Mechanical impulses can control metaphase progression in a mammalian cell

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Chromosome segregation machinery is controlled by mechanochemical regulation. Tension in a mitotic spindle, which is balanced by molecular motors and polymerization-depolymerization dynamics of microtubules, is thought to be essential for determining the timing of chromosome segregation after the establishment of the kinetochore-microtubule attachments. It is not known, however, whether and how applied mechanical forces modulate the tension balance and chemically affect the molecular processes involved in chromosome segregation. Here we found that a mechanical impulse externally applied to mitotic HeLa cells alters the balance of forces within the mitotic spindle. We identified two distinct mitotic responses to the applied mechanical force that either facilitate or delay anaphase onset, depending on the direction of force and the extent of cell compression. An external mechanical impulse that physically increases tension within the mitotic spindle accelerates anaphase onset, and this is attributed to the facilitation of physical cleavage of sister chromatid cohesion. On the other hand, a decrease in tension activates the spindle assembly checkpoint, which impedes the degradation of mitotic proteins and delays the timing of chromosome segregation. Thus, the external mechanical force acts as a crucial regulator for metaphase progression, modulating the internal force balance and thereby triggering specific mechanochemical cellular reactions.

chromosome segregation | mechanobiology | mitotic force | mitotic spindle | spindle assembly checkpoint

The hierarchical organization of cells is strongly influenced by changes in physical environments. In complex living tissues and organisms, cells have to adapt to compressive forces exerted by multiple surrounding cells. These mechanical interactions, which are intricately involved in cellular processes both mechanically and biochemically, thus have a strong impact on cellular functions (1–4). Mechanical forces play an especially important role in chromosome segregation machinery, which is controlled by mechanochemical regulation (5–7). During cell division, the premature rupture of intracellular mechanochemical links leads to errors in chromosome segregation, resulting in severe developmental defects, cancerous changes in normal cells, and so on (8, 9). However, there remain questions relating to the mechanochemical regulation of chromosome segregation during development and growth, such as: “Does the applied mechanical force modulate the internal force balance, in particular the tension between sister chromatids?” and “How does the applied mechanical force affect the chromosome segregation process?” These remain unsolved.

Metaphase progression towards the chromosome segregation carried out by a macromolecular machine, a mitotic spindle (9, 10), should be precisely controlled via the spindle assembly checkpoint (SAC) and the degradation of mitotic proteins (11–14). The SAC is a signaling pathway by which kinetochores that are not attached to the spindle emit diffusible ‘wait’ signals to delay anaphase, so the kinetochore-microtubule attachment state is one of the determinants for the timing of chromosome segregation. On the other hand, it is generally believed that kinetochores in all eukaryotes

control the timing of anaphase in a force-dependent manner, but little direct evidence supports this view. The most direct evidence came from classic experiments by R. B. Nicklas and his associates, in which glass needles were used to manipulate chromosomes in meiotic intact grasshopper spermatocytes, demonstrating that the modulation of tension by forces applied to single mis-attached chromosomes contributes to the regulation of the timing of chromosome segregation (15). Using physical (16, 17) and biochemical perturbations (18, 19), it was also reported that tension changed the molecular dynamics in kinetochores implicated in the mitotic progression. Thus, metaphase progression is considered to be highly sensitive to changes in tension. However, it remains debated whether tension balance in the mitotic spindle determines the timing of chromosome segregation in human cells. To test the possibility that the externally exerted mechanical perturbations, either increasing or decreasing tension within the mitotic spindle, may control metaphase progression in a mammalian cell, we applied precisely controlled mechanical impulses of varying magnitude and in different directions to the metaphase HeLa cells.

