المملكة العربية السعودية
جامعة الملك سعود
كلية العلوم
قسم الكيمياء الحيوية

Biochemistry of Specialized Tissues

Fibrous proteins

a- Collagen

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Fibrous vs. Globular Proteins

Globular	Fibrous
1. Compact protein structure	Extended protein structure
2. Soluble in water (or in lipid	Insoluble in water (or in lipid bilayers) bilayers)
3. Secondary structure is complex with a mixture of a-helix, b-sheet and loop structures	Secondary structure is simple based on one type only
4. Quaternary structure is held together by noncovalent forces	Quaternary structure is usually held together by covalent bridges
5. Functions in all aspects of metabolism (enzymes, transport, immune protection, hormones, etc).	Functions in structure of the body or cell (tendons, bones, muscle, ligaments, hair, skin)

Fibrous Proteins

Fibrous proteins have high α-helix or β-sheet content. Most are structural proteins.

Examples include:

- Collagen
- Elastin
- Keratin
- Fibroin

Fibrous Proteins

Characteristics of the fibrous proteins:

- Most of the polypeptide chain is parallel to a single axis

- Fibrous proteins are often mechanically strong & highly cross linked
- Fibrous proteins are usually insoluble in water
- They usually play a structural role

Collagen

What makes collagen a strong tensile protein?

Questions?

- How would you define the structure of a collagen molecule?
- What are the dimensions of a collagen molecule?
- What are the dimensions of a collagen fibril?
- State the most important aminoacids in collagen and explain their importance.
- What is the periodicity of collagen? Why does it happen?

Collagen background

- It is the most abundant protein in mammals, up to 30% of all proteins.
- It occurs in connective tissues where tensile strength is needed.
- Responsible for functional integrity of tissues such as cartilage, skin, tendon.
- More than 15 collagen types present in human tissues.

Collagen background

The tensile strength results from:

a- The triple helix secondary structure.

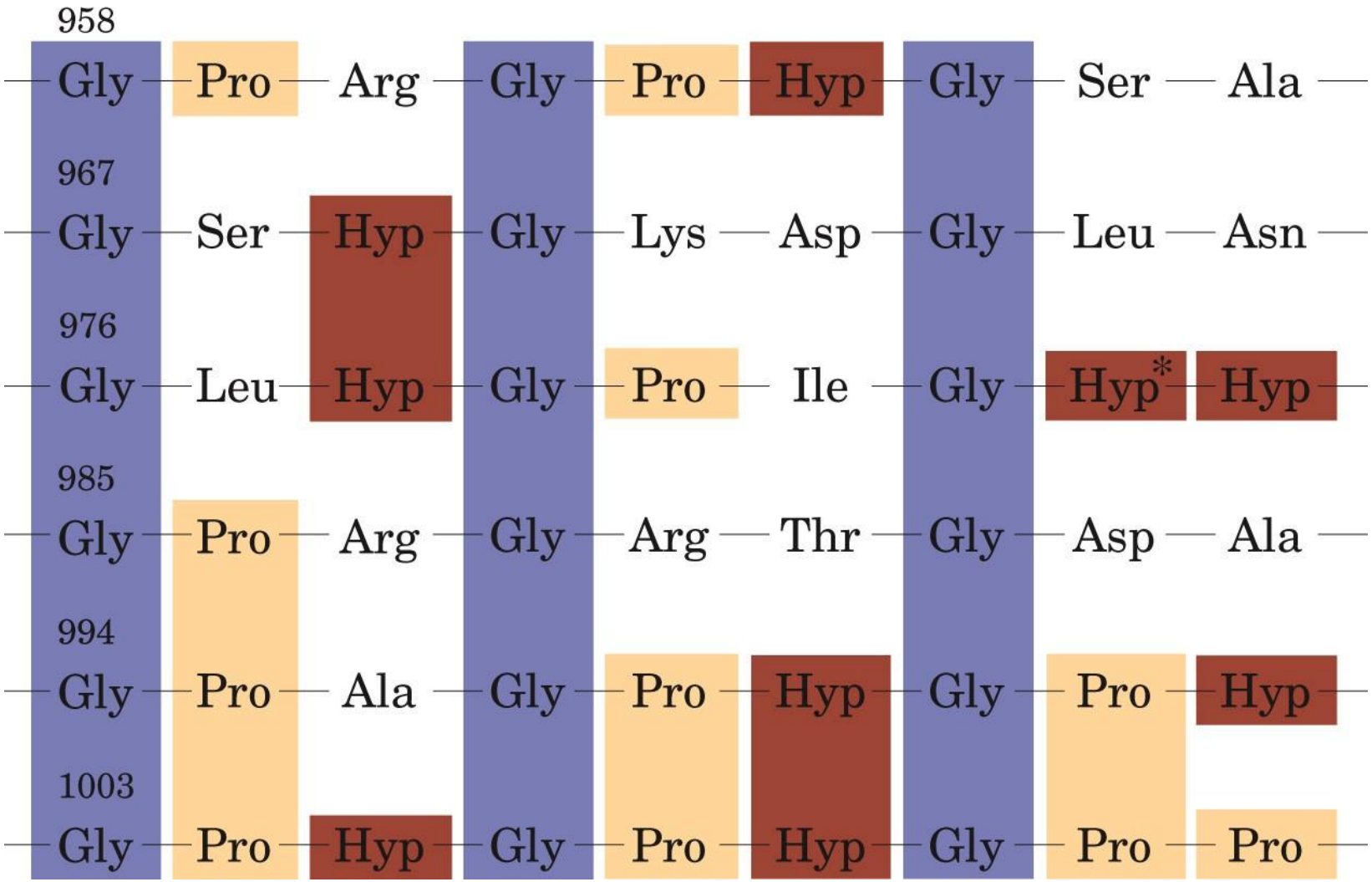
b- The assembly of tropocollagen subunits into fibers.

c- The chemical cross-linking between fibrils to form fibers.

Collagen - A Triple Helix

- Collagen A is the principal component of connective tissue (tendons, cartilage, bones, teeth)
- Its basic unit is tropocollagen which is composed of:
  - three intertwined polypeptide chains (1050 amino acid residues each
  - Mwt = 285,000 Dalton
  - its length is 300 nm long and the diameter 1.4 nm
  - unique amino acid composition

Collagen Amino Acid Sequence



Characteristics of Collagen Amino Acid Composition

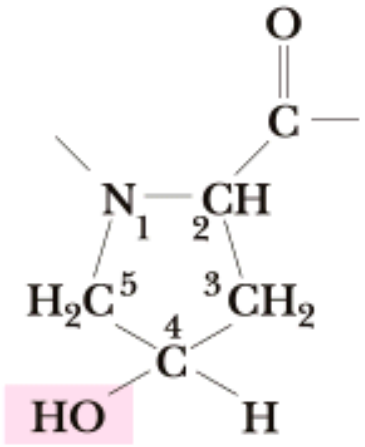
- Nearly one residue out of three is Glycine
- The typical structure contains Gly-X-Y
- Presence of many modified amino acids like:

  4- hydroxyproline

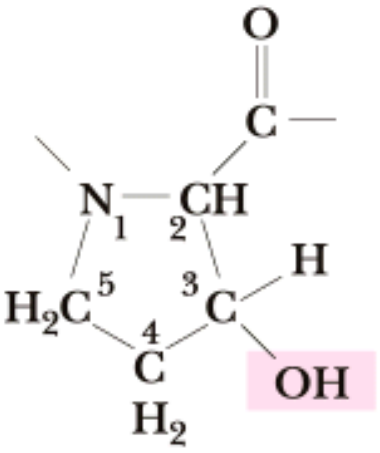
  3- hydroxyproline

  5- hydroxylysine
- Proline and hydroxyproline together represent 30% of residues

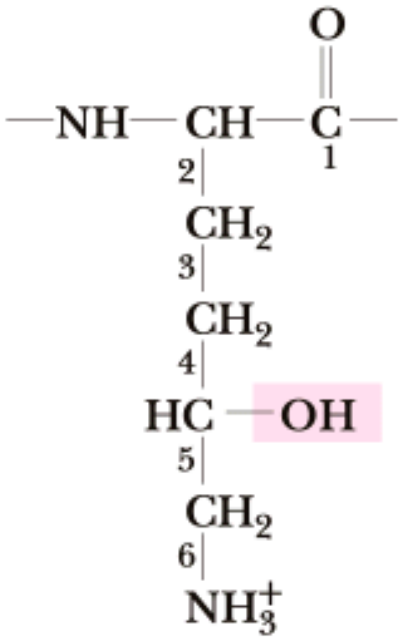
Hydroxylated residues found in collagen



4-Hydroxyprolyl residue (Hyp)



3-Hydroxyprolyl residue



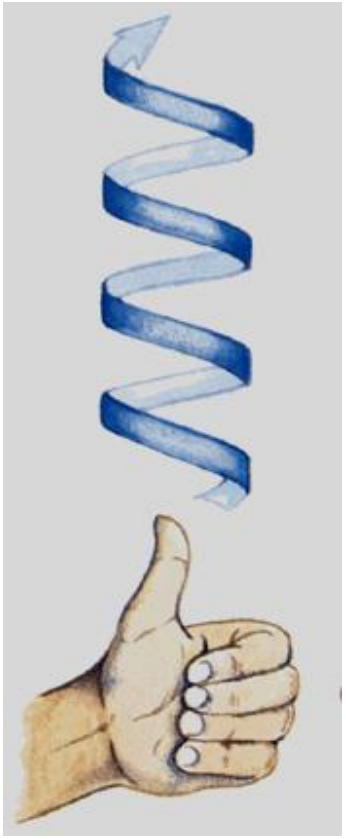
5-Hydroxylysyl residue (Hyl)

