# Molecular Cell Biology

Microtubule

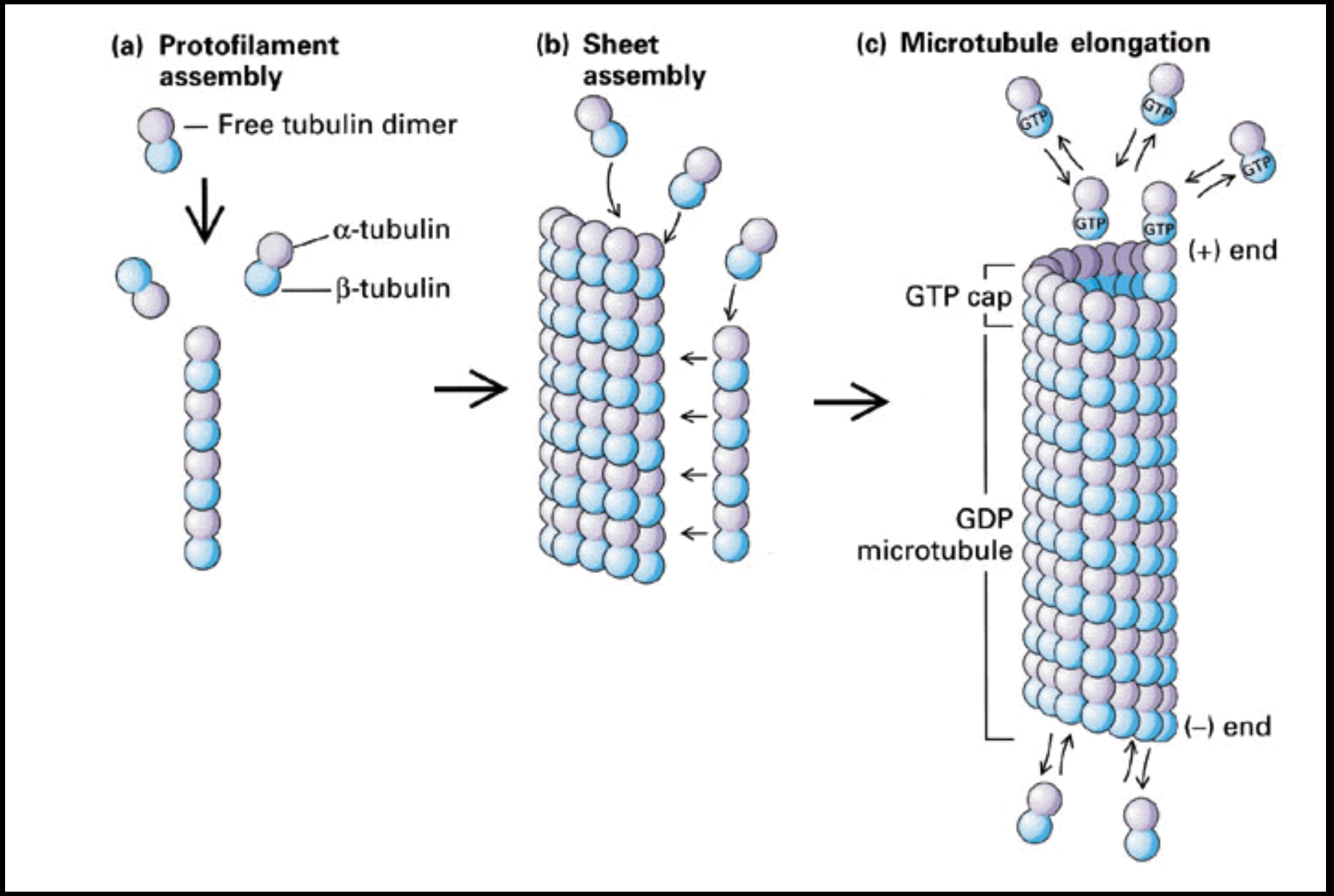
# Microtubules and their Motors

- Intro
- Vesicle Trafficking
- Cilia
- Mitosis

# Microtubule Structure

- Cross-section
  - Hollow tube
  - 24 nm wide
  - 13-15 protofilaments
- Helical structure
- Polar
  - Plus ends generally distal
  - Minus ends generally proximal (at MTOC)
- Composed of Tubulin ⟨/® Heterodimer

