Control of calcium metabolism.

MUNI
MED

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

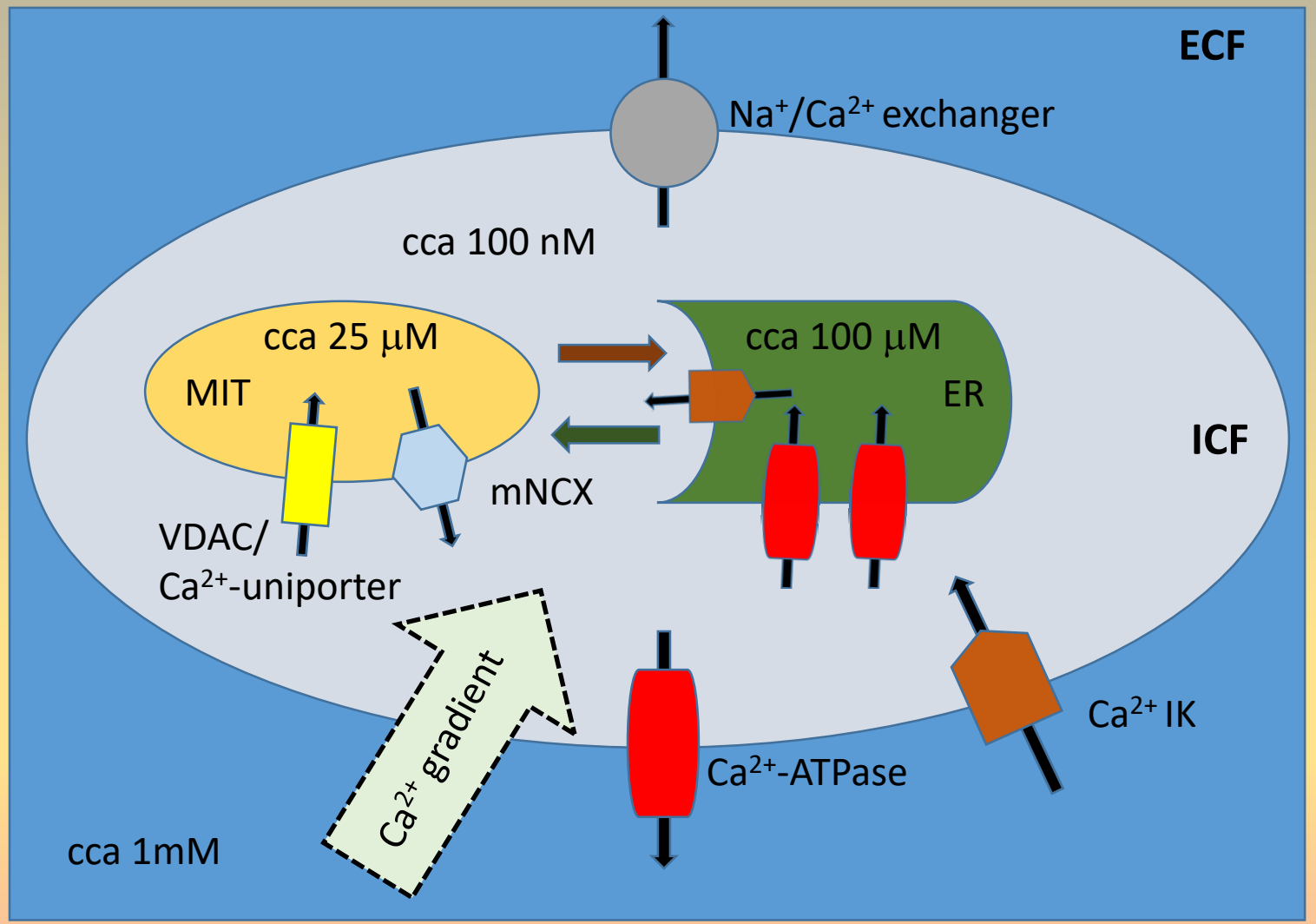
Bone tissue
- 99 % of overall Ca, of it 99 % in mineral component
- 1 % - quickly mobilizable and convertible (ICF - ECF)

	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

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

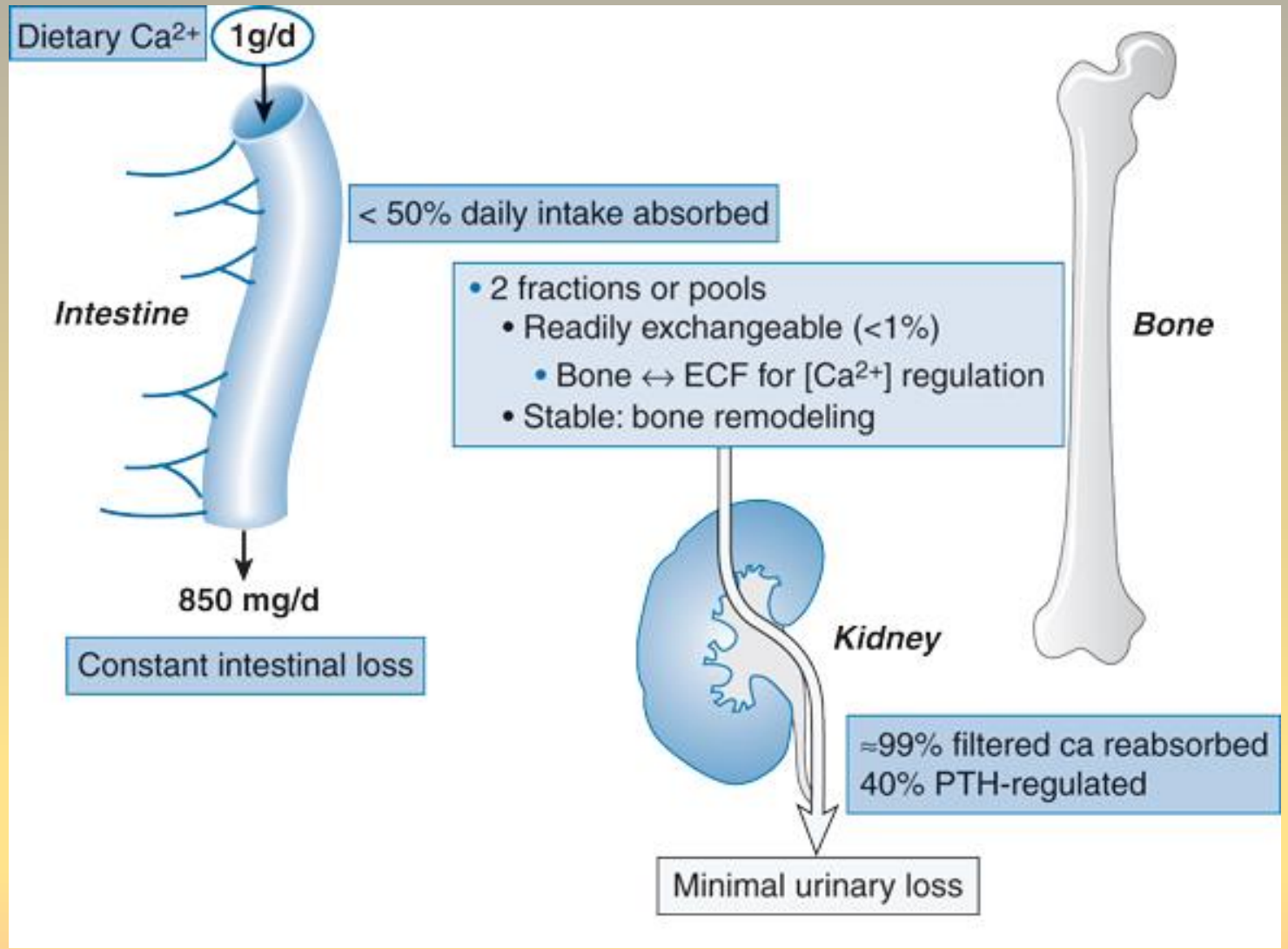


Calcium and its intake

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



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

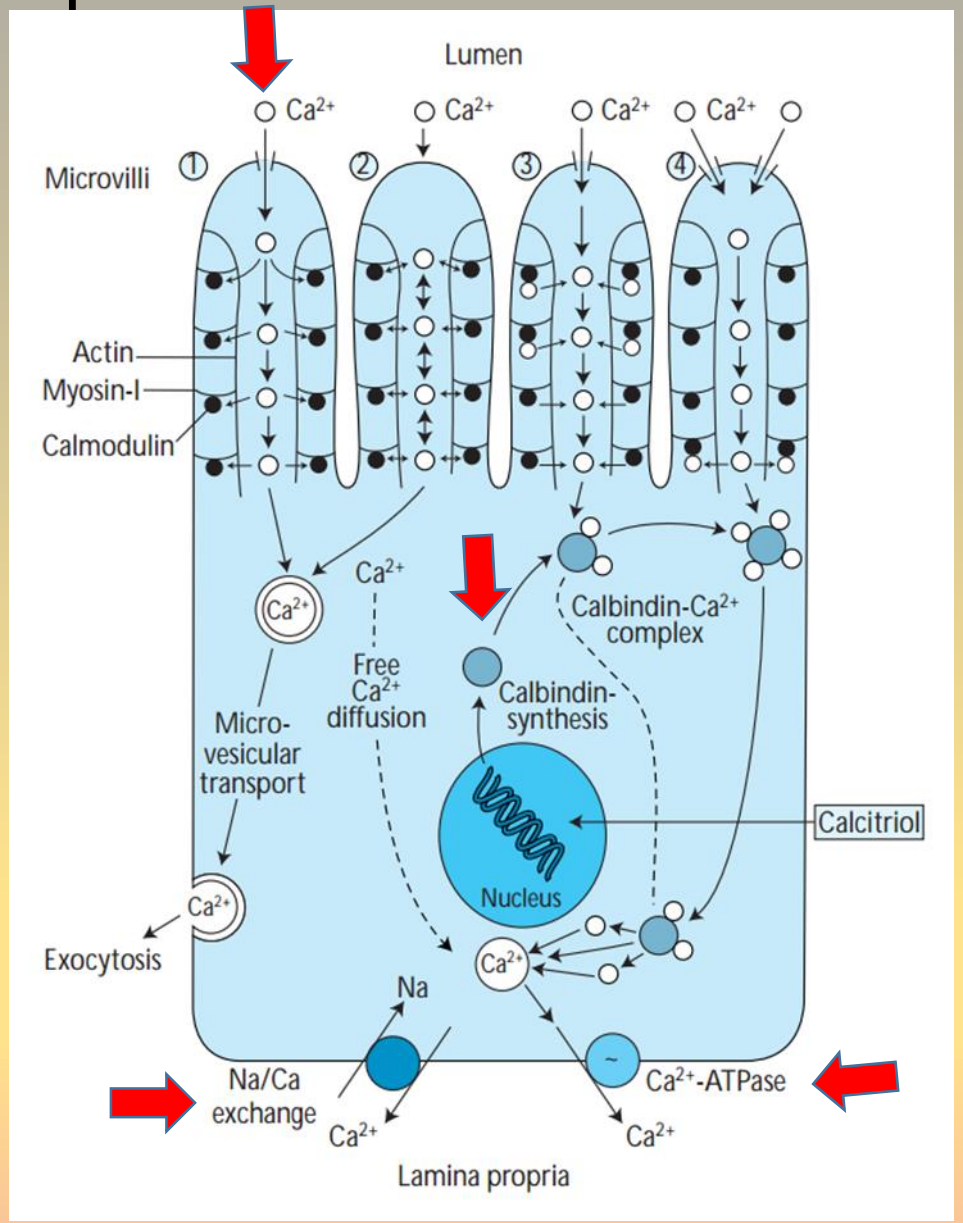
Mechanisms of calcium absorption

Vitamin D

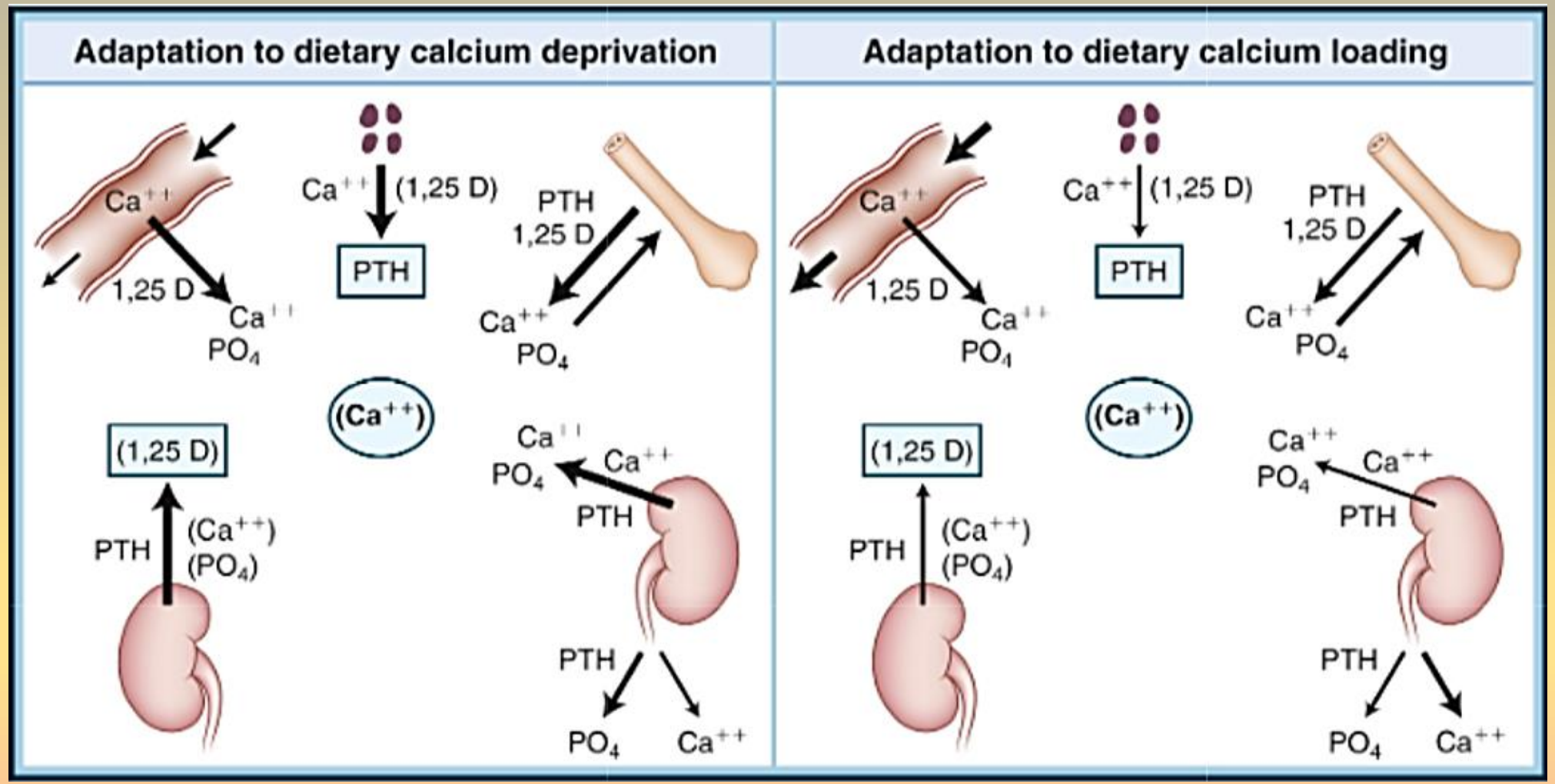
Paracellular
- Luminal electrochemical gradient
- Integrity of intercellular connections
- Claudins and their role in paracellular transport

