Biology of Bone

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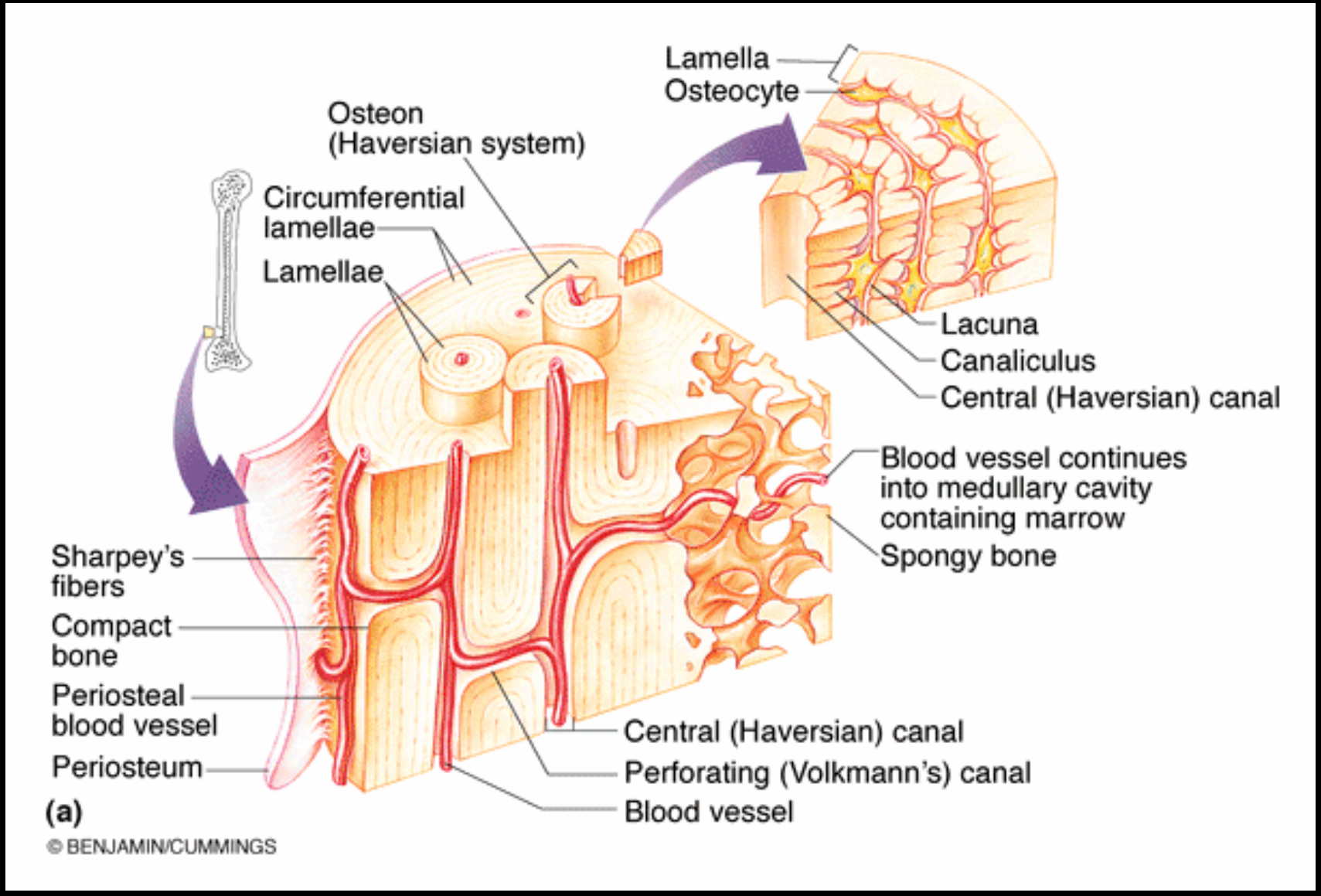
- Background
- Modelling / Remodelling of Bone
- Controlling bone cell activity
- The role of mechanical load
- Coordinating bone cell activity
- Biology in Orthopaedics


Lamella
Osteocyte
Osteon
(Haversian system)
Circumferential
lamellae
Lamellae
Lacuna
Canaliculus
Central (Haversian) canal
Blood vessel continues
into medullary cavity
containing marrow
Spongy bone
Sharpey's
fibers
Compact
bone
Periosteal
blood vessel
Periosteum
Central (Haversian) canal
Perforating (Volkmann's) canal
Blood vessel
(a)
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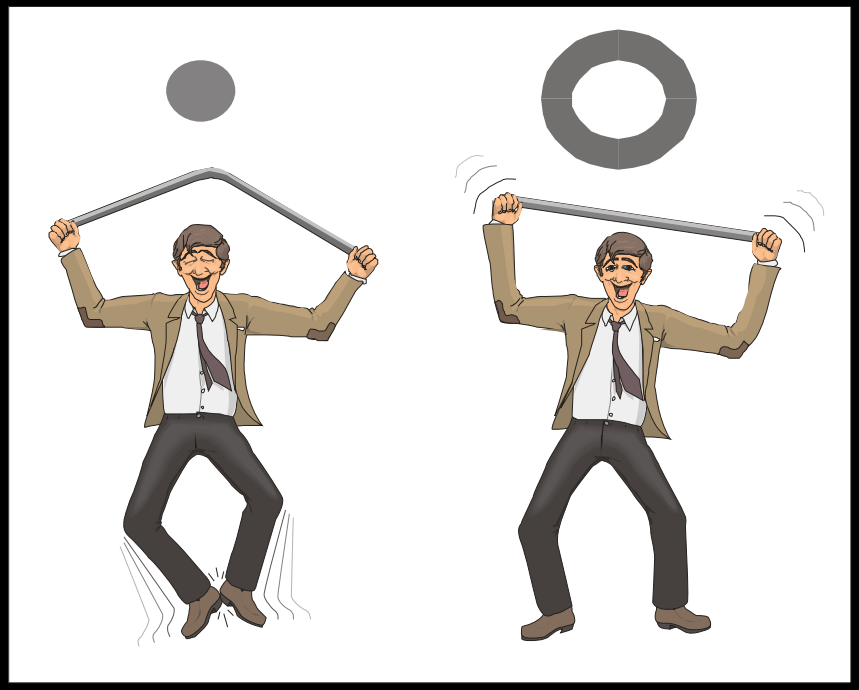
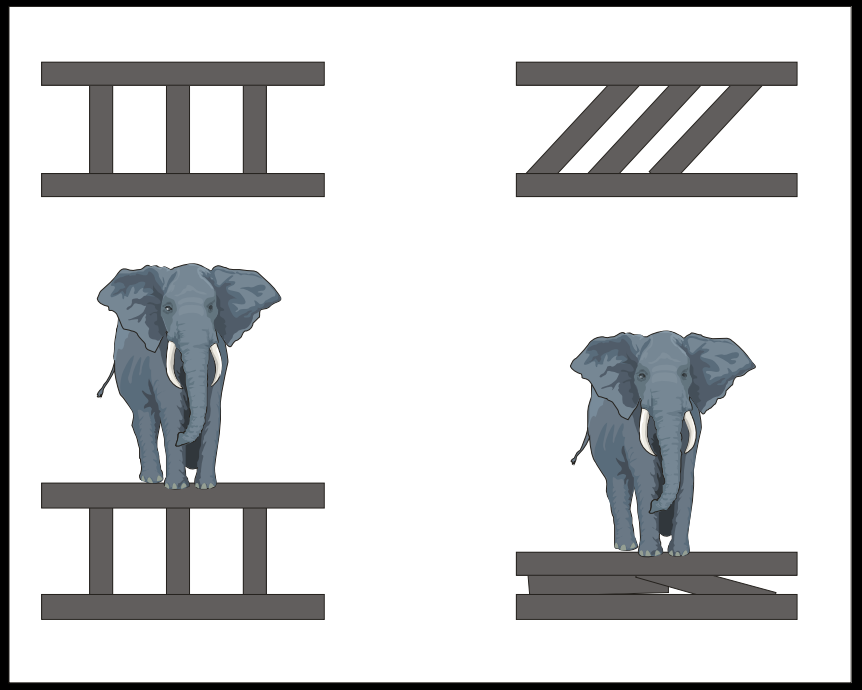
To meet mechanical need



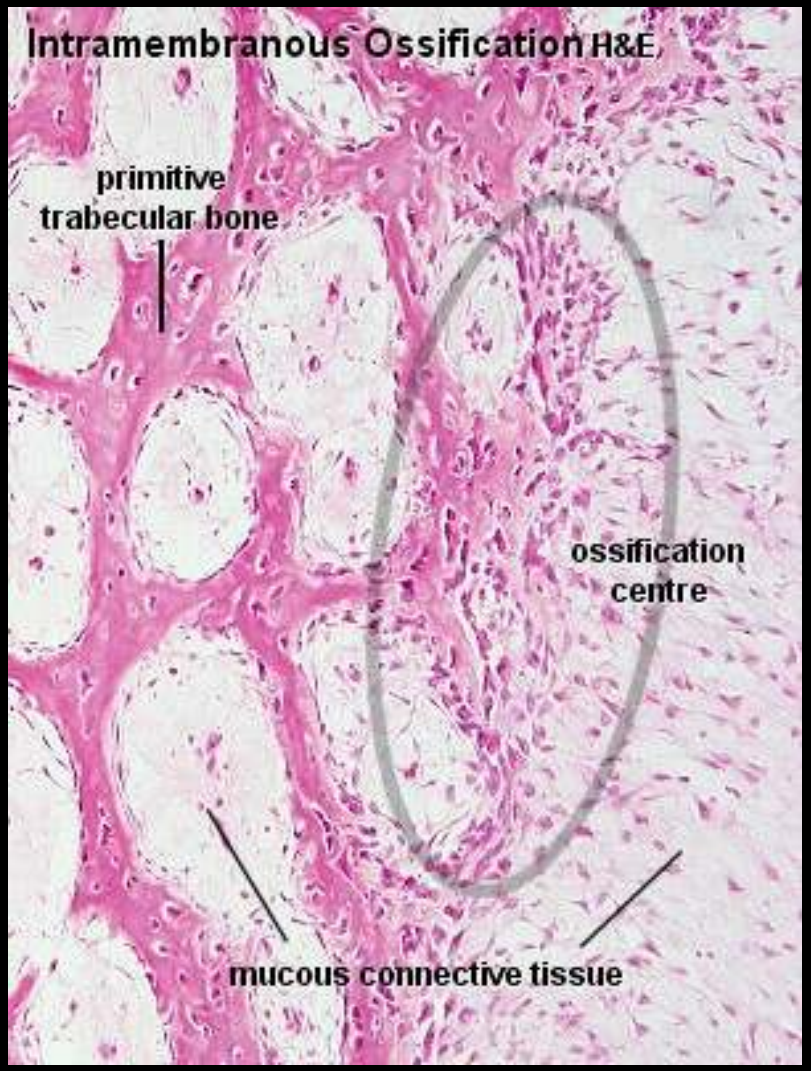


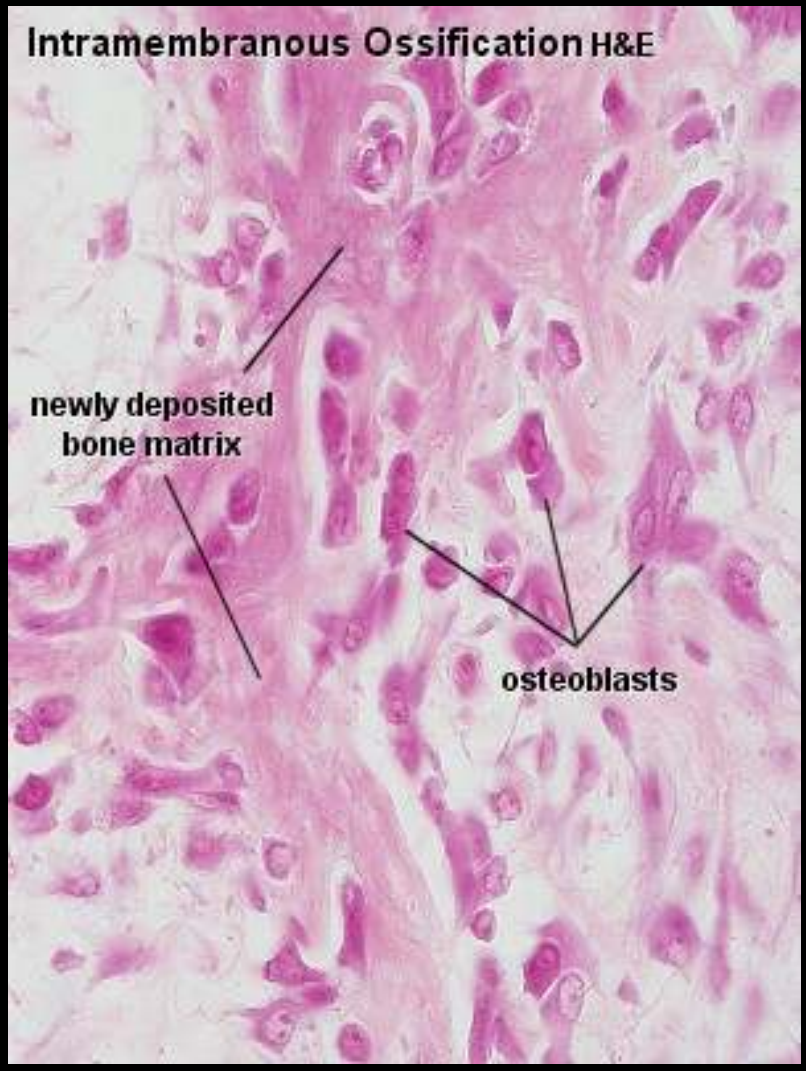
Bone formation

- 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

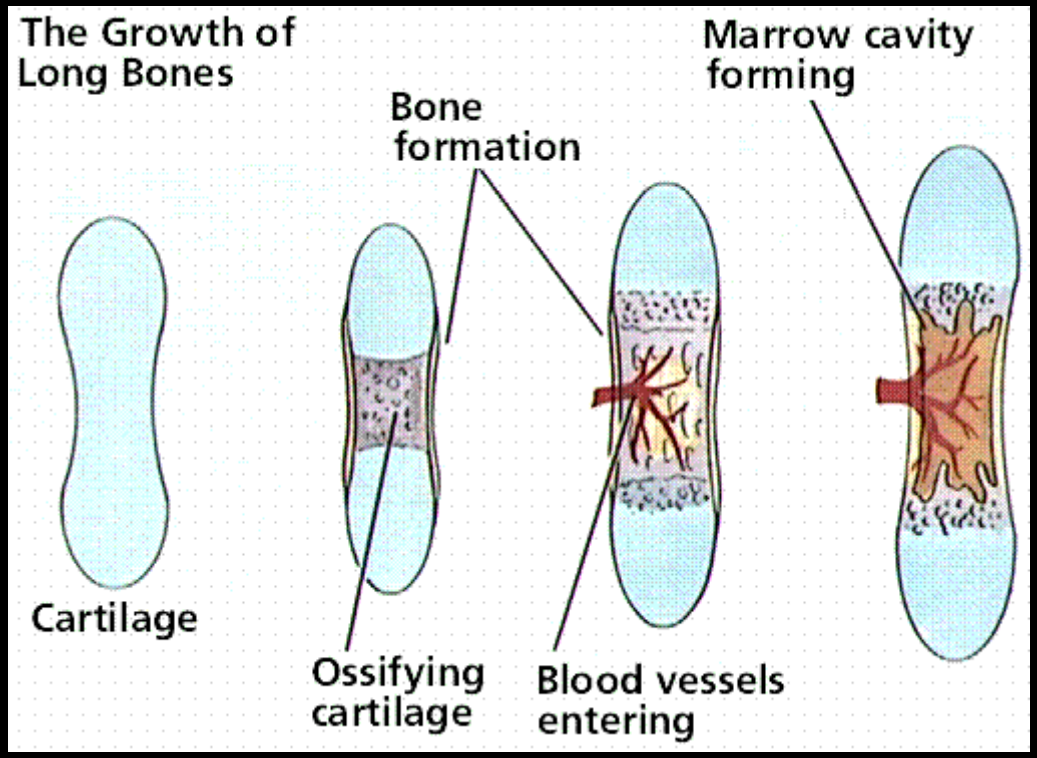
- Bone formation of the surface skull bones and clavicles
- Osteoblasts cluster around the centre of ossification
  - Here, osteoblasts secrete a collagenous matrix to form a framework for mineralization
  - The collagenous matrix is then calcified by the deposition of hydroxyapatite
  - The osteoblasts 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

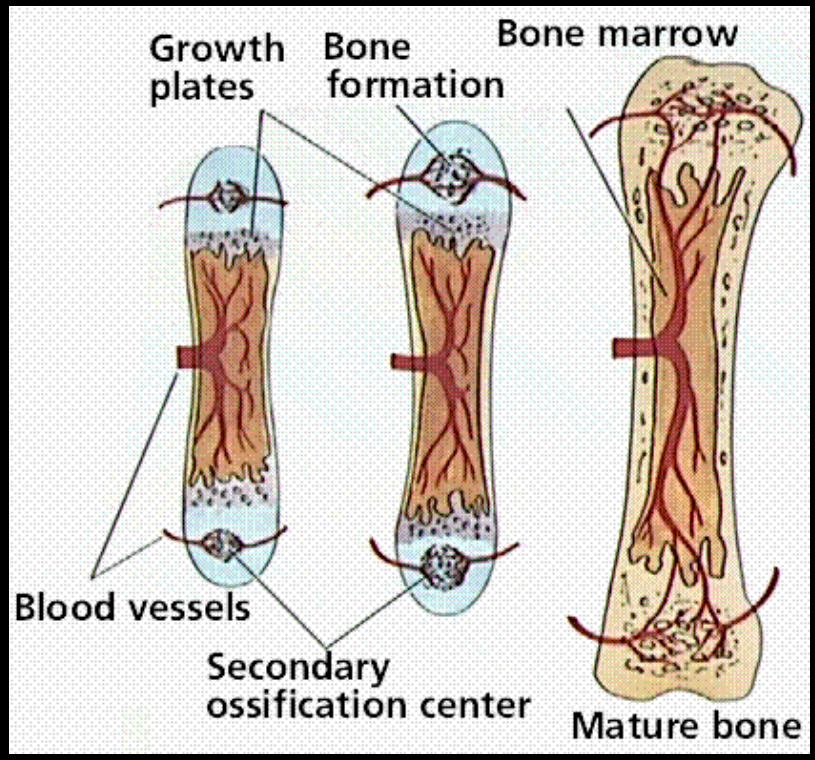

Intramembranous Ossification H&E
primitive
trabecular bone
ossification
centre
mucous connective tissue

