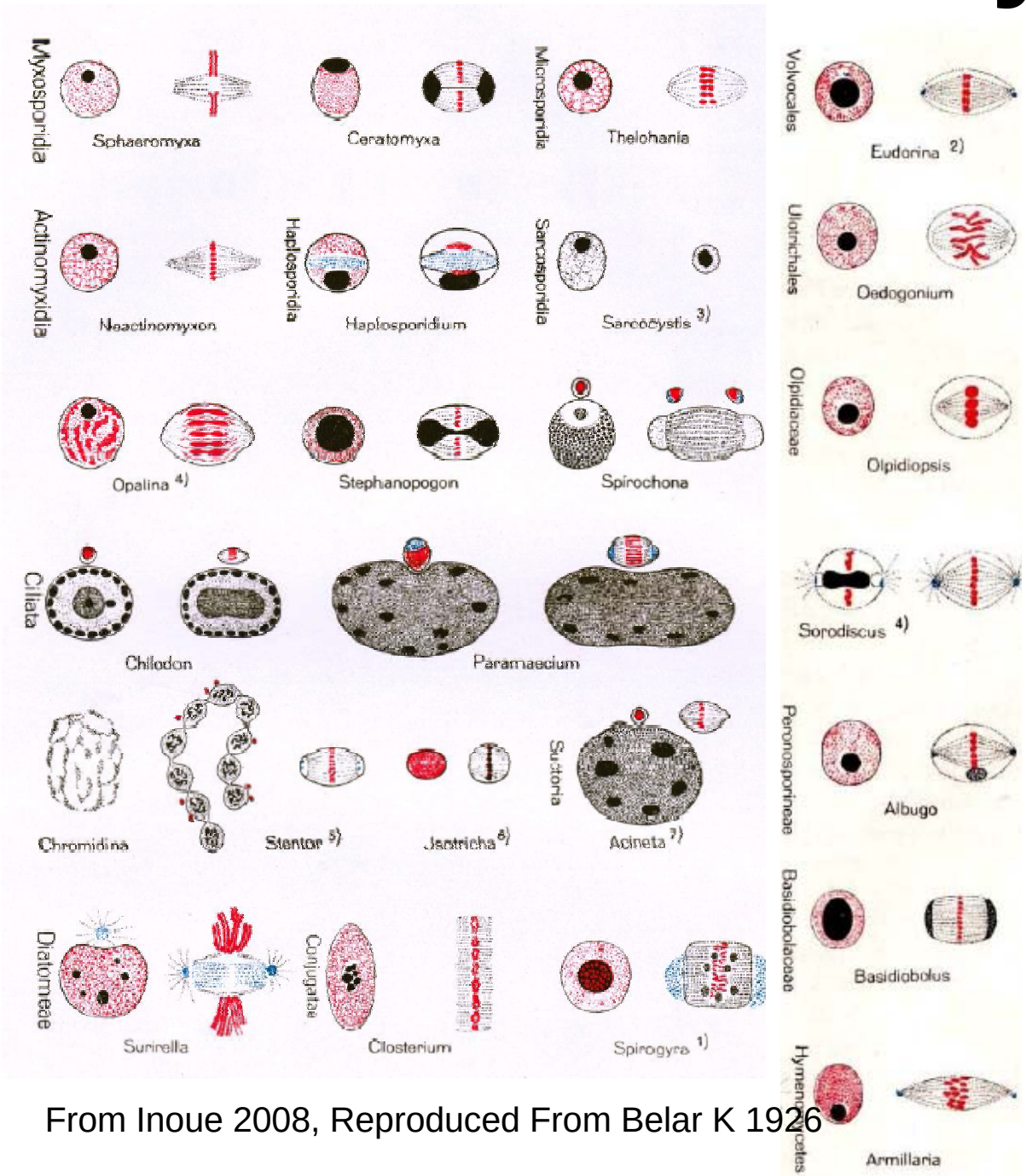
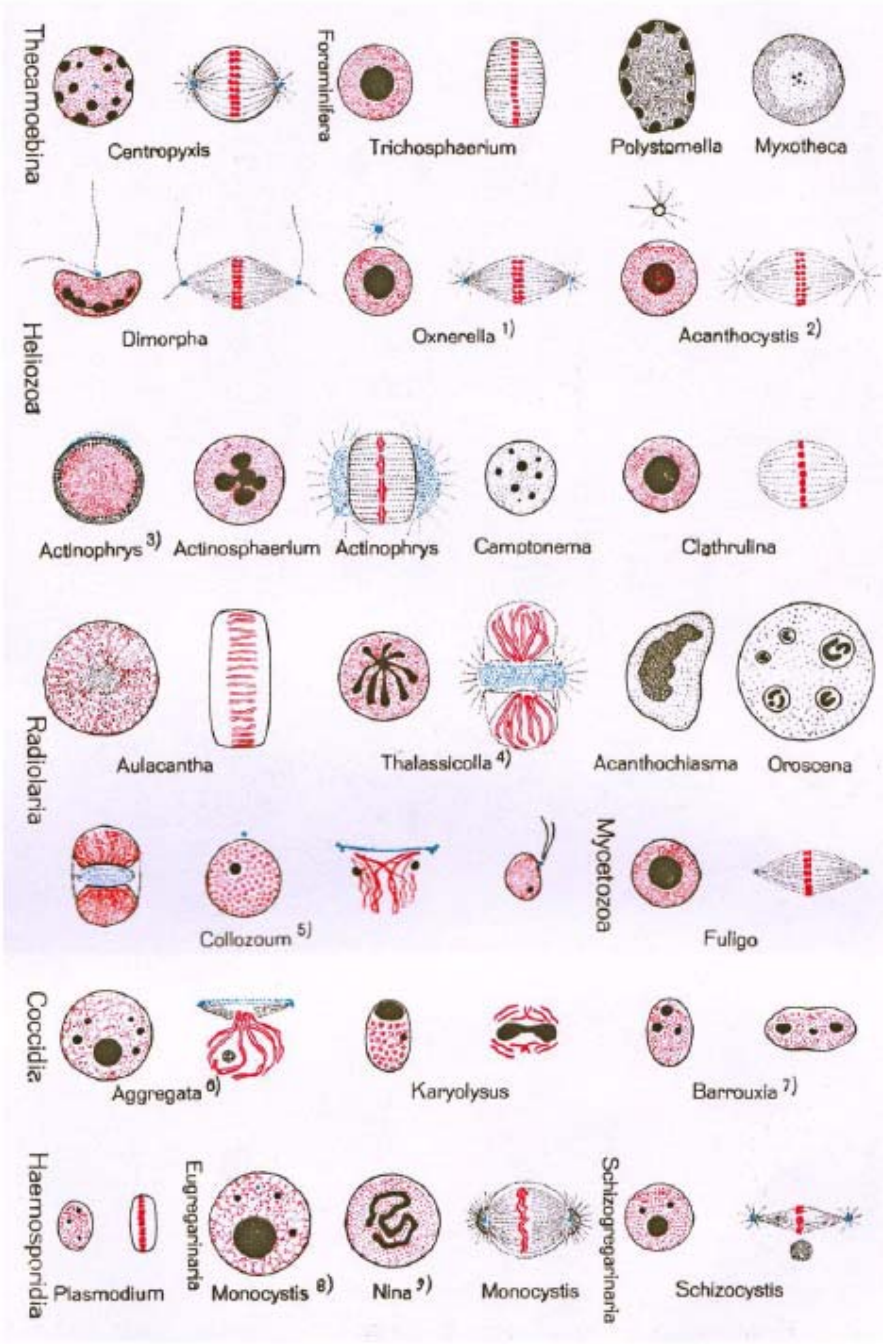
The Diversity of Division





From Inoue 2008, Reproduced From Belar K 1926

My Motivation:

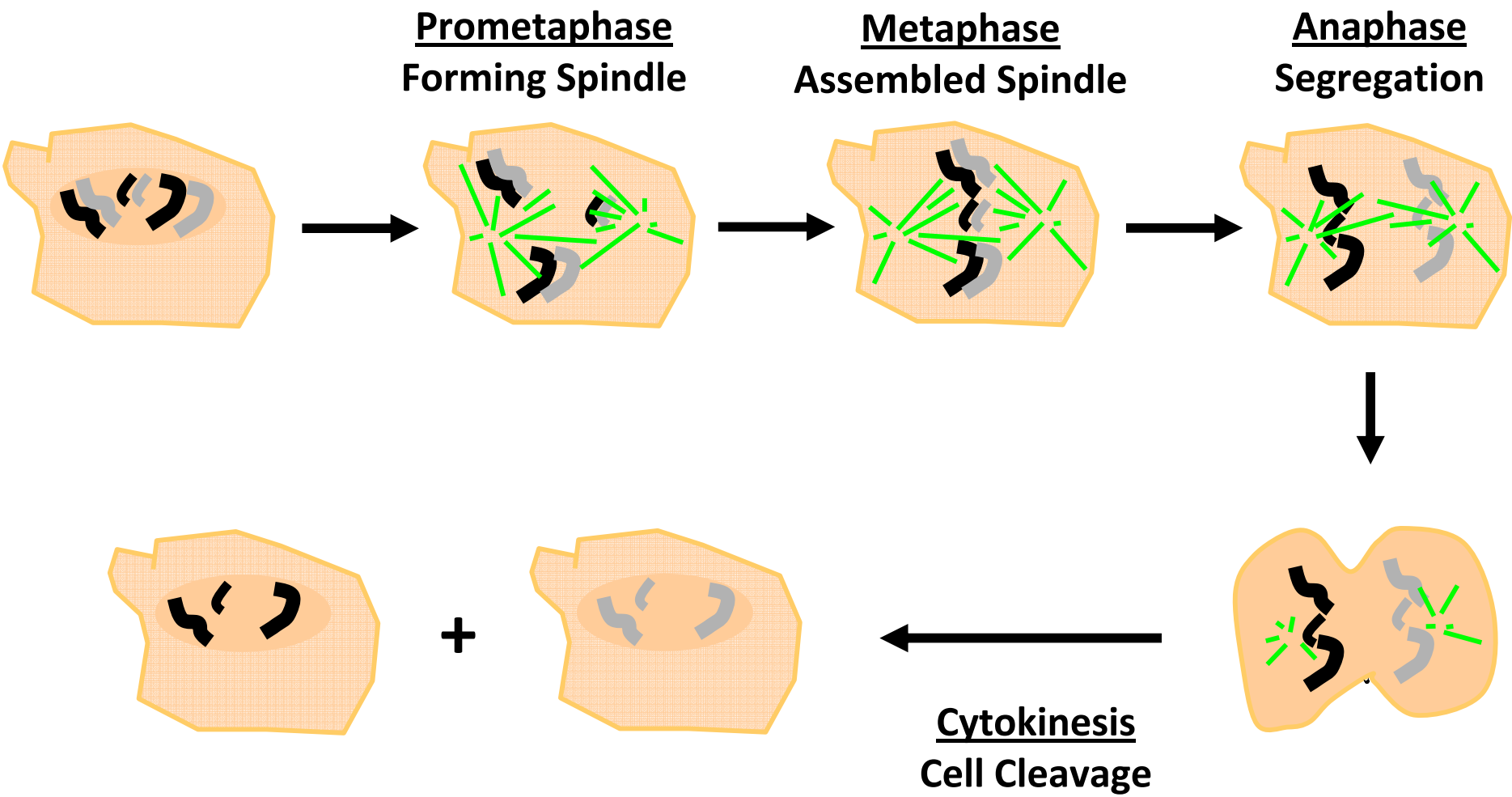
1) What Aspects of Spindle Self-Organizatio
Biologically Important?

---

2) How to Explain Existing Diversity of Spir
Organization and Dynamics?

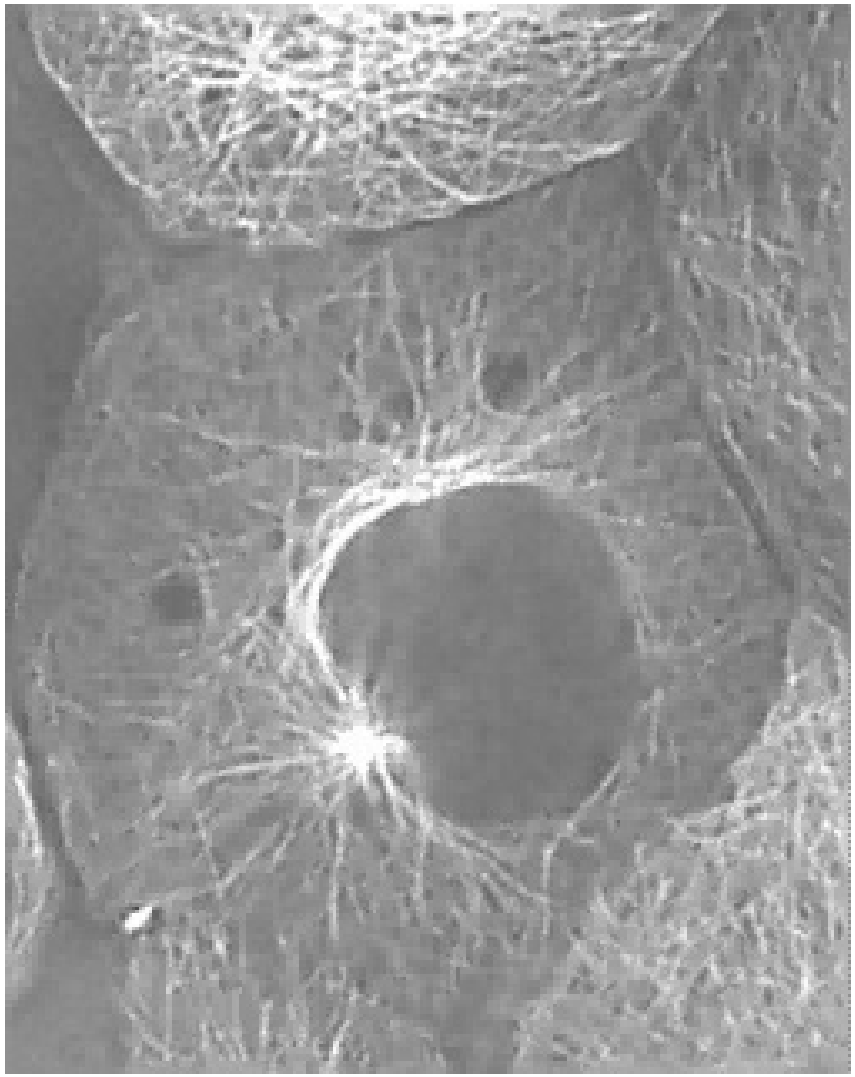
3) How to Think about the Evolution of
Self-Organizing Structures?

