

# 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
<b>Extracellular</b>		
Concentration		
total, in serum	$2.5 \times 10^{-3} \text{ M}$	$1.00 \times 10^{-3} \text{ M}$
free	$1.2 \times 10^{-3} \text{ M}$	$0.85 \times 10^{-3} \text{ M}$
Functions	Bone mineral Blood coagulation Membrane excitability	Bone mineral
<b>Intracellular</b>		
Concentration	$10^{-7} \text{ M}$	$1-2 \times 10^{-3} \text{ M}$
Functions	<b>Signal for:</b> <ul style="list-style-type: none"> <li>• Neuron activation</li> <li>• Hormone secretion</li> <li>• Muscle contraction</li> </ul>	<ul style="list-style-type: none"> <li>• Structural role</li> <li>• High energy bonds</li> <li>• Regulation of proteins by phosphorylation</li> </ul>

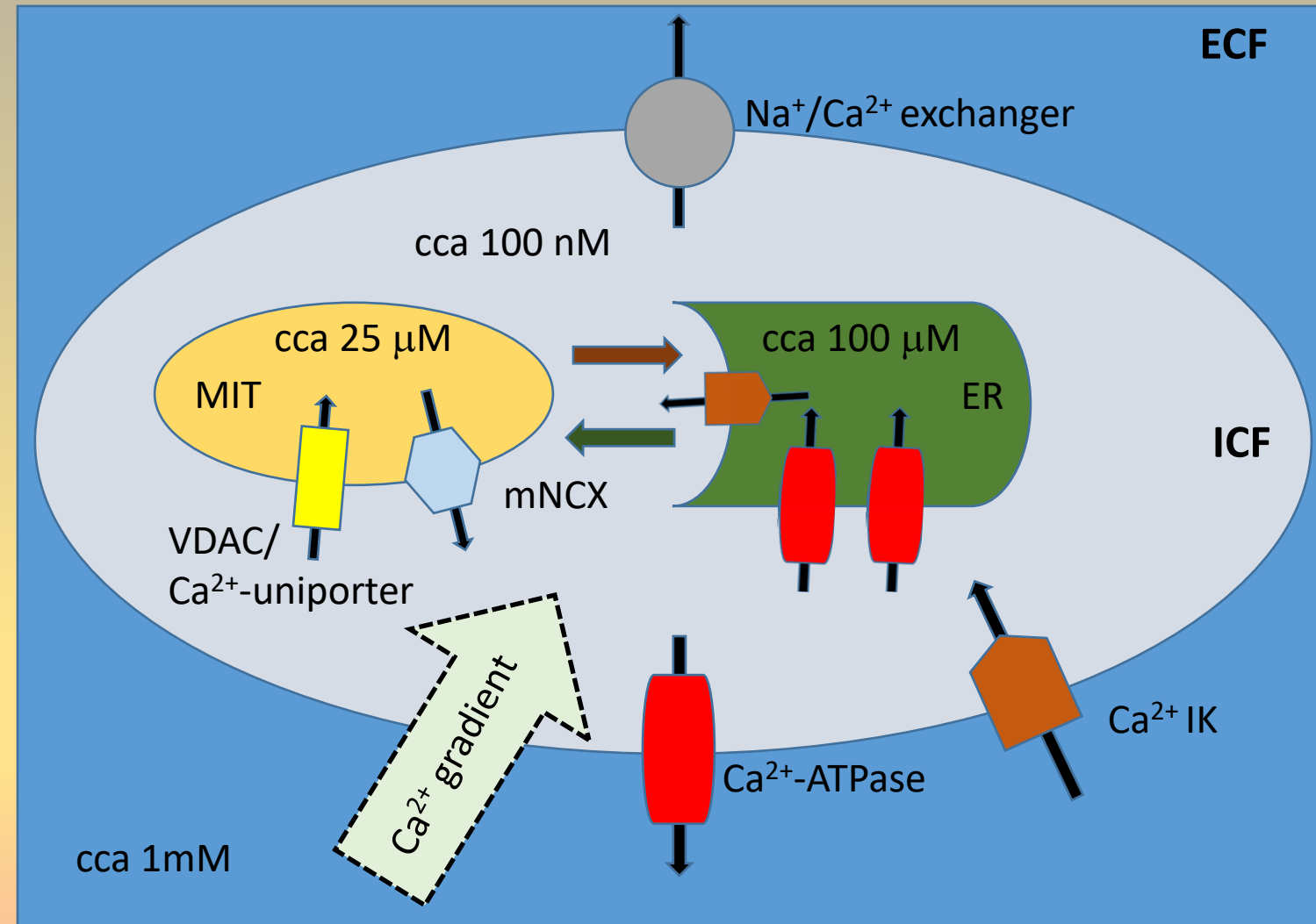
# Extra- and intracellular calcium

## 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

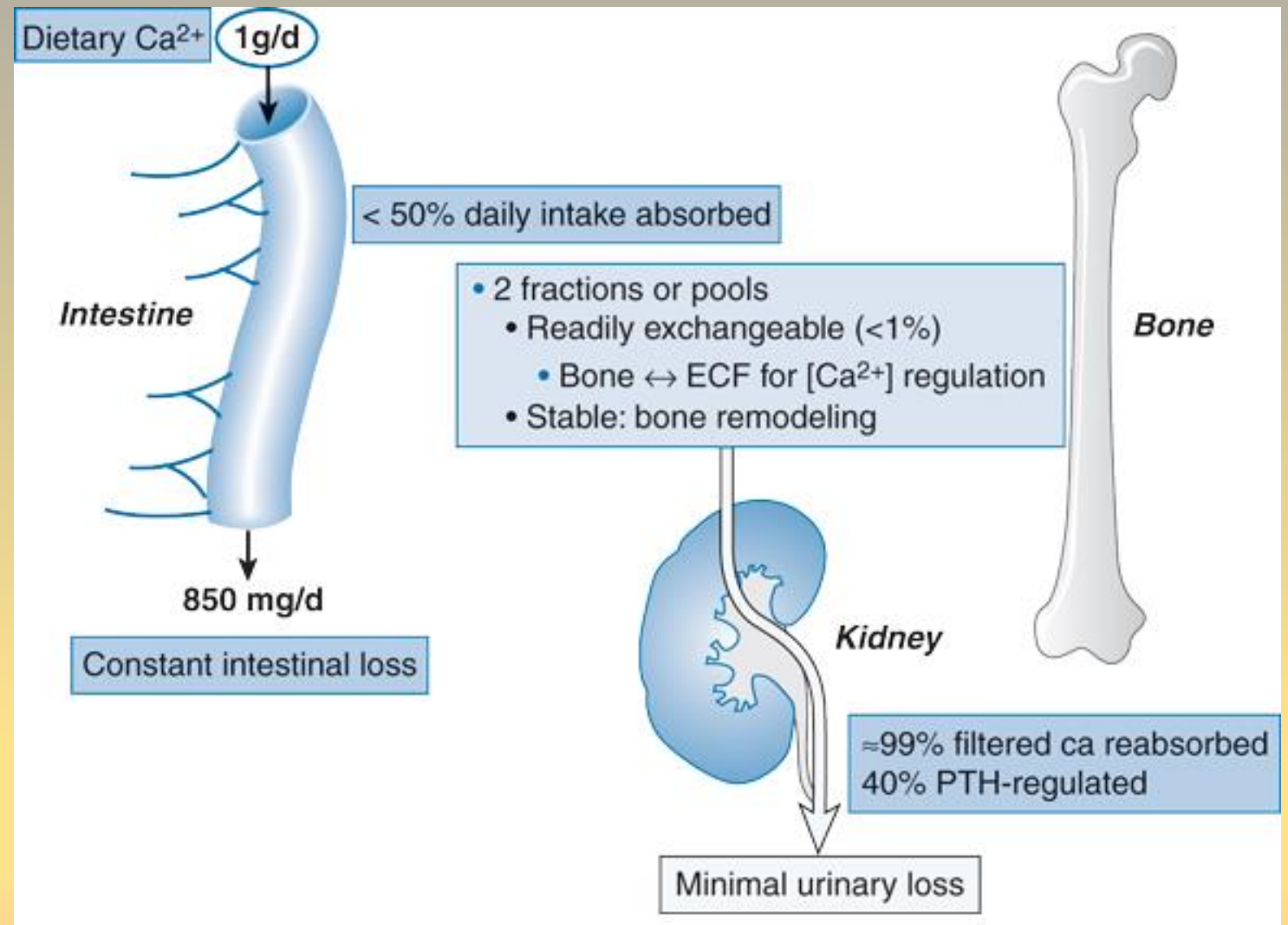
- 25 – 60 %
- Age
- Dietary habits and calcium content in diet
- Bone tissue requirements
- Vitamin D

