Stress fiber anisotropy contributes to force-mode dependent chromatin stretching and gene upregulation in living cells

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Living cells and tissues experience various complex modes of forces that are important in physiology and disease. However, how different force modes impact gene expression is elusive. Here we apply local forces of different modes via a magnetic bead bound to the integrins on a cell and quantified cell stiffness, chromatin deformation, and DHFR (dihydrofolate reductase) gene transcription. In-plane stresses result in lower cell stiffness than out-of-plane stresses that lead to bead rolling along the cell long axis (i.e., alignment of actin stress fibers) or at different angles (90° or 45°). However, chromatin stretching and ensuing DHFR gene upregulation by the in-plane mode are similar to those induced by the 45° stress mode. Disrupting stress fibers abolishes differences in cell stiffness, chromatin stretching, and DHFR gene upregulation under different force modes and inhibiting myosin II decreases cell stiffness, chromatin deformation, and gene upregulation. Theoretical modeling using discrete anisotropic stress fibers recapitulates experimental results and reveals underlying mechanisms of force-mode dependence. Our findings suggest that forces impact biological responses of living cells such as gene transcription via previously underappreciated means.
It is well established that living cells and tissues respond to mechanical force stimulation such as shear stresses in the blood or interstitial flow, tractional forces at cell–matrix contacts, and cell–cell contacts. Mechanical properties of the living cells and tissues are also known to be critical in regulating their biological responses in physiology and diseases. However, how different modes of forces impact gene expression is elusive. For example, while it is well known that shear stresses at the cell apical surface produce different signals and cellular effects than do stretching forces at the base or sides of endothelial cells, as vessel diameter changes during dilation or constriction, the underlying mechanisms of how gene expression is altered by various force modes remain unclear. There are only a few well-characterized technologies that can apply controlled local mechanical forces to a single living cell. One such method is the atomic force microscopy (AFM) that in general applies an indentation force on the apical surface of the cell. Another method is the optical tweezer that uses a focused laser light to trap a micrometer-sized particle to apply a lateral force on the cell apical surface. The third method is the method of magnetic twisting cytometry (MTC) or magnetic gradient pullers. In magnetic gradient pullers, an electromagnetic inhomogeneous field is generated at the tip of the tweezer to pull on a magnetic bead attached to the apical surface of the cell. In MTC, a ferromagnetic bead is magnetized with a strong magnetic field pulse in the horizontal direction (e.g., along Y-axis) and then a weak homogenous magnetic field is applied in the vertical direction (along Z-axis) to rotate the bead about the X-axis. This early version of the MTC can only rotate the magnetic bead about one axis—the X-axis and is thus called one dimensional (1D) MTC. Because of the technical difficulty to compare mechanical responses of the living cell to various mechanical probes, it is not clear how surface force modes influence cellular responses and functions. Here we describe a strategy of using the three-dimensional MTC (3D MTC) to apply forces in any desired directions to the same living cell by rotating the magnetic bead about any axes. We find that for a given stress amplitude, a living cell responds to a local in-plane stress differently from responding to a local out-of-plane complex stress by stretching chromatin and upregulating gene transcription to different levels. Experimental results in living cells and theoretical modeling analyses using discrete anisotropic elements reveal that stress fiber anisotropy determines force-mode dependent cell stiffness and chromatin stretching and thus regulates gene upregulation.

Results
A strategy of applying local in-plane stress. The 1D MTC generated a bead rotation that was out-of-the X–Y plane and thus generated a complex stress on the cell surface: as the bead rotated in the Y–Z plane, the bead edge that moved upward stretched the cell membrane and the bead edge that moved downward compressed the cell membrane (Fig. 1a, b). In contrast, the 3D MTC could magnetize a magnetic bead in any desired direction (X, Y, or Z) and applied a homogeneous twisting field in any desired direction (X, Y, or Z) (Fig. 1c). When a magnetic bead was magnetized in Z direction and then twisted in the Y direction or in the X direction, an out-of-plane complex stress was applied as the bead rotated about the X-axis or the Y-axis, respectively (Fig. 1c). Here we describe a strategy to apply local in-plane stresses with the 3D MTC: when a magnetic bead was magnetized in X direction and then twisted in Y direction (Fig. 1d, left), a local stress was applied to the cell surface as the bead rotated about the Z-axis (Fig. 1d, middle, right).

