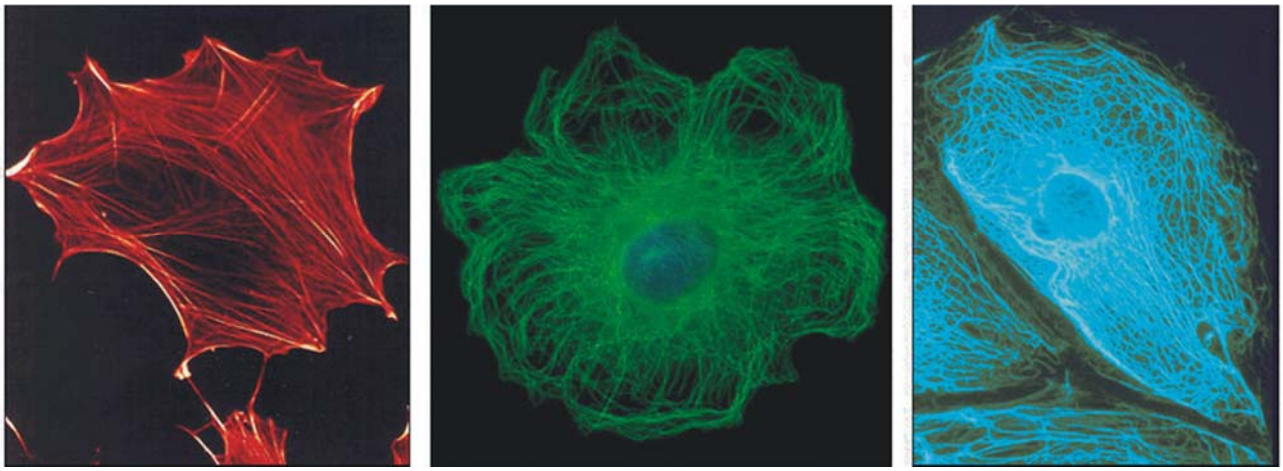


Cytoskeleton

1

Cytoskeleton

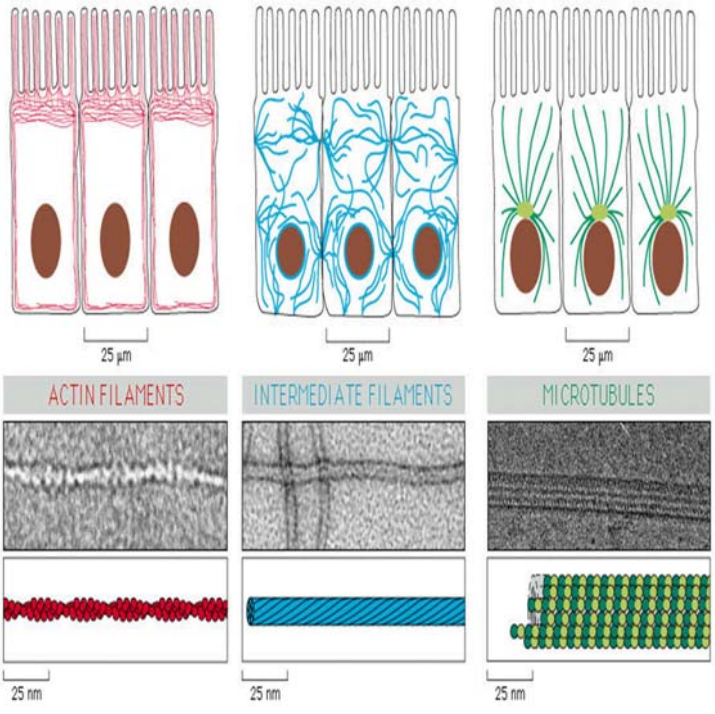
- www.ncbi.nlm.nih.gov/books/bv.fcgi?call=bv.View..ShowSection&rid=**cell**
- All images are form the source mentioned above, unless otherwise stated

Cytoskeleton

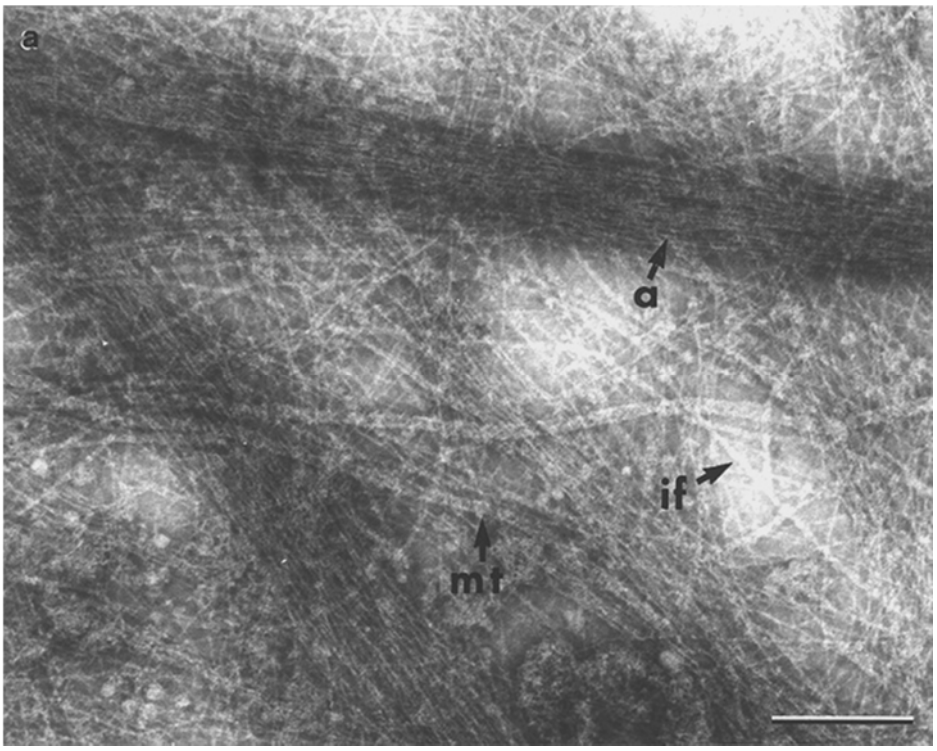
- What is the cytoskeleton
- What is it made of
- Functions of each of its components
- Striated muscle contraction

Cytoskeleton

– Three well defined filamentous structures
  - Microfilaments
  - Intermediate filaments
  - Microtubules



Cytoskeleton in EM



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Cytoskeleton

- Protein polymers that form *protofilaments*
- Protofilaments associate to form specific filaments or microtubular elements

- Cytoskeletal proteins are ATP/GTP-binding proteins and exert ATP-/GTP-ase activity

- Protein-ATP = 
- Protein- ADP= 

6

Polymerization of cytoskeletal components

Stages:

- 1. Nucleation
- 2. Elongation
- 3. Steady-state

Polymerization of cytoskeletal components (animation)

https://www.youtube.com/watch?v=VVgXDW_8O4U

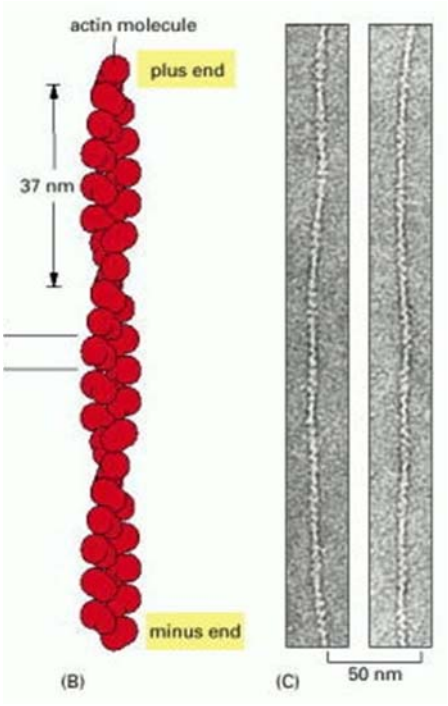
Cytoskeleton

- Cytoskeletal components involved in motility have **polarity!**

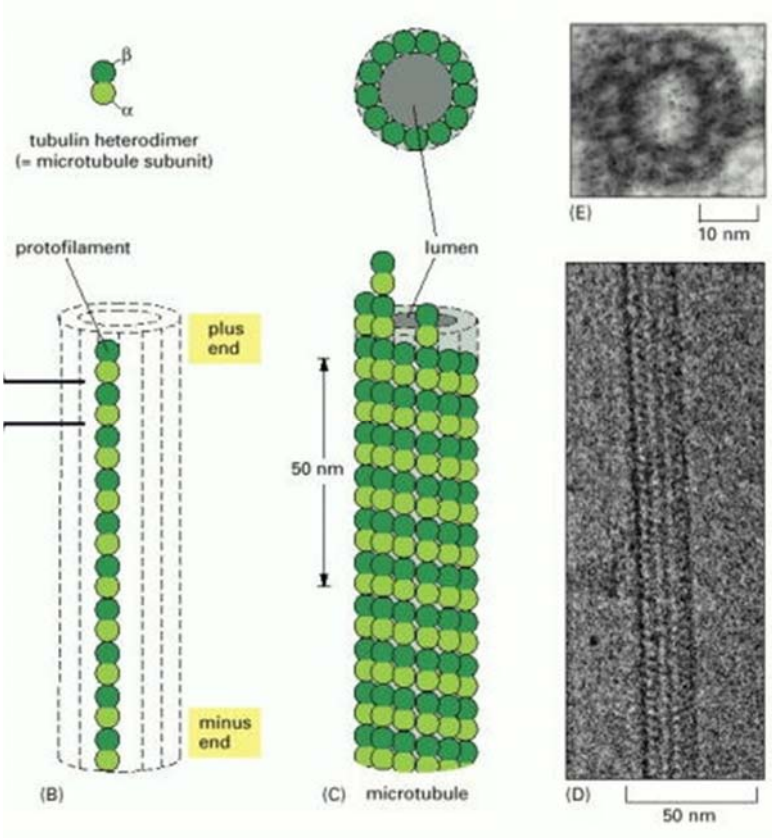
- plus end (+) – new monomers are added
- minus end (-) – monomers are removed

Polarity of filaments guides ***motor proteins*** (proteins interacting with cytoskeleton elements to generate active movements)

