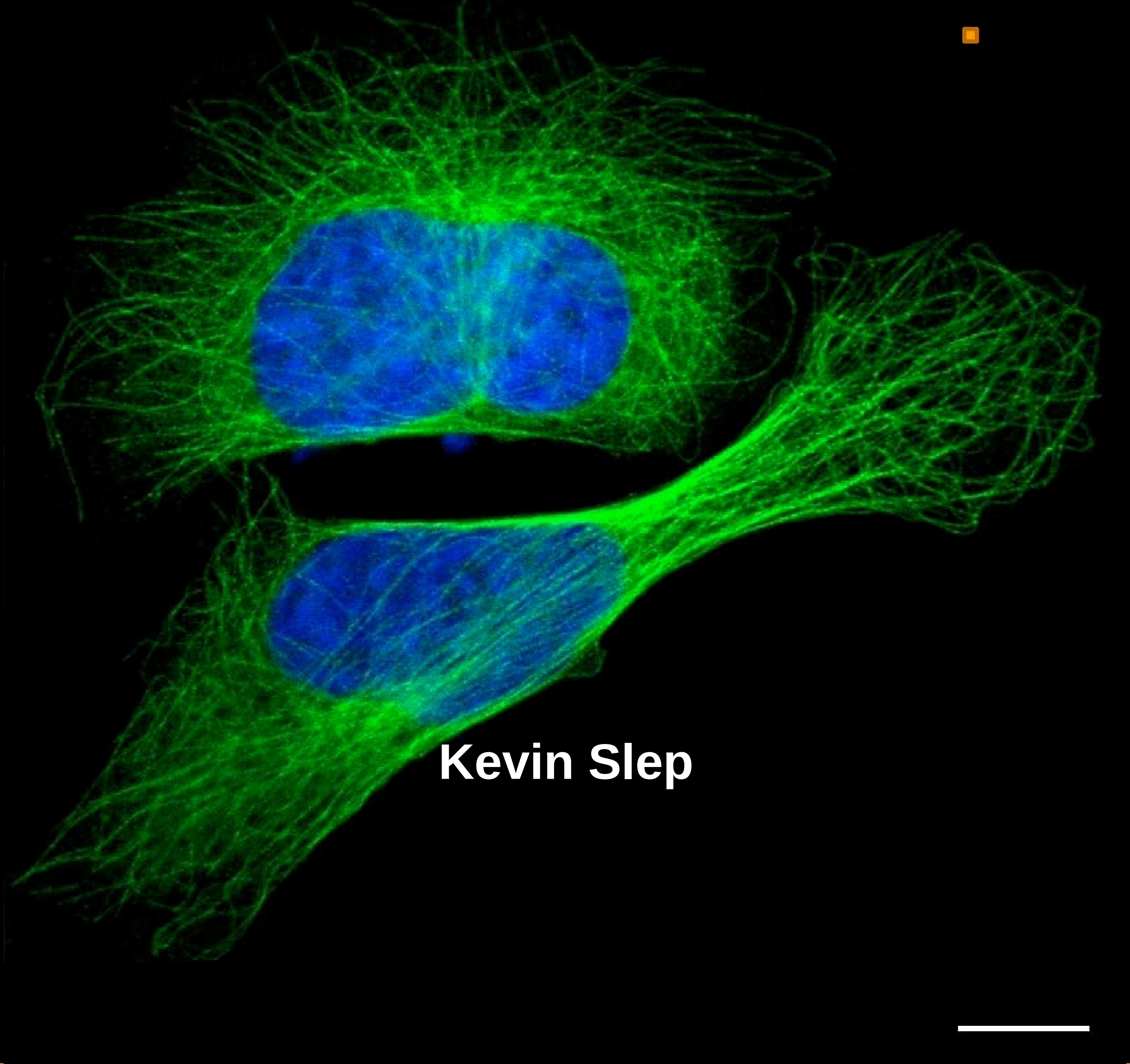
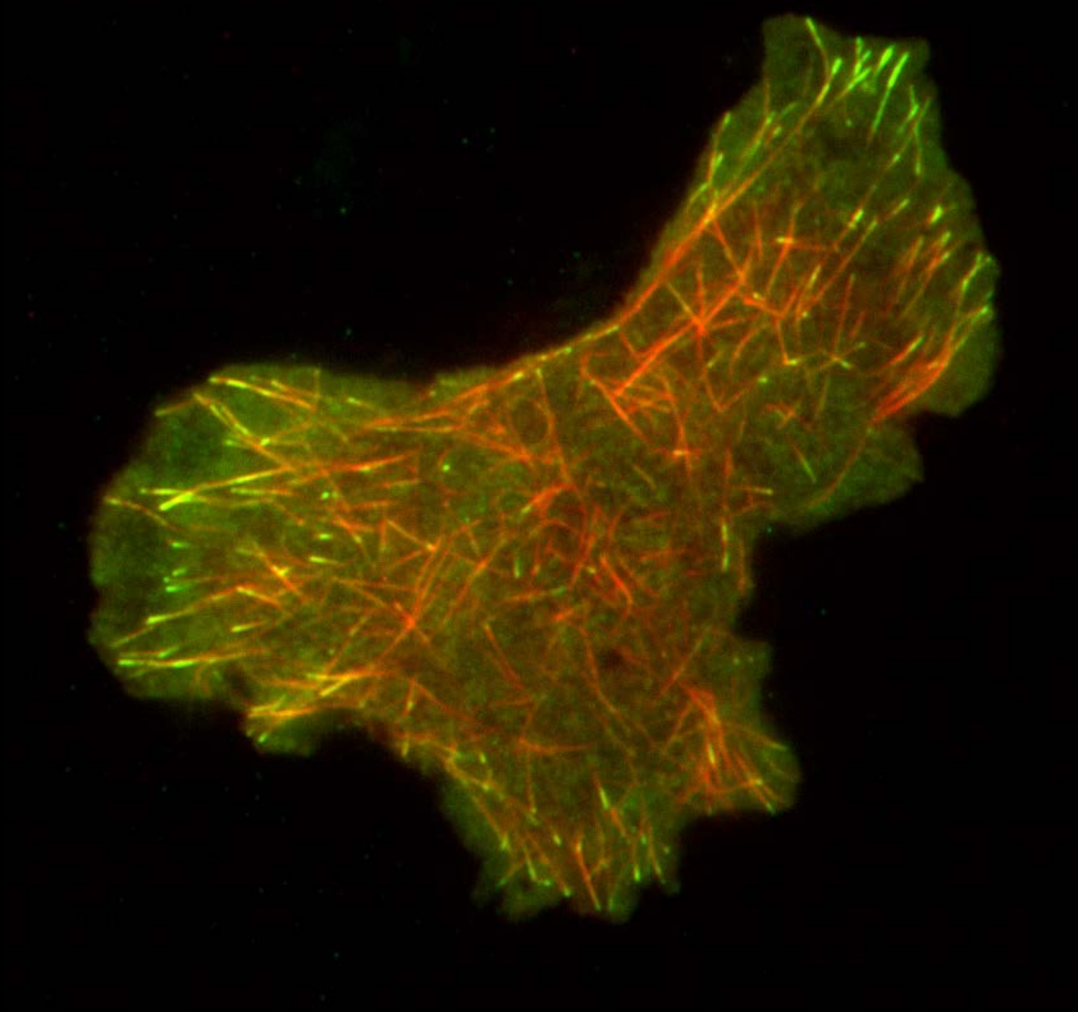
Microtubule Forces



Kevin Slep

Microtubules are a Dynamic Scaffold



Microtubules in red, XMA215 family MT polymerase protein in green

Some Microtubule Functions

Cell Structure

Polarized Motor Track
(kinesins and dynein)

Cilia structure
(motile and sensory)

Mitotic and meiotic spindle structure

Cell polarity

Coordinate cell motility with the F-actin network

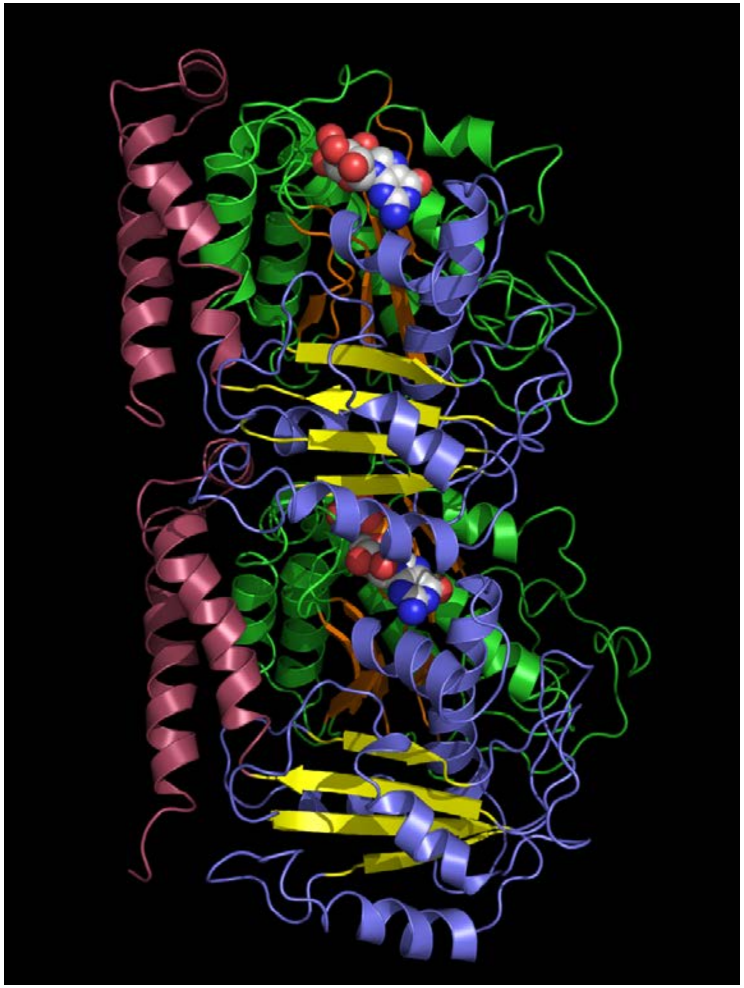
Architecture of Tubulin and the Microtubule

α/β-Tubulin: The Microtubule Building Block

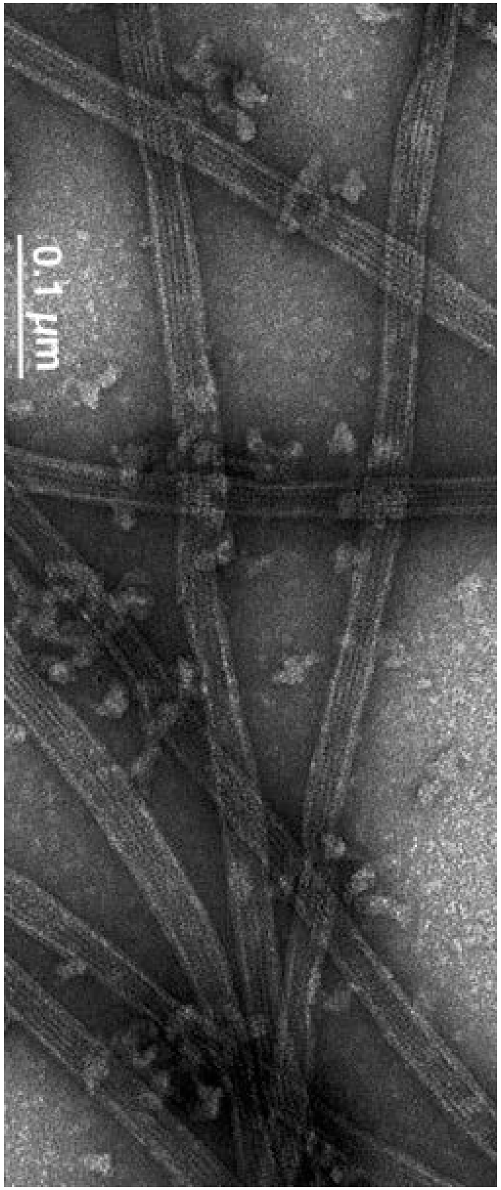
Tubulin is a heterodimer composed of α and β tubulin

□α and β tubulin are each approximately
• 55 kD and are structurally very similar to
•each other.

•Each tubulin binds GTP: The α GTP is non-exchangeable and the dimer is very stable, Kd = 10^{-10}; the β GTP is exchangeable in the dimer



The Microtubule Architecture



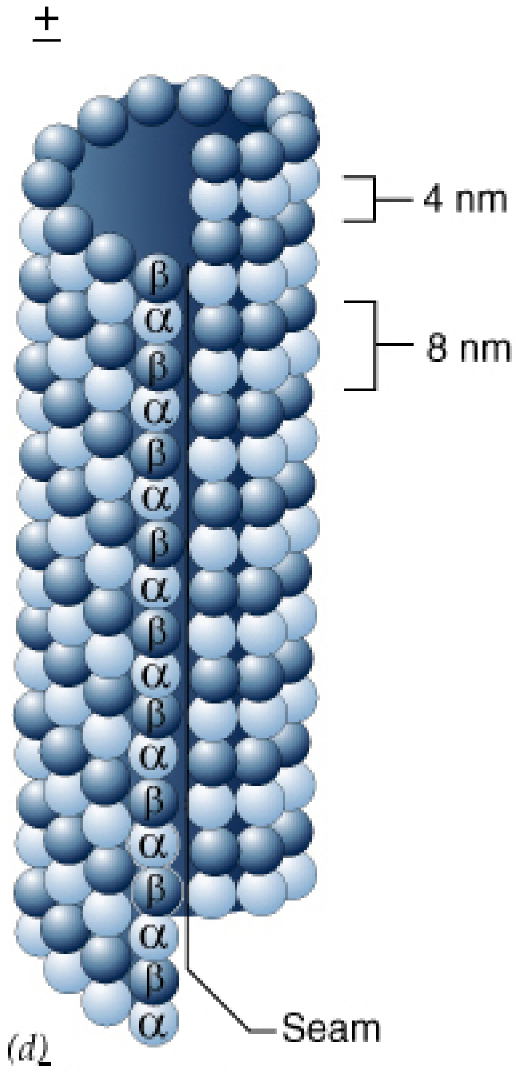
Tubulin binds head-to-tail along protofilaments, forming LONGITUDINAL interactions.

Longitudinal interactions complete the active site for GTP hydrolysis

13 protofilaments form a hollow tube-the microtubule: 25 nm OD, 14 nm ID (protofilaments interact via LATERAL interactions)

The MT is a left-handed helix with a seam, it rises 1.5 heterodimers per turn (α and β form lateral interactions)

