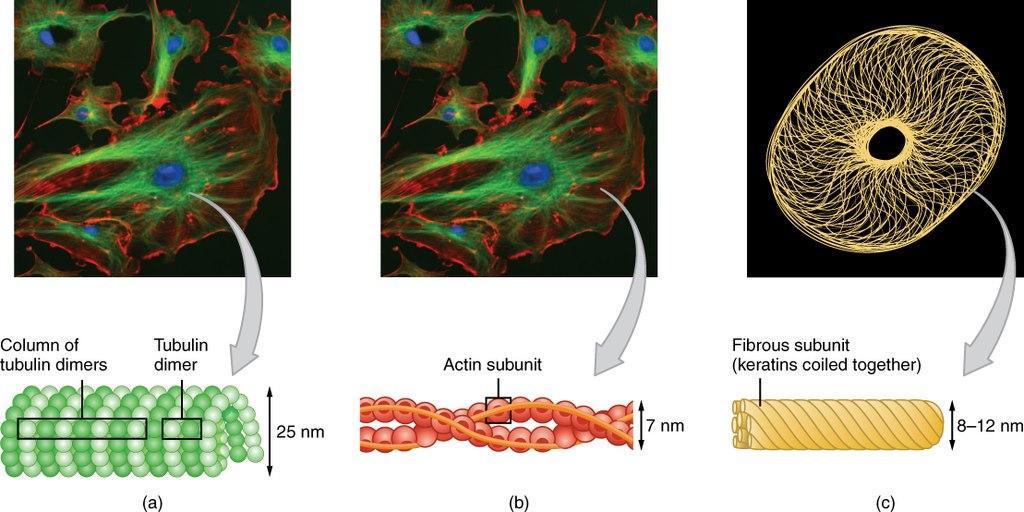
Chapter 1: Cytoskeleton

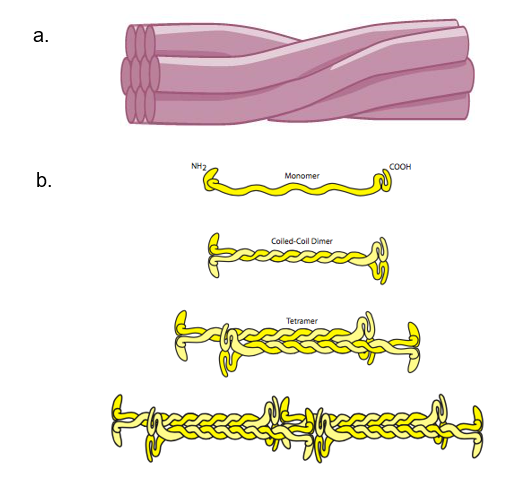
The cytoskeleton is a network of different protein fibers that provide many functions. It maintains or changes the shape of the cell, secures some organelles in specific positions, enables the movement of cytoplasm and vesicles within the cell, and enables the cell to move in response to stimuli. There are three types of fibers within the cytoskeleton: microfilaments (also called actin filaments), intermediate filaments, and microtubules. Some of the cytoskeletal filaments work in conjunction with molecular motors that move along the filaments within the cell to carry out a diverse set of functions (Fig 1-1).



**Figure 1-1: The three types of cytoskeletal filaments**. Images showing the location within cells (top) and structure (bottom) of individual cytoskeletal filaments. (a) microtubules (green in the fluorescent image), (b) microfilaments/actin (red in the fluorescent image) and, (c) intermediate filaments.

## Intermediate Filaments

Intermediate filaments are made of several strands of fibrous proteins that are wound together. They are the most diverse group of cytoskeletal elements. There are many types of intermediate filaments, which collectively have diameters between those of microtubules and microfilaments. This is why they are called "intermediate” filaments. One example of an intermediate filament is **keratin**, which has a diameter of 8-12nm (Fig 1-1). Keratin is the fibrous protein that strengthens your hair, nails, and the epidermis of the skin. Intermediate filaments are described as rope-like proteins, made of filamentous monomers that twist together to provide strength (Fig 1-2a).