## 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
- Recycling of TRPV6
- Alternative mechanisms?

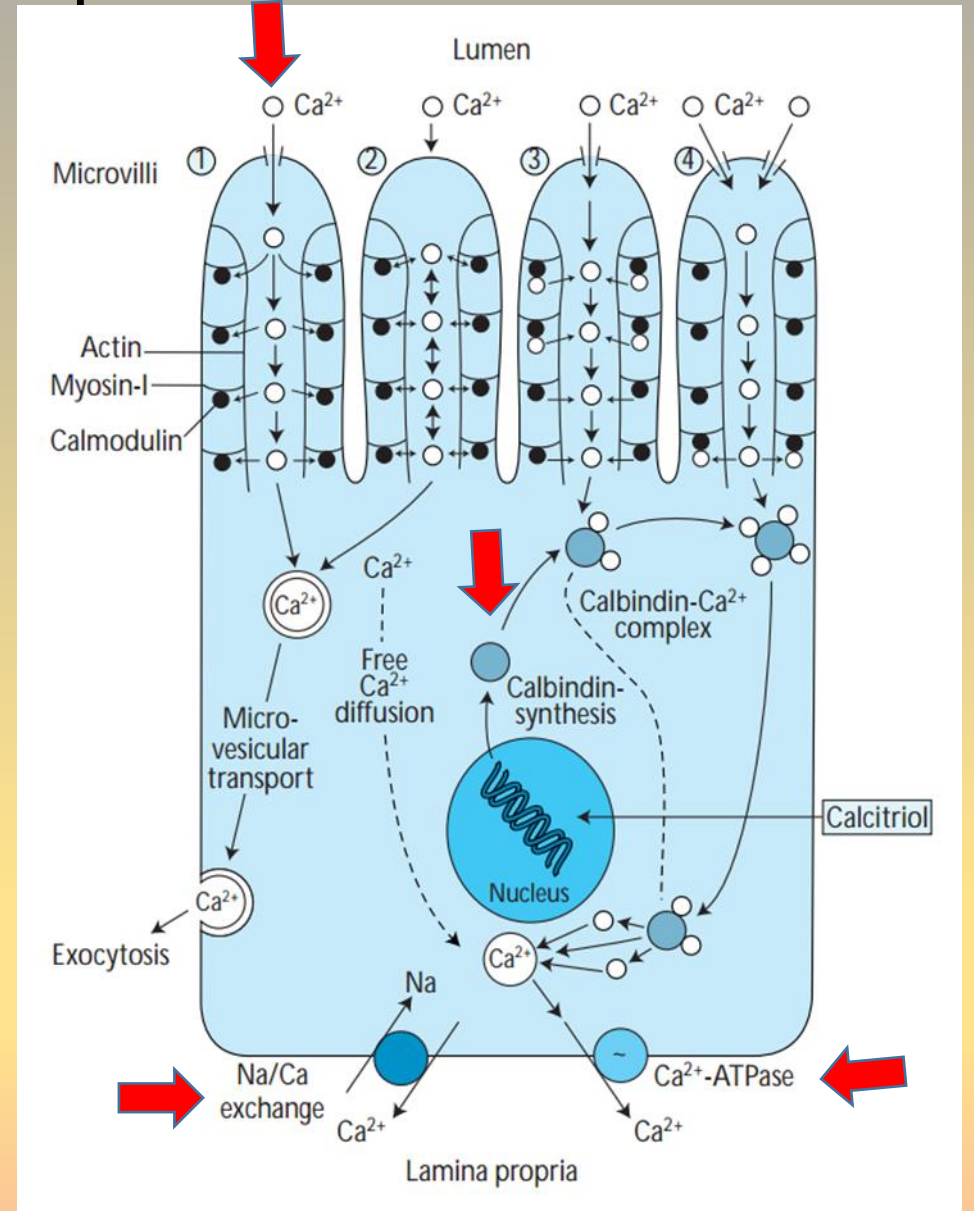
Glucocorticoids



Estradiol



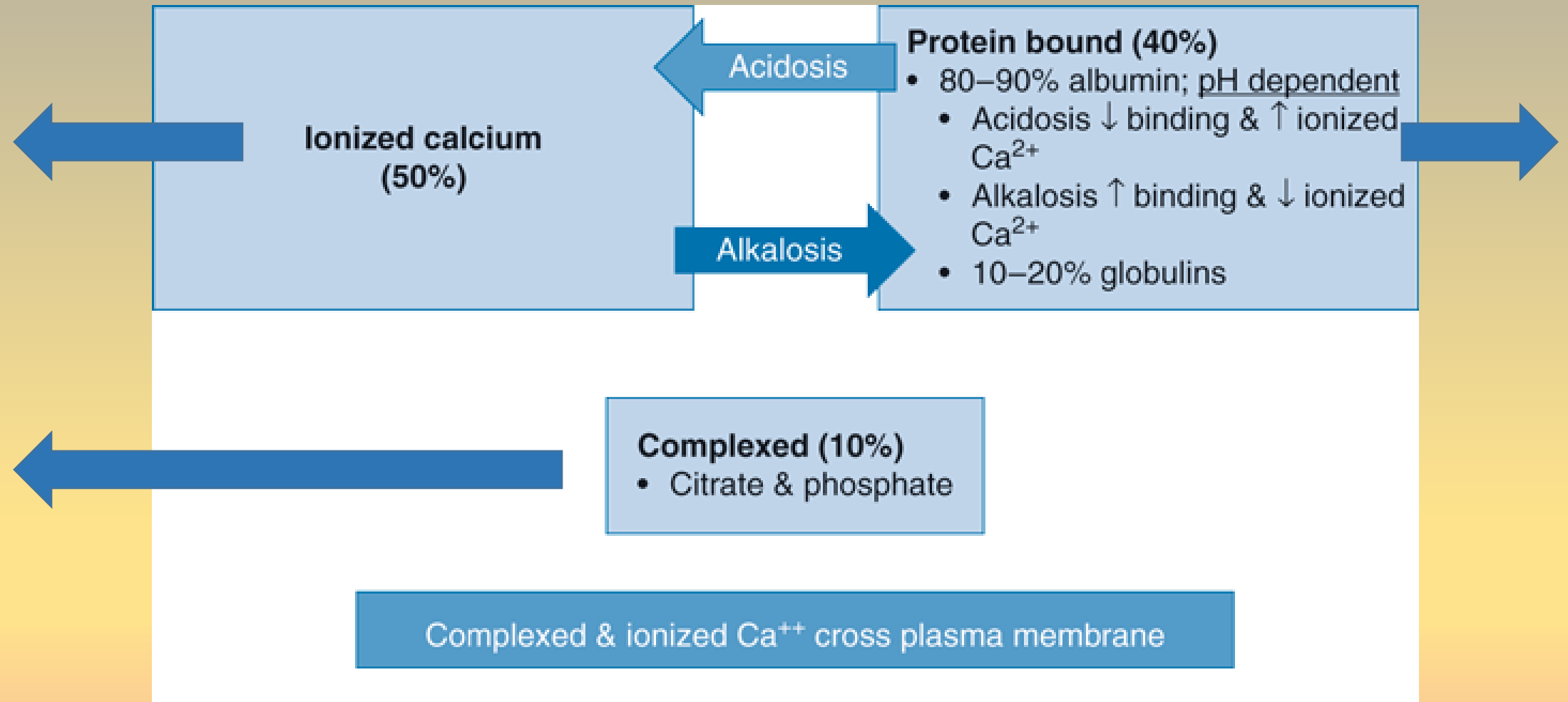
Prolactin



# Calcium on blood (calcemia)

Glomerular filtration YES

Endocrine control

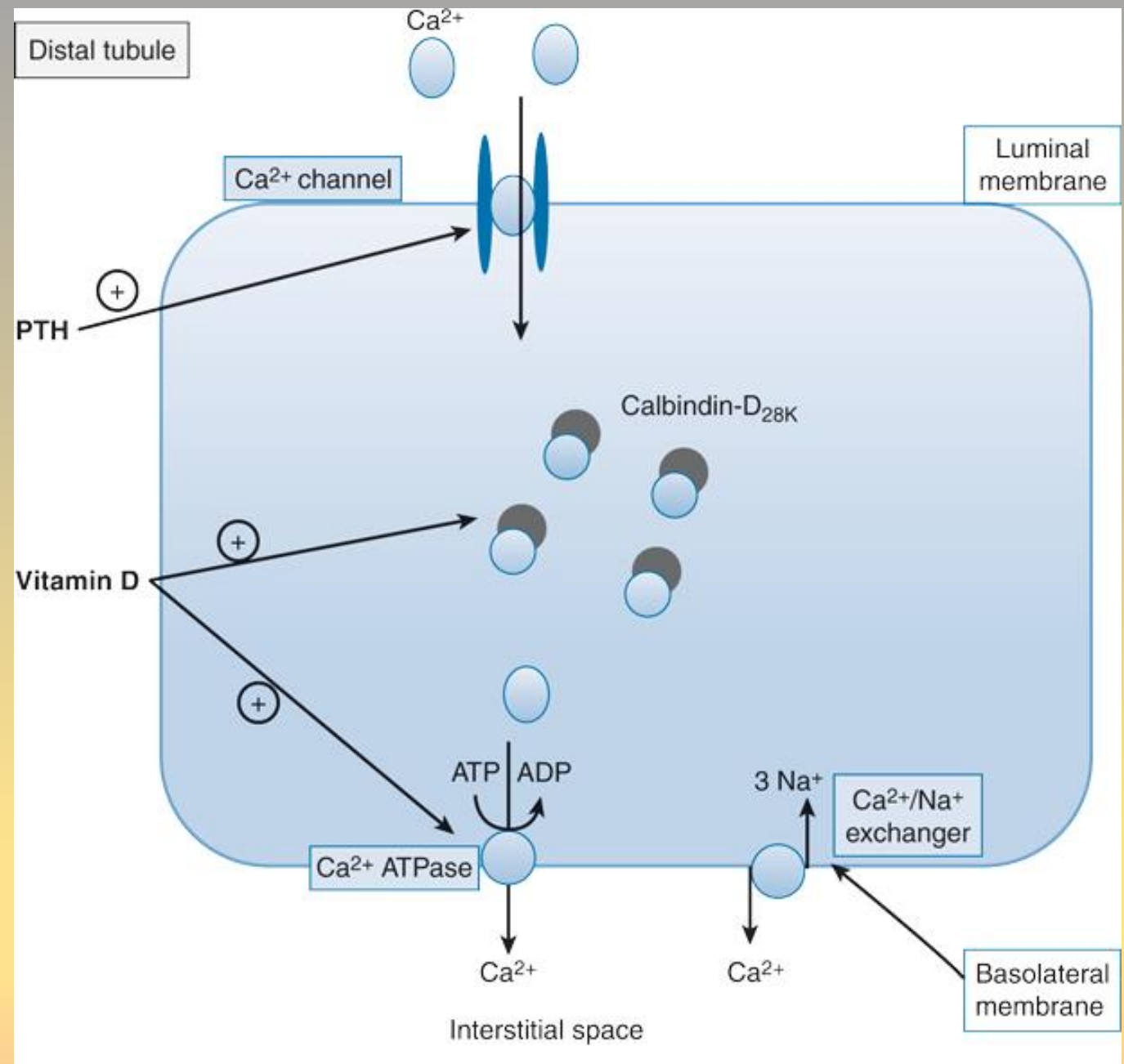


Glomerular filtration NO

