# Control of calcium metabolism.

# 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

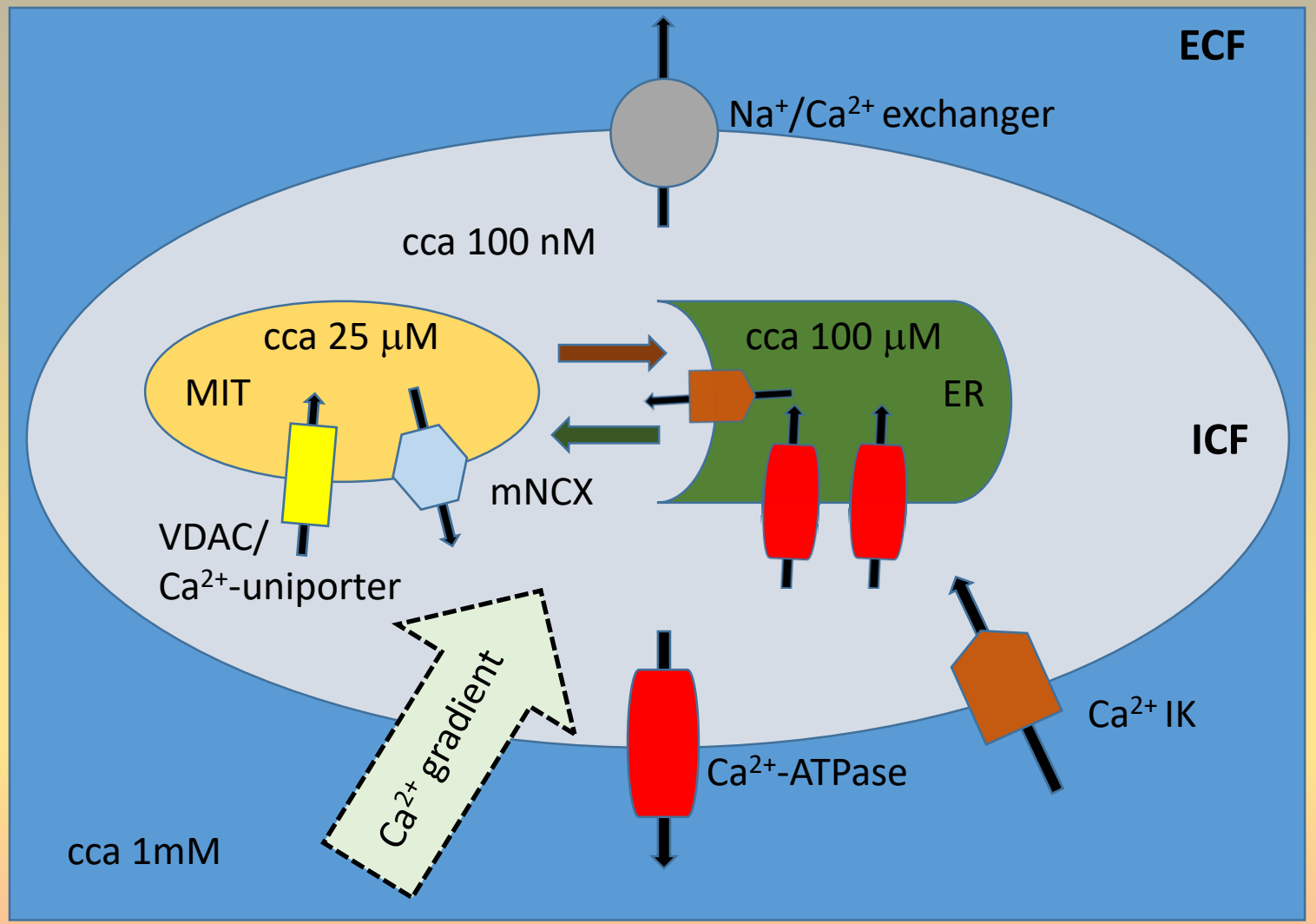
Bone tissue
- 99 % of overall Ca, of it 99 % in mineral component
- 1 % - quickly mobilizable and convertible (ICF - ECF)

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

# Extra- and intracellular calcium

Extracellular calcium
- Cartilage and bone mineralization
- Cofactor of enzymes including proteins of coagulation cascade
- „Source“ of intracellular calcium
- Excitable tissues

Intracellular calcium
- Signaling role
- Contractility
- Excitability
- Neurosecretion
- Endocrine and exocrine secretion
- Cell differentiation and proliferation
- Cell death and its regulation

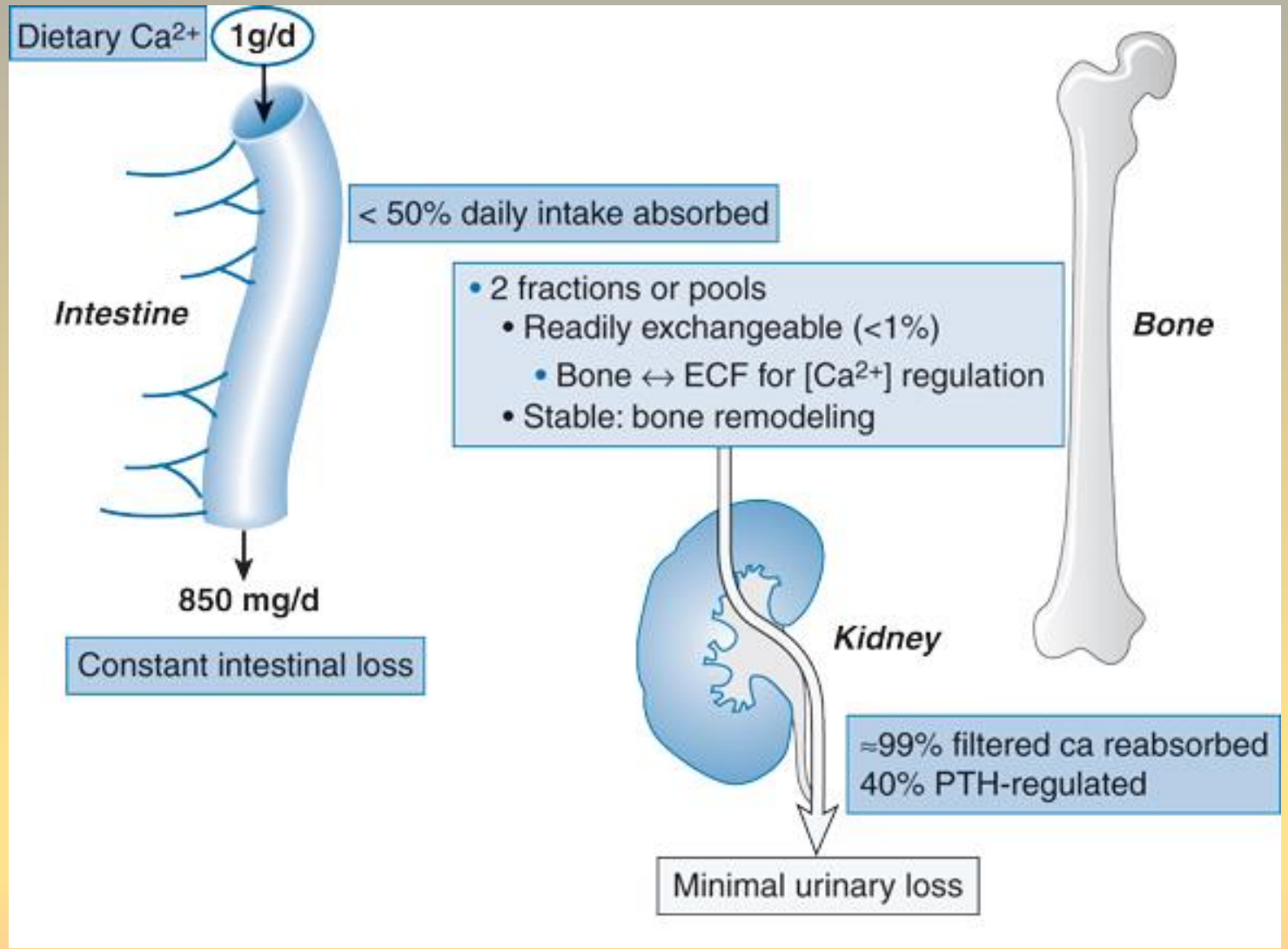


# Calcium and its intake

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 and jejunum – 90 %
- Adaptive intake – duodenum and ileum




**Age-related negative calcium balance is an osteoporosis risk factor.**

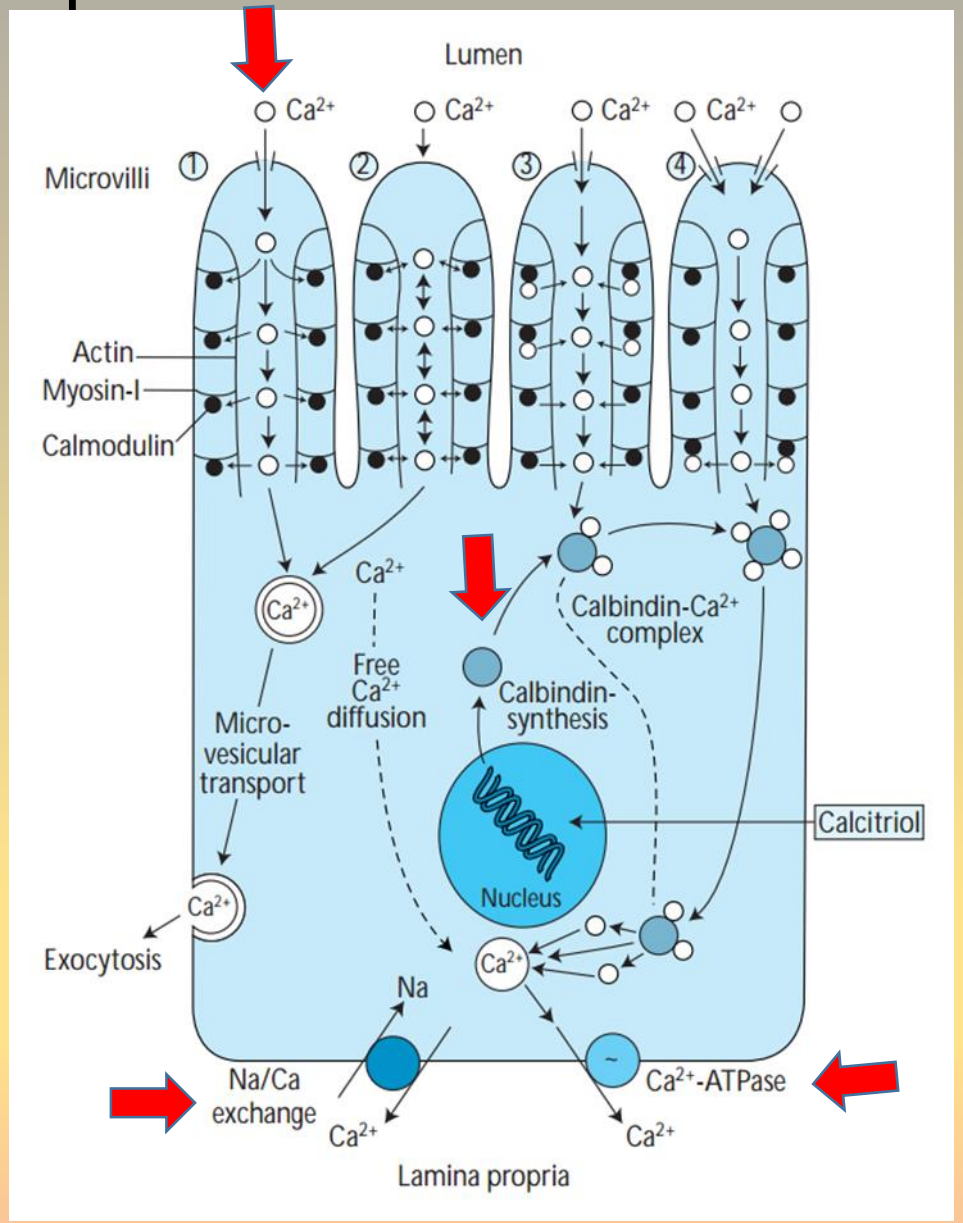
# Mechanisms of calcium absorption

**Vitamin D**

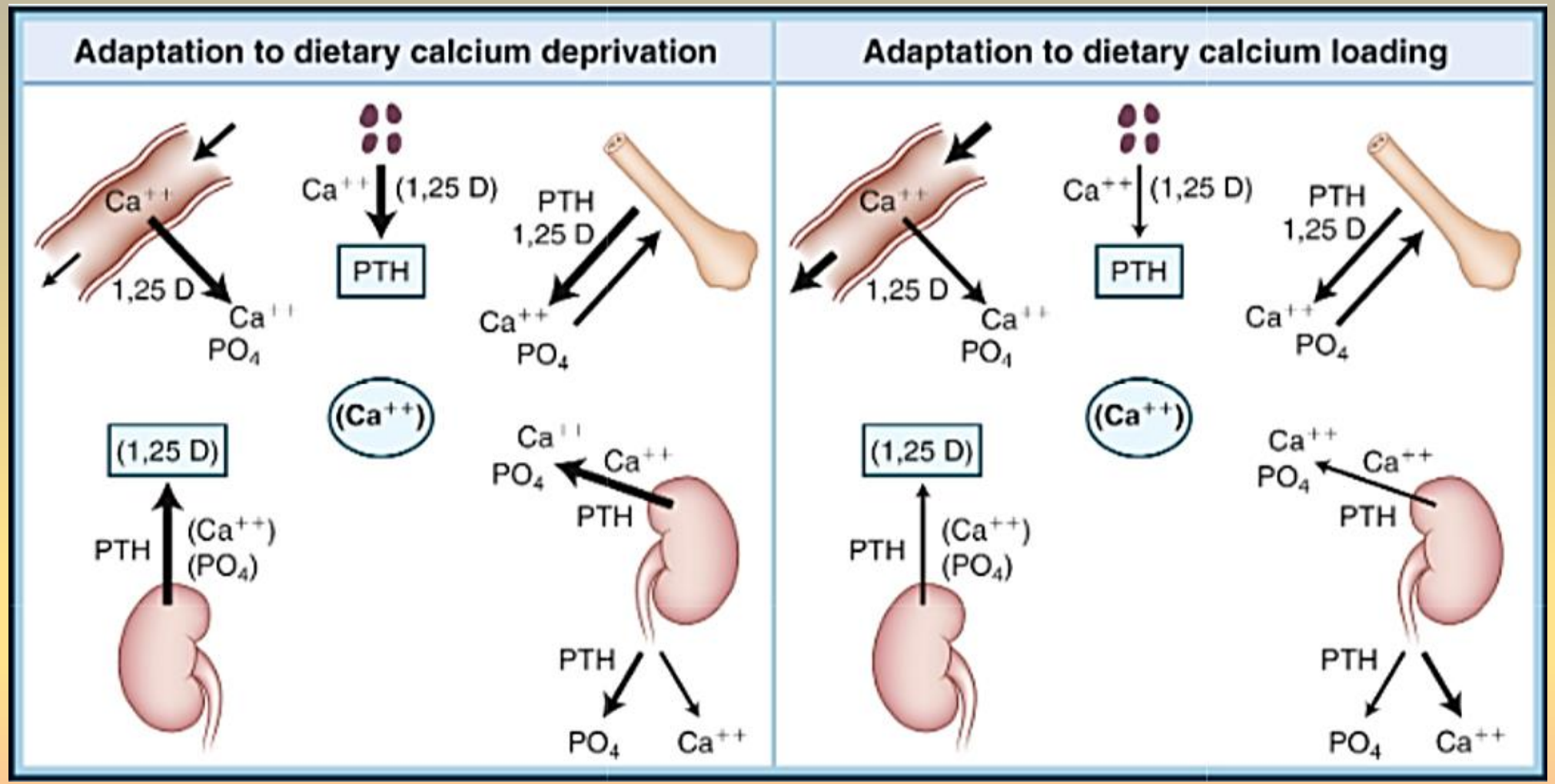
Paracellular
- Luminal electrochemical gradient
- Integrity of intercellular connections
- Claudins and their role in paracellular transport

