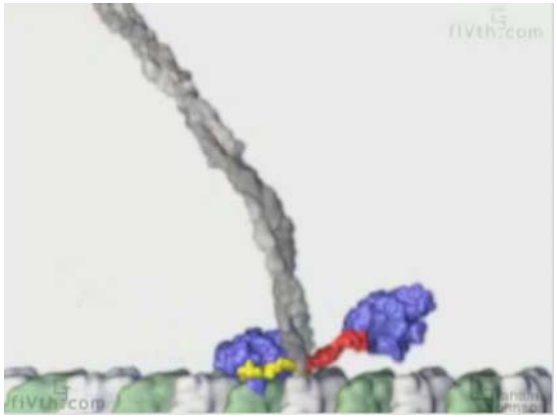
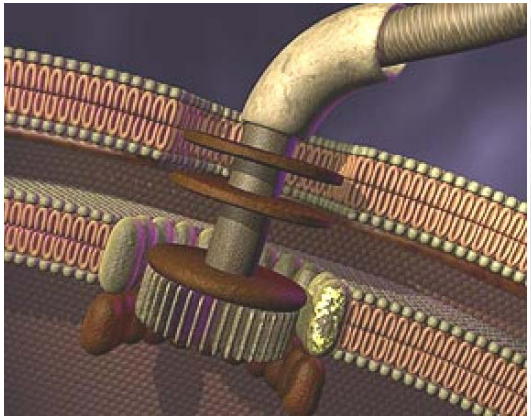
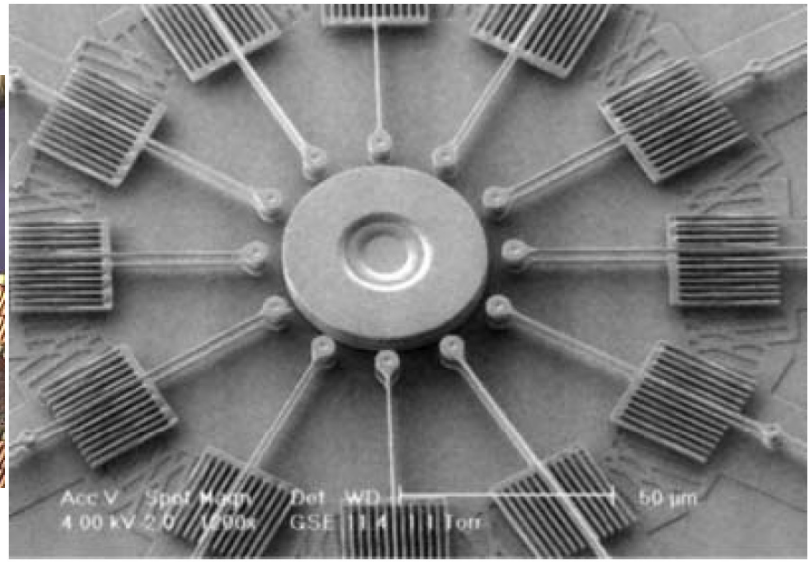
Biological Machines, Cell Mechanics and Nanotechnology







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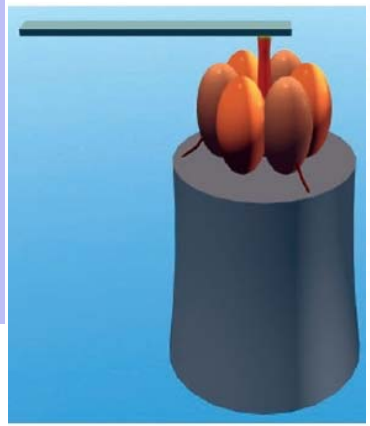
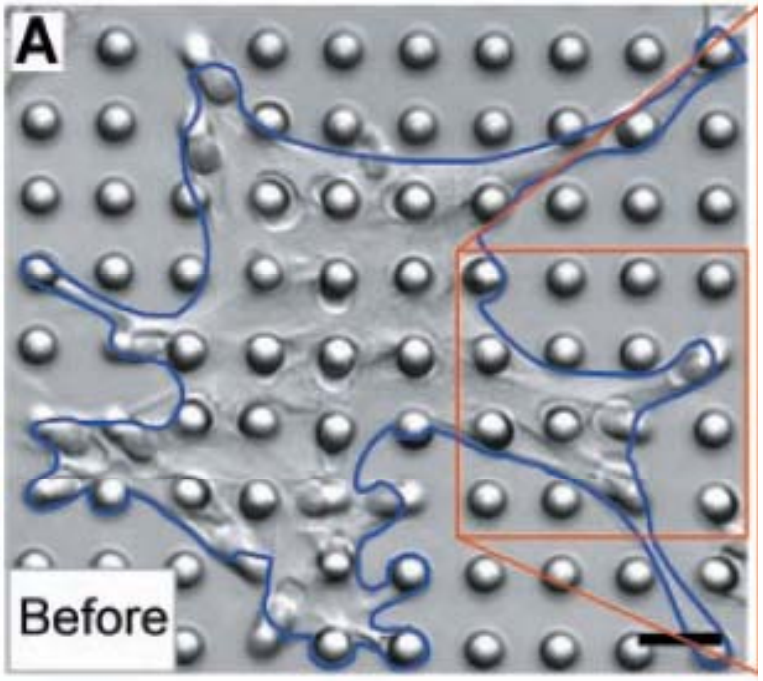
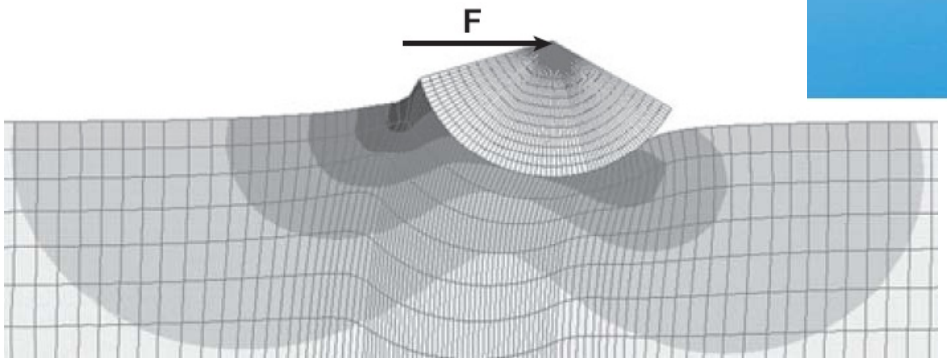
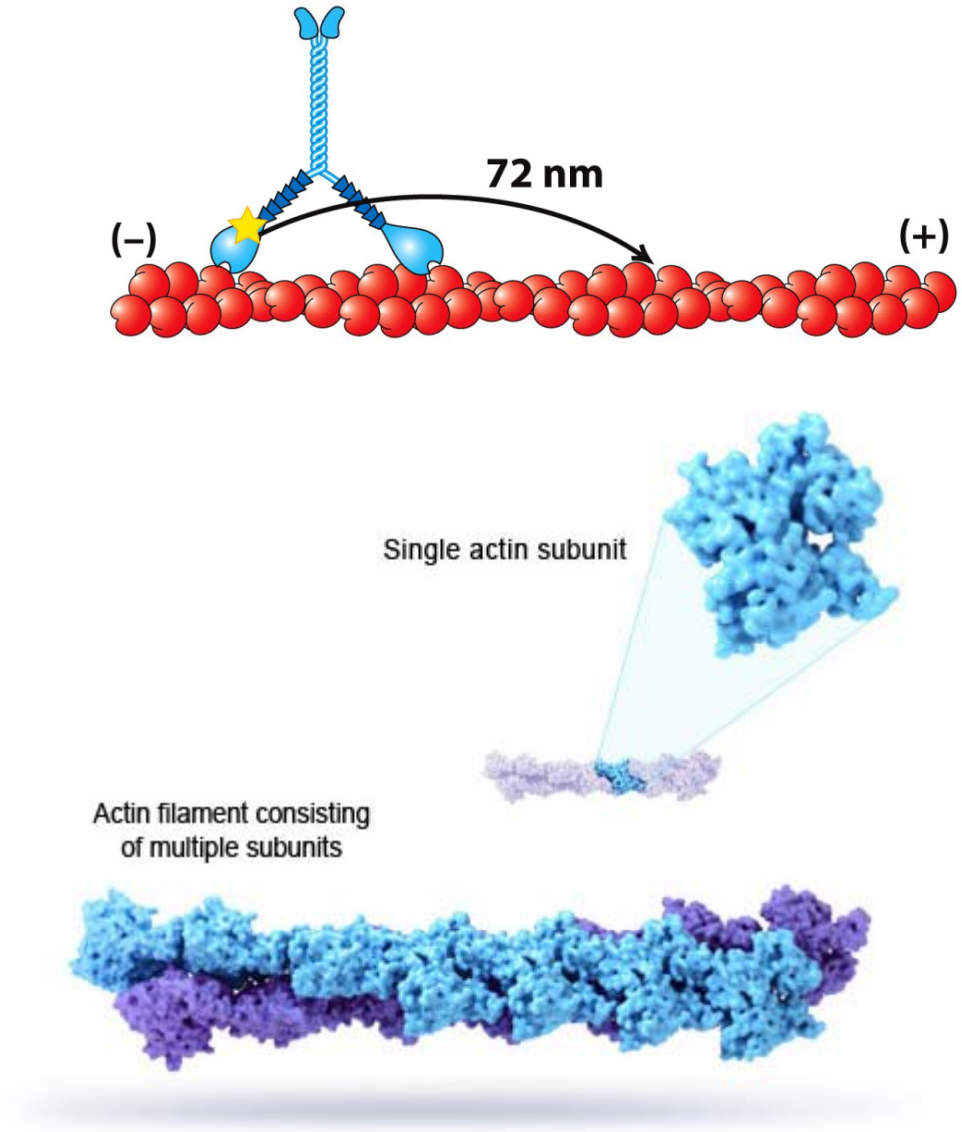




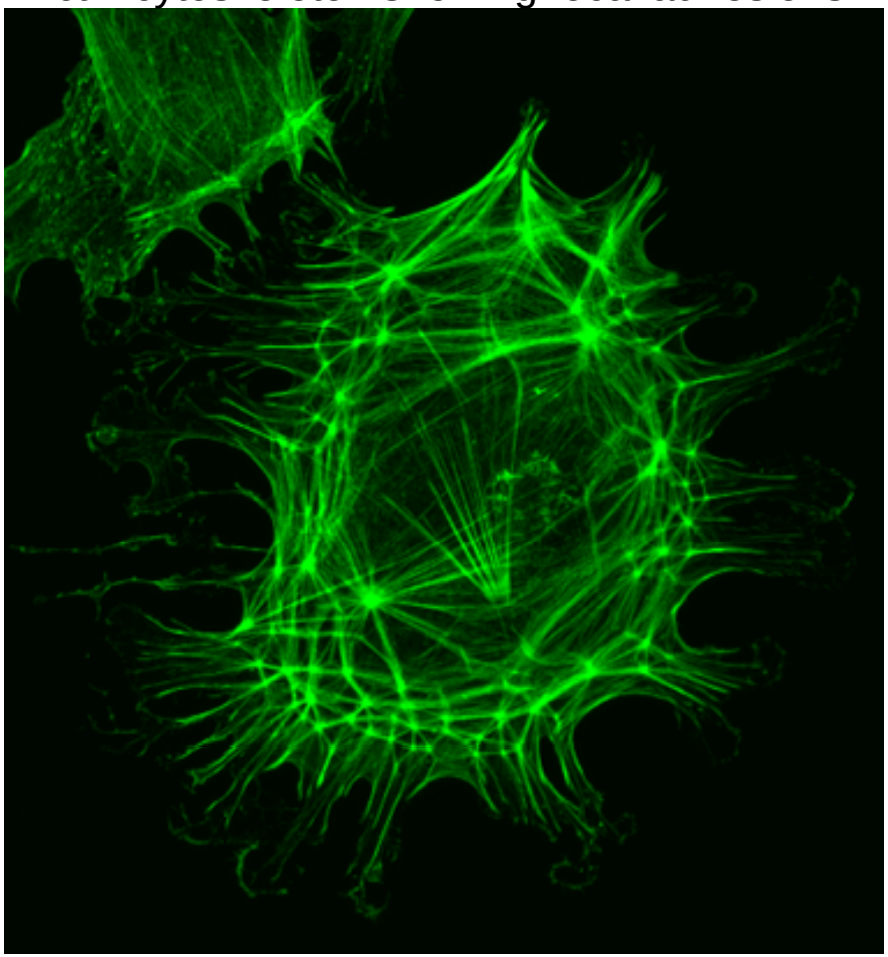


Actin-based motors

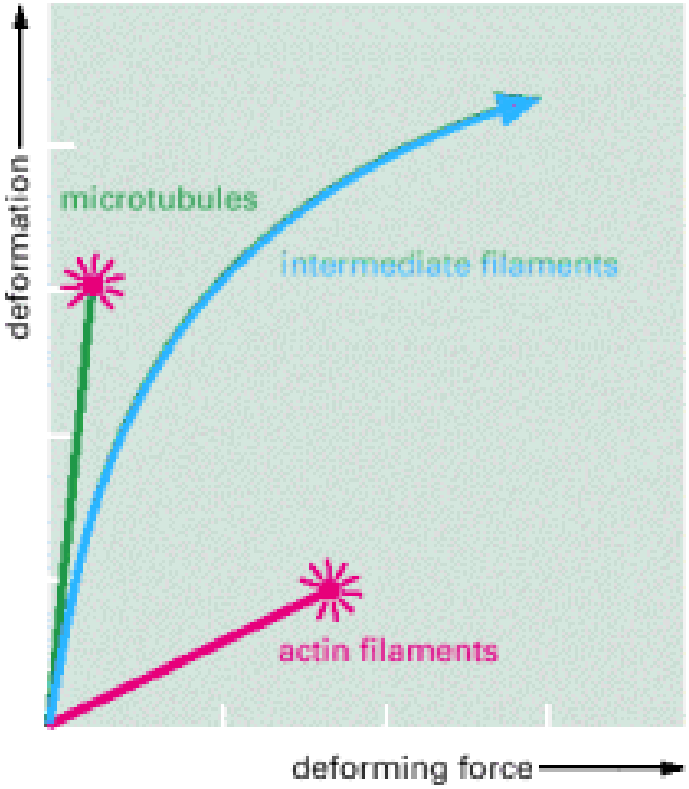
Besides stabilizing cells and fixing them to the substrate, actin acts as a **highway** for molecular motors of type **myosins**.



Actin cytoskeleton showing focal adhesions

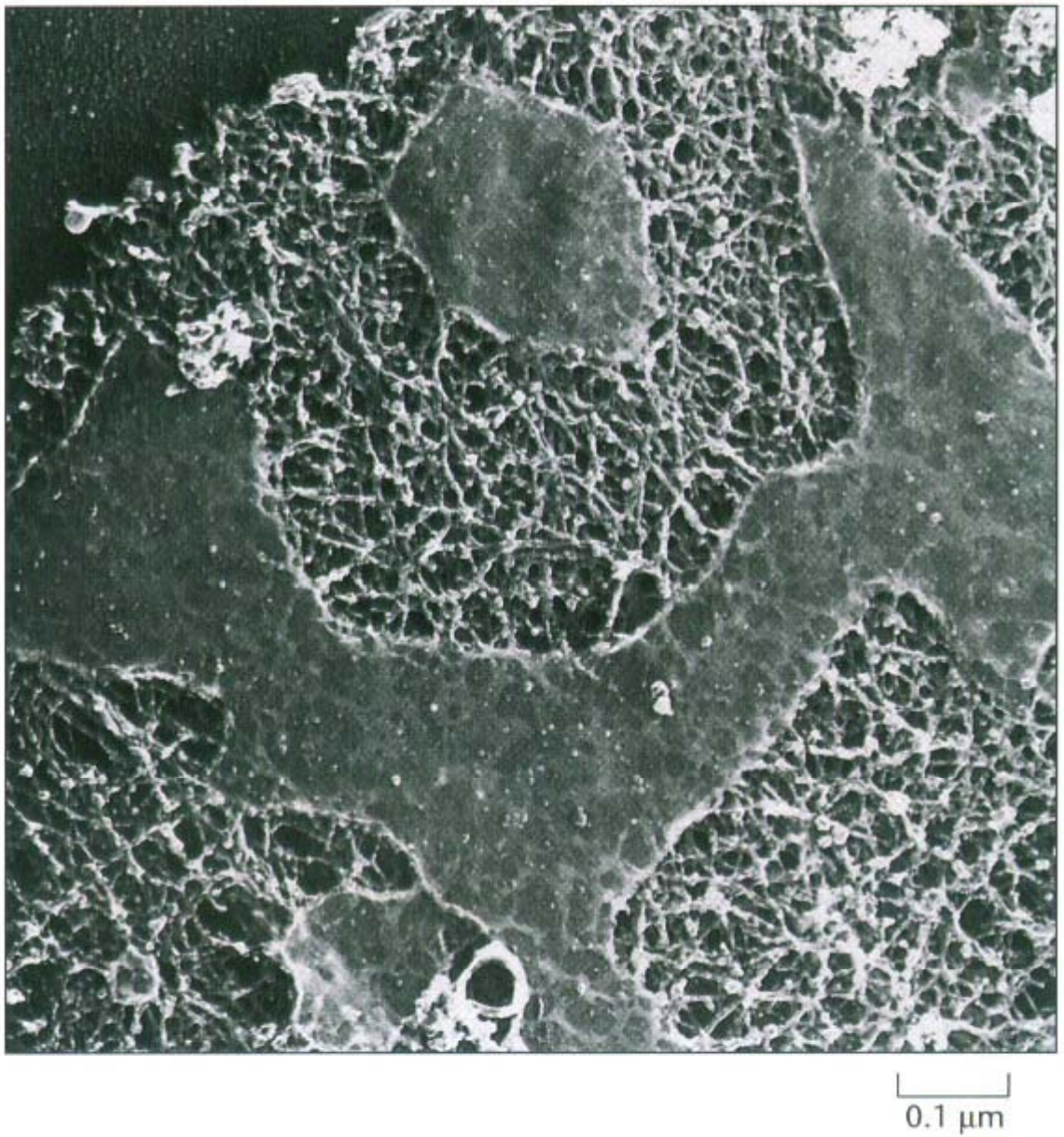


Actin filaments are more resistant to deformation than MTs



P. Janmey, *JCB*, 1991

Cell cortex: gel-like and highly elastic

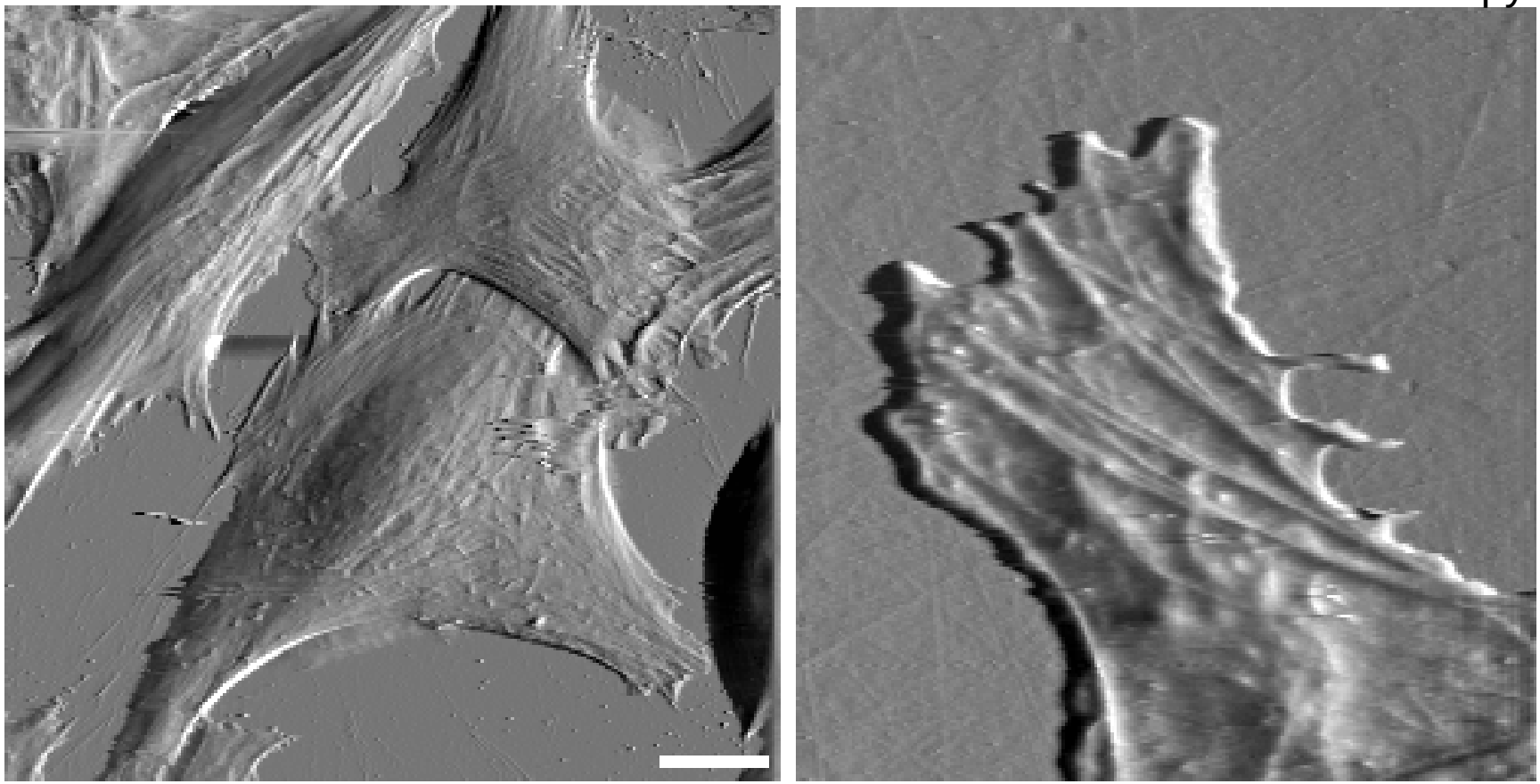


Fibroblast treated with **mild detergent** to partial remove plasma membrane

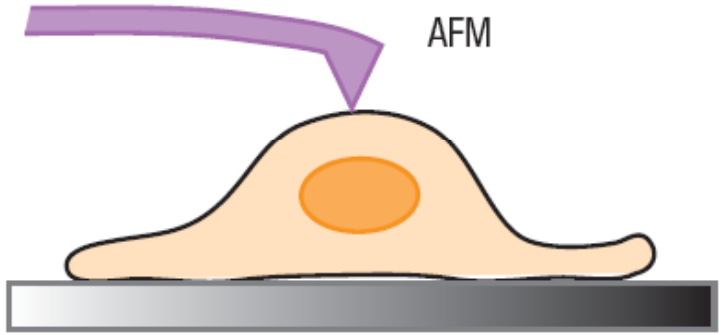
Fast-freeze, deep-etch electron microscopy reveals actin-network linked by α-actinin and filamin

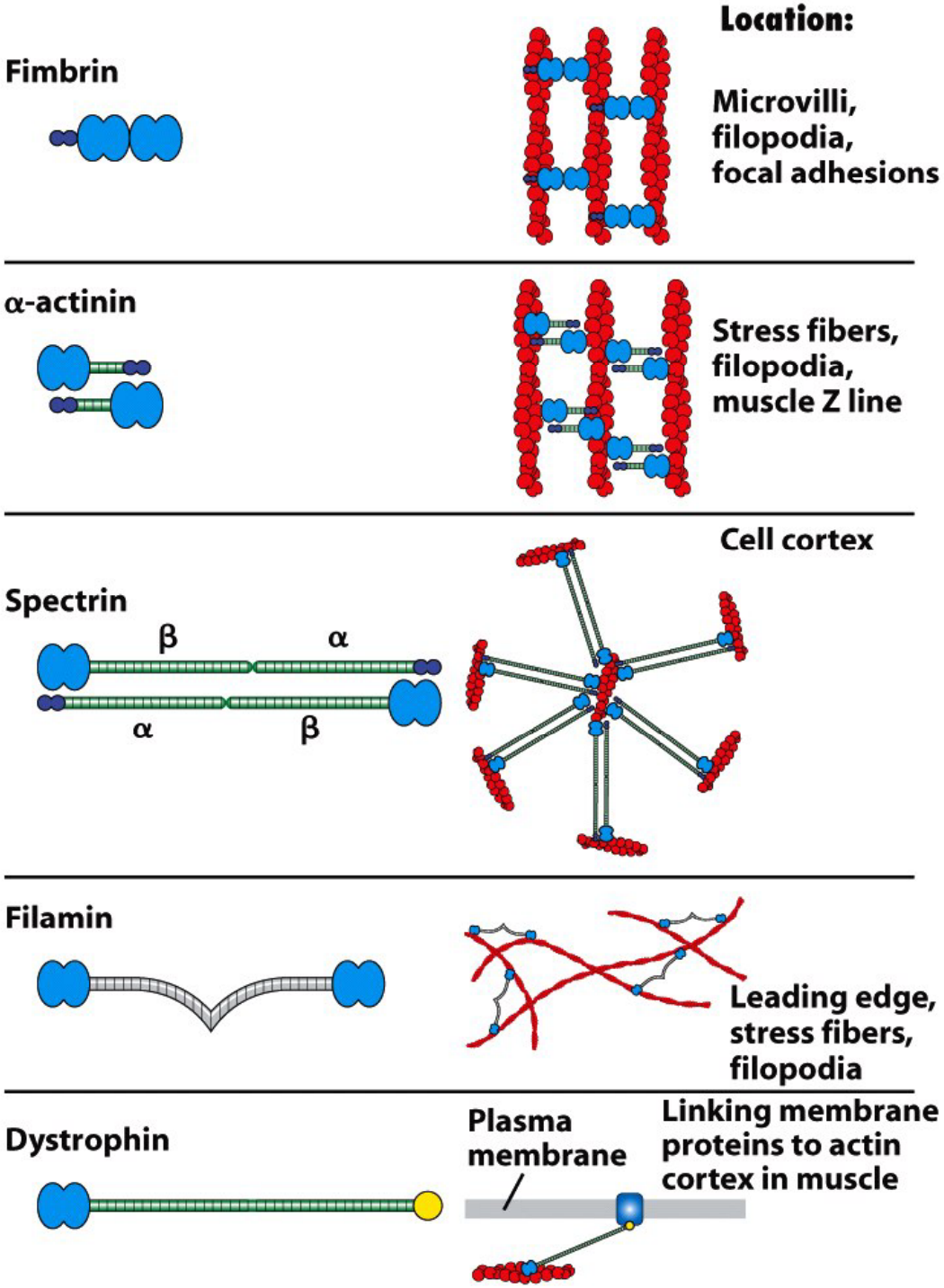
Elastic actin-bundles in fibroblasts made visible by AFM

Atomic Force Microscopy



Parak et al., *Biophys. J.*, 1999

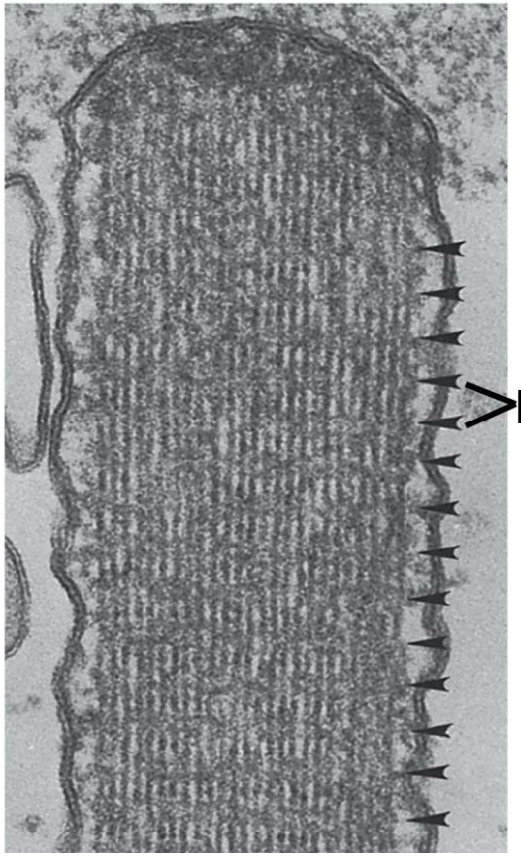




Understanding Cell Mechanics

Actin cross-linking proteins can force the actin cytoskeleton into diverse configurations with **different mechanical (viscoelastic) properties**
For example: they can bundle actin (e.g., microvilli) or form a network of actin (e.g., cell-cortex or leading edge)