# 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 % -  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$
- Organic and inorganic form in ICF and ECF
- Age, sex, growth

## Blood

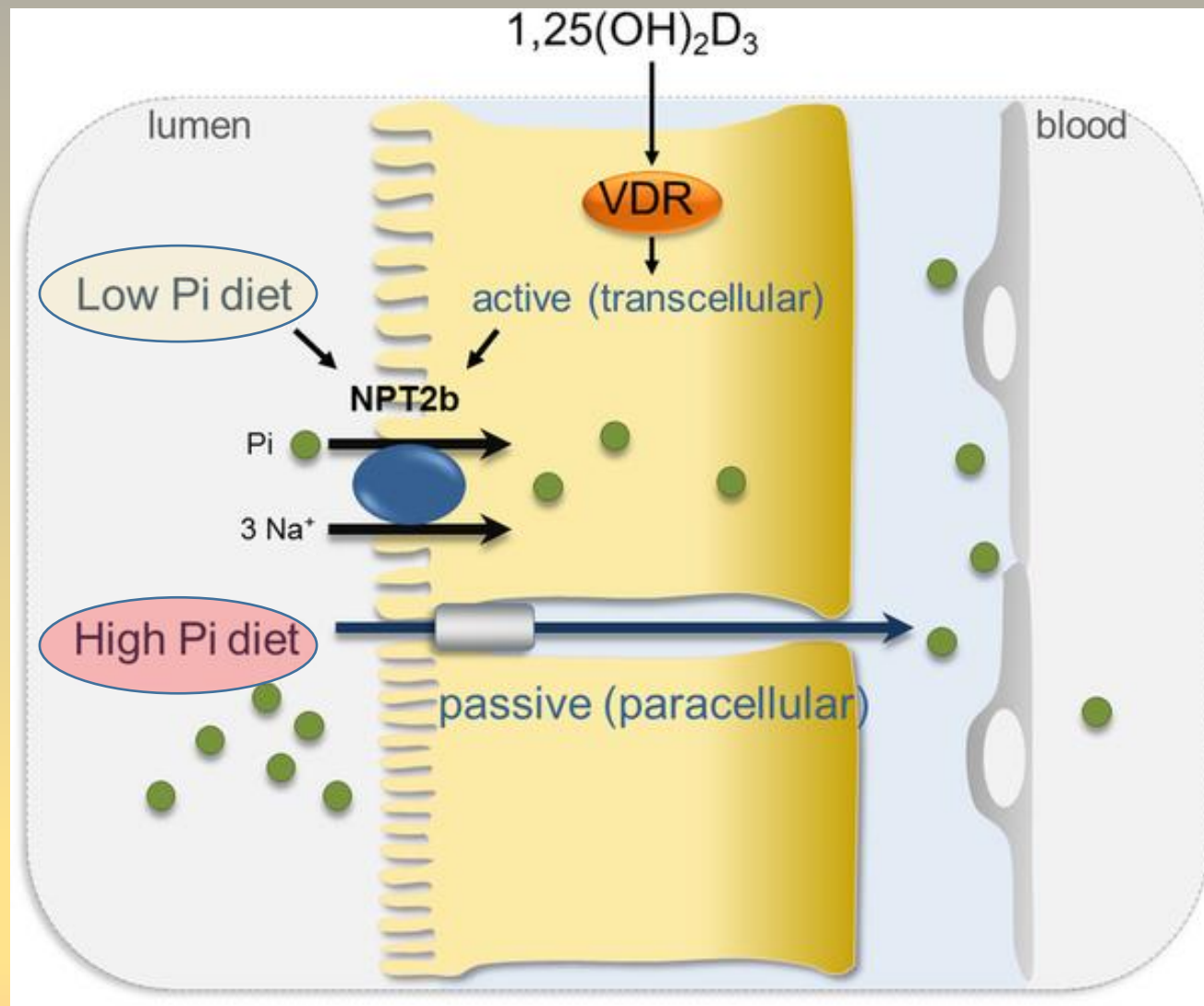
- Concentration 1 mM (serum)
- Ionized form ( $\text{HPO}_4^{2-}$ ,  $\text{H}_2\text{PO}_4^-$ )
- 12 % protein complexes
- Intracellular concentration approximately same as extracellular
- Cotransport with sodium

