University of Jordan
Faculty of Medicine

GENETICS & Molecular Biology

Number: 25

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Subject: Intermediate filaments & ECM

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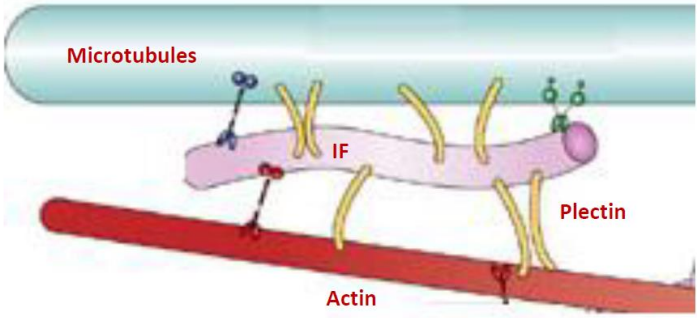
بسم الله الرحمن الرحيم

In the last lecture we talked about different types of cellular junctions and intermediate filaments (intermediate in size between actin & microtubules filaments and connect them by the help of plectin).

Plectin: type of linking plakin proteins , these proteins connects the intermediate filaments to other structures & filaments within the cell.

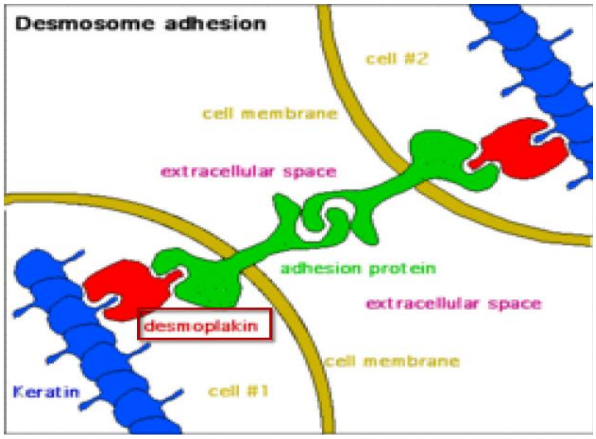


Plectin bridges microtubules to actin filaments and stabilizing them and increasing the mechanical stability of the cell.

We said also that intermediate filaments are part of cellular junctions like desmosomes & hemi-desmosomes :

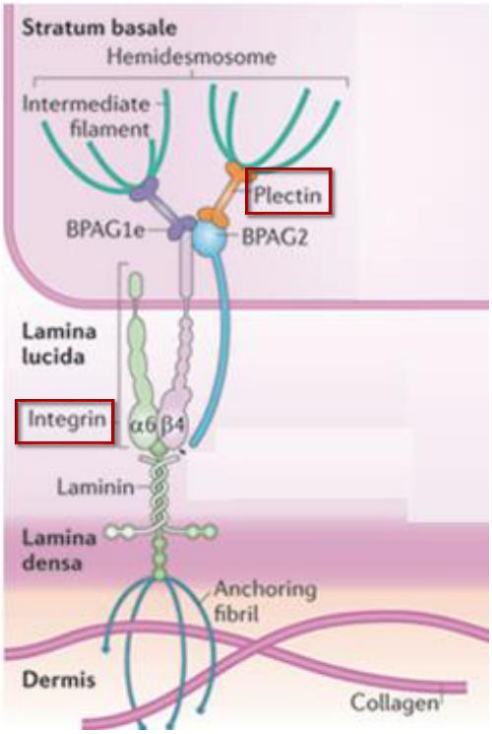
For example, in this figure, the adjacent cells are connected together via trans-membrane proteins, these trans-membrane proteins are connected to protein called desmoplakin (one of the plakins) and these are connected to intermediate filaments.

This connection provides **stability & strength** to the junction.



