

# Cytoplasm

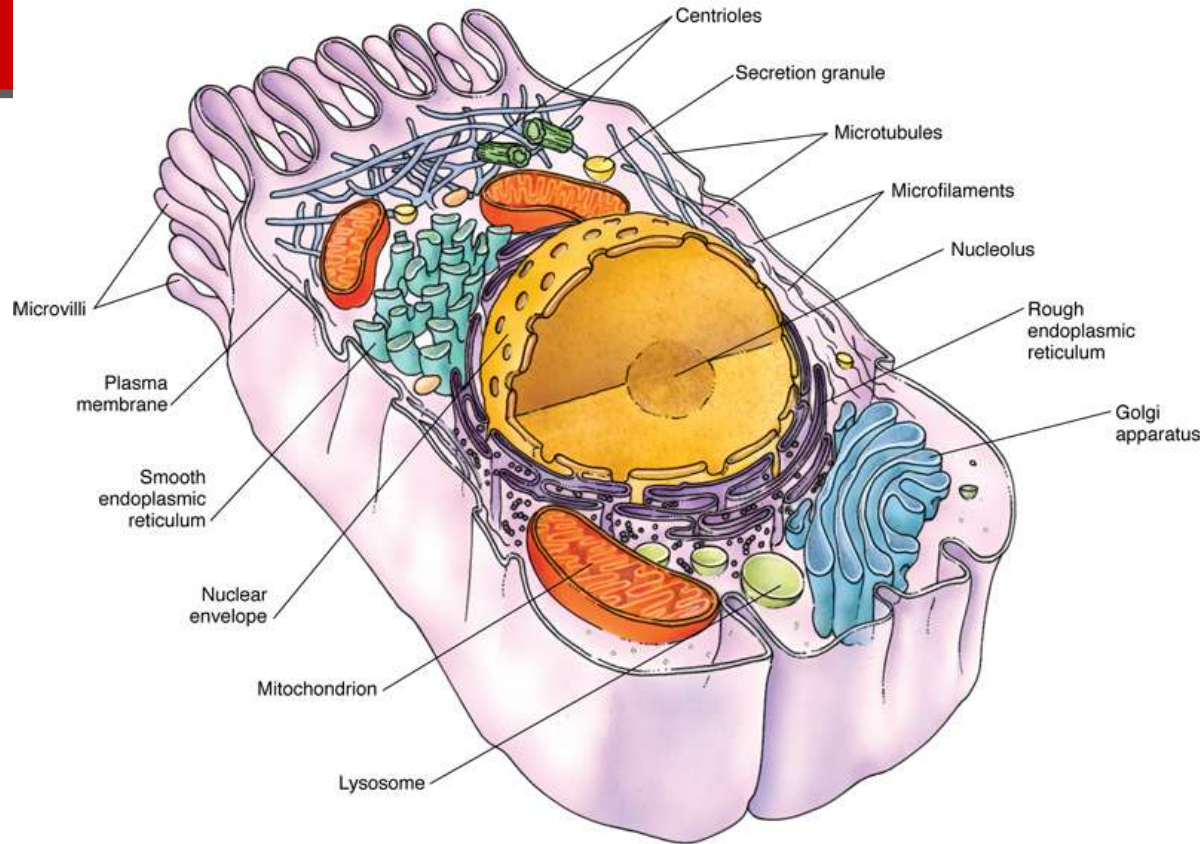
Textbook of Histology, 4th ed.

*Gartner*

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# The Cell



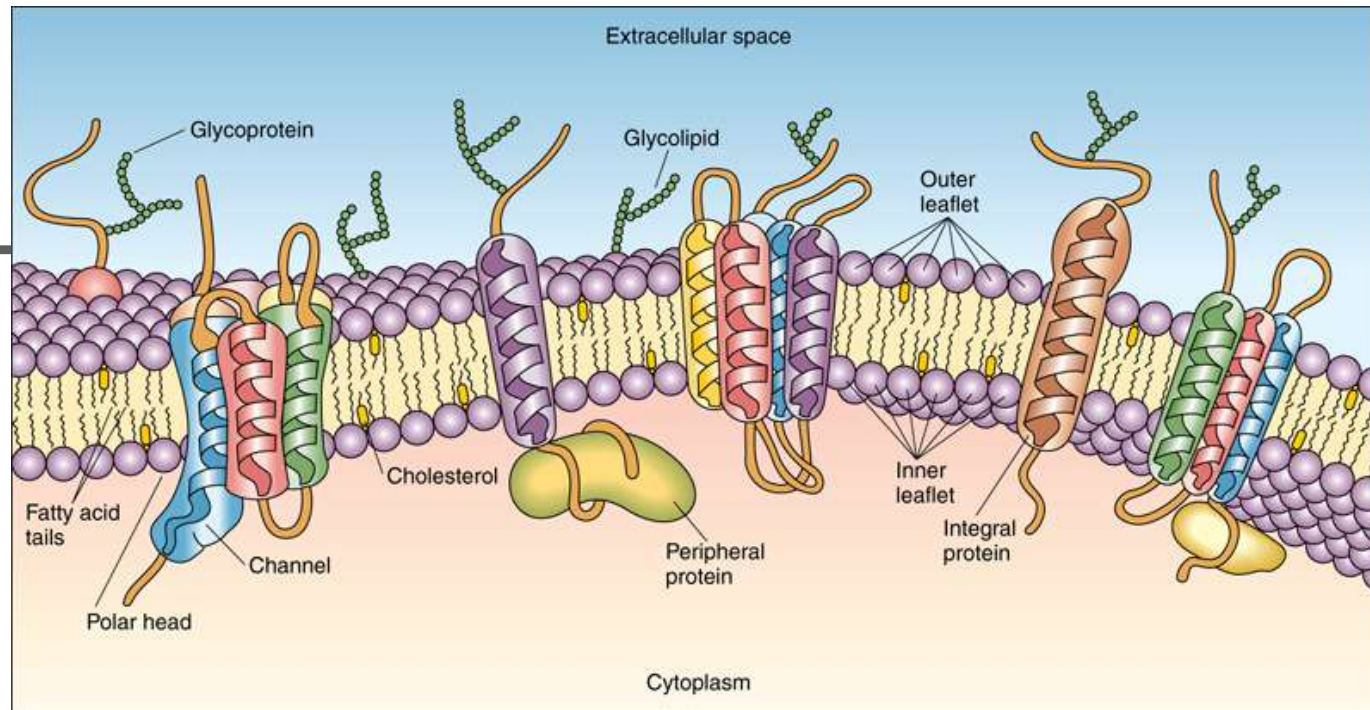
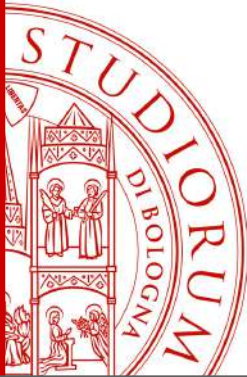
A cell, is subdivided into two compartments: **cytoplasm**, extending from the plasma membrane to the nuclear envelope and **karyoplasm (nucleoplasm)**, the substance forming the contents of the nucleus.

The bulk of the cytoplasm is **water**, in which various inorganic and organic chemicals are dissolved and/or suspended.

This fluid suspension, the **cytosol**, contains **organelles**, metabolically active structures that perform distinctive functions.

Three-dimensional illustration of an idealized cell, as visualized by transmission electron microscopy. Various organelles and cytoskeletal elements are displayed.

# Cell Membrane



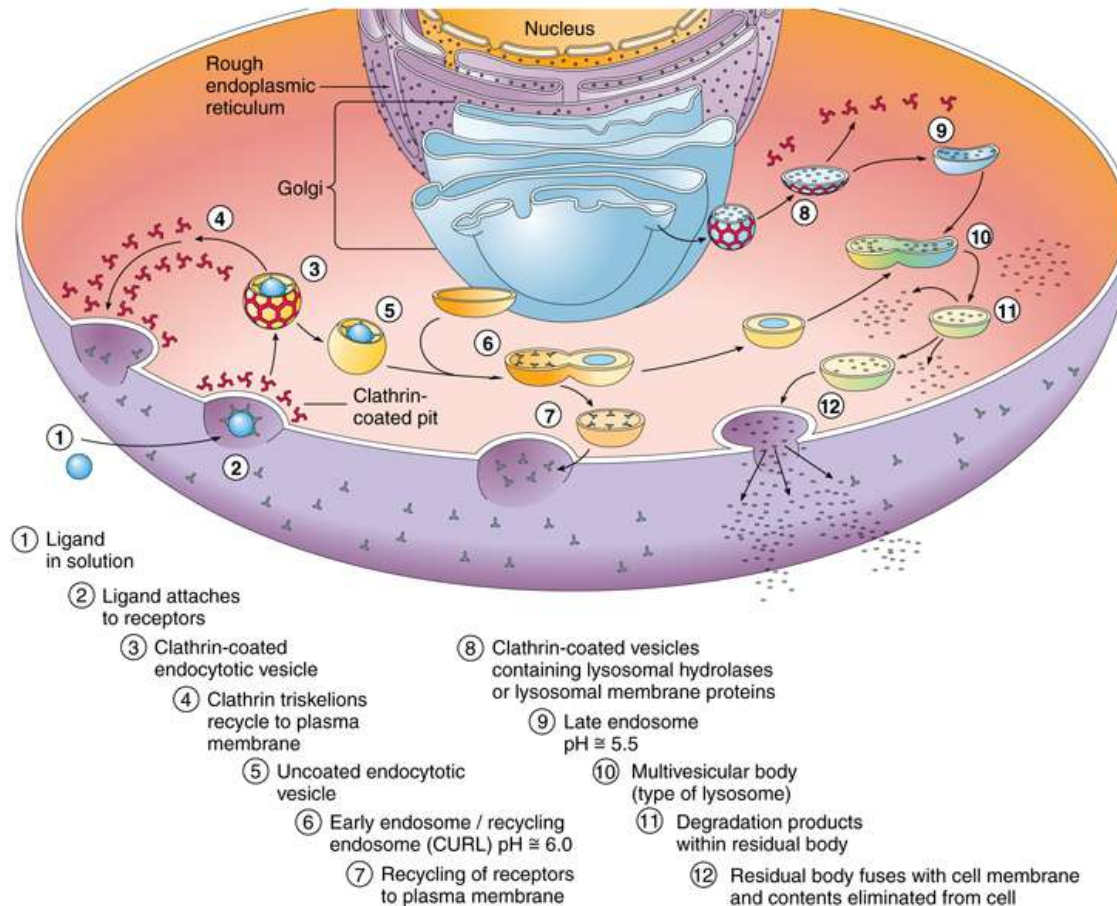
A fluid mosaic model of the cell membrane.

Each cell is bounded by a **cell membrane** (also known as the **plasma membrane**) that functions in:

- Maintaining the structural integrity of the cell
- Controlling movements of substances in and out of the cell (selective permeability)
- Regulating cell-cell interactions
- Recognition, via receptors, antigens, and foreign cells as well as altered cells
- Acting as an interface between the cytoplasm and the external milieu
- Establishing transport systems for specific molecules
- Transducing extracellular physical or chemical signals into intracellular events.



# Endocytosis and the Endosomal System of Vesicles



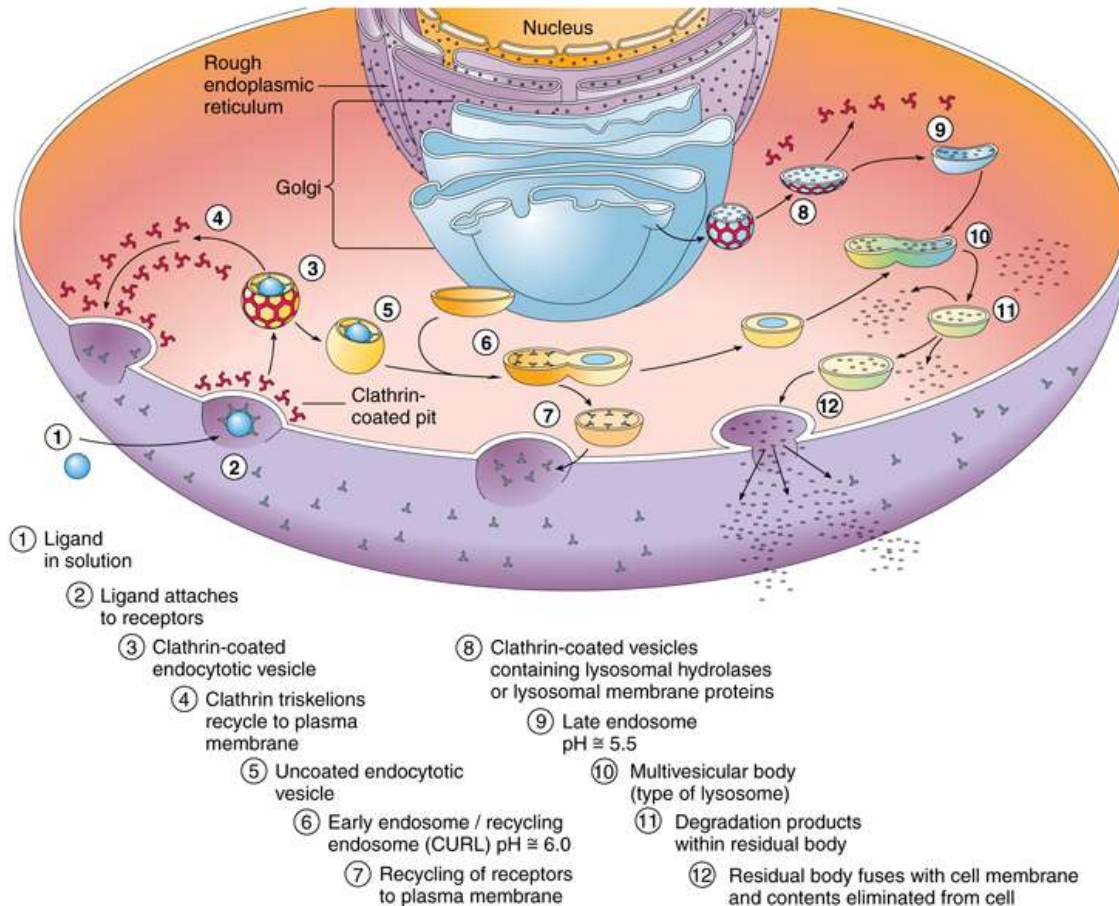
The process whereby a cell ingests macromolecules and other substances from the extracellular space is referred to as **endocytosis**.

