

# BME 1532-CELL BIOLOGY

## Cytoskeleton and Extracellular Matrix

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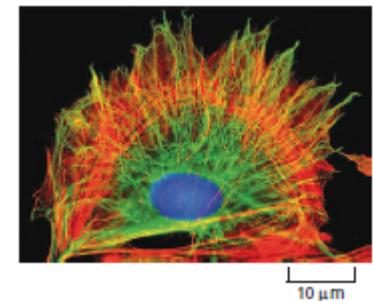
Yıldız Technical University  
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# Cytoskeleton

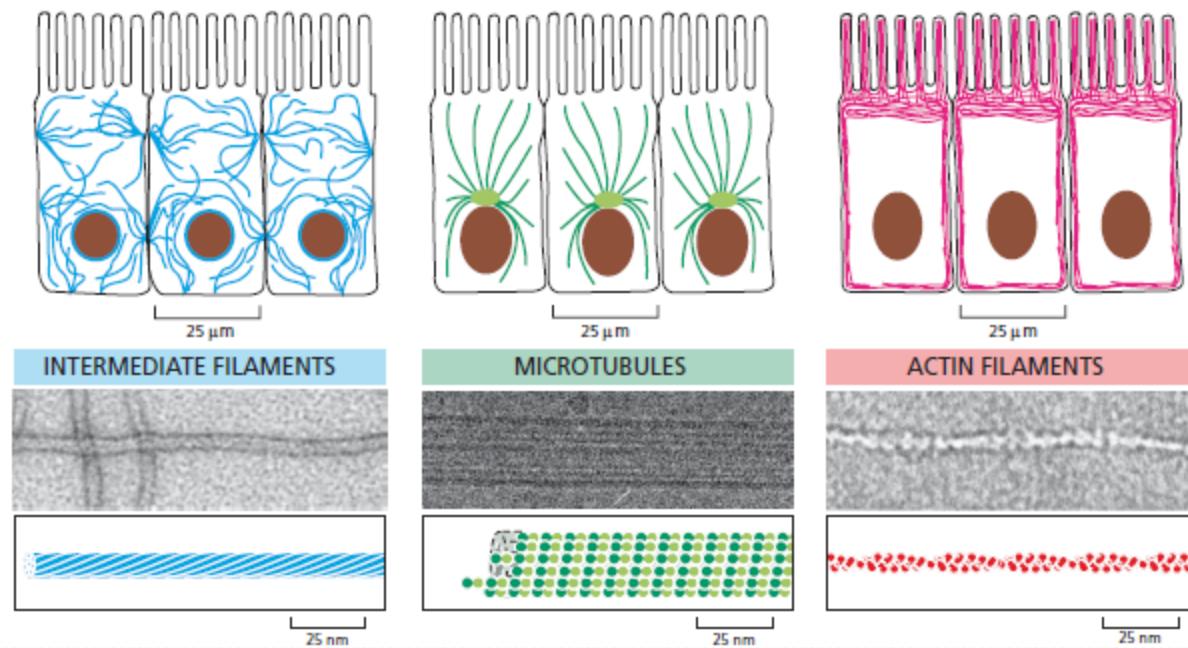
- Cytoskeleton is an intricate network of protein filaments that extends throughout the cytoplasm.
- The ability of eukaryotic cells to adopt a variety of shapes, organize the many components in their interior, interact mechanically with the environment, and carry out coordinated movements depends on the cytoskeleton.
- This filamentous architecture helps to support the large volume of cytoplasm, a function that is particularly important in animal cells, which have no cell walls.

- Unlike our own bony skeleton, however, the cytoskeleton is a highly dynamic structure that is continuously reorganized as a cell changes shape, divides, and responds to its environment.
- The cytoskeleton is not only the “bones” of a cell but its “muscles” too, and it is directly responsible for large-scale movements, including the crawling of cells along a surface, the contraction of muscle cells, and the changes in cell shape that take place as an embryo develops.
- Without the cytoskeleton, wounds would never heal, muscles would not contract, and sperm would never reach the egg.

- The cytoskeleton is built on a framework of three types of protein filaments: *intermediate filaments*, microtubules, and *actin filaments*.
- Each type of filament has distinct mechanical properties and is formed from a different protein subunit.
- A family of fibrous proteins forms the intermediate filaments; globular *tubulin* subunits form microtubules; and globular *actin* subunits form actin filaments.
- In each case, thousands of subunits assemble into fine threads that sometimes extend across the entire cell.



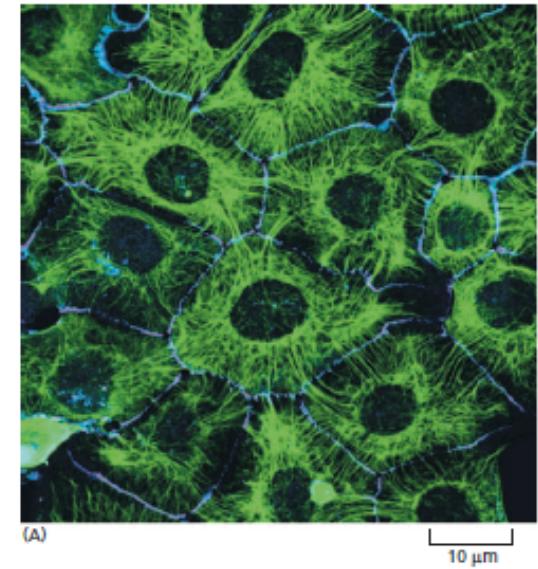
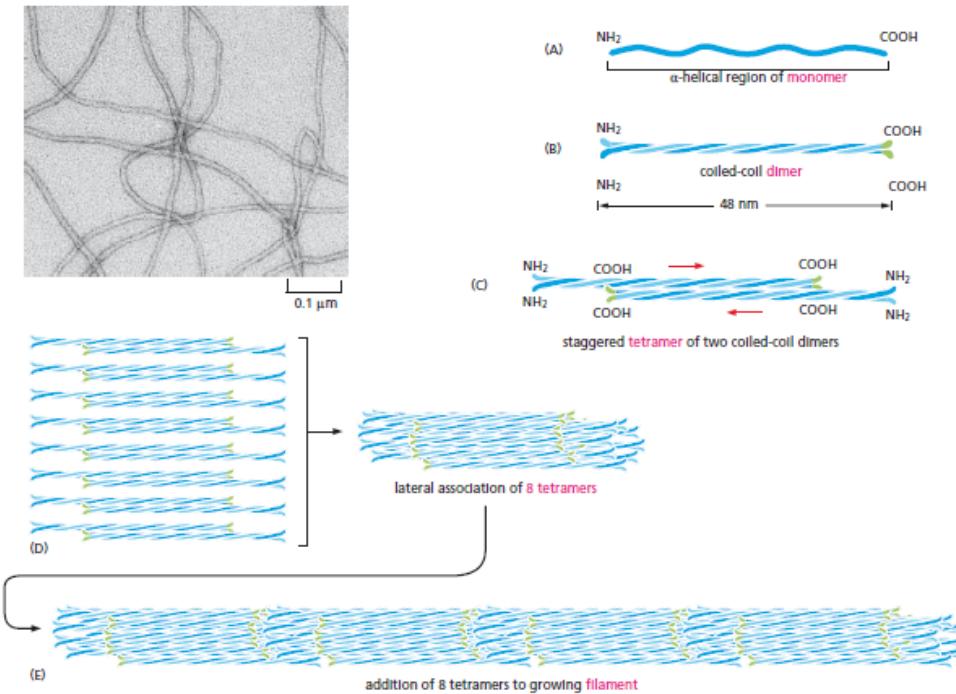
Microtubules (*green*) and the actin filaments (*red*). Where the two filaments overlap, they appear *yellow*. The DNA in the nucleus is labeled in *blue*.



