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

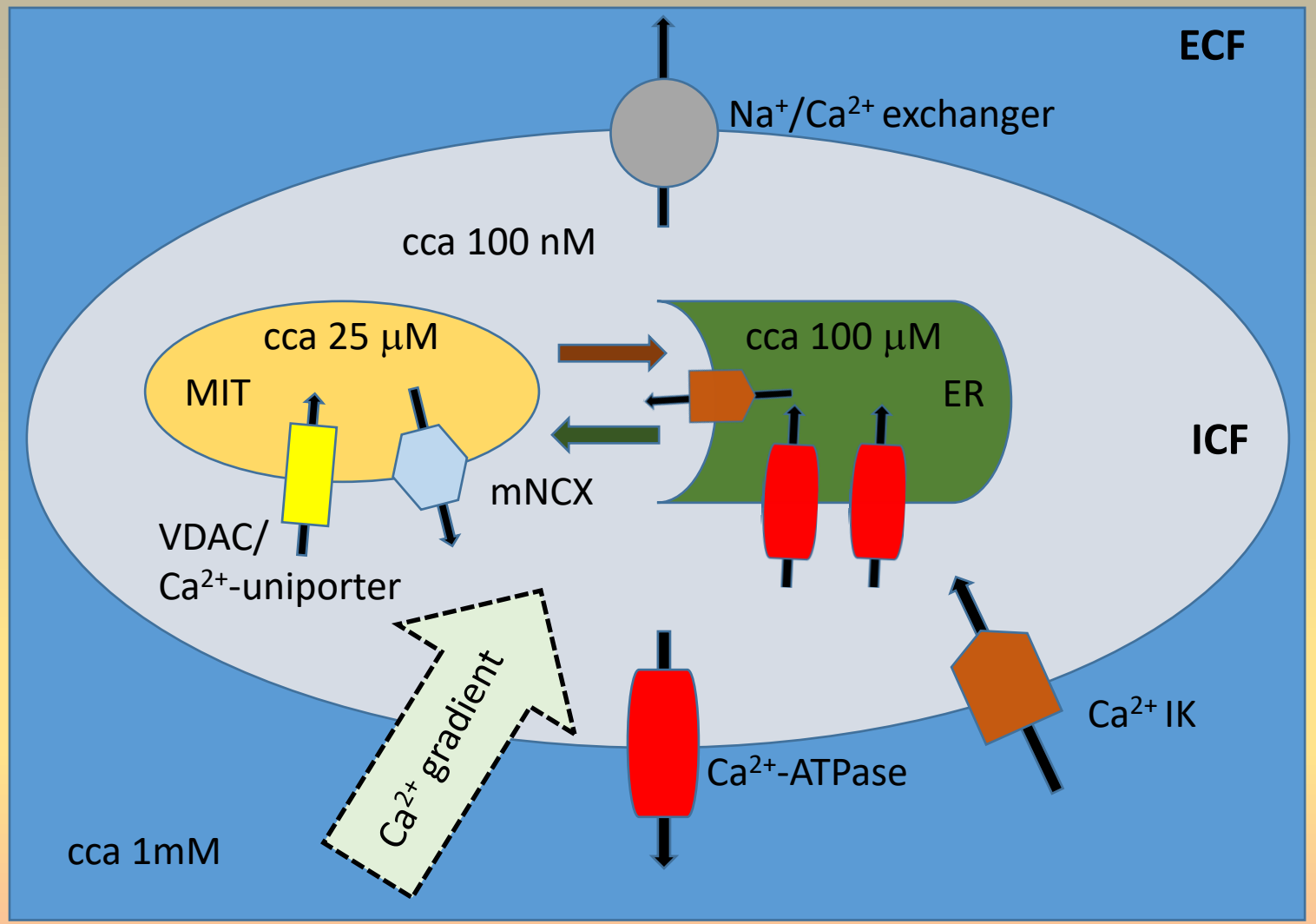
Bone tissue
- 99 % of overall Ca, of it 99 % in mineral component
- 1 % - quickly mobilizable and convertible (ICF - ECF)

	Calcium ions	Phosphate ions
Extracellular		
Concentration		
total, in serum	2.5×10^{-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