MTs are polar-they have a plus end and a minus end

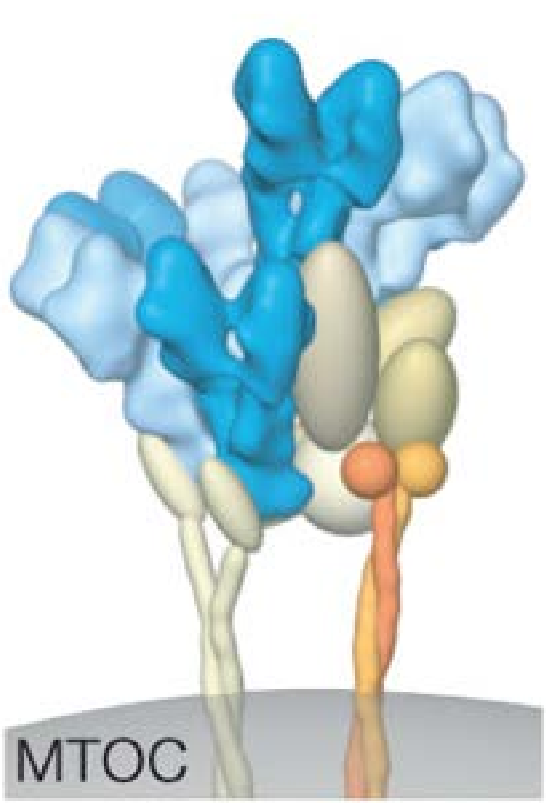


The γTubulin Ring Complex (γTuRC) forms a lockwasher to nucleate MTs

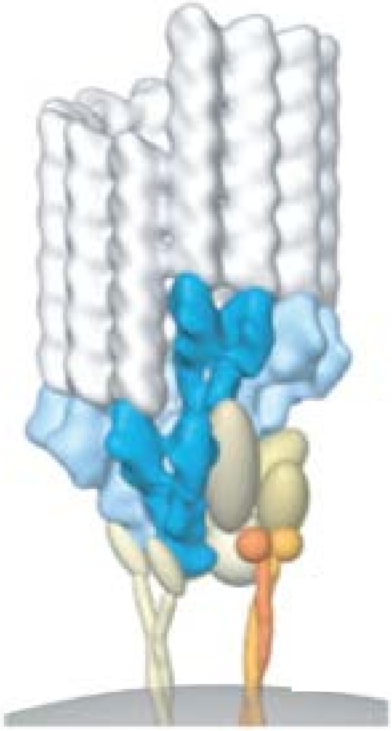
Axial view

Side View

γTuRC positions
13 γTubulins in a ring



γTuRC attachment



nucleated
microtubule

The Centrosome is a Microtubule Organizing Center (MTOC) rich in γTuRC

MTOC's control where microtubules are formed

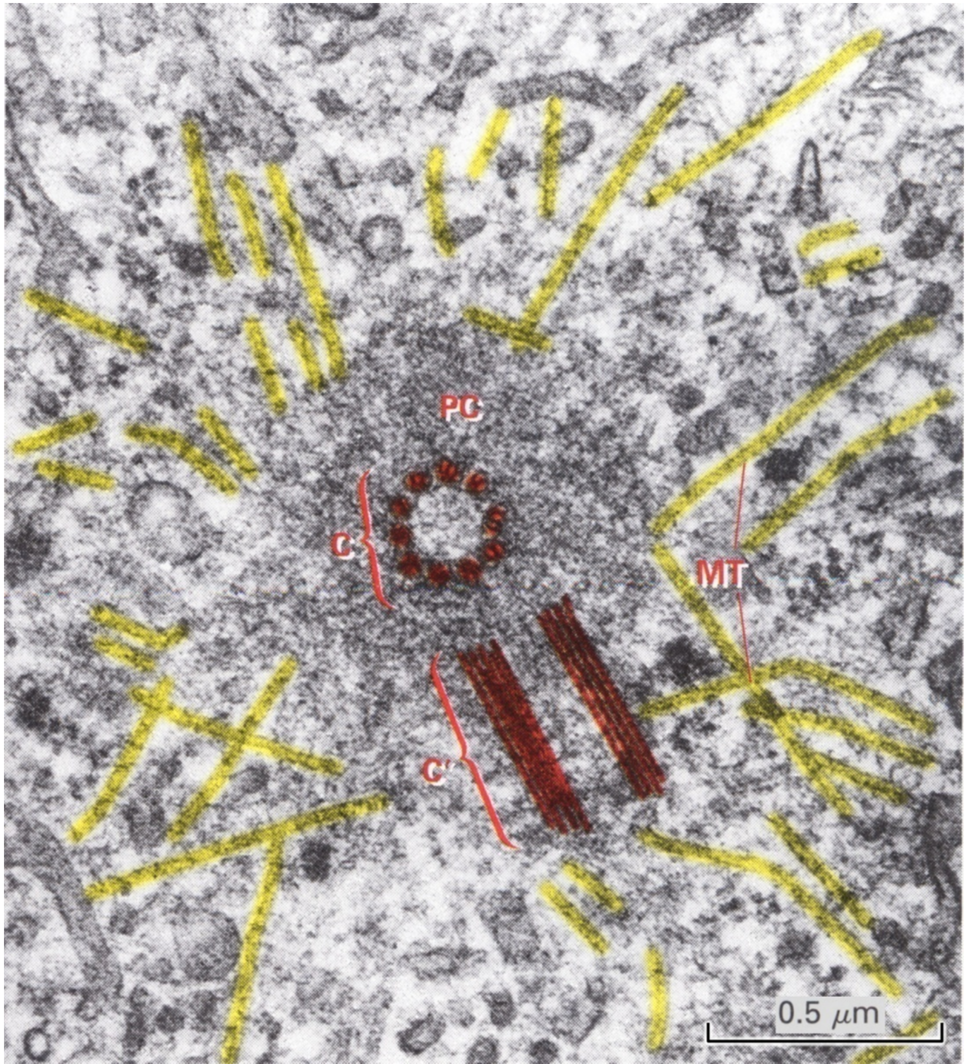
Centrosomes contain peri-centrosomal material (PCM) surrounding a pair of centrioles

γTuRC nucleation complexes are localized to the PCM

Centrioles within centrosomes become basal bodies, which are nucleation centers for cilia (motile and primary) and flagella

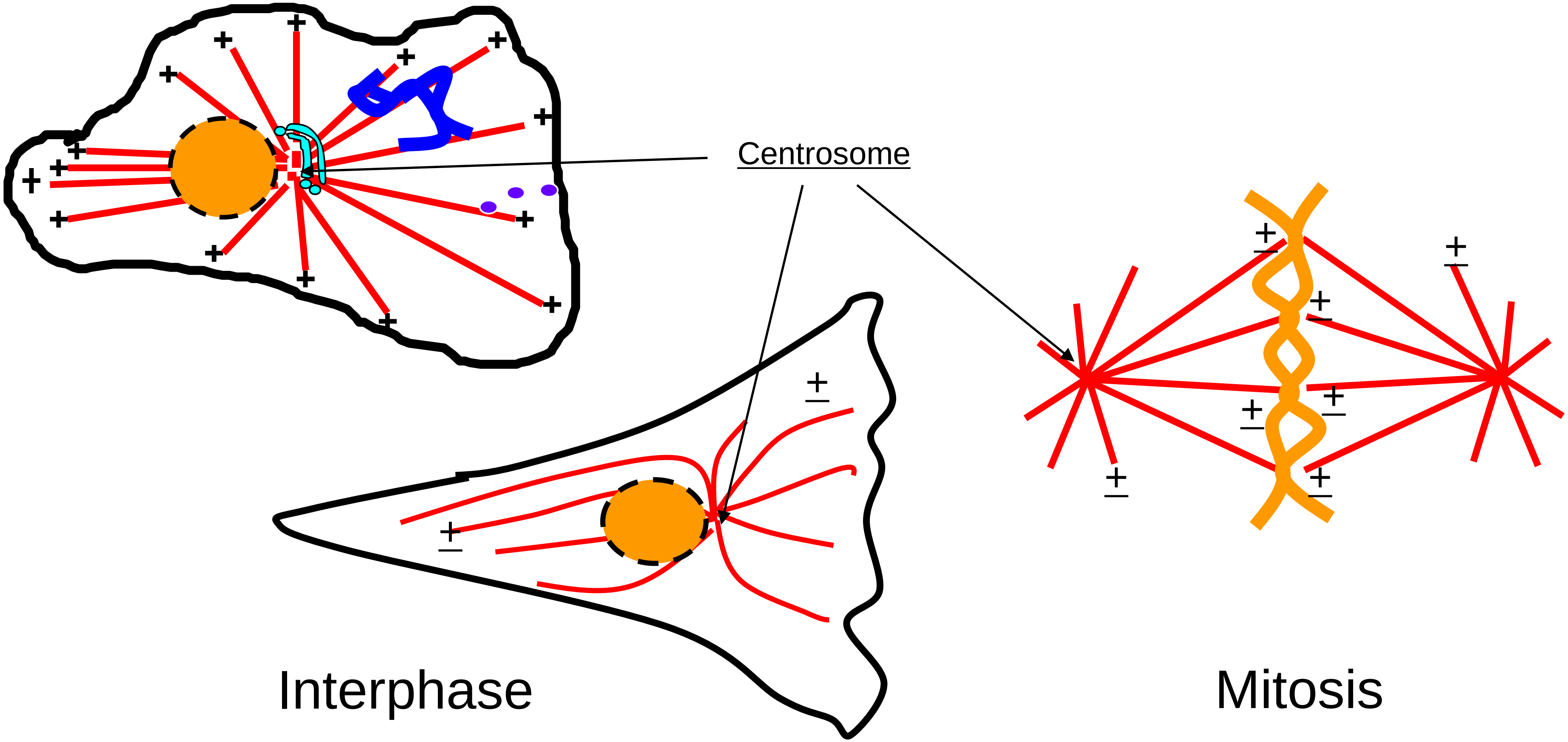
Centrosomes duplicate once per cell cycle

Mother centriole nucleates growth of a daughter centriole with an orthogonal orientation

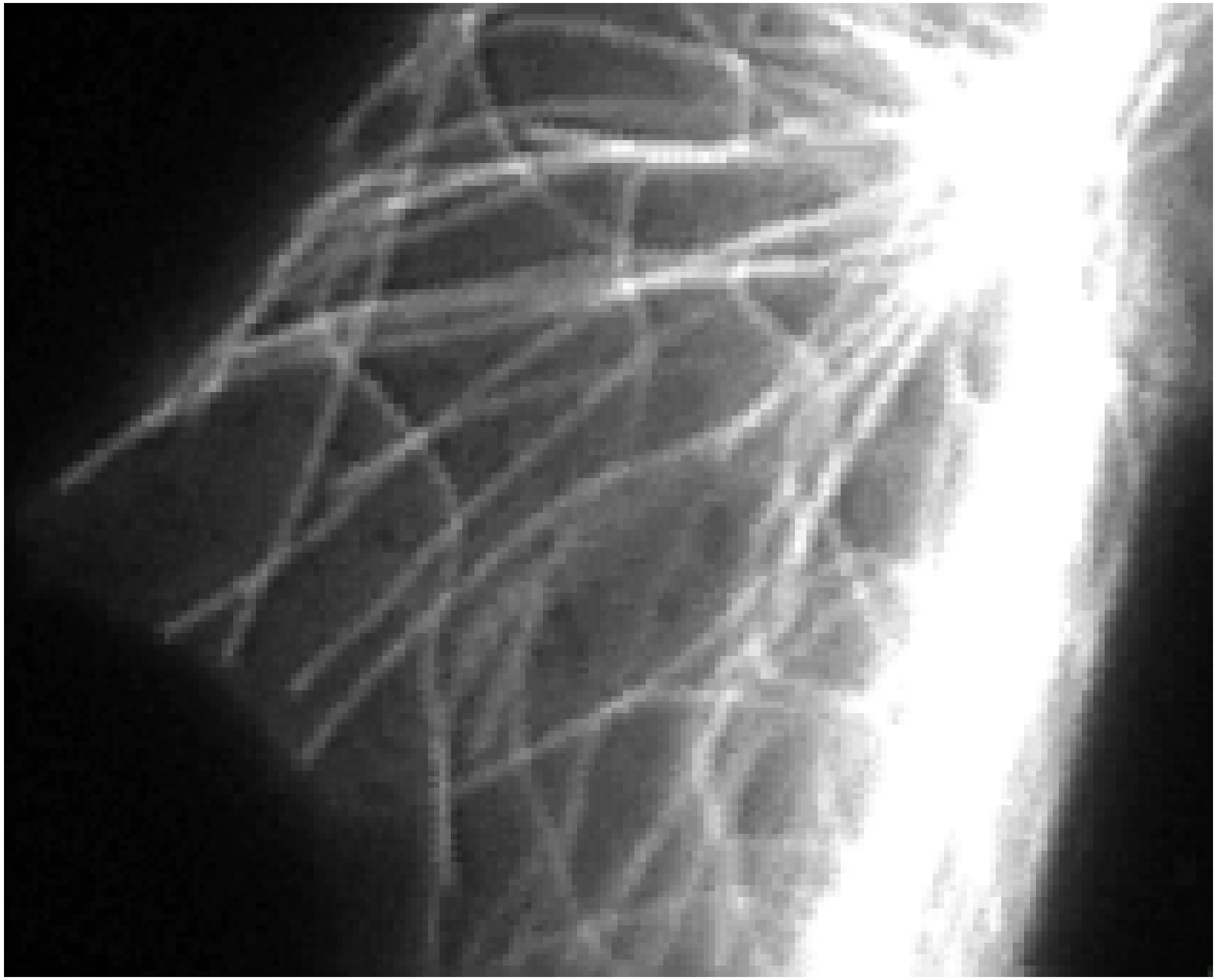


Microtubule Polarity and Dynamics

Polarized Microtubule Organization in Vivo

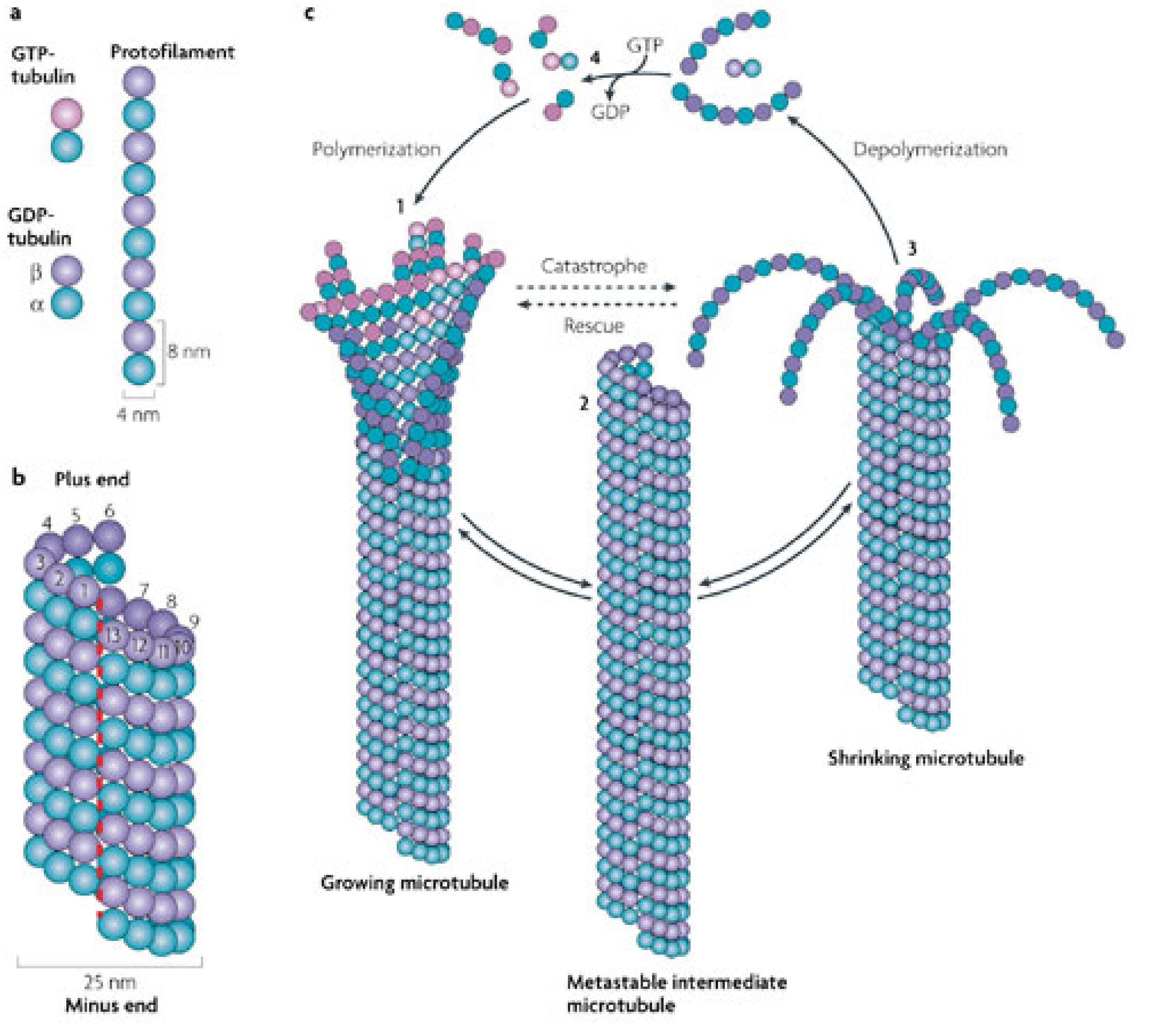


Microtubules are Dynamic



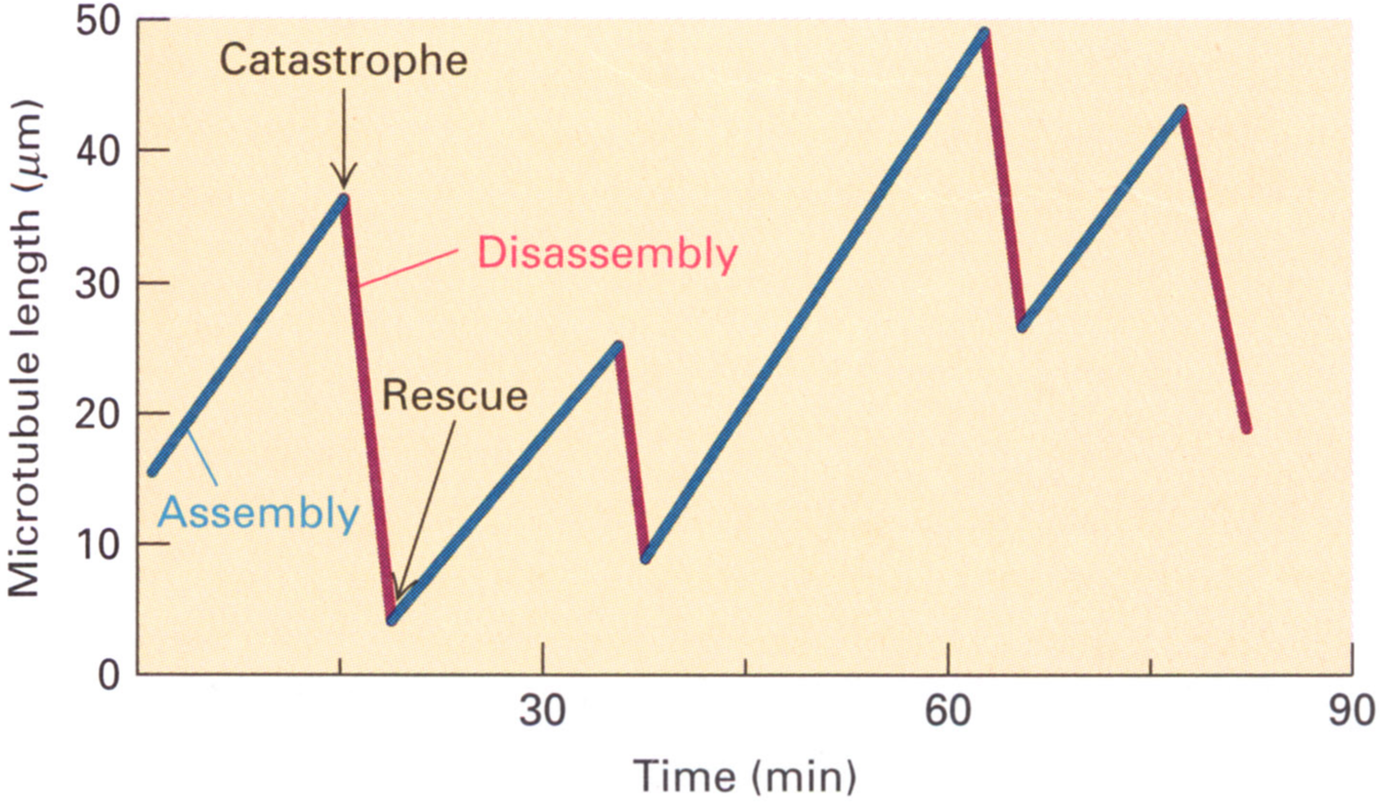
Fish melanophore injected with Cy3-tubulin
Vorobjev, I.A. et al., J. Cell Sci. 112 (1999)
Gary Borisy’s Lab

