Cytoskeleton


• All images are from 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

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Cytoskeleton

– Three well defined filamentous structures
  • Microfilaments
  • Intermediate filaments
  • Microtubules
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= 😞 😞
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)

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**Actin filaments**

- [Image of actin filaments]

**Microtubules**

- [Image of microtubules]

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Cytoskeleton

- Microfilaments
  - Actin and actin-associated proteins
- Microtubules
  - Hollow tubes
  - Tubulins
- Intermediate filaments
  - Ropelike fibers

Microfilaments

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

- Dynamic structures regulated by actin-associated proteins
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)
ACTIN- binding proteins

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
Actin filaments and cell shape

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

Actin filaments and cell shape - transient processes

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

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.
Stereocilia

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

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)
**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

**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
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.

Contraction

- \(\text{Ca}^{2+}\) 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
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
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

Cytoskeleton

- α-tubulin
  - Bound GTP
  - Binds on + end
- β-tubulin
  - Bound GDP
  - Binds on – end
- γ-tubulin – promoter of polymerization
Microtubule dynamics

- 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
Microtubules – centrosome

• Organizing center of microtubules, near nucleus
• Formed by 2 centrioles at right angle to each other
• Centriole: 9 microtubule triplets
Microtubules in cell division (I)

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

Microtubules in cell division (II)

![Image of microtubules in cell division](image_url)
Microtubules – cilia

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

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
Microtubules – basal bodies

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

Non-motile cilia

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

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
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<sub>i</sub>
      » Binding of new ATP
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*

Cytoskeleton

- Motor proteins
  - Kinesin-mediated organelle transport
    - Kinesins aligned with plus ends away from nucleus
    - Tend to move organelles in anterograde direction
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
**Intermediate 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
Intermediate filaments
general organization (II)

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

- cytoplasmatic components of desmosomes, hemidesmosomes

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
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)