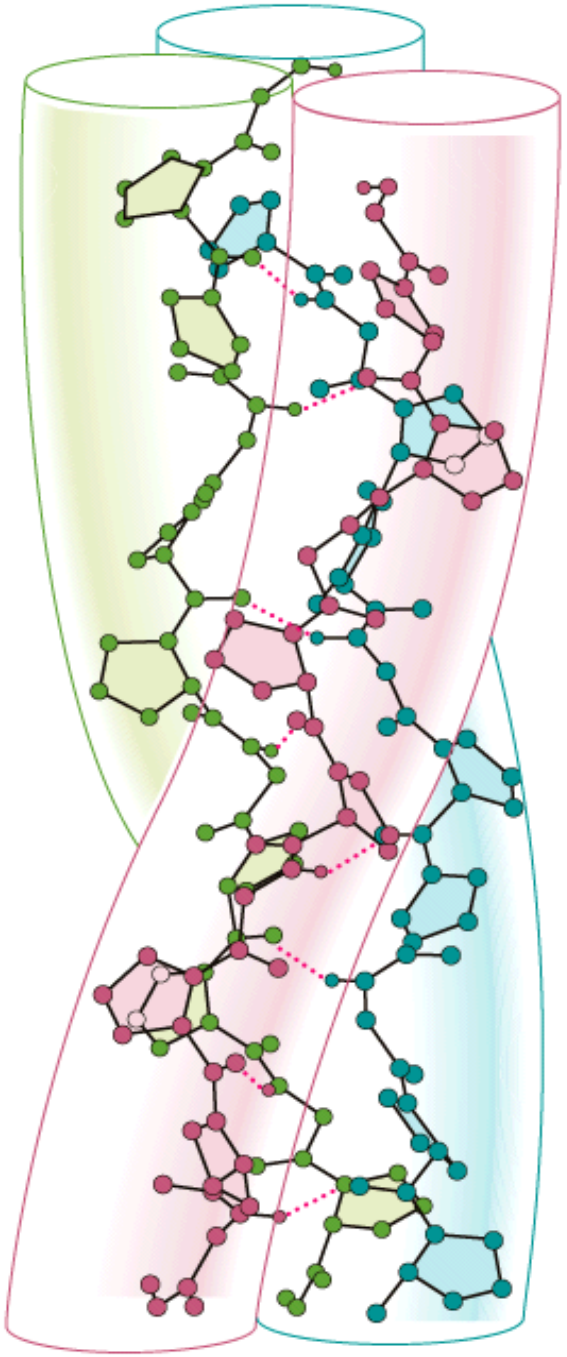
Collagen Secondary Structure: The Triple Helix

- Collagen is formed from tropocollagen subunits. The triple helix in tropocollagen is highly extended and strong.
- Features:
  - Collagen triple helix is formed from three separate polypeptide chains arranged as a left-handed helix (note that α-helix is right-handed).
  - 3.3 amino acid residues per turn
  - Each chain forms hydrogen bonds with the other two to make it more strong

The Collagen Triple Helix

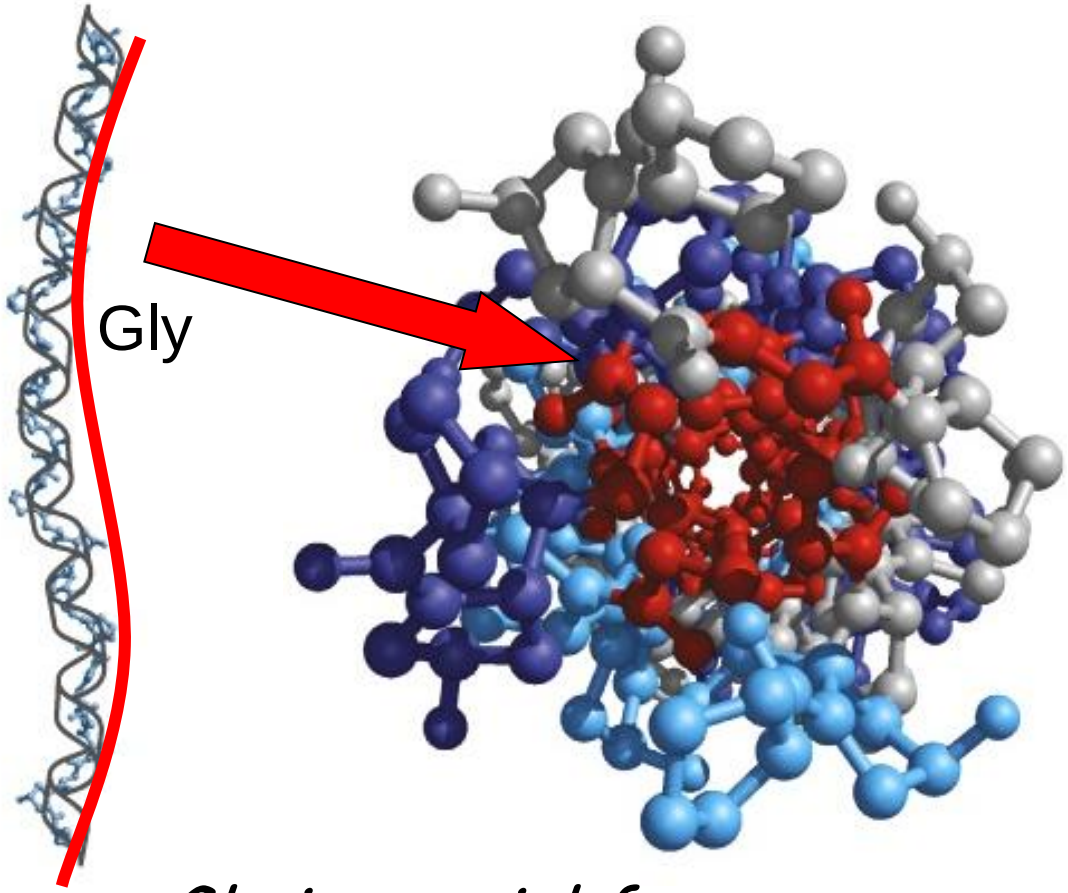
- The unusual amino acid composition of collagen is not favorable for alpha helices OR beta sheets
- But it is ideally suited for the collagen triple helix: three intertwined helical strands
- It is much more extended than alpha helix, with a rise per residue of 2.9 A°
- There are long stretches of Gly-Pro-Pro/HyP





In collagen triple helix, H-bonds form between separate chains. In alpha helix H-bonds formed between residues of the same chain.

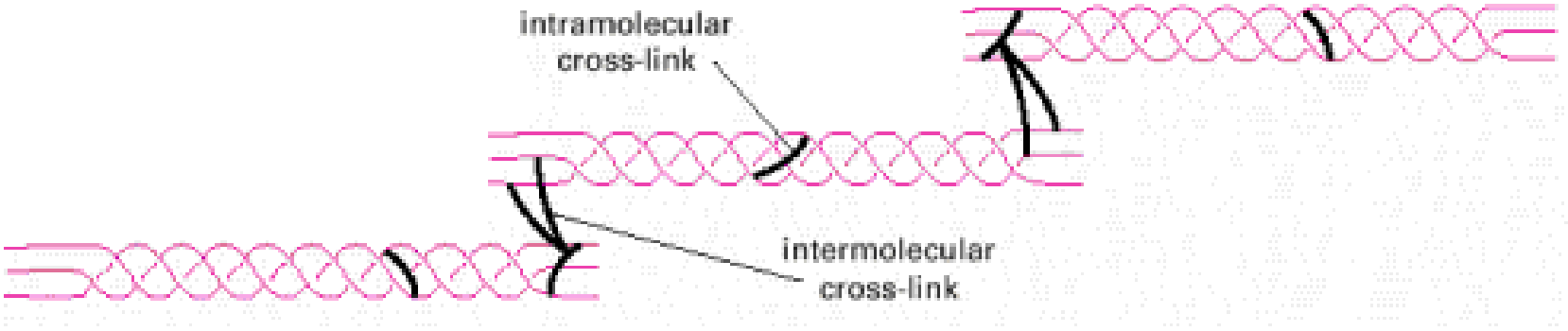
Collagen



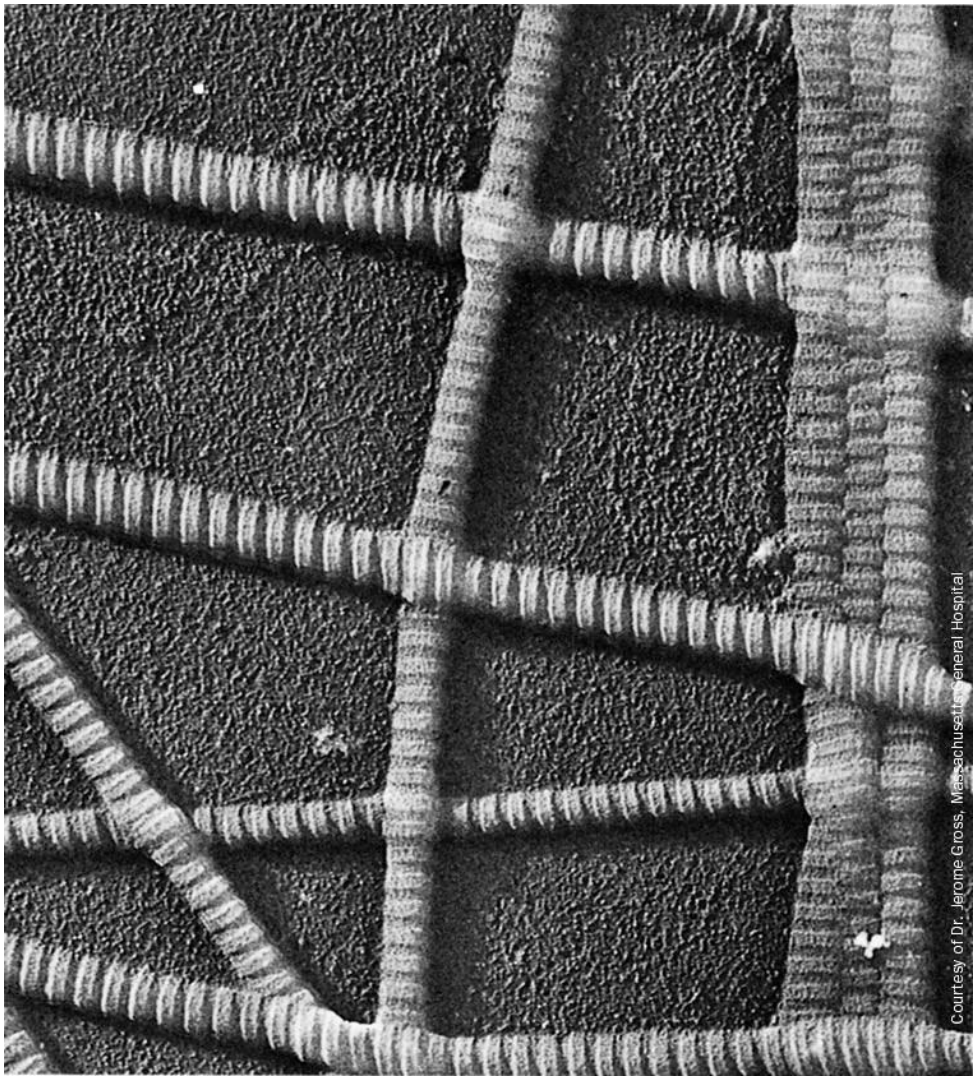
Gly is crucial for forming collagen structure.