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

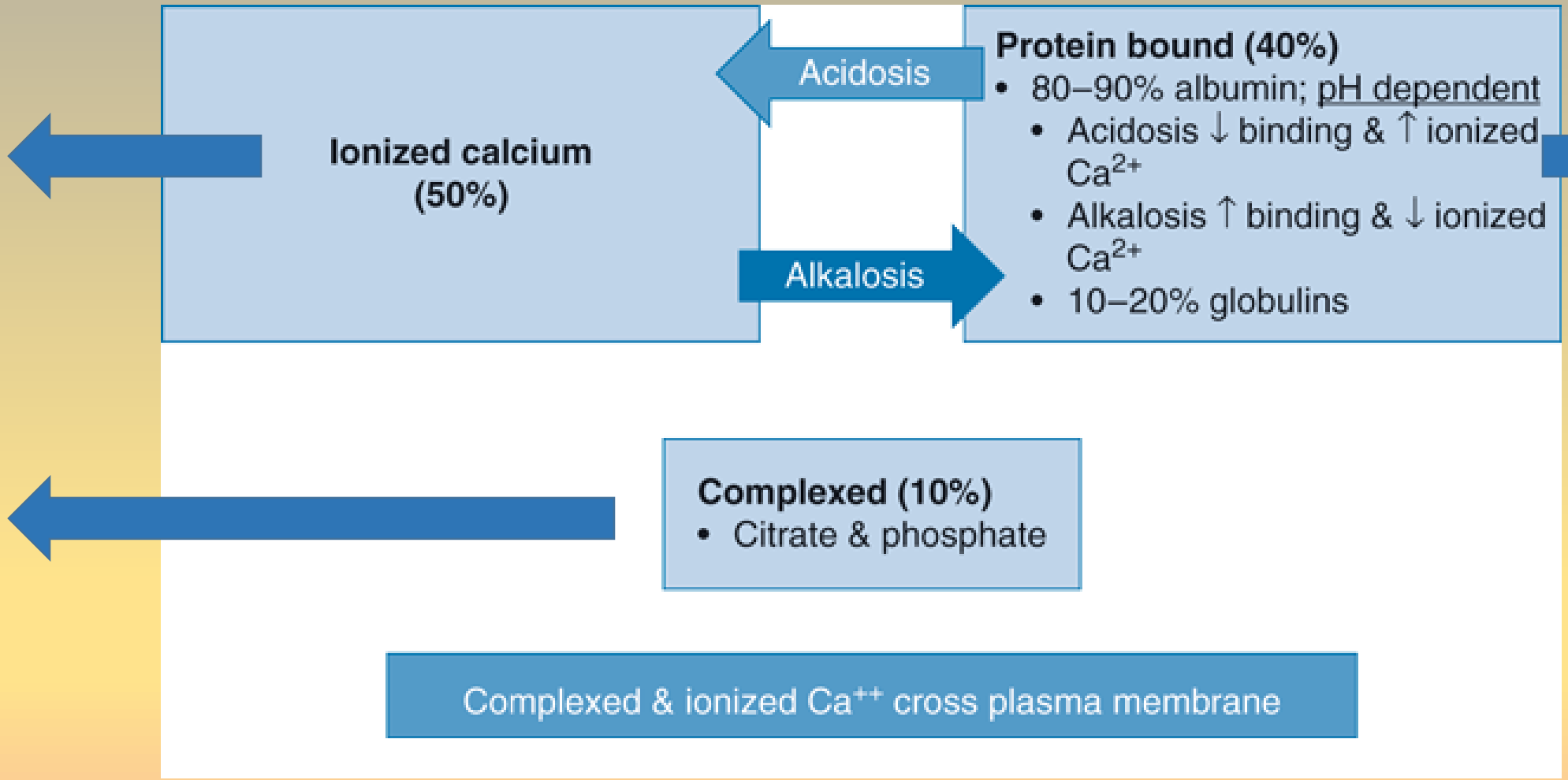
Glucocorticoids	Estradiol	Prolactin
-	+	+



Adaptation to dietary calcium levels



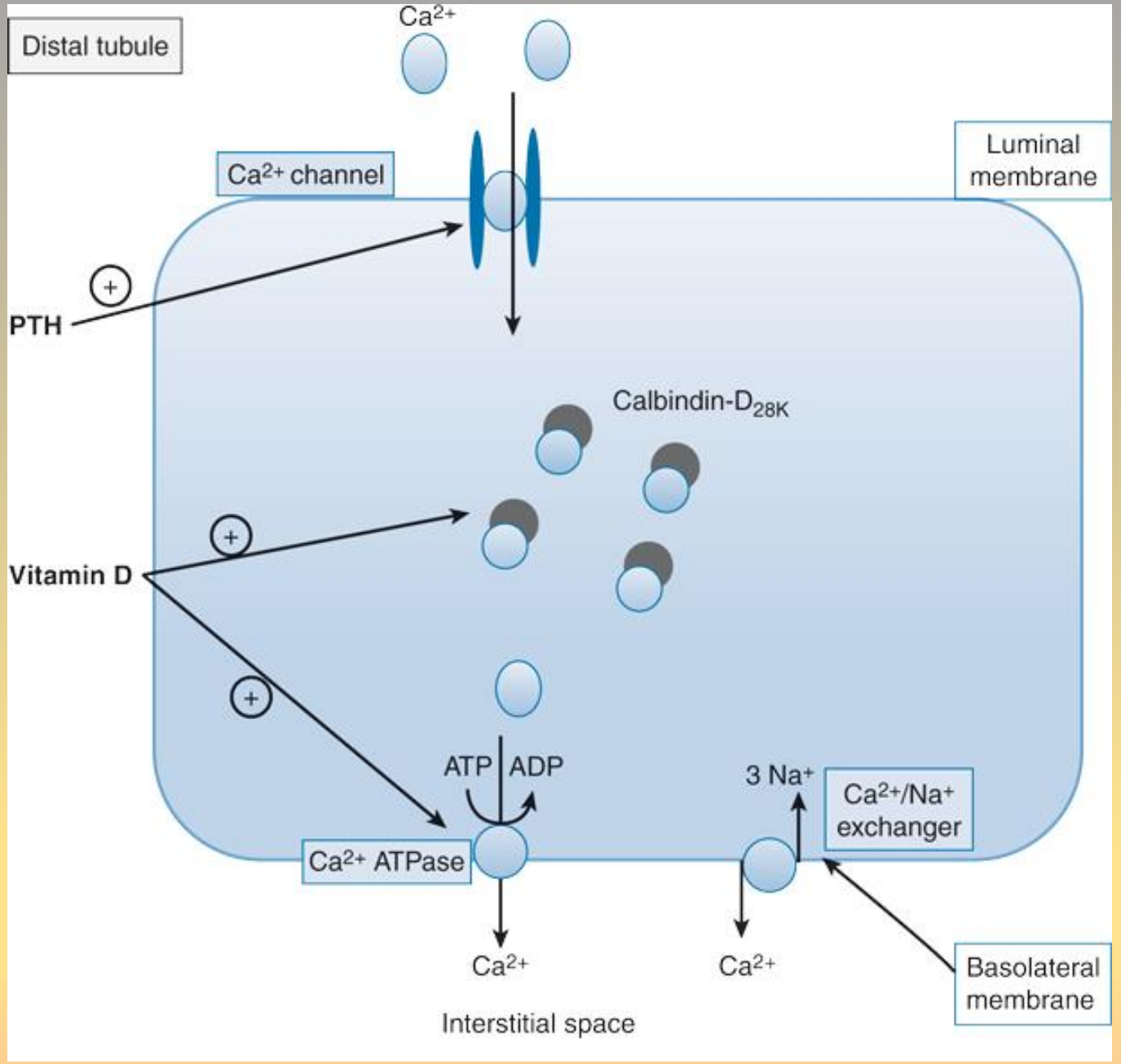
Calcium on blood (calcemia)



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



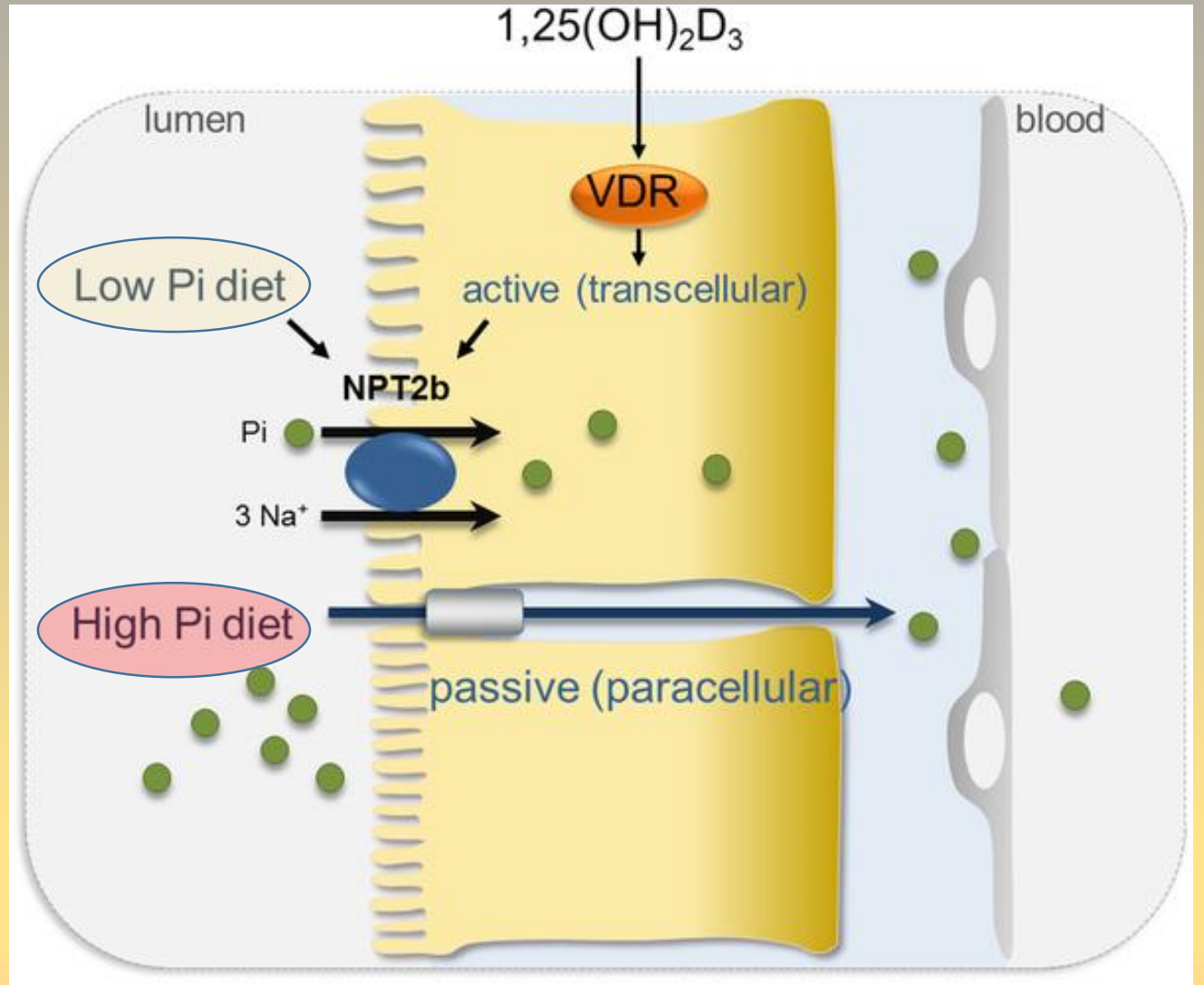
Phosphorus

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

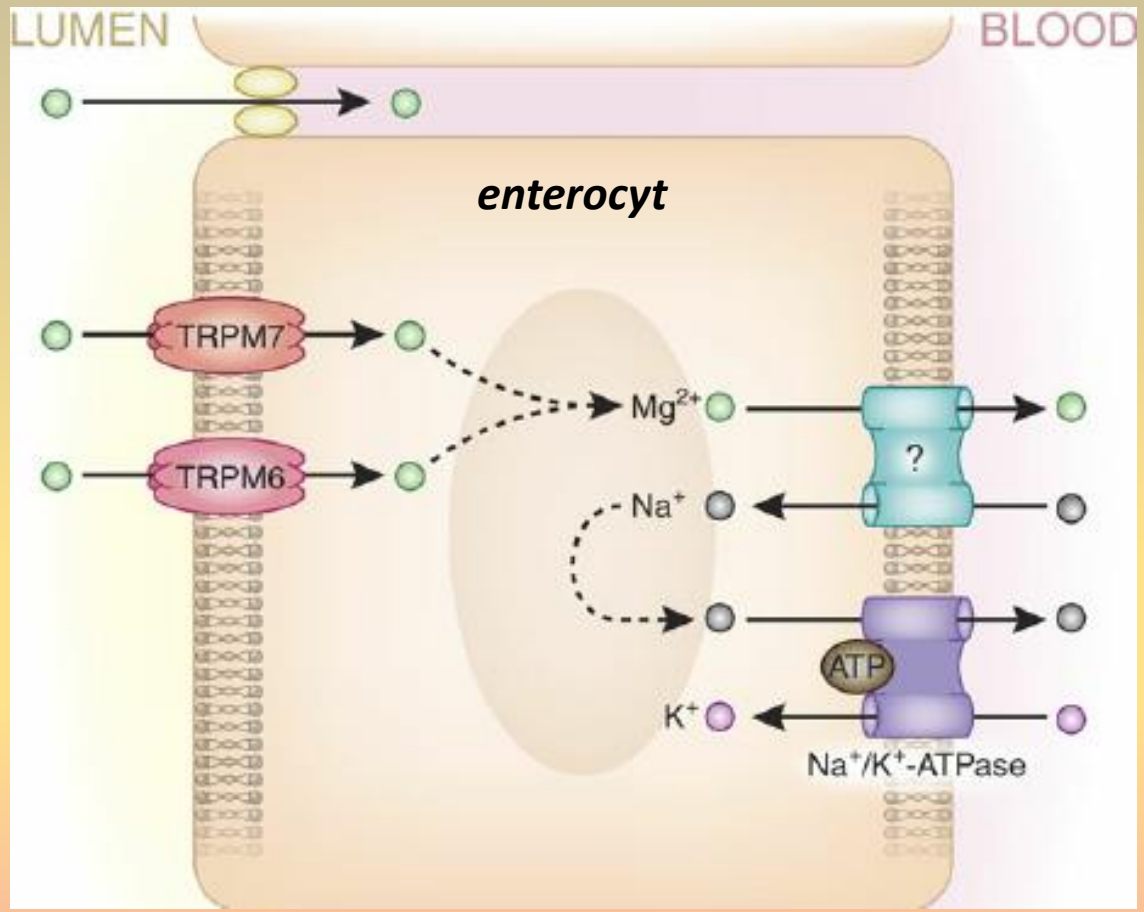
Distribution
- 1 mol - bones approx. 54 %, muscles and soft tissues approx. 56 %
- ECF – 0.5 mM