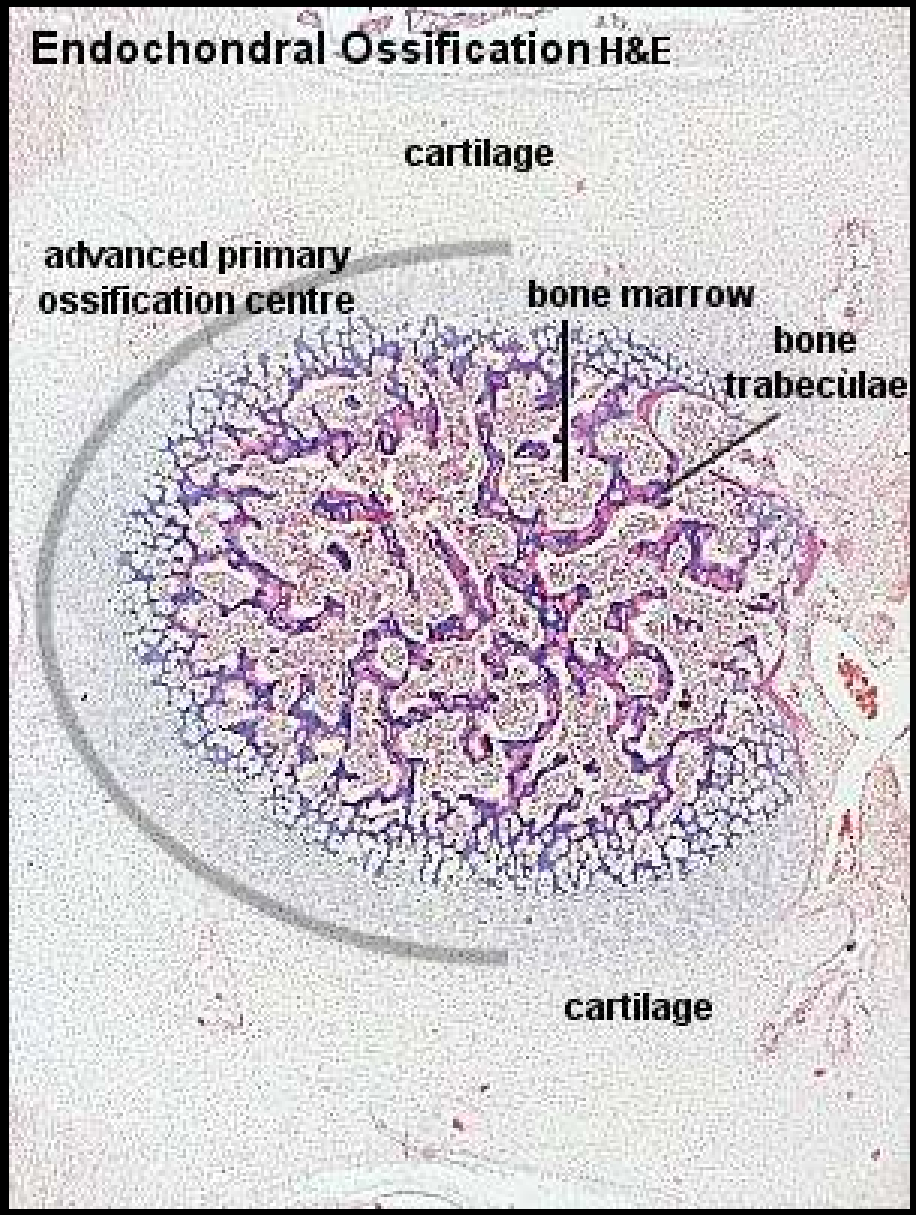

Intramembranous Ossification H&E
newly deposited
bone matrix
osteoblasts

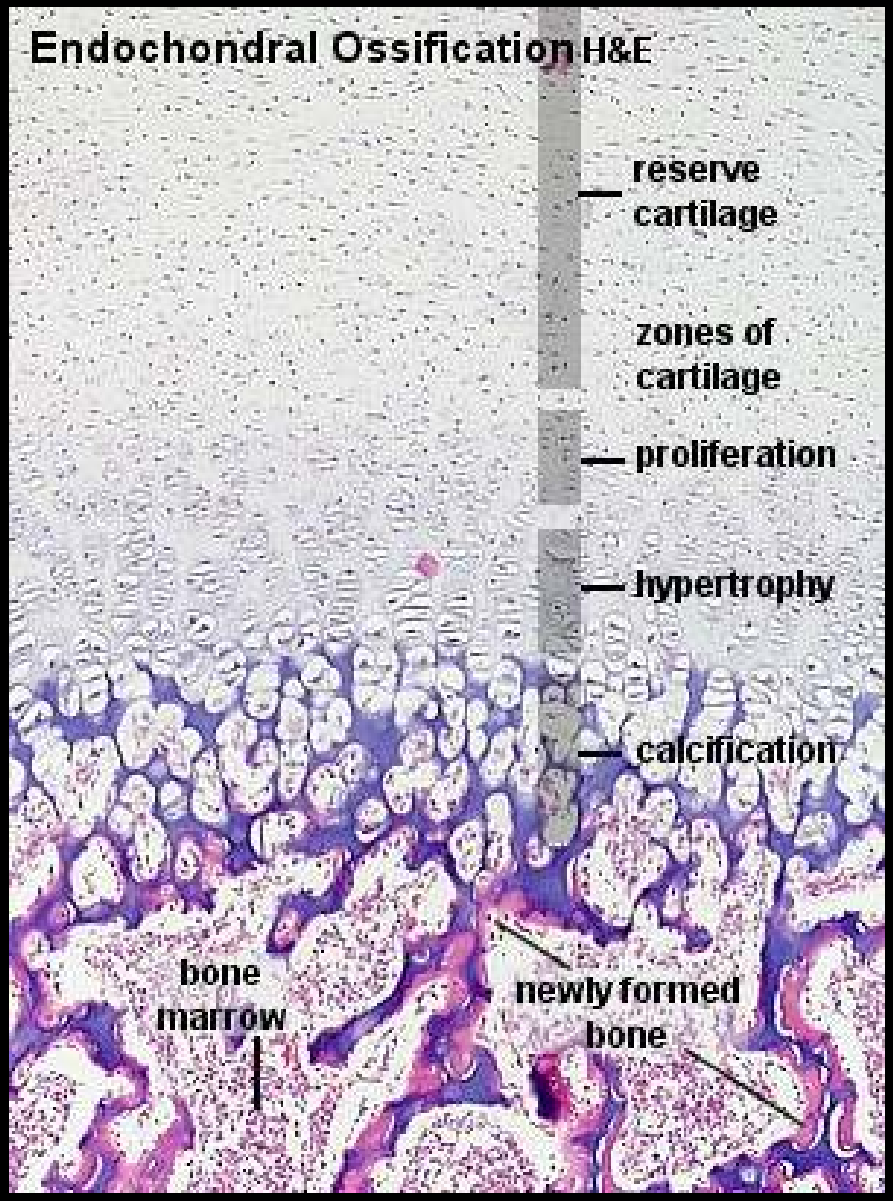
Endochondral Ossification

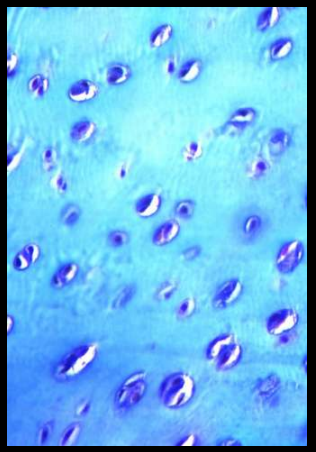
- Replacement of cartilage with bone
- Primary ossification process for most bones of the body
  - Best exemplified in *long bones*
- During embryonic development, a cartilage model, or ***perichondrium***, is laid down
  - Compact bone then forms around this area and is called the ***periosteum***
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

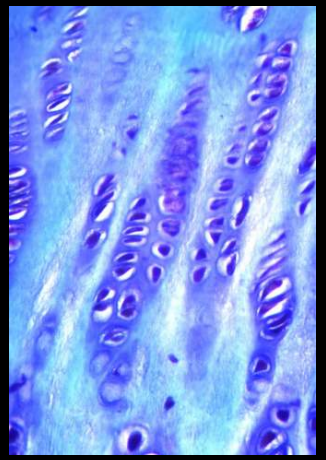

The Growth of Long Bones
Bone formation
Marrow cavity forming
Cartilage
Ossifying cartilage
Blood vessels entering

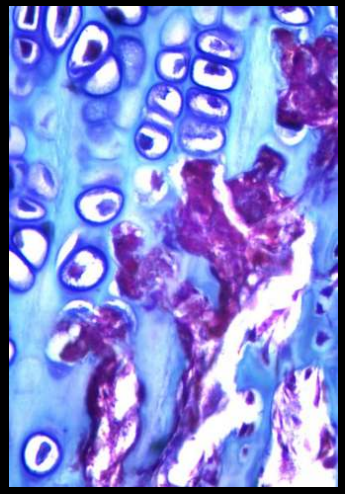

Growth plates
Bone formation
Bone marrow
Blood vessels
Secondary ossification center
Mature bone


Endochondral Ossification H&E
cartilage
advanced primary
ossification centre
bone marrow
bone
trabeculae
cartilage

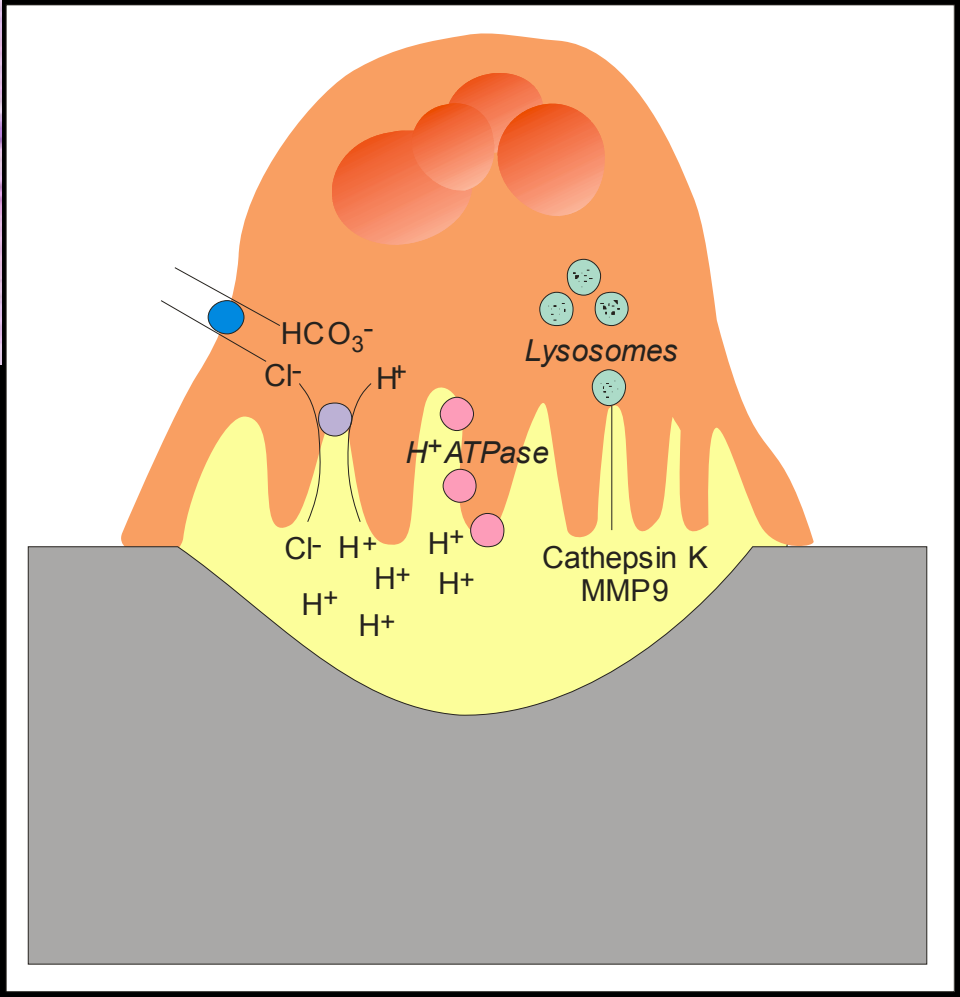

Endochondral Ossification H&E
reserve cartilage
zones of cartilage
proliferation
hypertrophy
calcification
bone marrow
newly formed bone

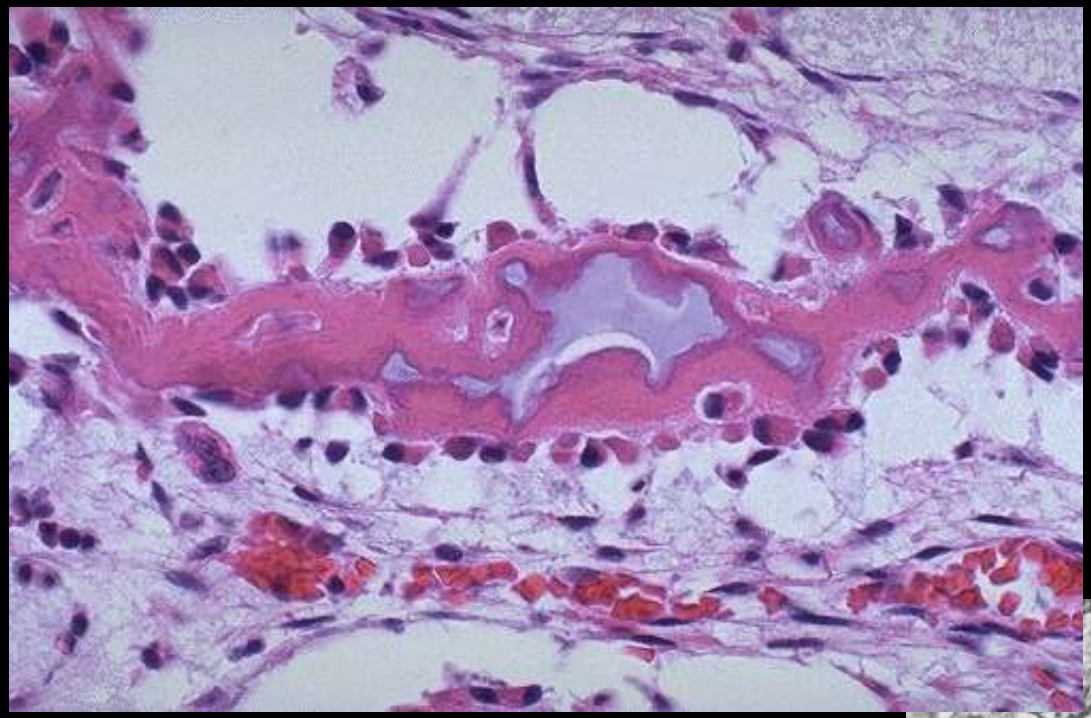


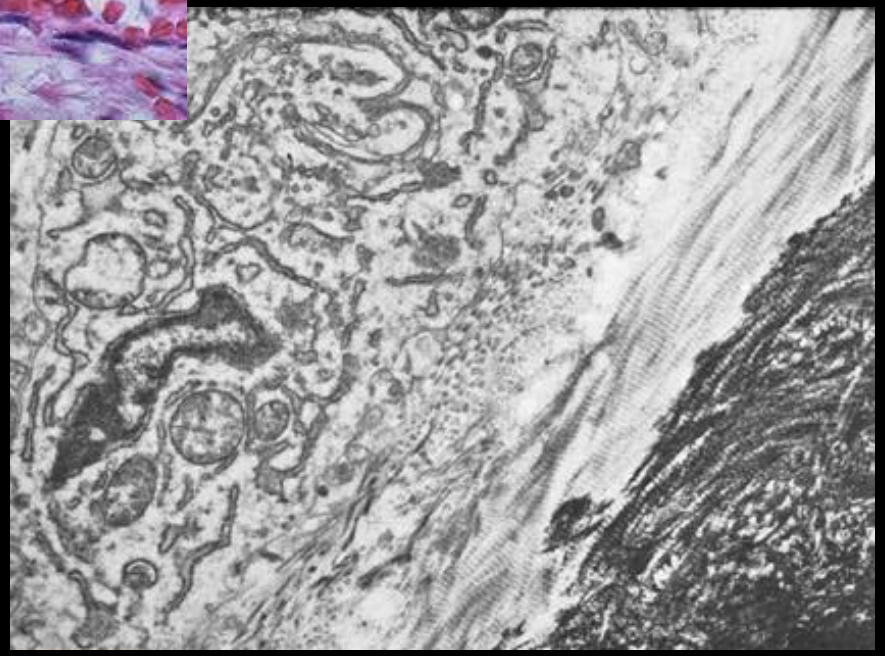


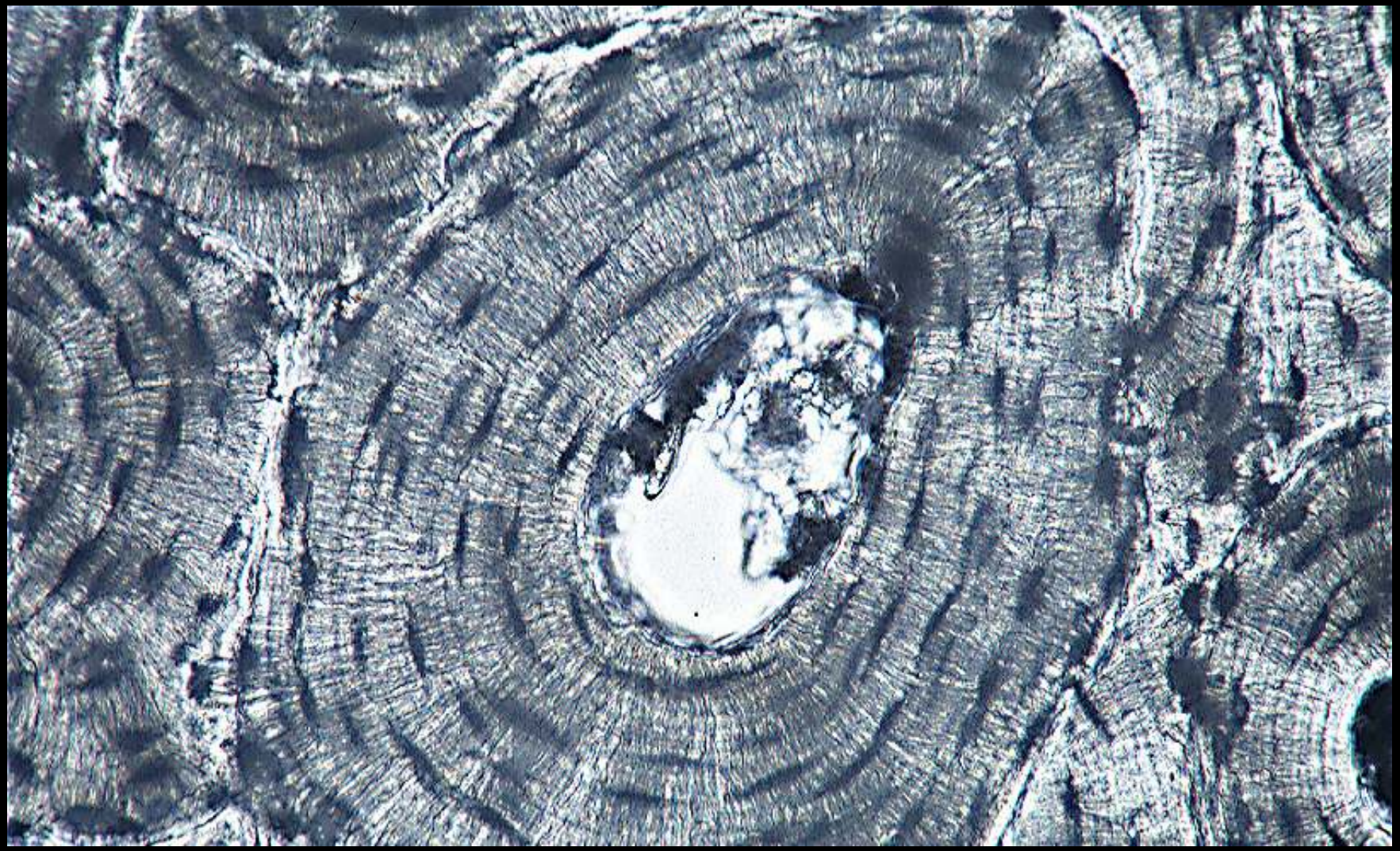



resorption
bay
osteoclast
bone

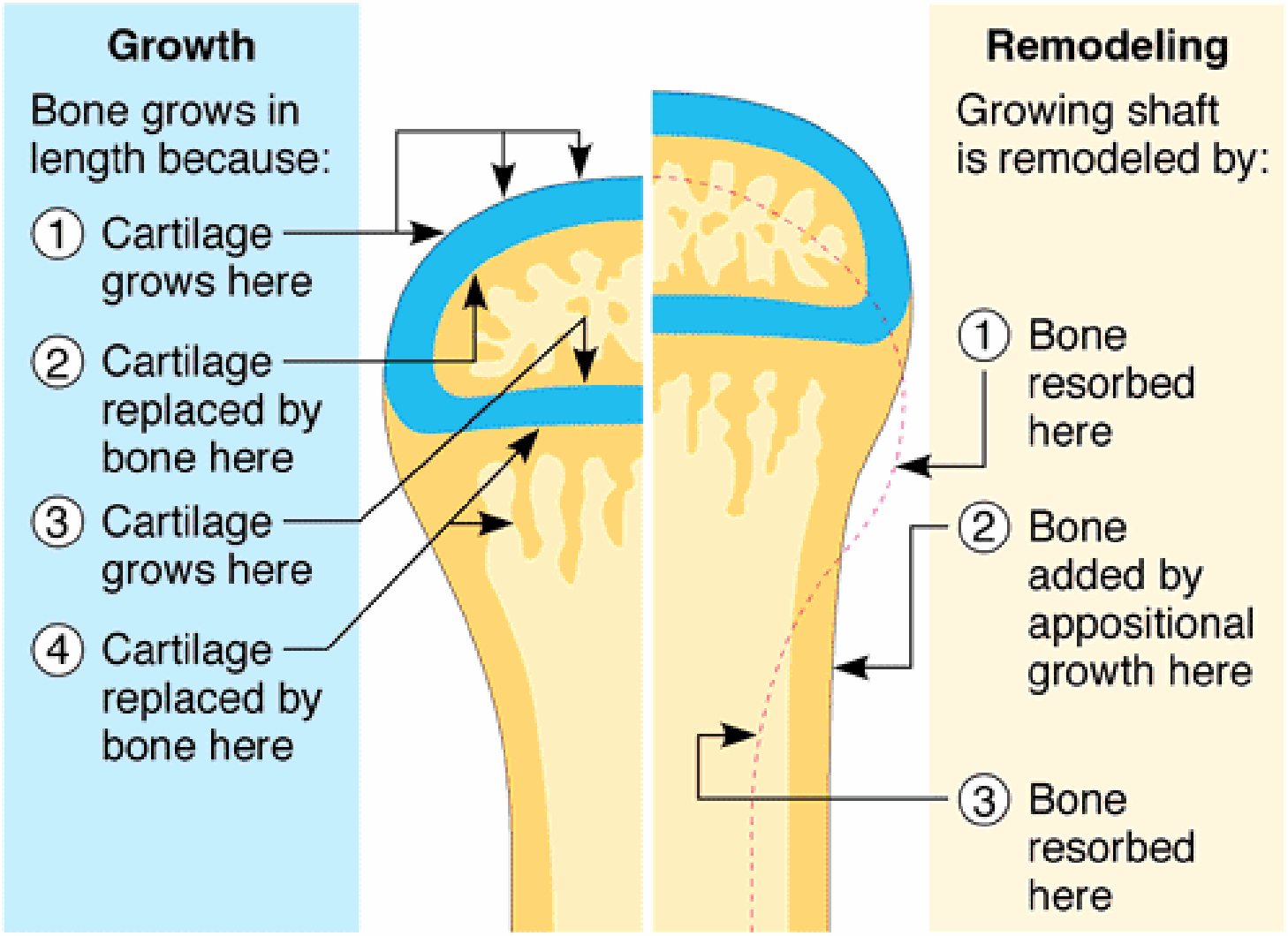

HCO_3^-
Cl^-
H^+
Lysosomes
H^+ ATPase
Cl^-
H^+
H^+
H^+
H^+
H^+
H^+
Cathepsin K
MMP9

