CYTOSKELETON

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I. GENERALITIES

- The cytoskeleton is a **complex network** of filaments and tubules located in the cytoplasm of eukaryotic cells.
- It is composed of **three filamentous** structures : microfilaments, microtubules, and intermediate filaments.
- These are **polymers** of protein subunits held together by weak non-covalent bonds and exhibit structural and functional differences.
- Each component of the cytoskeleton has its own specific properties :
  - **Microtubules** are long, hollow, unbranched tubes made of a protein called tubulin.
  - **Microfilaments** are thin, solid structures, often organized into a branched network, composed of the protein actin.
  - **Intermediate filaments** are strong, rope-like fibers made of various structurally related proteins.
- The cytoskeleton is **dynamic** and can rapidly reorganize to meet the functional needs of the cell.
- It plays a fundamental role in maintaining cell structure, enabling cell movement, and supporting various intracellular functions.

II. MICROFILAMENT

Microfilaments have a diameter of approximately 7 nm and are composed of globular subunits of a protein called actin. In the presence of ATP, actin monomers polymerize to form a flexible helical filament. Each actin subunit has polarity, and since all subunits in an actin filament are oriented in the same direction, the resulting microfilament is polarized.

Polymerization does not occur at the same rate at both ends of an assembled microfilament. The (+) end, where polymerization is rapid, elongates faster than the (–) end, where polymerization

is slower. Actin represents 5% of total proteins in non-muscle cells and 20% in muscle cells. Generally, actin is distributed throughout the cytoplasm. At the periphery of animal cells, within the cell cortex, microfilaments are arranged in bundles.
Cortical microfilaments have a mechanical role: the shape of the cell depends on the distribution, organization, and density of the actin network, which pushes organelles toward the endoplasm.

Actin-binding proteins (ABP) are proteins that bind to microfilaments and regulate their polymerization and depolymerization, their organization within the cell, and their interactions with the plasma membrane. Microfilaments also associate with myosin II molecules within contractile bundles. Myosins constitute a family of motor proteins (types I to XVIII). Myosin is associated with actin in muscle cells as well as in other cell types.
Contractile bundles may insert into the plasma membrane at focal adhesion sites, form contractile rings, or be associated with junctional complexes. Contraction depends on ATP-dependent sliding movements of microfilaments relative to one another, which are generated by myosin II.

- **Focal adhesions** are sites where actin contractile bundles interact with the plasma membrane, which at this level adheres to the extracellular matrix.
- **Contractile rings** form at the end of mitosis in animal cells and during cytokinesis. A submembranous ring composed of actin microfilaments and myosin II constricts to separate the mitotic cell into two daughter cells.
- **Adherens junctions** are junctional zones located near the surface of epithelial cells (such as enterocytes) that encircle the cell and are connected by contractile bundles.

Microfilaments, together with myosin I, participate in organelle transport and in sliding movements of actin filaments. Myosin I, which has a single head and a tail, is responsible for movement along microfilaments. Myosin I binds organelles via its tail and binds to a microfilament via its head. Movement occurs from the negative end toward the positive end of the filament, that is, toward the plasma membrane.
In skeletal muscle fibers, actin microfilaments and myosin II are organized into myofibrils. Muscle contraction results from the sliding of actin microfilaments relative to myosin myofilaments. Microfilaments associate with the basal lamina surrounding each striated muscle fiber through dystrophin.

III. MICROTUBULES

Microtubules are relatively rigid tubular structures present in eukaryotic cells. They appear as cylinders with an external diameter of 25 nm and an internal lumen of 15 nm. Their length can vary from a fraction of a micrometer to several tens of micrometers. The microtubule wall consists of globular proteins arranged in longitudinal rows called protofilaments, aligned parallel to the long axis of the tubule. In cross-section, microtubules contain 13 protofilaments arranged in a circle. Each protofilament is assembled from dimeric blocks composed of one α-tubulin and one β-tubulin globular subunit. Tubulin dimers assemble linearly along protofilaments. Because each assembly unit contains two different elements (heterodimers), the protofilament is asymmetric: one end terminates with α-tubulin and the other with β-tubulin. All protofilaments share the same polarity, giving the entire microtubule structural polarity.

