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Cytoskeleton dynamics: Fluctuations within the network

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Abstract

Out-of-equilibrium systems, such as the dynamics of a living cytoskeleton (CSK), are inherently noisy with fluctuations arising from the stochastic nature of the underlying biochemical and molecular events. Recently, such fluctuations within the cell were characterized by observing spontaneous nano-scale motions of an RGD-coated microbead bound to the cell surface [Bursac et al., Nat. Mater. 4 (2005) 557–561]. While these reported anomalous bead motions represent a molecular level reorganization (remodeling) of microstructures in contact with the bead, a precise nature of these cytoskeletal constituents and forces that drive their remodeling dynamics are largely unclear. Here, we focused upon spontaneous motions of an RGD-coated bead and, in particular, assessed to what extent these motions are attributable to (i) bulk cell movement (cell crawling), (ii) dynamics of focal adhesions, (iii) dynamics of lipid membrane, and/or (iv) dynamics of the underlying actin CSK driven by myosin motors.

Keywords: Cytoskeleton; Remodeling; Lipid dynamics; Airway smooth muscle

The cytoskeleton (CSK) is fundamental to many cellular processes, including proliferation, migration, and contraction [7,20,21]. To perform these functions, cells orchestrate a complex cascade of signals and molecules that lead to robust structural changes in the underlying CSK [15,21]. This ability of the CSK to disassemble, to reform, and to stabilize provides, for example, a basic framework for metastasis of a cancer cell as it explores, crawls, and invades other tissues [5,41,45]. As such, progression of cancer as well as pathogenesis of many chronic disorders is now thought to be associated with abnormalities in the stability and dynamics of the underlying CSK [16,34,37,39].

The CSK is a network of actin filaments, microtubules, and intermediate filaments that are bound together by associated cross-linkers and driven by motor proteins [8,21,27]: the cell interior is a crowded environment [11,12] and, at the same time, is far from an equilibrium system [18]. Such complexity of the network and its out-of-equilibrium dynamics are the focus of much attention in the fields as diverse as condensed matter physics [10,17,28] as well as biophysics of the living cell [13,25,26,35,38,42]. Most recently, we have made a series of phenomenological observations in a number of different cell types and reported a functional assay that probes molecular level fluctuations within the living cell [1,3,6]. This assay is based on spontaneous nano-scale movements of an individual microbead that is coated with a peptide

Abbreviations: ASM, airway smooth muscle; CSK, cytoskeleton; MSD, mean square displacement; acLDL, acetylated low-density lipoprotein; PLL, poly-L-lysine; RGD, arginine–glycine–aspartic acid.

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containing the sequence Arg-Gly-Asp (RGD); such beads bind to cell surface integrin receptors [43], form focal adhesions [29,30], and become well integrated into the cytoskeletal scaffolding [13,19,29,33]. Accordingly, these bead motions may reflect ongoing remodeling events of the underlying cytoskeletal network [1,3,6], but the structural origin of such motions and forces that drive these dynamics in a living cell remain to be elucidated.

Like the CSK of many cell types, that of the airway smooth muscle (ASM) cell is a dynamic structure that is in a continuous state of remodeling [1,4,6,24,31,39]. Using the ASM cell as a model and, using multiple well-defined cell microenvironments, we provide here strong evidence that spontaneous nano-scale motions of an individual RGD-coated microbead report ongoing molecular level reorganization of the underlying actin CSK driven by myosin motors.

Materials and methods

Materials. Tissue culture reagents were obtained from Sigma (St. Louis, MO). The synthetic Arg-Gly-Asp (RGD) containing peptide was purchased from American Peptide Company, Inc. (Sunnyvale, CA) and acetylated low-density lipoprotein (acLDL) was purchased from Biomedical Technologies (Stoughton, MA). The micropatterned (50 μm × 50 μm) substrates were a generous gift from Dr. Phillip DeLuc (Boston, MA). All other reagents and drugs were obtained from Sigma with the exception of jasplakinolide, which was purchased from CalBiochem (La Holla, CA). Jasplakinolide and cytochalasin-D were prepared in sterile dimethylsulfoxide (DMSO). Histamine and N^6,2′-*O*-dibutyryladenosine 3′,5′-cyclic monophosphate (db-cAMP) were reconstituted in sterile distilled water. On the day of experiments, all drugs were diluted to final concentrations in serum-free media, yielding less than 0.1% DMSO in final volume.

