Light induced, localized, and abrupt force relaxations in fibroblast cells on soft substrates (Short title for Extreme Mechanics: Nanomechanics of single cells)

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Abstract

Exposure to fluorescent excitation light is known to adversely affect living cells. Here, we provide experimental evidence showing fibroblast cells relax abruptly during illumination with fluorescent excitation light. Cells were plated on polyacrylamide (PA) gels embedded with fluorescent microbeads. The beads served as fiducial markers to track substrate displacements induced by cell force. When the cells adhere, they apply traction forces to their underlying surface, causing the substrate to contract inward with respect to the cell boundary. We find that contractile cell forces are sporadically interrupted by abrupt force relaxation events, characterized by outward “jumps” in displacement (≥ 30 nm in 0.5 s) of the underlying substrate during exposure to fluorescent light. Jumps occur more frequently on softer substrates. Additionally, the speed and direction of displacements of particles before and after jumps are conserved, suggesting that the jumps do not involve any change in cytoskeletal or adhesion structure of the cell, and are possibly mediated by disengagement and re-engagement of myosin motors with actin filaments. Interestingly, we find that the frequency of jumps is also modulated by the energy introduced by the excitation light source. We propose a mechanistic hypothesis to explain the observations.

Keywords

Fluorescence, excitation light, displacement dynamics, relaxation, jumps

1. Introduction

Living cells respond to their surrounding mechanical microenvironment. Factors such as substrate stiffness [1-4], applied tension [5-7], and extracellular matrix composition [7-11] affect cell behavior. Cells are also known to respond mechanically to their surroundings, by altering the forces they apply in response to environmental conditions. Recently it has been shown that cells modulate their contractile forces in response to various illumination conditions [12]. This mechanical response manifests within seconds of exposure to fluorescent excitation light and consists of contractile pulling of the substrate interrupted by sporadic instances of large-scale force relaxation.

Previous studies have reported similar intermittent force relaxation events in various biological contexts. In reconstituted systems containing actin and myosin molecules, actomyosin interactions exhibit periodic relaxation composed of intermittent myosin-motor detachment verified through computational and experimental studies [13, 14]. Additionally, focal adhesion slippage has been identified during periodic extension and retraction of lamellipodia in migrating epithelial cells [15]. Filopodia forces in epithelial cells have been shown to stall abruptly during retraction [16], exhibiting an interruption to contractile forces. Other studies have identified stiffness-dependent load-and-fail dynamics exhibited by a molecular clutch that transmits force from a cell to its underlying substrate [17]. Small-scale force fluctuations have also been identified within

individual focal adhesions. One study reported the location and magnitude of peak traction force within a focal adhesion stalls and occasionally abruptly snaps outward, representing intermittent relaxation [18]. These fluctuations were also shown to mediate rigidity sensing within the cell, representing a mechanosensitive process.

These studies provide contextual precedent for abrupt relaxation events in adherent cells. However, no prior study has investigated the effect of excitation light on abrupt relaxation events, to our knowledge. Living cells are known to respond adversely to illumination by fluorescent excitation light. Photo-induced cellular responses include blebbing [19], mitochondrial swelling [20], and stimulation or interruption of cell division [21, 22]. Furthermore, photo-induced force relaxation yields nearly complete detachment of cells from their substrate, compromising cell viability [12]. Because excitation light is commonly used in experimental studies, it is critical to understand the process by which cells alter their forces in response to observation with light. This highlights the need to further characterize the mechanical response of adherent cells to fluorescent excitation light.

In this study, we have utilized a common experimental platform for measuring cell traction forces in order to study the effect of excitation light on abrupt relaxation events in fibroblast cells. Cells were plated on polyacrylamide (PA) hydrogels embedded with a monolayer of fluorescent microbeads [23]. Adherent cells apply forces to their underlying surface [24, 25], resulting in deformation of the substrate and motion of the embedded microbeads [26, 27]. Displacement of the microbeads serves as a measure of cell force dynamics. We illuminated cells with fluorescent excitation light in order to induce cell force relaxation, and quantified the displacement of the beads as previously reported [12]. Intermittent outward (relative to the cell area centroid) jumps in displacement indicate abrupt relaxation events during illumination. Our findings demonstrate that increasing substrate rigidity reduces jump frequency, indicating that the mechanical microenvironment influences relaxation events. Jumps occur in localized spatial regions ($< 5 \mu\text{m}$ from non-jumping neighboring particles), and particles maintain the same contractile-pulling speed and direction before and after a jump event. This finding suggests the substrate adhesion linkage is maintained and a disruption to the internal cell force-generating machinery is responsible. Finally, we show that modulation of excitation light intensity alters jump frequency patterns, indicating that the observed force-disruption process is energy-dependent. Understanding the broad context within which abrupt changes to force-generating mechanisms in cells has been studied previously, we propose a hypothesis by which displacement jumps occur as a result of excitation light intensity.