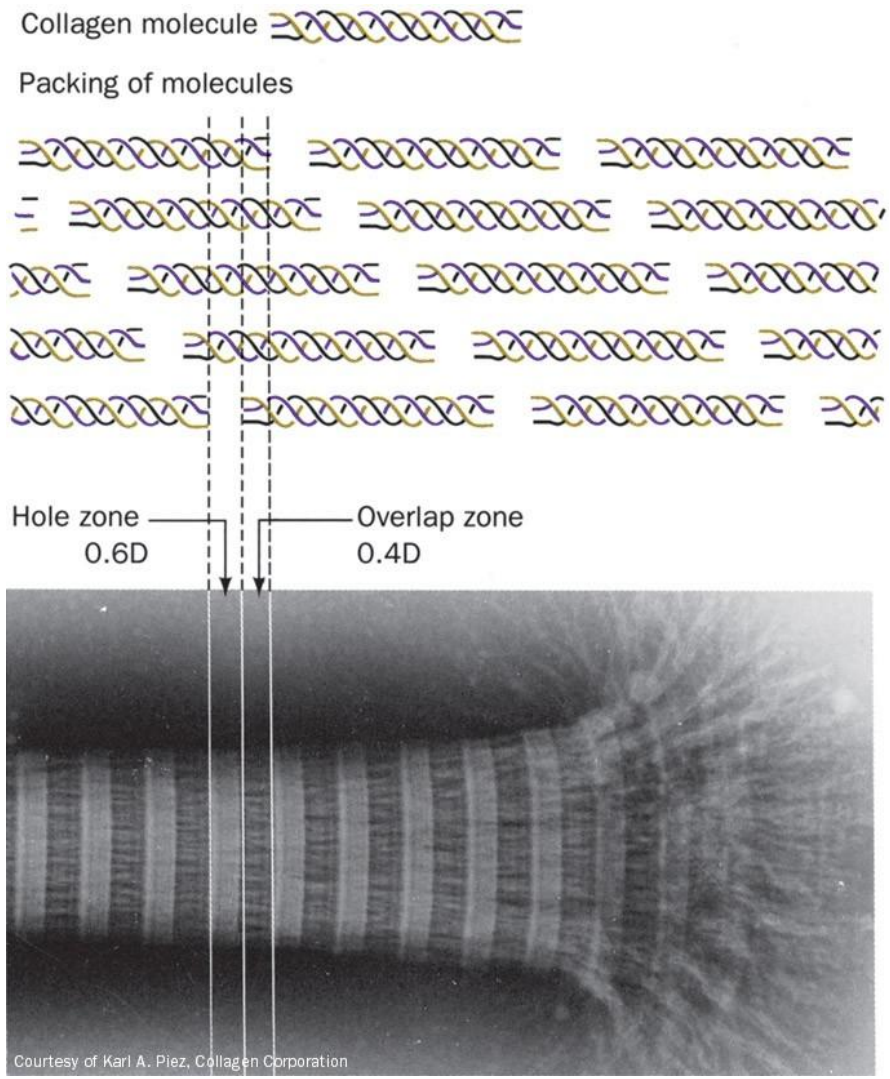
Collagen Fibers

- *Fibers are formed by staggered arrays of tropocollagens*
- Banding pattern in EMs with 68 nm repeat
- Since tropocollagens are 300 nm long, there must be 40 nm gaps between adjacent tropocollagens (5x68 = 340 A°)
- 40 nm gaps are called "hole regions" - they contain carbohydrate and are thought to be nucleation sites for bone formation



Electron *Micrographs of Collagen* fibers showing band pattern





Structural basis of the collagen triple helix

- Every third residue faces the crowded center of the helix - only Gly fits
- Pro and HyP suit the constraints of phi and psi
- Interchain H-bonds involving HyP stabilize helix
- Fibrils are strengthened by intrachain lysine-lysine and interchain hydroxypyridinium crosslinks

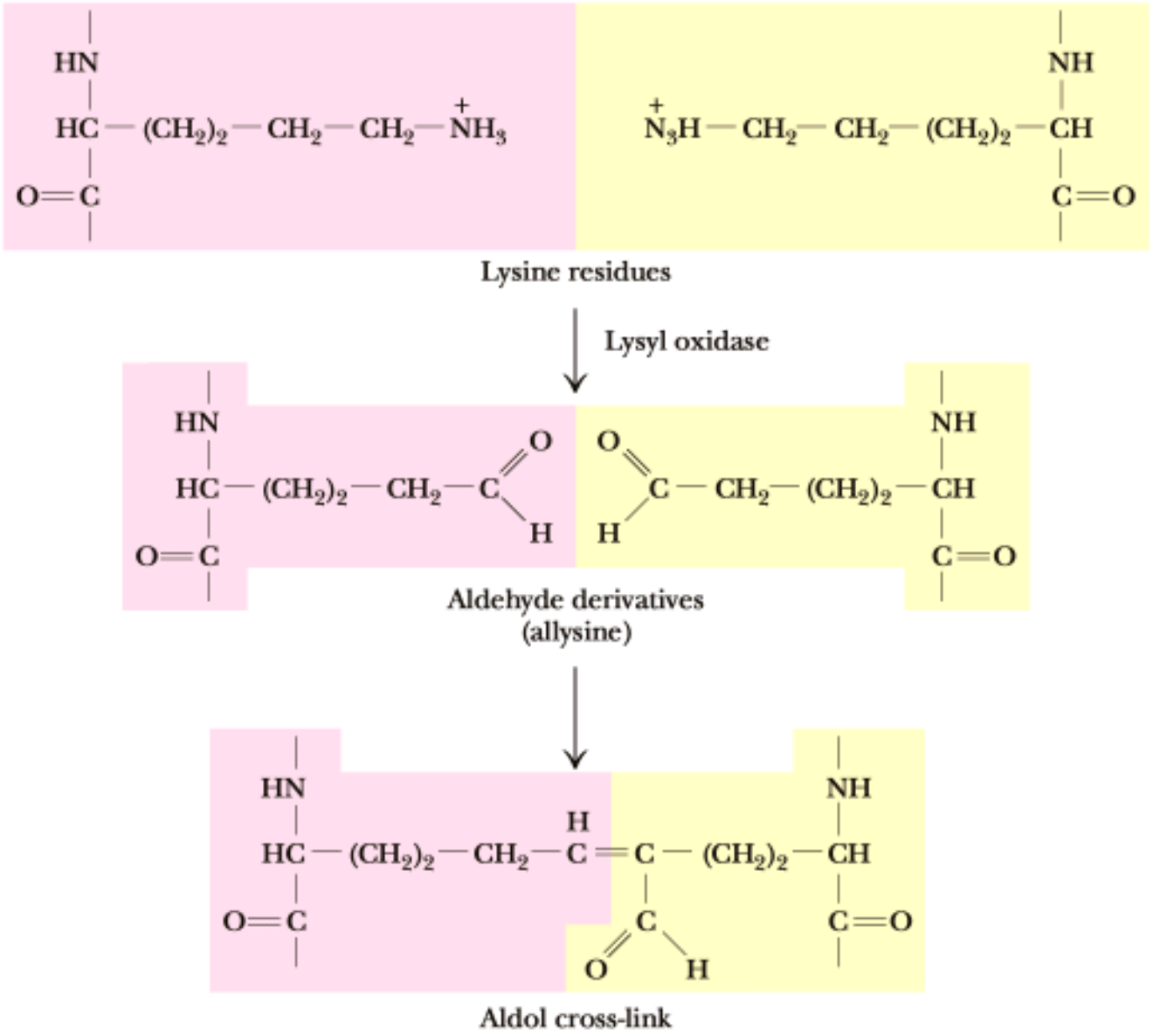
Polypeptide Chains

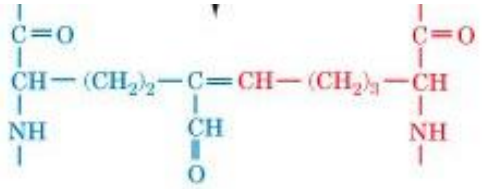
- Triple-helix conformation
  - **Gly must occupy every third residue**
    - Side chain is a hydrogen atom only
    - Small enough to fit inside the helix
  - **Gly aligned with X residue of one chain and Y residue of third chain**
    - Staggered arrangement in helix by one residue
  - **Mutation that causes replacement of Gly leads to defective molecules and disease**
    - Osteogenesis Imperfecta

Polypeptide Chains

- Typically
  - X-position occupied by Proline (Pro)
  - Y-position occupied by Hydroxyproline (Hyp)
- Function
  - Pro and Hyp: triple-helix stability
  - Other
    - Intermolecular binding
    - Binding to extracellular matrix molecules

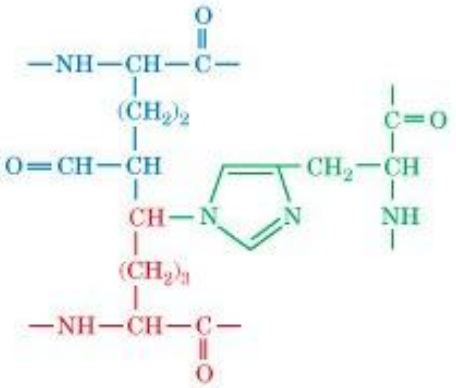
Aldol condensation cross-links in collagen





