

Today's class:

Active transport in the cell

This lecture follows the Chapter 16 in the book ‘Physical Biology of the Cell’ by Philips et al. and parts of chapter 17 in the book ‘The Molecules of Life’ by Kuriyan et al.

Long distance travels in a the cell is done by active transport

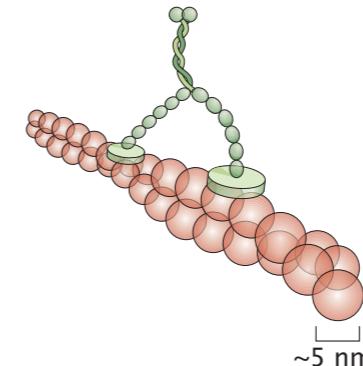
Diffusive transport is inefficient for transporting molecules across long distances, like ~ cm or more

Active transport

Active transport is an energy-consuming process through which molecules are moved between different parts of cells. The term "active transport" is also used to mean movement of molecules across membranes against a concentration gradient, as was discussed in Chapter 11.

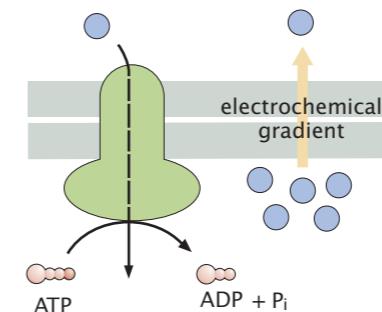
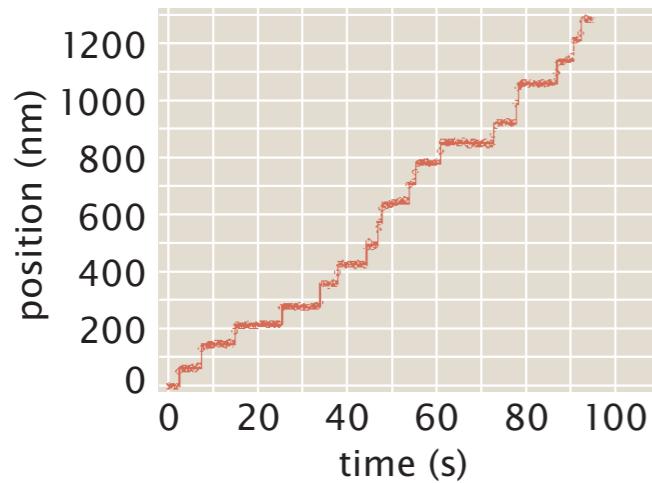
Cell employs two kinds of active transport mechanisms

→ Transport through molecular motors



→ Transport against a conc gradient through pumps

Active transport is 'directional'



Ion-specific channels also provide directional transport but the motion is diffusive

Active transport through molecular motors

There are four broad classes of motors

Translational motors

1-D linear movement on a substrate 'track'.
E.g. myosin, helicases

Polymerization motors

Effective force generating systems through filament (de)polymerization. E.g. actin or microtubule assembly systems. They also provide tracks for translational motors

Rotatory motors

Membrane embedded motors generating torque by rotational elements. E.g. bacterial flagellar motor

Translocation motors

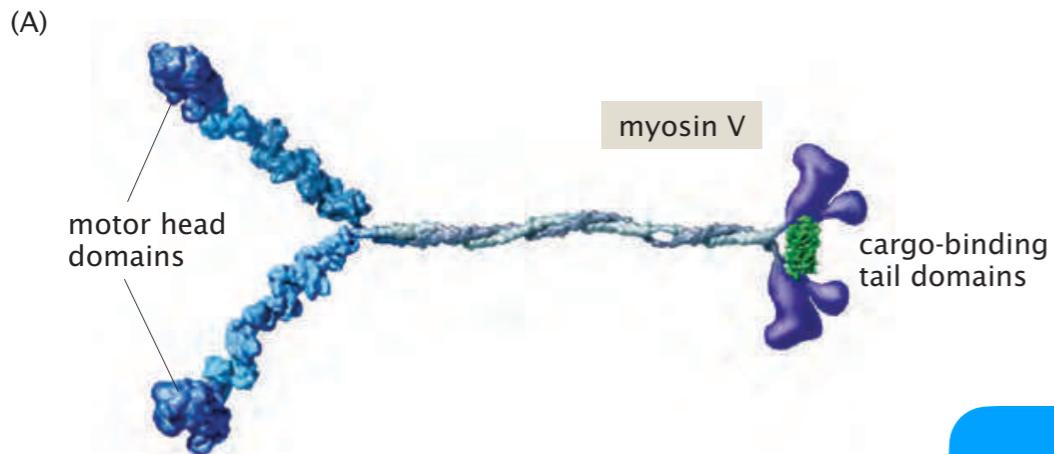
Motors that push or pull DNA/protein across pores. E.g. bacterial DNA translocation motor

Translational motors

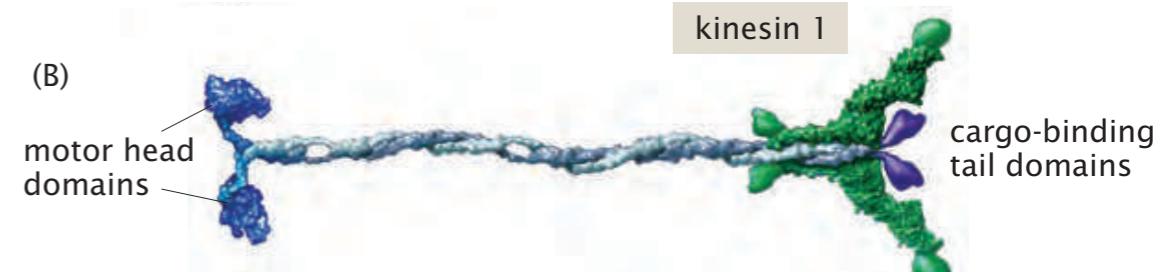
The most important translational motors are associated with the cytoskeleton - actin and microtubules