The endocytosed material is engulfed in a vesicle appropriate for its volume.

If the vesicle is large (>250 nm in diameter), the method is called **phagocytosis** (cell eating) and the vesicle is a **phagosome**.

The endosomal pathways. CURL, compartment for *uncoupling of receptor and ligand*.

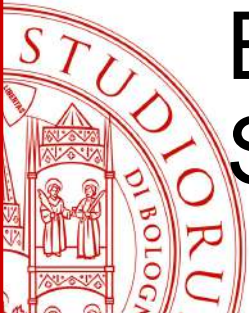
# Endocytosis and the Endosomal System of Vesicles



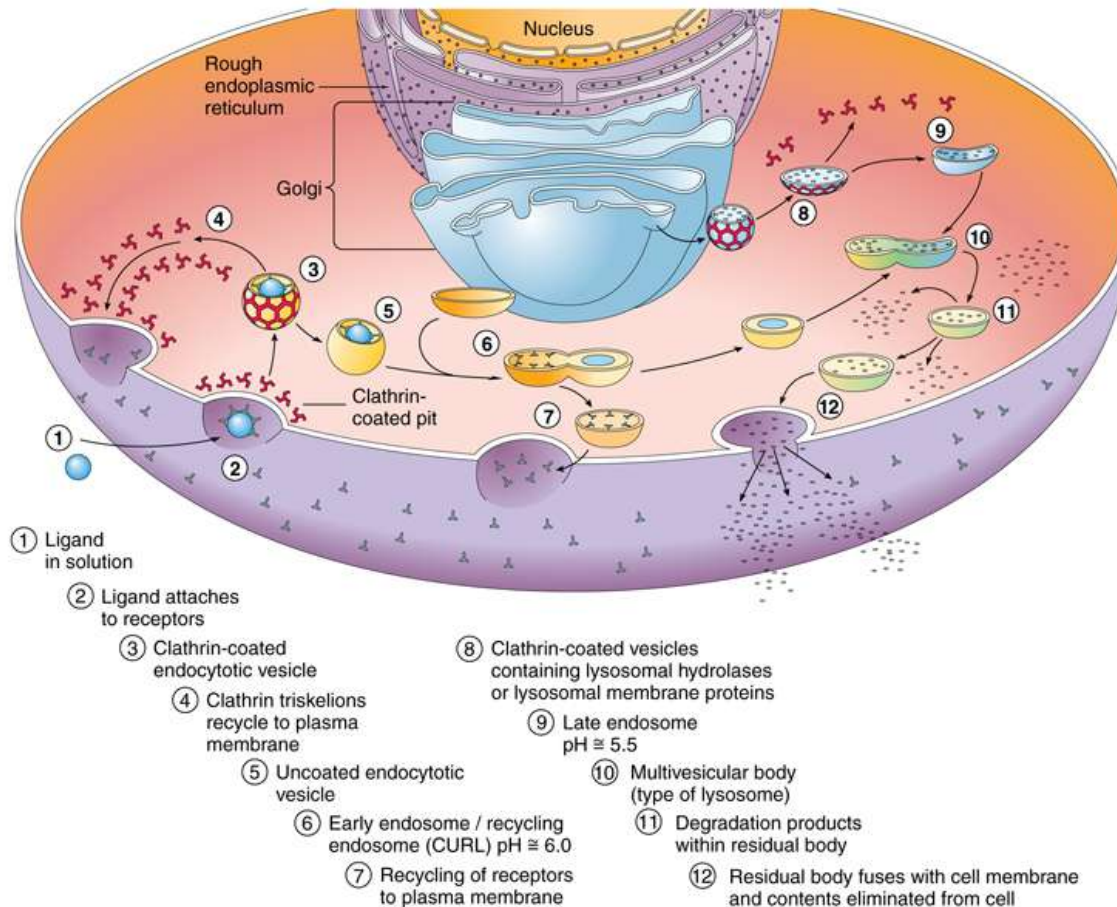
If the vesicle is small (<150 nm in diameter), the type of endocytosis is called **pinocytosis** (cell drinking) and the vesicle is a **pinocytotic vesicle**.

Shortly after their formation, pinocytotic vesicles lose their clathrin coats (which return to the pool of clathrin in the cytosol) and fuse with **early endosomes**, a system of vesicles and tubules located near the plasma membrane.

The endosomal pathways. CURL, compartment for *uncoupling of receptor and ligand*.



# Endocytosis and the Endosomal System of Vesicles



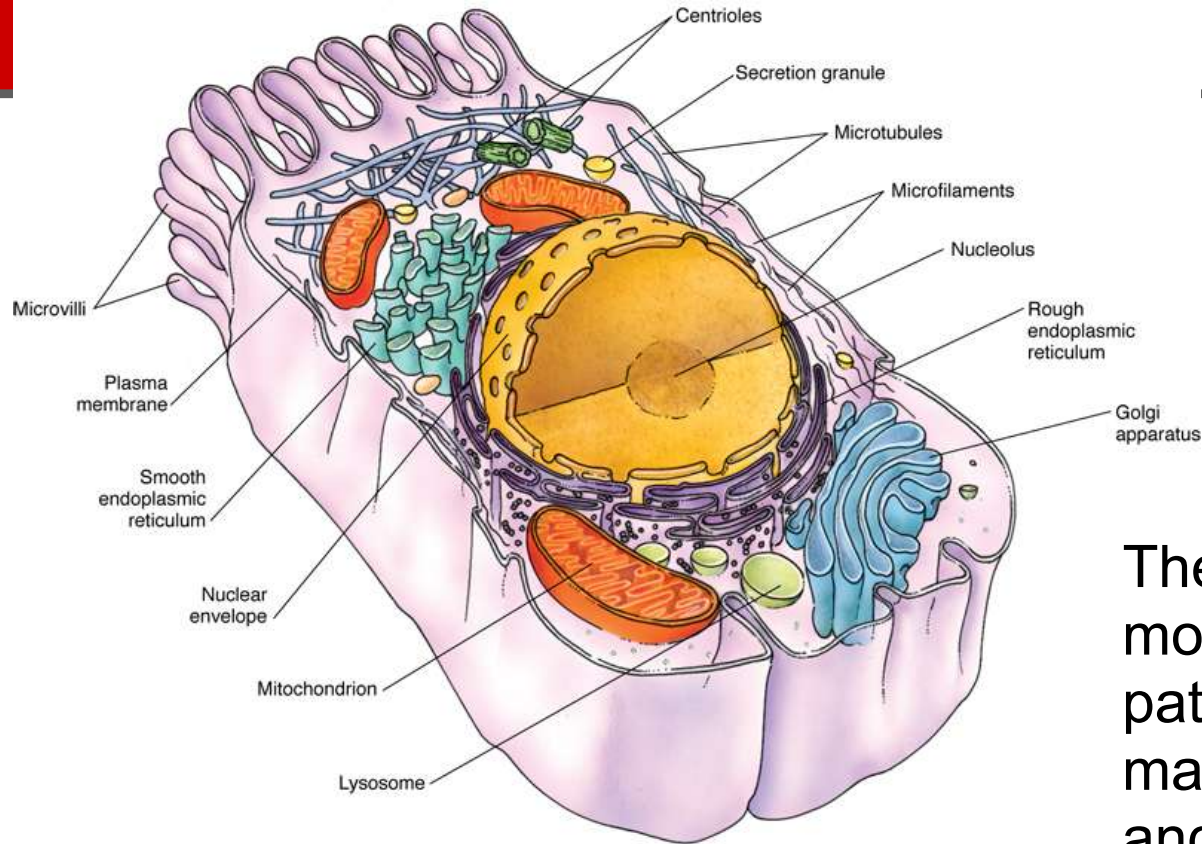
If the entire contents of the pinocytotic vesicle requires degradation, the material from the early endosome is transferred to a **late endosome**.

This similar set of tubules and vesicles, located deeper in the cytoplasm near the Golgi apparatus, helps to prepare its contents for eventual destruction by lysosomes.

The endosomal pathways. CURL, compartment for uncoupling of receptor and ligand.

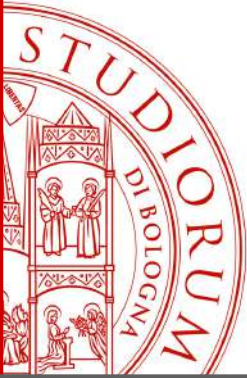


# Cytoskeleton



The shapes of cells, their ability to move, and the intracellular pathways within cells are maintained by a system of tubules and filaments known as the **cytoskeleton**.

Three-dimensional illustration of an idealized cell, as visualized by transmission electron microscopy. Various organelles and cytoskeletal elements are displayed.



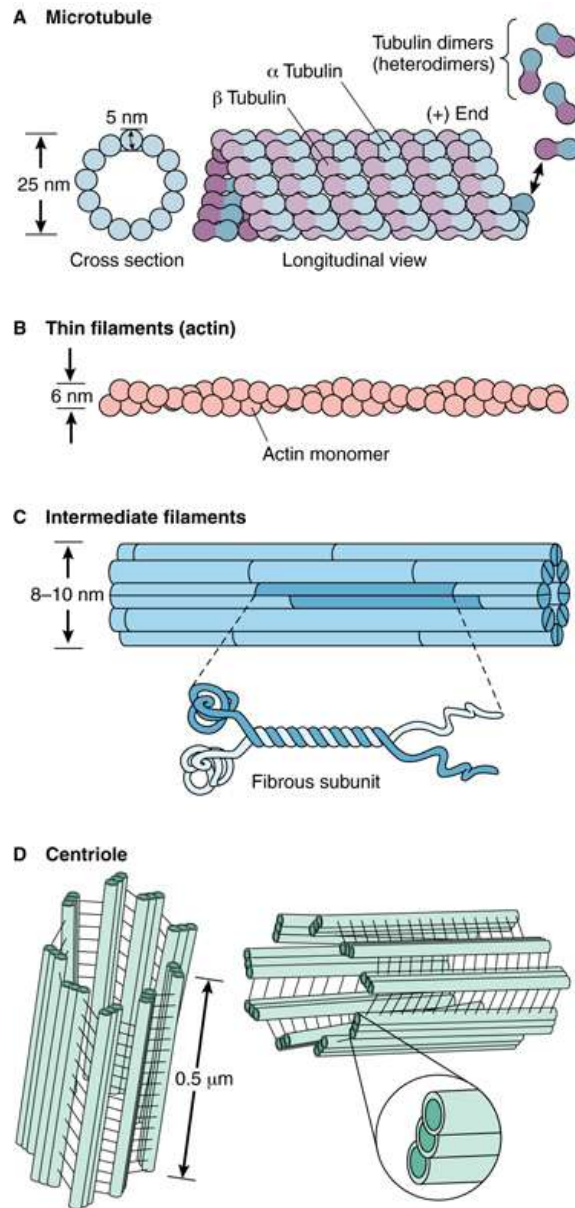
# Cytoskeleton

Three dimensional network of protein distributed throughout the cytoplasm with roles:

- **Cell movement** (blood cells along the blood vessels walls, migration of fibroblasts during wound healing and in the embryonic development)
- **Support and strenght for the cell**
- **Phagocytosis**
- **Cytokinesis**
- **Cell-cell and cell-extracellular matrix adherence**
- **Change in cell shape**

Three-dimensional illustration of an idealized cell, as visualized by transmission electron microscopy. Various organelles and cytoskeletal elements are displayed.

# Cytoskeleton



The component of the **cytoskeleton** were originally identified by electron microscopy as cytoplasmic “cables” that fell into 3 size groups

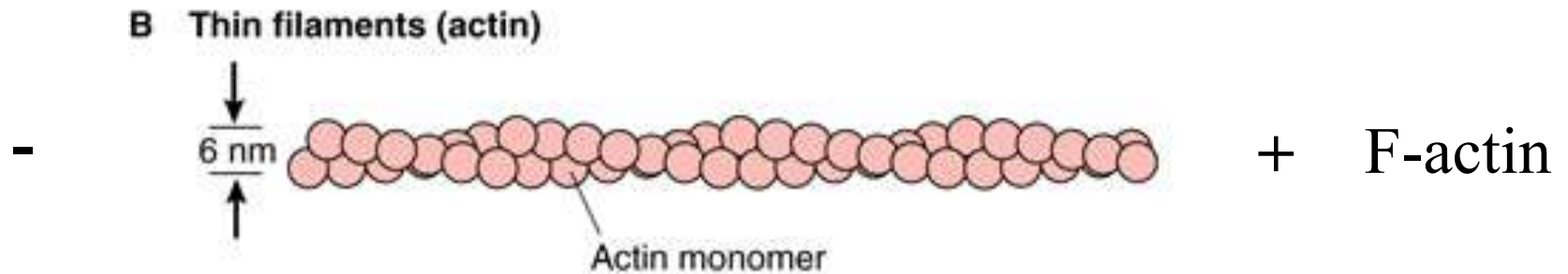
The cytoskeleton has three components

- (A) microtubules**
- (B) thin filaments or microfilaments**
- (C) intermediate filaments**

The only organelle that is composed of cytoskeletal elements is the centriole, a structure assembled from **microtubules**.

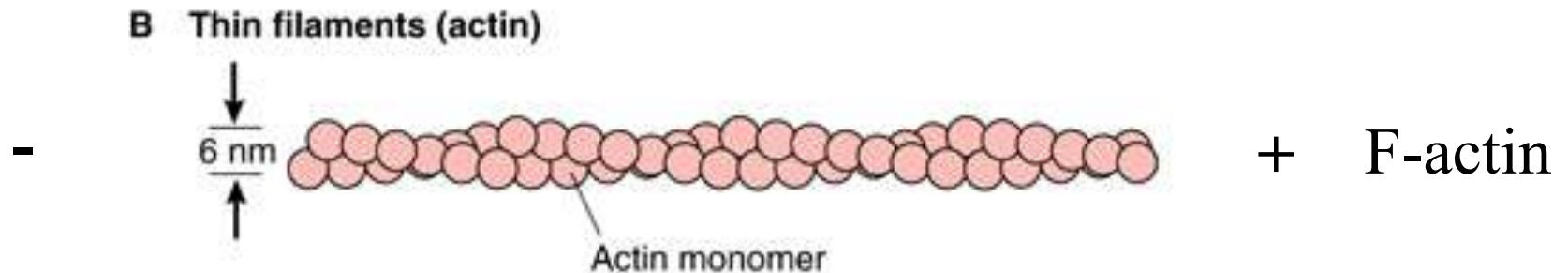
Elements of the cytoskeleton and centriole. **A**, Microtubule; **B**, thin filaments (actin); **C**, intermediate filaments; **D**, centriole.

