activated state                                                           |                 |
| Ratio of undeformed nucleus length to cell length                         | \( \beta = 0.1 \) |
| Dimensionless cytoskeletal stiffness                                       | \( E_C = 0.001 - 0.1 \) |
| Dimensionless amplitude of length oscillation                             | \( \epsilon = 0.01, 0.04 \) |
| Ratio of cross-bridge properties to cell level properties                  | \( \alpha_i = 0.004 \) |
| Loss tangent or hysteresivity parameter                                   | \( \tau = 0.001, 0.02 \) |
| Dimensionless time at which oscillations are imposed                      | \( \tilde{t} = 20 \) |

\[ (\eta_A, \eta_B, \rho_A, \rho_B) = \begin{cases} (1, 1, 1, 1), & 0 \leq t < \tilde{t}, \\ (2, 0.67, 0.5, 0.5) & t \geq \tilde{t}. \end{cases} \]  

(ii)

Cytoskeletal stiffness is characterized by the following two cases:

(A)  
\[ E_C = 0.001 \text{ or } 0.1; \]  
(B)  
\[ E_C = a e^{bL} \] where \( a = 1.0 \times 10^{-27} \) and \( b = 58 \).

The constants \( a \) and \( b \) are chosen to give the range of cytoskeletal stiffness in Table 1 when the cell length is within \( \pm 10\% \) of its reference length.

The time-dependent dimensionless equations (2.17) and (2.18) together with (A.1) and (A.6) are solved by recasting the equations into a form which may be solved computationally (see Appendix) using a Godunov scheme (Harten et al., 1983; Roe, 1986). This scheme combines an upstream differencing finite volume method with a method-of-characteristics approach.

2.6  Parameter values

Dimensional parameter values are given in Table 1, and the resulting dimensionless parameters are listed in Table 2.

We determine the extensional viscosity \( \mu_D^* \) in Table 1 from reported values in the literature. Deng et al. (2006) report values of shear storage and loss moduli \( (G' \text{ and } G'') \) of ASM cells that vary with frequency. The hysteresivity, or loss tangent \( \tan \delta = G''/G' \), is reported to remain fairly constant at \( \sim 0.2 \) over frequencies between 0.1 and 10 Hz. Typically for a Kelvin–Voigt viscoelasticity model, in which an elastic element of stiffness \( E^* \) is in parallel with a dashpot of viscosity \( \mu^* \), \( \tan \delta = \mu^* \omega^*/E^* \). The reported value and the nucleus stiffness in this expression yields an extensional viscosity \( \mu_D^* \) as given in Table 1, and corresponds to the larger value of the dimensionless viscosity parameter \( \tau \) in Table 2.
This value for viscosity, which is based on the experiment on whole individual cells, is likely to be an effective value that combines the contributions of both cross-bridge mechanisms and cytoskeletal structure changes. In the simulations, we find that the smaller value of $\tau$ provides closer agreement of simulated force–length loops with experiment. The value used in the simulations therefore probably reflects just the cytoskeletal contribution to whole cell viscosity (see Section 4).

To estimate $\rho^*_i$, we assume that binding sites are distributed uniformly along the actin filament with a minimum distance of $h^*$ between them. Then under the assumption of one-to-one correspondence of cross-bridges with binding sites, we use the sarcomere length $Y^*_i$ to determine an approximate lower bound for $\rho^*_i$ as given in Table 1.