Three main families of cytoskeletal motors

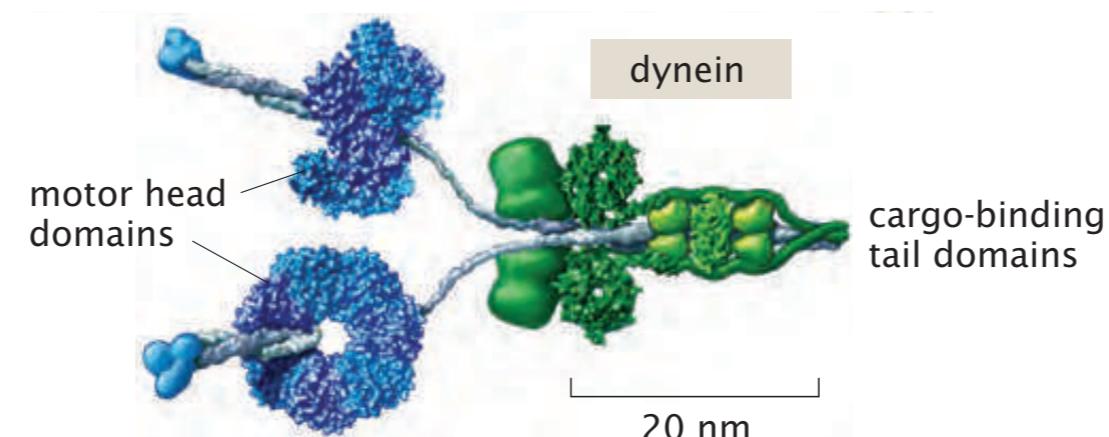
Myosin



Kinesin



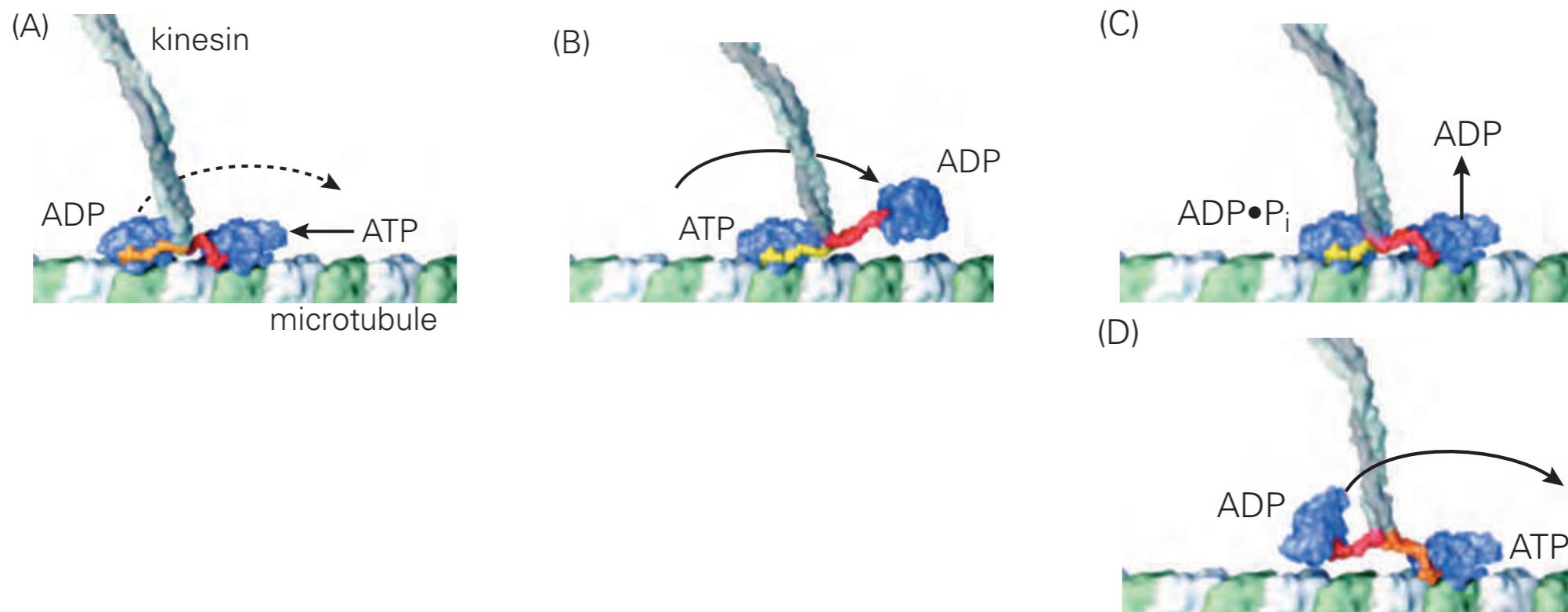
Dynein



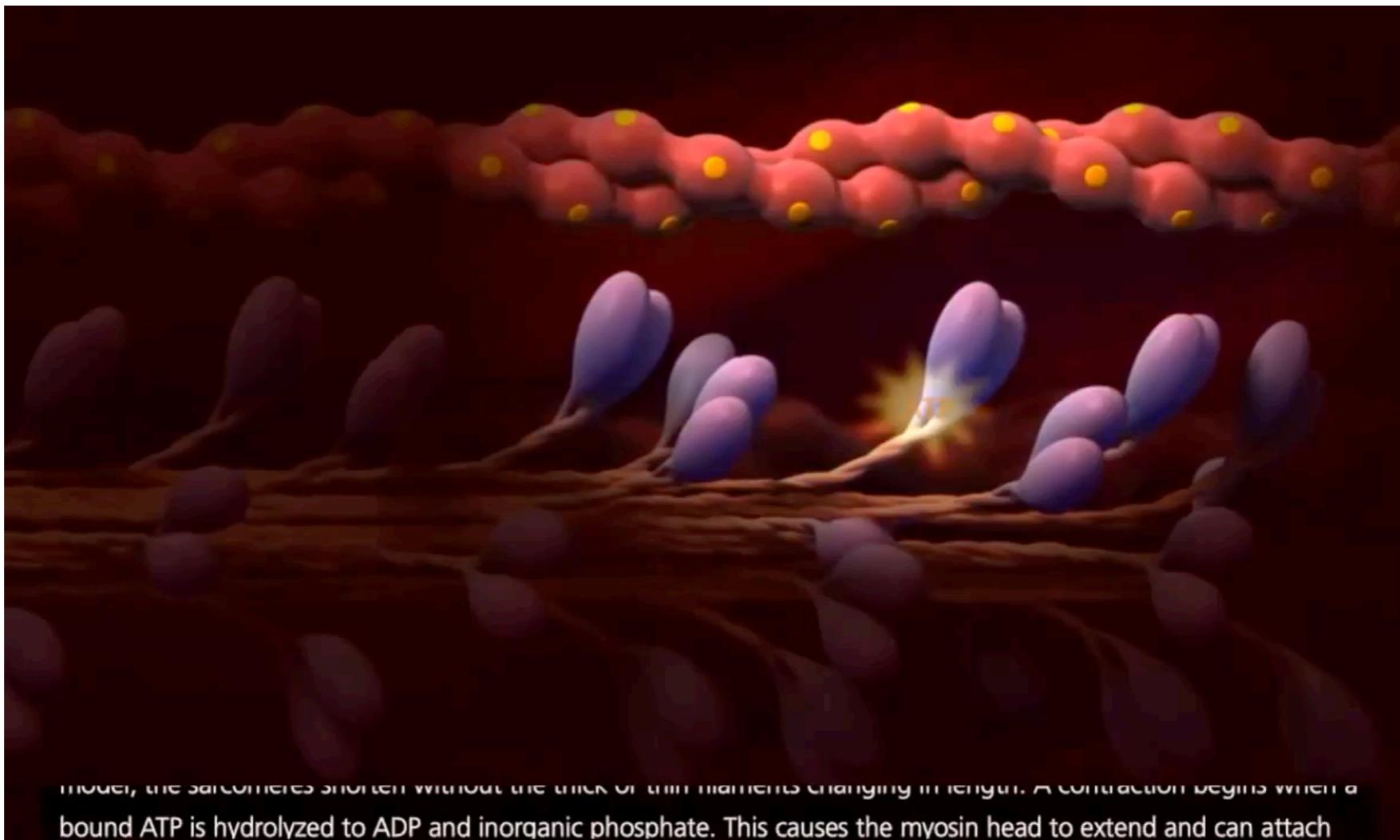
All cytoskeletal motors follow similar dynamic cycle

