Cell

Probing the Stochastic, Motor-Driven Properties of the Cytoplasm Using Force Spectrum Microscopy

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SUMMARY

Molecular motors in cells typically produce highly directed motion; however, the aggregate, incoherent effect of all active processes also creates randomly fluctuating forces, which drive diffusive-like, non-thermal motion. Here, we introduce force-spectrum-microscopy (FSM) to directly quantify random forces within the cytoplasm of cells and thereby probe stochastic motor activity. This technique combines measurements of the random motion of probe particles with independent micromechanical measurements of the cytoplasm to quantify the spectrum of force fluctuations. Using FSM, we show that force fluctuations substantially enhance intracellular movement of small and large components. The fluctuations are three times larger in malignant cells than in their benign counterparts. We further demonstrate that vimentin acts globally to anchor organelles against randomly fluctuating forces in the cytoplasm, with no effect on their magnitude. Thus, FSM has broad applications for understanding the cytoplasm and its intracellular processes in relation to cell physiology in healthy and diseased states.

INTRODUCTION

The cytoplasm of living cells is not a static environment but is instead subjected to a wide variety of forces (Howard, 2001). For example, molecular motors such as kinesin and dynein generate forces that directionally transport cargo along microtubule tracks, while myosin II motors actively contract actin filaments (Vale, 2003). These active processes all have clearly established functions in the cell, and their individual forces have been precisely quantified (Svoboda and Block, 1994; Vale, 2003). Collectively, these forces have important consequences in the cytoplasm: several motors operating coherently can generate large forces for directional transport (Hendricks et al., 2012; Rai et al., 2013). On an even larger scale, the cooperative activity of a large number of motors and other active processes collectively drive critical functions at the level of the whole cell, such as division, migration, and contraction (Doyle and Yamada, 2010; Dufrêne et al., 2011; Grashoff et al., 2010; Gundersen and Worman, 2013; Heisenberg and Bellaïche, 2013). However, the aggregate effect of all the motors and active processes also contribute an incoherent background of fluctuating forces, and the ensemble aggregate of the forces from the incoherent effects of all cellular activities is directly associated with the functional efficiency and the overall metabolic state of the cell (Doyle and Yamada, 2010). In the cytoplasm, these fluctuating forces can give rise, for instance, to random motion of vesicles, mitochondria, and signaling proteins (Ananthanarayanan et al., 2013; Brangwynne et al., 2008a; del Alamo et al., 2008; Hammar et al., 2012; Han et al., 1999; Jaqaman et al., 2011; Kyoung and Sheets, 2008) and may drive an even broader range of intracellular dynamics.

Because the motion that arises from fluctuating forces in the cytoplasm is random, little previous effort has been made to quantify these forces. Indeed, many researchers have interpreted random cytoplasmic motion as arising primarily from thermally-induced diffusion, not recognizing the role of overall, aggregate forces. The ensemble forces from overall cellular activity are likely to have a large effect on overall motion within the cytoplasm and would change as the biochemical processes giving rise to these forces are altered during different cell conditions. These forces could thus be a critical readout of the dynamic state of the cell. Because of this, we sought out a direct way to measure aggregate forces within the cytoplasm, aiming to devise methodology for quantifying these forces and testing how they impact different cell states and control motion of cytoplasmic components.

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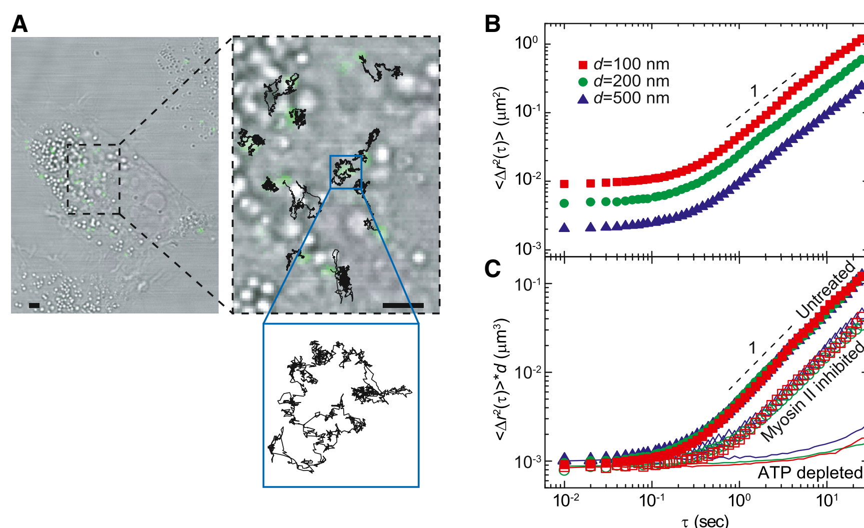

Figure 1. Movements of Microinjected Tracer Particles in Living Cells

(A) Bright-field image of an A7 cell with microinjected 200-nm-diameter fluorescence particles (green) and 2 min trajectories (black) superimposed on top. PEG-coated particles are microinjected into cells grown on collagen-I-coated, glass-bottom dishes. Particle trajectories in the cytoplasm look very similar to thermal Brownian motion. Scale bar, 5 μm.