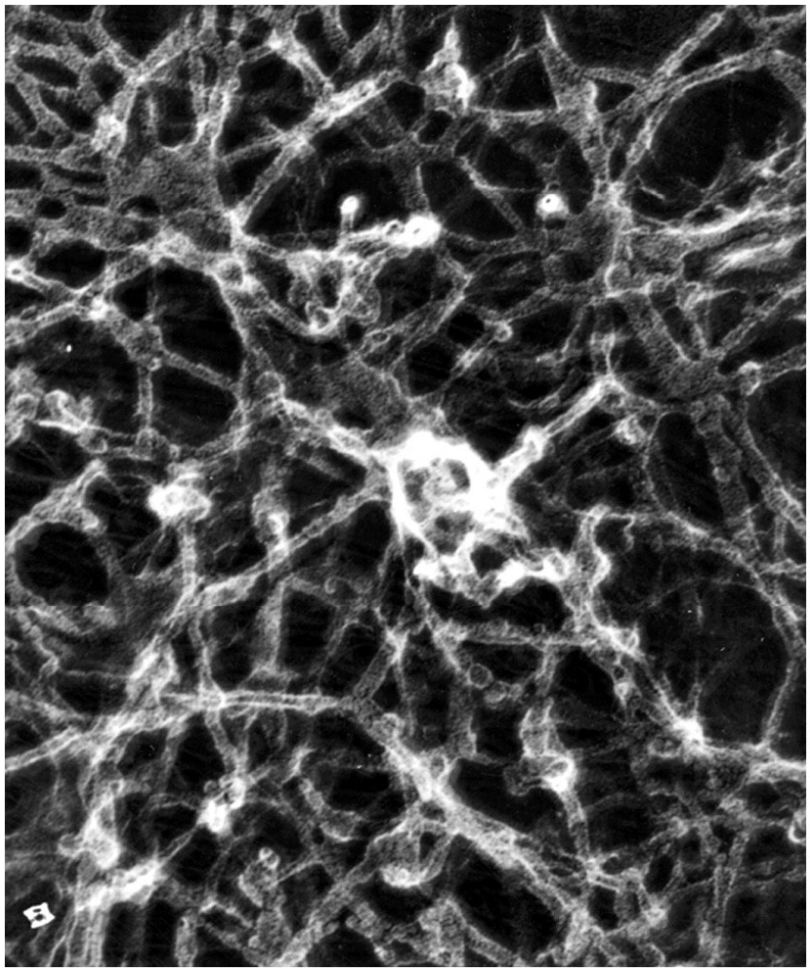
Fimbrin: **actin-binding sites** are very close to each other: F-Actin tightly **bundled**



Microvilli

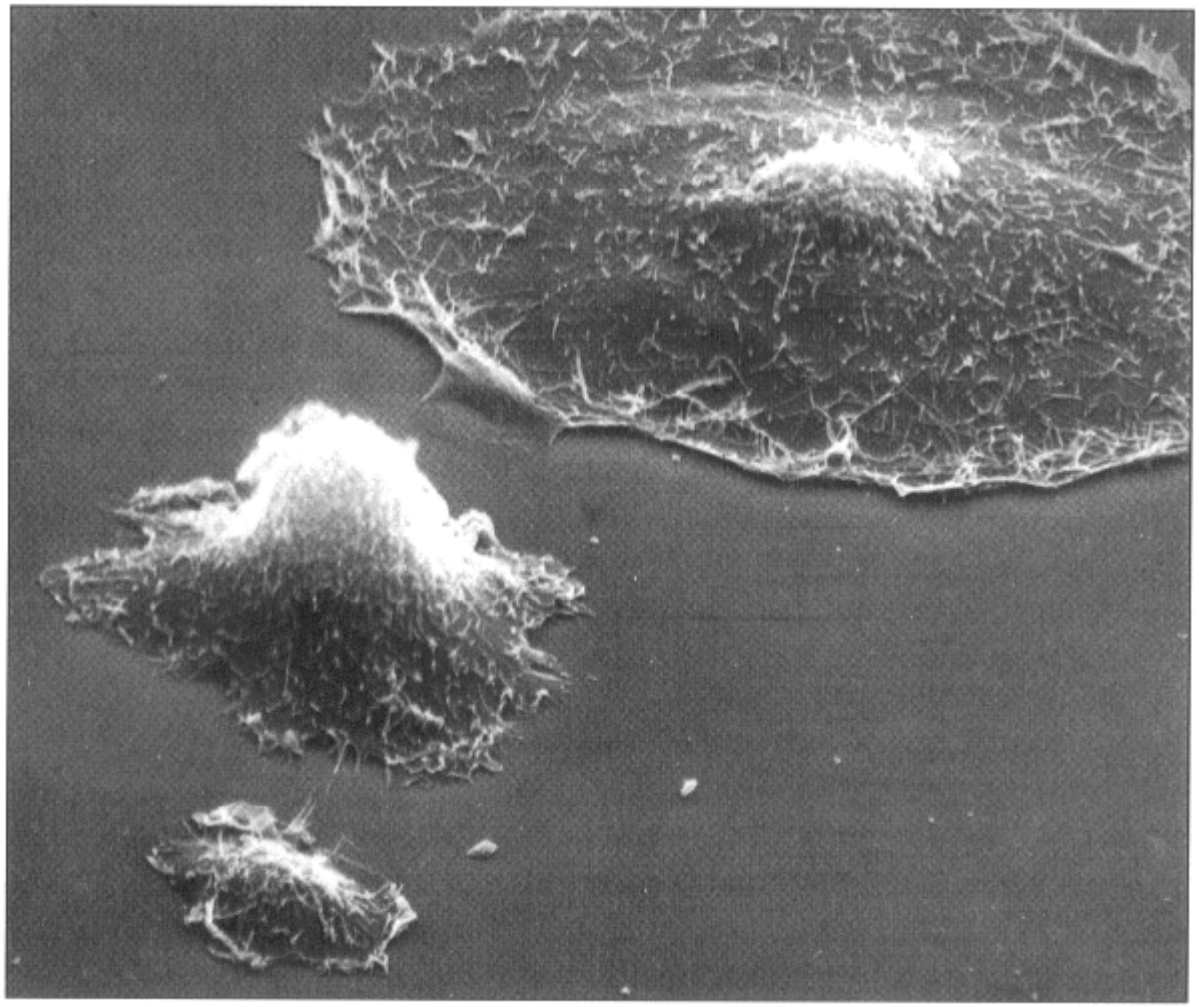
Filamin: **actin-binding sites** are largely spaced apart: F-actin more fluffy in a **network**

Filamin formed actin-networks contribute largely to the **gel-like** character of the **cytoplasm**



fast-acting gel/sol transition to power cell movement

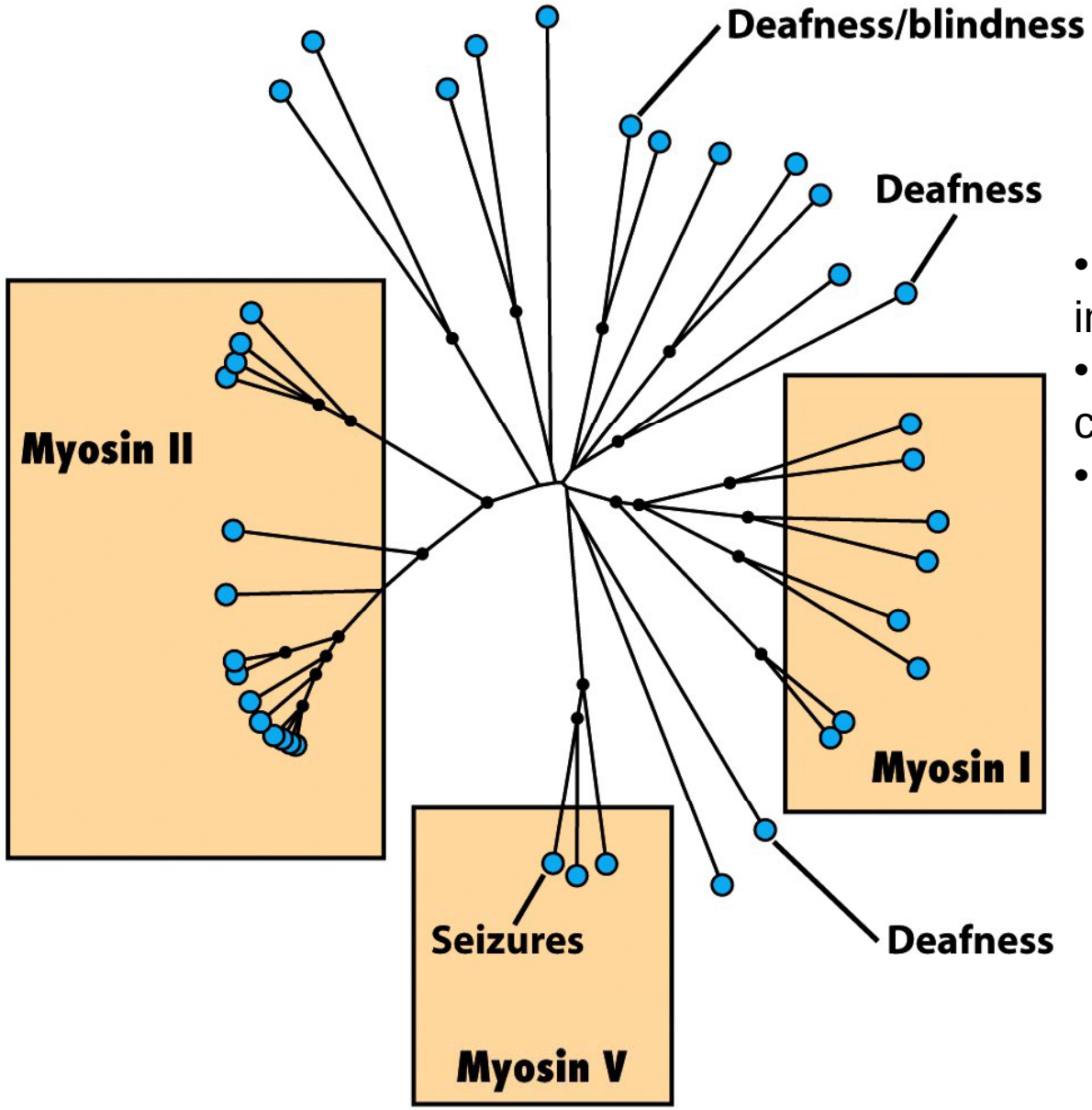
Is the cell-cytoskeleton an autonomous organ?



- Large cell fragments can be achieved using cytochalasin and trypsin treatment
- These fragments remain intact and **can form lamellipodia** and **filopodia** and migrate over surfaces for many hours

Bray, Cell Movements, 2nd Ed.

Myosins make up a large family of actin-based motor proteins

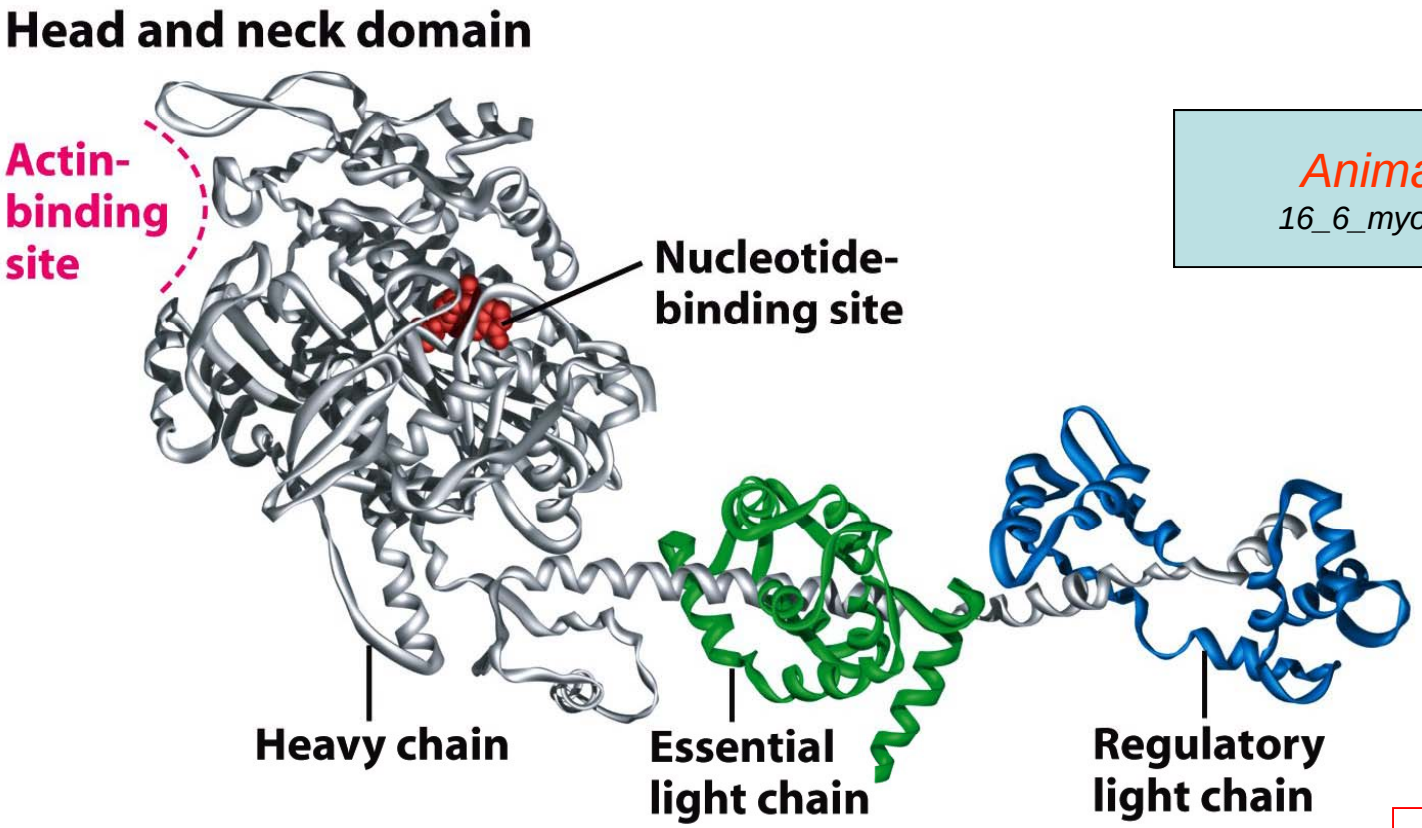


- **40 myosin genes** found in the human genome
- Loss of specific myosins can cause a disease
- 3 important classes:
  - Myosin I (**endocytosis**)
  - Myosin II (**muscle contract.**)
  - Myosin V (**cargo transport**)

Myosins walk on actin filaments

Myosins consists of **several light** and **heavy chains**
⇒ **light chains** have regulatory function
⇒ **heavy chains** form the motor head (**actin-binding** and **ATP-binding**),
a flexible neck and a tail domain (with “cargo-binding” function)

Tail consists of α**-helical coiled-coils** that **form** (the rod-like) myosin **dimer**

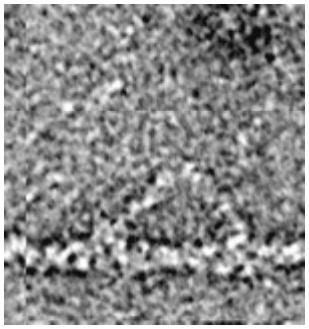


Animation
16_6_myosin.mov

Myosin monomer

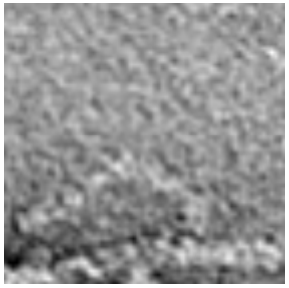
Mechanical cycle of myosin V

EM imaging and TIRFM (total interference reflection microscopy) revealed the stepping mechanisms of mysin V



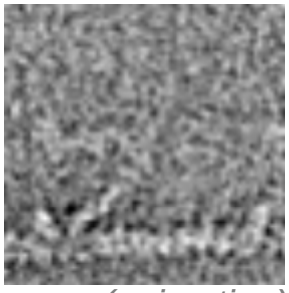
(animation)

Myosin-V **head and neck** at low [ATP] on F-actin visualized by EM



(animation)

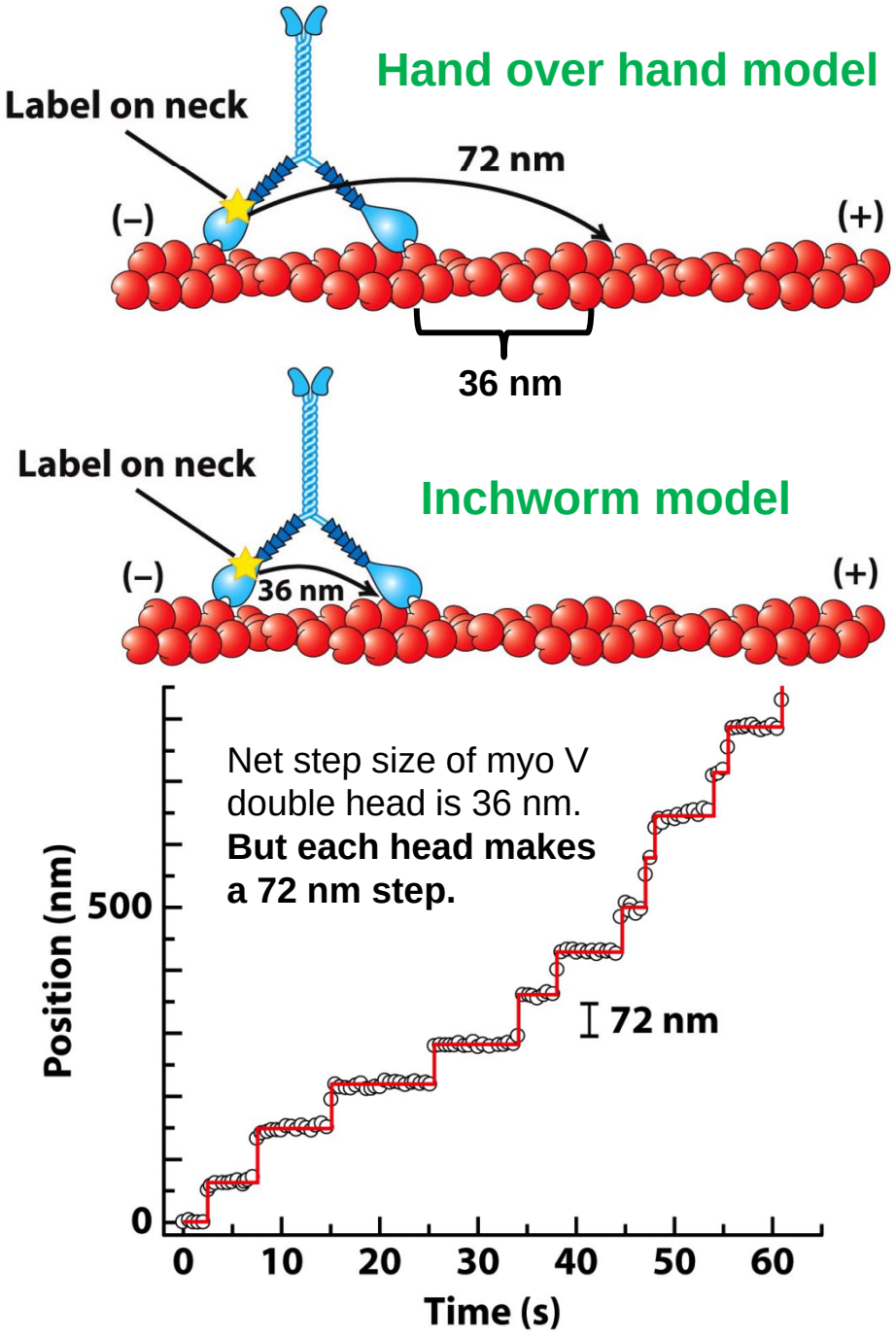
Pre-power stroke attachment



(animation)

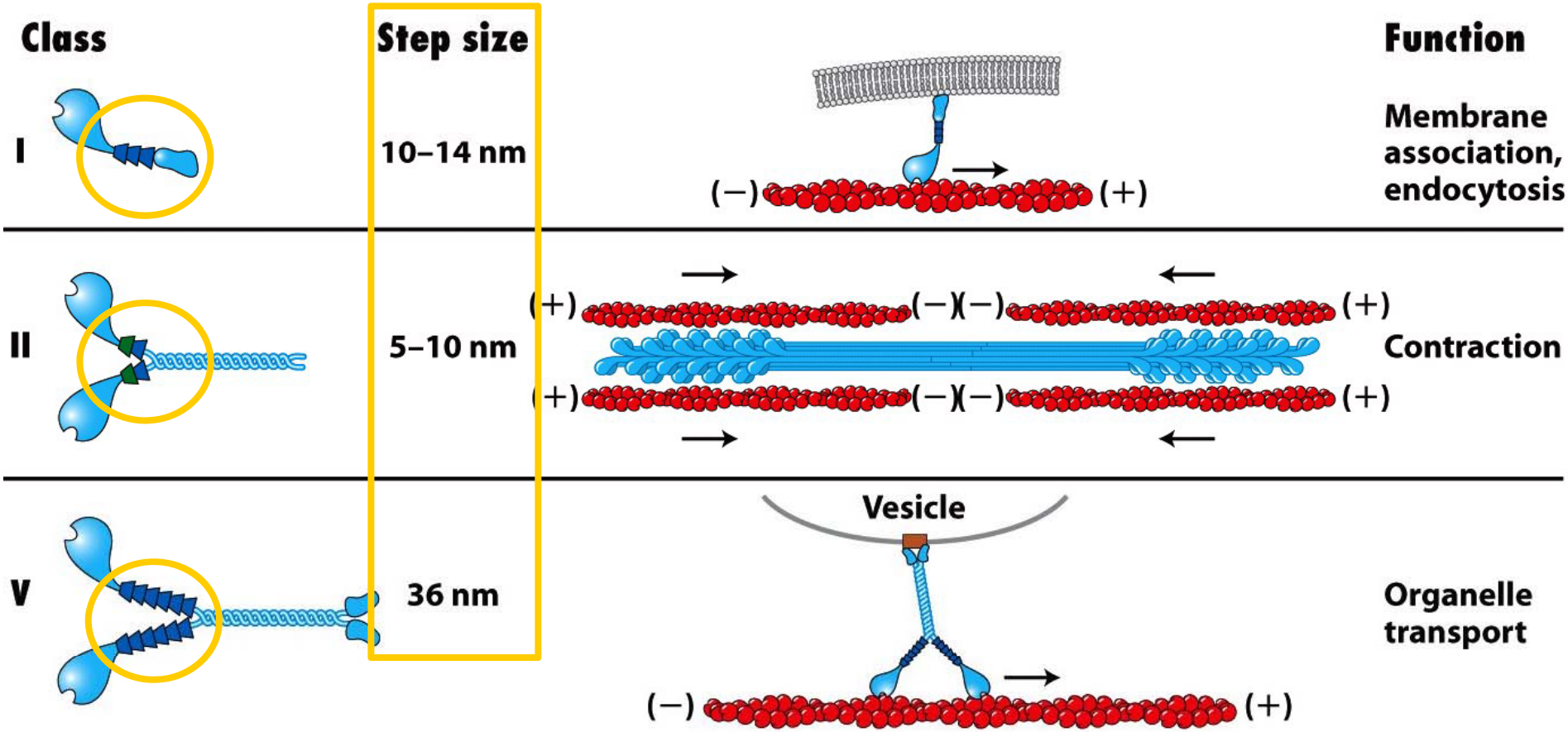
Post-power stroke attachment

Walker et al., Nature, 2000



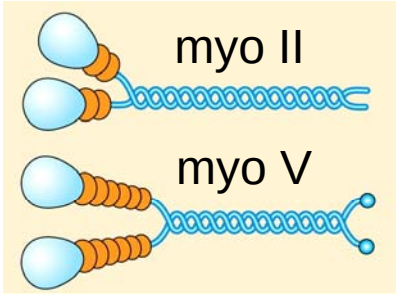
Cellular and intracellular movements depend on myosins

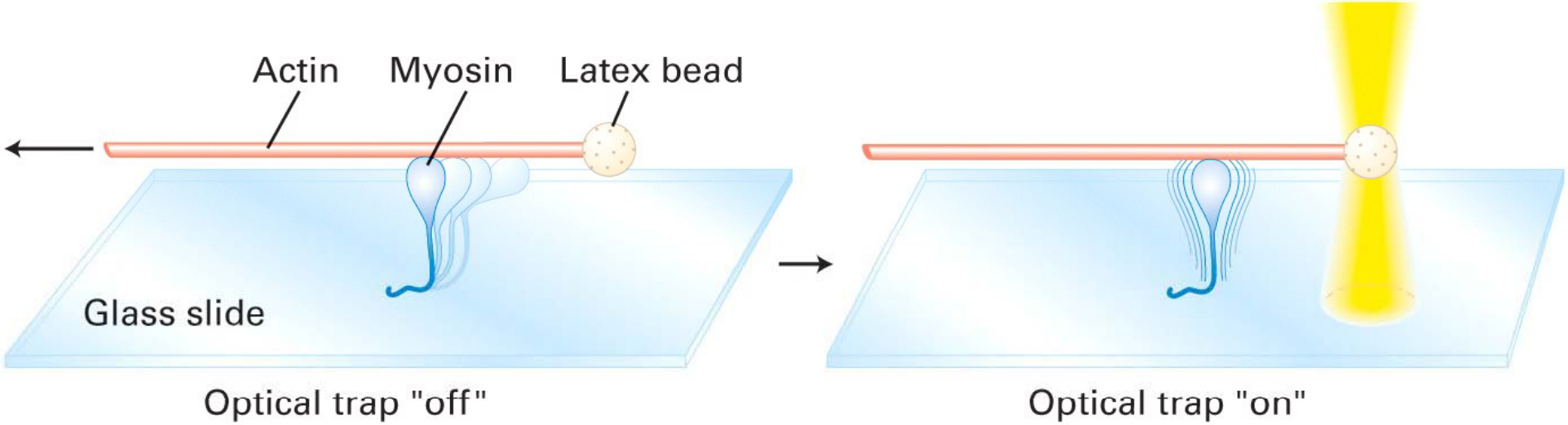
- **Muscle cells**: <u>contraction</u> based on filament sliding between F-actin and **myosin II**
- **Vesicle transport**: **myosin V**
- **Endocytosis (membrane invagination)**: monomeric **myosin I**