Key features related to functioning of these motors

- Here each ‘head’ domain has an ATP binding site and substrate binding site(s)
- ATP hydrolysis triggers a conformational change that allows substrate binding
- Binding is followed by a power stroke that is equivalent to ‘walking/sliding’
- ADP is released and a new ATP binds as the head domain unbinds from substrate and next cycle starts
- The head domain is largely conserved within a family but the cargo-binding tail domain varies



Motor action cycle of myosin in muscles



How much force does a single motor exert on the substrate?

Force exerted during a single motor step

Kinesin motor moves about 8 nm per ATP hydrolysis event

If all force exerted is converted from chemical energy then

$$F_{max} = \frac{\text{free energy of ATP hydrolysis}}{\text{step size}}$$

$$\approx \frac{20 k_B T}{8 \text{ nm}} \approx \frac{20 \times 4 \text{ pN nm}}{8 \text{ nm}} = 10 \text{ pN}$$

Comparable to
 $2 - 3k_B T$!

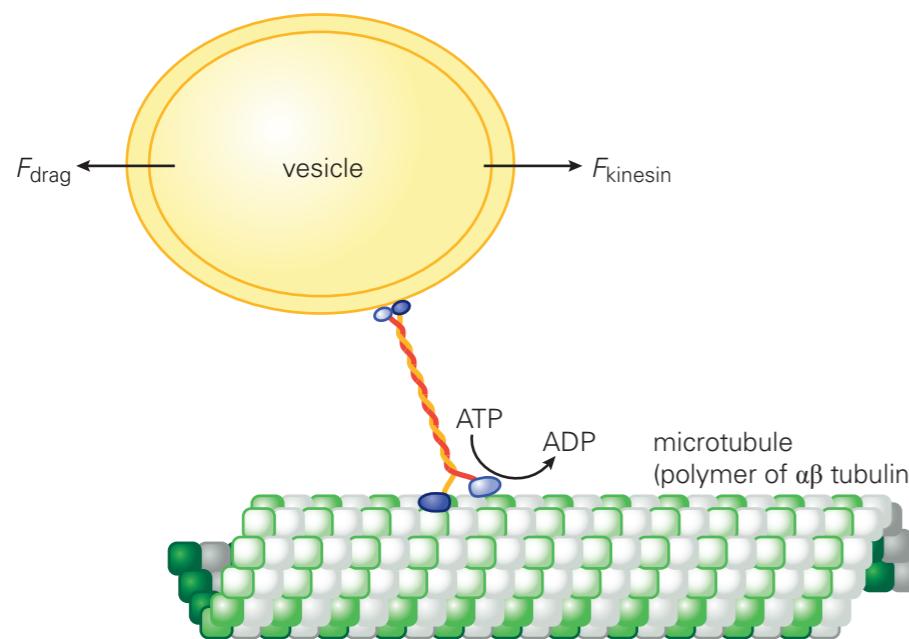
In reality the motors work with less efficiency. Let's estimate that using an example of cargo transport.

Motor processivity: ability of motors to move continuously along the substrate track in bound state

If a motor falls off from the track then its less processive which reduces the mean speed

Myosin or kinesin is much less processive than RNA polymerase as the latter creates entire mRNA in one single run

Cargo transport dynamics by kinesin



If kinesin transports a cargo at a speed $\sim 1 \mu\text{m s}^{-1}$

The force required to move this through cytoplasmic fluid
is balanced by the drag force

$$F_{\text{drag}} = f \times v \quad \text{Where } f = \text{friction factor}$$

For a spherical cargo of radius 100 nm

$$F_{\text{drag}} = 6\pi\eta av = 6\pi(1 \text{ cP}) \times 100 \text{ nm} \times 1 \mu\text{m s}^{-1}$$

$$F_{\text{drag}} \approx 2 \times 10^{-10} \text{ dyne} = 2 \times 10^{-15} \text{ N}$$

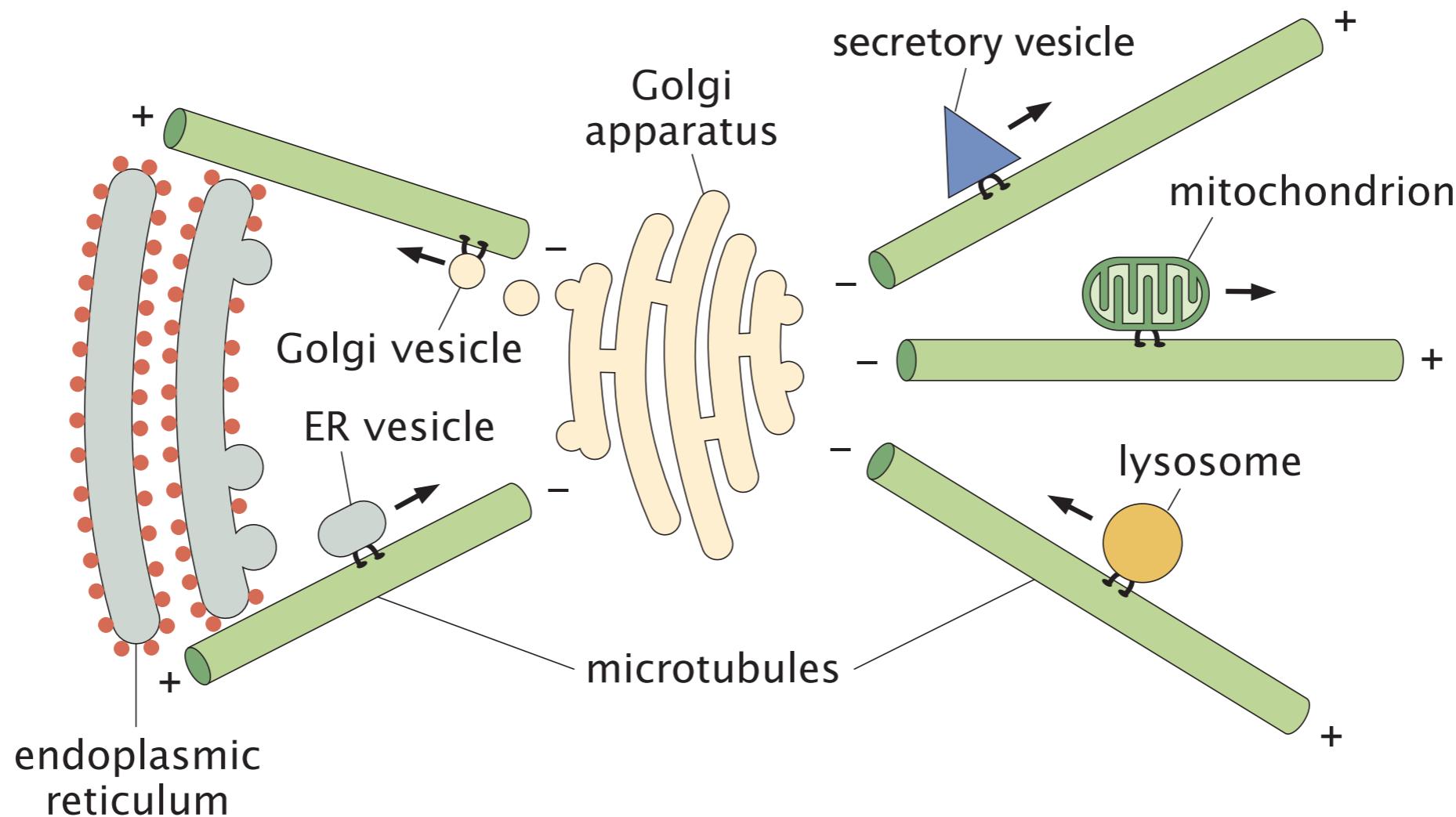
So the energy required to move $1 \mu\text{m s}^{-1}$ $\implies F_{\text{drag}} \times 1 \mu\text{m} = 2 \times 10^{-21} \text{ J}$

