بسم الله الرحمن الرحيم

CYTOLOGY

MID – Lecture 10

The ECM and cell-cell interactions

﴿ وَإِن تَتَوَلَّوْا يَسْتَبْدِلْ قَوْمًا غَيْرَكُمْ ثُمَّ لَا يَكُونُوا أَمْثَالَكُم ﴾

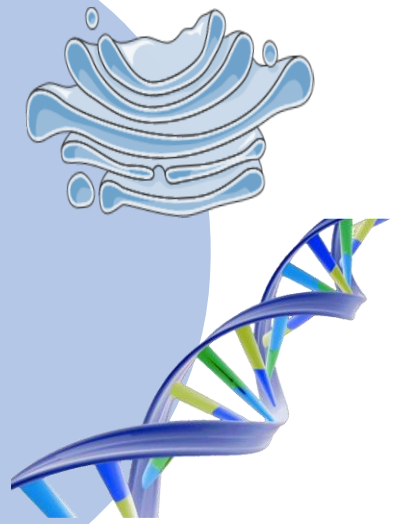
اللهم استعملنا ولا تستبدلنا

Written by:

- Laith Alhuniti
- Mahmood Al-Absi

Reviewed by:

- Laith Joudeh
- Waleed Darawad



Lecture 7: the extracellular matrix and cell-cell interaction

Prof. Mamoun Ahram

School of Medicine

Second year, Second semester, 2024-2025

The extracellular matrix

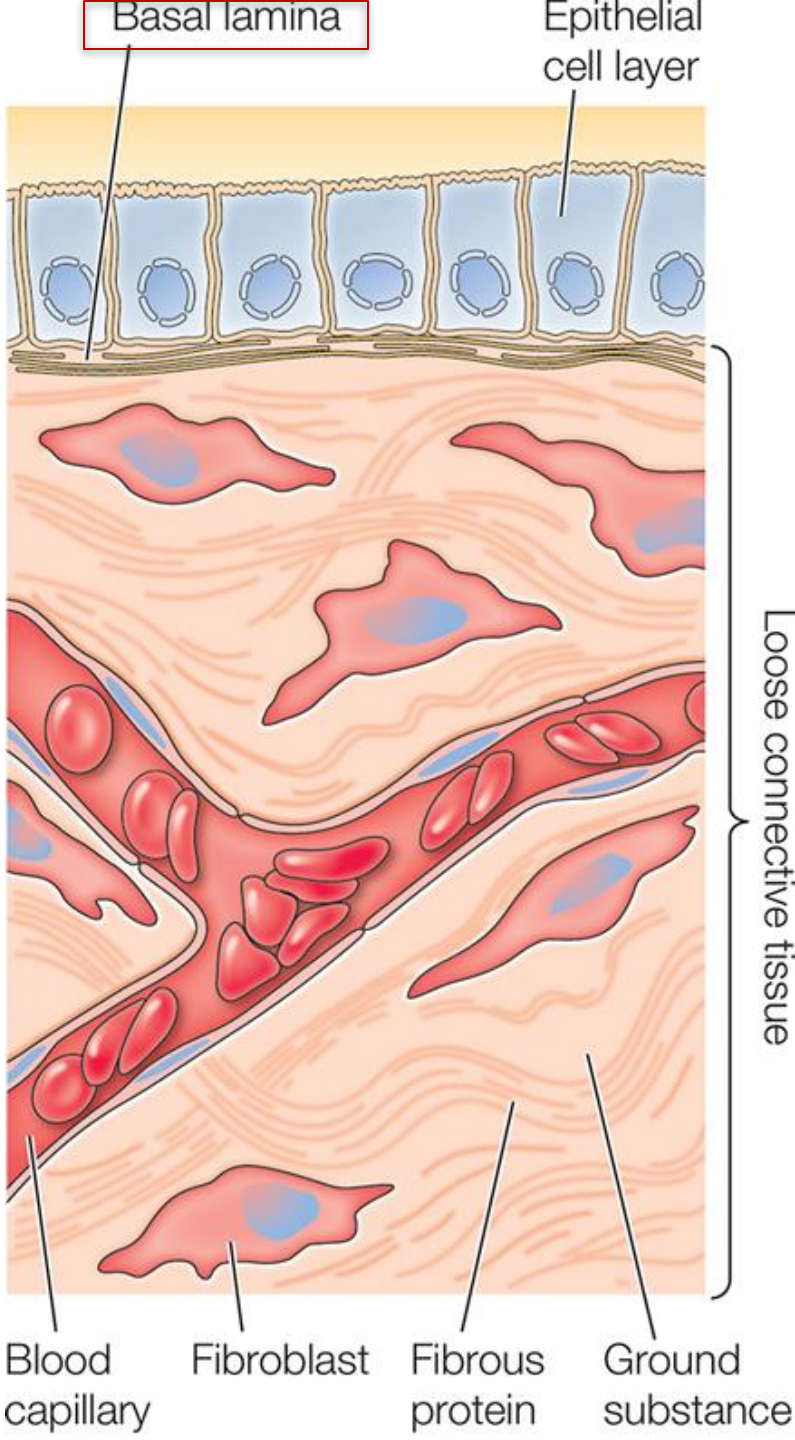
What is it?

- The extracellular matrix fills the spaces between cells, binds cells, and forms tissues.

The basal lamina (singular of basal laminae) forms a barrier between epithelial cells and the underlying extracellular matrix (connective tissue), which contains matrix proteins and various cell types, such as fibroblasts, immune cells, and newly formed blood vessels.

- Types:
  - Basal laminae: thin, sheet-like, structure upon which layers of epithelial cells rest
    - It supports the sheets of epithelial cells
    - It surrounds muscle cells, adipose cells, and peripheral nerves.
  - Connective tissues: Loose network of proteins and carbohydrates (and combinations of glycosaminoglycans, proteoglycans,....) underneath the epithelial cell layers where fibroblasts are distributed.
    - Others (other types of connective tissue): bone, tendon, and cartilage (which is negatively charged).

The basal laminae contain matrix components that differ from those in the connective tissues.



When a tumor forms from an epithelial cell with mutations, uncontrolled cell proliferation leads to the development of a cell mass known as carcinoma in situ. Carcinoma in situ, which refers to 'cancer in place,' describes a localized tumor that has not spread beyond its original site. In solid tumors, once cancer cells become malignant, they degrade the basal lamina, allowing them to invade the surrounding tissue matrix. From there, cancer cells can further spread to other areas.

Components of ECM

- Matrix proteins
  - Examples: Collagen, elastin
    - Tough fibrous proteins embedded in a gel-like polysaccharide ground substance.

Elastin is tough, but it also provides elasticity and flexibility to the tissue.

- Adhesion proteins
  - Examples: Fibronectin, laminin
    - These proteins link components of the matrix to one another and to the cells.

They are found in the basal lamina.

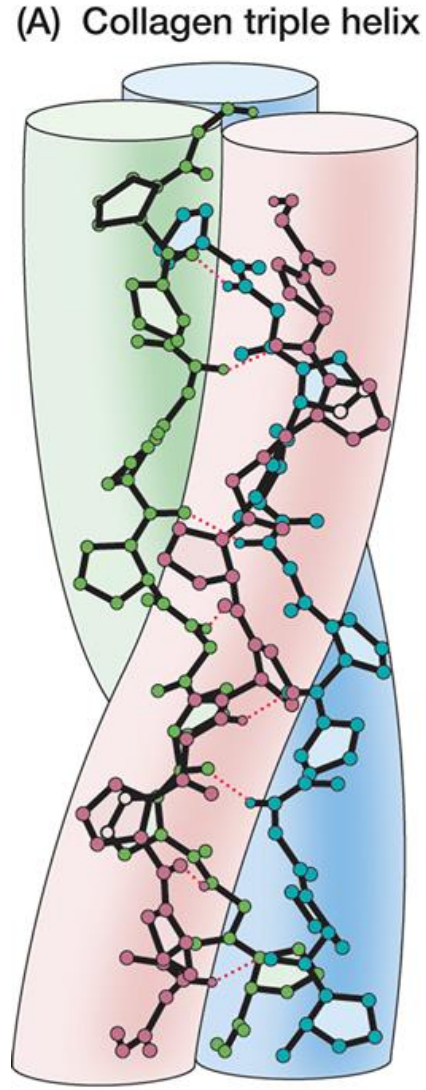
- Glycosaminoglycans.

Remember from biochemistry course (**EXTRA**): Glycosaminoglycans (GAGs) are long, negatively charged polysaccharides in the extracellular matrix that provide structural support, retain water, and regulate cell signaling in connective tissues.

The collagens

- The most abundant proteins in mammals (25% of the total protein mass).
- Long, stiff, triple-stranded helical structure made of three α chains (These α chains can either be identical or different (α1, α2, α3, α4, etc), they can make different combinations (α1, α1, α2 for example)).
- A basic unit of mature collagen is called tropocollagen.
- Rich in glycine (33%), proline (13%), and hydroxyproline (9%)
- It contains hydroxylysine (attachment of polysaccharides)
- Crosslinking of chains via lysine and aldolysine via the action of lysyl oxidase Making the molecule tough.

