Biochemistry of Connective Tissue

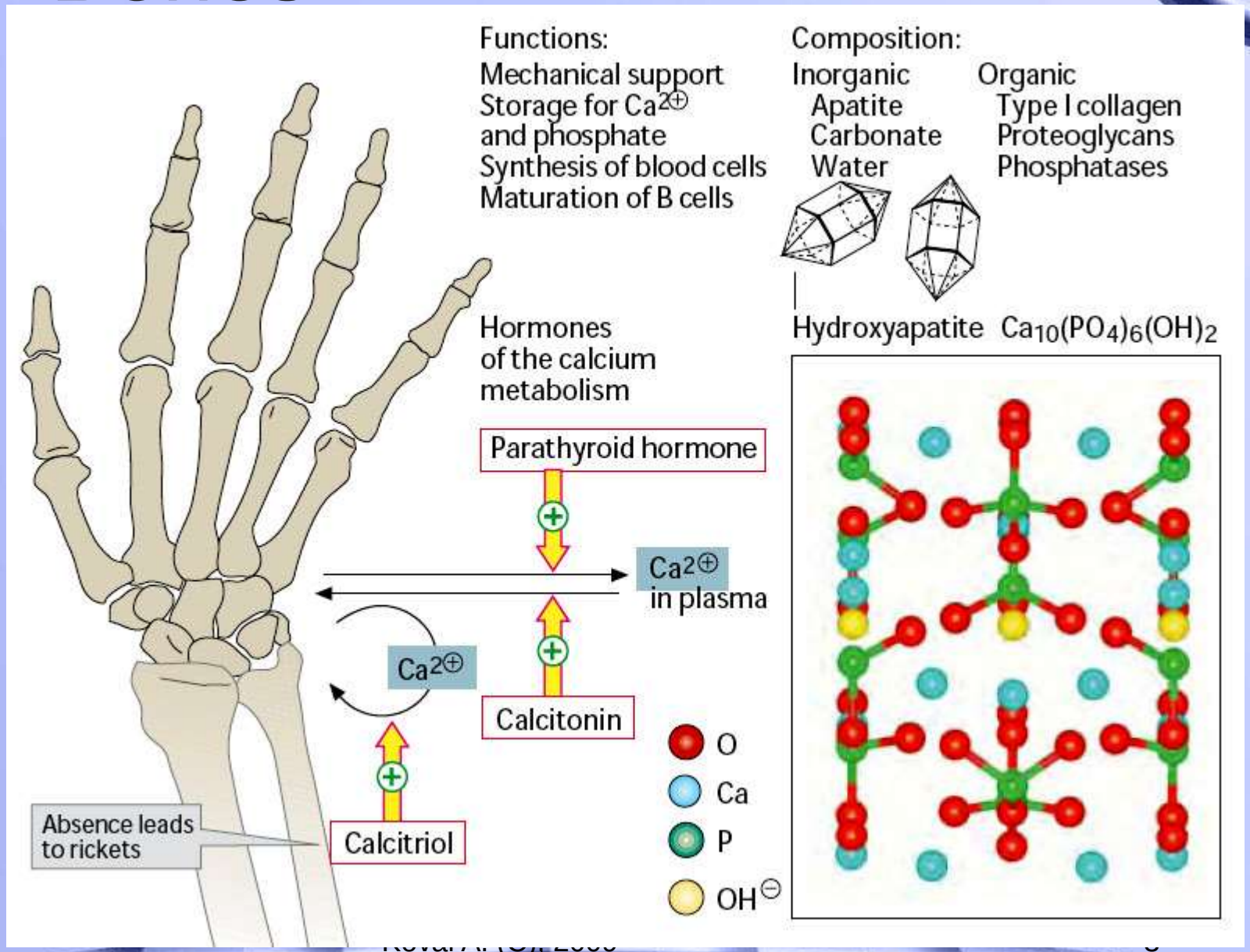
Lecture # 34

Lecturer Alexander Koval

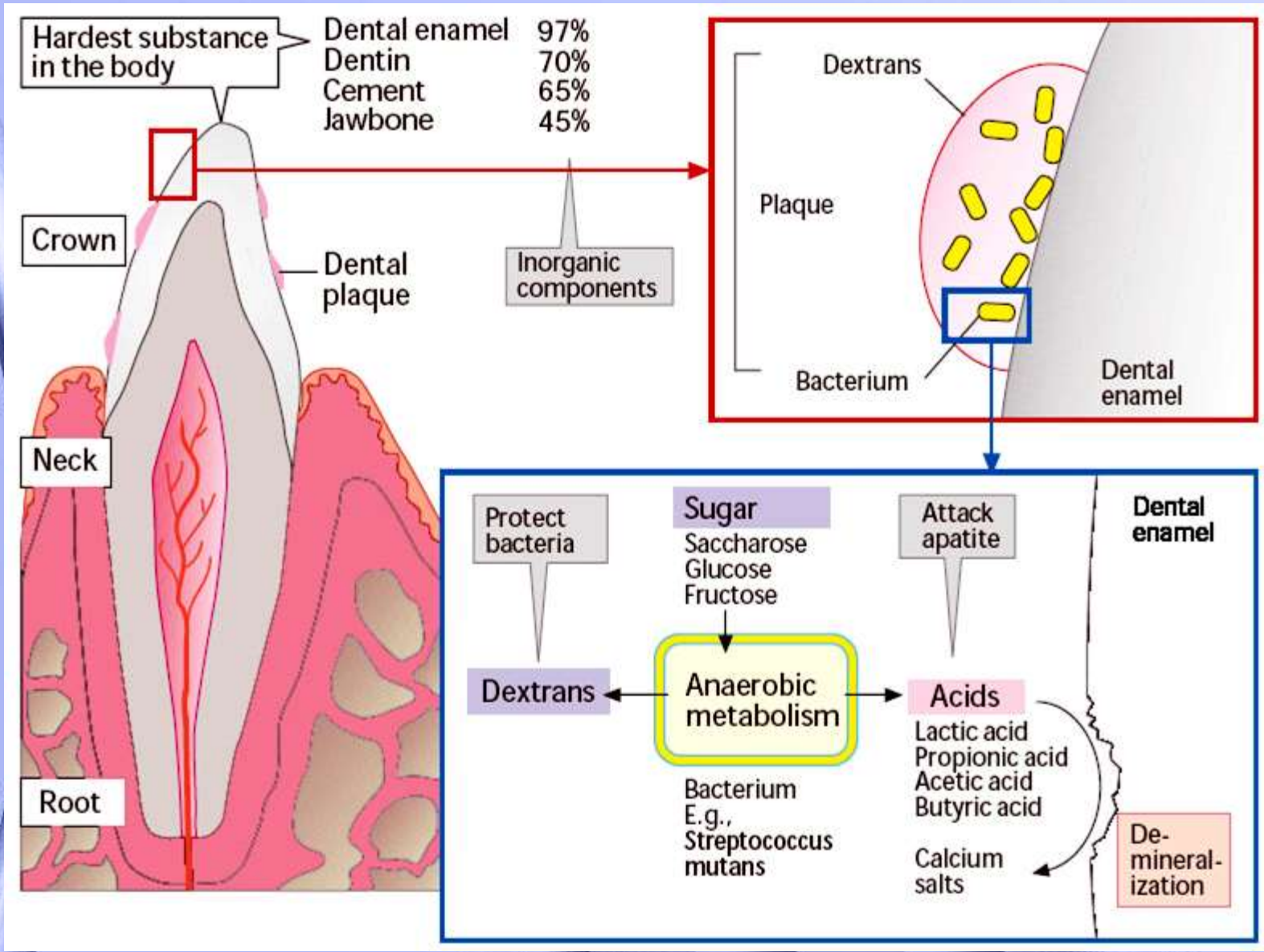
Introduction

- The family of connective-tissue cells includes **fibroblasts, chondrocytes** (cartilage cells), and **osteoblasts** (bone-forming cells).
- They are specialized to secrete extracellular proteins, particularly collagens, and mineral substances, which they use to build up the **extracellular matrix**.
- By contrast, **osteoclasts** dissolve bone matter again by secreting H^+ and collagenases.