Textbook Mitosis



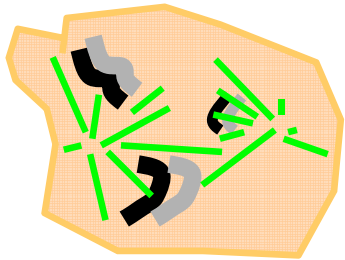
Textbook Mitosis

LLC-PK1 Cells (Pig epithelial tissue culture)
GFP-tubulin

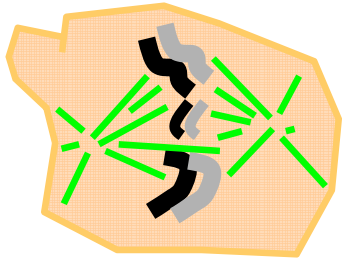


Patricia Wadsworth
UMass Ahmerest

Prometaphase



Metaphase



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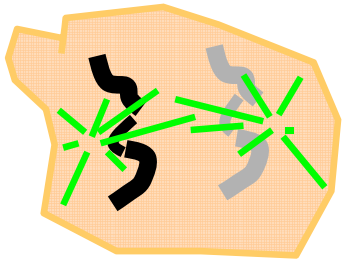
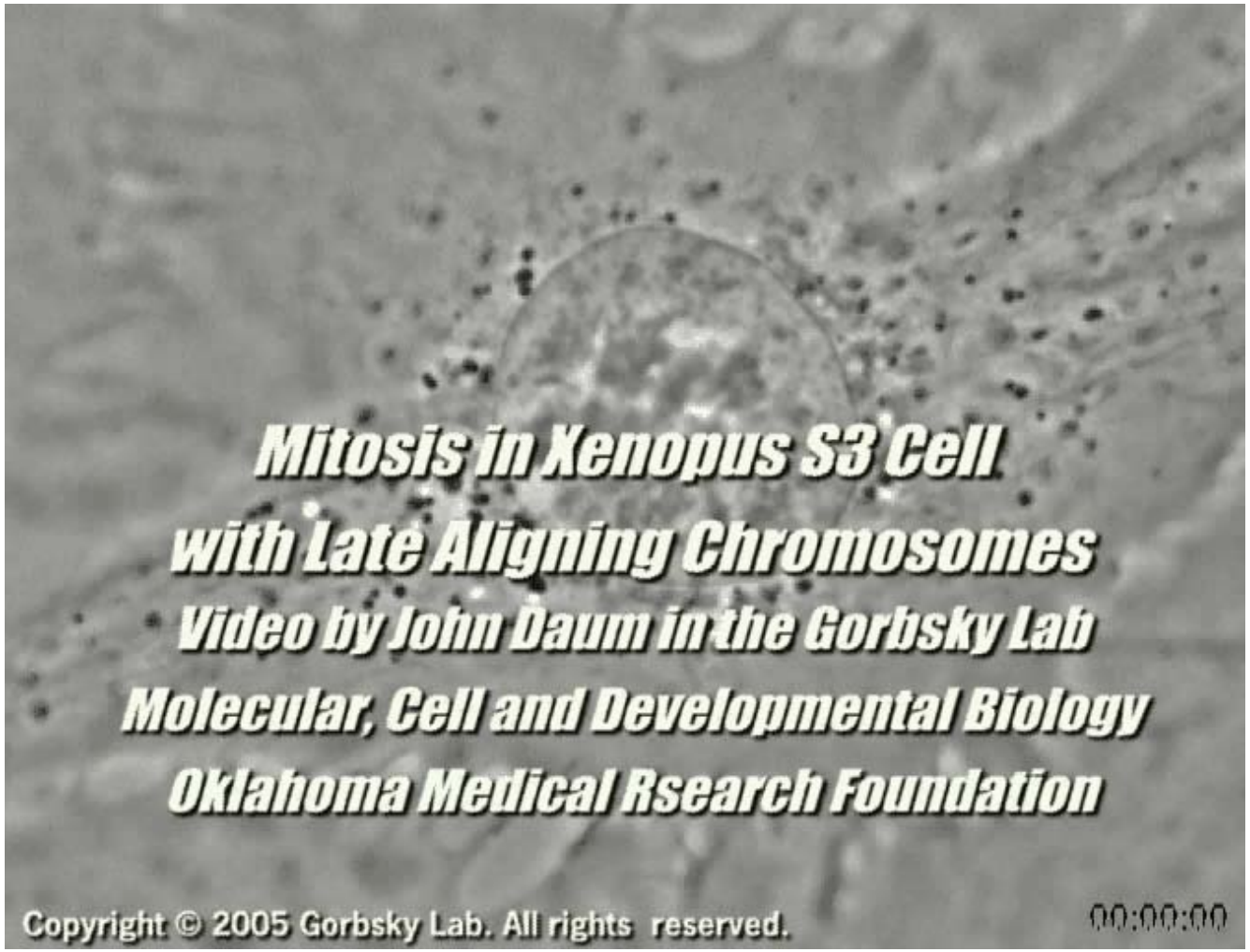


Textbook Mitosis



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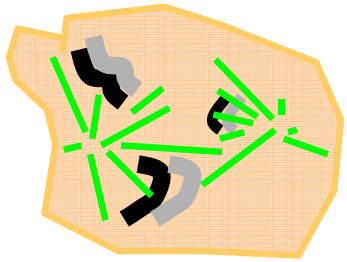

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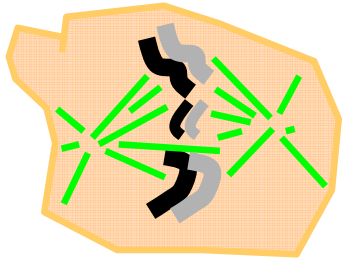

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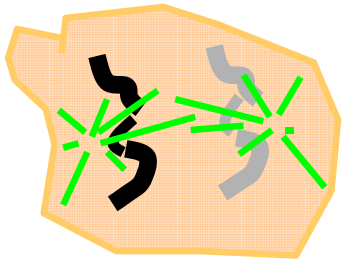
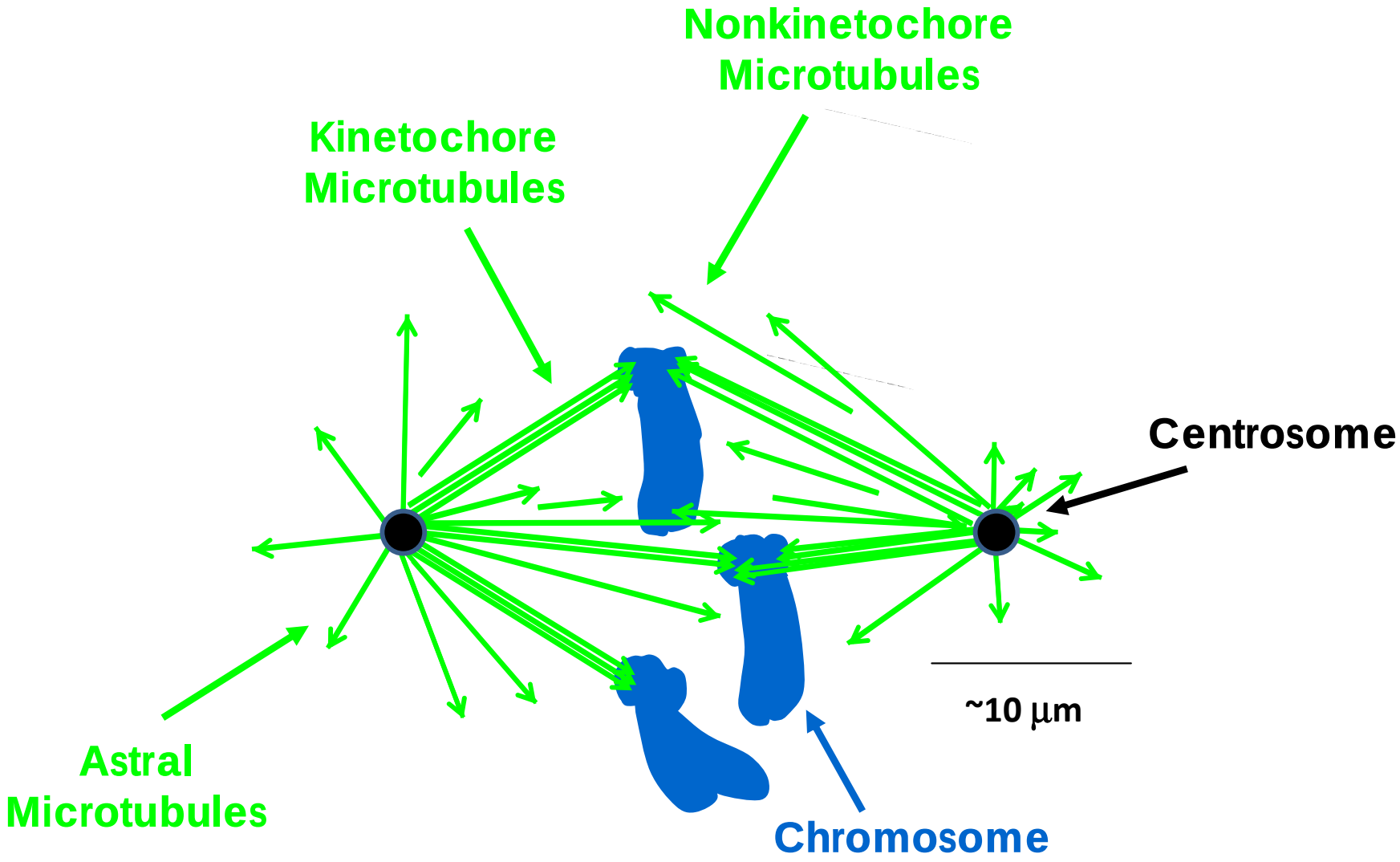


By John Daum (Gorbsky Lab)
Xenopus Tissue Culture

Textbook Mitosis

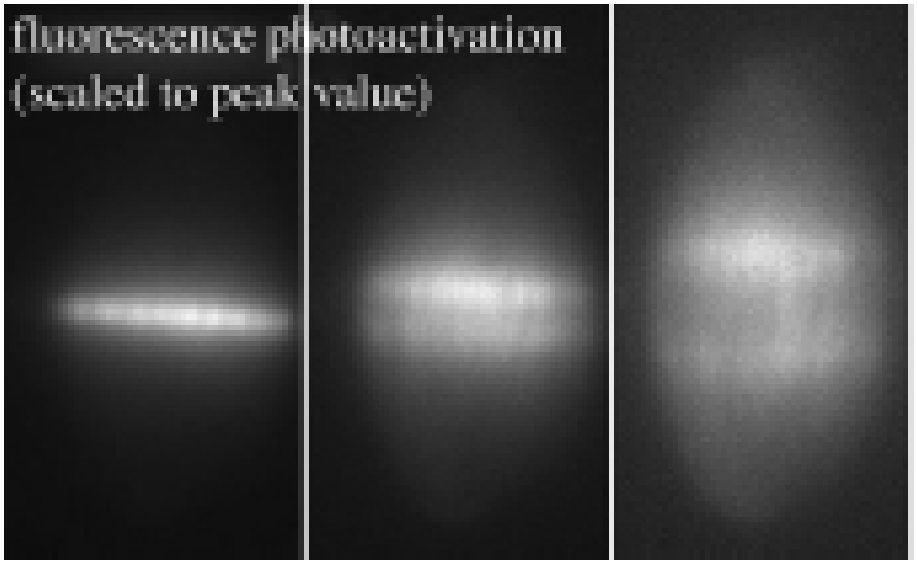
The Metaphase Spindle



Textbook Mitosis

The Metaphase Spindle is Highly Dynamic

Tubulin:



Mitchison, JCB, 1989, 109, 637

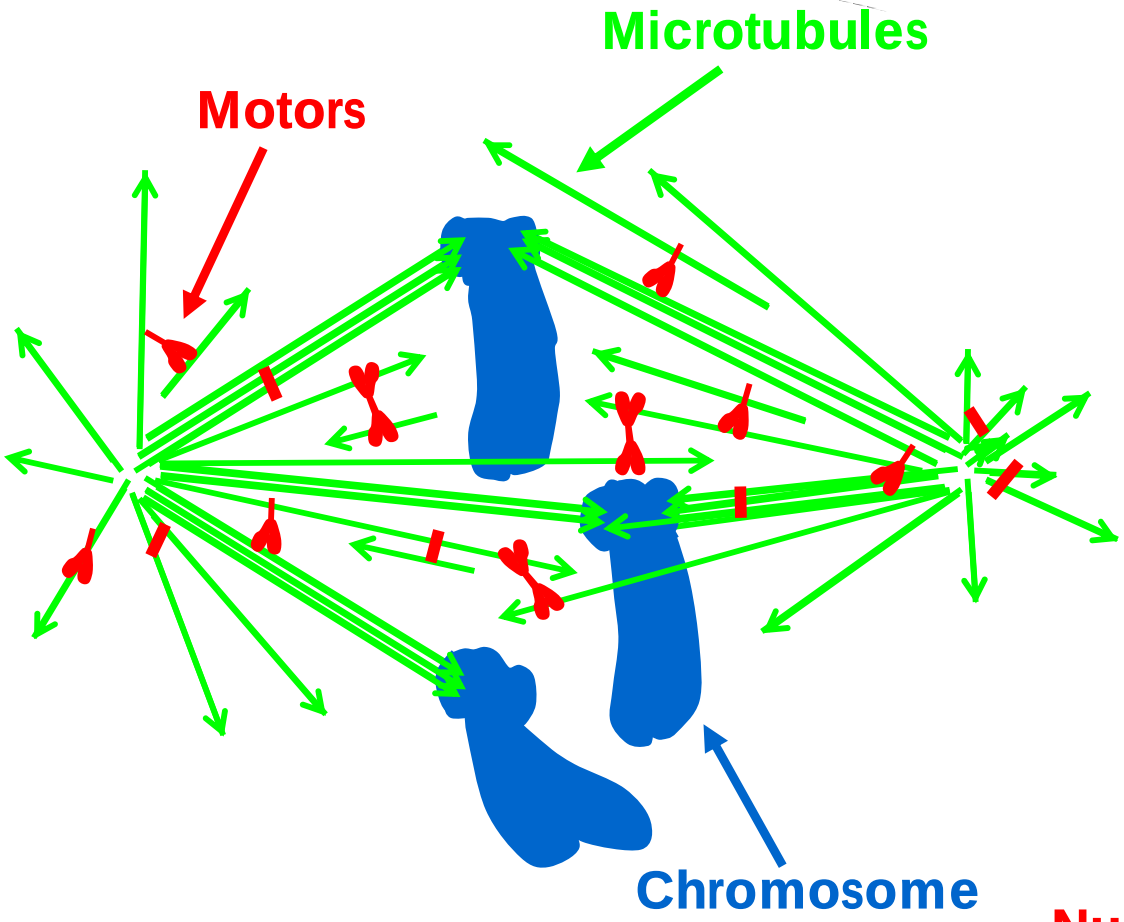
The Metaphase Spindle Can Exist at Steady-State for Hours but. . .

