

On the origin of shape fluctuations of the cell nucleus

Fang-Yi Chu^a, Shannon C. Haley^a, and Alexandra Zidovska^{a,1}

^aCenter for Soft Matter Research, Department of Physics, New York University, New York, NY 10003

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The nuclear envelope (NE) presents a physical boundary between the cytoplasm and the nucleoplasm, sandwiched in between two highly active systems inside the cell: cytoskeleton and chromatin. NE defines the shape and size of the cell nucleus, which increases during the cell cycle, accommodating for chromosome decondensation followed by genome duplication. In this work, we study nuclear shape fluctuations at short time scales of seconds in human cells. Using spinning disk confocal microscopy, we observe fast fluctuations of the NE, visualized by fluorescently labeled lamin A, and of the chromatin globule surface (CGS) underneath the NE, visualized by fluorescently labeled histone H2B. Our findings reveal that fluctuation amplitudes of both CGS and NE monotonously decrease during the cell cycle, serving as a reliable cell cycle stage indicator. Remarkably, we find that, while CGS and NE typically fluctuate in phase, they do exhibit localized regions of out-of-phase motion, which lead to separation of NE and CGS. To explore the mechanism behind these shape fluctuations, we use biochemical perturbations. We find the shape fluctuations of CGS and NE to be both thermally and actively driven, the latter caused by forces from chromatin and cytoskeleton. Such undulations might affect gene regulation as well as contribute to the anomalously high rates of nuclear transport by, e.g., stirring of molecules next to NE, or increasing flux of molecules through the nuclear pores.

nuclear envelope | chromatin | nuclear lamina | active materials

The cell nucleus changes its shape and size dramatically during the cell cycle (1, 2). Such changes correspond to, e.g., decondensation of chromosomes in a newly formed nucleus, or, later, chromosome duplication, and occur on time scales of hours (3). Recently, small oscillations of nuclear area were found for stem cells and fibroblasts at time scales of minutes and attributed to local changes in chromatin compaction and cytoskeletal forces (4, 5). Shape fluctuations at even shorter time scales, “flickering,” were previously studied only in membranous systems, e.g., lipid vesicles, plasma membrane of red blood cells, and macrophages (6–8), where they were found to be largely thermally driven. Flickering of the cell nucleus, which has a more complex architecture, remains to be shown and characterized.

The shape of the cell nucleus is defined by the nuclear envelope (NE), a complex structure that confines chromatin, forming a physical boundary between the nucleoplasm and cytoplasm. NE comprises two lipid bilayers separated by a perinuclear space of 20 nm to 40 nm and supported from inside by nuclear lamina, a 50- to 80-nm-thick network formed by intermediate filament lamins (9, 10), which are presumed to provide NE with its structural support (11–13). Furthermore, NE is perforated by thousands of nuclear pores, gigantic protein complexes with diameter ~ 120 nm and depth ~ 45 nm, which aid the molecular transport into and out of the cell nucleus (14, 15). The presence of nuclear pores in the NE is integral to the NE’s mechanical properties (16, 17).

Since NE is sandwiched between two active systems inside the cell—cytoskeleton on the cytoplasmic side and chromatin on the nucleoplasmic side—both could contribute to the nuclear shape fluctuations. Moreover, cytoskeleton as well as chromatin maintain direct links with NE (e.g., LINC complex, emerin, MAN1) (19, 20). Recent studies in yeast showed that microtubules were involved in the nuclear shape fluctuations over minutes, while chromatin tethering to NE influences nuclear deformability (21). In mammalian cells, cytoskeletal perturbations lead to changes

in nuclear deformability (5), whereas chromatin interacts with NE via hundreds of lamina-associated domains, which reshuffle stochastically inside the nucleus (22). In fact, chromatin’s interaction with NE is implicated in regulation of some genes (23–25).

