

# «Αρθρώσεις-αρθρικός υμένας-Αρθρικός χόνδρος (Δομή και φυσιολογία)»

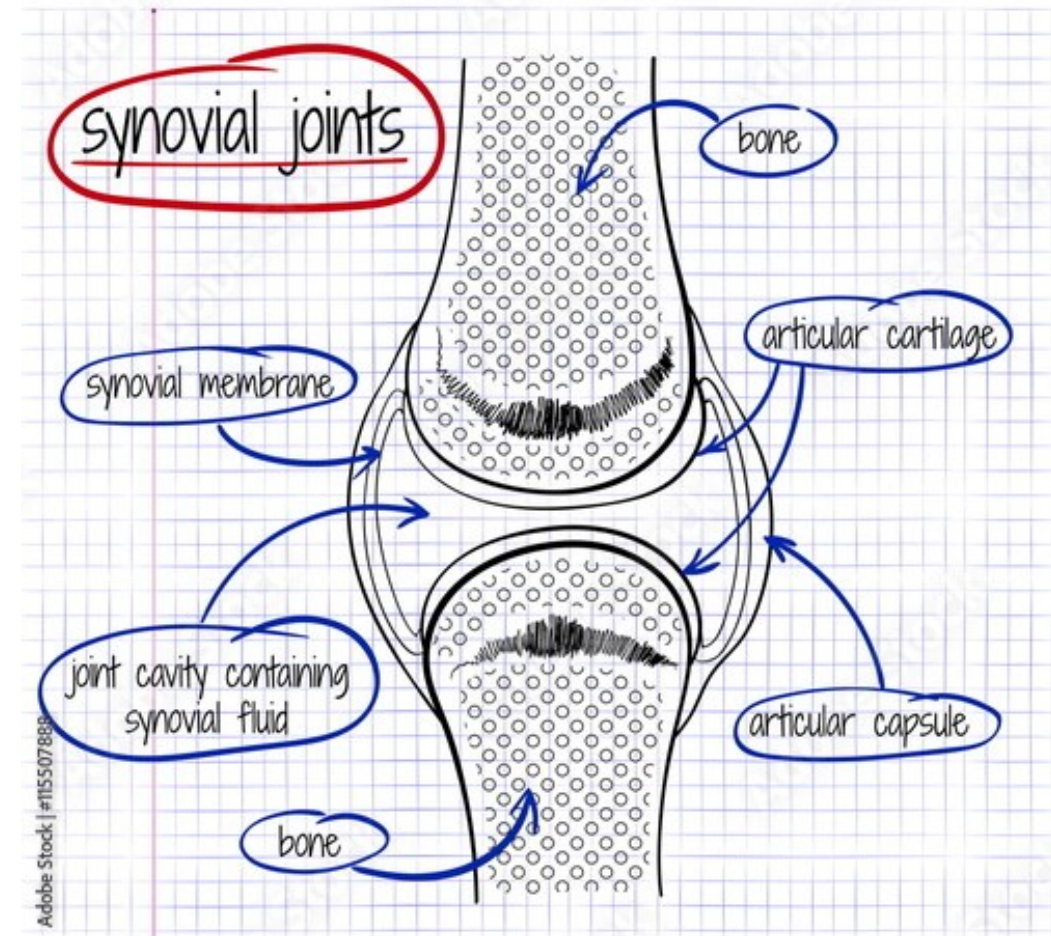
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# Εκπαιδευτικοί στόχοι

- Αρθρικός υμένας & Χόνδρος:
  - κύτταρα – φυσιολογία – παθολογία

# Normal synovial joint

- The synovial joint comprises opposing bones with articular surfaces that are covered by cartilage.
- The main protein in bone is **type I collagen**, whereas cartilage comprises mainly **type II collagen and proteoglycan molecules**.
- The non-articulating surfaces of synovial joints are lined by a thin adventitious layer known as the synovium.
- Normal synovial tissue comprises **one to three layers of specialized columnar fibroblasts-like synovioocytes (FLSs)** that are interspersed with **macrophages**
- The entire structure is enclosed by a **fibrous capsule and, together with ligaments, muscles and tendons**, the fibrous capsule confers strength and stability to the joint.



# Joint Remodeling - Homeostasis

- The joint is a dynamic environment that is subject to minor trauma continually — owing to movement and, in some joints, compression due to weight bearing — and is therefore **subject to continued wound healing and repair processes.**
- Continual remodeling of the articular cartilage and adjacent bone is therefore necessary, and this process requires **a balance of anabolic and catabolic enzyme activity in both cartilage and bone.**
- Carefully regulated proteolytic enzymes are responsible for maintaining the balance between anabolic and catabolic processes within the joint and cartilage.

# Function of the synovium

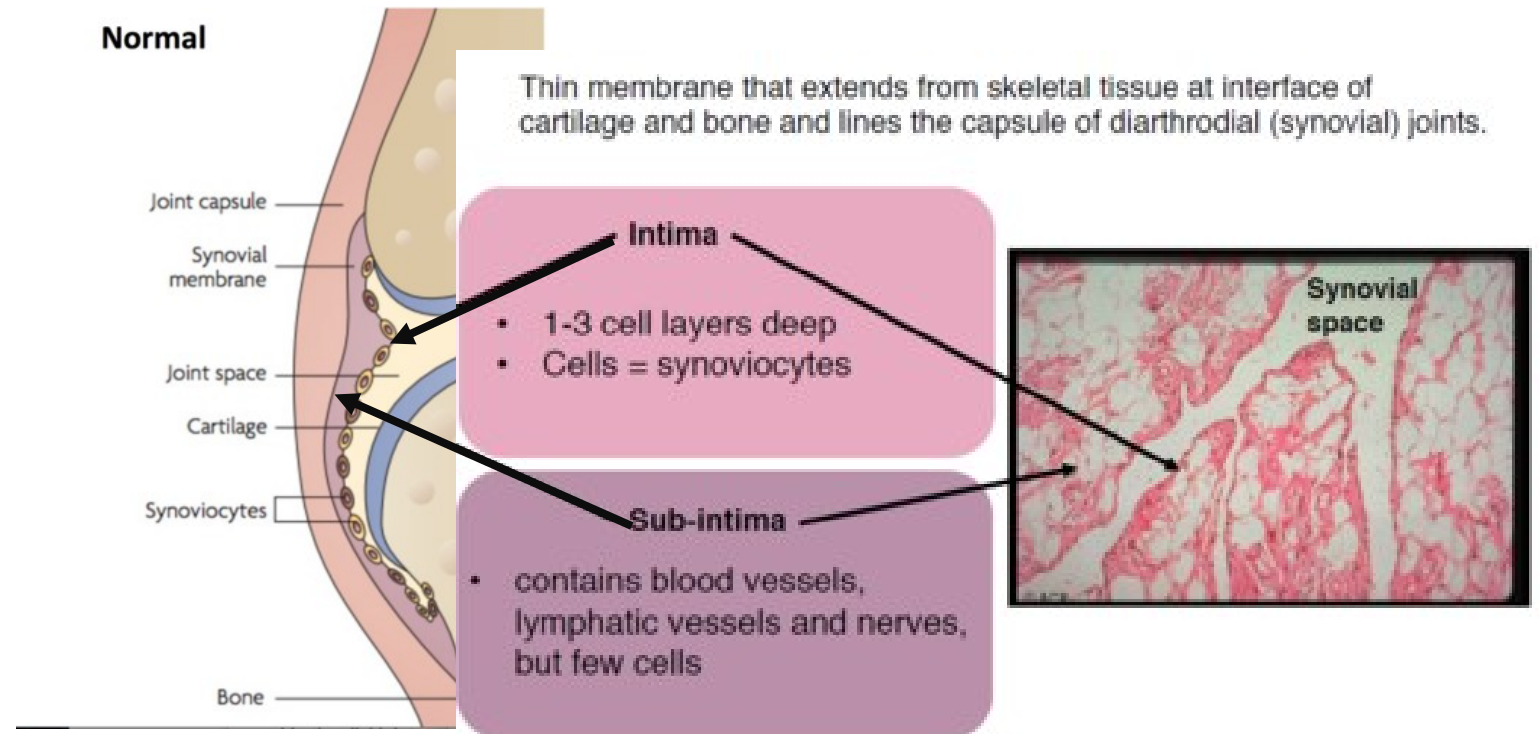
## 1. Maintenance of intact, non-adherent surface of the joint

- Hyaluronan (HA) (glycosaminoglycan-polysaccharide)
  - Produced by FLS
  - Inhibits adhesion
  - “Trapped” in the synovium”