Measuring the force generated by single myosin heads using the **optical trap**

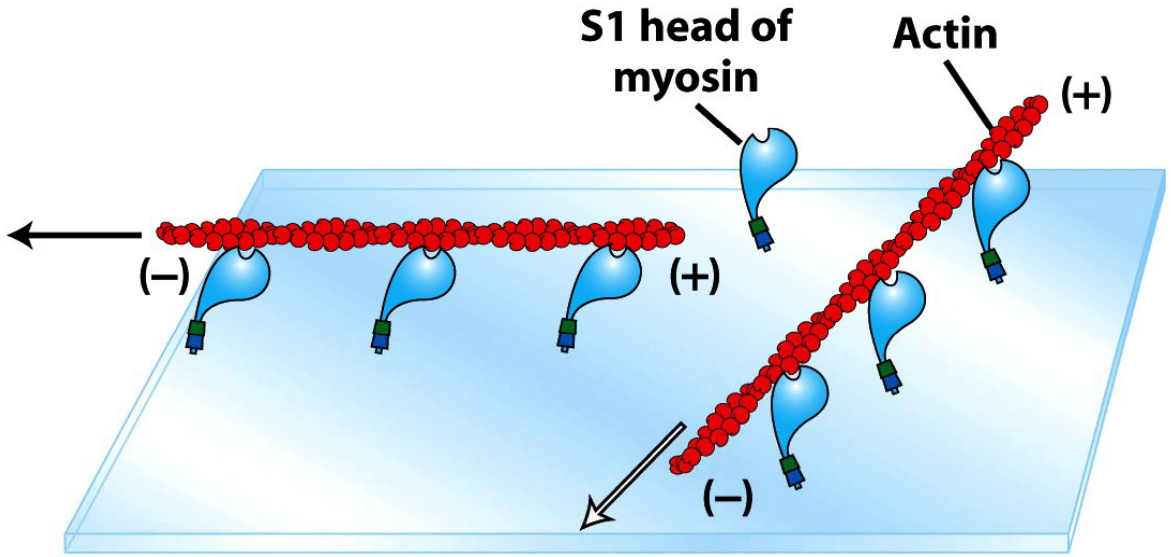
- An infrared beam is used to immobilize a bead attached to an **actin filament**
- During the myosin's **power stroke** the actin filament is hold in position
- The force generated by the myosin head is determined by measuring the bead displacement => for myosin II about **3-5 pN** (piconewton)
- The **step-size** and force depends on the length of the **lever arm**:
  - myosin II = **5-10 nm**
  - myosin V = **36 nm**





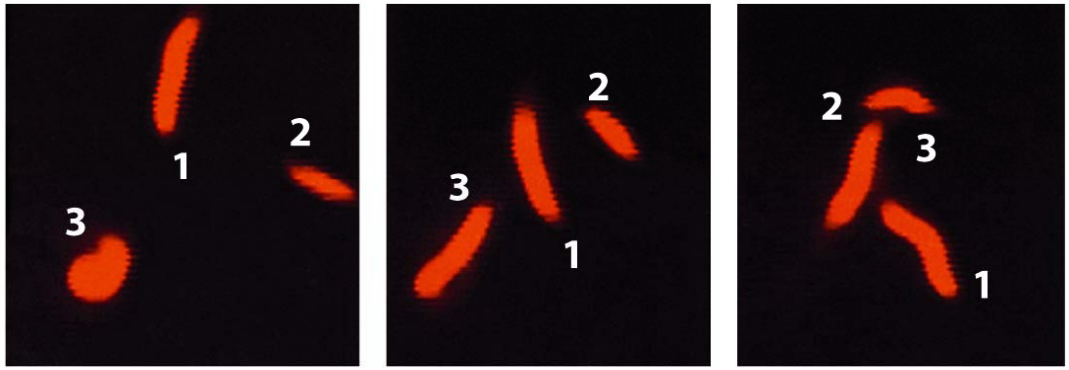
In vitro motility assay to determine forces and motor steps in the nano-range

- Movement of fluorescently-labeled **actin filaments** on immobilized **myosin heads** on a cover glass is observed using a fluorescence microscope
- Addition of ATP triggers the **movement of actin filaments** along the fixed myosins
- Upon ATP-binding, myosin heads dissociate from the filament while the head tilts towards the (+) end (no power-stroke yet)
- The **upcoming power-stroke** pushes the filament with the (-) end in the lead



Animation 1
a19-03-in_vitro_motility.swf

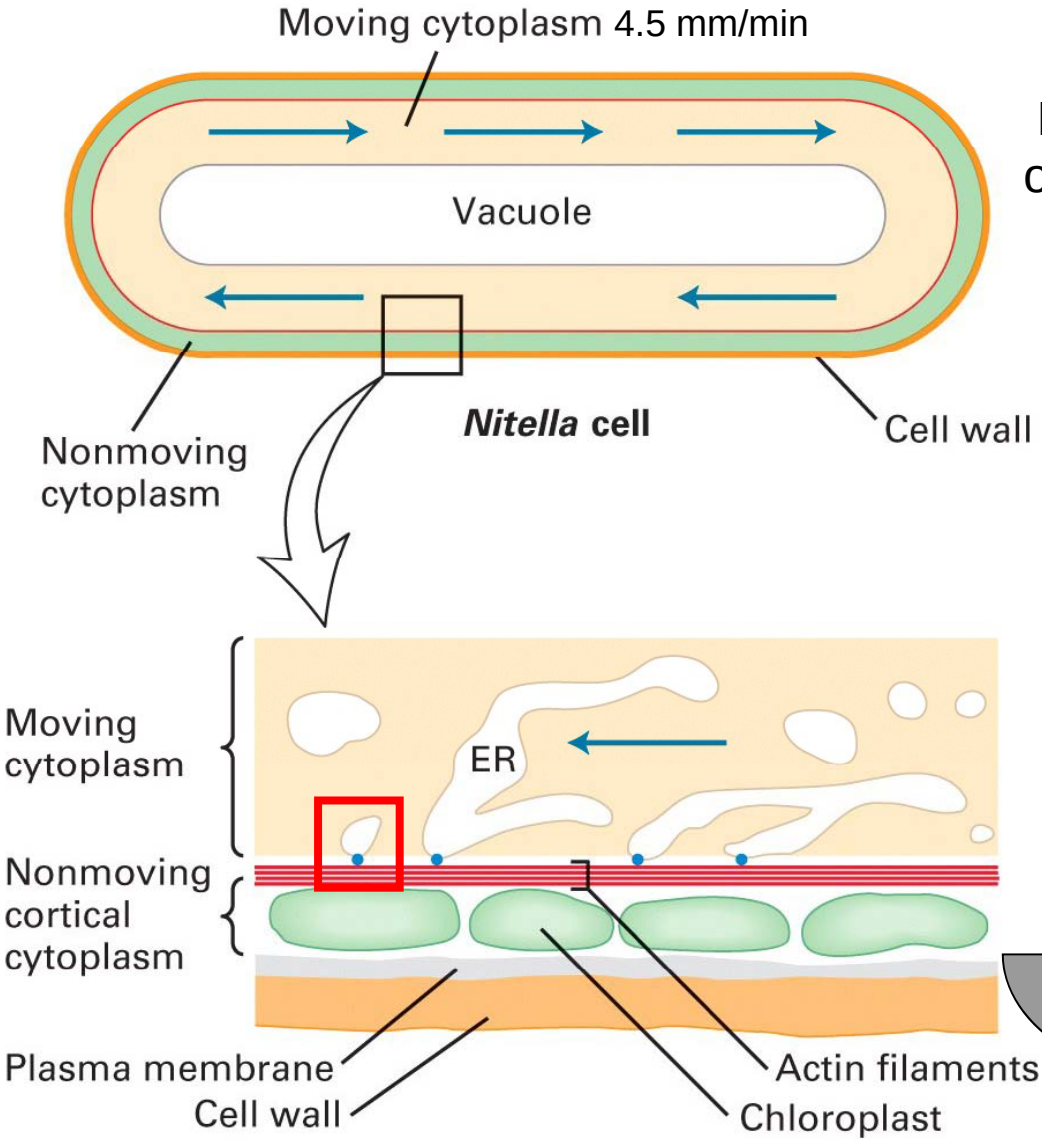
Animation 2
16_8_actin myosin in vitro assay.mov



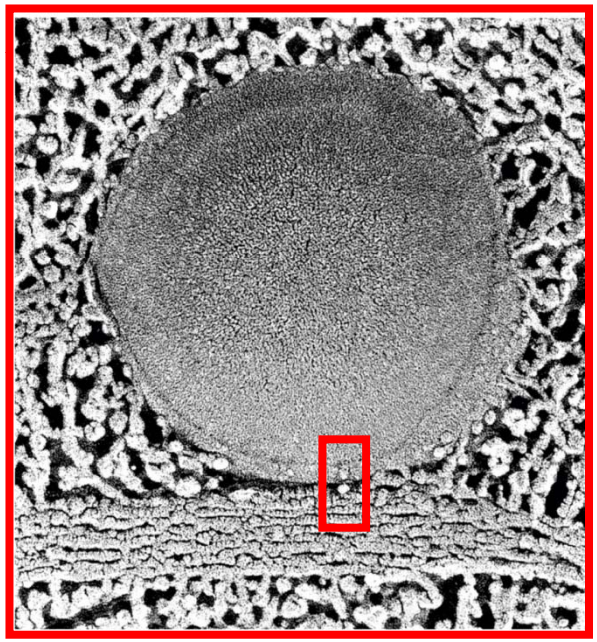
Rhodamine-phalloidin labeled actin filaments. One frame each 30s.

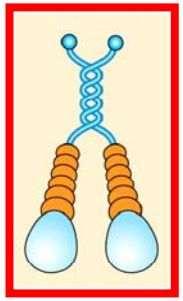
Myosins in intracellular trafficking: cytoplasmic streaming in *nitella*

- In the cell, actin tracks are immobilized while myosins are free to move
- The myosin tail binds to the cargo and the head connects to the actin filament



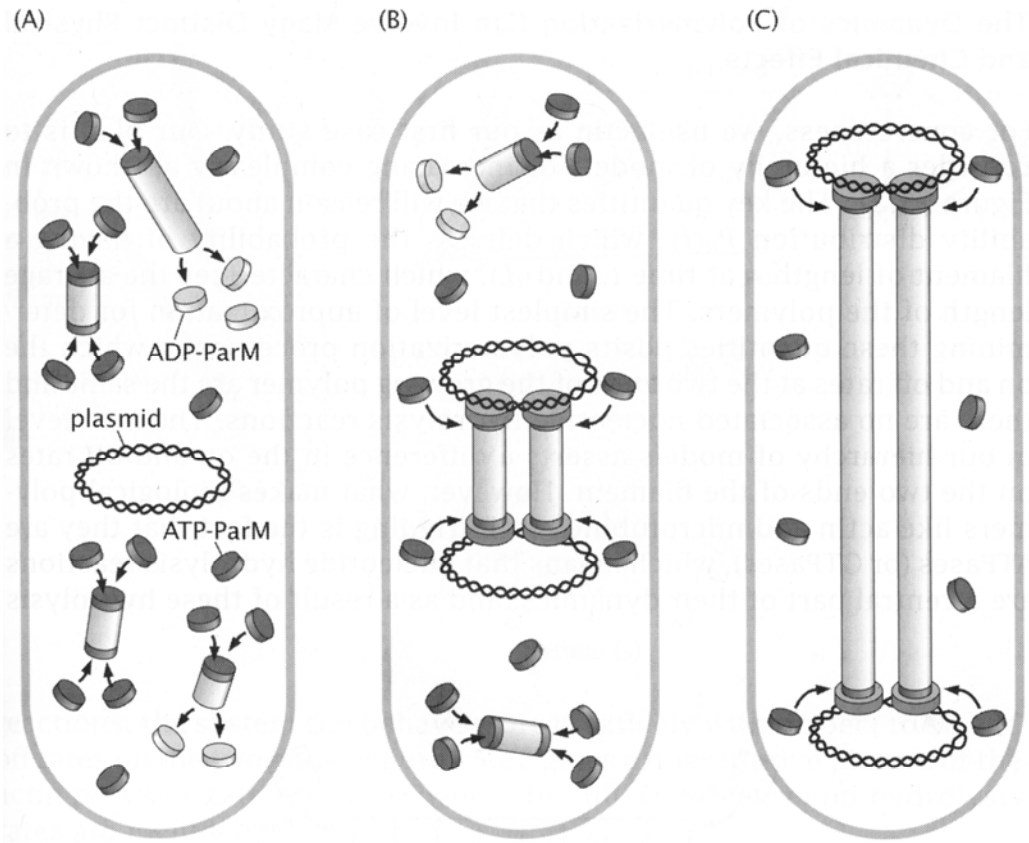
In plant cells **myosin XI** binds parts of the ER causing a flow of cytoplasm

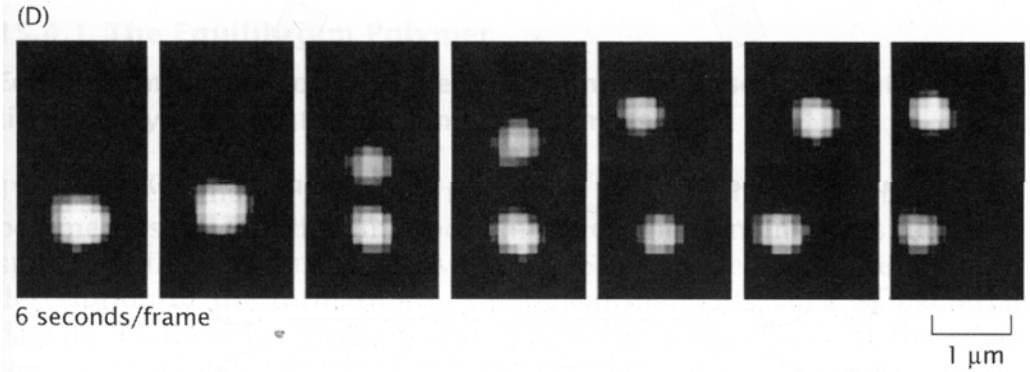




EM

Actin-driven plasmid segregation in bacteria





- **ParM** is an actin homolog in bacteria
- Some plasmids exist in low copy number that carry antibiotic resistance genes (A)
- These plasmids have to duplicate and segregate similar to chromosomes before bacterial division
- ParM filaments are extremely unstable but become more stable when bound to plasmids (B)
- The growth of the filaments pushes the two copies of the plasmids to the opposing poles of the bacteria (C)

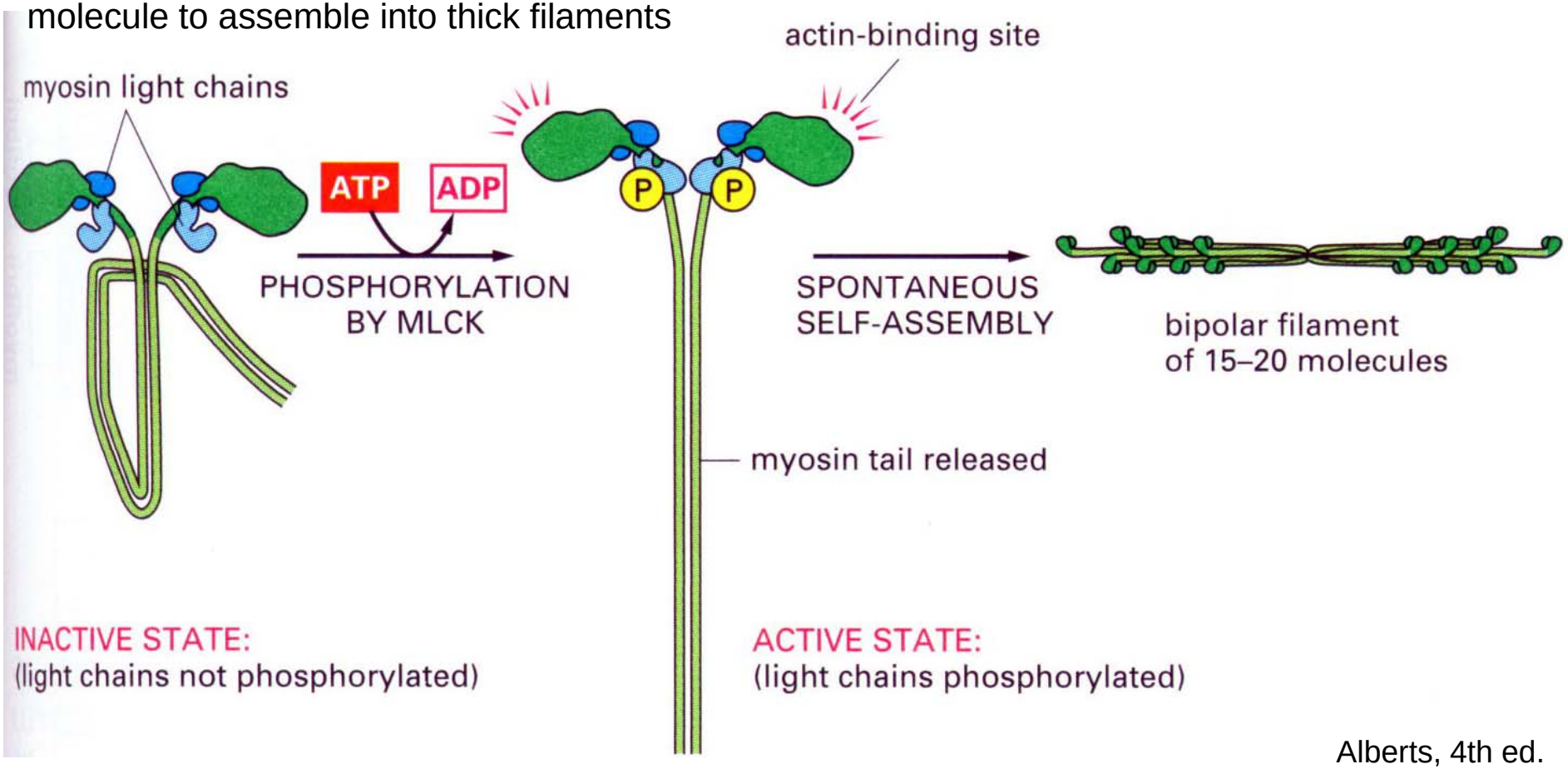
Time lapse imaging showing segregation of fluorescently labeled plasmids in *E. coli*

Garner et al., *Science*, 2004

Actin-myosin contraction in non-muscle or smooth muscle cells

- **Smooth muscle** cells are present in the gut, respiratory tract and blood vessels
- Smooth muscle and non-muscle cell contraction is regulated by **turning myosin on and off** (compare *striated muscle cell*: actin is turned on and off by tropomyosin!)
- Loosely aligned actin-myosin bundles contract when myosin light chain (**LC**) is **phosphorylated by** myosin LC kinase (**MLCK**)

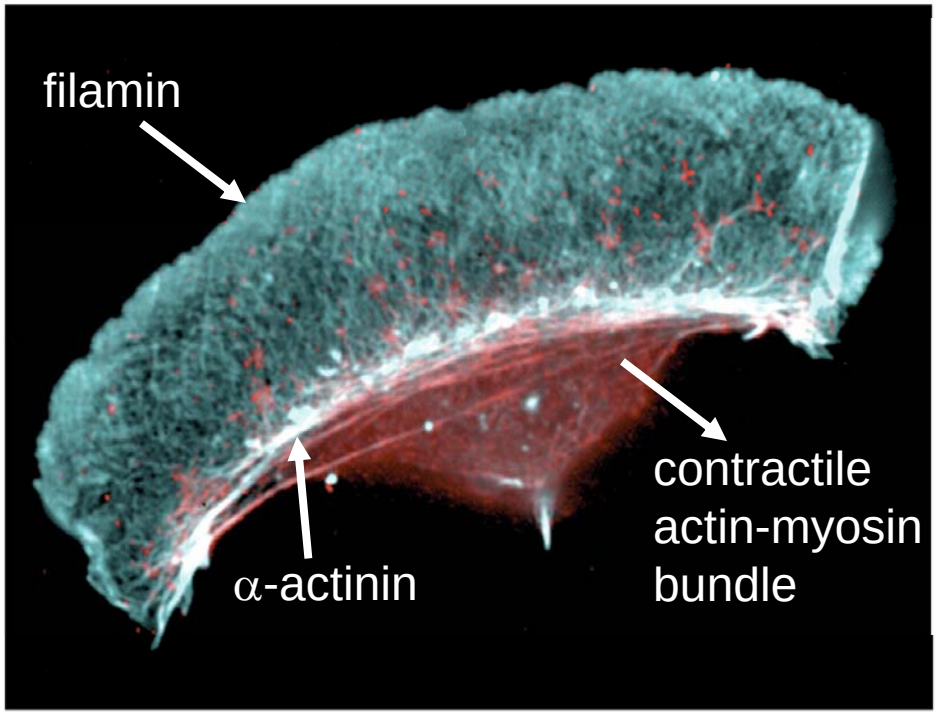
Phosphorylation of myosin LC releases the myosin tail from its "sticky" patch" allowing the myosin molecule to assemble into thick filaments

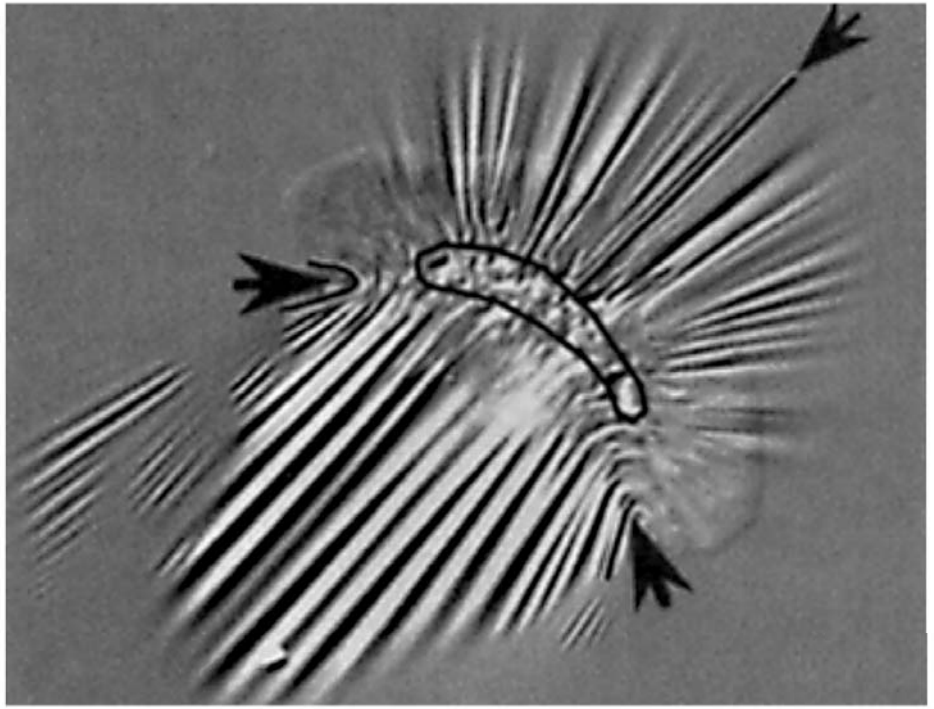


Alberts, 4th ed.

Forces generated by contractile bundles made visible on thin silicon substratum

As a keratinocyte moves forward, the generated force by the actin-myosin bundle in the center of the cell causes the silicon rubber to wrinkle



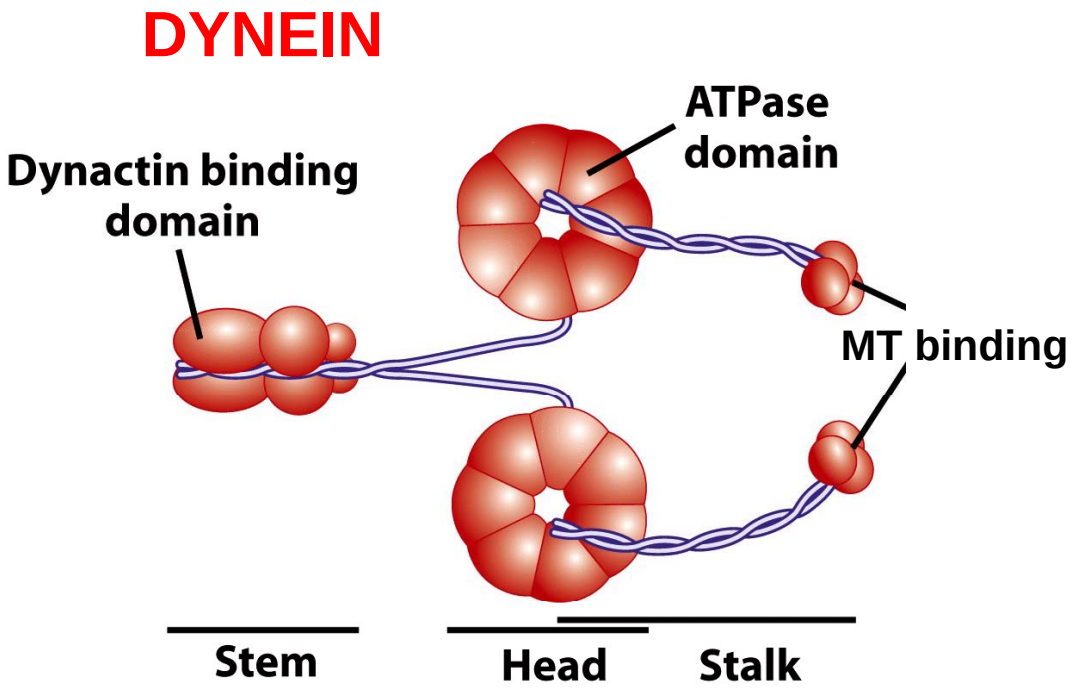


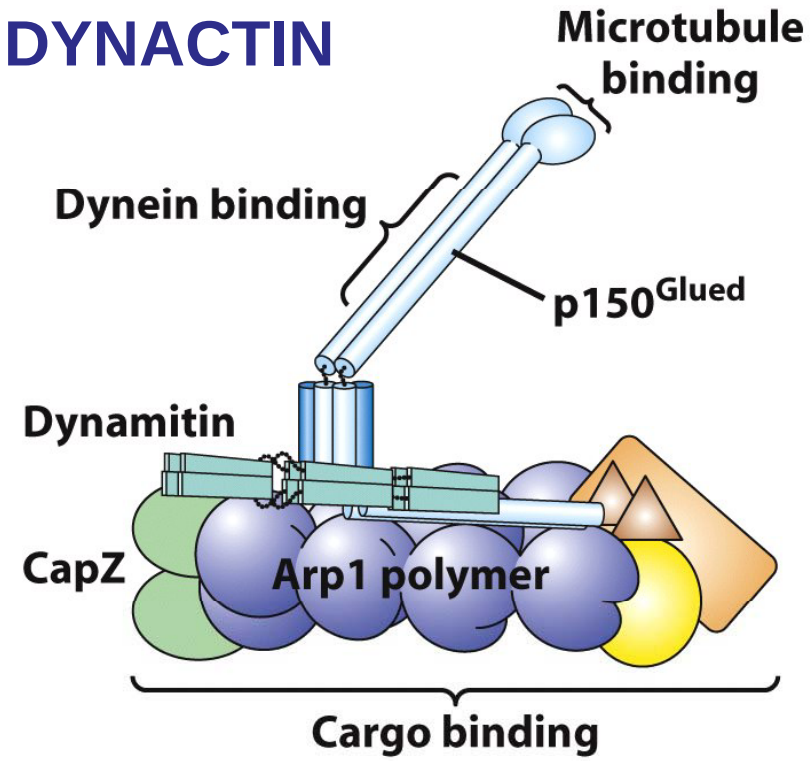
Movie
16_4_heart muscle cell on rubber substrate.mov

