

Molecular motors

Intracellular transport and cell movement



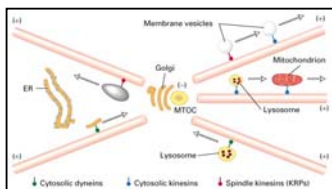
Cellular motors

- Change chemical energy of ATP into force, later converted to molecular motion
- Do it by conformational change in the head domain
- Use tracks (cytoskeletal filaments) to move cargo or to produce cell shape changes (movement)



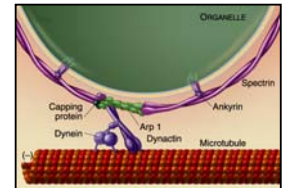
Transport of vesicles

- Along microtubules (free cellular microtubules are singlets)
 - Antrograde – toward periphery
 - Retrograde – toward the middle of the cell
- Direction is determined by a motor



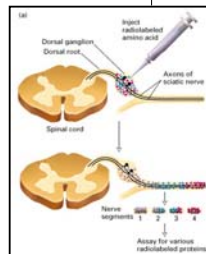
Transport of vesicles

- Kinesins directly bind to vesicles by their tail domain
- Dyneins can not directly bind and use a complex called dynactin
 - p150 Glued binds to intermediate chain of dynein
 - On the other end to Arps and through them to vesicle cytoskeleton



Fast axonal transport

- Associated with axonal growth and renewal of synaptic vesicles
 - Anterograde - synaptic vesicles towards synapse
 - Retrograde - "old" membranes toward lysosomes
- Transport along microtubules
- Different proteins are transported at different rates



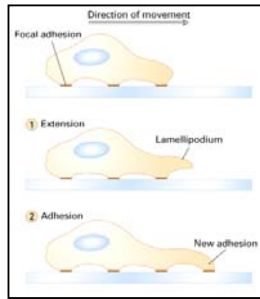
Membrane bound myosins cause vesicle translocation in the active zone

- Involves myosin I and V
- Movement along actin filaments
- Mostly translocation of Golgi vesicles between membrane compartments



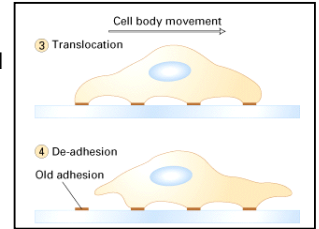
Changes in cell shape during migration

- Extension of the cell membrane
- Adhesion of the extended edge to the substratum



Changes in cell shape during migration

- Translocation
- Retraction of the cell body (de-adhesion)



Force-generating processes underlying cell movement

- Actin polymerization and rearrangement of actin filaments
- Interactions of myosins with actin filaments
 - Stress fibers – contractile bundles used in cell adhesion
 - Cortical contraction by myosin II
 - Myosin I and V generated movement

Cell movement

- Membrane is extended forward by the pushing action of the polymerizing actin filaments
- Actin bundles at the leading edge quickly develop into focal adhesions
 - Anchoring of the cell
 - Preventing retraction

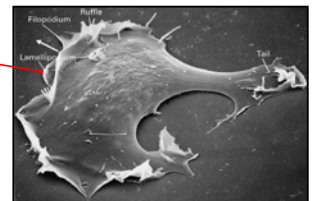
Cell movement

- Myosin dependent cortical contraction moves the cytoplasm forward
 - Organelles are attached to the cytoskeleton
- De-adhesion - contraction of stress fibers in the tail

Lamellipodium

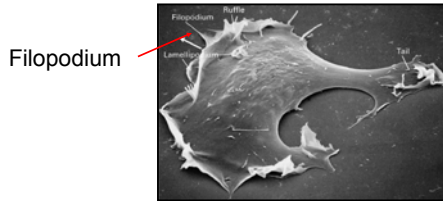
- Large, broad membrane protrusion
- Typical for fast moving cells