Cell stiffness and chromatin stretching under different stress modes. Using the strategy of in-plane stress, we applied local stresses to the cell surface via specific receptors like integrins using an Arg-Lyn-Asp (RGD) peptides coated magnetic bead (Fig. 2a). The peak amplitude of the sinusoidal wave was kept at 15 Pa and loading frequency was kept at 0.3 Hz. We measured the bead rotation angles (radians) when the in-plane stress was applied (Fig. 2b). We also measured 2D projection of center displacements of the bead in the X–Y plane on the surface of the same cell as the bead rolled either along the long axis of the cell (0° stress mode) or transverse the long axis of the cell (90° stress mode) when the out-of-plane complex stress was applied (Fig. 2b). As expected from the anisotropic mechanical behaviors of the living cell, the bead displacements were much less for the 0° mode (i.e., along the long axis of the cell and thus the direction of most stress fibers) than for the 90° mode (Fig. 2b). Using the published method of computing cell stiffness by taking into account the bead–cell contact area, we computed cell stiffness of the same living cell under the condition of different stress modes. Cell stiffness was twice as much for the 0° mode as for the 90° mode (Fig. 2c). Interestingly, cell stiffness was lowest when the in-plane stress was applied (Fig. 2c). To determine how the local surface stresses deform the chromatin, we quantified deformation of chromatin domains where green fluorescent protein (GFP) labeled transgene DHFR (dihydrofolate reductase) resided (Fig. 2d). Mean Square Displacements (MSDs) of the GFP spots (Fig. 2e) and the changes in distances between any two GFP spots (chromatin deformation) (Fig. 2f) were highest for the 90° stress mode, intermediate for the in-plane stress mode, and lowest for the 0° mode. We then computed stretching of the chromatin domain containing the DHFR gene by quantifying the tensile strains and the shear strains of the chromatin and found that the in-plane stress mode resulted in the strains that were higher than the 0° mode and lower than the 90° mode (Fig. 2g, h). The data showed that tensile strains were about twice as much as the shear strains, suggesting that the dominant form of the chromatin deformation was tensile (i.e., stretching) for the in-plane mode. This result was unexpected. Since in contrast to the out-of-plane stress modes that resulted in predominantly normal strains for the 0° mode and similar magnitudes of normal and shear strains for the 90° mode at the cell cortex, the in-plane stress mode caused mostly shear strains at the cell cortex (Supplementary Fig. 1; Supplementary Table 1), but inside the nucleus the chromatin domain deformation via the in-plane mode was mainly tensile, suggesting that the complex structural arrangements of the cytoskeleton, linker of nucleoskeleton and cytoskeleton, and the nuclear lamins propagate the surface stress into the nucleus as a complex stress to result in mainly tensile deformation in the chromatin. Next we examined how stress amplitudes of the in-plane mode would impact chromatin deformation and cell stiffness. MSDs of individual GFP spots increased with the stress amplitude (Supplementary Fig. 2a–d). The bead twisting angle increased linearly with stress amplitude within the range of stress that we had applied (Supplementary Fig. 2e). As a result, cell stiffness did not change with the amplitude of the in-plane stress (Supplementary Fig. 2f). Furthermore, chromatin deformation increased with the in-plane stress amplitude (Supplementary Fig. 2g), suggesting that the structural changes in the chromatin domain are direct mechanical responses. Together these data show that in-plane stress induces different responses on cellular mechanical properties (i.e., cell stiffness) and chromatin deformation from the out-of-plane complex stresses.

Gene upregulation varies with stress modes. Next we examined how DHFR gene transcription might change in response to different stress modes at the same stress amplitude and frequency. To minimize contributions of potential confounding factors in
Out-of-plane complex stress

**Fig. 1 Strategies of applying different stress modes to a cell.** a Schematic of the cell (blue color) with its nucleus (yellow color); the black big dot was the ferromagnetic bead. The cell was placed in a culture dish in the center of the coils and the focal plane of the microscope. Not drawn to scale. b Schematic of one-dimensional magnetic twisting device (1D MTC) to apply force on the cell. The bead rotated in the Y-Z plane about the X-axis (torque was in the x direction) and generated a local complex stress. c Schematic of three-dimensional magnetic twisting device (3D MTC) to apply force on the cell. The bead rotated in the X-Z plane (out-of-plane) about the Y-axis (the right-hand rule), the Y-Z plane (out-of-plane) about the X-axis, or the X-Y plane (in-plane) about the Z-axis. d Schematic of magnetizing the magnetic bead in the X direction and twisting it in the Y direction. The bead rotated in the X-Y plane (about the Z-axis) which was parallel to the substrate surface of cell spreading and generated a local stress.

the cytoplasm to DHFR transcription, we employed a published strategy of using fluorescently labeled 5′-end probes that could detect the transcription of the first 1700 bps DHFR mRNA. This way we could detect processes of transcription by reading the partial transcripts as early as seconds to a few minutes before the full transcript of DHFR was completed. DHFR gene is 34 kb long and it takes ~10 min to complete one transcript. When the stress was applied at 15 Pa and 0.3 Hz for 2 min, DHFR transcription was upregulated from the baseline: the in-plane stress mode caused an upregulation that was higher than the 0° mode and lower than the 90° mode (Fig. 3a). Extending the duration of stress application to 60 min, we found that all three stress modes resulted in a time-dependent elevation of transcription of DHFR (Fig. 3b). In line with the published results, the in-plane mode also led to a stress-amplitude dependent elevation of DHFR transcription (Fig. 3c). Since the extent of the chromatin deformation and DHFR upregulation under the in-plane mode was between those under the 0° mode and the 90° mode, we wondered if the 45° stress mode (i.e., the bead rotation was along the direction that was diagonal between the long axis and the short axis of the cell) would cause similar responses from the cell as the in-plane mode. It is interesting that although cell stiffness probed via the 45° out-of-plane mode was ~3 times that via the in-plane mode (Fig. 3d), the chromatin deformations were very similar (Fig. 3e). In addition, there was no difference in DHFR transcription upregulation between the 45° mode and the in-plane mode, either after 2 min stress or after longer stress application (Fig. 3f, g), suggesting that although these two modes are quite distinct in cell surface deformation (one is out-of-plane and the other is in-plane) that led to different stiffness values of the same cell, their impacts on the chromatin deformation and DHFR transcription were similar.

