The extracellular matrix

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Learning Objectives

- Describe types, functions and components of the extracellular matrix (ECM)
- Describe the structure and functions of major proteins of ECM
- Describe the structure and functions of proteoglycans and glycoproteins of ECM
- Describe the disorders associated with components of ECM

- The extracellular space in the tissues of multicellular animals is filled with gel like material, called **extracellular matrix (ECM).**
- ECM is also called **ground substance**.
- ECM often referred to as **“connective tissue”,** that provide structural and biochemical support for surrounding cells.

Types, Functions And Components Of The Extracellular Matrix (ECM)

- The extracellular matrix (ECM) is secreted by cells. It is a three-dimensional network of extracellular macromolecules, such as **collagen**, **enzymes**, and **glycoproteins**.
- ECM holds the cells together and provides a porous pathway for diffusion of **nutrients** and **oxygen** to individual cells.

- The composition of ECM varies between multicellular structures; however, **cell adhesion**, **cell-to-cell communication** and **differentiation** are common functions of the ECM.

Types Of The Extracellular Matrix

The ECM has two basic forms:

1. **Basement membrane:** The basement membrane is a thin layer of ECM that forms between the epithelial and endothelial cells. It surrounds muscle, fat and nerve cells. It provides **mechanical structure**, **separates different cell types**, and **signals for cell differentiation**, **migration**, and **survival**.

2. **Interstitial matrix:** Interstitial matrix is present between various animal cells. Gels of polysaccharides and fibrous proteins fill the interstitial space and act as a **compression buffer** against the stress placed on the ECM.

Functions of the Extracellular Matrix

- ECM holds the cells together and provides a porous pathway for diffusion of nutrients and oxygen to individual cells.
- ECM protects the organs and also provides elasticity where required, for example, in blood vessels, lungs, and skin.
- ECM regulates cell processes such as **growth**, **migration** and **differentiation**.

- Extracellular matrix directs **morphology** of a tissue by interacting with cell-surface receptors and by binding to the surrounding growth factors which then stimulate signaling pathways.
- The proteins and glycosaminoglycans of ECM form a gel like structure which provides a flexible mechanical support and functions as a cushion and lubricant against mechanical shocks.

- The extracellular matrix support growth and wound healing. For instance, bone growth depends on the extracellular matrix since it contains the minerals needed to harden the bone tissue.
- The extracellular matrix has an important role in tissue repair which can be utilized as a therapeutic target.

Components of the Extracellular Matrix

- Components of the ECM are produced intracellularly by cells and secreted into the ECM via exocytosis.
- Once secreted, they then aggregate with the existing matrix.

The extracellular matrix contains:

1. Water
2. Fibrous proteins: The main fibrous proteins that build the extracellular matrix are **collagen** and **elastin**
3. Glycoproteins: The main glycoproteins are **fibrillin, fibronectin**, and **laminin**s
4. Proteoglycans (glycosaminoglycans, GAGs): The major proteoglycans are, **hyaluronic acid**, **chondroitin sulfate**, **keratan sulfate**, **heparan sulfate** and **heparin**

The main fibrous proteins of ECM

The main fibrous proteins of ECM are:

- Collagen
- Elastin

- **Collagens** are the major structural component of the ECM. In the matrix, collagen will give the cell **tensile strength** and facilitate **cell-to-cell adhesion** and **migration**. Collagens provide scaffolding for the attachment of laminin, proteoglycans and cell surface receptors.

- **Elastins** in contrast to collagens, give elasticity to tissues, allowing them to stretch when needed and then return to their original state. This is useful in blood vessels, the lungs, in skin, and the ligamentum nuchae. These tissues contain high amounts of elastins.

Glycoproteins of ECM

The main glycoproteins are

- Fibrillin,
- Fibronectin, and
- Laminins

- **Fibrillin** is a glycoprotein, which is essential for the formation of elastic fibers found in connective tissue. Fibrillin is secreted into the extracellular matrix by fibroblasts and becomes incorporated into the insoluble microfibrils, which appear to provide a scaffold for deposition of elastin.

- **Fibronectins** are glycoproteins that connect cells with collagen fibers in the ECM, allowing cells to move through the ECM. Fibronectins also help at the site of tissue injury by binding to platelets during blood clotting and facilitating cell movement to the affected area during wound healing.

- The **laminin** is a glycoprotein. They are secreted and incorporated into cell-associated extracellular matrix. Laminin is vital for the maintenance and survival of tissues. Defective laminins can cause muscles to form improperly, leading to a form of muscular dystrophy, lethal skin blistering disease (junctional epidermolysis bullosa) and defects of the kidney filter (nephrotic syndrome).

Proteoglycans

- Glycosaminoglycans (GAGs), attached to extracellular matrix proteins (except hyaluronic acid) to form proteoglycans.
- Proteoglycans have a net negative charge that attracts positively charged sodium ions (Na^+), which attracts water molecules via osmosis, keeping the ECM and resident cells hydrated.
- Proteoglycans may also help to trap and store growth factors within the ECM.

Clinical significance of ECM

- Biologic scaffold materials composed of extracellular matrix (ECM) have been used in a variety of surgical and tissue engineering/regenerative medicine applications.
- Extracellular matrix has been found to cause regrowth and healing of tissue.
- ECM is associated with remodeling properties including **angiogenesis**, and **stem cell recruitment**.