The rate of tubulin association (polymerization) or dissociation (depolymerization) varies depending on the cell's needs. Each microtubule has a **plus (+) end** (terminating in β-tubulin), located near the plasma membrane, and a **minus (–) end** (terminating in α-tubulin), located in the central region of the cell, in the **microtubule-organizing center (MTOC)** or centrosome near the nucleus and the Golgi region. Microtubule structural polarity plays an essential role in their growth and in their participation in oriented mechanical activities.

Microtubule-associated proteins (MAPs) interact with microtubules and are classified into stabilizing/binding proteins (e.g., CLIP-170) and motor proteins (kinesins, dyneins). MAPs typically have one domain that binds laterally to the microtubule and another filamentous domain extending outward. MAPs enhance microtubule stability and promote their assembly.

Two main types of microtubules exist :
a. cytosolic microtubules, and
b. microtubules of centrioles, cilia, and flagella.

a. Cytosolic microtubules

These microtubules are unstable polymers that continuously polymerize and depolymerize at their ends. They are in dynamic equilibrium with free tubulin dimers in the cytosol.

Cytosolic microtubules have several functions in association with specific proteins:

- They contribute to the maintenance and development of cell shape.
- They participate in intracellular transport.

There are two major protein complexes involved in microtubule-based transport:

a. Kinesins: centrifugal or anterograde transport toward the cell periphery.
b. Dyneins: centripetal or retrograde transport toward the cell center.

These complexes mediate the movement of molecules, vesicles, and organelles along microtubules (e.g., migration of endocytic vesicles and secretory granules, mRNA transport).

Transport of endocytic vesicles:
Pinocytic vacuoles and phagosomes bind to microtubules through CLIP-170 (Cytoplasmic Linker Protein). Dyneins then take over and transport vesicles toward the minus end of microtubules, accumulating them near the Golgi apparatus and lysosomes.

Directed transport of mRNA:
Microtubules and motor MAPs transport ribonucleoprotein complexes containing mRNA. mRNAs encoding cytoskeletal proteins are transported to their site of use via microtubules through the MAP 1A protein.

b. Microtubules of centrioles, cilia, and flagella

These structures contain stabilized microtubule polymers with much lower rates of polymerization and depolymerization than cytosolic microtubules. They serve several essential functions:

- **Cell movement:**
  Cilia and flagella contain microtubules that enable motility (e.g., sperm cells, paramecia).
- **Chromosome movement:**
  One of the functions of centrioles is to organize microtubule subunits into the mitotic spindle. Chromosome displacement depends on spindle microtubules.

IV. INTERMEDIATE FILAMENT

Intermediate filaments (IFs) are tough, rope-like protein fibers with a diameter of 8–12 nm, intermediate between microfilaments and microtubules. They provide mechanical strength to cells subject to physical stress, such as neurons, muscle cells, and epithelial cells.

Intermediate filaments are polymers of fibrous proteins. Although protein composition varies by location and cell type, the basic filament structure is conserved. The basic unit is a monomer composed of a central α-helical rod domain with amino- and carboxy-terminal ends. Two monomers coil together to form a dimer. Two dimers arranged antiparallel associate to form a

tetramer. Longitudinal assembly of tetramers forms a protofilament. Eight protofilaments assemble laterally into a cylindrical, non-polar intermediate filament. Intermediate filaments are often interconnected with other cytoskeletal elements by thin filamentous linkers. In many animal cells, these linkers are formed by an elongated protein called plectin (a member of the plakin family). Plectin has binding sites for intermediate filaments and, depending on the isoform, binding sites for microtubules or actin microfilaments.

Intermediate filaments are made of a large variety of proteins expressed in specific cell types. Five major categories of IFs are distinguished based on their constituent proteins:

- **Cytokeratin filaments** in epithelial cells
- **Neurofilaments** in neuronal axons
- **Desmin filaments** in muscle cells
- **Vimentin filaments** in mesenchymal cells
- **Lamin filaments** in the nucleoplasm

In epithelial cells, intermediate filaments are distributed around the nucleus and beneath the plasma membrane. They anchor to membrane proteins through cytoplasmic linker proteins. They extend from the perinuclear region to the cell periphery. In epithelial cells, they attach to the cytoplasmic plaques of desmosomes (macula adherens), providing mechanical strength and stability.