If the cell is interacting with **an extracellular component**, this will form hemi-desmosome:

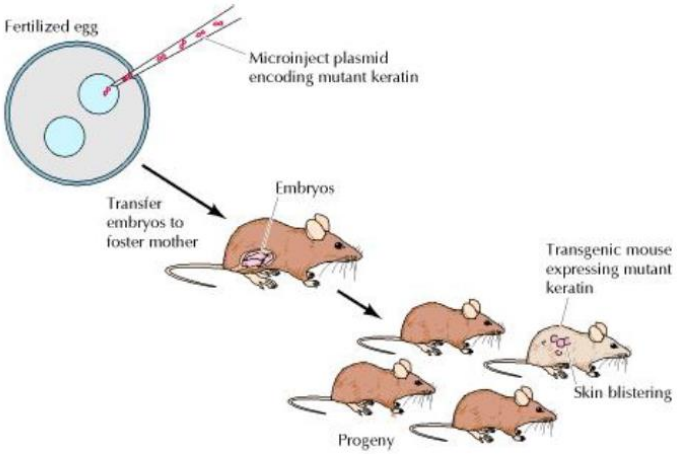
In this figure, integrin proteins (have two polypeptide chains :α & β) are trans-membrane proteins that interact with plactin protein (of the plakins) that interacts with the intermediate filaments within the cell. Integrins can interact with several proteins that interact with components of the ECM. We call this junction hemi-desmosomes –half of a desmosome- because one cell is connected to the ECM.



Intermediate filaments & diseases:

Mutations in intermediate filament, like vimentin in fibroblasts, don't affect cell growth or movement, studying their effect they generate "trans-genic mice"

HOW?? They injected a plasmid with mutated intermediate filaments gene (the gene that is responsible for assembly of keratin filaments) to a fertilized egg, so this gene became part of the fertilized egg genome resulting with a mouse that has both original & abnormal gene.



Some of the pups of that mouse had mutations resulting in skin blisters after any minor mechanical trauma! , in human this disease is called **Human epidermolysis bullosa simplex.**

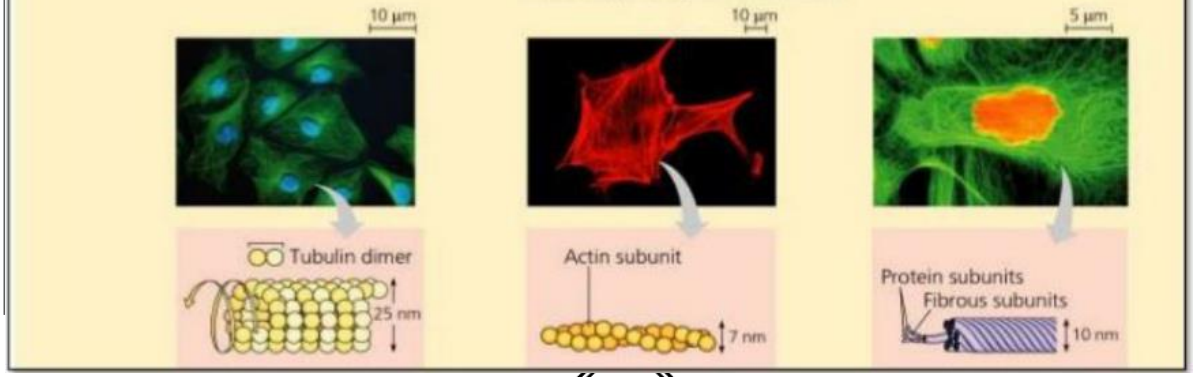
Human epidermolysis bullosa simplex is caused by keratin gene mutations that interfere with the normal assembly of keratin filaments causing skin blisters after minor trauma.

Other disease associated with intermediate filaments **is Amyotrophic lateral sclerosis (ALS)** due to mutations of <u>neurofilaments</u> (type of intermediate filaments).