**Stress fiber anisotropy determines chromatin deformation and gene upregulation.** Next we set out to determine what dominates anisotropy of cell stiffness, chromatin deformation, and gene transcription upregulation. Since filamentous actin (F-actin) is a major player in cell stiffness, we used a specific F-actin inhibitor, Latrunculin A (LatA), to treat the cells for various times to disrupt actin stress fibers. Actin stress fibers number decreased when the cells were treated with LatA for 2 min; stress fibers became short, disorganized, aggregated structures after 5 min LatA treatment in the absence of obvious cell rounding (Supplementary Fig. 3a–c). As a result, cell stiffness decreased gradually at 2 min for all stress modes; by 5 min of LatA treatment, the differences in cell stiffness between various stress modes disappeared (Fig. 4a). The cell stiffness data are consistent with the loss of structural integrity of the stress fibers. Interestingly, MSDs of chromatin GFP spots, a measure of chromatin deformation, increased after 2 min of LatA treatment (compare Fig. 4c with Fig. 4b), likely due to the fact that stress fibers were only partially disrupted (Supplementary Fig. 3b) and that the remaining stress fibers were able to transmit forces to the nucleus that was softened by the reduction in the F-actin in the cytoplasm. Five minutes after LatA treatment, however, force-induced chromatin deformation completely disappeared under all stress modes (Fig. 4d), likely because stress fiber integrity and the force-transmission pathway to the chromatin were completely disrupted. Interestingly, the gene upregulation under various stress modes after LatA treatment followed the same trend as the chromatin deformation: 2 min after LatA, transcription was increased when compared to that without LatA treatment for all stress modes and there was anisotropy in changes in transcription under different stress modes; however, no transcription upregulation was observed after 5 min LatA treatment under any stress.
Shear strains were computed from the same chromatin deformation. When the stress (15 Pa at 0.3 Hz) was applied at 0° or 90° or in-plane mode. No stress data represent the spontaneous GFR spots movements in the sinusoidal magnetic fields were maintained at 15 Pa and 0.3 Hz. Each triangle or dot represents one cell. Cell stiffness computed with different stress modes. $P = 0.0079$ between 0° and in-plane stress modes; $P < 0.001$ between 0° and 90°, 0° and in-plane stress modes. d Fluorescent image of the three GFP spots in the same chromatin of a representative cell. Scale bar, 1 μm. e Normalized mean squared displacement (MSD) of all individual GFP spots when the stress (15 Pa at 0.3 Hz) was applied at 0° or 90° or in-plane mode. No stress data represent the spontaneous GFR spots movements in the absence of force application. f Chromatin deformation (i.e., changes of distances between any two GFP spots in the same chromatin domain) depends on stress modes. $P < 0.001$ between each different stress modes. g Tensile strains of the chromatin were computed from chromatin deformation. $P = 0.0055$ between 0° and in-plane stress modes; $P = 0.0017$ between in-plane and 90° stress modes; $P < 0.001$ between 0° and 90° stress modes. h Shear strains were computed from the same chromatin deformation. $P = 0.00701$ between 0° and in-plane stress modes; $P < 0.001$ between in-plane and 90° stress modes; $P < 0.001$ between in-plane and 90° and 0° and 90° stress modes. For b, c, and e-h, mean ± s.e.m.; n = 39 cells, 29 independent experiments; **P < 0.01; ***P < 0.001. P values were calculated and corrected using two-tailed Student’s t-test and Bonferroni correction. Source data are provided as a Source data file.

Mechanisms of stress-mode dependence of cell stiffness and chromatin deformation. To elucidate the underlying mechanism of why the in-plane stress mode leads to the lowest cell stiffness of all the stress modes but induces chromatin deformation comparable to that by the 45° out-of-plane complex stress mode, we employed the approach of finite element model (FEM) (Fig. 5a, b). The FEM of a typical anisotropic cell, whose number of actin stress fibers along the cell long axis was twice as much as the short axis (Fig. 5a), generated stress-mode-dependent magnetic bead displacements and chromatin deformation (Fig. 5c–e). The displacement of the magnetic bead along the short axis of the cell (the 90° stress mode) was 1.6 times that along the long axis (the 0° stress mode), and the displacement of the bead at 45° stress mode was in-between these two values (Fig. 5d), indicating that the cell stiffness has distinct anisotropy. In addition, the rotation angle of the magnetic bead under the in-plane stress was ~0.5 rad, suggesting that the cell stiffness induced by the in-plane mode was lower than all out-of-plane stress modes. These results quantitatively reproduced what was measured experimentally in living cells (see Fig. 2 and Supplementary Fig. 4a). We then analyzed the effects of stress modes on chromatin deformation. We used the same approach as the live cell experiments, i.e., three points in the $X$-$Y$ plane of a given height inside the nucleus to represent the points in the same chromatin domain (Fig. 5c). We then calculated the change of the distance between each two points before and after loading, and took the average of the three values as the chromatin deformation, with which the tensile and shear strain were calculated. The FEM calculation qualitatively reproduced the live cell experimental measurements (compare Fig. 5e–g with Fig. 2f–h and with Supplementary Fig. 4). Tensile strains and shear strains exhibited anisotropy and the tensile strains were ~twice as large as the shear strains. In addition, the chromatin strains induced by the in-plane stress mode were similar to those by the 45° out-of-plane stress mode.

