

# Biology of Bone

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Musculoskeletal Research Group

The Medical School

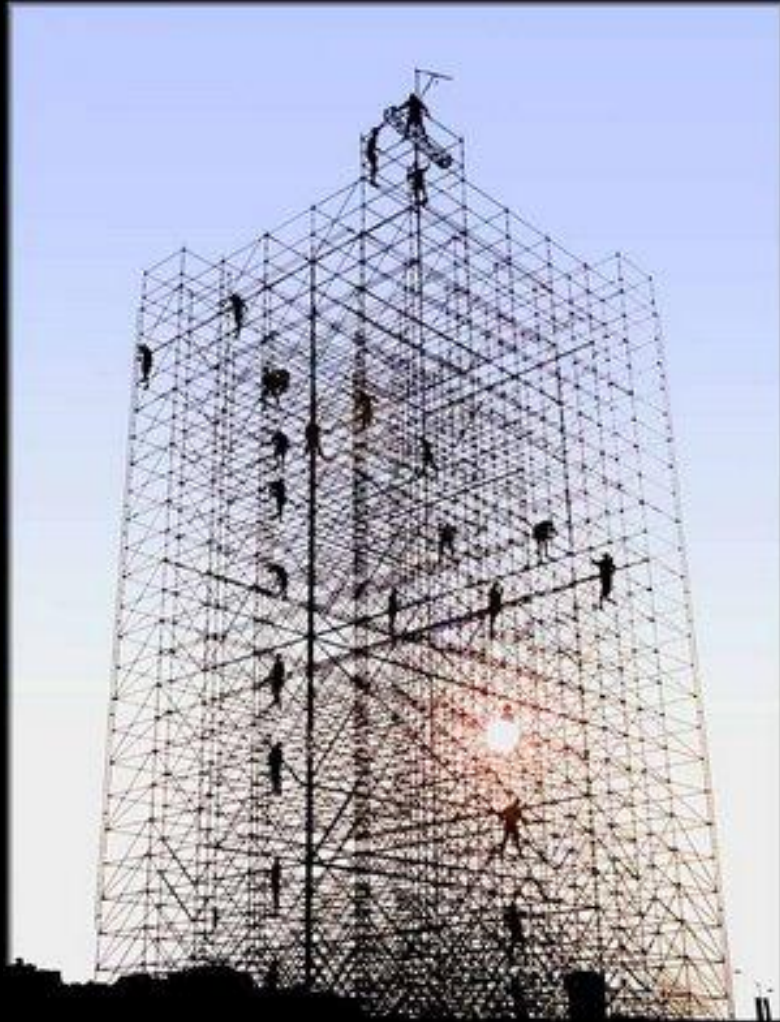
Newcastle University

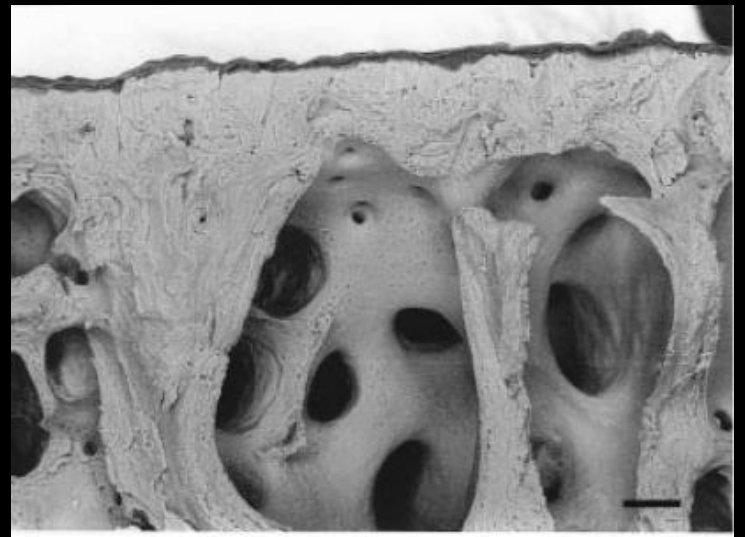
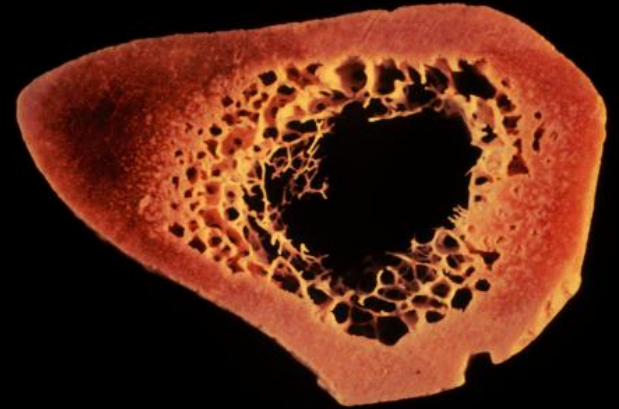
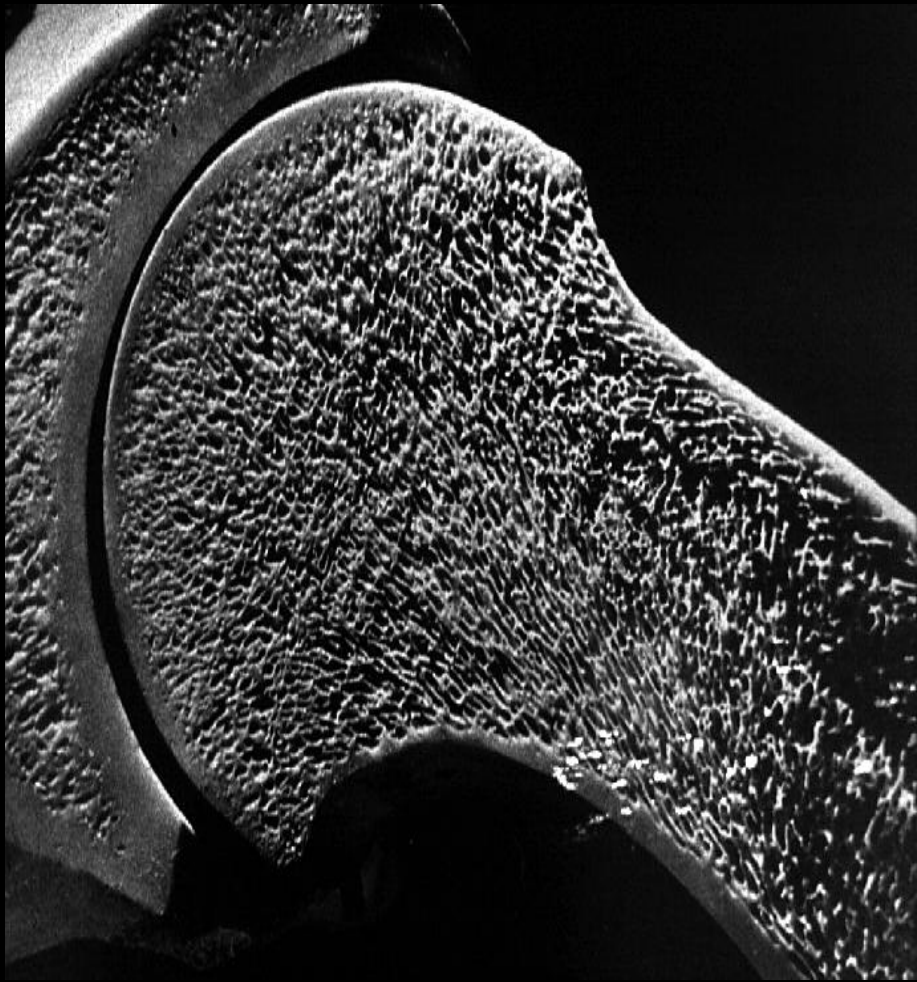
([mark.birch@ncl.ac.uk](mailto:mark.birch@ncl.ac.uk))

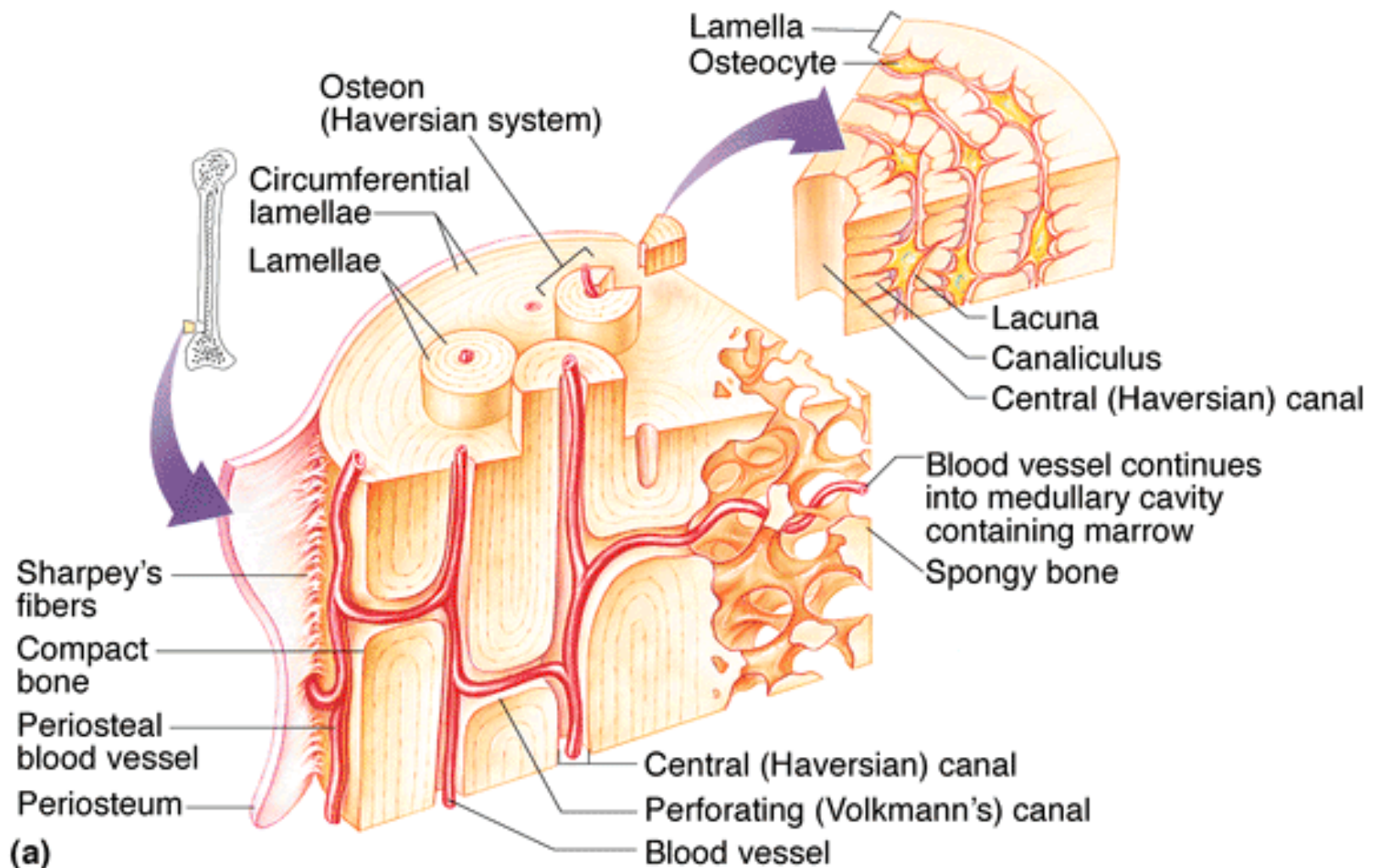
# Overview

- **Structure / function**
- **Modelling / Remodelling of Bone**
- **Controlling bone cell activity**
- **The role of mechanical load in regulating bone cells**
- **Coordinating bone cell activity**
- **Bone cell biology on orthopaedics**

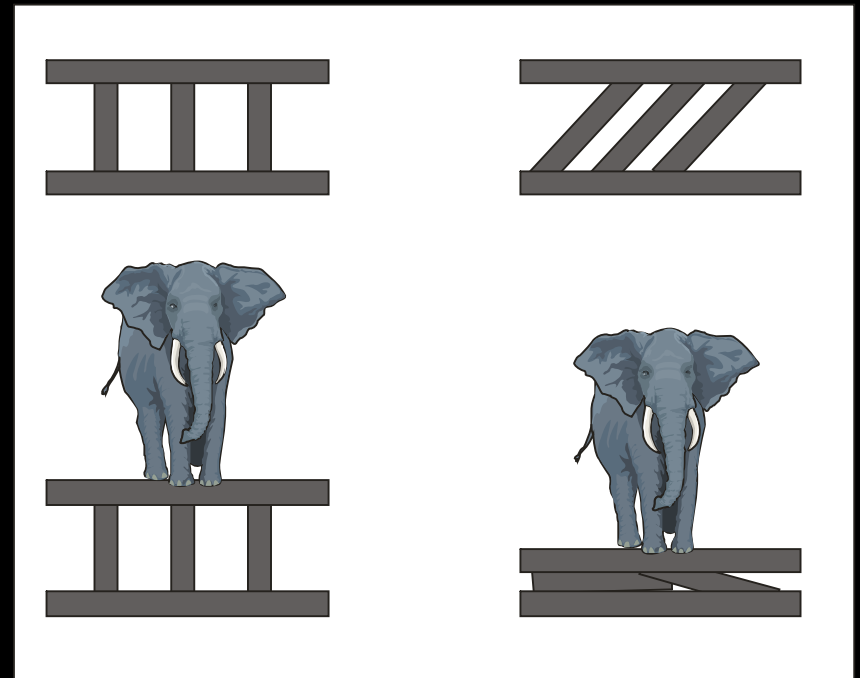
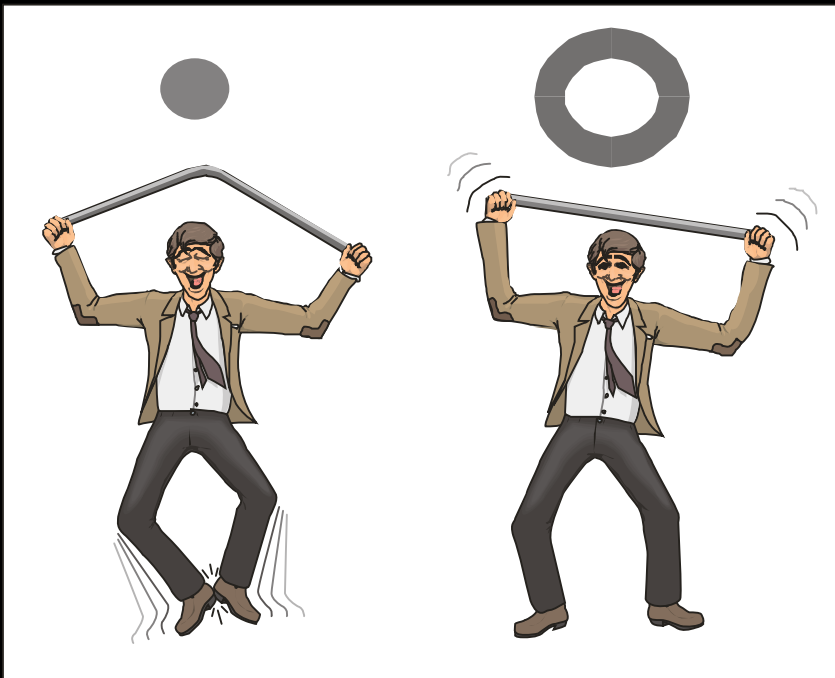
# Structure / Function

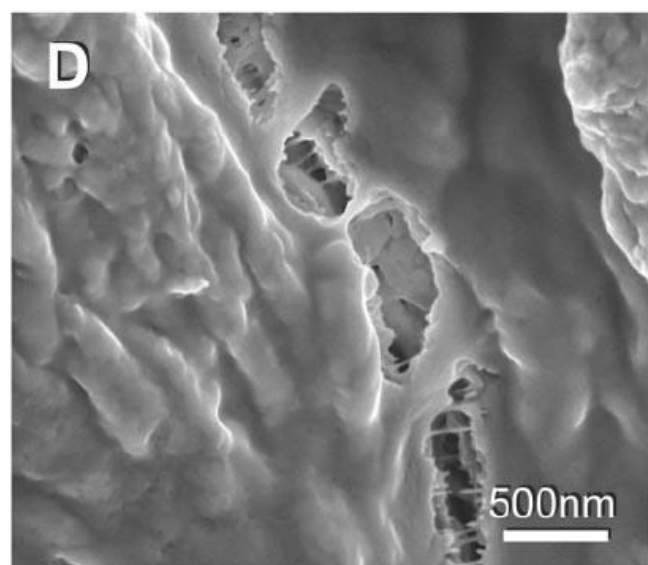
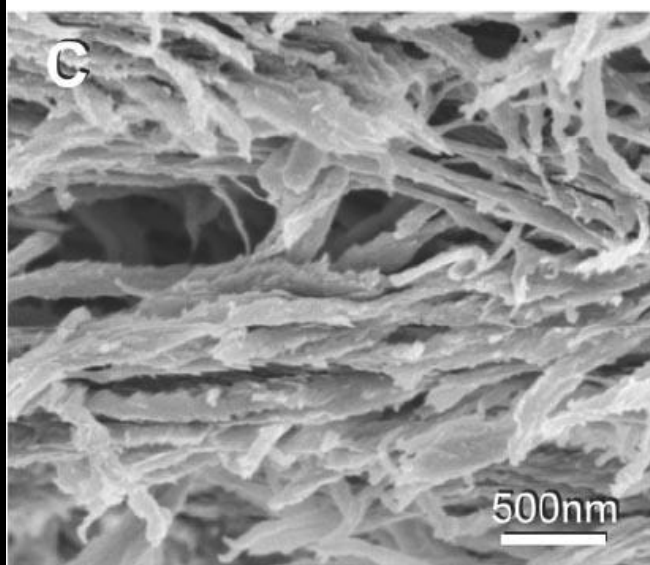
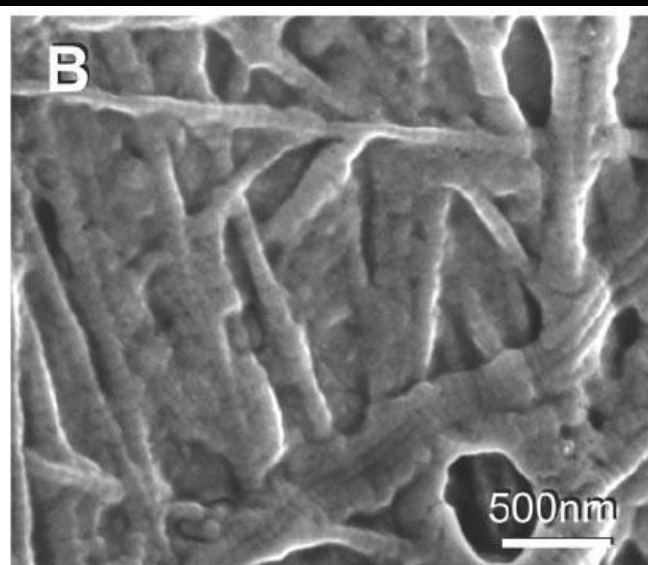
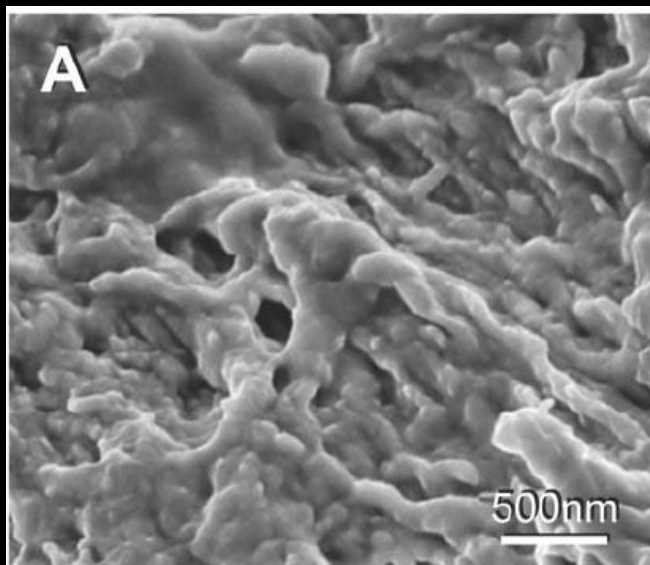






# To meet mechanical need





# Development



# ***Bone formation***

- Bone formation is termed “ossification” or “osteogenesis”
- The “early” skeleton in an embryo is composed of *fibrous membranes* and *hyaline cartilage*
- During the process of ossification (around the 6-7th week of embryonic development):
  - Chondroblasts form cartilage
  - Osteoblasts form bone (mineralization)

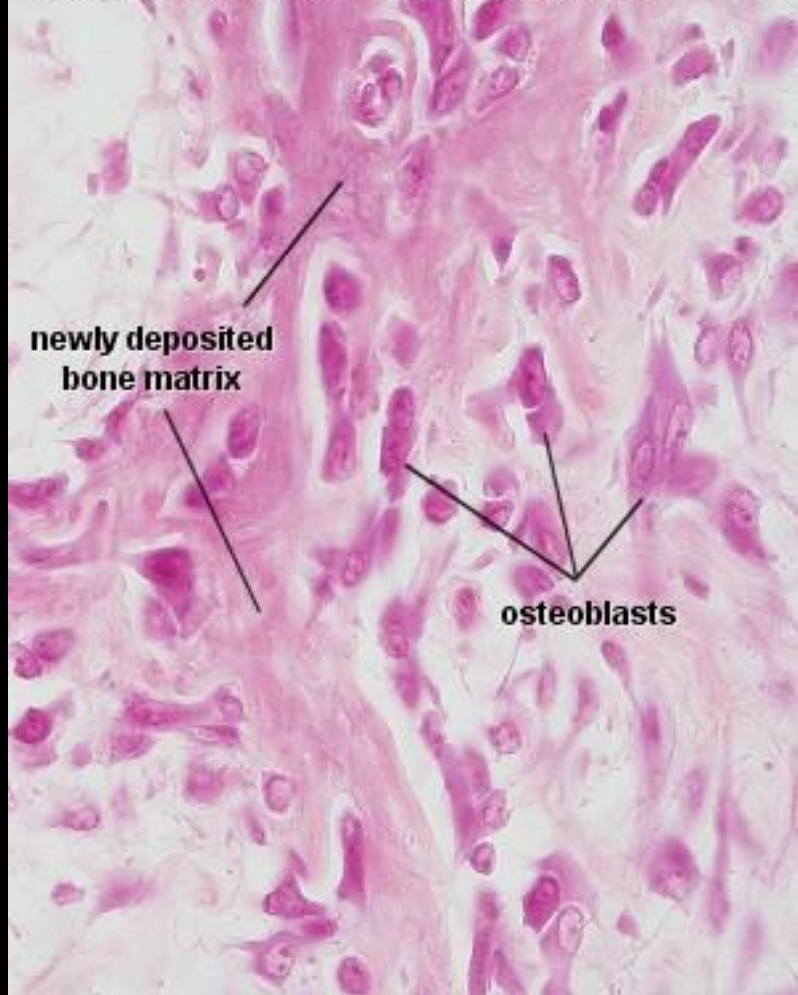
# *Intramembranous Ossification*

- Bone formation of the surface skull bones and clavicles
- Osteoblasts cluster around the centre of ossification
  - Here, osteoblasts secrete a collagenous matrix to form a framework for mineralization
  - The collagenous matrix is then calcified by the deposition of hydroxyapatite
  - The osteoblasts and their surrounding calcified matrix are now referred to as a trabecula
- Most of the trabeculae will be eventually destroyed and reformed to give a bone its final adult size and shape