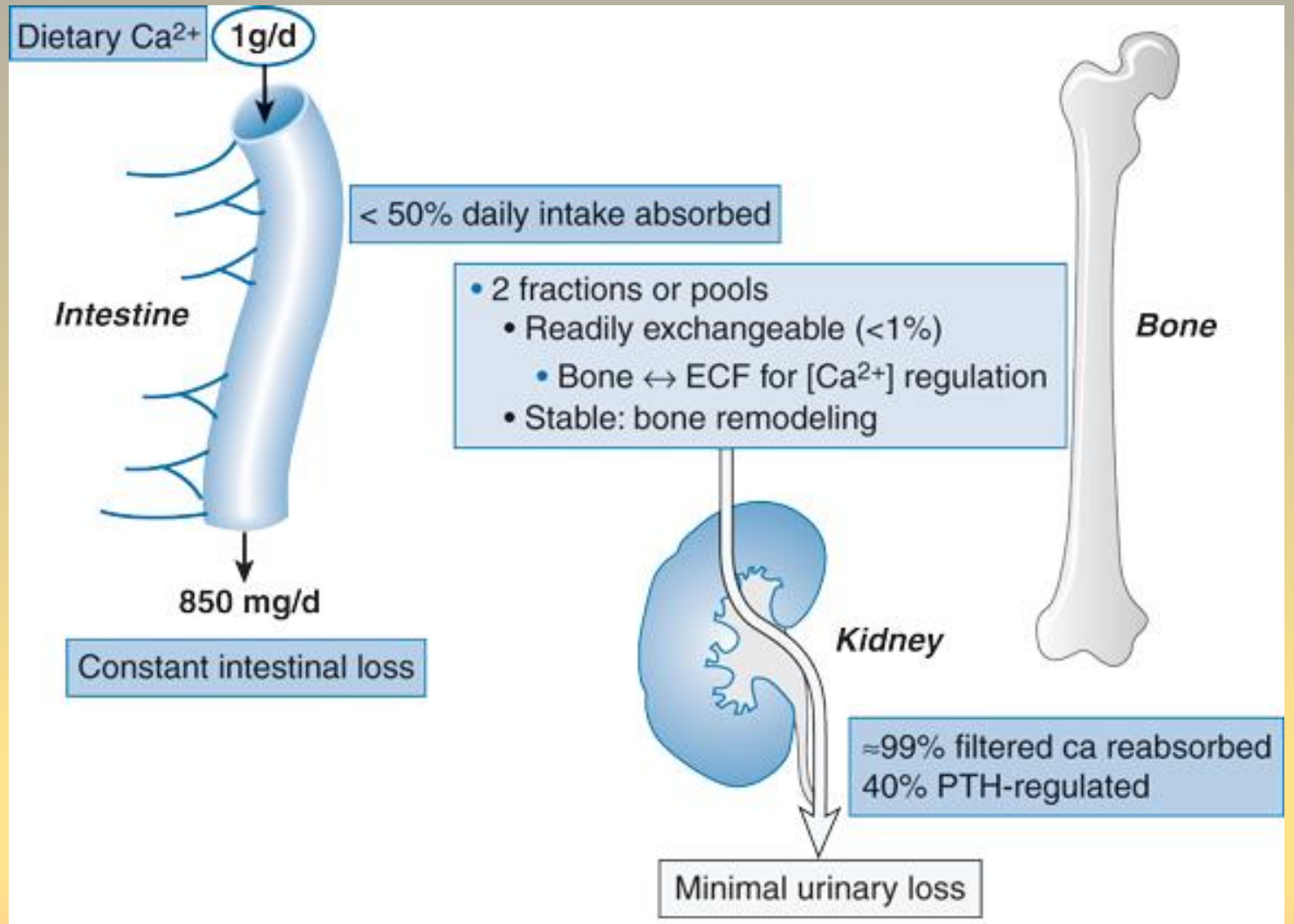


Calcium and its intake

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



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

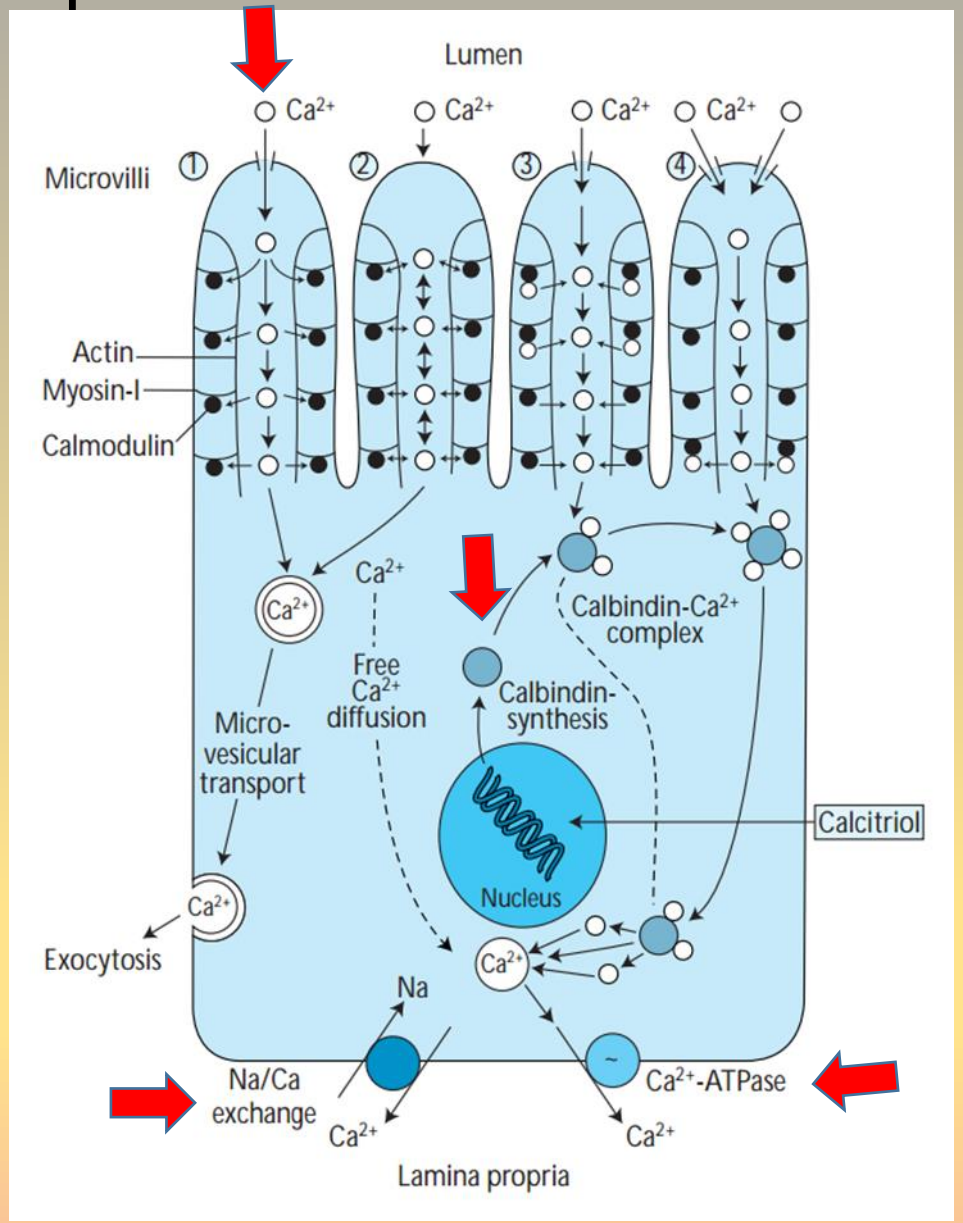
Mechanisms of calcium absorption

Vitamin D

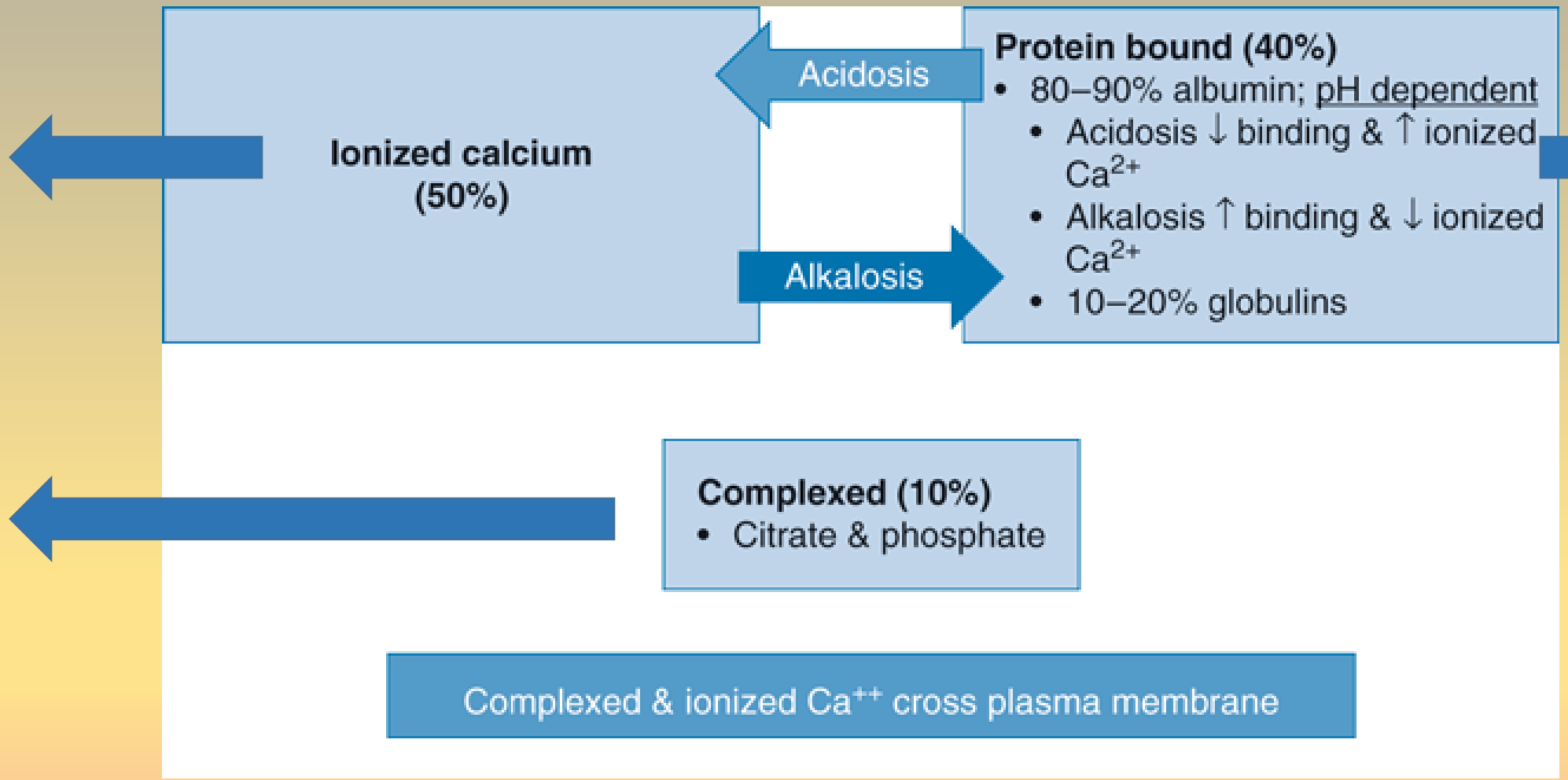