## 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

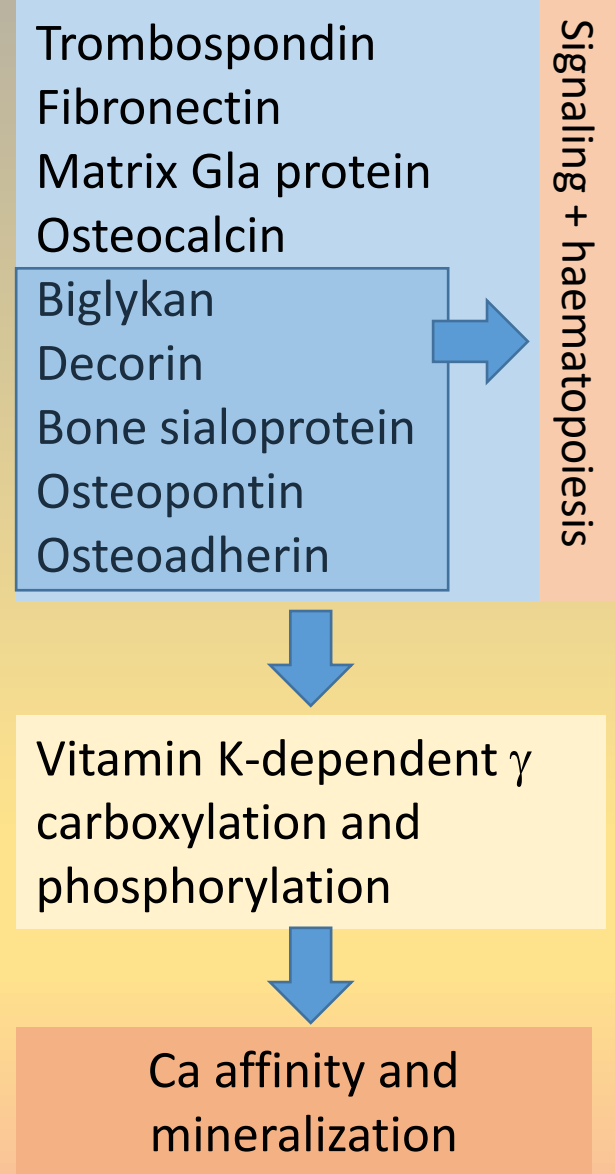
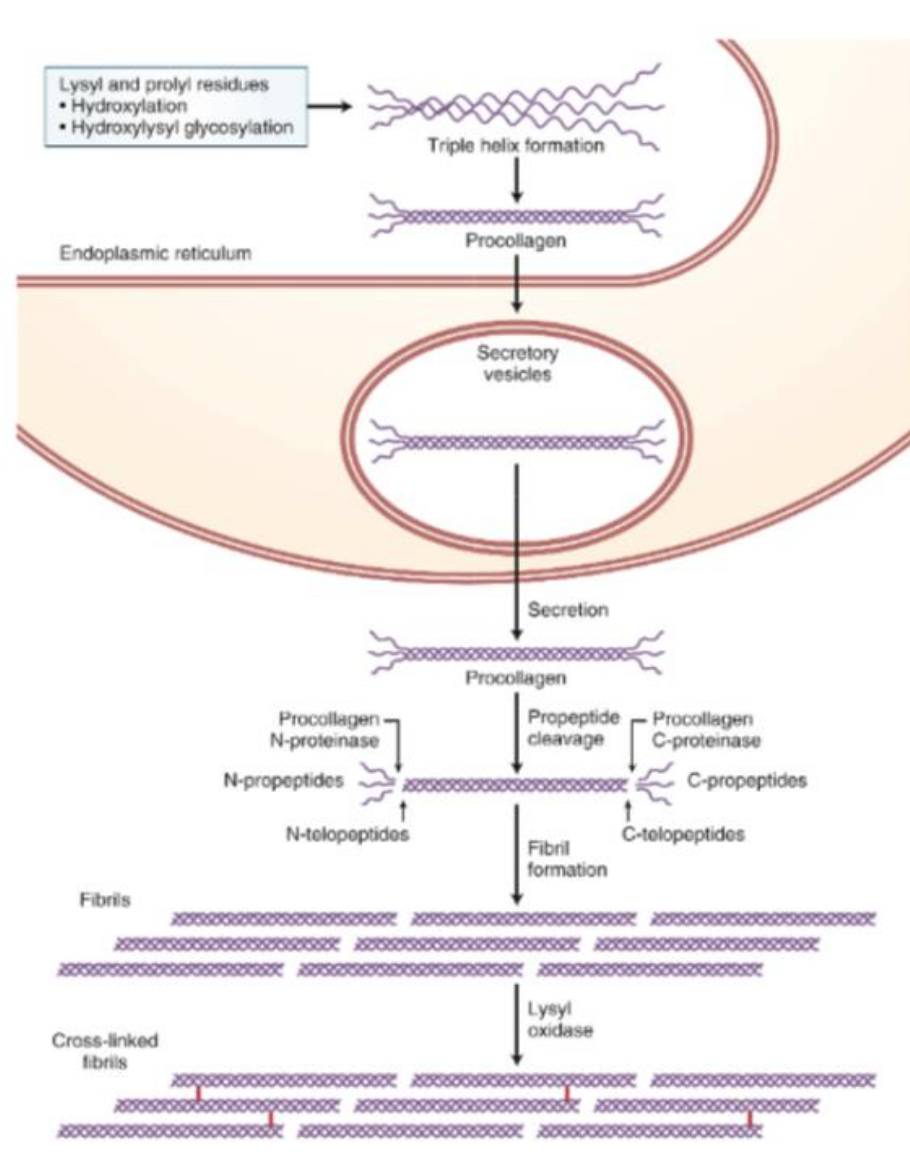
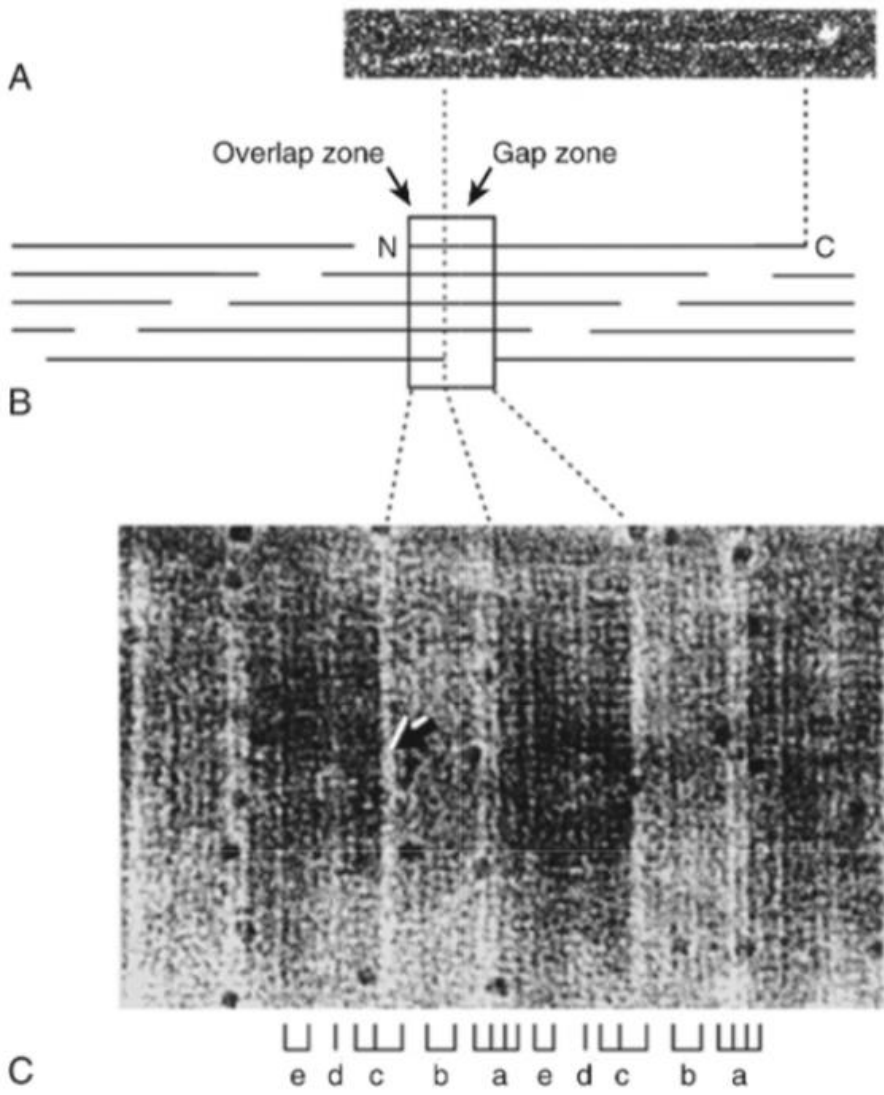
FGF23



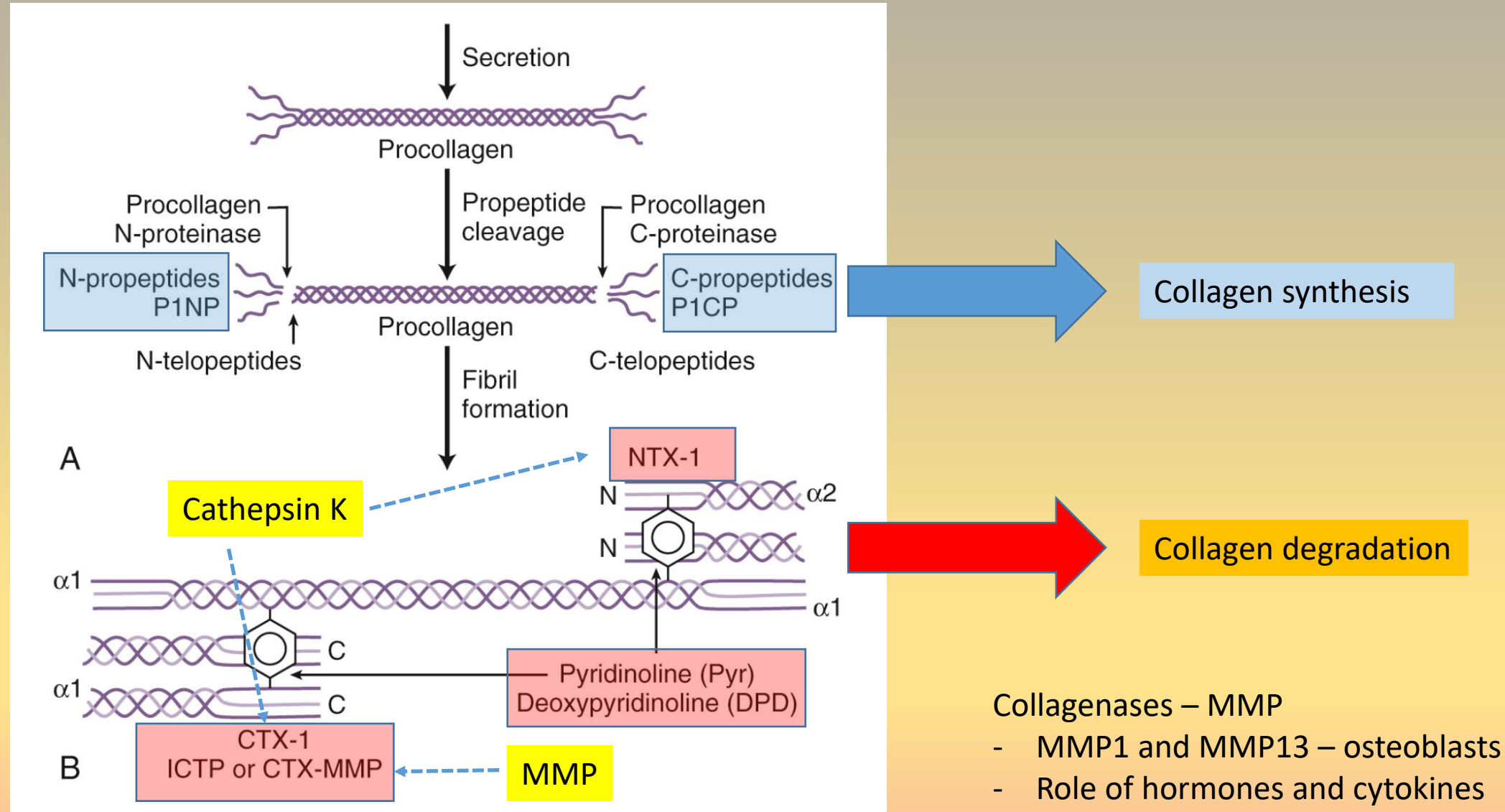
**Collagen type I = most important protein of bone matrix**

