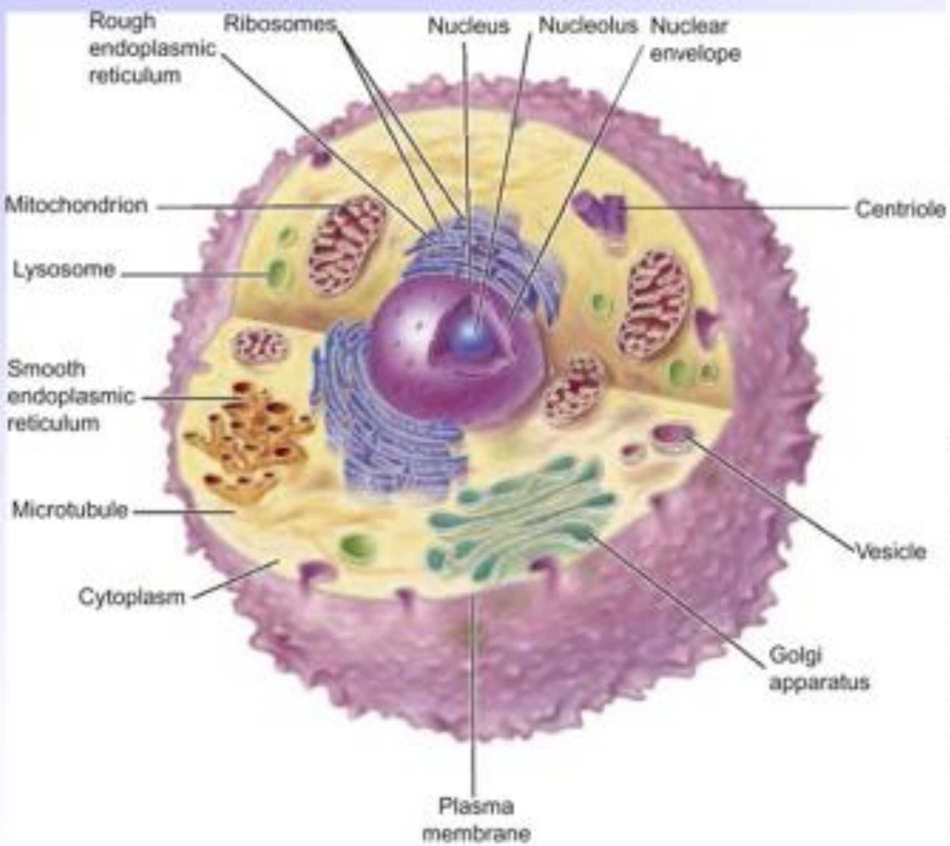
University of Teramo

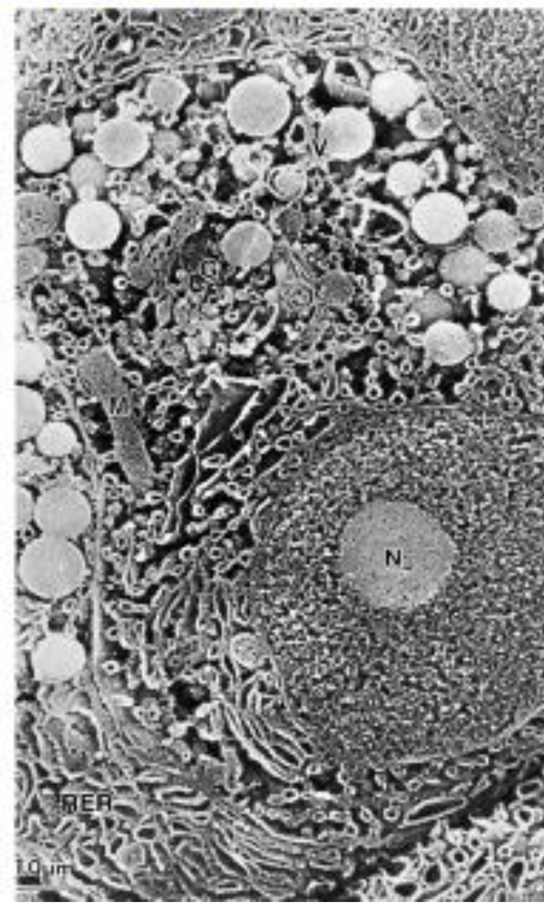
Degree Program in Biotechnology

COURSE OF CYTOLOGY AND HISTOLOGY
Prof. Mauro

CITOPLASM AND ORGANELLES

Organization of the protoplasm of a eukaryotic cell

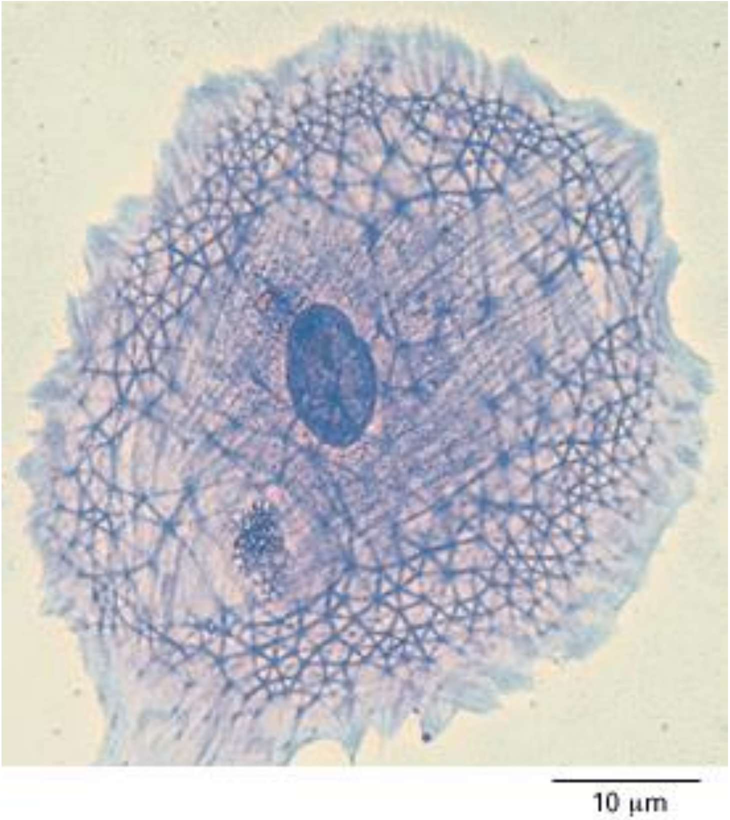




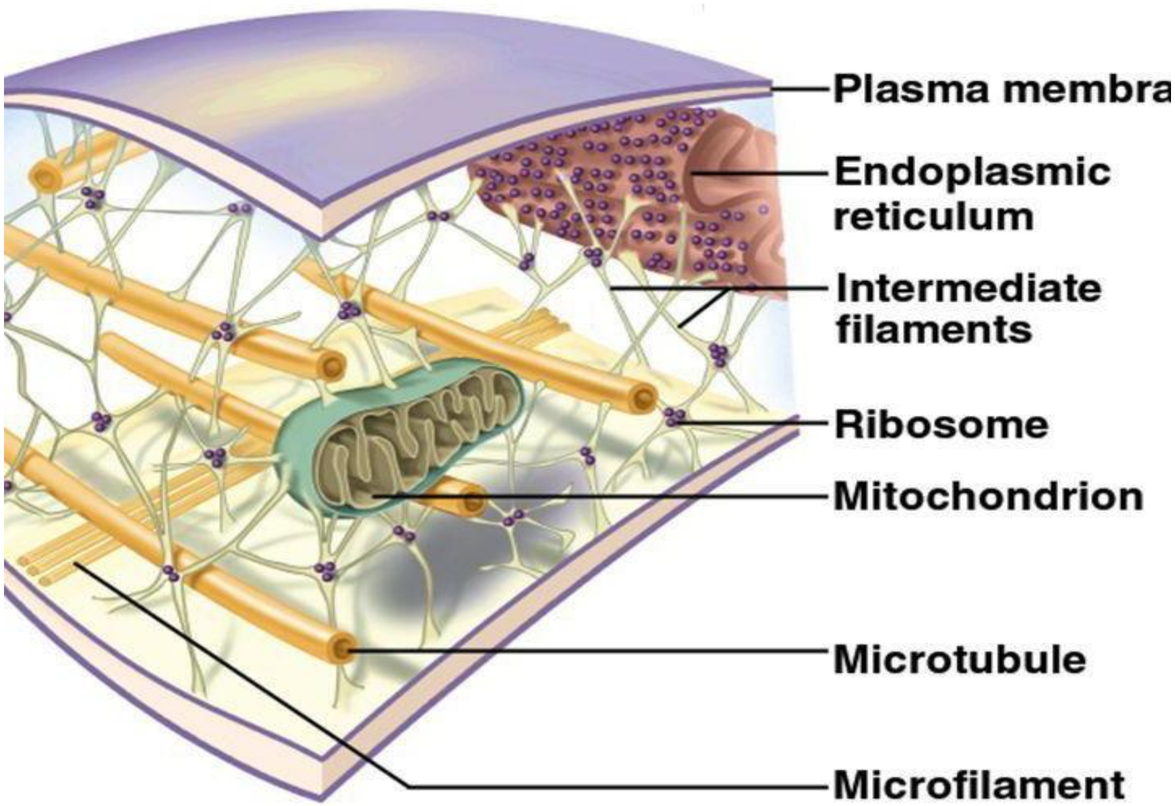
Cytoskeleton

Cytoskeleton

THE CYTOSKELETON IS A COMPLEX PROTEIN NETWORK OF INTERCONNECTED FILAMENTS AND TUBULES THAT EXTENDS THROUGH THE CYTOSOL, FROM THE INNER SURFACE OF THE PLASMA MEMBRANE TO THE NUCLEUS.
INSIDE THE NUCLEUS, IT FORMS THE NUCLEOSKELETON.



Cytoskeleton



It is protein-based and highly dynamic (cell motility) and-provides architectural structure (tensegrity structure) to eukaryotic cells-gives cells a high level of internal organization-allows them to take on and maintain complex shapes.

The Cytoskelet on is involved in

- **spatial organization of the cytoplasm**
- **intracellular movements of organelles**
- **segregation of chromosomes during cytokinesis in cell division**
- **movement of cells on the substrate**
- **muscle contraction**
- **cell signaling and adhesion processes**

It is characteristic of eukaryotic cells

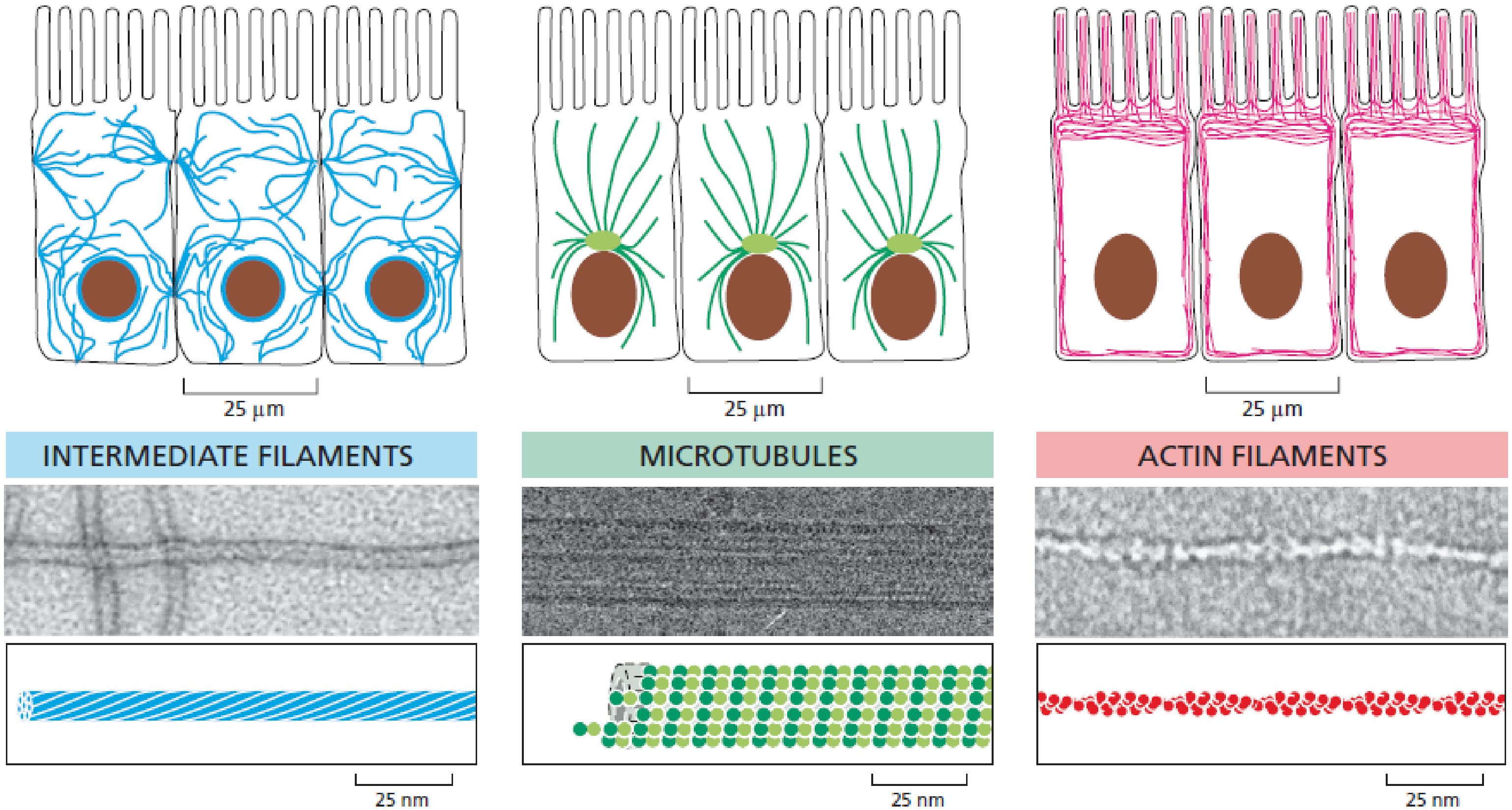
Cytoskeleton

The Cytoskeleton is built on a framework of three types of protein filaments:

- **Actin microfilaments**
- **Microtubules**
- **Intermediate filaments**

They are associated with accessory proteins (*bridge proteins*), essential for the assembly of cytoskeletal structures and their functioning.

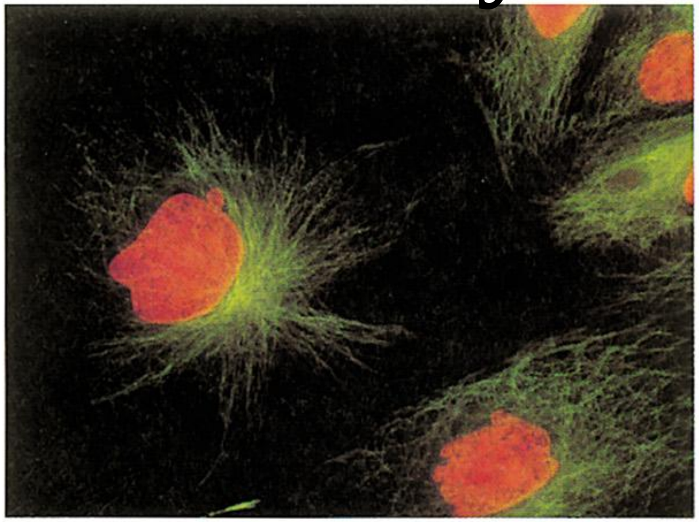
Cytoskeleton



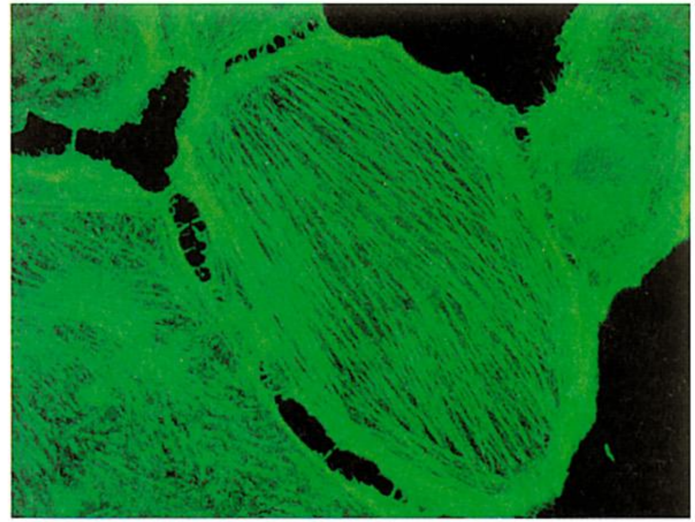
The three types of protein filaments that form the cytoskeleton differ in their composition, mechanical properties, and roles inside the cell.

They are shown here in epithelial cells, but they are all found in almost all animal cells.

