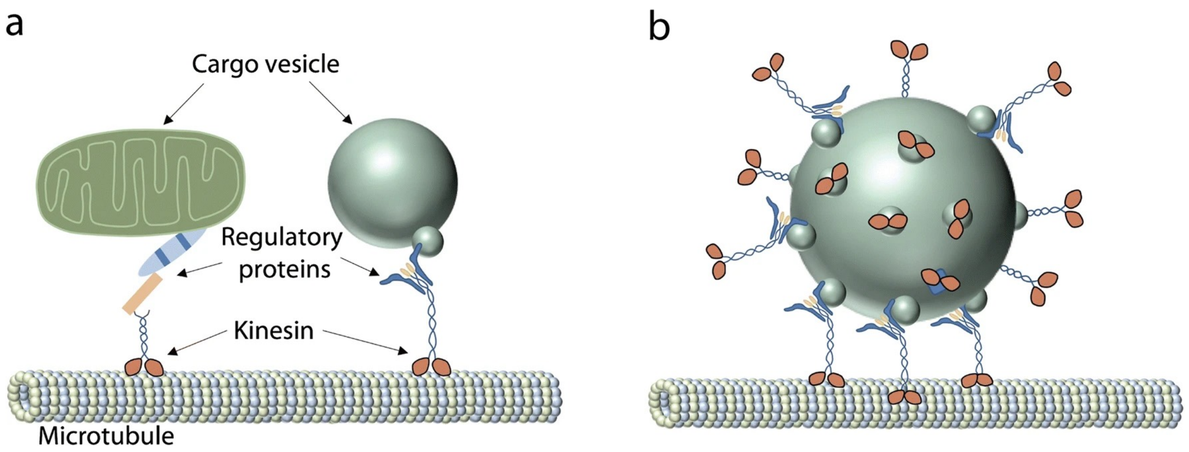
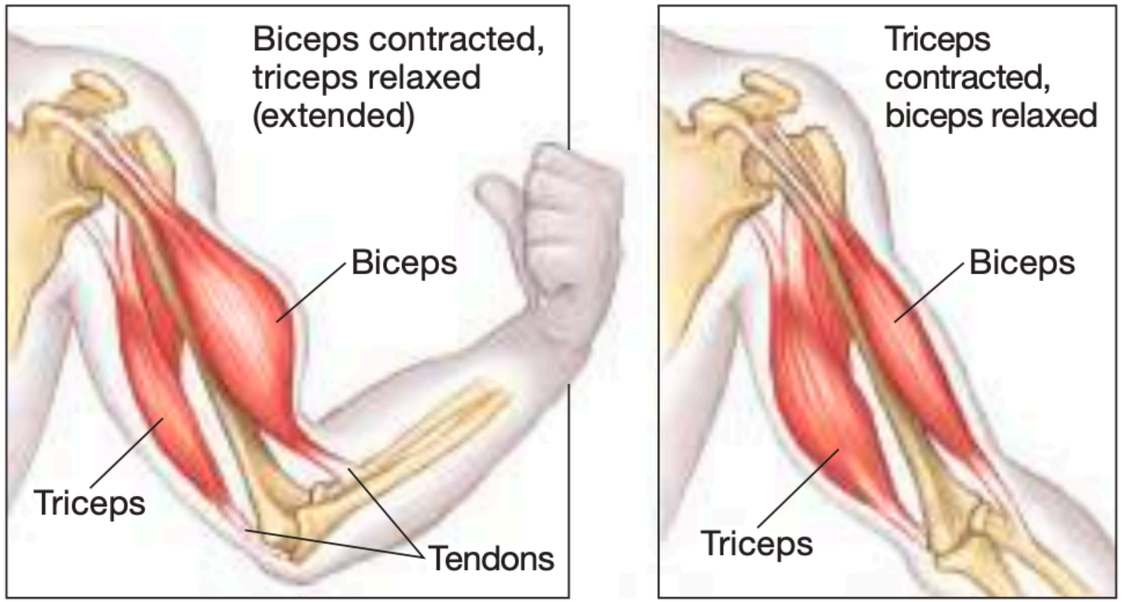
More machines, tracks, trucks, trains & cranes for movement, transport and force generation

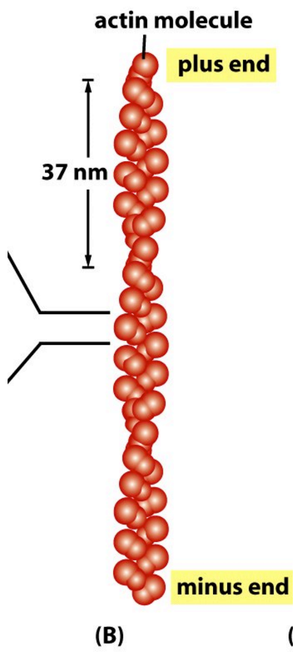


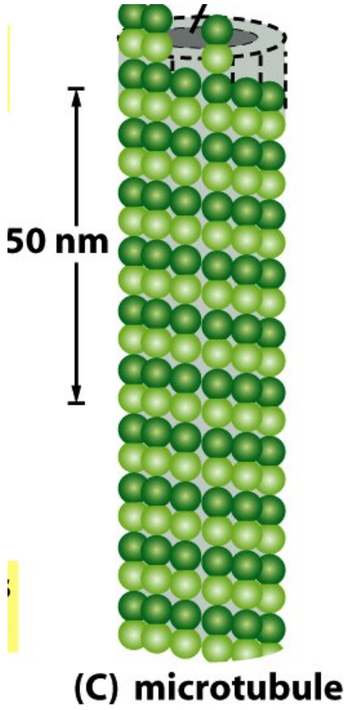


Changing color quickly like chameleons: how?

How does muscle work?

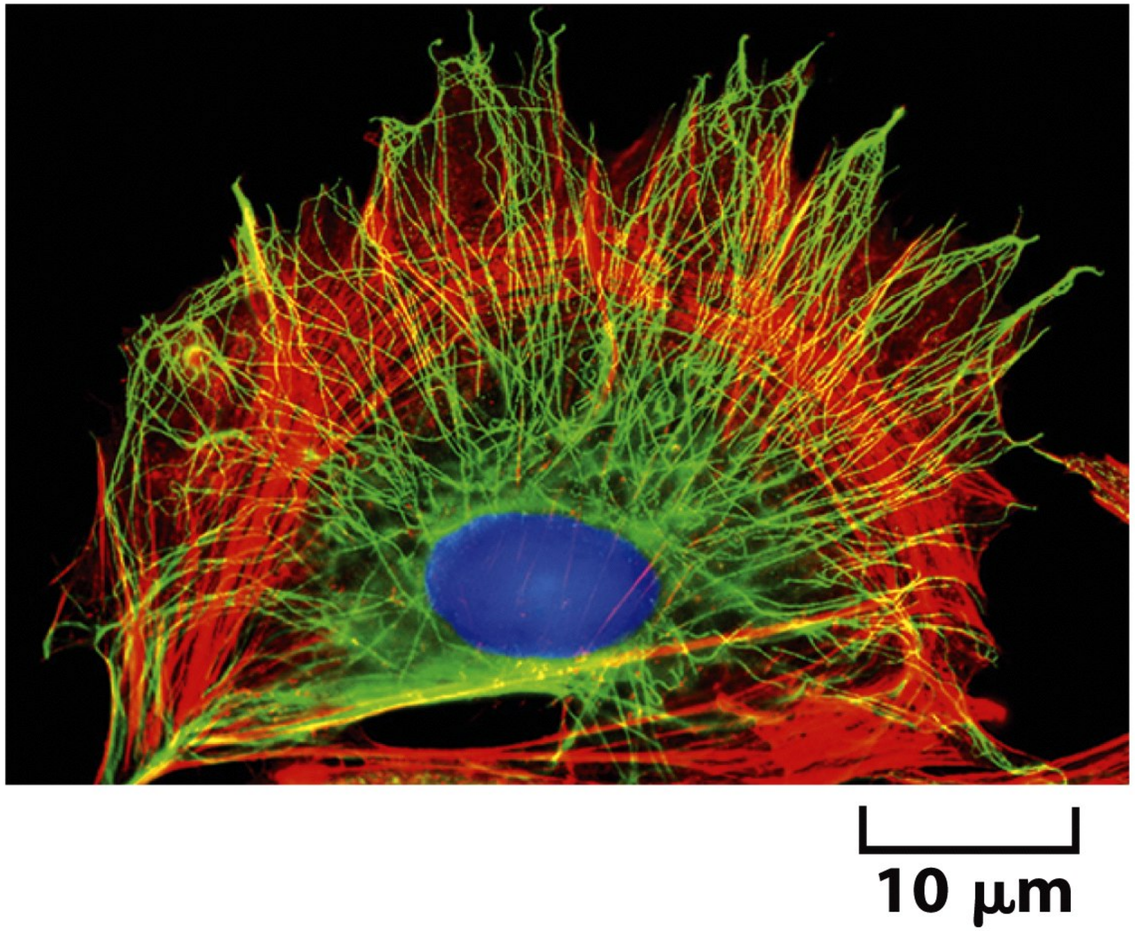
Similar to actin there is another filament: Microtubule



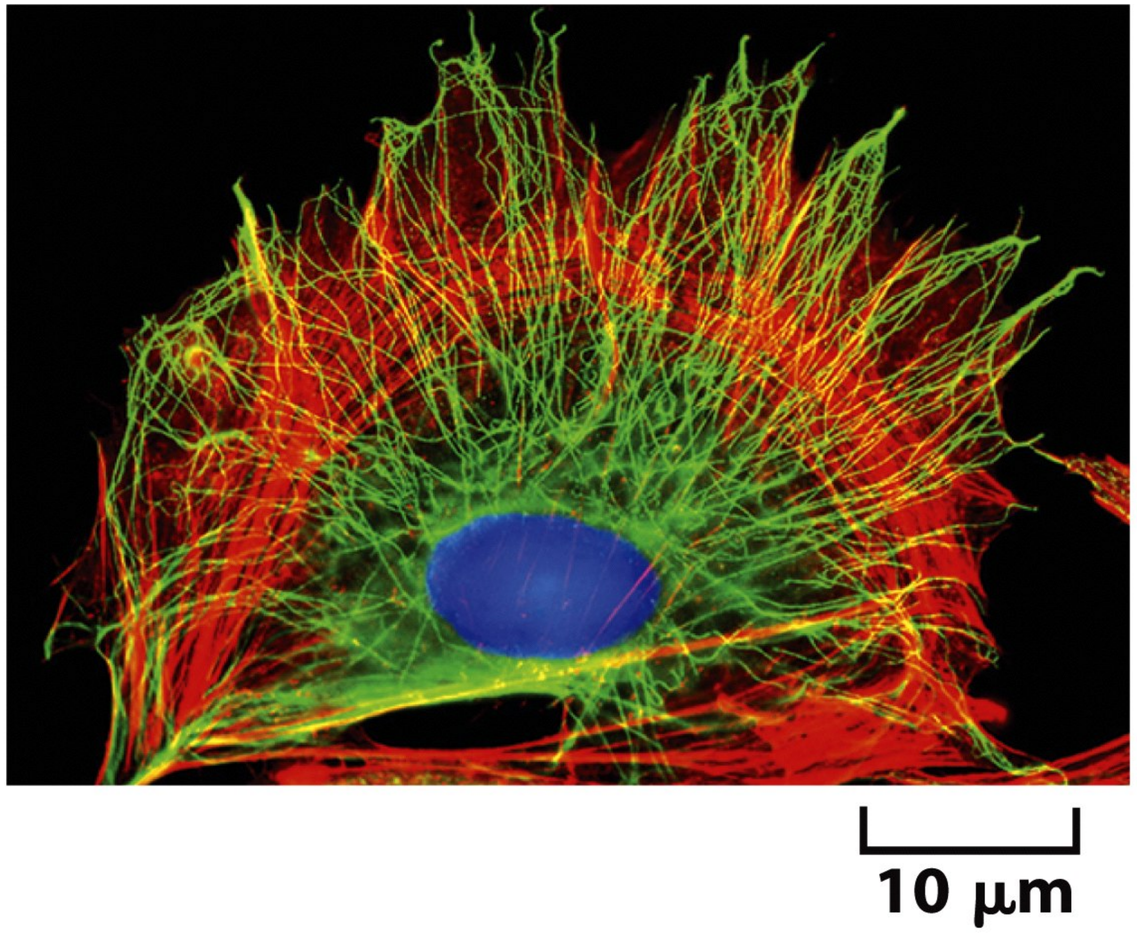


(Alberts et al)

Actin and Microtubule in a cell



We learned about actin force generation. But actin as multiple roles.

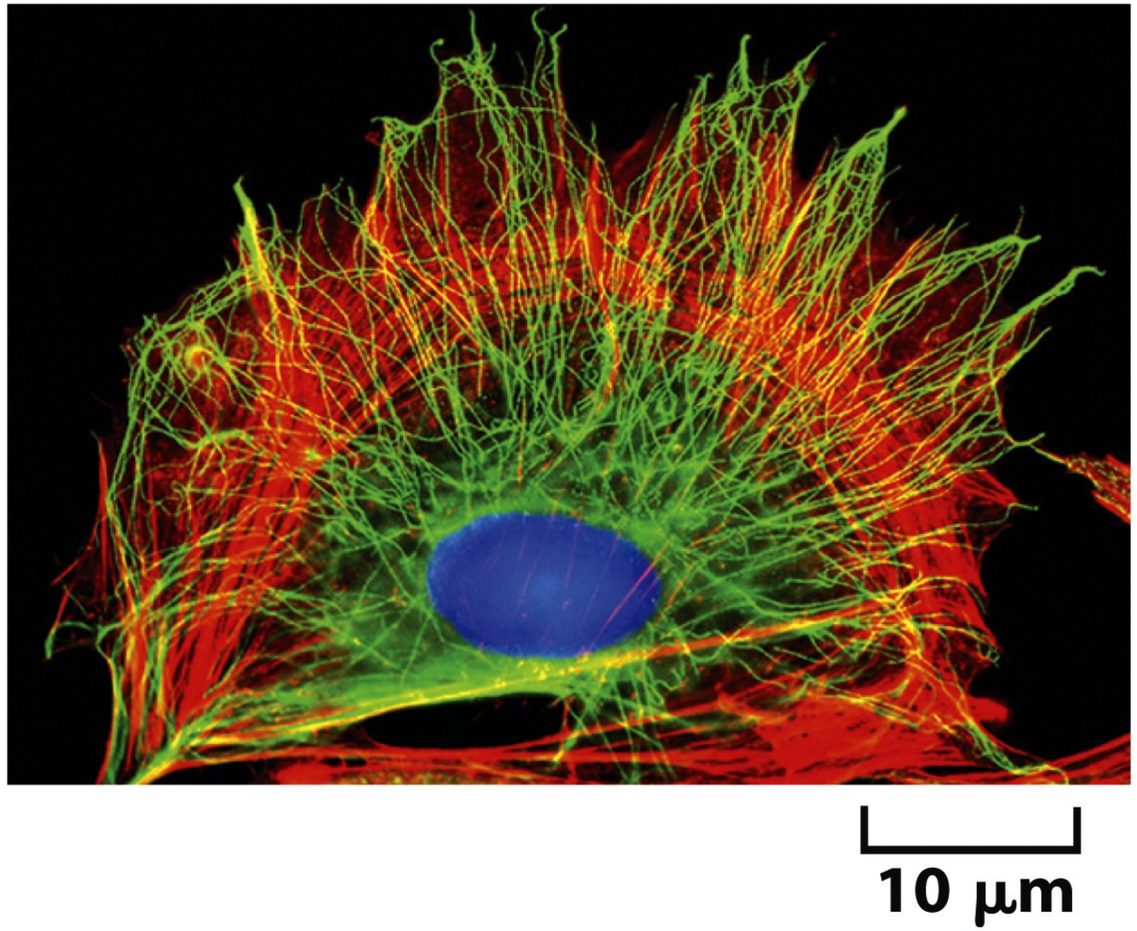


(1) It acts like skeletal beam, giving rigidity to cell,

(2) It pushes and generates force

- Actin serves like skeletal beam, giving rigidity to cell

- It pushes and generates force



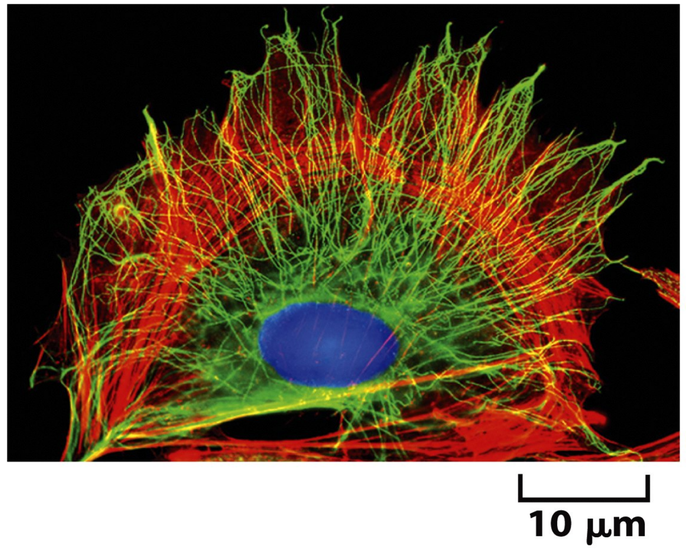
Beyond these machines that use energy,

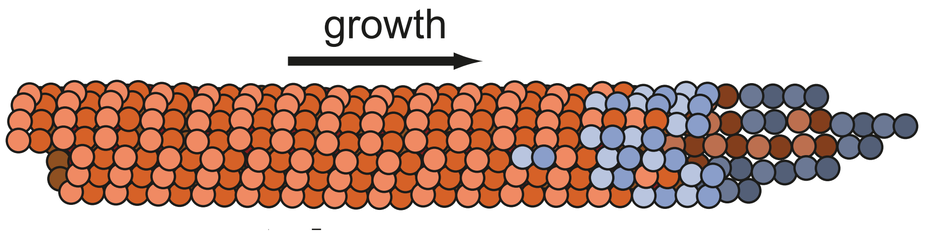
Biology is about how cells regulate these multiple roles at the right time at the right place!

