PNAS COMMENTARY

Spring-like behavior of cytoplasm holds the mitotic spindle in place

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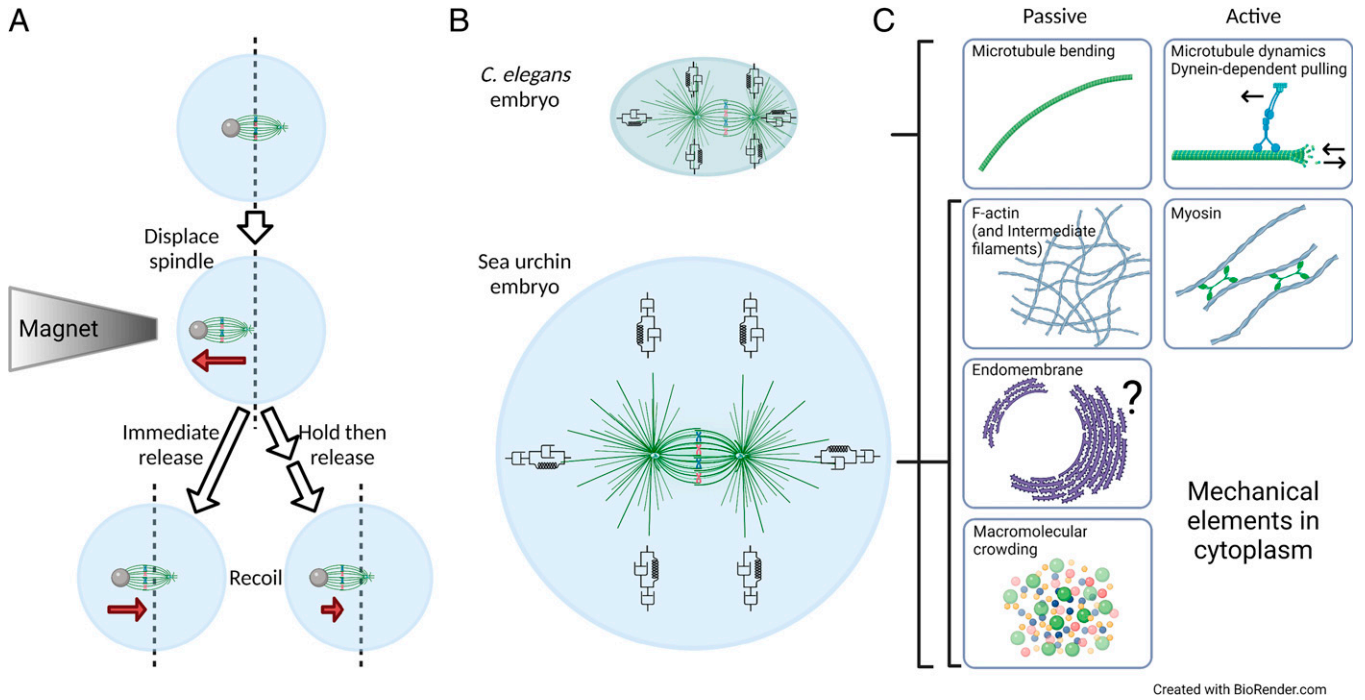

Fig. 1. Viscoelastic forces from bulk cytoplasm maintain the spindle position. (*A*) The experimental procedure for displacing mitotic spindles with magnetic tweezers. (*B*) Viscoelasticity modeled by spring–dashpot models. In small cells where microtubules touch the cortex, microtubules constitute the main elastic component. In large cells, in contrast, bulk cytoplasm generates viscoelastic restoring forces. (*C*) Cytoplasmic components implicated in force generation. Passive components resist perturbations, while active components generate force using chemical energy.

In cell biology textbooks, cytoplasm is often depicted as a homogeneous color that bathes the nucleus and organelles, leaving the impression that it consists of a liquid solution without structure or physical properties. In reality, cytoplasm is crowded with macromolecules (1) and organized by cytoskeletal networks (2), which endow it with both viscous and elastic properties (2), but the biological significance of these properties is often unclear. In PNAS, Xie et al. (3) use magnetic tweezers to displace the mitotic spindle in sea urchin embryos and find that it springs back when displaced. Remarkably, the elasticity that maintains spindle position does not depend on microtubules and only partly depends on actin, suggesting that the crowded nature of cytoplasm may contribute.

During cell division, eukaryotic cells assemble a mitotic spindle whose position determines cleavage geometry and sometimes, the developmental fate of daughter cells (4). In symmetric divisions, the spindle is positioned at the center of the cell. How it achieves this central location has been studied in multiple organisms. In most metazoans, the poles of the spindle are defined by radial arrays of microtubules called asters. Microtubules are nucleated at the aster center, and their growth is bounded by dynamic instability, leading to a maximum mitotic aster radius of ∼30 μm (5). If the cell radius is shorter than this, the spindle is positioned dynamically throughout mitosis by microtubules that touch the cortex, as in *Caenorhabditis elegans* embryos (6). If longer, as in *Xenopus* embryos, the spindle forms at a central location defined by microtubules in the preceding interphase (7, 8). In such large cells, the spindle has to remain in position throughout mitosis without connections to the cortex, and the mechanisms that keep it there have been unclear. The sea urchin embryos analyzed by Xie et al. (3) have a radius of ∼48 μm and a mitotic aster radius of ∼25 μm, so they are in the large cell regime and provide a system to probe the mechanisms used to maintain spindle location.

