PASSION ACADEMIC TEAM

YU - MEDICINE

MUSCULOSKELETAL SYSTEM

Sheet# 1 - Biochemistry

Lec. Title : Biochemical Structure & Bone Tissue .

Written By : Noor Hammouri

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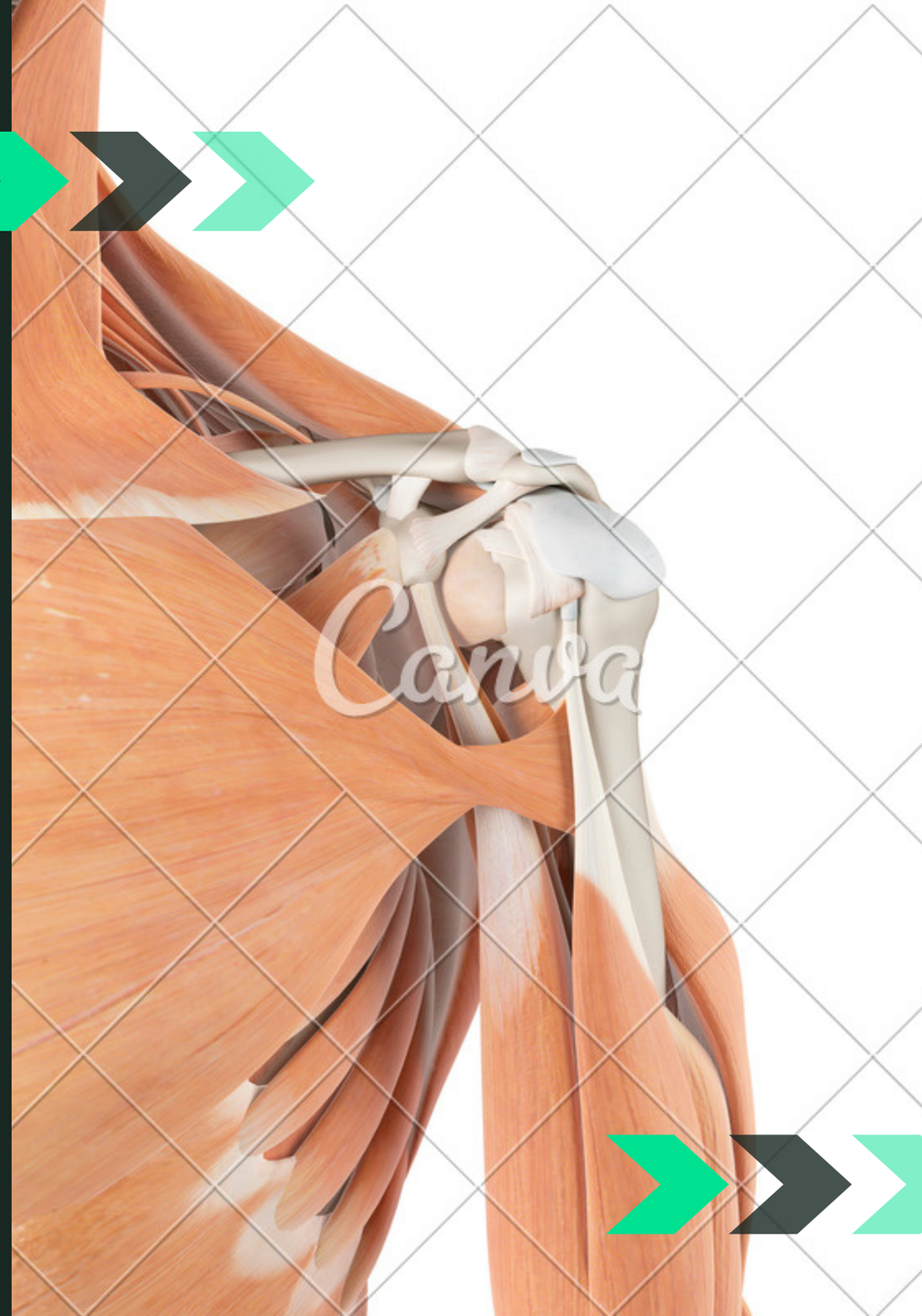


Biochemical structure of bone tissue, the collagen matrix and the hydroxyapatite cement

Dr. Mazhar Al Zoubi

Dec 2020

Harper's Illustrated Biochemistry

TWENTY-NINTH EDITION

CHAPTER

50

The Extracellular Matrix

Objectives:

1. Describe the biochemical structure of bone tissue, the collagen matrix and the hydroxyapatite cement.
2. List bone matrix proteins and describe their function.
3. Describe the Composition of calcified tissues, calcification in bones and teeth and formation of hydroxyapatite.
4. Understand the role of alkaline phosphatase, calcium and hosphate and vitamin D: 1,25-Dihydroxy-vit-D in bone formation and remodeling.
5. Review calcium and phosphate homeostasis.

BONE
IS A MINERALIZED CONNECTIVE TISSUE

The minerals are accumulated to form the rigidity of the bone

From recording:

- There are two major components of the bone:

1. Organic matter(proteins)
2. Inorganic matter(crystalline hydroxyapatite)

- The cells that are forming the bone are responsible for coordination of these components:

a) Production of proteins (collagen or non-collagen)

b) Mineralization process : how are these minerals deposited in order to form this structure.

TABLE 48–11 The Principal Proteins Found in Bone[1]

Proteins	Comments
Collagens	
Collagen type I	Approximately 90% of total bone protein. Composed of two α1(I) and one α2(I) chains.
Collagen type V	Minor component.
Noncollagen proteins	
Plasma proteins	Mixture of various plasma proteins.
Proteoglycans[2] CS-PG I (biglycan)	Contains two GAG chains; found in other tissues.
CS-PG II (decorin)	Contains one GAG chain; found in other tissues.
CS-PG III	Bone-specific.
Bone SPARC[3] protein (osteonectin)	Not bone-specific. For the connection in the bone
Osteocalcin (bone Gla protein)	Contains γ-carboxyglutamate (Gla) residues that bind to hydroxyapatite. Bone-specific.
Osteopontin	Not bone-specific. Glycosylated and phosphorylated.
Bone sialoprotein	Bone-specific. Heavily glycosylated, and sulfated on tyrosine.
Bone morphogenetic proteins (BMPs)	A family (eight or more) of secreted proteins with a variety of actions on bone; many induce ectopic bone growth.
Osteoprotegerin	Inhibits osteoclastogenesis

The **organic matter is mainly protein.**

GlycosAminoGlycans (GAGs):

- **Hyaluronic acid,**
- **Chondroitin sulfate,**
- **Keratan sulfates I and** II,
- Heparin,
- Heparan sulfate,
- Dermatan sulfate.

*** CS: chondroitin sulfate**

osteoclastogenesis Formation of osteoclasts

From recording: proteins types

- Collagen components:

- collagen proteins are mainly a triple alpha chains that connect to each other , so we classify collagen types depending on the type of alpha chains that compose it , for example collagen type 1(the main type in the bone) consists of two alpha-1 chains and one alpha-2 chain.

