Connective Tissue

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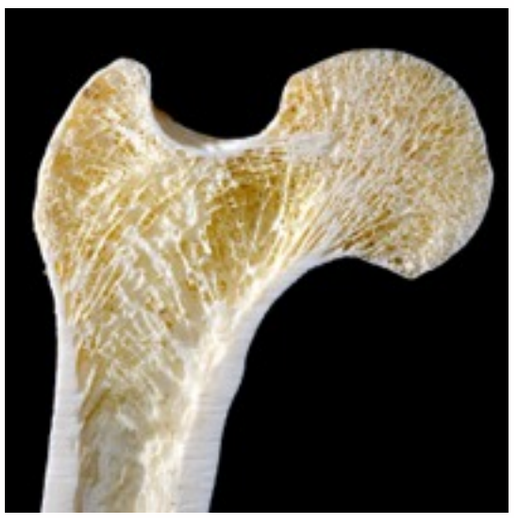
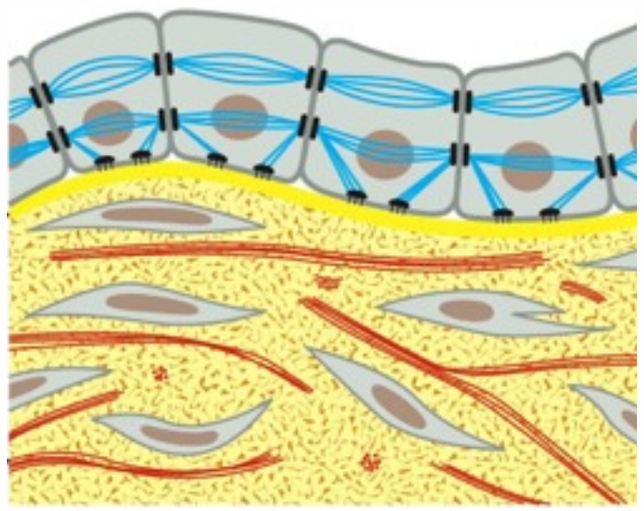
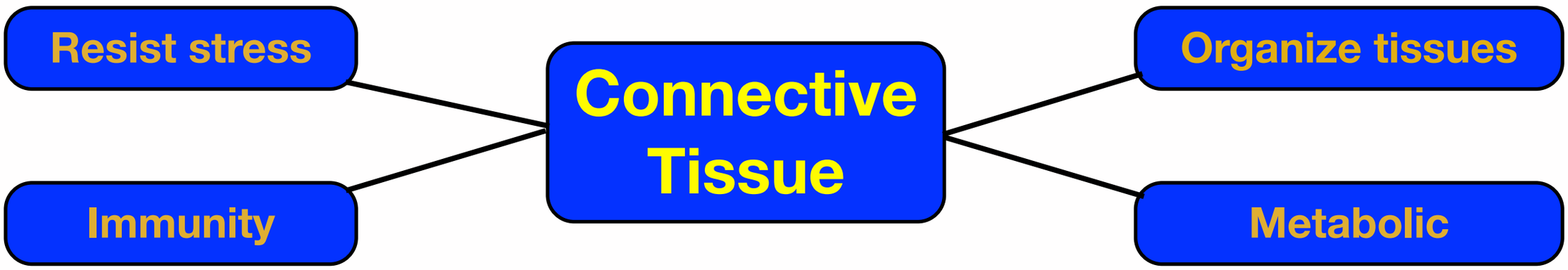
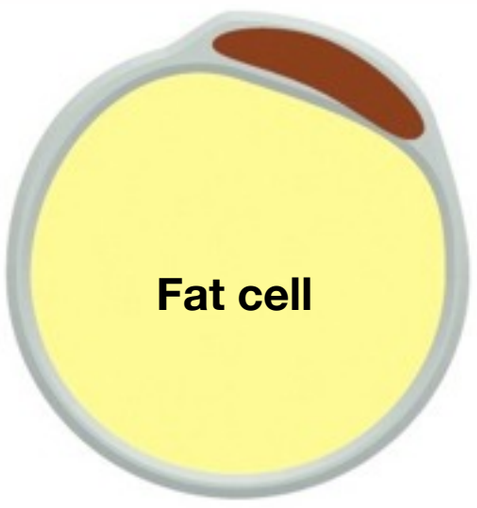
- Types and function
- Components: Collagen, Elastic Fibers, Glycosaminoglycan
- Cells of connective tissue

Connective tissue serves a variety of functions throughout the body.



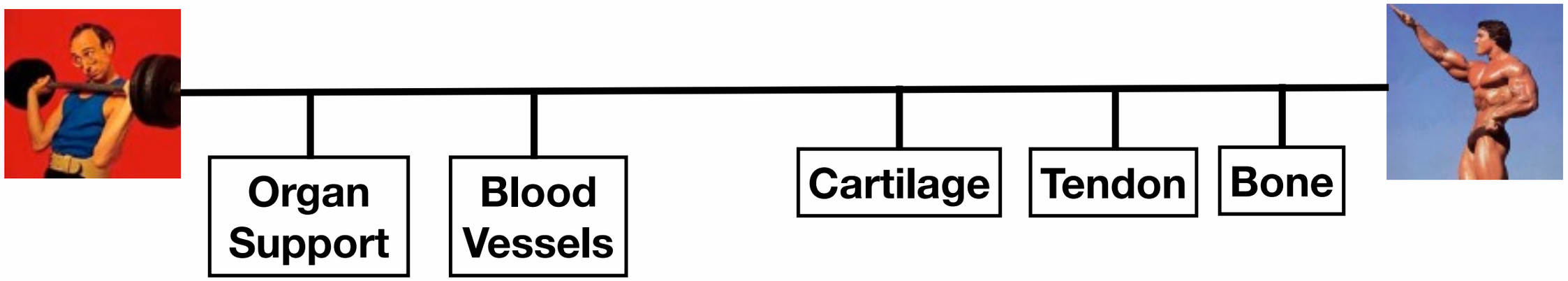






Connective tissue serves a number of important functions. It provides mechanical support to tissues and organs allowing them to resist tension and compression. It organizes cells into tissues by binding to surface receptors on cells and regulating their growth and morphology. It provides metabolic support in the form of growth factors, hormones, and high energy lipids through blood vessels. It contains a variety of cells that generate immune responses to foreign cells. These functions tend to be exclusive so that connective tissue that is mechanically robust offers less metabolic and immune support. In contrast, connective tissue that provides metabolic and immune support tends to be weaker.

Connective tissue can generate a range of mechanical strengths.



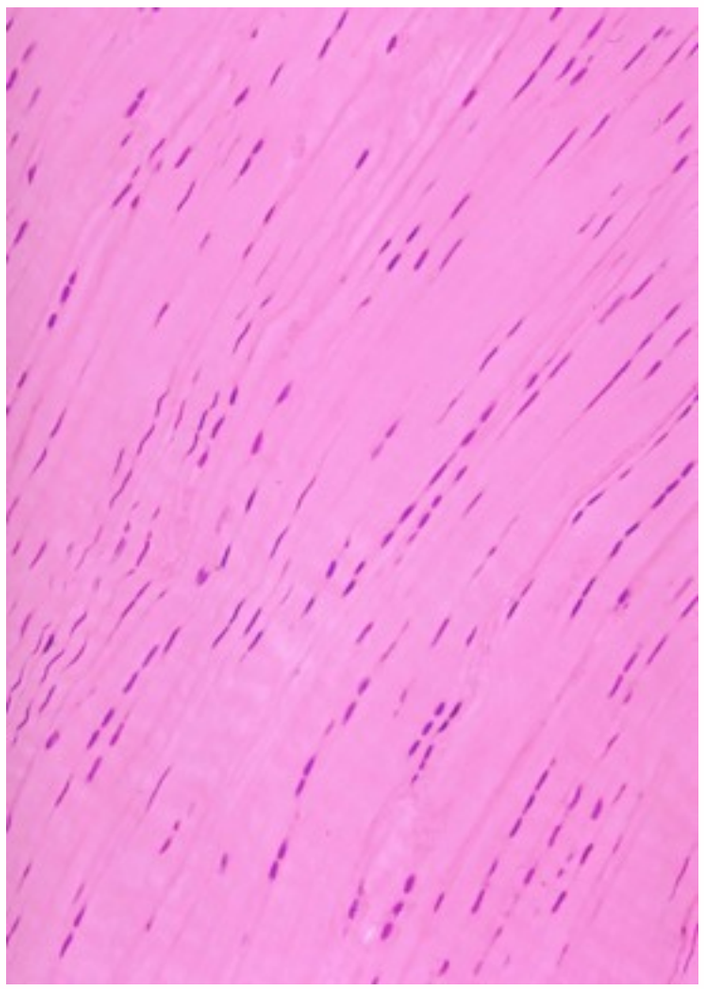
The mechanical strength of connective tissue varies widely, from the stiffness and hardness of bone to the squishiness of many organs. In between are types of connective tissue with different mechanical properties. Tendons resist tension and do not stretch making them ideal for linking muscle to bone. Cartilage resists compression. Large blood vessels can withstand stretch and recoil in response to changes in blood pressure. All of these mechanical properties are mediated by connective tissue.

Connective tissue resists tension and compression.

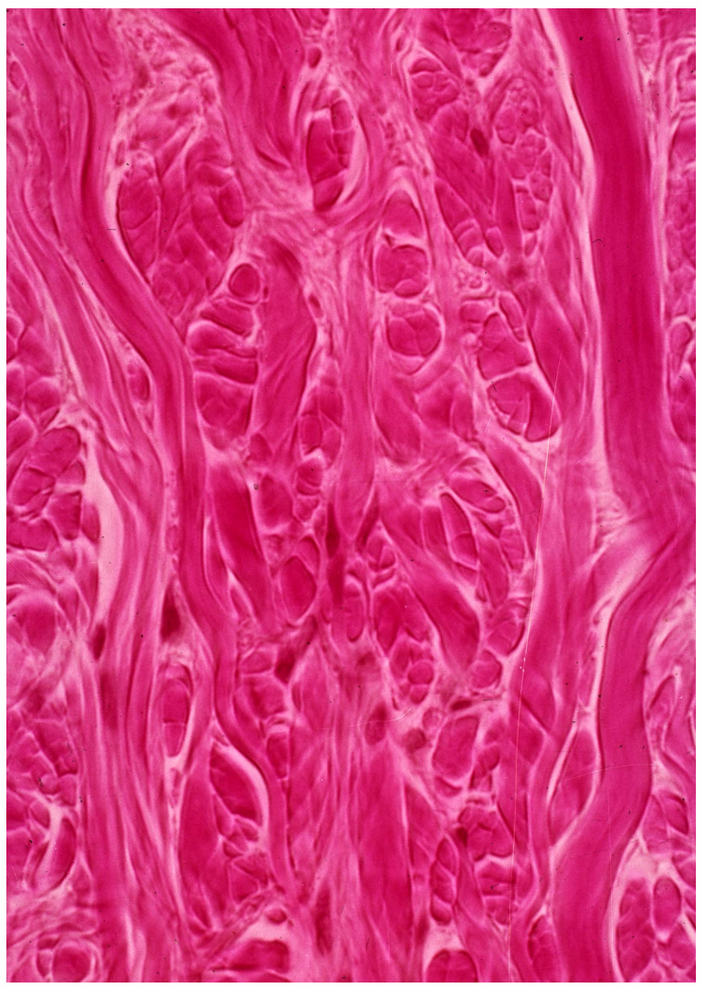
There are several important molecules that allow connective tissue to generate different mechanical properties. In general, these molecules either resist tensile and stretching forces or compressing forces. Collagen is the main component that resist tension. Elastin also resist tension but behaves similar to rubber in that it can be stretched and will recoil after the force is removed. On the other side are glycosaminoglycans that resist compressive forces. Glycosaminoglycans are long sugar polymers that occupy large volumes within connective tissue.

The density and organization of fibers determines the strength of connective tissue.

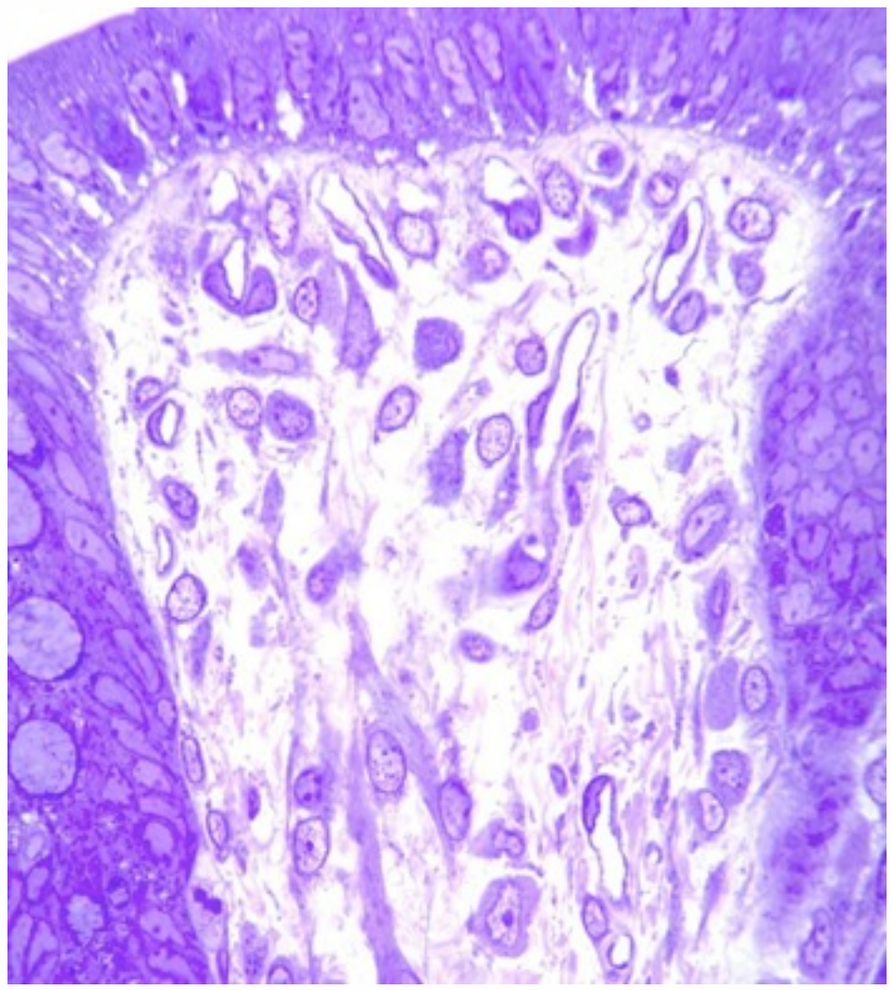
Dense Regular



Dense Irregular



Loose

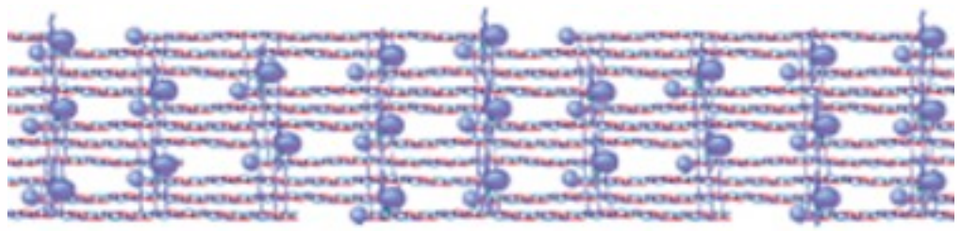


One way that connective tissue varies in mechanical strength is by the density and organization of its collagen fibers. Tendon contains a high ratio of collagen to number of cells and the collagen fibers are arranged in parallel arrays along the lines of tension. This provides maximal resistance to external forces. The dermis of skin contains a large amount of collagen fibers that are less organized and oriented in multiple directions. This allows skin to resist tension in different directions but sacrifices its overall mechanical strength. Connective tissue in organs contains much less collagen and is more cellular. Organs, such as the small intestine, are structurally weaker than tendon because they require connective tissue to provide metabolic and immune support so the connective tissue must contain blood vessels, macrophages, lymphocytes. These cells are absent in tendon allowing it to pack in more fibers and their presence in the connective tissue of most organs means fewer fibers.