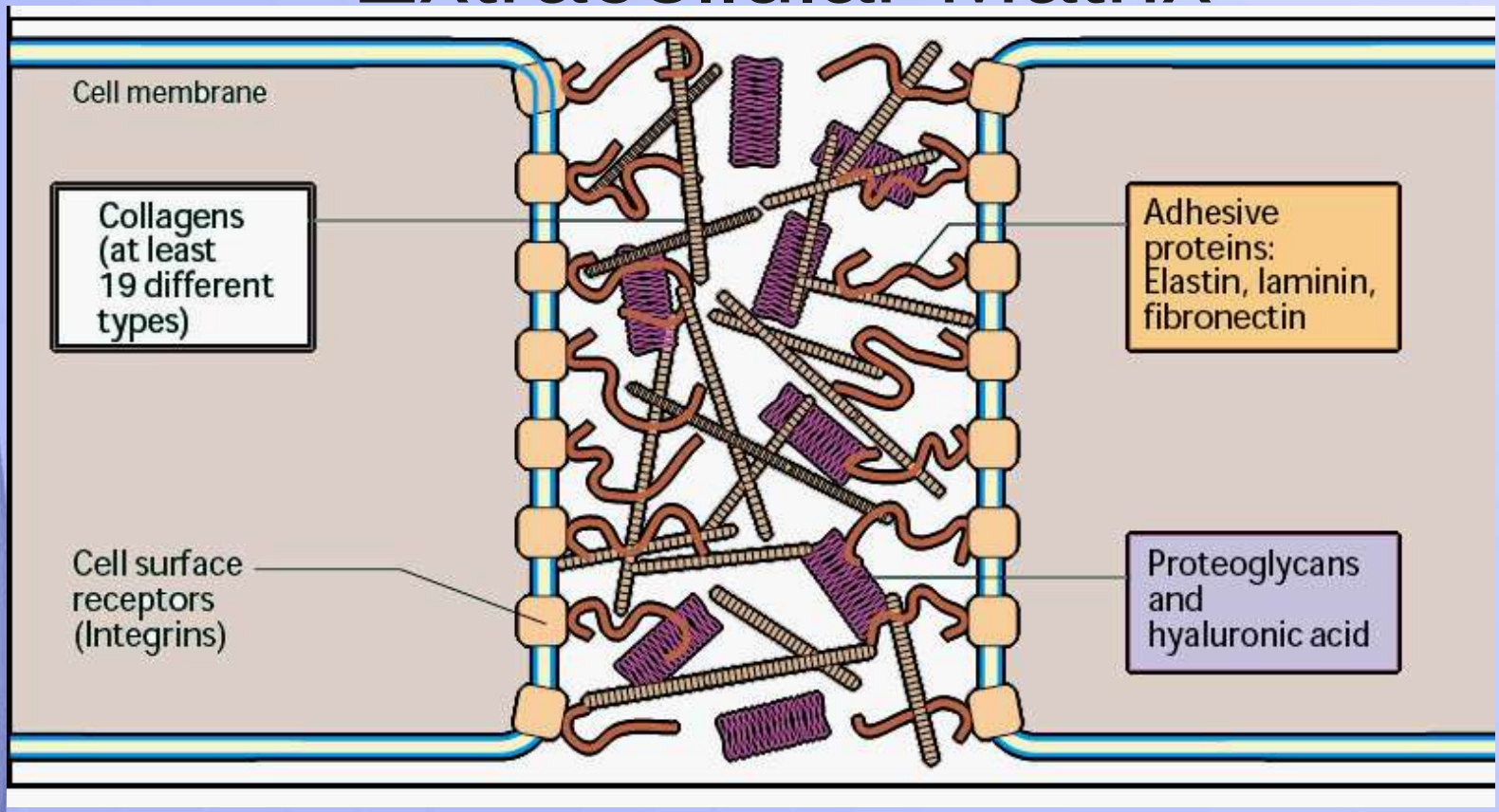
Bones


Functions:
Mechanical support
Storage for $Ca^{2\oplus}$ and phosphate
Synthesis of blood cells
Maturation of B cells
Composition:
Inorganic
Apatite
Carbonate
Water
Organic
Type I collagen
Proteoglycans
Phosphatases
Hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$
Hormones of the calcium metabolism
Parathyroid hormone
$Ca^{2\oplus}$ in plasma
$Ca^{2\oplus}$
Calcitonin
Calcitriol
Absence leads to rickets
O
Ca
P
$OH^{\ominus}$

Teeth


Hardest substance in the body
Dental enamel 97%
Dentin 70%
Cement 65%
Jawbone 45%
Crown
Dental plaque
Inorganic components
Neck
Root
Dextrans
Plaque
Bacterium
Dental enamel
Protect bacteria
Sugar
Saccharose
Glucose
Fructose
Attack apatite
Dental enamel
Dextrans
Anaerobic metabolism
Acids
Lactic acid
Propionic acid
Acetic acid
Butyric acid
Bacterium
E.g.,
Streptococcus mutans
Calcium salts
De-mineral-ization

Extracellular Matrix

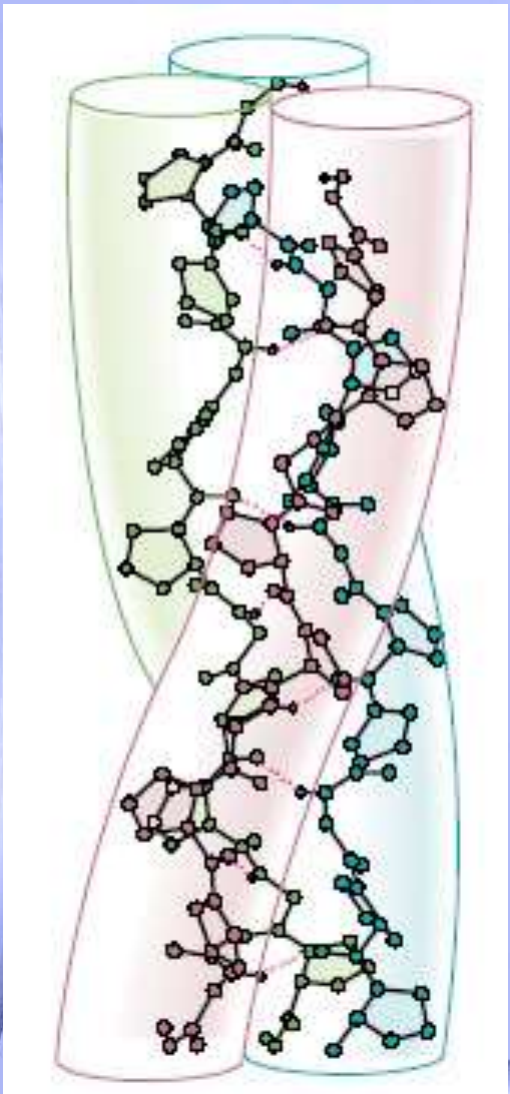


- The extracellular matrix (ECM) is a complex structural entity surrounding and supporting cells that are found within mammalian tissues.
- The ECM is often referred to as the connective tissue.

Extracellular Matrix (cont'd)

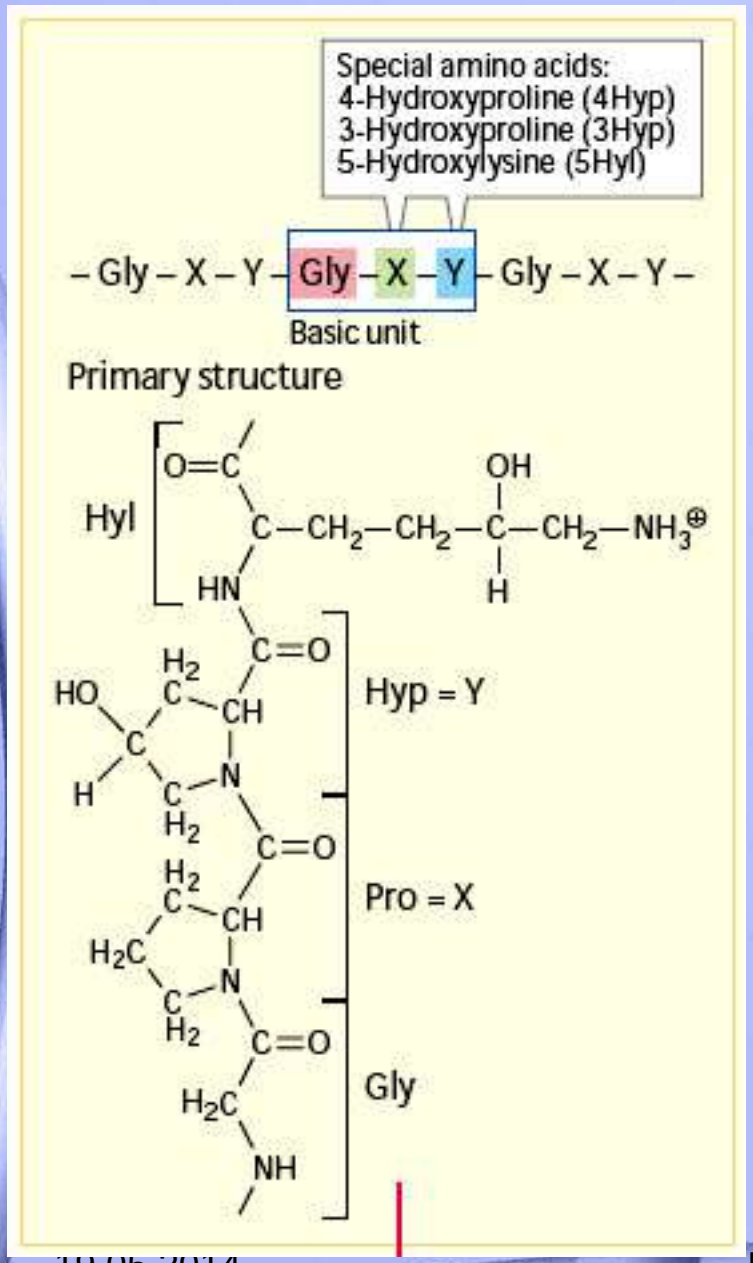
- The ECM is composed of 3 major classes of biomolecules:
  1. Structural proteins: collagen and elastin.
  2. Specialized proteins: e.g. fibrillin, fibronectin, and laminin.
  3. Proteoglycans: these are composed of a protein core to which is attached long chains of repeating disaccharide units termed of glycosaminoglycans (GAGs) forming extremely complex high molecular weight components of the ECM.

