Control of calcium metabolism

MUNI MED

Lékařská fakulta

Calcium and phosphorus homeostasis

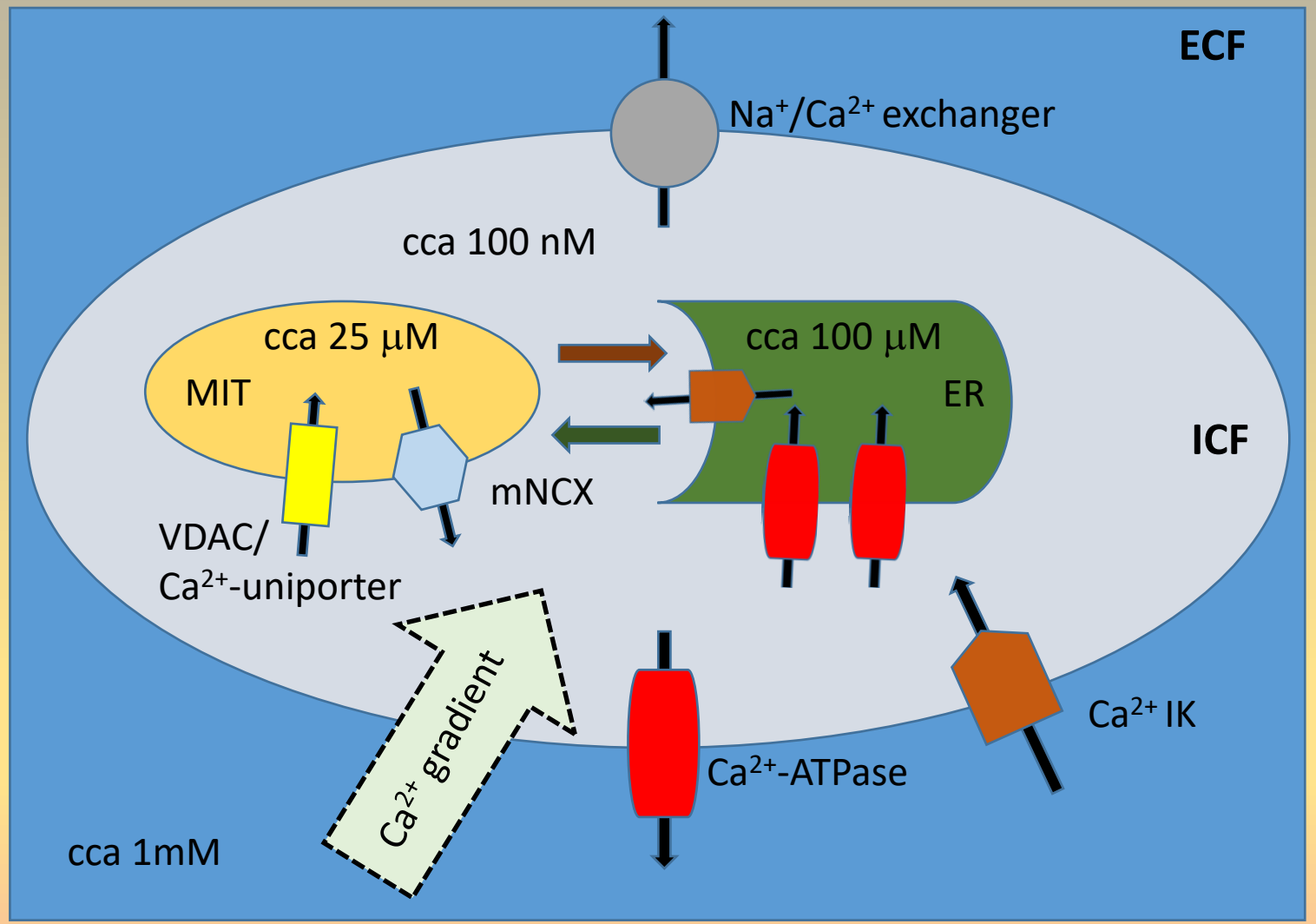
Primary elements of blood tissue are calcium (Ca) and phosphorus (P).

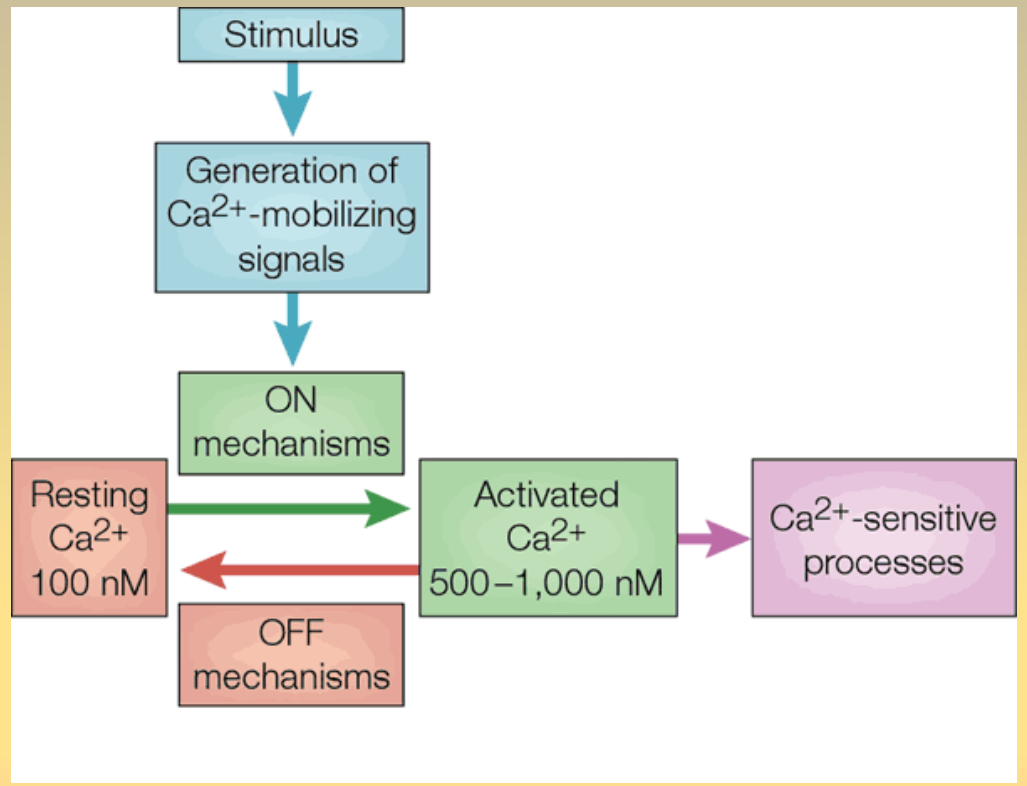
- up to 65 % of bone weight
- almost all Ca and P supply, half of supply of Mg in human body
- Essential role of these elements in physiological processes

Bone tissue
- 99 % of overall Ca, of it 99 % in mineral component
- 1 % - quickly mobilizable and convertible (ICF - ECF)

	Calcium ions	Phosphate ions
Extracellular		
Concentration		
total, in serum	2.5×10^{-3} M	1.00×10^{-3} M
free	1.2×10^{-3} M	0.85×10^{-3} M
Functions	Bone mineral Blood coagulation Membrane excitability	Bone mineral
Intracellular		
Concentration	10^{-7} M	$1-2 \times 10^{-3}$ M
Functions	**Signal for:** • Neuron activation • Hormone secretion • Muscle contraction	• Structural role • High energy bonds • Regulation of proteins by phosphorylation

Extra- and intracellular calcium



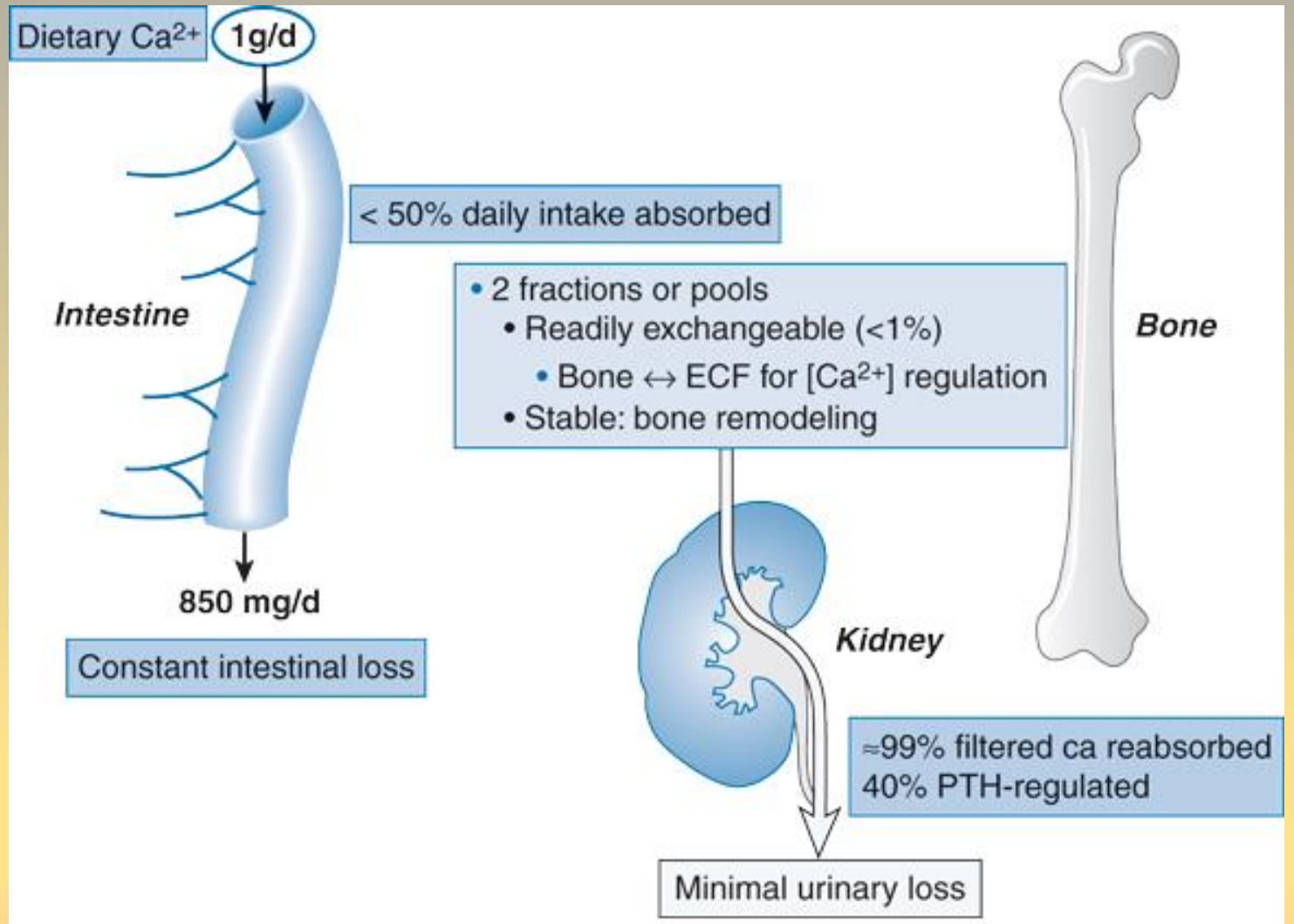


Calcium and its intake

Calcium absorption
- 25 – 60 %
- Age
- Dietary habits and calcium content in diet
- Bone tissue requirements
- Vitamin D

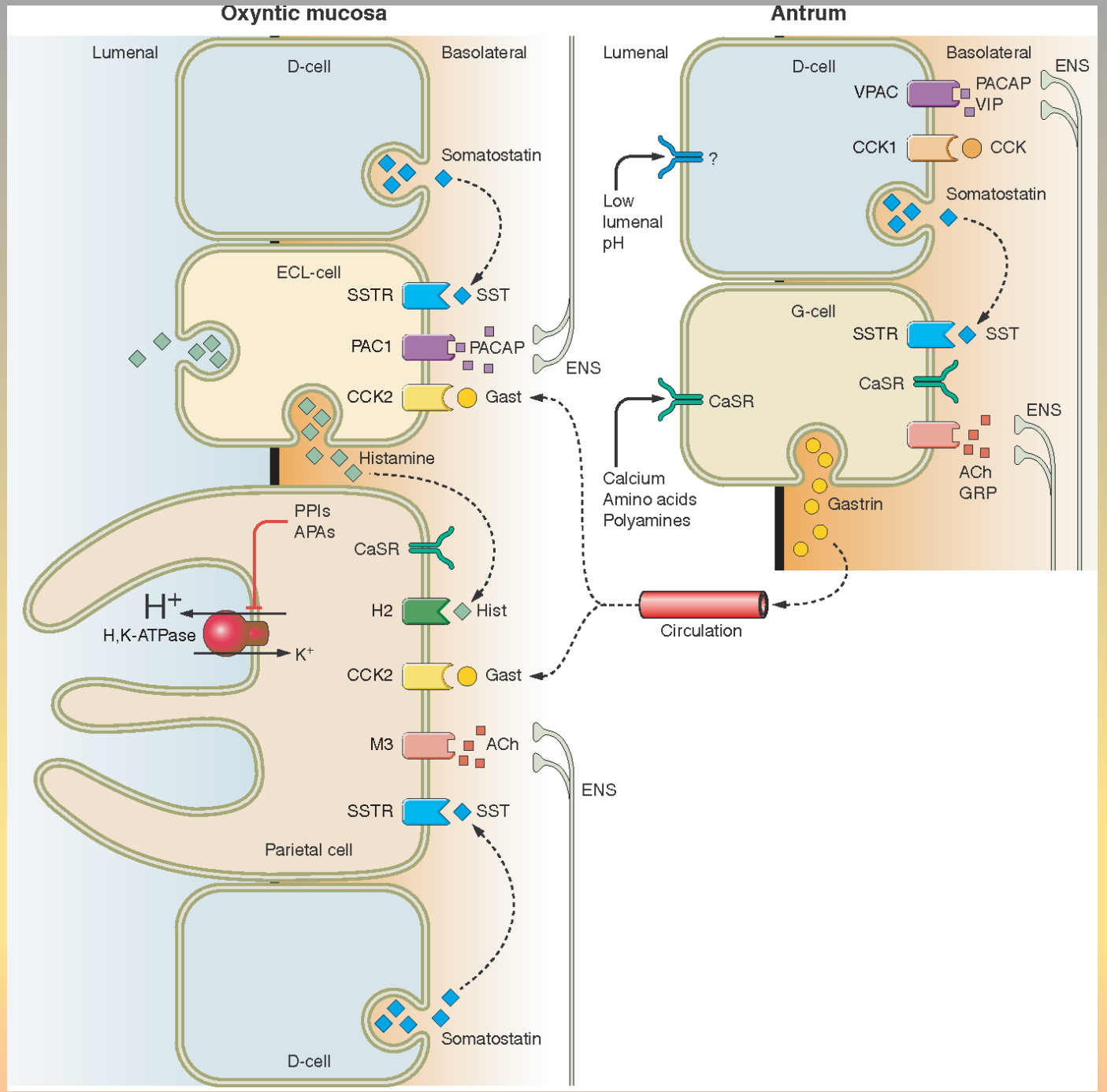
Stomach
- Gastric juice and role of HCl
- Signalization connected to HCl production

Small intestine
- Duodenum a jejunum – 90 %
- Adaptive intake – duodenum and ileum



Bone tissue is crucial calcium and phosphorus storage tissue. Age-related negative calcium balance is an osteoporosis risk factor.

Calcium absorption and stomach



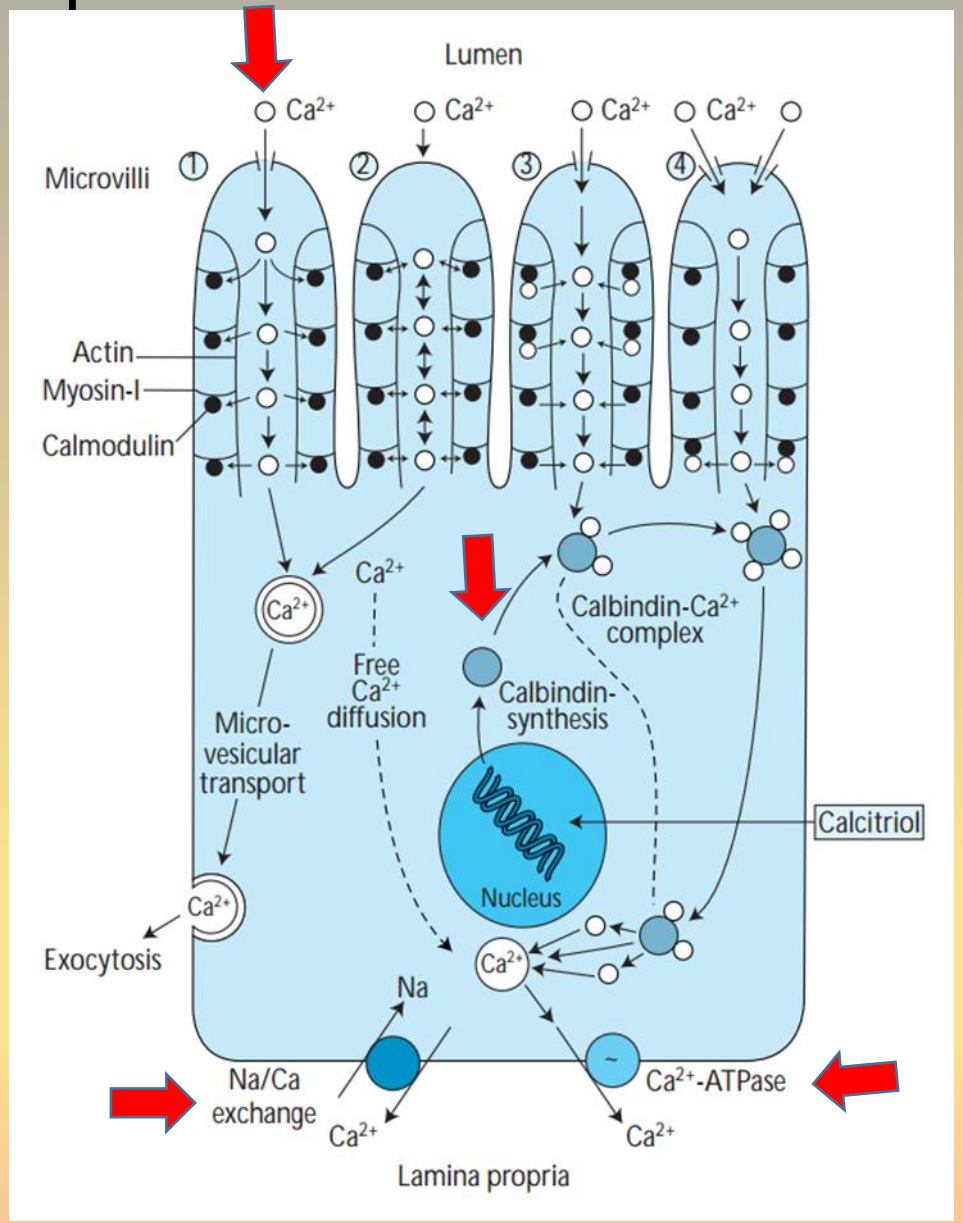
Mechanisms of calcium absorption

Vitamin D

Paracellular
- Luminal electrochemical gradient
- Integrity of intercellular connections
- Claudins and their role in paracellular transport

Transcellular
- TRPV6 and associated proteins
- Recyclation of TRPV6
- Alternative mechanisms?