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

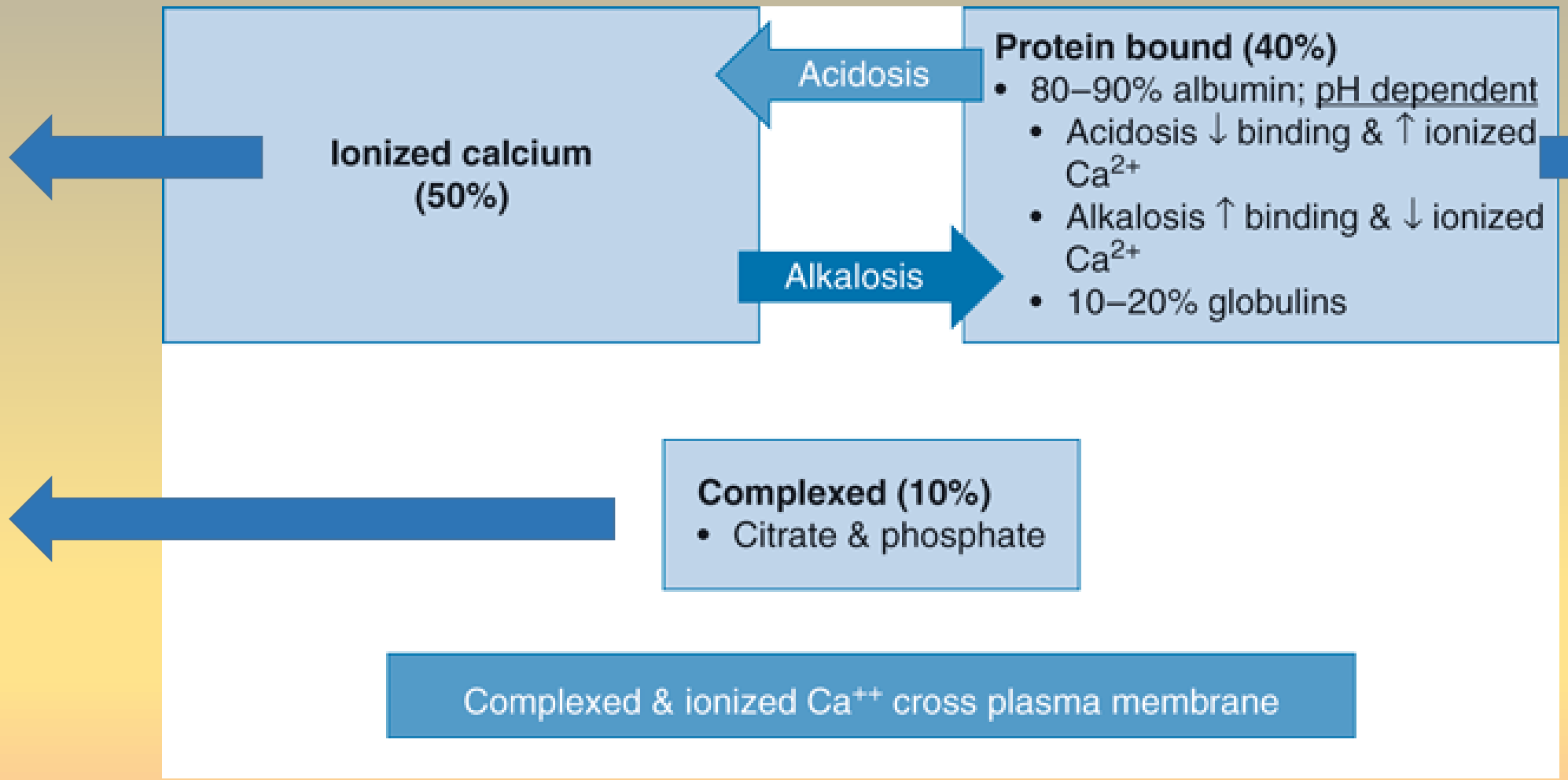
| Glucocorticoids | Estradiol | Prolactin |
|---|---|---|
| - | + | + |



# Adaptation to dietary calcium levels



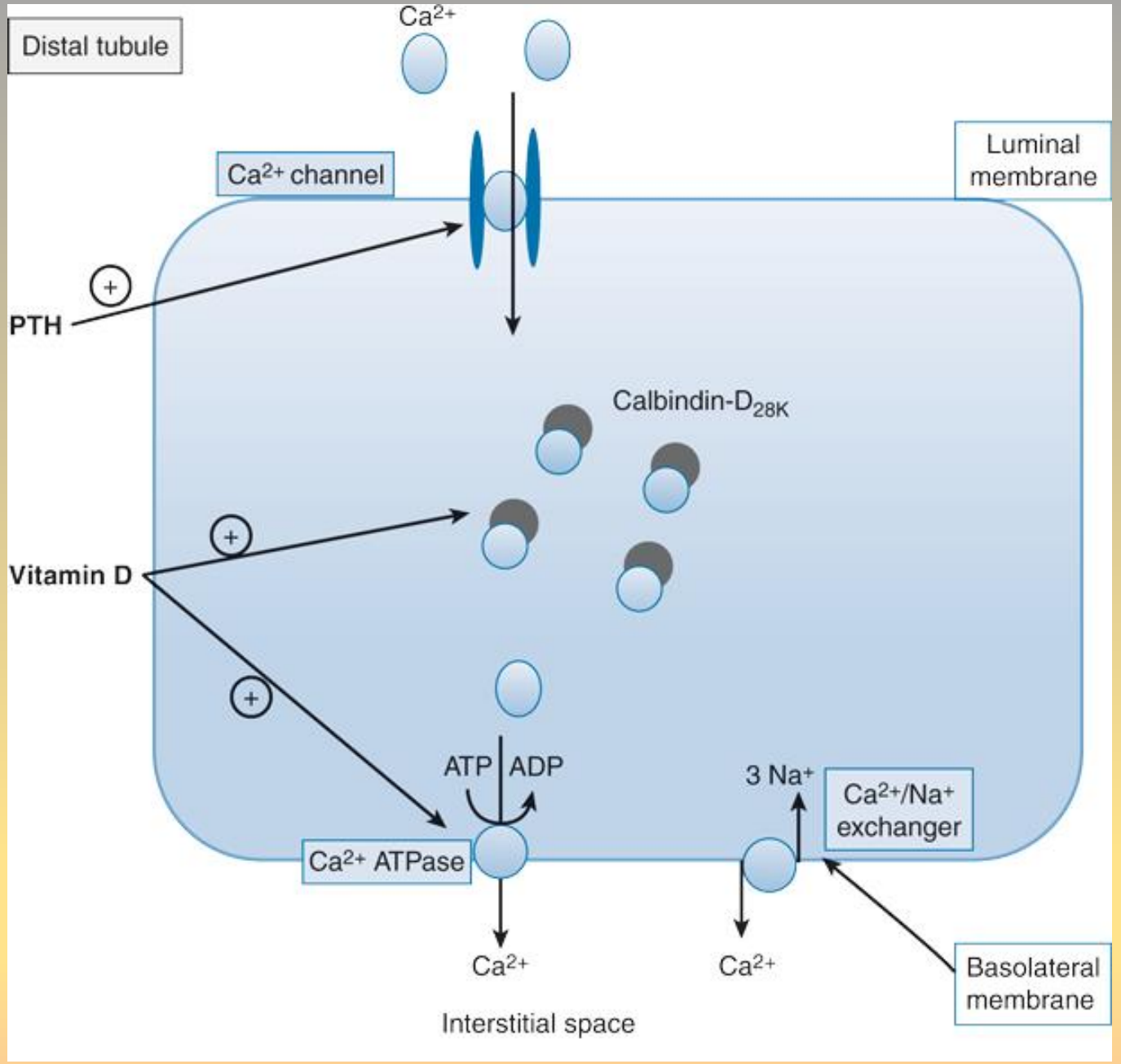
# Calcium on blood (calcemia)



# 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



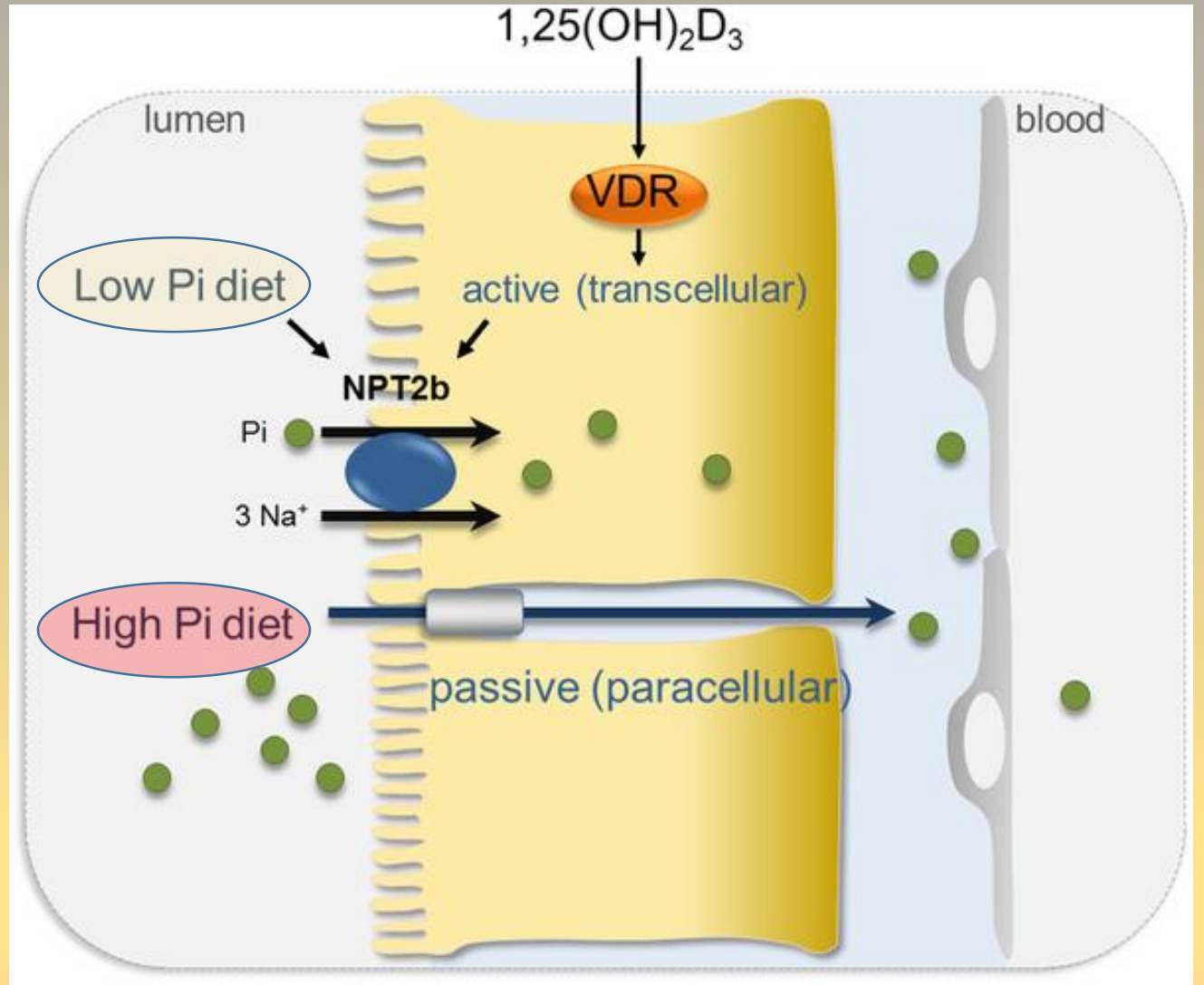
# Phosphorus

Distribution
- Bones cca 45 % - $Ca_{10}(PO_4)_6(OH)_2$
- Organic and inorganic form in ICF and ECF
- Age, sex, growth

Blood
- Concentration 1 mM (serum)
- Ionized form ($HPO_4^{2-}$, $H_2PO_4^-$)
- 12 % protein complexes
- Intracellular concentration approximately same as extracellular
- Cotransport with sodium

Functions
- Structural – NA, phospholipids
- Modified saccharides, phosphoproteins, cofactors, G proteins
- Macroergic compounds (ATP)
- Regulatory role – signaling cascade, energetic processes

Kidneys
- Reabsorption - proximal tubule (85 %) – *Npt1-3*




Vitamin D

PTH

IGF-1

FGF23

# Magnesium

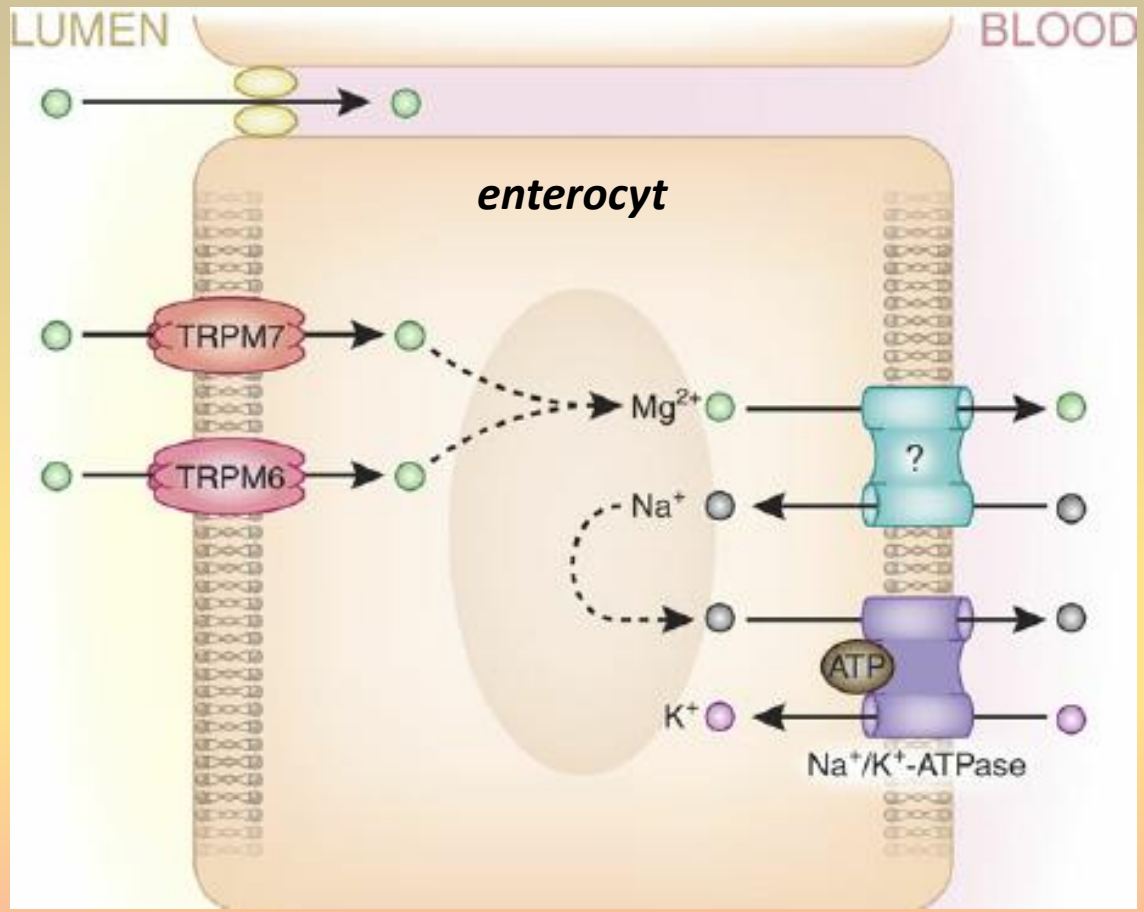
Distribution
- 1 mol - bones approx. 54 %, muscles and soft tissues approx. 56 %
- ECF – 0.5 mM

Blood
- 0.7 – 1 mM
- Approx. 30 % in protein complexes
- 15 % in phosphate and low molecular weight anion complexes
- 55 % free

Cell
- 95 % in ATP and similar molecules
- Concentration 0.5 mM
- Ion channels?

Kidneys
- 95 % of filtered amount is reabsorbed
- 15 % PT, 70 % cortical TAHL, 10 % DT
- Regulation – magnesemia, calcamia, ECF volume

Functions
- Cofactor (glycolytic, kinase and phosphatase systems)
- Stabilizing function (DNA, RNA, ribosomes)
- Activator of ATP transporters
- Neuromuscular excitability



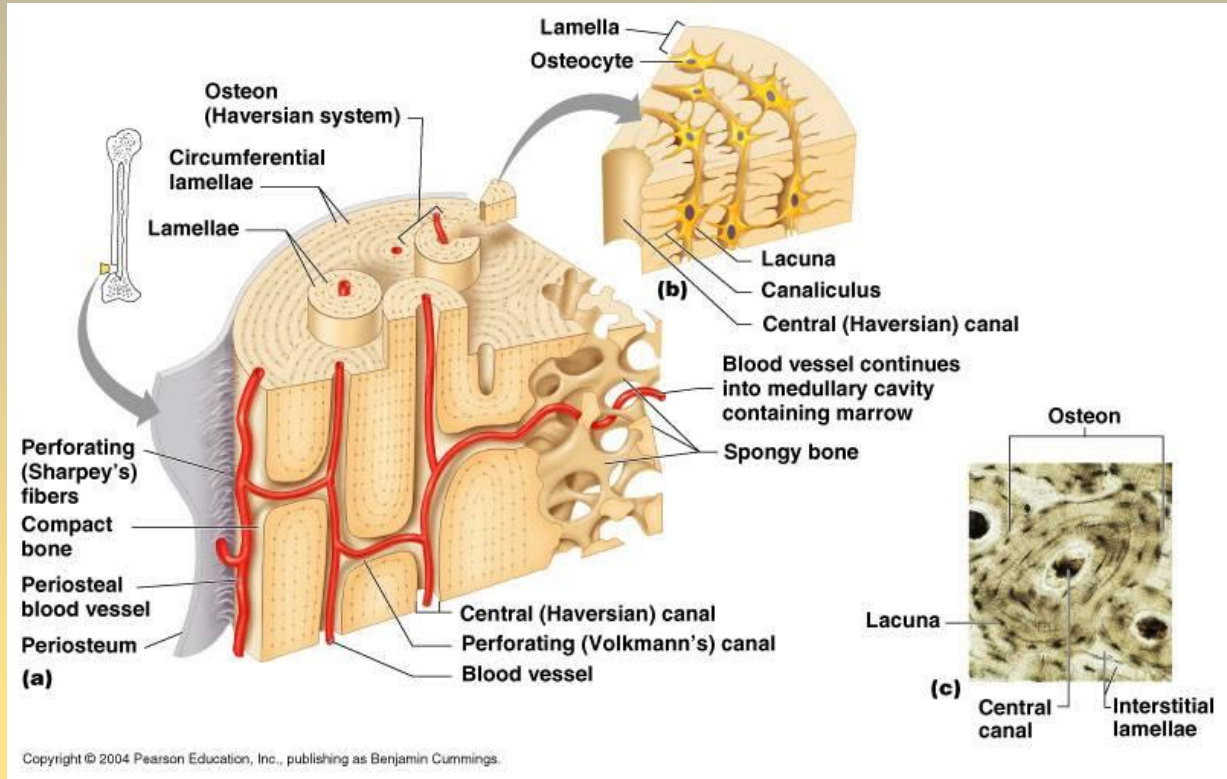
# Bone tissue physiology

**Compact (cortical) bone** – approx. 80 %

- Low surface-to-volume ratio, osteocytes in resting state
- Haversian canals with concentric layers of collagen – osteons (Haversian systems)
- Collagen matrix impregnated with bone mineral crystals
- 20 x 3-7 nm, mainly hydroxyapatite