Lamellipodium



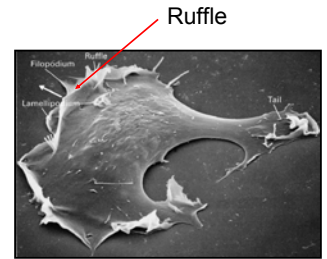
Filopodia

- Slender, spiky membrane protrusion
- Typical for slow moving cells



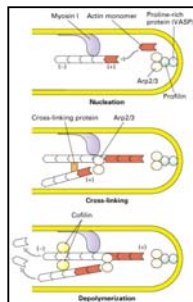
Membrane ruffle

- A membrane protrusion that did not form focal adhesions
- Projects upward



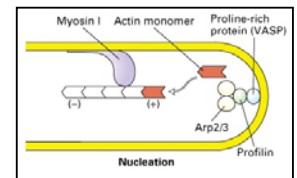
Molecular events at the leading edge of a moving cell

- Forward movement is created by rapid actin polymerization
- Aided by actin binding proteins
- Myosin I helps anchor actin filaments to the membrane



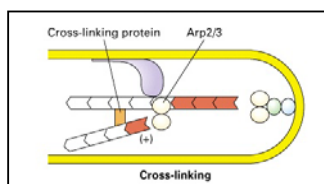
Nucleation

- Polymerization of actin at barbed end
- Stimulated by profilin
- Arp2/3 and VASP direct the assembly
 - VASP binds to profilin and releases actin monomer that can be added to a filament
- Outward motion



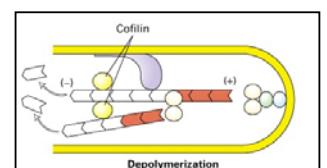
Cross-linking

- Cross-linking proteins stabilize actin filaments in network and bundles



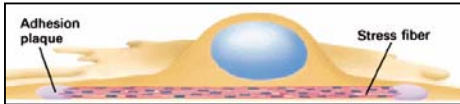
Depolymerization

- Cofilin induces the loss of actin at (-) end



Adhesion

- Formation of focal adhesions happens at the end of polymerizing actin filaments
- Actin filaments and myosin II form contractile bundles - stress fibers

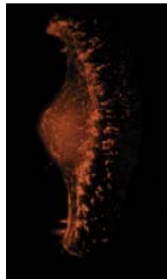


Adhesion

- Stress fibers
 - Have sarcomere-like organization
 - Lie along ventral surface of cultured cells
 - Myosin and α actinin are distributed in patches
- Ends of stress fibers terminate in focal adhesions

Cell translocation and de-adhesion

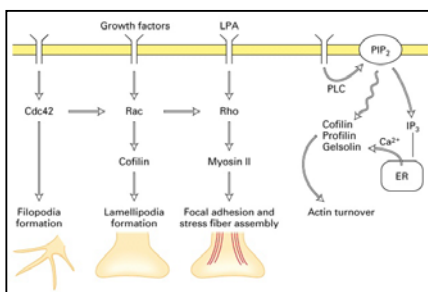
- Myosin II is localized between cell body and lamellipodia (cortical zone)
- Cortical contractions translocate the cytoplasm with organelles attached to the cytoskeleton
- Contraction of stress fibers breaks the focal adhesions at the rear end of the cell



Modulation of cell locomotion and adhesion

- External stimuli can affect cell locomotion through the activation of surface receptors
 - Growth factors
 - Serum

Signal-transduction pathways that control cell locomotion



Second messenger pathways that control cell locomotion

Monomeric proteins from Rho family

- Rac controls the assembly of lamellipodia
- Rho A controls the stress fibers formation
- Cdc42 stimulates the assembly of filopodia

Integration of cell movement

- Activation of surface receptors
- PIP_2 hydrolysis by phospholipase C
- Release of profilin, cofilin from the membrane
- IP_3 (a product of PIP_2 breakdown by phospholipase C) stimulates Ca^{2+} release from intracellular stores



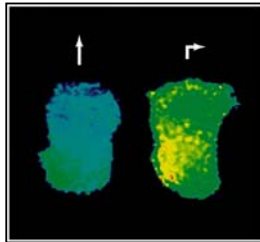
Integration of cell movement

- Ca^{2+} activates myosin II and gelsolin activity
- In addition Ca^{2+} is a strong chemotactic molecule directing cell translocation



Chemotactic molecules

- Cytosolic Ca^{2+} is a sensor for chemotaction
- Most of actin-binding proteins are regulated by Ca^{2+}