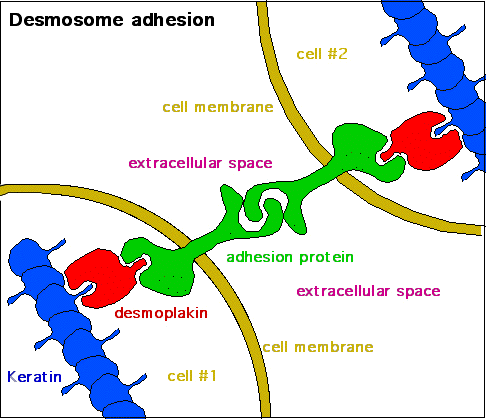


**Figure 1-2: Structure of intermediate filaments**. a) Rope-like polymers of intermediate filaments are made from several filamentous protein monomers wrapped together. b) Intermediate filaments are made from several filamentous proteins staggered and wrapped together.

Intermediate filaments have no role in cell movement. Their function is purely structural. They bear tension, providing **tensile strength**, which is the strength to help a cell withstand mechanical stress. Therefore, they help to maintain the shape of the cell and anchor the nucleus and other organelles in place. The basic unit of intermediate filament structure is a dimer of monomers, called a coiled-coil. Dimers further aggregate to form tetramers and larger filament bundles. The dimers are formed and then are staggered together, generating tetramers, which then bind together, resulting in twisted strands of protein that provide tensile strength to cells (Fig 1-2b).

**Intermediate Filaments: Keratin**

Skin cells have a high concentration of the intermediate filament keratin. Keratin forms a mesh-like network throughout cells. Additionally, these keratin networks connect to keratin networks of adjacent cells via bundles of keratin fibers in a cellular adhesion structure called a **desmosome** (Fig 1-3). Desmosomes are necessary for the structural integrity of epithelial layers and are the most common cell-cell junction in such tissues. Due to desmosomes, the pressure that might be able to burst a single cell can be spread out over many cells, sharing the burden, and thus protecting each member. Keratin, therefore, provides tensile strength to skin cells, allowing them to move together as a sheet, rather than separating in response to mechanical stressors (like a pinch).



**Figure 1-3: Desmosomes**. Desmosomes are cell adhesion structures between two neighboring cells, which connect keratin from one cell to the keratin of a neighboring cell, using a series of adaptors and adhesion proteins.

Loss-of-function mutations in keratins lead to the inability of each individual cell to maintain structural integrity under pressure. Mutations in keratin genes can lead to conditions collectively termed *epidermolysis bullosa*, in which the skin is extraordinarily fragile, blistering, and breaking down with only slight contact. This compromises the patients' first line of defense against infection, the skin (Fig 1-4).



**Figure 1-4: Defects in keratin.** Image of a patient with *epidermolysis bullosa* suffering from skin lesions due to mutations in keratin genes.

**Intermediate Filaments: Lamins**

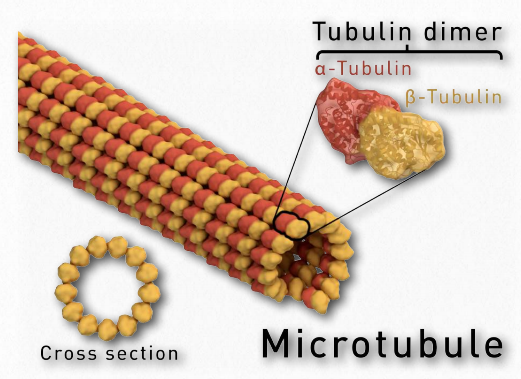
Another type of intermediate filaments are **lamins**. They make up structural elements of the **nuclear lamina**, a kind of nucleoskeleton. The nuclear lamina is located just inside of the nuclear envelope and is essential in maintaining its structure. The nuclear lamina also plays an important role in cellular functions including mitosis, during which the nuclear envelope breaks down during prophase and reforms during telophase. Notably, defects in nuclear lamin proteins have been associated with progeria. Figure 1-5 demonstrates the structure of the nuclear lamin from healthy patients (top right) as compared with those isolated from progeria patients (bottom right). As shown, progeria patients have irregularly-shaped nuclear envelopes. These irregularities are associated with defects in mitosis, which cause premature aging in patients with these defects in lamins (left panel).