Glucocorticoids	Estradiol	Prolactin
-	+	+



Calcium on blood (calcemia)

Glomerular filtration YES
Endocrine control

Ionized calcium (50%)

Acidosis

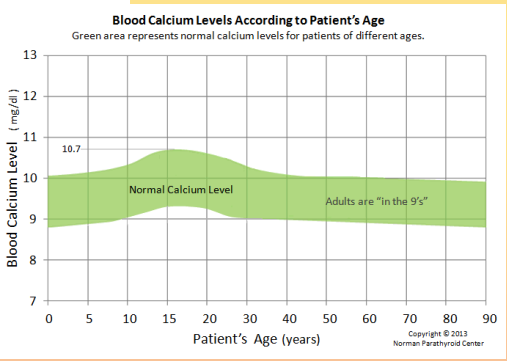
Alkalosis

Protein bound (40%)
- 80–90% albumin; pH dependent
  - Acidosis ↓ binding & ↑ ionized Ca^{2+}
  - Alkalosis ↑ binding & ↓ ionized Ca^{2+}
- 10–20% globulins

Glomerular filtration NO

Complexed (10%)
- Citrate & phosphate

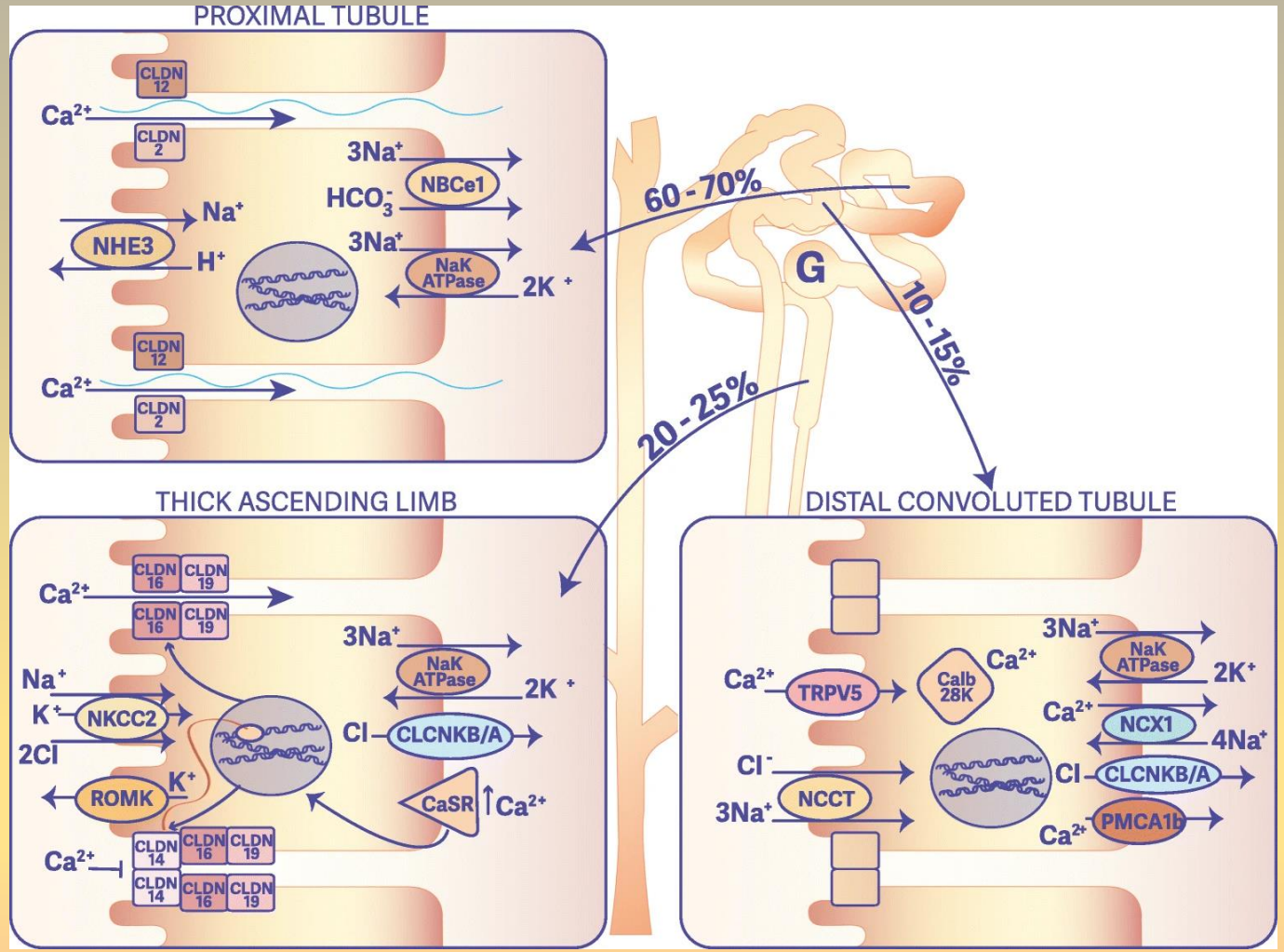
Complexed & ionized Ca^{++} cross plasma membrane



Calcium excretion

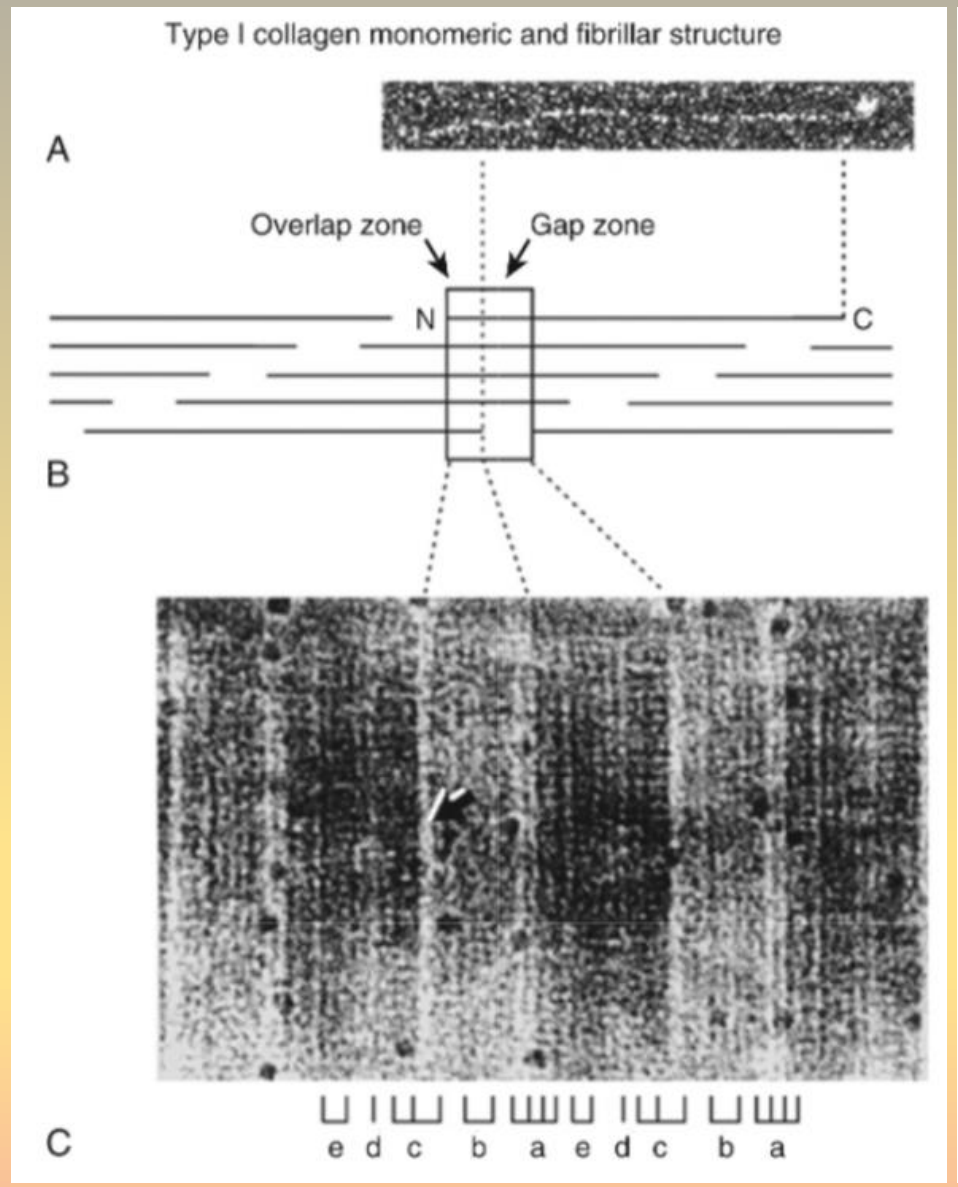
- **98 % of filtered Ca is reabsorbed**
- 70 % proximal tubule
- 20 % thick ascending limb of HL
- 5 % collecting duct
- 2 % urine

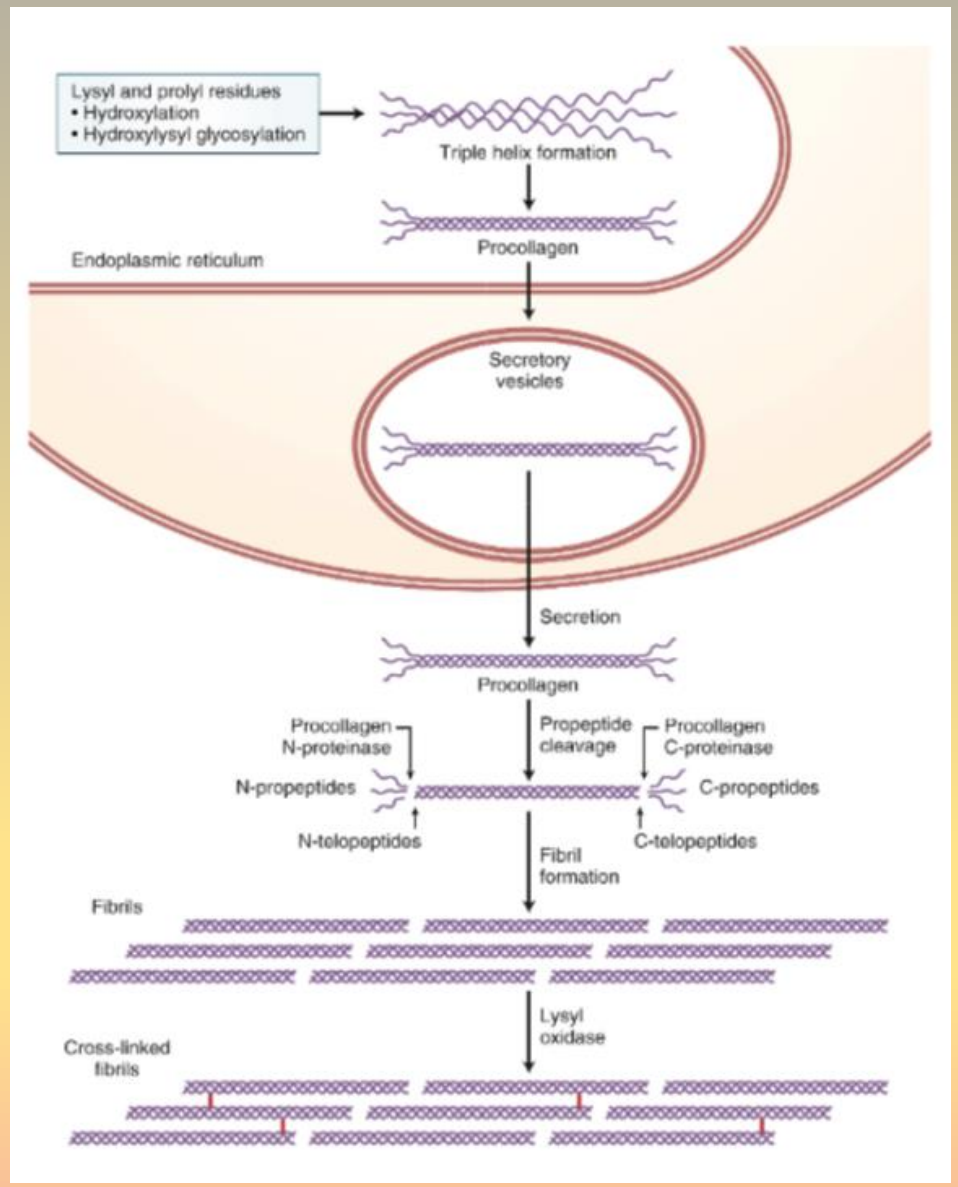
- CaSR (TALH)
- Paracellin-1
- PTH



Collagen type I = most important protein of bone matrix

Bone matrix and bone mineral





Trombospondin
Fibronectin
Matrix Gla protein
Osteocalcin
Biglykan
Decorin
Bone sialoprotein
Osteopontin
Osteoadherin

Signaling + haematopoiesis

Vitamin K-dependent γ carboxylation and phosphorylation

Ca affinity and mineralization

Mineralization

= production of small hydroxyapatite crystals (Ca, phosphates, carbonates, Mg, Na, K)

External mechanism – alkalic phosphatase

Internal mechanism – phospho1 (Phosphoethanolamine/ phosphocholine phosphatase)

Endopeptidases, PHEX – FGF23

Ca, P, and AF availability

Vesicle formation (matrix)

Collagen and its arrangement

Cleavage of pyrophosphate

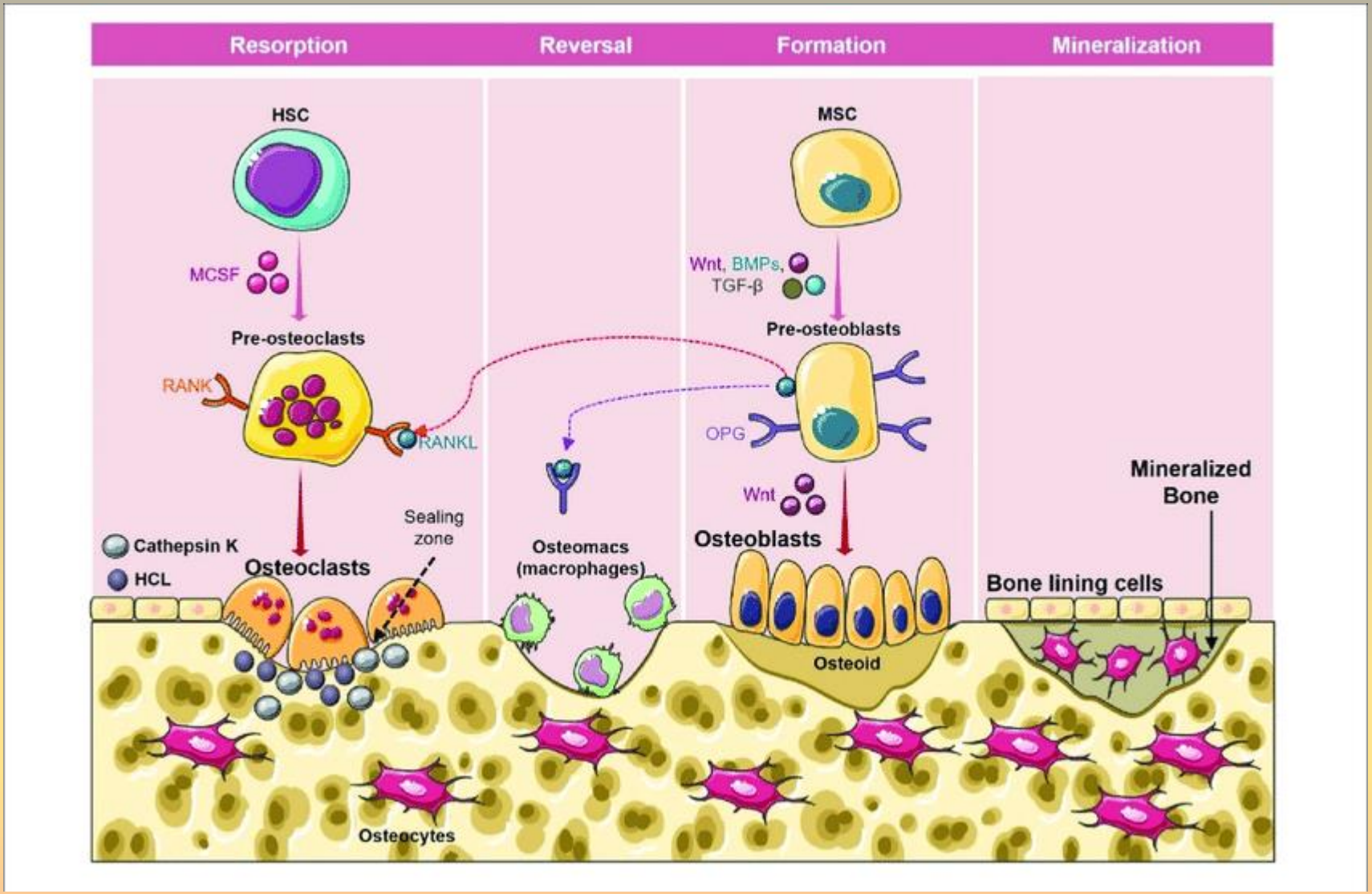
Phosphate availability for mineralization

Diet, calcium in diet, calcium/phosphorus in ECF

SIEBLINGs
- Osteopontin, DMP-1 (OC)
- Bone sialoprotein, MEPE

Deposition of calcium

Bone tissue and its remodeling



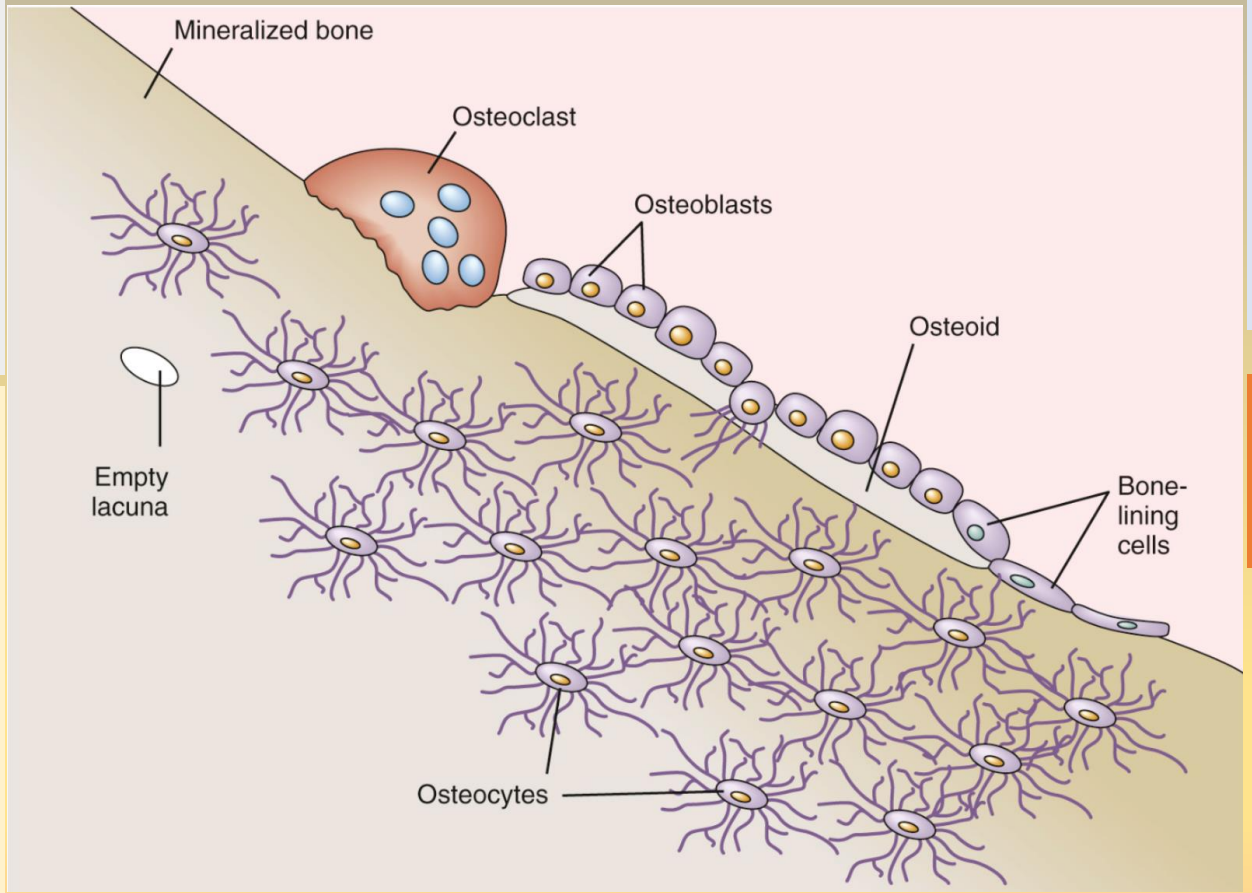
Bone remodeling cycle. Bone remodeling occurs in four phases: (1) Activation phase: In this phase, M-CSF and RANKL promote the differentiation of osteoclast precursors into osteoclasts. (2) Resorption phase: During this phase mature osteoclasts with unique ruffled borders induce resorption of bone by secreting cathepsin K, and H + in the sealing zone. After resorption osteoclasts detach from the surface of the bone and undergo apoptosis. (3) Reversal phase: In the reversal phase osteoblasts' precursors get differentiated into mature osteoblasts and are recruited to the resorption site. (4) Formation phase: In this phase osteoblasts get occupied in the resorbed lacuna and start depositing the bone matrix. After the formation phase, osteoid gets mineralized and bone surface returns to resting phase with bone lining cells.