**Trabecular (spongy) bone** – cca 20 %

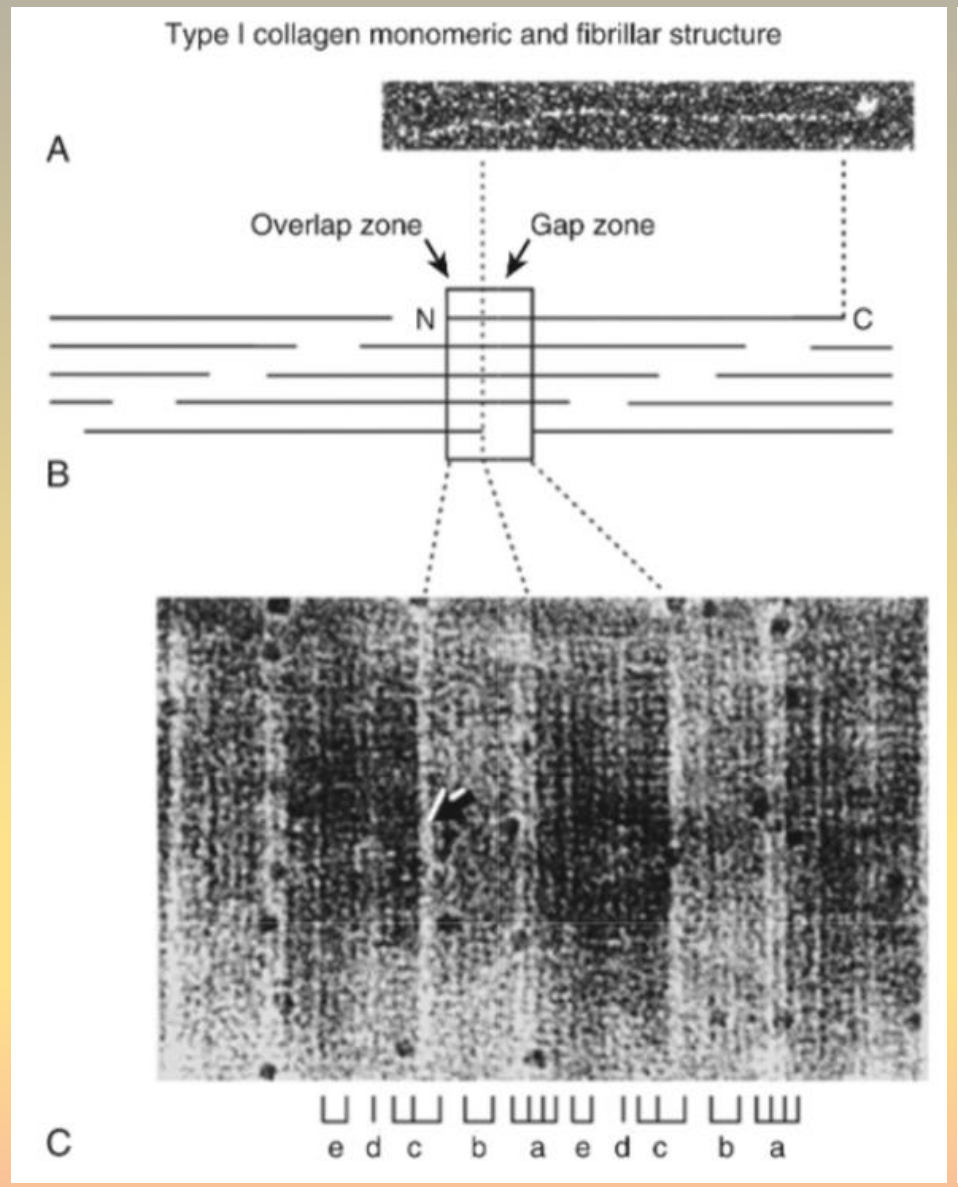
- High surface-to-volume ratio
- High metabolic activity
- Nutrients diffuse from ECF to trabecules

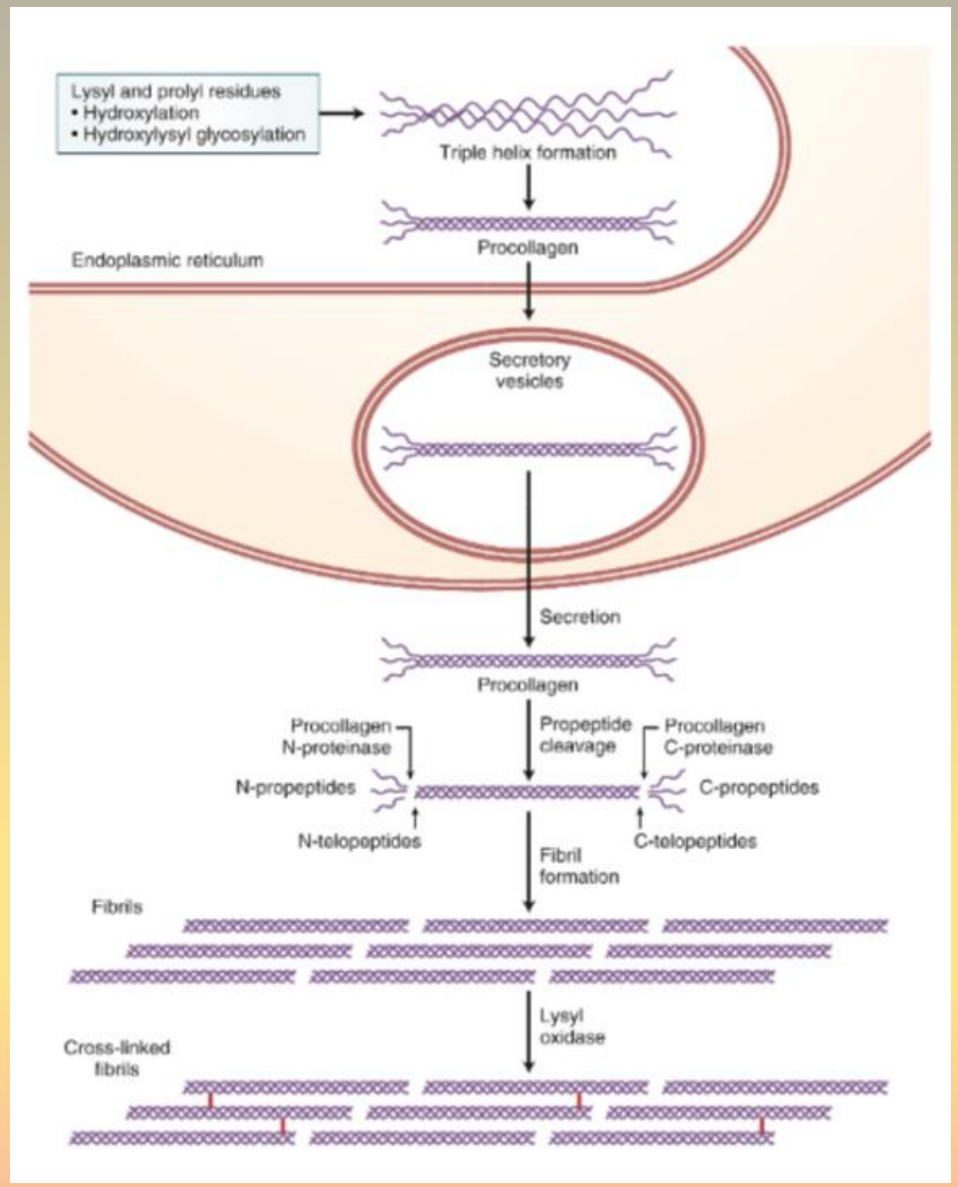



www.creab.org - Human Body Anatomy - Online anatomy atlas. Viktoria Ruppel. 14. 3 2015

**Collagen type I = most important protein of bone matrix**

# Bone matrix and bone mineral



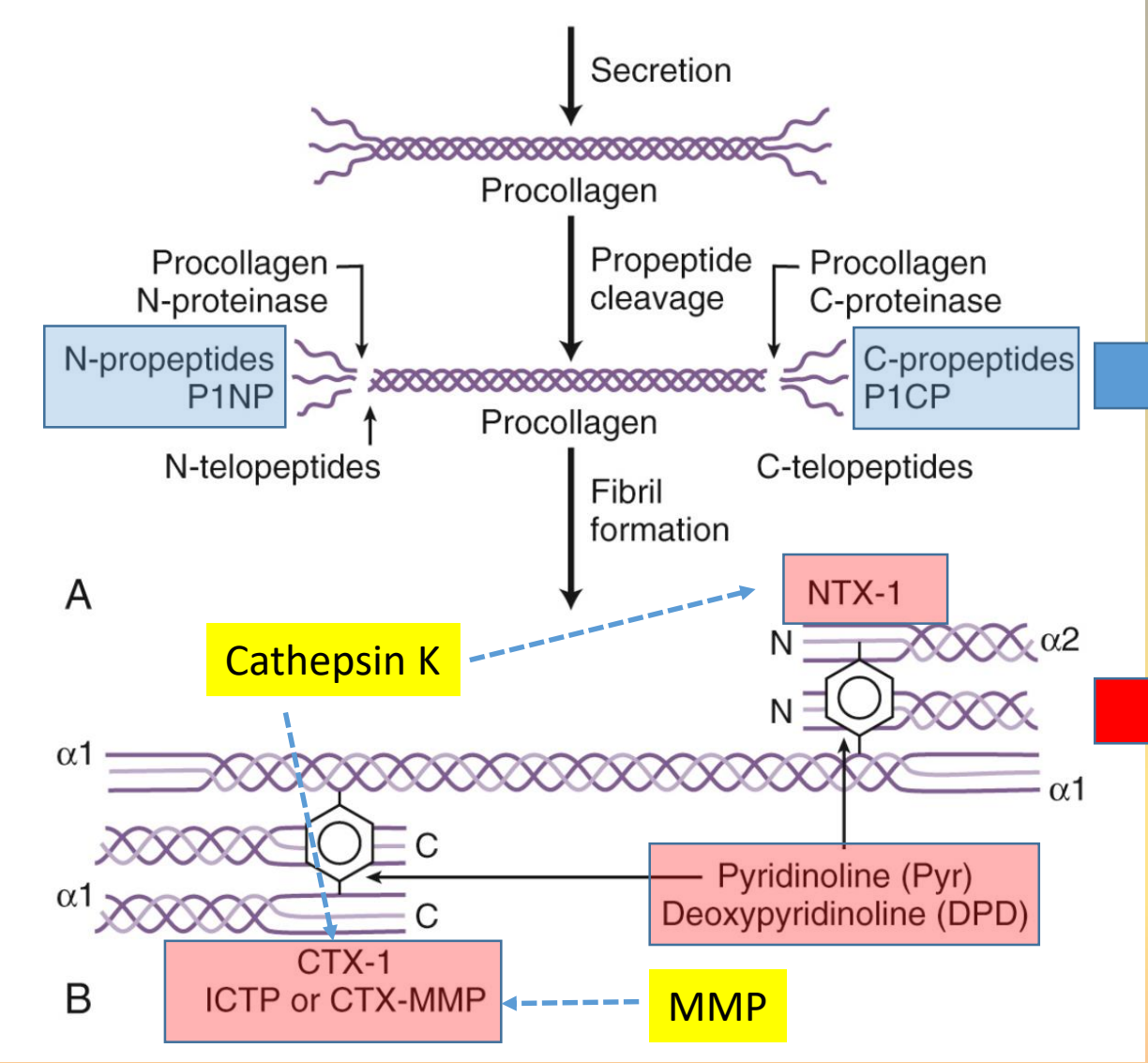

Trombospondin
Fibronectin
Matrix Gla protein
Osteocalcin
Biglykan
Decorin
Bone sialoprotein
Osteopontin
Osteoadherin