# Intermediate Filaments

- Intermediate filaments have great tensile strength, and their main function is to enable cells to withstand the mechanical stress that occurs when cells are stretched.
- Intermediate filaments are the toughest and most durable of the cytoskeletal filaments.
- Intermediate filaments are found in the cytoplasm of most animal cells.
- They typically form a network throughout the cytoplasm, surrounding the nucleus and extending out to the cell periphery. There they are often anchored to the plasma membrane at cell-cell junctions.
- Intermediate filaments are also found within the nucleus of all eukaryotic cells. There they form a meshwork called the *nuclear lamina*, which underlies and strengthens the nuclear envelope.

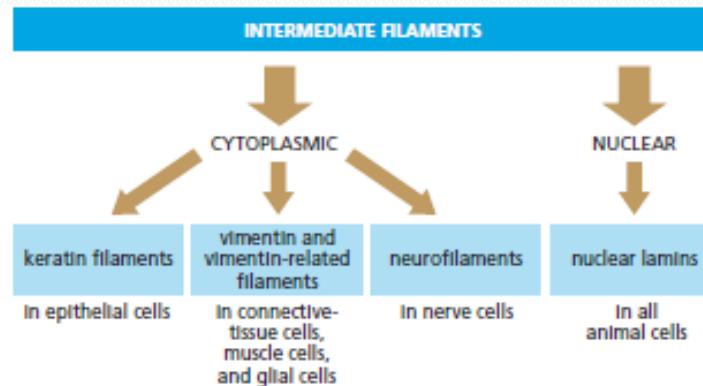
- An intermediate filament is like a rope in which many long strands (fibrous proteins) are twisted together to provide tensile strength.
- The strands of this cable are made of fibrous subunits.
- The rod domain enables pairs of intermediate filament proteins to form stable dimers by wrapping around each other in a coiled coil configuration.
- Two of these coiled-coil dimers, running in opposite directions, associate to form a staggered tetramer.
- The tetramers associate with each other side-by-side and then assemble to generate the final ropelike intermediate filament.



- Because the two dimers point in opposite directions, the two ends of the tetramer are the same, as are the two ends of assembled intermediate filaments.
- This distinguishes these filaments from microtubules and actin filaments, whose structural polarity is crucial for their function.
- All the interactions between the intermediate filament proteins depend solely on noncovalent bonding; it is the combined strength of the overlapping lateral interactions along the length of the proteins that gives intermediate filaments their great tensile strength.
- The central rod domains of different intermediate filament proteins are all similar in size and amino acid sequence, so that when they pack together they always form filaments of similar diameter and internal structure.
- By contrast, the terminal domains vary greatly in both size and amino acid sequence from one type of intermediate filament protein to another.
- These unstructured domains are exposed on the surface of the filament, where they allow it to interact with specific components in the cytoplasm.

# Intermediate filaments

- Intermediate filaments are particularly prominent in the cytoplasm of cells that are subject to mechanical stress such as neurons, muscle cells etc.
- Intermediate filaments can be grouped into four classes: (1) *keratin filaments* in epithelial cells; (2) *vimentin* and *vimentin-related filaments* in connective-tissue cells, muscle cells, and supporting cells of the nervous system (glial cells); (3) *neurofilaments* in nerve cells; and (4) *nuclear lamins*, which strengthen the nuclear envelope.
- The first three filament types are found in the cytoplasm, whereas the fourth is found in the nucleus.
- Filaments of each class are formed by polymerization of their corresponding intermediate filament subunits.

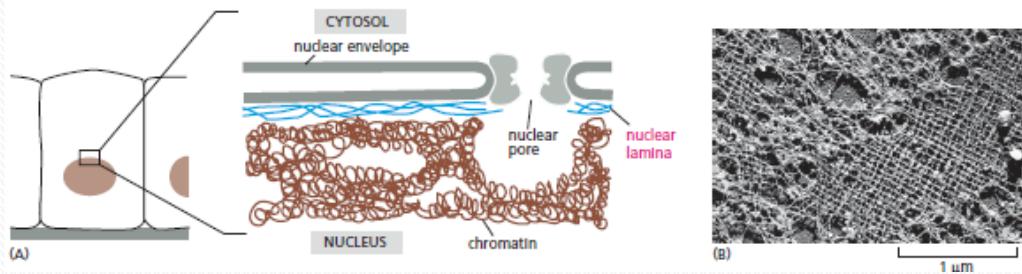


# Keratin

- The keratin filaments are the most diverse class of intermediate filament.
- Every kind of epithelium in the vertebrate body—whether in the tongue, the cornea, or the lining of the gut—has its own distinctive mixture of keratin proteins. Specialized keratins also occur in hair, feathers, and claws.
- Keratin filaments typically span the interiors of epithelial cells from one side of the cell to the other, and filaments in adjacent epithelial cells are indirectly connected through cell-cell junctions.
- This cabling of high tensile strength, formed by the filaments throughout the epithelial sheet, distributes the stress that occurs when the skin is stretched.
- If keratin filaments did not exist the skin would be highly vulnerable to mechanical injury, and even a gentle pressure can rupture its cells, causing the skin to blister.

# Nuclear Lamins

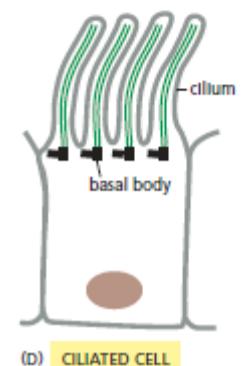
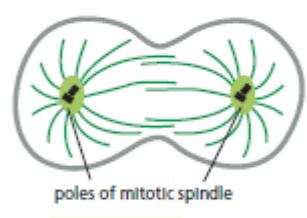
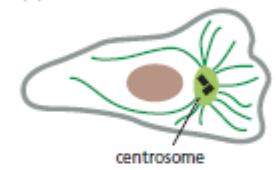
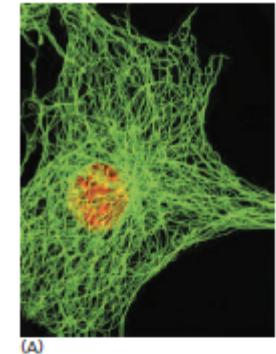
- Although cytoplasmic intermediate filaments form ropelike structures, the intermediate filaments lining and strengthening the inside surface of the inner nuclear membrane are organized as a two-dimensional meshwork.
- The intermediate filaments that form this tough nuclear lamina are constructed from a class of intermediate filament proteins called *lamins*.
- Defects in a particular nuclear lamin are associated with certain types of *progeria*—rare disorders that cause affected individuals to age prematurely.
- Children with progeria have wrinkled skin, lose their teeth and hair, and often develop severe cardiovascular disease by the time they reach their teens.
- Although exact mechanism is not known, it may be that the resulting nuclear instability leads to impaired cell division, increased cell death, a diminished capacity for tissue repair, or some combination of these.