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



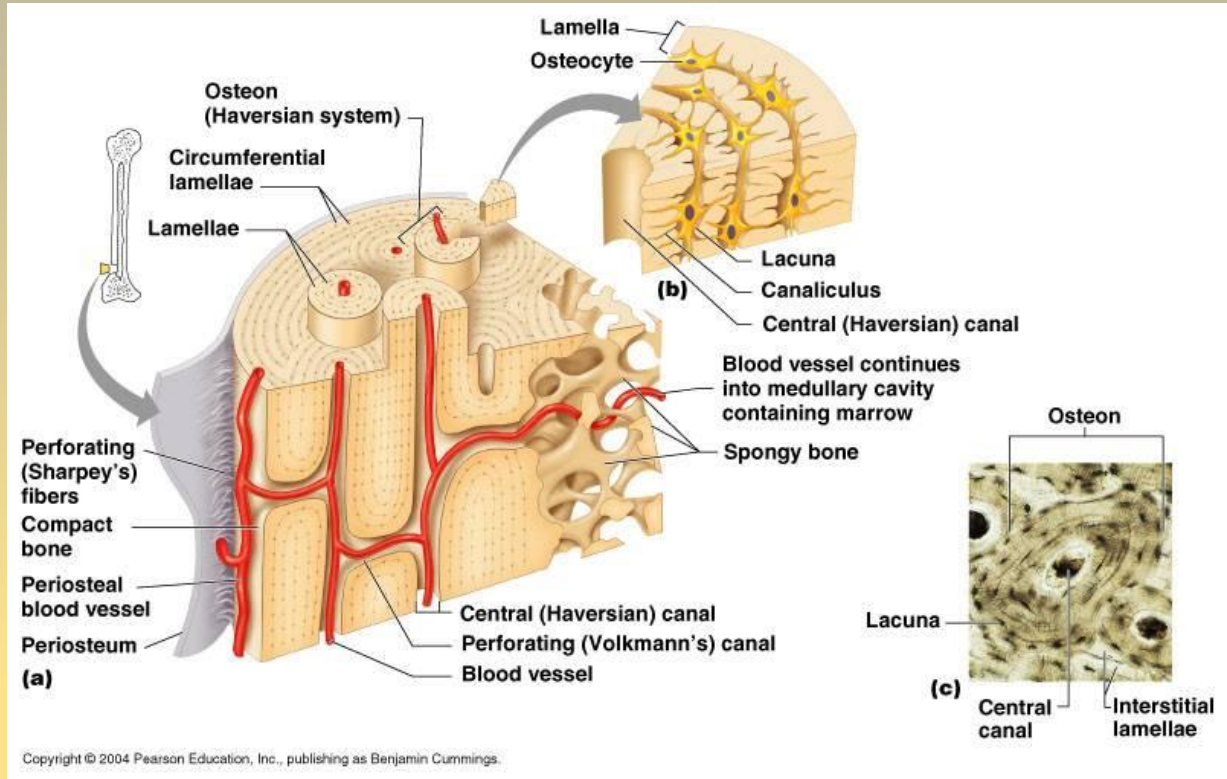
Bone tissue physiology

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 %

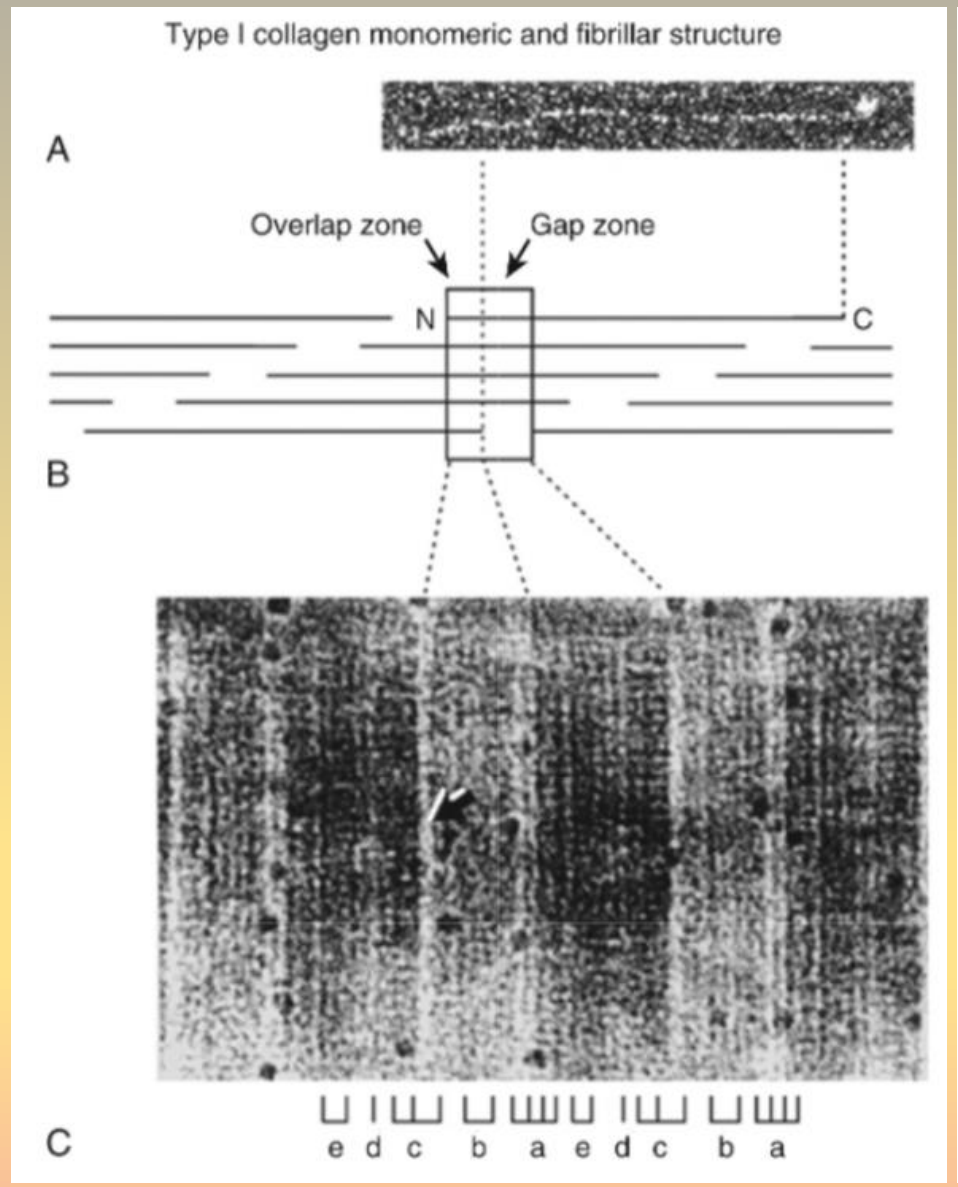
- High surface-to-volume ratio
- High metabolic activity
- Nutrients diffuse from ECF to trabecules

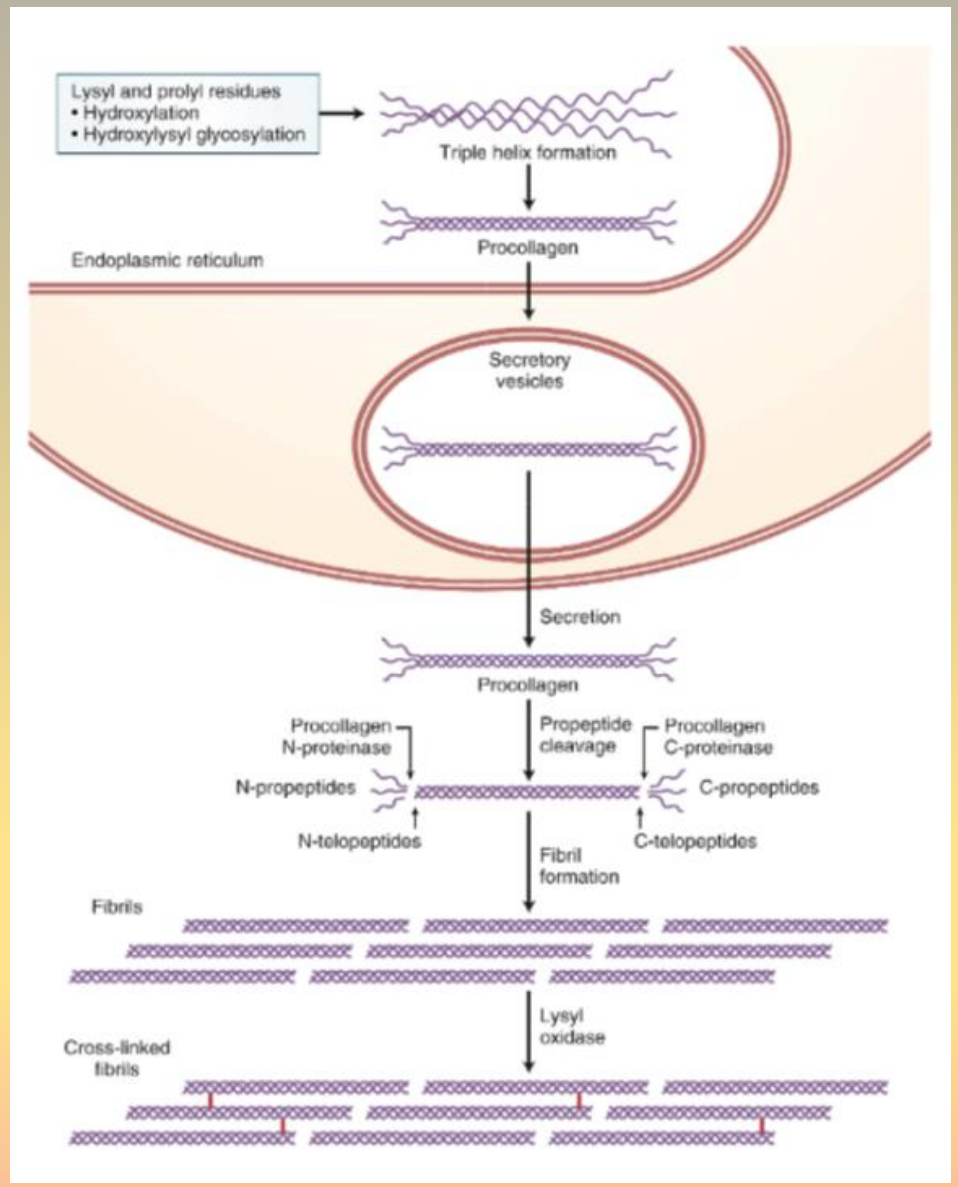


www.creab.org - Human Body Anatomy - Online anatomy atlas. Viktoria Ruppel. 14. 3 2015

Collagen type I = most important protein of bone matrix

Bone matrix and bone mineral





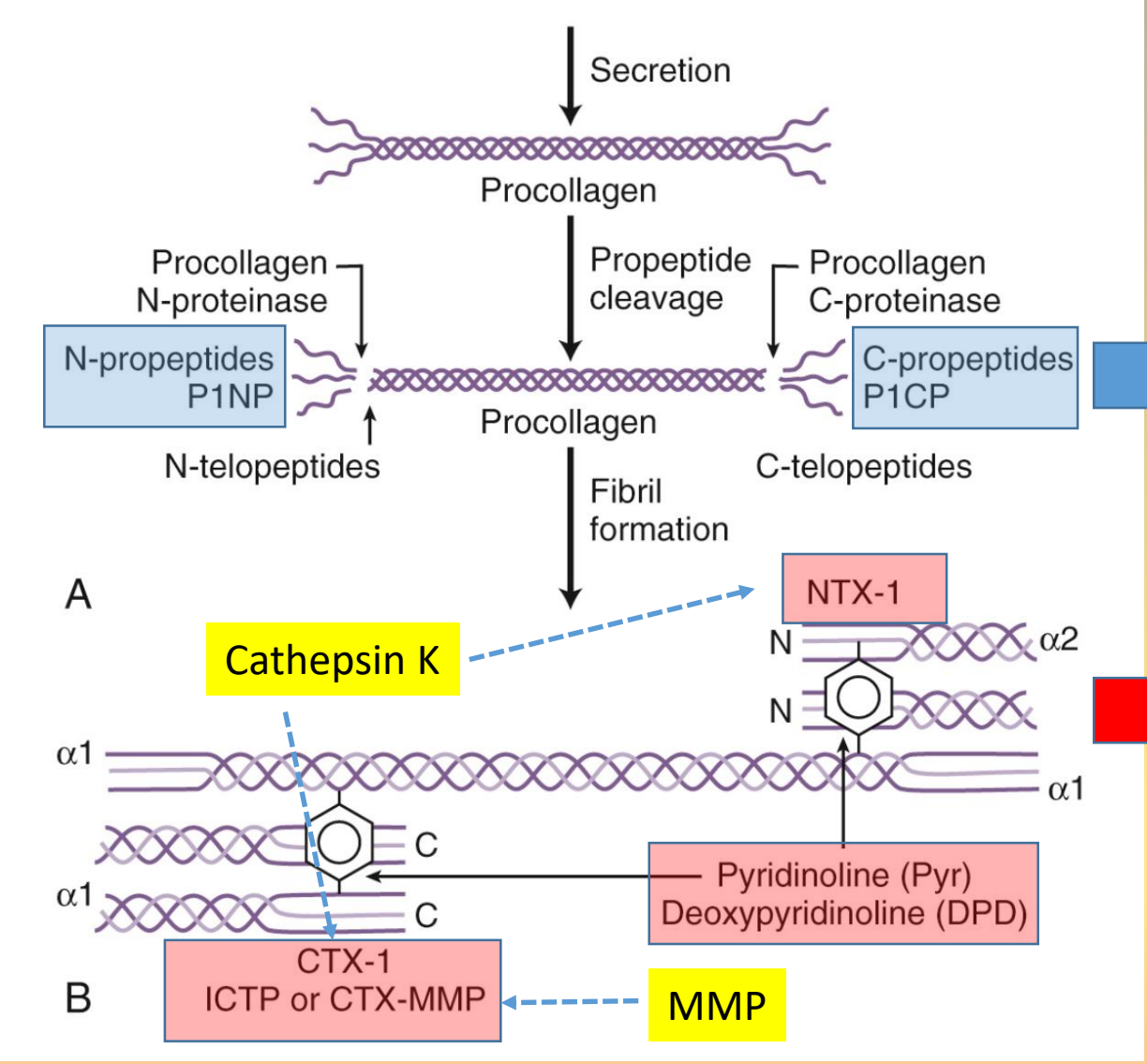
Trombospondin
Fibronectin
Matrix Gla protein
Osteocalcin
Biglykan
Decorin
Bone sialoprotein
Osteopontin
Osteoadherin