(B) Two-dimensional ensemble-averaged mean-square displacement (MSD) $<\Delta r^2(\tau)>$ of PEG-coated tracer particles of various sizes are plotted against lag time on a log-log scale, in living A7 cells. Red, green, and blue symbols and lines represent particles that are 100, 200, and 500 nm in diameter, respectively. Dashed lines indicate a logarithmic slope of 1. Measurements are done with more than 200 tracer particles in about 25 individual cells for each particle size.

(C) Ensemble-averaged MSD scaled with particle diameter, in untreated (solid symbols), blebbistatin treated (open symbols), and ATP-depleted (solid lines) A7 cells.

See also Figure S1 and Movie S1.

Toward this end, we introduce force spectrum microscopy (FSM), an approach that probes the frequency dependence of the aggregate, incoherent cytoplasmic forces within a cell. To accomplish this, we combine independent measurements of the intracellular fluctuating movement of injected particles with measurements of the mechanics of the cytoplasm performed with active microrheology using laser tweezers. With these measurements, we determine the temporal spectrum of the ensemble of the random, fluctuating forces, demonstrating that this ubiquitous fluctuating motion in cells is not thermally induced, but is instead a consequence of random forces. We then exploit FSM to probe the intracellular micromechanical behavior of malignant and benign cells, and show that cancer cells exhibit a significantly enhanced level of forces, albeit with the same frequency dependence, as predicted by our model. Moreover, we show that these active forces strongly dominate thermal Brownian forces in the cellular interior, impacting motion of objects from nanometers to microns in scale, and providing a fundamental mechanism for transport of objects of all scales. Thus, FSM is a valuable new tool for characterizing the dynamic state of a cell.

RESULTS

Random Intracellular Movement Appears Diffusive

To measure the fluctuating motion in the cytoplasm of eukaryotic cells, we microinjected submicron colloidal particles into A7 melanoma cells (Cunningham et al., 1992) and measured their time-dependent motion with confocal microscopy. The particles were rendered inert by attaching a short polyethylene-glycol (PEG) brush layer to their surface to eliminate interactions with biopolymers or proteins (Valentine et al., 2004). Moreover, because they were microinjected, the particles were not surrounded by a lipid membrane. Unlike smaller tracers that travel freely through the cytoskeletal network, the injected submicron particles were larger than the typical cytoskeletal mesh size, which is about 50 nm (Luby-Phelps, 2000; Luby-Phelps et al., 1987). Thus, their motion reflects the fluctuations of the cytoplasm itself. To avoid cell-boundary effects, we imaged particles that are greater than ~1 μm deep within the cell; we also imaged particles away from both the thin lamellar region and the nucleus to avoid any interactions with the mechanically distinct cell cortex and nucleus (Extended Results). Particle centers were determined in each image with an accuracy of 22 nm. We tracked their trajectories and calculated the time- and ensemble-averaged mean-square displacement (MSD), $<\Delta r^2(\tau)>$, where $\Delta r(\tau) = r(t+\tau)-r(t)$.

At shorter timescales ($t \leq 0.1$ s), the MSD of the probe particles was nearly constant in time; however, the fluctuations were always at least five times larger than the noise floor. At longer timescales ($t \geq 0.1$ s), the MSD increased approximately linearly with time, as shown in Figure 1 and Figure S1, available online. Particles of different size, d, exhibited a similar time dependence, as shown in Figure 1B. Moreover, the amplitude of the fluctuations scaled as $1/d$, as shown in Figure 1C; this is consistent with motion in the continuum viscoelastic environment of the cytoplasm (Hoffman et al., 2006). Such motion is often interpreted as thermal Brownian motion (Baker et al., 2010; del Alamo et al., 2008; Gupton et al., 2005; Hale et al., 2009; Wu et al., 2012; Yamada et al., 2000). However, a MSD that increases linearly with time is only consistent with Brownian motion in a purely viscous liquid and at thermal equilibrium, neither of which applies to the cytoplasm (Brangwynne et al., 2008a; Bursac et al., 2005; Hoffman et al., 2006; MacKintosh, 2012; Wilhelm, 2008). As the cytoplasm is neither a pure viscous liquid or at thermal equilibrium, we concluded that the observed cytoplasmic fluctuations must derive from some other sources other than thermal-based diffusion.

Cytoplasmic Diffusive-like Movement Results from Active Processes

To clarify the active character of the cytoplasmic fluctuating motion, we examined the effect of inhibiting myosin II activity

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