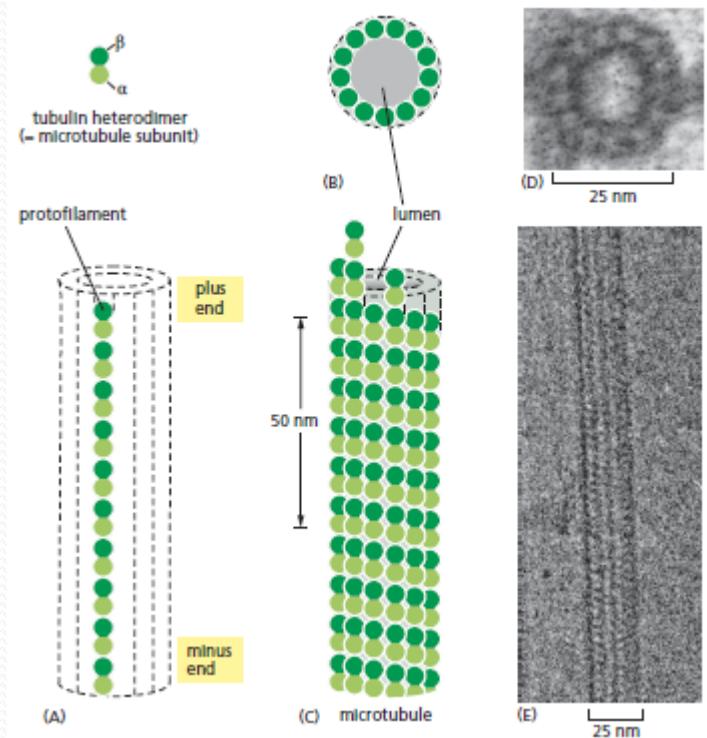
# Microtubules

- Microtubules have a crucial organizing role in all eukaryotic cells.
- In a typical animal cell, microtubules grow out from a small structure near the center of the cell called the *centrosome*.
- Extending out toward the cell periphery, they create a system of tracks within the cell, along which vesicles, organelles, and other cell components can be transported.
- These 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.

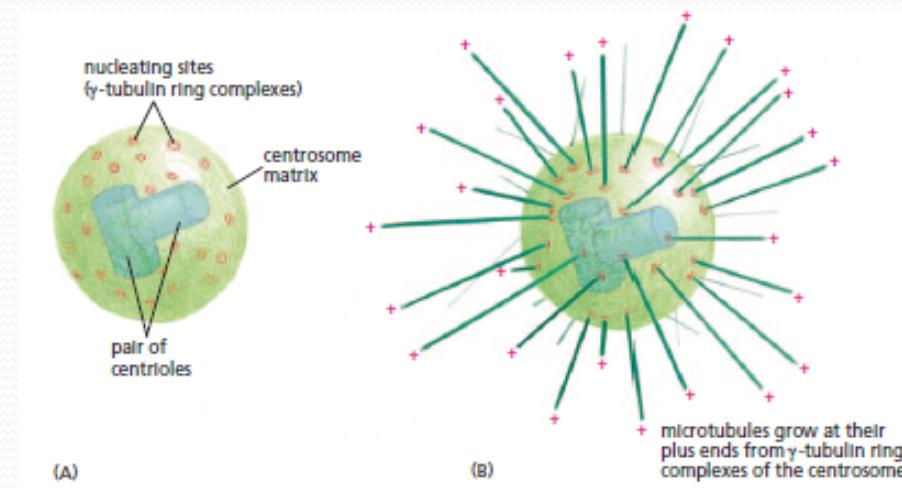
- They also have important roles in cell division.
- When a cell enters mitosis, the cytoplasmic microtubules disassemble and then reassemble into an intricate structure called the *mitotic spindle*.
- The mitotic spindle provides the machinery that will segregate the chromosomes equally into the two daughter cells just before a cell divides.
- Microtubules can also form stable structures, such as rhythmically beating *cilia* and *flagella*.



- Microtubules are built from subunits—molecules of tubulin—each of which is itself a dimer composed of two very similar globular proteins called  $\alpha$ -tubulin and  $\beta$ -tubulin, bound tightly together by noncovalent interactions.
- The tubulin dimers stack together, again by noncovalent bonding, to form the wall of the hollow cylindrical microtubule.
- This tubelike structure is made of 13 parallel protofilaments, each a linear chain of tubulin dimers with  $\alpha$ - and  $\beta$ -tubulin alternating along its length.
- Each protofilament has a structural polarity, with  $\alpha$ -tubulin exposed at one end and  $\beta$ -tubulin at the other, and this polarity gives a structural polarity to the microtubule as a whole.
- One end of the microtubule, thought to be the  $\beta$ -tubulin end, is called its *plus end*, and the other, the  $\alpha$ -tubulin end, its *minus end*.
- The polarity of the microtubule—the fact that its structure has a definite direction, with the two ends being chemically and functionally distinct—is crucial, both for the assembly of microtubules and for their role in intracellular transport.



- Inside cells, microtubules grow from specialized organizing centers that control the location, number, and orientation of the microtubules.
- In animal cells the **centrosome**—which is typically close to the cell nucleus when the cell is not in mitosis—organizes an array of microtubules that radiates outward through the cytoplasm.
- The centrosome consists of a pair of centrioles, surrounded by a matrix of proteins. The centrosome matrix serves as the starting point, or *nucleation site*, for the growth of one microtubule.
- The  $\alpha\beta$ -tubulin dimers are added in a specific orientation in centrosome matrix, with the result that the minus end of each microtubule is embedded in the centrosome, and growth occurs only at the plus end that extends into the cytoplasm because tubulin dimers add more rapidly to the plus end than to the minus end.



# Dynamic Instability of Microtubules

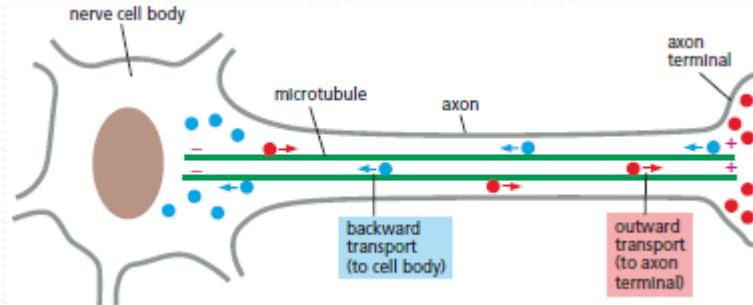
- Once a microtubule has been nucleated, it typically grows outward from the organizing center for many minutes by the addition of  $\alpha\beta$ -tubulin dimers to its plus end.
- Then, without warning, the microtubule can suddenly undergo a transition that causes it to shrink rapidly inward by losing tubulin dimers from its free plus end.
- It may shrink partially and then, no less suddenly, start growing again, or it may disappear completely, to be replaced by a new microtubule.
- This remarkable behavior—switching back and forth between polymerization and depolymerization—is known as dynamic instability.
- It allows microtubules to undergo rapid remodeling, and is crucial for their function.

