in ring assembly is unclear. To examine their roles, we mutated them and modified the concentrations of alpha-actinin Ain1 and fimbrin Fim1 in vivo. By titrating Ain1 and Fim1, we find that node condensation rates depend on their concentrations, with stable meshworks forming instead of rings at high concentrations and nodes clumping in deletion mutants. In order to better understand the role of actin filament cross-linkers in ring formation, we further developed the SCPR model. We modeled actin filaments as semi-flexible polymers and allowed transient filament bundling (representing cross-linking) and myosin movement damping along cross-linked actin bundles. With these additional mechanisms we were able to reproduce morphological features that matched experimental observations. By decreasing the degree of cross-linking the model generates structures that transition from stable branched meshworks, as in cross-linker over expression assays, to node clumps, as in cross-linker deletion assays. Our work supports a hierarchical process of ring self-organization involving components drawn together from distant parts of the cell followed by progressive stabilization by cross-linking proteins.

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Imaging FtsZ Rings In Vitro by Negative-Stain EM

Sara L. Milam, Masaki Osawa, Harold P. Erickson.
Duke University, Durham, NC, USA.

In prokaryotes, the major cytoskeleton protein involved in cell division is FtsZ, a homolog of tubulin. In vivo, light microscopy studies have shown that FtsZ forms a ring (Z ring) at midcell during cell division. However, the substructure of the Z ring remains a mystery. In vitro, FtsZ assembles into short, single-stranded protofilaments (pfs), but it is unknown how these 120 nm long pfs come together to form a ring that encompasses the ~3000 nm circumference of the cell. One possibility is that they associate by lateral contacts to make ribbons of protofilaments. Another is that the pfs are not in direct contact but communicate via distortions of the membrane. We have developed a lipid tubule system that has enabled us to image the Z-ring substructure by negative-stain EM. Here we took advantage of the self-assembling lipid, DC 8,9, PC, which spontaneously forms 500 nm diameter tubules in solution. We added membrane-targeted FtsZ (mts-FtsZ), which has been shown to form Z rings inside or outside of tubular liposomes. The mts-FtsZ assembled characteristic Z rings imaged by fluorescence light microscopy. Negative-stain EM showed that these Z rings consist of ribbons of pfs, which were one-subunit thick. Fourier transform analysis of these images confirmed the close packing (~5 nm) of individual pfs in the Z ring. Overall, the data suggest that lateral contacts are important for Z ring assembly. In addition, these results were similar for FtsZ from E. coli and M. tuberculosis, suggesting that lateral contacts are an intrinsic property of Z rings.

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Superresolution Investigation of In Vivo Structure and Dynamics of E. coli FtsZ Structures during Cytokinesis

Carla Coltharp, Guo Fu, Tao Huang, Jackson Buss, Zach Hensel, Jie Xiao.
The Johns Hopkins University School of Medicine, Baltimore, MD, USA.

Control of E. coli cell division is achieved by a network of proteins that organize cytokinesis in space and time. FtsZ, a tubulin-like GTPase that forms head-to-tail protofilaments, localizes to the midcell and assembles into a ring-like structure known as the Z-ring. The Z-ring serves as an essential scaffold to recruit all other division proteins and generates contractile force for cytokinesis, but its supramolecular structure and contraction mechanism remain unknown. We have used photoactivated localization microscopy (PALM) to characterize the in vivo structural dynamics of the Z-ring in E. coli at a spatial resolution of ~35 nm.

Superresolution imaging revealed that the Z-ring is ~110nm wide and is composed of a loose 3D bundle of FtsZ protofilaments that overlap with each other in both the longitudinal and radial directions in the cell. These 110nm rings were observed in either a single-ring conformation at midcell or in a multiple-ring conformation reminiscent of a tight helix. The tight helix conformation was very rarely observed in cells with a visible septation, suggesting that the Z-ring fluctuates between the single-ring and tight helix conformation before cytokinesis begins, but then adopts only the single-ring conformation during constriction. We also observed non-ringlike FtsZ structures emanating from the midcell in cells at later stages of constriction, indicating Z-ring disassembly. These disassembling structures were observed in cells with 200-600nm septum diameters, suggesting that Z-ring disassembly occurs gradually throughout the constriction process. We also observed that the bandwidth distribution of the Z-ring did not change with varying expression levels, cell size, or during the progression of cytokinesis, suggesting that the factors that regulate Z-ring thickness are not affected by FtsZ concentration or changes in cell shape.