2. Materials and Methods

2.1 Substrate fabrication and cell culture

PA gel substrates were fabricated with red (100 nm-dia., 580/605 nm ex/em) or dark red (200 nm-dia., 580/605 nm ex/em) fluorescent microbeads (Fluospheres®, Life Technologies, Carlsbad, CA) embedded just below ($\leq 1.6 \mu\text{m}$) the cell culture surface [23]. Substrates were fabricated with elastic moduli 2, 5, and 10 kPa by mixing appropriate proportions of acrylamide and bisacrylamide according to a well-established protocol [28]. The extracellular matrix protein, fibronectin, was linked to the surface using *N*-hydroxysuccinimide (NHS) and 1-Ethyl-3-[3-

dimethylaminopropyl]carbodiimide hydrochloride (EDC) (Thermo Scientific, Waltham, MA) [29]. Cell culture media was formulated with Dulbecco's Modified Eagle's Medium (Corning, Corning, NY) (4.5g/L glucose, 4mM L-glutamine), 10% fetal bovine serum (Sigma-Aldrich, St. Louis, MO), and 1% Penicillin Streptomycin (Corning, Corning, NY).

2.2 Experimental procedure

Monkey Kidney Fibroblast cells (ATCC, Manassas, VA) were plated at an approximate density of 2,500 cells/cm² on PA gel substrates and allowed to adhere (attaining a fully spread state) for 4 to 6 hours. Individual cells were identified and illuminated for 60 s with fluorescent excitation light. During the illumination period, a video of the embedded beads underlying the cell was recorded at a sampling rate of 10 Hz. All experiments utilized an Olympus IX81 and 40x UApo N340 water immersion objective (NA 1.15) (Olympus America Inc., Center Valley, PA) mounted on a vibration isolation table (Newport Corporation, Irvine, CA). The microscope imaging platform was enclosed by an environmental chamber, and culture conditions were maintained throughout experimentation (5% CO₂, 60% humidity and 37° C). All image acquisition was conducted with a Neo sCMOS camera (active pixels 1392 x 1040, 165 nm/pixel) (Andor Technology, Belfast, Northern Ireland). Three fluorescent excitation light sources were employed to evaluate the effect of varying levels of light intensity on displacement dynamics: (1) A wide field fluorescent metal halide lamp (X-Cite® 120, Excelitas Technologies, Waltham, MA) coupled with an mCherry filter (Semrock Brightline mCherry-M-OMF, $\lambda = 540\text{-}585/600\text{-}682$ nm ex/em, $I = 12.5$ W/m², Rochester, NY), (2) a neutral density filter (32ND25, Olympus America Inc., $I = 3.0$ W/m², Center Valley, PA) coupled with the light source listed in (1), and (3) a deep red collimated LED ($\lambda = 625\text{-}650/650$ (high pass) nm ex/em, $I = 1.9$ W/m²) (Thorlabs, Inc., Newton, NJ). Throughout the remainder of this paper, the light sources will be referred to as follows (1) mCherry, (2) ND25, and (3) LED.

2.3 Displacement and jump analyses

Cell-induced substrate deformation was quantified using a single-particle tracking algorithm [30] with ± 10 nm spatial resolution [12]. Displacements of embedded particles represent changes in cell force dynamics. Particles were classified as either inside or outside of the cell spread area boundary. Motion of particles outside the cell boundary represents measurement noise. Drift was approximated as an average of all particle motion outside the cell area at a given time during illumination, and was removed from all displacements. Particle motion was characterized based on its direction relative to the cell area centroid. Motion toward the centroid (inward) is negative and represents cell contraction; motion away from the centroid (outward) is positive and represents cell relaxation. We define a displacement “jump” as positive motion ≥ 30 nm within 0.5 s. The displacement threshold was set as 50% above the noise window (20 nm). The time threshold was determined based on assessment of empirical results. A post-processing software analysis of all tracked particles was used to identify jumps in particle trajectories. Analysis of particle trajectories utilized a Heaviside step function with a rolling window (no overlap) to identify positive motion ≥ 30 nm during 0.5 s time intervals. The analysis is most comprehensive for 2 kPa gels, where average

displacements are largest. Displacements of stiffer gels ($E = 5$ and 10 kPa) are smaller, thus additional jumps undetectable by this system may occur below the noise level.