Microtubule Dynamic Instability

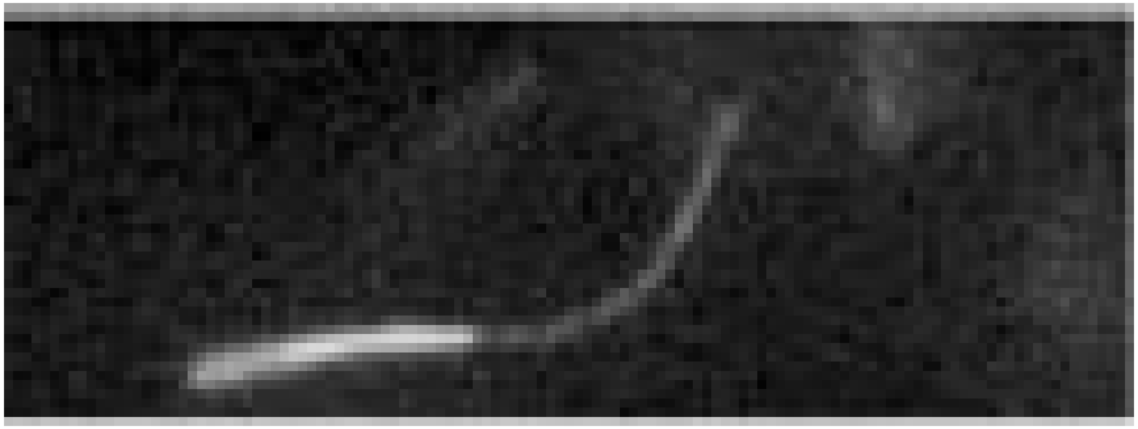


Nature Reviews | Molecular Cell Biology

Microtubule Ends Exhibit Dynamic Instability
Not Simple Equilibrium Assembly



In Vitro Microtubule Dynamic Studies



Defining Microtubule Polarity: the use of markers

Axonemes

Longer microtubules grow from the axoneme’s plus end

Polarity Marked MTs

GMPCPP MTs , crosslinked and labeled with a different fluor, or a higher stoichiometry of the fluor

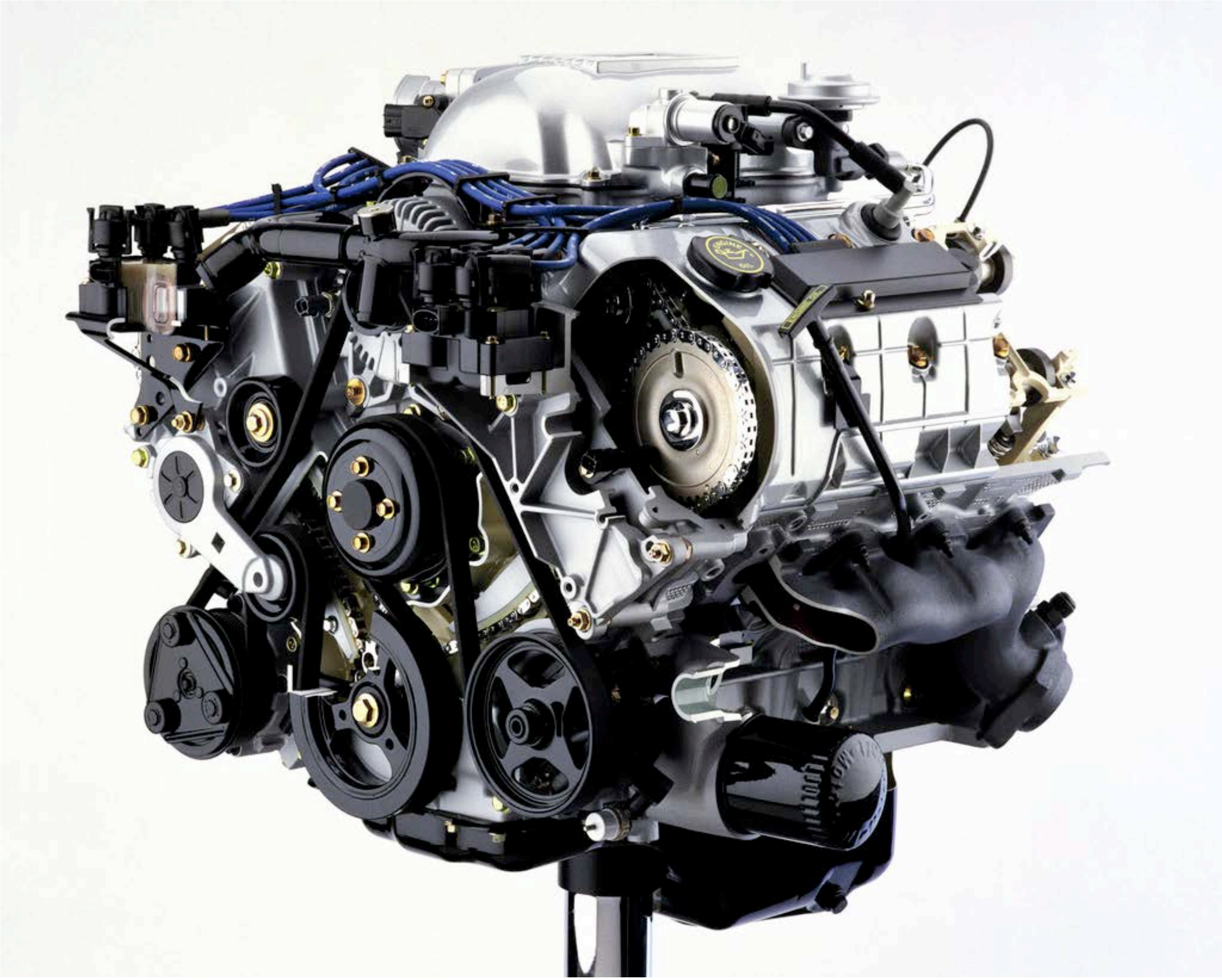
Microtubule Associated Proteins (MAPs) Modulate Microtubule Dynamics

Depolymerization and Polymerization Factors:

- Depolymerization:
  Stathmin
  Depolymerizing Kinesins (KinI)
  Spastin, Katanin (generates MT breaks)

- Polymerization:
  Stabilizing MAPs: MAP2, Tau)
  Many Plus End Tracking proteins:
  EB1, CLIP-170, CLASP, XMAP215

Force Generating Proteins:
Microtubule Motor Proteins



Microtubule Motor Functions

- Cilia and Flagella Motility
- Vesicle Transport
- Protein transport, RNA transport
- Polarized Organization of Nucleus, Golgi, ER, Mitochondria and Other Organelles in Cells
- Microtubule, actin filament and intermediate filament transport
- Microtubule Catastrophe Factors
- Spindle Assembly
- Motility of Kinetochores and Chromosome Arms
- Intra-flagella transport for cilia, flagella, photoreceptor and chemoreceptor function

Overview

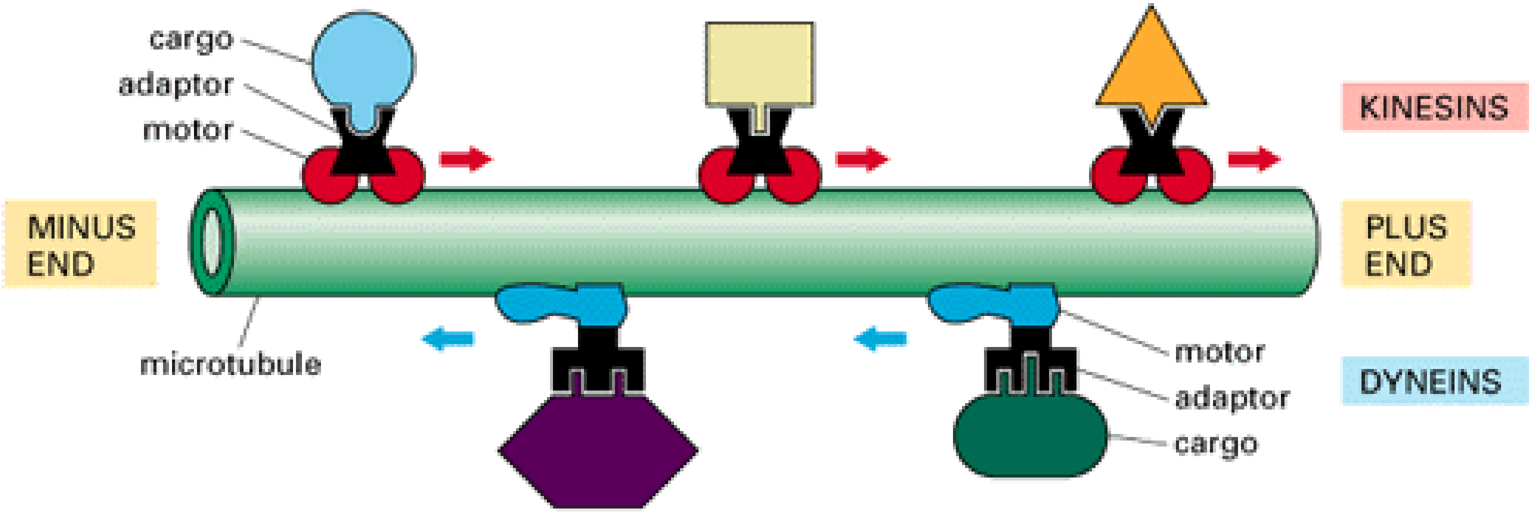
1. Kinesins and Kinesin-Related Proteins (cytoplasmic)

2. Cytoplasmic dyneins

3. Axonemal dyneins (cilia and flagella)

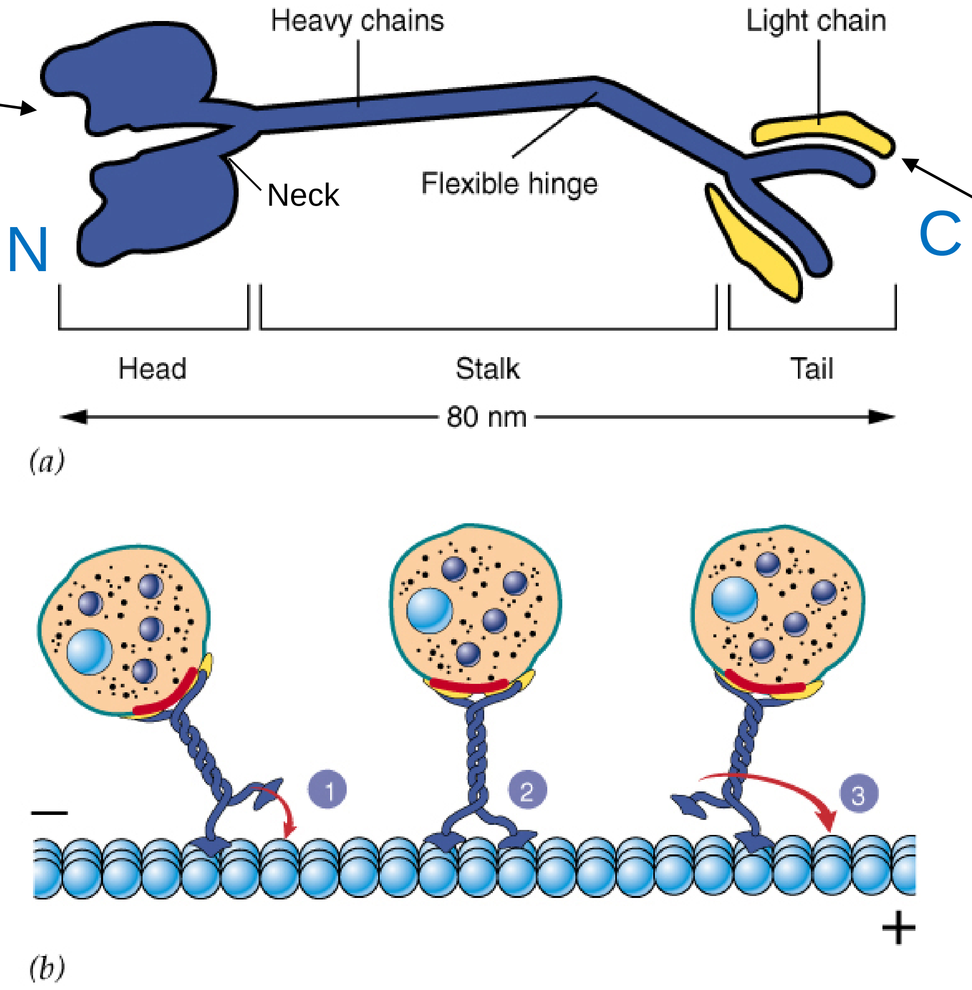
Cytoplasmic MT-based motors

Kinesin / kinesin-related proteins and cytoplasmic dyneins:



Kinesin I (conventional kinesin)

Head: Motor Domain
ATP Hydrolysis
MT Binding



Tail: Cargo Binding
(Vesicles, etc.)

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How kinesin (and dynein) motility is studied:

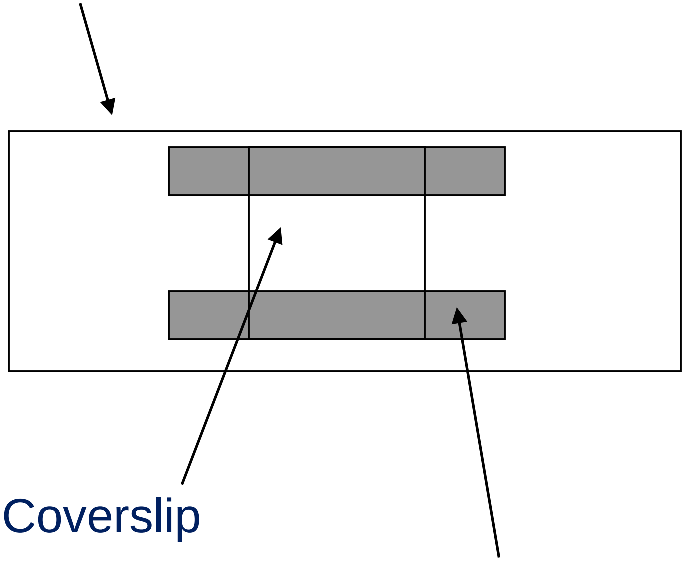
Motility assays:

(1) MT gliding assays
(2) bead assays
(3) single molecule fluorescence: FIONA

Types of imaging:

(1) DIC
(2) fluorescence
(3) optical trap
(4) atomic force microscopy

Preparations for Motility Assays

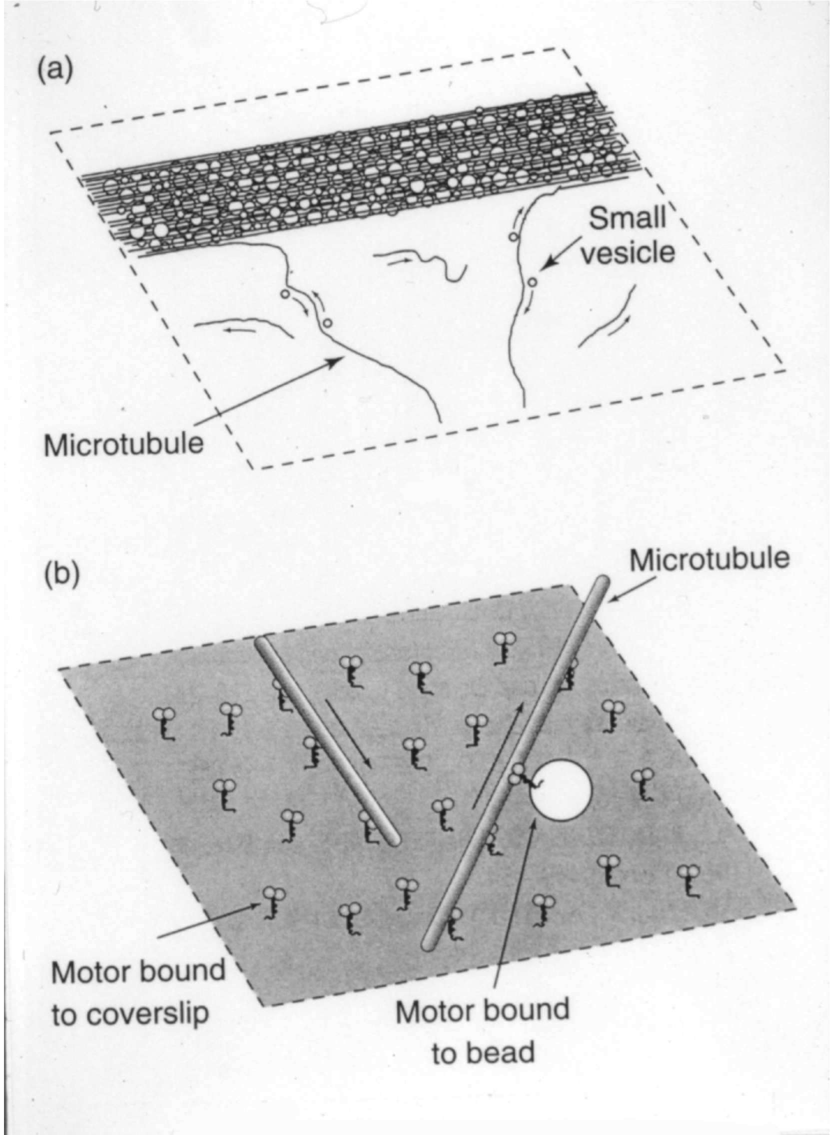


Slide

Coverslip

70mm Thick
Double-Stick Tape

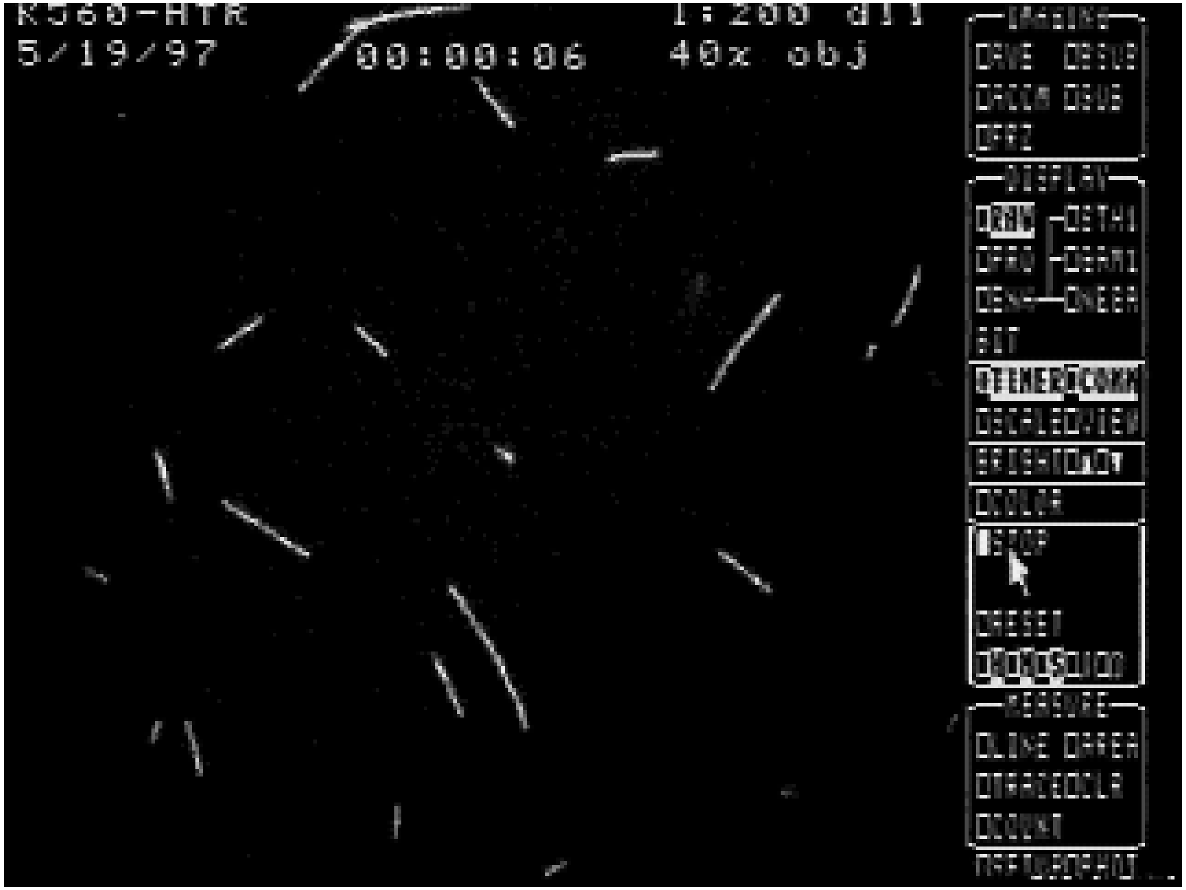
Perfusion Chamber



Imaging MT motility using fluorescent MTs

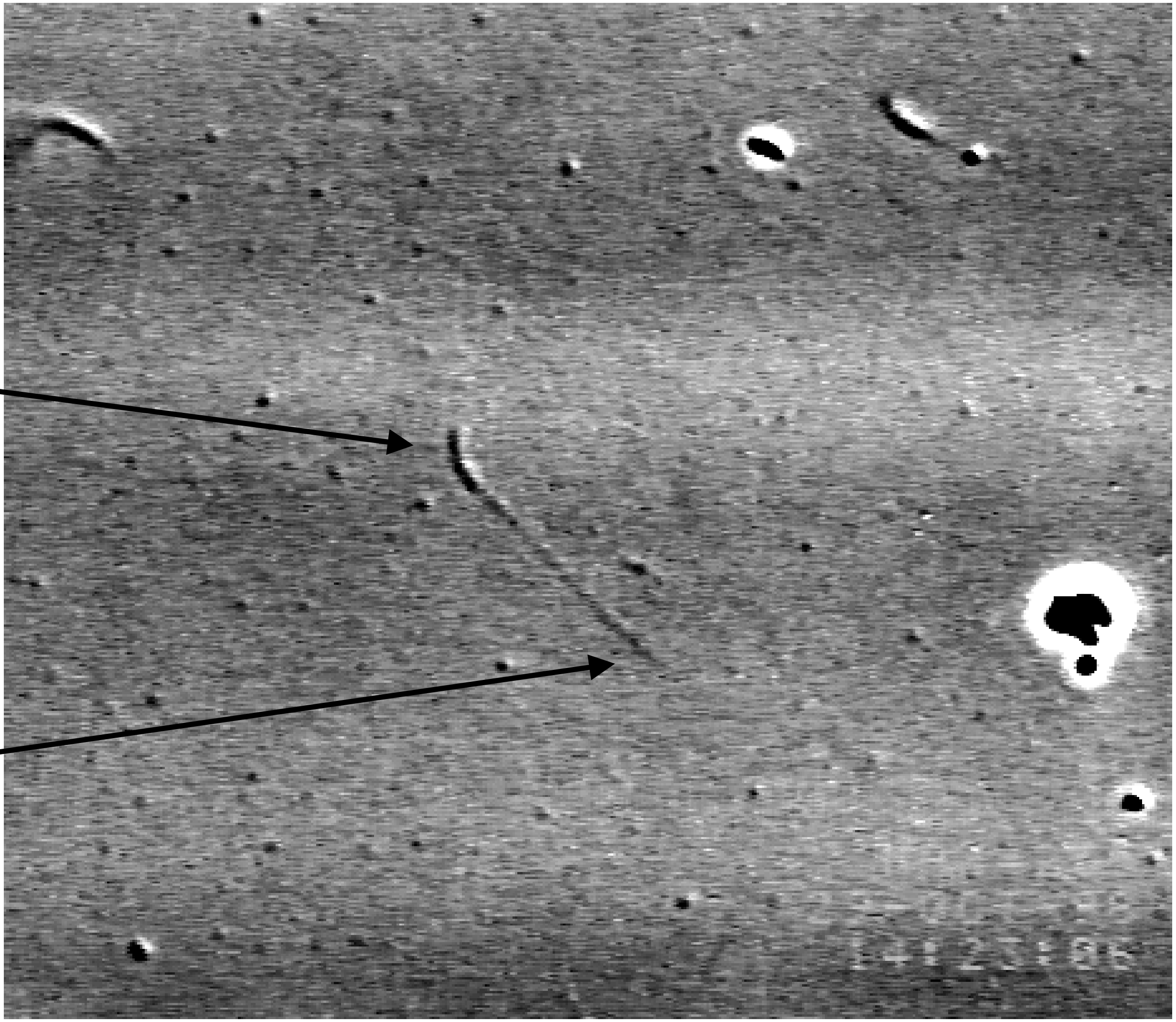
Microtubules assembled with rhodamine tubulin -usually stabilized by taxol

Kinesin bound to surface of coverslip



Time: 30x

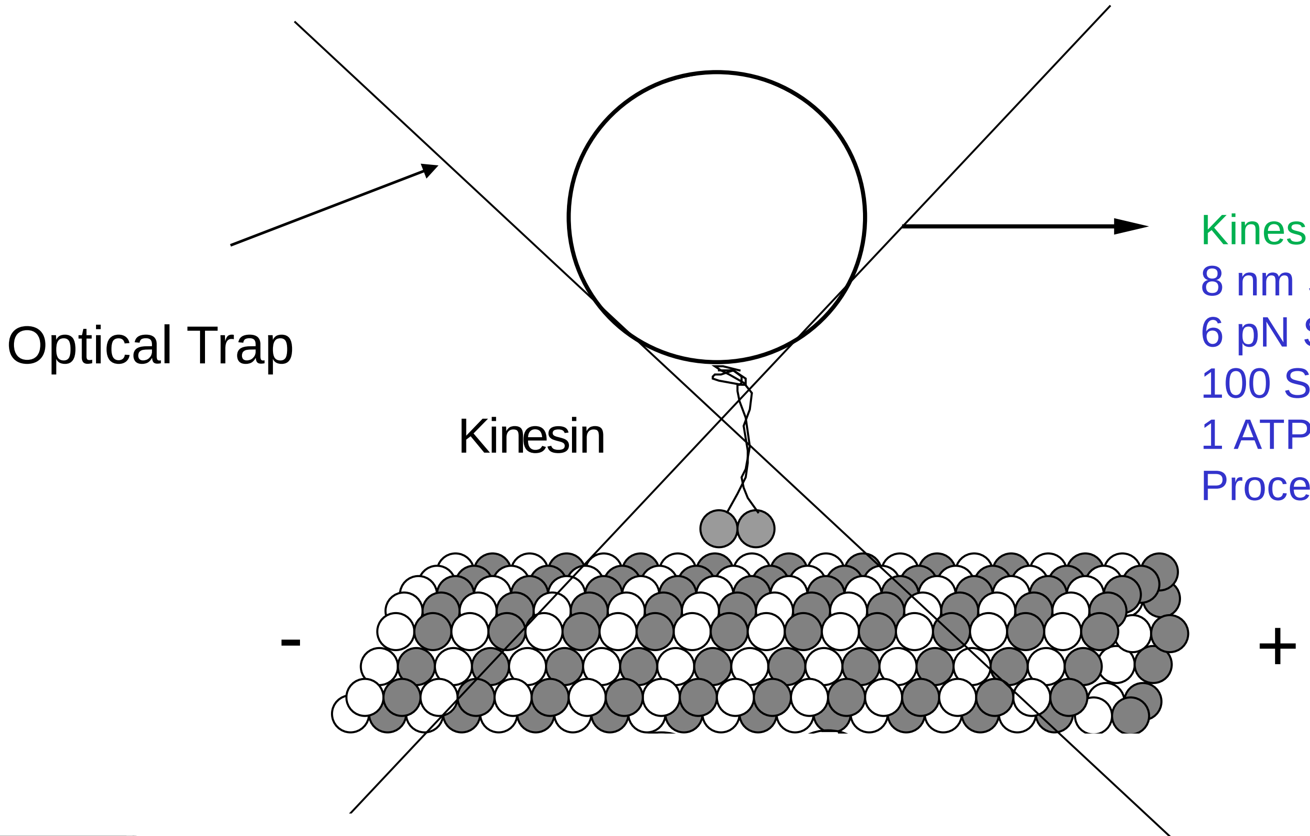
Polarity marked MT imaged by VE-DIC



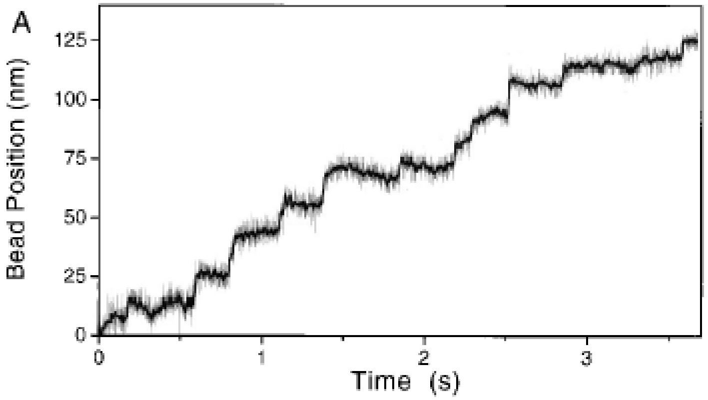
Axoneme

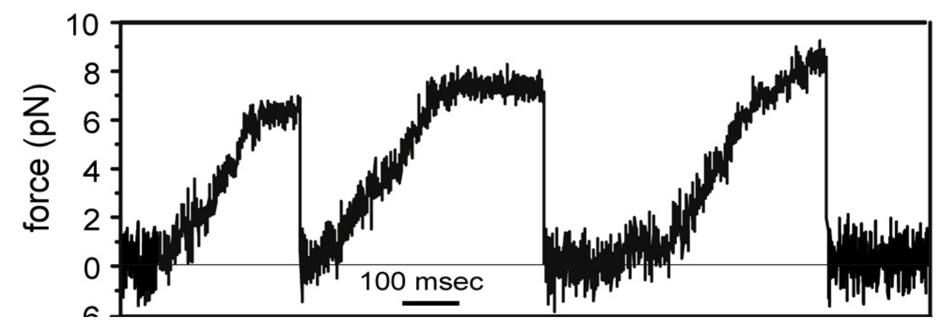
MT plus end

Microtubule motor driven organelle motility

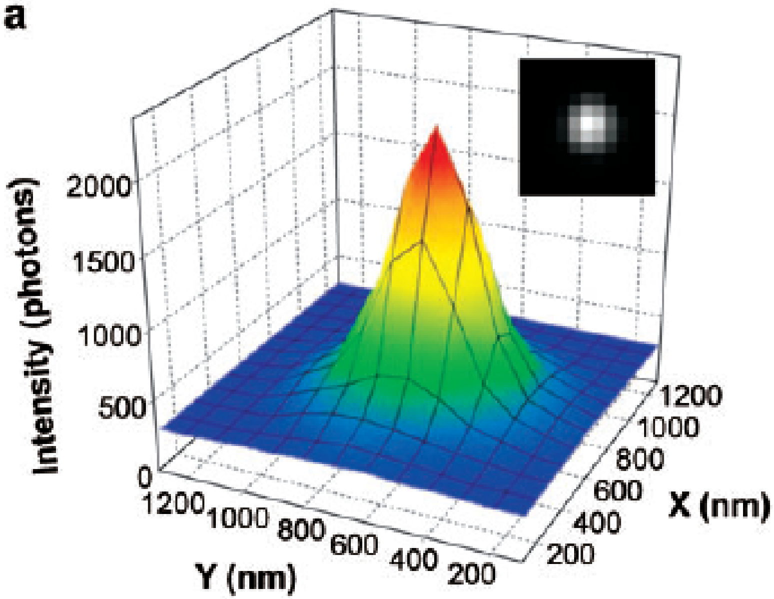


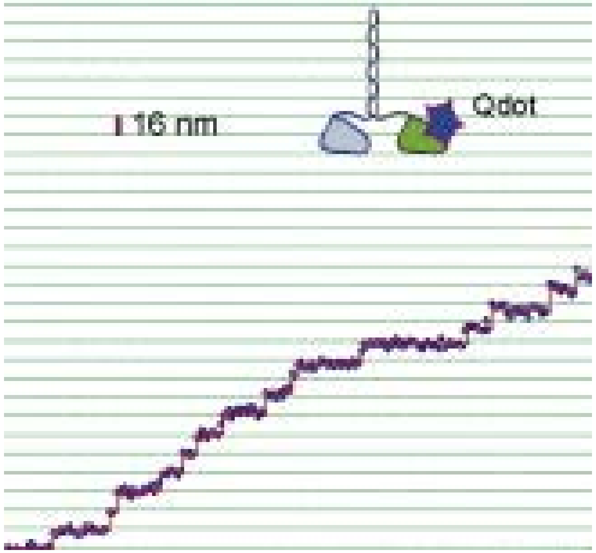
Kinesin 1:
8 nm Step
6 pN Stall Force
100 Steps/sec at no Load
1 ATP Hydrolyzed per step
Processive





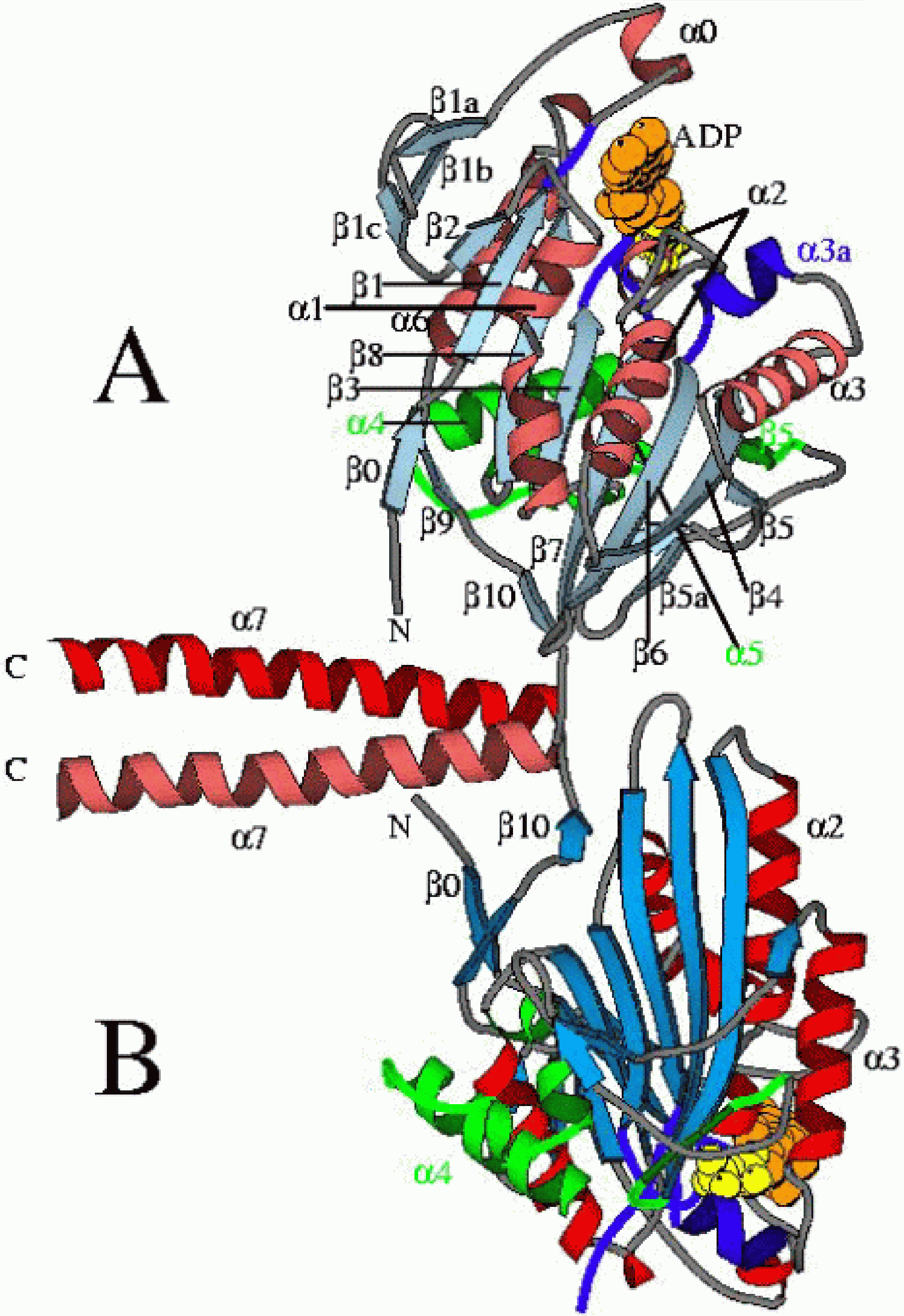
FIONA: Fluorescence Imaging with One-Nanometer Accuracy