Actin filaments



Microtubules



Cytoskeleton

- Microfilaments
  - Actin and actin-associated proteins
- Microtubules
  - hollow tubes
  - Tubulins
- Intermediate filaments
  - ropelike fibers

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Microfilaments

- Actin
- Monomeric actin = G (globular) actin
- Polymerized actin = F (filamentous) actin

- Dynamic structures regulated by actin-associated proteins

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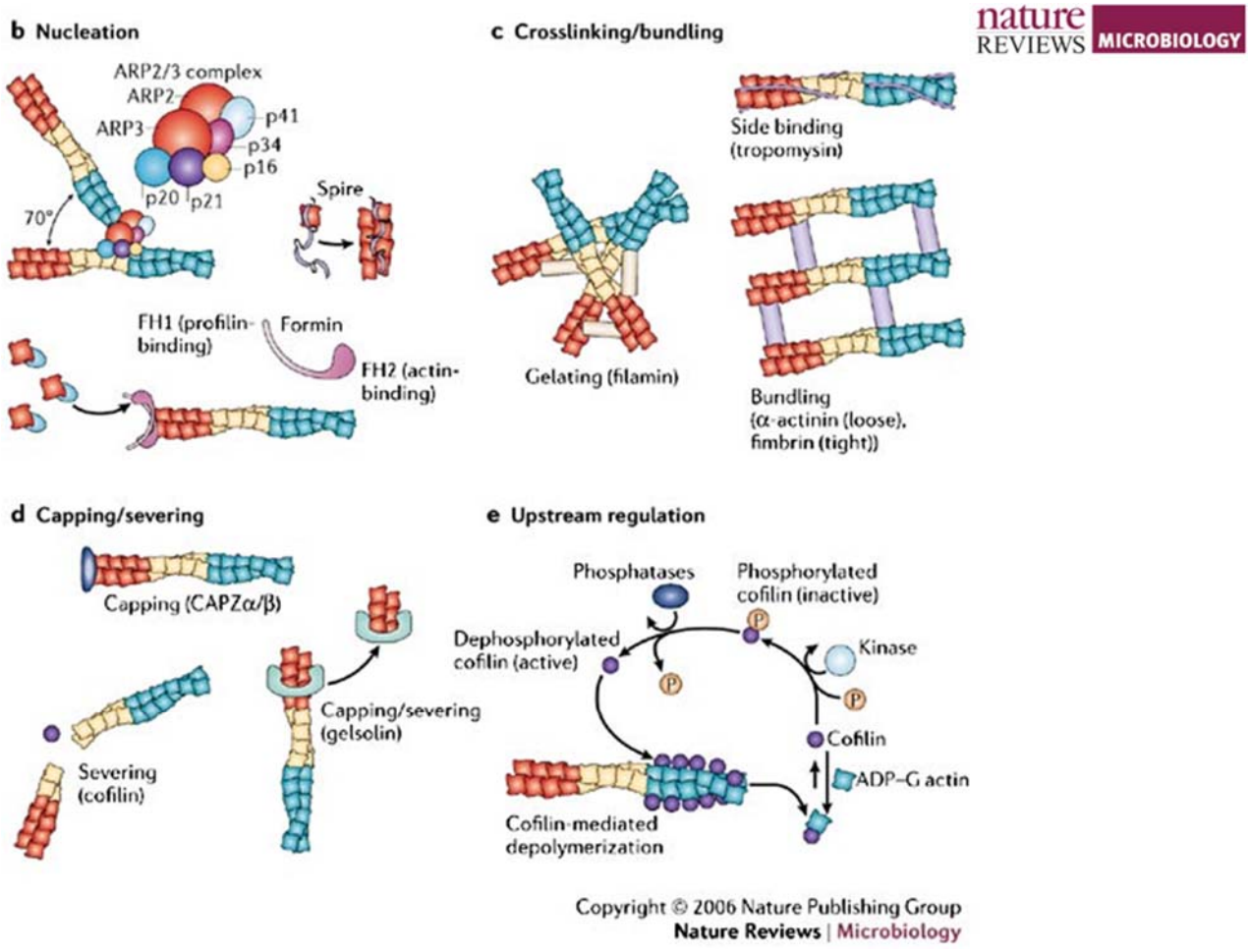
Actin-associated proteins

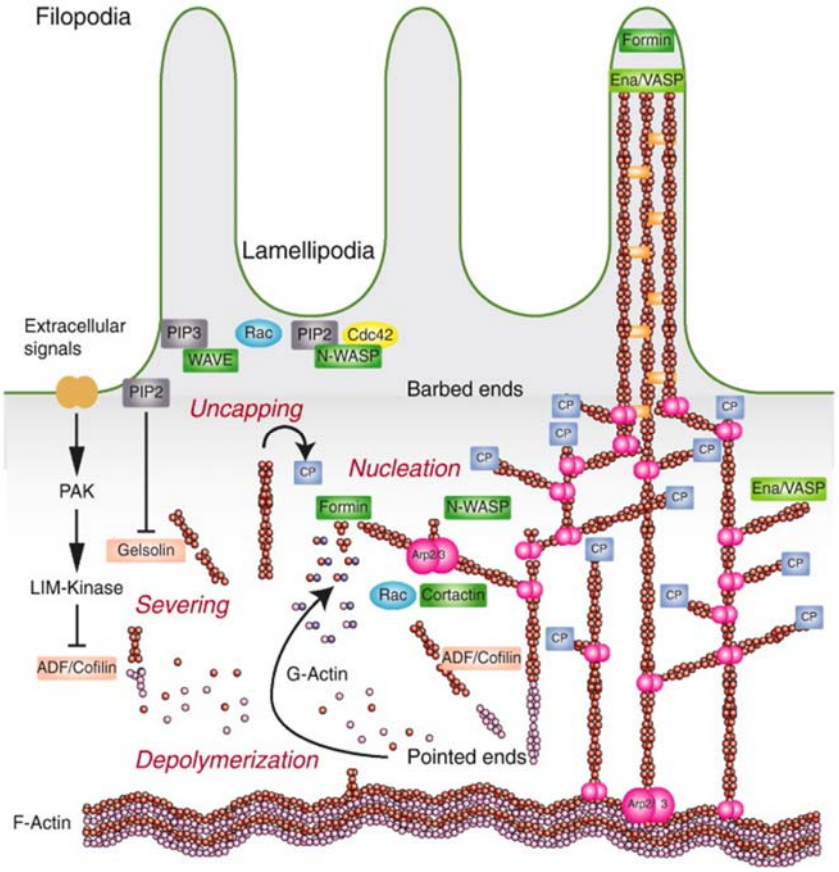
- **Modulate filament organization**
  - Nucleating proteins – promote filament growth
  - Capping proteins – stabilize/depolymerize filaments
  - Monomer sequestering proteins
  - Severing proteins

- **Dictate 3D organization of filaments**
  - Parallel bundles (microvillus axis, stress fibers)
  - Networks (terminal web)



Baum *et al. Nature Reviews Microbiology* **4**, 621–628 (August 2006) | doi:10.1038/nrmicro1465

ACTIN- binding proteins



Biochimica et Biophysica Acta (BBA) – Biomembranes. Volume 1778, Issue 3, March 2008, Pages 670–691

Functions of actin filaments

- Cell shape
- Organization of some cell junction
- Motility (cell motility, intracellular component motility)
- Cell division

Actin filaments and cell shape

- Transient cellular processes
  - Pseudopodia
  - Filopodia
  - Lamellipodia
- Permanent cellular processes
  - Microvilli
  - Stereocillia

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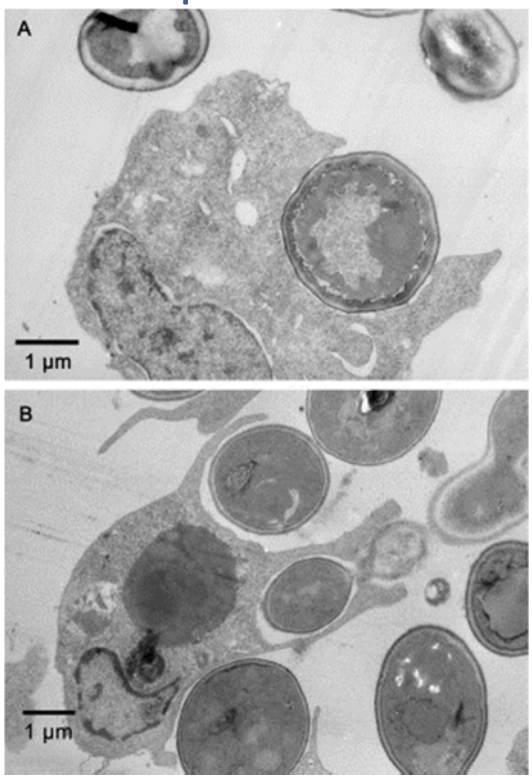
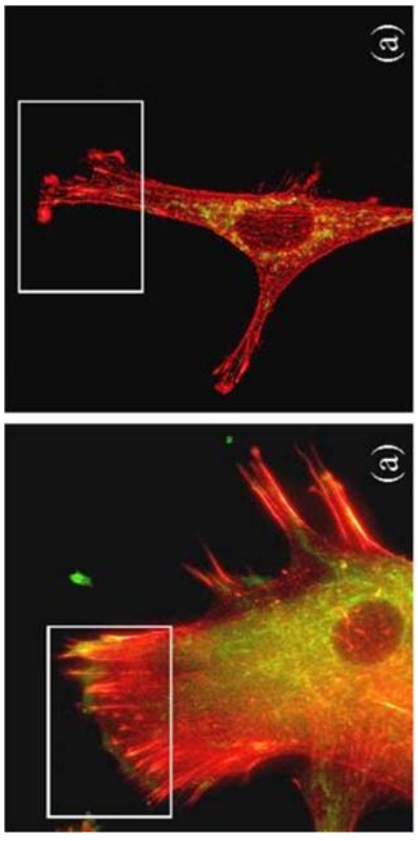
Actin filaments and cell shape

- Pseudopods



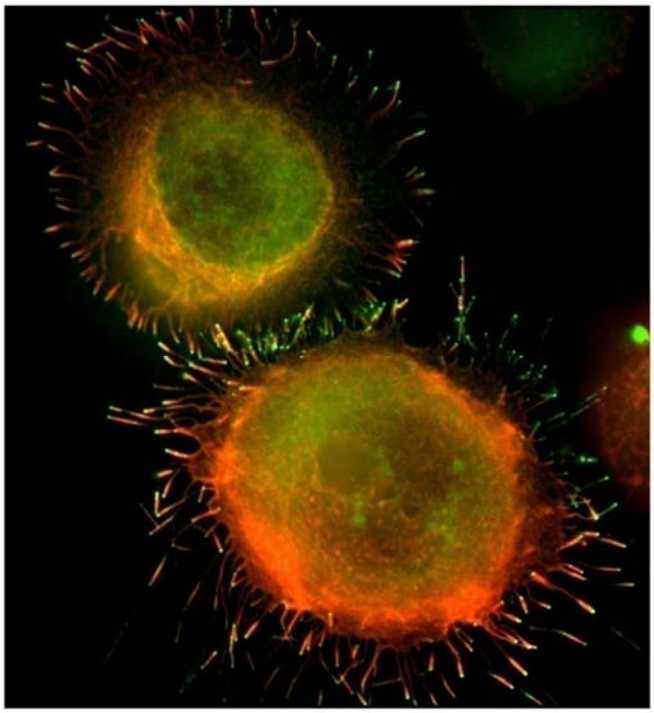
Molecular Immunology Volume 49, Issues 1–2, October–November 2011, Pages 211–218



Acta Biomaterialia Volume 6, Issue 2, February 2010, Pages 591–597

Actin filaments and cell shape

Filopodia are long thin transient processes that extend out from the cell surface.