1) Constant Motion (flux) of Tubulin from Chromosomes to Poles

2) Rapid Turnover of Microtubules Half-life ~20 seconds

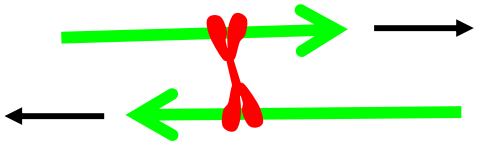
Textbook Mitosis

A Wide Variety Proteins Organize Microtubules in the Spindle

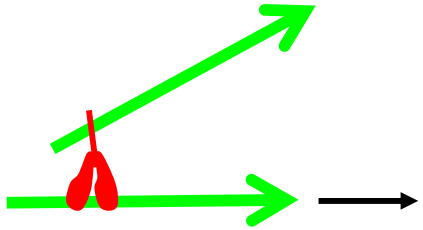


Motors

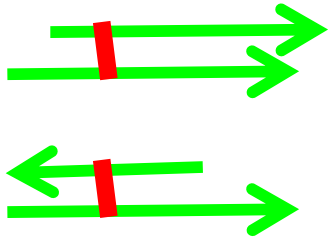
Slide Anti-Parallel Microtubules



Slide Parallel Microtubules

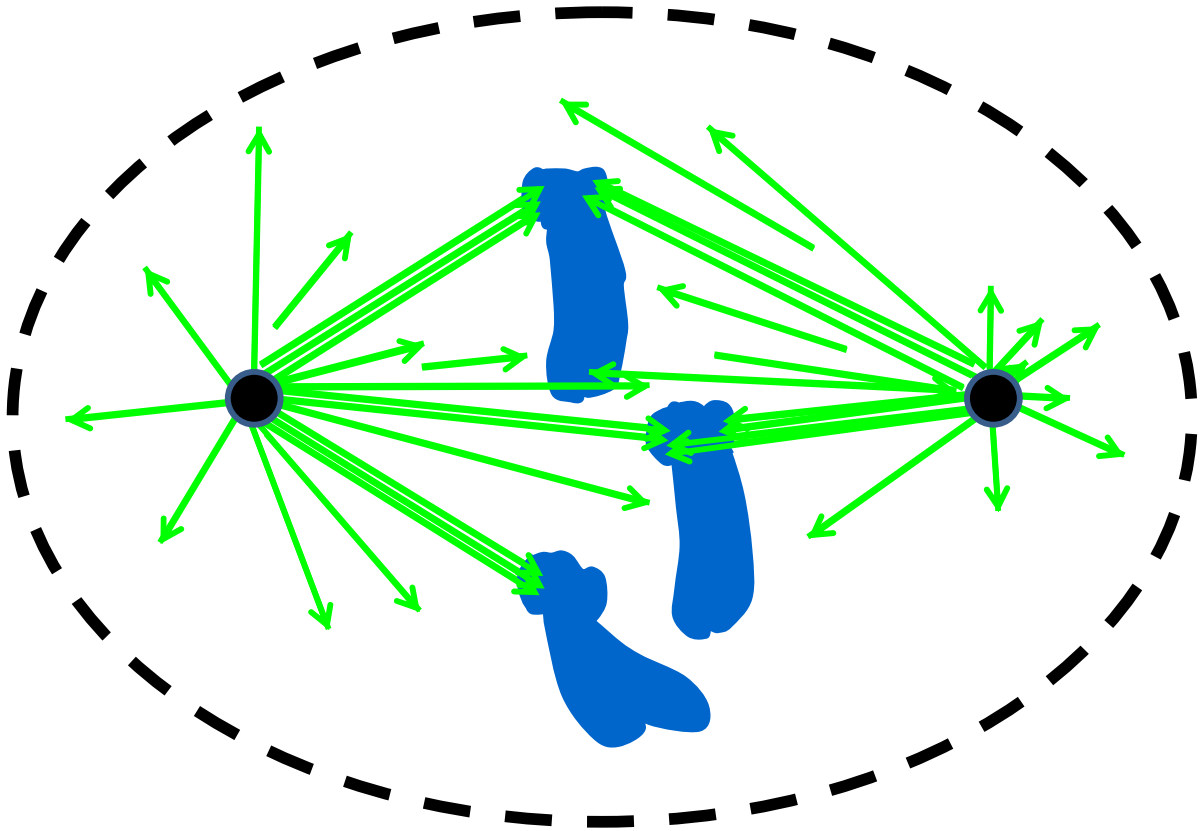


Cross-Linkers



Nucleators, Stabilizers, Destabilizers

Variation in Nuclear Envelope Breakd



Variation in Nuclear Envelope Breakd

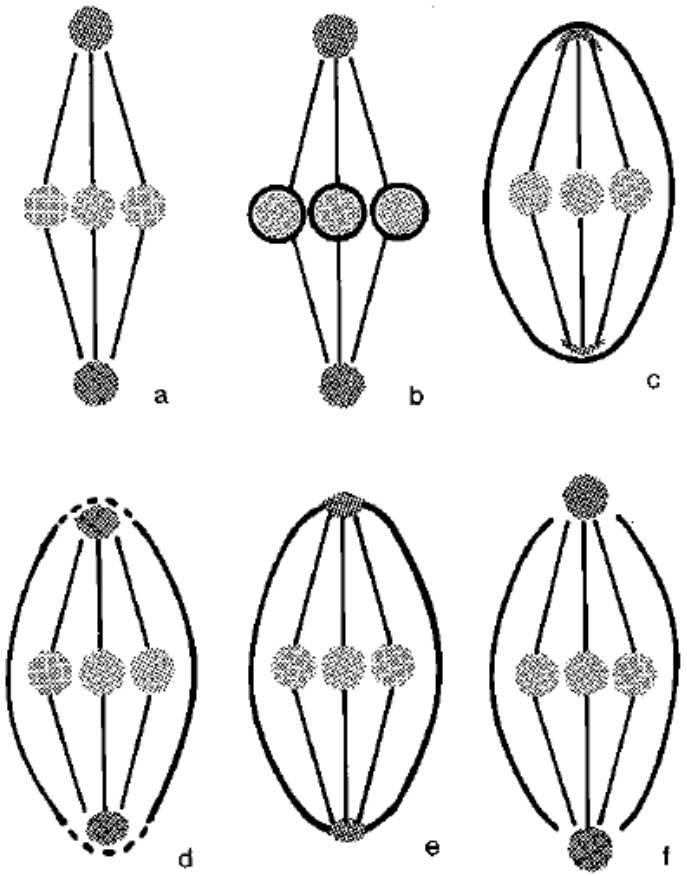


FIG. 1. Behavior of the nuclear envelope during mitosis. Light stippled circles represent the chromosomes, heavy stippled circles the polar structures, straight lines the spindle, and the heavy line the nuclear envelope. In (a) the envelope disperses, usually at prophase, to give an open spindle (see "di P, M, A, or T" in Table II); (b) represents a rare situation, only reported for *Stylocephalus*, in which the nuclear envelope disperses, but forms individual envelopes around each chromosome throughout mitosis; (c) and (e) are closed divisions with intranuclear spindle organizing or polar structures and membrane inserted NAOs respectively (see "intact" in Table II); (d) illustrates the "oc" behavior of Table II in which the membrane seems to transiently open to admit the NAO, then reseals around it during mitosis; (f) represents the polar fenestrate ("pf" in Table II) type of behavior where the cytoplasmic NAOs lie in large openings of the nuclear envelope through which the spindle was formed.

Heath 1980

Open

Complete Nuclear Envelope Breako

Textbook Metazoan

Closed

Spindle Inside Nucleus

S. cerivisia, S. pombe

Other

Many, many varieties

In myxomycetes, both closed and open division occur, depending on the stage of the life cycle

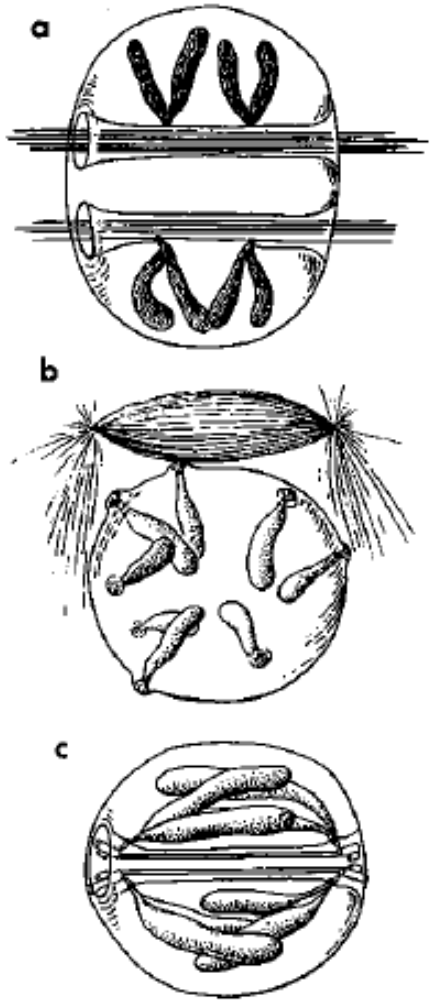


FIG. 1. The various forms of extranuclear spindles. (a) In dinoflagellates extranuclear microtubules occur in several parallel cytoplasmic channels which traverse the nucleus. Chromosomes are attached to the nuclear envelope surrounding channels. In some species a dense knoblike differentiation is found in the region of chromosome-membrane contact, and a single microtubule terminates at this structure (as illustrated in lower channel; cf. Fig. 5). In other species no such indications of microtubule-chromosome contact have been found (upper channel). (b) In hypermastigotes extranuclear microtubules are massed in a well-defined spindle. During a significant portion of chromosome movement, kinetochores are enclosed in pouchlike evaginations of the intact nuclear envelope and are not in contact with microtubules (cf. Figs. 2 and 3). (c) In *Syndinium* sp. the extranuclear spindle occurs within a single cytoplasmic channel which traverses the nucleus. Kinetochores protrude through porelike openings in the nuclear envelope and are connected to centrioles via chromosomal microtubules. Chromosome movement is produced as intercentriolar microtubules elongate (cf. Fig. 4).

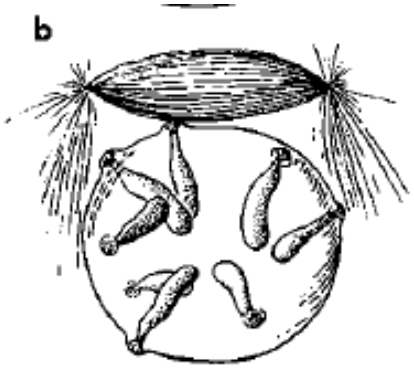
Dinoflagellates

Microtubules Outside Nucleus
Chromosomes Inside Nucleus
No Traditional Spindle

Hypermastigotes

Spindle Outside Nucleus
Chromosomes Inside Nucleus

From DF Kubai 1975



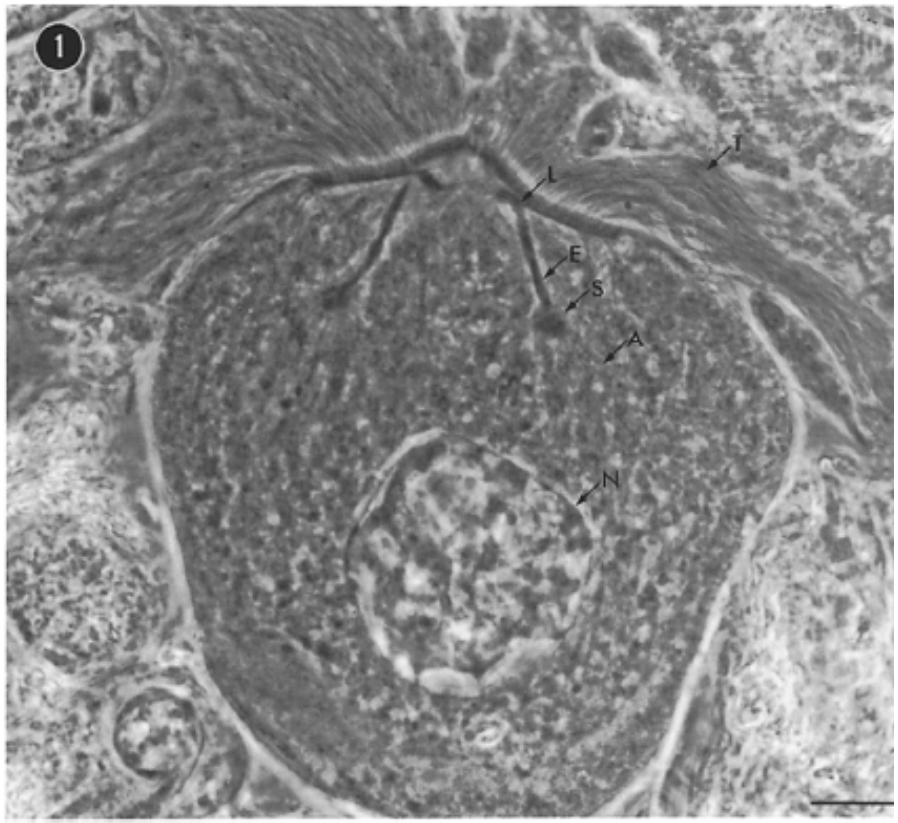


FIGURE 1 Phase-contrast micrograph of *Barbulanympha* very early in division. The nucleus (*N*) still lies some distance away from the two bulbous centrosomes (*S*). The centrosomes cap the distal tips of the elongate-centrioles (*E*) which are seen as prominent twisted rods at the anterior end of the organism. Asters (*A*) radiate from the centrosomes. The converging proximal ends of the two elongate-centrioles bend medially and merge with the parabasal axostylar lamellae (*L*), seen in this optical section as thin dark lines running at the base of the flagellar kinetosomes. The left and right flagellar tufts (*T*) have not yet started to separate. Many species of protozoa cohabiting the wood cockroach intestine crowd around the *Barbulanympha*. Bar, 10 μm. × 1,370.