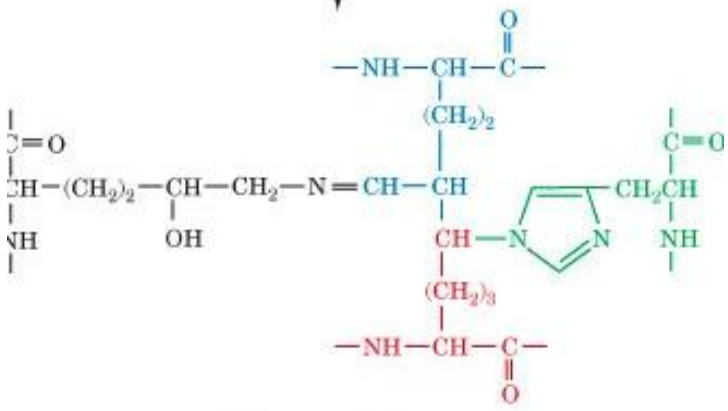
Allysine aldol

His



Aldol-His

5-Hydroxy-Lys

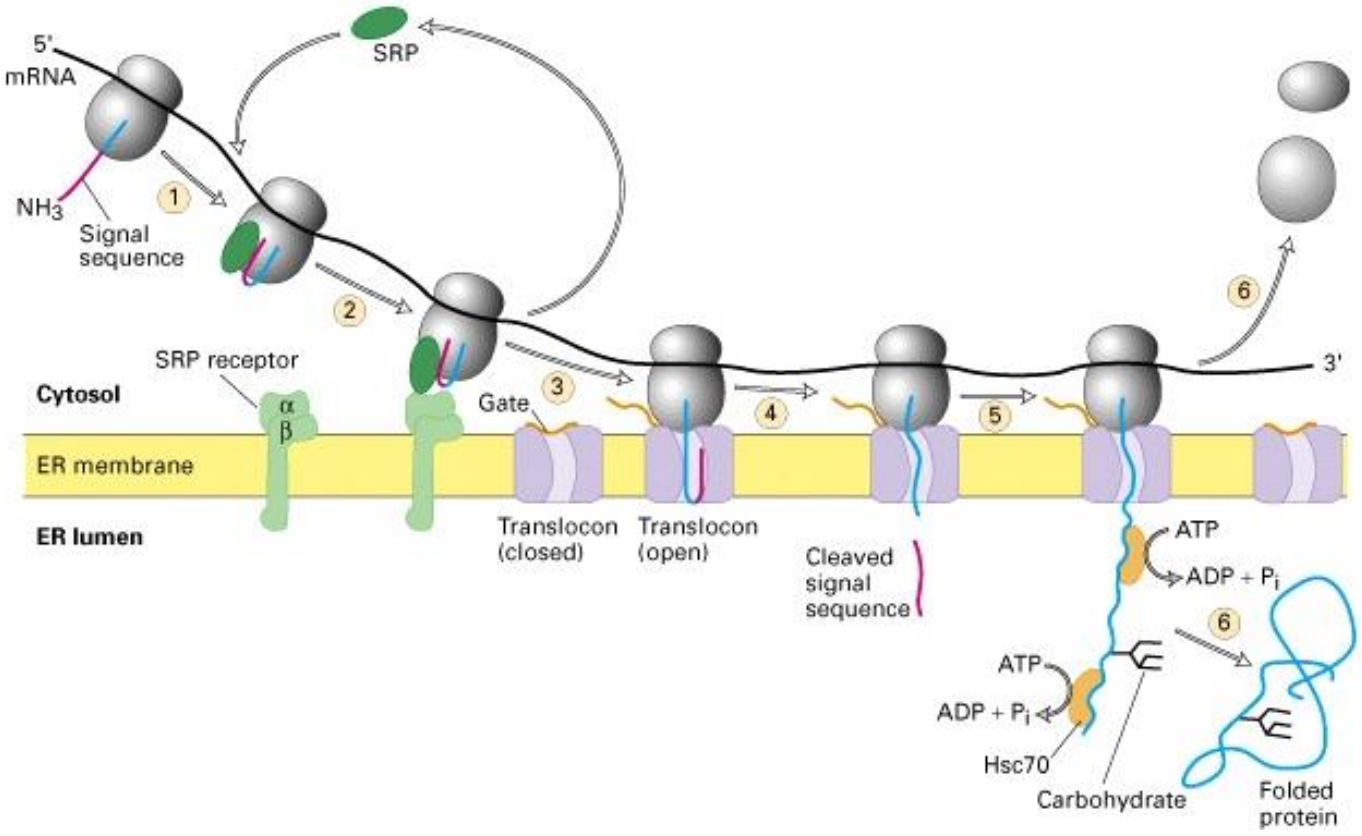


Histidinodehydrohydroxy-
merodesmosine

Formation of crosslinks between Lys, His, and hydroxy-Lys residues in collagen.

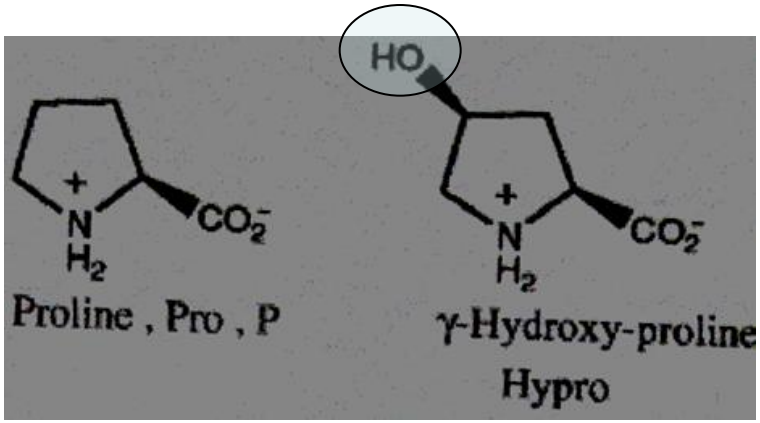
How is collagen made?

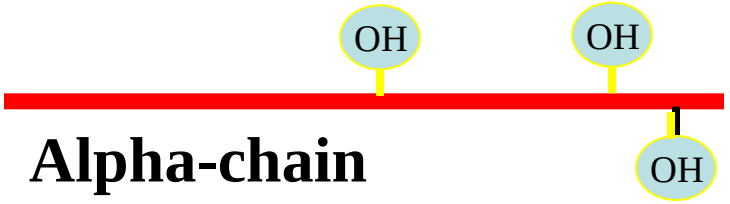
- **STEP 1:** Synthesis of α-chains of pre-procollagen on ribosomes and its transfer to endoplasmic reticulum



How is collagen made?

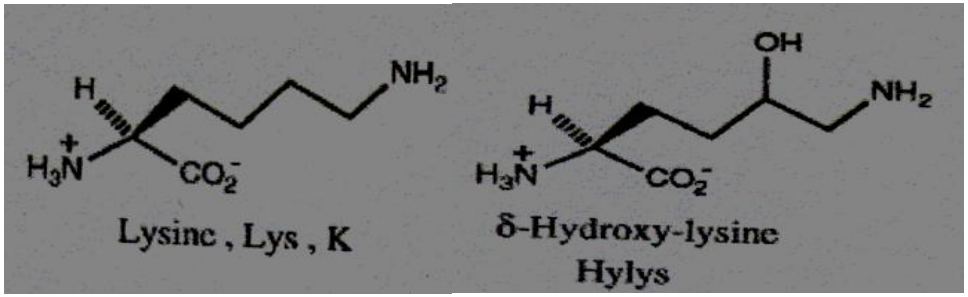
- **STEP 2: Hydroxylation of proline residues to obtain hydroxyproline (an aminoacid unique to collagen).**
  - a reaction that substitutes a hydroxyl group, OH, for a hydrogen atom, H, in the proline
  - the hydroxylation reaction secures the chains in the triple helix of collagen
  - hydroxylation is catalyzed by the enzyme prolyl-4-hydroxylase
  - Vitamin C and α-keto glutarate are essential for enzyme action,

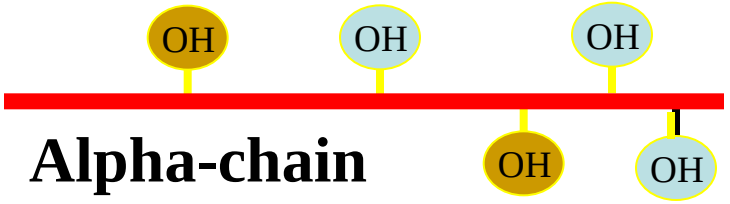




How is collagen made?

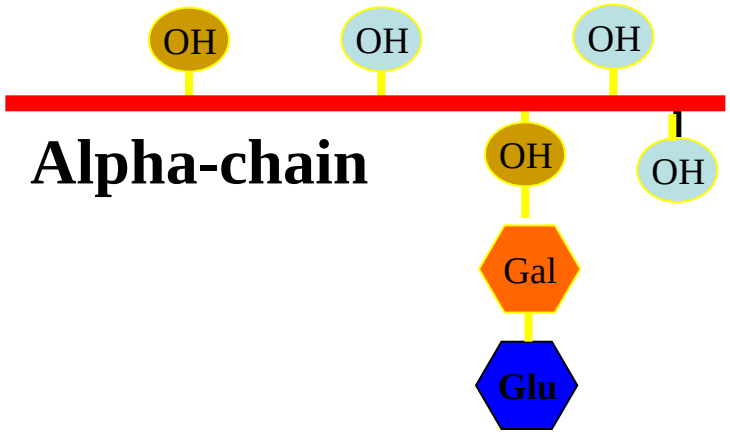
- **STEP 3: Hydroxylation of lysine residues to obtain hydroxylysine**
  - hydroxylysine is needed to permit the cross-linking of the triple helices into the fibers
  - the enzyme peptidyl proline hydroxylase is essential





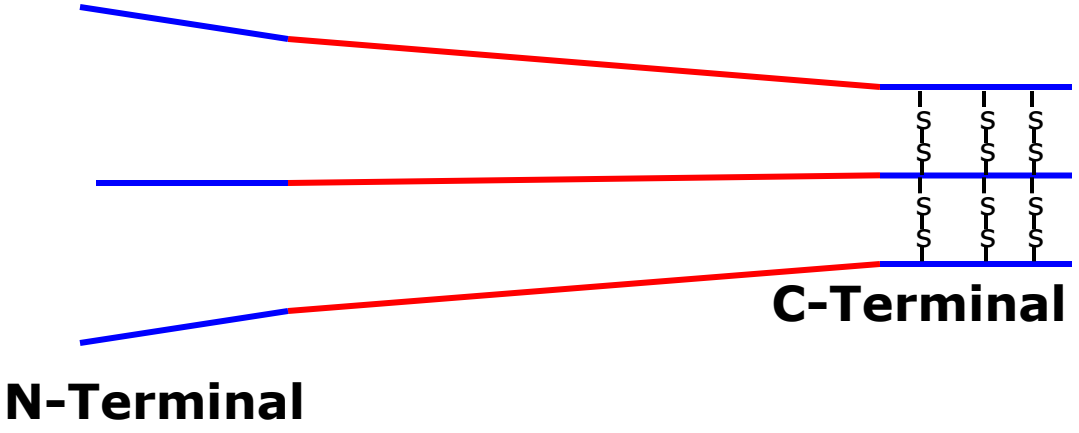
How is collagen made?