Signaling + haematopoiesis

Vitamin K-dependent γ carboxylation and phosphorylation

Ca affinity and mineralization

# Collagen and its synthesis




Collagen synthesis

Collagen degradation

Collagenases – MMP

- MMP1 and MMP13 – osteoblasts
- Role of hormones and cytokines

# 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

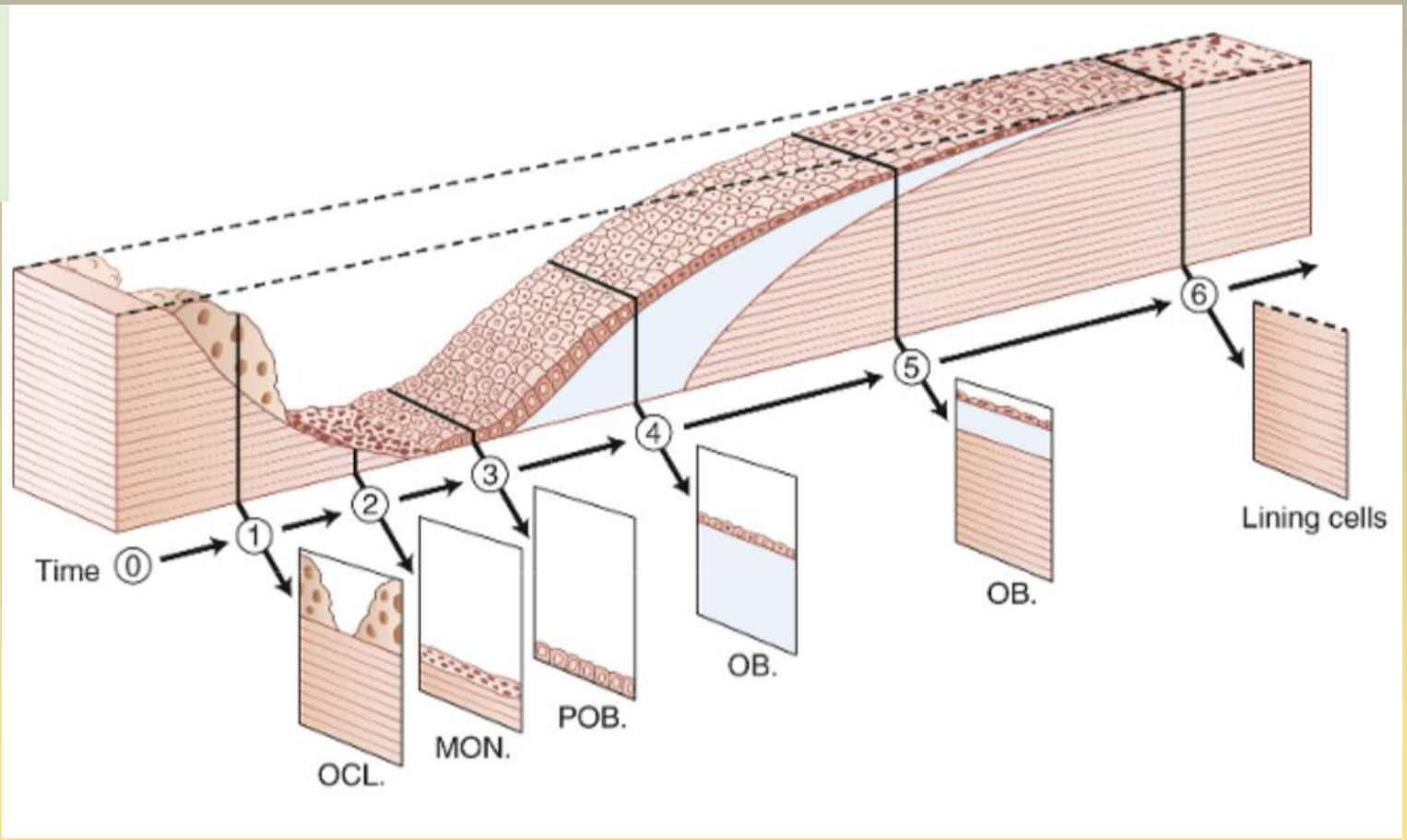
# Bone tissue and its remodeling

**Modeling**
**versus remodeling**
**of bone tissue**

**REMODELING UNIT - BMU**

osteoblasts | osteoclasts | osteocytes

Bone lining cells




**Bone reabsorption**

**Building of bone tissue**

osteoclasts | mononuclear cells

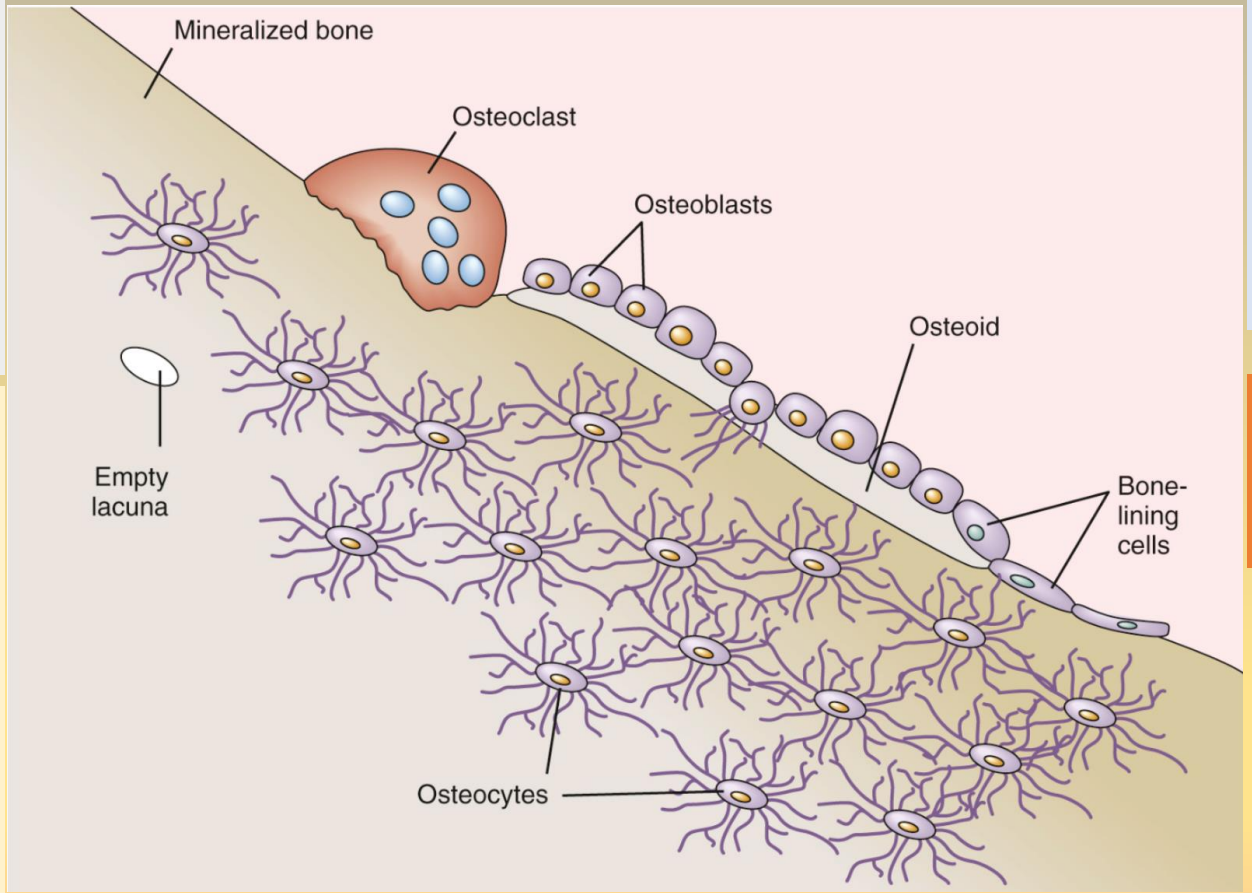
preosteoblasts

osteoblasts

# 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

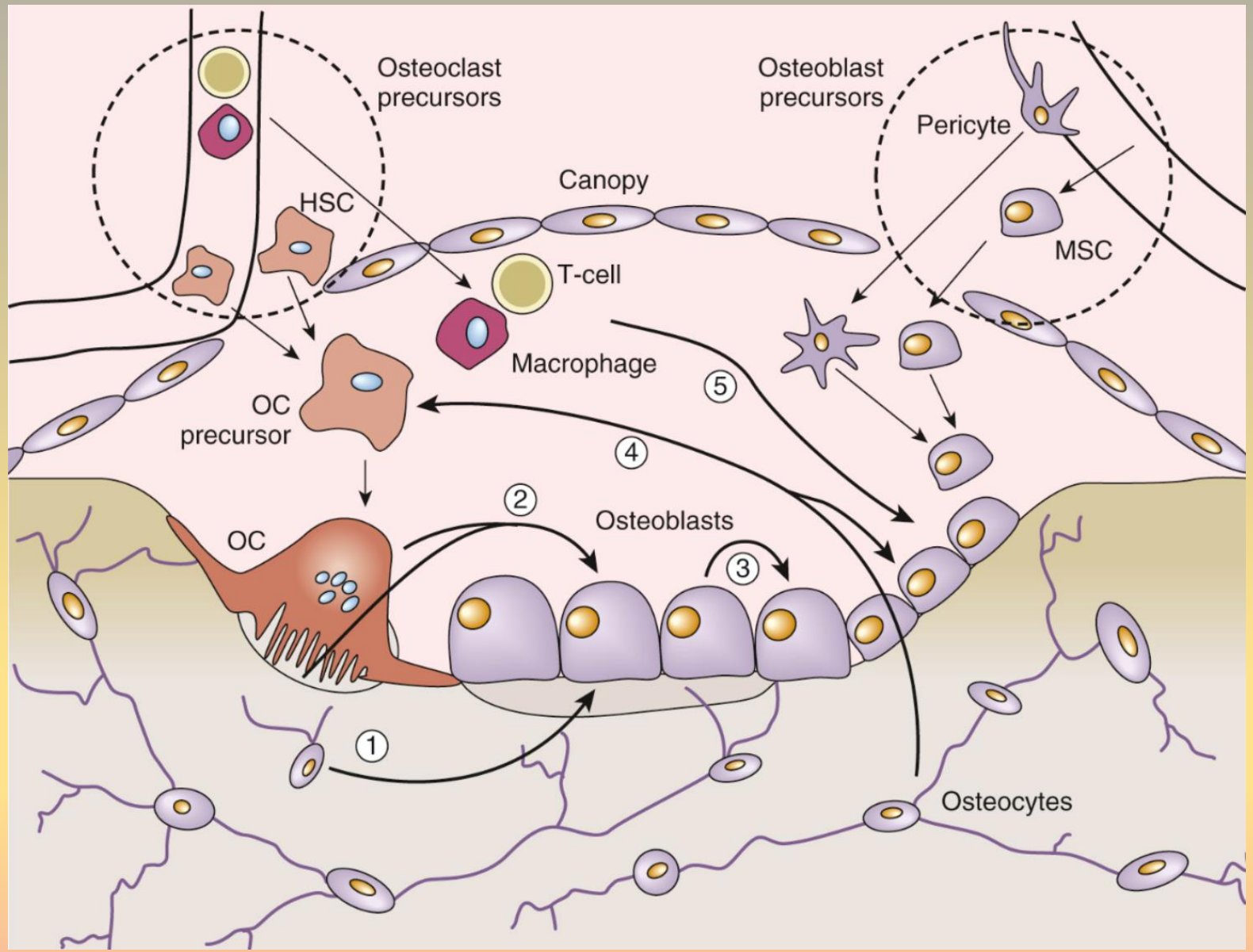



Lining cells
- Stimulation of OB differentiation
- OC communication
- Differentiation to OB stimulated by PTH

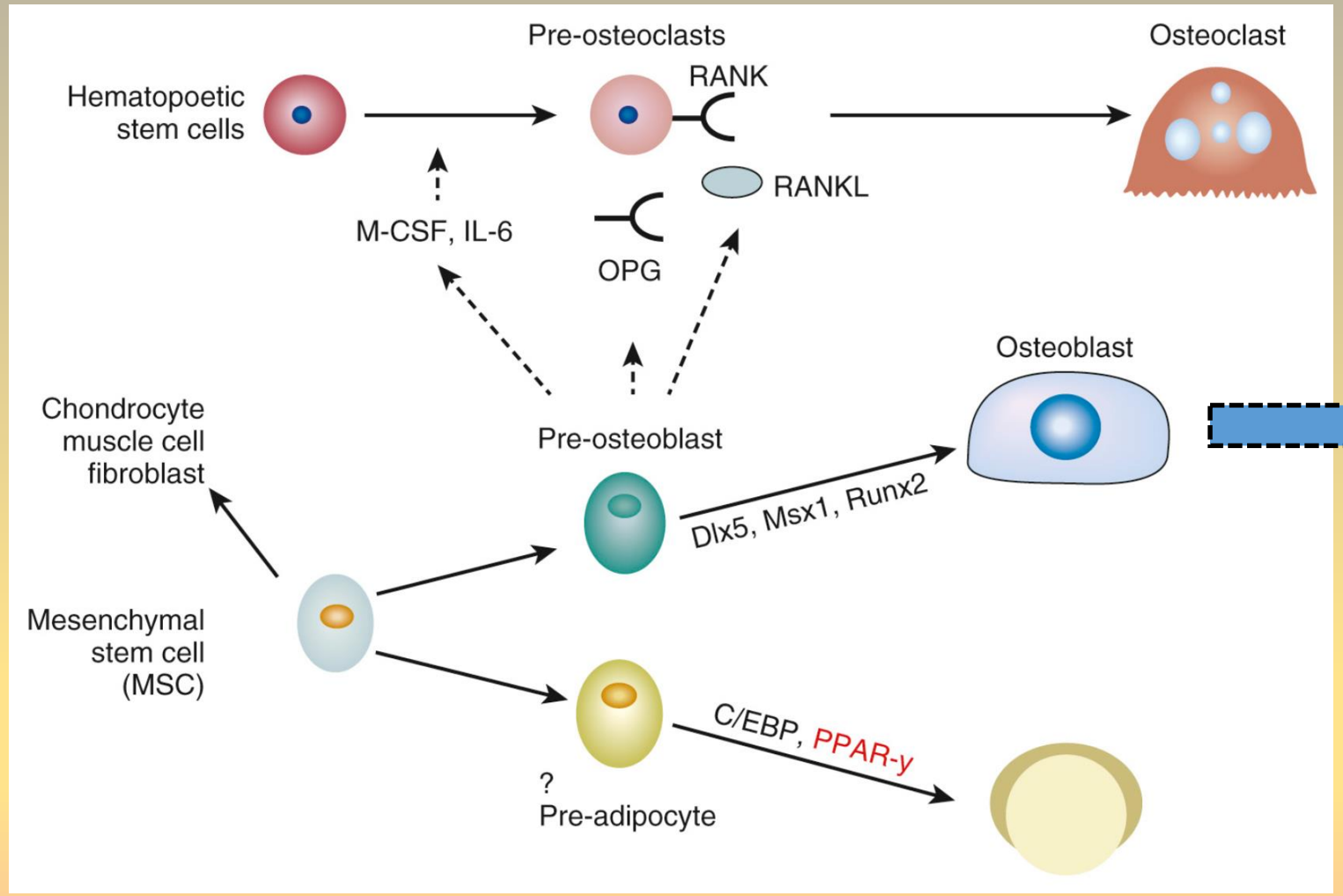
Osteoclasts (OK)
- Bone tissue reabsorption

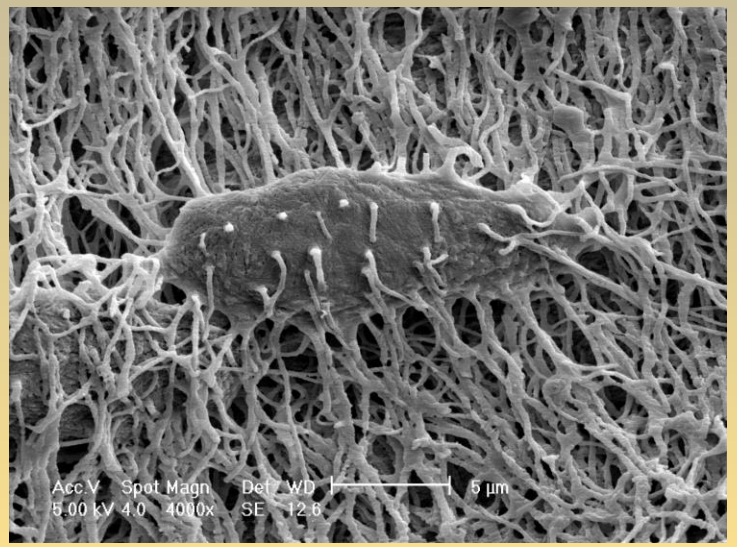
# Remodeling unit - BMU

1. Stimulatory and inhibitory signals of osteocytes (oncostatin M - OSM, sclerostin, PTHrP)
2. Stimulatory and inhibitory signals of osteoclasts to osteocytes (TGF-β, IGF-1, cardiotropin-1, Sema4D – semaforin 4D, sfingosin-1 phosphate)
3. Signalling between osteoblasts (ephrinB2, EphB4, Sema3a, PTHrP, OSM)
4. Stimulatory and inhibitory signals between osteoblasts and osteoclasts and their derivatives (RANKL, Sema3B, Wnt5a, osteoprotegerin - OPG)
5. Signalling between haematopoietic stem cells and osteoblasts (macrophage-produced OSM, IL produced by T-cells, RANKL)



# Osteocyte origin




Osteocyte

- Changes in metabolic activity
- Formation of „projections“ – communication
- Communication with other osteocytes (syncytium – OC + OB)

# Osteoclasts

**Key factor regulating bone resorption is RANKL/OPG ratio.**

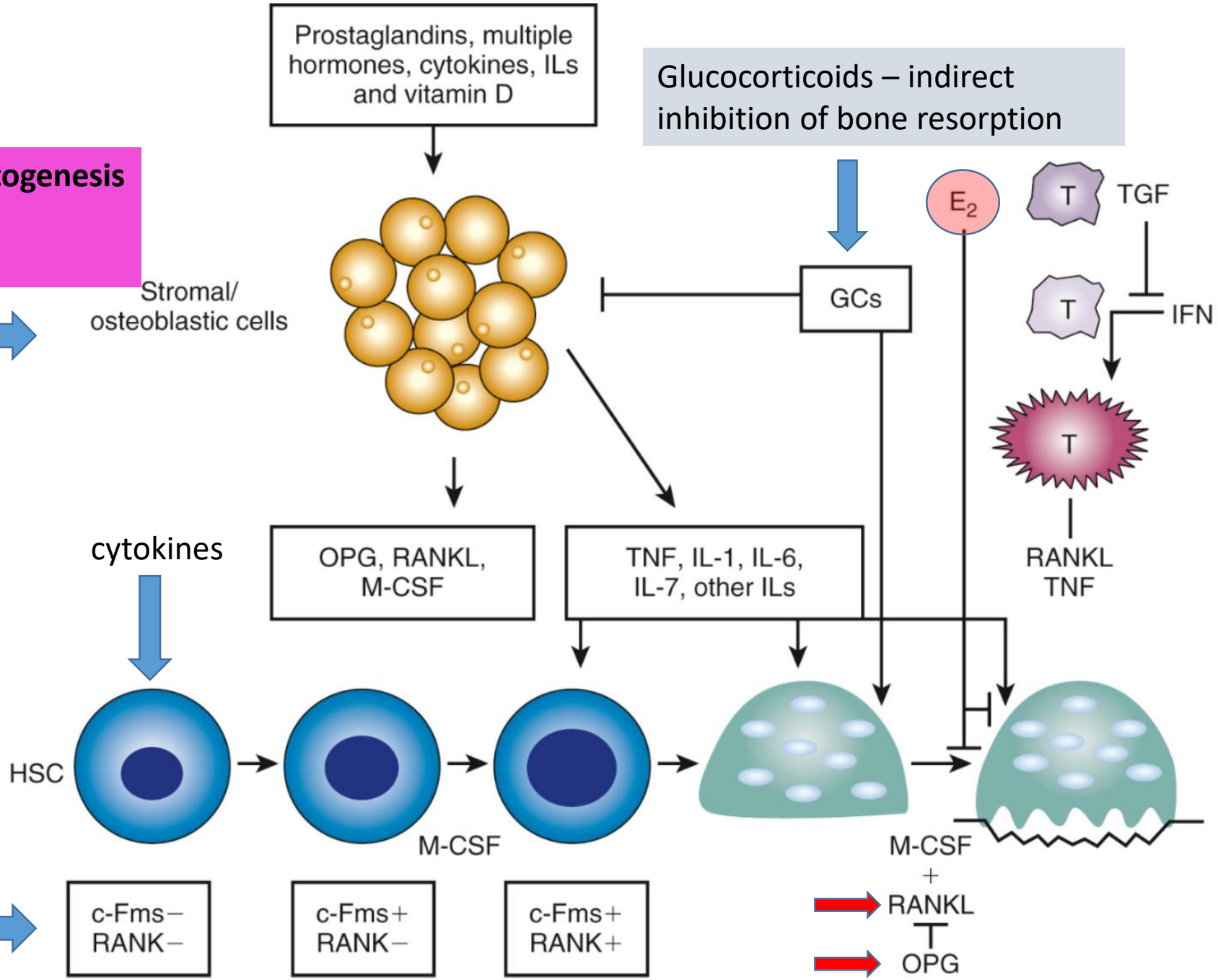
**Osteoclastogenesis**
**(+) RANKL**
**(-) OPG**

Production of mixture of pro- and anticlastogenic factors (differences in time)

