Learning objectives

- Describe in detail the three major motor protein families
- Describe in detail the complex structure of sarcomeres
- Understand the mechanism of muscle contraction
- Describe how kinesin carries cargo within cells
- Describe how rotary motors drive bacterial motion
Movement

Whole organisms
Move to adapt to changes in the environment i.e. to keep warm
Navigating towards food
Moving away from danger

Cells
Not static
Assemblies of moving proteins, nucleic acids, organelles along defined paths inside cells

*Biochemical mechanism is the same*
Motion within cells
Molecular motors

• Operate by small increments and convert changes in protein conformation into directed motion

• Directed, orderly motion requires a track that steers the motion of the motor assembly

• Tracks consist of Actin and microtubules – protein filaments composed of repeating identical sub-units

• Motor proteins cycle between forms having high or low affinity for the filament tracks in response to ATP binding and hydrolysis
Most molecular motor proteins are members of the P-Loop NTPase superfamily

- Convert chemical energy in the form of ATP into kinetic energy
- Proteins are all members of the same protein family – the P-loop NTPases
- Catalyse the hydrolysis of ATP thus providing free energy for movement.
3 Major families of motor proteins

• MYOSINS
• KINESINS
• DYNEINS
Myosins

• Characterised on the basis of its role in muscle movement
• Moves along filaments of the protein actin
• Consist of two copies of a heavy chain (87kd)
• An essential light chain
• Regulatory light chain
Myosins

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• Consist of two copies of a heavy chain (87kd)
• An essential light chain
• Regulatory light chain
• Human genome encodes > 40 distinct myosins
• Mostly function in muscle contraction
KINESINS

• Role in protein, vesicle and organelle transport along microtubules i.e. chromosome segregation

• Consist of two copies each of a heavy chain (40kd) and a light chain

• Human genome encodes > 40 kinesins
Kinesin

(a) Structure of kinesin

- Tail
- Stalk
- Head

Light chains
Head domain of kinesin

Nucleotide-binding site

P-loop
Dyneins

• Larger
• Heavy chains molecular mass > 500kd
• Form multimers
• Human genome encodes approximately 10 dyneins
• Powers the motion of cilia and flagella
• Vesicular transport
• Mitosis
Dynein
Myosin
Myosin dissection
S1 fragment

- Essential light chain
- Regulatory light chain
- P-loop
- Actin-binding site
- Nucleotide-binding site
LMM
ATP binding and hydrolysis induces changes in the conformation and binding affinities of Motor proteins

- Feature of P-loop NTPases is that they undergo structural changes induced by ATP binding and hydrolysis

- Structural changes induce altered affinities for binding partners
S1 fragment (scallop muscle)
Relay helix

Position of lever arm when ADP is bound

Position of lever arm when ADP–VO$_4^{3−}$ is bound

Relay helix

Switch II

P-loop

VO$_4^{3−}$

Switch I
• In presence of ADP the LEVER ARM has rotated nearly 90° relative to the ATP position

• Occurs due to regions around the nucleotide binding site adopting different positions when the γ phosphate group of ATP is absent

• Binding of ATP DECREASES the affinity of myosin head for actin filaments
ATP – increases affinity of Kinesin to its track (microtubule)
<table>
<thead>
<tr>
<th>Protein</th>
<th>Bound to</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>NTP</td>
</tr>
<tr>
<td>Myosin (ATP or ADP)</td>
<td>Low</td>
<td></td>
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<tr>
<td>Affinity for actin</td>
<td></td>
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<tr>
<td>Kinesin (ATP or ADP)</td>
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<tr>
<td>Affinity for microtubules</td>
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<tr>
<td>Heterotrimeric G protein (α subunit)</td>
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<td>Low</td>
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<tr>
<td>(GTP or GDP)</td>
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<tr>
<td>Affinity for βγ dimer</td>
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<tr>
<td>Affinity for effectors</td>
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</table>
Myosins move along actin filaments

• Myosins, kinesins and dyneins move by cycling between different affinities for long polymeric molecules that serve as their tracks