- **Matrix-bound nanovesicles (MBVs)** of ECM are a key player in the healing process.
- In human fetuses, for example, the extracellular matrix works with stem cells to grow and regrow all parts of the human body, and fetuses can regrow anything that gets damaged in the womb.

- In terms of injury repair and tissue engineering, the extracellular matrix serves two main purposes.
    1. First, it prevents the immune system from triggering from the injury and responding with inflammation and scar tissue.
    2. Next, it facilitates the surrounding cells to repair the tissue instead of forming scar tissue

- Extracellular matrix proteins are commonly used in cell culture systems to maintain stem and precursor cells in an undifferentiated state during cell culture and function to induce differentiation of epithelial, endothelial and smooth muscle cells in vitro

Structure and Function of Collagen

- Collagen is the main protein of connective tissue and the most abundant protein in mammals.
- It has great tensile strength and is present to some extent in nearly all organs and serves to hold cells together in discrete units.

Structure of Collagen

- All collagen types have triple helical structure.
- The basic structural unit of collagen is **tropocollagen**, which consists of **three polypeptide** chains called α-**chains**.
- These three polypeptide chains twisted around each other in a **triple helix** forming a rope like structure, which has great tensile strength

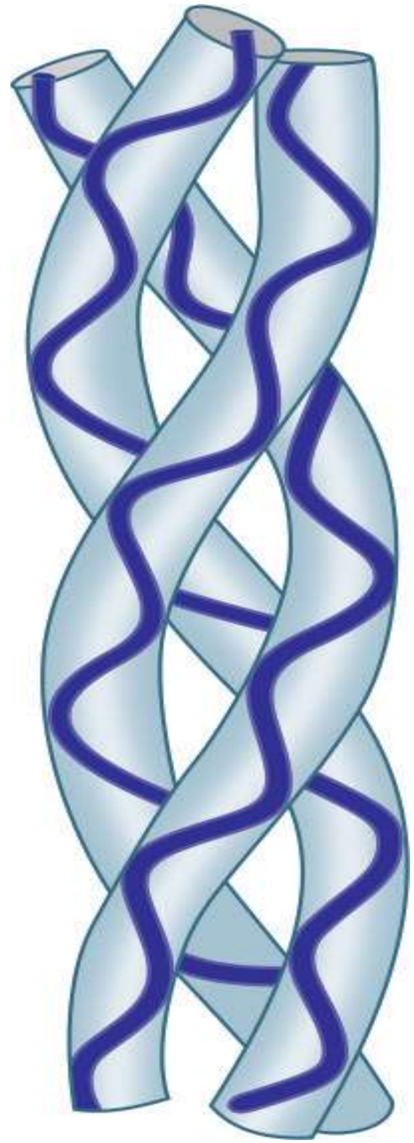


Figure 31.1: Right-handed collagen triple helix formed from three left-handed α-chains.

- The three helically interwind polypeptides are of equal length, each having about 1000 amino acids residues.
- The three polypeptide chains are held together by **hydrogen bonds** between chains.
- Multiple types of collagen in human tissues arise from different triple helical combinations of polypeptides.
- In human tissues, 19 distinct types of collagen have been identified.

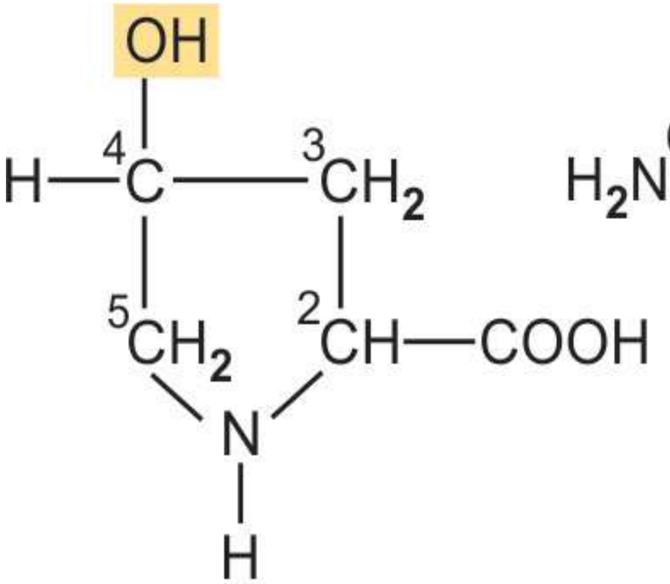
TABLE 31.1: Most abundant types of collagen found in human tissues and their distribution.

Types of collagen	*Distribution*
I	Skin, tendon, bone, cornea
II	Articular cartilage, intervertebral disk, vitreous body
III	Fetal skin, cardiovascular system, reticular fibers
IV	Basement membrane
V	Placenta, skin