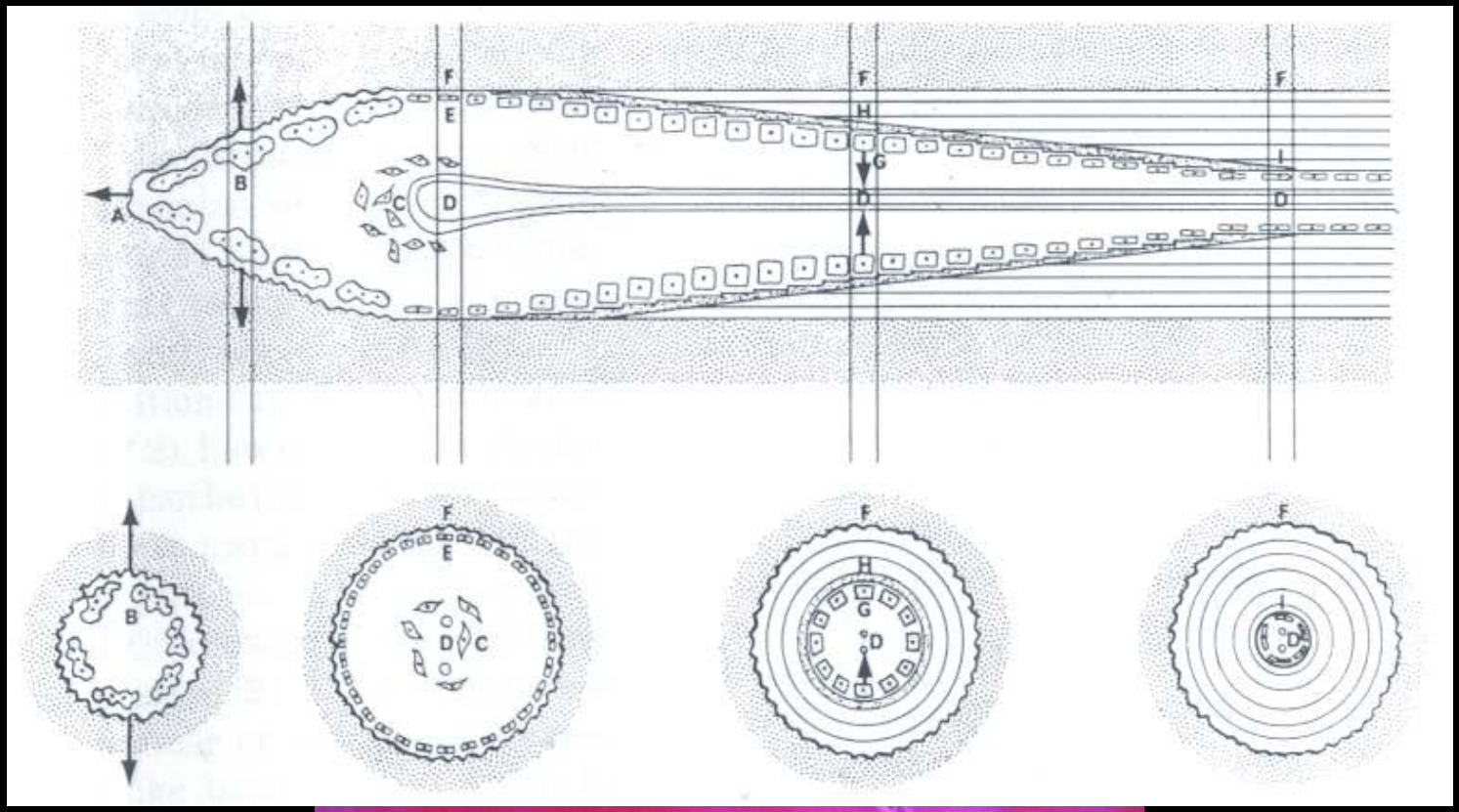


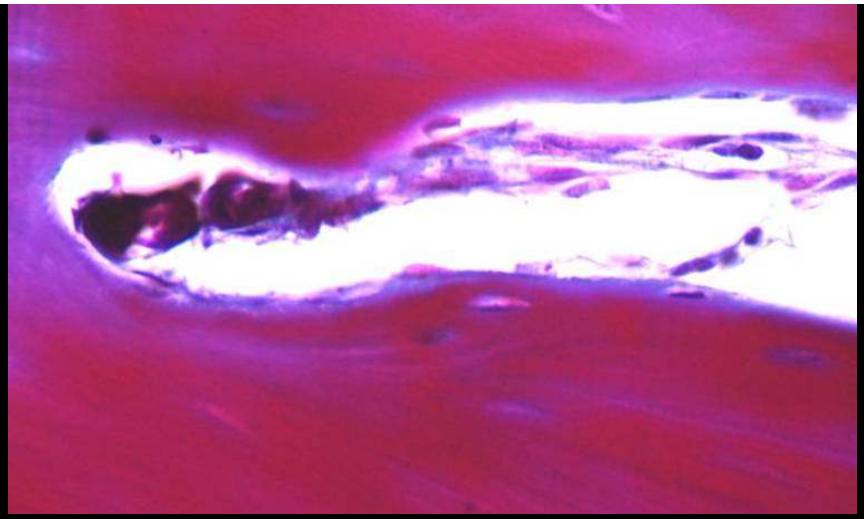


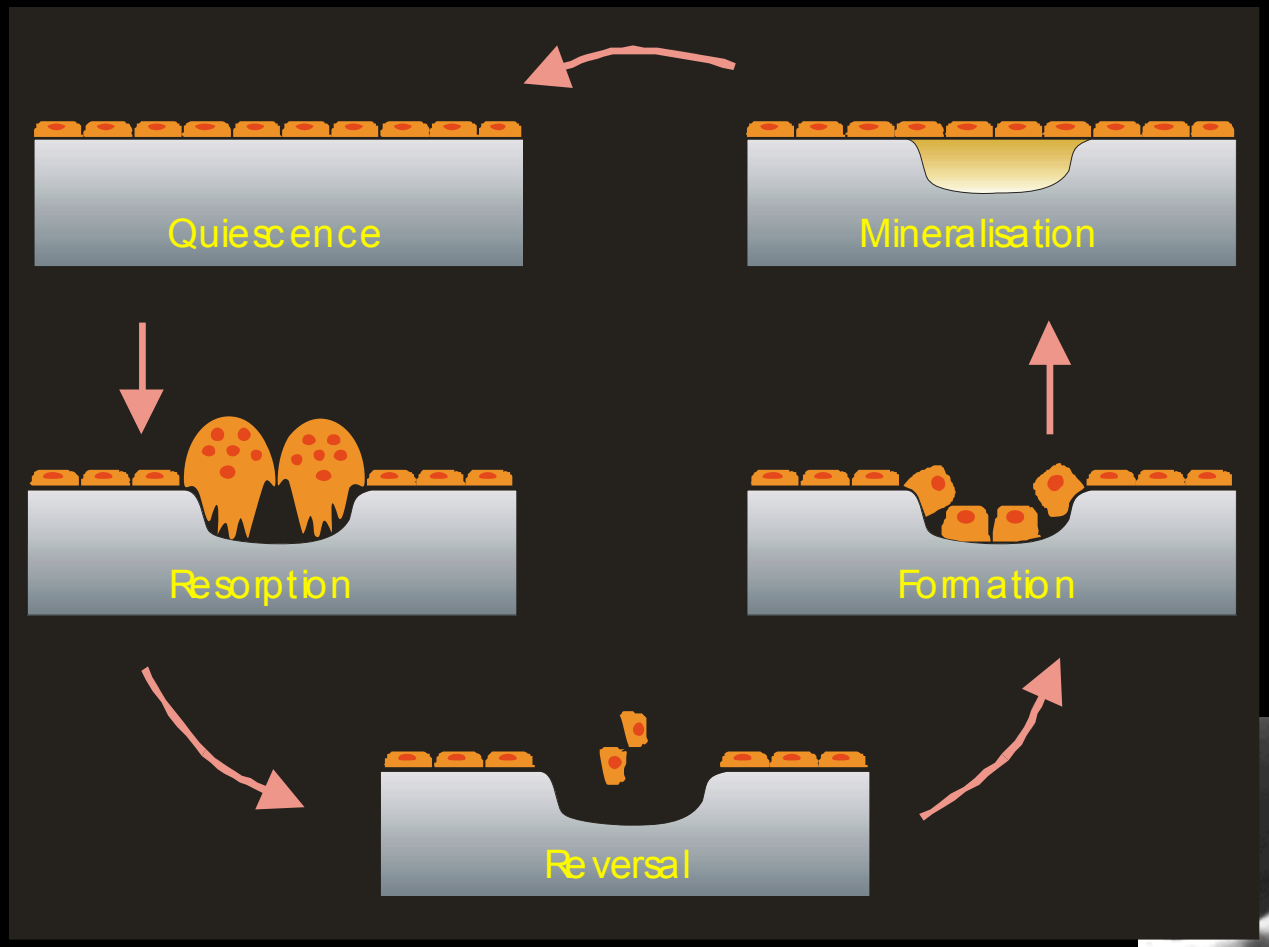


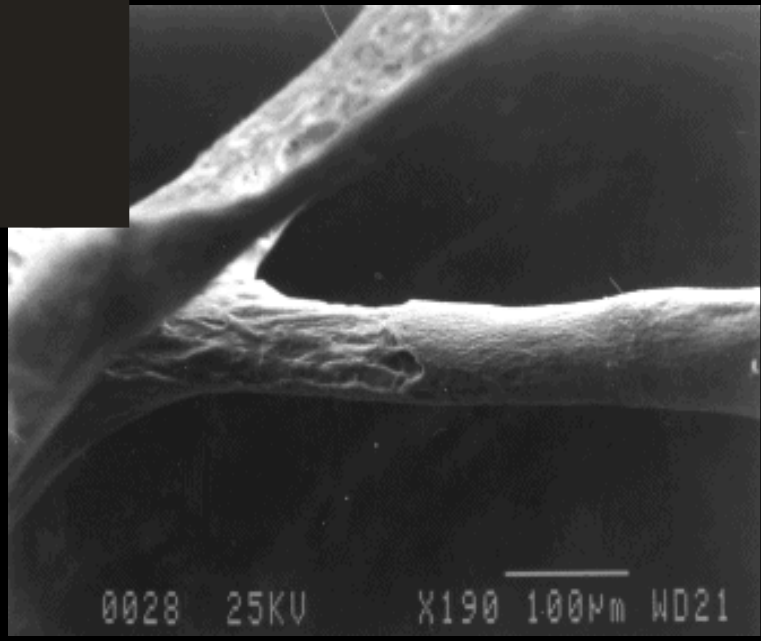
Modelling / Remodelling of Bone




A
B
C
D
E
F
G
H
I



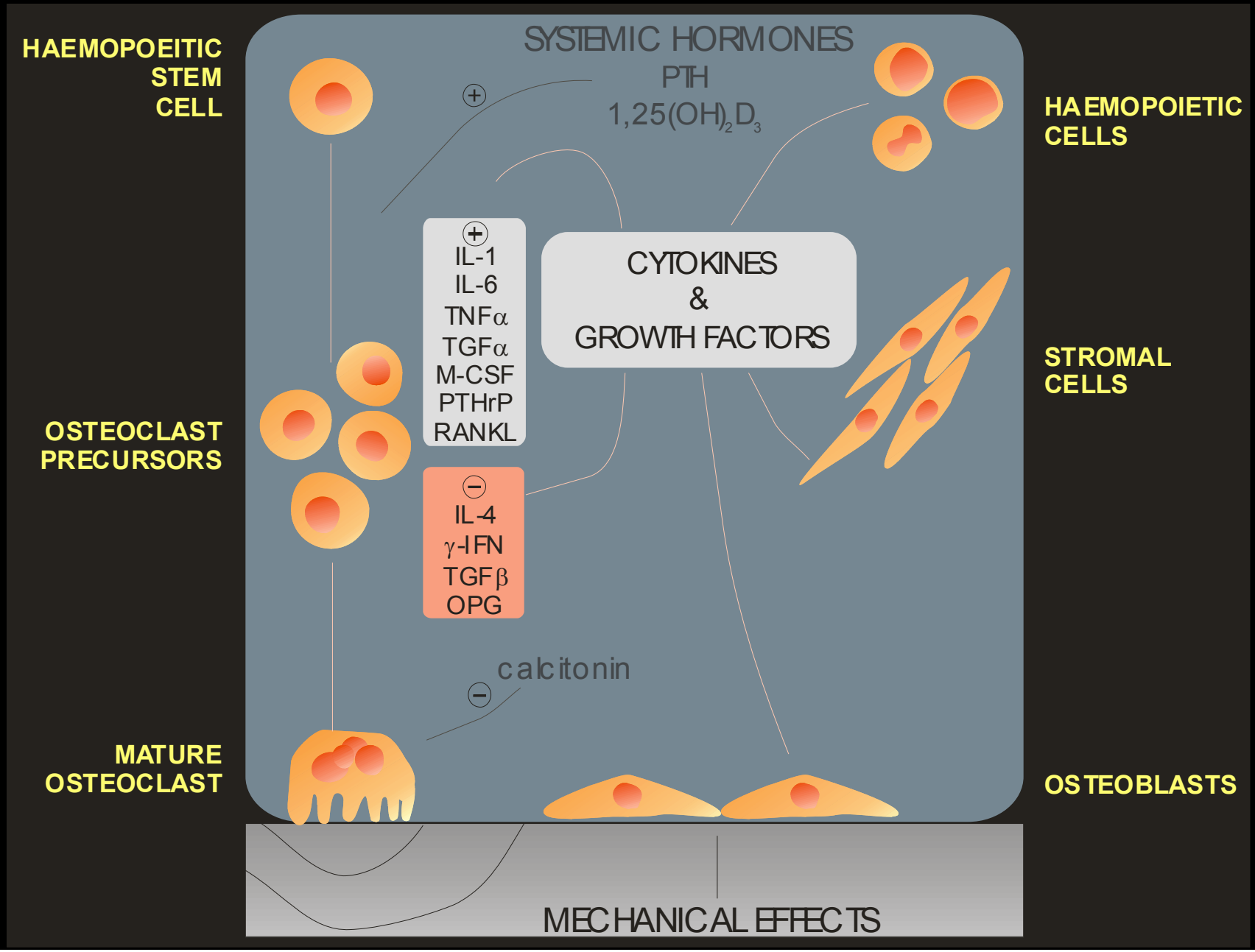

Quiescence
Mineralisation
Resorption
Formation
Reversal


0028 25KV X190 100µm WD21

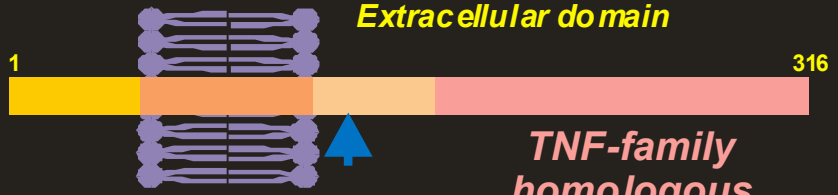
Controlling bone cell activity

- Osteoclast

Osteoclastogenesis

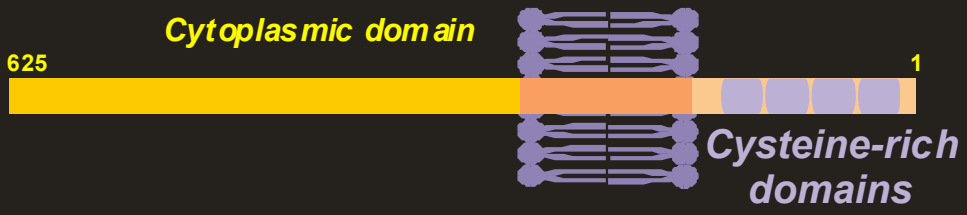


RANK / RANKL & OPG

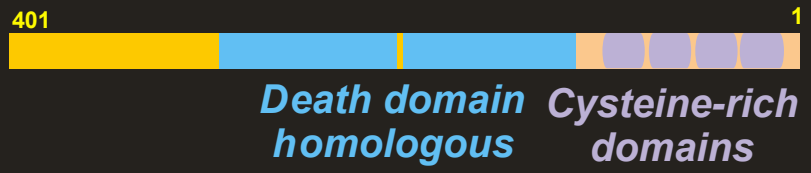


Receptor activator of NF-κB ligand (RANKL)

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



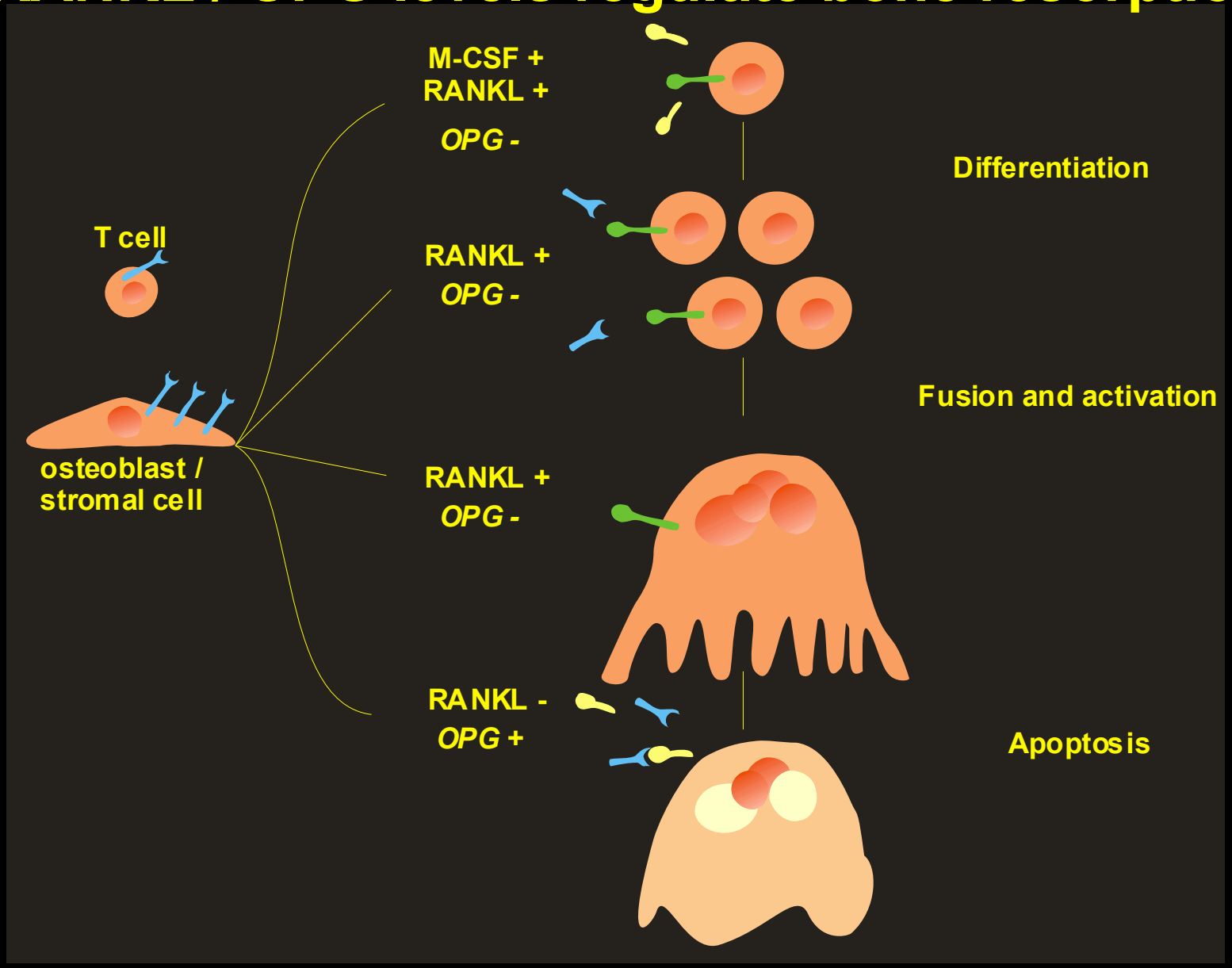
Receptor activator of NF-κB (RANK)



