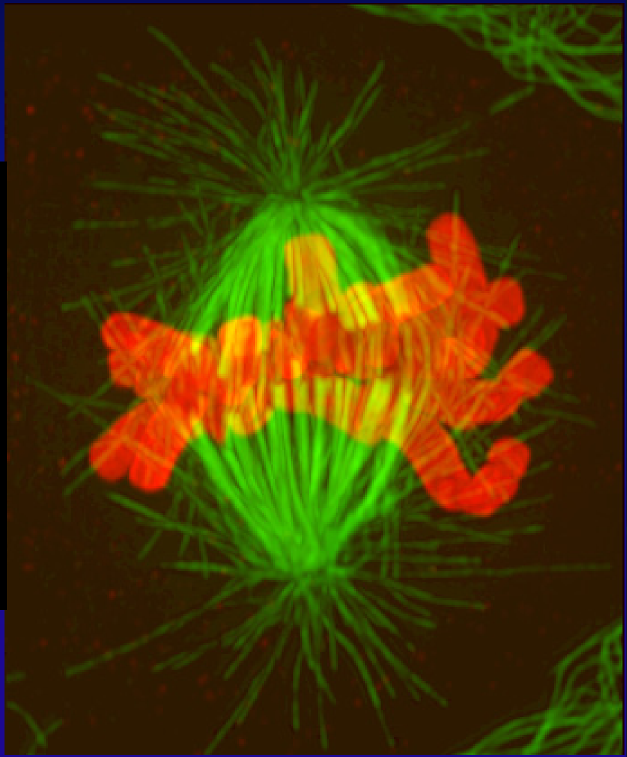
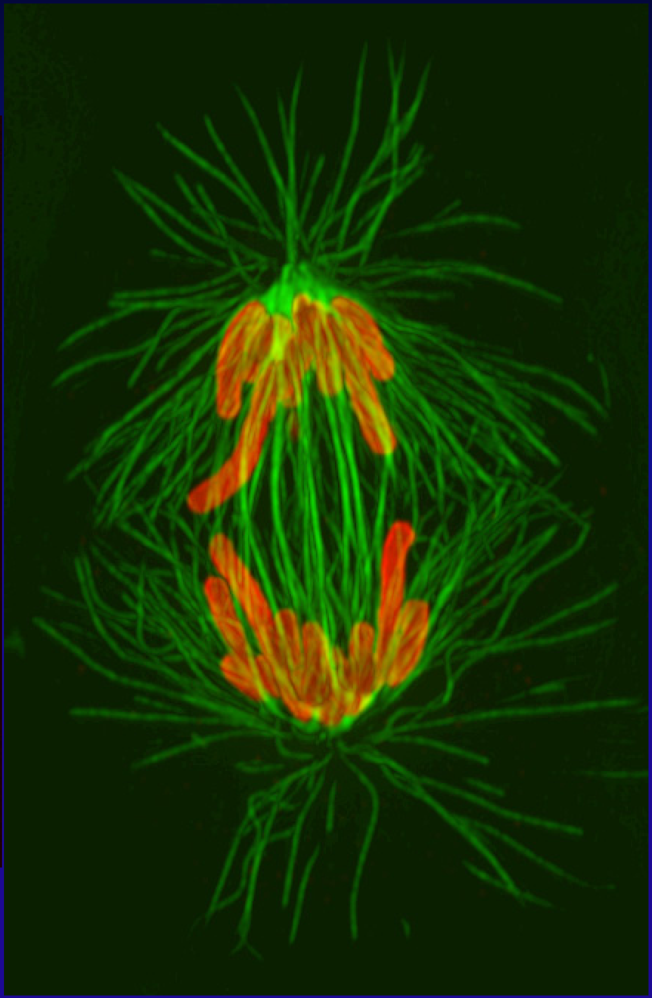
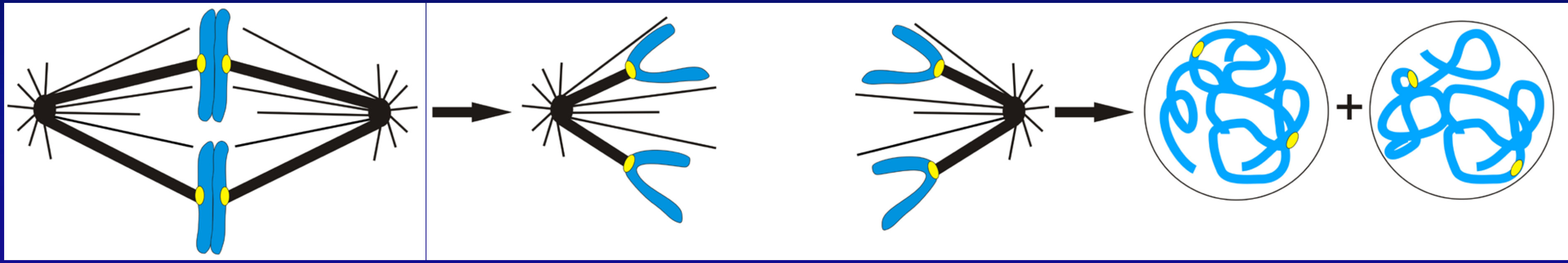
Mitosis: The accurate segregation of chromosome sets





Kerry Bloom, UNC Chapel Hill May 23, 2011 KITP Biopoly Conference

Chromosome Segregation



Metaphase
Mitotic Spindle

Chromosomes bi-orient, cohesin holds sisters together

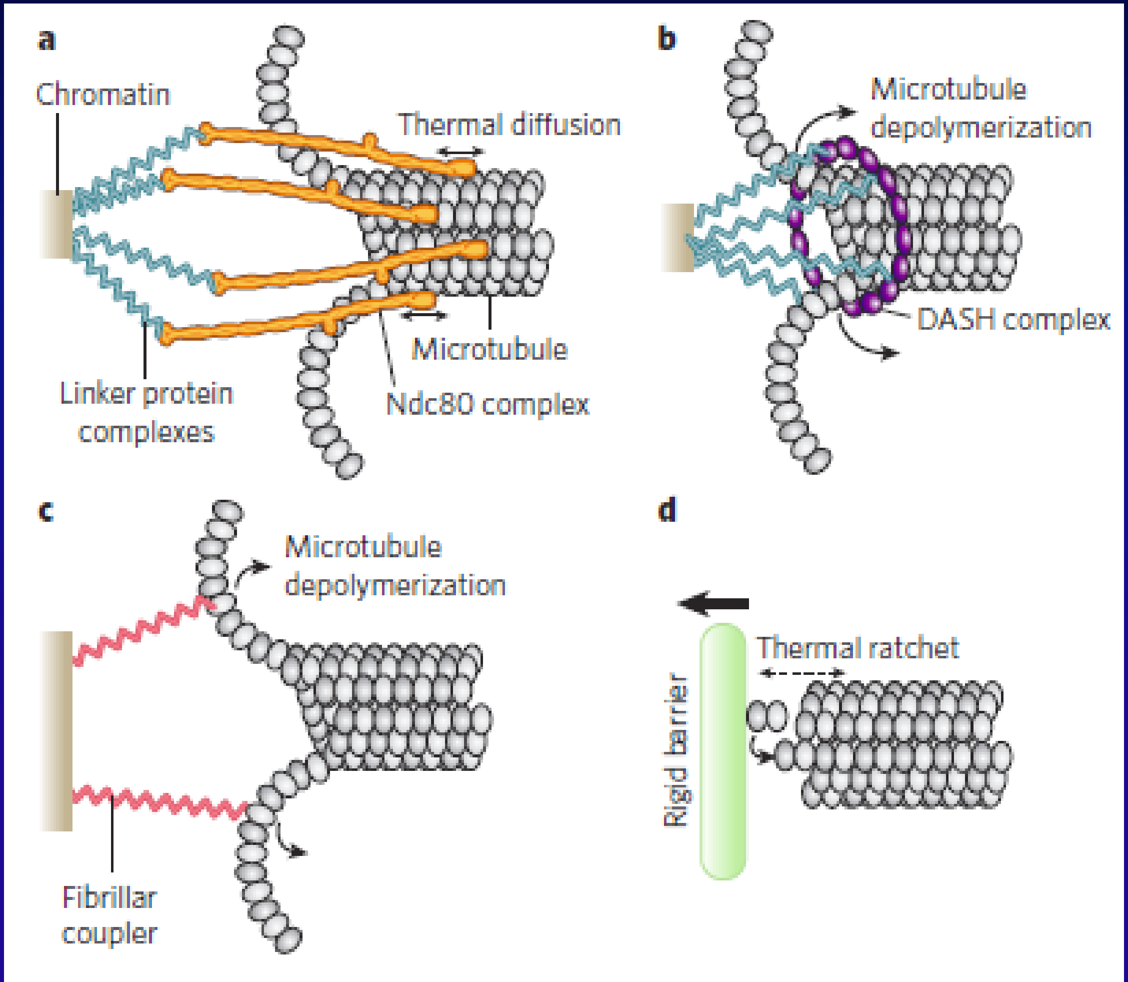
Anaphase
Mitotic Spindle

Chromosomes segregate, cohesin is removed by proteolysis

Telophase
Mitotic Spindle

Chromosomes decondense

Models of Chromosome Segregation



Force estimates from micromanipulation measurements

Table 1 Specific power output (erg s^{-1}/cm^3)

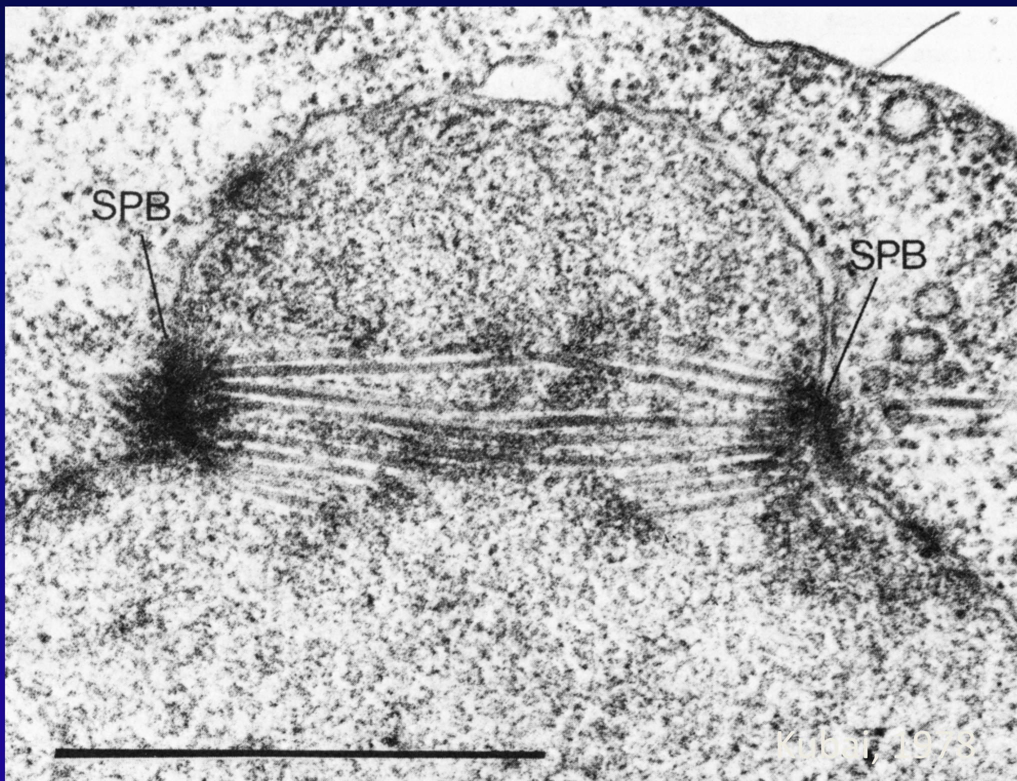
Source	Power output
Gas turbine engine[a]	8×10^7
Race car engine[a]	2×10^7
Passenger car engine[a]	3×10^6
Flagellum[a]	3×10^5
Striated muscle[a]	2×10^6
Cytokinetic furrow[a]	3×10^2
Spindle motor (grasshopper)/chromosome[a]	6
Spindle motor (yeast)/16 chromosomes[b]	7