To examine the role of structural integrity of F-actin in cell stiffness anisotropy and chromatin deformation, actin stress fibers were gradually disassembled in the FEM to simulate the inhibitory effect of LatA on F-actin in the live cell experiments. Based on the experimental results from live cells, when treated with LatA for 2 min, actin stress fibers were only partially disassembled, but when treated with LatA for 5 min, the stress fibers were completely destroyed (Fig. 6a–c). The displacements of the magnetic bead after LatA treatment under the three out-of-

Fig. 2 Mechanical anisotropy of the cell and of the chromatin induced by different force modes. a A representative image of adherent and elongated CHO (Chinese hamster ovary) cell with the GFP labeled chromatin domain (green dots; see the enlarged image within the dashed white lines). A 4-μm RGD-coated ferromagnetic bead (the solid black ball) was attached to the cell surface via integrins. Theta represents the angle of bead rolling direction with respect to the long axis of the cell (this notation applies to all cells in all figures). The bead and GFP spots in the white box were enlarged and shown on the right. Scale bar, 3 μm. The in-plane ($X$-$Y$ plane) rotation has a direction along the $Z$-axis and the two out-of-plane rotations have directions along the $X$- and $Y$-axes, using the right-hand rule (see Fig. 1). b Peak 2D displacements of the center of the magnetic bead in the $X$-$Y$ plane at stress angles 0° or 90° for the out-of-plane stress mode or the peak bead twisting angles for the in-plane stress mode in the same cell. In all stress modes, the peak amplitudes of the sinusoidal magnetic fields were maintained at 15 Pa and 0.3 Hz. Each triangle or dot represents one cell. c Cell stiffness computed with different stress modes.
Stress was applied to each cell only once at one particular angle for all FISH experiments. Fluorescently labeled 5′-end probes were used to detect expression of the first 1700 bps DHFR mRNA. Controls (No stress) were the cells in the same dish without attachment of magnetic beads. The stress was applied for 2 min at 15 Pa and 0.3 Hz. P < 0.001 between each different stress modes. Mean ± s.e.m.; n = 115, 97, 344, and 85 cells for no stress, 0°, in-plane, and 90° stress modes, respectively, in nine separate experiments; ***P < 0.001. b DHFR transcription upregulation depends on stress (15 Pa at 0.3 Hz) duration for both in-plane and out-of-plane modes. P = 0.016 between 30 and 60 min under 0° stress mode; P = 0.025 between 15 and 30 min and P = 0.0015 between 30 and 60 min under in-plane stress mode; P = 0.0039 between 30 and 60 min under 90° stress mode; P < 0.001 between other conditions under each stress mode. Mean ± s.e.m.; 0°: n = 21, 33, and 52 cells at 15, 30, and 60 min; in-plane: n = 38, 66, and 60 cells at 15, 30, and 60 min; 90°: n = 26, 25, and 32 cells at 15, 30, and 60 min. Three independent experiments. *P < 0.05; **P < 0.01; ***P < 0.001. The dashed line was the no stress control. c DHFR gene upregulation depends on stress amplitudes of the in-plane mode. All stresses were applied at 0.3 Hz for 30 min. n = 71 cells for 0° stress; n = 47, 50, 68, and 66 cells at 6, 9, 12, and 15 Pa, respectively. P = 0.032 between 0 stress and 6 Pa; P = 0.0033 between 6 and 9 Pa; P = 0.014 between 9 and 12 Pa; P < 0.001 between other different stress amplitudes. Mean ± s.e.m.; *P < 0.05; **P < 0.01; ***P < 0.001. d Cell stiffness computed from different stress modes. P = 0.036 between 0° and 45° stress modes; P = 0.039 between 45° and 90° stress modes; P = 0.037 between 90° and in-plane stress modes; P < 0.001 between 0° and in-plane stress modes as well as 45° and in-plane stress modes. Mean ± s.e.m.; n = 11 cells, eight independent experiments. *P < 0.05; **P < 0.001. e Chromatin deformation (chromatin deformation, i.e., changes of distance between GFP spots) with different stress modes. No difference was found between the 45° mode and the in-plane mode. P = 0.97 between in-plane and 45° stress modes; P < 0.001 between other different stress modes. Mean ± s.e.m.; n = 33 GFP spots; 11 cells, eight independent experiments. ***P < 0.001; ns = not significantly different. f Comparison of DHFR upregulation (quantified with 5′-end probes) between the out-of-plane 45° mode and the in-plane mode. The stress was applied for 2 min at 15 Pa and 0.3 Hz. P = 0.069 between in-plane and 45° stress modes. Mean ± s.e.m.; n = 344 and 140 cells for in-plane and 45° stress modes; nine separate experiments; ns = not significantly different. The dashed line was the baseline transcription level in the absence of force. g DHFR upregulation was similar at longer durations of stress (15 Pa and 0.3 Hz) between the 45° out-plane mode and the in-plane mode. P = 0.44, 0.14, and 0.4 between in-plane and 45° stress modes under 15, 30, and 60 min, respectively. Mean ± s.e.m.; 45°: n = 21, 29, and 39 cells at 15, 30, and 60 min, respectively; in-plane: n = 38, 66, and 60 cells at 15, 30, and 60 min, respectively. Three independent experiments; ns = not significantly different. The dashed line was the baseline transcription level in the absence of force. P values were calculated and corrected using two-tailed Student’s t-test and Bonferroni correction. Source data are provided as a Source data file.