3. Results

Figure 6 shows the shortening velocities of the two sarcomere types $V_A$ and $V_B$ and corresponding contractile forces $F_A$ and $F_B$ plotted against time. In the isometric phase (inset of Fig. 6a), row A sarcomeres undergo shortening with a velocity $V_A$ that increases in magnitude to a maximum, after which it decreases to zero upon reaching maximal force generation (inset of Fig. 6b). This velocity is several orders of magnitude smaller than that imposed by the subsequent length fluctuations (Fig. 6a). In the oscillatory phase, the velocities of the two sarcomere types in architecture (i) are similar, the presence of the nucleus modifying that of the row A sarcomeres slightly as indicated by the red and blue curves in Fig. 6a. The difference, however, is more exaggerated in architecture (ii) where $V_A$ is increased significantly compared with $V_B$ due to the decreased numbers of sarcomeres in row A. This suggests that reorganization of the internal architecture could result in changes in shortening velocity as observed experimentally (Jackson et al., 2004; Jensen et al., 2001; Ma et al., 2002) and in model simulations (Bullimore et al., 2011). In contrast to the model simulations of Bullimore et al. (2011) the shortening velocity changes in our model as a result of changes in strain rate; cross-bridge cycling rates $f_p$, $g_p$ and phosphorylation rate $k_1$ are not modified.

The contractile forces $F_i$ corresponding to the shortening velocities in Fig. 6a are plotted against time in Fig. 6b. Force-generation during the isometric phase is identical for both row A and B sarcomeres and for both architectures (inset of Fig. 6b). At the onset of length oscillations, A and B sarcomeres in architecture (i) generate almost identical force, as would be expected by the similar shortening velocities seen in Fig. 6a. However, the amount of force that can be generated by the different sarcomere types in the two different architectures becomes evident (Fig. 6b). The significant drop in contractile force of row A and row B sarcomeres, as the architecture is modified, is particularly striking; this is a result of the reorganized contractile apparatus restricting the availability of cross-bridges. Additionally, the timescale associated with the reduction in contractile force on application of length fluctuations appears to be different for the two sarcomere types for architecture (ii); an exponential fit suggests that force generated by row A sarcomeres decays slightly quicker than row B sarcomeres (transients not shown).

Figure 7 shows the contractile force $F_i$, plotted against sarcomere length $Y_i$, where $i = A, B$, for the two architectures (i) and (ii) for the simulations shown in Fig. 6. For architecture (i) (Fig. 7a and b) the force–length loop for row A sarcomeres is only slightly different from that of the row B sarcomeres. B sarcomeres do not undergo length change during isometric force generation (indicated by the straight vertical black curve at $Y_B = 1$) while row A sarcomeres contract from their relaxed length $Y_A(0)$ (given in (A.7)) to 1. At the onset of length oscillations, the slight difference in the force–length loops between row A and row B sarcomeres is then attributed entirely to the presence of the nucleus. The force–length loops for architecture (ii) are shown in Fig. 7c and d. The straight horizontal sections of the transient curves are a result of the abrupt increase (decrease) in length of the row A (B) sarcomeres from their
isometric length due to the abrupt change in $N_A$ ($N_B$) imposed at $t = \tilde{t}$. Thereafter, there is a significant difference between A and B sarcomeres in terms of operating length as well as the amount of force generated; the latter is a result of fewer cross-bridges ($\rho_i$) available relative to the maximally contracted equilibrium solution.

The cross-bridge populations $n_i$ are plotted against cross-bridge position $x$ at $t = 40$ in Fig. 8 for architectures (i) and (ii). At this time point (denoted by black asterisks in the force–length loops of Fig. 7), the sarcomere lengths are close to their minimum value and the contractile force is approximately equal to the mean. As expected, the differences in the population densities between row A and row B sarcomeres for architecture (i) is small (Fig. 8a). In contrast, the difference is significant for architecture (ii) as shown in Fig. 8b. In the latter case (architecture (ii)), the row A cycling cross-bridges ($AM_{pA}$) and latch bridges ($AM_A$) undergo more significant displacement due to the larger amplitude of oscillation. This can be seen more clearly in Fig. 9; the attached cross-bridge populations ($AM + AM_p$) for the row A sarcomeres (Fig. 9a) at various time points through the oscillatory cycle have very different shapes, are more significantly displaced and have lower peak values compared with the row B sarcomeres (Fig. 9b). Thus, within the same cell, depending on the architecture of the contractile machinery, it is possible to have a wide range of both rapidly cycling and latch-bridge populations.

