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Nuclear and Division-Plane Positioning Revealed by Optical Micromanipulation

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Summary

The position of the division plane affects cell shape and size, as well as tissue organization. Cells of the fission yeast *Schizosaccharomyces pombe* have a centrally placed nucleus and divide by fission at the cell center. Microtubules (MTs) are required for the central position of the nucleus [1–4]. Genetic studies lead to the hypothesis that the position of the nucleus may determine the position of the division plane [5–10]. Alternatively, the division plane may be positioned by the spindle or by morphogen gradients or reaction diffusion mechanisms [7, 11]. Here, we investigate the role of MTs in nuclear positioning and the role of the nucleus in division-plane positioning by displacing the nucleus with optical tweezers. A displaced nucleus returned to the cell center by MT pushing against the cell tips. Nuclear displacement during interphase or early prophase resulted in asymmetric cell division, whereas displacement during prometaphase resulted in symmetric division as in unmanipulated cells. These results suggest that the division plane is specified by the predividing nucleus. Because the yeast nucleus is centered by MTs during interphase but not in mitosis, we hypothesize that the establishment of the division plane at the beginning of mitosis is an optimal mechanism for accurate symmetric division in these cells.

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Results and Discussion

Displacing the Nucleus with Optical Tweezers

A direct way to study nuclear and division-plane positioning employs mechanical perturbation of the nuclear position. We displaced the nucleus with optical tweezers by trapping a granule naturally present in the cytoplasm [12] and using it as a handle to push the nucleus (Figures 1A and 1B; see Movie S1 in the Supplemental Data available with this article online). Without inducing significant damage or heating of the cell [13], optical manipulations offer several advantages over methods, such as centrifugation, previously used to displace cell organelles [14]: (1) Organelles can be displaced selectively; (2) a single cell can be observed both before and after the manipulation—thus, the manipulation can be performed exactly at a chosen phase of the cell cycle; and (3) displacements as small as a few hundred nanometers can be detected because the state of the cell after the manipulation can be compared directly to that before the manipulation.

Microtubule Pushing Moves a Displaced Interphase Nucleus Back to the Cell Center

We assessed the mechanism of nuclear centering by moving the nucleus away from its natural central position with optical tweezers. We followed simultaneously the dynamics of MTs with tubulin tagged with the green fluorescent protein (GFP) [15] and the position of the nucleus and of the spindle pole body (SPB) by GFP-tagged chromodomain protein Swi6p, which gives a diffuse signal through the whole nucleus, and several fluorescent spots in the nucleus including the bright and motile centromere spot [16], which is associated with the SPB [17]. The cells were observed by a two-photon microscope [18, 19]. After the transient displacement of the nucleus, the MT number, length, and brightness remained similar to that before the manipulation in a subset of cells (n = 5 out of 15 cells), whereas other cells showed short and/or faint MTs. It is therefore possible that in some cells, MTs were affected by the micromanipulation. The displaced nucleus returned to the geometric cell center in all the cells with unaffected MTs, as well as, either partially or completely, in a few cells with affected MTs (~50% of all nuclei returned). We explored the positioning of the nucleus in cells with unaffected microtubules. In these cells, the nucleus was displaced by 0.5–0.9 μm, whereas natural changes in the position of the unmanipulated nuclei do not exceed 0.2 μm during a 10 min interval (n = 5). After nuclear displacement, the centromere/SPB returned to the geometric center of the cell within 5–15 min (Figure 1C). Subsequently, the SPB oscillated around the cell center, as in unmanipulated cells (n = 10 cells; Figure S1). The fast large movements of the SPB toward one cell end occurred when an MT extending from the SPB reached the opposite cell end and continued growing (Figure 1C; Movie S2). The direction of the SPB movement away from the tip touched by the microtubule