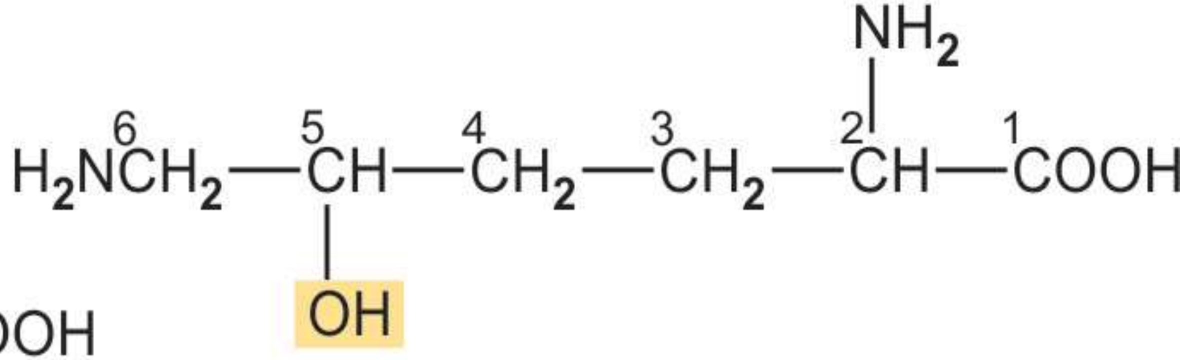
Structure of α-chain of Collagen

- Collagen has an unusual amino acid composition with 33% of the total residues being **glycine (Gly)**, 10% **proline (Pro)**, 10% **hydroxyproline (Hyp)** and 1% **hydroxylysine (Hyl)**.
- Two amino acids that are found in collagen, 4-**hydroxyproline** and 5-**hydroxylysine** are not resent in most proteins.

Figure 31.2: Two special amino acids found in collagen.



4-hydroxyproline



5-hydroxylysine

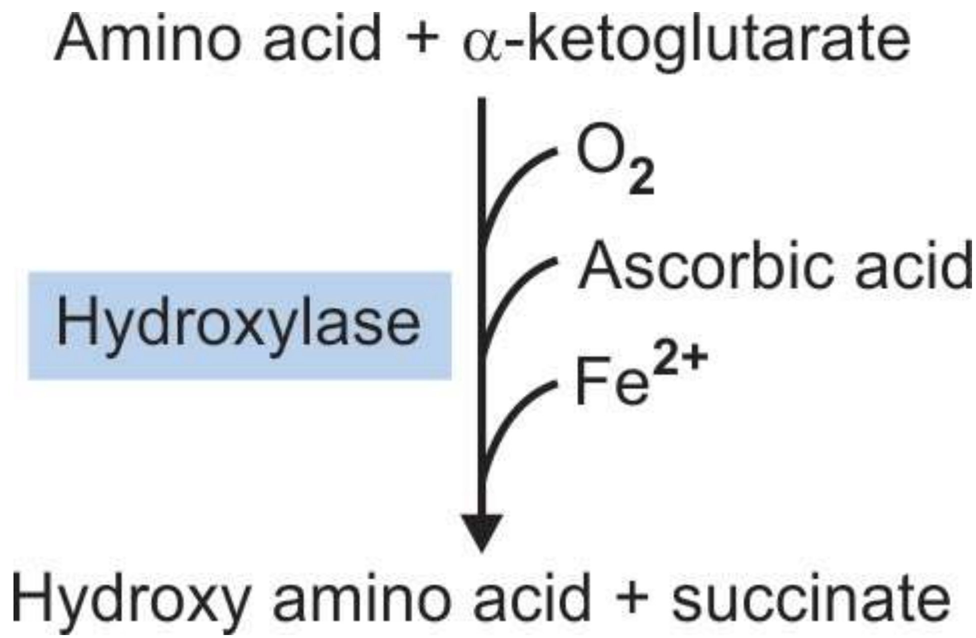
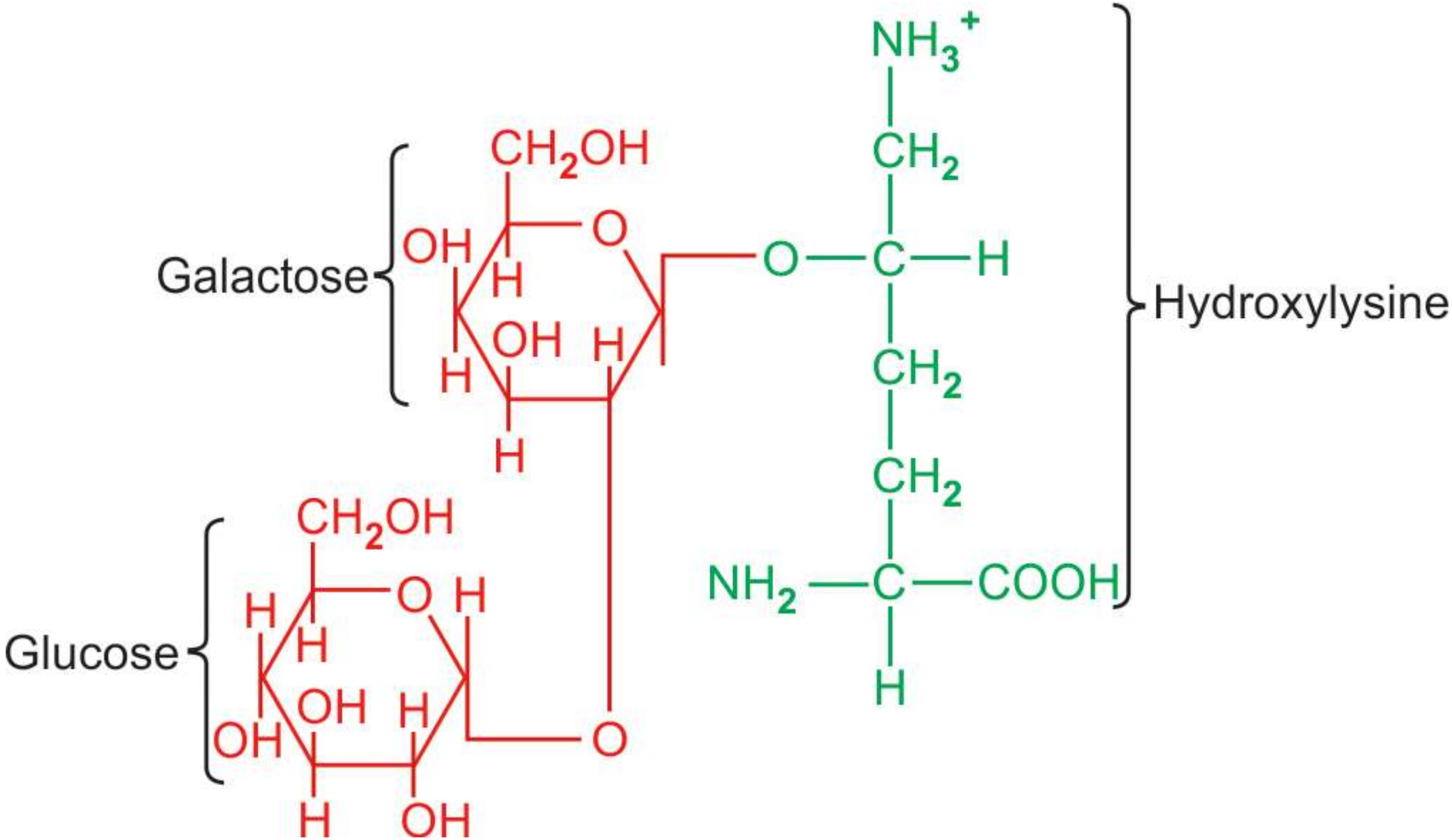


