The Cytoskeleton

Our cells need support too!

Part II

BIOL366

April 22, 2025

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Learning Objectives for Today's Lecture:

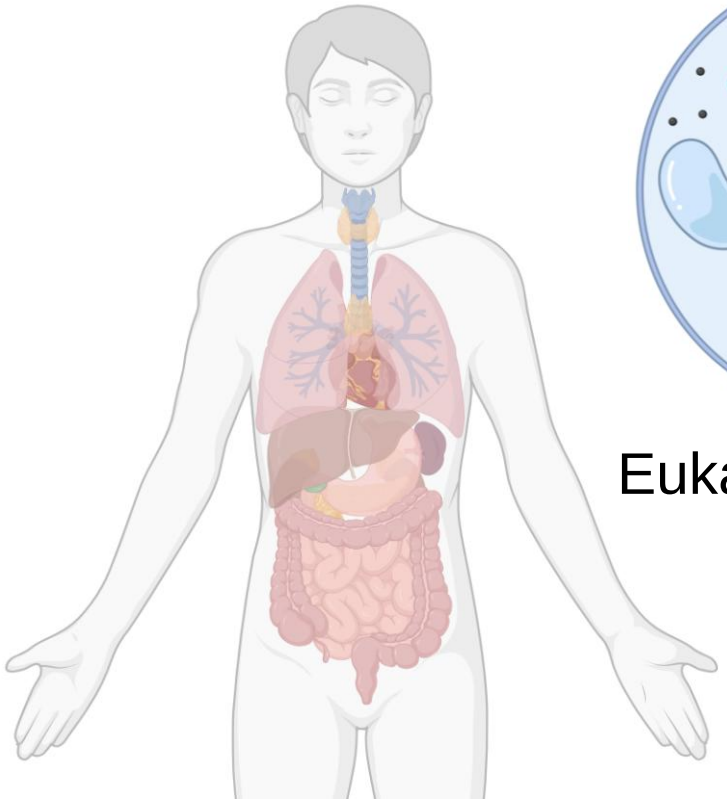
Upon completing this module, **you should be able to**:

- Describe the role of **intermediate filaments** in the eukaryotic cytoskeleton
- Explain the dynamic process of **actin filament** assembly (i.e., treadmilling)
- Understand the roles actin filaments play in **cellular movement** and **muscle contraction**

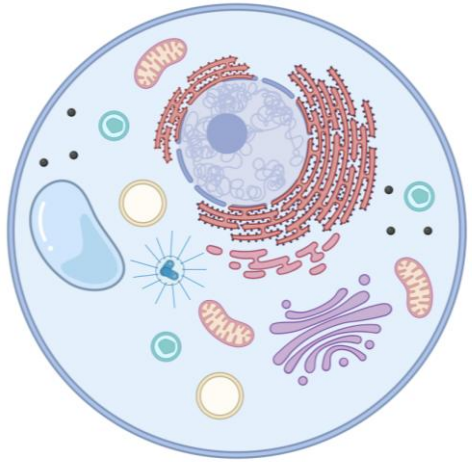
Key Terms

- **Intermediate filament**: Key component of the cytoskeleton, providing structural support to cells and resistance to mechanical stress
- **G-actin:** Globular, monomeric actin
- **F-actin:** Filamentous actin polymers
- **Treadmilling:** Dynamic process by which actin filaments are constantly disassembles at one end while being assembled at the other end
- **Lamellipodium:** Membrane protrusion at the leading edge of moving cells
- **Filopodium:** Thin, cytoplasmic projections of the cell membrane that help cells explore their environment and adhere to surfaces
- **Myosin:** A molecular motor protein which converts chemical energy in the form of ATP into mechanical energy for the generation of force and movement
- **Sarcomere:** Smallest functional unit of a striated muscle tissue, composed of actin and myosin filaments

The Cytoskeleton provides structural support and maintains the shape of cells

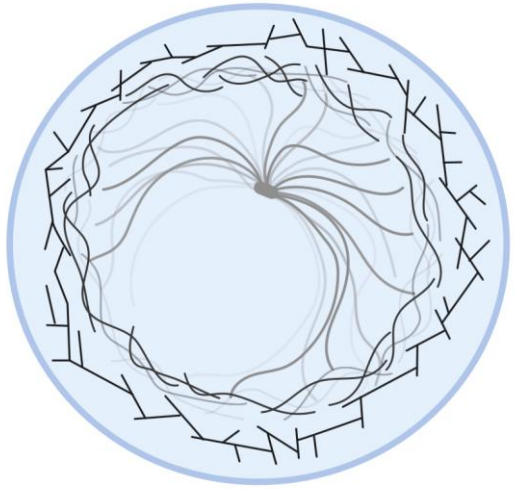


Human body with organs



Eukaryotic cell with organelles

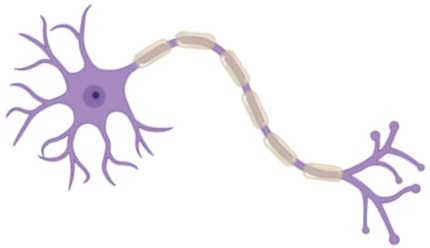
Human skeletal system



Eukaryotic cytoskeleton

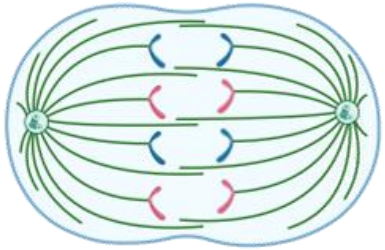
Functions of the Cytoskeleton

Muscle contraction

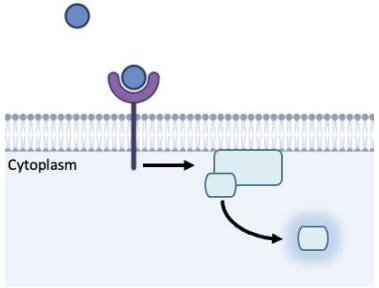


Cell shape maintenance and support

Cell movement



Chromosome segregation during cell division

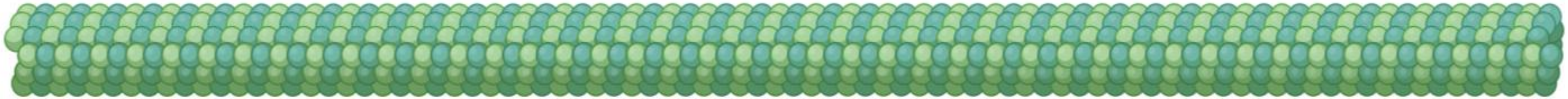


Aid in signal transduction

Transport of vesicles and organelles

The 3 essential components of the Cell's skeleton

Microtubules



Intermediate filaments

Microfilaments

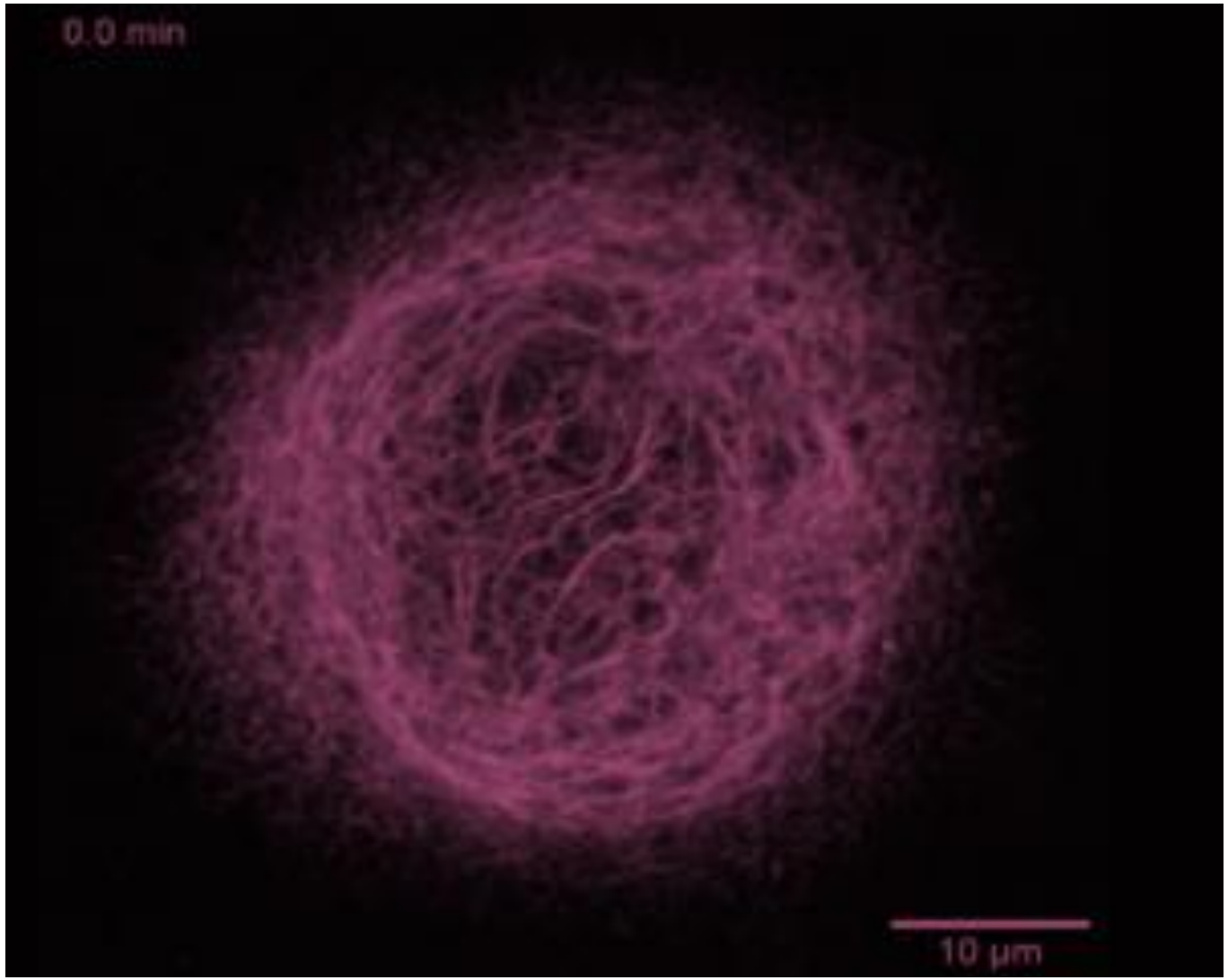
The 3 main components of the Cell's skeleton

Microtubules

Intermediate filaments

Microfilaments

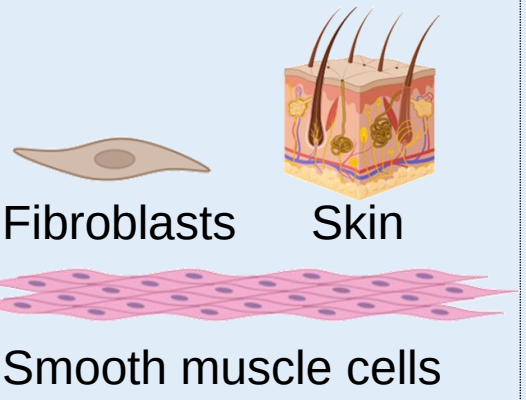
Intermediate filaments are the strongest component of the Cytoskeleton

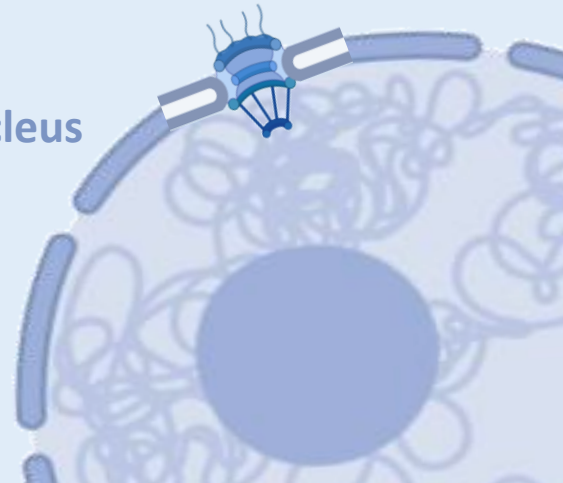


- **Rope-like** filaments that have **high tensile strength** and stability
- **Less dynamic** compared to **microtubules** and **actin filaments**
- Average diameter: ~15nm

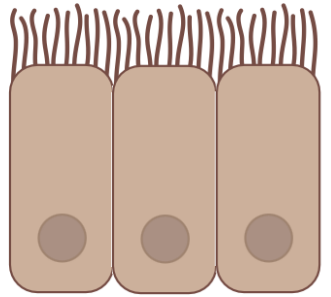
Intermediate Filaments are composed of a variety of proteins

Intermediate filament class	(1) Keratin filaments	(2) Vimentin and vimentin-related filaments	(3) Neurofilaments	(4) Nuclear lamins
Protein members	Keratins	Vimentin, Glial fibrillary acidic protein (GFAP)	Syncoilin, Alpha-internexin	Lamins
Tissue distribution	Hair, Nails, Skin	Fibroblasts, Skin, Smooth muscle cells	Neurons	Nucleus