Force–length loops for the whole cell for the simulations above are shown in Figs. 10–12. Simulations for architecture A(i) with baseline parameters as indicated by the caption (Fig. 10) are plotted in all three figures for reference. First, for these baseline parameter simulations (low $E_C$, $\tau = 0$ and relatively...
stiff nucleus compared with cross-bridge stiffness (low $\alpha_i$), nonlinearity, due entirely to the cross-bridge mechanics, is immediately obvious in both architectures (Fig. 10a). The peak force in architecture A(ii) is lower than in A(i). The areas contained within the closed loops (Fig. 10b) are an indication of the magnitude of hysteresis in the system and hence the viscoelasticity that arises as a result of cycling cross-bridges. Thus, hysteresis, due entirely to cross- and latch-bridges (with $\tau = 0$) is lower for architecture A(ii) than in A(i). The loops, however, do not resemble the banana-shaped loops of Bates et al. (2009) (Fig. 1).

We now turn to the effect of changing internal cell architecture. One of the key parameters associated with this is $\alpha_i$, increases in which may be interpreted as increased cross-bridge stiffness $K^*$ or longer actin or myosin filament lengths, via $\rho_i^*$, since a greater number of cross-bridges could be associated with larger overlap lengths that would stem from having longer filaments. Additionally, increased $\alpha_i$ could also be interpreted as increased number of rows of type $i$. Increasing the stiffness of either the row A or B cross-bridges (by increasing $\alpha_A$ or $\alpha_B$) causes the cell to generate significantly higher peak force as well as increased hysteresis relative to the baseline cases in both architectures (Fig. 10a). While an increase in peak force would be expected as a result of increased cross-bridge stiffness, increased $\alpha_A$ or $\alpha_B$ could also result from longer actin and myosin filament lengths. This could explain why,
surprisingly, hysteresis is also significantly increased in both cases (Fig. 10b). Indeed it has been found that actin and myosin filaments lengths within the cell are distributed normally about a mean length and that both mean and variance can depend on degree of activation (J. Solway, personal communication). Differences between architectures (i) and (ii) are particularly striking for higher $\alpha_A$ or $\alpha_B$ (Fig. 10b); the drop in force for the rearranged architecture in case A(ii) is more significant for higher $\alpha_i$, suggesting that contractile apparatus rearrangement has a much greater effect in cells with either long filaments or stiff cross-bridges.

Increasing cell viscosity $\tau$, generates slightly greater peak forces in cases A(i) and A(ii) (Fig. 11a) and, as expected, slightly greater hysteresis (Fig. 11b). Increased cytoskeletal stiffness (with $\tau = 0$) generates significantly greater peak forces in both A(i) and A(ii) and interestingly also a significant phase shift; an additional increase in $\tau$ generates only a slight increase in peak force and hysteresis. Thus, changes in cell viscosity appear to have slightly greater impact on a more compliant cytoskeleton. The most striking feature in Fig. 11 is the significant change in shape of the force–length loops; the nonlinearity exhibited by the cross-bridge dynamics appears to be swamped by the linear cytoskeletal mechanics, although hysteresis is still evident. In all variations investigated here, peak forces and hysteresis for A(ii) were always found to be lower than those for A(i). Very small values of $\tau$ are used here to illustrate the role of the cytoskeletal viscoelasticity. Simulations for values of $\tau$ as large as those reported in Table 2 (not shown) suggest that cell hysteresis dominates the mechanical behaviour completely, with force–length loops becoming almost circular. As mentioned above, the reported value of viscosity in Table 2, which is based on experiment on whole individual cells, is likely to be an effective value that combines the contributions of both cross-bridge mechanisms and cytoskeletal structure changes. The smaller values of $\tau$ reflects just the cytoskeletal contribution. It is interesting to note that increases in $\alpha_i$ (Fig. 10) cause an increase in peak force but do not cause a decrease in minimum force so that the mean force is greater for increased cross-bridge stiffness. On the other hand, an increase in cytoskeletal stiffness increases the range of total force but the mean force varies only a little relative to the baseline cases (Fig. 11).
Fig. 9. Total attached cross-bridge populations $AM + AMp$ for (a) row A sarcomeres and (b) row B sarcomeres plotted against cross-bridge position $x$ at specific time points through one oscillatory cycle for architecture (ii).