**Intramembranous Ossification H&E**



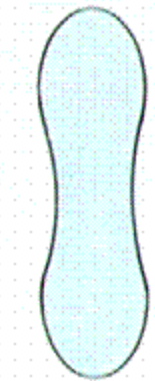
**Intramembranous Ossification H&E**



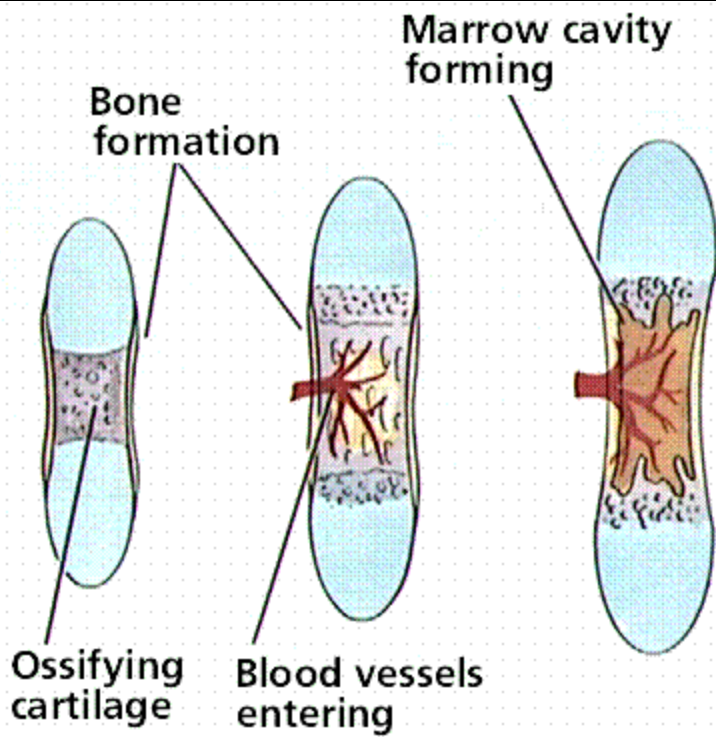
# ***Endochondral Ossification***

- Replacement of cartilage with bone
- Primary ossification process for most bones of the body
  - Best exemplified in *long bones*
- During embryonic development, a cartilage model, or ***perichondrium***, is laid down
  - Compact bone then forms around this area and is called the ***periosteum***
    - Periosteal collar
- Cartilage grows outward from it's center and is gradually calcified into bone tissue
  - Primary ossification centre: diaphysis
  - Secondary ossification centre: epiphysis
  - Two areas remain uncalcified cartilage: articulations, growth plate

# The Growth of Long Bones



Cartilage

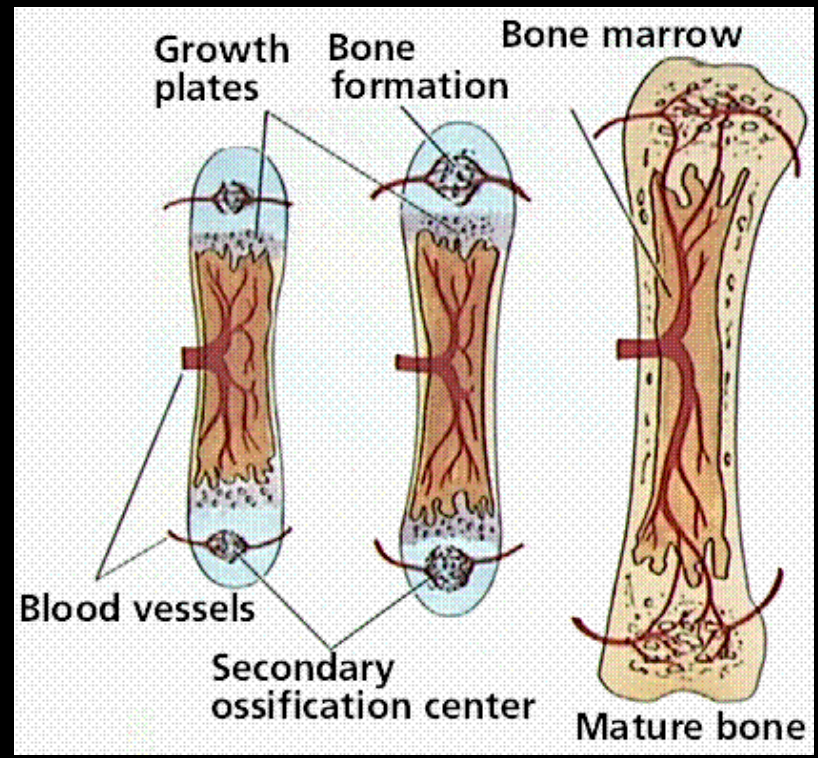


Bone formation

Ossifying cartilage

Blood vessels entering

Marrow cavity forming



Growth plates

Bone formation

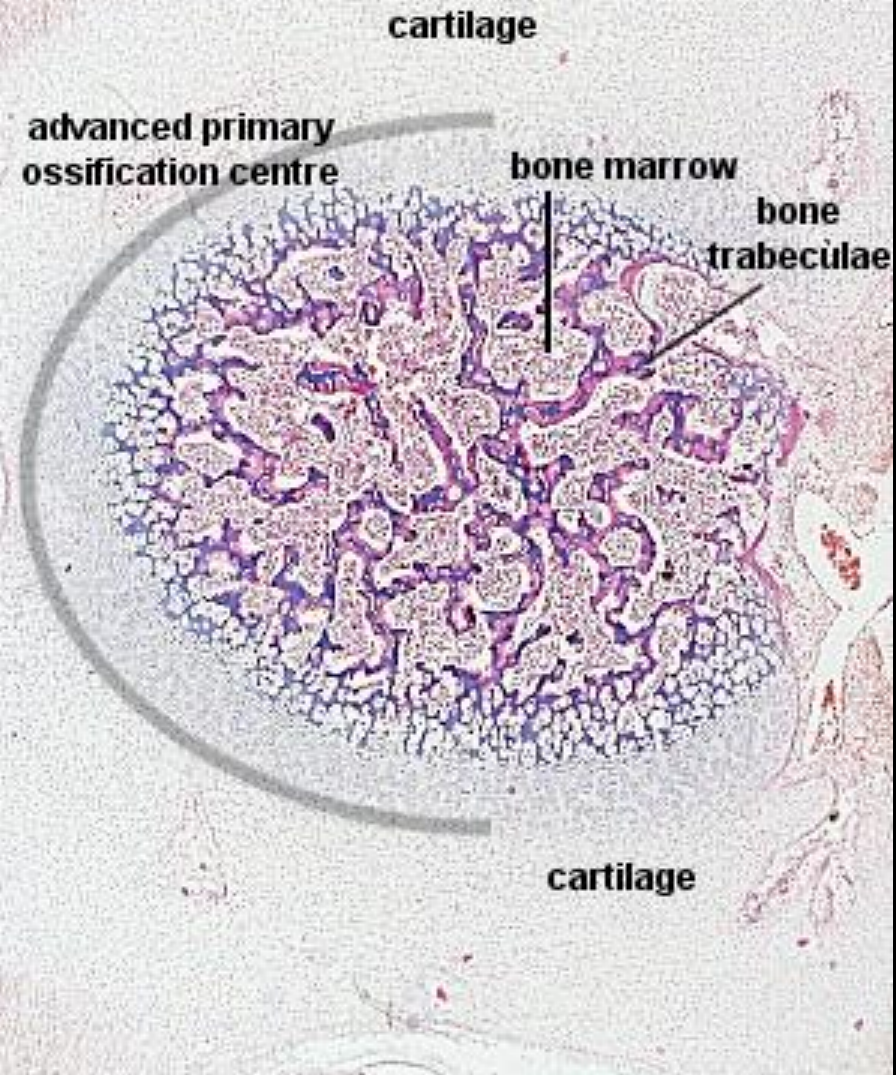
Bone marrow

Blood vessels

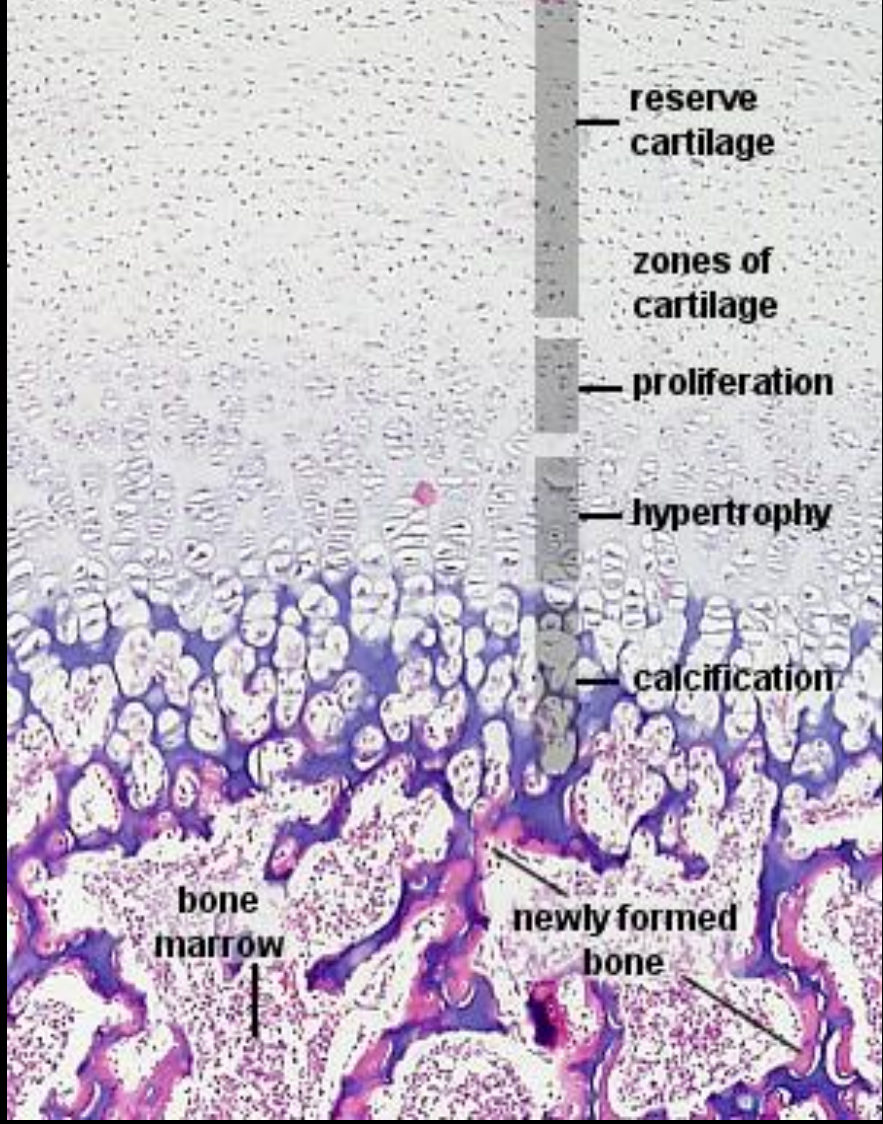
Secondary ossification center

Mature bone

## Endochondral Ossification H&E



# Endochondral Ossification H&E



reserve cartilage

zones of cartilage

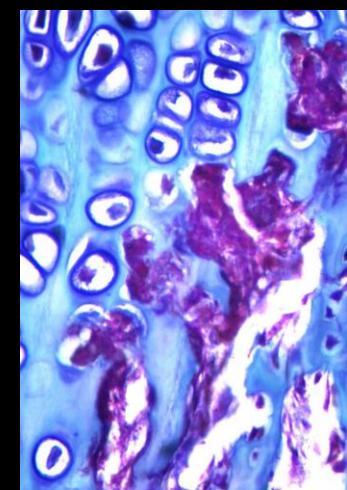
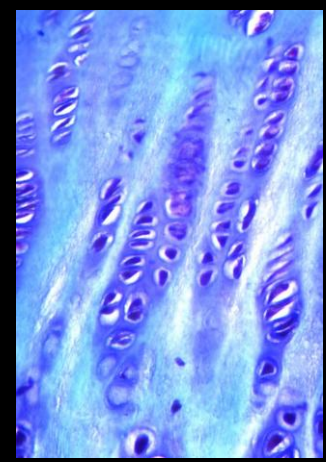
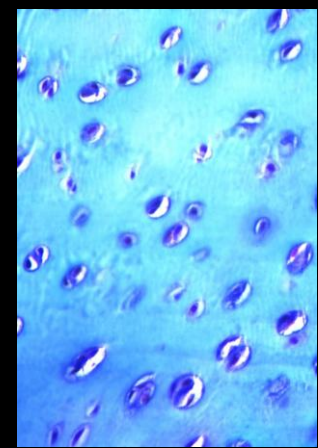
proliferation

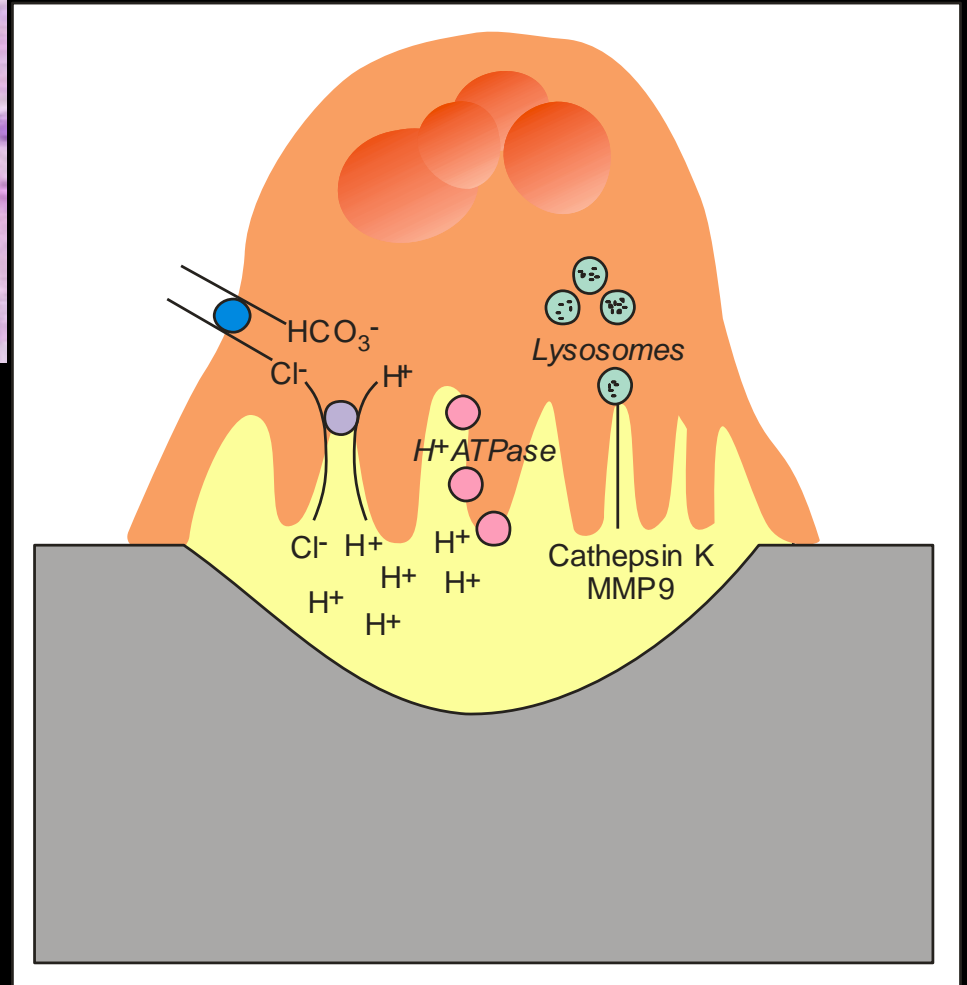
hypertrophy

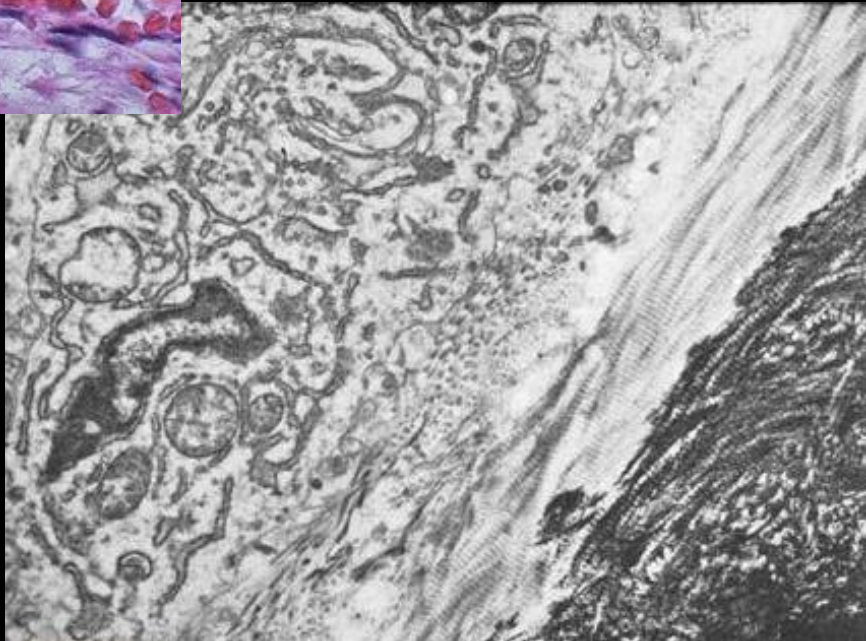
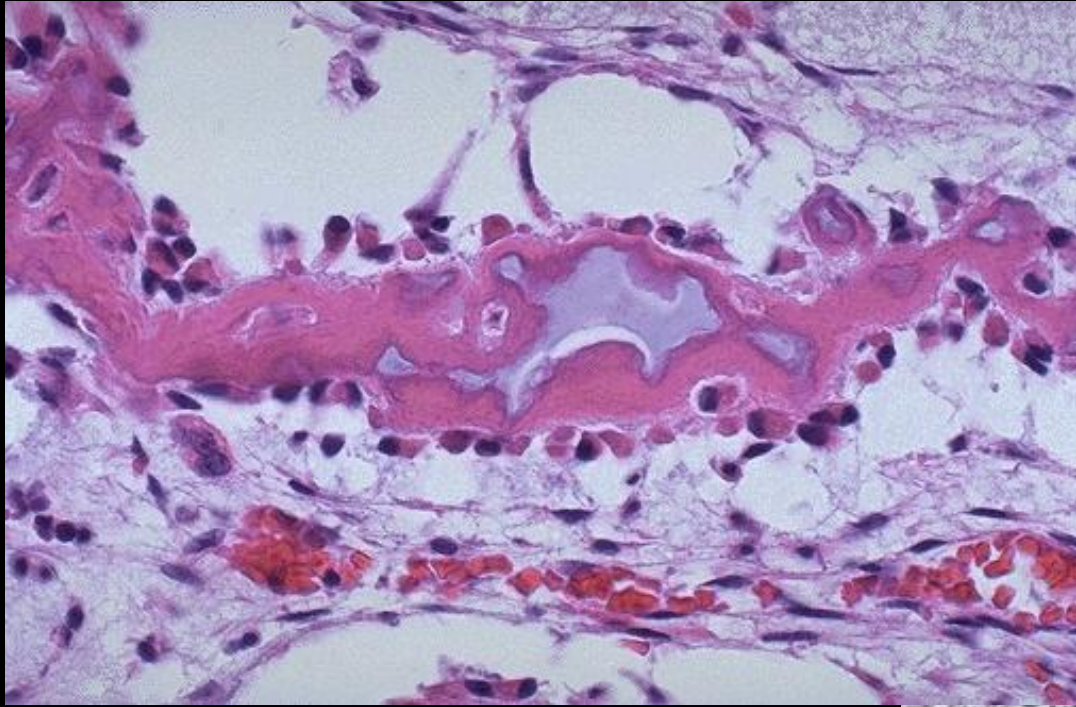
calcification