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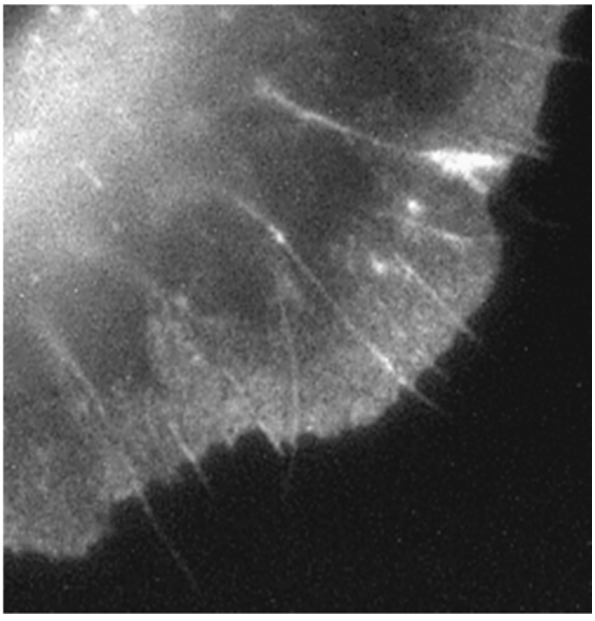
Actin filaments and cell shape - transient processes

- **Lamellipodia** are thin but broad projections at the edge of a mobile cell.



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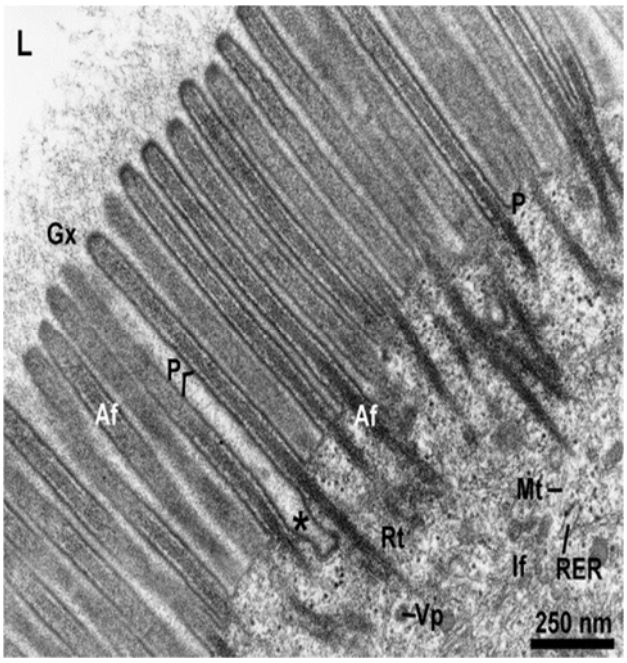
F-Actin filaments and cell shape – permanent processes

Microvilli are shorter and more numerous protrusions of the cell surface found in some cells (enterocytes, nephrocytes).



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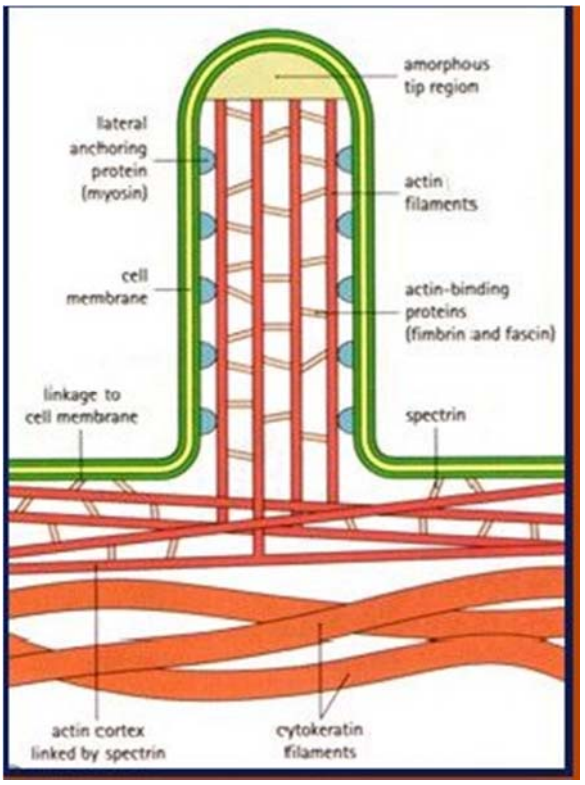
Microvilli

– Tightly bundled actin filaments are located within these structures as well, with plus ends oriented toward the tip.

– The small cross-linking proteins **fimbrin** and **villin** bind actin filaments together within microvilli.



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Stereocilia

- non-motile, permanent actin-organised processes, longer than microvilli
- found in: male genital tract, inner ear

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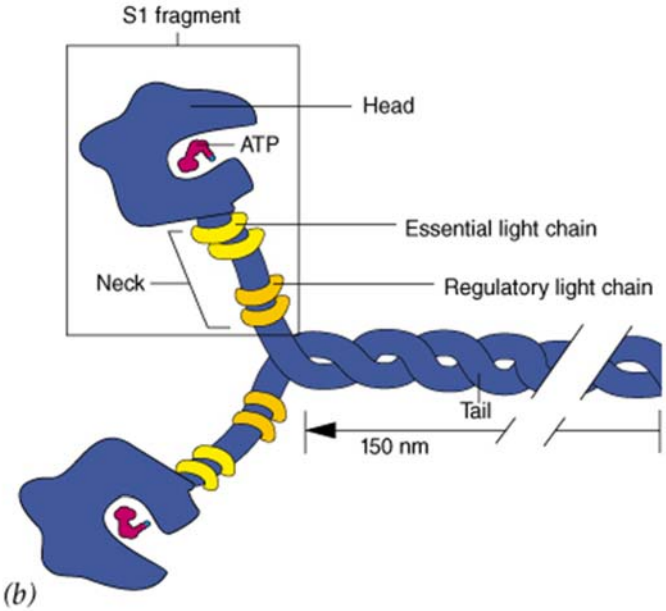
Myosin

- MOTOR PROTEIN found mostly in muscle cells, involved in muscular contraction (muscle myosin – organizes filaments)
- non-muscle myosin in other cell types (enterocytes, fibroblasts)
- structure:
  - 2 heavy chains
  - 4 light chains (2 essential + 2 regulatory)



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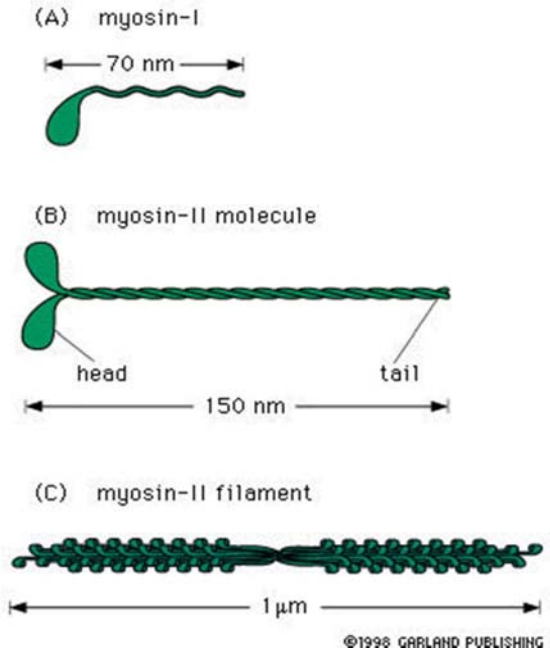
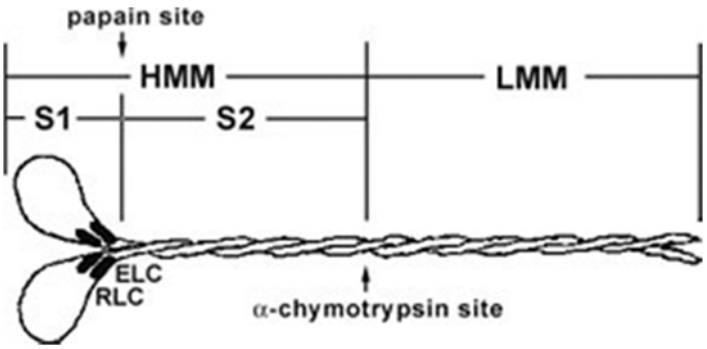
Myosin

- muscular myosin may be digested with chymotrypsin – 2 fragments
  - HMM = heavy meromyosin – ATPase activity
  - LMM = light meromyosin – associates with other myosin molecules