Results

Application of Mechanical Impulses Using the Microfabricated Cantilever System. For compressing a cell, we used plate-like microfabricated cantilevers (20, 21) (Fig. 1*A*), between which a metaphase HeLa cell expressing EGFP-tagged histone H2B was sandwiched (Fig. 1*B*). We either used a pair of stiff cantilevers or, for the force measurements, replaced one of the stiff cantilevers with a flexible one. The contact area between cantilevers and a cell was sufficient for compressing the whole cell, which is spherical during metaphase, without causing local membrane indentation. Using micromanipulators, cantilevers were positioned on either side of a metaphase cell, in which chromosomes were aligned at the metaphase plate. We could apply the external force to compress the sandwiched cell in various directions and with different velocities (Fig. 1*C*).

To probe the effect of external forces exerted over a short period of time on metaphase progression, single mechanical impulses on the order of several tens of milliseconds were applied to a cell by precisely displacing one stiff cantilever using a piezo actuator (Fig. S1*A* and *B*). The amplitude of impulses was 5 μm during the force measurements [when the other cantilever was flexible, which resulted in an actual cell compression of approximately 3 μm (approximately 15% of the cell diameter)], and 8 μm when a cell was compressed by two stiff cantilevers [in which case

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Fig. 1. Mechanical perturbation of a metaphase cell. (A) Schematics showing the experimental setup for manipulating a metaphase cell. The temperature of the medium was maintained at 37 °C using the heaters for the stage and the objective lens. The movement of the stiff cantilever producing the mechanical impulse (MI) was controlled by piezo actuator. (F/S), flexible or stiff cantilever; (S), stiff cantilever. (B) Merged bright-field image of a cell and the fluorescent image of EGFP-histone H2B (green) in a mitotic HeLa cell sandwiched between the parallel plate-like microfabricated cantilevers. Black regions on the sides are the parts of a stiff (S) and a flexible (F) cantilever. Scale bar, 5 μ m. (C) Directions of the mechanical perturbation and its expected effect on the long-axis length of a mitotic spindle assembled in a HeLa cell. The directions correspond to the angles between the surface of the cantilevers and the long axis of the chromosome array in the range of 0° $\pm$ 30° (0°), 45° $\pm$ 15° (45°), and 90° $\pm$ 30° (90°). (D) Images of the mitotic spindle before, during, and after the mechanical impulse (MI). The 8- μ m mechanical impulse in the 0° and 90° directions was applied to cells expressing EGFP-EB1. Different cells were used for each experiment. Scale bars, 5 μ m.

cell compression was equal to the amplitude of the impulse (approximately 40% of the cell diameter)]. The compression of cells depended on the directional stiffness of each cell and also on the compression rate when the flexible cantilever was used, because of its deflection during compression. Using a flexible cantilever, we estimated the magnitude of the external force applied to the cells to be on the order of tens of nanonewtons, which is an order of magnitude weaker than the force generated between neighboring cells (22) (see Materials and Methods). These impulsive mechanical perturbations neither caused the plastic change of the cell shape nor significantly displaced the chromosome position (Fig. S1C).

The External Force Alters the Balance of Forces Within the Mitotic Spindle. The forces generated inside and outside of the mitotic spindle are finely balanced through the action of molecular motors and microtubule dynamics to maintain the constant shape and size of a mitotic spindle and its orientation within a metaphase cell (23). To ascertain whether external mechanical force influences the balance of forces within the mitotic spindle, we examined the morphological alteration of a mitotic spindle by the 8- μ m impulses. The orientation of the pole-to-pole axis of the mitotic spindle assembled in metaphase HeLa cells is controlled by retraction fibers anchoring it to the surface of a glass-bottom