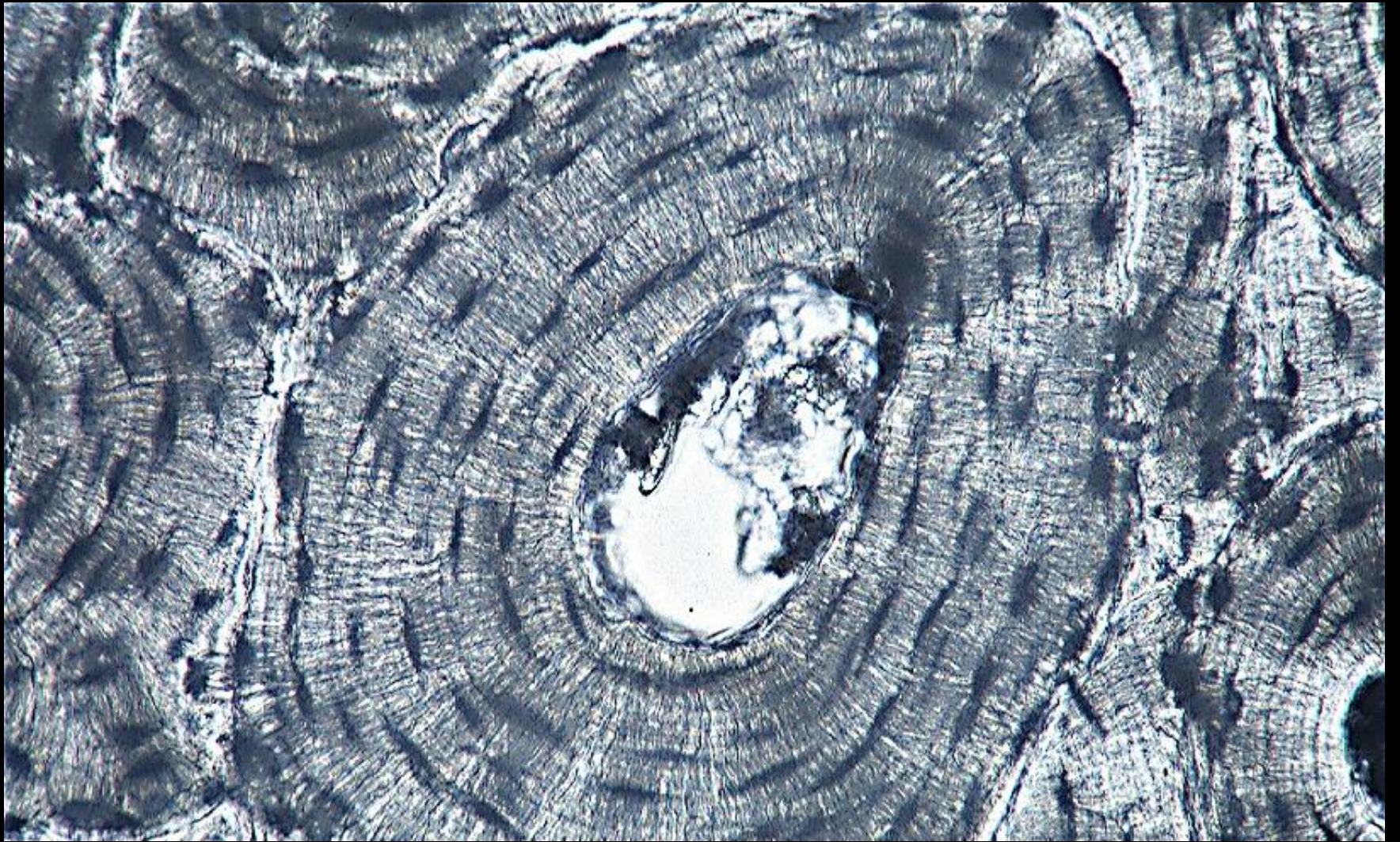
bone marrow

newly formed bone









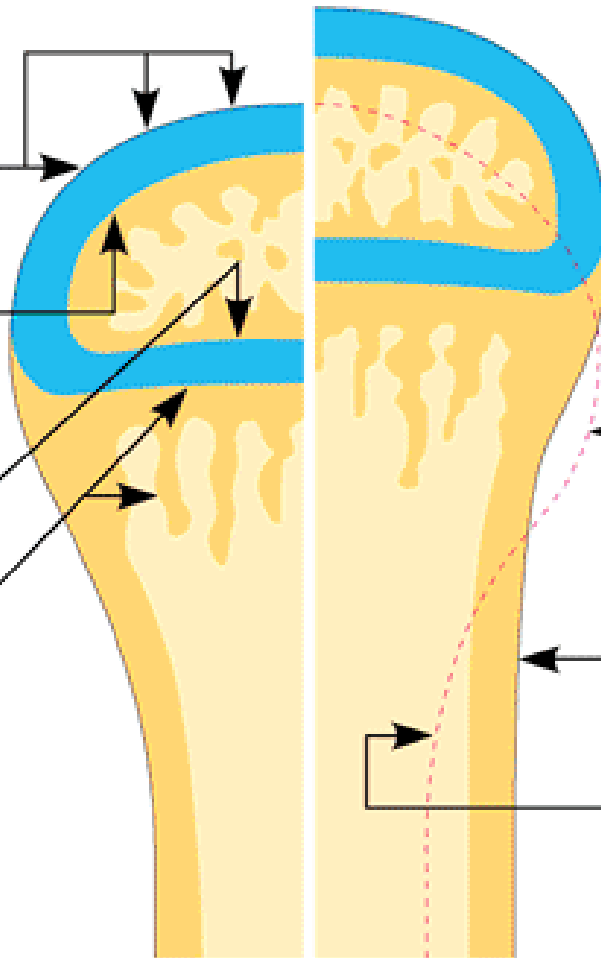
# Modelling / Remodelling



## Growth

Bone grows in length because:

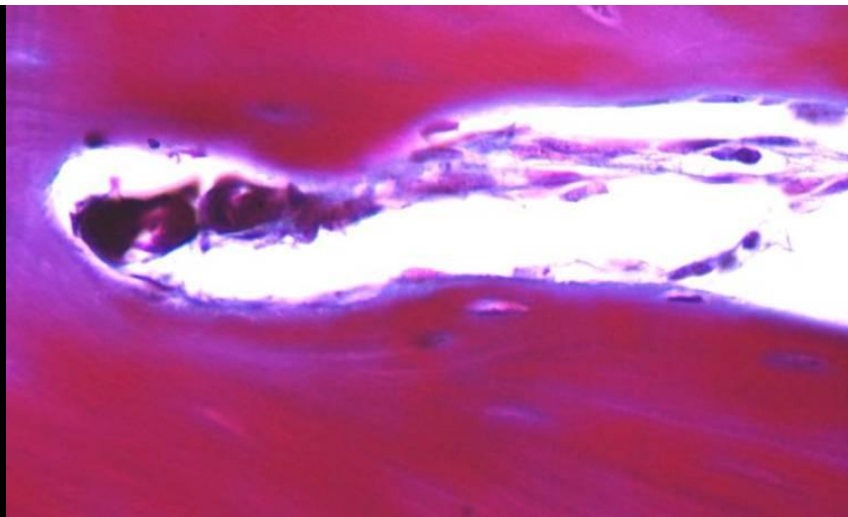
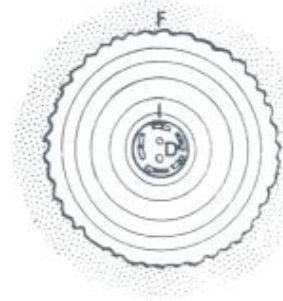
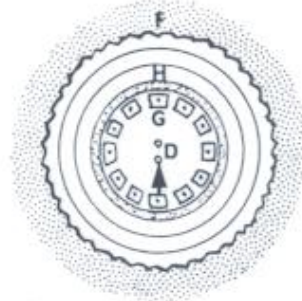
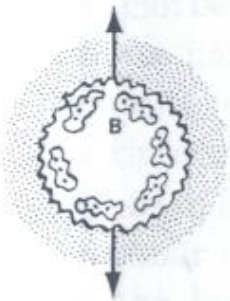
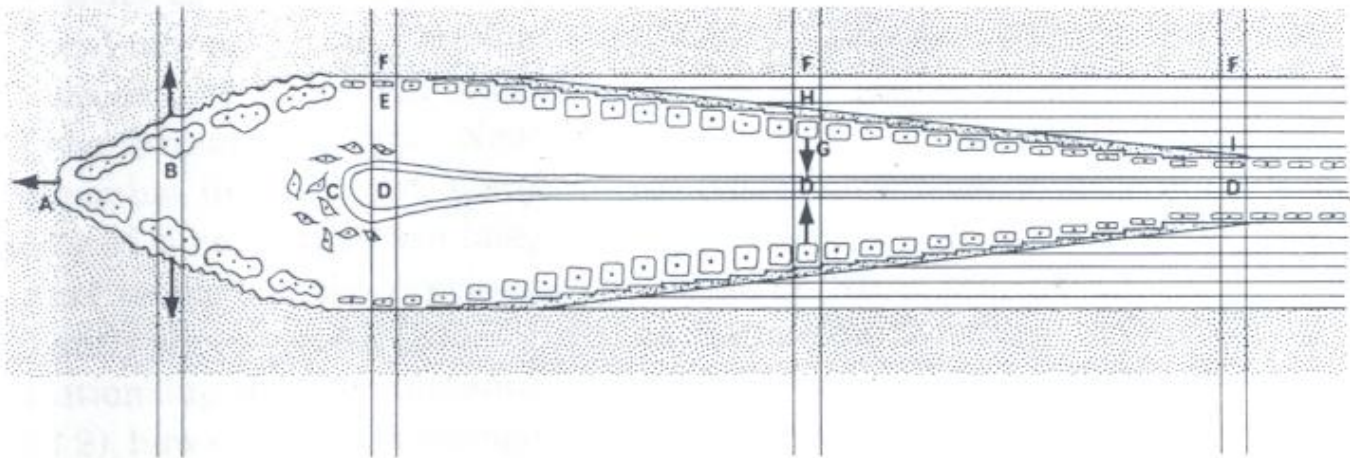
- ① Cartilage grows here
- ② Cartilage replaced by bone here
- ③ Cartilage grows here
- ④ Cartilage replaced by bone here

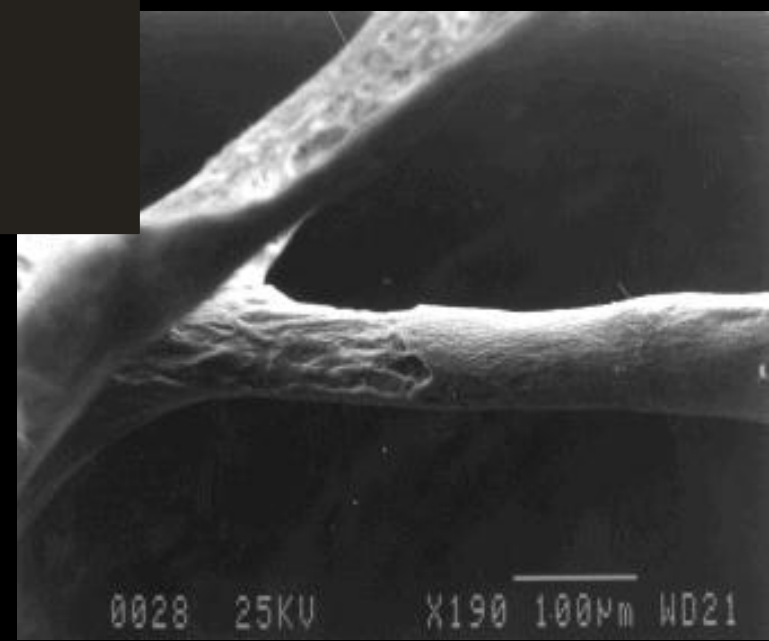
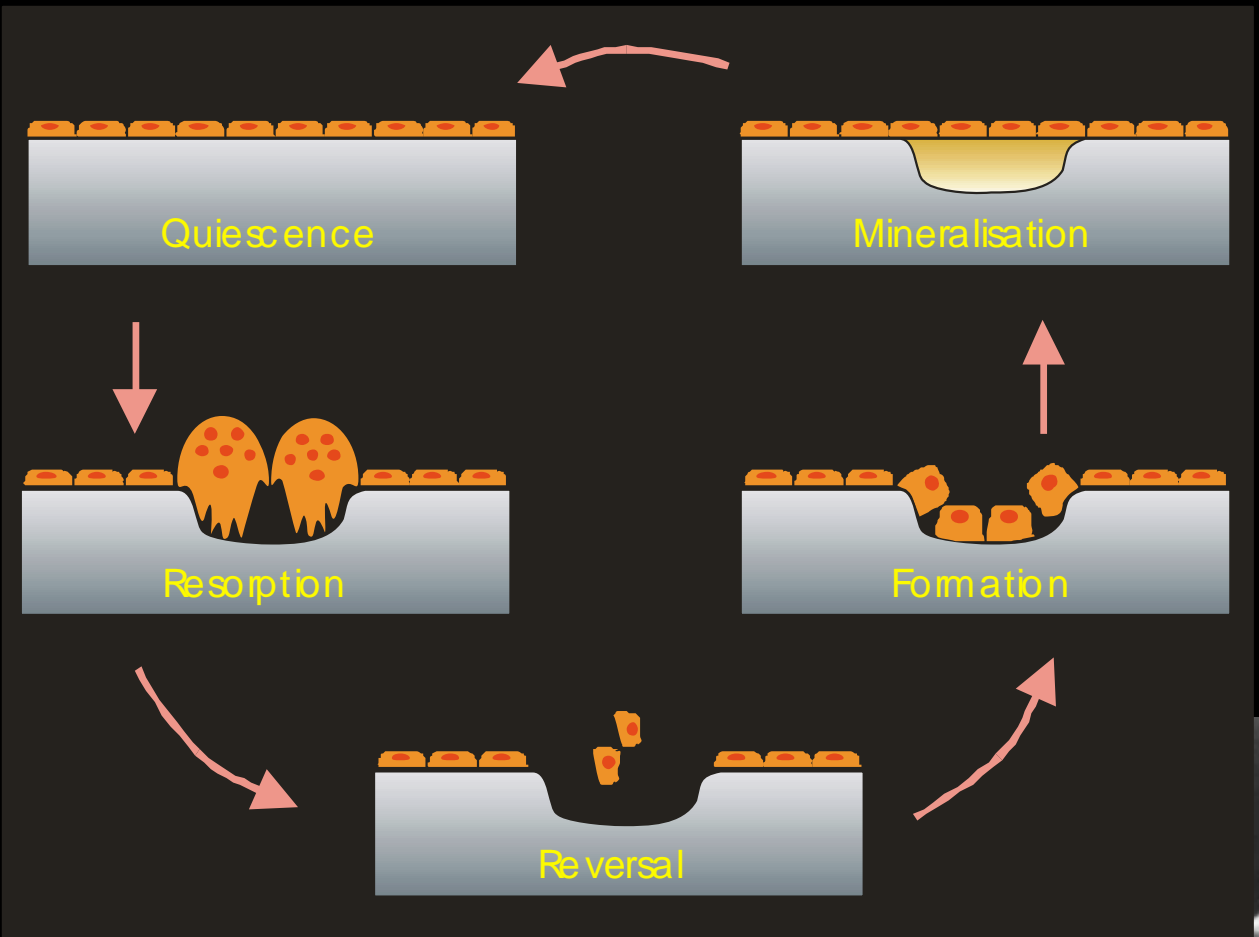


## Remodeling

Growing shaft is remodeled by:

- ① Bone resorbed here
- ② Bone added by appositional growth here
- ③ Bone resorbed here





# Controlling bone cell activity



# Osteoclast



# Osteoclastogenesis

HAEMOPOEITIC  
STEM  
CELL

SYSTEMIC HORMONES

PTH  
1,25(OH)<sub>2</sub>D<sub>3</sub>

HAEMOPOIETIC  
CELLS

OSTEOCLAST  
PRECURSORS

⊕  
IL-1  
IL-6  
TNF $\alpha$   
TGF $\alpha$   
M-CSF  
PTHrP  
RANKL

CYTOKINES  
&  
GROWTH FACTORS

STROMAL  
CELLS

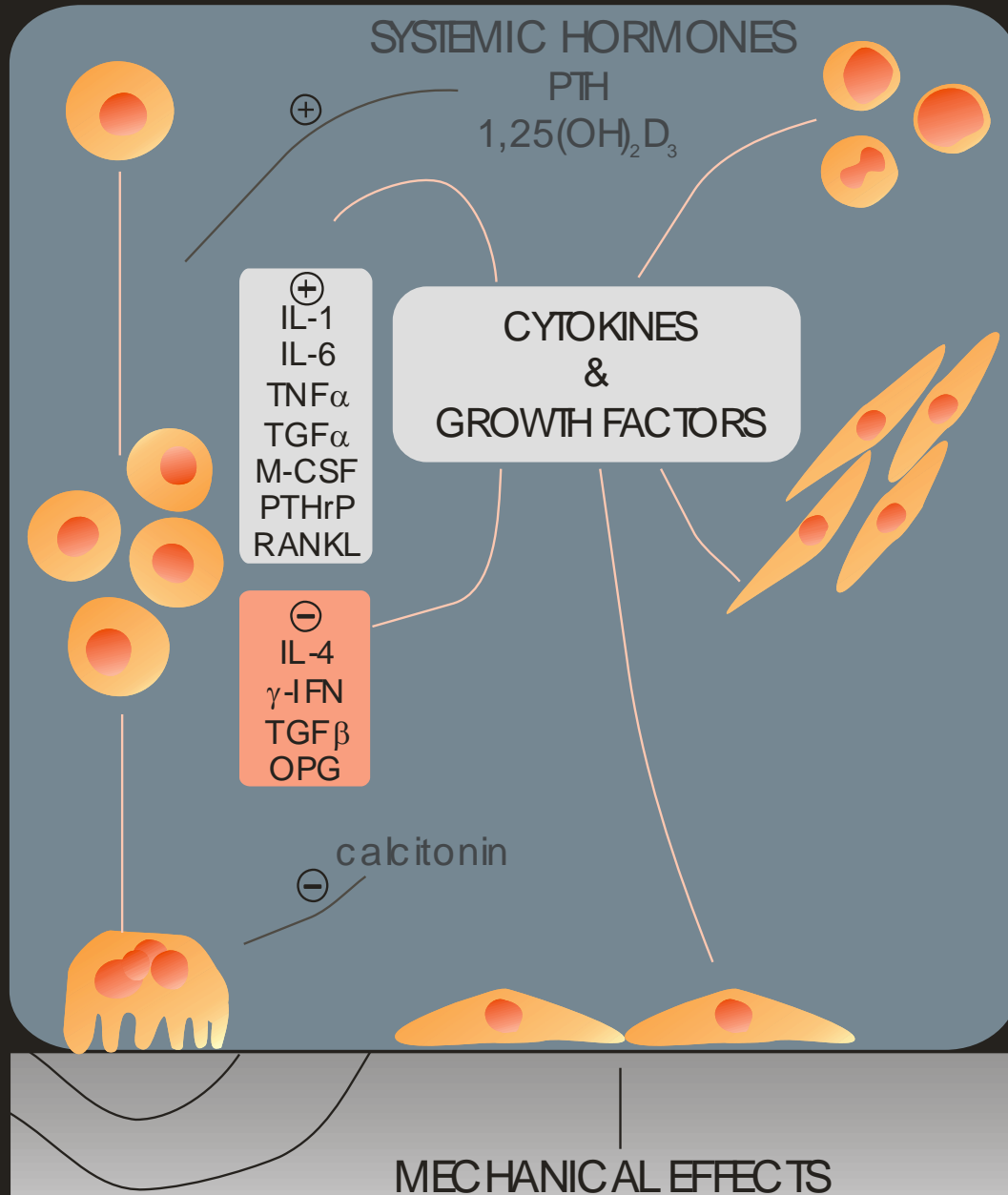
⊖  
IL-4  
 $\gamma$ -IFN  
TGF $\beta$   
OPG

MATURE  
OSTEOCLAST

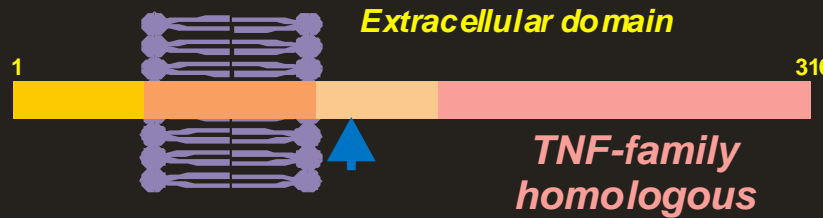
calcitonin

OSTEOBLASTS

MECHANICAL EFFECTS

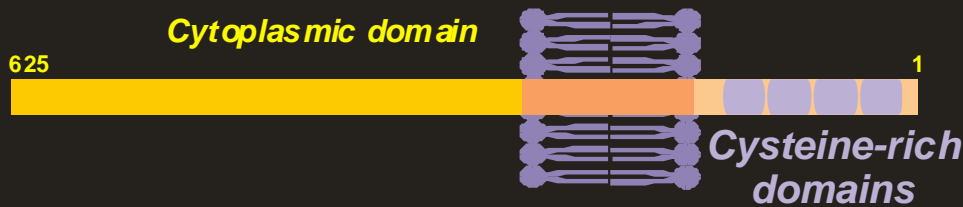


# RANK / RANKL & OPG



Receptor activator of NF- $\kappa$ B ligand (RANKL)

Osteoclast differentiation factor (ODF)  
Osteoprotegerin ligand (OPGL)  
TNF-related activation-induced cytokine (TRANCE)



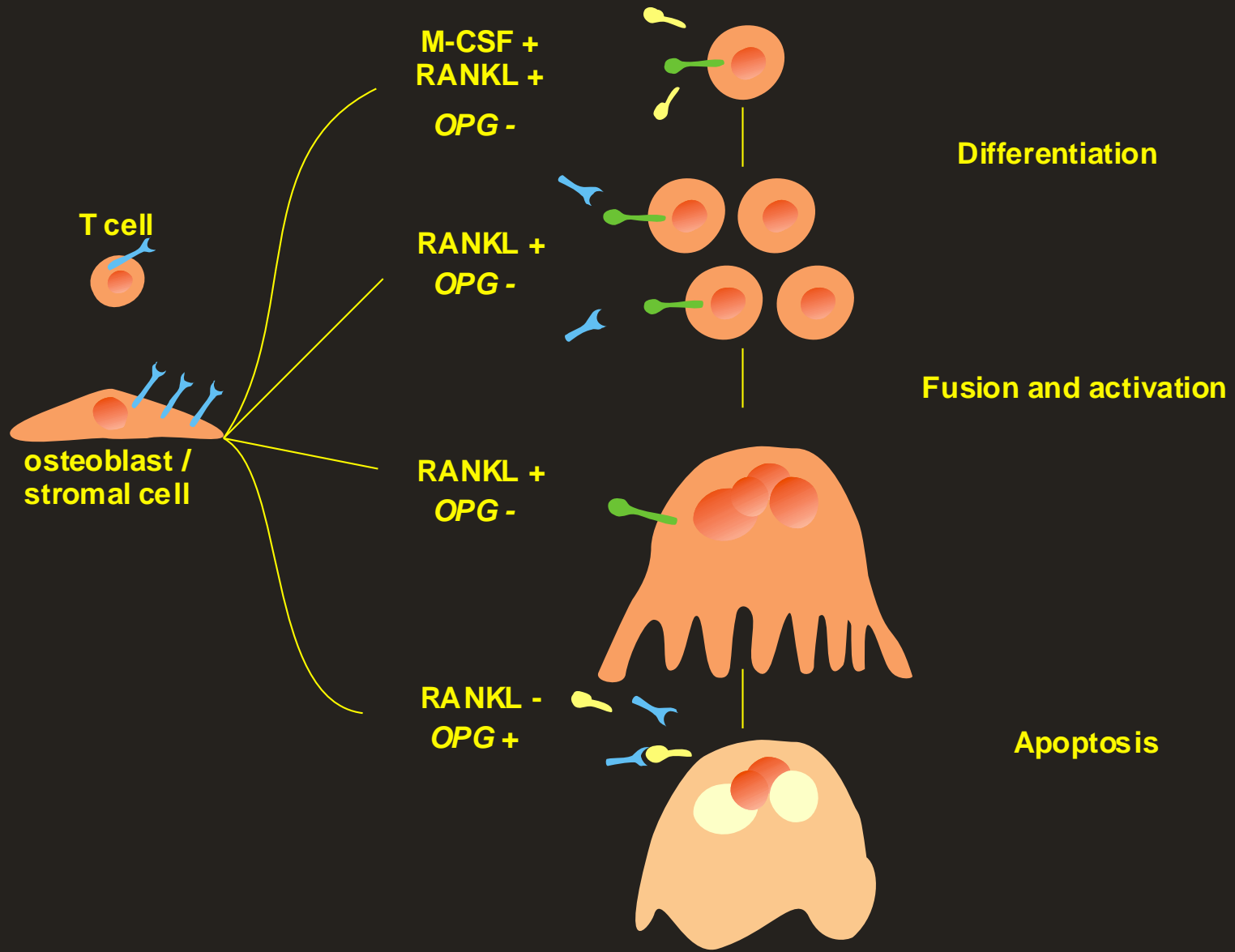
Receptor activator of NF- $\kappa$ B (RANK)