• Myosin binds to ACTIN a 42kd protein

• Actin filaments involved in the reshaping of the cytoskeleton and the cell itself
Muscle is a complex of myosin and actin

- Vertebrate muscle which is under voluntary control is banded (Striated)
- Contains many parallel myofibrils (1\(\mu\)M in diameter)
- Functional unit is a SARCOMERE
Skeletal muscle
Skeletal Muscle

Structure of a Skeletal Muscle

- Bone
- Perimysium
- Blood vessel
- Muscle fiber
- Tendon
- Epimysium
- Endomysium
- Fascicle
Sarcomere

• Consists of two kinds of interacting protein filaments

• Thick filaments (diameter 15nM) composed primarily of myosin

• Thin filaments (diameter 9nM) contains actin, tropomyosin and troponin complex

• Muscle contraction is achieved by thin filaments sliding along the length of thick filaments, driven by hydrolysis of ATP

• Tropomyosin and troponin regulate this sliding in response to nerve impulses which increase Ca$^{2+}$ concentration
(A) Diagram showing the muscular sarcomere with regions labeled as I band, A band, and H zone, with Z lines indicating the boundaries.

(B) Diagram illustrating the arrangement of thick and thin filaments within the sarcomere. The caption indicates that there are thick filaments only, thick and thin filaments, and thin filaments only.
Figure 34-14b
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Actin is a polar, self assembling, dynamic power

• Actin monomers (G-actin i.e. Globular) come together to form actin filaments (F-actin)

• 4 domains which surround a bound nucleotide either ATP or ADP

• Oriented in the same direction along F-actin filament resulting in polarity

• Two ends
  - Barbed End (+) – towards Z-line
  - Pointed end (-)
Actin structure

- Dynamic
- Hydrolysis of ATP to ADP favours depolymerisation
Phosphate release triggers the myosin power stroke

• Addition of ATP to a complex of actin and myosin results in dissociation of the complex
• ATP binding and hydrolysis is therefore not directly responsible for power stroke
How does myosin travel along actin?
Myosin head moves 110Å
Summary

• Myosin-ADP bound to actin. Release of ADP and binding of ATP results in dissociation of myosin from actin

• Binding of ATP leads to significant conformational change amplified by the lever arm

• Moves myosin head along the actin filament 110Å

• ATP in myosin is hydrolysed. ADP and Pi remain bound to myosin.

• Myosin then binds to surface of actin causing dissociation of Pi.
• Phosphate release causes conformational change increasing affinity of myosin head for actin and allows lever arm to move back to original position

• The conformational change due to release of Pi corresponds to the power stroke

• After release of Pi myosin remains bound to actin and the cycle can begin again
• Actin filaments associate with each head rich region with barbed ends of actin towards the z-line

• Presence of normal levels of ATP most myosin heads are detached from actin

• Each head can independently
  Hydrolyse ATP
  Bind to actin
  Release Pi
  Undergo power stroke

• Having many myosin heads independently attaching and moving an actin filament allows for much greater speed than could be achieved by a single motor protein >80,000Å/s
Muscle innervation

Axons

Motor end plates

Myofibers
REGULATION OF MUSCLE CONTRACTION BY CALCIUM

In resting muscle Ca^{2+} is pumped into the sarcoplasmic reticulum (SR) by an ATP-driven active transporter. A nerve impulse leads to the release of Ca^{2+} from the SR. The rise in [Ca^{2+}] from 1\,\mu\text{m} to 10\,\mu\text{m} causes a conformational change in the \textit{troponin complex} which is transmitted to the \textit{tropomyosin}.

At low Ca^{2+} tropomyosin sterically blocks the binding of S1 to actin, but not at high Ca^{2+}.