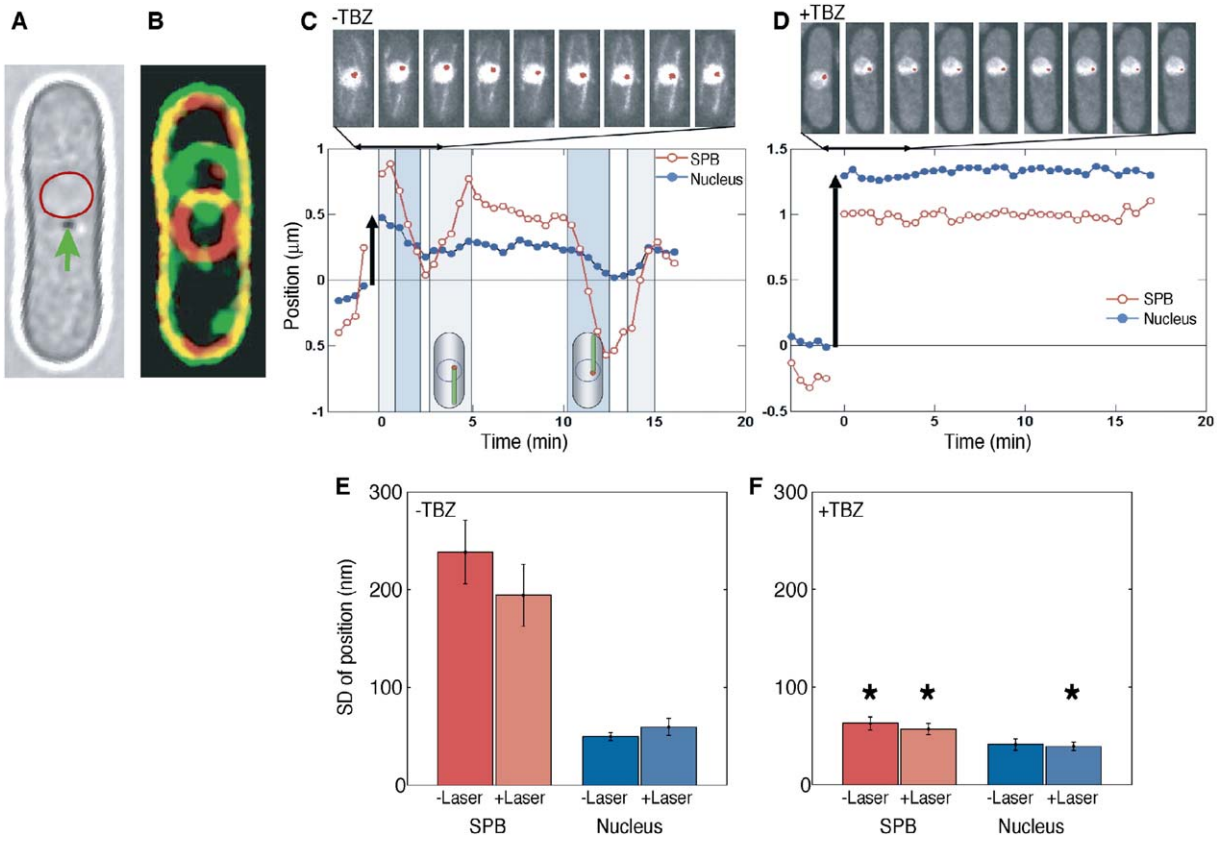

Figure 1. Displaced Interphase Nucleus Returns to the Cell Center by Microtubule Pushing

(A) For displacement of the nucleus, a granule (arrow) is optically trapped close to the nucleus (red circle) and used as a handle to push the nucleus.

(B) Superposition of two images of a single cell: before (red) and after (green) the manipulation of the nucleus. The nuclear envelope and cell membrane were marked with GFP.

(C) Top: A time-lapse sequence of images of a cell with GFP-tubulin and GFP-Swi6p (SPB is colored red). The nucleus was displaced after the first image. Bottom: Position of the center of the nucleus and the SPB along the major axis of the cell (zero represents the cell center), before and after the displacement (arrow). Periods in which one microtubule extends upward from the SPB (dark-blue areas) or downward (light-blue areas) correlate with the movement of the SPB downward and upward, respectively. The motion of the nucleus is correlated with that of the SPB. The error on each data point is ~50 nm; n = 5 cells.

(D) The displaced SPB and nucleus do not return to the cell center in cells treated with TBZ. Legend as in (C); n = 6.