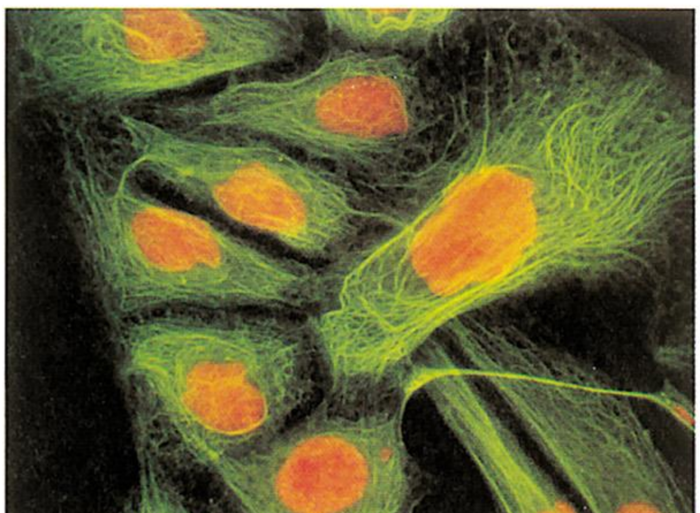
Cytoskeleton



(a) **Microtubules**

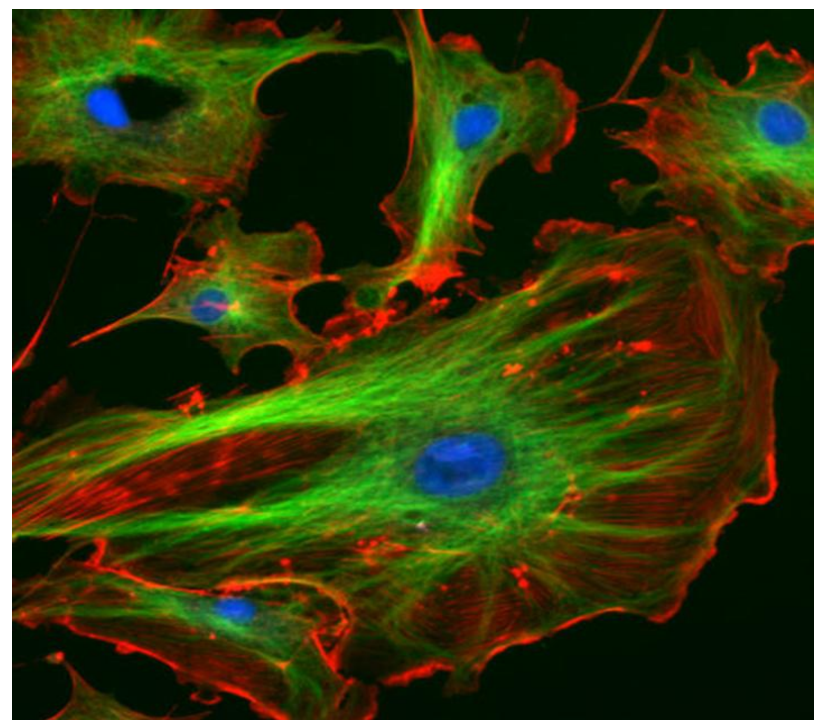


(b) **Actin microfilaments**

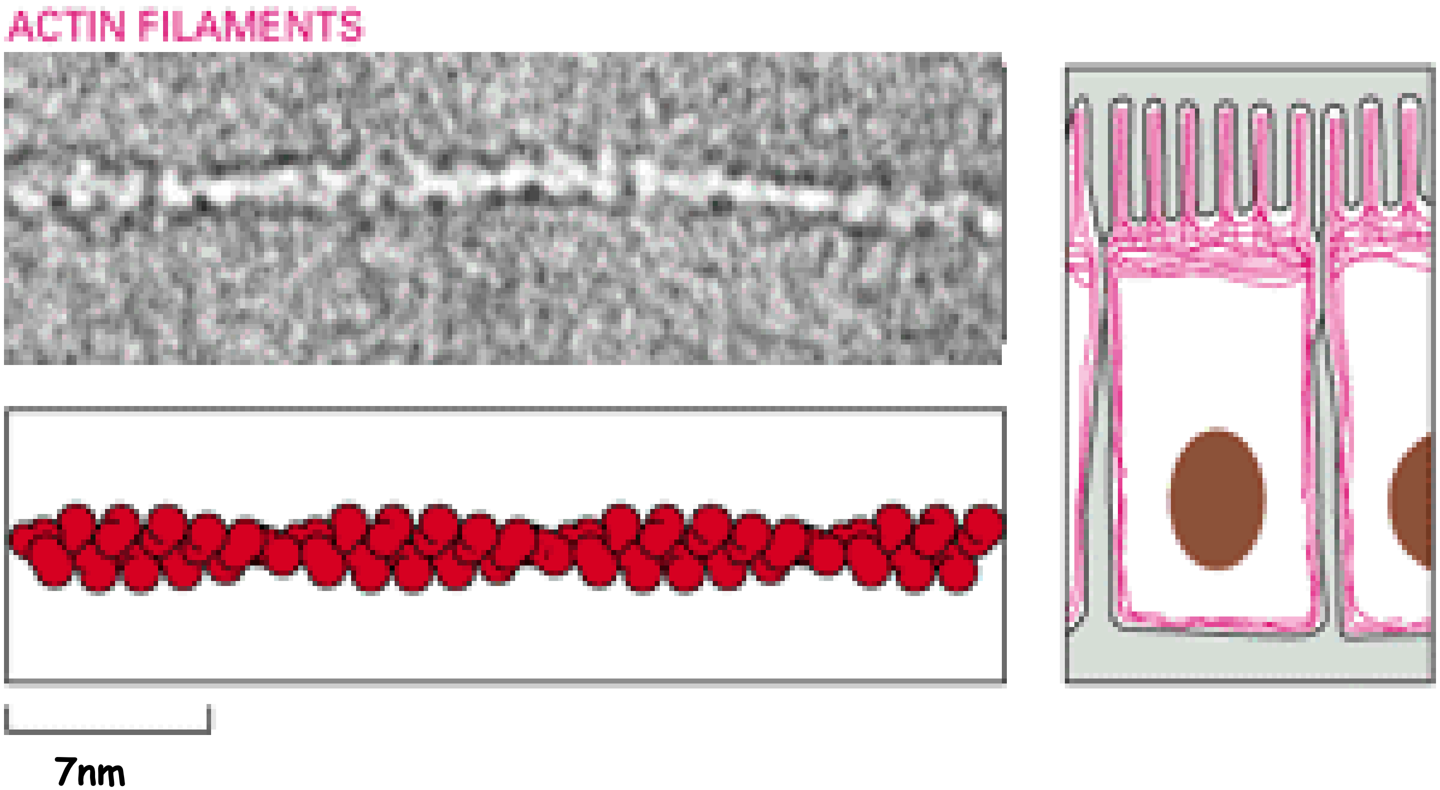


(c) **Intermediate filaments**

5 μm

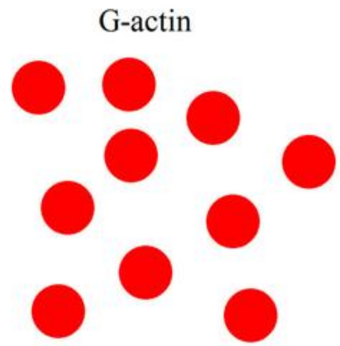


Actin microfilaments

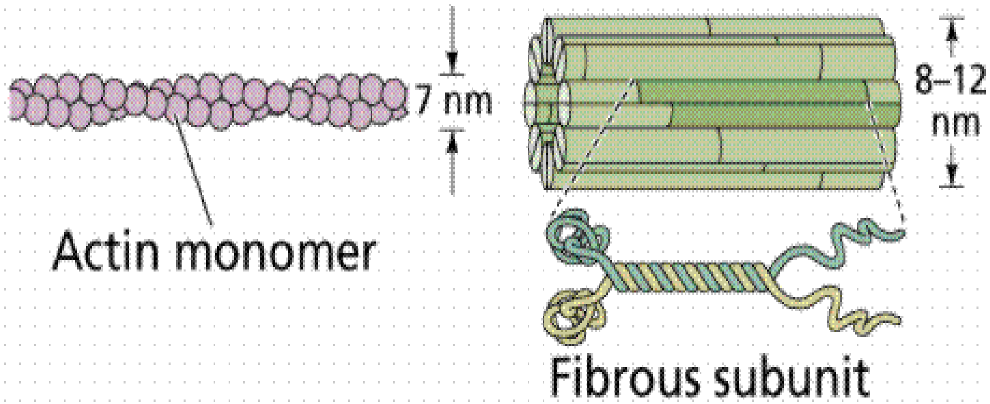


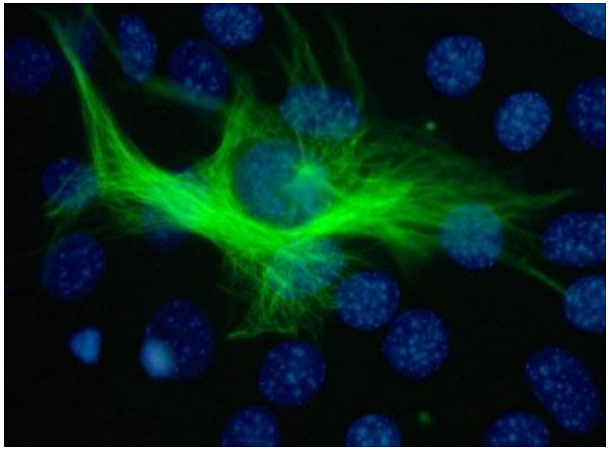
MICROFILAMENTS, with a diameter of 7 nm, are polymers of the globular protein ACTIN (protein 42.3 kDa): responsible for cell structure and movement.

Actin microfilaments



Actin globular protein (G-Actin) is present in all eukaryotic cells and accounts for 15%–20% of cellular proteins.

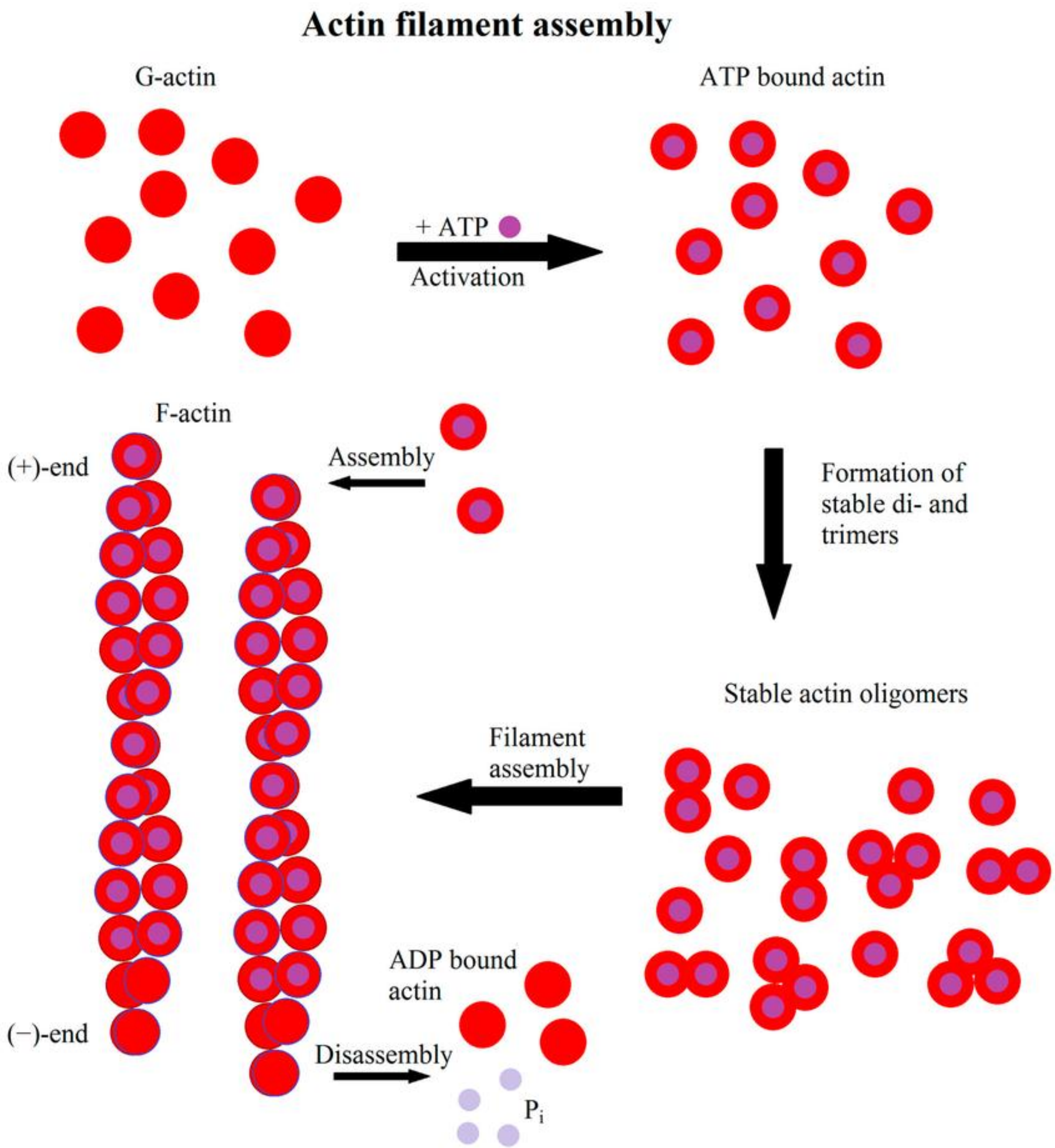




Consisting of two chains of actin globular subunits (G-actin) that spiral around each other to form filaments (F-actin)
They form bundles of varying lengths depending on the functions that the cell must perform.

E.g. movement of organelles and vesicles, exocytosis, endocytosis, pod emission, cell migration

Actin microfilaments



4 stages of filament assembly:
-monomer activation,
-nucleation,
-elongation,
-annealing

Actin polymerization requires Mg^{2+}, K^{+}, and ATP.

Actin microfilaments

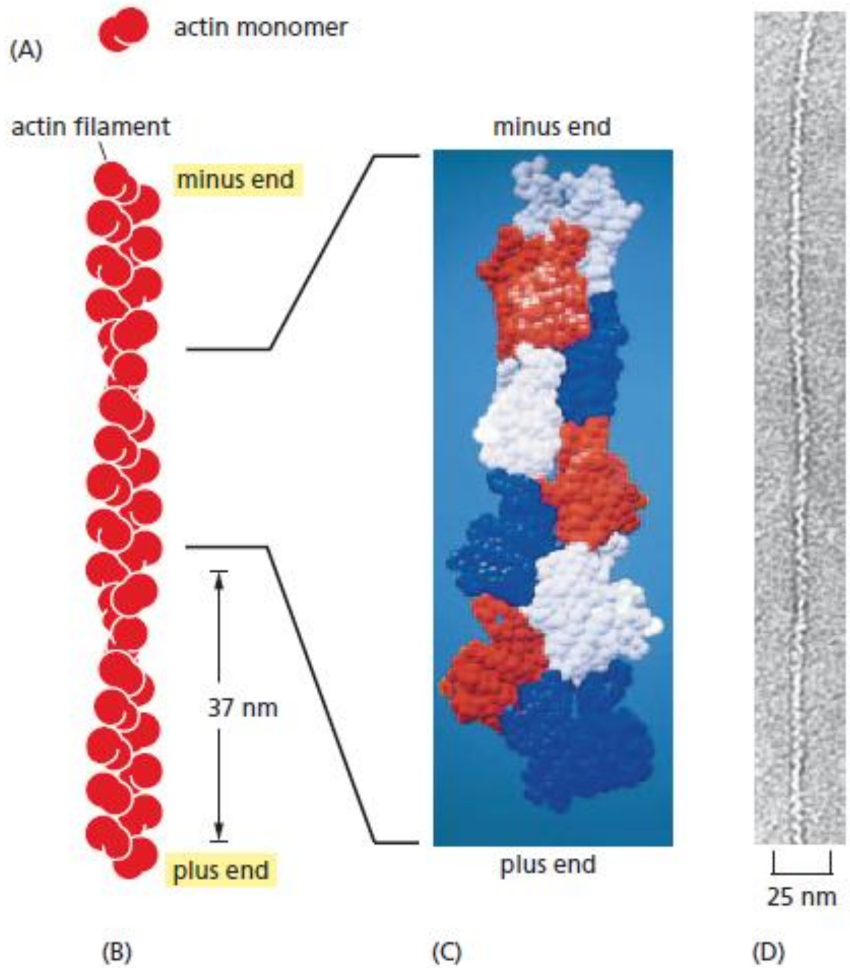


Figure 17–29 Actin filaments are thin, flexible protein threads. (A) The subunit of each actin filament is an actin monomer. A cleft in the monomer provides a binding site for ATP or ADP.
(B) Arrangement of actin monomers in an actin filament. Each filament may be thought of as a two-stranded helix with a twist repeating every 37 nm. Multiple, lateral interactions between the two strands prevent the strands from separating.
(C) Close-up view showing the identical subunits of an actin filament in different colors to emphasize the close interactions between each actin molecule and its four nearest neighbors.
(D) Electron micrograph of a negatively stained actin filament.