<u>Summary:</u>

Table 7.2 The Structure and Function of the Cytoskeleton

Property	Microtubules	Microfilaments (Actin Filaments)	Intermediate Filaments
Structure	Hollow tubes; wall consists of 13 columns of tubulin molecules	Two intertwined strands of actin	Fibrous proteins supercoiled into thicker cables
Diameter	25 nm with 15-nm lumen	7 nm	8–12 nm
Protein subunits	Tubulin, consisting of α-tubulin and β-tubulin	Actin	One of several different proteins of the keratin family, depending on cell type
Main functions	Maintenance of cell shape (compression-resisting "girders") Cell motility (as in cilia or flagella) Chromosome movements in cell division Organelle movements	Maintenance of cell shape (tension-bearing elements) Changes in cell shape Muscle contraction Cytoplasmic streaming Cell motility (as in pseudopodia) Cell division (cleavage furrow formation)	Maintenance of cell shape (tension-bearing elements) Anchorage of nucleus and certain other organelles Formation of nuclear lamina



Extracellular matrix (ECM) :

The ECM is what surrounds the cell, it is composed of proteins & polysaccharides that interact with each other to provide structure for the ECM, interacting with cells & providing means for connection and interaction between different cell types. There are different types of ECM surrounds different types of cells.

One example of ECM is **basal lamina**, it is what anchors epithelial cells and separate them from the surrounding connective tissue. Also, it surrounds muscle cells, adipose and peripheral nerves. Another example is **connective tissue**,(i.e, bone, cartilage, tendons BUT NOT the blood, ECM is where we find fibroblasts).

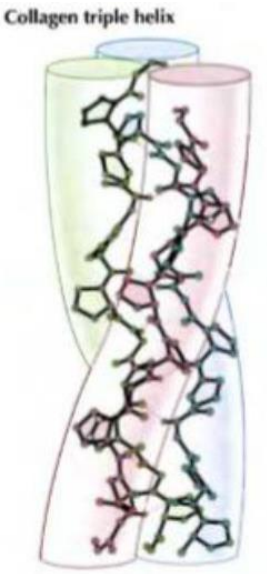
-different types of ECM have different composition to provide their function but there are some **common components** like:

- **Fibrous proteins**: provide structural integrity. They are embedded in a gel-like polysaccharide ground substance.
- **Adhesion proteins**: connect the protein fibers to other molecules (like polysaccharides, cell receptors,…etc.), an example of those are integrins (in cell-cell & cell-ECM connections).

-Differences in the type and amount of different components make different types of ECM. Bone for example is mostly composed of ECM, and this is rich with inorganic minerals (calcium & phosphate crystals mainly → this provide strength) and cells are scattered in it. On the other hand, cartilage needs some flexibility and this is provided by polysaccharides (like proteoglycans that have the sugar component of GAGs –glycosaminoglycan-, these are negatively charged and attract water molecules providing cushioning effect).Also, tendons have high proportion of fibrous proteins.

In glaucoma the tissue that regulates the eye pressure is called trabecular meshwork, the cells of this meshwork respond to any increase in eye pressure all the time (changes in pressure happen all the time). The tissue adapts to the pressure by intra ocular pressure homeostatic response. **HOW??** By changing the composition of ECM (by digestion some of the GAGs to remove some of the spikes out of the way opening more roads or aqueous humor to exit the eye) thus decreasing the eye pressure.

Fibers of ECM:



The most important type is **collagen fibers** (the most abundant protein in the body).

Collagen is a triple helices (three alpha chains) that can assemble into fibrils and then into fibers. A basic unit of mature collagen is called tropocollagen. It is characterized by the presence of **Glycine, Proline, and hydroxyl-Proline**. The process of hydroxylation is *post translational* modifications that happen in the ER. The purpose of hydroxylation is cross linking, because OH groups can form hydrogen bonds with each other, so they help in establishing these non-covalent interactions and providing strength of the structure of collagen. Collagen also contains **hydroxyl-Lysine** that attaches to polysaccharides.