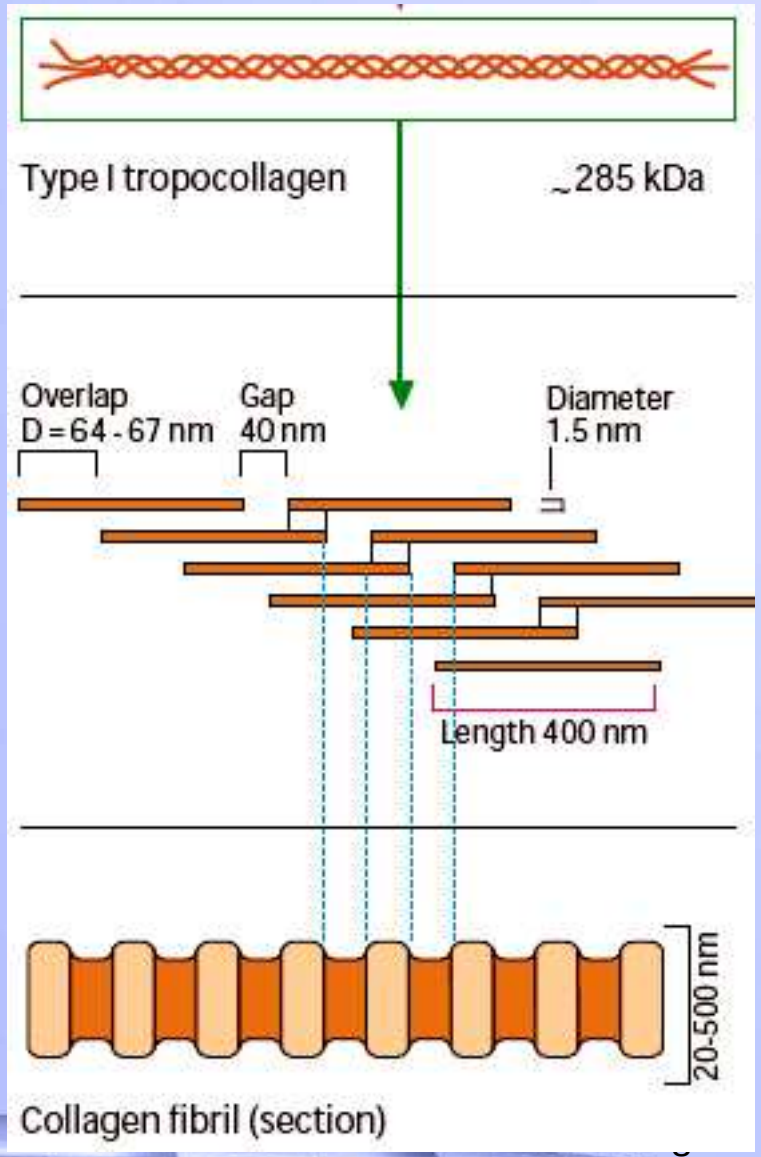
Structure of Collagens



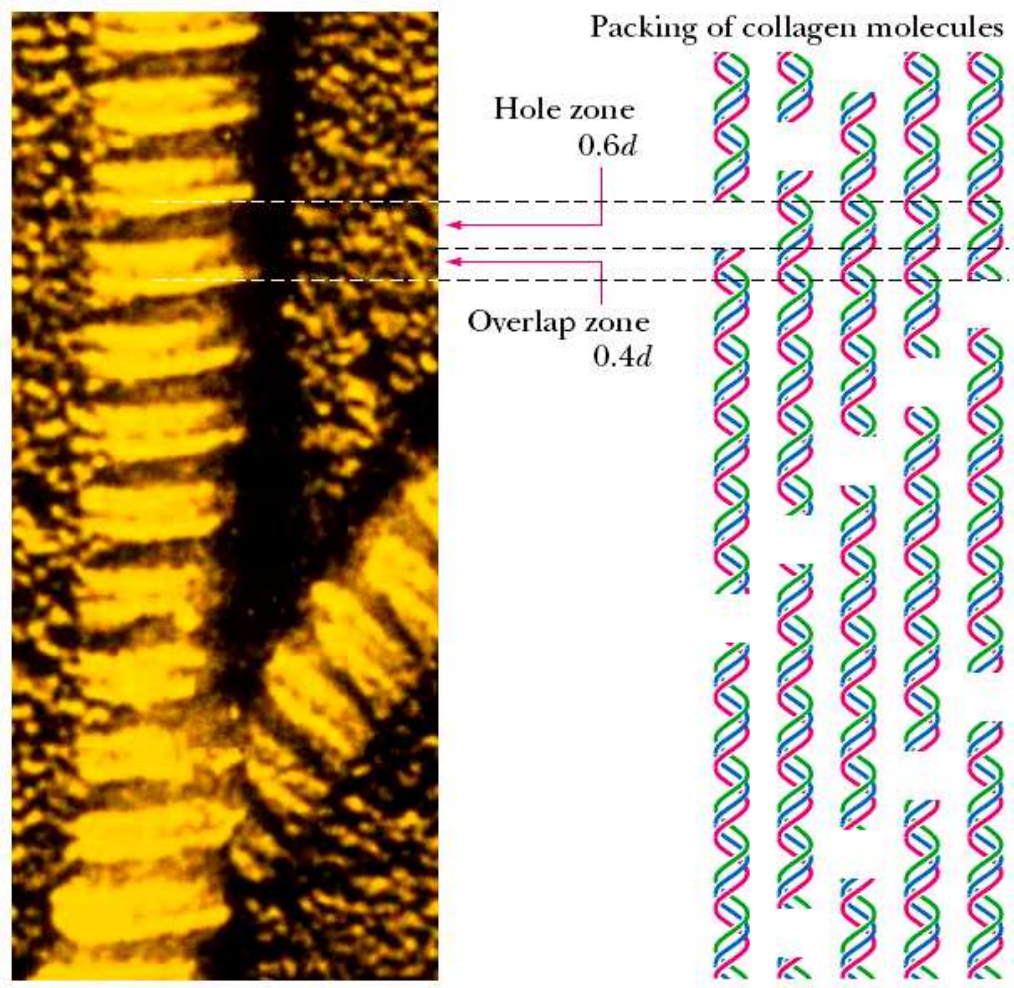
- The fundamental higher order structure of collagens is a long and thin diameter rod-like protein.
  - Type I collagen for instance is 300nm long, 1.5nm in diameter and consists of 3 coiled subunits composed of two α1(I) chains and one α2(I) chain.
  - Each chain consists of 1050 amino acids wound around each other in a characteristic right-handed triple helix.
    - There are 3 amino acids per turn of the helix and every third amino acid is a G.
  - Collagens are also rich in proline and hydroxyproline. The bulky pyrollidone rings of proline reside on the outside of the triple helix.

Collagens





Formation of Collagen Fibrils



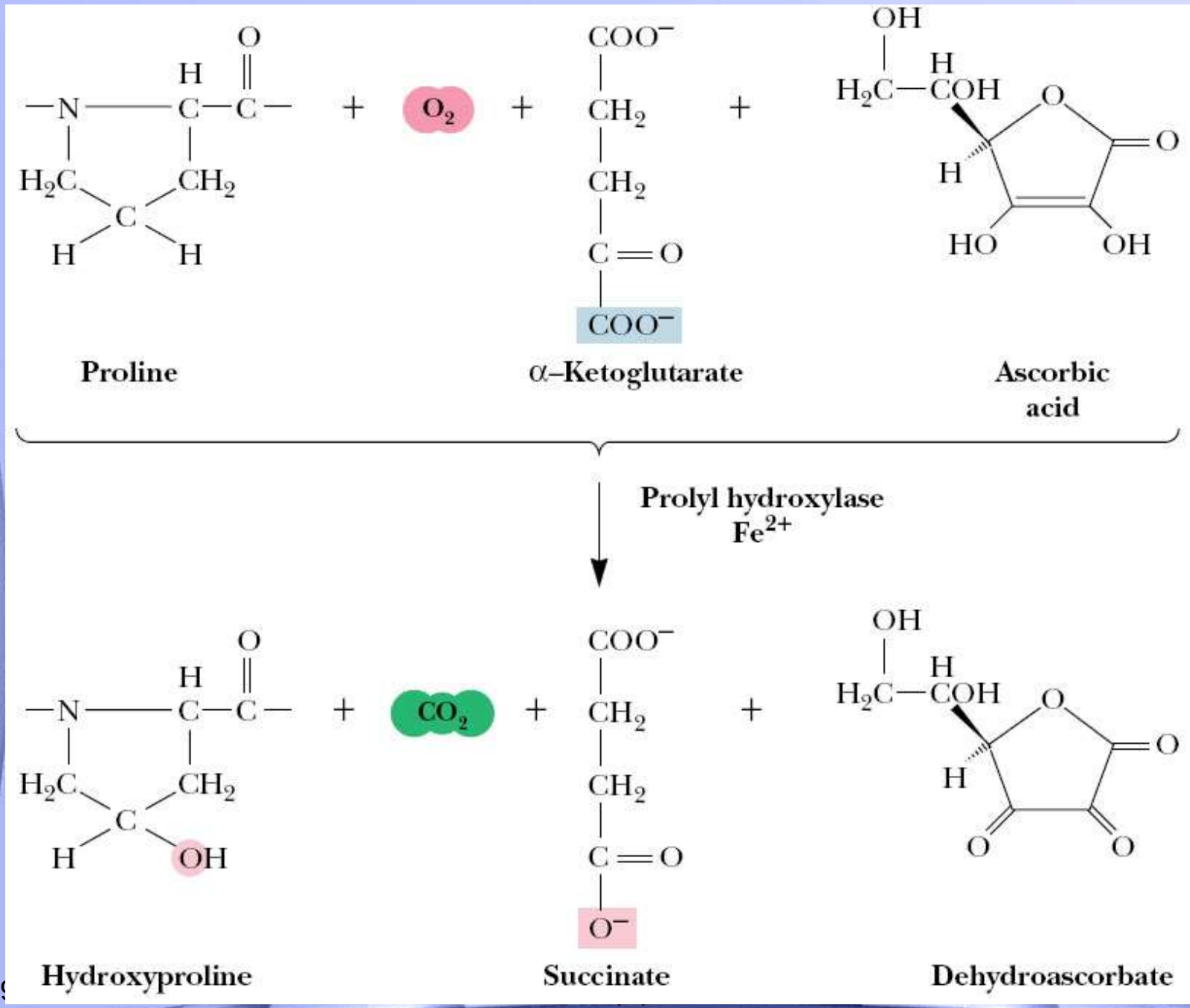
- Lateral interactions of triple helices of collagens result in the formation of fibrils roughly 50nm diameter.
- The packing of collagen is such that adjacent molecules are displaced approximately 1/4 of their length (67nm).
  - This staggered array produces a striated effect that can be seen in the electron microscope.

Procollagens

- Collagens are synthesized as longer precursor proteins called **procollagens**.
- Type I procollagen contains an additional 150 amino acids at the N-terminus and 250 at the C-terminus.
  - These pro-domains are globular and form multiple intrachain disulfide bonds.
  - The disulfides stabilize the proprotein allowing the triple helical section to form.

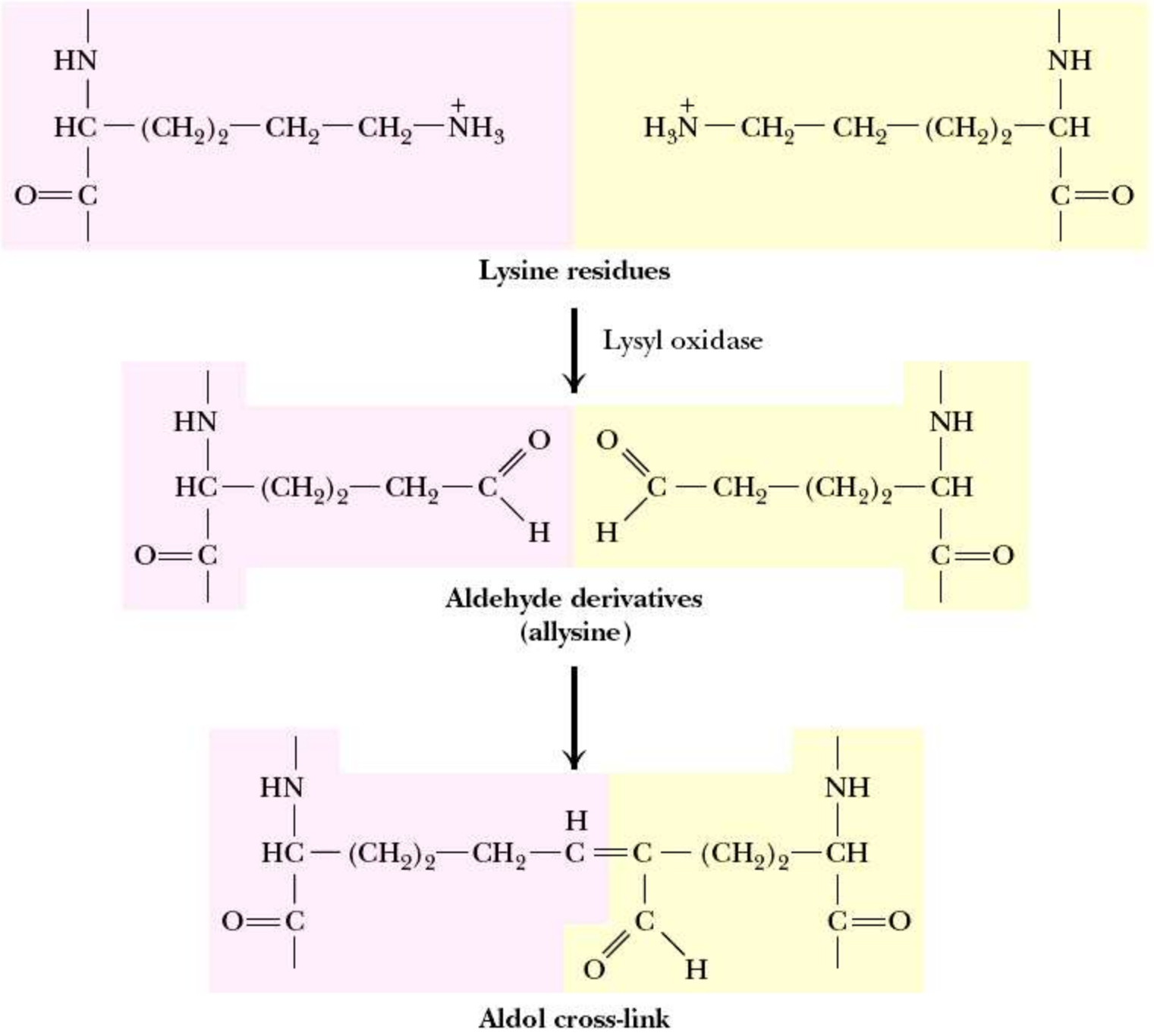
Collagen processing (1)