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**Figure 1-5: Progeria**. Image of a patient with progeria (left) and an image of the nuclear lamina (green) in healthy patients (top right) and progeria patients (bottom right).

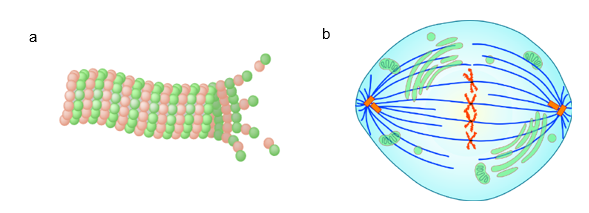
## Microtubules

Microtubules are the thickest type of cytoskeleton with a diameter of 25nm (Fig 1-1) and are found throughout the cytoplasm. These polymers are made up of globular protein subunits called **α-tubulin** and**β-tubulin** that attach to the polymer as tubulin dimers. The tubulin dimers stack to form individual **protofilaments**, thirteen protofilaments associate to form the hollow tube of the final polymerized microtubule (Fig 1-6).



**Figure 1-6: Structure of microtubules.** Image of the fully assembled microtubule made up of dimers of α-tubulin and β-tubulin stacked to form protofilaments that assemble to form a hollow tube structure.

Both the α-tubulin and β-tubulin subunits bind to GTP. When bound to GTP, the formation of the microtubule can begin, this is called the **nucleation event**. As more GTP tubulin dimers assemble onto the filament, GTP is slowly hydrolyzed by β-tubulin to form GDP. Tubulin bound to GDP is less structurally robust and can lead to disassembly of the microtubule. Microtubules have a distinct polarity that is critical for their biological function. Tubulin polymerizes end to end, with the β-subunits of one tubulin dimer contacting the α-subunits of the next dimer. These differences lead to different subunits being exposed on the two ends of the filament. The ends are designated the "(−)" and "(+)" ends. Microtubules can elongate at both the "(+)" and "(-)" ends, but elongation is significantly more rapid at the "(+)" end. The ability of the microtubule to assemble and disassemble, thereby changing its length is termed **dynamic instability**. Note that dynamic instability is a feature specific to microtubules and microfilaments/actin. The breakdown and reformation of nuclear lamins is not a type of dynamic instability. Fig 1-7a demonstrates the disassembly, or shortening, or the microtubules as the protofilaments that make up the hollow microtubule "peel" back.



**Figure 1-7: Dynamic instability of microtubules.** a) Image showing that microtubules display dynamic instability and have the ability to disassemble, whereby protofilaments peel back from the structure, and then dimers are released. b) Image showing that microtubules are involved in mitosis during the cell cycle when sister chromatids (orange) attach to spindle fibers (blue) during metaphase for separation during mitosis.

This dynamic instability is integral for some cellular functions. One example of this is the movement of chromosomes to opposite poles of a dividing cell during mitosis, which is dependent on polymerization and de-polymerization of microtubules, called spindle fibers, that originate from the microtubule organizing center (MTOC) (Fig 1-7b). In addition to their role in mitosis, microtubules facilitate cellular transport by providing pathways for vesicles to be carried through the cell. Microtubules are also the primary structural elements of flagella, cilia, and centrioles.

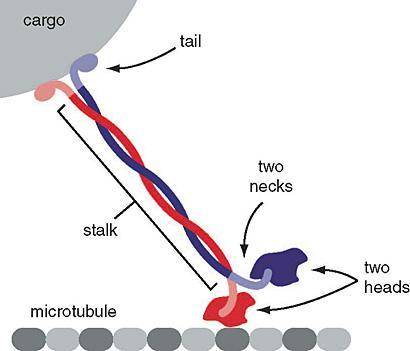
**Microtubule Motor Proteins**

As previously noted, one function of microtubules is to move cellular components from one part of the cell to another. These cellular components are called "cargo" and are often stored within a vesicle for transport. You can think of the cytoskeleton as "railroad tracks," providing support and directionality inside of the cell.