There are different **types of collagen**:

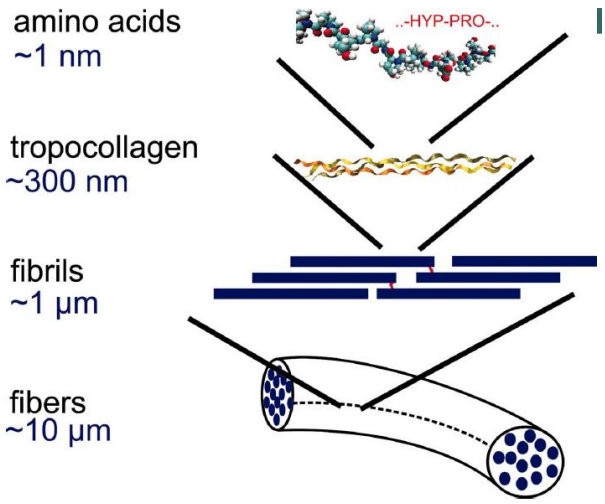
- **Fibril-forming (fibrillary) collagens** such as **collagen I**
- **Fibril-associated collagens** (link collagen fibrils to each other and to ECM components) such as **collagen types IX and XII** link fibrils to one another and to other components in the ECM).
- **Network-forming collagens**, flexible because they are interrupted by non helical short domains e.g. **type IV** is a constituent of the basal laminae.
- **Anchoring fibrils** associate network-forming collagens to fibrillarcollagens.
- **Transmembrane collagens** participate in cell matrix interactions

Assembly of fibrillary collagen:

1.three **polypeptide chains** (large number of amino acids) assemble together forming **tropocollagen** (mature collagen / triple helix).

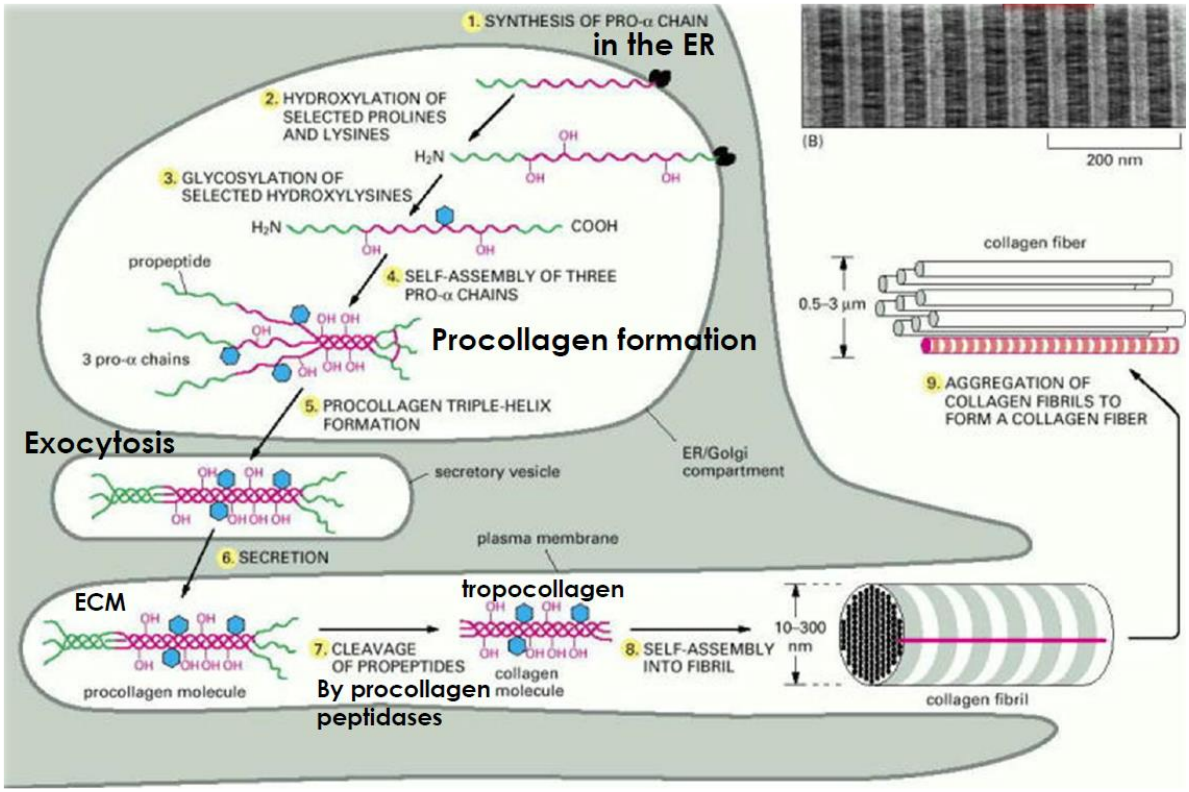
2. tropocollagen molecules interact with each other forming **fibrils** with small overlap in between, this overlap provides a better cross linking.