## 2. Cartilage lubrication

- Lubricin

## 3. Chondrocyte nutrition



# Function of the synovium

## 4. Control of synovial fluid (SF) volume - composition

SF= plasma + HA

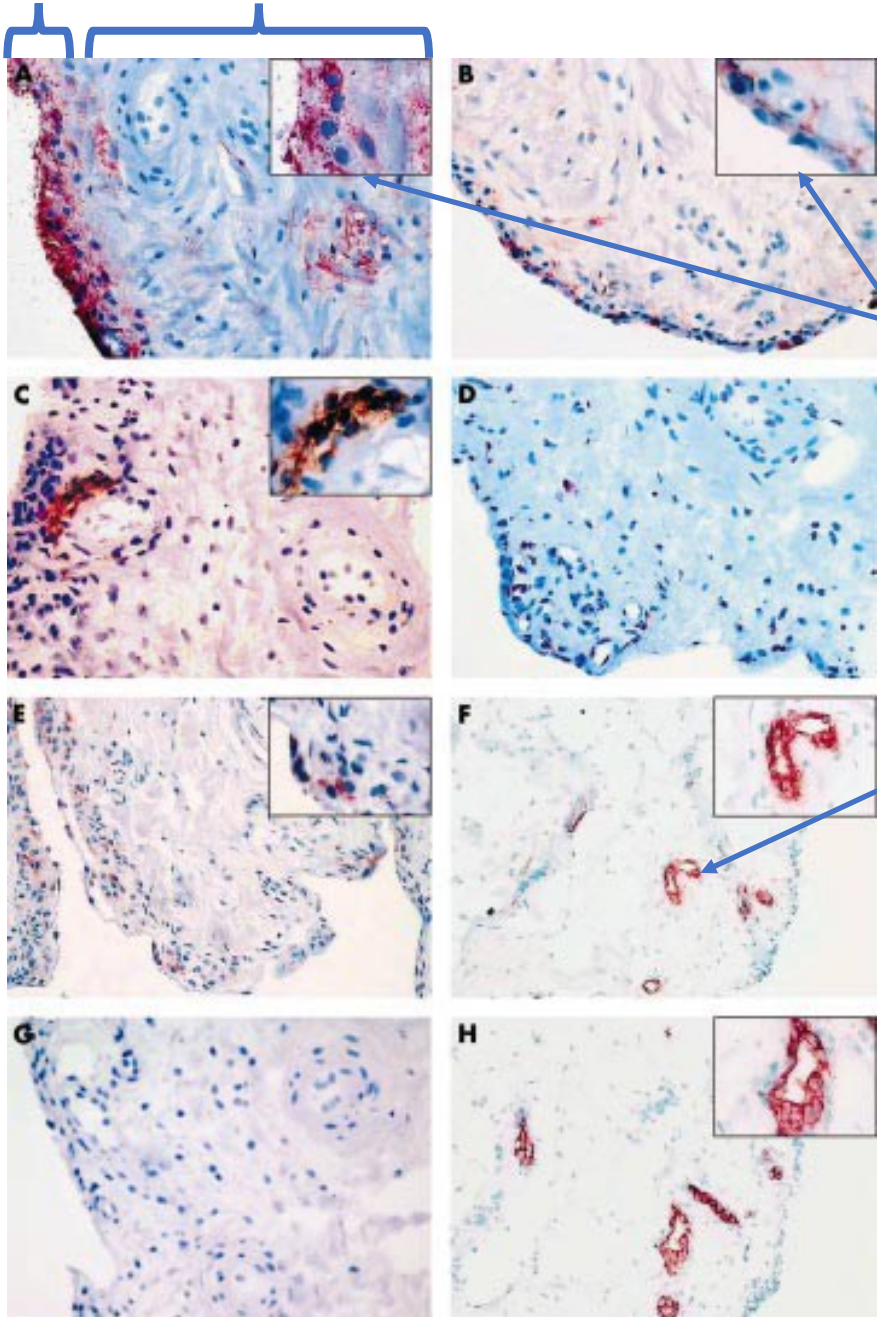
- SF Volume:
  - increased by hyaluronan
  - decreased by (–)ve hydrostatic pressure

### ✓ Mechanism of effusions

1. Mechanical irritation (*friction forces or debris of cartilage*) of FLS →  
↑HA → ↑volume
2. Inflammation → increase vascular permeability → ↑ volume

Lining (Intima)      Sub-Lining

# Normal synovium - Synovial cells



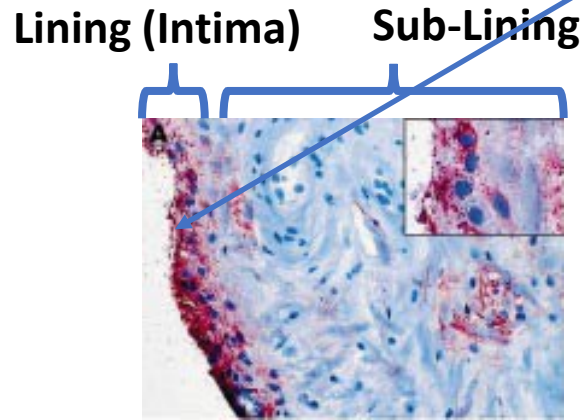
Immunohistochemical labelling of a representative **normal synovial** membrane stained with:

- (A) anti-CD55 (fibroblast-like synoviocytes - FLS)
- (B) anti-CD68 (macrophages)
- (C) anti-CD3 (T cells)
- (D) anti-IL1b
- (E) anti-interleukin 1 receptor antagonist (anti-IL1Ra)
- (F) anti-factor VIII (endothelial cells)
- (G) anti-RANKL
- (H) monoclonal antibody for OPG.

1. Lining (Intima) → FLS & Mφ
2. Sublining layers

# Normal synovium - Synovial cells

## Lining (Intima)

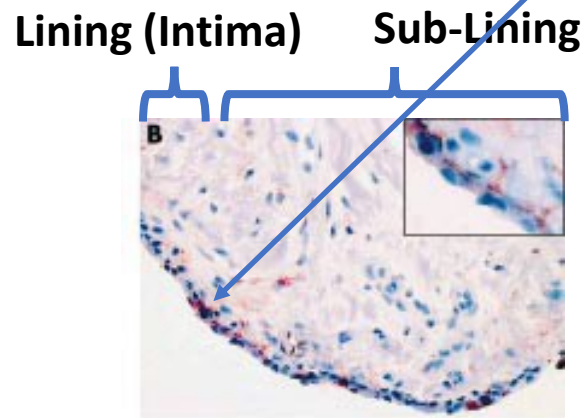


- **Fibroblast-like synoviocytes – FLS**

- UDPGD (UDP-glucose to UDP-glucuronate Hyaluronan production)
- CD55
- ICAM1
- VCAM1 (allow PMNs to traffic to SF BUT NOT monocytes)

- **Macrophages**

- CD163
- CD68
- CD14
- FcγRI (Ig receptor (ACPA/RF) local inflammation)

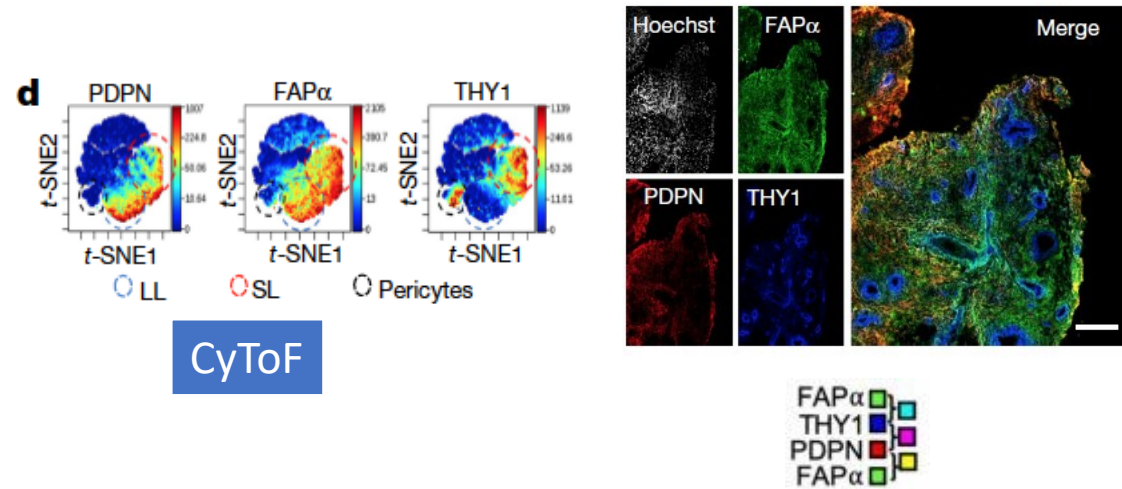


- **Matrix**

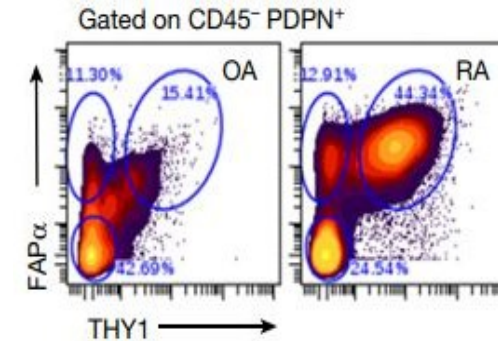
- No basement membrane
- **Rich in hyaluronan**
- **Lubricin (glycoprotein)**