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To study the mechanics by which the spindle is held at the center of the cell, Xie et al. (3) inject magnetic beads into one-cell sea urchin embryos. The beads recruit dynein, which transport them to the aster centers, allowing the authors to exert calibrated forces on the poles of the spindle using a magnetic tweezer. The spindles behave as rigid objects that translocate or rotate in response to magnetic forces. The authors first measure displacement in response to constant pulling force and observe that velocity slows as displacement increases, which is characteristic of a viscoelastic response. When the force is removed, the spindles partially recoil back to their original location (Fig. 1*A*), also consistent with viscoelastic forces. When spindles are held off center for increasing times and then released, recoiling gradually decreases, which indicates dissipation of stored elastic energy over time as well as lack of dynamic repositioning (Fig. 1*A*). To test if restoring forces act specifically on spindles, Xie et al. (3) inject 30-μm oil droplets and again observe viscoelastic resistance to displacement. Interestingly, both displacement and recovery of oil droplets occur ∼10 times faster than spindles, suggesting that oil droplets feel much less viscous drag, for unknown reasons. The authors employ a Jeffreys model (9), with one spring and one dashpot in parallel and one dashpot in series, to describe viscoelastic properties of the cytoplasm (Fig. 1 *B*, *Lower*) and estimate a bulk elastic modulus of ∼0.3 Pa.

Magnetic tweezers have a long history as probes of cellular mechanics and rheology. As early as the 1940s, by rotating phagocytosed magnetic particles in cultured chick fibroblasts, Crick and Hughes (10) observed recoil of magnetic particles after force removal, showing that cytoplasm is elastic as well as viscous. In the 1960s, Hiramoto (11, 12) injected magnetic beads into the cytoplasm of sea urchin eggs and also concluded that it is viscoelastic, with mechanical properties that changed across cell cycle (12). Half a century later, manipulating magnetic particles in cells remains a powerful approach for interrogating cytoplasmic mechanics in living cells (2, 13). Most studies measured forces acting on individual magnetic particles, yielding estimations of shear elastic modulus on the order of 10^1 to 10^2 Pa, while fewer studies deformed or translocated subcellular assemblies. Garzon-Coral et al. (14) investigated spindle positioning using magnetic tweezers in *C. elegans* one-cell embryos, where astral microtubules reach the cortex. The spindle exhibited viscoelastic response to deformation, with up to 80% recoil when force was removed. By knocking down genes involved in cortical force generation and microtubule dynamics, the authors argued that the spindle is dynamically recentered by polymerization of microtubules that touch the cortex (Fig. 1 *B*, *Upper*). Previous authors rather implicated cortical dynein in dynamic recentering, but whatever the mechanism, all agreed on a central role for microtubules in maintaining spindle position in cells where astral microtubules reach the cortex (6, 15).

To test if microtubules maintain spindle position in sea urchin embryos, Xie et al. (3) partially depolymerize them and conclude that they contribute little to the mechanics, consistent with a lack of direct spindle–cortex connections in large cells. Depolymerizing F-actin decreases stiffness by ∼37% and drag by ∼50%, consistent with actin networks contributing to bulk mechanics (2, 16). Concentrating the cytoplasm by osmotic removal of water increases both restoring stiffness and viscous drag of the spindle; diluting it has the opposite effects. One possible interpretation of this perturbation is that viscoelastic forces can arise simply from the crowded nature of cytoplasm.

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Reviewing Xie et al. (3) and previous work, the force-producing mechanisms that position spindles can be divided into passive and active categories (Fig. 1*C*). Passive forces oppose displacement but cannot actively center spindles, while active forces can. Starting with the active forces, microtubule polymerization dynamics and cortical dynein have been implicated in positioning spindles in many small cell systems (6). Actomyosin also contributes to actively positioning spindles (17, 18), although how it is spatially regulated is unclear. Moving to the passive elements, microtubules and actin filaments can oppose displacement by bending, stretching, and entropic effects (16). Intermediate filaments can also exert passive forces (19), but it is not clear if they are present in sea urchin embryos. The endoplasmic reticulum (ER) constitutes a network of tubules and cisternae that densely accumulate around mitotic spindles (3). This might act as an elastic element, although literature estimations suggest that ER is much softer than cytoskeleton (20). Lastly, macromolecular crowding could, by itself, generate passive forces. From a pure physics point of view, emulsions as simple as oil in water as well as suspensions with either hard or deformable particles in liquid exhibit tunable rheological properties, including viscoelasticity (21, 22). The physiological concentration of macromolecules in cytoplasm is reportedly close to the jamming transition (23). Concentrating such a colloidal suspension, as in Xie et al.'s osmotic compression (3), can induce a glass-like state (23, 24), with concomitant increases in both elasticity and viscosity (23–25). If this interpretation is correct, Xie et al.'s work (3) implicates cytoplasmic crowding in generating biologically relevant forces at the micrometer scale.

Xie et al. (3) have made a highly original contribution in characterizing the passive forces that maintain spindle position in the large cell regime. Related forces are likely to participate in all aspects of subcellular organization at the micrometer scale. What questions remain? The difference between the viscoelastic response to moving spindles vs. oil droplets or magnetic beads is fascinating and deserving of further study. It is possible that considering the mechanics of the spindle as equivalent to that of a solid object is oversimplified. The higher drag experienced by spindles might reveal microtubule-specific connections to passive mechanical elements. How the spindle is positioned at the

center of large cells by microtubule asters in the preceding cell cycle is also unclear (8). In the prevailing model, asters are pulled through the cytoplasm by dynein anchored on organelles (7). A recent study proposed that all components of the cytoplasm move collectively with the aster (26). If confirmed, this observation requires new models for aster-centering forces in large cells. Finally, it would be interesting to consider how much cytoplasmic mechanics contributes to spindle positioning in smaller cells compared with microtubule-dependent forces. The mechanics of bulk cytoplasm is an enduring mystery, and much remains to be learned. Xie et al.'s work (3) shows that it will be important to integrate active and passive forces in future models.

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