CELL MECHANICS

LECTURE 6

1. Introduction
2. Physical principles
3. Mechanics of motor proteins and cytoskeleton
4. Experimental techniques to study cell mechanics and mechanotransduction
5. Lab visit – experimental session

3. Mechanics of motor proteins and cytoskeleton

3.1. Motor proteins (types, working principles)

- Motor proteins classification
- Actin- and microtubule- based motor proteins.
- Force generation by Motor Proteins
- Muscle contraction
- Experiment example: a myosin II synthetic nanomachine

3.2. Cytoskeleton structure and Force generation by the cytoskeleton.

- Cytoskeleton filaments: types, structure, localization and function
- Cytoskeleton filaments polymerization and depolymerization.
- Forces generated by polymerization / depolymerization
- Cell motility induced by cytoskeleton filaments, examples.

NO

1. Howard Book, Ch. 9 Polymerization of Cytoskeleton Filaments; Ch. 11 Active polymerization; Ch 10 Force generation by cytoskeletal filaments
2. Alberts Book Ch 16. Self assembling

3.1. Motor proteins

3.1.1.Motor proteins classification

3.1.2. Actin- and microtubule- based motor proteins.

3.1.3. Force generation by Motor Proteins

3.1.4. Motor proteins and cellular functions

3.1.5. Muscle contraction

References:

1. Molecular Biology of the Cell. 4th edition. Alberts B, Johnson A, Lewis J, et al. New York, Garland Science; 2002. https://www.ncbi.nlm.nih.gov/books/NBK21054/ - Chapter 16
2. Articles e.g. For 3.1.1: 2013 Kolomeisky_J._Phys. _Condens._Matter_Molecular motors review 1

Motor proteins are very tiny engines (mechanochemical enzymes) that consume fuel (energy of biochemical reactions) to produce mechanical work useful for their biological functions .

Their working conditions are very different from the environment of macroscopic engines:

motor proteins operate in stochastic non-equilibrium isothermal systems that are also crowded by a large number of other chemically active biological molecules.

Motor proteins are nature's solution for directing movement at the molecular level. They display a very efficient and robust performance, and any major malfunction in them lead to cell death.

The application of advanced spectroscopic and microscopic methods have allowed researchers to visualize and manipulate motor proteins with a single molecule precision and high temporal resolutions, providing important information on how molecular motors operate.

Motor proteins can be divided into three main groups:

(1) Cytoskeleton filaments motor proteins

(2) Nucleic acids motor proteins

(3) Rotary motor proteins

(1) Cytoskeleton filaments motor proteins:

as **dyneins, kinesins** and **myosins**, are motor proteins that work along the cytoskeleton filaments: **actin filaments** and **microtubules.**

These motor proteins (MP) utilize the energy of hydrolysis of ATP (adenosine triphosphate) or related compounds. They are the main players in cellular transport processes.

Cytoskeleton MP are the most studied systems from the dynamic and structural points of view, and our current understanding of the mechanisms of energy transduction in molecular motors comes mainly from experiments on kinesin and myosin motor proteins.

(2) Nucleic acids motor proteins,

such as **helicases** and **polymerases,** usually function by associating with **DNA** and **RNA** molecules.

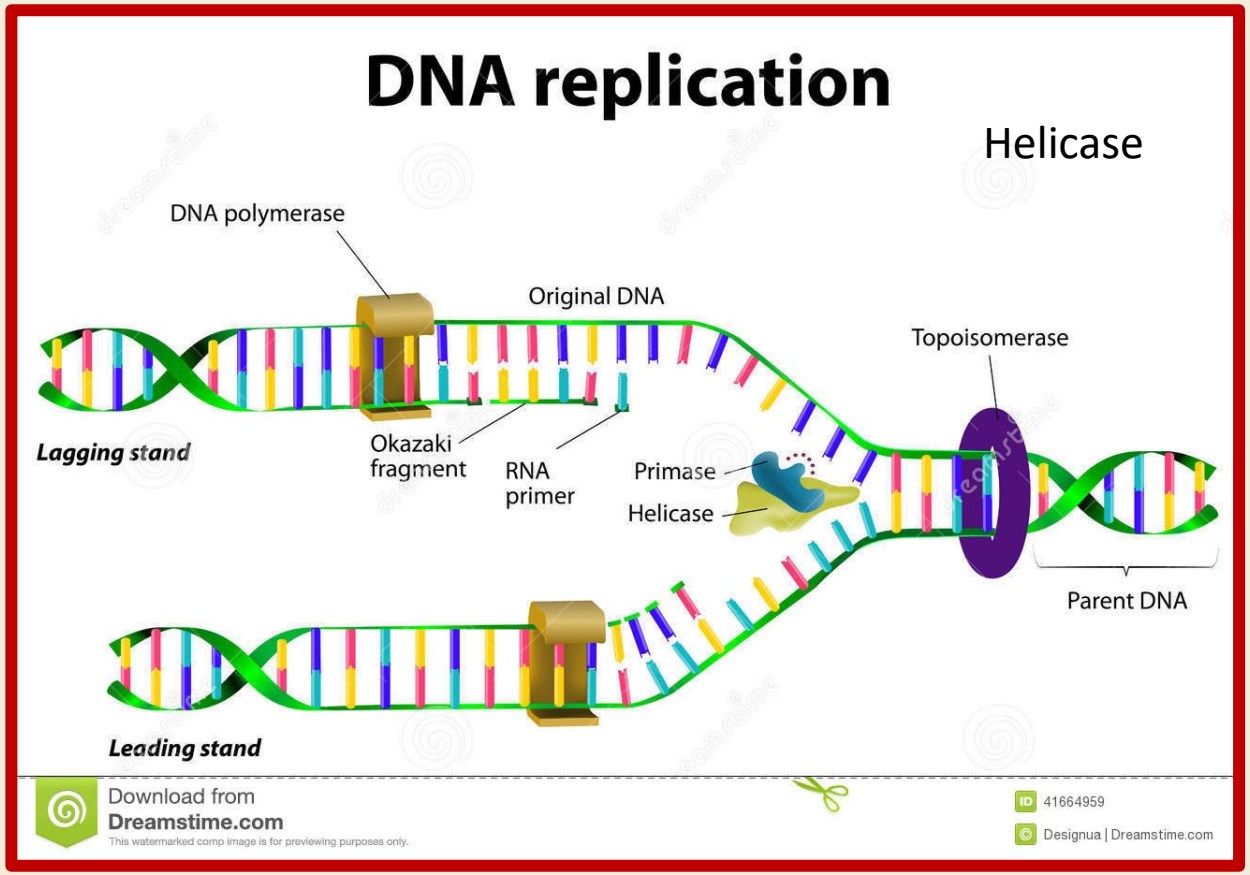
The source of chemical energy for these motors is the polymerization reactions of nucleic acids, synthesis of proteins and/or ATP hydrolysis.

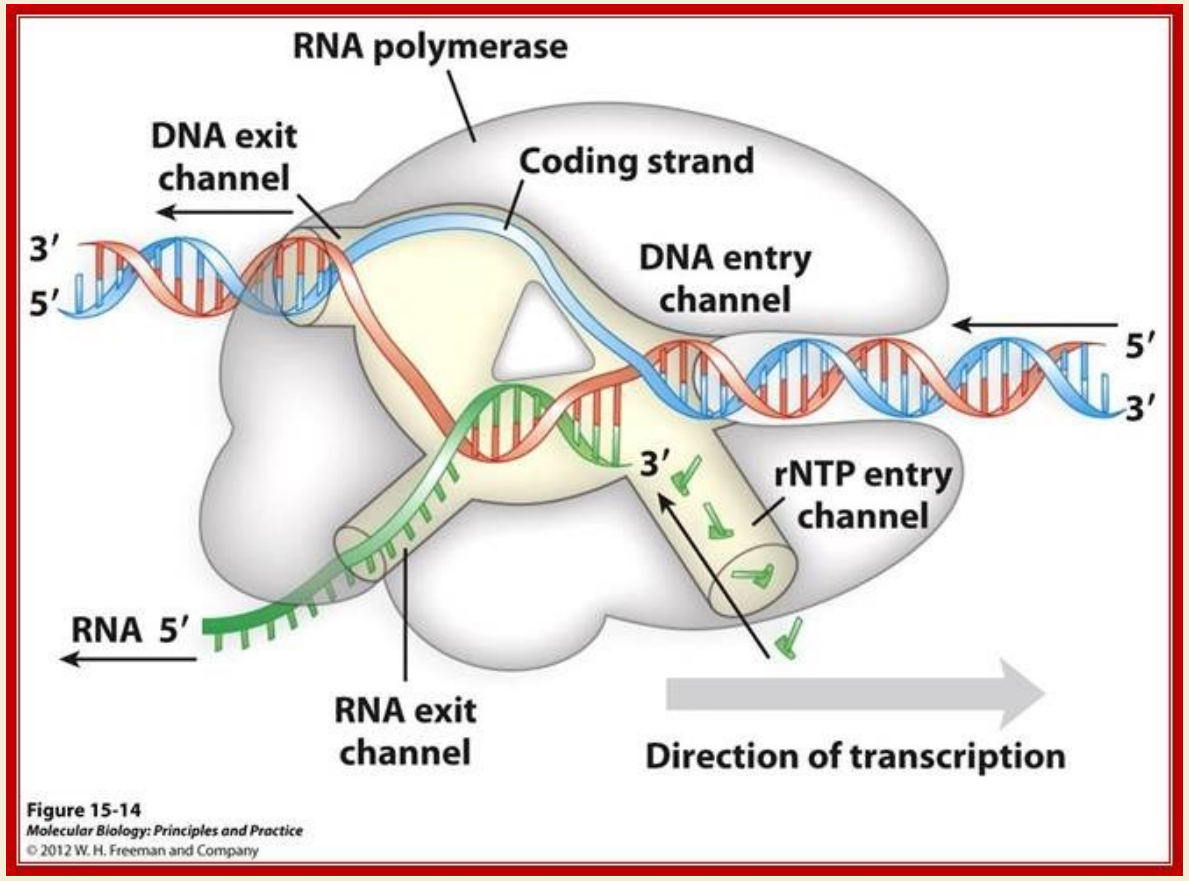
These MPs are important for the maintenance and processing of genetic information, as well as for the synthesis of all protein molecules in cells.

Helicase: MP that unpackage the organism's genes.

Polymerase : MP that synthesizes long chains of polymers or nucleic acids. DNA polymerase and RNA polymerase are used to assemble DNA and RNA molecules

DNA transcription





https://www.yourgenome.org/facts/what-is-dna-replication/

(3) Rotary motor proteins,

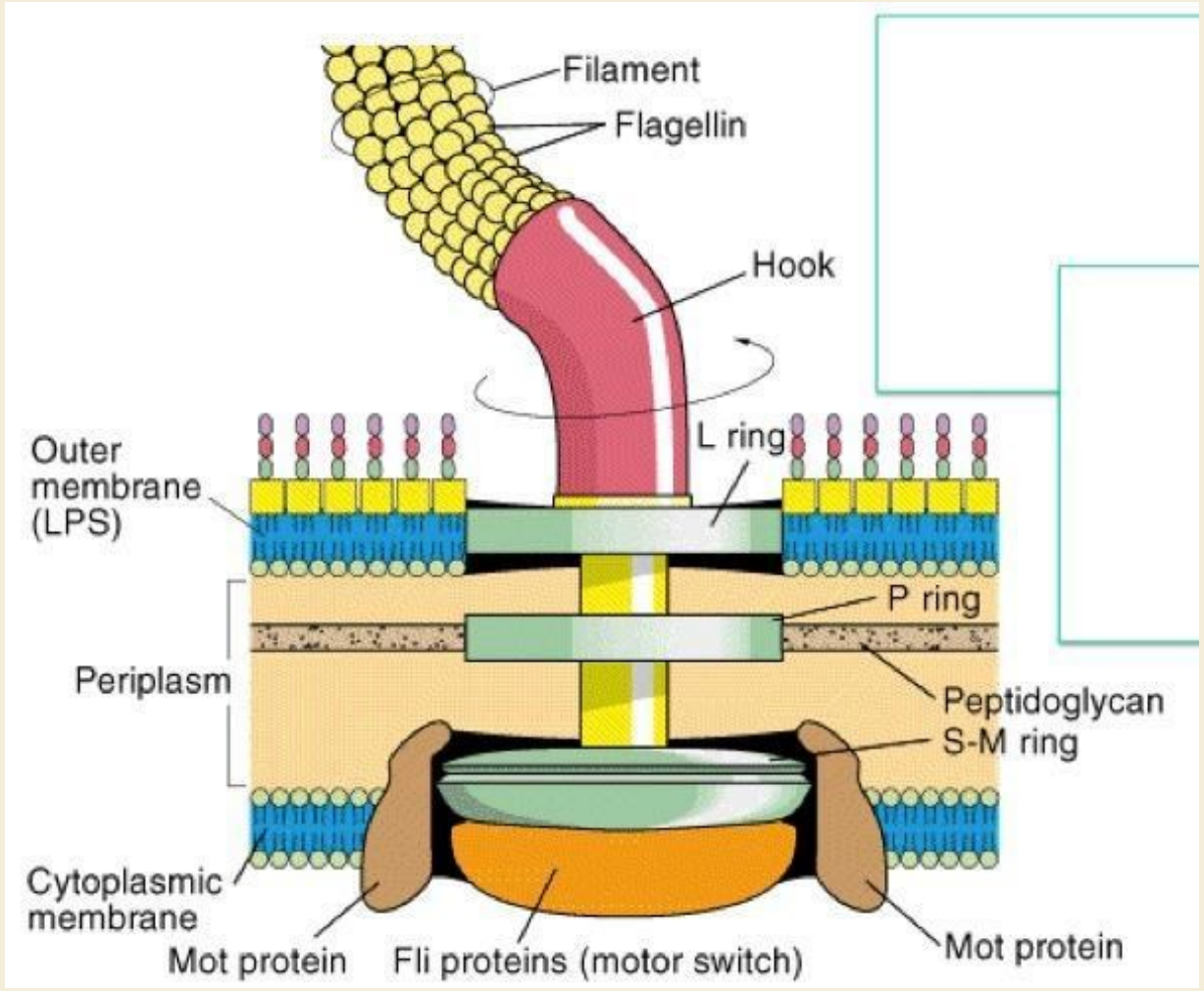
such as **bacterial flagella** (essential for bacterial migration) and **ATP synthase** (which is used to synthesize ATP molecules in mitochondria, are usually bound to cellular membranes.

Rotary MP are involved in circular motions in the membrane or outside of the cell.

Some of rotary motors utilize the electrochemical energy of various ion gradients that exist across cellular membranes.

These molecular motors are very important for cell motility and chemotaxis (i.e., for the cellular motion in the direction of available nutrients), but the mechanisms of these rotary MPs are understood much less in comparison with linear MPs.

Bacterial flagella structure - schematic



Nakamura , Biomolecules 2019, 9, 279; doi:10.3390/biom9070279

Processive and non-processive molecular motors

depending on **how many steps can be performed before detaching from the track**

Processive molecular motors:

motor proteins that, similar to railway locomotives or heavy tracks on highways, translocate along cytoskeleton filaments or nucleic acids in preferred directions by repeatedly hydrolyzing ATP molecules or polymerizing DNA, RNA or other protein molecules, taking hundreds of discrete steps before finally dissociating from the cytoskeleton filament.

Examples:

- **kinesin** and **dynein**, which move in opposite directions **along microtubule filaments**.
- **myosins V and VI motor proteins** translocate in opposite directions **along actin filaments**.
- **RNA and DNA polymerases** move with a high fidelity over long distances **along DNA molecules**.

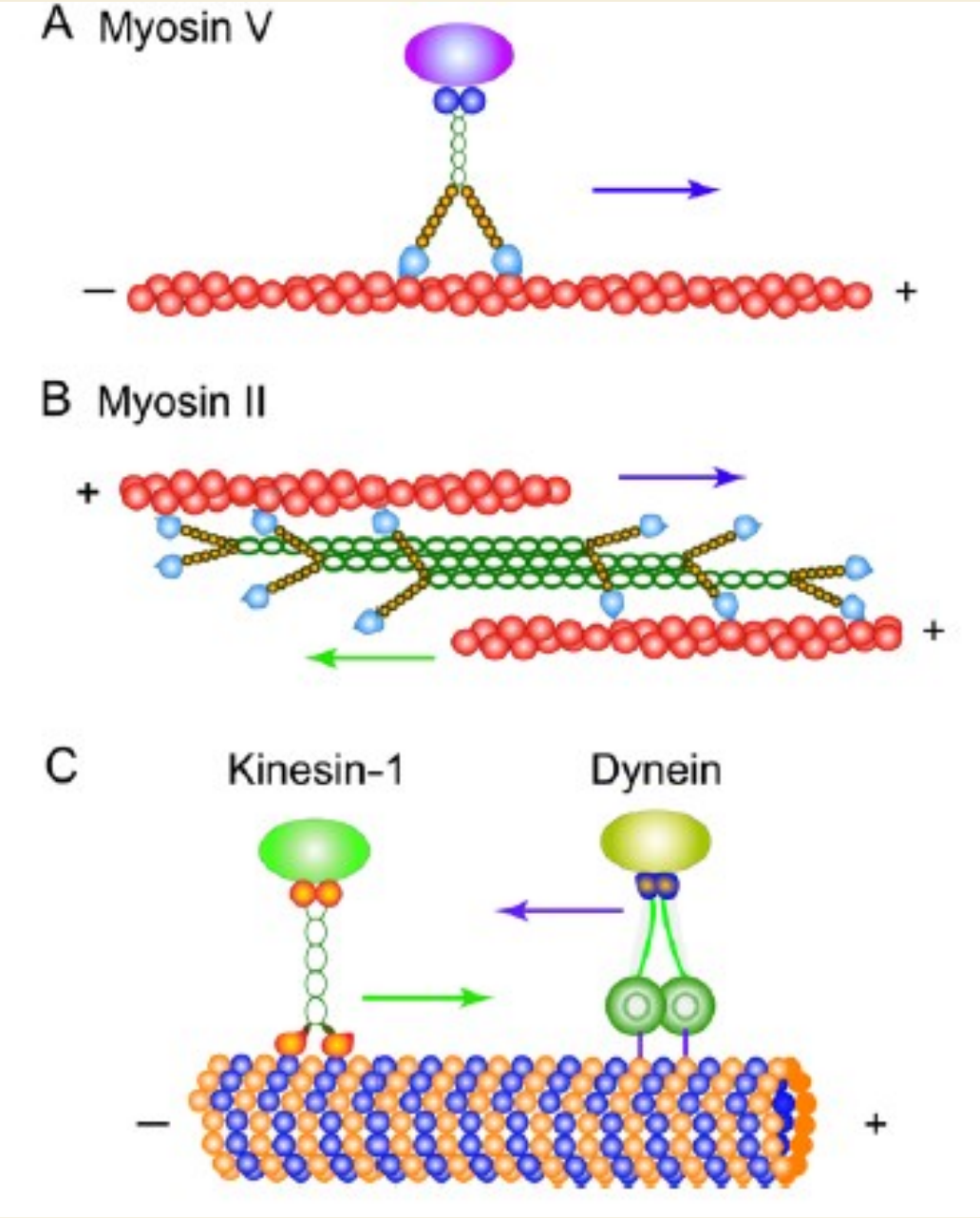
Note that protein filaments, as well as nucleic acids, can be viewed as polar tracks for motor proteins, effectively breaking the symmetry and specifying the preferred direction of the motor's motion.