[a] Nicklas (1984, 1988) [b] Bloom (Chromosoma 2008)

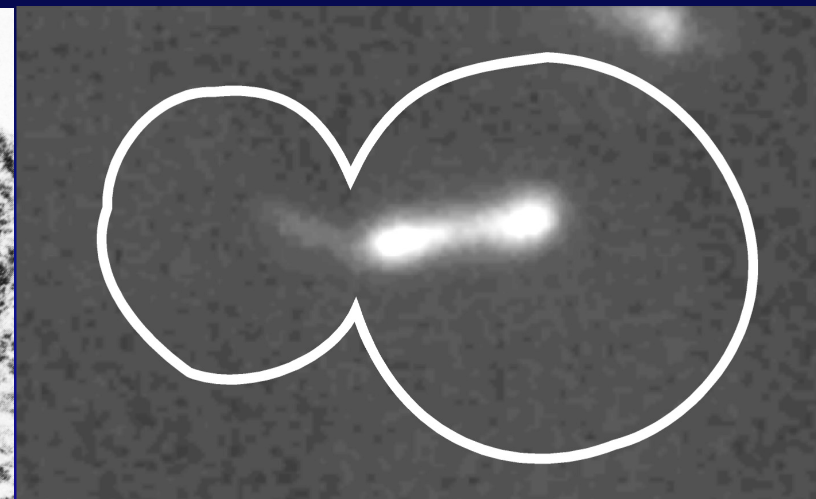
Nicklas estimated that the hydrolysis of 25 ATP's is sufficient for chromosome movement (energy to overcome viscous resistance)