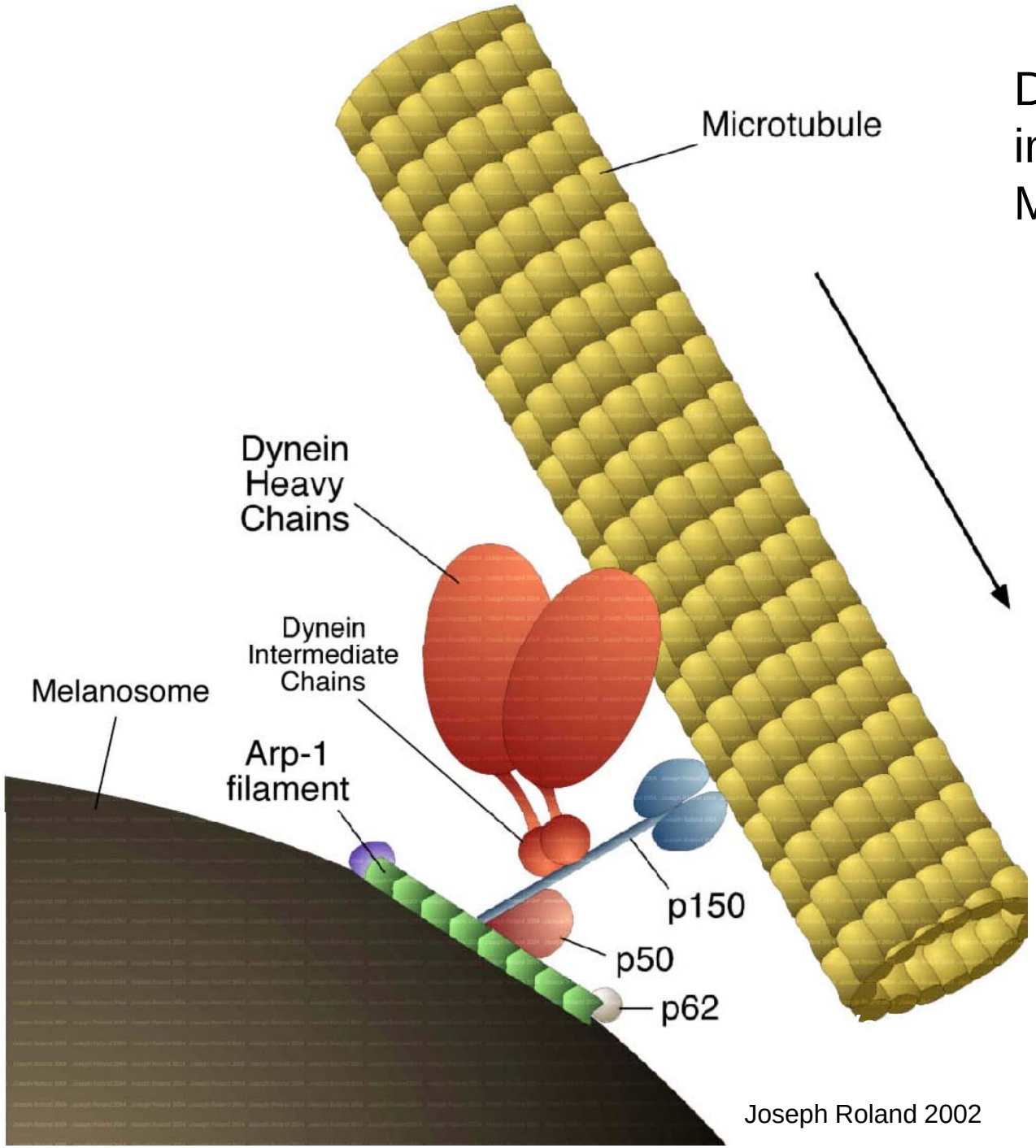
"beating" heart muscle cell on thin silicon substratum

Dyneins move retrograde

- **Dyneins** move cargo retrograde: from plus-end to minus-ends
- Dyneins are large molecules (**1 MDa**) associated to a complicated protein complex named **dynactin** which mediates cargo *and* MT binding

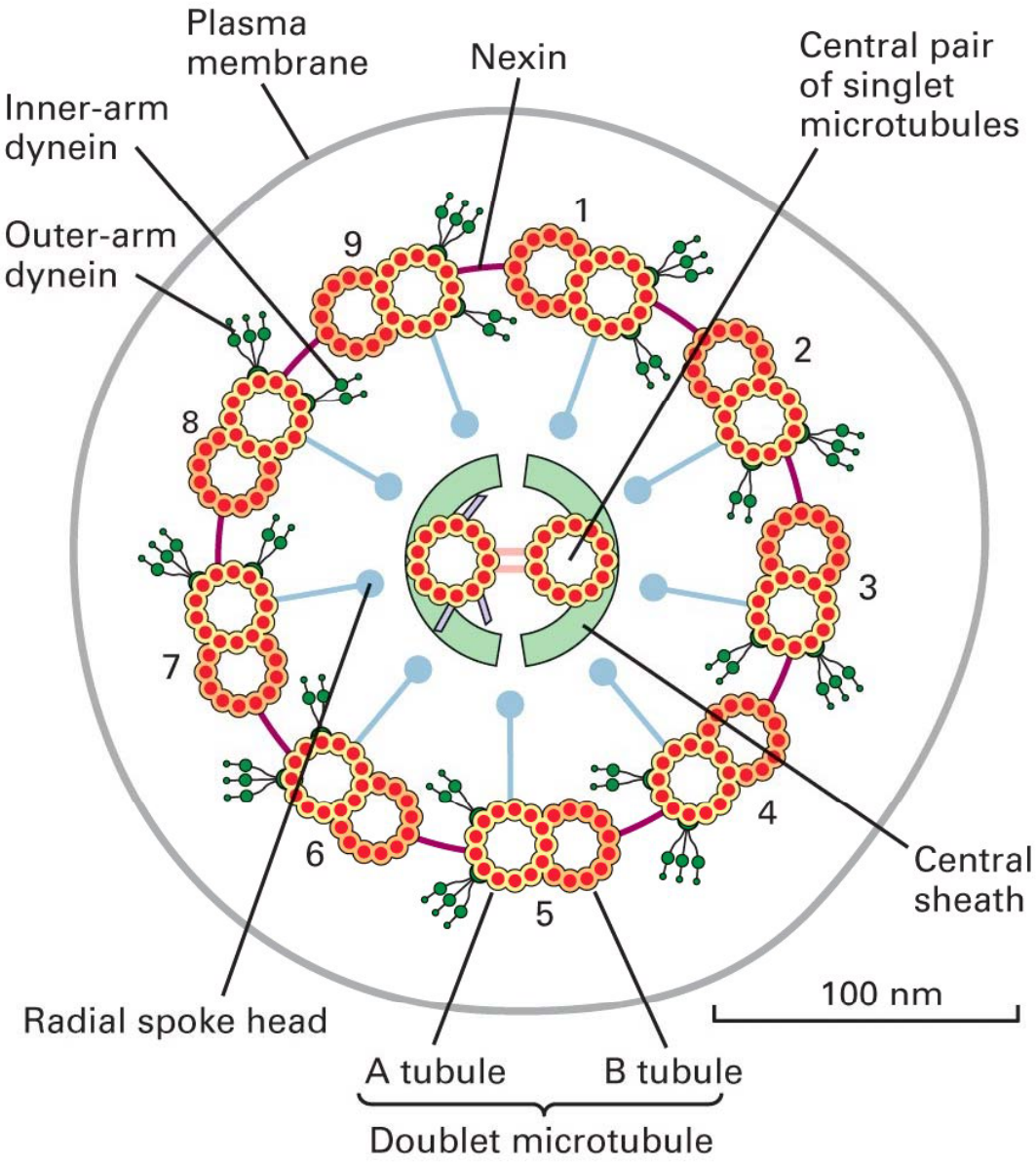




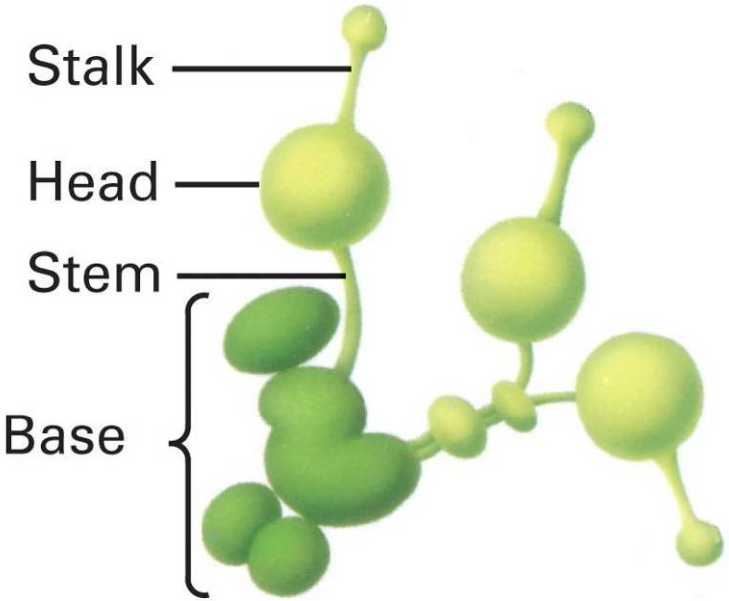


Dynactin mediates interactions between MT, dynein and cargo

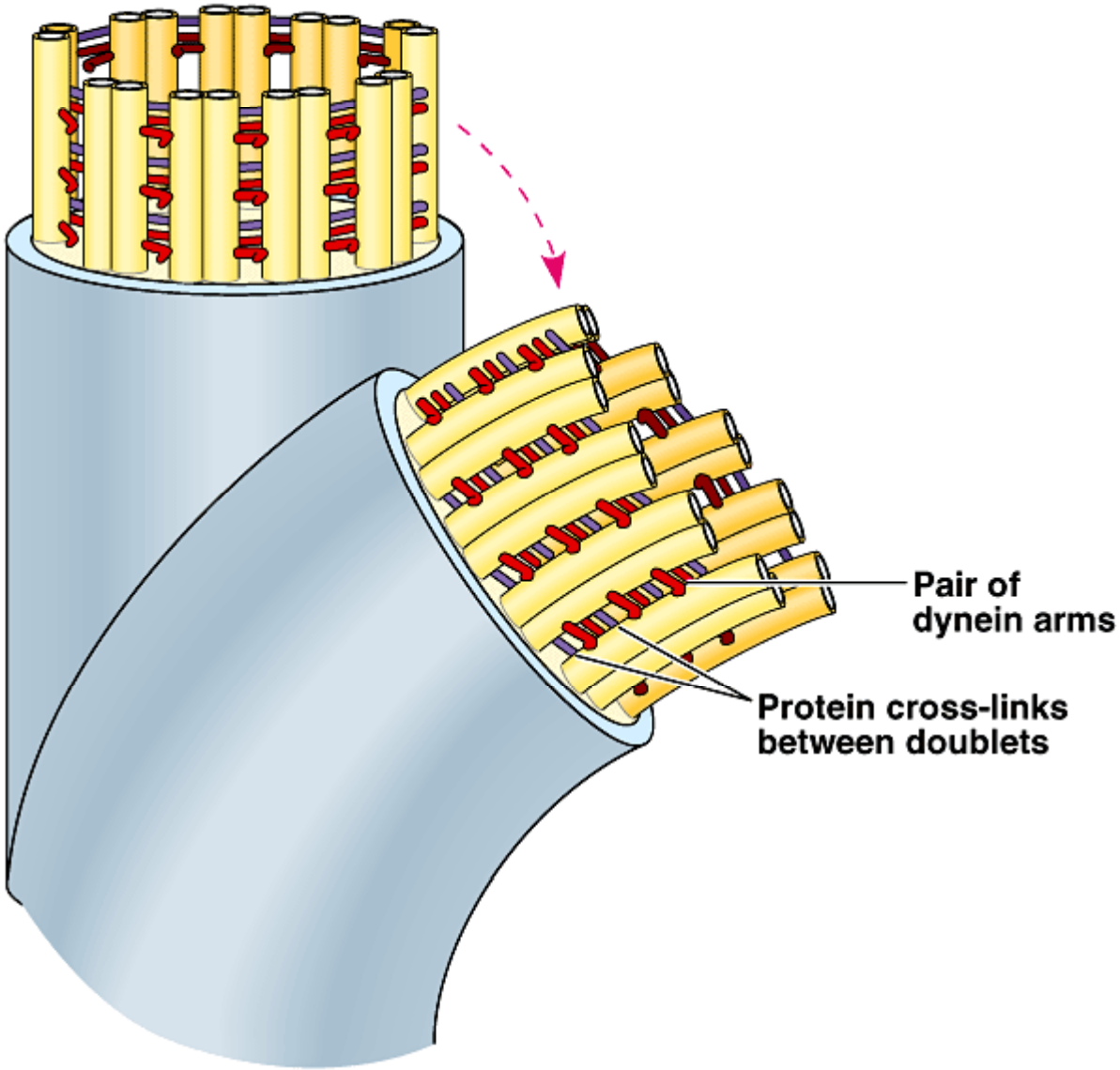
Flagella bending is generated by MTs sliding past each other powered by dynein

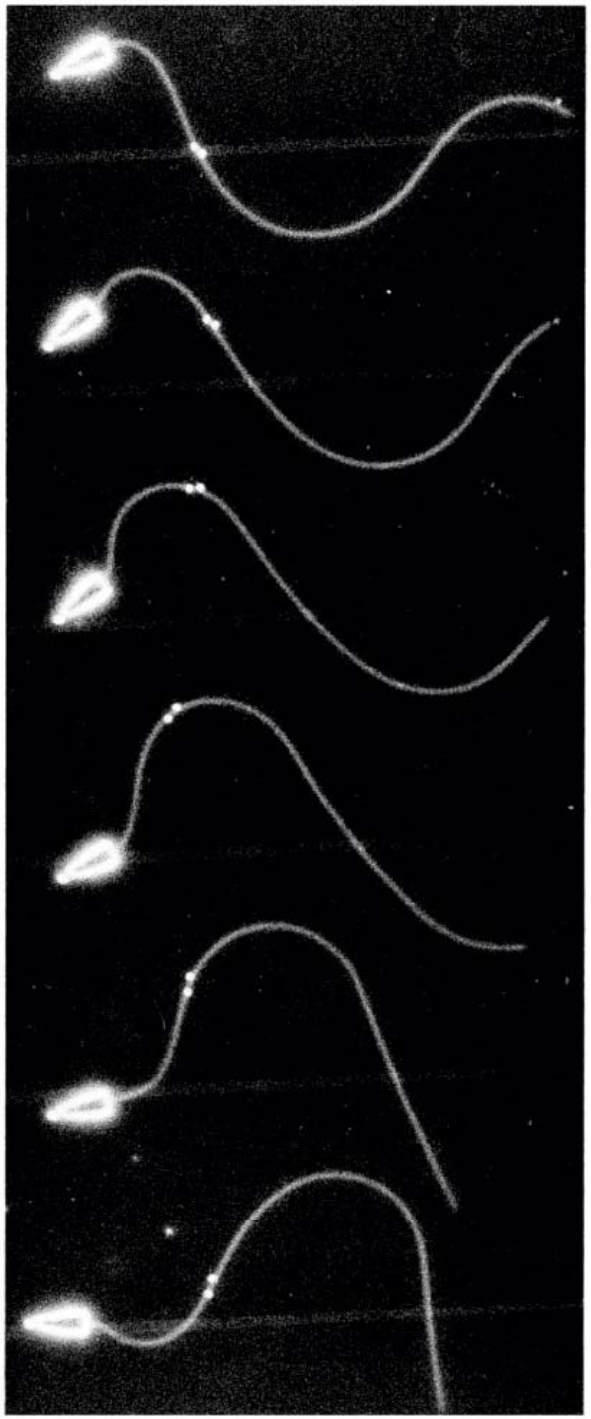


Cross-section thru a cilia



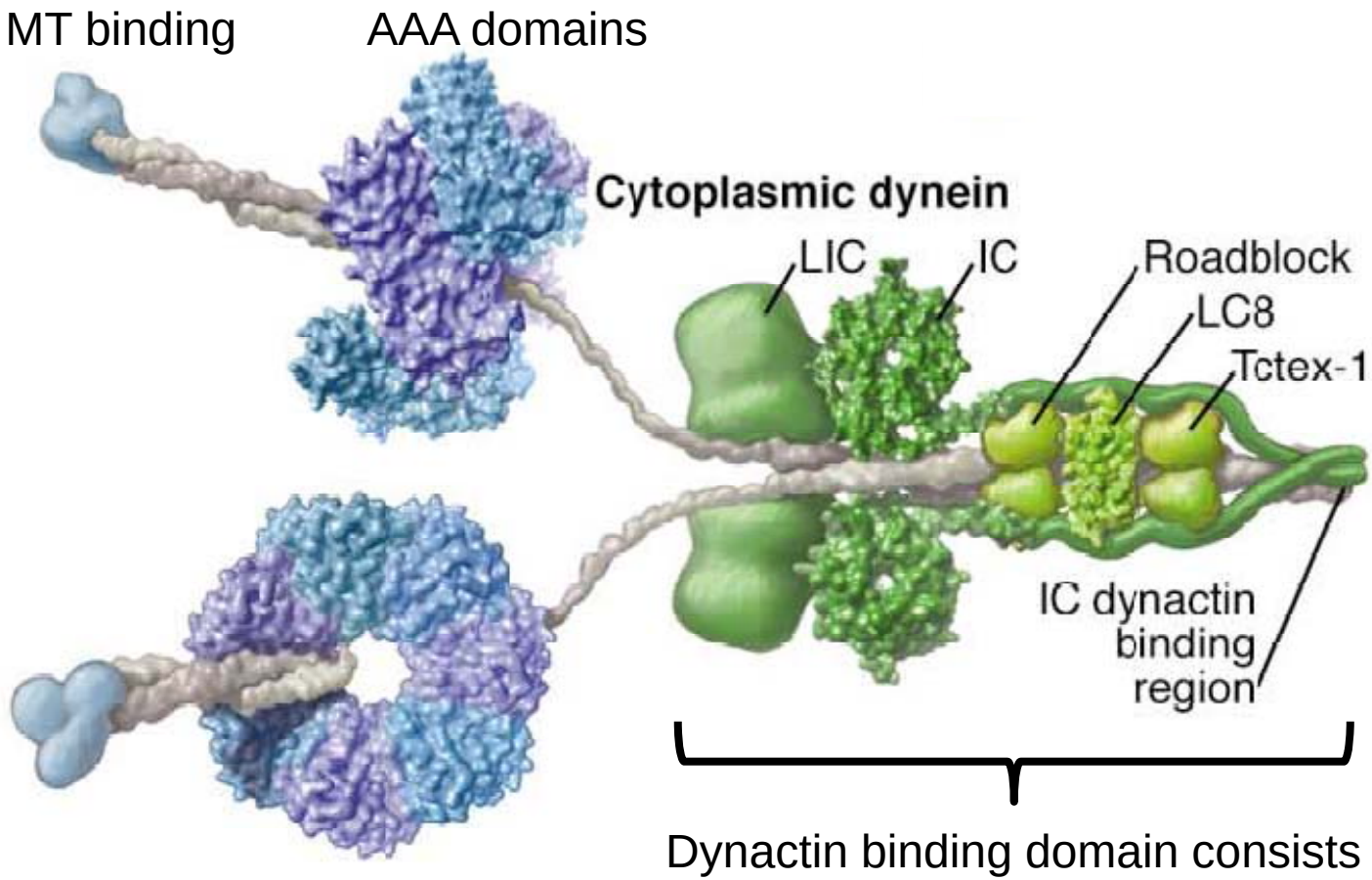
Flagella bending generated by MTs sliding past each other powered by dynein





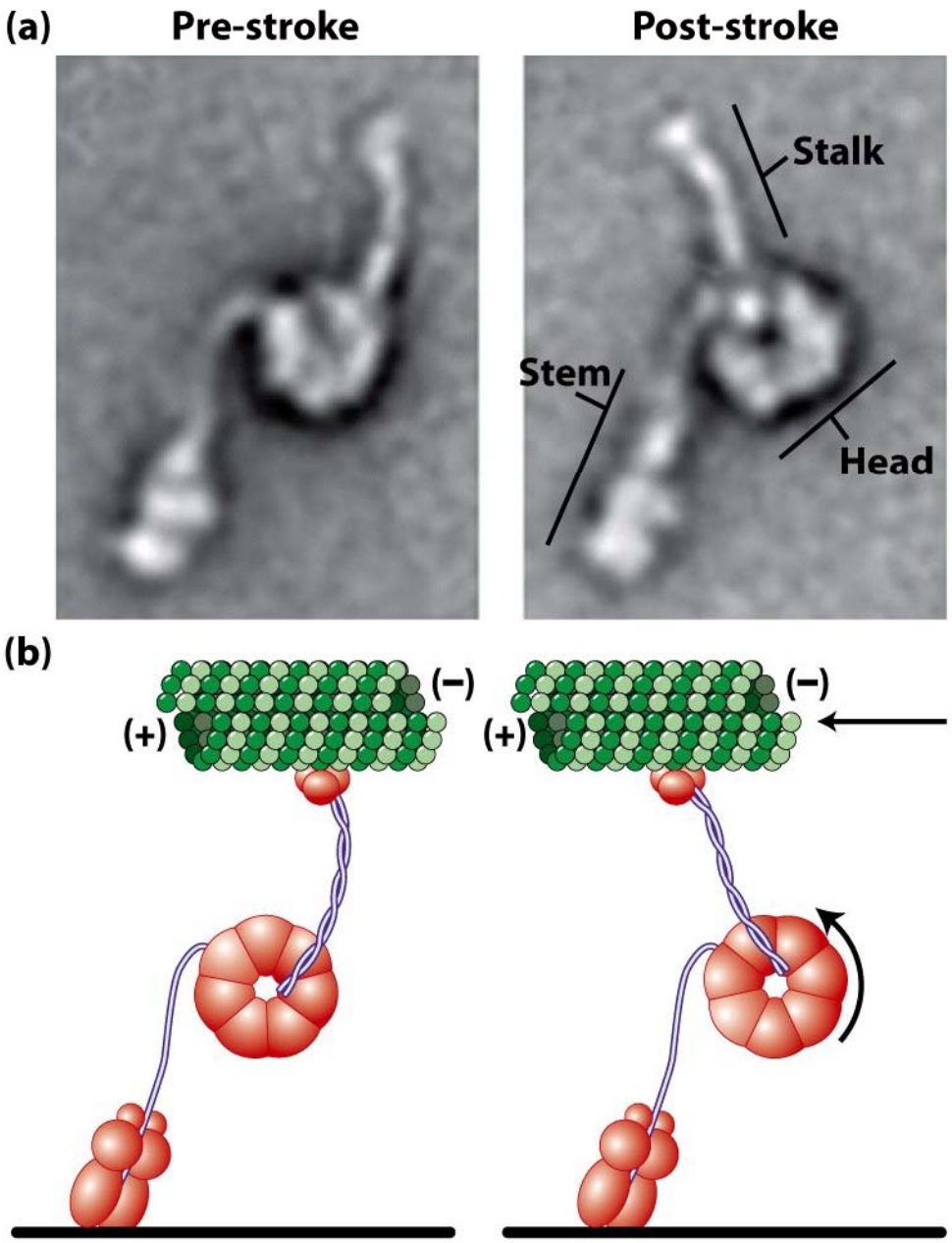
Detailed model of dynein

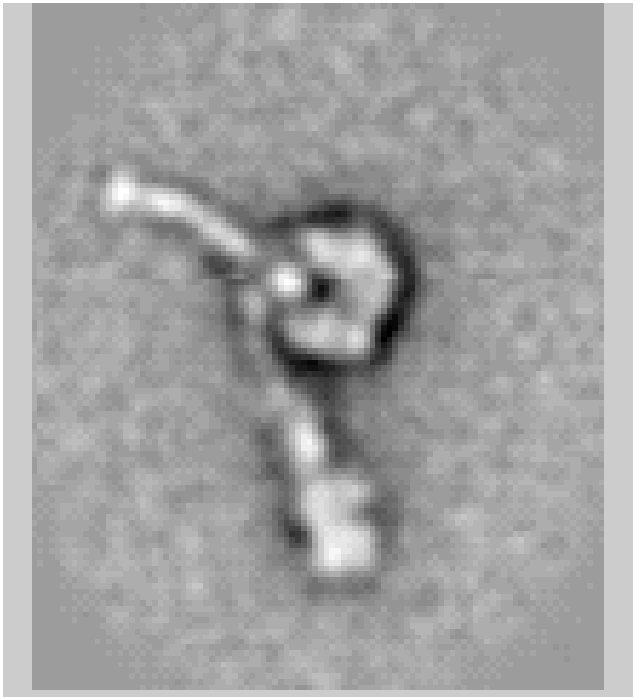
• Dynein heavy chain (360 kDa) consists of 6 **AAA** (***A***TPases ***a***ssociated with cellular ***a***ctivities) **domains**
• Four of the six AAA domains are able to bind ATP while only one domain can hydrolyze ATP



Dynactin binding domain consists of several subunits (green)

Conformational change of dynein revealed by EM

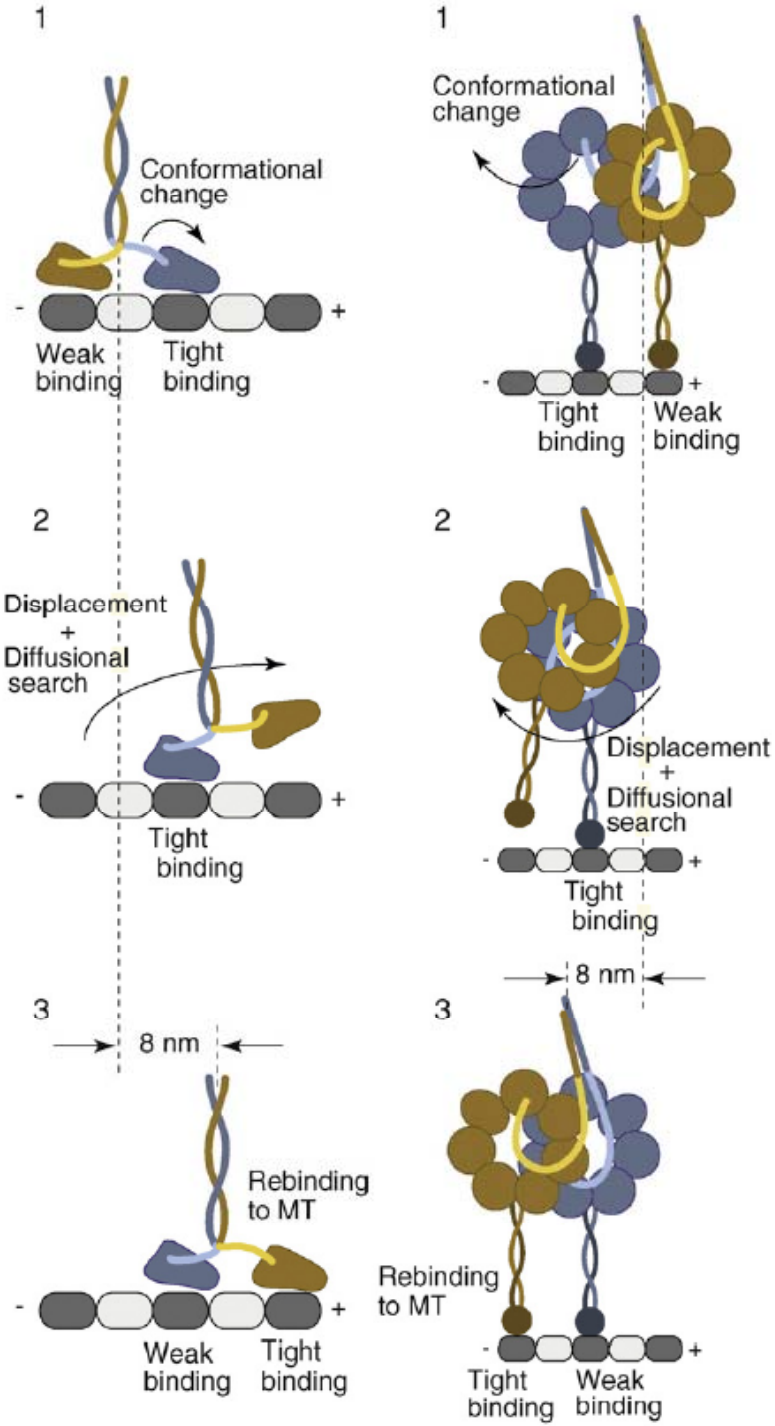




animation

During ATP hydrolysis, the dynein head undergoes a conformational change showing that a poststroke follows a prestroke

