EXTRACELLULAR MATRIX 3

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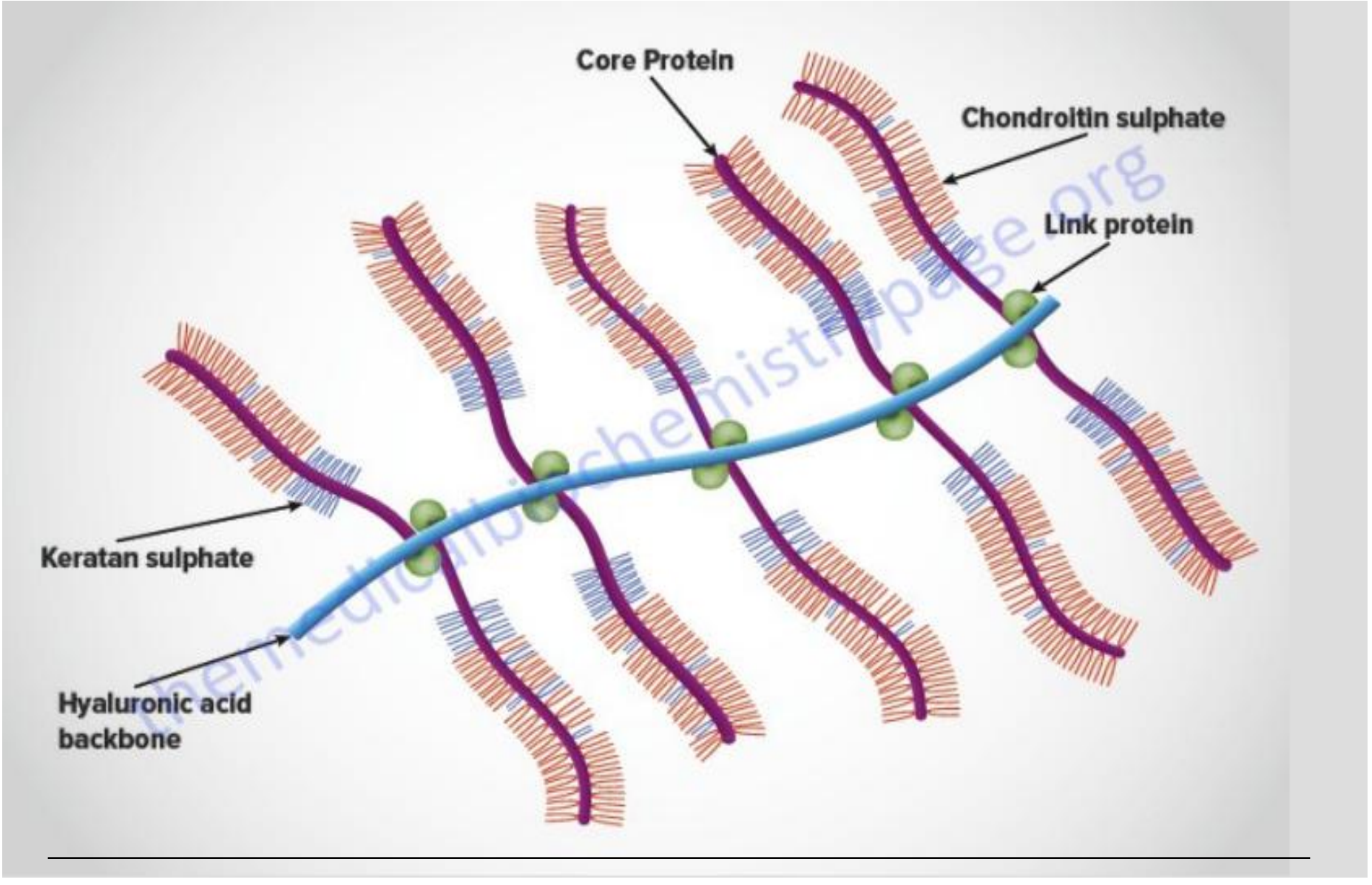
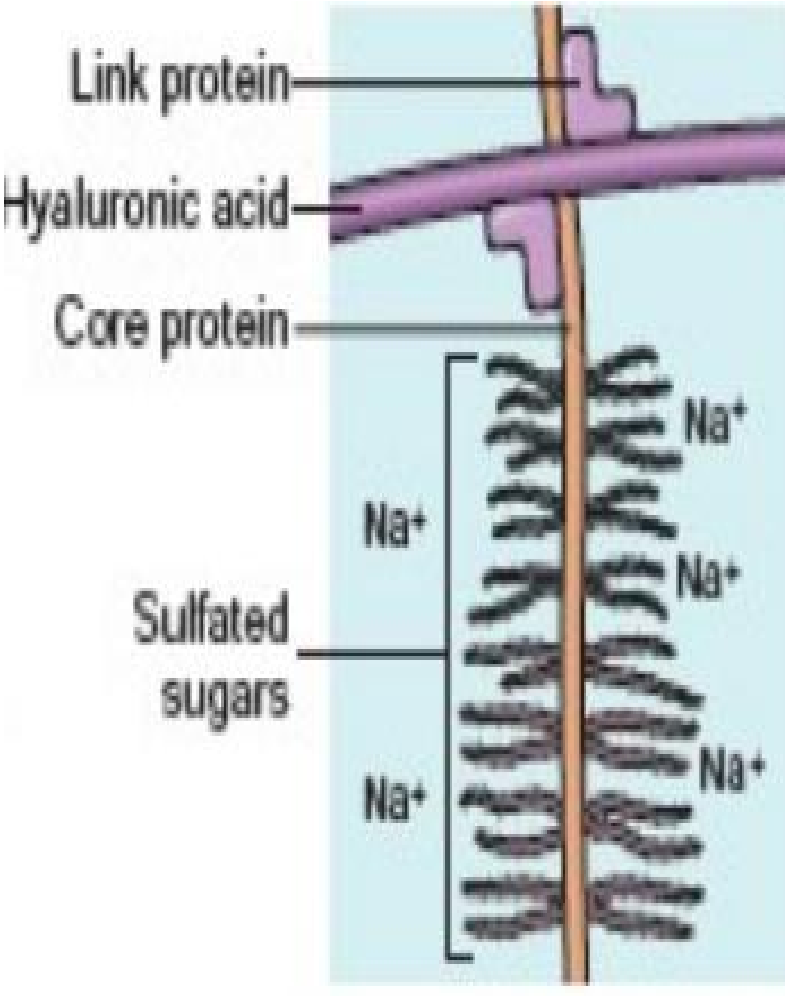
PROTEOGLYCAN

- A proteoglycans consists of a core protein bound covalently to GAGs, and these units form large complexes with other components of the extracellular matrix, such as hyaluronic acid or collagen.
- GAGs consist of repeating disaccharide subunits.
- Proteins linked covalently to glycosaminoglycans (GAGs). Carbohydrates make up about 95% of its weight.
- Proteins bound covalently to GAGs are called core proteins.
- Many have been classified; they vary in tissue of origin, function, core protein types.
- Examples include aggrecans, syndecan, betaglycan, serglycan

PROTEOGLYCAN



- The highly negatively charged sulfated sugars on the proteoglycan "bristles" recruit sodium and water to generate a viscous but compressible matrix.
- They have diverse role in regulating connective tissue structure and permeability (ie regulates movement of molecules through matrix).



- They also serve as reservoir of growth factors (eg FGF & HGF), they act as modulators of cell growth and differentiation.
- In joint cartilage they also provide layer of lubrication between bony surfaces.
- Some are integral part of cell membrane & have roles in cell proliferation, migration and adhesion
- Highly hydrated compressible gels that confer resistance to compressive forces.

	Proteoglycans	Glycoproteins
Structure	Core protein covalently attached to one or more glycosaminoglycan chains	Oligosaccharide chains covalently attached to proteins
Location	Connective tissues	Cell surface
Function	Combine with collagen to form cartilage, modulation of cellular development	Cell-to-cell recognition and signaling
Carbohydrate Content	50–60%	10–15%
Charge	Carbohydrate chains of proteoglycans are negatively charged	Carbohydrate chains of glycoproteins may or may not be negatively charged
Significance	The water associated with proteoglycans provides the cushion function of cartilage. The inability to sufficiently break down proteoglycans is linked to several genetic disorders and leads to other disease symptoms.	Carbohydrate modifications are essential to proper functioning of proteins. Changes in glycosylation patterns are common in cancer cells. Carbohydrates can also affect the performance of therapeutic antibodies.
Types	Chondroitin sulfate, dermatan sulfate, heparan sulfate, keratan sulfate, others	Collagens, mucins, transferrin, immunoglobulins, others

Glycoaminoglycans

- Unbranched polysaccharide chains composed of repeating dissacharide units.
- Negatively charged under physiological conditions (due to the occurrence of sulfate and uronic acid groups)
- Disaccharide subunits are:

1. Uronic acid

   D-glucuronic acid or

   L-iduronic acid

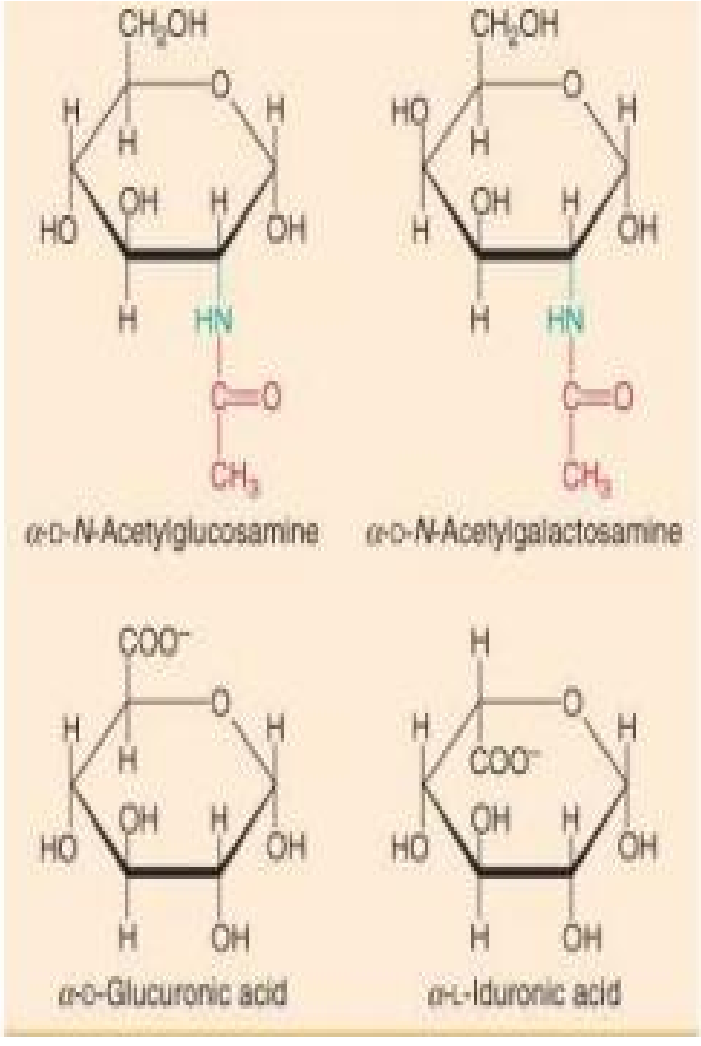
2. Aminosugar

   N-acetyl glucosamine (GlcNAc) or

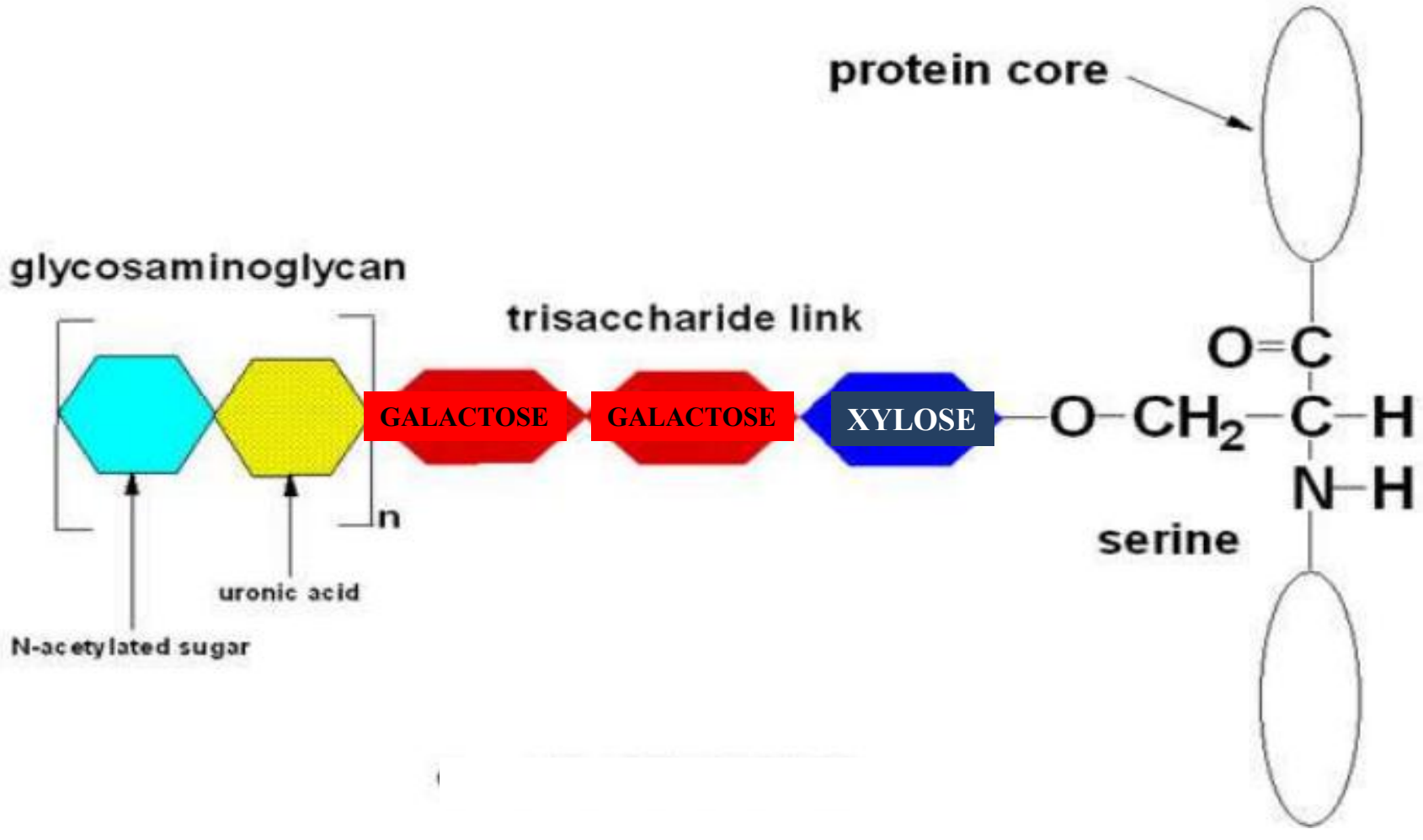
   N-acetyl galactosamin (GalNAc)

Glycoaminoglycans

- Amino sugars and uronic acids are the most common building blocks of the glycosaminoglycans.
- amino sugars -OH at C-2 is replaced by an amino group. This amino group is most often acetylated and sometimes sulfated.
- uronic acids C-6 of the hexose is oxidized to a carboxyl group.



Linkage of GAGs to protein core by specific trisaccharide linker



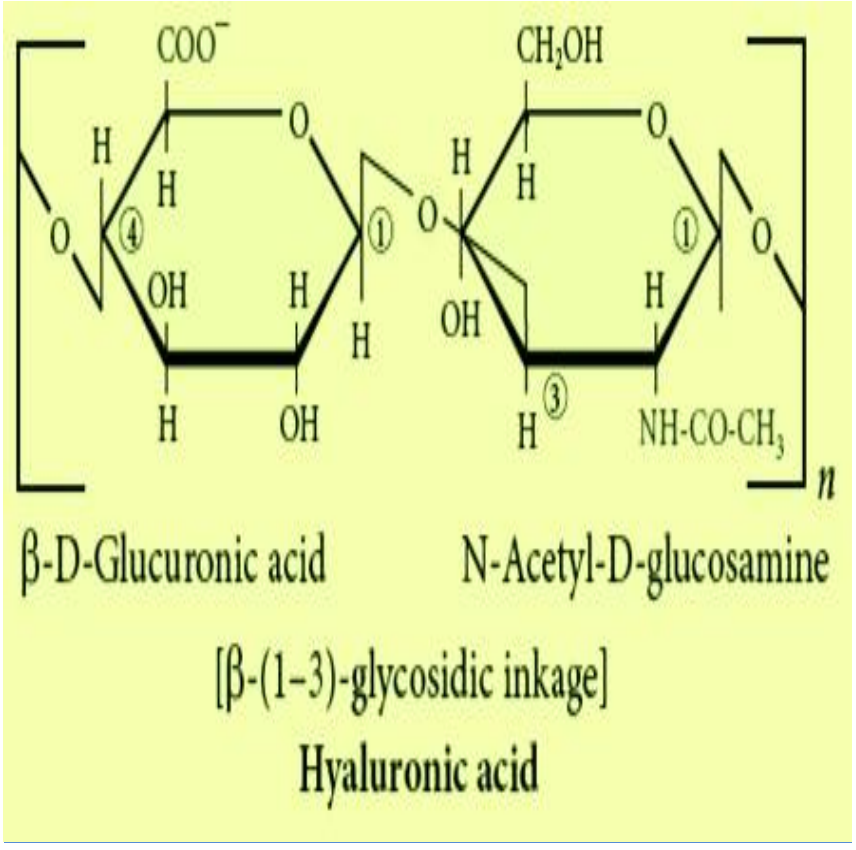
Types of GAGs

Seven types of GAGs

1. Hyaluronan
2. Chondroitin sulfate
3. Dermatan sulfate
4. Heparin
5. Heparan sulfate
6. Keratan sulfate I
7. Keratan sulfate II

1. Hyaluronan

- Made up of Unbranched, repeating units of GlcUA and GlcNAc
- It tends to have enormous carbohydrate chain
- Not covalently attached to a core protein



- The carbohydrates are not sulfated

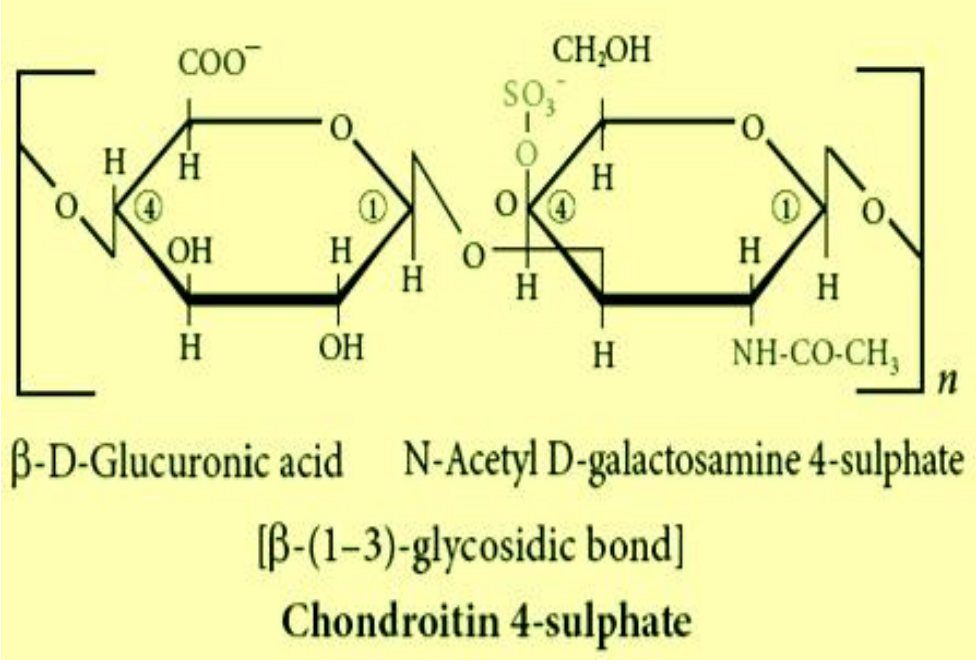
- Especially high in concentration in highly hydrated tissues such as skin and umbilical cord, and in bone, cartilage, joints (synovial fluid) and in vitreous humor in the eye, as well as in embryonic tissues

Hyaluronan

- permitting cell migration during morphogenesis
- Important in wound healing
- Its ability to attract water into the ECM triggers loosening of the matrix
- The high concentrations of hyaluronic acid together with chondroitin sulfates present in cartilage contribute to its compressibility
- Hyaluronidase an enzyme secreted by some bacteria helps with their invasion of tissues

2. Chondroitin sulfate

- Repeating unit of GlcUA and GalNAc
- Attached to a core protein through xyl-serine
- Sulfated carbohydrates
- Tends to have shorter polymers



β-D-Glucuronic acid N-Acetyl D-galactosamine 4-sulphate

[β-(1–3)-glycosidic bond]

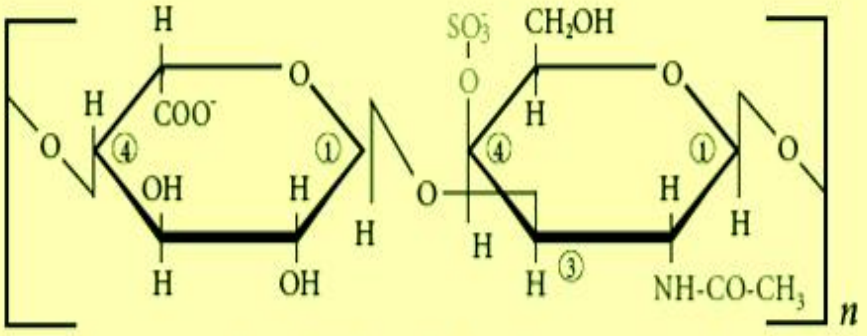
Chondroitin 4-sulphate

Chondroitin sulfate

- They are located at sites of calcification in endochondral bone and are a major component of cartilage.
- Provides tensile strength to cartilage, tendons, ligaments and walls of aorta
- Thought to act as signaling molecules in the prevention of the repair of nerve endings after injury.