dish (7), making it almost parallel to the surface of the dish (24). Therefore, we surmised that mechanical perturbations in the nanonewton and micrometer range externally applied to a cell could either compress or extend a mitotic spindle depending on the direction of compression relative to the spindle's long axis (Fig. 1C). HeLa cells expressing EGFP-tagged End Binding protein-1 (EB1), which is one of the microtubule plus-end tracking proteins, were imaged with a spinning-disk confocal fluorescence microscope in order to observe the deformation of a mitotic spindle. Compression of the cell in the 0° direction resulted in the reduction of the pole-to-pole distance with a simultaneous increase in the spindle width (Fig. 1D, *Top*; Movie S1). On the other hand, the application of mechanical impulse in the 90° direction increased the spindle length and narrowed its width (Fig. 1D, *Bottom*; Movie S2). In both cases, the shape of the mitotic spindle almost recovered to its original shape as the compression was released (from 12.76 $\pm$ 0.88 to 12.58 $\pm$ 0.87 μ m, mean $\pm$ SD, n = 18 spindles in the 0° direction; from 12.81 $\pm$ 0.98 to 12.76 $\pm$ 0.95 μ m, mean $\pm$ SD, n = 16 spindles in the 90° direction). These results indicate that the externally applied force is efficiently transmitted to the mitotic spindle inside a cell.

Next, we evaluated whether the compression or the extension of a mitotic spindle induces changes in tension between sister chromatids, which are stabilized by a multisubunit cohesin complex (25). To do this, we analyzed the force-induced changes in the distance between sister chromatids, which reflect variations in tension. We examined HeLa cells expressing EGFP-tagged CENP-A, a centromere-specific protein marker. The intercentromere distance responded reversibly to the mechanical impulse, similarly to the deformation of the mitotic spindle (Fig. 2A; Movies S3 and S4). The intercentromere distances before and during mechanical impulses were measured for the centromeres that did not move out of focus (see yellow arrowheads in Fig. 2A and Fig. S2). For all directions of the applied mechanical impulses the response was nonuniform, that is, the external force caused some pairs to move closer, whereas the others separated farther. However, the average change in centromere distance obtained by the 8- μ m compression in the 0° direction was found to be -0.14 ± 0.21 μ m (mean $\pm$ SD, n = 79 pairs of centromeres from 22 cells; Fig. 2B, *Top*), whereas the 8- μ m compression in the 90° direction increased the intercentromere distance by 0.14 ± 0.18 μ m (mean $\pm$ SD, n = 78 pairs of centromeres from 22 cells; Fig. 2B, *Bottom*). This clearly manifested that mechanical impulses can modulate the intercentromere distance in a direction-dependent manner. Reinforcing this conclusion, weaker (5 μ m) compression produced smaller changes in the intercentromere distance [-0.09 ± 0.13 μ m (mean $\pm$ SD, n = 79 pairs of centromeres from 20 cells)] in the 0° direction and 0.08 ± 0.14 μ m (mean $\pm$ SD, n = 78 pairs of centromeres from 19 cells) in the 90° direction (Fig. 2C, *Top* and *Bottom*). The changes in the intercentromere distance were less significant for the compression in the 45° direction (Fig. 2B and C, *Middle*). Therefore, we conclude that the directional mechanical perturbation is able to physically control tension between centromeres in metaphase cells in a direction- and magnitude-dependent manner.

Mechanical Impulse Can Control Metaphase Progression. To examine the effects of the directional mechanical perturbation on metaphase progression, we performed time-lapse observation of chromosome dynamics over 30 min (Fig. 3A). Cell cycles were not synchronized, so the timing of the application of mechanical impulse to a metaphase cell (within approximately 0.5 min after a proper cell was found) was not precisely defined. The time of anaphase onset was determined from the time course of the distance between a pair of sister chromatids and was defined as the moment at which the distance started to increase (Fig. 3B and Fig. S3A and B). The frequency of control metaphase cells that reached the anaphase within 30 min was 89.3% (Fig. 3C,