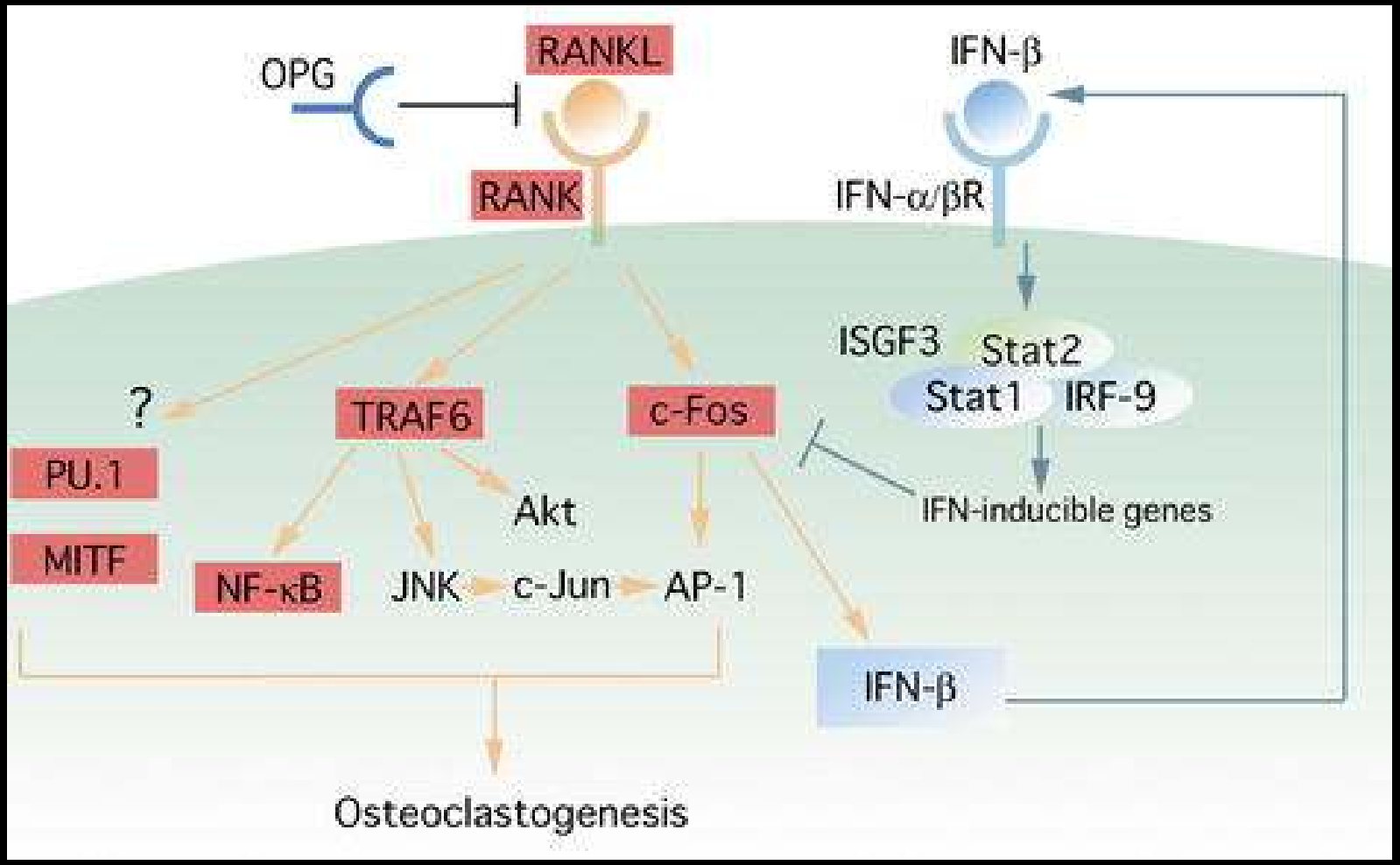
Osteoprotegerin (OPG)

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

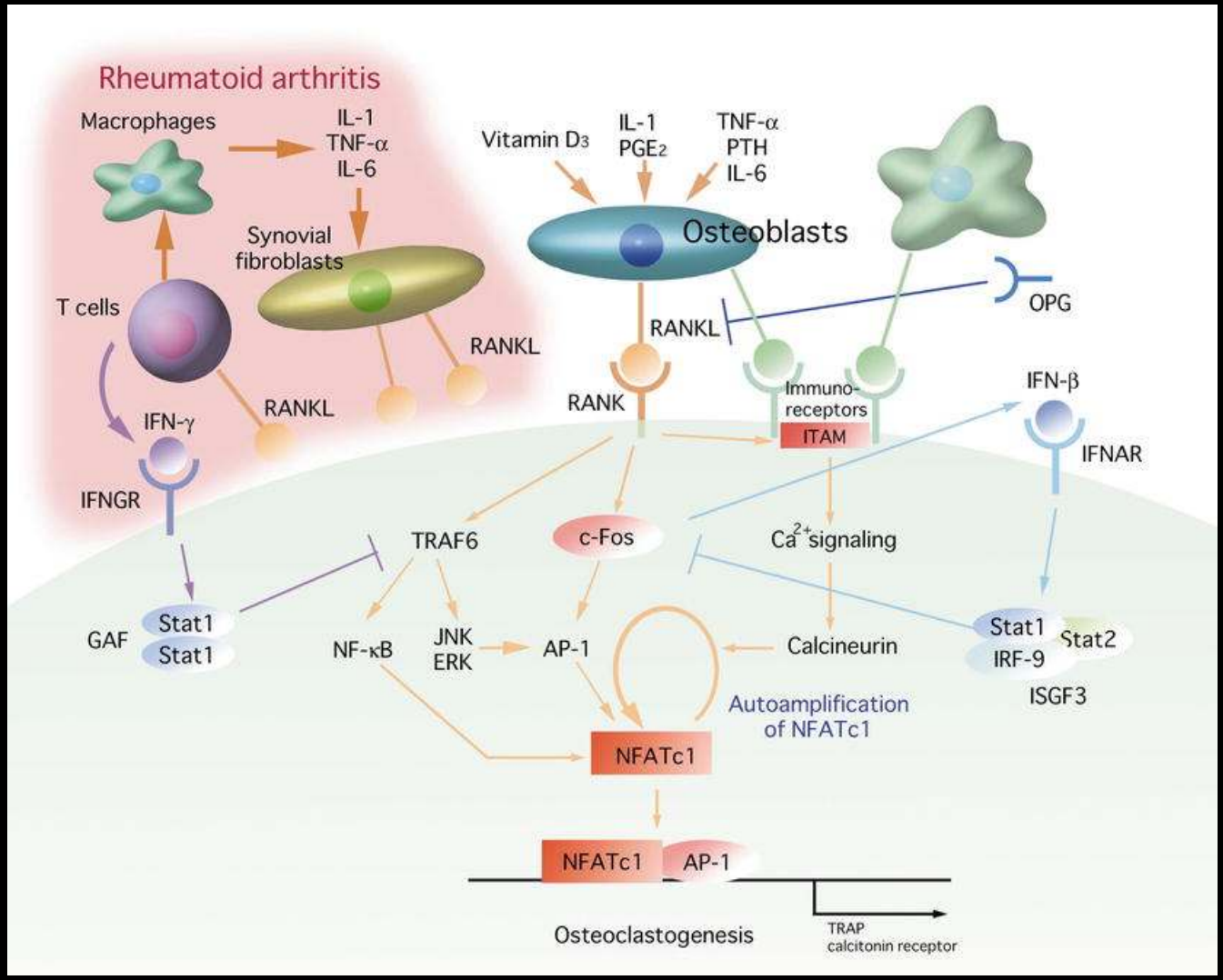
RANKL / OPG levels regulate bone resorption



RANKL intracellular signalling cascades

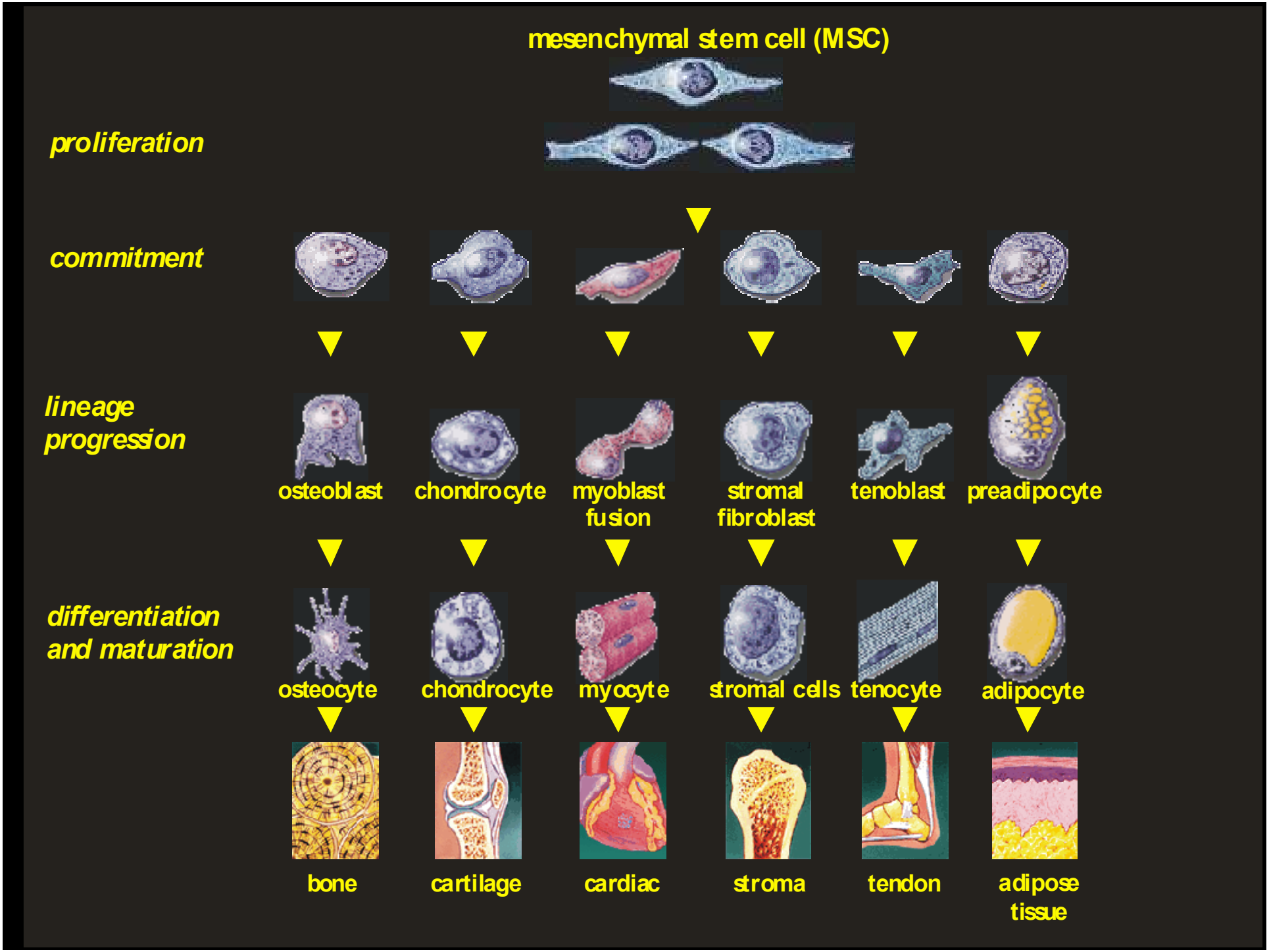


Crosstalk between immune and skeletal cells

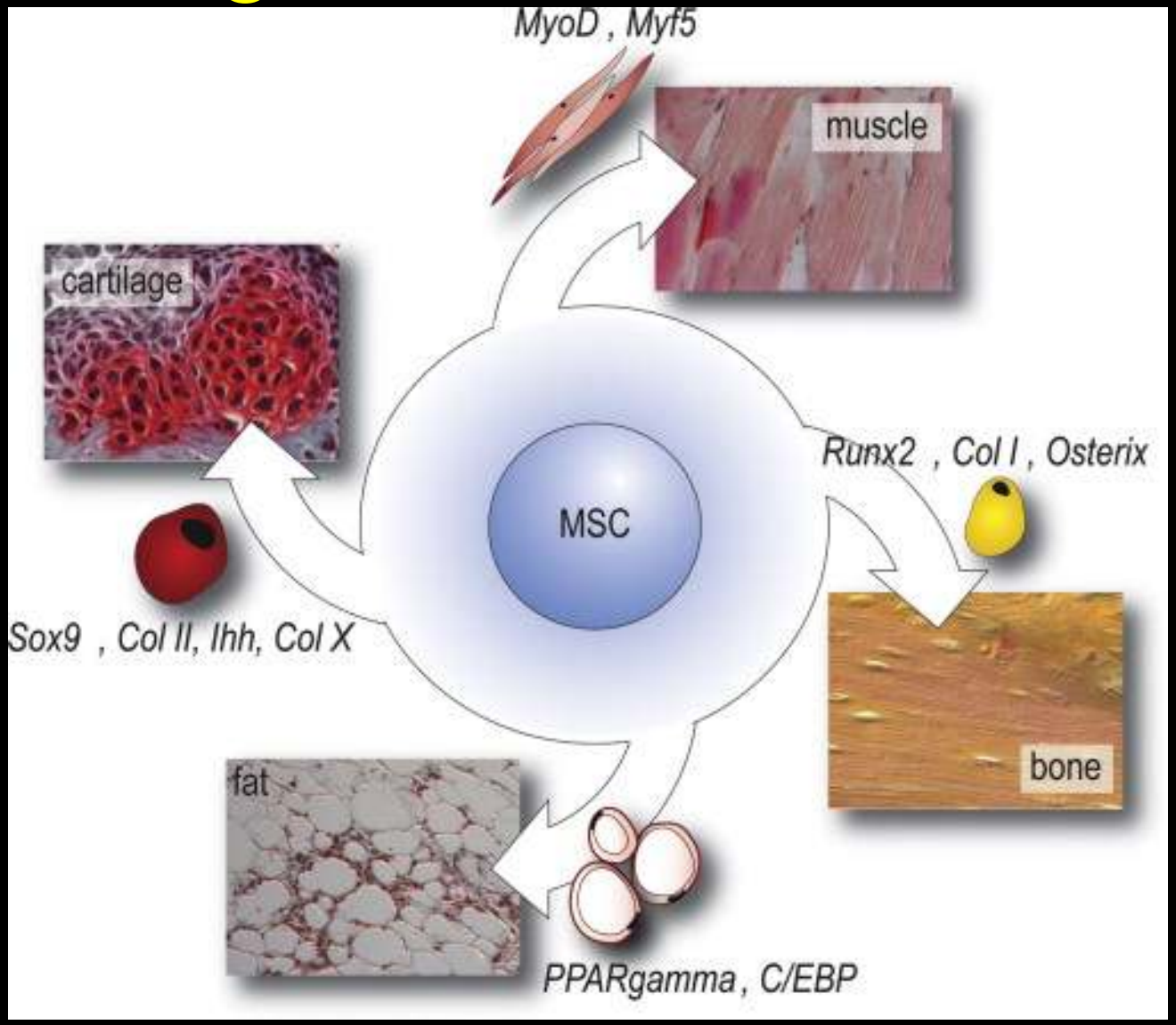


Controlling bone cell activity

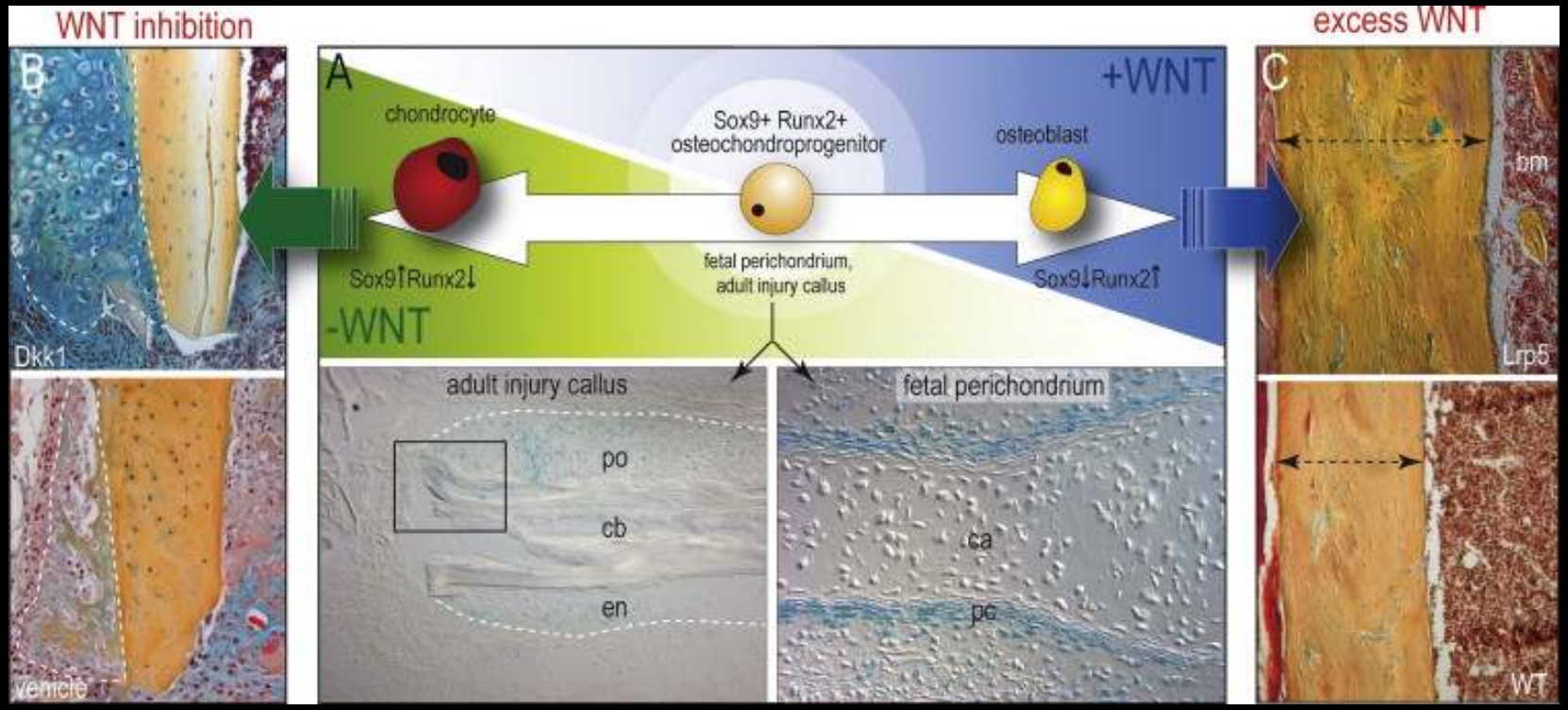
- Osteoblast

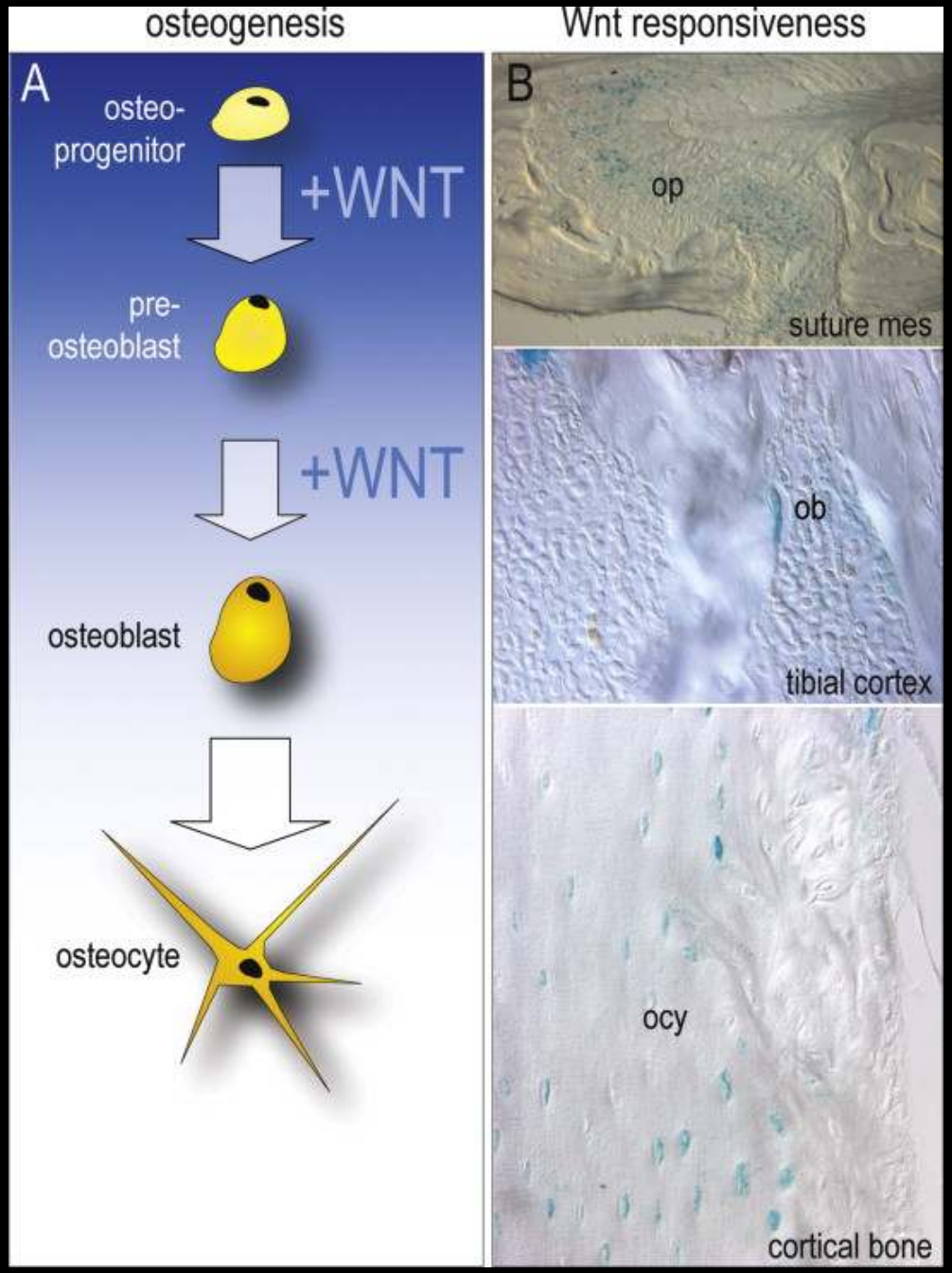

mesenchymal stem cell (MSC)
proliferation
commitment
lineage progression
differentiation and maturation
osteoblast
chondrocyte
myoblast fusion
stromal fibroblast
tenoblast
preadipocyte
osteocyte
chondrocyte
myocyte
stromal cells
tenocyte
adipocyte
bone
cartilage
cardiac
stroma
tendon
adipose tissue