Bhardwaj A, Sapra L, Tiwari A, Mishra P, Sharma S, Srivastava R: **"Osteomicrobiology": The Nexus Between Bone and Bugs.** ***Frontiers in Microbiology 2022, 12.***

Bone tissue and its remodeling

Osteocytes (OC)
- Metabolic activity
- PTH receptors
- Communication with bone surface
- Mechanic sensing
- RANKL production
- Direct degradation of bone tissue (osteocytic osteolysis)
- Adaptive remodeling

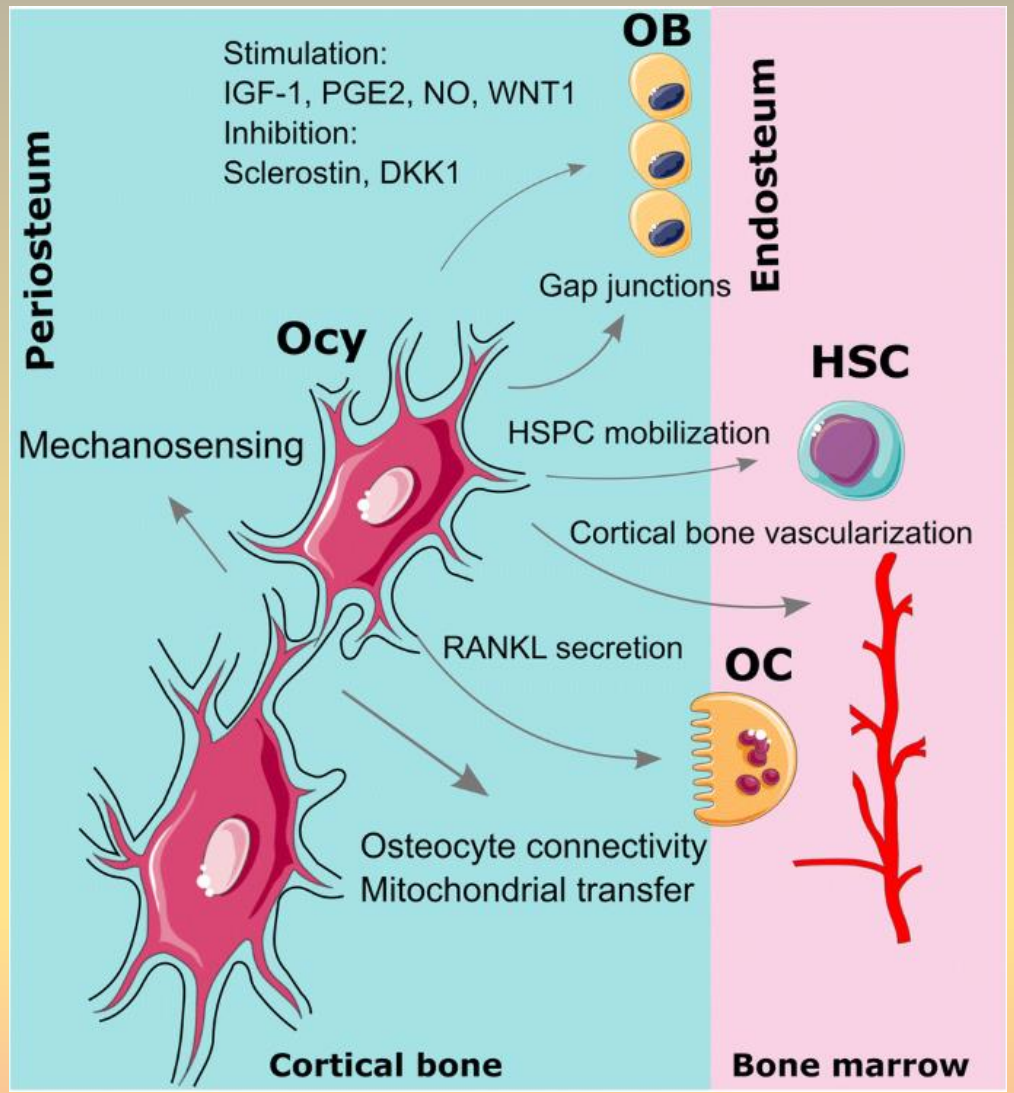
Osteoblasts (OB)
- Bone matrix production
- Production of collagen and non-collagen peptides + their orientation
- Regulation by hormones, local factors and cytokines
- Differentiation and further fate – apotosis, osteocytes, lining cells
- „recruitment“ of other cells – IGF-1, IGF-2, TGF-β

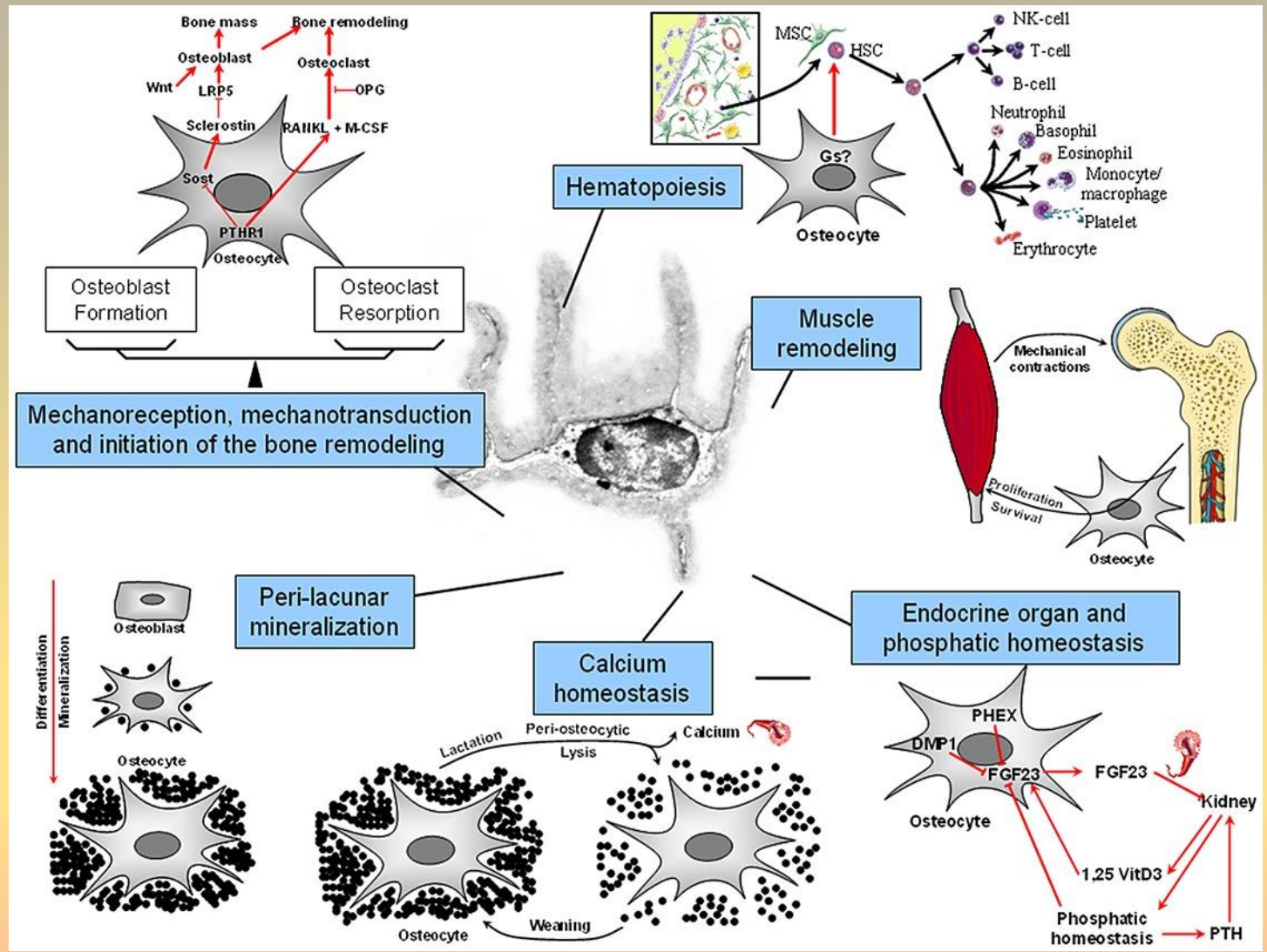


Lining cells
- Stimulation of OB differentiation
- OC communication
- Differentiation to OB stimulated by PTH

Osteoclasts (OK)
- Bone tissue reabsorption

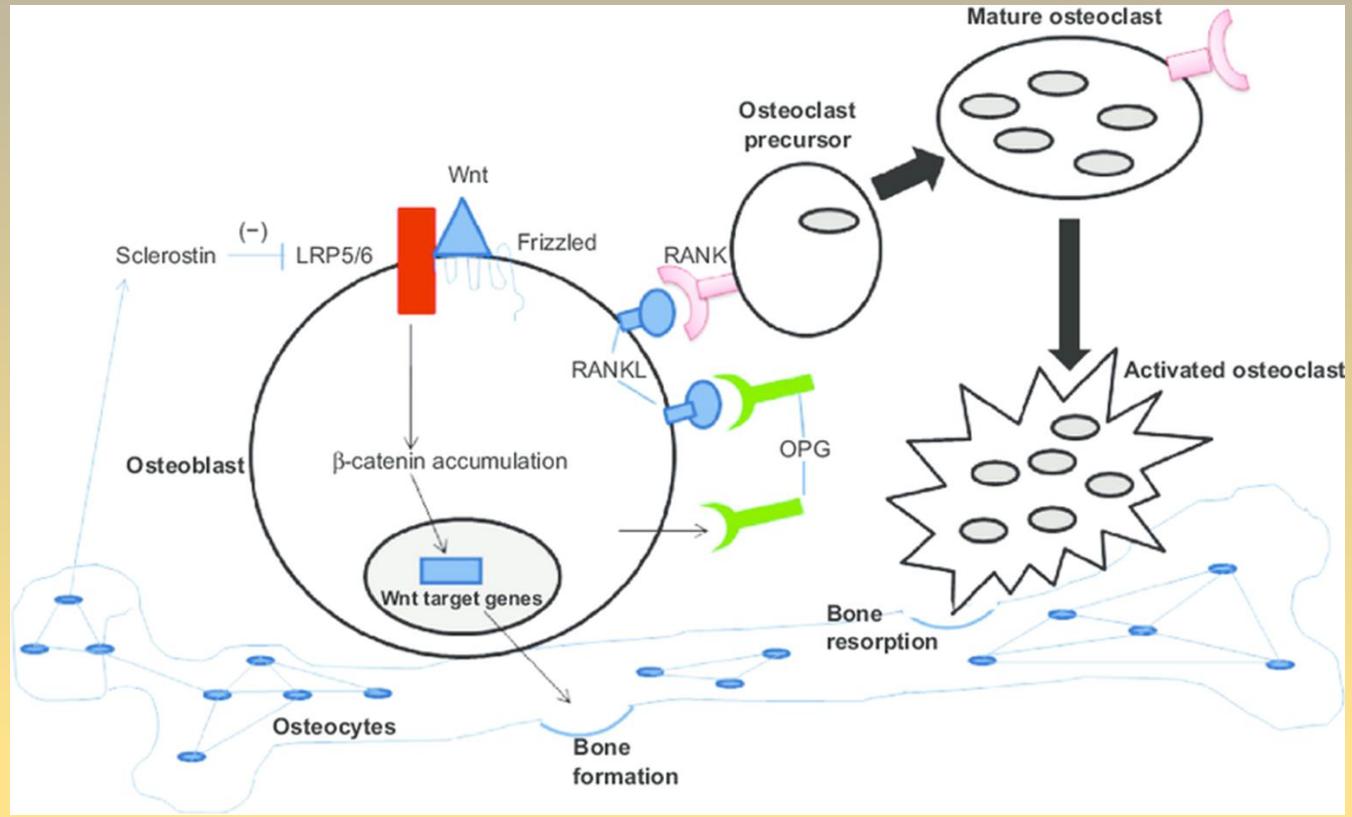
Bone mechanosensing





RANK/RANKL

Key factor regulating bone resorption is RANKL/OPG ratio.



Osteoclastogenesis
(+) RANKL
(-) OPG

Wnt is synthesized, subjected to Porc-mediated lipidation by palmitoleic acid, and is secreted from cells; Porc is an acyltransferase found in the endoplasmic reticulum. Wls is involved in the extracellular secretion of Wnt. Lipidation by palmitoleic acid is required for the binding of Wnt to Wls. Wls-deficient cells failed to secrete all Wnt ligands. Wnt ligands activate β-catenin-dependent canonical and -independent non-canonical signals. β-catenin-dependent canonical signal induces bone formation through promotion of osteoblastogenesis and OPG expression. β-catenin-independent non-canonical signals enhance LRP5/6 expression, thereby promoting osteoblast differentiation. OPG: osteoprotegerin, Porc: porcupine, Wls: wntless.

RANK/RANKL

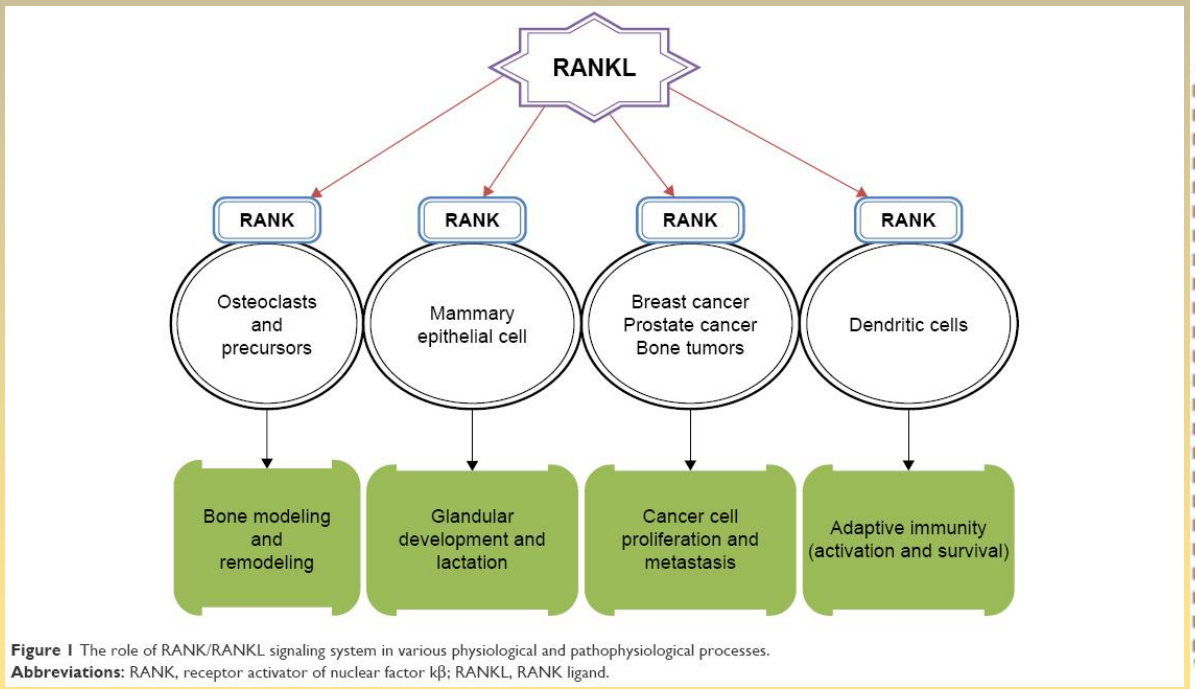
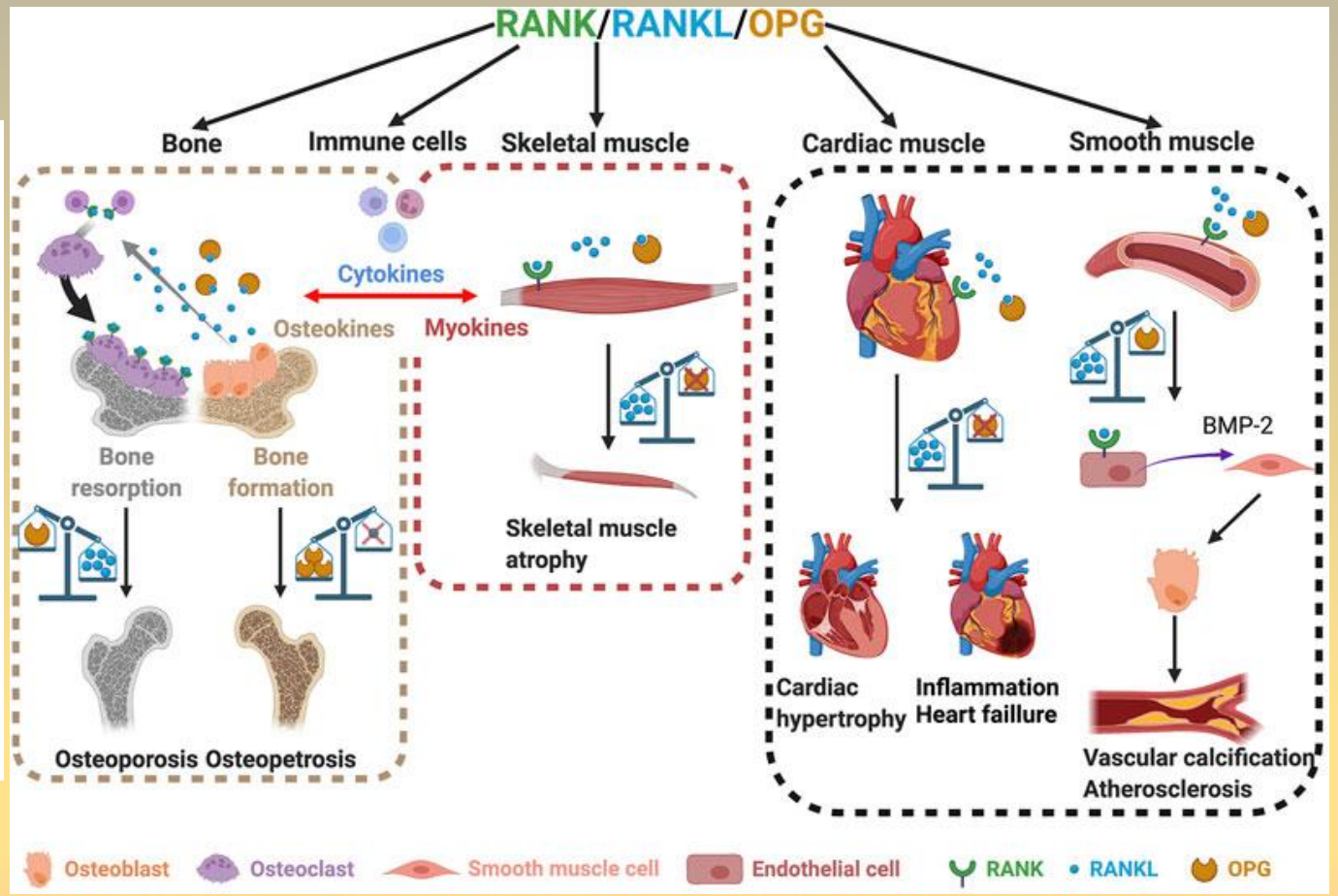
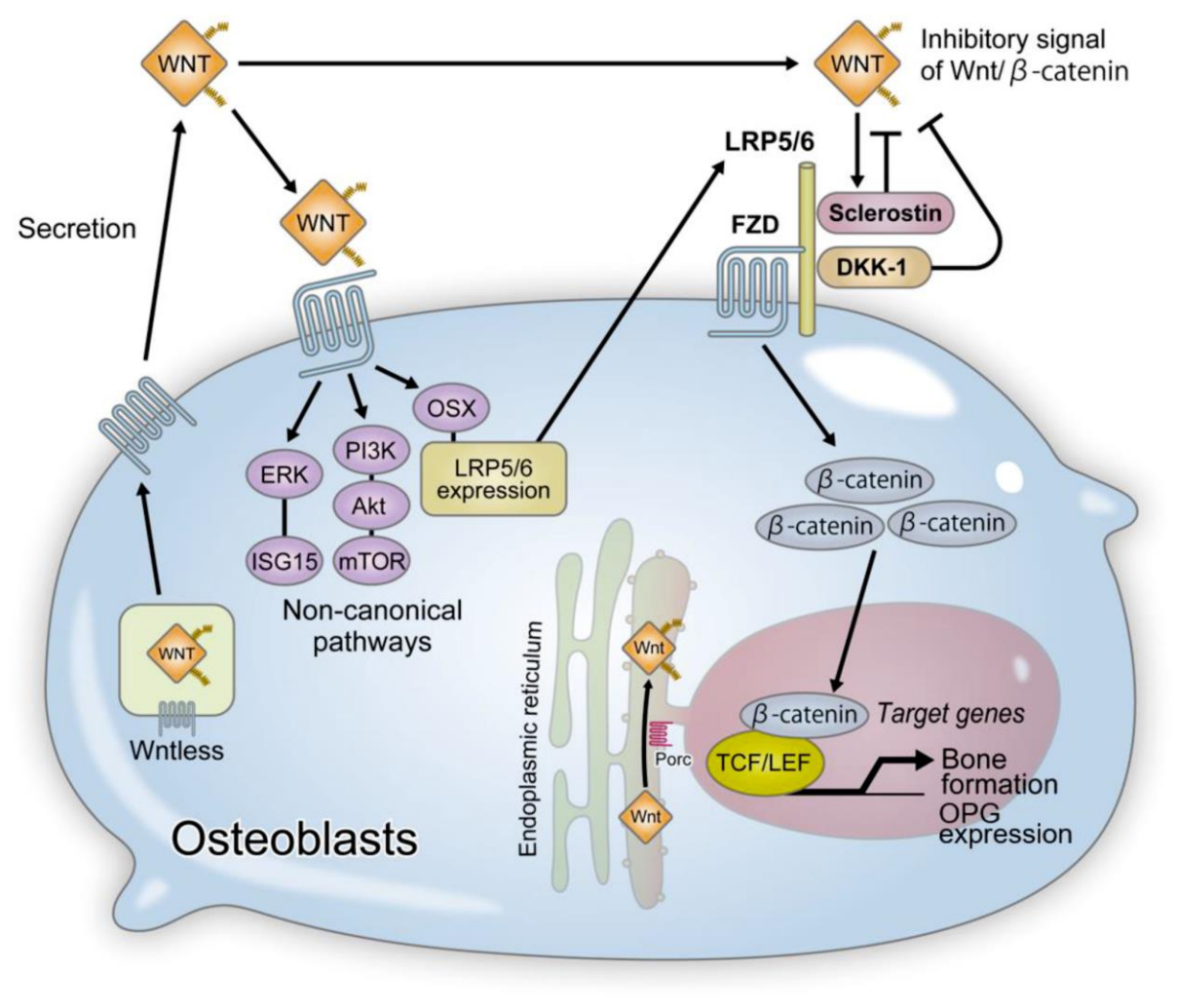
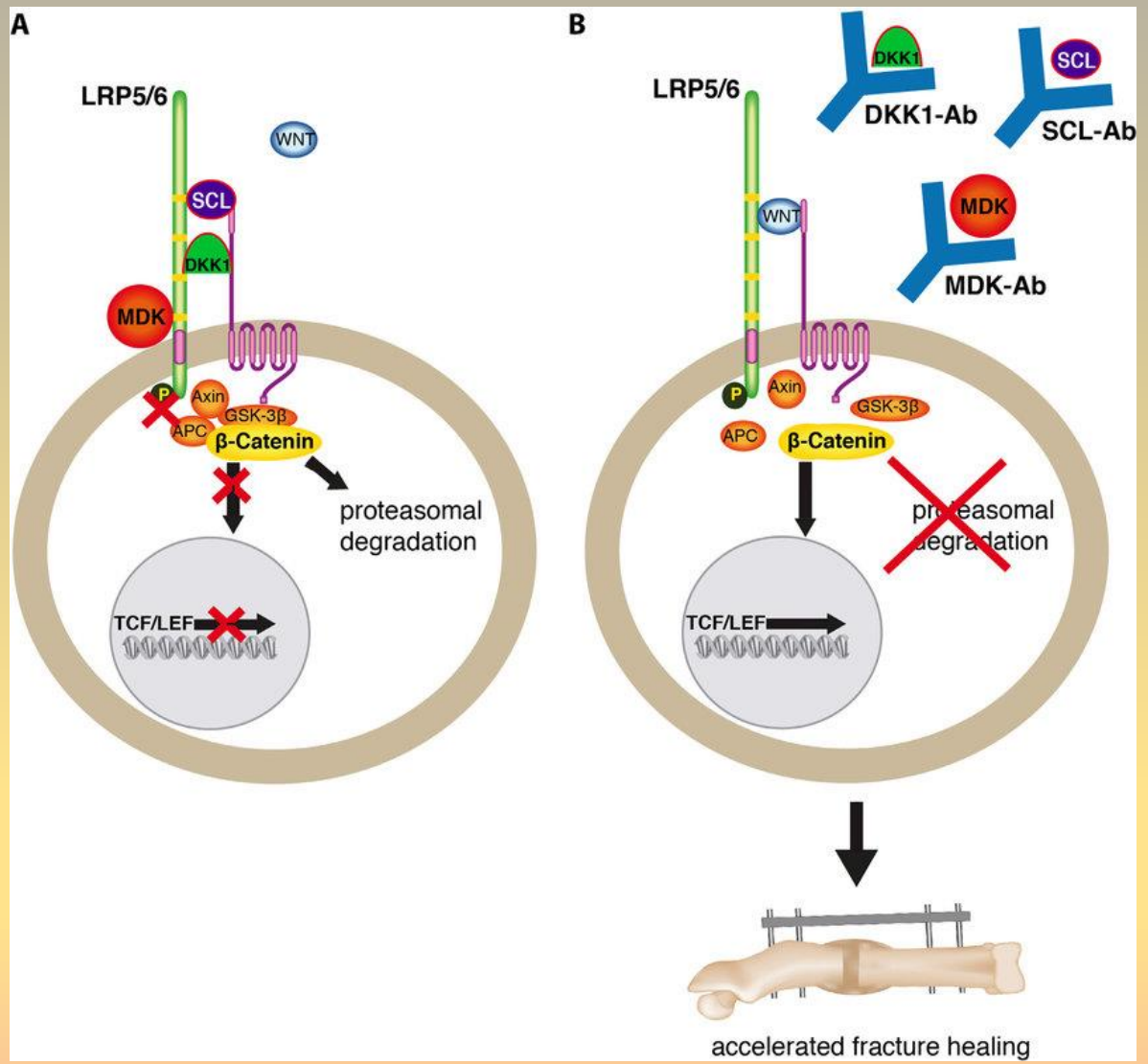