Fig. 2. Directional mechanical impulse modulates the distance between centromeres. (A) Images of the centromeres before, during, and after the mechanical impulse (MI). The 8-μm mechanical impulse in the 0° and 90° directions was applied to cells expressing the EGFP-CENP-A. Different cells were used for each experiment. The arrowheads indicate the centromeres analyzed in B. Scale bars, 1 μm. (B and C) Distribution of the difference in intercentromere distance before and during the 8-μm (B) or 5-μm (C) mechanical impulses. The average change (mean ± SD) is shown by black vertical arrows. Gray areas correspond to a decrease in the intercentromere distance. * $p < 0.05$, ** $p < 0.001$. (Distributions of the intercentromere distances before and during MI were compared.) Data for 8-μm impulse, 0°, $p = 6 \times 10^{-9}$; 45°, $p = 0.20$; 90°, $p = 3 \times 10^{-8}$. Data for 5-μm impulse, 0°, $p = 2 \times 10^{-8}$; 45°, $p = 0.03$; 90°, $p = 1 \times 10^{-6}$.

Left). The 5-μm impulses (Fig. S14) induced neither a disruption of aligned-chromosome array nor mis-separation (Fig. 3D; Movie S5), so chromosome segregation in compressed cells occurred similarly to that which occurred in control cells (Fig. S3A and B).

Most remarkably, the effect produced by the mechanical impulse strongly depended on its direction (Fig. 3E and F and Fig. S3C). Compression in the 0° direction decreased the occurrence frequency of anaphase within 30 min (82.9%), compared with other directions (93.5% after compression at 45° and 94.6% at 90°), and slightly prolonged the average time of anaphase onset (14.69 ± 1.67 min after a 5-μm impulse, mean ± SEM). On the other hand, compression in the 90° direction shortened the average time of anaphase onset (10.27 ± 1.35 min, mean ± SEM). Although the changes in the intercentromere distance showed a mirror-image relationship between the 0° and 90° directions (Fig. 2), the directional mechanical impulse did not induce symmetric responses in the modulation of metaphase progression. As

a result, the average time of anaphase onset in compressed metaphase cells combined for all directions (12.16 ± 0.84 min, mean ± SEM; Fig. S3D) was slightly shorter than in control cells (13.01 ± 0.84 min, mean ± SEM; Fig. 3C). The frequency of chromosome segregation that occurred within 30 min in compressed metaphase cells was 90.7% (Fig. S3D), whereas the mechanical impulse applied to cells with unaligned chromosomes did not induce chromosome segregation during prometaphase (Fig. S3G). These results indicate that applied mechanical forces can control metaphase progression.

We also examined the dependence of the produced effects on the rates of compression and release. When a stiff cantilever was displaced slowly (at 1 μm/s) during either compression or release (Fig. S4A and D), the reduction of the time of anaphase onset was less pronounced than it was after the fast compression-fast release impulse (100 μm/s; Fig. S4B and E). A slower mechanical impulse slightly decreased the occurrence of anaphase within 30 min (80.4% by the fast compression-slow release and 84.5% by the slow compression-fast release). Importantly, however, fast compression noticeably delays the time of anaphase onset in the 0° direction and accelerates it in the 90° direction, whereas slow compression apparently does not produce these effects (Fig. S4C and F; Table S1). Consistent with the changes in anaphase onset after slow compression, the changes in the intercentromere distance were smaller (Fig. S4G). These results suggest that fast compression, which transiently modulates tension exerted between sister chromatids, efficiently modulates chromosome segregation.