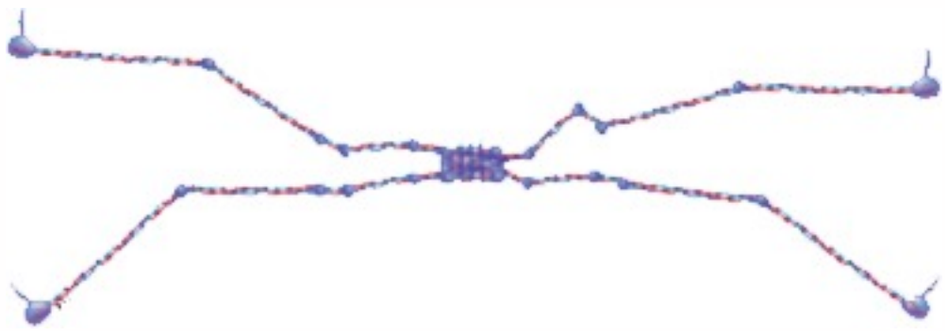
Collagen

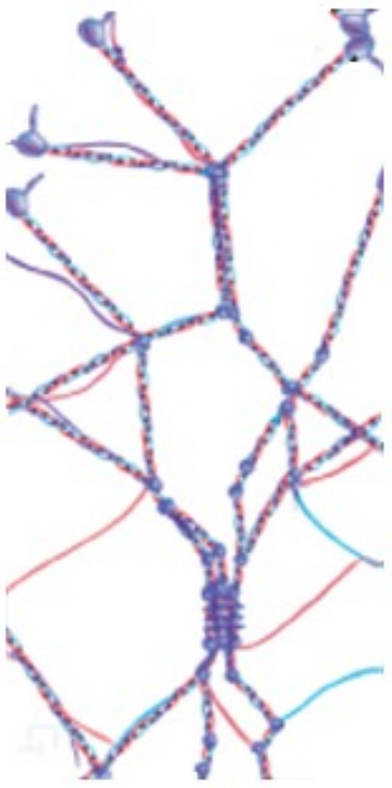
Collagens are a large family of proteins that form fibers or networks.

Fibrillar: type I, type II, type III



Network: type IV





Collagen is the most abundant class of proteins and pound for pound some are as strong as steel. There are several different types of collagens and their locations within the body varies. Most collagens, about 80-90% of total collagen, form fibers that provide the most mechanical strength. Aggregation and lateral interactions between the individual fibers increase the mechanical strength. Type IV collagen forms a sheet-like network instead of fibers. This collagen is an important component of the basal lamina that underlies epithelia and muscle cells. The different types of collagens are usually found in different tissues and organs. For example, type I collagen is the strongest and is found in bone, ligament, skin, tendon, etc. Type II collagen is thinner than type I and is found almost exclusively in cartilage. Type III makes up reticular fibers that form a network that helps organize cells within some organs.

Collagens are a large family of proteins that form fibers or networks.

Type	Structure	Tissue Distribution
I	Fibril	*Bone*, *ligament*, *skin*, *tendon*, artery walls, cornea
II	Fibril	*Cartilage*
III	Fibril	*Reticular fibers*
IV	Sheetlike network	*Basement membrane*

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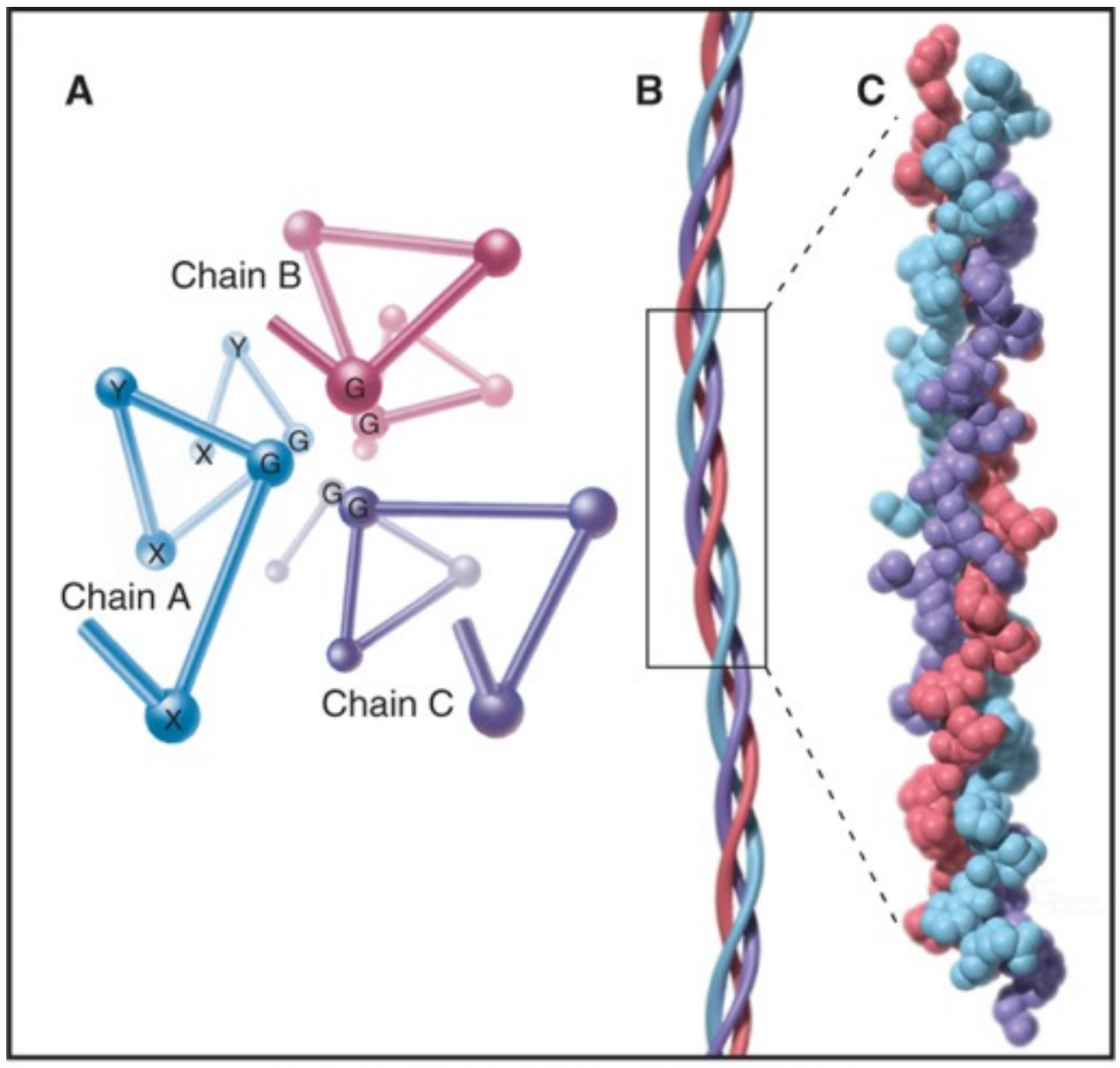
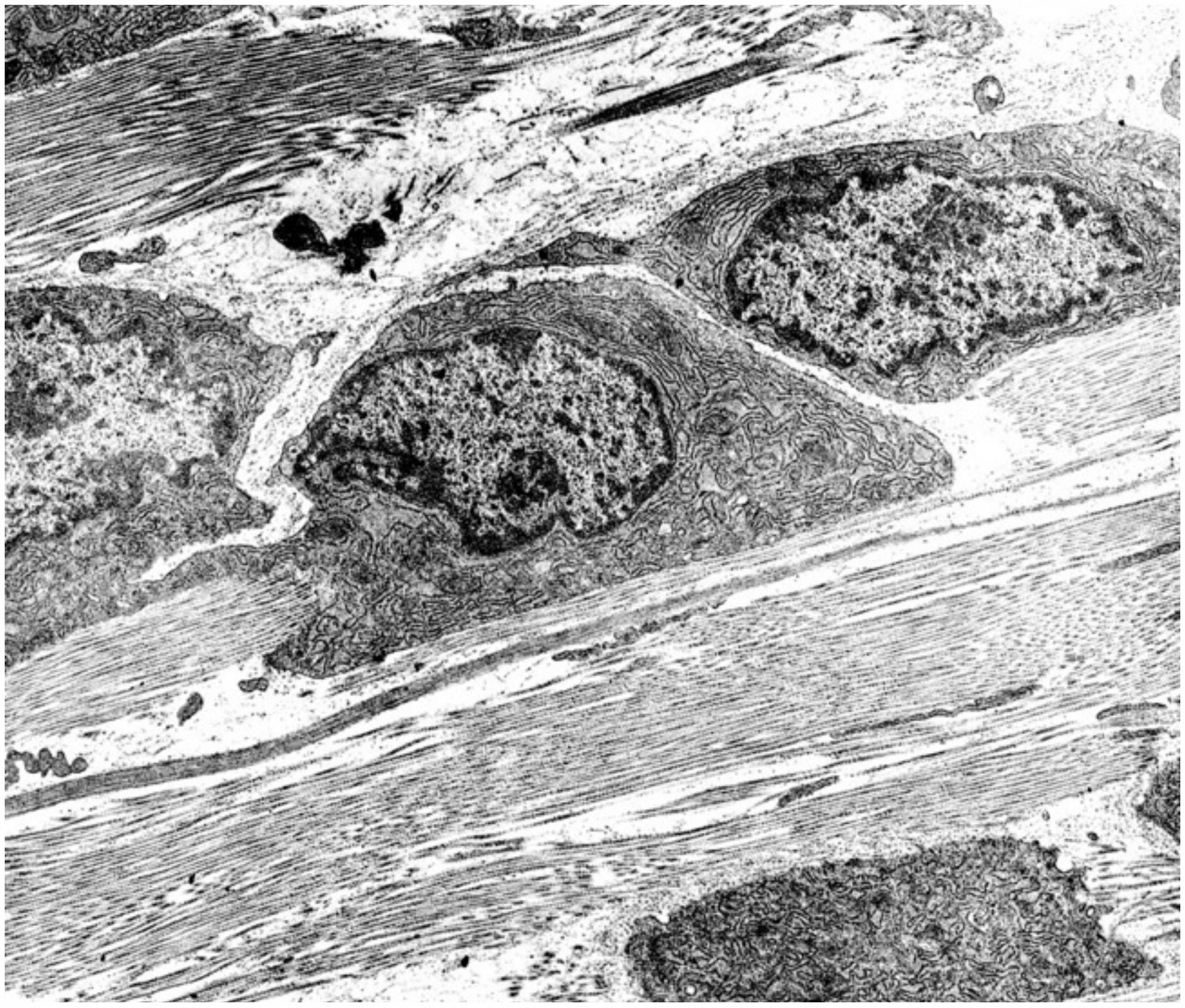
Three collagens polypeptides associate to form rope-like structures.



Pollard et al. *Cell Biology 2nd Edition*

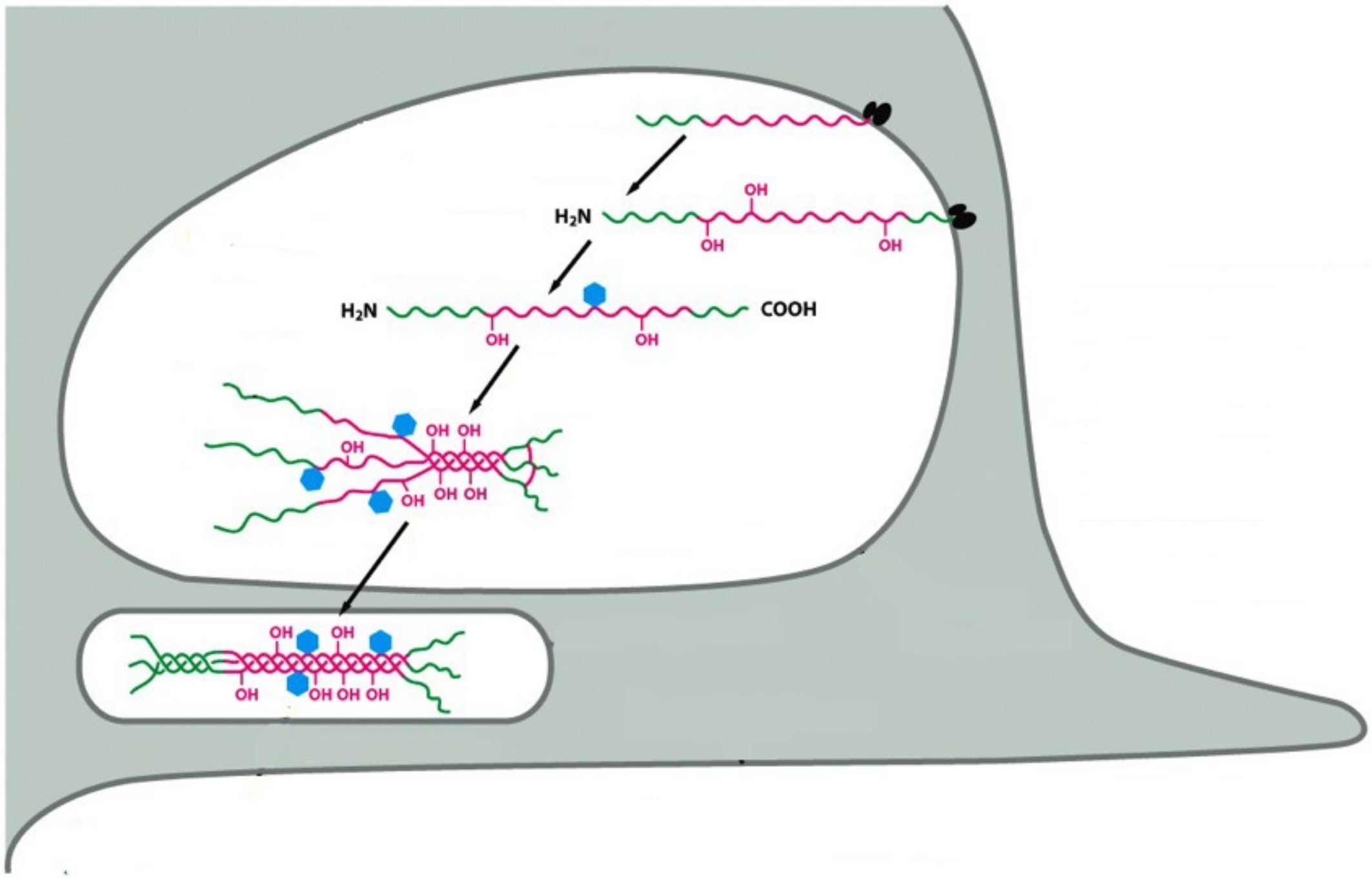
All collagens regardless of type are composed of three polypeptide chains. Each of these polypeptides can be over 1000 amino acids giving the trimer an overall length of 300 nm and a width of 1.5 nm. The polypeptides wrap around each other and form coiled coil interactions. The sequence is a repeat of 3 amino acids: glycine and usually proline and lysine. Glycine which is the smallest amino acid allows for tight packing of the polypeptides. Note the extensive lateral interactions that give the structure its mechanical strength.