plane stress modes were shown in Fig. 6d: as stress fibers gradually disassembled, the differences of the bead displacements among the three stress modes were substantially reduced. These results recapitulate the cell experimental results that stress fibers play a key role in the anisotropy of cell stiffness and the average cell stiffness.

It is known from our earlier published reports that the long transmission distance of force into the nucleus depends on actin stress fibers.21,23 In the FEM, stress fibers between the nucleus and the cell surface were partially (~30%) disrupted after 2 min LatA, leading to weakening the constraints on the nucleus to increase chromatin deformation, which was a result of the elevated stress concentration on the remaining stress fibers to propagate stresses to deform chromatin (Fig. 6e). In contrast, stress fibers between the magnetic bead and the nucleus in the FEM after 5 min LatA treatment were completely disrupted, no longer being able to transfer the load into the nucleus. Hence the chromatin deformation reduced dramatically and the impact of different stress modes on chromatin deformation no longer existed (Fig. 6e). The modeling data therefore are consistent with the experimental results from living cells (see Fig. 4b–d).

Our FEM simulation results showed that under the out-of-plane complex stress modes most of the actin stress fibers were directly stretched or compressed by the rolling motion of the magnetic bead. In contrast, under the in-plane stress mode, most of the stress fibers were not directly stretched. Instead, a tangential deflection was produced which allowed a large rotation of the magnetic bead, resulting the larger extent of cell deformation and thus lower cell stiffness than those by out-of-plane stress modes.

Why chromatin deformation under the in-plane stress mode is similar to that under the 45° out-of-plane stress mode is not
that are propagated into the nucleus by the in-plane and the 45° different modes are quite distinct at the cell cortex, the stresses Fig. 1c, d; Supplementary Table 1). These simulation results higher shear strains than the 45° stress mode (Supplementary in-plane stress mode whereas the in-plane stress mode induced contrast, at the cell cortex near the magnetic bead, the 45° out-of- plane stress mode induced much higher normal strains than the two cases were very similar and the nuclear strains near the site of nuclear deformation magnitudes and distributions between the 45° stress modes (Supplementary Fig. 5a, b), we found that the underlying mechanism, we performed additional FEM simula- structures and not by a continuous material. To determine the tions. Comparing nuclear deformation between the in-plane and obvious. The fact that the two different stress modes (i.e., 45° out- of-plane and in-plane) have similar impacts at a distance, i.e., and 45° stress modes (Supplementary Fig. 5a, b), we found that the nuclear deformation magnitudes and distributions between the two cases were very similar and the nuclear strains near the site of loading were similar as well (Supplementary Fig. 5c, d). In contrast, at the cell cortex near the magnetic bead, the 45° out- of-plane stress mode induced much higher normal strains than the in-plane stress mode whereas the in-plane stress mode induced higher shear strains than the 45° stress mode (Supplementary Fig. 1c, d; Supplementary Table 1). These simulation results suggest that although the cell deformations induced by these two different modes are quite distinct at the cell cortex, the stresses that are propagated into the nucleus by the in-plane and the 45° out-of-plane stress modes are quite similar, possibly due to the fact that all stresses in the cytoplasm have to be concentrated into the nucleus to deform the chromatin, leading to similar chromatin deformation.

Discussion

In the current study we compared the effects of applying (cell surface) out-of-plane complex stresses with those of applying a (cell surface) in-plane stress on cell mechanical behaviors and biological responses such as gene transcription. We find that there is substantial anisotropy in cell stiffness, chromatin deformation, and gene transcription under different stress modes. For out-of-plane stress modes, rolling the magnetic bead along the short axis of the cell (i.e., 90° from the direction of the most stress fibers) results in larger cell deformation than that along the long axis of the cell (0°) or 45° from the cell long axis. In contrast, applying an in-plane stress leads to more cell deformation than all of the out-of-plane stresses. FEM simulation shows that this is due to the fact that in-plane mode causes little compression or stretching of the stiff actin stress fibers and mainly tangential
The cross-sectional illustration of cell deformation near the nucleus and medium nuclear strain magnitudes. In contrast, the 90° mode resulted in small stress fiber deformation and high nuclear strain magnitudes. These modeling data demonstrate that chromatin deformation at the nucleus to deform the chromatin and to increase gene expression differently.

From the current study and a previously published report, it is known that the level of rapid DHFR gene upregulation depends on the extent of chromatin stretching or chromatin tensile strain. Together these findings suggest that because the majority of the stress fibers align along the long axis of the cell, which results in an anisotropy of cell stiffness, different force modes cause different stress fiber deformation at the nucleus to deform the chromatin and to increase gene expression differently.