- Collagen fibers begin to assemble in the ER and Golgi complexes.
- The signal sequence is removed and numerous modifications take place in the collagen chains.
  - Specific proline residues are hydroxylated by prolyl 4-hydroxylase and prolyl 3-hydroxylase.
  - Specific lysine residues also are hydroxylated by lysyl hydroxylase.
  - Both prolyl hydraoxylases are **absolutely dependent upon vitamin C** as co-factor.
  - Glycosylations of the O-linked type also occurs during Golgi transit.

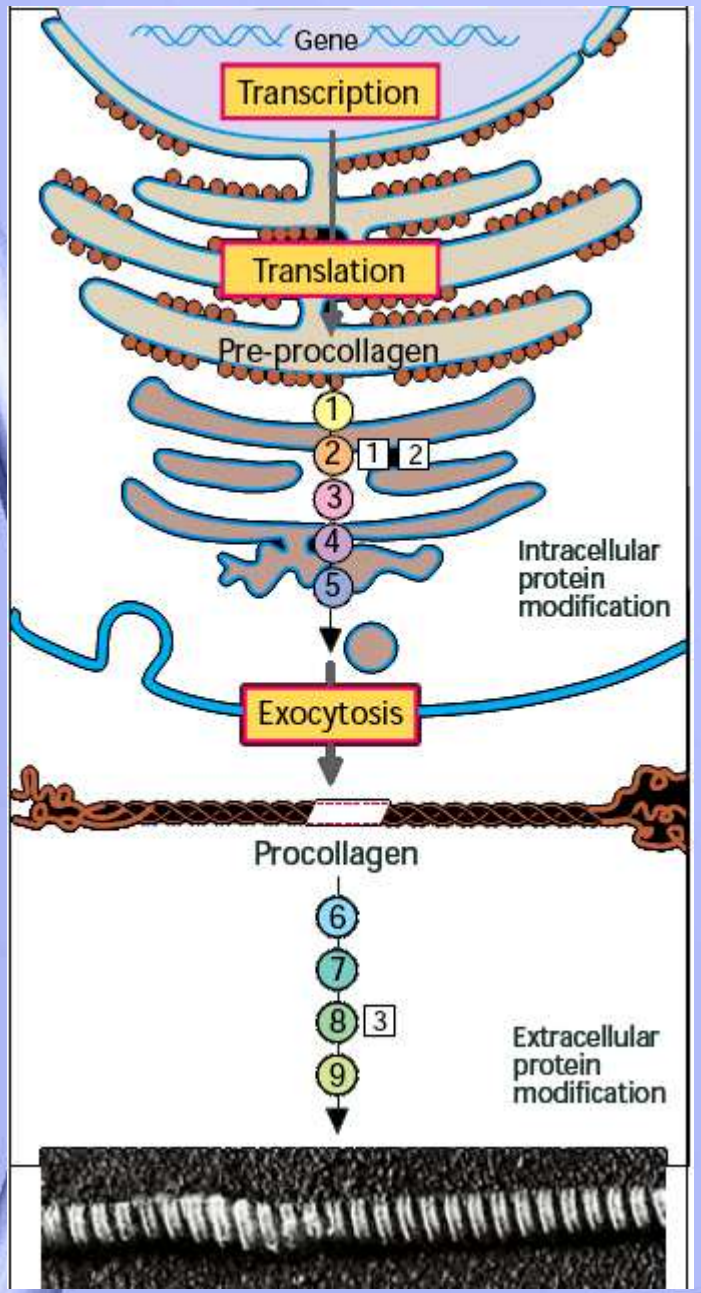

Proline
α–Ketoglutarate
Ascorbic acid
Prolyl hydroxylase
Fe2+
Hydroxyproline
Succinate
Dehydroascorbate

Collagen processing (2)

- Following completion of processing the procollagens are secreted into the extracellular space where extracellular enzymes remove the pro-domains.
  - The collagen molecules then polymerize to form collagen fibrils.
  - Accompanying fibril formation is the oxidation of certain lysine residues by the extracellular enzyme lysyl oxidase foming reactive aldehydes.
  - These reactive aldehydes form specific cross-links between two chains thereby, stabilizing the staggered array of the collagens in the fibril.


Lysine residues
Lysyl oxidase
Aldehyde derivatives
(allysine)
Aldol cross-link

Biosynthesis of Collagen: Overview



1. Removal of the prepeptide
2. Hydroxylation of Pro and Lys residues
3. Glycosylation of 5Hyl and Asn
4. Oxidation of Cys in propeptides
5. Assemblage to form triple helix
6. Removal of the propeptide
7. Staggered deposition to form fibrils
8. Oxidation of Lys and 5Hyl to aldehydes
9. Cross-linking to form supramolecules

[1] Procollagen-proline 4-dioxygenase 1.14.11.2 [ascorbate, Fe]

[2] Procollagen-lysine 5-dioxygenase 1.14.11.4 [ascorbate, Fe]

[3] Protein-lysine 6-oxidase 1.4.3.13 [Cu]

Types of Collagen (1)

Types	Chain Composition	Structural Details	Localization
I	$[\alpha1(I)]_2[\alpha2(I)]$	300nm, 67nm banded fibrils	skin, tendon, bone, etc.
II	$[\alpha1(II)]_3$	300nm, small 67nm fibrils	cartilage, vitreous humor
III	$[\alpha1(III)]_3$	300nm, small 67nm fibrils	skin, muscle, frequently with type I
IV	$[\alpha1(IV)_2[\alpha2(IV)]$	390nm C-term globular domain, nonfibrillar	all basal lamina
V	$[\alpha1(V)][\alpha2(V)][\alpha3(V)]$	390nm N-term globular domain, small fibers	most interstitial tissue, assoc. with type I
VI	$[\alpha1(VI)][\alpha2(VI)][\alpha3(VI)]$	150nm, N+C term. globular domains, microfibrils, 100nm banded fibrils	most interstitial tissue, assoc. with type I
VII	$[\alpha1(VII)]_3$	450nm, dimer	epithelia

Types of Collagen (2)

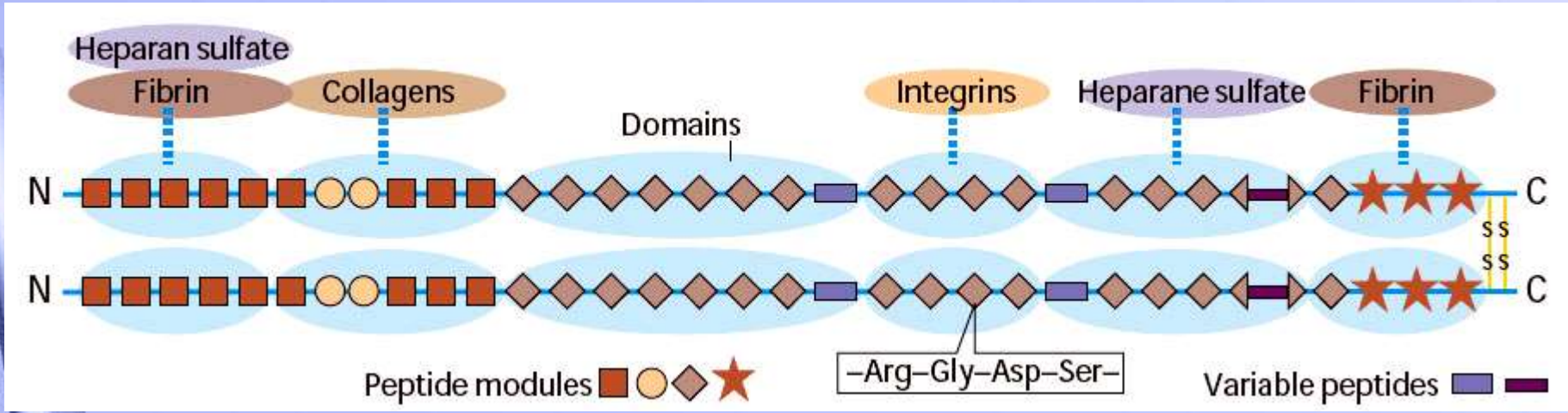
Types	Chain Composition	Structural Details	Localization
VIII	$[\alpha 1(VIII)]_3$	?, ?	some endothelial cells
IX	$[\alpha 1(IX)][\alpha 2(IX)][\alpha 3(IX)]$	200nm, N-term. globular domain, bound proteoglycan	cartilage, assoc. with type II
X	$[\alpha 1(X)]_3$	150nm, C-term. globular domain	hypertrophic and mineralizing cartilage
XI	$[\alpha 1(XI)][\alpha 2(XI)][\alpha 3(XI)]$	300nm, small fibers	cartilage
XII	$\alpha 1(XII)$	?, ?	interacts with types I and III