3.Dermatan sulfate

- Made up of repeating IdUA and GalNAc.
- May also contain GlcUA
- Attached to a core protein through xyl-serine

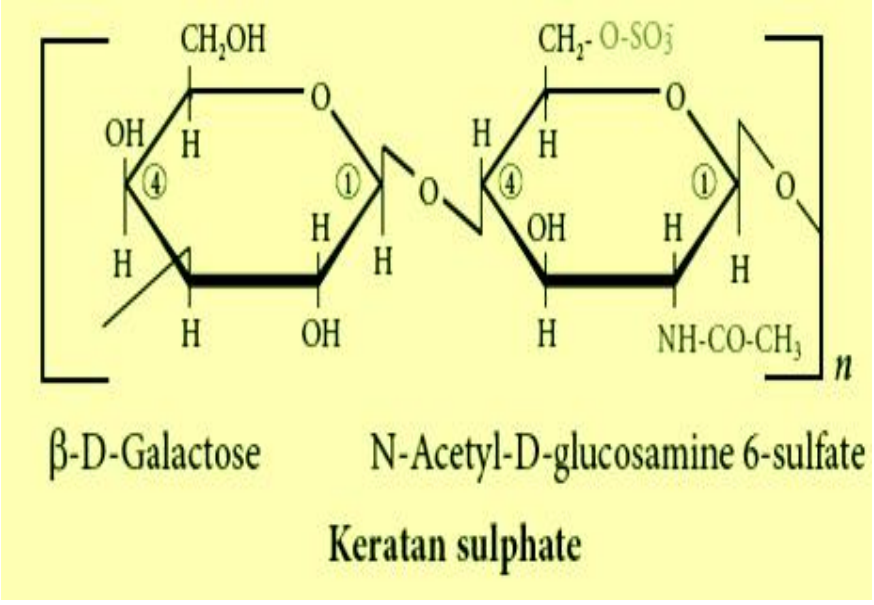


Dermatan sulphate

- Widely distributed troughout the body.
  Contributes to the pliability of the skin
- Evidence suggests it may play a part in blood coagulation, wound repair and resistance to infection

Keratan Sulfate (KS) I and II

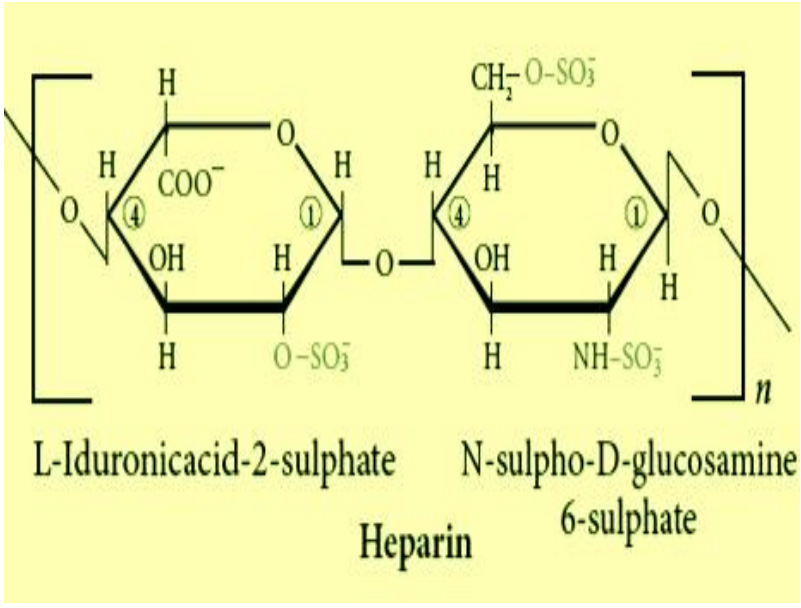
- Repeating units of Gal and GlcNAc
- KS I is attached to core protein through GlcNAc-Asp
- KS II is attached through GalNAc-Thr
- Present mainly in cornea, cartilage, bone



- In the eye, they lie between collagen fibrils and play a critical role in corneal transparency

Heparin

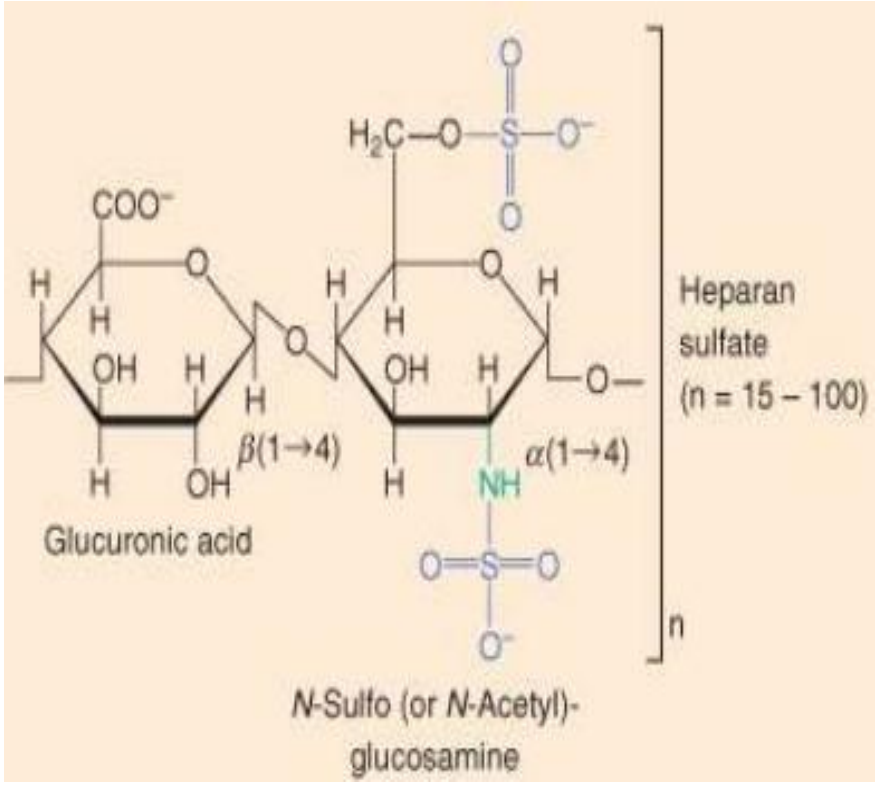
- Repeating units of GlcN (mostly sulfated but sometimes acetylated) and either of the gluconic acids mostly iduronic acid
- Heparin is linked to its core protein (mostly glycine and serine) through a bond with serine
- Heparin is mostly intracellular unlike rest of GAGs-in mast cells



- Involved in anticoagulation by binding factor factor IX, XI and Plasma antithrombin III
- Binds lipoprotein lipase in endothelial cell walls and puts them into circulation

Heparan sulfate

- Made up of GlcN and uronic acid predominantly glucoronic acid
- Attached to its core protein through xyl-serine
- Mainly extracellular



- Associated with the plasma membrane of cells, may act as receptors and may also participate in the mediation of the cell growth and cell - cell communication
- This proteoglycan is also found in the basement membrane of the kidney along with type IV collagen and laminin where it plays a major role in determining the charge selectiveness of glomerular filtration.

Synthesis of proteoglycans

- Starts with core protein synthesis from ribosomes on the RER
- The addition of GAGs takes place in the Golgi Apparatus
- The addtions of the GAGs to their core protein is of three types:
  1. O-glycosidic linkage between xylose and serine (xyl-gal-gal-glcua)
  2. O-glycosidic linkage between GalNAc and serine eg in Keratan sulfate II
  3. N-glycosylsamine bond between GlcNAc and asparagine

Elongation

- The units in the saccharide chains are elongated in alternating acidic/amino sugars, donated from UDP derivatives through specific glycosyl transferases

Further modifications

- Epimerization of glucoronic acid to iduronic acid catalysed by epimerases
- Sulfation of the amine sugars are catalysed by sulfo-transferases

Function of Proteoglycans

- organize water molecules
  - resistent to compression
  - return to original shape
  - repel negative molecules
- occupy space between cells and collagen
- high viscosity - lubricating fluid in the joints
- specific binding to other macromolecules

Function of Proteoglycans

- link to collagen fibers - form network - in bone combine with calcium salts (calcium carbonate, hydroxyapatite)
- cell migration and adhesion - passageways between cells
- anchoring cells to matrix fibers

Degradation of GAGs and Inborn Errors of Metabolism

- GAGs are degraded by specific lysosomal enzymes including exo and endoglycosidases, sulfatases
- Inborn error of metabolism affecting any of these enzymes results in accumulation of GAGs in lysosome mucupolysaccharidoses Eg. Hurler's and Hunter's syndrome