Collagen's rigidity is due to several factors. The presence of proline and hydroxyproline stabilizes its helical structure, and three helices wrap around each other to form a triple-helix. This triple-helical structure, combined with covalent cross-linking, enhances collagen's rigidity. Additionally, collagen's hydrophobic nature allows the molecules to pack closely together, aided by glycine—a small amino acid that minimizes the distance between collagen helices.



Types of collagens

According to their structure and function.

- More than 40 types of collagen that resist tissue stretching.
- Types:
  - Fibrillar collagens
  - Fibril-associated collagens
  - Network-forming collagens
  - Anchoring fibrils
  - Transmembrane collagens

Types of fibrillar collagens

- Type I: most connective tissues (long, aligned in parallel to each other in a regular staggered array to form fibrils), and rigid (fit to be in bone structure) It is also found in many other types of connective tissue, but what gives bone its solid structure? The presence of additional molecules, such as hydroxyapatite, contributes to its rigidity.
- Type II: cartilage and vitreous humor
  - Smaller in diameter than type I and oriented randomly in the viscous proteoglycan matrix
  - Rigid macromolecules, but compressible (to resist large deformations in shape and absorb shocks)

Type I collagen consists of three α1 chains, whereas Type II collagen contains three α chains that are distinct from those in Type I. This variation in α chain composition differentiates the collagen types from one another.

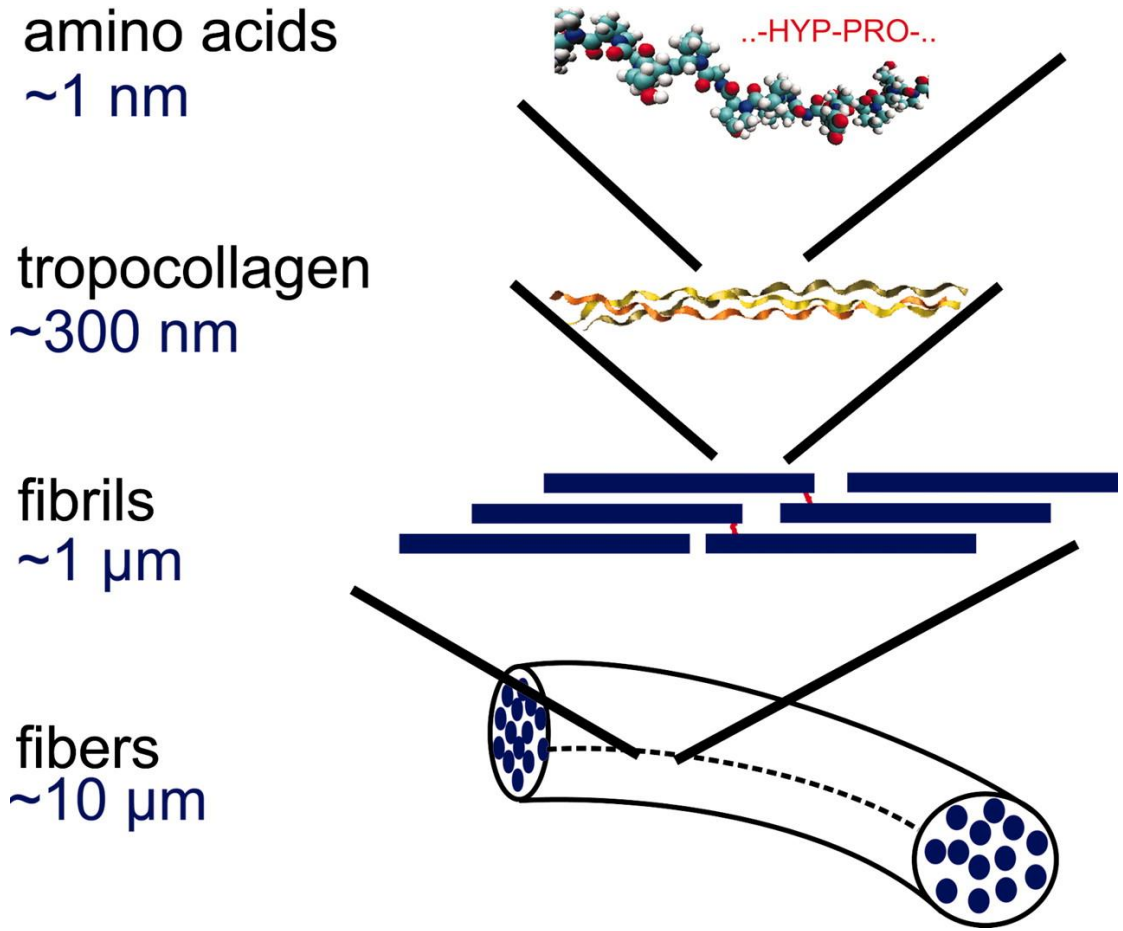
- *Type III: extensible tissues (skin and lung)*
- *Type XI: cartilage*
- *Type XXIV: bone and cornea*
- *Type XXVII: eye, ear, and lung*

Types in grey color are **not required**.

Assembly of fibrillar collagens

- After secretion, they assemble into collagen fibrils, which aggregate into collagen fibers (larger, cable-like bundles.

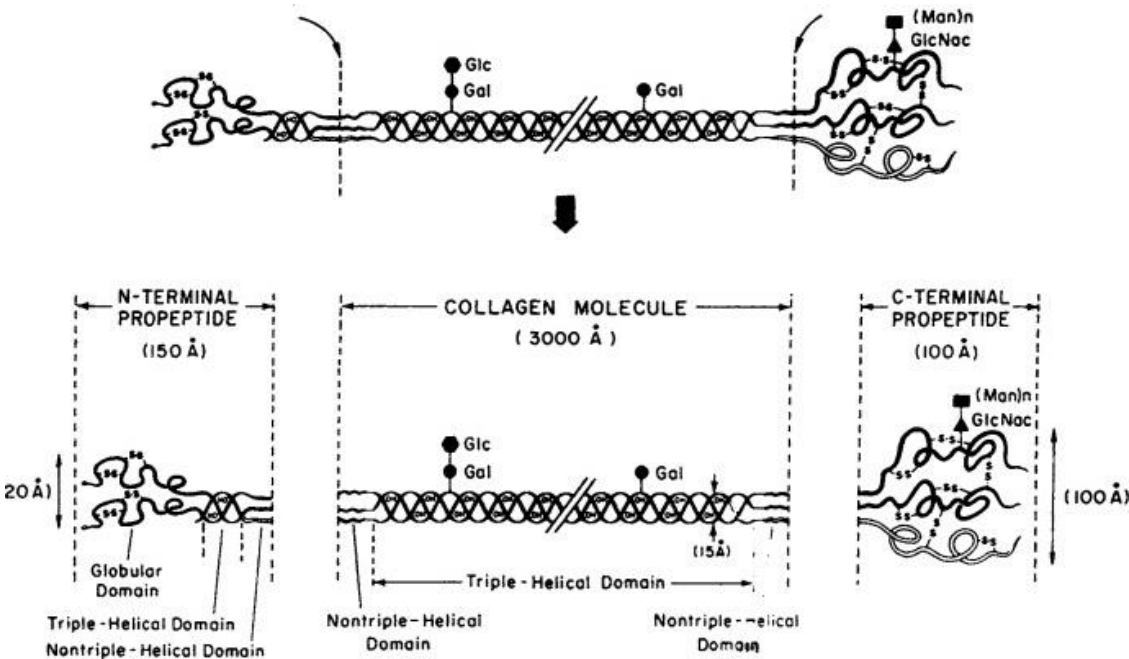
The synthesis pathway of collagen is as follows: it begins with a single α chain, followed by the formation of a triple-stranded molecule, which serves as the basic structural unit. **Outside the cell**, these molecules assemble into fibrils, which are stabilized by cross-linking. Cross-linking can occur either within a single tropocollagen molecule (between its three strands) or between different tropocollagen molecules. Fibrils can be observed under an electron microscope. These fibrils then aggregate to form fibers, which are large enough to be seen under a light microscope.



Synthesis of collagen

- The molecule is synthesized as a procollagen where the N-terminal and C-terminal propeptides inhibit intracellular fibril formation preventing the catastrophic assembly of fibrils within the cell.
  - Lysyl oxidase, which catalyzes formation of reactive aldehydes, is an extracellular enzyme (another protective measure).
- Following exocytosis, the procollagen peptidases remove the propeptides.

- Procollagen I Intact N-Terminal (PINP) is considered the most sensitive marker of bone formation and bone resorption, and it is useful for monitoring bone resorption therapies.