Paracellular
- Luminal electrochemical gradient
- Integrity of intercellular connections
- Claudins and their role in paracellular transport

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

Glucocorticoids	Estradiol	Prolactin
-	+	+



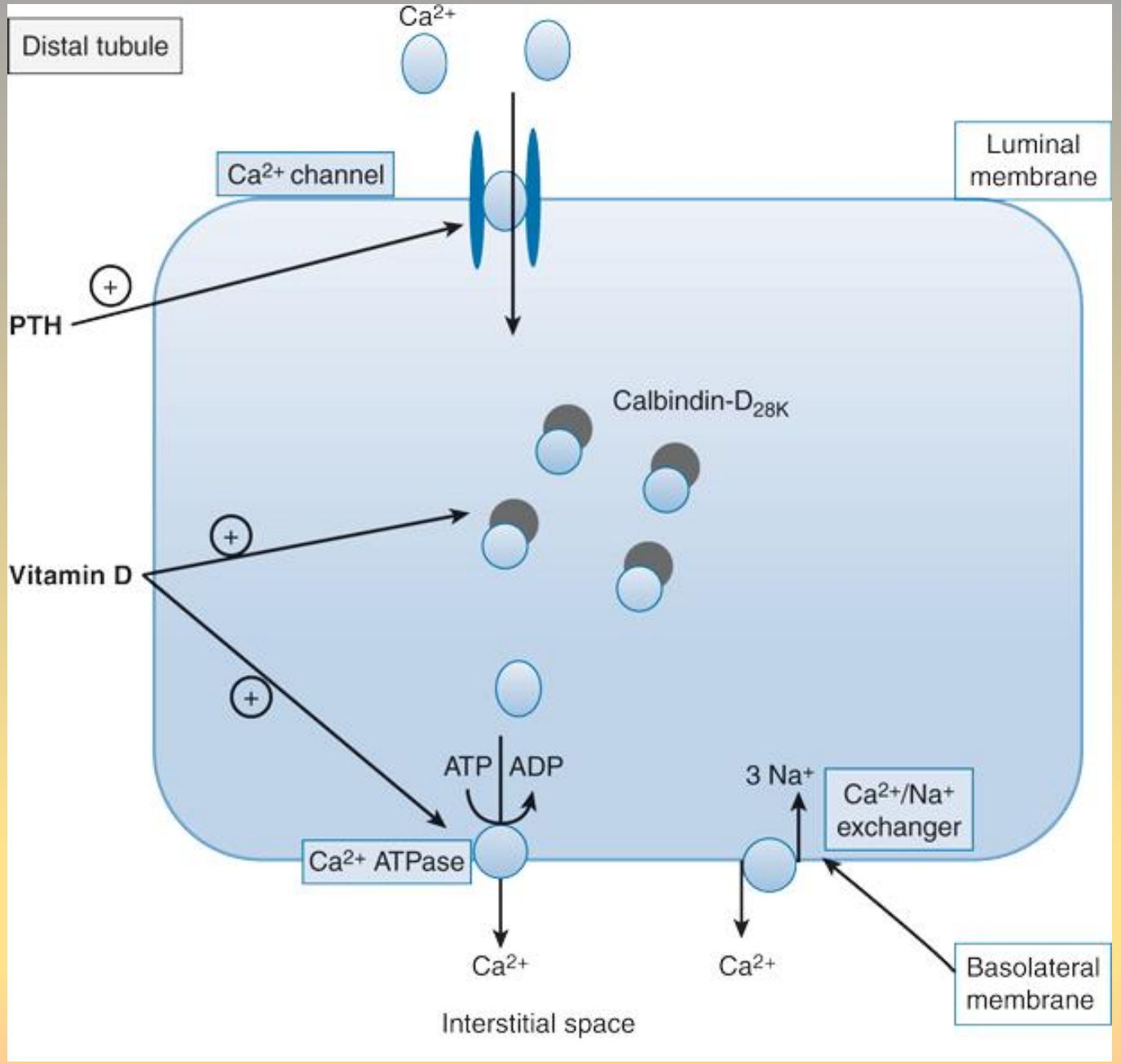
Calcium on blood (calcemia)