Regulation of cell fate

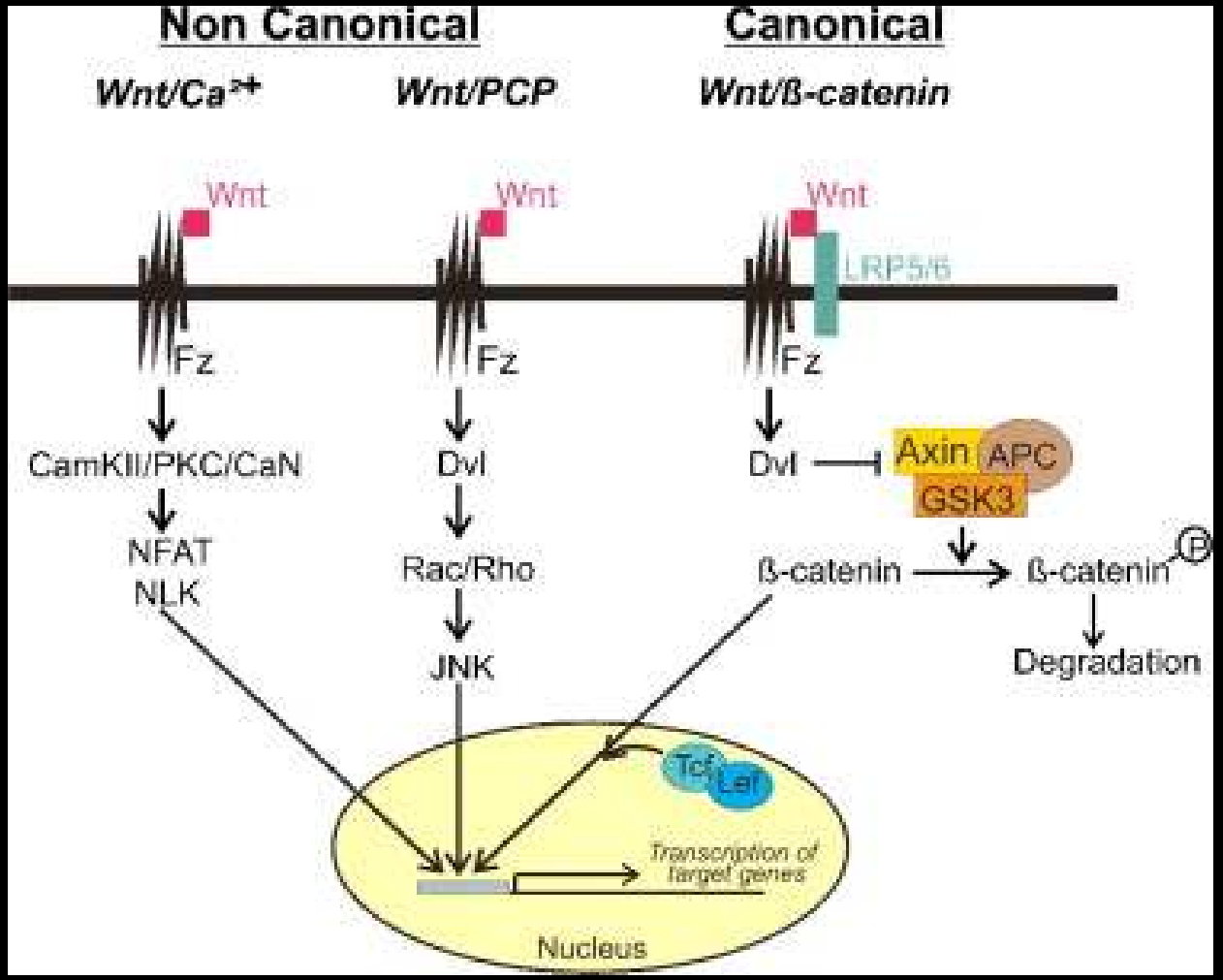


Role of Wnt in skeletal patterning

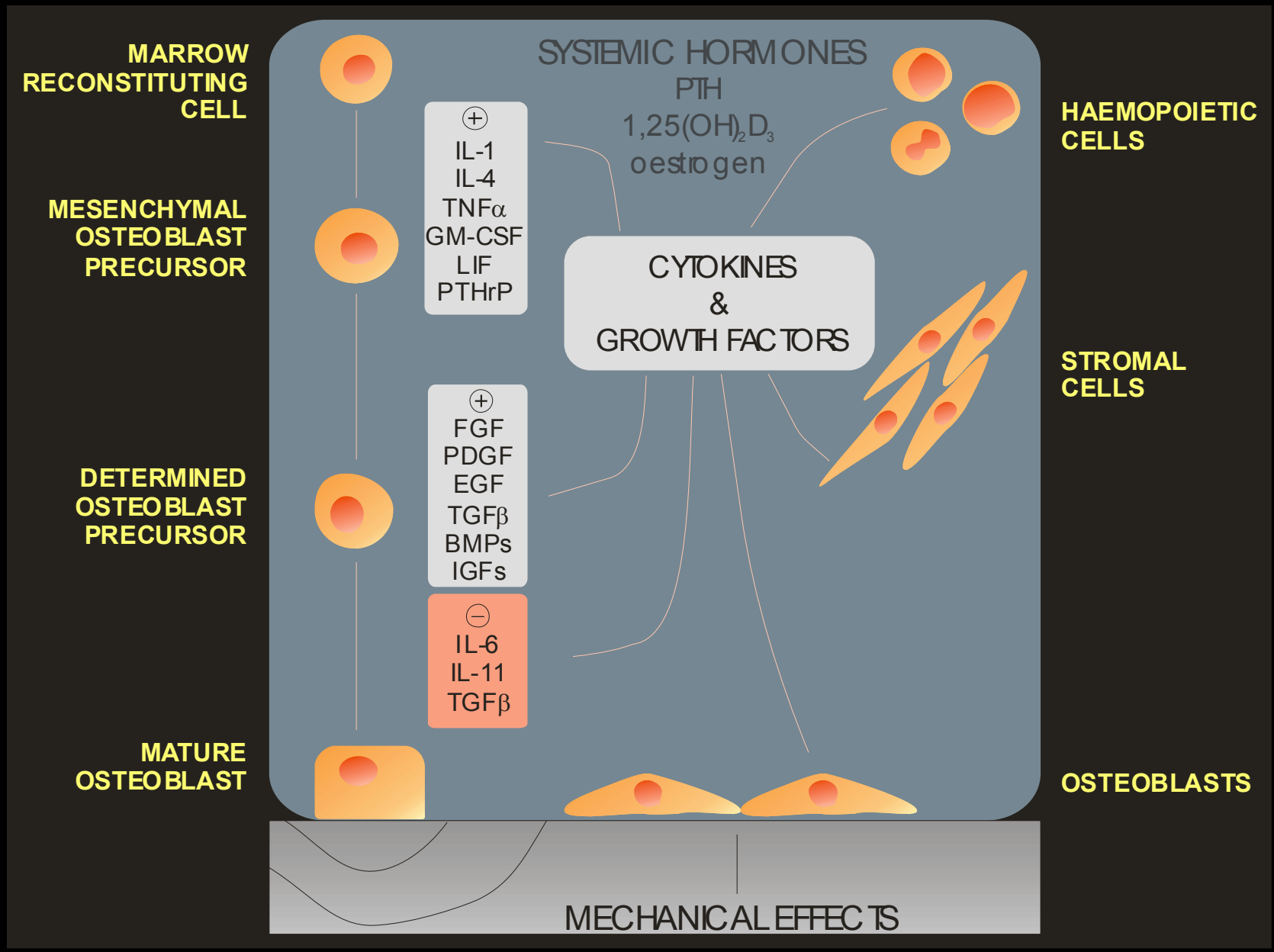



osteogenesis
Wnt responsiveness
A
osteo-
progenitor
+WNT
pre-
osteoblast
+WNT
osteoblast
osteocyte
B
op
suture mes
ob
tibial cortex
ocy
cortical bone

Intracellular signalling in response to Wnt



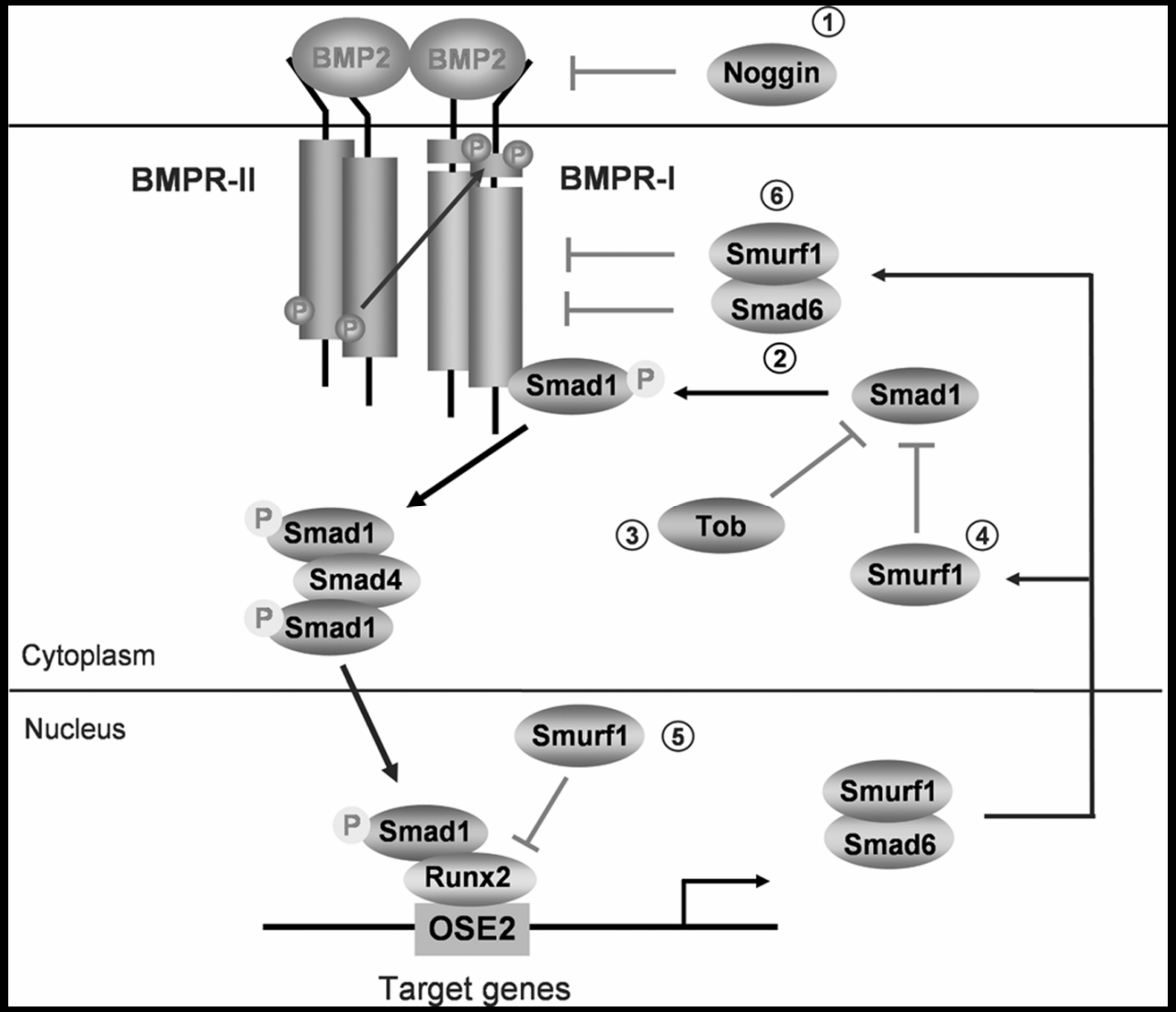
Osteoblast Differentiation



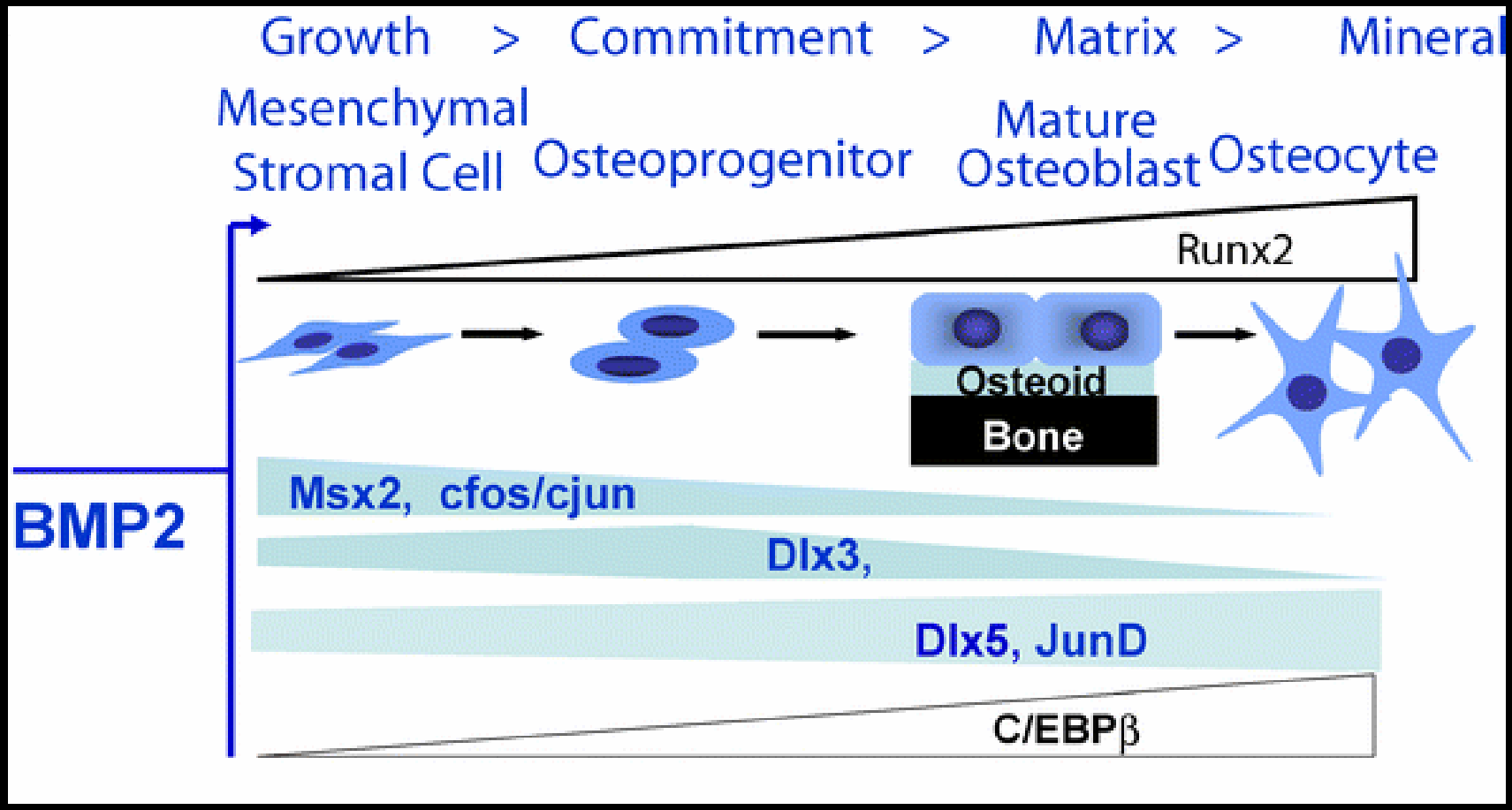
Regulating Bone formation

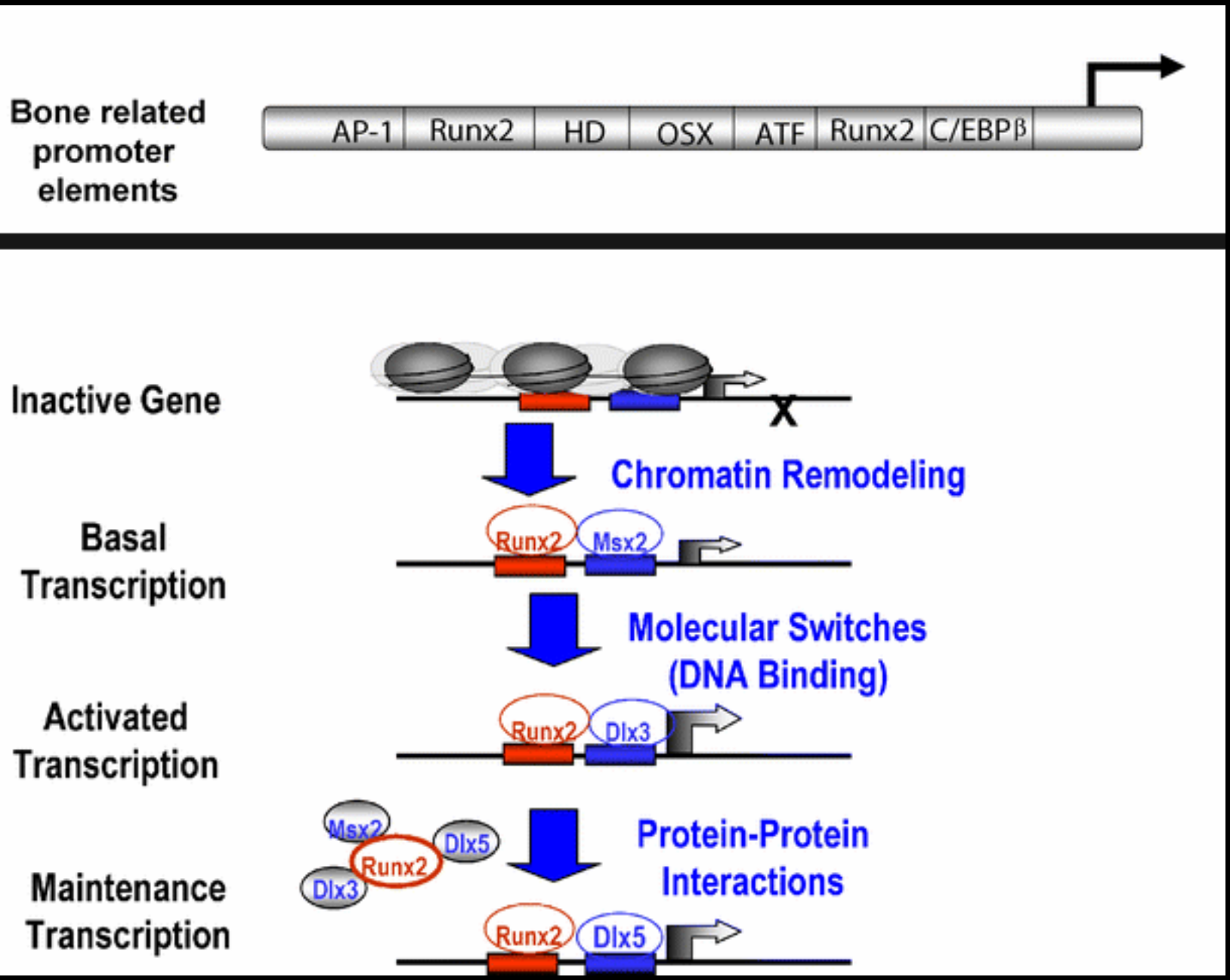
BMP Subfamily	***Genermic Name***	***BMP Designation***
BMP2/4	BMP-2A	BMP-2
	BMP-2B	BMP-4
BMP-3	Osteogenin	BMP-3
	Growth/differentiation factor-10(GDF-10)	BMP-3B
OP-1/BMP-7	BMP-5	BMP-5
	Vegetal related-1(Vgr-1)	BMP-6
	Osteogenic protein-1	BMP-7
	Osteogenic protein-2	BMP-8
	Osteogenic protein-3	BMP-8B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