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

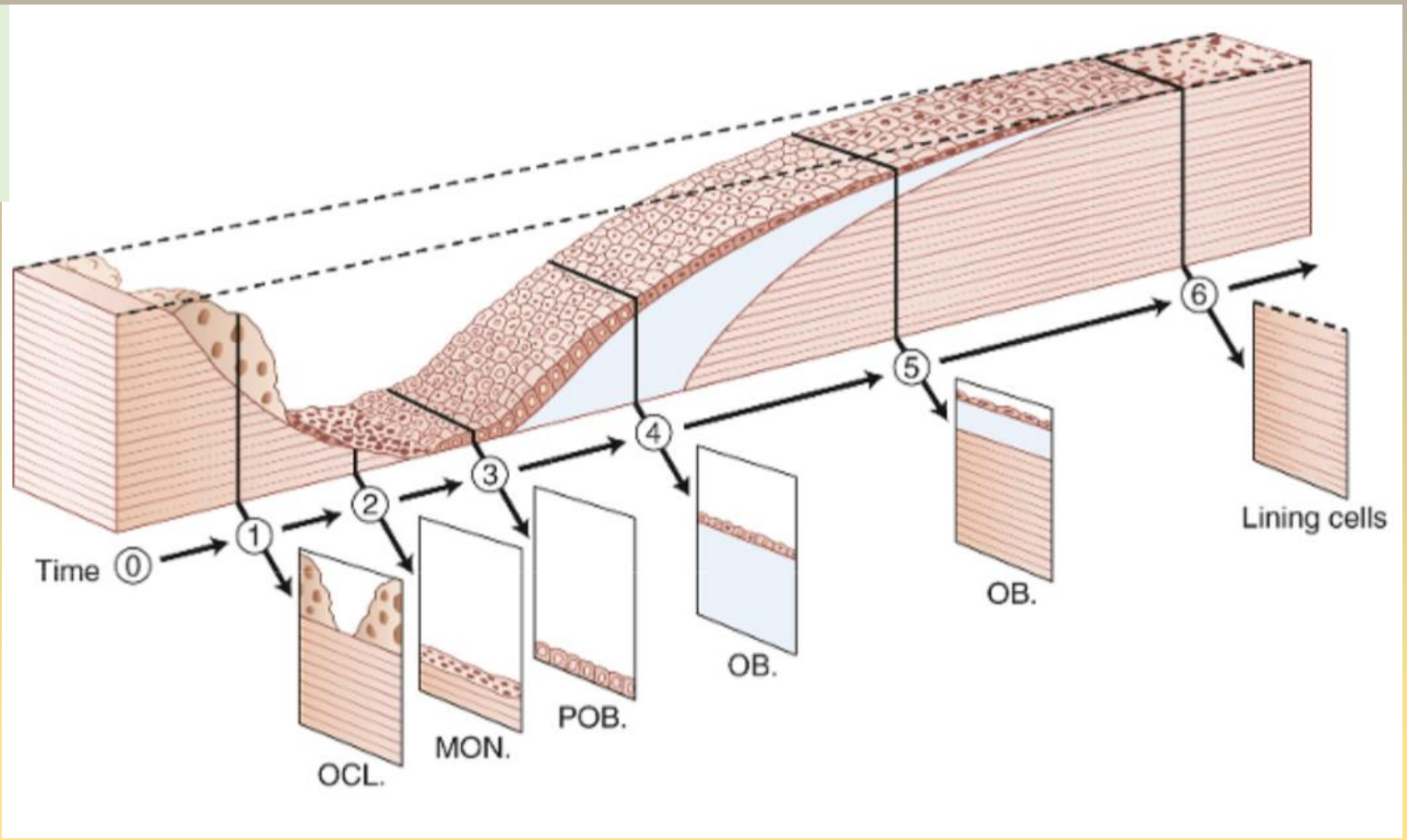
Bone tissue and its remodeling

Modeling
versus remodeling
of bone tissue

REMODELING UNIT - BMU

osteoblasts | osteoclasts | osteocytes

Bone lining cells



Bone reabsorption

Building of bone tissue

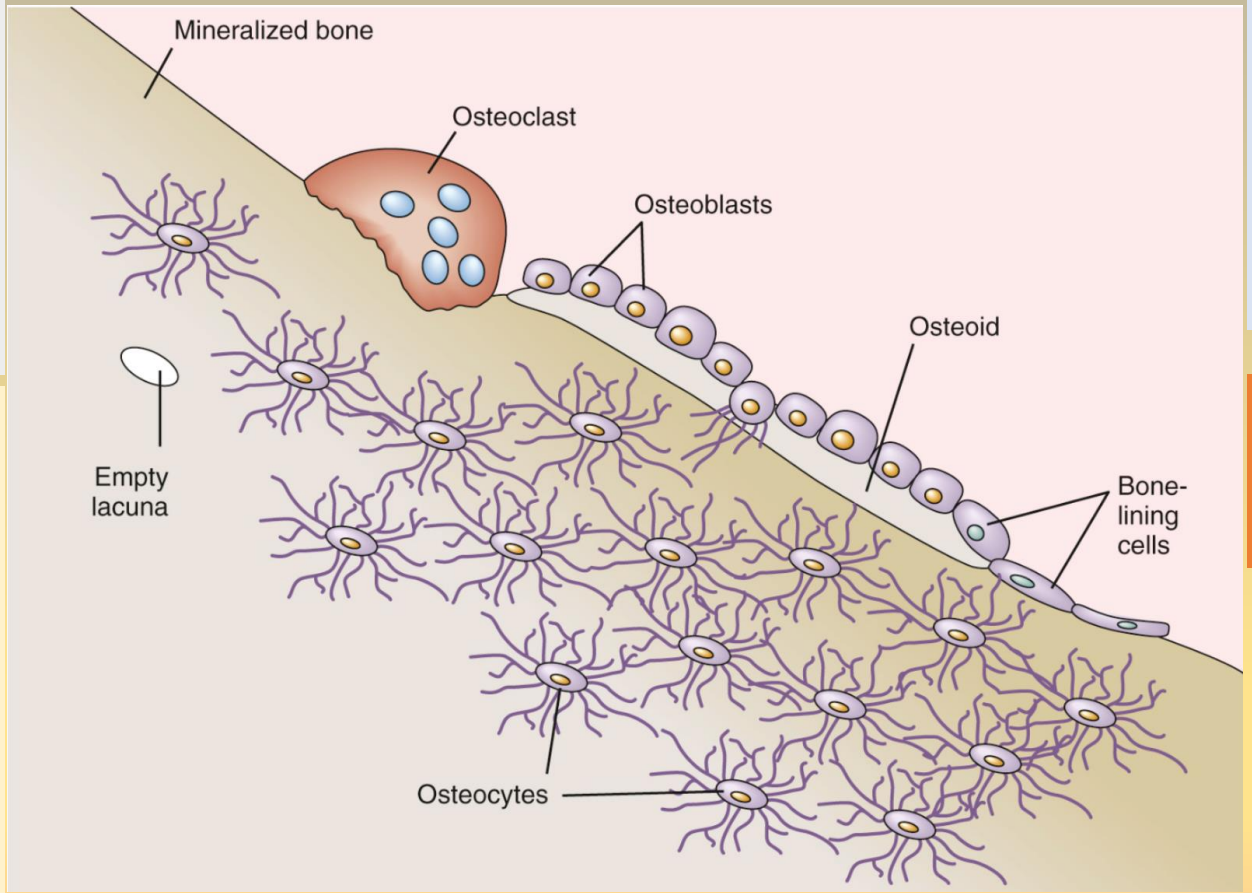
osteoclasts | mononuclear cells

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

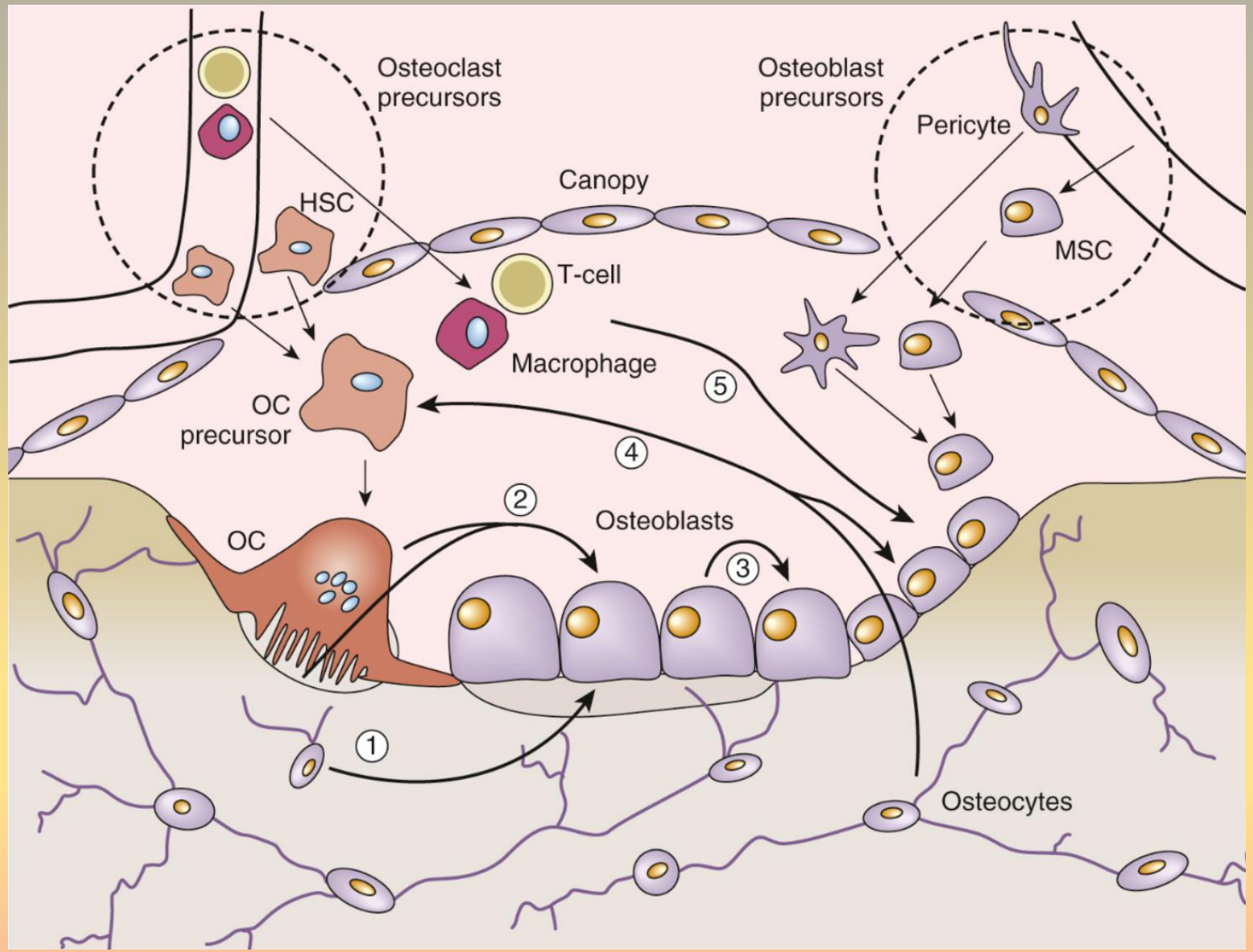


Lining cells
- Stimulation of OB differentiation
- OC communication
- Differentiation to OB stimulated by PTH

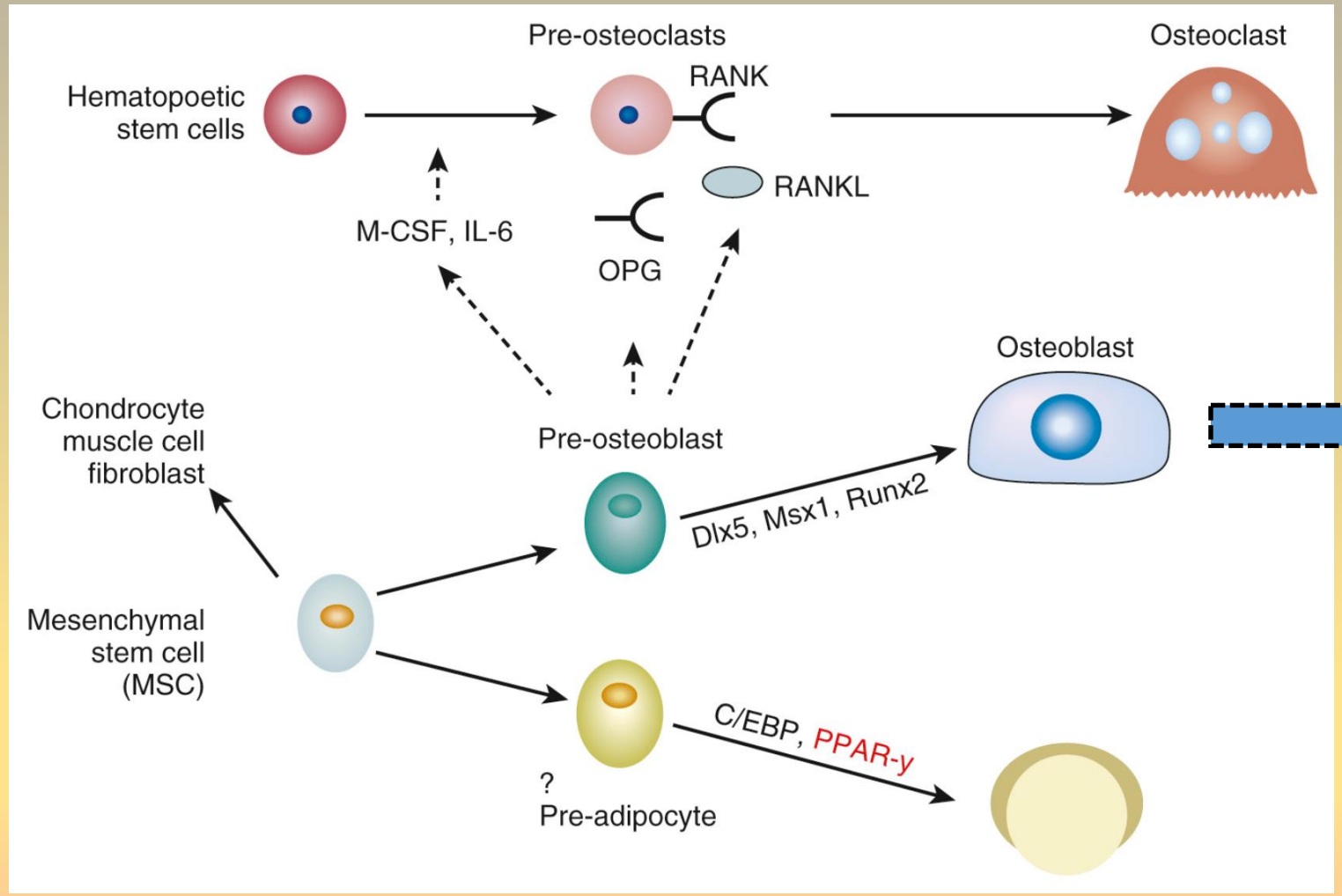
Osteoclasts (OK)
- Bone tissue reabsorption

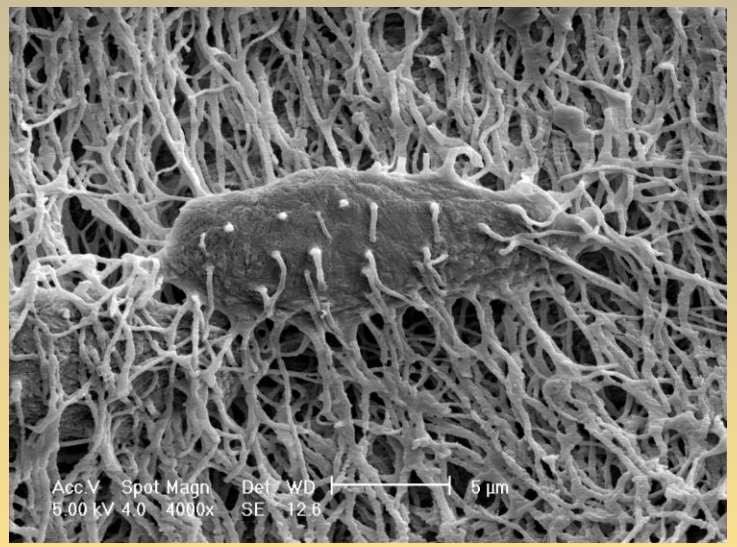
Remodeling unit - BMU

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.