(E and F) The SPB's (red) and nucleus's (blue) degrees of motion, measured as standard deviation (SD) of their position over 5 min intervals (10 data points). For each bar, 12–19 intervals from 4–6 cells were used. Micromanipulation did not significantly change the extent of motion (light bars, "+Laser," versus dark bars, "–Laser"), suggesting that the positioning mechanism was not affected. Disruption of microtubules by TBZ ([F] versus [E]) decreased the motion of the SPB and of the manipulated nucleus significantly (asterisk; $p < 5\%$, t test). The error bars represent the SEM.

suggests that the SPB is being pushed, as opposed to pulled, by the attached microtubule. The nucleus moved in a manner correlated with the movement of the SPB, typically with the SPB at the forward-facing side of the nucleus [20, 21]. These observations are consistent with the hypothesis that the SPB-attached MTs push against the cell ends and thus move the SPB, which leads the movement of the bulk of the nucleus.

The Displaced Nucleus Does Not Return to the Cell Center in Cells Treated with TBZ

We asked whether MTs are necessary for the movements of the SPB and the nucleus. In cells treated with the MT-depolymerizing agent thiabendazole (TBZ), the displaced nucleus and the SPB did not return to the cell center within the time course of the experiments (n = 6 out of 6 cells; Figure 1D; Movie S3). The excursions of the SPB decreased ~3.5 times after disruption of MTs, both in manipulated and unmanipulated cells (n = 4–6 cells in each group; Figures 1E and 1F). The movements of the nucleus also decreased significantly after MT disruption in manipulated cells. The decrease was not significant in unmanipulated cells, most likely because the nuclear motion is minimal in those cells because the nucleus is in its equilibrium position at the cell center. Manipulation of the nucleus did not significantly change the extent of nuclear motion. This is consistent with the MTs moving the nucleus by the same amount during short periods of time (5 min), irrespective of whether the nucleus had been displaced by the

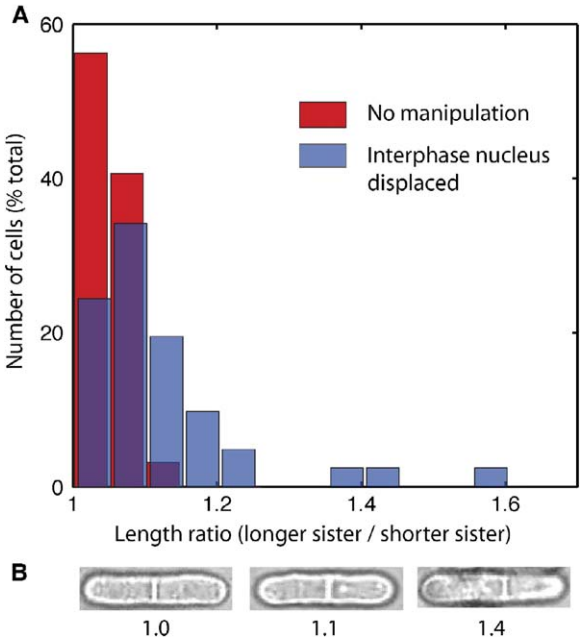

Figure 2. Displacement of the Nucleus during Interphase Can Result in an Asymmetric Cell Division

(A) Histogram of the length ratio of the longer and shorter sister cell in intact (red, n = 32) and micromanipulated cells (blue, n = 41). Intact cells divide symmetrically, whereas some micromanipulated cells show asymmetric division.

(B) Examples of septating cells with various length ratios.

laser (by a moderate distance of up to 1 μm) or not, thus suggesting that the MT-based positioning mechanism was not affected by the laser manipulation. We conclude that MTs are required for large excursions of the SPB, as well as for the return of the displaced SPB and the nucleus toward the cell center. Our data provide direct evidence in favor of the model of nuclear centering by MT pushing forces, which are most likely generated by MT polymerization at the cell ends [4].

Displacement of the Nucleus during Interphase Can Result in an Asymmetric Cell Division

Next, we investigated the spatiotemporal coordination of nuclear events (chromosome segregation) and cortical activities (cytokinesis). Is the spatial cue for the division site provided by the position of the nucleus? If the division site is established by a signal from the nucleus at a defined period of the cell cycle, then displacing the nucleus at an earlier time would result in a corresponding displacement of the division plane, whereas a later displacement would not affect the normal position of the division plane at the cell center. To study the effect of the displacement of the nucleus during interphase, we took advantage of the fact that in ~50% of the cells, the displaced nucleus did not return to the cell center. Intact cells divide symmetrically: 97% of the cells (n = 32) produce two equally long daughter cells, within a 10% difference. When the cells with optically manipulated nuclei divided, the two daughter cells were equally long in only 59% of the cases (n = 41; Figure 2). The distribution of the length ratio of the two sister cells was significantly different between the group of unmanipulated and the group of manipulated cells (p = 0.0024 with a Kolmogorov-Smirnov [K-S] test for comparing the distribution of two samples [22]; t test was not appropriate because both distributions were non-normal). This result supports the correlation between the position of the premitotic nucleus and the position of the division plane.