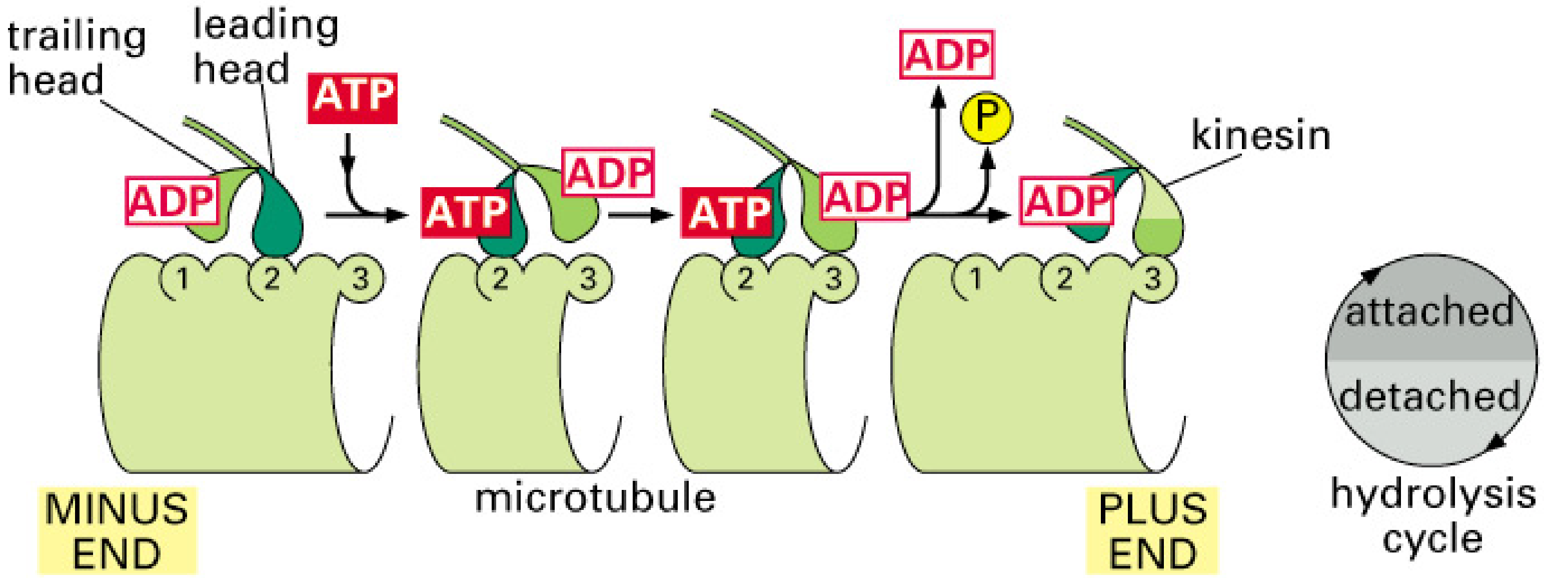
Each motor head takes 16 nm steps,
The center of mass moves 8 nm/step = length of a tubulin heterodimer

How does kinesin use ATP to generate force for movement?



Crystal structure of the kinesin motor domain: two heavy chains are dimerized via the stalk regions

How does kinesin use ATP hydrolysis to generate force for movement?



** ATP hydrolysis cycle regulates MT affinity and docking of the neck linker onto the motor domain through conformational change

** **Nucleotide Free**: head is bound to MTs and the linker is mobile
** **ATP binds**: linker docks into the motor domain, throwing the back head forward
** **ATP hydrolysis**: lowers kinesin's affinity for the MT, kinesin detaches the linker from the motor domain

Kinesin is a processive motor

A single motor can travel for hundreds of ATPase cycles along a MT without dissociating

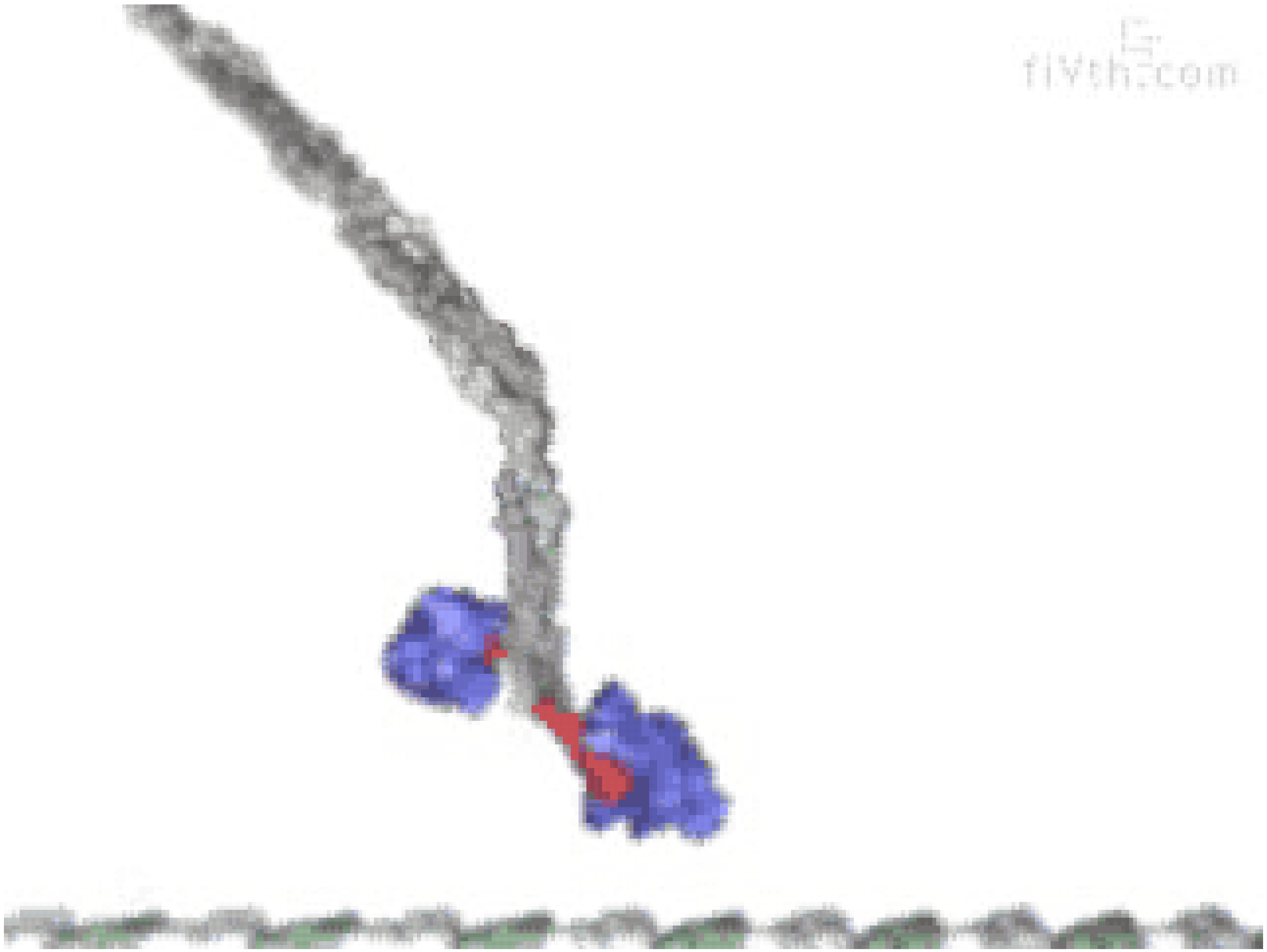
WHY?

1. **cycles of the motor heads are coordinated with each other so that one kinesin doesn’t let go until the other one binds the MT (hand-over-hand motion)**

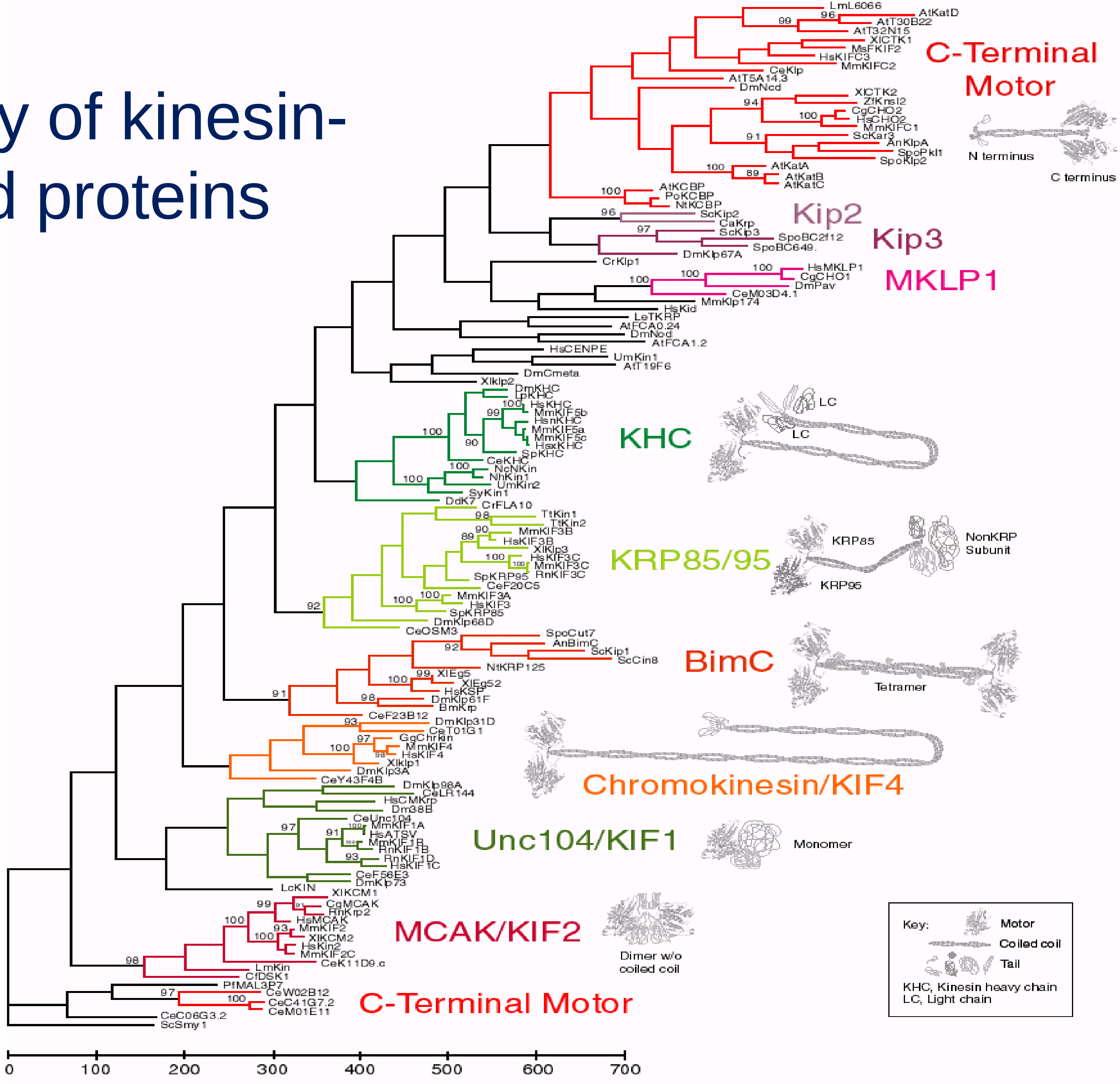
2. **kinesin spends half of its cycle in the MT-bound state (vs. ~5% for myosin)**

The ATP hydrolysis cycle and Kinesin Processivity

Ron Milligan’s Web site, Scripps

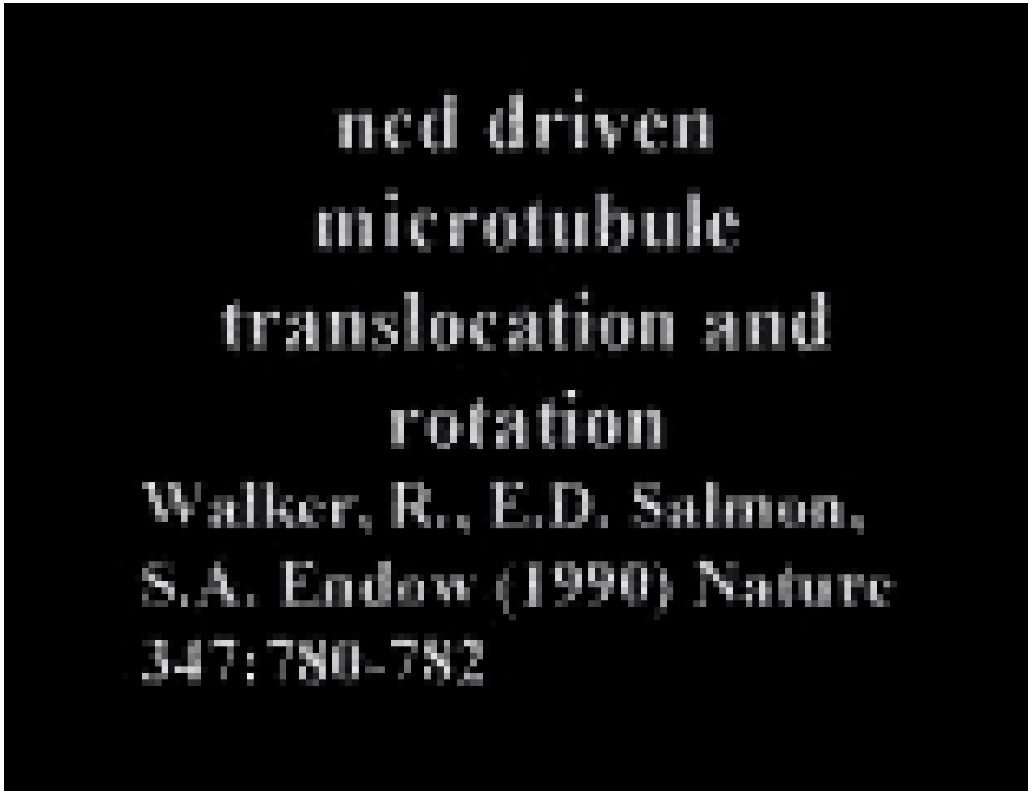


Discovery of kinesin-related proteins



A. J. Kim & S. A. Endow April 2000

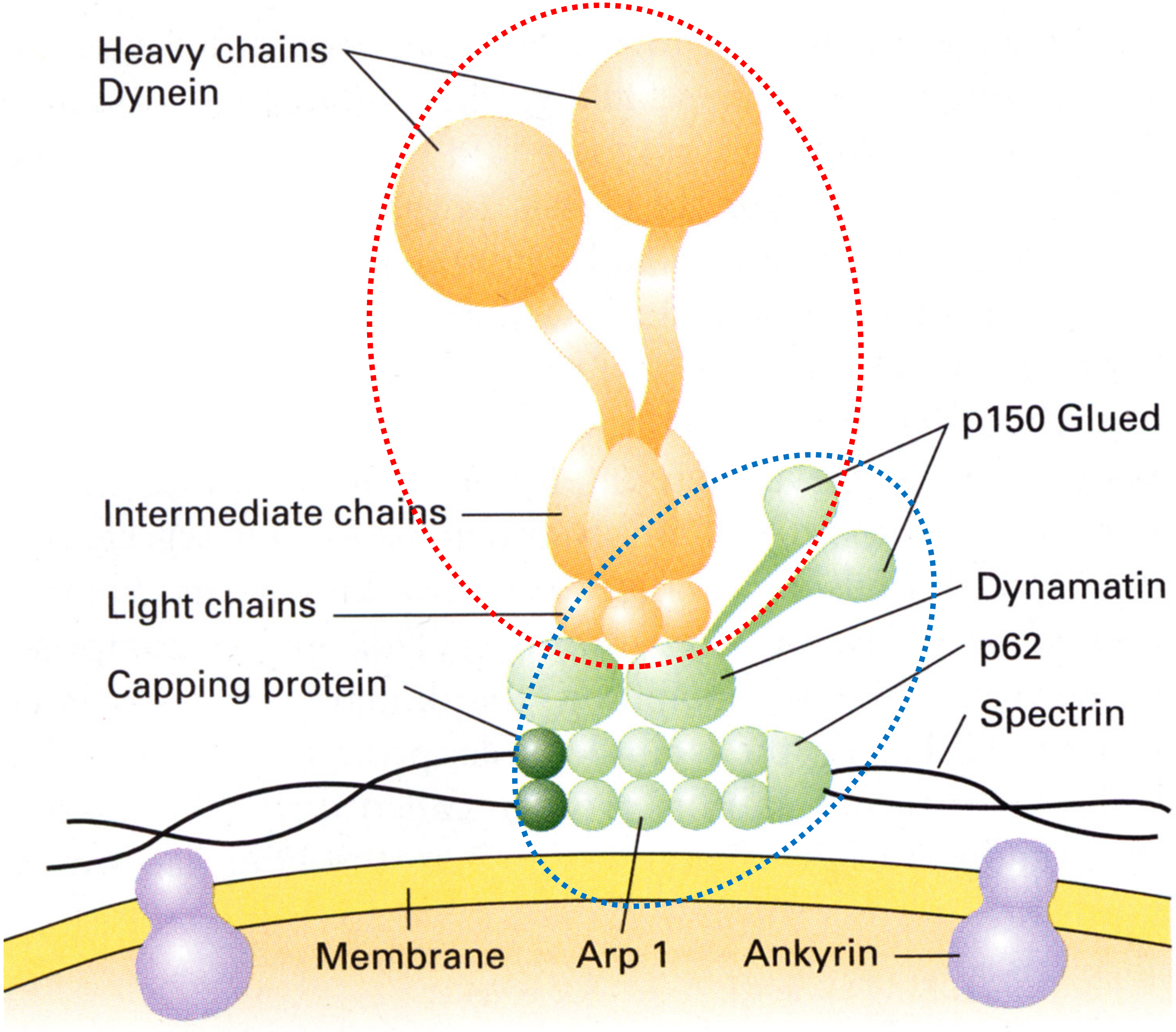
VE-DIC Microtubule Motility Assay: Ncd is a Minus End-directed MT Motor