Now this energy comes from hydrolysis of how many ATP? $N_{\text{ATP}} = \frac{1 \mu\text{m}}{8 \text{ nm}} \approx 100$

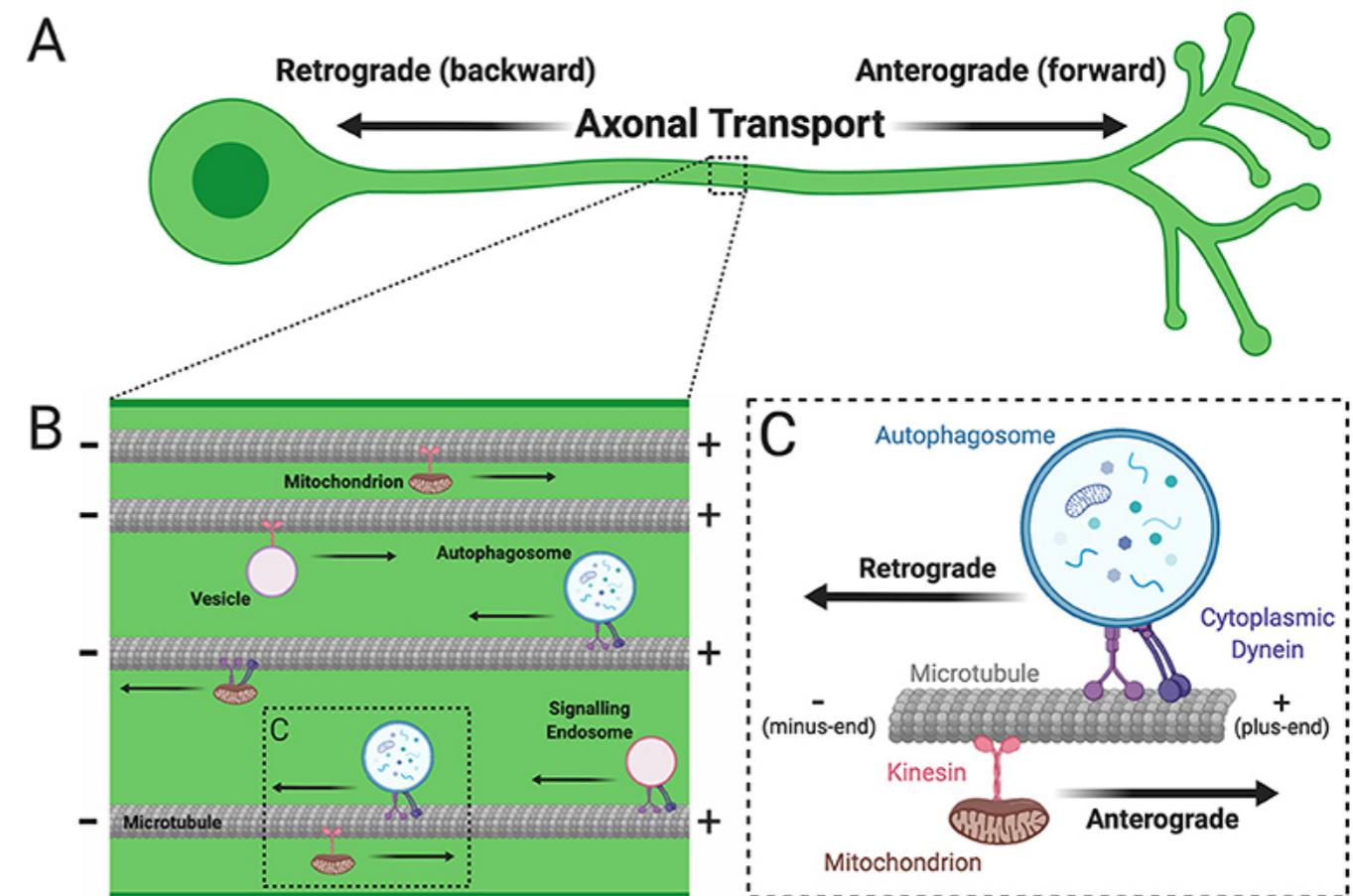
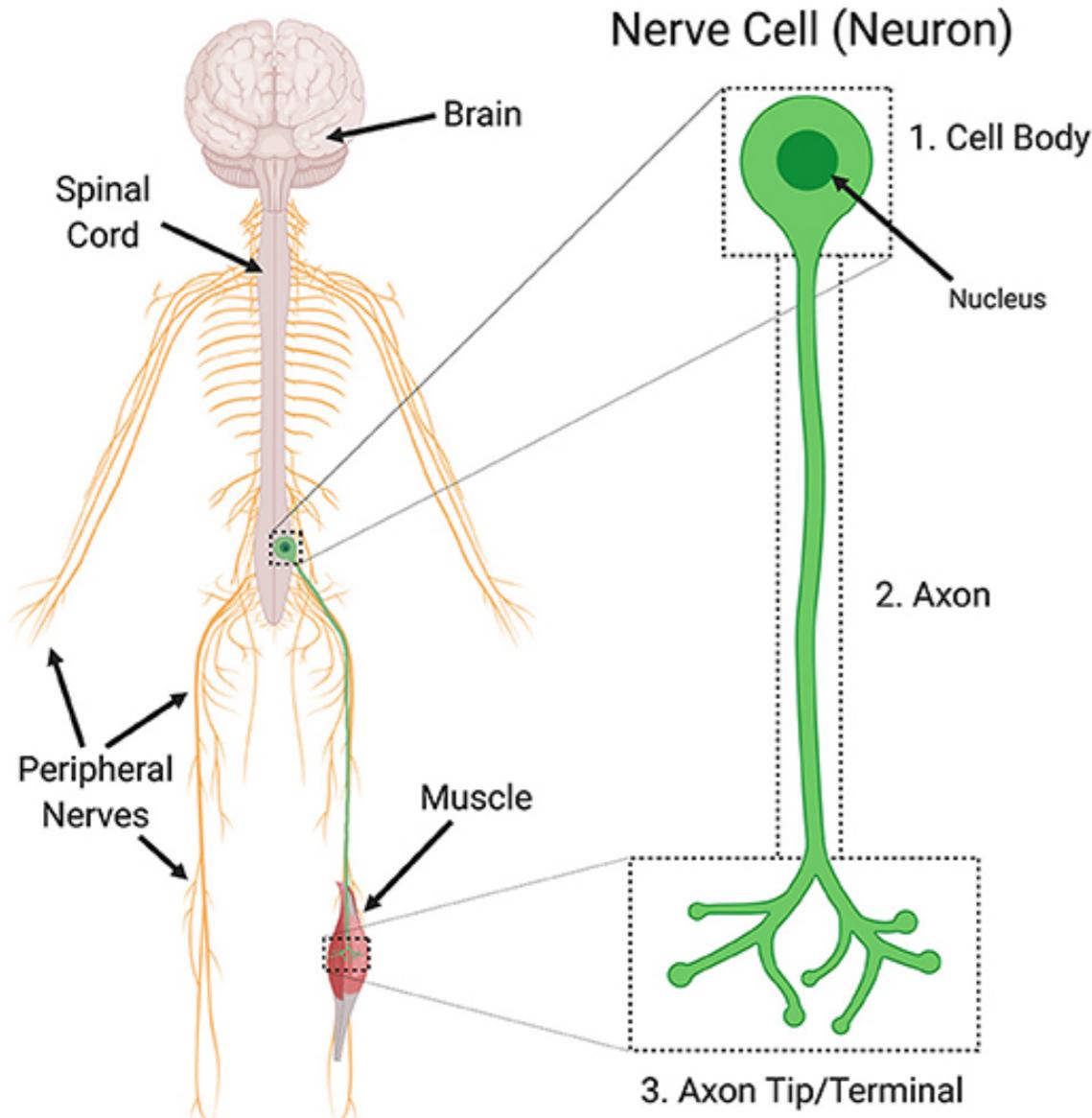
Energy for hydrolysing this many ATP $= N_{\text{ATP}} \times 20 k_B T \approx 100 \times 20 \times 4 \text{ pN nm} = 8 \times 10^{-18} \text{ J}$