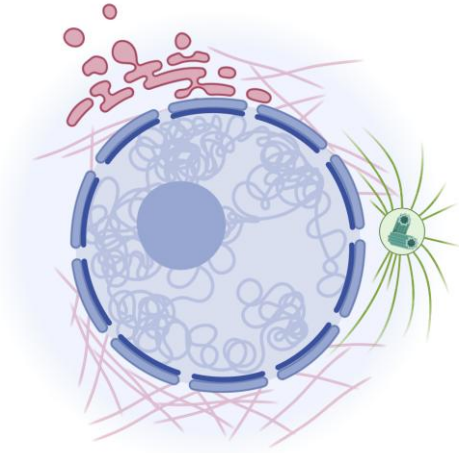


Provides mechanical support to cells

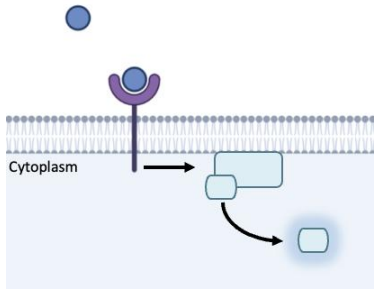


Tissue cohesion

Functions of the **Cytoskeleton** (Intermediate filaments)

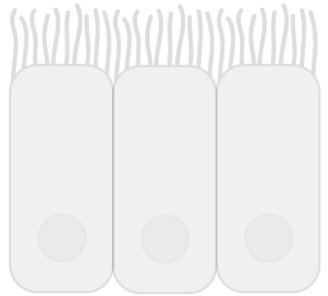


Nuclear lamina formation



Aid in signal transduction

Provides mechanical support to cells



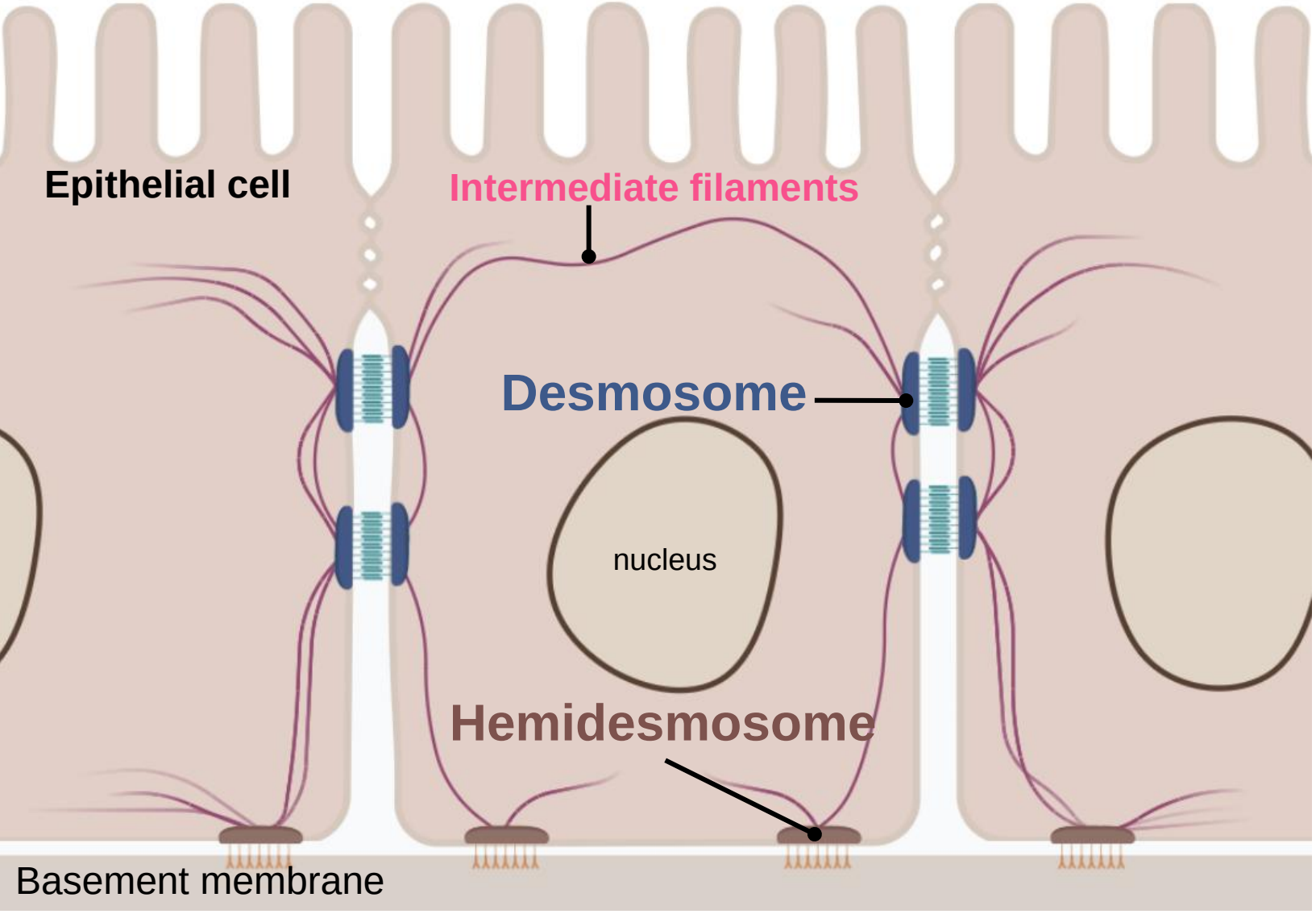
Tissue cohesion

Functions of the **Cytoskeleton** (Intermediate filaments)

Nuclear lamina formation

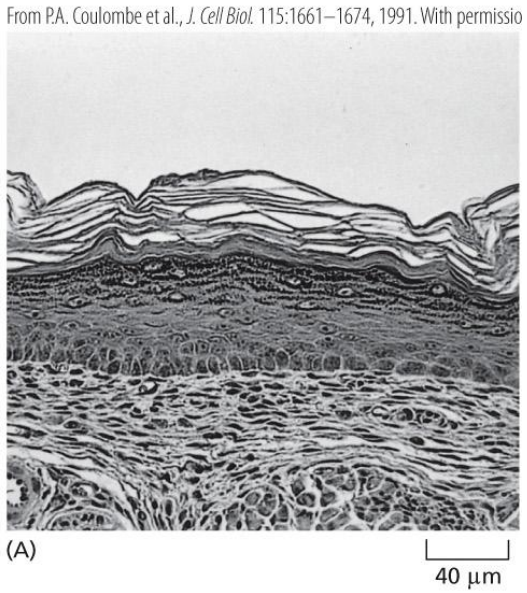
Aid in signal transduction

Intermediate filaments are anchored to the cell membrane by adhesive proteins

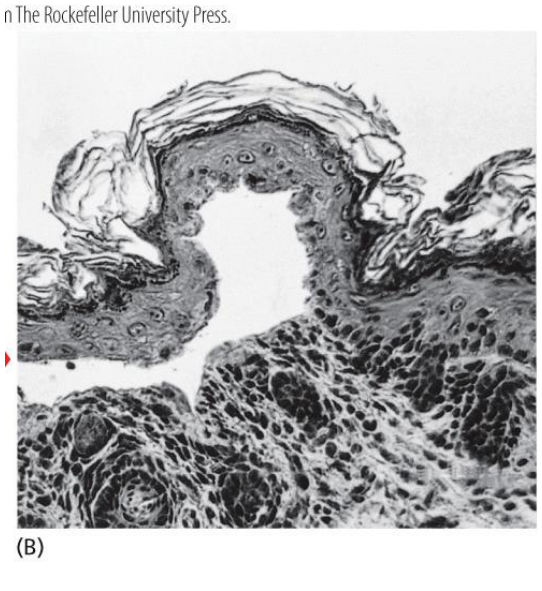


- **Desmosomes** attach to intermediate filaments to mediate **cell-cell adhesion**
- **Hemidesmosomes** attach to intermediate filaments to the **basal surface of epithelial cells**
- **Desmosomes** and **hemidesmosomes** help cells resist mechanical forces

Intermediate Filaments allow cells to withstand significant stretching and compression forces without rupturing



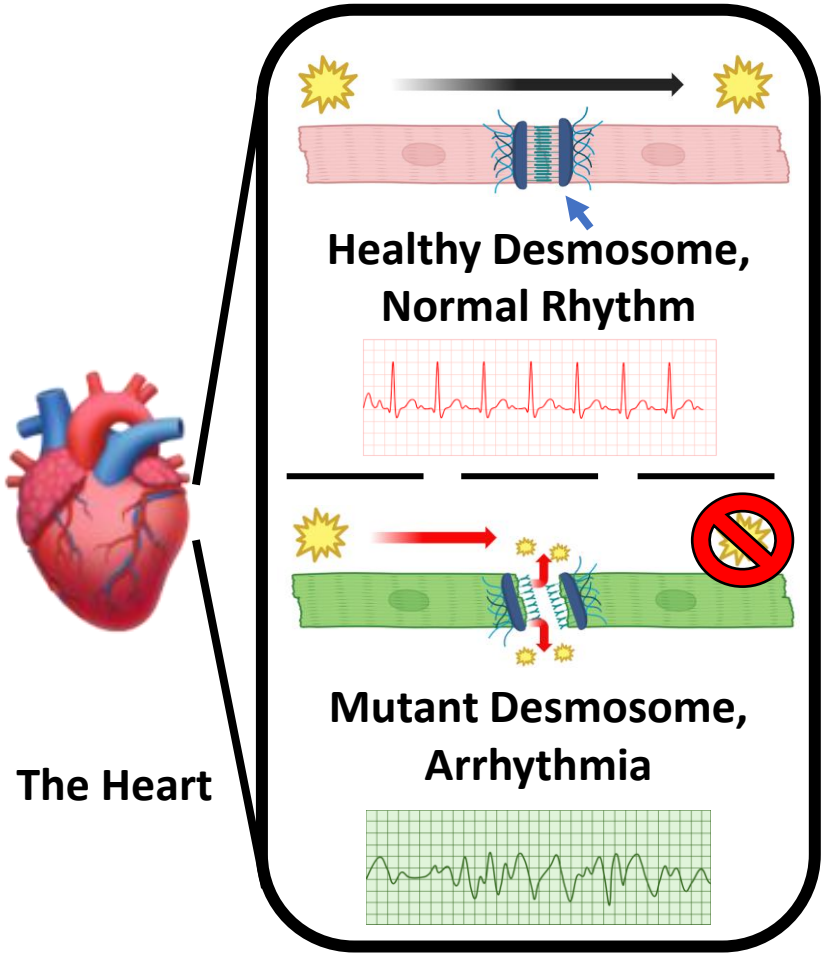
Normal mouse skin is resistant to mechanical pressure

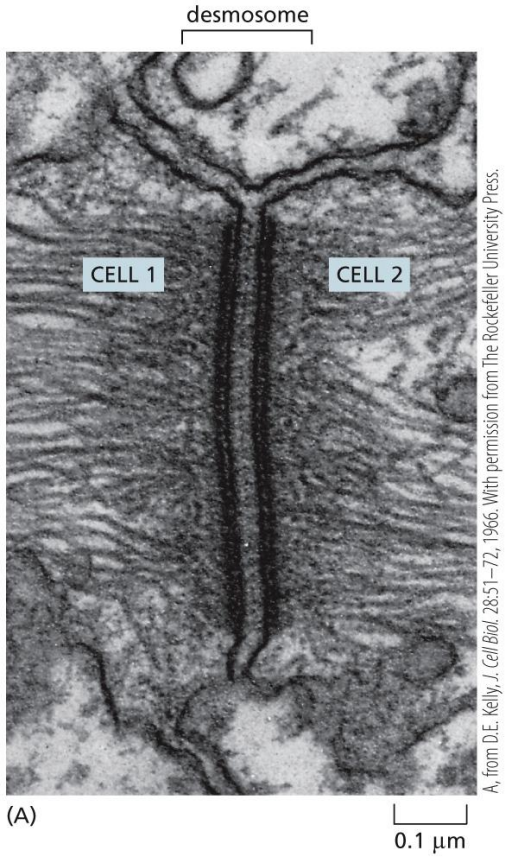


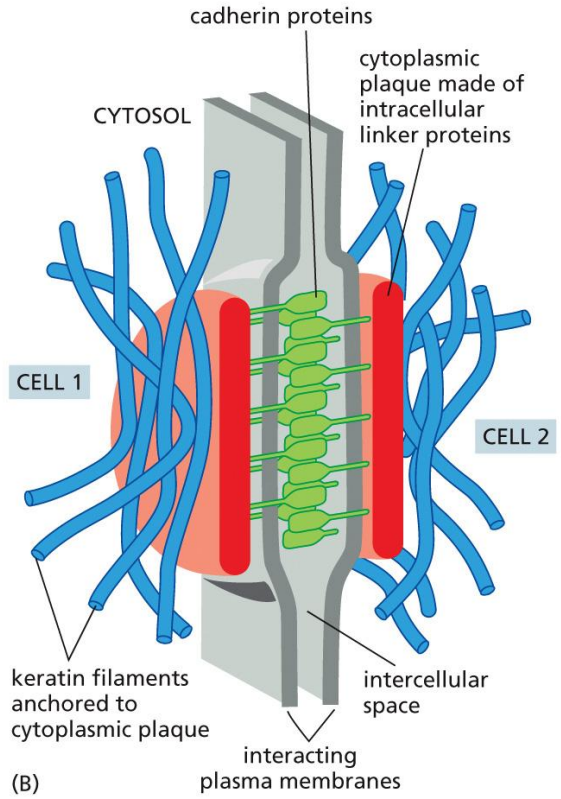
Mutation in keratin causes blistering skin in mice