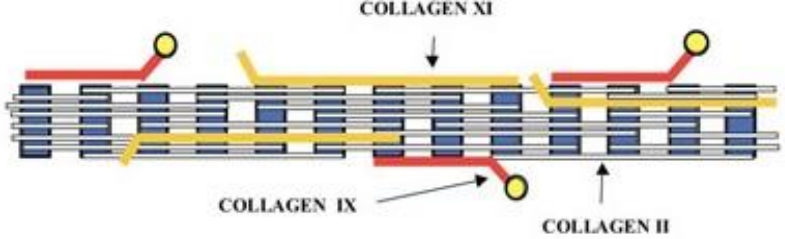


Further Elaboration:

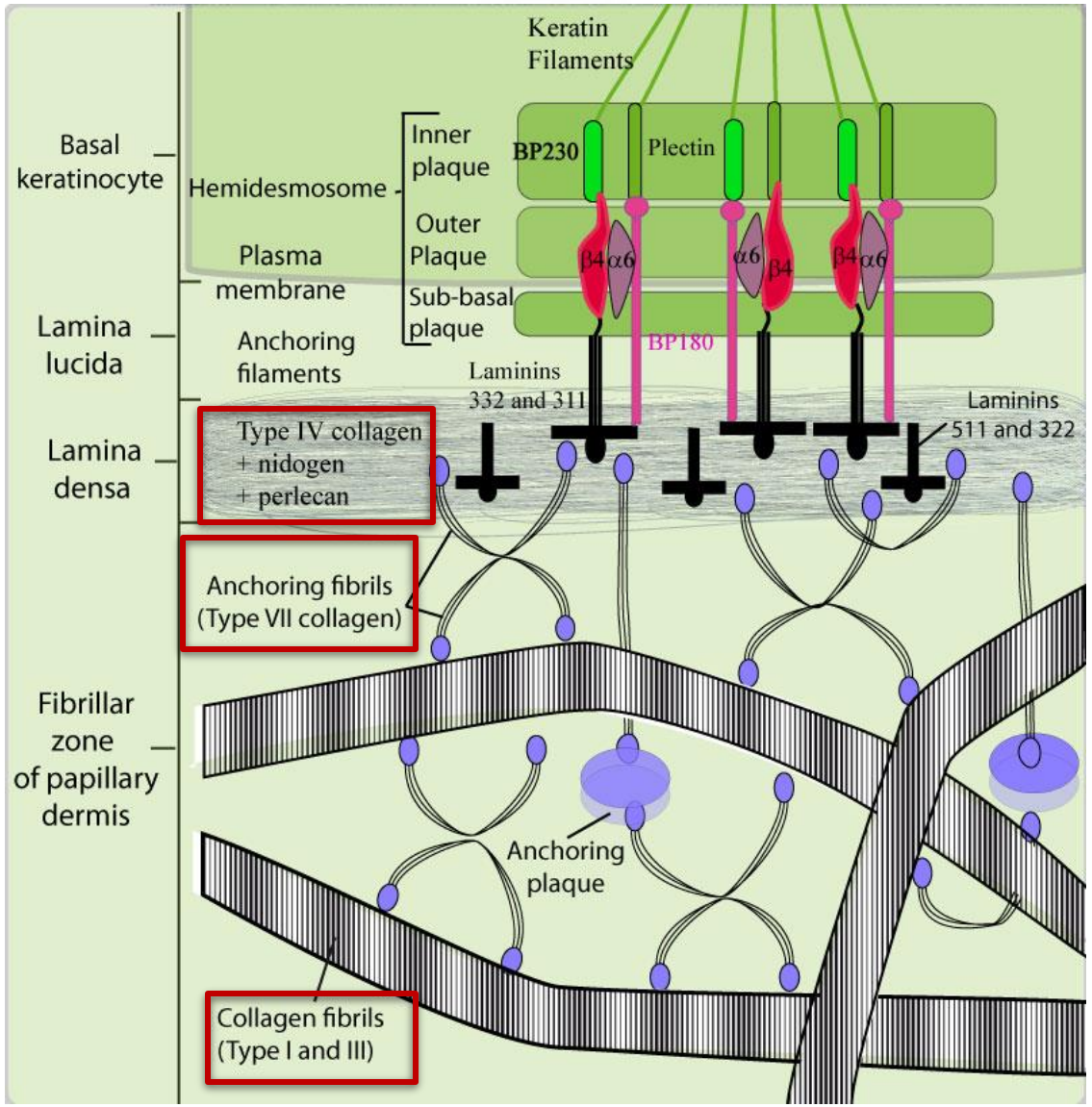
- Collagen synthesis starts in the endoplasmic reticulum (ER) and continues with modifications in the Golgi apparatus (GA), where it is released as an inactive precursor called procollagen. Similar to zymogens, procollagen requires extracellular enzymatic cleavage by peptidase at the C- and N-termini to become active. This activation outside the cell is crucial because cells cannot manage large collagen fiber assembly internally. Additionally, the enzyme lysyl oxidase assists in cross-linking collagen fibers extracellularly, preventing premature formation inside the cell.
- Normally, bone undergoes continuous remodeling through the balanced processes of bone formation and bone resorption, as it serves as the body's reservoir for calcium and phosphate. This balance is maintained by the activity of osteoblasts (bone-forming cells) and osteoclasts (bone-resorbing cells). In many elderly people, especially postmenopausal women but also in some men, osteoporosis occurs when bone resorption exceeds bone formation. To monitor the dynamics of bone formation, we measure the level of Procollagen Type I N-Terminal Propeptide (PINP). Elevated levels of PINP indicate increased bone formation, while lower levels suggest reduced bone formation, which may imply that resorption is outpacing formation.

Others

These collagens are linked to other fibrillar collagens and serve to connect different types of collagen to one another, as illustrated in the figure.

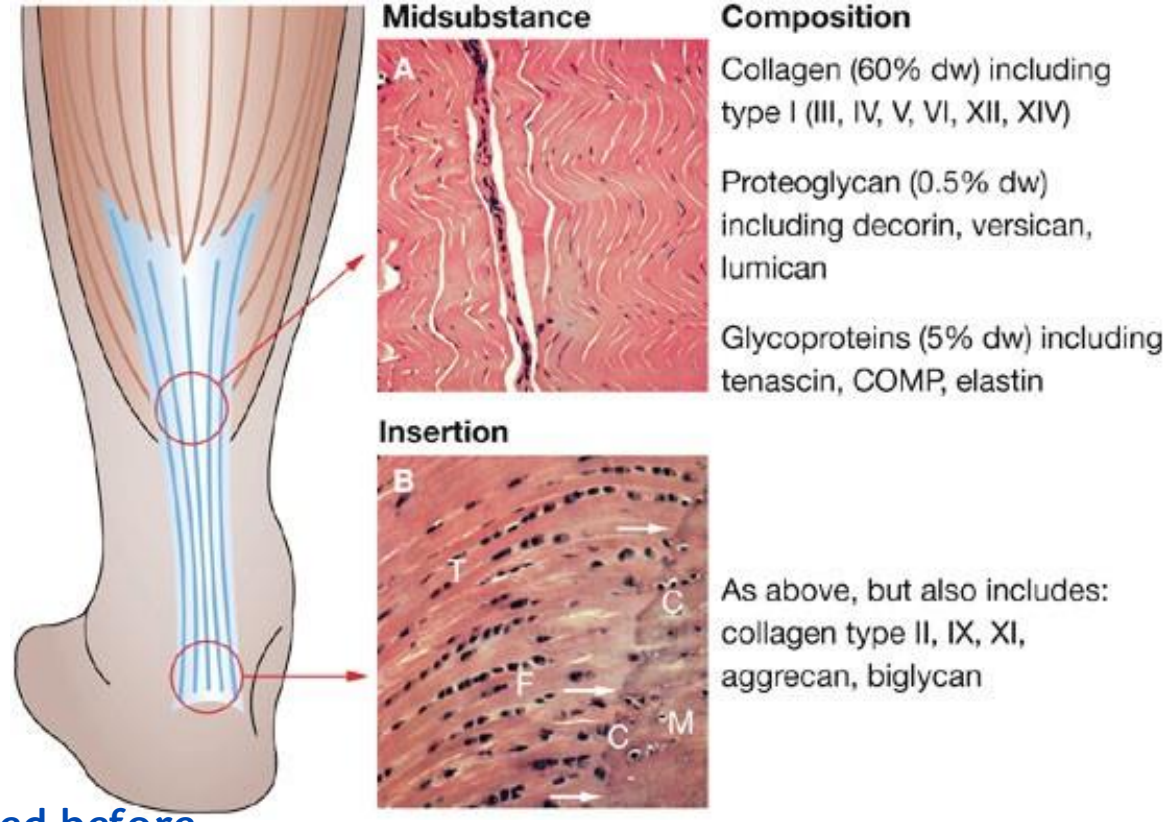


- Fibril-associated collagens
  - The Gly-X-Y repeats interrupted by short nonhelical sequences making it flexible.
  - Collagens type IX and XI link fibrils to one another and to other components in the ECM.
- Network-forming collagens
  - Types IV: constituent of the basal laminae.
- Anchoring fibrils (type VII): link basal laminae to underlying connective tissues (network-forming collagens (in basal laminae) to fibrillar collagens (in connective tissue)).