Non-processive motor proteins function in cells only working **cooperatively** in large groups.

Non-processive motors typically make only one or few steps before detaching from their tracks.

Details of the **cooperative mechanisms** of these non-processive molecular motors are much less understood. It is widely believed that the specific processivity or non-processivity of motor proteins is closely related to their structure.

Non-processive molecular motors are often monomers, while **processive** motors mostly exist in multi-domain dimeric or oligomeric forms.



Linear MPs typically convert chemical energy to translocate along the protein filaments and/or nucleic acids in an effectively 1D fashion.

Schematic view of some of the most important linear MP

(A) Dimeric myosin V motor proteins step unidirectionally along actin cytoskeleton filaments.

(B) A group of monomeric myosin-II motor proteins combined in the filament can move together along several actin filaments.

(C) Conventional kinesin motor proteins translocate along the microtubules in the positive direction, while the dynein motors step along the microtubules in the opposite direction.

2013 Kolomeisky_J._Phys. _Condens._Matter_Molecular motors review 1

All motor proteins have a **multi-domain (*) structure** to support their successful functioning in the complex cellular environment. The regions of the molecule where biochemical reactions are catalyzed are known as **motor domains**. There are also **domains** that are responsible for **binding to cellular cargoes**, as well as regions that provide necessary **mechanical flexibility** and **chemical stability**.

For some motor proteins various domains might strongly interact with each other, modifying their individual enzymatic activities. For example, for some kinesins and myosins, partial unfolding of a cargo-binding domain might completely inhibit the catalytic activities of the motor head domains, i.e. these nanoscale trucks move only when there is an available load, and there is little futile consumption of the fuel from the cellular point of view.

Full structural information is very important for understanding the microscopic details of motor protein functioning. Mechanics experiments in physiological conditions at single molecule and single cell level are complementary to structural investigations and are important as well.

* A **protein domain** is a conserved part of a given protein sequence (and tertiary structure) that can evolve, function, and exist independently of the rest of the protein chain.

Motor proteins working along the **Actin** filaments **: Myosins** family

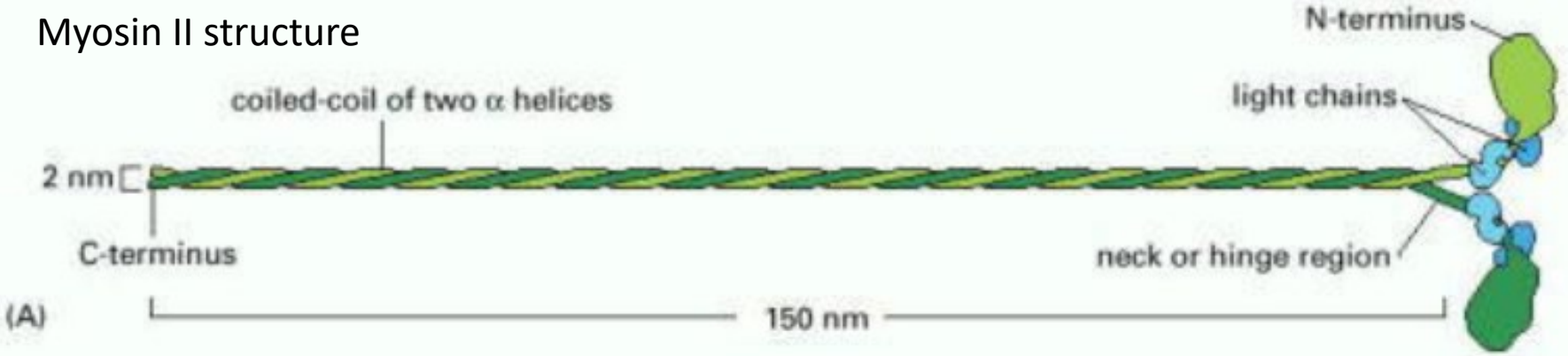
Motor proteins working along the **microtubules: Kinesins** and **Dyneins** families

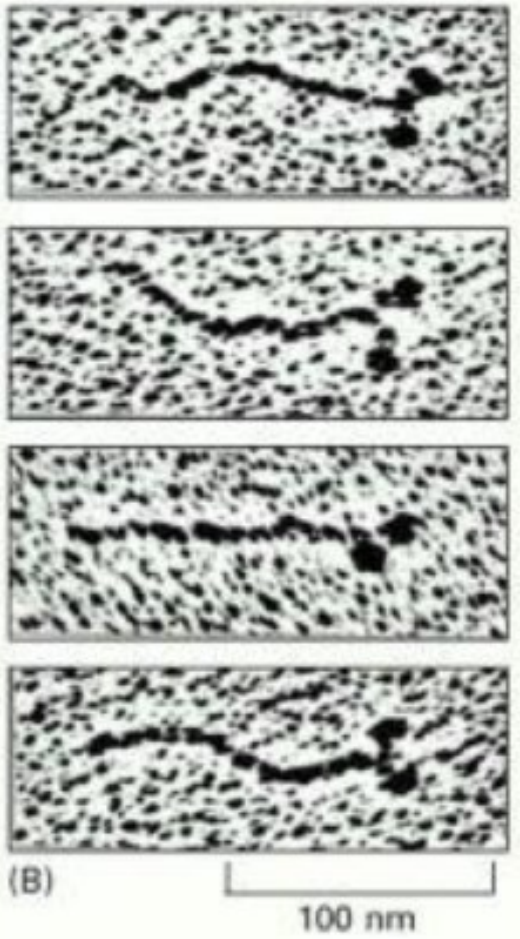
Actin-based motor proteins are members of the **myosin** superfamily – see the slides at the end

Here we discuss one of the most important: the **skeletal muscle myosin**, called **myosin II**

Myosin II is responsible for generating the force for muscle contraction.

Myosin II structure





EM myosin molecules shadowed with platinum

Myosin II is formed of two heavy chains (green) and four light chains (blue).

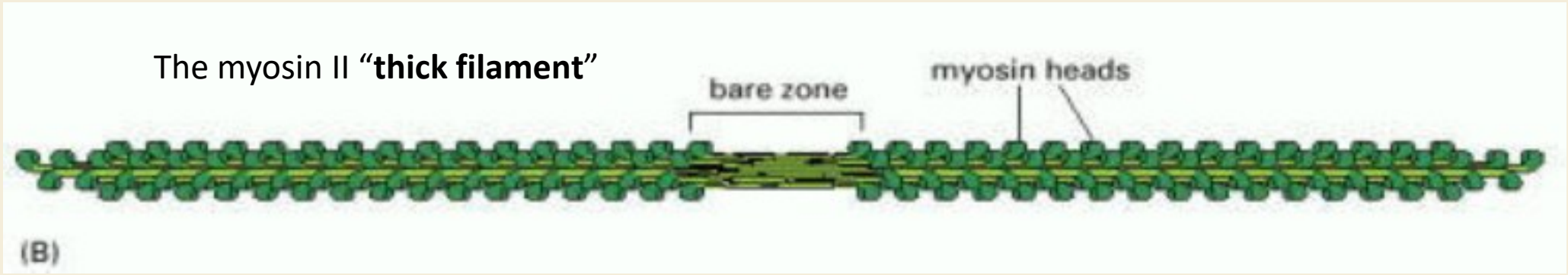
Each of the heavy chains has a globular head domain (approx. 900 amino acids, 90 kDa MM) at its N-terminus that contains the force-generating machinery.

The two light chains bind close to the N-terminal head domain.

The two α helices of the heavy chains wrap around each other to form a coiled-coil: the tail

The long coiled-coil tail bundles itself with the tails of other myosin molecules, resulting in large bipolar "**thick filaments**" that have several hundred myosin heads.

The myosin II "**thick filament**"



Each myosin head binds and hydrolyses ATP, using the energy of ATP hydrolysis to walk toward the plus end of an actin filament.

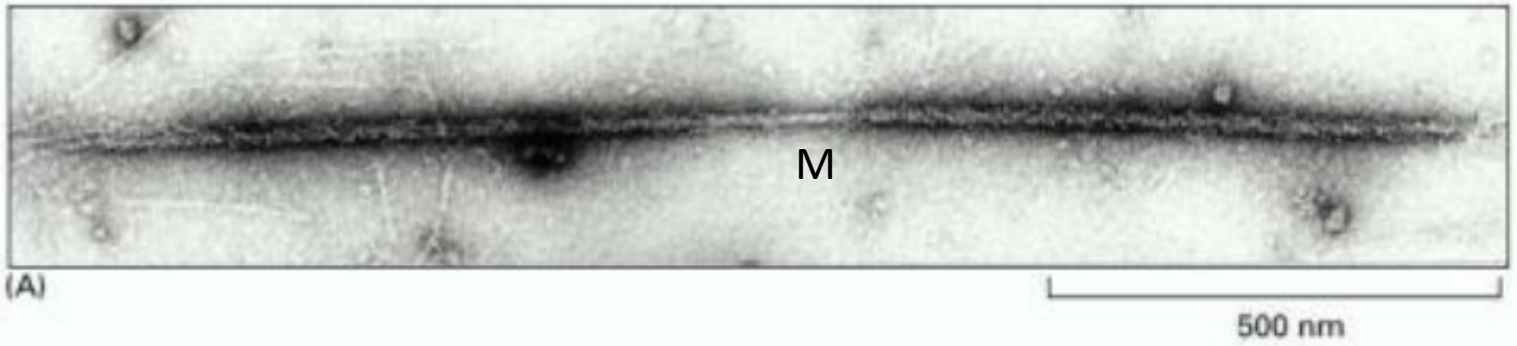
The opposing orientation of the heads in the thick filament makes the filament efficient at sliding pairs of oppositely oriented actin filaments past each other.

In skeletal muscle, in which carefully arranged actin filaments are aligned in "thin filament" arrays surrounding the myosin thick filaments, the ATP-driven sliding of actin filaments results in muscle contraction.

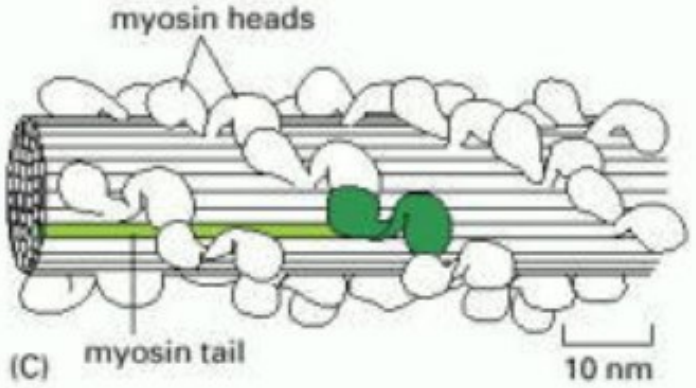
Plus end:
the end of a microtubule or actin filament at which addition of monomers occurs most readily;
(the "fast-growing" end of a microtubule or actin filament)

The myosin II bipolar “thick filament”

Electron micrograph



Schematic diagram.



Small section of a myosin II filament as reconstructed from EM

The myosin II molecules aggregate by means of their tail regions, with their heads projecting to the outside of the filament.

The bare zone in the middle (M) of the filament consists entirely of myosin II tails.

Microtubule Motor Proteins: **Kinesins** and **Dyneins**

Kinesin is a motor protein associated and working along microtubules.

Most kinesins work toward the plus end, but there are also few working toward minus end.

Kinesin is similar structurally to myosin II in having two heavy chains and two light chains per active motor, two globular head motor domains, and an elongated coiled-coil responsible for heavy chain dimerization.

The C-terminal domain forms a tail that attaches to cargo, such as a membrane-enclosed organelle.

Like myosin, kinesin is a member of a large protein superfamily, for which the motor domain is the only common element.

Dyneins are a family of minus-end-directed microtubule motors, but they are unrelated to the kinesin superfamily. They are composed of two or three heavy chains (that include the motor domain) and a large and variable number of associated light chains.

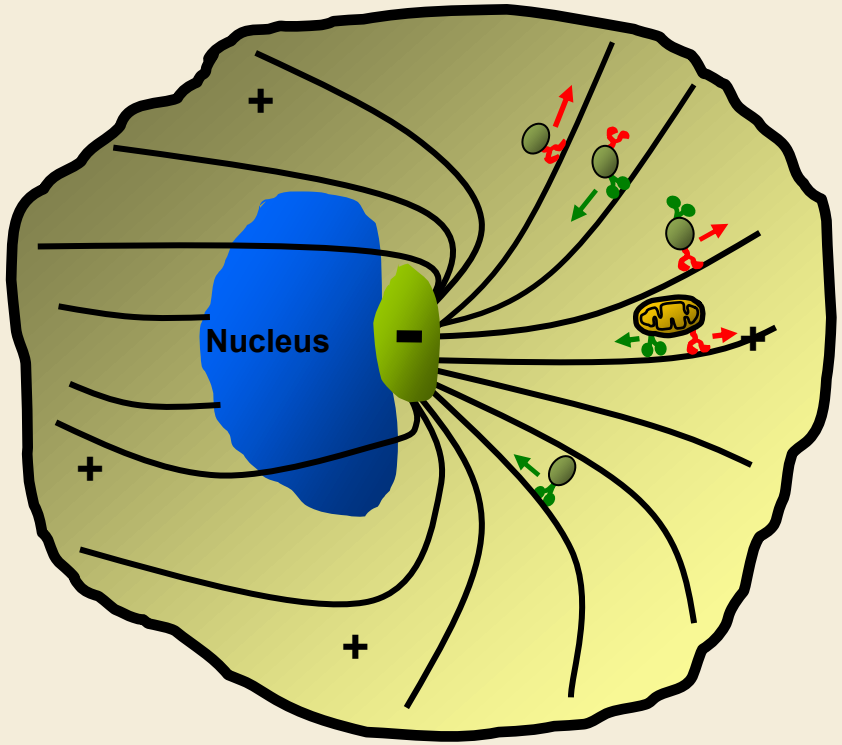
The dynein family has two major branches:

Cytoplasmic dyneins, which are typically heavy-chain homodimers, with two large motor domains as heads. Cytoplasmic dyneins are found in all eukaryotic cells, and they are important for vesicle trafficking, as well as for localization of the Golgi* apparatus near the center of the cell.

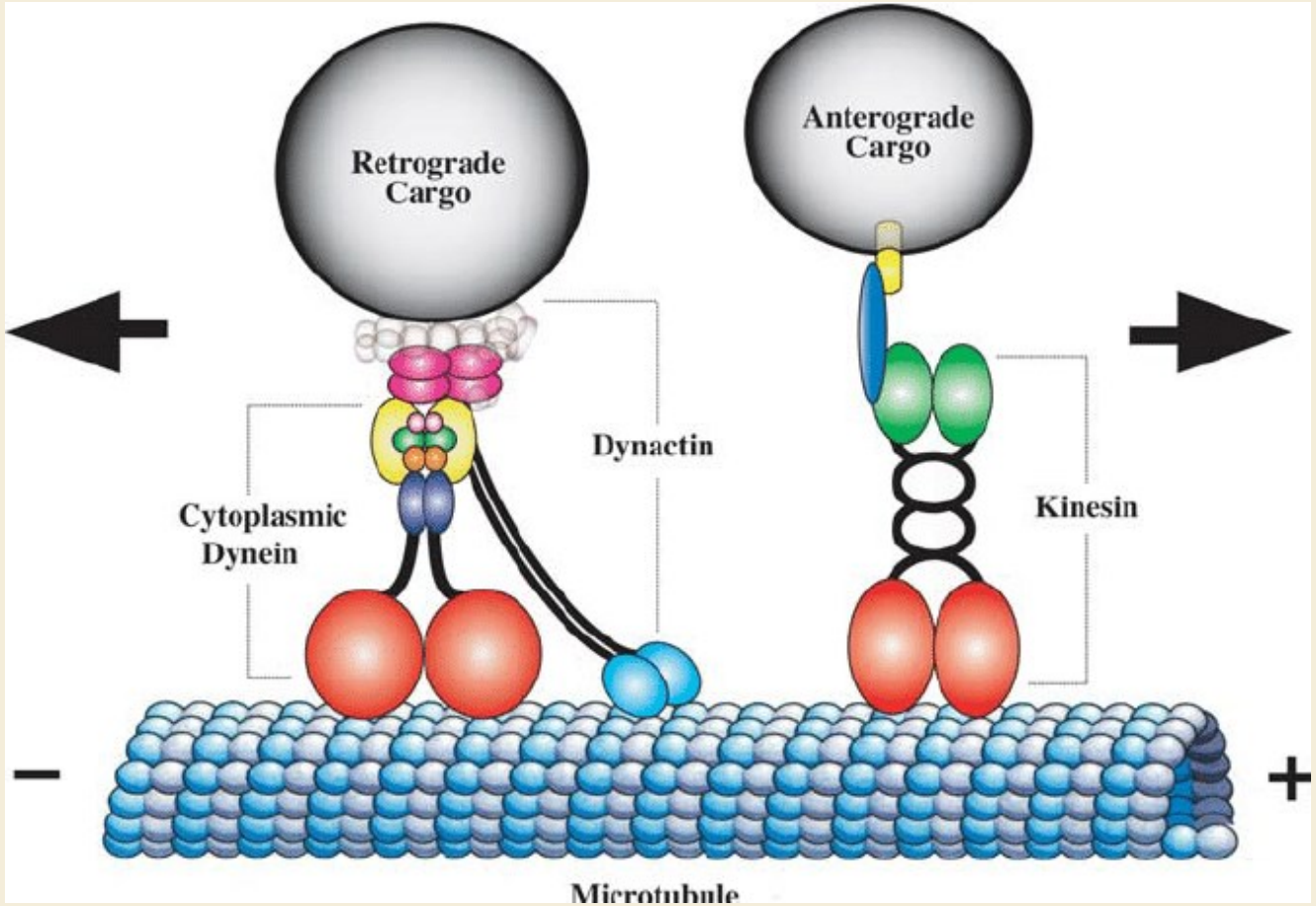
Axonemal dyneins, include heterodimers and heterotrimers, with two or three motor-domain heads, respectively. They are highly specialized for the rapid and efficient sliding movements of microtubules that drive the beating of cilia and flagella.

* The Golgi apparatus is responsible for transporting, modifying, and packaging **proteins** and **lipids** into vesicles for delivery to targeted destinations.