Comparison of kinesin's and dynein's stepping

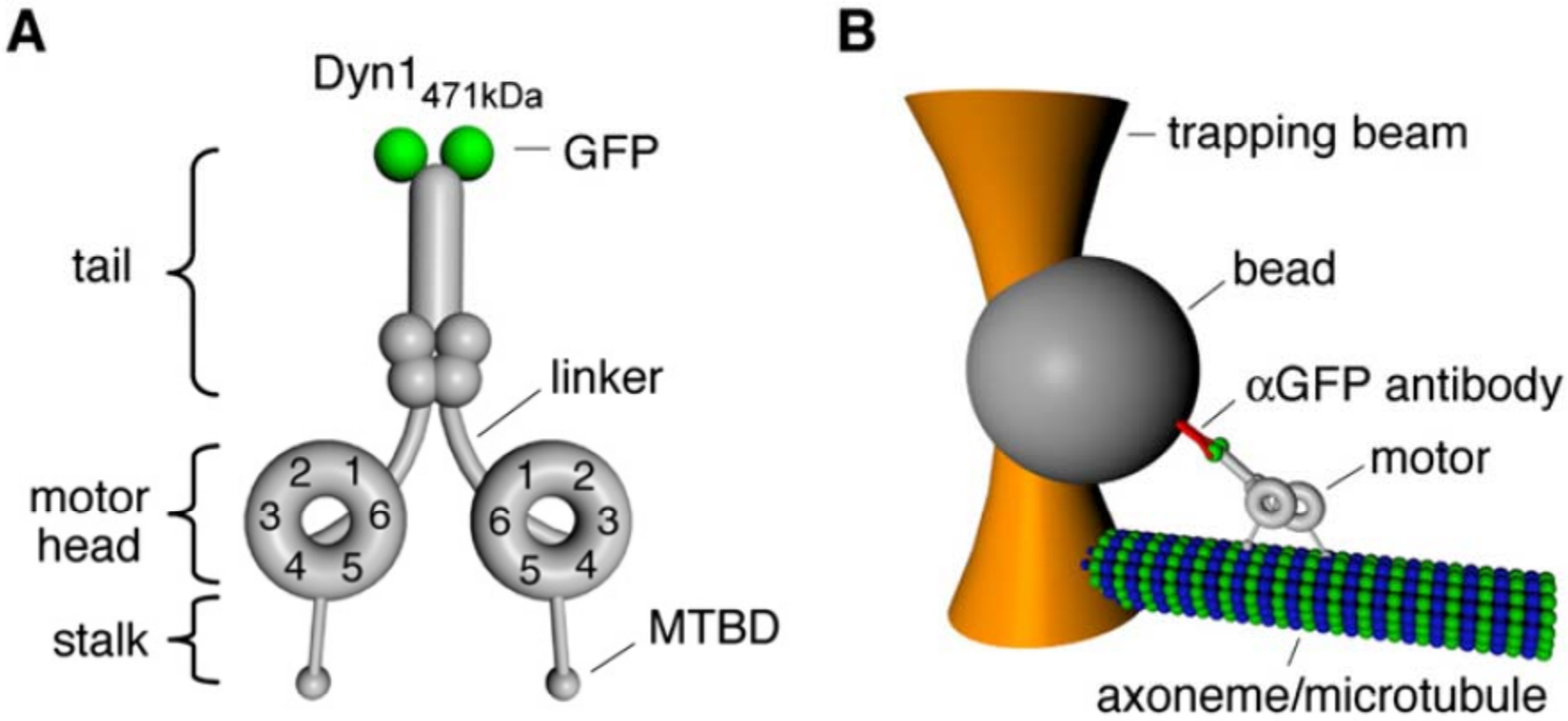


- Kinesin's **rear head** (brown) makes a 16 nm step, however, the net advancement of kinesin is 8 nm
- A conformational change of a **linker element** in the dyneins **front head** (blue) displaces the connected **partner head** (brown) towards the minus-end of the MT

Gennerich and Vale, Curr. Opin. Cell Biol., 2009

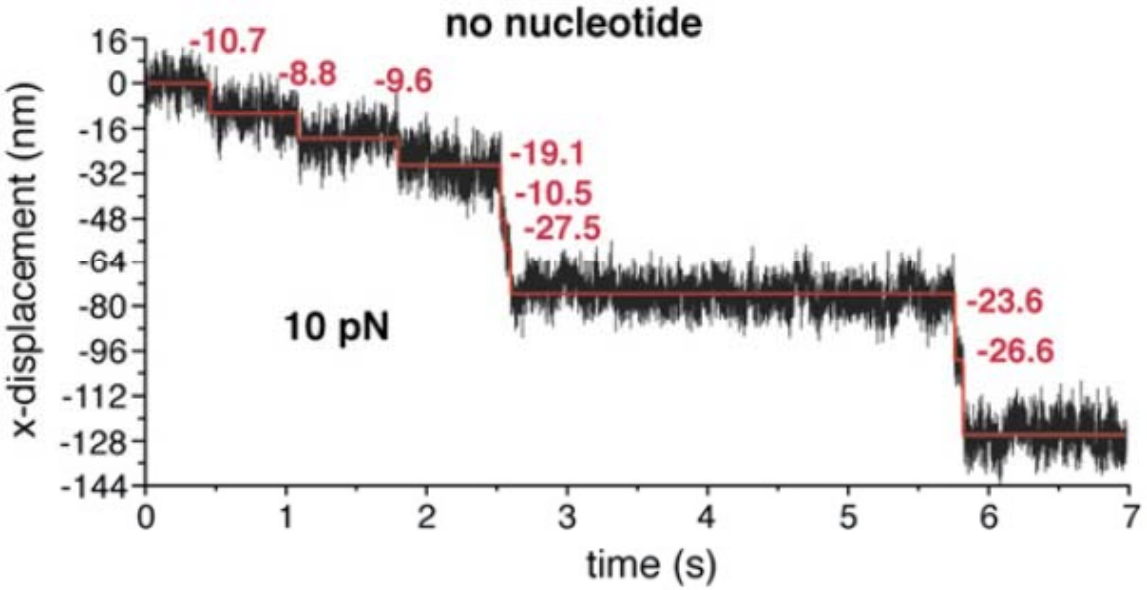
Special features of dynein revealed by optical trapping

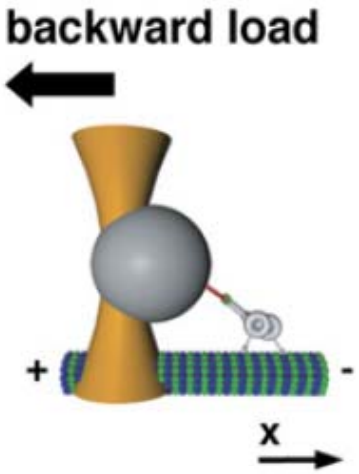
- A **GFP** (green fluorescent protein) can be fused to the dynein tail domain and tagged (detected) with an antibody
- Bead is covered with an anti-GFP antibody resulting in tight binding of dynein
- Bead can be used to exert **pulling or a pushing force** on dynein

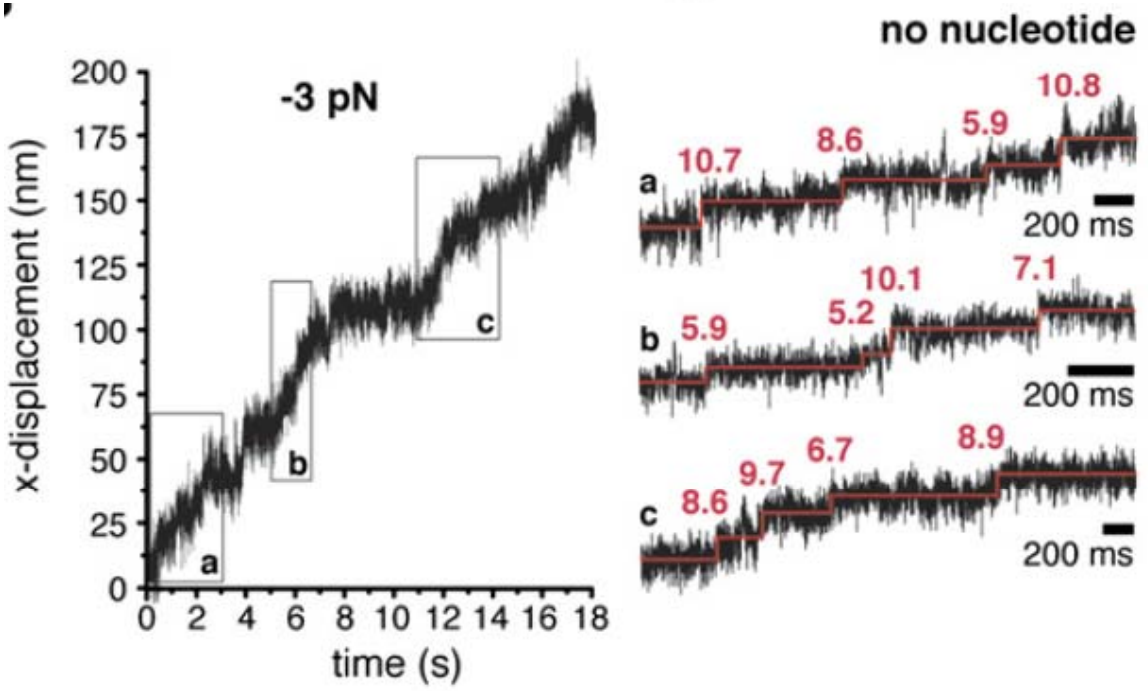


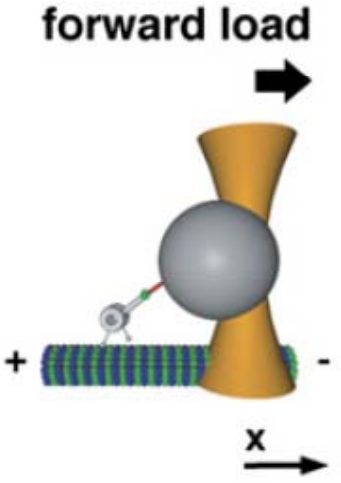
Gennerich et al., 2007, Cell

If dynein is pulled/pushed by bead it can make discreet steps even **without ATP**







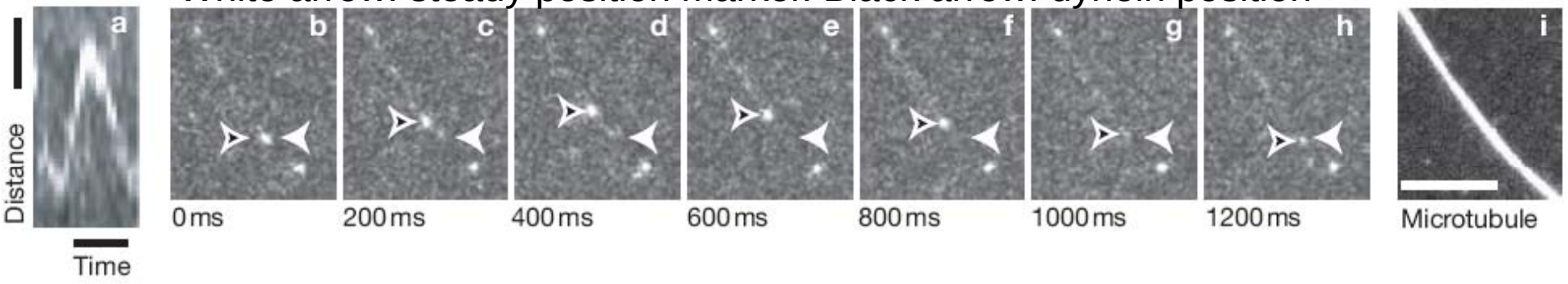


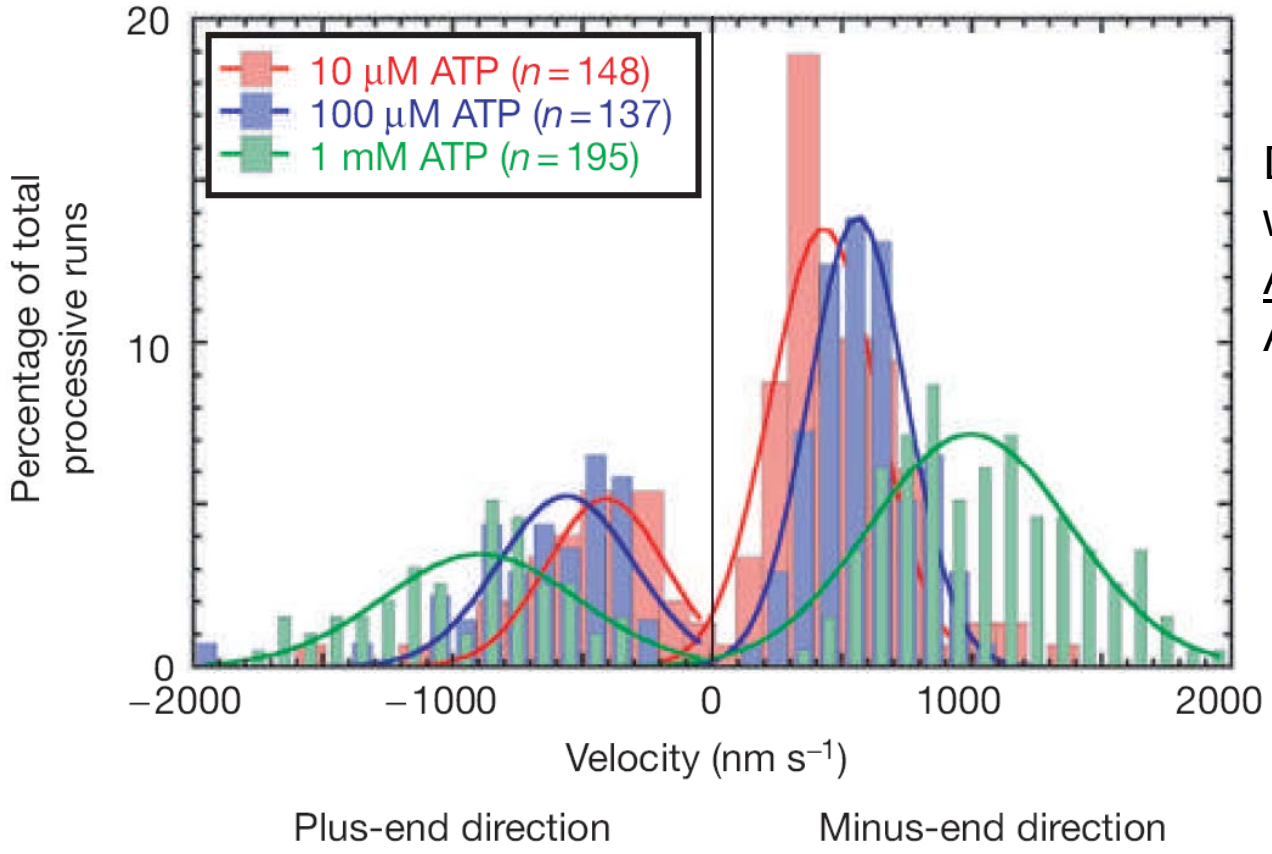
Dynein's 8 nm-stepping **depends on the load** <u>and not on</u> the presence of <u>ATP</u>

Gennerich et al., 2007, Cell

Optical trap studies reveals bidirectional movement of dynein

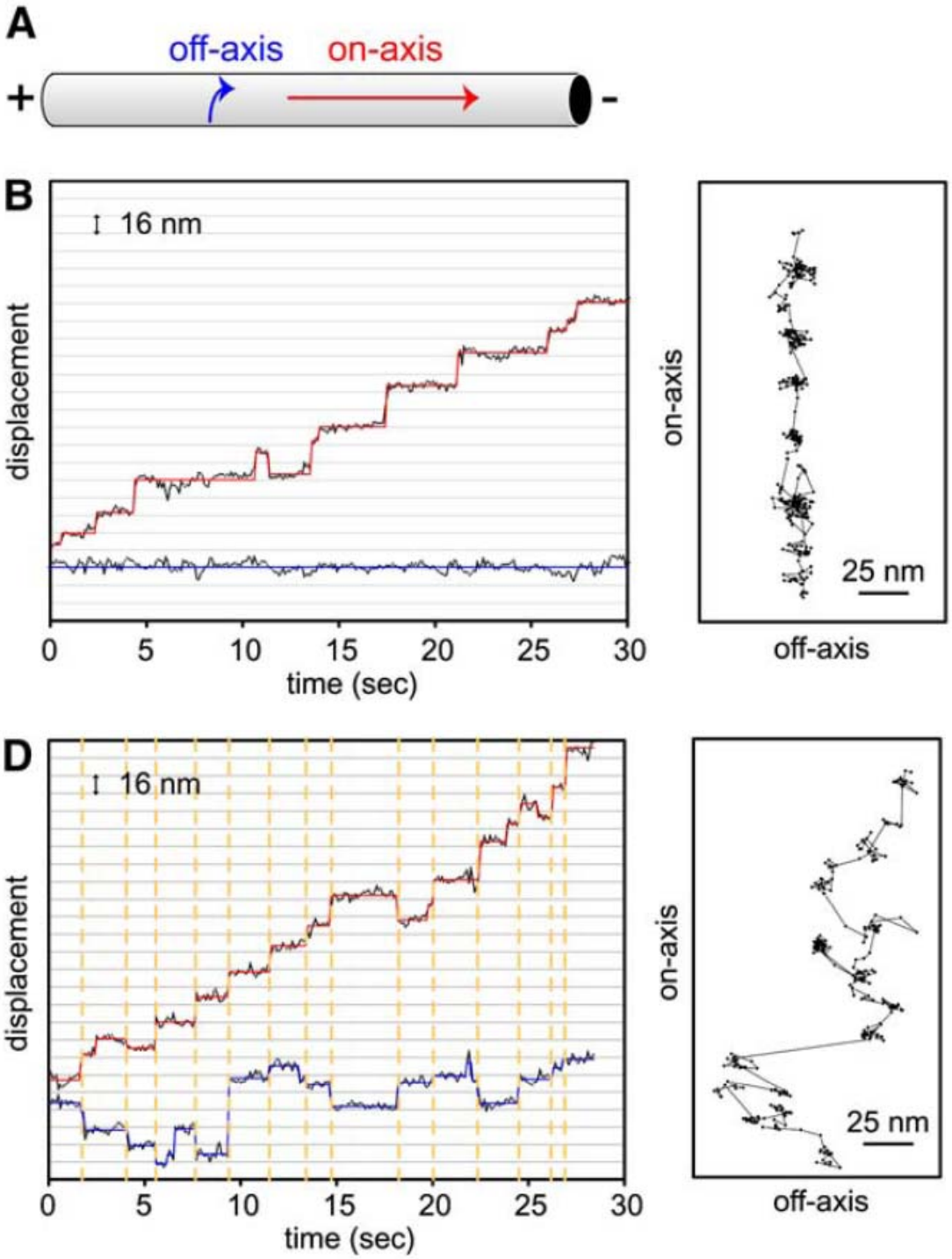
White arrow: steady position marker. Black arrow: dynein position





Dynein **moves bidirectional** while the <u>speed depends</u> on <u>ATP concentration</u> (the more ATP the higher the speed)

Ross et al., 2006, *Nat Cell Biol*

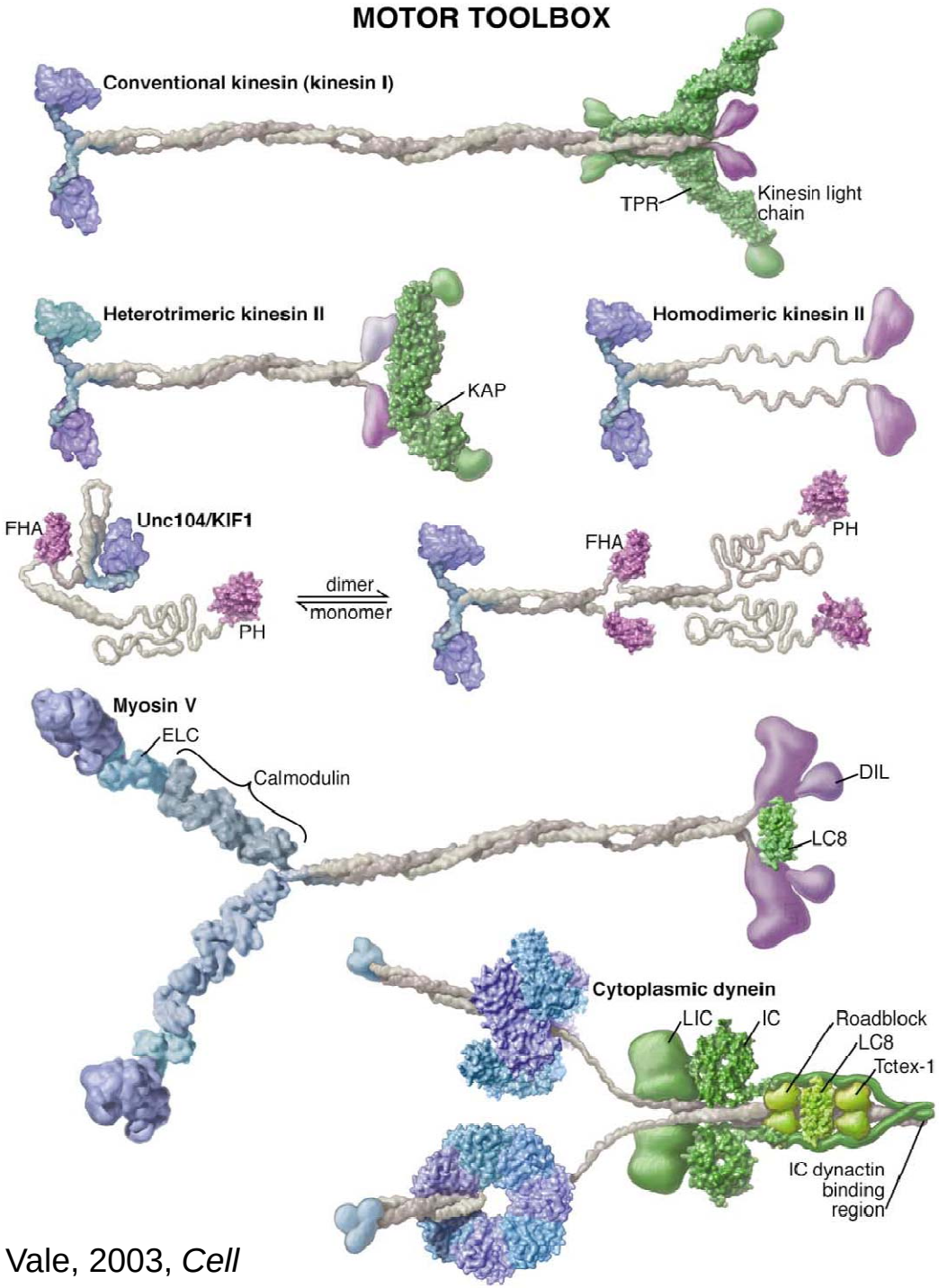


Dynein can make side steps (onto other protofilaments)

Example of no off-axis stepping

Example of **frequently observed** **off-axis stepping**

Reck-Peterson, 2006, Cell

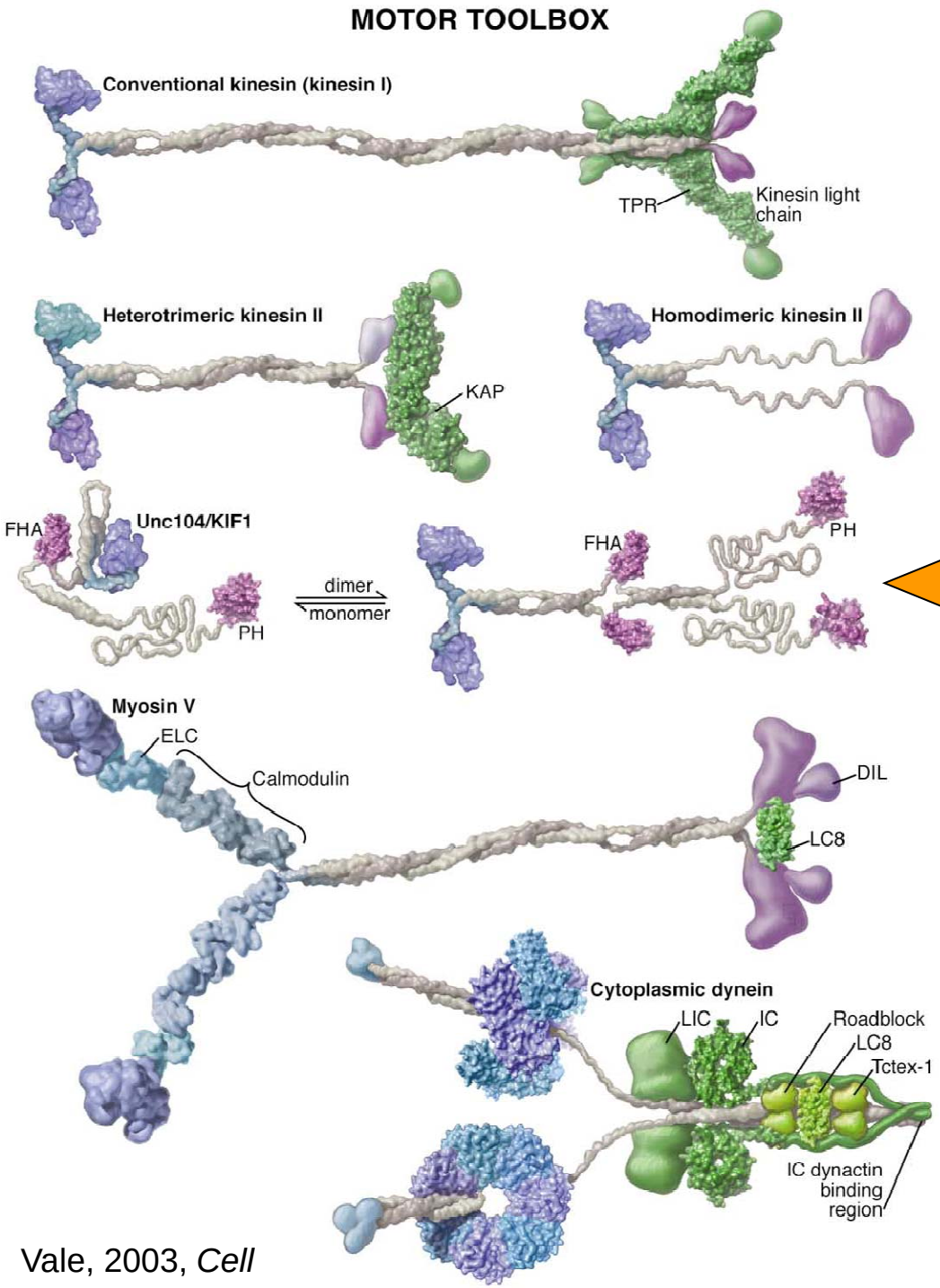


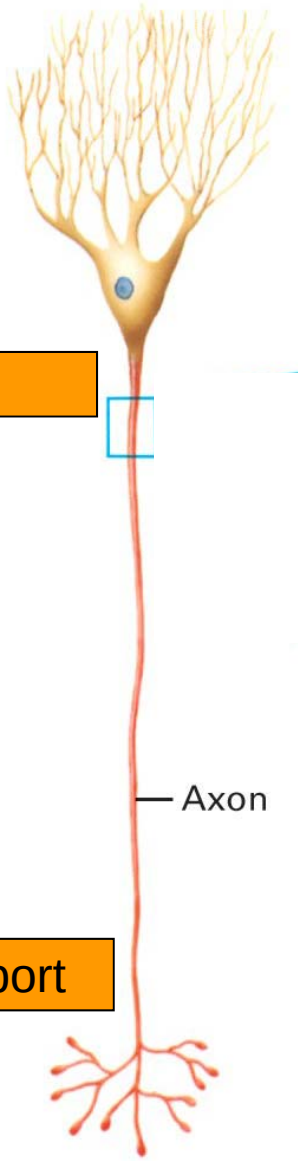
Vale, 2003, *Cell*

Molecular motors are a diverse class of proteins

- **Motor domains** = blue
- **Mechanical amplifiers** (neck linkers, lever arms) = light blue
- **Tail domains** = purple
- **Regulatory subunits** = green