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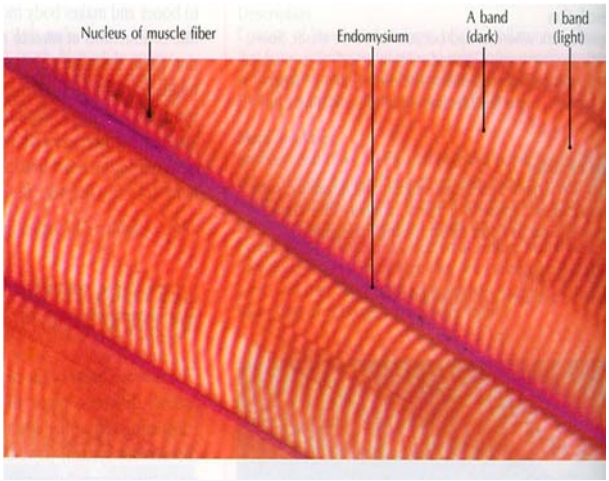
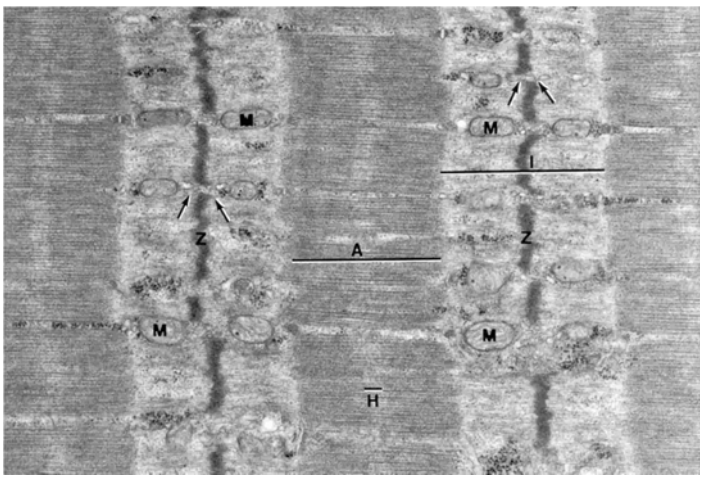
Actin and myosin in muscular fiber

- Striated muscle fibers are highly organized as repetitive patterns of dark (A) and light (I) bands, due to presence of *myofibrils*

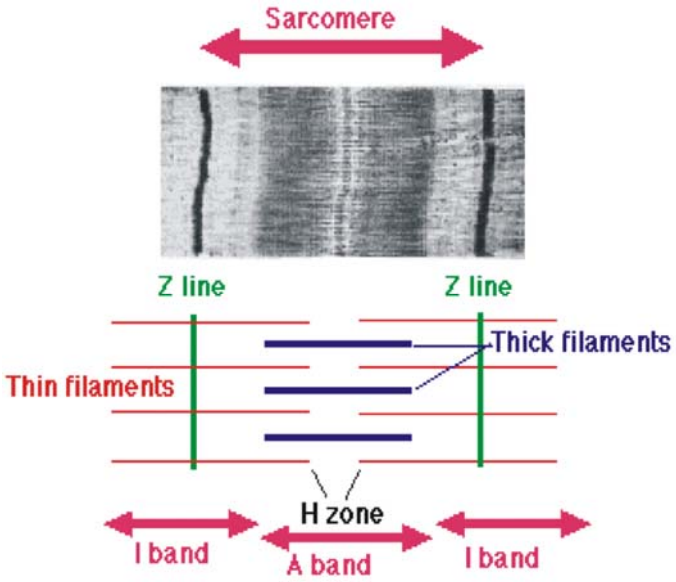




- *Myofibrils* = thin + thick filaments
  - Thin = Actin filaments + associated proteins
  - Thick = myosin filaments

Actin and myosin in muscular fiber – the sarcomere

- The smallest repetitive subunit of the contractile apparatus = *sarcomere*
- Sarcomere = part of a myofibril between two Z lines
  - Z line = dark line



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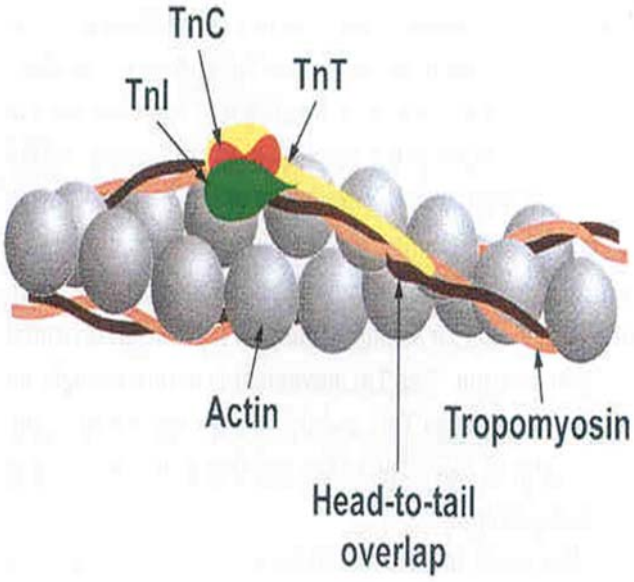
Actin-associated proteins in sarcomere

- Tropomyosin
- Troponin
  - T – attaches to tropomyosin
  - C – binds Ca ions
  - I – inhibits the A-M interaction



In resting muscle, tropomyosin-troponin complex covers A-M interaction site

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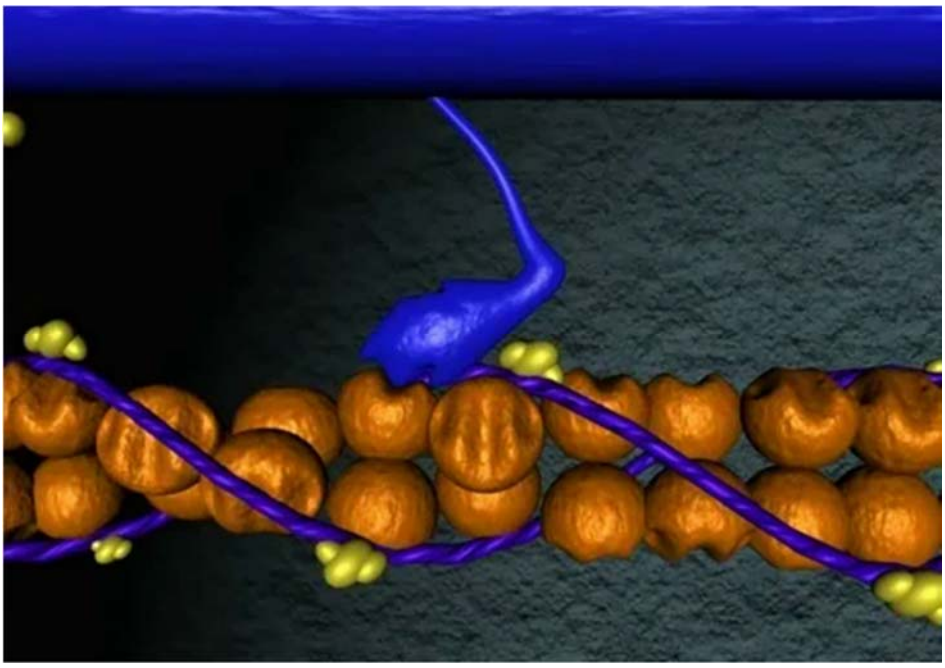
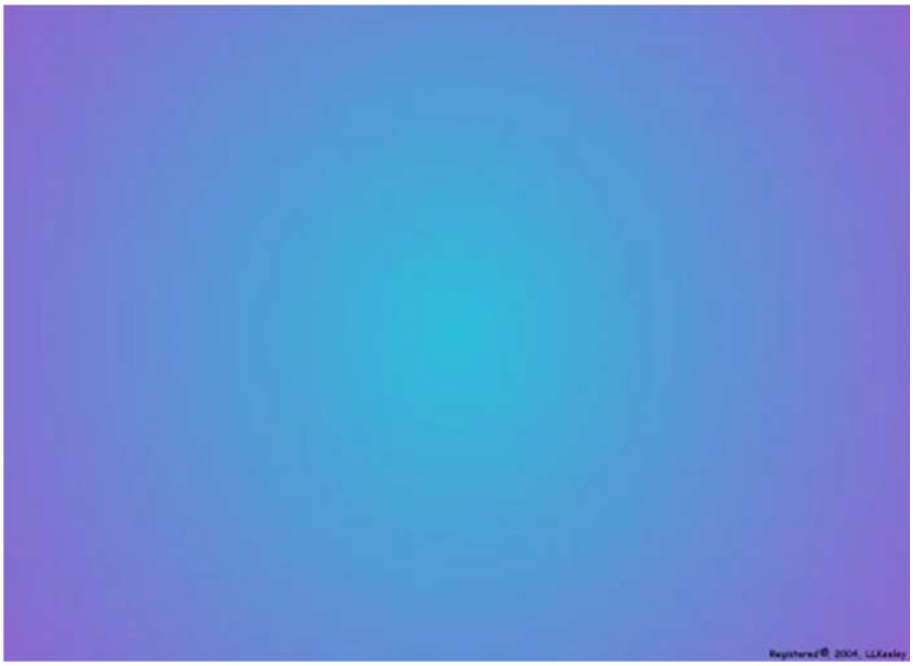
Muscle contraction



https://www.youtube.com/watch?v=gJ309LfHQ3M

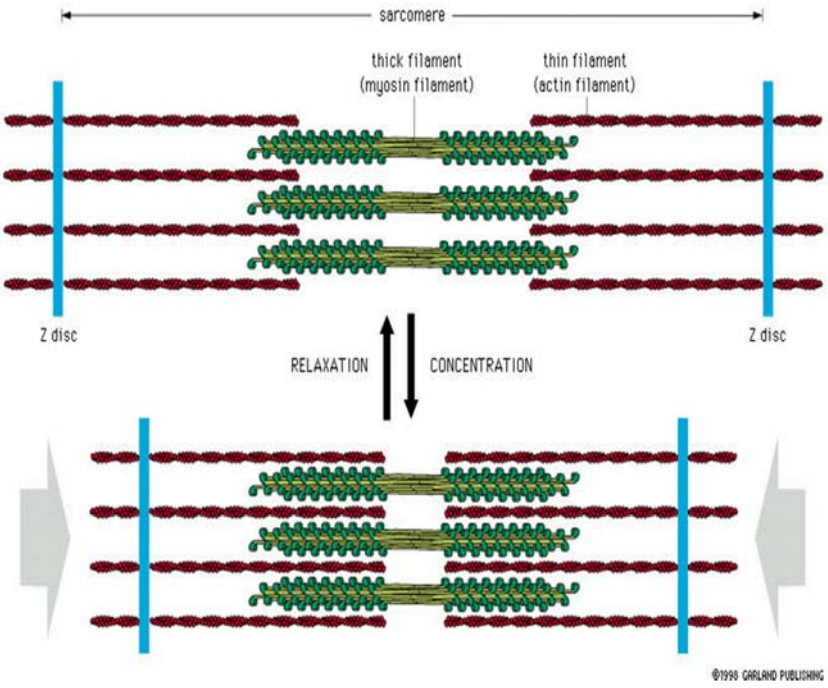
Muscle contraction



https://www.youtube.com/watch?v=zQocsLRm7_A

Actin and myosin in muscular fiber

- During contraction, thick myosin filaments slide through thin actin filaments, shortening the sarcomere