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

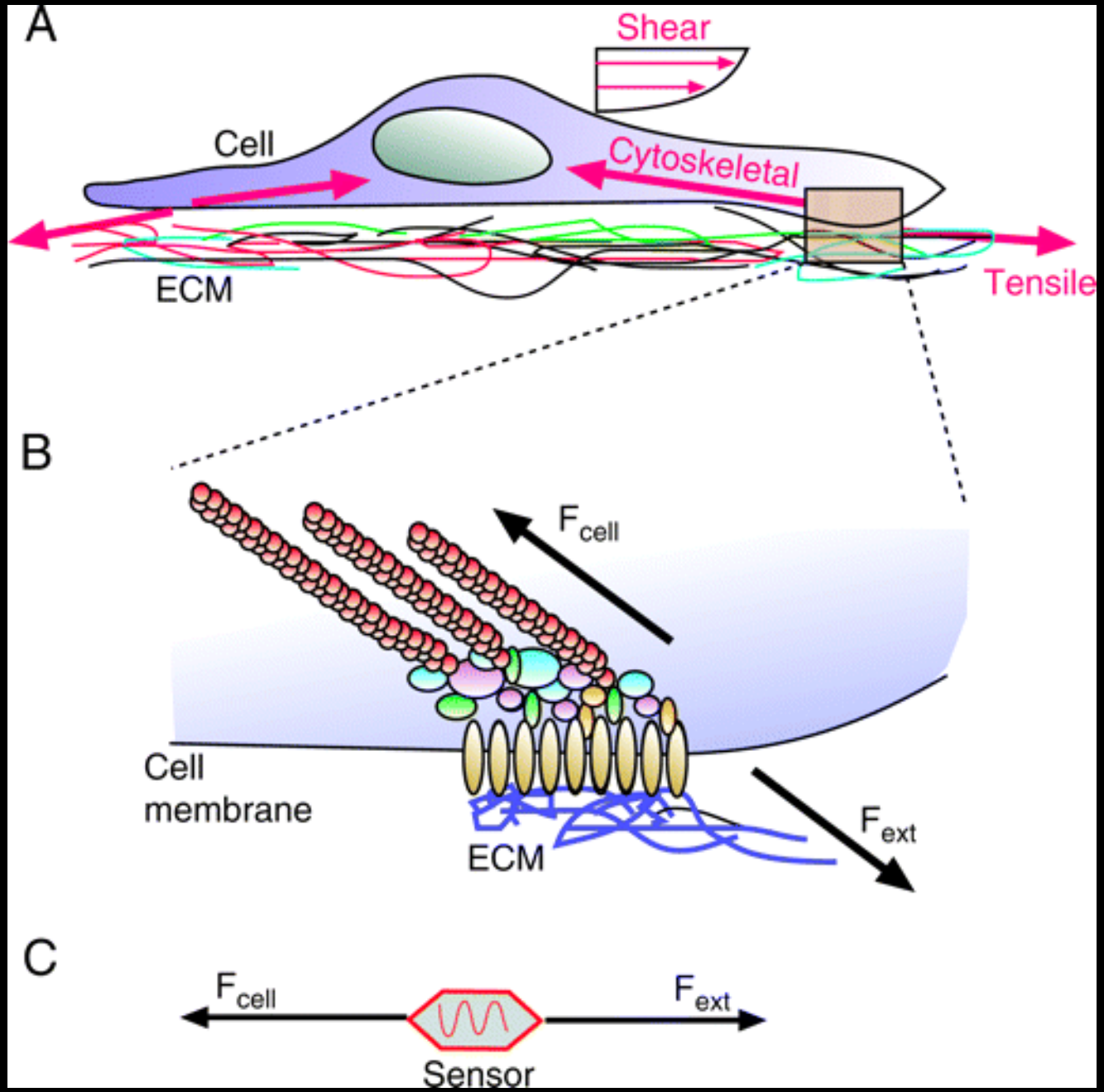


Control of osteoblast differentiation by transcription factors

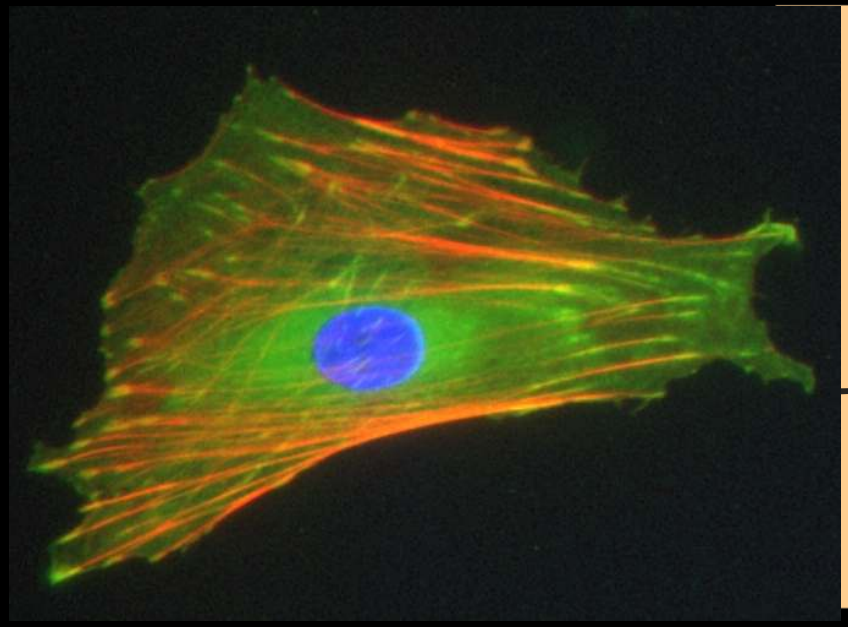


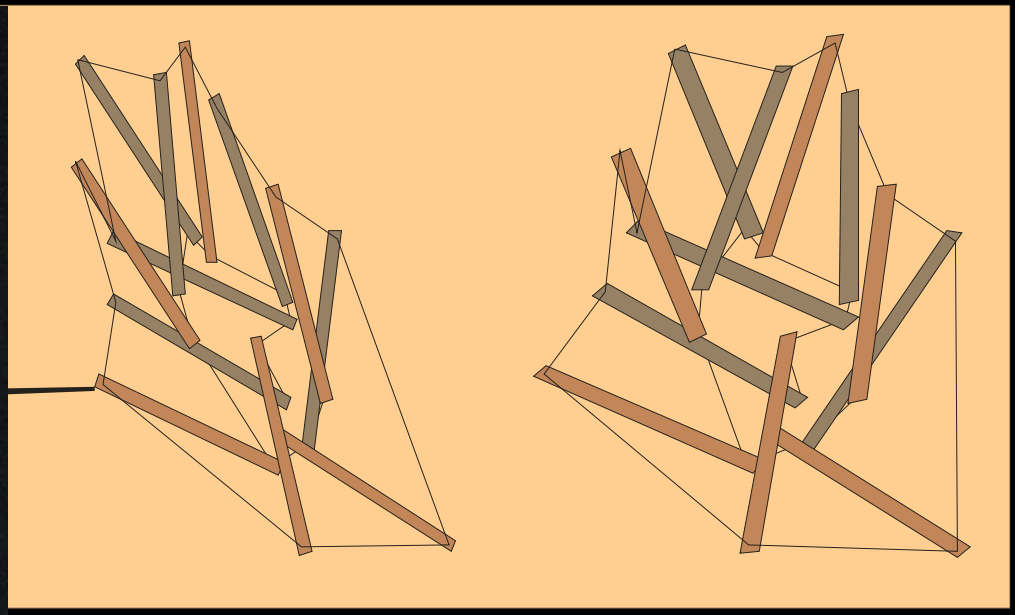

Bone related promoter elements
AP-1
Runx2
HD
OSX
ATF
Runx2
C/EBPβ
Inactive Gene
X
Chromatin Remodeling
Basal Transcription
Runx2
Msx2
Molecular Switches (DNA Binding)
Activated Transcription
Runx2
Dlx3
Msx2
Dlx5
Runx2
Dlx3
Protein-Protein Interactions
Maintenance Transcription
Runx2
Dlx5

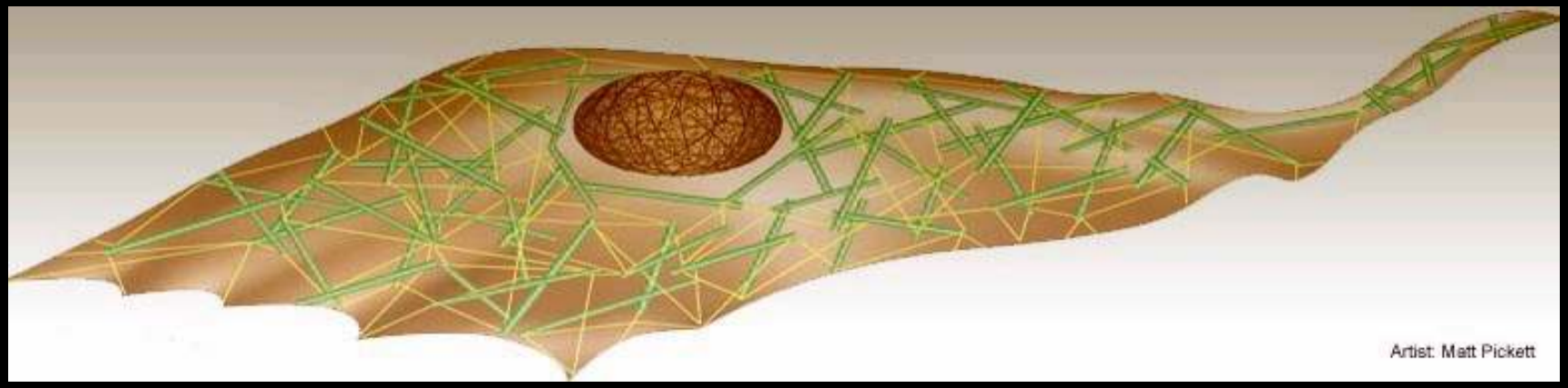
Mechanical load regulates bone cell activity and bone mass


A
Shear
Cell
Cytoskeletal
ECM
Tensile
B
F_{cell}
Cell
membrane
ECM
F_{ext}
C
F_{cell}
F_{ext}
Sensor