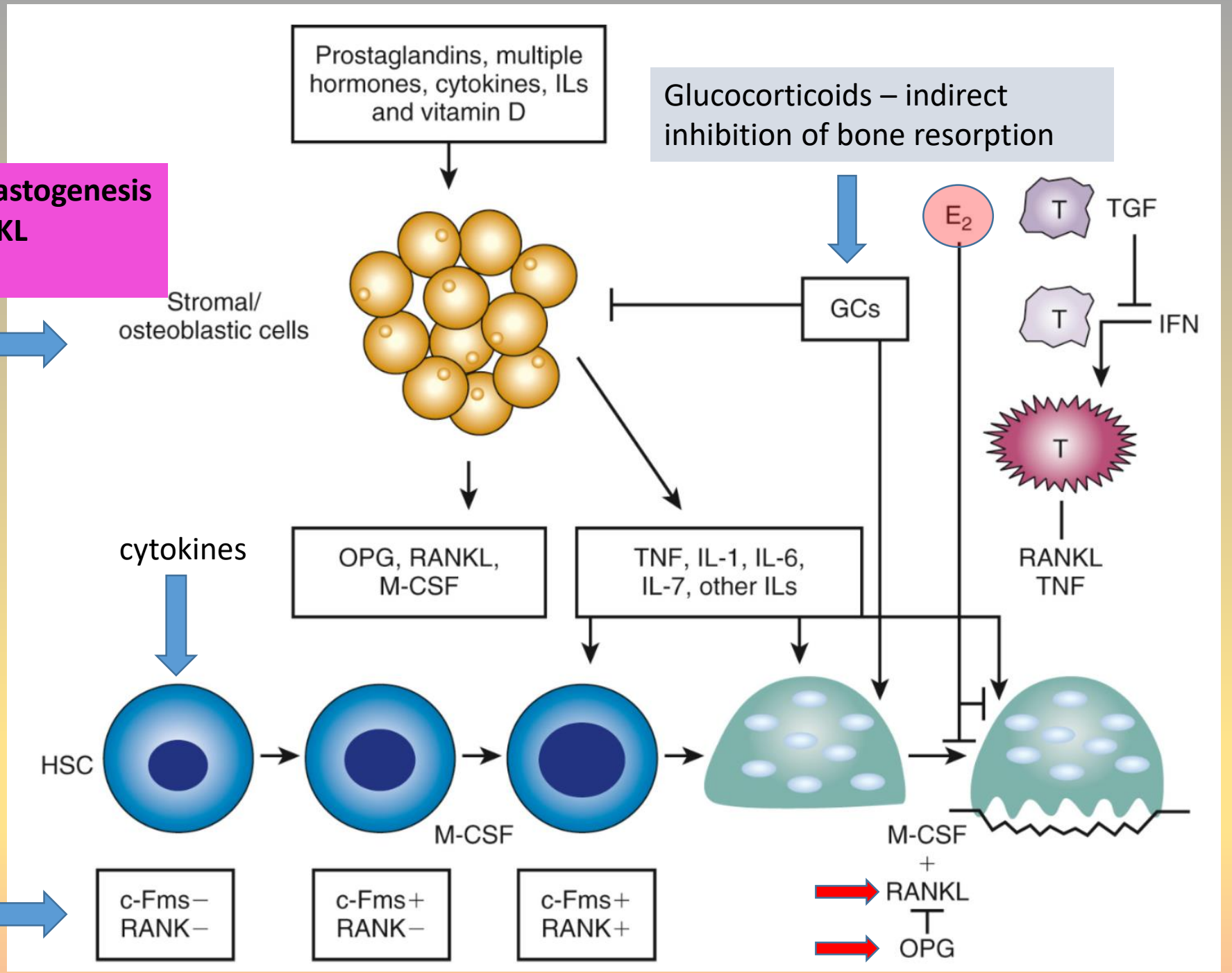
Osteoclastogenesis
(+) RANKL
(-) OPG

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

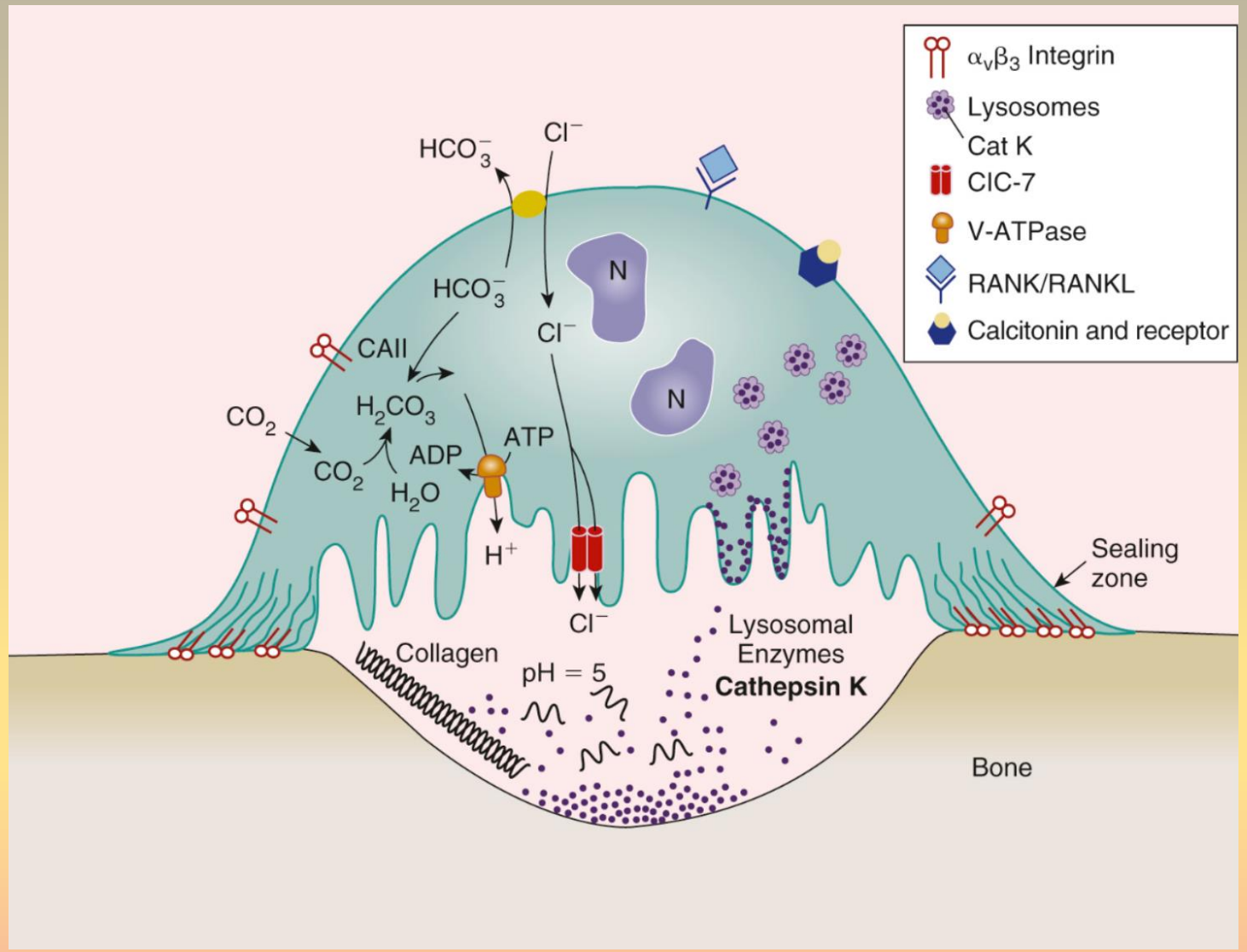
E2 (estrogens) – inhibition of T cell activation= inhibition of RANKL and TNF-α secretion

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

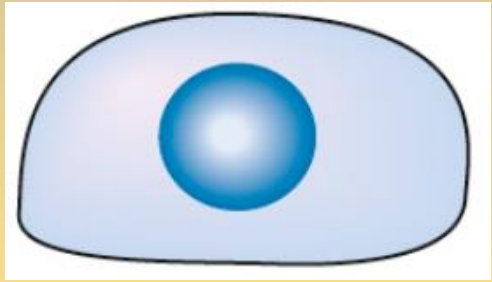
Factors influencing bone tissue remodeling

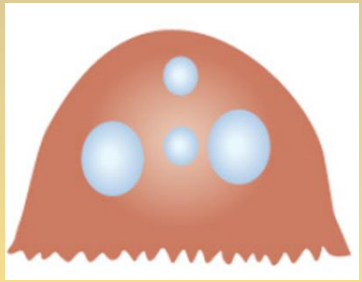
Resorption takes approx. 2 weeks
Mineralization and formation approx. 12 weeks

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

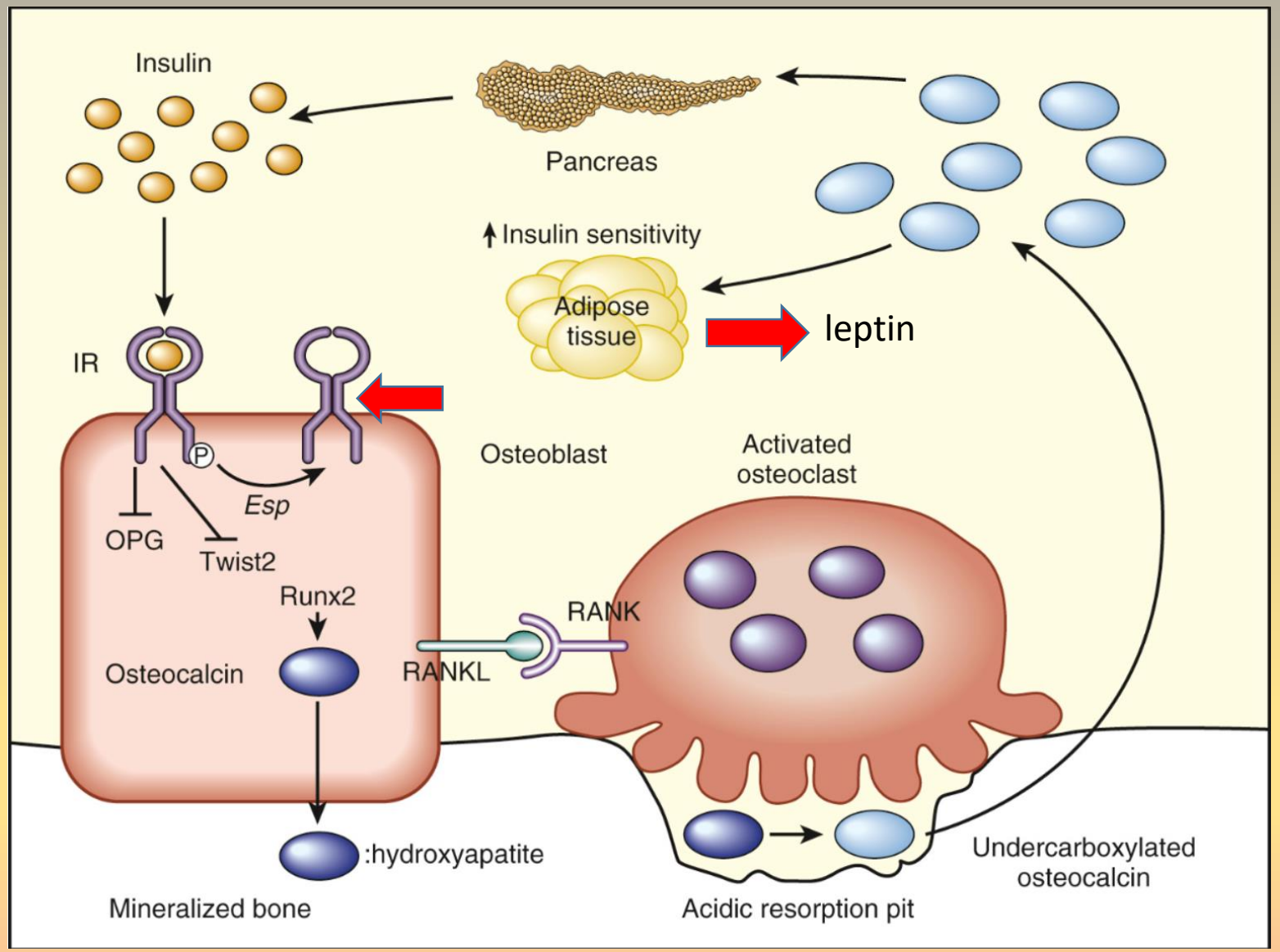
−	+
Cytokines - IL-1α, IL-1β, TNF-α, TNF-β, proinflammatory IL (7, 15, 17)	Cytokines - IL-4, IL-13, IL-10, IL-18
TGF-α and EGF, FGF21, FGF23	Prostaglandins
Prostaglandins	VEGFA, HIF-1α (+/-)
PDGF	IGF-1 (endo-/paracrine)
	BMPs (OB, autocrine)

Endocrine regulation of bone tissue

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

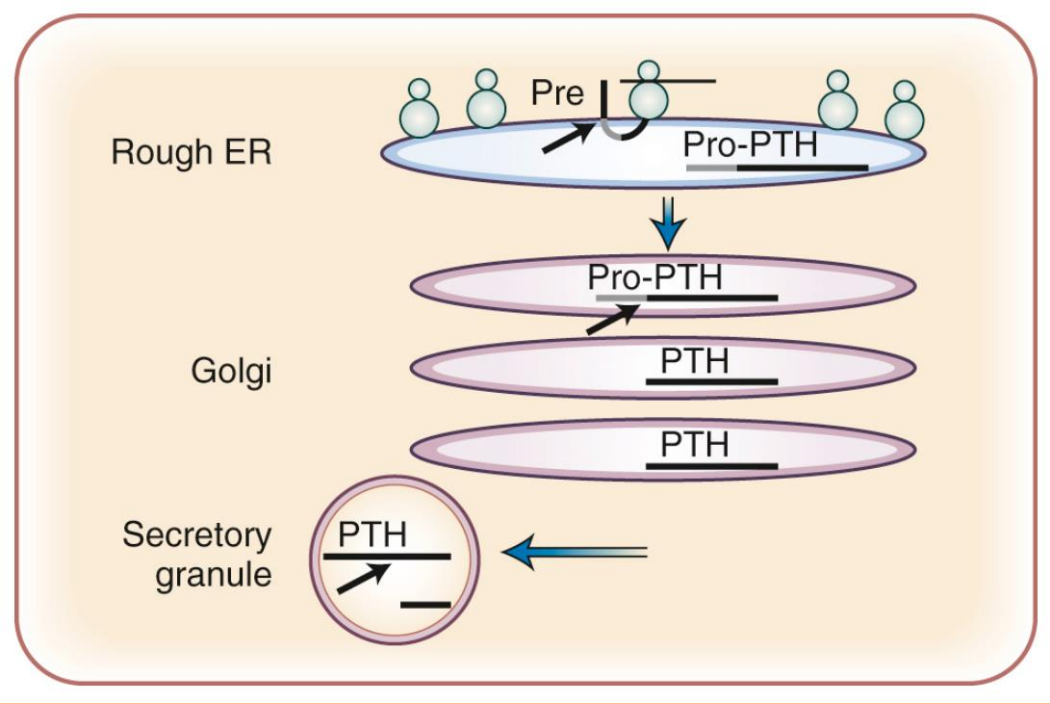
HPLC – high performance liquid chromatography; ELISA – enzyme-linked immunosorbent assay; RIA – radio immuno assay; ECLIA – electrochemiluminiscence immunoassay; ICMA – immunochemiluminometric assay

Clinical relevance

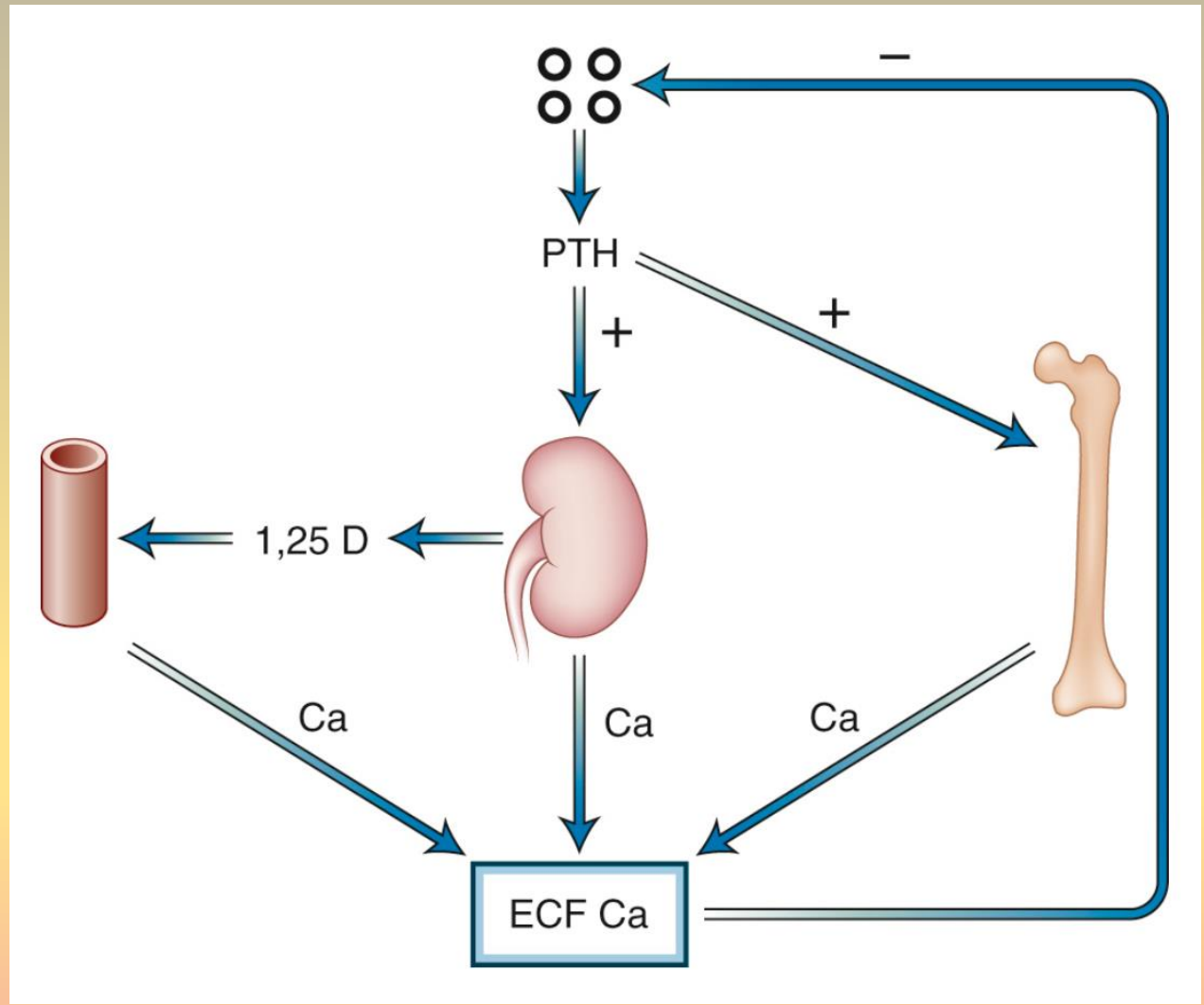
- Osteogenesis imperfecta
- Osteopetrosis
- Osteomalacia
- Rachitis
- Osteopenia – T score -1 – -2.5
- Osteoporosis – T score under -2.5

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.