ncd driven
microtubule
translocation and
rotation

Walker, R., E.D. Salmon,
S.A. Endow (1990) Nature
347:780-782

2. Cytoplasmic Dynein/Dynactin Complex

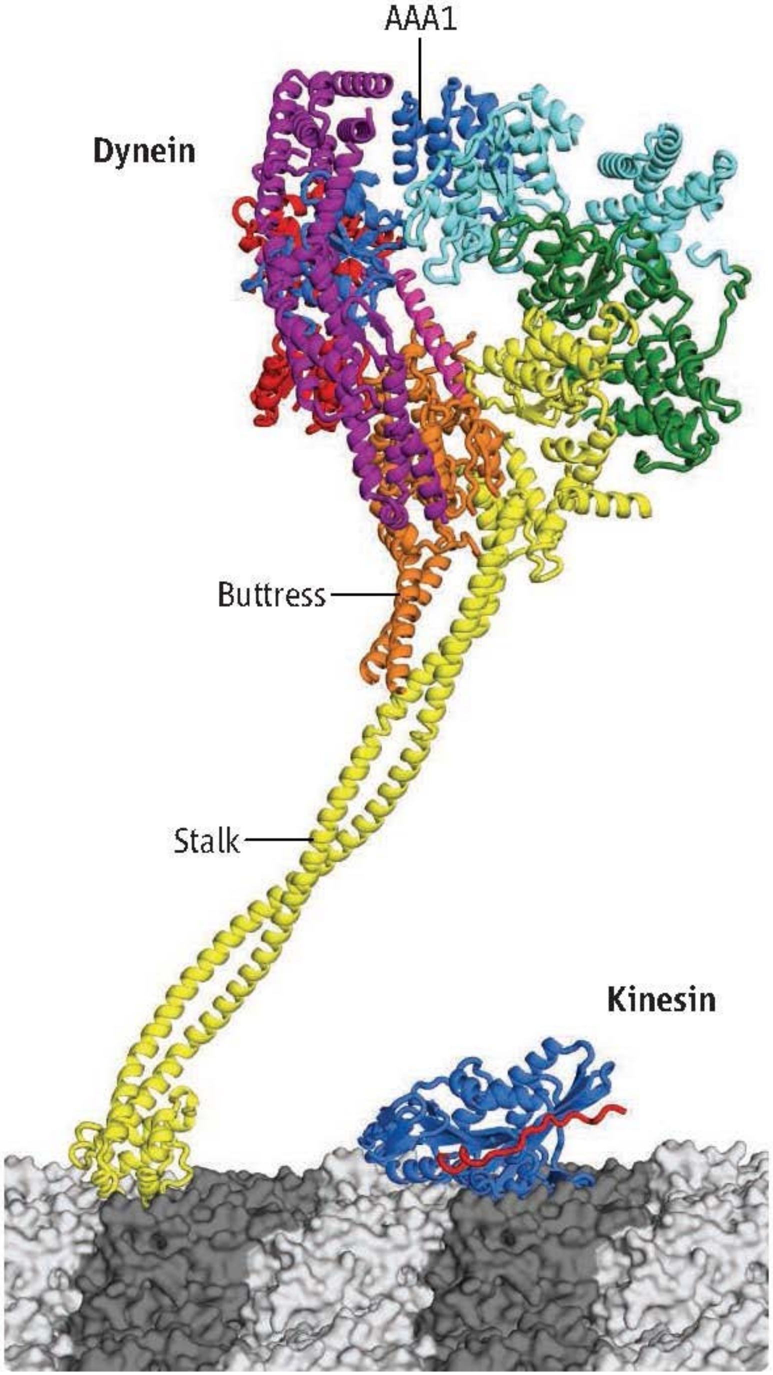


Roles for dynein:

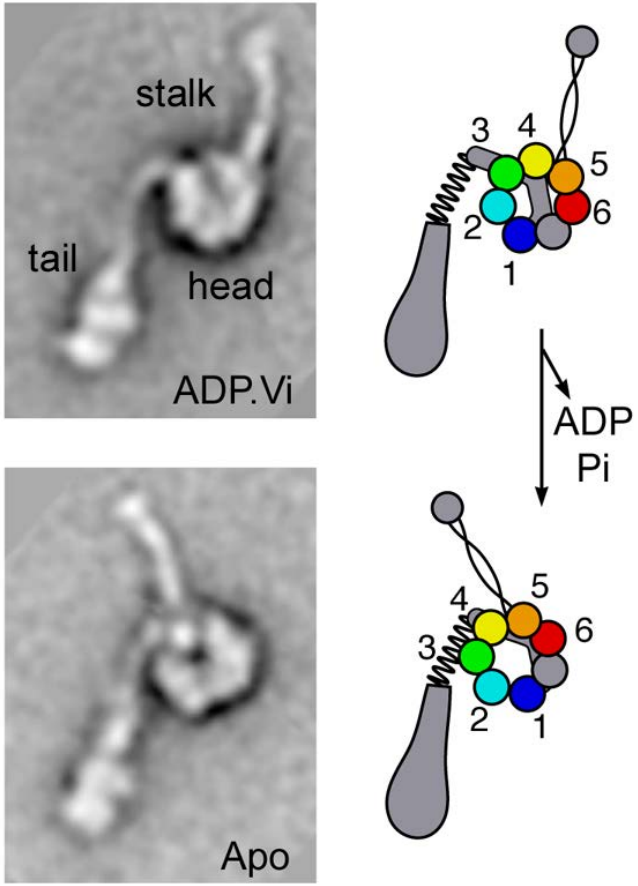
-Minus end-directed vesicle trafficking

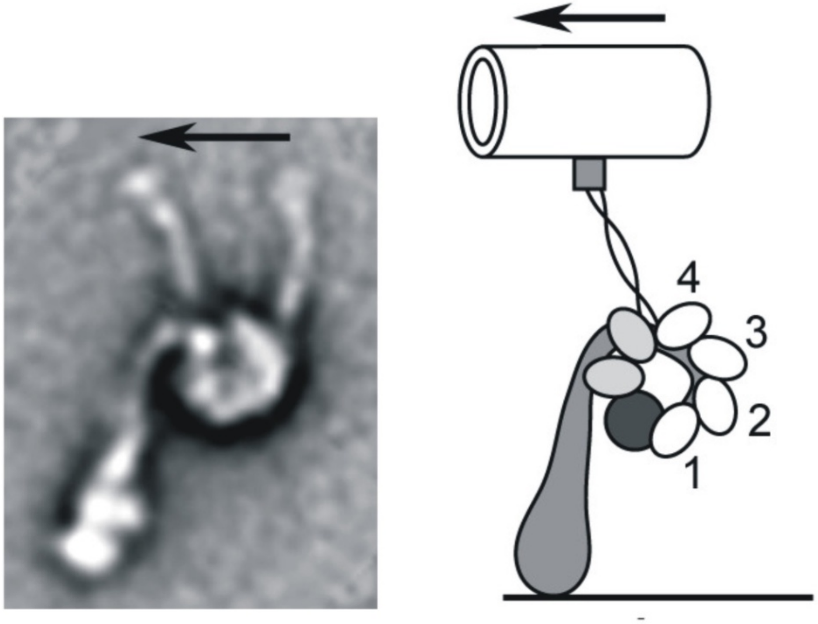
-Localization of the Golgi at cell’s center

-Mitotic spindle assembly and dynamics

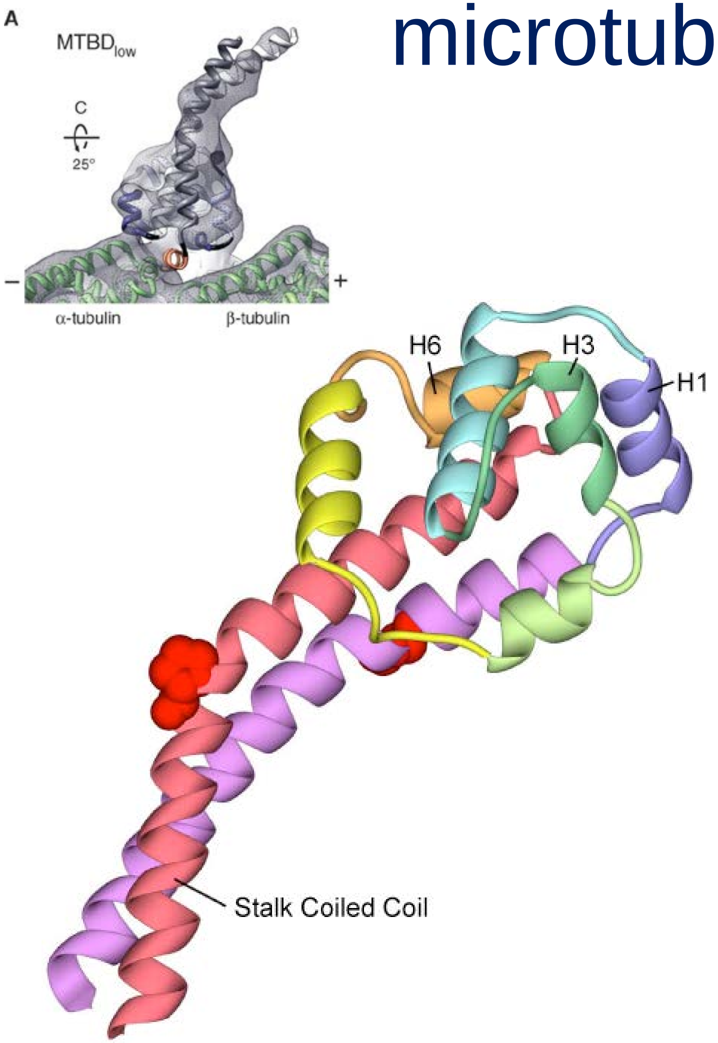


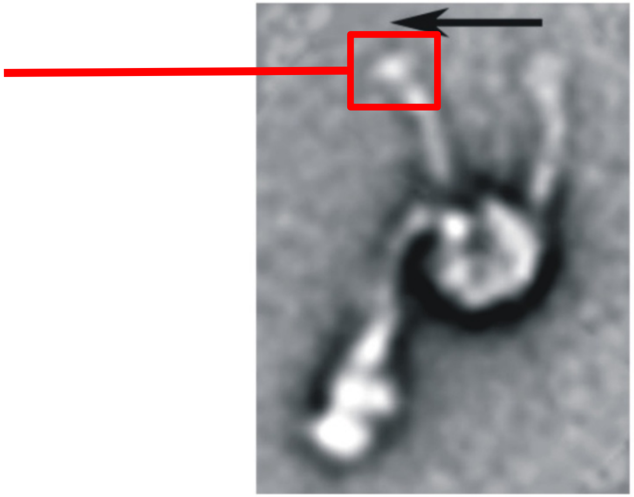
ATP-dependent structural changes in Dynein

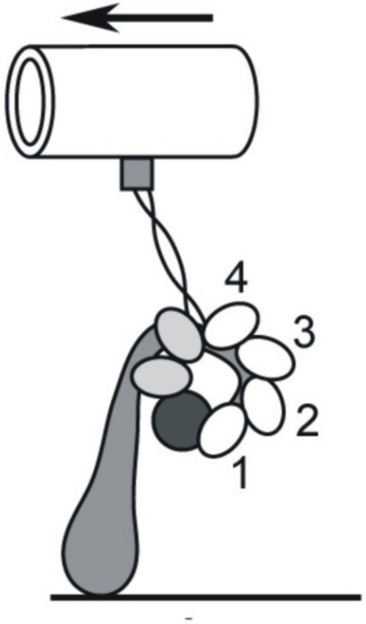




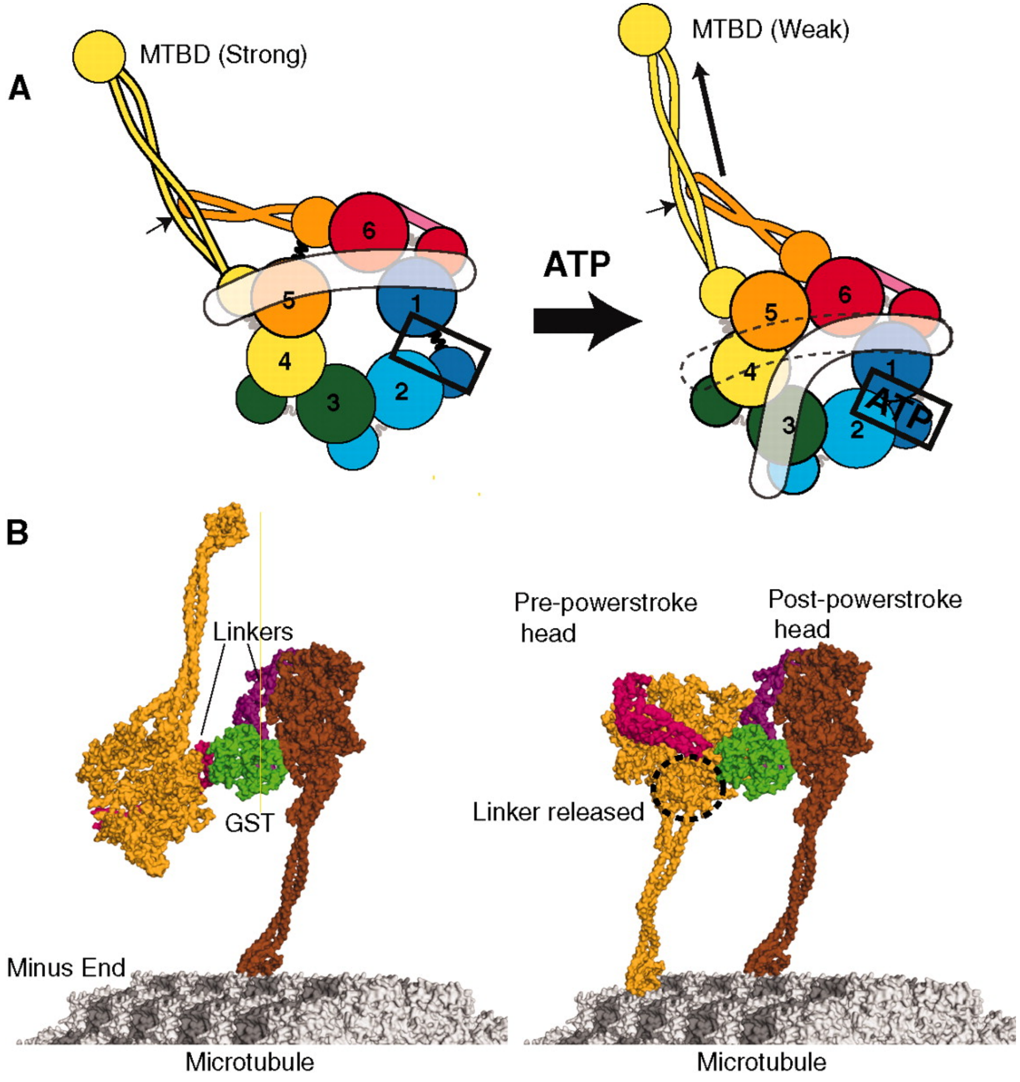
Structural changes must be propagated to the microtubule binding domain