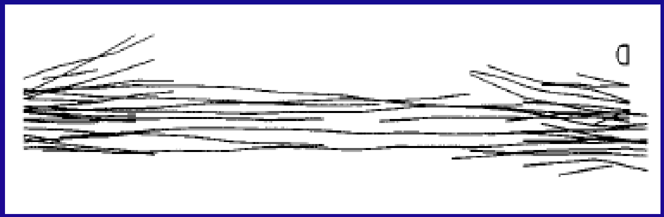
Yeast Mitotic Spindle Structure

EM



Tub-GFP



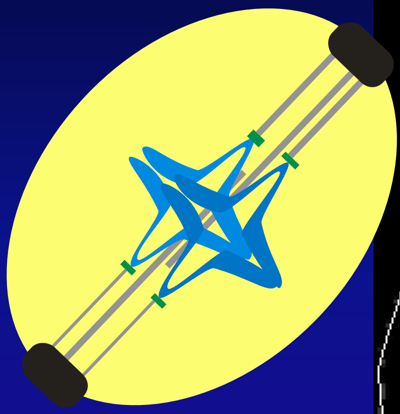


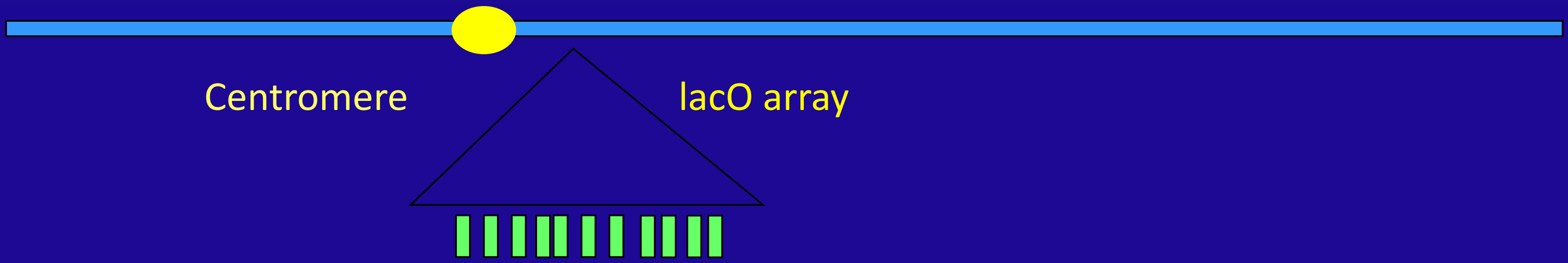
Tomographic reconstruction

40 microtubules in the mitotic spindle

Chromosome Movement in Yeast

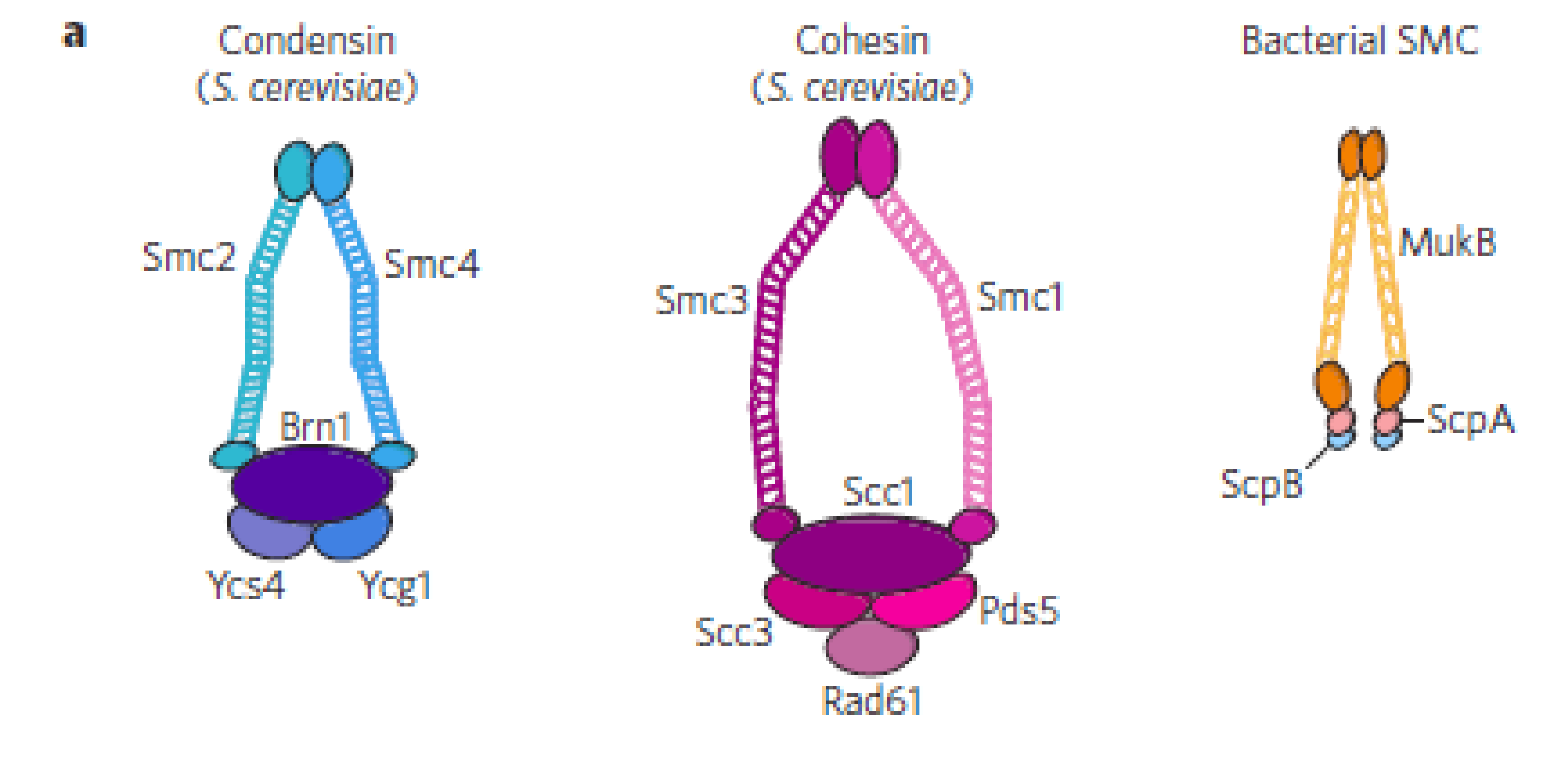
Mitotic spindle



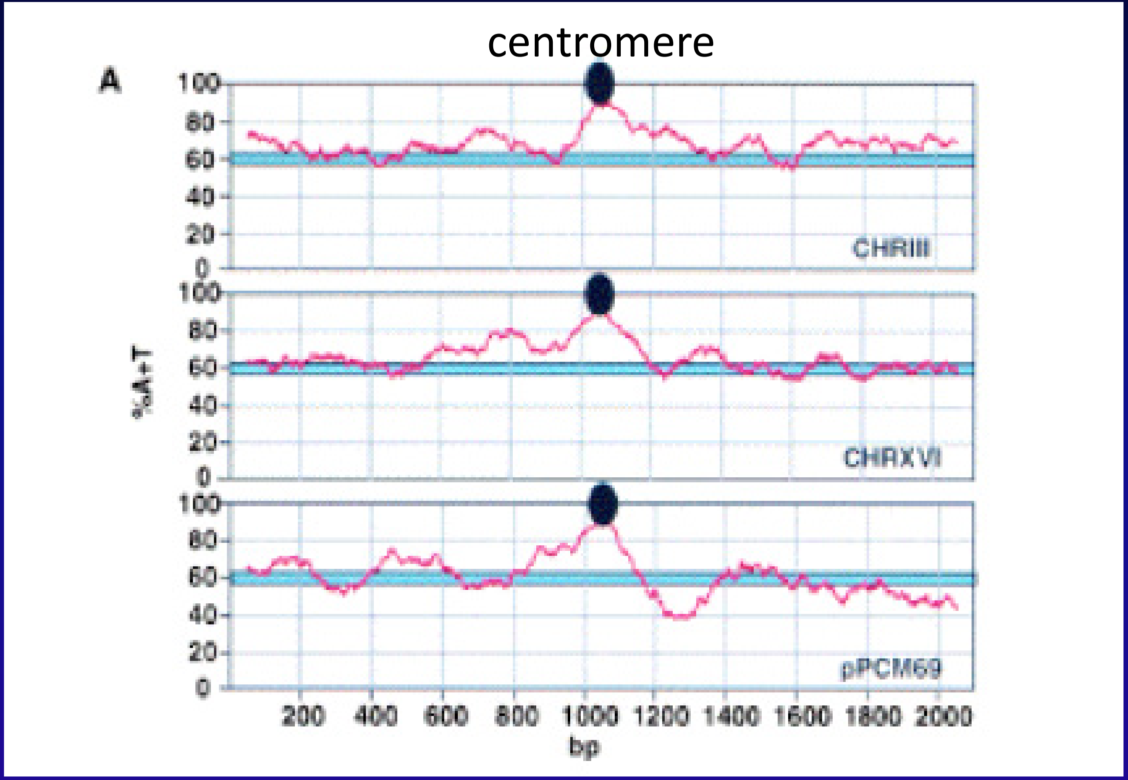


Structure of the Topology Adjusters: Cohesin and Condensin

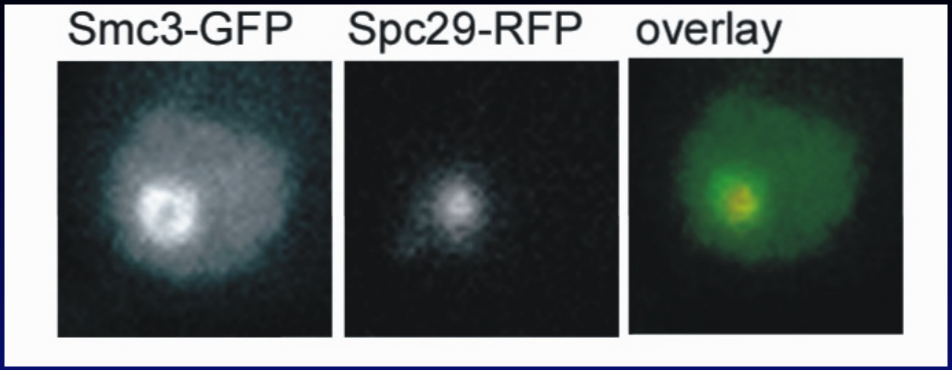
Cohesin encircles two DNA strands to promote ***cohesion***, Condensin facilitates DNA looping to condense the chromosome

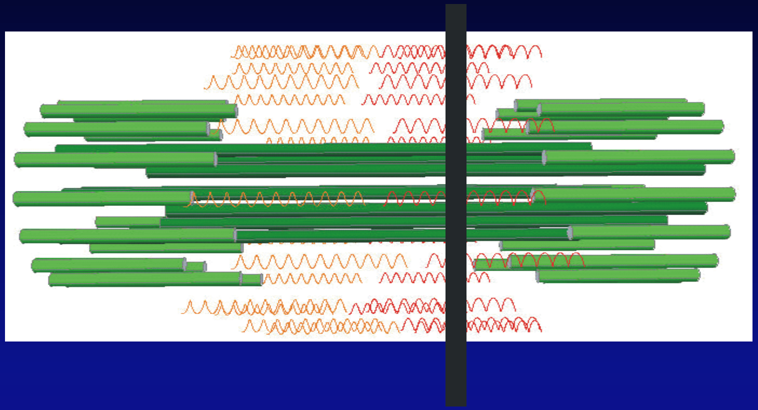


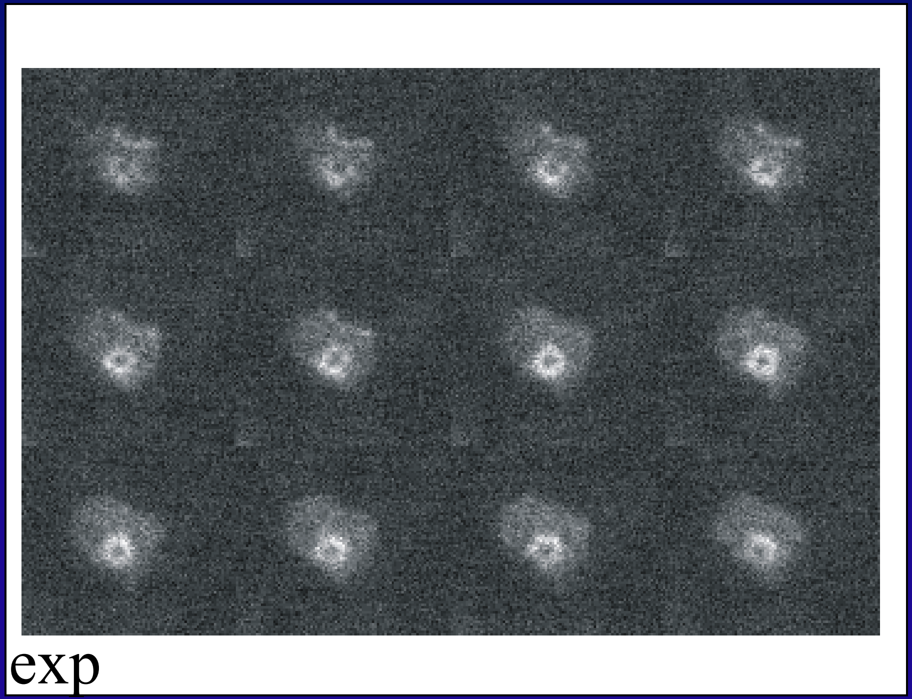
Paradox: Cohesin and condensin concentrated at centromeres, but centromeres are separated *in vivo*

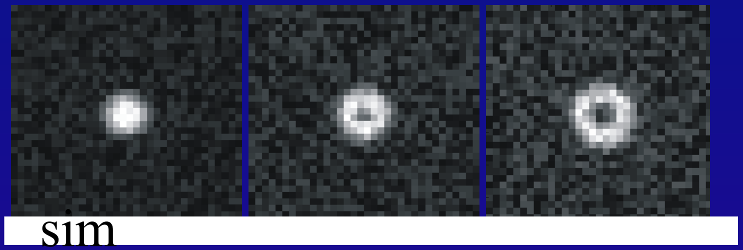


What is the distribution of cohesin *in vivo*?
What is the function of cohesin *in vivo*?







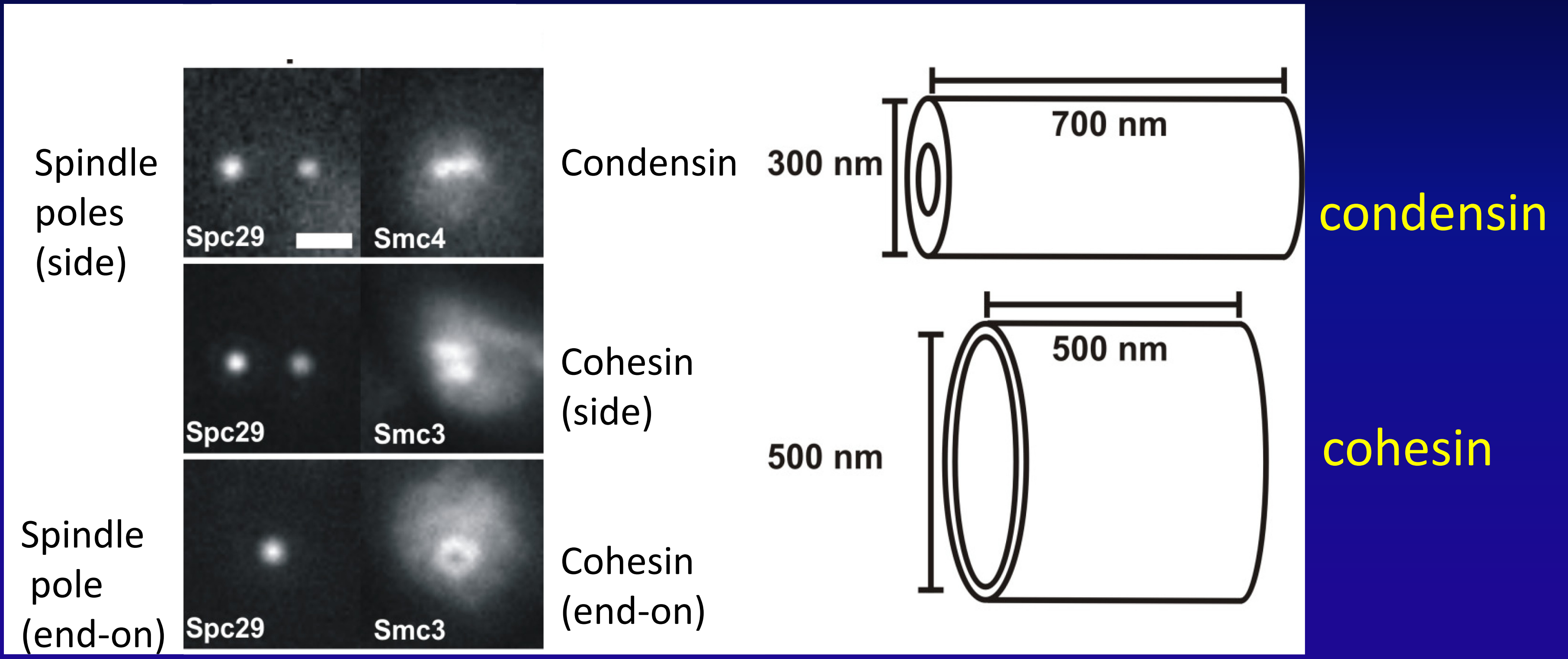


125nm 175nm 225 nm

Dimension (radius)

