The Incredible Shrinking Spindle

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https://doi.org/10.1016/j.devcel.2018.05.007

As cell size decreases during the reductive divisions of early development, intracellular structures must shrink to fit. In this issue of *Developmental Cell*, Lacroix et al. (2018) identify a conserved mechanism of spindle scaling in nematode and sea urchin embryos whereby spindle microtubule polymerization rates decrease as development proceeds.

In dividing cells of all eukaryotes, the microtubule-based mitotic spindle functions to segregate replicated chromosomes to daughter cells. However, spindle size and morphology vary dramatically among different species and cell types to ensure chromosome segregation fidelity, as well as proper spindle positioning under diverse circumstances. Early animal development provides a striking example of spindle size scaling, because rapid divisions in the absence of overall growth can decrease cell volume several orders of magnitude within a matter of hours. The correlation between cell size and spindle size was first documented over 100 years ago (Wilson, 1897) and is conserved across animal phyla, but the molecular mechanisms underlying this scaling phenomenon are poorly understood. In this issue of *Developmental Cell*, Lacroix et al. (2018) provide important new evidence for cell size-dependent changes in microtubule growth rates, providing a robust mechanism to scale spindle size with cell size.

Spindle microtubules are highly dynamic, turning over with a half-life of less than a minute. Four parameters define microtubule dynamic behavior: the rate of growth, the rate of shrinkage, and the frequency at which they transition between these two states (termed catastrophe and rescue). Spindle scaling phenomena are thought to be mediated by changes in the complement of proteins that determine microtubule dynamics and motor-dependent organization. By imaging fluorescently labeled microtubules *in vivo*, Lacroix et al. (2018) demonstrated that as *C. elegans* and *P. lividus* embryos develop, spindle microtubule growth rates decreased in parallel with decreases in spindle length and cell size. In contrast, the other microtubule dynamic parameters remained relatively constant during these early mitoses. Importantly, microtubule growth rate and spindle size did not appear to be developmentally regulated. Instead, they correlated with changes in cell volume as development progressed. These results suggested that the reduced growth rate of microtubules is causative in scaling spindle size to cell size (Figure 1A).

Leveraging the power of the C. *elegans* system, Lacroix et al. (2018) tested the validity of their model in two ways. First, they utilized a mutant *C. elegans* strain with increased cell and embryo size to show that both microtubule growth rate and spindle length scaled up. Second, they depleted the ortholog of mammalian CLASP, a microtubule-associated protein known to increase microtubule polymerization rate, and showed that as a result, both microtubule growth rate and spindle length scaled down. Furthermore, by building 3D simulations of the mitotic spindle, the authors showed that alteration of microtubule growth rates was sufficient to explain the relationship between cell volume and spindle scaling during development. Remarkably, these scaling relationships were also evident in sea urchin embryos, even though they are about 20 times larger in volume than *C. elegans* embryos.

These experiments provide strong circumstantial evidence that cell volume and microtubule polymerization rate impact spindle length, but the question of exactly what factor or factors sense cell size to modulate microtubule growth remains open. Interestingly, previous work examining microtubule dynamic parameters in cytoplasmic extracts prepared from embryos of the frog *Xenopus laevis* containing four large-cell (stage 3) or several thousand small-cell (stage 8) embryos did not reveal a decrease in microtubule growth rate, but rather an increase in catastrophe frequency that could be linked to the activity of kif2a, a kinesin-13 that peels apart microtubule protofilaments to induce depolymerization (Wilbur and Heald, 2013; Figure 1B). Independent of the biochemical change driving a decrease in spindle size during *Xenopus* development, a second mechanism was shown to operate based on cell volume. Encapsulating *Xenopus* egg or embryo cytoplasm in droplets of different sizes revealed that spindle size scaled with compartment size (Good et al., 2013; Hazel et al., 2013). By this mechanism, components such as tubulin subunits themselves were calculated to become limiting at small cell sizes. However, microtubule dynamics have not been measured in different-sized droplets, so it remains unclear whether physical cell size-dependent mechanisms alter microtubule polymerization rate, catastrophe frequency, or both.

