

Displacement Field of the Cytoskeleton in Response to a Local Load

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Abstract-Mechanical stresses acting on the apical cell surface are transmitted to the anchoring sites of the cell via cytoskeletal polymers, but details of the stress-, strain- or deformation field within the cell are largely unknown. Here we have measured the deformation field within cultured smooth muscle cells in response to small stresses. Stresses were applied to integrin receptors on the cell surface via magnetic microbeads (4.5 μm diameter). The beads were torqued in a sinusoidally varying magnetic twisting field (specific torque amplitude of 90 Pa, frequency 0.3 Hz). Cells were transfected to express either fluorescent mitochondria, microfilaments, or microtubules. 10 images were taken during each of 10 or more twisting cycles, from which we computed the deformation field within the cell. Our results confirm that mechanical stresses in cells can be transmitted via focal adhesions on the apical cell surface to the internal cytoskeleton. Importantly, cytoskeletal deformations in most cells decayed to below the resolution limit within a short distance ($\sim 5\mu\text{m}$) from the locus of stress application.

Keywords - mechanical stress, cytoskeleton, magnetic twisting

I. INTRODUCTION

It has been well established that mechanical stresses acting on the surface of adherent, eukaryotic cells are transmitted through the cell via cytoskeletal polymers [1-4]. However, important details of how those mechanical stresses are spatially distributed remain largely unknown. In this study we asked whether displacements of the internal cytoskeleton are localized to the neighborhood of the stress source, or whether they can be transmitted over large distances along stress fibers.

II. METHODOLOGY

Living human airway smooth muscle (HASM) cells were adenovirus-transfected with yellow or green fluorescent protein-labeled mitochondria, microfilaments, or microtubules. Stresses were applied to integrin receptors on the cell surface via magnetic microbeads (4.5 μm diameter). The beads were permanently magnetized in the horizontal direction and then torqued vertically in a sinusoidally varying magnetic twisting field (amplitude of 5 mT, frequency 0.3 Hz). The resulting specific torque (the ratio of magnetic torque to bead volume) was 90 Pa. We also measured cytoskeletal deformations at specific torque amplitudes of 18 Pa and 45 Pa, and under influence of the contractile agonist histamine (100 μM). Image acquisition was phase-locked to the twisting field; 10 images were taken during each twisting cycle. To reduce noise caused by spontaneous cytoskeletal movements, we averaged images taken during the same twisting phase over at least 10 cycles (32 seconds). The averaged images were cropped to a size

of 32 μm square, with the bead in the center. The images were then subdivided into arrays of 11x11 pixels (2.2 μm x 2.2 μm). The arrays overlapped by 5 pixels (1.0 μm). We computed the cytoskeletal deformation 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 (0.01 μm) 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.

III. RESULTS

Displacements of the magnetic beads and the fluorescently labeled mitochondria, microfilaments or microtubules, followed the sinusoidal twisting field. Bead displacement amplitude was 0.37 ± 0.03 μm , while the displacement amplitude of the mitochondria beneath the bead was 0.12 ± 0.08 μm (mean \pm SE, $n=130$ cells). On average, cytoskeletal displacements were largest immediately beneath the bead, and decayed to below the noise floor of our measurements (0.01 μm) within a radius of 5 μm . When cells were stimulated with the contractile agonist histamine (100 μM), both bead and cytoskeletal displacements decreased within 1 minute to 65% of baseline amplitude. When probed at different twisting fields, cytoskeletal displacements were proportional to the applied torque.

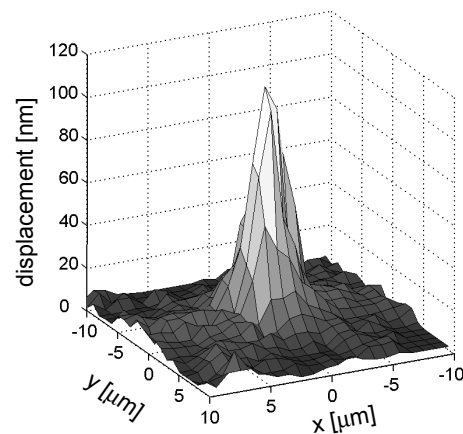


Fig. 1: Displacement amplitude of mitochondria motion (mean values of 130 human airway smooth muscle cells), measured at a height of $\sim 2\mu\text{m}$ from the basal cell surface, in response to a specific torque application on the apical cell surface of 90 Pa. The coordinate ($x=0$, $y=0$) denotes the center of the magnetic bead. The maximum displacement amplitude was 0.12 μm .

IV. DISCUSSION

In this study, we investigated the deformations of the internal cytoskeleton in living cells that are caused by mechanical stresses applied to the apical cell surface. By applying periodic stresses at a frequency of 0.3 Hz and using phase-locked image acquisition, we could markedly reduce the effects of spontaneous cytoskeletal movements and reorganization [5]. Our results indicate that cytoskeletal deformations linearly increase with the magnitude of external stresses. Further, we found that cytoskeletal deformations are proportional to changes of cell stiffness caused by contractile agonists, as gauged by the bead motion amplitude. This also confirms that beads coated with integrin receptor ligands are tightly linked to the internal cytoskeleton. Most noteworthy, we found that cytoskeletal deformations are negligible beyond 5 μm from the bead center (Fig. 1), indicating that there is sufficient coupling within the cytoskeleton that mechanical stresses decay within short distances. We noted in some cells, however, that cytoskeletal movements synchronous to the twisting field could spread well beyond a distance of 10 μm from the bead center. We hypothesize that this might be related to larger spatial separations between focal adhesions on the basal cell surface.

V. CONCLUSION

We have developed a method to measure the displacement field of the cytoskeleton in response to a local load. Our results confirm that mechanical stresses in cells can be transmitted from focal adhesions on the apical cell surface to the internal cytoskeleton. Deformations of the cytoskeleton decay within a short distance from the locus of stress application.

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