# Distinct FLS subsets drive inflammation & damage in RA: Lining & sub-lining FLS

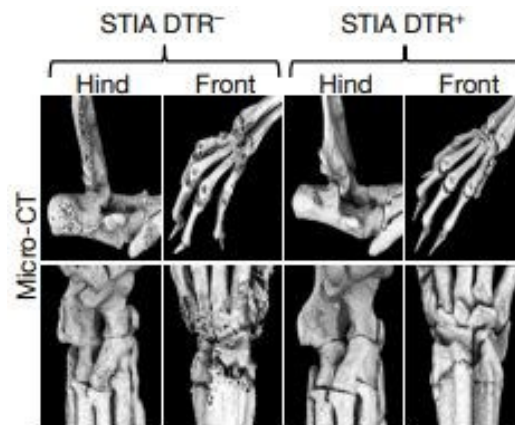
## Human RA synovium: 3 subtypes of FLS



## Expansion of THY1+ FLS in human RA synovium



## FLS depletion ameliorates synovitis in RA mouse models

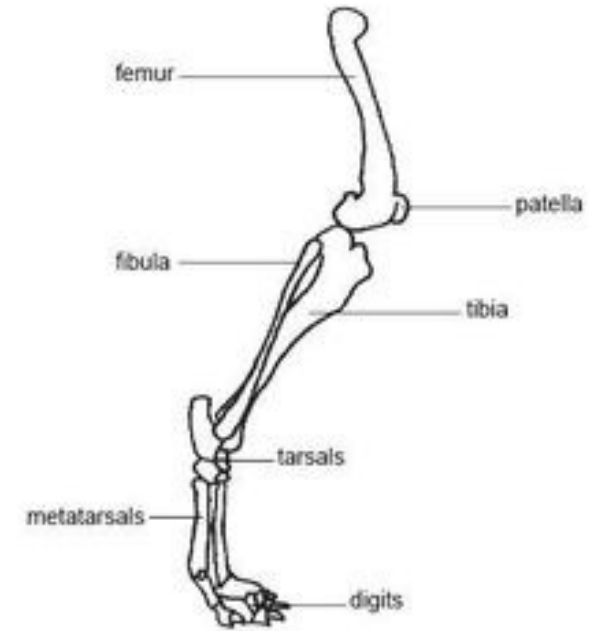
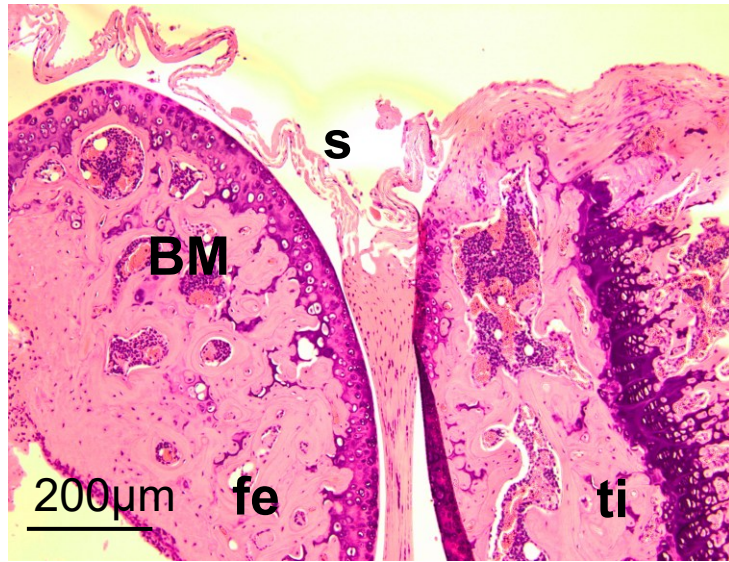


Adoptive FLS transfer experiments documented differential function of sub-lining (SL) & lining (LL)

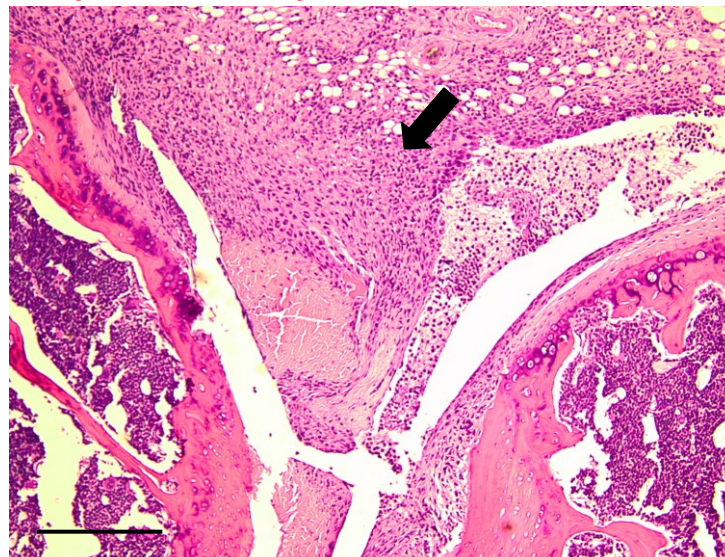
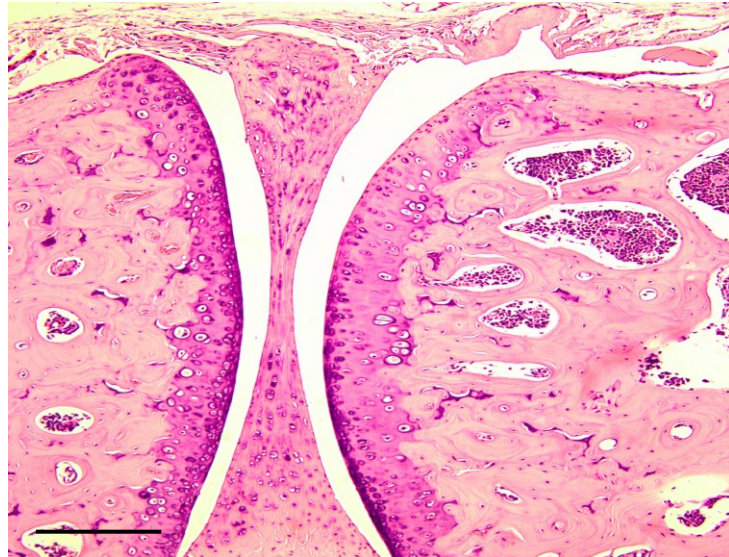
- ✓ THY1 (CD90) + (SL): **INFLAMMATION**
- ✓ THY1 (CD90) – (LL): **DAMAGE**

# H&E in FFPE mouse joint sections

Normal limb (Score = 0)



Arthritic limb (Score = 25)

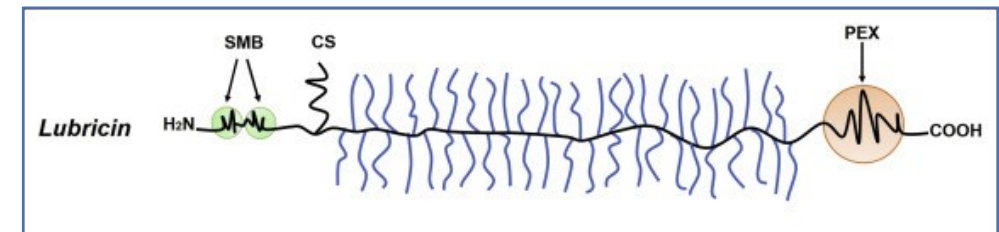
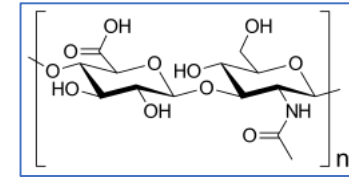