Actin microfilaments

Ancillary proteins of monomeric actin:
Profilins, β-thymosins, DNase I, Vitamin D-binding protein, depactin, actaphorin

Ancillary proteins of the microfilament:
End-blocking protein, barbed-end capping protein, and pointed-end capping protein, spectrin, dystrophin, α-actinin, etc.

Actin-mediated motility:
Propulsive movements
Retractive movements

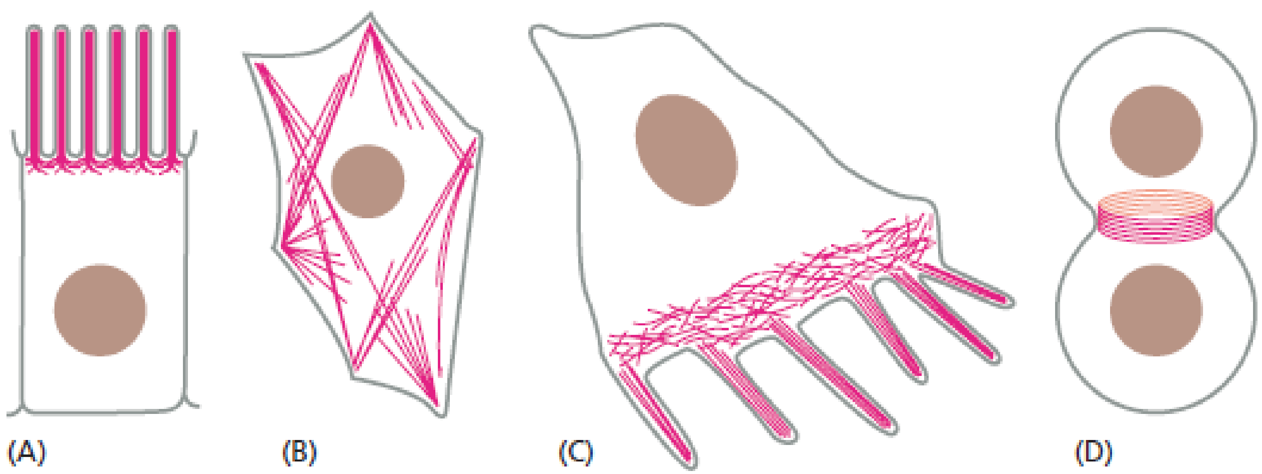
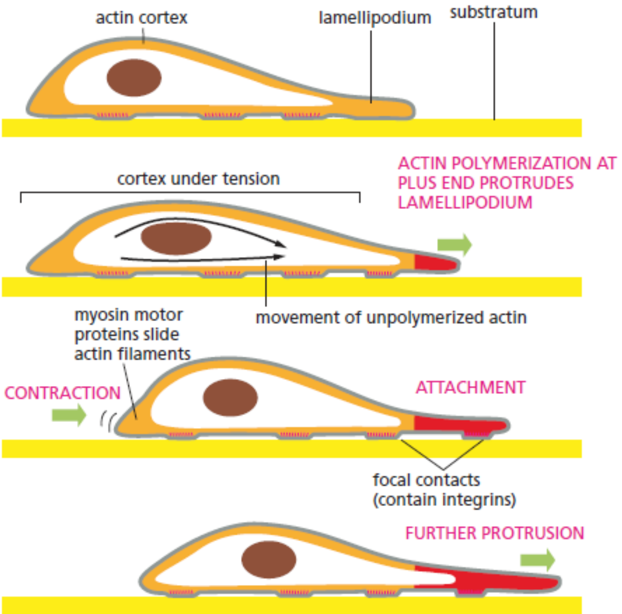


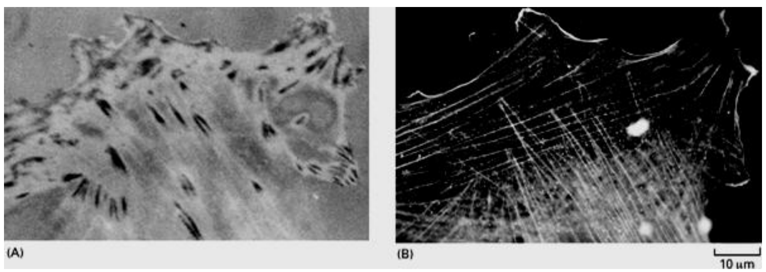
Figure 17–28 Actin filaments allow animal cells to adopt a variety of shapes and perform a variety of functions. The actin filaments in four different structures are shown here in red:
(A) microvilli;
(B) contractile bundles in the cytoplasm;
(C) fingerlike filopodia protruding from the leading edge of a moving cell;
(D) contractile ring during cell division.

Actin microfilaments

- **Actin filaments allow animal cells to migrate**
- **Cell Crawling Depends on Cortical Actin**



Focal contacts



Lamellipodia and Filopodia

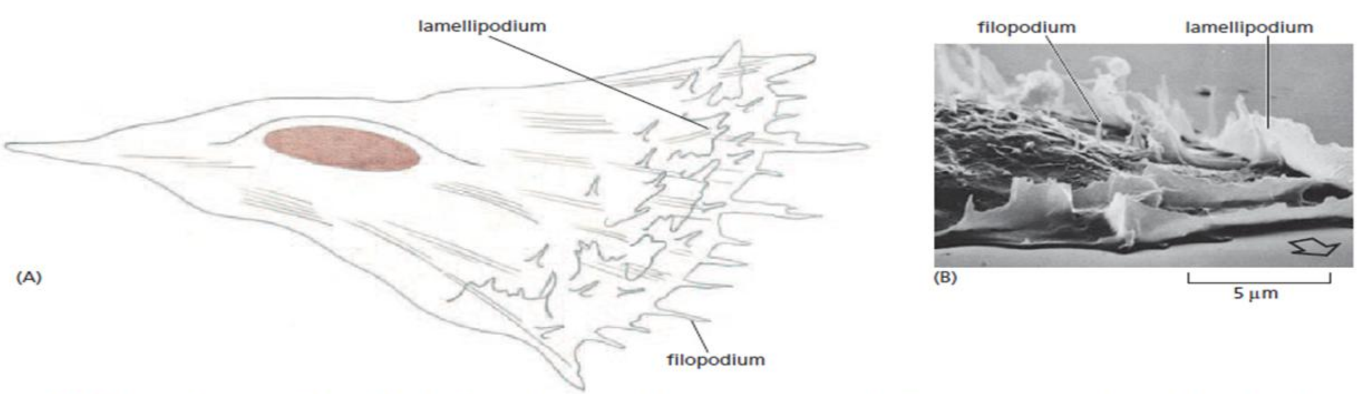
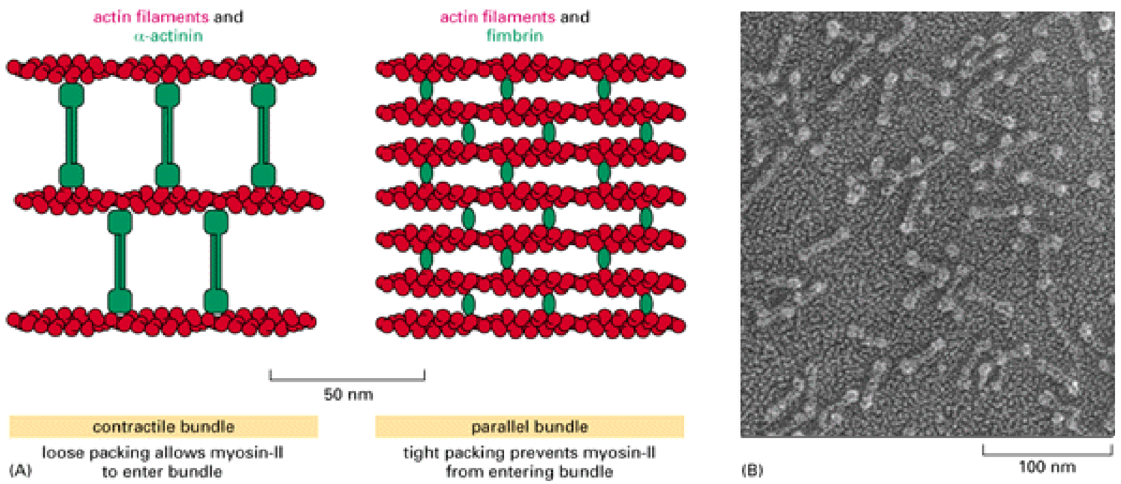


Figure 17–34 Actin filaments allow animal cells to migrate. (A) Schematic drawing of a fibroblast, showing flattened lamellipodia and fine filopodia projecting from its surface, especially in the regions of the leading edge. (B) Scanning electron micrograph showing lamellipodia and filopodia at the leading edge of a human fibroblast migrating in culture; the arrow shows the direction of cell movement. As the cell moves forward, the lamellipodia that fail to attach to the substratum are swept backward over the upper surface of the cell— a movement referred to as ruffling. (B, courtesy of Julian Heath.)

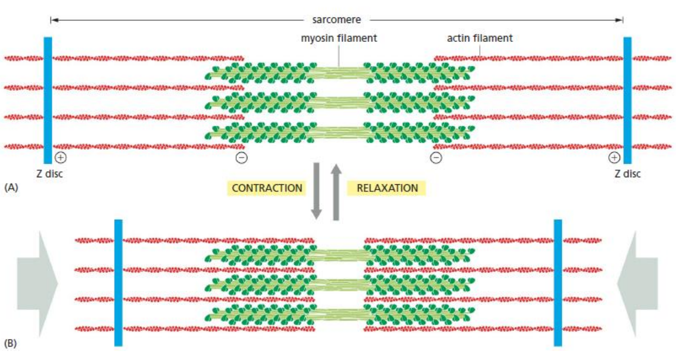
Actin microfilaments

Different accessory proteins are associated with actin microfilaments to form the following structures in various cells

parallel bundles
network structures
contractile structures.



Actin Associates with Myosin II to Form Contractile Structures in skeletal muscle cells