Structural and functional errors of the NE lead to a large number of developmental and inherited disorders, so-called nuclear envelopopathies, such as cardiomyopathy, muscular dystrophy, mandibuloacral dysplasia, and Hutchinson–Gilford progeria, as well as cancer (20, 26). Two major working hypotheses for these diseases are defects in gene regulation and abnormalities in nuclear architecture (27). Thus, elucidating mechanics of the nuclear shape fluctuations might contribute to the efforts to understand the NE in health and disease.

In this work, we study the flickering of the cell nucleus, i.e., nuclear shape fluctuations at short time scales of tens of seconds with temporal resolution of 250 ms. We monitor dynamics of both chromatin and nuclear envelope (NE) in live human cells. Using spinning disk confocal microscopy (*Materials and Methods*), we recorded high-resolution streams of live cells and studied the shape fluctuations for both the chromatin globule surface (CGS) visualized by GFP-tagged histone H2B (H2B-GFP) and the NE visualized by GFP-tagged lamin A (LMNA-GFP) (Fig. 1).

Results and Discussion

In Vivo Measurements of Nuclear Shape Fluctuations. To measure the shape fluctuations of CGS and NE as a function of time, we detected the contours of CGS (Fig. 1*B*) and NE (Fig. 1*E*) in every frame of the streams (*Materials and Methods*) and analyzed their dynamics. As shown in Fig. 1*B* and *E*, *Insets*, the contours exhibit pronounced local shape fluctuations in time with amplitudes u of 100 nm to 200 nm in both directions (Fig. S1). We calculated u , which is the deviation of the instantaneous contour $r(\phi, t)$ from the average contour $r_0(\phi)$, by $u(\phi, t) = r(\phi, t) - r_0(\phi)$ at different times t . To focus on the amplitude of both inward and outward fluctuations, we computed u^2 for

Significance

While it is known that the shape and size of the cell nucleus change dramatically during the cell cycle, we find that the cell nucleus also exhibits subtle, but measurable, fast shape fluctuations at seconds. We find that the amplitude of these fluctuations systematically decreases during the cell cycle, thus serving as a reliable cell cycle stage indicator. Our findings show that the nucleus undergoes both thermally and actively driven undulations, the latter caused by forces from chromatin and cytoskeleton. Such undulations might affect gene regulation as well as aid the nuclear transport. Understanding the mechanism behind such undulations could prove critical for illuminating dynamic behavior of the nucleus in health and disease.

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¹To whom correspondence should be addressed. Email: alexandra.zidovska@nyu.edu.

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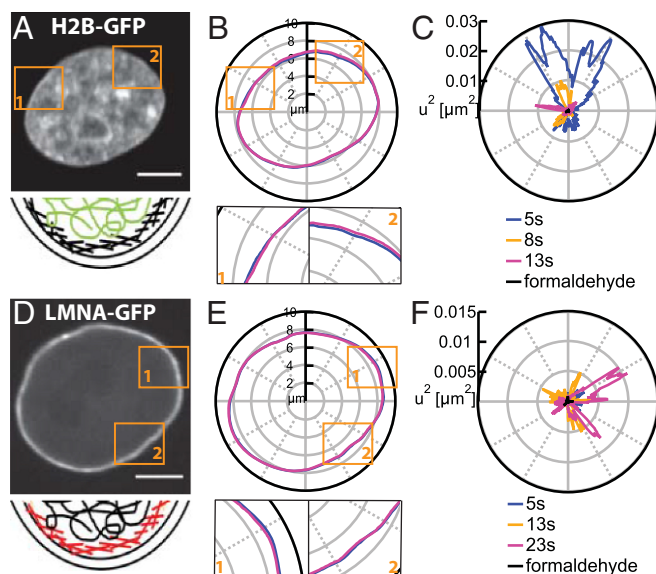


