STRESS DISTRIBUTION IN THE CYTOSKELETON

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ABSTRACT

We mapped the distributions of displacements and computed the stress field within the cytoskeleton (CSK) of smooth muscle cells, using fluorescently tagged mitochondria as fiducial markers of intracellular structures. A localized oscillatory load was applied with a magnetic bead bound to integrin receptors. We found displacements and stresses were concentrated at discrete sites quite remote from the localized load; the distributions of displacements and stresses were lumpy. This finding represents a major departure from predictions of existing continuum models of cell mechanics. The stress lumps were abolished when the microfilament lattice was disrupted. The distributions of the displacement and stress lumps were altered when the level of tension generation in the CSK was changed. These data suggest that stress lumps could in part be attributable to the heterogeneous distribution of the prestress in the CSK.

INTRODUCTION

A fundamental question in the field of mechanotransduction is how mechanical stresses present at the cell surface are transmitted into the cell body. Despite ample evidence that mechanical forces are critical for many cell functions such as growth, proliferation, protein synthesis, and gene expression, the specific pathways of mechanical force transmission and transduction remain elusive. Published work suggests that integrin receptors are one of the primary molecular pathways for mechanical force transmission and transduction across the cell membrane to the CSK. However, other models have been proposed that favor the general deformation of the membrane as the primary mode of force transmission and subsequent diffusion-based processes as the means of signal transduction. Even in the case of mechanical force transmission across the integrin receptors, some investigators suggest that it is the diffusion of signaling molecules that dominates the signaling cascades beyond the cytoplasmic domain of the focal adhesion complex and they question the role of the deep cytoplasmic CSK structures in force transmission and transduction. Therefore it is crucial to determine to what degree deep cytoplasmic structures (e.g., the CSK) transmit mechanical stresses. It follows that in order to determine potential specific loci of mechanotransduction inside the cell, one must first determine the distribution of stress in the cytoplasm in response to external mechanical stimuli.

METHODS

Human airway smooth muscle cells were isolated from tracheal muscle of lung transplant donors. Adenovirus vectors were added for 2 days to transiently transfect YFP-cytochrome C oxidase into the mitochondria of cells after the cells (passage 3-8) reached 70-80% confluency. Then the cells were plated on type 1 collagen-coated dishes overnight before experiments.

We used an inverted microscope (Leica) with a 40X objective (NA=0.55). A progressive scan, triggerable black and white CCD camera with pixel-clock synchronization (JAI CV-M10, Glostrup, Denmark) was attached to the microscope. Image acquisition was phase locked to the sinusoidal twisting field. Images were digitized and transferred to the PC memory using a frame grabber. Optical magnetic cell twisting was used for applying twisting torques to cells in a dish under a microscope and for detection of cellular deformations [1]. A pair of twisting coils and a pair of magnetizing coils were mounted to the microscope stage. The microscope stage was heated to maintain 37^0C. The twisting current was driven by a current source controlled by a micro-controller. Ferromagnetic beads (4.5-μm diameter) coated with Arg-Gly-Asp (RGD)-containing peptides (specific ligands for integrin receptors) were bound the surface of the adherent cells for 15 min. The beads were magnetized by a strong (1,000 G) and short (<0.1 ms) magnetic field pulse oriented in the horizontal direction. A sinusoidally varying, homogeneous magnetic "twisting" field was applied in the vertical direction, and resulting bead displacements induced by bead rotation were determined by quantifying the bead center movement using an intensity-weighted-center-of-mass algorithm. The specific torque applied to the beads was calibrated by rotating beads in a viscosity standard. This method could be used to calculate the corresponding dynamic modulus. Image acquisition was phase-locked to the twisting field such that 10 images were taken during one twisting cycle. To reduce noise caused by

spontaneous cytoskeletal movements, we averaged images taken during the same twisting phase over 3 to 10 cycles (10-30 seconds). The images were then subdivided into arrays of 11x11 pixels (corresponding to 2.2 μm x 2.2 μm). The arrays overlapped by 5 pixels. We computed the displacement field by comparing corresponding arrays between two images taken at different phases during the twisting cycle. We shifted the arrays of the second image by sub-pixel increments (10 nm) in the Fourier-domain until the mean square differences of the pixel-intensities between the shifted array and the corresponding array from the first image reached a minimum. The resolution of the displacement measurements was 10 nm.