The motor toolbox for intra-cellular transport





Dendritic vesicles

Axonal vesicles

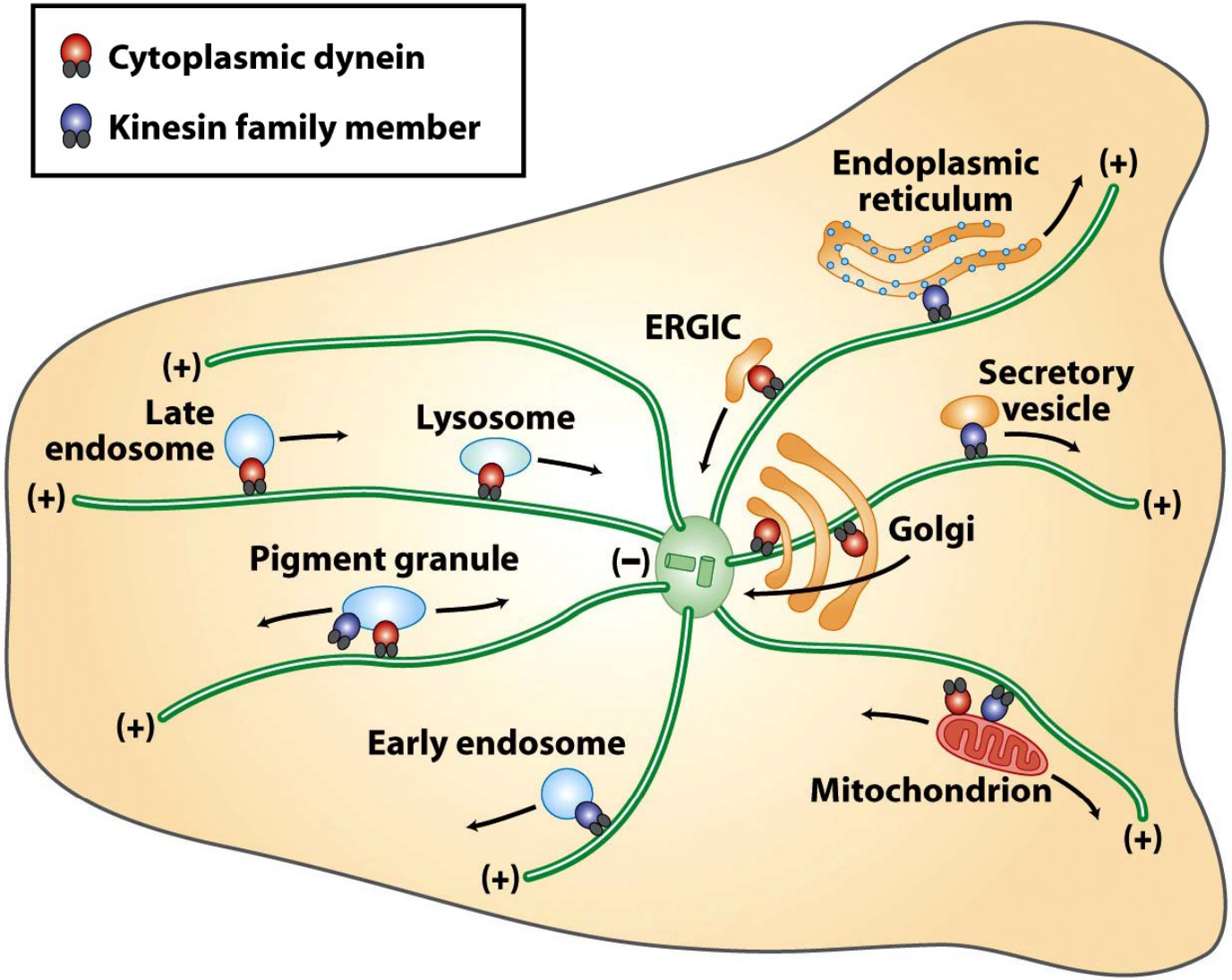
Backward transport

Vale, 2003, *Cell*

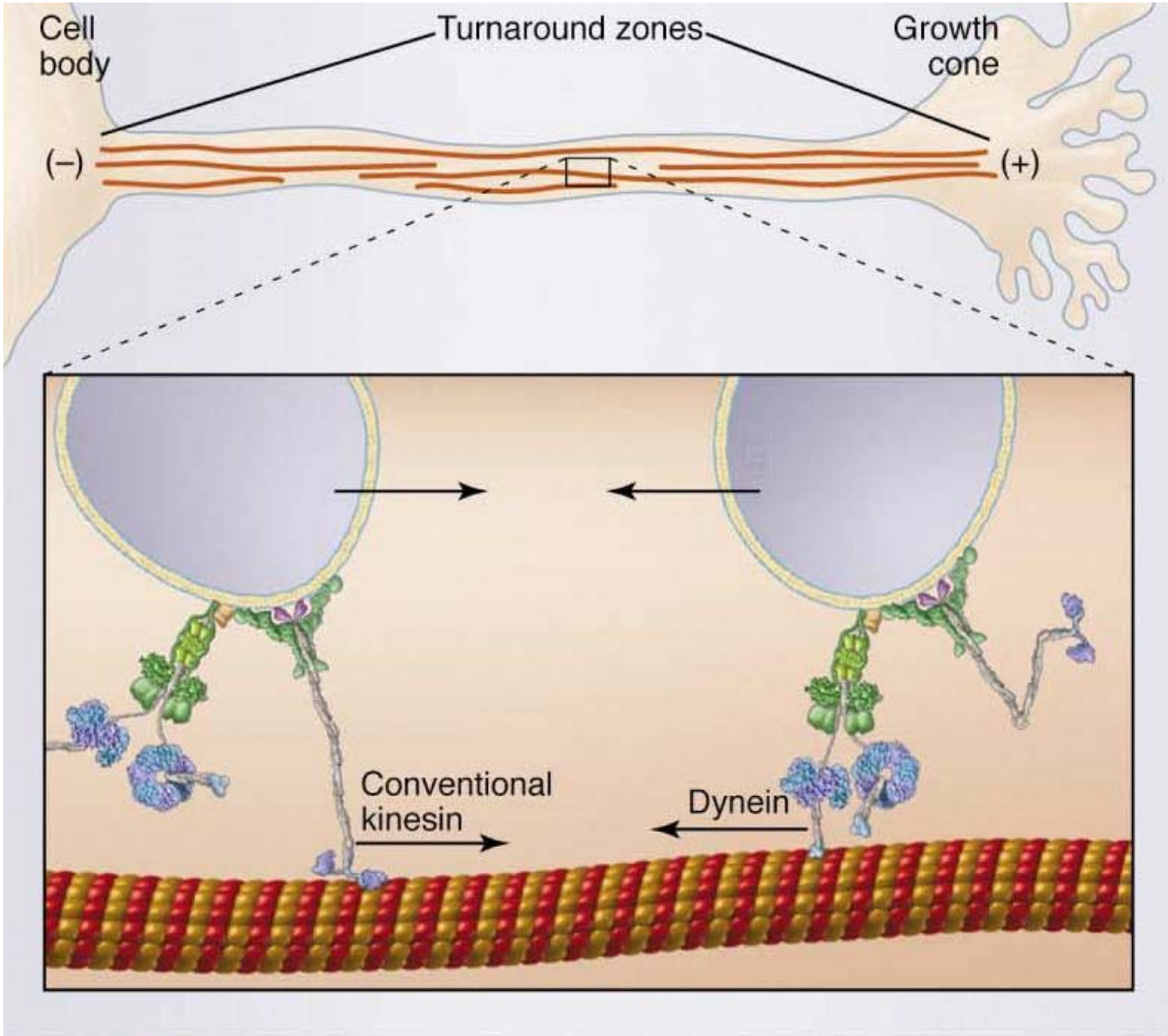
How can vesicles move bidirectional?

• Many vesicles exhibit **bidirectional movements**: probably dynein and kinesins are attached to the cargo at the same time and their activity is regulated

• Recent studies show that kinesin directly interacts with dynactin => directional switches probably due to **swapping between two opposing motors**



How can vesicles move bidirectional?

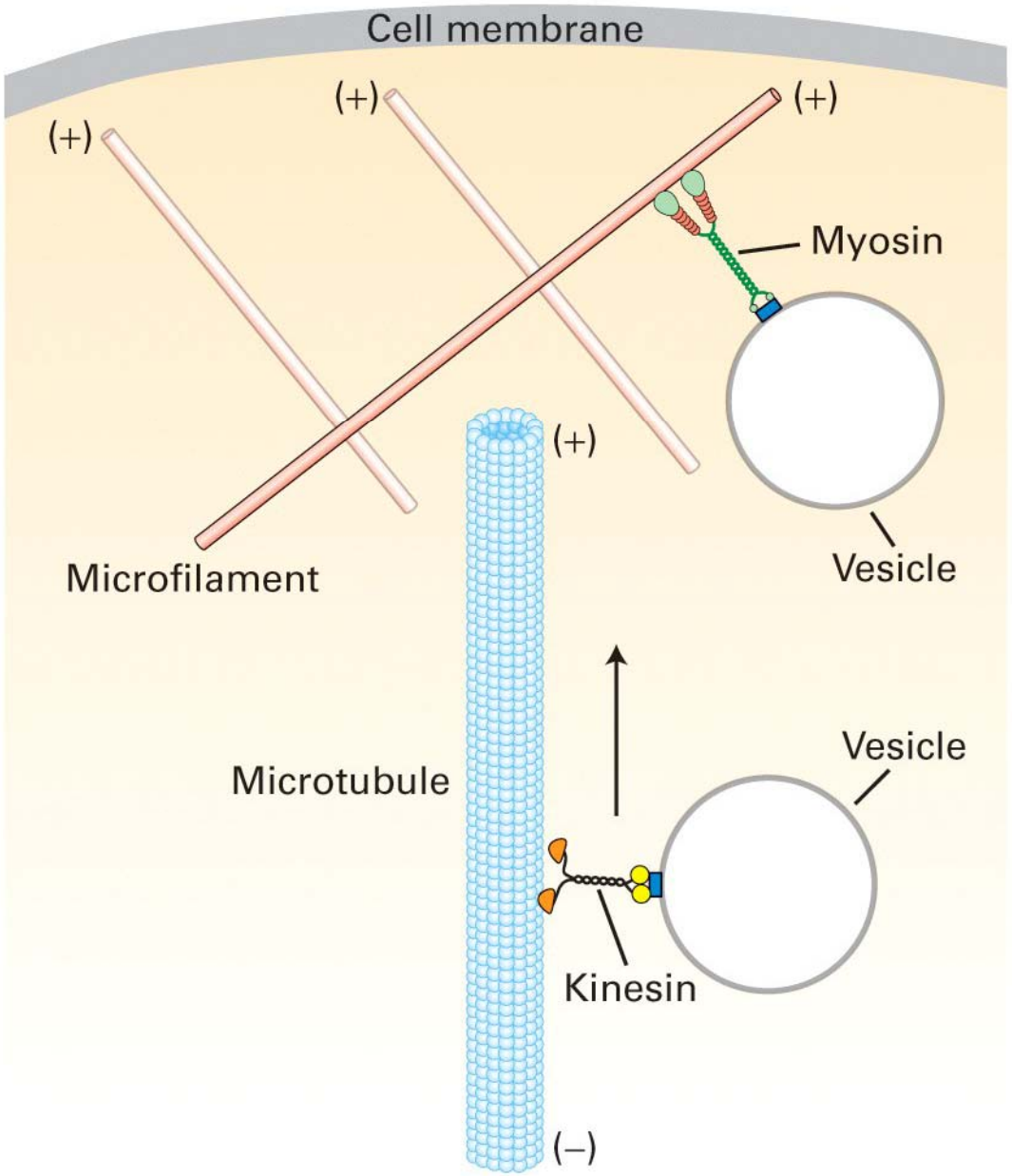


Coordinated activity of two types of motors on the same vesicle? (by phos-phorylation or adaptor proteins)

Pollard, 1st ed.

Bidirectional movement: tug-of-war between antagonistic motors?

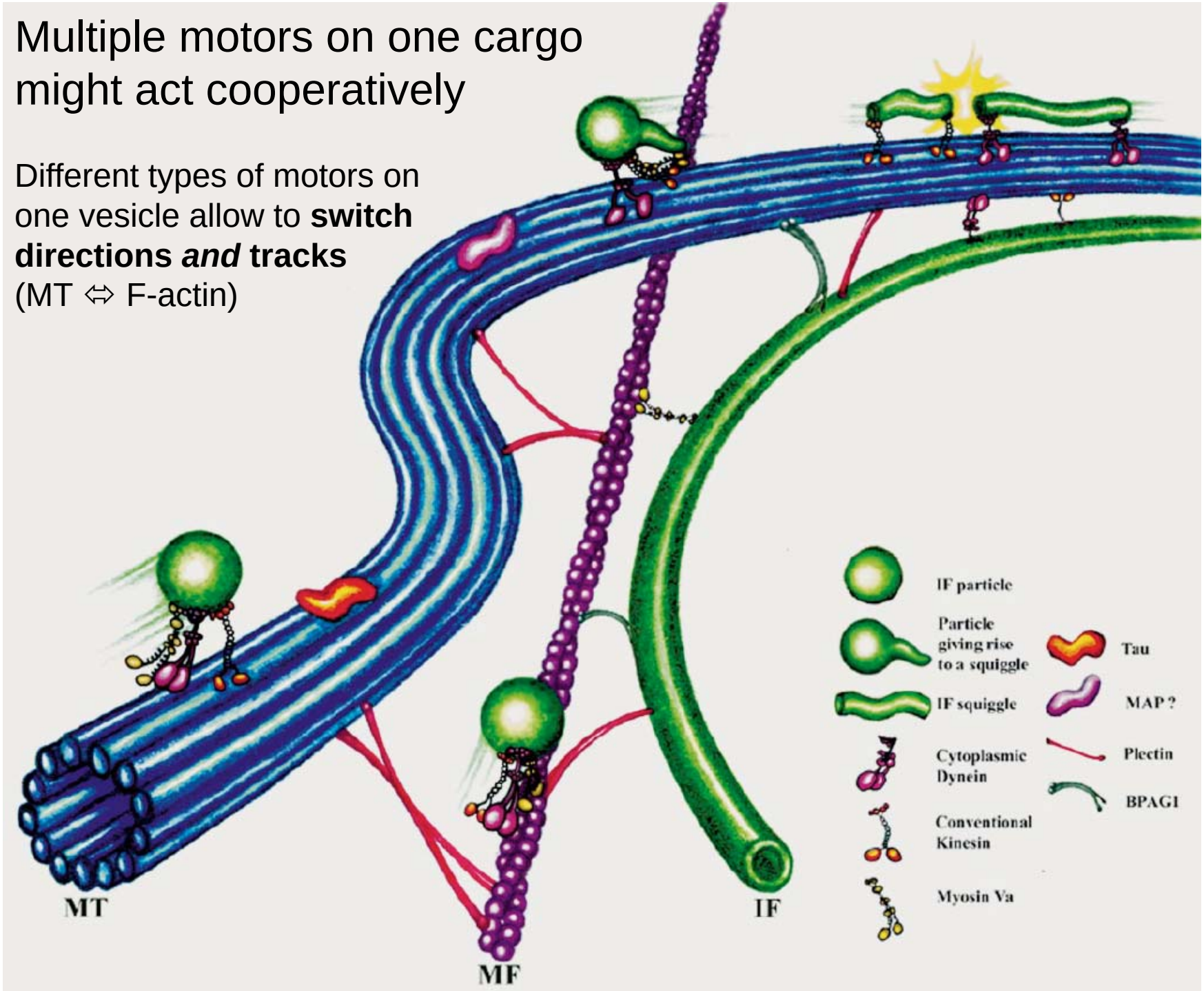
Changing tracks: myosins and kinesins may work cooperative



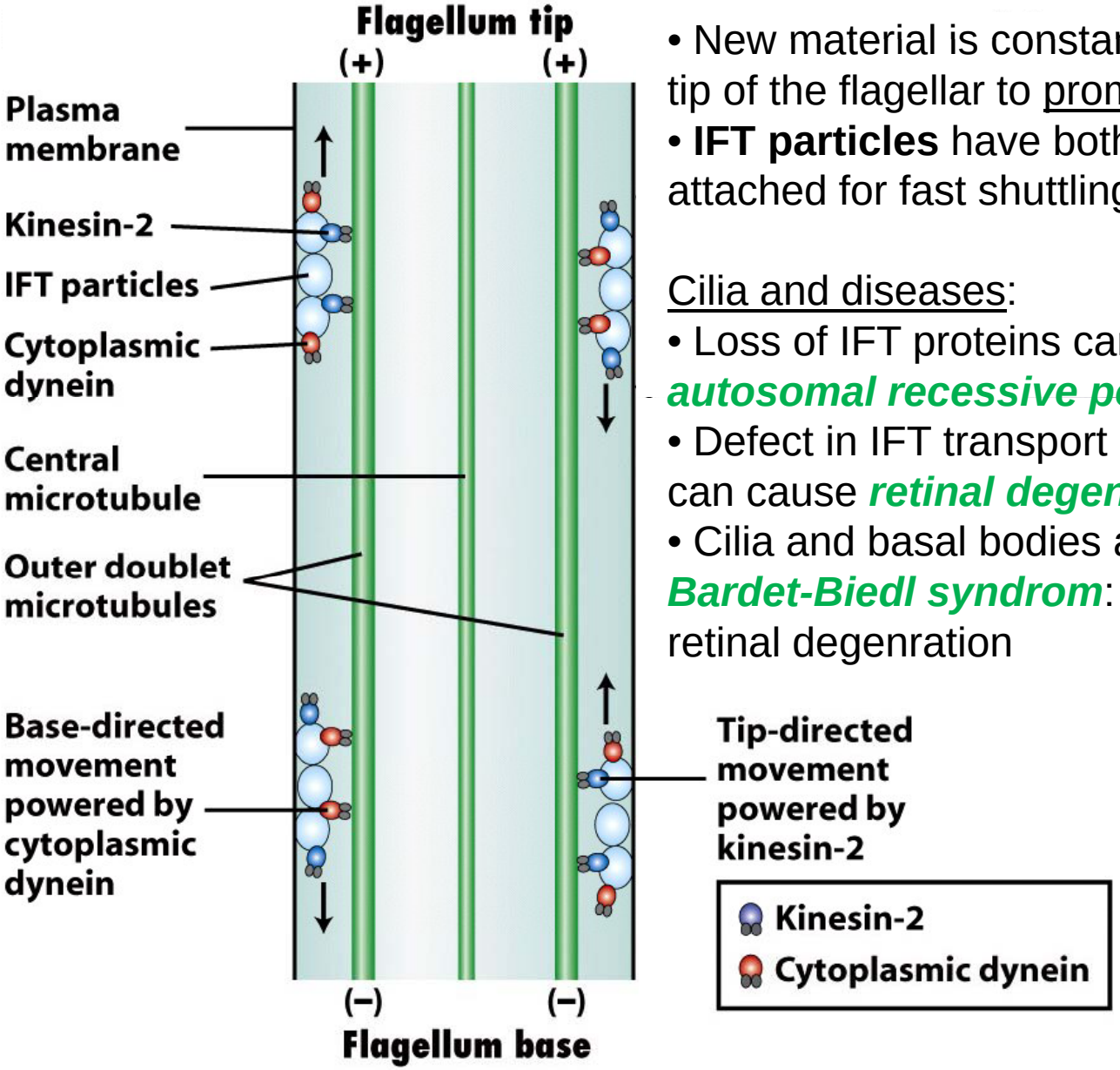
A vesicle might be moved from deep inside the cell to the periphery by a **kinesin** while at the cell periphery a **myosin** takes over this vesicle

Multiple motors on one cargo might act cooperatively

Different types of motors on one vesicle allow to **switch directions *and* tracks** (MT ⇔ F-actin)



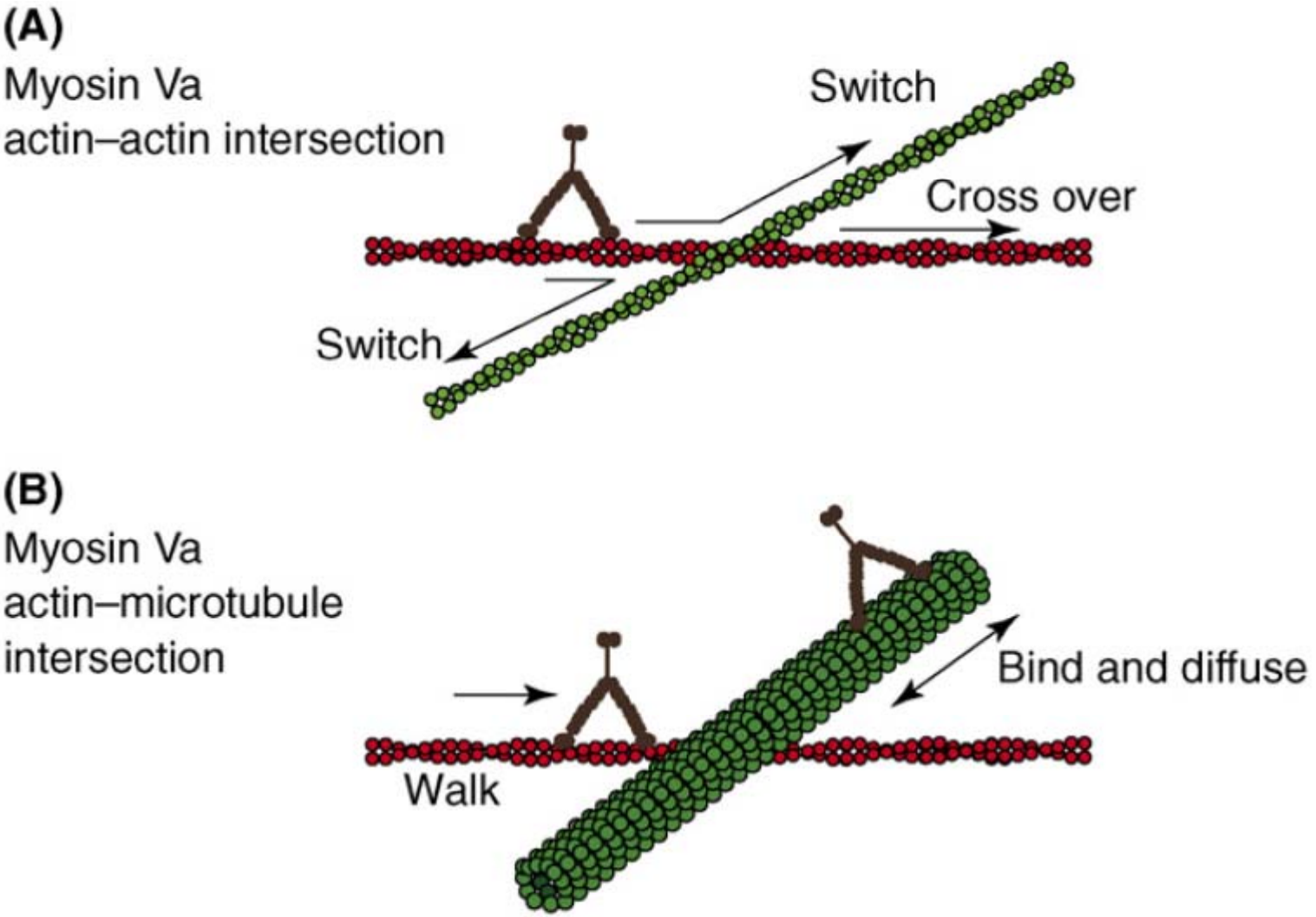
Cooperative motor activity in the intraflagellar transport (IFT)



• New material is constantly transported to the tip of the flagellar to promote microtubule growth
• **IFT particles** have both, **kinesins** and **dyneins** attached for fast shuttling cargo in **two directions**

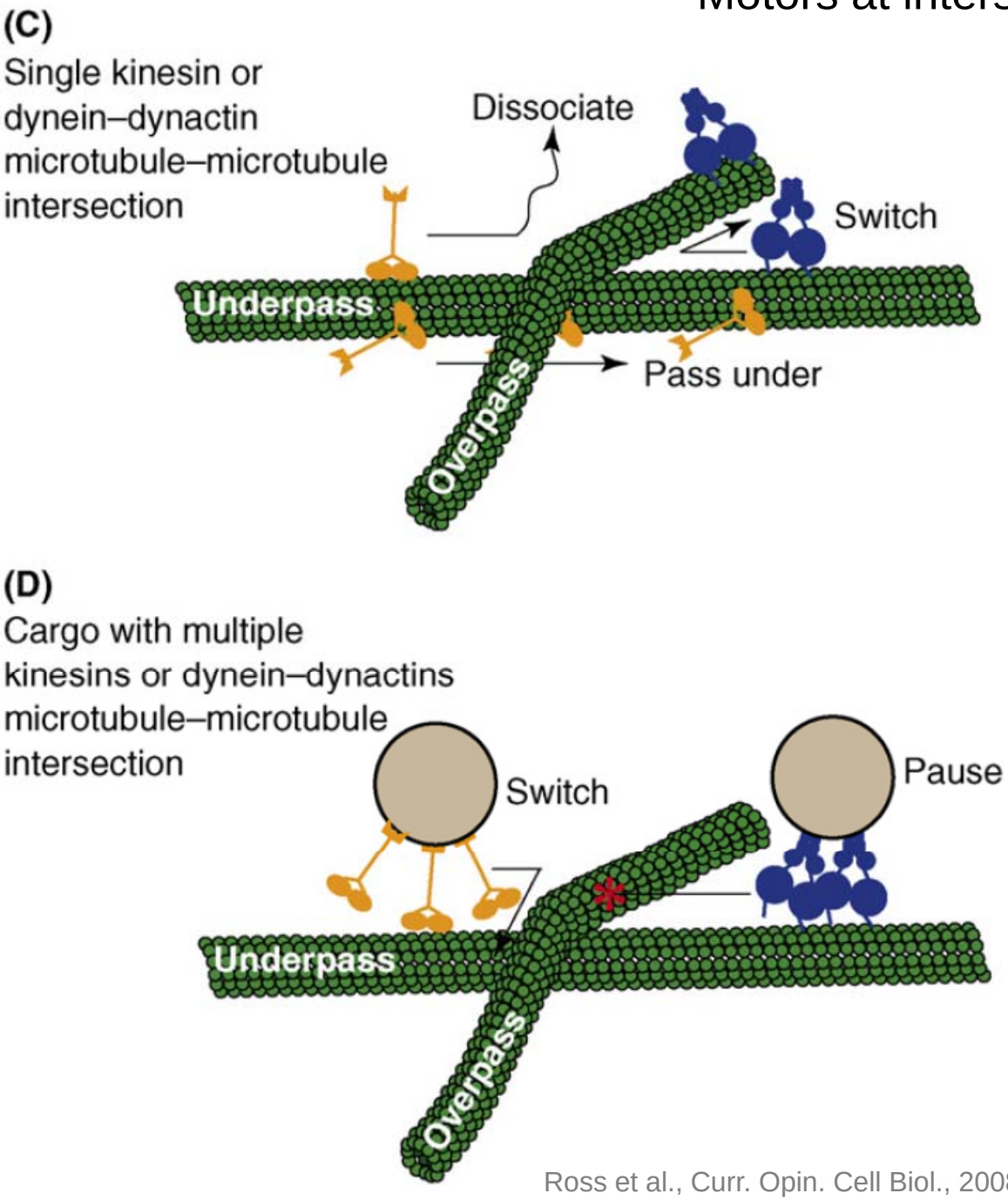
Cilia and diseases:
• Loss of IFT proteins can cause **ADPKD** = ***autosomal recessive polycystic kidney disease***
• Defect in IFT transport of **photoreceptor cilia** can cause ***retinal degeneration***
• Cilia and basal bodies are affected in the ***Bardet-Biedl syndrom***: loss of ability to smell and retinal degenration