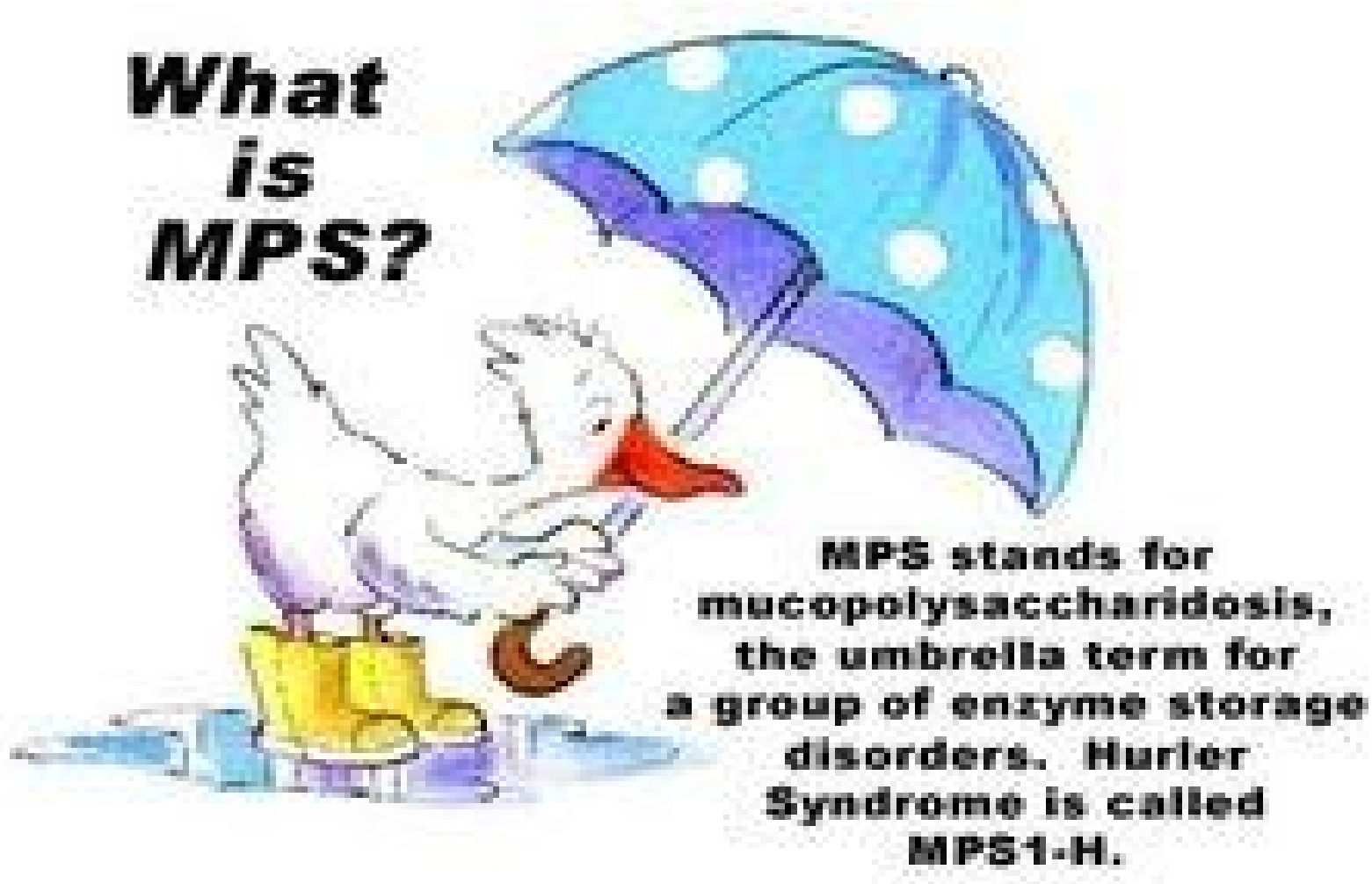


TABLE 50–8 The Mucopolysaccharidoses

Disease Name	Abbreviation[a]	Enzyme Defective	GAG(s) Affected	Symptoms
Hurler-, Scheie-Hurler-Scheie syndrome	MPS I	α-L-Iduronidase	Dermatan sulfate, heparan sulfate	Mental retardation, coarse facial features, hepatosplenomegaly, cloudy cornea
Hunter syndrome	MPS II	Iduronate sulfatase	Dermatan sulfate, heparan sulfate	Mental retardation
Sanfilippo syndrome A	MPS IIIA	Heparan sulfate-*N*-sulfatase[b]	Heparan sulfate	Delay in development, motor dysfunction
Sanfilippo syndrome B	MPS IIIB	α-*N*-Acetylglucosaminidase	Heparan sulfate	As MPS IIIA
Sanfilippo syndrome C	MPS IIIC	α-Glucosaminide *N*-acetyltransferase	Heparan sulfate	As MPS IIIA
Sanfilippo syndrome D	MPS IIID	*N*-Acetylglucosamine 6-sulfatase	Heparan sulfate	As MPS IIIA
Morquio syndrome A	MPS IVA	Galactosamine 6-sulfatase	Keratan sulfate, chondroitin 6-sulfate	Skeletal dysplasia, short stature
Morquio syndrome B	MPS IVB	β-Galactosidase	Keratan sulfate	As MPS IVA
Maroteaux-Lamy syndrome	MPS VI	*N*-Acetylgalactosamine 4-sulfatase[c]	Dermatan sulfate	Curvature of the spine, short stature, skeletal dysplasia, cardiac defects
Sly syndrome	MPS VII	β-Glucuronidase	Dermatan sulfate, heparan sulfate, chondroitin 4-sulfate,chondroitin 6-sulfate	Skeletal dysplasia, short stature, hepatomegaly, cloudy cornea
Natowicz syndrome	MPS IX	Hyaluronidase	Hyaluronic acid	Joint pain, short stature

Causation of a mucopolysaccharidosis

Mutation(s) in a gene encoding a lysosomal hydrolase involved in the degradation of one or more GAGs

Defective lysosomal hydrolase

Accumulation of substrate in various tissues, including liver, spleen, bone, skin, and central nervous system

Mucopolysaccharidoses (MPSs)

- Autosomal recessive (exception Hunter disease, X-linked recessive
- Hurler and Hunter syndromes (most widely studied)
- Chronic and progressive and affect multiple organs.

Many patients exhibit

- Organomegaly (eg, hepato- and splenomegaly)
- Severe abnormalities in the development of cartilage and bone
- Abnormal facial appearance
- Mental retardation.
- In addition, defects in hearing, vision and the cardiovascular system may be present.

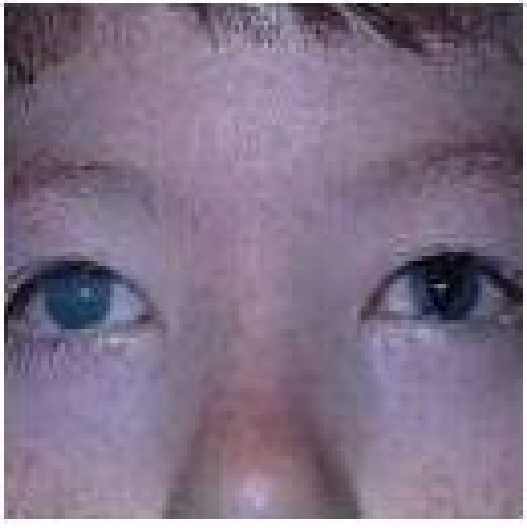
MPS I (Hurler)

- Deficiency of α-L-iduronidase
- Is a severe, progressive disorder with multiple organ and tissue involvement that results in premature death, usually by 10 years of age

Clinical features:

- Corneal clouding
- Hepatosplenomegal y
- Cardiomyopathy
- Dysostosis multiplex
- Mental retardation
- Coarse facial features

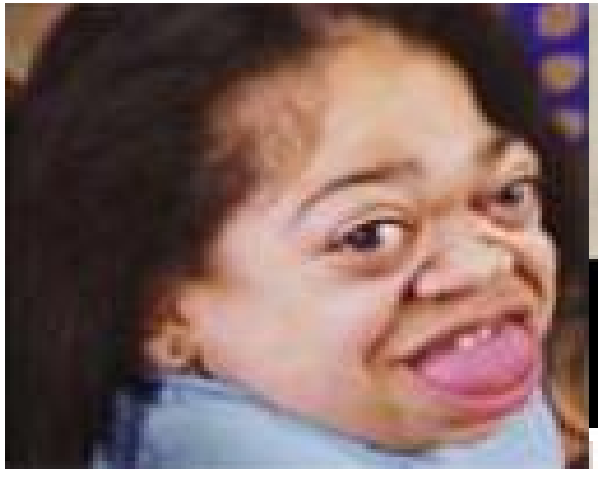
Coarse facial features
(flat nasal bridge, thick lips and large tongue, Prominent forehead)



Corneal clouding

Intraoral picture showing decayed teeth and macroglossia

skeletal dysplasia known as dysostosis multiplex



Large tongue

Short stature

Joint stiffness

Hurler Disease

BIOCHEMISTRY OF BONE

- Bone is made up of the matrix and the cells

Matrix

- Bone matrix is made up of organic and inorganic matter.
- Organic matter makes up about 20-40%

Inorganic matter - 60%

Water makes about 10%

Cellular Part

i. Osteoblast
ii. Osteoclast
iii. Osteocytes
iv. Osteoprogenitor

Matrix

Organic

Collagen Type I - 90-95%

Collagen Type V

Osteonectin

Osteocalcin

Proteoglycans (Biglycan, Decorin)

Inorganic

Hydroxyapatite – $Ca_{10}(PO_4)_6(OH)_2$

Octacalcium phosphate - $Ca_8H_2(PO_4)_6 .5H_2O$

Brusite – $CaHPO_4 .2H_2O$

Amorphouse calcium phosphates – $Ca_9(PO_4)_6$

Magnesium

Fluoride

Sodium

Metabolism

- Bone is a dynamic structure
- Undergoes remodelling in form of resorption and deposition of new bones
- Remodelling is under the influence of hormones and physical demands (eg weight bearing)
- Resorption of bones is performed by osteoclast
- Deposition of bones is performed by osteoblast
- Approximately 4% of compact bone and 20% of trabecular gets renewed annually

Osteoblast and bone deposition

- Osteoblast are mononucleated
- Descendants of mesenchymal marrow cells
- Lays down bone matrix (osteoid) - collagen, osteocalcin, osteonectin.

  Collagen type I and V

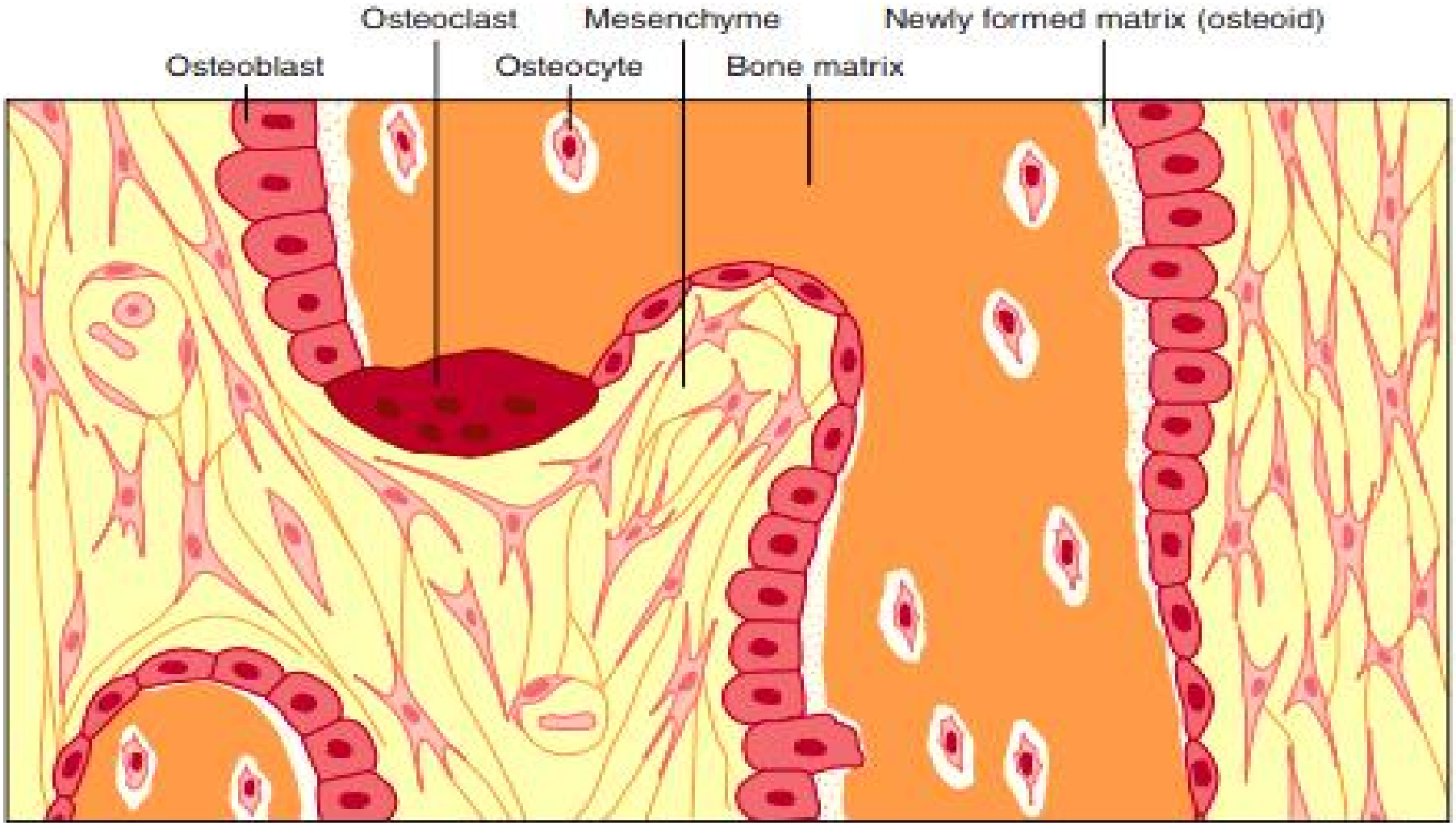
Osteocalcin

Protein with carboxylated glutamate with help of Vit K.