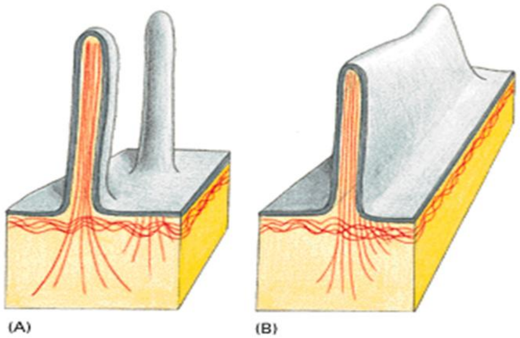


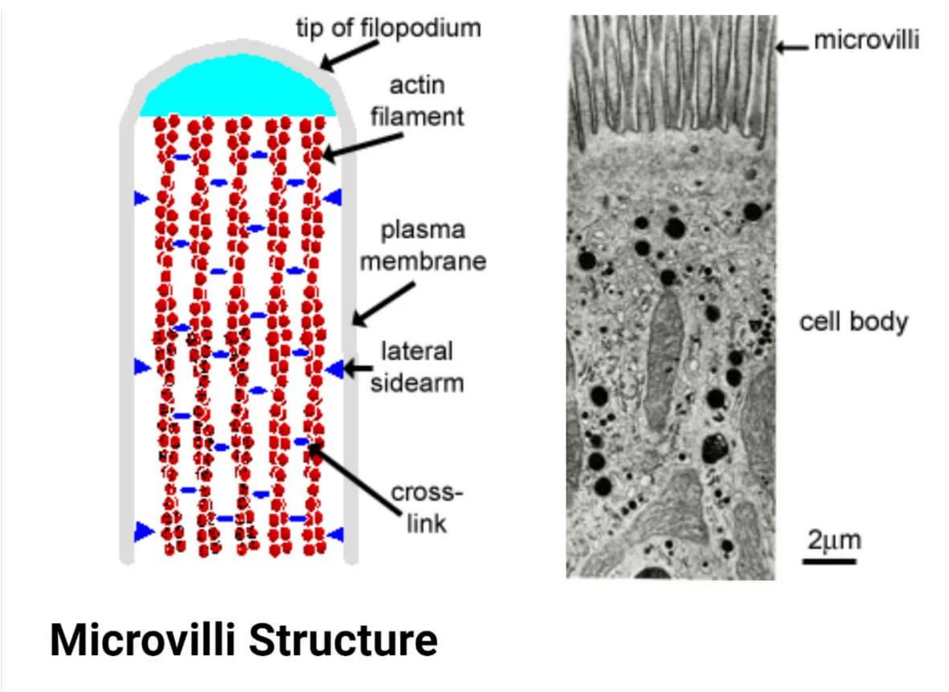
sarcomere

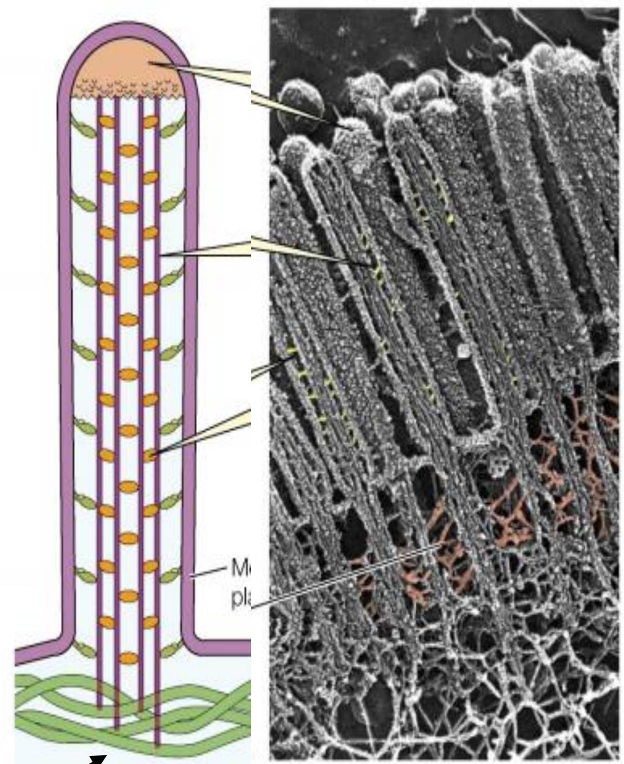
Actin microfilaments

present in the apical specializations of some epithelial cells

MICROVILLI

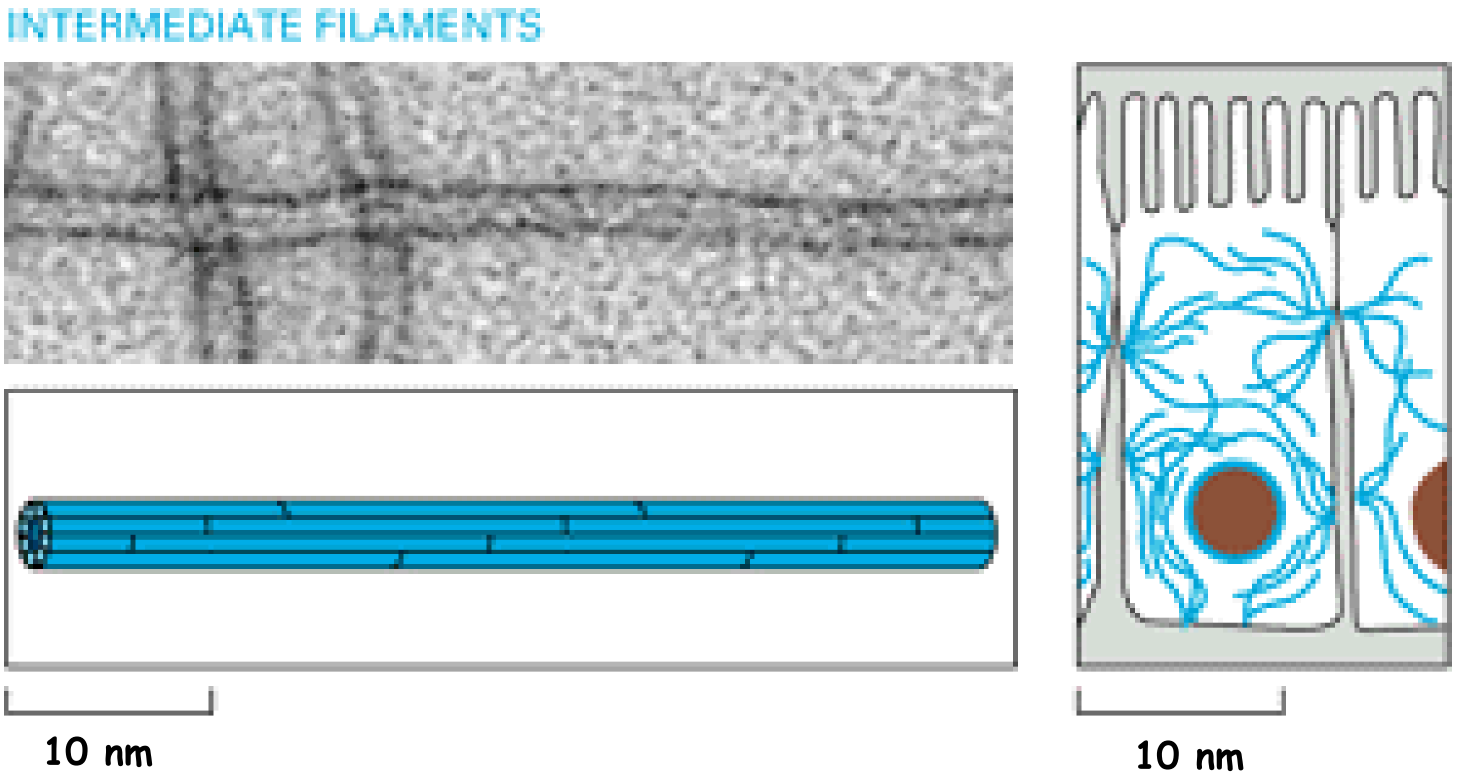






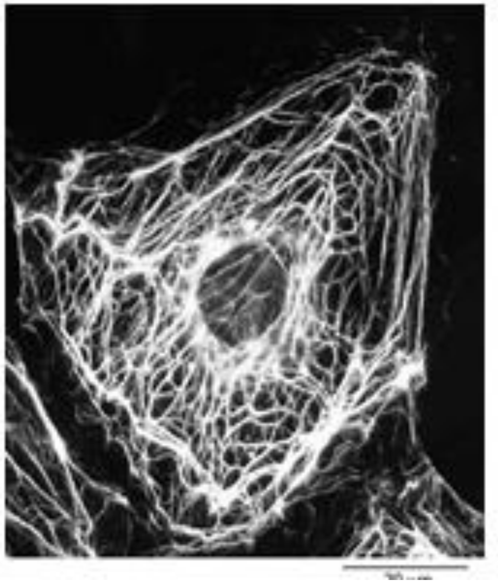
intermediate filaments

Intermediate filaments

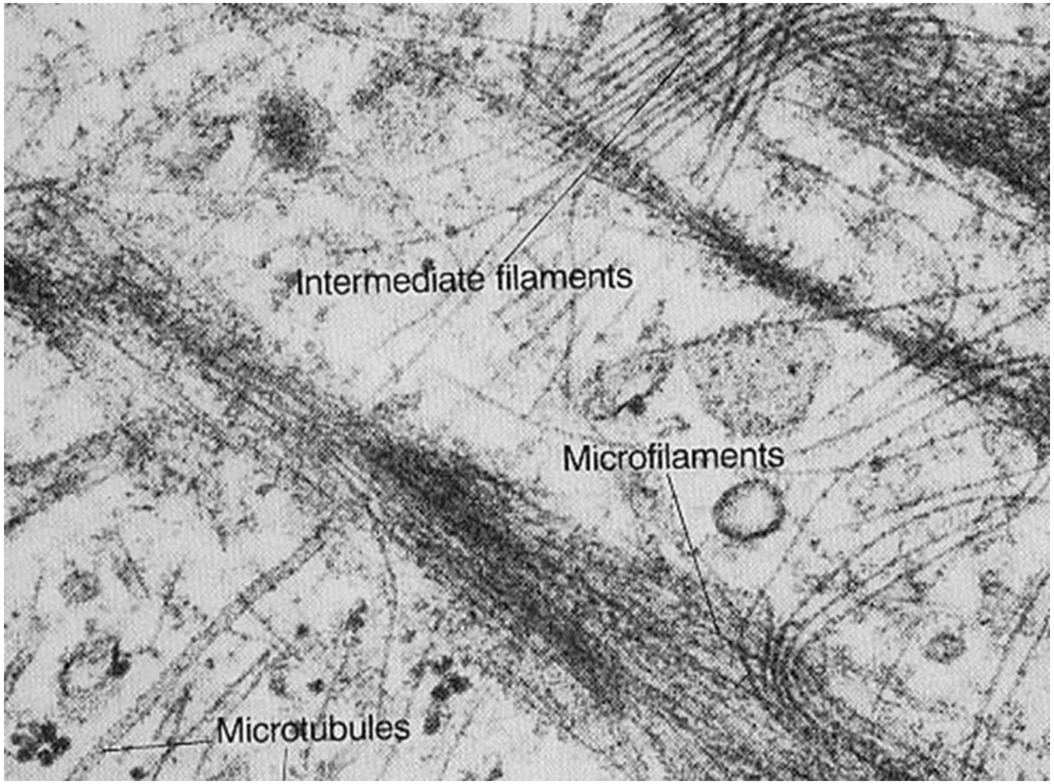


The INTERMEDIATE FILAMENTS in each cell type are formed by POLYMERS OF DIFFERENT PROTEINS, but all similar in size and structure.

Intermediate filaments



They typically form a network throughout the cytoplasm, surrounding the nucleus and extending out to the cell periphery

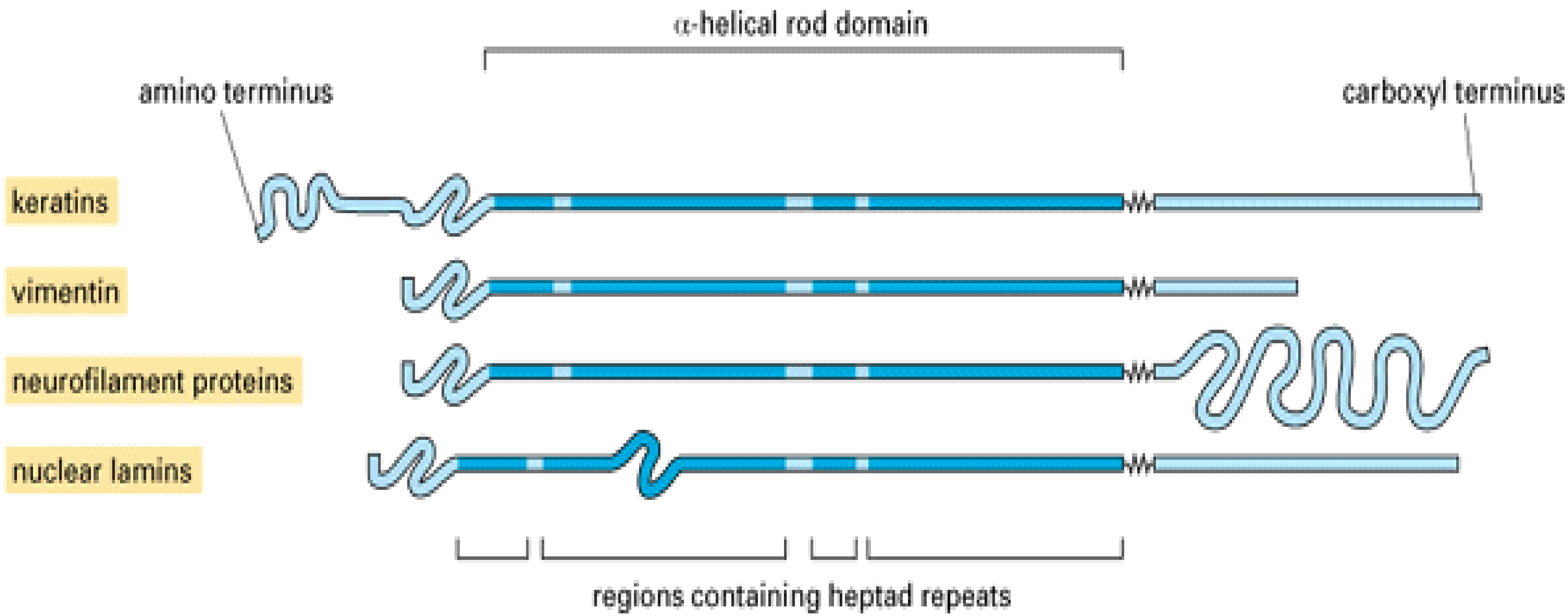


Intermediate filaments

Intermediate filaments (IFs) have a diameter of approximately 8-12 nm and are organized into strong, durable protein bundles.
They play a fundamental structural role in supporting cell tension. Intermediate filaments are the most stable and least soluble structures of the cytoskeleton (they remain intact even in concentrated saline solutions and non-ionic detergents).

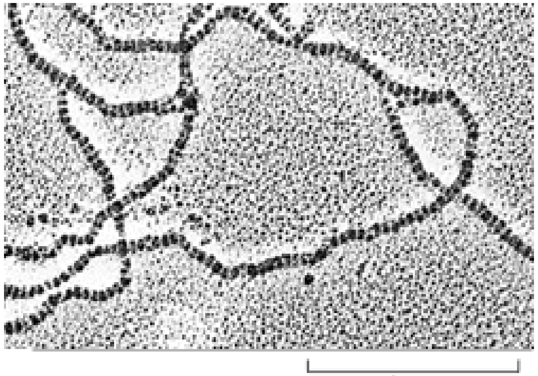
The TYPING OF INTERMEDIATE FILAMENTS also serves as a **diagnostic tool in medicine** and is particularly useful in the diagnosis of tumors, as tumor cells retain the intermediate filaments characteristic of the tissue of origin, regardless of the location of the tumor in the body.

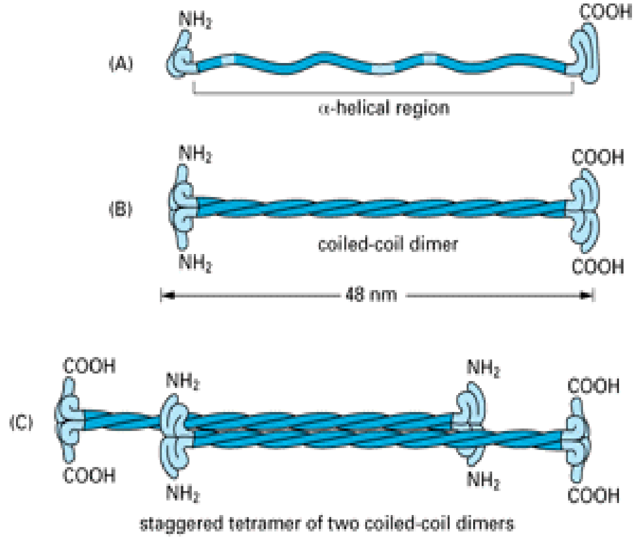
Intermediate filaments

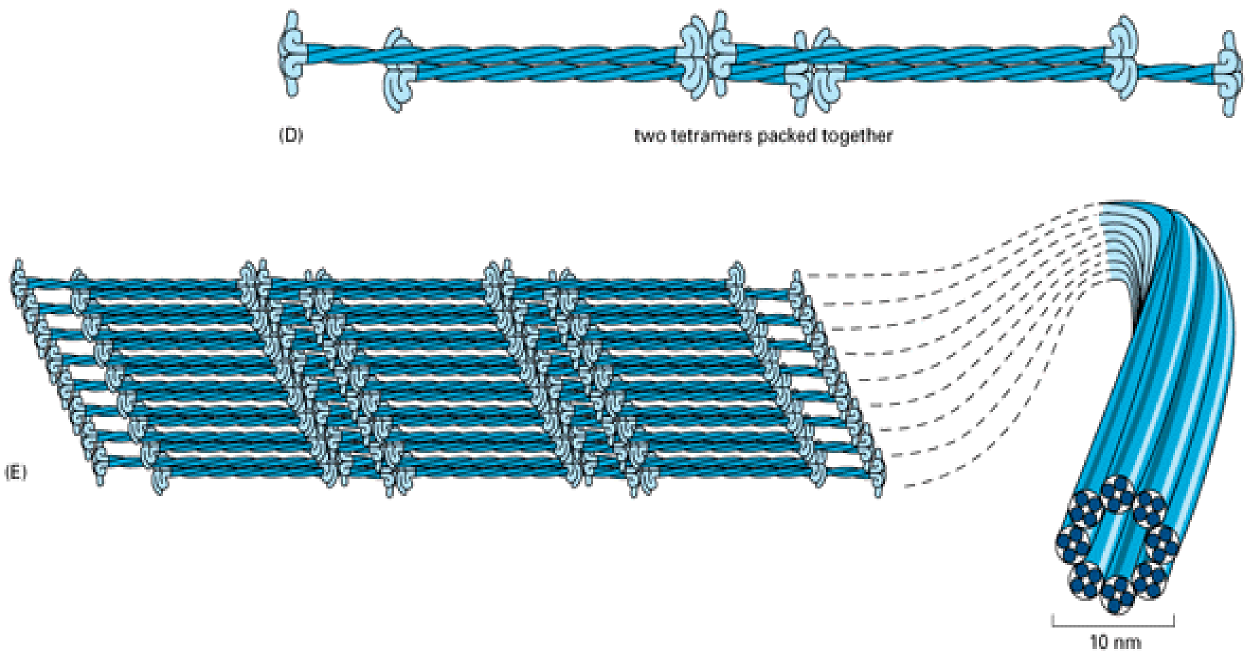


Intermediate filament proteins are fibrous and characterized by a central alpha-helix domain, **a rod formed by approximately 310-318 amino acids**, which is conserved in size, secondary structure, and in some cases also in sequence..

Intermediate filaments







filament monomer consists of an α-helical central rod domain (A) with unstructured regions at either end (not shown).

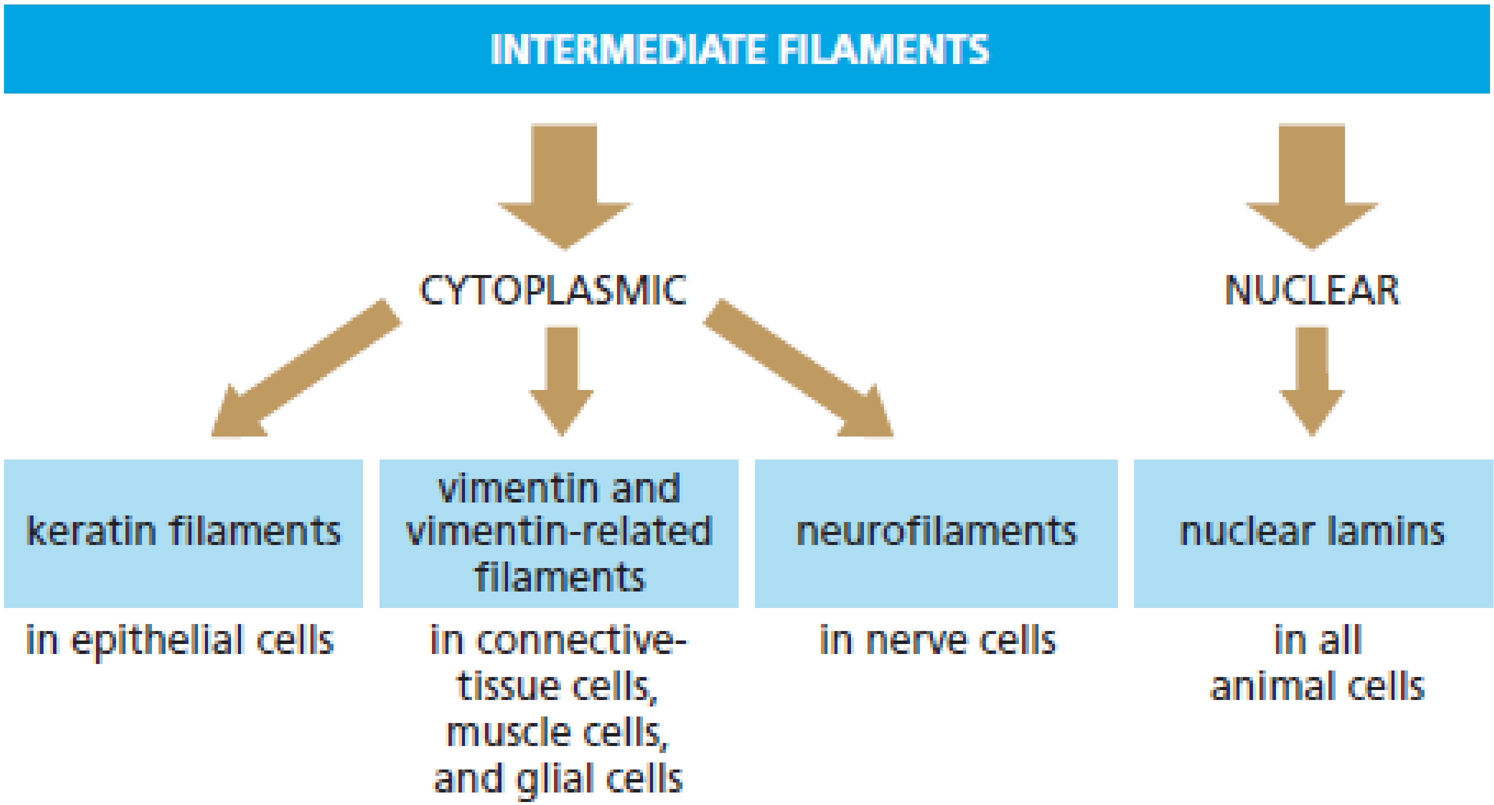
Pairs of monomers associate to form a dimer (B), and two dimers then line up to form a staggered, antiparallel

tetramer (C). Tetramers can pack together into a helical array containing eight tetramer strands (D), which in turn

assemble into the final ropelike intermediate filament (E)

Intermediate filaments

Intermediate filaments (IFs) can be grouped into four classes



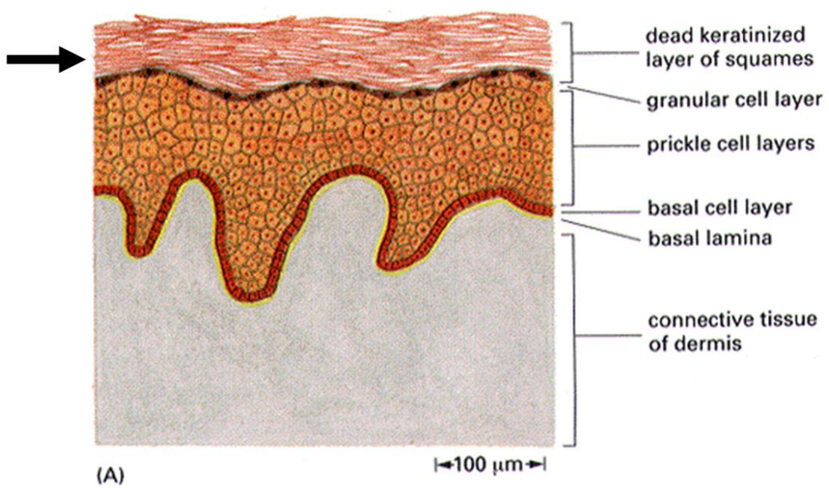
Intermediate filaments

Class I: keratin filaments proteins form the tonofilaments of epithelial cells covering the body surface and its cavities.