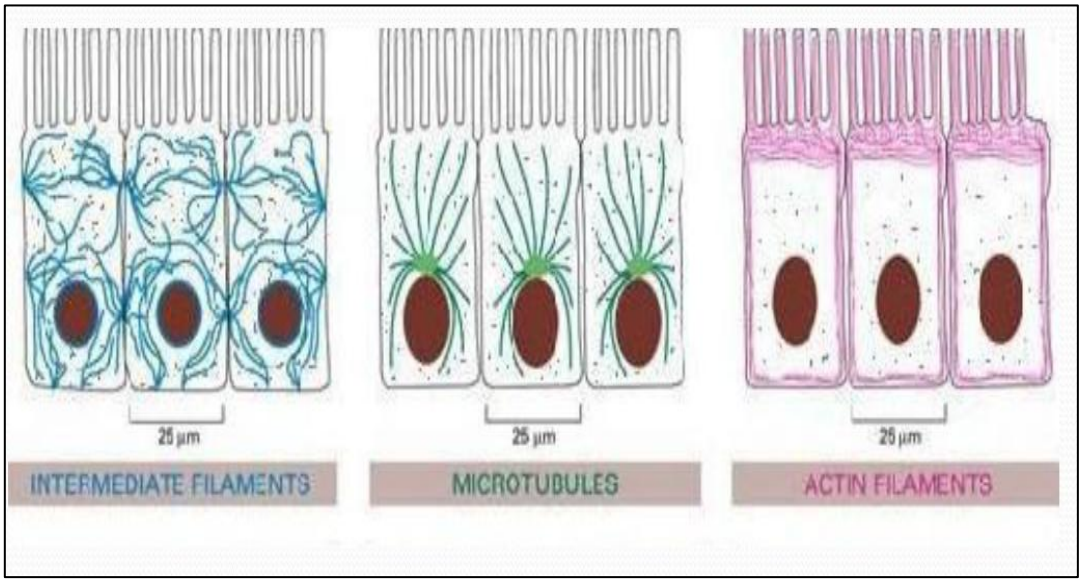
In neuronal axons, neurofilaments form a supportive framework contributing to rigidity and resistance.

In striated skeletal muscle cells, desmin filaments connect the inner face of the plasma membrane to myofibrils and participate in transmitting the forces generated during contraction or relaxation.

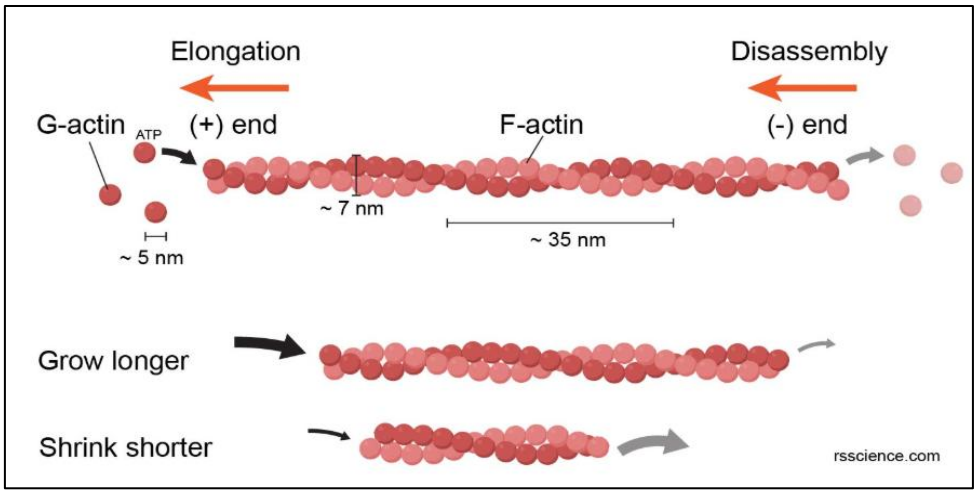
In the nucleus, lamins are located beneath the inner nuclear membrane, forming the nucleoskeleton. Their anchoring depends on specific receptors on the nuclear envelope. Lamins maintain nuclear shape and serve as anchoring sites for DNA. They are involved in mitosis: during mitosis, lamins attach to specific binding sites on mitotic chromosomes, allowing them to participate in nuclear reassembly, particularly reconstruction of the nuclear envelope.

