Supporting Information

Itabashi et al. 10.1073/pnas.1116749109

SI Materials and Methods.

Cell Cultures. HeLa cells stably expressing EGFP-tagged histone H2B, EGFP-CENP-A, EGFP-E1B, mCherry-Cyclin B1 (1) (kind gift from T. Hirono) or EGFP-Mad2 (2) (kind gift from T. M. Kapoor) were grown in Dulbecco’s modified Eagle’s medium (Nissui Pharmaceuticals) supplemented with 10% fetal bovine serum (FBS) and L-glutamine (final concentration 2 mM) (Invitrogen) at 37°C in 5% CO2. EGFP-BubR1 for the analysis of the spindle assembly checkpoint was transfected into HeLa cells using Lipofectamine LTX (Invitrogen). For the micromanipulation experiments, cells were plated on 35-mm glass-bottom dishes (Iwaki). The medium was exchanged to CO2-independent Leibovitz’s L-15 medium including 10% FBS at least 24 h prior to the measurements. In the experiments with nocodazole, cells were treated with low concentration of nocodazole (5 ng/mL, Sigma-Aldrich). For inhibiting cell progression to anaphase, a proteasome inhibitor MG132 (Sigma-Aldrich) was added (final concentration 1 μM) 1.5 h before the experiment. For the analysis of mCherry-cyclin B1 degradation and the SAC using EGFP-Mad2 or EGFP-BubR1, chromosomes were stained with 0.1 μg/mL Hoechst 33342 (Dojindo).

Mechanical Perturbation, Live Cell Imaging, and Analysis. A glass-base dish was placed on the microscope stage, the temperature of which was maintained at 37°C by the stage heater (Tokai Hit) and the dish was placed on the microscope stage, the temperature of which was maintained at 37°C by the stage heater (Tokai Hit). To measure the applied force during smaller compression, we replaced one of the stiff cantilevers with a flexible one and compressed a cell by displacing the stiff cantilever controlled by the piezo actuator (Fig. S1). The mechanical perturbation was completed within 0.5 min after finding a metaphase cell. Then, the time-lapse images of the chromosome dynamics were recorded, either until the chromosomes had sufficiently separated or for 30 min if the segregation did not occur.

Live cell imaging of EGFP-H2B and mCherry-Cyclin B1 cells was performed with iXon™ + DU-888 back-illuminated EMCCD (Andor Technology) on an inverted microscope (IX70; Olympus) equipped with an objective heater (Bioptechs). For the imaging of EGFP-E1B, EGFP-CENP-A, EGFP-Mad2, and EGFP-BubR1 (Figs. 1 and 2 and Fig. S5), a spinning-disk confocal unit (CSU-10) was combined with the above microscopy setup. Images were collected at 0.15–0.23 s intervals during a mechanical perturbation, followed by the time-lapse imaging at 5-s intervals, using the Andor iQ software (Andor Technology).

The distance between sister centromeres that remained in focus before and during the mechanical perturbation was analyzed by ImageJ software (http://rsb.info.nih.gov/ij/) by measuring the distance between two focal centers of EGFP-marked centromeres. Chromosome images were analyzed to determine the time of anaphase onset (TAO), which corresponds to the time at which chromosome segregation begins. Here the time moment 0 (t = 0) was defined as the moment at which the suitable metaphase cell was found under the microscope. To calculate the average time of anaphase onset under various conditions, for “undivided cells,” i.e., those that did not divide within 30 min, it was set to be 30 min. The direction of mechanical perturbation was defined as the angle between the flat surface of cantilevers and the long axis of chromosomes array (metaphase plate), which was measured from a chromosome image obtained just before the mechanical perturbation (Fig. 1B and C).

Paired student’s t-tests were used to examine statistical difference of intercentromere distance before and during the application of a mechanical impulse (Fig. 2 and Fig. S4G). The statistical significance of the time of anaphase onset obtained under each experimental condition was compared using the Mann-Whitney test (Figs. 3 and 4 and Figs. S3, S4, S6, and S7).


Fig. S1. Displacement of a stiff cantilever to produce the fast compression-fast release mechanical impulse. (A and B) Velocity of the stiff cantilever was controlled by piezo actuator and fixed at 100 μm/s. The displacement was 5 μm (A) or 8 μm (B). A cell was sandwiched between a set of a flexible and a stiff cantilevers (A) or a pair of stiff cantilevers (B). In the former case, the flexible cantilever deflected by approximately 2 μm, and the cells were compressed by approximately 3 μm. (C) Images of a cell (bright-field micrographs) and chromosomes (fluorescence micrographs) before, during and after the compression. In the bright-field images, the black region on the right is a part of a stiff cantilever (S), and the small black region on the left, attached to a cell, is a flexible cantilever (F). Left Cell’s morphological changes. Right Changes in the chromosome position. The cells in the left and the right columns were different. Scale bar, 5 μm.