There are 20 types of soft keratins and 8 hard keratins, the latter being specific to hair and nails.

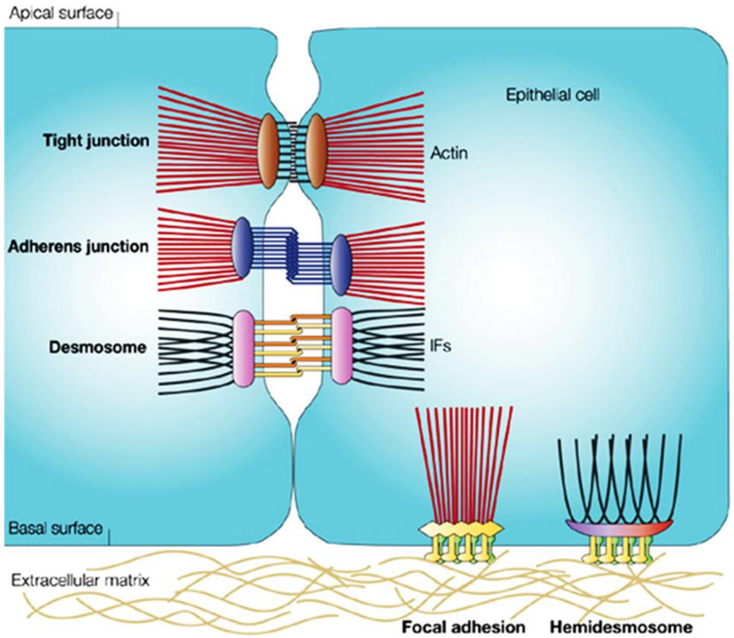
They are distinguished as acidic keratins and basic keratins, which assemble into heterodimers.

arrow indicates the most superficial layer of the epidermis, with cells rich in keratin



Intermediate filaments

CELL JUNCTIONS



The membranes of adjacent cells exhibit a series of specialized structures formed by Intermediate Filaments, called **CELL JUNCTIONS** that allow close contact and, in some cases, exchanges between cells.

Two main classes of **CELL JUNCTIONS** are distinguished:

ADHERING JUNCTIONS:

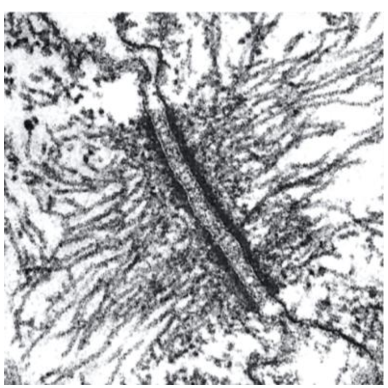
- Desmosomes
- Hemidesmosomes
- Adherens junctions
- Tight junctions

COMMUNICATING JUNCTIONS also known as gap junctions.

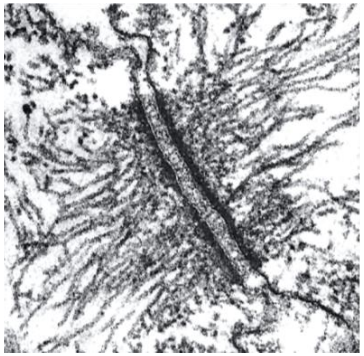
Adhering junctions hold cells together and act as “anchors,” keeping cells in fixed positions within tissues. Desmosomes strengthen areas subjected to mechanical stress.
Tight junctions close the intercellular spaces to prevent the free passage of ions and molecules, forming a barrier that controls diffusion.

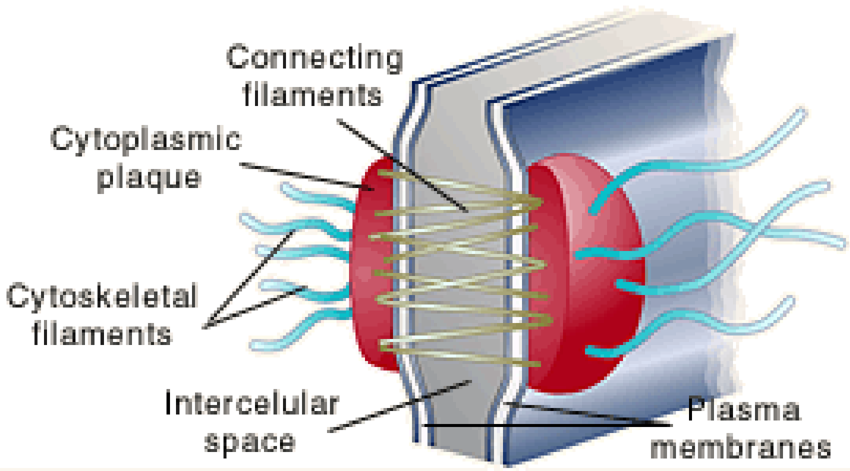
Gap junctions, on the other hand, form channels between the plasma membranes of adjacent cells, allowing the diffusion of ions and small molecules from one cell to another.

Desmosomes



Intermediate filaments





DESMOSOMES or MACULAE ADHERENTES

These are the most widespread and complex junctional systems, especially in epithelial tissues.

They have a circular or elliptical shape;

in the region containing a desmosome, a PLAQUE can be observed , a thick layer of dense material located beneath the plasma membrane.

From the plaque, extending toward the cytoplasm, arise intermediate filaments composed of keratin or vimentin. These are called TONOFILAMENTS, and they serve as anchoring structures for the junction.

Hemidesmosomes are observed when cells are anchored to extracellular material rather than to other cells.

They are also found in cultured cells *in vitro*, where they serve as anchoring points to plastic or glass surfaces.

Intermediate filaments

Class II: Vimentin and vimentin-related filaments

This Class of IFs include vimentin, desmin, and glial fibrillary acidic protein (GFAP).

Vimentin is located in connective tissues and other cell types of non-epithelial origin.

Desmin is found in muscle cells.

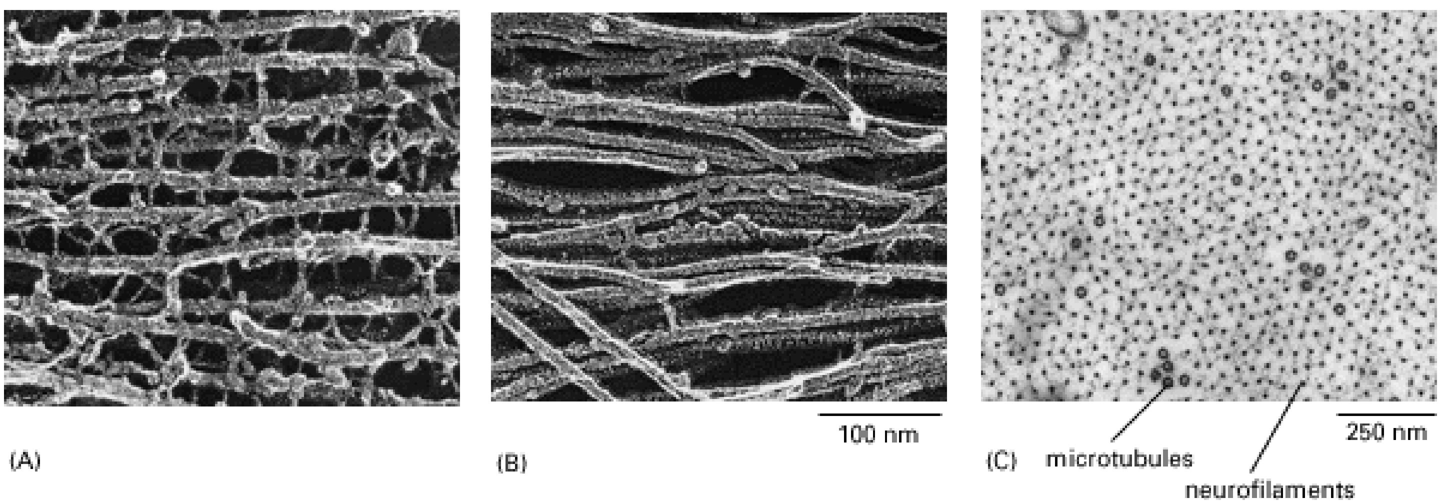
GFAP is characteristic of glial cells that surround and insulate nerve cells.

Intermediate filaments

Class III: Neurofilaments

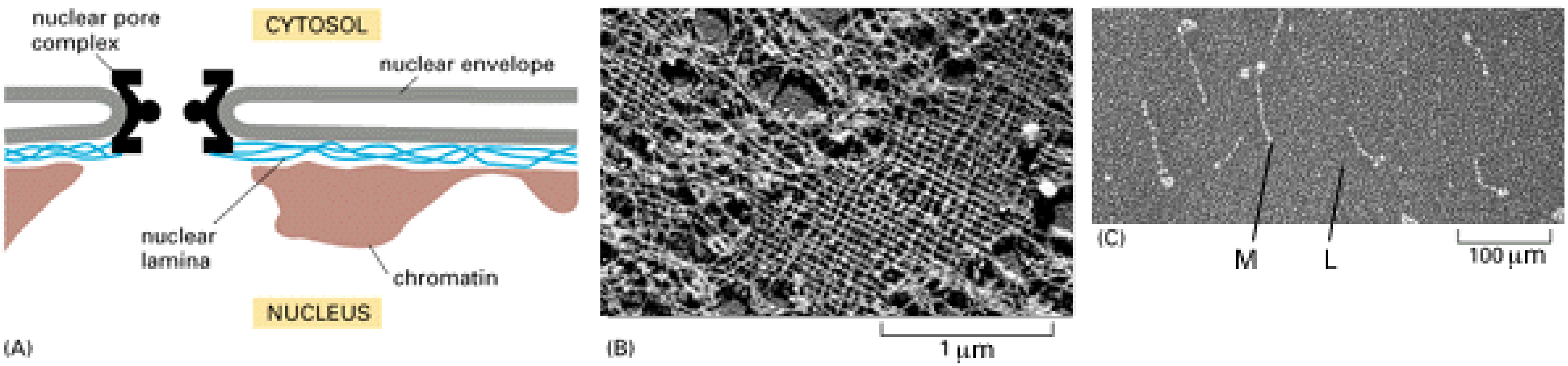
Intermediate Filaments composed of neurofilament (NF) proteins, which provide structural support to axons and the extensions of nerve cells.

Specific IFs are also neurofilament proteins, found in the embryonic nervous system, and are composed of nestin.



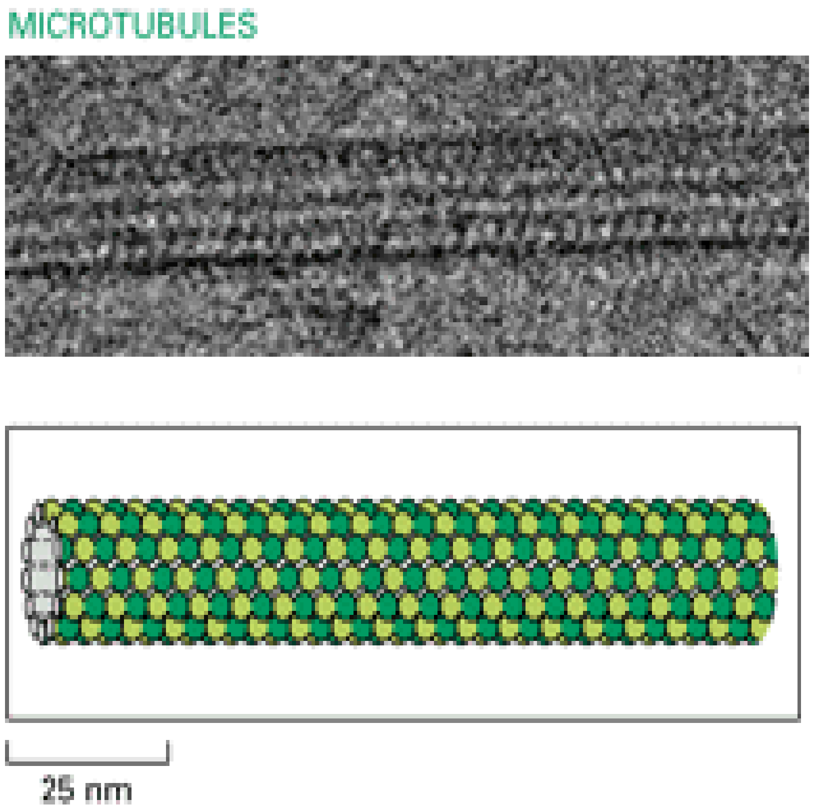
Intermediate filaments

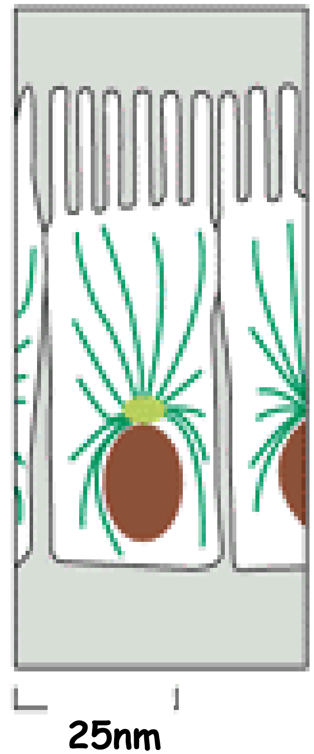
Class IV: Nuclear lamins

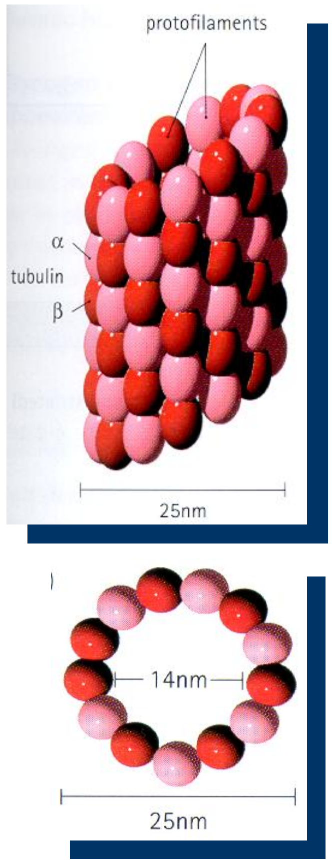


This Class consists of nuclear lamin A, B, and C, which form a filamentous network along the inner surface of the nuclear membrane of all eukaryotic cells. They have a nuclear localization signal.
The formation of the network is dynamic during cell division.