The effect of changes in cytoskeletal stiffness with cell length (Kim et al., 2011) for architectures B(i) and B(ii) is shown in Fig. 12. The strain-stiffening behaviour, applied phenomenologically, so that cytoskeletal stiffness varies from $E_C = 0.001$ to $E_C = 0.1$ (to match the simulation of A(i) and A(ii) in Fig. 11) gives rise to highly nonlinear behaviour. We recall that strain-stiffening is an instantaneous elastic response characterized by a nonlinear stress–strain relationship as distinct from fluidization of cells observed immediately after transient stretch. In both architectures B(i) and B(ii), for low strains the force–time curves follow the baseline (for which $E_C = 0.001$, Fig. 12a) but then generate increased peak force upon stiffening of the cell as it increases in length (Fig. 12b). The nonlinear behaviour more
closely matches the banana-shaped loops of the experimental observations in Fig. 1. There are however discrepancies between the two, specifically in that the narrow end of the loops with the sharp cusp occurs at lower values of force in the experiments compared with present simulations. It is however interesting to note that only a nonlinearity in $E_C$ (and not in $\tau$) was required to generate the banana-shape loops. This is in contrast to the findings of Bates et al. (2009) in which nonlinearities in both quantities were required. This may have been partly due to the fact that their models for contractile force did not explicitly account for cross-bridge mechanisms but rather used an approximate kinetic scheme.

4. Discussion

The manner in which contractile, cytoskeletal and associated proteins interact within the cell so as to produce integrated mechanical effects (e.g. cellular contraction, stiffness or remodelling) remains a major open question (Gunst & Fredberg, 2003). In an attempt to understand these interactions, we have here developed a mechanistic model of an ASM cell that accounts for basic elements of its internal architecture, rearrangements of its contractile apparatus and changes in cytoskeletal stiffness and cell viscosity. The model is based on the conceptual models proposed by Kuo & Seow (2004), Gunst & Fredberg (2003), Herrera et al. (2005) and Lavoie et al. (2009). Contractile force generation is predicted via the HHM model (described by Mijailovich et al., 2000) for a single contractile unit, with contractile units arranged both in series and in parallel within the cell. In this study, stretching was assumed to induce a single rapid rearrangement of this architecture, changing contractile unit numbers between serial and parallel configurations. The model predicts the net force generated depending on the cellular architecture, and the force–length relationships for a single cell determined by contractile and cytoskeletal components. Results from model simulations are compared against length-fluctuation tissue-strip experiments that have been extensively used (e.g. Fredberg et al., 1997) and in particular the simulation results are compared with data of Bates et al. (2009) shown in Fig. 1.
By taking a mechanistic approach to modelling a single ASM cell, we are able to investigate the effect of varying properties of different components within the model that have a direct physical correspondence with real cell components. The tissue-level model of Bates et al. (2009) takes into account elastic and visco-elastic elements and a single contractile element (which does not explicitly include cross-bridge mechanisms) in an attempt to understand the observed behaviour of activated tissue-strips undergoing length-fluctuations. Their conclusion was that both nonlinear elastic and viscoelastic components were required to generate the banana-shaped force–length loops observed in experiment. While they were able to reproduce the experimental results it is not clear what gives rise to nonlinearity or
hysteresis at the cell level in their model. Given that most experimental treatments applied to cells involve manipulation of contractile apparatus or cytoskeletal structures, (e.g. Latrunculin B, which promotes actin depolymerization, Dowell et al., 2005), it is desirable to understand the contribution of these various cell-level components to observed phenomena and how different components may be targeted to obtain a particular outcome. The results from this model demonstrate a number of important cell-level interactions that could play an important role in molecular-level force generation mechanisms.