- Non collagen components:

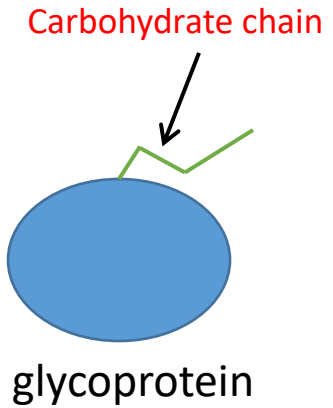
1. Plasma proteins: are located there as a sequence of blood flow in the bone(living tissue).
2. Proteoglycans:

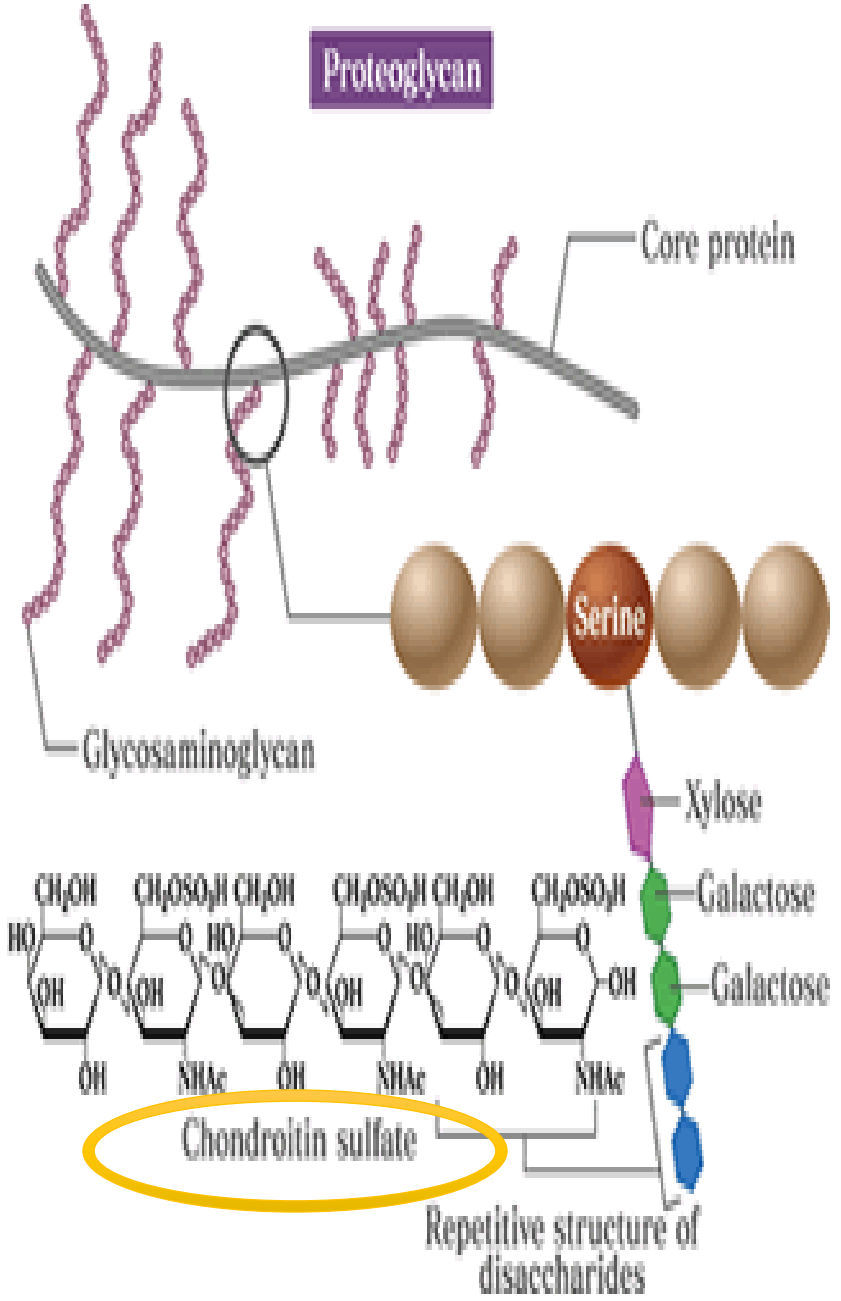
- They consist of huge amounts of carbohydrates that are conjugated to the protein in a certain manner. (more details are discussed in the next slides)
- The difference between them and the glycoproteins is that glycoproteins are proteins that have a simple branch of carbohydrate
- Biglycan and decorin are bone non-specific proteins , while CS-PGIII is bone specific so we use it clinically to detect any disorder in the bone.

- Proteoglycan is a core protein that is connected with branches of carbohydrate chains called GAGs.

- If we zoom in these GAGs we will find that they are polysaccharides consists of heterogeneous structure of sugars (xylose galactose etc.) you don’t have to memorize the name of them, I’ve mentioned them just for understanding

- The reason that we have multiple types of proteoglycans is the heterogeneous structure of sugars that made up GAGs.





The **inorganic or mineral component is mainly crystalline hydroxyapatite** $\mathbf{Ca_{10}}(PO_4)_6(OH)$. (the formula is important to be memorized)

- With sodium, magnesium, carbonate, and fluoride
- 99% of the body's calcium is contained in bone.
- Bone is a **dynamic structure:** (because it's a living tissue so there will be turn over of the cells)
  - Resorption followed by Deposition of new bone tissue
  - This remodeling system is adapted to physiological and hormonal signaling by:
    - **Osteoclasts** (Resorption) → degradation
    - **Osteoblasts** (Deposition)→ production

From recording:

- Osteoblast maturation will lead to osteocytes formation (bone cells)
- Osteoclasts are multinucleated cells.
- Mesenchyme is an early forming bone.
- Bone matrix is the mature bone.
- Osteoid is the mineralized component of the bone.
- Collagen type I is formed by osteoblasts.