Figure 31.3: Hydroxylation of amino acid to hydroxyamino acid.

- In addition to hydroxylation reactions, the formation of stable triple helices requires **glycosylation**. A few hydroxylysine residues of collagen are covalently bound to carbohydrate units, mostly a **disaccharide of glucose and galactose**

Figure 31.4: Glycosylated hydroxylysine.



- In collagen, **tyrosine** is present in low amounts and the essential amino acid **tryptophan** is absent. **Cysteine** also is absent and, thus, no disulfide cross-links are present.
- Hydroxyproline and hydroxylysine are derived from proline and lysine in enzymatic process of post-translational modification.

- The primary structure of collagen is unusual in that, collagen has regular arrangement of amino acids in each of the α-chains of the tropocollagen.
- The sequence generally follows the pattern **(Gly-X-Y)**, where Gly for glycine and X and Y, for any amino acid residues.
- Most of the time X is for proline and Y is for hydroxyproline.

- Thus, glycine, the smallest amino acid is found in every third position of the polypeptide chain.
- This is necessary because *glycine is the only amino acid small enough to be accommodated in the limited space available in the central core of the helix*

Figures 31.5A and B:

(A) Representation of primary structure of α-chain of collagen;

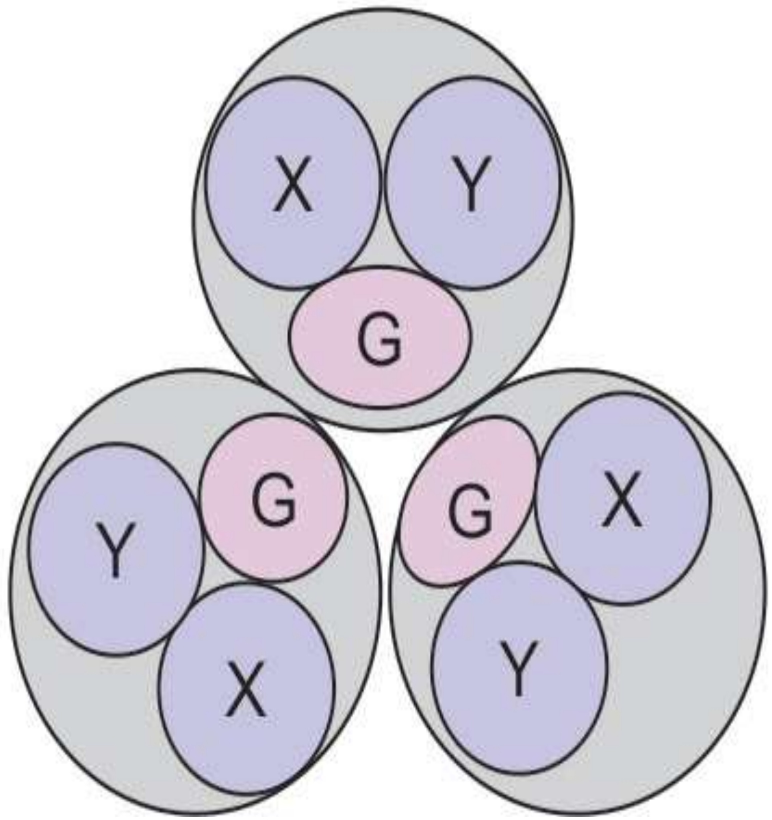
(B) Cross-section of triple helical structure showing position of glycine in the central core of the helix of collagen,

where G = glycine,

X and Y = any other amino acid mostly proline and hydroxyproline.