3. Results

3.1 Illumination-induced dynamics exhibit intermittent displacement jumps

Continuous illumination of fibroblast cells induces changes in small-scale cell force dynamics. Previous work has shown that exposure to fluorescent excitation light causes force relaxation within time periods as short as 60 s [12]. We find that illumination-induced relaxation primarily manifests in two ways: (1) continuous relaxation, characterized by a steady, positive displacement trajectory slope (beads move away from the cell center monotonically with time) or (2) continuous contractile motion (beads moving towards cell center) interrupted by short, sudden, outward displacements (“jumps”) of substrate-embedded particles. A sample representative cell exhibiting jumps is shown in Fig. 1. Jumps (blue arrows in Fig 1 b) are sporadic, and seem to occur randomly for any given particle and any given cell. The direction of jumps is always positive, meaning the particle moves away from the cell centroid, representing a short, abrupt, force relaxation event.

Fig. 1. Representative cell exhibiting jumps. (a) Fibroblast cell plated on polyacrylamide gel ($E = 2$ kPa). (b) Subset of displacement trajectories for beads embedded in the substrate underlying the cell. Select jumps are indicated by blue arrows.

3.2 Substrate stiffness affects jump prevalence and jump distance

Although jumps seem to occur randomly, our results suggest that substrate stiffness affects the likelihood of jumps. We find that the number of jumps per cell during 60 s of fluorescent illumination is higher for cells on soft substrates. The number decreases with increasing substrate stiffness (Fig. 2). It is known that actomyosin contractility contributes to cell traction forces applied on the underlying substrate [31]. However, the jump heights (distance traversed by a given particle during a jump) are independent of substrate stiffness. At least half of all particles traverse the same distance (30 to 35 nm) during a jump on each tested substrate stiffness (Fig. 3 a). The distribution of jump heights is similar for all tested substrates. Note that jumps less than 30 nm could not be detected with confidence. It is thus unclear whether the jump height distribution remains stiffness independent below 30 nm. This result is expected, as cell force has been shown

to increase linearly with increasing substrate stiffness (Fig. 3 b) [2]. In order for force to scale linearly with substrate stiffness, displacement remains constant. This trend is true for cell forces applied to substrates comprised of micropillars or continuous flexible hydrogels [2].

Fig. 2. Effect of substrate stiffness on the frequency of displacement jumps. Number of jumps as a function of substrate stiffness. For each stiffness a population of $n=17$ cells were tested.

Fig. 3. Effect of substrate stiffness on jump displacement distribution. (a) Distribution of jump distances occurring on different stiffness substrates. For each stiffness a population of $n=17$ cells were tested. (b) Plot of the mean force exerted by fibroblasts as a function of the equivalent Young's modulus, E_{eff} . Square (■) markers represent the forces measured on micropillar substrates and round (●) markers correspond to the forces obtained by previous studies on flexible continuous gels. The straight line is a linear fit for the values <90 kPa (reprinted from *Soft Matter*, 2008, 4, 1836-1843 with permission from Royal Society of Chemistry publishing company).

3.3 Spatial location of jumps coincides with cell boundary

To elucidate the context in which internal cell machinery contributes to abrupt relaxation events, we characterized the jumps based on their spatial location relative to the cell boundary. The geometric edge of a spread cell was examined because it coincides with the location of highest traction forces [32] as well as the highest density of adhesion molecules that transmit forces to the underlying surface [33]. We find that jumps mainly occur at the periphery of the cells' spread area (Fig. 4 a). This finding is consistent across

Fig. 4. Spatial location of jumps relative to the cell edge. (a) Locations of jump occurrences for representative cell (red markers). (b) Distance of jumping particles from the cell edge. (c) Jump distance as a function of the spatial location of the particle. Inset shows enlarged view of highest density region containing jumps less than six microns from the cell edge. Results represent $n=17$ cells on PA gels of all tested substrate stiffnesses, though the number of jumps is significantly less in the case of 5 and 10 kPa substrates. Over 75% of jumps occur within 4 μm of the cell edge (Fig. 4 b). We also find that jump height decreases with increasing distance from the cell edge towards the cell center (Fig. 4 c).