A lot here is yet to be discovered

You have the opportunity to go and discover these things!

Microtubule is another force-generating machine

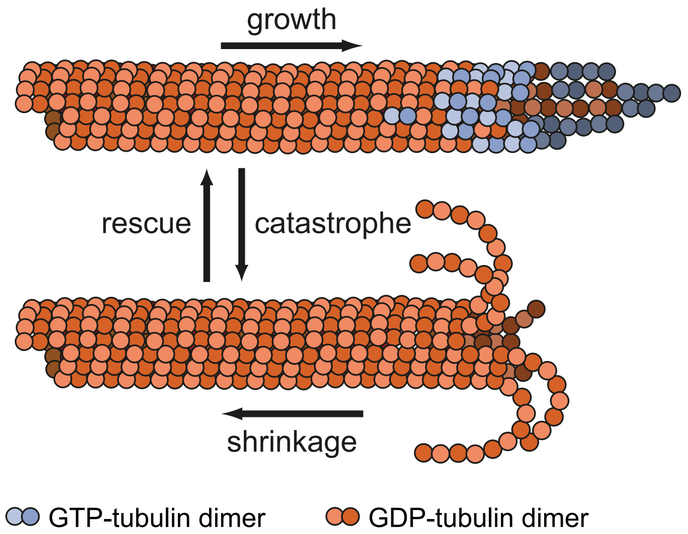




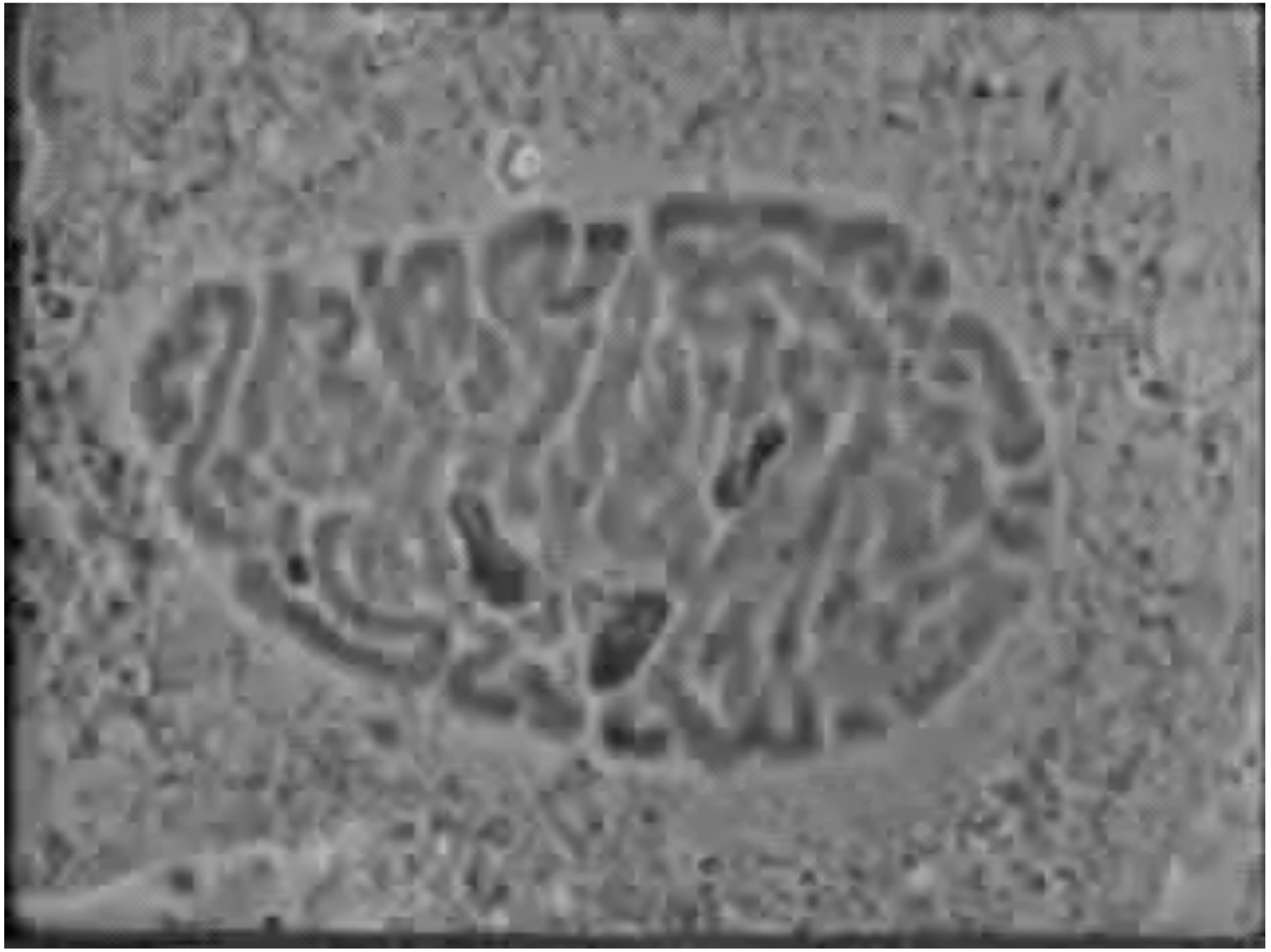
Some similarity and many differences with actin

Microtubule also has multiple roles

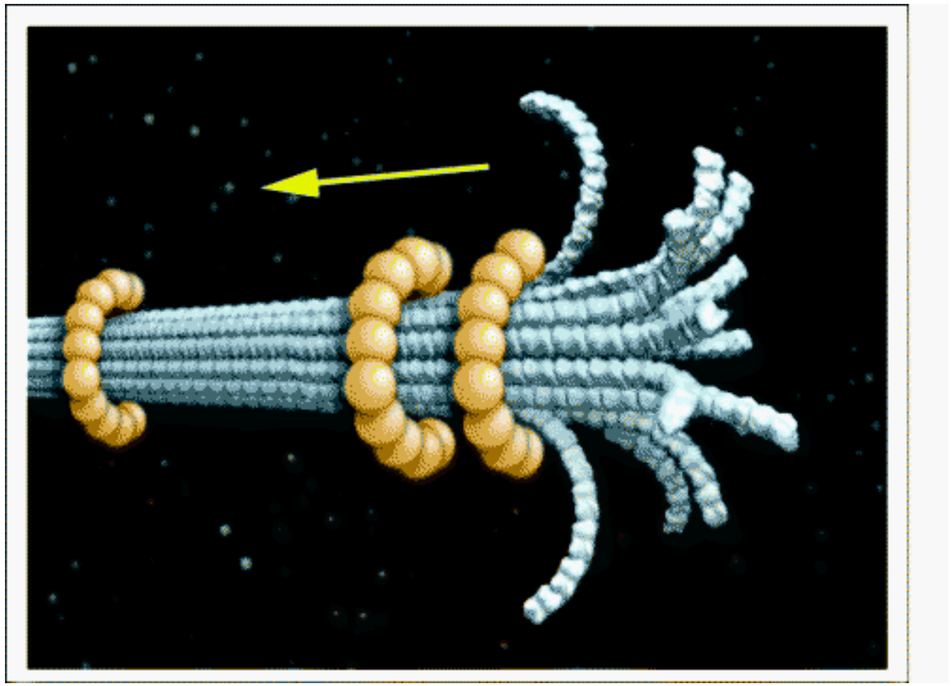
- It generates force by pushing
- It searches for chromosomes as search engine!
- It pulls chromosomes
- It acts like a railway track inside cells for molecule motors (cargo trains) to move
- How can it enact these multiple roles at the right place and at the right time?



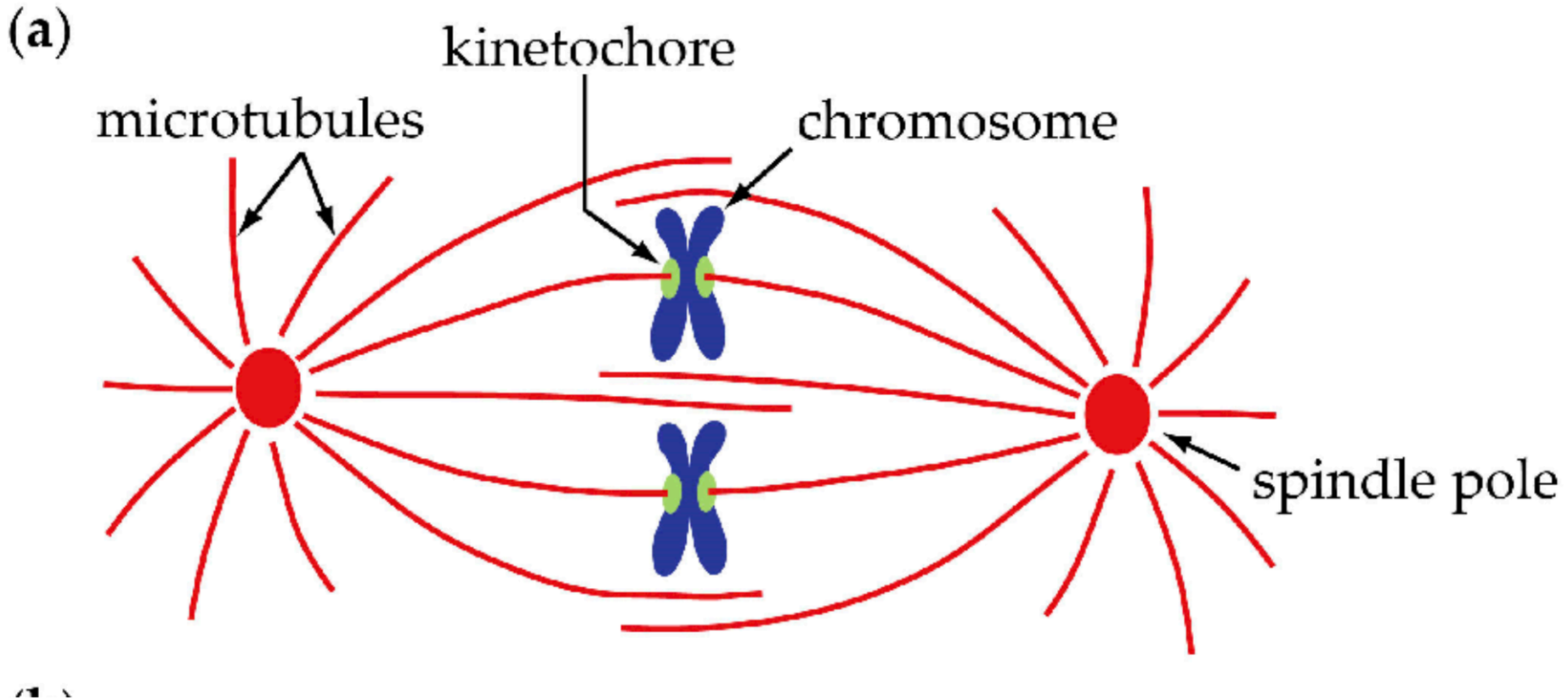
Cell division and chromosome segregation: the machinery involved