Synthesizing type I collagen

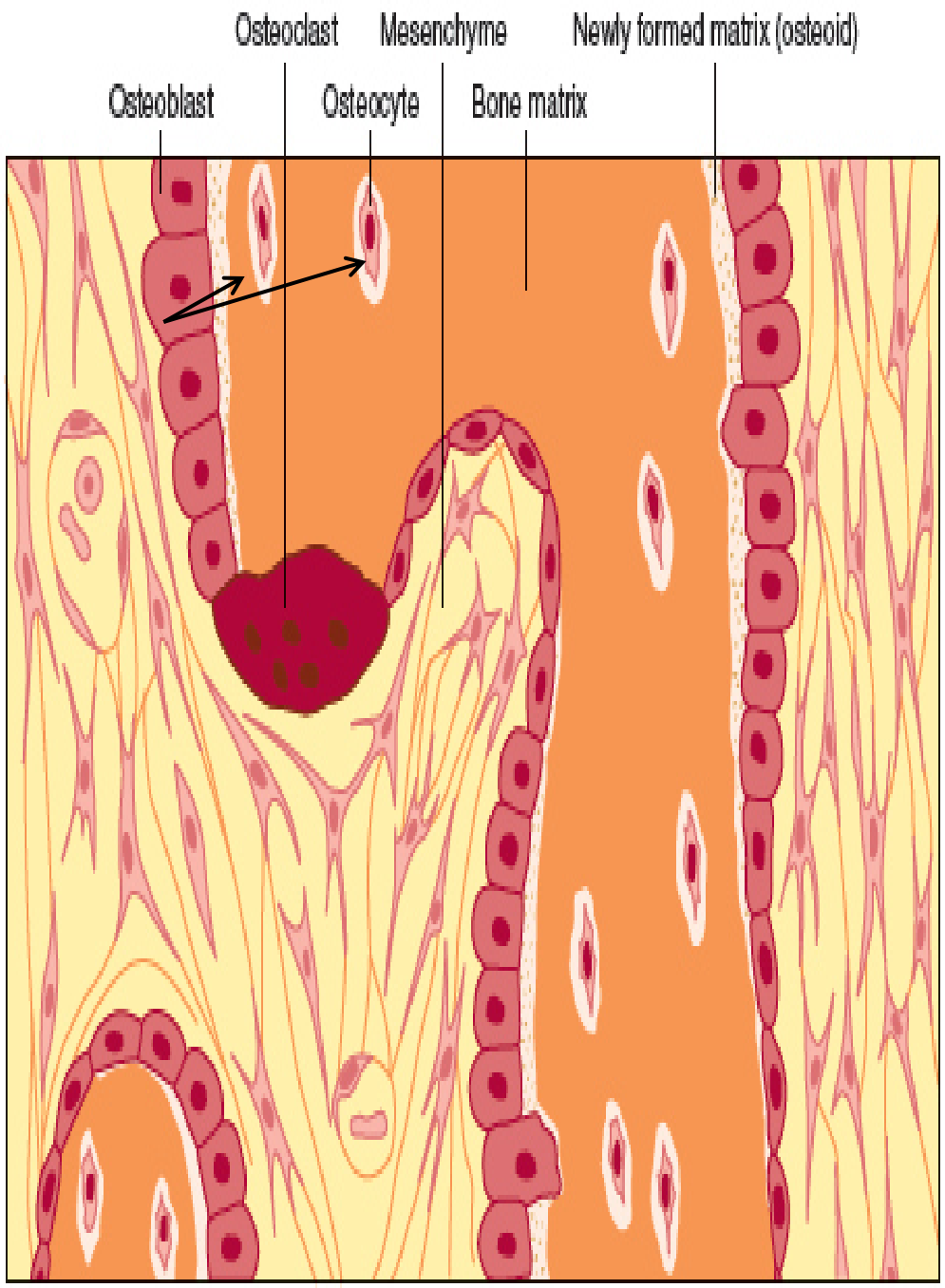


FIGURE 48–11 Schematic illustration of the major cells present in the membranous bone. Osteoblasts (lighter color) are synthesizing type I collagen, which forms a matrix that traps cells. As this occurs, osteoblasts gradually differentiate to become osteocytes. (Reproduced, with permission, from Junqueira LC, Carneiro J: *Basic Histology: Text & Atlas*, 10th ed. McGraw-Hill, 2003.)

Osteoclasts

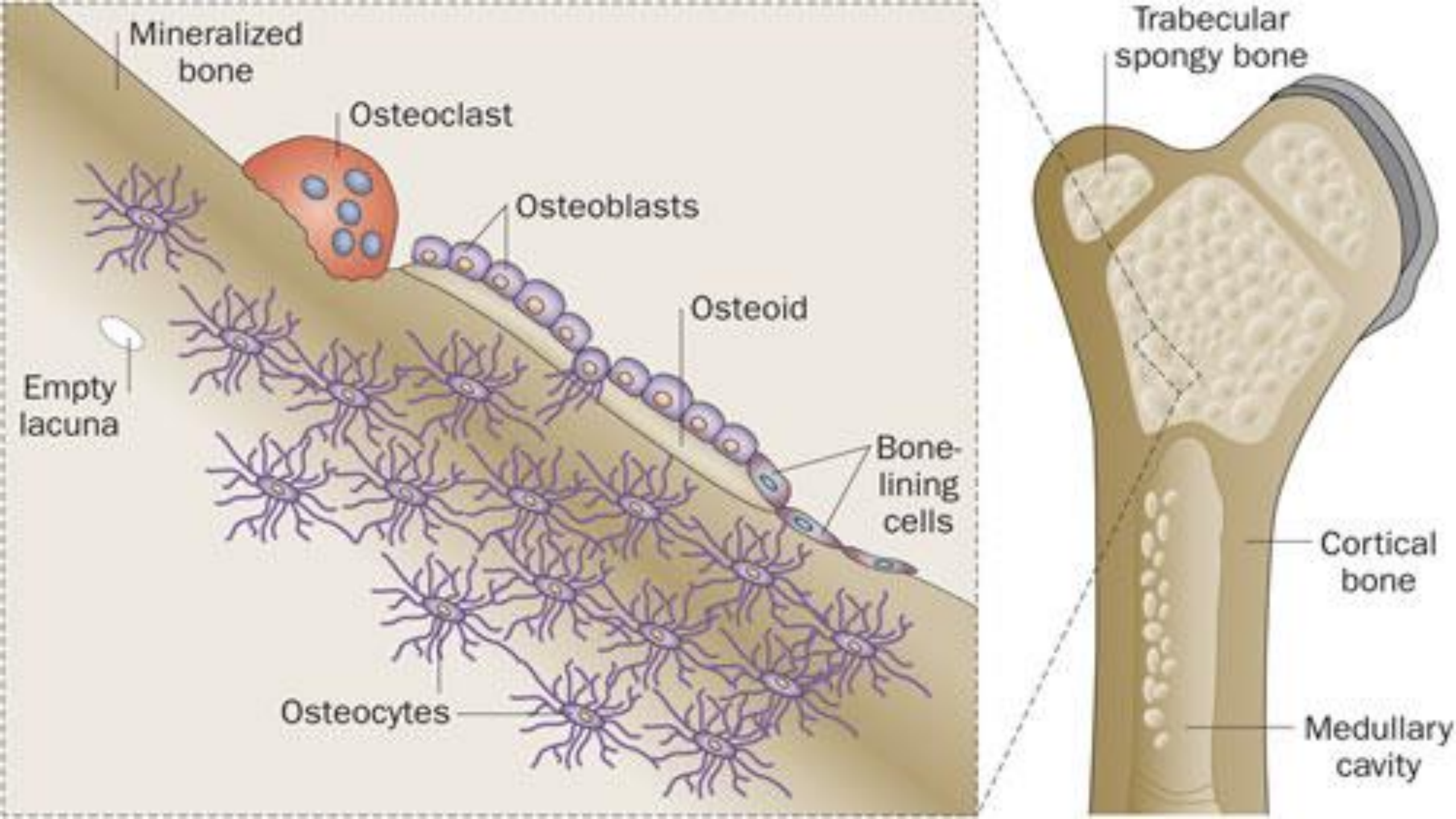
❖ **Multinucleated cells derived from pluripotent hematopoietic stem cells.(from the bone marrow ,and it comes from the same origin that RBCs come from)**

❖ **Possess** an apical membrane domain, exhibiting a **ruffled border** that plays a key role in bone resorption. (ruffled border are vesicles that contain acidic environment (4 ph)

❖ Low pH (4.0) or less, increases the solubility of **hydroxyapatite** and allowing demineralization to occur.

↓

Cement component of the bone


Mineralized bone
Osteoclast
Osteoblasts
Osteoid
Empty lacuna
Bone-lining cells
Osteocytes
Trabecular spongy bone
Cortical bone
Medullary cavity