3.4 Jumps of neighboring particles exhibit autonomy, simultaneity in localized regions

We find that the jumps of particles embedded in the gel either do not affect the dynamics of nearby (within 2 μm) particles, i.e., the jumps are localized, or multiple nearby

Fig. 5. Neighboring particles exhibit autonomy through jump dynamics. (a) Three neighboring particles (circled in yellow; $< 2 \mu\text{m}$ p-p distance) below a fibroblast cell (on PA gel, $E = 10 \text{ kPa}$). Inset in top left shows enlarged view of three neighboring beads. Scale bar = 2 μm . (b) Displacement trajectories corresponding to each bead as indicated by color.

particles jump simultaneously. Figure 5 shows an example of the former case. Here, one of the three nearby particles jumps (blue trajectory), but the others continue to move at

steady rates. In other cases, nearby particles jump in quick succession, exhibiting a cascade effect (Fig. 6 c). Figure 6 d shows two nearby particles jumping simultaneously.

Fig. 6. Neighboring particles exhibit a cascade effect and simultaneity through jump dynamics. (a-b) Two sets of neighboring particles ($< 3 \mu\text{m}$ p-p distance, circled in yellow) below a Fibroblast cell. Enlarged image of bead layout in (b) indicates corresponding trajectories in (c) and (d). Corresponding displacement trajectories in (c) and (d) exhibit a cascade effect and simultaneity, respectively, during the force release exemplified by the displacement jump.

3.5 Displacement speed and direction maintained after a jump

Displacement trajectories of particles that exhibit jumps were analyzed immediately preceding and following the jump. We find that the speed and direction of motion of particles before and after jumps remain unaffected by the jumps, indicating that the sudden relaxation events do not interfere with the rate and direction of local cell force contractility (Fig. 7). This result is consistent across all tested substrate stiffnesses. This suggests that the contractile machinery involving actomyosin structure and adhesion with the substrate are unperturbed by the intermittent, catastrophic failure that causes the abrupt relaxation event.

Fig. 7. Speed and direction of bead dynamics is maintained following displacement jumps. (a) Speed and (b) angle, θ , of displacement before ($|V|_b$, θ_b) versus after ($|V|_a$, θ_a) a jump occurrence as a function of substrate stiffness for ten representative cells on PA gels of various stiffnesses. Outliers for 2 kPa trajectories in (b) are believed to be a result of the jump occurring close to the onset of the video, where limited displacement history information is available.

3.6 Jump frequency increases with increasing intensity of excitation light

While fluorescent excitation light has been shown to affect cell force dynamics by inducing global relaxation, we aimed to determine the effect of illumination on the presence of jumps. We find that excitation light affects the frequency of jump events. Three different excitation light sources were utilized, with respective intensities of 12.5, 3.0, and 1.9 W/m² as described in the Materials and Methods section. Jump frequency depends on the excitation light source with which the cells are illuminated, and the average quantity of jumps per cell increases with increasing exposure intensity (Fig. 8). The effect of light intensity on jump frequency was exclusively tested on substrates of stiffness $E = 2$ kPa due to the consistently high prevalence of jumps on soft substrates. These results suggest that excitation light affects jump frequency in a dose-dependent manner.

Fig. 8. Effect of excitation light on jump frequency. Average number of jumps per cell based on 60 s of illumination by each of three excitation light sources (mCherry, mCherry + ND25 filter, or LED with intensities 12.5, 3.0, and 1.9 W/m², respectively).

4. Discussion

We explored the abrupt relaxation in fibroblast contractility on elastic hydrogel substrates during fluorescent illumination. The relaxation manifests as jumps in displacements of fiducial markers embedded in the substrate. The direction of jumps is radially outward with respect to the cell area centroid, while immediately before and after a jump, the direction of the displacement is *inward*. Contractile cell forces yield inward motion of the underlying substrate, while force relaxation induces outward motion. The displacement jumps are followed by a return to contractile force, when the fiducial markers continue to move inward along the same direction and speed as they did prior to the jumps. Conservation of contractility (direction and rate of increase) suggests that the jumps preserve the cytoskeletal and adhesion structures, and that the jumps are generated by sudden, disengagement of myosin motors with the actin fibers.