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



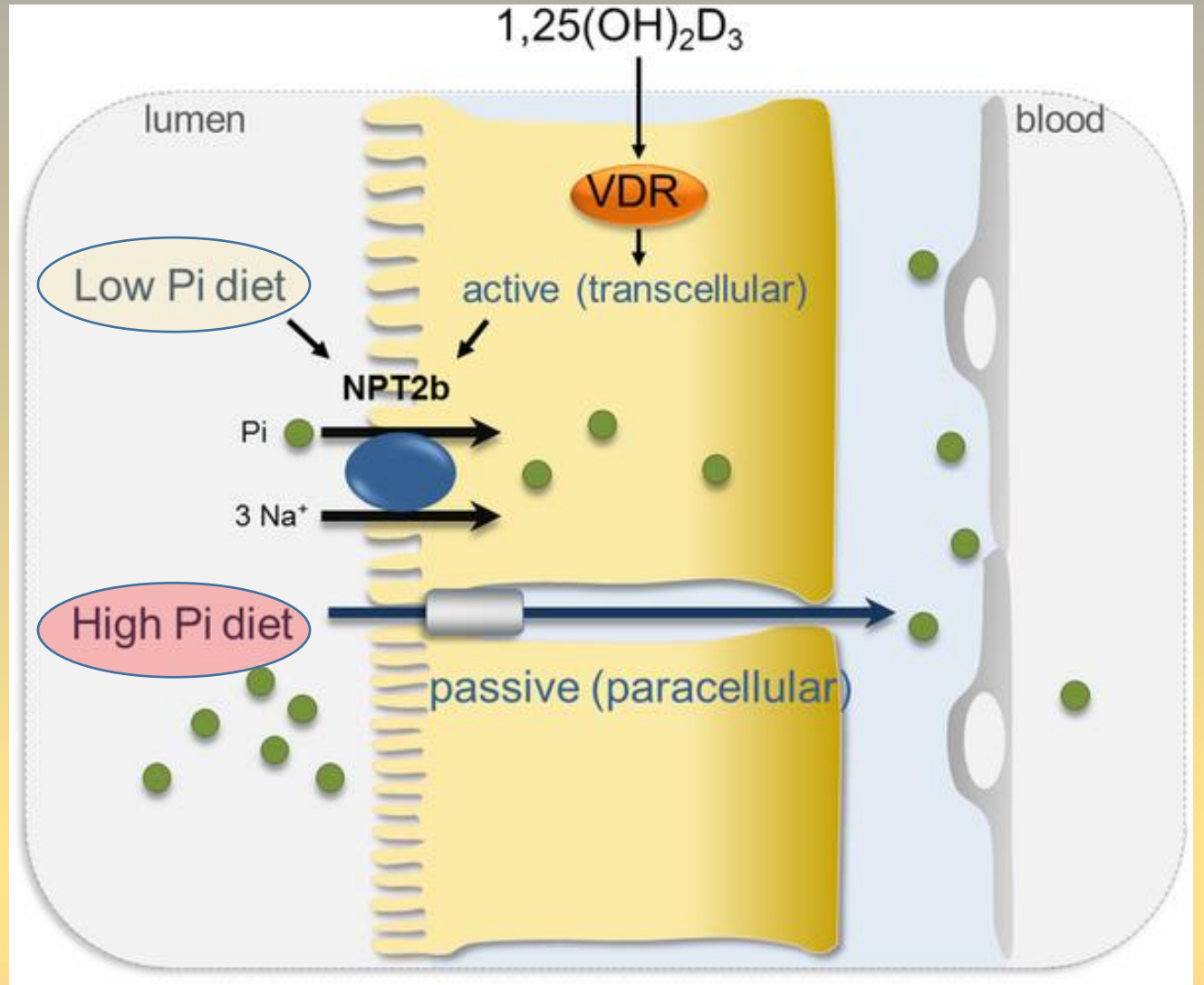
Phosphorus

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

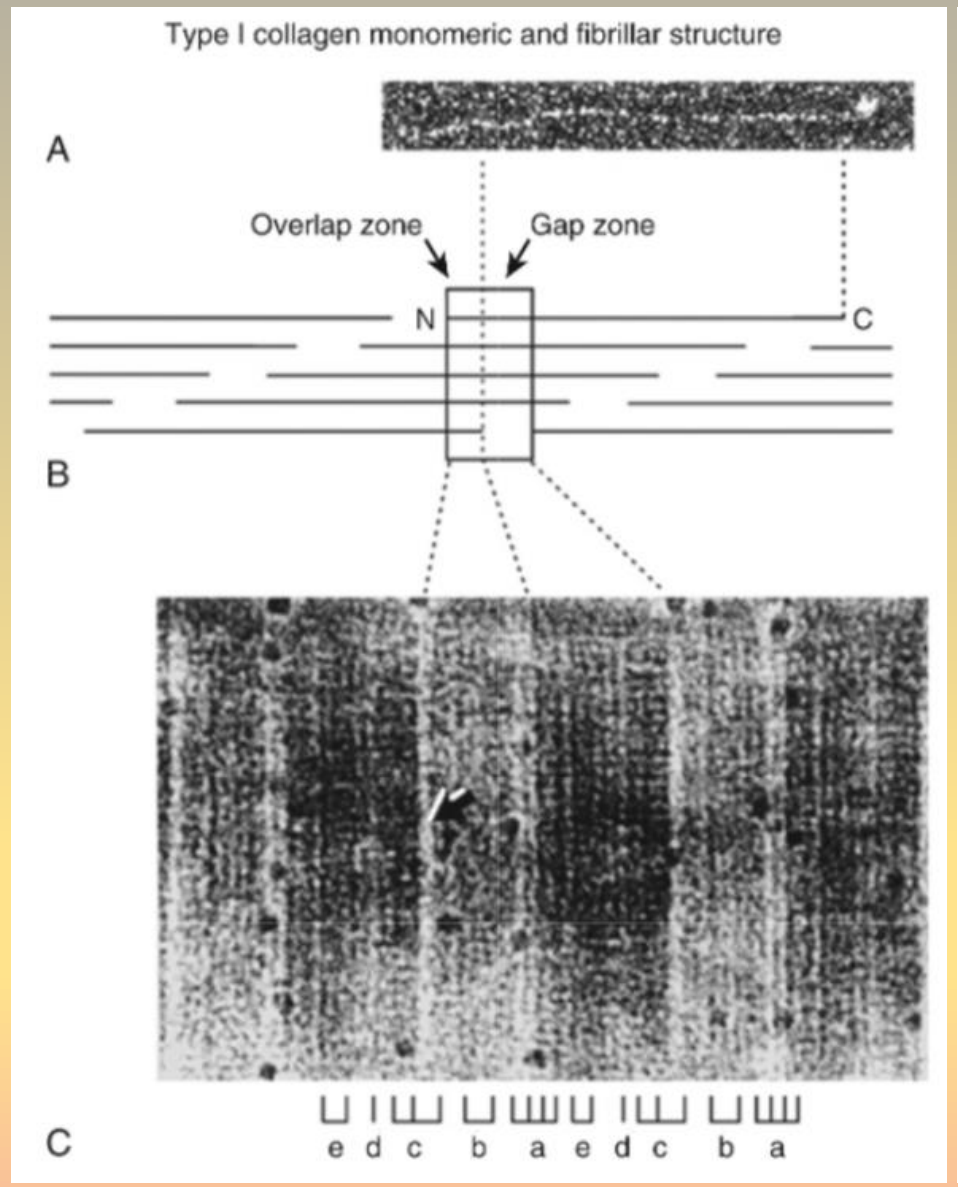
PTH

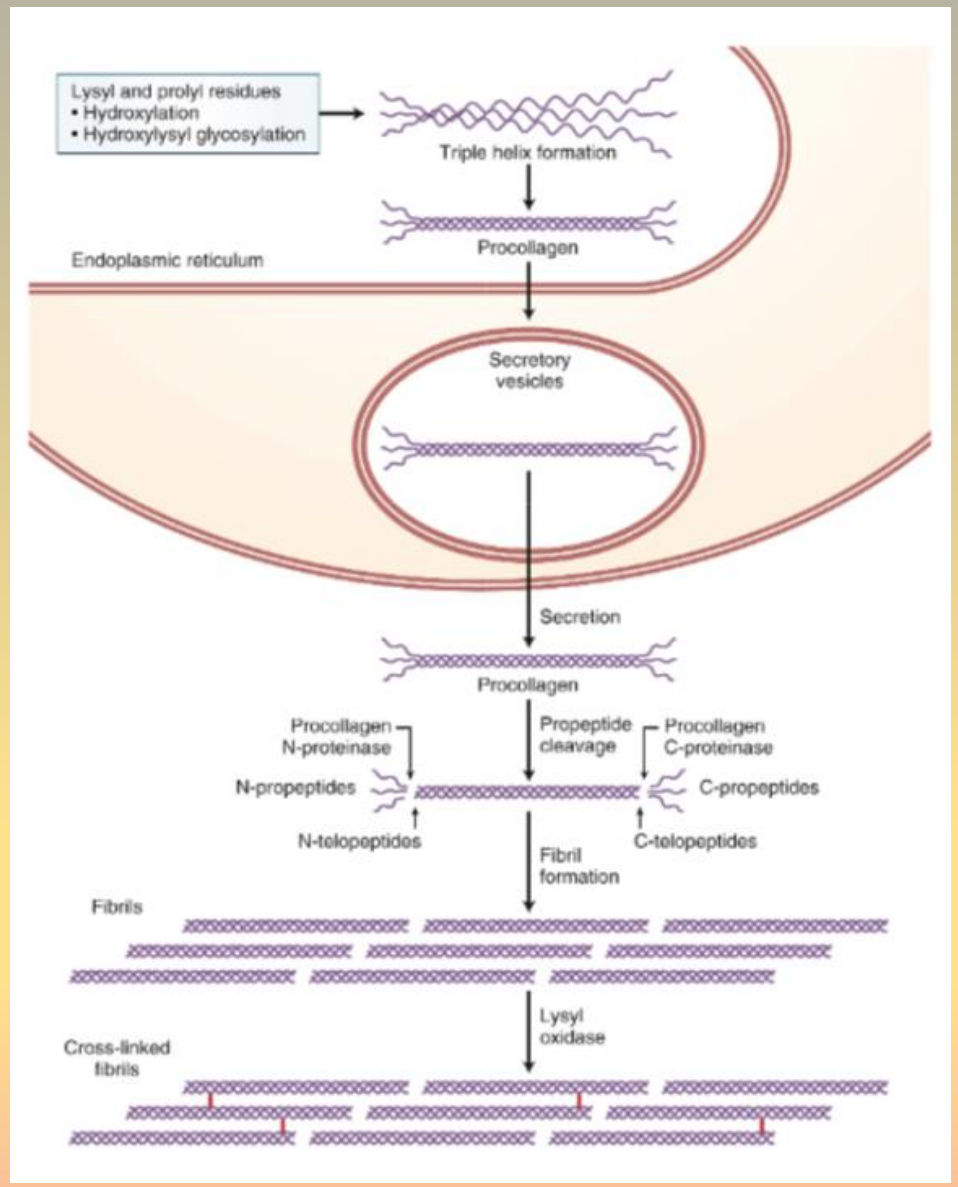
IGF-1

FGF23

Collagen type I = most important protein of bone matrix

Bone matrix and bone mineral





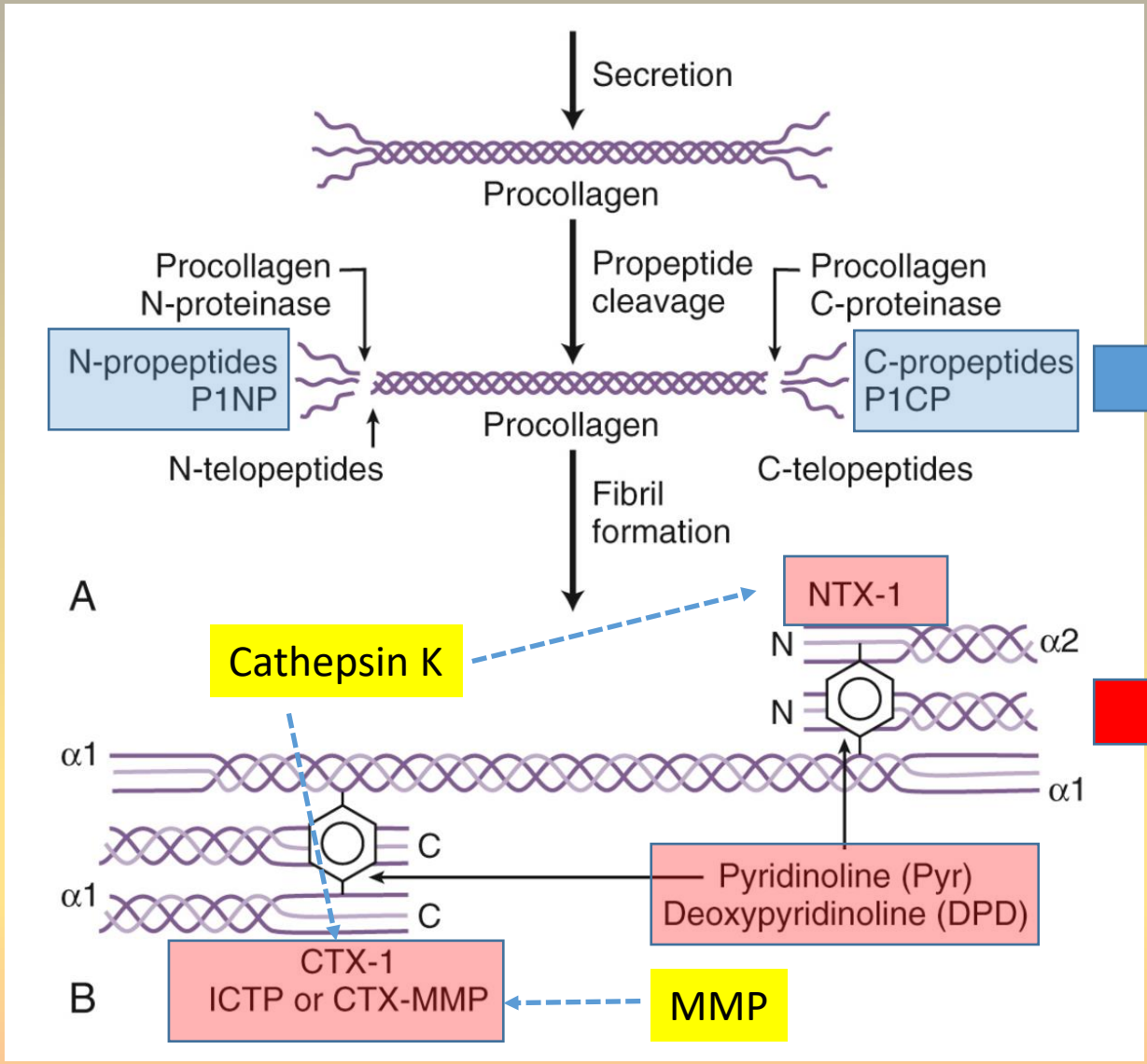
Trombospondin
Fibronectin
Matrix Gla protein
Osteocalcin
Biglykan
Decorin
Bone sialoprotein
Osteopontin
Osteoadherin