- Drugs that prevent the polymerization or depolymerization of tubulin dimers can have a rapid and profound effect on the organization of microtubules—and thereby on the behavior of the cell.
- Consider the mitotic spindle, the microtubule-based apparatus that guides the chromosomes during mitosis.
- If a cell in mitosis is exposed to the drug **colchicine**, which binds tightly to free tubulin dimers and prevents their polymerization into microtubules, the mitotic spindle rapidly disappears, and the cell stalls in the middle of mitosis, unable to partition the chromosomes into two groups.
- This finding, and others like it, demonstrates that the mitotic spindle is normally maintained by a continuous balanced addition and loss of tubulin subunits: when tubulin addition is blocked by colchicine, tubulin loss continues until the spindle disappears.
- The drug **Taxol** has the opposite effect. It binds tightly to microtubules and prevents them from losing subunits. Because new subunits can still be added, the microtubules can grow but cannot shrink. However, despite this difference in their mechanism of action, Taxol has the same overall effect as colchicine—arresting dividing cells in mitosis.

# Cancer drugs target microtubules

- The inactivation or destruction of the mitotic spindle eventually kills dividing cells.
- Because cancer cells divide in a less controlled way than do normal cells of the body, they can sometimes be killed preferentially by microtubule-stabilizing drugs such as *Taxol* or microtubule-destabilizing antimitotic drugs such as *colchicine*.

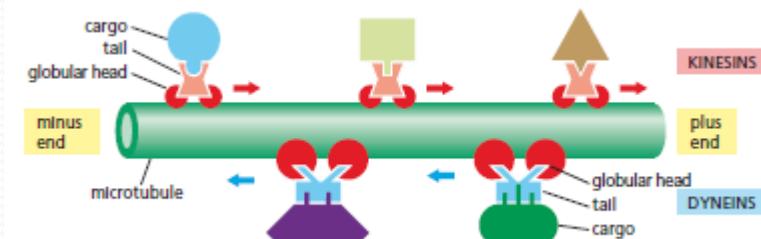
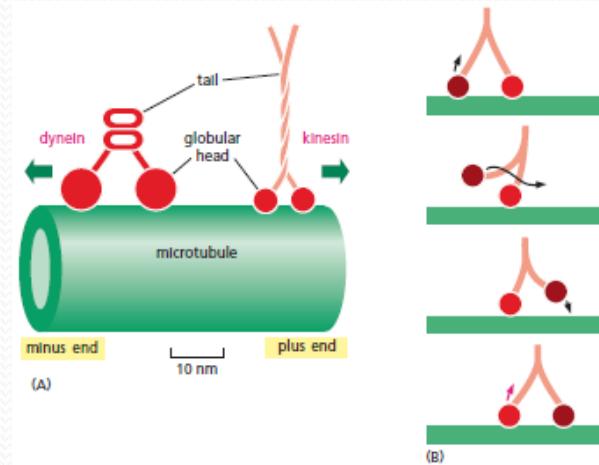
- During mitosis microtubules disassemble rapidly and then reassemble into the mitotic spindle.
- On the other hand, when a cell has differentiated into a specialized cell type, the dynamic instability of its microtubules is often suppressed by proteins that bind to either the ends or the sides of the microtubules and stabilize them against disassembly.
- The stabilized microtubules then serve to maintain the organization of the differentiated cell.
- Most differentiated animal cells are polarized; that is, one end of the cell is structurally or functionally different from the other. Nerve cells, for example, put out an axon from one end of the cell and dendrites from the other. Cells specialized for secretion have their Golgi apparatus positioned toward the site of secretion, and so on.
- The cell's polarity is a reflection of the polarized systems of microtubules in its interior, which help to position organelles in their required location within the cell and to guide the streams of vesicular and macromolecular traffic moving between one part of the cell and another.
- In the nerve cell, for example, all the microtubules in the axon point in the same direction, with their plus ends toward the axon terminals; along these oriented tracks, the cell is able to transport organelles, membrane vesicles, and macromolecules—either from the cell body to the axon terminals or in the opposite direction.



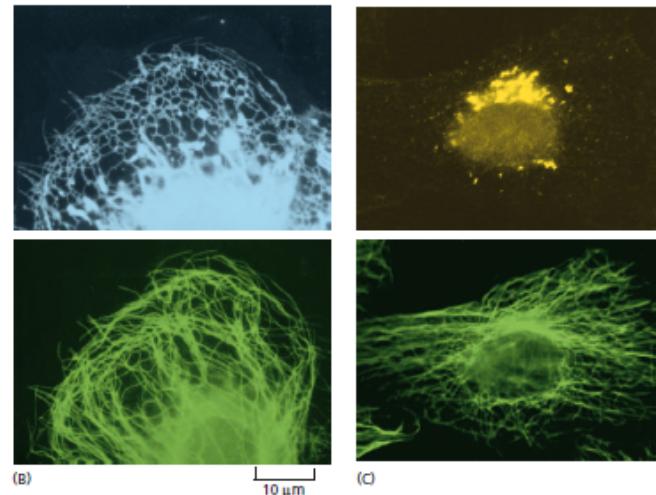
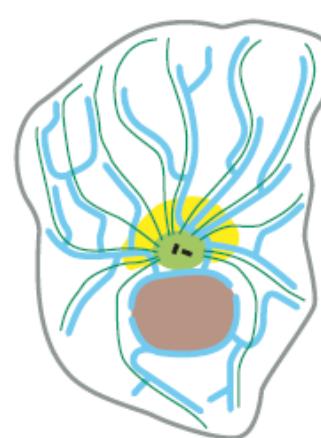
# Motor Proteins

- Movement guided by microtubules is faster and more efficient than movement driven by free diffusion.
- Motor proteins are responsible for actively transporting organelles, vesicles, and other macromolecules along microtubules.
- Motor proteins attach both the cytoskeletal filaments and to other cell components as they transport the cargo along the filaments.
- There are dozens of different motor proteins; they differ in the type of filament they bind to, the direction in which they move along the filament, and the cargo they carry.

- The motor proteins that move along cytoplasmic microtubules belong to two families: the kinesins generally move toward the plus end of a microtubule (outward from the cell body) and the dyneins move toward the minus end (toward the cell body).
- Both kinesins and dyneins are generally dimers that have two globular heads and a single tail.
- The heads interact with microtubules and the tail of a motor protein generally binds stably to some cell component, such as a vesicle or an organelle, and thereby determines the type of cargo that the motor protein can transport.