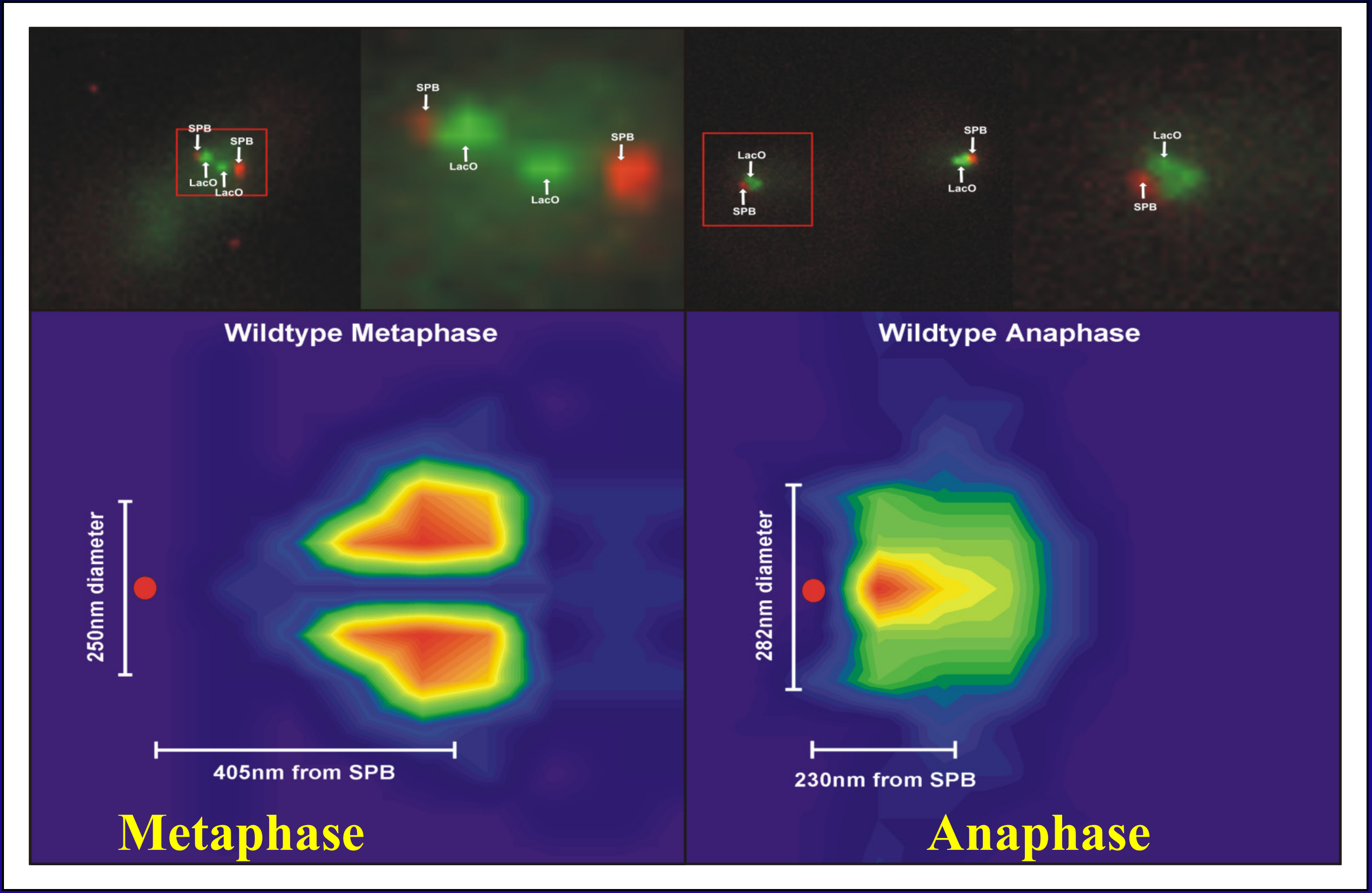
End-on view reveals the cylindrical array of cohesin surrounding polar microtubules

Cohesin is cylindrically arrayed around the spindle ~500nm diameter
Condensin lies proximal to the spindle with a diameter ~300nm



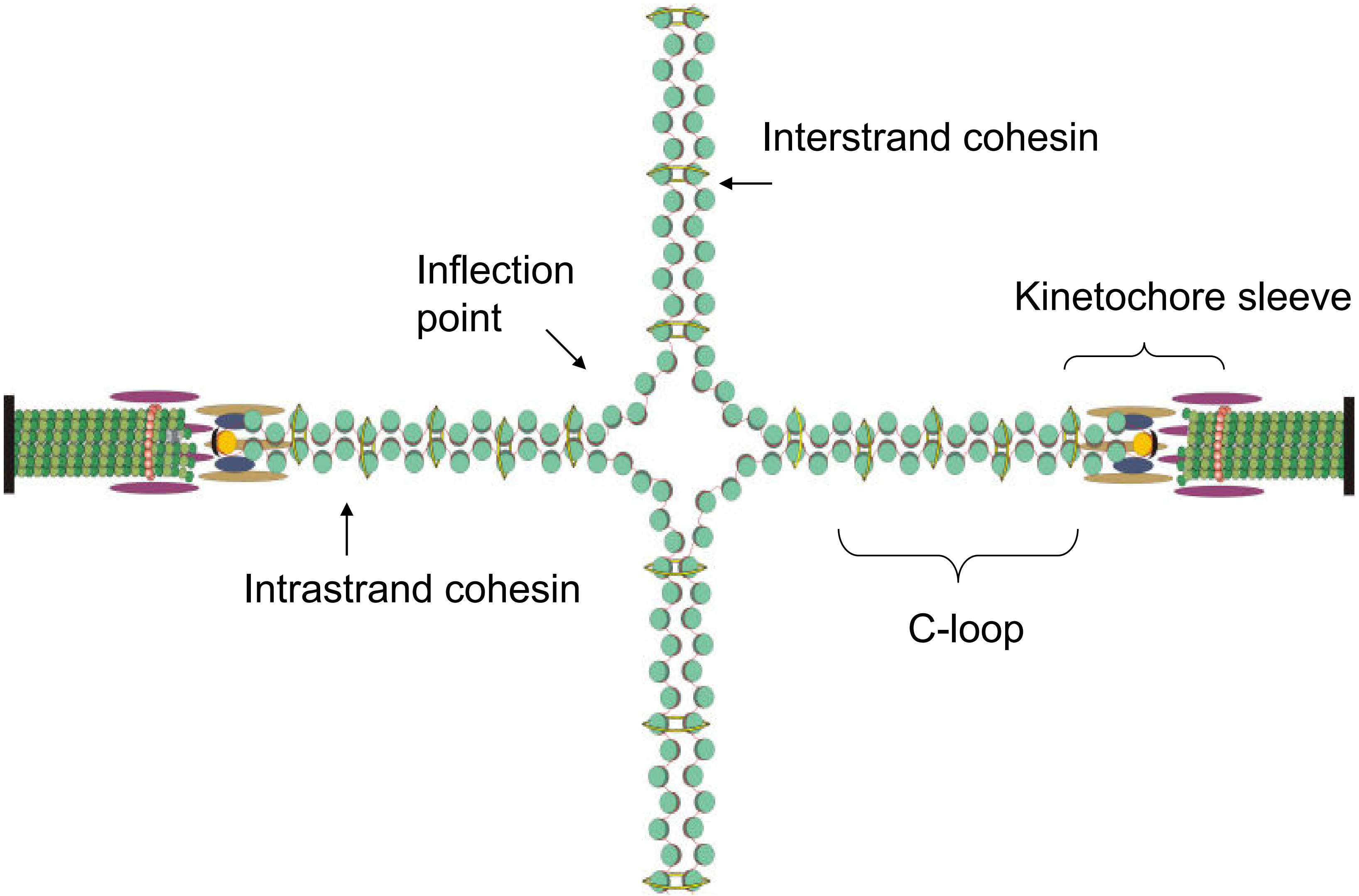
Yeh et al., Curr Biol 2008;
Stephens et al., JCB in press 2011

Mapping the spatial distribution of DNA in the spindle: Statistical probability maps



LacO repeats are radially displaced from the mitotic spindle axis

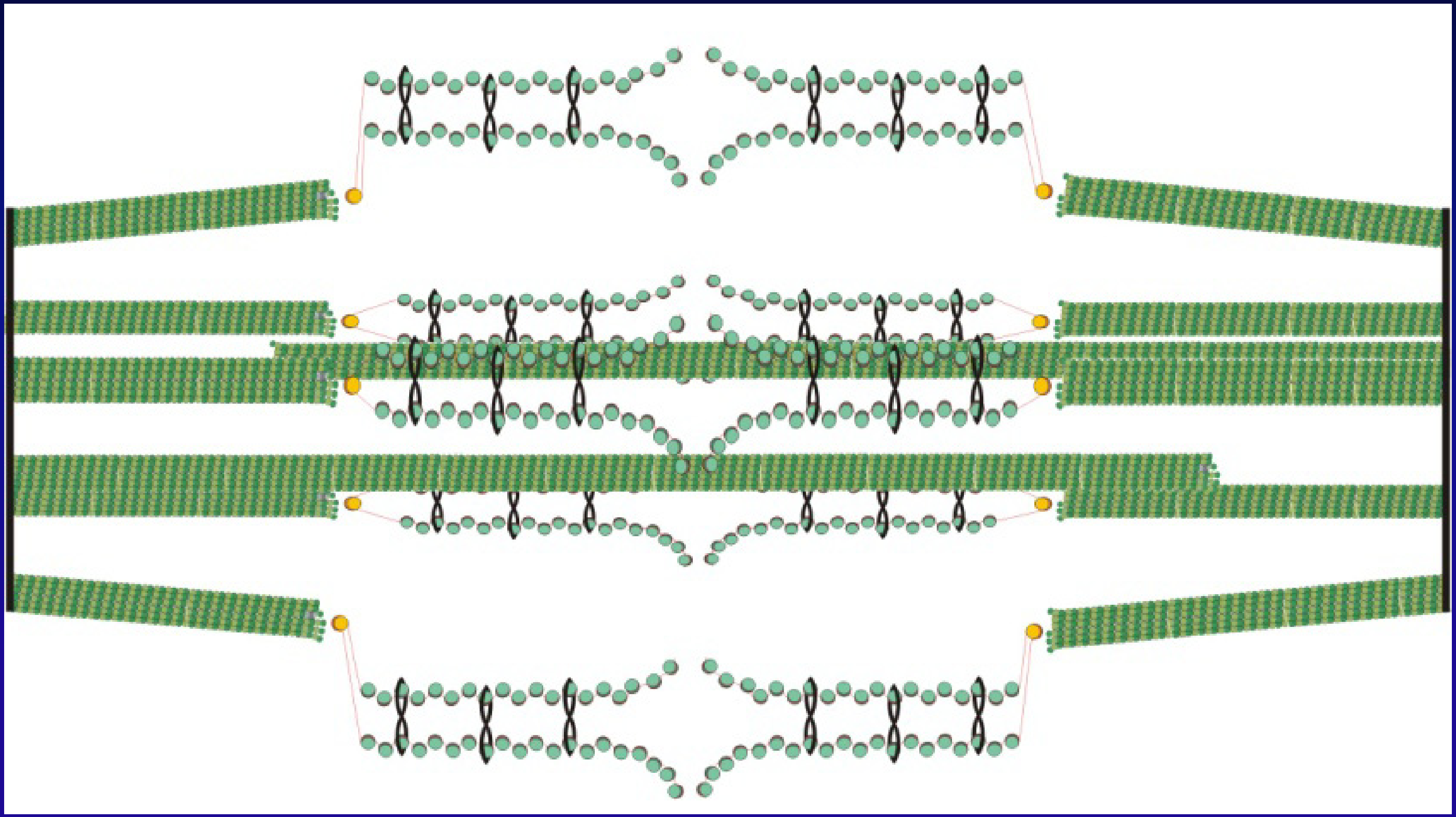
Anderson et al., MBOC 2009



Proposed Path of Centromere DNA in a Eukaryotic Kinetochore: C-loop

Yeh et al., Curr Biol 2008

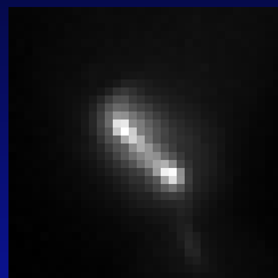
Proposed structure of pericentric chromatin within yeast spindle

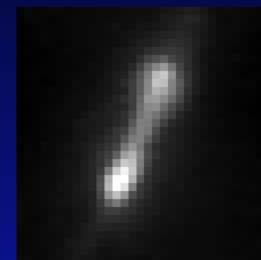


There is ~10X the DNA concentration in this barrel relative to the cell nucleus

The pericentric DNA, cohesin and condensin constitutes a molecular spring in mitosis