Collagen-related diseases

- Collagen is highly crosslinked in tissues where tensile strength is required such as in Achilles tendon.
- If lysyl oxidase is mutated and crosslinking is inhibited, the tensile strength of fibers is greatly reduced, collagenous tissues become fragile, and collagen structures tend to tear (skin, tendon, and blood vessels).



The Achilles tendon is strong but can still rupture under excessive stress or repetitive strain. Issues with collagen crosslinking may further weaken the tendon, increasing the risk of injury.

Like we mentioned before, issues with hydroxyproline formation (due to deficiencies or mutations affecting prolyl hydroxylase) can lead to collagen fragility.

Osteogenesis imperfecta (Brittle-bone disease)

Bones are easily
fractured and broken.

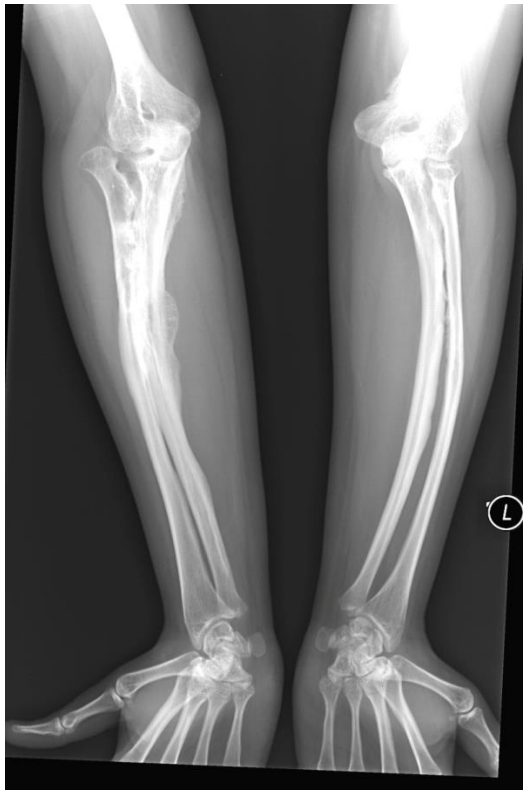
- “Osteogenesis imperfecta" = imperfect bone formation
- A genetic disorder of several forms that cause fragile, soft, brittle, and easily broken bones.
- Four types of osteogenesis imperfecta designated as type I through type IV (type 1 2 3 4)
  - Type I: the mildest form of the condition.
  - Type II: the most severe form that results in death in utero or shortly after birth.
  - Milder forms generate a severe crippling disease.

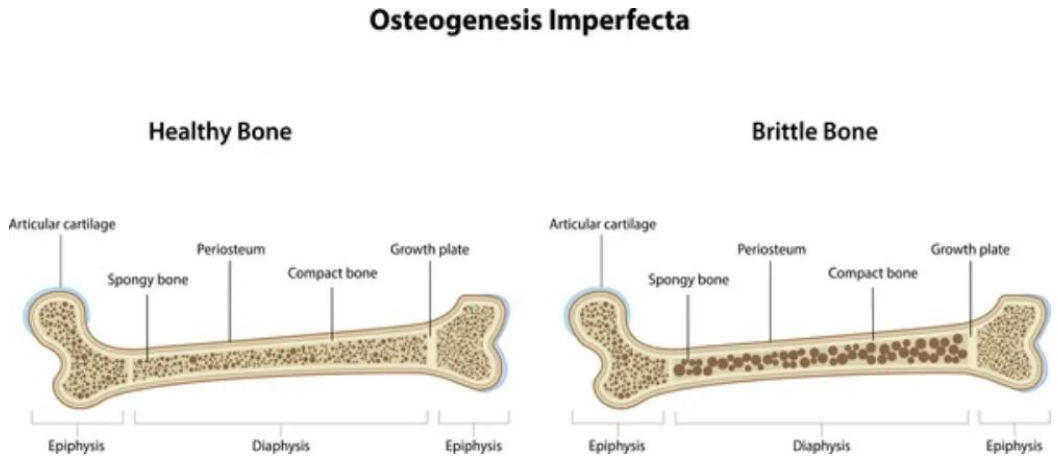
Mutations of OI

Causes weak and fragile bones.

Components of type 1 collagen.

- Mutations in the COL1A1 and COL1A2 genes (and others) interfere with the assembly of **type I collagen**.
- Defective collagen weakens connective tissues, particularly bone, resulting in the characteristic features of OI.

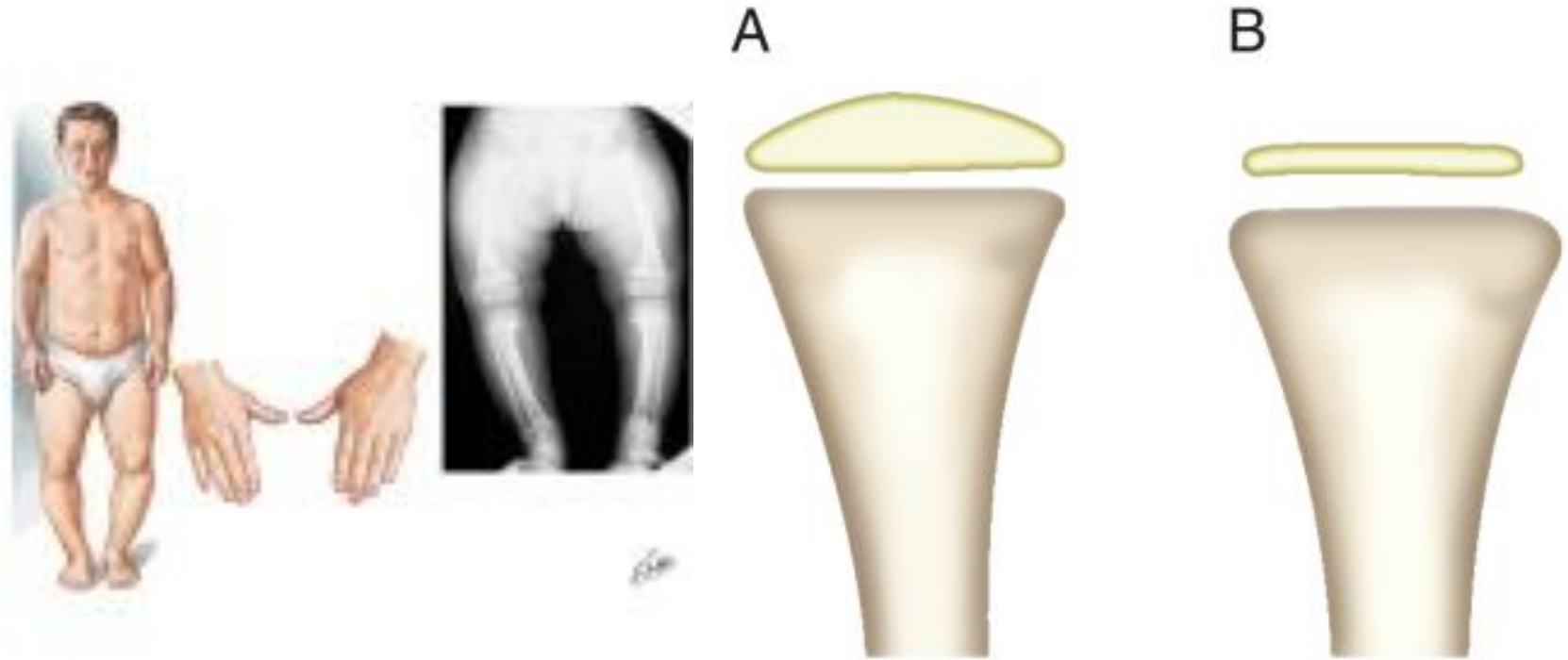




Chondrodysplasias

Remember: Type 2 collagen is abundant in cartilage; therefore affecting bones and joints.

- Mutations affecting **type II collagen** cause chondrodysplasias, characterized by abnormal cartilage, which leads to bone and joint deformities.