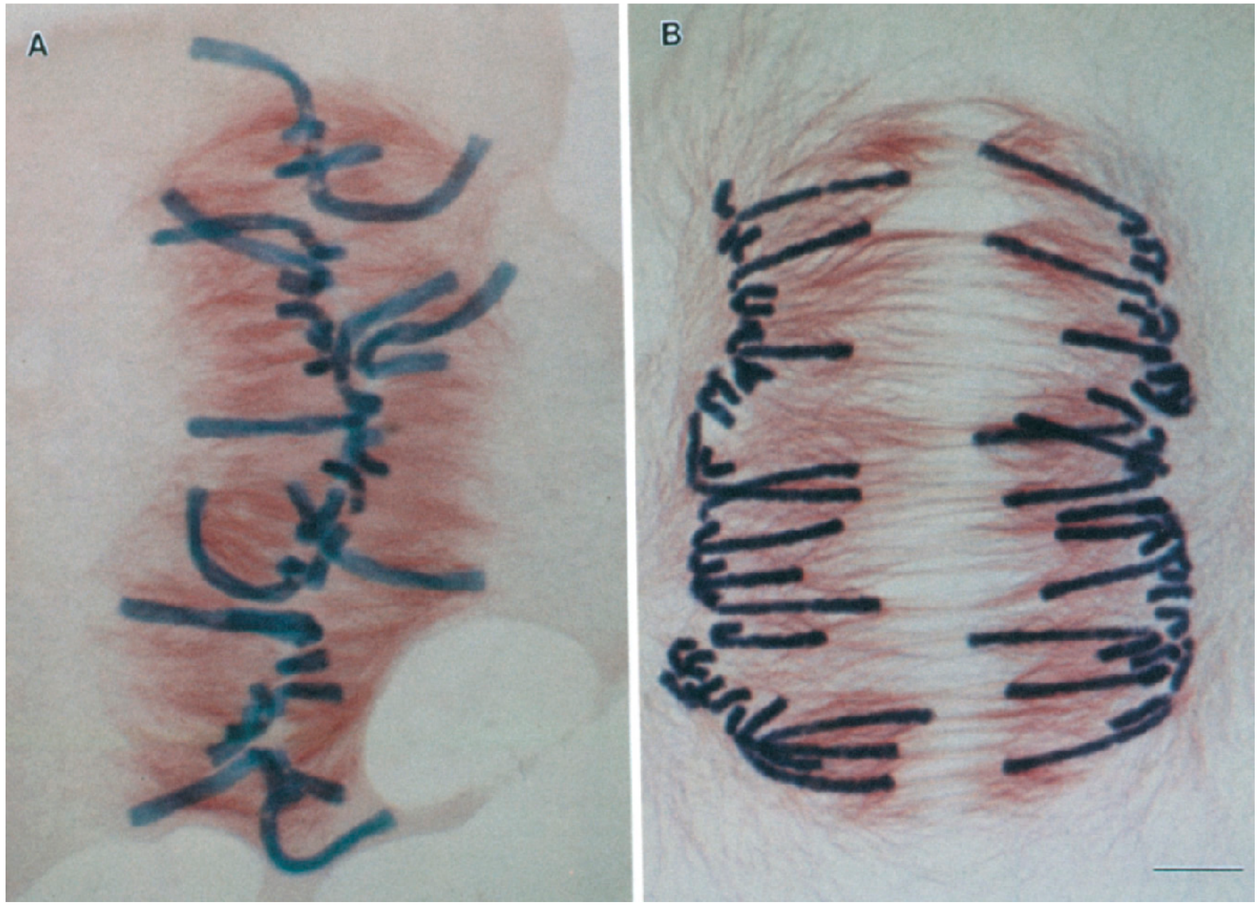
Pulling machine



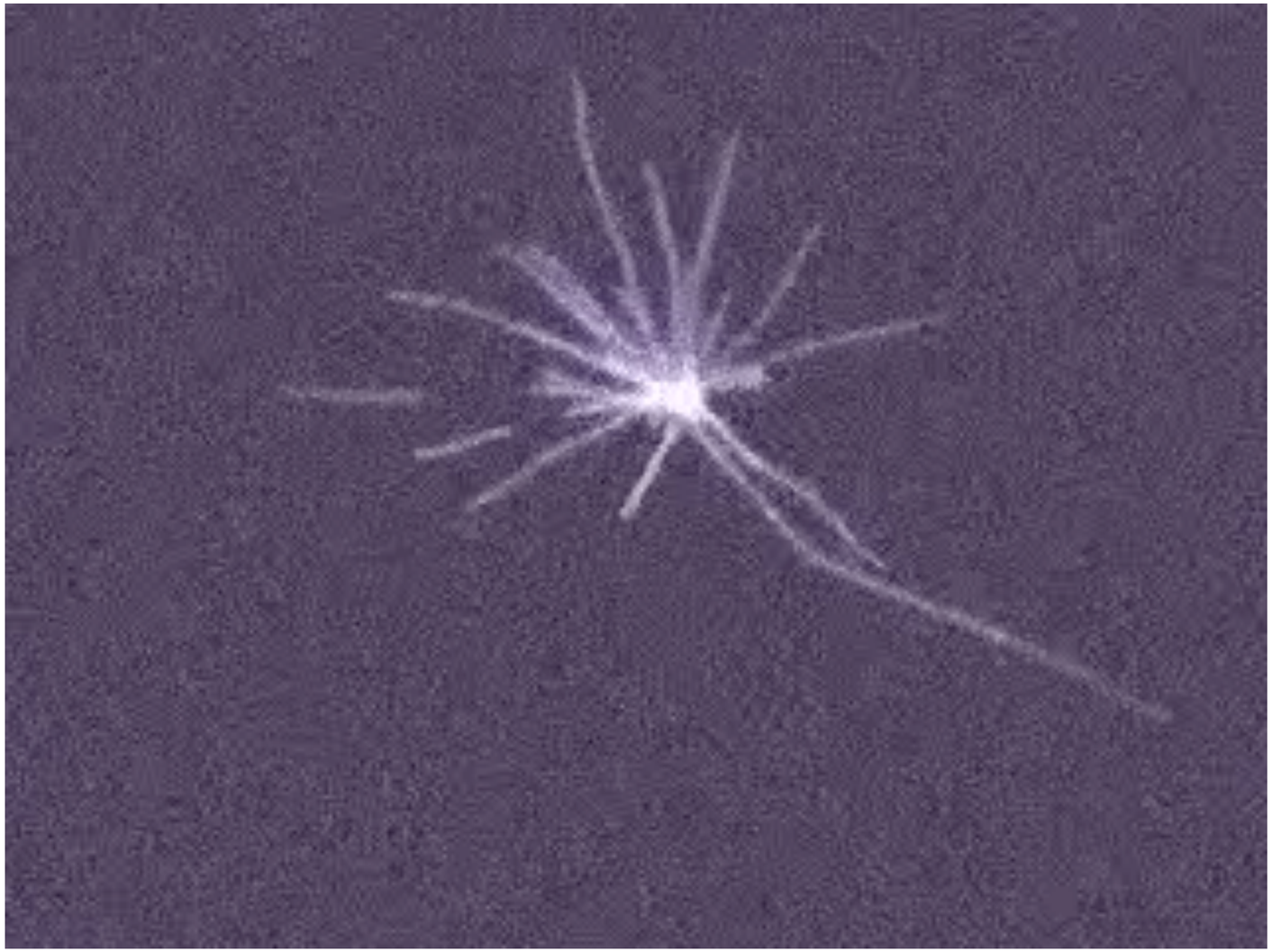
Microtubule search and capture chromosomes



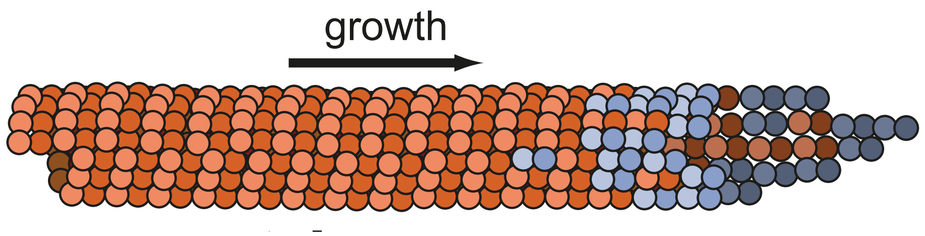
And then pull them apart to two cells, curing cell division



To search, Microtubules grow and shrink



GTP-bound Tubulin dimers polymerize to form a cylinder



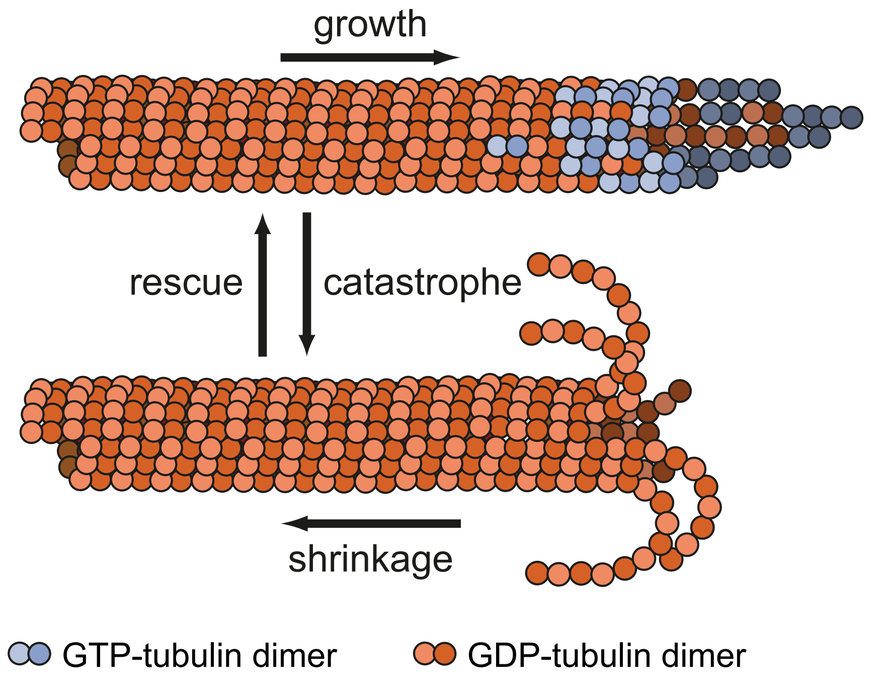
Cylinder made of 13 “proto-filaments”

GTP-tubulin dimer GDP-tubulin dimer

GTP hydrolyses to GDP on the filament

Picture courtesy: Howard & coworkers, Bioessays, (2013)

GTP-bound tubulin protofilaments are “straight”



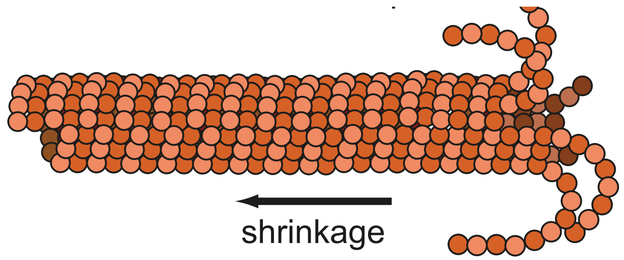
GDP-bound tubulin protofilaments are intrinsically curved

Picture: Howard & coworkers (2013)

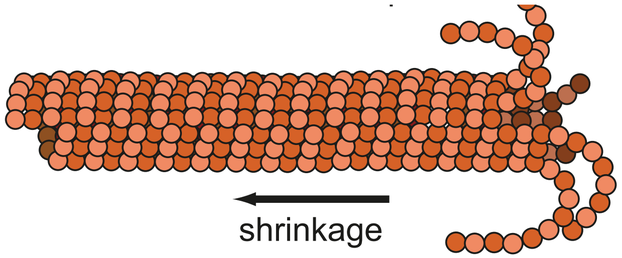
Stability of a microtubule cylinder and shrinkage

Lateral bonds between protofilaments hold MT stable

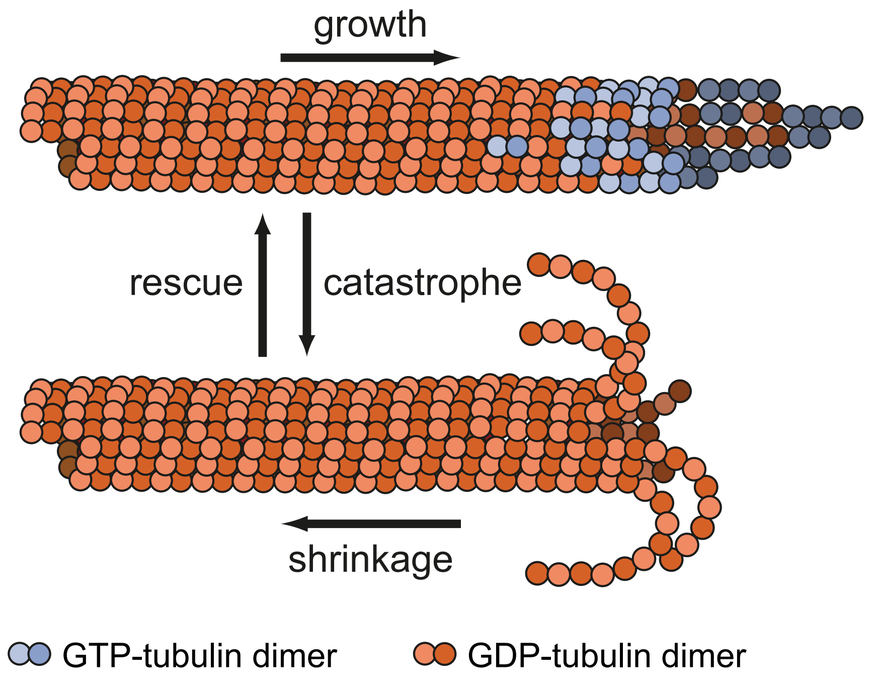
GDP protofilaments would want to curve out destabilizing MT



This “power struggle” between inter-protofilament interaction and bending elasticity decides the stability of MT

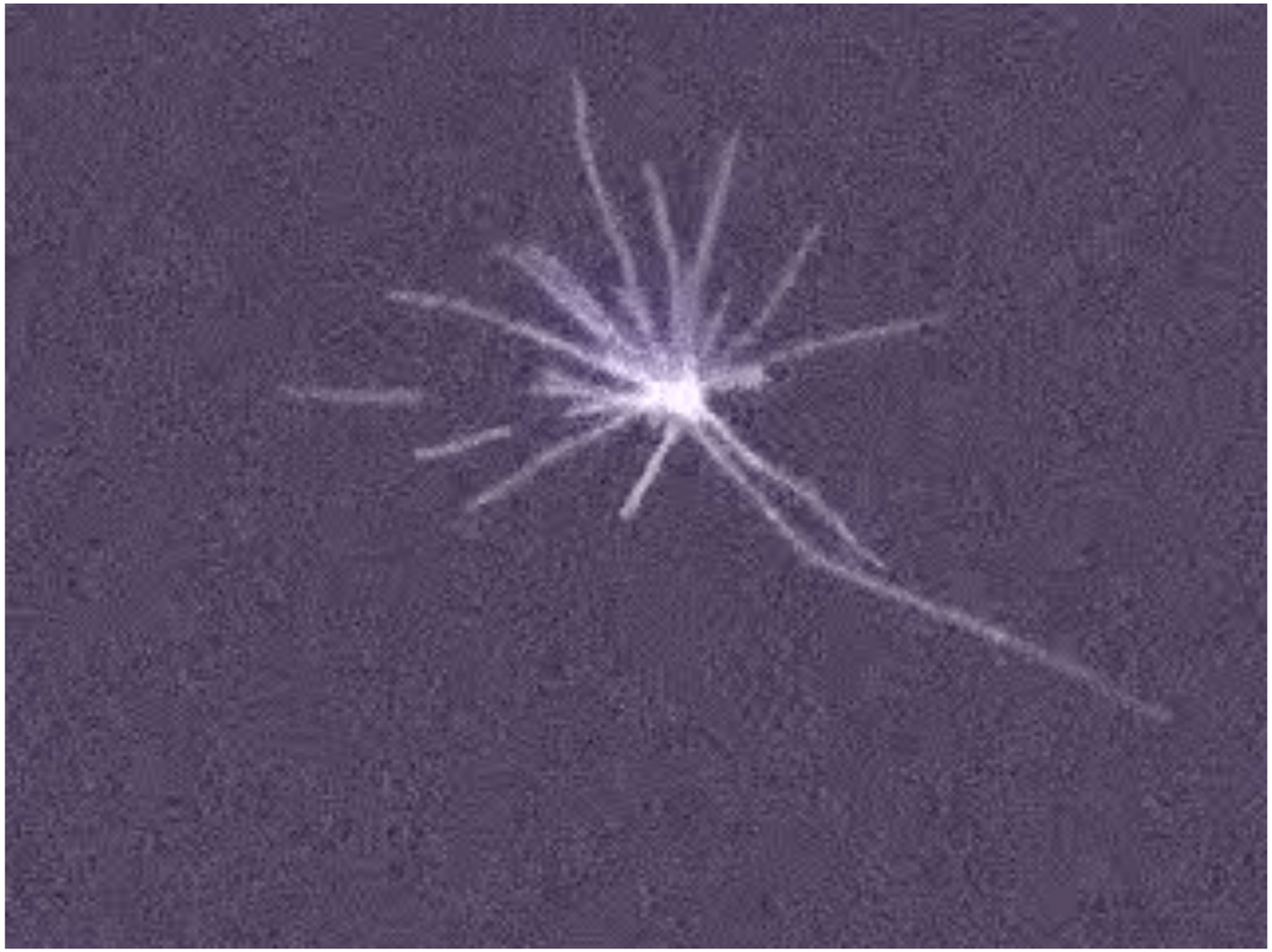


This transition results in a phenomenon: where microtubule grow slowly and rapidly shrink, then grow again

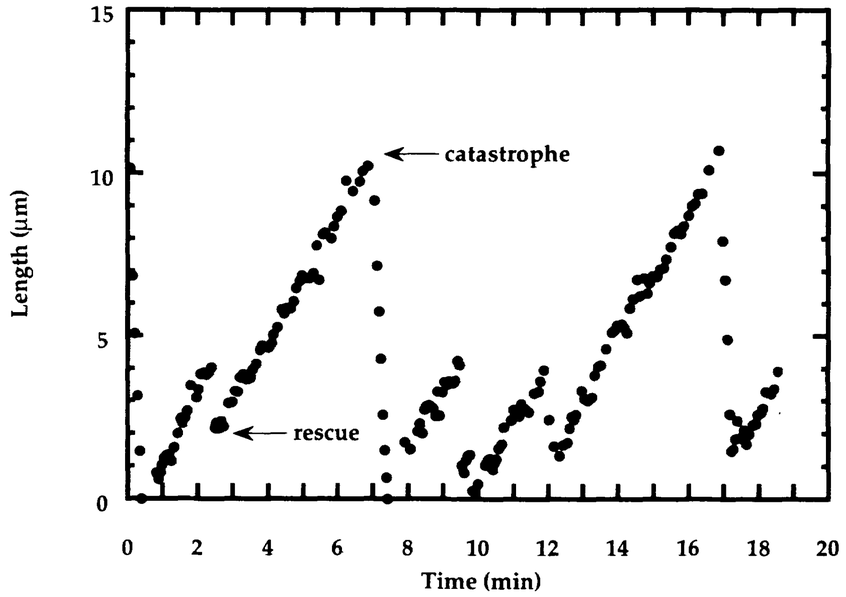


This phenomenon: Dynamic Instability.
Helps in searching chromosomes or changing directions

Dynamic instability

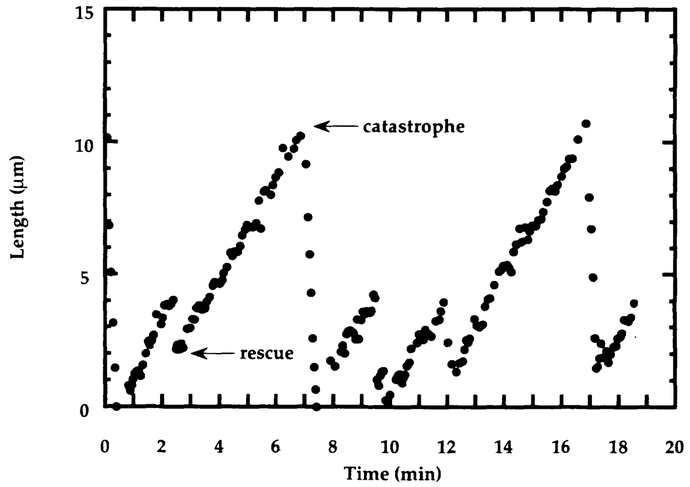


Dynamic instability: slow growth and rapid shrinkage



Plot Length of the microtubule versus time

Growth, Catastrophe, Shrinkage and Rescue



$$\text{Growth velocity}, v_{+}$$
$$\text{Shrinkage velocity}, v_{-}$$
$$\text{Catastrophe frequency}, f_{+-}$$
$$\text{Rescue frequency}, f_{-+}$$

These four parameters can describe MT properties

Advanced (optional) question to ponder: can you write a dynamic equations that include catastrophe and rescue?

To predict dynamics of microtubules.

What equations will you use? Newton's equation will not work!