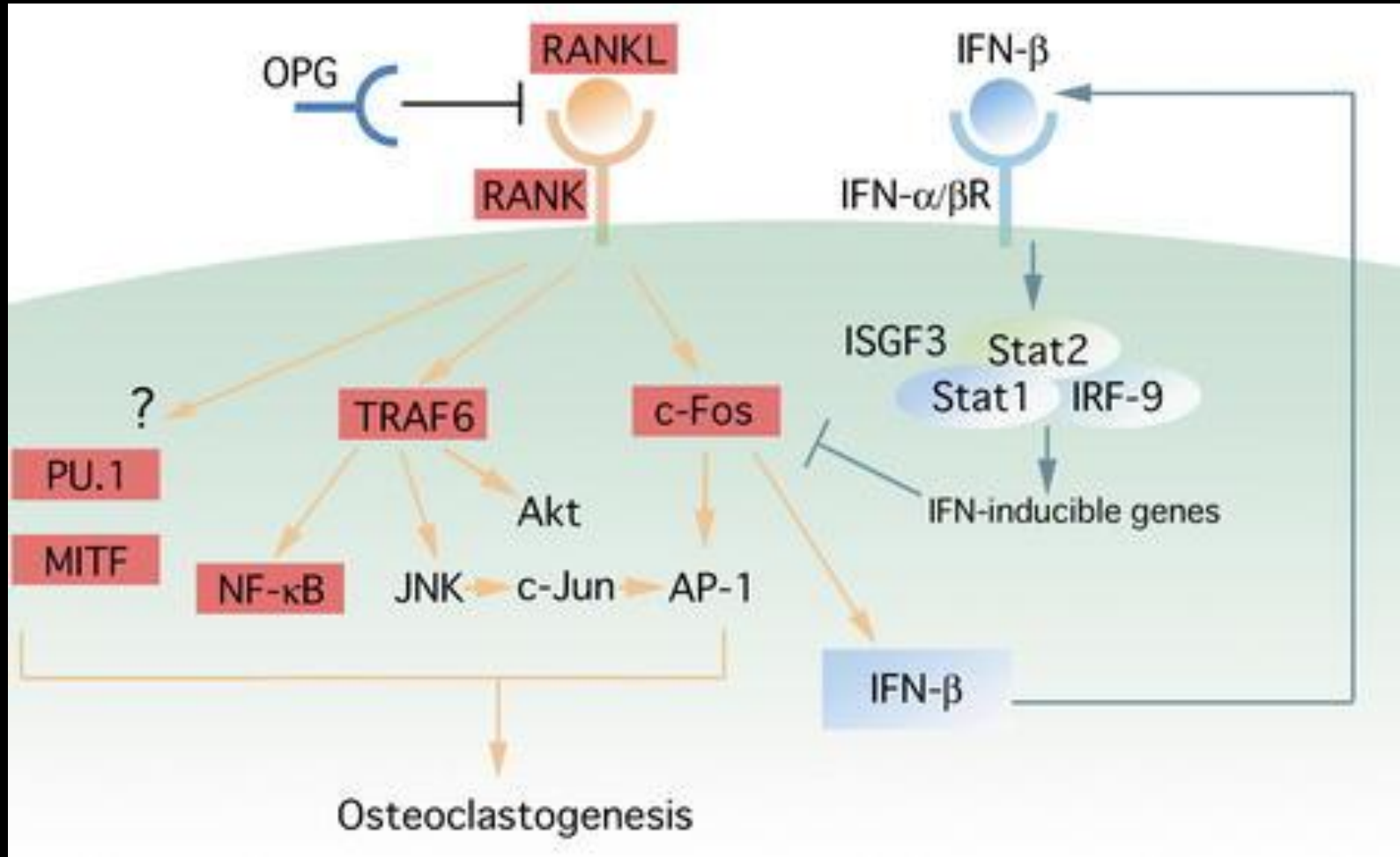
Osteoprotegerin (OPG)

Osteoclastogenesis inhibitory factor (OCIF)  
TNF receptor-like molecule 1 (TR1)

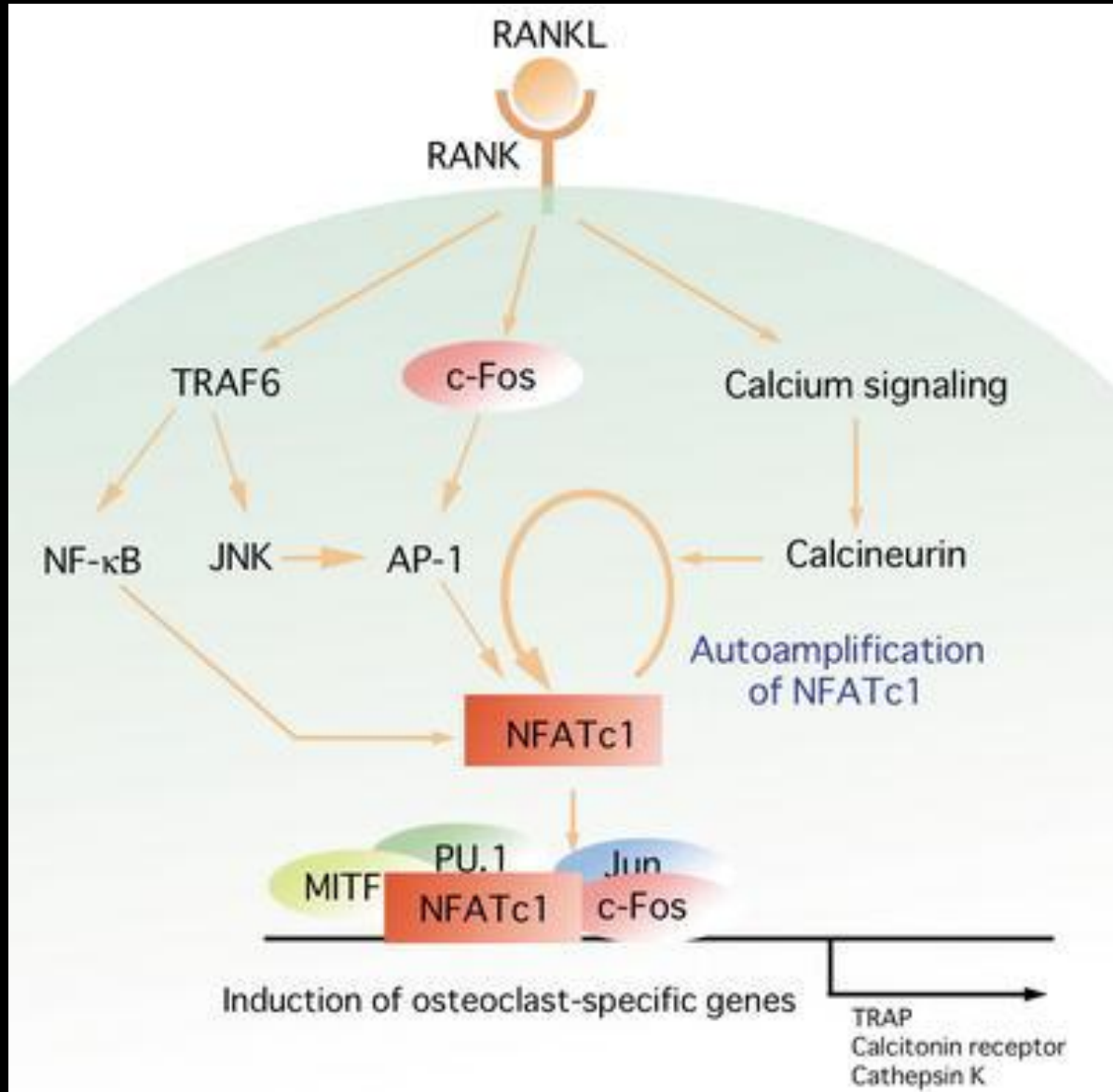
# RANKL / OPG levels regulate bone resorption



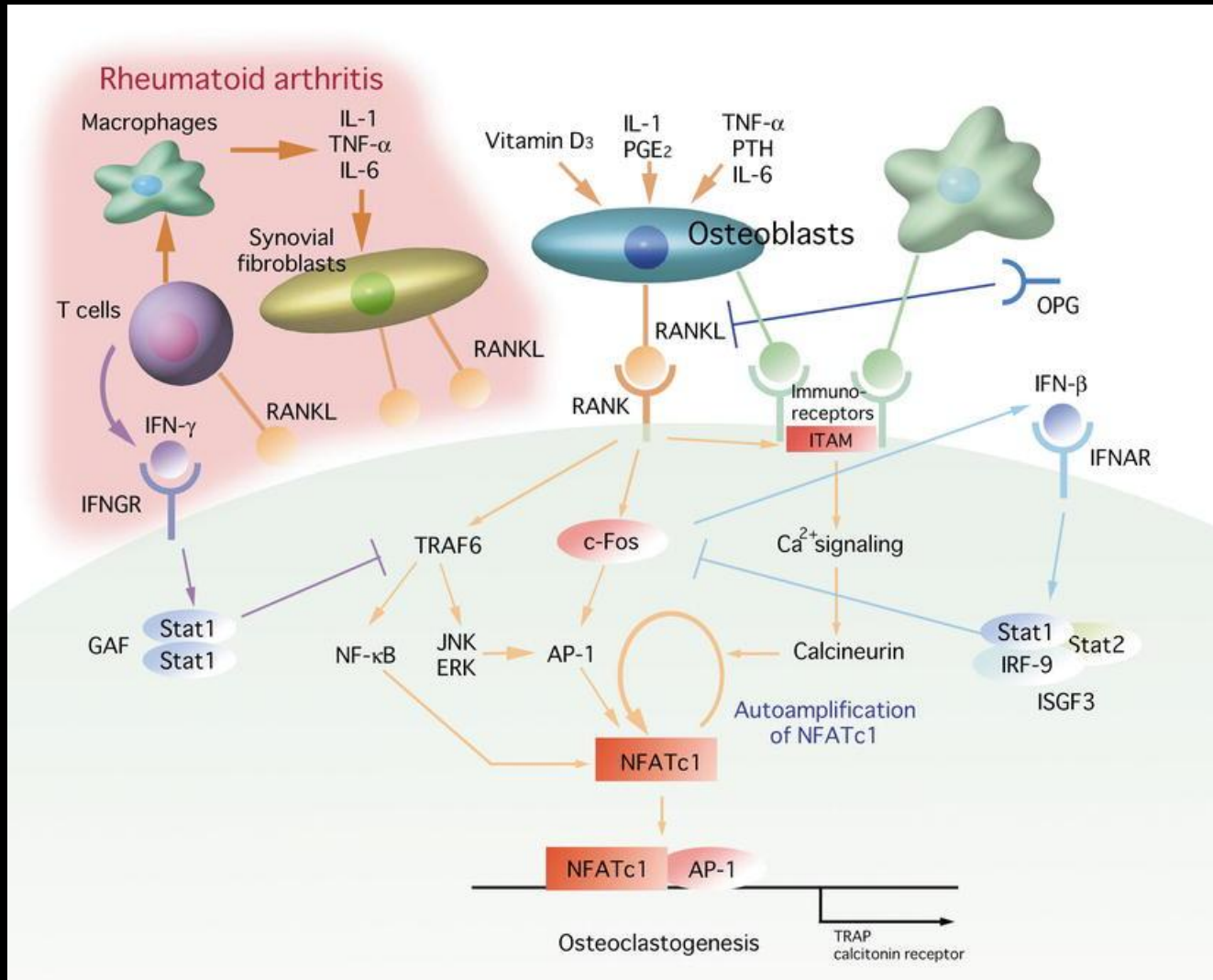
# RANKL intracellular signalling cascades



# Integration of RANKL signalling by NFATc1



# Crosstalk between immune and skeletal cells



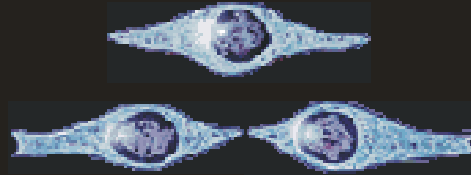
# Controlling bone cell activity

## - Osteoblast

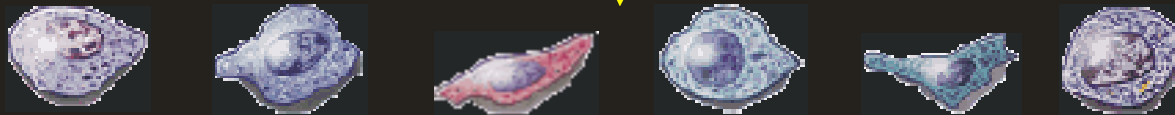


# mesenchymal stem cell (MSC)

*proliferation*



*commitment*



*lineage progression*



*differentiation and maturation*



**bone**



**cartilage**



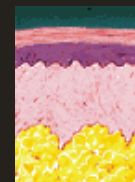
**cardiac**



**stroma**

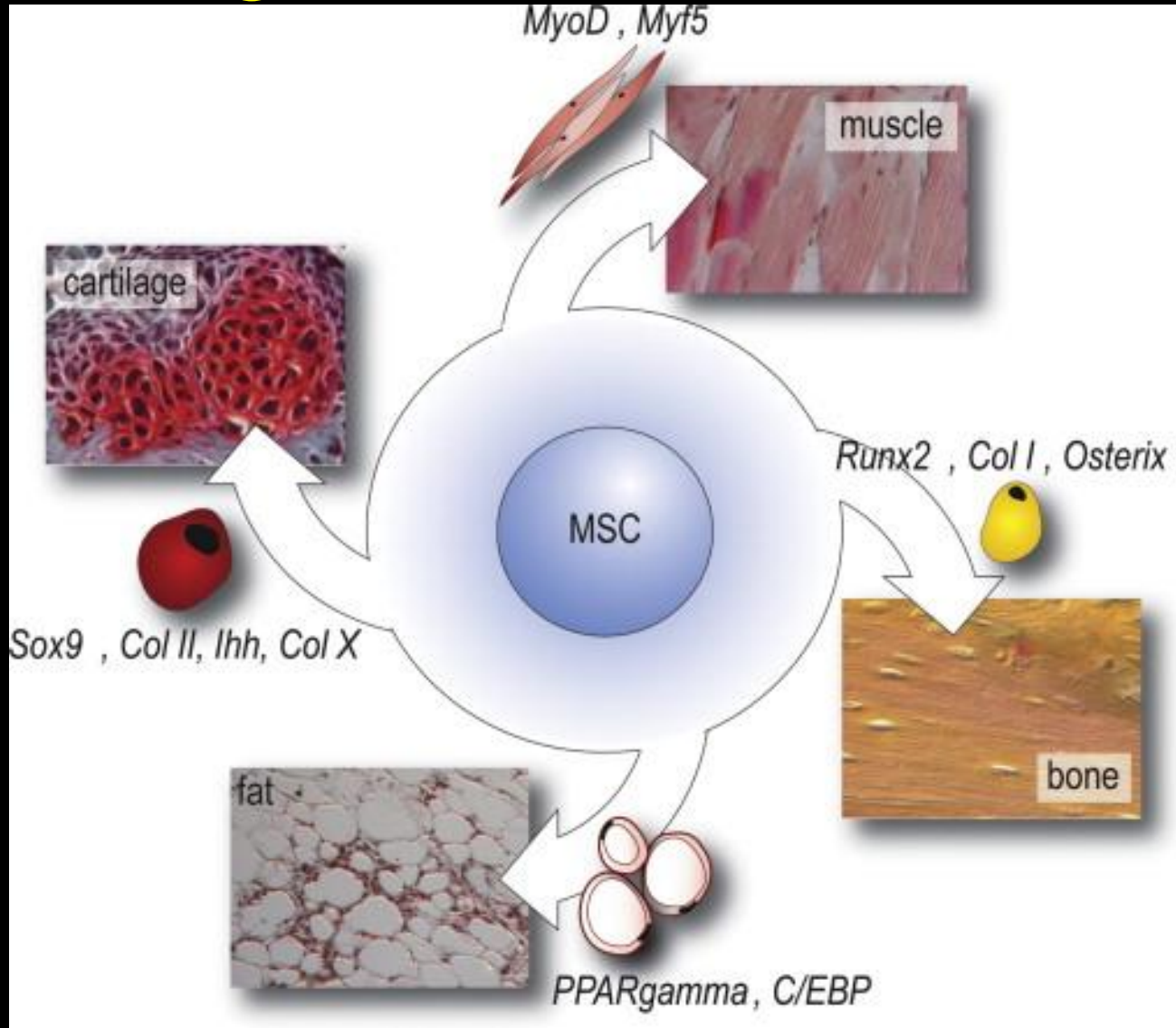


**tendon**

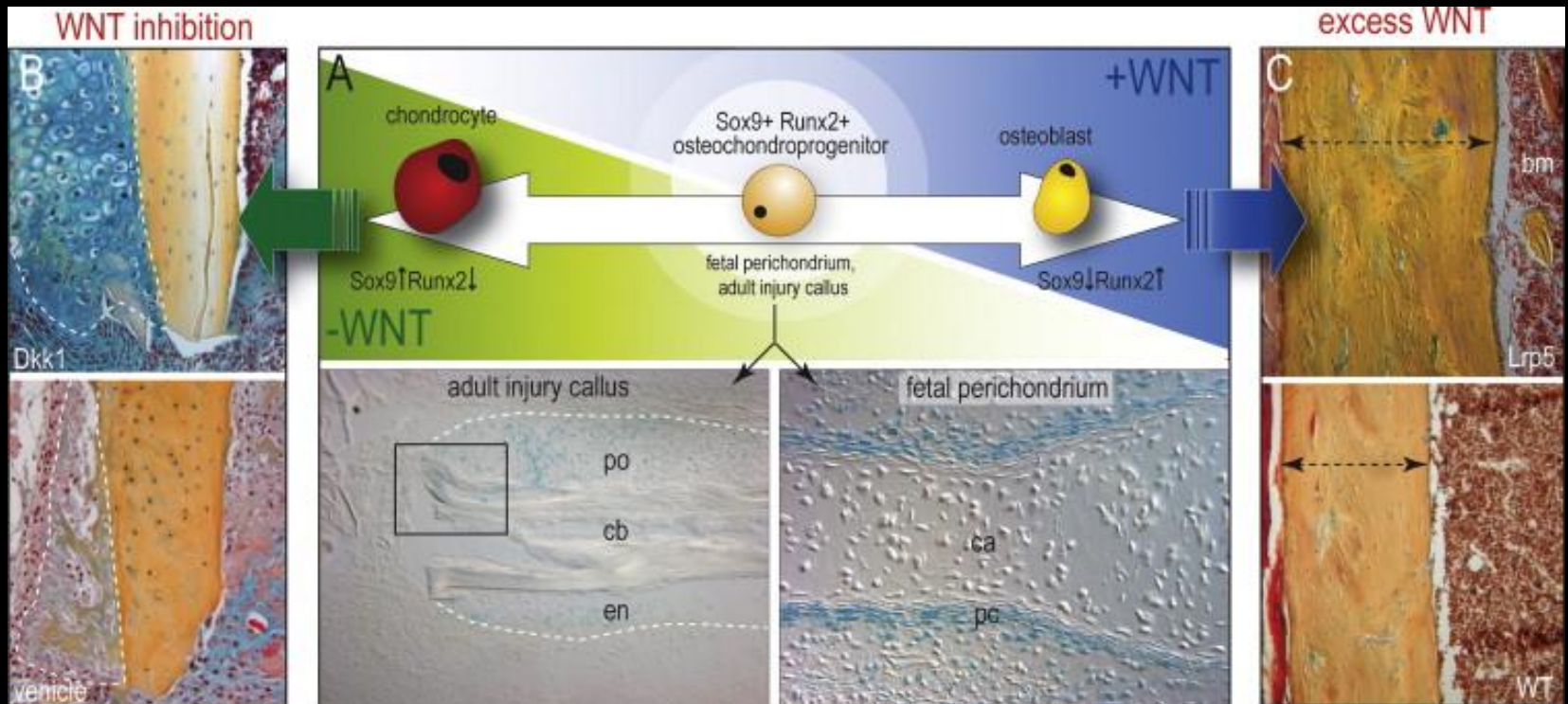


**adipose tissue**

# Regulation of cell fate

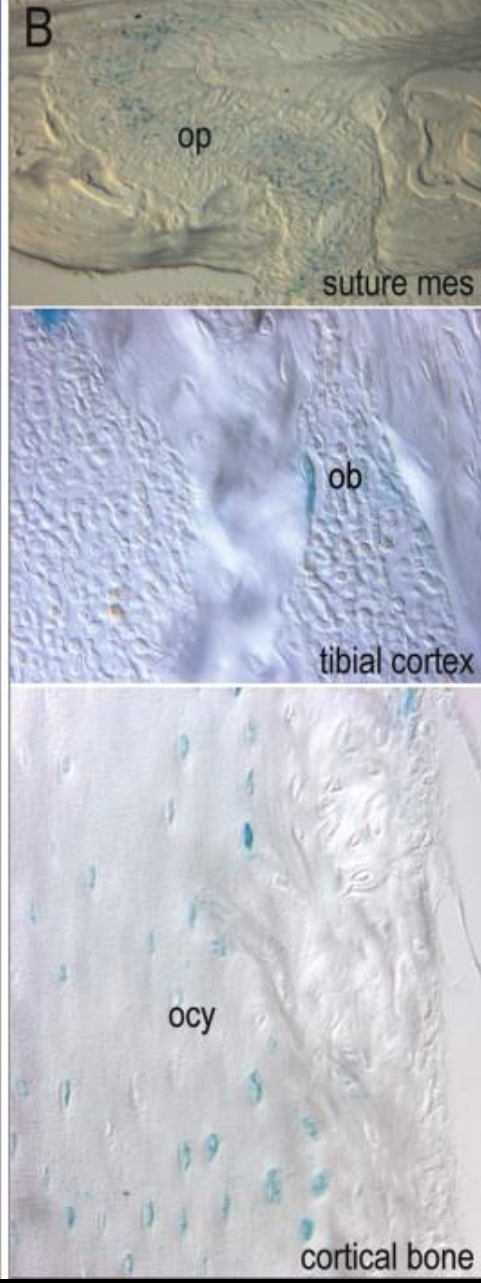
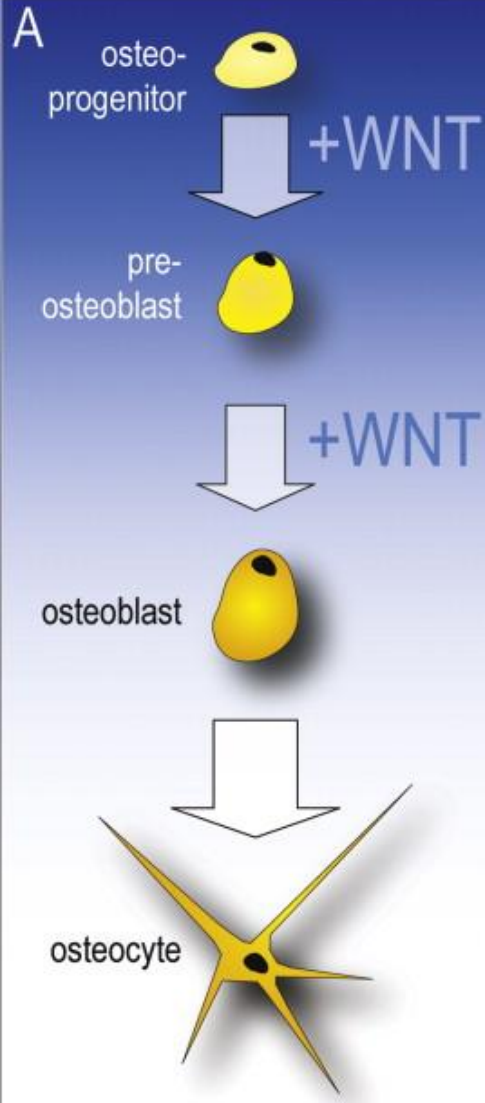