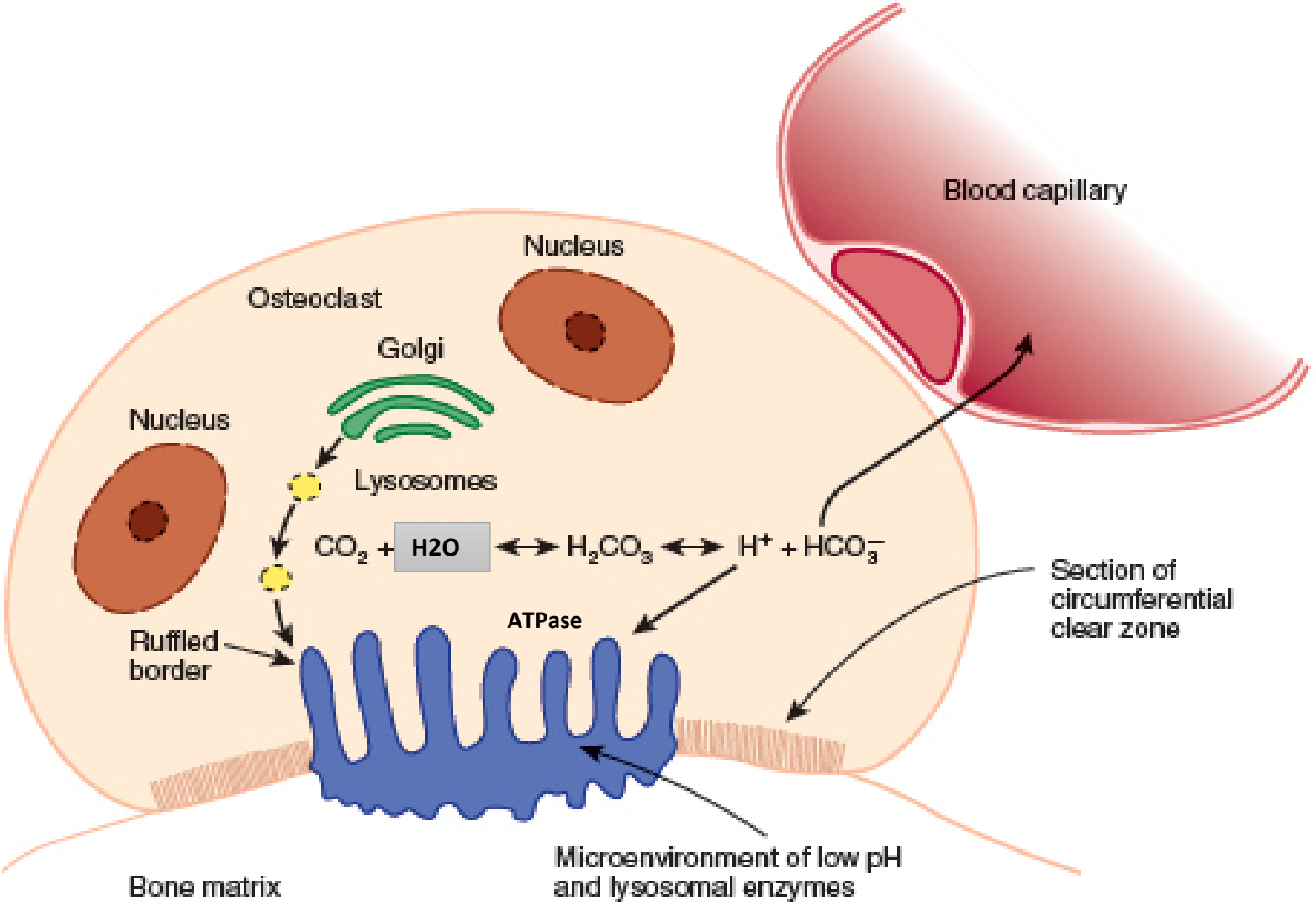


FIGURE 48–12 Schematic illustration of some aspects of the role of the osteoclast in bone resorption. Lysosomal enzymes and hydrogen ions are released into the confined microenvironment created by the attachment between the bone matrix and the peripheral clear zone of the osteoclast. The acidification of this confined space facilitates the dissolution of calcium phosphate from bone and is the optimal pH for the activity of lysosomal hydrolases. The bone matrix is thus removed, and the products of bone resorption are taken up into the cytoplasm of the osteoclast, probably digested further, and transferred into capillaries. The chemical equation shown in the figure refers to the action of carbonic anhydrase II, described in the text. (Reproduced, with permission, from Junqueira LC, Carneiro J: *Basic Histology: Text & Atlas,* 10th ed. McGraw-Hill, 2003.)

Regarding to the previous slide:

- Ruffled border is a vesicle that produced by accumulation of lysosomes which has 5 PH.

- Now there is reaction occurs in the osteoclast cells which is :

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^-$$

- On the surface of ruffled border there is an ATPase pump that transform H+ which was produced from the previous reaction into the vesicle so PH will be lower and it reaches 4.

- Then the vesicle will slowly release these acids into bone and causes a slow resorption.

Osteoblasts

❖**Mononuclear cells derived from pluripotent mesenchymal** precursors. **(Mesenchymal precursor: the early embryonic cells in the mesenchyme of the bone)**

❖Synthesize most of the proteins found in bone.

❖ Produce **growth factors and cytokines**

❖ Deposits new bone matrix (**osteoid**) and its subsequent mineralization

❖ **Mineralization is controled by regulating the passage of calcium and phosphate ions across their surface** membranes.

❖ **Alkaline phosphatase (ALP) is used to generate phosphate ions from organic** phosphates.

Type I collagen appears to be necessary, with mineralization being first evident in the gaps between successive molecules

Regarding to osteoblasts:

- Osteoid: the first stage of bone formation and mineralization
- Mineralization: deposition of minerals (Ca+2 and phosphate)
- Osteoblasts are rich in ALP enzyme , it’s used to convert organic phosphate that are conjugated to proteins into inorganic ones by dissociation process in order to do mineralization.
- We can use ALP as a marker to detect disorders in the bone, even if it’s non bone specific but it’s very common to be find in osteoblasts.

- **Acidic phosphoproteins, such as bone <u>sialoprotein</u>, acting** as sites of **nucleation.**

- These proteins contain motifs (eg, poly-Asp and poly-Glu stretches) that bind calcium and may provide an initial scaffold for mineralization. **Take a look to the next slide**

- Certain proteoglycans and glycoproteins, can also act as **inhibitors of nucleation.**

approximately 4% of compact bone is
renewed annually
whereas
approximately 20% of trabecular bone is replaced.

Regarding to the previous slide:

- Acidic proteins are named so because the contain acidic amino acids like aspartic acid and glutamic acid which are negatively charged molecules.

- Now why these proteins are important in bone formation and mineralization?
- Because as we said the contain negatively charged amino acid so the total charge of the protein is negative

- When you have a negative nuclei it will be very easy to attract positively charged molecules like Ca+2 and increase the deposition of it.

❑ ***Osteoblasts stimulated by:***

- ✓*parathyroid hormone*
- ✓*1,25dihydroxycholecalciferol*

❖***Inhibited by:***

- ✓*Corticosteroids*

very important

❑ ***Osteoclasts stimulated:***

- ✓ *Parathyroid hormone*
- ✓*1,25dihydroxycholecalciferol*

❖***Inhibited by:***

- ✓*Calcitonin*
- ✓*Estrogens*

BONE IS AFFECTED BY MANY METABOLIC & GENETIC DISORDERS

TABLE 48–12 Some Metabolic and Genetic Diseases Affecting Bone and Cartilage

Disease	Comments
Dwarfism	Often due to a deficiency of growth hormone, but has many other causes.
Rickets	Due to a deficiency of vitamin D during childhood.
Osteomalacia	Due to a deficiency of vitamin D during adulthood.
Hyperparathyroidism	Excess parathormone causes bone resorption.
Osteogenesis imperfecta (eg, OMIM 166200)	Due to a variety of mutations in the *COL1A1* and *COL1A2* genes affecing the synthesis and structure of type I collagen.
Osteoporosis (OMIM 166710) Due to activation of osteoclasts	Commonly postmenopausal or in other cases is more gradual and related to age; a small number of cases are due to mutations in the *COL1A1* and *COL1A2* genes and possibly in the vitamin D receptor gene
Osteoarthritis	A small number of cases are due to mutations in the *COL1A* genes
Several chondrodysplasias	Due to mutations in *COL2A1* genes
Pfeiffer syndrome[1] (OMIM 101600)	Mutations in the gene encoding fibroblast growth receptor 1 (FGFR1)
Jackson-Weiss (OMIM 123150) and Crouzon (OMIM 123500) syndromes[1]	Mutations in the gene encoding FGFR2
Achondroplasia (OMIM 100800) and thanatophoric dysplasia[2] (OMIM 187600)	Mutations in the gene encoding FGFR3 ***

Excess amount will activate osteoclast more the osteoblast cells

due to mutation in collagen expression genes

Occurs in cartilage disorders (COL2 mutation)

Mainly due to FGFR mutations

Osteogenesis imperfecta (brittle bones): Fragility of bones. Also called OI

✓Thin and translucent sclera and may appear blue owing to a deficiency of connective tissue.