3.fibrils then assemble to form **fibers**.



Synthesis of collagen:

Re-synthesis, hydroxylation & glycosylation of the pro-alpha chain takes place in the ER.



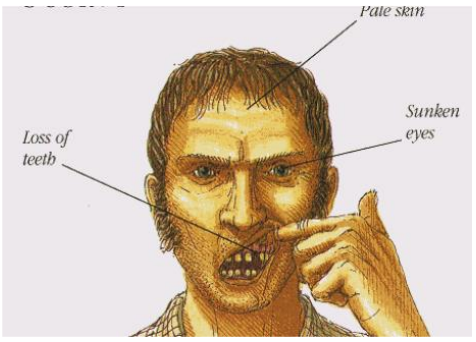
The produced pro-collagen fibers are packaged into vesicles and exported out of the cell (to the ECM where it is going to be assembled).

In pro-collagen molecules -even after modifications in the ER- there is still some structures that need to be cut (by pro-collagen peptidases).

-NOTE: Final assembly doesn't happen in the cell because the final product is really huge; so collagen molecules (mature/cut & modified collagen) assemble in the ECM.

Collagen & diseases:

►**Scurvy** → mainly it is vitamin C deficiency disease, this affects hydroxylation of Proline, thus affects the structural integrity and stability of collagen fibers → more unstable triple helices (tropocollagens) that will be targeted for degradation→ the tissue becomes week.



Symptoms include: -skin and gum lesions & laceration.

-weak blood vessels.

►**Osteogenesis imperfecta (OI)** → in this disease the formation of bone is not perfect, as the name implies, due to mutation in **collagen 1A1 & 1A2** genes resulting in collagen I deficiency, remember that collagen I is a major component of ECM.

There are four types of OI, type1 is the mildest whereas type2 is the most severe.

OI is **autosomal dominant** inherited disease means that one gene hit is enough to have it. Also, patients are heterozygous because homozygous fetus dies early in embryo life.

► **Chondrodysplasias** → here, a mutation in **collagen of the type II**.

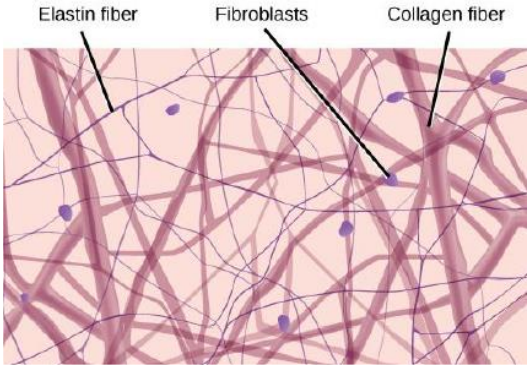
-NOTE: The problem in Achondroplasia is that collagen can't be transformed to bone whereas in Chondrodysplasia there is deficit & abnormality in the shape & cartilage formation process. Both conditions results in dwarfism.