Classification of Collagens, Based Primarily on the Structures That They Form

Class	Type
Fibril-forming	I, II, III, V, and XI
Network-like	IV, VIII, X
FACITs	IX, XII, XIV, XVI, XIX
Beaded filaments	VI
Anchoring fibrils	VII
Transmembrane domain	XIII, XVII
Others	XV, XVIII

Murray, 2006

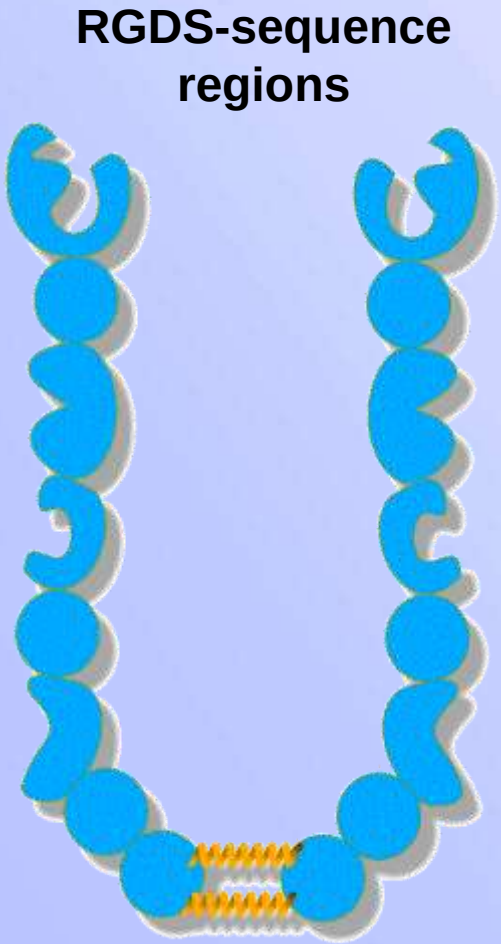
Fibronectins

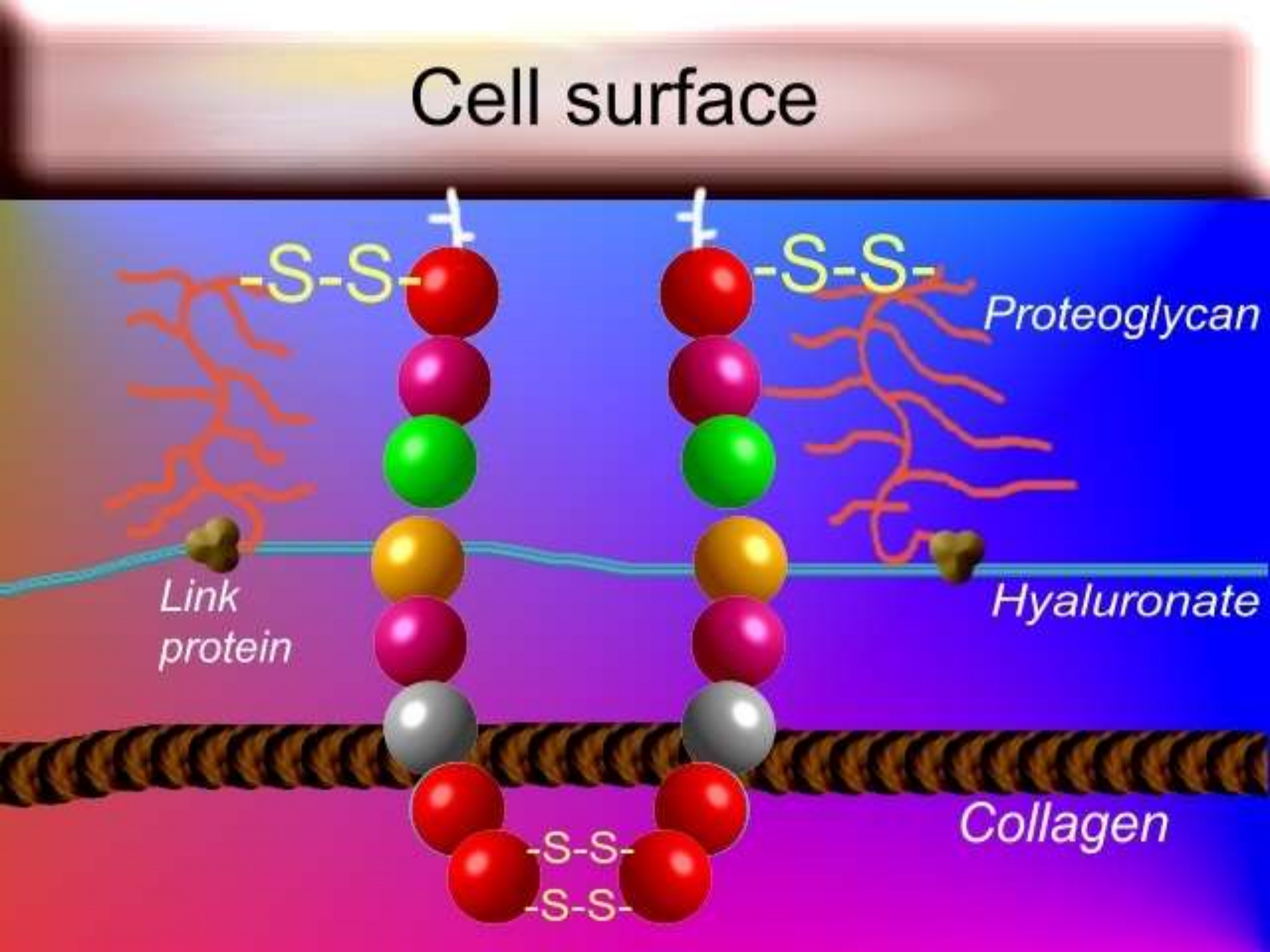


- Fibronectins contain 6-8 tightly folded domains each with a high affinity for a different substrate such as **heparan sulfate**, **collagen** (separate domains for types I, II and III), **fibrin** and **cell-surface receptors**.

Fibronectins

- The cell-surface receptor-binding domain contains a consensus amino acid sequence, **RGDS**.

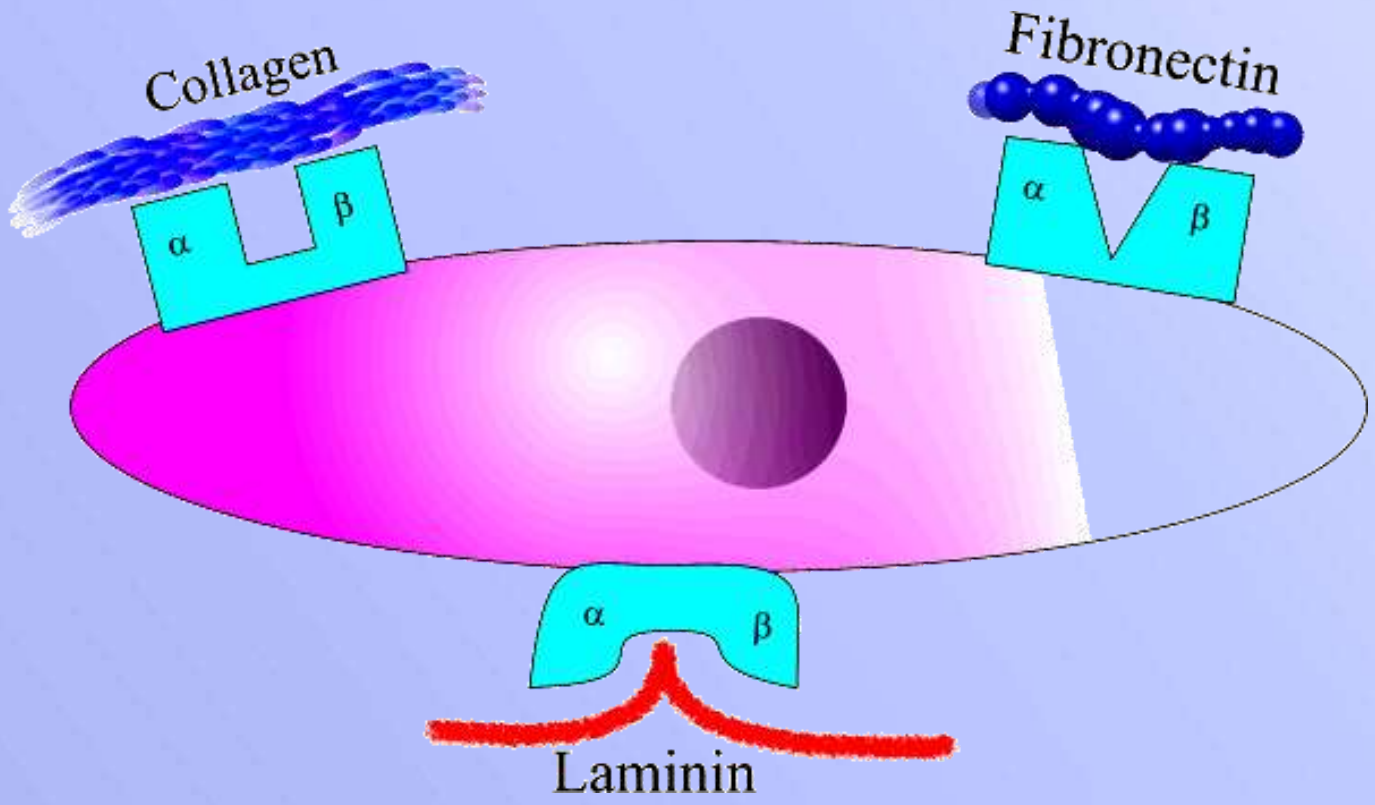



Cell surface
-S-S-
-S-S-
Proteoglycan
Link
protein
Hyaluronate
Collagen
-S-S-
-S-S-