Acts as a dock for Ca^{2+} which finally reacts with phosphates to form hydroxyapatite

Osteonectin

osteoid protein that makes contact collagen I and hydroxyapatite



Schematic illustration of the major cells present in the membranous bone. Osteoblasts are synthesizing type I collagen, which forms a matrix that traps cells. As this occurs, osteoblasts gradually differentiate to become osteocytes.

- Osteoblast synthesize most of the proteins found in bones as well as growth factors and cytokines needed for mineralization.
- Osteoblast synthesize the new bone matrix called osteoid and cause its mineralization.
- They contain alkaline phosphatase in their apical surface which releases phosphates from organic phosphates.
- Bone proteins such as bone sialoproteins (e.g. tyrosin rich acid matrix proteins or TRAMP) and osteopontin bind calcium through their structural motiffs rich in aspartate and glutamate.

- These proteins provide the initial side of nucleation for mineralization which is facilitated by localized high calcium and phosphate concentration

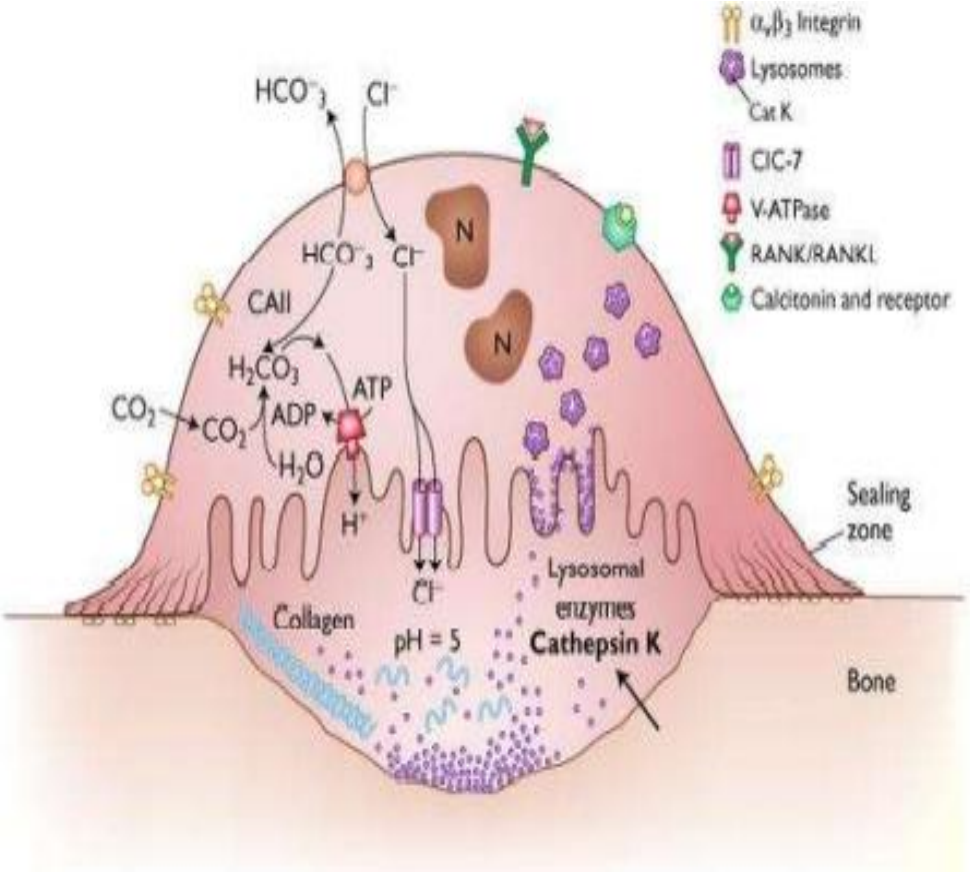
- Ionic product of Ca^{+2} and PO_4^{-3} of 70 or more (average = 40) stimulate mineralization.

- Osteoblast subsequently differentiate into osteocytes to maintain matrix.

OSTEOCLAST AND BONE RESORPTION

- Multinucleated cells, interspersed between osteoblast
- Cause resorption of bones
- These cells have ruffled border in their apical membrane which is in contact with bone matrix.
- Protons and lysosomal enzymes such as acid proteinase released into this area create a micro environment of low pH (below 4.0)
- The hydroxyapatite crystal solubilizes in this environment and bone proteins in matrix are degraded leading to bone resorption.
- Products of the bone resorption are taken up in the cytoplasm of osteoclasts for further digestion and transferred into capillaries

- Osteoclast seals off matrix to be resorbed
- H/K ATPase pump- pumps H+ into the matrix (p^{H}=4) increasing the solubility of hydroxyapatite
- Lysosomal acid hydrolases (acid phosphatases, collagenases, sulfatases, Cathepsin K) exocytosed into the matrix to hydrolyse the matrix
- HCO_3^- is extruded out of the cell to maintain intracellular pH



Regulation of bone metabolism

- Many factors are involved in the regulation of bone metabolism.
- Glucocorticoids – inhibition of bone formation.
- Growth hormone (GH) – stimulation of bone formation through somatomedins (growth factors IGF-1 and IGF-2).
- Insulin – stimulation of synthetic activity of osteoblasts.
- Thyroid hormones – stimulation of osteoclasts, activation of bone remodelation.

Regulation of bone metabolism

- Estrogens – inhibition of bone resorption (inhibition of osteoclastic activity through specific local factors).
- Catecholamines – antagonists of calcitonin.
- Prostaglandins – different classes of prostaglandins have different effect, which is dependent on concentration (10^{-9} – 10^{-7} mol/l stimulates synthesei of collagen, 10^{-6} inhibits collagen synthesis.

Calcium homeostasis

A. Parathyroid hormone (parathyroid)

- Released by low plasma calcium.
- Stimulates bone resorption.
- Prevents calcium excretion by kidneys.
- Stimulates calcitriol synthesis.

B. Calcitriol (1,25-diOH-Vit. D)

- 25-hydroxylation in liver
- 1-hydroxylation in kidney
- Stimulates bone resorption.
- Stimulates intestinal calcium absorption

Calcium homeostasis

C. Calcitonin (thyroid)

- Is released by high plasma calcium.
- Acts on bone osteoclasts to reduce bone resorption.
- Net result of its action is a decline in plasma calcium & phosphate.

CARTILAGE

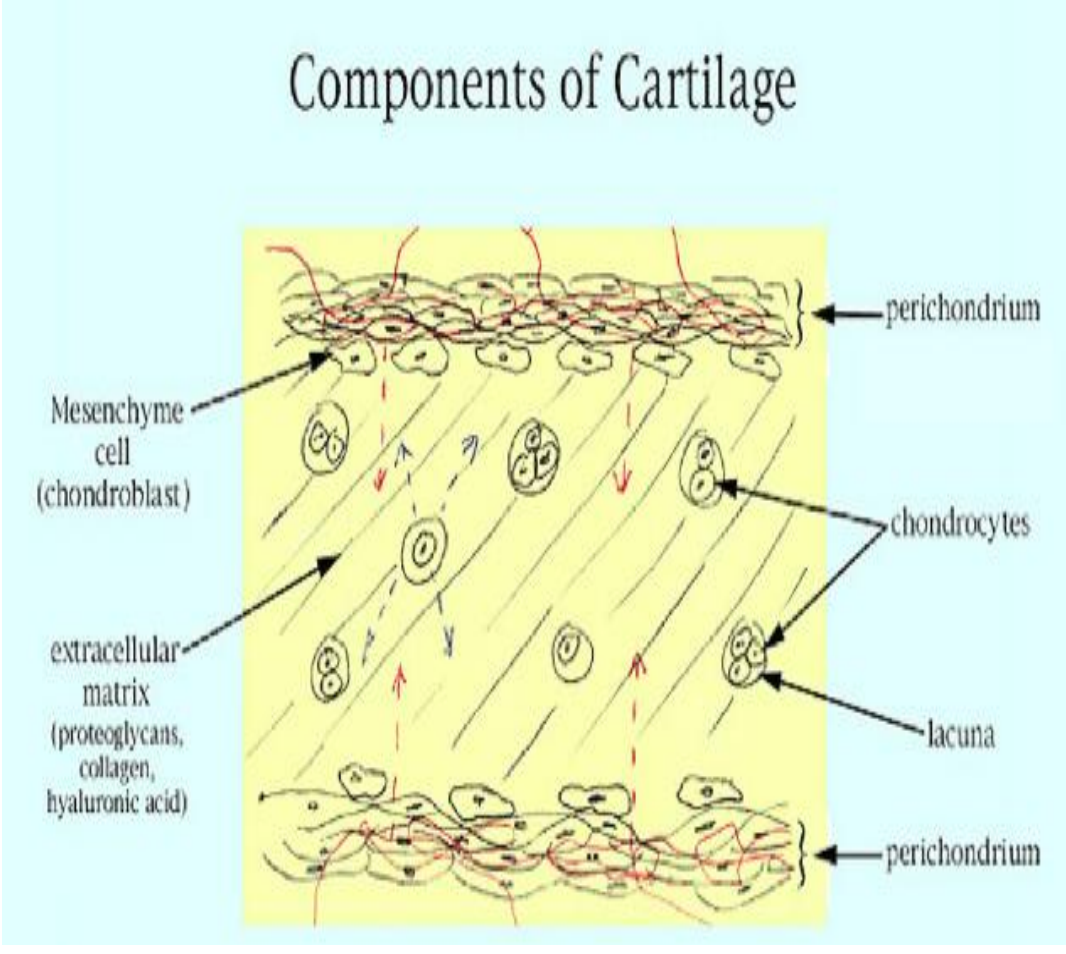
1. Hyaline

- Flexible and resilient

– Chondrocytes appear spherical

– Lacuna – cavity in matrix holding chondrocyte

– Collagen the only fiber



2. Elastic

- highly- bendable
- Matrix with elastin as well as collagen fibers
- Epiglottis, larynx and outer ear