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Contraction

- Ca^{2+} binds to troponin C (TnC)
- Spatial configuration of Tn changes, inducing adjustment of tropomyosin configuration
- Revealing of myosin-binding site on actin, and
- Myosin head binds to actin, pulling in “hinge-like” manner

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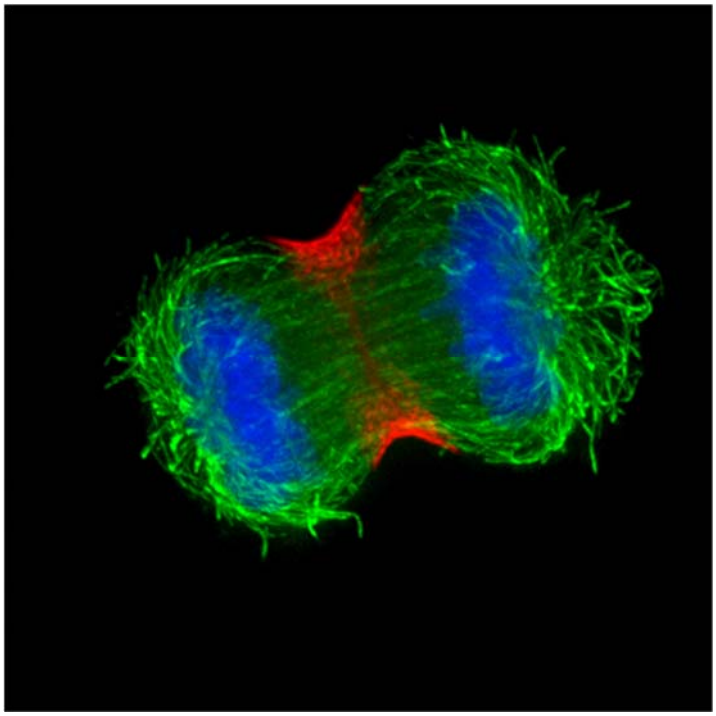
Actin filament functions

- **Contractile ring**
  in cytokinesis



Cytoskeleton

- Eukaryotic cell Skeletal System
  - Microfilaments
    - Actin
  - Microtubules
    - Rigid tubes
    - Tubulin
  - Intermediate filaments
    - Ropelike fibers
    - Many related proteins

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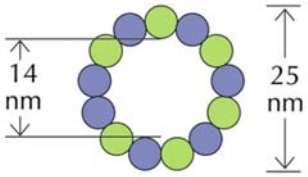
Cytoskeleton - *Microtubules*

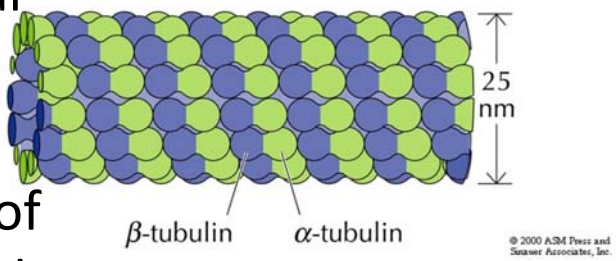
Components of a diverse array of structures

- Mitotic spindle
- Core of flagella and cilia

Structure: tubes of globular proteins (tubulins)

- Protofilaments
- Cross-section – a circle of 13 rows of protofilaments
  - Dimer building blocks – α-tubulin and β-tubulin





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Cytoskeleton

– α-tubulin
  - Bound GTP
  - Binds on + end

– β-tubulin
  - Bound GDP
  - Binds on – end

– γ-tubulin – promoter of polymerization

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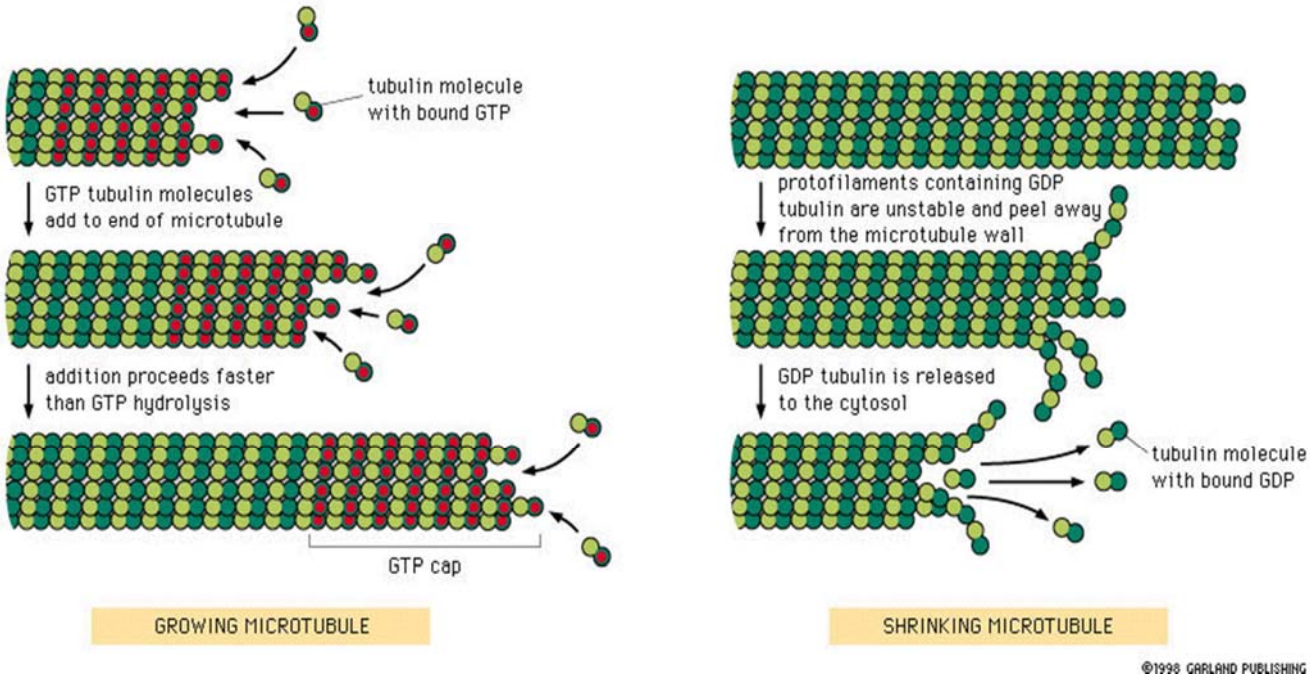
Microtubule dynamics



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Microtubules – structural functions

- Cell division
- Determine cell shape
- Internal organization
- Intracellular transport of vesicles
  - Axonal transportation
    - Materials moved from cell body, along axon, toward axonal terminal
      - » Anterograde
    - From axon to cell body – endocytosis
      - » Retrograde

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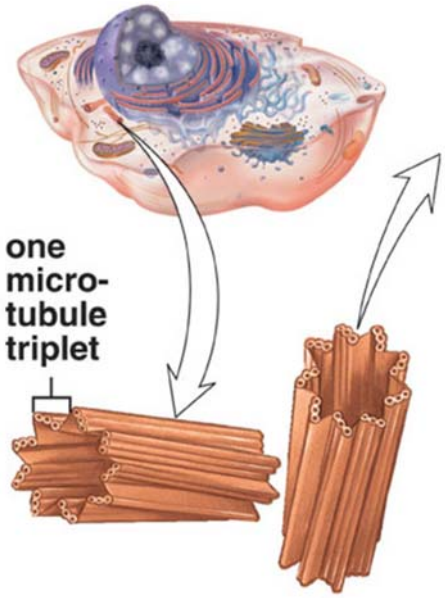
Microtubules – centrosome

- Organizing center of microtubules, near nucleus
- Formed by 2 centrioles at right angle to each other
- Centriole: 9 microtubule triplets



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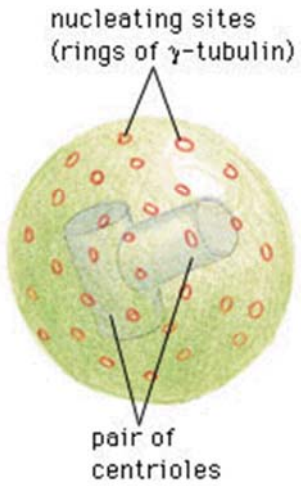
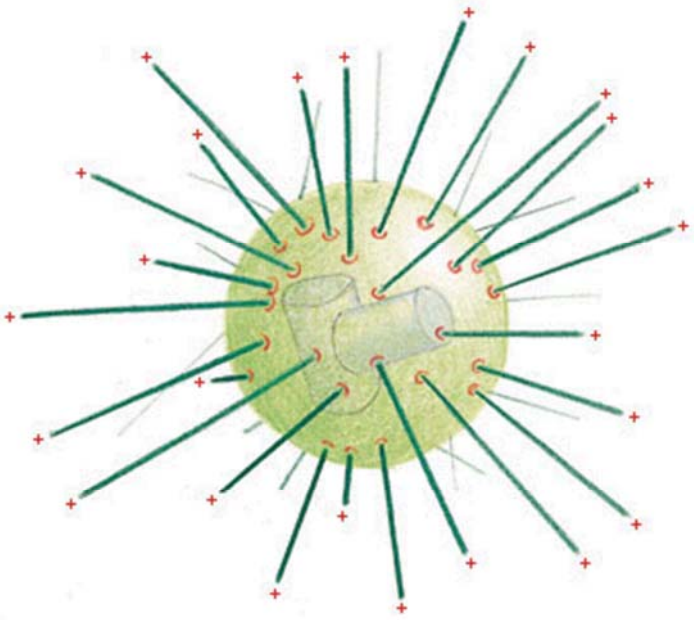
Microtubules – centrosome