Wild-type

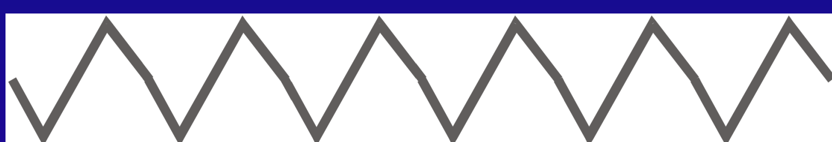




Mutant cells lacking cohesin or condensin

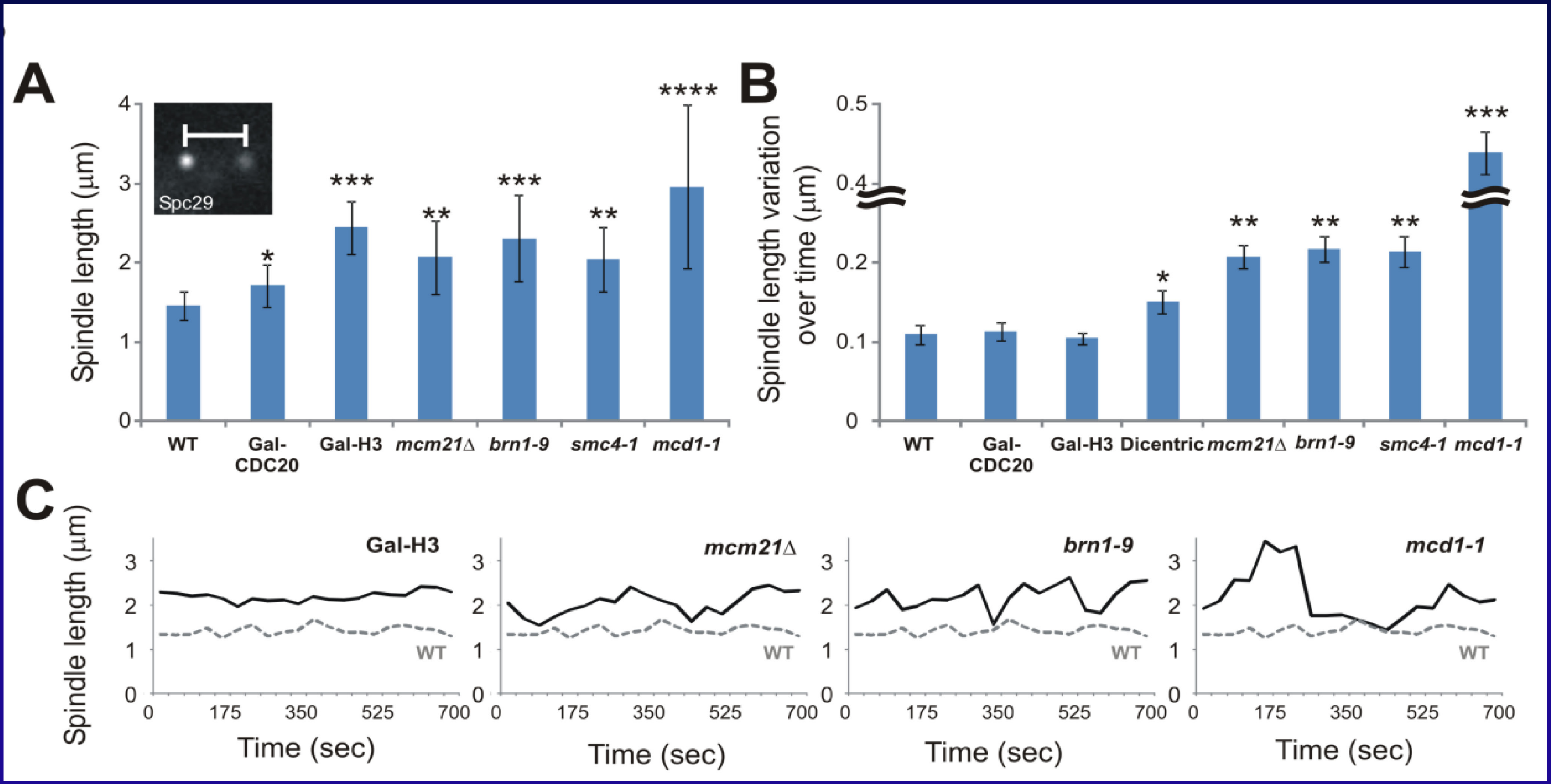
Changing spring constant

Changing rest length



Deplete outward force
(reduce microtubule motor)

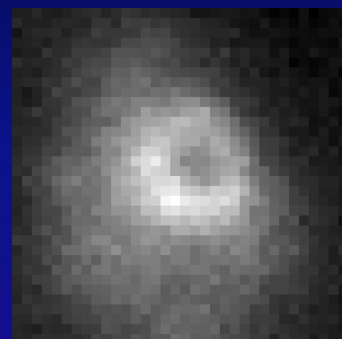
Cohesin, condensin, histone chromatin protein contribute to spindle dynamics and length regulation



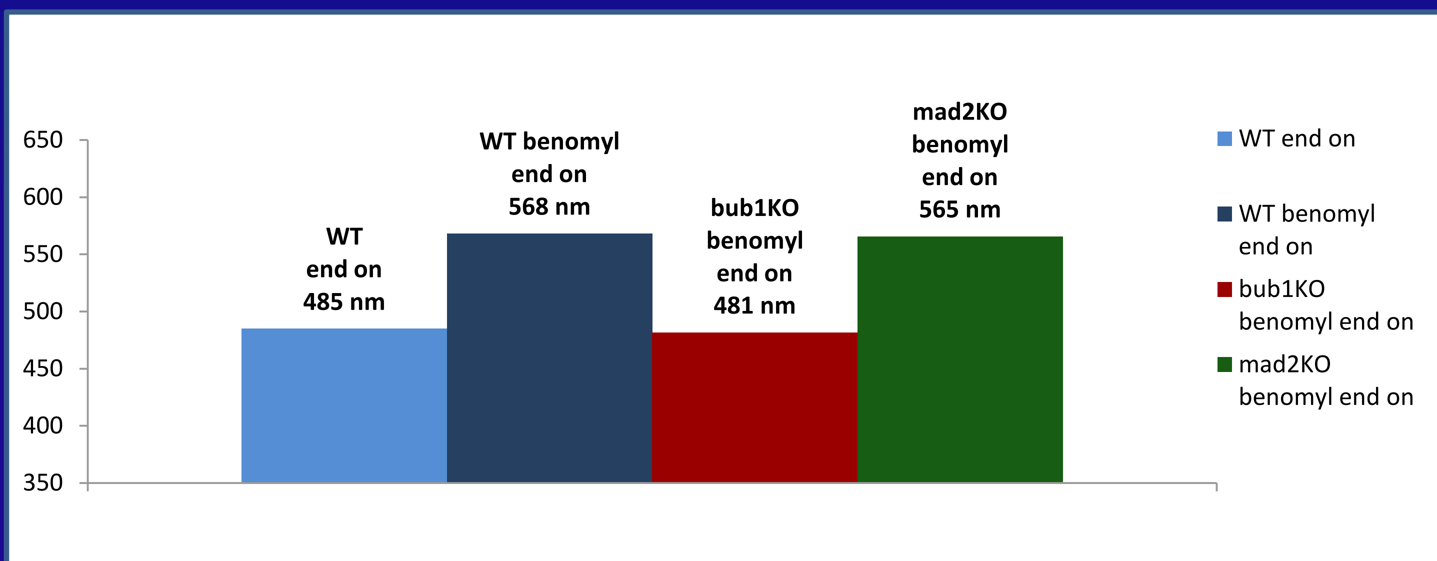
Bouck and Bloom, Curr Biol., 2007
Stephens et al., JCB in press 2011

Is there a rheostat (or clutch) to regulate the spring?