G—X—Y—G—X—Y—G—X—Y—G—X—Y........

Primary structure of α-helix of collagen



Strike a Note

- Collagen synthesized in absence of ascorbic acid (vitamin C) is insufficiently hydroxylated and because proline and lysine residues are not hydroxylated hydrogen bonding with triple helices and cross-linking between triple helices cannot occur and collagen molecules cannot stabilize. This abnormal collagen cannot properly form fibers and thus, causes the skin lesions, blood vessel fragility, loose teeth, and bleeding gums in scurvy.
- Mutation of a single glycine residue in collagen leads to connective tissue disorder which can be lethal, e.g. **osteogenesis imperfecta.**

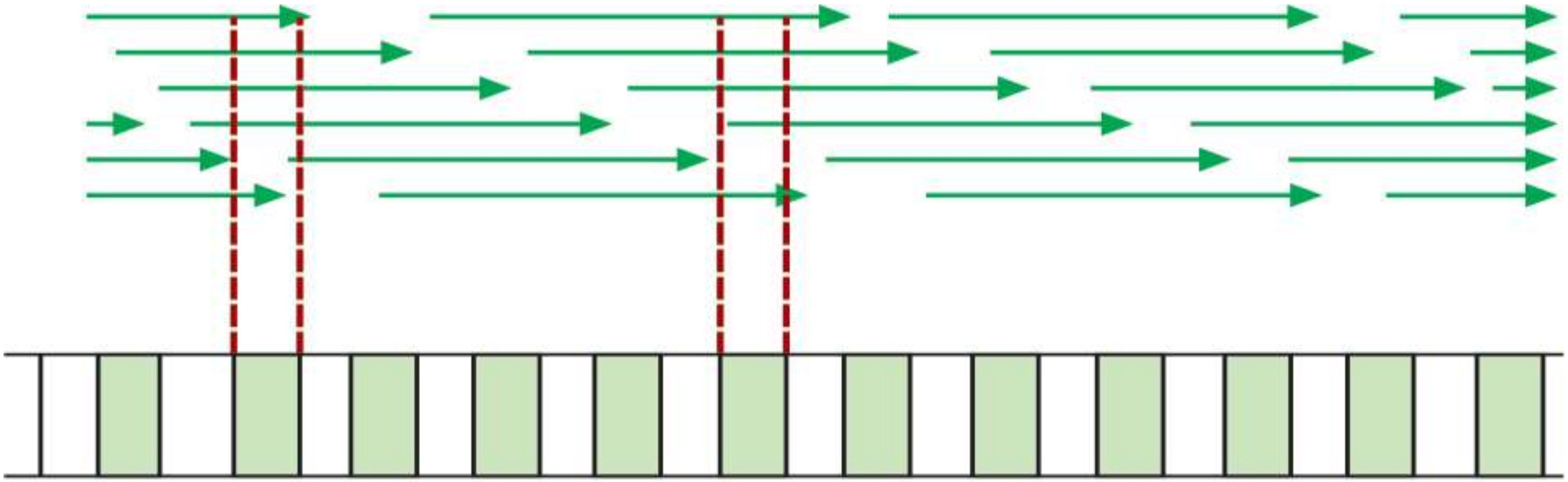
- Collagen is unique in its high content of helix destabilizing amino acids, **proline**, **hydroxyproline**, and **glycine**. These prevent the formation of the usual α-helical and β-pleated structure. Instead, it forms a **triple helical** secondary structure.
- Destruction of elastin by **elastase** is normally inhibited by α-**trypsin**, the genetic deficiency of which can result in **emphysema**.