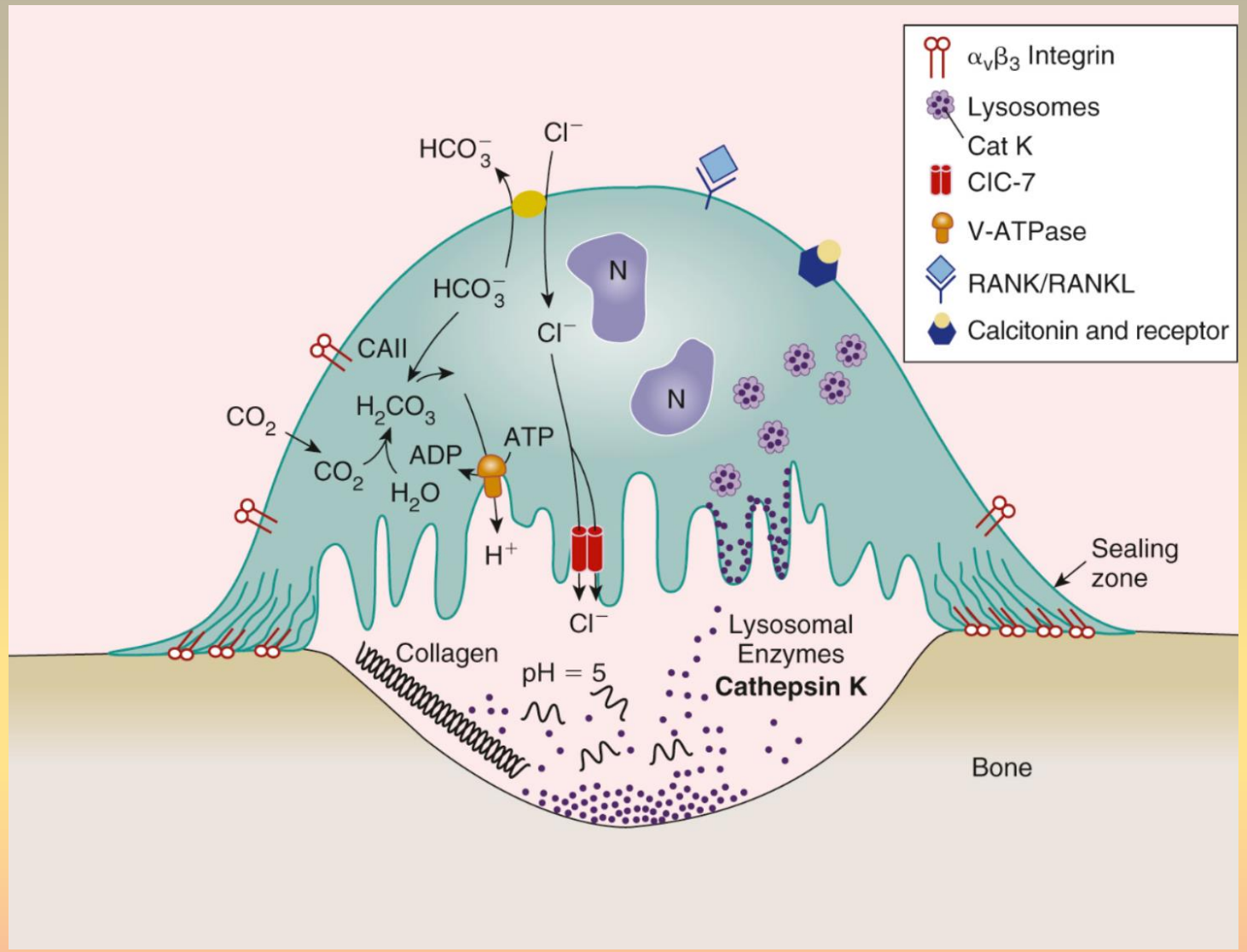
E2 (estrogens) – inhibition of T cell activation= inhibition of RANKL and TNF-α secretion

Sex hormones – regulation of osteoblasts and osteoclasts differentiation, including length of their life

Expression of different receptors in time (effect of various stimuli)



# 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

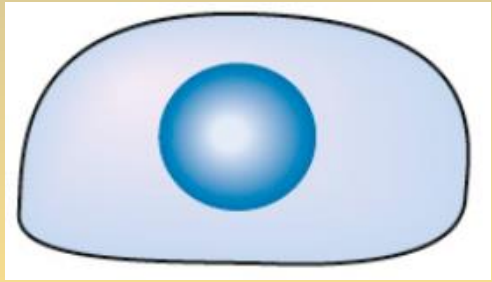
# Factors influencing bone tissue remodeling

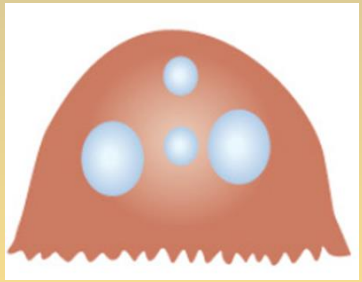
Resorption takes approx. 2 weeks
Mineralization and formation approx. 12 weeks

**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

Immediate calcium need - homeostasis

Ensuring mechanical requirements

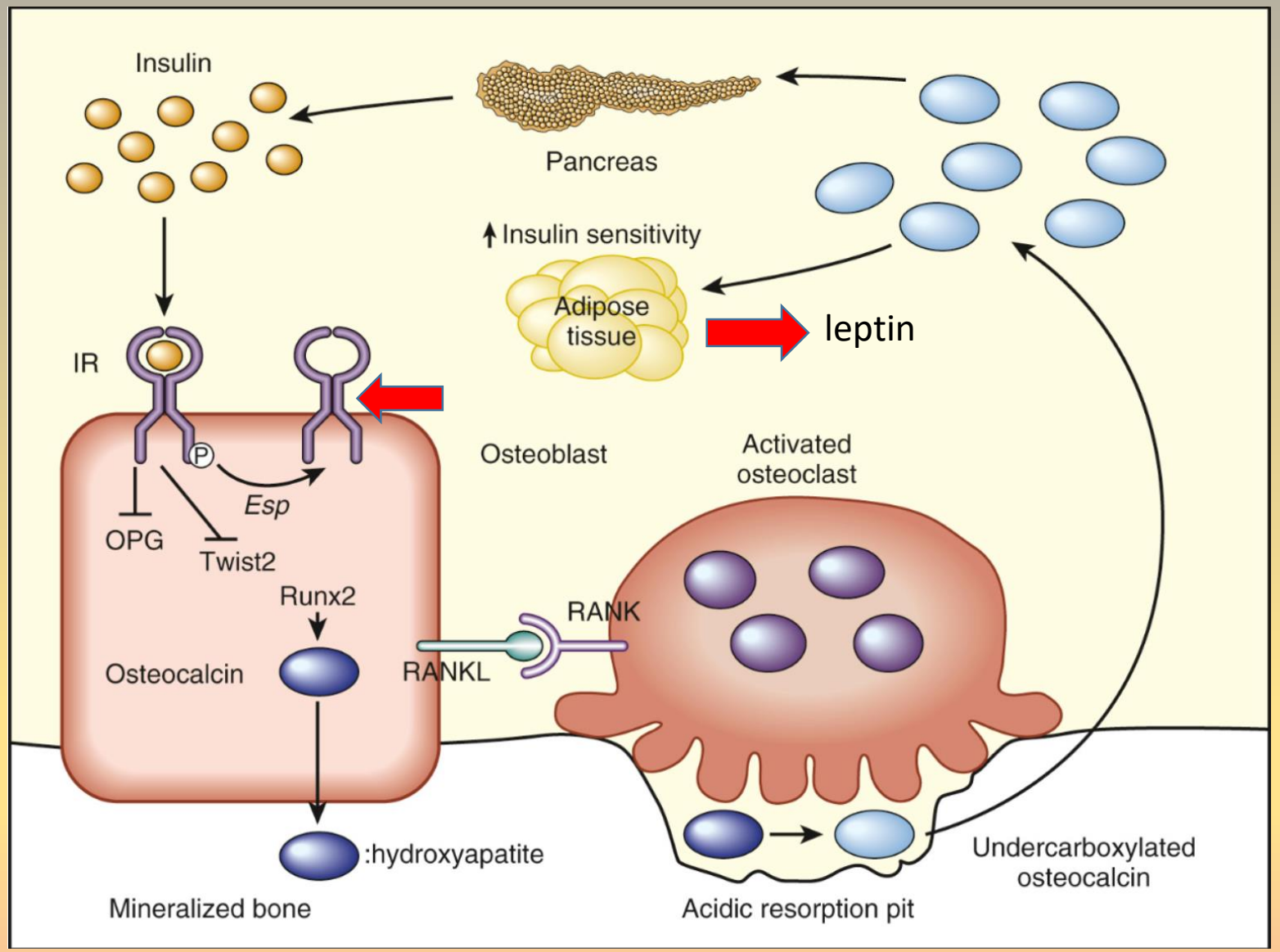
| − | + |
|---|---|
| Cytokines - IL-1α, IL-1β, TNF-α, TNF-β, proinflammatory IL (7, 15, 17) | Cytokines - IL-4, IL-13, IL-10, IL-18 |
| TGF-α and EGF, FGF21, FGF23 | Prostaglandins |
| Prostaglandins | VEGFA, HIF-1α (+/-) |
| PDGF | IGF-1 (endo-/paracrine) |
| | BMPs (OB, autocrine) |

# Endocrine regulation of bone tissue

| Hormone | Effect | Target cells |
|---|---|---|
| **PTH** | - Stimulation of resorption (long-term effect)<br>- Stimulation of bone formation (pulsatile effect)<br>- Stimulation of local secretion of IL-1 and IL-6 | Osteoblasts, lining cells, osteocytes |
| **Vitamin D** | - Stimulation of resorption (higher concentration)<br>- Inhibition of mineralization (higher concentration)<br>- Stimulation of bone formation (low concentrations, with PTH) | Osteoblasts (primarily) |
| **Calcitonin** | - Inhibition of resorption<br>- Regulation of bone tissue remodeling | Osteoclasts |
| **Growth hormone IGF-1** | - Stimulation of bone turnover<br>- Stimulation of osteoblast proliferation and differentiation<br>- Increased synthesis of collagen and other proteins | Osteoblasts – primarily GH<br>Osteoblasts and osteoclasts – IGF-1 |
| **Glucocorticoids** | - Decreased absorption of Ca in GIT<br>- Induction of osteoclastogenesis<br>- Increased bone resorption (+ RANKL)<br>- Suppressed remodeling of bone tissue<br>- Induction of apoptosis in osteoblasts and osteocytes<br>- Inhibition of IGF-1 synthesis | Osteoblasts, osteocytes, osteoclasts |
| **Thyroid hormones** | - Children – Stimulation of mineralization and epiphyseal maturation<br>- Adults – increased resorption<br>- Chondrocyte growth and proliferation (permissive effect on growth hormone)<br>- Increased transcription of collagenase and gelatinase | Osteoblasts, osteoclasts (also indirect through TSH) |
| **Insulin** | - Stimulation of bone tissue formation and mineralization<br>- Increased collagen synthesis<br>- Stimulation of IGF-1 secretion | Primarily osteoblasts |
| **Sex hormones** | - Epiphyseal closure (E)<br>- Inhibition of RANKL secretion<br>- Changes in speed of bone resorption and formation (stimulation of formation and mineralization) | Primarily osteoblasts, also other bone cells |
| **Prolactin** | - Indirect effect | |

# Insulin – osteocalcin axis

**insulin**




**Decarboxylated OCN (Glu13-OCN)**

# Bone tissue metabolism markers

| | |
|---|---|
| Ionized calcium | 8.5 – 10.5 mg/dL |
| Plasmatic phosphates | 3 – 4.5 mg/dL |
| PTH | 10 – 65 pg/mL |
| Vitamin D | 30 – 100 ng/mL |

| Marker | Tissue origin | Analytical sample | Analytical method |
|---|---|---|---|
| **Hydroxyproline, total and dialyzable (OH-Pro, OHP);** specific for all fibrilar collagens and a part of collagen proteins, including Ciq and elastin; present in newly synthesized and mature collagen | bone, skin, cartilage, soft tissues | urine | colorimetry, HPLC |
| **Pyridinoline (PYD, Pyr);** high concentrations in cartilage and bone collagen: not present in skin; present only in mature collagen | bone, tendon, cartilage | urine | HPLC, ELISA |
| **Deoxypyrindoline (DPD, d-Pyr);** high concentrations only in bone collagen: not present in cartilage or in skin; present only in mature collagen | bone, dentine | urine | HPLC, ELISA |
| **Cross-linked C-terminal telopeptide of type I collagen (ICTP);** high proportion from bone collagen in type I collagen; can partly originate from newly synthesized collagen | bone, skin | serum | RIA |
| **Cross-linked C-terminal telopeptide of type I collagen (fragments alpha-CTX, beta-CTX);** in type I collagen; probably high proportion from bone collagen | all tissue containing type I collagen | urine, serum | ELISA, RIA, ECLIA |
| **Cross-linked N-terminal telopeptide of type I collagen (fragments NTX);** in type I collagen; big proportion from bone | all tissue containing type I collagen | urine (alpha/beta), serum (only beta) | ELISA, RIA, ICMA |
| **Hydroxylysine-glycosides (Hyl-Glyc);** collagens and collagen proteins; glucogalactosyl- hydroxilysine is highly represented in soft tissue collagens and C1q; galactosil-OHLys is highly represented in bone collagen | bone, skin, soft tissue, serum complement | urine | HPLC, ELISA |
| **Bone sialoprotein (BSP);** synthesized by active osteoblasts and lay in extracellular bone matrix; it seems to express osteoclast activity | bone, dentine, hypertrophic catrilage | serum | RIA, ELISA |
| **Tartarat-resistant acid phosphatase (TR-ACP);** osteoclasts, thrombocytes, erythrocytes | bone, blood | plasma/serum | colorimetry, RIA, ELISA |
| **Free gamma carboxyglutamin acid (GLA);** resulted from bone proteins (e.g. osteocalcin, matrix Gla protein) and from coagulation factor | blood, bone | serum/urine | HPLC |

HPLC – high performance liquid chromatography; ELISA – enzyme-linked immunosorbent assay; RIA – radio immuno assay; ECLIA – electrochemiluminiscence immunoassay; ICMA – immunochemiluminometric assay

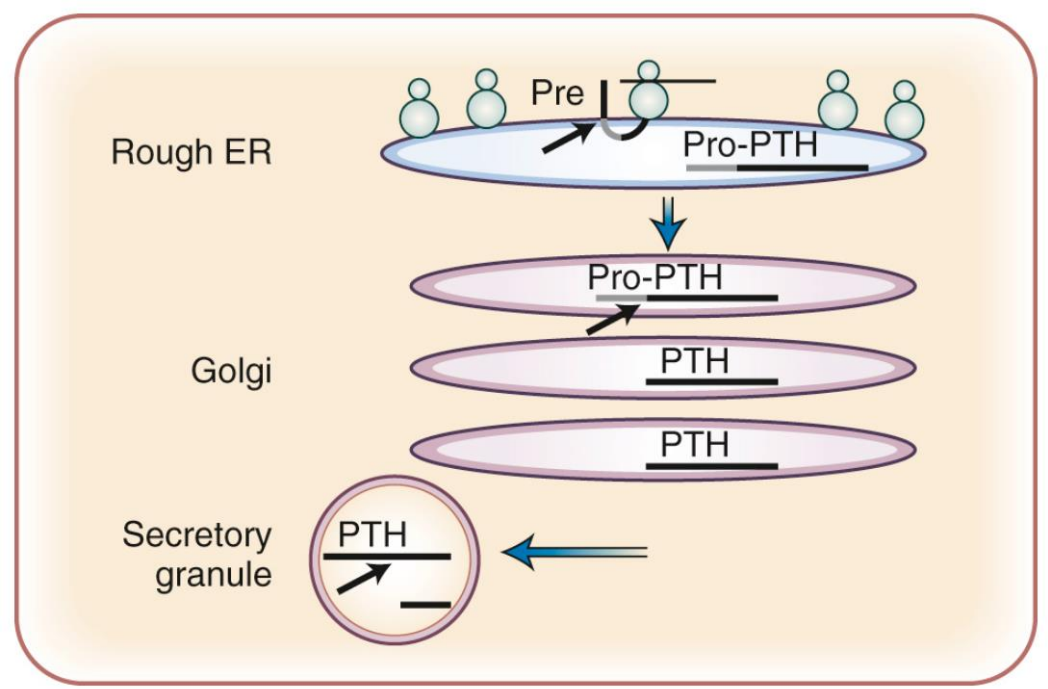
# Clinical relevance

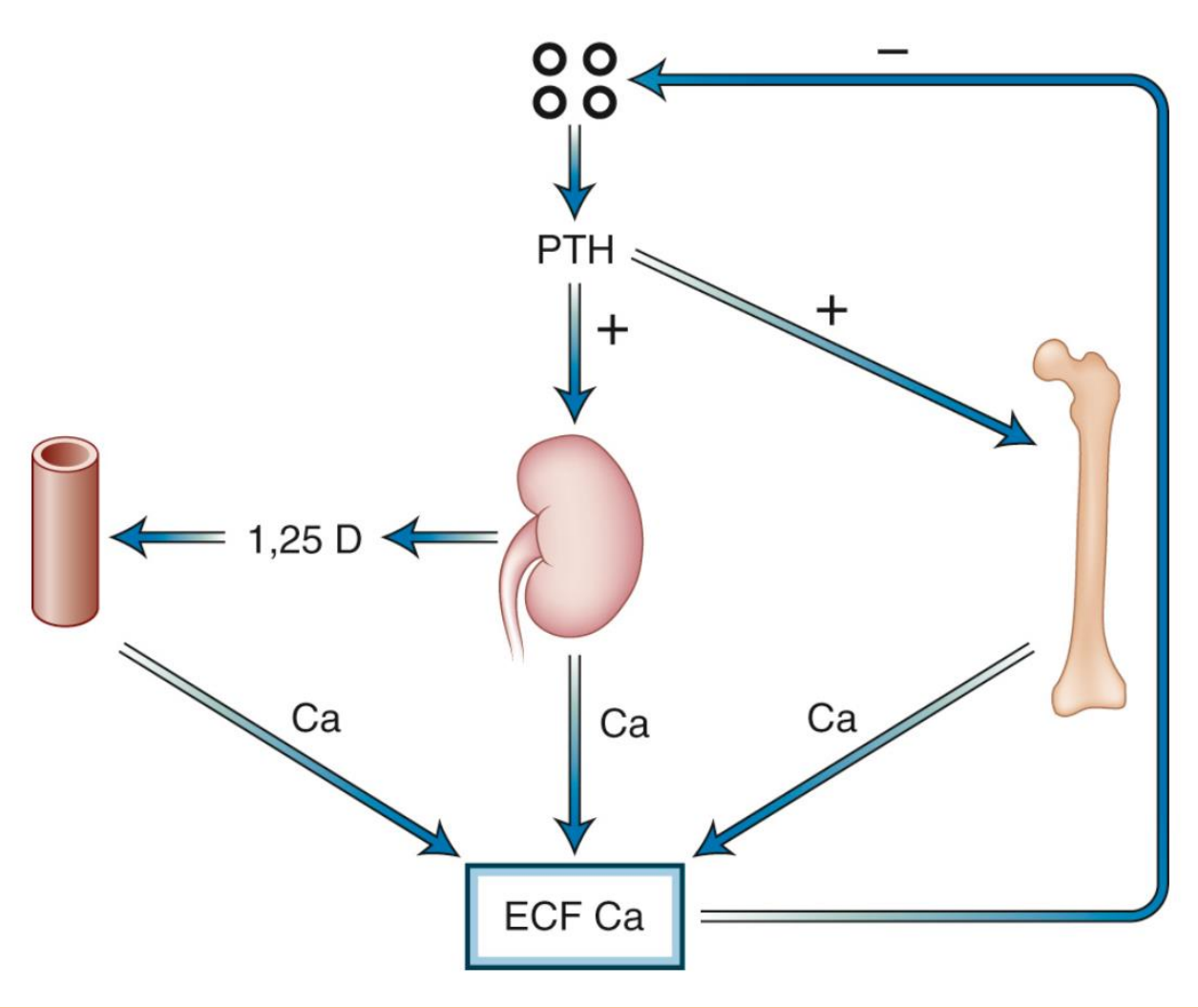
- Osteogenesis imperfecta
- Osteopetrosis
- Osteomalacia
- Rachitis
- Osteopenia – T score -1 – -2.5
- Osteoporosis – T score under -2.5