Larger Compression More Efficiently Accelerates Metaphase Progression. To examine whether metaphase progression is accelerated more effectively by larger directional mechanical impulses, we compressed the metaphase cells by 8-μm impulses (Fig. 3G and H; Movie S6). The larger impulse strongly deformed the metaphase cell, but did not prevent chromosome segregation in 94.3% of all cells (Fig. S3F), irrespective of the direction of compression (92.5% at the 0° direction, 90.0% at 45°, and 100% at 90°) (Fig. 3G, and H and Fig. S3E). The most noticeable result was that the produced effects strongly depended on the direction of compression. Specifically, the compression in the 0° direction did not produce noticeable effects (Fig. 3G) whereas the impulses applied in the 90° direction reduced the average time of anaphase onset almost twofold to 6.89 ± 0.95 min (mean ± SEM) (Fig. 3H), and more than half of compressed cells reached anaphase within 5 min (Fig. 3C and H). Also, the frequency of prompt chromosome segregation, which occurred within approximately 1 min after the application of an impulse (i.e., approximately 1.5 min after finding a metaphase cell) in the 90° direction (18.6%; a yellow bar in Fig. 3H), increased approximately sevenfold compared to the 5-μm impulse (2.7%; Fig. 3F) and the control cells (2.7%; Fig. 3C). These results indicate that the mechanical impulse producing larger compression, which exerts larger tension on sister chromatids, more efficiently accelerates metaphase progression.

Taken together, our data demonstrate that the directional mechanical impulse modulates the timing of anaphase onset depending on its magnitude (Fig. 3) and compression rate (Table S1). This suggests that transient changes in forces balanced within the mitotic spindle may induce mechanochemical reactions that either positively or negatively control metaphase progression.

Mechanochemical Regulation of Metaphase Progression by the Application of a Mechanical Impulse. It is generally believed that the chromosome segregation machinery is controlled by the cell-cycle regulatory proteins (such as cyclin B) and SAC. To clarify in more detail how the applied force affects the mechanochemical processes in metaphase progression, we first examined whether the directional mechanical impulses activate the SAC, which is a crucial mechanism ensuring accurate chromosome segregation. For the activation of the SAC, the SAC proteins, such as BubR1, Mad2,

etc., are localized to kinetochores (26). We found that the mechanical impulse in the 0° (tension-decreasing) direction (Fig. S5A), but not in the 90° (tension-increasing) direction (Fig. S5B), induces the accumulation of BubR1 [which indicates the reduced tension (18)] within minutes. On the other hand, we did not detect the accumulation of Mad2 to kinetochores [which is a sensitive indicator of defects in the kinetochore-microtubule attachment (27)] after the application of a mechanical impulse in any direction (Fig. S5 C–E). Although the effect of mechanical impulse in the 0° direction on the timing of chromosome segregation was modest (Fig. 3 E and G), the above result indicates that only tension-decreasing force induces the evident activation of the SAC.

The activation of the SAC is coupled with an inactivation of the anaphase-promoting complex/cyclosome (APC/C), i.e., ubiquitin ligase (28). Therefore, to clarify how the mechanochemical molecular pathways are involved in the mechanical modulation of the time of anaphase onset, we examined the kinetics of the degradation of mitotic proteins in HeLa cells stably expressing mCherry-cyclin B1 protein (Fig. S6A) (29). A decrease in the mCherry-cyclin B1 level accurately correlates with the time to anaphase onset, which we used to gain more insights into the link between metaphase progression and the kinetics of its determinants (30). Anaphase onset in compressed cells was slightly delayed in the 0° direction ($p = 0.10$, Fig. S6A) and occurred slightly earlier in