Beyond Newton's equations

Probabilistic equations that can compute changes in time and space:

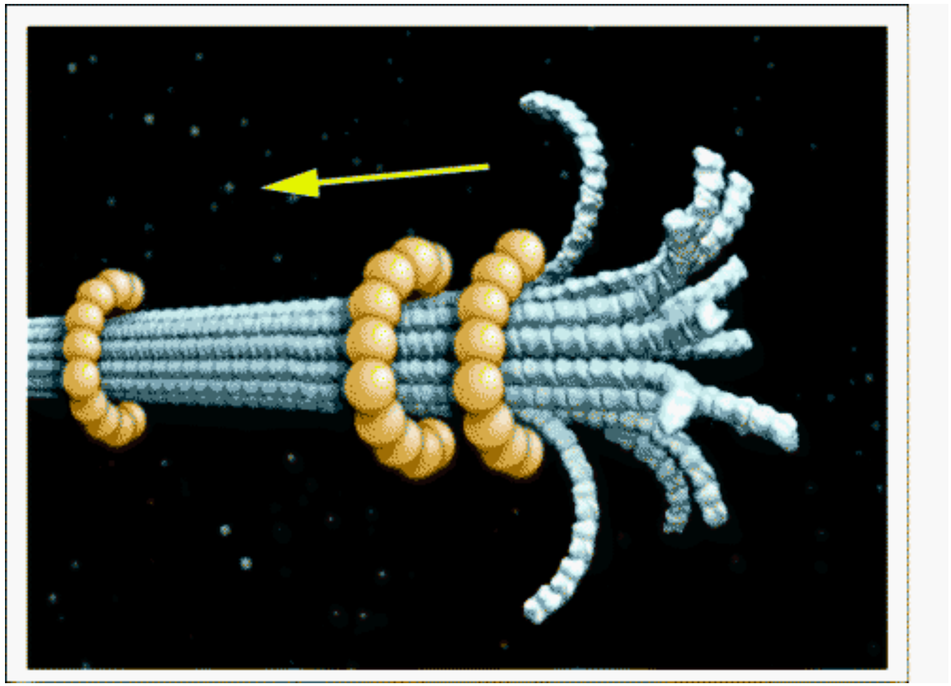
Master equations, Fokker-Planck equations

The parameter values

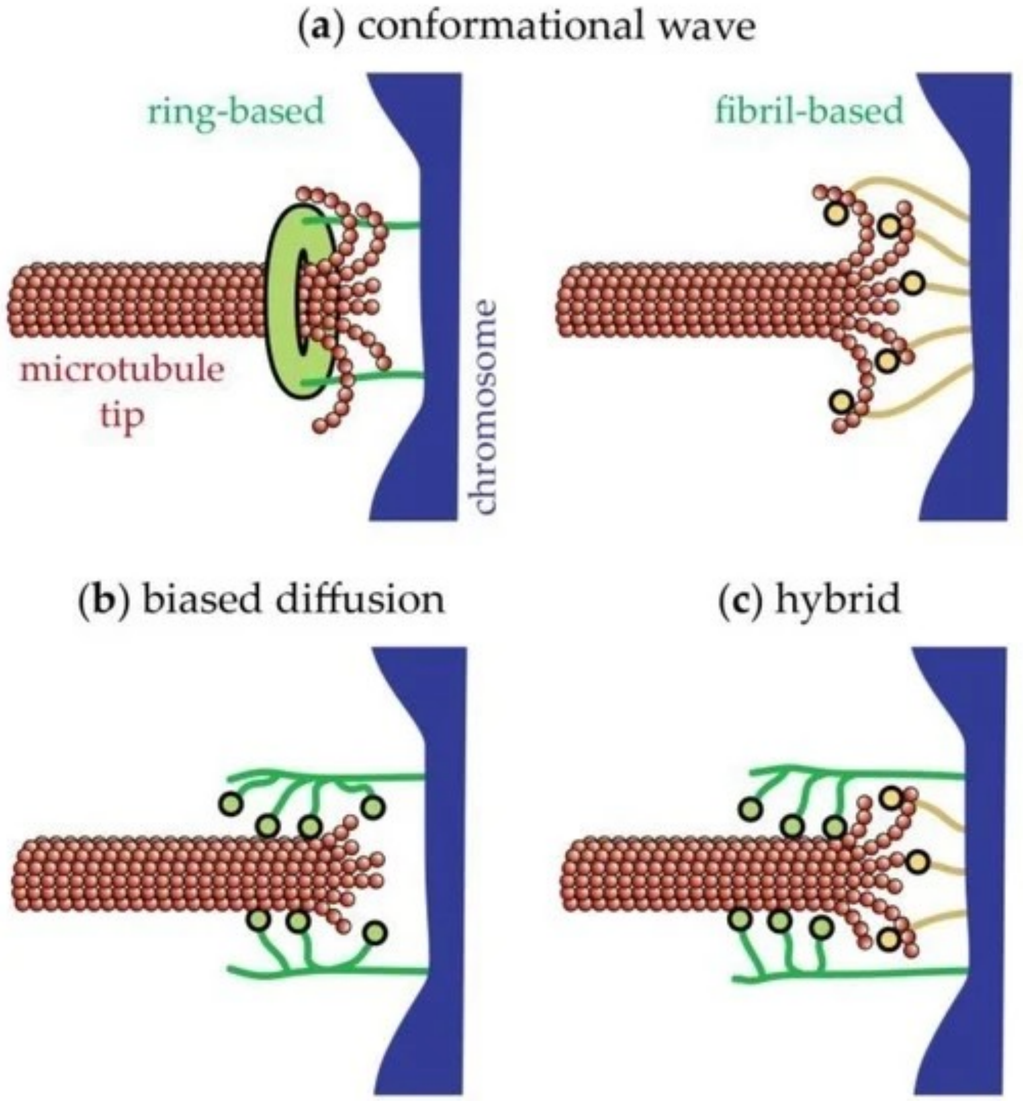
$$\text{Growth velocity}, v_+ \approx 1\mu\text{m/min}$$
$$\text{Shrinkage velocity}, v_- \approx 30\mu\text{m/min}$$
$$\text{Catastrophe frequency}, f_{+-} \approx 1/4 \text{ min}$$
$$\text{Rescue frequency}, f_{-+} \approx 10/\text{min}$$

Tough: these values are determined by a combination of chemical kinetics and filament elasticity, far away from thermal equilibrium

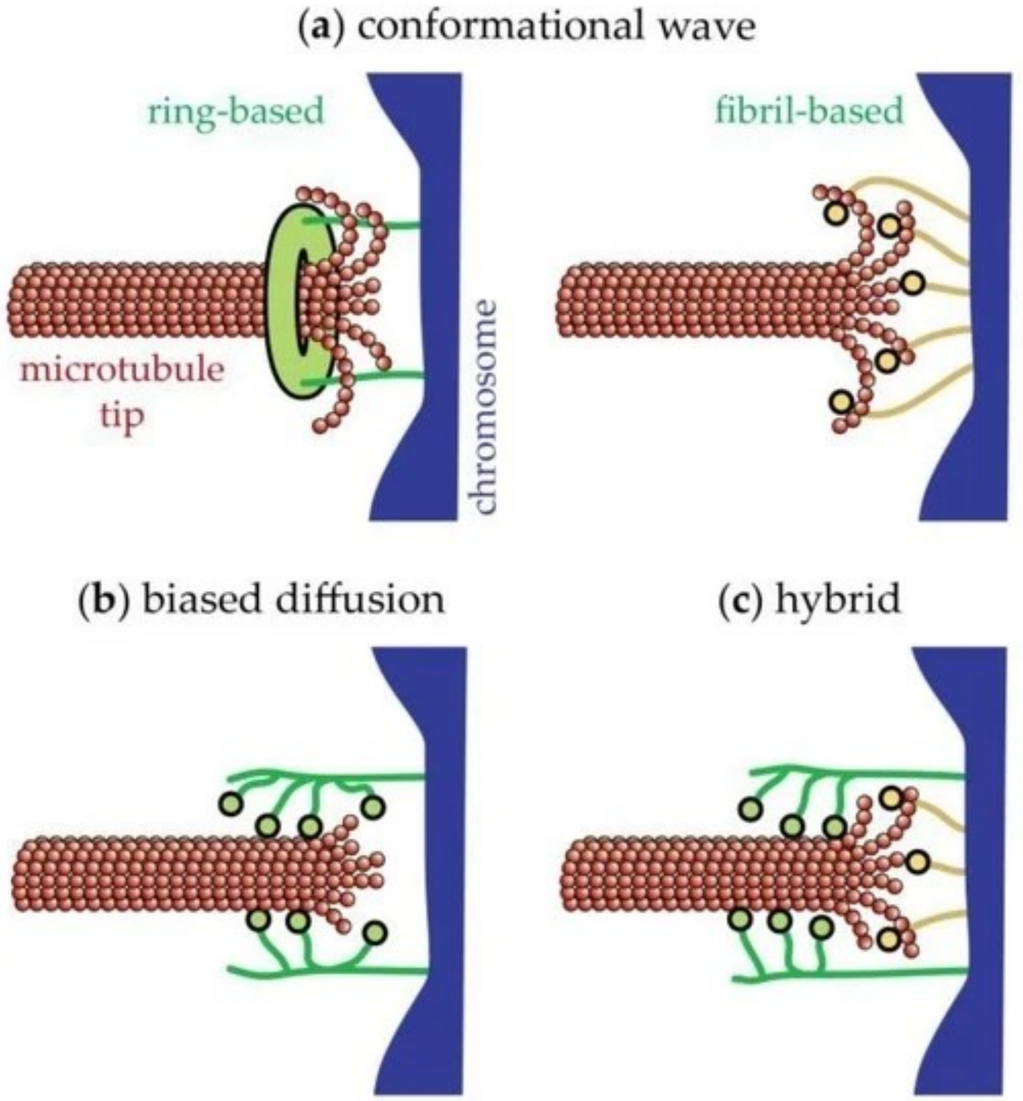
Shrinking can be converted to work by a ring-like protein complex



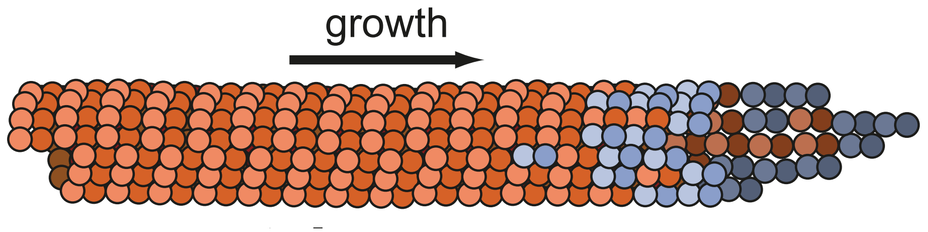
Bending and dynamics together can pull a ring



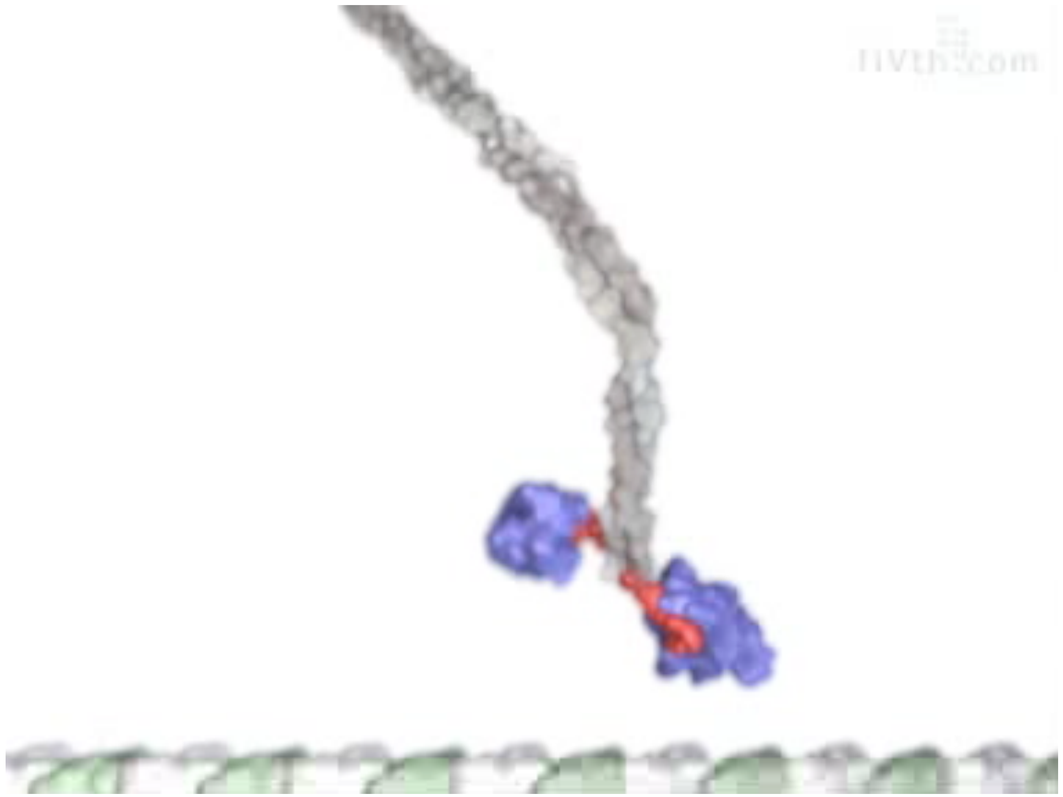
In human cells and many other cells, pulling is not done by this ring



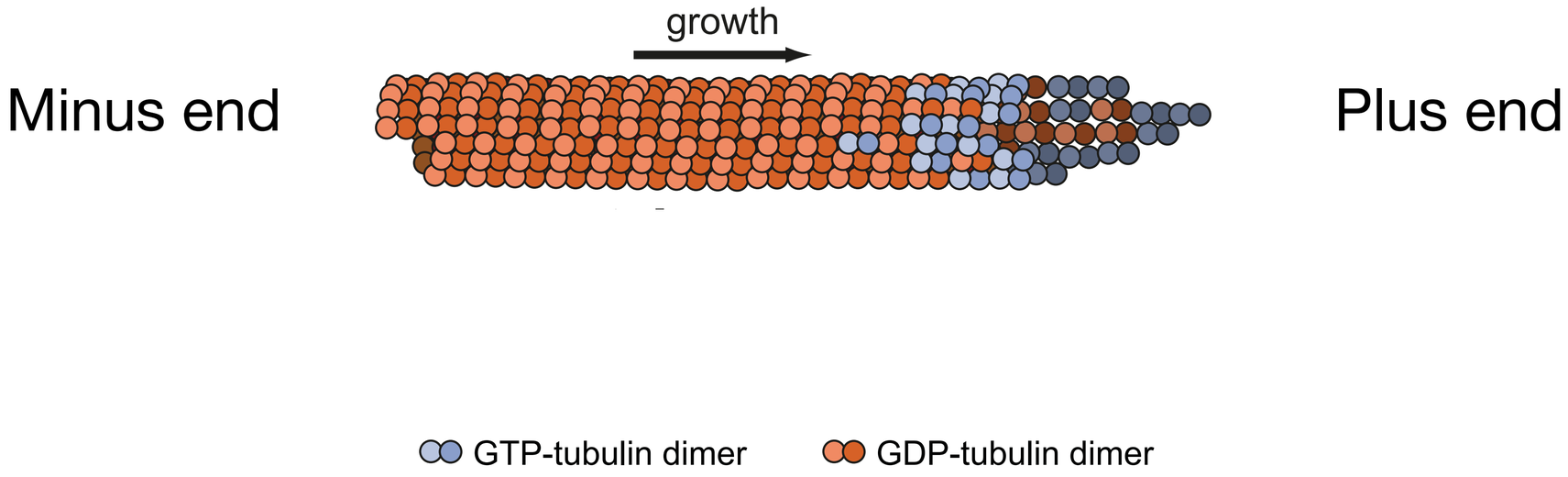
Microtubule acts like a railway track for some engines/motors to move



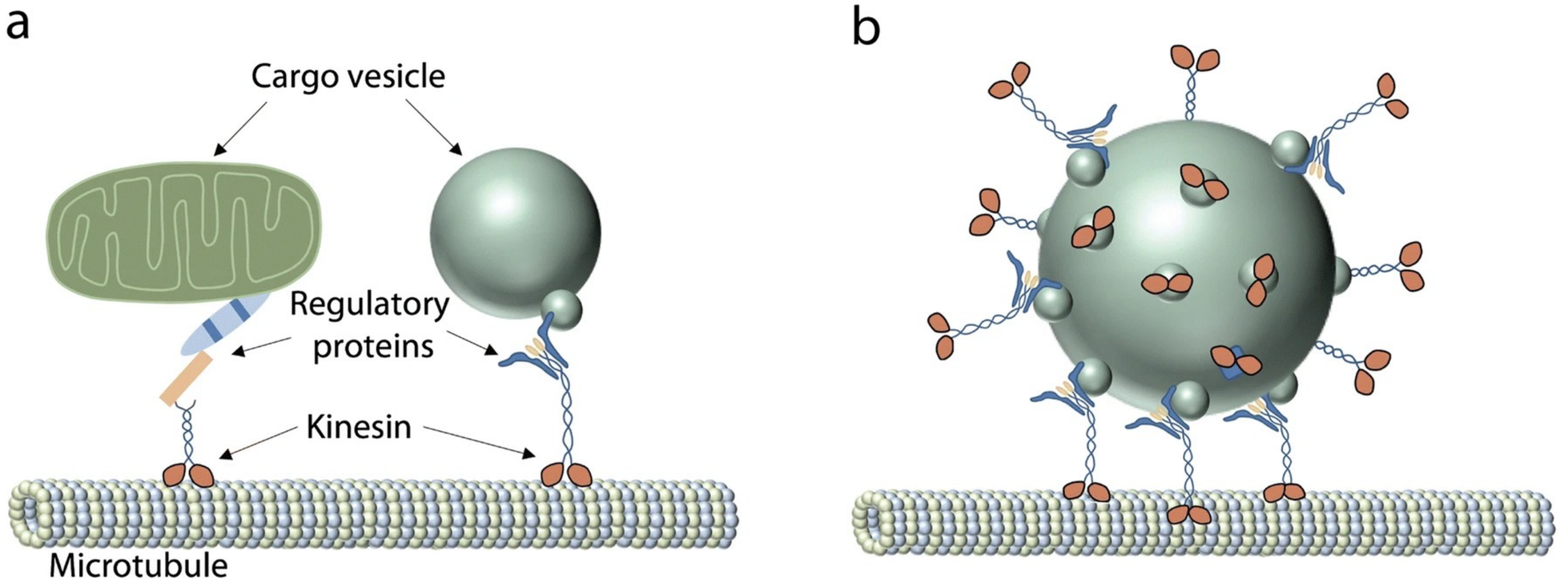
Kinesin: Molecular motor that walks along microtubule