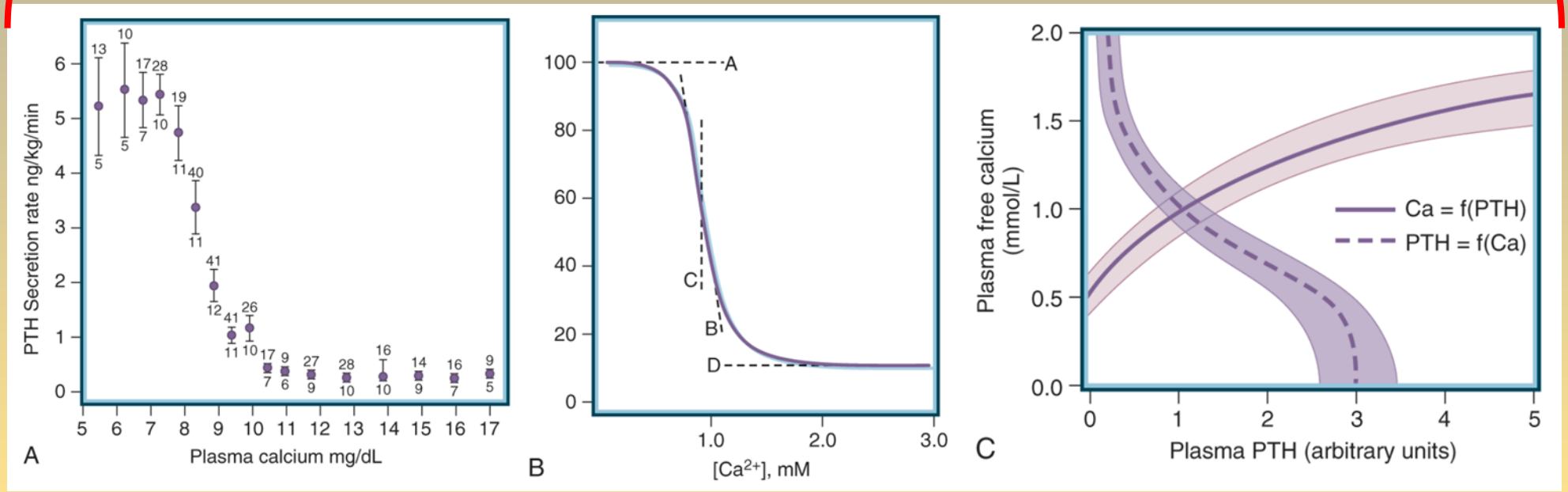
PTH secretion

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



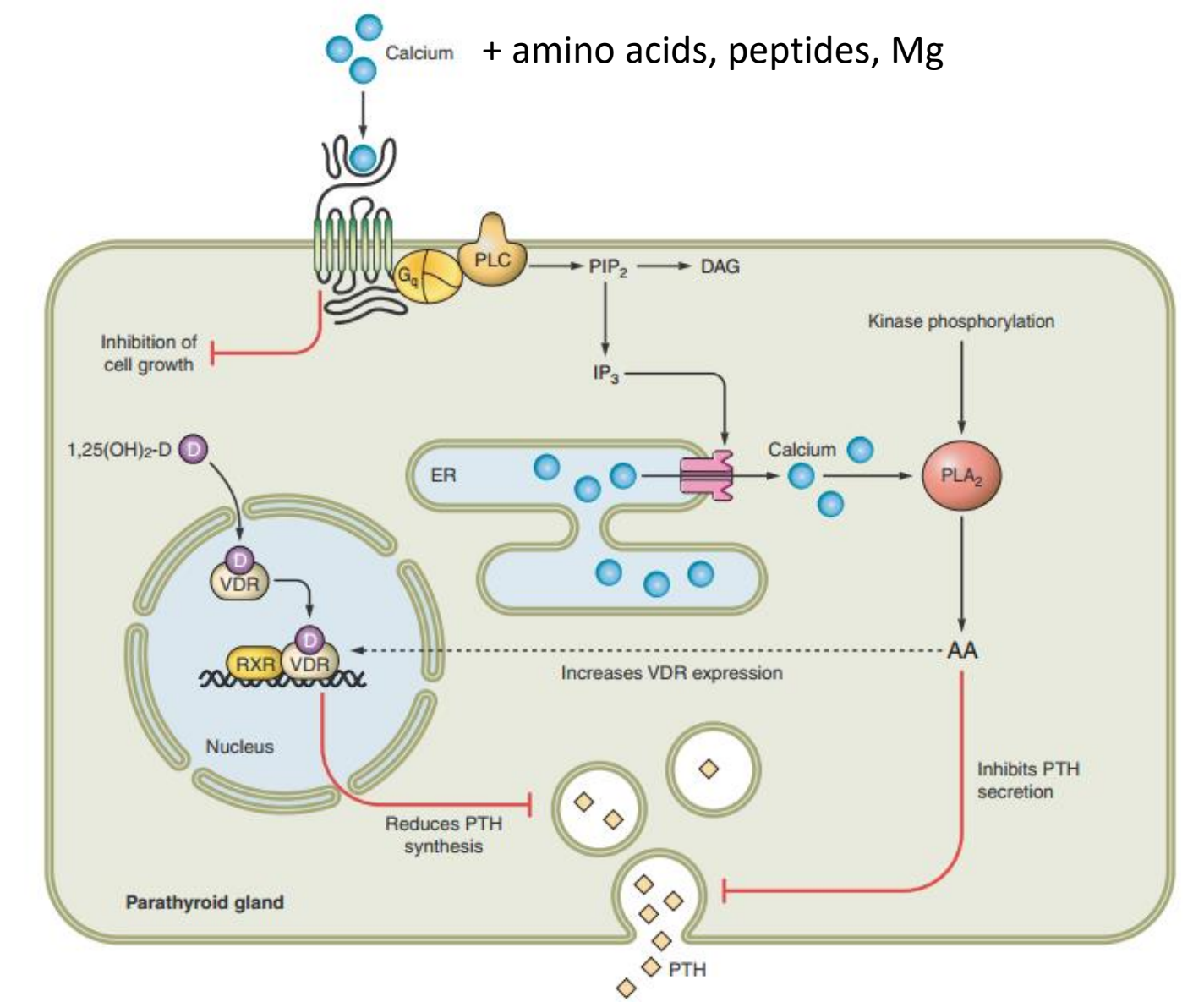
Level of ionized calcium in blood is a key parameter for PTH secretion.

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

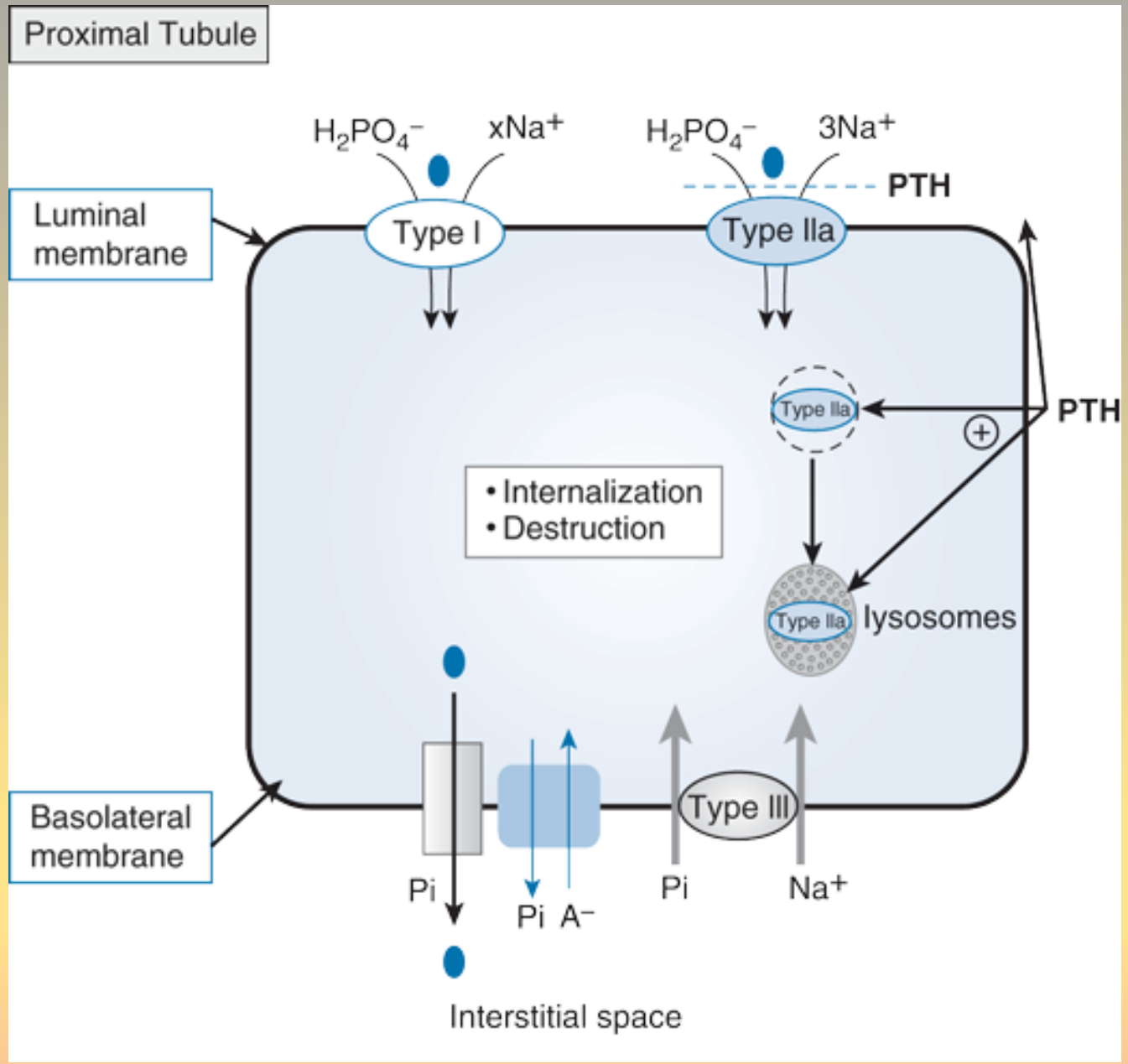
Clinical aspects
- Mutation – inactivation/activation
- familial hypocalciuric hypercalcemia (in.)
- Familial hypoparathyroidism with hypercalciuria (ac.)
- Calcimimetics – inhibition of PTH secretion

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

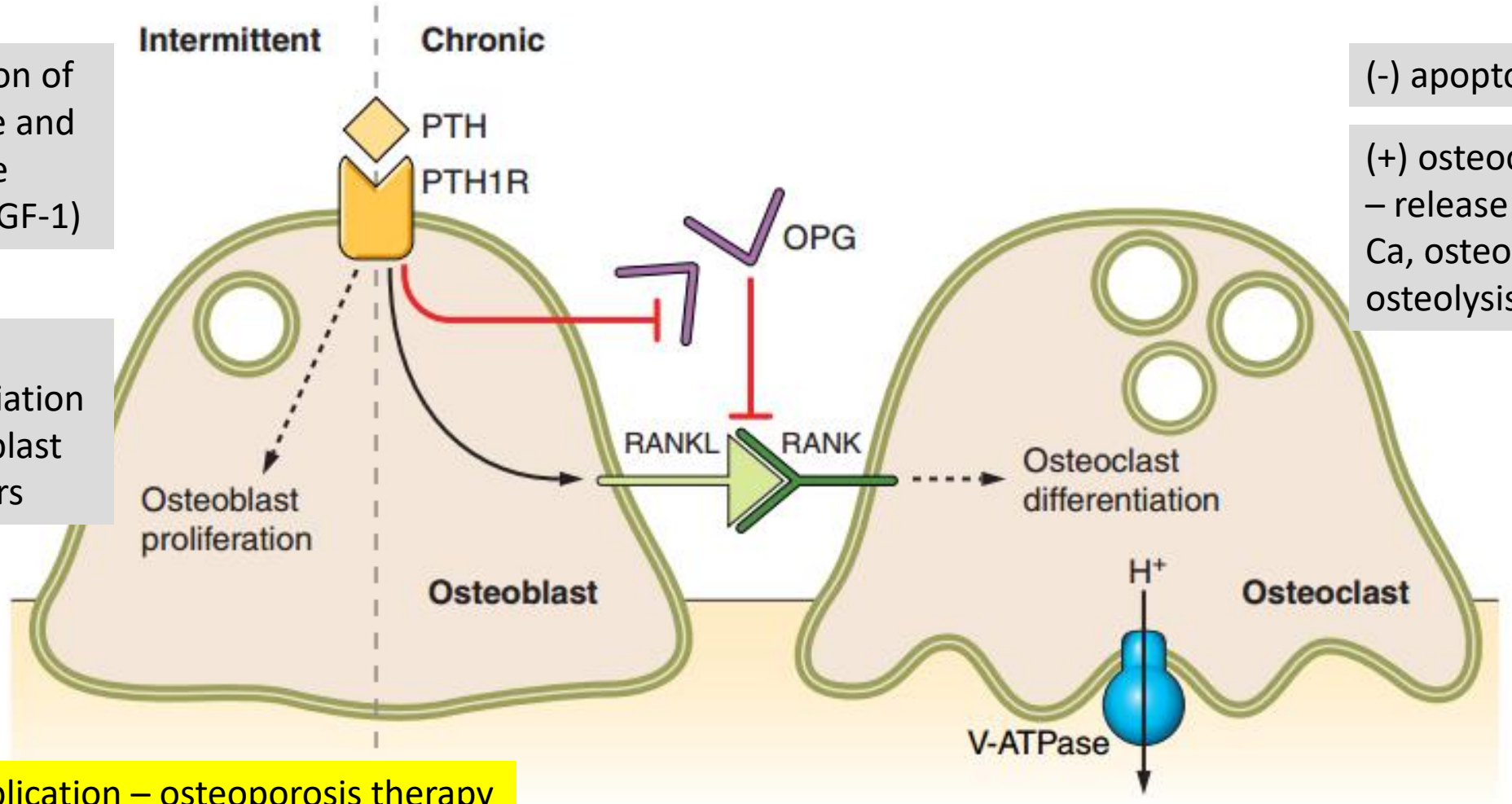
(+) activity of 1α-hydroxylase - PT
(-) resorption of Na, water and bicarbonate – PT
(-) Na^+/K^+-APTase (basolateral membrane)
(+) gluconeogenesis – PT
(-) GFR - podocytes