Fibroblasts synthesize and process collagen.



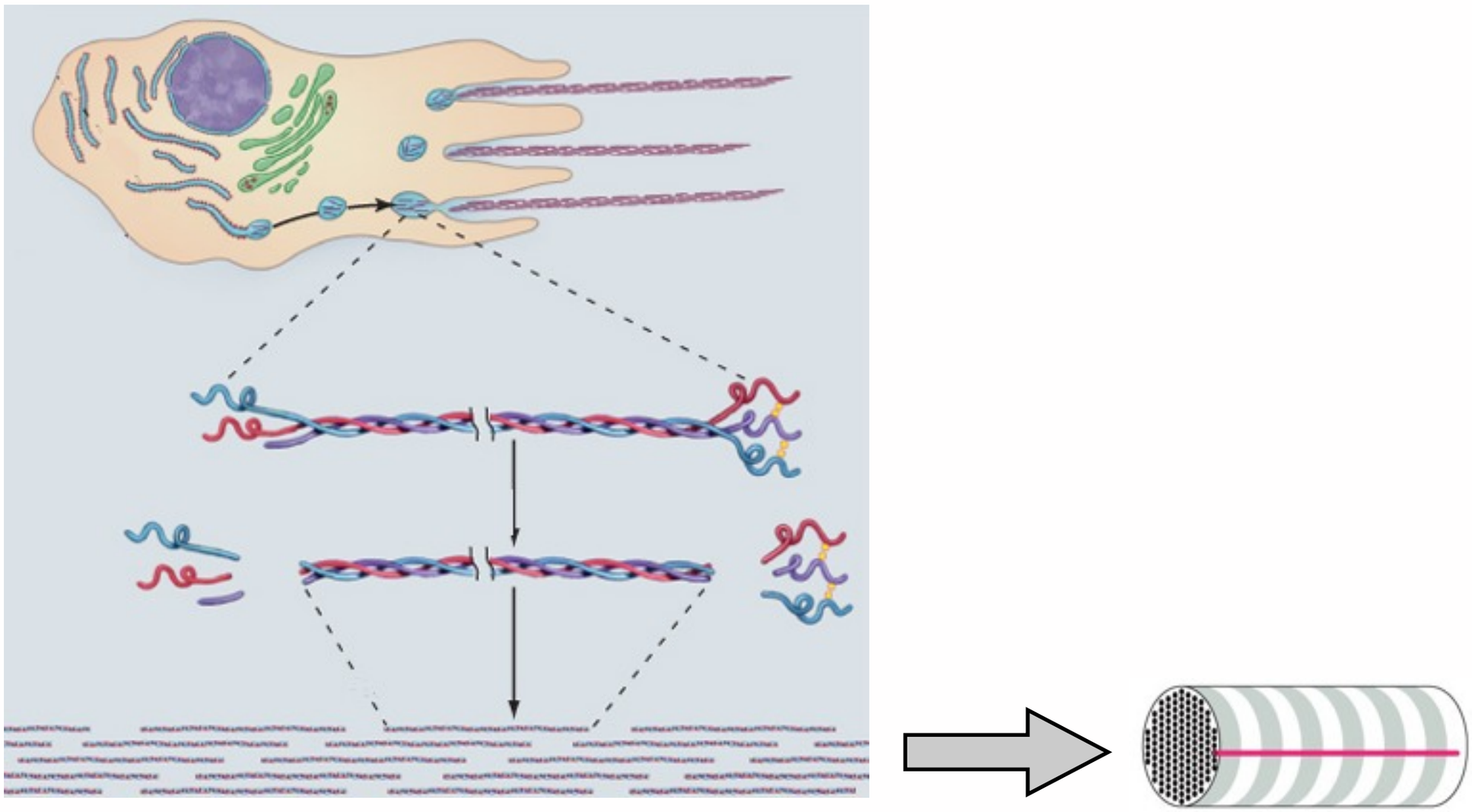
Fibroblasts within the connective tissue are responsible for synthesizing and secreting collagen.

Fibroblasts synthesize and secrete collagen via constitutive pathway.



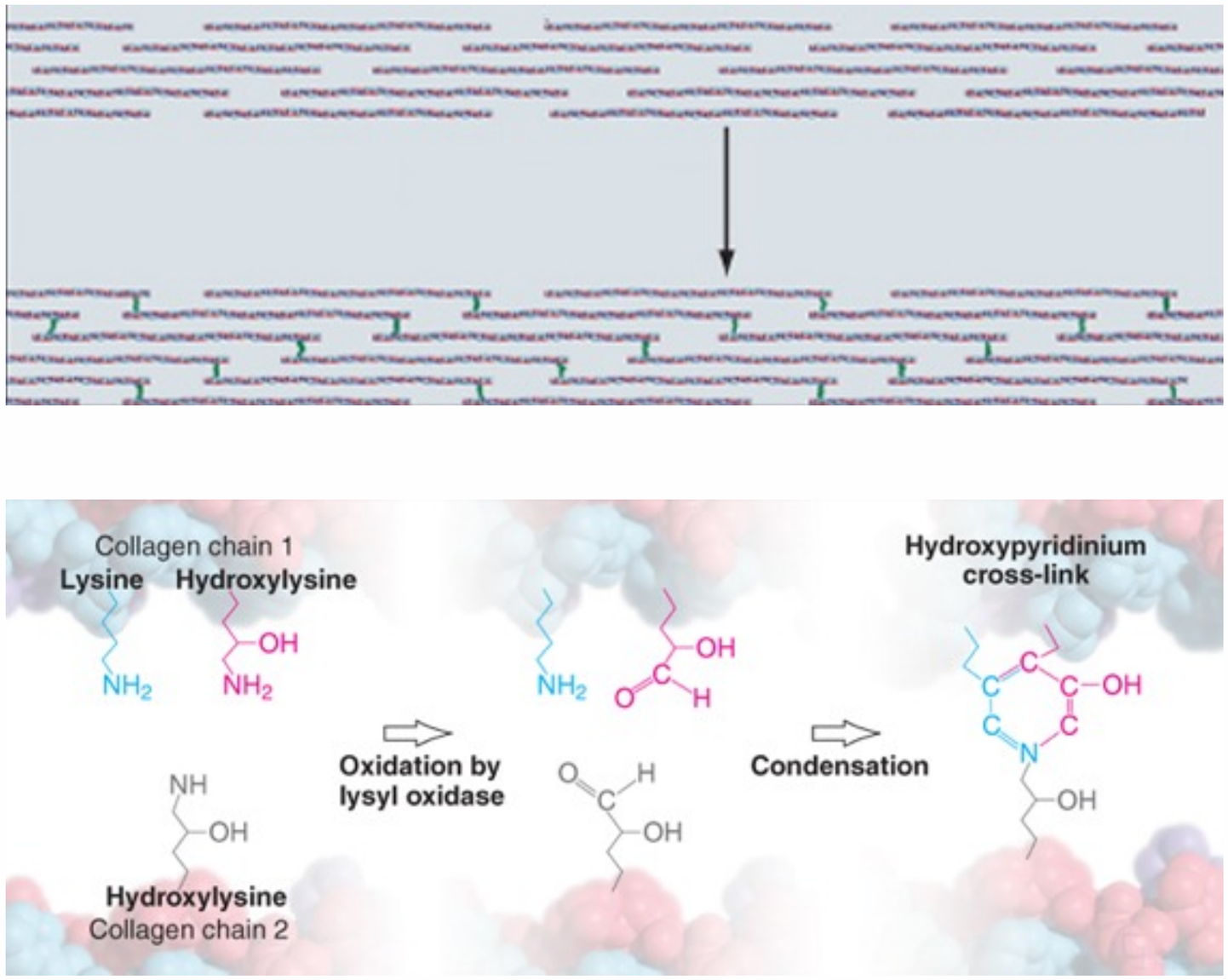
There are a number of important steps in the synthesis of collagen inside fibroblasts that allow for its final assembly into collagen fibers. Collagen is synthesize on ER-bound ribosomes and crosses the ER membrane during translation similar to other secreted proteins. In the ER two important modifications take place. First, certain prolines and lysines are hydroxylated. These modifications will allow for assembly into trimers and covalent crosslinks between collagen trimers outside the cell. Second, disulfide bonds between collagen polypeptides mediates their assembly into trimers by facilitating interaction between correct collagen proteins. One other feature of intracellular collagen is that it contains extra sequence at its N and C-termini called prodomains. These prevent collagen trimers from assembling into fibrils inside the cell which would be catastrophic for the cell.

Removal of prodomains allows collagens to assemble into fibrils.



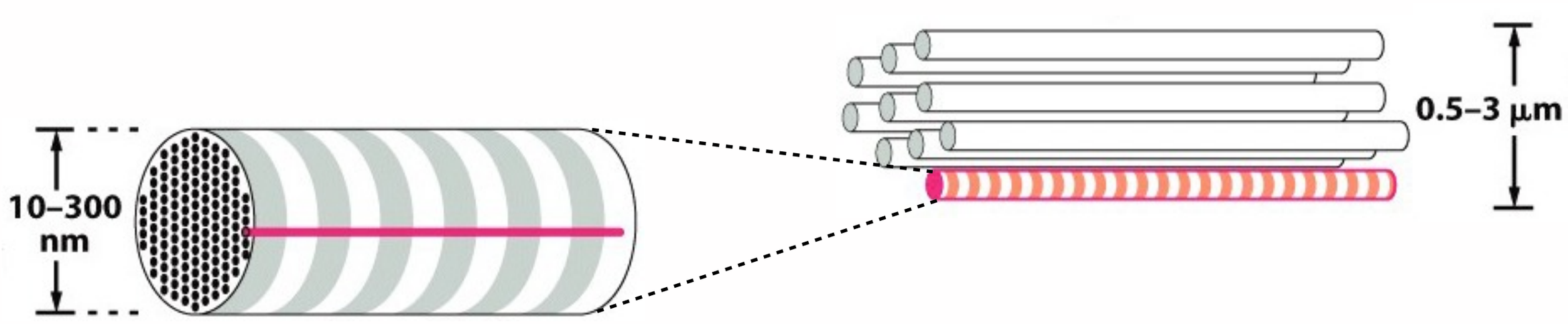
1. Once secreted, the prodomain are removed from the procollagen by proteases outside fibroblasts to produce the mature collagen molecule.
2. Collagen the self-assembles into fibrils -> entropy-driven process.

Lysyl oxidase crosslinks collagen trimers in fibrils.



Covalent crosslinks between lysines and hydroxlysines are formed to generate a much stronger fibril. Lysyl oxidase catalyzes reaction. Mutations that affect hydroxylation of lysines generate weaker collagen.

Collagen fibrils aggregate to form fibers.



All collagens are trimers but fibrous collagens assemble into parallel arrays to increase mechanical strength. Fibrils then aggregate into large bundles called fibers -> type I. This gives type I collagen three levels of interactions:
Trimer -> assembles inside fibroblasts.
Fibrils -> aggregation of trimers and crosslinking; outside fibroblasts.
Fibers -> aggregation of fibrils.

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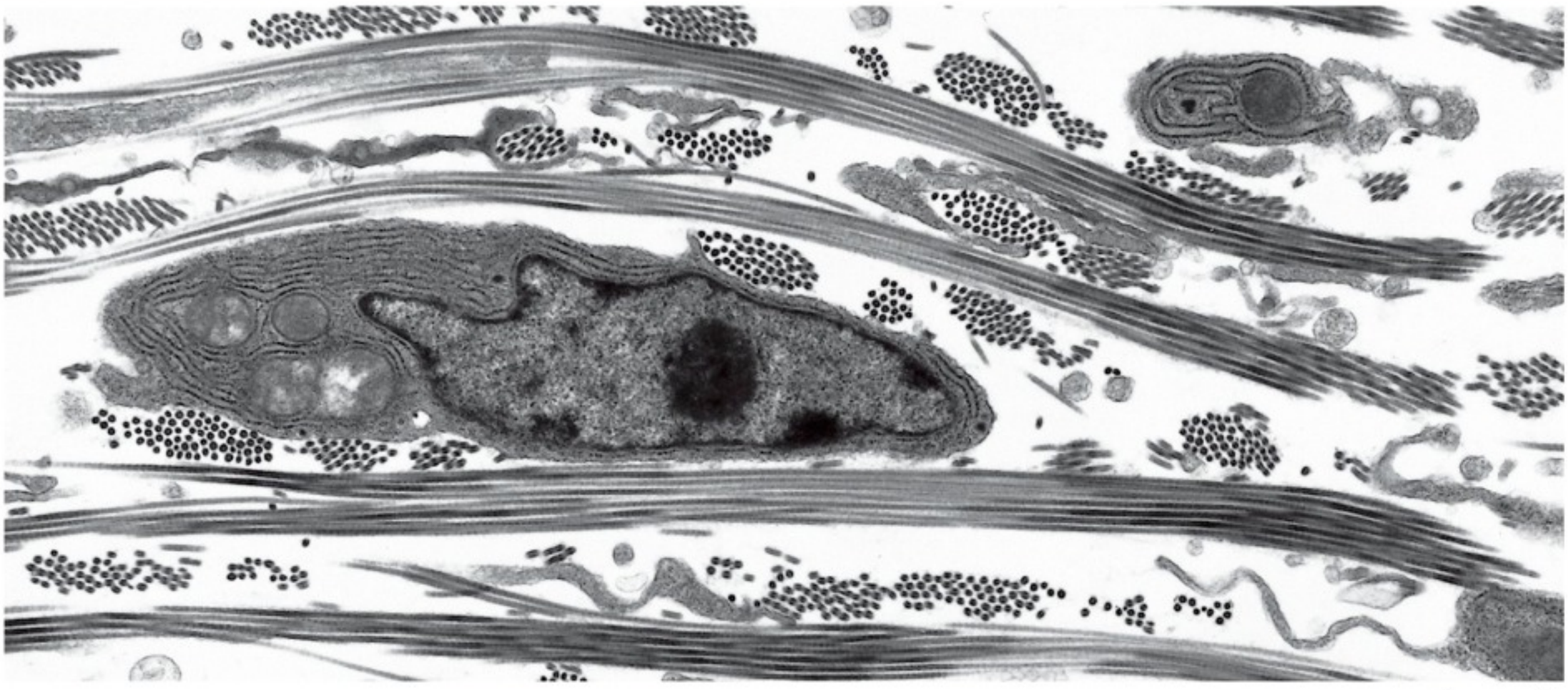