Therefore, lot more energy is available for even faster cargo transport.
The limited speed is due to properties of kinesin itself.

Diverse trafficking by cytoskeletal motors in the cell



Axonal transport by cytoskeletal motors



Sleigh J (2020) Axonal Transport: The Delivery System Keeping Nerve Cells Alive. *Front. Young Minds.* 8:12.

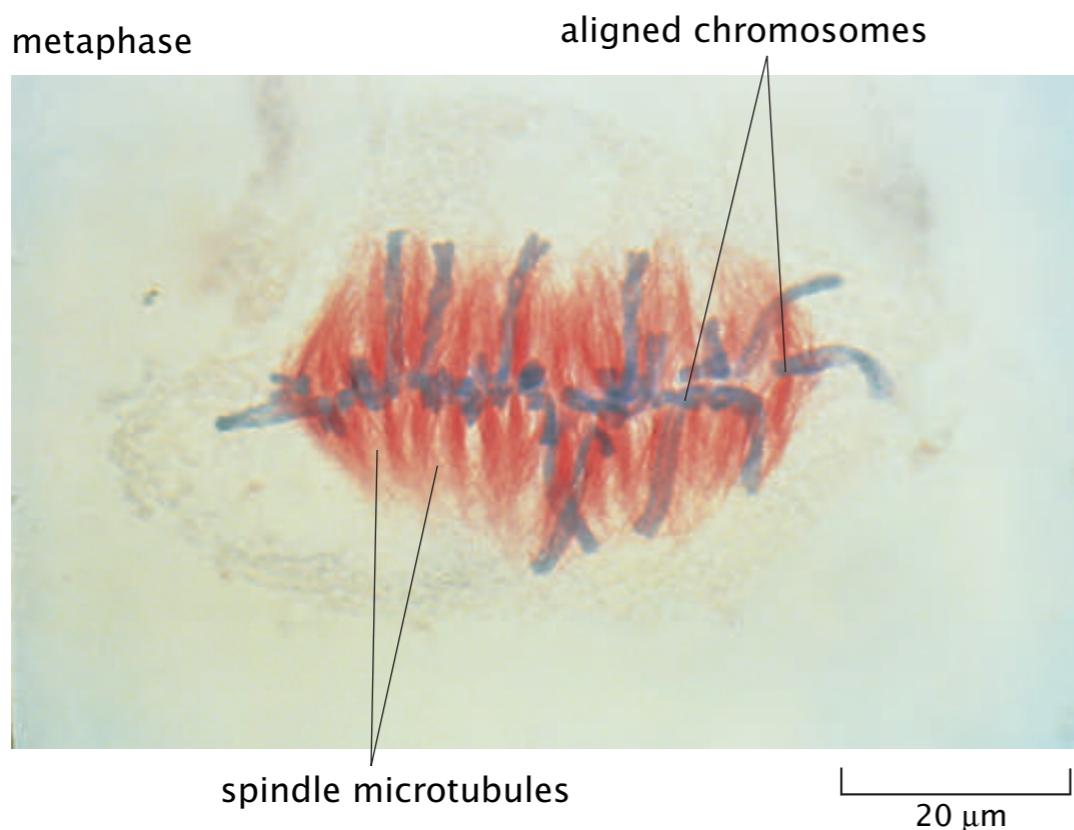
For giraffes the nerve cells can be several meters long - the axonal transport happens by motors @ 20-40 cm/day

Also note that the microtubules are polar with defined + and - ends. So, cytoskeletal motors are also directional.