# Bone matrix and bone mineral

Type I collagen monomeric and fibrillar structure



# Collagen and its synthesis



# Mineralization

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

External mechanism – alkalic phosphatase

Endopeptidases, PHEX – FGF23

Internal mechanism – phospho1 (Phosphoethanolamine/ phosphocholine phosphatase )

Deposition of calcium

Ca, P, and AF availability

Vesicle formation (matrix)

Collagen and its arrangement

Cleavage of pyrophosphate

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

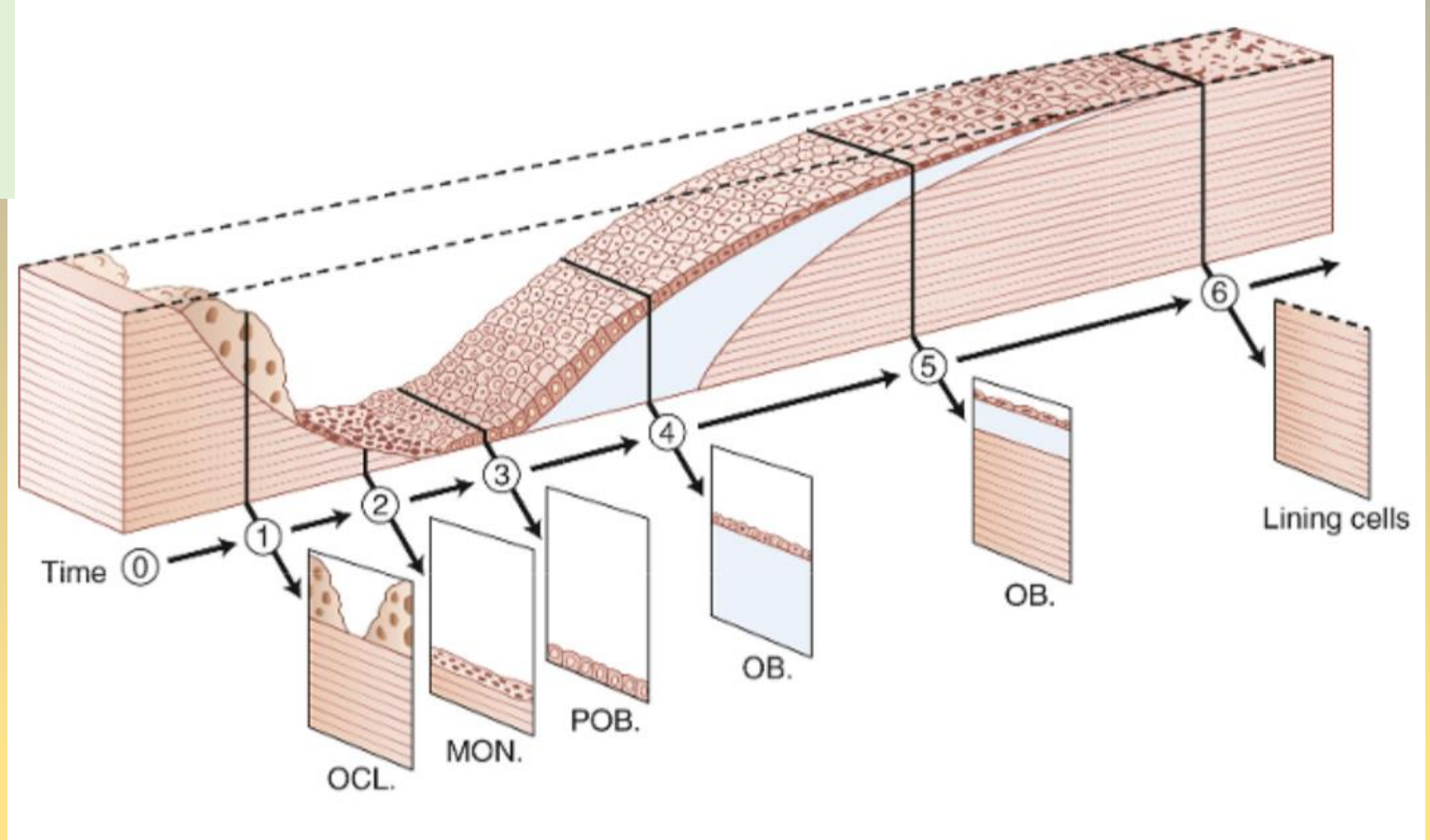
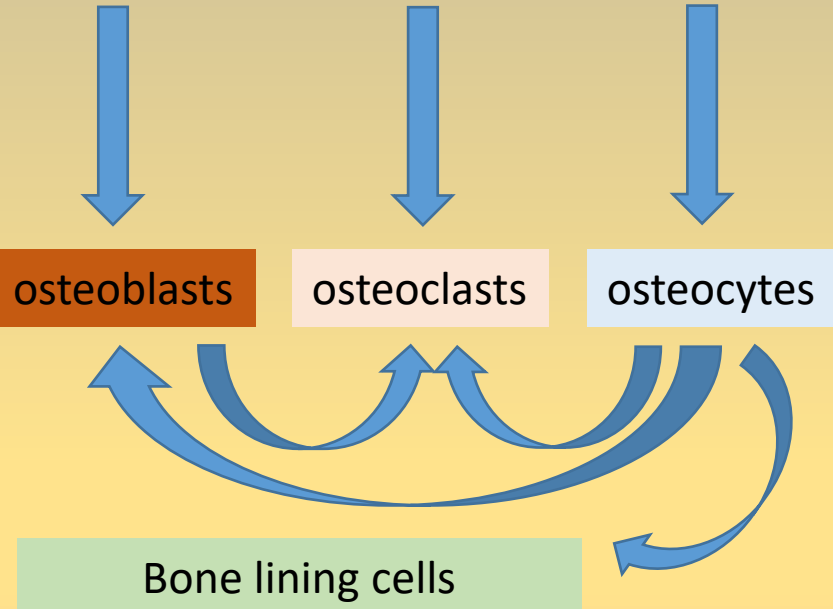
Phosphate availability for mineralization

Diet, calcium in diet, calcium/phosphorus in ECF

# Bone tissue and its remodeling

**Modeling**  
versus remodeling  
of bone tissue

**REMODELING UNIT - BMU**



**Bone reabsorption**

**Building of bone tissue**

osteoclasts

mononuclear cells

preosteoblasts

osteoblasts



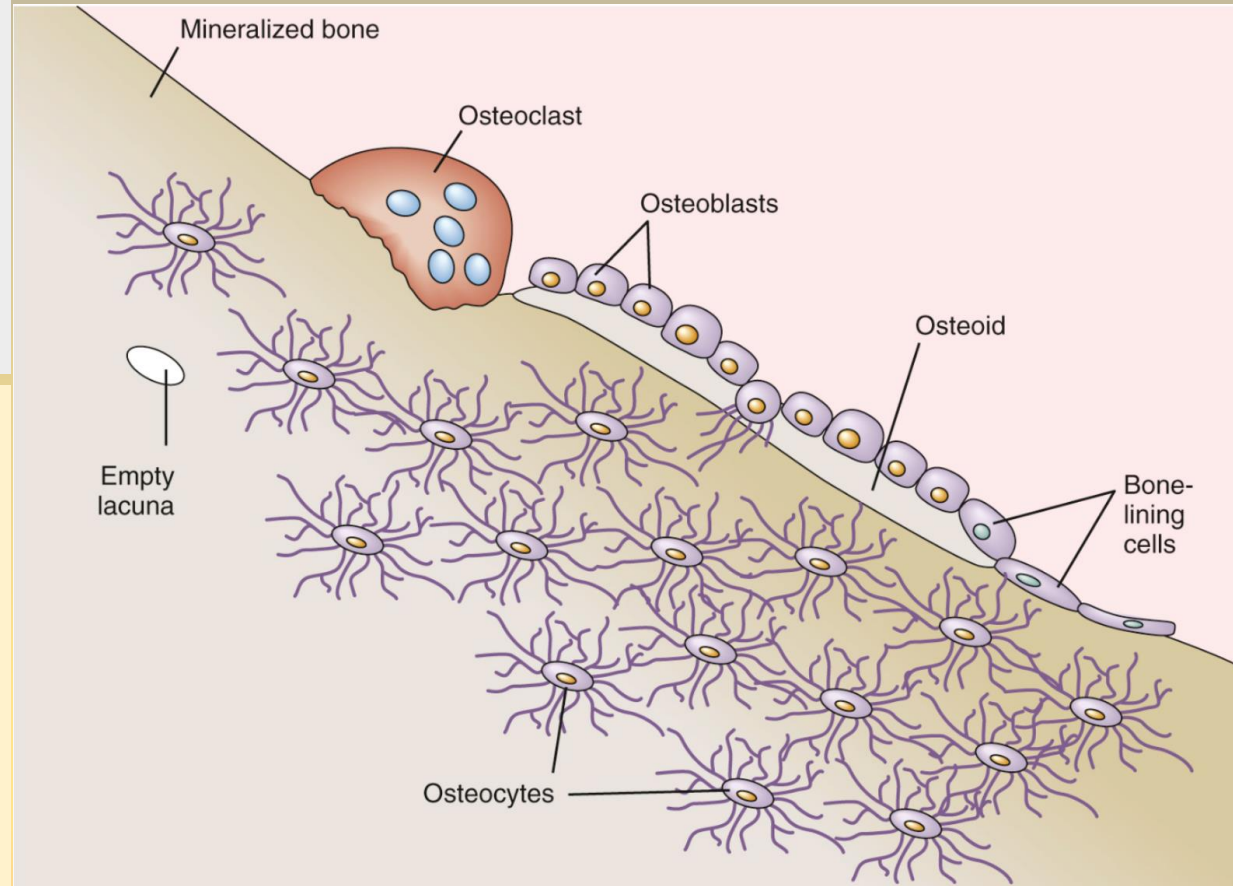
# Bone tissue and its remodeling

## 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 – apoptosis, osteocytes, lining cells
- „recruitment“ of other cells – IGF-1, IGF-2, TGF- $\beta$