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

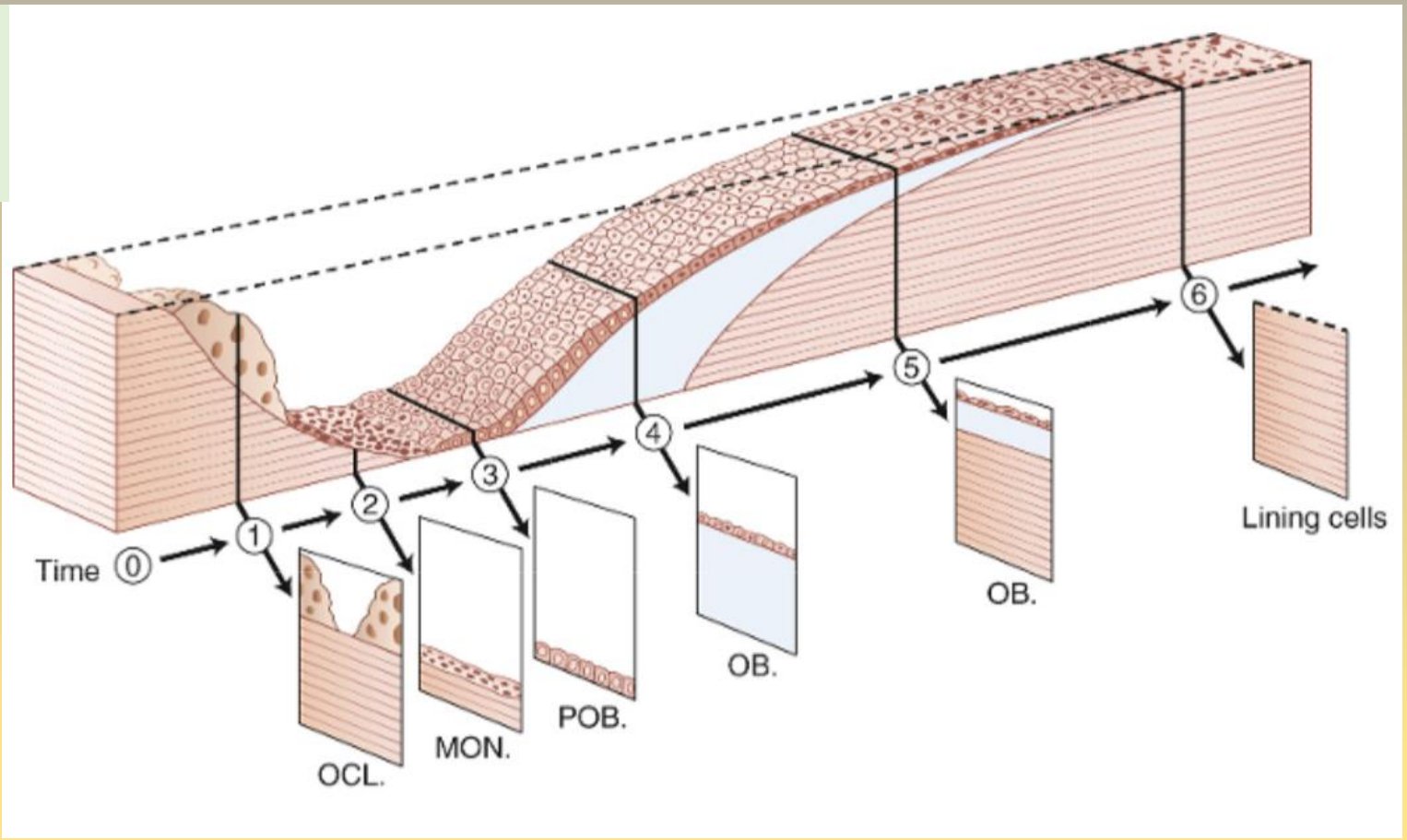
Bone tissue and its remodeling

Modeling
versus remodeling
of bone tissue

REMODELING UNIT - BMU

osteoblasts | osteoclasts | osteocytes

Bone lining cells



Bone reabsorption

Building of bone tissue

osteoclasts | mononuclear cells

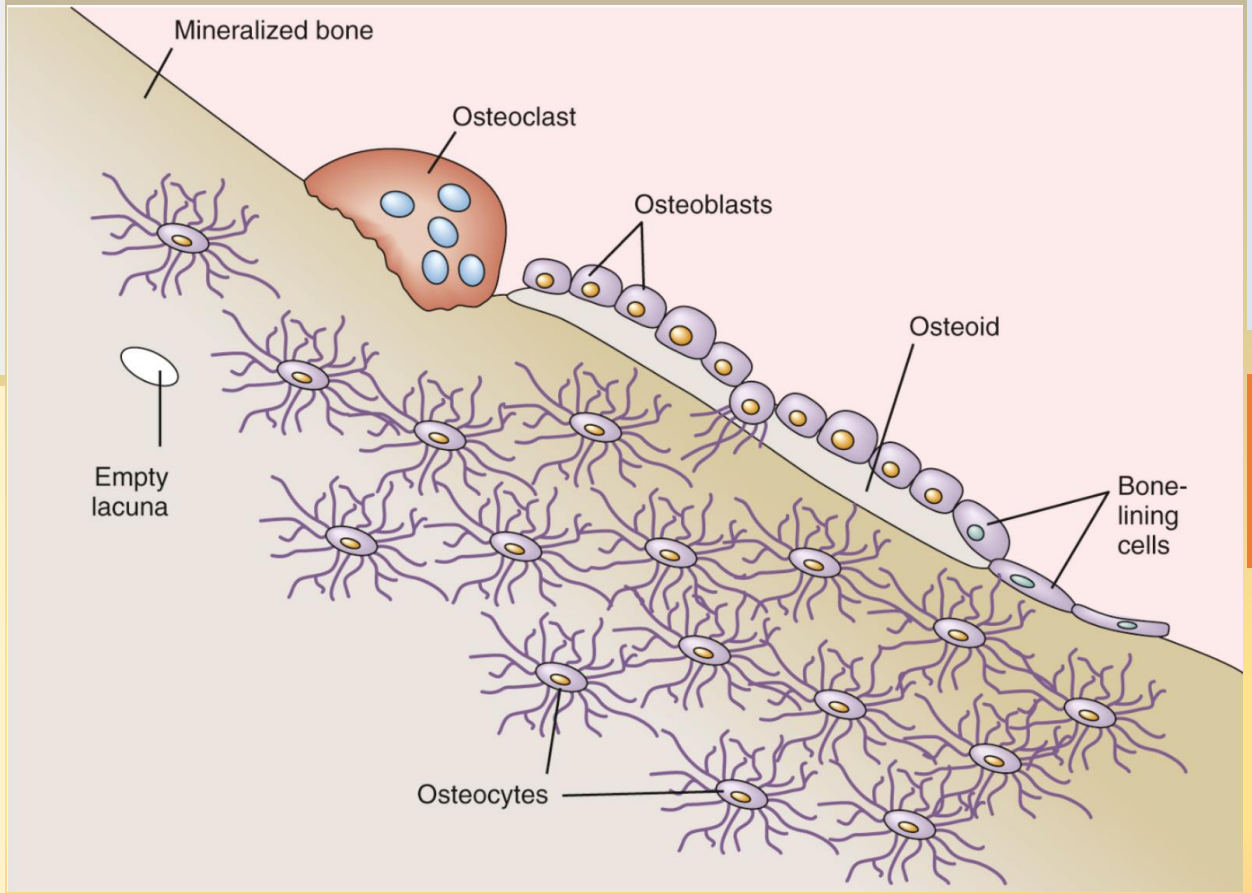
preosteoblasts

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



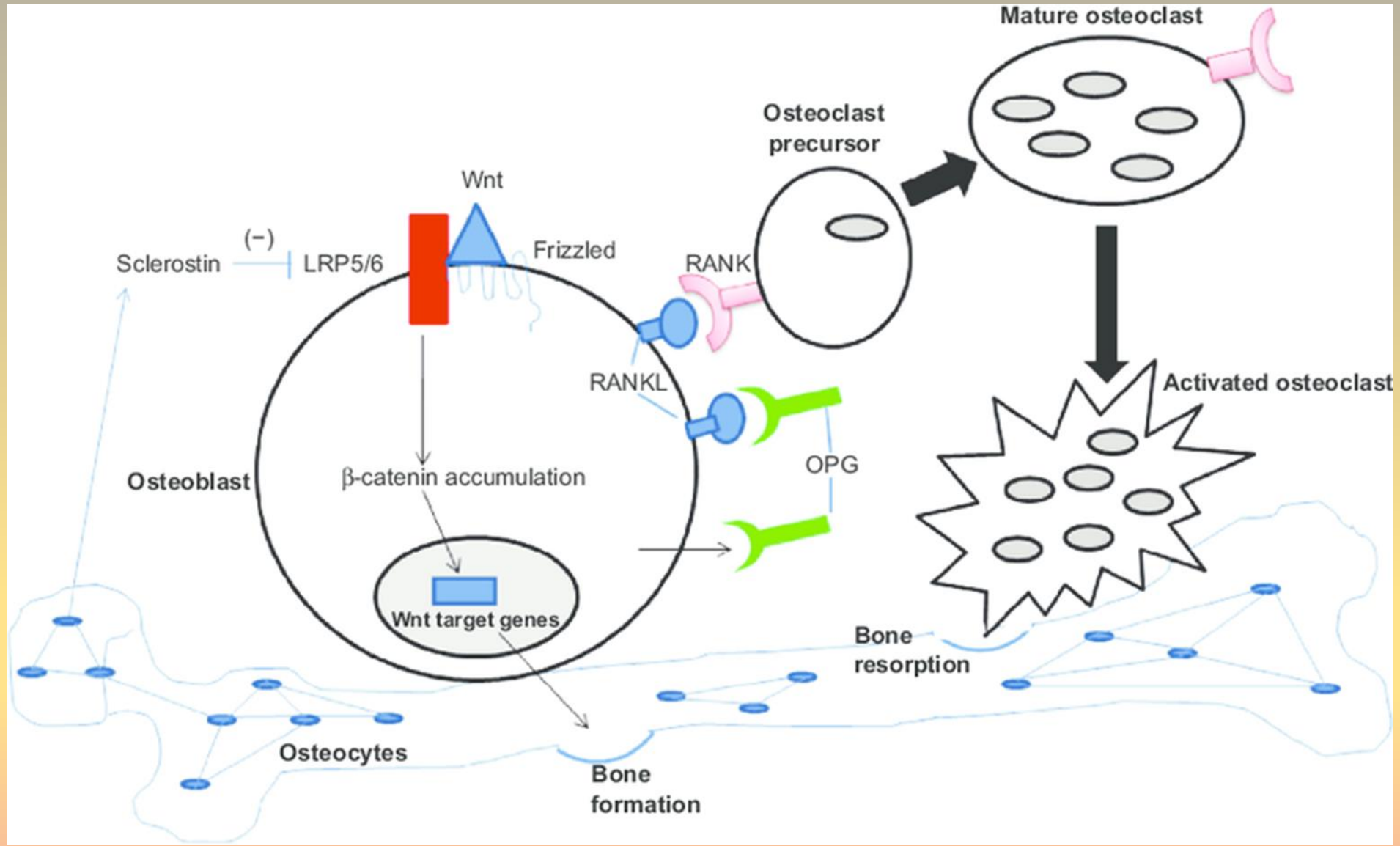
Lining cells
- Stimulation of OB differentiation
- OC communication
- Differentiation to OB stimulated by PTH

Osteoclasts (OK)
- Bone tissue reabsorption

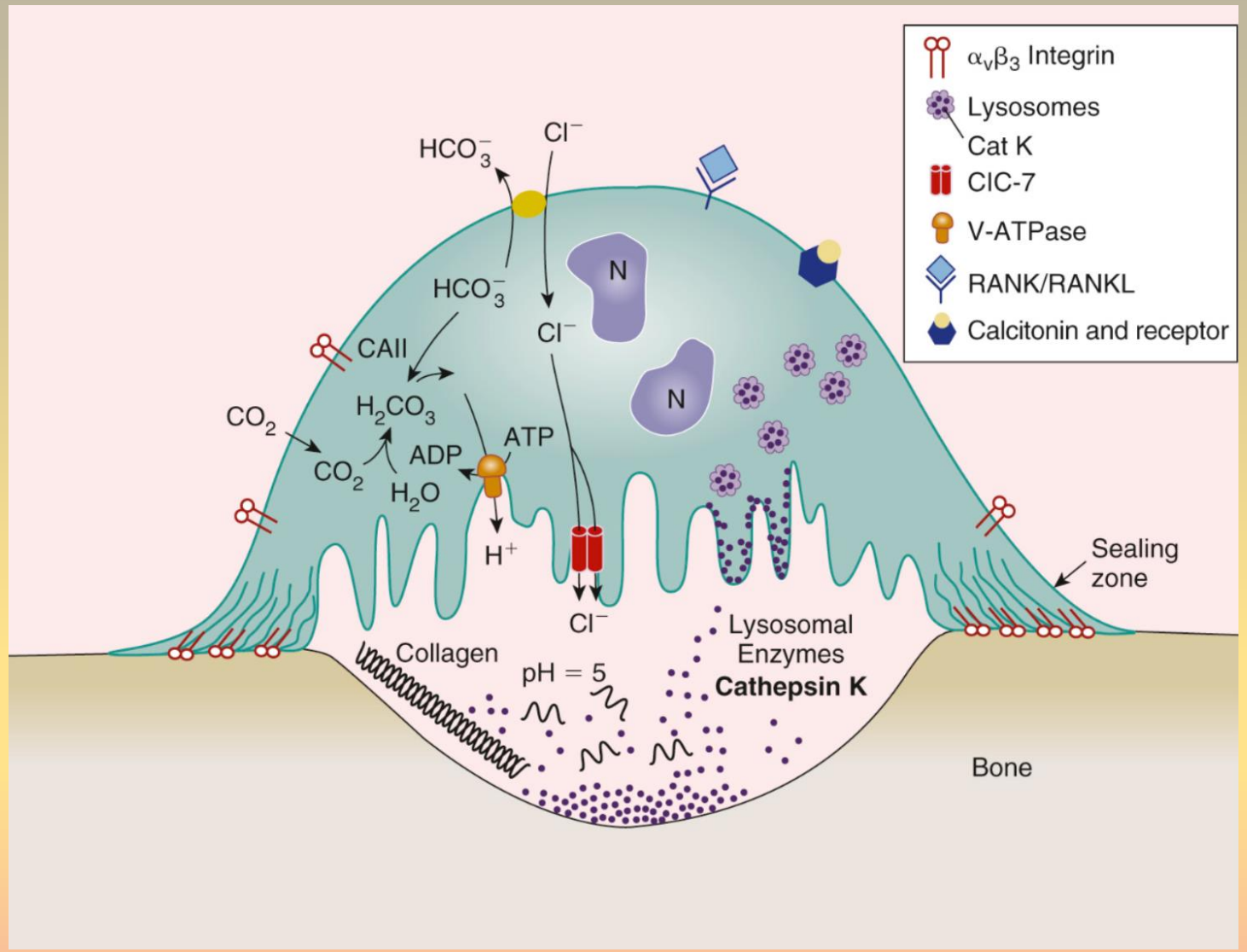
Osteoclasts

Key factor regulating bone resorption is RANKL/OPG ratio.