Is the out-of-plane complex stress or in-plane stress physiologically relevant modes of force application? It is well known that endothelial cells lining the blood vessels experience fluid shear stresses on their apical surfaces and these stresses are balanced at the basal surfaces at the focal adhesions. In contrast, blood vessels experience blood pressure induced stretching that can cause complex strains in the cells of the vessels along the length of the vessels. It is also known that the myosin-II mediated cellular tractional forces at the focal adhesions are complex stresses with both in-plane and out-of-plane components. Therefore, exploring the effects of different stress modes on cellular functions is both physiologically relevant and important in understanding how forces impact living cells and tissues.

A living cell’s cytoskeleton consists of three major filament systems: actin filaments, microtubules, and intermediate filaments.
The actin filaments are bundled together to form actin stress fibers with myosin-II. We have identified the dominant role of actin stress fibers in anisotropic responses of the cells. Microtubules are known to play mechanical roles in cell functions. However, when the cells were treated with colchicine, there were no observable changes in cell stiffness or chromatin deformation (Supplementary Fig. 6), suggesting that microtubules play a minor role, if any, in rapid gene upregulation under different stress modes in these CHO cells. Intermediate filaments of the cytoskeleton also play roles in mechanical and biological functions of living cells. However, it is known that intermediate filaments play significant roles in cell stiffness only at relatively large deformations. In the future, it will be interesting to explore the potential impact of intermediate filaments in cell responses under different stress modes. CHO cells, generally elongated and have numerous stress fibers after adhesion and spreading, are an epithelial cell line. It remains to be determined in the future whether our findings can be applied to other types of cells.

When the stress fibers are disrupted by LatA, changes in geometry (i.e., bead–cell contact area) and/or bead–cell adhesion might influence cell stiffness measurement. We employed an approach of infecting living cells with talin-GFP, which labeled focal adhesions and focal complex-like structures at the cell basal surface (Supplementary Fig. 7) to examine this possibility. Bead embedding was calculated using the largest diameter of the bead on the cell surface (Supplementary Fig. 7) to examine this possibility. Bead embedding and the bead–cell contact area were measured. When the cells were treated with colchicine, there were no observable changes in cell stiffness or chromatin deformation. However, 5 min after LatA treatment to disrupt stress fibers, the cell stiffness exhibited the opposite trend: the cell stiffness was reduced dramatically for all force modes (see Fig. 4a). These data suggest that the change in cell stiffness after LatA treatment is most likely due to the increase in cell deformation as a result of stress fiber disruption and not due to changes in the bead–cell contact area. On the issue of the adhesion between the RGD-bead and integrins, it is known that a single ligand bond yields force is ~100 pN. There are hundreds of integrin receptors that interact with one 4-μm magnetic bead, which is coated with a saturating amount of RGD peptides, suggesting the bonds between the bead and the cell surface are strong. Moreover, we did not observe any bead peeling off the cell surface after stress fiber disruption, suggesting that most bonds are stable. However, it is reported that the characteristic adhesion stress decreases by ~25% after F-actin disruption with cytochalasin D treatment for 5 min. It will be interesting in the future to investigate whether the characteristic adhesion stress changes in various force modes and whether its change after stress fiber disruption impacts on cell stiffness measurement. Furthermore, the stretching distance of the RGD-bead and integrins is only on the order of 0.1–1 nm before the bond is broken, too small to account for the measured bead displacements of several hundred nanometers in live cells (see Fig. 2b). The bead displacements increased by several

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**Fig. 6 FEM simulation of the effects of disrupting stress fibers on cell and chromatin deformation.** a) No LatA control. b) +LatA 2 min. c) +LatA 5 min. Cell shapes and the bead are not drawn to scale. d) Effects of the LatA treatment on magnetic bead displacements under different stress modes. e) Effects of LatA treatment on chromatin deformation under different stress modes.
adhesion alteration. From published articles\textsuperscript{14,17,20} twice as many stress from the bead to the cell under each force mode. The middle images were the stress under different force modes. For visual clarity, the bead was removed from each image. The images on the right were chromatin strains under various force modes. Each wiggly line represents a stretched chromatin domain containing the DHFR gene locus (the pink dot), which was located at \(-2\,\mu\text{m}\) from the edge of the nuclear envelope\textsuperscript{37}. From the color scale bar of the nuclear strain and the chromatin domain location, it was estimated that the chromatin domain strain was low (-5\%) for the 0° mode (the bead rolling along the cell long axis), high (-25\%) for the 90° mode, and medium (-10\%) for both the 45° mode and the in-plane mode, comparable with the experimental data from living cells (see Figs. 2 and 3). This model suggests that the extent of chromatin stretching (tensile strain) and thus the level of gene upregulation (the number of the plus sign represents relative levels of gene upregulation) depend on nuclear strain, which is caused by different stress fiber deformation at the nucleus under different force modes. The color scale applies to all images.

folds when stress fibers are disrupted by LatA; these changes in bead displacements cannot be explained by the bead–cell bond adhesion alteration. From published articles\textsuperscript{14,17,20,22}, it is known that when the RGD-bead is attached to the cell surface, the bead is attached to the cell via the integrin–actin linkages and the bead is tethered by tensile F-actin bundles (i.e., stress fibers), providing majority of the resistance to bead rotation (or displacement) under stress. This resistance is an index of cell stiffness. When F-actin bundles are disrupted, this tethering is abolished and hence the resistance to bead rotation (or displacement) under applied stress is substantially reduced and the cell stiffness is dramatically decreased. Together all these suggest that the bead–cell contact area and adhesion play a minor role in cell stiffness after stress fiber disruption.