## Lining cells

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

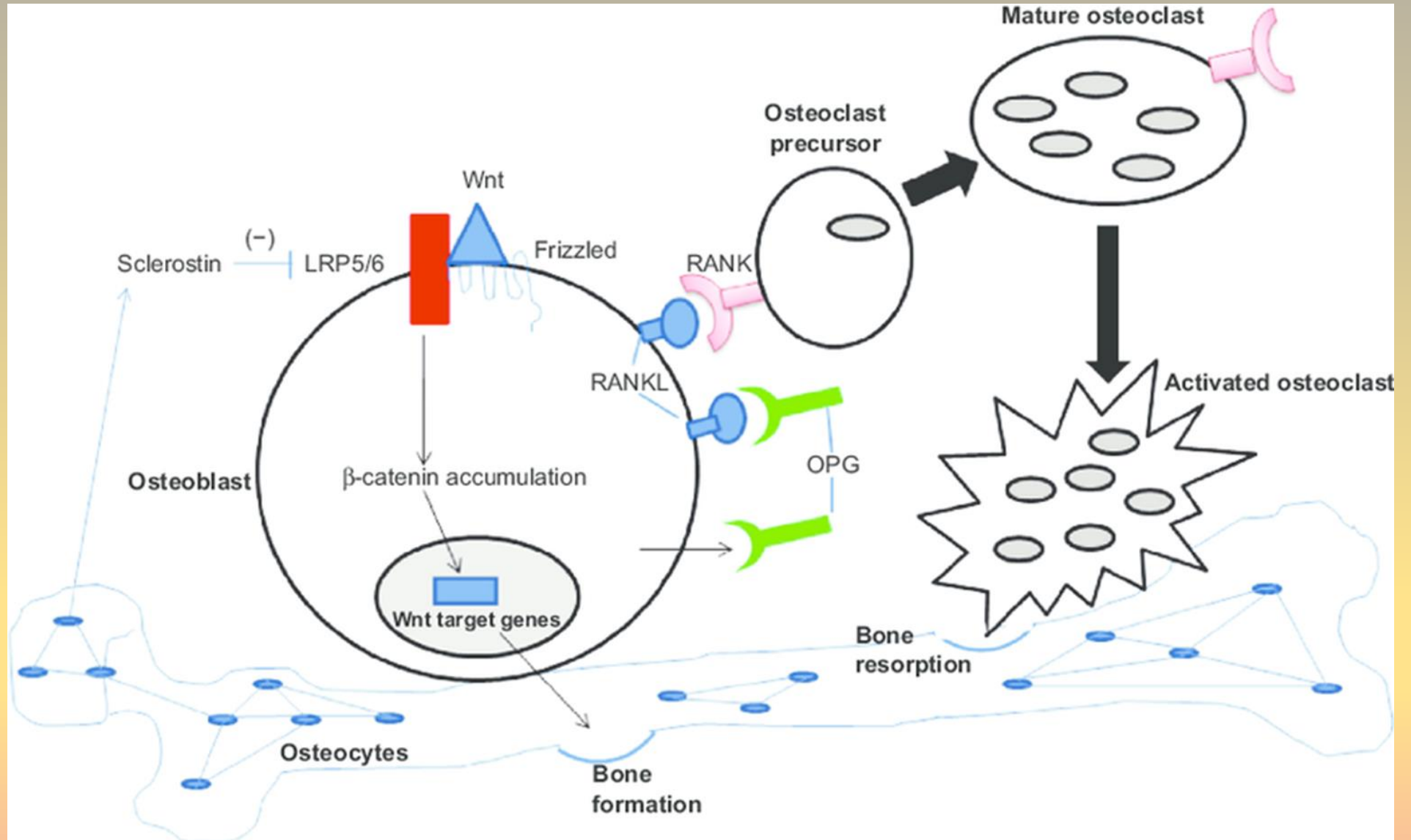
## Osteoclasts (OK)

- Bone tissue reabsorption

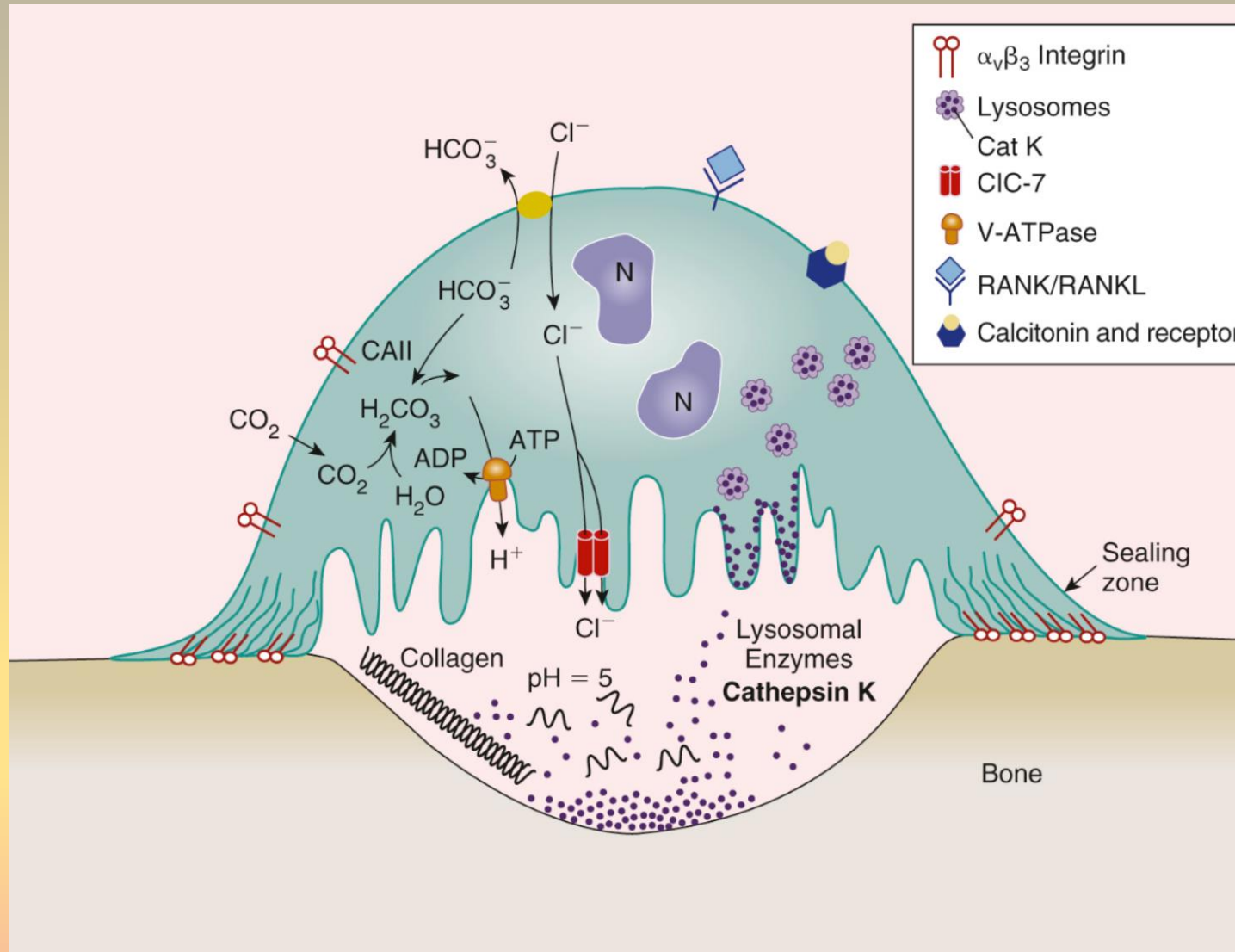
# Osteoclasts

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.**

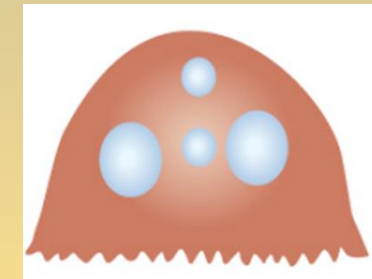
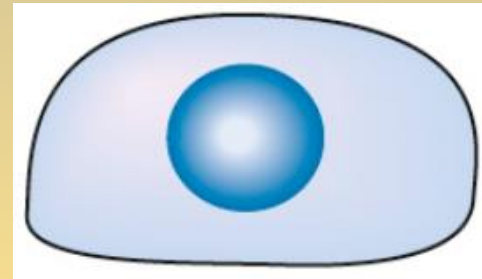
Systemic signals