Fig. S2. The changes in the intercentromere distance induced by mechanical impulses. (A and B) Relationship between the intercentromere distance before and during the 8-μm (A) or 5-μm (B) mechanical impulses (MI). The yellow stars indicate the average intercentromere distance. Gray areas correspond to a decrease in the intercentromere distance. Data for 8-μm impulse, 0°, \( p = 6 \times 10^{-5} \); 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°, \( p = 1 \times 10^{-6} \).
Fig. S3. Effects of the mechanical impulse on the transition from metaphase to anaphase. (A) Time courses of the changes in the chromosome separation distance in control cells. (B) Time courses of the changes in the chromosome separation distance after the 5-μm mechanical impulse in the cell sandwiched between a set of a flexible and a stiff cantilevers. (C–F) Percentage of cells undergoing chromosome segregation (Left), and histograms of the time of anaphase onset (Right) after 5-μm (C and D) or 8-μm (E and F) mechanical impulses. Data for the 45° direction are shown in (C and E). Total data for all directions are shown in (D and F). Yellow bars show cells that reached anaphase within approximately 1 min after the application of mechanical impulse. Black bars show cells in which chromosome segregation did not occur within 30 min. The average time of anaphase onset (mean ± SEM; “undivided cells” are included) is indicated. Data for the approximately 3-μm compression, 45° versus control, 45° versus 0°, 45° versus 90°, p = 0.05. Data for the 8-μm compression, control versus 45°, p = 0.94; 45° versus 0°, p = 0.59; 45° versus 90°, p = 0.005. (G) Percentage of cells undergoing chromosome segregation within 30 min. Compressed metaphase cells (n = 118) and compressed prometaphase cells (n = 8) were examined. Impulsive mechanical perturbation with 5-μm amplitude at 100 μm/s (fast compression-fast release as in Fig. S1) was applied to the cells, which were sandwiched between the flexible and the stiff cantilevers. Direction of the mechanical impulse to prometaphase cells was not identified due to an incomplete formation of the metaphase plate. For the directions in metaphase cells, see Fig. 3 E and F and Fig. S3C.
Fig. S4. Effects of the slower compression or release. (A) Time course of the displacement of stiff cantilever during the fast compression-slow release mechanical impulse. Velocity of the stiff cantilever was controlled by piezo actuator and set at 100 μm/s for fast compression and 1 μm/s for slow release. (B and C) Histograms showing the distribution of the time of anaphase onset in the cells \( (n = 107) \), to which the fast compression-slow release mechanical impulse was applied after sandwiching a cell between a set of a flexible and a stiff cantilevers. Total data for all directions are shown in B, and data for the 0°, 45°, and 90° directions are shown in C. Yellow bars show cells that reached anaphase within approximately 1 min after the application of mechanical impulse. Black bars show cells in which chromosome segregation did not occur within 30 min. The average time of anaphase onset (mean ± SEM; “undivided cells” are included) and the percentage of cells undergoing chromosome segregation within 30 min are indicated in each histogram. 0° versus control, \( p = 0.23 \); 0° versus 45°, \( p = 0.76; 0° \) versus 90°, \( p = 0.08 \); 45° versus control, \( p = 0.15 \); 45° versus 90°, \( p = 0.04 \); 90° versus control, \( p = 0.19 \). (D) Time course of the displacement of a stiff cantilever during the slow compression-fast release mechanical impulse. Velocity of the stiff cantilever was controlled by piezo actuator and set at 100 μm/s for slow compression and 1 μm/s for fast release. (E and F) Histograms showing the distribution of the time of anaphase onset in cells \( (n = 110) \), to which the slow compression-fast release mechanical impulse was applied after sandwiching a cell between a set of a flexible and a stiff cantilevers. Total data for all directions are shown in E, and data for the 0°, 45°, and 90° directions are shown in F. Yellow bars show cells that reached anaphase within approximately 1 min after the application of mechanical impulse. Black bars show cells in which chromosome segregation did not occur within 30 min. The average time of anaphase onset (mean ± SEM; “undivided cells” are included) and the percentage of cells undergoing chromosome segregation within 30 min are indicated in each histogram. 0° versus control, \( p = 0.58 \); 0° versus 45°, \( p = 0.64 \); 0° versus 90°, \( p = 0.76 \); 45° versus control, \( p = 0.88 \); 45° versus 90°, \( p = 0.34 \); 90° versus control, \( p = 0.33 \). (G) The changes in intercentromere distance induced by the 5-μm slow compression-fast release mechanical impulse (0° direction, \( n = 70 \) pairs of centromeres from 20 cells; 45° direction, \( n = 68 \) pairs of centromeres from 25 cells; 90° direction, \( n = 74 \) pairs of centromeres from 27 cells). Top Relationship between the intercentromere distance before and during mechanical impulse. The yellow stars indicate the average intercentromere distance. Bottom Distribution of the difference in the intercentromere distance before and during a mechanical impulse. The average change (mean ± SD) is shown by black vertical arrows. Gray areas correspond to a decrease in the intercentromere distances. * \( p < 0.05 \) (Distributions of the intercentromere distances before and during MI were compared.) 0°, \( p = 0.03 \); 45°, \( p = 0.93 \); 90°, \( p = 0.83 \).
Mechanical impulse in the 0° direction induced the accumulation of EGFP-BubR1 (indicated by yellow arrows) at kinetochores (A) (n = 13). No noticeable accumulation of EGFP-BubR1 was detected after the mechanical impulse in the 90° direction (B) (n = 14). (C–E) HeLa cells stably expressing EGFP-Mad2 were imaged with a spinning-disk confocal fluorescence microscope. In each image, the time after the application of directional 8-μm mechanical impulse (C and D) or after the addition of nocodazole (E) is shown (min:sec). Scale bars, 5 μm. No significant accumulation of EGFP-Mad2 was detected after the application of mechanical impulse in either direction (C and D) (0° direction, n = 16; 90° direction, n = 16). The addition of nocodazole induced the accumulation of EGFP-Mad2 at the kinetochores (E).