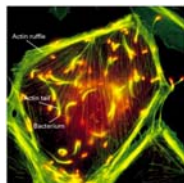
Chemotactic molecules

- Cytosolic Ca^{2+} is a sensor for chemotaction
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Intracellular movements of bacteria and viruses

- Bacteria and viruses use actin polymerization to move inside the eukaryotic cell
 - Ex: *Listeria monocytogenes*
 - Vaccinia



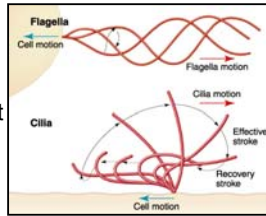
Intracellular movements of bacteria and viruses

- Actin polymerizes into filaments at the base of the bacterium that pushes bacterium ahead
- Profilin interacts with bacterial membrane protein and promotes polymerization



Cilia and flagella

- Flexible membrane extensions
- Usually few long flagella and multiple short cilia
- Same structure, different beating pattern

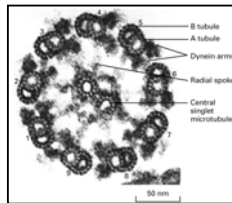


Cilia and flagella

- Beating is characterized by a series of bends
- Movement originates at the base
 - Flagellar beating propels cells forward
 - Ciliary beating sweeps materials across tissues

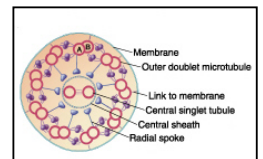
Structure of cilium and flagellum

- Both have same structure called axoneme
- Axoneme - a bundle of microtubules covered by the membrane
 - 9 doublets
 - 2 singlets in the middle
 - Accessory proteins to hold it together



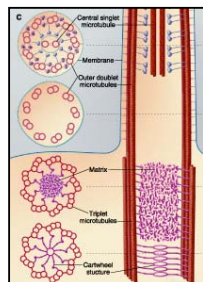
Axoneme arrangement

- Dyneins bound to outer doublets generate force
 - Dyneins attached to A microtubule
 - Head domains directed toward B tubule of another doublets
- Structure stabilized by radial spokes



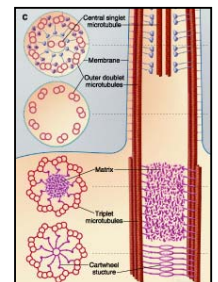
Basal body

- Microtubule organizing center
 - Modified centriole except the outer tubules are continuous
- Basal body anchors cilium or flagellum to the cell



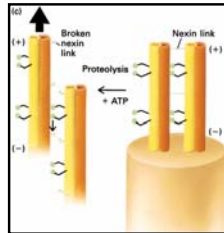
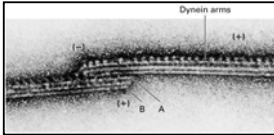
Basal body

- Cylindrical structure of 9 triplet microtubules
- Filled up with matrix proteins
- Initiates growth of axoneme



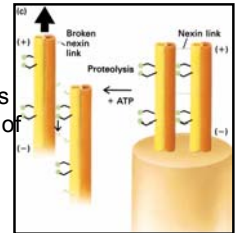
How do cilia and flagella move?

- Flagellar beating are produced by sliding of outer doublets relative to each other while nexin prevents microtubules from sliding



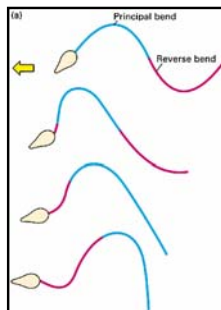
Model for sliding of doublet microtubules

- Dynein arms attached to A microtubule "walk" along the B microtubule
- "Walking" dynein generates the force
- Since microtubules are cross linked to each other instead of sliding they bend



Flagellar motions in sperm

- Flagella are stiff during the initial part of movement
- Effective stroke - stiff flagella
- Recovery stroke



REMEMBER

- The only thing eukaryotic and bacterial flagella have in common is a name
- Bacterial flagella are made of flagellin not microtubules
- They rotate not bend
- They are powered by movement of protons