# Role of Wnt in skeletal patterning

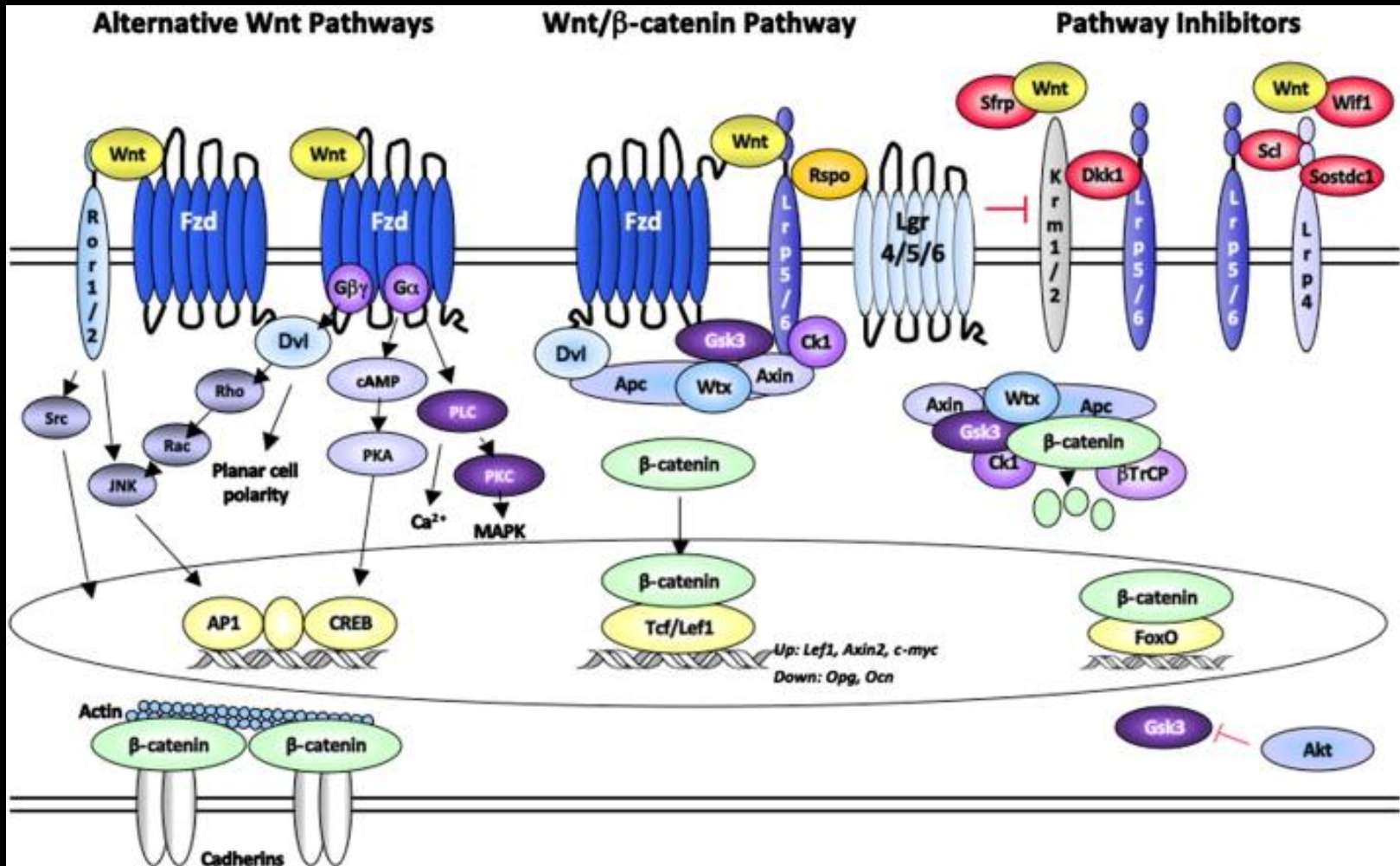


osteogenesis

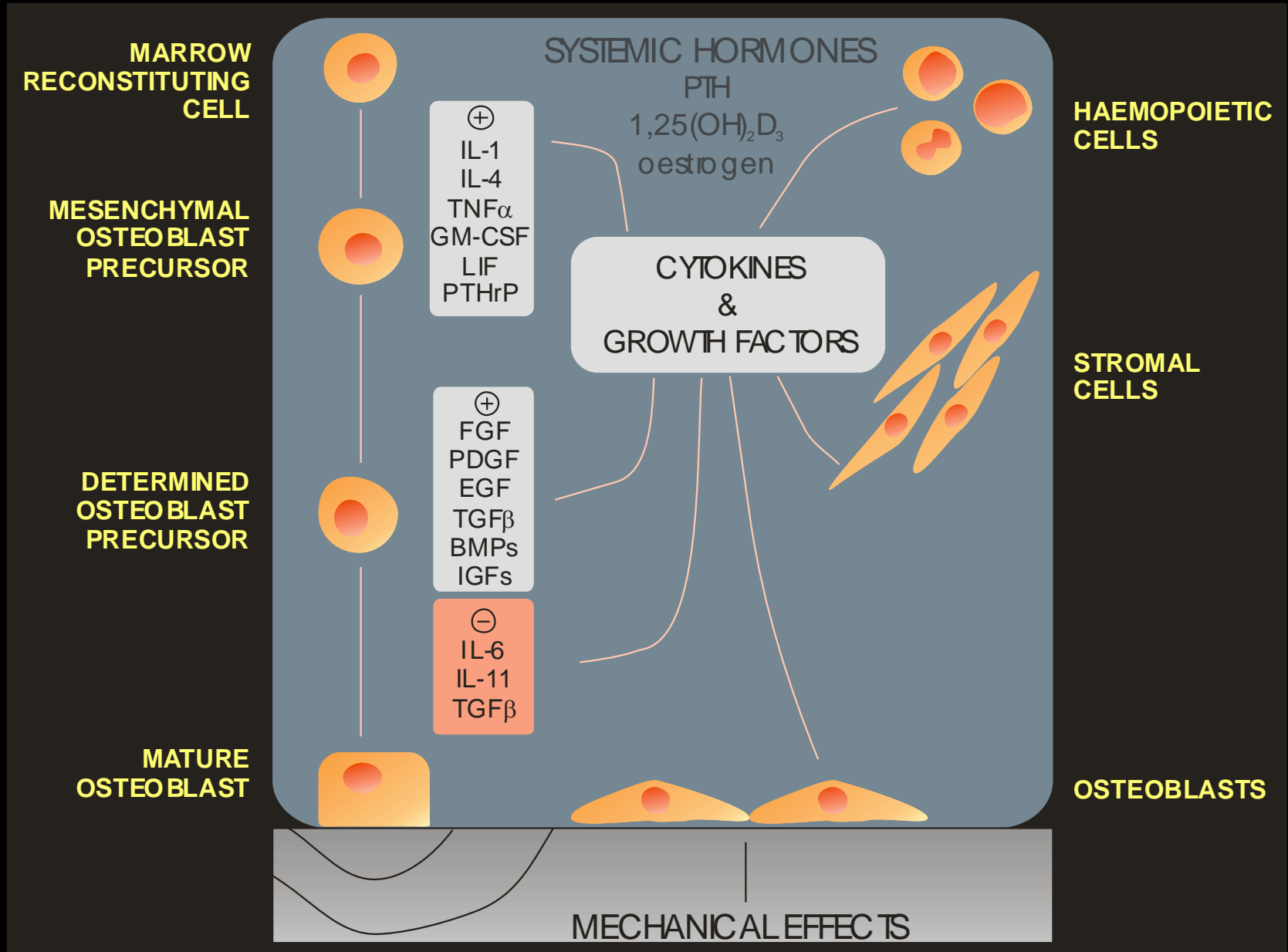
Wnt responsiveness



# Intracellular signalling in response to Wnt



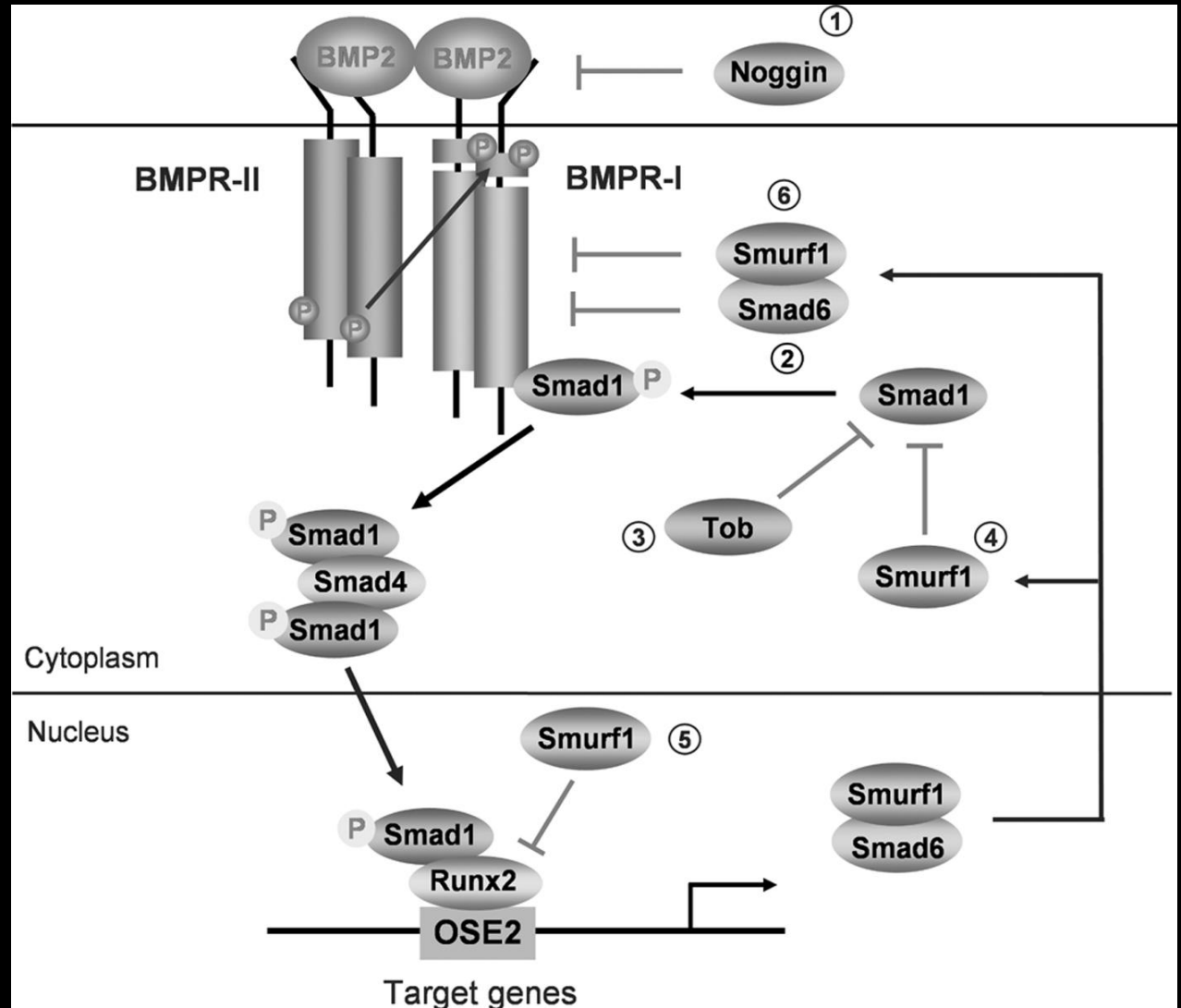
# Osteoblast Differentiation

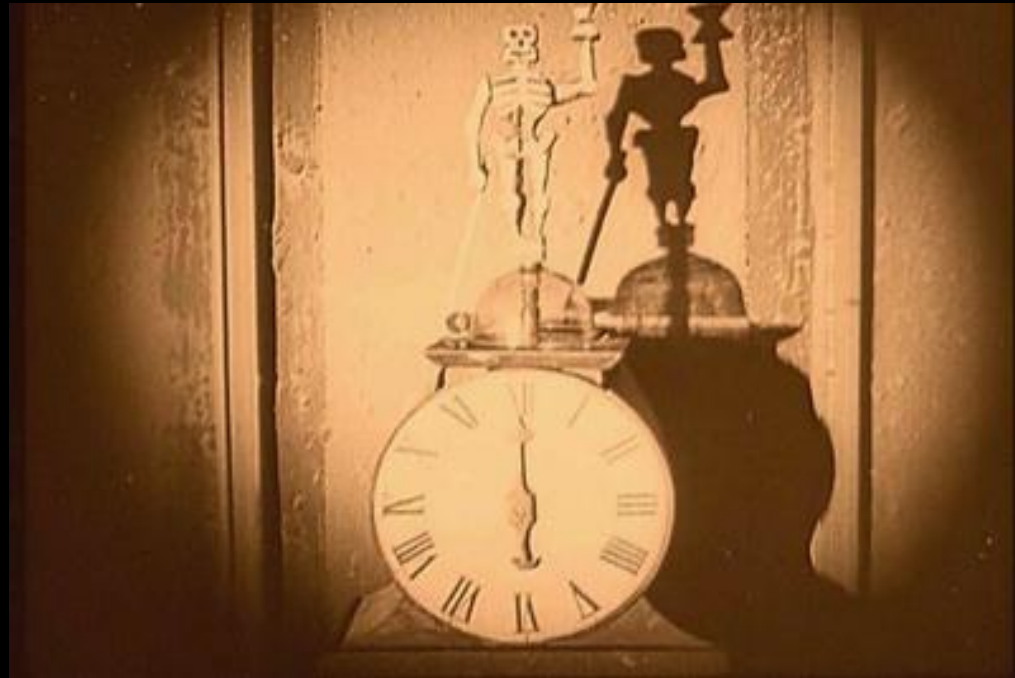


# Regulating Bone formation

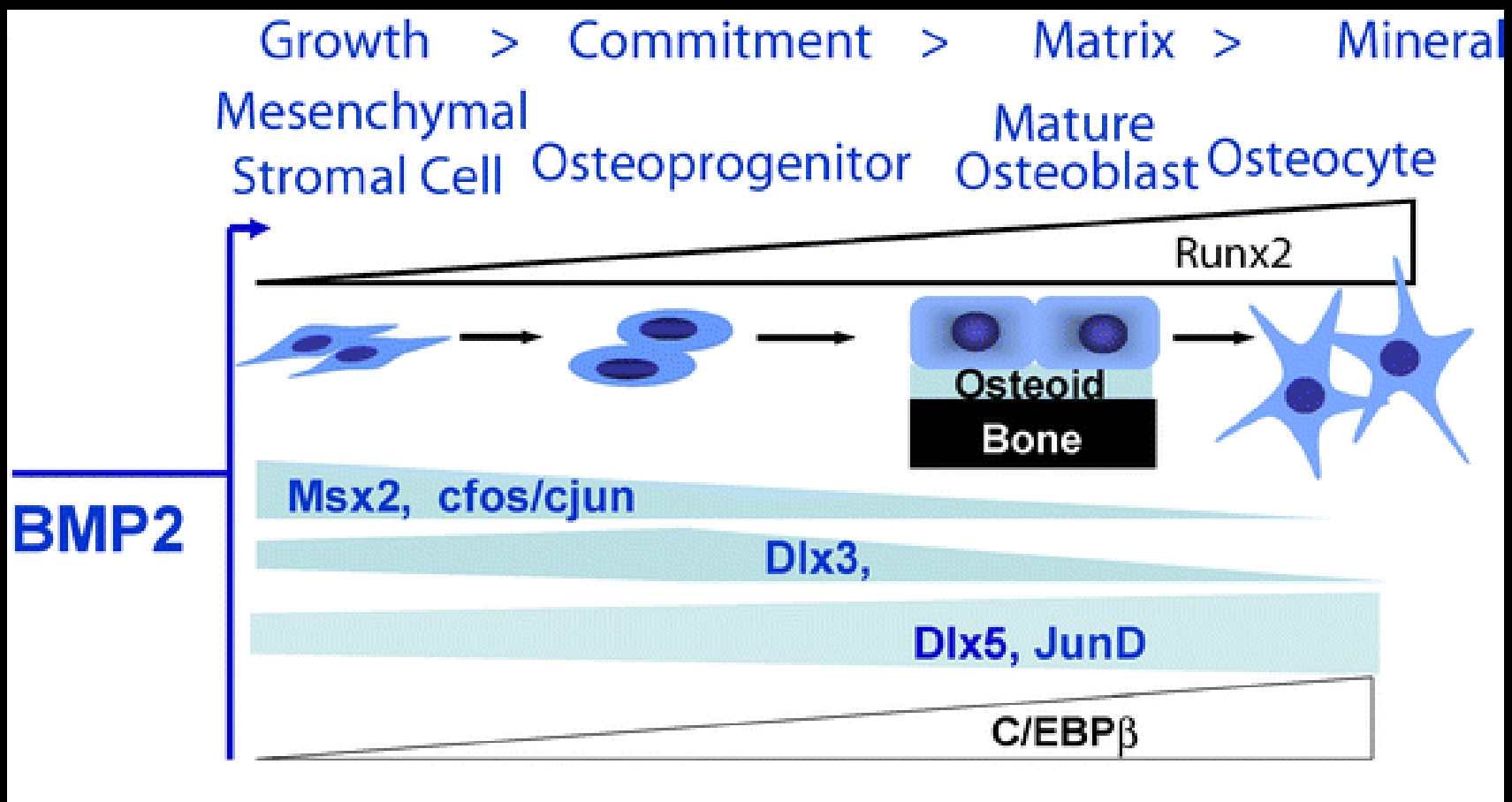
<u>BMP Subfamily</u>	<u>Genermic Name</u>	<u>BMP Designation</u>
BMP2/4	BMP-2A	BMP-2
	BMP-2B	BMP-4
BMP-3	Osteogenin	BMP-3
	Growth/differentiation factor-10(GDF-10)	BMP-3B
OP-1/BMP-7	BMP-5	BMP-5
	Vegetal related-1(Vgr-1)	BMP-6
	Osteogenic protein-1	BMP-7
	Osteogenic protein-2	BMP-8
	Osteogenic protein-3	BMP-8B
Others	BMP-9	BMP-9
	BMP-10	BMP-10
	Growth/differentiation factor-11 (GDF-11)	BMP-11
CDMP	Cartilage-derived morphogenetic protein-1 (CDMP-1)	BMP-14
	growth/differentiation factor-5 (GDF-5)	
	Cartilage-derived morphogenetic protein-1 (CDMP-2)	BMP-13
	growth/differentiation factor-5 (GDF-6)	
Cartilage-derived morphogenetic protein-1 (CDMP-3)	BMP-12	
growth/differentiation factor-5 (GDF-7)		
Others	BMP-15	BMP-15
	BMP-16	BMP-16