Cell culture. Human ASM cells were provided by Dr. Reynold Panettieri (University of Pennsylvania, PA) and rat ASM cells were prepared as previously described [1,2]. Cells were grown until confluence at 37 °C in humidified air containing 5% CO_2 and passaged with 0.25% trypsin–0.02% EDTA solution every 10–14 days. In the present study, we used cells in passages 3–7. Unless otherwise specified, serum-deprived post-confluent cells were plated at 20,000 cells/cm^2 on plastic wells (96-well Removawell, Immulon II: VWR International, West Chester, PA) previously coated with type I collagen (Vitrogen 100; Cohesion, Palo Alto, CA) at 500 ng/cm^2. Cells were maintained in serum-free media for 24 h at 37 °C in humidified air containing 5% CO_2. These conditions have been optimized for seeding cultured cells on collagen matrix and for assessing their mechanical properties [2,13,43].

Bead coating. Ferrimagnetic microbeads were coated with RGD, acLDL, or PLL as described previously [9,13,33,43]. Unless otherwise noted, approximately 5×10^4 beads were added to individual sample wells and incubated for 20 min. Unbound beads were then removed by washing cells with serum-free media.

Characterization of spontaneous bead motions. Under microscopic observation, we visualized spontaneous displacements of an individual microbead (approximately 40–100 beads per field-of-view) and recorded its positions at frequency of 12 frames/s for $t_{max} \sim 330$ s as previously described [6]. Bead positions were corrected for the effects of microscope stage drift; the stage drift was estimated from changes in the mean position of all beads within a field of view [6]. We defined mean square displacement (MSD) of an individual bead as

$$\mathrm{MSD}_b\langle \Delta t\rangle = \langle (r(t+\Delta t) - r(t))^2 \rangle \quad (1)$$

where $r(t)$ is the bead position at time t, Δt is the time lag, and brackets indicate an average over many starting times t [6]. The limit of resolution in our system was in the order of ~10 nm, but for $\Delta t \sim 4$ s most beads had displaced a much greater distance. Accordingly, we analyzed data for time lags greater than 4 s and up to t_{max}. MSD of most beads increased with time according to a power-law relationship.

$$\mathrm{MSD}(\Delta t) = D^*(\Delta t/\Delta t_o)^{\alpha} \quad (2)$$

The coefficient D^* and the exponent α of an individual bead were estimated from a least-square fit of a power–law to the MSD data for Δt between 4 s and $t_{max}/4$. The upper cut-off of $t_{max}/4$ was chosen arbitrarily to increase statistical accuracy of the estimated D^* and α. We took Δt_o to be 1 s and expressed D^* in units of nm^2.

In the present study, we quantified individual bead motions both before and after each drug treatment by MSD(Δt). To modulate actin polymerization, cells were treated either with actin disrupting agent cytochalasin-D (1 μM) for 30–60 min or with actin polymerizing agent jasplakinolide (1 μM) for 10 min. To modulate actomyosin interactions, cells were contracted for 5 min with histamine (100 μM) or relaxed for 15 min with db-cAMP (1 mM).

Optical magnetic twisting cytometry (OMTC). To estimate the stiffness of structures bound to the bead, we measured bead displacements under applied torque as previously described [13]. In brief, ferrimagnetic microbeads were first magnetized horizontally (parallel to the surface on which cells were plated) and then twisted in a vertically aligned homogenous magnetic field (20 G) at a frequency of 0.75 Hz. The resulting lateral bead displacements in response to the oscillatory torque were detected optically, and the ratio of specific torque to lateral bead displacements was computed and expressed as the cell stiffness in units of Pa/nm.

Results and discussion

Characterization of spontaneous bead motions

Spontaneous motions of each RGD-coated bead (4.5 μm in diameter) bound to the surface of the ASM cell were random and consisted of relatively small steps (Fig. 1A); over the course of 5 min, bead trajectories amounted to only a small fraction of the bead diameter. Such trajectories, however, appeared elongated or directed, suggesting a certain degree of positive correlation between incremental bead steps.

For each bead, we characterized its spontaneous nano-scale motions by calculating mean square displacement (MSD_b) (Eq. 1); MSD_b varied by two orders of magnitude, but MSD_b of most beads increased with time according to a power-law relationship (Fig. 1B). These motions were further characterized by fitting a power–law to individual MSD_b to estimate diffusion coefficient D^* and the exponent α. The probability density of the diffusion coefficient D^*, between individual beads, showed monophasic and almost lognormal distributions with a maximum of 50 nm^2, whereas that of the exponent α exhibited monophasic and almost normal distributions with a maximum of 1.6 (Fig. 1C). Accordingly, ensemble average of all MSD_b (MSD) demonstrated superdiffusive behavior ($\alpha > 1$), whereby the MSD increased with time as $\sim t^{1.6}$ (Fig. 1B, inset). Taken together, unlike a simple diffusive thermal Brownian motion that increases its MSD linearly with time [23], spontaneous motions of an individual RGD-coated