Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 360 (2007) 797–801
BBRC
www.elsevier.com/locate/ybbrc

Directional memory and caged dynamics in cytoskeletal remodelling

Guillaume Lenormand *, Julien Chopin, Predrag Bursac, Jeffrey J. Fredberg, James P. Butler

Molecular and Integrative Physiological Sciences, Department of Environmental Health, School of Public Health, Harvard University, 665 Huntington Avenue, Building I, Room 1305, Boston, MA 02115, USA

Received 15 May 2007
Available online 5 July 2007

Abstract

We report directional memory of spontaneous nanoscale displacements of an individual bead firmly anchored to the cytoskeleton of a living cell. A novel method of analysis shows that for shorter time intervals cytoskeletal displacements are antipersistent and thus provides direct evidence in a living cell of molecular trapping and caged dynamics. At longer time intervals displacements are persistent. The transition from antipersistence to persistence is indicative of a time-scale for cage rearrangements and is found to depend upon energy release due to ATP hydrolysis and proximity to a glass transition. Anomalous diffusion is known to imply memory, but we show here that memory is attributed to direction rather than step size. As such, these data are the first to provide a molecular-scale physical picture describing the cytoskeletal remodelling process and its rate of progression.

Keywords: Magnetic twisting cytometry; Soft glassy rheology; Jamming; Spontaneous bead motion; Actin dynamics; Human airway smooth muscle

The network of biopolymers that confers shape stability and mechanical integrity to the living cell is called the cytoskeleton (CSK). The CSK is highly dynamic and in a constant state of remodelling. This CSK remodelling, in turn, is fundamental to a wide variety of cellular functions including locomotion, invasion, contraction, and wound healing, but the physical laws governing underlying molecular-scale structural rearrangements within the CSK remain quite unclear. Recently, we have reported a striking but controversial analogy between the dynamics of the CSK and that of inert out-of-equilibrium systems, especially soft glassy materials [1–5]. From forced motions of a microbead tightly bound to the CSK of the living cell, we established scale-free rheology [4,5], mechanical aging [1], and shear-induced fluidization [1,2], and from spontaneous nanoscale motions of the same bead we established intermittent dynamics, slow relaxation, approach to kinetic arrest, and breakdown of the fluctuation–dissipation theorem [1,2]. These out-of-equilibrium dynamics, taken together, are strongly reminiscent of those that are characteristic of inert soft glassy materials [6].

Nonetheless, the extent to which the CSK properly belongs to the class of soft glassy materials remains a question of some debate [7–9]. In a soft glassy material, slow relaxation and approach to kinetic arrest are thought to reflect approach to a glass transition and associated confinement of a constitutive particle by a cage formed by its neighbours [6,10,11]. It is the structural rearrangement of this cage that is thought to lead to the final slow structural relaxation that limits the rate of particle diffusion through the sample.

Caged dynamics have been suggested in the past based upon observations of the mean square displacement, $\langle r^2(t)\rangle$, of sequential tracer displacements during anomalous diffusion [1,10,11], but because the mean square displacement cannot distinguish between memory of tracer step size versus memory of step direction, that measure of anomalous diffusion is merely suggestive of caged motion (Online supplement 1). Based upon correlations of tracer sequential angular displacements, by contrast, we report here the first direct evidence of directional memory and caged dynamics at the molecular level. At short time intervals, Δt, displace-

* Corresponding author. Fax: +1 617 432 4710.
E-mail address: glenorma@hsph.harvard.edu (G. Lenormand).

0006-291X/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2007.05.228

ments are found to be antipersistent; when a step is in one direction, the following step is more likely to be in the opposite direction, and therefore this behaviour provides the first direct evidence of molecular trapping and caged dynamics. At longer Δt, however, displacements are found to be persistent; when a step is in one direction, the following step is more likely to be in the same direction.

Materials and methods

Microbead coating, cell preparation, and mean square displacement measurements used in this paper are described in detail elsewhere [1,12,13] and are summarized below.

Microbead and living cell preparation. Ferrimagnetic microbeads (4.2 μm in diameter) are produced in our laboratory, and coated with a peptide containing the sequence Arg-Gly-Asp (150 μg ligand/mg microbeads) by overnight incubation at 4 °C in carbonate buffer (pH 9.4).

Human airway smooth muscle (HASM) cells in passage 6–7 are serum-deprived for 36 h; serum deprivation arrests the cell cycle in the G_1/G_0 phases. Plastic wells (6.4 mm, 96-well Removawells, Immunlon, IL) are coated with collagen I at a density of 500 ng/cm^2 for at least 12 h. Cells are harvested with trypsin and incubated overnight in collagen-coated wells at confluence (~20,000 cells/well). Prior to each measurement, approximately 10,000 microbeads are added to an individual well and incubated for 20 min. The well is then rinsed twice with serum-free medium at room temperature to remove unbound microbeads.

Reagents. Tissue culture reagents are obtained from Sigma (St. Louis, MO, USA), with the following exceptions: Trypsin–ETDA solution, which is purchased from Gibco (Grand Island, NY), Type I rat tail collagen (Vitrogen Collagen) from Cohesion Technologies (Palo Alto, CA), RGD peptide (Peptide 2000) from Telios Pharmaceuticals (San Diego, CA), jasplakinolide from CalBiochem (La Holla, CA). Jasplakinolide (Jasp) and cytochalasin-D are prepared in sterile dimethylsulfoxide (DMSO). Histamine and dibutyryl adenosine 3′,5′-cyclic monophosphate (DBcAMP) are reconstituted in sterile distilled water. On the day of experiments, all drugs are diluted to final concentrations in serum-free media, yielding less than 0.1% DMSO in final volume.