Comparison between the three components of the cytoskeleton

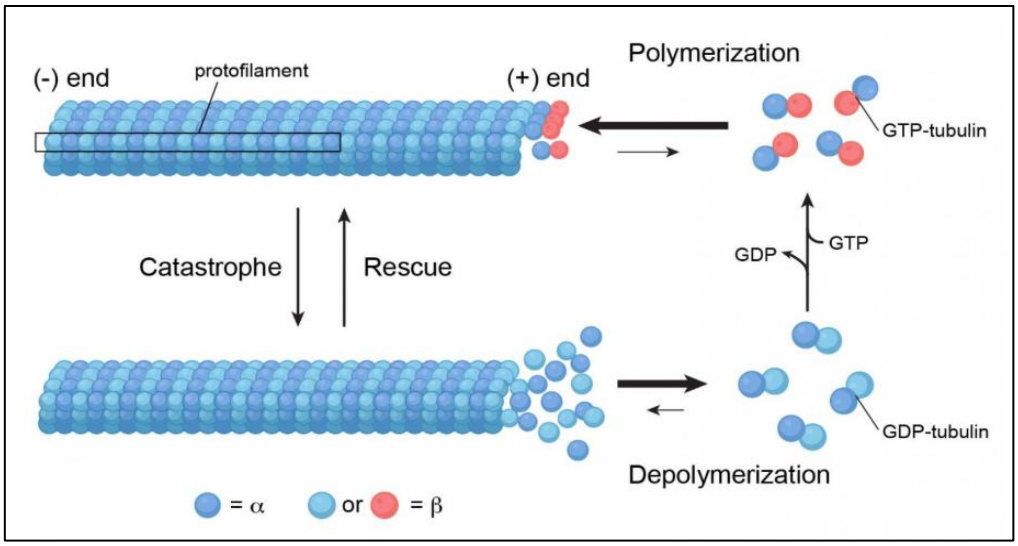
	Microtubules	Microfilaments	Intermediate Filament
Structure	Hollow, rigide tube	Flexible helical filament	Resistant fibers
Dimensions	25 nm	7 nm	10-12nm
Distribution	All eukaryotes	All eukaryotes	Animal cells
polarity	Yes	Yes	No
Enzymatic activity	GTPase	ATPase	None
Motor Protein	Kinesin, Dynein	Myosins	None
Main Associated Protein Group	MAPs (associated proteins)	ABPs (Actin-binding proteins)	Plakins



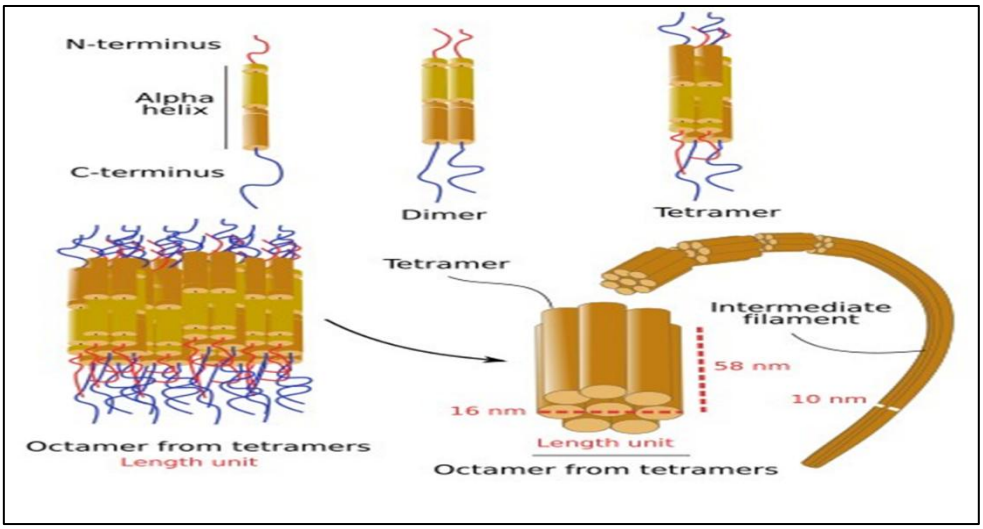
Distribution of the cytoskeleton within the cell.