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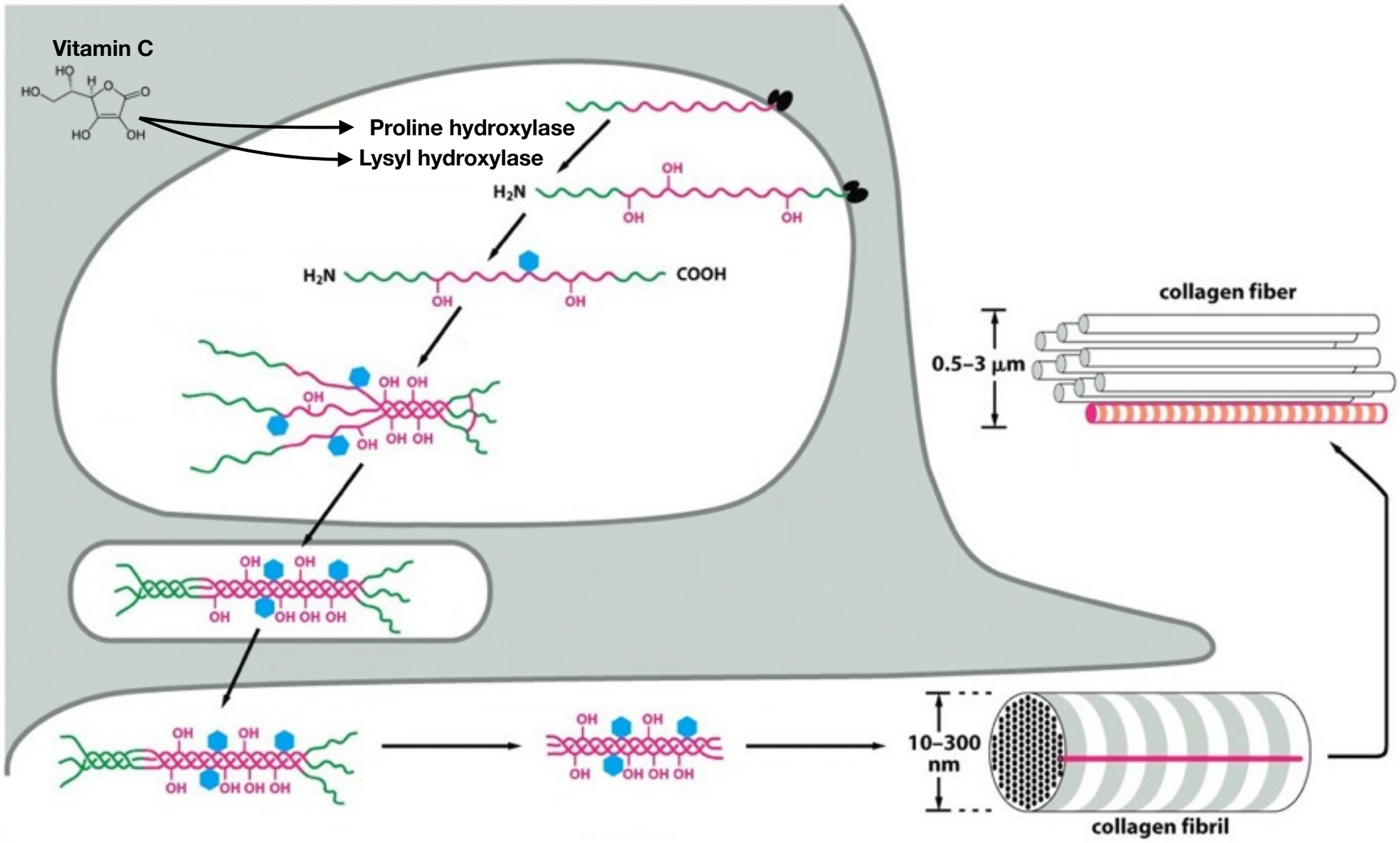
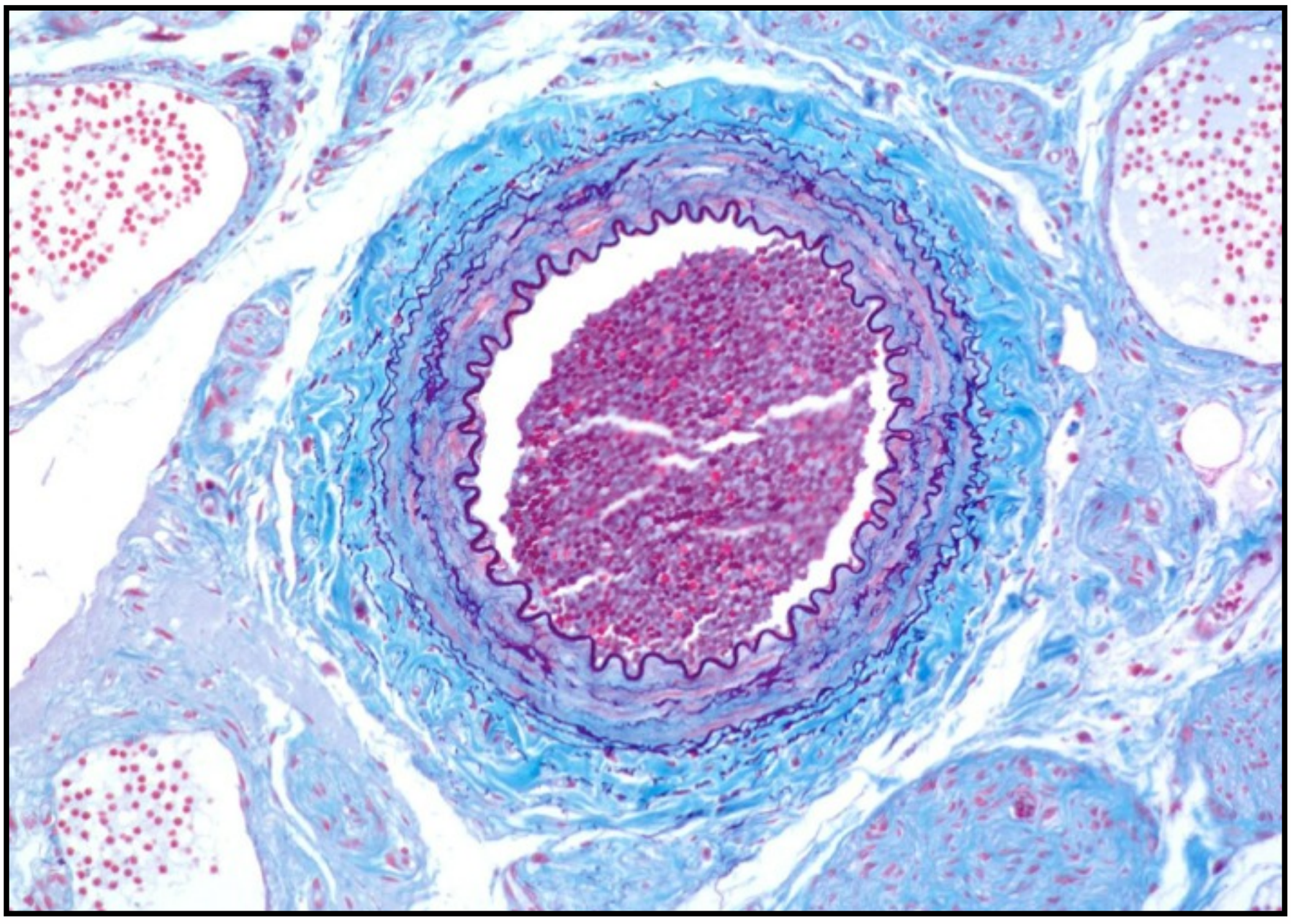
A 40 year old, homeless man arrives at the Neighborhood Health Project in New Haven.



The proper assembly of collagen is dependent upon two enzymes that act immediately after collagen is synthesized in the ER. Proline and lysyl hydroxylases convert proline and lysine into hydroxylated versions. These hydroxyl groups will later be used to crosslink trimers in collagen fibrils. Both enzymes require vitamin C as a cofactor and people who don’t consume enough vitamin C will produce collagen that lacks hydroxylated lysines and prolines. Because these trimers cannot be crosslinked, the collagen fibrils will be weaker leading to tissues that are more prone to damage.

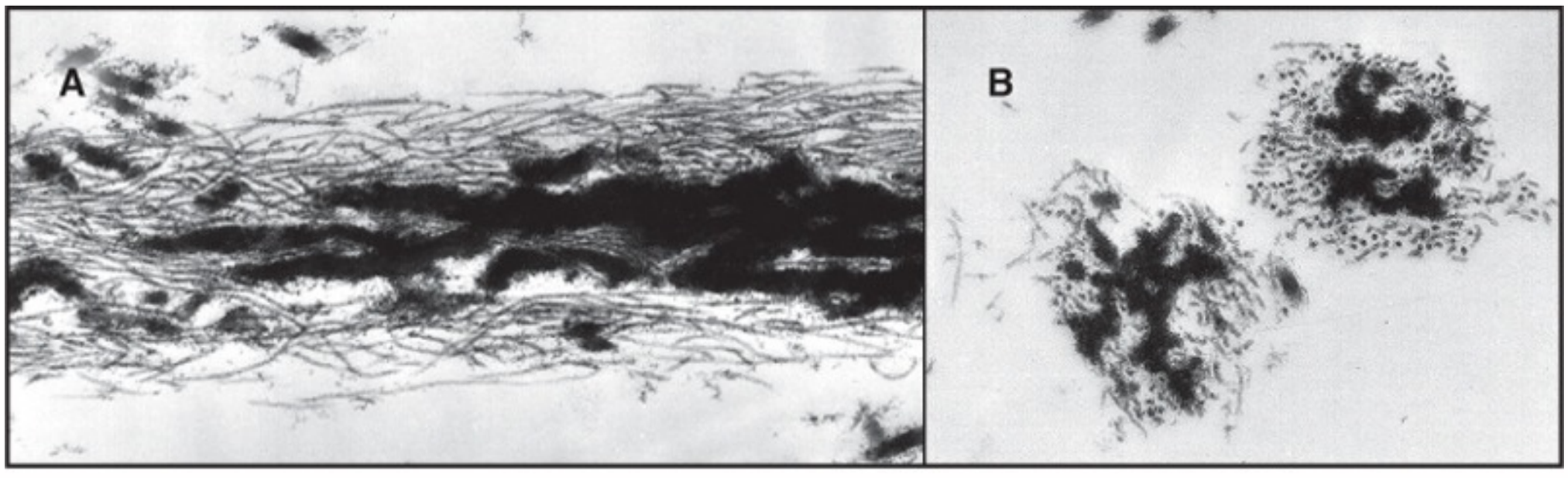
Elastic Fibers

Elastic fibers allow tissues to stretch and recoil.



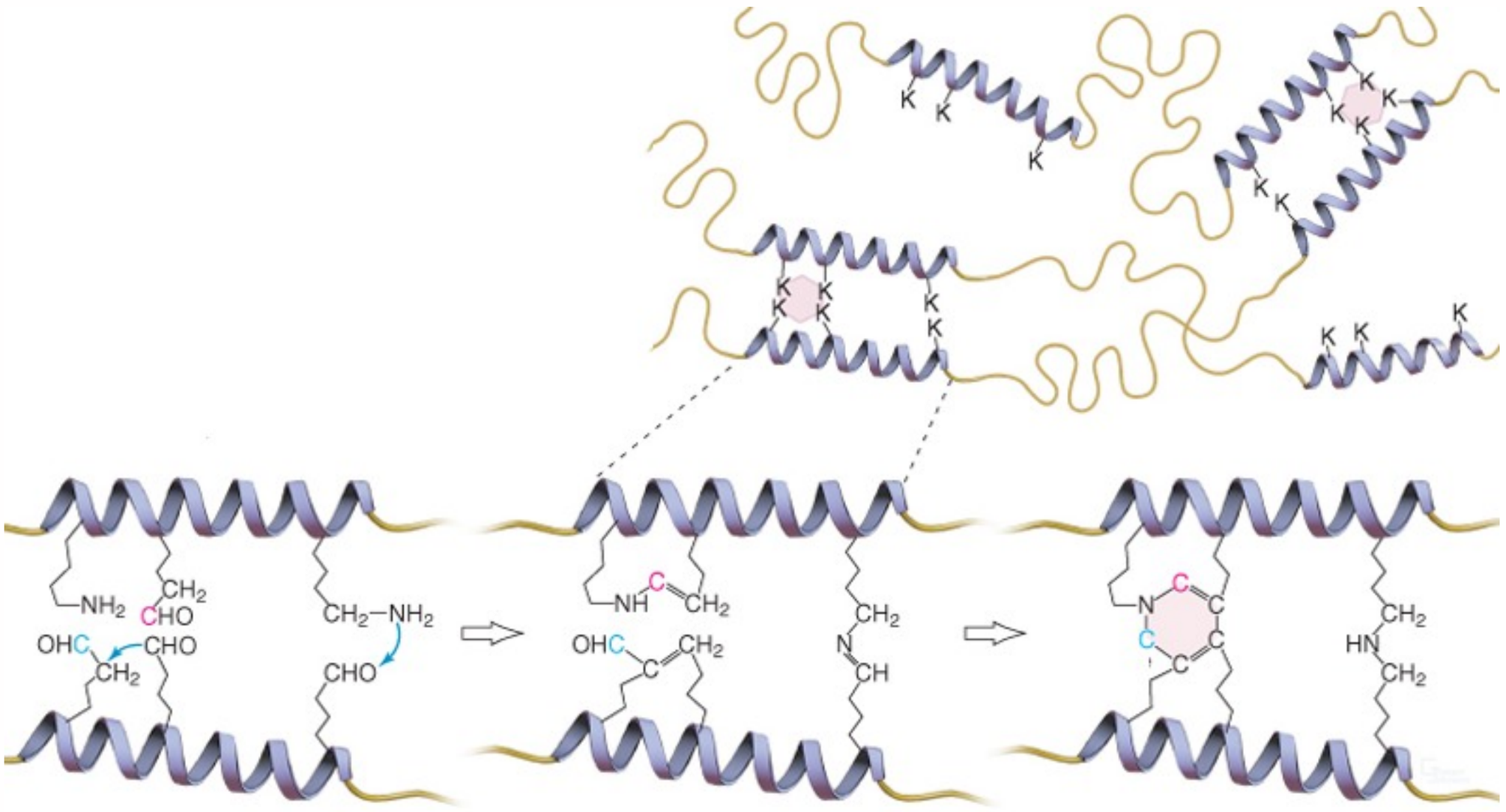
Next, we will examine elastic fibers that are often found enmeshed with collagen fibers as shown in this cross section of an artery. The elastic fibers stain dark blue whereas the collagen stains light blue. Elastic fibers have different mechanical properties from collagen. They allow for stretching of tissues under external force, but generate a recoil force when the external force is removed. Elastic fibers are prominent in the walls of arteries especially the aorta. The elastic fibers stretch to allow the aorta to accommodate a large volume of blood during systole. When the pressure drops during diastole, the fibers recoil pushing blood into the circulatory system. Because of elastic fibers a constant blood pressure is maintained throughout the circulatory system, even though the pumping of the heart delivers blood in a pulsatile fashion.

Elastic fibers are a composite of elastin and fibrillin.