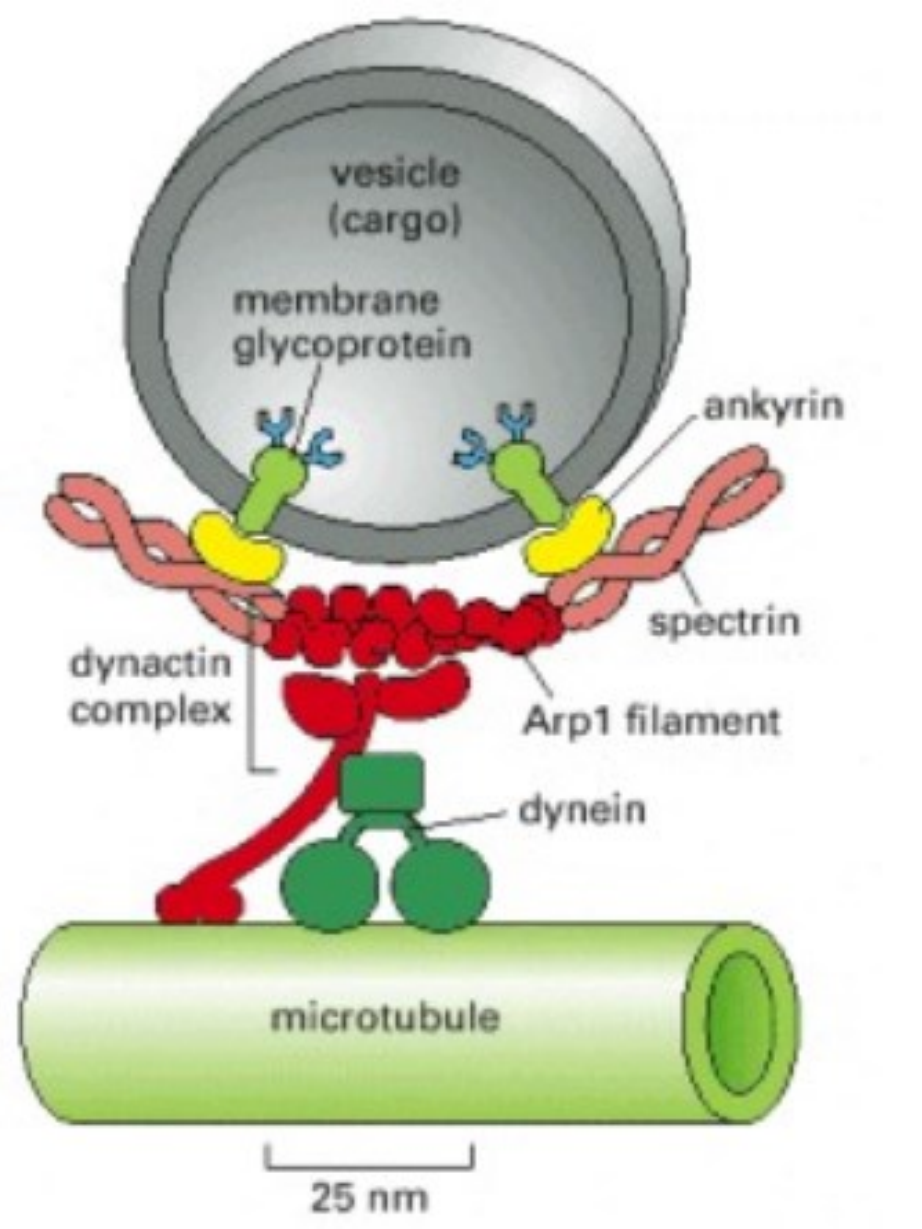
Traffic in the cell



Binaries, motors, cargos



How the cargo/organelle attaches to the motor ?



Model for the attachment of dynein to a membrane-enclosed organelle

Dynein requires the presence of a large number of **accessory proteins** to associate with membrane-enclosed organelles.

Dynactin is a large complex *(red)* that includes components that bind weakly to microtubules, components that bind to dynein itself, and components that form a small actin like filament made of the **actin-related protein Arp1**.

It is thought that the **Arp1 filament** may mediate attachment of this large complex to membrane-enclosed organelles through a network of **spectrin** and **ankyrin**, similar to the membrane-associated cytoskeleton of the red blood cell.

Kinesin – processive motor. Kinesin has two heads that step along the microtubule, and it dissociates from the microtubule after about 100 steps. This action requires energy, which is provided by ATP binding and hydrolysis.

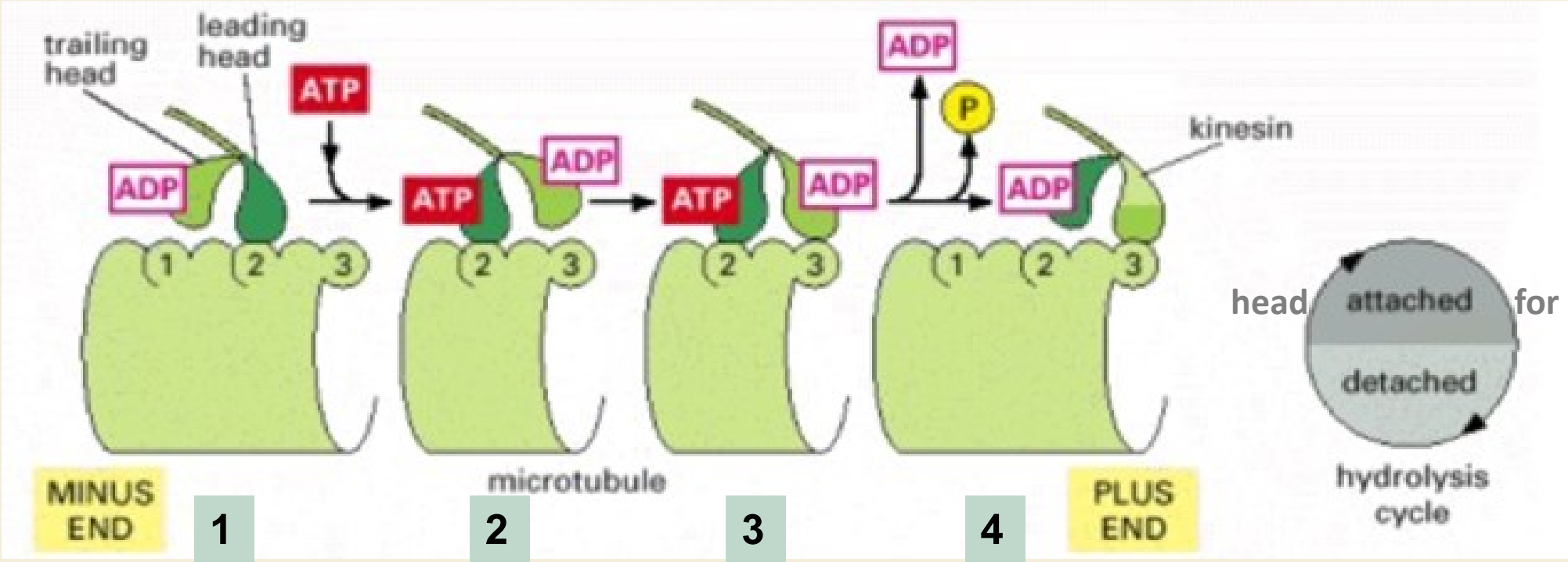
Each of the two heads has one binding site for microtubule and one for ATP/ADP.

Specific for kinesin is **that binding of ATP increases strongly the affinity of kinesin for microtubule** (tubulin dimer).

ATP binding triggers a conformational change in the head domain, locking it on the tubulin and repositioning the second head dimer to next tubulin dimer at a distance of about 8 nm toward the plus end of the microtubule.

The intrinsic ATPase activity of the first head hydrolyzes the ATP. When the second head domain binds to the microtubule, the first head releases ADP and binds ATP.

The coupling between ATP hydrolysis and conformational changes for kinesin



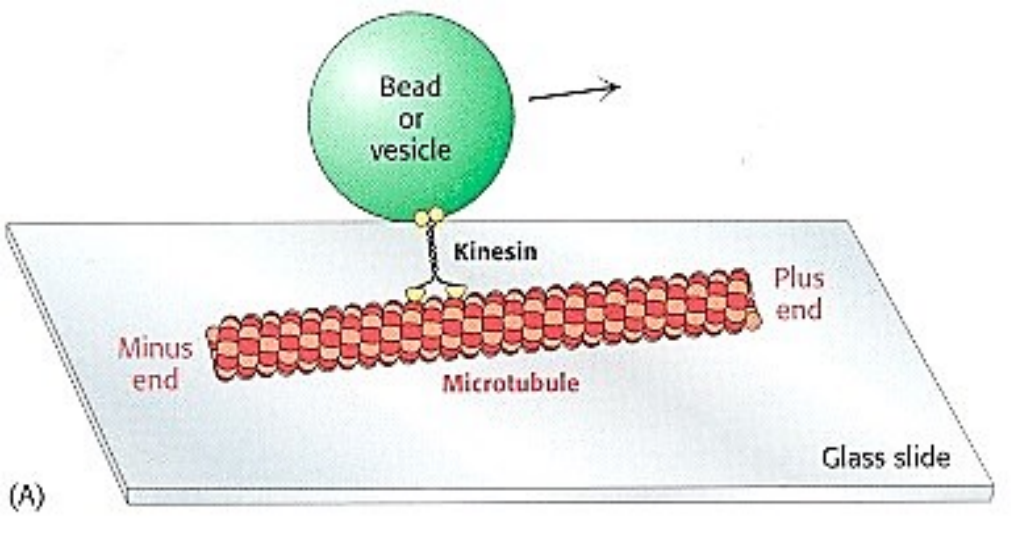
1. The leading head is bound to the microtubule, with trailing head detached.

2-3. Binding of ATP to the front head causes the rear head to be thrown forward, past the binding site of the attached head, to another binding site further toward the plus end of the microtubule.

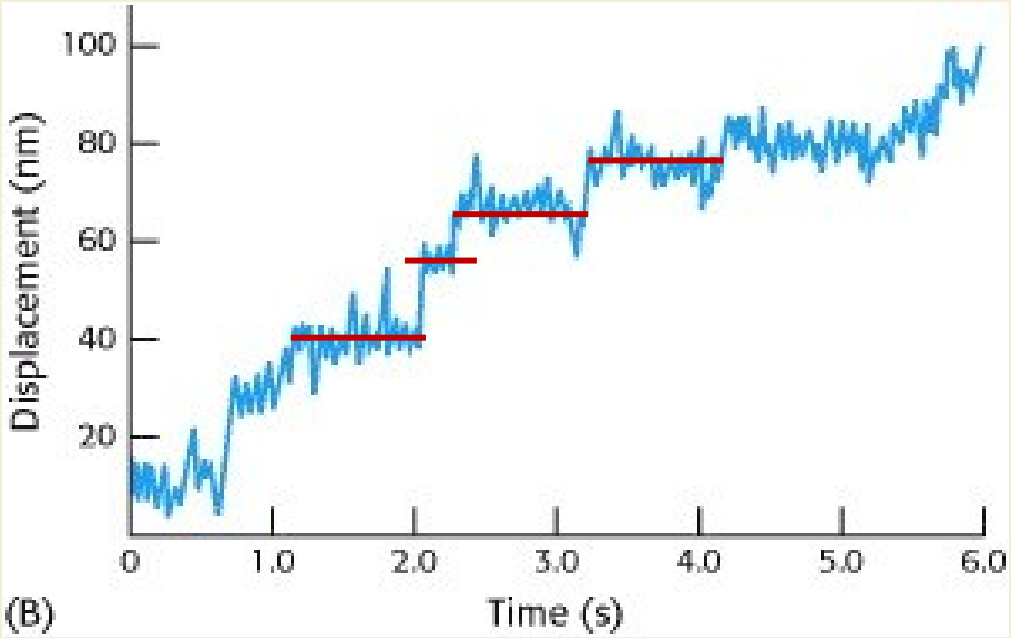
4. Release of ADP from the second head (now in the front) and hydrolysis of ATP on the first head (now in the rear) brings the dimer back to the original state, but the two heads have switched their relative positions, and the motor protein has moved one step along the microtubule.

DUTY RATIO: 50 %; SPEED of MOTOR 640 nm / s ; step size 8 nm; WORKING DISTANCE about 100 steps before detaching – 8000 A

Monitoring Movements Mediated by Kinesin



(A) The movement of beads or vesicles, carried by individual kinesin dimers along a microtubule, can be directly observed attaching a microbead or vesicle – interferometry detection of displacement in optical microscope, and force measured by optical tweezers.



(B) A trace showing the displacement of a bead carried by a kinesin molecule.

The average step size is about 8 nm.

K. Svoboda, C. F. Schmidt, B. J. Schnapp, and S. M. Block. *Nature* 365(1993):721

Muscle contraction, ATP and the cytoskeletal motor protein Myosin II.

The motion of muscle shortening occurs as myosin heads bind to actin and pull the actin inwards. This action requires energy, which is provided by ATP through hydrolysis.

The Myosin head has two binding sites: one for actin and one for ATP.

ATP binding causes myosin to release the actin filament, allowing actin and myosin to detach from each other.

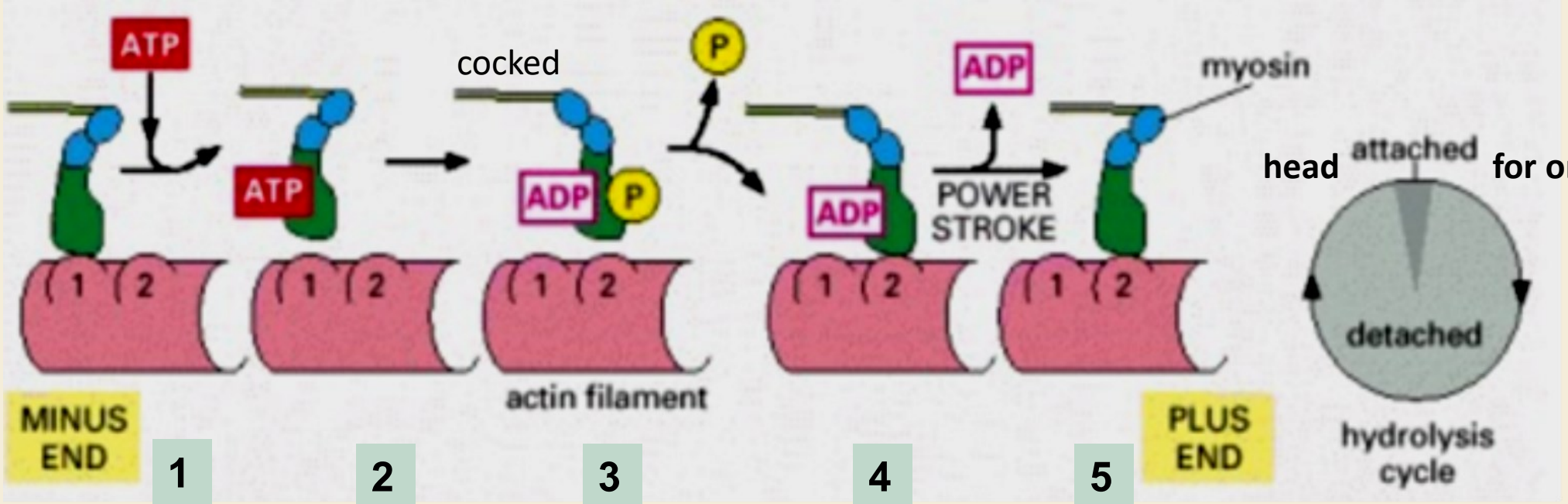
After this happens, the newly bound ATP is converted to ADP and inorganic phosphate, Pi.
The energy released during ATP hydrolysis changes the angle of the myosin head into a “cocked” position.

The myosin head is then in a position for further movement, possessing potential energy, but ADP + Pi are still attached.

If actin binding sites are covered and unavailable, the myosin will remain in the high energy configuration with ATP hydrolyzed, but still attached.

If the actin binding sites are exposed, a **cross-bridge** will form; i.e, the myosin head spans the distance between the actin and myosin molecules. Pi is then released, allowing myosin to expend the stored energy as a conformational change.

The coupling between ATP hydrolysis and conformational changes for myosin II



1. Myosin is tightly bound to the actin filament, with no ATP associated, the so-called “rigor” state.

2. ATP binding releases the head from the filament.

3. ATP hydrolysis occurs while the myosin head is detached from the filament, causing the head to assume a **cocked** conformation, although both ADP and inorganic phosphate remain tightly bound to the head.

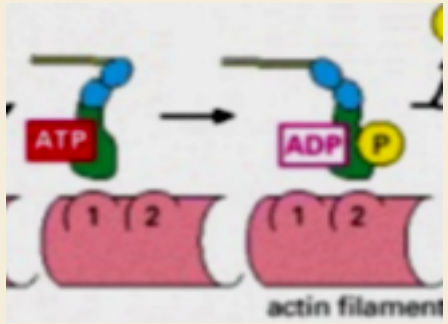
4. When the head rebinds to the filament, the release of phosphate, followed by the release of ADP, trigger the **power stroke** that moves the filament relative to the motor protein.

5. ATP binding releases the head to allow the cycle to begin again.

DUTY RATIO 5% , SPEED of MOTOR 2-60 um /s; WORKING DISTANCE : 5.5 nm

See also Houdusse and Sweeney, Trends in Biochemical Sciences, 2016, Vol. 41, No. 12

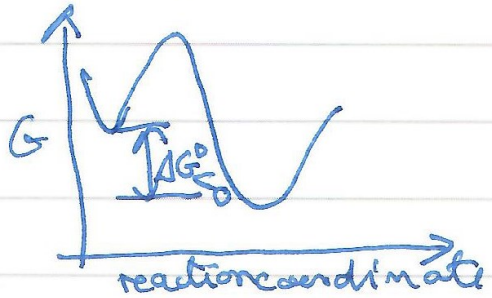
Role of myosin-crossbridge in the contraction of the muscle



There is an interplay between chemical and "mechanical" reactions

Coupling ATP hydrolysis to conformational change (get to "chocked" configuration) 2→3

ATP hydrolysis



$ATP + H_2O \rightleftharpoons ADP + P_i + H_+ + OH$

conc. 1M for all → standard

$$K^{\circ}_{ATP} = \exp\left[-\frac{\Delta G^{\circ}}{RT}\right] =$$

$$= \exp\left[\frac{30\times10^{3}}{2.4\times10^{3}}\right] \simeq 3.10^{5}$$

$R \cong 8.3\ J\ mol^{-1}\ k^{-1}$

$T \cong 300\ K$

$\Delta G^{\circ} \cong -30\ kJ\ mol^{-1}$

In cell, due to presence of Mg ions, pH etc → $K_{ATP} = 10^{8}$ much bigger

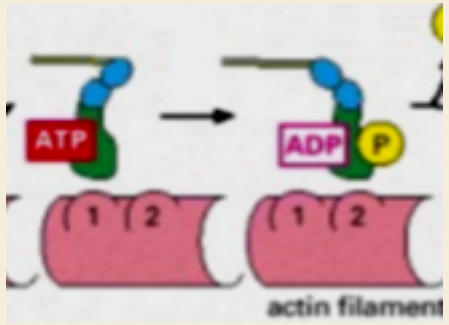
1 kcal / mole ~ 4.184 kJ / mole ~ 1.6 KT / molecule

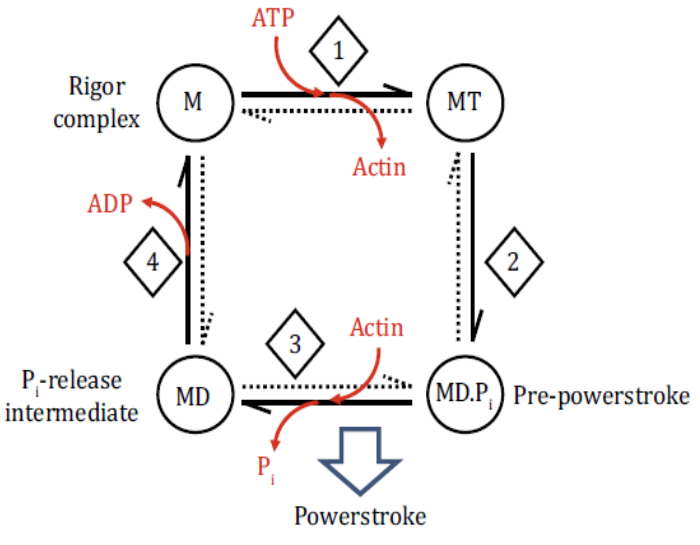
1 KT ~ 4.1×10^{-21} J 1 J ~ 2.5×10^{20} KT

NA ~ 6×10^{23}

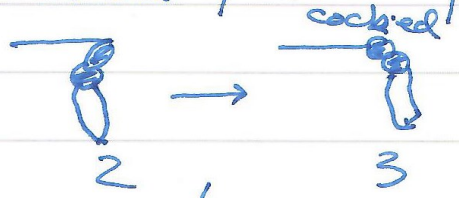
RT= KT x NA

Role of myosin-crossbridge in the contraction of the muscle





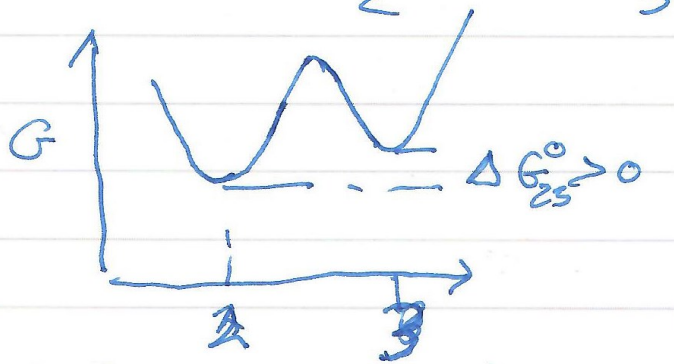
Let us consider protein conformational change!