Example: Loss of keratin in the skin leads to blister formation

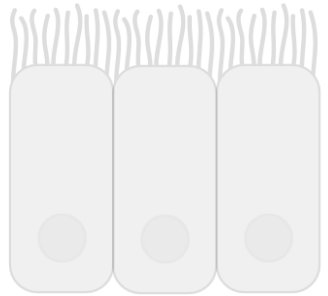
Intermediate Filaments are responsible for the generation of a normal sinus rhythm in the heart





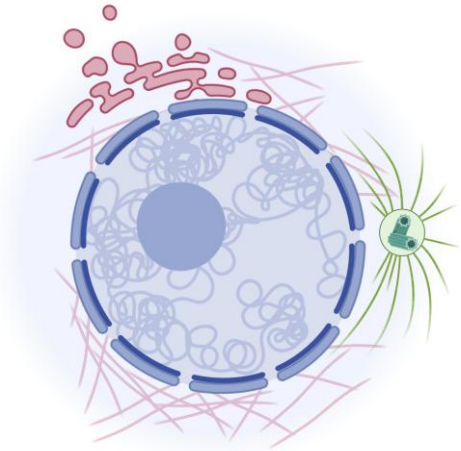


Provides mechanical support to cells



Tissue cohesion

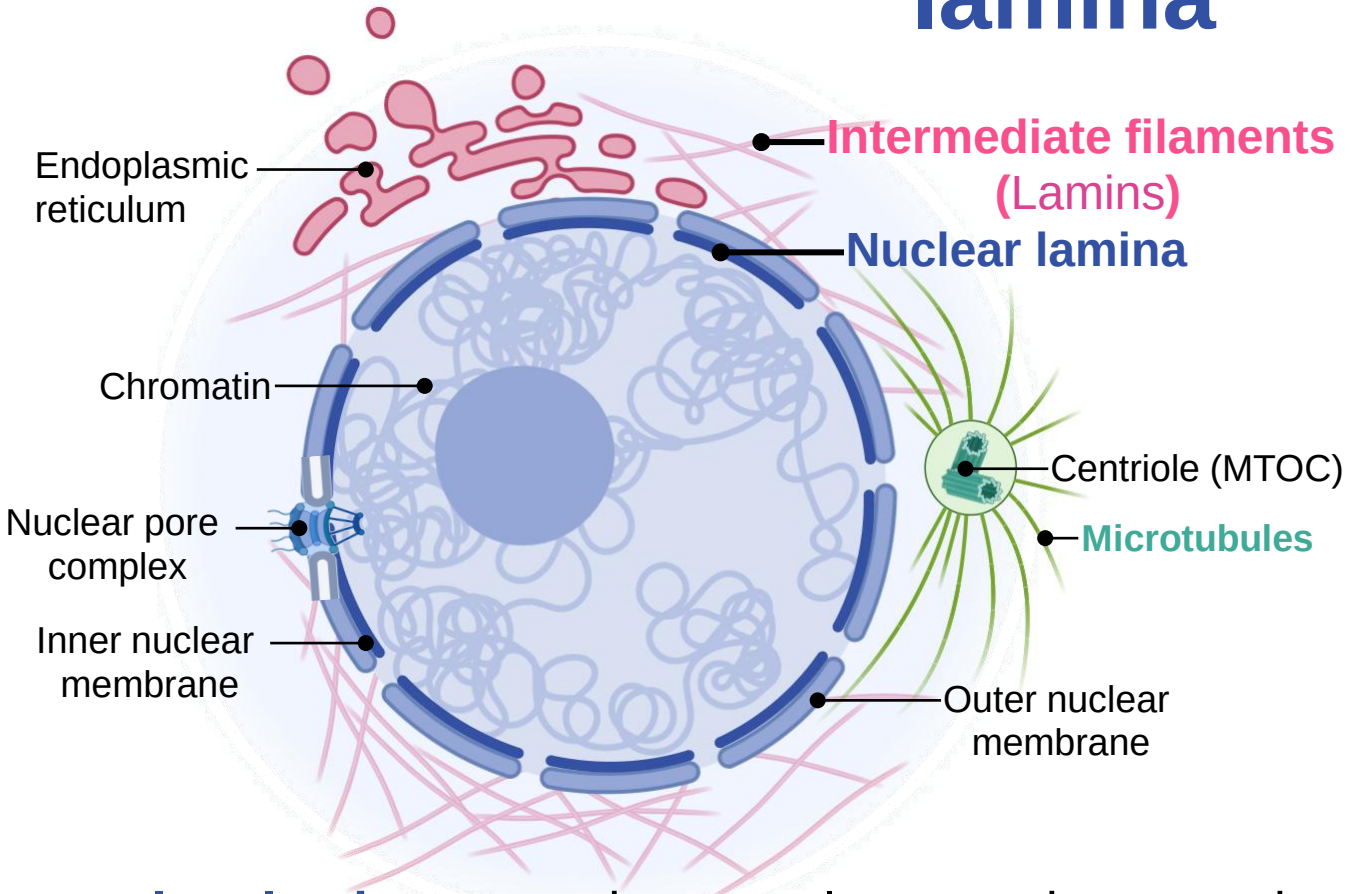
Functions of the **Cytoskeleton** (**Intermediate filaments**)



Nuclear lamina formation

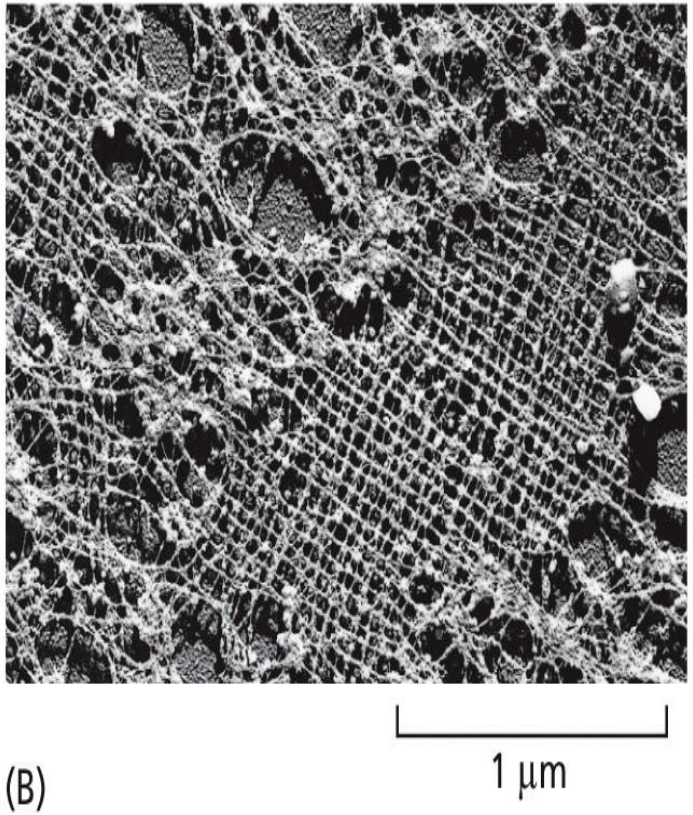
Aid in signal transduction

Nuclear intermediate filaments form the nuclear lamina



Nuclear lamina of a frog egg

From U. Aebi et al., *Nature* 323:560–564, 1986. With permission from Macmillan Publishers Ltd.



- The **nuclear lamina** strengthens nuclear membrane and serves as the attachment sites for chromosomes
- **Intermediate filaments** are reinforced by accessory proteins called plectins that crosslink them into bundles and connect them to **microtubules** and **actin filaments**

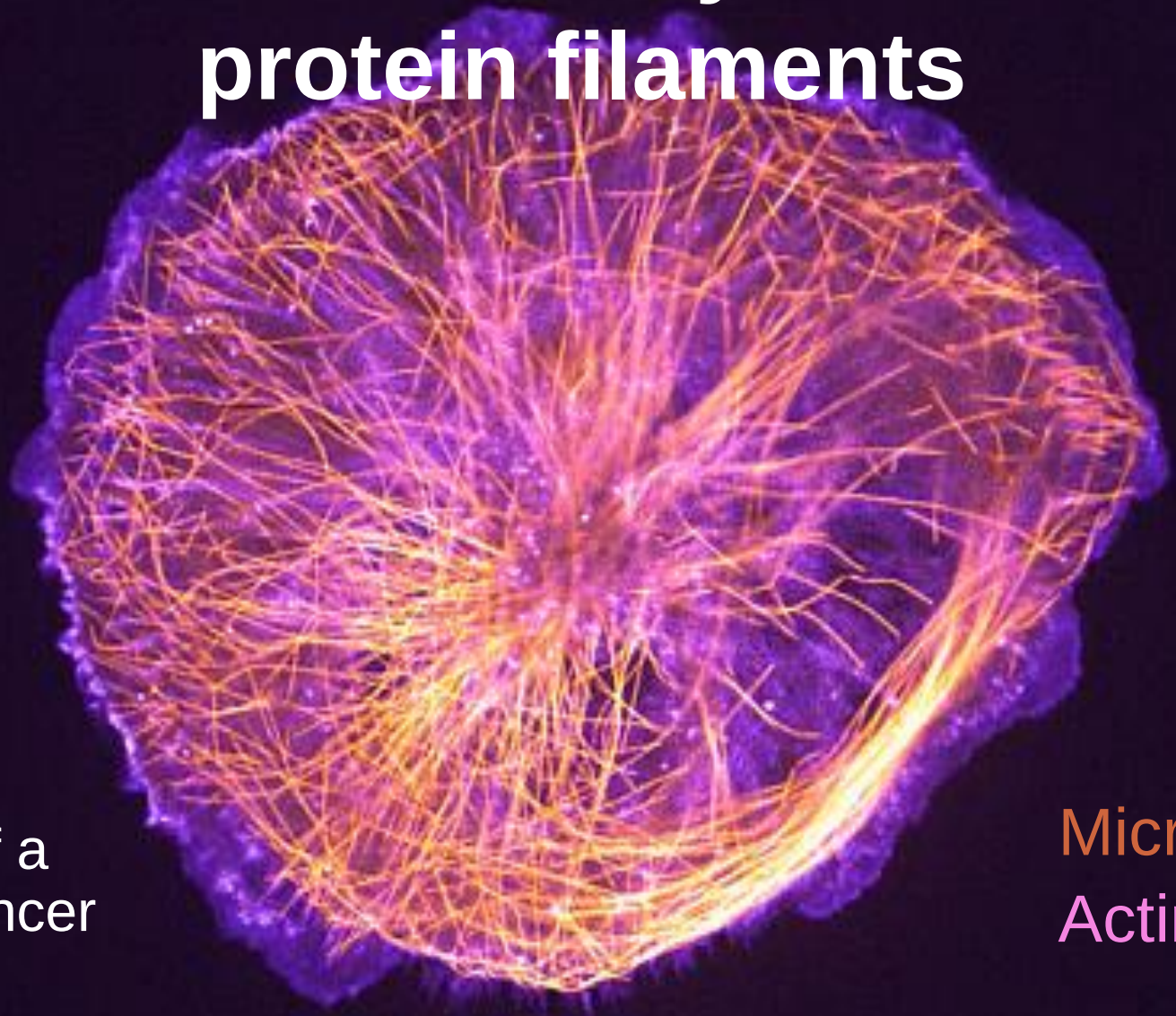
The 3 essential components of the Cell's skeleton

Microtubules

Intermediate filaments

Actin filaments

The Cytoskeleton is a dynamic network of protein filaments

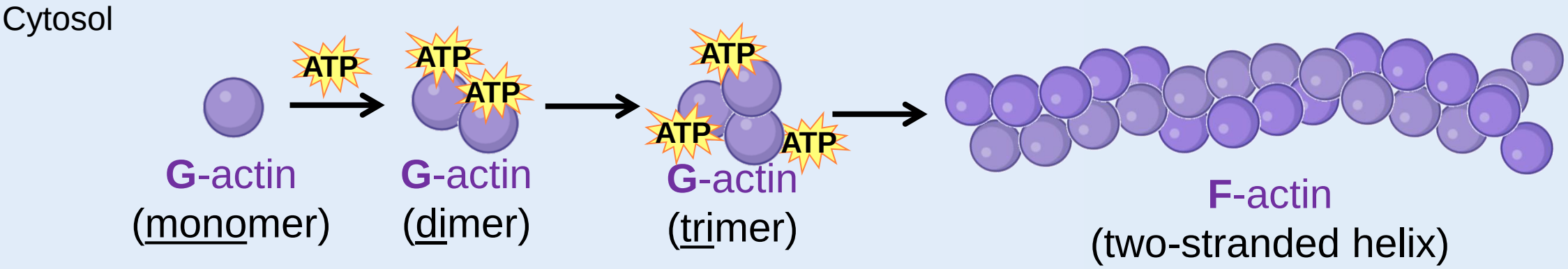


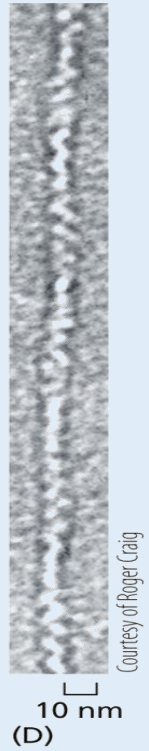
Cytoskeleton of a human breast cancer cell line