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Integrating High Resolution Bioimaging Techniques to Unravel Spatio-Temporal Organization of Podosomes

Koen van den Dries[1], Marjolein Meddens[1], Samantha Schwartz[2], Diane S. Lidke[2], Keith A. Lidke[3], Carl G. Figdor[1], **Alessandra Cambi**[1].
[1]Nijmegen Centre for Molecular Life Sciences, Nijmegen, Netherlands, [2]Department of Pathology, University of New Mexico, Albuquerque, NM, USA, [3]Department of Physics and Astronomy, University of New Mexico, Albuquerque, NM, USA.

Podosomes are highly dynamic adhesion structures which consist of a dense actin core surrounded by a ring of integrins and adaptor proteins (Fig. 1A), typically associated with focal adhesions (FAs). Interestingly, while it is well established that myosin mediated tension plays an essential role in regulating adaptor protein recruitment to FAs, it is unknown whether similar mechanisms control podosome assembly. Here, by combining FRAP with Image Correlation Spectroscopy, we identify myosin-independent mechanisms that recruit adaptor proteins during podosome assembly (Fig. 1B). Moreover, we provide evidence that the cortical actin network creates the necessary tension eliciting the recruitment of adaptor proteins during podosome assembly. Combined with super-resolution STORM images of individual podosomes, our studies provide novel detailed insight into podosome spatio-temporal organization. A model integrating these results will be presented.

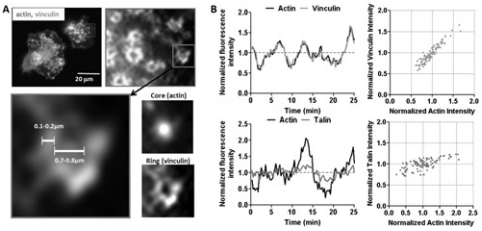

Figure 1. Organization and dynamics of podosome components in antigen-presenting cells. **A)** Podosomes are adhesion structures containing a dense actin core and a ring of vinculin, talin and other cytoskeletal components. **B)** Correlated fluoresence intensities of actin and cytoskeletal adaptor proteins at a single podosome in time.

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Moving Towards Self-Assembling Machines: Harnessing Spindle Components for Biosynthetic Devices

Timothy D. Riehlman, Zachary T. Olmsted, Carmen Branca, Ani Levine, Laura Patrick, **Janet L. Paluh**.
CNSE, UAlbany-SUNY, Albany, NY, USA.

The remarkably dynamic and complex manufacturing environment of the eukaryotic cell is currently unrivaled by manmade systems. Through dissection and harnessing of biological machines and underlying processes, and merger with synthetic materials, we hope to scale manufacturing into realms currently restricted from physical manipulation. Spindle assembly is the first critical step in chromosome segregation and a primary target for anti-cancer therapeutics. An assemblage of nanoscale components and communication networks must be integrated for spindle formation and function. We are applying nanotechnology in parallel with traditional methods of yeast genetics, timelapse microscopy, AFM and biochemistry. Dramatic reorganization of interphase microtubules into a bipolar organization for chromosome segregation requires focused nucleation from spindle pole body microtubule organizing centers (MTOCs). The γ-tubulin small complex (γ-TuSC) MTOC associates with growing microtubules and is part of a larger ring complex (γ-TuRC). The complex controls microtubule nucleation, organization and dynamics. Three conserved kinesin-like protein (Klp) families in fission yeast contribute in critical roles in spindle assembly. Kinesin-14 Pkl1 and Kinesin-5 associate with γ-TuSC at poles and provide counterbalanced roles in microtubule nucleation and organization, while Kinesin-6 acts on overlapping anti-parallel microtubules. The coupled action of the MTOC and Klps enables multiple levels of control. By purifying the γ-TuSC and γ-TuRC complexes from human and fission yeast cells using superose 6 FPLC chromatography, co-immunoprecipitation with magnetic beads and western analysis and attachment of these complexes to functionalized surfaces we are analyzing minimal components and frameworks for generating anti-parallel, bipolar and more complex microtubule array structures in the presence of multiple Klps. Our goal is to incorporate the MTOC, tubulin and multiple Klp families in different biosynthetic platforms to better understand self-assembling machines and transitional dynamic architectures thereby refining both in vivo models and in vitro advanced applications.