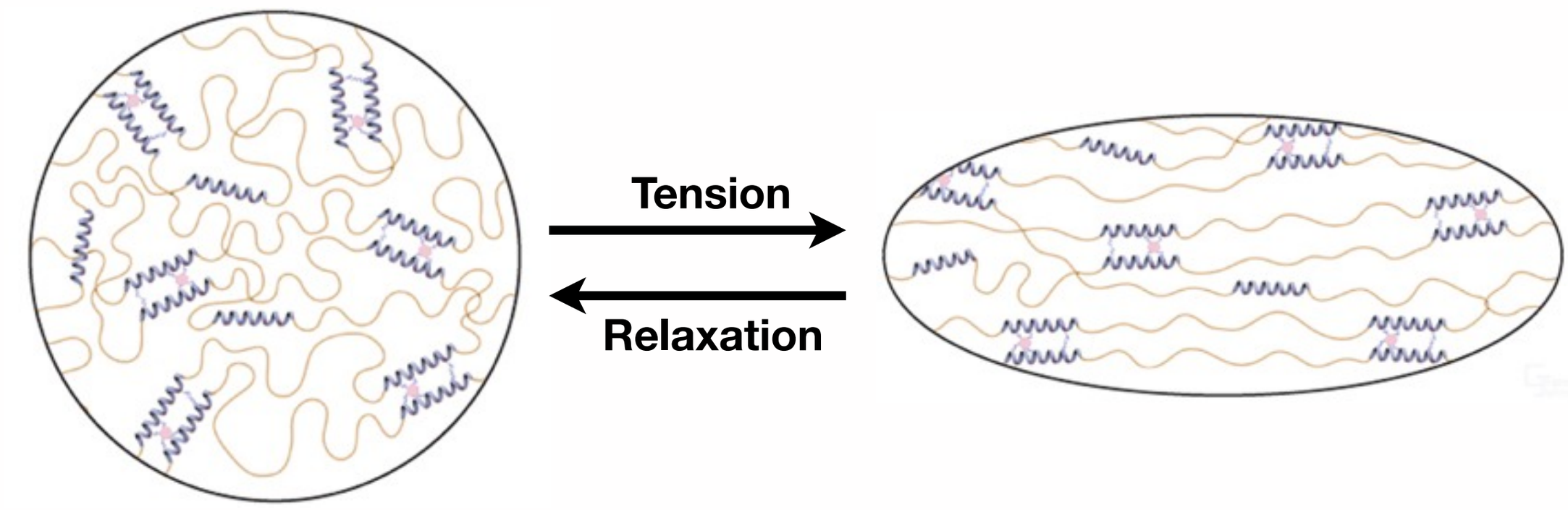
Elastic fibers are a composite material composed of two primary components: elastin and fibrillin. Both are synthesized by fibroblasts and secreted into the surrounding tissue where they assemble into elastic fibers. Fibrillin fibers are thin and arranged in more or less parallel arrays. They are required for correct assembly of elastic fibers. Elastin appears as a amorphous substance. Elastin is what gives elastic fibers its characteristic mechanical properties of stretching and recoiling.

Elastin is an unstructured protein that is crosslinked into networks.



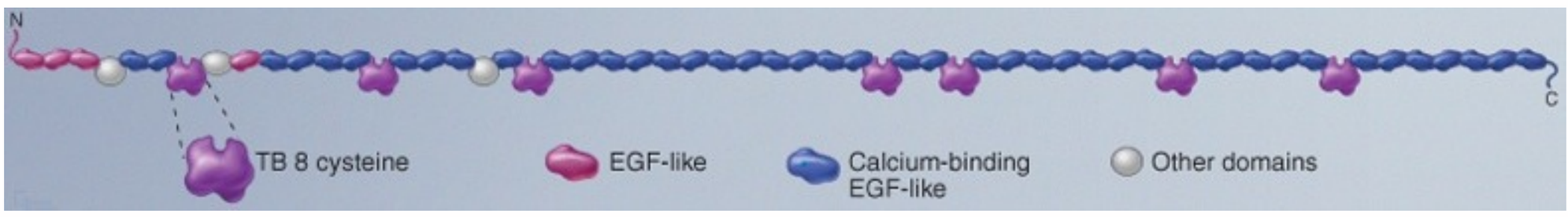
Elastin is the main structural component of elastic fibers. It is largely unstructured compared to collagen. It has a hydrophobic domain and coiled coil domain. Lysyl oxidase crosslinks elastins by a similar mechanism as it does for collagen trimers.

Tension generates order in elastin networks that provides energy for recoil.



Relaxed fibers largely unstructured and disordered. The hydrophobic domains cluster to avoid water. Tension stretches elastin generating order in hydrophobic domains. When tension is removed, elastin returns to its lower energy state of disordered fibers, causing the elastin network to recoil.

Fibrillin is a multidomain protein that assembles into microfibrils with periodicity.



Fibrillin contains an array of different domains. Fibrillin monomers polymerize in head to tail fashion to form 10 nm microfibers with a repeating pattern.

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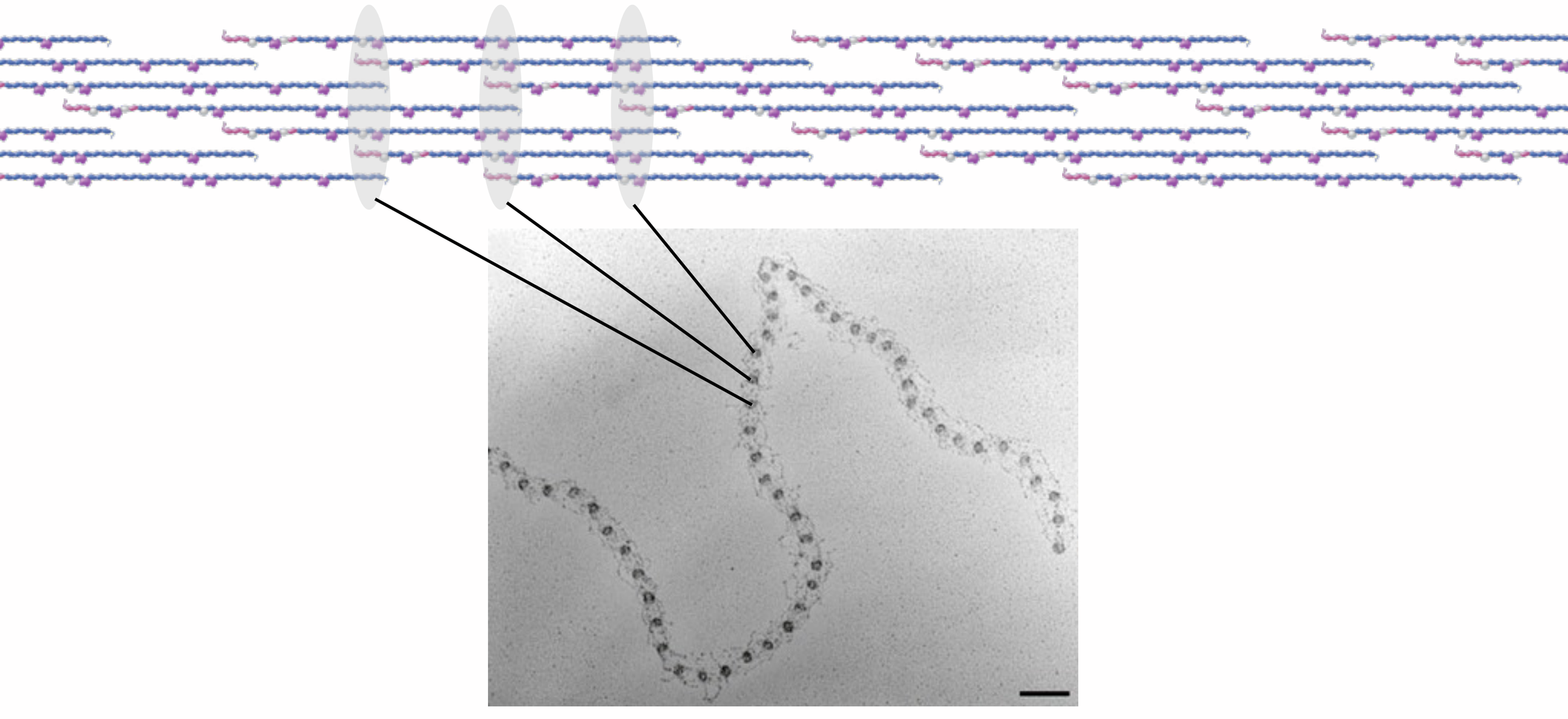
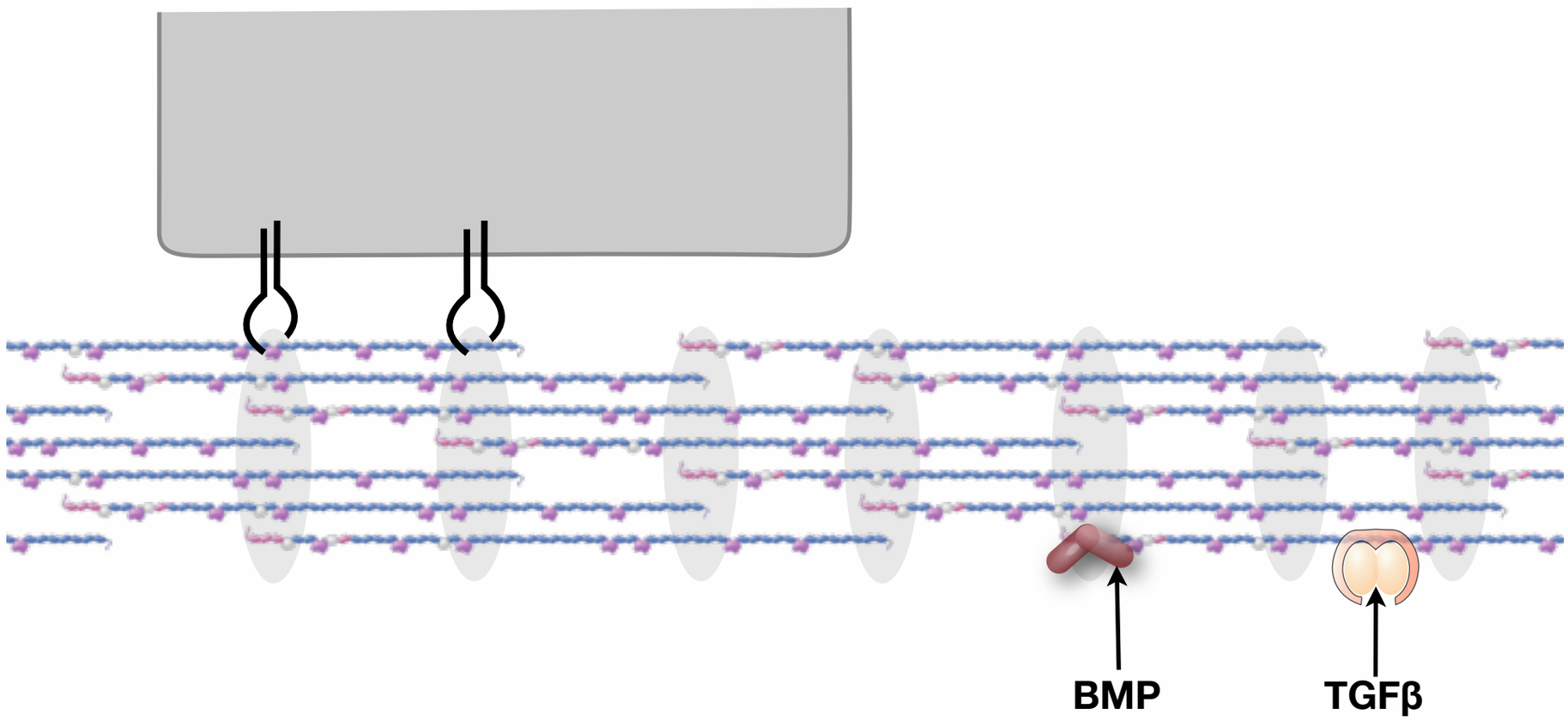


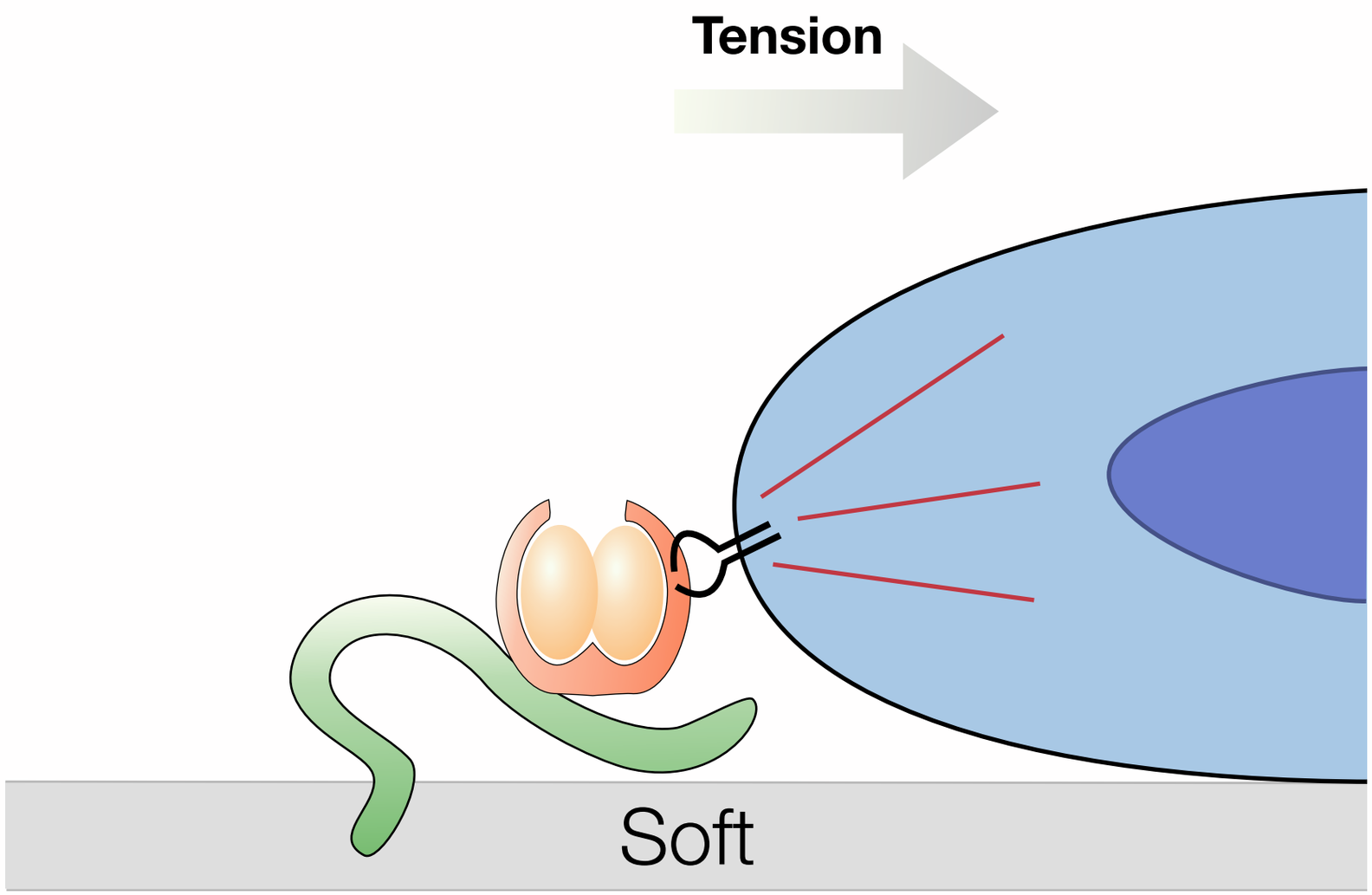
Fibrillin contains an array of different domains. Fibrillin monomers polymerize in head to tail fashion to form 10 nm microfibers with a repeating pattern.

Fibrillin interacts with integrins and key growth factors TGFβ and BMP.



Fibrillins form a number of interactions. They associate with integrins on cells. Recent work has found that fibrillin also interacts with key signaling molecules such as, BMP , an anti-mitogen and TGF-beta, an anti-mitogen that alters cell behavior and stimulate production of components of connective tissue.