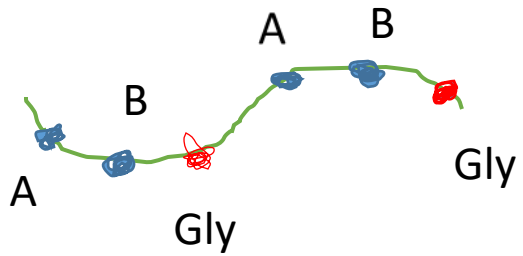
✓**Four types:** (mild, extensive, severe, and variable) **we have 4 types because there are more than 100 mutations can occur in COL1A1 and COL1A2 genes**

- Affected infants may be born with multiple fractures and not survive.
- Over 90% of patients with osteogenesis imperfecta have mutations in the ***COL1A1*** *and* ***COL1A2*** *genes, encoding pro* 1(I) and pro 2(I) chains.
- Over **100 mutations** in these two genes have been documented.
- Take a look to the next slide to clearly understand the next points:
- Most frequent type: **Replacement of glycine by another bulkier amino acid, affecting formation of the triple helix.**

"procollagen suicide" is an example of a dominant negative mutation, when a protein consists of multiple different subunits. resulting in enzymatic degradation

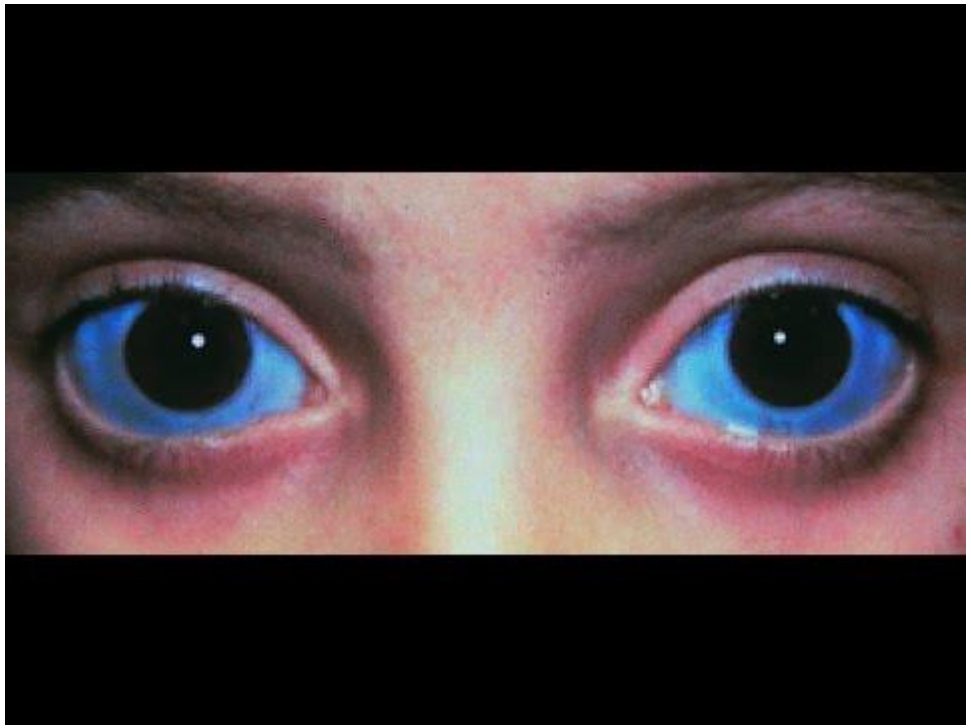
Regarding to the previous slide:

- The structure here indicate one alpha chain in collagen (a and b indicate different types of amino acids while gly is the amino acid glycine which forms 1/3 of the total structure of collagen)

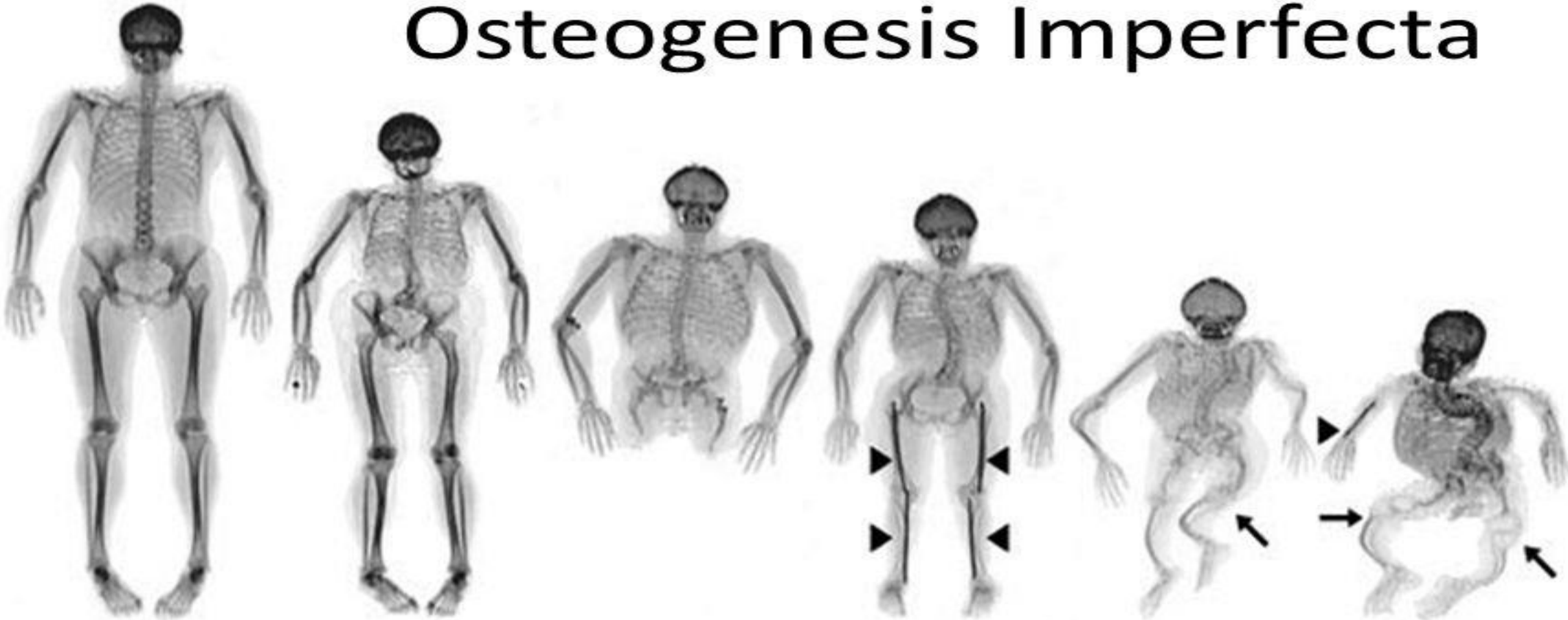


- Now any replacement of gly amino acid will cause a sever mutation and sever case of OI disease because gly is a small amino acid and the replacement by bulkier one will lead to something called **procollagen suicide** which means the protein will be degraded inside the cell.

- Mutation in the other amino acids will cause less sever conditions.