Osteoclastogenesis
(+) RANKL
(-) OPG



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

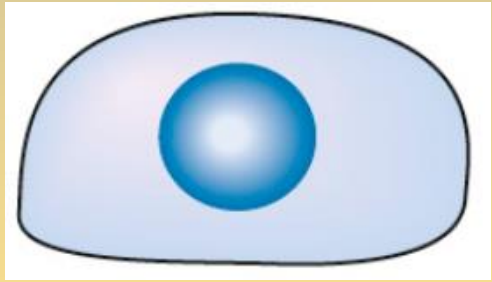
Factors affecting bone tissue remodeling

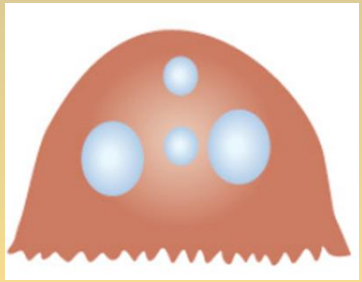
Resorption takes approx. 2 weeks
Mineralization and formation approx. 12 weeks

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

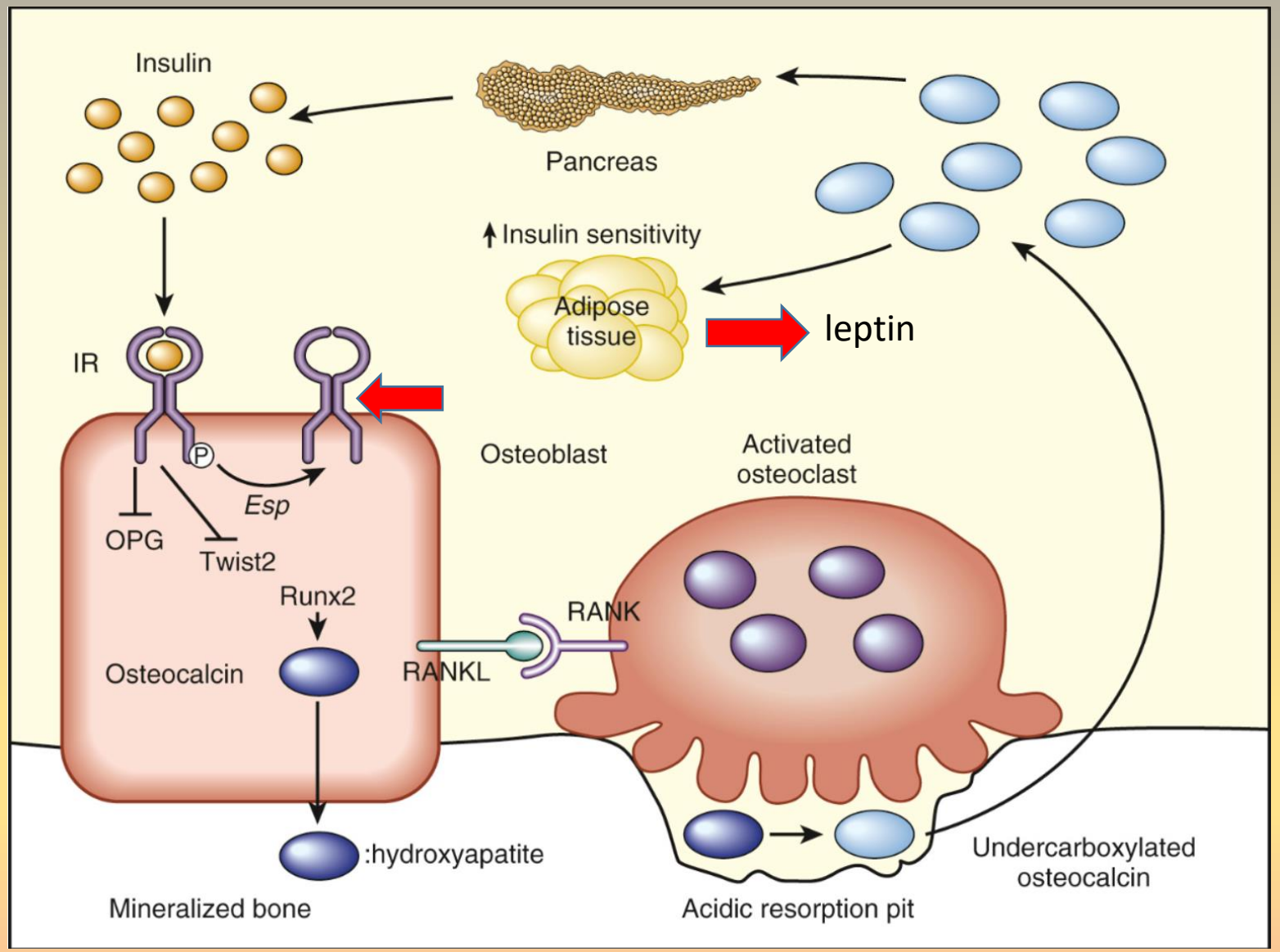
Ensuring mechanical requirements

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)

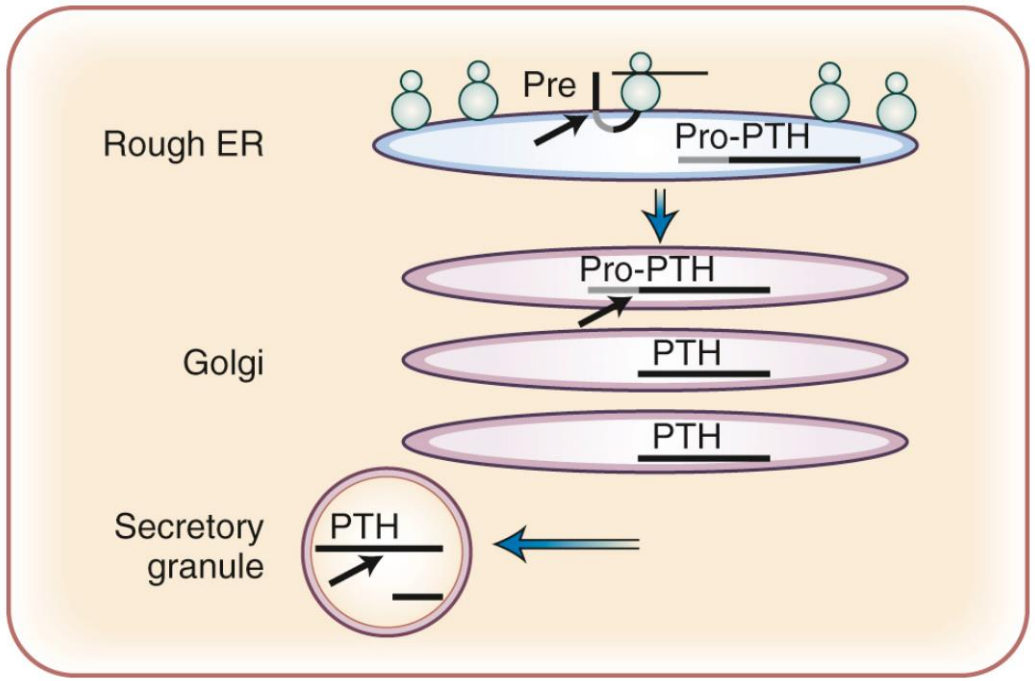
Clinical relevance

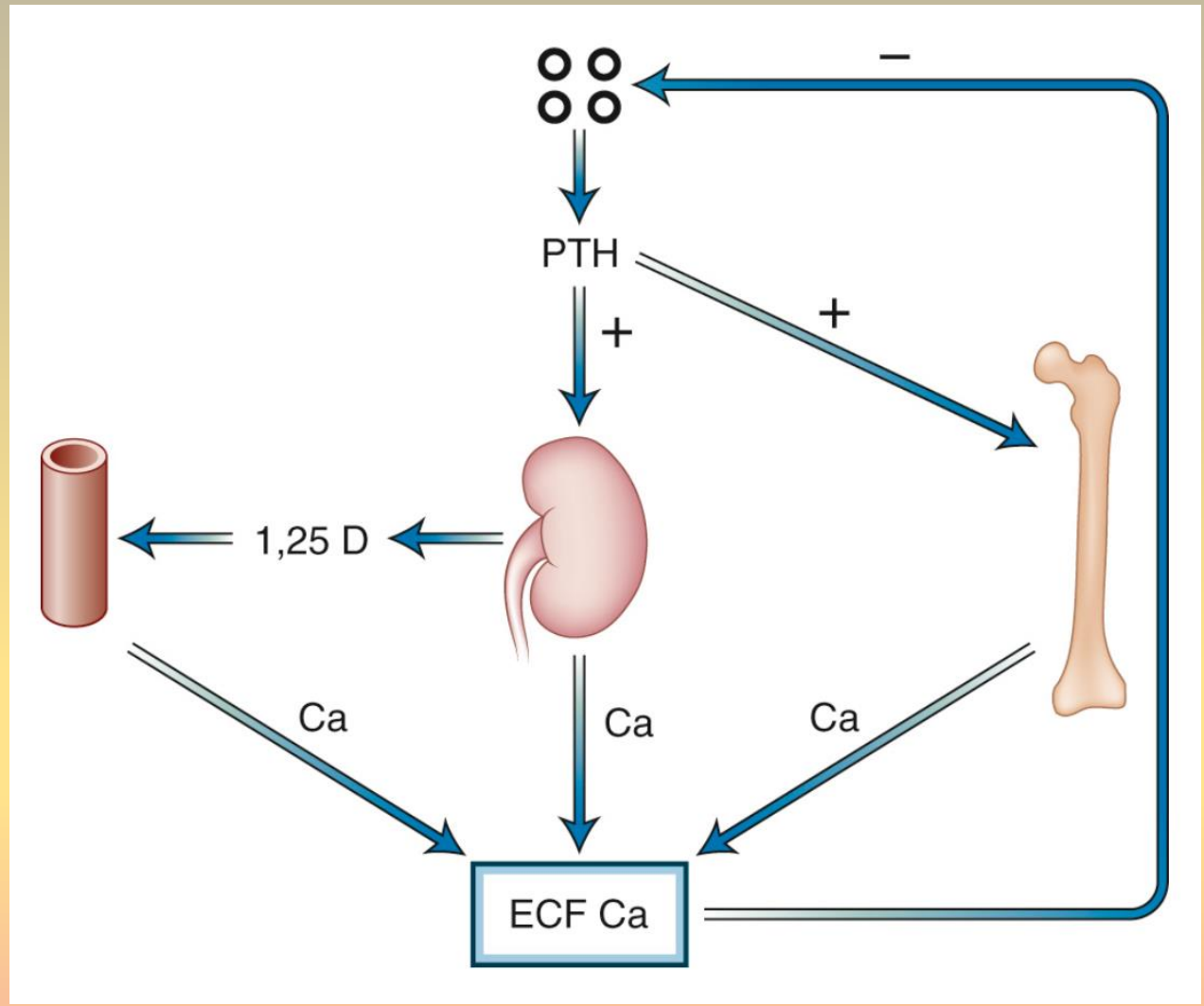
- Osteogenesis imperfecta
- Osteopetrosis
- Osteomalacia
- Rachitis
- Osteopenia – T score -1 – -2.5
- Osteoporosis – T score under -2.5

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.