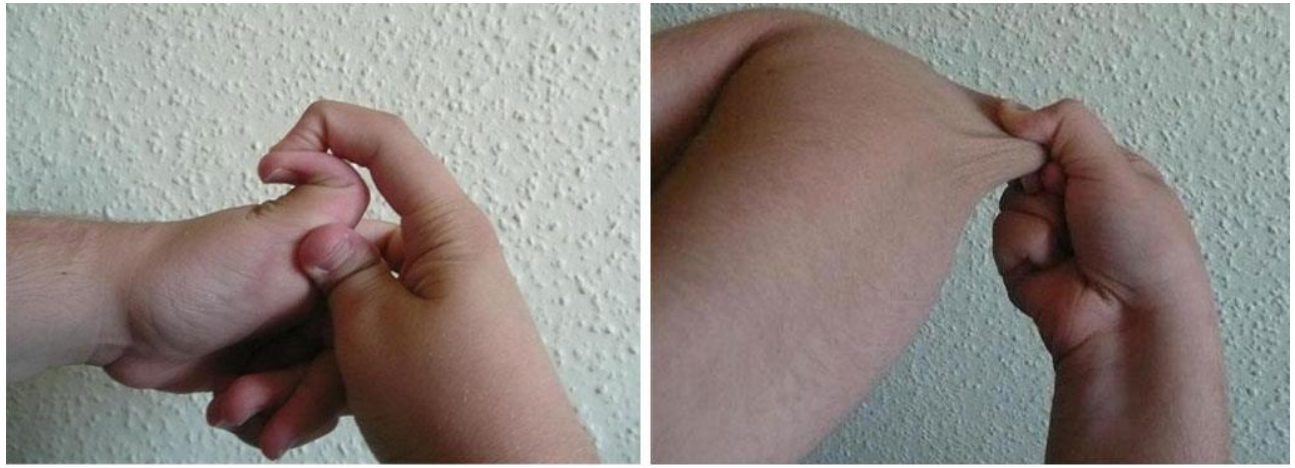
Ehlers-Danlos syndrome

In this case, the deficiency of collagen combined with an abundance of elastic fibers results in highly stretchable tissues, as they lack the tensile strength typically provided by collagen.

- A heterogeneous group of disorders that affect the skin, bones, blood vessels, and other organs.
- The signs and symptoms vary from mild to life-threatening.
- All result from defects in collagen synthesis and/or processing.
  - Mutations in collagens type I, III, or V or in the collagen processing enzymes like procollagen N-peptidase or lysyl hydroxylase

These are extracellular enzymes.

Major manifestations are skin fragility and hyperextensibility and joint hypermobility.



Type III EDS

- The most clinically important mutations are found in the gene of type III collagen.
- Since **type III collagen** is a major component of arteries, mutations affecting it result in fragile blood vessels.

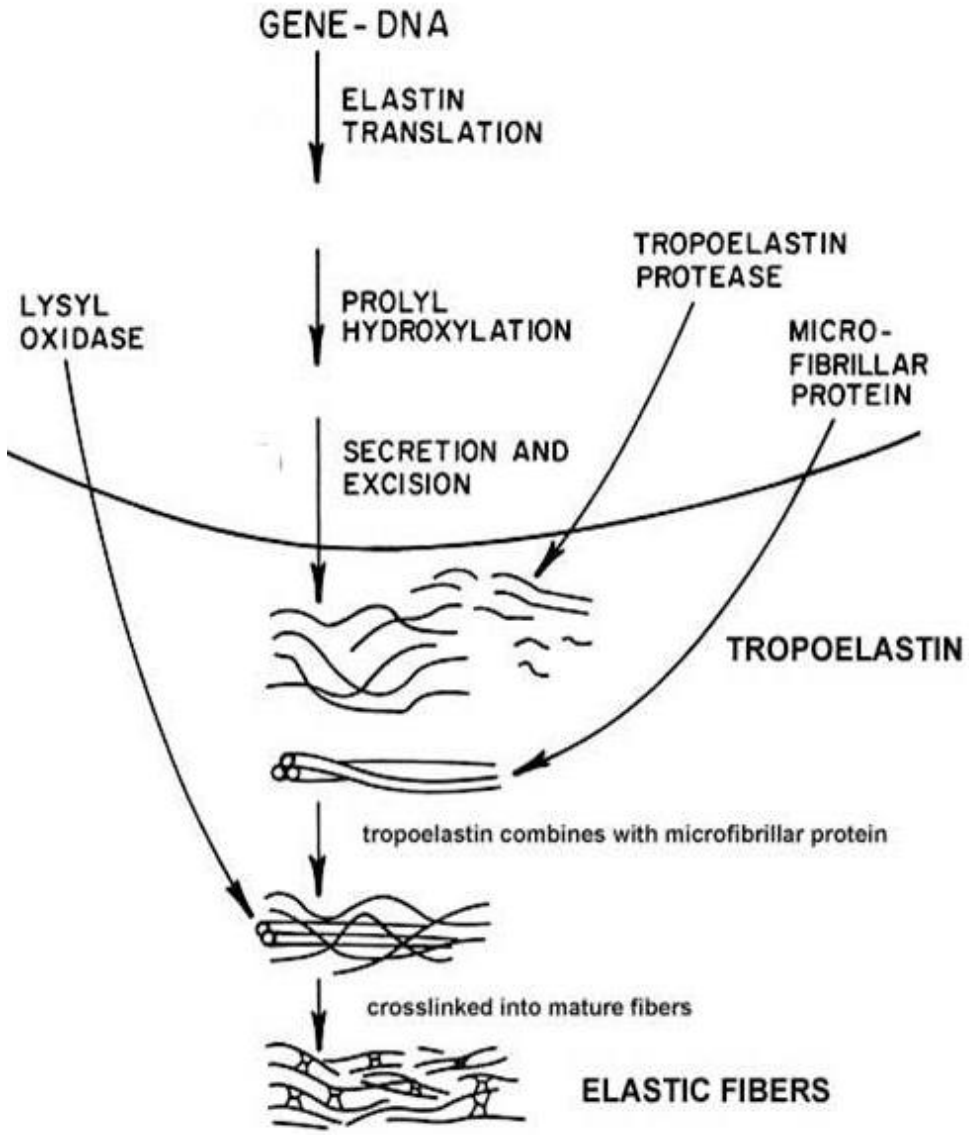
- Other symptoms include stretchy skin and hypermobile joints.

Factors Influencing Collagen-Related Disorders

- The consequences of a mutation determine the resulting disease, which is why a type I collagen deficiency may lead to conditions like Ehlers-Danlos syndrome, osteogenesis imperfecta, and others. The outcome depends on factors such as which enzymes are affected, the nature and location of the impact, the specific tissues involved, and the timing (e.g., fetal stage, childhood, adulthood). Additionally, some tissues may compensate by expressing other collagen types, and the severity of symptoms also depends on the tissue affected.

Elastin

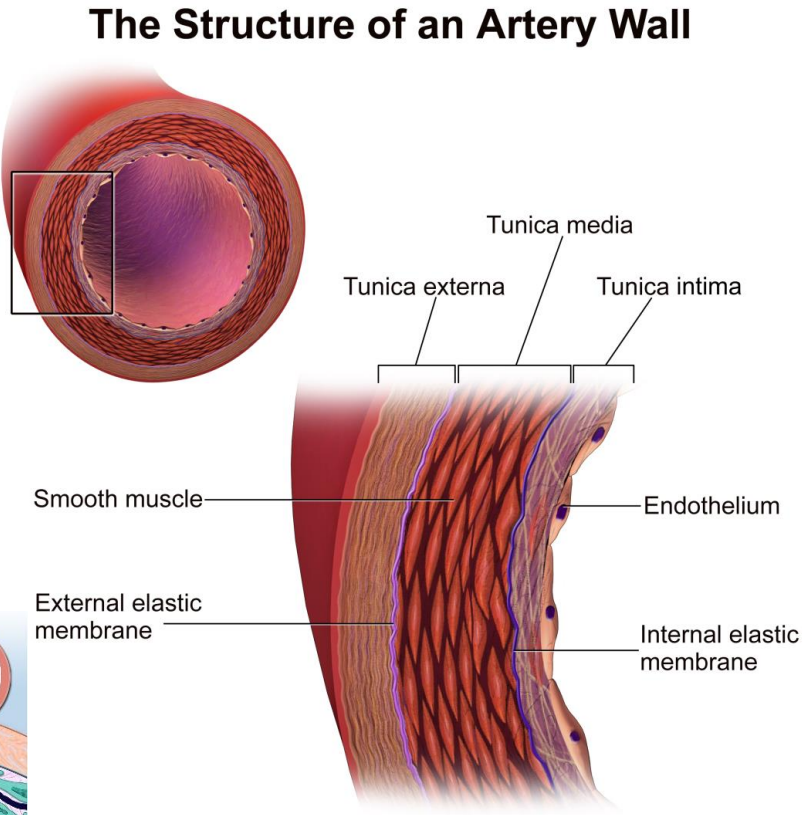
- The main component of elastic fibers is elastin
- Highly hydrophobic
- Rich in proline and glycine.
- Contains some hydroxyproline, but no hydroxylysine
- Not glycosylated
- Secretion of tropoelastin
- Assembly into elastic fibers
- Crosslinking via lysines



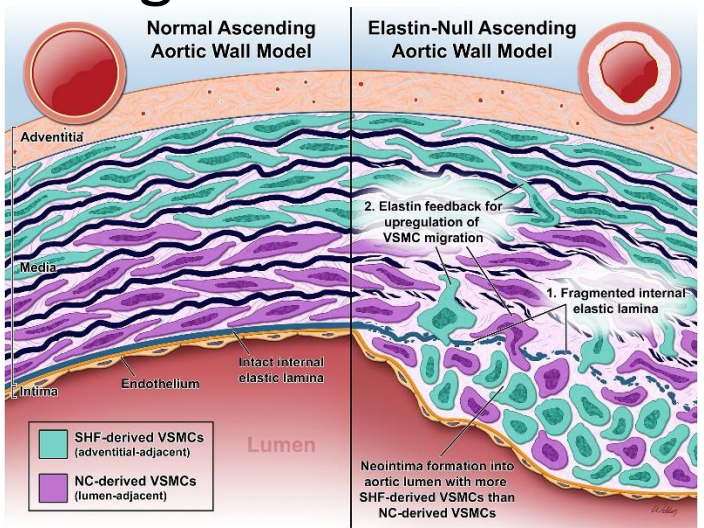
Function of elastic fiber

Tissues are stretchable due to elastin, while the presence of collagen prevents them from rupturing.