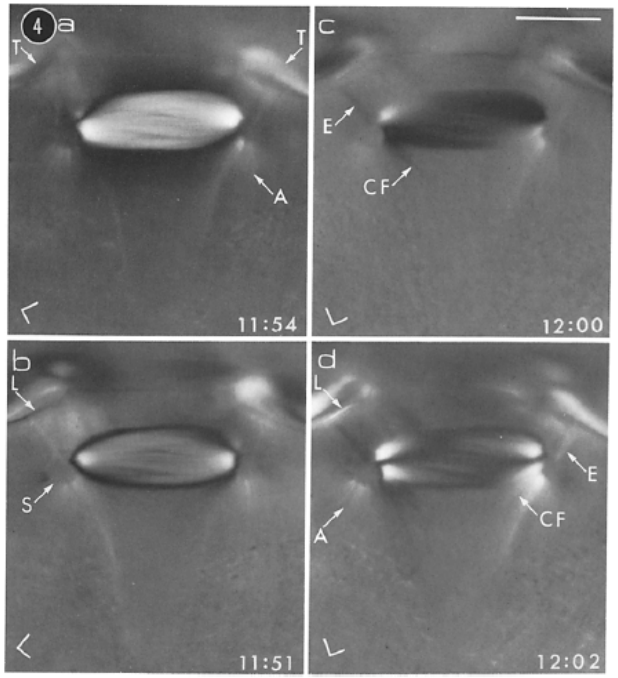
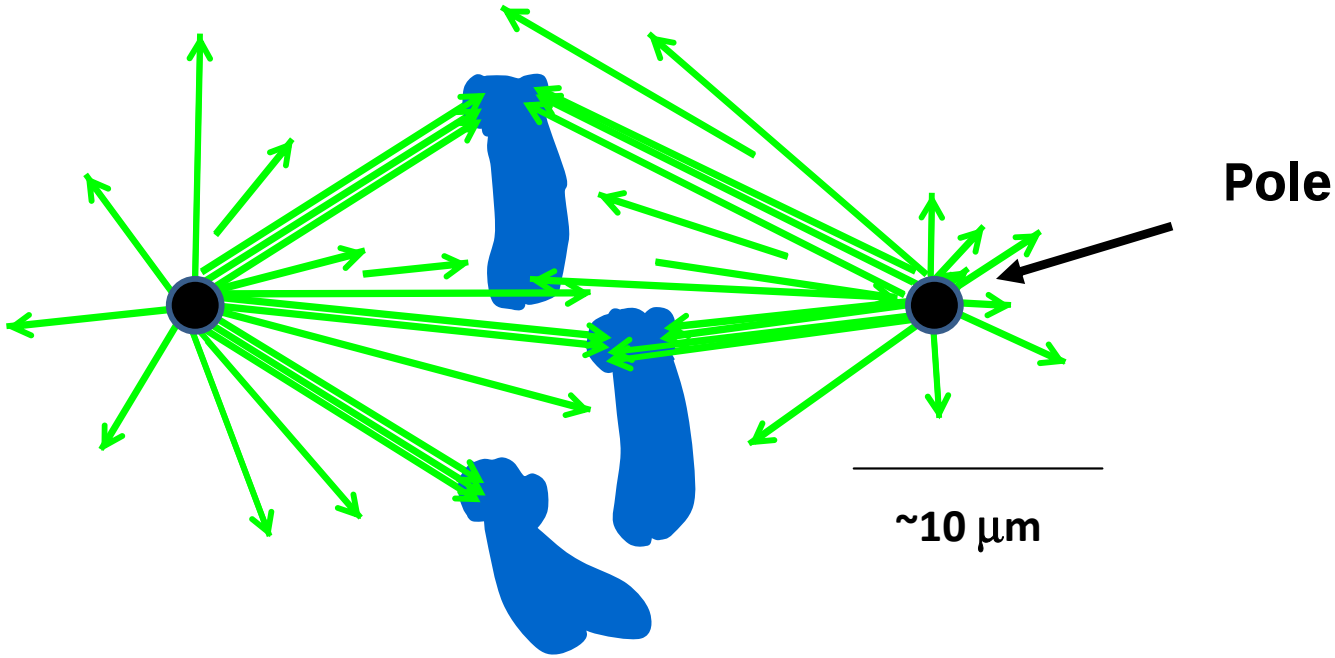


FIGURE 4 Birefringence of mature, central spindle in prophase. Flagellar tufts (*T*) and their basal structures are now sufficiently separated so that the elongate-centrioles (*E*) diverge anteriorly. All four pictures were taken with Nikon 40x rectified polarized light objectives, with crossed polars and compensator axes oriented variously relative to the spindle axis. *L*, Axes of crossed polarizer and analyzer and quadrant in which compensator slow axis is oriented. These photographs show how the central spindle is clearly composed of birefringent fiber bundles which are somewhat twisted relative to the spindle axis. Compare with electron micrograph Fig. 5. Where the concentration of fibers is greatest, adjacent to the centrosomal surface, individual fibers are not resolved and the birefringence is highest. Both spindle and aster birefringence appear to terminate at the surface (*S*) of the spherical centrosome, but in fact a weak, radially positive birefringence exists within the centrosomes (cf. Fig. 2*b*). The dark conical tip seen at the left spindle pole in (*b*) is in fact made up of birefringent fibers which continue from the central spindle through the centrosome and terminate at the tip of the elongate-centriole. (Also, see in Part II, Figs. 2*a*, *b*, *f* and 14*a*, *c*, *e*, and *f*). In (*b*), the fibers of the central spindle outside of the centrosome are more birefringent; they possess the same sign of birefringence, and also lie in the same orientation as the fibers within the centrosome, but they overcompensate and appear bright. In (*b*), the distal portion of the left hand elongate-centriole appears in bright compensation. In (*a*), (*c*), and (*d*), it appears in dark compensation. The distal portion of the right hand elongate-centriole which is mostly out of focus appears in bright compensation in (*a*), (*c*), and (*d*). Long astral rays (*A*), some running tangential to the teardrop-shaped nucleus, are seen in bright compensation in (*a*), (*b*), and (*d*). In (*c*) and (*d*), what appear to be short astral rays which converge postero-medially towards the spindle axis are in fact chromosomal spindle fibers (*CF*). They terminate abruptly on kinetochores which are permanently embedded in the nuclear envelope. Although difficult to photograph, a miniature, radially positive spherulite is visible in the rectified polarizing microscope at the tip of each of these astral rays. These spherulites are in fact the kinetochores. Time in h:min of day on 74g26. Bar, 30 μm. × 630.

Barbulanympha: a hypermastigote
an anaerobic symbiont in cockroach gu
From Ritter, Inoue, Kubai, JCB, 1978

Variation in Spindle Poles



Variation in Spindle Poles

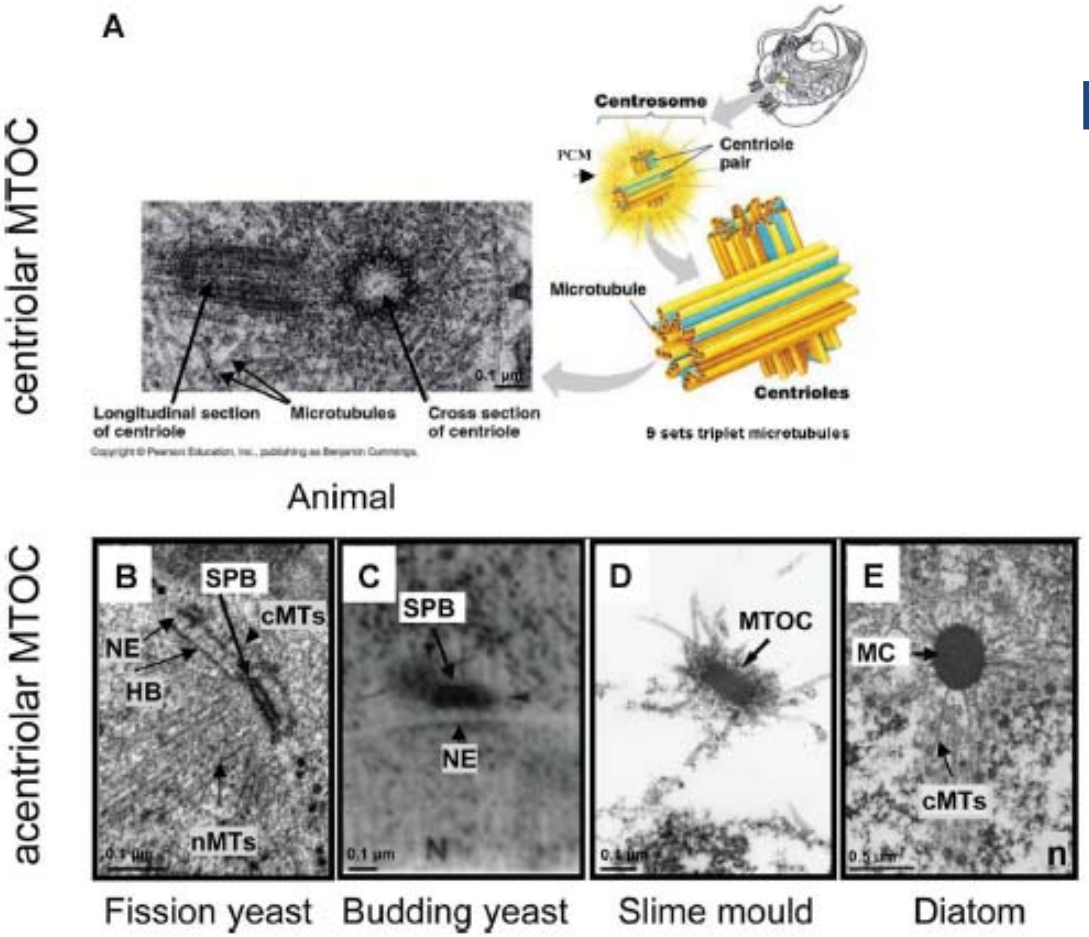


Figure 1. Diatom MTOC ultrastructure in an interphase cell compared to other MTOC models. **A**: Electron micrograph of animal centrosome (left) and schematic representation of the pair of centrioles surrounded by a pericentriolar matrix (PCM) (right).[23] Reprinted with permission from © Pearson Education, Inc., publishing as Benjamin Cummings (Campbell N. A. and Reece J. B., Biology, © 2002). **B–E**: Electron micrographs of acentriolar MTOC of yeast (SPB) (B and C), slime mould (D) and pennate diatom (E). The SPB in *S. cerevisiae* (B) is a disc-like structure embedded in the NE connected to cytoplasmic MTs (cMTs) and nuclear MTs (nMTs). The half bridge (HB) is the site of the new SPB assembly.[22] Reprinted with permission from the Annual Review of Cell and Developmental Biology, Volume 20, © 2004 by Annual Reviews, www.annualreviews.org. The SPB of *S. pombe* (C) is a single dense layer beside the NE (arrow).[23] Reproduced with permission from Ding *et al.*, 1997, Mol Biol Cell, 8 1461–1479, © The Biochemical Society. *D. discoideum* MTOC (D) is a matchbox-shaped three-layered structure.[110] Reprinted with permission from the Molecular Biology of the Cell, Volume 10, © 1999 by the American Society of Cell Biology. Pennate diatom (*Surirella ovalis*) MC (E) is an extranuclear structure, appearing as a well-defined dark granule from which MTs radiate around the interphase nucleus (n) and in all directions.[30] Reprinted with permission from Rockefeller University Press, © Tippit and Pickett-Heaps, 1977. Originally published in The Journal of Cell Biology. 73: 705–727.

Microtubule Organizing Cente

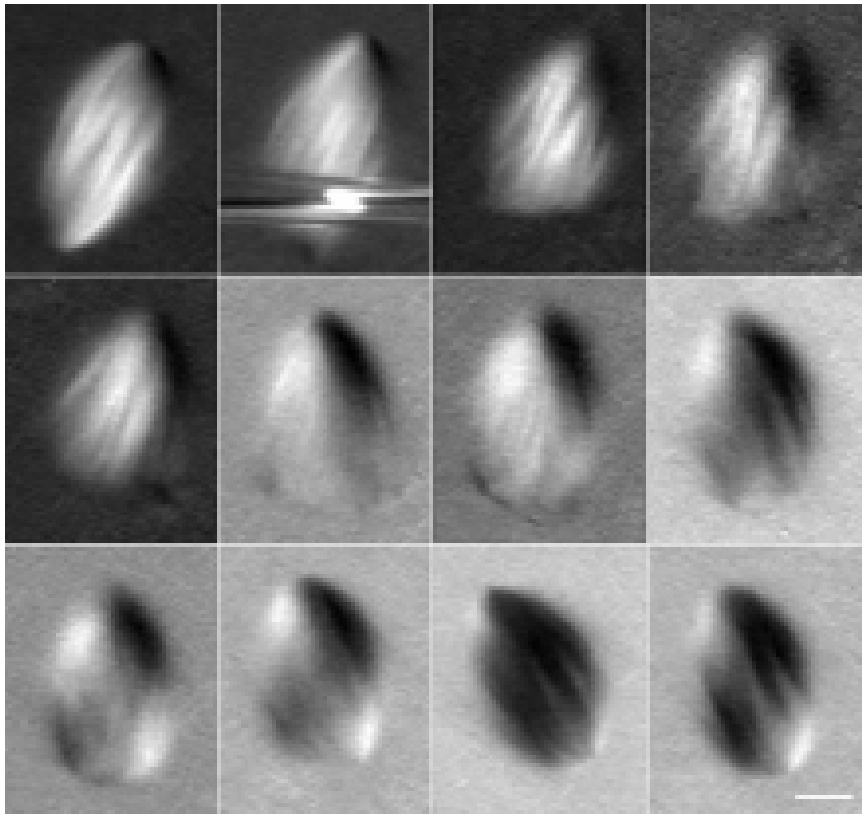
1) Centrioles

2) Acentriolar

3) Self-Organized

Self-Organized Spindle Poles

Xenopus laveis (Meiotic Extract)



Tirnauer et al, MBC, 2004, 15 1776

10 µm

Variation in Spindle Poles

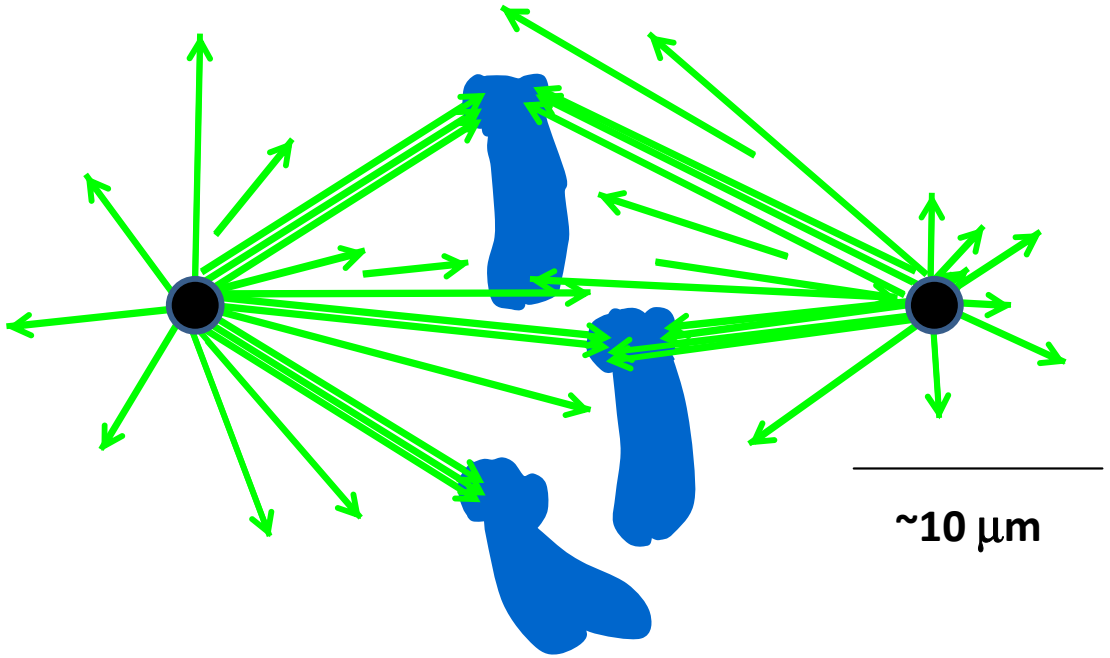
Variation In Importance of Centrioles in Spindle Fu