$\Delta G^\circ_{23} = 10\ kJ\ mol^{-1}$

$K_{2-3} = \exp\left[\frac{-10\times10^3}{2.4\times10^3}\right] =$

$= \exp[-4] = 2\cdot10^{-2}$



$\Delta G^\circ_{23} = 10\ kJ\ mol^{-1}$

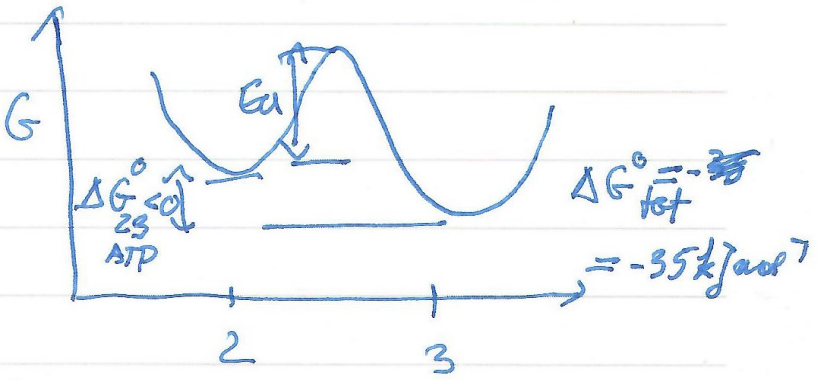
Coupling:

$ATP \rightleftarrows ADP + P_i$

$A \rightleftarrows B$

$K_{tot} = K_{ATP} \cdot K_{23} = 2\cdot10^6$!

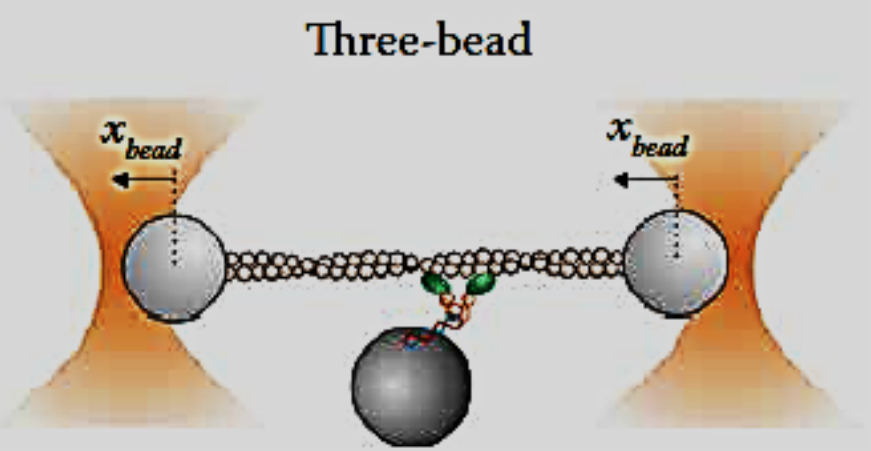
! An enzyme does not chang ΔG in reaction but lowers the ~~Energy of acti~~ Activation Energy E_a



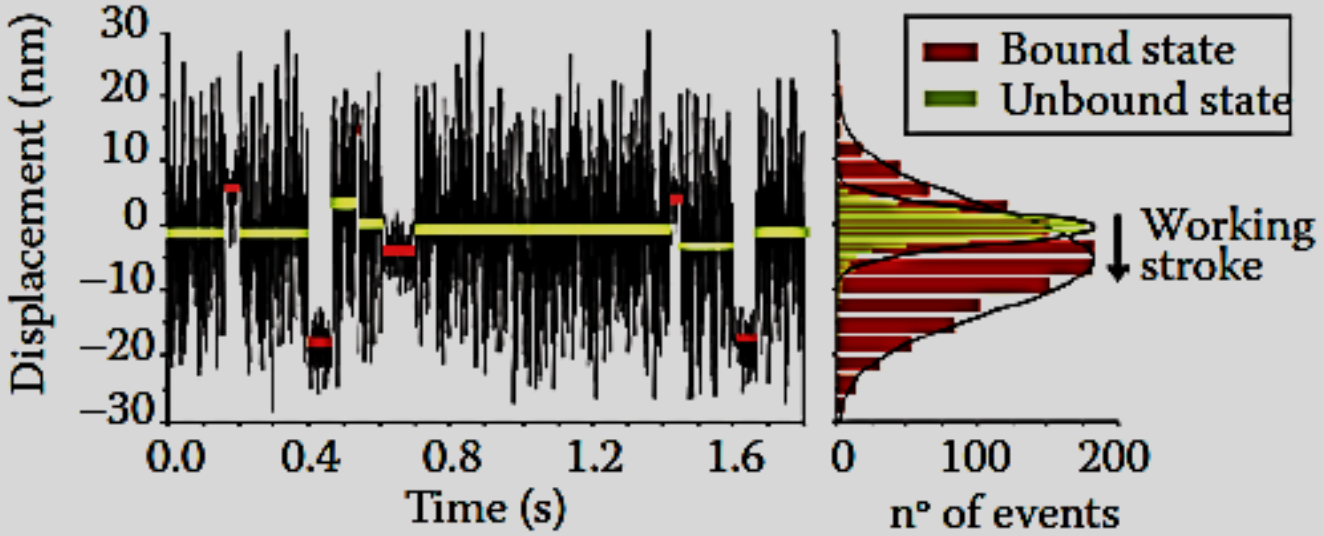
Role of myosin-crossbridge in the contraction of the muscle

https://www.dnatube.com/video/1306/Role-of-myosin-crossbridge-in-the-contraction-of-muscle

Three-bead assay. Example: working stroke of myosin



Actin filament connected to polystyrene beads held by optical tweezers.



Position recording while myosin is interacting with actin.

A single myosin molecule is attached onto a third bead stuck to the coverslip surface.
Movements of the actin filament produced by the attached myosin are measured through bead displacements (x_{bead})

Position recording while myosin is interacting with actin.
Red and green lines indicate the average position of bound and unbound events, respectively.
Distributions of the average position of bound and unbound events of a 100 s position recording containing several hundreds of interactions.
The working stroke is obtained from the displacement between the centers of the two distributions

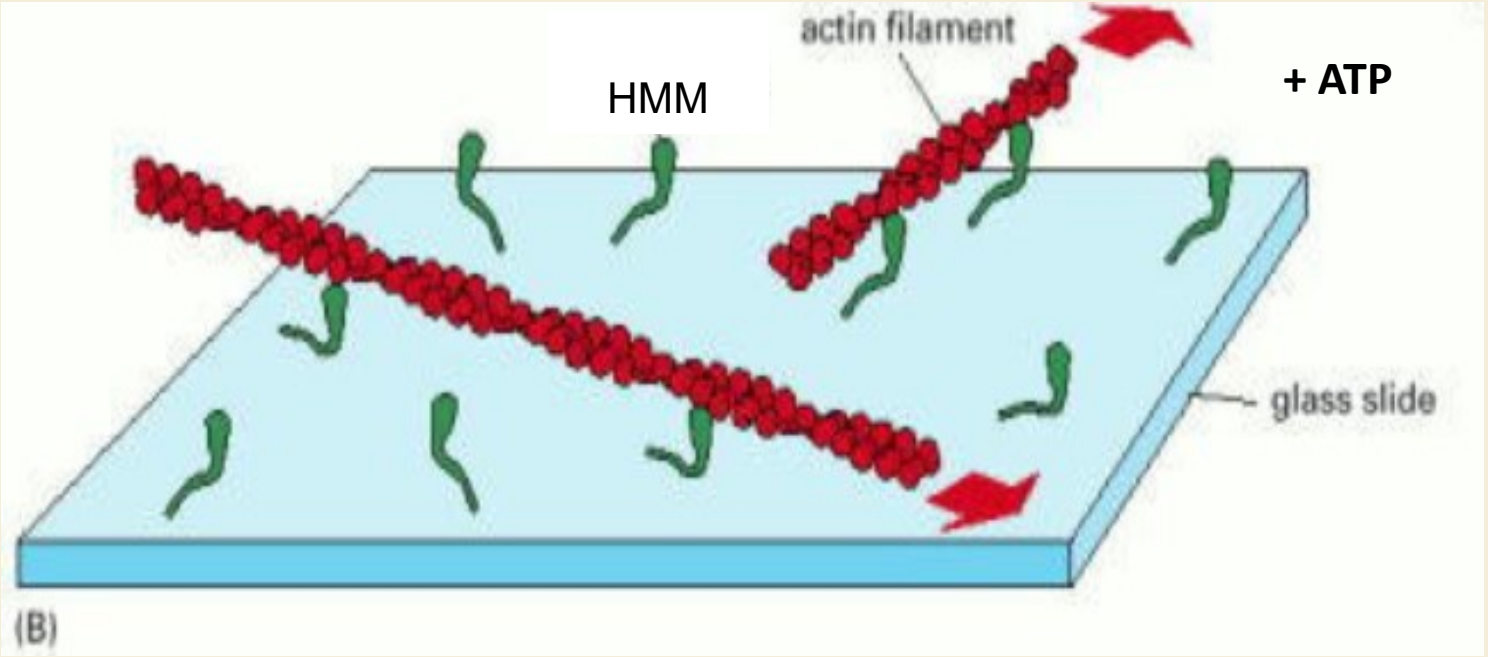
Capitanio, M., et al., Micr. Res. Tech., 65, 194–204, 2004

Direct evidence for the motor activity of the myosin head

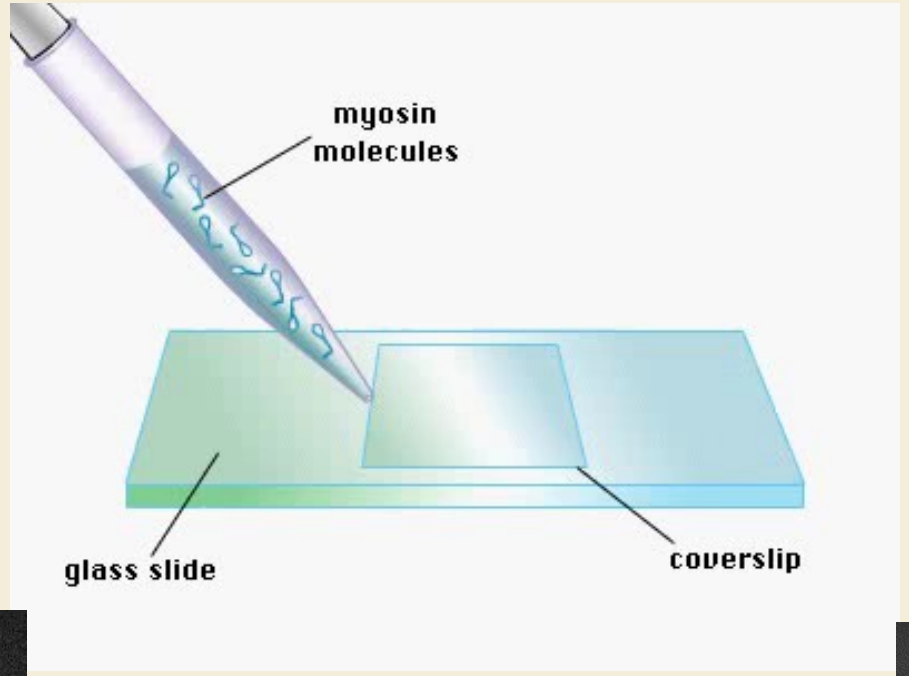
In Vitro Motility (IVM) assay

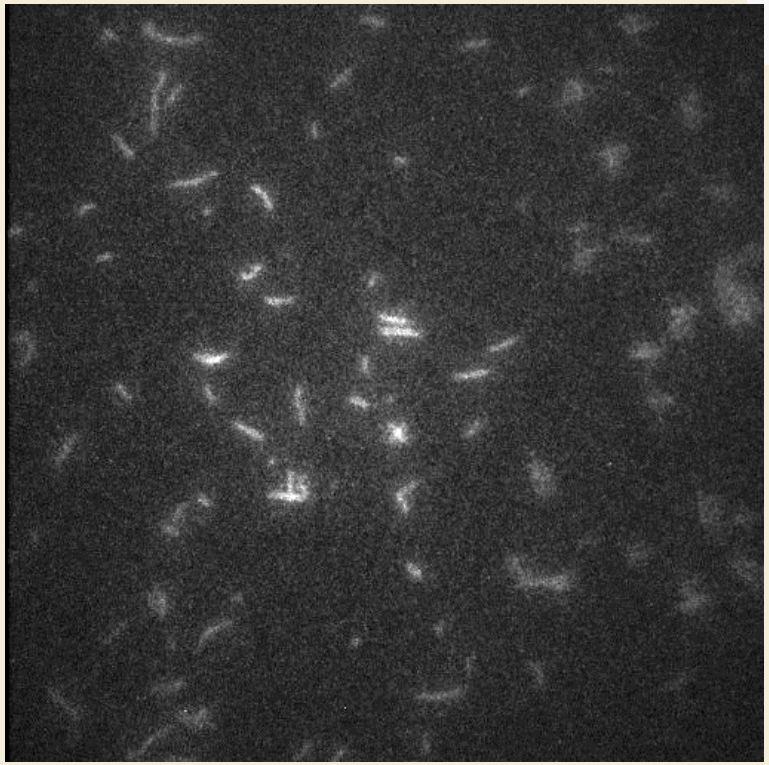
When a muscle myosin is digested by chymotrypsin and papain, the head domain is released as an intact fragment called heavy meromyosin (HMM).

The S1 fragment alone can generate filament sliding in vitro, proving that the motor activity is contained completely within the head



In Vitro Motility (IVM) assay





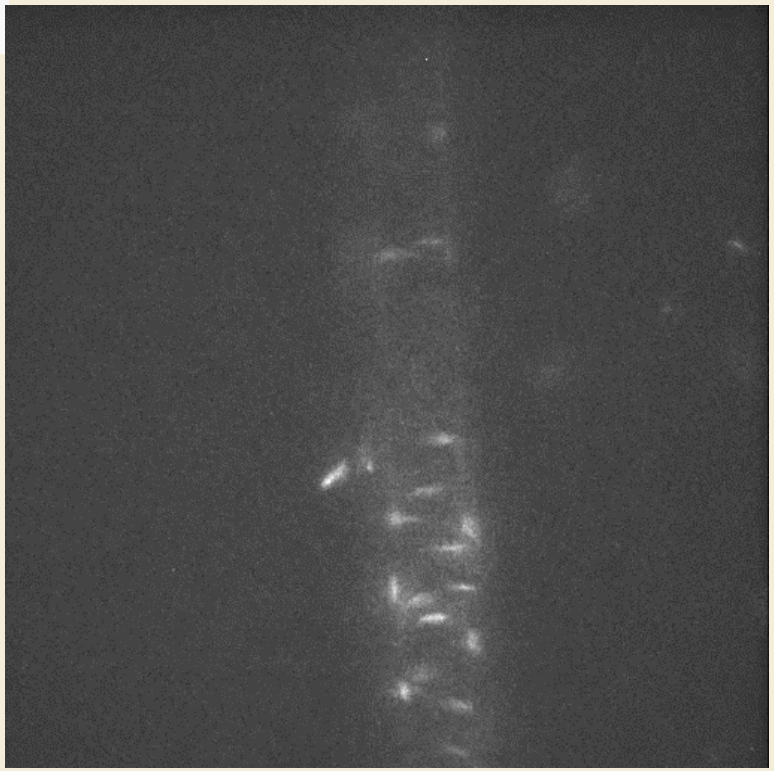
IVM assay

Frog HMM

<-- Glass slide

Glass Fiber →

5 um



Myosin and kinesin undergo analogous mechanochemical cycles but the coupling between the mechanical and chemical cycles is different:

- **Myosin** without ATP is tightly bound to its actin track, in a so-called “rigor” state, and it is **released from this track by the association of ATP**.

while

- **Kinesin** forms a rigor-like tight association when ATP is bound to tubulin, and **hydrolysis of ATP promotes release** of the motor from its track.

Motor protein kinetics are adapted to cell functions: some considerations on processivity

The motor proteins in the myosin and kinesin superfamilies exhibit a remarkable diversity of motile properties, well beyond their choice of different polymer tracks. A single dimer of conventional **kinesin moves in a highly processive fashion**, traveling for hundreds of ATPase cycles along a microtubule without dissociating.

Skeletal muscle **myosin II, in contrast, cannot move processively** and makes just one or a few steps along an actin filament before letting go. These differences are critical for the motors' various biological roles.

A **small number of kinesin molecules must be able to transport a mitochondrion all the way down a nerve cell axon,** and therefore require a high level of processivity.

Skeletal muscle myosin, in contrast, never operates as a single molecule but rather as part of a huge array of myosin II molecules. **Processivity would inhibit biological function**, since efficient muscle contraction requires that each myosin head perform its power stroke and then quickly get out of the way, to avoid interfering with the actions of the other heads attached to the same actin filament.

Within each motor protein class, speeds vary widely: **0.2 to 60 µm/sec** for **myosins;** **0.02 to 2 µm/sec** for **kinesins**.

These differences arise from a fine-tuning of the mechanochemical cycle.

The number of steps that an individual motor molecule can take in a given time, and thereby the velocity, can be increased by:

- increasing the motor protein's intrinsic ATPase rate
- decreasing the proportion of cycle time spent bound to the filament track.