The jump phenomena we observe are reminiscent of sudden force relaxation events reported in literature [17] where forces of filopodial growth cones on soft substrates were found to oscillate with time, although the effect of light was not considered. Here, cells are thought to use a “motor-clutch” mechanism to transmit traction forces to their underlying substrate via filamentous actin (F-actin). The study simulated molecular clutch activity on soft substrates and reported abrupt F-actin/clutch decoupling in which all clutches (transmembrane molecular protein complexes) disengage (from an F-actin bundle), causing the substrate to unload and snap back to its initial rest position. The model predicts oscillatory “load-and-fail” traction force dynamics characterized by periods of increasing tension interrupted by abrupt relaxation. Experimental results from the same study support the model prediction, in the absence of continuous light exposure. Here, substrate-embedded marker beads deflect towards the nucleus as a result of filopodial traction force, but occasionally snap back toward their rest position [17]. Our results are consistent with this finding, however such jumps reported in this study are stimulated by light exposure.

Increasing the intensity of fluorescent excitation is known to increase force relaxation in adherent cells [12]. In our experiments, the displacement jumps, indicative of sudden force relaxation, follow this trend. We find, for a given substrate stiffness, jump frequency increases with light intensity, and for a given light exposure, jump frequency increases with decreasing substrate stiffness. In all cases, the distribution of jump distance remains the same. How can we interpret these observations, particularly the two apparently paradoxical findings (jump frequency (probability) is stiffness dependent, but the jump distance is not)? We propose a simple hypothetical model to resolve this paradox.

Consider the model of a localized adhesion and the associated actomyosin machinery in Fig 9. It depicts two actin filaments linked by myosin motors. One of the actin fibers is attached to the substrate through the focal adhesion complex. The other is linked with the intracellular cytoskeletal structure with stiffness k_c . The substrate is represented by a Hookean spring with stiffness k_s . Myosin motors walk hand over hand on F-actin “tracks” pulling them toward each other [34]. This results in a traction force on the substrate and on the cytoskeletal springs, and consequently they both deform. In the case of the cell on a flexible substrate, the deformation of the substrate manifests through the displacement of the embedded fiducial markers. In the case of the model, the deformation of the substrate spring is represented by x_0 .

We now institute the following assumptions: (1) the force generated by all motors is equal, and is given by f . In the presence of n motors, the total force is $F_s = nf = k_s x_0$. This

Fig. 9. Model of a cell contractile machinery adhered to the substrate and the cytoskeletal structure. The stiffness of the substrate and the cytoskeletal structure are represented by k_s and k_c , respectively. Displacement, x_0 , of the substrate occurs upon contraction of the actin filaments by myosin motors.

equal-force hypothesis can be rationalized as follows: the actin fiber cannot sustain compression, but can resist tension. The time for a motor to take a step on a relaxed fiber is much shorter (μs - ms) than the observation times (sec) of force generation [35, 36]. Hence, if there is a slack in the actin fiber between two motors, one with force f , and the other with force less than f , then the latter will walk faster than the observation period to tighten the fiber until it reaches f . The motor force, f , is much smaller than the stall force, f_{stall} [37, 38], so that f increases with time almost linearly [39]. (2) Each motor is stiff, i.e., their deformation due to force f is negligible compared to x_0 , (3) the motors tend to detach from the actin fibers randomly (off rate) by overcoming an activation barrier, E_{detach} . This value has been quantified in various previous studies [40-42]. Force f decreases the effective activation barrier to $E_{\text{detach}} - fd_0$, where d_0 is a characteristic length scale. As f approaches f_{th} , $E = E_{\text{detach}} - fd_0$ approaches zero, making conditions more favorable for detachment to occur. Thus the probability of detachment increases with f . As f approaches f_{th} , the probability of detachment approaches unity. We speculate that light exposure decreases E_{detach} and hence $f_{\text{th}} = E_{\text{detach}}/d_0$ by a yet unknown mechanism. Light induced f_{th} might be less than f_{stall} .