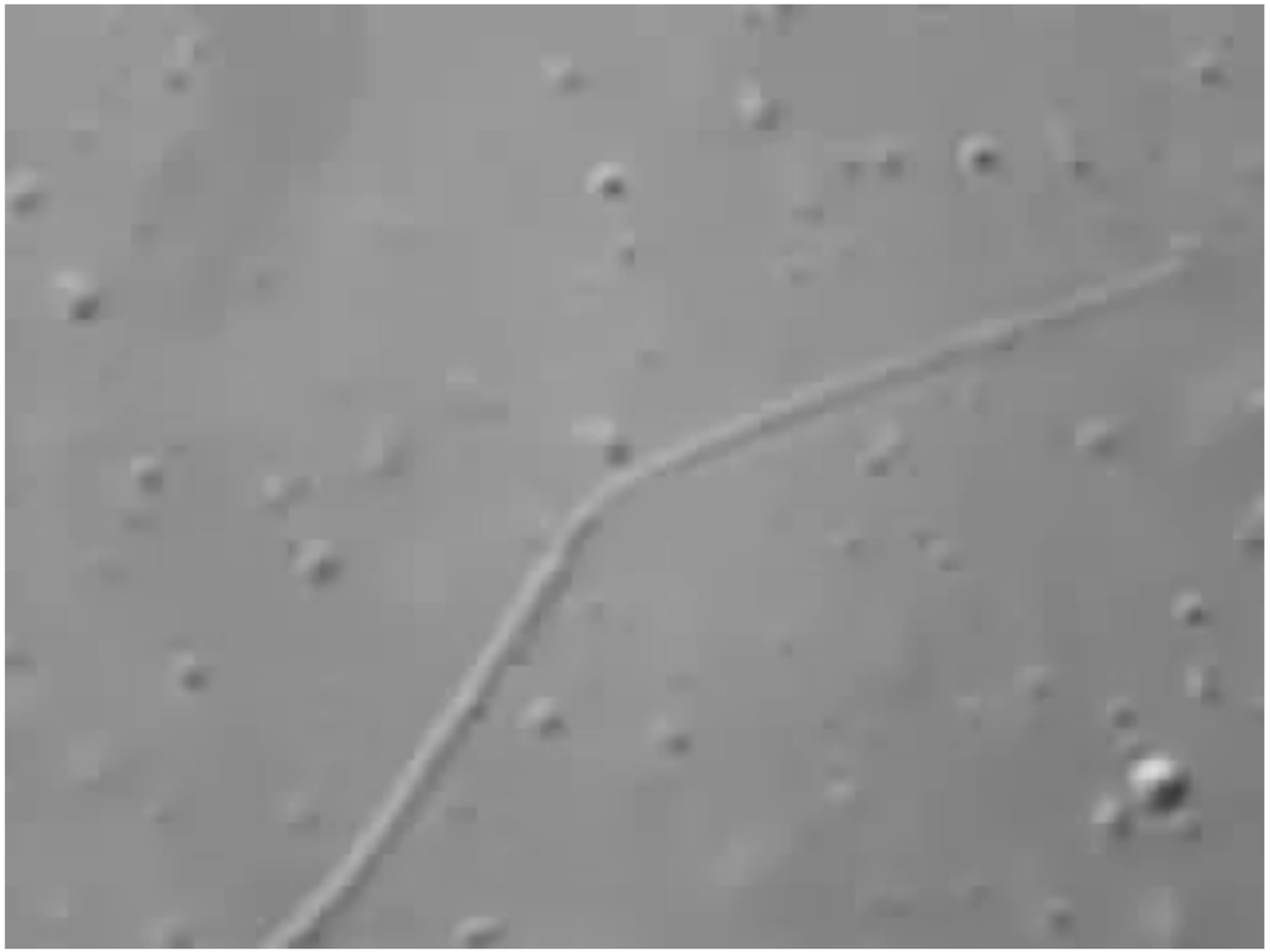
Kinesin moves towards the plus end of the microtubule (GTP end)



These motors carry a cargo of organelles (like a vesicle bag containing many things inside)



What you see under a microscope: cargo movement



Book:
Alberts et al
Molecular
Biology of
the Cell

Molecular motors hydrolyse ATP to move

It is a family of motors

Many different types

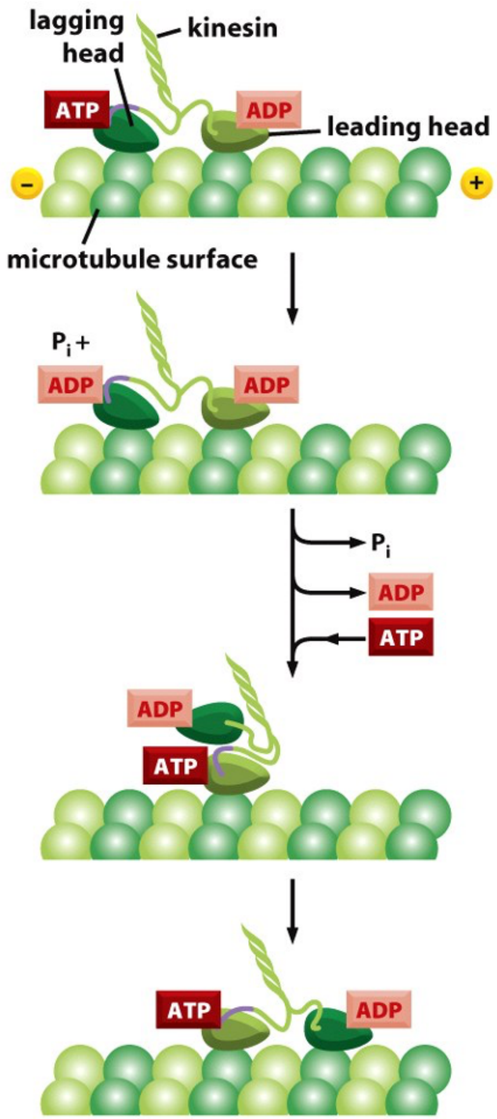
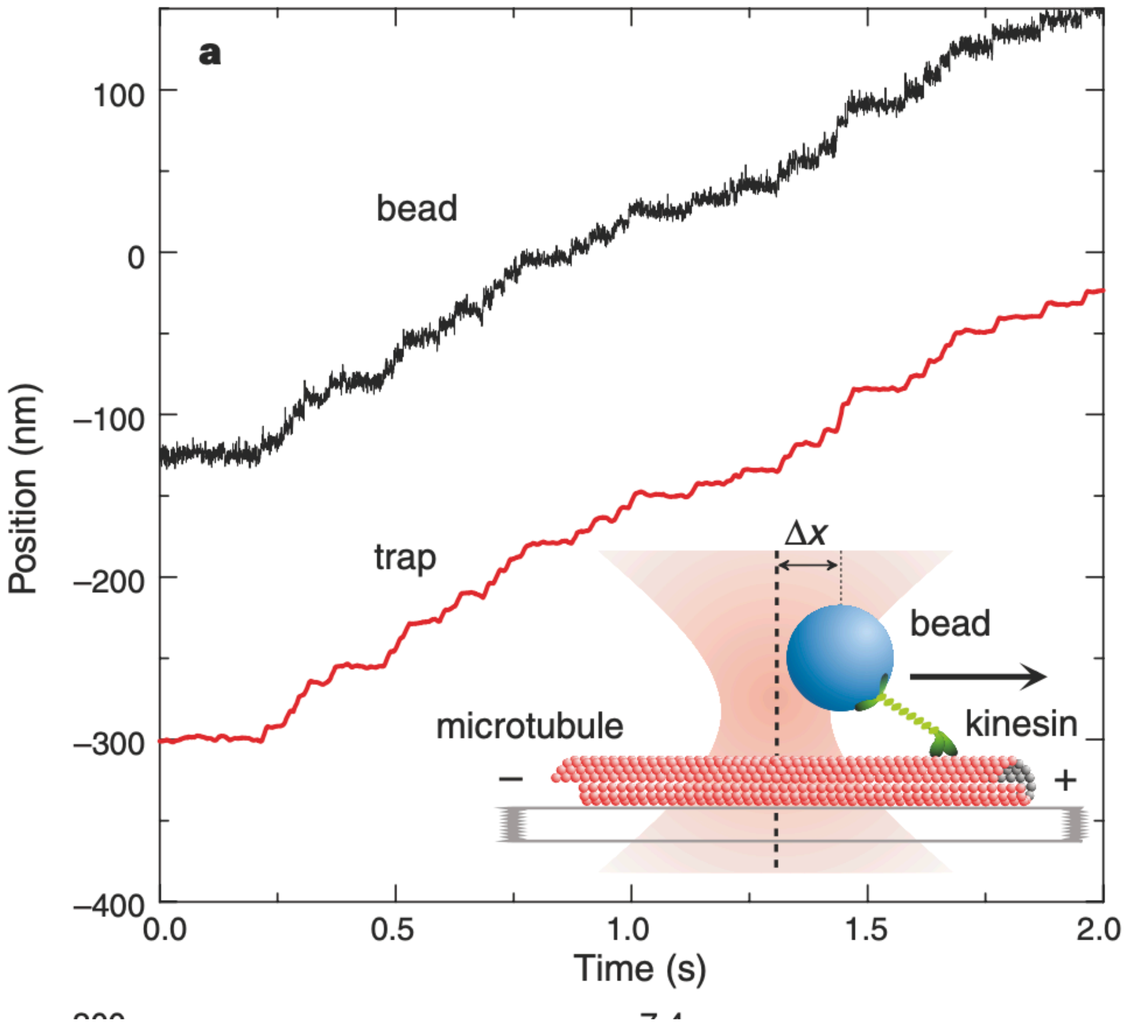
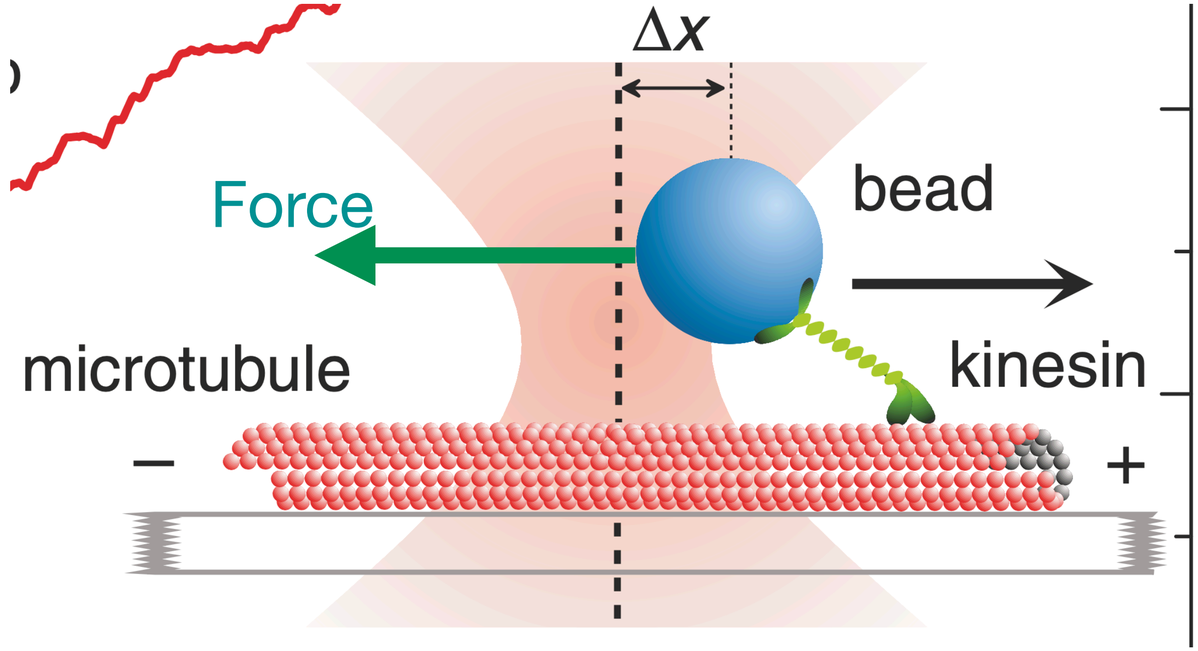


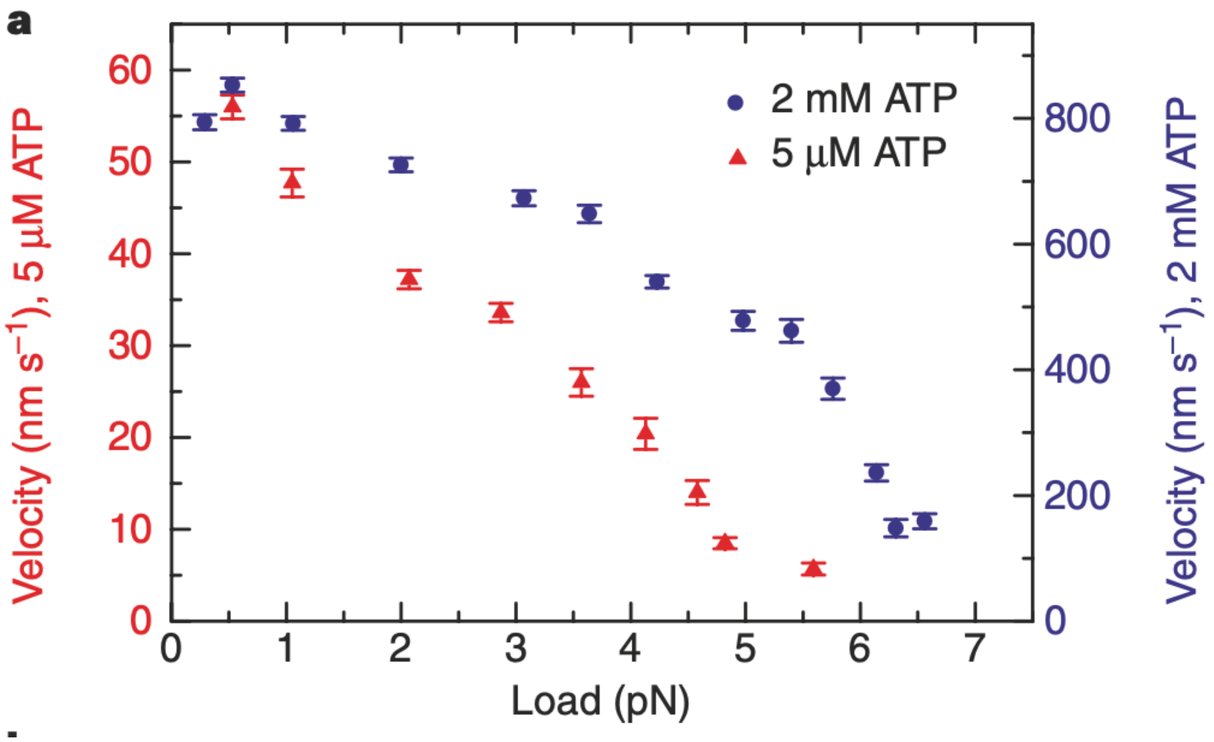
Figure 16-62 *Molecular Biology of the Cell* (© Garland Science 2008)