# Microtubule Structure & Assembly



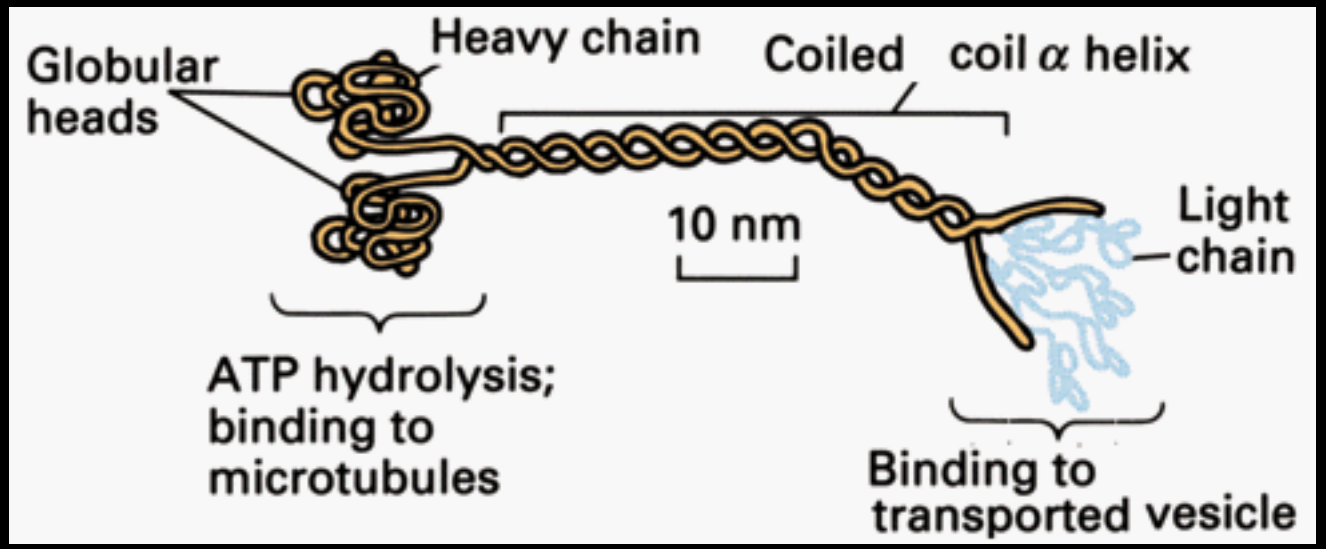
# Microtubule Motors

- Definition
  - Microtubule-stimulated ATPase
  - Motility along MT's
  - Sequence of known motor
- Dynein
  - Moves to Minus End of Mt
  - Large, multi-subunit protein
- Kinesin
  - Moves to Plus End of Mt
  - Exception - Ncd/Kar3

# Discovery of Kinesin

- Search for Motor for Axonal Transport
  - Development of Video-enhanced DIC Imaging
- Movement Requires ATP
- AMPPNP Freezes Particles
- Microtubule Affinity Chromatography
  - Bind in AMPPNP, Release in ATP