Fig. 1. In vivo measurements of shape fluctuations of CGS and NE. (A) A micrograph of cell nucleus expressing H2B-GFP and schematics of the localization of chromatin (green) relative to the NE (black). (B) Contours of CGS at $t = 5$ and 13 s. Insets 1 and 2 present a zoomed in view. (C) Fluctuations u^2 of CGS at $t = 5, 8$, and 13 s and for nucleus fixed with formaldehyde (black dot at the origin) demonstrating that our measurements are well above the noise floor. (D) A micrograph of cell nucleus expressing LMNA-GFP and schematics of the localization of lamins (red) relative to chromatin (black) and two lipid bilayers (black) comprising the NE. (E) Contours of the NE at $t = 5$ and 23 s. Insets 1 and 2 present a zoomed in view. (F) The u^2 of NE at $t = 5, 13$, and 23 s. (Scale bar, $5 \mu\text{m}$.)

CGS and NE, respectively. Fig. 1 C and F shows u^2 for CGS and NE at three different time points, demonstrating their dynamic behavior. As a negative control, we measured u^2 for cells fixed in formaldehyde and found the shape fluctuations to be eliminated (Fig. 1 C and F), showing that our measurements are well above the noise floor.

Further, to determine the wavenumber-dependent shape fluctuations of the CGS and NE, we performed the Fourier transformation of $u(\phi, t)$ at every time t following $u_q(t) = 1/2\pi \int_0^{2\pi} u(\phi, t) e^{-iq\phi} d\phi$ and calculated the mean-square amplitude $\langle u_q^2 \rangle$ as described in ref. 7 (SI Materials and Methods). We computed $\langle u_q^2 \rangle$ as a function of modal wavenumber q for both CGS and NE. Fig. 2A shows $\langle u_q^2 \rangle$ averaged over 47 nuclei for both CGS and NE, which are similar in their amplitude and q dependence (Fig. S2). As a negative control, we carried out the same calculation for cells fixed in formaldehyde (Fig. 2A), which shows elimination of shape fluctuations upon fixation.

To gain insight into the population average $\langle u_q^2 \rangle$ from Fig. 2A, we reviewed the $\langle u_q^2 \rangle$ distribution at every q separately. Fig. 2B shows examples of histograms for $\langle u_q^2 \rangle$ at $q = 2$ and $q = 6$ for both CGS and NE. We found the $\langle u_q^2 \rangle$ distributions to have quite complex shapes for both CGS and NE, suggesting a presence of more than one contributing population. For example, $\langle u_q^2 \rangle$ at $q = 2$ for NE shows two modes, one at low and one at high $\langle u_q^2 \rangle$. A careful inspection of the $\langle u_q^2 \rangle$ distributions revealed that different modes correspond to different sizes of the cell nuclei. To further explore this finding, we sorted the nuclei by their area into four groups (with n being the number of nuclei in the group) for CGS— $A_1 = 140 \mu\text{m}^2$ to $170 \mu\text{m}^2$ ($n = 11$), $A_2 = 170 \mu\text{m}^2$ to $190 \mu\text{m}^2$ ($n = 11$), $A_3 = 190 \mu\text{m}^2$ to $210 \mu\text{m}^2$ ($n = 16$), and $A_4 = 210 \mu\text{m}^2$ to $240 \mu\text{m}^2$ ($n = 9$)—and for NE— $A_1 = 160 \mu\text{m}^2$ to $210 \mu\text{m}^2$ ($n = 10$), $A_2 = 210 \mu\text{m}^2$ to $240 \mu\text{m}^2$ ($n = 10$), $A_3 =$

$240 \mu\text{m}^2$ to $260 \mu\text{m}^2$ ($n = 11$), and $A_4 = 260 \mu\text{m}^2$ to $345 \mu\text{m}^2$ ($n = 16$). Fig. 2C shows the average $\langle u_q^2 \rangle$ for all four groups for CGS (green) and NE (red). Remarkably, there was a clear decrease of $\langle u_q^2 \rangle$ with increasing nuclear size, while we found no correlation with the nuclear shape (Fig. S3). Thus, we hypothesized that, since the nuclear size is known to be cell cycle-dependent (1, 2), the $\langle u_q^2 \rangle$ is cell cycle-dependent.