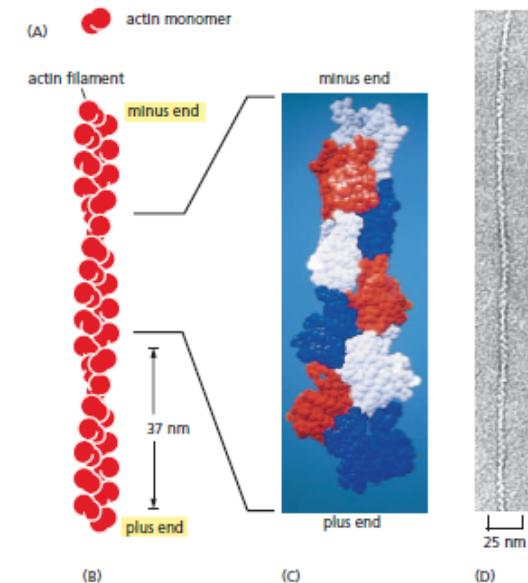
- Microtubules and motor proteins play an important part in positioning organelles within a eukaryotic cell.



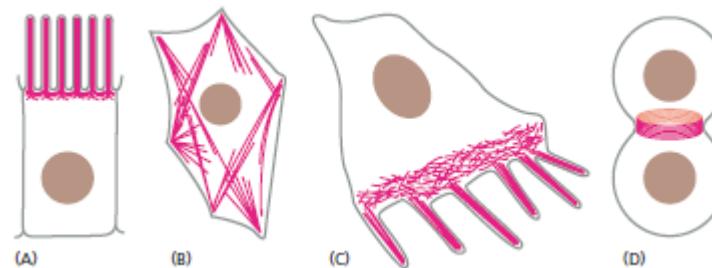
The typical arrangement of cytoplasmic microtubules (*green*), endoplasmic reticulum (*blue*), and Golgi apparatus (*yellow*).

# Actin Filaments

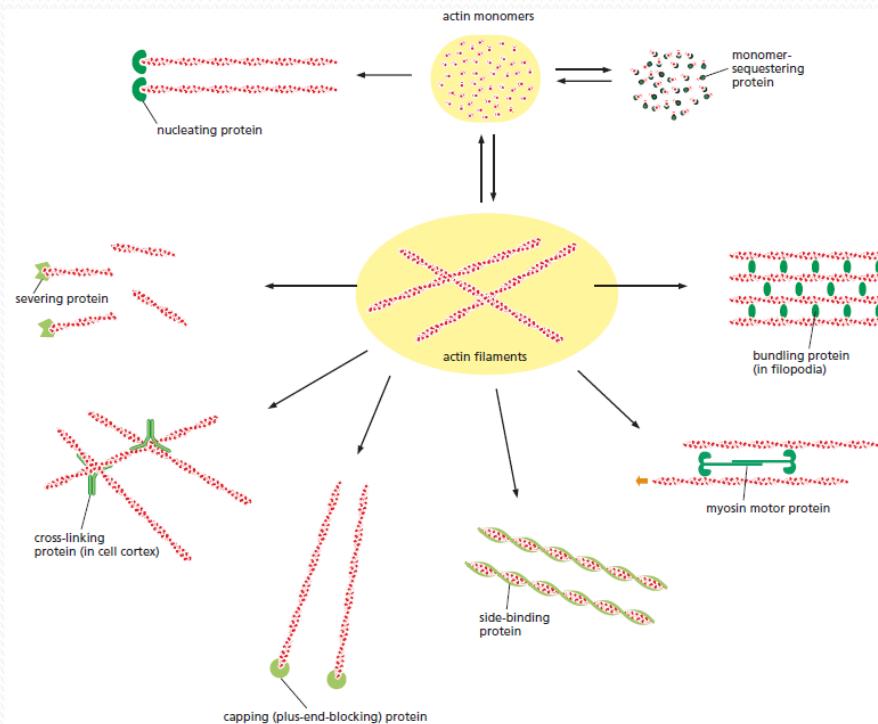
- Actin filaments, polymers of the protein actin, are present in all eukaryotic cells and are essential for many of the cell's movements, especially those involving the cell surface.
- Without actin filaments, for example, an animal cell could not crawl along a surface, engulf a large particle by phagocytosis, or divide in two.
- Actin filaments are thinner, more flexible, and usually shorter than microtubules.
- Each actin filament is a twisted chain of identical globular actin monomers, all of which “point” in the same direction along the axis of the chain.
- Like a microtubule, therefore, an actin filament has a structural polarity, with a plus end and a minus end.



- Depending on which of these proteins they associate with, actin filaments can form stiff and stable structures, such as the *microvilli* on the epithelial cells lining the intestine (A) or the small *contractile bundles* that can contract and act like tiny muscles in most animal cells (B).
- They can also form temporary structures, such as the dynamic protrusions formed at the leading edge of a crawling cell (C) or the *contractile ring* that pinches the cytoplasm in two when an animal cell divides (D).

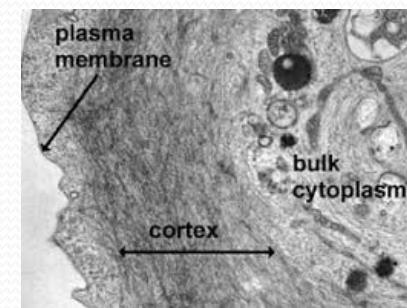
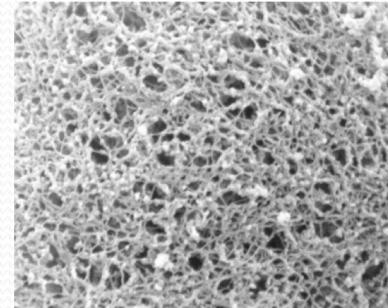
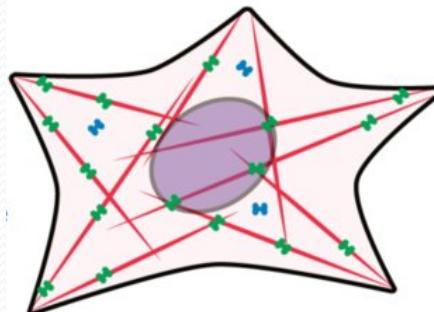


- Actin filaments can grow by the addition of actin monomers at either end. However, like microtubules, their rate of growth is faster at the plus end than at the minus end.
- There are a great many actin-binding proteins in cells many of which bind to assembled actin filaments rather than to actin monomers and control the behavior of the intact filaments.