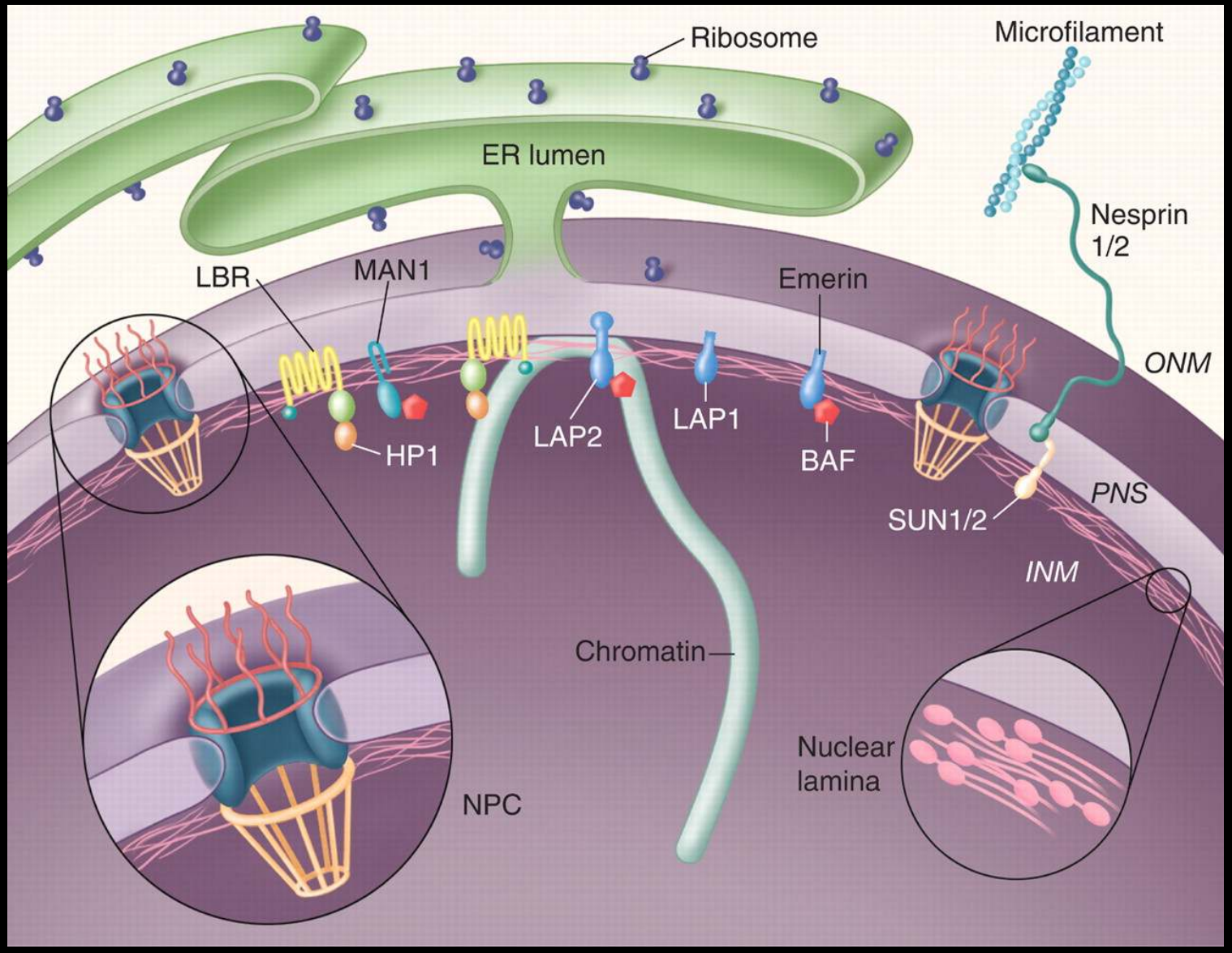
Tensegrity



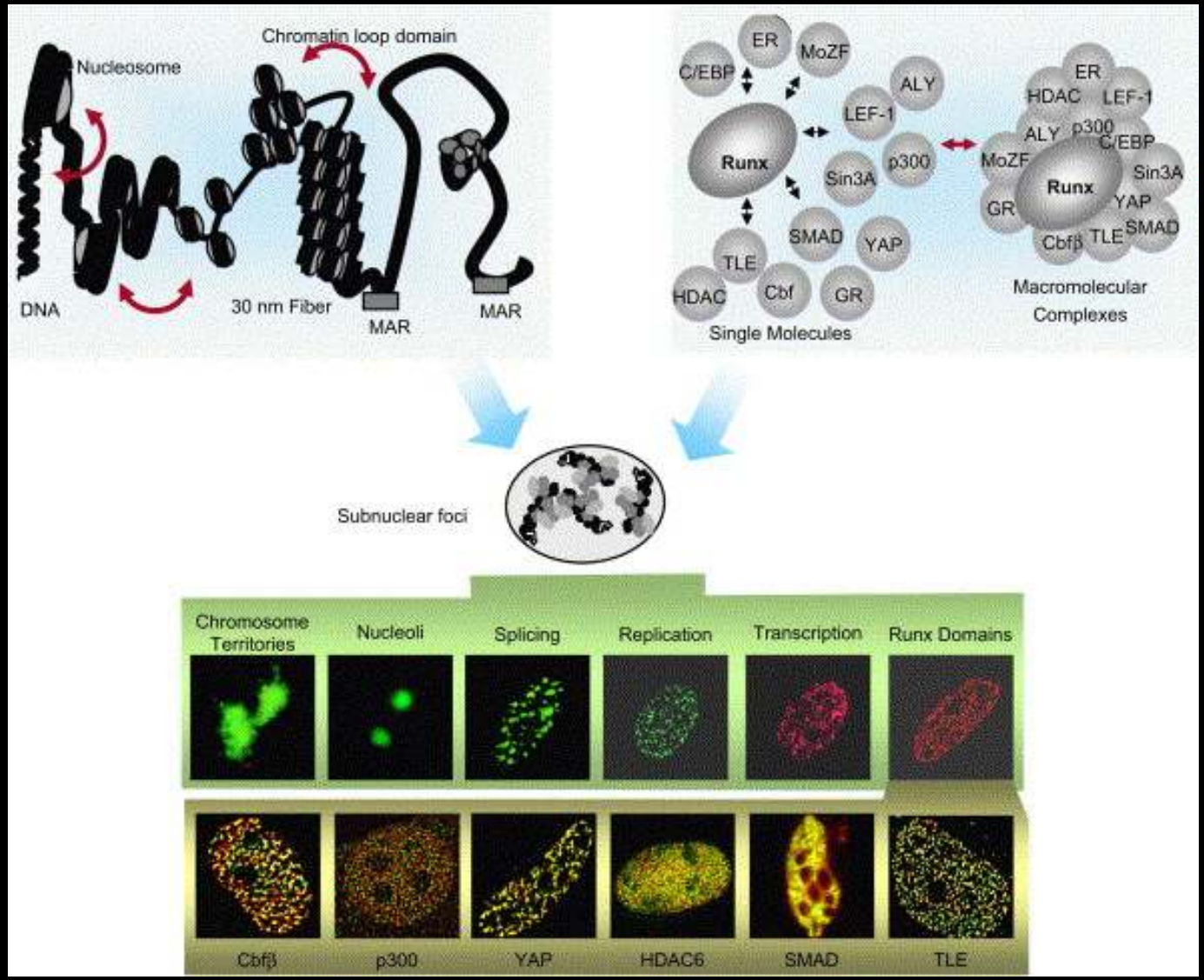


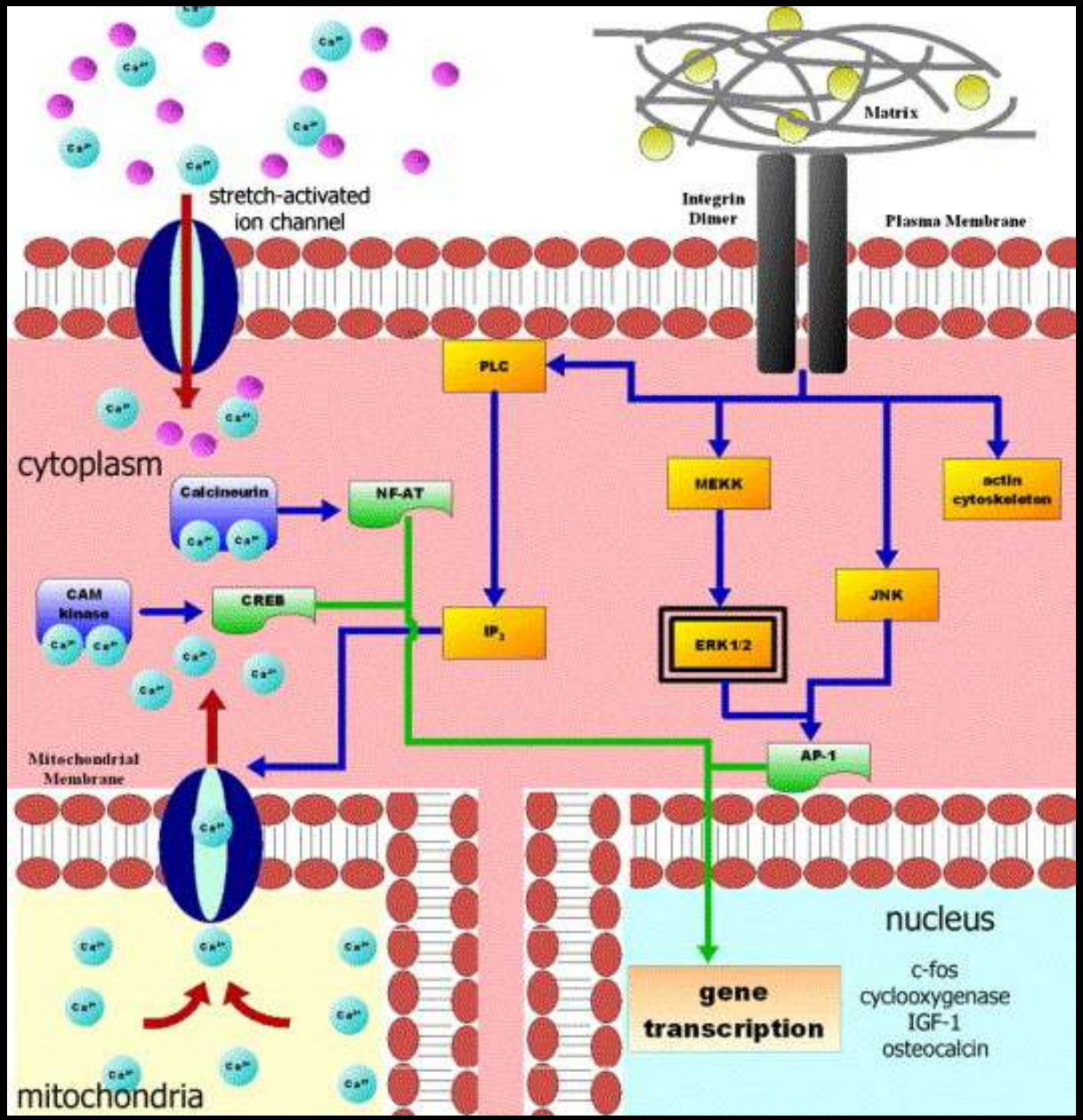


Nuclear architecture

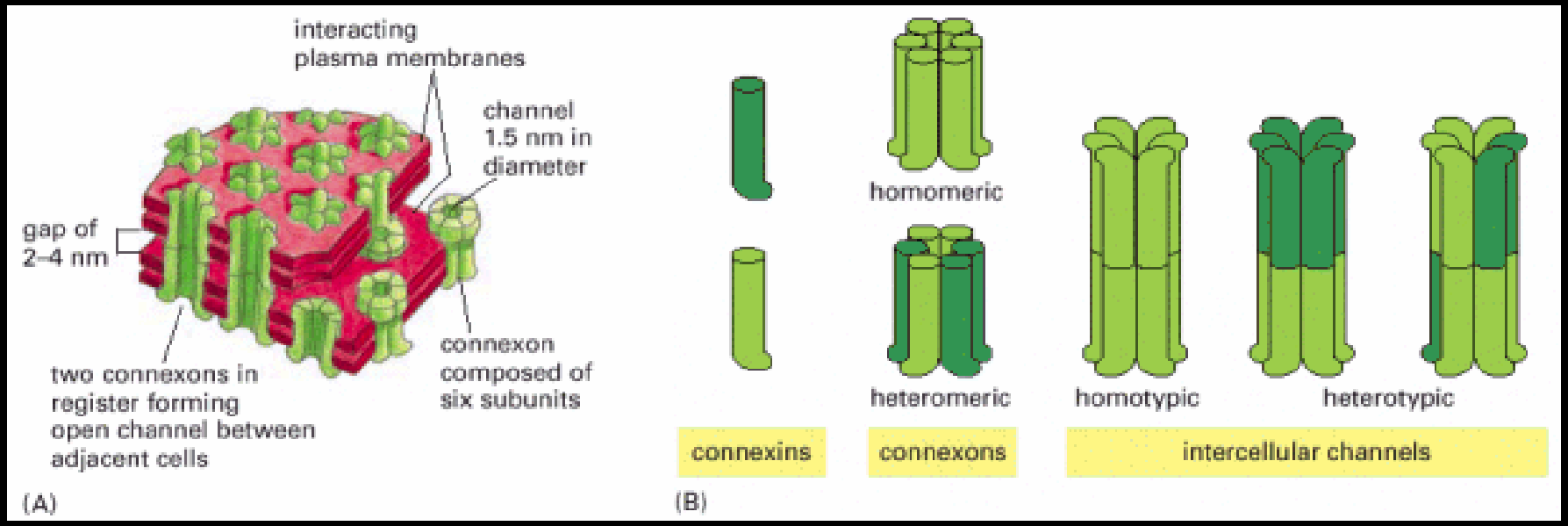


Levels of nuclear organisation

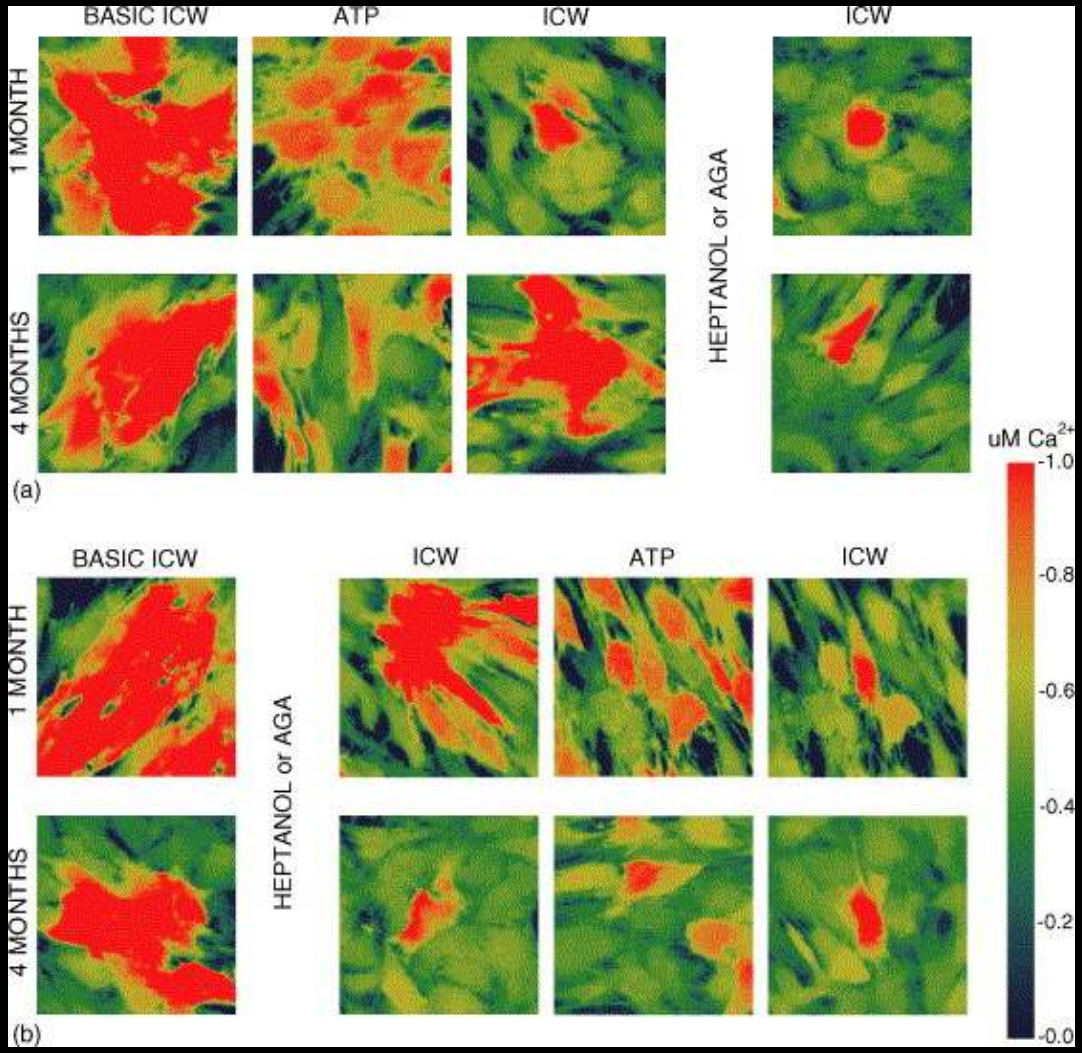



Matrix
Integrin Dimer
Plasma Membrane
stretch-activated ion channel
PLC
cytoplasm
Calcineurin
NF-AT
MEKK
actin cytoskeleton
CAM kinase
CREB
IP3
ERK1/2
JNK
AP-1
Mitochondrial Membrane
nucleus
gene transcription
c-fos
cyclooxygenase
IGF-1
osteocalcin
mitochondria

Propagation of signals



Intercellular calcium wave propagation during osteoblast differentiation



Henriksen Z, et al. Cell Calcium. 2006; 39(5):435-44

Coordinating bone cell behaviour

Mechanostat theory

Disuse Range

Resorption > Formation

- modeling
- remodeling

Physiological Range

Resorption = Formation

homeostasis

Overload Range

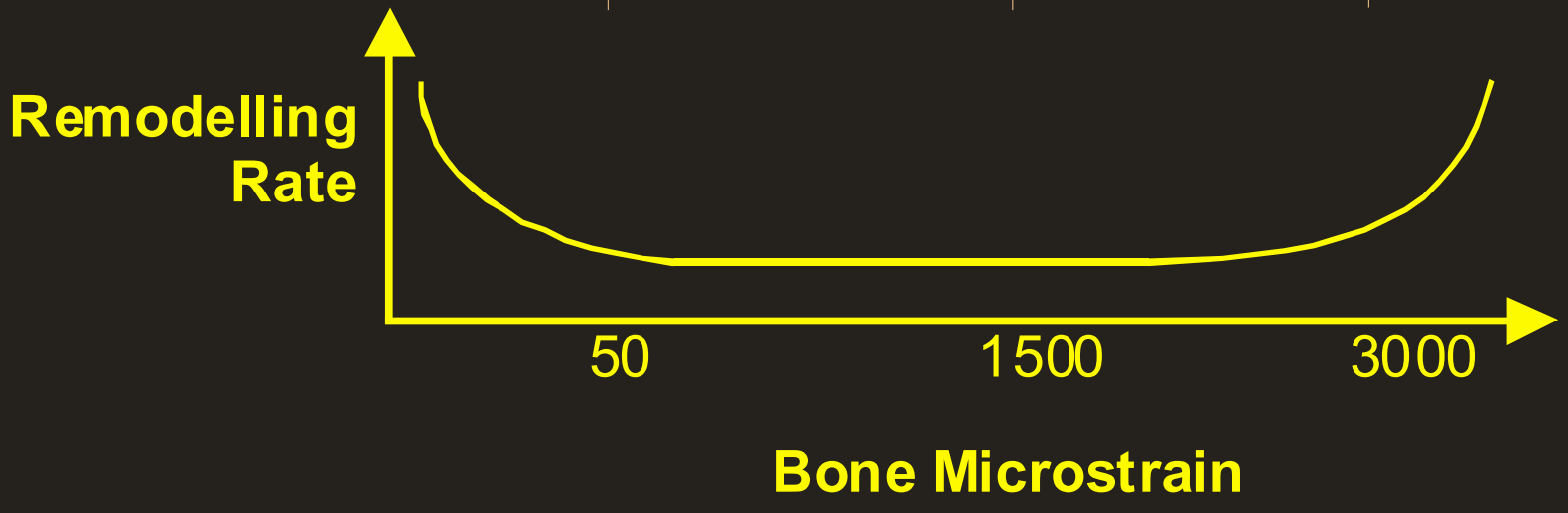
Formation > Resorption

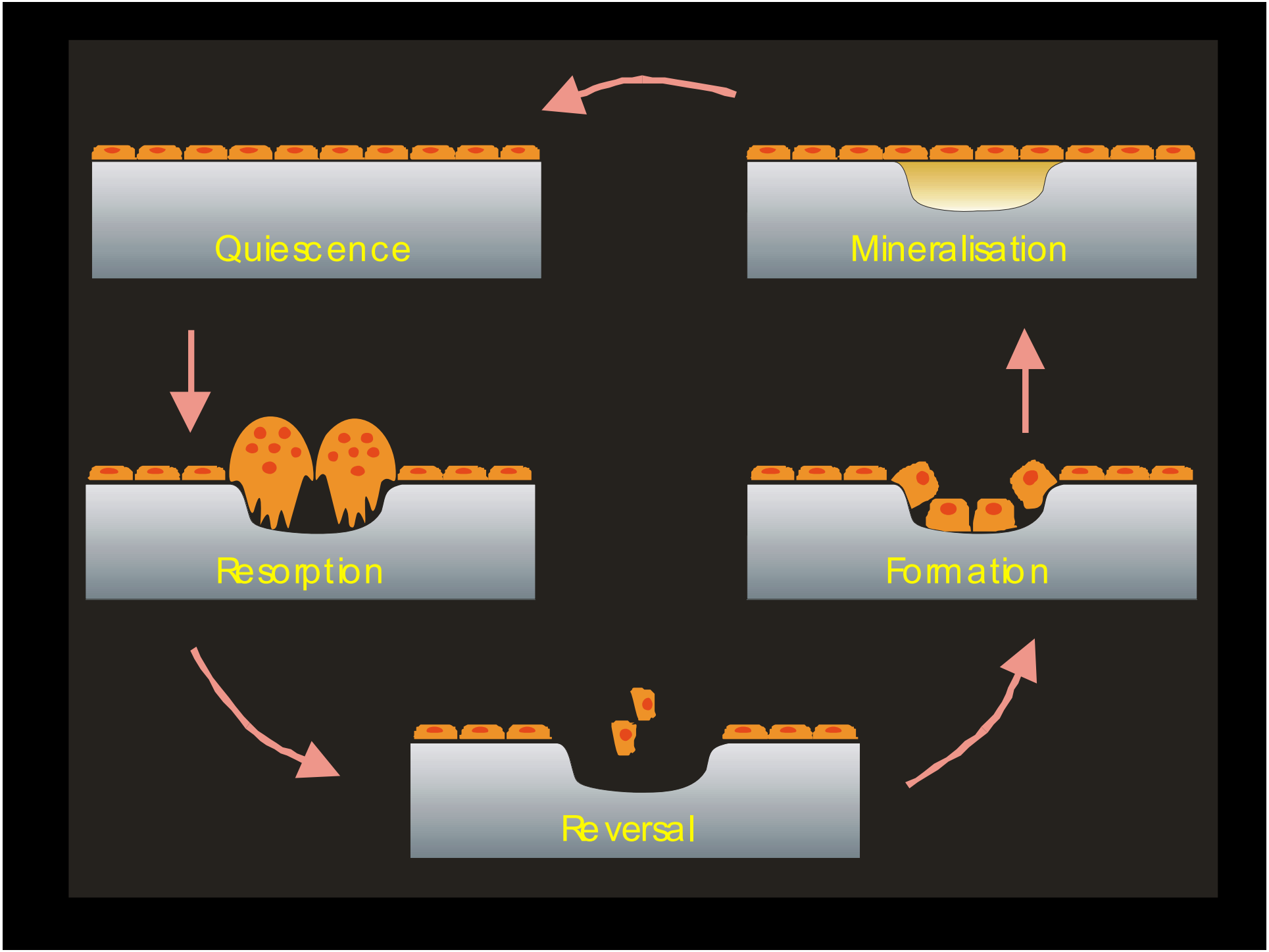
- modeling
- remodeling

Pathological Overload Range

Formation > Resorption

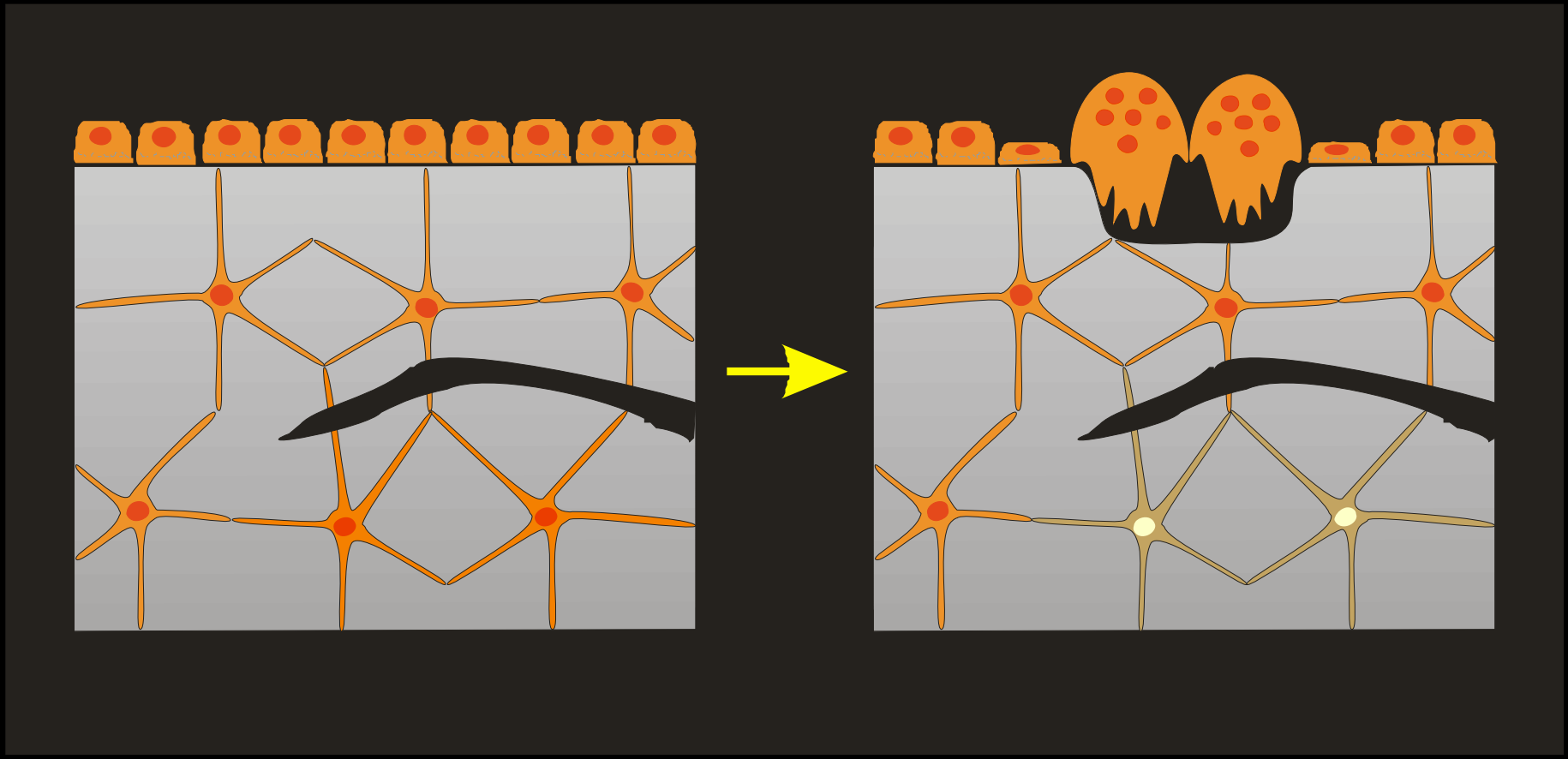
- modeling
- remodeling




Quiescence
Mineralisation
Resorption
Formation
Reversal

Time & Space

Initiation of bone remodelling..