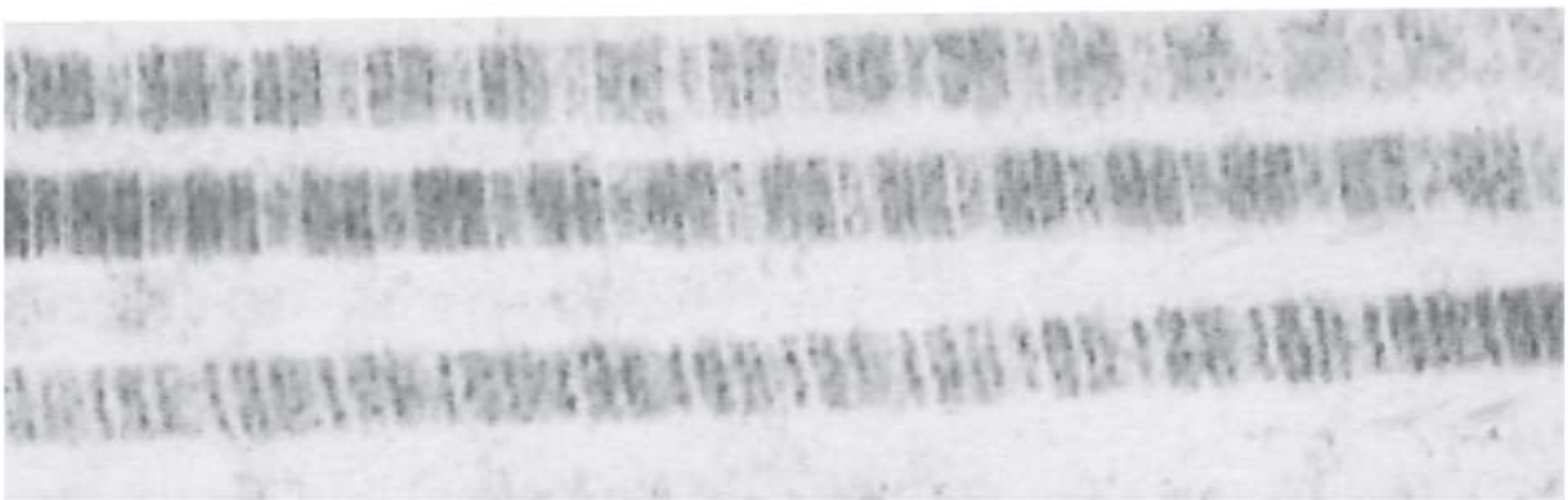
Formation of Collagen Fibrils

- Individual tropocollagen molecules spontaneously laterally aggregate to form fibril.
- They arrange themselves under physiological conditions into staggered, parallel and overlapping array structures.
- Each tropocollagen molecule overlaps its neighbour by a length approximately three quarters of a molecule
- The regularity of gaps and overlaps is responsible for the banded appearance of these fibers in connective tissues.

Figures 31.6

- The quarter staggered, parallel, and overlapping array structural arrangement of tropocollagen to form collagen fiber;
- The regularity of gaps and overlaps generates the banded appearance in the collagen fiber;
- Electron micrographic banded appearance of fibrillar collagen.

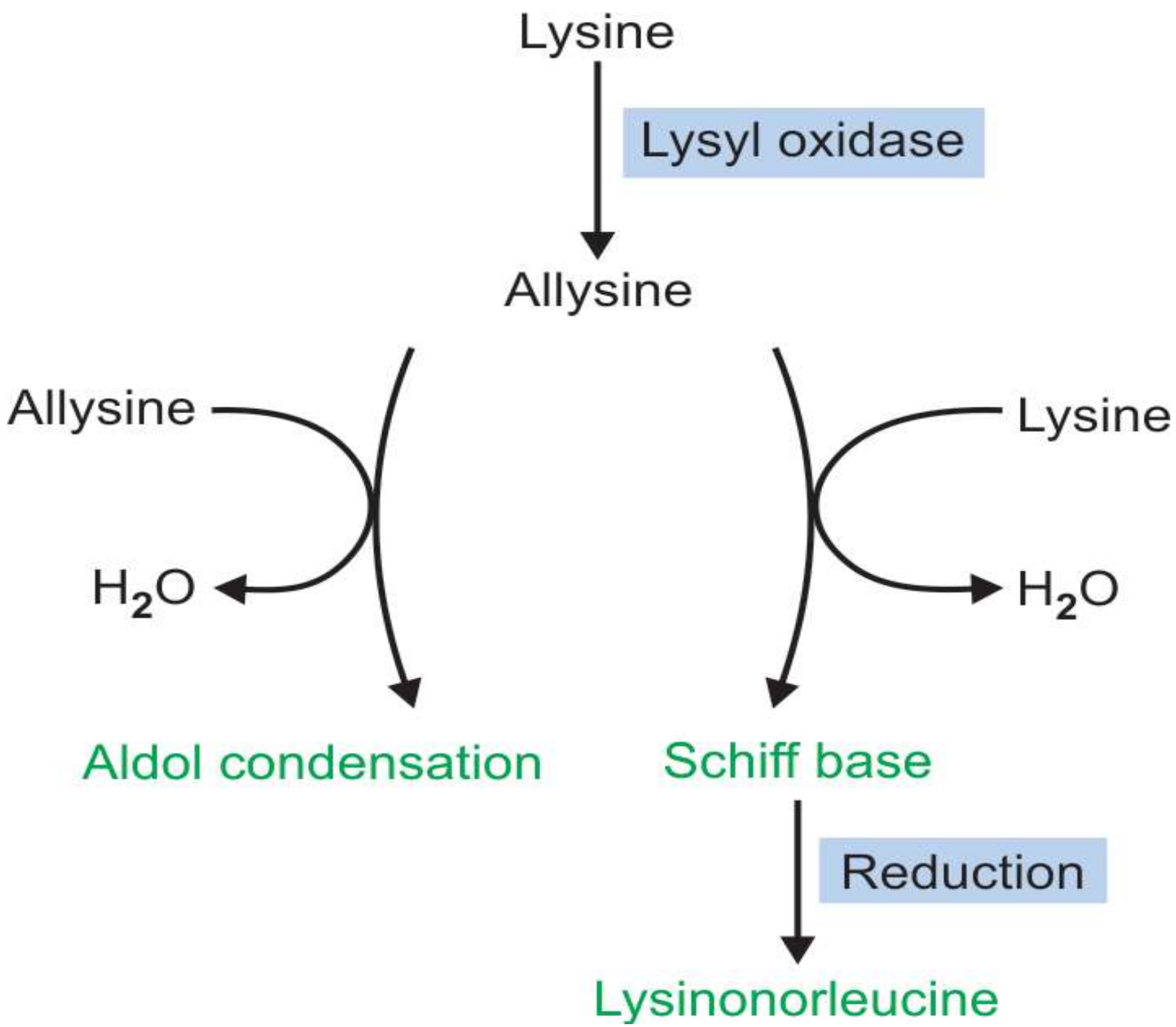




- These fibriller arrays of tropocollagen molecules become connected and subsequently stabilized by intra- and intercovalent cross-links through action of copper requiring enzyme **lysyl oxidase**.
- Three types of inter- or intramolecular cross-links that stabilize the collagen fibril are

    1. Aldol condensation.
    2. Schiff base.
    3. Lysinonorleucine

Figure 31.7: Types of cross-links in collagen.