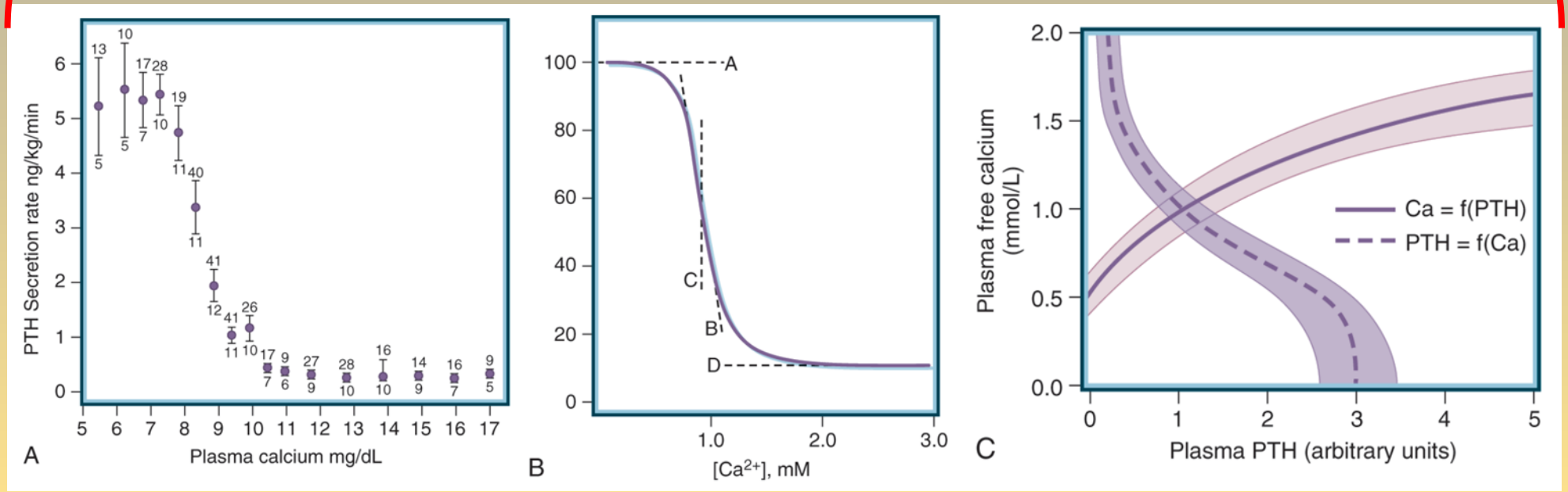
PTH secretion

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



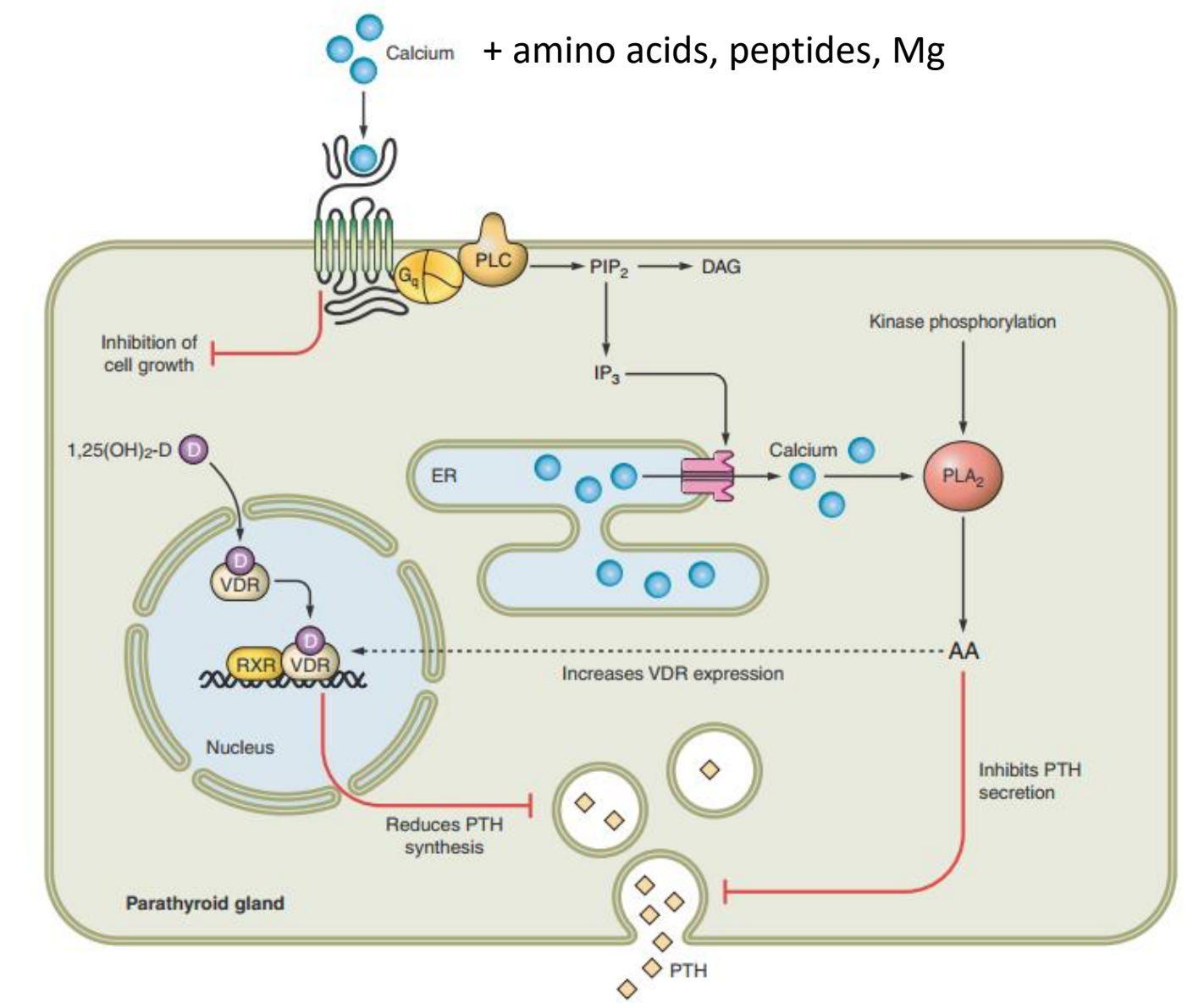
Level of ionized calcium in blood is a key parameter for PTH secretion.

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

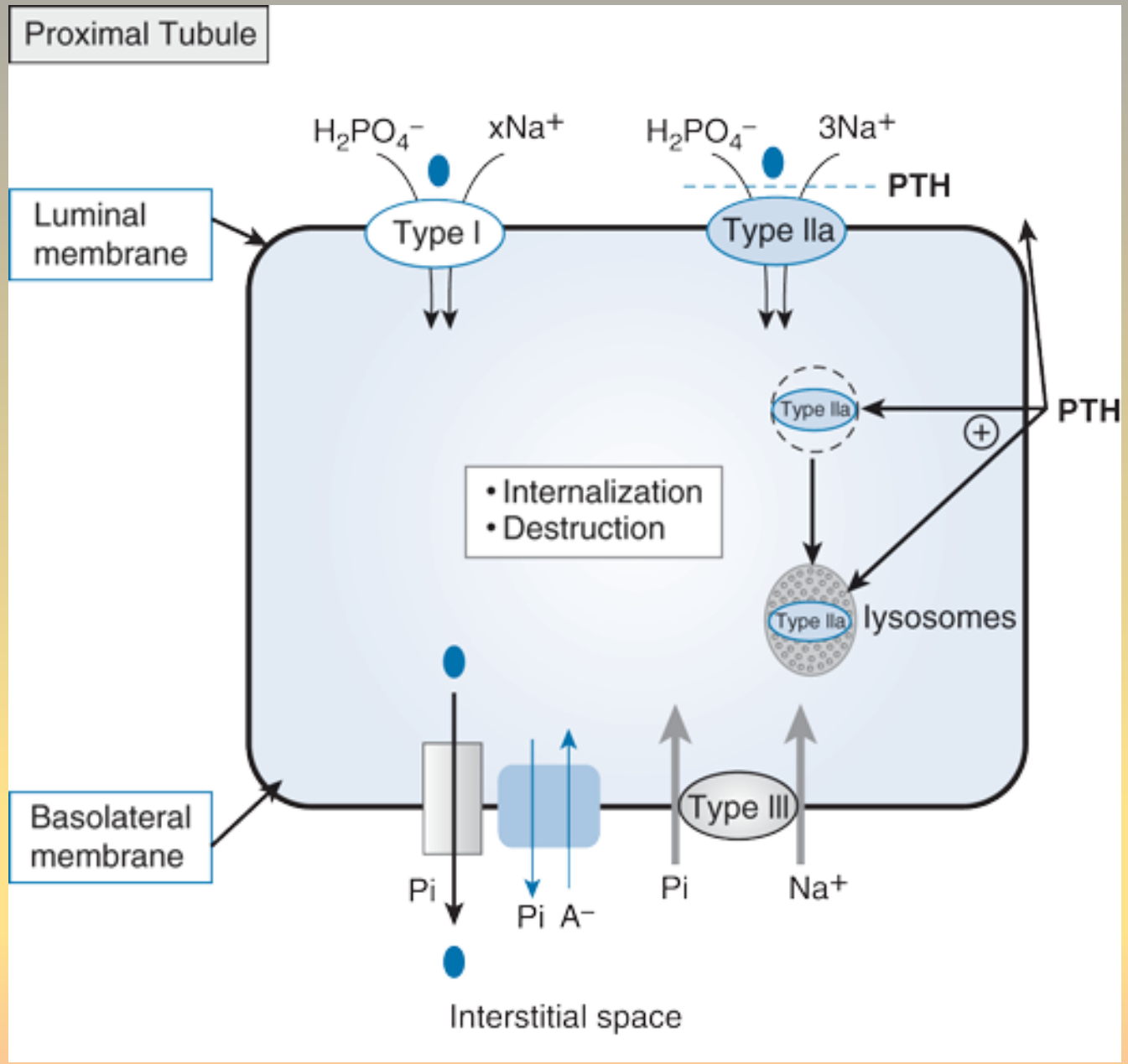
Clinical aspects
- Mutation – inactivation/activation
- familial hypocalciuric hypercalcemia (in.)
- Familial hypoparathyroidism with hypercalciuria (ac.)
- Calcimimetics – inhibition of PTH secretion