# Microfilaments or Thin Filaments – 6-7 nm (thick)



F-actin is a versatile and abundant cytoskeletal component forming static and contractile bundles. F actin anchors to the plasma membrane, thus it contributes to the shape of the cells.

# Microfilaments or Thin Filaments – 6-7 nm (thick)



Thin Filaments are actin filaments that interact with myosin to allow cellular movement

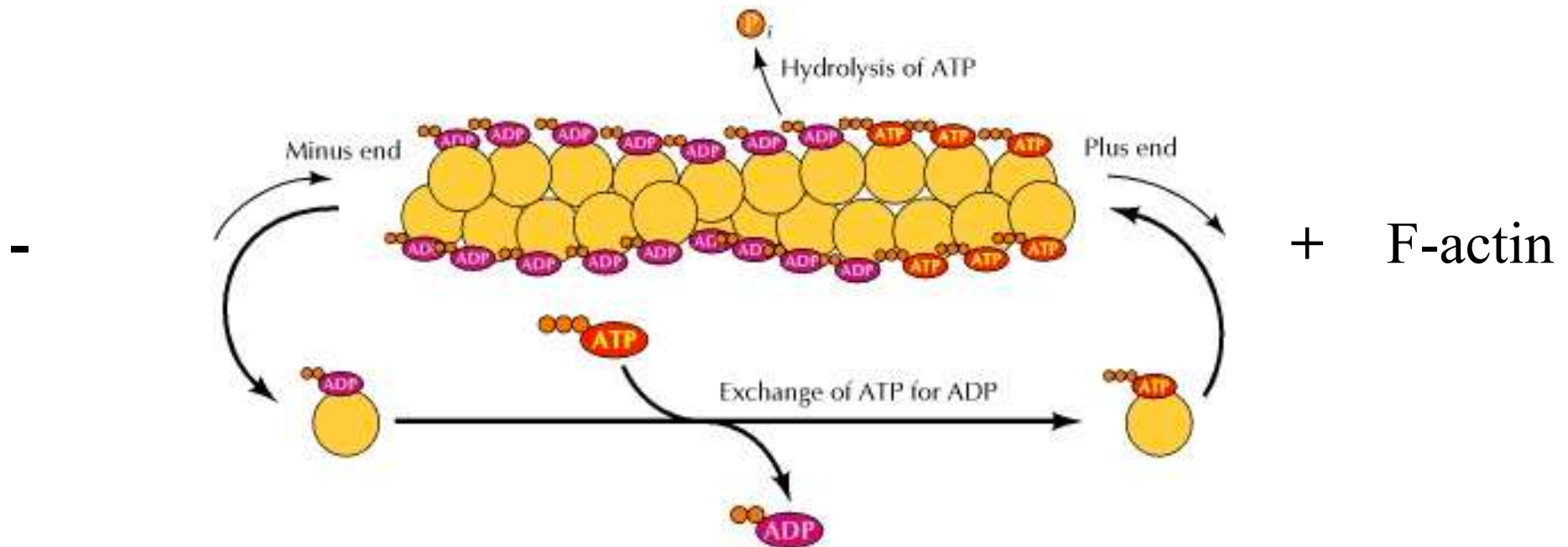
Actin filaments are polar

Thin Filaments are composed of two chains of globular subunits, G-actin, coiled around each other to form a filamentous protein, **F-actin**.

Thin filaments are 6-7 nm thick and possess a faster growing **plus end** and a slower-growing **minus end**.

**Capping proteins** attach to the plus end, terminating the lengthening of the filaments

# Thin Filaments or Microfilaments – Treadmilling

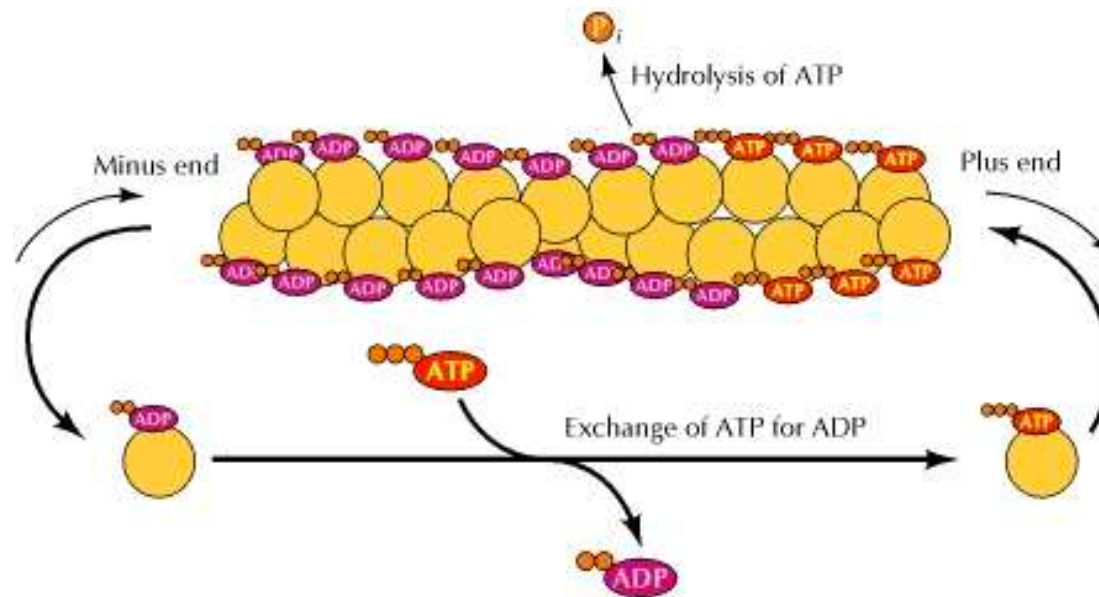


One interesting property of thin filaments is treadmilling. Treadmilling refers to the dynamic process by which actin monomers are added to one end of a filament (known as the "plus end") while simultaneously being removed from the other end (known as the "minus end"). This results in the overall movement of the filament towards the plus end while maintaining a constant length.

The minus ends (*pointed end*) grow less rapidly than the plus ends (*barbed end*) of actin filaments.

**gActin bound to ATP associates with the rapidly growing plus ends, and the ATP bound to actin is then hydrolyzed to ADP.**

# Treadmilling



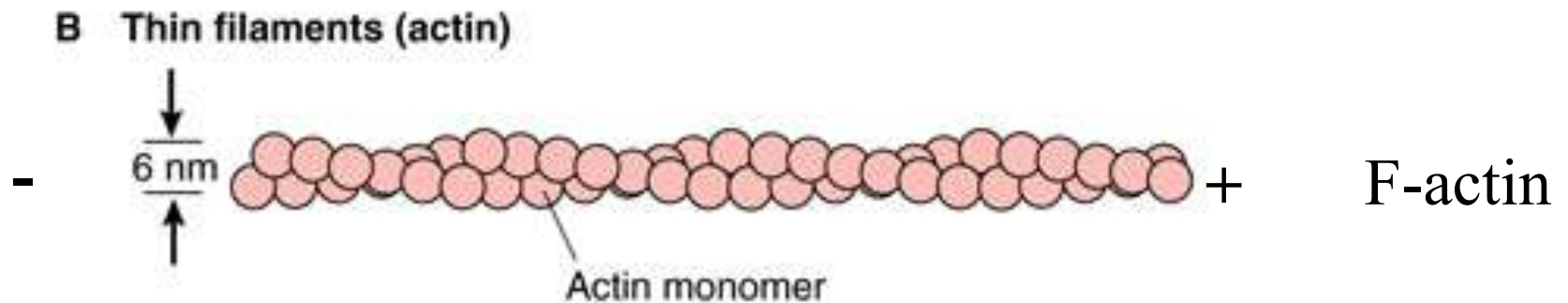
During treadmilling, the addition and removal of actin monomers are regulated by various proteins that bind to the filament ends.

1- the **protein profilin, binds G-actin** and helps to add actin monomers to the plus end

2- while the **protein cofilin, an actin depolymerizing factor** helps to remove actin monomers from the minus end, this replenishes the pool of G-actin for subsequent network assembly.

- When the actin filament reaches the desired length, member of a family of *capping proteins* (such as **gelsolin**), attach to the plus-end, terminating the lengthening of the filament.
- The cell membrane poly-phospho-inositide removes the actin cap gelsolin, permitting elongation of the actin filament.

# Thin Filaments or Microfilaments



There are 3 classes of actin:

- a-actin → muscle
- b-actin and gamma actin → no muscle cells

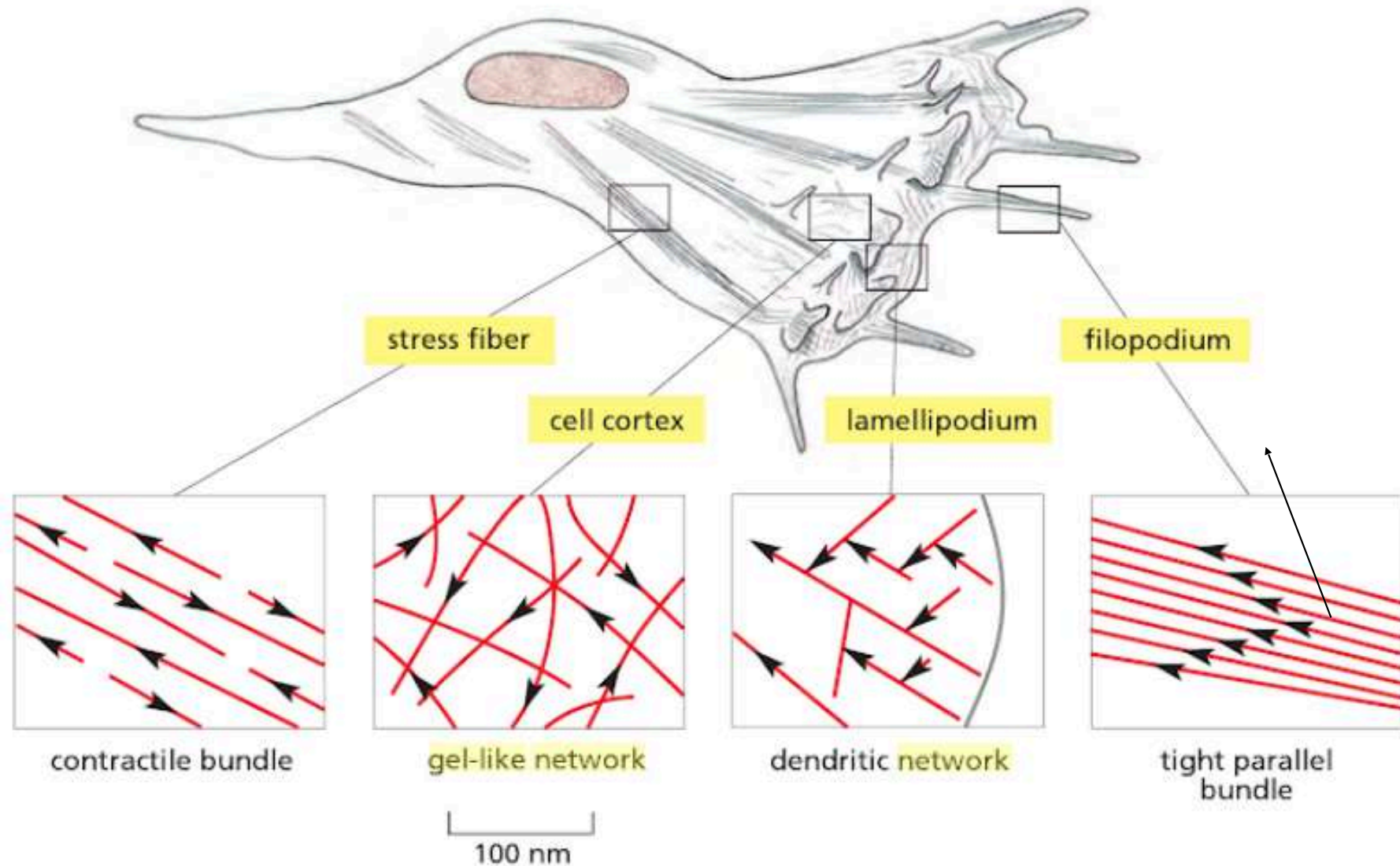
Responsible for motility via association with different actin binding protein. The most commonly known is **myosin**.

**Gelsolin** is a cap protein, stabilizing the end of the filaments

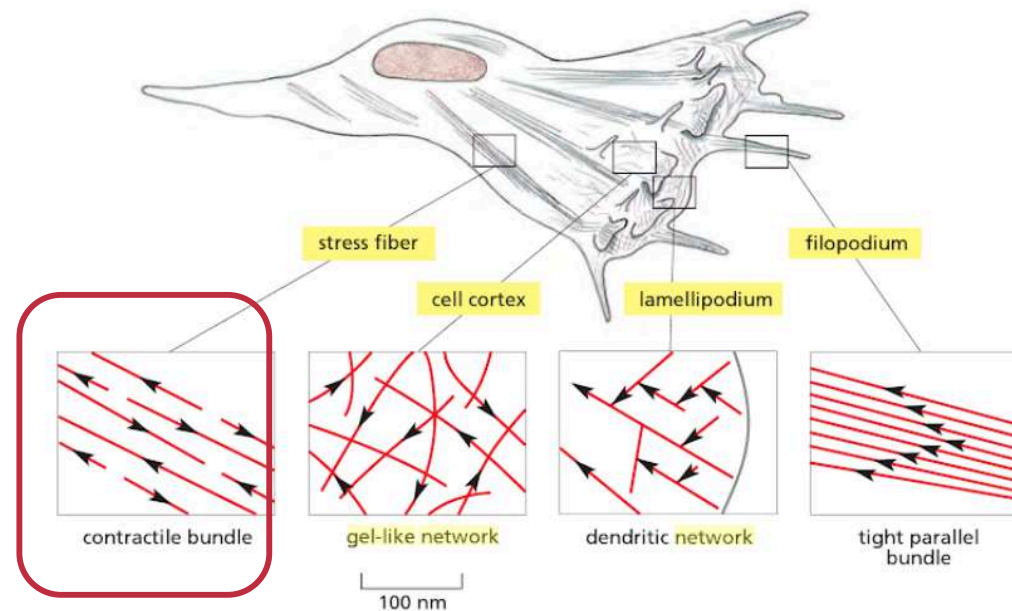
**Cofilin**: induces depolymerization by the removal G-actin at the minus end.