(A)



(B) microtubules growing from nucleating sites on centrosome

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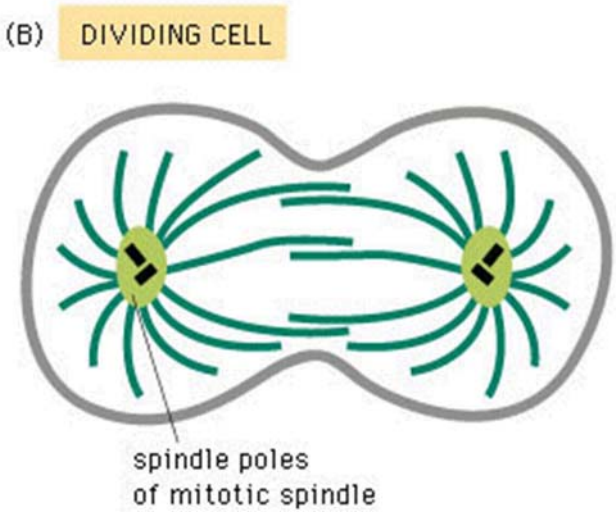
Microtubules in cell division (I)

- Just before cell division, the centrosome divides, resulting 2 pairs of centrioles that migrate to opposite ends of cell
- between them – mitotic spindle will form, supporting migration of chromosomes



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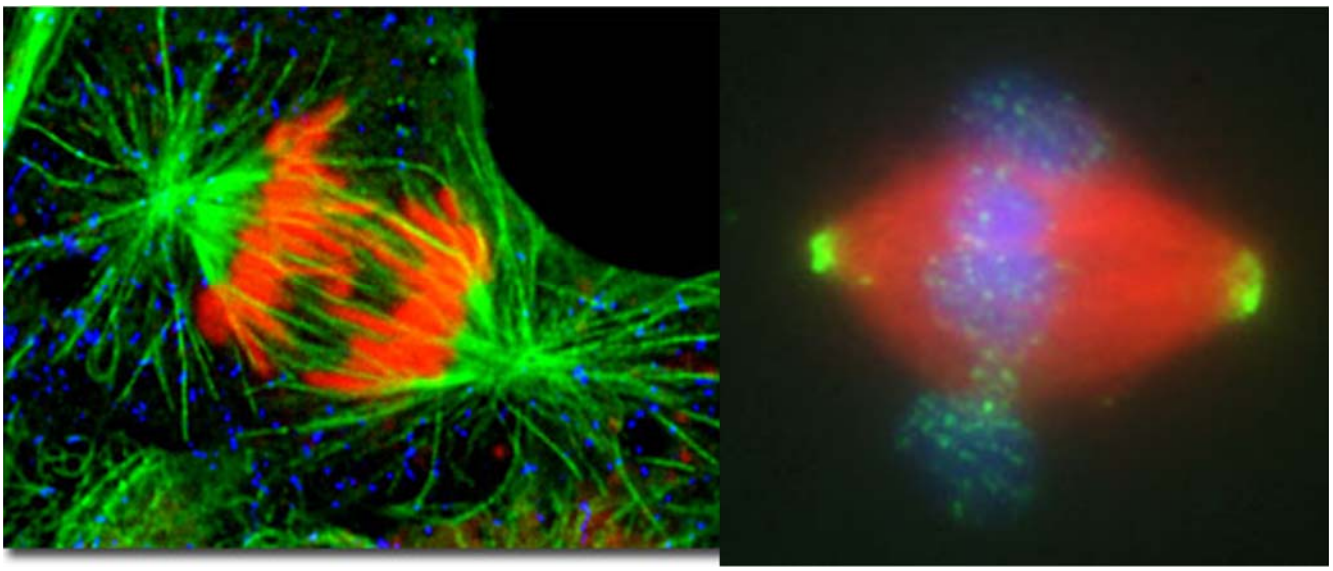
Microtubules in cell division (II)



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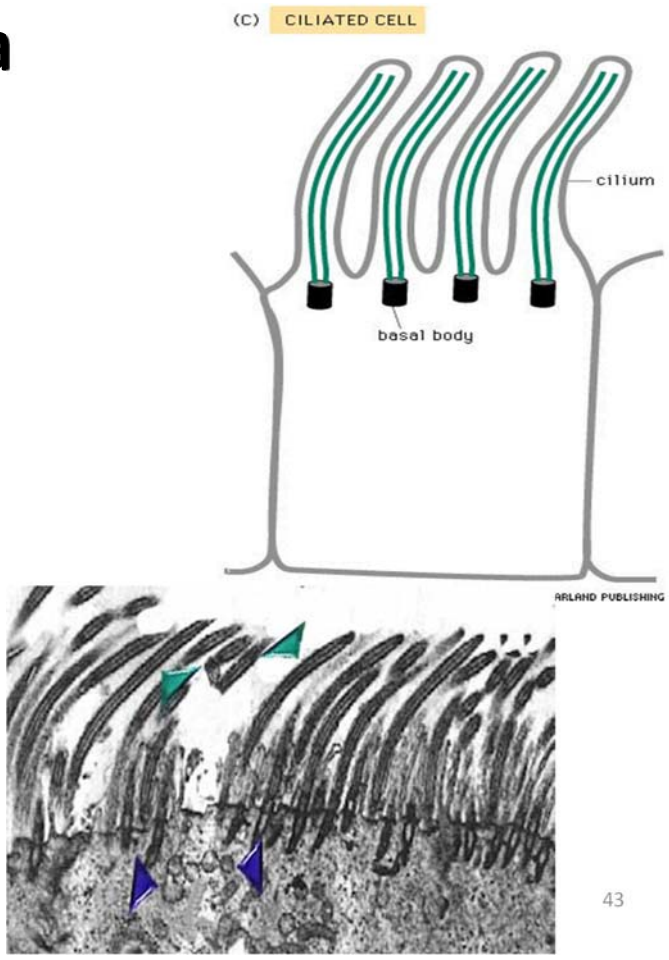
Microtubules – cilia

- Permanent motile processes of cell surface
- Found in respiratory tract, both male and female genital tract
- formed by basal body



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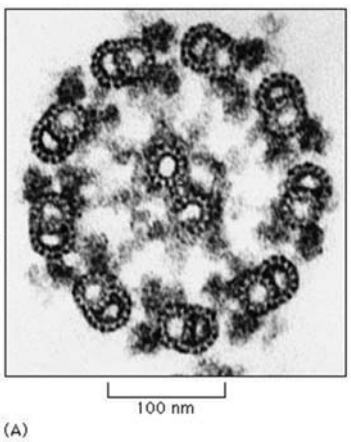
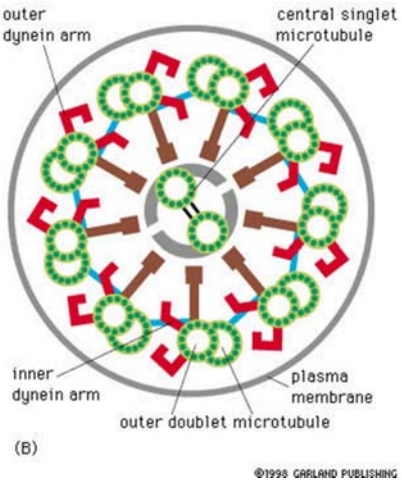
Microtubules – cilia

- 2 central microtubules, surrounded by 9 microtubule doublets
- The central doublet is surrounded by a sheet of nexin
- Peripheral doublets are stabilized by dynein arms





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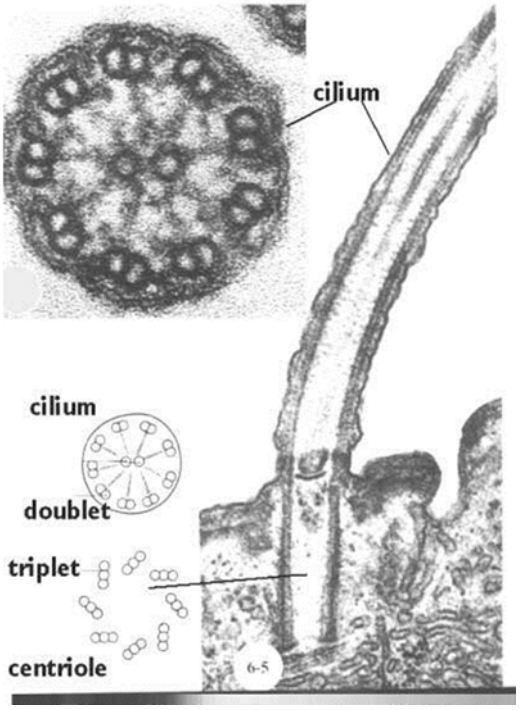
Microtubules – basal bodies

- location: base of each cilium
- function: organisation and motility of cilium



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Non-motile cilia

- a solitary and non-motile element that projects out of the cell and resembles to a flagellum.
- It has sensory and mechano-sensory properties, and is also a key ultrastructure for mediating signal transduction