Osteocyte-mediated initiation of bone resorption

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

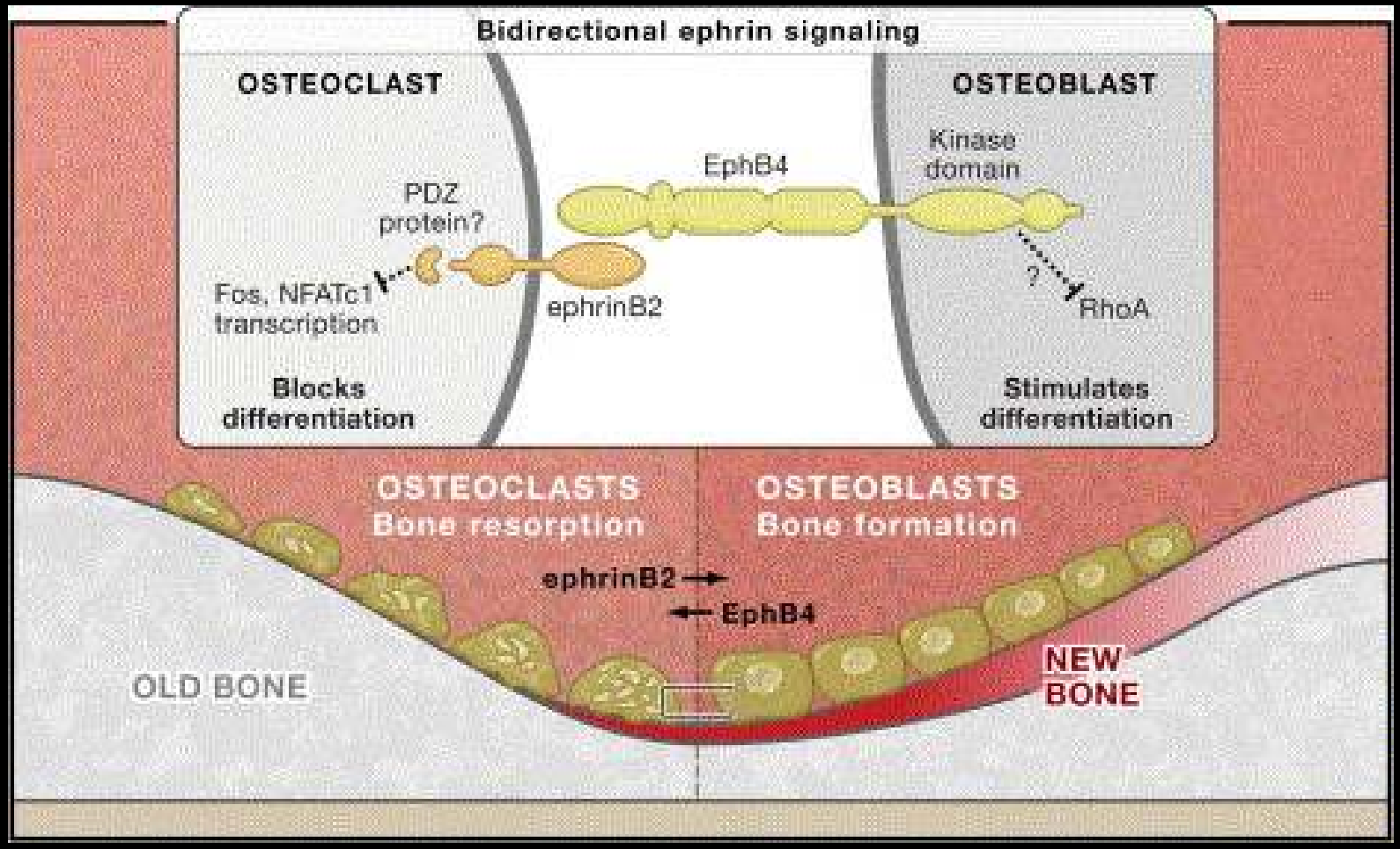
Osteoclast recruitment

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

Reversal / Transition

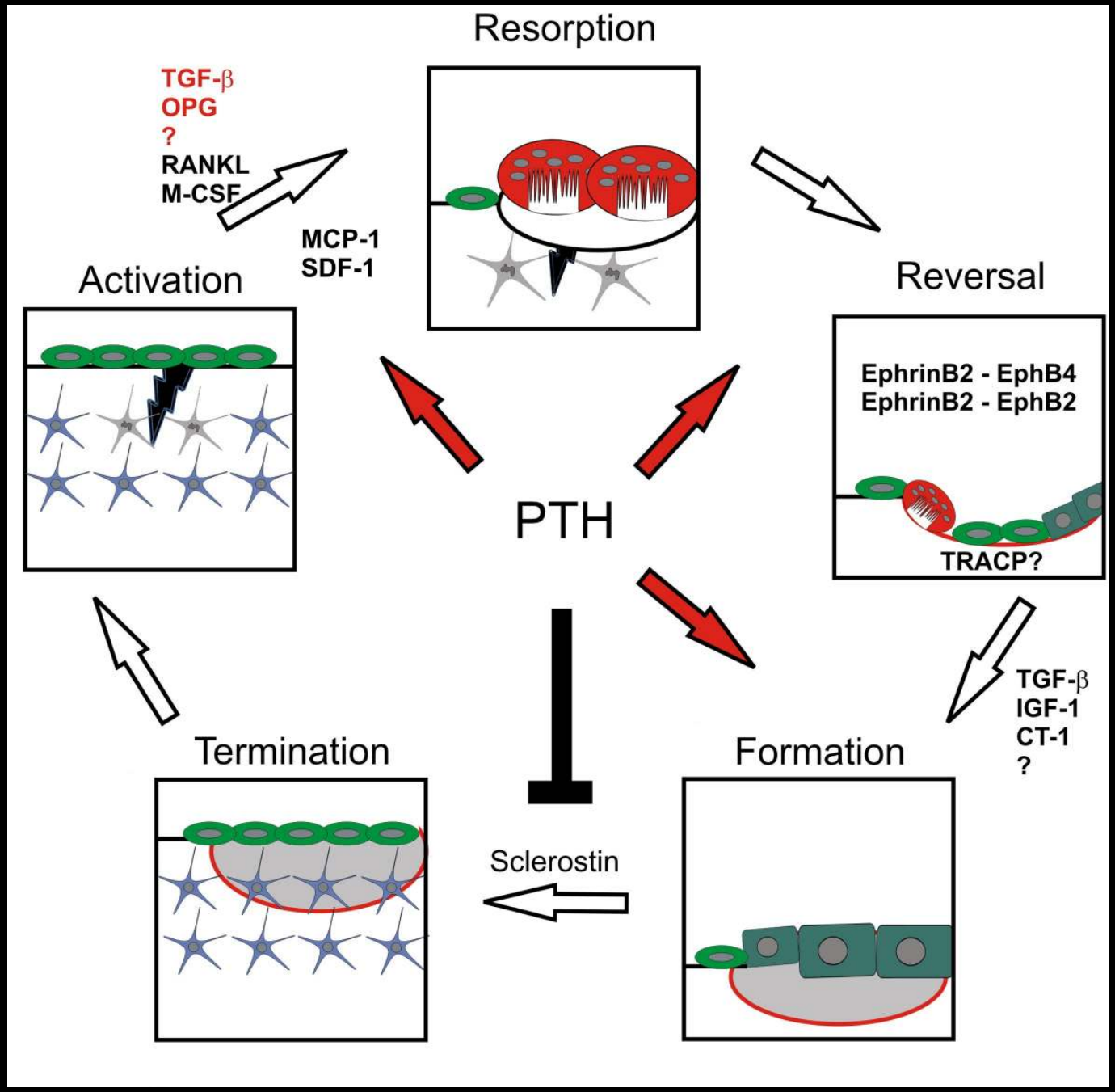
- Factors released from the bone matrix
  - IGF-1, BMP-2, TGFβ, PDGF
- Factors released by osteoclasts
  - Cardiotrophin-1 (CT-1)

Cell:cell interactions



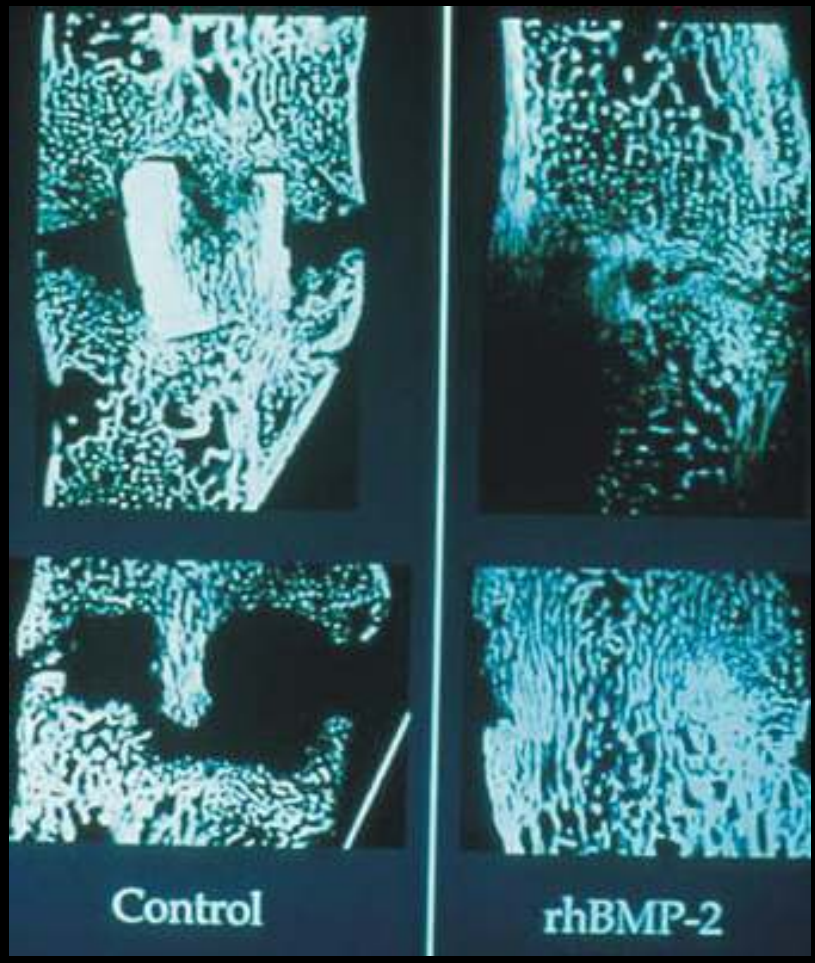
Termination of bone formation

- 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

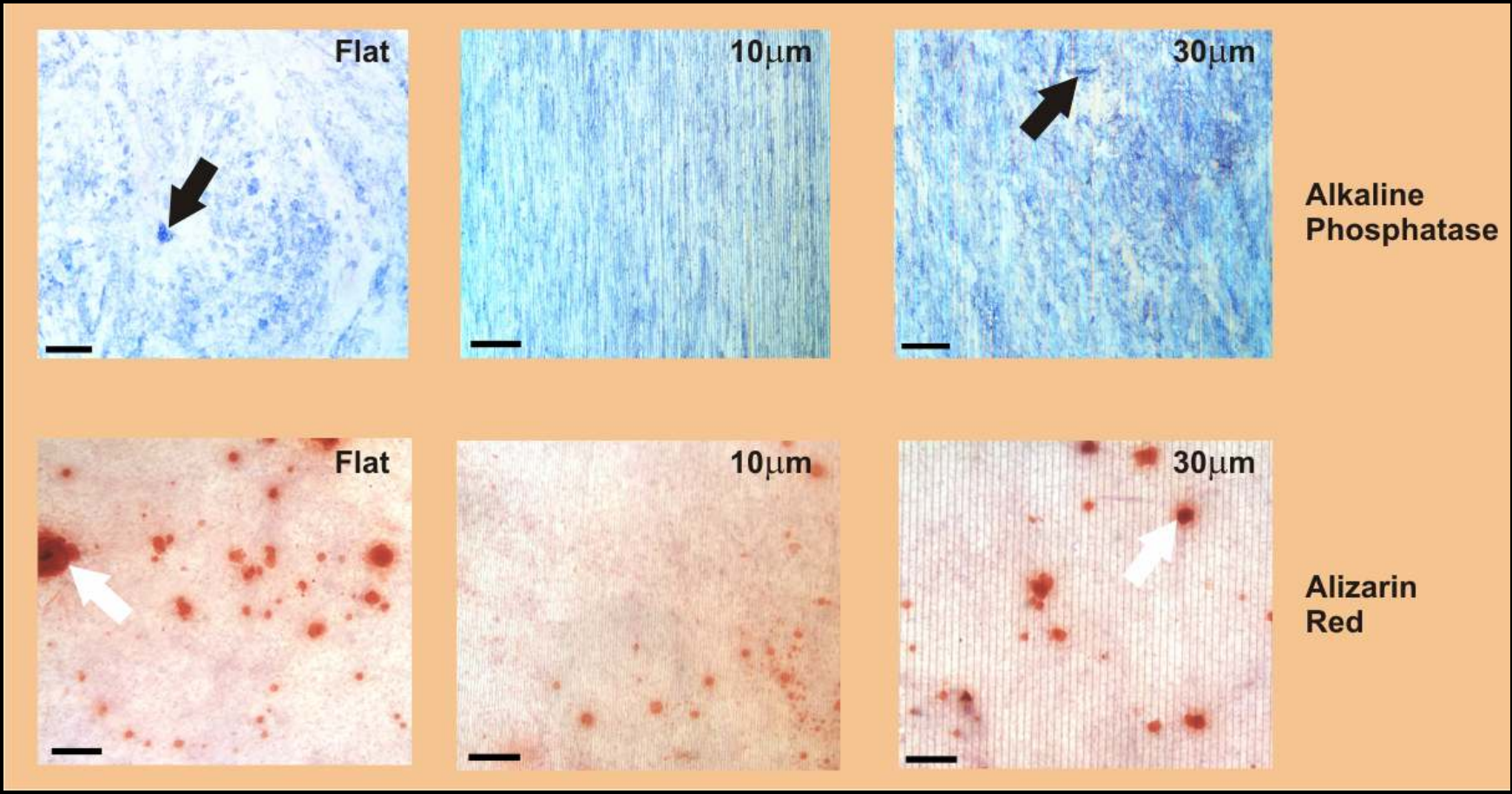

Resorption
TGF-β
OPG
?
RANKL
M-CSF
MCP-1
SDF-1
Activation
Reversal
EphrinB2 - EphB4
EphrinB2 - EphB2
TRACP?
PTH
TGF-β
IGF-1
CT-1
?
Termination
Formation
Sclerostin

Biology in Orthopaedics

- Biologics
- Biomaterial engineering
- Cell therapies

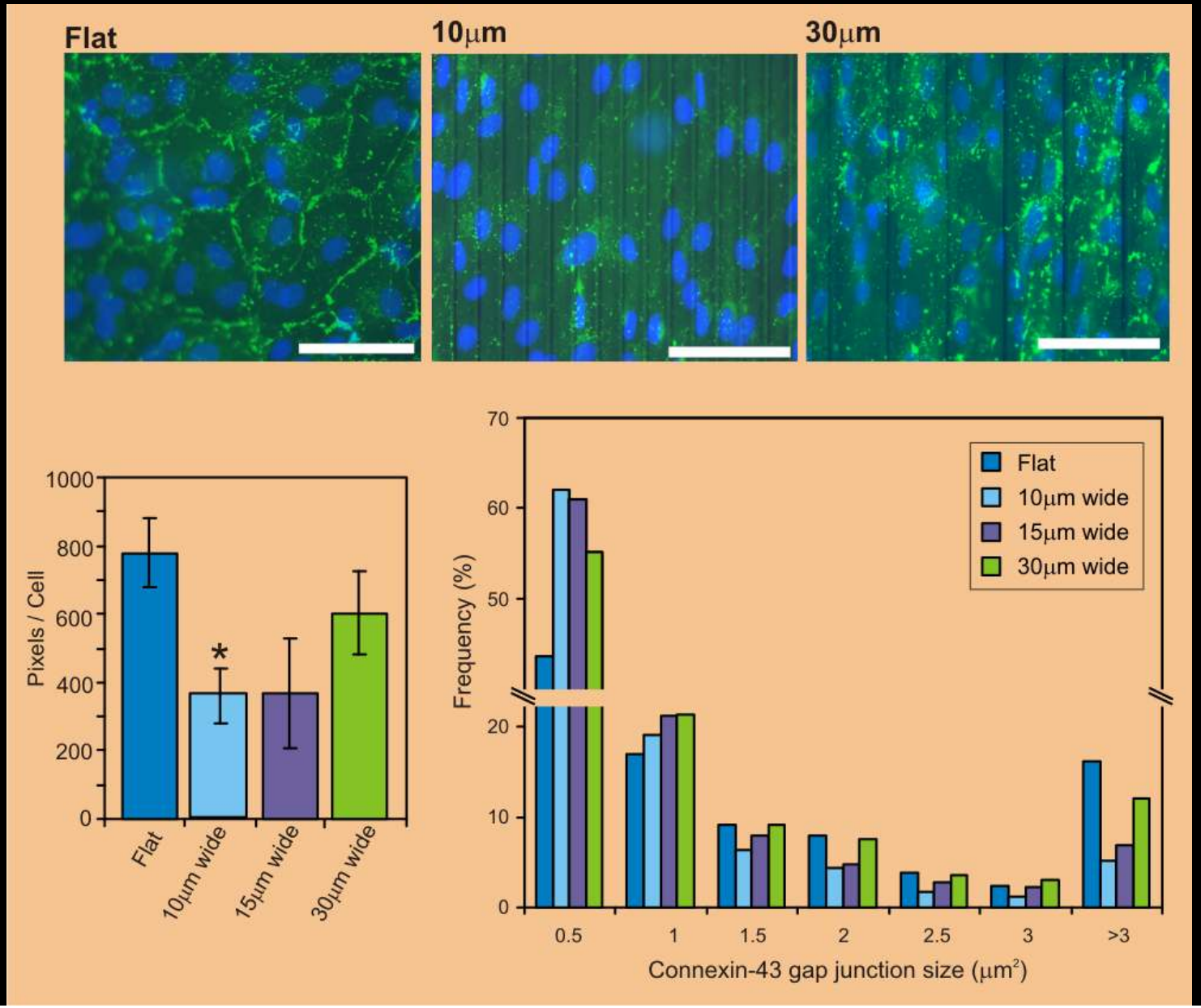


Osteogenesis on 10-30μm grooved surfaces

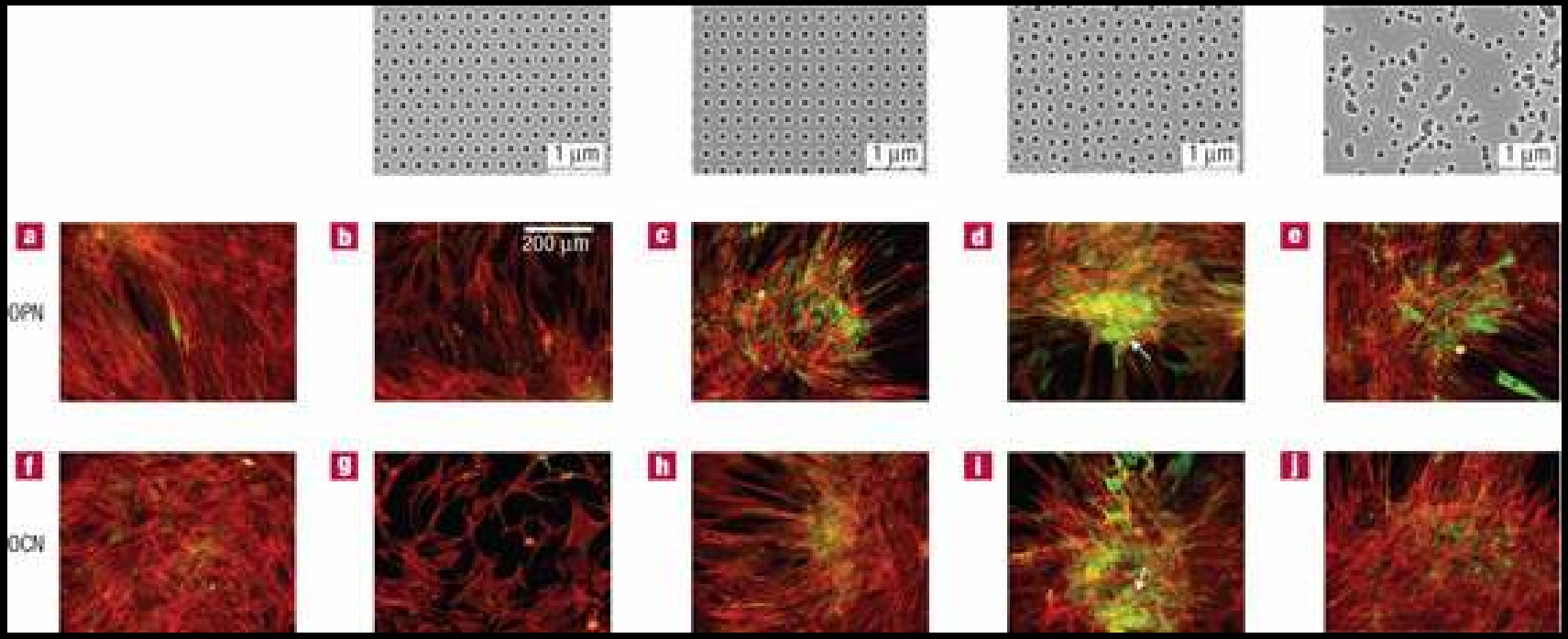


Kirmizidis G, Birch MA. Microfabricated grooved substrates influence cell-cell communication and osteoblast differentiation *in vitro*. Tissue Eng Part A. 2009 15(6):1427-36.

Cell:cell interactions on grooved substrates

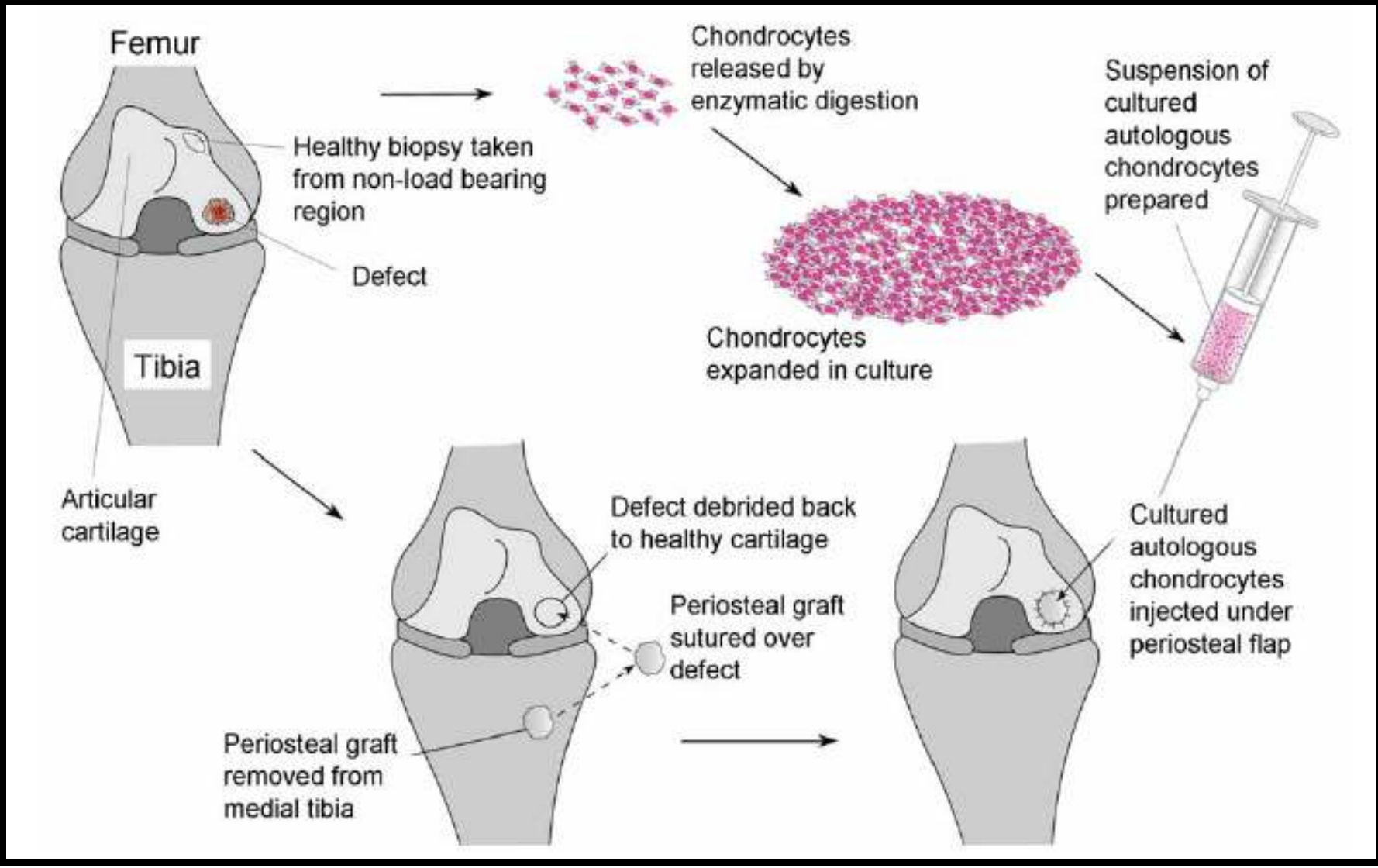


Nanoscale disorder regulates osteoblast differentiation



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



Any questions?