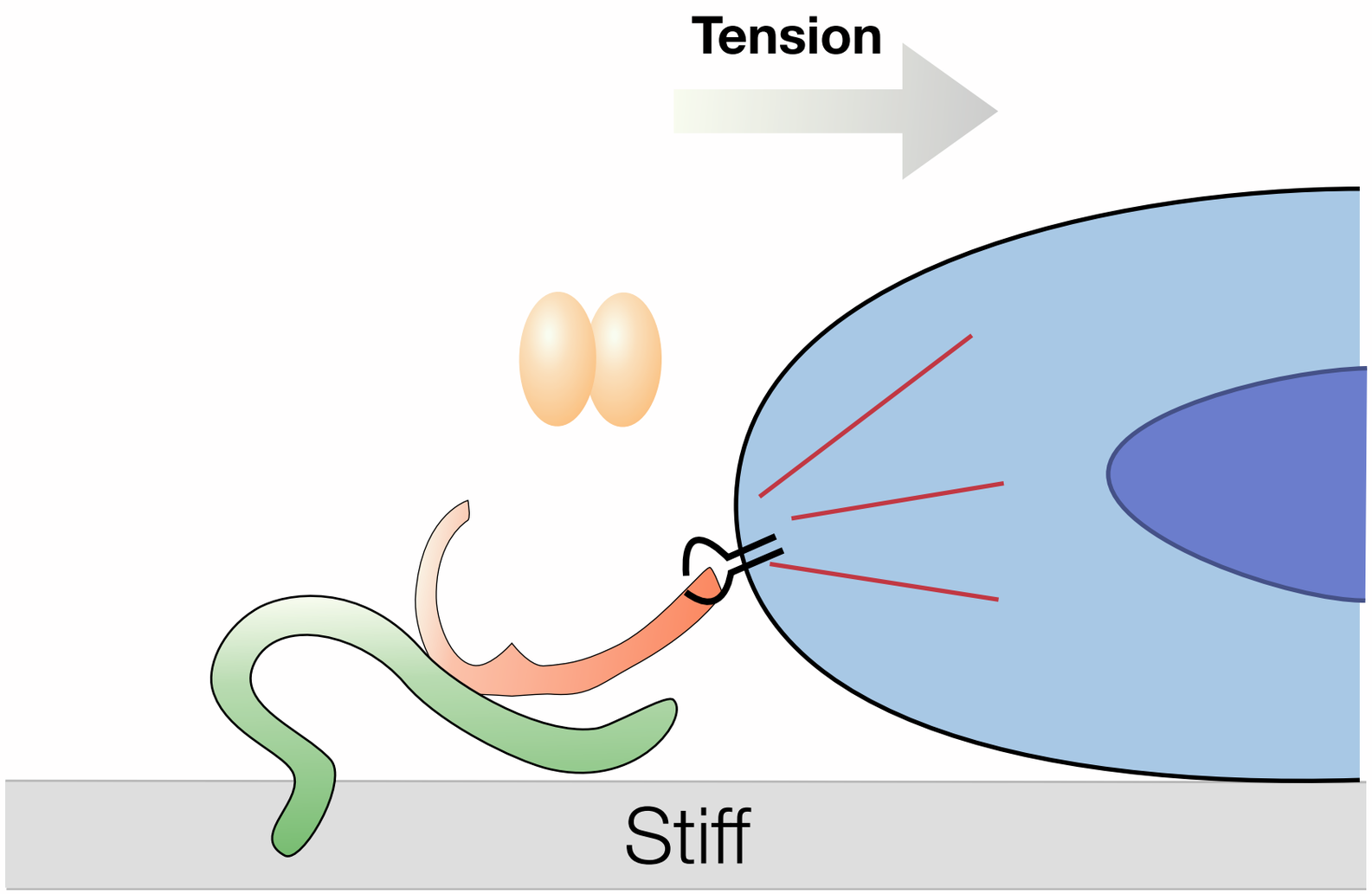
Fibrillin regulates the release of active signaling molecules.



Fibrillin acts to keep TGF-beta concentrations low. It associates with a complex of proteins called the latent complex that sequester TGF-beta and keep it inactive. In the absence of fibrillin, the latent complex can be digested to release TGF-beta.

Cells can release TGF-beta from the latent complex by generating tension. Integrins on cells bind the latent complex. By using myosin filaments to pull on the actin filaments associated with the integrins, cells can exert force on the latent complex. If the connective tissue is to soft, the tension will merely move the complex.

Fibrillin regulates the release of active signaling molecules.



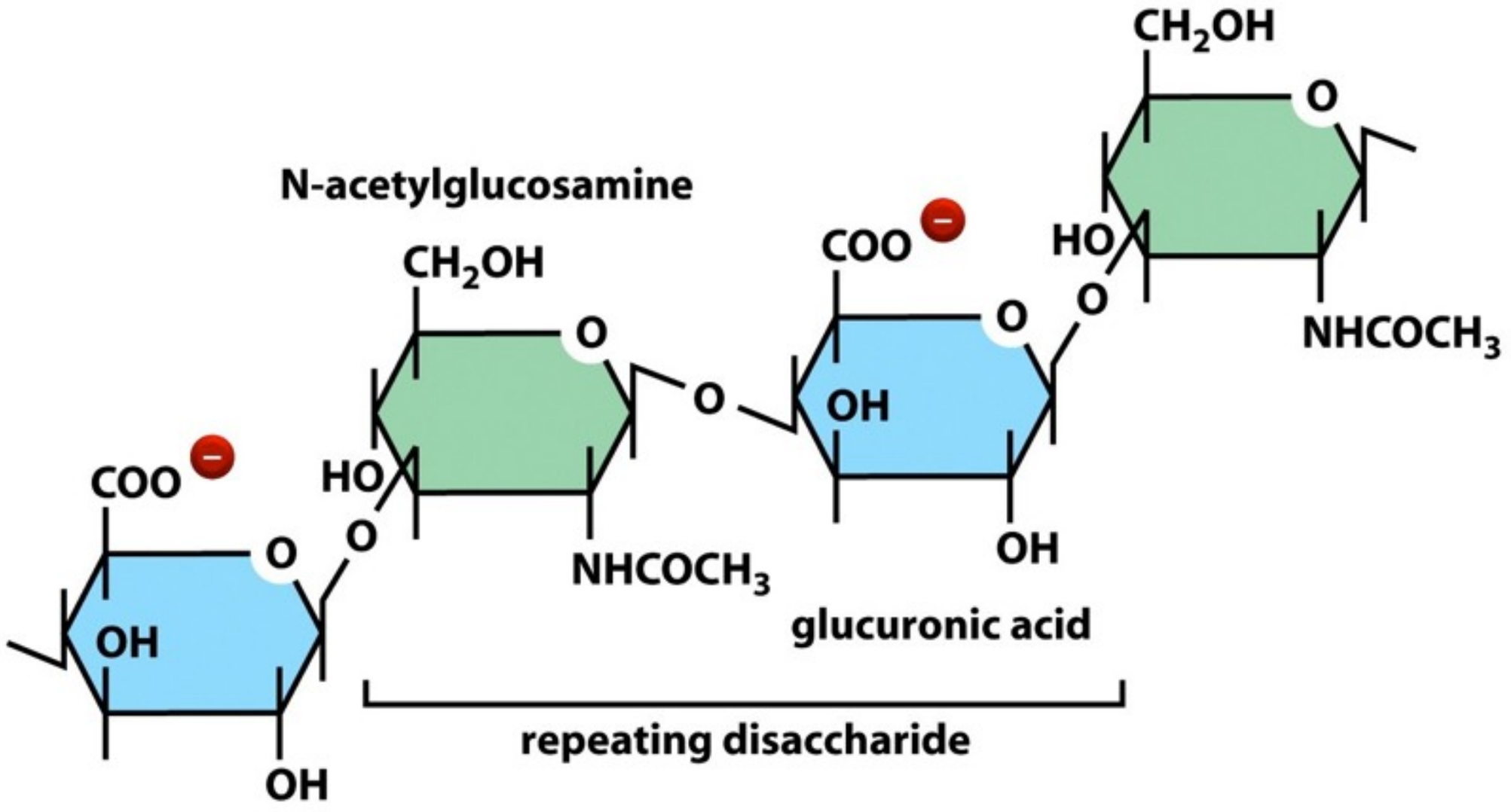
If the connective tissue is stiff, then the tension will open the latent complex and release TGF-beta. The medical importance is that mutations in fibrillin lead to several different diseases. The most well-known is Marfan's syndrome. Patients with Marfan's syndrome are often unusually tall with long limbs. The major medical consequences of Marfan's are defects in the cardiovascular system, including the heart and aorta. Most severe is an increased likelihood of an aortic aneurysm or dissection that can lead to sudden death. Originally, the defect in fibrillin leading to these symptoms was its structural role in elastic fibers. However, recent research has shown that the changes in the cardiovascular system are likely due to fibrillin's role in sequestering TGF-beta and that an excess amount of TGF-beta in patients with Marfan's leads to the clinical symptoms.

Glycosaminoglycans

Glycosaminoglycans (GAGs) in connective tissue resist compression by retaining water.

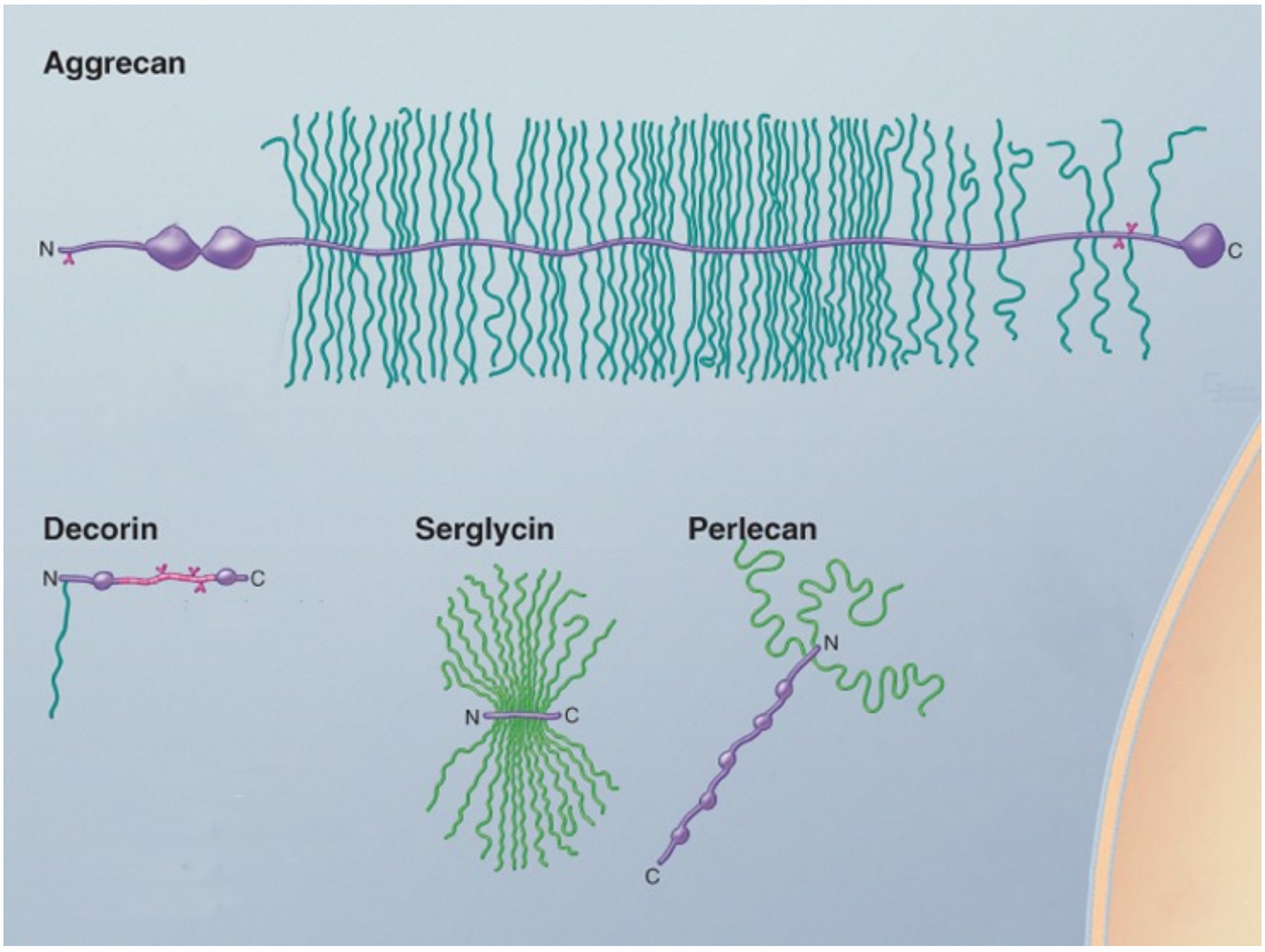
Tissue must not only accommodate tensile or pulling forces but they must also resist compression. The primary component in connective tissue that responds to compression are a family of molecules called glycosaminoglycans. GAGs resist compression by occupying a large volume and retaining water, similar to a plastic bottle filled with water. An air filled bottle collapses under applied force because the force expels the air from the bottle. In contrast, a bottle filled with water that is sealed so that it retains water, resists compression from an outside force. Several components generate the ability to retain water and resist compression and they have in common a large amount of glycosaminoglycans.

Glycosaminoglycans are long chains of repeating disaccharides.



The base component of GAGs is a disaccharide of two different sugars. These disaccharides are joined into polymers that can contain 1000s of disaccharides. The sugars that make up the disaccharides differ between GAGs, but the main feature is that they are negatively charged. Thus, GAGs are long, negatively charged polymers that that attract high concentrations of sodium. Osmosis attracts and retains large amounts of water.

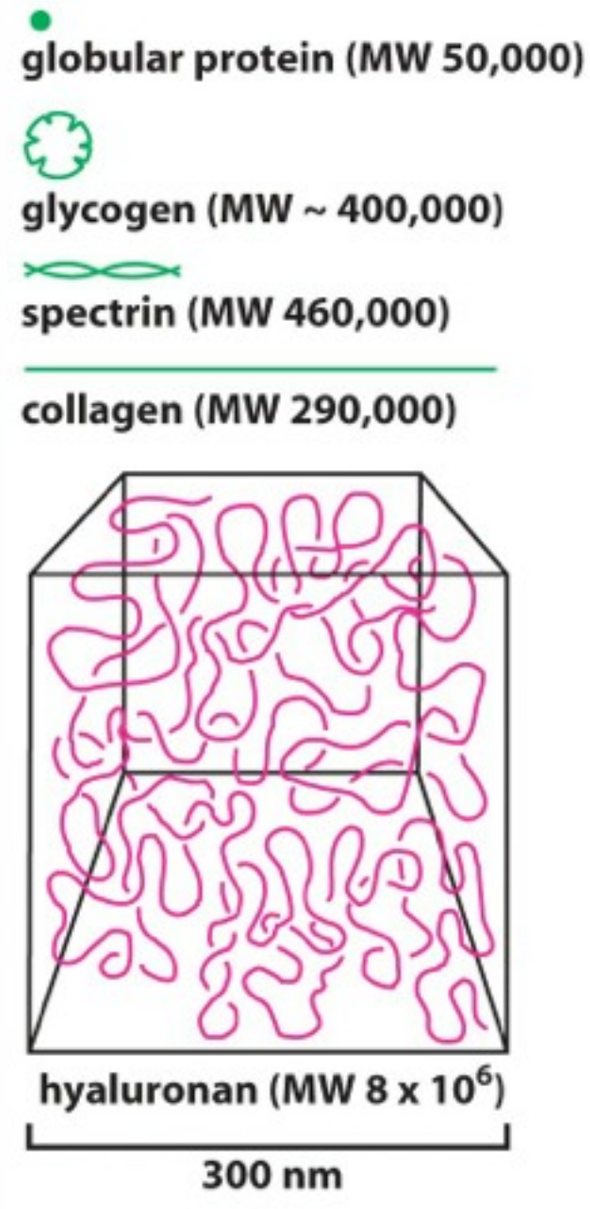
Proteoglycans are single polypeptide with several attached glycosaminoglycans.