Moreover, the size of each step can be changed by

- changing the length of the lever arm (e.g., the lever arm of myosin V is about three times longer than the lever arm of myosin II)
- changing the angle through which the helix swings.

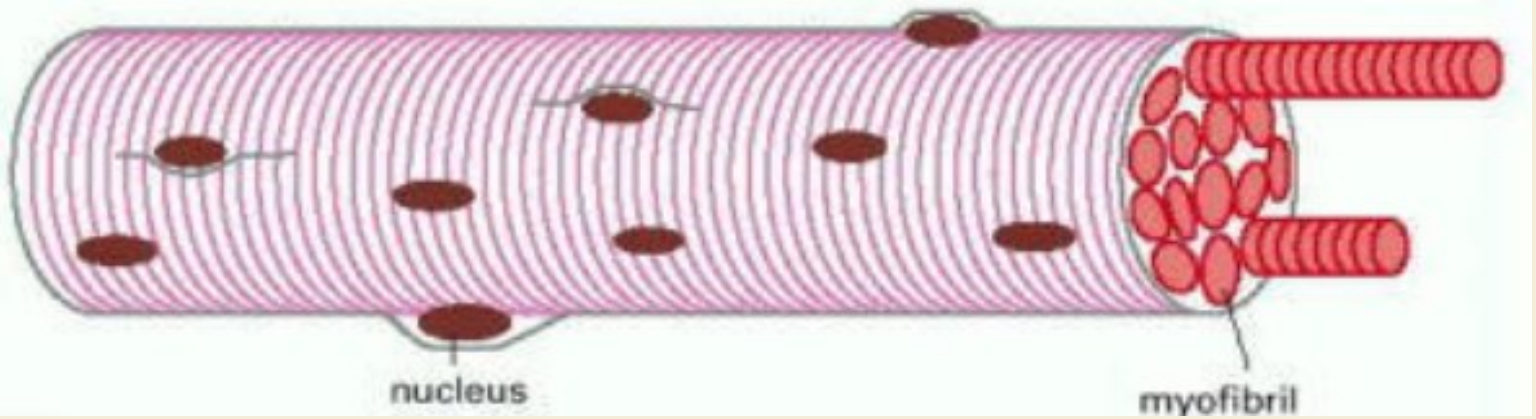
Muscle contraction depends on the sliding of myosin II and actin filaments

In vertebrates, running, walking, swimming, and flying all depend on the rapid contraction of skeletal muscle on its scaffolding of bone, while involuntary movements such as heart pumping and gut peristalsis depend on the contraction of cardiac muscle and smooth muscle, respectively.

All these forms of muscle contraction depend on the ATP-driven sliding of highly organized arrays of actin filaments against arrays of myosin II filaments.

Skeletal muscle cells (also called muscle fibers)

In an adult human, a **muscle cel**l is typically **50 µm** in diameter and can be up to several **centimeters long.**



The long thin muscle fibers of skeletal muscle are actually single multinucleated cells (in plasma membrane).

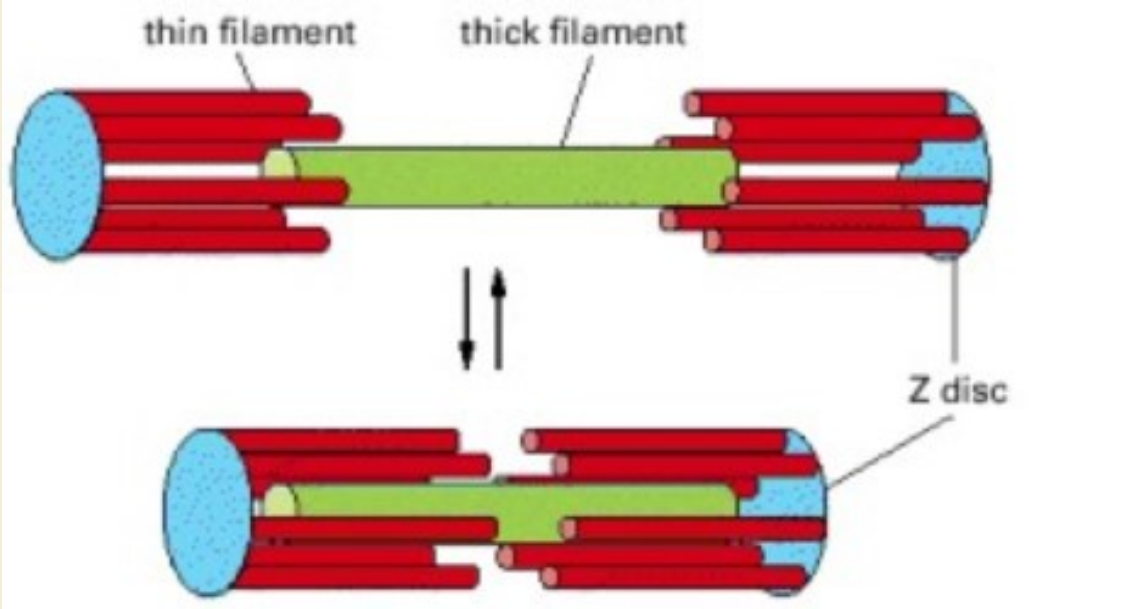
The bulk of the cytoplasm inside is made up of **myofibrils:** the basic contractile elements of the muscle cell.

A **myofibril** is a cylindrical structure **1–2 µm in diameter** that is often as long as the muscle cell itself.

It consists of a long repeated chain of tiny contractile units—called **sarcomeres**, each about **2.2 µm long**, which give the vertebrate myofibril its striated appearance.

The sliding-filament model of muscle contraction

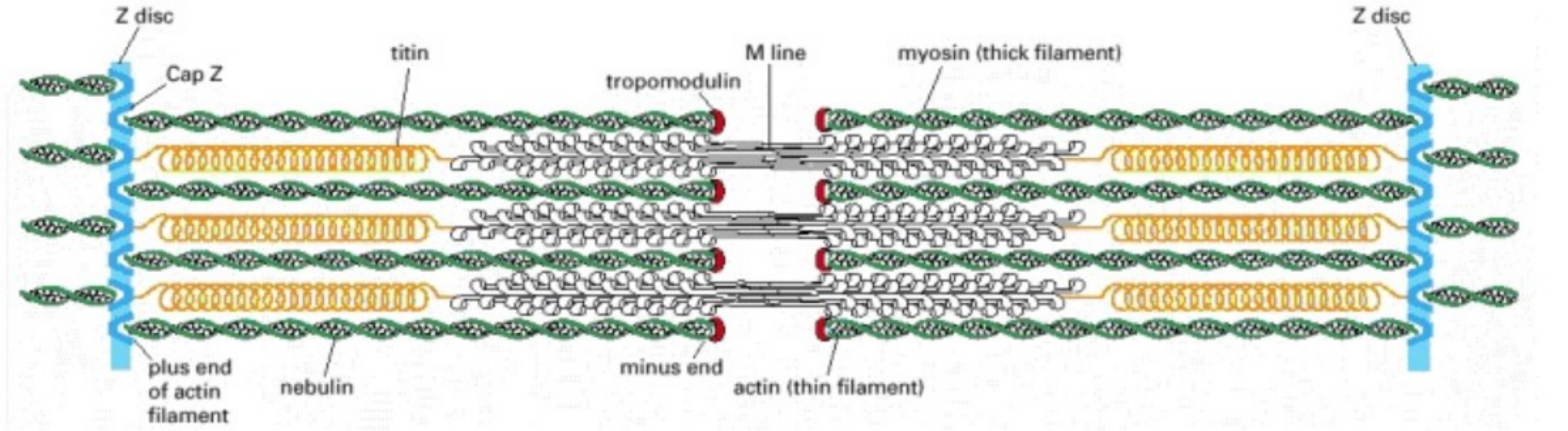
The actin *(red)* and myosin *(green)* filaments in a sarcomere slide past one another without shortening.



Each myosin **thick filament has about 300 heads**, and **each head cycles about 5 times/second** in the course of a rapid contraction—sliding the myosin and actin filaments past one another at **rates of up to 15 µm/sec** and enabling the **sarcomere to shorten by 10% of its length in less than 20 ms !**

The rapid synchronized shortening of the thousands of sarcomeres lying end-to-end in each myofibril gives skeletal muscle the ability to contract rapidly enough for running and flying, and even for playing the piano. ☺

Accessory proteins in a sarcomere



Titin molecules extend from the Z disc to the M line (distance > 1 μm). Part of each titin molecule is closely associated with a myosin thick filament, the rest of the titin molecule is elastic and changes length as the sarcomere contracts and relaxes.

Nebulin molecules which have the length of thin filaments.

The actin filaments are also coated with **tropomyosin** and **troponin** (not shown) and are capped at both ends:

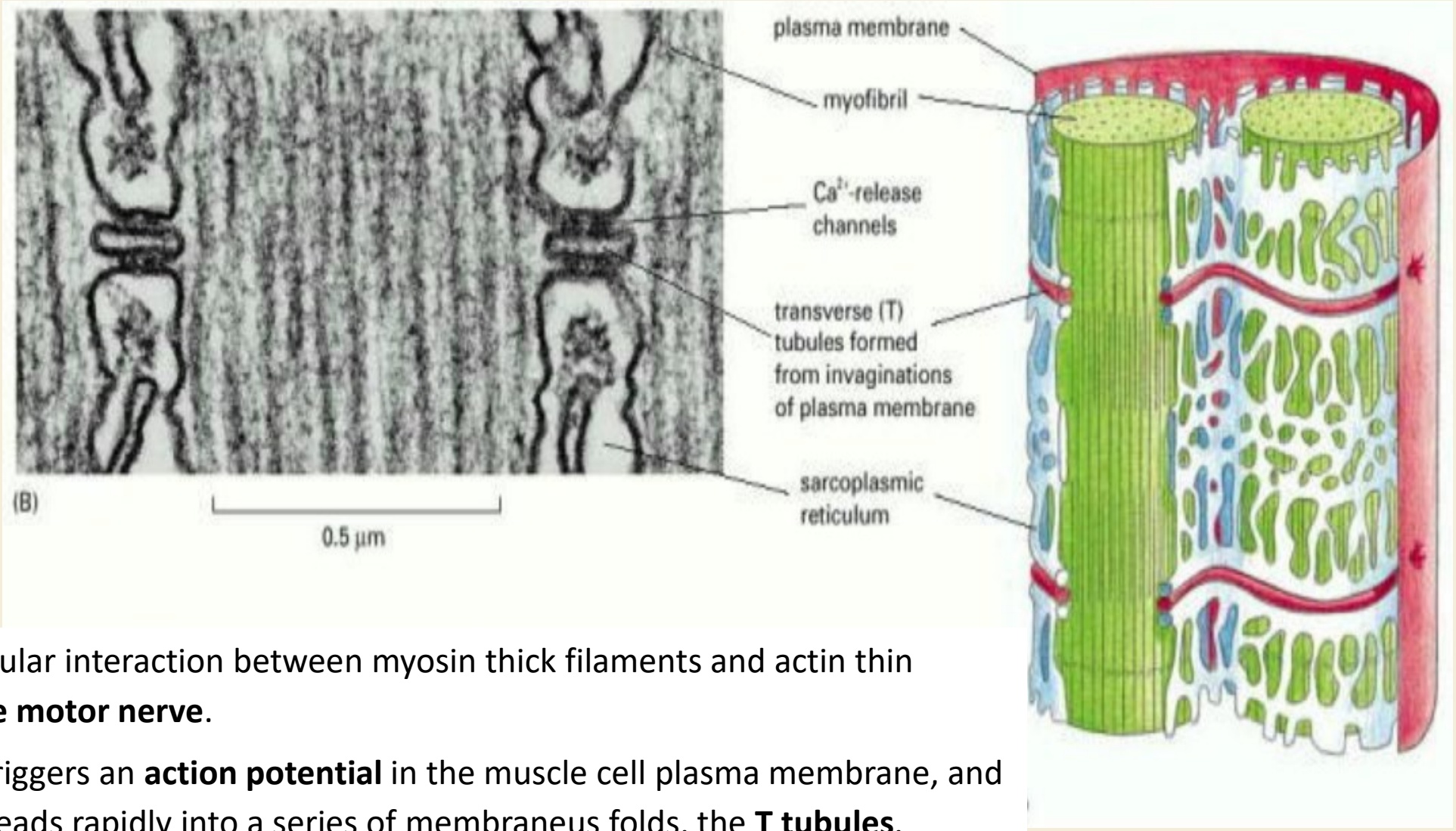
Tropomodulin caps the minus end of the actin filaments

CapZ anchors the plus end at the Z disc, which also contains **α-actinin**.

Accessory proteins govern the remarkable uniformity in filament organization, length, and spacing in the sarcomere

Triggering the Muscle contraction → rise the cytosolic Ca^{2+} concentration

T tubules and the sarcoplasmic reticulum



The force-generating molecular interaction between myosin thick filaments and actin thin filaments is triggered **by the motor nerve**.

The signal from the nerve triggers an **action potential** in the muscle cell plasma membrane, and this electrical excitation spreads rapidly into a series of membraneus folds, the **T tubules**.

The signal is then relayed across a small gap to the **sarcoplasmic reticulum**, an adjacent that surrounds each myofibril like a net stocking.

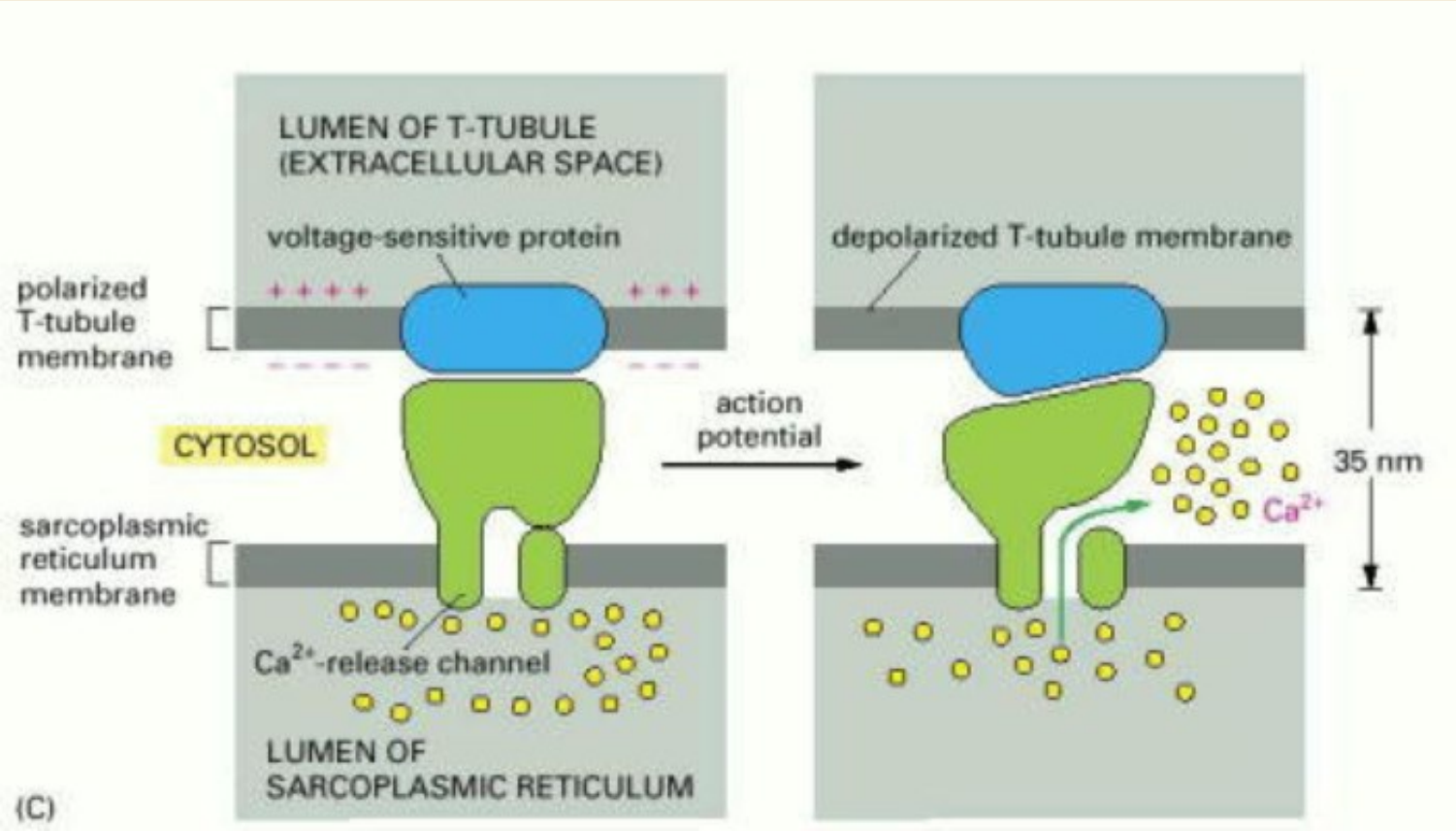
Voltage-sensitive channels/proteins in the T-tubule are activated by incoming action potential →

trigger the opening of Ca2+-release channels in the **sarcoplasmic reticulum.**

Ca2+ flooding into the cytosol then initiates the contraction of each myofibril.

Because the signal from the muscle-cell plasma membrane is passed within milliseconds (via the T tubules and sarcoplasmic reticulum) to every sarcomere in the cell, all of the myofibrils in the cell contract at the same time.