Cells with the Spindle Displaced at Prometaphase Divide at the Cell Center

In contrast to interphase, the nuclei displaced during mitosis did not return spontaneously to their normal location at the cell center (n = 26 out of 26 nuclei displaced by more than 0.5 μm). This can be explained by the absence of MTs, which could center the nucleus along the long cell axis: Cytoplasmic MTs are absent during prometaphase [23], whereas in anaphase, astral MTs center the nucleus along the short cell axis [24]. The cells with spindles displaced in prometaphase divided symmetrically, as do unmanipulated cells (within a 10% difference; n = 15 out of 15 manipulated cells; Figures 3A, 3B, and 3F). Indeed, the hypothesis that the values of the sister cells' length ratio in unmanipulated cells (n = 32) and in cells manipulated in prometaphase (n = 15) are drawn from the same distribution could not be rejected (p = 0.24, K-S test). This result suggests that the division site is determined before prometaphase.

Cells with the Spindle Displaced at the Time of SPB Separation Can Divide Off-Center

When the manipulation was performed in early prophase, at the time of SPB separation, the cells divided either close to the cell center (n = 8 out of 15 cells) or close to the new position of the spindle after displacement (n = 7 out of 15 cells; Figures 3C–3E). The distribution of the distance between the cell center and the division plane of the cells micromanipulated in early prophase was significantly different from that of the cells manipulated in prometaphase (p = 0.0011, K-S test). We conclude that the division plane is specified by the position of the nucleus simultaneously with SPB separation in early prophase. Alternatively, the division plane may be positioned by an MT-based mechanism acting independently of the nucleus position, which might have been disturbed by the laser treatment. This possibility is unlikely because the interphase MTs are to a large extent disassembled by the time of SPB separation. Our observations are related to the studies of M. Girbardt, who showed that displacement of the nucleus with a needle in another fungus, *Trametes versicolor*, resulted in a corresponding displacement of the septum if the nucleus was moved before prometaphase, but if the nucleus was moved after this stage, the septum formed where the nucleus was during early mitosis [25].

Proteins such as Mid1p, Pom1p, and Plo1p were hypothesized to link the cell division site to the nuclear position [5, 6, 8, 9]. Mid1p migrates from the nucleus to a broad cortical band overlying the nucleus. The transfer of Mid1p starts more than 90 min before mitosis and ends 2 min after SPB separation [26]. We propose that