Fig. 3. Directional mechanical impulse can control the metaphase progression. (A) A flow chart showing the timeline of an experiment. After the metaphase cell with all chromosomes aligned at metaphase plate was found ($t = 0$), the cantilevers were quickly positioned at its both sides (Fig. 1B), and within 0.5 min after finding a cell the mechanical impulse was applied to the cell by displacing a stiff cantilever for 5 μ m (Fig. S1A) or 8 μ m (Fig. S1B). The time-lapse images of chromosome dynamics were then taken at 5-second intervals, either until the chromosomes were sufficiently separated (t_{TAO} = time of anaphase onset) or for 30 min if the segregation did not occur. (B) Time courses of the changes in the chromosome separation distance in control cells (Bottom). Data for 43 representative control cells, chosen so that the shape of the distribution of the time of anaphase onset in these cells was roughly identical to that for all 112 cells shown in C. Black trajectories show cells in which chromosome segregation did not occur within 30 min (Top). Fluorescence micrographs showing the chromosome dynamics. The red lines indicate the chromosome separation distance for one of the control cells, shown by red trajectory in the bottom panel, at the time points indicated by black vertical arrows. Scale bar, 5 μ m. (C) Percentage of cells undergoing chromosome segregation (Left), and histograms of the time of anaphase onset (Right) in control cells. Yellow bars show cells reaching anaphase within approximately 1.5 min after finding a metaphase cell. Black bars show cells in which chromosome segregation did not occur within 30 min. The average time of anaphase onset (mean $\pm$ SEM) is shown in histograms (for the “undivided cells,” it was set to be 30 min). (D) Sequential images of a mitotic HeLa cell, from the application of the mechanical impulse to the completion of cytokinesis. The time of anaphase onset (TAO) was 14.93 min. In each image, the time after the cell was found under the microscope is shown (min: sec). Scale bar, 5 μ m. (E–H) Percentage of cells undergoing chromosome segregation (Left), and histograms of the time of anaphase onset (Right) in compressed cells. (E and F) Data for approximately 3- μ m compression, (G and H) Data for 8- μ m compression. Data for the 0° and 90° directions are shown in E and F and G and H, respectively. Data for the 45° direction are shown in Fig. S3 C and E. Total data for all directions are shown in Fig. S3 D and F. Yellow and black bars are the same as in C. The average time of anaphase onset including the “undivided cells” is also shown (mean $\pm$ SEM). * $p < 0.001$ compared with the control cells in C. Data of the approximately 3- μ m compression, control versus 0°, $p = 0.41$; control versus 90°, $p = 0.07$; 0° versus 90°, $p = 0.05$. Data for the 8- μ m compression, control versus 0°, $p = 0.56$; control versus 90°, $p = 0.0001$; 0° versus 90°, $p = 0.005$.

the 90° direction ($p = 0.07$, Fig. S6A) compared with the control. Time courses of the change in the mCherry-cyclin B1 level were biphasic, consisting of the initial phase, during which the cyclin B1 level remained relatively constant, and the second phase, during which cyclin B1 was degraded rapidly (Fig. 4 A–C and Fig. S6B). The steepness of the second phase of the cyclin B1 degradation, which manifests the SAC inactivation, seemed to be unaffected by the mechanical perturbation (Fig. S6C, see also Fig. 4 A–C). Interestingly, the fluorescence intensity of mCherry-cyclin B1 at anaphase onset after 90°-compression was 3.060×10^5 A.U. (67% of the initial level) contrary to 2.635×10^5 A.U. (60% of the initial level) in control cells ($p = 0.008$, Fig. 4 A–C, Right), which indicates that the chromosome segregation occurred even when cyclin B1 was not sufficiently degraded.

Discussion

Here we demonstrated that external mechanical impulses can modulate the internal force balance in the mitotic spindle, resulting in the alteration of the timing of chromosome segregation in a mammalian cell. Up to the present, the glass needle technique was used to demonstrate the tension-dependent control of the SAC in intact grasshopper spermatocytes (15–17), but it may produce too severe effects on cells when attempting to alter force balance within the mitotic spindle in human cells. Biochemical

Fig. 4. Mechanochemical modulation of cyclin B1 degradation by the directional mechanical impulse. (A–C) Time courses of the change in fluorescence intensity (F.I.) of mCherry-cyclin B1 (Middle) in control (A) or compressed (B and C) cells. Data for the 8- μ m mechanical impulse in the 0° and 90° directions are shown in B and C, respectively. (See Fig. S6B for the 45° direction.) Time 0 ($t = 0$) is the time of anaphase onset. Histograms on the left and right show, respectively, the distribution of fluorescence intensity of mCherry-cyclin B1 at the beginning of the observation and at anaphase onset. The average fluorescence intensity (A.U.) of mCherry-cyclin B1 is shown in histograms. * $p < 0.01$ compared with the control in A. Data for initial F.I., control versus 0°, $p = 0.62$; control versus 90°, $p = 0.43$; 0° versus 90°, $p = 0.98$. Data for F.I. at anaphase onset, control versus 0°, $p = 0.30$; control versus 90°, $p = 0.008$; 0° versus 90°, $p = 0.18$. (D) Schematic summary describing the mechanochemical regulation of the timing of chromosome segregation (for details, see text).