**fe:** femur  
**ti:** tibia  
**BM:** bone marrow  
**s:** synovial membrane

Courtesy: Elpida Neofotistou

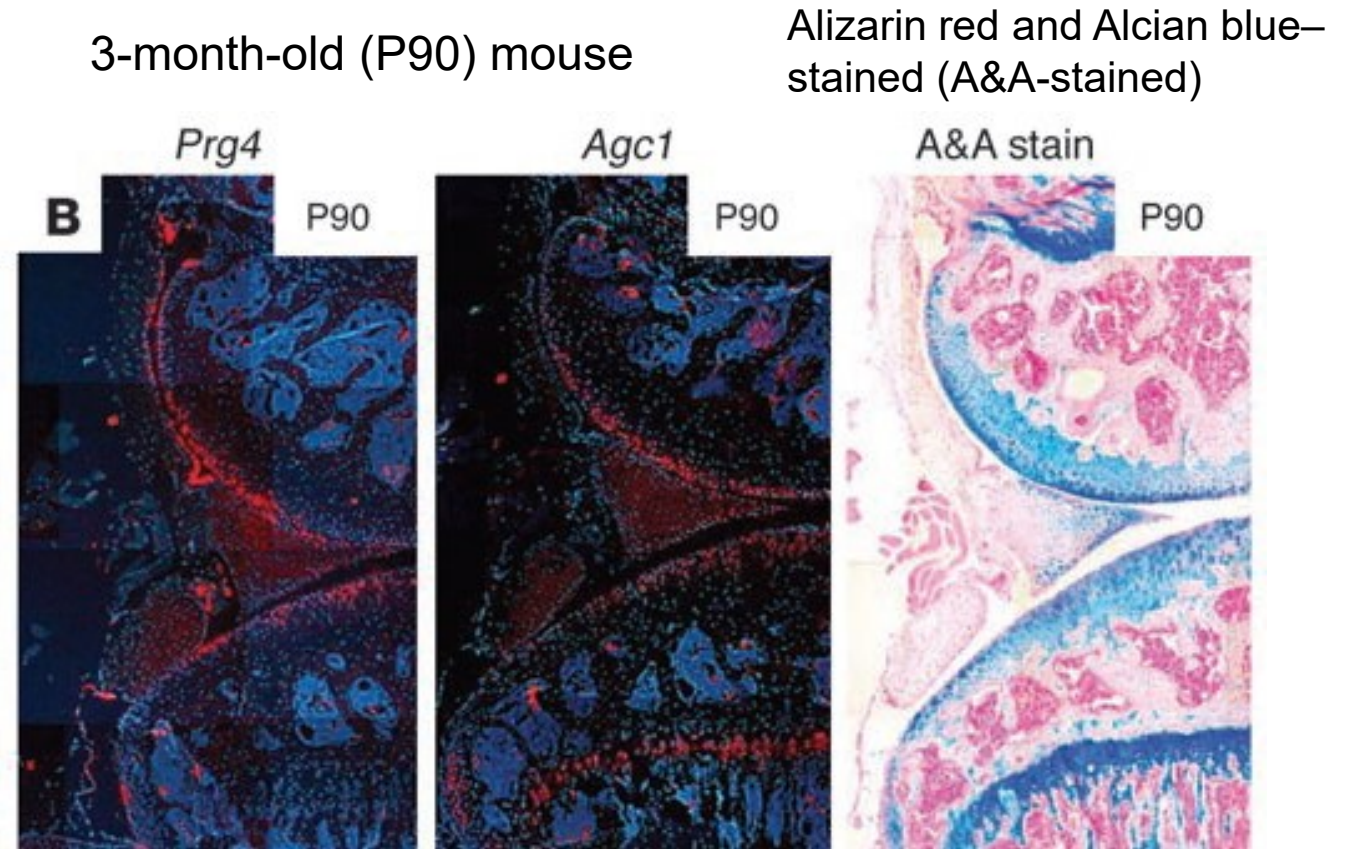
# Major molecules of SF-synovium: Hyaluronan (HA) & Lubricin (PRG4)

- ✓ **Hyaluronan (HA)** [simplest glycosaminoglycan (negatively charged polysaccharides)] produced predominantly by fibroblast-like synoviocytes
  - Inhibits synovial adhesion
  - Lubrication
  - It protects joint tissues inhibiting the stimulatory effect of pro-inflammatory cytokines on the production of matrix metalloproteinases
- ✓ **Lubricin [proteoglycan 4 (PRG4)]** (glycoprotein) is secreted by synoviocytes as well as chondrocytes in the superficial layer of articular cartilage.



# Lubricin

- Lubricin, a product of the gene proteoglycan 4 (PRG4) is a major component of synovial fluid
- It is an O-linked glycosylated protein that is highly expressed by synoviocytes
- Participates in the boundary lubrication of synovial joints

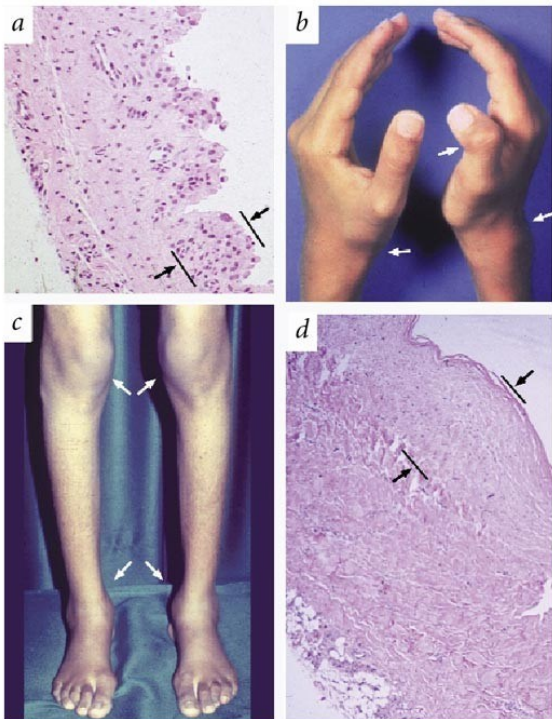


# Lubricin mutations

## HUMAN

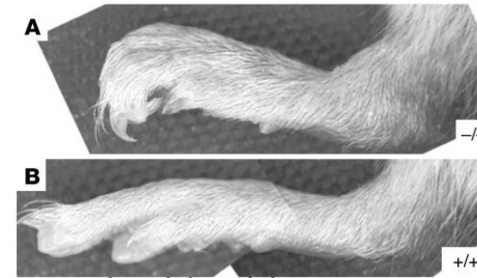
### Congenital or early-onset camptodactyly (CAMP)

- ✓ loss-of-function mutations in PRG4
- ✓ childhood-onset noninflammatory arthropathy
- ✓ synovial hyperplasia.
- ✓ pro-gressive coxa vara deformity
- ✓ noninflammatory pericardial effusion.

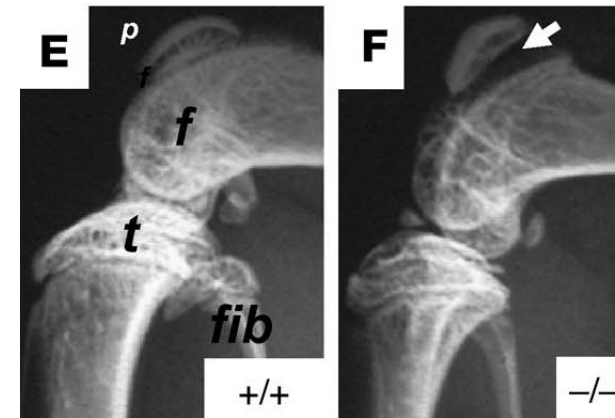


## Prg4<sup>-/-</sup> mice.

Hind paws of 6-monthold Prg4<sup>-/-</sup> (A) and wild-type (B) mice.



- Lateral knee x-ray of a 4-month-old wild-type mouse (E) or Prg4<sup>-/-</sup> mouse.
- Increased joint space between the patella and femur (arrowheads) and osteopenia of the patella, femoral condyles, and tibial plateau.