# Thin Filaments or microfilaments

branching F-actin into the leading edge: lamellopodia involved into motility and interaction with other cells

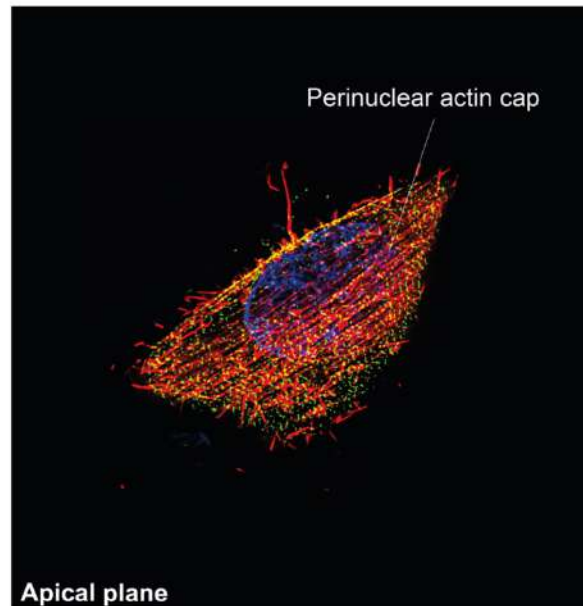
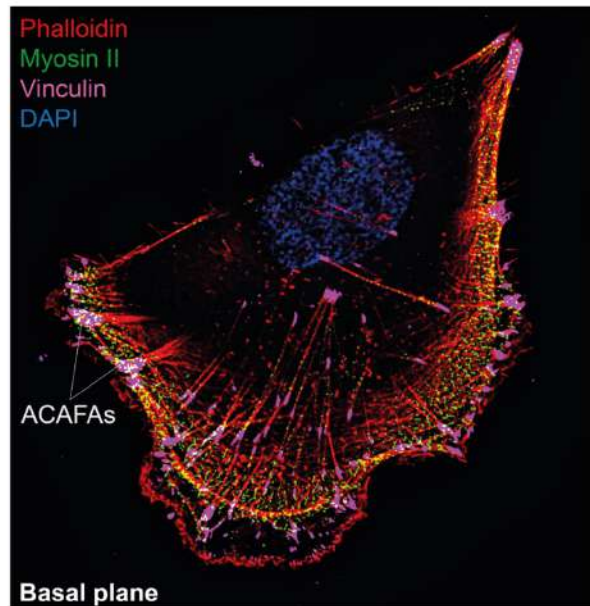
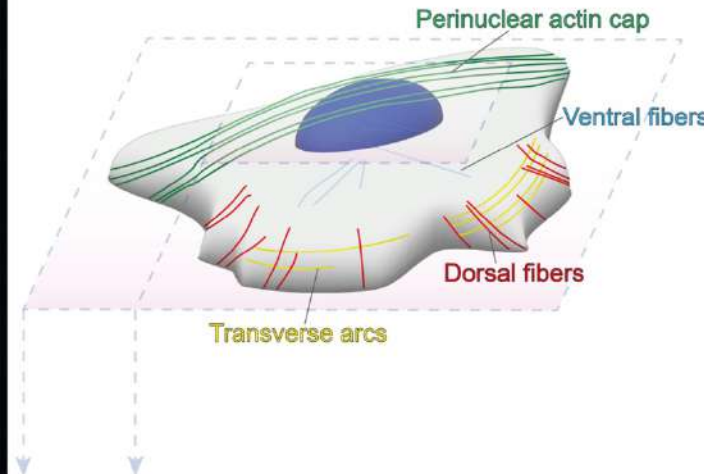
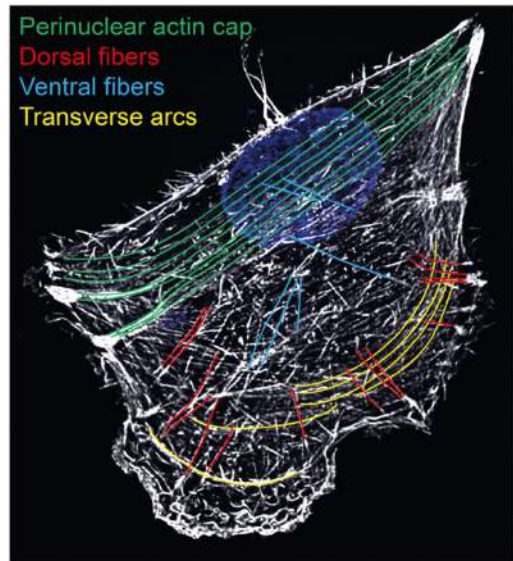


# Contractile bundles



- Are associated with myosin
- The actin filaments are arranged loosely, parallel to each other, with plus and minus end alternating in direction
- Role: organelles and vesicles movements within the cells
- Endo and Exocytosis
- Contractile process and muscle contraction

# Spatial organization of actin stress fibers in adherent cells.



Super resolution images of Normal cells stained with phalloidin highlights distinct stress fiber subtypes (color-coded): dorsal stress fibers, transverse arcs, ventral stress fibers and the perinuclear actin cap (**top left**).

A schematic 3D representation of the same cell illustrates their spatial arrangement within the cell body (**top right**).

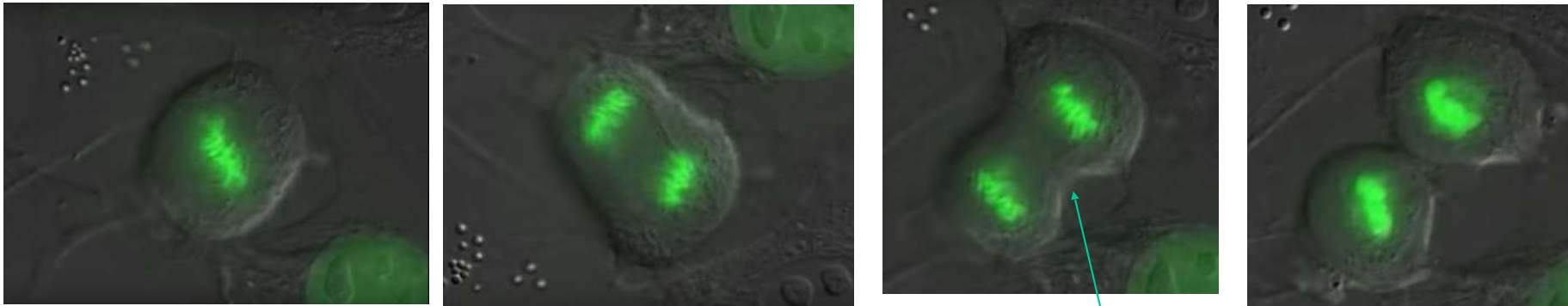
Basal (**bottom left**) and apical (**bottom right**) planes show actin together with myosin II and vinculin, highlighting actomyosin contractility and adhesion-associated structures, including actin cap-associated focal adhesions (ACAFA). Nuclei are counterstained with DAPI.

# Thin Filaments or Microfilaments

Form contractile bundles responsible for the formation of contractile rings during mitotic division, associated with myosin.

<https://www.youtube.com/watch?v=AG0kYOLYzhA>

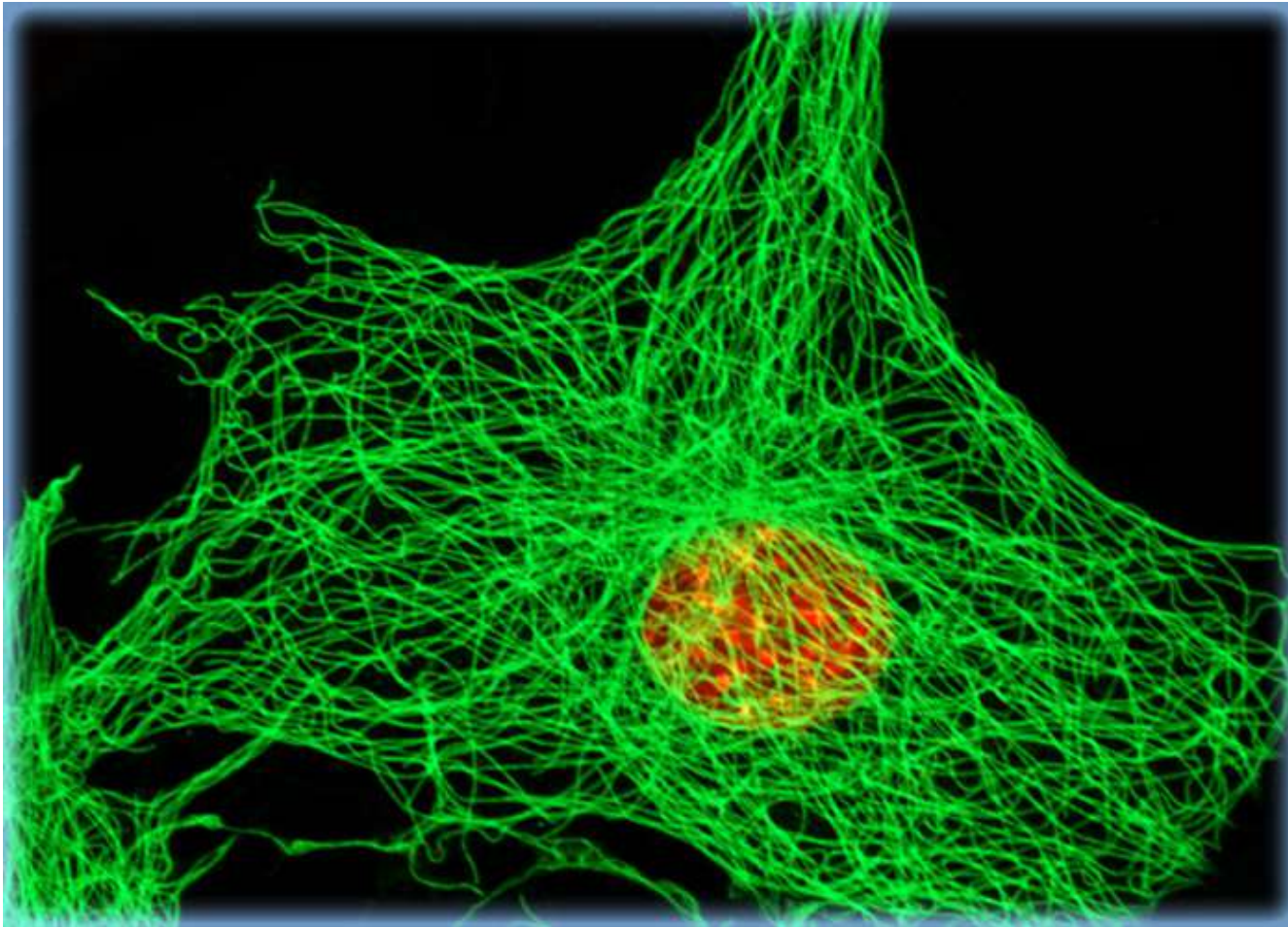
[https://www.youtube.com/watch?v=AG0kYOLYzhA&ab\\_channel=RateMyScience](https://www.youtube.com/watch?v=AG0kYOLYzhA&ab_channel=RateMyScience)



Contractile ring

**Martin Chalfie Nobel Prize 2008-GFP**

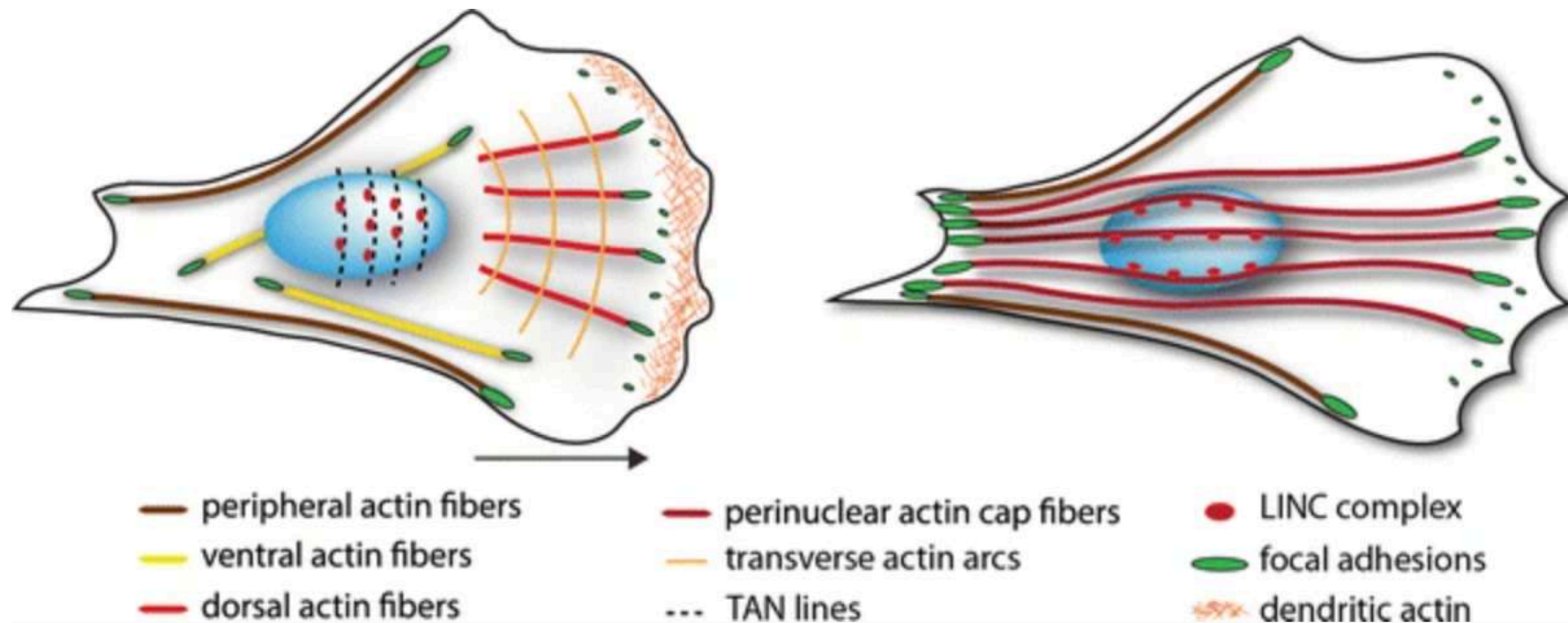
# Spatial organization of actin stress fibers in adherent cells