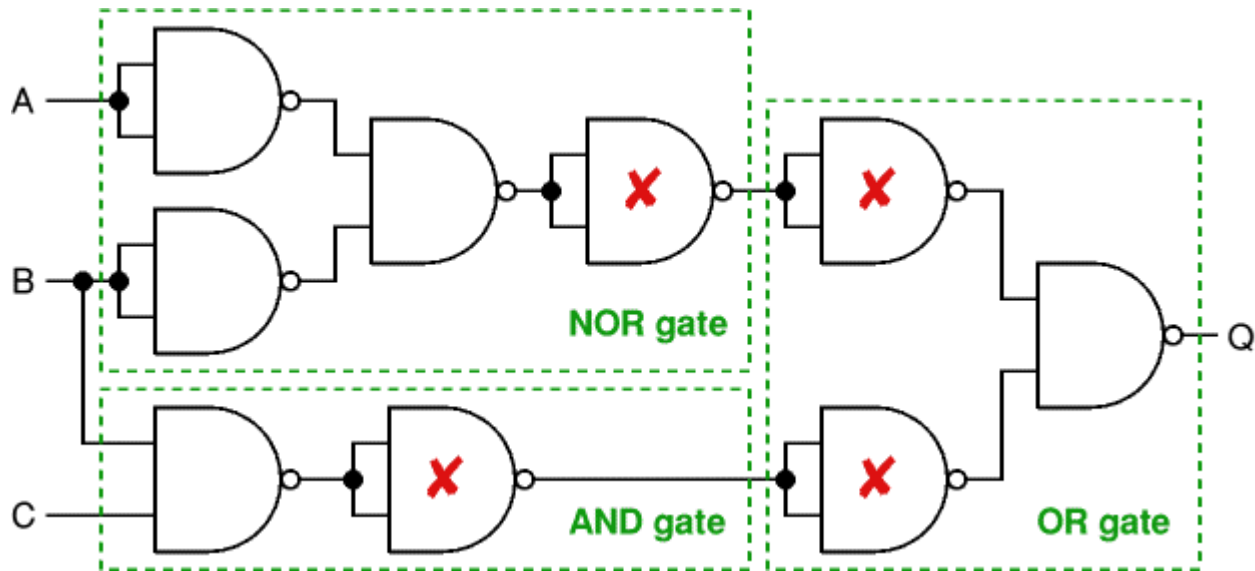
Signal transduction events mediate the action of BMPs leading to regulation of osteogenic genes





# Control of osteoblast differentiation by transcription factors

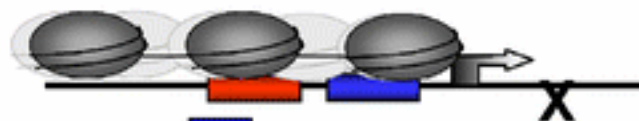




Bone related promoter elements



Inactive Gene



Chromatin Remodeling

Basal Transcription



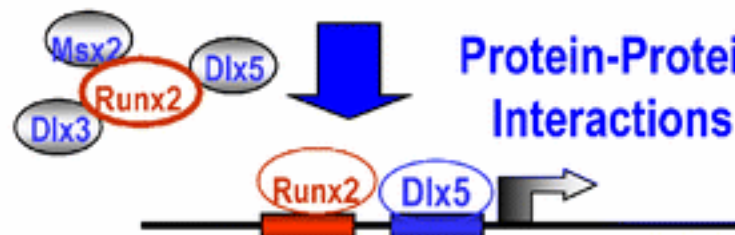
Molecular Switches (DNA Binding)

Activated Transcription



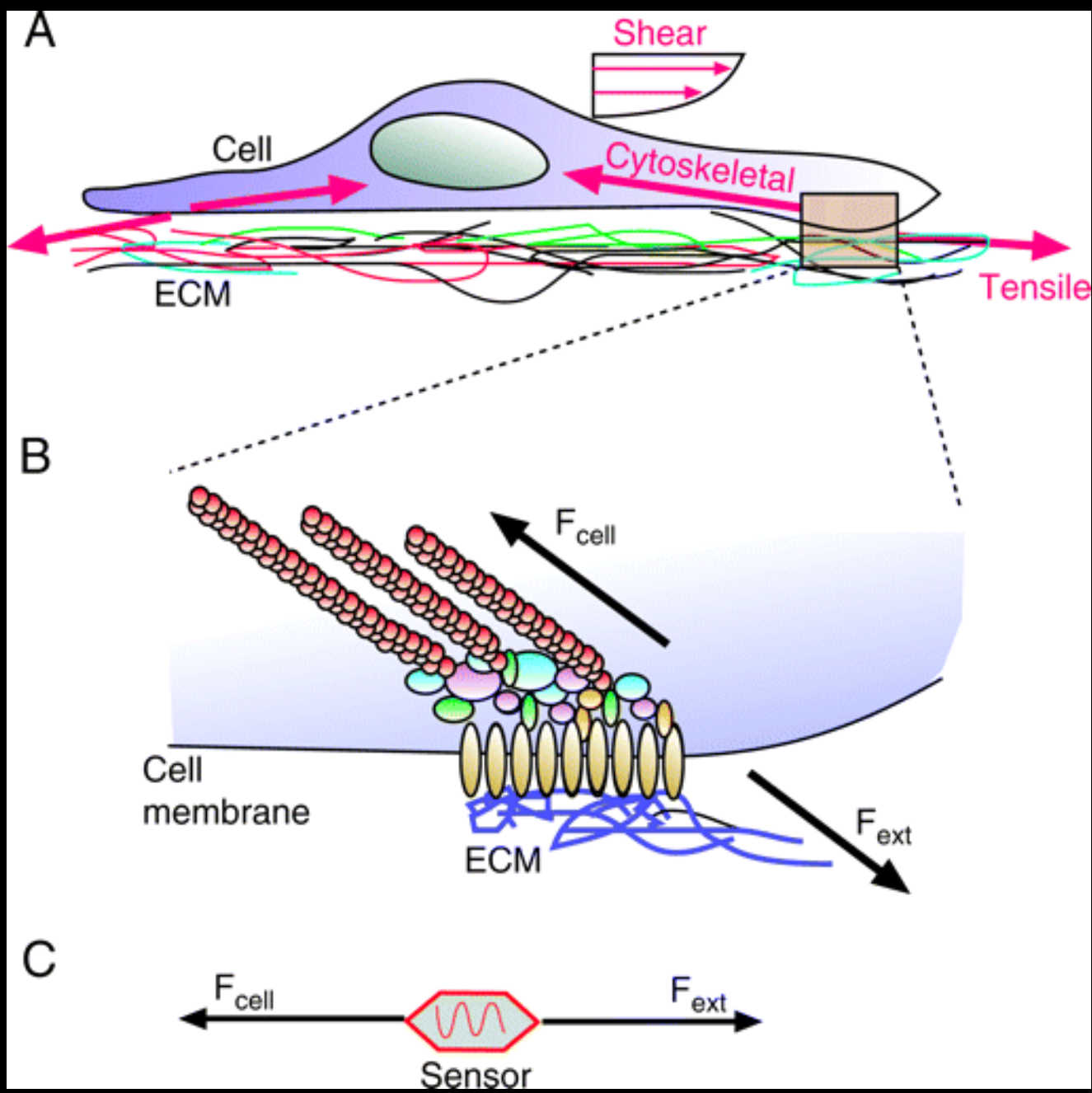
Protein-Protein Interactions

Maintenance Transcription

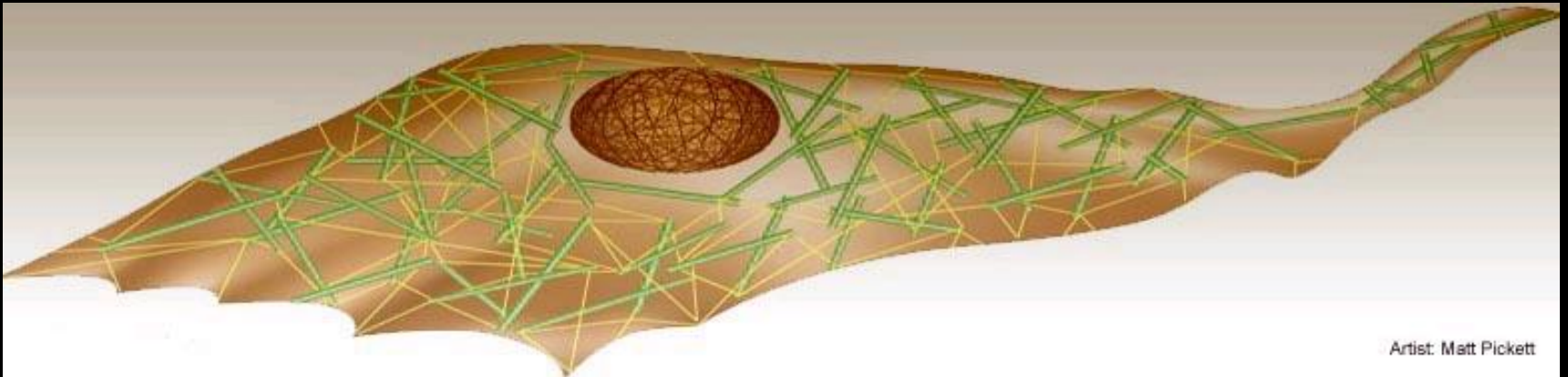
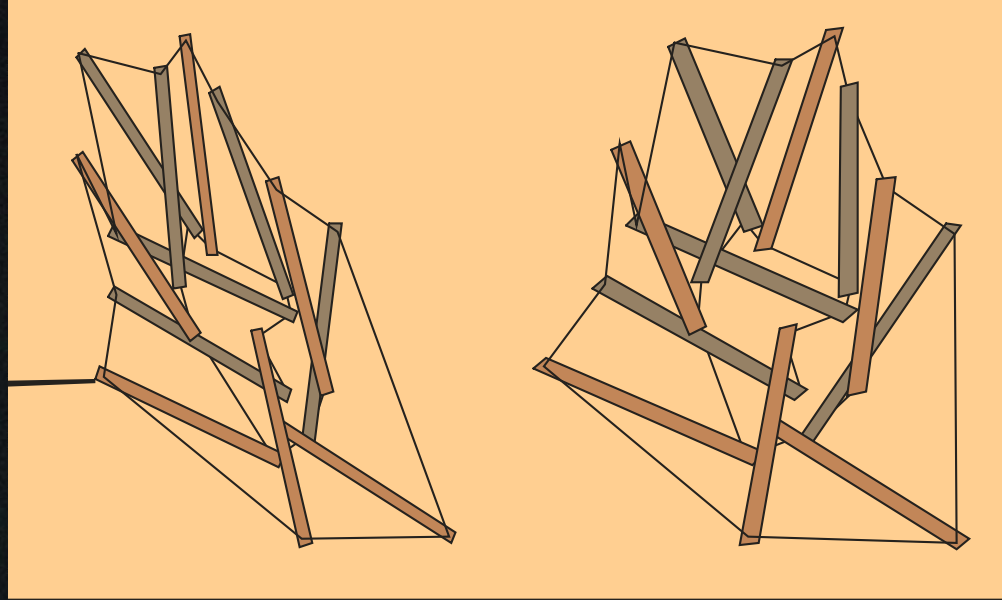
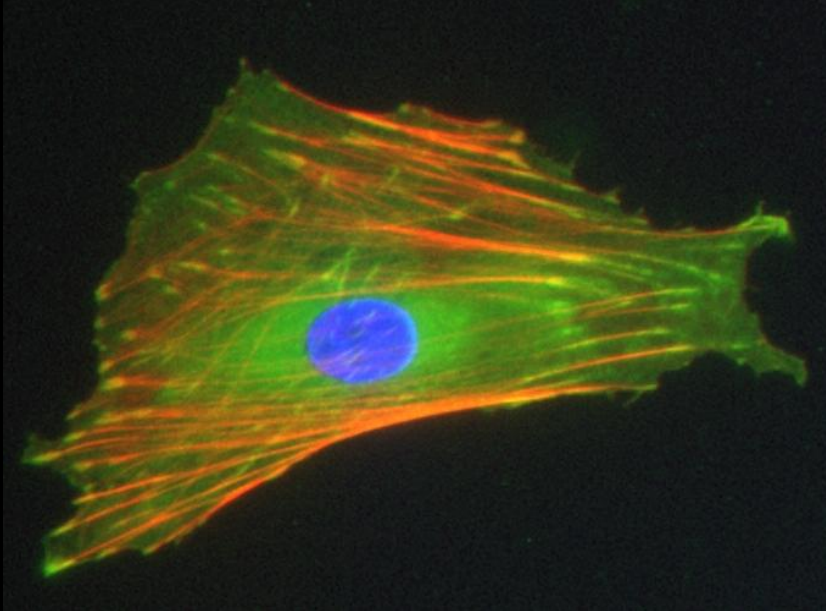