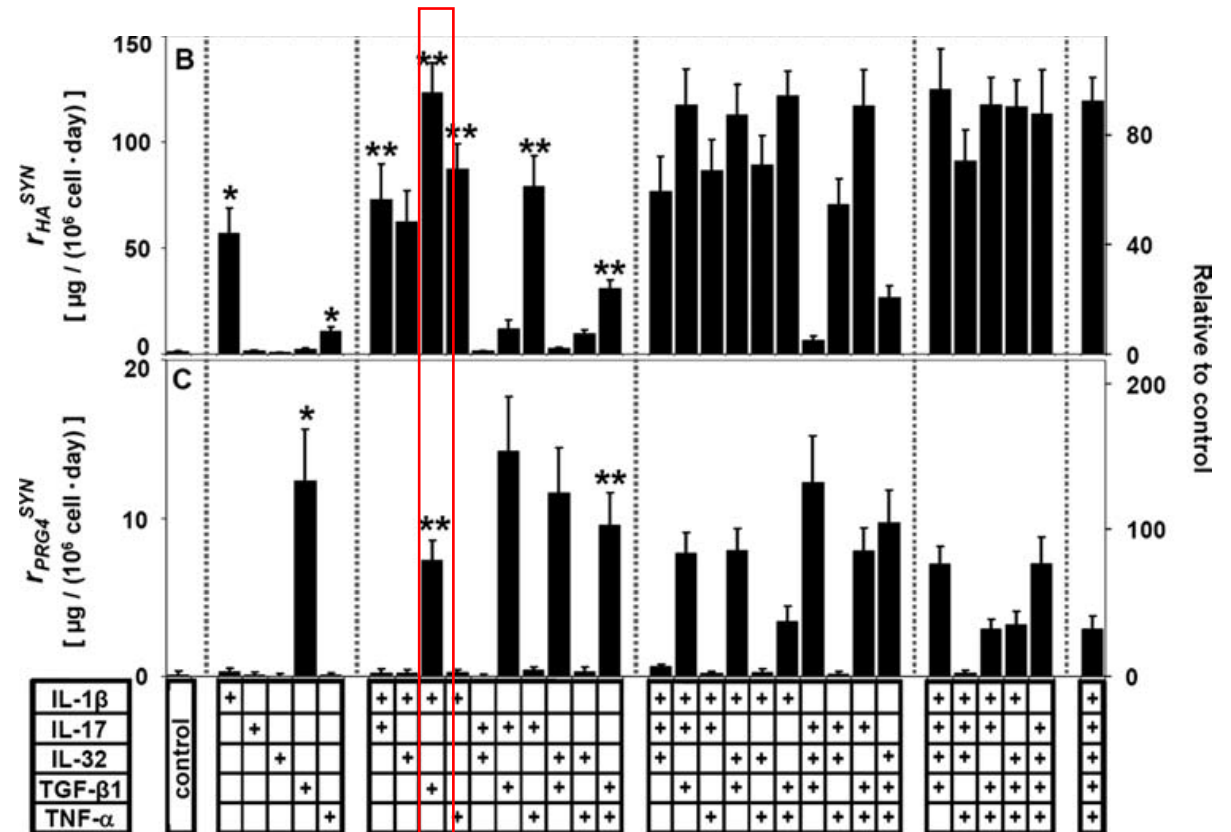
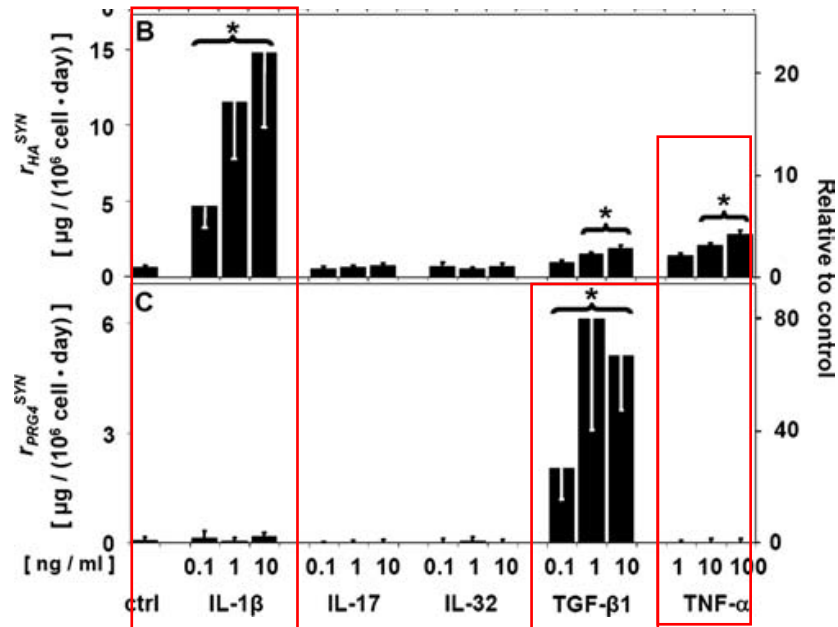
patella (p), femoral condyle (f), tibial plateau (t), and fibula (fib) (F)

# Role of Synovium in the maintenance of normal homeostasis in the synovial joint

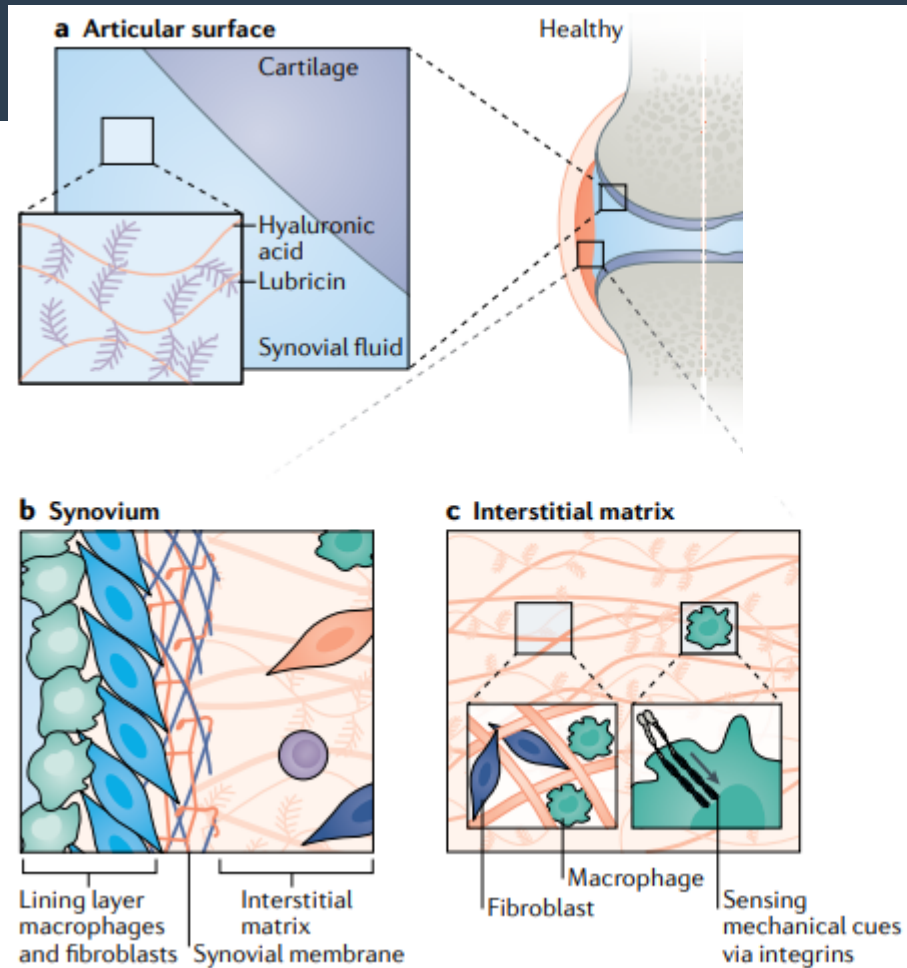
- Expression of the protective **lubricin**
- Secretion of **matrix metalloproteinases (MMPs)** by FLSs
- **Immune sentinel roles** of resident **macrophages** and **FLSs**
- **Regulated entry and exit of leukocytes** involved in immune surveillance
- **Local regulation** by cytokines and growth factors.

# Cytokines affects synthesis and molecular weight of HA and PRG4

- HA secretion rates were increased:
  - \*40-fold by IL-1b
  - \*80-fold by the combination of IL-1b + TGF-b1 or TNF-a + IL-17.
- PRG4 secretion rates were increased
  - \*80-fold by TGF-b1
  - this effect was counterbalanced by IL-1b and TNF-a.