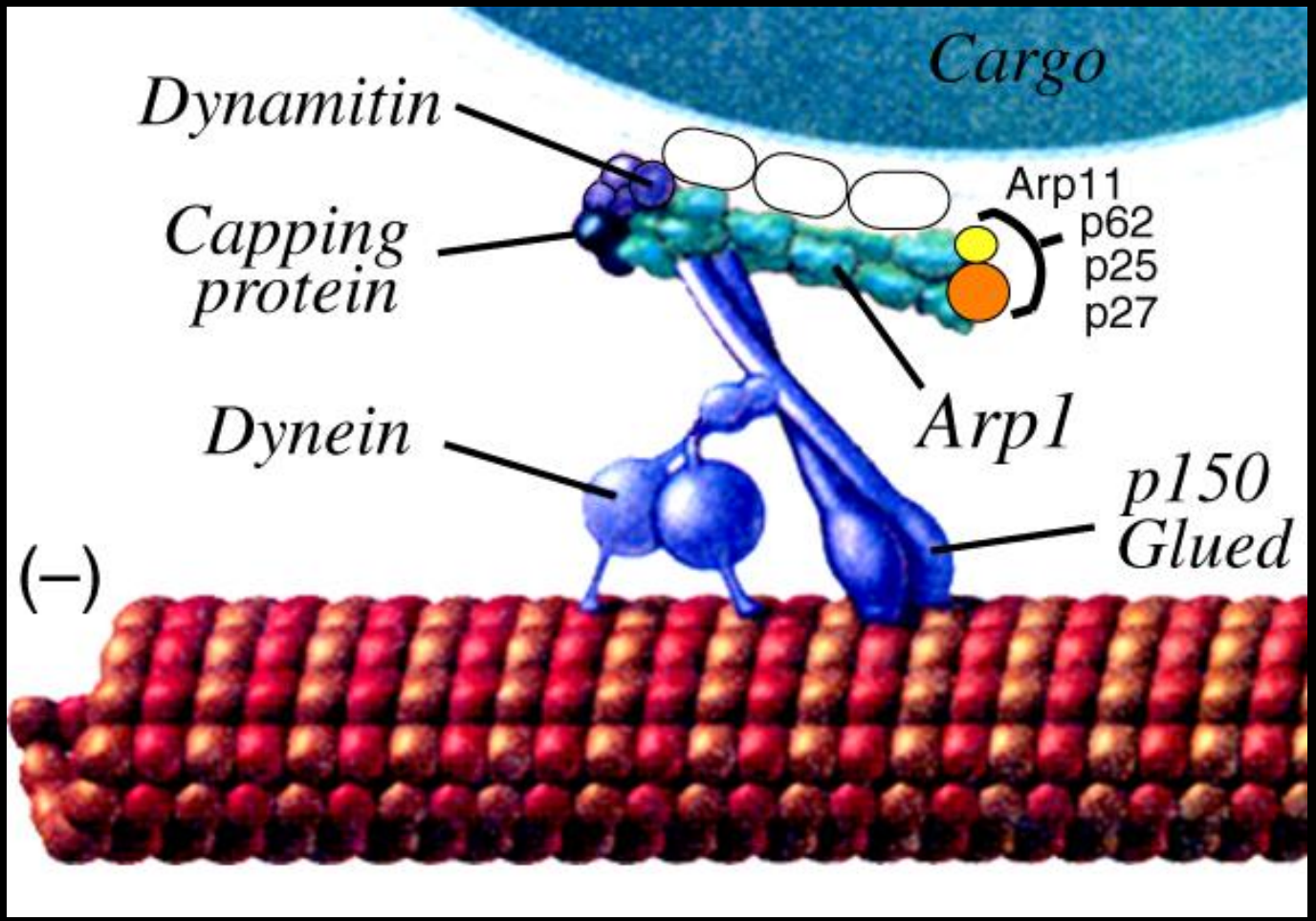
# Kinesin Structure



# Cytoplasmic Dynein

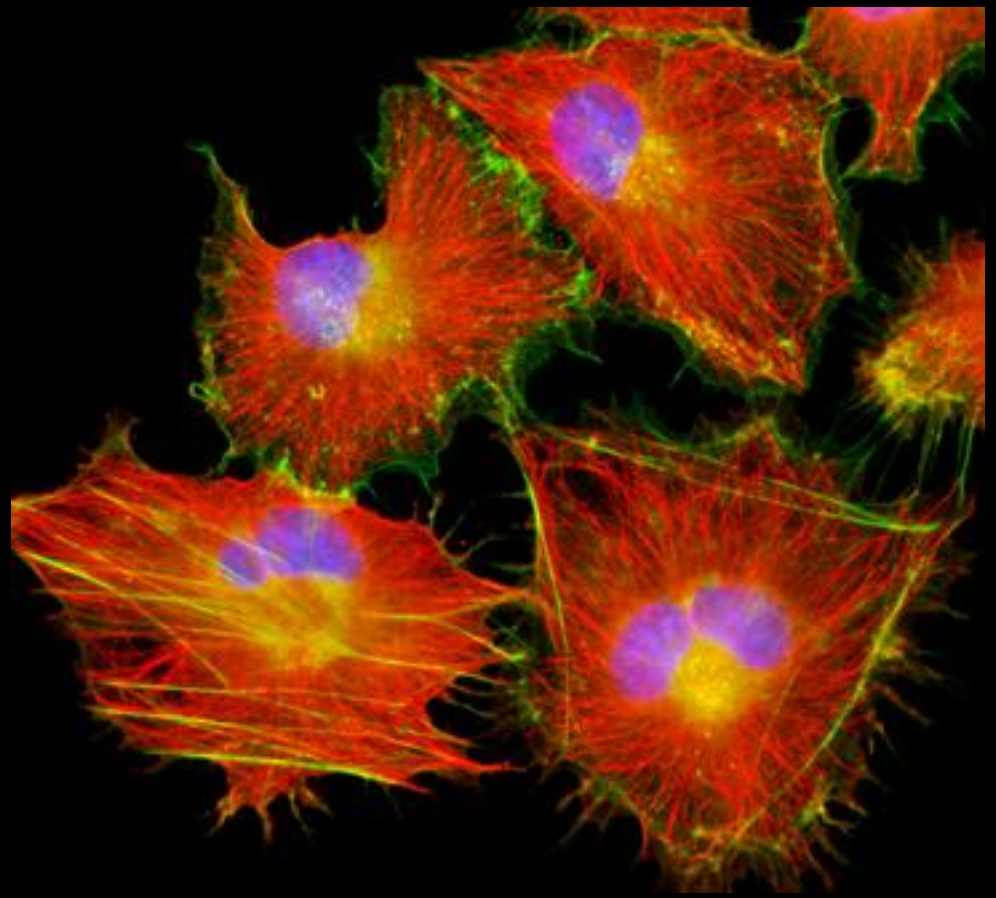
- Discovered  Biochemically
- Minus End Motor for Vesicle Transport
- Requires Dynactin Complex for Function
- Moves the Mitotic Spindle

