the presence of different regulatory proteins. Furthermore, we are able to follow the fate of Arp2/3 after debranching. Contrary to previous observations, we find specific physiological conditions in which Arp2/3 can remain attached to the mother filament after branch departure. Unexpectedly, in this type of debranching, the remaining Arp2/3 complex is able to nucleate a new branch and contribute to the turnover of the network.

814-Plat
Myosin-I facilitates symmetry breaking and promotes the growth of actin 'comet tails'
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Myosin-Is are single-headed, membrane associated members of the myosin superfamily that participate in crucial cellular processes related to membrane morphology and trafficking. Recent studies show that myosin-I isoforms frequently concentrate on membranes in areas of Arp2/3 complex-mediated actin polymerization that affect membrane shape and dynamics. To investigate how myosin-Is affect actin assembly, we performed a ''comet tail assay'' where branched actin networks were nucleated by Arp2/3 complex from a bead surface coated with a nucleation promoting factor (NPF). Actin filaments first formed a cloud around the bead, which transitioned into a polarized comet tail after symmetry breaking. We site-specifically coupled a range of densities of myosin-Is to the bead surface and assessed their effects on actin polymerization, network architecture, and symmetry breaking. We found that high myosin densities prevented comet tail formation. Instead, an extremely sparse actin cloud was created surrounding the bead. Decreasing the myosin density resulted in an actin network that broke symmetry more rapidly and formed a polarized comet tail. This actin comet tail was able to elongate from the bead at a faster rate and frequently showed smooth, rather than pulsatile motion. Myosin also changed the architecture of actin networks in comet tails. Compared with the coherent actin networks from non-myosin-coated beads, actin networks emerging from myosin-coated beads were sparser and more disordered, which might be due to the gliding and rotating effect of myosin. Strikingly, under low capping protein concentrations, where bead was embedded in a highly-dense actin shell and symmetry breaking was completely inhibited, myosin-coated beads were able to overcome this inhibition to break symmetry and form a comet tail. These studies show synergy between myosin activity and actin polymerization to power morphological changes at the cell membrane.

815-Plat
Actin filament sliding in CaMKII-actin bundles
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During learning and memory formation, neurons form synapses in the brain by connecting the axons of presynaptic neurons to the dendrites of postsynaptic neurons. Small protrusions in the postsynaptic neuron dendrites, called dendritic spines, form when the presynaptic neurons send high-frequency signals in a process known as long-term potentiation. The Calcium/calmodulin-dependent protein kinase II, CaMKII, is a critical protein in long-term potentiation, as it can decode the signals, trigger a phosphorylation cascade, and interact with actin filaments forming blunt-ended bundles. In this project, we modeled actin filaments using a previously reported coarse-grained model of 4 particles per actin protomer. We also modeled CaMKII and actin filaments based on docking simulations done in our group. We compare CaMKII bundles with bundles formed with other crosslinkers, such as α-actinin and fascin, and show that the geometry of CaMKII allows it to slide along actin filaments. We show how the sliding of the CaMKII monomers depends on the affinity and geometry of the CaMKII-actin interaction. Finally, we propose a mechanism that explains how CaMKII sliding may lead to the formation of blunt-ended actin bundles.

816-Plat
Midzone microtubule dynamics in anaphase cells
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During anaphase, antiparallel overlapping midzone microtubules elongate, contributing to chromosome segregation and specify of the location where the contractile ring will form. Midzone microtubules are dynamic in early anaphase, but not in late anaphase, when they appear to be stabilized. The kinetics and mechanisms of this stabilization are not completely understood. Here, we quantify of midzone microtubule dynamics using a photoactivatable-GFP tubulin expressed in LLCPk1 cells. We find that immediately after anaphase onset, a single highly dynamic population of microtubules is present, but as anaphase progresses, both a dynamic and more stable population of microtubules coexist, with a gradual increase in the dissipation half-time for the slower population. By the middle of cytokinesis, only static microtubules are detected. We examined the microtubule dynamics during furrowing examining the effects of actin and midzone-associated proteins. Blocking furrow ingression using either C3 or latrunculin treatment resulted in two spatially distinct microtubule populations in telophase cells: peripheral microtubules that were highly dynamic and central microtubules with fast and slow populations that never transition to a static array. Depletion of midzone microtubule-associate proteins, PRC1 or Kif4a, did not block furrow ingression in most cells and prevented formation of a static array in telophase cells. Although microtubules were partially stabilized similar to mid-anaphase cells. Quantification of the length of PRC1 decorated microtubule overlaps showed that static arrays are characterized by short highly compacted zones. These results demonstrate that dynamic turnover and sliding of midzone microtubules is gradually reduced as anaphase progresses and microtubules are further stabilized during cytokinesis. These data reveal the importance of the PRC1/KIF4A module and midzone microtubule compaction in generating a static midzone.

817-Plat
Chromosome size affects alignment efficiency in mammalian mitosis
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The kinetochore connects chromosomes to spindle microtubules during cell division. Accurate chromosome segregation requires sister kinetochores to biorient, attaching to opposite spindle poles. To achieve biorientation, the kinetochore destabilizes incorrect attachments and stabilizes correct ones. How it discriminates correct from incorrect attachments is not clear. Here, we test the model that tension serves as the stabilizing cue at correct attachments and how that model affects chromosomes of different sizes. Live imaging of mitotic PtK2 cells reveals long chromosomes align at the metaphase plate more slowly than short chromosomes. Using laser ablation to shorten long chromosome arms—reducing polar ejection forces on them—we show that chromosomes align faster after ablation, indicating chromosome size affects alignment efficiency. Finally, artificially enriching for incorrect attachments using STLC washouts and imaging error correction live, we show that long chromosomes exhibit a delay in correcting errors, rather than simply in attachment formation. We propose a model where increased polar ejection forces on long chromosomes stabilize not only correct but also incorrect attachments, delaying their biorientation and alignment. As such, long chromosomes may experience more challenges correcting errors and, as a result, higher missegregation rates.

818-Plat
Self-organized Rho and F-actin patterning in an artificial cell cortex
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The cell cortex, comprised of the plasma membrane and an underlying meshwork of filamentous actin (F-actin), is remodeled during a variety of essential biological processes. Previous work has shown that prior to large-scale remodeling the cell cortex is dynamically patterned with subcellular waves of F-actin assembly and disassembly, a phenomenon termed ''cortical excitability''. In developing embryos, cortical excitability is generated through coupled positive and negative feedback, with rapid activation of F-actin assembly driven by the small GTPase Rho, followed by inhibition of Rho activity. These feedback loops are proposed to serve as a mechanism for amplification of active Rho signaling at the cell equator to support furrowing during cytokinesis, while also maintaining flexibility for rapid error correction. Investigating the mechanisms that support and regulate cortical patterning is currently limited by a lack of technical approaches that can bridge our understanding of biochemical feedback signaling and cortical pattern formation, including the molecular regulation of signaling