1) Not Present - Not Important
   meiosis in most metozan females

2) Present - Not (Very) Important
   mammalian tissue culture cells
   drosophila

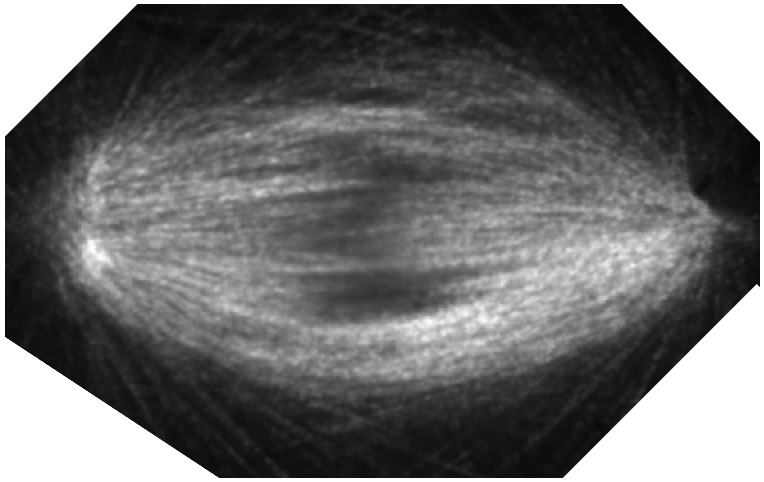
3) Present - Crucial
   C. elegans 1st mitosis

Variation in Spindle Structure And Microtubule Behaviors

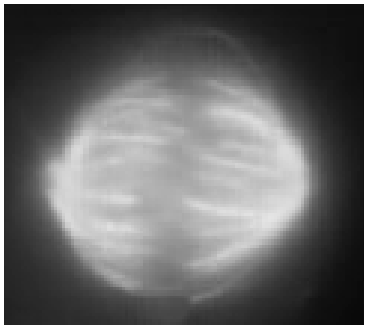


Spindles Have Different Shapes a

Xenopus
Egg Extract

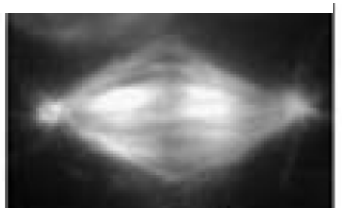


Human
Tissue Culture



Sauer, et al, Mol. Cel. Prot. 4.1,35, 2005

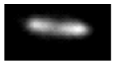
Drosophila
Tissue Culture



~10 μm

Goshima, et al, Curr. Bio. 15:1979, 2005

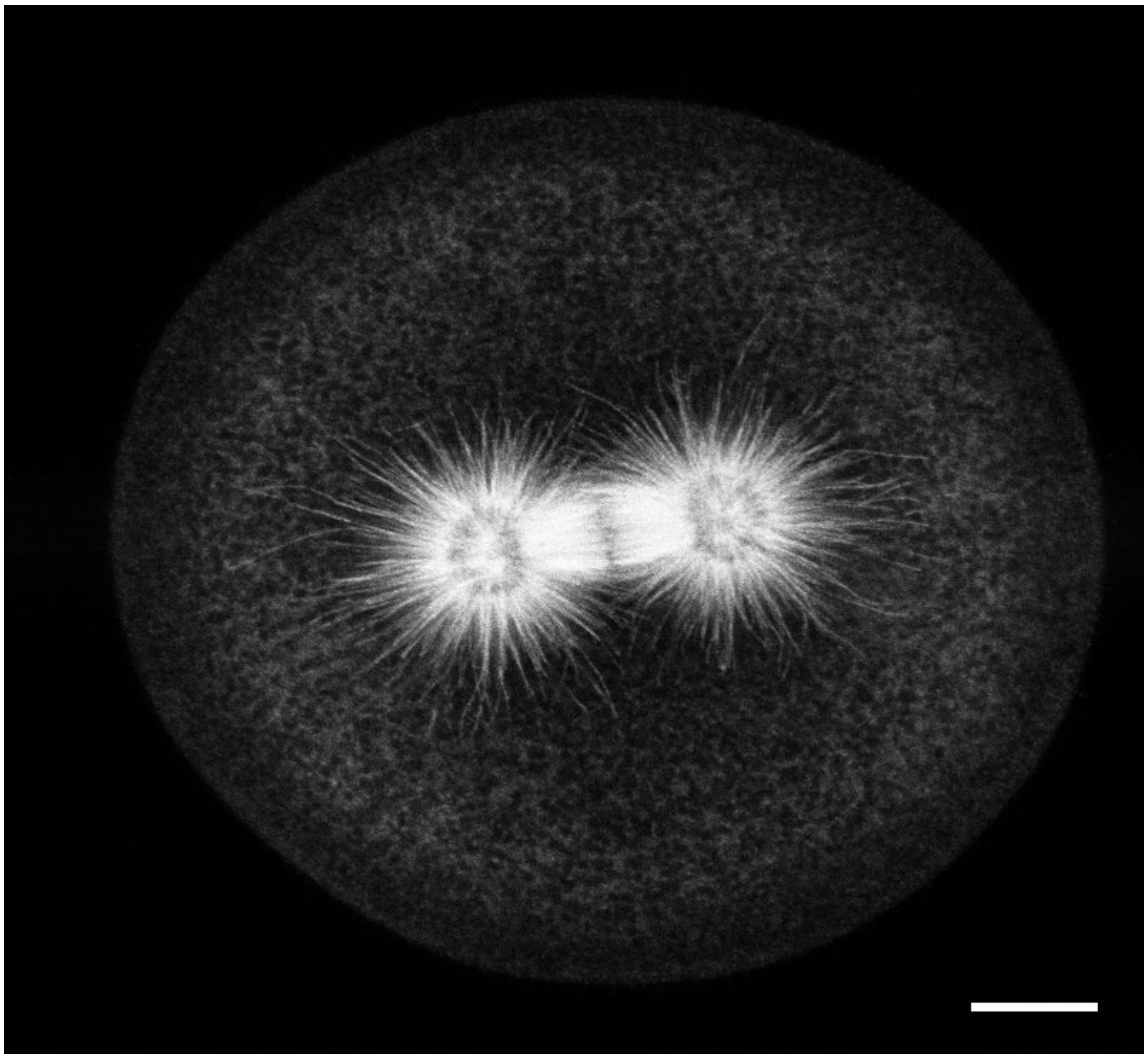
Yeast



Pearson, et al, Mol. Bio. Cell. 17:4069, 2006

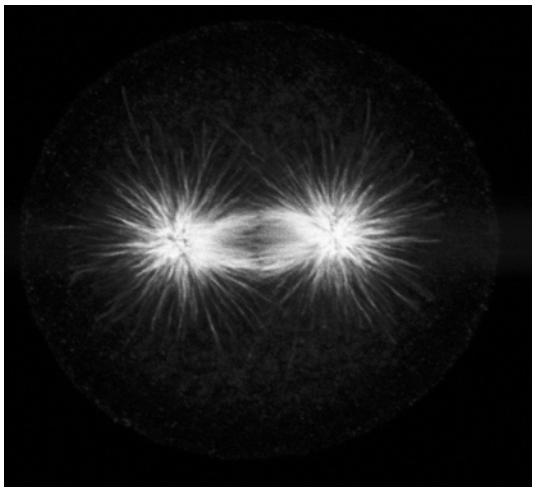
sely Related Organisms Can Have Different Spin

S. droebachiensis
“green urchin”



20 μm

S. purpuratus
“purprle urchin”



Victoria Foe
Center for Cell Dynam

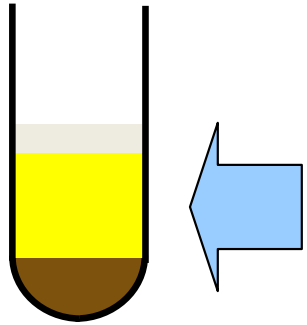
ely Related Organisms Can Have Different Spin

Cell Extracts

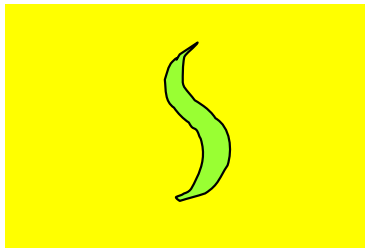
Xenopus laevis

Eggs

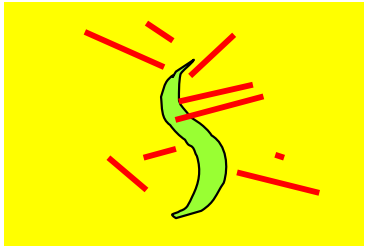
Egg Extracts



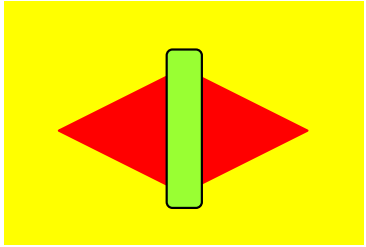
Add DNA



Microtubules Grow



Spindles Assemble



Adopted from Ryoma Ohi

Closely Related Organisms Can Have Different Spindles

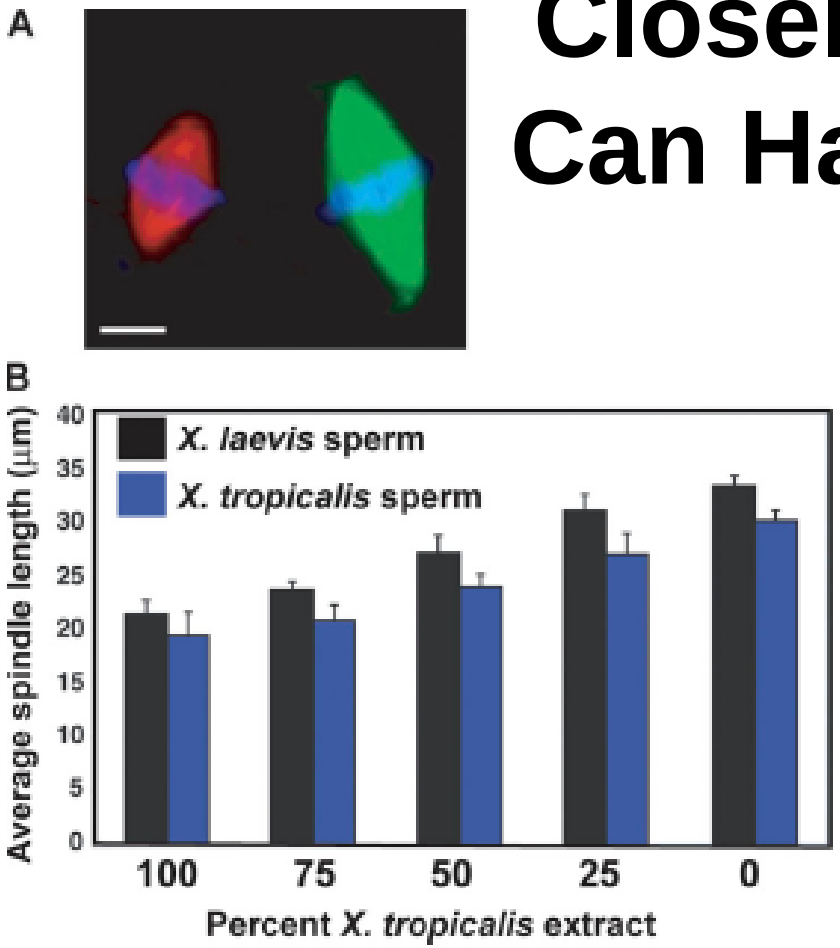


Figure 3. **Comparison of spindle length between *X. tropicalis* and *X. laevis*.** (A) Spindles assembled around *X. laevis* sperm nuclei in either *X. laevis* or *X. tropicalis* egg extracts were visualized using Hoechst dye (blue, DNA) and the incorporation of X-rhodamine tubulin (red microtubules, *X. tropicalis*), or Alexa Fluor 488 tubulin (green microtubules, *X. laevis*). Bar, 10 μm. (B) Mixed reactions with the indicated proportion of *X. tropicalis* extract were combined with *X. laevis* or *X. tropicalis* sperm nuclei. Spindle length was measured from pole to pole. A linear relationship was observed between the proportion of *X. laevis* extract present and spindle length. Error bars are the SD.

Xenopus tropicals

Eggs ~0.6 mm diameter
Genome ~1.7 x 10^9 bp
Meioses II Spindle Length ~ 20 micro

Xenopus laevis

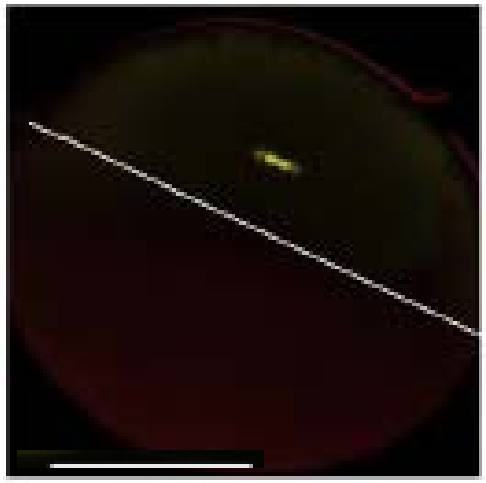
Eggs ~1.2 mm diameter
Genome ~3 x 10^9 bp
Meioses II Spindle Length ~ 35 micror

Xenopus tropicalis egg extracts provide insight into scaling of the mitotic spindle

Katherine S. Brown,[1] Michael D. Blower,[1] Thomas J. Maresca,[1] Timothy C. Grammer,[2] Richard M. Harland,[2] and Rebecca Heald[1]

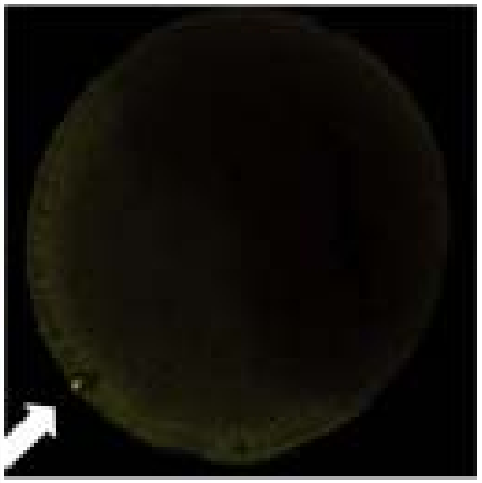
fferent Spindles Within the Same Organism Are Differe