Motors at intersecting cytoskeletal filaments



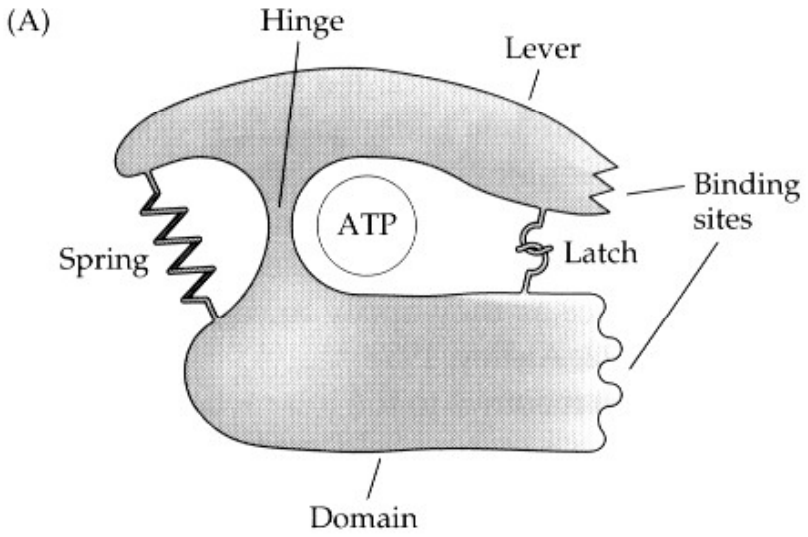
Ross et al., Curr. Opin. Cell Biol., 2008

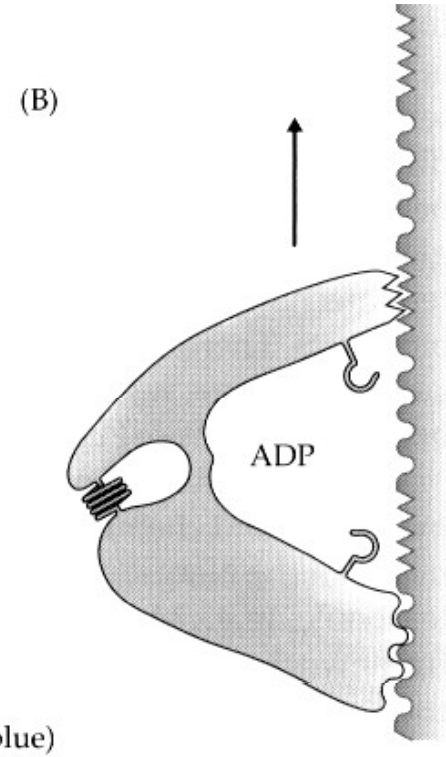
Motors at intersecting cytoskeletal filaments

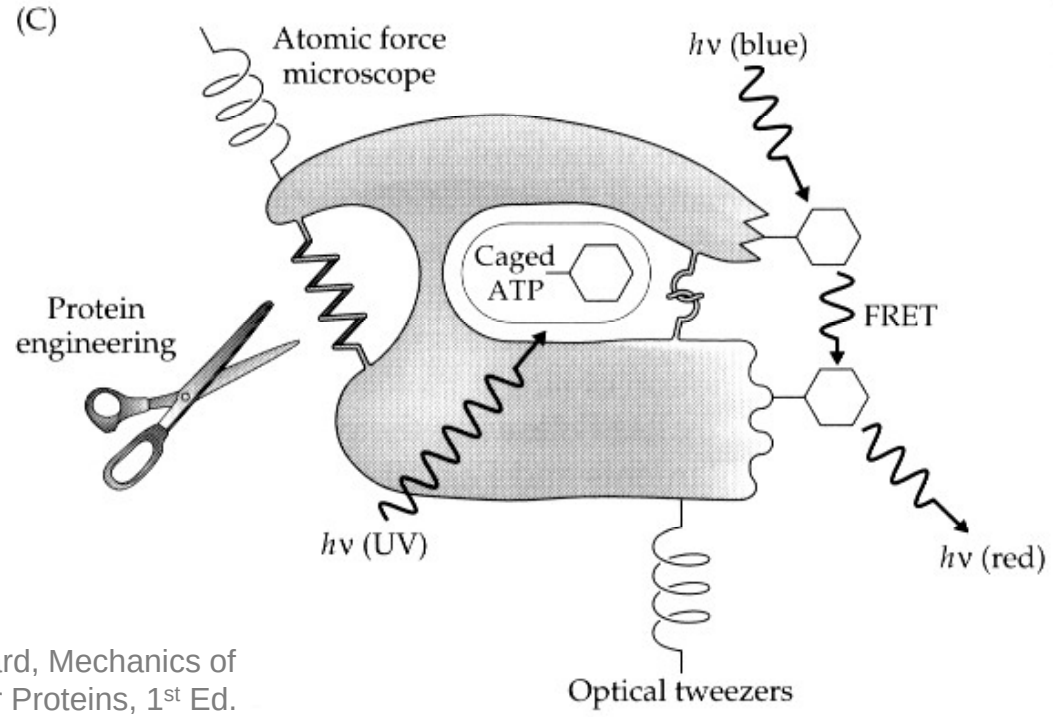


Dynein can pass at low motor densities (on the bead) but pause at high motor densities

Ross et al., Curr. Opin. Cell Biol., 2008





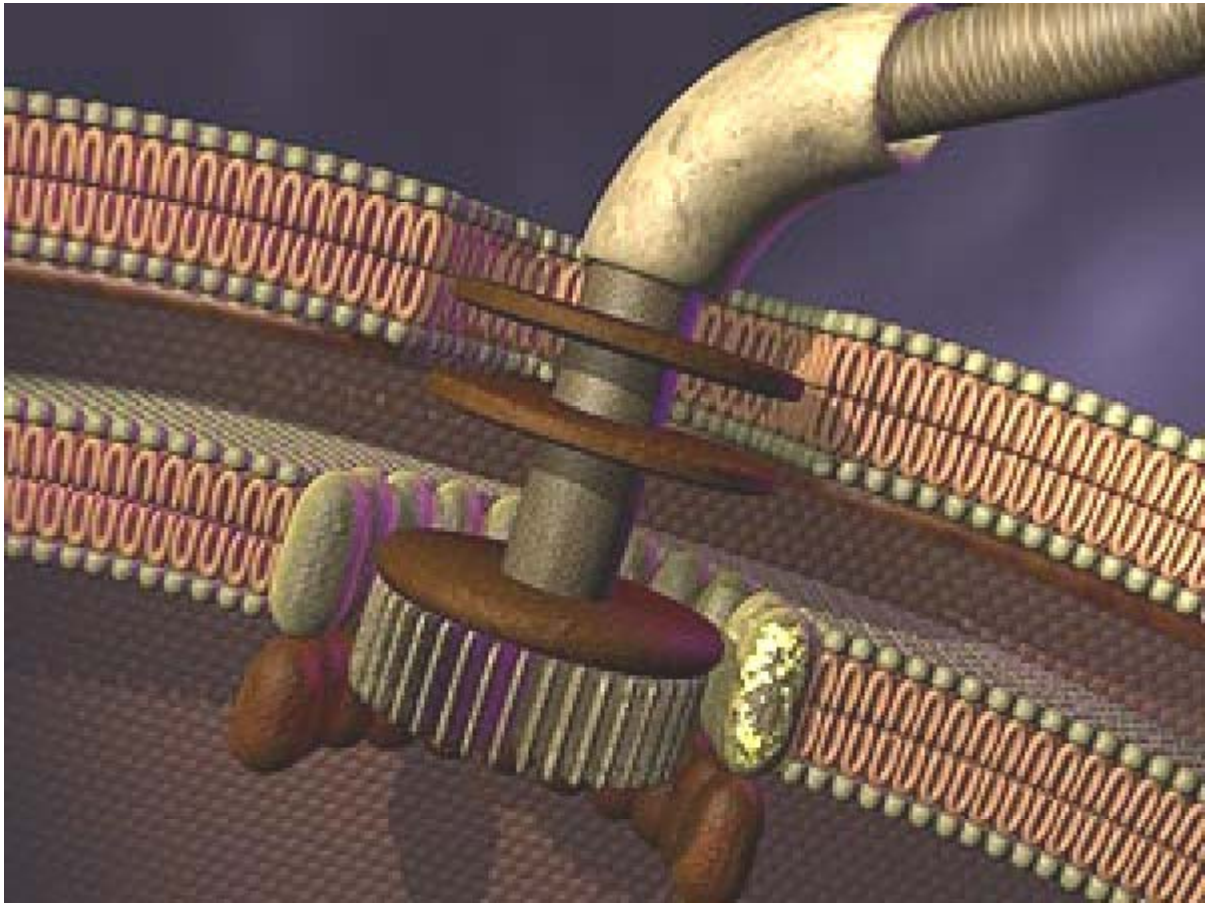


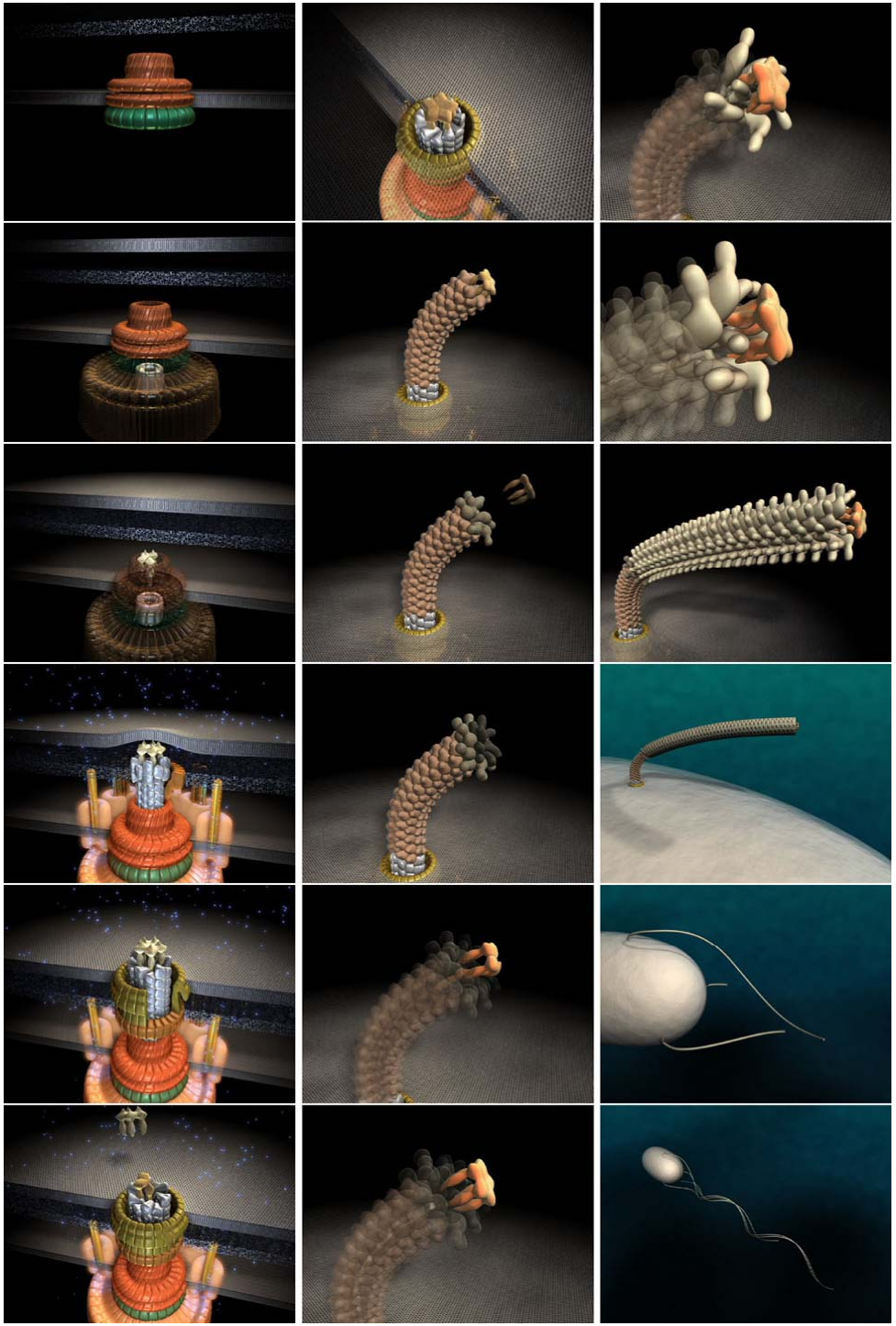
Howard, Mechanics of Motor Proteins, 1st Ed.

Protein as a machine

- Motion is produced by **cleft opening** (after ATP hydrolysis) assisted by a **spring** (and the **hinge**)
- The rigidity of a biological nanomachine is similar to that of **hard plastic**
- Still, compared to a machine made out of plastic, molecular motors response million times faster to an applied force
- Release of **caged ATP** can be measured and its binding to a domain
- Movement of protein domains relative to each other can be measured by **FRET**
- **AFM** or **optical tweezers** can exert force to measure mechanical properties
- **Site-directed mutagenesis** for functional protein analysis

The bacterial flagellum motor

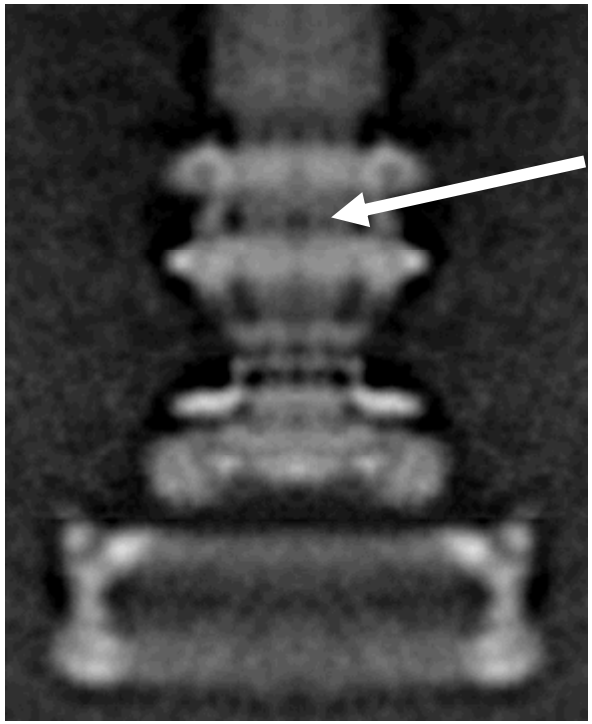




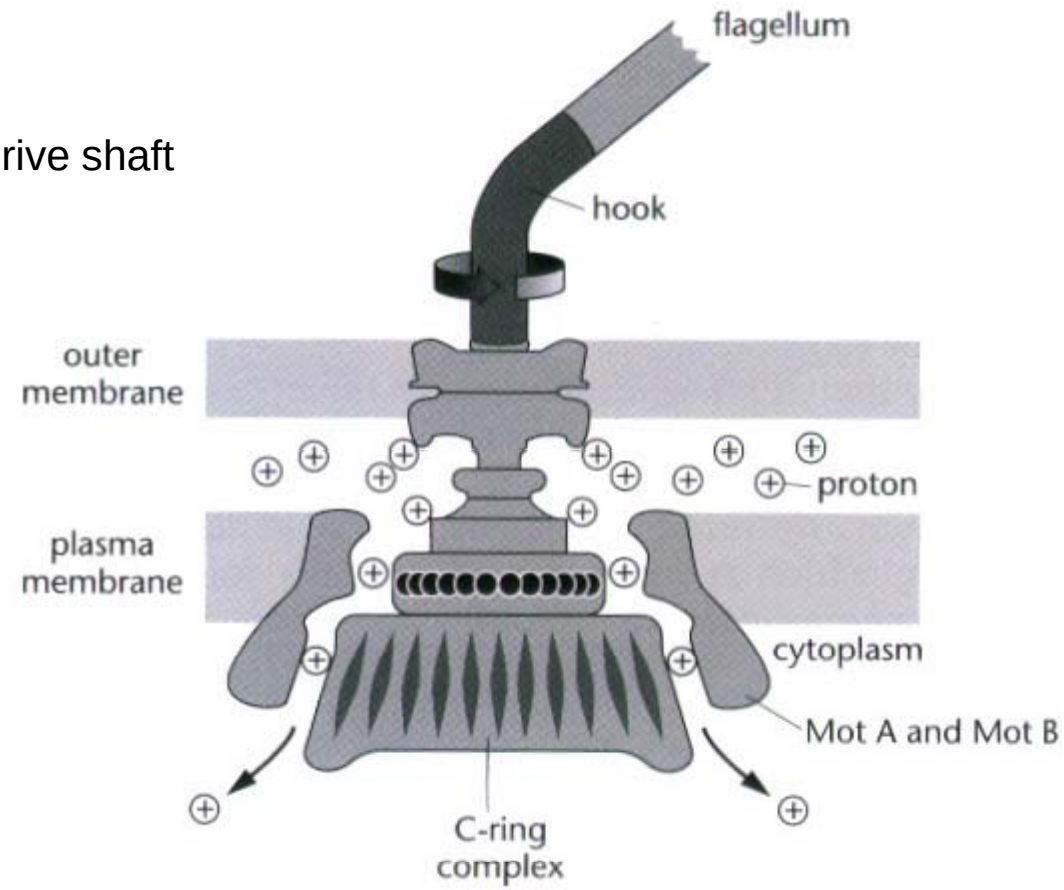
The bacterial flagellum motor

- Motor composed of **20 proteins**
- **40 genes** needed to make the motor and its flagellum
- **8 torque** (turning) **generators** driven by proton motion force (no ATP required)
- **1200 protons** needed per turn
- **100 revolutions** per second
- **18,000 RPM** (300 Hz)
- Directional reverse within 1/10 of second
- Efficiency: < 5%
- Power output: 10^{-15} Watt (2-3 more efficient than ATP motors)

A proton gradient drives the motor like water drives a turbine



Low-dosage EM and
image-averaging techniques

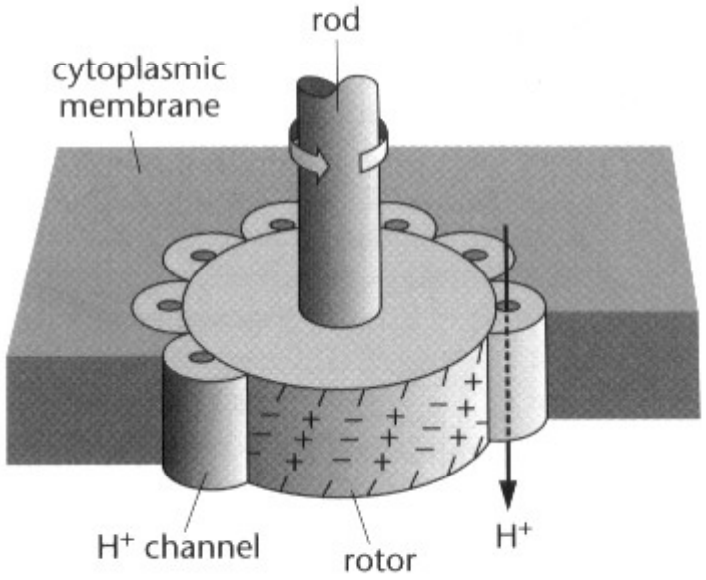


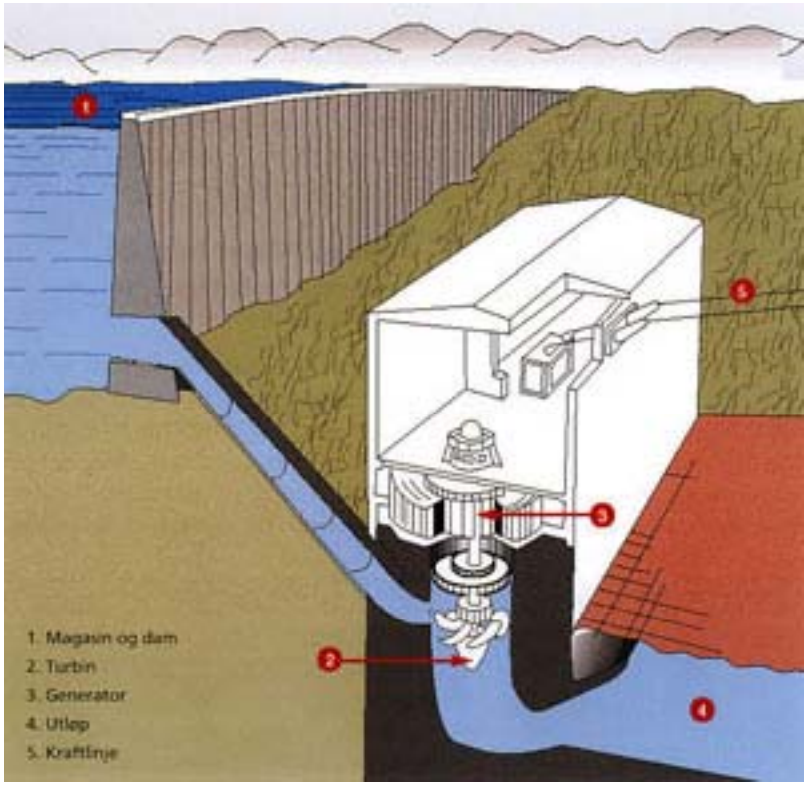
- **Drive shaft** passes thru two rings in the outer membrane. The rings act as bearings. Not involved in force generation
- **C-ring** (cytoplasmic ring) is important for force production and directional reversal
- **MotA** and **MotB** rings form the proton channels

Howard, Mechanics of Motor Proteins, 1st Ed.

Electrostatic model of the flagellum motor

Rotor-stator (motor) interaction generates the torque (turning):





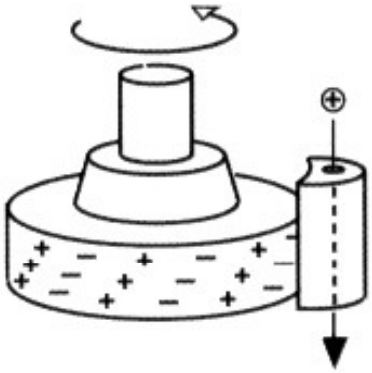
A water-flow driven turbine

Alternating charges on the rotor might be used to **drive the motor**:

- The lines of charges on the rotor are tilted with respect to the channels
- As **positive protons** move thru the channels, they **attract negative charges** on the rotor
- These electrostatic attraction forces might turn the motor

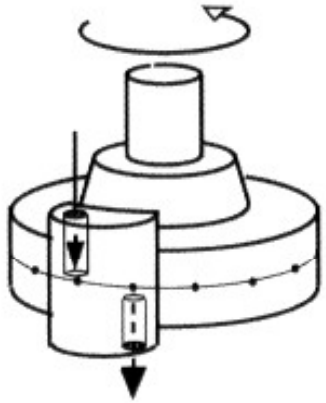
Howard, Mechanics of Motor Proteins, 1st Ed.

Conceptual models of rotor-stator interactions



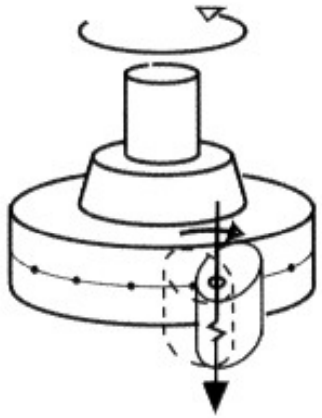
Turbine

Protons driven thru the stators **interact with charged components** of the rotor: alternate negative or positive charges attract or repeal transient protons generating a rotation of the MS-ring



Turnstile

Similar to the F_0 ATP-synthase motor: one proton can only completely pass if the motor has moved a certain distance (by ion-ion interactions or thermal fluctuation). However, extensive mutagenesis has so far delivered **no proof** for an proton-binding site of the rotor.



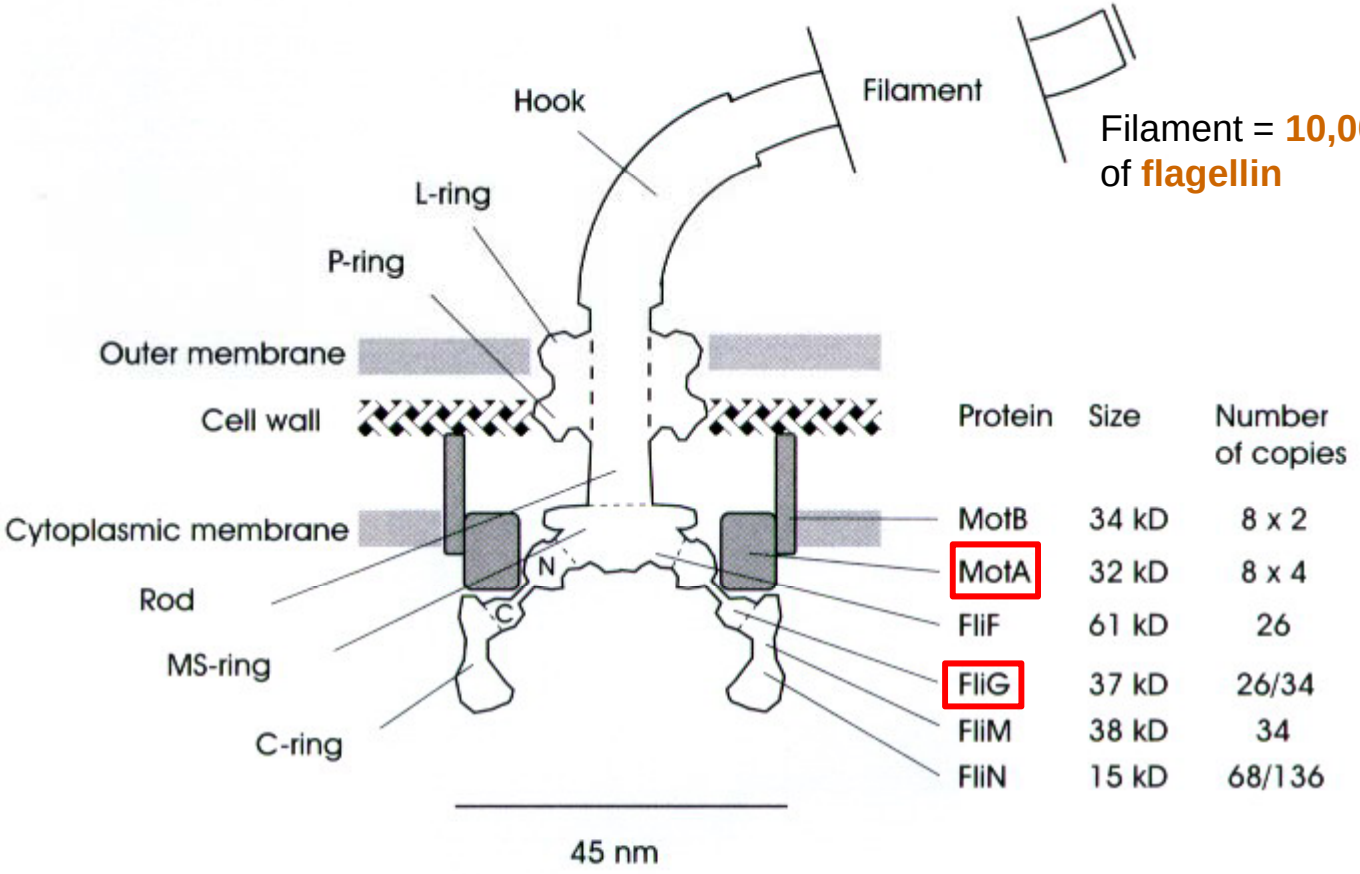
Crossbridge

Stator is tightly bound to the rotor. Incoming proton causes conformational change in the stator forcing the rotor to turn. Indeed, it has been shown that protonation of MotA leads to conformational changes.