4.1 Cell properties may be inferred from force–length loops

The fact that cross-bridge cycling contributes to the hysteresis observed in experimental force–length loops has been known since the HHM model was developed. For example, Mijailovich et al. (2000) showed that cycling at higher frequencies or smaller amplitudes reduces the degree of hysteresis. Our model incorporates two main sources of hysteresis: cross-bridge cycling and cell viscosity. However, they affect hysteresis very differently. Increased hysteresis with increased peak tensile force without increased peak compressive force suggests either relatively high cross-bridge stiffness or large filament (or overlap) length (Fig. 10). If the cell also strain-stiffens, the width of the force–length loop is maintained at low strains, generating a sharp cusp at peak tensile force (Fig. 12). Increased cell viscosity has a different effect; at low cytoskeletal stiffnesses there is an overall increase in the width of the loop (Fig. 11), with increases in peak tensile and compressive forces. The increased width is also maintained at low strain when the cell strain-stiffens, so that the cusp at peak tensile force is maintained (Fig. 12). The other difference between these two hysteresis-generating mechanisms is that nonlinearity, clearly evident from the cross-bridge cycling, is further exaggerated for large cross-bridge stiffness or filament length, but gets swamped by large cell viscosity, with force–length loops becoming circular. The components we have included in the model so far are not able to reproduce the cusp at the low-strain end of the force–length loop (Fig. 1). Model simulations also suggest that cytoskeletal viscosity (\( \tau \)) may be estimated from a knowledge of whole cell extensional viscosity and prediction of hysteresivity from the cross-bridge mechanisms. A further potential source of hysteresis may be length adaptation that results from sarcomere or cytoskeletal reorganization, which has not been accounted for here.

Cytoskeletal stiffness is also evident from the force–length loops of the whole cell. Increases in both peak tensile and peak compressive forces indicate increases in cytoskeletal stiffness, without strain-stiffening (Fig. 11). The nonlinearity due to cross-bridge cycling can be swamped by the large linear cytoskeletal stiffness, although hysteresis is maintained (Fig. 12). Phase-shifts in the force–time plot are also related to increases in cytoskeletal stiffness (Fig. 11). Neither the changes in cross-bridge stiffness nor changes in strain-independent stiffness are capable of generating the force–length loops resembling the experimentally observed banana-shape (Fig. 12). One way in which the current model is able to generate force–length loops that more closely resembles the banana-shaped loops is through the incorporation of a nonlinear stress–strain relationship to characterize instantaneous changes in cytoskeletal stiffness. Furthermore, the strain-stiffening behaviour also accounts for the change in force–length loop shapes as the amplitude of length fluctuations is varied, as observed by Fredberg et al. (1997) (results not shown). There are numerous additional possible sources of nonlinearity that we have not included here. Inferences about strain-stiffening may have to be modified upon further exploration of these additional elements.

4.2 Transitions from serial to parallel configurations play a quantifiable role in force generation

The change in architecture in this model is conceived in response to observations of Lavoie et al. (2009), who proposed that length fluctuations may disrupt actin–myosin connectivity. The present
model accounts for such internal rearrangement brought about instantaneously at the onset of oscillation. Despite the total number of contractile units being conserved between both architectures (i) and (ii), the cell’s contractile force is modified significantly by contractile apparatus rearrangement when the cytoskeleton is relatively compliant, the cross-bridges are stiffer than the nucleus, and filament lengths are relatively long. Additionally the length about which the sarcomeres oscillate is perturbed significantly from its equilibrium value (Figs 7c and d). A drop in the number of row A sarcomeres, and hence also the number of available cross-bridges, results in a smaller force being generated by these sarcomeres. The associated increase in row B sarcomeres and consequent reduction in number of available cross-bridges also results in a decrease in contractile force. Thus, the overall effect is a drop in the total contractile force. Creation of additional rows would further affect the total contractile force with a relative pay-off between increased parallel sarcomeres versus decreased available cross-bridges. Although we have assumed only two rows, our model can represent the possibility of increased rows provided the parameters $\alpha_i$ and $\rho_i$ are chosen appropriately.