ATP is depleted by incubation with 2 mM deoxyglucose and 2 mM NaN_3. Residual ATP is assayed using a standard luciferase-based ATP determination kit (Sigma), and concentrations after depletion are from 2% to 7% of that in control samples.

Mean square displacement measurements. When a RGD-coated microbead is incubated on the surface of a HASM cell, it ligates integrin receptors and forms focal adhesion complexes. Mechanical responses of such a bead have a precise locus that remains equivocal [9,14–17], but these responses have been shown in an unequivocal manner to be highly sensitive to manipulations of actin filaments, myosin motors, vinculin, heat shock proteins 20 and 27, cell spreading, cell stretching, cytoskeletal tension, and depletion of ATP [1,2,4,18–25]. We reasoned, accordingly, that the bead can move spontaneously only if the cytoskeletal structures to which it is attached rearrange; therefore, spontaneous bead motions report ongoing CSK remodelling in space and time [18], whereby CSK we mean any structure or molecule that contributes appreciably to the integrated mechanical properties of the cell.

Spontaneous bead motions are measured by identifying the position of the centre of mass of each bead (approximately 20 beads per field-of-view) at the rate of 12 frames per second (0.082 s per frame) at 40× magnification for 5 or 10 min. Bead positions are corrected for the effects of microscope stage drift; the stage drift is estimated from changes in the mean position of all beads within a field of view. We define mean square displacement, $\langle r^2(t)\rangle$, as $\langle r^2(\Delta t)\rangle = \langle (r(t+\Delta t) - r(t))^2\rangle$, where $r(t)$ is the bead position at time t, Δt is the time lag, and brackets indicate an average over t and over all beads [1,12].

Elastic modulus measurements. To estimate the stiffness of structures bound to the bead, microbeads are first magnetized horizontally (parallel to the surface on which cells were plated) and then twisted in a vertically aligned homogenous magnetic field at different frequencies—from 0.1 to 10^3 Hz. The resulting lateral bead displacements in response to the oscillatory torque are measured optically. The cell elastic modulus, g', is defined as the real part in Fourier space of the ratio of specific torque to lateral bead displacements; g' is expressed in units of Pa/nm.

Results and discussion

We begin by quantifying $\langle r^2(\Delta t)\rangle$ on the nanometre scale [1] (Online supplement 2). For short Δt, $\langle r^2(\Delta t)\rangle$ increases only modestly with Δt, roughly following a power-law with exponent β significantly less than one (Fig. 1), indicating subdiffusive behaviour. By contrast, for large Δt, $\langle r^2(\Delta t)\rangle$ increases much faster, approximating a power-law with β significantly greater than one, indicating superdiffusive behaviour. The amplitude of $\langle r^2(\Delta t)\rangle$ as well as the transition time from subdiffusive to superdiffusive behaviour depend upon thermodynamic temperature (Fig. 1A), CSK treatments, and ATP depletion (Fig. 1B).

But to what extent is the motion in some particular direction correlated with continued motion in that direc-

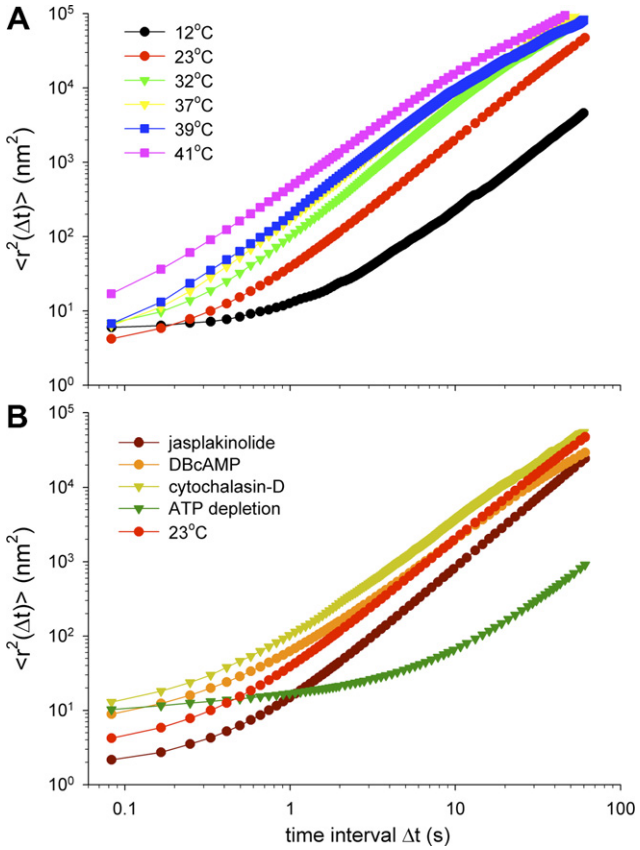

Fig. 1. Mean square displacements, $\langle r^2(\Delta t)\rangle$, computed from spontaneous motion of RGD-coated beads (400–720 beads per group). (A) $\langle r^2(\Delta t)\rangle$ at different thermodynamic temperatures, from 12 to 41 °C. (B) $\langle r^2(\Delta t)\rangle$ under baseline condition (23 °C), and after challenge with DBcAMP (decreases cell contractility, 1 mM), jasplakinolide (stabilizes actin, 1 μM), cytochalasin-D (disrupts actin filaments, 2 μM), and after depleting ATP. ATP concentration after depletion was from 2% to 7% of that in control samples. Noise level on bead position is discussed in Bursac et al. [1] and in Online supplement 2.