Nuclear Shape Fluctuations During Cell Cycle. To test our hypothesis, we obtained $\langle u_q^2 \rangle$ as a function of cell cycle progression. We synchronized both HeLa H2B-GFP and HeLa LMNA-GFP cells (Materials and Methods) and measured $\langle u_q^2 \rangle$ as well as nuclear size for 31 h, where $t = 0$ h corresponds to metaphase. We collected data at six time points: $t = 1, 7, 13, 19, 25$, and 31 h. Fig. 2D presents histograms of nuclear area for CGS and NE for different time points in the cell cycle, which clearly show the increase in the nuclear size with cell cycle progression. Fig. 2E presents four micrographs of the same HeLa H2B-GFP nucleus at four different time points, showing the monotonic increase in the size of the cell nucleus (corresponding data for HeLa LMNA-GFP are shown in Fig. S4). In addition, after we computed $\langle u_q^2 \rangle$ for the six time points, we found that $\langle u_q^2 \rangle$ clearly decreases with progressing cell cycle (Fig. 2F and G), which is consistent with our original hypothesis. To quantify this phenomenon, we normalized $\langle u_q^2 \rangle$ at every time point by $\langle u_q^2 \rangle$ at t_1 , for $q = 2$ and 5 , for both CGS and NE. Fig. 2H shows a more than fivefold reduction of relative $\langle u_q^2 \rangle$ during the cell cycle for both CGS and NE, while the relative nuclear area A , i.e., A at different times normalized by A at t_1 , shows almost a twofold increase, with the biggest changes found in G1 phase. Thus, strikingly, measuring $\langle u_q^2 \rangle$ allows us to determine the cell cycle stage in live cells. Using $\langle u_q^2 \rangle$ as a cell cycle stage reporter is noninvasive and requires only one fluorescent tag (labeling either NE or CGS).

The pronounced decrease of $\langle u_q^2 \rangle$ with the progress of cell cycle may be due to two main reasons: change of NE material properties (e.g., increase in its stiffness) or reduction of the forces driving the shape fluctuations. The former is consistent with our and others' observations of gradual deposition of lamin A into NE over hours after mitosis, which would make NE stiffer (Fig. S4 and ref. 28). This is supported by an observed cell cycle-dependent decrease of $\langle u_q^2 \rangle$ at high q , the part of the spectra that follows q^{-4} (Fig. S4), which would be expected from Helfrich theory for bending rigidity dominated thermal fluctuations (29). Furthermore, the forces driving the nuclear shape fluctuations might very well change with the cell cycle.

Coupling of CGS and NE Shape Fluctuations. Similar dynamic behavior of CGS and NE as evidenced by $\langle u_q^2 \rangle$ suggests that CGS and NE are dynamically closely related, possibly coupled. To investigate how the dynamics of CGS and NE are related, we visualize the shape fluctuations of CGS and NE simultaneously by transfecting HeLa LMNA-GFP cells with H2B-mCherry (Fig. 3A and Materials and Methods). We evaluated the extent of fluctuations by calculating the ratio $r_f = n_f/n_{tot}$ determined by counting the fluctuation sites n_f , where $u_{ne} \neq 0$ or $u_{cgs} \neq 0$, along the contour and normalized by the length of the contour (i.e., total number of sites n_{tot}); u_{cgs} and u_{ne} are the shape fluctuations of CGS and NE, respectively. We find that, at a given time, two thirds of the contour exhibits shape fluctuations. We measured the duration of the fluctuation events τ_f and found two types of events: $\tau_{f,1} < 1$ s and $\tau_{f,2} \approx 4$ s to 5 s. Further, we computed the rate of the fluctuation activity $k_f = \sum(\tau_{f,i}/t_{tot})$ for a fluctuation site to be, on average, 0.22 , which we calculated as the time a fluctuation site is fluctuating per unit time, where t_{tot} is the length of the measurement (Fig. S5).