Xenopus laveis

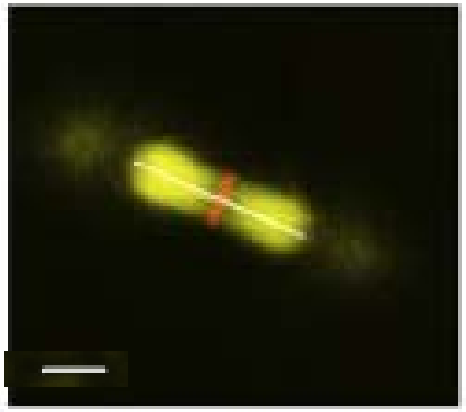


500 µm

Mitosis 2



Meiosis II



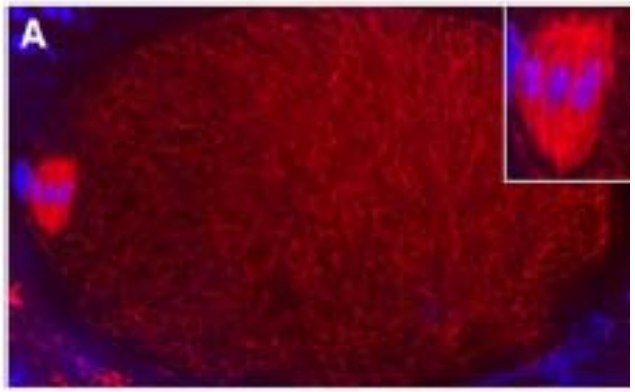
20 µm

Evidence for an Upper Limit to Mitotic Spindle Length

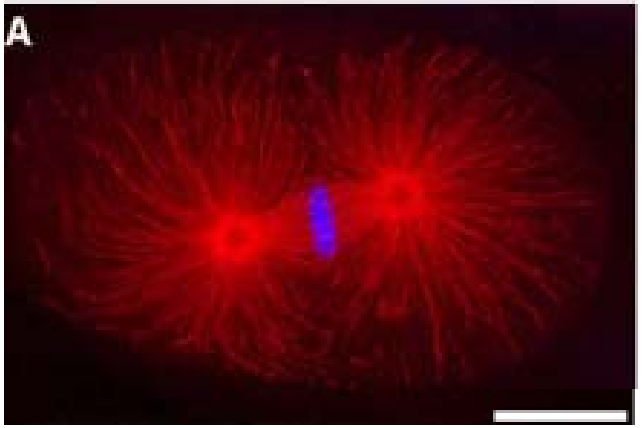
Martin Wühr,[1,*] Yao Chen,[2] Sophie Dumont,[1,3] Aaron C. Groen,[1] Daniel J. Needleman,[1] Adrian Salic,[2] and Timothy J. Mitchison[1]

C. elegans

Meiosis



Mitosis 1



10 µm

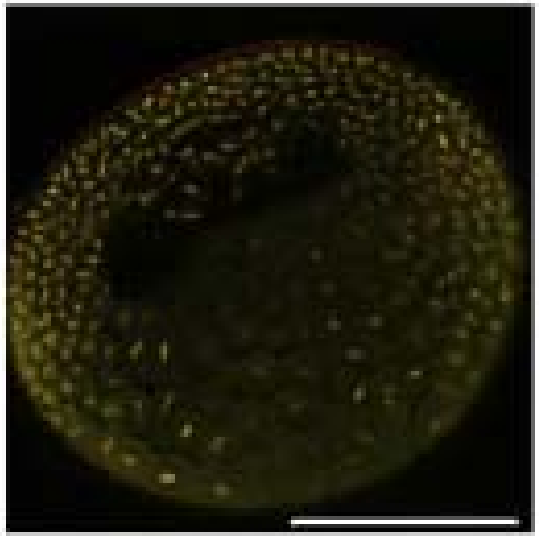
Katanin Disrupts the Microtubule Lattice and Increases Polymer Number in *C. elegans* Meiosis

Martin Srayko,[1,3] Eileen T. O'Toole,[2,3] Anthony A. Hyman,[1] and Thomas Müller-Reichert[1,*]

were distributed throughout th
(Figure 1C, white spheres; see

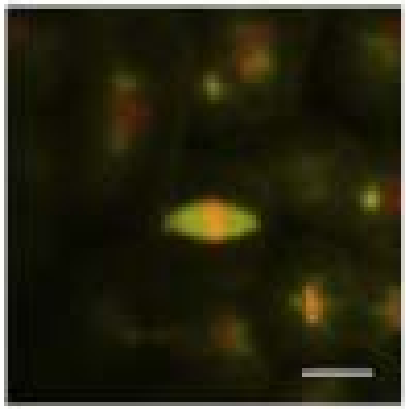
fferent Spindles Within the Same Organism Are Differe

Xenopus laveis

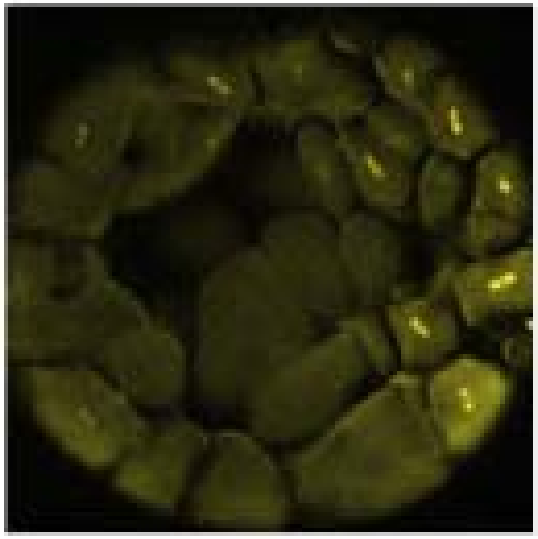


Stage 8

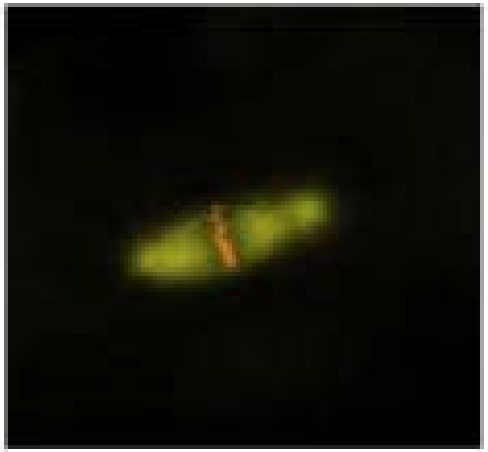
500 μm

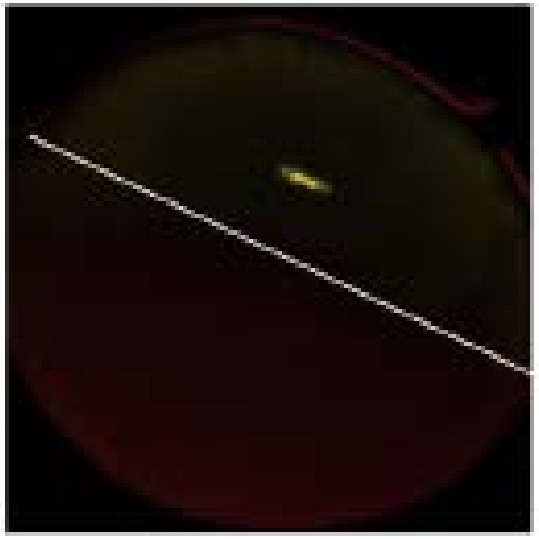


20 μm

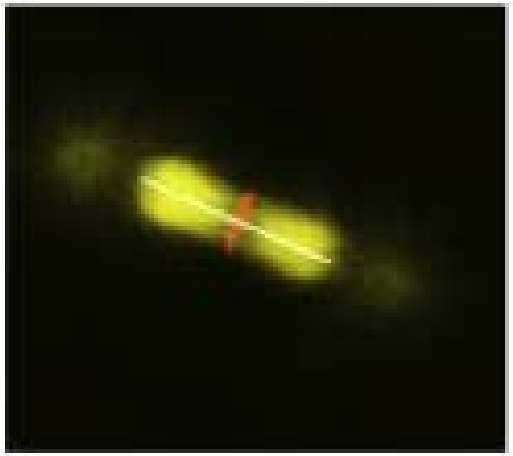


Mitosis 7





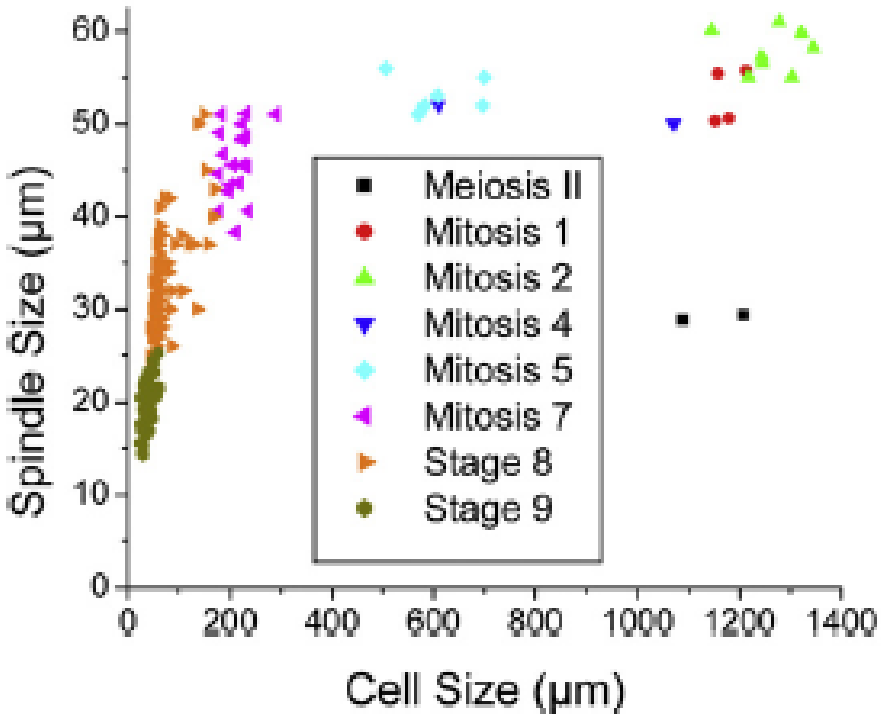
Mitosis 2



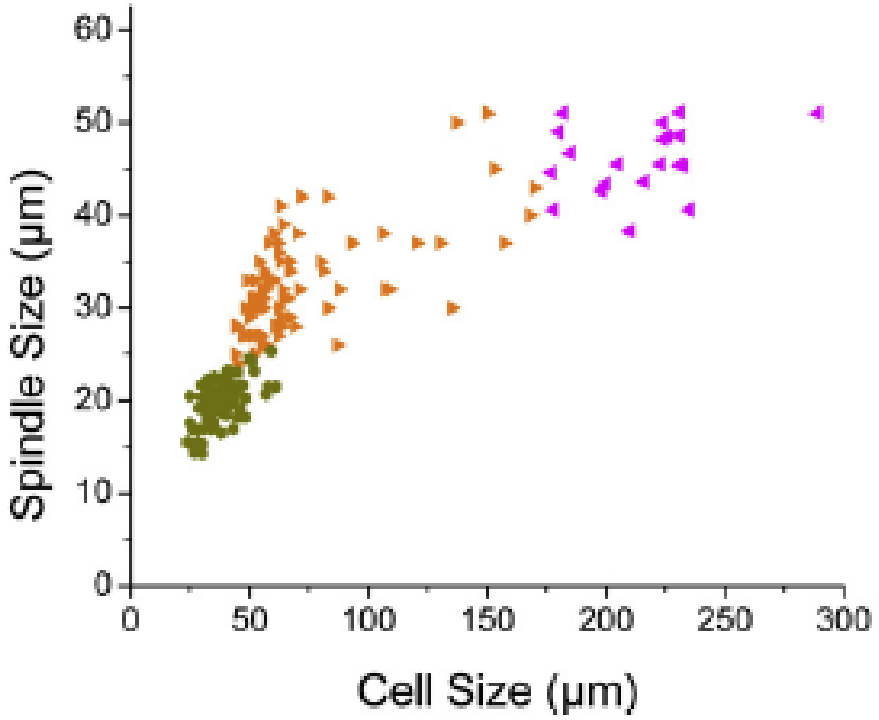
fferent Spindles Within the Same Organism Are Differe

Xenopus laveis

Spindle Length



Cell "Diameter"

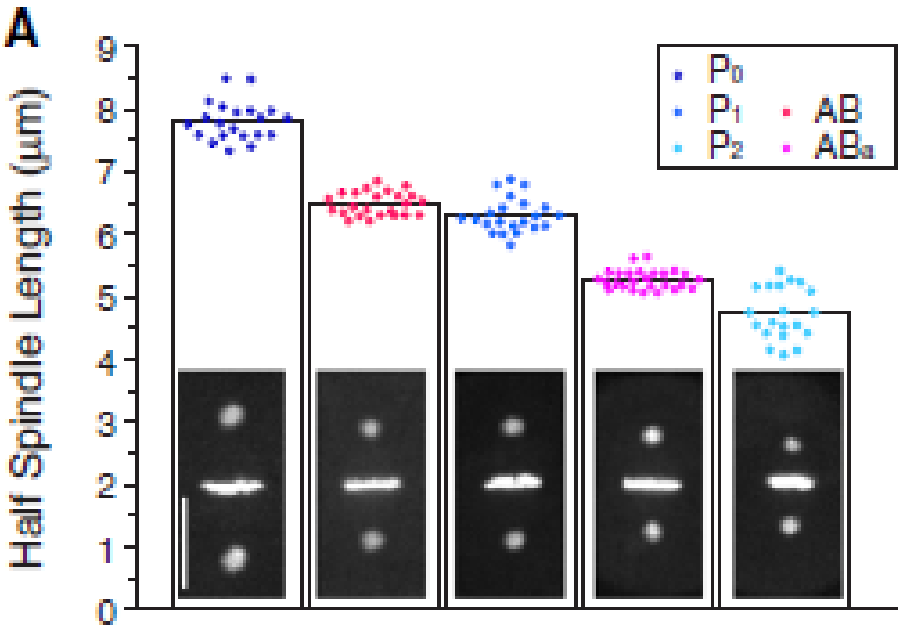


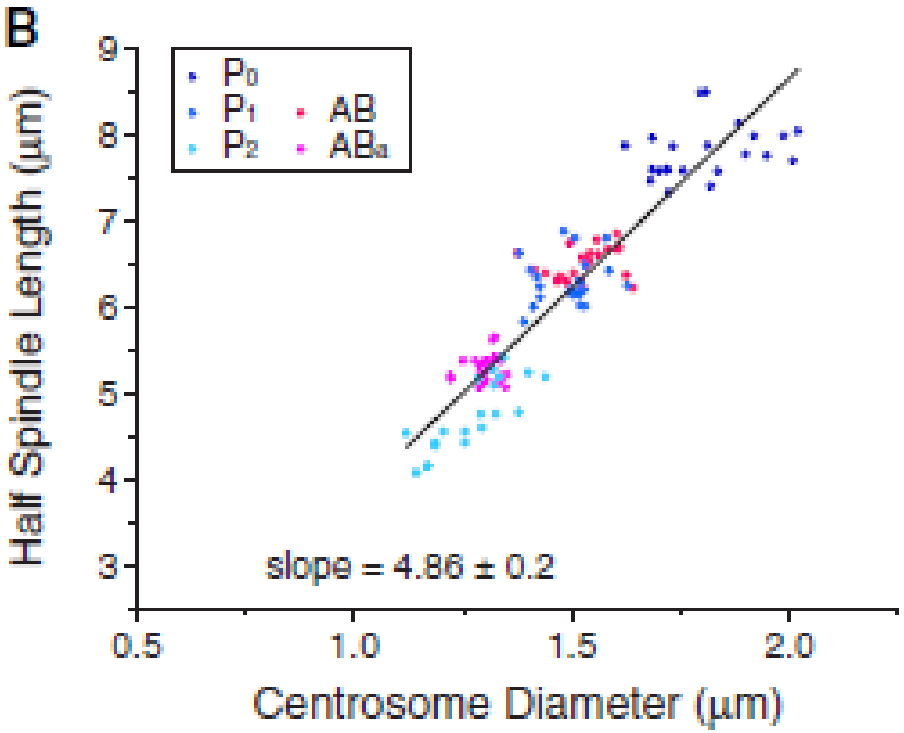
Cell "Diameter"