- Elastin is the dominant extracellular matrix protein in arteries.
- The normal elasticity of an artery restrains the proliferation of smooth muscle cells.
- Abnormal or deficiency of elastin results in excessive proliferation of smooth muscle cells in the arterial wall and narrowing of the aorta.



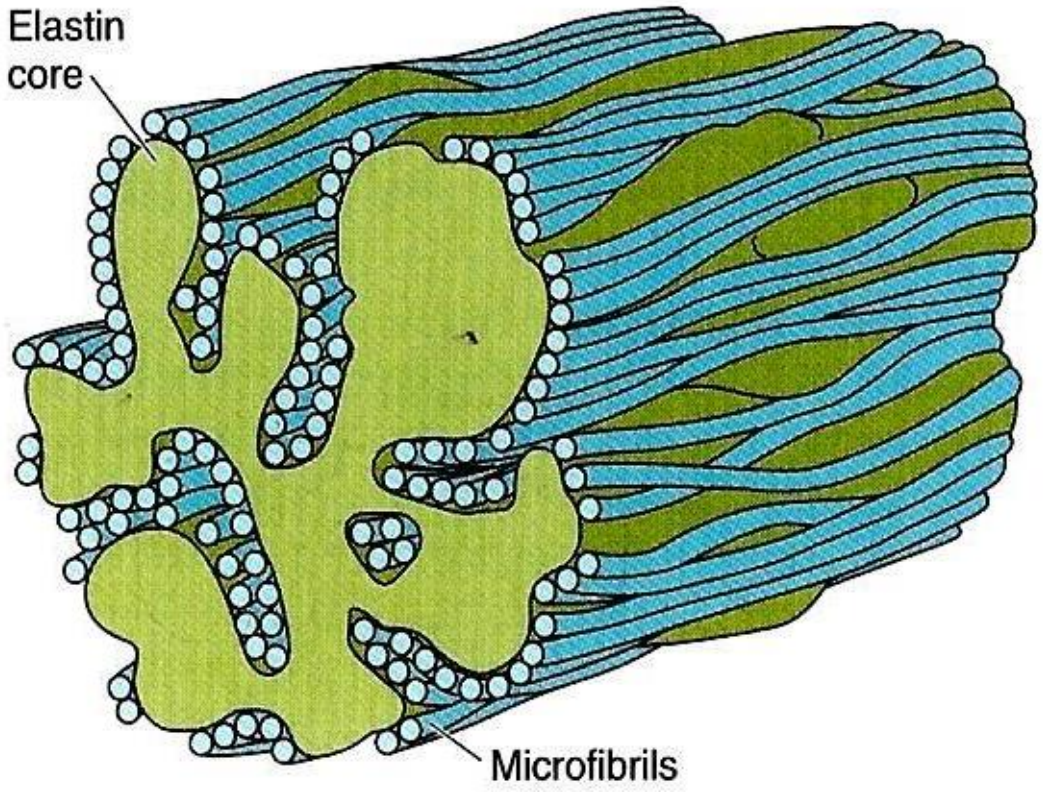
Elastin is present in arteries, surrounding smooth muscle cells, and plays a strict role in controlling their proliferation. If an elastin abnormality occurs, these cells may continue to grow unchecked, leading to vessel narrowing.



Microfibrils and fibrillin

- The elastin core is covered with a sheath of microfibrils, which are composed of a number of glycoproteins, including the large glycoprotein fibrillin.
- Fibrillin binds to elastin and is essential for its integrity.

Fibrillin keeps the integrity and strength of elastic fibers.



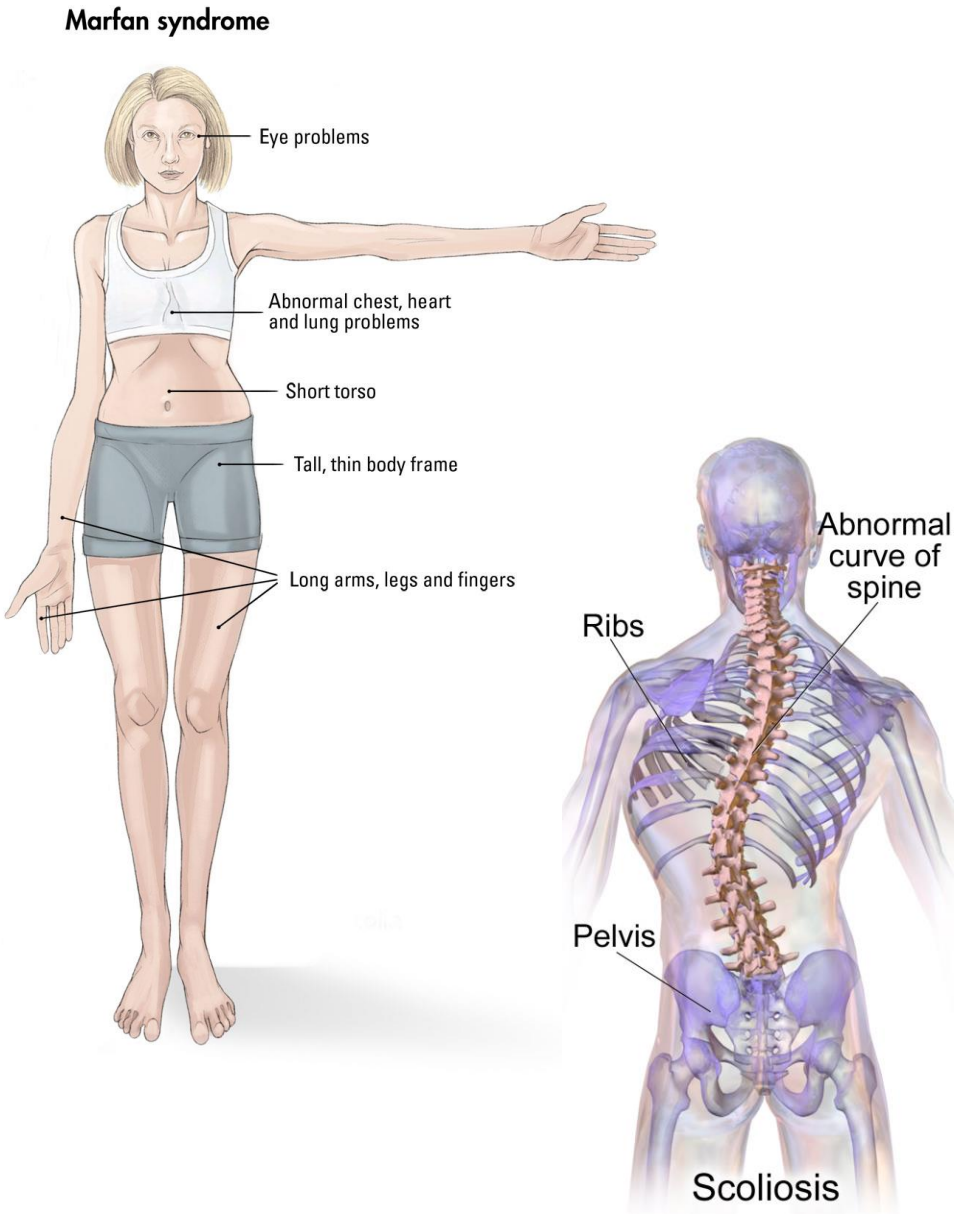
Marfan's syndrome

- Due to mutated fibrillin
- Rupture of aorta.

Elastic fibers can stretch too much and rupture.