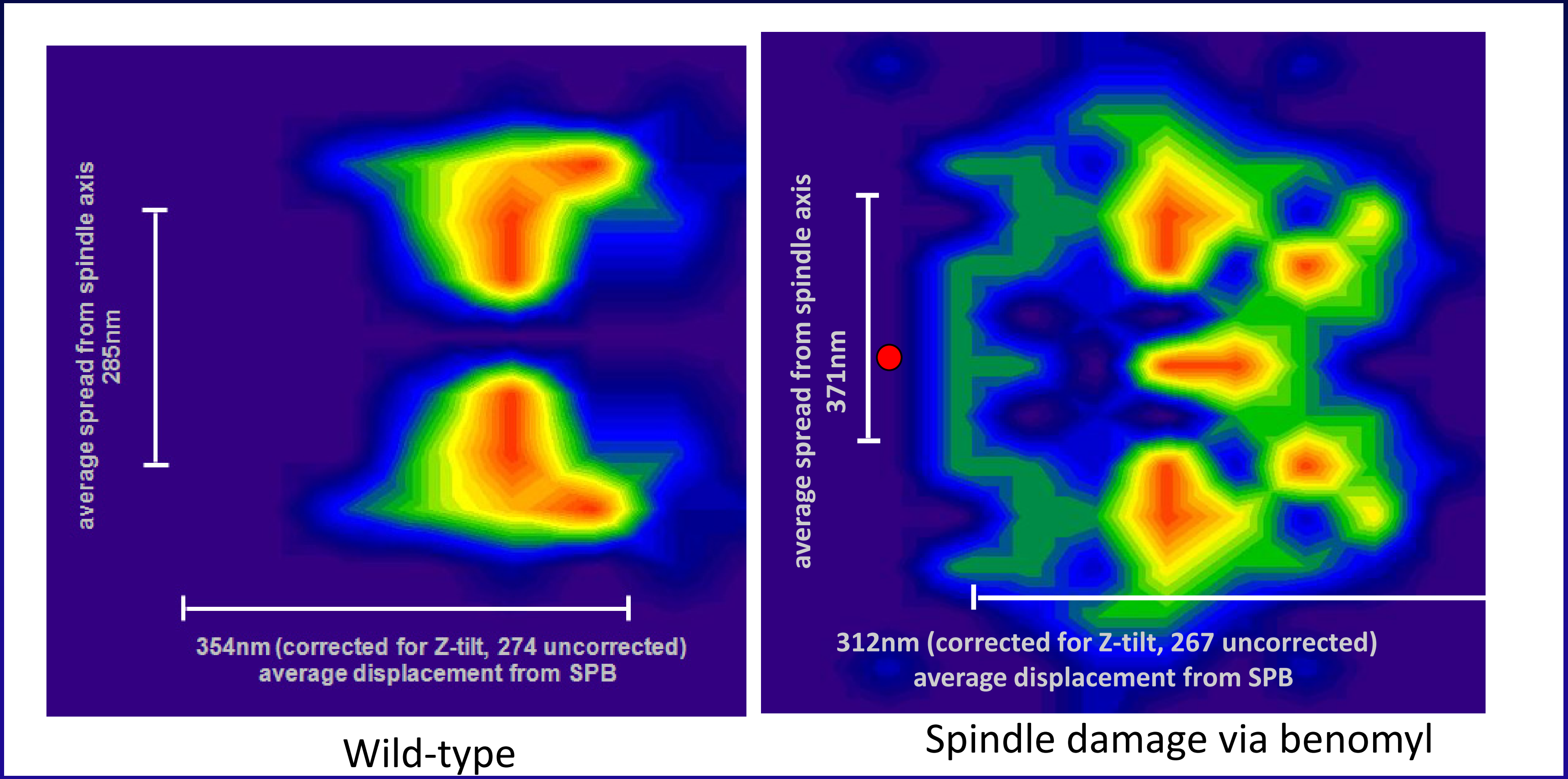
End-on view of cohesin barrel



The barrel expands and contracts when spindle damage is incurred

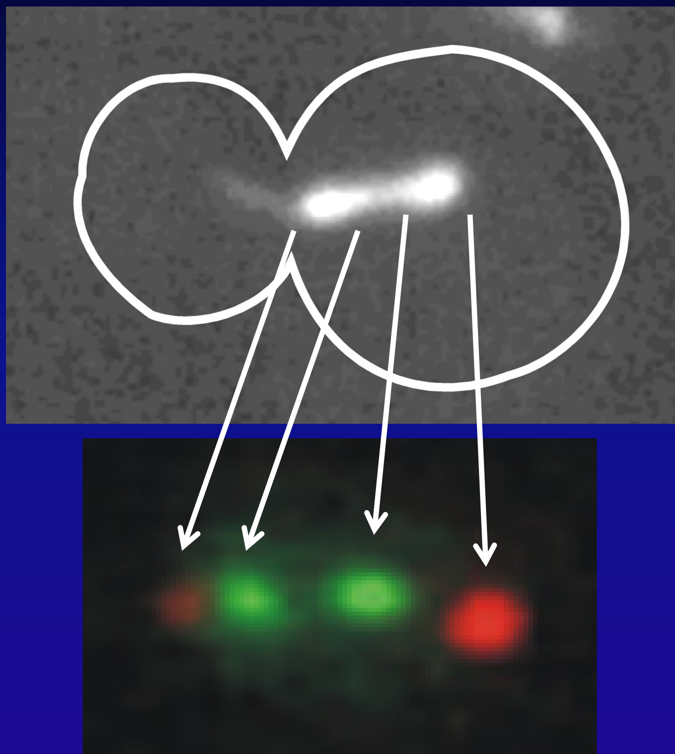


Statistical distribution of pericentric lacO relative to spindle pole (red circle)

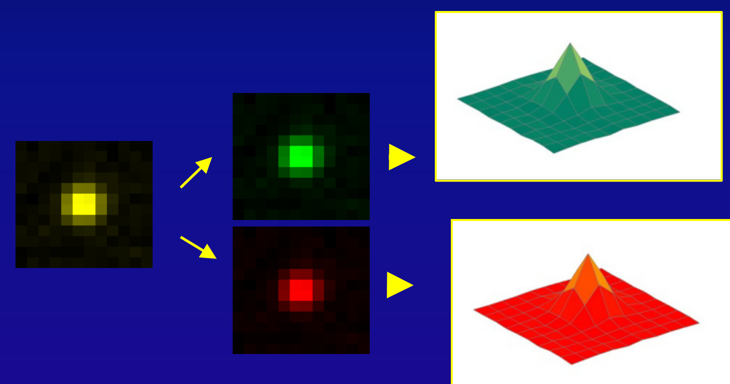


Pericentric chromatin expands its spatial range in cells with larger cohesin barrels

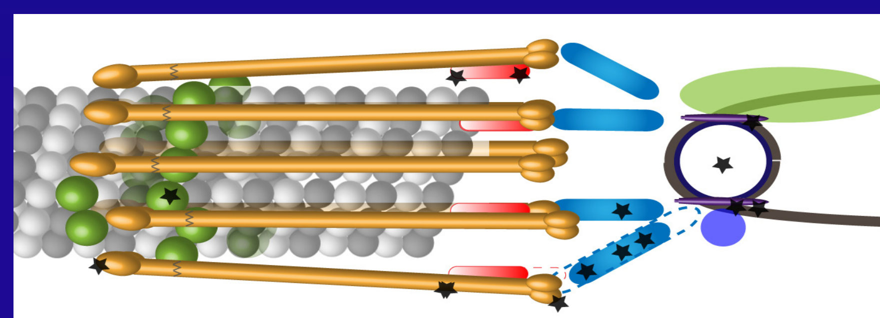
The inner kinetochore contracts along the spindle axis



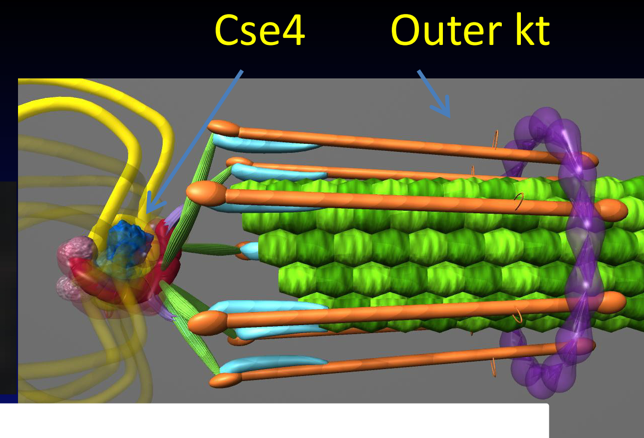
Yeast spindle visualized with Tubulin-GFP   1.5 µm

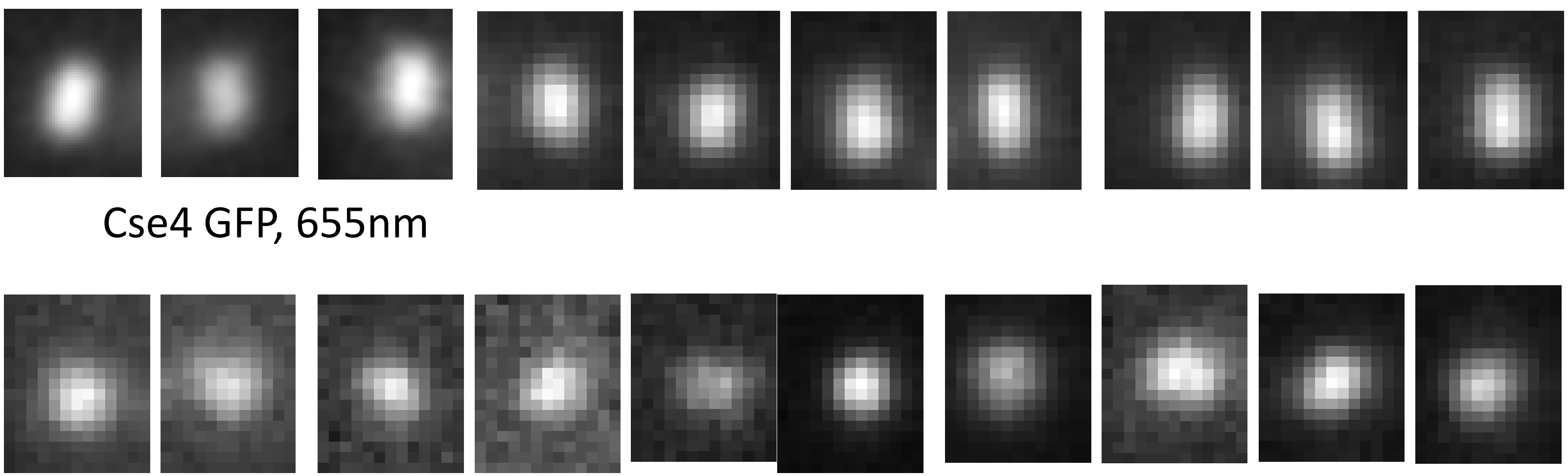


Fluorescent proteins fused to spindle poles (red) or kinetochores (green)



Cse4 Anisotropy in metaphase





Cse4 GFP, 655nm

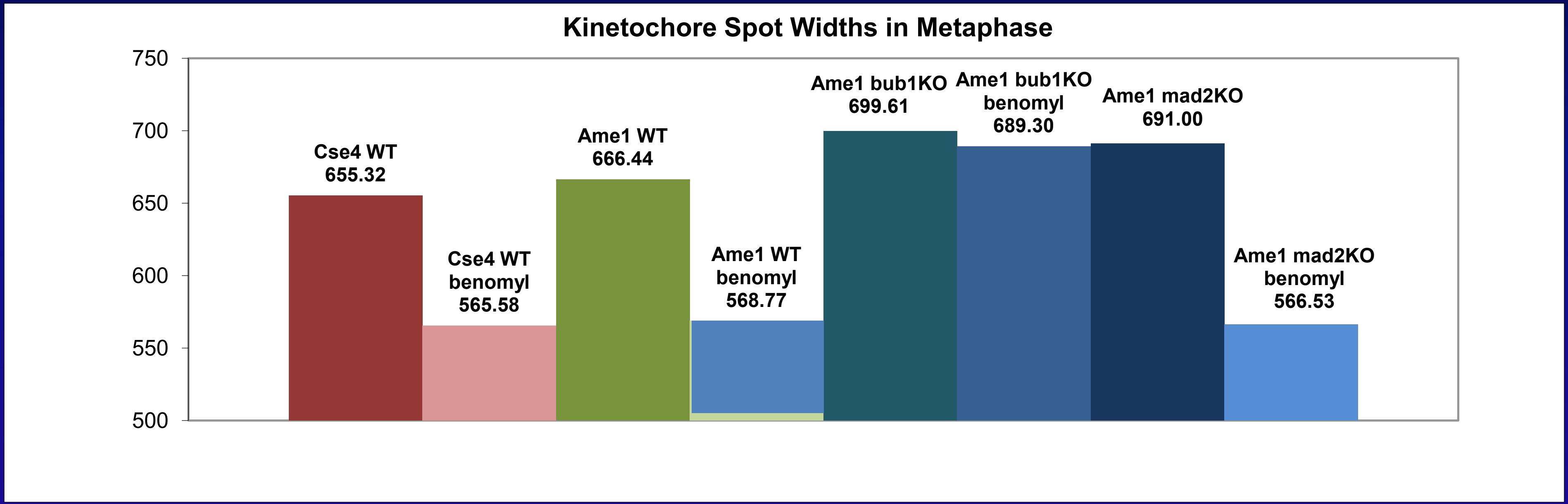
Outer kinetochore GFP, 581nm

Anisotropy - Not homogeneous is all directions

Cse4 is wider perpendicular to the spindle axis and narrow parallel to the spindle axis

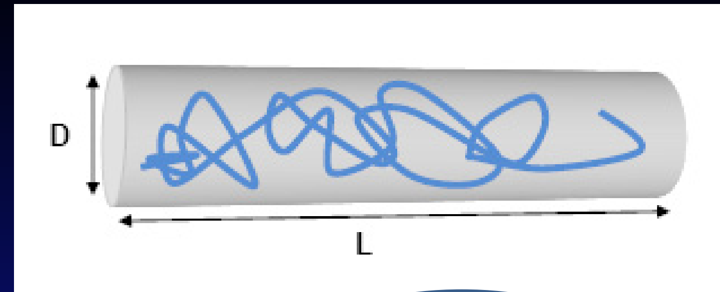
Outer kinetochore proteins are isotropic in metaphase