We note that constant $\rho_i$ is effectively the small-deformation limit of situations in which $\rho_i$ may vary with overlap length. Large deformations cause large changes in overlap length between actin and myosin filaments, thus reducing the total number of cross-bridges available for binding. Additionally, the oscillation amplitudes applied here generate a significant difference in rapidly cycling and latch bridge populations in row A compared with those of row B (Fig. 8b). The simple conservation law (2.2) assumes that the distance between binding sites is much greater than $h^*$. The larger the amplitude of oscillation (particularly in row A sarcomeres), the more unrealistic this assumption becomes, since latch-bridges take large excursions out of the power-stroke region. We have assumed here that the transition from row A to row B occurs instantaneously. An extension to this model could allow for these transitions to occur over some finite time. Further refinement of the model will thus demonstrate the effect of dynamic changes by incorporating a more realistic mechanism that allows for contractile units to disassemble and reassemble.

4.3 Contractile apparatus rearrangement causes significant change in shortening velocity

A relationship between increased ASM shortening velocity and airway hyper-responsiveness has been suggested both experimentally (Bullimore et al., 2011; Jackson et al., 2004; Jensen et al., 2001; Ma et al., 2002) and via the use of model simulations (Bullimore et al., 2011). Other experimental observations suggest a relationship between shortening velocity and myosin filament density (Smolensky et al., 2005). We have shown that the shortening velocity of sarcomeres that attach to the cytoskeleton via the nucleus is modified both by the mechanical properties of the cell nucleus and by changes in the architecture of the cell (Fig. 6a). The modifications are a result of changes in strain rate; cross-bridge cycling rates $f_p, g_p$ and phosphorylation rate $k_1$ are not modified here. In contrast, it is these rates that are modified to generate changes in shortening velocity in the model simulations of Bullimore et al. (2011) to investigate the relationship between increased shortening velocity and hyper-responsiveness. Interestingly, and in contrast to their findings, the architecture change chosen for these simulations, which caused an increased shortening velocity of row A sarcomeres (Fig. 6a), was accompanied by a reduction in corresponding contractile force (Fig. 6b). Nucleus deformation in an activated ASM cell is evident from electron microscopy of its ultrastructure (Kuo & Seow, 2004) and so is likely to have an effect on the shortening velocity of type A sarcomeres in the manner we have outlined. If the nucleus is sufficiently stiff, however, or stiffens instantaneously with imposed stretch, then the shortening velocity of row A sarcomeres will approximate the cell shortening velocity. Here, we have assumed a relatively compliant nucleus and for this, along with an increase in row A shortening velocity, the
shortening velocity of row B sarcomeres drops relative to that of architecture (i). In this model, we are able to apply length changes and predict the force generated. The inverse problem of applying force oscillations, and predicting length changes is a non-trivial initial value problem, for which a numerical solution has been proposed (Bullimore et al., 2011) and could be used in future work. At this time, however, we are not able to investigate how force-oscillation-driven internal remodelling affects shortening velocity. We have shown however, that architecture changes affect shortening velocity of some of the sarcomeres and suggest that architecture changes may thus contribute to the factors that cause airway hyper-responsiveness.

In summary, we have combined a number of conceptual models into a mathematical model of an individual ASM cell. We have shown how remodelling of the internal and external structures of the cell significantly modifies the mechanical behaviour of the cell. Given the very different effects of the contractile apparatus and cytoskeletal behaviour and their interaction, we propose ways in which cell properties may be inferred from force–length loops. We show that contractile force, shortening velocity and sarcomere operating lengths vary as the internal cell architecture is altered, and that force generation and hysteresis characteristics depend strongly on interactions between internal and external structures. We believe this bottom-up approach that accounts for cell structure is crucial in understanding how molecular mechanisms within the ASM cell affect airway properties in the diseased lung.