Figure 1 The role of RANK/RANKL signaling system in various physiological and pathophysiological processes.
Abbreviations: RANK, receptor activator of nuclear factor kβ; RANKL, RANK ligand.



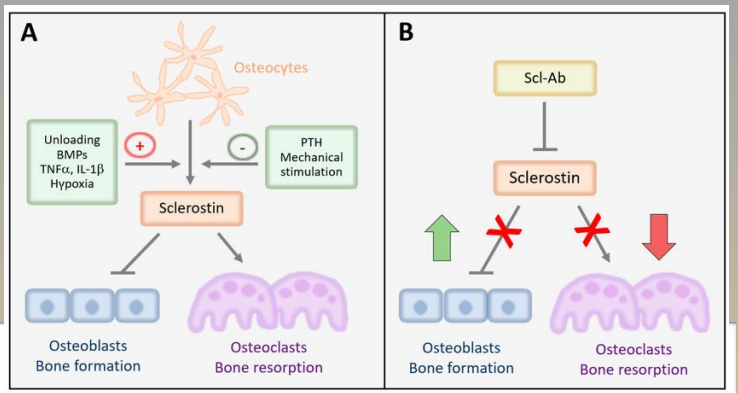
Wnt signaling pathway

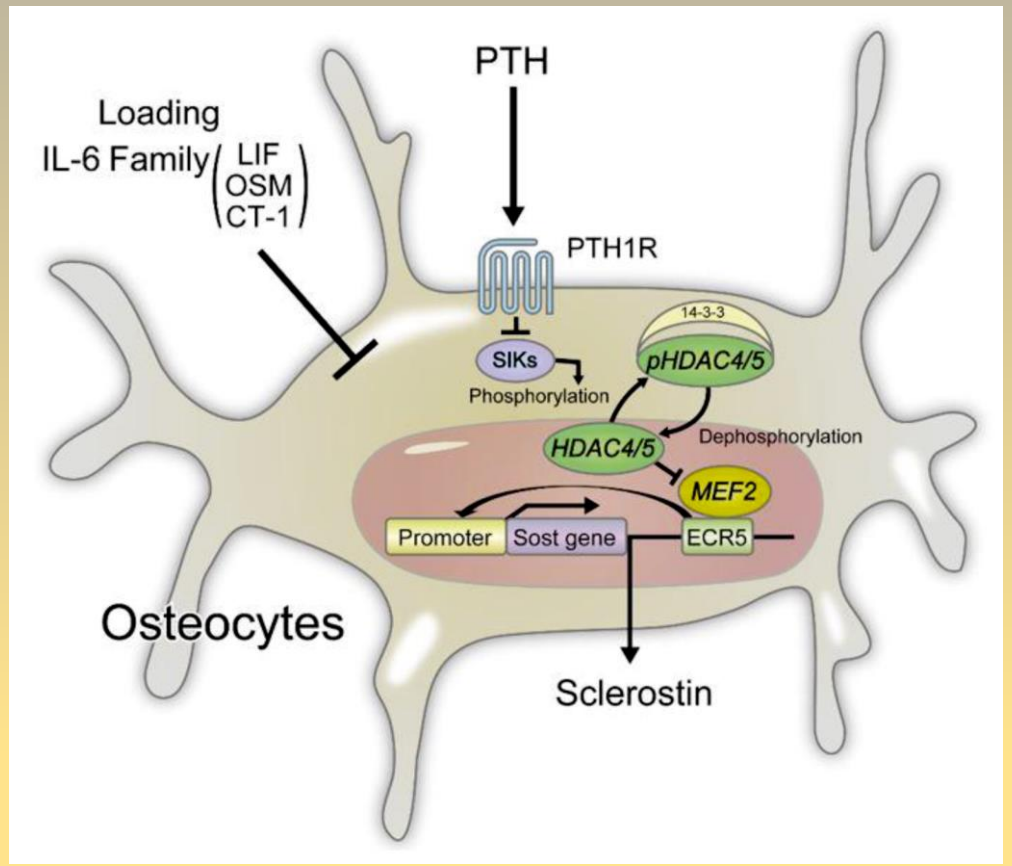


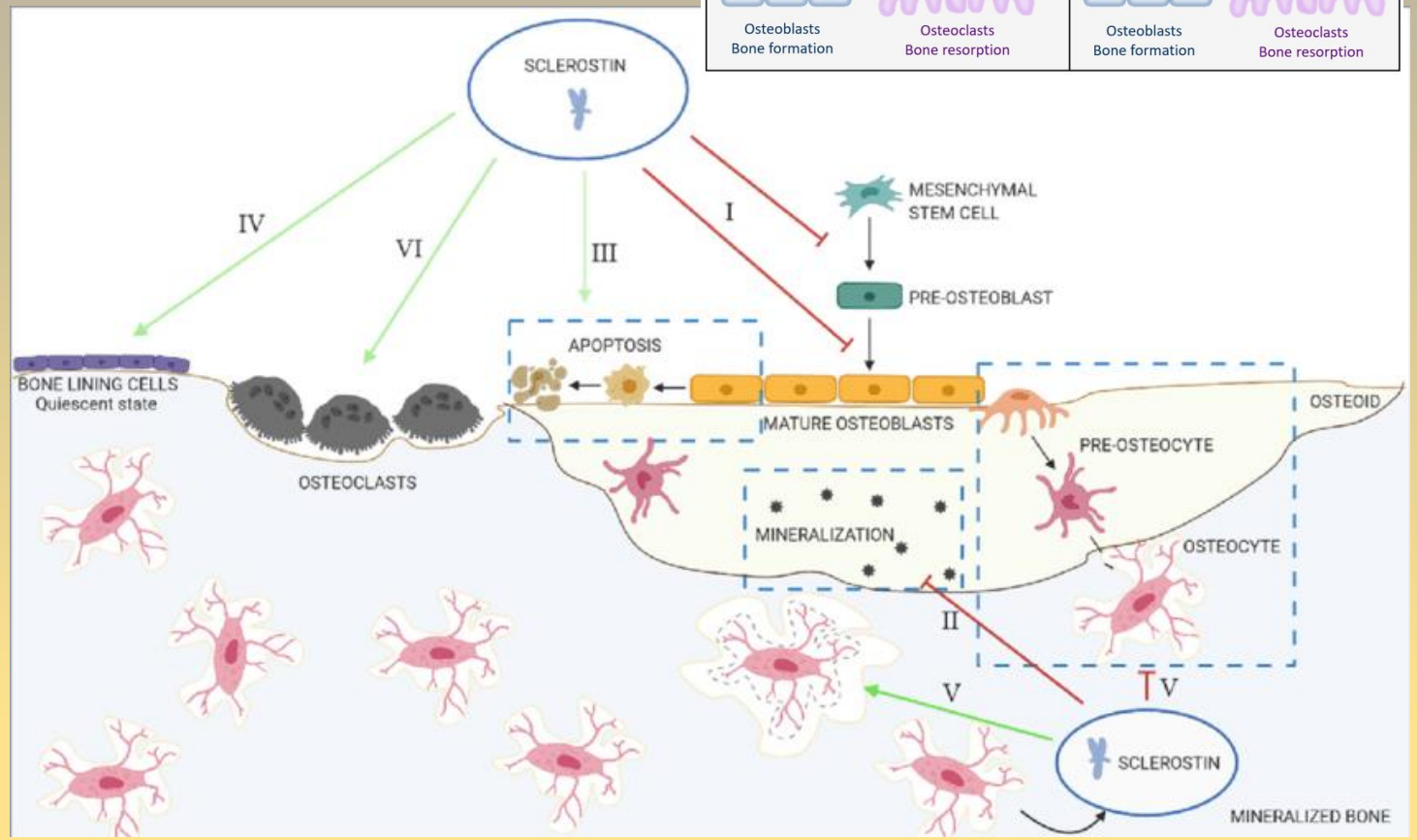


Haffner-Luntzer M: Experimental agents to improve fracture healing: utilizing the WNT signaling pathway. Injury 2020, 52.

Sclerostin

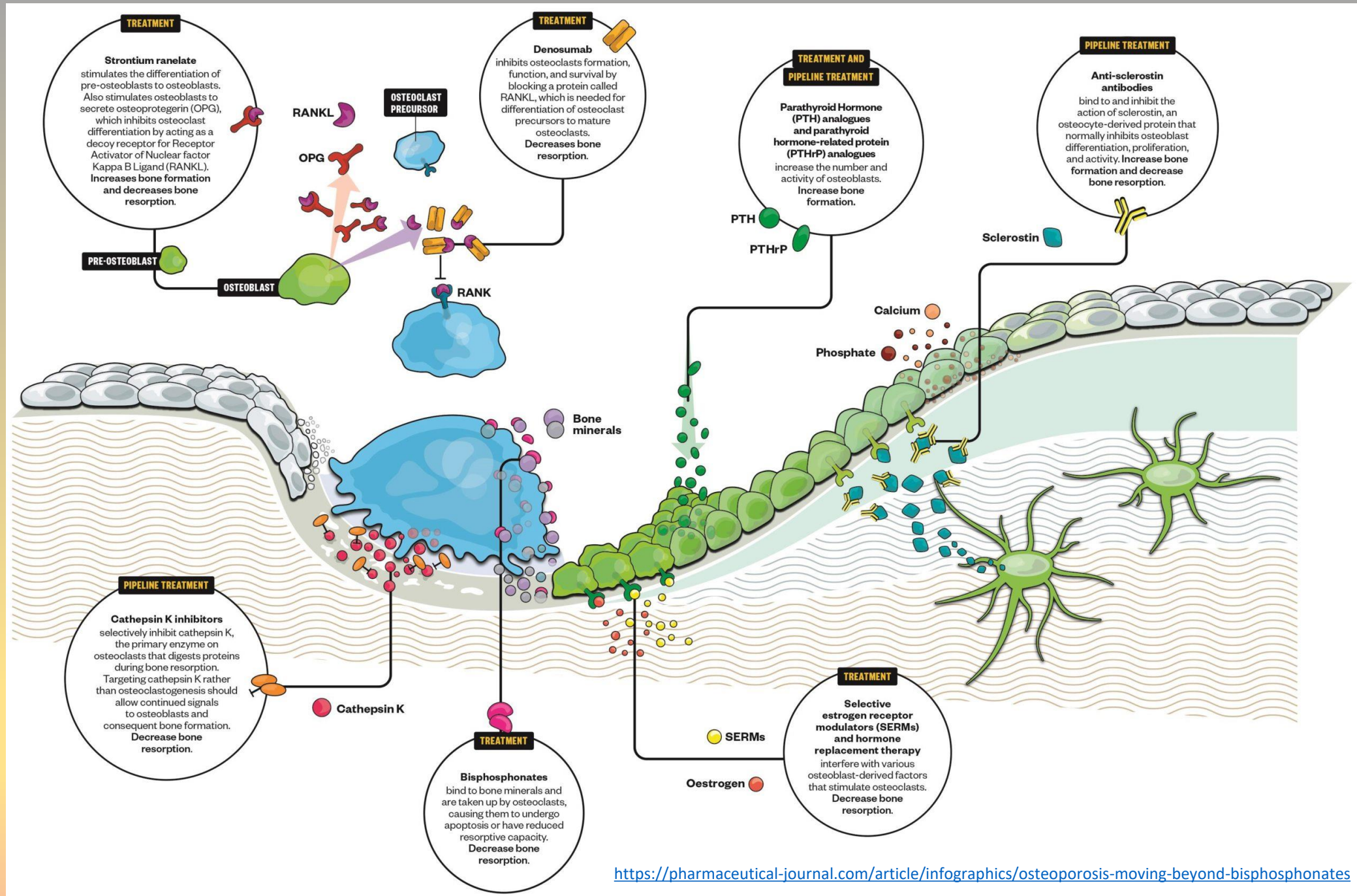






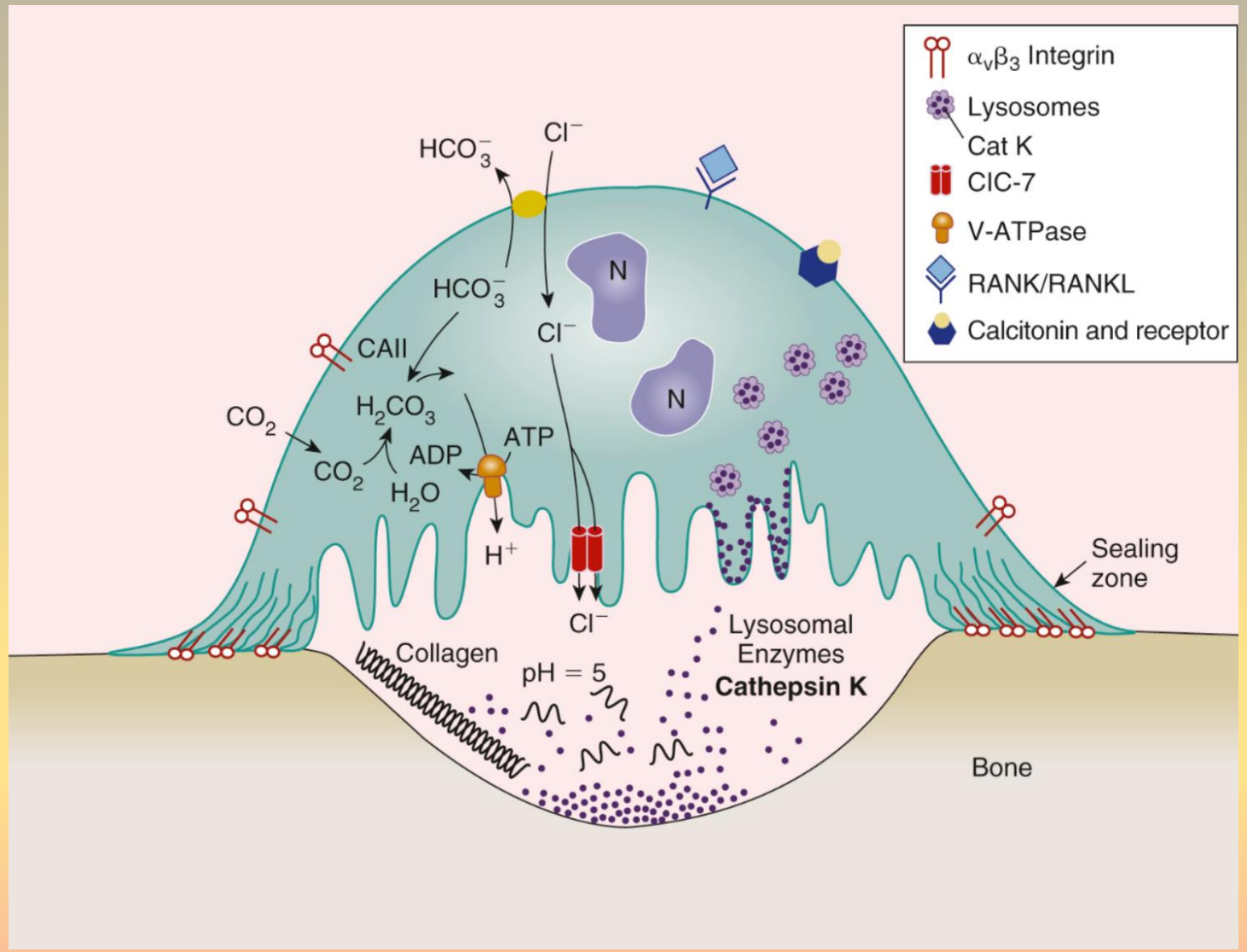
I: Inhibition of proliferation and differentiation of osteoprogenitor/pre-osteoblastic cells, as well as decreased activation of mature osteoblasts; II: decreased mineralization; III: increased apoptosis of the osteogenic cells; IV: maintenance of bone lining cells in their quiescent state; V: regulation of osteocyte maturation and osteocytic osteolysis; VI: stimulation of bone resorption.

Rauner M, Taipaleenmäki H, Tsourdi E, Winter L: **Osteoporosis Treatment with Anti-Sclerostin Antibodies—Mechanisms of Action and Clinical Application.** ***Journal of Clinical Medicine 2021, 10:787.***



https://pharmaceutical-journal.com/article/infographics/osteoporosis-moving-beyond-bisphosphonates

Bone tissue resorption by osteoclasts



Role of compartmentalization in bone resorption - *podosomes*

Resorption and secretion of bone resorption products - transcytosis

Essential role of pH for bone tissue resorption

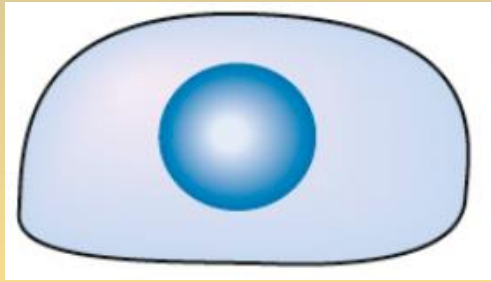
Factors affecting bone tissue remodeling

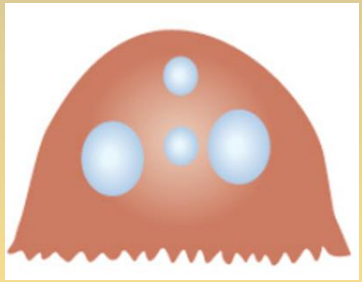
Resorption takes approx. 2 weeks
Mineralization and formation approx. 12 weeks

In pathophysiologic conditions is disrupted the continuity of bone tissue resorption and formation.