# Parathormone

Characteristics
- Parathyroid glands – chief cells
  - Synthesis and storage of PTH
  - Very quick secretion of PTH
  - Ability to proliferate during long-term stimulation

PTH
- Synthetized as pre-pro-PTH
- Several types of secretion granules (PTH; PTH+cathepsin B, H)
- Very quick metabolization (70 % liver, 20 % kidneys) – 2 min
- Presence of several types of fragments
- PTHR1, PTHR2, PTHR3 – G prot.



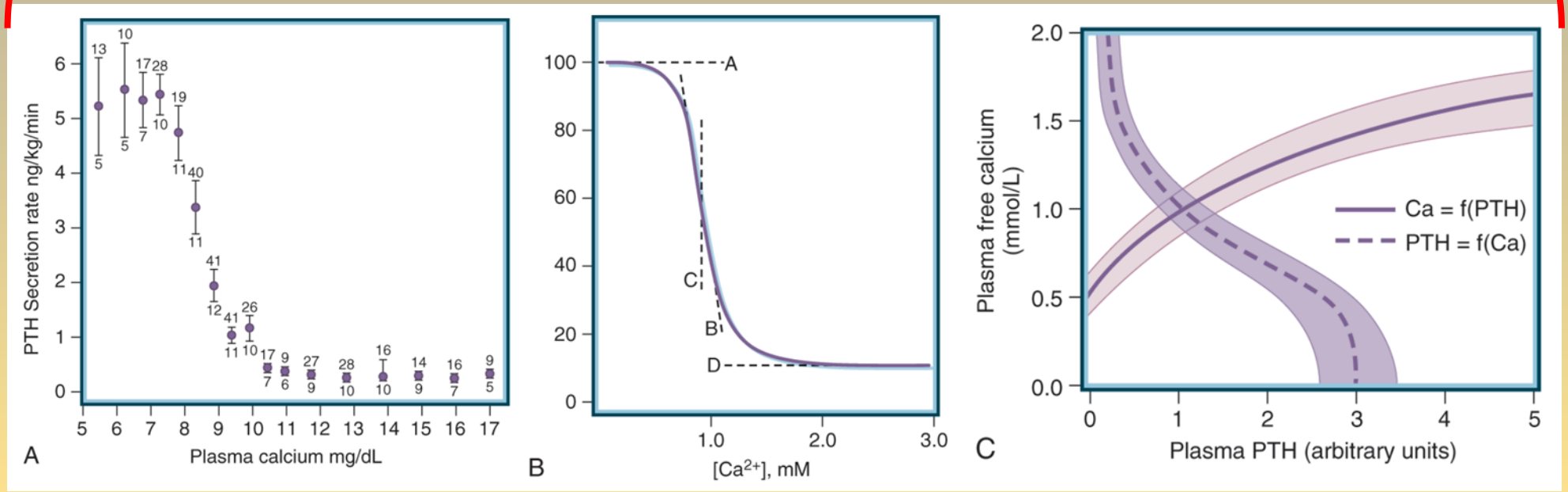
# PTH secretion

Cell proliferation of chief cells is an important adaptive mechanism for:

- Hypocalcemia
- Low levels of vitamin D($1,25(OH)_2D_3$)
- Hyperphosphatemia (uremia)
- Neoplastic growth

Maximal secretion (reserve capacity)

Minimal secretion



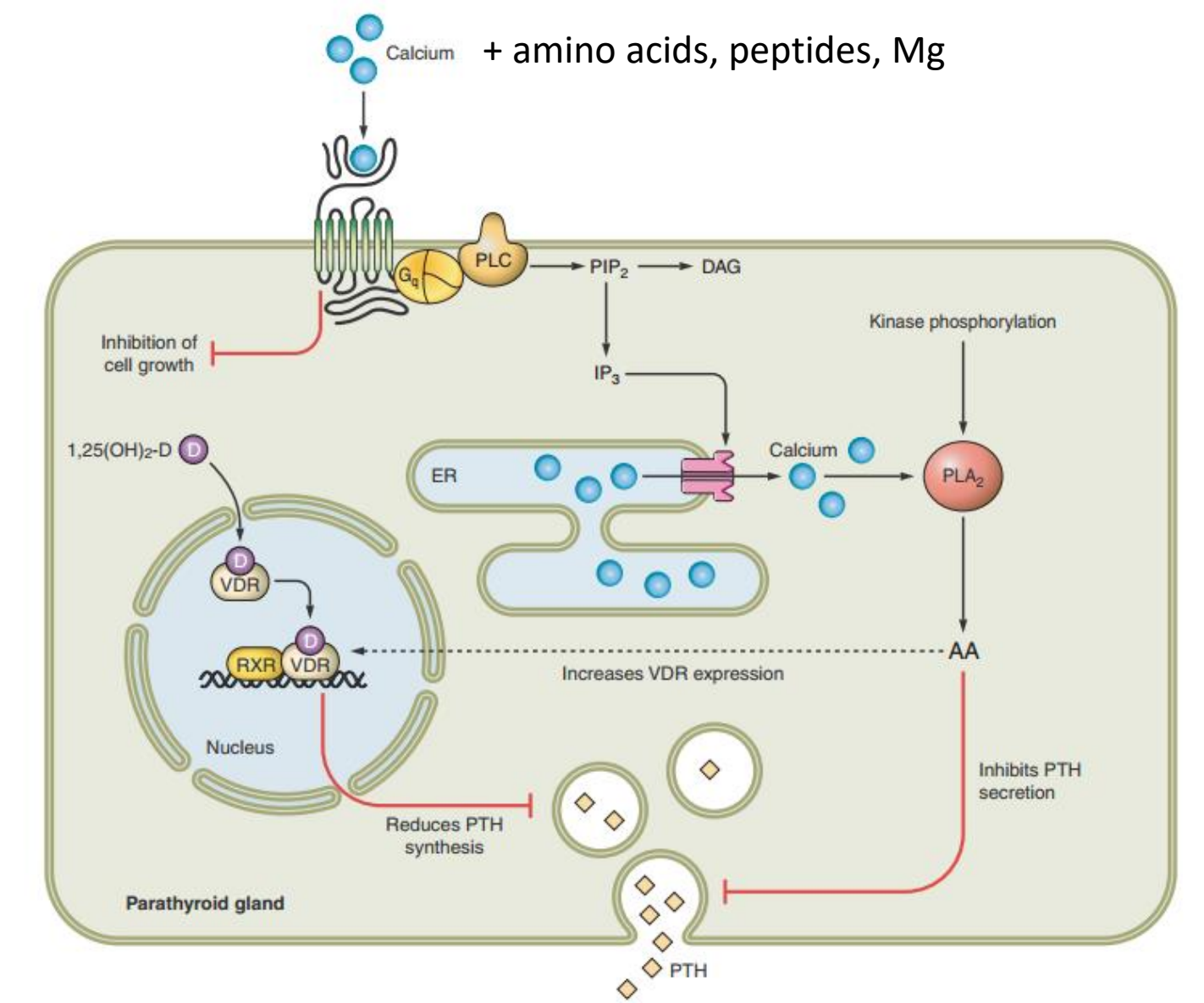

**Level of ionized calcium in blood is a key parameter for PTH secretion.**

**During sudden decrease of ionized calcium is PTH secretion increased.**

**Vitamin D decreases PTH secretion (inhibits expression and production of PTH), NOT during chronic hypocalcemia**

**Phosphates stimulate production and secretion of PTH with delay.**

# 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

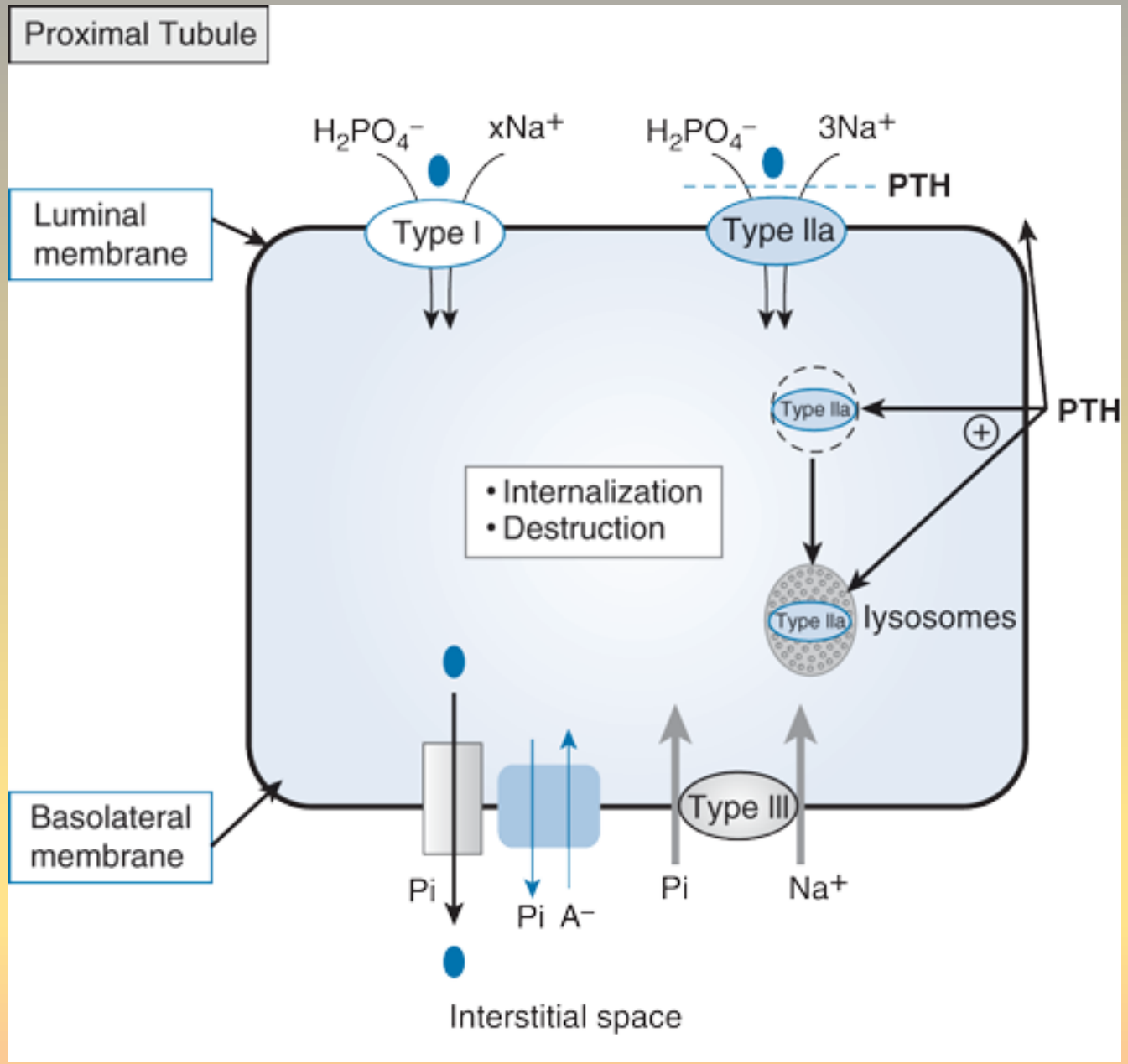
Clinical aspects
- Mutation – inactivation/activation
- familial hypocalciuric hypercalcemia (in.)
- Familial hypoparathyroidism with hypercalciuria (ac.)
- Calcimimetics – inhibition of PTH secretion

# Main effects of PTH

(+) calcium resorption
- cTAHL, **DT**
- transcellular and paracellular transport
- TRPV5 and TRPV6 – $Ca^{2+}$ inhibition
- Calbindin-D28K
- NCX1 and PMCA

(+) phosphate excretion
- PT and DT
- Inhibition of resorption
- NaPi cotransporters – internalization, degradation

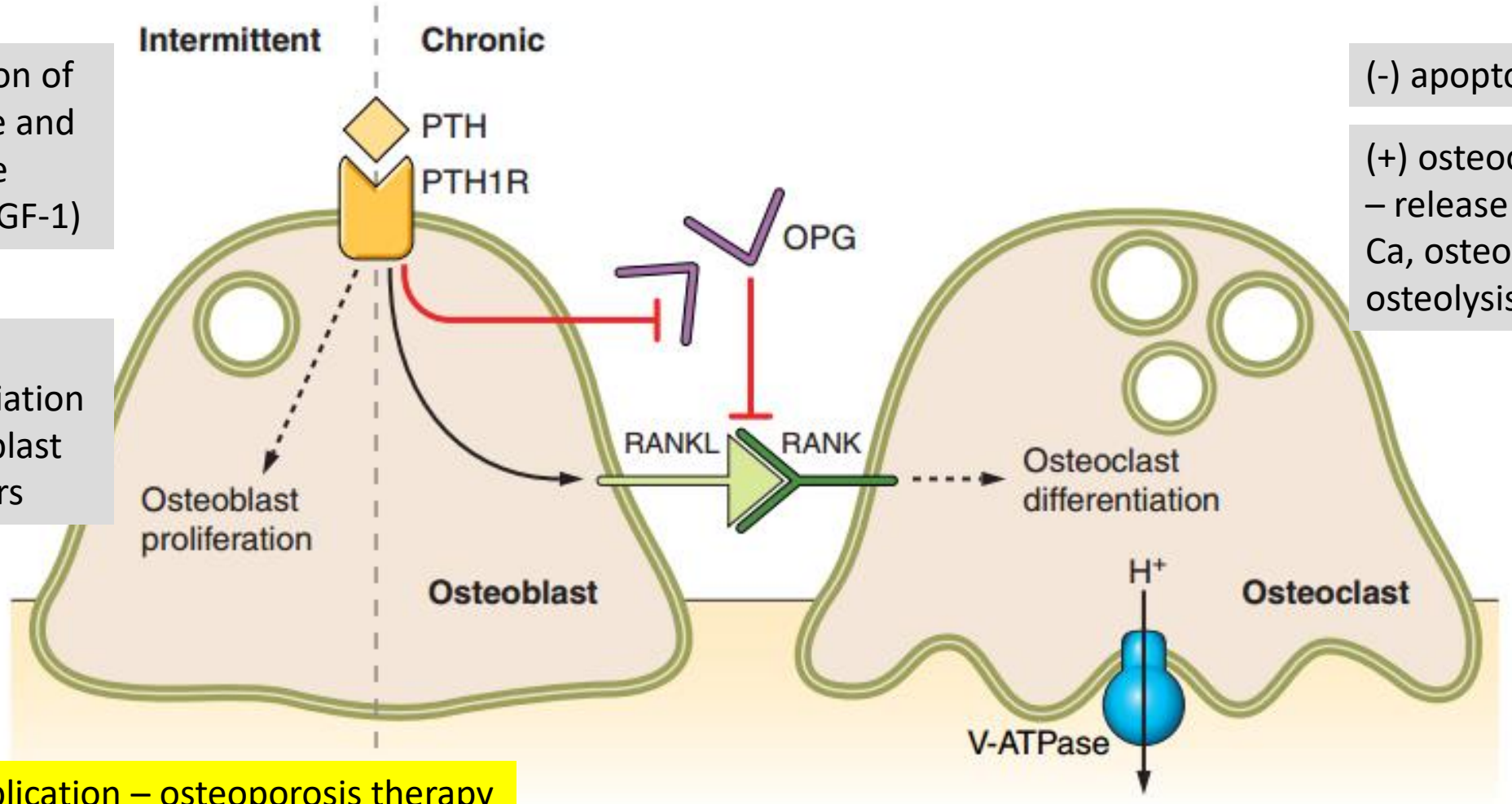
(+) activity of 1$\alpha$-hydroxylase - PT
(-) resorption of Na, water and bicarbonate – PT
(-) $Na^+/K^+$-APTase (basolateral membrane)
(+) gluconeogenesis – PT
(-) GFR - podocytes