Mechanism Unclear: **Confinement?**
Altered Biochemistry?

fferent Spindles Within the Same Organism Are Differe

C. elegans





Centrosome Size Sets Mitotic Spindle Length in *Caenorhabditis elegans* Embryos

Garrett Greenan,[1,2] Clifford P. Brangwynne,[1,2,3] Steffen Jaensch,[1] Jöbin Gharakhani,[2,3] Frank Jülicher,[2,3,*] and Anthony A. Hyman[1,2,*]

embryonic divisic
between kinetocl
($p > 0.05$), or

Microtubule Behaviors Are Different In Different Spindles: Turnover

Xenopus laveis (Meiotic Extract)

FRAP

Fluorescence Recovery After Photobleaching

1) Photobleach Fluoresc Tubulin

2) Measure Time Scale o Recovery

(many problems and caveat

Microtubule Behaviors Are Different In Different Spindles: Turnover

Yeast, S. pombe — 40-100 seconds
FRAP (Gardner, 2006)

Yeast, S. cerevisiae — ~50 seconds
FRAP (Maddox, 2000)

Xenopus lavies (meiotic extract) — ~20 seconds
FRAP, Photoactivation, Single Molecule Dynamics

Drosophila melanogaster (tissue culture) — ~20 seconds
FRAP (Buster, 2007)

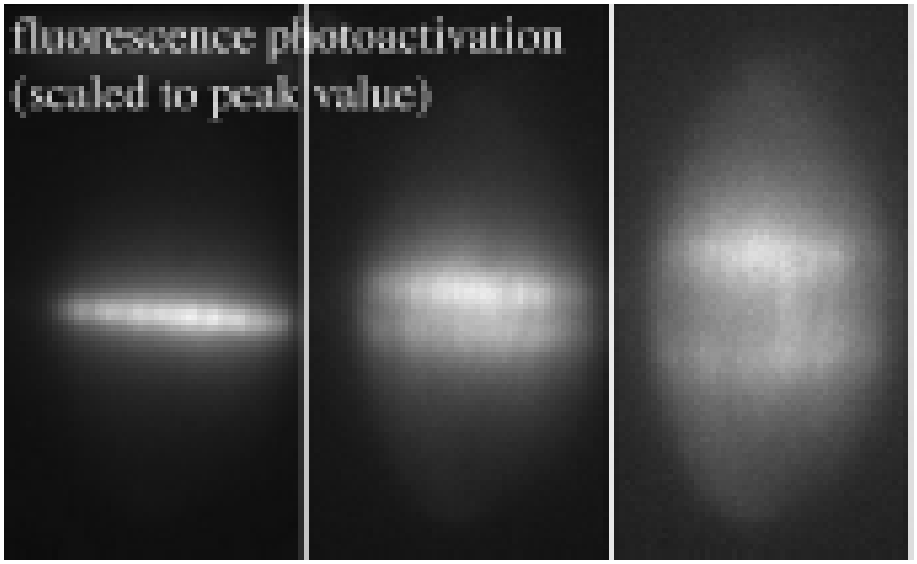
Sea Urchin Embryo — ~20 seconds
FRAP (Salmon, 1984)

PTK2 (kangaroo rat kidney tissue culture) — ~10 seconds
FRAP (Rizik, 2009)

Drosophila melanogaster (embryo) — ~7 seconds
FRAP (Cheerambathur, 2007)

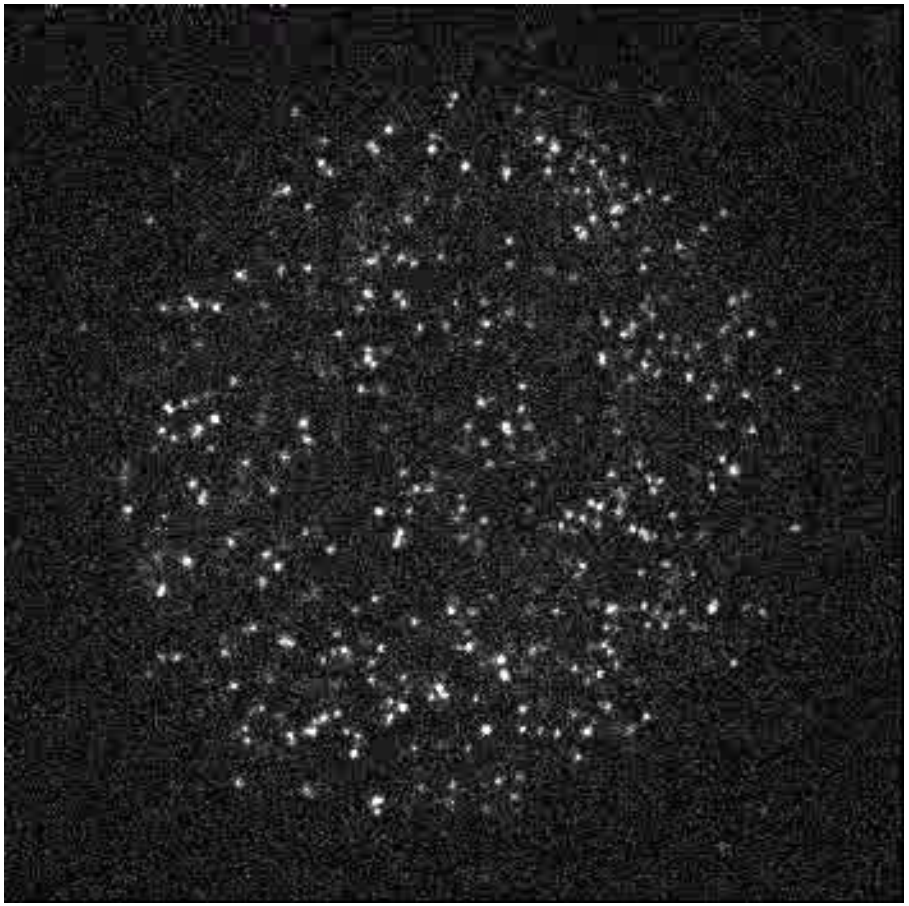
Microtubule Behaviors Are Different In Different Spindles: Flux

Photoconvertible Tubulin
(PTK2, Marsupial Tissue Culture)



Mitchison, JCB, 1989, 109, 637

Single Molecule Tubulin
(Xenopus laveis, meiotic extract)



Microtubule Behaviors Are Different In Different Spindles: Flux

Xenopus lavies (meiotic extract) ~ 2 μm/min
FRAP, Photoactivation, Single Molecule Dynamics, Speckle

Arabidopsis (plant) ~ 2 μm/min
FRAP (Dhonukshe, 2006)

Drosophila melanogaster (embryo) ~ 1.5 μm/min
Speckle (Brust-Mascher, 2002)

PTK2 (kangaroo rat kidney tissue culture) ~0.5 μm/min
Photoconversion (Mitchison, 1989)

U2OS (human tissue culture) ~0.5 μm/min
Photoconversion (Ganem, 2005)

Yeast, S. pombe Not Measurable
FRAP (Gardner, 2006)

Yeast, S. cerevisiae Not Measurable
FRAP, Speckle (Maddox, 2000)

C. Elegans (1st mitotic) Not Measurable
FRAP

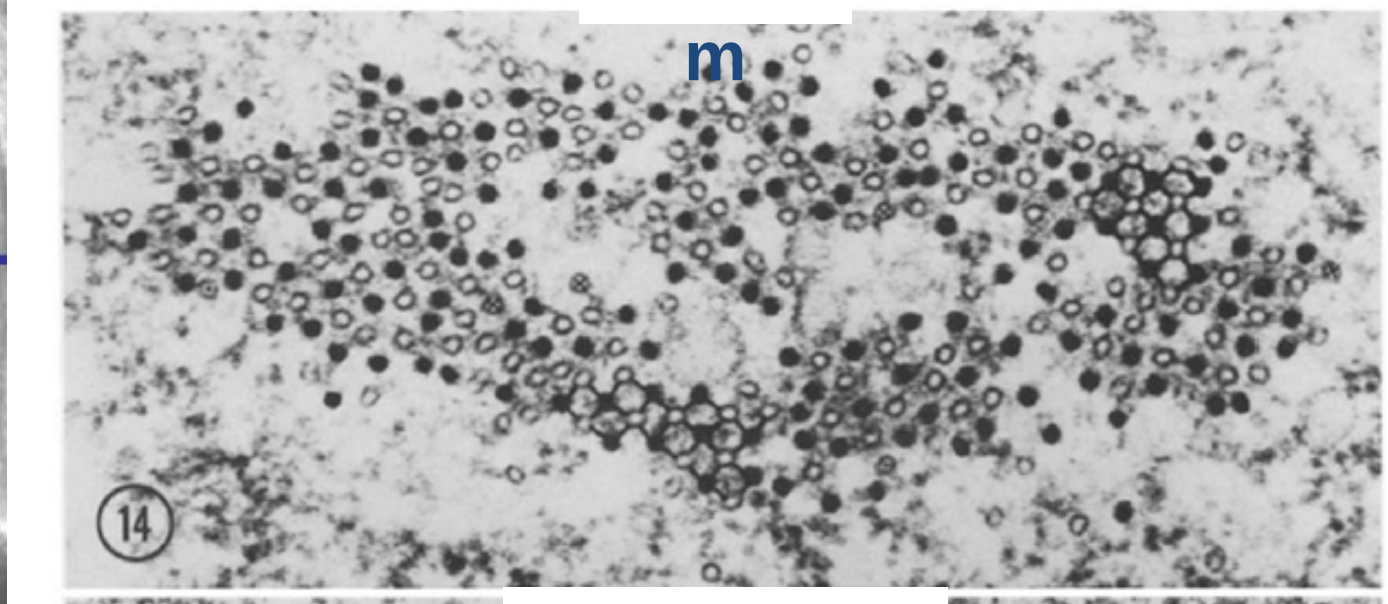
Microtubule Behaviors Are Differ

Diato m

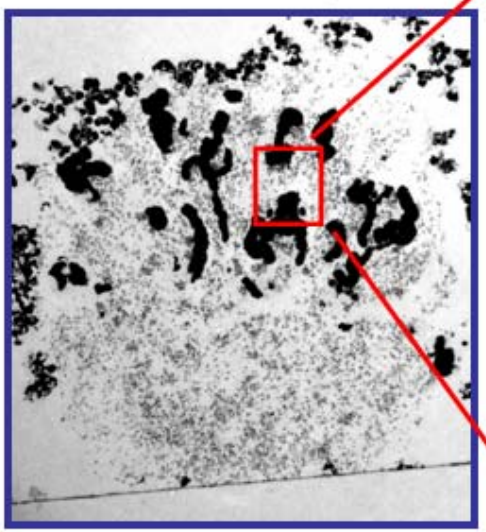
mber

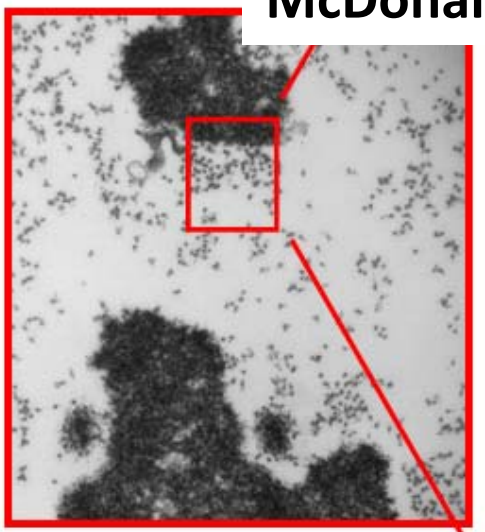
Extract

Lab)