Local signals



Remodeling of bone tissue



Osteoblasts  
 Lining cells

Osteoclasts

Trabecular bone

Immediate calcium need - homeostasis

Ensuring mechanical requirements

**-**  
 Cytokines - IL-1 $\alpha$ , IL-1 $\beta$ ,  
 TNF- $\alpha$ , TNF- $\beta$ ,  
 proinflammatory IL (7,  
 15, 17)

TGF- $\alpha$  and EGF, FGF21,  
 FGF23

Prostaglandins

PDGF

**+**  
 Cytokines - IL-4, IL-13, IL-  
 10, IL-18

Prostaglandins

VEGFA, HIF-1 $\alpha$  (+/-)

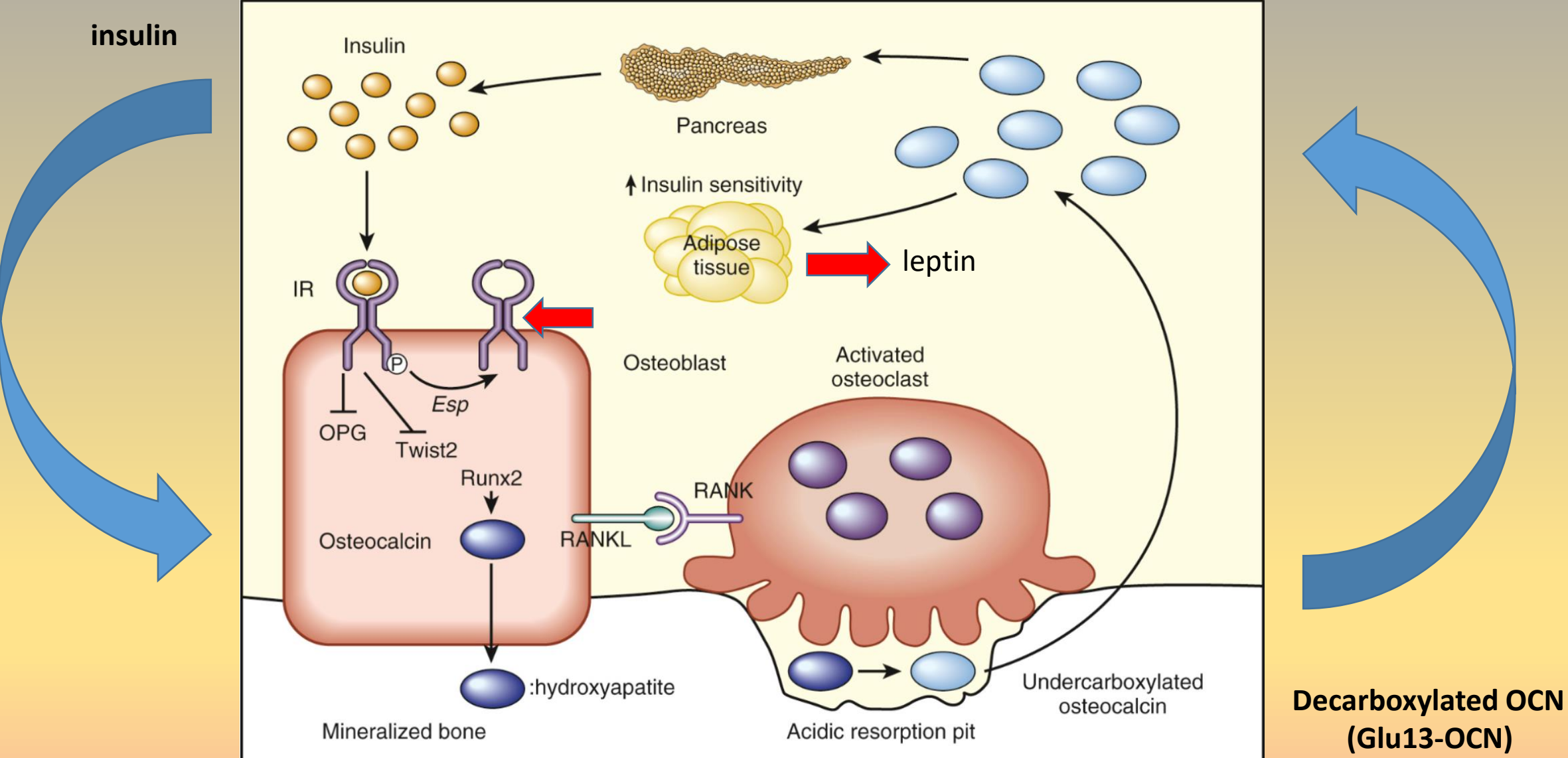
IGF-1 (endo-/paracrine)

BMPs (OB, autocrine)

# Endocrine regulation of bone tissue

Hormone	Effect	Target cells
<b>PTH</b>	<ul style="list-style-type: none"> <li>- Stimulation of resorption (long-term effect)</li> <li>- Stimulation of bone formation (pulsatile effect)</li> <li>- Stimulation of local secretion of IL-1 and IL-6</li> </ul>	Osteoblasts, lining cells, osteocytes
<b>Vitamin D</b>	<ul style="list-style-type: none"> <li>- Stimulation of resorption (higher concentration)</li> <li>- Inhibition of mineralization (higher concentration)</li> <li>- Stimulation of bone formation (low concentrations, with PTH)</li> </ul>	Osteoblasts (primarily)
<b>Calcitonin</b>	<ul style="list-style-type: none"> <li>- Inhibition of resorption</li> <li>- Regulation of bone tissue remodeling</li> </ul>	Osteoclasts
<b>Growth hormone IGF-1</b>	<ul style="list-style-type: none"> <li>- Stimulation of bone turnover</li> <li>- Stimulation of osteoblast proliferation and differentiation</li> <li>- Increased synthesis of collagen and other proteins</li> </ul>	Osteoblasts – primarily GH Osteoblasts and osteoclasts – IGF-1
<b>Glucocorticoids</b>	<ul style="list-style-type: none"> <li>- Decreased absorption of Ca in GIT</li> <li>- Induction of osteoclastogenesis</li> <li>- Increased bone resorption (+ RANKL)</li> <li>- Suppressed remodeling of bone tissue</li> <li>- Induction of apoptosis in osteoblasts and osteocytes</li> <li>- Inhibition of IGF-1 synthesis</li> </ul>	Osteoblasts, osteocytes, osteoclasts
<b>Thyroid hormones</b>	<ul style="list-style-type: none"> <li>- Children – Stimulation of mineralization and epiphyseal maturation</li> <li>- Adults – increased resorption</li> <li>- Chondrocyte growth and proliferation (permissive effect on growth hormone)</li> <li>- Increased transcription of collagenase and gelatinase</li> </ul>	Osteoblasts, osteoclasts (also indirect through TSH)
<b>Insulin</b>	<ul style="list-style-type: none"> <li>- Stimulation of bone tissue formation and mineralization</li> <li>- Increased collagen synthesis</li> <li>- Stimulation of IGF-1 secretion</li> </ul>	Primarily osteoblasts
<b>Sex hormones</b>	<ul style="list-style-type: none"> <li>- Epiphyseal closure (E)</li> <li>- Inhibition of RANKL secretion</li> <li>- Changes in speed of bone resorption and formation (stimulation of formation and mineralization)</li> </ul>	Primarily osteoblasts, also other bone cells
<b>Prolactin</b>	<ul style="list-style-type: none"> <li>- Indirect effect</li> </ul>	

# Insulin – osteocalcin axis



# Clinical relevance

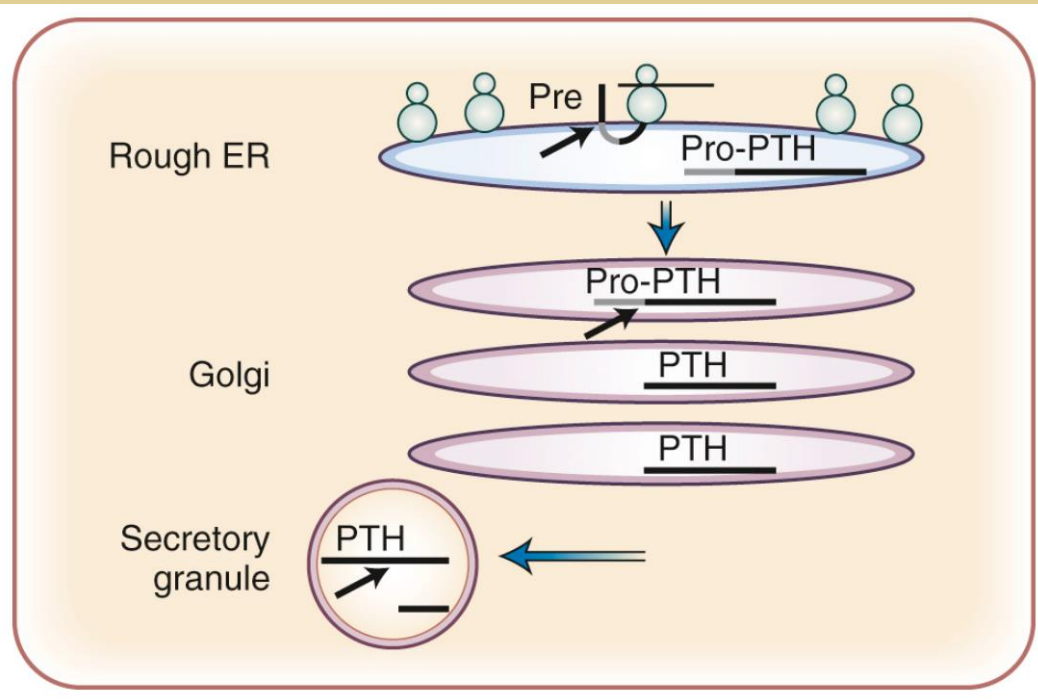
- Osteogenesis imperfecta
- Osteopetrosis
- Osteomalacia
- Rachitis
- Osteopenia – T score -1 – -2.5
- Osteoporosis – T score under -2.5