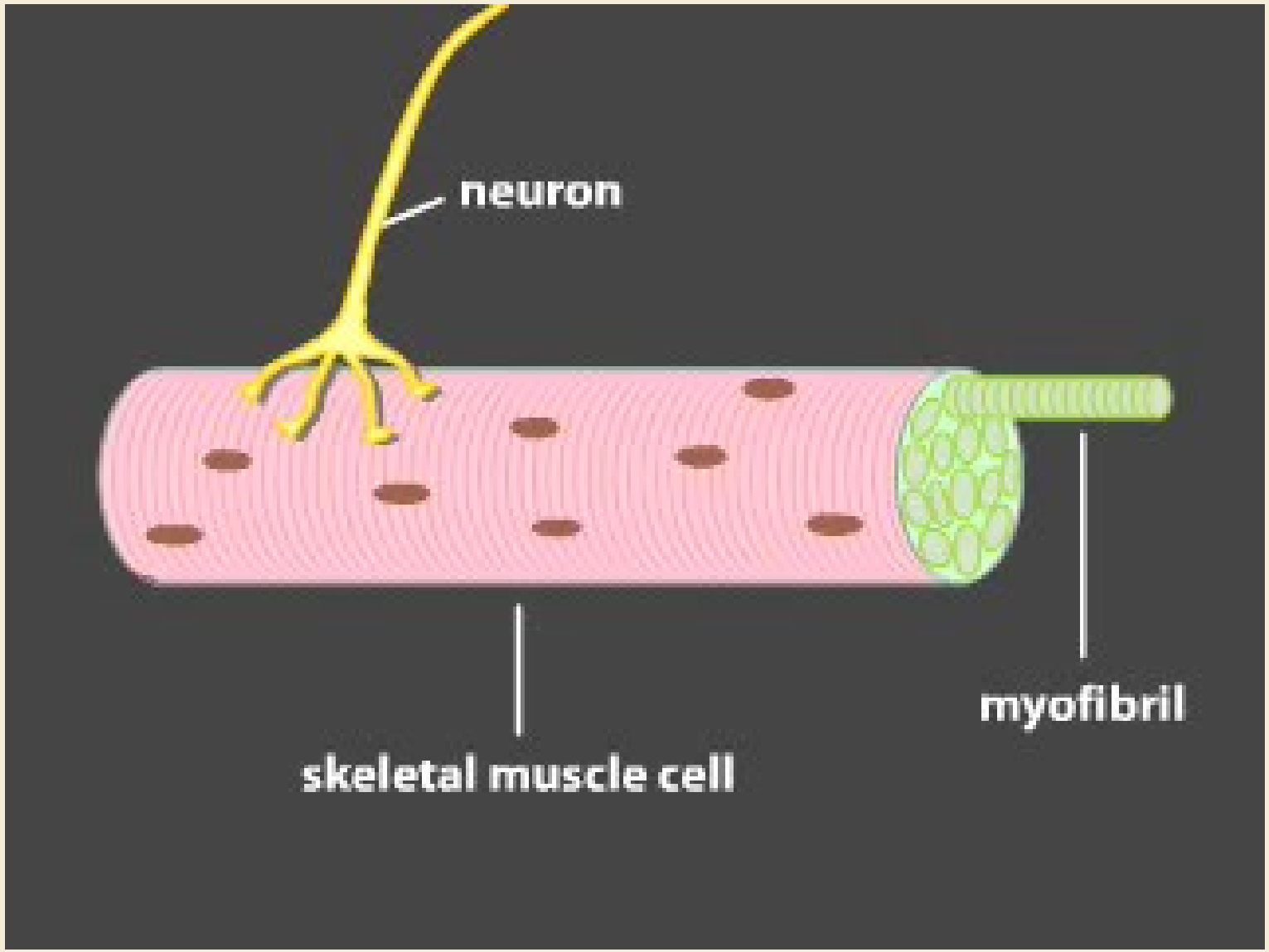
Schematic diagram showing how a Ca^{2+}-release channel in the sarcoplasmic reticulum membrane is thought to be opened by a voltage-sensitive transmembrane protein in the adjacent T-tubule membrane.



The Ca2+ dependence of skeletal muscle contraction, and hence its dependence on motor commands transmitted via nerves, **is due entirely to a set of specialized accessory proteins** that are closely associated with the actin thin filaments : **tropomyosin**, **troponin**,

See slide **The control of skeletal muscle contraction by troponin tropomyosin** at the end

Contraction of a muscle cell - movie



https://youtu.be/CepeYFvqmk4

https://www.dnatube.com/video/1306/Role-of-myosin-crossbridge-in-the-contraction-of-muscle

Experimental Example:

A myosin II nanomachine mimicking the striated muscle

Goal:

Build a nanomachine which contains the minimum number of motor molecules needed to reproduce the collective mechanics of muscle myosin II in situ and fits the performance of mammalian skeletal muscle

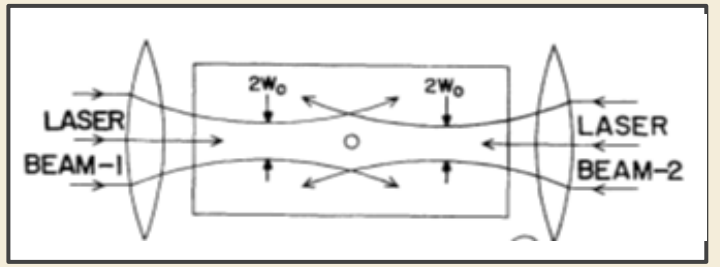
New:

- Developed and characterized a synthetic 1-D nanomachine with myosin II dimers from rabbit psoas muscle, which performs isometric and isotonic contractions at 2mM ATP, delivering a maximum power of 5 aW.
- Kinetic model fitted to the performance of mammalian skeletal muscle, showing that the condition for the motor coordination that maximises the efficiency in striated muscle is a minimum of 32 myosin heads sharing a common mechanical ground.

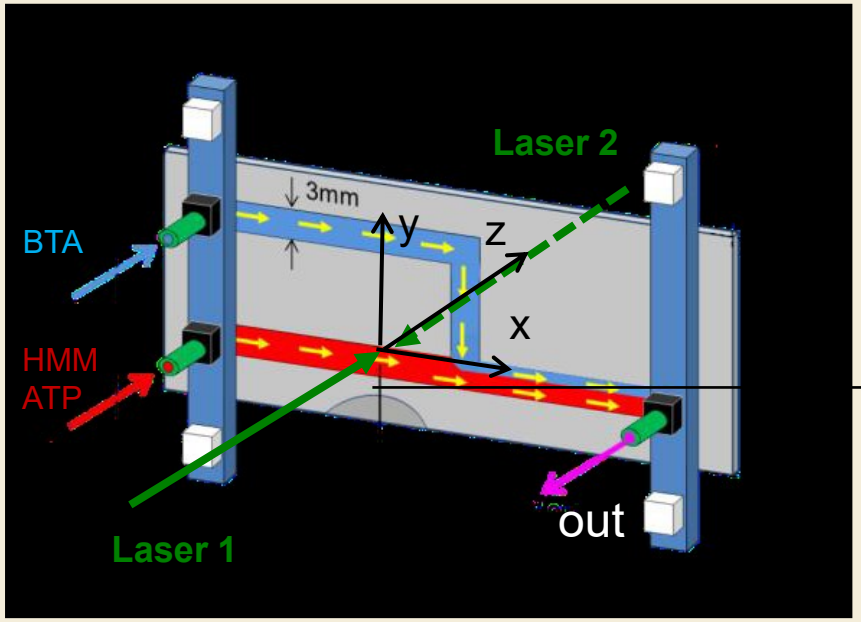
Main difficulties: assemble multiple motors and couple them in conditions close to physiological conditions.

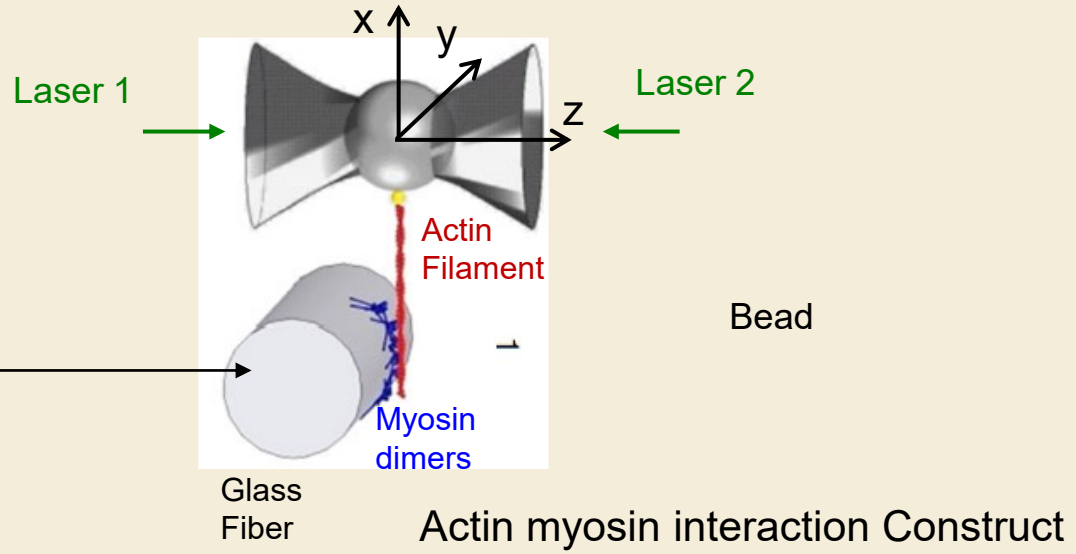
Pertici et al., *Nat. Comm.* (2018) 9:3532.

Setup based on Dual Laser Tweezer (counterpropagating beams)



Schematic representation of the experimental chamber.





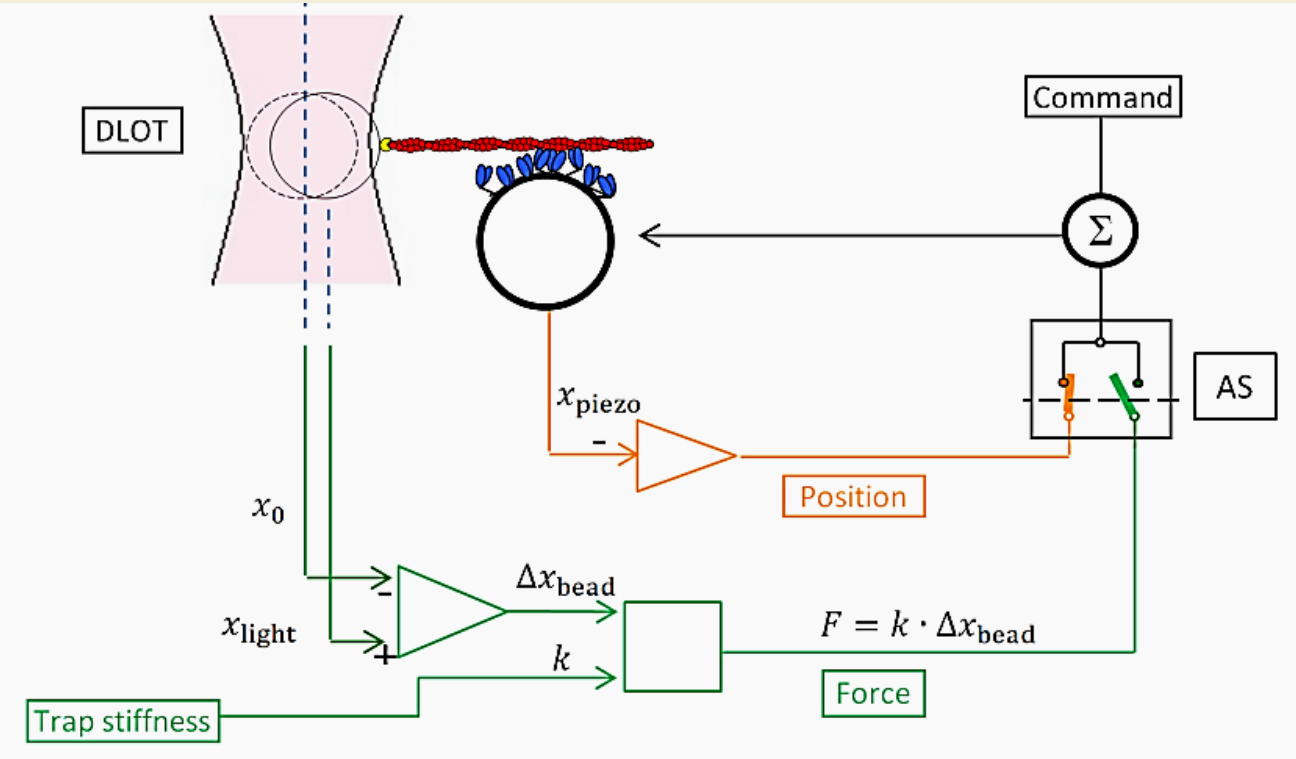
Actin myosin interaction Construct

The chamber: two coverslip slides separated by a parafilm layer (180-μm), two channels that converge in their final tract.

HMM motors deposited on a single mode optical fiber (d~4 μm) coated with methylcellulose for HMM attachment.

HMM and ATP are flowed through the lower channel, BTA (BeadTailActin) through the upper channel. A BTA, captured by the laser trap in the upper (blue) compartment, is brought in the proximity of the myosin array on the fiber by moving the chamber positioned on a nanopiezo system.

Position clamp vs force clamp implementation



Block diagram of the system for recording/control the nanomachine mechanics.

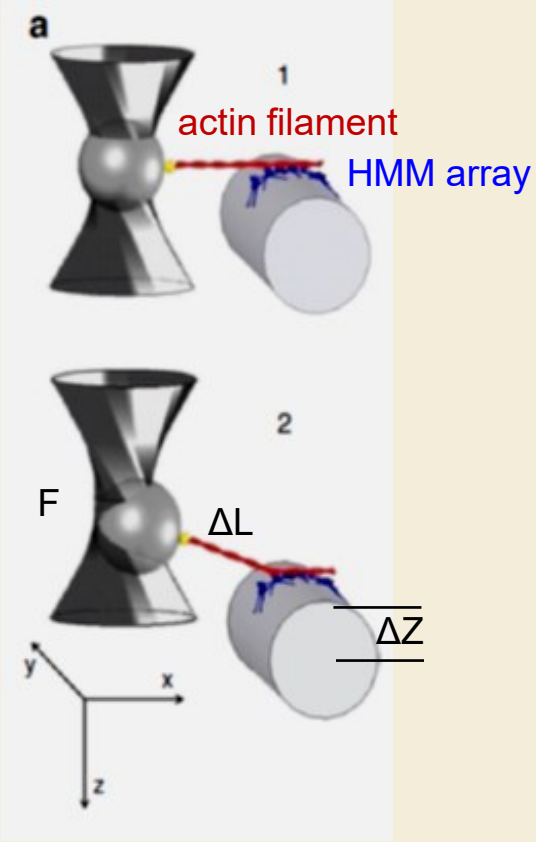
The analogue switch (AS) selects the signal that feeds the summing amplifier (Σ) to be compared with the command (black):

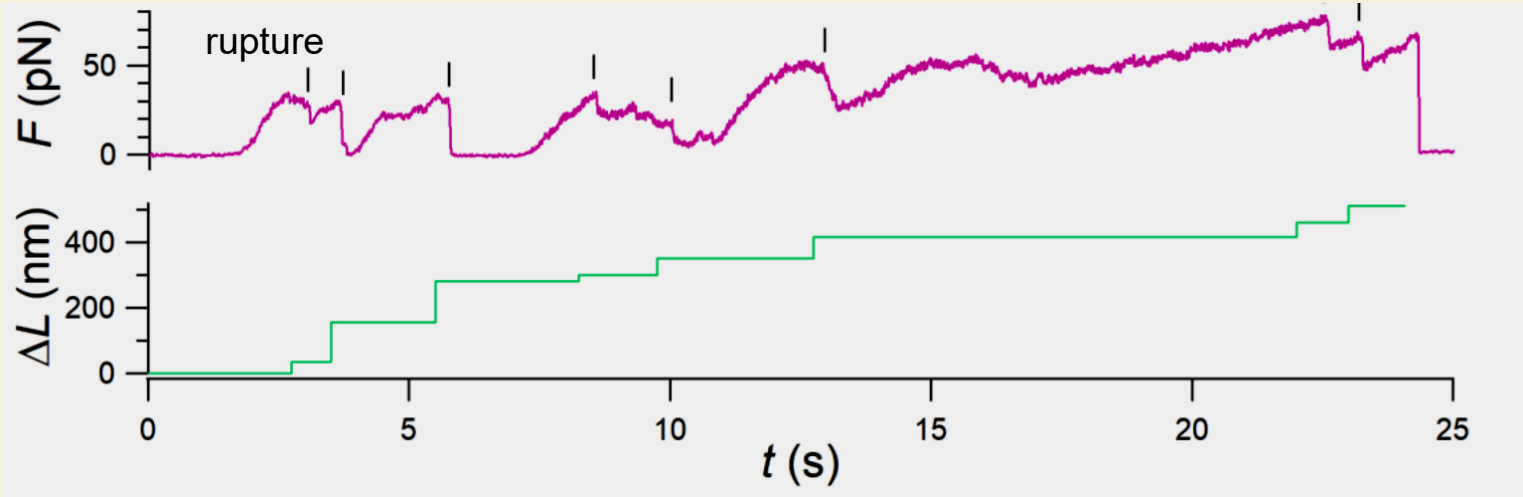
position feedback or force feedback.

Force is calculated as:

$F = k_{OT} \Delta x$ k_{OT} = 0.1 - 0.4 pN/nm; ΔX= 0 – 600 nm

Rigor experiment: How many HMM are available for acto-myosin interaction ?



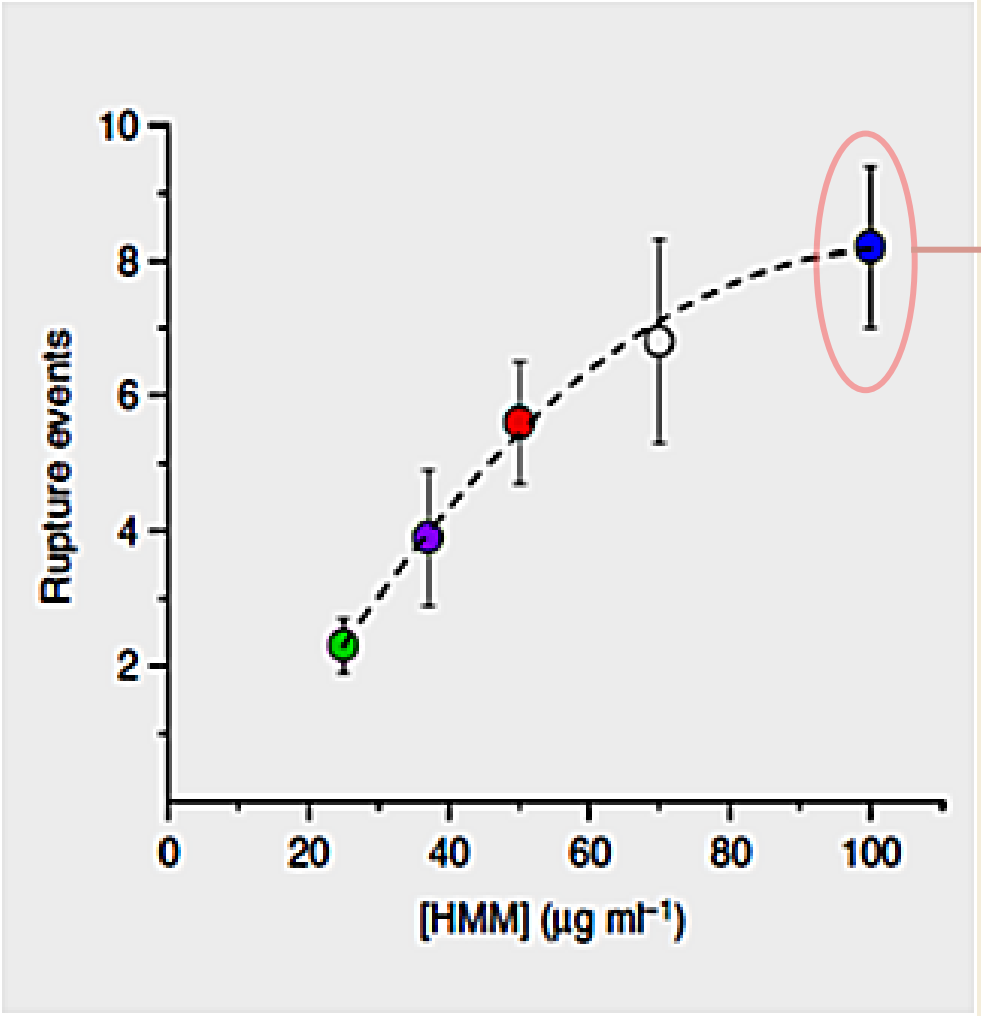


The number of HMMs dimers available for actin interaction is determined from rigor rupture events:

after the contact with actin filament is established, the fiber with HMM is moved in Z, orthogonal to the actin–myosin interface and the number of ruptures of HMM from actin is counted.