The Role of F**ibronectins**

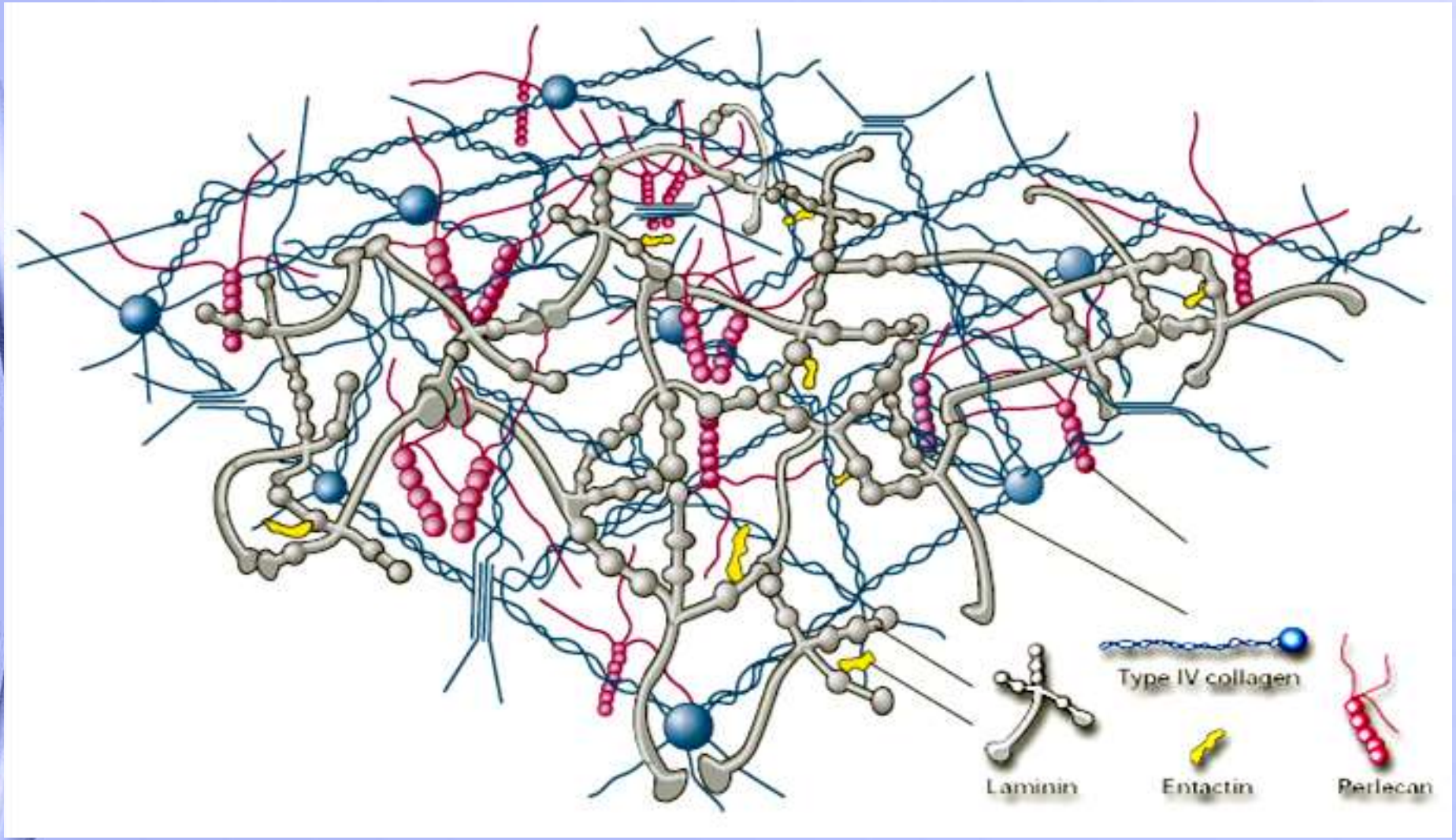
- The role of **fibronectins** is to attach cells to a variety of extracellular matrices.
  - Fibronectin attaches cells to all matrices except type IV that involves **laminin** as the adhesive molecule.
  - Fibronectins are dimers of 2 similar peptides. Each chain is 60-70nm long and 2-3nm thick.
  - At least 20 different fibronectin chains have been identified that arise by alternative RNA splicing of the primary transcript from a single fibronectin gene.

Integrins



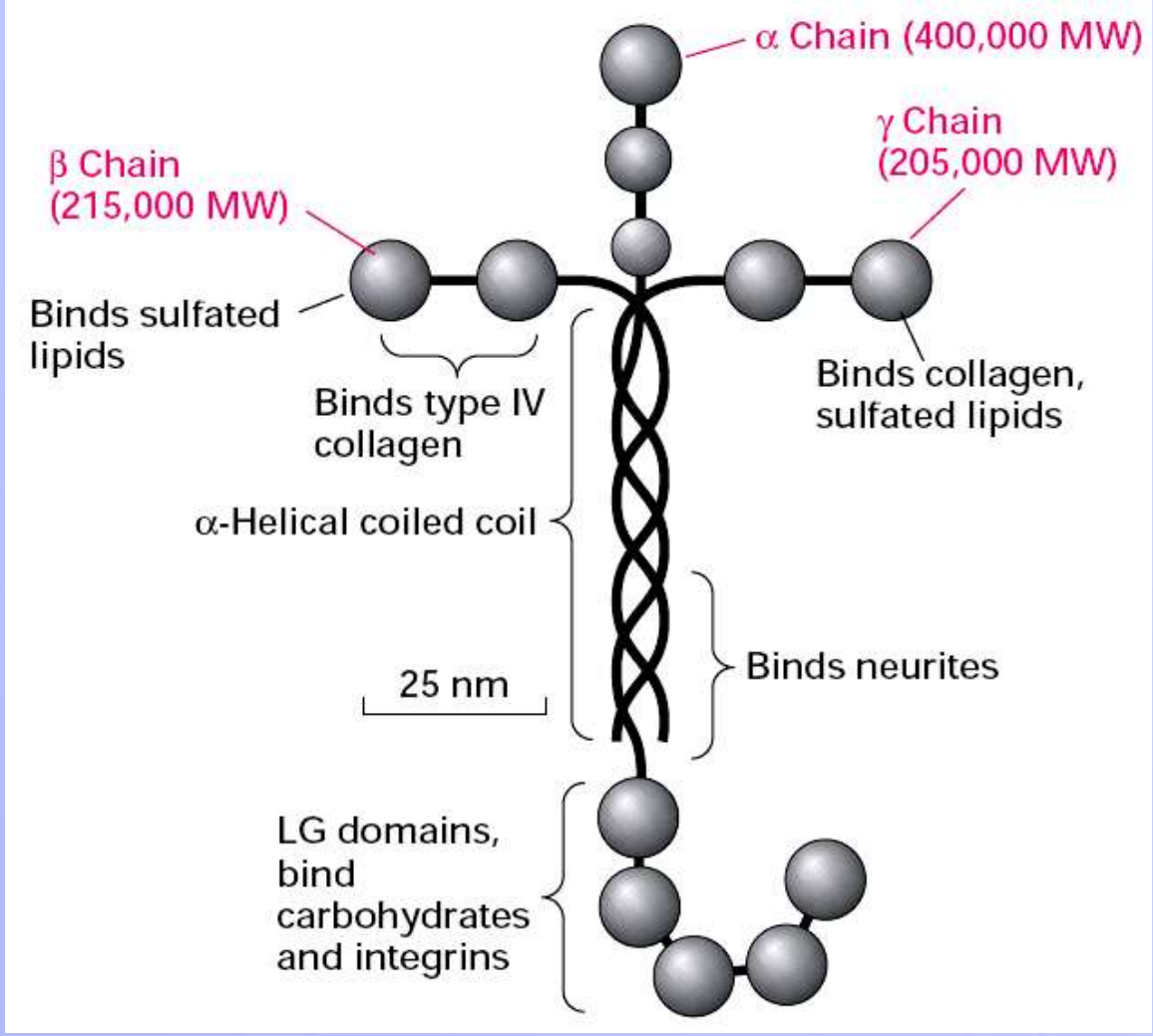
- Integrins are heterodimers, containing various types of α and β polypeptide chains.

Major Components of the Basal Lamina



Basal Lamina Components: Laminin

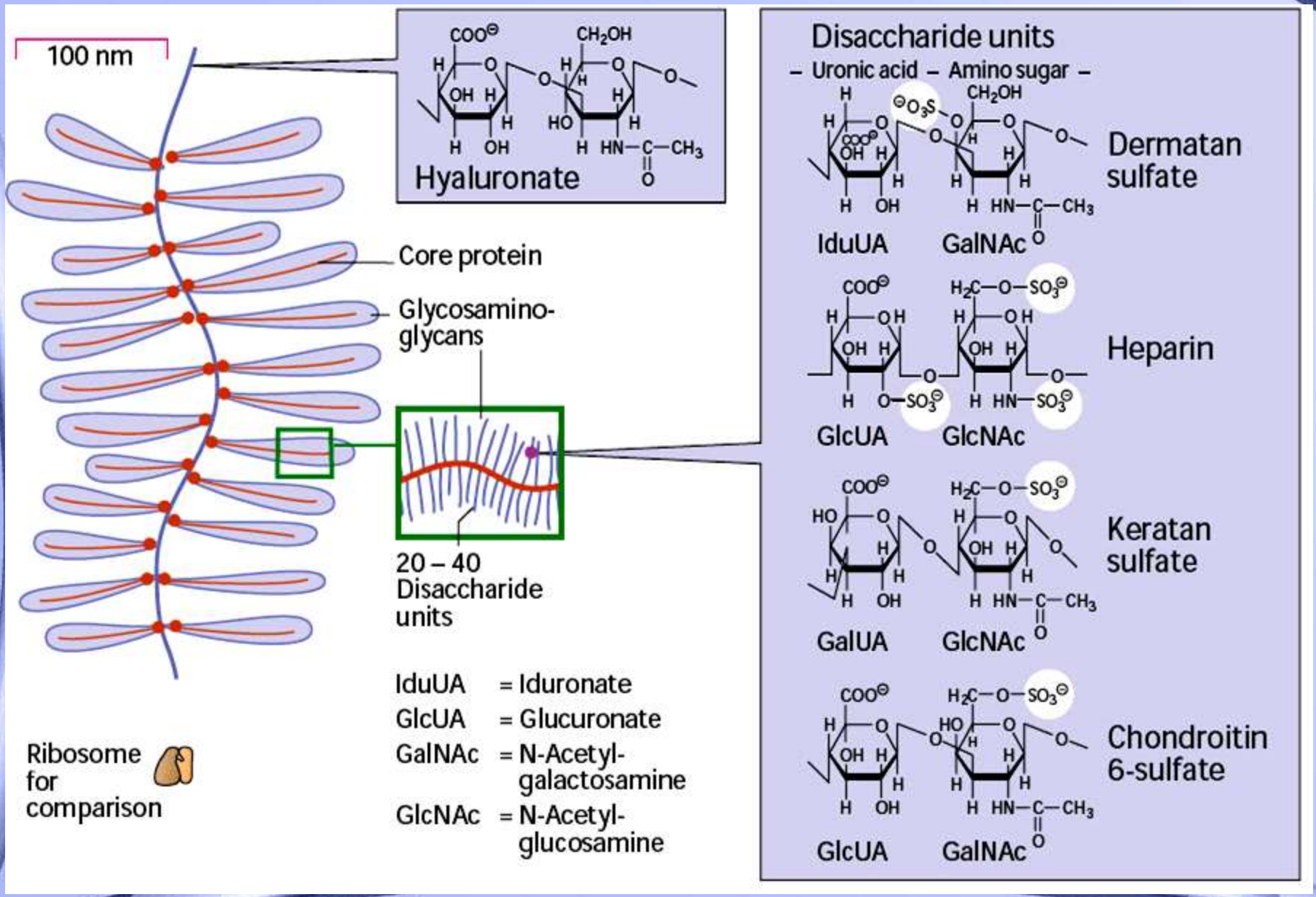
- All basal laminae contain a common set of proteins and GAGs.
- These are type IV collagen, heparan sulfate proteoglycans, perlecan, entactin and laminin.
  - The basal lamina is often refered to as the type IV matrix.
  - Each of the components of the basal lamina is synthesized by the cells that rest upon it.
  - Laminin anchors cell surfaces to the basal lamina.



