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Meiotic Spindle Has a Soft Spot

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The spindle relies on forces exerted by microtubules and motor proteins to align and segregate chromosomes. In this issue of *Developmental Cell*, Takagi et al. (2019) show that meiotic spindle microtubules respond differently to forces at different spindle locations, depending on microtubule organization and motor proteins that crosslink them.

The spindle depends upon creating and maintaining dynamic arrangement of microtubule arrays in order to generate forces necessary for chromosome congression to the metaphase plate and segregation of chromatids in anaphase (Pavin and Tolić, 2016). Forces generated in the spindle, as well as external forces, can affect spindle length, shape, and overall stability (Dumont and Mitchison, 2009). How the spindle responds to forces while preserving its integrity is not well understood. To address this question, Takagi et al. (2019), in this issue of *Developmental Cell*, performed microneedle-based manipulations together with microtubule tracking on *Xenopus laevis* meiotic spindles. The approach based on microneedles to study forces in the spindle was pioneered by Nicklas in grasshopper spermatocytes (Nicklas, 1983) and later expanded by others (Gatlin et al., 2010; Shimamoto et al., 2011). Takagi et al. (2019) combined microneedle-based perturbations with simultaneous imaging and tracking of fluorescent tubulin speckles that label single microtubules to monitor their motion (Yang et al., 2007). They performed single and double microneedle assays in a systematic way, probing different parts of the spindle in order to investigate how individual microtubules respond to mechanical perturbation.

In the first set of experiments, a microneedle was inserted into the spindle and a sinusoidal force was applied along the longitudinal axis of the spindle (Figure 1A, top). This oscillatory force resulted in oscillatory movement of tubulin speckles, showing the movement of spindle microtubules. Speckles in the vicinity of the needle oscillated with the highest amplitude, whereas the oscillations diminished with distance from the needle. Analysis of speckle amplitudes at different distances from the needle, along the longitudinal and transversal spindle axis, provided information on microtubule coupling and spindle stiffness in that region. The authors inserted the microneedle in the pole region, at the equator and in the middle of the spindle half. Interestingly, the region of high amplitude of speckle movement was larger when the needle was inserted at the poles or the equator, whereas in the middle of the spindle half, the oscillations subsided closer to the needle (Figure 1A, compare middle and bottom). Using this approach, the authors concluded that the meiotic spindle is a heterogeneous structure consisting of microtubule arrays with different mechanical properties. At the spindle poles and the equator, microtubules are mechanically coupled, making these regions stiff, whereas in the region between the pole and the equator, the coupling is weaker, making this part more compliant.

In the second set of experiments, Takagi et al. (2019) explored how microtubules in different regions of the spindle react to spindle stretching. The authors inserted two microneedles into the spindle, one of which was fixed to pin down the spindle, while the other was moved away to stretch the spindle (Figure 1B, top). The spindle elongated by about 20% at a constant velocity, which was accompanied by the movement of tubulin speckles mostly parallel to the applied force. Remarkably, the stretch of the spindle was found to result from microtubules sliding mainly in the mechanically compliant regions between the poles and equator (Figure 1B, middle and bottom). Conversely, microtubules in the equatorial and polar regions resisted the pulling force, in agreement with single-microneedle experiments.

To explore the molecular mechanisms involved in the force response of spindle microtubules, the authors inhibited the activity of the motor proteins kinesin-5 and dynein. They found that microtubule coupling at the poles, where microtubules are organized in parallel arrays, is under influence of both kinesin-5 and dynein. Kinesin-5 was found to be crucial for the coupling of antiparallel microtubules within the equatorial region. The mechanical response of the middle part of the spindle half, where microtubules are mainly parallel, was unaffected by these inhibition experiments, indicating that other motor or non-motor crosslinking proteins regulate their mechanical properties. Upon inhibition of kinesin-5 and dynein, the force response of different spindle regions became more homogeneous, implying that the crosslinking of microtubules in the pole regions and at the equator is involved in maintaining the difference in force response. However, this difference was not completely abolished, suggesting a role of additional molecular players in generating heterogeneous mechanical properties of the spindle.

An intriguing question that remains is the biological role of the mechanical heterogeneity of the spindle. The role of the strong microtubule coupling at the pole may be to ensure tight pole focusing and thus spindle bipolarity. Strong coupling at the equator, on the other hand, may be important for keeping sister kinetochore fibers aligned with the longitudinal spindle axis, allowing only for subtle changes in their orientation when forces act on the spindle, which in turn may ensure robustness of the direction of forces driving kinetochore segregation. Finally, weakly coupled microtubules in the middle of the spindle half might serve as a cushion, allowing the spindle to change its size and shape while maintaining kinetochore alignment.