PTH and bone tissue physiology

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

+ differentiation of osteoblast precursors



(-) apoptosis

(+) osteocytes – release of Ca, osteocytic osteolysis

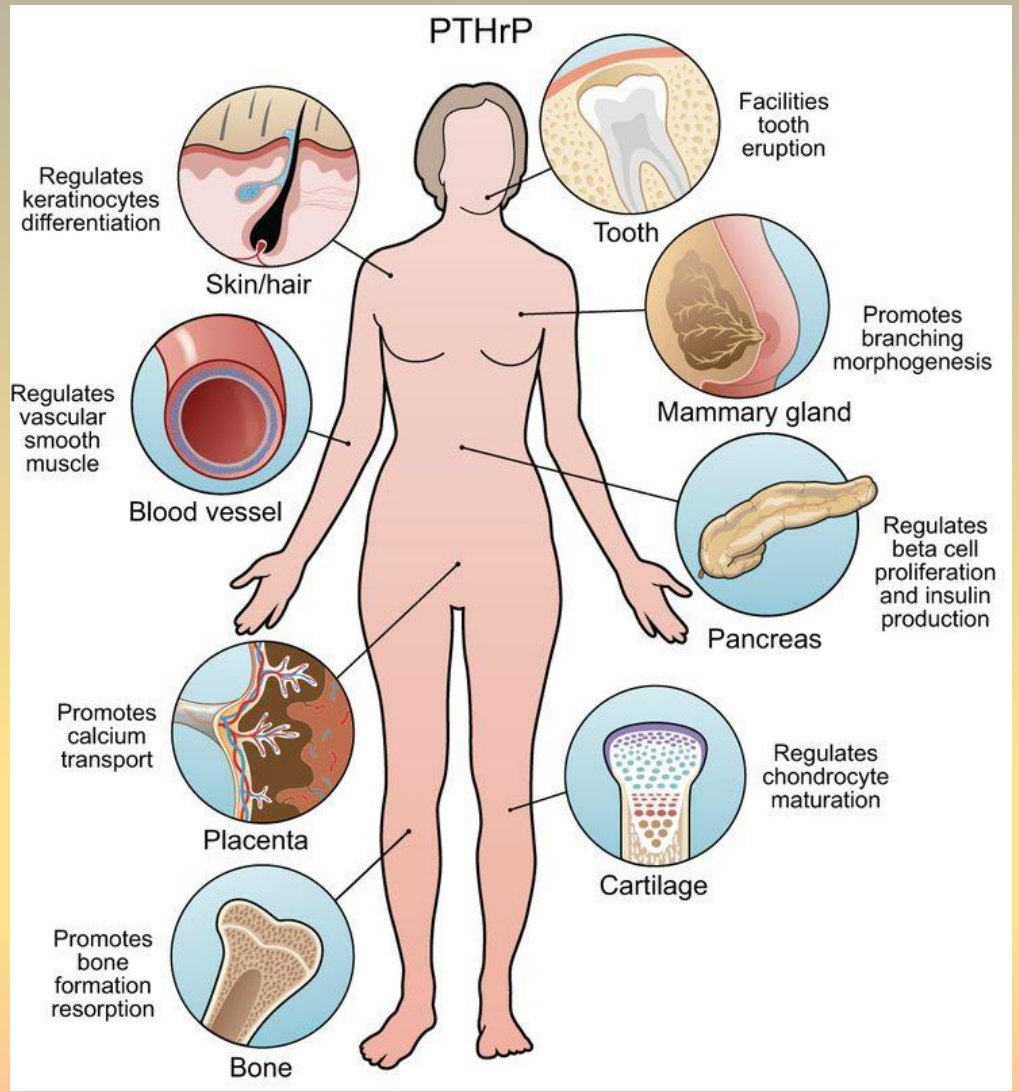
Clinical application – osteoporosis therapy

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

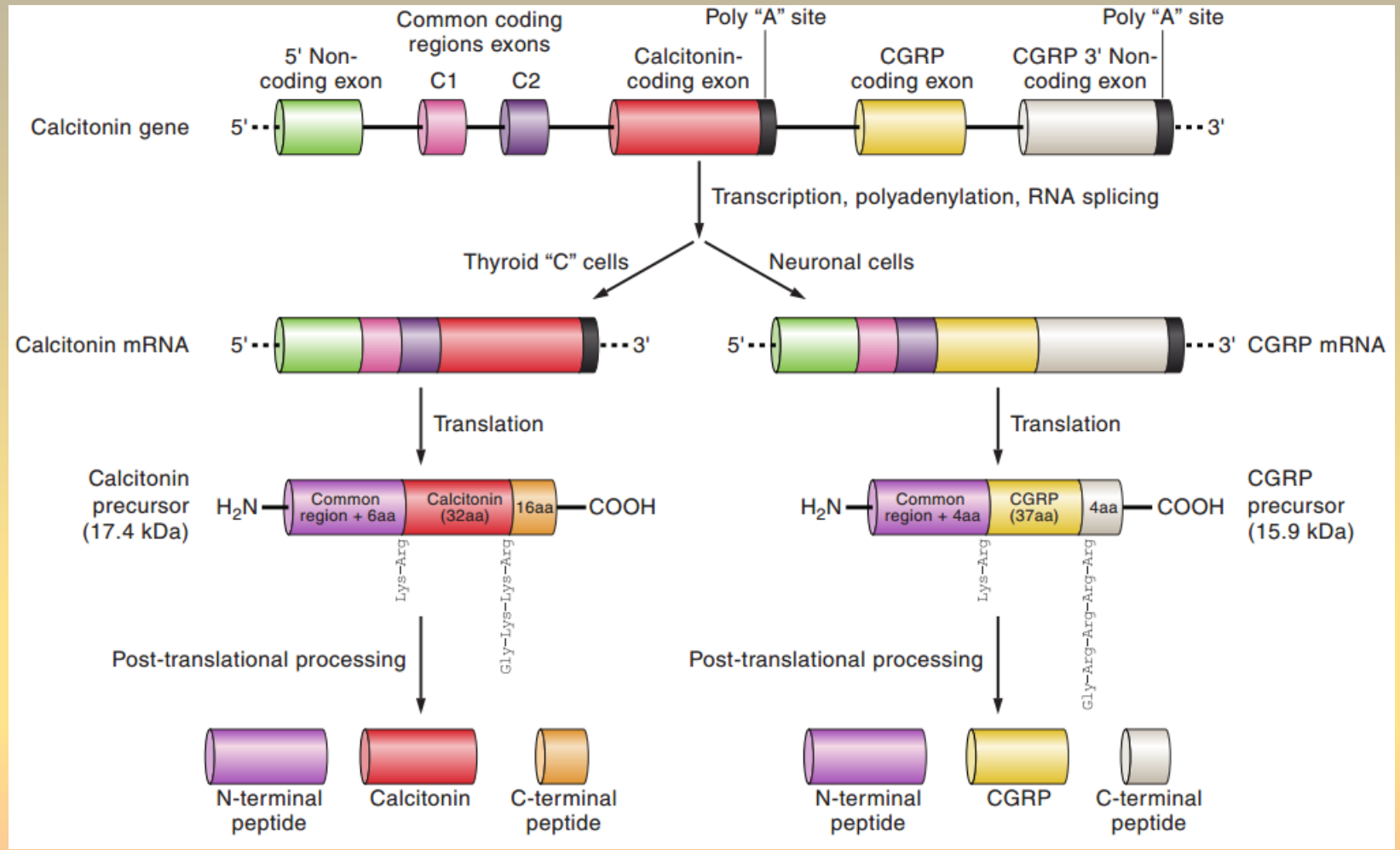
  } Function unclear
- Inhibition of secretion - somatostatin

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

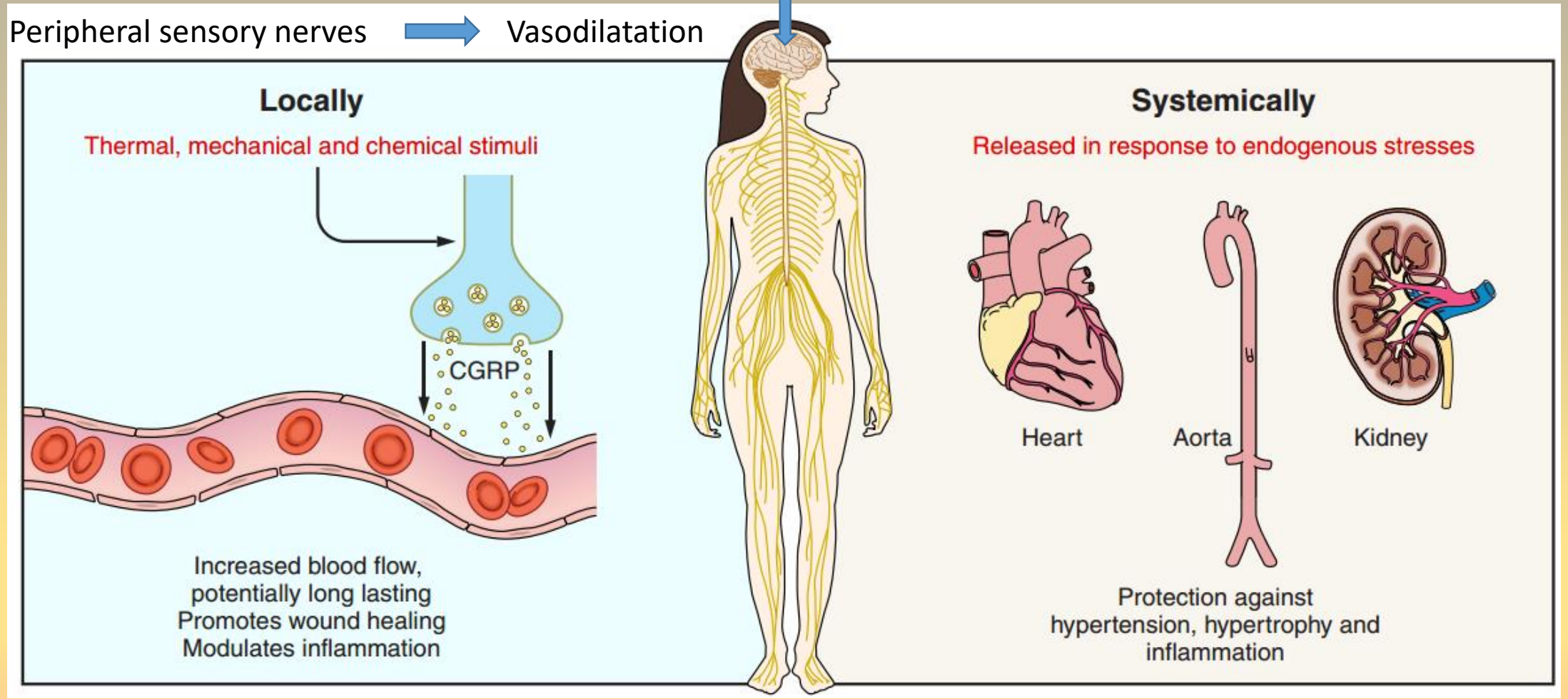
C cells



CNS and neurons in general

Calcitonin gene-related peptide - CGRP

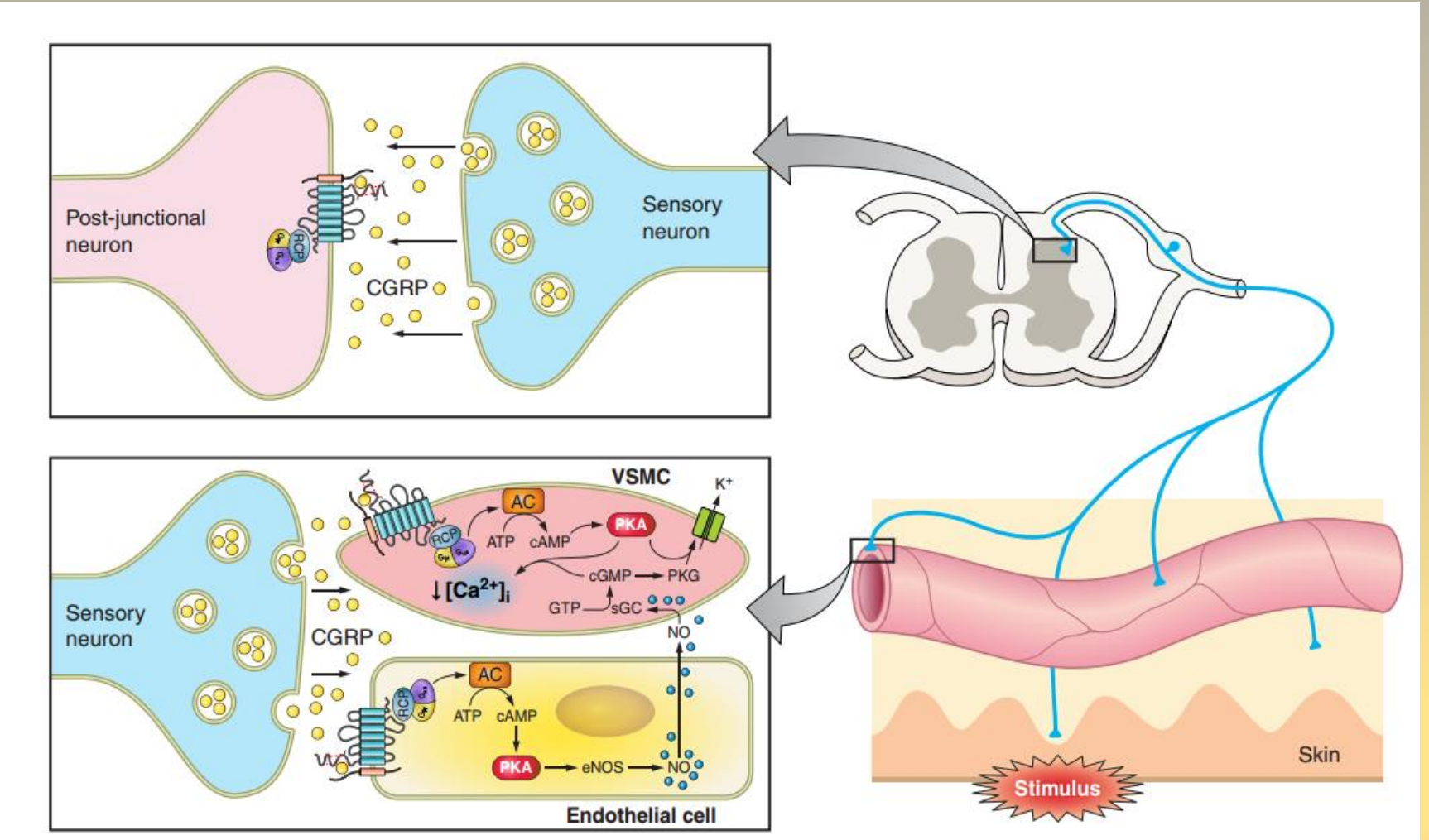
Neuropeptide – sensoric and integrative motoric functions