- **STEP 4:** Glycosylation of some hydroxlysine residues
  - glucose and galactose are added by enzymes galactosyl transferase and glycosyl transferase



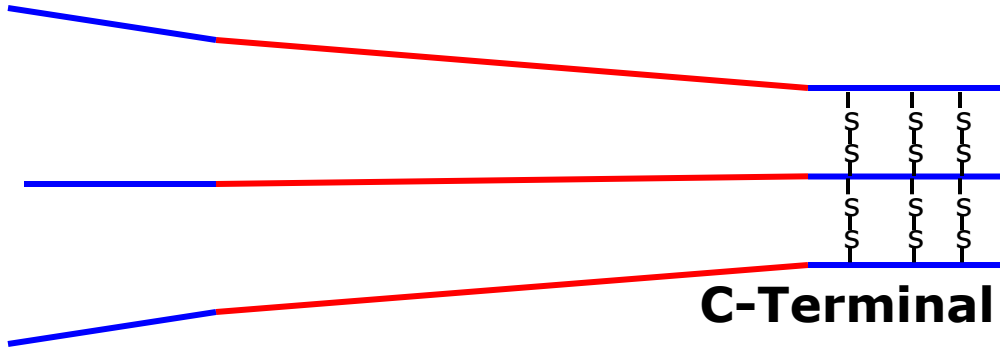
How is collagen made?

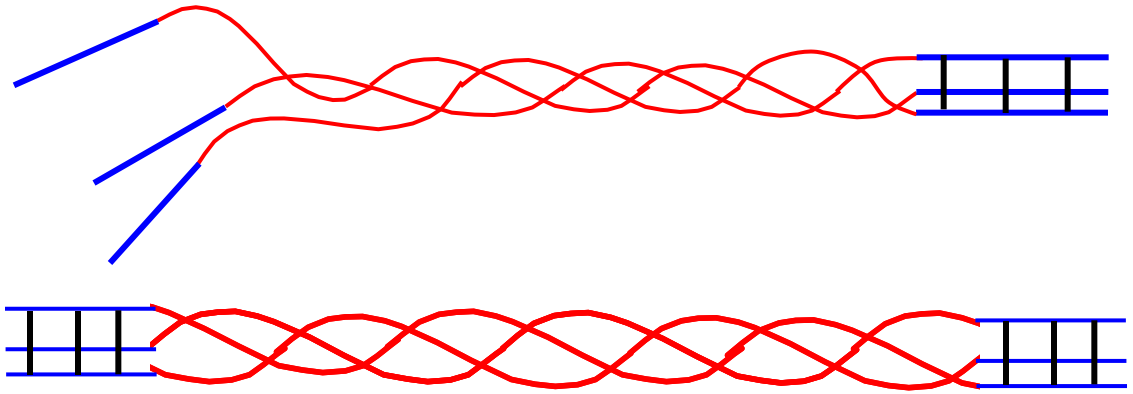
- **STEP 5:** Assembly of the three alpha chains to from procollagen
  - formation of disulphide bonds between parts of the polypeptide chains known as ***registration peptides*** at the C-terminal
  - three chains associate, align and the triple helix forms in a zipper-fashion giving procollagen



How is collagen made?

- **STEP 5 (cont):**

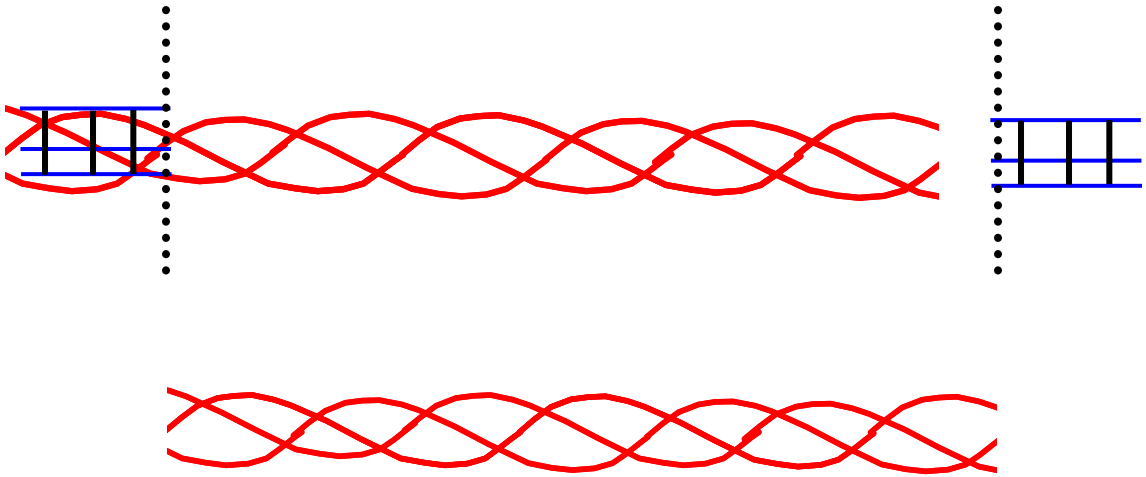




PROCOLLAGEN

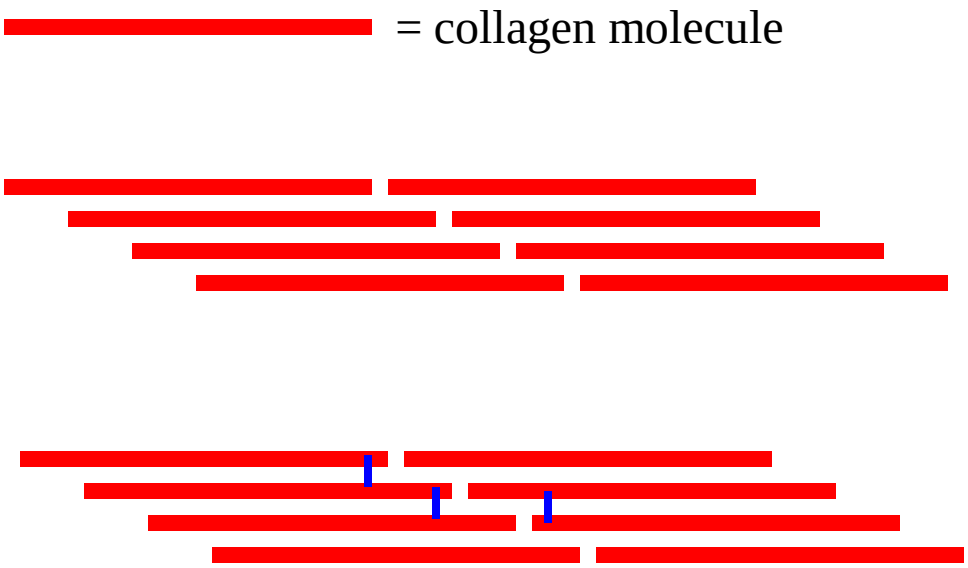
How is collagen made?

- **STEP 6:** Secretion of procollagen molecules by exocytosis into the extra cellular space
- **STEP 7:** Cleavage of registration peptides in the extra cellular space, by procollagen peptidases.
- The resulting molecule is collagen (finally!)



How is collagen made?

- **STEP 8:** Self-assembly or polymerization of collagen molecules form collagen fibrils.
- **STEP 9:** Cross-linkage between adjacent collagen molecules that stabilizes the fibrils.

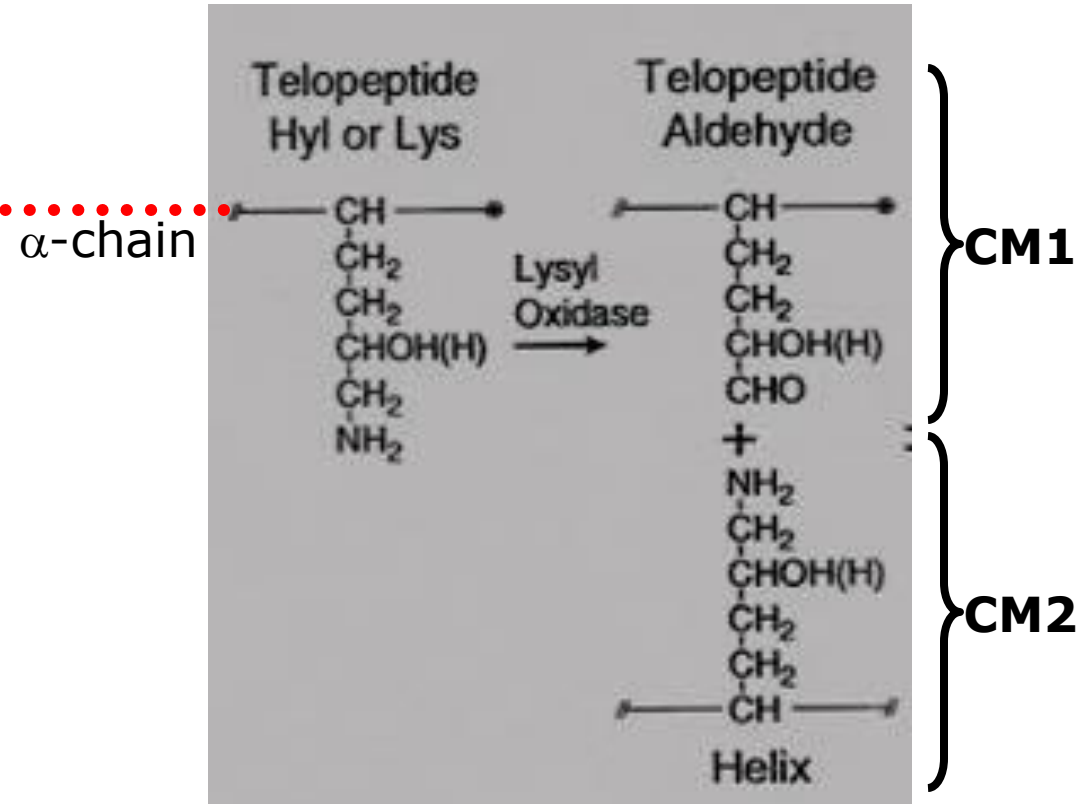


How is collagen made?