\textit{Actin and myosin have contractile roles in nearly all eukaryotic cells.}
Troponin and tropomyosin

Myosin binding sites
CYTOSKELETON AND MICROTUBULES
• Cytoskeleton includes other components along with actin
• Microtubules - serve as tracks for two classes of motor proteins
    Kinesins
    Dyneins
• Kinesins moving along microtubules usually carry cargo such as organelles or vesicles
• Dyneins – important in sliding microtubules relative to one another during the beating of cilia and flagella on the surface of some eukaryotic cells
Microtubules

- Consist of two kinds of homologous 50kd subunits called α and β tubulin
- Assemble in a helical array of alternating tubulin types to form the wall of a hollow cylinder
Microtubules

• Consist of two kinds of homologous 50kd subunits called α and β tubulin

• Assemble in a helical array of alternating tubulin types to form the wall of a hollow cylinder

• Outer diameter is 30nM

• Polar structures
  
  minus end - anchored near the centre of the cell

  plus end – extends towards the surface of the cell
Microtubules

- GTP bound
- Dynamic instability

P-Loop NTPase domain
• Microtubules are key components of cilia and flagella

• Sperm propel themselves through the motion of flagella containing microtubules

• Important in determining the shape of cells and in separating daughter chromosomes during mitosis

• Dynamic structures that grow through addition of $\alpha$ and $\beta$ tubulin to the ends of existing structures

• Tubulins bind and hydrolyse nucleoside triphosphates (GTP) through their P-loop NTPase cores

• Dynamic instability

• Taxol
Flagella

- Bundle of microtubules called axenome surrounded by a membrane continuous with the plasma membrane
- Composed of a peripheral group of 9 microtubule pairs surrounding two single microtubules
- Motif is often called a 9 + 2 array
Flagellar axoneme
Flagella

• Bundle of microtubules called axenome surrounded by a membrane continuous with the plasma membrane

• Composed of a peripheral group of 9 microtubule pairs surrounding two single microtubules

• Motif is often called a $9 + 2$ array

• Dynein drives motion of one member of each pair relative to the other – structure bends
Cross-sectional diagram of the Chlamydomonas flagellar axoneme

- Inner arm dynein
- Outer arm dynein
- Nexin link
- Radial spoke
- Microtubule doublet
- Central pair microtubules
- B tubule
- A tubule
- 50 nm
Kinesin motion is processive

• Motor proteins that move along microtubules
• Two head groups of kinesin molecule operate in tandem. One binds then the next one etc
• Single kinesin molecule can take 100 steps toward the plus end before the molecule becomes detached
• ATP strongly INCREASES the affinity of kinesin for microtubules (CONTRAST WITH MYOSIN WHICH CAUSES DISSOCIATION FROM ACTIN)
• Two headed kinesin molecule in ADP form is dissociated from microtubule

• Neck linker binds the head when ATP is bound and released when ADP is bound

• Interaction of head domain with tubulin dimer on a microtubule stimulates release of ADP and subsequent binding of ATP

• ATP triggers conformational change

  Affinity of head domain for microtubules increases-locking into place

  Neck linker binds to head domain

• This change repositions the other head domain
• Second head now links up with a second tubulin dimer 80Å along microtubule in direction of plus end
• ATPase activity of first head domain hydrolyses ATP to ADP + Pi
• Second head domain binds microtubule, first head releases ADP and binds ATP
• ATP binding favours conformational change which pulls first head domain forward
• Continues for many cycles until both heads are simultaneously bound with ADP when kinesin dissociates from microtubule
• Kinesin hydrolyses ATP at 80 molecules per second
• Step size = 80Å per ATP
• Kinesin molecule moves along microtubule at 6400Å per second
• Slower than myosin which maximises speed of motion
• Kinesin functions to achieve steady, but slower transport in one direction along a filament
Rotary Motors
5.5 subunits per turn
40 distinct proteins

MS (membrane and supramembrane)
Flagella motor components

Charged amino acids
Proton transport-coupled rotation of the flagellum

(A) Outer half channel
MotB
MotA
Inner half channel
Peri
Cyto

(B) Proton uptake through outer half-channel
Counterclockwise rotation of MS ring
Proton release through inner half-channel
• Video showing bacteria switching between runs and tumbles
Bacterial motion
Changing direction

a-c
Chemotaxis signalling pathway