Cytoskeletal motors orchestrate chromosome segregation

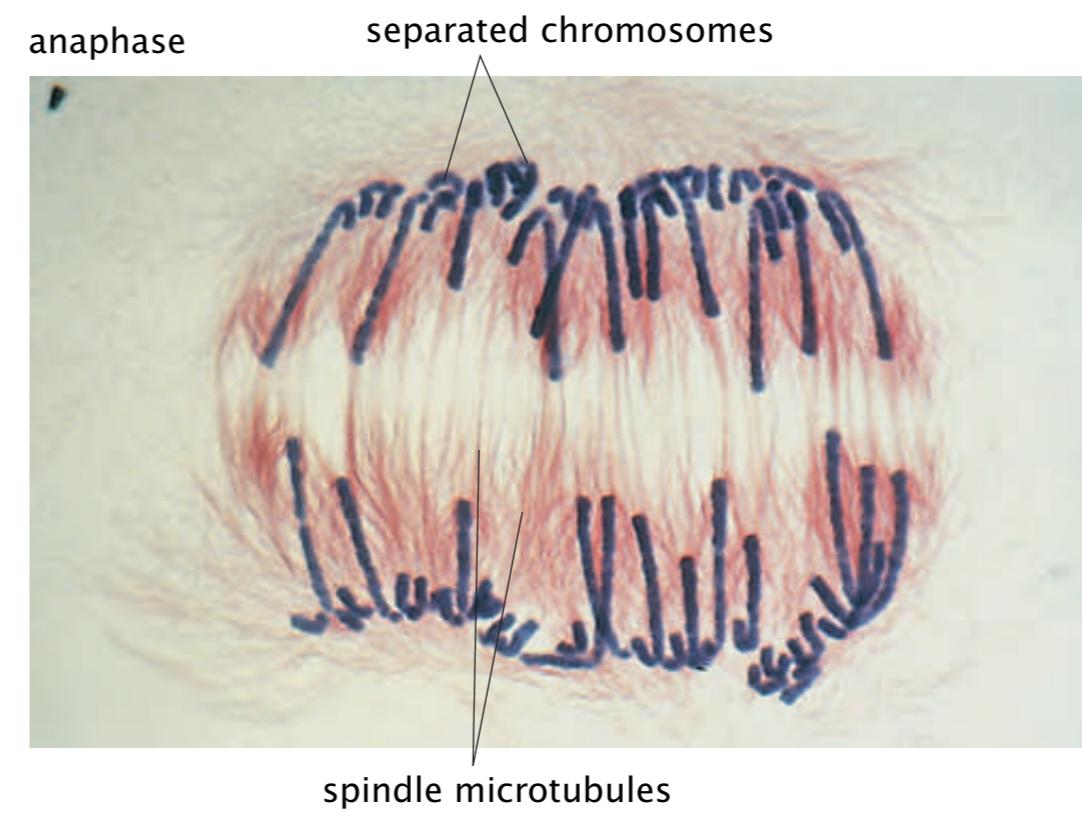
(A)

metaphase

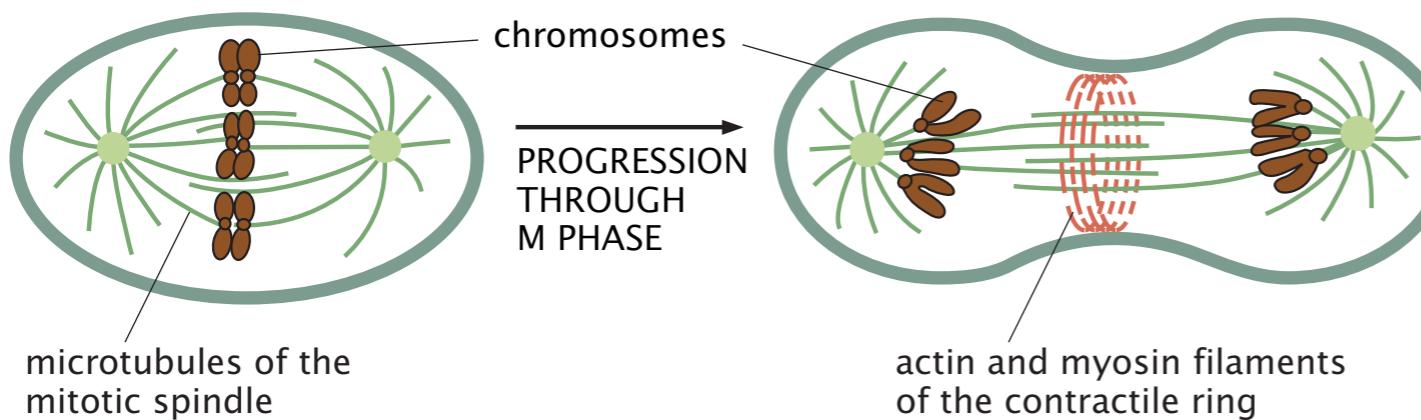


aligned chromosomes

anaphase

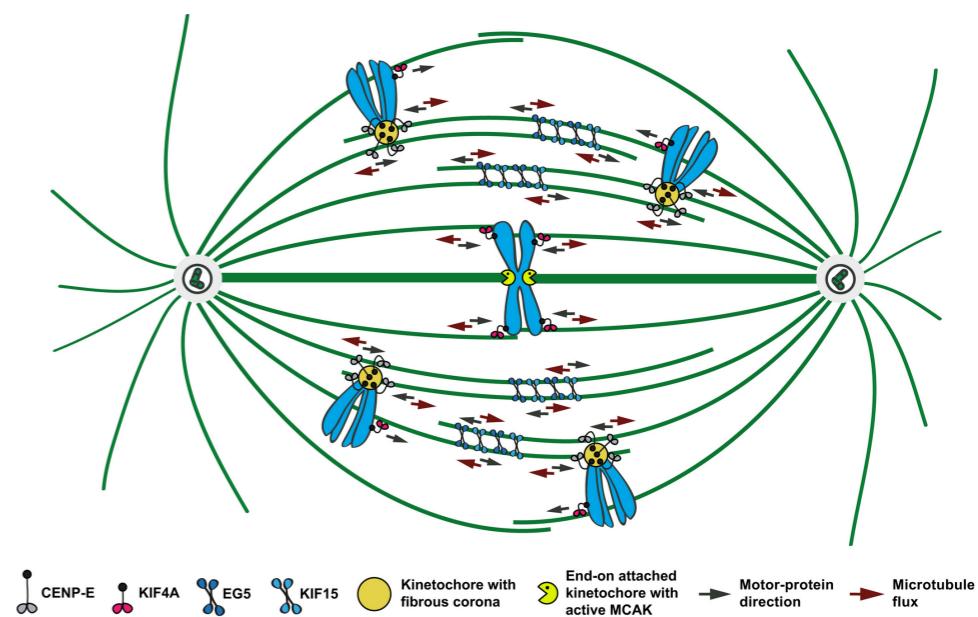


(B)