# Cell Cortex

- Although actin is found throughout the cytoplasm of a eukaryotic cell, in most cells it is highly concentrated in a layer just beneath the plasma membrane.
- In this region, called the cell cortex, actin filaments are linked by actin-binding proteins into a meshwork that supports the plasma membrane and gives it mechanical strength.
- For plants, yeasts, and bacteria, the cell's shape and mechanical properties are conferred by a rigid cell wall—a meshwork of proteins, sugars, and other macromolecules that encases the plasma membrane.
- By contrast, the plasma membrane of animal cells is stabilized by a meshwork of fibrous proteins, called the cell cortex, that is attached to the underside of the membrane. The meshwork of cortex is connected to the membrane through intracellular attachment proteins that link them to specific transmembrane proteins.
- Actin filaments become cross-linked into a three-dimensional meshwork, which governs cell shape and the mechanical properties of the plasma membrane: the rearrangements of actin filaments within the cortex provide much of the molecular basis for changes in both cell shape and cell locomotion.



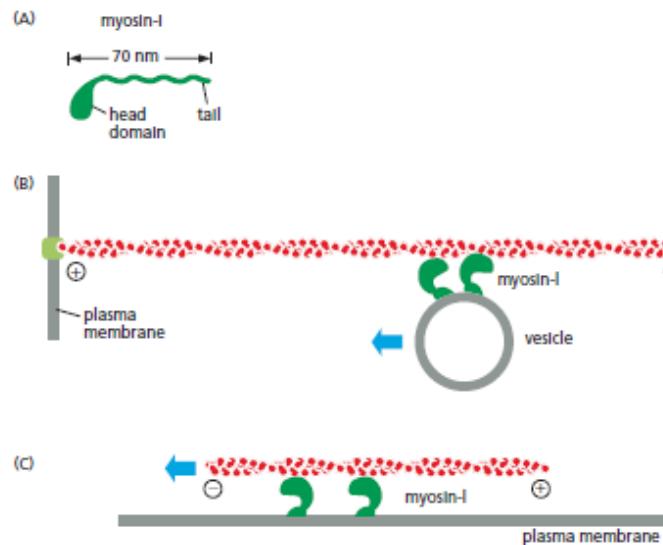
- Many eukaryotic cells move by crawling over surfaces, rather than by swimming by means of beating cilia or flagella.
- This crawling includes 3 steps:
  1. the cell pushes out protrusions at its “front,” or leading edge;
  2. these protrusions adhere to the surface over which the cell is crawling;
  3. the rest of the cell drags itself forward by traction on these anchorage points.
- All three processes involve actin filaments and are driven by actin polymerization and myosin motor proteins.

# Myosin Proteins

- All actin-dependent motor proteins belong to the myosin family.
- There are various types of myosins in cells, of which the myosin-I and myosin-II subfamilies are the most abundant.
- Myosin-I is present in all types of cells, whereas muscle cells make use of a specialized form of myosin-II.

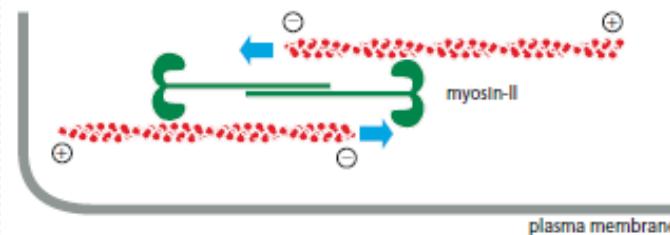
# Myosin-I

- Myosin-I molecules have a head domain and a tail.
- The head domain binds to an actin filament and has the ATP-hydrolyzing motor activity that enables it to move along the filament in a repetitive cycle of binding, detachment, and rebinding.
- The tail varies among the different types of myosin-I and determines what type of cargo the myosin drags along. For example, the tail may bind to a particular type of vesicle and propel it through the cell along actin filament tracks, or it may bind to the plasma membrane and pull it into a different shape.



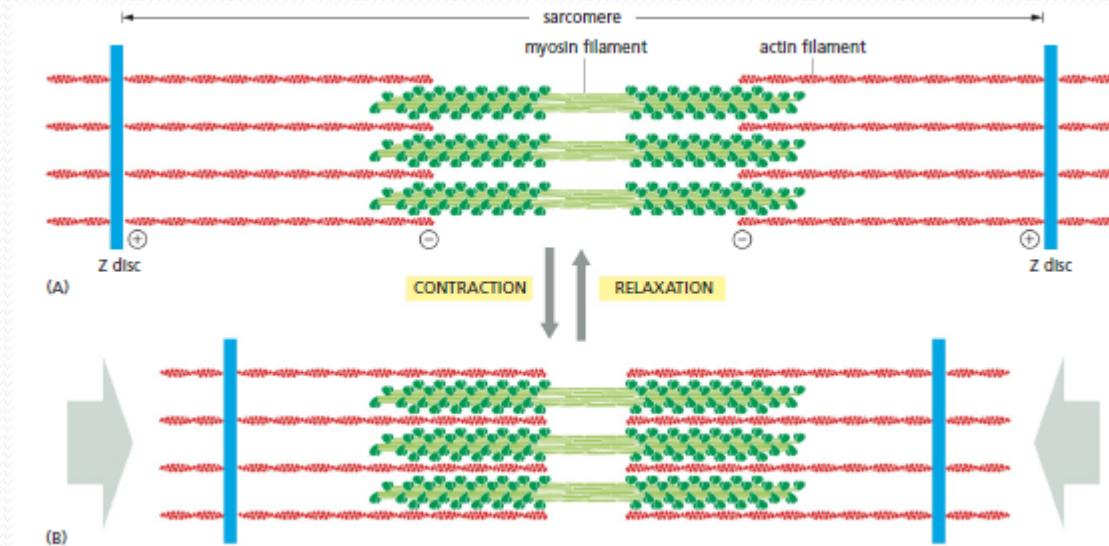
# Myosin-II

- Muscle myosin belongs to the myosin-II subfamily of myosins, all of which are dimers, with two globular ATPase heads at one end and a single coiled-coil tail at the other.
- Clusters of myosin-II molecules bind to each other through their coiled-coil tails, forming a bipolar myosin filament from which the heads project.
- The myosin filament is like a double-headed arrow, with the two sets of myosin heads pointing in opposite directions, away from the middle. One set binds to actin filaments in one orientation and moves the filaments one way; the other set binds to other actin filaments in the opposite orientation and moves the filaments in the opposite direction. As a result, a myosin filament slides sets of oppositely oriented actin filaments past one another.



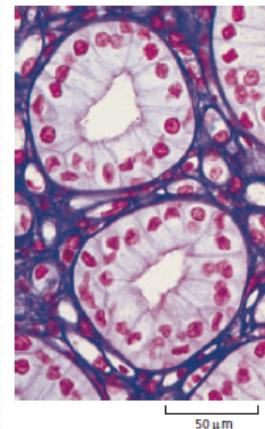
# Muscle Fibers

- Actin filaments and myosin filaments are organized together in a bundle, the bundle can generate a strong contractile force.
- This is seen most clearly in muscle contraction.