Effect of aging on collagen

- The cross-links are made slowly but continuously throughout life. As the number of cross-links increases, collagen beings to lose elasticity.
- As we become older, more and more covalent cross-links form in and between tropocollagen molecules, rendering the collagen fibrils in our connective tissues more rigid and brittle. Its increasing brittleness and rigidity on aging alter the mechanical properties of tendons and cartilage which makes bones more brittle and causes the cornea of the eye to be less transparent.

Structure and function of Elastin

- Elastin occurs with collagen in connective tissues.
- Elastin is a **rubber-like protein**, which can stretch to several times their length and then rapidly return to their original size and shape when the tension is released.
- Elastin is present in large amounts, particularly in tissues that require these physical properties, e.g. lung, blood vessels and ligaments.

- Smaller quantities of elastin are also found in skin, ear cartilage and several other tissues.
- Elastin differs from collagen in several properties.
- In contrast to collagen, there is only one genetic type of elastin

TABLE 31.2: Differences between collagen and elastin.

Collagen	*Elastin*
Many different genetic types	One genetic type
It has no capacity to stretch	It has capacity to stretch an subsequently to recoil
Primary structure has repeating (Gly-X-Y) sequences	Primary structure has no repeating (Gly-X-Y) sequenc
Formation of triple helical secondary structure	No formation of triple helix
Presence of hydroxylysine	No hydroxylysine present
Glycosylated hydroxylysine is present	No glycosylated hydroxylys present
Formation of intramolecular aldol cross-links	Formation of intramolecula desmosine cross-links

Structure of Elastin

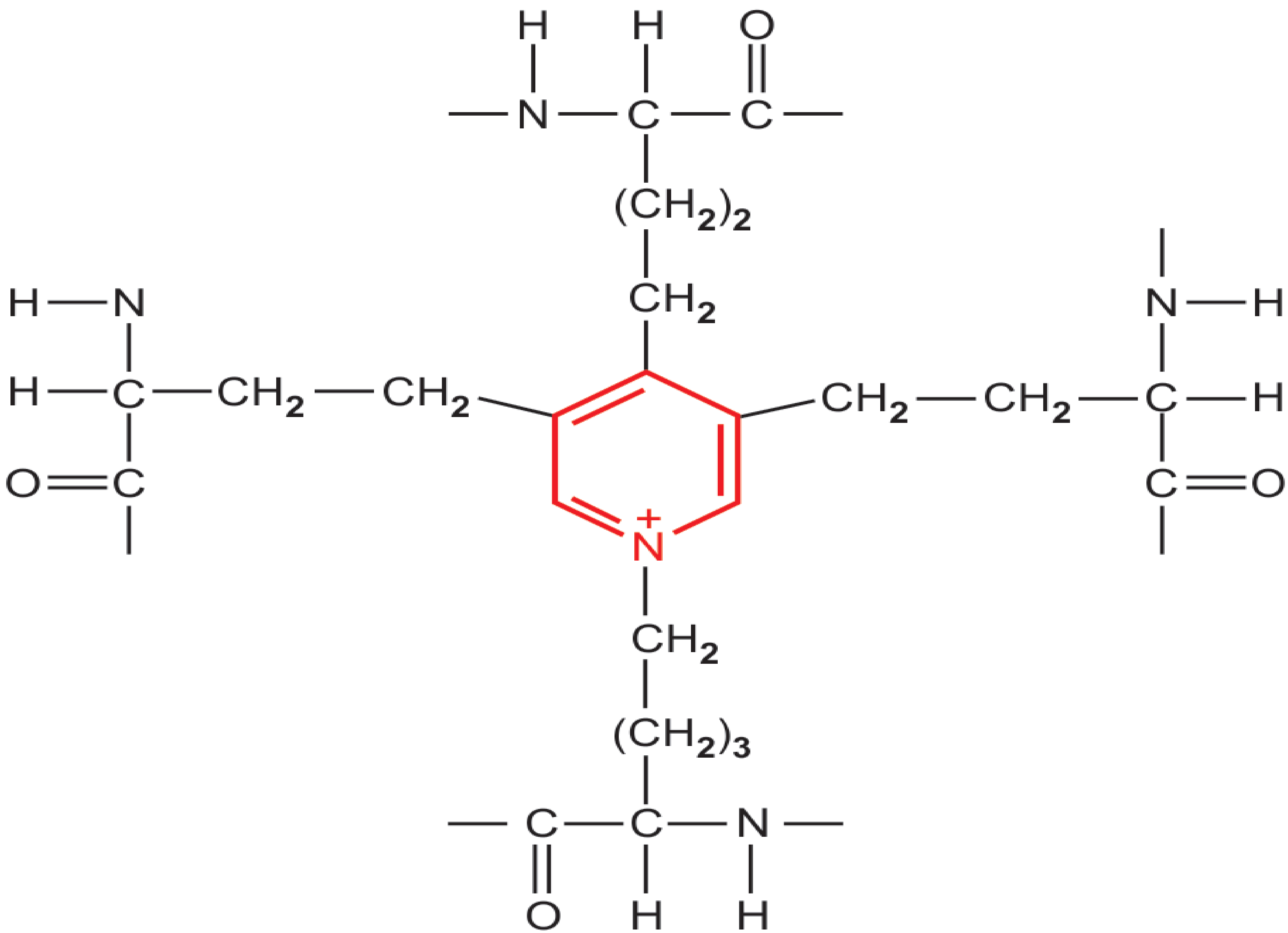
- The basic subunit of elastin fibrils is **tropoelastin** which contains about 800 amino acid residues.
- Unlike collagen, elastin does not contain repeat (Gly-X-Y) sequences.
- Although elastin and collagen contain similarly high amounts of glycine and proline and both lack cysteine and tryptophan elastin contains less hydroxyproline and no hydroxylysine and glycosylated hydroxylysine.
- Elastin has very high content of **alanine** and other nonpolar aliphatic residue, i.e. **valine**, **leucine** and **isoleucine**.