In addition to differences in microtubule growth rate and catastrophe frequency that can drive spindle scaling during development, other mechanisms have been identified that contribute to differences in spindle size among species. The frog *Xenopus tropicalis* scales smaller than *Xenopus laevis* at the organismal, cellular, and subcellular levels, and spindle size differences observed in egg extracts have been attributed to two molecular scaling factors. First, microtubule stability is reduced in *X. tropicalis* meiotic spindles due to increased activity of the microtubule severing factor katanin (Loughlin et al., 2011; Figure 1C). Second, *X. tropicalis* egg extracts possess a higher concentration of TPX2, a spindle assembly factor that regulates the localization

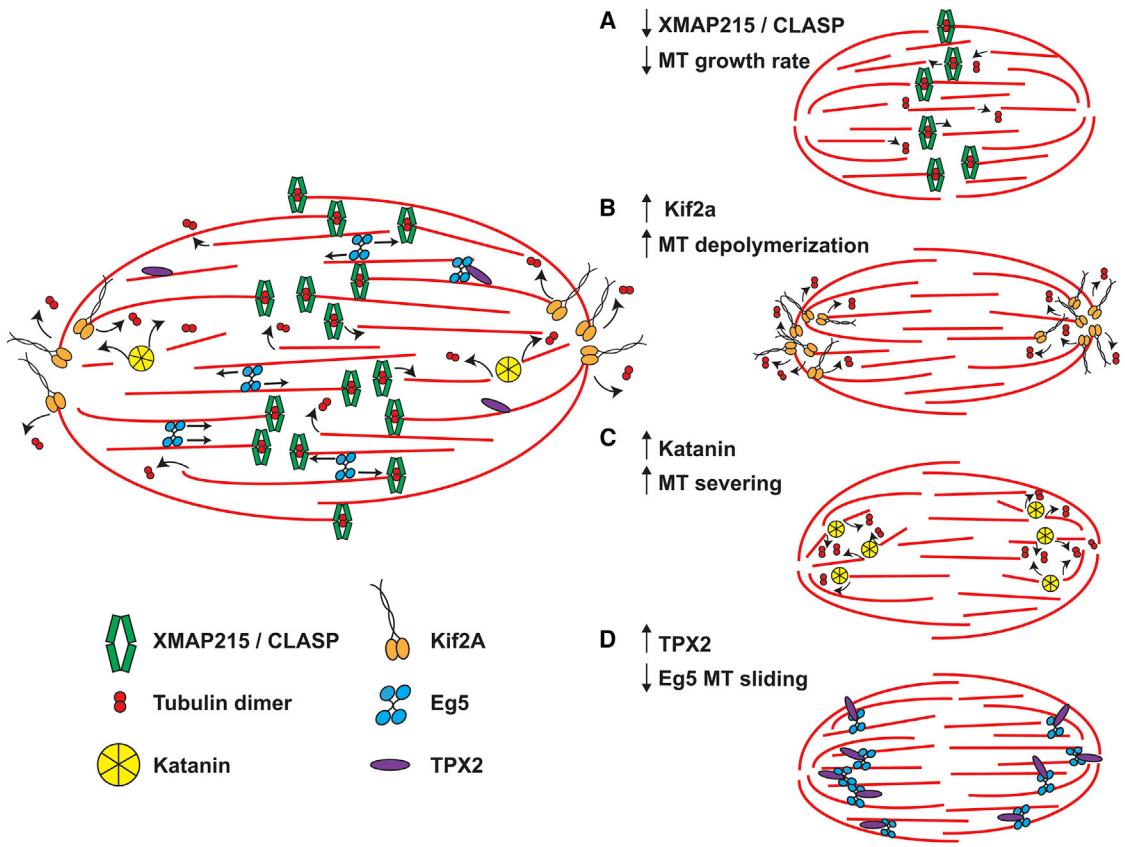

Figure 1. Models for Mechanisms Operating to Decrease Spindle Size
(A) A decrease in the activity of microtubule polymerizing factors such as XMAP215 and CLASP decreases microtubule growth rate, microtubule length, and spindle length. (B) An increase in the activity of the catastrophe-promoting factor kif2a decreases microtubule stability and spindle length. (C) An increase in the activity of the microtubule-severing enzyme katanin similarly decreases microtubule stability and spindle length. In (B) and (C), average microtubule length may or may not be affected. (D) An increase in TPX2 levels targets the kinesin-5 motor Eg5 to spindle poles, decreasing its antiparallel sliding activity and spindle length without necessarily affecting microtubule length.

and activity of the kinesin-5 antiparallel microtubule sliding motor, Eg5 (Helmke and Heald, 2014; Figure 1D). Therefore, in addition to microtubule dynamic parameters, factors affecting the force-balance of microtubule sliding can contribute to the control of spindle length (Goshima and Scholey, 2010).