The consequence of the above assumptions is that if a motor detaches at force f , there is a sudden overload on each remaining motor. This overload might still be less than f_{stall} , such that the motor may not walk backwards to relax. Even if the overload is higher than f_{stall} , the motor will walk backwards in a time dependent manner, but will need to instantaneously survive the overload. In the case of n motors, this overload is $F_s/(n-$

$l)=[n/(n-1)]f$. Thus, the overload increases with decreasing n . This overload has two possible effects. One possibility is that all motors fail in a catastrophic fashion if $nf/(n-1)$ approaches f_{th} , which depends on light exposure. Consequently, the force on the spring drops to zero and the tracer bead jumps. The other possible effect is that the overload is not high enough to induce a catastrophic failure, and the $(n-1)$ motors continue to walk and increase the force until catastrophic failure occurs or the motors reach stall force. There is of course the third possibility in which the detached single motor re-engages and new motors join the actomyosin machinery and increase the spring force. We hypothesize that after the catastrophic failure, n number of motors re-engage and begin to increase the force on the substrate spring and repeat the cycle. The catastrophic failure gives rise to the jumps of the tracer particles, and the re-engagement induces force generation and displacement of the particles towards the cell centroid after a jump.

What determines n ? To develop insight on this question, we return to the reported literature. We note that cell traction force and cell stiffness increase with substrate stiffness, almost linearly for a substrate modulus below 10 kPa (Fig. 3 b) [2]. Additionally, more actomyosin machinery is involved in cells on stiffer substrates [43-46]. In our simplified model, this implies that n increases linearly with substrate stiffness k_s , and cytoskeletal stiffness k_c is proportional to k_s . Thus, with increasing k_s , cell force $F_s = nf = k_s x_0$ increases, and x_0 remains independent of k_s . The assumption that n is proportional to k_s , implies $nf = Ck_s f = k_s x_0$, where C is a constant. Furthermore, since n increases with k_s , force overload, $fn/(n-1)$, decreases with increasing k_s . This reduces the frequency of catastrophic failures for a given light exposure. However, since $x_0 = nf/k_s = C$, the jump heights remain the same with increasing stiffness. This resolves the paradox. For a given substrate stiffness, $f_{th} = E_{detach}/d_0$ decreases with increasing light intensity, and the frequency of jumps increase. It is thus expected that jump distance $x_0 \sim Cf_{th}$ will decrease with increasing light intensity on a given substrate.

The analysis of abrupt relaxation phenomena we present here further characterizes the effect of fluorescent excitation light on cell force dynamics. Our findings suggest that a change in the internal force generating machinery contributes to the relaxation events we have identified through displacement jumps. Jumps predominantly occur near the cell periphery, where cells are known to apply the largest traction forces [32, 33]. Additionally, focal complex molecules that transmit force to and from the cell to its substrate are most dense near the cell boundary. Thus, it is not surprising that the location for sporadic interruptions in contractile force aligns with the cell periphery.

Additionally, the jump phenomena appear to be highly localized and are well within the size scale of individual focal adhesions (1 – 5 μm) [47]. Most often, beads as near as 1.5 μm from their closest neighbor exhibit far different displacement trajectories: a jump of one bead leaves its nearest neighboring bead(s) undisturbed (Fig. 5). This suggests force relaxation in a small (sub micrometer scale) region such as those studied previously wherein the location and magnitude of maximum traction force shifts within a single focal adhesion [18]. In contrast, we also find neighboring beads jumping simultaneously (Fig. 6) pointing to force-relaxation events upstream from the cell-substrate contact.

5. Conclusion

Previous work has shown that excitation light affects cell force dynamics, inducing relaxation during short periods of fluorescent illumination. In this study, we employed a well-established experimental platform to characterize cell force relaxation as a result of excitation light. Our findings demonstrate that photo-induced cell force relaxation consists of short, sudden relaxation events. These events are characterized by jumps in displacement of substrate-embedded particles away from the cell centroid. Jumps occur near the periphery of cells, and occur most frequently on soft substrates. Additionally, jumps occur in localized spatial regions, and are preceded and succeeded by motion with the same speed and direction, suggesting myosin detachment from actin as a possible mechanism of abrupt force relaxation when the cytoskeletal and adhesion structure is preserved. Furthermore, illumination with increasing excitation light intensity increases the frequency of jump occurrences. Our findings suggest that excitation light mediates abrupt local force relaxation in a dose-dependent manner. These results could be useful for identifying adverse effects of light during studies of mechanical cell forces, as well as in elucidating the mechanism behind illumination-induced relaxation.

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