Mechanochemical regulation of cyclin B1 degradation. (A) Time courses of the change in the fluorescence intensity of mCherry-cyclin B1 with/without the application of an 8-μm mechanical impulse to the cell sandwiched between two stiff cantilevers. Yellow dots show the timing of chromosome segregation; its distribution is shown in the histogram above, where the “undivided cells” are included in the calculated average time of anaphase onset (mean ± SEM). 0° versus control, p = 0.10; 0° versus 45°, p = 0.30; 0° versus 90°, p = 0.01; 45° versus control, p = 0.36; 45° versus 90°, p = 0.05; 90° versus control, p = 0.07. (B) Time courses of the change in the fluorescence intensity (F. I.) of mCherry-Cyclin B1 after the 8-μm mechanical impulse in the 45° direction applied to the cell sandwiched between two stiff cantilevers. *p < 0.01 compared with the control. Data for initial F.I., 45° versus control, p = 0.11; 45° versus 0°, p = 0.41; 45° versus 90°, p = 0.51. Data for F.I. at anaphase onset, 45° versus control, p = 0.004; 45° versus 0°, p = 0.10; 45° versus 90°, p = 0.95. (C) Time course of the average degradation rate (A.U./min) of mCherry-cyclin B1 in control (black circles) and compressed (0°, blue circles; 45°, green circles; 90°, red circles) cells. Time moment 0 (t = 0) is the time of anaphase onset (TAO). Error bars show the width (SD) of the distribution of average degradation rates obtained at each time point. The average degradation rate is the average of the distribution of degradation slopes, which is a rate of change in the fluorescence intensity [=(Df1)/(Dt); Dt = 2 min] estimated for each cell.
Mechanical impulse triggers chromosome segregation in metaphase-arrested cells. (A) Time courses of the change in the fluorescence intensity (F.I.) of mCherry-cyclin B1 in the control (n = 38) and compressed (n = 32) cells in the presence of MG132. The treatment with MG132 abolished the degradation of mCherry-cyclin B1 by APC/C-dependent proteolysis, so the cells remained arrested in metaphase. Then, the mechanical 8-μm impulse was applied to a cell sandwiched between two stiff cantilevers. Yellow dots show the timing of chromosome segregation, which occurred in none out of 38 control cells and in 5 out of 32 compressed cells. Histograms (Left) show the distribution of fluorescence intensity of mCherry-cyclin B1 at the start of observation. The average fluorescence intensity (A.U.) of mCherry-cyclin B1 is shown in histograms. Data for initial F.I., control versus compressed, p = 0.89. (B) Percentage of cells undergoing chromosome segregation within 30 min in the control MG132-treated cells (n = 93; Left) and the compressed MG132-treated cells (n = 86), to which the fast compression-fast release 8-μm mechanical impulse was applied (Right). The number of compressed cells undergoing chromosome segregation was 2 out of 31 for 0° direction, 4 out of 28 for 45° direction, and 7 out of 27 for 90° direction. (C) Sequential images of a compressed MG132-treated HeLa cell. Chromosome segregation began at 3.84 min (TAO), but the cleavage furrow ingression was not observed even after the chromosomes had sufficiently separated. Mis-segregated chromosomes are indicated by an arrowhead. In each image, the time after the cell was found under the microscope is shown (min:sec). Scale bar, 5 μm.