Systemic signals + Local signals

Remodeling of bone tissue





Osteoblasts
Lining cells

Osteoclasts

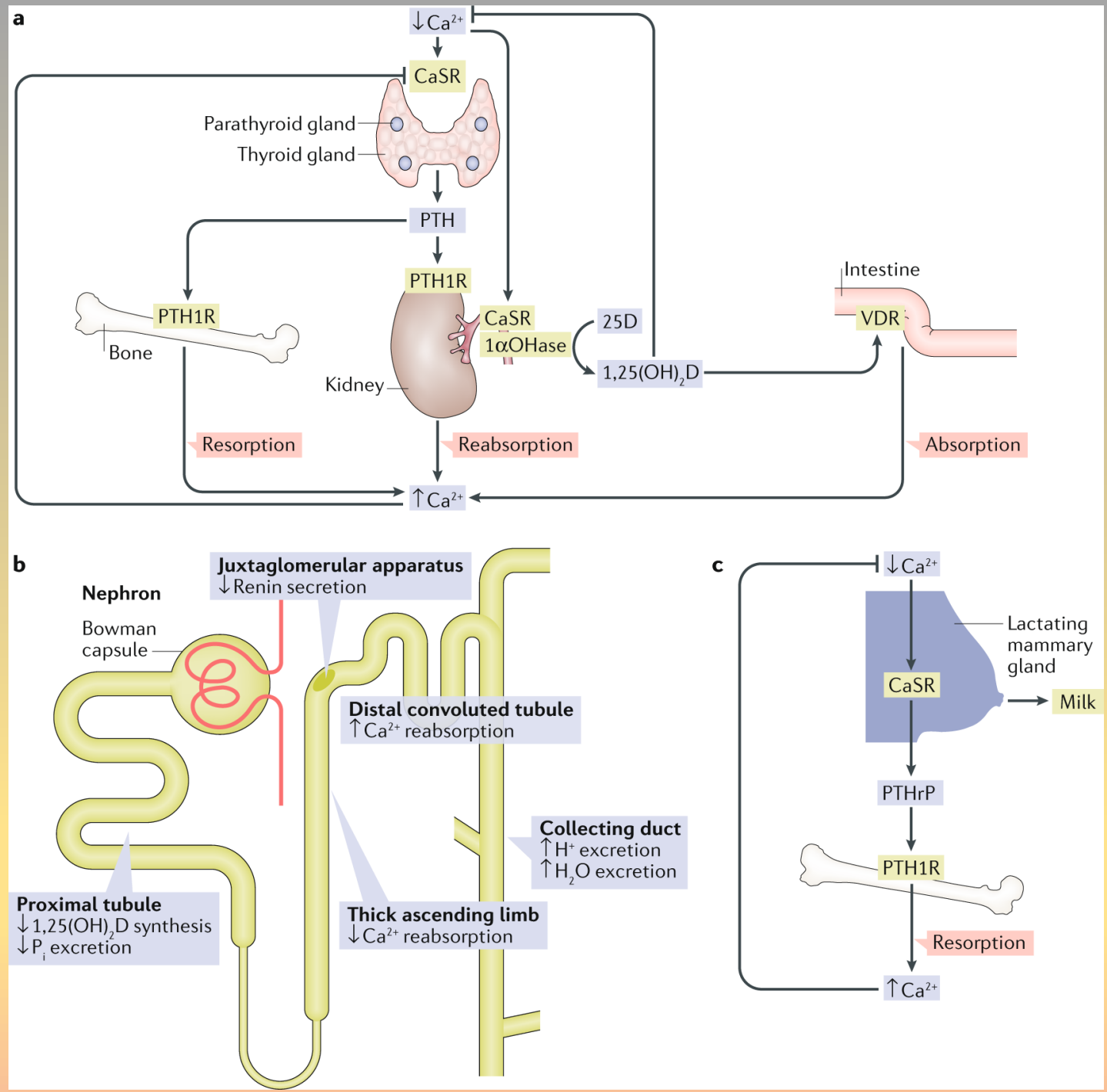
Trabecular bone

−	+
Cytokines - IL-1α, IL-1β, TNF-α, TNF-β, proinflammatory IL (7, 15, 17)	Cytokines - IL-4, IL-13, IL-10, IL-18
TGF-α and EGF, FGF21, FGF23	Prostaglandins
Prostaglandins	VEGFA, HIF-1α (+/-)
PDGF	IGF-1 (endo-/paracrine)
	BMPs (OB, autocrine)

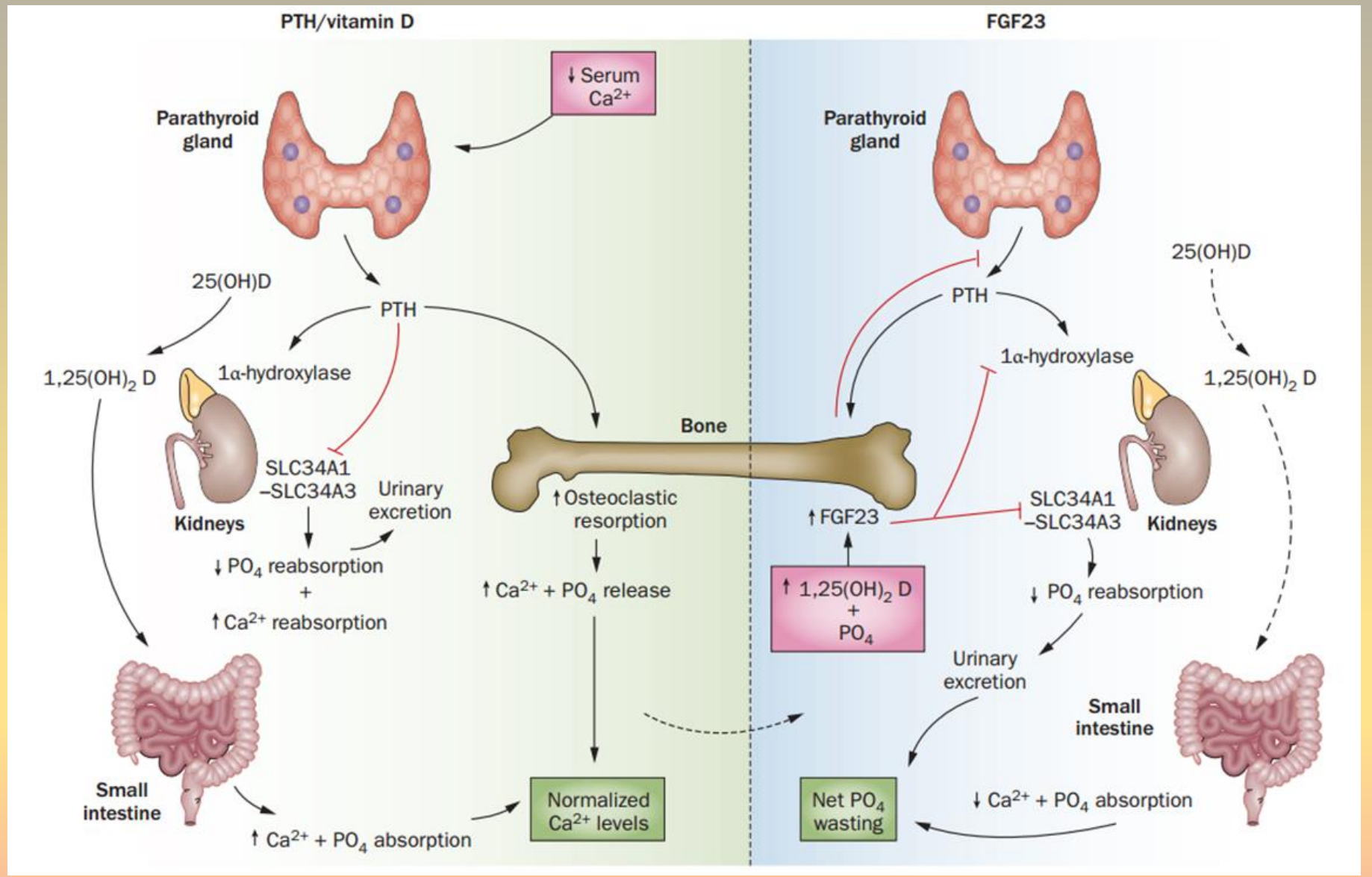
Immediate calcium need - homeostasis

Ensuring mechanical requirements

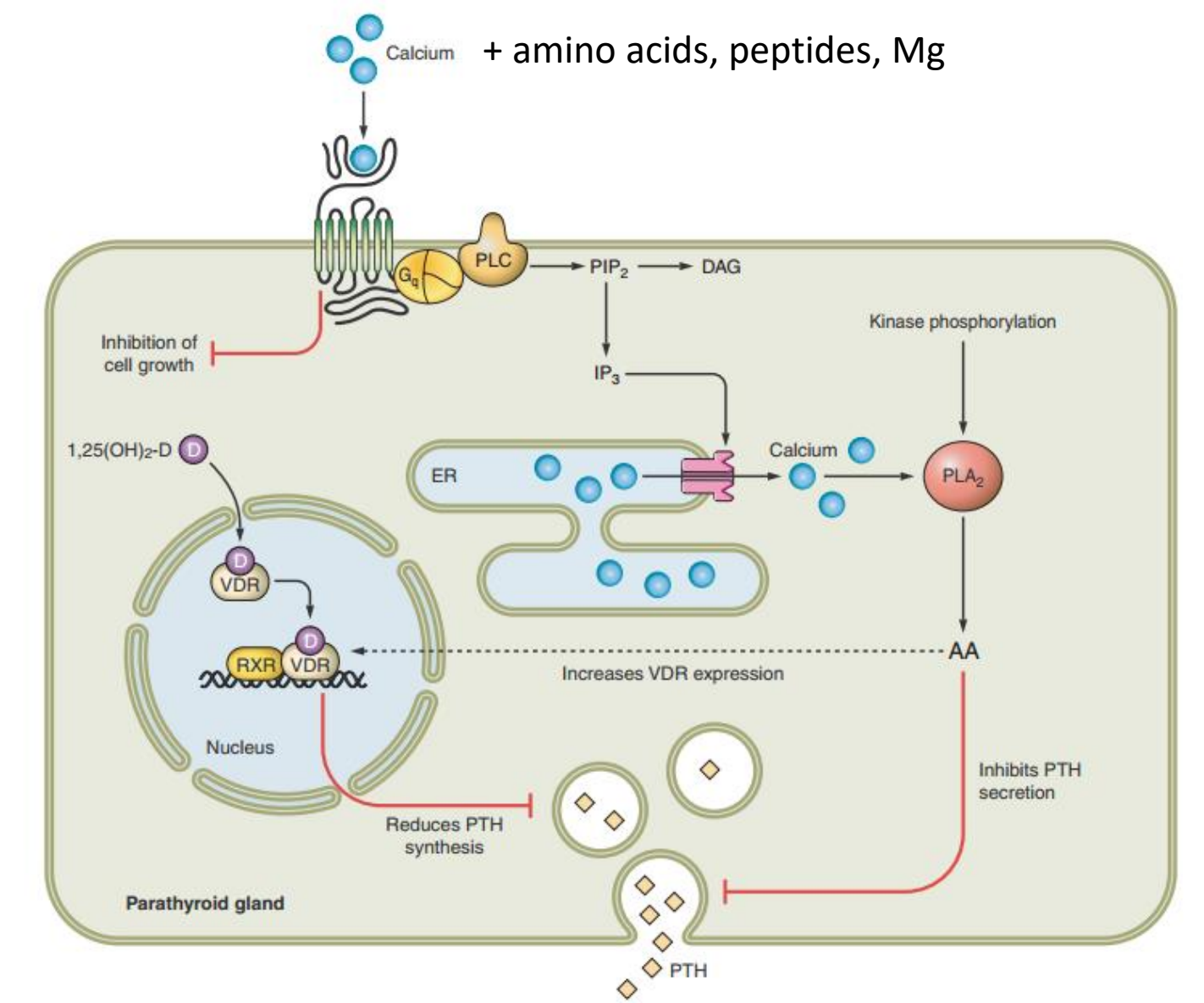
Calcium sensing receptors (CASRs)



Endocrine regulation of bone tissue – PTH,vitamin D, FGF23



Calcium sensing receptors - CaSR - and PTH secretion



CaSR – G-protein coupled receptor
- Activation of PLC
- Inhibition of cAMP production

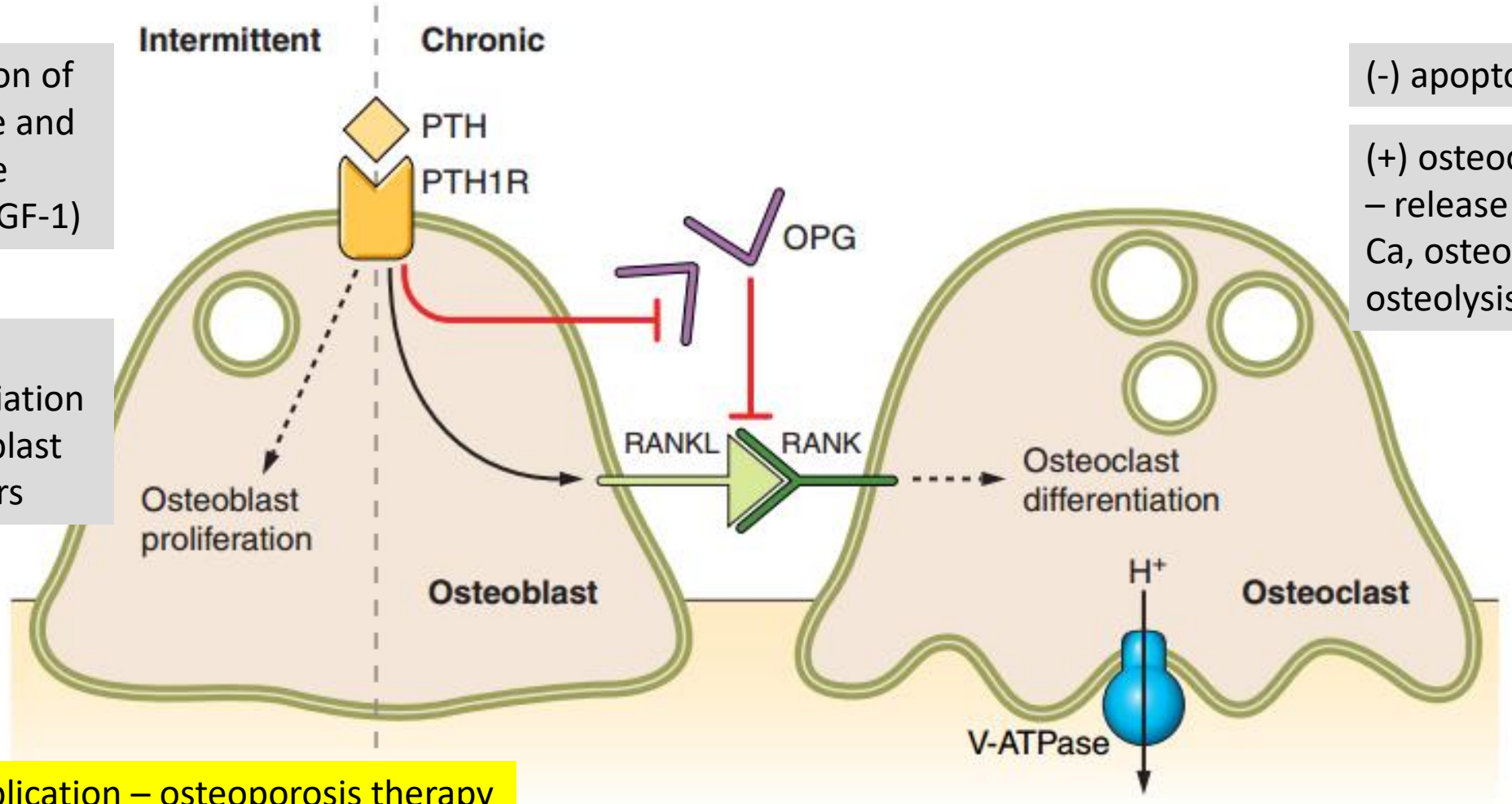
Various distribution in tissues – all tissues participating in calcium homeostasis
- Parathyroid glands
- Kidneys
- Skin
- GIT epithelium, enterocytes
- G cells of stomach
- CNS

Clinical aspects
- Mutation – inactivation/activation
- familial hypocalciuric hypercalcemia (in.)
- Familial hypoparathyroidism with hypercalciuria (ac.)
- Calcimimetics – inhibition of PTH secretion

PTH and bone tissue physiology

+ secretion of paracrine and autocrine factors (IGF-1)

+ differentiation of osteoblast precursors



(-) apoptosis

(+) osteocytes – release of Ca, osteocytic osteolysis

Clinical application – osteoporosis therapy

Effect of PTH on osteoclasts is indirect. Pulsatile secretion stimulates osteoblasts, chronic continual osteoclasts.

PTHrP

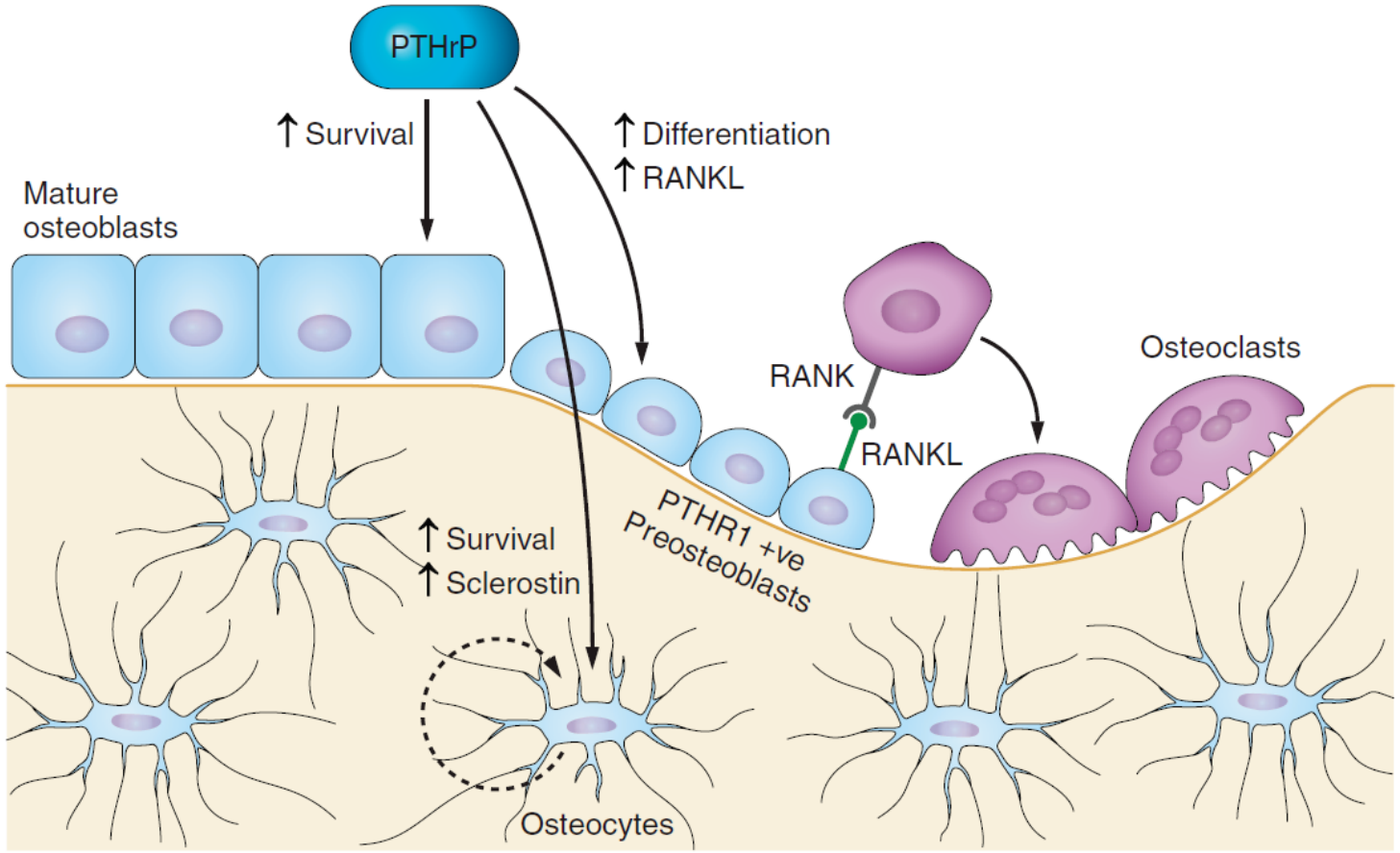


FIGURE 12. Paracrine actions of PTHrP in bone remodeling. PTHrP produced by cells early in the osteoblast lineage acts on cells of the lineage that have differentiated to the stage of possessing the PTH1R, promoting their differentiation and therefore bone formation, as well as increasing production of RANKL and osteoclast formation. PTHrP also inhibits apoptosis of mature osteoblasts, of earlier cells, and of osteocytes (see text for details).

