the kinetochore microtubules are extremely short resulting in a small mitotic spindle at anaphase onset. To determine whether the short mitotic spindle or TPXL-1 depletion itself causes defects in aster-based suppression, we increased spindle length in *tpxl-1* mutants by depleting the kinetochore component *hcp-4*. Rescuing spindle length in *tpxl-1* mutants did not rescue the defects in aster-based suppression, suggesting that TPXL-1 has a direct role in this process. Next we tested whether aster-based clearing of anillin depends on the ability of TPXL-1 to activate aurora A kinase. To this end we generated wild type (TPXL-1^{WT}) and Aurora A binding-defective (TPXL-1^{FD}) RNAi-resistant TPXL-1 transgenes. We find that in the absence of endogenous TPXL-1, TPXL-1^{WT} but not TPXL-1^{FD} supports clearing of anillin from the cell poles. Our findings suggest that aurora A kinase activation by TPXL-1 is essential for the removal of contractile ring components from the cell poles. In summary, we identified TPXL-1 and aurora A kinase as the first molecular components of the aster-based mechanism that inhibits the accumulation of contractile ring proteins at the cell poles during cytokinesis.

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Polo-like kinase-1 and Aurora B act in redundant signaling pathways that drive cytokinesis initiation.

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Cytokinesis drives the physical separation of the daughter cells at the end of mitosis. It starts in anaphase with the formation of an actomyosin-based contractile ring at the equatorial cortex. Assembly and contractility of the contractile ring is governed by the local activation of the small GTPase RhoA, and hence strict spatial and temporal regulation of RhoA activity is necessary to coordinate cytokinesis with nuclear division. Despite extensive research in various model systems it remains incompletely understood how localized RhoA activation is achieved and regulated in anaphase. Here we delineated the contribution of the central spindle, centralspindlin complex (Mklp1 and mgcRacGAP), Aurora B and Plk1 to cytokinesis initiation in human cells. By disrupting the central spindle via knock-down of the microtubule bundling protein PRC1, we identified two redundant signaling pathways involved in the activation of RhoA and the initiation of cleavage furrow ingression: One dependent on the central spindle and Plk1 as previously described, and a second pathway depending on cortical Aurora B activity and centralspindlin oligomerization, and operating independently of the central spindle and Plk1. We further show that Plk1 inhibition in PRC1-proficient cells sequesters Mklp1 and MgcRacGAP onto the central spindle making centralspindlin unavailable for Aurora B-dependent phosphorylation and oligomerization at the equatorial cortex. We propose that Plk1 activity at the central spindle allows the dynamic exchange of centralspindlin between the central spindle and equatorial cortex allowing centralspindlin to function as a regulator of central spindle formation and activator of RhoA at the equatorial cortex.

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Structure and constriction mechanism of the actomyosin ring.

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Cytokinesis is orchestrated by a contractile actomyosin ring, but its structure and mechanism remain elusive. We visualized the 3D structure of the ring in frozen-hydrated dividing yeast cells by electron

cryotomography (ECT). Detailed arrangements of actin filaments within the ring and with respect to the membrane were seen for the first time, providing a crucial spatial constraint for the constriction mechanism of the ring. Using the ECT data and input from the current literature we then explored sixteen mechanistic models by coarse-grained simulations at the 3D molecular details, revealing plausible mechanisms for preventing membrane distortion and protein aggregation. We found that, in the model that best fits experimental data, both bipolar and membrane-attached unipolar myosins exist in the ring, reconciling two different views in the field regarding the myosin configuration. In this model, ring tension is generated primarily by interactions between bipolar myosins and actin, and transmitted to the membrane via unipolar myosins. This model recapitulates a broad distribution of distances from actin filaments to the membrane observed in our tomograms and separation of two different myosin isoforms into the outer and inner subdomains of the ring reported in a previous fluorescence microscopy study. Further, it rationalizes how bundles of actomyosin were able to separate from the membrane in fluorescence microscopy experiments of the same previous study.

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Modeling contractile ring dynamics in the Caenorhabditis elegans zygote.

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Cytokinesis is required for cell proliferation with failures potentially leading to aneuploidy and cancer. The actomyosin contractile ring is a dynamic structure responsible for driving cytokinesis. Proper cytokinesis requires assembly of contractile ring components at the cell equator, in the cortex. Actin, myosin, crosslinkers, and regulators then reorganize as the ring matures from a wide band to a tight cable, becoming contractile. Previous work has provided insight into the changes that occur in ring and cell structure. However, less is known about changes on the mesoscopic and molecular scales due to imaging limitations. Several models have given insight to mechanisms of contractility, including actin treadmilling and minifilament adaptive response to force-load, but the predictive power of these is limited by the simplification of myosin minifilament and actin dynamics. Herein we set out to further bridge the gap between the models of cytokinetic ring components and quantitative cell biology, by first establishing a model of contractility where myosin minifilaments are modeled. We developed this model of cytokinesis using the software Cytosim, which provides unparalleled resolution on a molecular scale. In our model actin treadmilling and shortening, both of which have been implicated in models of contractility, provide for a dynamic actin meshwork. We built upon previous models depicting the catch-slip bond nature of myosin II motors binding to actin filaments and built full minifilaments as multiple motor heads protruding off either end of 300nm rods. To further refine our model we queried the changes in protein concentration of several components of the ring including myosin, actin, anillin, and septin. Previous methods for visualizing cytokinesis result in uneven illumination and detection of structures across the cytokinetic ring, with the illumination plane orthogonal to the contractile ring plane; making quantitative analysis of the ring less precise. To this end we used custom chambers to position *C. elegans* zygotes such that the entire contractile ring forms in the illumination plane of a focused light sheet. This setup allowed us to quantify the contractile ring densities of components over the length of cytokinesis. Using this system, we generate contractile rings, on the scale of *C. elegans* zygote contractile rings, that exhibit protein density dynamics like those measured in our in vivo data and show a model for how these rings may contract. Our initial estimates for all major structural components yield simulated rings that close at biologically-relevant timescales, exert force that constricts a “membrane,” and form mesoscopic contractile foci.