Movie S1. Changes in the structure of a mitotic spindle by mechanical perturbation in the 0° direction. The 8-μm mechanical impulse in the 0° direction was applied to a HeLa cell expressing EGFP-EB1, which was sandwiched between a pair of stiff cantilevers. Scale bar, 5 μm.

Movie S1 (MOV)
Movie S2. Changes in the structure of a mitotic spindle by mechanical perturbation in the 90° direction. The 8-µm mechanical impulse in the 90° direction was applied to a HeLa cell expressing EGFP-EB1, which was sandwiched between a pair of stiff cantilevers. Scale bar, 5 µm.

Movie S2 (MOV)

Movie S3. Changes in the intercentromere distance after mechanical perturbation in the 0° direction. The 8-µm mechanical impulse in the 0° direction was applied to a HeLa cell expressing EGFP-CENP-A, which was sandwiched between a pair of stiff cantilevers. Arrows show the timing and the direction of the mechanical impulse. Scale bar, 1 µm.

Movie S3 (MOV)

Movie S4. Changes in the intercentromere distance after mechanical perturbation in the 90° direction. The 8-µm mechanical impulse in the 90° direction was applied to a HeLa cell expressing EGFP-CENP-A, which was sandwiched between a pair of stiff cantilevers. Arrows show the timing and the direction of the mechanical impulse. Scale bar, 1 µm.

Movie S4 (MOV)
Movie S5. Small mechanical perturbation of a metaphase cell. The mechanical impulse with 5-μm amplitude was applied to a HeLa cell expressing EGFP-histone H2B, which was sandwiched between the set of a flexible and a stiff cantilevers. Scale bar, 5 μm.

Movie S5 (MOV)

Movie S6. Large mechanical perturbation of a metaphase cell. The mechanical impulse of an 8-μm amplitude was applied to a HeLa cell expressing EGFP-histone H2B, which was sandwiched between a pair of stiff cantilevers. Scale bar, 5 μm.

Movie S6 (MOV)

Movie S7. Induction of chromosome segregation by the mechanical impulse in an MG132-treated cell. After the cell expressing EGFP-histone H2B was treated with MG132 for 1.5 h, the 8-μm mechanical impulse was applied to a cell sandwiched between a pair of stiff cantilevers. Chromosome segregation was triggered by the mechanical impulse, but the cell failed to achieve cytokinesis. Scale bar, 5 μm.

Movie S7 (MOV)
Table S1. Summary of effects produced by both symmetrical and asymmetrical 5-μm mechanical impulses (approximately 3 μm compression)

<table>
<thead>
<tr>
<th>Direction of compression</th>
<th>TAO (min)</th>
<th>% Cells</th>
<th>TAO (min)</th>
<th>% Cells</th>
<th>TAO (min)</th>
<th>% Cells</th>
<th>TAO (min)</th>
<th>% Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast compression-fast release</td>
<td>12.16 ± 0.84</td>
<td>90.7 (n = 118)</td>
<td>14.69 ± 1.67</td>
<td>82.9 (n = 35)</td>
<td>11.77 ± 1.32</td>
<td>93.5 (n = 46)</td>
<td>10.27 ± 1.35</td>
<td>94.6 (n = 37)</td>
</tr>
<tr>
<td>Fast compression-slow release</td>
<td>14.19 ± 0.98</td>
<td>80.4 (n = 107)</td>
<td>15.30 ± 1.62</td>
<td>77.5 (n = 40)</td>
<td>16.12 ± 1.84</td>
<td>72.7 (n = 33)</td>
<td>11.04 ± 1.52</td>
<td>91.2 (n = 34)</td>
</tr>
<tr>
<td>Slow compression-fast release</td>
<td>12.62 ± 0.94</td>
<td>84.5 (n = 110)</td>
<td>12.58 ± 1.59</td>
<td>85.0 (n = 40)</td>
<td>13.31 ± 1.61</td>
<td>84.8 (n = 33)</td>
<td>12.06 ± 1.69</td>
<td>83.8 (n = 37)</td>
</tr>
<tr>
<td>Control</td>
<td>13.01 ± 0.84</td>
<td>89.3 (n = 112)</td>
<td>-</td>
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</table>

Data for the average time of anaphase onset (TAO) (mean ± SEM) and the percentage of cells undergoing anaphase (% cells) are shown.