Microtubules

Actin filaments

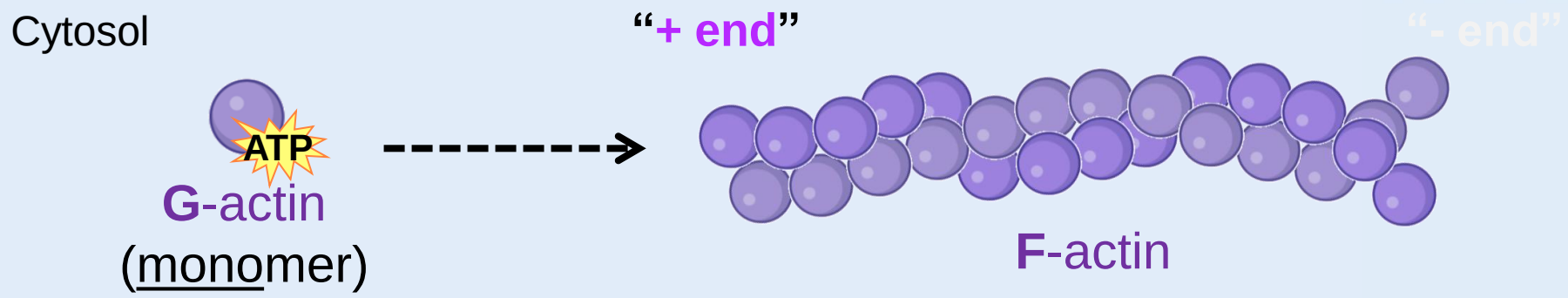
Actin filaments are composed of globular actin monomers





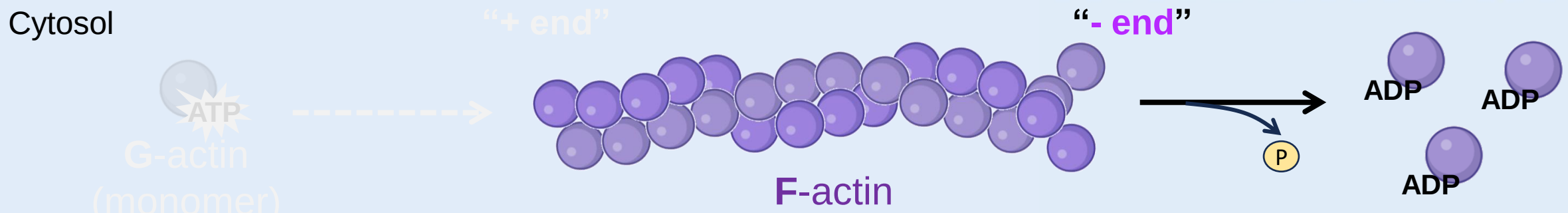
- **G**-actin (**g**lobular actin) binds to ATP for assembly
- **G**-actin assembles to create **F**-actin (actin **f**ilaments)
- Monomers will hydrolyze ATP to ADP after incorporation into the filament (in contrast with **microtubules**, which use GTP)
- **Actin filaments** are the **smallest component** of the **Cytoskeleton** (~ 7 nm)

Actin filament polarity determines its growth rate



- "**+ end**" allows for rapid **growth**
- "**+ end**" is the primary site for **G**-actin assembly (**polymerization**)

Actin filament polarity determines its growth rate

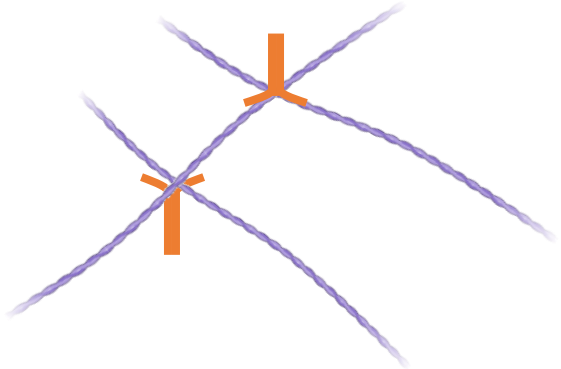


- "**- end**" allows for slow **growth**
- "**- end**" is where **depolymerization** primarily occurs; **ADP** binds to **G**-actin

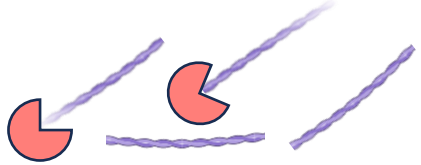
- In contrast to **microtubule** dynamic instability which predominantly occurs at the "+ end," actin filaments demonstrate **treadmilling**, as monomers are lost on the "- end" while new ones are being added to the "+ end"

Actin-binding proteins control the dynamic nature of Actin

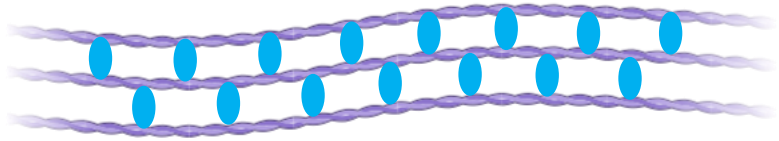
Cross-linking proteins organize actin filaments into complex structures

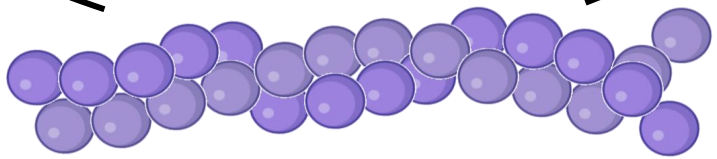


Severing proteins break actin filaments and help reorganize the cytoskeleton



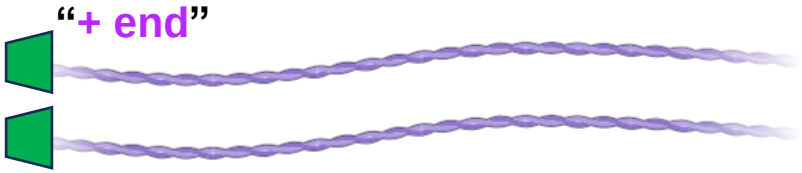
Bundling proteins allow for actin bundles



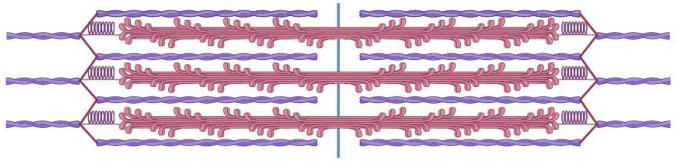


F-actin

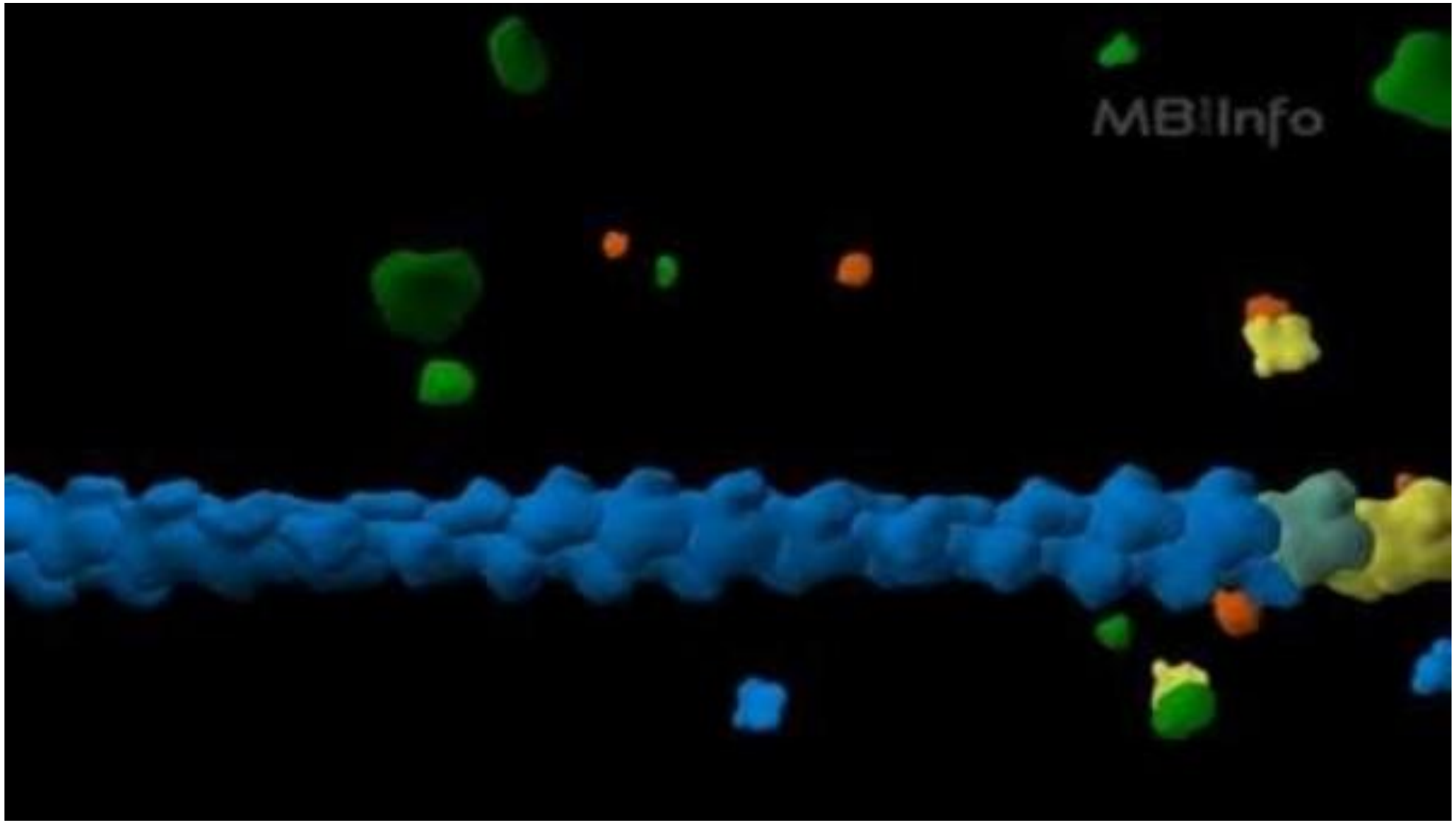
Capping proteins help regulate actin polymerization and depolymerization



Myosin moves along actin filaments to cause muscle contraction



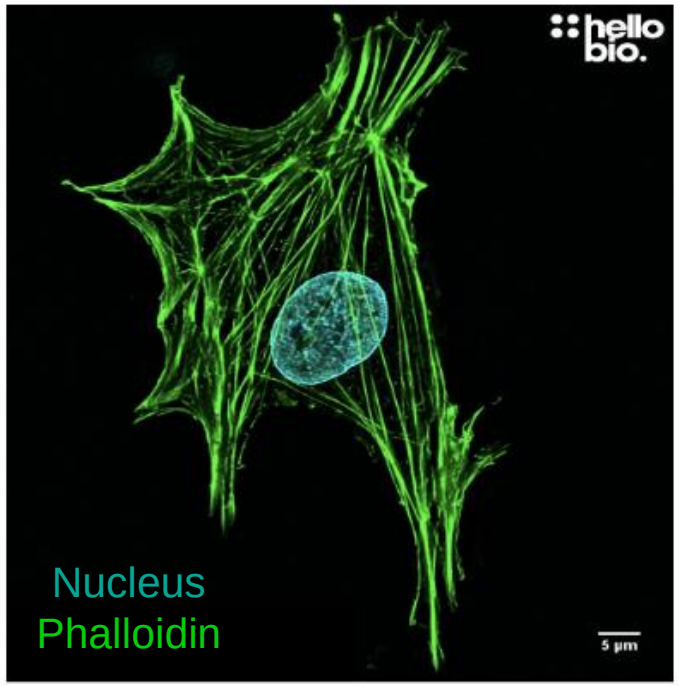
Actin filament assembly video



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

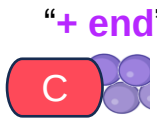
Actin dynamics can be modified by drugs

Phalloidin



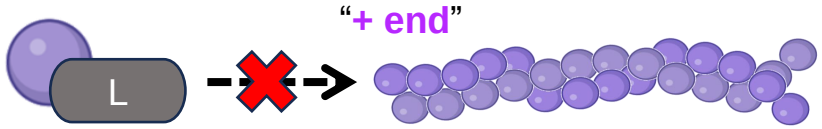
Binds to actin filaments (**F**-actin) and prevents depolymerization; stabilizes actin filaments

Cytochalasin



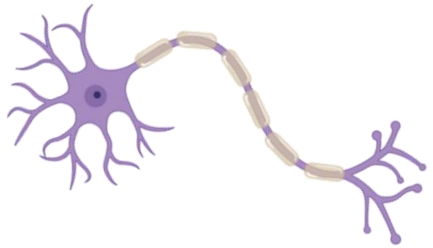
Caps actin filament (**F**-actin) "**+ end**" which prevents polymerization