- **STEP 9 (cont):**
  - Types of crosslinks based on the number of molecules involved
    - bivalent (reducible): linking the N or C terminal (i.e. telopeptides) of one molecule to the helical region on another
    - trivalent (stable or mature crosslink): linking the N or C terminals of two molecules to the helical region of the third

How is collagen made?

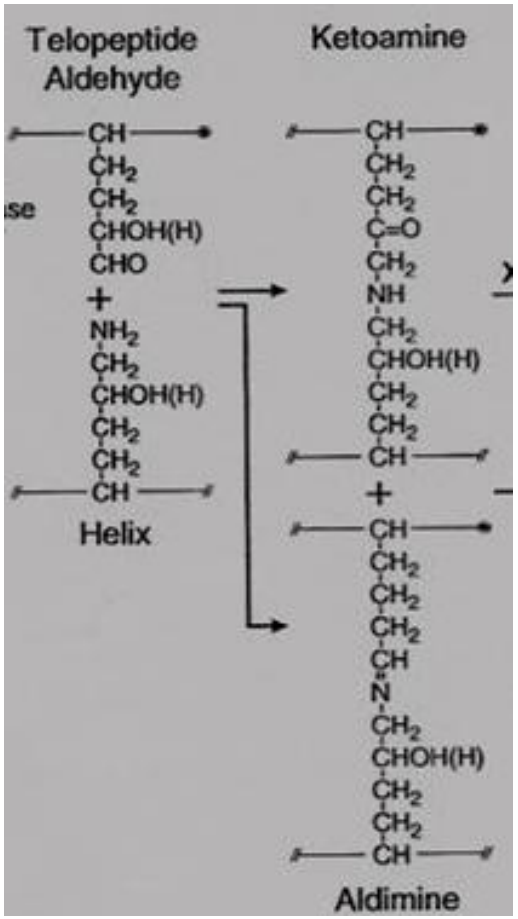
- **STEP 9 (cont):**



- Lysyl oxidase deamines (removes NH_2) the lysine or hydroxylysine residue on the telopeptide of collagen molecule 1 (CM1) ➔ aldehyde forms
- Aldehyde links with the lysine or hydroxylysine residue on the helical region of another collagen molecule (CM2) to form bivalent crosslink (see the next slide)

How is collagen made?

- **STEP 9 (cont):** Bivalent crosslinks



CM1+CM2

Ketoamine crosslink

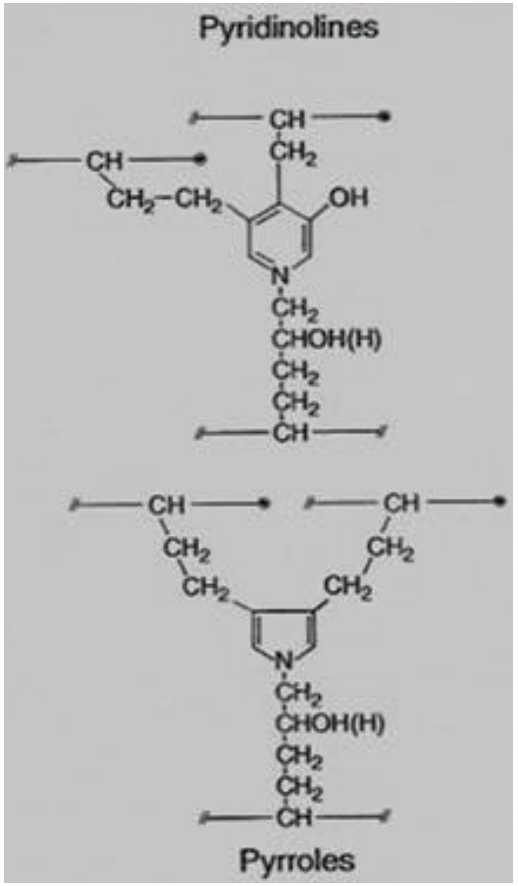
<u>OR</u>

CM1+CM2

Aldimine crosslink

How is collagen made?

- **STEP 9 (cont):** A third telopeptide can be added to the bivalent xlink to obtain stable trivalent xlink



CM1+CM2+CM3

Pyridinoline (lysylpyridinoline if helical residue is lysyl; hydroxypyridinoline if helical residue is hydroxylysl