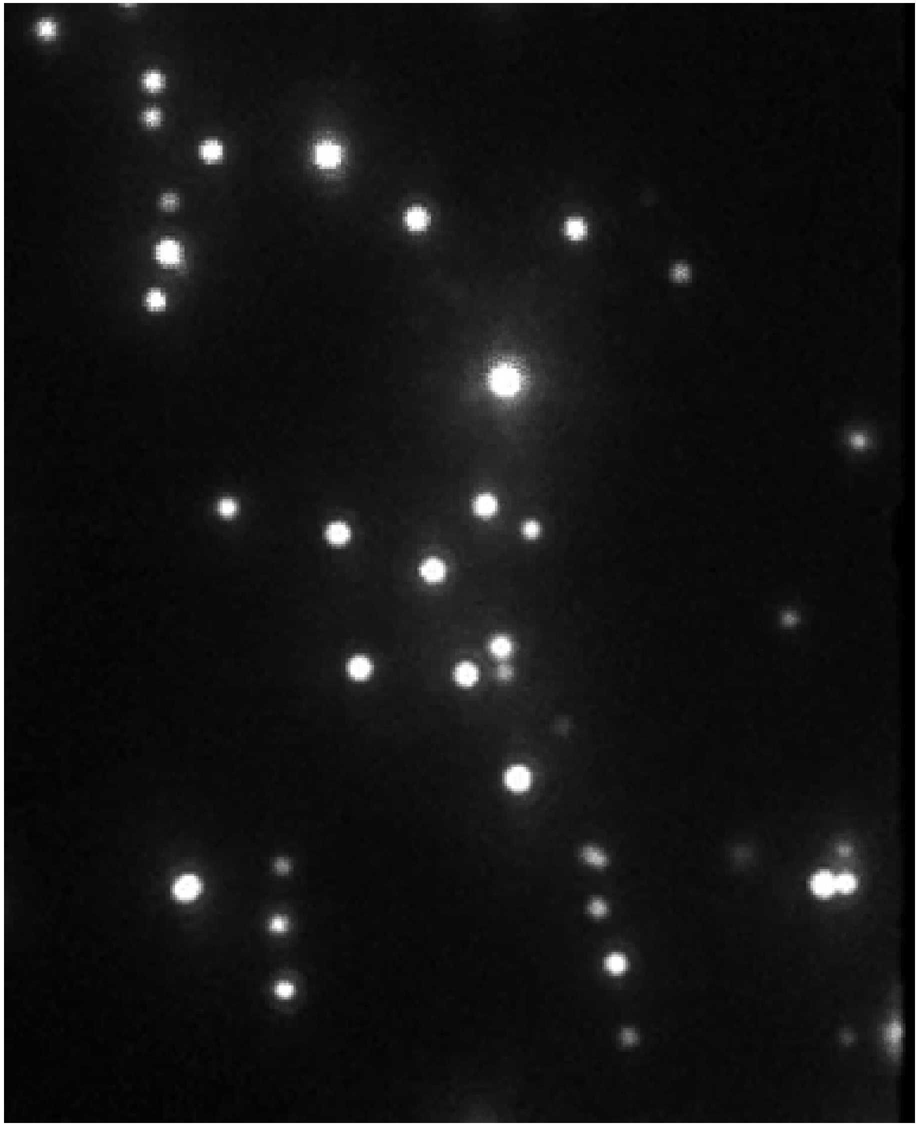
Structures of Cytoplasmic Dynein



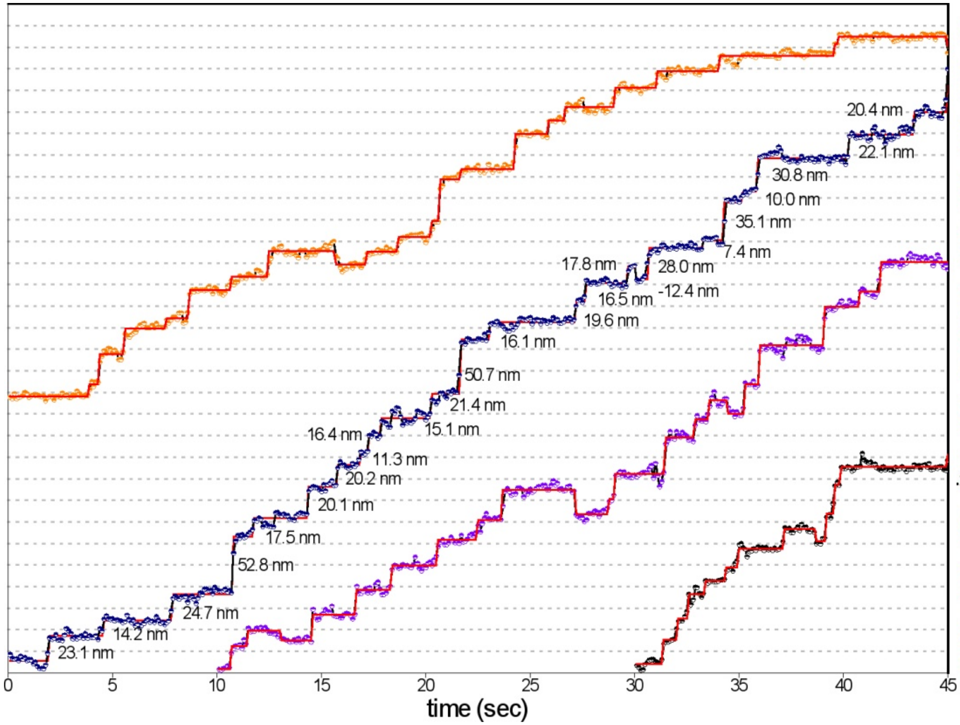
Crystal Structure

Two-Head Bound Model

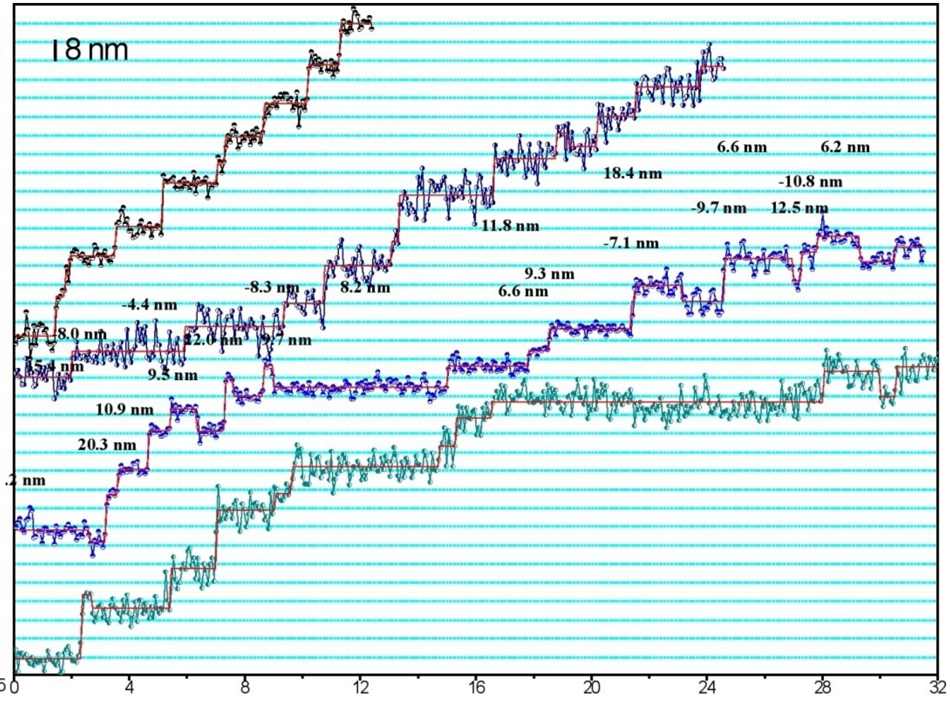
Imaging dynein motility using Quantum Dots



Dynein Step Size (FIONA): Fluorophore on the Motor or Tail

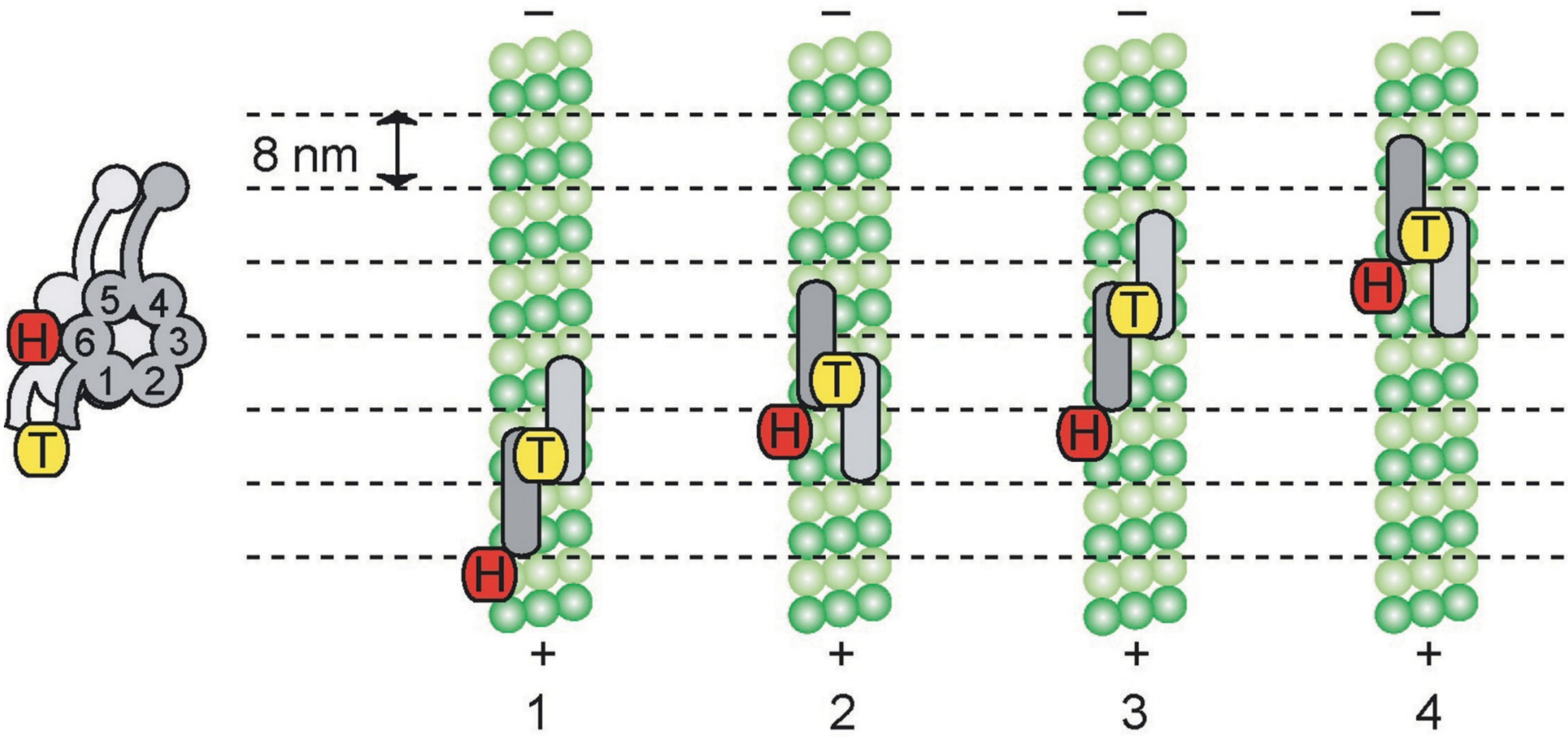


Motor-Labeled
16 nm steps

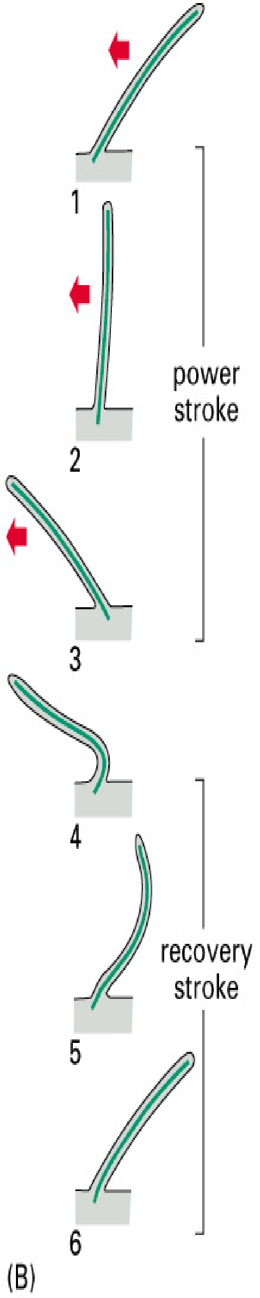


Tail-Labeled
8 nm steps

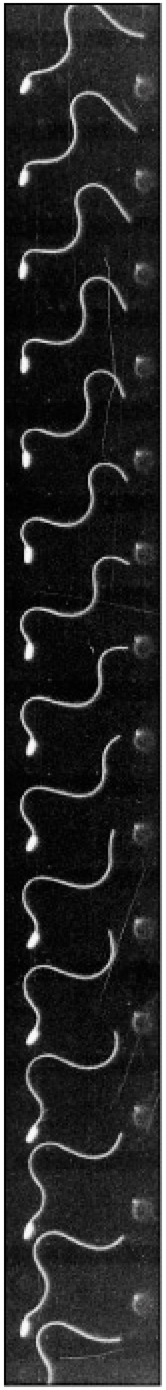
Dynein: Each head (motor): 16nm steps
Center of mass (tail): 8nm steps



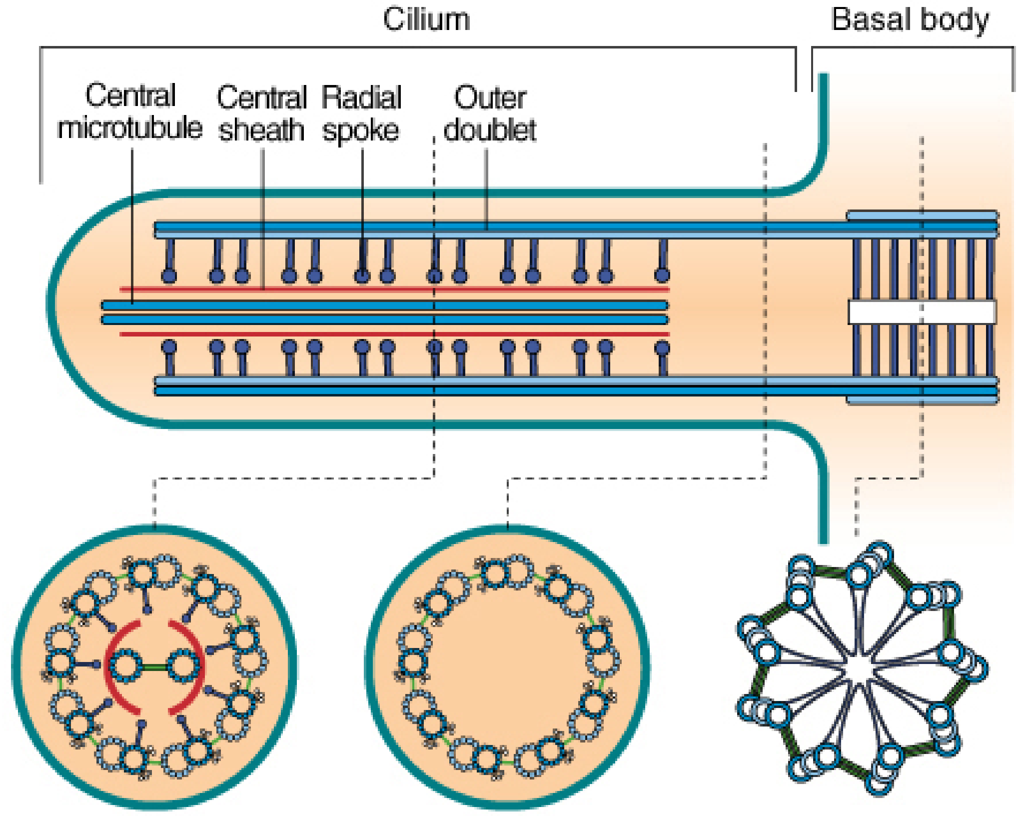
3. Axomenal Dyneins
Cilia vs. Flagella



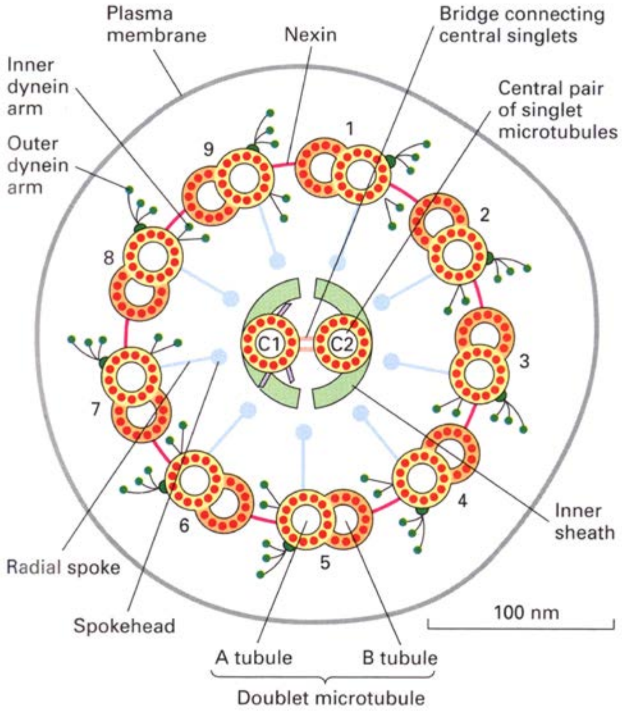
Cilia: move single cells or move fluid over cells	Flagella: motor sperm and protozoa through liquid
Oar-like Power stroke and Recovery	Multiple beating patterns
Shorter (~10 μm)	Longer (>100 μm)
Many working together: Coordinated movement	Cells typically have one or two



Movement of both cilia and flagella is generated by the bending of their core: the axoneme



(b)