perturbations modulating the tension between sister chromatids have also been used to indirectly study the relationship between the persistent loss of tension and chromosome dynamics during mitosis. However, it is difficult to produce reversible transient perturbations on the order of 1–100 msec by biochemical techniques (e.g., even when using caged microtubule-destabilizing drugs) (31). Here, using precisely controlled mechanical perturbations by a pair of cantilevers, we made it possible to investigate how a human mitotic cell mechanochemically responds to an external mechanical impulse. In contrast to physical manipulation of flattened cells that have various shapes during cell division (16, 32), metaphase HeLa cells, which become spherical during

mitosis due to a “rounding up” force (6), enabled us to not only compress but also stretch the mitotic spindle by applying the external force from orthogonal directions. Although morphological changes in the mitotic spindle were indirectly induced by the mechanical perturbation applied from the outside of the cell, the deformed shape of the spindle was similar to that obtained by directly compressing the meiotic spindle assembled in the cytoplasmic extracts (21). Moreover, the changes in the intercentromere distance depended on the direction and the extent of compression. This result suggests that the applied force is transmitted into the interior of the mitotic spindle, probably via astral microtubules linked to the cell membrane, and to the kinetochores that link the chromosomes to the spindle. This implies that the internal force balance existing within this supramolecular complex, composed of a mitotic spindle and astral microtubules, is dynamically altered by the applied mechanical force and reaches a new state of mechanical balance.

Our technique examines the effects produced by the applied mechanical force without direct inhibition of the essential molecular components. Therefore, it should greatly contribute to our understanding of the fundamental links between the determinant of anaphase onset by cell-cycle regulatory molecules and the impact of mechanical forces on the essential molecules (33). It is generally accepted that degradation of mitotic proteins, such as cyclin B and securin, is completed prior to the transition from metaphase to anaphase (30, 34). During metaphase, APC/C must be activated to promote both the degradation of cyclin B1 and the loss of sister chromatid cohesion, which determine timing of the chromosome segregation. Therefore, the acceleration or deceleration of metaphase progression that we observed could be correlated with the APC/C activity coupled with the SAC. We detected the mechanochemical regulation of mitotic processes through the tension-sensitive cellular reactions depending on the direction of compression. Specifically, the deceleration of the degradation kinetics of cyclin B1, which was induced by the mechanical impulses in the tension-decreasing direction, is attributable to the activation of the SAC manifested by the BubR1 localization. Recent studies suggested that the intercentromere tension that we analyzed does not correlate with the intrakinetochores tension, which has an impact on the SAC function (14, 29, 35). Although a decrease in tension induces loss of kinetochore microtubules (36), the impulsive force did not induce detectable Mad2 localization, implying that the intrakinetochores tension, but not the microtubule attachment, might be affected. Therefore, these results provide experimental evidence suggesting that in human cells the SAC can directly respond to tension. In addition to the SAC-mediated inhibition of separase, the protease that breaks the linkages between sister chromatids (25), the delay in cyclin B1 degradation after the mechanical impulse may explain, at least partly, the associated delay in anaphase onset, since cyclin B directly inhibits separase (37). These results suggest that the transient reduction of tension within the mitotic spindle induces activation of the SAC, which hinders degradation of mitotic proteins and delays the timing of chromosome segregation (Fig. 4D).