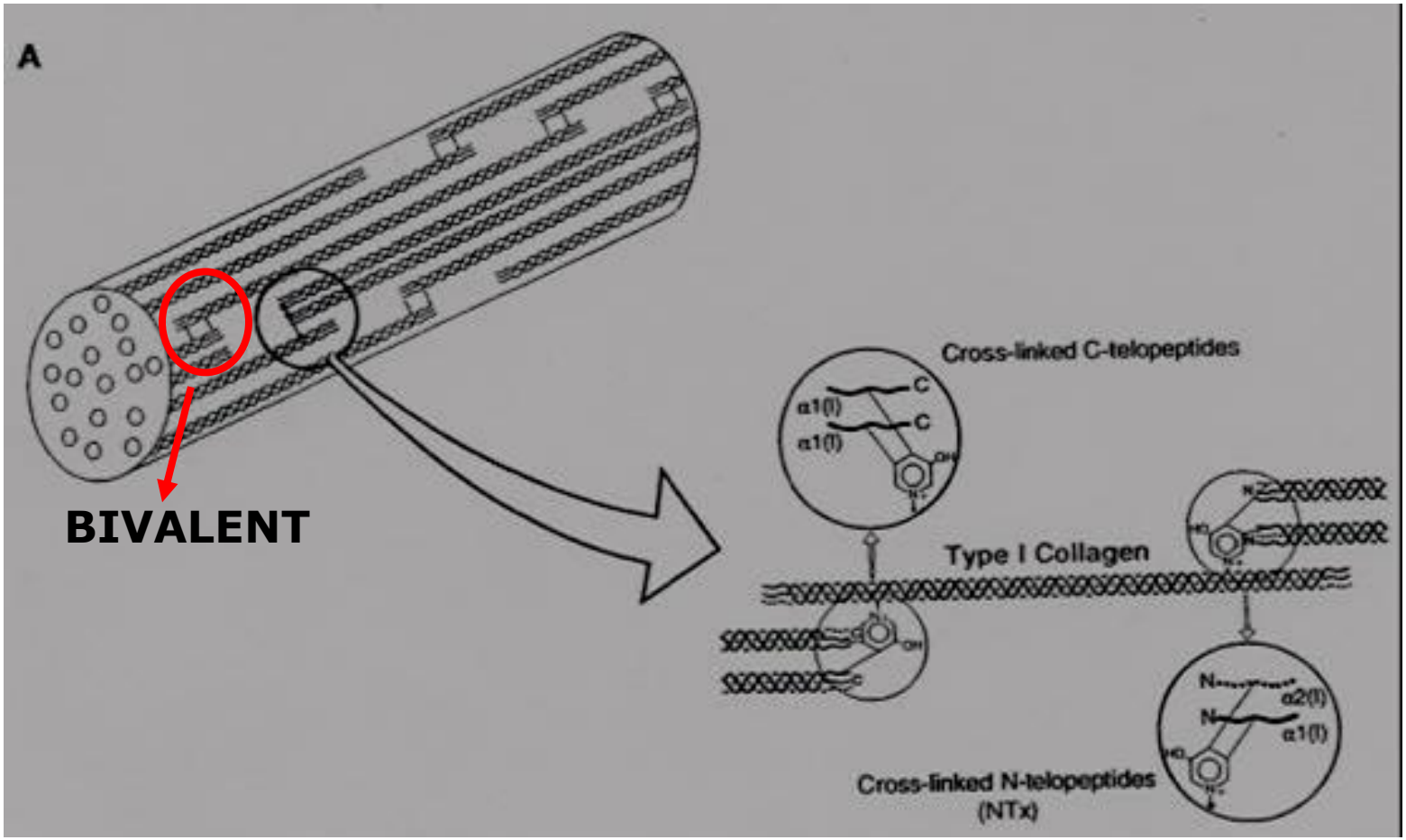
<u>OR</u>

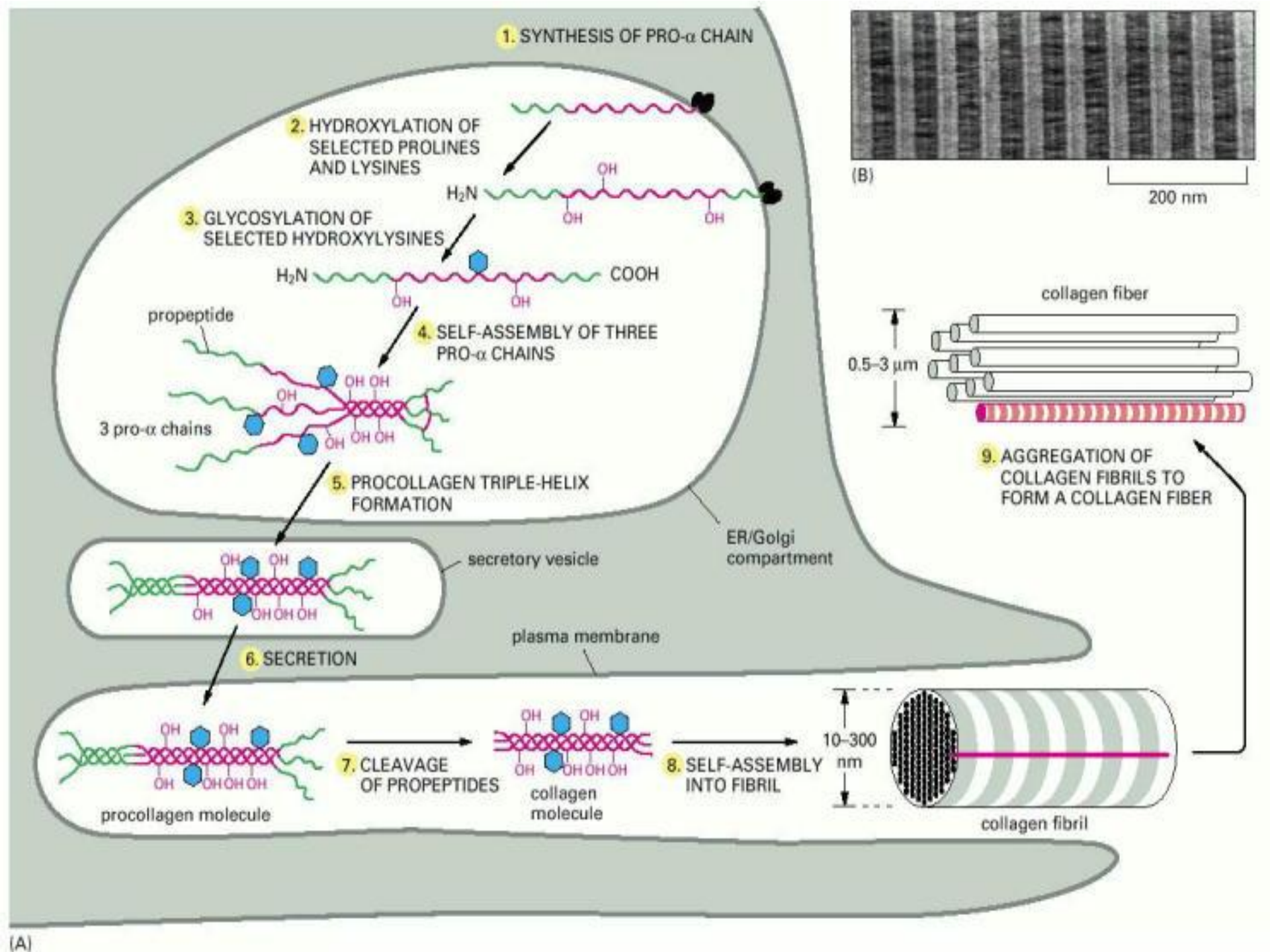
CM1+CM2+CM3

Pyrrole crosslink

How is collagen made?

- **STEP 9 (cont):** How it looks in 3D…




1. SYNTHESIS OF PRO-α CHAIN
2. HYDROXYLATION OF SELECTED PROLINES AND LYSINES
3. GLYCOSYLATION OF SELECTED HYDROXYLYSINES
4. SELF-ASSEMBLY OF THREE PRO-α CHAINS
5. PROCOLLAGEN TRIPLE-HELIX FORMATION
6. SECRETION
7. CLEAVAGE OF PROPEPTIDES
8. SELF-ASSEMBLY INTO FIBRIL
9. AGGREGATION OF COLLAGEN FIBRILS TO FORM A COLLAGEN FIBER
propeptide
3 pro-α chains
secretory vesicle
ER/Golgi compartment
plasma membrane
procollagen molecule
collagen molecule
collagen fibril
collagen fiber
0.5–3 μm
10–300 nm
(A)
(B)
200 nm

Steps of tropocollagen synthesis

-Inside the cell

In the cytoplasm, Syntheis of peptide chains in ribosomes and their transfer along the Rough Endoplasmic Reticulum (RER)

Inside RER, Peptide chains are sent into the lumen of the RER ,

- **Cleavage of signal peptides.**
- **Hydroxylation of Y- prolyl and some Y-lysyl .**
- **Glycosylation of specific hydroxy lysyl residues.**
- **Formation of intra and inter chain disulfide bonds.**
- **Formation of the helical structure starting from the C-terminal.**

Inside Golgi apparatus, Procollagen is shipped to the Golgi apparatus, where it is packaging and secretion of the procolagen.

- **Outside the cell**
  - **Cleavage of pro sequence .**

Formation of fibrils followed by fibers.

Assembly of collagen fibers in quarter-staggered alignment.

Collagen is attached to cell membranes via several types of protein, including fibronectin and integrin .

Disorders of Collagen Deposition

- Disorders of collagen deposition
  - insufficient collagen content
  - presence of chemically and/or morphologically abnormal collagen
  - excessive collagen content
  - insufficient collagen resorption
  - excessive collagen resorption

Disorders of Collagen Deposition

- Genetic abnormalities of collagen
  - mutations that lead to aminoacid deletions or additions
  - deficient synthesis of a portion
  - disorders in post-translational modification (hydroxylation of lysine, hydroxylation of proline)
  - defects in enzymes essential for post-translational modification

Disorders of Collagen Deposition

- Collagen is the building block; thus, its disorders lead to significant deterioration in the mechanical integrity of tissues
- Several disorders
  - Ehlers-Danlos syndrome (ED)
  - Osteogenesis Imperfecta (OI)
  - Marfan syndrome (MS)

Ehlers-Danlos

- Clinical manifestations
  - Joint hypermobility
  - skin hyperextensibility
  - skin tends to split with minor trauma
  - nodules
  - tendency to bruise

Ehlers-Danlos: Type I

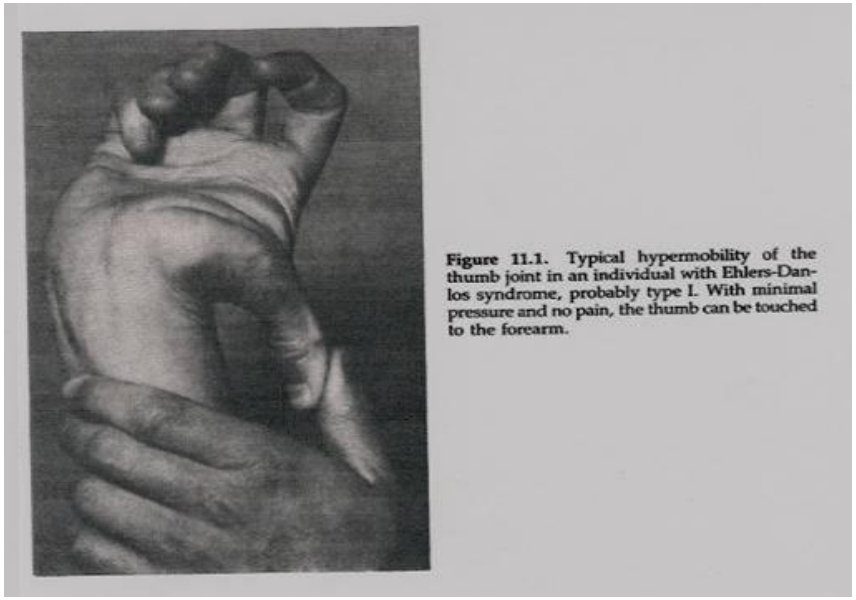


Figure 11.1. Typical hypermobility of the thumb joint in an individual with Ehlers-Danlos syndrome, probably type I. With minimal pressure and no pain, the thumb can be touched to the forearm.

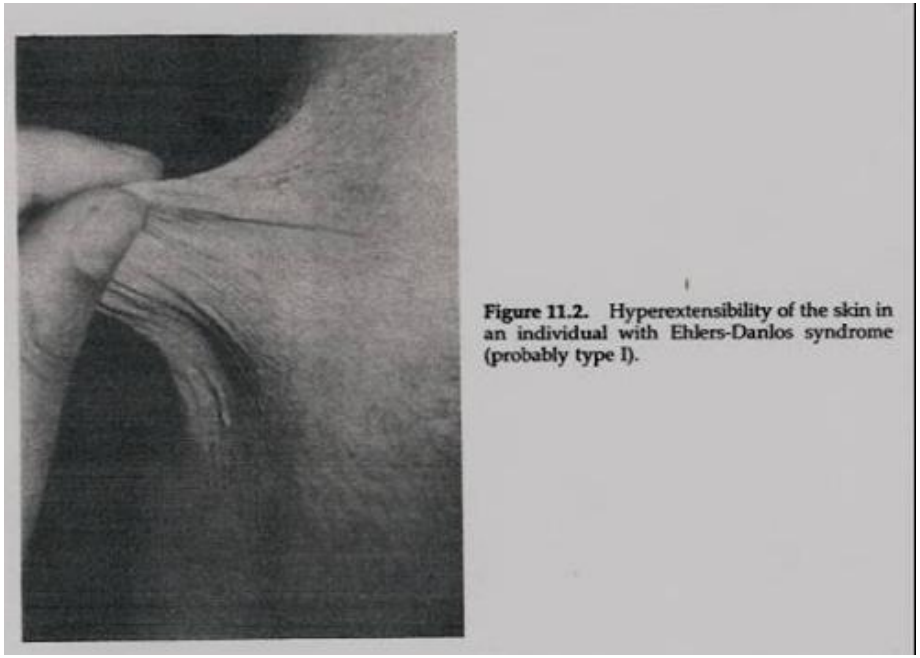


Figure 11.2. Hyperextensibility of the skin in an individual with Ehlers-Danlos syndrome (probably type I).