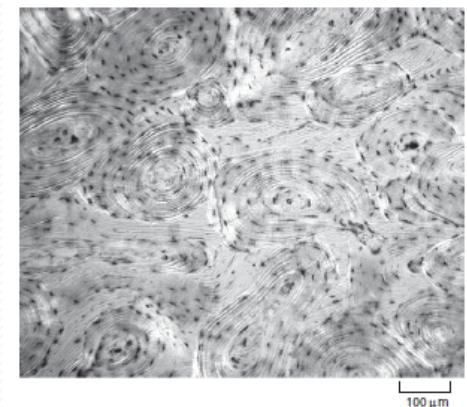
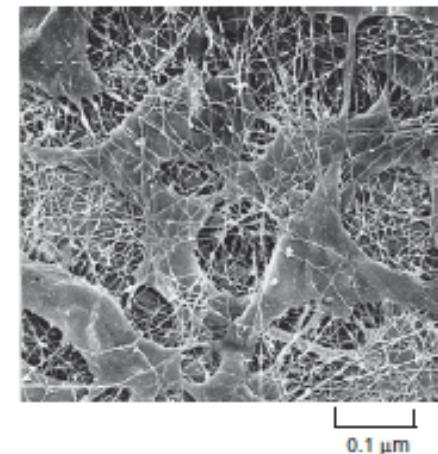


# Extracellular Matrix

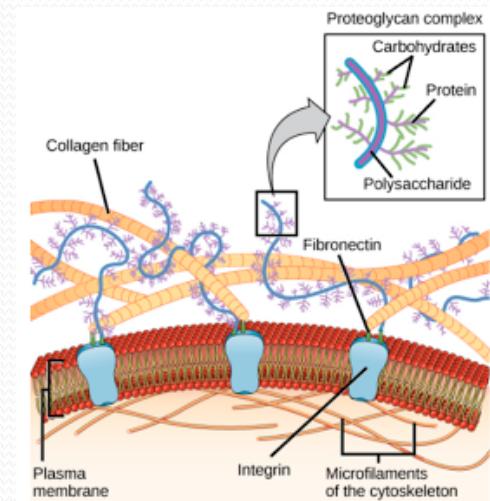
- Cells are the building blocks of multicellular organisms.
- Most of the cells in multicellular organisms are organized into cooperative assemblies called tissues, such as the nervous, muscle, epithelial, and connective tissues found in vertebrates.
- Tissues are composed not only of cells, with their internal framework of cytoskeletal filaments, but also of extracellular matrix (ECM), which cells secrete around themselves.



- ECM is a well-organized network which is not only a physical scaffold into which cells are embedded but also regulates many cellular processes including growth, migration, differentiation, survival, homeostasis, and morphogenesis.
- The ECMs consist of a large variety of matrix macromolecules whose precise composition and specific structures vary from tissue to tissue.
- In connective tissues, ECM is plentiful and carries the mechanical load.
- In other tissues, such as epithelia, ECM is scanty, and the cells are directly joined to one another and carry the mechanical load themselves.



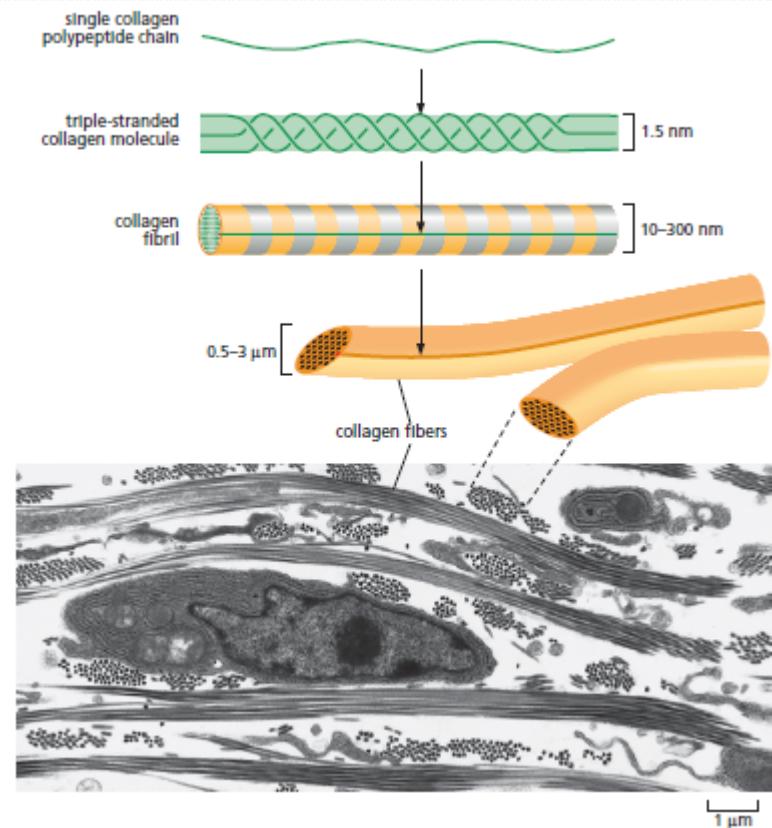
- The macromolecules that constitute the extracellular matrix are mainly produced locally by cells in the matrix. As we discuss later, these cells also help to organize the matrix: the orientation of the cytoskeleton inside the cell can control the orientation of the matrix produced outside.
- Two main classes of extracellular macromolecules make up the matrix: :
  - polysaccharide chains of the class called ***glycosaminoglycans (GAGs)***, which are usually found covalently linked to protein in the form of ***proteoglycans***,
  - fibrous proteins, including ***collagen***, ***elastin***, ***fibronectin***, and ***laminin***, which have both structural and adhesive functions.



# Collagen

- Collagen is the most abundant fibrous protein within the interstitial ECM and constitutes up to 30% of the total protein mass of a multicellular animal.
- Collagens, which constitute the main structural element of the ECM, provide tensile strength, regulate cell adhesion, support chemotaxis and migration, and direct tissue development.

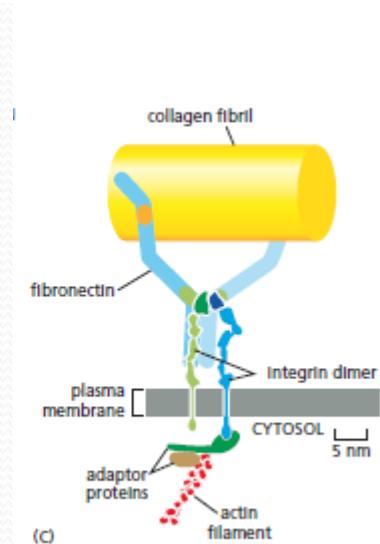
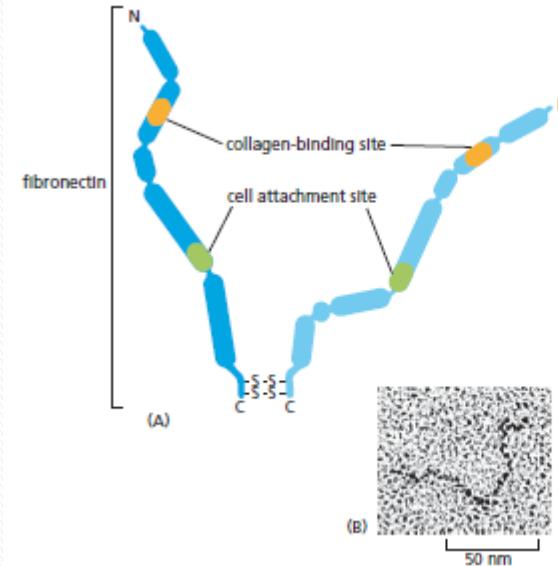
- The characteristic feature of a typical collagen molecule is its long, stiff, ***triple-stranded helical structure***, in which three collagen polypeptide chains are wound around one another in a ropelike superhelix.
- Collagen molecules in turn assemble into ordered polymers called ***collagen fibrils***, which are thin cables 10–300 nm in diameter and many micrometers long; these can pack together into still thicker ***collagen fibers***.
- Some types of collagen molecules decorate the surface of collagen fibrils and link the fibrils to one another and to other components in the extracellular matrix.