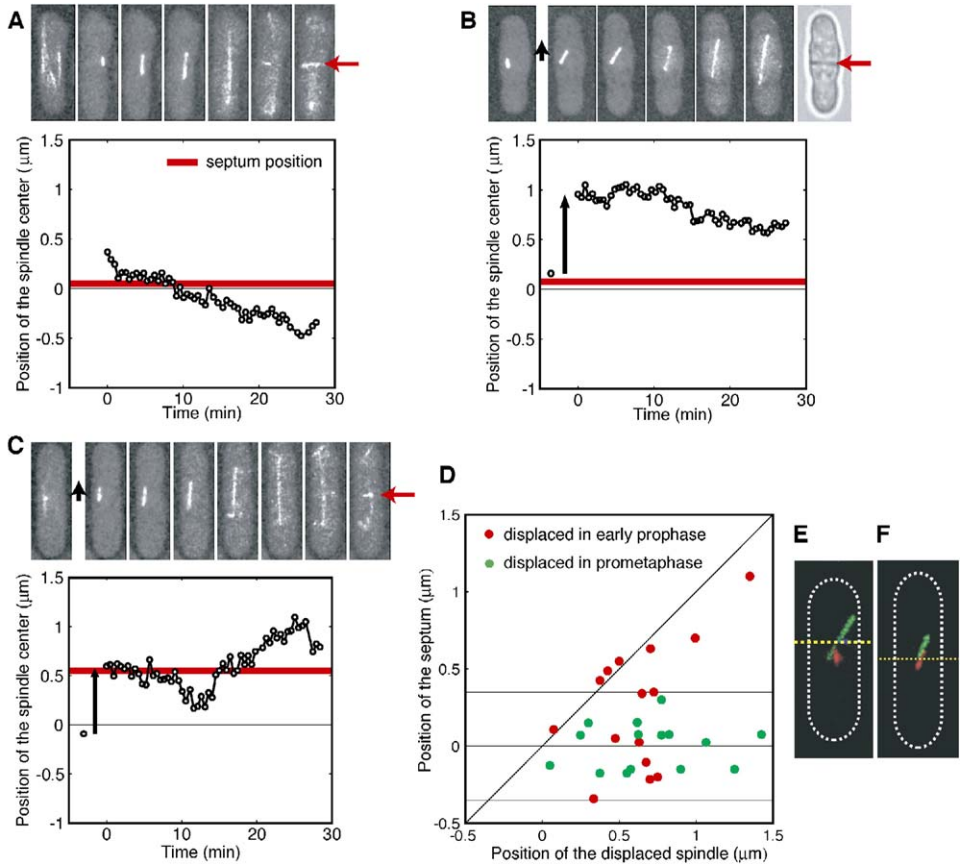

Figure 3. Division Plane Is Determined in Early Prophase

(A) Control cell in which trapping was performed without a displacement of the nucleus. Top: Images of the dividing cell with GFP-tagged tubulin, separated by 10 min, are shown. The cell divides at the center (red arrow points to the equatorial MT-organizing center, which coincides with the septum position [29, 30]). Bottom: The position of the spindle center with respect to the cell center (zero) is shown as a function of time. The red line marks the position of the division plane.

(B) A cell with the spindle displaced at prometaphase (black arrows) divides at the cell center (red arrow points to the septum in a bright-field image).

(C) A cell with the spindle displaced at the time of SPB separation (black arrows) divides off-center.

(D) Position of the division plane versus the position of the spindle center after the displacement of the nucleus. The spindles displaced in prometaphase (spindle length 1–2.5 μm; green) divide within 350 nm (horizontal lines) away from the geometric cell center, equivalent to a ~10% variation of the sister-cell length (n = 15/15). The spindles displaced early in prophase at the time of SPB separation (red) divide either close to the cell center (n = 8/15) or close to the position of the spindle after displacement (n = 7/15).

(E and F) Each panel is a superposition of two images of a single cell: immediately before (red) and after (green) the manipulation of the nucleus. (E) A cell with a nucleus displaced in early prophase divided off-center (yellow line). (F) A cell with a nucleus displaced in prometaphase divided at the center.

upon completion of Mid1p transfer, the position of the broad band is fixed at the cell cortex. Compaction of the broad band into a tight ring determines the final position of the division plane. It is possible that among the cells in which the nucleus was displaced in early prophase, Mid1p export finished earlier in the cells that divided symmetrically than in those that divided asymmetrically. Correlation between the position of the nucleus, the Mid1p cortical domain, and the septum could be tested by optical micromanipulation in cells expressing fluorescently labeled Mid1p. Furthermore, displacing the nucleus in cells with a variety of labeled contractile-ring proteins should provide new mechanistic details of the exact sequence of events that lead to specification of the division plane.

Determination of the Division Plane by the Predividing Nucleus May Be an Optimal Mechanism for Fission-Yeast Fission

Successful cell division requires that the division plane intersect the spindle axis, so that the daughter nuclei are found on opposite sides of the division plane. Although fission yeast and higher eukaryotes share the main molecular mechanisms of cytokinesis, the relative timing of the spindle positioning and the division-plane selection is different, as well as the signal that determines the division-plane position. Here, we have shown directly that the division-plane selection in fission yeast occurs before spindle formation, by the position of the nucleus. In animal cells, on the contrary, the spindle is positioned first, and the division plane is selected later

by a signal from the spindle [27, 28]. Because the yeast nucleus is centered by microtubules during interphase but not in mitosis, the establishment of the division plane at the beginning of mitosis may be an optimal mechanism for accurate symmetric division in these cells.

Supplemental Data

Supplemental Data including three movies, one figure, and detailed Experimental Procedures are available at http://www.current-biology.com/cgi/content/full/15/13/1212/DC1/.

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