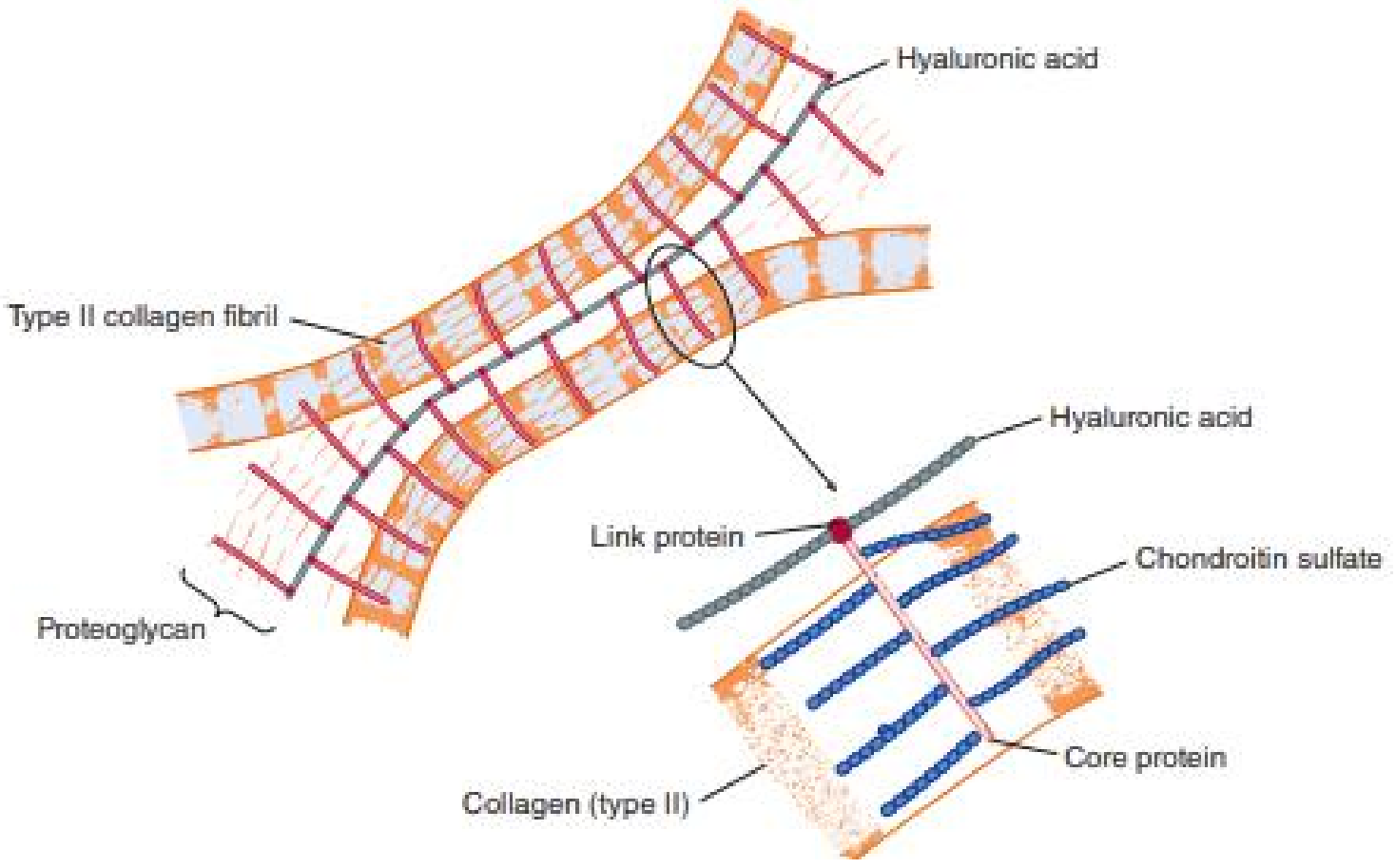
3. Fibrous

-resists compression and tension

- Rows of thick collagen fibers alternating with rows of chondrocytes (in matrix)
- Knee menisci and annunulus fibrosis of intervertebral discs

The Principal Proteins Found in Cartilage

Proteins	Comments
Collagen proteins	
Collagen type II	90–98% of total hyaline cartilage collagen. Composed of three α1(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.
Noncollagen proteins	
Cartilage oligomeric matrix protein (COMP)	An important structural component of cartilage. Regulates cell movement and attachment.
Aggrecan	The major proteoglycan of cartilage.
DS-PG I (biglycan)[a]	Similar to CS-PG I of bone.
DS-PG II (decorin)	Similar to CS-PG II of bone.
Chondronectin	Promotes chondrocyte attachment to type II collagen



Schematic representation of the molecular organization in the cartilage matrix.

Link proteins noncovalently bind the core protein (red) of proteoglycans to the linear hyaluronic acid molecules (gray). The chondroitin sulfate side chains of the proteoglycan bind to the collagen fibrils, forming a cross-linked matrix

CHONDROCYTES

- Progenitor cells arise in marrow
- Progenitor cells differentiate into chondroblast
- Chondroblast-secrete chondrin the primary substance in cartilage for building and repairing cartilage
- When chondroblast get completely surrounded by matrix-chondrocytes
- Chondrocytes in gaps called lacunae
- Functions to produce and maintain the extracellular matrix
- CHONDRONECTIN is involved in the attachment of type II collagen to chondrocytes (the cells in cartilage)

- Cartilage is an avascular tissue and obtains most of its nutrients from synovial fluid.
- It exhibits slow but continuous turnover.
- Various proteases (eg, collagenases and stromelysin) synthesized by chondrocytes can degrade collagen and the other proteins found in cartilage.
- Interleukin-1 (IL-1) and tumor necrosis factor α (TNFα) stimulate the production of such proteases.
- Whereas transforming growth factor β (TGFβ) and insulin-like growth factor 1 (IGF-I) generally exert an anabolic influence on the cartilage.

Cartilage Matrix

Composition

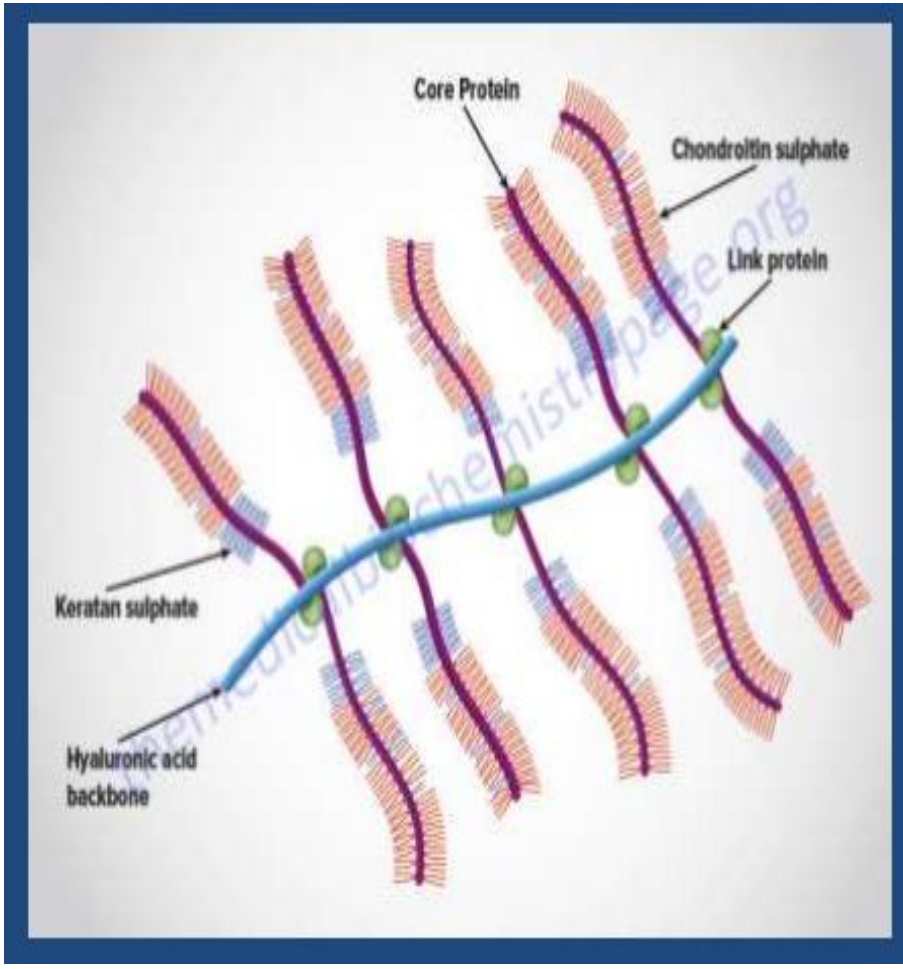
- Collagen –Type II (main matrix collagen) and I
- Elastin and fibrous cartilages contain elastin and type II collagen respectively
- Proteoglycans-

  Aggrecan is the main one.

  Others include chondronectin.