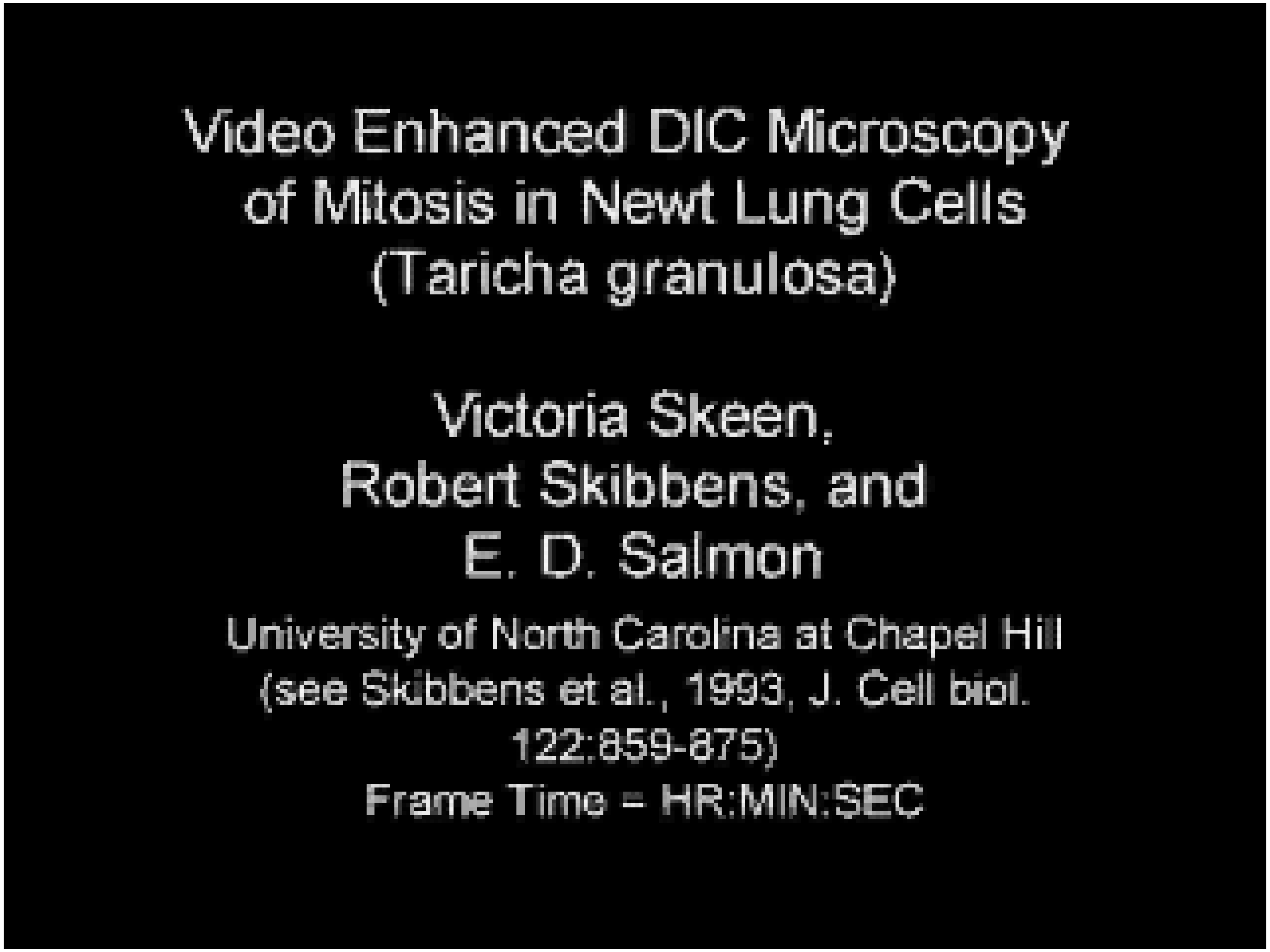
Kinesin vs. Dynein

	Kinesin	Dynein
Direction	Plus End (exception: NCD)	Minus End
Step Size	8 nm (16 nm single head)	varies (8, 12, 16 nm, etc.)
Oligomer	Dimer	Dimer
Nucleotide	ATP	ATP
Fold	G-protein-like	AAA domains
Family	numerous members	cytoplasmic (homodimer) axonemal (heterotrimer)
Subunits	Associated chains (cargo)	Associated chains (cargo)

Mitosis:

Achieving Accurate Chromosome Segregation

Time-Lapse of Mitosis

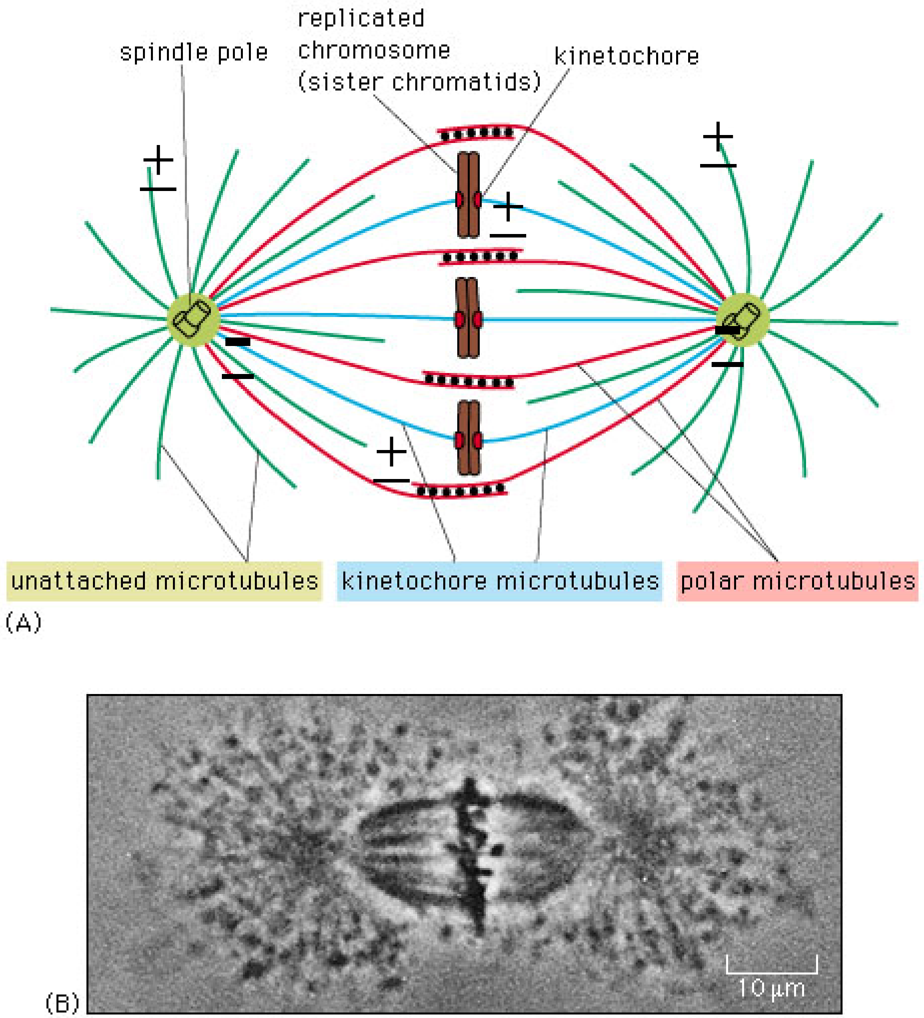


Video Enhanced DIC Microscopy
of Mitosis in Newt Lung Cells
(*Taricha granulosa*)

Victoria Skeen,
Robert Skibbens, and
E. D. Salmon

University of North Carolina at Chapel Hill
(see Skibbens et al., 1993, J. Cell biol.
122:859-875)
Frame Time = HR:MIN:SEC

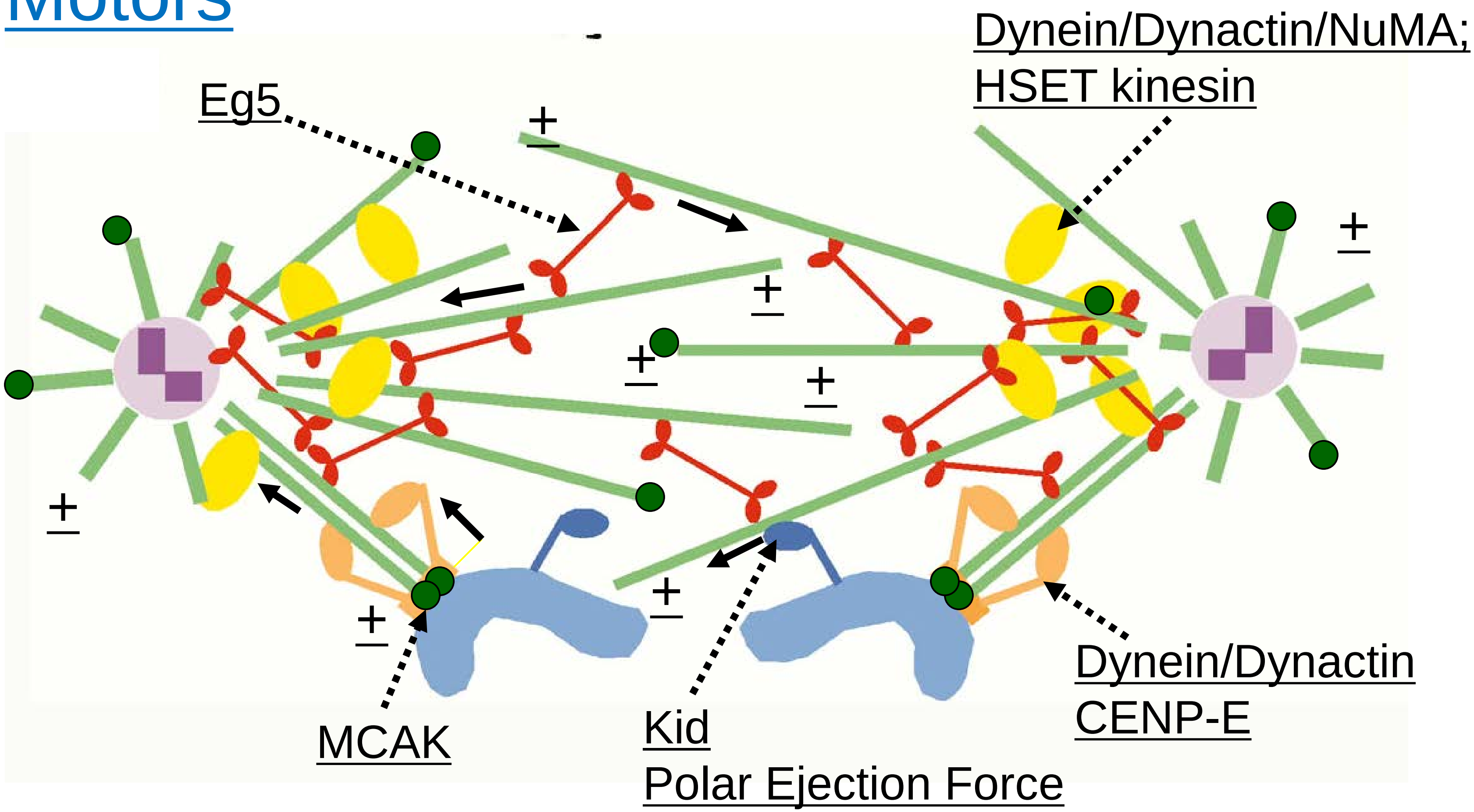
Microtubule Organization Within the Bi-Polar Metaphase Mitotic Spindle



Chromosomes Segregate Toward MT Minus Ends at Spindle Poles

The Mitotic Spindle is a Dynamic Assembly of Microtubules (MTs) and MT Motor Proteins

Motors



Eg5

Dynein/Dynactin/NuMA;
HSET kinesin

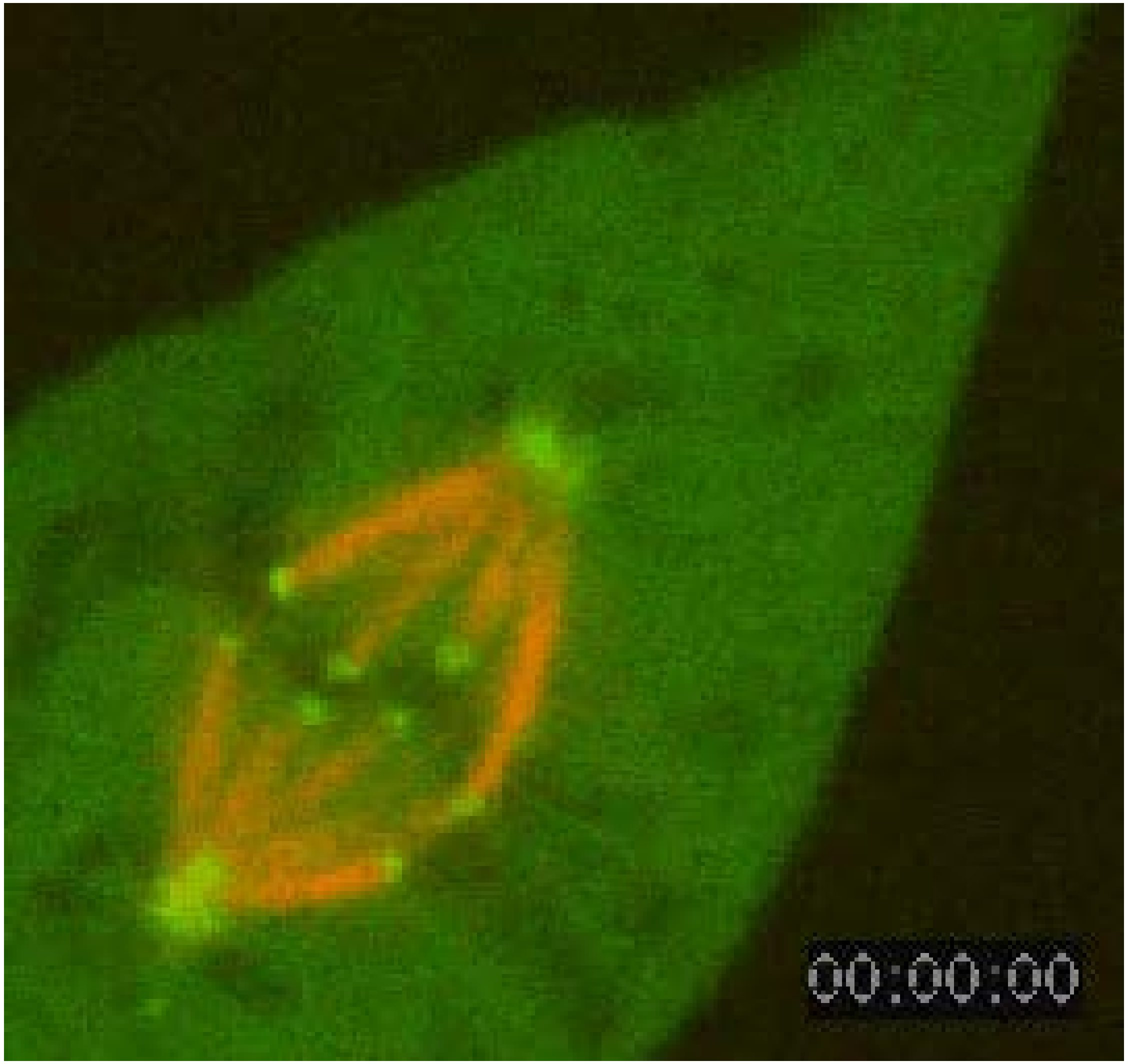
MCAK

Kid
Polar Ejection Force

Dynein/Dynactin
CENP-E

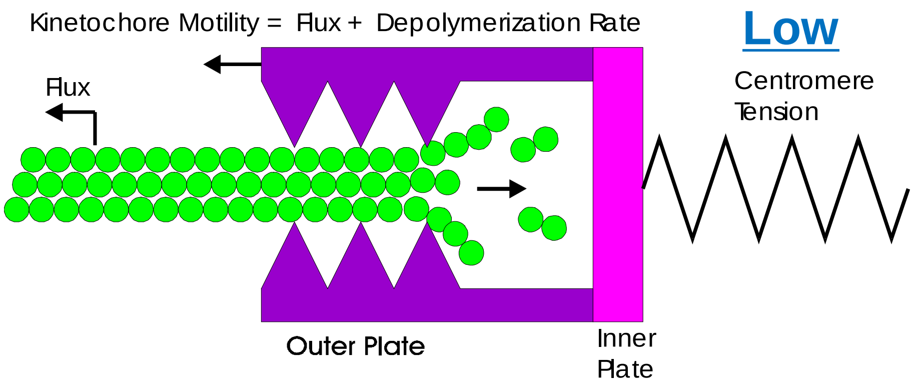
Mitchison and Salmon, 2001, Nat. Cell Biol. 3:E17-E21

In Tissue Cells, Kinetochores Exhibit Directional Instability



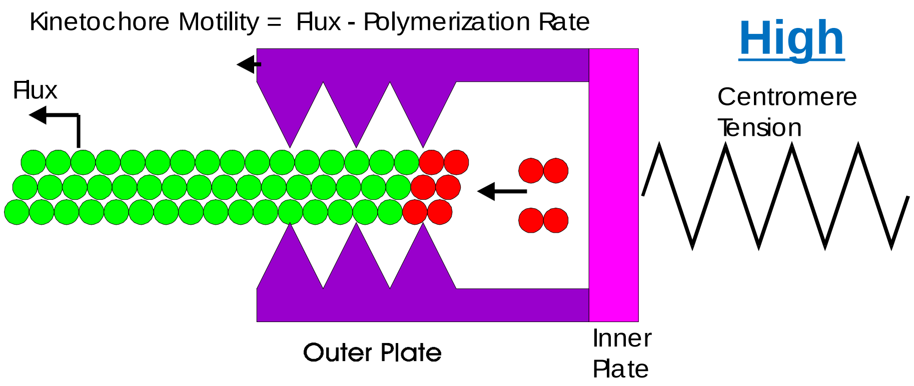
States of Kinetochore Bi-Stability
Depolymerization and the Slip-Clutch

Depolymerizing State, Force Generating



- -Kinetochore generates pulling force coupled to depolymerization
- -Kinetochore force and flux force additive

Polymerization State,Resistive



- -MT is pulled out of kinetochore by flux forces and centromere tension
- -Kinetochore resists sliding at the lattice interface to produce tension
- -Polymerization at sliding velocity prevents detachment

🔴🔴 GTP-Tubulin 🟢🟢 GDP-Tubulin