Thus, evolution has provided an arsenal of factors that could be modulated to mediate spindle scaling, but how are their activities linked to cell size during embryogenesis in the near-absence of gene expression? One likely scenario involves titration or partitioning of spindle regulatory factors. Although the biochemical composition of early embryos is defined by its maternal content, each reductive division is accompanied by replication of the chromosomes and centrosome, as well as an increase in the cell surface area-to-volume ratio that could serve to redistribute or sequester spindle size regulators (Wilbur and Heald, 2013). Lacroix et al. (2018) propose an interesting model in which regulators of microtubule plus-end growth become limiting as the average microtubule length (calculated from the dynamic parameters), but not microtubule number (and therefore plus ends), decreases with cell size. This would fit well with the dose-dependent effects of microtubule polymerizing factors such as CLASP and XMAP215 on both growth rate and spindle size (Lacroix et al., 2018; Reber et al., 2013). However, due to the high density of spindle microtubules, direct measurement of their lengths and number requires 3D electron tomography (Müller-Reichert et al., 2018), and further support for this model awaits analysis of different-sized spindles *in vivo*.

In summary, the work of Lacroix et al. (2018) highlights important differences in the way various eukaryotes achieve spindle scaling in response to changes in cell size. Whereas some species rely on changes in microtubule catastrophe frequency to regulate spindle scaling during early development, others rely predominantly on changes in microtubule growth rates. Crucially, these changes do not depend upon developmental stage per se, but rather on the size of the cells within those stages. These findings provide a strong foundation for future mechanistic studies elucidating how microtubule dynamic changes are linked to cell size to control spindle scaling.

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Circuit Wiring: Neurite Speed Dating versus Stable Synaptic Matchmaking

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https://doi.org/10.1016/j.devcel.2018.05.008

Understanding the mechanisms establishing the complex but precise pattern of connectivity characterizing neural circuits remains an immense challenge. In a recent issue of *Neuron*, Mao and colleagues (2018) provide new insights by showing that the activation kinetics of EphB2, a transmembrane receptor tyrosine kinase, control whether dendritic filopodia makes a synapse with candidate axons.

Over the past 30 years, the field of developmental neuroscience has gone from a mostly cellular description of the mechanisms patterning neuronal connectivity by pioneers such as Cajal or Sperry to the identification of some of the key molecules underlying the guidance of axons and dendrites, their local branching pattern (Sanes and Zipursky, 2010), and—even more recently—the molecular mechanisms underlying synaptic specificity (de Wit and Ghosh, 2016), i.e., the ability of specific subsets of axons and dendrites to form synapses in a cell-type-specific and/or subcellular-specific way (Sanes and Zipursky, 2010). The model that emerges from this impressive body of work suggests that the adult pattern of connectivity characterizing a given functional circuit emerges through a series of steps progressively "simplifying" the complexity of the wiring diagram. First, axons, and to some extent their target dendrites, are guided by specific cues present at intermediate guideposts and/or within their final target. Second, once they reach their target structure(s), which still represents a vast potential "postsynaptic space," local branching of axons significantly reduces the number of potential postsynaptic dendrites with which these axons can form synapses. However, it has become apparent that the mere proximity of axons and dendrites is not sufficient to explain the specificity of synaptic connections. A major step toward answering this problem came from the identification of a large number of synaptic adhesion molecules that can (1) serve as *trans*-synaptic bridges between the correct pre- and postsynaptic partners while (2) directly or indirectly recruiting pre- and postsynaptic organizing molecules such as neurotransmitter receptors and the neurotransmitter release machinery (de Wit and Ghosh, 2016).

However, our understanding of the molecular mechanisms allowing the key transition between axon and dendrite guidance/branching and synaptogenesis is still fragmentary. One key limitation here is to improve our ability to image and ultimately understand the molecular mechanisms differentiating unfruitful contacts between axon and dendrites and contacts that lead to the formation of functional synapses.

To tackle this issue, in a recent issue of *Neuron*, Mao and colleagues (2018) conducted technically challenging experiments to image the localization and activity of the kinase EphB2 at developing dendritic filopodia as they scan their environment for potential axonal partners. EphrinBs and their EphB receptor tyrosine kinases (RTKs), including EphB2, are multifaceted, bi-directionally signaling transmembrane proteins known to control not only axon guidance and local axon branching (Kania and Klein, 2016) but also dendritic filopodia motility and stabilization of nascent spines by *trans*-synaptic interactions with ephrinB ligands (Kayser et al., 2008). This *trans*-synaptic interaction leads to presynaptic differentiation (Kayser et al., 2006), and postsynaptically, EphB2 also binds to and regulates