We found that, while CGS and NE typically fluctuate in phase, they do exhibit sites where they move out of phase, which we termed “separation sites.” Fig. 3B and C shows an example of

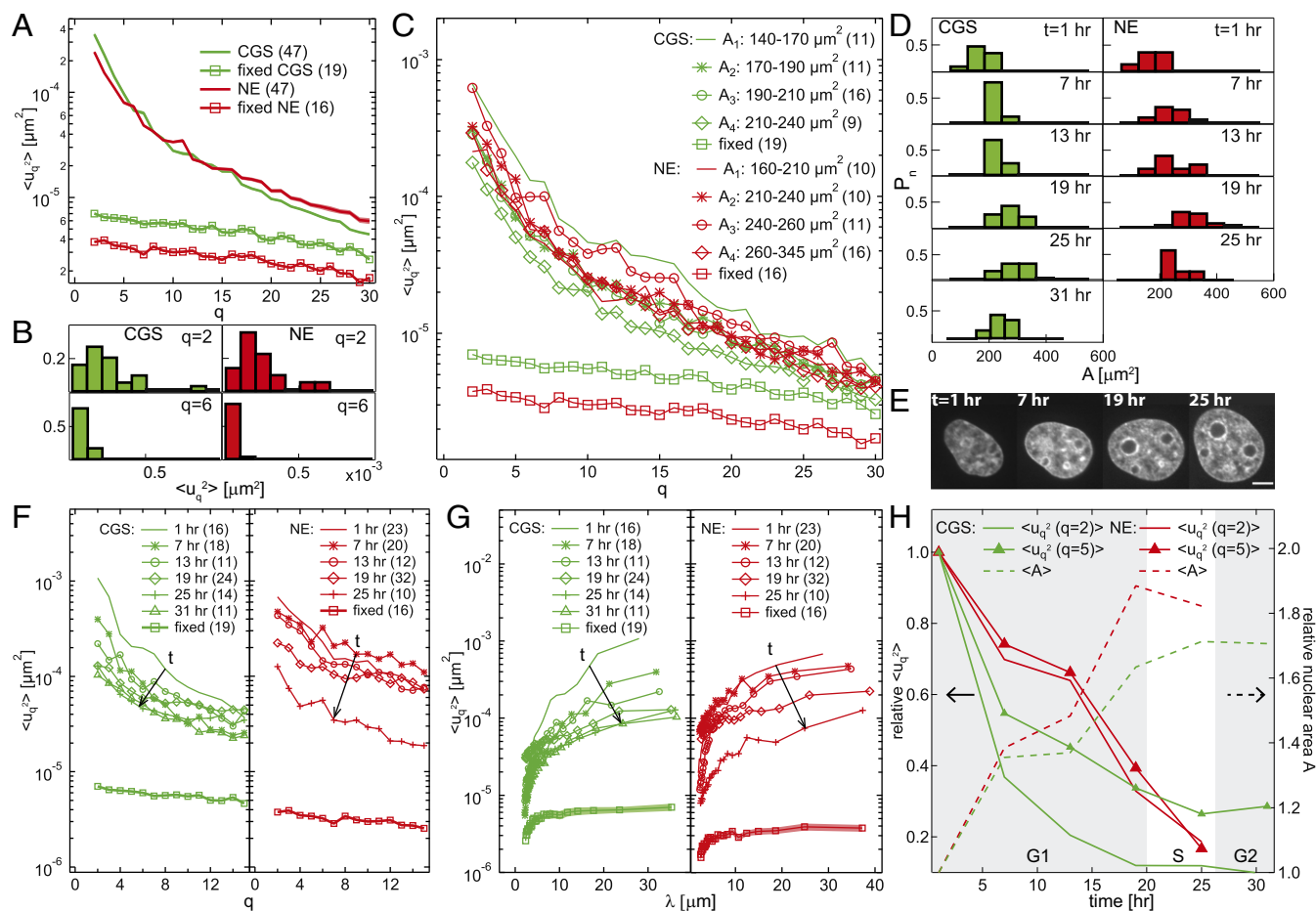


Fig. 2. Shape fluctuations of CGS and NE are cell cycle-dependent. (A) Wavenumber-dependent fluctuations $\langle u_q^2 \rangle$ for CGS (H2B-GFP, green line, $n = 47$) and NE (LMNA-GFP, red line, $n = 47$). As a negative control, we calculated $\langle u_q^2 \rangle$ for both CGS (green square markers, $n = 19$) and NE (red square markers, $n = 16$) after fixing with formaldehyde. (B) Histograms of $\langle u_q^2 \rangle$ for CGS and NE, at $q = 2$ and 6 , respectively. (C) The $\langle u_q^2 \rangle$ of CGS and NE calculated separately for four groups based on their nuclear area A for CGS (green) and NE (red); $\langle u_q^2 \rangle$ for CGS decreases with the increasing A . (D) Histograms of nuclear area A measured for a synchronized cell population at $t = 1, 7, 13, 19, 25$, and 31 h after metaphase. The nuclear size increases for both CGS and NE with the progressing cell cycle. (E) Micrographs of the same nucleus at four different times, showing its size increase during the cell cycle. (Scale bar, $5 \mu\text{m}$.) (F) The $\langle u_q^2 \rangle$ of CGS (green) and NE (red) measured at different times during the cell cycle; $\langle u_q^2 \rangle$ for both CGS and NE exhibits a monotonic decrease with increasing time (highlighted by the black arrow) with p value less than 0.05 for $t = 1, 13, 25$, and 31 h. (G) $\langle u_q^2 \rangle$ of CGS (green) and NE (red) from F plotted as a function of wavelength $\lambda = L_c/q$, where L_c is the contour length. (H) Relative $\langle u_q^2 \rangle$, i.e., $\langle u_q^2 \rangle$ at different times normalized by $\langle u_q^2 \rangle$ at $t = 1$ h, for $q = 2$ and 5 , for CGS (green) and NE (red) decreases during G1, S, and G2 (timing from ref. 18), while the relative nuclear area A , i.e., the nuclear area at different times normalized by the nuclear area at $t = 1$ h, increases. Error bars for A, C, and F–G are shown in Fig. S2.