Latrunculin

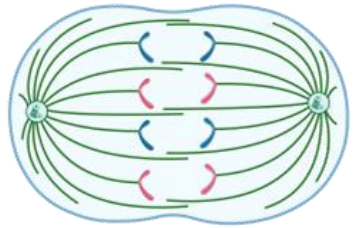


Binds to **G**-actin monomers to prevent polymerization

Functions of the Cytoskeleton (Microfilaments)

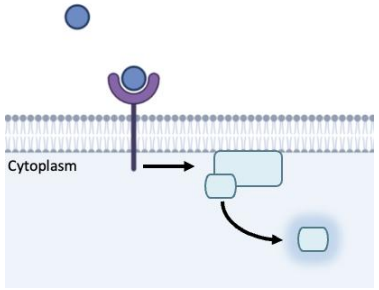


Cell shape maintenance and support



Contractile ring formation during cell division

Cell Movement (Cytokinesis)



Aid in signal transduction

Muscle contraction

Functions of the **Cytoskeleton** (Microfilaments)

Cell Movement (Cytokinesis)

Aid in signal transduction

Muscle contraction

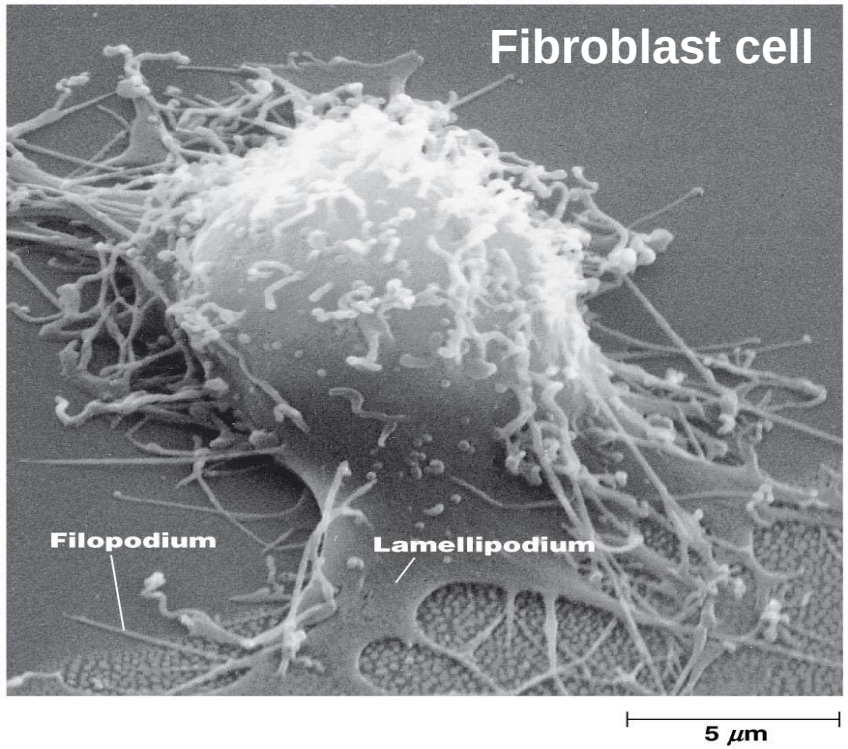
Contractile ring formation during cell division

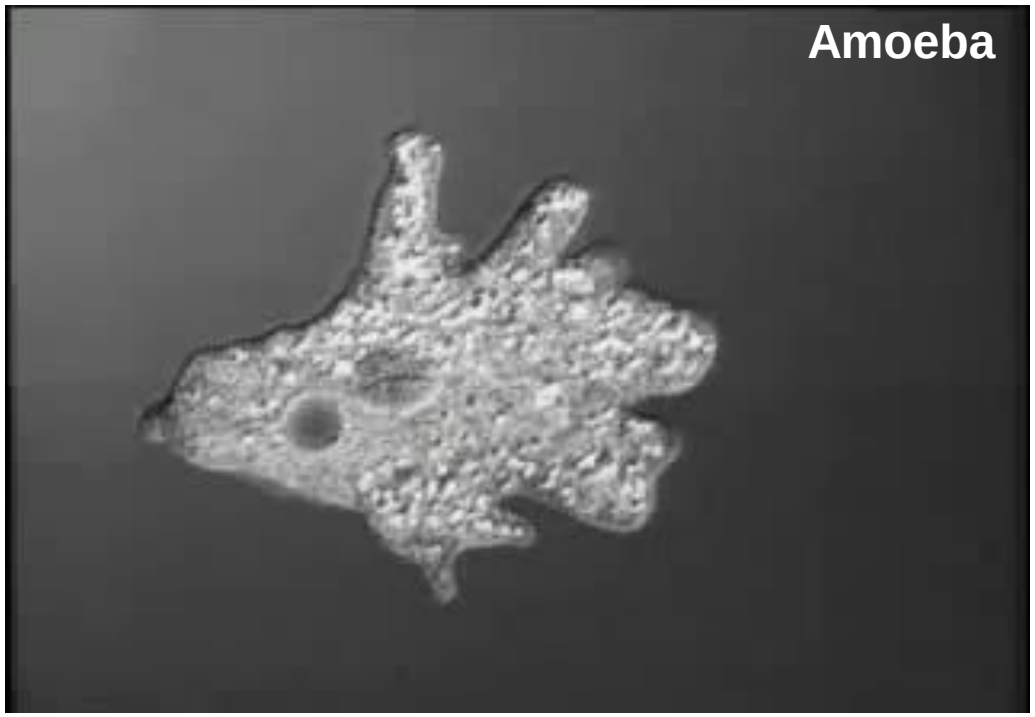
Cell shape maintenance and support

Cell crawling depends on Actin filaments

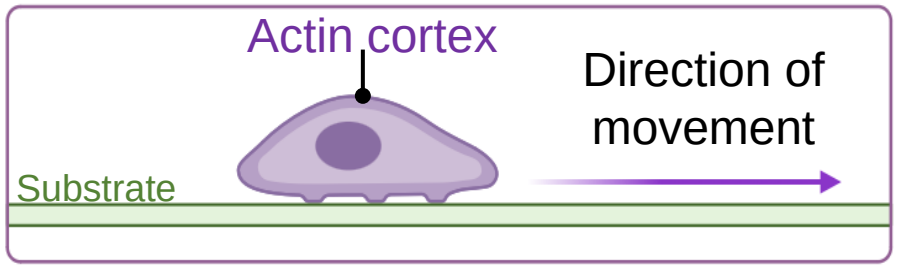
Many cells are capable of **crawling** using either:

- **Lamellipodia**: thick bundles of **actin** at the **leading edge of the cell**
- **Filopodia**: thin-pointed protrusions of **actin** bundles at the **leading edge of the cell**