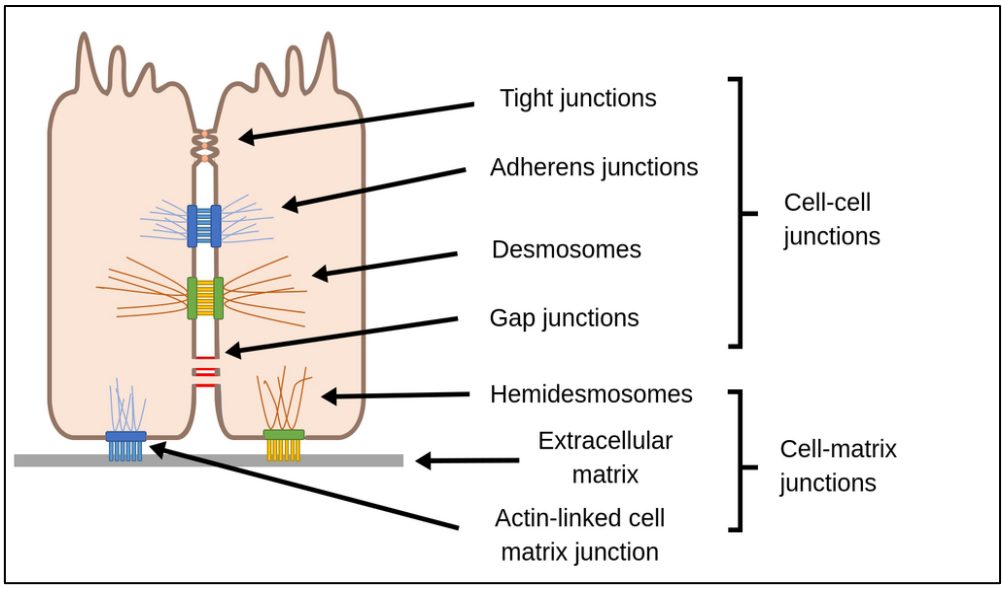
Polymerization of G-actin units into a F-actin filament.



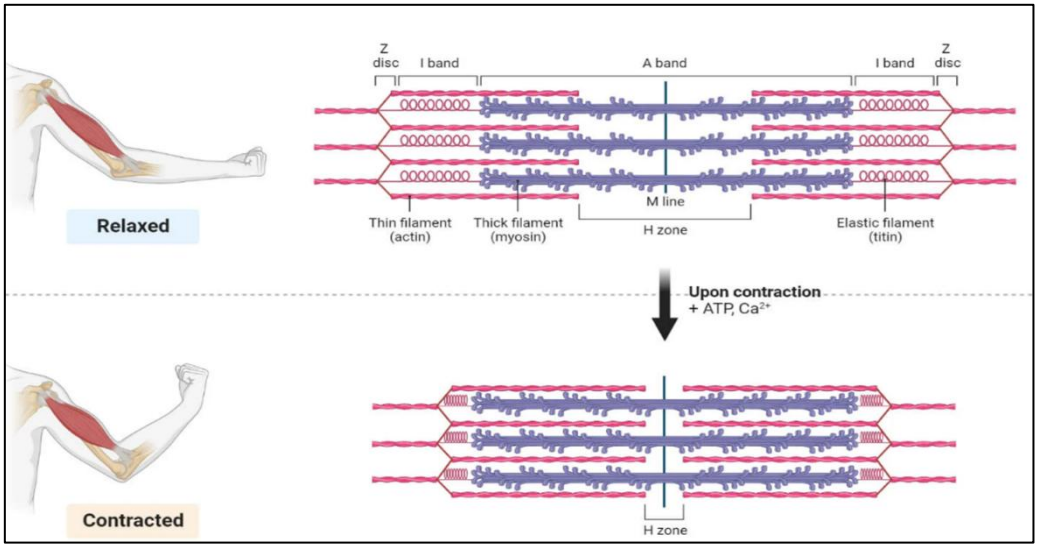
Polymerization and depolymerization of Microtubules.