a separation site, which occurred at $t = 10$ s, but is no longer present at $t = 22$ s. The separation sites can be detected by comparing the simultaneous contours of CGS and NE (Fig. 3D, highlighted by blue arrow) as well as in the shape fluctuation amplitudes u^2 (Fig. 3E).

To analyze the extent and the temporal evolution of a separation site, we evaluated relative shape fluctuations of CGS and NE with respect to each other, which we defined as $(u_{ne} - u_{cgs})^2$. Fig. 3F shows a plot of $(u_{ne} - u_{cgs})^2$ as a function of time t and polar angle ϕ , which visualizes the temporal evolution of separation sites along the nuclear contour $r(\phi, t)$. The plot shows only the $(u_{ne} - u_{cgs})^2$ above the noise floor, which was determined by measurements in fixed cells. The separation sites undergo spurts of separation, which we named “separation events” and characterized by their duration time τ . The separation site in Fig. 3F shows several separation events of variable duration $\tau = 2$ s to 5 s.

We evaluated two types of separation ratios, $r_{s,tot} = n_s/n_{tot}$ and $r_{s,f} = n_s/n_f$, determined by counting the number of separation sites n_s along the contour and normalized by n_{tot} and n_f ,

respectively. We find that, at any given time, approximately 20% of the CGS and NE can fluctuate out of phase, as evidenced by $r_{s,tot}$, which corresponds to $\sim 30\%$ of the fluctuation sites, as evidenced by $r_{s,f}$. The rate of separation activity $k_s = \sum (\tau_{s,i}/t_{tot})$ was found to be about 0.07 , which we calculated as the time a separation site is actually separated ($u_{ne} - u_{cgs} \neq 0$ above the noise floor) per unit time.