# PTH and bone tissue physiology

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

+ differentiation of osteoblast precursors




(-) apoptosis

(+) osteocytes – release of Ca, osteocytic osteolysis

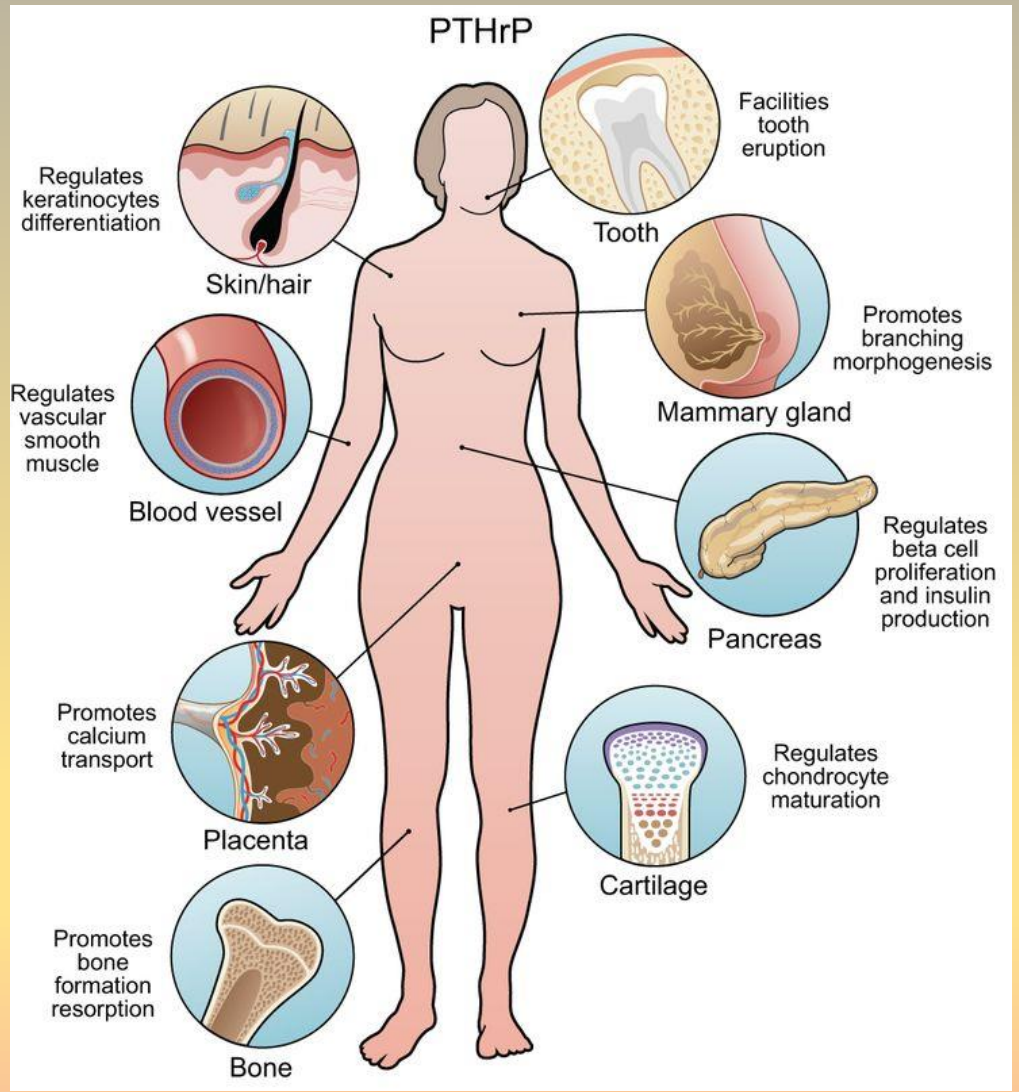
Clinical application – osteoporosis therapy

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

# Parathyroid Hormone-Related Peptide - PTHrP

Characteristics
- First as a peptide produced by tumors – endocrine effect – kidneys + bones
- Also paracrine – local increase of Ca concentration
- Later discovered in many tissues

Functions
- Calciotropic hormone
- Fetal development - proliferation and differentiation
- Lactation – (+) resorption of bone tissue without possibility to affect by Ca supplementation
- Skin – proliferation and differentiation
- GIT, bladder, uterus – (+) smooth muscles relaxation
- CNS - neuroprotection
- Para-/auto-/intracrine effect



# 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

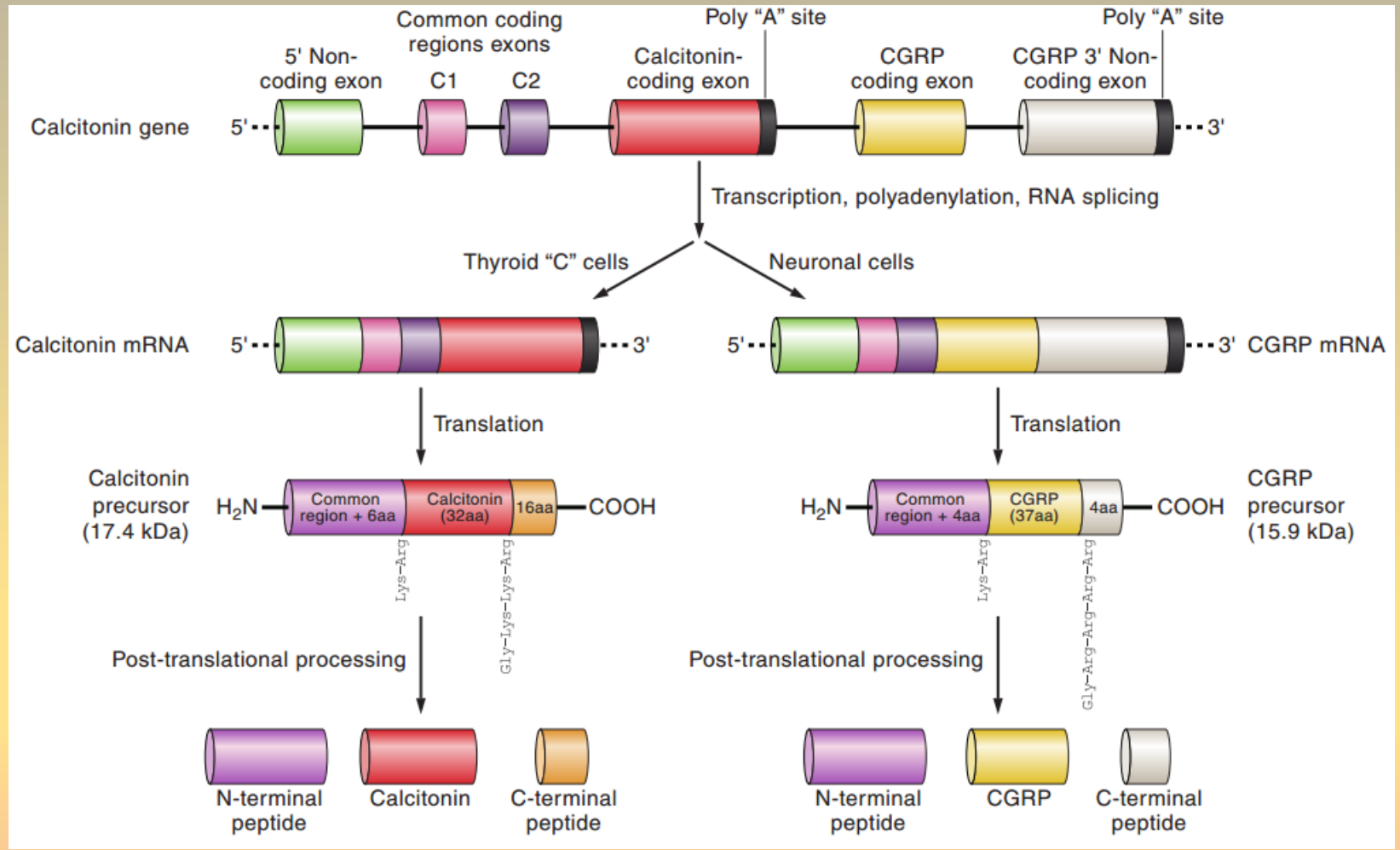
  } Function unclear
- Inhibition of secretion - somatostatin

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 gene, mRNA splicing and posttranslational modifications

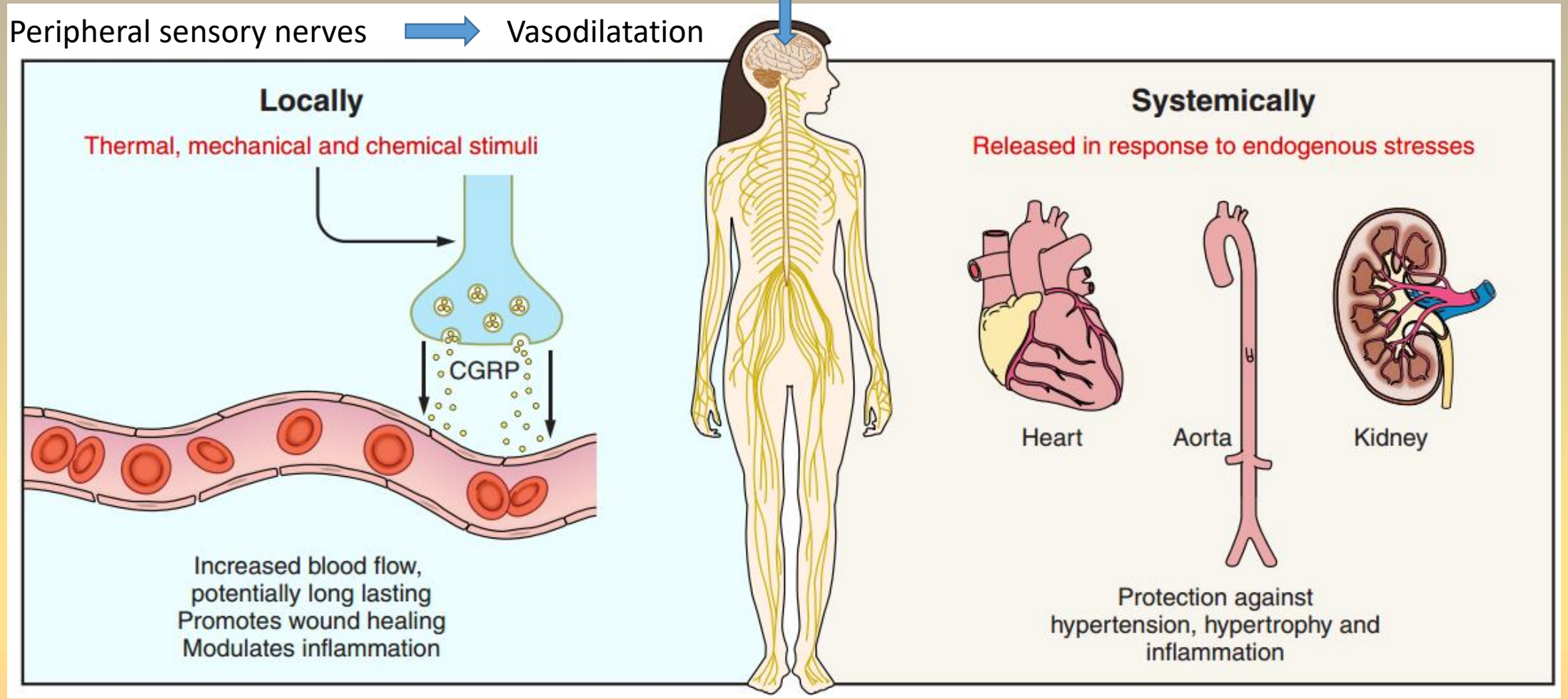
C cells




CNS and neurons in general

# Calcitonin gene-related peptide - CGRP

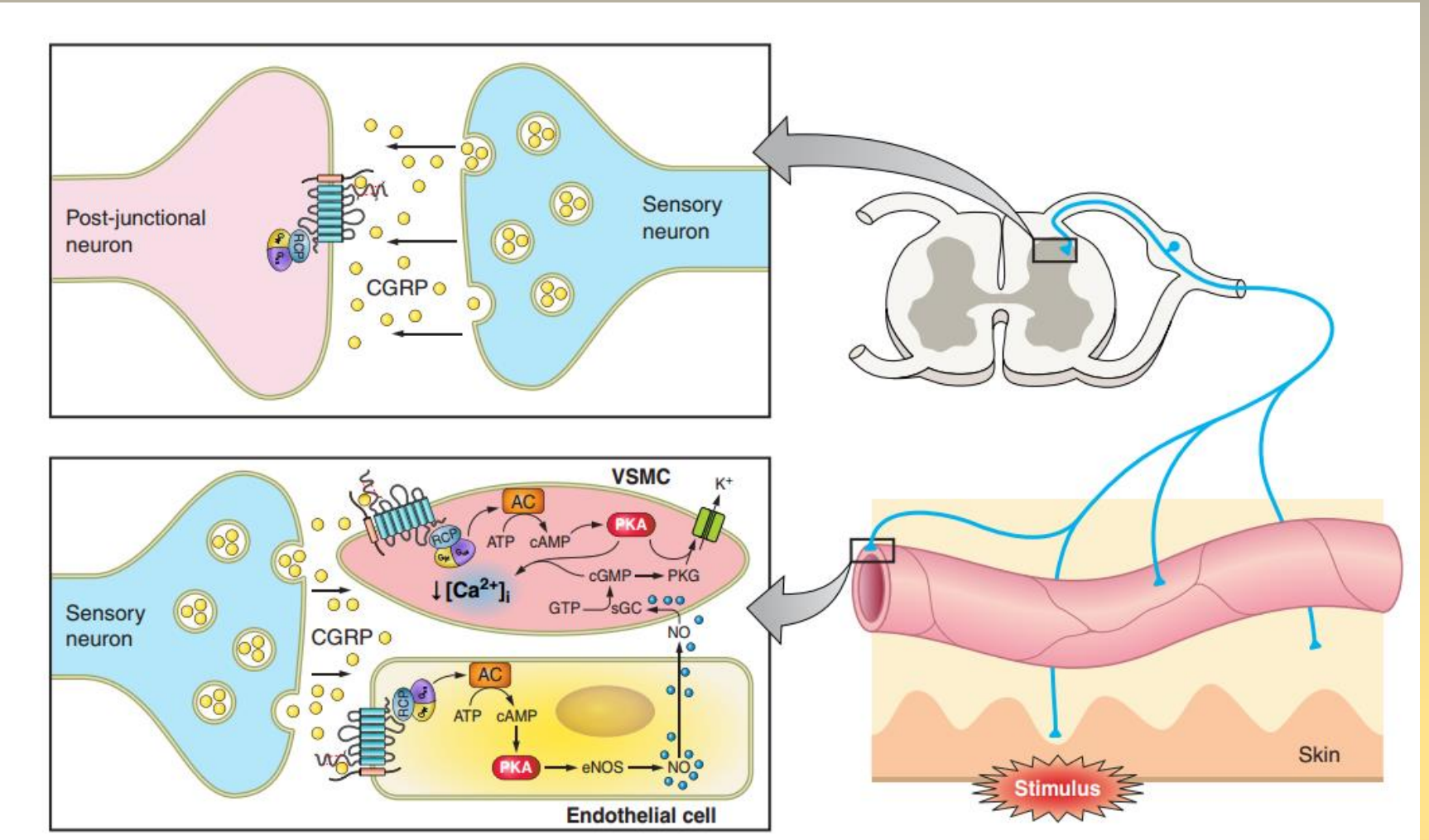
Neuropeptide – sensoric and integrative motoric functions




Russell FA, King R, Smillie SJ, Kodji X, Brain SD: **CALCITONIN GENE-RELATED PEPTIDE: PHYSIOLOGY AND PATHOPHYSIOLOGY.** ***Physiol Rev 2014, 94(4):1099-1142.***