Calcitonin

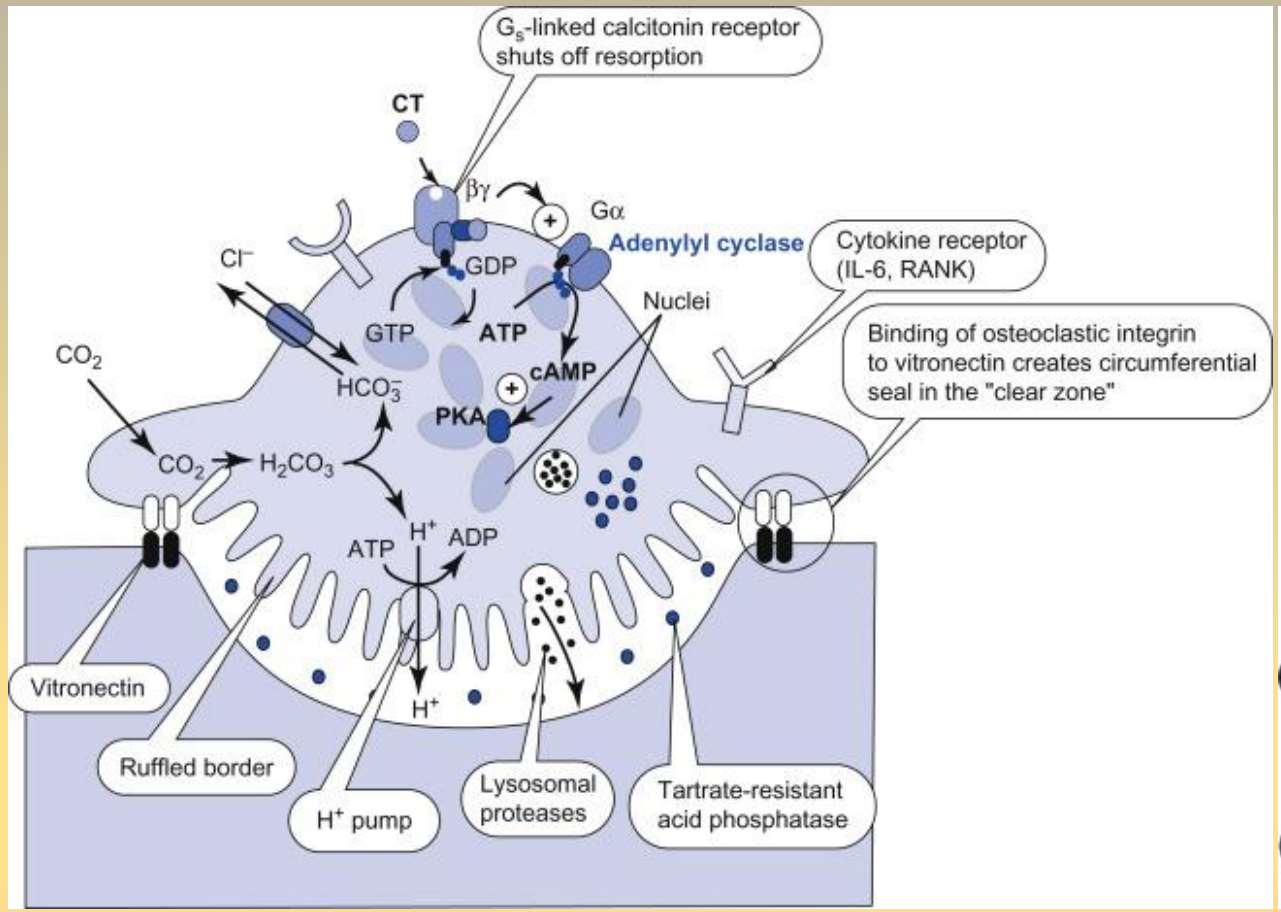
Characteristics
- C cells of thyroid gland
- Family of peptides (amylin, CGRPs, adrenomedulin)
- Different distribution in various tissues
- Secretion is determined by level of ionized calcium (CaSR)
- Stimulation of secretion:
  - Glucocorticoids
  - CGRP
  - Glucagon
  - Enteroglucagon
  - Gastrin
  - Pentagastrin
  - Pancreozymin
  - β-sympatomimetics

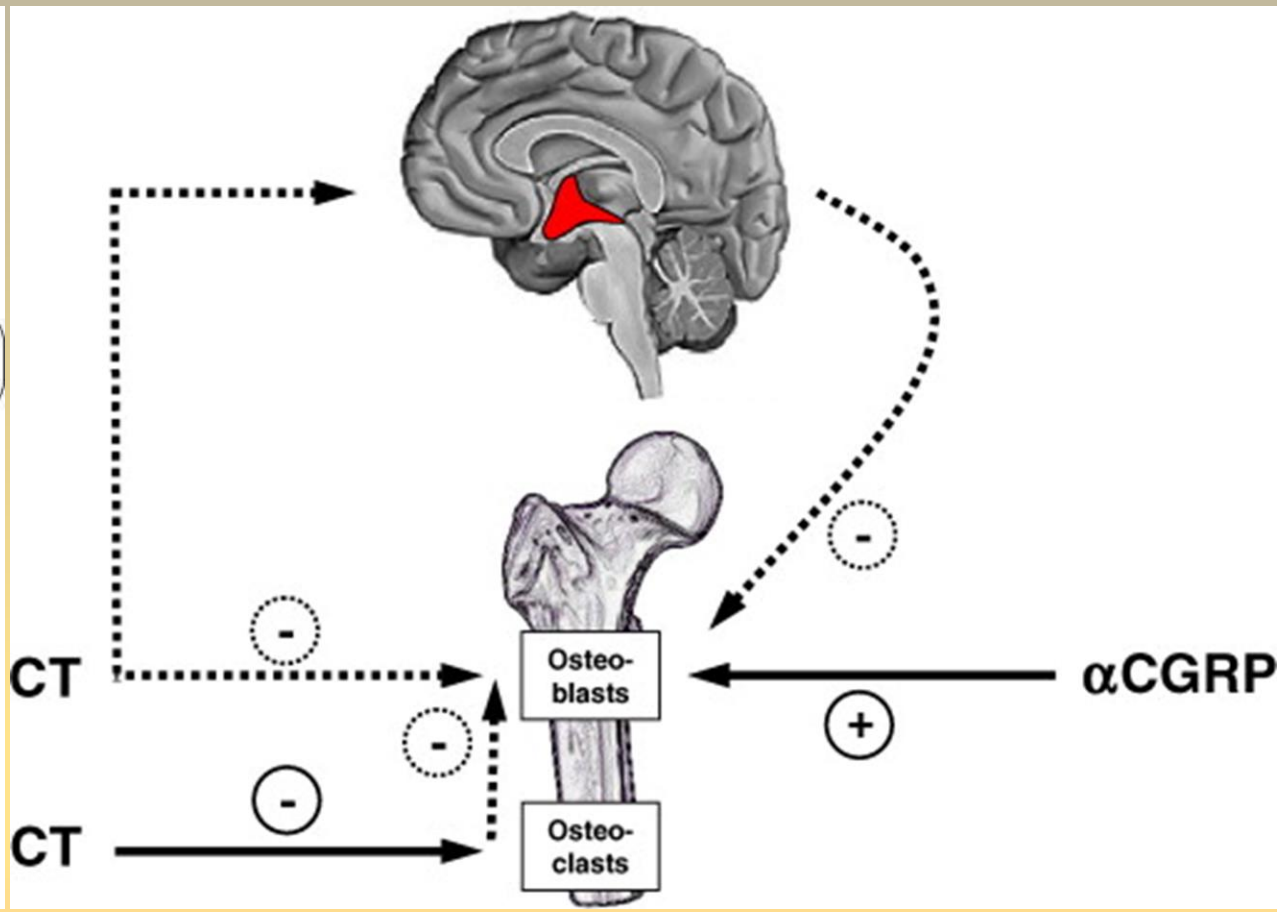
  } Function unclear
- Inhibition of secretion - somatostatin

Functions
- Bone tissue
  - Inhibition of osteoclast motility and differentiation
  - Inhibition of osteoclast secretion
  - ATPase inhibition
- Kidneys
  - Increased excretion of Ca – inhibition of resorption (Ca^{2+} ion channels – LS, Na^{+}/Ca^{2+} - BM)
- Skeleton development?
- Skeleton protection during pregnancy?

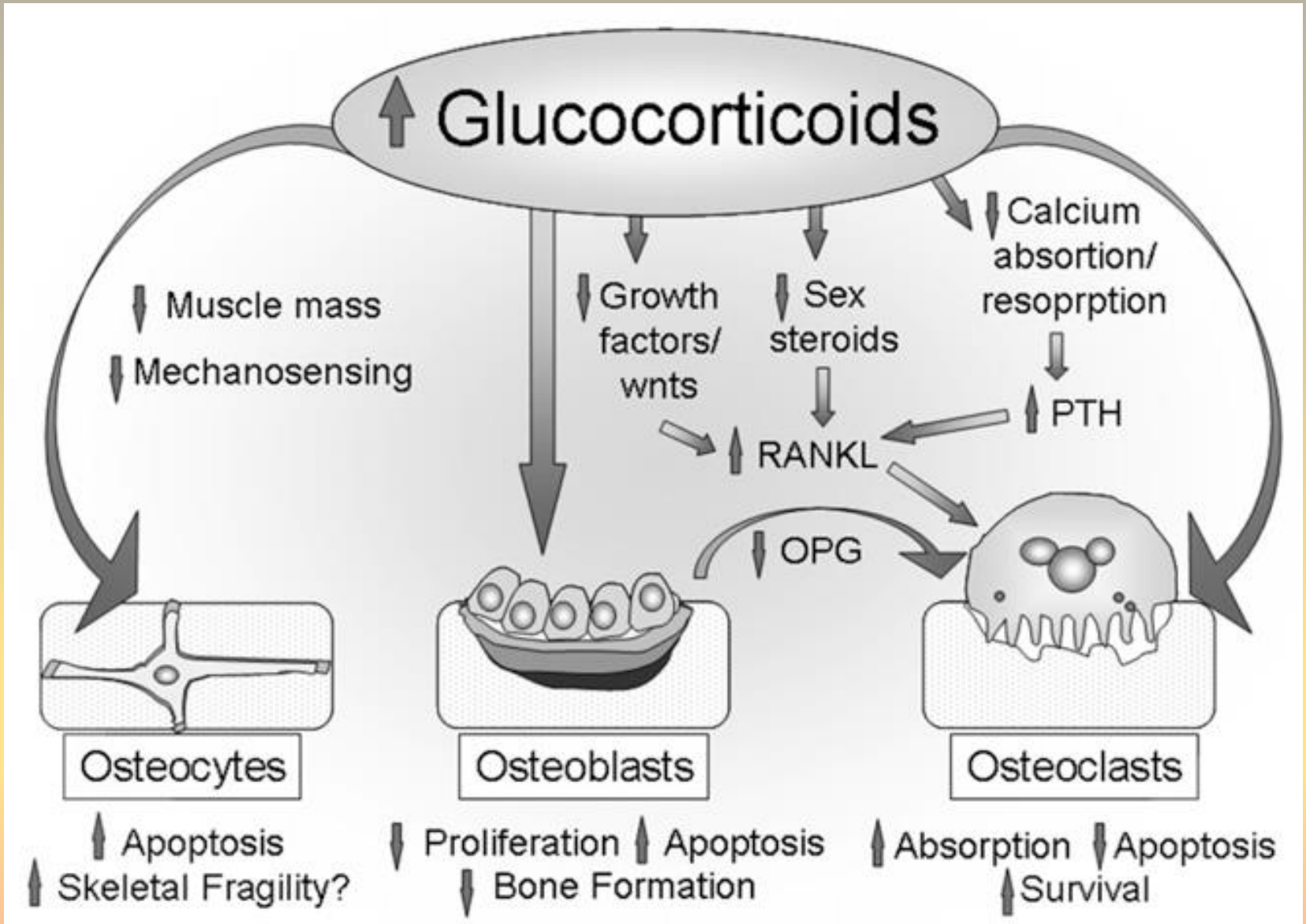
Clinical relevance
- Osteoporosis therapy
- Paget disease therapy
- Treatment of pain (bones metastases)
- ! Increased risk of cancer