- To do their job, collagen fibrils must be correctly aligned.
- In skin, for example, they are woven in a wickerwork pattern, or in alternating layers with different orientations so as to resist tensile stress in multiple directions.
- In tendons, which attach muscles to bone, they are aligned in parallel bundles along the major axis of tension.
- Collagen associates with elastin, another major ECM fiber.

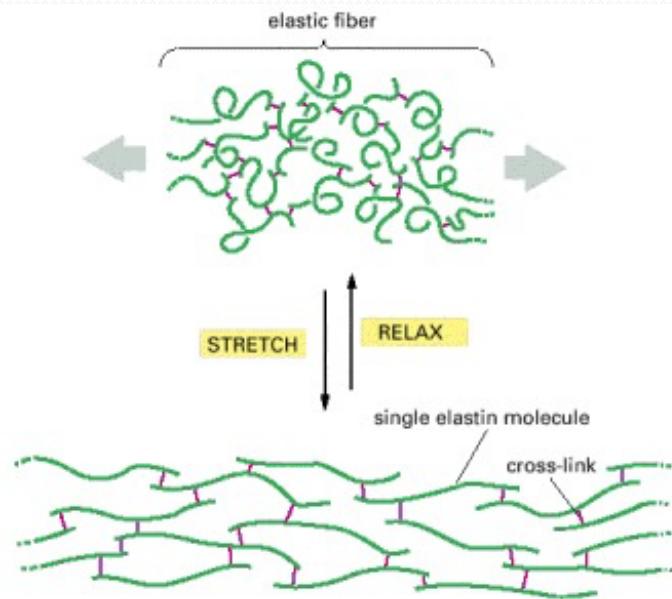
# Fibronectin

- Fibronectin, provides a linkage between ECM and cells: part of the fibronectin molecule binds to collagen, while another part forms an attachment site for a cell.
- A cell attaches itself to fibronectin by means of a receptor protein, called an **integrin**, which spans the cell's plasma membrane.
- When the extracellular domain of the integrin binds to fibronectin, the intracellular domain binds (through a set of adaptor molecules) to an actin filament inside the cell.



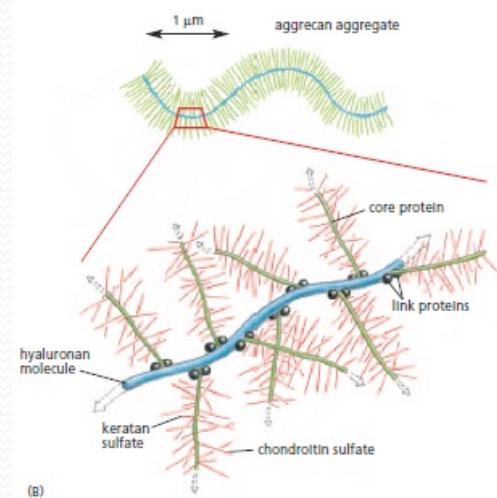
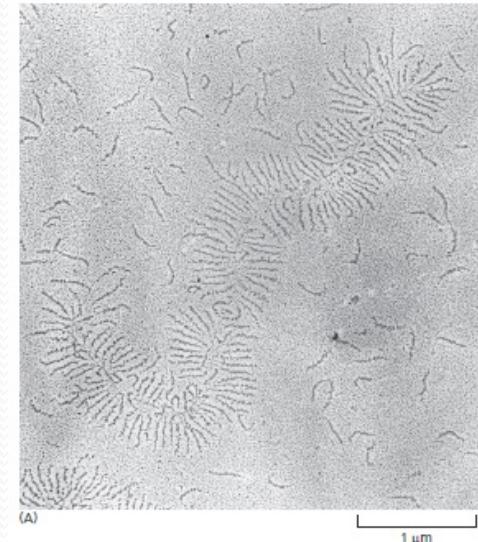
# Elastin

- Many vertebrate tissues, such as skin, blood vessels, and lungs, need to be both strong and elastic in order to function.
- A network of elastic fibers in the extracellular matrix of these tissues gives them the required resilience so that they can recoil after transient stretch.
- The elastin protein is composed largely of two types of short segments that alternate along the polypeptide chain: hydrophobic segments, which are responsible for the elastic properties of the molecule, and segments that form cross-links between adjacent molecules.



# Glycosaminoglycans

- While collagen provides tensile strength to resist stretching, a completely different group of macromolecules in the extracellular matrix of animal tissues provides the complementary function, resisting compression.
- These are the glycosaminoglycans (GAGs), negatively charged polysaccharide chains made of repeating disaccharide units GAGs are usually covalently linked to core proteins to form proteoglycans, which are extremely diverse in size, shape, and chemistry.
- Typically, many GAG chains are attached to a single core protein, which may in turn be linked at one end to another GAG, creating an enormous aggregate resembling a bottlebrush, with a molecular weight in the millions.



- In general, GAGs are strongly hydrophilic and tend to adopt highly extended conformations, which occupy a huge volume relative to their mass.
- Thus GAGs act as effective “space fillers” in the ECM.
- Even at very low concentrations, GAGs form hydrophilic gels: their multiple negative charges attract a cloud of cations, such as  $\text{Na}^+$ , that are osmotically active, causing large amounts of water to be sucked into the matrix.
- This gives rise to a swelling pressure, which is balanced by tension in the collagen fibers interwoven with the proteoglycans. When the matrix is rich in collagen and large quantities of GAGs are trapped in its meshes, both the swelling pressure and the counterbalancing tension are enormous.
- Such a matrix is tough, resilient, and resistant to compression.
- The cartilage matrix that lines the knee joint, for example, has this character: it can support pressures of hundreds of kilograms per square centimeter.

- Proteoglycans perform many sophisticated functions in addition to providing hydrated space around cells.
- They can form gels of varying pore size and charge density that act as filters to regulate the passage of molecules through the extracellular medium.
- They can bind secreted growth actors and other proteins that serve as extracellular signals for cells.
- They can block, encourage, or guide cell migration through the matrix.
- In all these ways, the matrix components influence the behavior of cells, often the same cells that make the matrix—a reciprocal interaction that has important effects on cell differentiation and the arrangement of cells in a tissue.