Schliwa, Molecular Motors, 1st Ed.

Detailed view of the bacterial flagellar motor

- **White parts** in the diagram below are rotation parts; **gray parts** are fixed (**stators**)
- Important domains for torque generation are **MotA** and **FliG** (rotor-stator interaction)
- **MS-ring** is composed of a single protein FliF
- **C-ring** is composed of FliG, FliM and FliN



Filament = **10,000 copies** of **flagellin**

Protein	Size	Number of copies
MotB	34 kD	8 x 2
MotA	32 kD	8 x 4
FliF	61 kD	26
FliG	37 kD	26/34
FliM	38 kD	34
FliN	15 kD	68/136

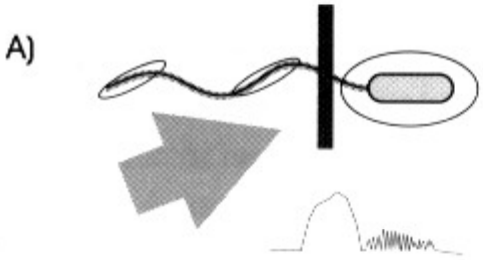
Torque-speed relationships

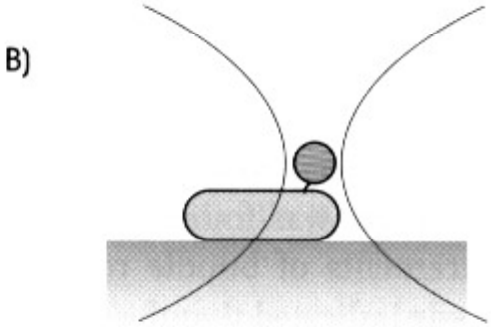
Methods to measure rotation of single motors:

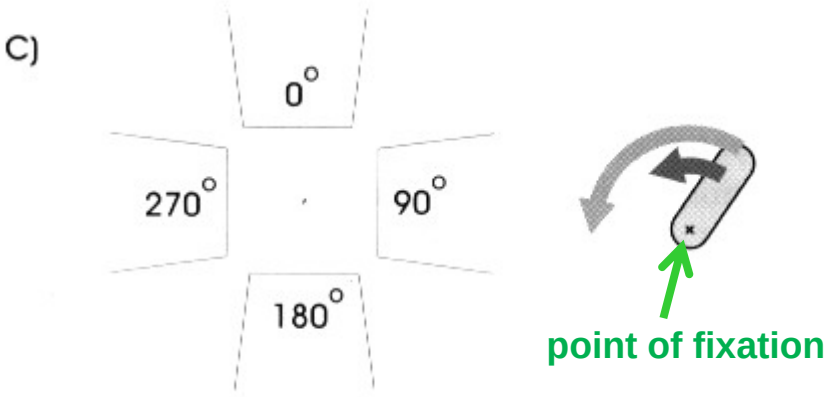
A) Laser illumination: flagellum illuminated by a laser thru a small slit (dark bar)

B) Polystrene beads: beads are attached to flagella and deflection monitored by laser

C) Oscillating voltage: fixed bacterium are exposed to an oscillating (90°) voltage field

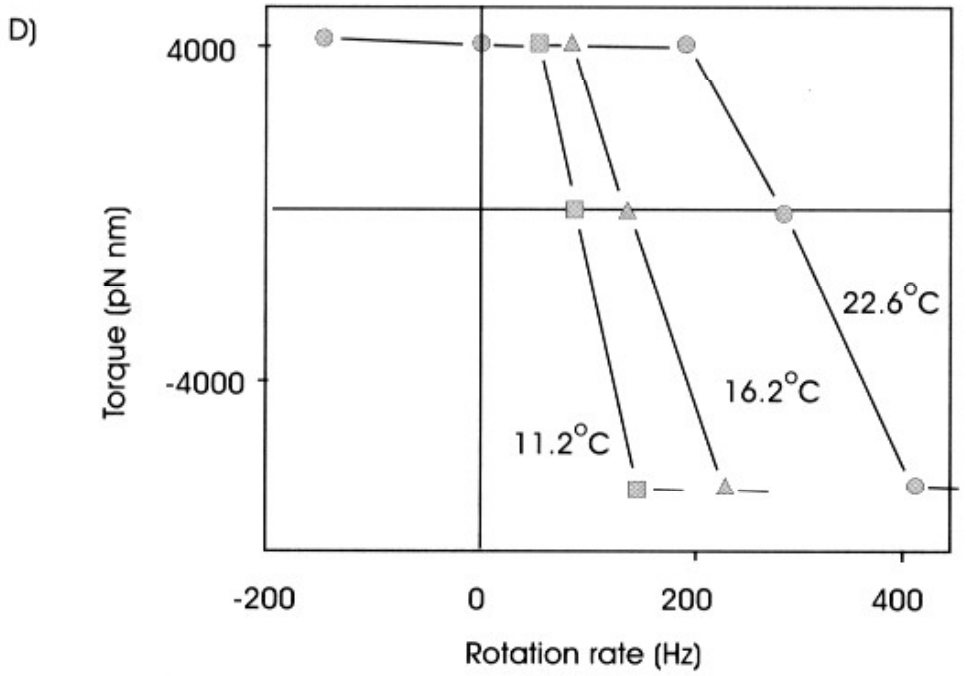






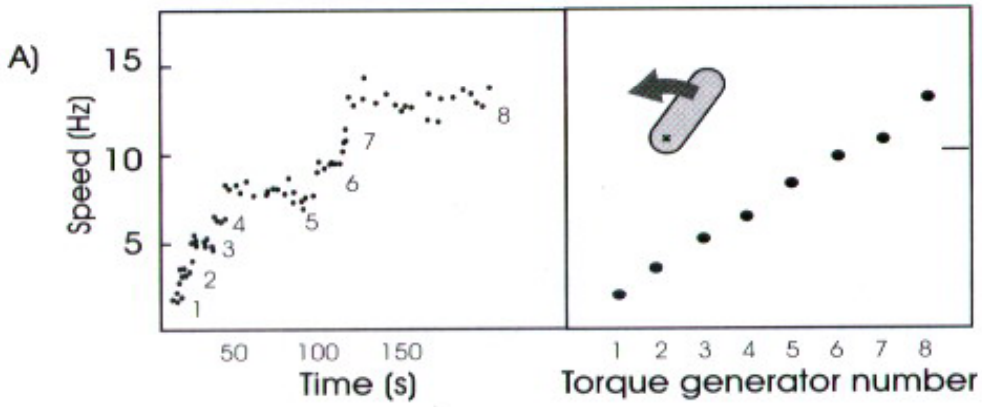
Rotating (90°) electric field around bacterium generates torque in flagellar

Result: Flagellar motor generates constant torque up to a certain speed (depend on temperature). At higher speeds the motor torque decreases until it stops

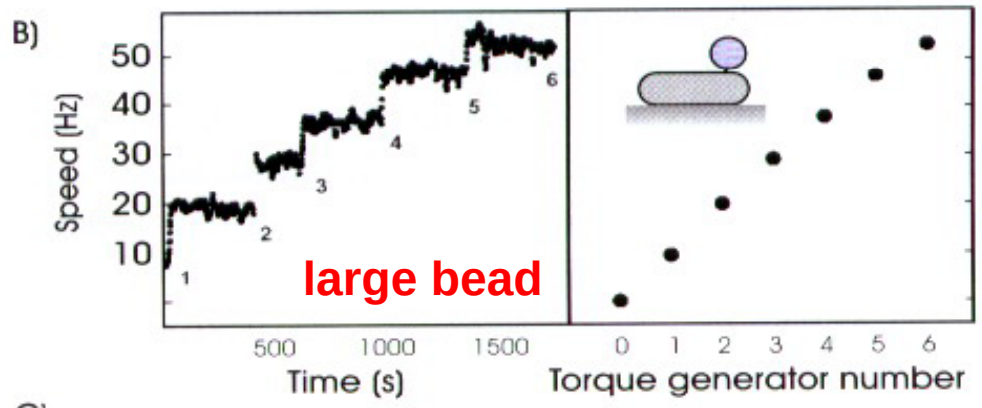


Schliwa, Molecular Motors, 1st Ed.

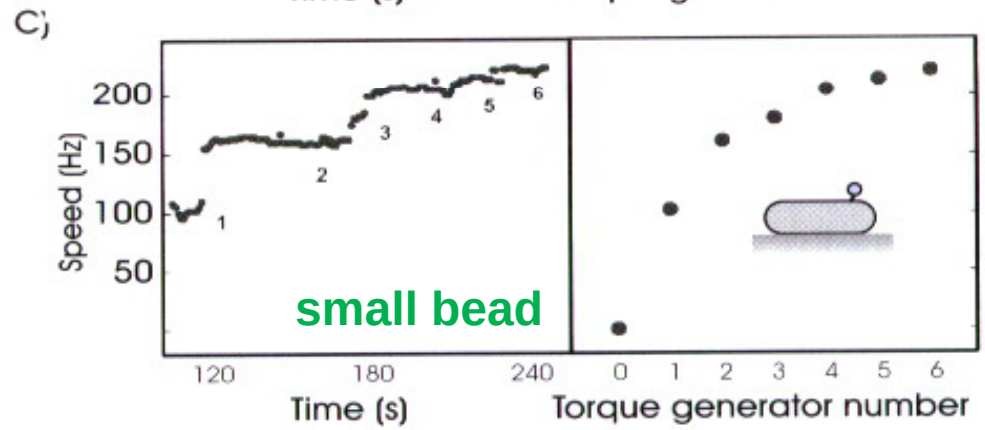
Torque generators work independently

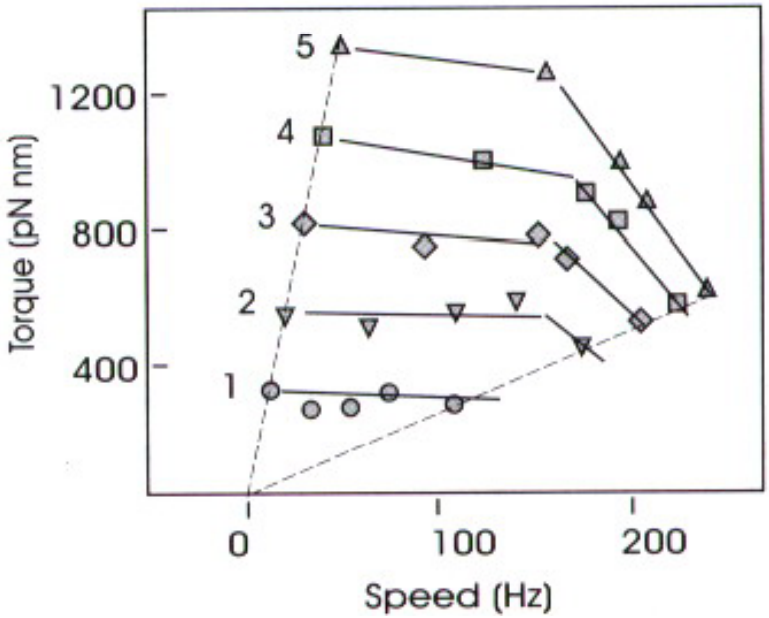


Sequentially expression of all 8 MotA/B:
• Bacterial <u>rotation appears stepwise</u> in **discrete speed increments** (upon expression of each MotA/B protein)
• **Maximum number** of <u>speed increments</u> = **eight** (steady motor speed)



<u>Torque-speed curves</u> show <u>similar pattern</u> for all speed increments indicating that all <u>8 generators work independently</u>

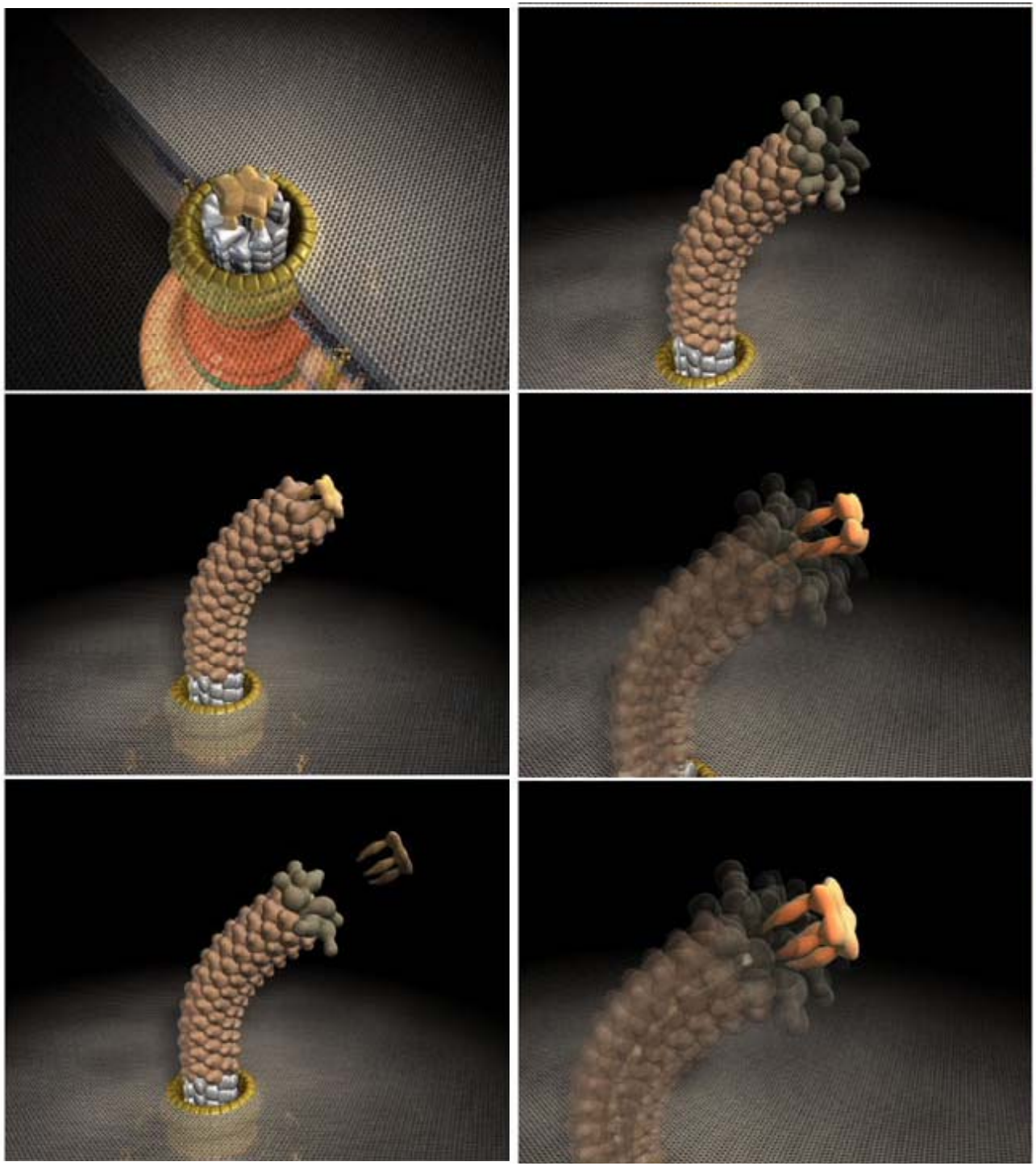




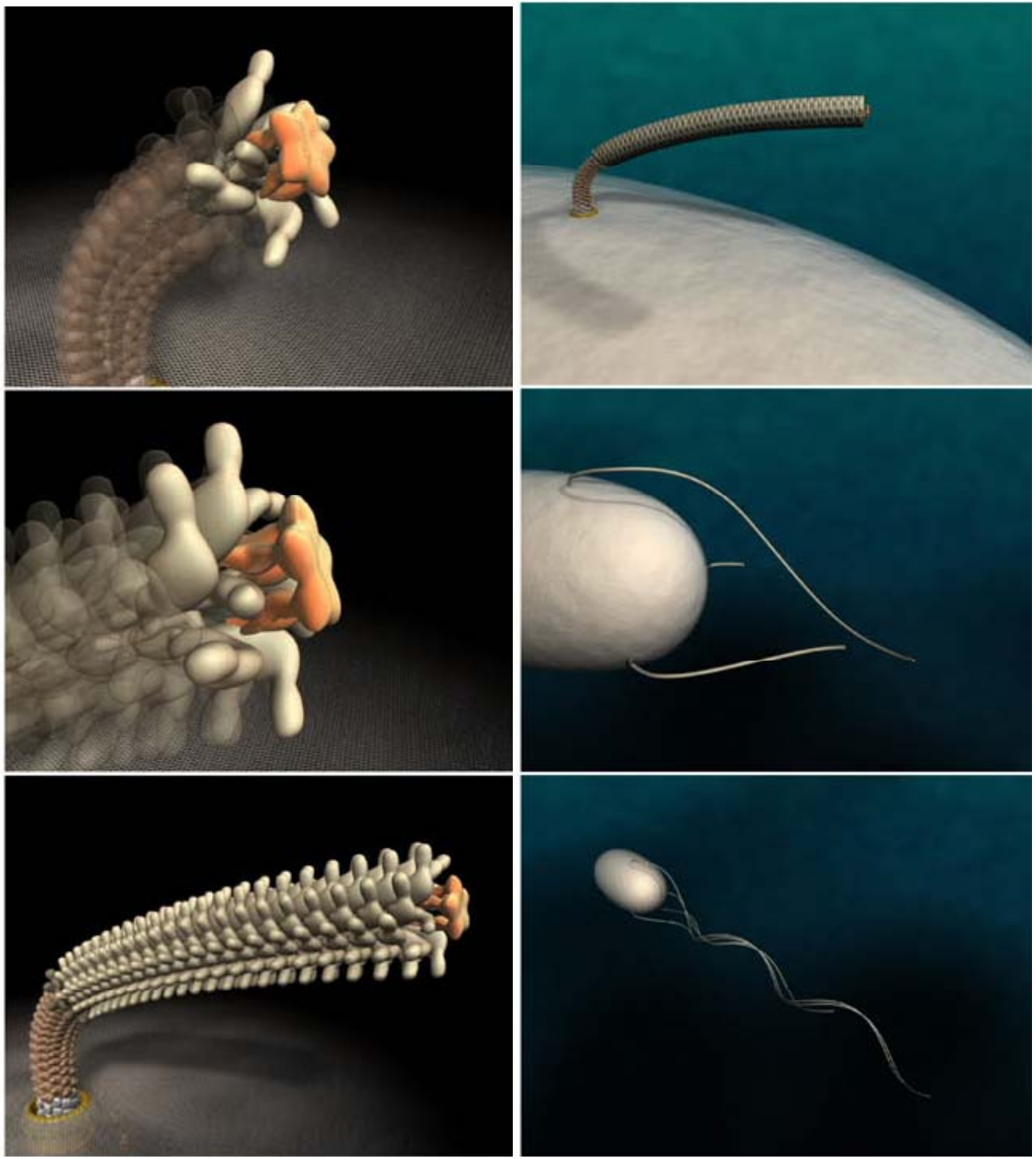
Schliwa, Molecular Motors, 1st Ed.

• Flagellar motor made of **20 different proteins**
• It spans across three layers of membranes: **outer membrane**, **peptidoglycan layer** and **cytoplasmic membrane**
• It consists of various components, such as a rotor, **stators**, a drive shaft, a **plug socket**, a rotation-switch regulator, and so on.

OM
PL
CM



• Rotary motor embedded at the base of a **helical filament**
• A short segment (55 nm) connects the motor and the helical propeller = **hook** (universal joint)



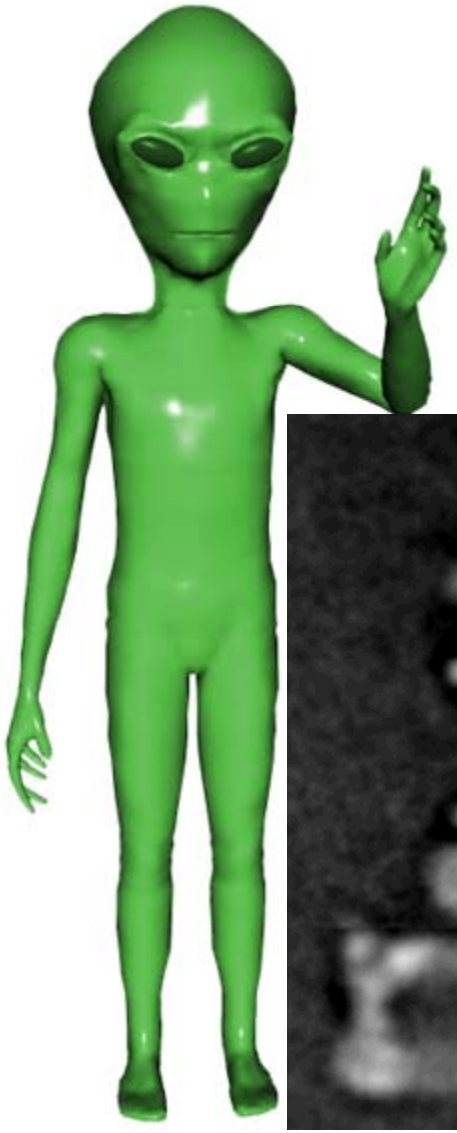
• **Flagellin** molecules (synthesized in the cytoplasm) transported to the end of the filament through the channel
• Flagellin binding is coordinated by a **rotating cap** (always preparing only one flagellin binding site)
• Cap movements looks like climbing up the helical stairs step by step.

Movie
bacterial_motor.wmv

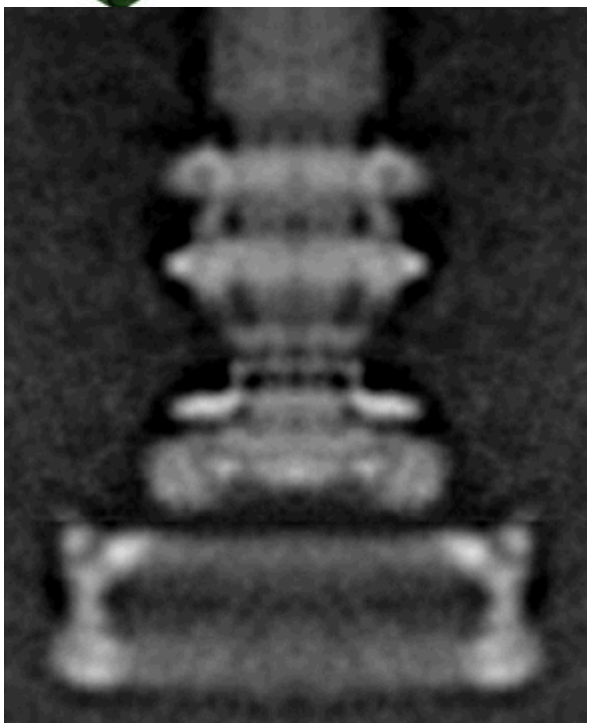
18:42

The bacterial flagella motor: Evolution or Intelligent design?

Intelligent design (ID) is the concept that some aspects of the natural universe are better explained by an **intelligent cause** rather than by an undirected process such as **natural selection**



- "The bacterial flagellum is an **irreducible** complex system" (a minimal, not reducible system)
- "This **complexity** could not have arisen through gradual variation or natural selection"
- "This system is so complex that it can only function when all of their components are present" (could not evolve from a simpler assemblage containing a fully functionary machinery)



nature REVIEWS MICROBIOLOGY

PERSPECTIVES

SCIENCE AND SOCIETY

From *The Origin of Species* to the origin of bacterial flagella

Mark J. Pallen and Nicholas J. Matzke

Protein	Location	Function	Indispensable?	Homologies*
FlgA	P ring	Chaperone?	Absent from Gram-positive bacteria	CpaB‡
FlgBCFG	Rod	Transmission shaft	Yes	FlgBCEFGK§
FlgD	Hook	Hook cap	Yes	
FlgE	Hook	Universal joint	Yes	FlgBCEFGK
FlgH	L ring	Bushing	Absent from Gram-positive bacteria	None yet known
FlgI	P ring	Bushing	Absent from Gram-positive bacteria	None yet known
FlgJ	Rod	Rod cap; muramidase	FlgJ N-terminal domain absent from some systems	None yet known
FlgK	Hook–filament junction	Hook-associated protein 1	Yes	FlgBCEFGK§
FlgL	Hook–filament junction	Hook-associated protein 3	Yes	FliC§
FlgM	Cytoplasm and exterior	Anti-σ factor	Absent from *Caulobacter*	None yet known
FlgN	Cytoplasm	Chaperone	Undetectable in some systems	None yet known
FlhA	T3SS apparatus	Protein export	Yes	LcrD/YscV‖
FlhB	T3SS apparatus	Protein export	Yes	YscU‖
FlhDC	Cytoplasm	Transcriptional regulator	Absent from many systems	Other activators†
FlhE	Unknown	Unknown	Mutant retains full motility	
FliA	Cytoplasm	σ factor	Absent from *Caulobacter*	RpoD, RpoH, RpoS‖
FliB	Cytoplasm	N-methylase	Absent from *Escherichia coli*	
FliC	Filament	Flagellin	Yes	FlgL§, EspA¶
FliD	Filament	Filament cap; hook-associated protein 2	Absent from *Caulobacter*	None yet known
FliE	Rod/basal body	MS ring–rod junction	Yes	None yet known
FliF	T3SS apparatus	Protein export	Yes	YscJ§
FliG	Peripheral	Motor	Yes	MgtE¶
FliH	T3SS apparatus	Regulates FliI	Mutant retains some motility	YscL*, AtpFH¶
FliI	T3SS apparatus	ATPase for protein export	Yes	YscN‖, AtpD‖, Rho‖
FliJ	Cytoplasm	Chaperone	Undetectable in some systems	YscO¶
FliK	Hook/basal body	Controls hook length	Yes	YscP¶
FliL	Basal body	Unknown	Mutant retains full motility	None yet known
FliM	T3SS apparatus	Protein export	Yes	FliN‡, YscQ†
FliN	T3SS apparatus	Protein export	Yes	FliM‡, YscQ†
FliO	T3SS apparatus	Protein export	Undetectable in some systems	None
FliP	T3SS apparatus	Protein export	Yes	YscR‖
FliQ	T3SS apparatus	Protein export	Yes	YscS‖
FliR	T3SS apparatus	Protein export	Yes	YscT‖
FliS	Cytoplasm	FliC chaperone	Absent from *Caulobacter*	None yet known
FliT	Cytoplasm	FliD chaperone	Absent from many systems	None yet known
FliZ	Cytoplasm	Regulator	Absent from many systems	None yet known
MotA	Inner membrane	Motor	Yes	ExbB†, TolQ†
MotB	Inner membrane	Motor	Yes	ExbD†, TolR†, OmpA†

- Only 50% of components are really necessary (=> indispensable)
- There is not "*the*" bacterial flagellum: thousands if not millions different bacterial flagellum systems exist
- Some are **redundant** and non-function and used for functions others than motility
- Several proteins have sequence homology indicating a **common ancestor**
- The flagellum evolved starting from just two proteins (e.g., **proto-flagellin**)
- Similarities between flagellar and non-flagellar systems exist
- Proto-flagellum could **easily arose** from pre-existing modules as the ATPase, polymerized filaments, ion-channels and domains of the chemotaxis apparatus

The bacterial flagella motor: Evolution or Intelligent design?