Representative matrix types produced by vertebrate cells

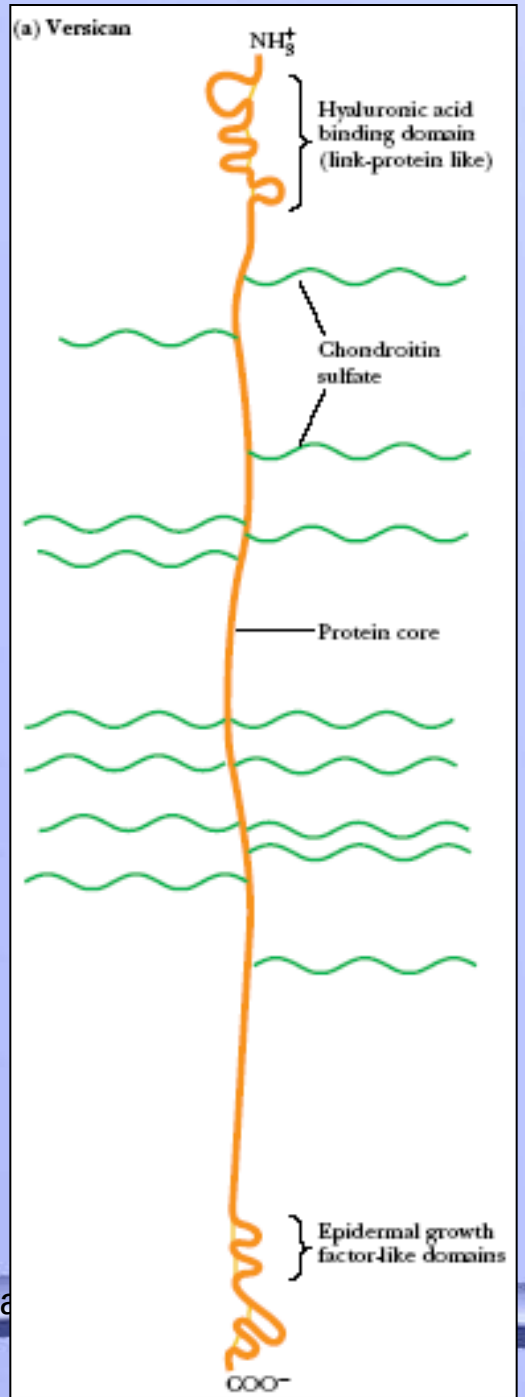
Collagen	Anchor	Proteoglycan	Cell-Surface Receptor	Cells
I	fibronectin	chondroitin and dermatan sulfates	integrin	fibroblasts
II	fibronectin	chondroitin sulfate	integrin	chondrocytes
III	fibronectin	heparan sulfate and heparin	integrin	quiescent hepatocytes, epithelial; assoc. fibroblasts
IV	laminin	heparan sulfate and heparin	laminin receptors	all epithelial cells, endothelial cells, regenerating hepatocytes
V	fibronectin	heparan sulfate and heparin	integrin	quiescent fibroblasts
VI	fibronectin	heparan sulfate	Iitegrin	quiescent fibroblasts

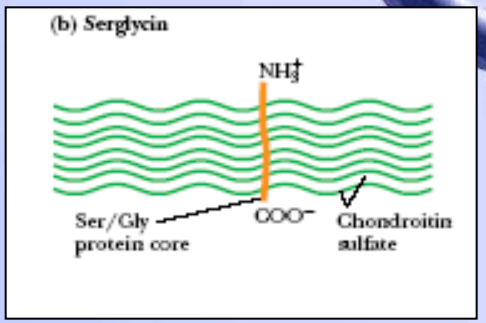
Proteoglycans

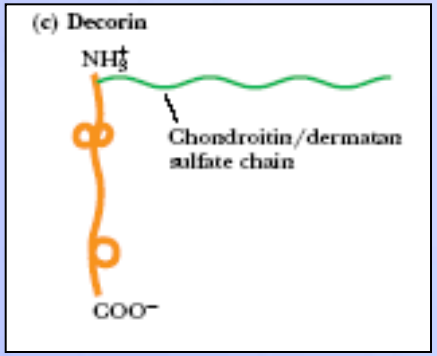


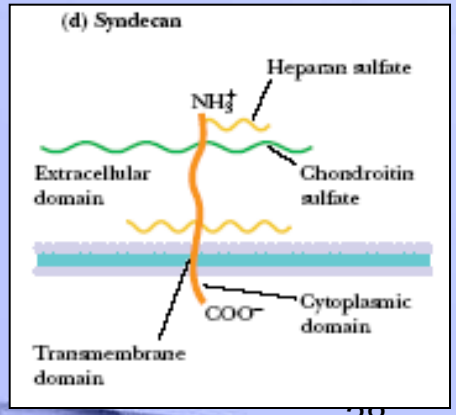
Some Proteoglycans

- The known proteoglycans include a variety of structures.
- The carbohydrate groups of proteoglycans are predominantly glycosaminoglycans O-linked to serine residues.
- Proteoglycans include both soluble proteins and integral transmembrane proteins.



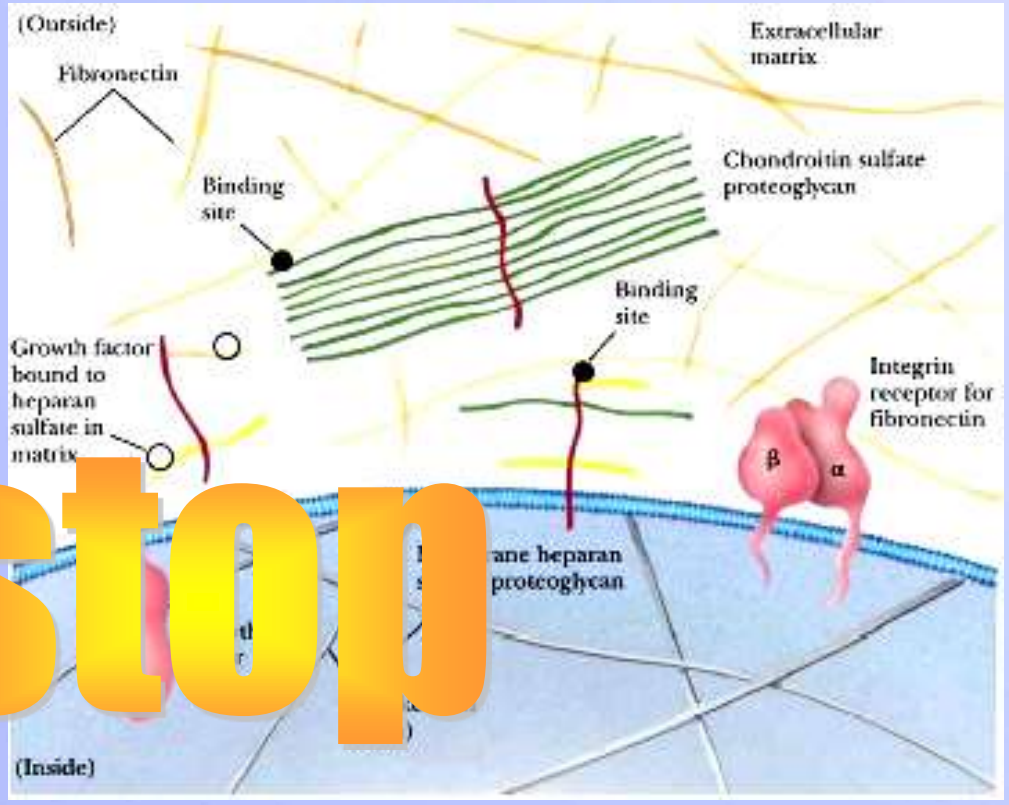






Role of Proteoglycans

- Proteoglycans serve a variety of functions on the cytoplasmic and extracellular surfaces of the plasma membrane.
- Many of these functions appear to involve the binding of specific proteins to the glycosaminoglycan groups.



stop

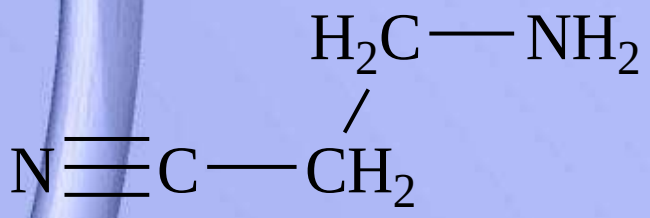
Pathology of Connective Tissue

Collagen-Related Diseases

- Collagen provides an ideal case study of the molecular basis of physiology and disease.
  - The nature and extent of collagen cross-linking depends on the age and function of the tissue.
  - Collagen from young animals is predominantly *uncrosslinked* and can be extracted in soluble form, whereas collagen from older animals is highly cross-linked and thus insoluble. The loss of flexibility of joints with aging is probably due in part to increased cross-linking of collagen.

Lathyrism

- Several serious and debilitating diseases involving collagen abnormalities are known.
- **Lathyrism** occurs in animals due to the regular consumption of seeds of *Lathyrus odoratus,* the sweet pea, and involves weakening and abnormalities in blood vessels, joints, and bones.
  - These conditions are caused by **β-aminopropionitrile** (see figure), which covalently inactivates **lysyl oxidase** and leads to greatly reduced intramolecular cross-linking of collagen in affected animals (or humans).