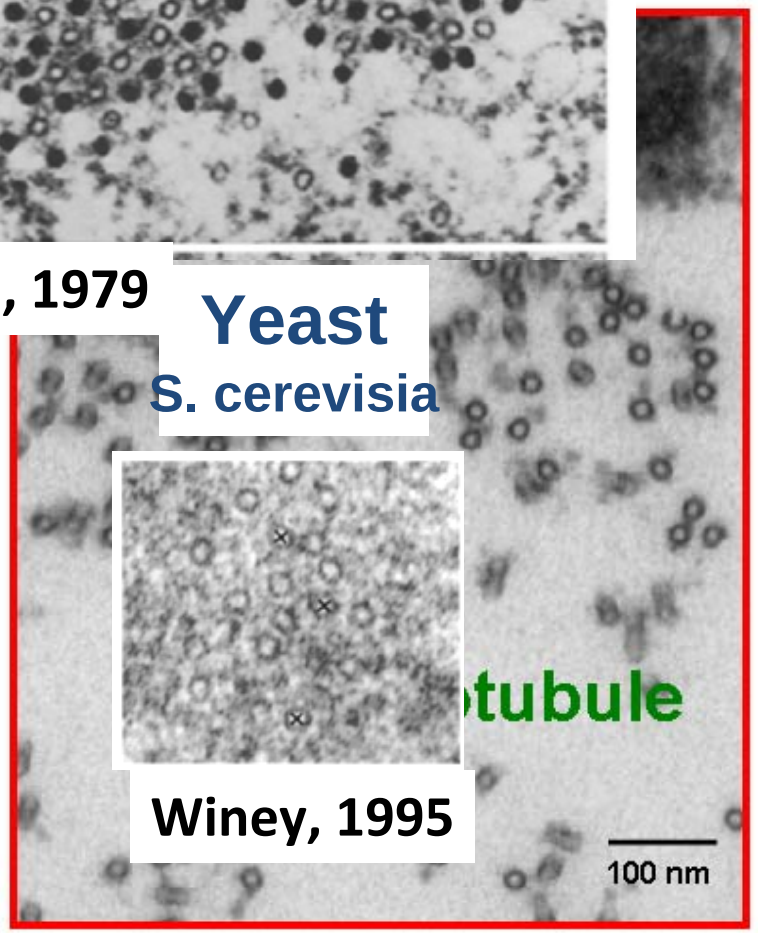
McDonald, 1979





Yeast

S. cerevisia



Winey, 1995

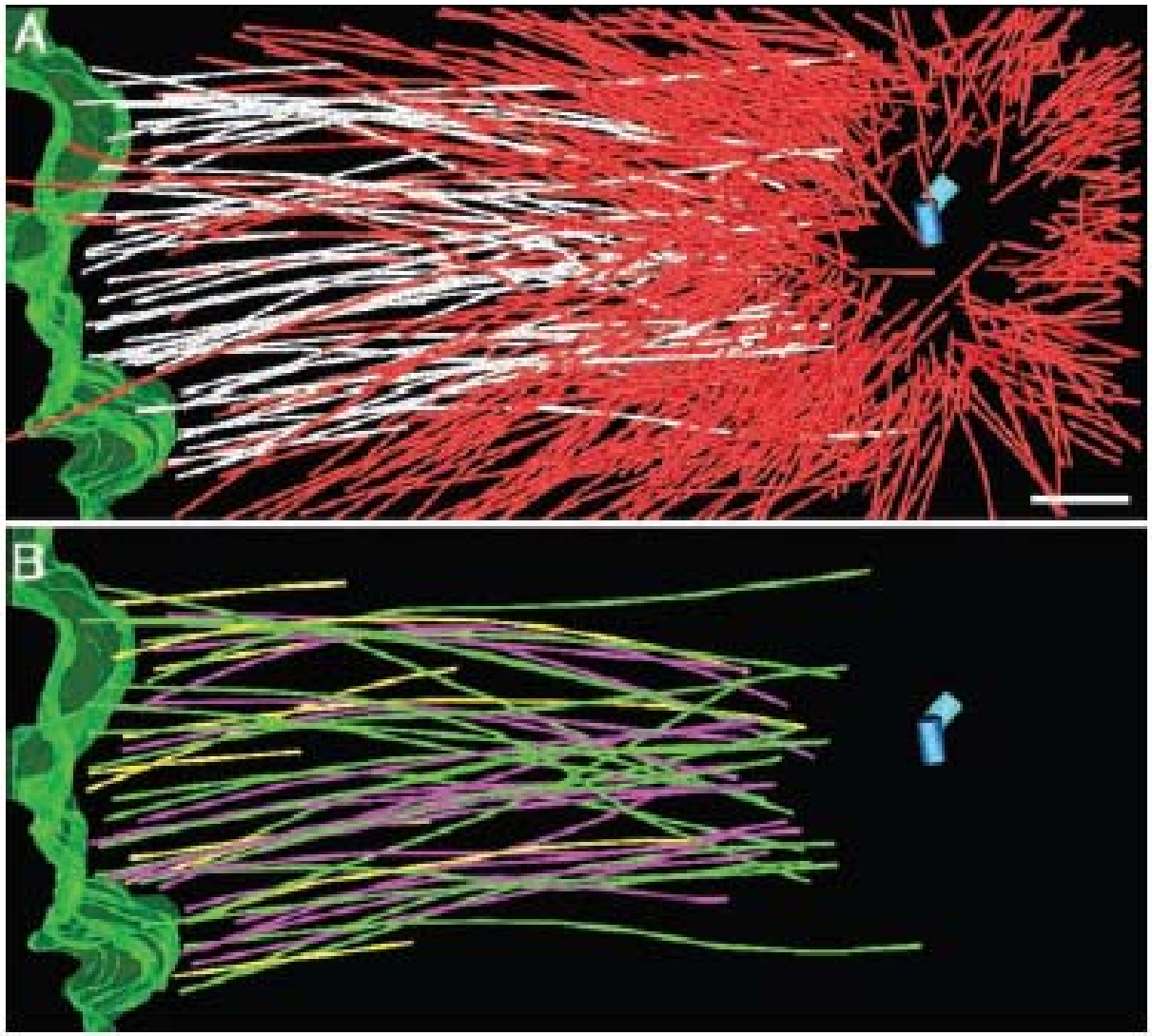
Microtubule Behaviors Are Different In Different Spindles: Number

Xenopus lavies (meiotic extract)	~ 100,000 ?
HeLa (human tissue culture) McIntosh, 1971	~10,000 ?
PTK2 (kangaroo rat kidney tissue culture) Mastronarde, 1995	~5,000 ?
Diatoma vulgare McIntosh, 1979	~1,000
Yeast, S. cerevisiae Winey, 1995	~40
Yeast, S. pombe Ding, 1993	~40

Microtubule Behaviors Are Different
n Different Spindles: Lengths and Locatior

C. Elegans mitotic (partial)

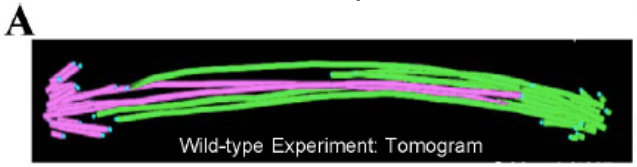
O'Toole, 2003



500 nm

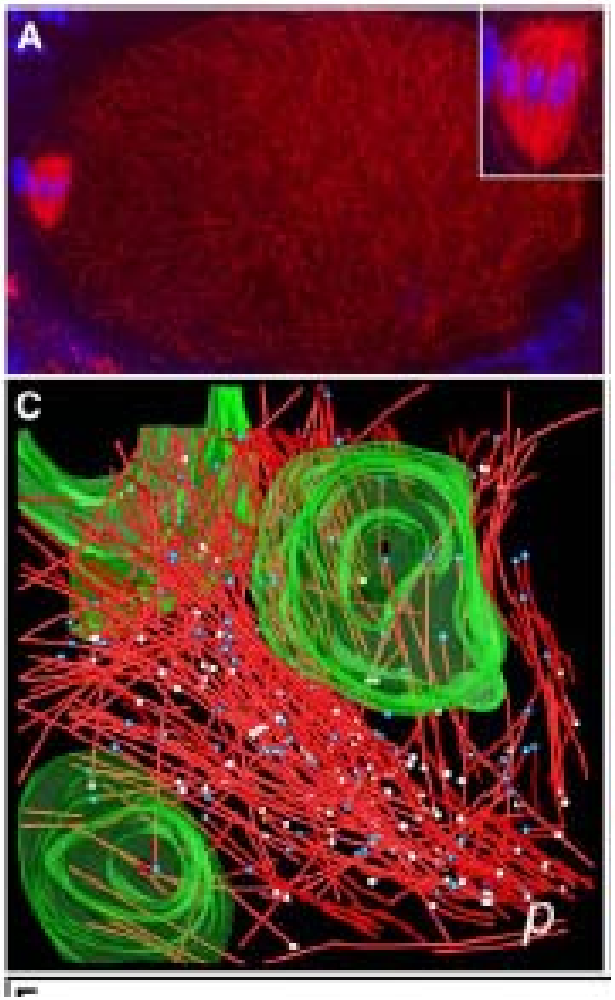
Yeast, S. cerevisiae

Gardner, 2008

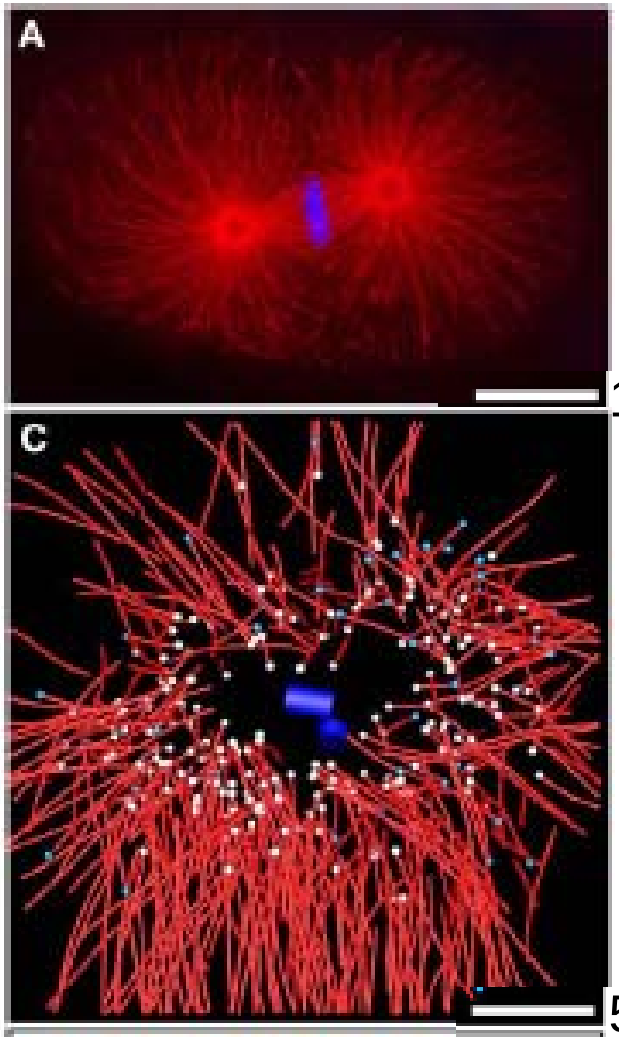


Microtubule Behaviors Are Different
n Different Spindles: Lengths and Locatior

C. elegans
meiosis



C. elegans
1st mitotic



Meiosis

- Short Microtubules
-Plus and Minus Ends Spread Throughout

1st Mitotic

- Long Microtubules
-Minus Ends Located Near Centrosome

Microtubule Behaviors Are Different
n Different Spindles: Lengths and Location

C. Elegans (1st mitotic)	All Minus Ends At Pole "Long" Microtubules
Yeast, S. pombe	All Minus Ends At Poles "Long" Microtubules
Yeast, S. cerevisiae	All Minus Ends At Pole "Long" Microtubules
PTK2 (kangaroo rat kidney tissue culture)	Minus Ends Thro "Short" Microtubules
C. Elegans (meiotic)	Minus Ends Thro "Short" Microtubules
Xenopus laveis (meiotic extract) (indirect data)	Minus Ends Throughou "Short" Microtubules

Microtubule Behaviors Are Differen
In Different Spindles: Kinetochores

Microtubules per Kinetochore

Yeast, S. cerevisiae Winey, 1995	~1
Yeast, S. pombe Ding, 1993	~3
Human Tissue Culture	~20
PTK2 (kangaroo rat kidney tissue culture) Mastronarde, 1995	~20
Xenopus lavies (meiotic extract)	~ 20
C. elegans (1st mitotic)	100s ? (holocer

Variation in Molecular Processes

No Mitotic Kinesisn Are Universally Conserved

A Holistic Kinesin Phylogeny

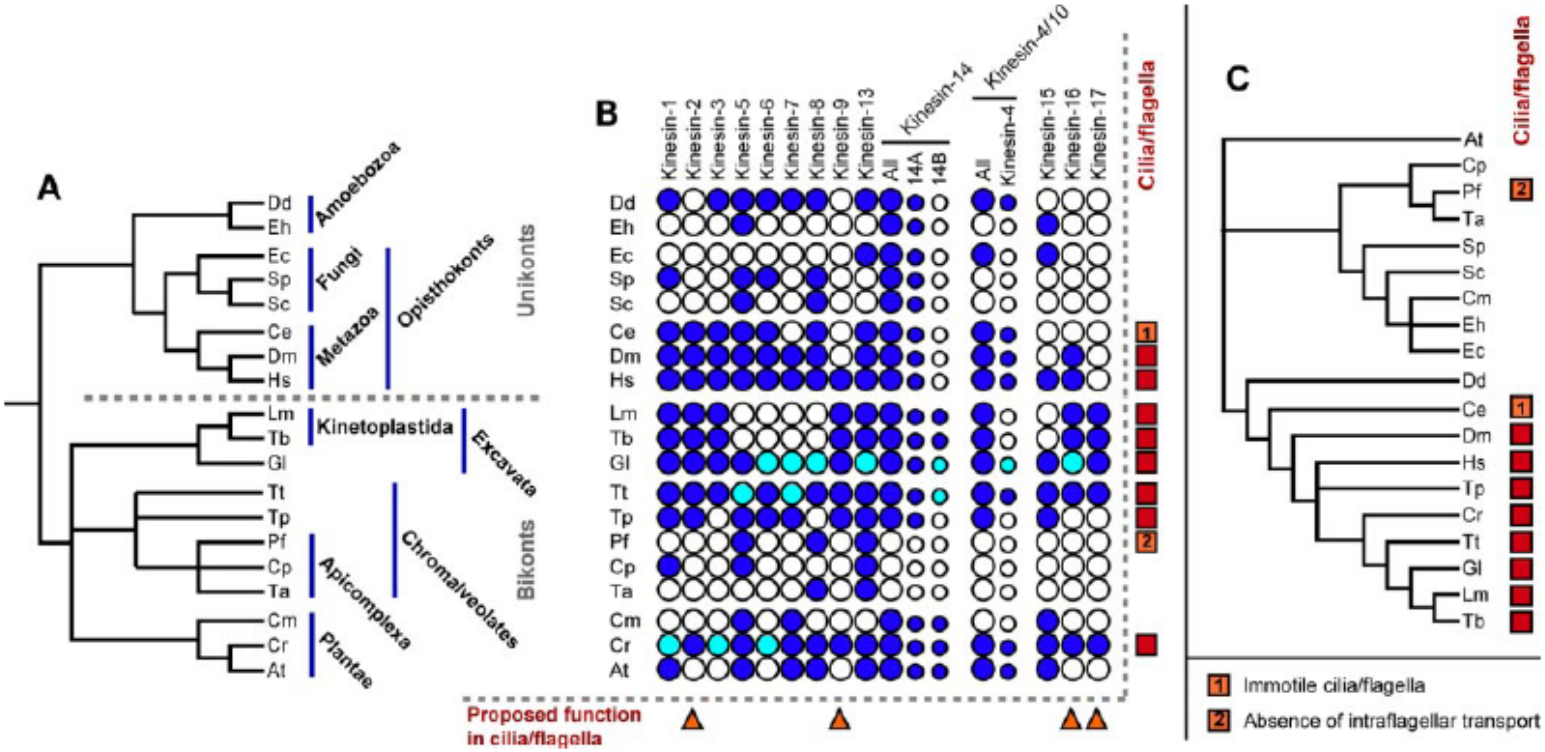


Figure 3. Distribution of kinesin families among eukaryotes. (A) Cladogram showing the probable evolutionary relationships of the 19 organisms analyzed. (B) Taxonomic distribution of kinesin families: presence of paralogue (dark blue dot), absence of a paralogue from an incomplete genome (light blue dot), and absence from a complete genome (open dot). Subfamilies are represented by smaller dots. Kinesin families with a proposed role in cilia/flagella are indicated (triangle), as are organisms that build cilia/flagella (square). (C) Consensus of the 10 most parsimonious trees accounting for the observed kinesin paralogue distribution using family presence/absence as a binary character. See legend to Figure 2 for organism abbreviations.

But inhibiting specific kinesins can lead to similar phenot

Variation in Molecular Processes

Dynein is Absent in Flowering Plants

Ran-pathway Crucial for Microtubule Nucleation In Many Spindles

Checkpoint

No checkpoint in early development

Importance of checkpoint different for different organisms
Null mice die
Null flies are okay
Null C elegans are pretty good, but cannot survive dauer

No metaphase in many organisms: yeast, protists

Time of mitosis (from Heath 1980), strongly temperature dependent
Chilomonas and Fusarium = 5 minutes
Tradescantia (plant) = 340 minutes
Human Tissue Culture ~ 30 minutes

How accurate is chromosome segregation?