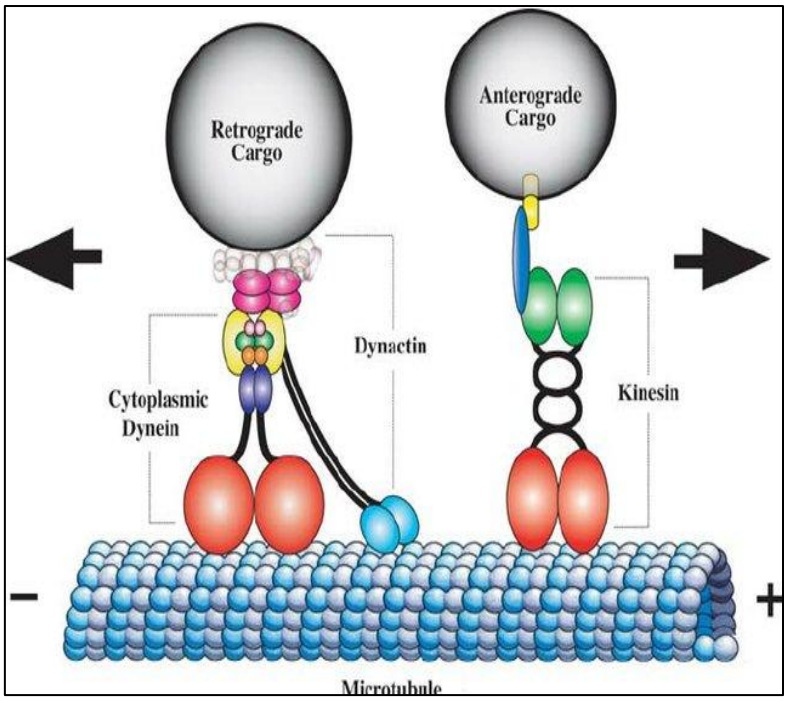
Assembly of Intermediate filament.



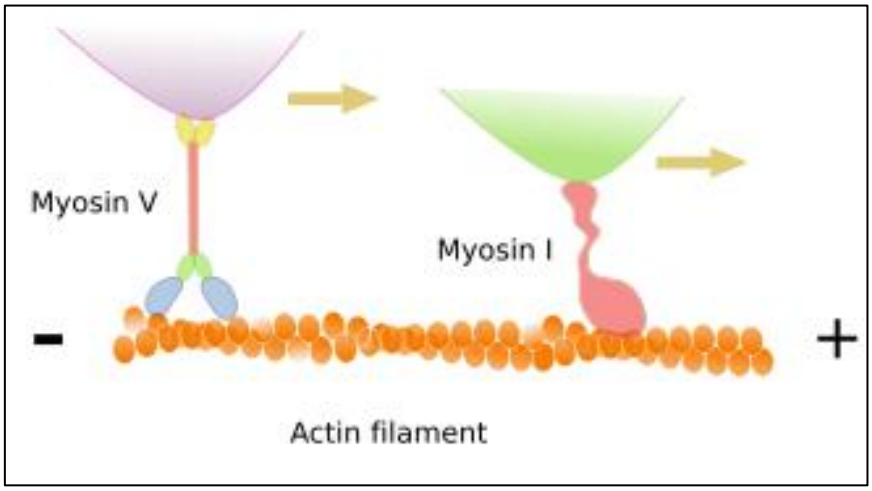
Adhesion contacts (cell-cell or cell-MEC) involving microfilaments and intermediate filaments.



Muscle contraction.



Motor protein of Microtubules : Dynein and kinesin.



Motor protein of Microfilament : Myosin.