GAGs come in two different types. One type is covalently linked to proteins and called proteoglycans. Proteoglycans are a single polypeptide that contains a few or many GAGs attached as side chains. Addition of GAGs to the protein occurs in the secretory pathway. Proteoglycans have proteins of different lengths and differ in the number and type of GAGs that are attached.

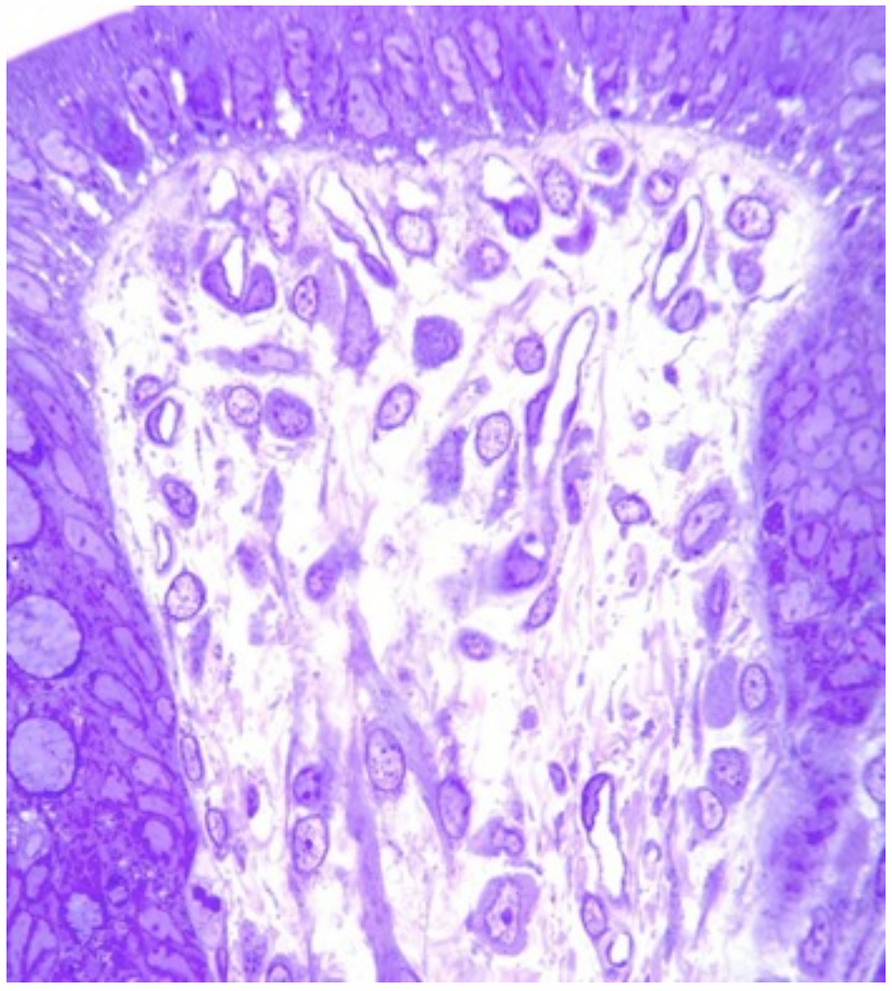
Beside resisting compression, proteoglycans also play a role in signaling. There is evidence that the GAG side chains bind different signaling molecules and therefore, proteoglycans may control the extent or spread of a signaling event. Remember paracrine signaling in which the signaling molecule only affects cells in the immediate surrounding environment and does not enter the blood stream. Proteoglycans limit the diffusion of the signaling molecule by binding it with their GAG side chains.

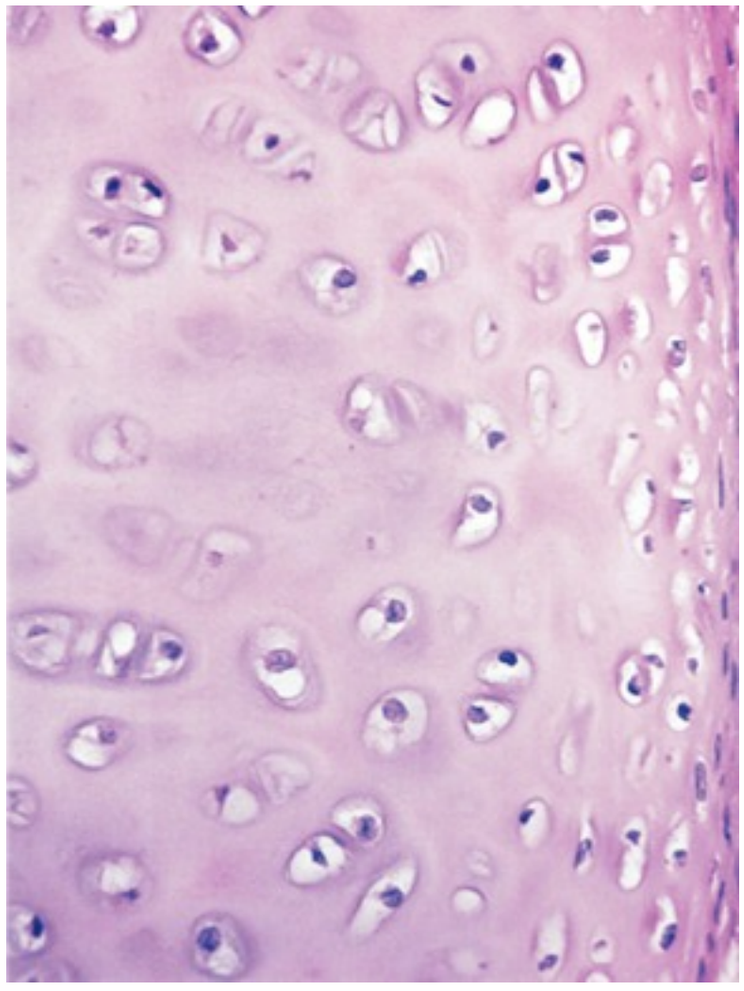
Hyaluronan is a long polymer of dissaccharides that occupies a large volume.



Hyaluronan is another glycosaminoglycan and is the primary compression-resisting component of connective tissue. Unlike proteoglycans, hyaluronan does not contain protein. Instead, it is a long polymer of repeating disaccharides. Hyaluronan can contain up to 25000 repeats and reach a length of 20 μm, the size of an average cell. Hyaluronan lacks the structure of most proteins and contains many regions of that form random, flexible coils. Remember that the sugars in hyaluronan are negatively charged and repel each other. That generates a lot of space within the hyaluronan and allows it to occupy an incredibly large volume. In addition, hyaluronan like other GAGs bind and retains water. So hyaluronan functions as that water filled bottle to resist compression.

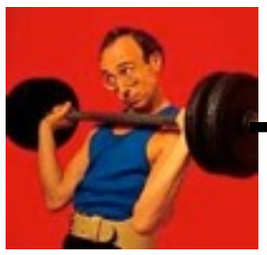
Proteoglycans and hyaluronan are found primarily in loose connective tissue and cartilage.

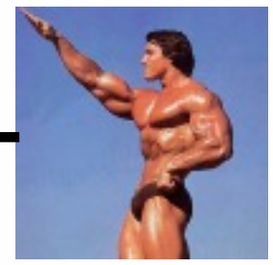




GAGs are found predominantly in loose connective tissue and cartilage. In loose connective tissue it is often referred to as ground substance in histological samples. Usually it makes up the white space because the GAGs stain poorly by H&E.

Varying composition of collagen, GAGs and elastic fibers generate different mechanical properties.





Organ Support

Collagen +
GAGs +
Elastic Fibers +
Cells +++

Blood Vessels

Collagen +++
GAGs +
Elastic Fibers + to +++

Cartilage

Collagen ++
GAGs ++++
Elastic Fibers ++++

Tendon

Collagen +++++

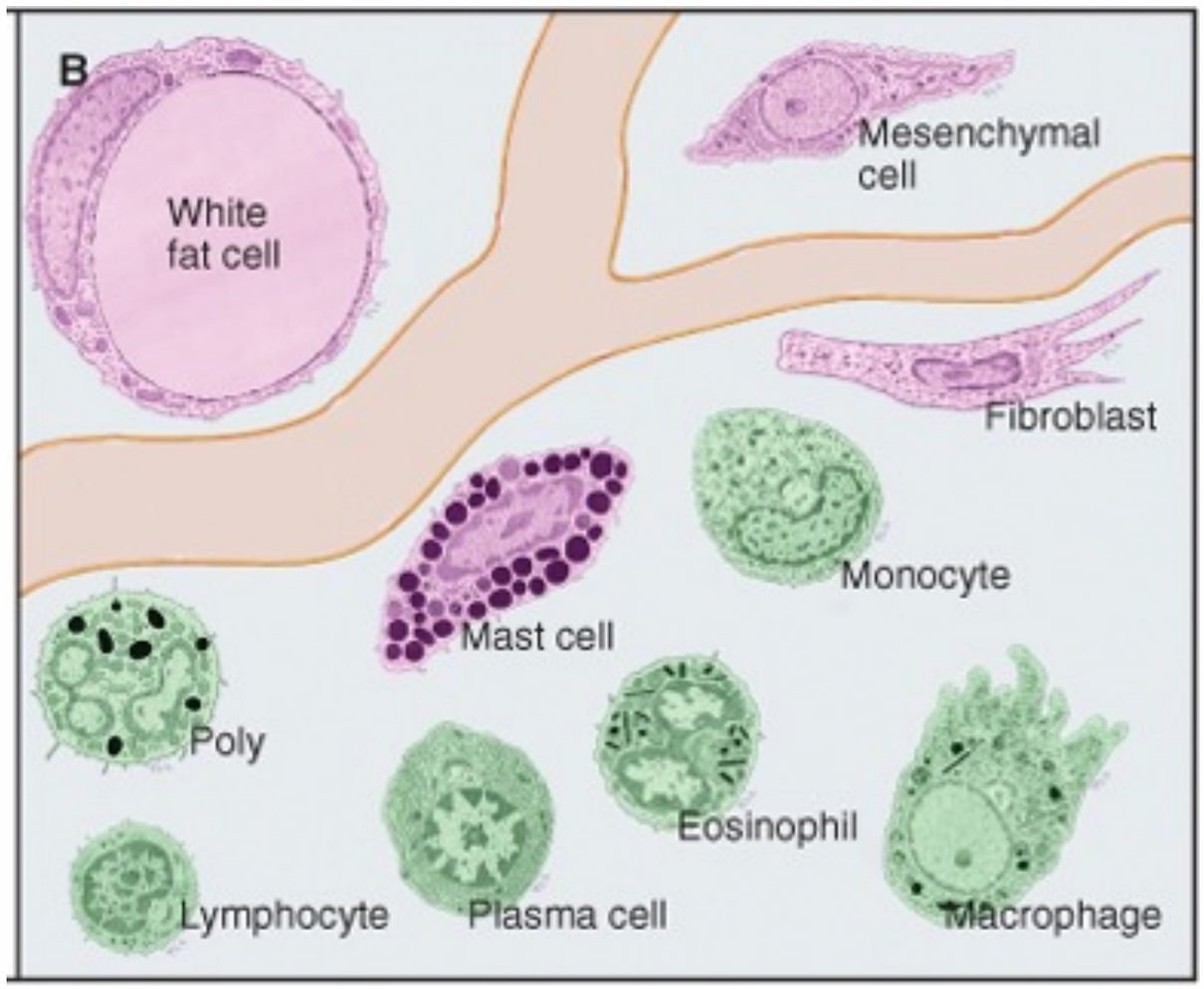
Bone

Collagen +++++
Mineral +++++

Cells of connective tissue

Connective tissue contains resident and transient cells.

Resident



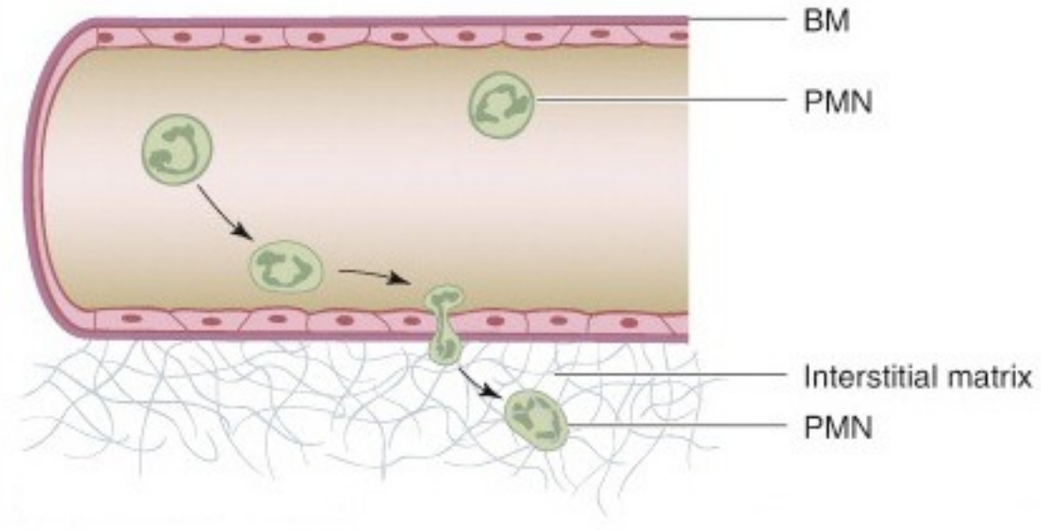
Pollard et al. *Cell Biology 2nd Edition*

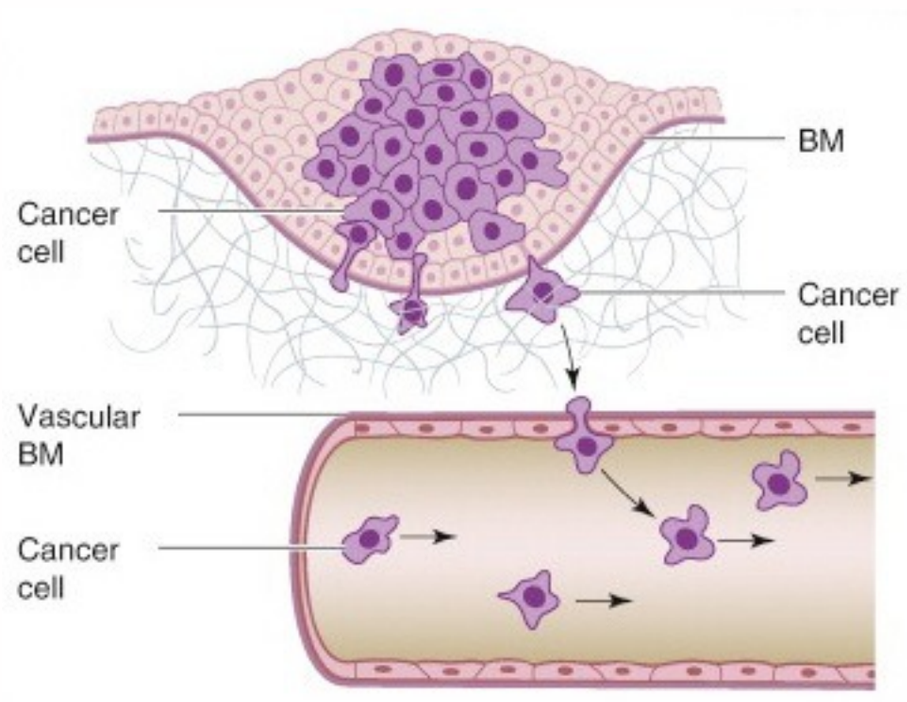
Transient

Cells in connective tissue can be divided into two camps: Cells that develop and remain in the connective tissue and cells that migrate from blood and stay transiently. The resident cells include fibroblasts that generate most of the structural components of connective tissue: collagens, elastin, GAGs. Fat cells are a source of energy and synthesize hormones. Mast cells mediate hypersensitivity reactions via release of inflammatory factors. Mesenchymal cells are the stem cells for the resident cells.