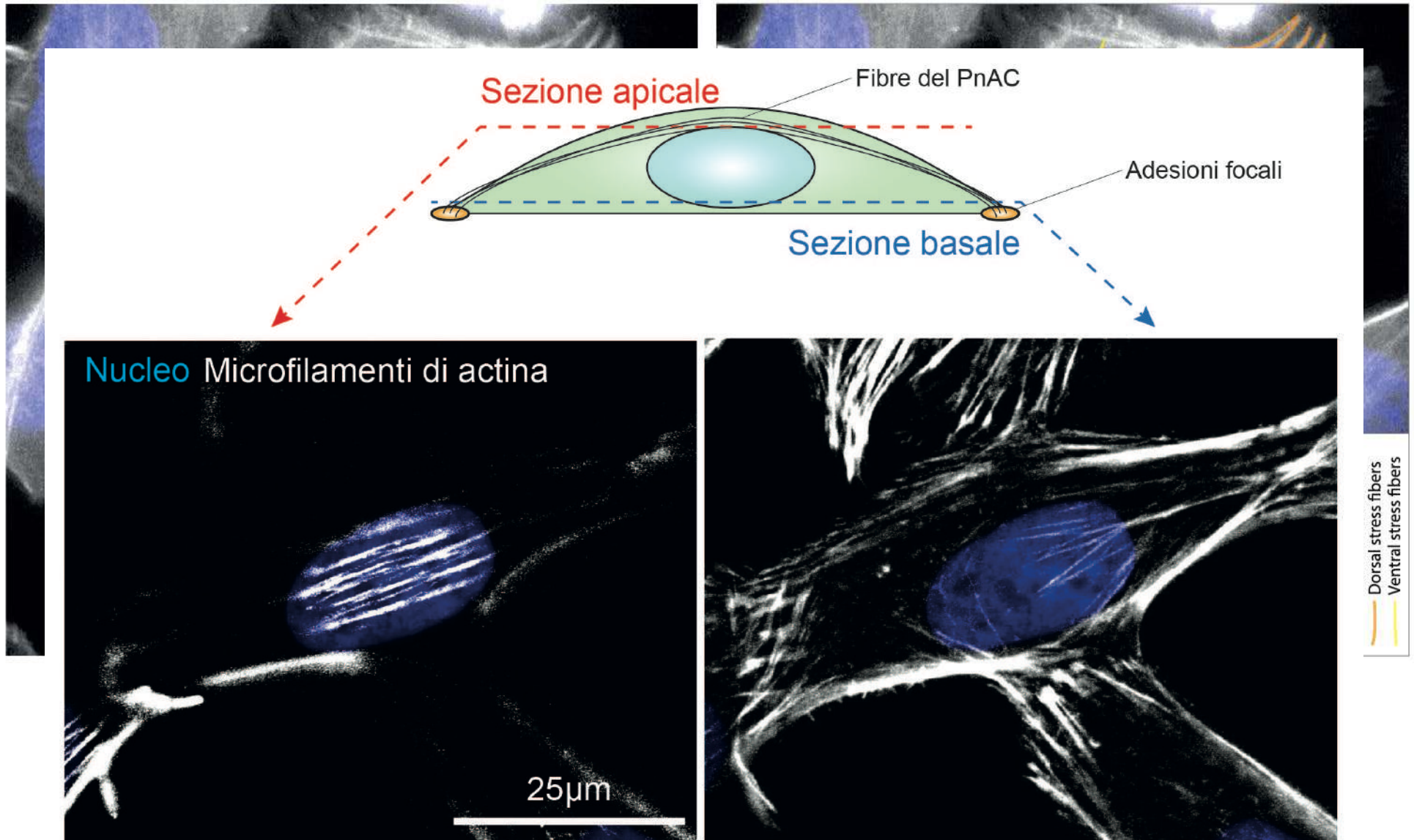
Ventral or basal actin stress fibers

# The assembly and function of perinuclear actin cap in migrating cells

Polarized cells display a conical shape with predominantly convex leading edge and concave cell sides. The nucleus (*blue*) is located at the cell rear with **longer nuclear axis aligned with the direction of cell migration** (*black arrow*). Actin forms different stress fibers that vary in their location and in the attachment to focal adhesions.



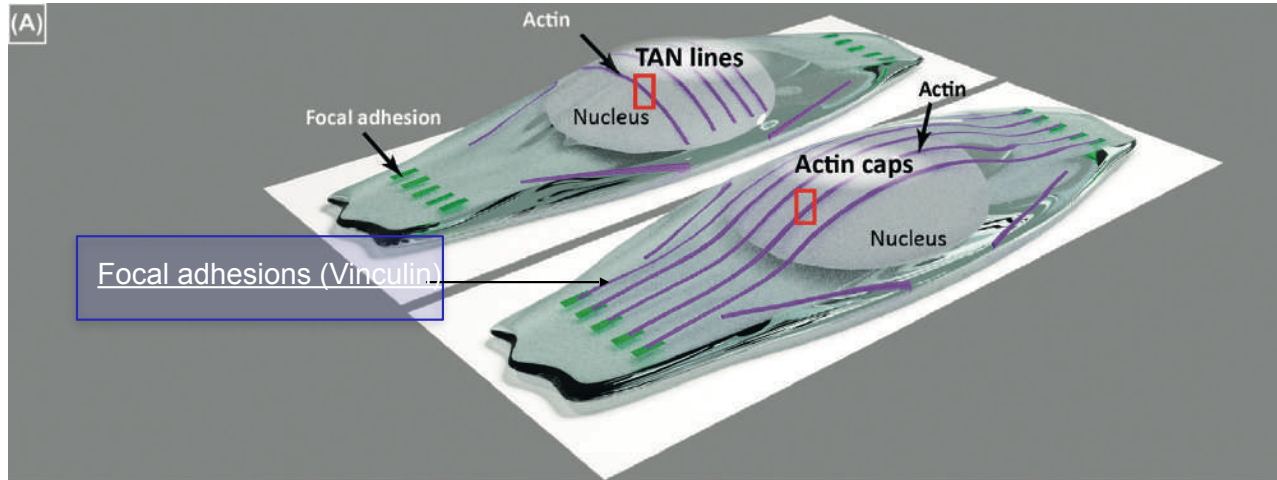
# Stress Fibers



a. Perinuclear actin cap (PnAC)

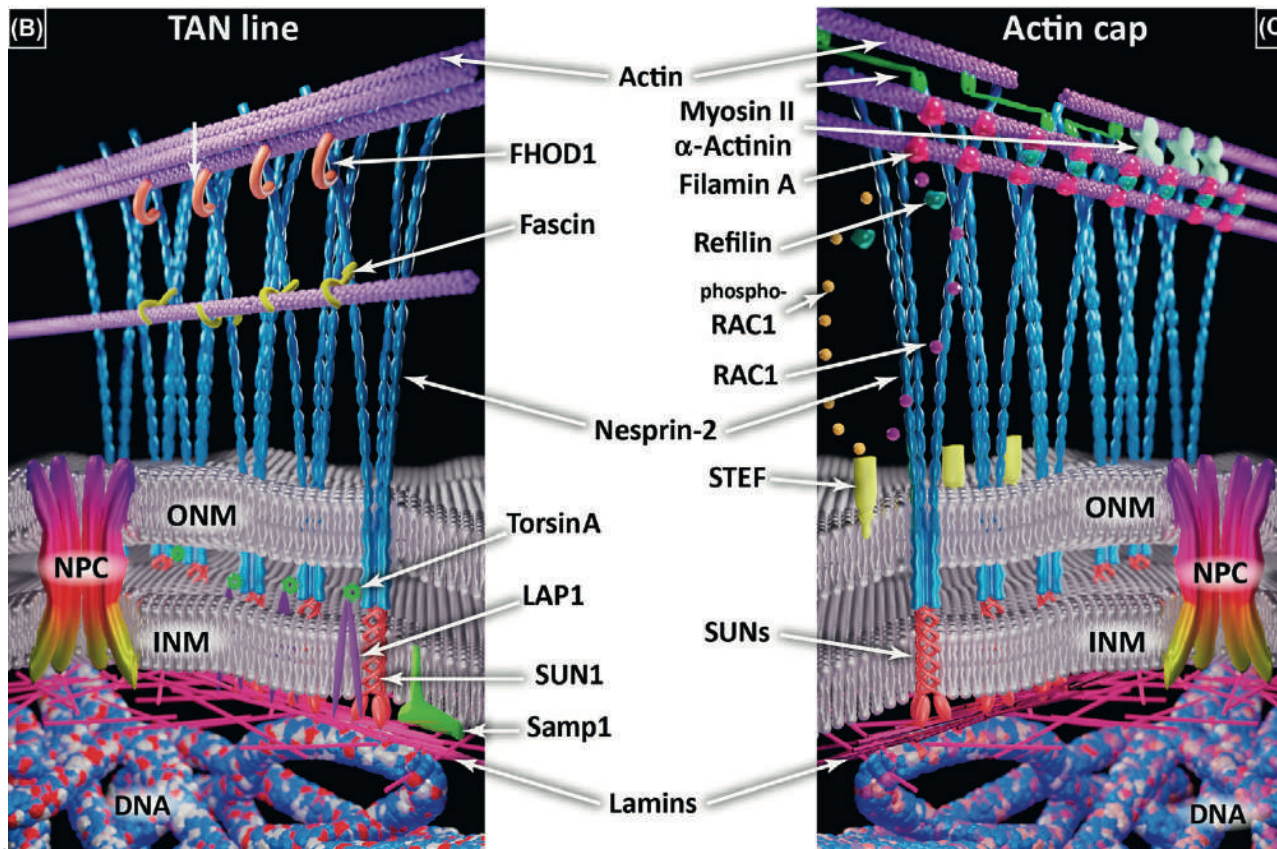
b. Stress fibers basali

# Actin cap known partners



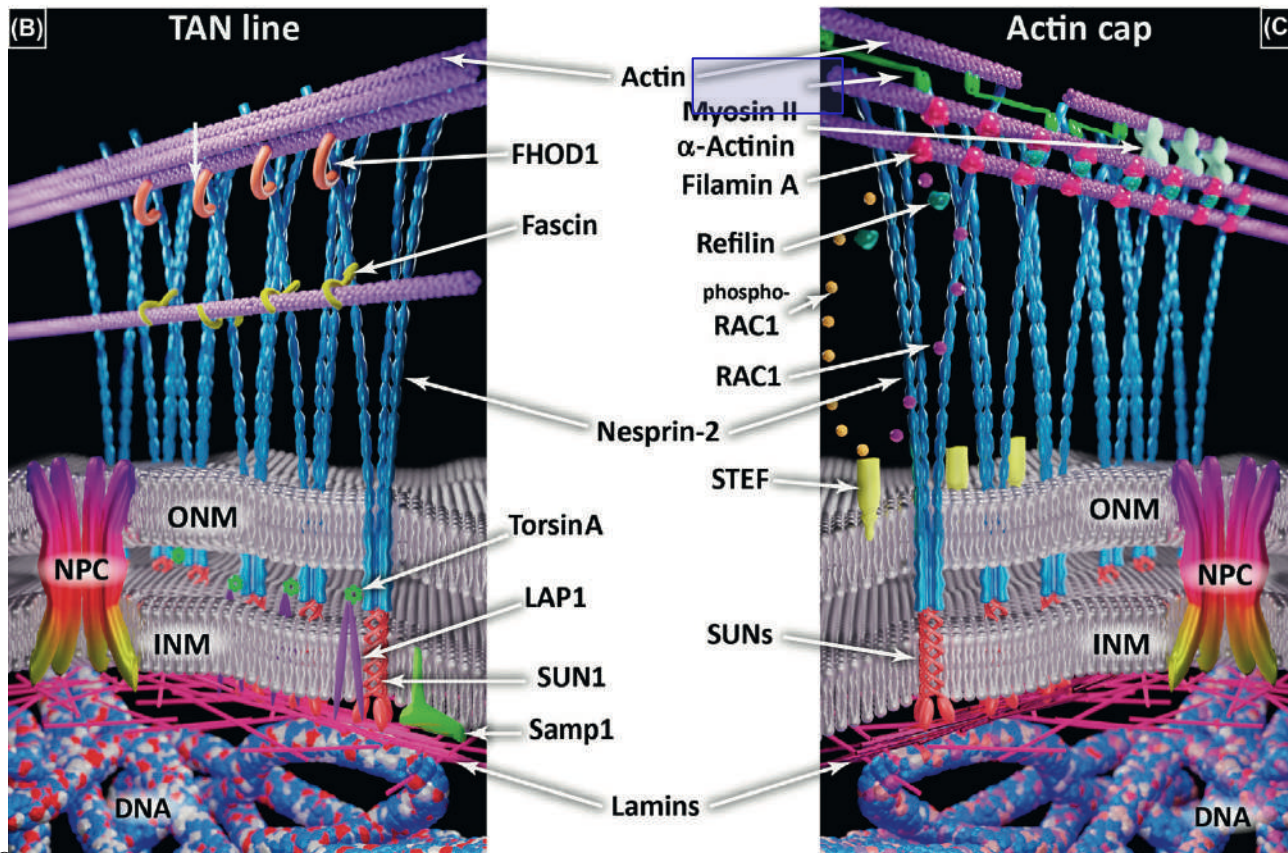
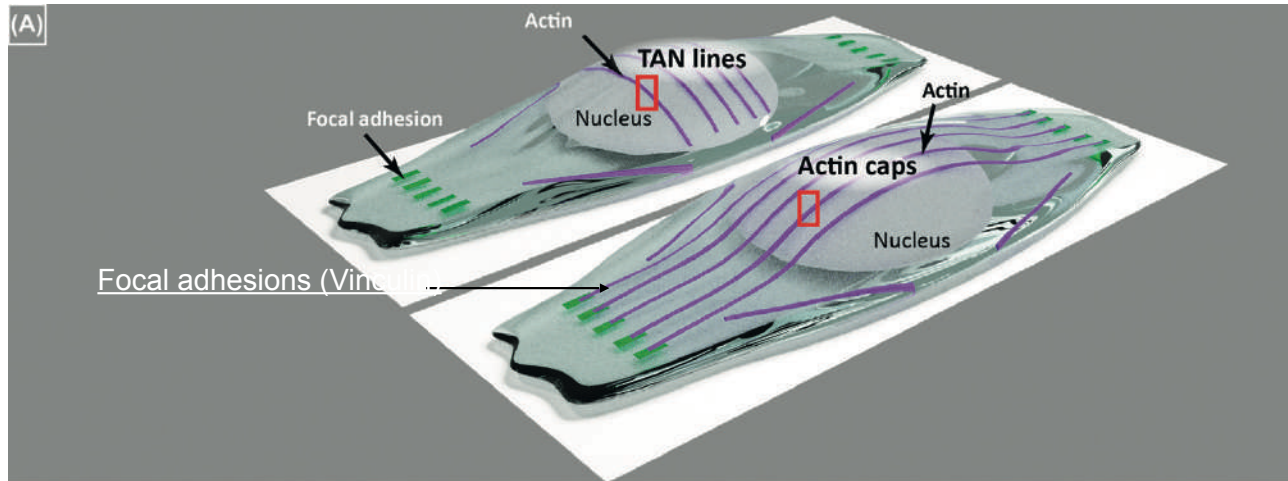
**Vinculin:** cytoskeletal protein associated with cell-cell and **cell-matrix junctions.**

it functions as one of several interacting proteins involved in anchoring F-actin to the membrane