Calcitonin and α-CGRP

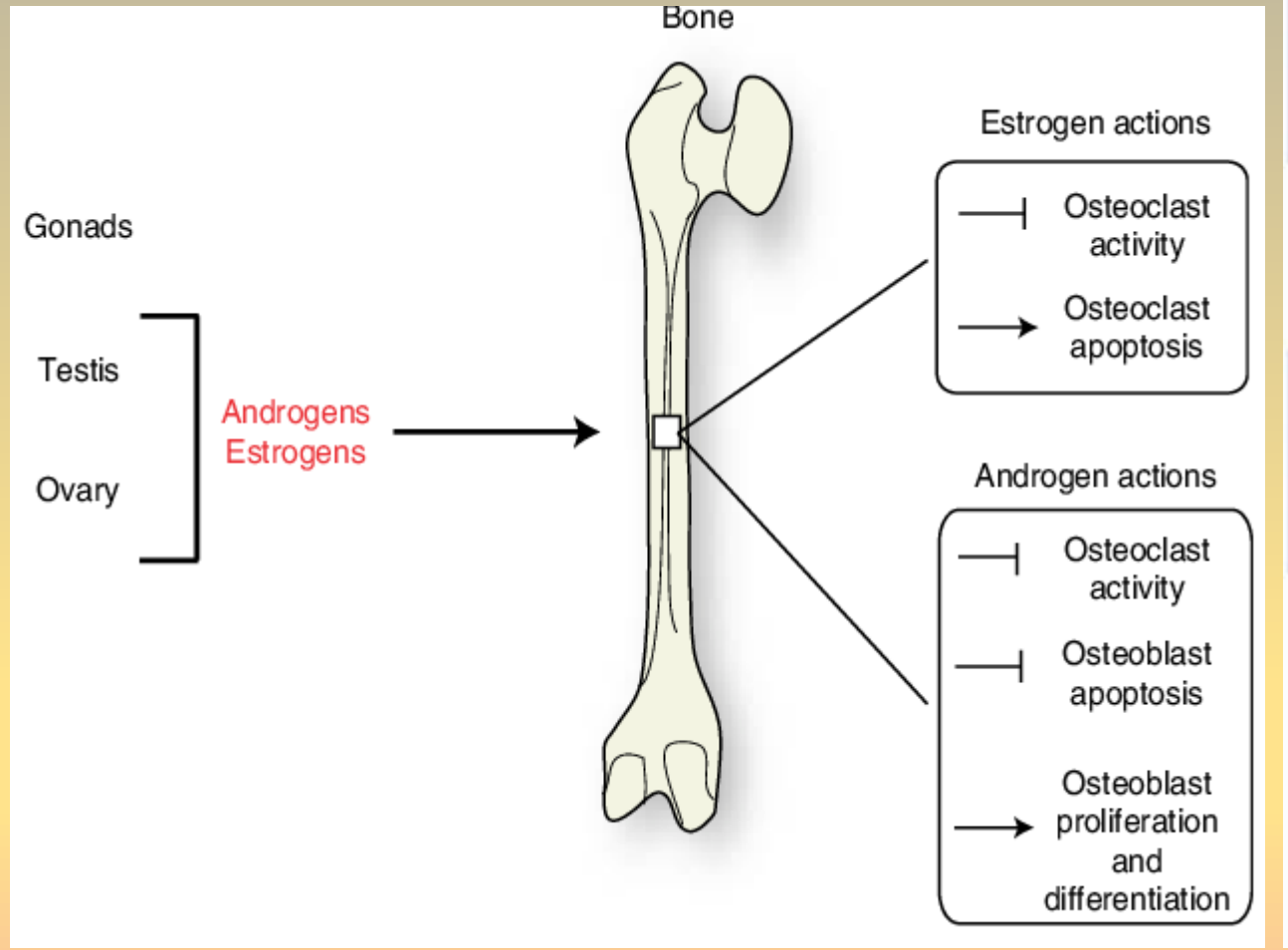


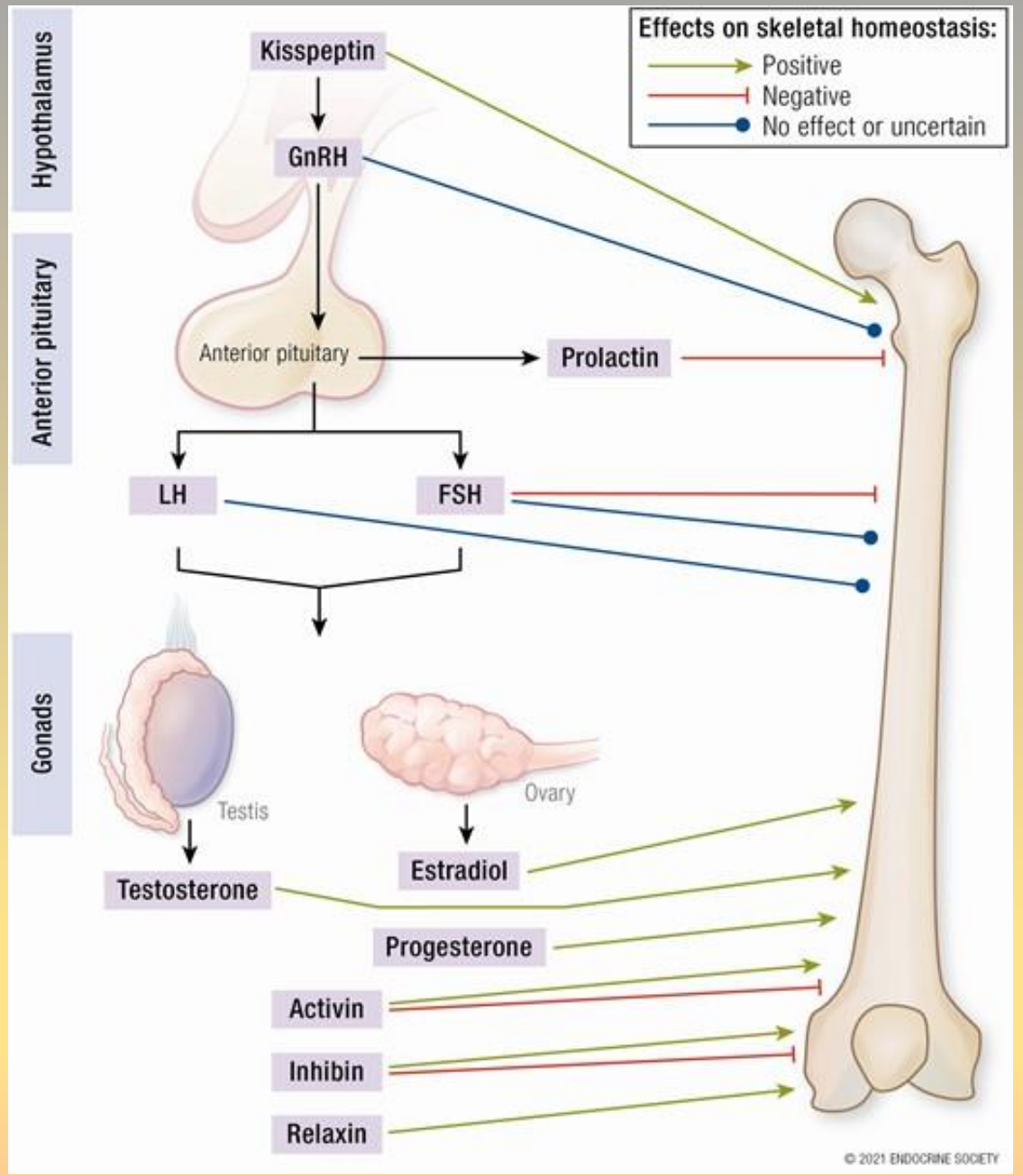


Glucocorticoids

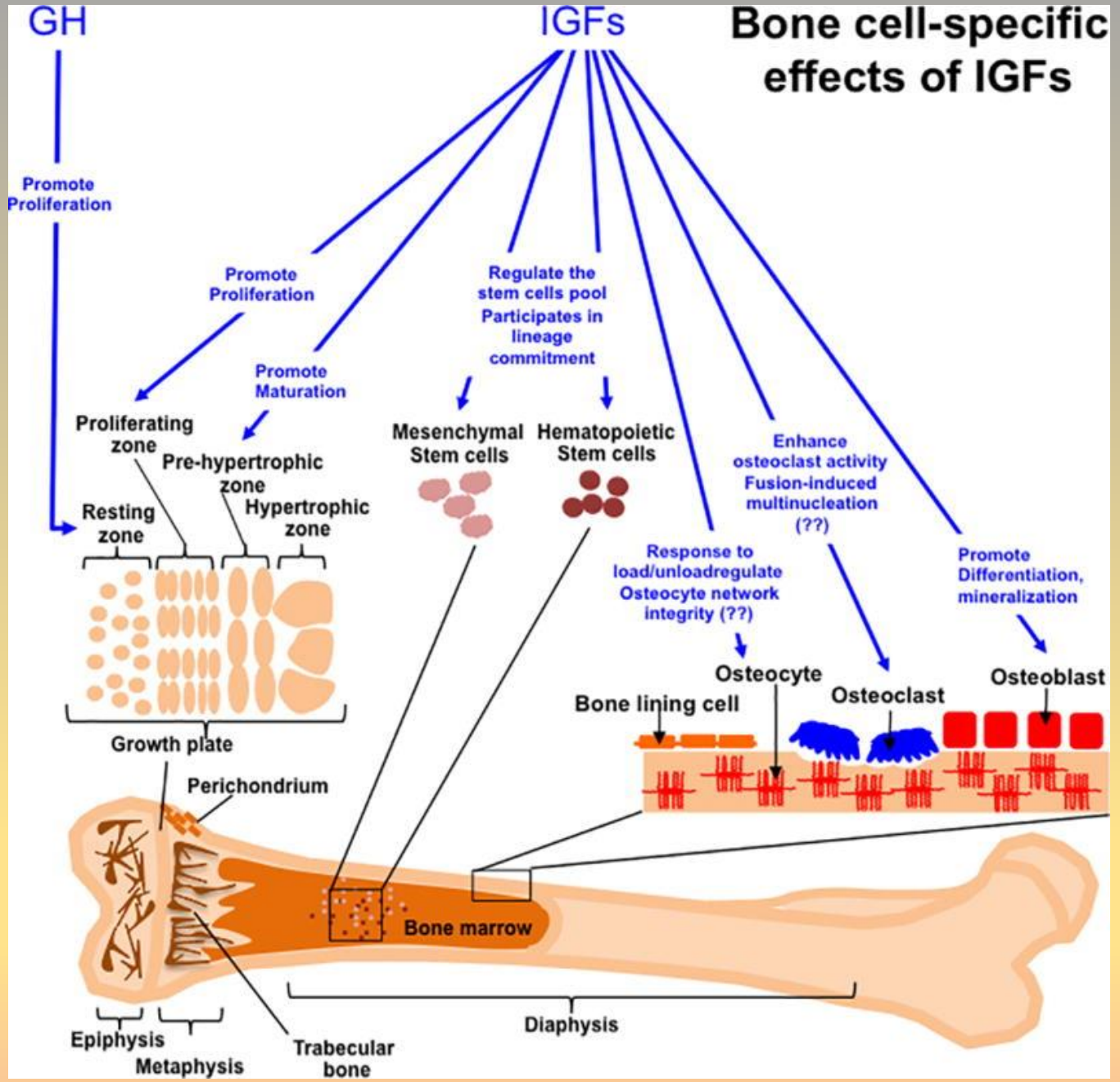


Sex hormones





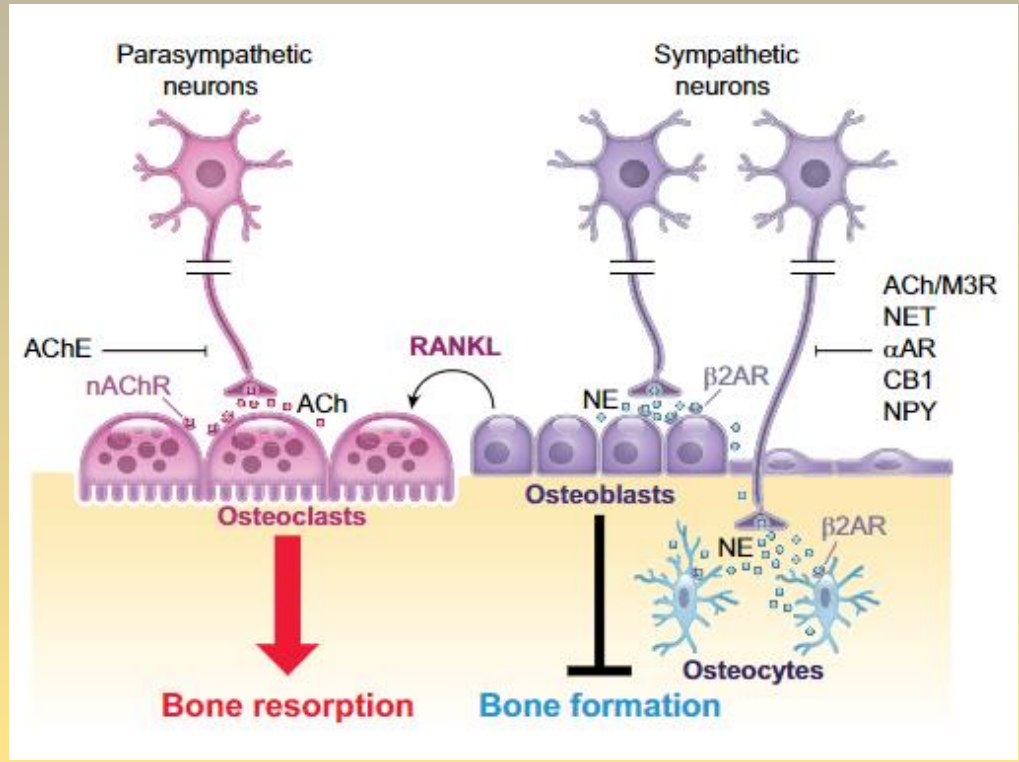
GH, IGF-1


GH
IGFs
Bone cell-specific effects of IGFs
Promote Proliferation
Promote Proliferation
Promote Maturation
Regulate the stem cells pool
Participates in lineage commitment
Proliferating zone
Pre-hypertrophic zone
Hypertrophic zone
Resting zone
Mesenchymal Stem cells
Hematopoietic Stem cells
Enhance osteoclast activity
Fusion-induced multinucleation (??)
Response to load/unloadregulate
Osteocyte network integrity (??)
Promote Differentiation, mineralization
Osteocyte
Bone lining cell
Osteoclast
Osteoblast
Growth plate
Perichondrium
Bone marrow
Epiphysis
Metaphysis
Trabecular bone
Diaphysis

ANS and bone physiology

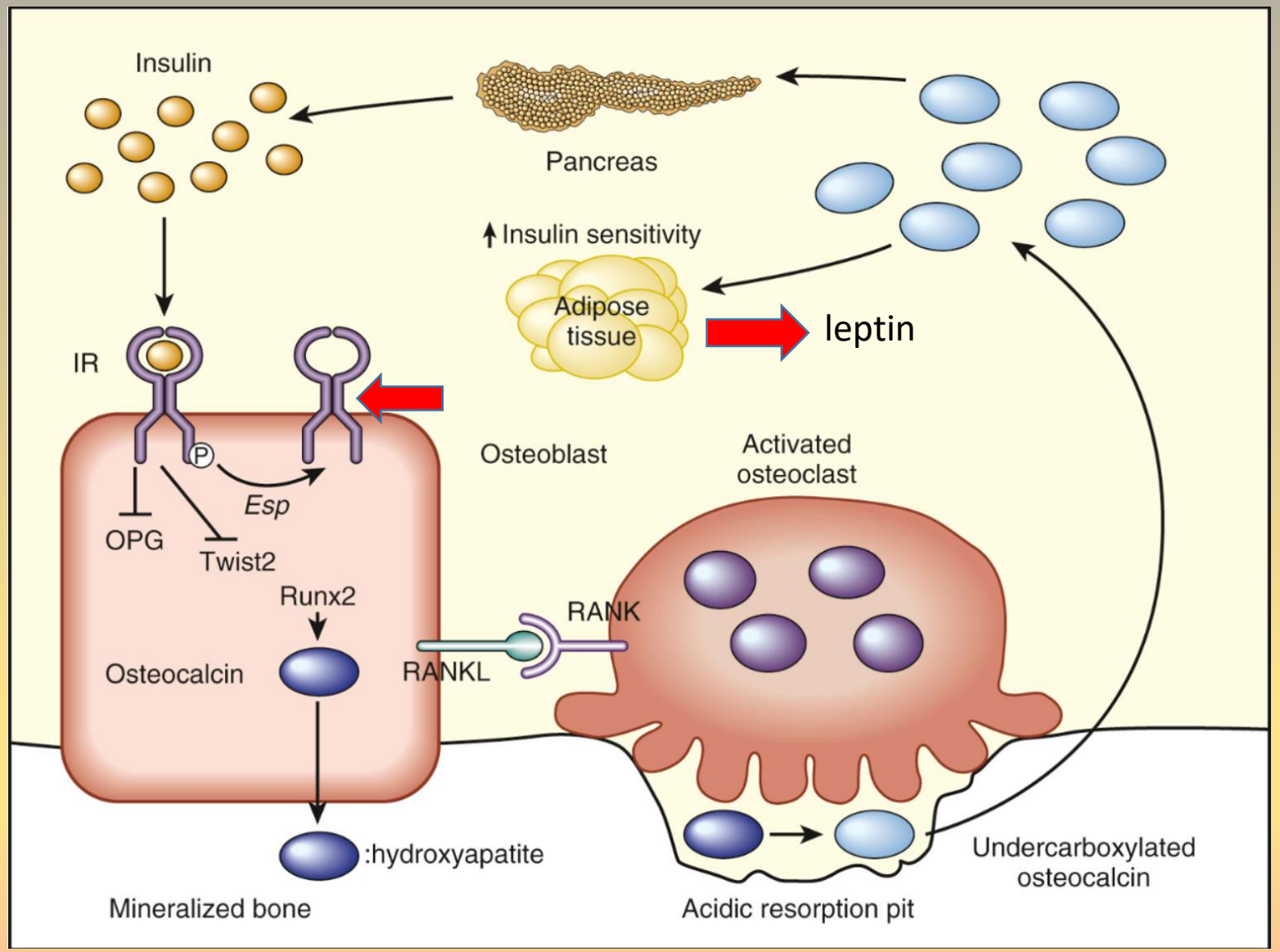
Table I. *Main adrenergic receptor and enzyme mRNAs expressed in bone cells*

Cell Lineage	Cells Investigated	Receptor/Enzyme
Osteoclasts	Differentiated bone marrow macrophages and Raw 264.7 cells	β2AR
		β2AR
Chondrocytes	Mouse growth plate chondrocytes (IHC)	α_{2A}AR and α_{2C}AR
Immature osteoblasts (undifferentiated)	Rat bone marrow mesenchymal cells	α_1AR, α_{1B}AR, $\alpha 1_D$AR
	Mouse sarcoma C3H10T1/2 cells	α_{1A}AR
	Mouse MC3T3 cells	α_{1A}AR and α_{1D}AR
	Mouse MC3T3 cells	β2AR, α_{2A}AR
	Human fetal long bone-derived osteoblasts	α_{1B}AR and β2AR
	Human osteosarcoma MG63 cells	β2AR
	Human osteosarcoma SaOS2 cells	β2AR, β1AR
	Human osteosarcoma TE-85 cells	β2AR, β1AR
	Human osteosarcoma OSH-4 cells	β1AR
	Rat ROS 17/2.8 cells	β2AR
	Human periosteum-derived osteoblastic SaM-1 cells	β2AR
	Human osteosarcoma HOS cells	β2AR
	Mouse calvarial osteoblasts	β2AR
	Mouse bone marrow stromal cells	α_{1D}AR
Differentiated osteoblasts	Mouse bone marrow stromal cells	α_{1A}AR and α_{1D}AR
Osteocytes	Mouse IHC	β2AR



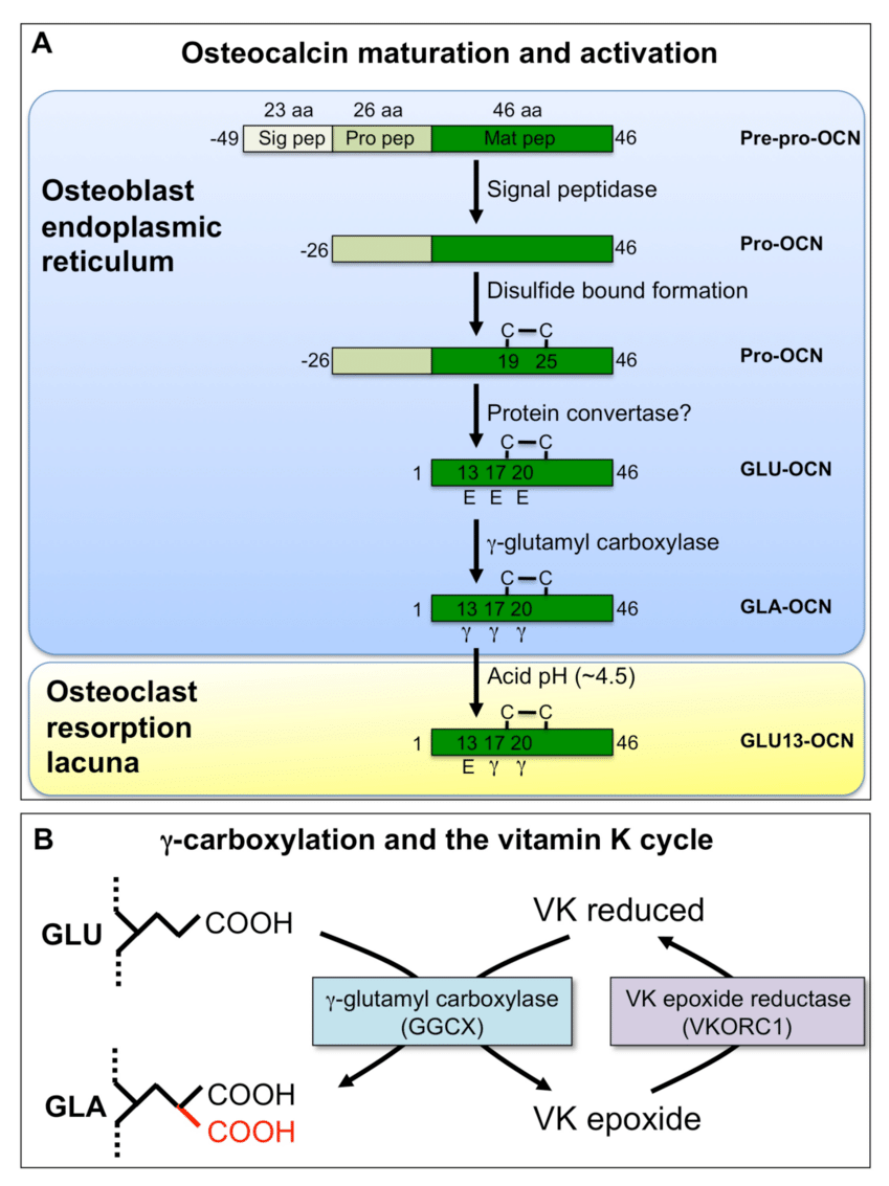
Bone as endocrine organ

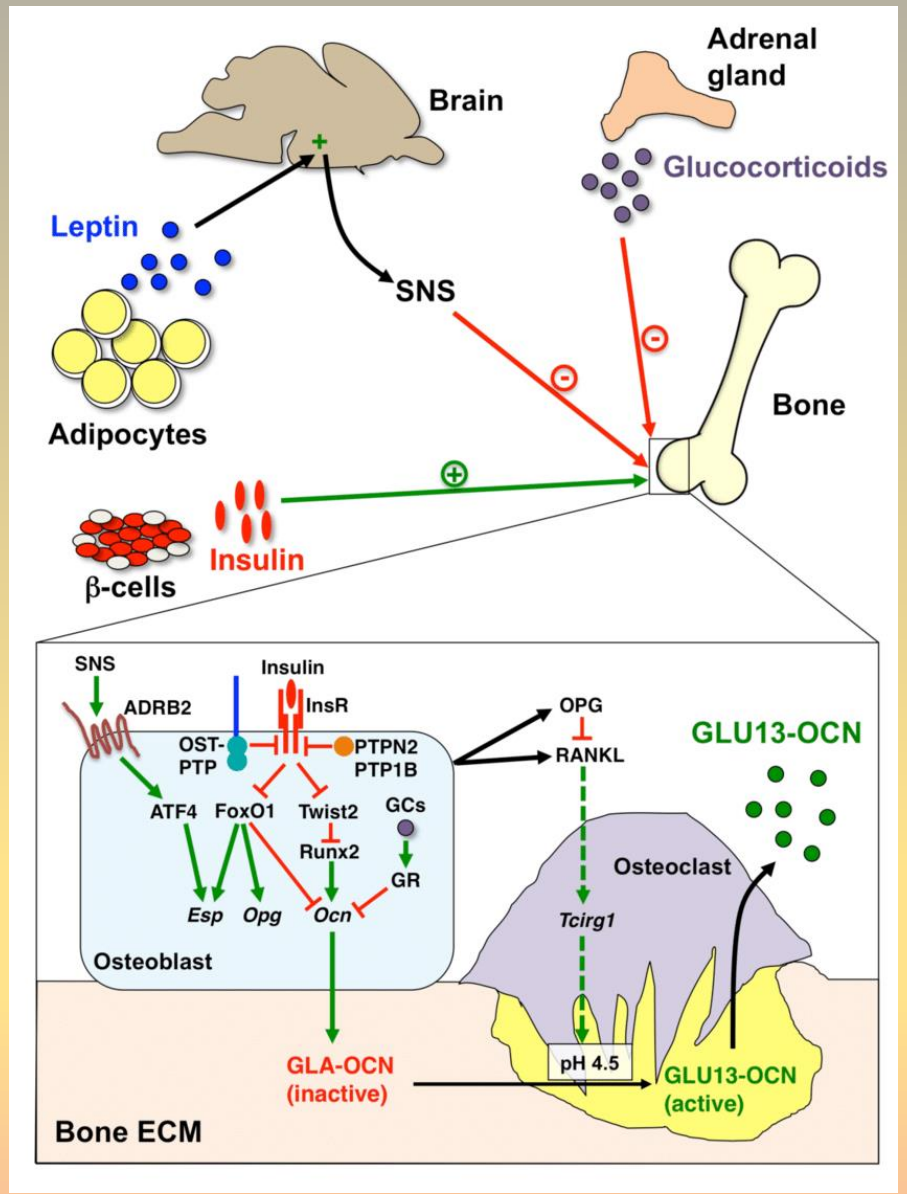
insulin



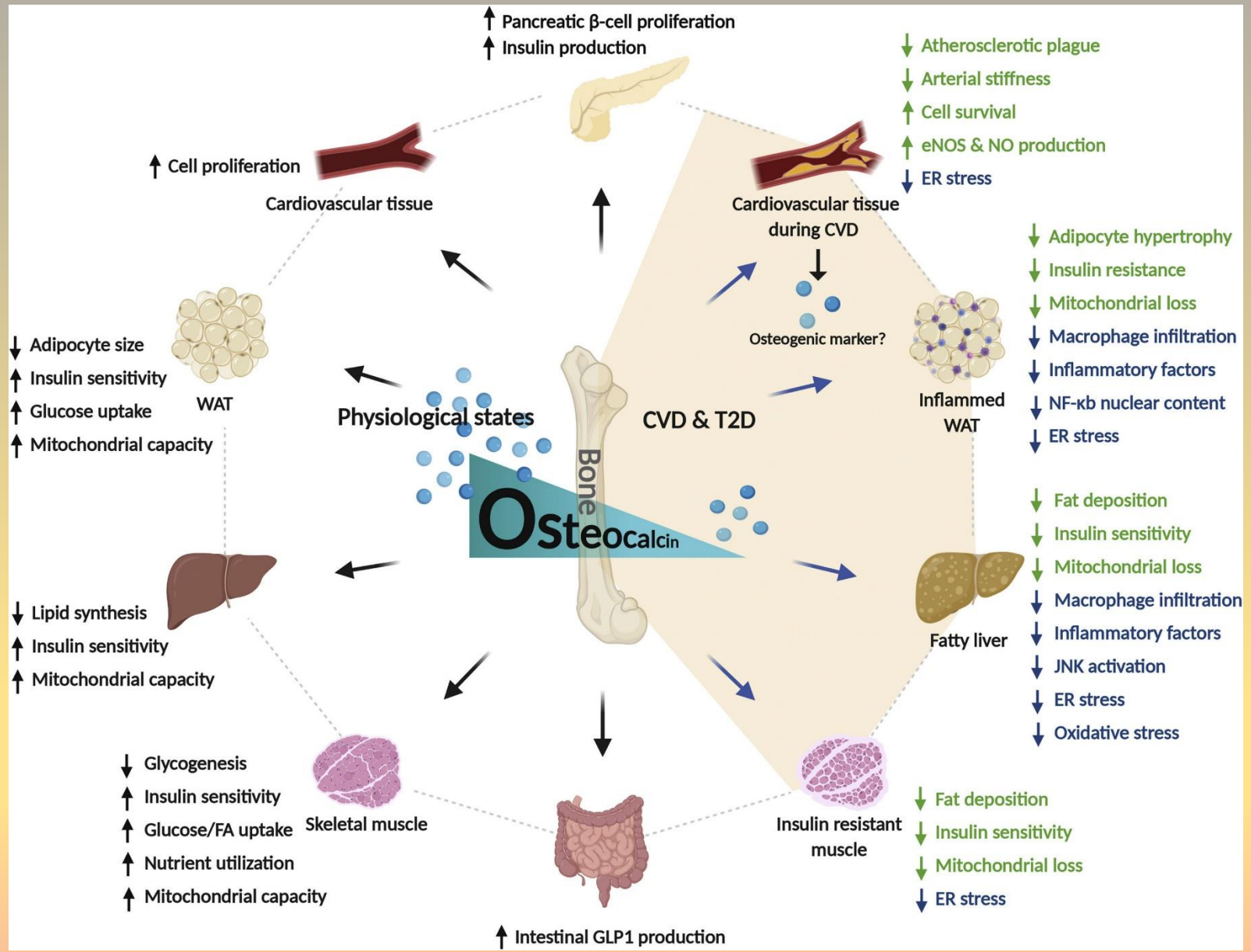
Decarboxylated OCN (Glu13-OCN)