RESULTS

To determine the displacement distribution in the cytoplasm, we used mitochondria that were fluorescently labeled by transfecting airway smooth muscle cells with YFP (yellow fluorescent protein)-cytochrome C oxidase. These mitochondria served as fiducial markers to quantify intracellular displacements that resulted from local external mechanical loads. Bright field images of magnetic beads and fluorescent images of the mitochondria were collected at a rate of 10 images per twisting cycle. Typically, we applied a magnetic twisting field with amplitude of 50 Gauss (specific torque=90 Pa) at a fixed frequency of 0.3 Hz.

The displacements of the mitochondria were high near the rotating magnetic bead and decayed away from the bead, as would be predicted by conventional models of cell mechanics. In many cells, however, we found that displacements of mitochondria did not decay quickly in space, with appreciable displacements more than 20 μm away from the bead center (Fig. 1 top panel). Rather than smooth, the distribution of displacements of the mitochondria was found to be lumpy at discrete sites.

From this displacement field, we computed the tractions acting on a cross-sectional surface (corresponding to a transversal section through the cell defined by the focal plane of the microscope) as described in [2, 3]. These tractions would be required to generate the measured displacement field and thus represent an index of the stress field inside the cell at the focal plane. Fig. 1 middle panel shows the stress field computed from the mitochondria displacement data. We used the bead lateral displacement to estimate the Young's modulus of the cell, assuming a Poisson ratio of 0.5.

We found that stresses were concentrated at different sites in a discrete manner that could be described as lumpy. This was surprising because existing models of cell mechanics predict that the stress distribution would decay smoothly. Furthermore, small areas with appreciable stress lumps could be seen at a distance more than 20 μm away from the maximum stresses. These findings call into question continuum models of cell mechanics that predict that the induced stress field decays rapidly as a function of $1/R^2$, with R being the distance from the external load. These data thus demonstrate force transmission to sites remote from a localized load.

We further tested if the level of contractile activation in those cells can contribute to the distribution of the stress lumps within the CSK. Activation of the cells with histamine, a contractile agonist that increases CSK tension by elevating acto-myosin interactions, caused the stress pattern to appreciably change (Fig. 1 bottom left). To determine the role of actin microfilaments in the stress distribution, we disrupted actin microfilament lattice integrity with cytochalasin D. This led to an almost complete abolishment of stress lumps in the cell (Fig. 1 bottom right). These results suggest that integrity of the actin lattice and the contractile stress carried by these actin microfilaments are at least partially responsible for the generation of stress lumps in the CSK far away from a localized load.

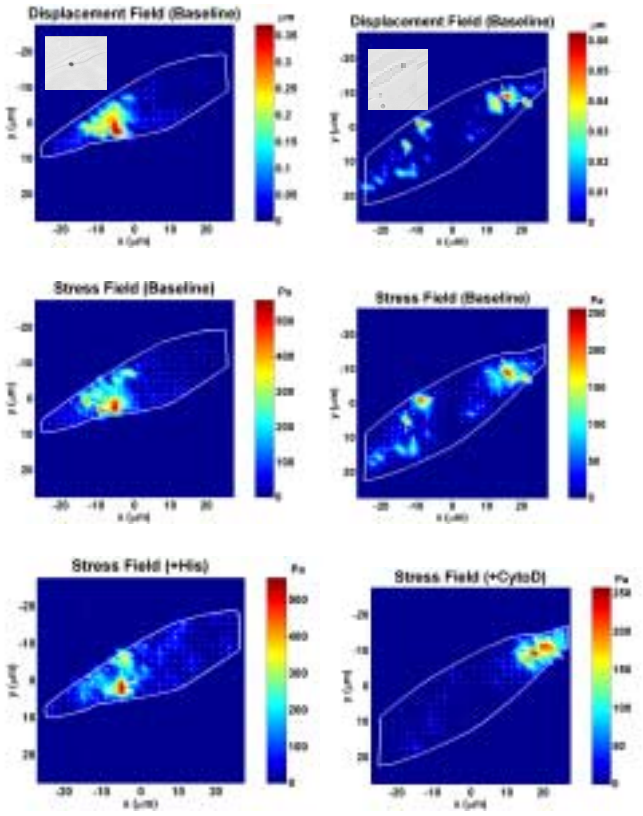

Fig. 1 The displacement fields (top) of two individual cells in response to a localized oscillatory load applied via a RGD-coated magnetic bead (inserts) and the computed stress fields under baseline (middle) and treated (bottom) conditions.

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