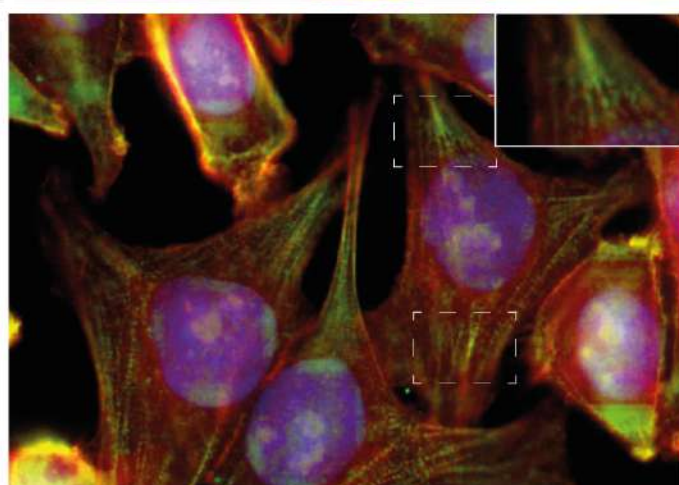
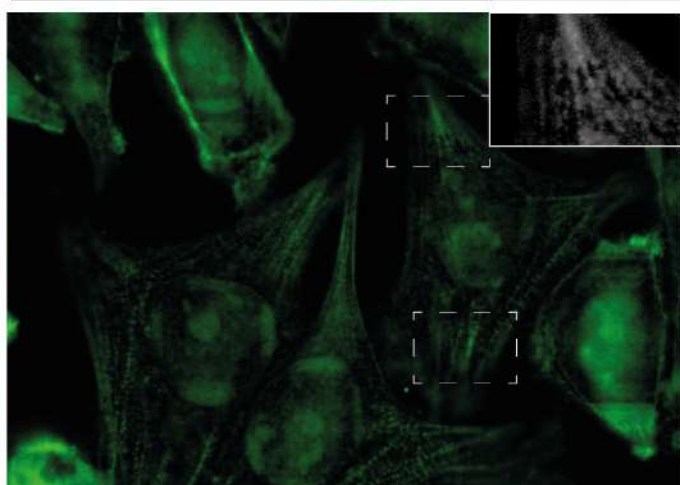
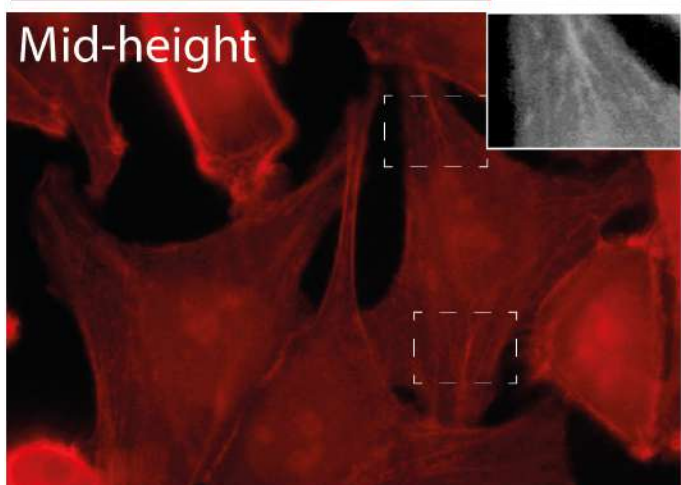
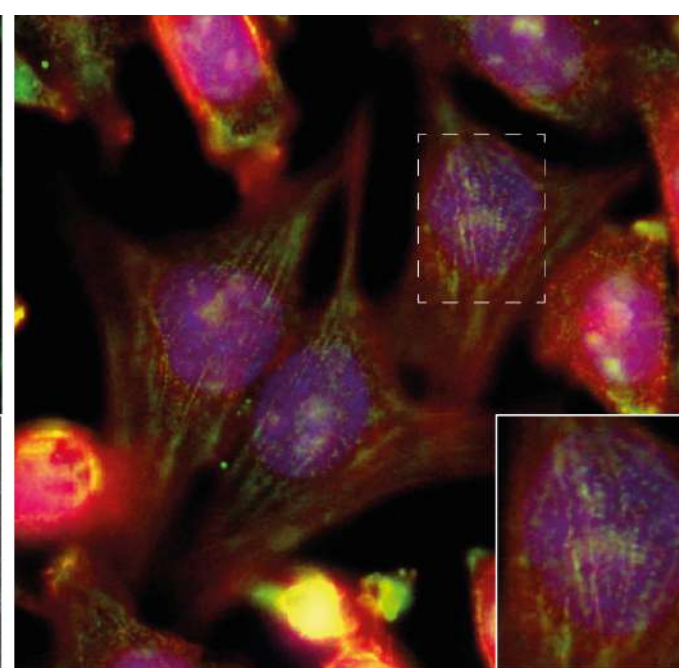
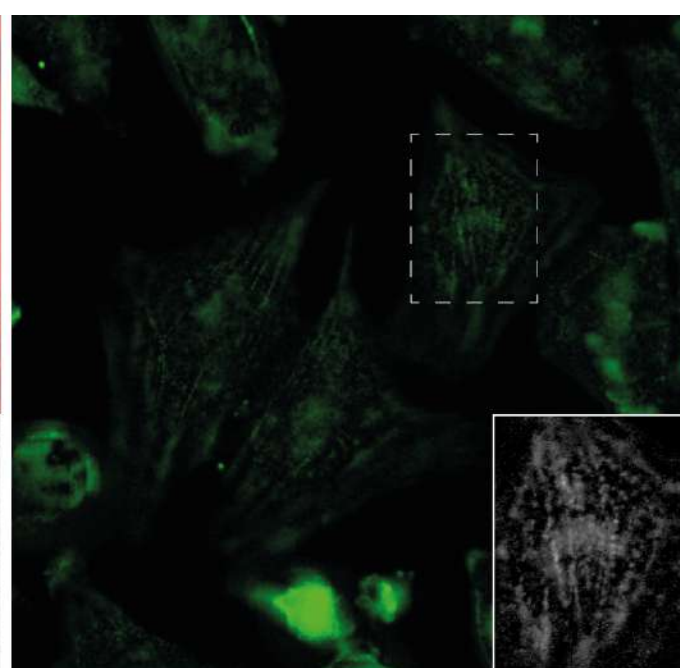
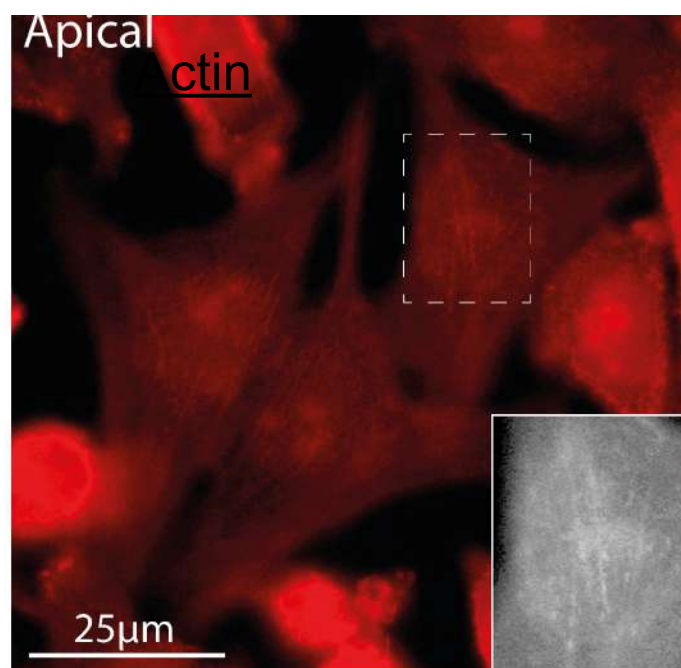
Davidson and Cadot «Actin on and around the Nucleus»  
Trends in Cell Biol, 2021

# Actin cap known partners

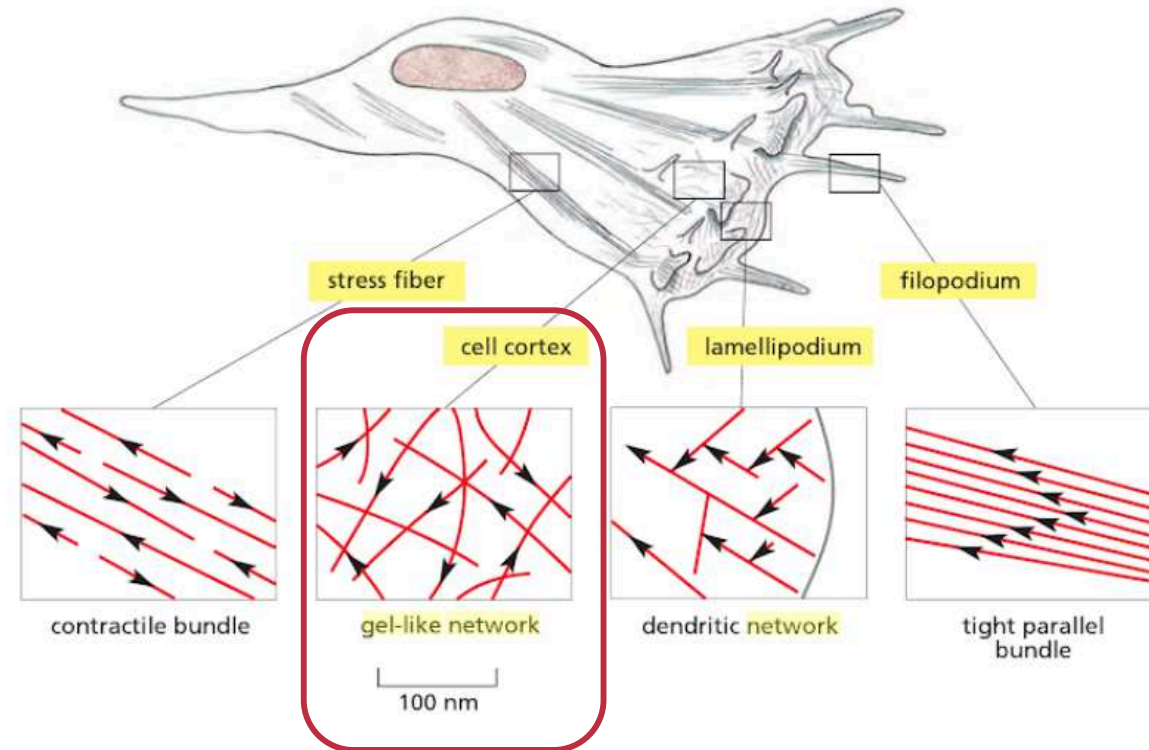


**Myosin II:** non-muscle myosin involved in cell motility, adhesion and cytokinesis → provides contractility to stress fibers

Davidson and Cadot «Actin on and around the Nucleus»  
Trends in Cell Biol, 2021



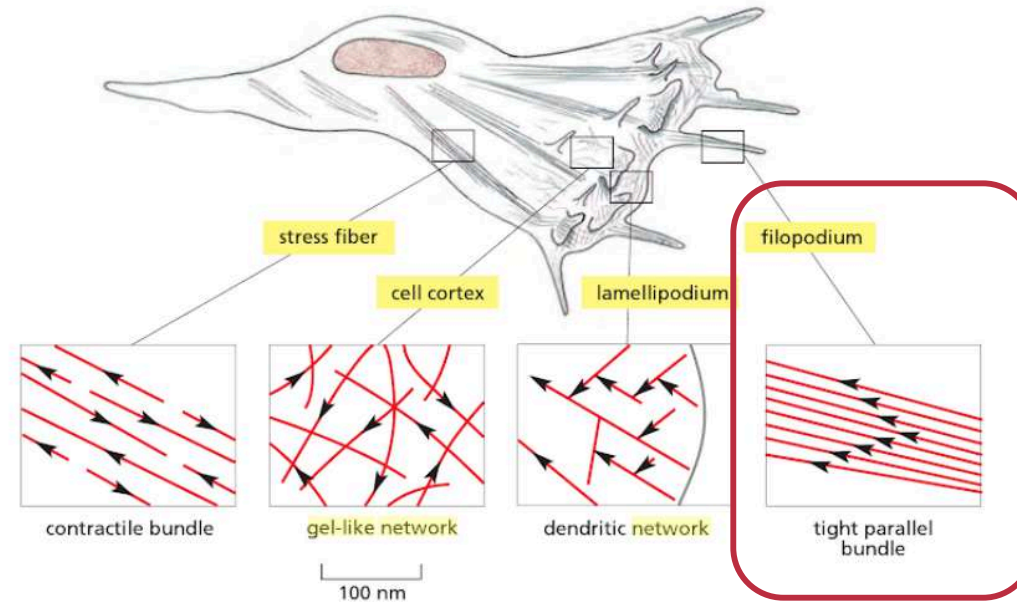
# The gel-like network



The gel-like network organization of actin filaments which form a cross-linked network similar to a gel.