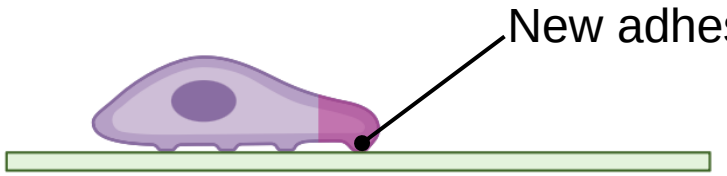


Actin filaments are crucial for cell movement

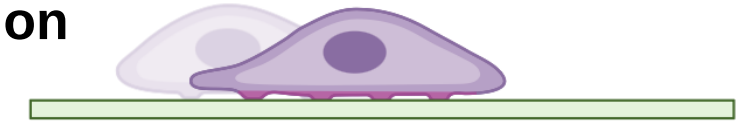


Extension

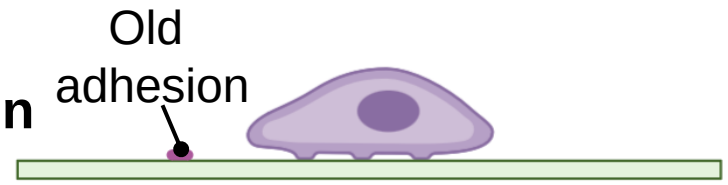
Adhesion

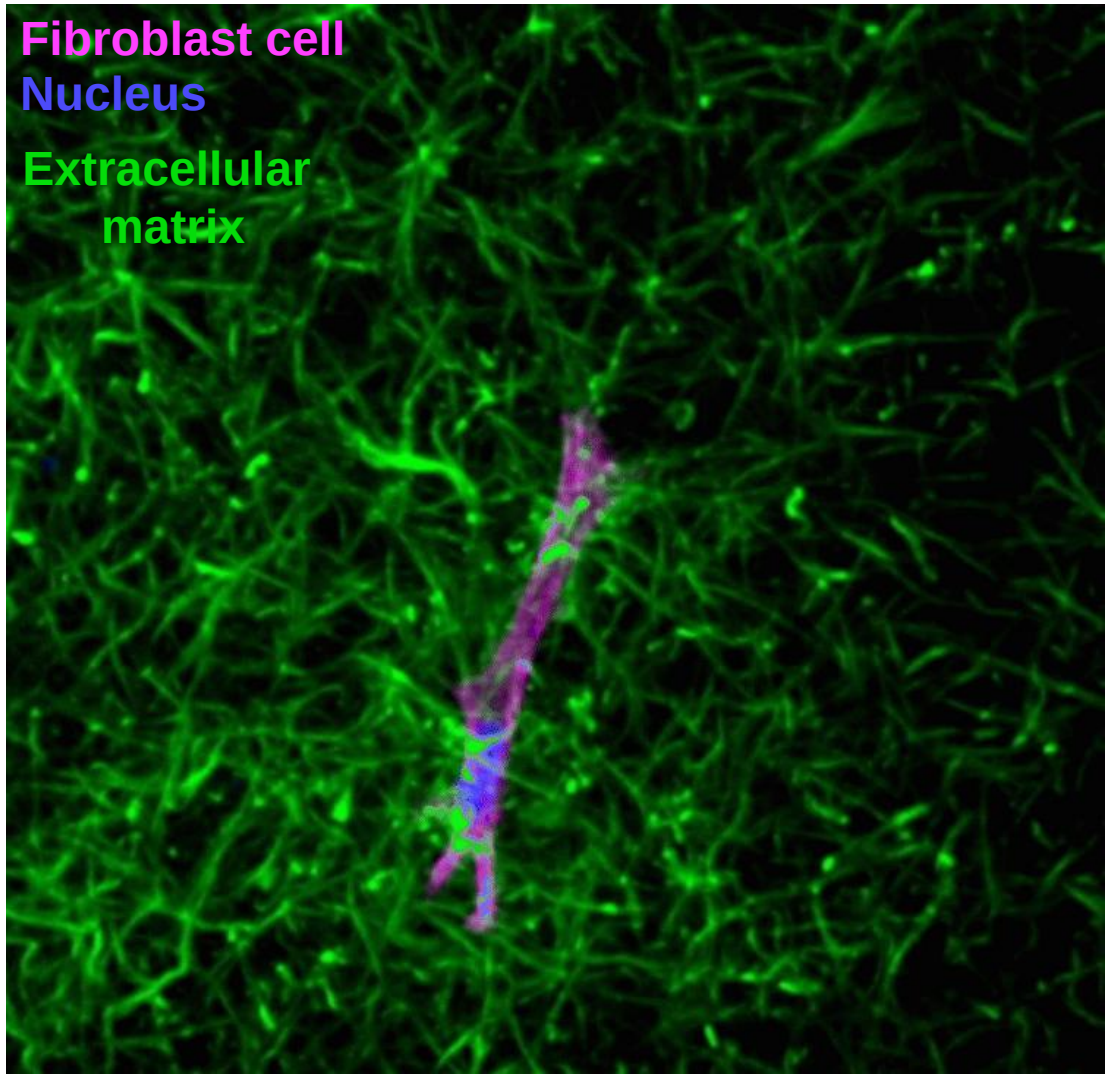


Translocation



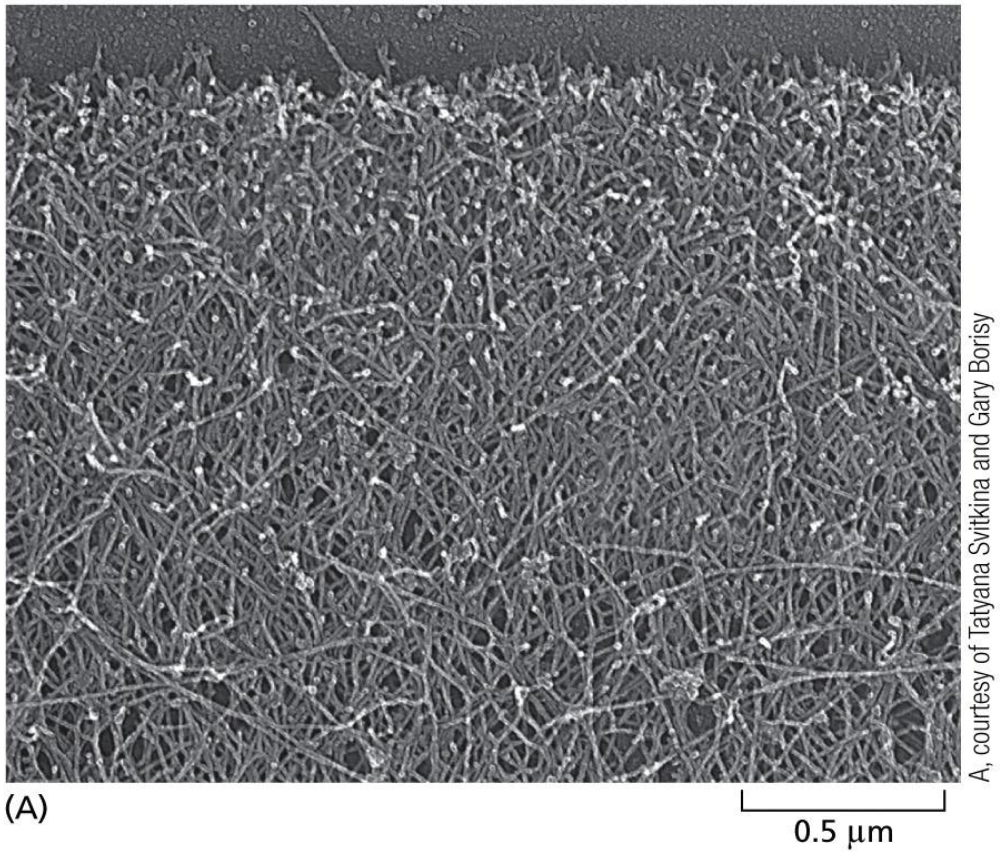
De-adhesion

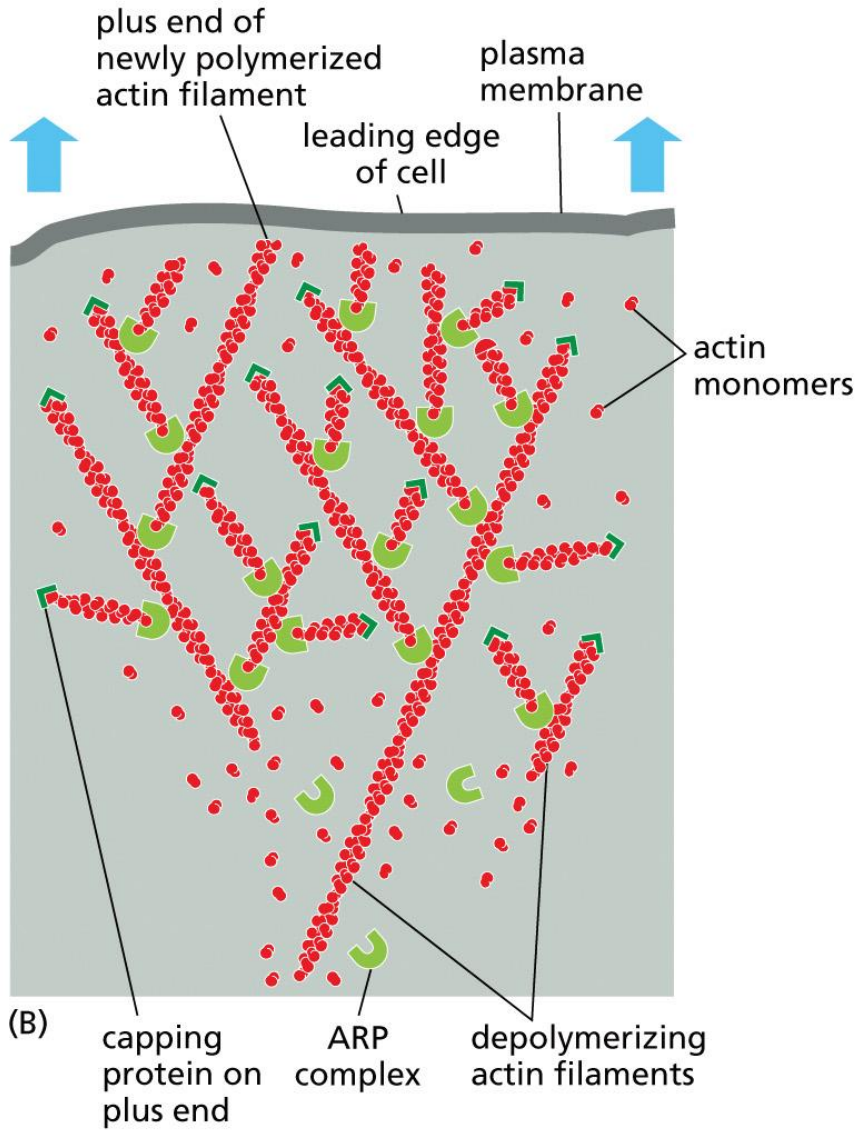




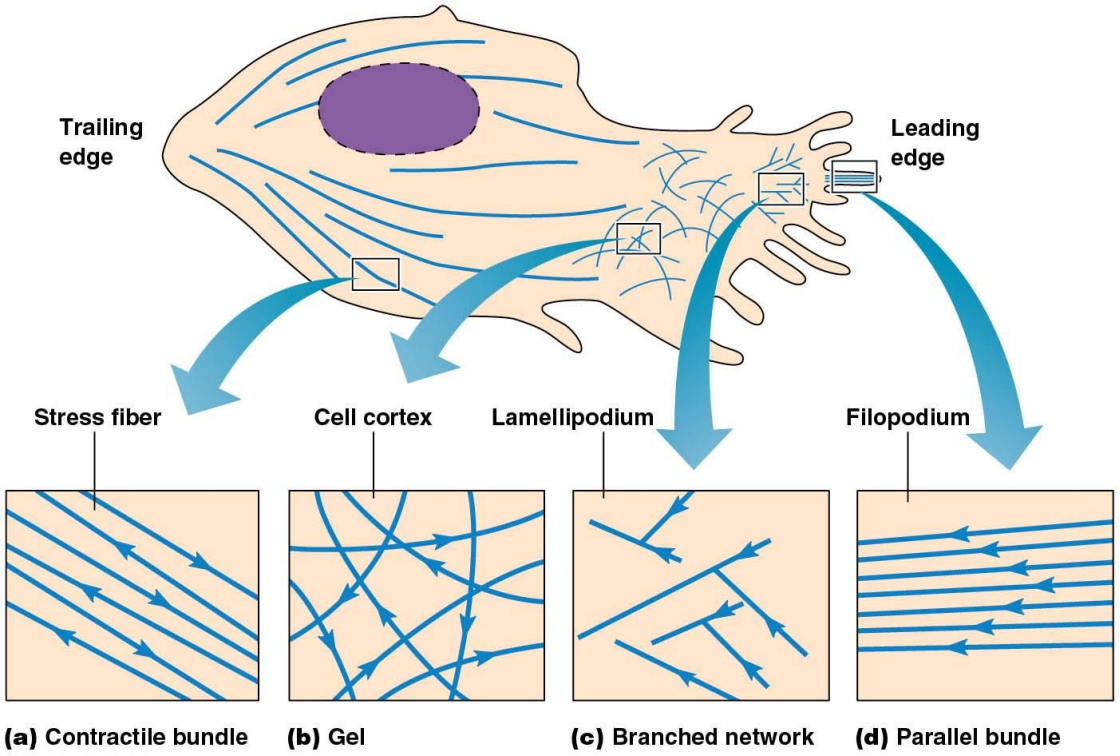
Laboratory of Dr. Ken Yamada, MD, Ph.D.
National Institutes of Health (NIH)

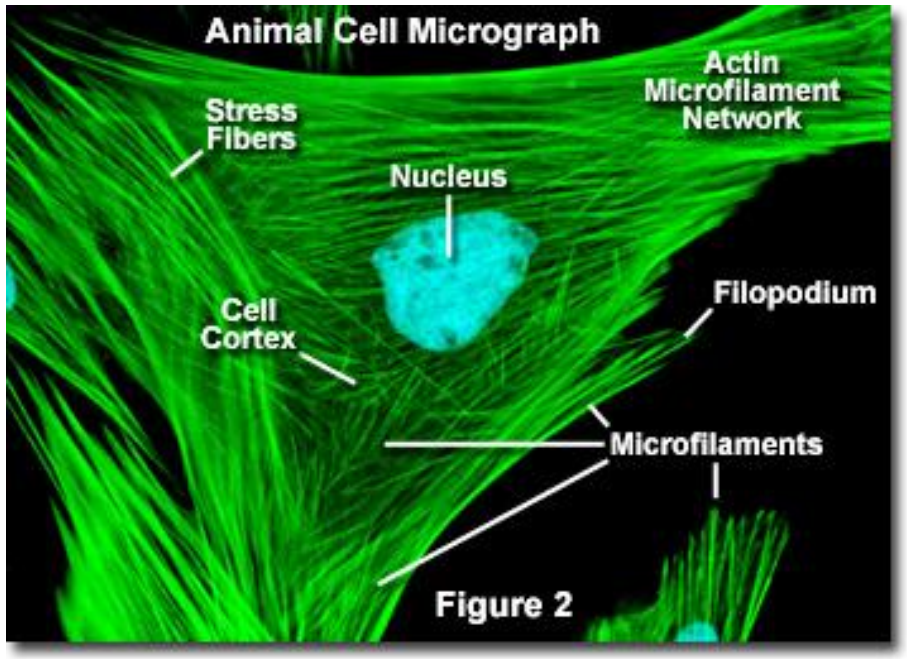
Web of polymerizing actin filaments pushes leading edge of cell





Cell crawling depends on actin-rich cortex





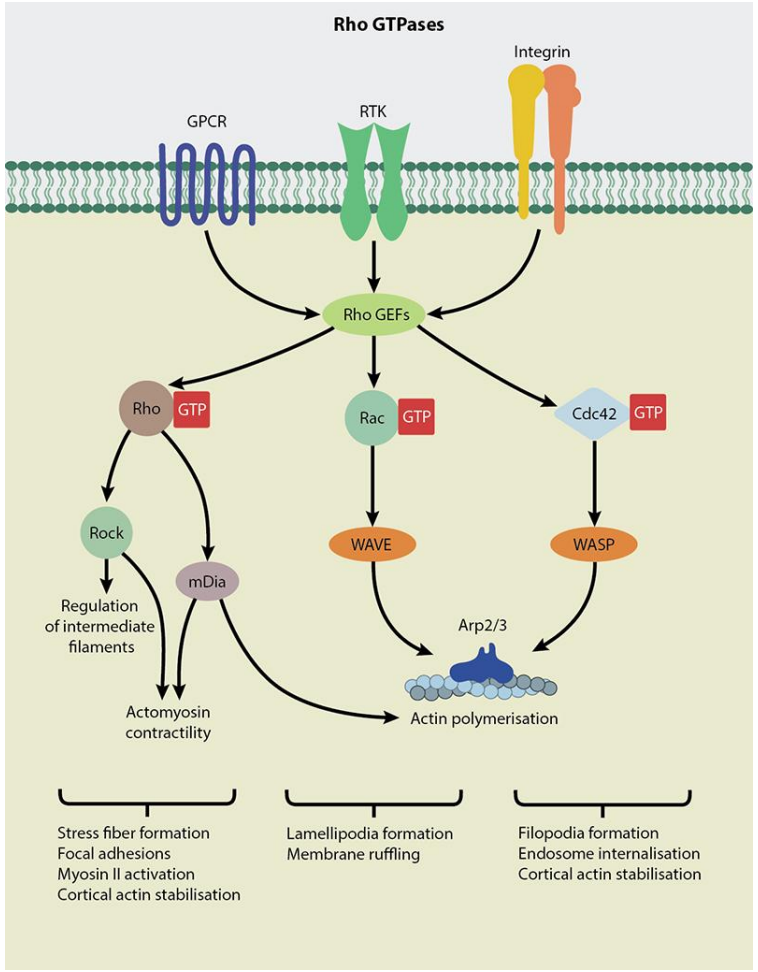
https://micro.magnet.fsu.edu/cells/microfilaments/microfilaments.html

- *Integrins* link cytoskeleton to outside surface by binding to both, creating anchorage points
- Permits *stress fiber assembly* that contain contractile bundles of actin filaments and myosin, which slides along actin filament, dragging the cell forward