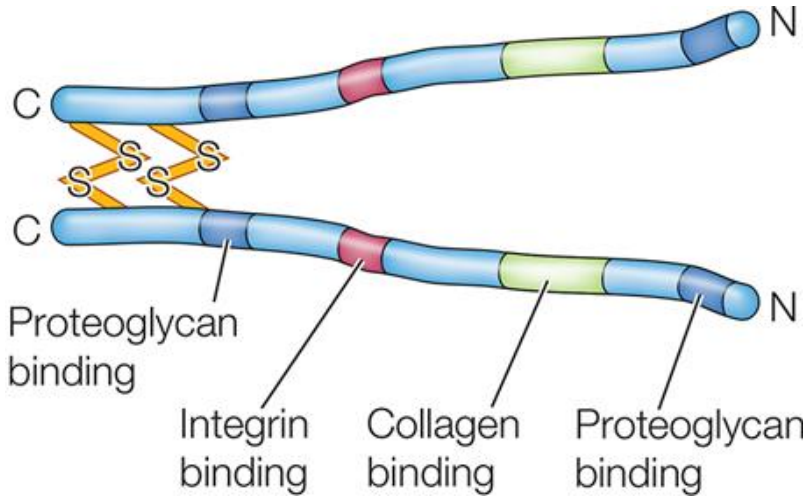
- **Others: A tall, thin build; Long arms, legs, fingers, and toes and flexible joints; Scoliosis, or curvature of the spine; A chest that sinks in or sticks out; Crowded teeth; Flat feet.**

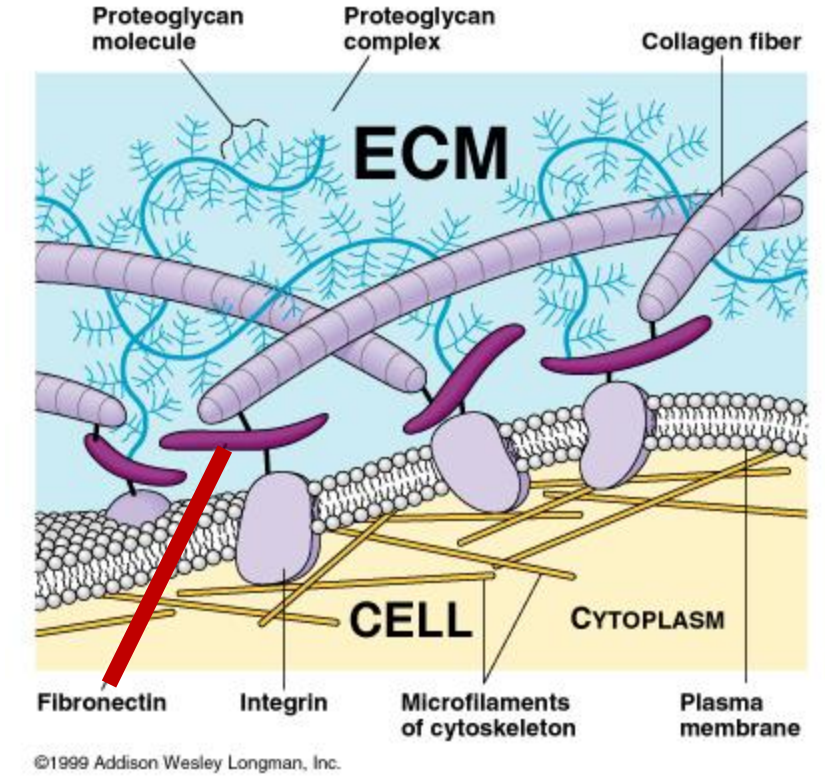
Good to know: The distance between the tips of both index fingers is greater than a person's height.



Matrix adhesion proteins

- They interact with collagen and proteoglycans and link matrix structural proteins with one another and to the surfaces of cells.
- Fibronectin: the principal adhesion protein of connective tissues.
  - A dimeric glycoprotein that is crosslinked into fibrils by disulfide bonds.
  - It binds to collagen and GAGs
  - It binds to cell surface proteins like integrins linking cells to the ECM

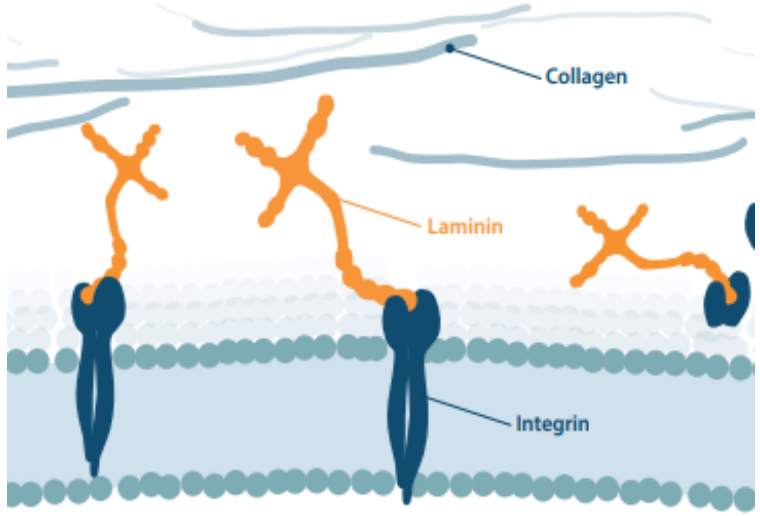


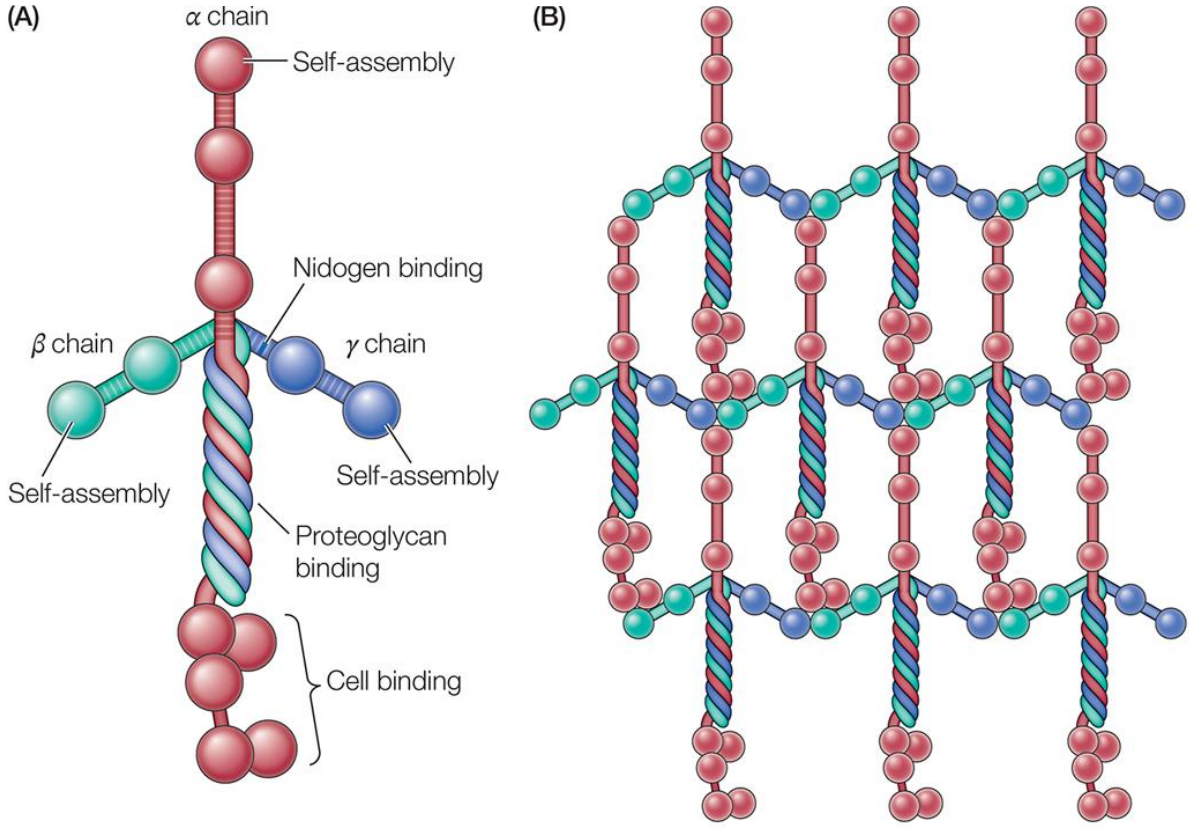


Laminin

- It is found in the basal laminae.
- It forms T-shaped heterotrimers with binding sites for cell surface receptors such as integrins, type IV collagen, and GAGs.

When found, it strengthens basal lamina.