To better understand the effect of long-range force propagation by the actin stress fibers, we performed simulation by treating the composite of cytoplasm and the actin cytoskeleton as anisotropic uniform elastic materials without explicitly modeling the actin cytoskeleton (denoted as continuous model) (Supplementary Fig. 9). In contrast, in the above analyses, we have employed an FEM (denoted as discrete model, see Fig. 5a) to simulate actin stress fibers as discrete stiff elements embedded in the cytoplasm and calculate their impact on cell and chromatin deformation. The boundary conditions and stress modes in the continuous model are the same as the discrete model. Our results show that although the continuous model could generate similar bead displacements or rotation angles (i.e., similar cell deformation) as the discrete model (compare Supplementary Fig. 9b with 9e) by choosing proper parameter values for the anisotropic properties of the cell, the magnitudes of the chromatin deformation in the continuous model are much smaller than those computed from the discrete model (compare Supplementary Fig. 9c, f), as a result of more localized deformation in the cytoplasm and the nucleus using the continuous model than the discrete model (compare Supplementary Fig. 9a, d). These results are mainly due to the fast decay of the deformation field in the uniform elastic body in the continuous model (stress decays rapidly as the square of the distance), as predicted by the St. Venant’s principle (i.e., a local force only causes local deformation), so that the load from the magnetic bead cannot be effectively transferred to the nucleus. In contrast, the stress fibers in the discrete model can effectively transfer the load to the nucleus to a long distance due to stress concentration, consistent with the previous report of a single stress fiber model\textsuperscript{23}. Therefore, the discrete model is more appropriate for simulating long distance force propagation and chromatin deformation of an intact, spread living cell. Besides cytoskeletal filament stiffness, it is known that myosin-II dependent endogenous prestress is critical in the long distance force propagation in the cytoplasm and to the nucleus\textsuperscript{20–23,35}. To examine the role of nonmuscle myosin-II, we treated the cells with blebbistatin to inhibit myosin-II. We found that for the same loading, cell stiffness decreased substantially after 50 \(\mu\text{M}\) blebbistatin treatment (Supplementary
Fig. 10a) and chromatin deformation decreased dramatically (Supplementary Fig. 10b–d), consistent with a finding that myosin-II inhibition reduces cell stiffness and earlier reports that prestress mediates long force propagation in the cytoskeleton and into the nucleus. Furthermore, gene upregulation by the applied stress was substantially inhibited when the myosin-II dependent endogenous stress was inhibited with blebbistatin for all force modes (Supplementary Fig. 10e), consistent with and extending the previous finding when myosin light chain kinase is inhibited by ML-7. In our current discrete FEM model, the impact of the endogenous prestress has been incorporated into the stress fibers to increase the stiffness of the stress fibers and thus is not explicitly shown. Complete disruption of stress fibers in the discrete FEM that removes the influence of myosin-II dependent prestress leads to almost total abolishment of chromatin deformation is consistent with the previous reports. Furthermore, it is well established that living cells are viscoelastic and they respond to loading frequencies. However, a recent report finds that loading frequencies between 0.3 and 6 Hz have only modest impacts on chromatin deformation and gene upregulation; this article reveals that force-induced gene upregulation in living cells does not follow the weak power law of rheology but depends on H3K9 demethylation. Although our current discrete FEM is only an elastic model, it has recapitulated the essential features of mechanical and transcriptional responses of living cells at a low loading frequency (0.3 Hz in the current study) and revealed the underlying mechanisms of cellular responses.

In the future, the FEM can be extended to include viscoelastic elements in order to better simulate responses under high loading frequencies (e.g., 10–20 Hz). Recently a 3D cell model has been reported to describe the crosstalk among cell adhesions, the cytoskeleton, and the nucleus. It will be interesting to find out if this model can simulate the effects of different stress modes on cell stiffness and direct chromatin stretching.

In summary, we demonstrate that stress fiber anisotropy contributes to force-mode dependent cellular mechanical responses, chromatin deformation, and gene transcription. Our current study is a first attempt to provide insights on better understanding how living cells respond to different modes of complex forces in physiology and disease.

Methods

Cell culture and reagents. CHO DG44 DHFR D10 cells (provided by Dr. Andrew Belmont of University of Illinois who created this cell line) were cultured in Ham’s F12 media without thymidine and hypoxanthine (Shanghai Basal) with 10% fetal bovine serum and into the nucleus. Furthermore, gene upregulation by the applied stress was substantially inhibited when the myosin-II dependent endogenous stress was inhibited with blebbistatin for all force modes (Supplementary Fig. 10e), consistent with and extending the previous finding when myosin light chain kinase is inhibited by ML-7. In our current discrete FEM model, the impact of the endogenous prestress has been incorporated into the stress fibers to increase the stiffness of the stress fibers and thus is not explicitly shown. Complete disruption of stress fibers in the discrete FEM that removes the influence of myosin-II dependent prestress leads to almost total abolishment of chromatin deformation is consistent with the previous reports.