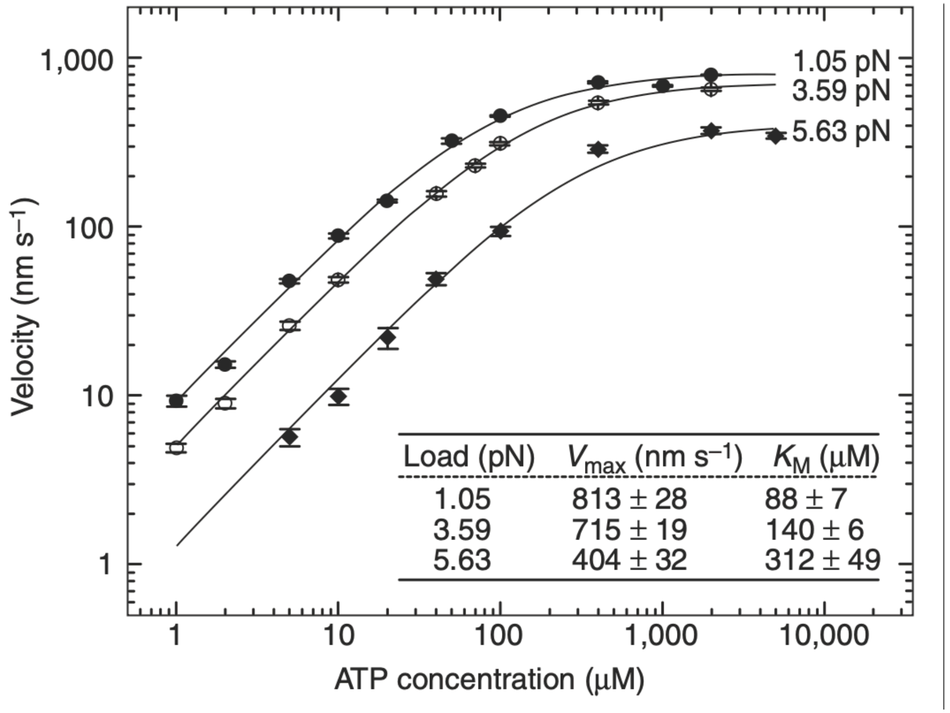
Measuring force generated by a molecular motor: Kinesin





Experiments measure velocity vs force or velocity vs ATP concentration



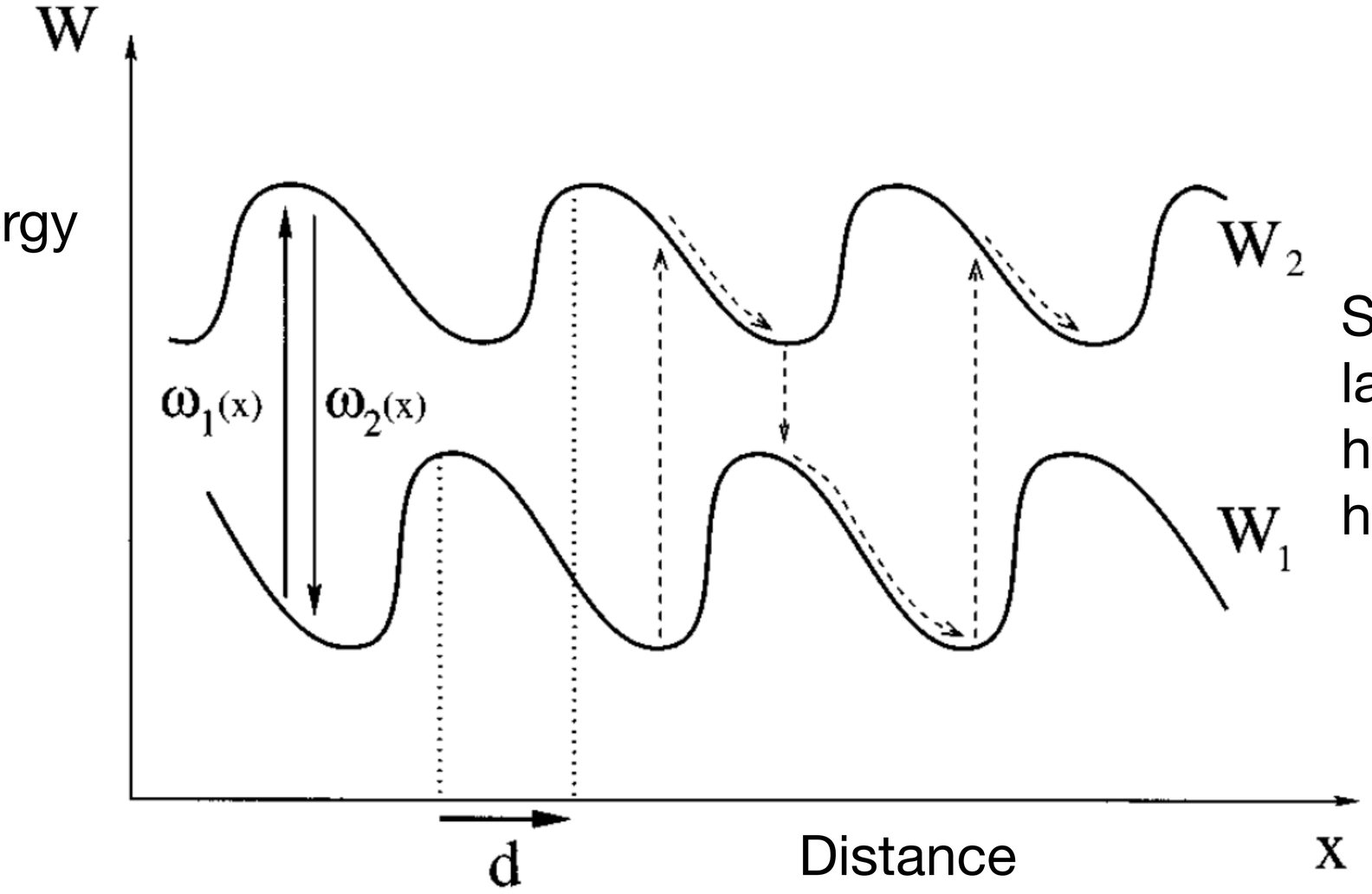


Details: https://www.nature.com/articles/22146

Kinesin cannot move without ATP

How to draw a plausible free energy diagram for an active motor moving forward burning ATP?

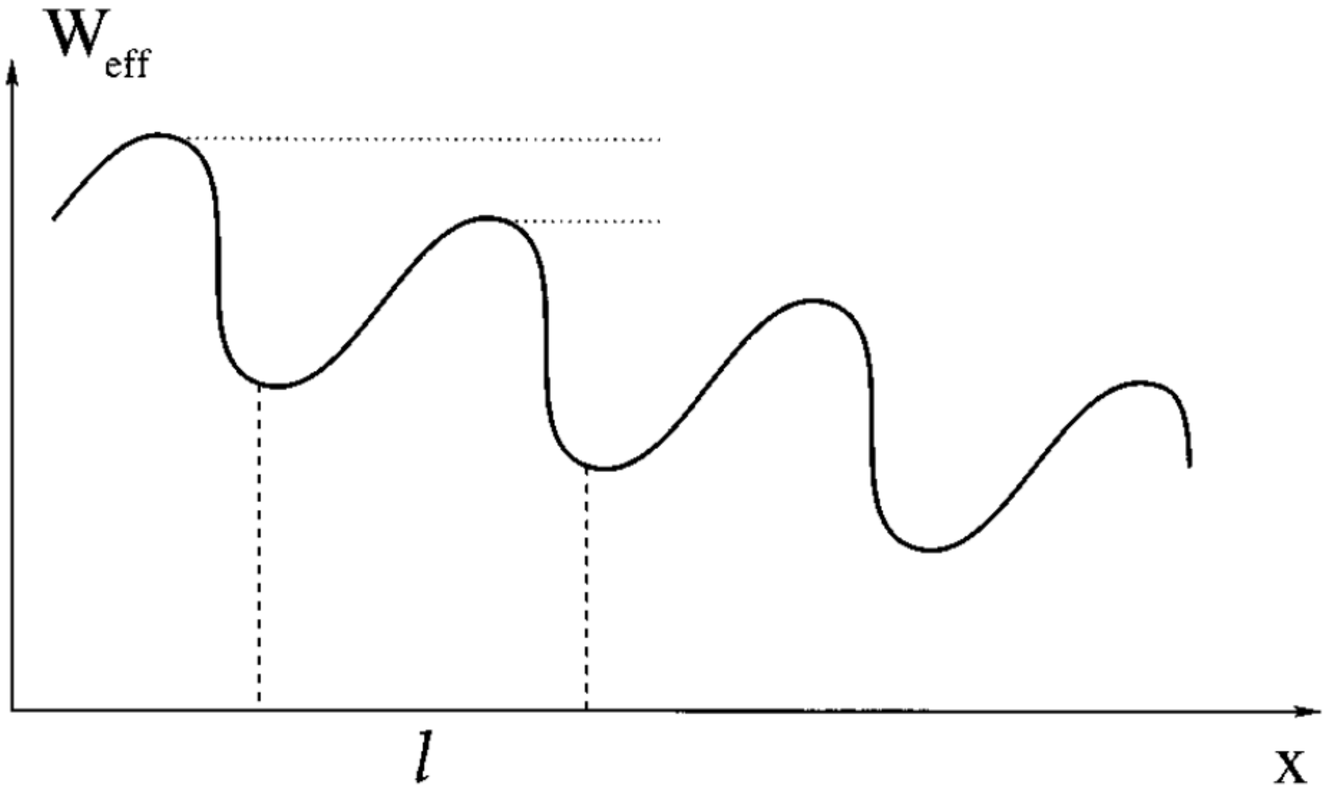
Energy-consuming active (non-thermal) motion: need to switch between two landscapes



Switching between landscapes can only happen with ATP hydrolysis

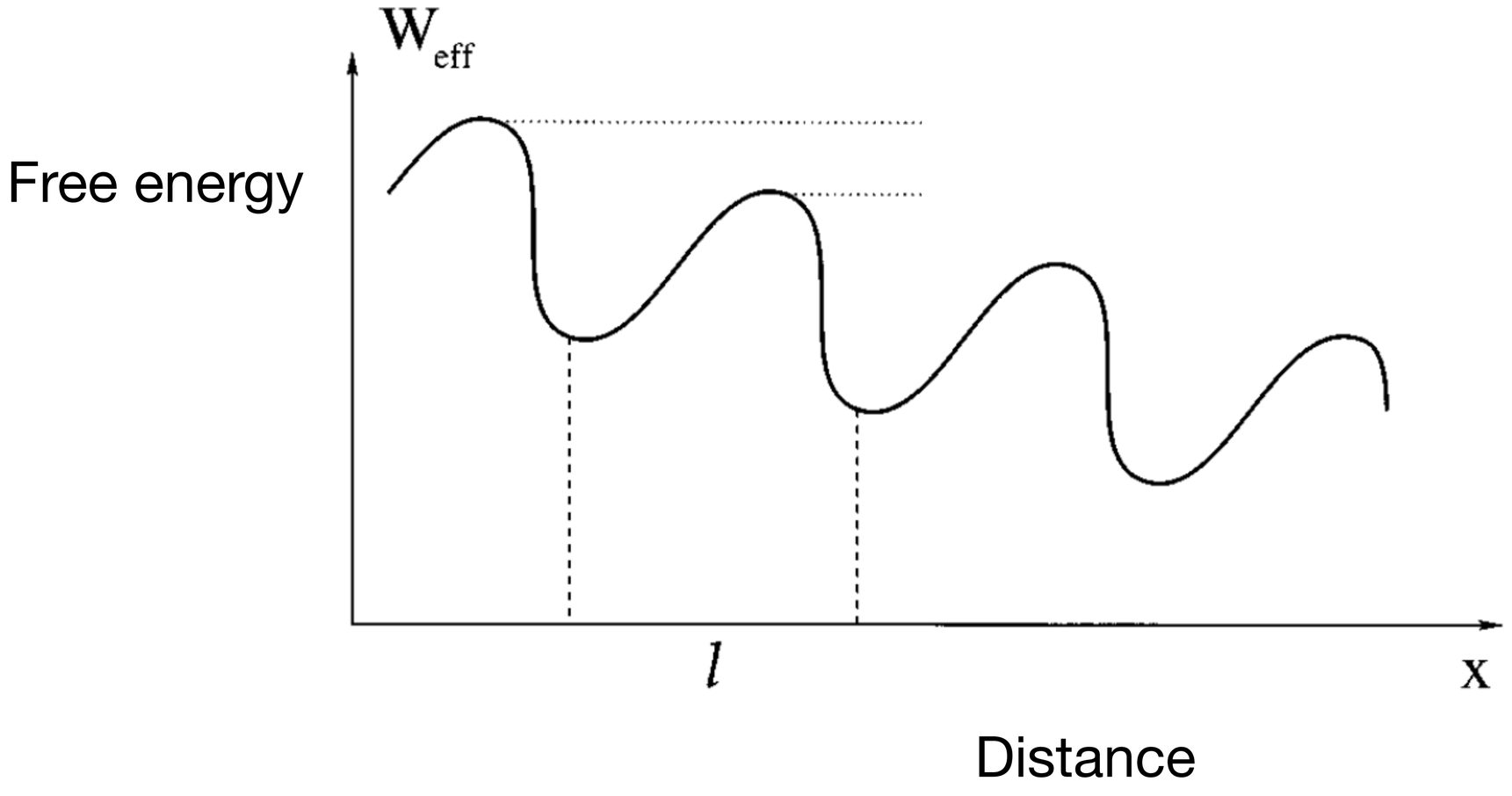
Will this landscape work?

Free energy

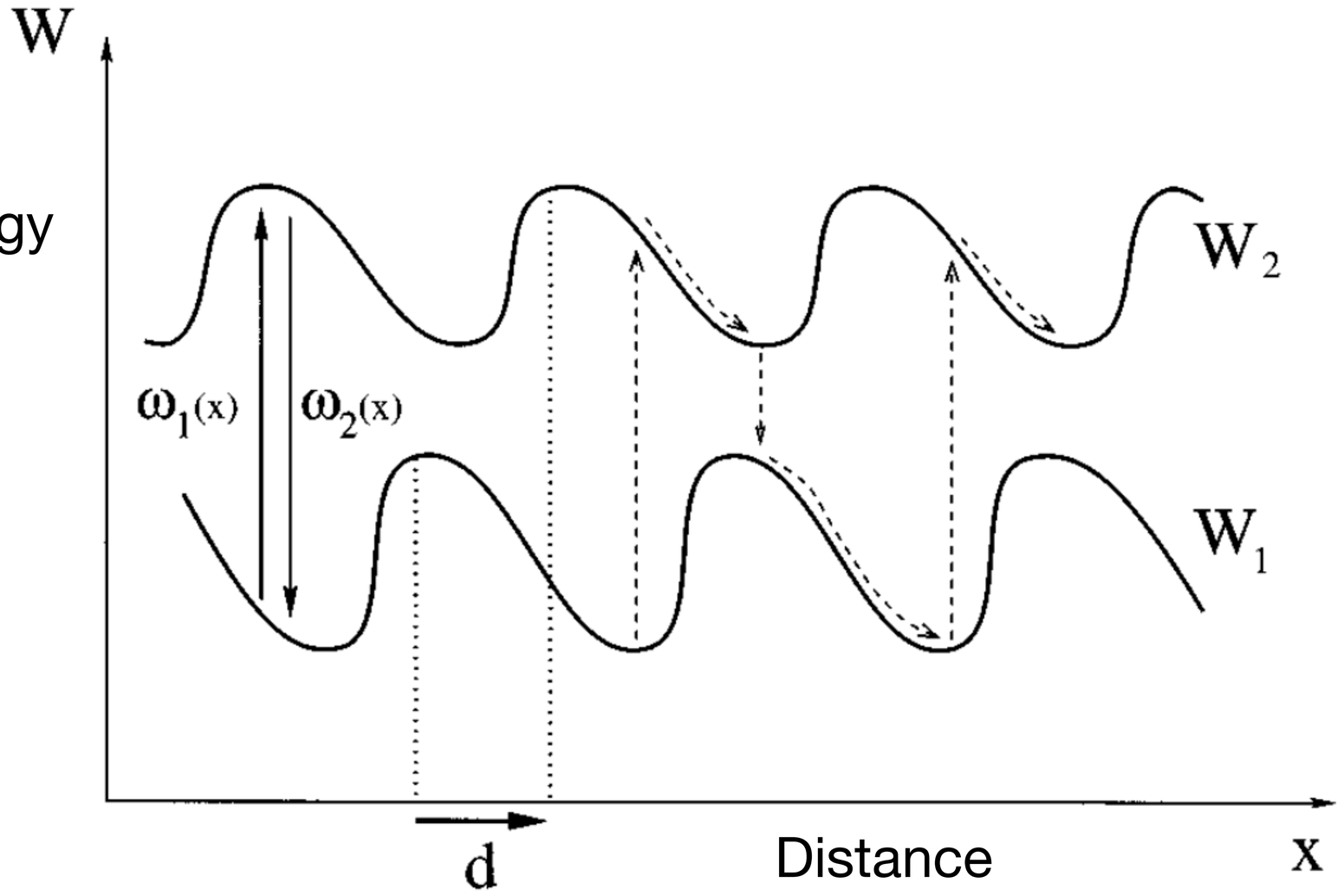


Distance

If you wait long enough, motor will move forward if this landscape was true. Velocity without ATP is not zero.