# Model for Interactions between Dynein, Dynactin Complex, Microtubules, and Cargo



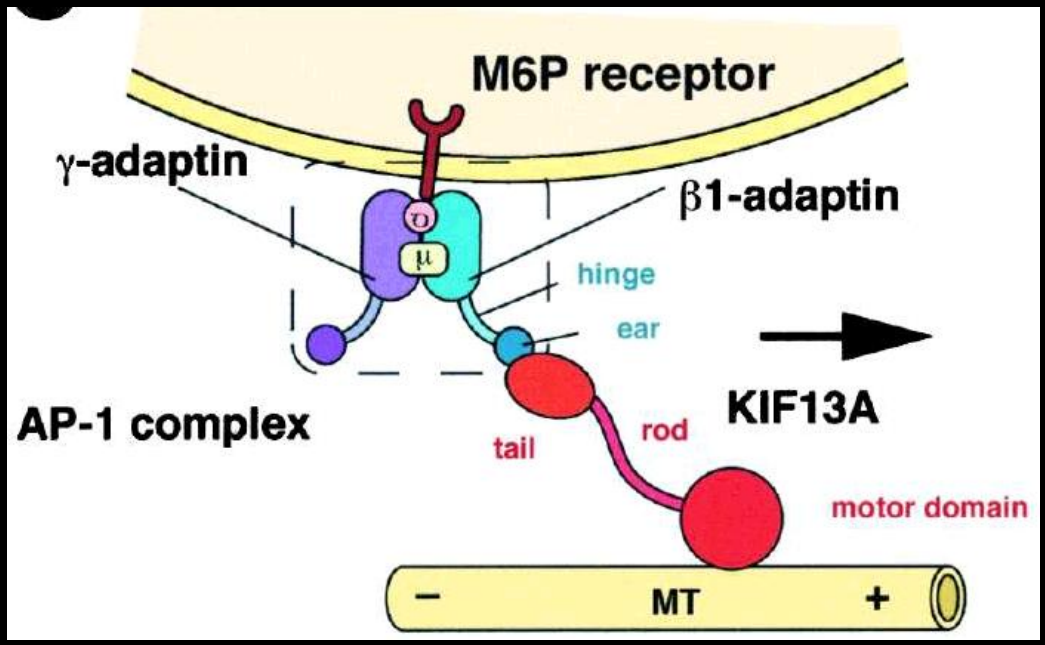
# Membrane Trafficking - ER and Golgi

- Positioning ER & Golgi
  - Golgi near MTOC
    - Minus Ends are at MTOC
    - Golgi Position Requires Dynein
  - ER
    - Tubular network spread about the cell
    - Kinesin moves the tubules peripherally

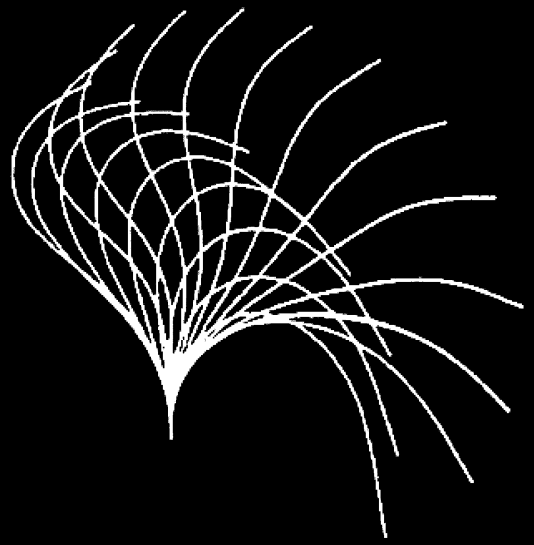


# Vesicle Traffic: Trans-Golgi to Plasma Membrane

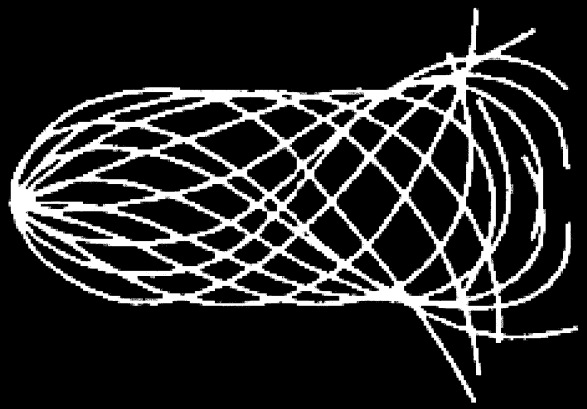
- Kinesin - “KIF13A”
  - Discovered by sequencing
  - Plus-end Directed, fast (0.3 μm/s)
  - Binds AP-1 (affinity chromatography) and mannose 6-P receptor
  - Inhibit function (express tail as dominant negative) -> less M6PR at cell surface