Furthermore, it is well established that living cells are viscoelastic and they respond to loading frequencies. However, a recent report finds that loading frequencies between 0.3 and 6 Hz have only modest impacts on chromatin deformation and gene upregulation; this article reveals that force-induced gene upregulation in living cells does not follow the weak power law of rheology but depends on H3K9 demethylation. Although our current discrete FEM is only an elastic model, it has recapitulated the essential features of mechanical and transcriptional responses of living cells at a low loading frequency (0.3 Hz in the current study) and revealed the underlying mechanisms of cellular responses.

In the future, the FEM can be extended to include viscoelastic elements in order to better simulate responses under high loading frequencies (e.g., 10–20 Hz). Recently a 3D cell model has been reported to describe the crosstalk among cell adhesions, the cytoskeleton, and the nucleus. It will be interesting to find out if this model can simulate the effects of different stress modes on cell stiffness and direct chromatin stretching.

In summary, we demonstrate that stress fiber anisotropy contributes to force-mode dependent cellular mechanical responses, chromatin deformation, and gene transcription. Our current study is a first attempt to provide insights on better understanding how living cells respond to different modes of complex forces in physiology and disease.
RNA FISH. Custom Stellaris FISH Probes were designed against DFFH mRNA (https://www.ncbi.nlm.nih.gov/nuccore/NM_010049.3) by using Stellaris RNA FISH probe Designer (Biorésearch Technologies Inc., Petaluma, CA) to detect the DFFH genes transcription. CHO DG44 cells were applied requisite stress amplitude with a certain time, then fixed in 3.7% formaldehyde for 30 min and permeabilized in 70% ethanol for 12 h at 4 °C. Samples were hybridized with DFFH Stellaris FISH Probes and incubated in dark humidified incubator for 12 h. Washing 2× in 1 mL wash buffer at 37 °C for 30 min, then proceed to imaging by Leica DMi6000B microscopy after adding 2 mL 2× SSC. Image J was used to analyze the FISH images.

Microscopy and live cell imaging. Leica DMi6000B with 63 × 1.4 NA oil-immersion objective (Leica, Cat. No. 15506350) was used to image FISH fluorescence via Leica MM AF software. Leica SP8-STOP microscopy with 100 × 1.4 NA oil-immersion objective was used to visualize chromatin GFP spots in CHO cells via Leica Application Suite X software. Chromatin deformation was measured following by quantifying changes of distances in the X–Y plane between chromatin GFP spots in the same chromatin domain. The Z-direction displacement was not measured and hence the chromatin deformation and chromatin strains were an underestimate of the total deformation. However, since the CHO cells were very spread (see Fig. 2a), the cell height in Z-axis was much less than the dimensions in X–Y plane, the changes in distances in Z was very small. Leica SP8-STOP microscopy excited GFP spots and Talin-GFP at 488 nm, SiR-actin at 633 nm.

Quantification of bead–cell contact area. The CHO cells were cultured for 48 h on glass-bottomed 35-mm dishes, precoated with 0.1% gelatin. Then 20 μL–1 medium of the reagent of CellLight™ Talin-GFP (Thermo, C10611, BacMam 2.0) that was a fusion construct of the c terminus of human talin and emGFPA were added to each 35-mm dish for 16 h before experiment. The fusion construct is packaged by the insect baculovirus, which does not replicate in mammalian cells, providing accurate and specific targeting to cellular talin-GFP, according to manufacturer's product description. Bead embedding was calculated using the largest diameter of the X–Y projected image of talin-GFP fluorescence as a measure of the largest bead–cell contact interface; bead–cell contact area was estimated by measuring the X–Y projected area times the average intensity of the largest fluorescence ring-like structure of talin-GFP surrounding the bead, which was an index of the bead–cell contact area, after taking into account of temporal controls without LaA of laser scanning induced photobleaching. Adjustment of thresholds was made at the same level for all images to eliminate the background noise. Since bead–cell contact area was varied among different cells, each bead–cell contact area after LaA treatment was normalized by that before the drug treatment.

Dynamic tracking and image analysis. MATLAB software for data analyzing and image processing can be downloaded from https://pan.baidu.com/s/1qXCGozy/. Source data are provided with this paper.

Data availability
Data supporting the findings of this paper are available from the corresponding authors upon reasonable request. A reporting summary for this Article is available as a Supplementary information file. Source data are provided with this paper.

Code availability
All codes used are available, free of charge, upon request to the corresponding authors. MATLAB software for data analyzing and image processing can be downloaded from https://pan.baidu.com/s/1qXCGozy/. Source data are provided with this paper.

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Author contributions
N.W. conceived the project. N.W., B.J., F.W., X.X., C.Z. and Y.L. designed the experiments. F.W., X.X., C.Z. and Y.L. carried out experiments and performed analyses. N.W., B.J., F.W., X.X., C.Z. and Y.L. all participated in writing the paper.

Competing interests
The authors declare no competing interests.

Additional information
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