Ehlers-Danlos: Type I

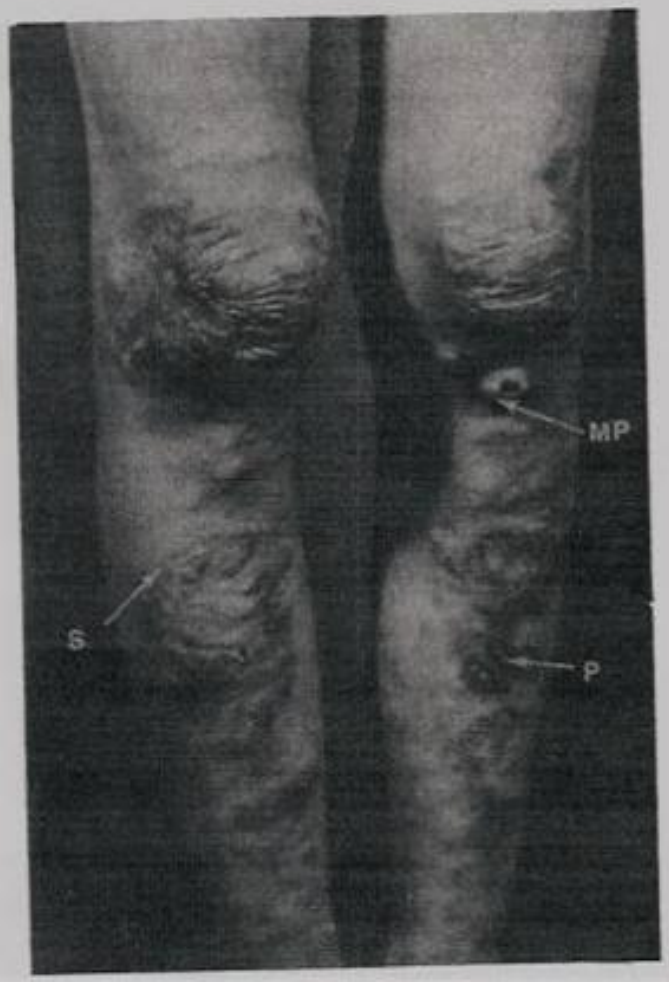
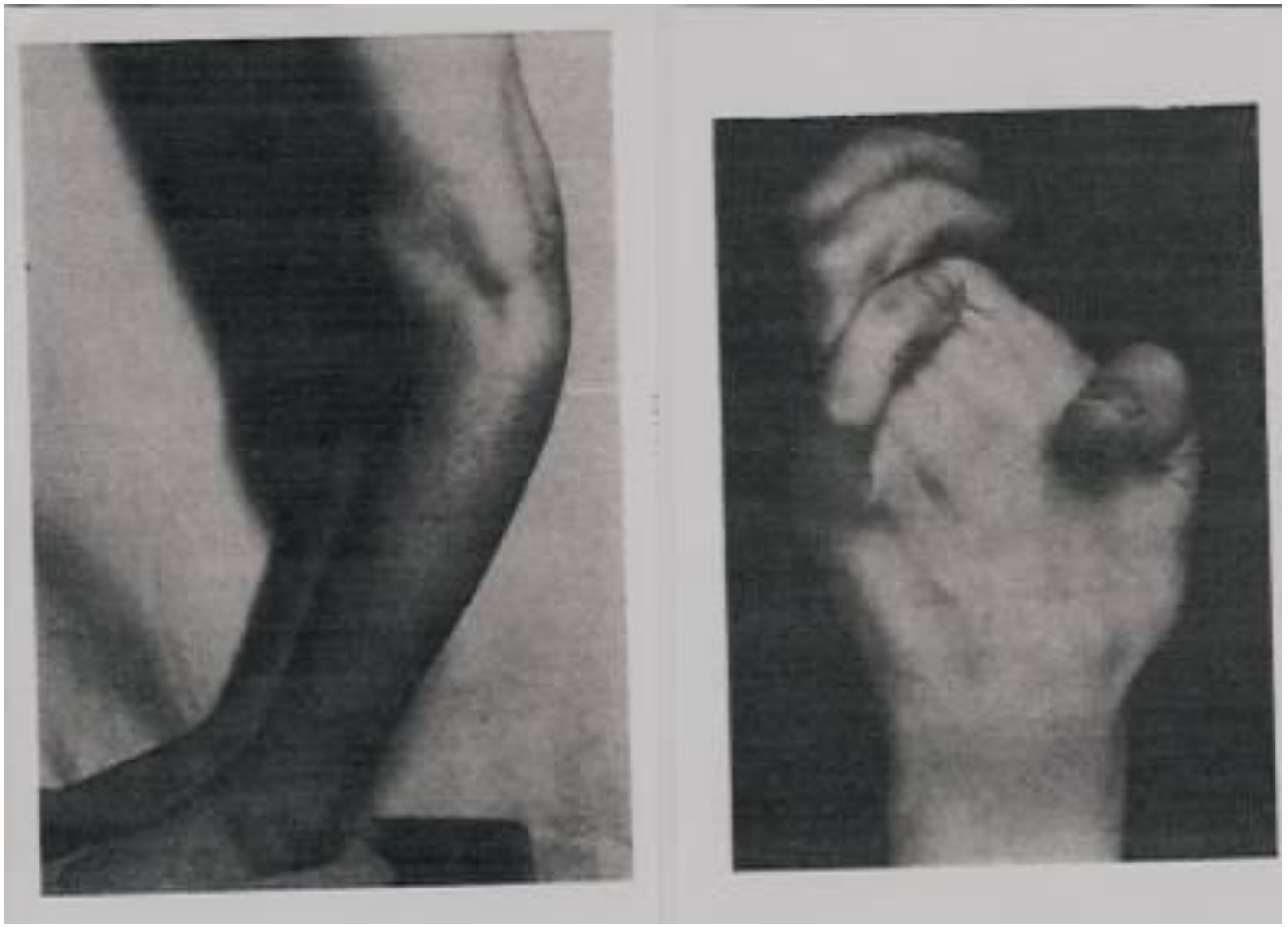


Figure 11.4. Extreme changes in the skin and subcutaneous tissues in a girl with type I Ehlers-Danlos syndrome. Note thin scar (*S*) and pigmentation (*P*) as a result of old hemorrhage, and markedly thin skin over the knees and nodules consistent with molluscoid pseudotumors (*MP*).

Ehlers-Danlos



Osteogenesis Imperfecta

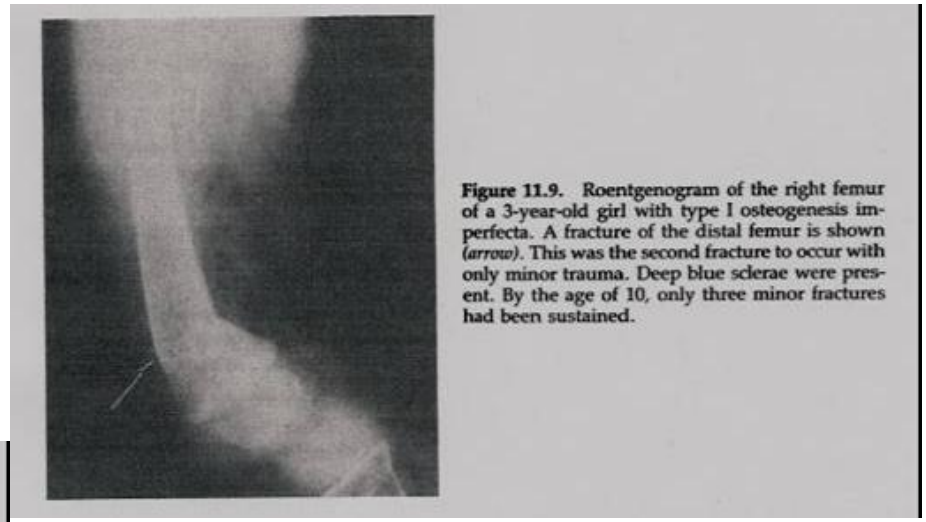


Figure 11.9. Roentgenogram of the right femur of a 3-year-old girl with type I osteogenesis imperfecta. A fracture of the distal femur is shown *(arrow)*. This was the second fracture to occur with only minor trauma. Deep blue sclerae were present. By the age of 10, only three minor fractures had been sustained.

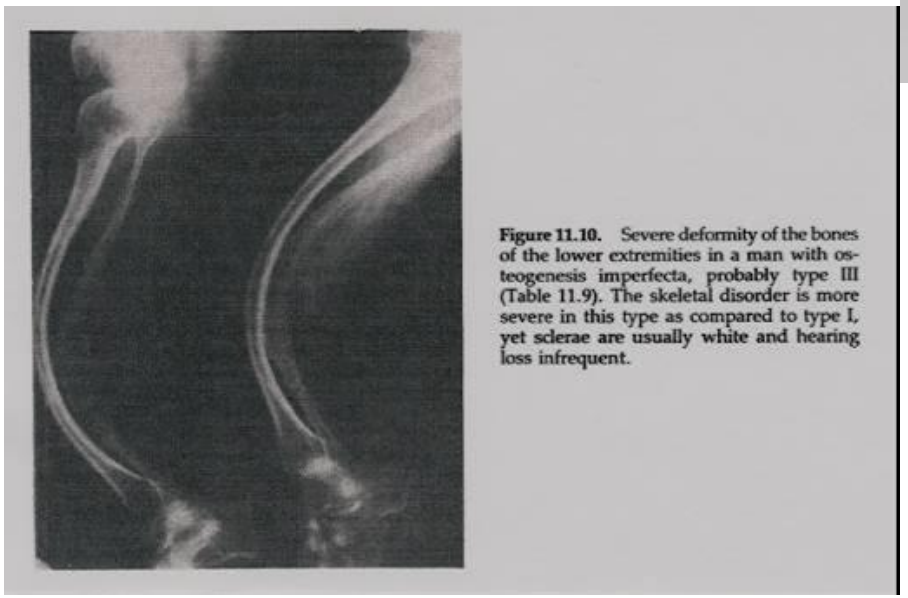


Figure 11.10. Severe deformity of the bones of the lower extremities in a man with osteogenesis imperfecta, probably type III (Table 11.9). The skeletal disorder is more severe in this type as compared to type I, yet sclerae are usually white and hearing loss infrequent.

Marfan Syndrome

- Clinical appearance:
  - long extremities
  - short trunk
  - chest is deformed
  - fingers are long and thin
- Defect in collagen crosslinks
  - misalignment of collagen molecules in collagen fibrils