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

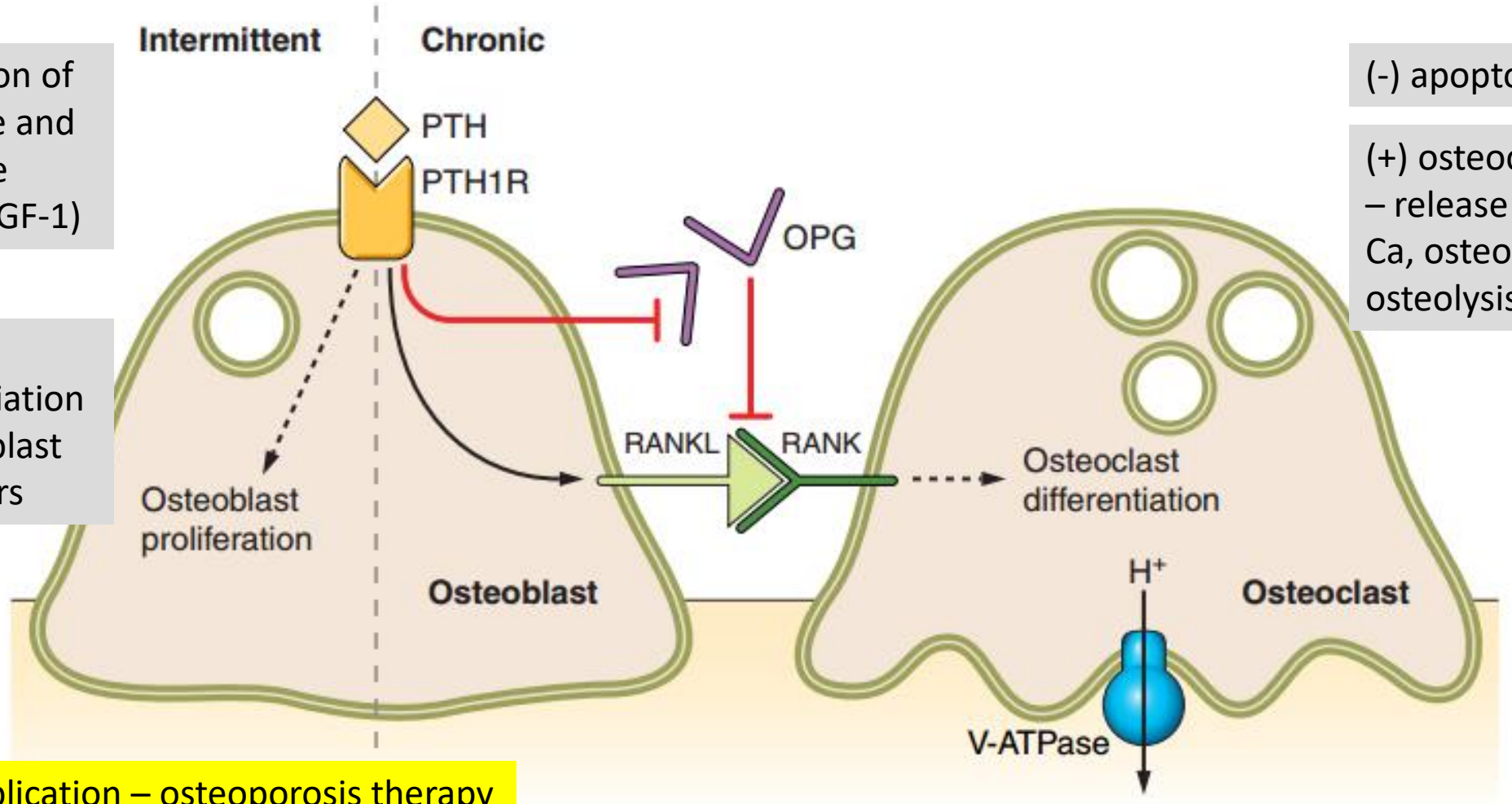
(+) activity of 1α-hydroxylase - PT
(-) resorption of Na, water and bicarbonate – PT
(-) Na^+/K^+-APTase (basolateral membrane)
(+) gluconeogenesis – PT
(-) GFR - podocytes



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.

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?

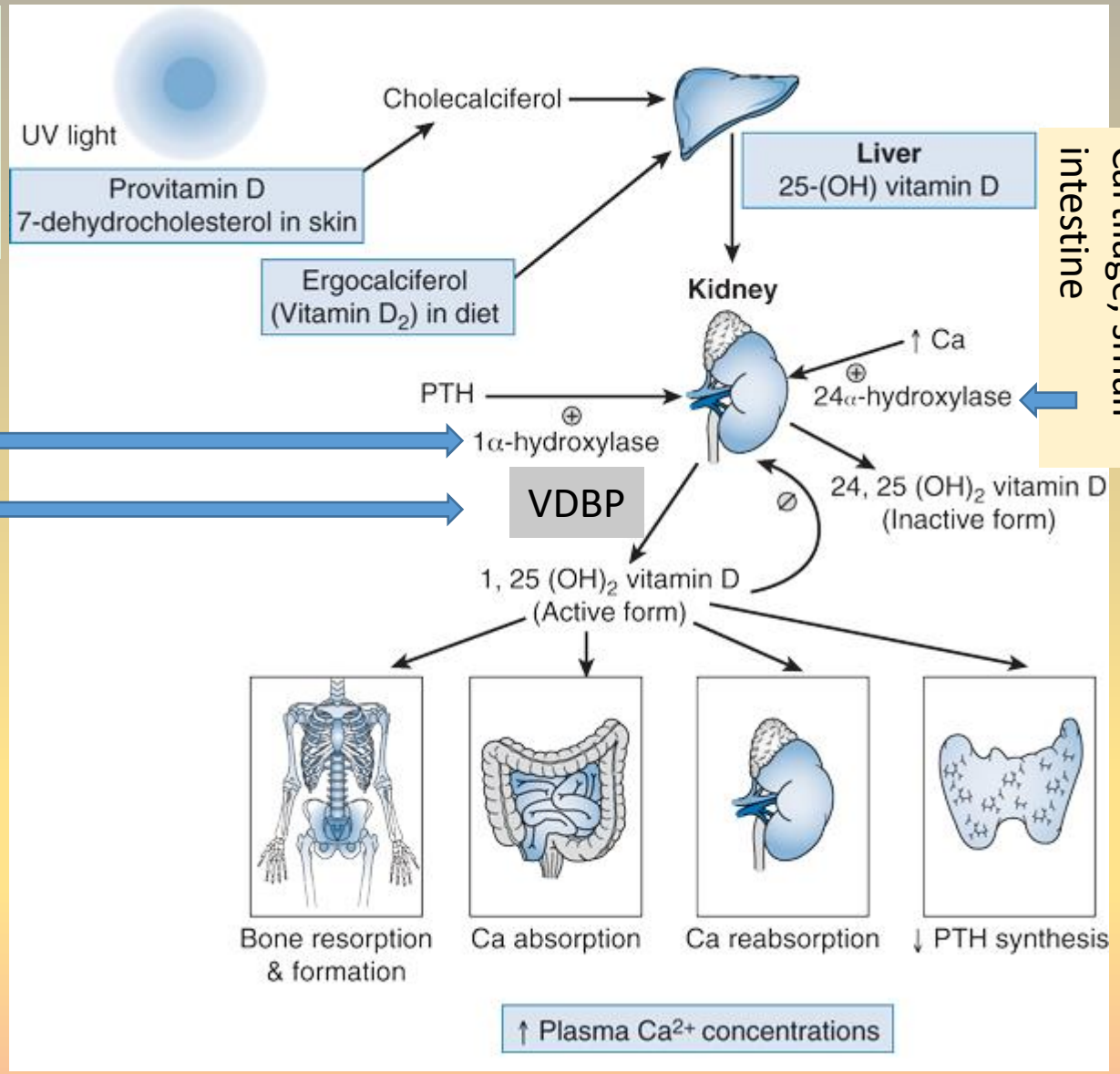
Clinical relevance
- Osteoporosis therapy
- Paget disease therapy
- Treatment of pain (bones metastases)
- ! Increased risk of cancer

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

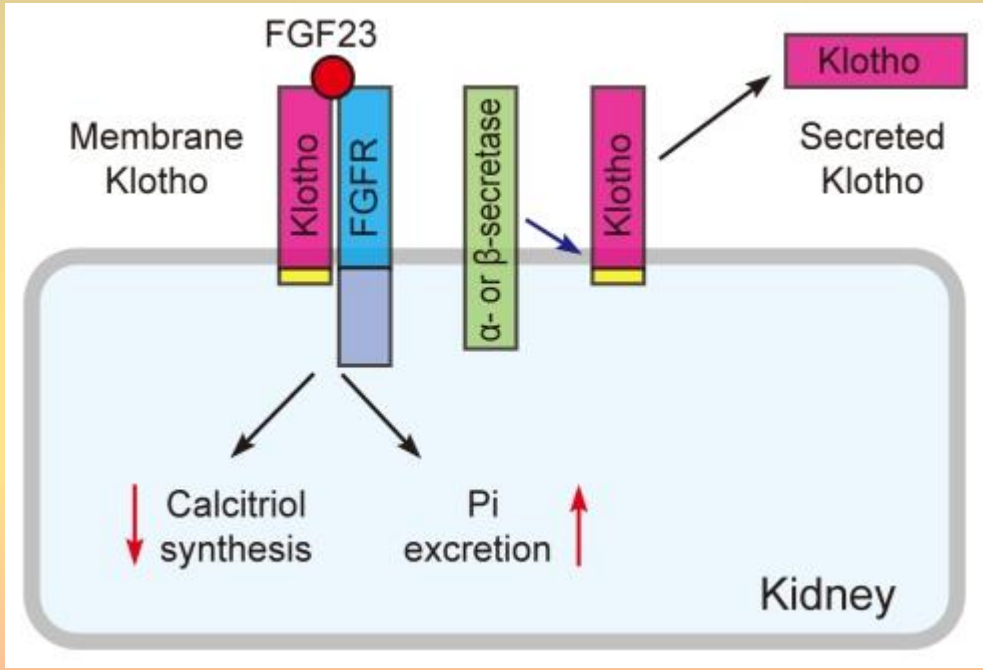
FGF23 – fibroblast growth factor 23

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



Calcium homeostasis – still just a simplified model