Of course, if there are "railroad tracks," there needs to be an engine that can both move on the tracks and pull or push cargo along. In this case, the engines are molecular motors, called **motor proteins** that can move along the tracks in a specific direction. There are two families of molecular motors associated with microtubules; **dyneins** and**kinesins**. These motor proteins and the cytoskeleton create a comprehensive network within the cell for moving vesicles from one organelle to another or from one organelle to the cell surface.

**Motor Proteins: Kinesin**

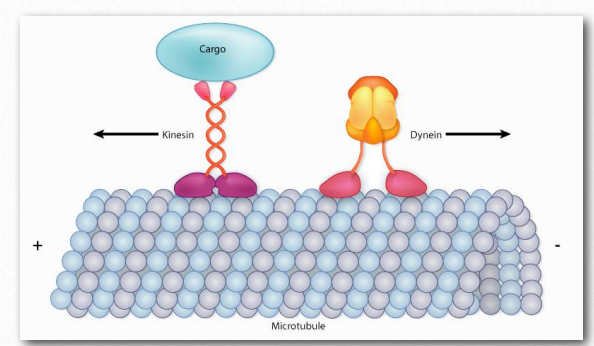
**Kinesins** are motor-protein complexes that "walk" along the microtubules and are involved in vesicle transport. The polarity of kinesin movement is from the "(-)" end of the microtubule to the "(+)" end using energy supplied by the hydrolysis of ATP, therefore they are called **plus end-directed motor proteins**. In most cells, this entails transporting cargo from the center of the cell towards the periphery. This form of transport is known as **anterograde transport**. The motor protein is made up of several parts, the tail regions of kinesins bind to the cargo, a vesicle, for example, the stalk region, and then two heads that interact with the microtubule itself (Fig 1-8). The globular heads "walk" along the microtubule towards the (+) end of the microtubule.



**Figure 1-8: Kinesin.** The motor protein kinesin (red and blue), has the head portion of the protein "walking" along the microtubule while the 'tails" are bound to vesicles carrying cargo proteins.

**Motor Proteins: Dynein**

**Dynein** is a second protein complex that functions as a molecular motor. In cells, it converts the chemical energy from ATP hydrolysis into the mechanical energy of movement to 'walk' along the microtubule while carrying a vesicle. Dyneins bind to microtubules and move or "walk" from the plus "(+)" end of the cytoskeletal microtubule filament to the minus "(-)" end of the filament, which is usually oriented towards the cell center. Thus, they are often referred to as **minus end-directed motor proteins**, moving in the opposite direction as kinesin (Fig 1-9). Cytoplasmic dynein moves progressively along the microtubule, hydrolyzing ATP with each "step" it takes along the microtubule

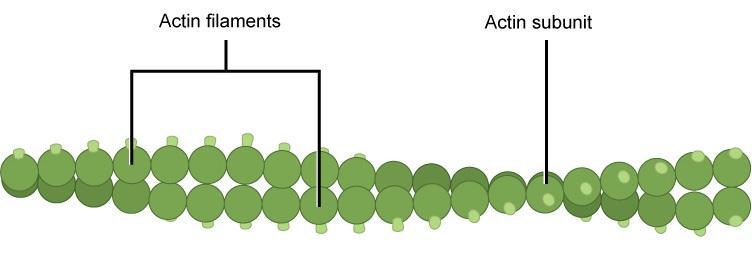


**Figure 1-9: Kinesin and dyenin**. The motor proteins associated with microtubules, kinesin and dynein, move in opposite directions along the microtubule. Kinesin is plus end-directed and moves towards the (+) end, while dynein is minus end-directed and moves towards the (-) end of the microtubule.

Cytoplasmic dyneins are used in many different processes. They are involved in organelle movement such as the positioning organelles in the cell and are used in the transport of cargo such as the movement of vesicles made by the endoplasmic reticulum, endosomes, and lysosomes.