Here velocity without ATP will be zero



Switching between landscapes can only happen with ATP hydrolysis

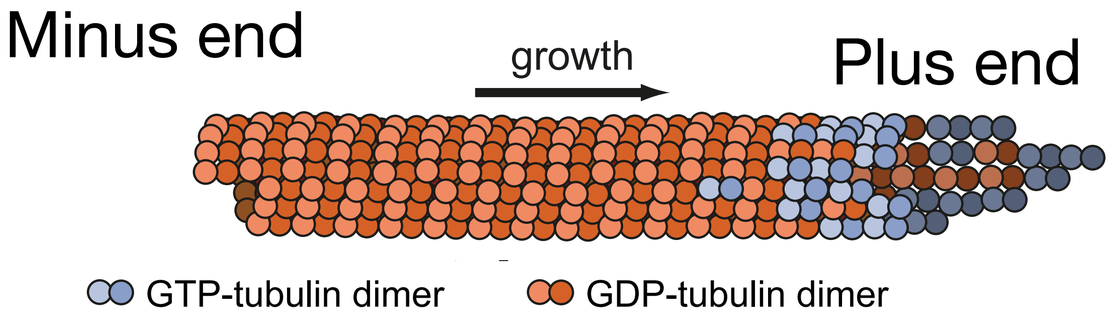
Advanced (optional) question to ponder: How to write dynamic equations for movements with switching landscapes

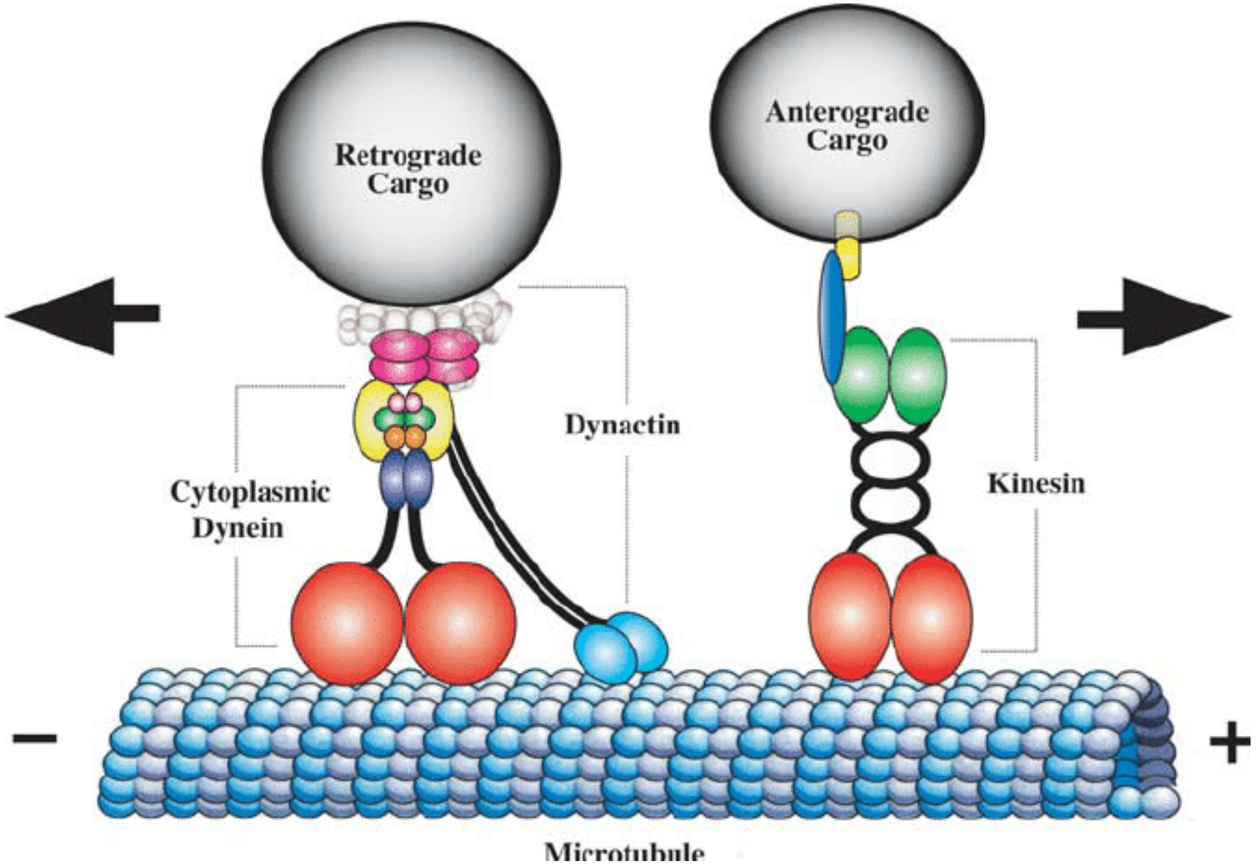
Beyond Newton's equations

Probabilistic equations that can compute changes in time and space:

Master equations, Fokker-Planck equations

Dynein: another motor that moves towards the minus end of the microtubule (GDP end)





One of the world's leading labs doing research on Dynein is here in IIT Bombay, Bio Department

Prof. Roop Mallik's Lab

BSBE building, Lab 406

NATURE | VOL 427 | 12 FEBRUARY 2004 | v

Cytoplasmic dynein functions as a gear in response to load

Roop Mallik[1], Brian C. Carter[1], Stephanie A. Lex[2], Stephen J. King[2] & Steven P. Gross[1]

Article

Cell

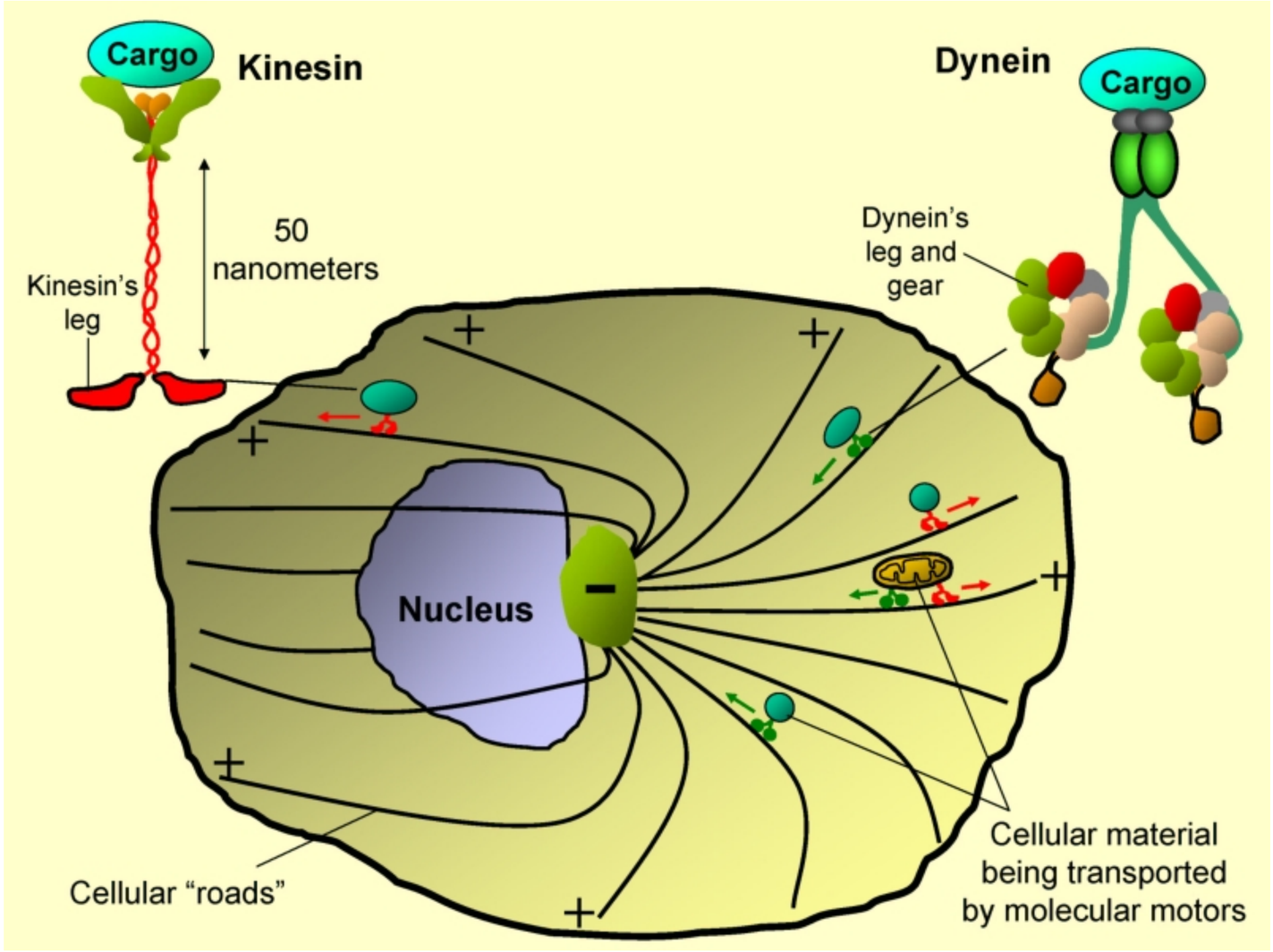
Dynein Clusters into Lipid Microdomains on Phagosomes to Drive Rapid Transport toward Lysosomes

Graphical Abstract

Authors

Ashim Rai, Divya Pathak, Shreyasi Thakur, Shampa Singh, Alok Kumar Dubey, Roop Mallik

Two motors travelling along microtubule



Outcome of this movement: quick control of spatial organization of molecules

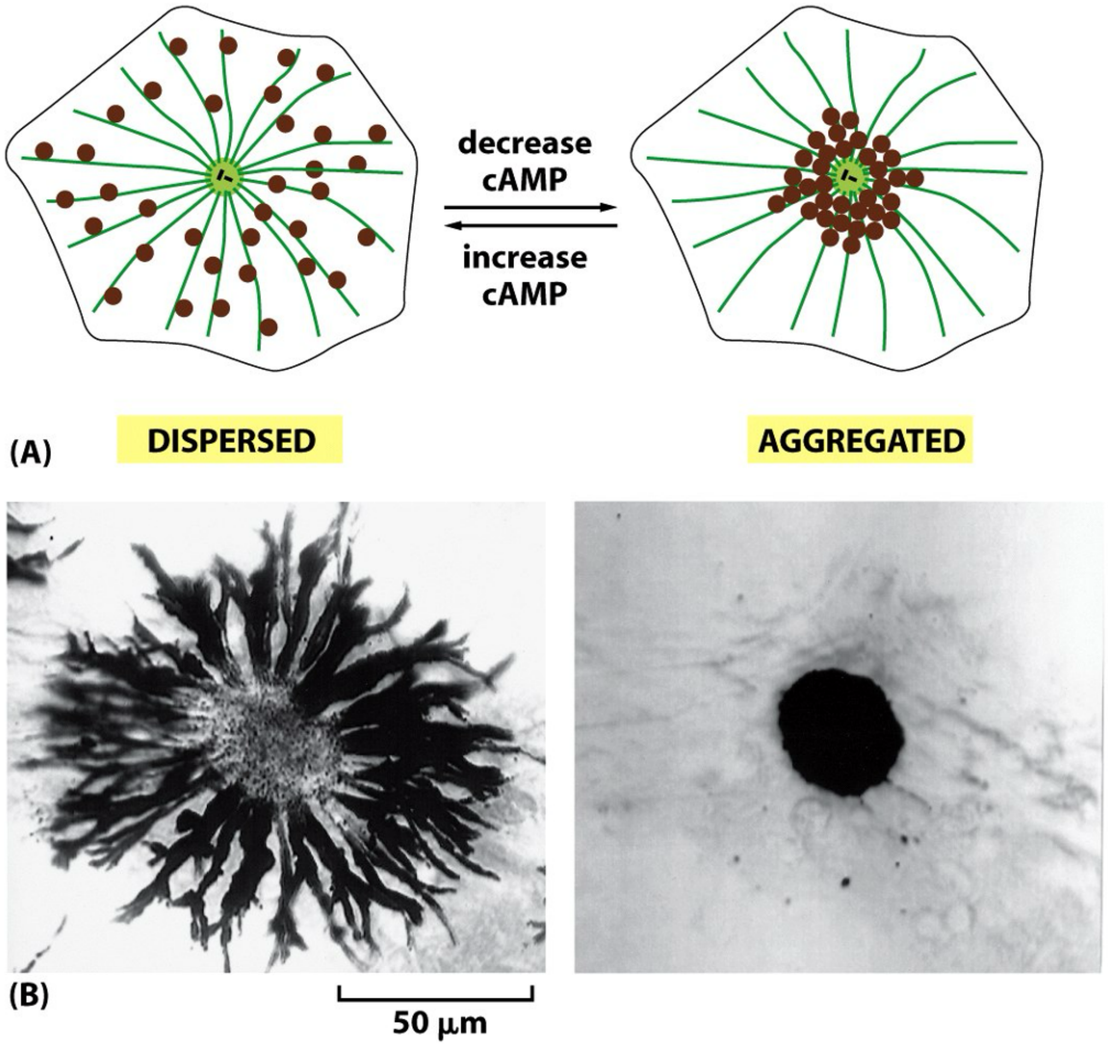
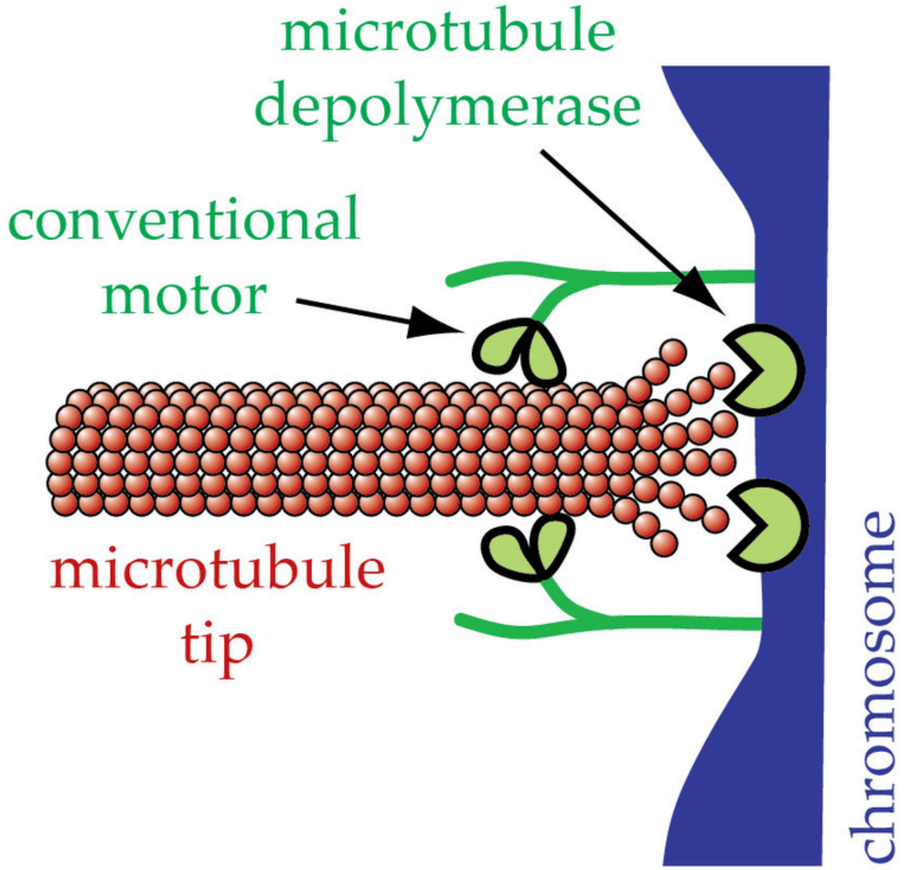


Figure 16-70 *Molecular Biology of the Cell* (© Garland Science 2008)

Figure 16–70 Regulated melanosome movements in fish pigment cells. These giant cells, which are responsible for changes in skin coloration in several species of fish, contain large pigment granules, or melanosomes *(brown)*. The melanosomes can change their location in the cell in response to a hormonal or neuronal stimulus. (A) Schematic view of a pigment cell, showing the dispersal and aggregation of melanosomes in response to an increase or decrease in intracellular cyclic AMP (cAMP), respectively. Both redistributions of melanosomes occur along microtubules. (B) Bright-field images of a single cell in a scale of an African cichlid fish, showing its melanosomes either dispersed throughout the cytoplasm *(left)* or aggregated in the center of the cell *(right)*. (B, courtesy of Leah Haimo.)

How chameleons change color?