# Mechanical load regulates bone cell activity and bone mass



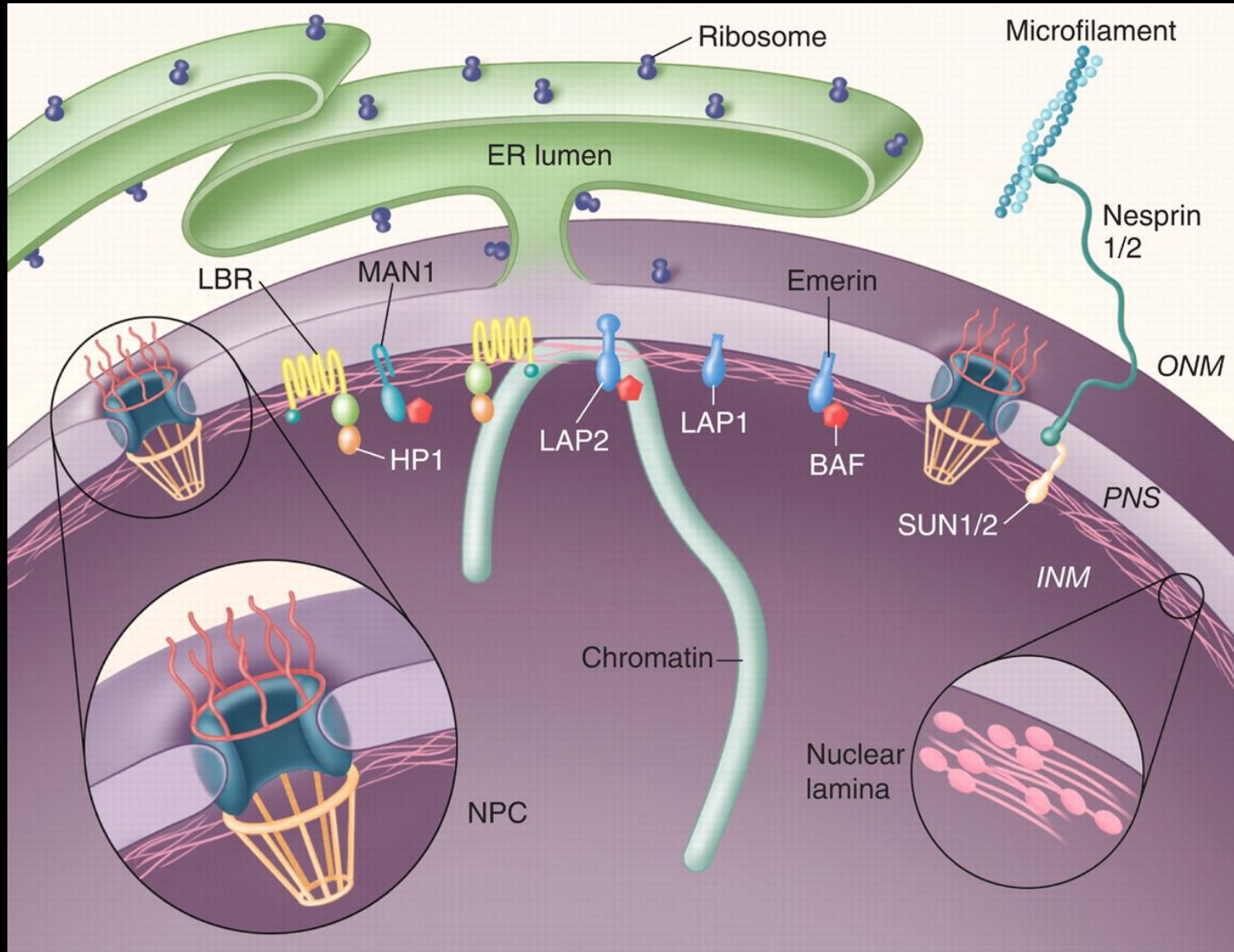


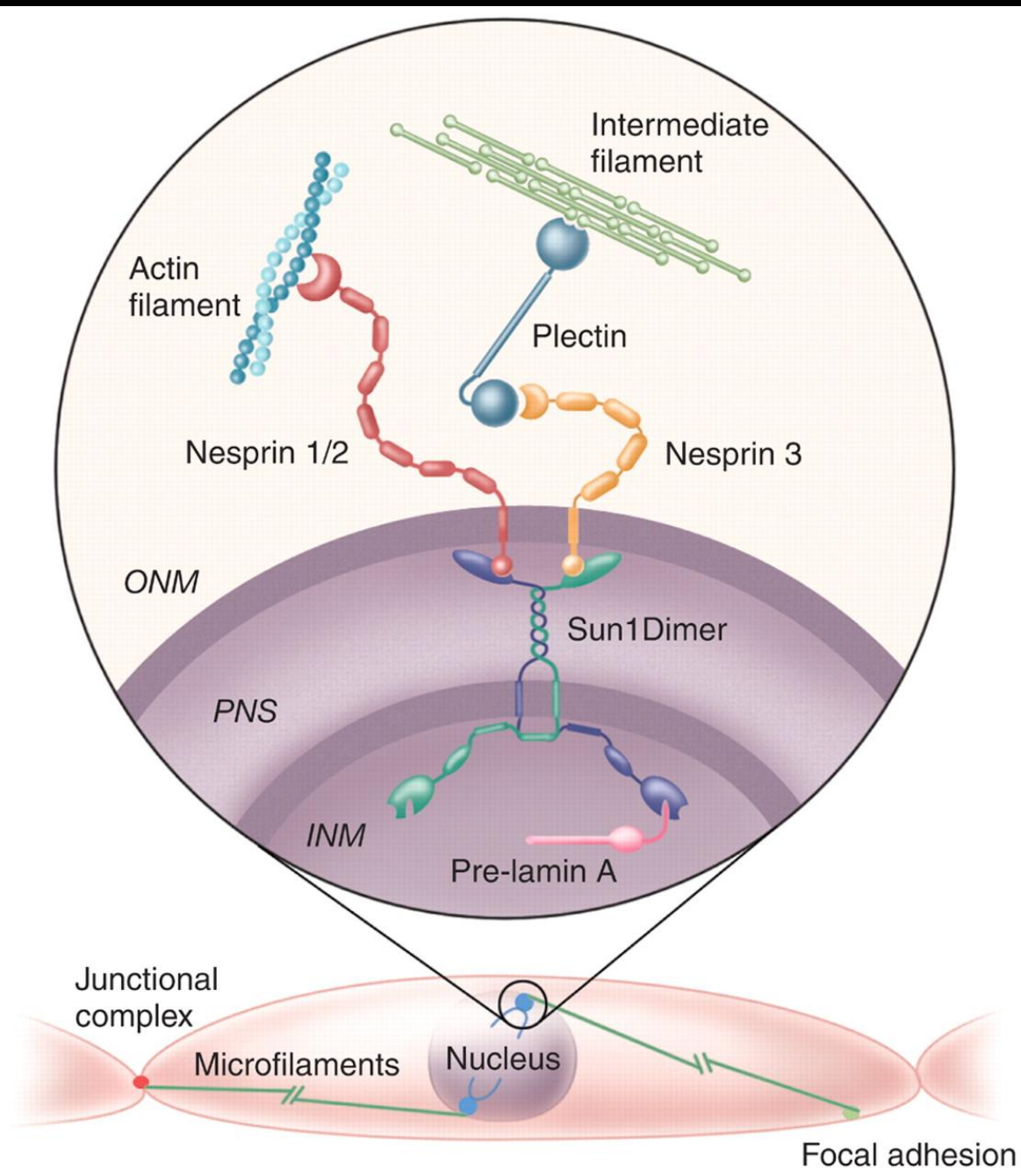
# Tensegrity



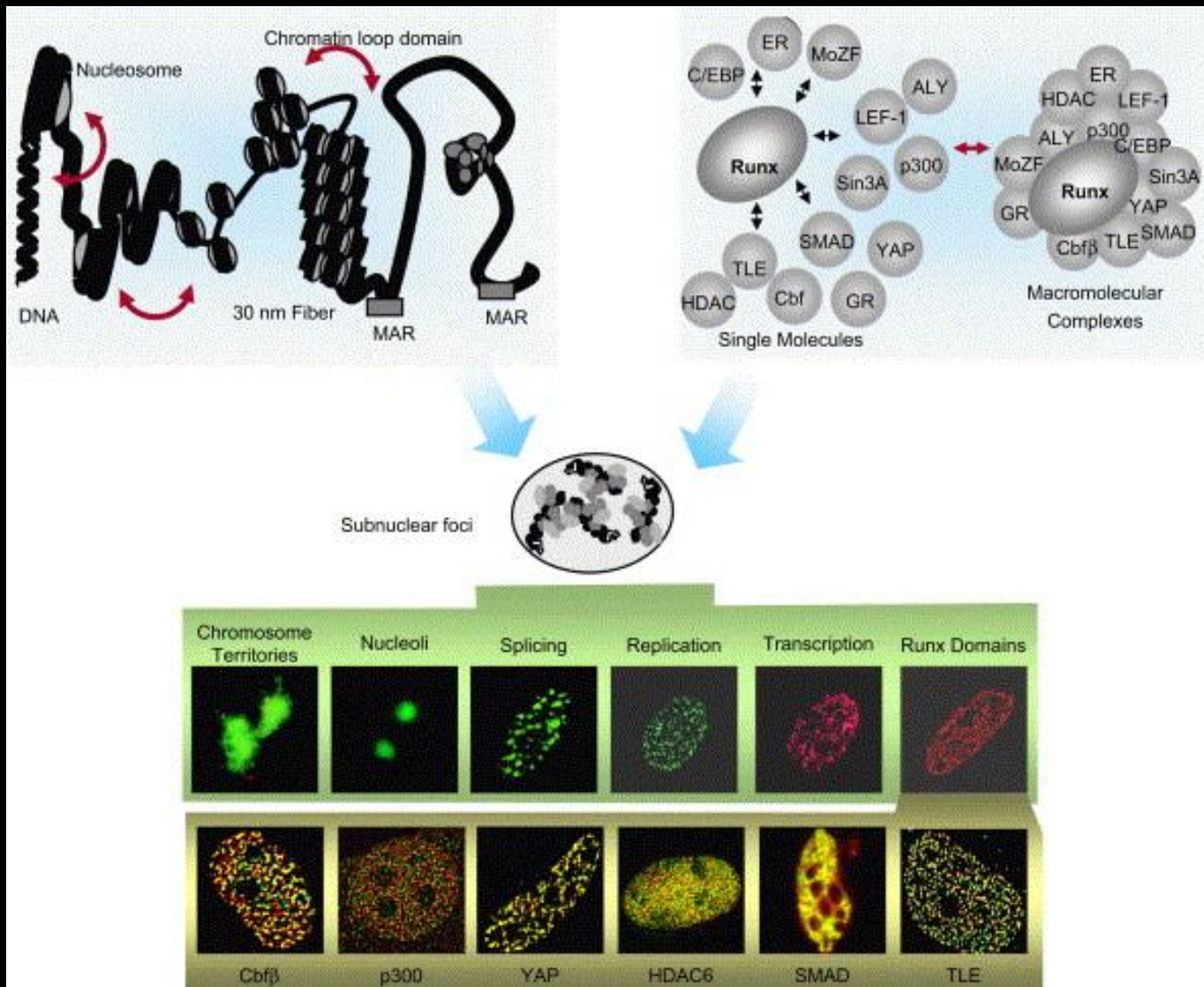
Artist: Matt Pickett

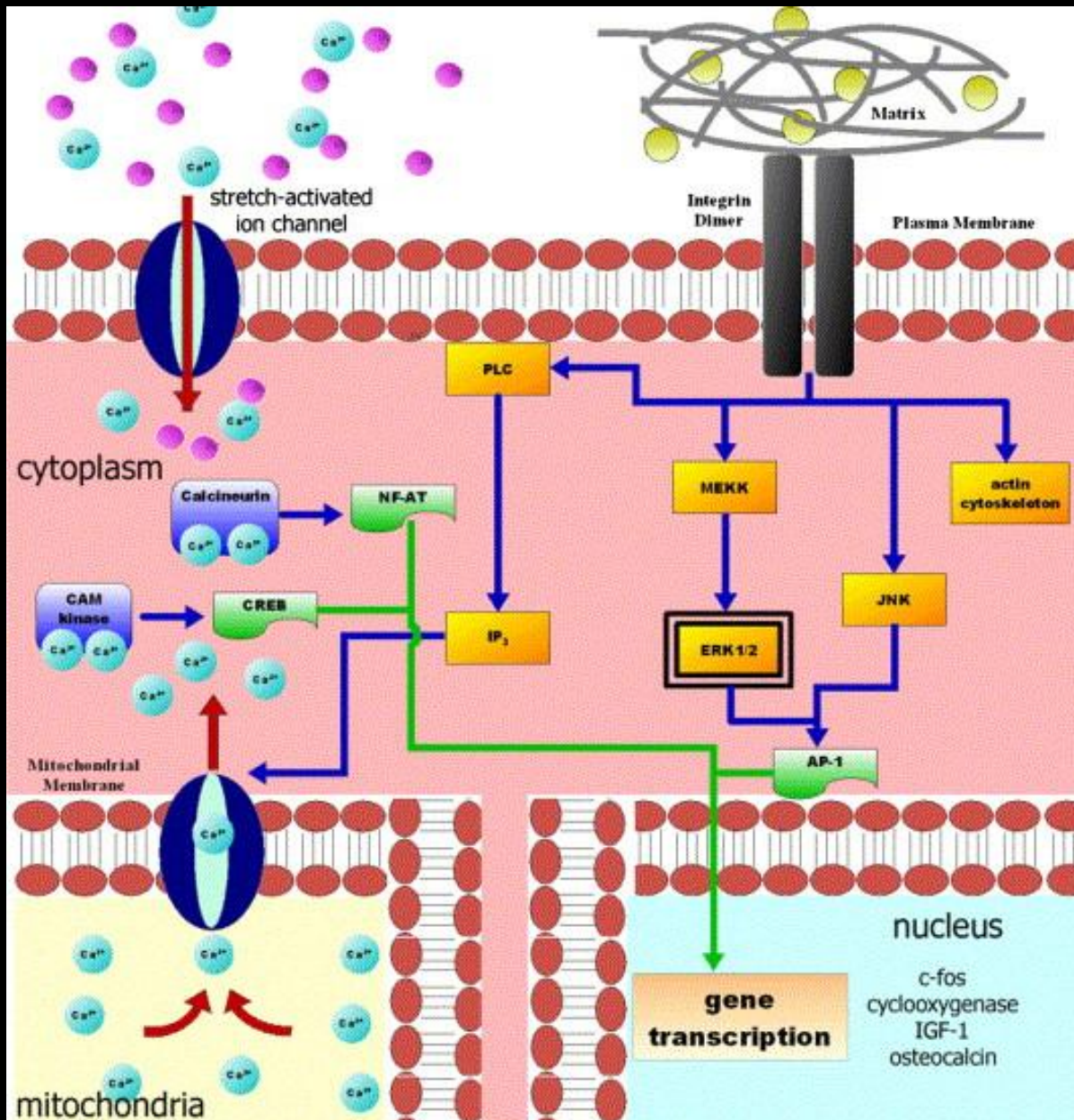
# Nuclear architecture





# Levels of nuclear organisation

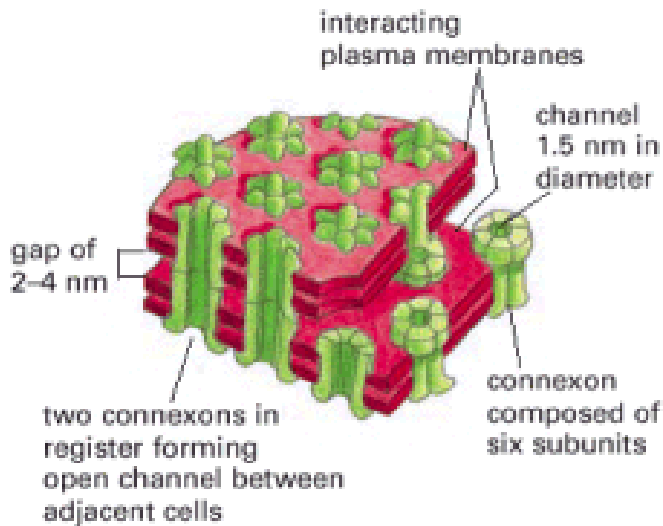




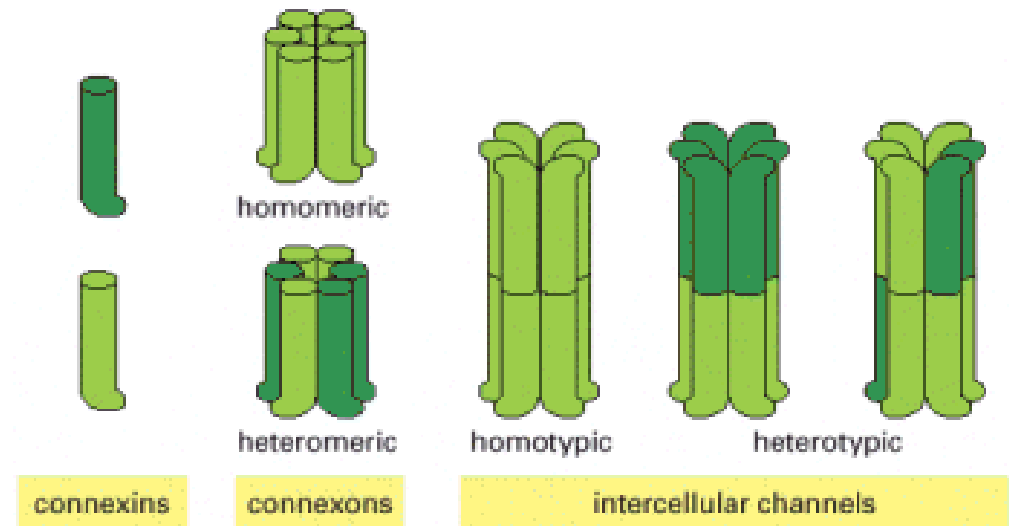
# Sharing the message



# Propagation of signals

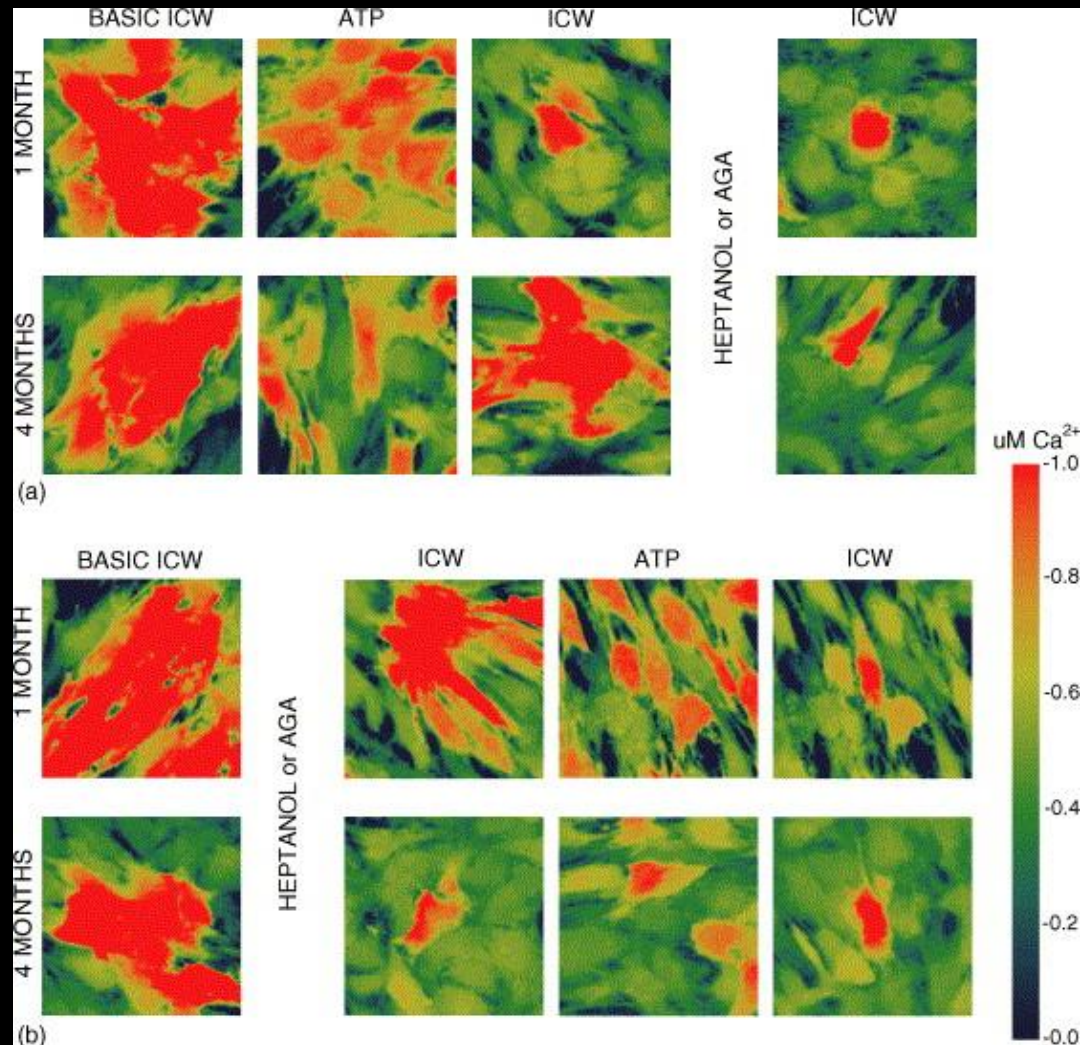


(A)

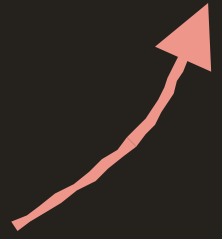
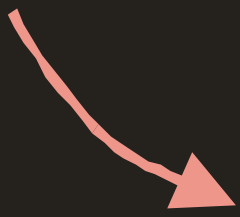
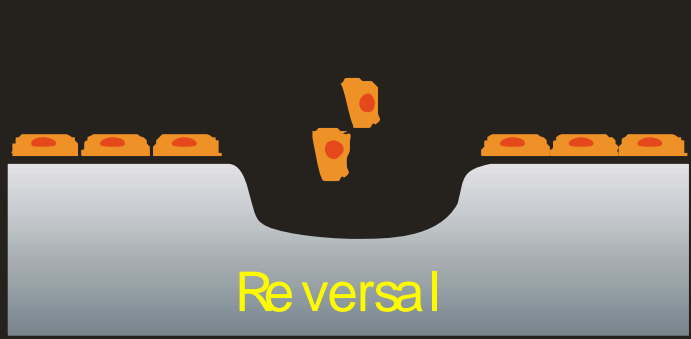
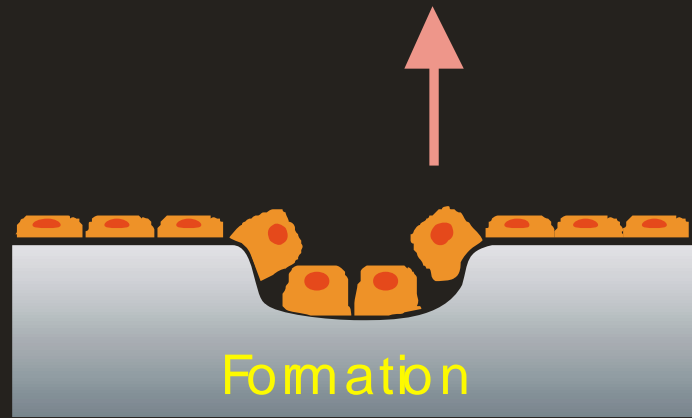
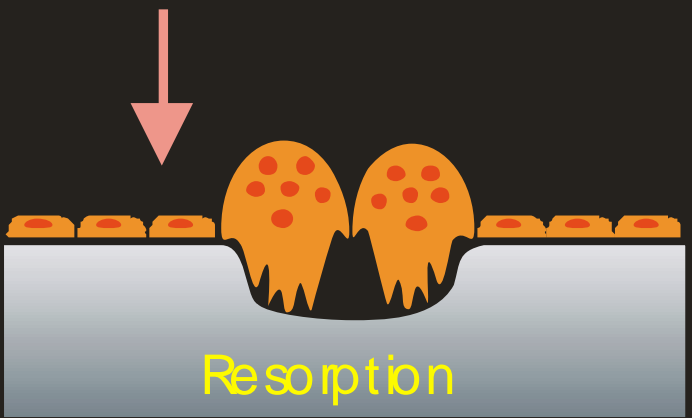
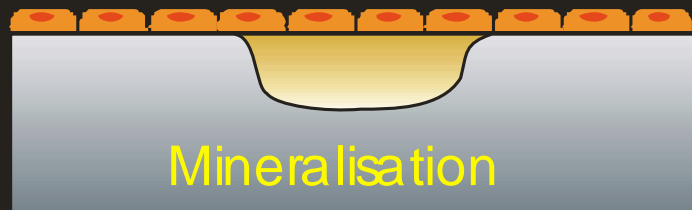


(B)

# Intercellular calcium wave propagation during osteoblast differentiation



# Coordinating bone cell behaviour

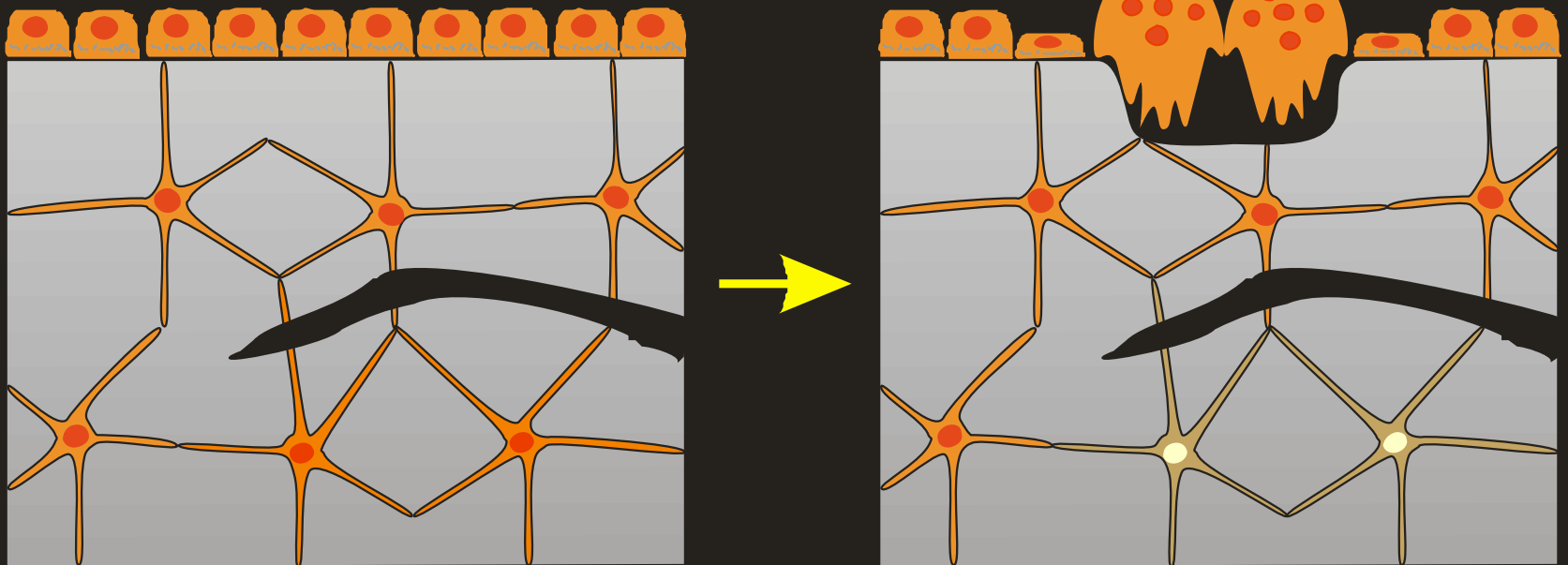


# Time & Space



[www.smallartworks.ca](http://www.smallartworks.ca)

# Initiation of bone remodelling..



# Osteocyte-mediated initiation of bone resorption

- Osteocytes prevent osteoclastogenesis – TGF $\beta$  / OPG
- Osteocytes produce RANKL / M-CSF
- The effects of osteocyte apoptosis are mediated by changes in the behaviour of bone-lining cells

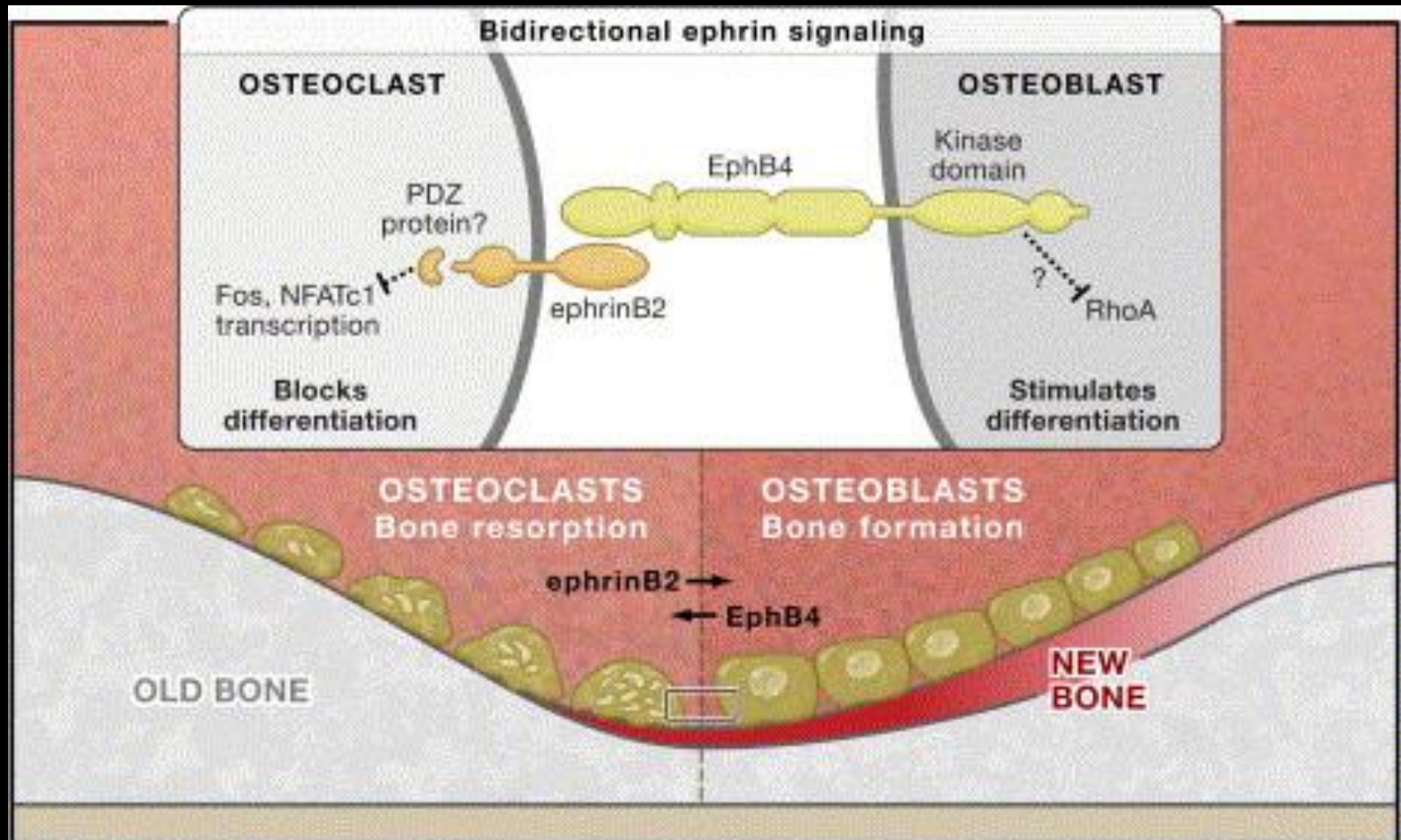
# Osteoclast recruitment

- Chemokines
  - Monocyte chemoattractant protein-1 (MCP-1, also known as CCL2)
  - stromal cell-derived factor (SDF-1, also known as CXCL12)