Cross-links of Elastin

- The cross-links in elastin are more complex than those in collagen.
- The major cross-links formed in elastin are the **desmosines**, which is derived from the condensation of three allysine (oxidized form of lysine) residues with lysine.
- These cross-links permit the elastin to stretch in two dimensions and subsequently recoil during the performance of its physiologic functions

Figure 31.8: Desmosine cross-link of elastin, formed from four lysine residues.

$$\text{3 Allysine + Lysine} \xrightarrow{\text{Condensation}} \text{Desmosine}$$


H
H
O
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C
C
(CH2)2
CH2
H
N
H
C
CH2
CH2
O
C
N
H
CH2
CH2
C
H
C
O
+
N
CH2
(CH2)3
C
C
N
O
H
H

Desmosine

Proteoglycans

- Proteoglycans are important in the structural organisation of the extracellular matrix.
- Proteoglycans are found in every tissue of the body, mainly in the ECM intracellular **ground substance**.
- Some proteoglycans bind ton collagen and others to elastin.

- The GAGs present in the proteoglycans are **negatively charged (anion)** and hence bind positively charged cations such as Na^+ and K^+.
- This Na^+ attracts water by osmotic pressure into extracellular matrix keeping the ECM and resident cells hydrated.
- GAGs also **gel** at relatively low concentrations.

- Because of the long extended nature of the polysaccharide chains of GAGs and their ability to gel, the proteoglycans can act as **sieves**, restricting the passage of large macromolecules into the ECM but allowing relatively diffusion of small molecules.
- As well as due to their extended structures and the huge macromolecular aggregation, they occupy a **large volume** of the matrix relative to proteins.

- The major glycosaminoglycans are:
  - Hyaluronic acid
  - Chondroitin sulfate
  - Keratan sulfate I and II
  - Heparan sulfate
  - Heparin

Glycoproteins

- Glycoproteins are proteins to which oligosaccharides are covalently attached.
- The main function of glycoprotein is to facilitate adhesion between various elements of connective tissue.

Some glycoproteins present in ECM are:

1. Fibrillins: Structural components of microfibrils
2. Fibronectin: an important glycoprotein involved in cell adhesion and migration
3. Lamin: Major protein component of **basal lamins**. Basal lamins are specialized areas of the ECM that surround epithelial and other cells, for example muscle cells.

Disorders Associated With Components Of ECM

- Mutations in genes that are responsible for production of collagen can lead to number of disorders. For example:
  - Osteogenesis imperfect,
  - Number of types of Ehlers-Danlos syndrome
  - Epidermolysis bullosa

- Marfan’s syndrome is due to mutations in genes coding for **fibrilin**, a glycoprotein.
- **Mucopolysaccharidoses** a group of genetic disorders is due to deficiencies of enzymes that degrade proteoglycans (the glycosaminoglycans; GAGs), a specific component of ECM.

TABLE 31.4: Types of mucopolysaccharidoses and their enzyme detects.

Mucopolysaccharidoses	*Alternative designation*	*Enzyme defect*	*Accumulated products*
Hurler	MPS I H	L-Iduronidase	Dermatan Sulfate Heparan Sulfate
Scheie	MPS I S	L-Iduronidase	Dermatan Sulfate
Hunter	MPS II	Iduronate Sulfatase	Dermatan Sulfate Heparan Sulfate
Sanfilippo A	MPS IIIA	Heparan Sulfate N Sulfatase	Heparan Sulfate
Sanfilippo B	MPS IIIB	N-Acetyl Glucosaminidase	Heparan Sulfate
Sanfilippo C	MPS IIIC	Glucosaminide N-acetyltransferase	Heparan Sulfate
Sanfilippo D	MPS IIID	N Acetylgucosamine Sulfatase	Heparan Sulfate
Morquio A	MPS IVA	Galactosamine 6 Sulfatase	Keratan Sulfate Chondroitin 6-Sulfate
Morquio B	MPS IVB	Beta Galactosidase	Keratan Sulfate
Maroteaux- Lamy	MPS VI	N Acetyl Galactosamine 4 Sulfatase (Aryl Sulfatase B)	Dermatan Sulfate
Sly	MPS VII	Beta Galactosidase	Dermatan Sulfate Heparan Sulfate

THANK YOU