Microtubules



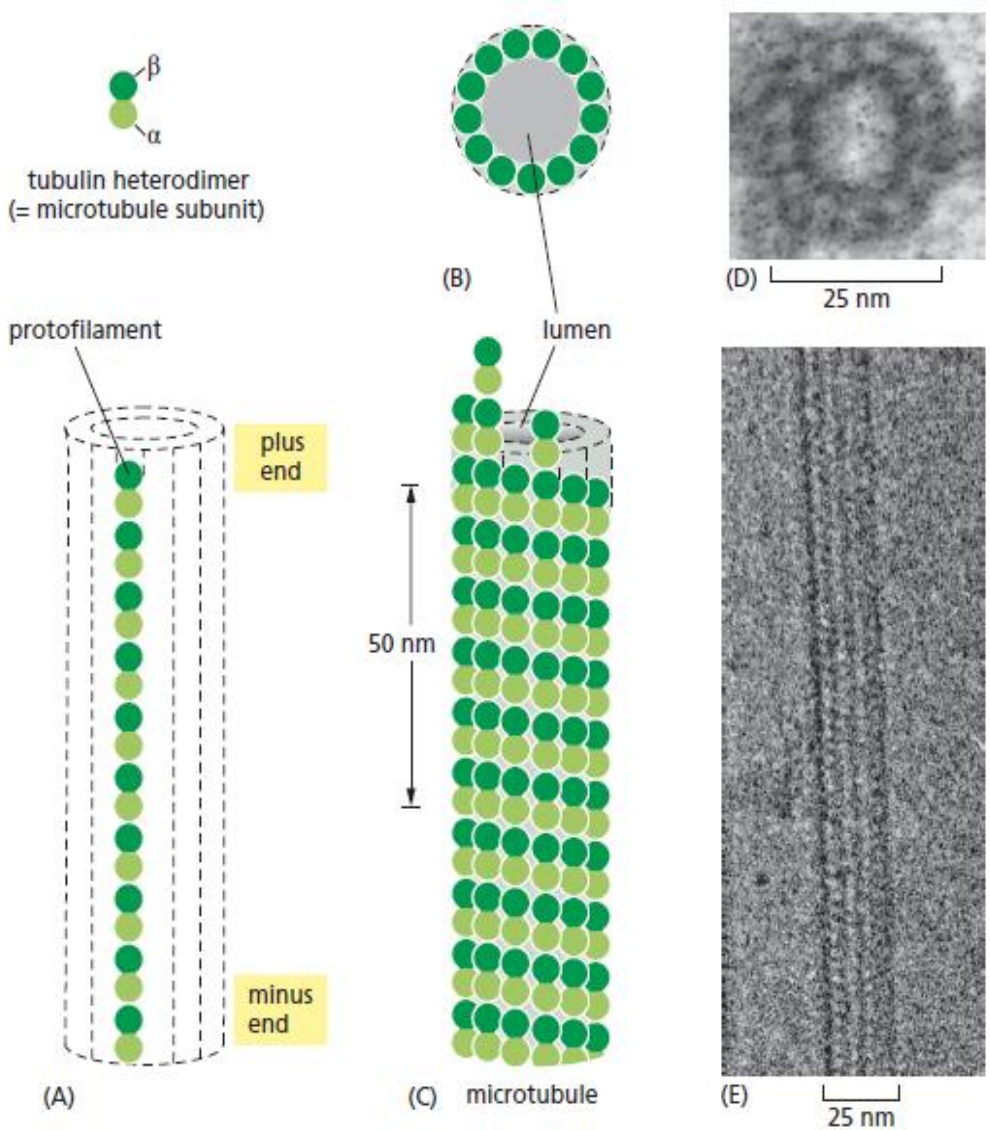




Microtubules are composed of the protein Tubulin and have a diameter of 25 nm.

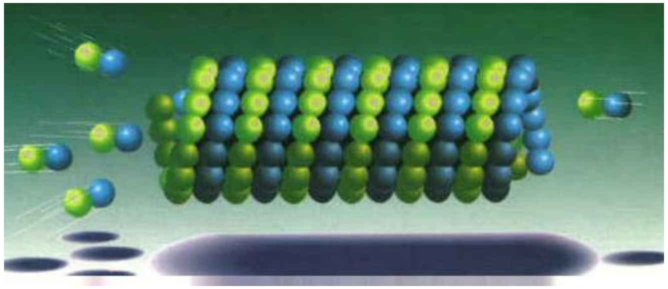
Microtubules

Microtubules Are Hollow Tubes with Structurally Distinct Ends



They are composed of two similar globular protein subunits, **β- and α-tubulin**, which associate to form α–β heterodimers.

These heterodimers aggregate in a helical arrangement to form the tubular structure..



structural polarity

Microtubules

Microtubule Organizing Center (MTOC)

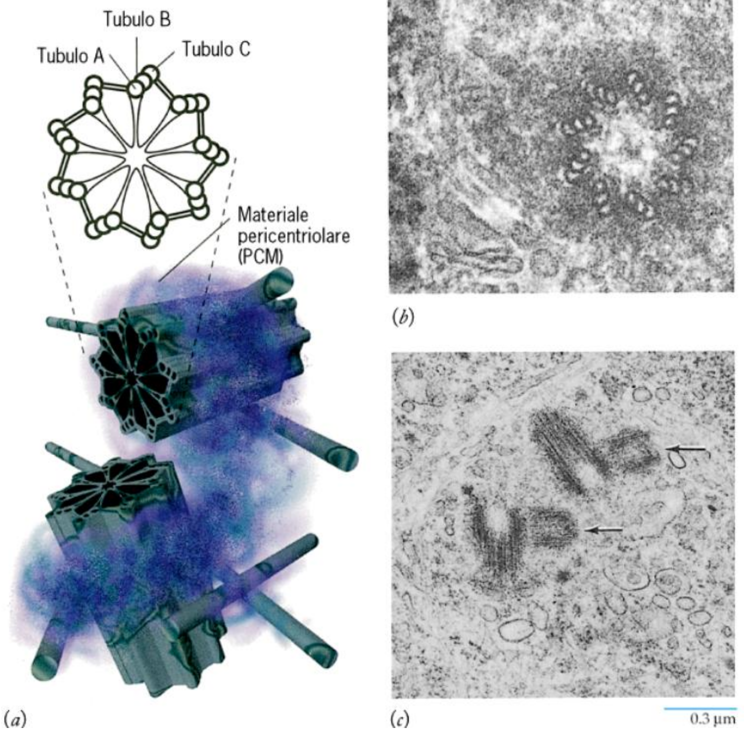
1. The microtubules in most cells extend outwards from a **microtubule organizing center** (**MTOC**) in which the (-) ends of microtubules are anchored.

2. In animal cells, the major MTOC is the **centrosome**, located adjacent to the nucleus near the centre of an interphase cell.

3. The centrosomes of most animal cells contain a pair of **centrioles** surrounded by amorphous **pericentriolar material.**

4. The proteins present: **pericentrin, centrin, γ-Tubulin** etc.

5. **γ-Tubulin**, **nucleates** microtubule assembly within the centrosome.



Microtubules

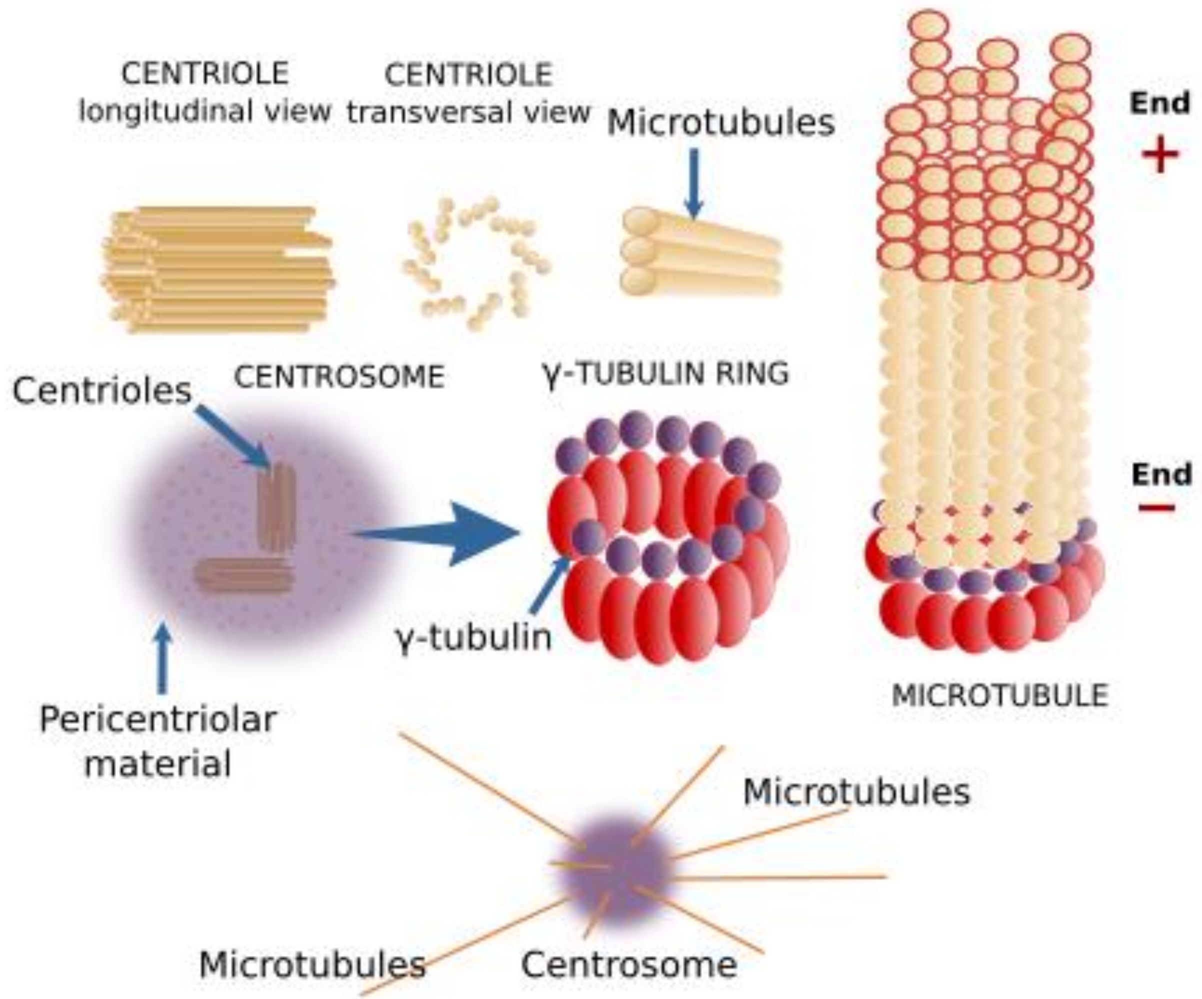
Microtubule Organizing Center

The pericentriolar material contains two proteins:

γ-Tubulin
Ring-shaped at the base of the nascent microtubule

Serves as a template for nucleation

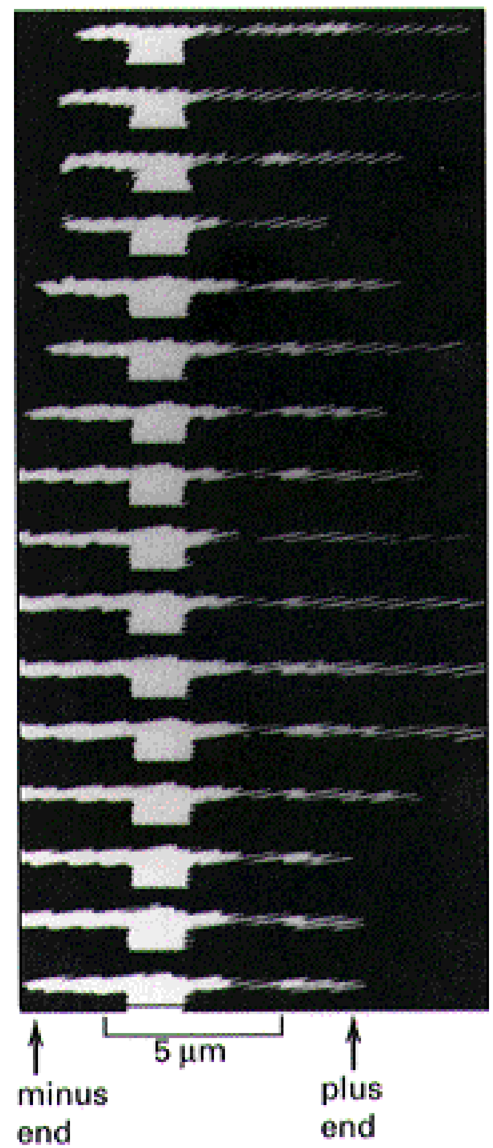
Pericentrin



Microtubules

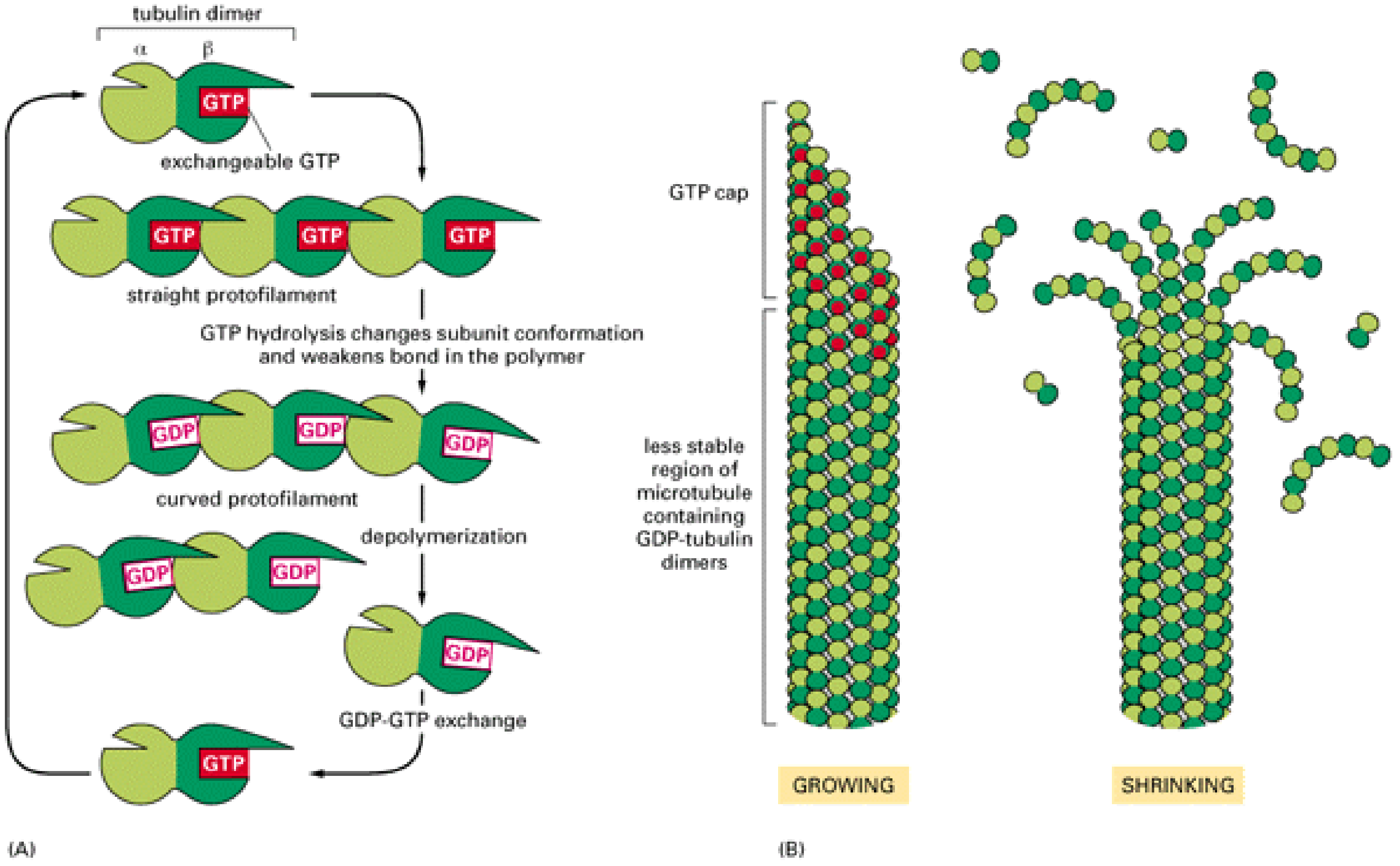
Microtubules continuously polymerize and depolymerize, with a half-life of approximately 10 minutes.

The term "**dynamic instability"** refers to the fluctuation in the length of a single microtubule (measurements taken at intervals of 1–2 minutes).