Osteogenesis Imperfecta



Type I
Female
Age, 38 yr
Height, 171 cm

Type I
Female
Age, 63 yr
Height, 137 cm

Type IV
Male
Age, 40 yr
Height, 90 cm

Type IV
Female
Age, 35 yr
Height, 124 cm

Type III
Female
Age, 27 yr
Height, 94 cm

Type III
Male
Age, 40 yr
Height, 84 cm

Osteopetrosis (marble bone disease)

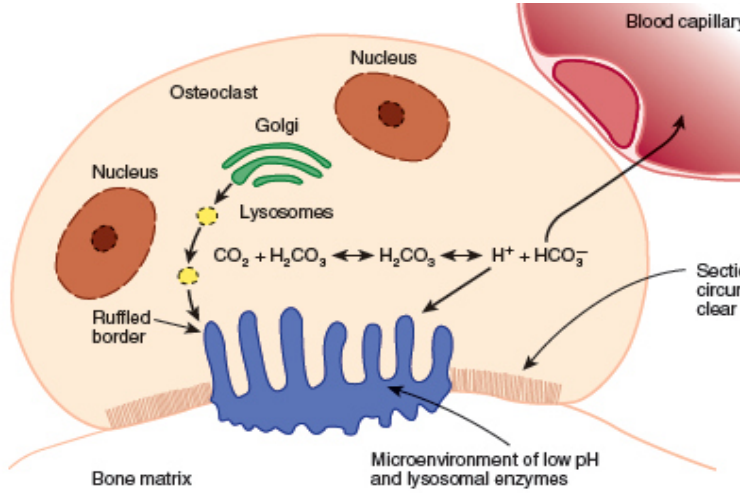
Solidification of the bone due to decrease of the activity of osteoclasts (not over activity of osteoblasts)

- Increased bone density due to inability to resorb bone.

- ~with renal tubular acidosis and cerebral calcification **(accumulation of Ca+2 in the nervous system, mainly the brain.**

- due to mutations in the gene (located on chromosome 8q22) encoding **carbonic anhydrase II (CA II)** **(the enzyme that is responsible for formation of H2CO3 in osteoclasts)**

- Mainly CA II not CA I which is the responsible for blood buffering system
- Decrease in CA II will decrease the production of H2CO3→decrease in H+→ increase in PH in ruffled border → decrease in bone resorption.



Osteoporosis

- Is a generalized progressive reduction in bone tissue mass per unit volume causing skeletal weakness.

- **Estrogens and the cytokines interleukins-1 and -6 appear to be intimately involved in the causation of** osteoporosis.

PASSION ACADEMIC TEAM YU - MEDICINE

MUSCULOSKELETAL SYSTEM

Sheet#2 (Part 1) - Biochemistry

Lec. Title : Biochemical (Part 2)

Written By : Roqaya Mahmoud +
Wasan Ababneh + Noor Hammouri

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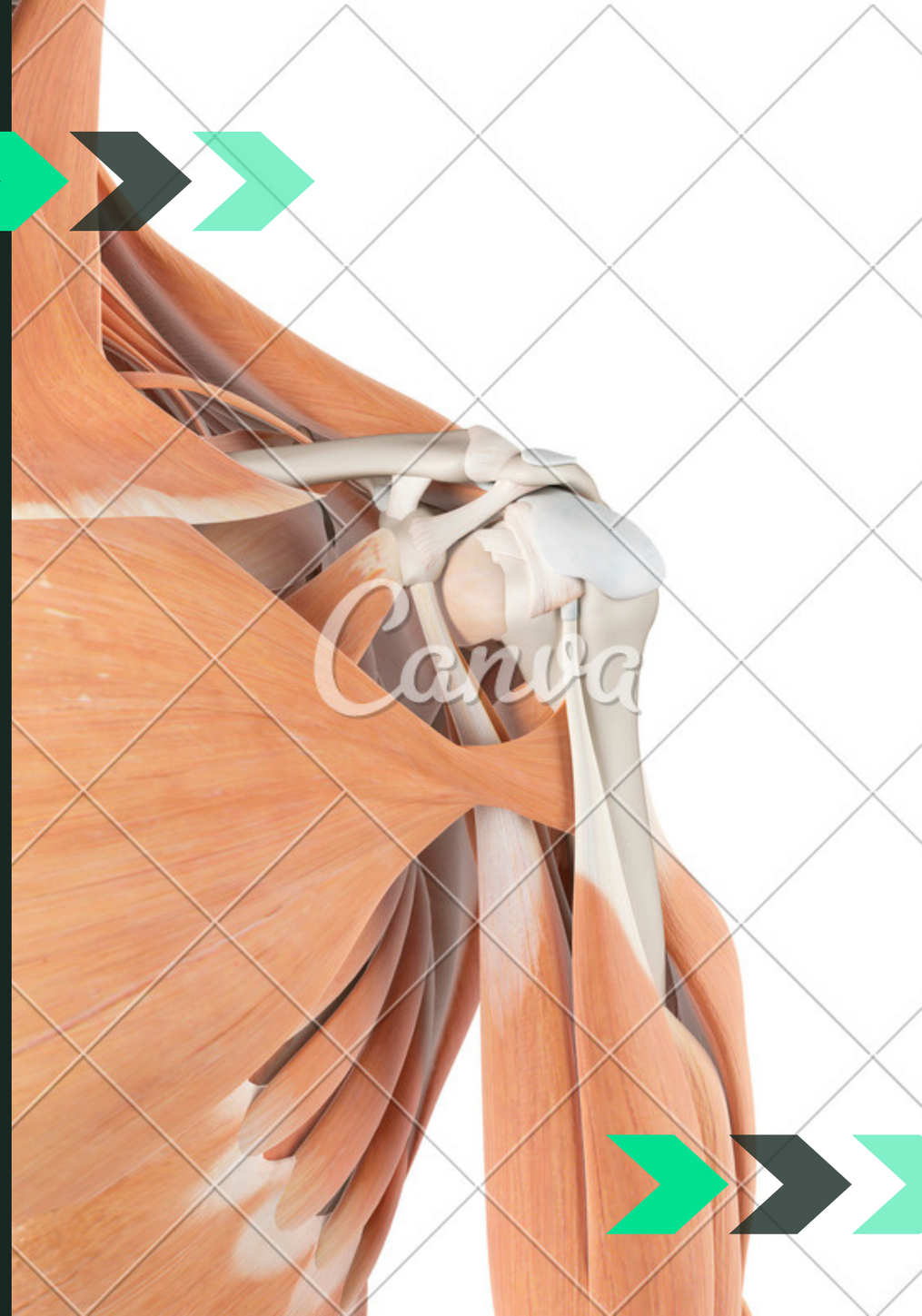


THE MAJOR COMPONENTS OF CARTILAGE ARE TYPE II COLLAGEN & CERTAIN PROTEOGLYCANS

Hyaline cartilage (the major type of cartilage)

Elastic cartilage contains elastin and fibroelastic cartilage contains type I collagen.

❖Cartilage is **an avascular** tissue and obtains most of its nutrients from synovial fluid.
Slow continuous **turnover.**

❖***Various proteases (eg, collagenases and stromalysin) synthesized by chondrocytes can degrade collagen and the other proteins found in cartilage.***

❖***(IL-1) and (TNF-α)*** *stimulate the production of such proteases.*

❖***Transforming growth factor-β (TGF-β) and insulin-like growth factor 1 (IGF-I) generally exert an anabolic influence on cartilage.***

TABLE 48–13 The Principal Proteins Found in Cartilage

Proteins	Comments الجدول مطلوب
Collagen proteins	
Collagen type II	90–98% of total articular cartilage collagen. Composed of three α 1(II) chains.
Collagens V, VI, IX, X, XI	Type IX cross-links to type II collagen. Type XI may help control diameter of type II fibrils.
Non-collagen proteins	
Proteoglycans	The major proteoglycan of cartilage.
Aggrecan	Found in some types of cartilage.
Large non-aggregating proteoglycan	
DS-PG I (biglycan)[1]	Similar to CS-PG I of bone.
DS-PG II (decorin)	Similar to CS-PG II of bone.
Chondronectin	May play role in binding type II collagen to surface of cartilage.
Anchorin C II	May bind type II collagen to surface of chondrocyte.