-in chondrodysplasia, the patient suffers from joint deformities & dwarfism.

►**Ehlers-Danlos syndrome** → characterized by hypermobile joints & hyperextensible. This syndrome is due to mutation in **collagen I,III OR V** (but mostly type III) genes or in collagen modifying enzymes genes.

-NOTE: Since type III collagen is a major component of arteries, mutations affecting type III collagen result in fragile blood vessels.

Another type of ECM fibers are **elastic fibers** that are made of elastin protein



This pic shows the difference in diameter between elastin (provides elasticity) & collagen fibers (provides strength).
Elastin fibers have **hyrdoxy-Pro residues** (just like collagen) But unlike collagen they **don't have hydroxy-Lys residues** at all and they are **not glycosylated.**

What makes them more flexible than collagen fibers?

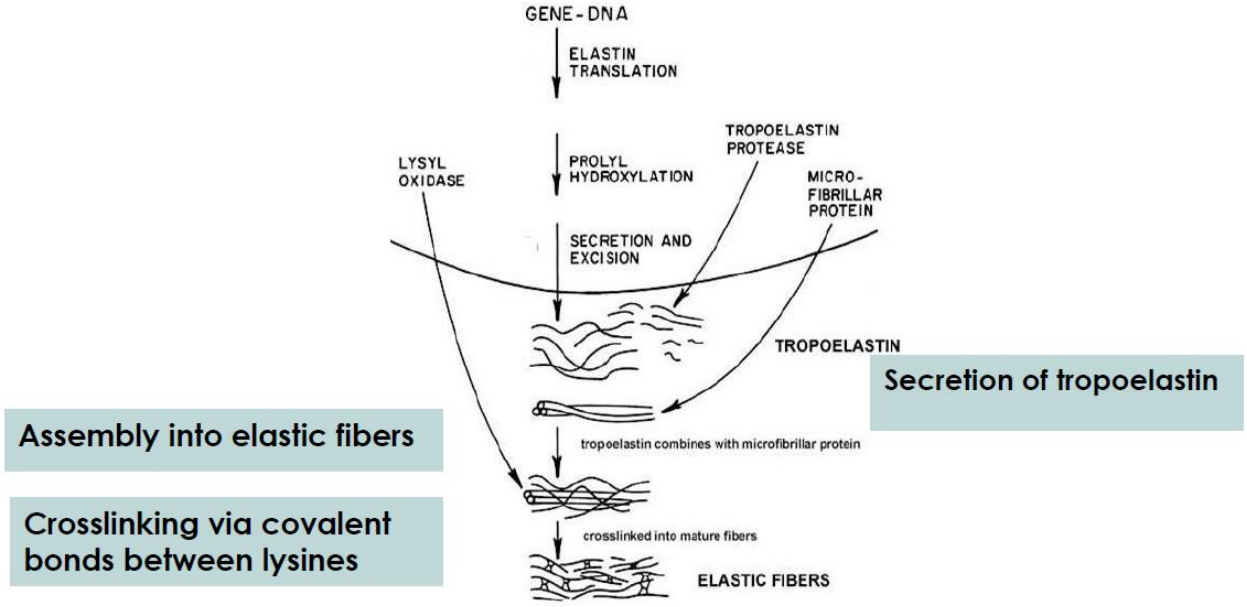
-**lesser diameter** than collagen fibers.

-**stretches of hydrophobic amino acids** (segments), these provide some flexibility because they hydrophobic amino acids don't provide cross linking→these segments provide helical structures that can't be cross linked.

Elastin fibers also have some sort of **strength** due to the presence of **Alanine-and lysine-rich a-helical segments**, which form cross-links between adjacent molecules.

Synthesis of elastin:

First of all, the gene is translated hydroxylation of Proline happen, as post translational modification, then pro-elastin molecules are released to ECM where they are further modified by the action of peptidases producing tropoelastin molecules (mature elastin), these assemble into elastin fibrils then into elastic fibers (by the presence of proteins that help in fibrils assembly).