The number of ruptures indicates the number of available HMM at a given concentration

Number of rupture events vs HMM concentration



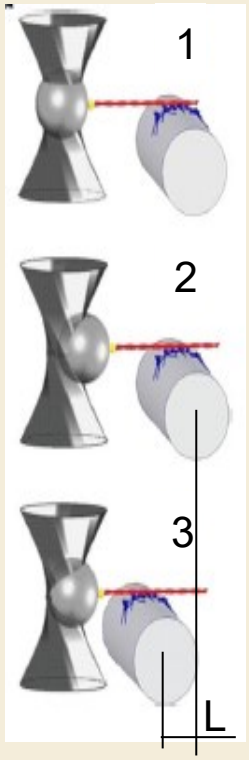
8 HMM dimers → 16 heads

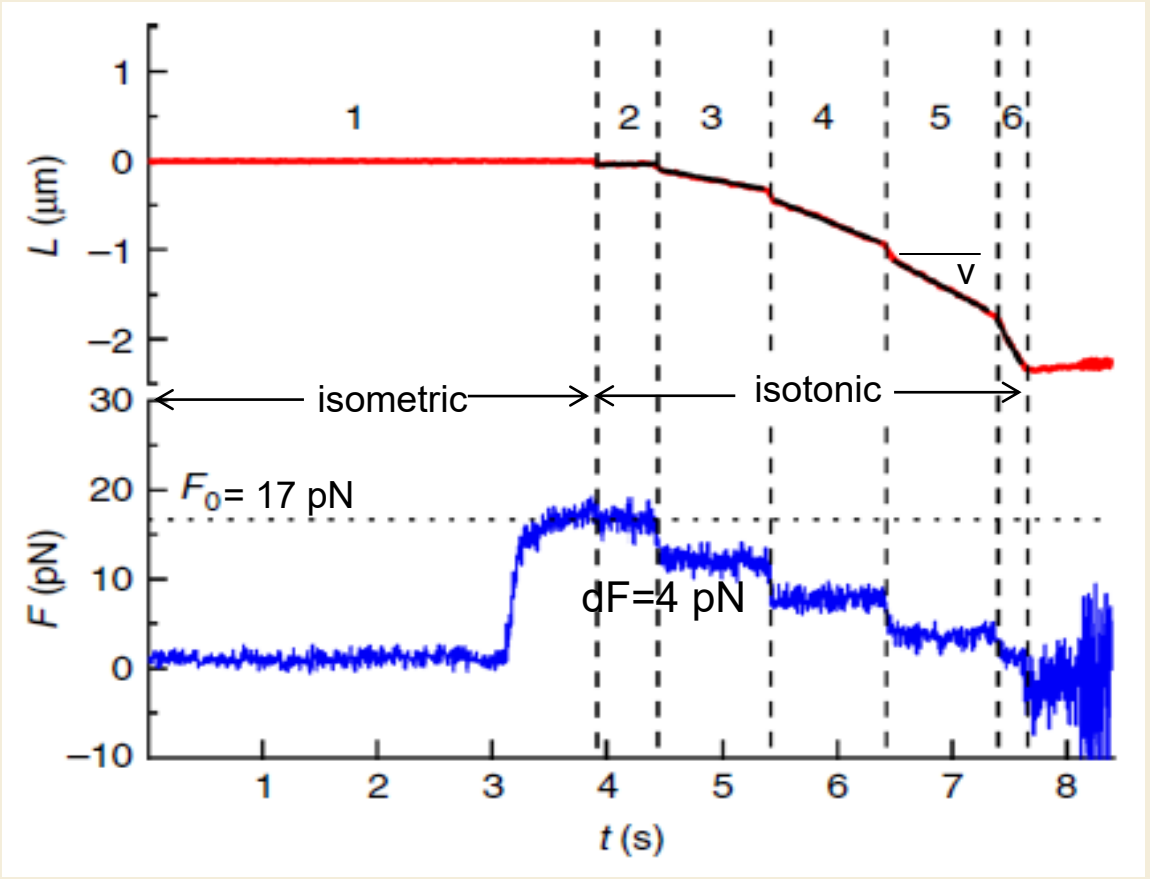
Mechanical output of the myosin-II nanomachine in 2mM ATP

Isometric and isotonic performance of the nanomachine

Position clamp

Force clamp





Isometric condition is first achieved (Force increases up to F0, due to the HMM motors work on actin) – position clamp

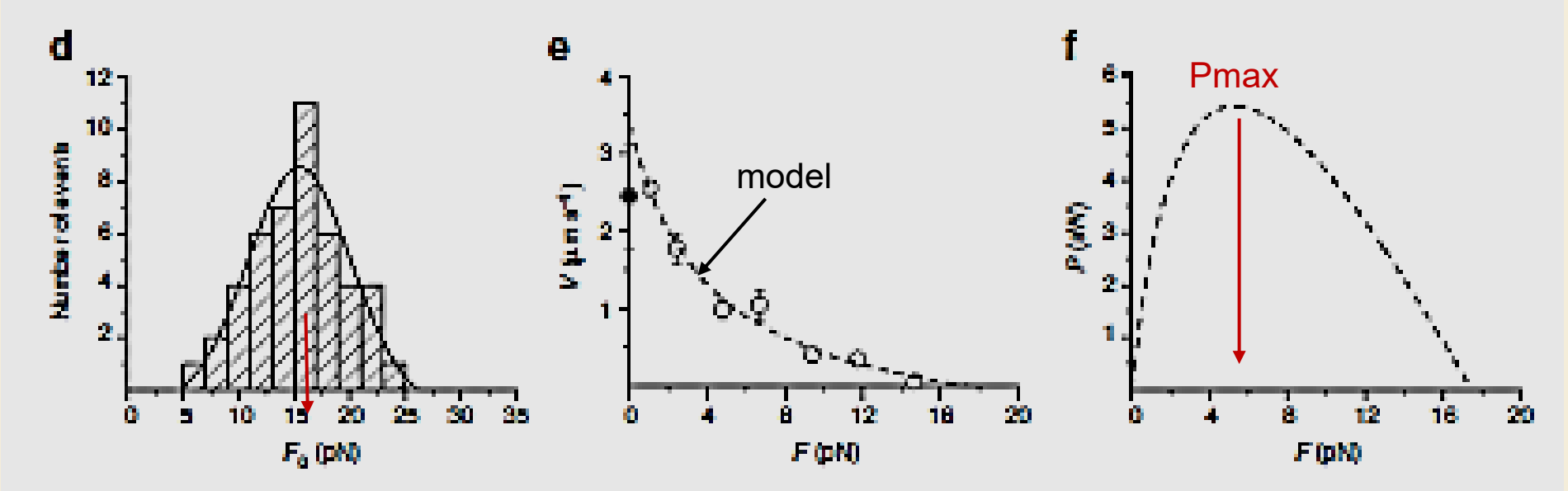
Force is kept constant for time t, length L is reduced at constant v (isotonic condition) Force is than lowered in steps of dF=4 pN, and the change of length measured again. Force clamp.

F_0 = 17 pN – maximum steady value (isometric condition)

Isometric: generate force without changing the length of the muscle
Isotonic: tension remains the same, whilst the muscle's length changes

Mechanical output of the myosin-II nanomachine in 2mM ATP

Steady Force F_0 | Force-velocity (F-V) relation | Power-force (P-F) relation



F_0 = 15.5 pN

P= F V

Pmax= 5.4 10^{-18} W = 5.4 aW

- A synthetic 1-D nanomachine with N= 16 motors which performs isometric and isotonic contractions at physiological concentration of ATP [ATP]= 2 mM, delivering a maximum power Pmax = 5.4 aW..
- The kinetic model fits the performance of mammalian skeletal muscle and show that efficiency is maximized when the number N of myosin heads is ***N > 32***

(some) Conclusions:

- A synthetic 1-D nanomachine with N= 16 motors which performs isometric and isotonic contractions at physiological concentration of ATP [ATP]= 2 mM, delivering a maximum power Pmax = 5.4 aW.

- Efficiency = Mechanical energy delivered by the nanomachine per ATP / ATP energy (fuel energy)

Eff = E_{NM} / E_{ATP}

Free energy for ATP hydrolysis E_{ATP} = 110 zJ (~ 25 KT)

Rate of ATP hydrolysis per myosin head k_{ATP} = 30 s^{-1}; for N= 16 motors, the total ATP split per second by the nanomachine at Pmax: k_{ATP}_tot= 30 s^{-1} x 16 = 480 s^{-1}.

The mechanical energy delivered by nanomachine per ATP: E_{NM}= Pmax / k_{ATP}_tot = (54000 zJ s^{-1} / 480 s^{-1}) = 11.2 zJ;

It results: Eff ~ 0.1 , which is about 3 times lower than the efficiency for fast mammalian muscle (Eff = 0.3)

(random orientation of molecules reduces the efficiency by a factor of 2, the difference comes from the reduced number of motors)

- A kinetic model elaborated on the basis of the results fits the performance of mammalian skeletal muscle and show that efficiency is maximized when the number N of myosin heads is ***N > 32***

Supplementary – optional - slides

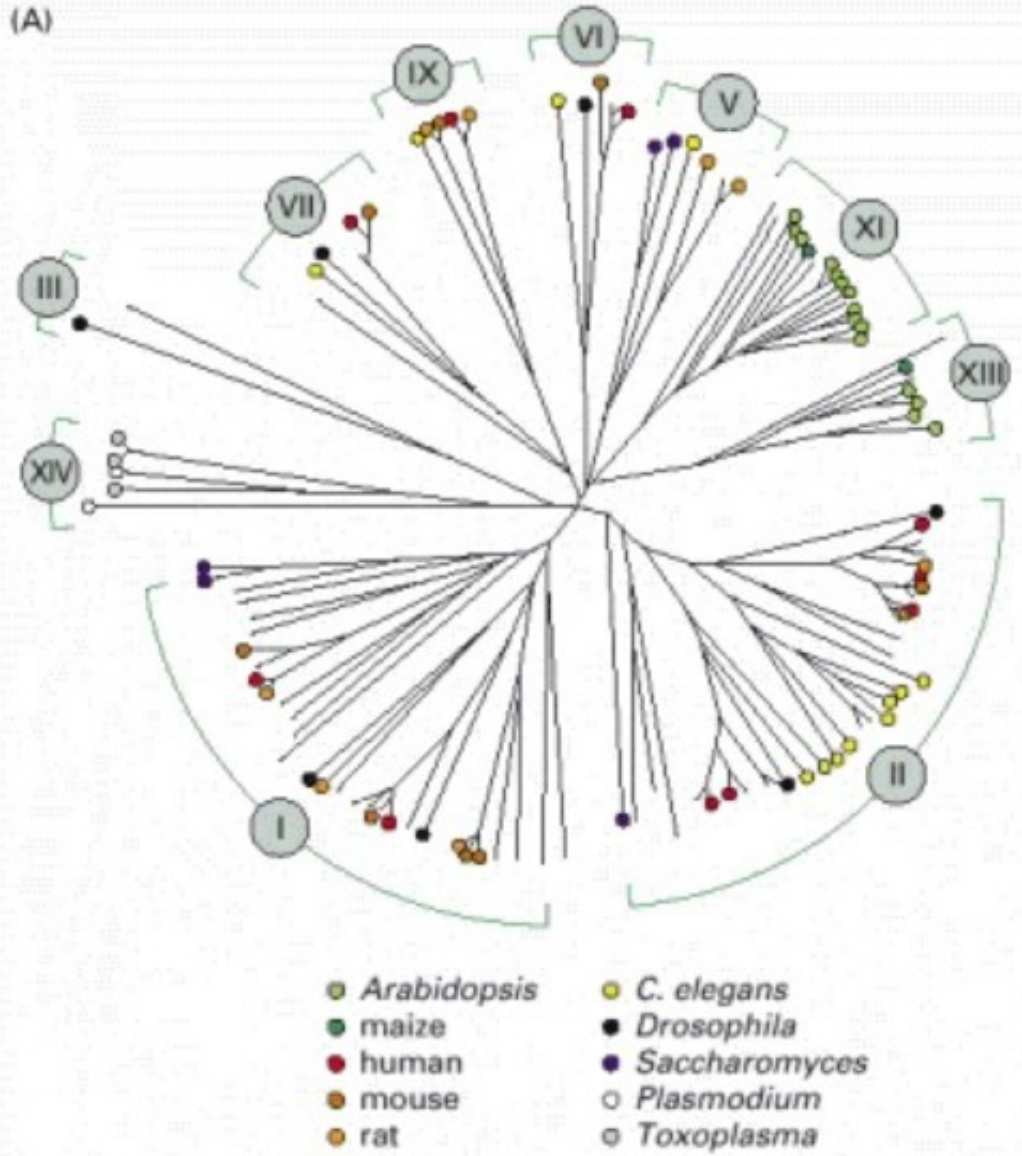
Myosin superfamily tree

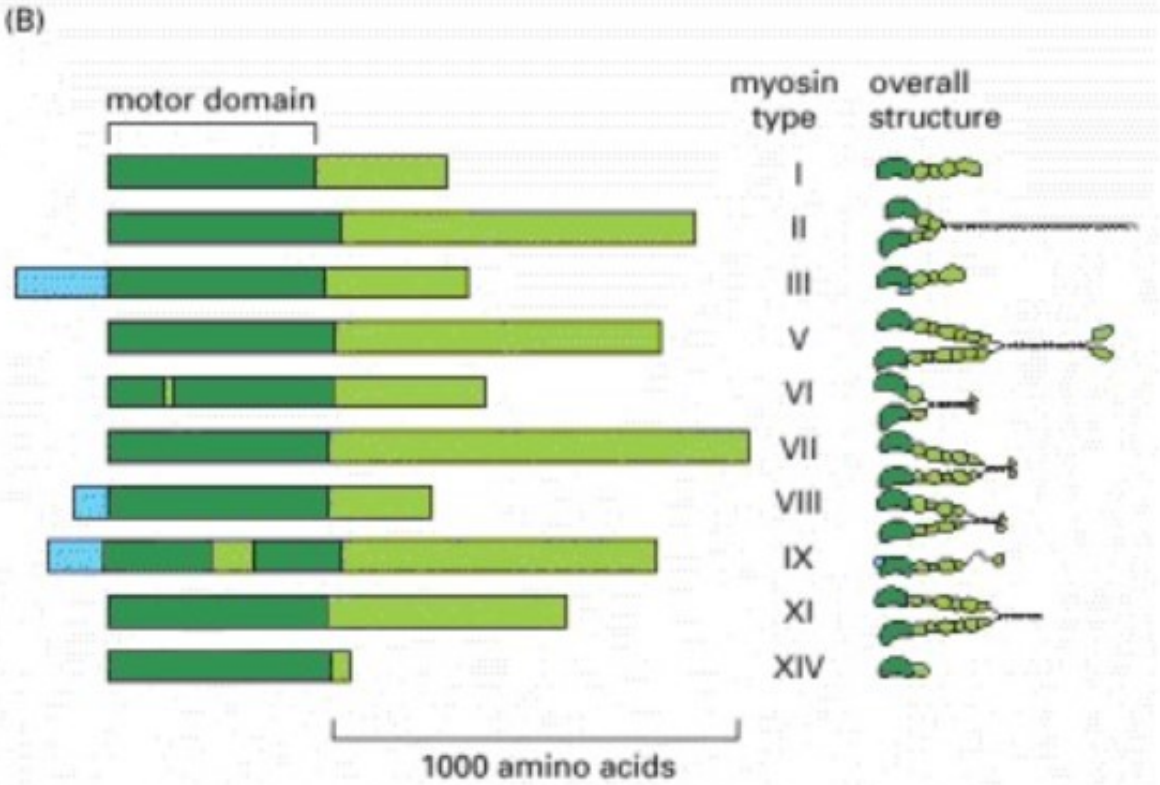
There are many other myosin types beside myosin II. The heavy chains generally start with a recognizable myosin motor domain at the N-terminus, and then diverge widely with a variety of C-terminal tail domains (Figure next slide).

The new types of myosins include a number of one-headed and two-headed varieties that are approximately equally related to myosin I and myosin II, and the nomenclature now reflects their approximate order of discovery (myosin III through at least myosin XVIII).

The myosin tails (and the tails of motor proteins generally) have apparently diversified during evolution to permit the proteins to dimerize with other subunits and to interact with different cargoes.

Myosin superfamily tree



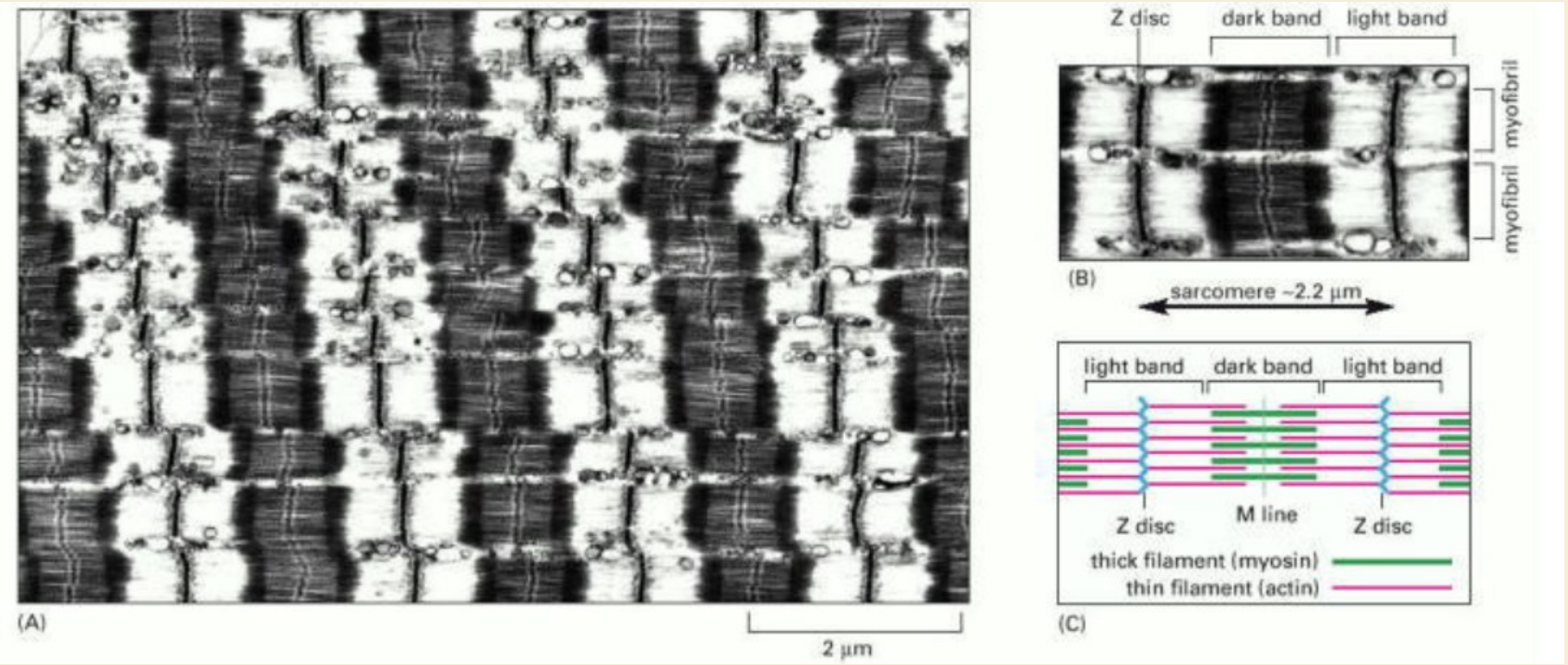


Comparison of the domain structure of the heavy chains of some myosin types. All myosins share similar motor domains (shown in dark green), but their C-terminal tails (light green) and N-terminal extensions (light blue) are very diverse.

