# Central spindle robustness by PRC1-centralspindlin interaction

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Mitotic apparatus (MA) plays central roles in cell division for both mitosis and cytokinesis. It achieves these mechanical tasks by changing its morphology under the control of cell cycle machinery. This relies on dynamic polymerization and depolymerization cycles of microtubules and their assembly into higher order structures such as bundles involving various microtubule regulators. A dramatic remodeling of the MA occurs at the metaphase to anaphase transition (Figure). Before this, two spindle poles are connected both by interpolar microtubules and by kinetochore microtubules attaching the unsegregated chromatids. After anaphase onset, the link via kinetochore microtubules and chromosomes disappears due to loss of chromosome cohesion. As a consequence, the interpolar microtubules, which have now developed into a more prominent structure termed the central spindle, become the sole mechanical link between the two poles (a in Figure). Metaphase-anaphase transition also promotes the growth of astral microtubules. Dynein anchored at the cell cortex interacts with the astral microtubules and generates mechanical forces (cortical pulling forces) that pull spindle poles towards the cell cortex [1]. In some cell types such as the *C. elegans* embryos, cortical pulling force is the major driving force for chromosome separation via elongation of the pole-to-pole distance (anaphase B). In this situation, the central spindle is dispensable for chromosome segregation; it rather works as a brake against the cortical pulling force. Indeed, in *C. elegans* embryos, chromosome separation is accelerated when the central spindle is severed by laser manipulation or by genetic perturbation [2]. So, why does a cell form the central spindle? Well, this is because it has an

important role in cytokinesis.

Cytokinesis in animal cells occurs through cleavage furrow ingression driven by the constriction of an actomyosin ring, followed by abscission that cuts the intercellular bridge. Both astral microtubules and the central spindle contribute to positioning of the cleavage furrow. The central spindle also promotes continuous ingression of the cleavage furrow. It is compacted by the ingressing furrow into midbody, which anchors the plasma membrane at the narrow intercellular channel and provides a platform for final abscission. Disruption of the central spindle leads to defective cytokinesis. Here, we find a dilemma that an anaphase cell has to face: how to reconcile cytokinesis, which relies on the central spindle, with anaphase B driven by cortical pulling forces, which is braked by the central spindle? For successful cytokinesis, a robust central spindle is needed. However, if it was made too rigid, it would interfere with spindle elongation and thus with chromosome segregation. It is not easy to timely establish the central spindle and maintain the interpolar link while continuously increasing its overall length in response to the external force.

A solution by the cell, which we recently discovered, is to pair up two different types of microtubule-bundling factors, PRC1 and centralspindlin [3]. In our paper, we first demonstrated that the central spindle is equipped with a resilient recovery mechanism against mechanical perturbations such as increased external force or reduced internal friction (b in Figure). Then, we discovered that a direct interaction between PRC1 and centralspindlin plays a key role in preventing the rupture of the central spindle that would have been caused by the tension originated from cortical pulling forces. PRC1 is a non-motor rod-like dimer that preferentially bundles microtubules in an anti-parallel manner. Centralspindlin is a 2:2 heterotetrameric complex of MKLP1 kinesin and CYK4 non-motor subunit, which also preferentially forms anti-parallel microtubule bundles [4]. Both serve as hubs in the protein-protein interaction network crucial for cytokinesis [5]. We found that the interaction between PRC1 and centralspindlin previously reported for human proteins [6] is conserved between *C. elegans* orthologs. Mutations

that disrupt this interaction, one of which had been previously identified in a forward genetic screen [7], caused central spindle rupture (c in Figure) and embryonic lethality in *C. elegans*. Interestingly, the spindle rupture phenotype was suppressed by reduction of the cortical pulling forces (d in Figure). This indicates that the interaction between PRC1 and centralspindlin is essential neither for microtubule bundling nor accumulation of centralspindlin to the spindle midzone but is crucial for the mechanical resilience of the central spindle against the tension applied by cortical pulling forces.

This work shed new light into a molecular mechanism underlying the mechanical integrity of the MA. To elongate the pole-to-pole distance while keeping the length of the central anti-parallel overlap relatively constant, anti-parallel sliding of the microtubules needs to be coupled with polymerization at the overlapping plus ends. Based on the behavior of wild-type centralspindlin upon accelerated anaphase B spindle elongation, we propose that robust central spindle maintenance involves leakage of centralspindlin and PRC1 from the central overlap due to microtubule sliding and their efficient return to the overlap zone by the plus-end directed motility, which would be promoted by the interaction between these microtubule bundlers. We speculate that this interaction is widely conserved and hence the same mechanism applies to the spindle integrity in a variety of cell types. Future research will reveal other mechanisms for mechanical robustness of the whole MA that ensure genome stability.

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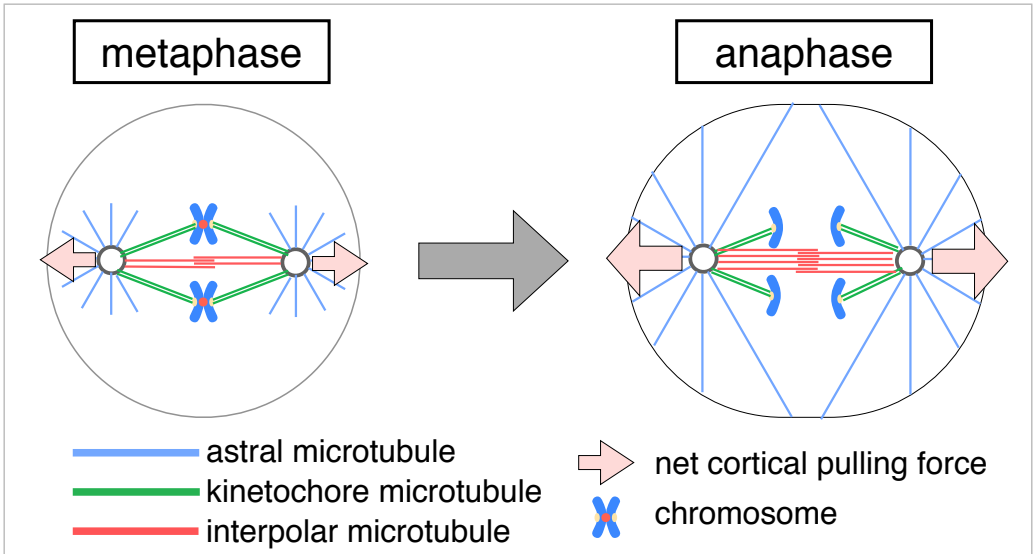


| case | cortical pulling force | centralspindlin-PRC1 interaction | central spindle in anaphase |
|---|---|---|---|
| i | Normal | + | |
| ii | Increased | + | |
| iii | Normal | – | |
| iv | Reduced | – | |