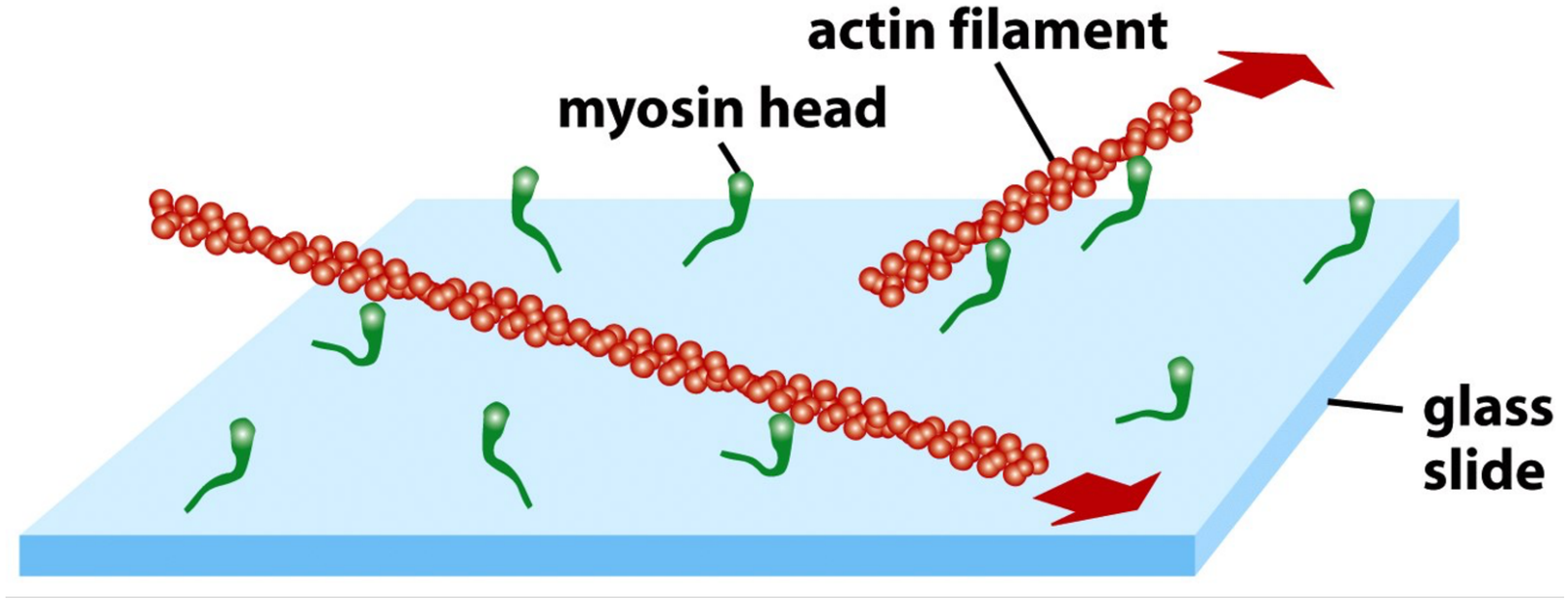
These motors pull chromosomes during cell division, in conjunction with microtubule depolymerisation



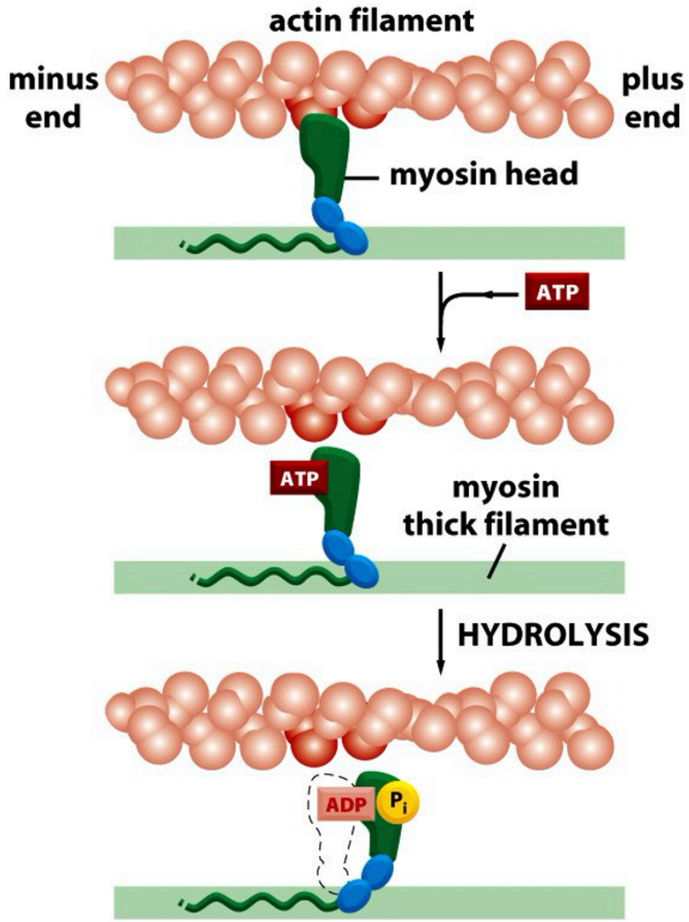
Summary

- Microtubule
- Kinesin
- Dynein
- Myosin
- Chromsome segregation
- Movement of cargo (pigments as example); also fat and other things

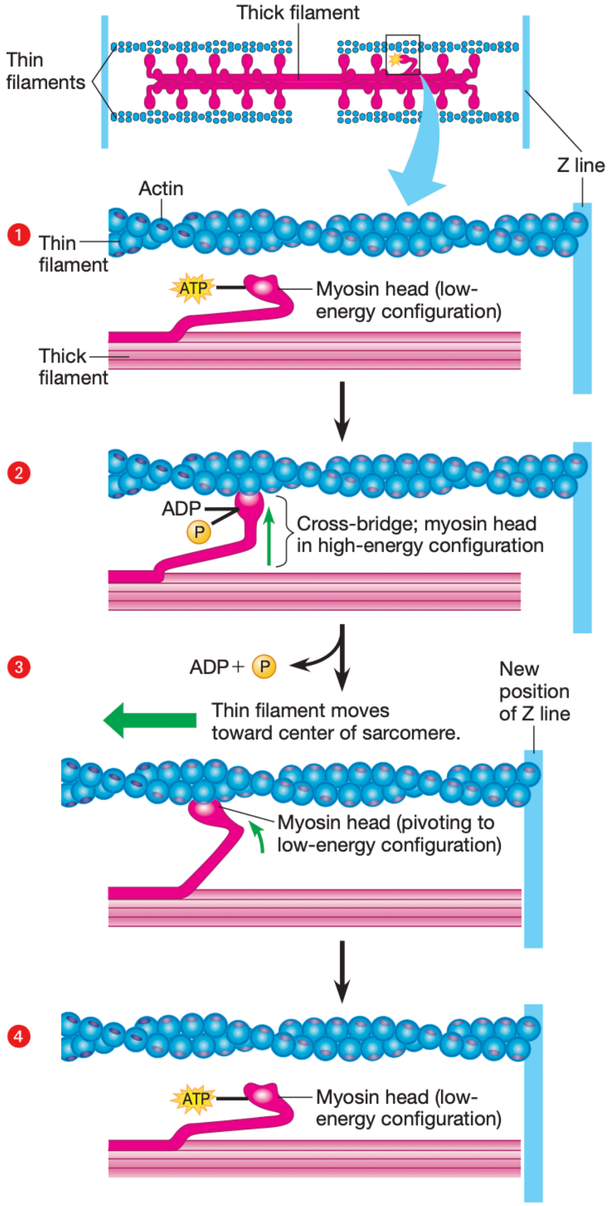
Myosin: a motor family that binds to actin



Myosin uses ATP hydrolysis and pushes Actin



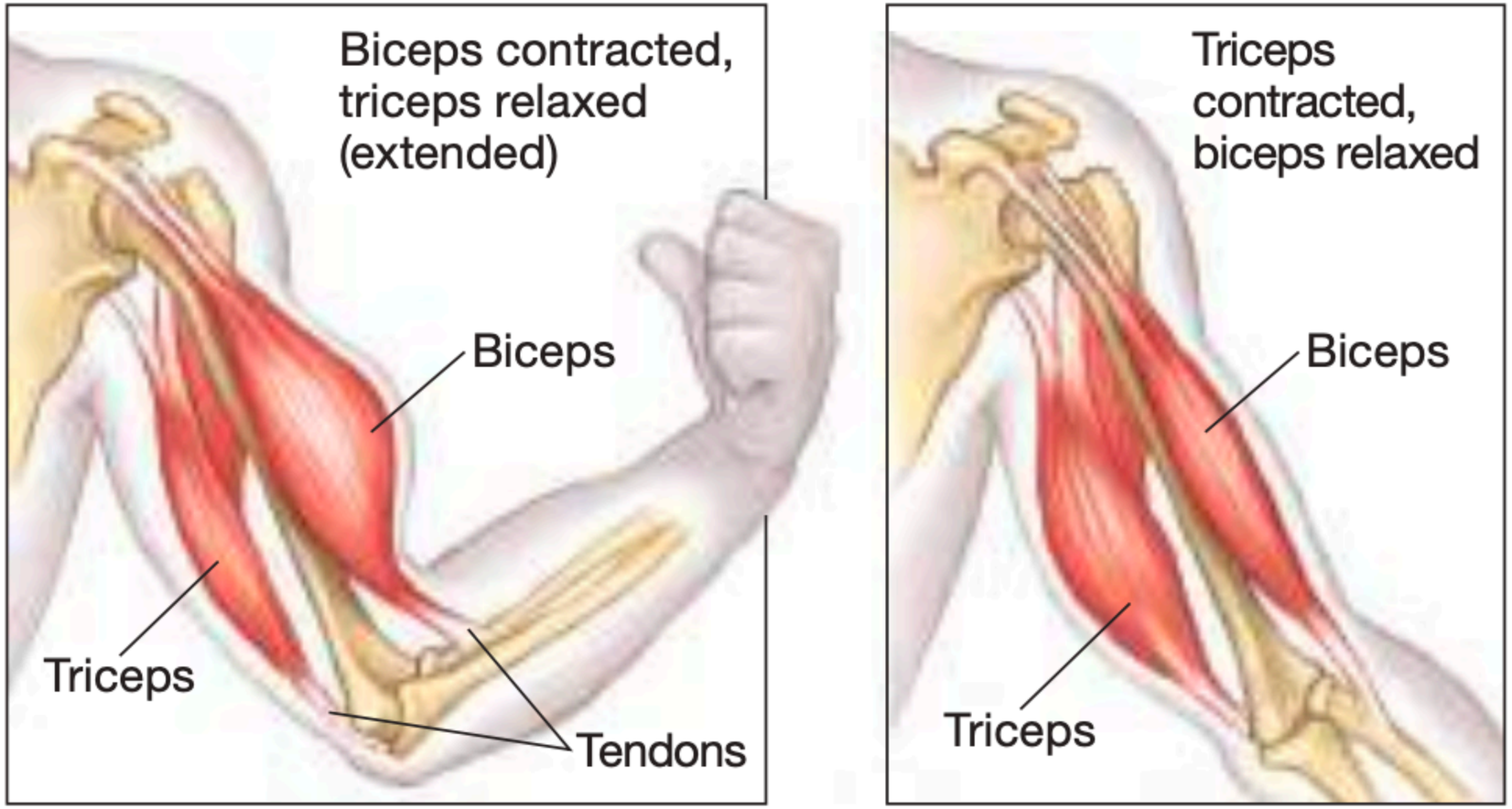
Myosin pushing actin



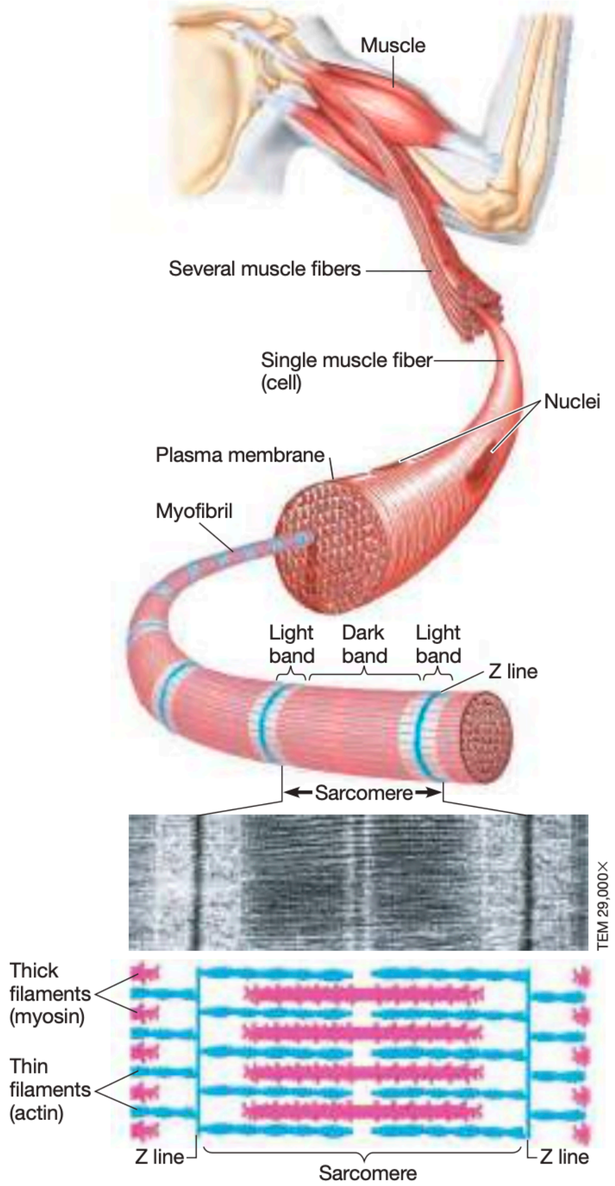
▲ **Figure 30.9B** The mechanism of filament sliding

TRY THIS At each step, describe in your own words the action of the myosin head.

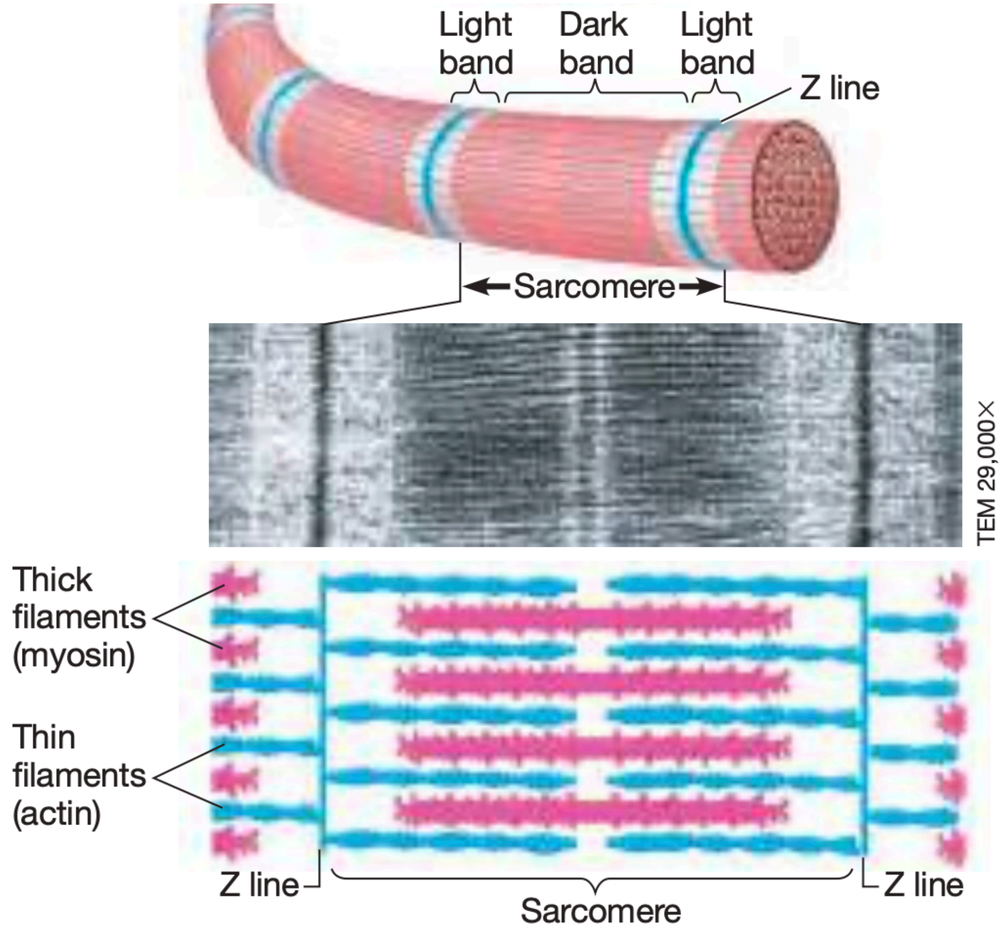
How do our muscles work? Generate force?



Actin and myosin together make the muscle work!



▲ **Figure 30.8** The contractile apparatus of skeletal muscle



▲ **Figure 30.8** The contractile apparatus of skeletal muscle

Summary

- Microtubule
- Kinesin
- Dynein
- Myosin
- Chromsome segregation
- Movement of cargo (pigments as example); also fat and other things
- Muscle