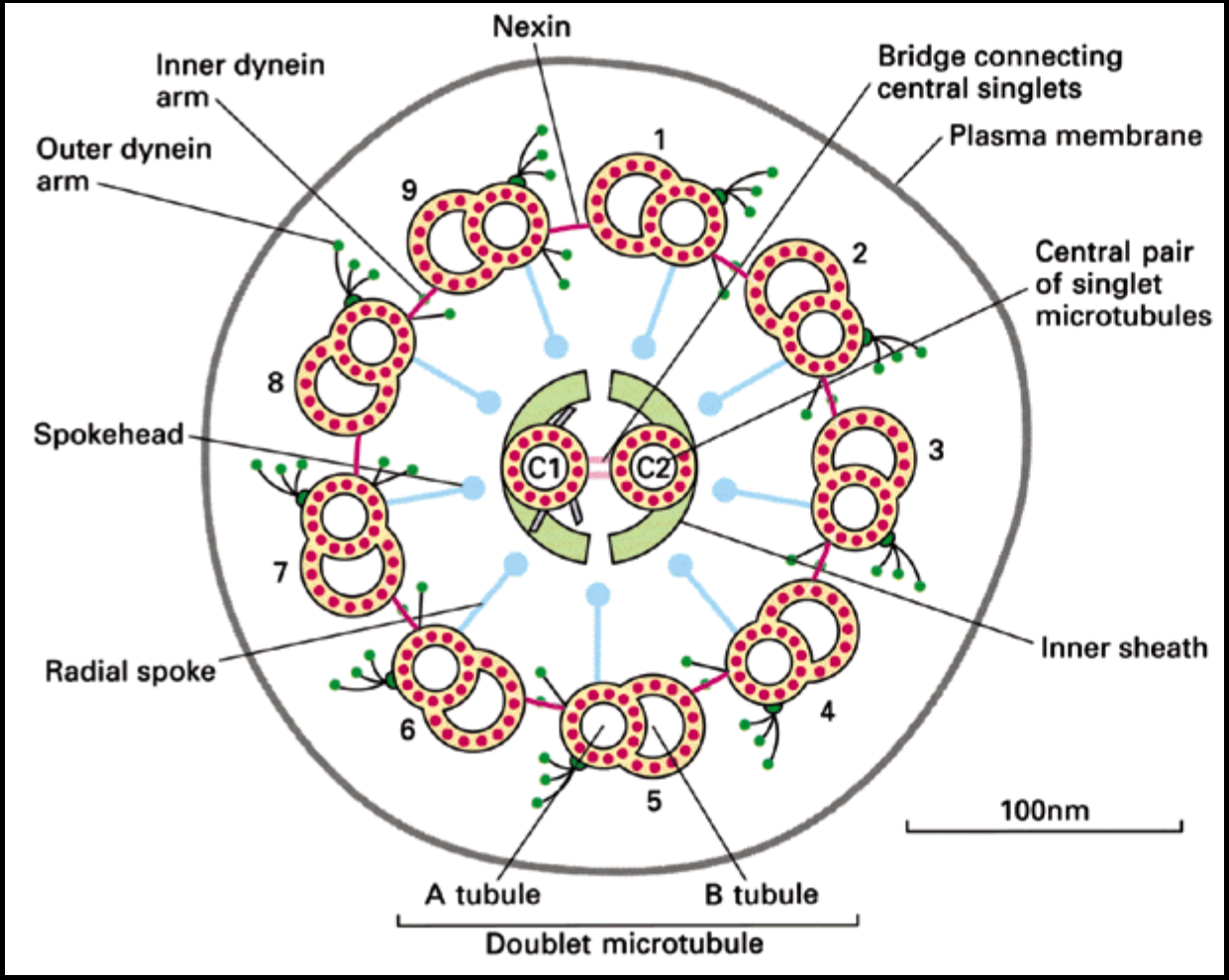
Chlamydomonas
Cilia

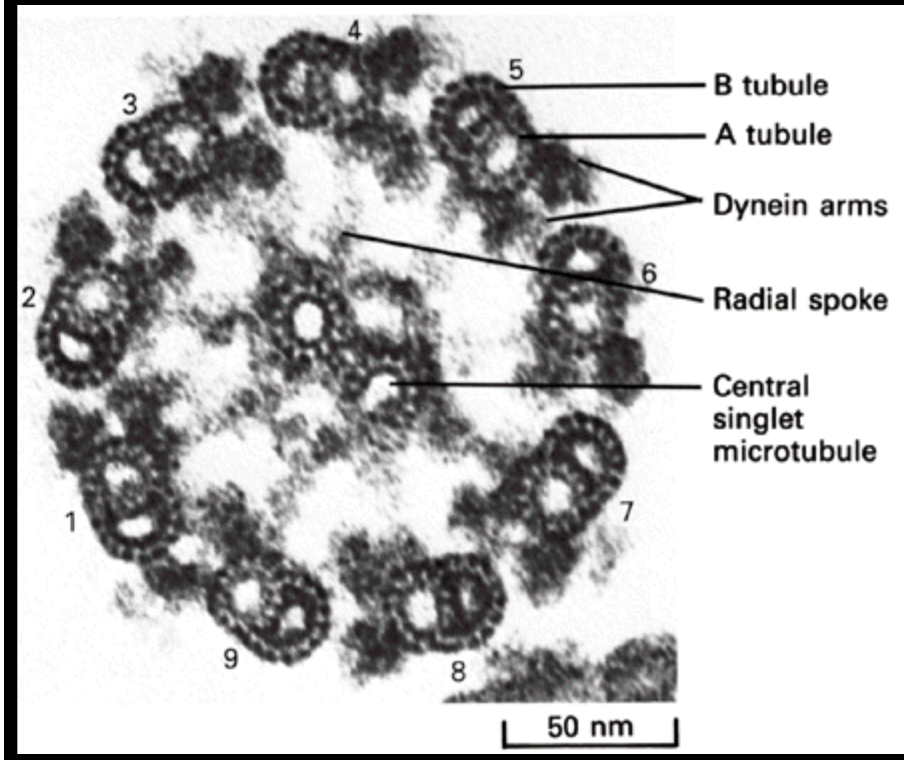


Sperm Flagellum



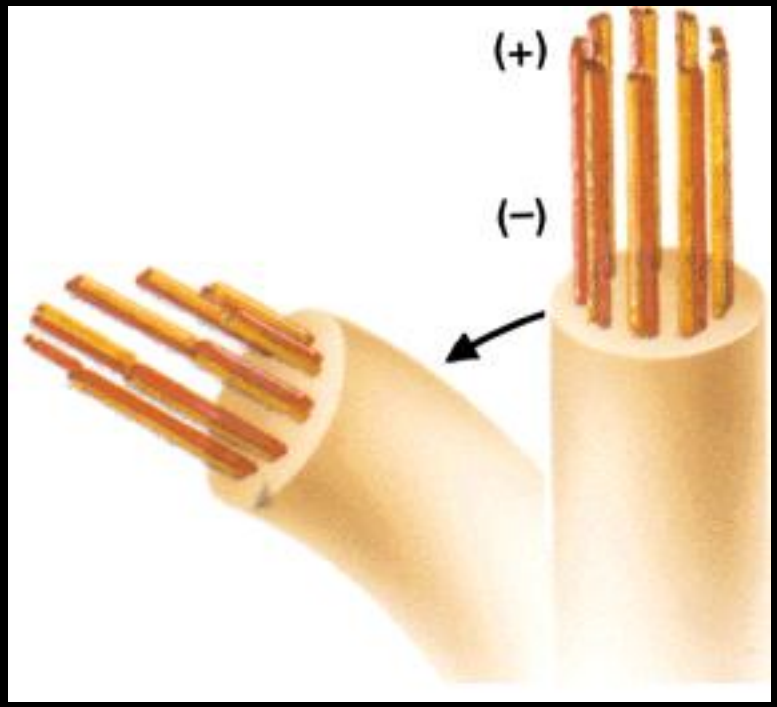
# Structure of Axoneme: Cross-section



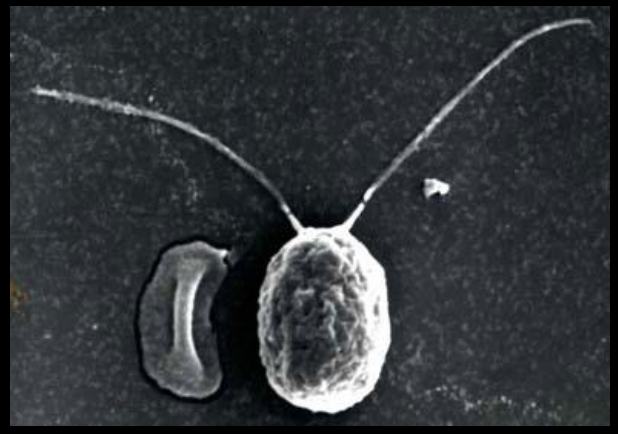
# Conversion of Sliding to Bending to Wave Formation

- Slide on only side of axoneme
- Propagate down the long axis



# Experimental Approaches to Study Cilia in Chlamydomonas

- Axoneme 2-D gel - 250 polypeptides!
- Mutants - Collect & Characterize
- What Structures and Polypeptides Missing?