# The tissue microenvironment: cartilage, synovium, matrix



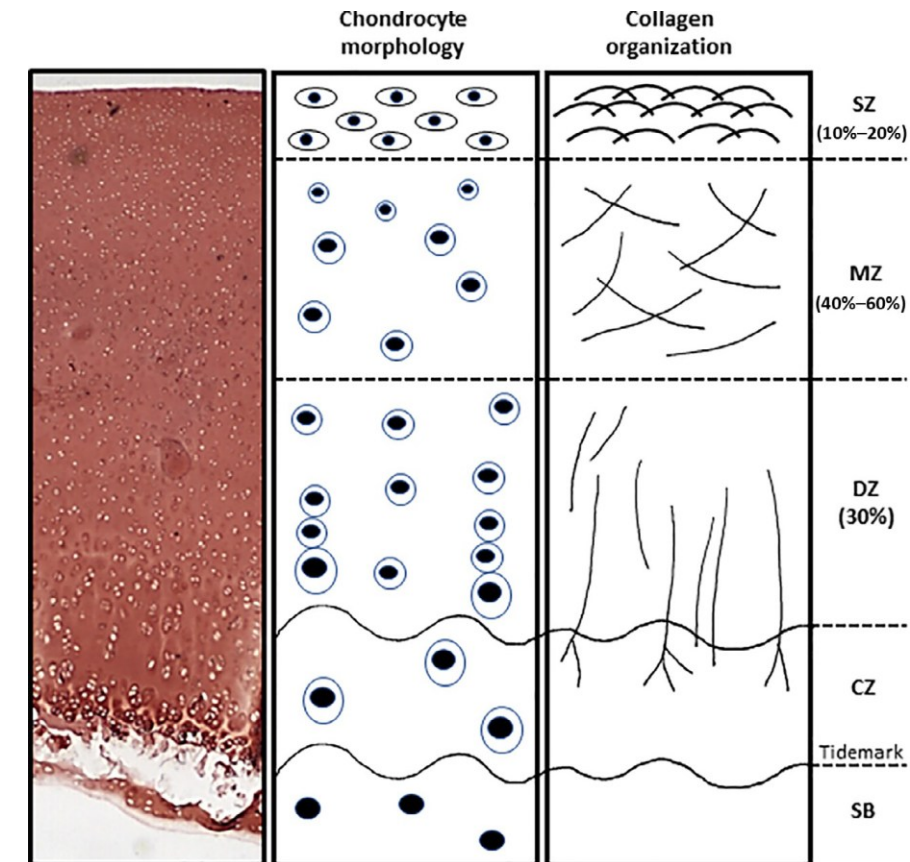
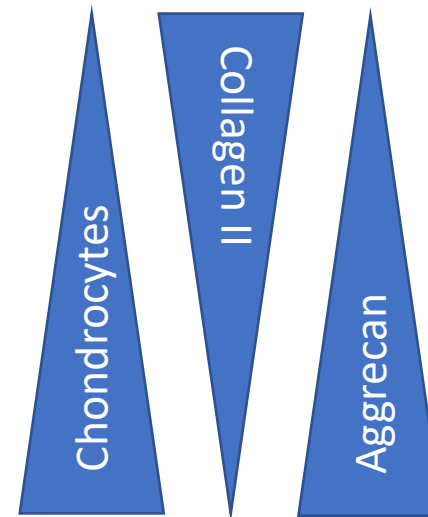
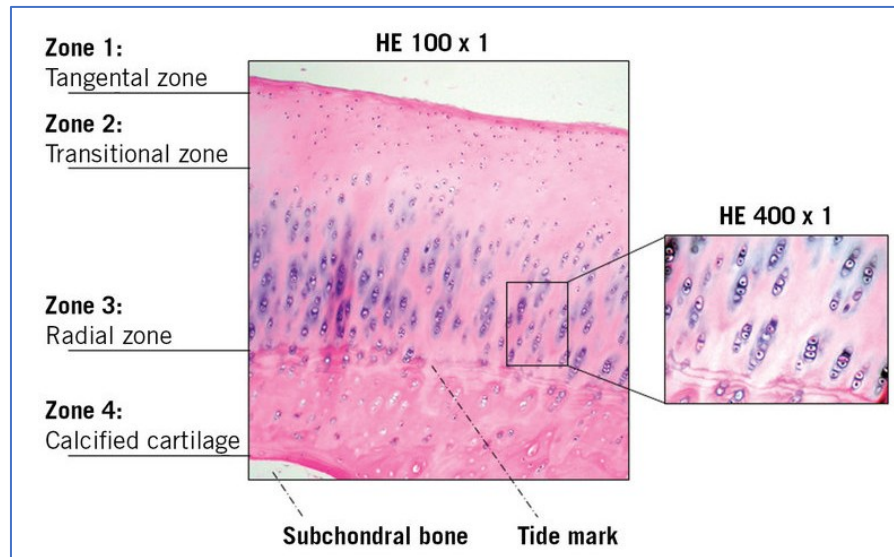
**Physical properties, mechanical cues**  
**Patterning of soluble factors**  
**Direct cell signalling**

# Cartilage

- In an articulating joint cartilage can be subjected to compressive and frictional forces several million times annually
- Cartilage is **avascular tissue** deriving its nourishment from **synovial fluid**, a filtrate of blood that has components added and removed by the joint capsule's intimal lining cells, the synoviocytes
- Adult **chondrocytes do not normally divide** in vivo and defects in cartilage surfaces are not self-repairing
- Therefore, for a joint to maintain its function throughout a lifetime of use, there must be biologic **mechanisms that help minimize damage** resulting from activities of daily living.
- Composition:
  - Water 70%
  - 90% of cartilage dry weight: collagen II & aggrecan
  - Chondrocytes: 1-2% of the volume

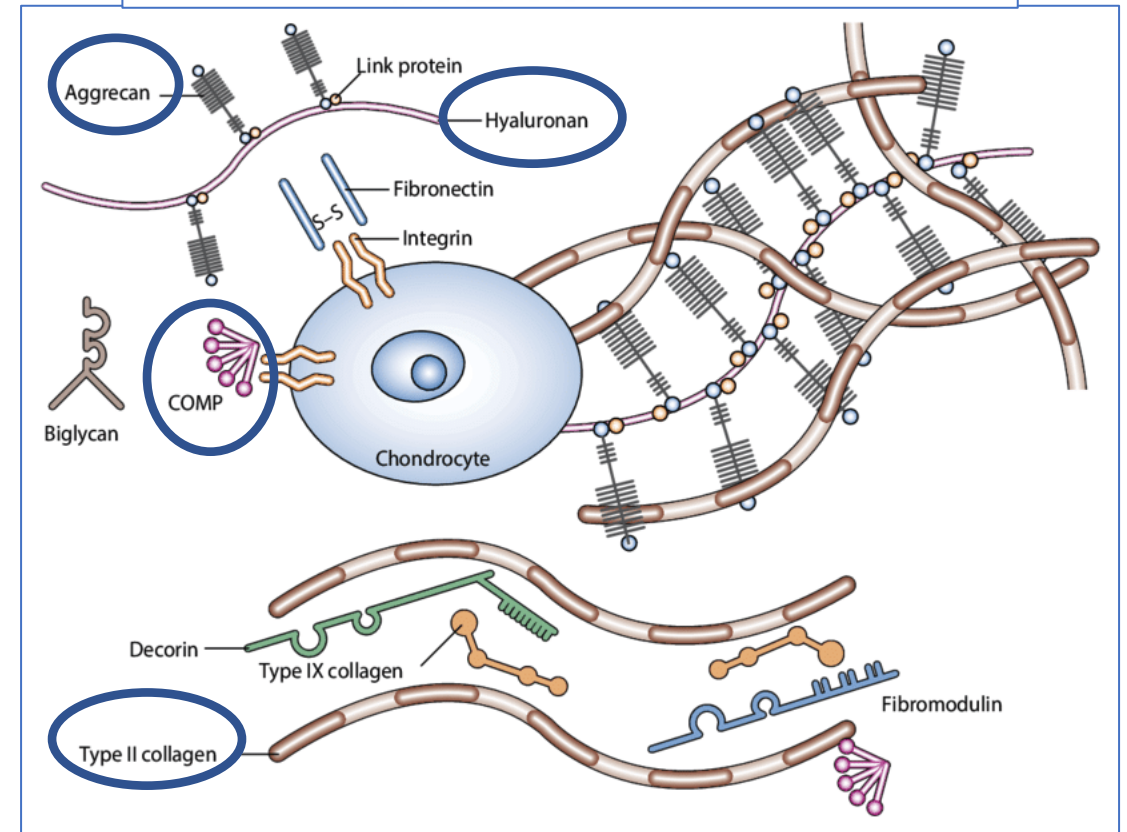
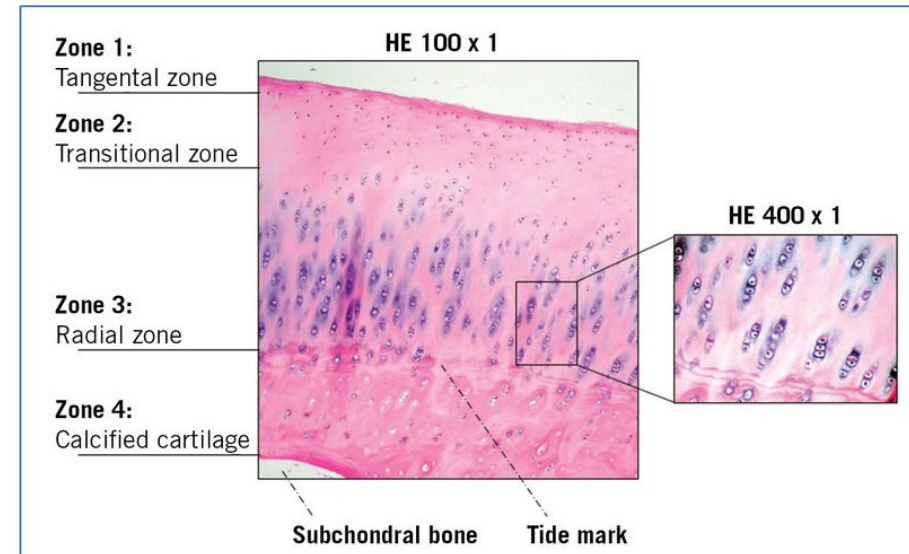
# Cartilage