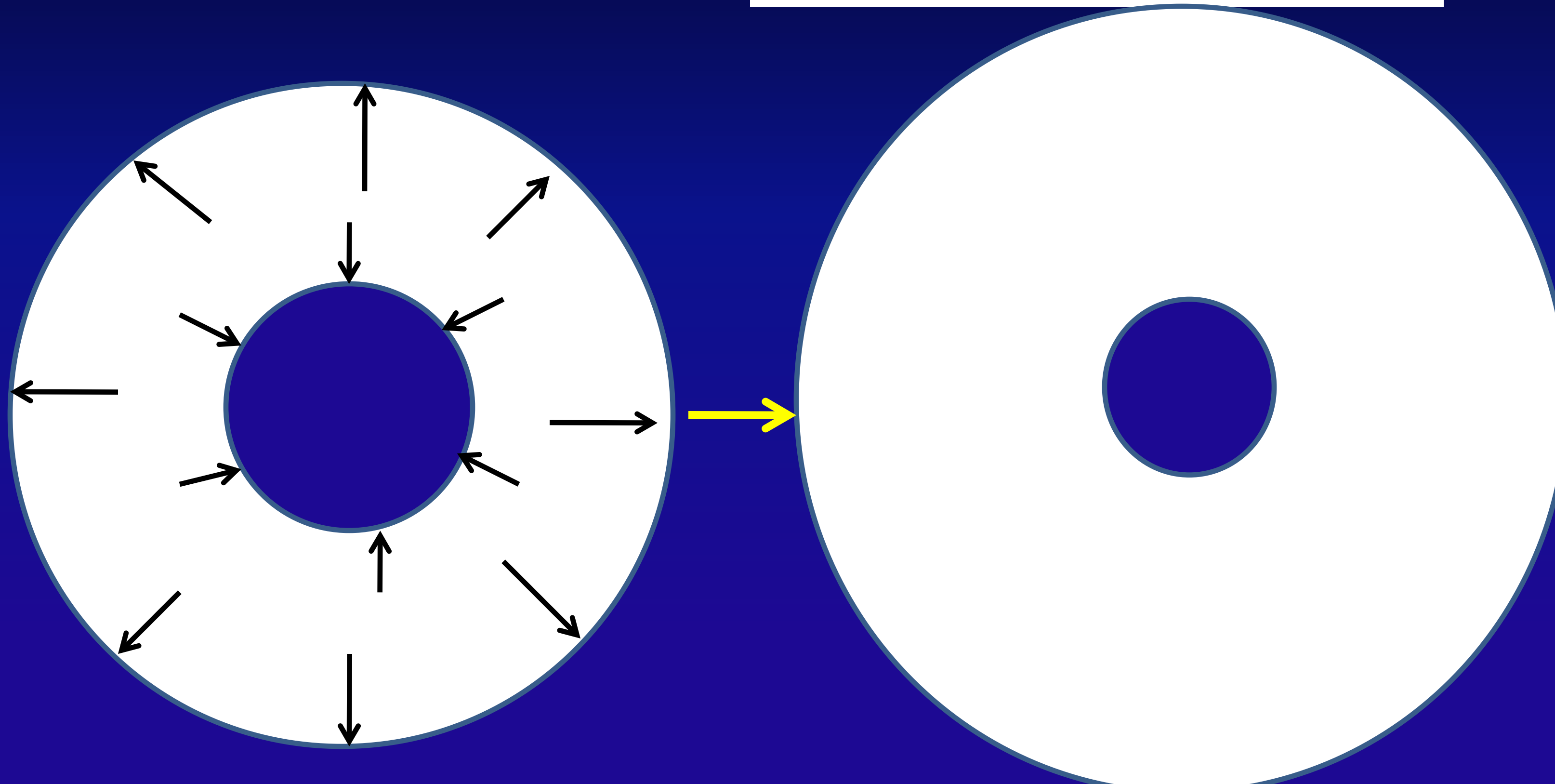
Contraction of inner kinetochore upon mitotic spindle damage



Both cohesin cylinder expansion as well as inner kinetochore contraction are dependent on the action of the Bub1 kinase that phosphorylates Histone H2A at position S121

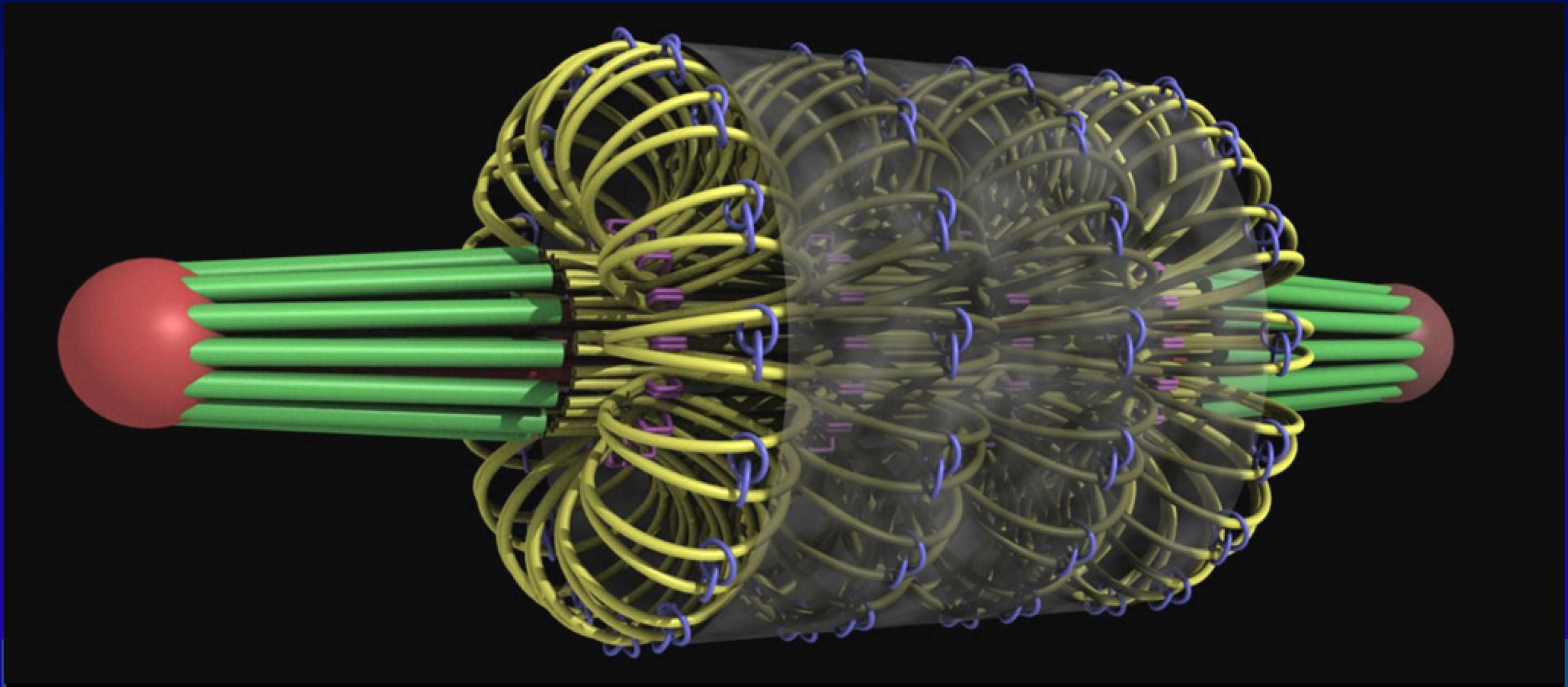
Change in cohesin and kinetochore following spindle damage





Increased pressure could result in increased outer diameter (cohesin) and decreased inner diameter (kinetochore)

Function of pericentric cohesin is to form a "skin" around the spindle and confine pericentric chromatin. This results in amplification of tension axially along the spindle



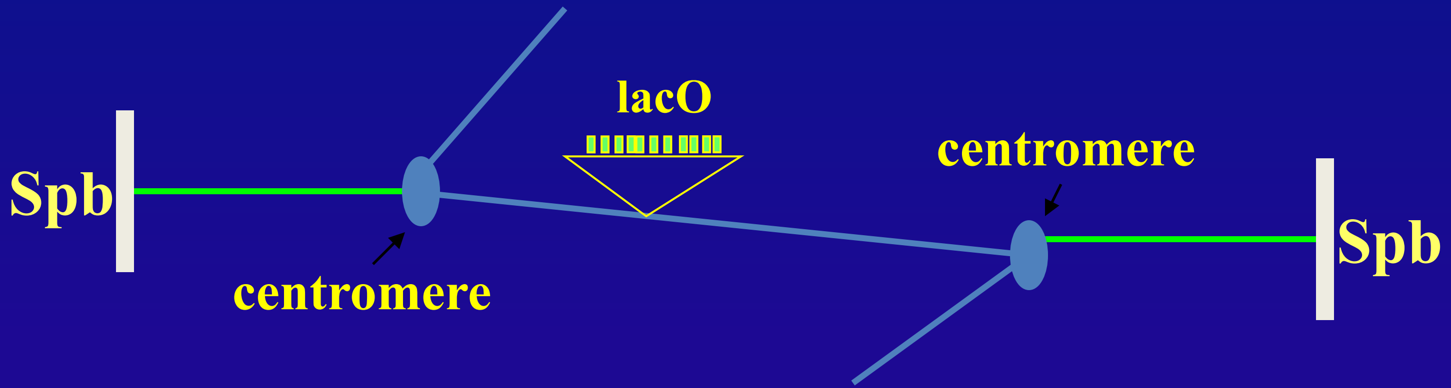
Spindle pole body (red), Microtubules (green), DNA (yellow loops), cohesin (blue rings), Condensin (purple rings)

Emergent Properties from Constraint: Polymer Repulsion

Are *in vivo* forces commensurate with a confined polymer model?

Single molecule DNA measurements *in vivo*

The dicentric chromosome: Harnessing force from the mitotic spindle to stretch DNA



Compare *in vivo* experiments to *in vitro* results with force microscope

Chromosome breakage in mitosis: Chromatin recoils to spindle pole. The rate of recoil is proportional to the spring constant

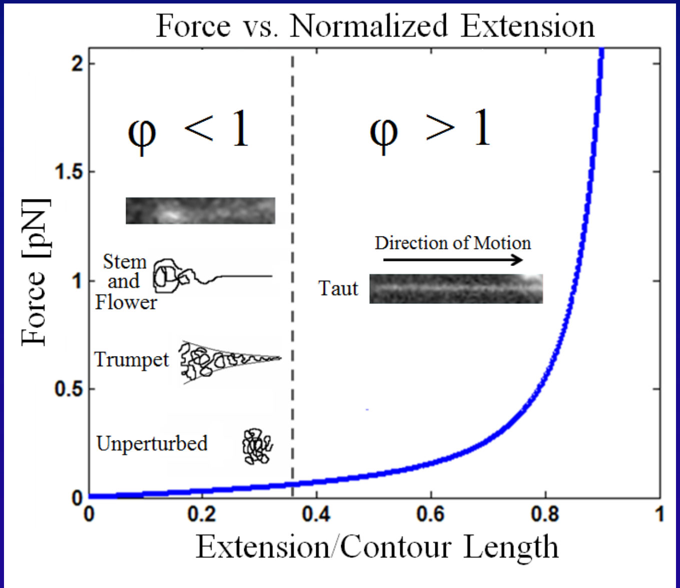
Tension force determination from *in vivo* relaxation