# Reversal / Transition

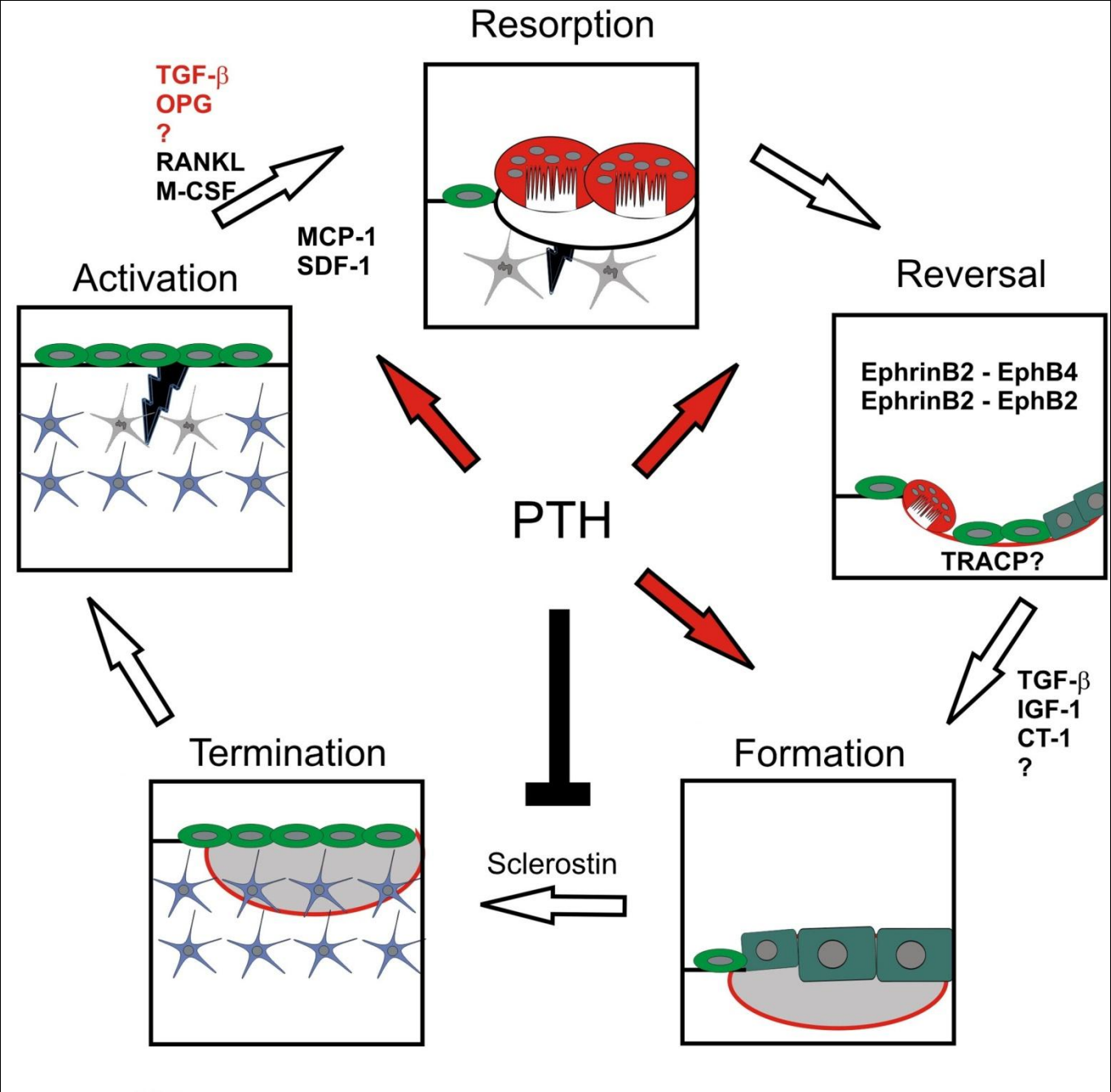
- Factors released from the bone matrix
  - IGF-1, BMP-2, TGF $\beta$ , PDGF
- Factors released by osteoclasts
  - Cardiotrophin-1 (CT-1)

# Cell:cell interactions



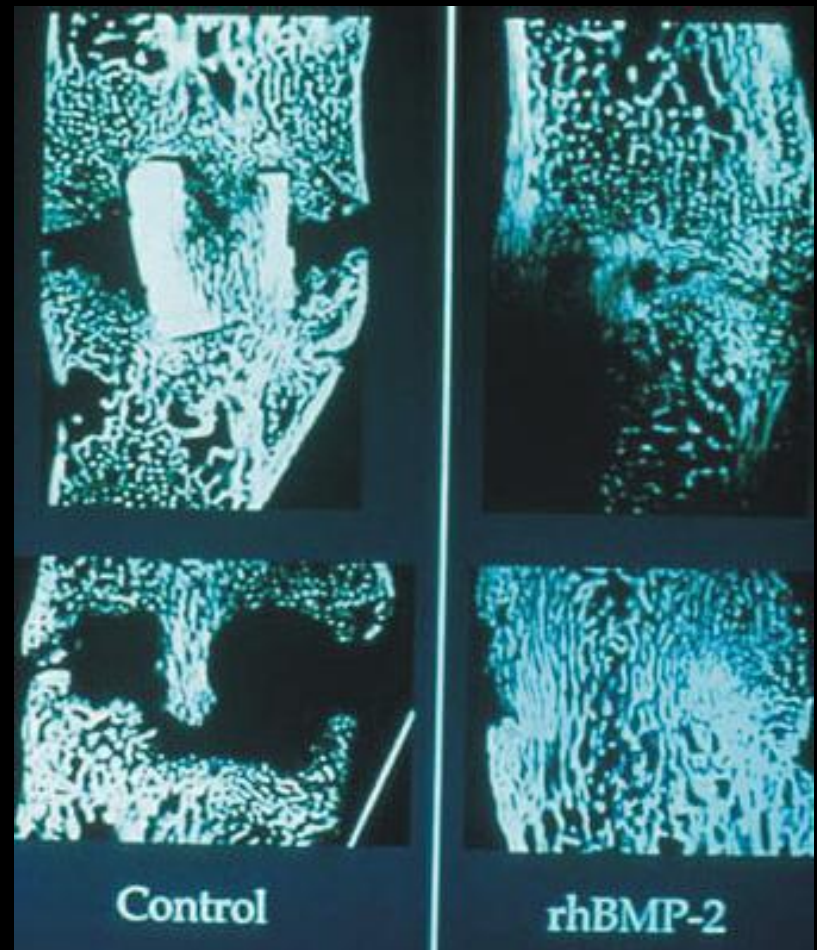
# Termination of bone formation

- Osteocytes produce Sclerostin
  - Ligand for LRP5 & therefore prevents Wnt activated bone formation
  - PTH treatment and mechanical load reduce sclerostin expression by osteocytes
  - Lack of sclerostin leads to high bone mass diseases, Van Buchem disease and sclerosteosis

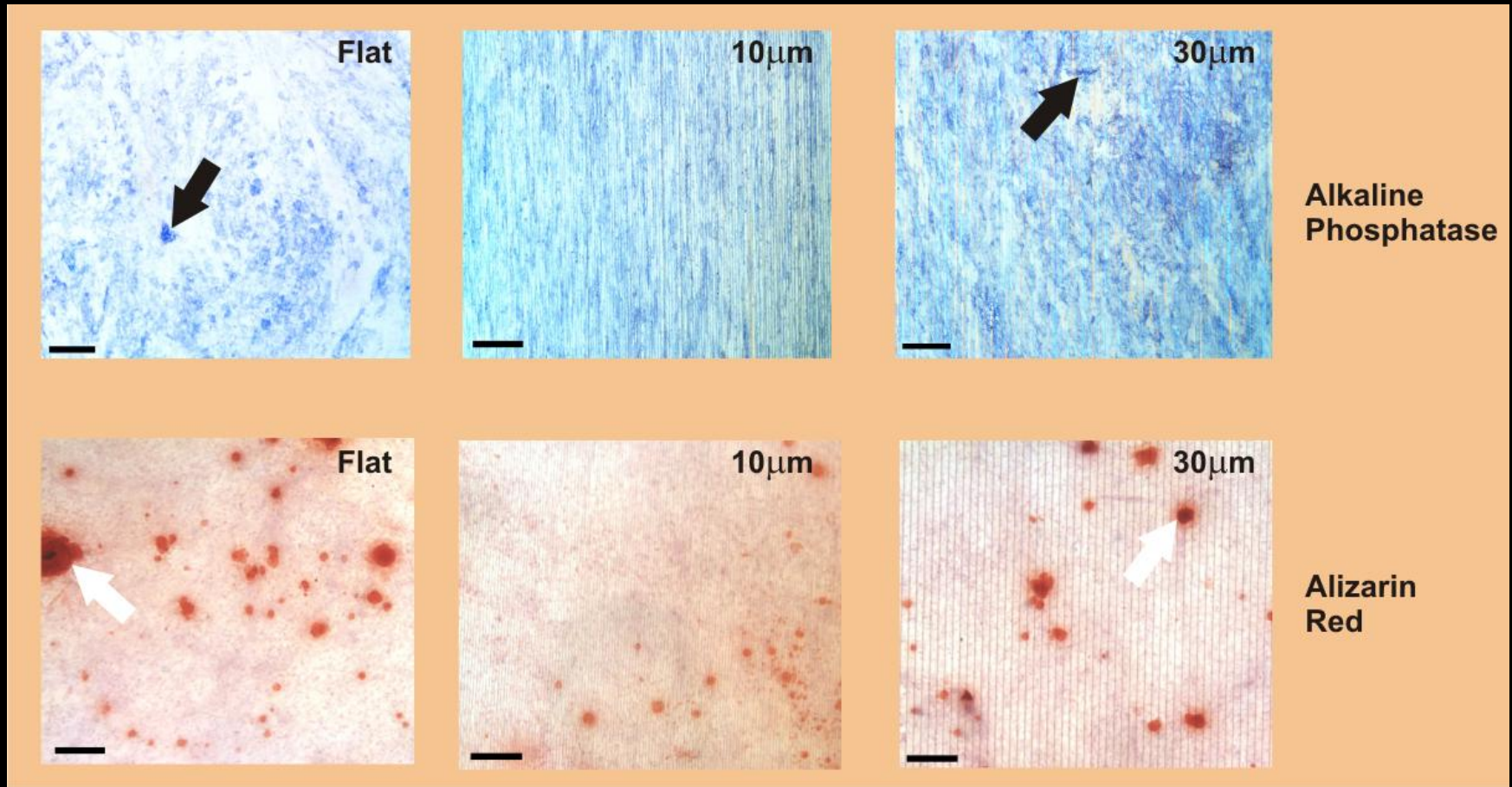


# Biology in Orthopaedics

- Biologics
- Biomaterial engineering
- Cell therapies

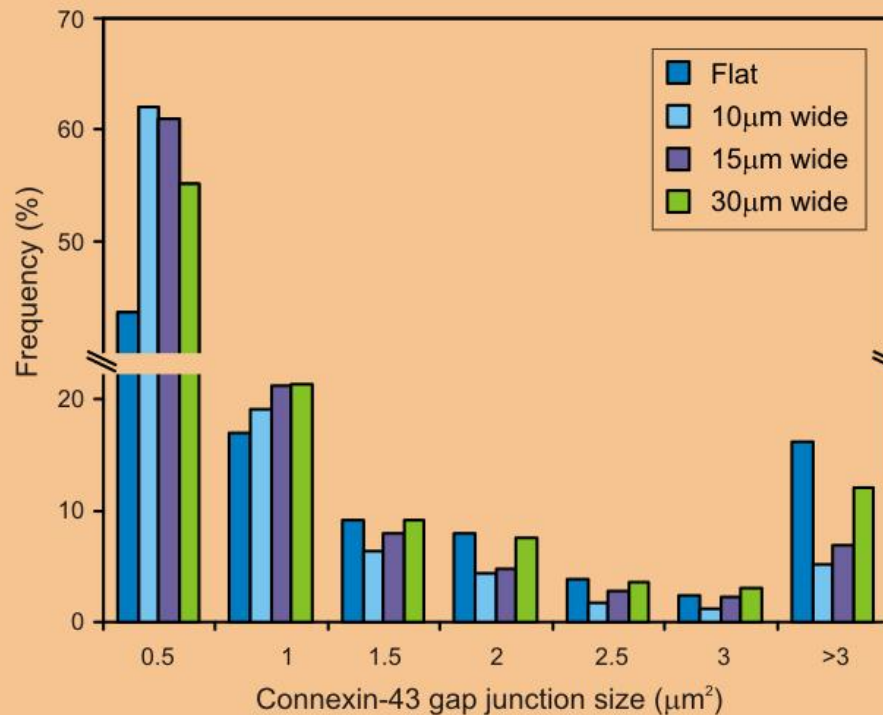
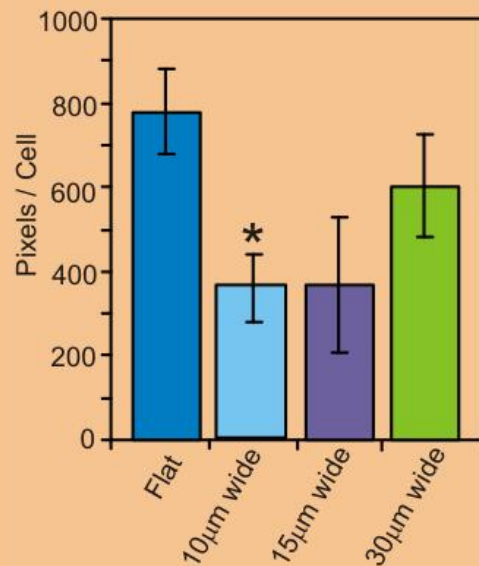
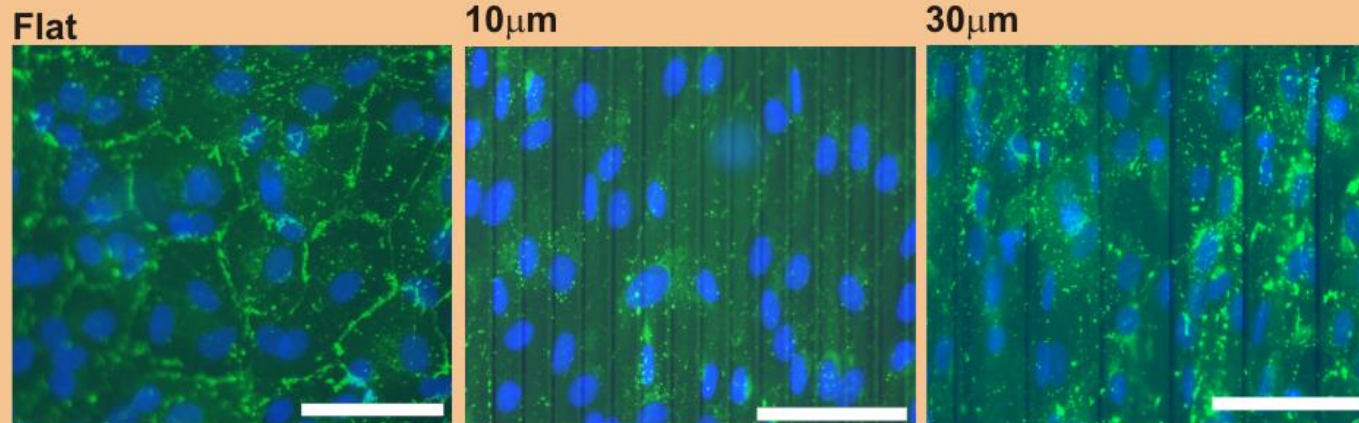


# Osteogenesis on 10-30 $\mu$ m grooved surfaces

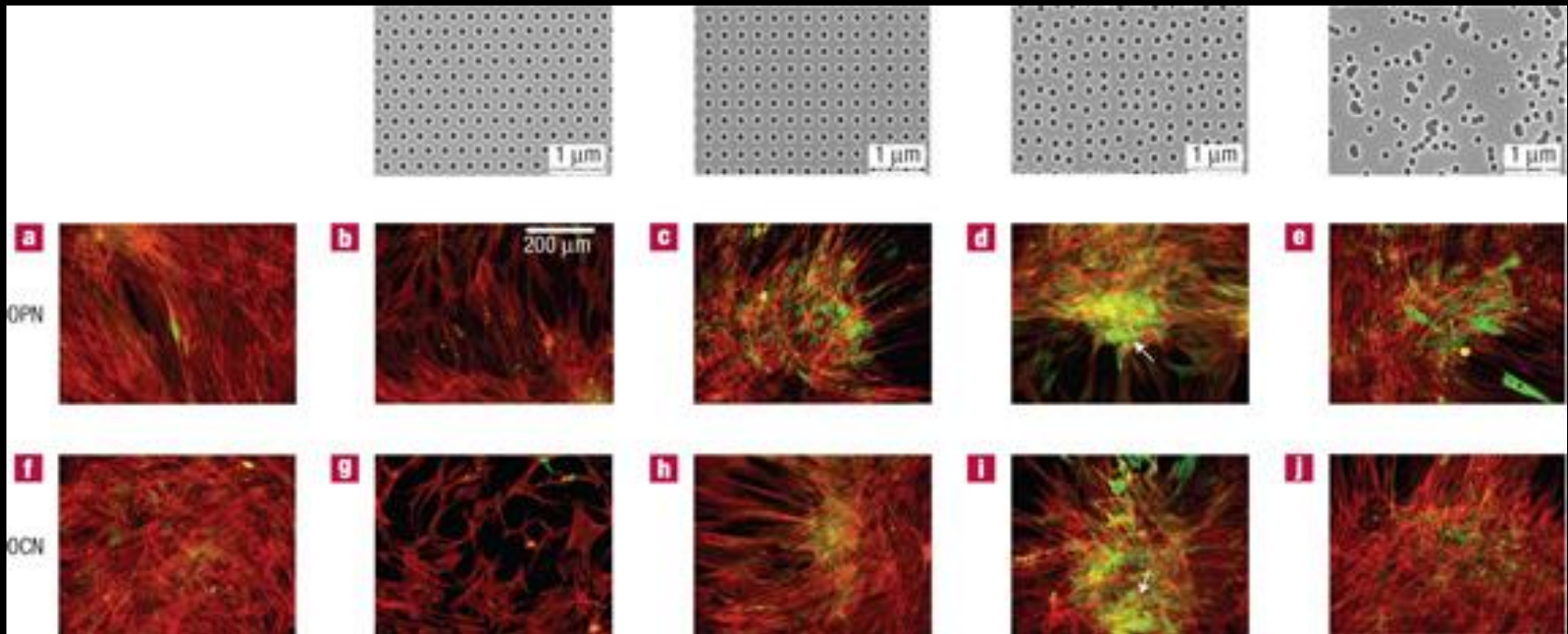


Kirmizidis G, Birch MA. Microfabricated grooved substrates influence cell-cell communication and osteoblast differentiation *in vitro*. Tissue Eng Part A. 2009 15(6):1427-36.

# Cell:cell interactions on grooved substrates

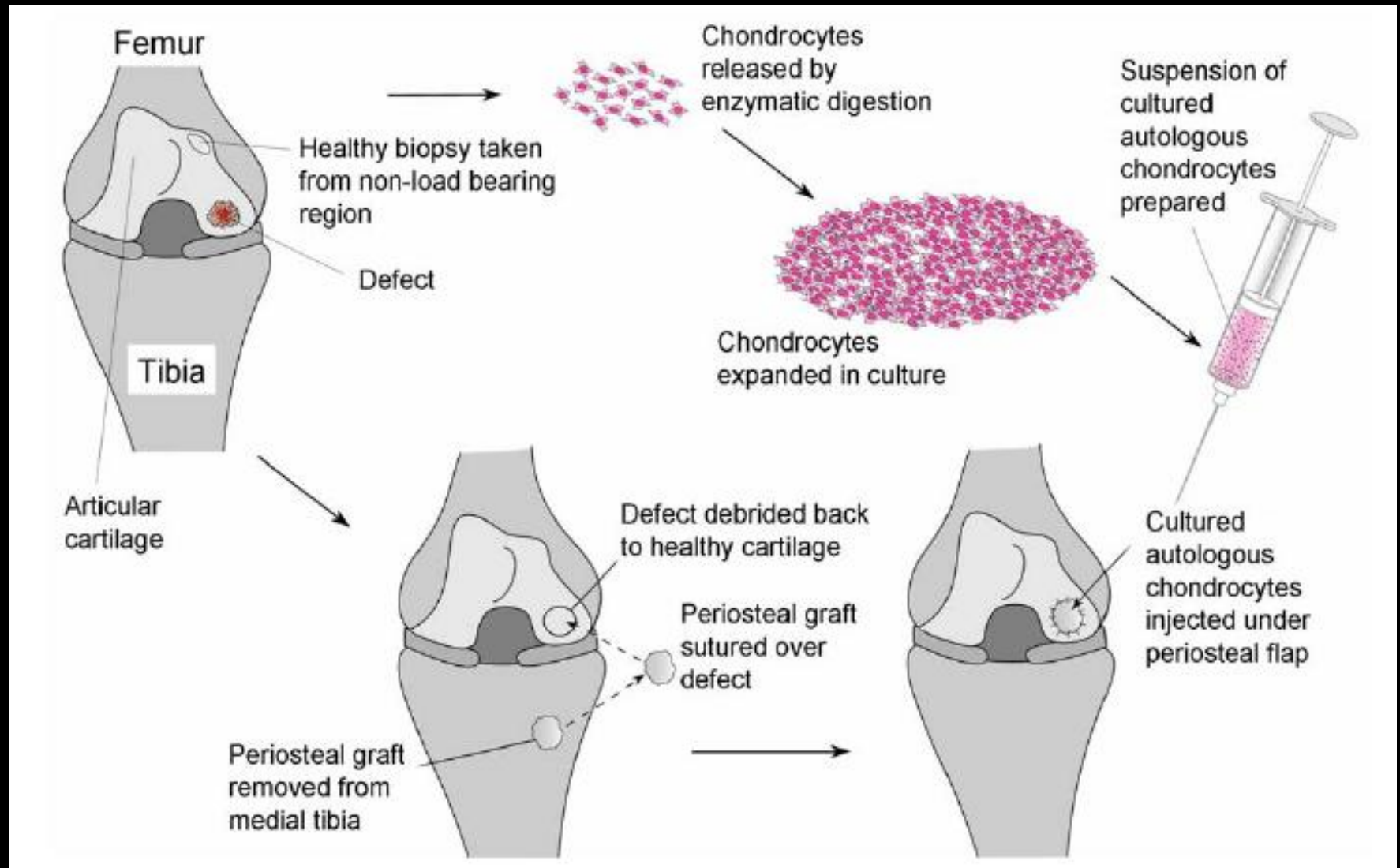


# Nanoscale disorder regulates osteoblast differentiation



Dalby M et al The control of human mesenchymal cell differentiation using nanoscale symmetry and disorder. *Nature Materials* 2007 6(12):997-1003.

# Autologous Chondrocyte Implantation



# Summary

- Bone is adapted to fulfill mechanical needs
- Coordinated activities of several cell types are required for the formation and maintenance of bone
- Bone cell activity is regulated by systemic hormones, cytokines / growth factors, and mechanical load
- Pro-inflammatory mediators tend to ↑ bone resorption
- Wnt signalling ↑ commitment of MSC → Osteoblasts
- BMPs drive Osteoblast differentiation
- Differentiation exhibits fine temporal control

Any questions?