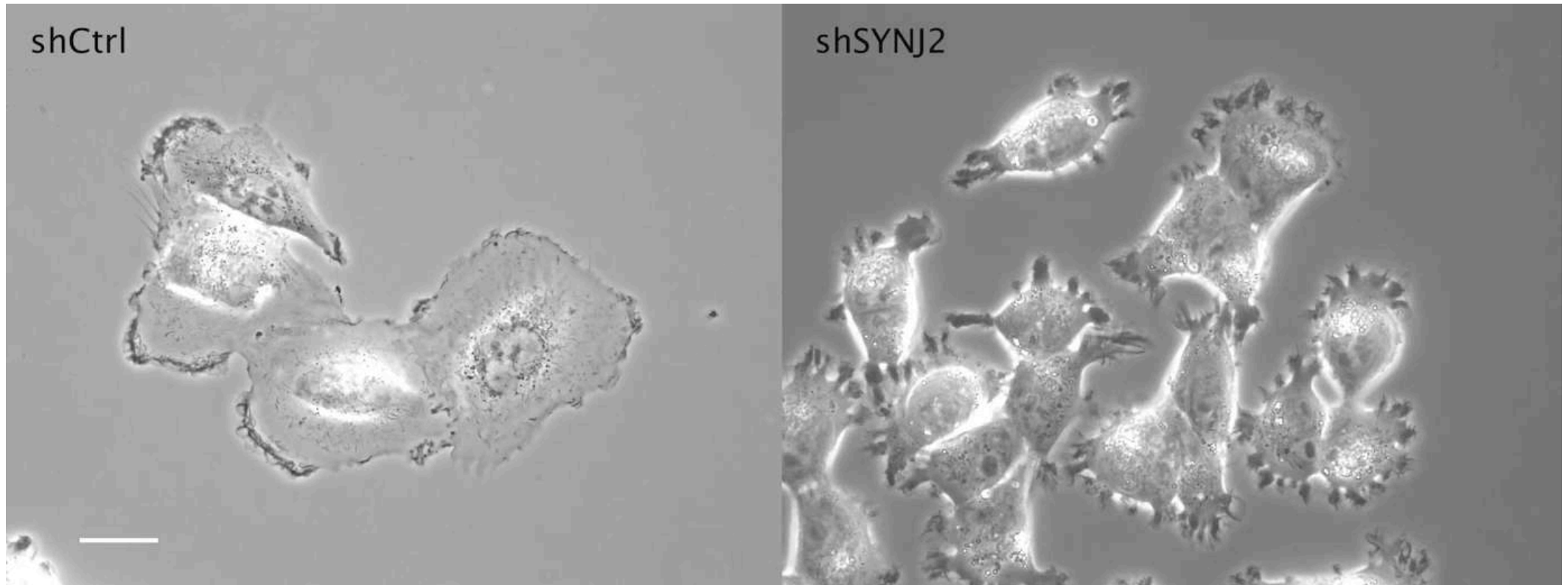
The stiffness is due to the protein **filamin**, that assists the formation of the actin network forming high-viscosity matrices such as those of the cell cortex

# Parallel Bundles

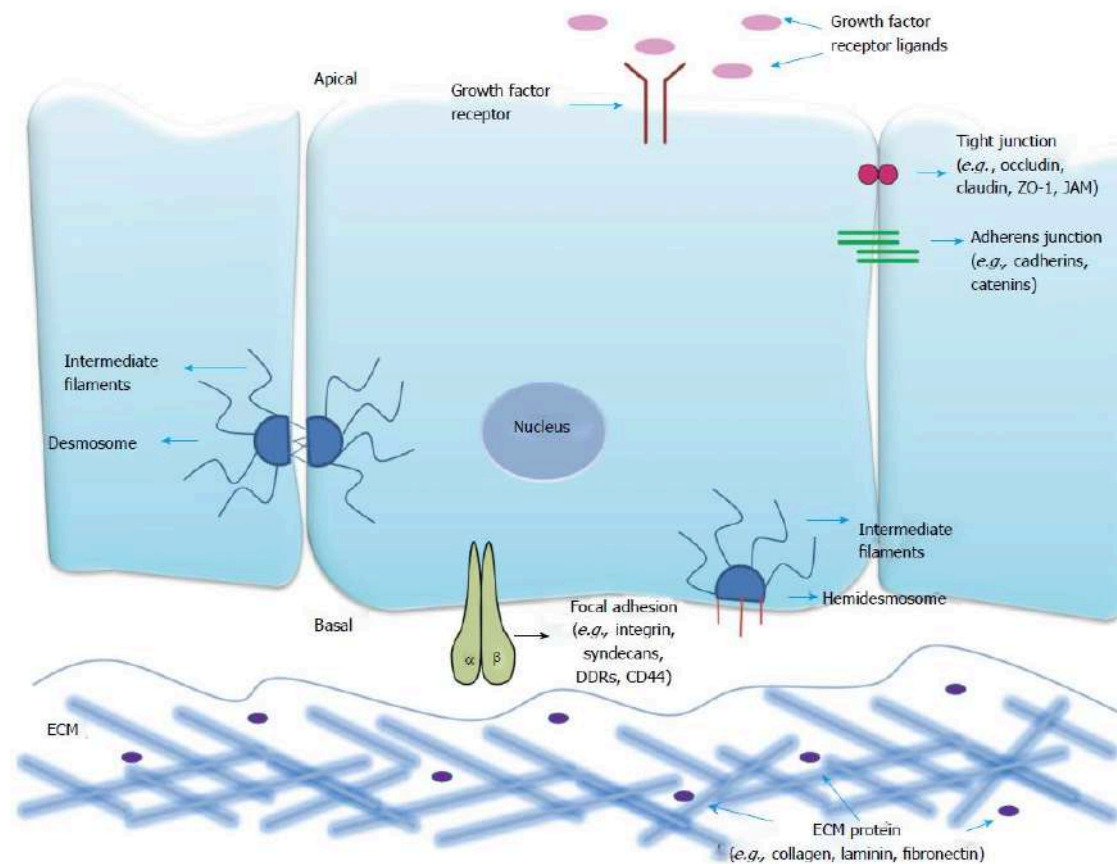


Are thin filaments associated with the proteins **fimbrin** and **villin**, which are responsible for forming closely packed parallel bundles of actin that form the core of microspikes (**filopodium**) and **microvilli**. These bundles are anchored to the terminal web - a region of the cortex composed of the network of intermediate filaments and the protein spectrin.

# Thin-Filaments in filopodia and lamellopodia in cell migration



# Actin in focal contacts

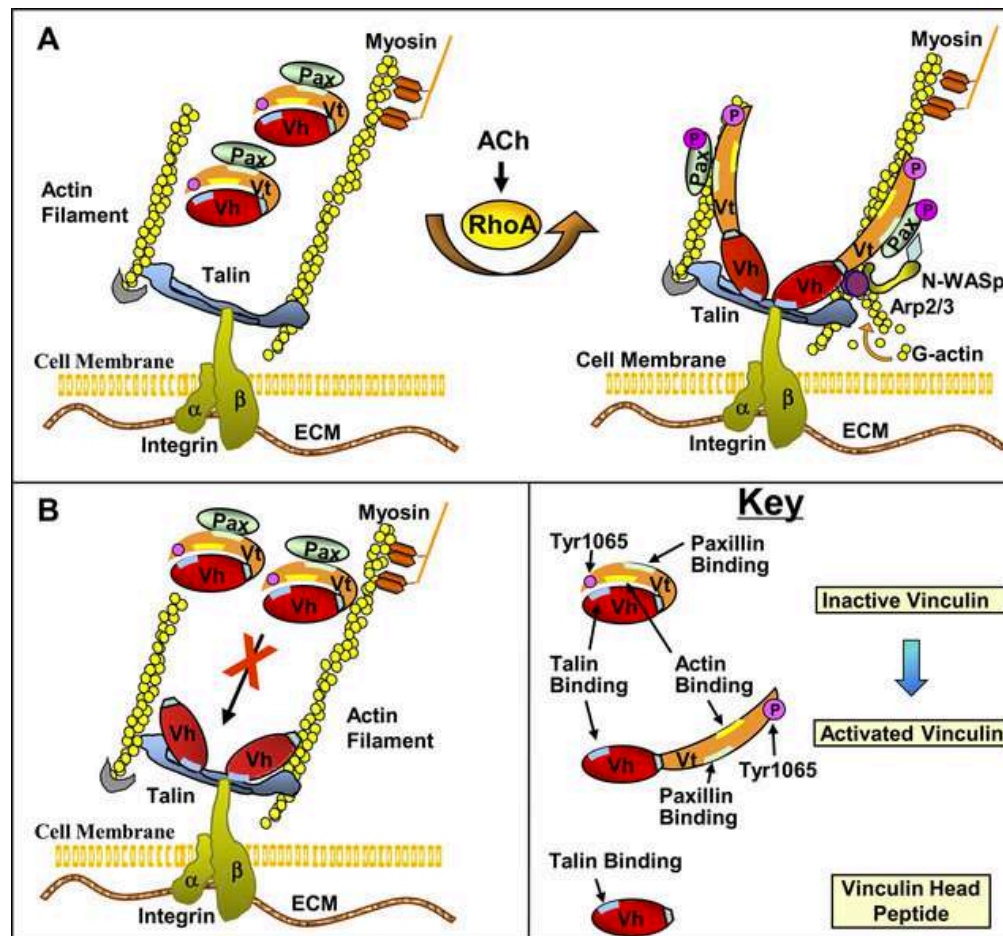


Actin is important in the **establishment of focal contacts** of the cells with the extracellular matrix.

At the focal contacts the transmembrane molecules integrins bind glycoproteins such as fibronectin in the ECM, permitting the cells to maintain the attachment.

# Actin in focal contacts

The intracellular region of the integrins contact the cytoskeleton and binds the **Talin**, which contacts both **actin filaments** and **vinculin**, forming contractile bundles, known as stress fibers.

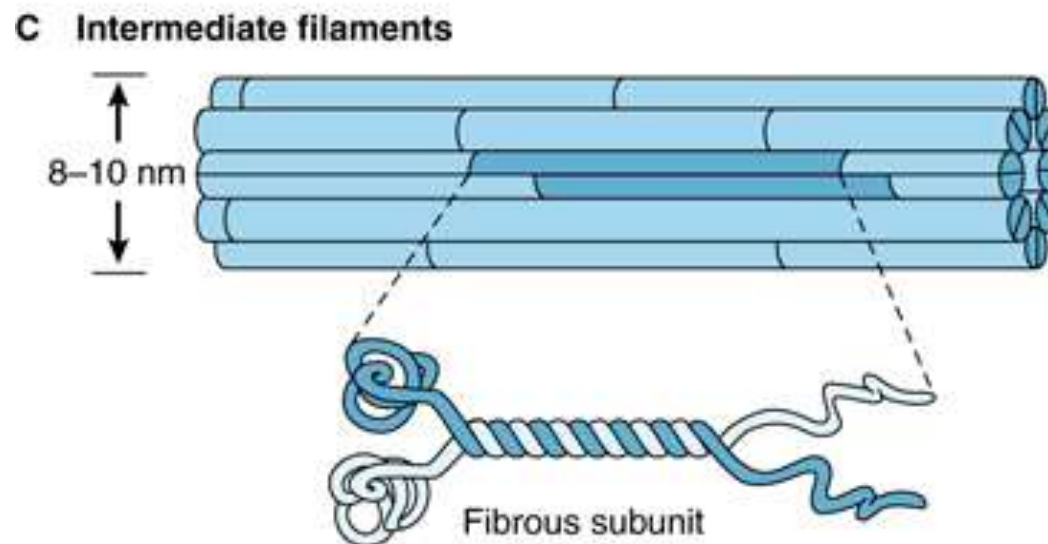


# Intermediate filaments 8-10nm

Intermediate filaments and their associated proteins assist in the establishment and maintenance of the three-dimensional framework of the cell

These filaments and their associated proteins:

- Provide structural support for the cell
- Form a deformable 3D structural framework for the cell
- Anchor the nucleus in place
- Provide an adaptable connection between the cell membrane and the cytoskeleton
- Maintenance of the nuclear envelope as well as its reorganization subsequent to mitosis



# Main function:

Protect the structural and functional integrity of the cells from external stresses by forcing modulations in the shape and location of cellular constituents

# Intermediate filaments

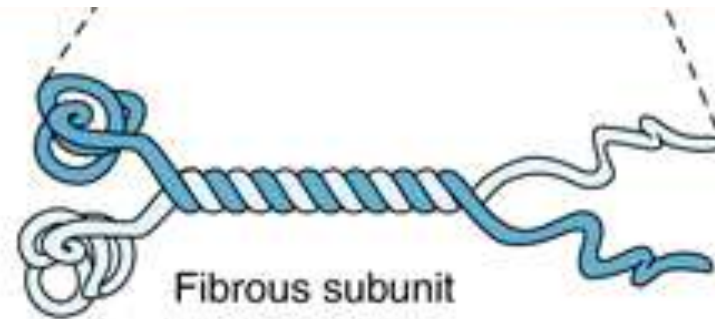
They are so named because their diameter is intermediate between the smaller actin filaments and the larger microtubules.

Intermediate filaments are found in the cells of most animal tissues, where they form a network of fibers that help to maintain the shape and integrity of the cell.

One of the important functions of intermediate filaments is to provide mechanical strength to cells and tissues. For example, in epithelial tissues such as the skin, keratin intermediate filaments provide structural support to the cells and help to prevent damage from mechanical stress or abrasion

# Intermediate filaments

The Intermediate Filaments are rope-like filaments that are constructed of 8 tetramers of proteins bundled into helices.