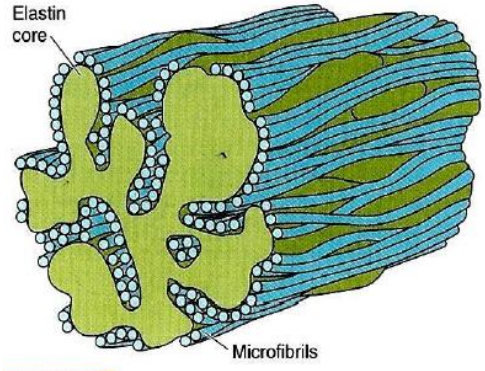


In the formation of elastic fibers **covalent bonds between Lys residues** are formed as cross links.

These fibers are present in different types of ECM in different structures like walls of arteries & smooth muscles.

If these is a discrepancy in the composition of elastic & collagen fibers, problems appear →example, if we have less elastic fibers in the arterial wall then the amount of smooth muscle cells will increase (respectively), and that will result in narrower vessel.

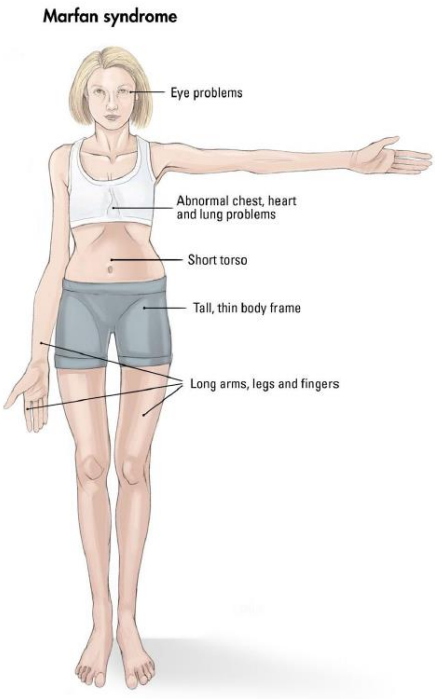
Elastic fibers are very thin, so to hold them and provide integrity they can provide a core (of elastic fibers) and covered by fibrils of glycoproteins, like fibrilin, to form what is known as microfibril. These microfibrils are found in hair and the tail of the sperm.



►**Marfan's syndrome** →A mutation in the **fibrilin glycoprotein** gene is the cause.

-Symptoms:

- ▪A tall, thin build with flexible joints
- ▪Long arms, legs, fingers, and toes
- ▪Scoliosis, or curvature of the spine
- ▪A chest that sinks in or sticks out
- ▪Crowded teeth and flat feet
- ▪**rupture of the aorta**



►**Emphysema (destructive lung disease)** →can be caused genetically due to a **dysfunctional alpha-1antitrypsin** leading to increased activity of elastase in lungs, BUT it <u>can be induced by smoking</u>.

What really happens ,genetically, is that lysine to-glutamate mutation causes protein misfolding, and this leads to formation of an aggregate and block of ER export causing deficiency in this enzymes, this decreases the elasticity of the alveoli so gas exchange efficiency is reduced and cyanosis happen.

_____(end of structural proteins of ECM)_____

<u>Glycosaminoglycans (GAGs):</u>

Sugar that has disaccharides repeated units (it's a **polysaccharide of disaccharide monomers**) , these disaccharide units are composed of **[N-acetylglucosamine OR N-acetylgalactosamine] & acidic unit [glucoronic acid OR iduronic acid].** These disaccharide units are **sulfated** (loaded with negative charge) and that helps in attracting water to produce cushioning effect.

GAGs attach to core protein forming proteoglycans (sugar is greater in amount).

GAGs are always in a form of proteoglycans as branches attached to a core protein except for **Hyaluronan (it is the only GAG with a single long polysaccharide chain).**

_____THE END