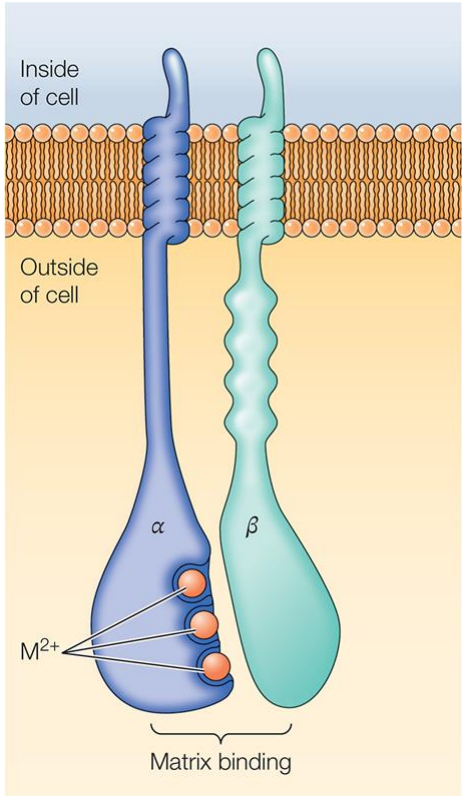


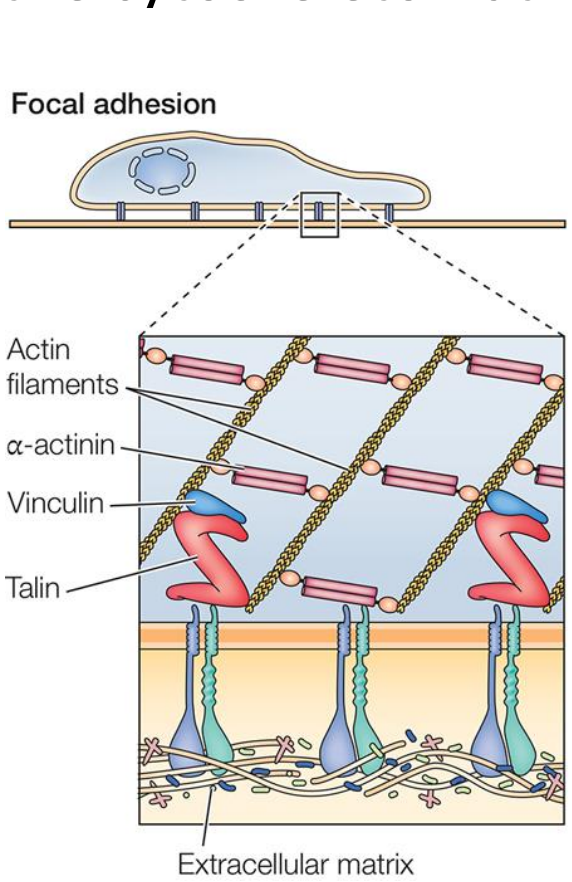


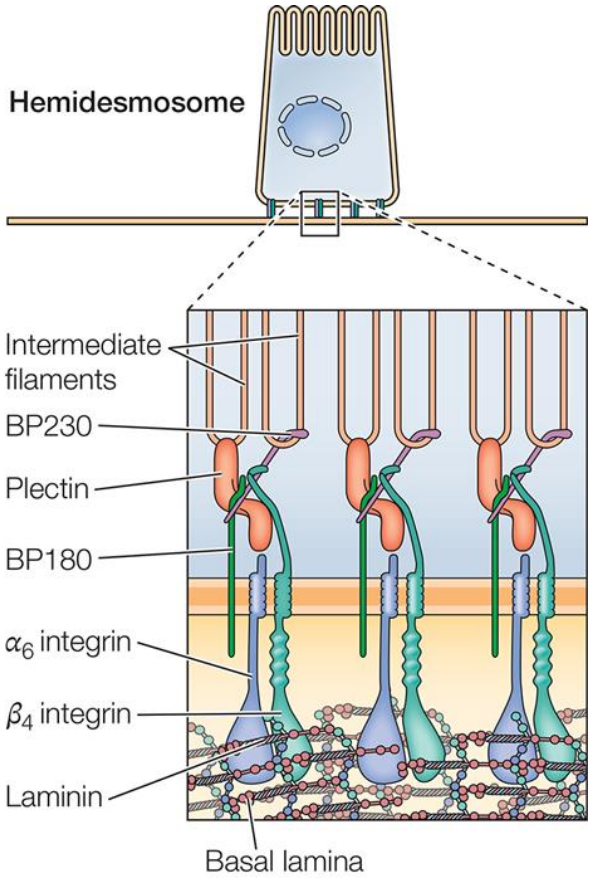
Cell-matrix interactions
Role of integrins

When laminin interacts with integrins—receptors that transmit signals into the cell—the cell's behavior is influenced by signals from the basal lamina. Thus, when a normal cell transforms into a cancer cell, its interaction with the basal lamina changes, impacting the cell's behavior.

- integrins are a family of transmembrane heterodimers (α and β) that bind to short sequences of ECM proteins and attach cells to ECM.
- They also anchor the cytoskeleton at focal adhesions and hemidesmosomes.

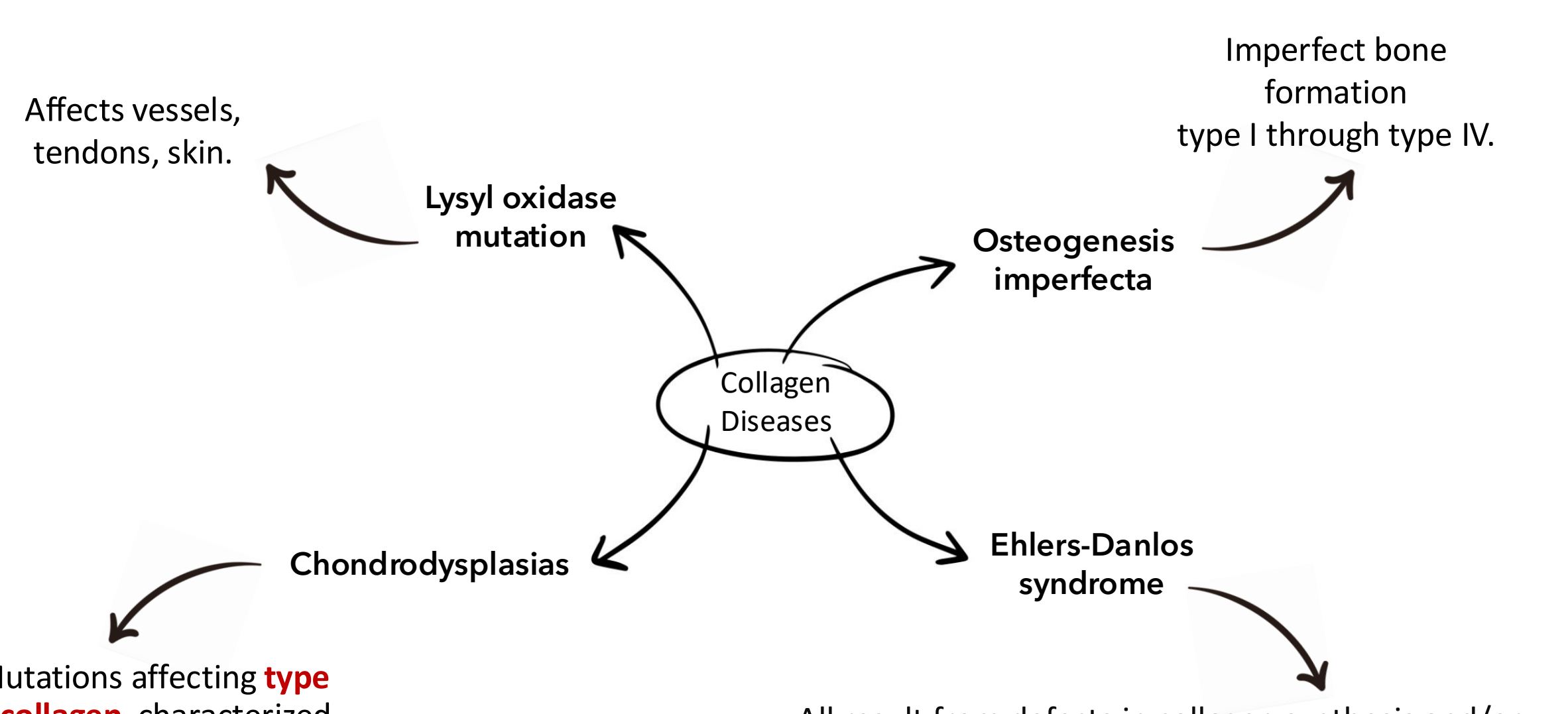






We have α and β
α1,α2, α3, α4..
β1, β2, β3...
With different combinations and with different interactions.

Integrins connect matrix proteins with actin filaments (focal adhesions) and intermediate filaments in hemidesmosomes.


Affects vessels, tendons, skin.
Lysyl oxidase mutation
Imperfect bone formation type I through type IV.
Osteogenesis imperfecta
Collagen Diseases
Chondrodysplasias
Mutations affecting type II collagen characterized by abnormal cartilage, which leads to bone and joint deformities.
Ehlers-Danlos syndrome
All result from defects in collagen synthesis and/or processing. collagens type I, III, or V or in the collagen processing enzymes like procollagen N-peptidase or lysyl hydroxylase.

For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1	#9, collagen type 1 subunits #7, glycine in collagen structure #14, Achilles tendon	2- Type I collagen consists of three identical α1 chains 3- between collagen molecules. 4- Achilles tendon is weak.	1- Deleted the extra summary slide 2- Type I collagen consists of two α1 chains and one α2 chain 3- between collagen helices. 4- Achilles tendon is strong.
V1 → V2			

Additional Resources:

رسالة من الفريق العلمي:

Reference Used:
(numbered in order as cited in the text)

1. The Cell: A Global Approach 8 th Ed., Chapter 11.1

اللهُم كُن لأهل غزة عوناً ونصيراً. اللهم انا نستودعك أهلنا في غزّة وفلسطين فانصرهم واحفظهم، واربط على قلوبهم وأمدهم بجُندك وأنزل عليهم سكينتك.