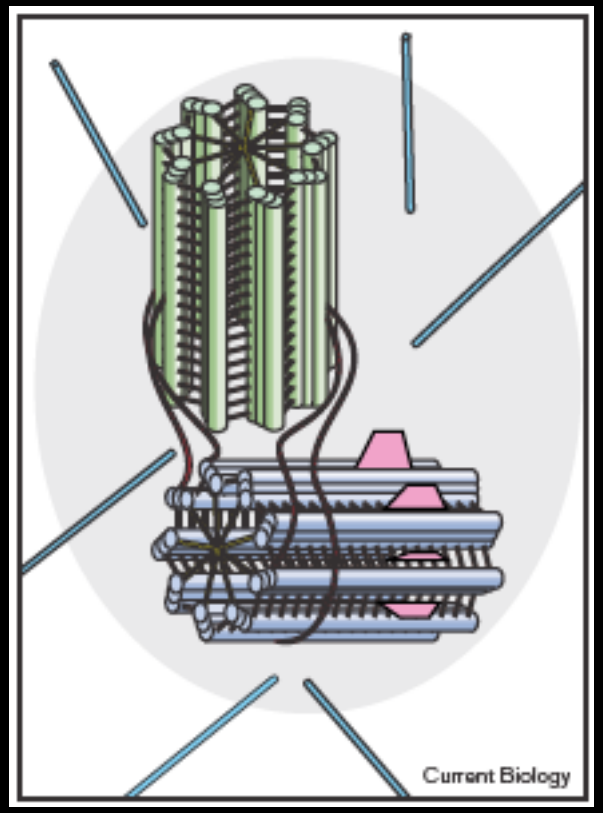
# Mitosis Background

- Names of Stages: Interphase, prophase, metaphase, anaphase, telophase
- Interphase MTs disassemble then reassembly as Spindle MTs

# Centrosomes

- Animals: Centriole Pair in Amorphous Cloud
- Ends of MT's in Cloud.No Relationship to Centrioles. Different from Relationship of Basal Body and Axoneme MT's.
- Flowering Plants: Lack Centrioles