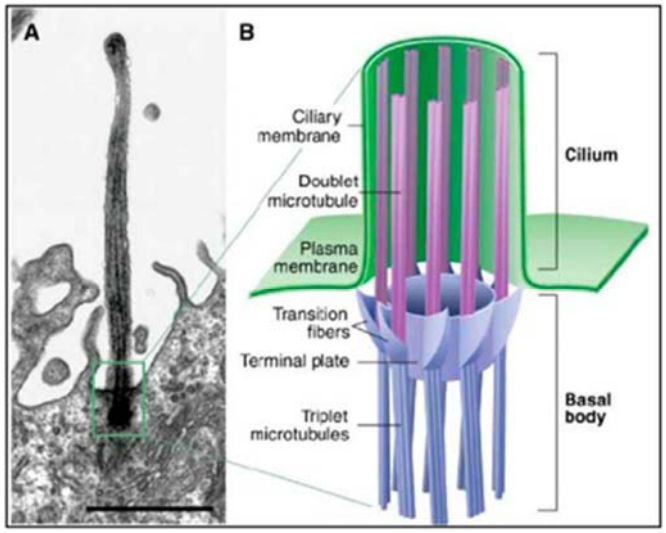
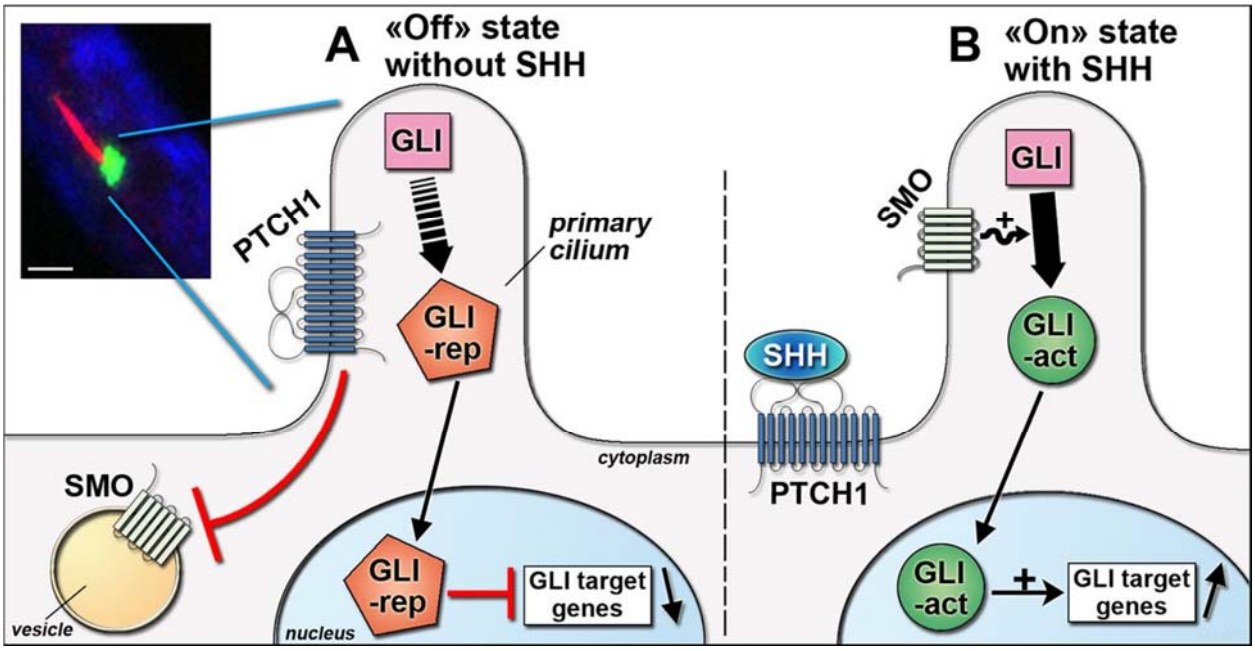


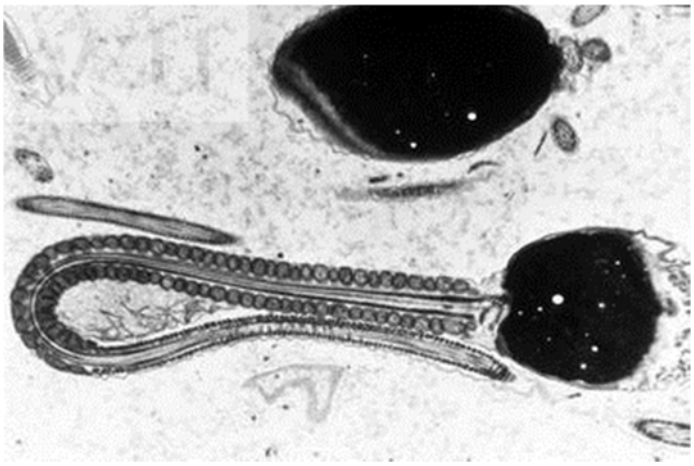
Fig. 2. Structure of a primary cilium.

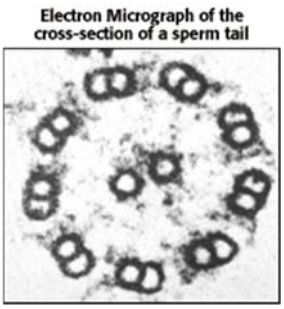
Non-motile cilia



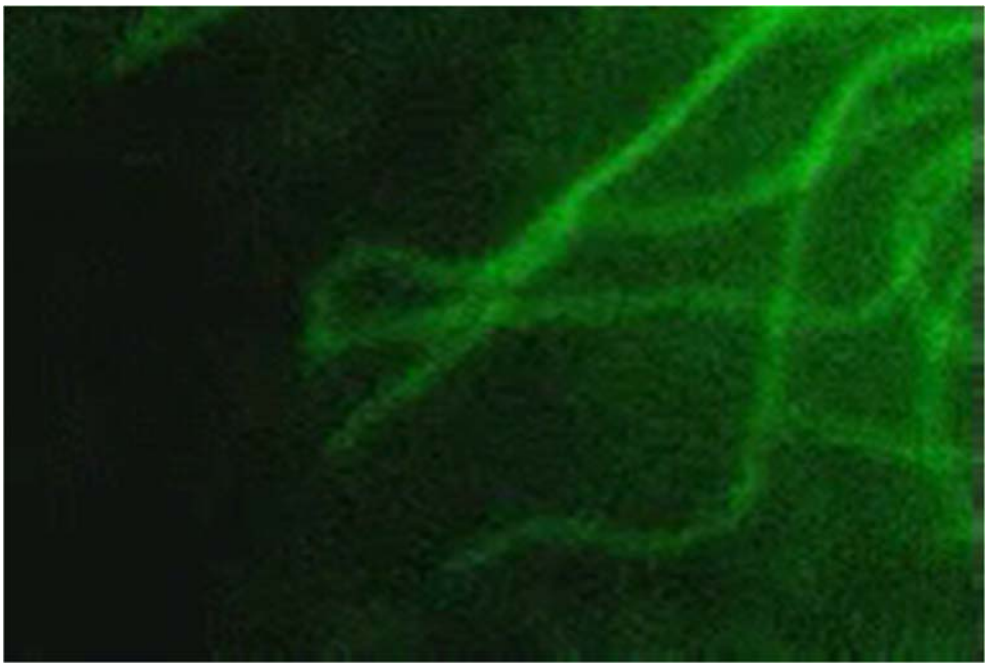
ajplung.physiology.org

Microtubules – flagella





Cytoskeleton – a dynamic organelle



https://www.youtube.com/watch?v=ZL3_BwrB6AM

Microtubules and intracellular transport

- Tracks for many *motor proteins*
- Motor proteins use ATP
  - Move cellular cargo
    - Vesicles, mitochondria, lysosomes, chromosomes
- Microtubule motor proteins
  - Kinesins
  - Dyneins
- Kinesins and Dyneins – move on microtubules

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Motor proteins

- Move unidirectionally
- Stepwise
- Series of conformational changes
  - A *mechanical cycle*
  - Coupled to chemical cycle – Energy
    - Steps –
      - » ATP binding to motor
      - » Hydrolysis of ATP
      - » Release of ADP and P_i
      - » Binding of new ATP

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http://www.cellimagelibrary.org/images/8082

Motor proteins – Kinesin

- Tetramer
  - 2 identical heavy and 2 identical light chains
- Functional domains
  - Pair of globular heads
    - » Bind microtubule
    - » ATP-hydrolysing
  - Neck / stem and tail
  - Tail binds cargo
- Move toward plus end of microtubule
  - *Plus end directed*

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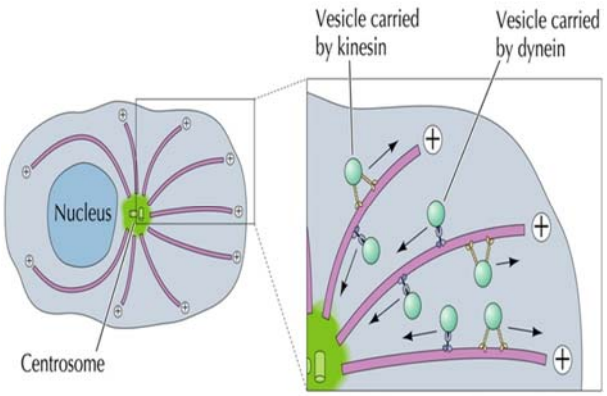
Cytoskeleton

- Motor proteins
  - Kinesin-mediated organelle transport
    - Kinesins aligned with plus ends away from nucleus
    - Tend to move organelles in anterograde direction



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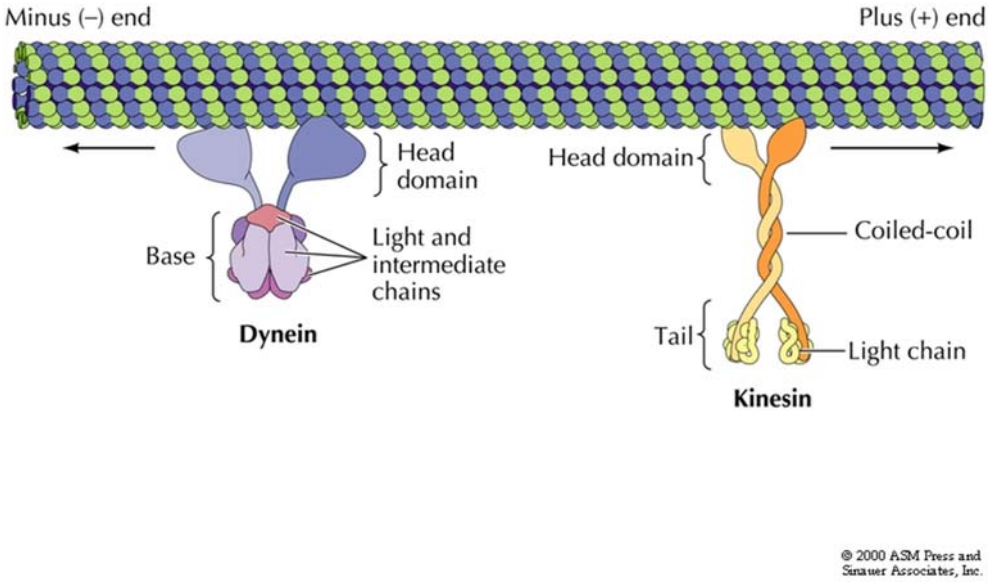
Cytoskeleton