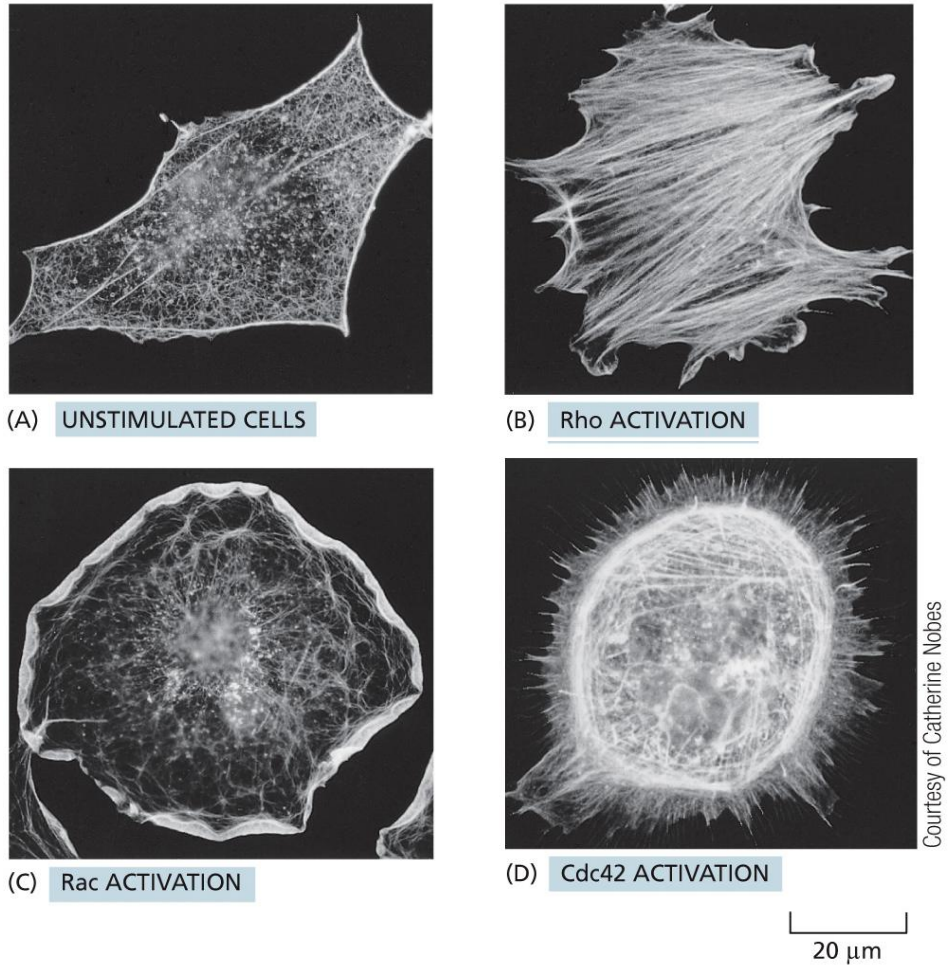
Extracellular signals regulate actin organization

- The Rho family of GTPases (Rho, Rac, Cdc42) induce actin remodeling when GTP is bound

- Rho's function is regulated by GEFs, GAPs and **GDIs** (GDP-dissociation inhibitors)
    - Sequesters GDP-bound Rho in the cytosol to *inhibit* actin remodeling



Experimental setup: microinject activated forms of Rho family proteins and visualize actin microfilaments using fluorescent label



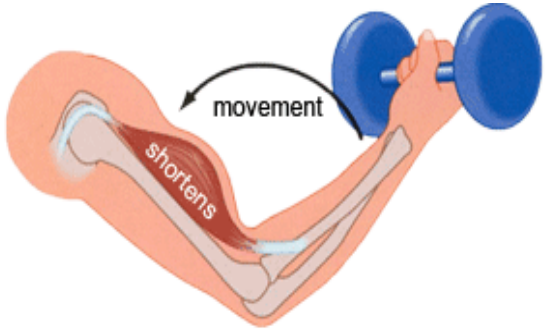
Cell shape maintenance and support

Functions of the **Cytoskeleton** (Microfilaments)

Contractile ring formation during cell division

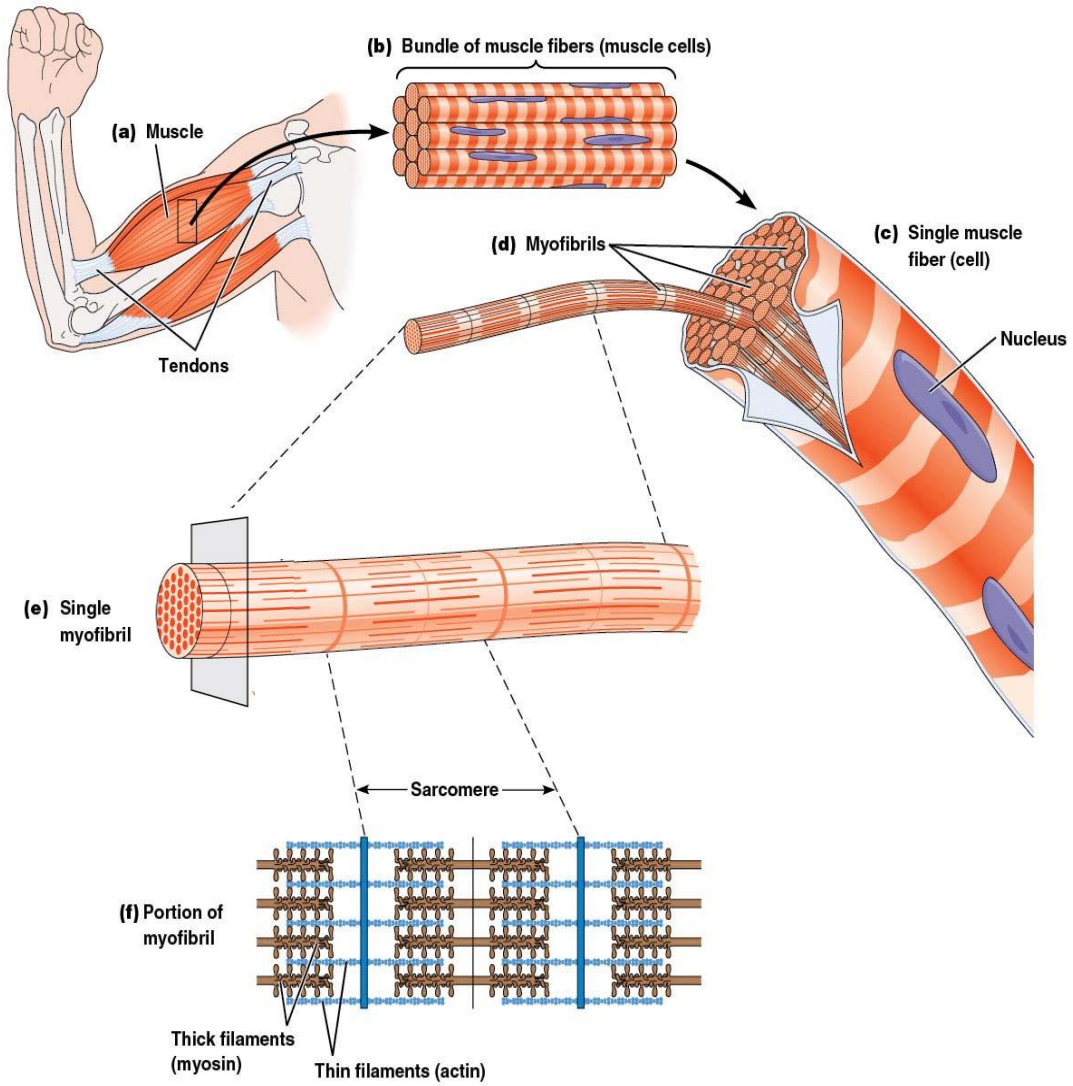
Cell Movement (Cytokinesis)

Aid in signal transduction



Muscle contraction

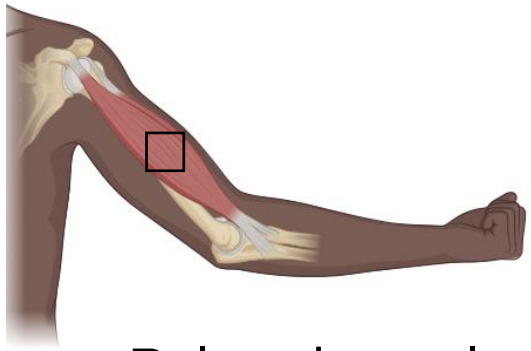
Skeletal Muscle Structure: Overview



- Muscles consists of bundles of muscle fiber (a single multinucleated cell)

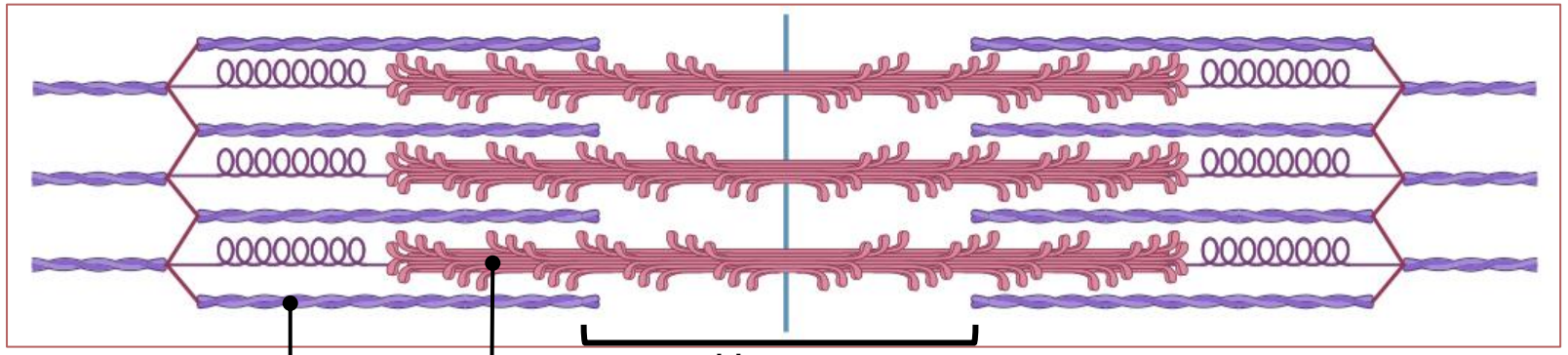
- Each muscle fiber contains myofibrils:
  - Divided along its length into repeating units called **sarcomeres**
  - Each sarcomere contains bundles of
    1. Thin filaments (actin)
    2. Thick filaments (myosin)

Muscle contraction depends on interacting filaments of Actin and Myosin



Relaxed muscle

Sarcomere



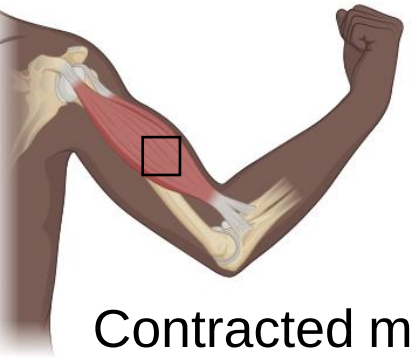
H zone

Actin

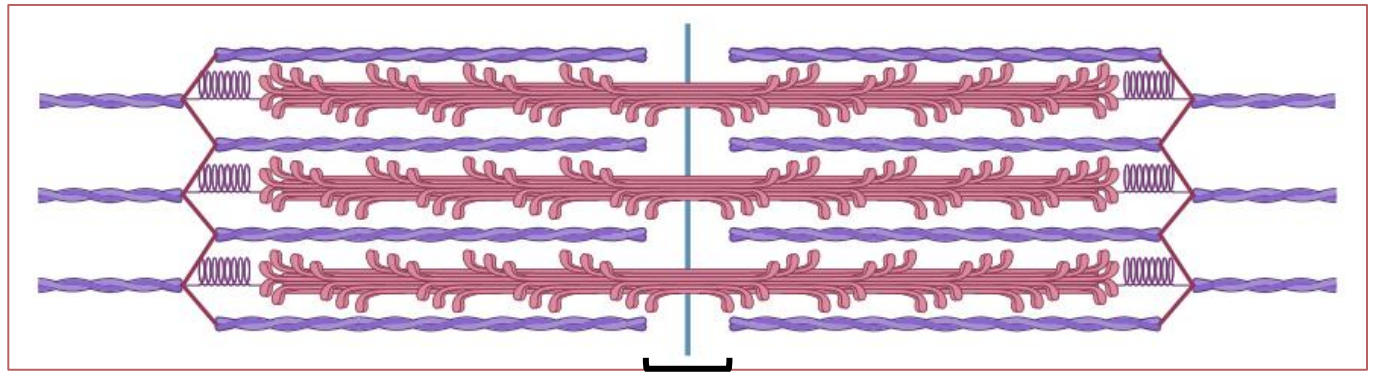
Myosin
(thick filaments)