  Attaches to Collagen type II

AGGRECAN



Clinical Correlation-Bone and Cartilage

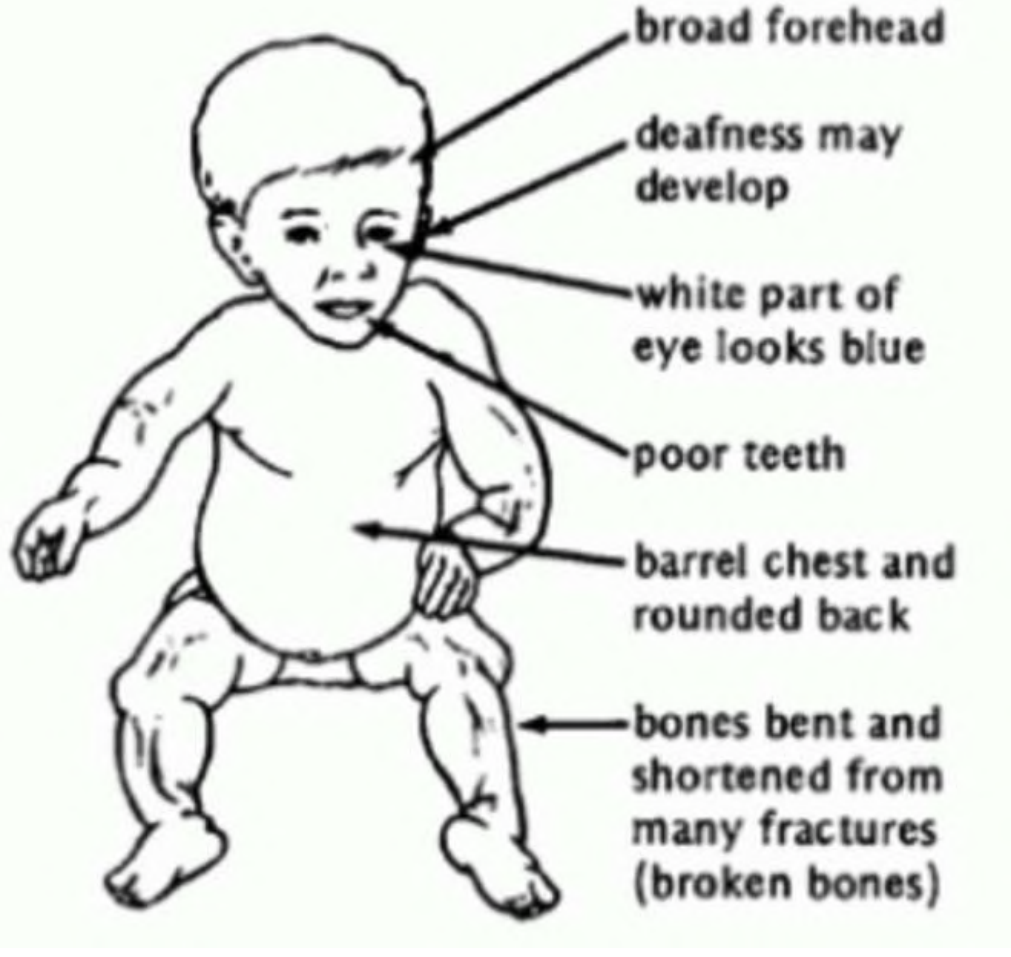
Osteogenesis imperfecta

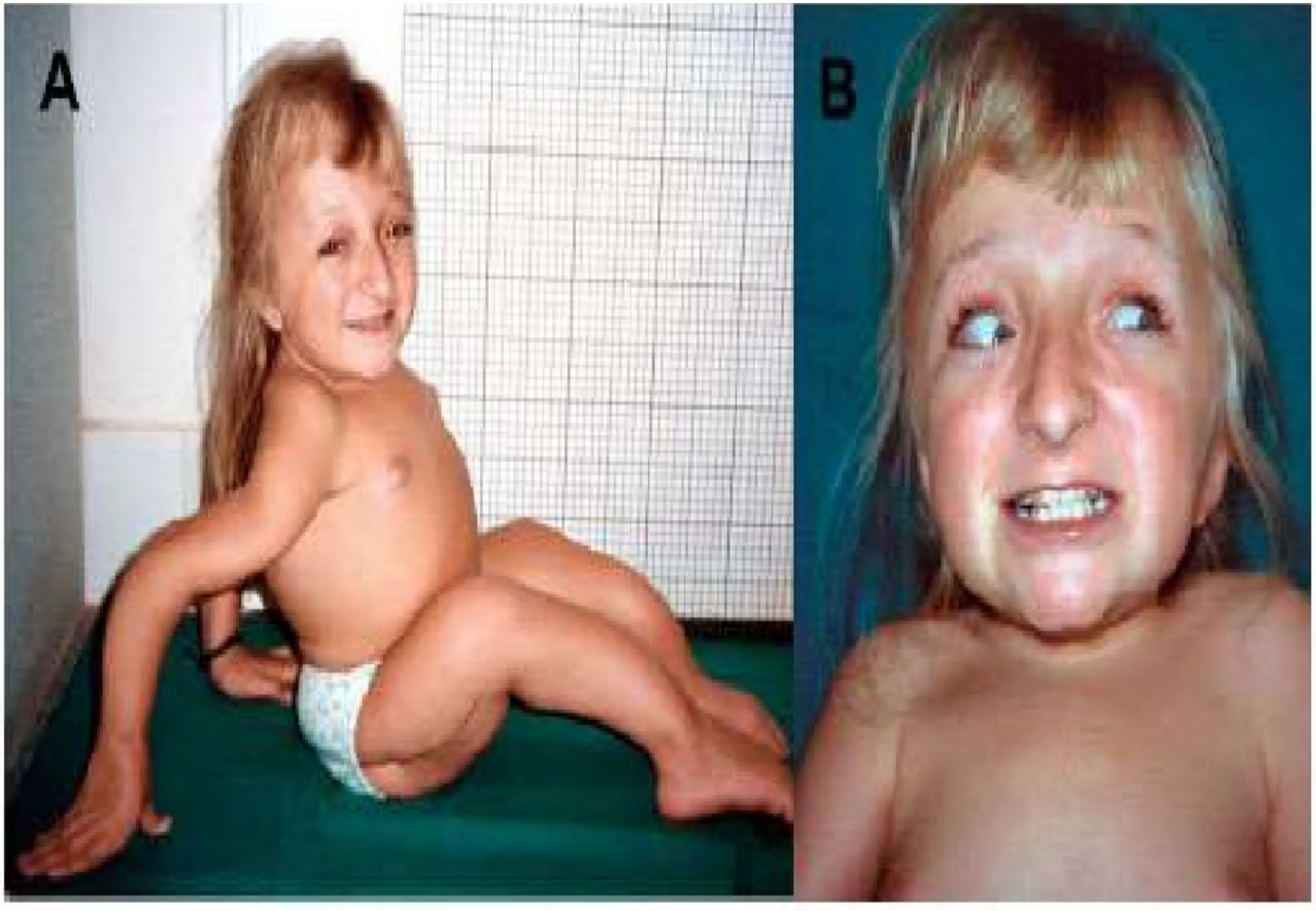
- Mutations in gene encoding type I collagen
- Leads to increased bone fragility
- scleras are often abnormally thin and translucent and may appear blue
- Severe forms-babies born with multiple fractures-mostly fatal
- Eight types (I-VIII) of this condition have been recognized.
- Types I to IV are caused by mutations in the COL1A1 or COL1A2 genes or both
- Mutation causes replacement of glycine by another bulkier amino acid, affecting formation of the triple helix.

Osteogenesis imperfecta

- These mutations result in decreased expression of collagen or in structurally abnormal pro chains that assemble into abnormal fibrils, weakening the overall structure of bone
- When one abnormal chain is present, it may interact with two normal chains, but folding may be prevented, resulting in enzymatic degradation of all of the chains. This is called "procollagen suicide
- Types V to VIII are less common and are caused by mutations in the genes for proteins involved in bone mineralization other than collagen

BRITTLE BONE DISEASE





Osteopetrosis (marble bone disease)

- Decreased ability to resorb bones
- Increased density of the bones
- Due to mutation in gene encoding carbonic anhydrase II.

- Deficiency of CA II in osteoclat prevent normal bone resorption, and osteopetrosis results.

- X-ray showing increased density in all the bones (bone in bone appearance)

BONE MODELLING AND REMODELLING

- MODELLING- during growth, skeleton increases in size by apposition of new bone tissue on outer surface of cortex.
- REMODELLING- It is a cellular process of bone activity by which both cortical and cancellous bone are maintained.
- Bone remodelling has two main functions-

  1. To repair micro damage within skeleton to maintain skeletal strength.

  2. To supply calcium to maintain serum calcium levels
- OSTEOPOROSIS results from bone loss due to age related changes in bone remodelling as well as extrinsic and intrinsic factors that exagerate this process.

Osteoporosis

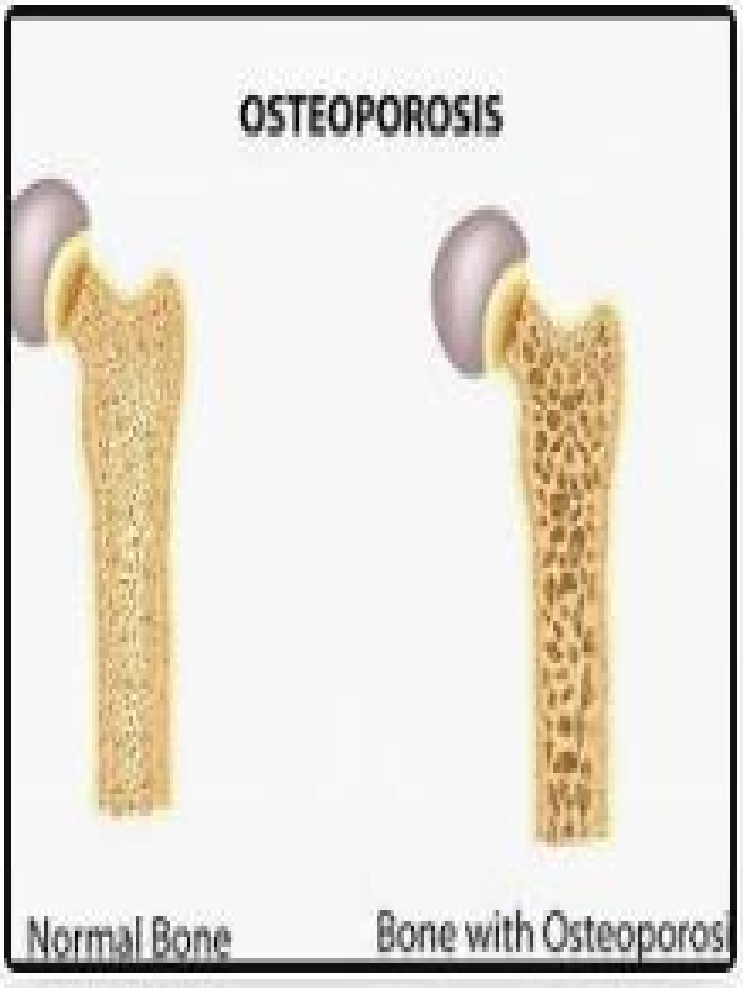
- Generalized progressive reduction in bone tissue mass per unit volume (densimetric studies) causing skeletal weakness.
- weak bones prone to fracture
- Resorption>deposition
- Primary- age related. Women>men
  1. Decrease in estrogen and androgen concentrations
  2. Reduced physical activity
  3. Insufficient vitamin D and calcium intake
  4. Reduced UV exposure, resulting in lower endogenous production of vitamin D
  5. Reduced renal function secondary to diabetes, arteriosclerosis, or analgesics abuse, resulting in insufficient 1-hydroxylation necessary to activate vitamin D

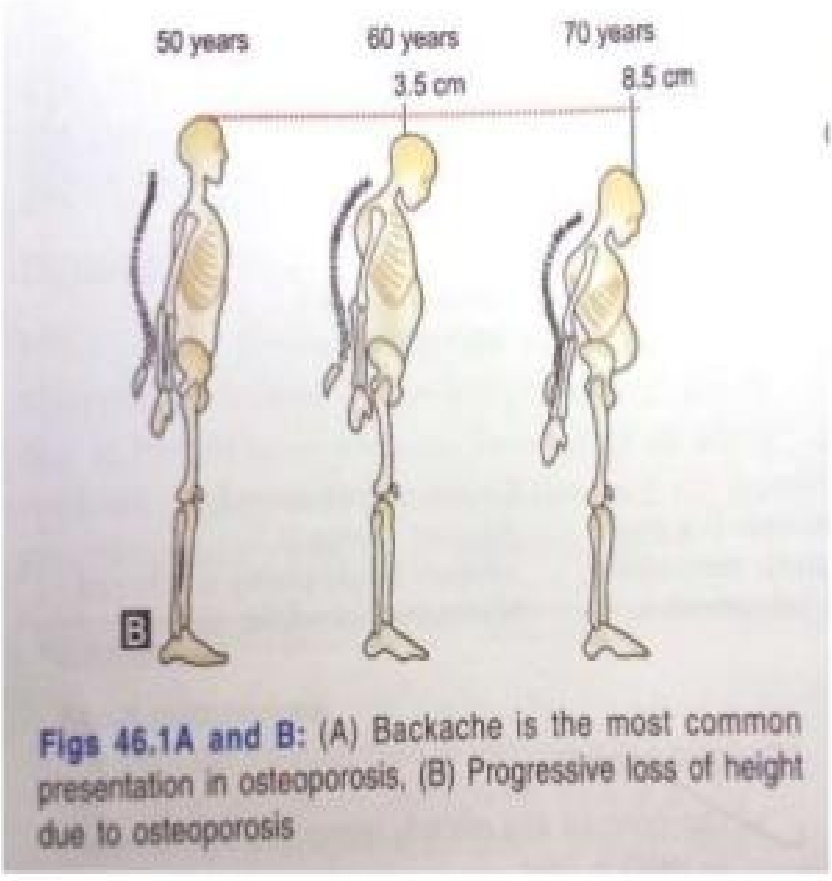
Dual-energy X-ray absorptiometry (DEXA)

- Gold standard method to determine bone mineral density.