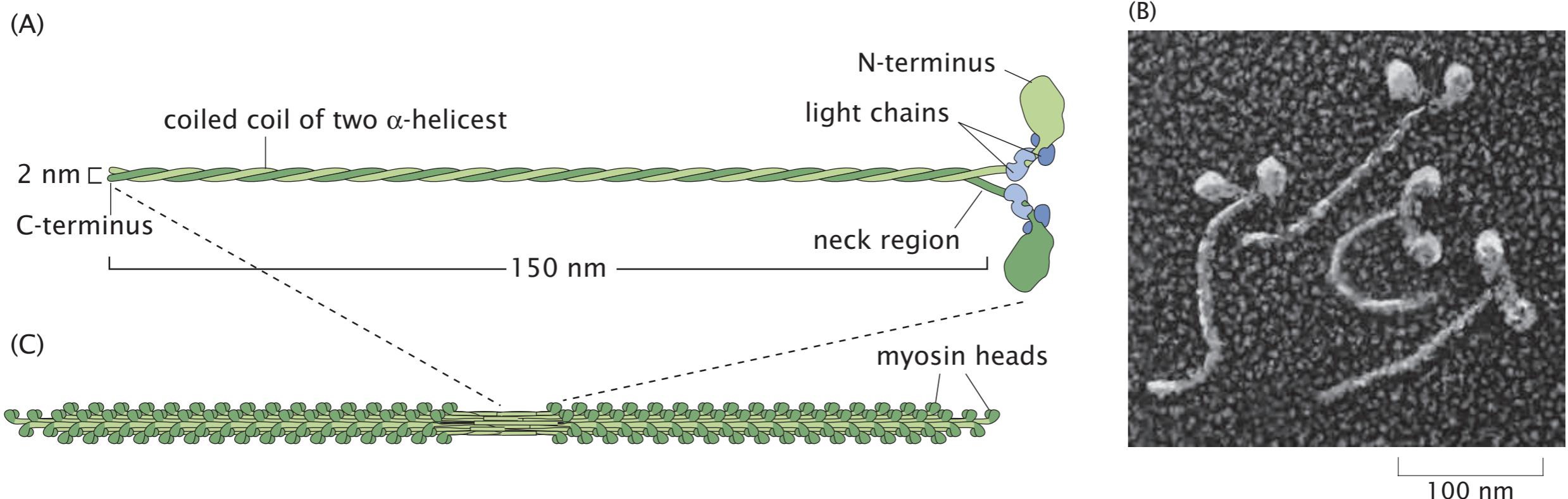
PROGRESSION THROUGH M PHASE

microtubules of the mitotic spindle



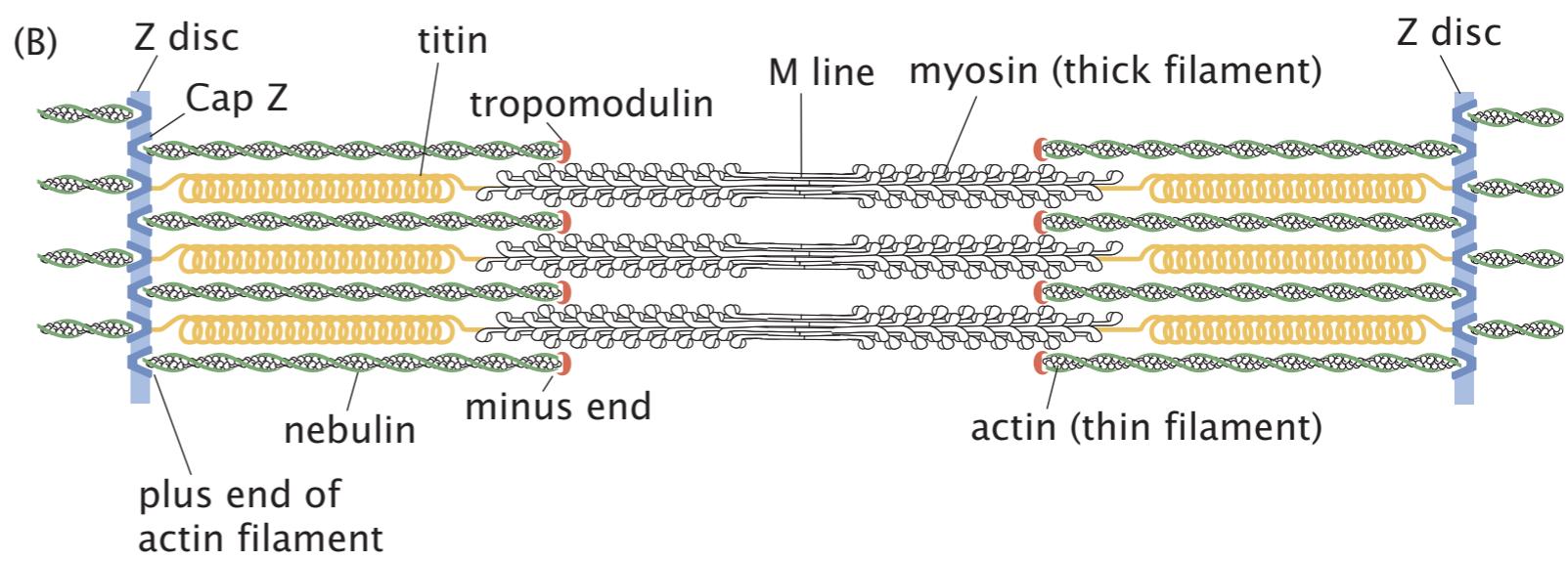
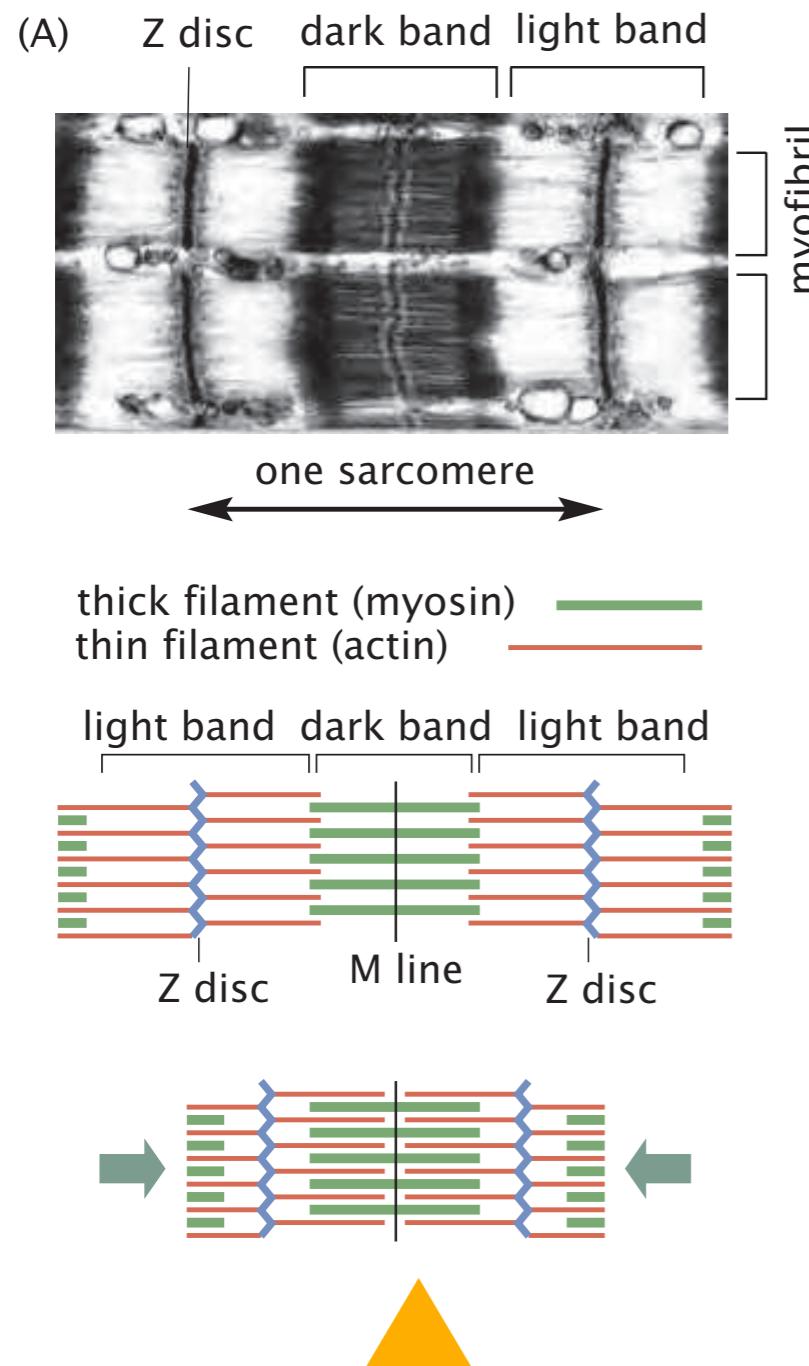
Dozen different kinesins and a dynein work together to drive the positioning and transport of chromosomes and microtubules