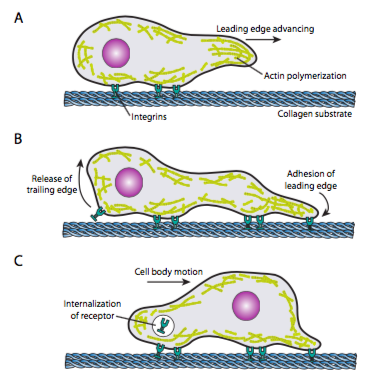
## Microfilaments (Actin Filaments)

**Microfilaments** (also called **actin filaments**) are cytoskeleton fibers composed of **actin** subunits. Microfilaments/actin filaments are the thinnest type of cytoskeleton with a diameter of 7nm (Fig 1-1) Actin is one of the most abundant proteins in eukaryotic cells. Actin can be present as either a free monomer that is globular in shape or bound together in a helical polymer (Fig 1-10).



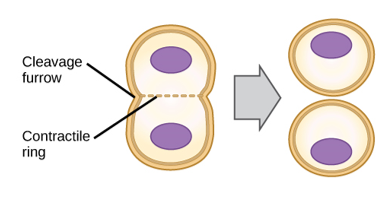
**Figure 1-10**: **Microfilaments/Actin**. Actin monomers polymerize to form a helical structure.

Actin must be bound to ATP to assemble into a polymer. The actin filament itself has structural polarity similar to microtubules. Again, this term "polarity" refers to the fact that there are two distinct ends to the filament. These ends are called the "(-)" end and the "(+)" end. At the "(+)" end, actin subunits are adding onto the elongating filament, and at the "(-)" end, actin subunits are disassembling or falling off of the filament. As seen in microtubules, the ability of the actin filament to assemble and disassemble, thereby changing its length, is termed dynamic instability. This dynamic instability is integral for some cellular functions, including cell crawling, which depends on rapid actin polymerization and de-polymerization. Fig 1-11 demonstrates the role of actin in cell crawling along a surface. Actin polymerization generates the leading edge of the cell, and the resulting cytoskeletal rearrangements allow movement of cells along a surface.



**Figure 1-11: Microfilaments/Actin in Cell Crawling**. Cells crawl by (a) extending the leading edge primarily through remodeling of the actin cytoskeleton, (b) forming new contacts at that leading edge while releasing contacts on the rear, and (c) movement forward to "catch up" with the leading edge.

Additionally, microfilaments participate in many cellular processes, including muscle contraction, vesicle and organelle movement, the maintenance of cell shape, and formation of the cleavage furrow during cytokinesis. During formation of the cleavage furrow, a contractile ring composed of actin filaments forms just inside the plasma membrane at the former metaphase plate. The actin filaments pull the equator of the cell inward, forming a fissure. This fissure, or "crack," is called the cleavage furrow. The furrow deepens as the actin ring contracts, and eventually, the membrane and cell are cleaved in two (Figure 1-12). Microfilaments are also included in cellular transport since microfilaments can serve as "railroad tracks" for a family of motor proteins called myosins.

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**Figure 1-12:** **The role of actin in cytokinesis**. Actin is involved in formation of the cleavage furrow and the contractile ring that helps separate the two daughter cells during cytokinesis

**Actin Motor Proteins: Myosin**

**Myosins** are a large class of motor proteins that interact with actin/microfilaments.Their movement also uses the energy of ATP hydrolysis to provide energy for the conformational changes needed for movement. While Myosin II is involved in muscle contraction, Myosin I is involved in vesicle movement along microfilaments using the head region of the protein to attach to the actin and the tail region to attach to the vesicle carrying the cargo. Myosin I is also considered a plus-end directed motor protein, carrying vesicles from the (-) end of actin towards the (+) end.

Associated Videos:

1. [Actin (white blood cell moving to chase bacteria)](https://youtu.be/AUih856vaQY)
2. [Comparison of cytoskeletal filaments](https://www.youtube.com/watch?v=YTv9ItGd050)
3. [Introduction to the cytoskeleton](https://www.youtube.com/watch?v=rvfvRgk0MfA)
4. [Microtubules and motor proteins](https://www.youtube.com/watch?v=Bxtgm5nMdJQ)
5. [Animation of a cytoplasmic dynein motor on a microtubule](https://www.youtube.com/watch?v=-7AQVbrmzFw)
6. [Mechanism of Kinesin moving on a microtubule](https://www.youtube.com/watch?v=YAva4g3Pk6k)
7. [A day in the life of a motor protein](https://www.youtube.com/watch?v=tMKlPDBRJ1E)