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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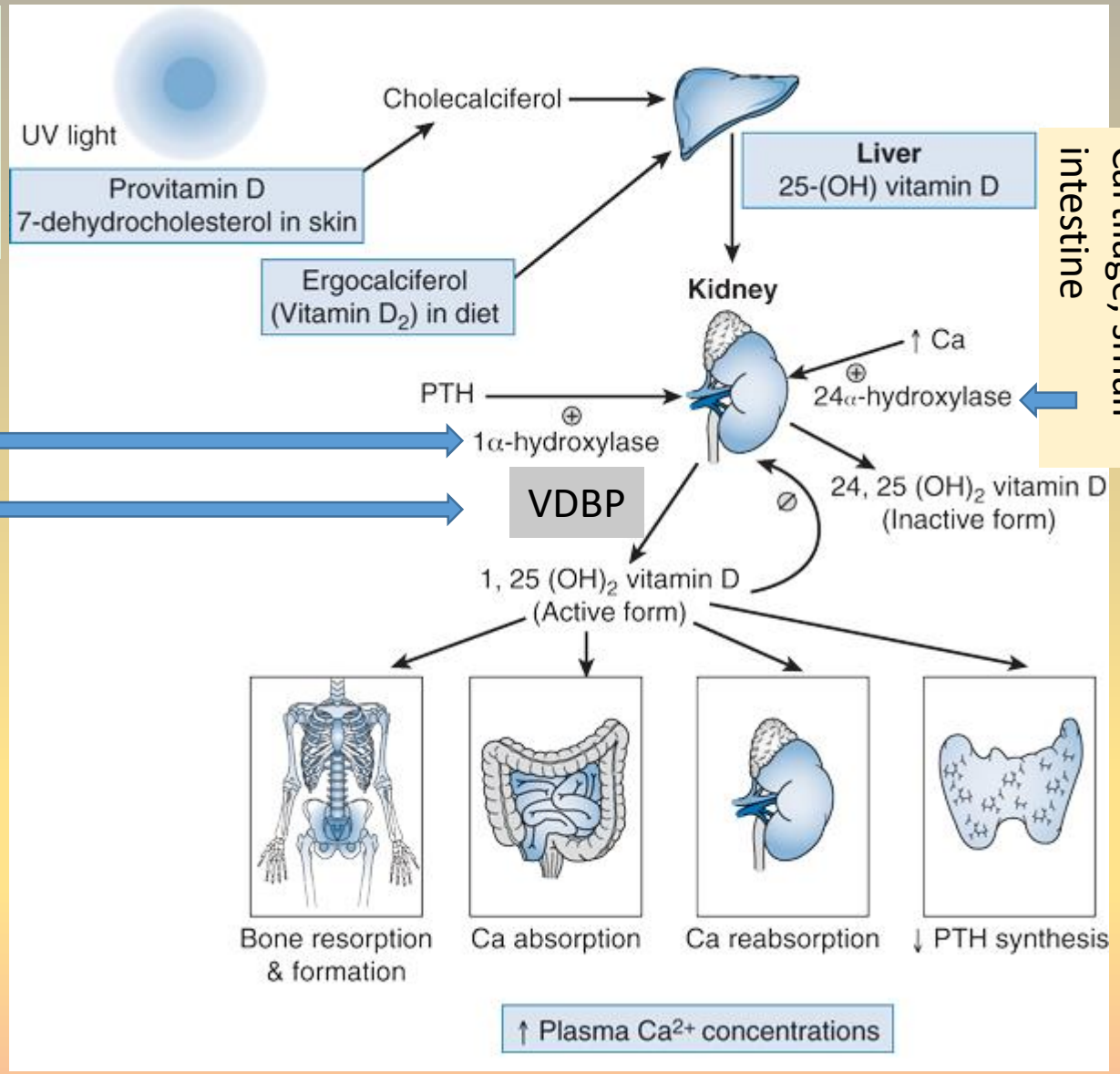
Vitamin D….hormone?...vitamin?

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$(OH)_2$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

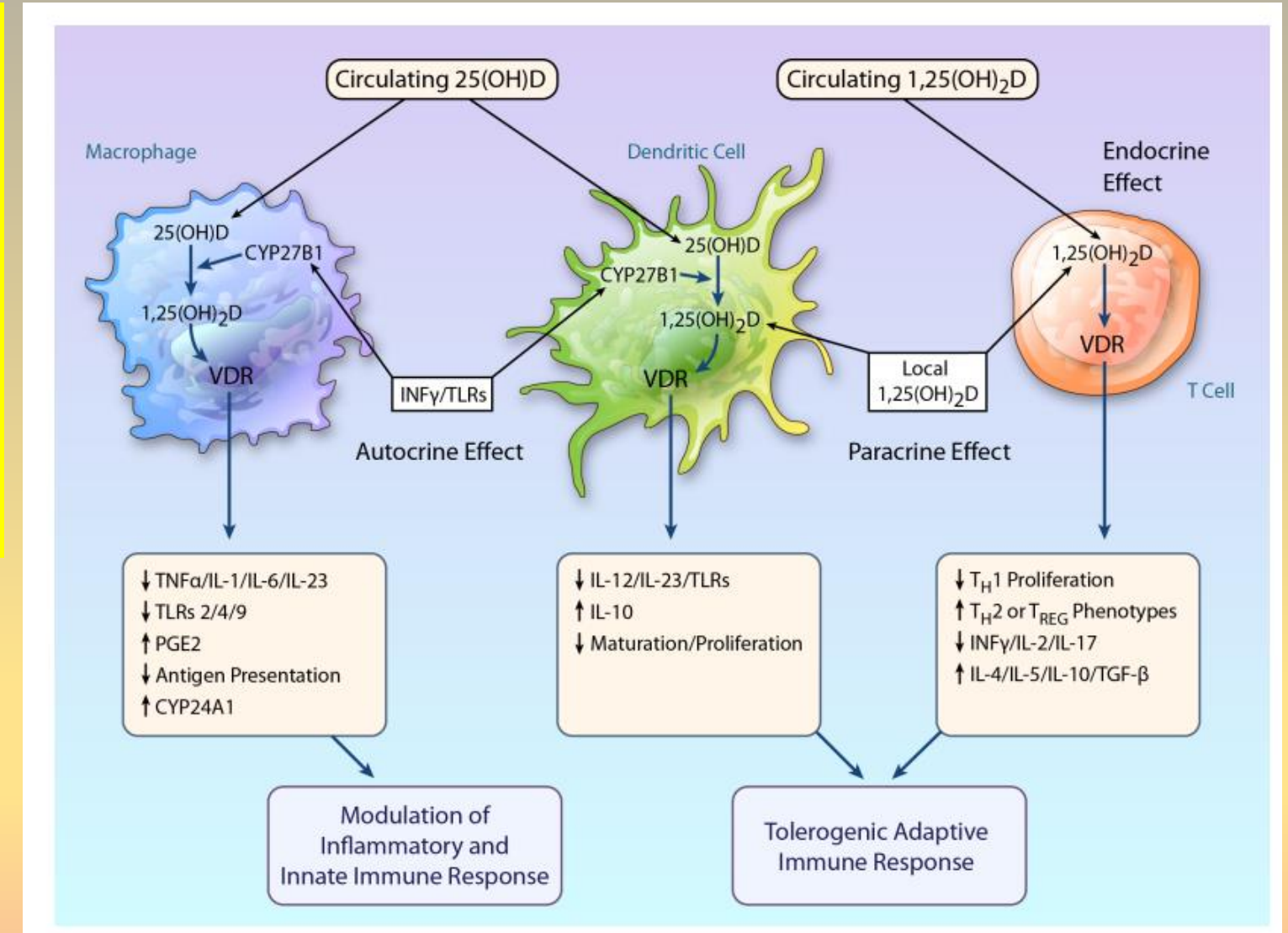
Bones and bone tissue
- (-) collagen synthesis
- (+) osteocalcin synthesis
- (+) osteoclasts differentiation – osteoclastogenesis
- (+) RANKL
- Main function – ensuring the stability of the bone microenvironment for mineralization by the standard intake and availability of Ca and phosphates

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 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

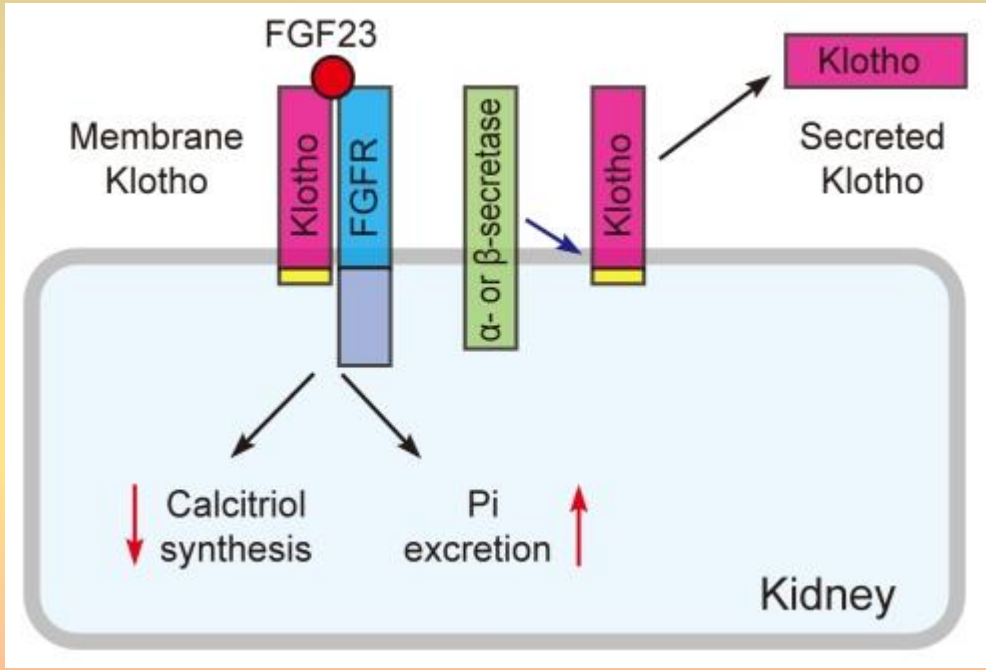
FGF23 – fibroblast growth factor 23

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



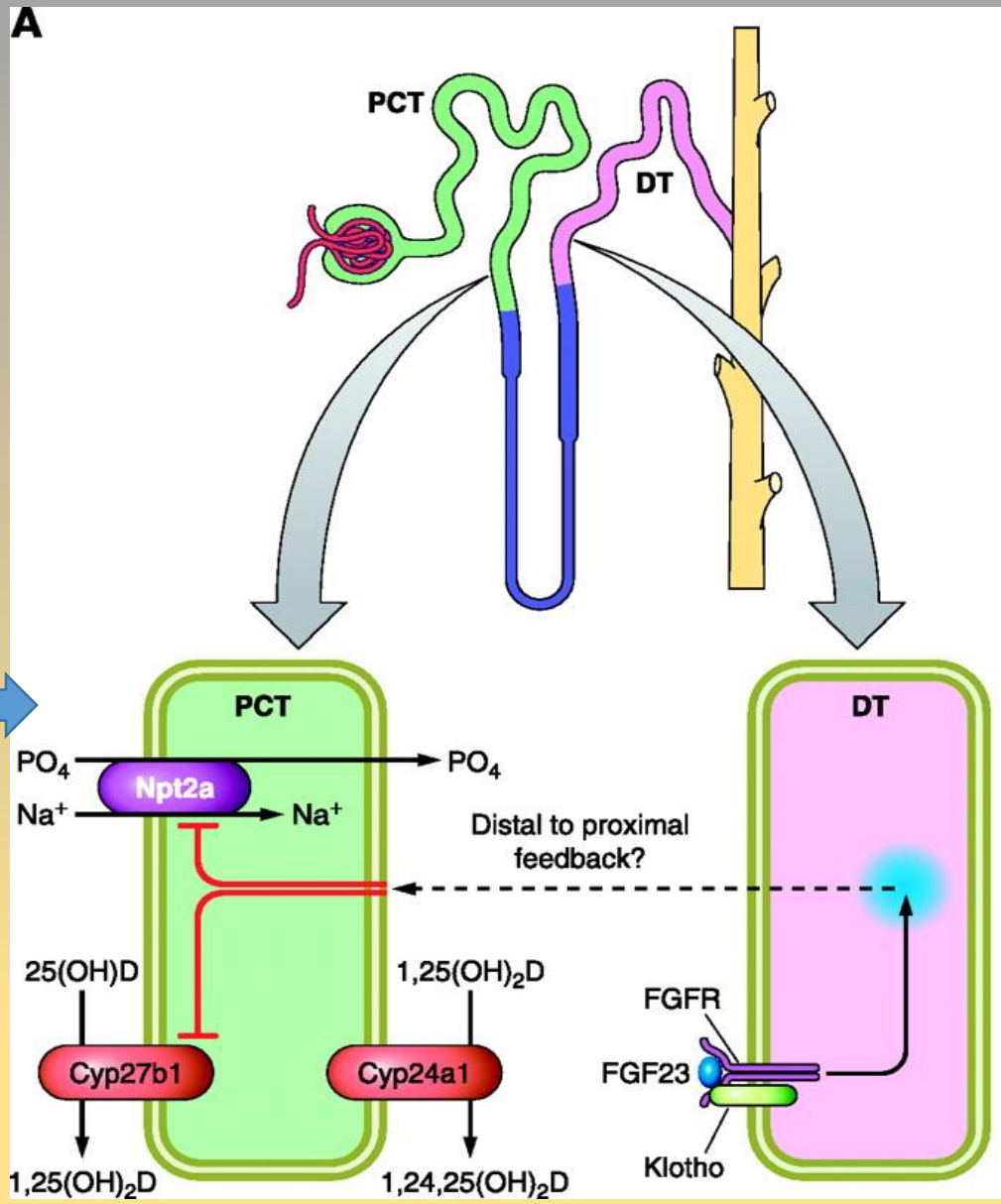
FGF23

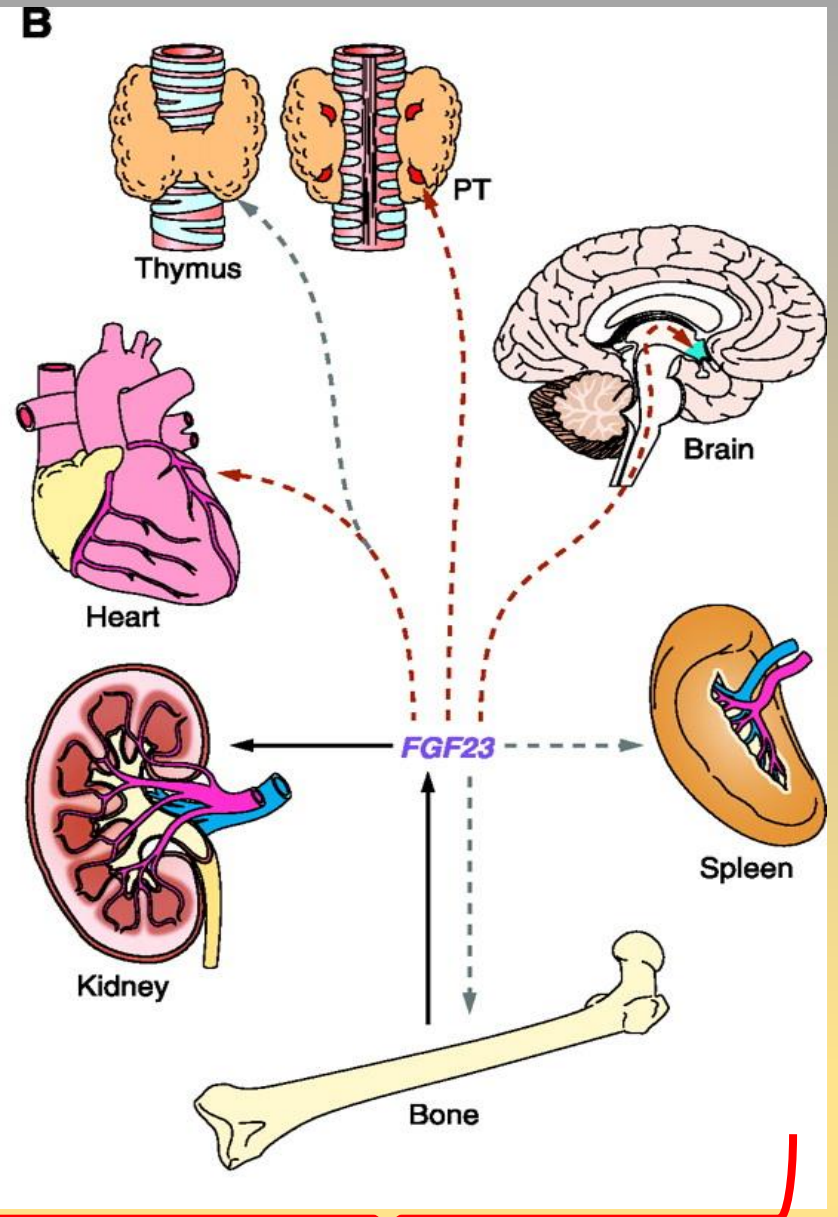
Pars convoluta PT

Inhibition

Cyp271b

Npt2a





1, $25(OH)_2D$

phosphate

Feedback mechanism between DT and PCT

endocrine, paracrine

Calcium homeostasis – still just a simplified model