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### Appendix A

#### A.1 Solution of governing equations

At any time, $t$ (2.17b) gives $Y_B$ as function of time so that the velocity imposed on the row B sarcomeres is given by

$$\dot{Y}_B = \begin{cases} 0, & 0 \leq t \leq \bar{t}, \\ \epsilon \eta_B \cos(t - \bar{t}), & t > \bar{t}. \end{cases} \quad (A.1)$$

Substituting this into (2.18) for $i = B$ allows determination of $\mathbf{n}_B(x, t)$ and hence $F_B(t)$. Rearranging (2.17a) for $Y_A$ and differentiating with respect to time gives

$$\dot{Y}_A = \eta_A \frac{\dot{L} - \alpha_A \dot{F}_A}{1 - \beta - \alpha_A \dot{F}_A}, \quad (A.2)$$

assuming $N_A$ is constant. Differentiating (2.18d) with respect to time gives

$$\dot{F}_A = \rho_A \int_{-\infty}^{\infty} x \mathbf{q} \cdot \left( -\gamma_A \dot{Y}_A \frac{\partial \mathbf{n}_A}{\partial x} + \mathbf{S}_A \mathbf{n}_A \right) dx, \quad (A.3)$$
using (2.18a) and assuming that $\rho_A$ remains constant. The first term in the integral can be integrated by parts giving
\[ \dot{F} = \rho_A (\gamma_A \dot{Y}_A H_2(t) + H_1(t)), \] (A.4)
where
\[ H_1(t) = \int_{-\infty}^{\infty} x q \cdot S_A n_A \, dx, \] (A.5a)
\[ H_2(t) = \int_{-\infty}^{\infty} q \cdot n_A \, dx. \] (A.5b)
Substituting for $\dot{F}_A$ into the expression for $\dot{Y}_A$ (A.2), and solving for $\dot{Y}_A$ yields
\[ \dot{Y}_A = \frac{\dot{L}(t) - \alpha_A \rho_A H_1(t)}{(1/\eta_A)(1 - \beta - \alpha_A F_A) + \alpha_A \rho_A \gamma_A H_2(t)}, \] (A.6)
where $\dot{L}(t) = \dot{Y}_B(t)/\eta_B$. This expression for $\dot{Y}_A$ then used in (2.18) for $i = A$ to determine $n_A(x, t)$ and $F_A(t)$. At any time $t$, having calculated $F_A(t)$, the sarcomere lengths for the A contractile units is given by
\[ Y_A(t) = \eta_A \frac{L - \beta - \alpha_A F_A}{1 - \beta - \alpha_A F_A}. \] (A.7)
Comparing this with the equation for $Y_B$ (2.17b), we note how the elasticity of the cell nucleus modifies the behaviour of the row A sarcomeres. It is then possible to determine the time-dependent tension on the cell via (2.17d) and (2.17c) yields $Z(t)$.

The time-dependent dimensionless equations (2.18) together with (A.1) and (A.6) are solved computationally subject to the initial and boundary conditions (2.19), using a Godunov scheme (Harten et al., 1983; Roe, 1986). This scheme combines an upstream differencing finite volume method with a method-of-characteristics approach. To ensure simulation results were accurate, the code was validated by comparing results obtained with the Godunov scheme on a single sarcomere, and corresponding results obtained using a predictor-corrector McCormack scheme; solutions were identical. The numerical artefacts in Fig. 6 appear in every cycle and are a result of having to apply a CFL stability condition for values of shortening velocity larger than some threshold value in the solution of the Godunov scheme. If applied to very small values of shortening velocity the corresponding time steps get very large, which, although allow stability for the PDE solution, causes problems with the chemical kinetics steps of the solution procedure.