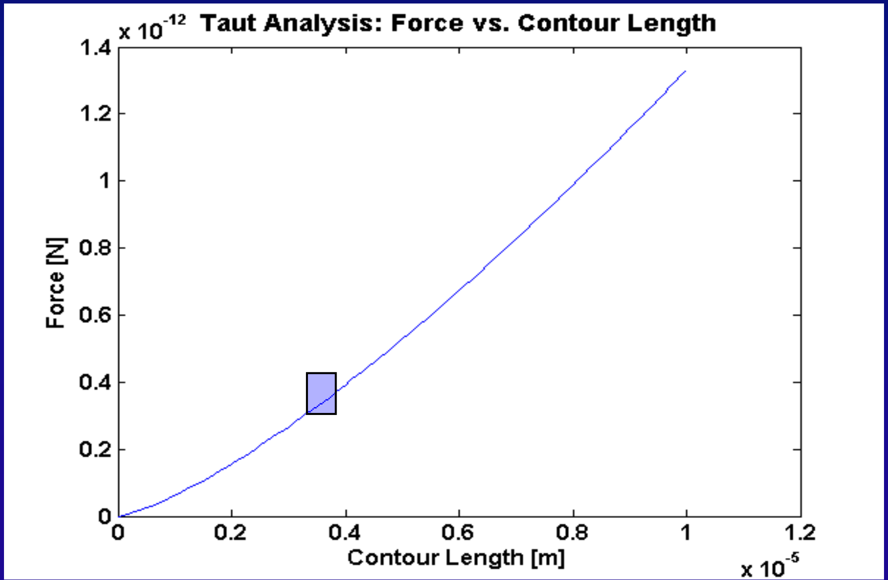
For a chain stretched under a uniform tension force:

$$F = L^{4/3}\, \eta^{2/3}\, l_{po}^{\;5/3} / \, k_B T^{5/3}\, \tau^{2/3}$$

L = chain length, η= viscous force, l_{po} = persistence length

P. de Gennes, Brochard-Wyart, R. Europhys. Lett., (1999) 47: 171

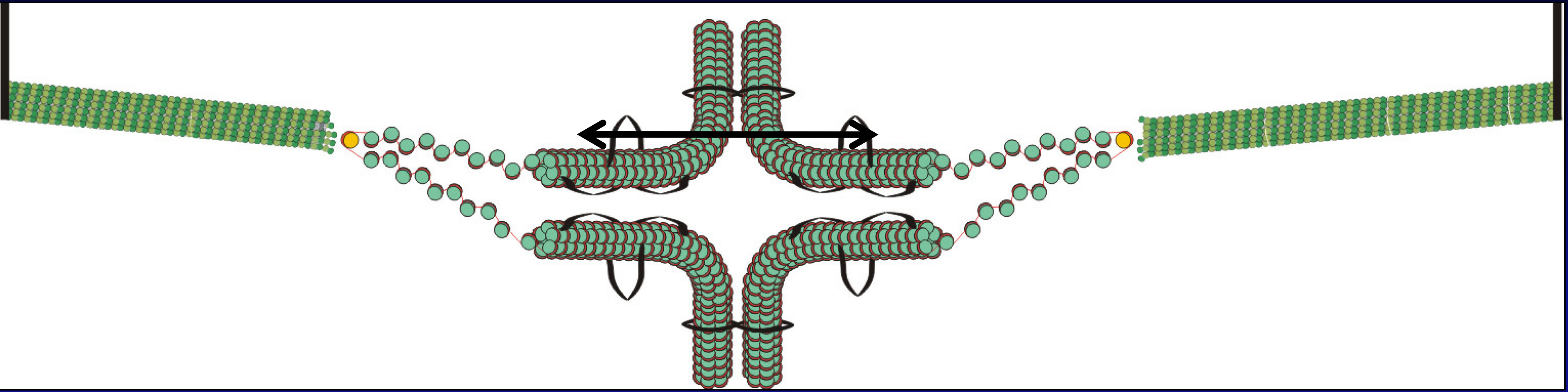


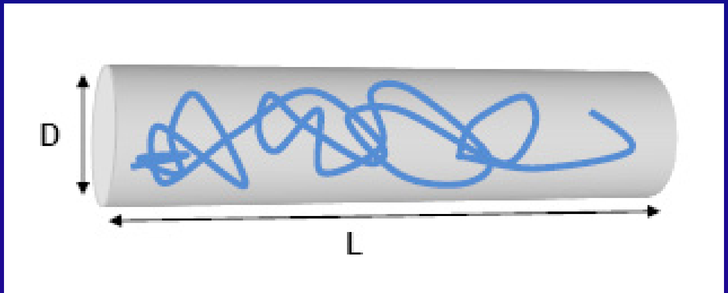


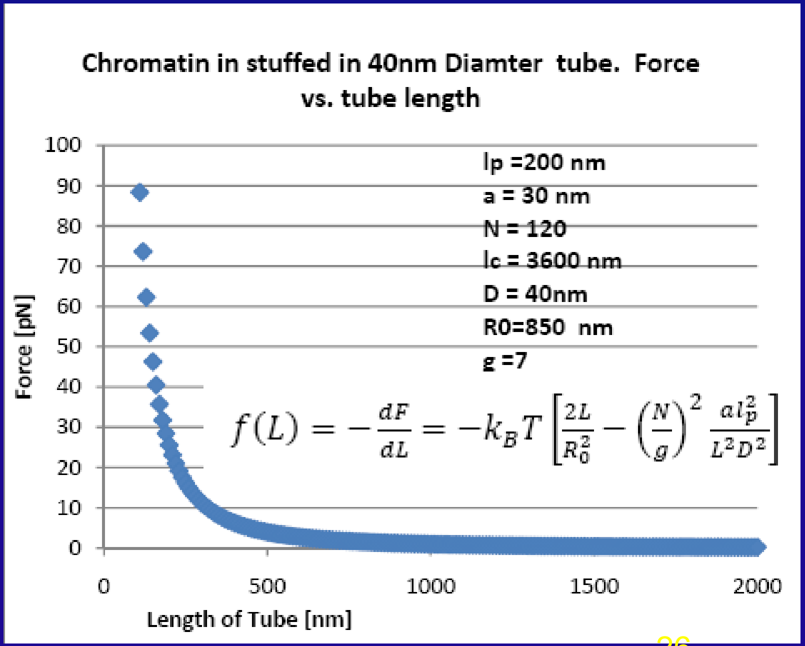
Tension Force = .3 pN (L = 3.3), over a range of parameter space

Fisher et al., PNAS 2009

Tension amplification due to confined geometry

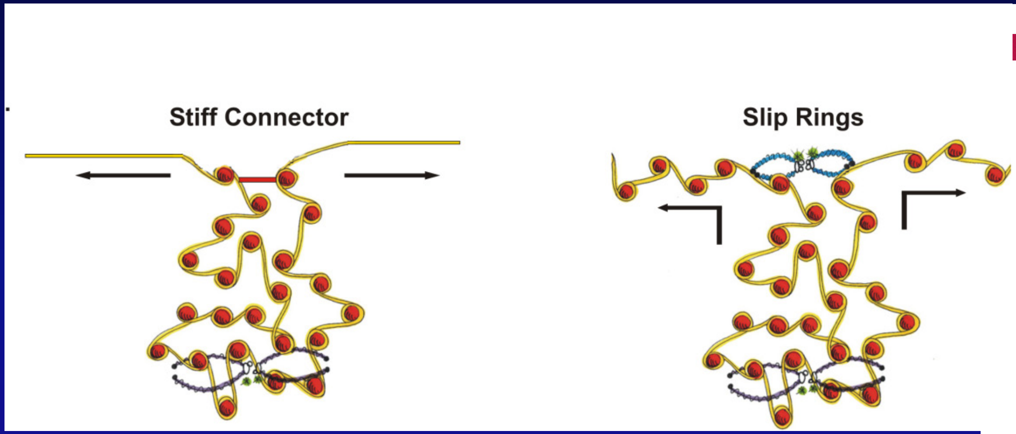


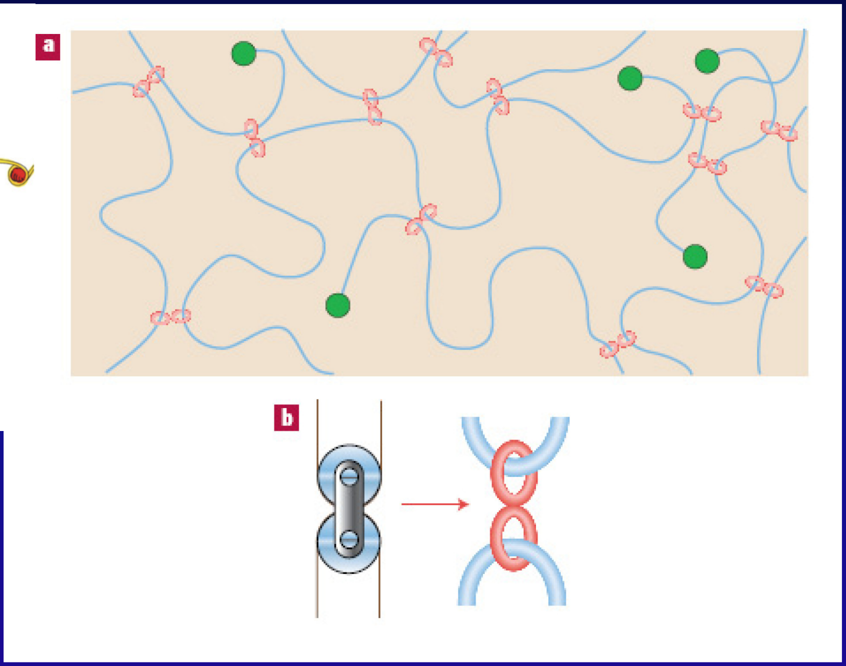




Brochard-Wyart, de Gennes, Langmuir 2005

The Polyrotaxane Gel: A topological gel of figure 8 cross-links





Do cohesin/condensin act as slip-rings providing a mechanism to distribute tension?

Okumura,Y. & Ito, K. *Adv. Mater* **13,** 485–487 (2001).
Granick and Rubinstein *Nat Mat.* 3 584-587 (2004)

DNA is an entropic spring (e.g. a rubber band)

There is a DNA based spring in the mitotic spindle

The spring can be tuned depending on cellular conditions

The tuning mechanism is chromatin modification via Bub1 phosphorylation of histone H2A-S121

There are very small (sub-piconewton) forces/tension *in vivo.*

The spindle may be gently guiding the centromere to the spindle pole and largely relying on thermodynamics of the system.

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Elaine Yeh

Postdoctoral Fellow
Ajit Joglekar

Graduate Students
Jolien Verdaasdonk
Andrew Stephens
Marybeth Anderson

Technician
Julian Haase

Undergraduates
Matt Larson
Fu Shih

Collaborators and Guidance

Biology
Ted Salmon

Physics
Mike Falvo
Jay Fisher
Rich Superfine

Chemistry
Michael Rubinstein

Computer Science
Leandra Vicci
Russell Taylor II

Research Associate

Elaine Yeh

Postdoctoral Fellow

Ajit Joglekar

Graduate Students

Jolien Verdaasdonk

Andrew Stephens

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Technician

Julian Haase

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Matt Larson

Fu Shih

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