The transient cell population are mostly cells that provide immune function. Neutrophils track and phagocytose bacteria and are short lived in connective tissue. Eosinophils increase during parasitic infection. Plasma cells produce antibody. T-cells initiate and regulate immune reactions. Macrophages ingest cell debris and antigens coated with antibody.

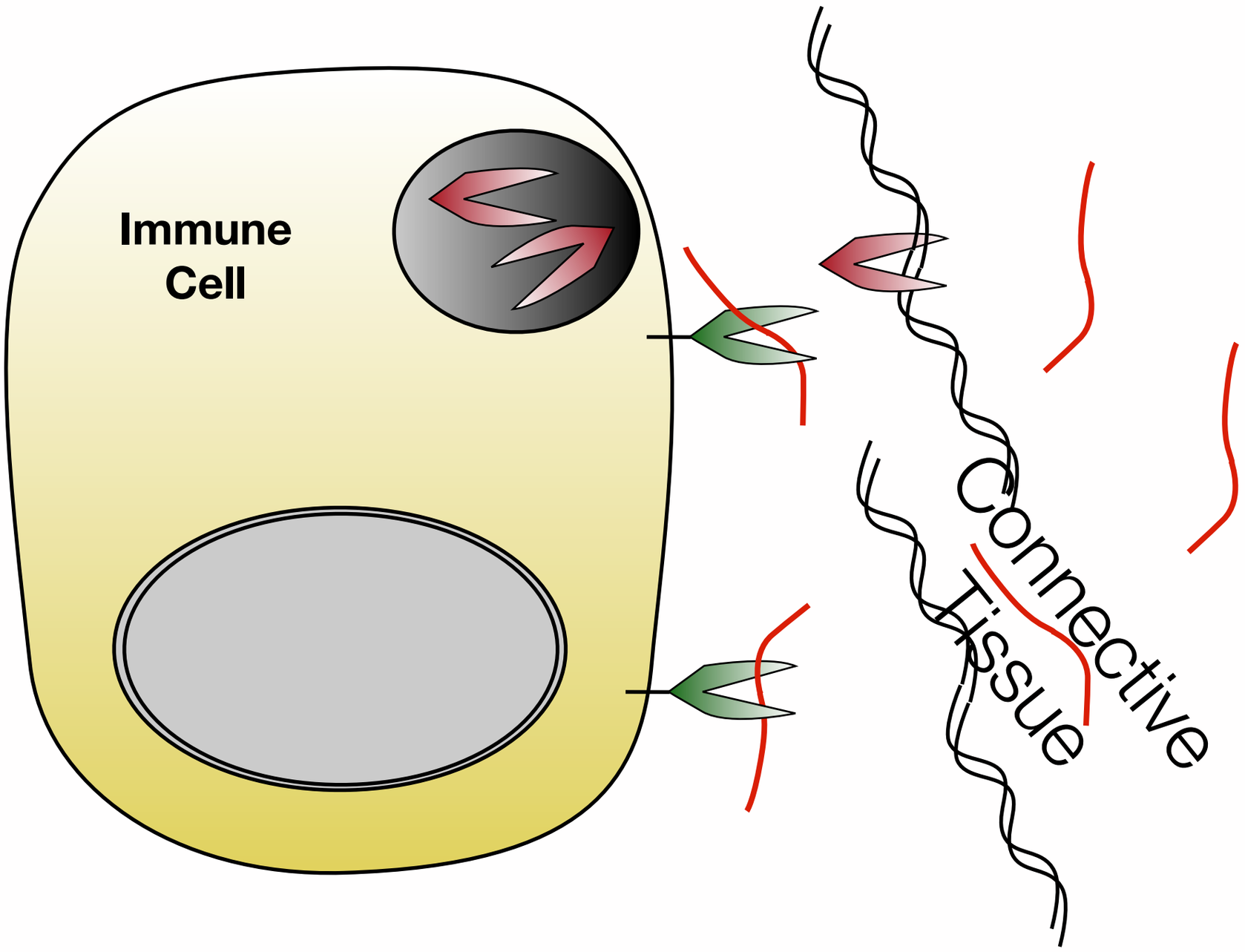
Transient cells digest connective tissue to aid motility and migration.





Because many cells in connective tissue come from blood, they must cross several barriers and migrate through connective tissue. First they must cross the endothelial cells, a process that will be discussed in a later lecture. They must also cross the basement membrane and migrate through the connective tissue. Most immune cells will digest components of connective tissue to open gaps through which cell can move. Metastatic cells develop a similar ability to escape a tumor and find their way to lymphatic or blood vessel.

Matrix metalloproteinases are a family of proteins that digest components of connective tissue.



Cells that navigate across basement membrane and through connective tissue utilize set of proteases to digest components called matrix metalloproteases (MMPs). MMPs require zinc as a cofactor and can be membrane-bound or secreted outside cell as soluble proteins. There are different types of MMPs that digest different types of collagen in connective tissue. Cells also secrete elastase which digests elastin.

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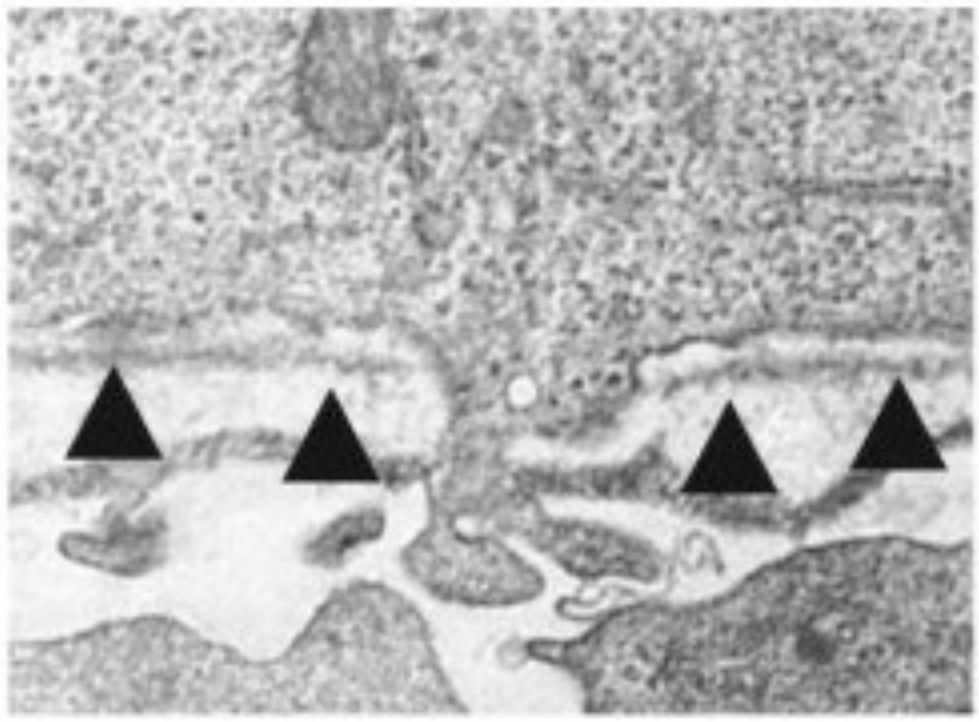

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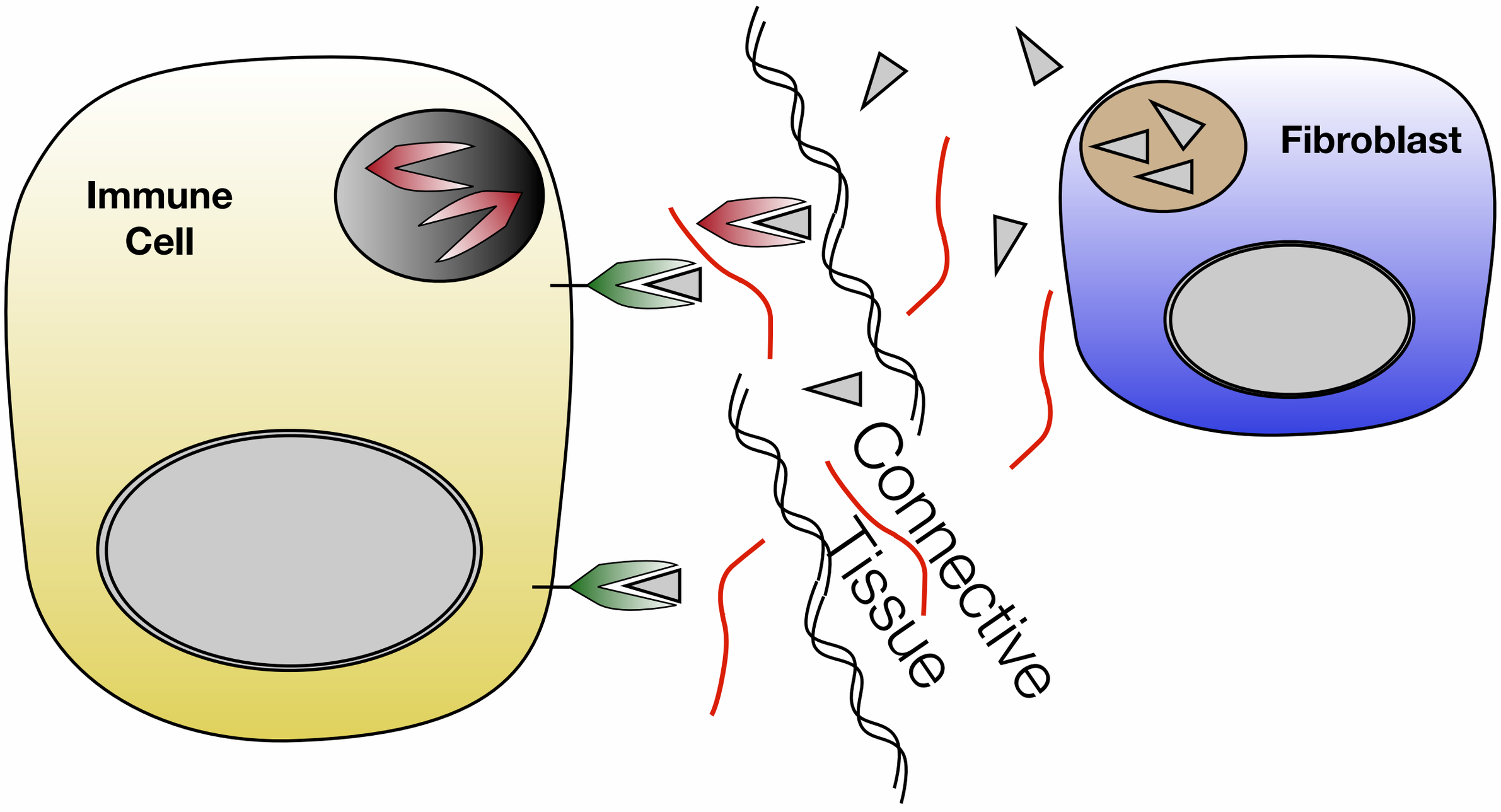
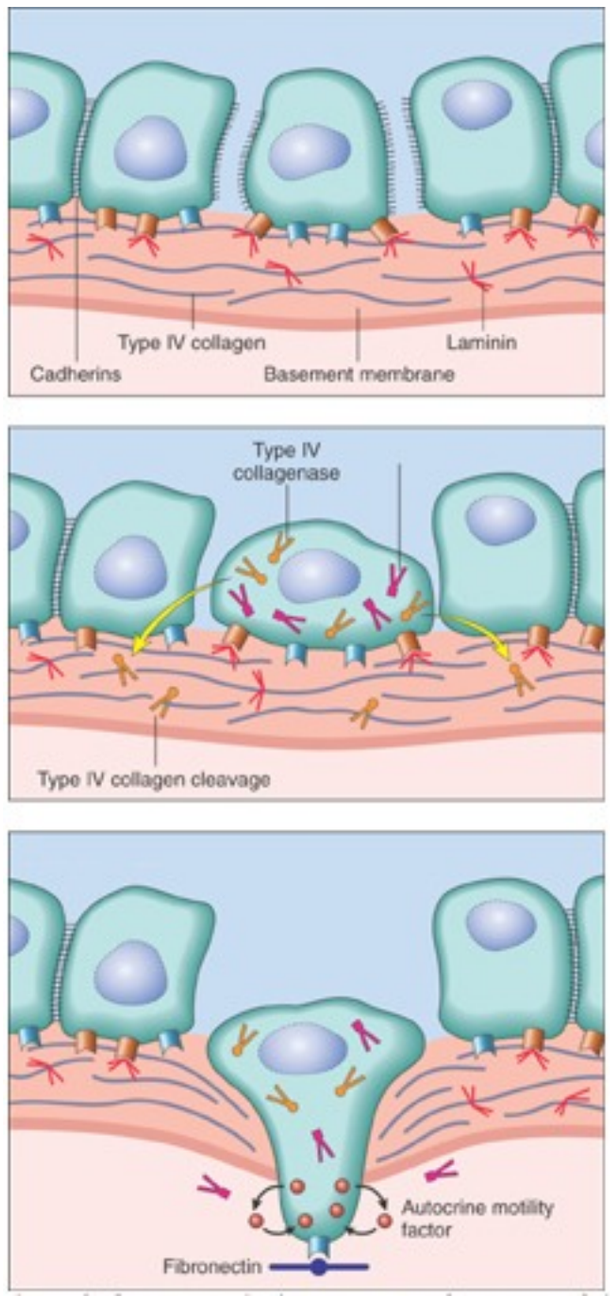
Connective tissue cells secrete tissue inhibitors of metalloproteases (TIMPs).



To inhibit the effects on MMPs, cells of connective tissue (e.g. fibroblasts) secrete inhibitors of MMPs called tissue inhibitors of metalloproteases (TIMPs). An imbalance between TIMPs and MMPs in a tissue can lead to digestion of connective tissue components and weakening of that tissue. The effects of rheumatoid arthritis on joints is thought be caused by an excess of MMPs over TIMPs and the destruction of connective tissue components in the cartilage of the joints.

Metastatic carcinomas use matrix metalloproteases to digest basement membrane.



Carcinomas use MMPs to digest components of the basement membrane. This allows carcinomas to migrate into the underlying tissue where blood vessels and lymphatic vessels reside. Several years ago inhibitors of MMPs were tested as potential inhibitors of metastasis of tumor cells. Although promising in animal models, the inhibitors did not have much effect on the progression of cancers in humans.

Take home points...

- Mechanical properties of connective tissue is determined by amount and arrangement of collagen, elastic fibers and glycosaminoglycans.

- Fibril collagens resist tensions and are formed from parallel arrays of collagen trimers.

- Elastic fibers allow tissues to stretch and recoil and contain elastin and fibrillin.

- Glycosaminoglycans contain large amounts of disaccharide chains and resist compression.

- Transient immune cells enter connective tissue by digesting its fibers.