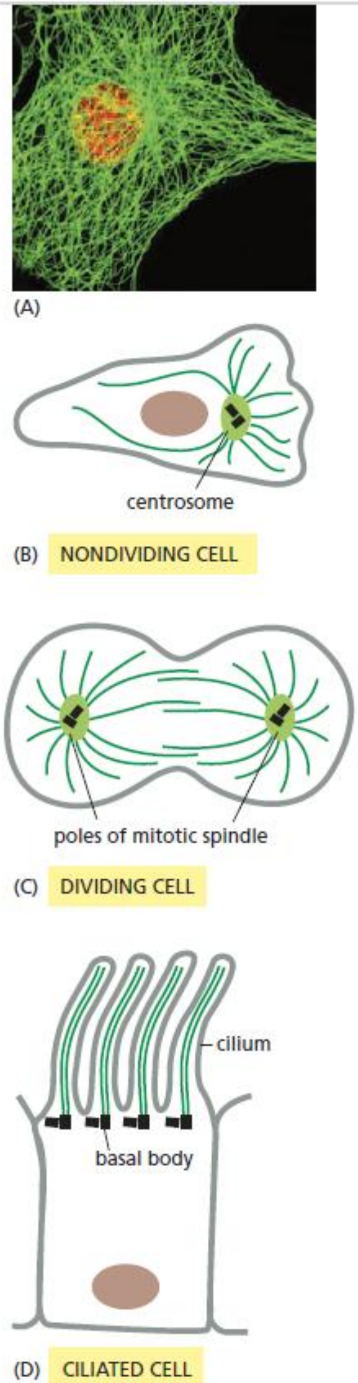
Microtubules

Dynamic Instability is Driven by GTP Hydrolysis



Microtubules

Main functions:



The cytoplasmic microtubules are the part of the cytoskeleton mainly responsible for transporting and positioning membrane-enclosed organelles within the cell and for guiding the intracellular transport of various cytosolic macromolecules..

The cytoplasmic microtubules disassemble and then reassemble into an intricate structure called the ***mitotic spindle***

Microtubules can also form stable structures, such as rhythmically beating **cilia** and **flagella**

Microtubules

Microtubules proteins associated (MAPs) Drive Intracellular Transport

The microtubules role in the movement of vesicles or cell organelles within the cell depends on the association with the **motor proteins** associated with them (MAPs)

Two classes of MAPs

Motor MAPs

Kinesin and dynein

Non-motor MAPs

Able to coordinate the organization of microtubules in the cytoplasm

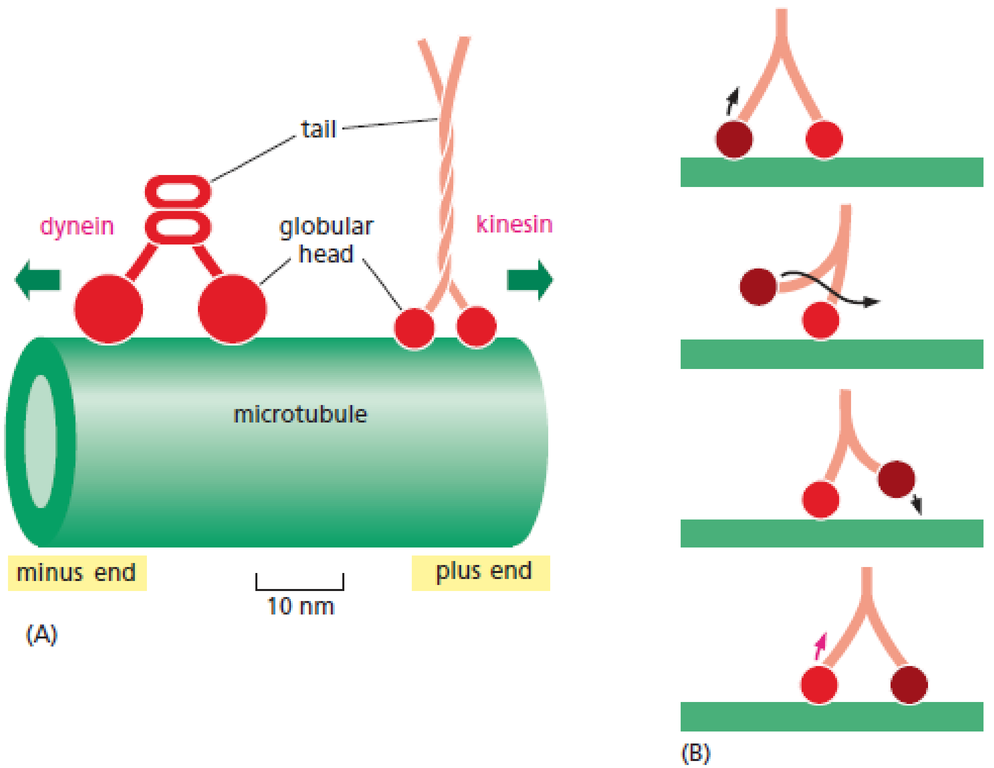
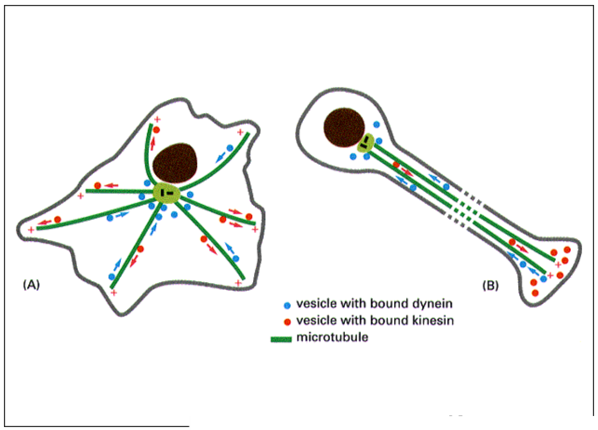
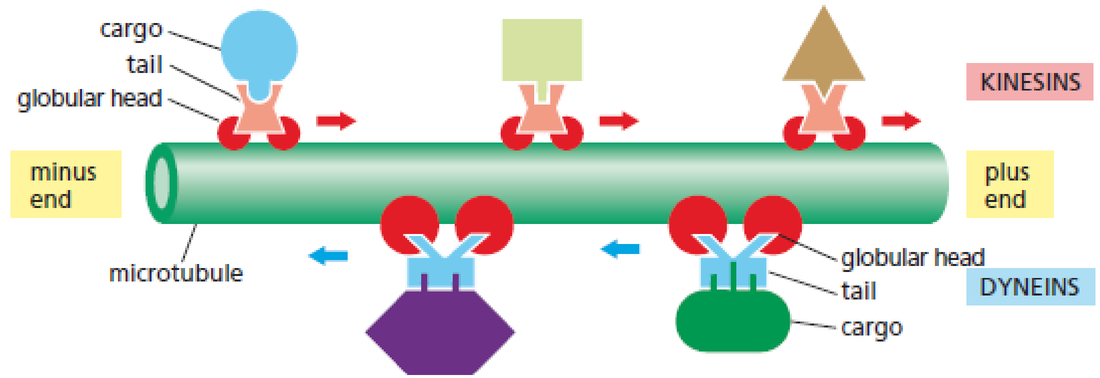


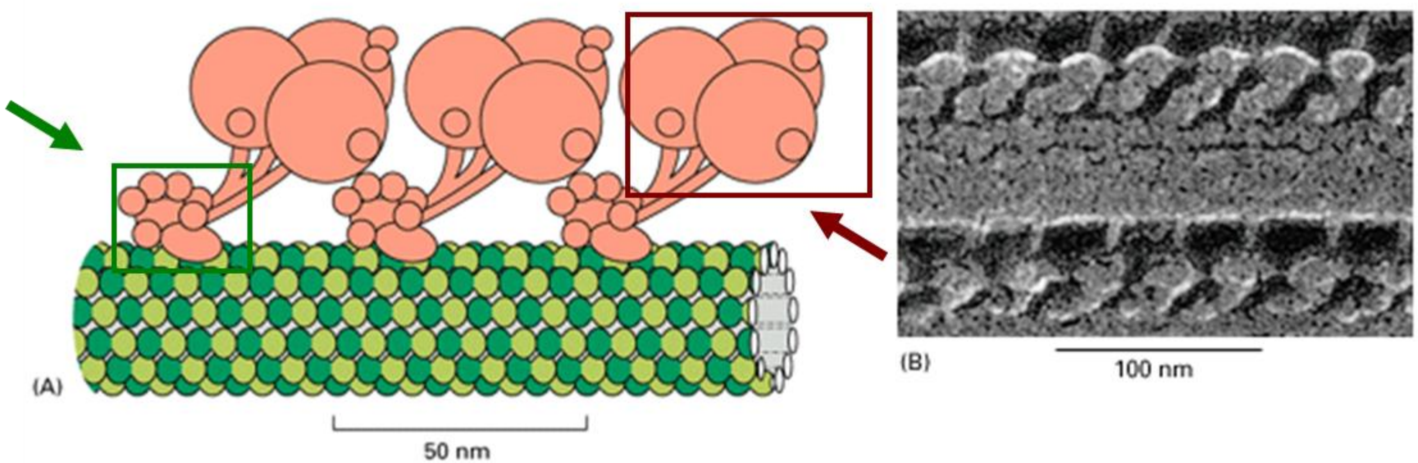
Figure: **Both kinesins and dyneins move along microtubules using their globular heads**.
(A) Kinesins and cytoplasmic dyneins generally move in opposite directions along a microtubule. Each of these proteins is a dimer composed of two identical subunits. Each dimer has two globular heads at one end, which bind and hydrolyze ATP and interact with microtubules, and a single tail at the other end, which interacts with cargo. (B) Schematic diagram of a generic motor protein "walking" along a filament.

Microtubules

Microtubules and Motor Proteins Position Organelles in the Cytoplasm







MAPs have a globular head that binds ATP and a rod-shaped tail. The heads are ATPase motors that attach to microtubules, while the tails attach to specific cellular components.

Microtubules

are present in the apical specializations of some cells

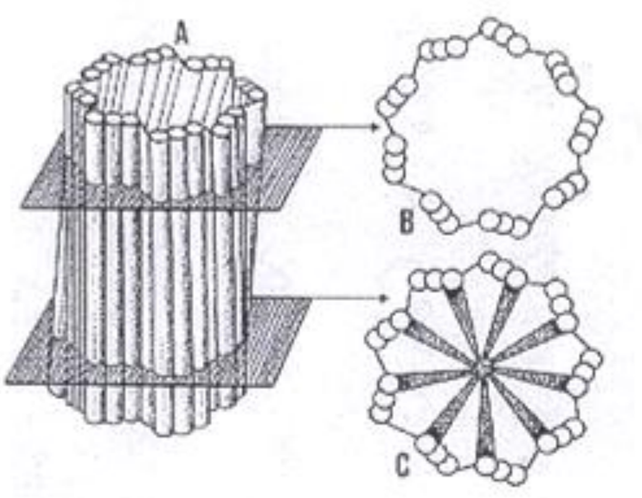
CILIA and FLAGELLA

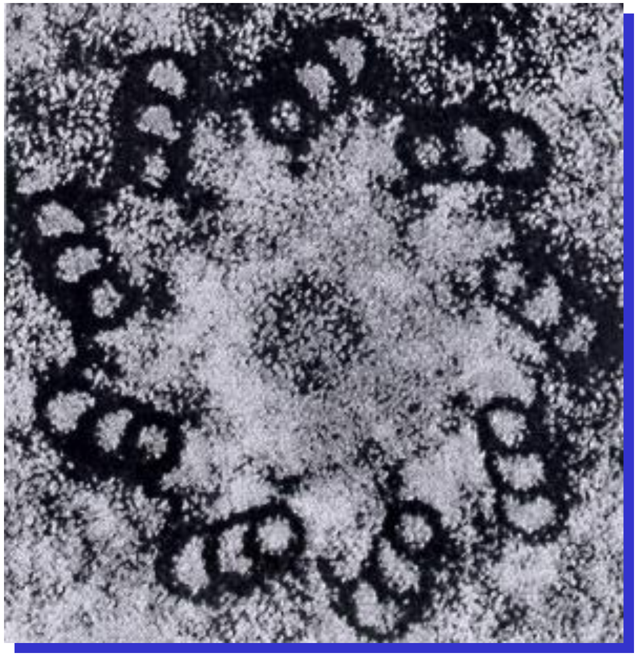
and in specific structures involved in cell division

CENTRIOLE and MITOTIC SPINDLE

Centrioles

small cylindrical structures, usually in pairs and arranged perpendicularly to each other and located in the centrosome near the Golgi apparatus.

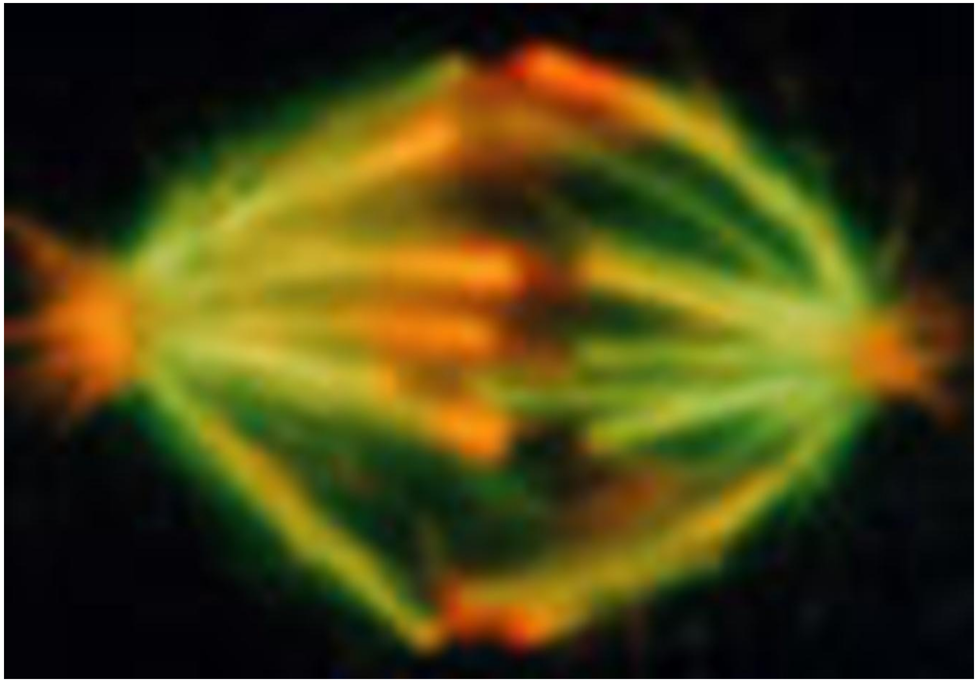




triplets of MICROTUBULES joined by connecting laminae and radial arms with the central dense material

Centrioles

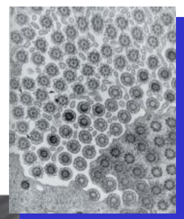
mitotic spindle

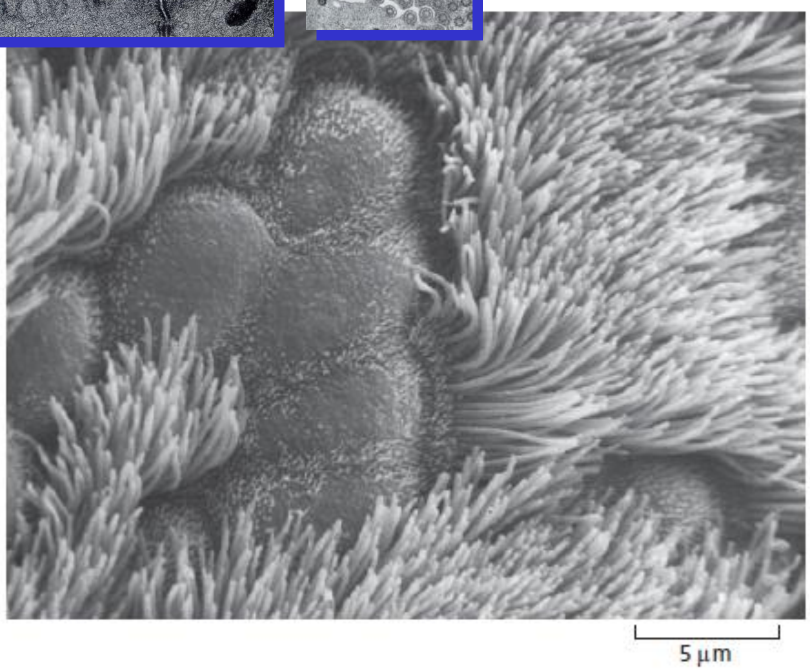


Cilia and Flagella

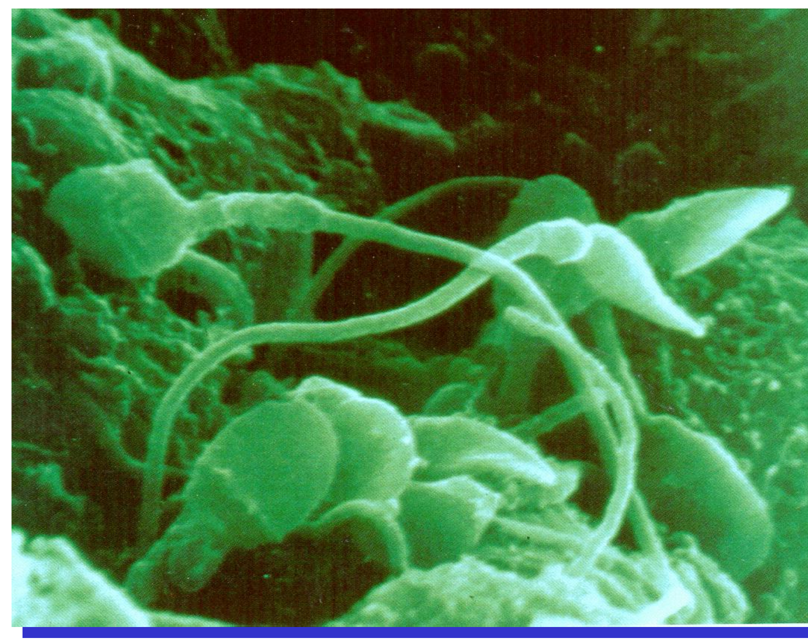
Cilia and **Flagella** Contain Stable Microtubules Moved by Dynein