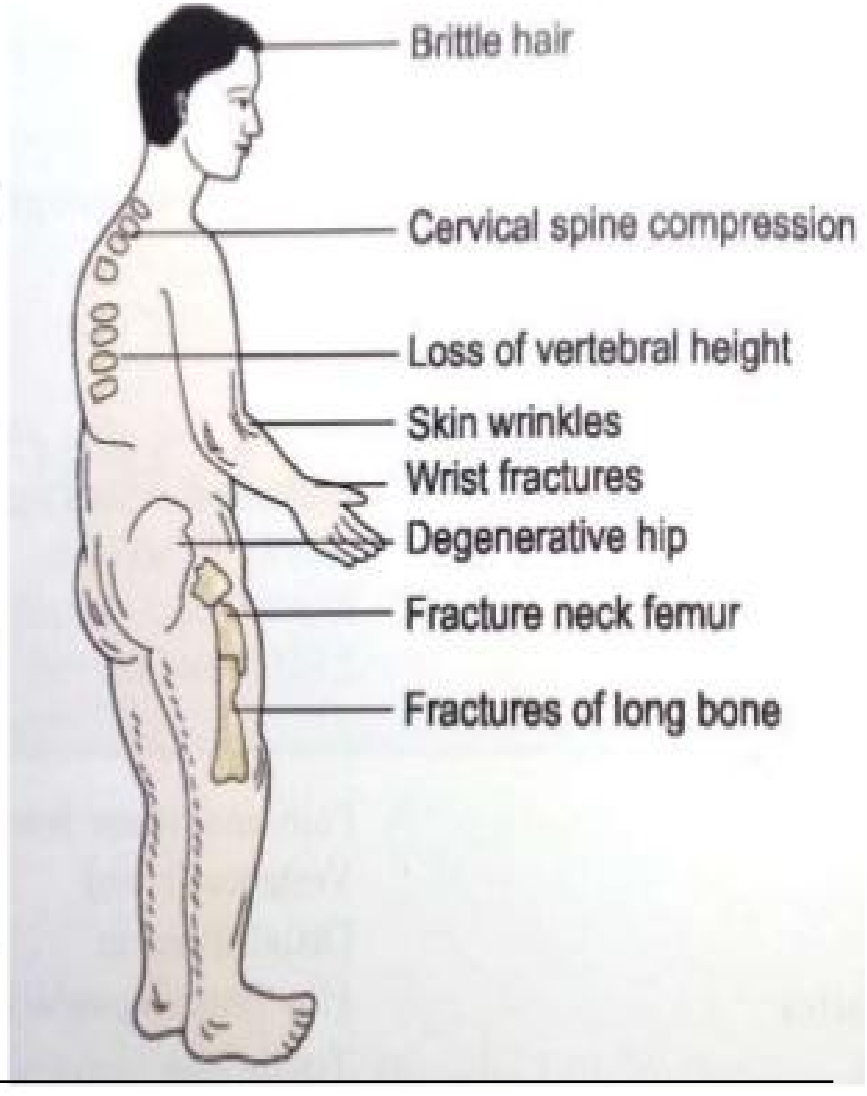
Advantages

1. Rapid and non invasive technique
2. Radiation exposure is minimal



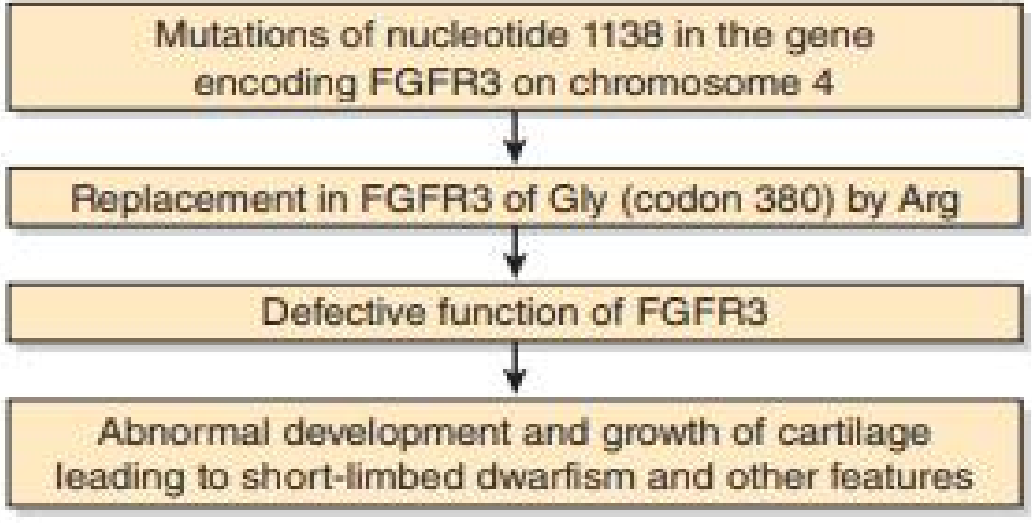


Figs 46.1A and B: (A) Backache is the most common presentation in osteoporosis, (B) Progressive loss of height due to osteoporosis



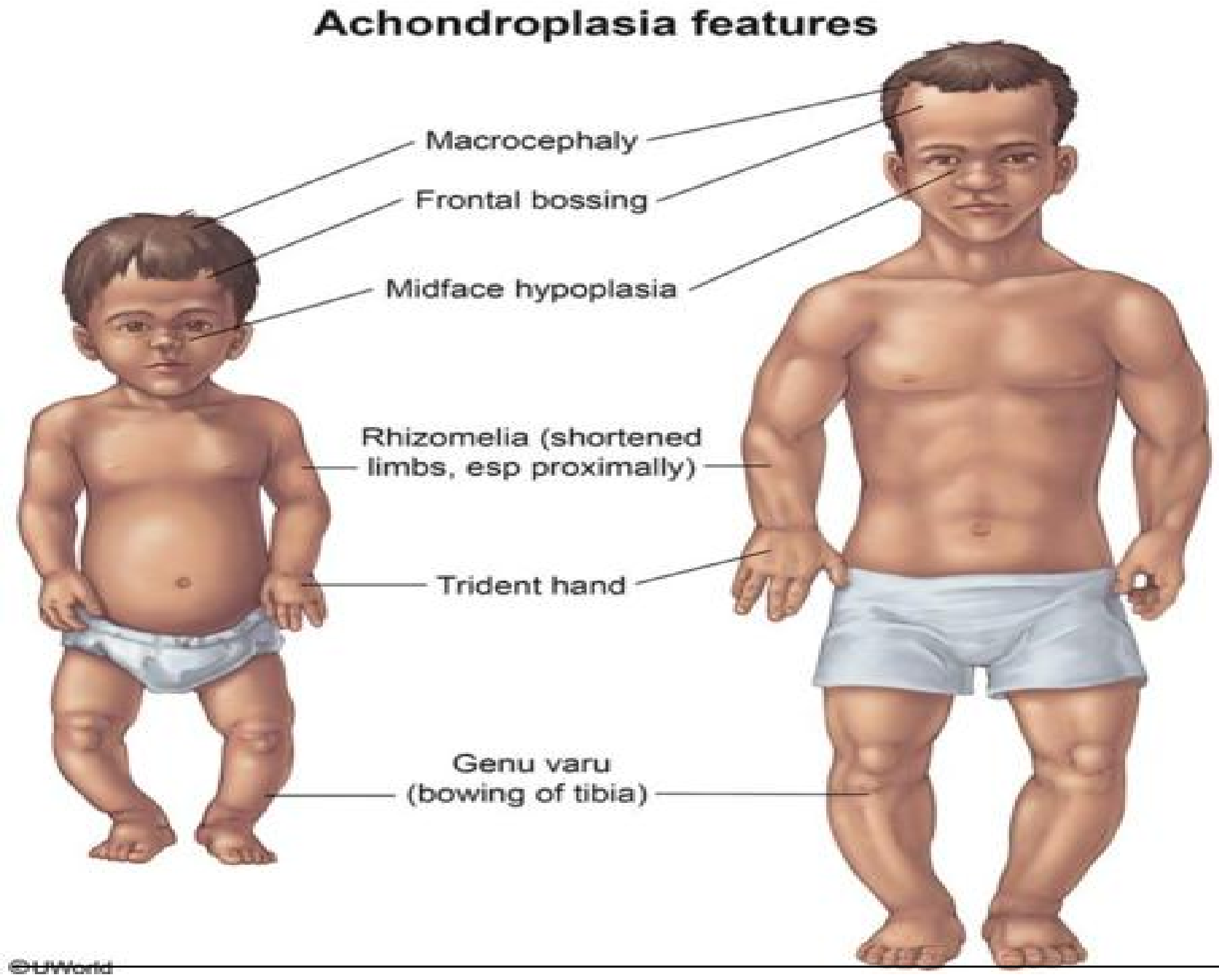
Dwarfism - Achondroplasia

- **Mutation in FGFR3 on chromosome 4 is responsible for achondroplasia .**
- The primary function of FGFR3 is to limit osteogenesis.
- Mutation causes enhancement in its function of limiting endochondral ossification. (↓ growth of proliferative zone of physis , ↓ thickness of hypertrophic cell zone → diminution in endochondral bone growth) .



Achondroplasia

- Affected individuals have short limbs, normal trunk size, macrocephaly, and a variety of other skeletal abnormalities.
- often inherited as an autosomal dominant trait,



Rickets

Lack of vitamin D in children

1. Bones of children are inadequately mineralized causing softened, weakened bones
2. Bowed legs and deformities of the pelvis, skull, and rib cage are common

Osteomalacia

Lack of vitamin D in adults

1. Bones are inadequately mineralized causing softened, weakened bones
2. Main symptom is pain when weight is put on the affected bone

10 important clinical features in Rickets