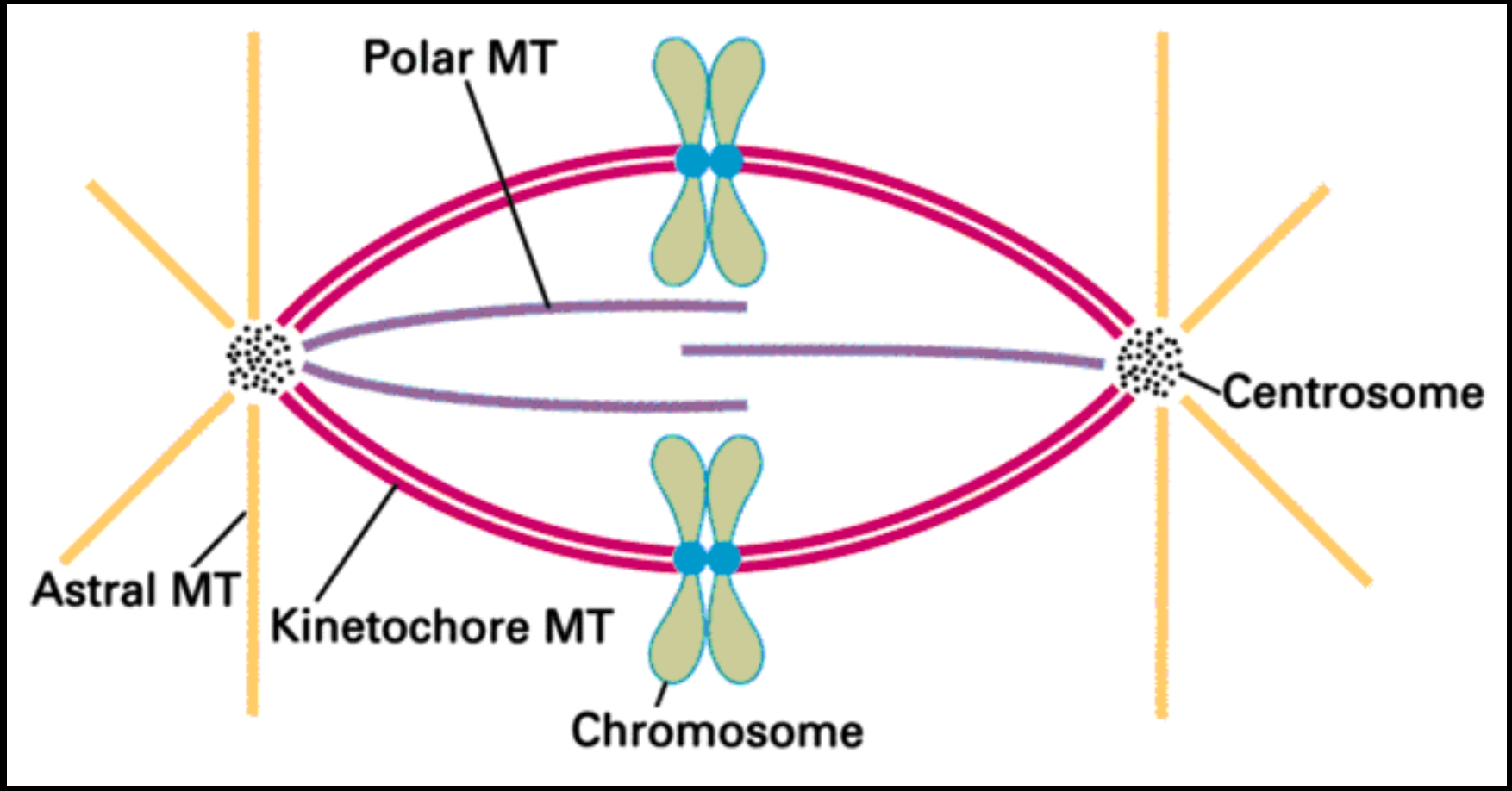
# Centriole Fine Structure



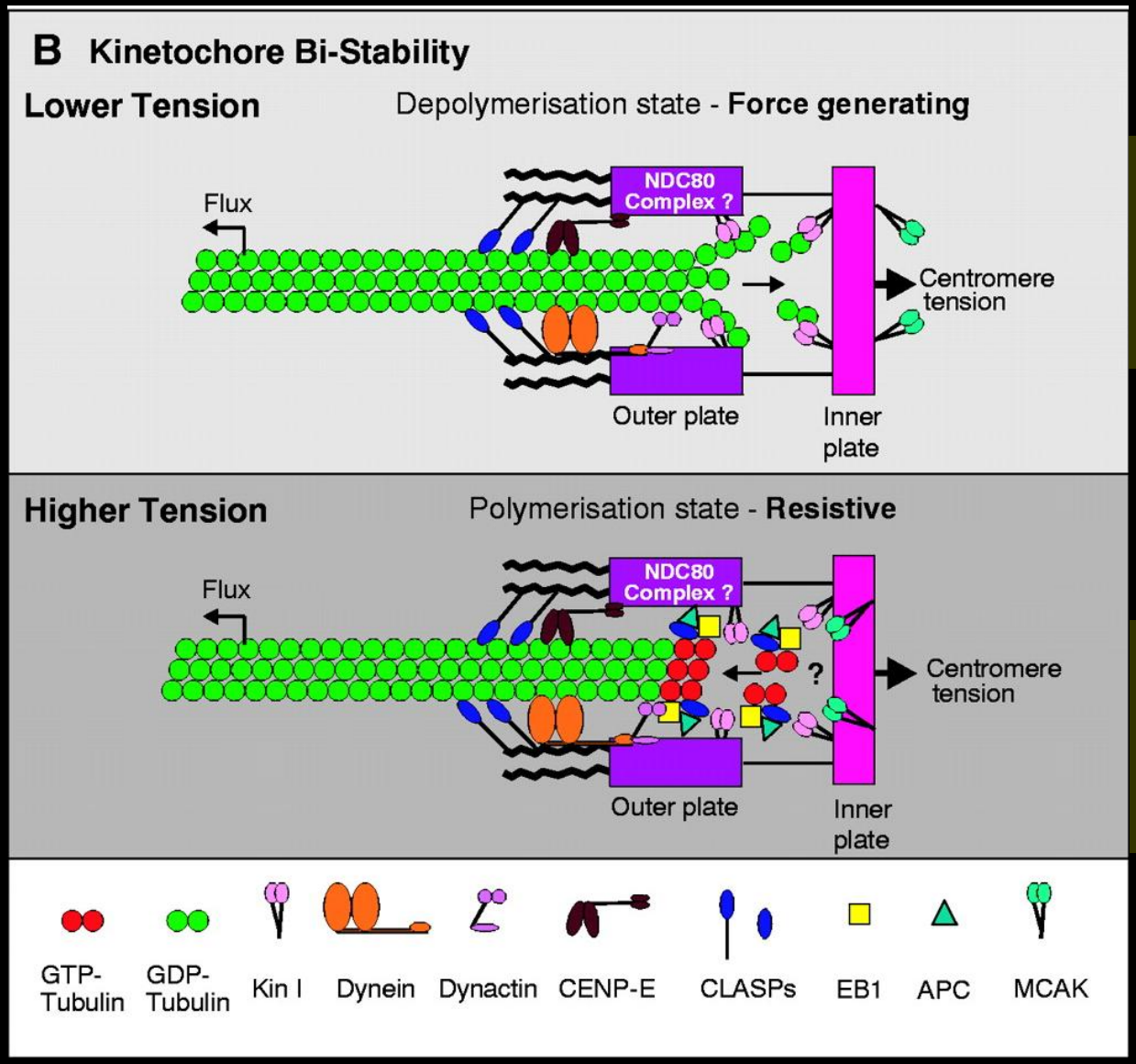
# Mitotic Spindle Assembly

- Centrosome duplicates and separates
- Nuclear envelope breakdown in animals
- MT's rearrange via dynamic instability

# Spindle MT's



# Kinetochore as a slip-clutch mechanism




Low tension:
Depolymerization generates force and movement

High tension:
Switch to polymerization to prevent detachment

End