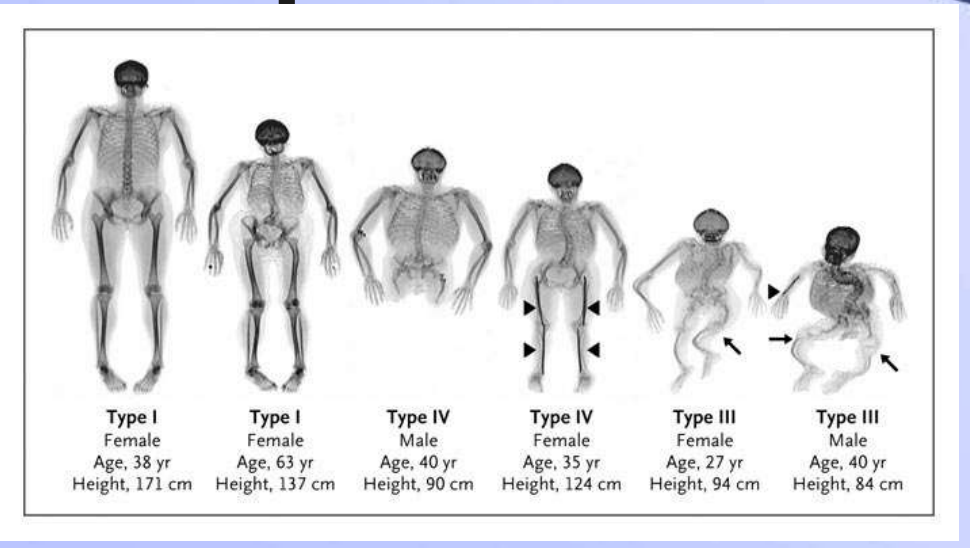


β-aminopropionitrile

Other Collagen Disorders

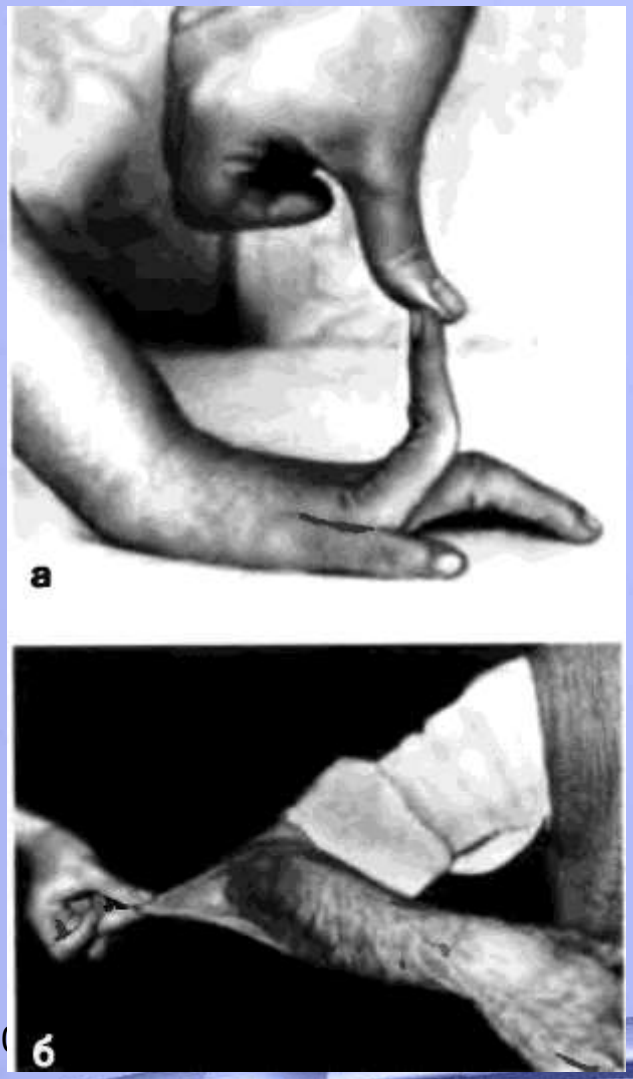
- Alterations in collagen structure resulting from abnormal genes or abnormal processing of collagen proteins results in numerous diseases, e.g. **Larsen syndrome**, **scurvy**, **osteogenesis imperfecta** and **Ehlers-Danlos syndrome**.

Osteogenesis Imperfecta



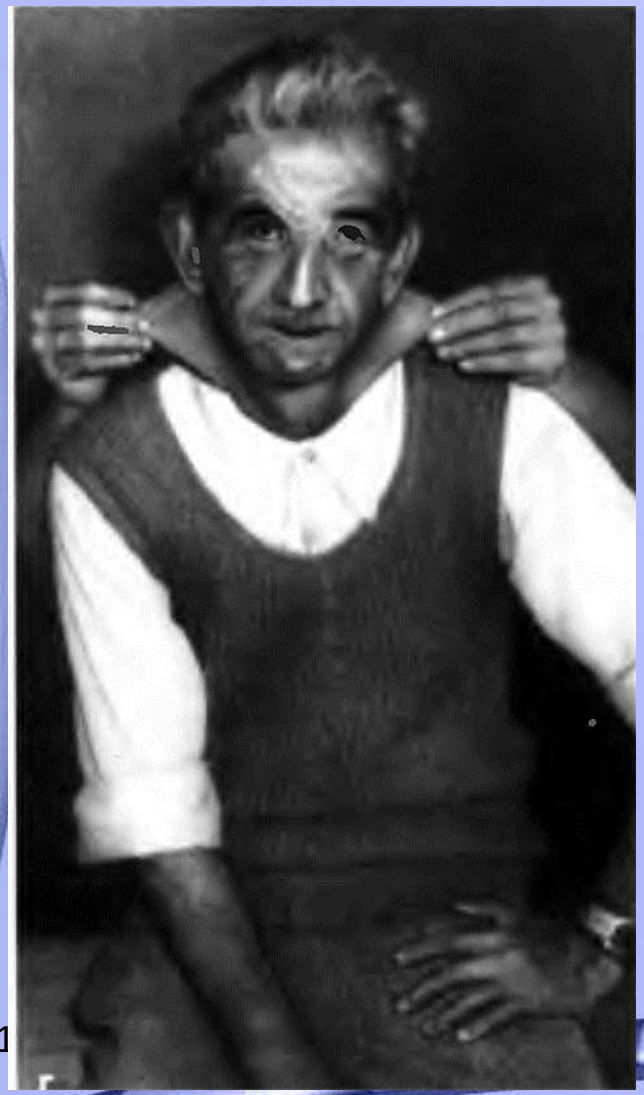
- **Osteogenesis imperfecta** also encompasses more than one disorder.
  - At least four biochemically and clinically distinguishable disorders have been identified all of which are characterized by multiple fractures and resultant bone deformities.

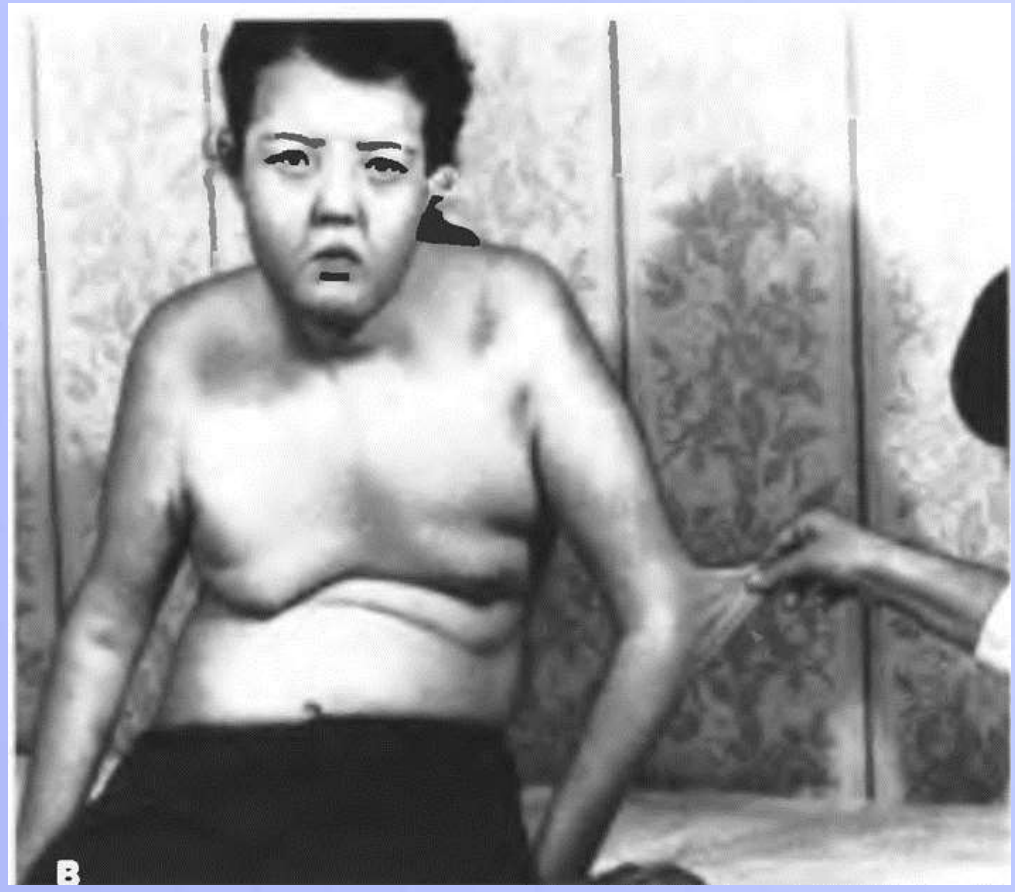
Ehlers-Danlos Syndrome



- **Ehlers-Danlos syndrome** is actually the name associated with at least ten distinct disorders that are biochemically and clinically distinct yet all manifest structural weakness in connective tissue as a result of defects in the structure of collagens.

Ehlers-Danlos Syndrome-2





Marfan's syndrome

- Marfan's syndrome manifests itself as a disorder of the connective tissue and was believed to be the result of abnormal collagens.
  - However, recent evidence has shown that Marfan's results from mutations in the extracellular protein, **fibrillin**, which is an integral constituent of the non-collagenous microfibrils of the extracellular matrix.

Disorders of Collagen

Disorder	Collagen Defect	Symptomology
Ehlers-Danlos IV	decrease in type III	arterial, intestinal and uterine rupture, thin easily bruised skin
Ehlers-Danlos V	decreased cross-linking	skin and joint hyperextensibility
Ehlers-Danlos VI	decreased hydroxylysine	poor wound healing, musculo-skeletal deformities, skin and joint hyperextensibility
Ehlers-Danlos VII	N-terminal pro-peptide not removed	easily bruised skin, hip dislocations, hyperextensibility
Oseteogenesis imperfecta	decrease in type I	blue sclerae, bone deformities
Scurvy	decreased hydroxyproline	poor wound healing, deficient growth, capillary weakness


Thank you
for your attention