- Function
  - ✓ Distribute the load to protect the underlying bone
    - ✓ Mechanism: high content of water which moves out and back into the cartilage
  - ✓ Provide low friction movements



# Cartilage

- Tissue organization
  - ✓ Cells: chondrocytes
  - ✓ Matrix
    - Collagen II: 50% of dry weight
    - Aggrecan (proteoglycan): 25 % of dry weight
    - COMP (Cartilage Oligomeric Matrix Protein)
    - Other organic and inorganic

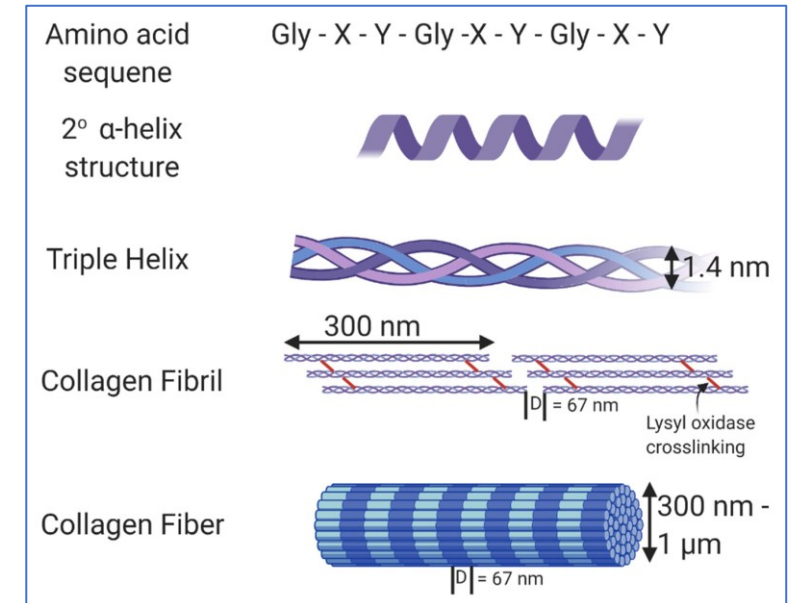
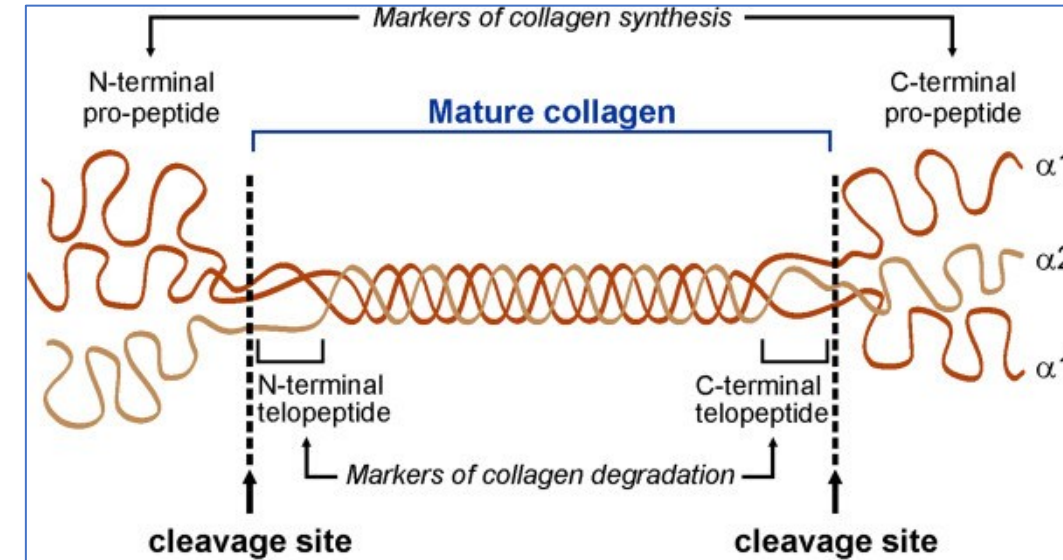


# Cartilage tissue (Matrix) structure: collagens

## Collagen II (*COL2A1*):

- ✓ Procollagen
- ✓ Collagen 3ple helix
- ✓ Collagen linked to other matrix molecules
  - COMP: role to stabilize collagen network
  - Decorin: binds to fibrin forming collagens
  - Fibromodulin: LRR protein
  - Collagen IX
- **Half-time:>100 years**

Other Collagens: IX/XI, VI, XII, XIV



# Pathologies associated with matrix proteins

- ✓ OA Pathology associated with:
  - Collagen :
    - ❑ ↑ destruction of IX = accelerated cartilage degradation
    - ❑ ↑ Coll III & IV
    - ❑ COMP: ↑ in early OA (repair mechanism)
- ✓ Pseudoachondroplasia, multiple epiphyseal dysplasia
  - COMP mutations

# Cartilage tissue structure: COMP

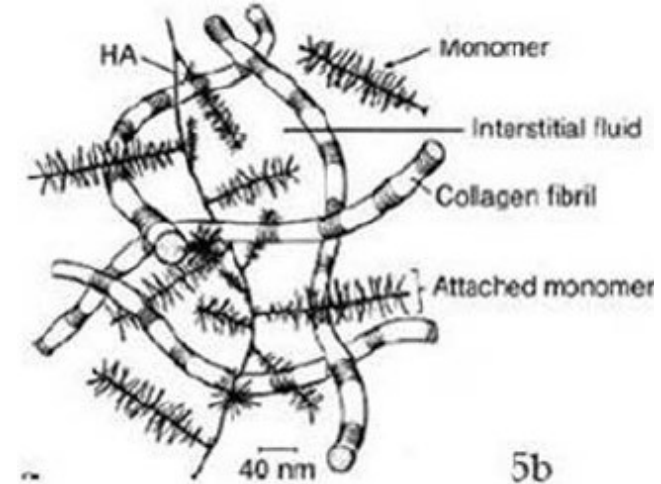
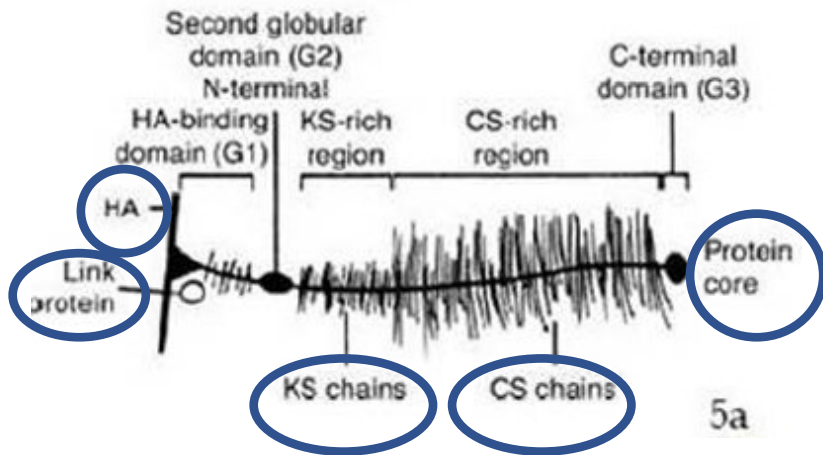
- Protein that links collagen I/II/IX
- Binds 5 collagen molecules
- in OA is upregulated (compensatory mechanism) and may be cleaved-released=measured
- Mutation of COMP
  - Pseudoachondroplasia
  - Multiple epiphyseal dysplasia

# Cartilage tissue structure: aggrecan

Proteoglycan aggrecan molecule composed of:

- ✓ a protein core
- ✓ glycosaminoglycans chondroitin (CS) and keratan sulfate (KS)
- ✓ link protein attached to hyaluronic acid (HA) chain

ECM structure of **collagen fibrils** intertwined in **aggrecan molecules**

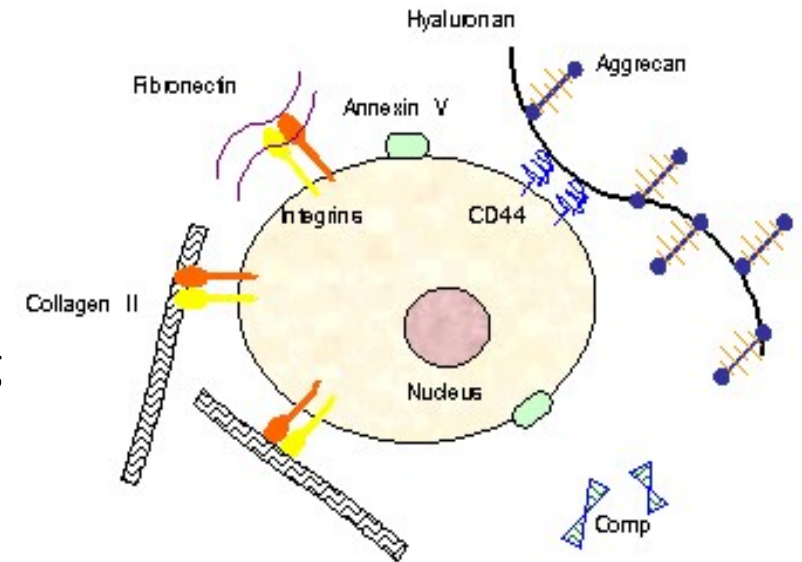


# Cartilage homeostasis

- ✓ Aggrecan production: chondrocytes high capacity for aggrecan replacement
- ✓ Aggrecan degradation
  - Aggrecanases
    - ADAMTS-4 & -5
- ✓ Pathologies associated to aggrecan:
  - ✓ Aging cartilage: Short aggrecan molecules, lack of glycosaminoglycans , only HA binding site,  
↓ *water content*

# Chondrocytes

- ✓ Mature chondrocytes respond to biochemical, mechanical stimuli
  - Producers of: matrix components, enzymes and enzyme inhibitors, GF, cytokines
  - Low producers of collagen
  - High producers aggrecan
- ✓ They maintain a steady-state metabolism secondary to equilibrium between anabolic and catabolic processes, resulting in the normal turnover of matrix molecules
- ✓ The mature articular chondrocyte embedded in its ECM is a resting cell with no detectable mitotic activity and a low rate of synthetic activity
- ✓ Energy metabolism depends strongly on glucose supply.
  - Facilitated glucose transport in chondrocytes is mediated by glucose transporter proteins (GLUTs)



# Chondrocyte's Receptors

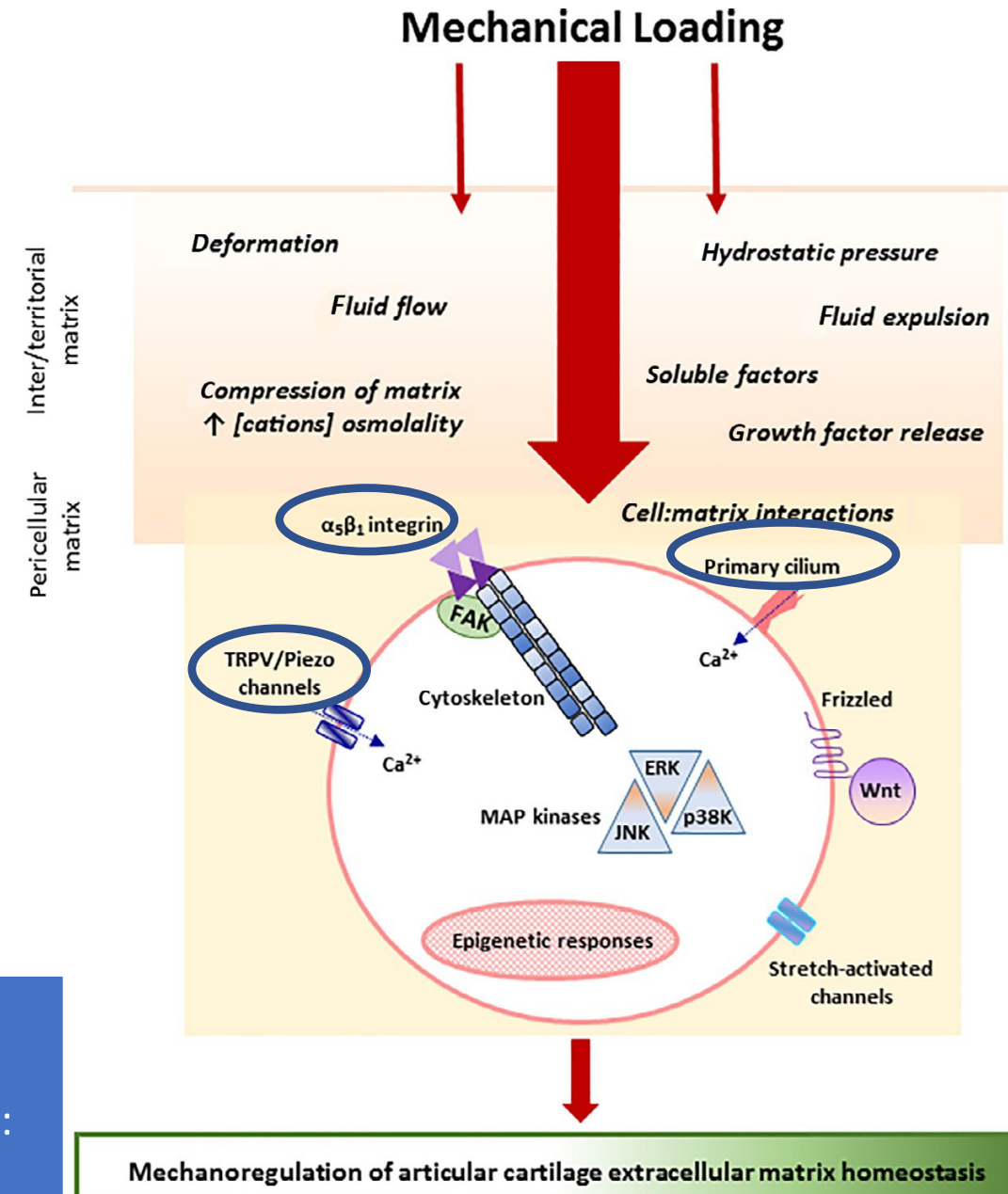
## ✓ Integrins:

- Cell to extracellular Matrix (ECM) link
- Mechanoreceptors

## ✓ Ion channels

- Mechanical stimulation regulates the activity of ion channels localized on the plasma membrane due to **perturbations in membrane tension and lipid bilayer distortion**.
  - TRPV4 is a  $\text{Ca}^{2+}$ -permeable

- ✓ **The primary cilium**, a microtubule-based structure extending from the cell surface and protruding into the Pericellular Matrix



## Example of interaction between ECM-receptors-chondrocyte metabolism:

Mechanical strain-induced  $\alpha_5\beta_1$ -mediated membrane hyperpolarization:  
 $\uparrow$  transcription of aggrecan and  $\downarrow$  transcription of MMP-3

*J Cell Biol* 1999;145(1):183–9.

# Matrix turnover, repair-degradation

- ✓ Collagen turnover: slow
- ✓ Proteoglycans: continuously produced
- ✓ MMPs – MMPs inhibitors
- ✓ Integrins: bind to collagen and:
  - Increase collagen synthesis OR increase degrading enzymes
- ✓ Fibronectin
  - Fragmented fibronectin stimulates chondrocytes to produce degrading enzymes
  - Upregulated in OA

# Joint Remodeling – MMPs – activators - inhibitors

- The most important of proteolytic enzymes since they can directly cleave collagen at a neutral pH are:
  - ✓ The collagenases MMP1 (interstitial collagenase), MMP3 (stromelysin-1), MMP8 (neutrophil collagenase), MMP13 (collagenase 3) and MMP18 (also known as MMP19)
- MMPs activators / inhibitors
  - ✓ Serine and cysteine proteinases are required to activate pro-MMPs after they are secreted.
  - ✓ Furthermore, inhibitors of these proteinases (such as tissue inhibitors of metalloproteinases (TIMPs) and inhibitors of serine proteinases (SERPINs)) are also present in the normal joint.