Each monomer has N-terminus (head) and C-terminus (tail), both folded into globular domains

## The categories of intermediate filaments

- Keratins → specific for epithelial cells
- Desmin → specific of all type of muscle cells
- Vimentin → specific of cells of mesenchymal origin, fibroblast and endothelial cells
- Neurofilaments → specific of neurons
- Nuclear Lamins

**TABLE 2-4** Predominant Types of Intermediate Filaments

Type	Filament	Polypeptide Component Size (Da)	Cell Type	Function
I	Acidic keratins	40,000–70,000	Epithelial cells	Support cell assemblies and provide tensile strength to cytoskeleton
II	Basic keratins	40,000–70,000	Cells of hair and nails	
III	Desmin	53,000	All types of muscle cells	Links myofibrils in striated muscle (around Z disks); attaches to cytoplasmic densities in smooth muscle
III	Vimentin	54,000	Cells of embryo as well as cells of mesenchymal origin: fibroblasts, leukocytes, endothelial cells	Surrounds nuclear envelope; is associated with cytoplasmic aspect of nuclear pore complex
III	Glial fibrillary acidic protein (GFAP)	50,000	Astrocytes, Schwann cells, oligodendroglia	Supports glial cell structure
IV	Neurofilaments		Neurons	Form cytoskeleton of axons and dendrites; assist in the formation of the gel state of the cytoplasm; cross-linking responsible for great tensile strength
	NF-L: low-molecular-weight	68,000		
	NF-M: medium-molecular-weight	160,000		
	NF-H: high-molecular-weight	210,000		
IV	Syncoilin	64,000	Skeletal muscle cells	Forms bonds with dystrobrevin in skeletal muscle
V	Nuclear lamins	65,000–75,000	Lining nuclear envelopes of all cells	Control and assembly of the nuclear envelope; organization of perinuclear chromatin
	A			
	B C			
VI	Phakirin and filensin	49,000	Lens fiber cells of the eyeball	Sustain the transparency of the lens
		94,000		

# Critical Thinking Question

## Tumor of Unknown Primary

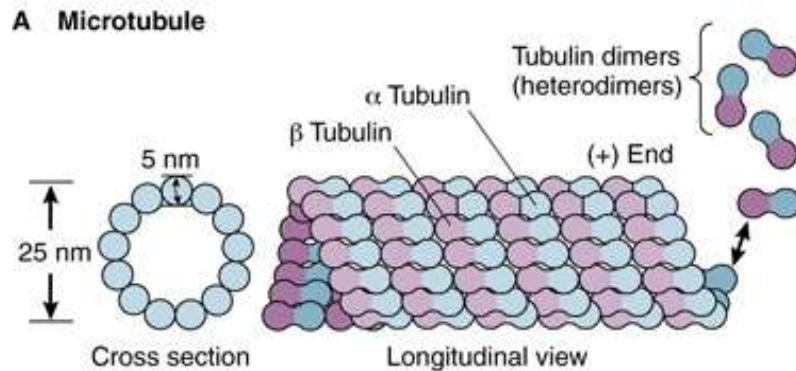
About 5-10% of cancer patients suffer from cancer of unknown primary (CUP).

The origin of these tumors assists not only in the diagnosis but also in devising effective treatment plans

Evaluation of.....

# Microtubules – 25 nm

- Long, straight and rigid cylindrical structures 25nm.
- Provides rigidity and maintains cell shape
- Regulate intracellular movement of organelles and vesicles
- Establish intracellular compartments
- Provide the **ciliary motion**



Monomers of tubulins (Alpha and Beta) are scattered into the cells, by forming heterodimers.

Microtubules are polarized having a plus end (**beta tub**) and a minus end (**alpha tub**) The minus end depolymerizes, thus shortening the microtubules.

# Microtubules – 25 nm

- The region where the gamma-tubulin ring complex begins the nucleation process is called **CENTROSOME**
- Centrosome is considered the **MICROTUBULES ORGANIZING CENTER** of the cells (MTOC)
- Microtubules are polarized, having a plus end (beta-tubulin) and minus end (alpha tubulin). MT are dynamic structures that change their length by undergoing growth burst and then become shorter.
- Alpha/beta tubulin heterodimers are assembled into protofilaments

# Microtubules

Microtubules make up two types of cellular appendages important for motion:  
cilia and flagella.

- **Cilia** are found on many cells of the body, including the epithelial cells that line the airways of the respiratory system. Cilia move rhythmically; they beat constantly, moving waste materials such as dust, mucus, and bacteria upward through the **airways**, away from the lungs and toward the mouth. Beating cilia on cells in the female fallopian tubes move egg cells from the ovary towards the uterus.
- <https://unibo.smartzoom.com/s1241/course1776/fl1777/i1789/>
- <https://unibo.smartzoom.com/s1241/course1776/fl1777/i1790/>
- A **flagellum** (plural = flagella) is an appendage larger than a cilium and specialized for cell locomotion. The only flagellated cell in humans is the sperm cell that must propel itself towards female egg cells.

# Navigator-3, a modulator of cell migration

NAV3 localizes to the plus ends of microtubules and enhances their polarized growth.

The NAV3 protein augments the ability of cells to adhere to a migration course, while also slowing down migration rates.

Published online: February 12, 2015

*Research Article*



SOURCE  
DATA



TRANSPARENT  
PROCESS



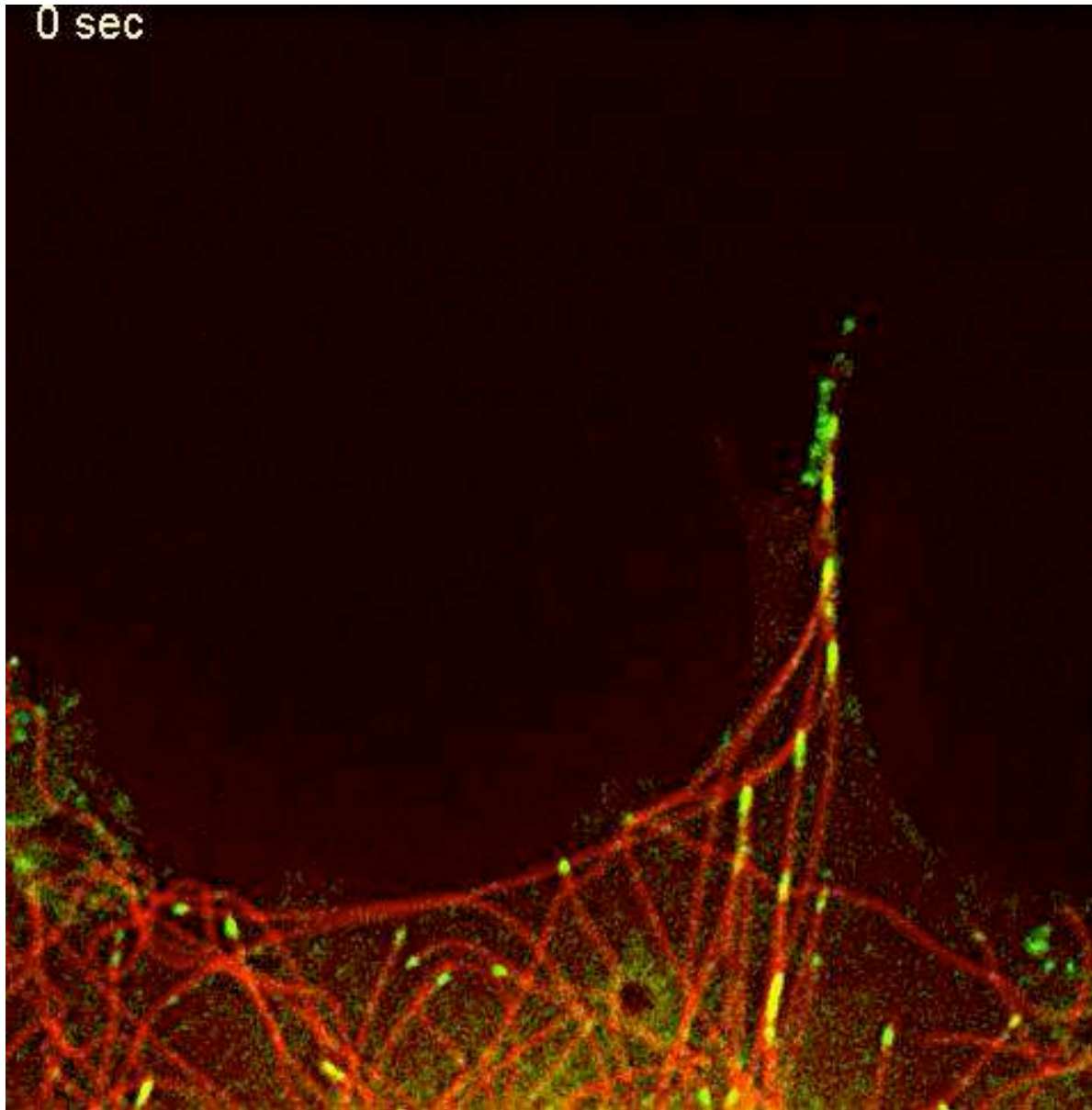
OPEN  
ACCESS

EMBO  
Molecular Medicine

## Navigator-3, a modulator of cell migration, may act as a suppressor of breast cancer progression

Hadas Cohen-Dvashi<sup>1</sup>, Nir Ben-Chetrit<sup>1</sup>, Roslin Russell<sup>2</sup>, Silvia Carvalho<sup>1</sup>, **Mattia Lauriola<sup>1,†</sup>**,  
Sophia Nisani<sup>1</sup>, Maicol Mancini<sup>1</sup>, Nishanth Nataraj<sup>1</sup>, Merav Kedmi<sup>1</sup>, Lee Roth<sup>1</sup>, Wolfgang Köstler<sup>1,†</sup>,  
Amit Zeisel<sup>3</sup>, Assif Yitzhaky<sup>3</sup>, Jacques Zylberg<sup>4</sup>, Gabi Tarcic<sup>1</sup>, Raya Eilam<sup>1</sup>, Yoav Wigelman<sup>1</sup>, Rainer  
Will<sup>5</sup>, Sara Lavi<sup>1</sup>, Ziv Porat<sup>6</sup>, Stefan Wiemann<sup>5</sup>, Sara Ricardo<sup>7</sup>, Fernando Schmitt<sup>7</sup>, Carlos Caldas<sup>2</sup> &  
Yosef Yarden<sup>1,\*</sup>

# Co-expression of GFP-NAV3 and mCherry- $\alpha$ -tubulin demonstrated dynamic tracking of the tips of growing MTs by NAV3

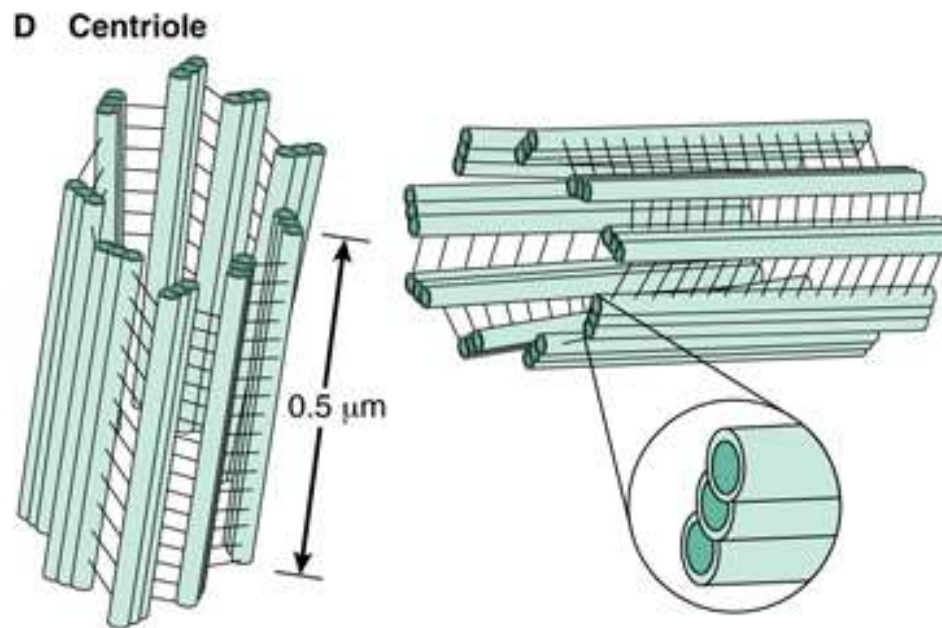


NAV3 marks the tips  
of the growing MT

# Centrioles

Centrioles are small, cylindrical structures, 0.2  $\mu\text{m}$  in diameter and 0.5  $\mu\text{m}$  in length.

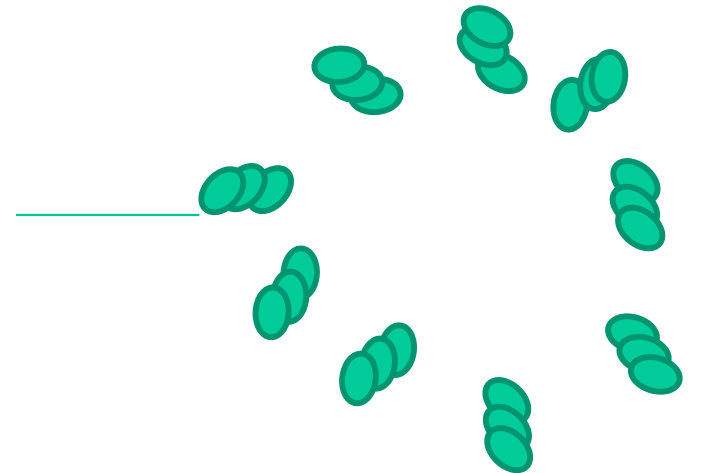
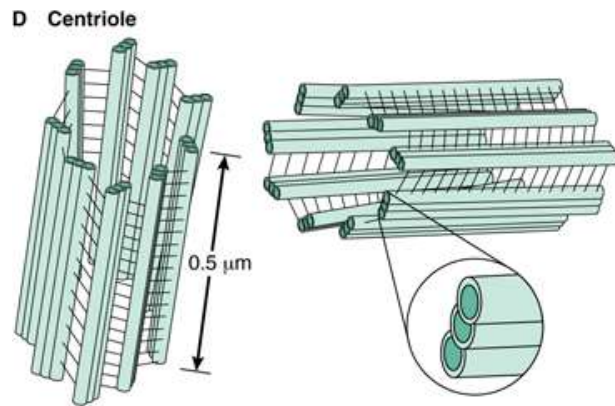
Centrioles are paired structures, arranged perpendicular to each other and are embedded in a matrix of pericentriolar material (Gamma Tub and pericentrin both interacting with the microtubules and anchoring them to the centrosome)



# Centrioles

Are composed of nine triplets of microtubules arranged around a central axis.

Each triplet is arranged so that it forms an oblique angles with the adjacent and a straight angle ( $90^\circ$ ) with the fifth triplet



During the S phase of the cell cycle, each centriol of the pair replicates, forming a procentriol in some unknown manner

# Microfilaments

**Select one:**

- a. are 6-7 nm thick
- b. are composed of two chains of globular subunits
- c. All of them
- d. are actin filaments that interact with myosin to allow cellular movement
- e. possess a faster growing plus end and a slower-growing minus end

# The protein filamin associates to:

**Select one:**

- a. Tubulin
- b. All of them
- c. Myosin II
- d. Membrane
- e. Gel-like networks

Select the wrong answer: Contractile bundles are

**Select one:**

- a. Are relevant for cell metabolism
- b. Are relevant for organelles and vesicles movements within the cells
- c. Are relevant for cell division
- d. Are relevant for cell proliferation
- e. Are relevant for cell migration