- Motor proteins

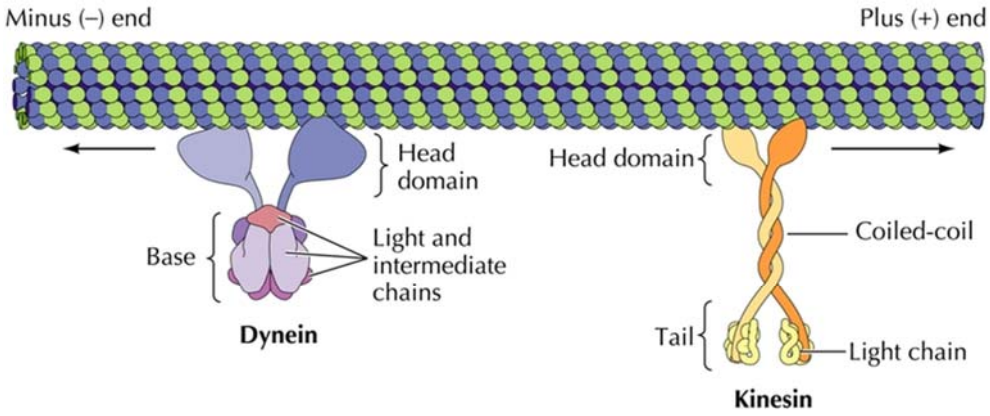
Dynein Kinesin



Motor proteins – cytoplasmic dynein

- Movement of cilia and flagella
- Ubiquitous motor protein in eukaryotic cells
- 2 identical heavy chains
- Many intermediate and light chains
  - *Minus end directed*

Motor proteins – Dynein



Cytoplasmic dynein roles:

- Force generation – spindle – mitosis
- Minus-end directed motor for Golgi Complex and vesicles

Cytoskeleton

- Eukaryotic cell Skeletal System
  - Microfilaments
    - Actin
  - Microtubules
    - Rigid tubes
    - Tubulin
  - Intermediate filaments
    - Ropelike fibers
    - Many related proteins

Intemediate filaments

- More stable structure of cytoskeleton (no obvious dynamics)
- Diameter in-between actin-filaments and microtubules
- Role
  - shape of the cell
  - strengthen cells that bind together
  - specific for different cellular types

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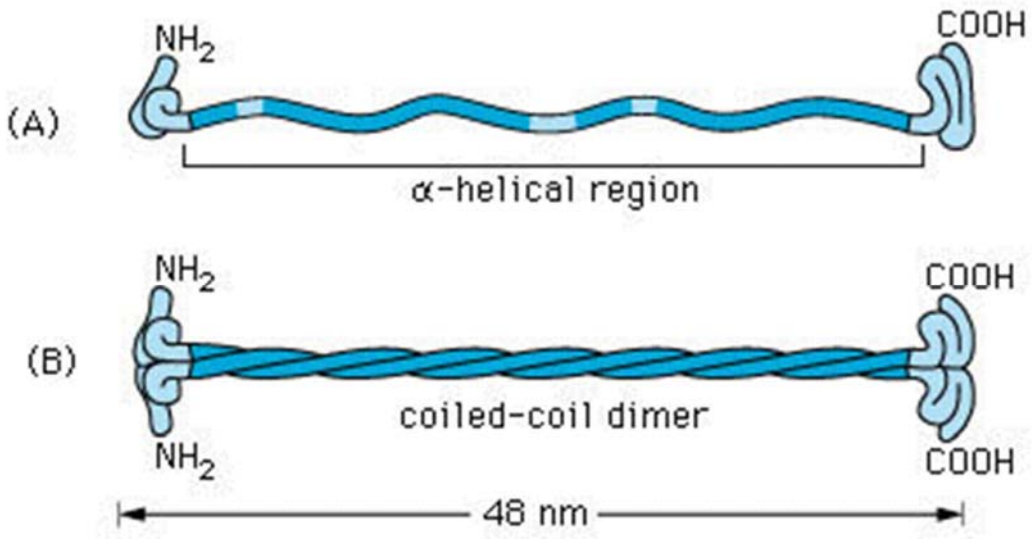
Intermediate filaments general organization (I)



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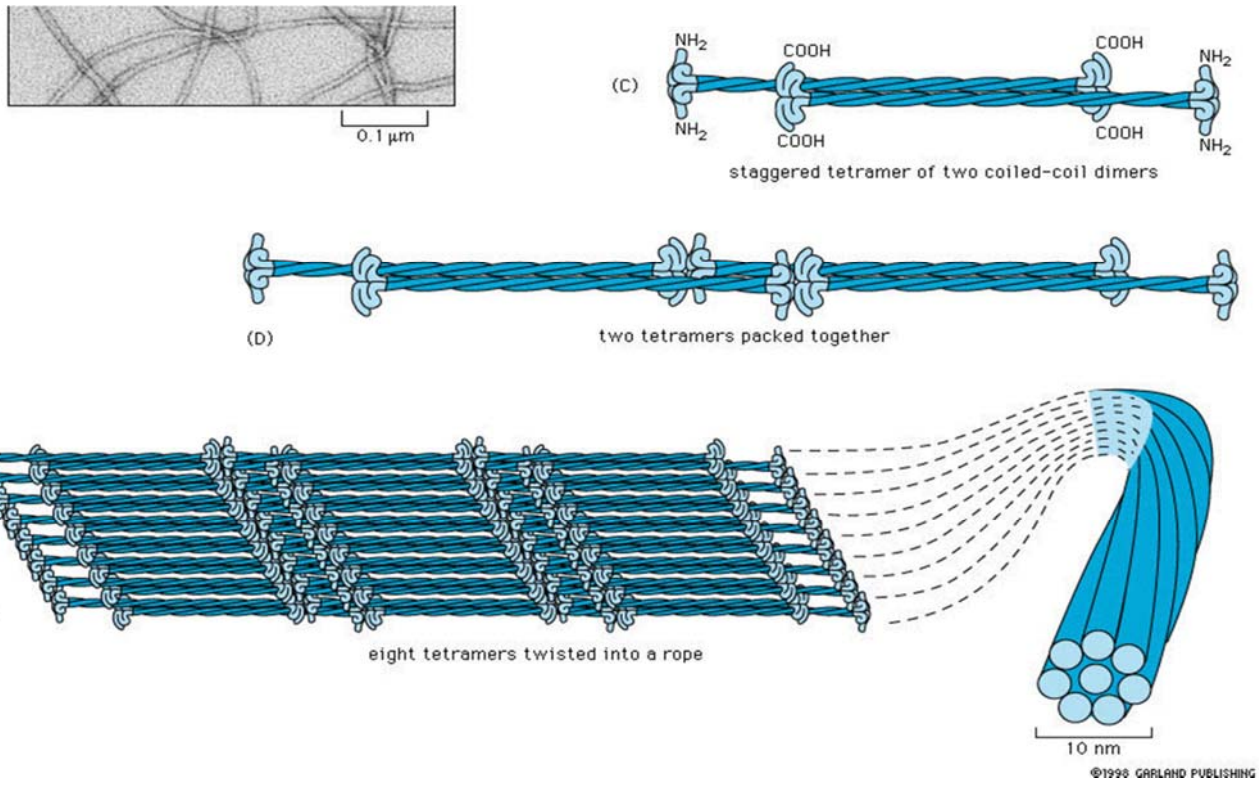
Intermediate filaments
general organization (II)



Intermediate filaments
cell specificity

Proteic components	Cell type
Keratin I Keratin II	Epithelial cells
vimentin	Mesenchymal cells
desmin	Muscle
neurofilament proteins: NF-L, NF-M, NF-H	Neurons

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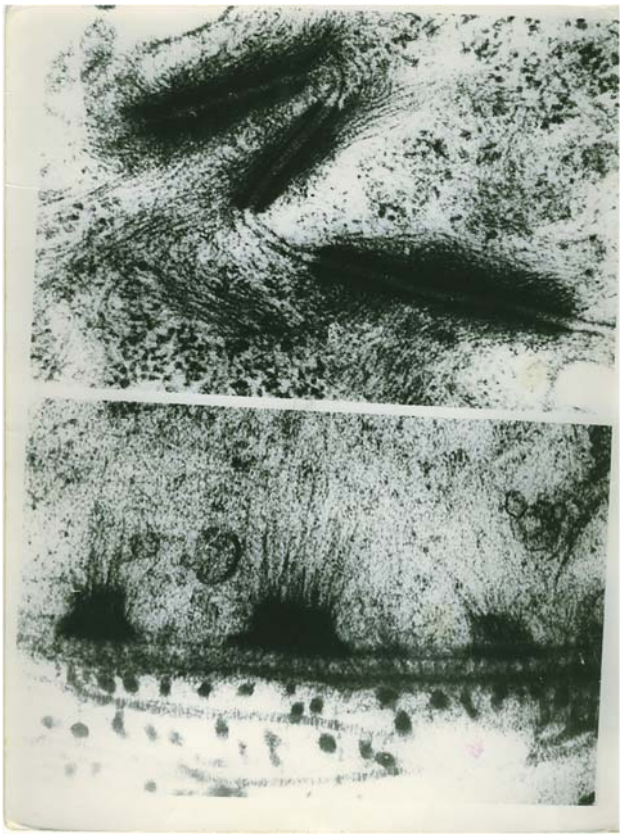
Intermediate filaments

- cytoplasmatic components of desmosomes, hemidesmosomes



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Intermediate filaments – associated proteins

- Involved in packing, stabilising intermediate filament bundles
  - Filaggrin – in skin
  - Plectin – also involved in connecting intermediate filaments with other components of cytoskeleton

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Conclusions (I)

-Cytoskeleton is a non-membrane bounded organelle

-Cytoskeleton is formed of 3 components: actin filaments, intermediate filaments and microtubules

-Cytoskeleton components are formed by polymerization

-Cytoskeleton components are involved in formation and maintenance of temporary and permanent cell structures, mostly cell protrusions

Conclusions (II)

- Roles of cytoskeleton
  - Cell division
  - Cell shape and motility
  - Internal organization of the cell
  - Intracellular transport of vesicles
  - Functions derived from each cytoskeleton-based element (e.g. absorption for microvilli)