On the right are depictions of the molecular structure for these family members. Many myosins form dimers, with two motor domains per molecule, but a few (such as I, IX, and XIV) seem to function as monomers, with just one motor domain.

OPTIONAL

Skeletal muscle myofibrils



(A) Low-magnification electron micrograph of a longitudinal section through a skeletal muscle cell of a rabbit, showing the regular pattern of cross-striations. The cell contains many myofibrils aligned in parallel (see previous figure).

(B) Detail of the skeletal muscle shown in (A), showing portions of two adjacent myofibrils and the definition of a sarcomere.

(C) Schematic diagram of a single sarcomere, showing the origin of the dark and light bands seen in the electron micrographs.

- The Z discs, at each end of the sarcomere, are attachment sites for the plus ends of actin filaments (thin filaments);
- the M line, or midline, is the location of proteins that link adjacent myosin II filaments (thick filaments) to one another.
- The dark bands, which mark the location of the thick filaments, are sometimes called A bands because they appear anisotropic in polarized light (that is, their refractive index changes with the plane of polarization).
- The light bands, which contain only thin filaments and therefore have a lower density of protein, are relatively isotropic in polarized light and are sometimes called I bands.

Each sarcomere is formed from a miniature, precisely ordered array of parallel and partly overlapping thin and thick filaments.

The *thin filaments* are composed of actin and associated proteins, and they are attached at their plus ends to a *Z disc* at each end of the sarcomere.

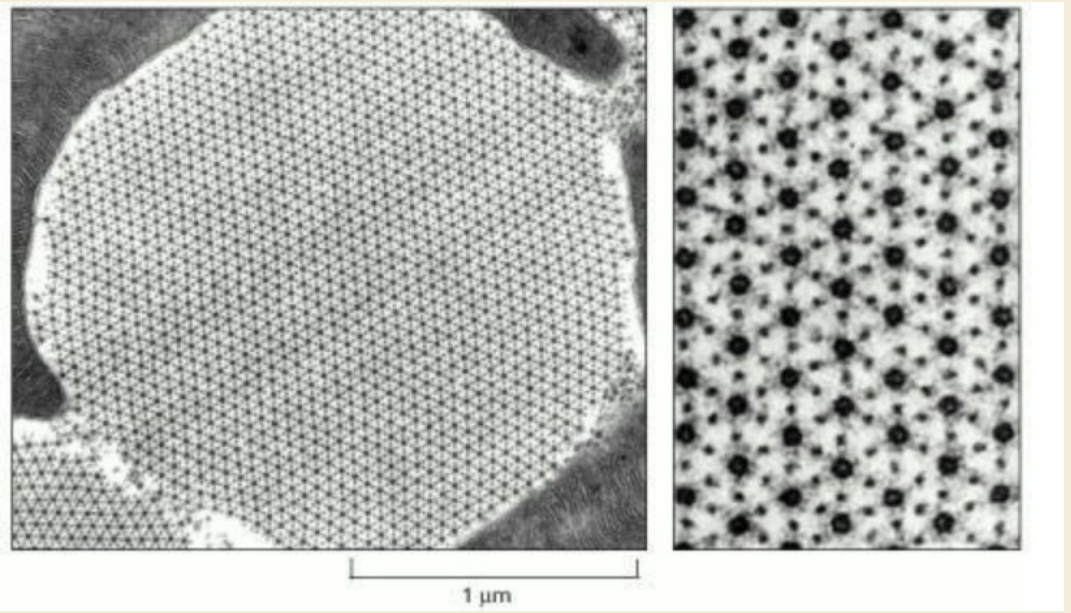
The capped minus ends of the actin filaments extend in toward the middle of the sarcomere, where they overlap with *thick filaments*, the bipolar assemblies formed from specific muscle isoforms of myosin II. When this region of overlap is examined in cross section by electron microscopy, the myosin filaments are seen to be arranged in a regular hexagonal lattice, with the actin filaments evenly spaced between them.

Cardiac muscle and smooth muscle also contain sarcomeres, although the organization is not as regular as that in skeletal muscle.

Electron micrographs of an insect flight muscle viewed in cross section

The myosin and actin filaments are packed together with almost crystalline regularity. Unlike their vertebrate counterparts, these myosin filaments have a hollow center, as seen in the enlargement on the right.

The geometry of the hexagonal lattice is slightly different in vertebrate muscle.



Motor Protein Kinetics Are Adapted to Cell Functions (4)

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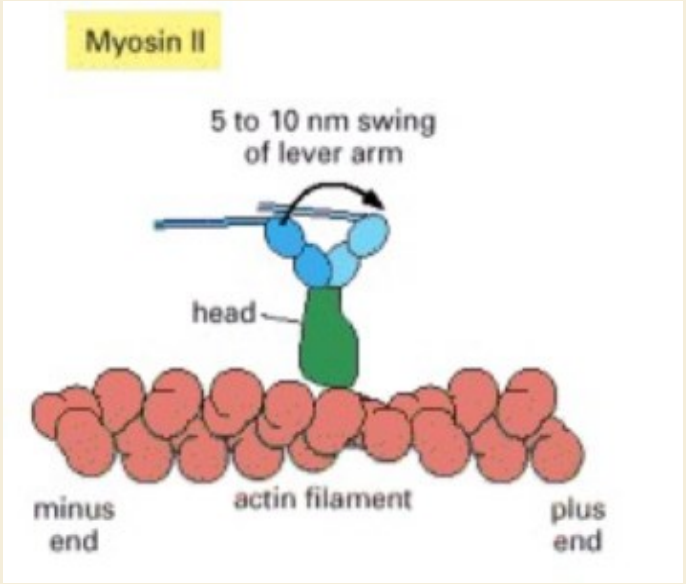
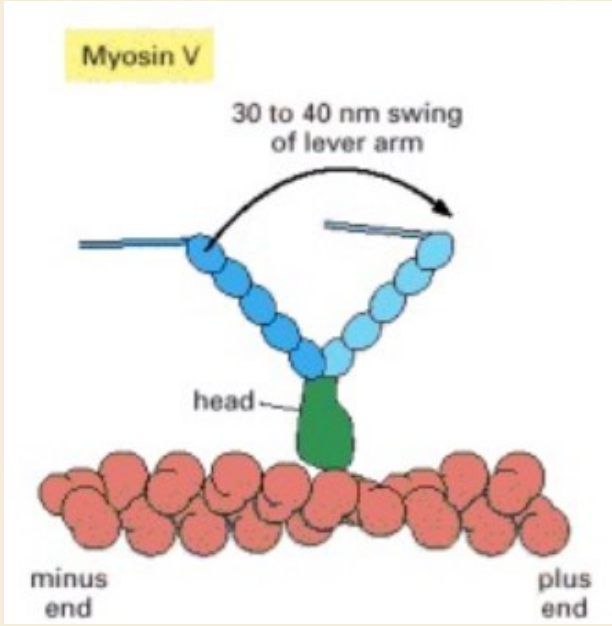


The effect of lever arm length on the step size for a motor protein

The lever arm of myosin II is much shorter than the lever arm of myosin V.

The power stroke in the head swings their lever arms through the same angle, so myosin V is able to take a bigger step than myosin II.



Each of these parameters varies slightly among different members of the myosin and kinesin families, corresponding to slightly different protein sequences and structures.

Structural comparison between Myosin and Kinesin

The motor domain of myosins is substantially larger than that of kinesins (about 850 amino acids compared with about 350 amino acids). They have no identifiable amino acid sequence similarities.

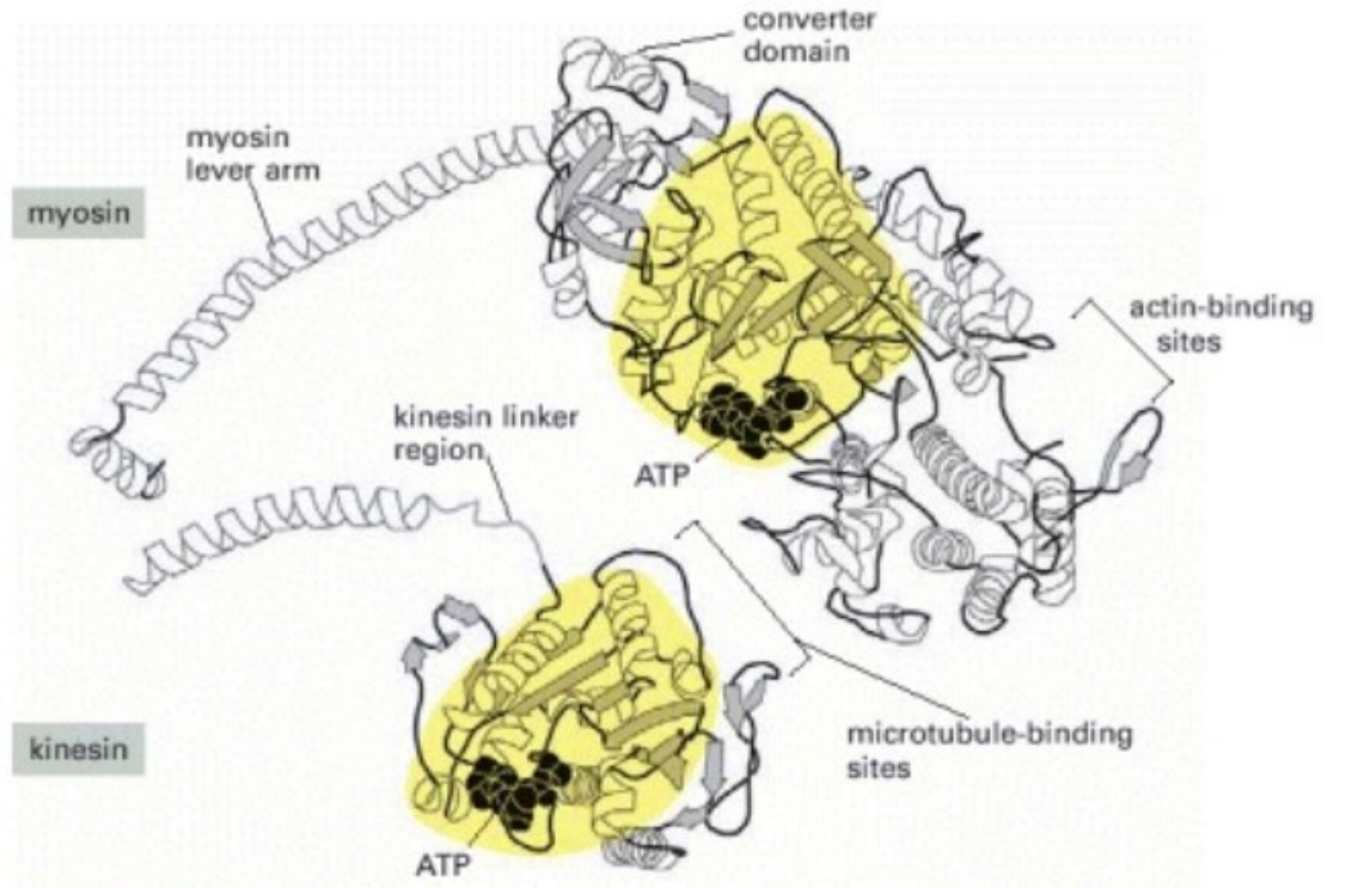
Myosin and kinesin work along **different filaments** and have **different kinetic properties.**

However, determination of the 3D structure of the motor domains of myosin and kinesin has revealed that these two **motor domains** are built around nearly **identical cores** (see slide X-ray crystal structures of myosin and kinesin heads at the end). The central force-generating element that the two types of motor proteins have in common includes the site of ATP binding and the machinery necessary to translate ATP hydrolysis into an allosteric conformational change.

The differences in domain size and in the choice of track can be attributed to large loops extending outward from this central core. These loops include the actin-binding and microtubule-binding sites, respectively.

Allosteric site: The place on an enzyme where a molecule that is not a substrate may bind, thus changing the shape of the enzyme and influencing its ability to be active.

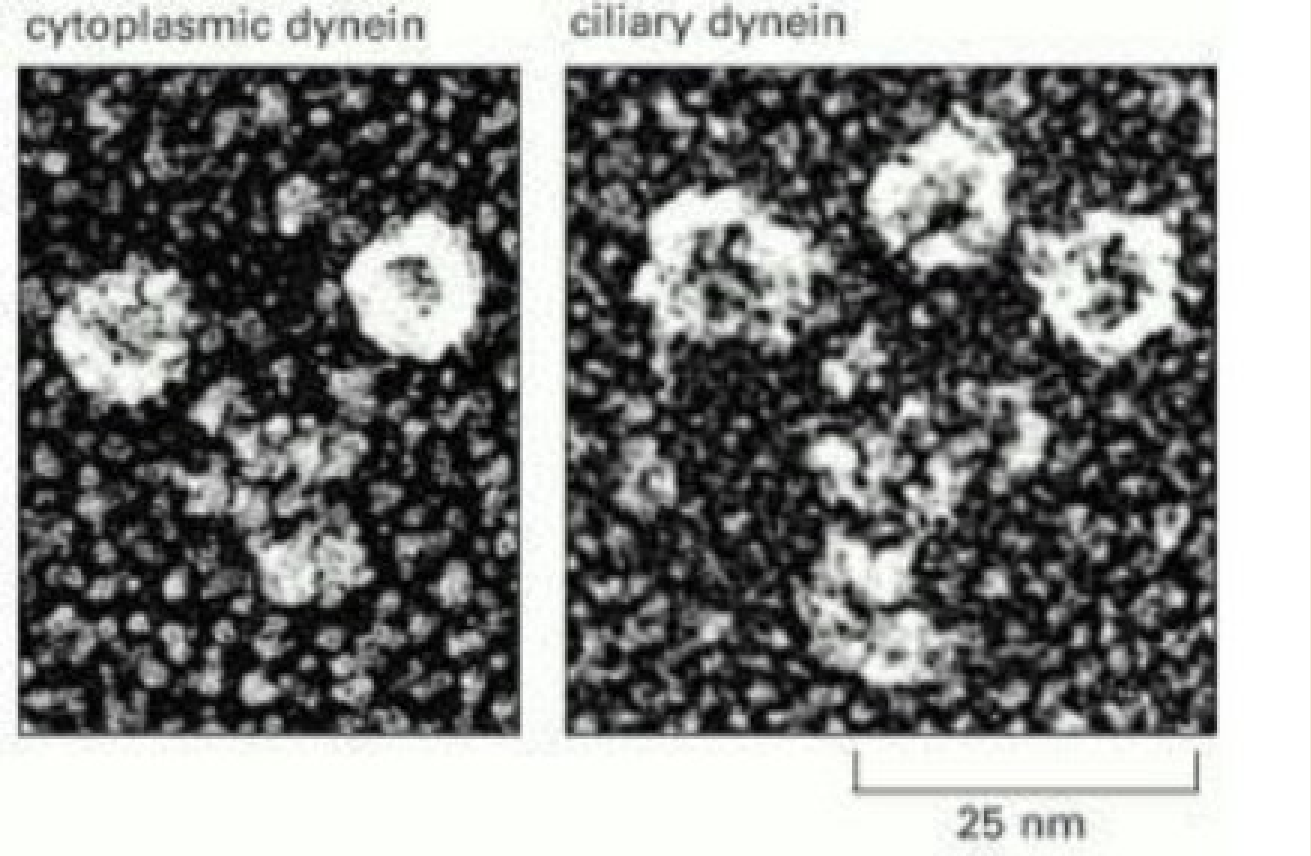
X-ray crystal structures of myosin and kinesin heads



The central nucleotide-binding domains of myosin and kinesin (shaded in yellow) are structurally very similar.

The very different sizes and functions of the two motors are due to major differences in the polymer-binding and force-transduction portions of the motor domain.

The Structural Similarity of Myosin and Kinesin Indicates a Common Evolutionary Origin



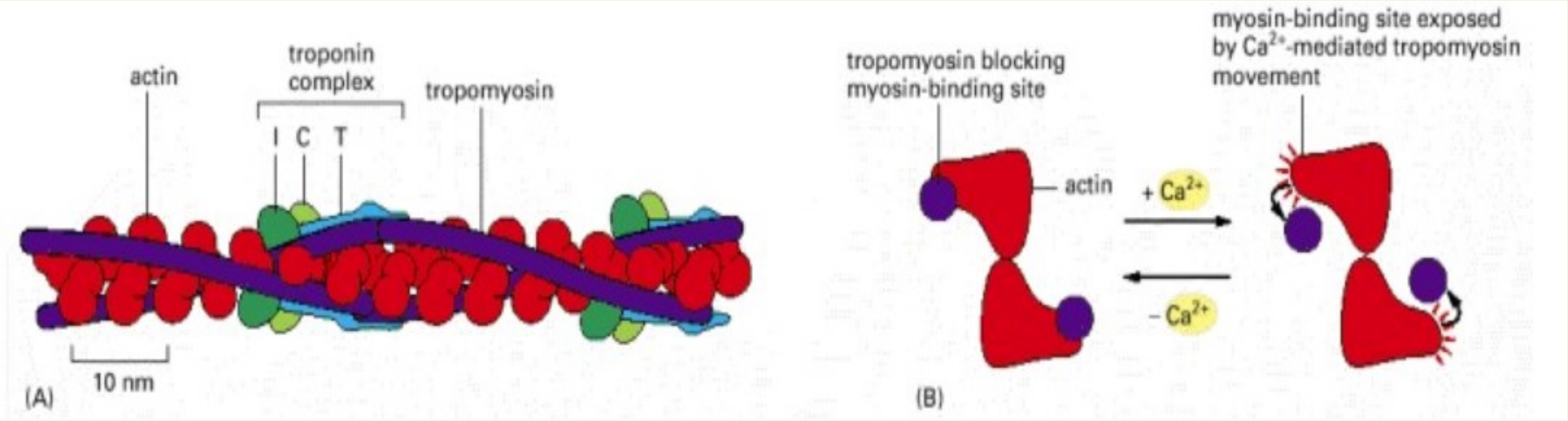
Electron Microscopy images

The dynein head is very large compared with the head of either myosin or kinesin.

Dyneins are the largest of the known molecular motors, and they are also among the fastest: axonemal dyneins can move microtubules in a test tube at the remarkable rate of 14 µm/sec.

In comparison, the fastest kinesins can move their microtubules at about 2–3 µm/sec.

The control of skeletal muscle contraction by troponin tropomyosin



(A) A skeletal muscle cell thin filament, showing the positions of tropomyosin and troponin along the actin filament. Each tropomyosin molecule has seven evenly spaced regions with similar amino acid sequences, each of which is thought to bind to an actin subunit in the filament.

(B) A thin filament shown end-on, illustrating how Ca^{2+} (binding to troponin) is thought to relieve the tropomyosin blockage of the interaction between actin and the myosin head.