On the other hand, although our findings accord well with the classic grasshopper experiments (15), it still remains debatable how the mechanical impulse can accelerate the timing of chromosome segregation. Tension stabilizes the attachment between kinetochore and microtubules (36, 38), thus, a transient increase in tension did not activate the SAC. Furthermore, the degradation kinetics of cyclin B1 in the compressed and control cells were indistinguishable (Fig. S6C), indicating that the APC/C activity was not altered by the mechanical impulses in the tension-increasing direction. Although it is conceivable that the tension-increasing force somehow causes an increase in separase activity independent of the levels of cyclin B1, we favor the interpretation that a mechanical impulse physically cleaves a part of the sister chromatid cohesion, equivalent to that manifested by the remaining

cyclin B1 (Fig. 4D, *Breakage of Cohesion*). At the same time, it does not modulate separase activity, so the cohesion reaches the threshold for chromosome segregation before the cyclin B1 is fully degraded (Fig. 4D). Consistent with this idea, the mechanical impulse alone could trigger chromosome segregation in cells, even when the degradation of mitotic proteins was inhibited by a proteasome inhibitor, MG132 (Fig. S7; Movie S7). Thus, the mechanical impulse can affect the timing of chromosome segregation independent of the changes in cyclin B1 levels. These results imply that the mechanical perturbation could add to the tension imposed on each cohesin complex, so that the effects of external force applied just before anaphase onset are synergistically enhanced as the number of cohesin complexes is decreased by separase activity. If this is the case, the applied force even in the tension-decreasing (0°) direction may have a similar effect. In practice, chromosome segregation was induced even in the presence of MG132 by the tension-decreasing compression (although only in 2 of 31 observations; Fig. S7 A and B). This may be the reason why the decelerating effect of the tension-decreasing mechanical impulse was not so evident compared with the accelerating effect of the tension-increasing mechanical impulse, in spite of the fact that the intercentromere distance changed in a mirror-image fashion during these two types of compression (Fig. 2). Further quantitative analysis will have significant implications for such asymmetric responses.

Physical forces generated inside or outside of the cells are transmitted through the cytoskeleton and the mechano-sensing proteins (mechano-sensors), affecting local mechanical properties and cellular behavior (motility, orientation, cell division, etc.) (1, 2, 39). The present results suggest that brief physical (mechanical) perturbation can directly control cellular functions

involved in epigenetic alternations that take place over substantially longer time scales.

Materials and Methods

The controlled mechanical perturbation of HeLa cells was performed using a dual cantilever-based system coupled with fluorescence microscopic imaging (21). The timeline of an experiment is shown in Fig. 3A. The impulsive mechanical perturbation (compression-release) of different magnitudes was applied to the cell from various directions, by displacing one of the cantilevers, within approximately 0.5 min after the metaphase cell was found. The velocity of the cantilever movement controlled by the piezo actuator was 100 $\mu\text{m/s}$, unless stated otherwise. By using a combination of a stiff and a flexible cantilever we measured the force applied to the cell by a few μm compression; the flexural rigidity of the flexible cantilever was 5–10 nN/ μm (20). The direction of mechanical impulse is defined as the angle between the flat surface of cantilevers and a long axis of the chromosome array (metaphase plate), which was measured from a fluorescent image of chromosomes obtained just before the mechanical perturbation. Next, the time-lapse images of chromosome dynamics were recorded either until the chromosomes had sufficiently separated or for 30 min, if the segregation did not occur. Chromosome images were analyzed to determine anaphase onset time, defined as the time at which chromosome segregation started. Mitotic spindle expressing EGFP-EB1, centromeres expressing EGFP-CENP-A, and spindle checkpoint proteins expressing either EGFP-Mad2 (40) or EGFP-BubR1 were observed inside cells by confocal microscopy. The kinetics of the degradation of mitotic proteins were examined in HeLa cells expressing mCherry-cyclin B1 protein (29) (SI Materials and Methods).

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