[1]The core proteins of DS-PG I and DS-PG II are homologous to those of CS-PG I and CS-PG II found in bone (TABLE 48–11). A possible explanation is that osteoblasts lack the epimerase required to convert glucuronic acid to iduronic acid, the latter of which is found in dermatan sulfate.

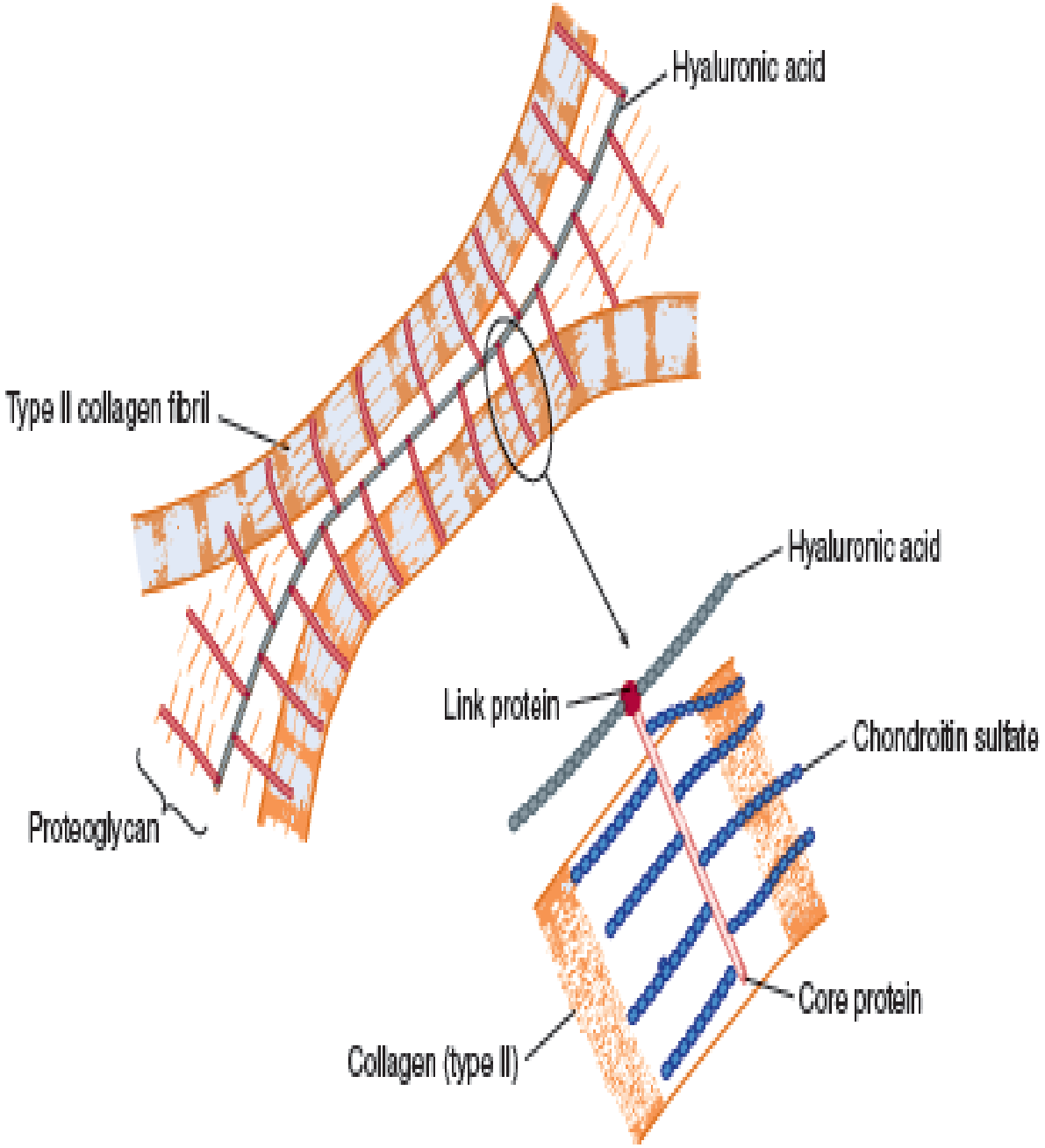
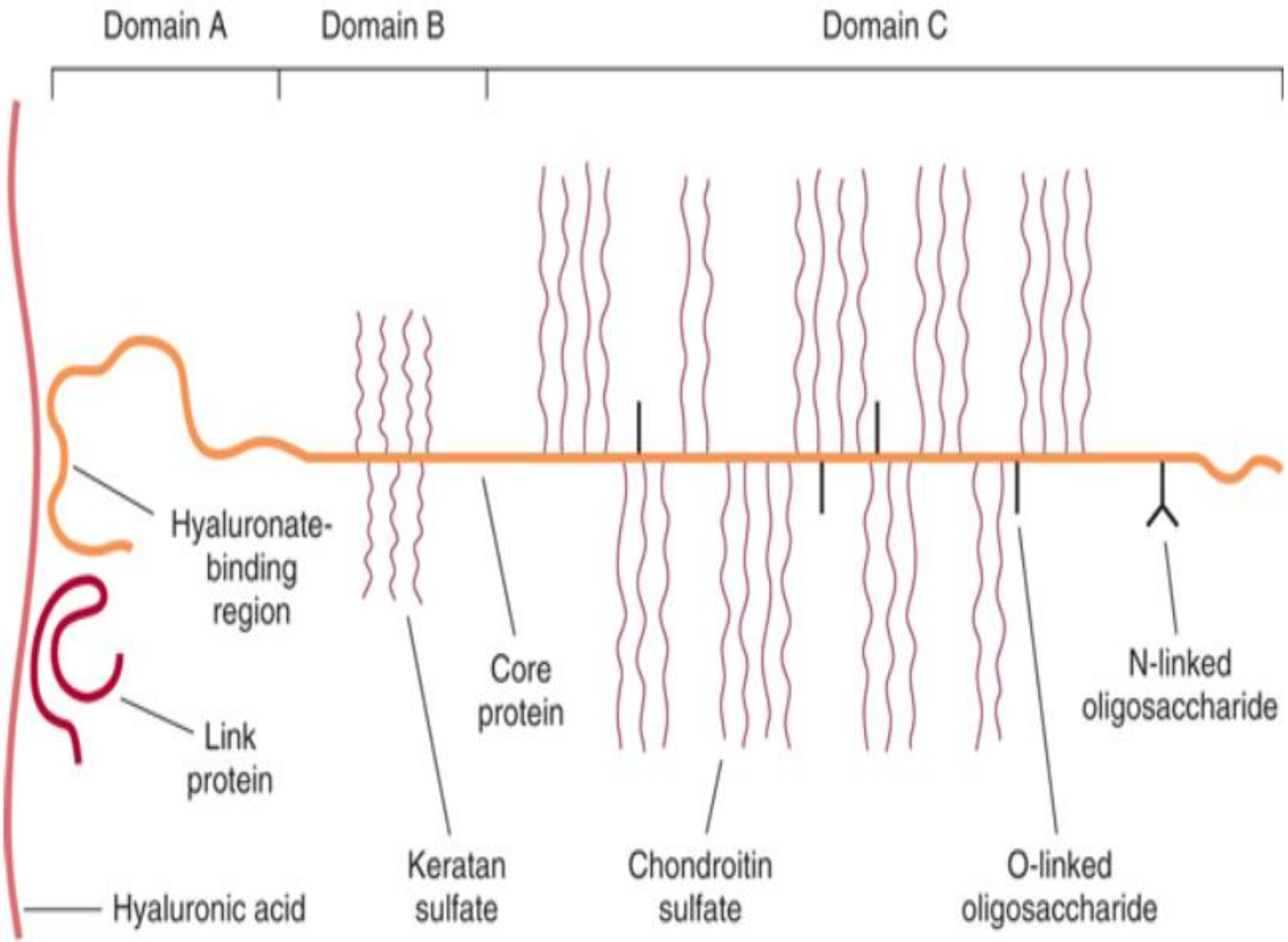


FIGURE 48–13 Schematic representation of the molecular organization in the cartilage matrix. Link proteins noncovalently bind the core protein (lighter color) of proteoglycans to the linear hyaluronic acid molecules (darker color). The chondroitin sulfate side chains of the proteoglycan electrostatically bind to the collagen fibrils, forming a cross-linked matrix. The oval outlines the area enlarged in the lower part of the figure. (Reproduced, with permission, from Junqueira LC, Carneiro J: *Basic Histology: Text & Atlas*, 10th ed. McGraw-Hill, 2003.)