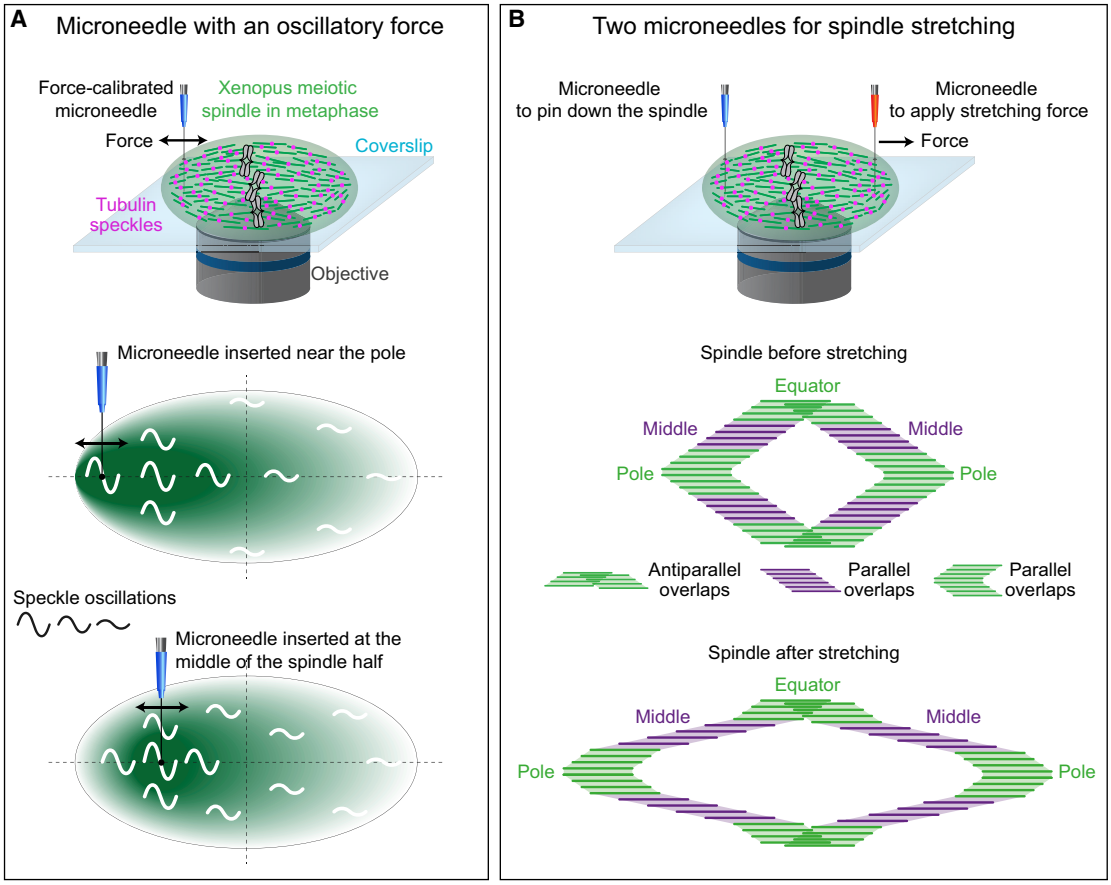

Figure 1. The Softest Part of the Spindle Is the Region between the Pole and Equator

(A) A microneedle is inserted into the spindle and a sinusoidal force is applied (top), resulting in sinusoidal movements of fluorescent speckles on microtubules (middle and bottom). High-amplitude speckle oscillations extend over a larger region around the needle when the needle is inserted at the spindle pole (middle) or at the equator, than in the middle of the spindle half (bottom).

(B) To stretch the spindle, two microneedles are inserted near the spindle poles, one of which is fixed while the other extends the spindle (top). During spindle stretching, the largest microtubule sliding occurs in the middle of the spindle half (middle and bottom).

It will be interesting to see whether a similar pattern of mechanical compliancy is present also in mitotic spindles. Recent work on mammalian mitotic spindles has shown mechanical coupling between kinetochore fibers and other spindle microtubules in the equatorial region of the spindle. In human cells, bundles of antiparallel microtubules termed bridging fibers link sister kinetochore fibers laterally in the form of a bridge (Kajtez et al., 2016). These fibers balance the tension on kinetochores during metaphase and provide tracks for kinetochore movements in anaphase as well as sliding forces that push sister kinetochore fibers apart (Vukušić et al., 2017). Similarly, crosslinks between kinetochore fibers and other spindle microtubules, which are important in load bearing, are present in PtK cells (Elting et al., 2017). These structures may have a role in reinforcing the microtubules in the vicinity of kinetochores and contribute to the mechanisms that enable the spindle to react to forces, while safeguarding correct chromosome segregation.

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