Cilia





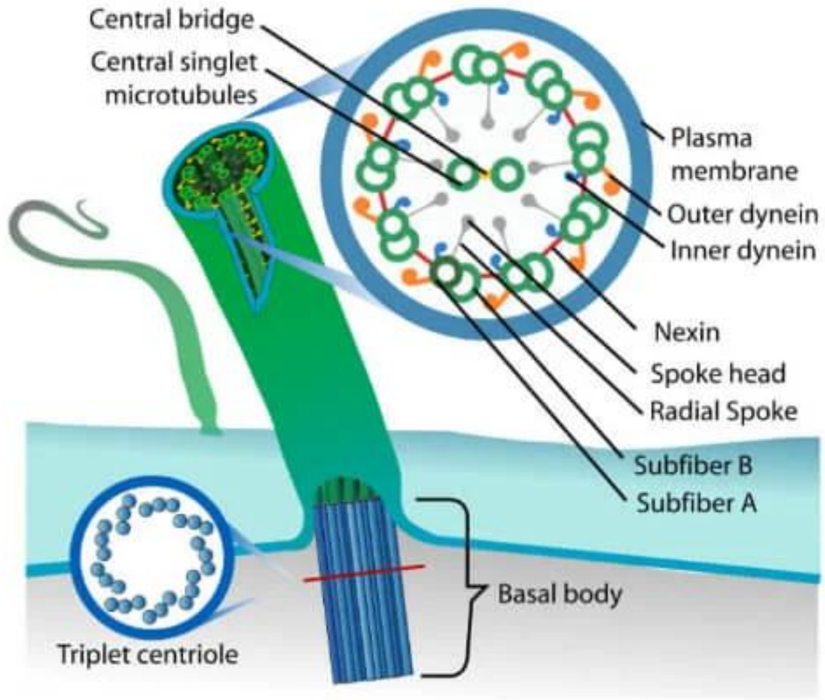
Flagella



Cilia and Flagella

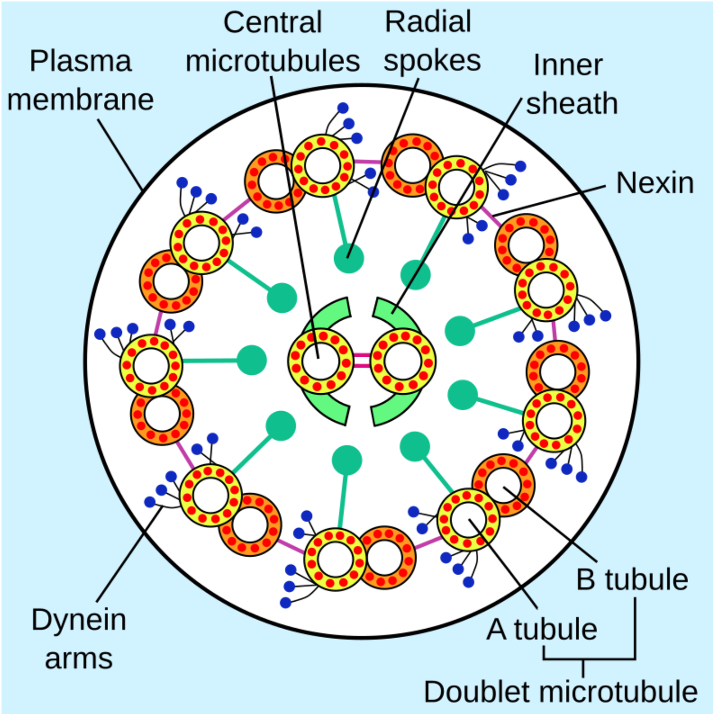
The microtubules in cilia and flagella are arranged in distinctive pattern

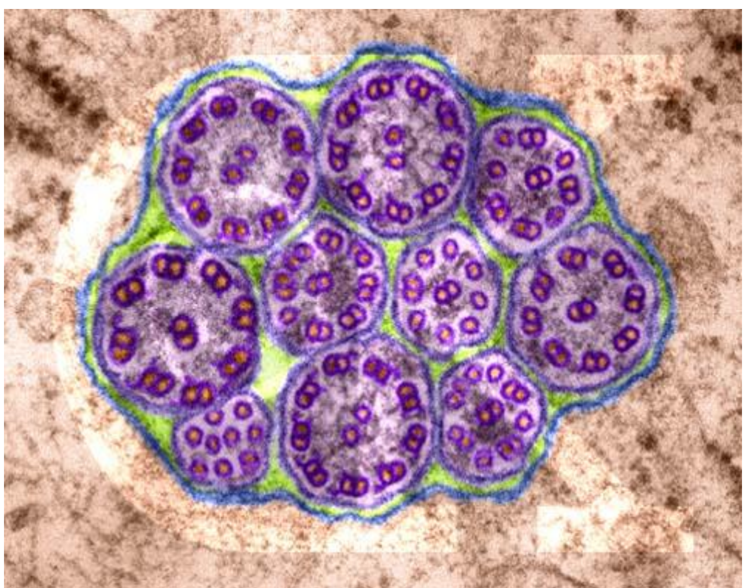
“9 + 2” array : the **Axoneme**

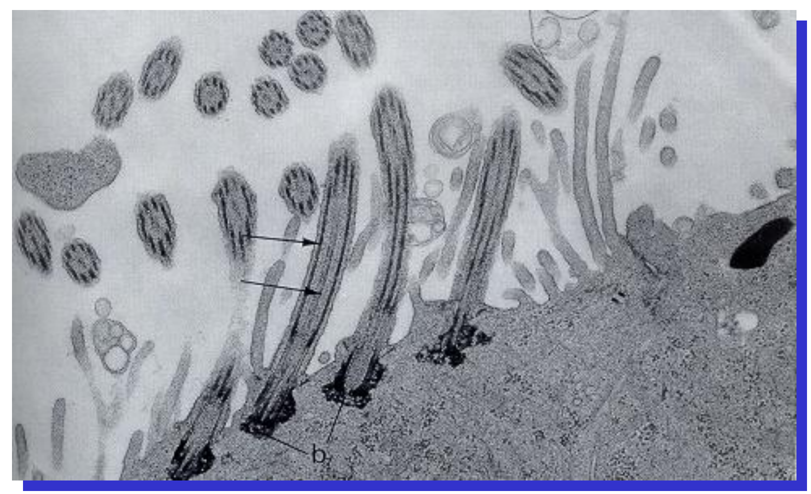


Structure of Eukaryotic Cilium

Cilia and Axoneme

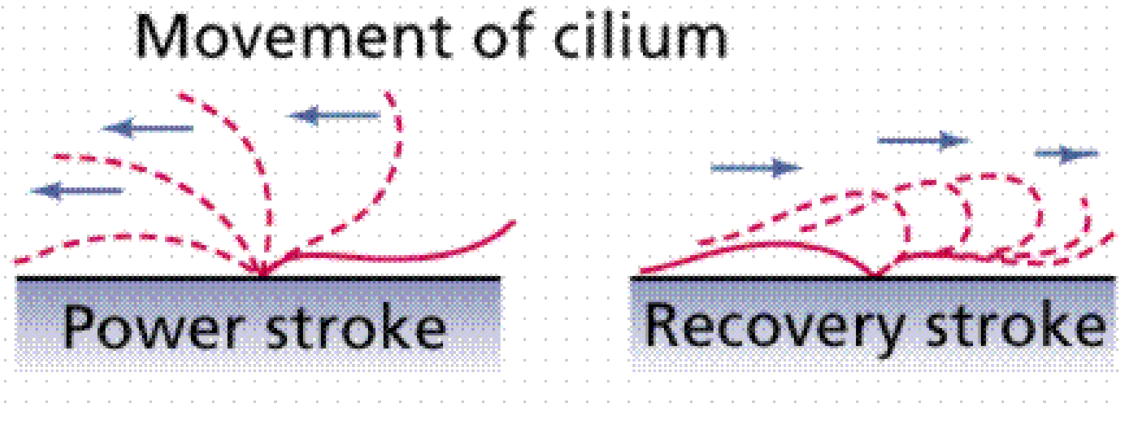


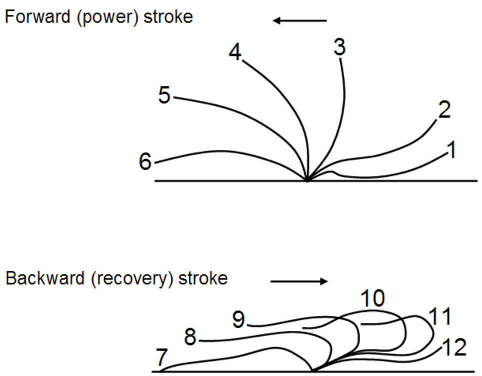




Cilia and Flagella

The ciliary beat cycle





1) **Power stroke**: The cilium is held straight and moves forcefully through an arc. This is the stroke that generates propulsion.

2) **Recovery stroke**: The cilium bends and sweeps back towards the cell surface in a more flexible, slower movement. The bending reduces the cilium's height and prevents it from interfering with the forward motion created by the power stroke.

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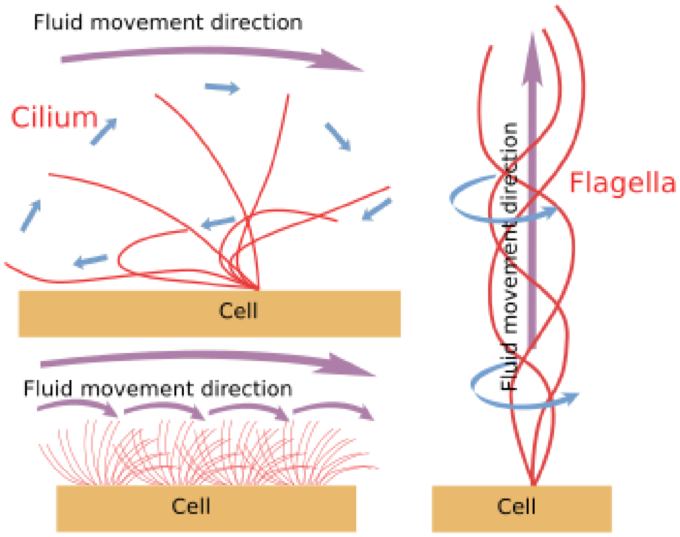
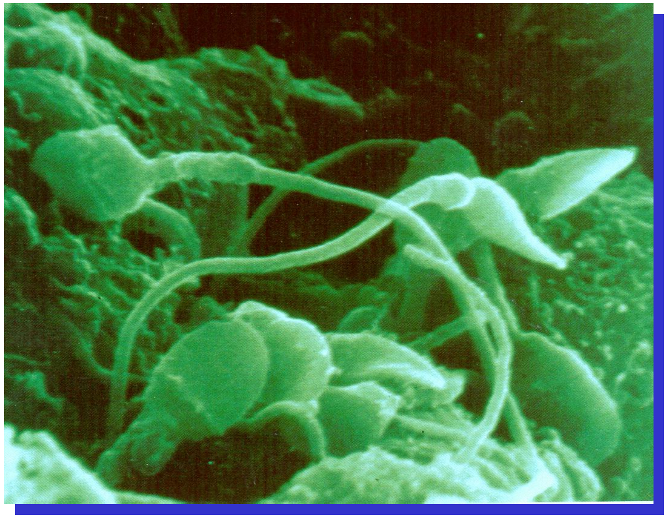
Cilia and Flagella

Flagella move like a propeller, rotating 360 degrees to propel a cell forward





Planar waves: The flagellum moves in an S-shaped wave, similar to an eel swimming.
Oarlike beating: The flagellum moves back and forth like an oar.
Three-dimensional waves: Complex, three-dimensional wave patterns are also possible.

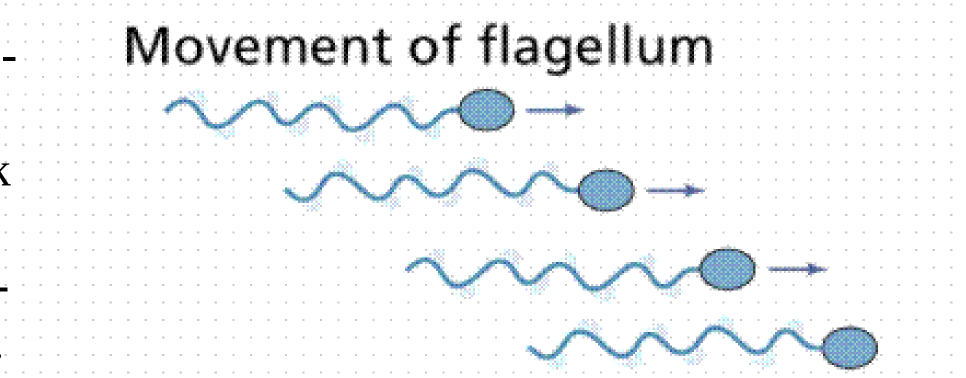
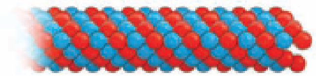


TABLE 2.4 Summary Characteristics of Three Types of Cytoskeletal Elements

	Actin Filaments (Microfilaments)	Intermediate Filaments	Microtubules
			
Shape	Double-stranded linear helical array	Rope-like fibers	Nonbranching long, hollow cylinders
Diameter (nm)	6–8	8–10	20–25
Basic protein subunit	Monomer of G-actin (MW 42 kDa)	Various intermediate filament proteins (MW ~50 kDa)	Dimers of α- and β-tubulin (MW 54 kDa); γ-tubulin found in MTOC is necessary for nucleation of microtubules; δ-, ϵ-, ζ-, η-tubulins are associated with MTOC and basal bodies
Enzymatic activity	ATP hydrolytic activity	None	GTP hydrolytic activity
Polarity	Yes; minus (−) or pointed end is slow-growing end Plus (+) or barbed end is faster-growing end	Nonpolar structures	Yes; minus (−) end is nongrowing end embedded in MTOC Plus (+) end is the growing end
Assembly process	Monomers of G-actin are added to growing filament Polymerization requires presence of K^+, Mg^{2+}, and ATP, which is hydrolyzed to ADP after each G-actin molecule is incorporated into the filament	Two pairs of monomers form two coiled-coil dimers; then two coiled-coil dimers twist around each other to generate a staggered tetramer, which aligns along the axis of the filament and binds to the free end of the elongating structure	At the nucleation site, α- and β-tubulin dimers are added to γ-tubulin ring Each tubulin dimer binds to GTP before it becomes incorporated into the microtubule in the presence of Mg^{2+} After polymerization, GTP is hydrolyzed to GDP
Source of energy required for assembly	ATP	N/A	GTP
Characteristics	Thin, flexible filaments	Strong, stable structures	Exhibit dynamic instability
Associated proteins	Variety of ABPs with different functions: fascin – bundling; gelsolin – filament severing; CP protein – capping; spectrin – cross-linking; myosin I and II – motor functions	Intermediate filament–associated proteins: plectins bind microtubules, actin, and intermediate filaments; desmoplakins and plakoglobins attach intermediate filaments to desmosomes and hemidesmosomes	Microtubule-associated proteins: MAP-1, -2, -3, and -4; MAP-τ; and TOG-ρ regulate assembly, stabilize, and anchor microtubules to specific organelles; motor proteins—dyneins and kinesins—required for organelle movement
Location in cell	Core of microvilli Terminal web Concentrated beneath plasma membrane Contractile elements of muscles Contractile ring in dividing cells	Extend across cytoplasm connecting desmosomes and hemidesmosomes In nucleus just beneath inner nuclear membrane	Core of cilia Emerge from MTOC and spread into periphery of cell Mitotic spindle Centrosome
Major functions	Provide essential components (sarcomeres for muscle cells)	Provide mechanical strength and resistance to shearing forces	Provide network ("railroad tracks") for movement of organelles within cell Provide movement for cilia and chromosomes during cell division

ABP, actin-binding protein; *ADP*, adenosine diphosphate; *ATP*, adenosine triphosphate; *GDP*, guanosine diphosphate; *GTP*, guanosine triphosphate; *kDa*, kilodalton; *MAP*, microtubule-associated protein; *MTOC*, microtubule-organizing center; *MW*, molecular weight; *N/A*, not applicable.