Origin of Nuclear Shape Fluctuations. To elucidate the origin of the shape fluctuations of CGS and NE, we used biochemical perturbations (Fig. 4). Specifically, to determine whether the observed dynamics of CGS and NE are actively driven, we depleted ATP (Materials and Methods). We found that both u^2 (Fig. 4B) and $\langle u_q^2 \rangle$ (Fig. 4C) were strongly reduced ($n = 24$), confirming that active processes indeed contribute to nuclear shape fluctuations. Furthermore, we explored the involvement of the cytoskeleton in the CGS and NE dynamics by measuring the shape fluctuations of CGS and NE upon inhibition of microtubule polymerization by nocodazole ($n = 18$), actin

1.5 (MatTek) for 24 h, and the medium was then replaced by Gibco CO₂-independent medium supplemented with L-glutamine (Invitrogen). Cells were then mounted on the microscope stage kept in a custom-built 37 °C microscope incubator enclosure with 5% CO₂ (vol/vol) delivery during the entire experiment. When specified, cells were synchronized using 10 μM RO-3306 (ALEXIS), which arrests cells at the G2/M checkpoint of the cell cycle, and were released, 1 h before imaging, by washing with CO₂-independent medium supplemented with L-glutamine. For fixation experiments, cells were fixed with 3.7% formaldehyde in PBS at room temperature for 20 min and then washed three times with PBS every 5 min. Coverslips were mounted on the glass microscope slides, using Prolong-Diamond antifade reagent (Invitrogen). HeLa cells expressing LMNA-GFP and H2B-mCherry were prepared by transient transfection of the stable HeLa LMNA-GFP cell line with H2B-mCherry plasmid using Lipofectamine 2000 (Invitrogen) following the manufacturer's protocol.

Biochemical Perturbations. To deplete ATP, cells were treated, 2 h before imaging, with 6 mM 2-deoxyglucose and 1 μM trifluoromethoxy-carbonyl-cyanide phenylhydrazone dissolved in CO₂-independent medium supplemented with L-glutamine. For cytoskeletal perturbations, 10 μM latrunculin A, 10 μM blebbistatin, or 10 μM nocodazole, and, for chromatin perturbations, 20 μg/mL α-amanitin, in CO₂-independent medium supplemented with L-glutamine, were added to cells 30 min before imaging. All chemicals are from Sigma-Aldrich.

Microscopy and Image Acquisition. Images were taken with a Yokogawa CSU-X1 spinning-disk confocal head with an internal motorized high-speed

emission filter wheel and Spectral Applied Research Borealis modification for increased light throughput and illumination homogeneity on a Nikon Ti-E inverted microscope equipped with a 100× Plan Apo NA 1.4 objective lens and the Perfect Focus System. The microscope was mounted on a vibration-isolation air table. LMNA-GFP and H2B-GFP fluorescence were excited by a 488-nm solid-state laser (controlled with an acousto-optic tunable filter) and collected with a 405/488/561/640 multiband-pass dichroic mirror (Semrock) and an ET525/50 emission filter (Chroma Technology). To image GFP and mCherry at the same time, we illuminated the sample simultaneously with two excitation wavelengths, 488 and 561 nm, produced by two distinct monochromatic solid-state lasers. The emission was separated by the W-View Gemini Image Splitter (Hamamatsu) using GFP/mCherry dichroic mirror (Chroma Technology), and further passed through an ET525/30 and an ET630/75m emission filter (Chroma Technology). The two fluorescent signals were allocated to the two halves of the image sensor, producing two distinct images. Images were obtained with a Hamamatsu ORCA-R2 cooled CCD camera controlled with MetaMorph 7 (Molecular Devices) software. The pixel size for the 100× objective was 0.065 μm. The observation duration was 25 s, with an exposure time of 250 ms. The streams of 16-bit images were saved as multi-tiff stacks. Images were converted to single-tiff images and analyzed in MatLab (The Math-Works). To detect a nuclear contour and analyze its shape fluctuations, we used a custom-written MatLab code (see *SI Materials and Methods* and Fig. S8).

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