# CGRP - functions

Vasodilatation induced by various mechanisms
- G prot.
- eNOS/NO

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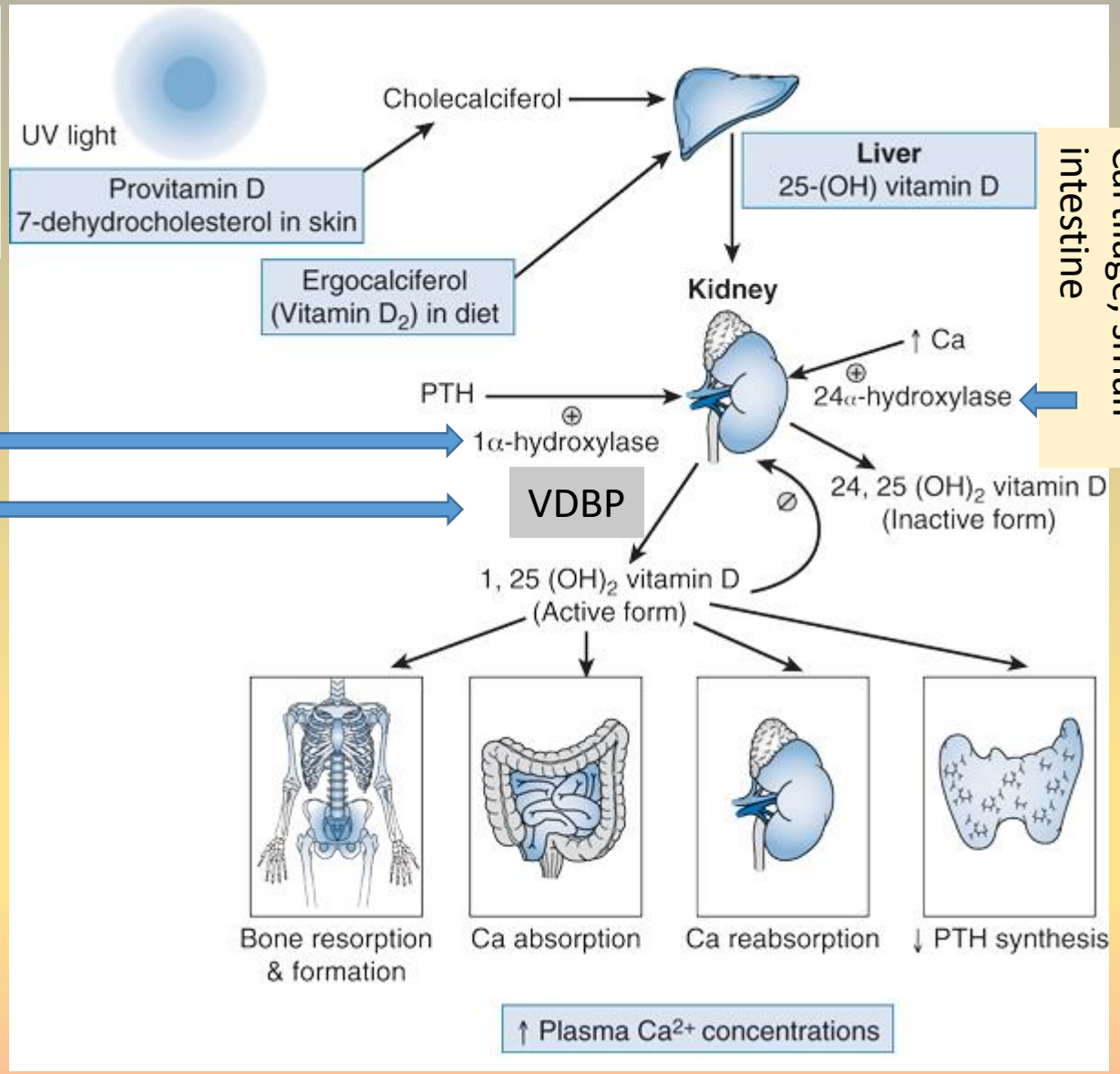
# Vitamin D….hormone?...vitamin?

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, c*alcitonin*, *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 $1,25(OH)_2D$
- Level of circulating $1,25(OH)_2D$
- 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

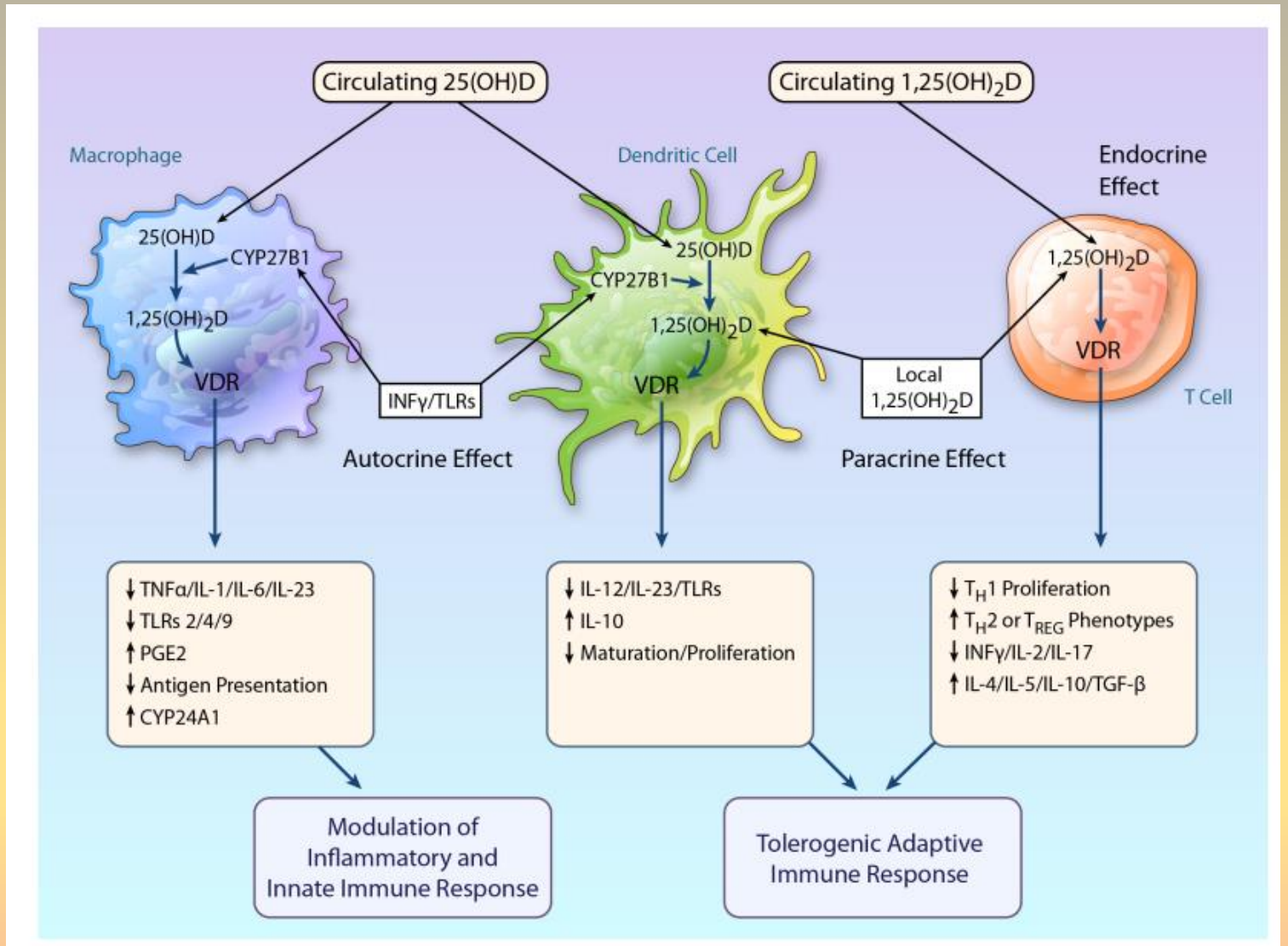
Bones and bone tissue
- (-) collagen synthesis
- (+) osteocalcin synthesis
- (+) osteoclasts differentiation – osteoclastogenesis
- (+) RANKL
- Main function – ensuring the stability of the bone microenvironment for mineralization by the standard intake and availability of Ca and phosphates

Vitamin D and Ca absorption/reabsorption
- (+) CBP, AP, $Ca^{2+}/Mg^{2+}$-ATPase
- (+) TRPV6 – absorption (GIT)
- (+/-) TRPV5 – reabsorption (kidneys)
- Calbindin-9K
- $1,25(OH)_2D$-inducible ATP-dependent $Ca^{2+}$ pump
- $Na^{+}/Ca^{2+}$ exchanger

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

# Vitamin D and immune system

Clinical relevance
- Analogue of vitamin D without ability to cause hypercalcemia
- Antiproliferative effect – treatment of cancer?
- Synergy with cyclosporin B – rejection of transplantates
- Suppression of PTH synthesis – 22-oxacalcitriol (hyperparathyroidismus)
- Psoriasis (clinical trials)




Macrophages
Dendritic cells
T cells

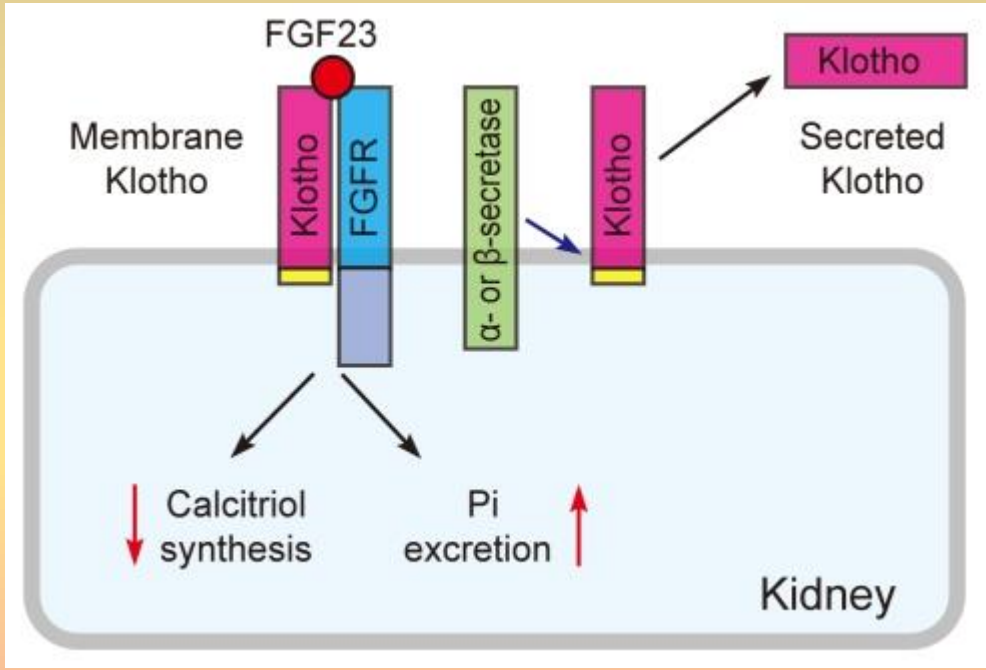
# FGF23 – fibroblast growth factor 23

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

Functions
- maintaining normophosphatemia and regulation of vitamin D metabolism
- Decreased expression of IIa, IIb, and IIc (NPT) – **phosphate transport**
- Increased 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



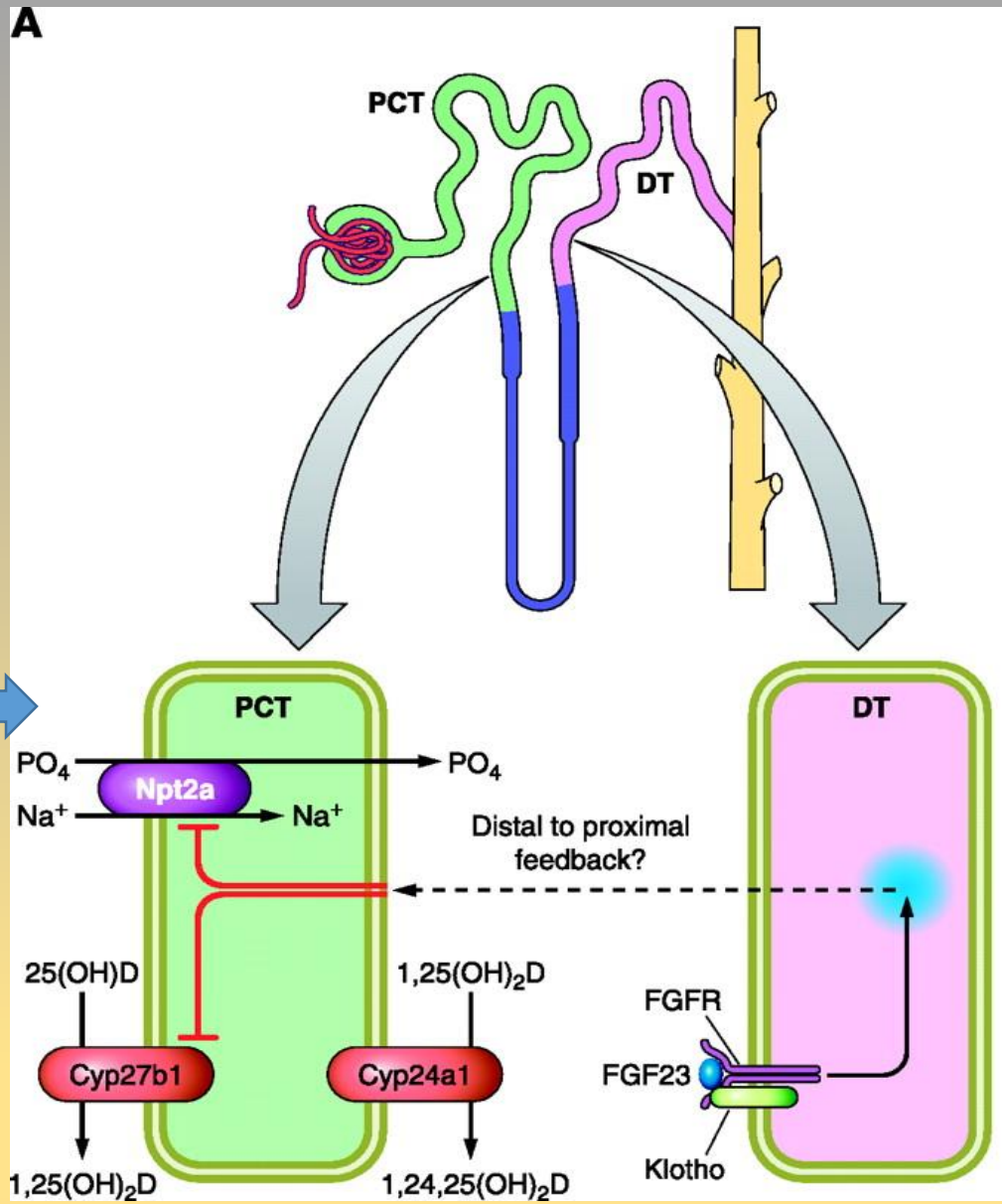
# FGF23

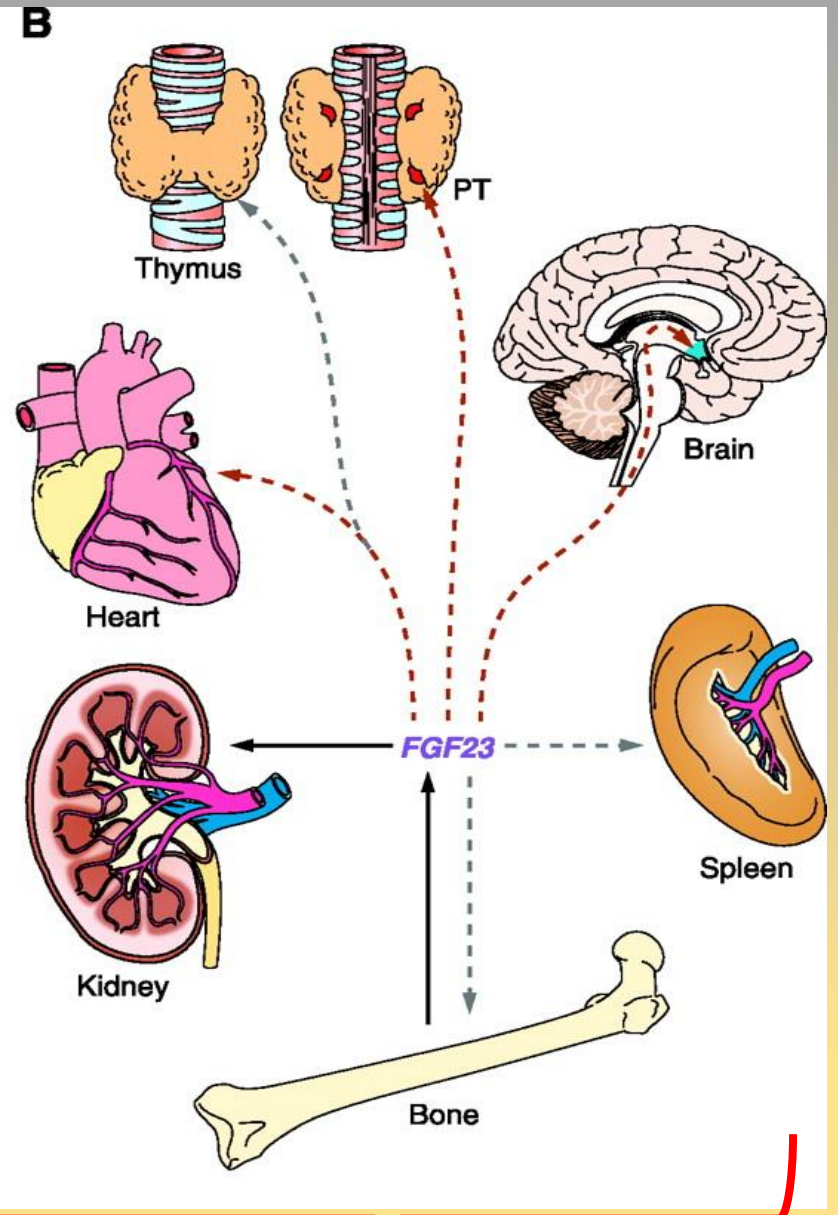
Pars convoluta PT

Inhibition

Cyp271b

Npt2a




$1, 25(OH)_2D$ phosphate Feedback mechanism between DT and PCT

**endocrine, paracrine**

# Calcium homeostasis – still just a simplified model