ATP

H zone:
composed of
Myosin



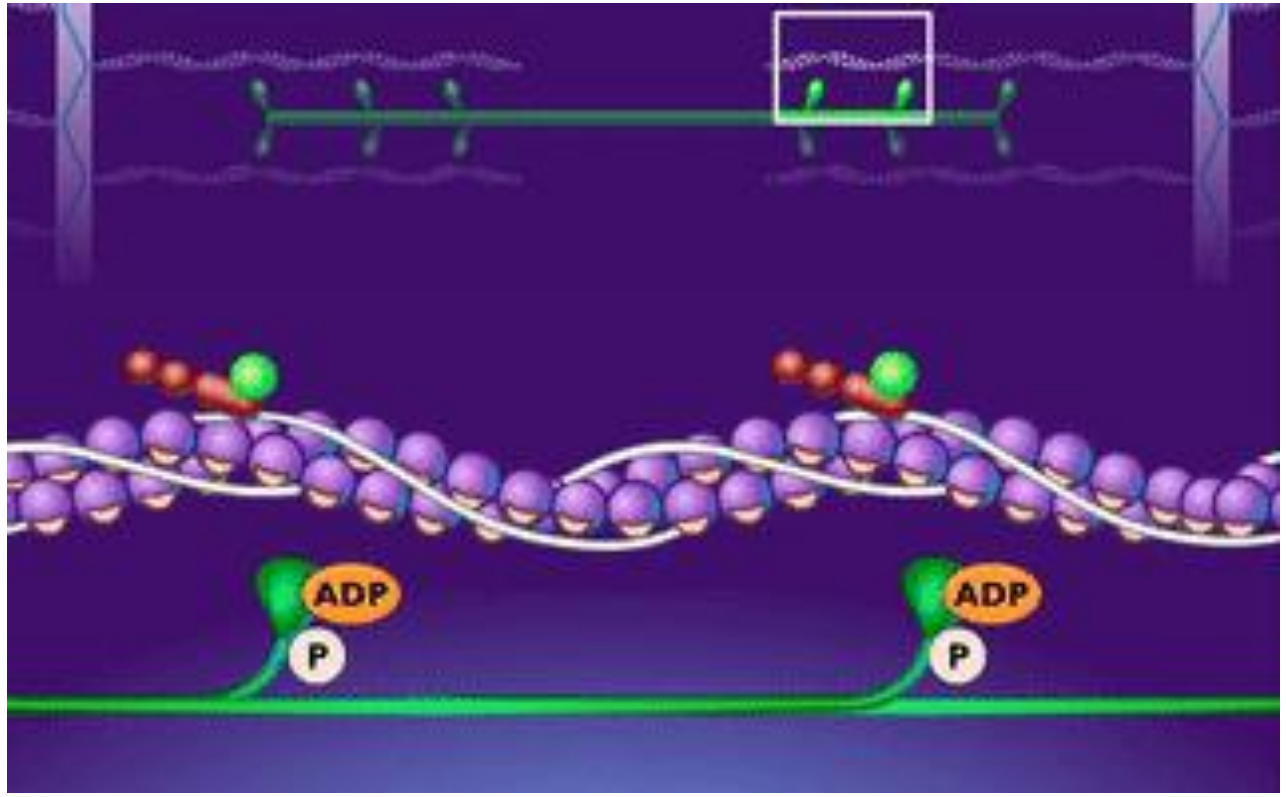
Contracted muscle



H zone

H zone:
shortens

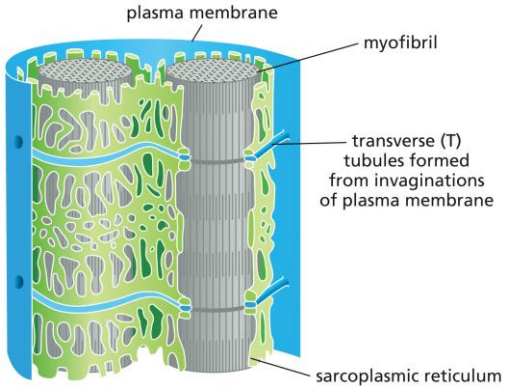
Muscle contraction requires ATP hydrolysis

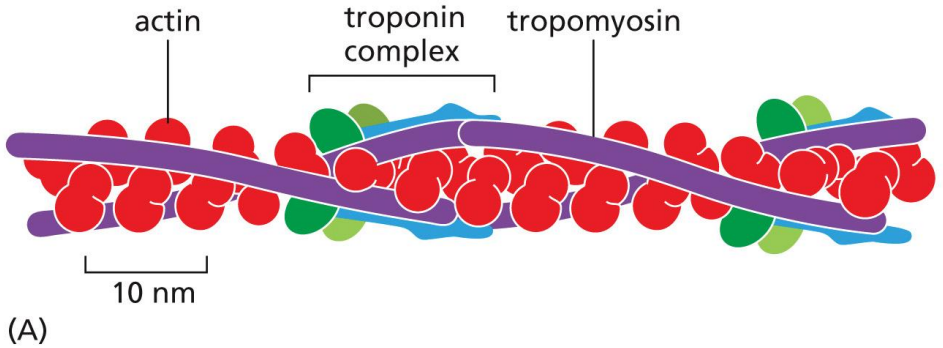


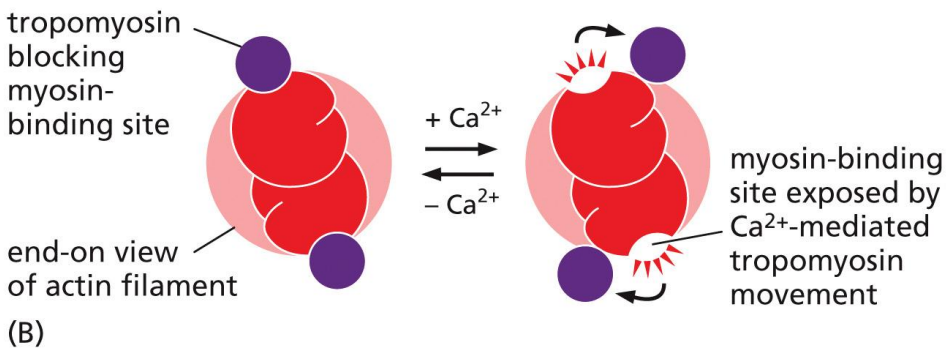
- **ATP** attaches to the **Myosin** head
- **ATP** is hydrolyzed to ADP and Ⓟ, releasing energy
- **Myosin** head binds to actin
- ADP and Ⓟ are released, causing the **Myosin** head to pull the actin filament towards the center of the sarcomere in a “power stroke” (**H zone shortens**)
- A new **ATP** molecule binds to the **Myosin** head, causing it to detach from the actin filament

Skeletal Muscle Contraction: Ca^{2+} is Key

- *Recall:* Ca^{2+} is a 2nd messenger used to relay messages to cells.
- Under normal conditions, tropomyosin blocks myosin heads from binding actin
- An electrical signal from motor neurons induces release of Ca^{2+} from the sarcoplasmic reticulum – a sheath that surrounds myofibrils
- In the presence of Ca^{2+}, the troponin complex (Ca^{2+} sensing) induces conformational change to induce release of tropomyosin
- Myosin can now bind to actin filament

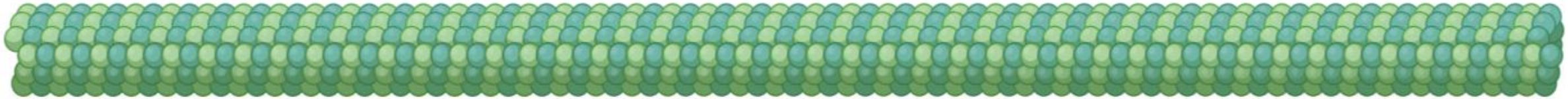






The 3 essential components of the Cell's skeleton

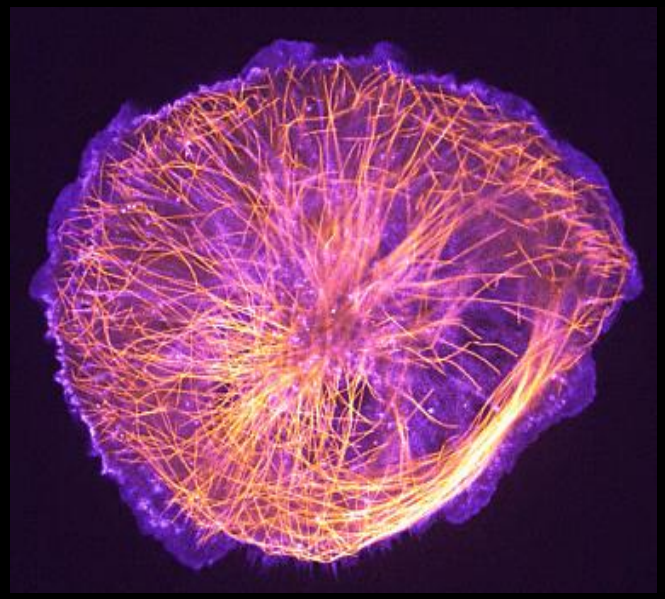
Microtubules

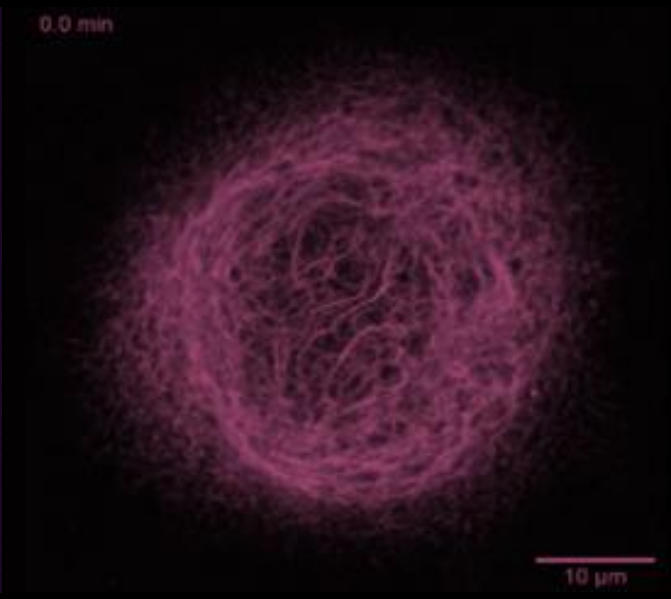


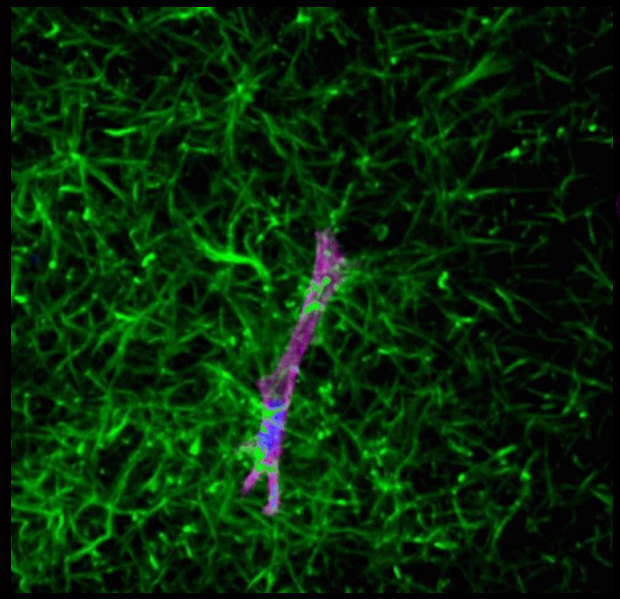
Intermediate filaments

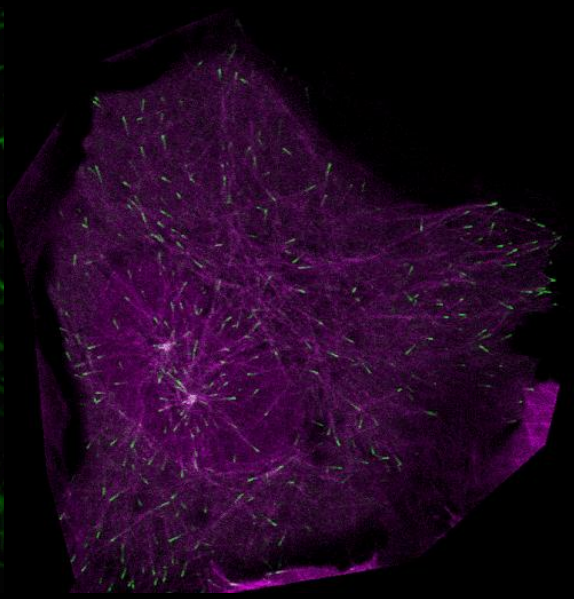
Actin filaments

The Cytoskeleton









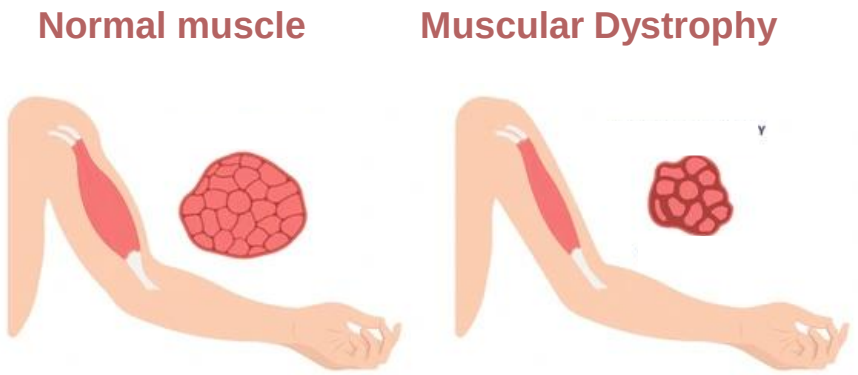
Microtubules, intermediate filaments, and **microfilaments**
work together to maintain cellular integrity

Cytoskeleton defects are associated with a variety of human diseases

Amyotrophic Lateral Sclerosis (ALS)

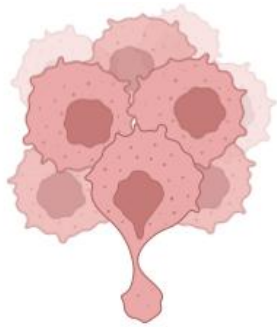
Cytoskeleton defects block axonal transport of cargo; impairs neuron development and synaptic function

Muscular Dystrophy



Cytoskeleton breakdown leads to muscle weakness, damage, and necrosis (death of the tissue)

Cancer



Abnormal cytoskeletal organization can facilitate cancer cell migration, invasion, and metastasis

Metacognitive Reflection Form