Osteocalcin

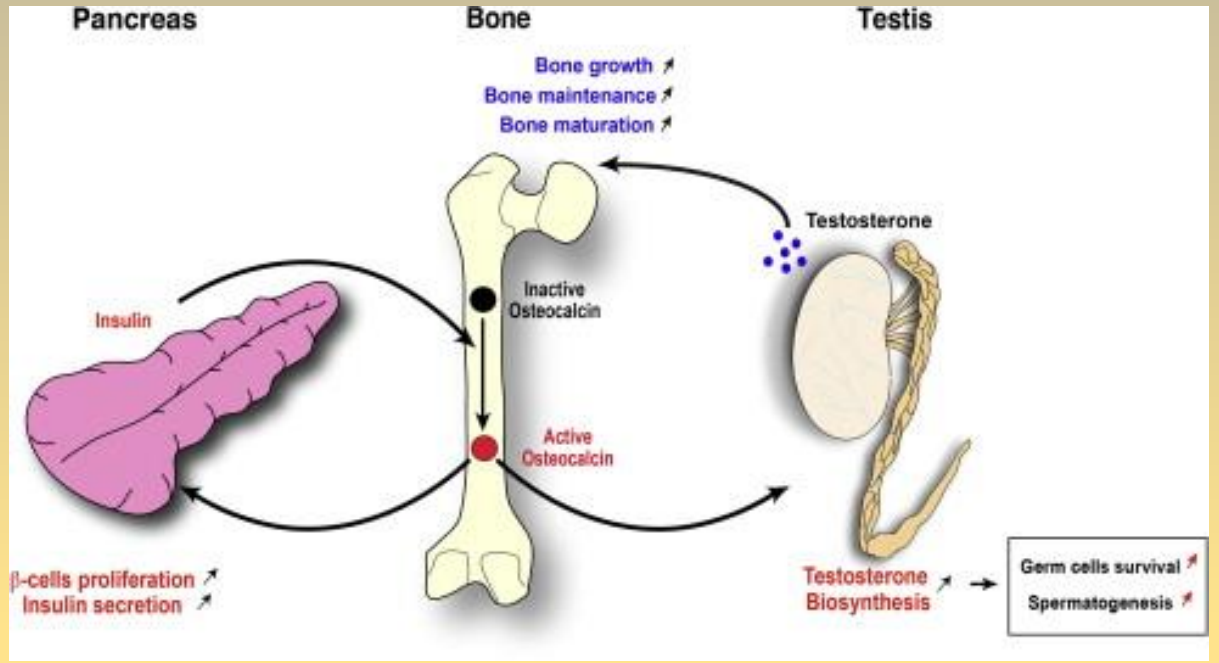


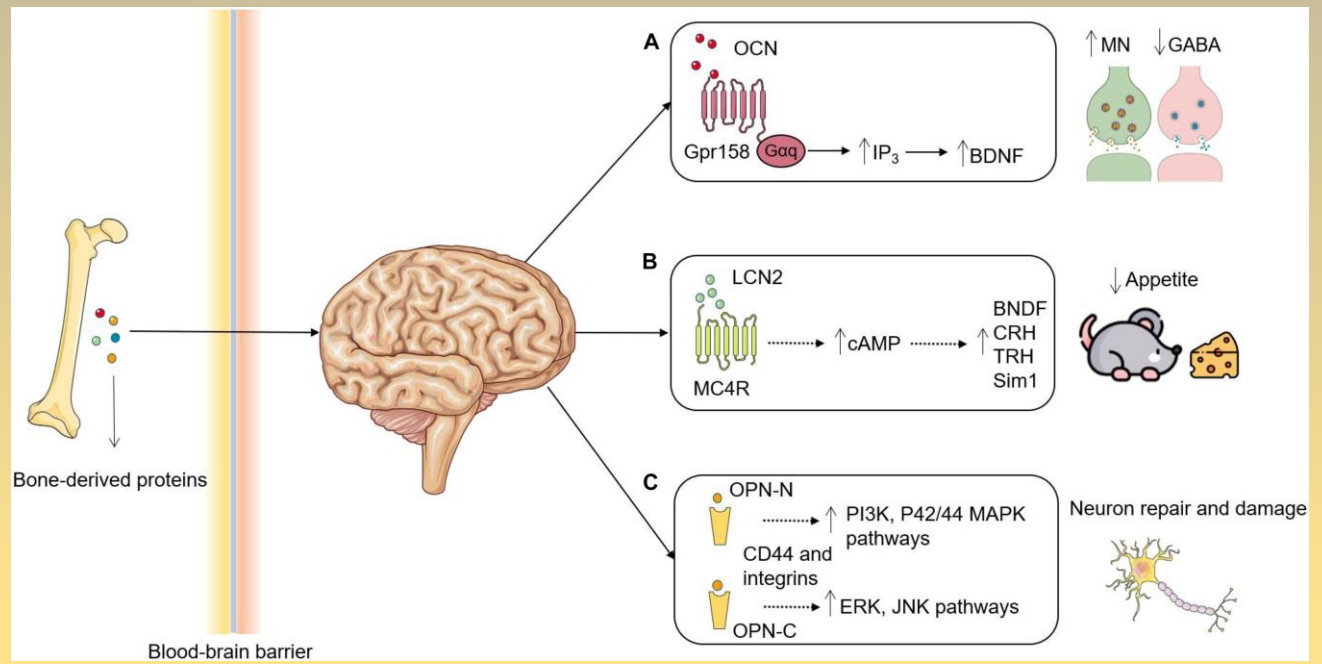


Osteocalcin



Osteocalcin



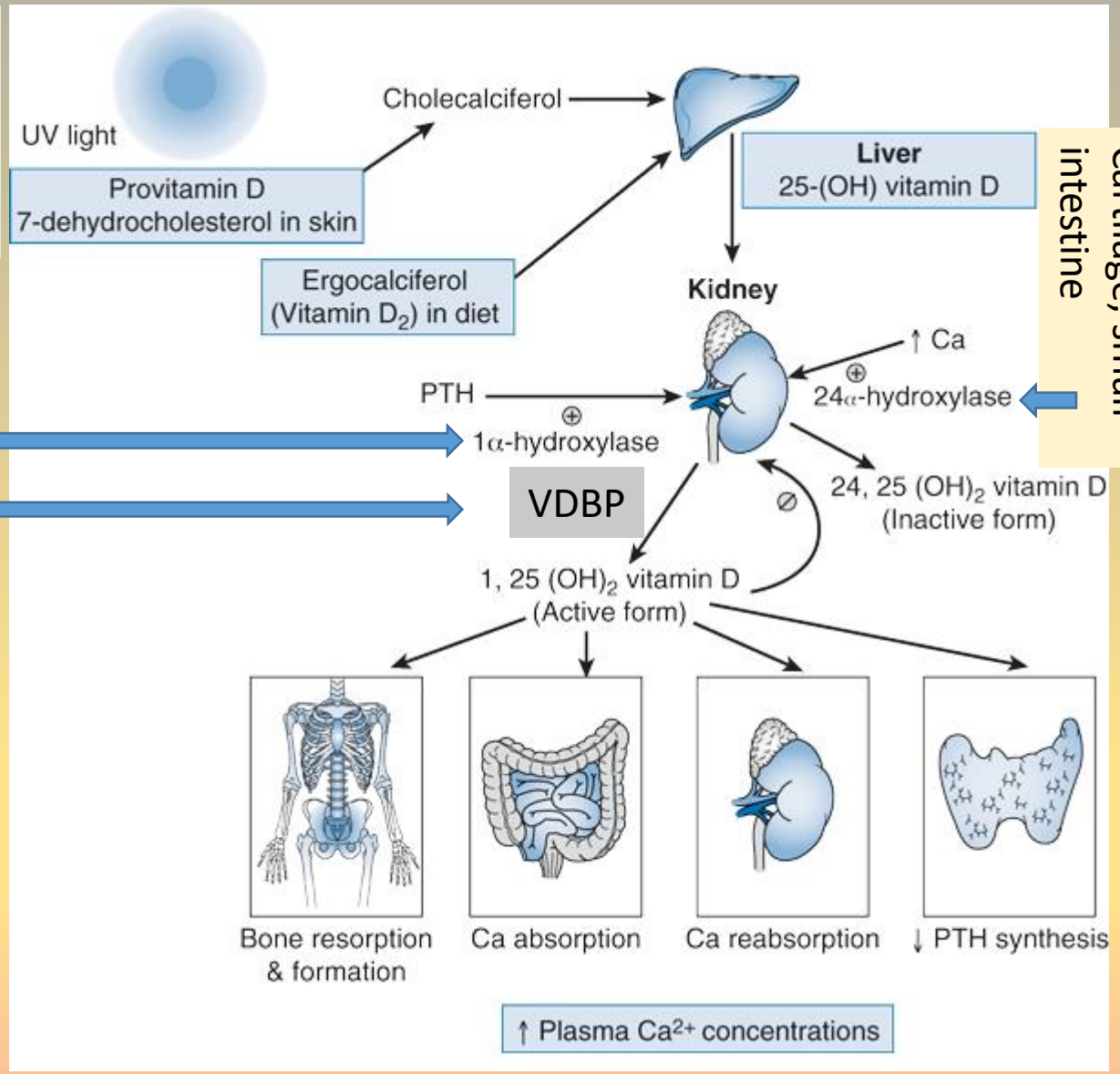


Vitamin D….hormone?...vitamin?

Characteristics
- Intake with diet or synthesized (UV)
- In blood bound to VDBP and albumin
- Very small free fraction 1,25$(OH)_2$D – cca 0,4 %

PTH, prolactin, *calcitonin*, *GH* (+)
T3/T4, metabolic acidosis (-)
Ca, phosphates, 1,25$(OH)_2$D, FGF23 (-)
Ketoconazole

Estrogens (+)



1α-hydroxylase
- Expression in various tissues
- Keratinocytes
- Placenta
- Macrophages

Different rate of feedback control

Different 1α-hydroxylase expression = local tissue homeostasis

Physiological effects of vitamin D

VDR
- High affinity to 1,25$(OH)_2$D
- Level of circulating 1,25$(OH)_2$D
- Heterodimer with RXR – coactivators, corepressors

Parathyroid glands
- Gene expression regulation
- Cell proliferation regulation
- (-) PTH gene transcription

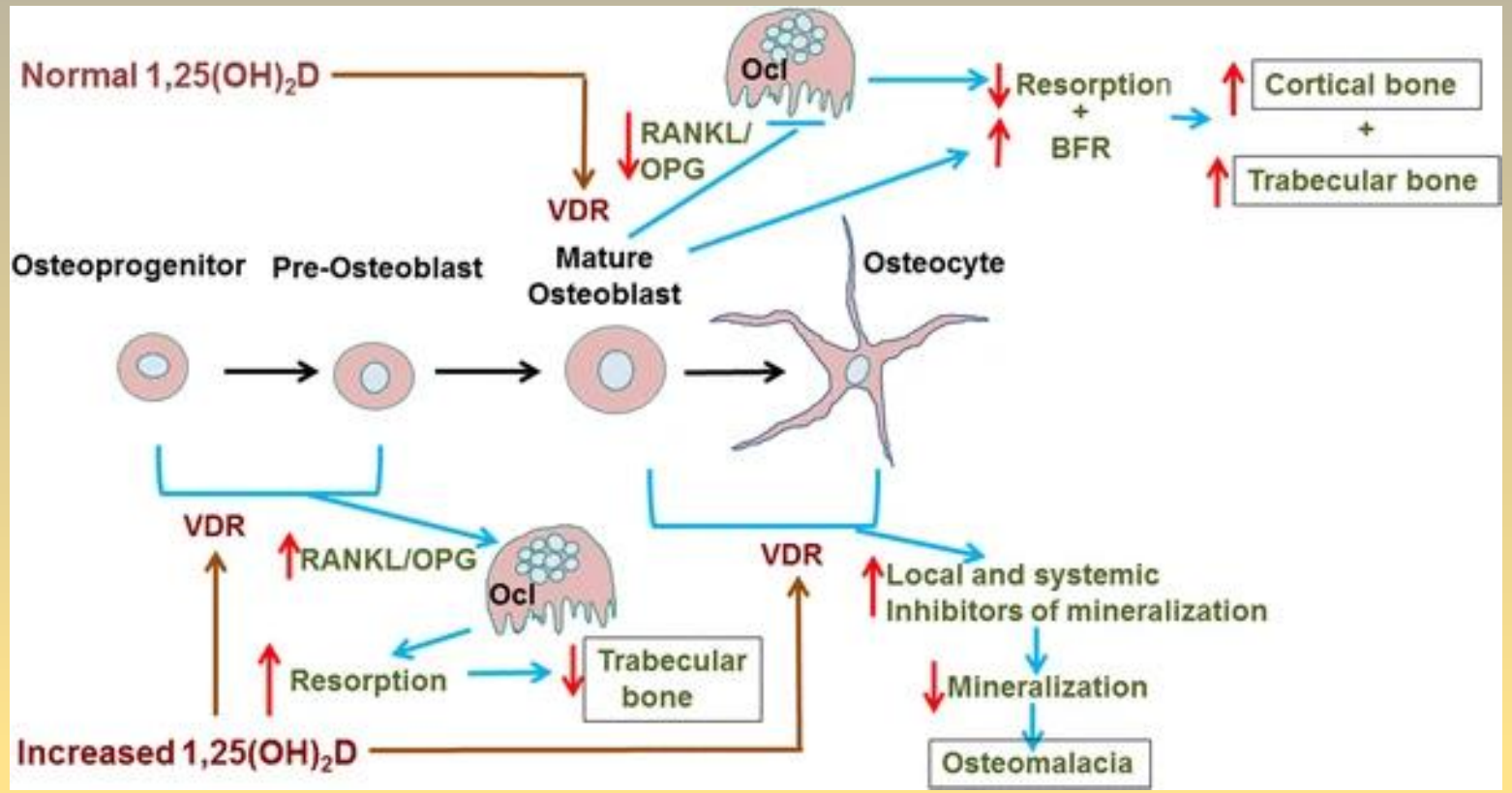
Non-genomic effects
- Rapid increase of intracellular Ca concentration
- PLC activation
- Opening of some Ca ion channels
- Required VDR presence

Bones and bone tissue
- (-) collagen synthesis
- (+) osteocalcin synthesis
- (+) osteoclasts differentiation – osteoclastogenesis
- (+) RANKL
- Main function – ensuring the stability of the bone microenvironment for mineralization by the standard intake and availability of Ca and phosphates

Vitamin D and Ca absorption/reabsorption
- (+) CBP, AP, Ca^{2+}/Mg^{2+}-ATPase
- (+) TRPV6 – absorption (GIT)
- (+/-) TRPV5 – reabsorption (kidneys)
- Calbindin-9K
- 1,25$(OH)_2$D-inducible ATP-dependent Ca^{2+} pump
- Na^+/Ca^{2+} exchanger

Muscle tissue
- (+) uptake AAs
- (+) troponin C
- Phospholipids metabolism

Vitamin D and bone resorption



Tentative scheme of direct actions of $1,25(OH)_2D$/VDR on bone. Normal levels of $1,25(OH)_2D$ act via the VDR in mature osteoblasts to decrease the ratio of RANKL/OPG and reduce osteoclastic bone resorption. As well, $1,25(OH)_2D$ action via the VDR in mature osteoblasts increases the bone formation rate (BFR). The net result is increased cortical and trabecular bone. Increased levels of $1,25(OH)_2D$ acting via the VDR in less mature osteoblasts may increase RANKL/OPG, stimulate osteoclastic bone resorption, and reduce trabecular bone. The action of high levels of $1,25(OH)_2D$ in mature osteoblasts and osteocytes can increase local and systemic inhibitors of osseous mineralization and decrease mineralization of bone leading to osteomalacia.

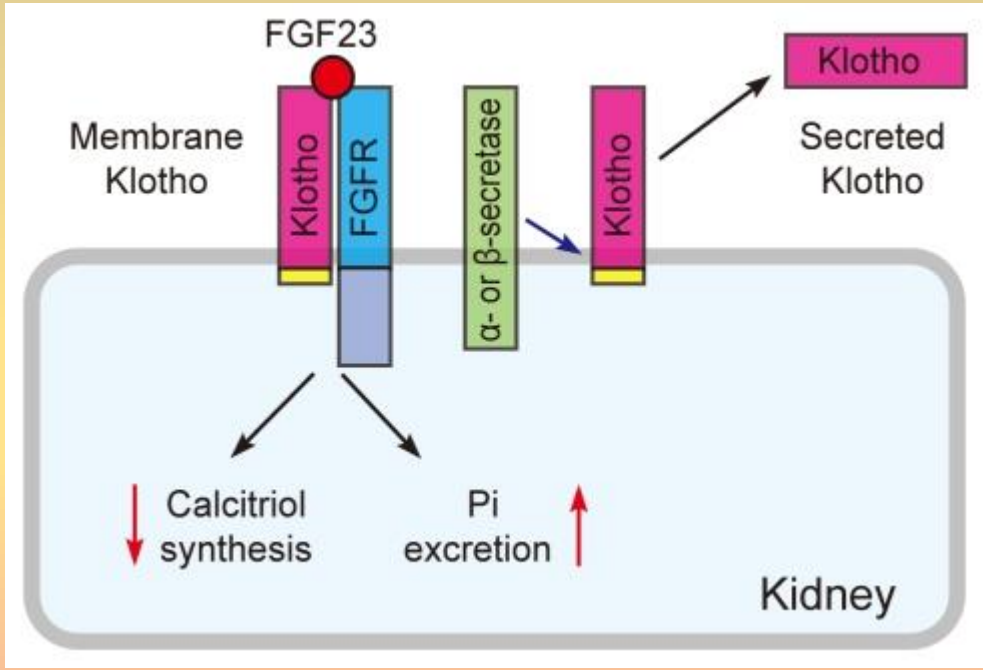
FGF23 – fibroblast growth factor 23

Characteristics
- New hormone?
- Overexpression = hypophosphatemia and decrease of 1α 25(OH)D hydroxylation

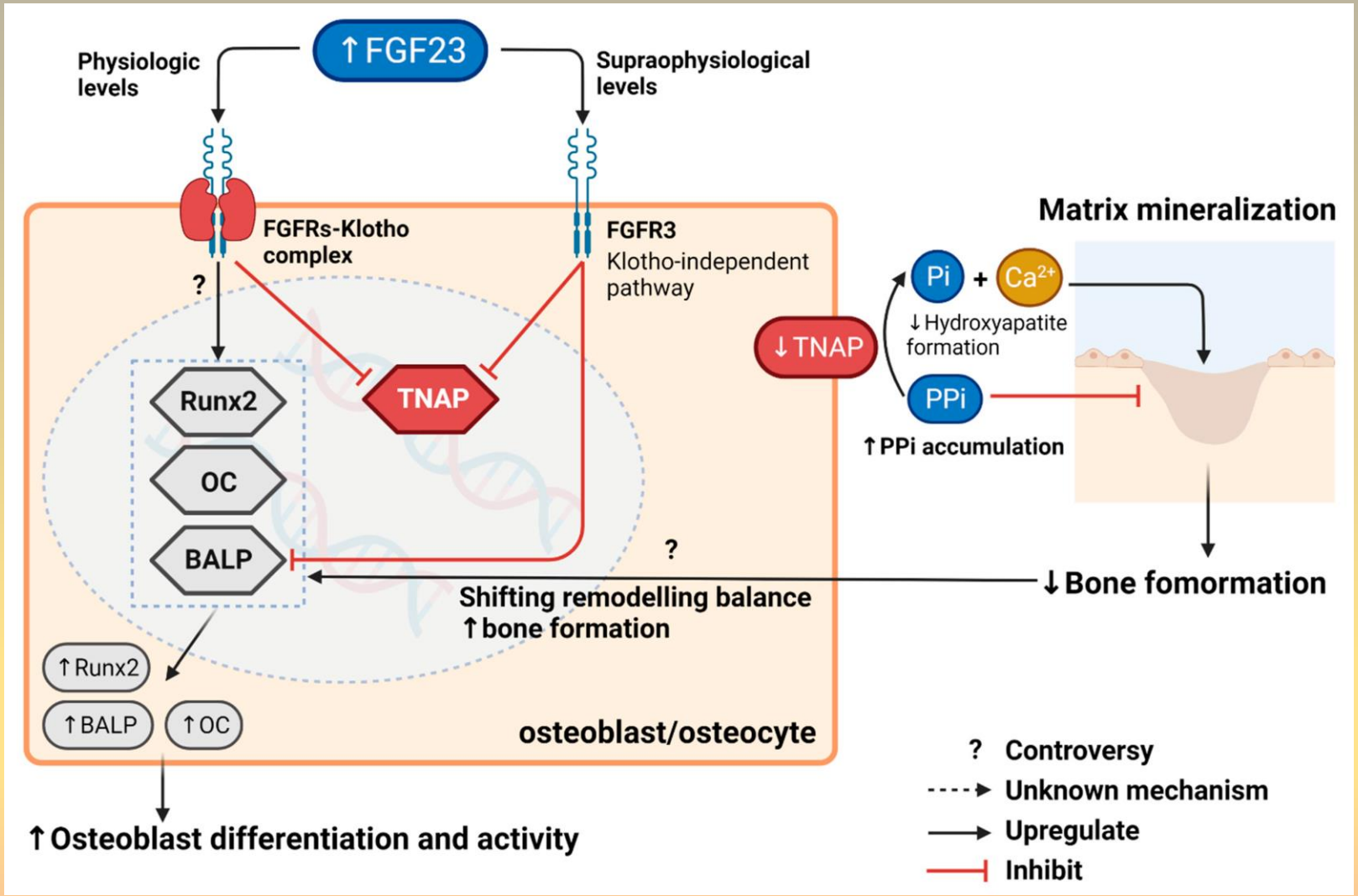
Functions
- maintaining normophosphatemia and regulation of vitamin D metabolism
- IncrDecreased expression of IIa, IIb, and IIc (NPT) – **phosphate transport**
- eased expression of 24-hydroxylase – **inactive form**
- Klotho = co-receptor

Regulation
- Phosphorus availability in diet (-)
- Serum phosphorus
- $1,25(OH)_2D$
- iron

Clinical relevance:
- Autosomal dominant hypophosphatemic rickets (ADHR)
- Tumor-induced osteomalacia (TIO)
- Klotho mutation
- Prediction of chronic kidney failure prognosis



FGF23 – fibroblast growth factor 23



Regulation of FGF23 and its autocrine/paracrine effects on bone formation. In supra physiologic conditions, FGF23 acts directly on FGFR3 in a Klotho-independent manner, thereby inhibiting bone formation. Increased FGF23 suppresses differentiated osteoblast activity and TNAP transcription, which subsequently causes PPi accumulation in the ECM and inhibits matrix mineralization. In physiological conditions, the actions of FGF23 on canonical receptors (FGFRs-Klotho complex) also downregulate TNAP, decreasing matrix mineralization. However, the upregulation of osteoblastic markers in these conditions may be caused by the shifting of remodelling balance toward bone formation or direct action of FGF23 via canonical receptors. The symbol "?" and dash lines denote issues of controversy and unknown mechanisms, respectively. This figure was generated with publication licensed by BioRender, Toronto, ON, Canada (Agreement number: VC237SOKSX, 19 November 2021). Abbreviations: BALP, Specific bone Alkaline phosphatase; FGF23, Fibroblast growth factor 23; Pi, Inorganic phosphate; PPi, Pyrophosphate; Runx2, Runt-related transcription factor 2; TNAP, Tissue nonspecific alkaline phosphatase; OC, Osteocalcin.