- Regarding to the previous slide:

- The doctor talked about proteoglycans that we have in cartilage tissue and how they make the connection between the fibers collagen type II and this is the fiber of collagen as well as the other fiber that we here they are connected together what's we called proteoglycan which is similar to proteoglycan that we have seen earlier in the bone, and if you take a cross section in this area we can see the presence of core protein as well as chondroitin sulfate (carbohydrate molecules)that are integrated in collagen type II in order to have complete structure here and there is a hyaluronic acid that is connected to carbohydrate molecule with protein.

Schematic diagram of the aggrecan from *bovine nasal cartilage*



- Regarding to the previous slide:

- the difference here between the cartilages and bone structure is the presence of this molecule known as aggrecan .Aggrecan molecule is created by a gene that has repeated sequence like:ACGTA and these sequences repeat many times. Aggrecan can be longer or shorter and the length of aggrecan itself connected with hyaluronic acid for example (link protein)

- so the aggrecan plays role in texture of collagen and now a days we are studying in YU THE relationship between the length of aggrecan and repeated sequence and the occurrence of certain degenerative disorder. So it could be a genetically deposit disorder. Link or structure of agrrecan can has a role in story of degenerative disorder.So,this example here is provided by bovine nasal cartilage as aggrecan structure .we can see core protein and see the extension of chondroitin sulfate(carbohydrate molecules) and also we have connection bonds and other thing could be connected with aggrecan molecule.

THE MOLECULAR BASES OF THE CHONDRODYSPLASIAS INCLUDE MUTATIONS IN GENES ENCODING TYPE II COLLAGEN & FIBROBLAST GROWTH FACTOR RECEPTORS FGFR

Chondrodysplasias: are a mixed group of hereditary disorders affecting cartilage.

A number of them are due to a variety of mutations in the ***COL2A1*** *gene, leading to abnormal forms of type II collagen.*

One example is ***Stickler syndrome****,* ***manifested*** by degeneration of joint cartilage and of the vitreous body of the eye.

The best-known of the chondrodysplasias is **achondroplasia**, **the most common cause of short-limbed dwarfism.**

- Regarding to the previous slide:

- aggrecan molecule makes a kind of network connection between the protein that we have in cartilage as well as in order to have well formed structure .As a matter of this order that can be associated with the cartilage structure. Some individual can develop what we called chondrodysplasias disorder that can be associated with type II collagen mutations and FGFR mutations. If there is a mutation in collagen type II (a structural protein),we could have abnormal chondrocytes structure as well as the development of chondrodysplasia which is abnormal growth of the cartilage.

- FGFR stands for fibroblast growth factor receptor ,specific type 3 when it is mutated means that there is problem in signaling pathway that will lead to the disorder. We have also other proteins can be mutated, but the main reason is abnormal structure of collagen type II specially alpha1 chain will lead to development of chondrodysplasia.

Rejection letters from more than 20 medical schools

A pediatric orthopedic surgeon at Johns Hopkins Hospital

Michael Ain

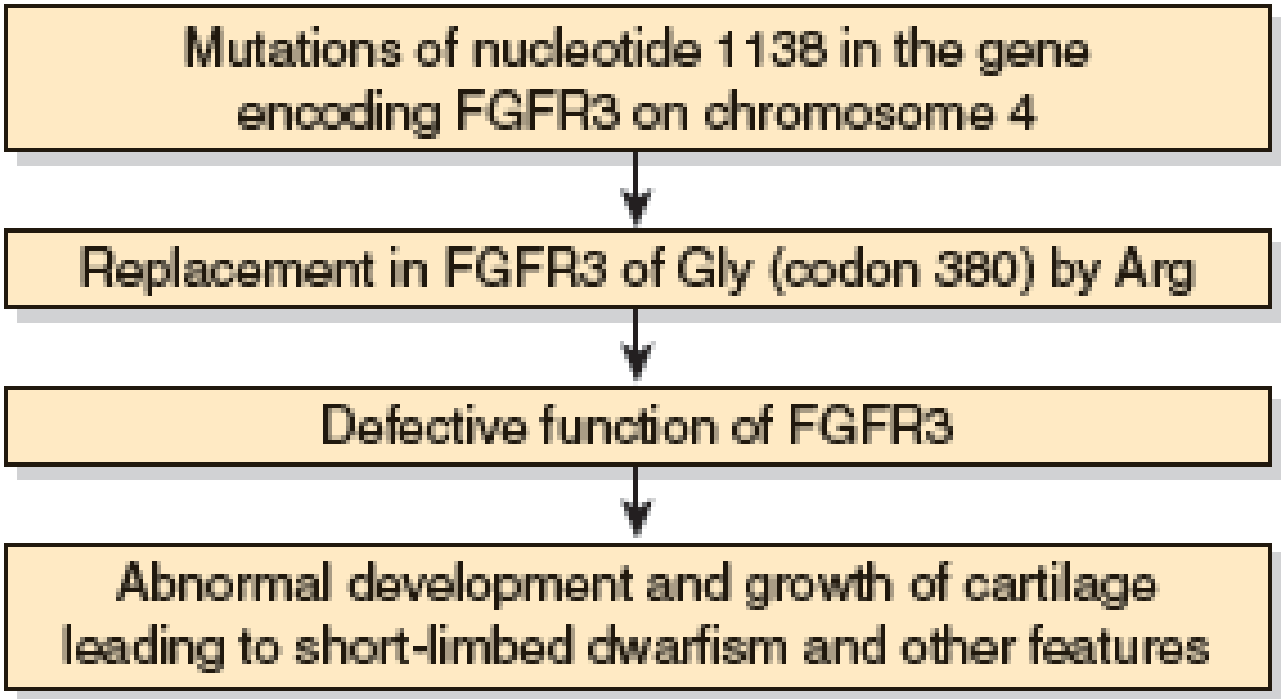


FIGURE 48–15 Simplified scheme of the causation of achondroplasia (OMIM 100800). In most cases studied so far, the mutation has been a G to A transition at nucleotide 1138. In a few cases, the mutation was a G to C transversion at the same nucleotide. This particular nucleotide is a real "hot spot" for mutation. Both mutations result in replacement of a Gly residue by an Arg residue in the transmembrane segment of the receptor. A few cases involving replacement of Gly by Cys at codon 375 have also been reported.

- Bone and cartilage are specialized forms of the ECM. Collagen I and hydroxyapatite are the major constituents of bone.

- Collagen II and certain proteoglycans are major constituents of cartilage.

- The molecular causes of a number of heritable diseases of bone (eg, osteogenesis imperfecta) and of cartilage (eg, the chondrodystrophies) are being revealed by the application of recombinant DNA technology.(important slide)