Muscle contraction is mediated by myosin motors



- (A) Myosin II from skeletal muscle is a hexamer consisting of two extremely large heavy chains and four much smaller light chains. The heavy chains include a long coiled-coil domain at the C-terminus and the actin-binding, force-generating motor head at the N-terminus.
- (B) Platinum replica imaging of individual myosin molecules reveals the beautiful regularity of their structure.
- (C) Several hundred individual myosin II hexamers can self-assemble to form a thick filament. In this cylindrical bundle, the myosin molecules in the left half are all pointing toward the left, and those in the right half are all pointing toward the right. This antiparallel orientation is critical for muscle contraction.

Muscle contraction is mediated by myosin motors



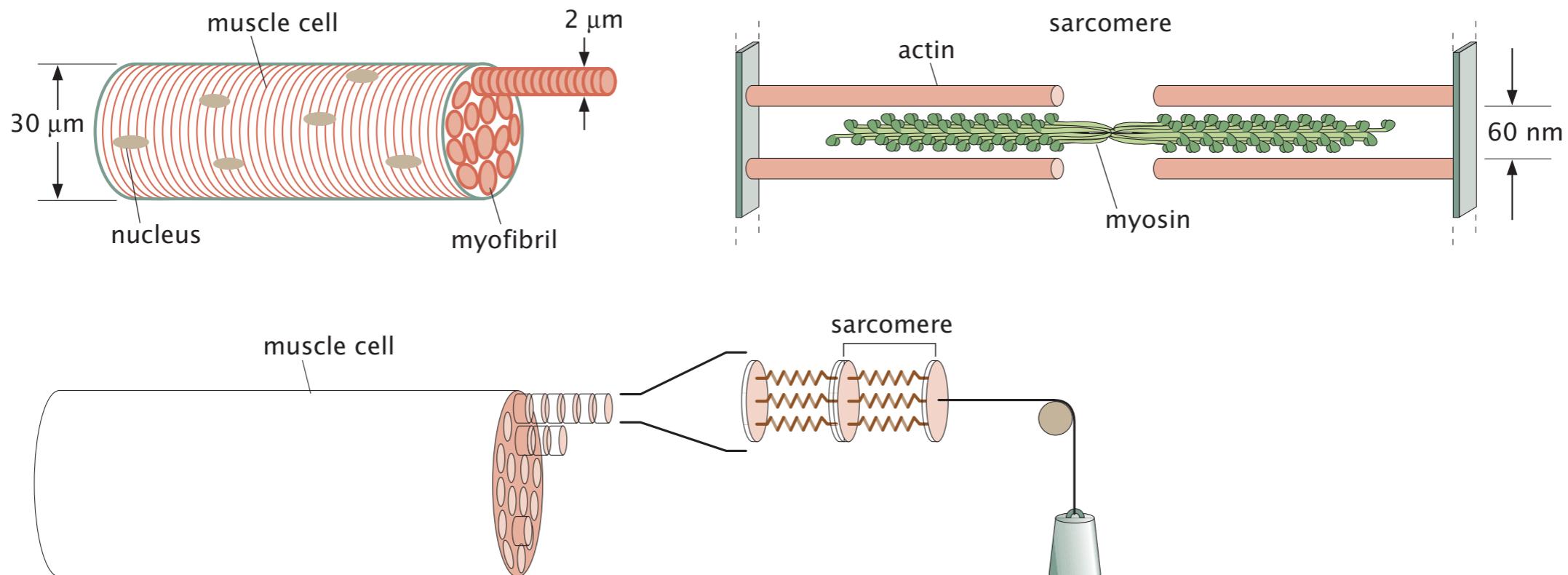
Z-discs provide anchor points for actin filaments so that all + ends point away from the myosin thick filaments in relaxed state

Contraction of a single sarcomere

Myosin forces in a muscle

How do we get the force exerted by a single myosin in the muscle which has arrays of myosins?

We consider an idealized representation of the muscle



Myosin forces in a muscle

$$N_{\text{myosin}} \approx \frac{\text{cross-sectional area of muscle}}{\text{cross-sectional area of thick filament}} \times N_{\text{myosin/thick filament}}$$

Typical cross section of muscles = 10-100 cm² and no. of myosin per thick filament is ~ 300

$$N_{\text{myosin}} \approx \frac{50 \text{ cm}^2}{\pi \times 60^2 \text{ nm}^2} \times 300 \approx 10^{14}$$

Let's assume the muscle is lifting 10 kg mass

So, force needed is $F = Mg = 10 \text{ kg} \times 10 \text{ m s}^{-2}$

If this force is equally partitioned among all the myosin in the muscle

$$F_{\text{myosin}} = \frac{Mg}{N_{\text{myosin}}} = \frac{100 \text{ N}}{10^{14}} = 1 \text{ pN}$$

This is same order of magnitude to
the value measured *in vitro*