# Parathormone

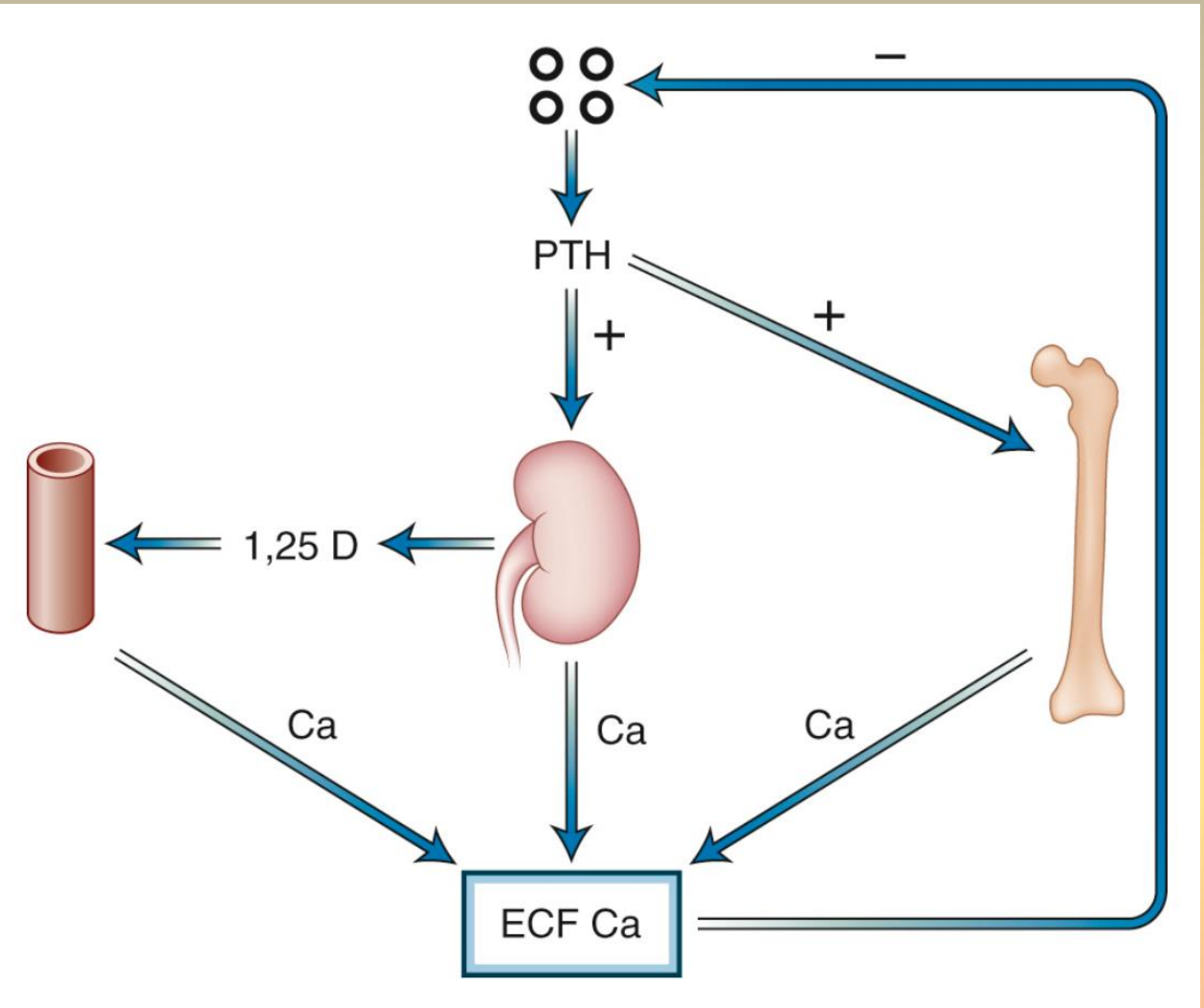
## Characteristics

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



## PTH

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





# PTH secretion

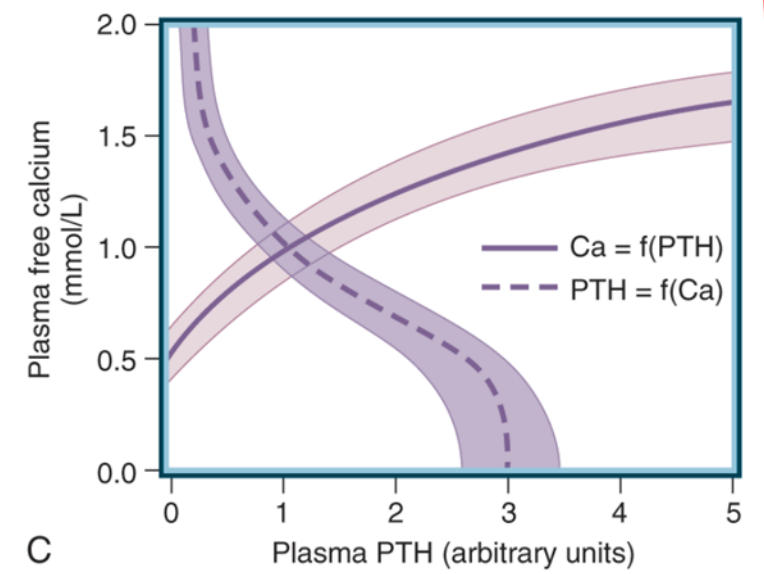
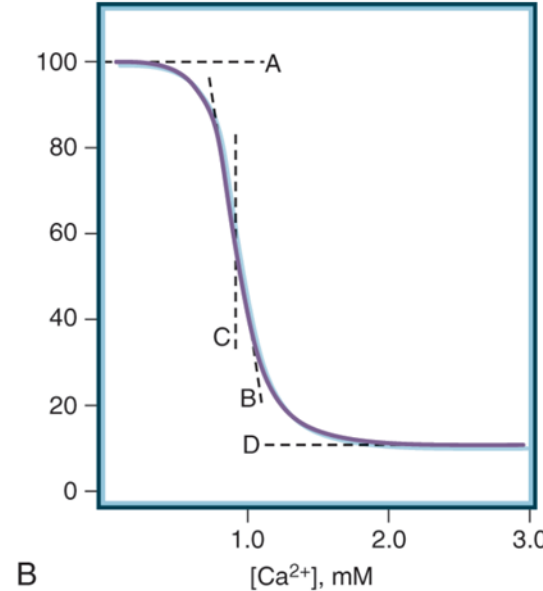
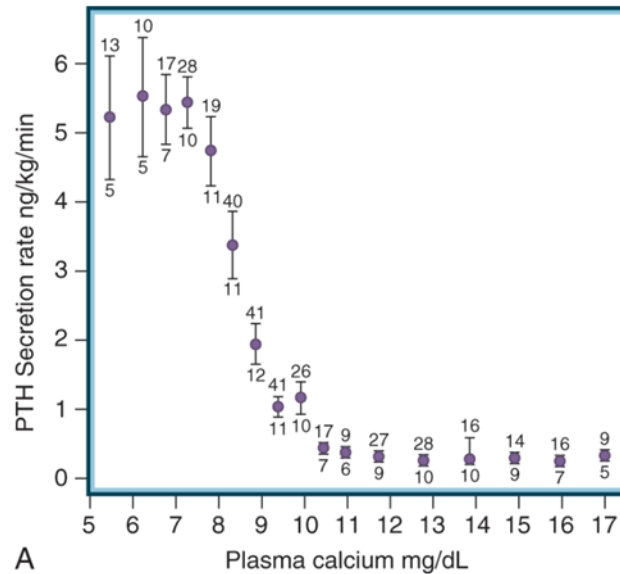
Cell proliferation of chief cells is an important adaptive mechanism for:

- Hypocalcemia
- Low levels of vitamin D( $1,25(\text{OH})_2\text{D}_3$ )
- Hyperphosphatemia (uremia)
- Neoplastic growth

Maximal secretion (reserve capacity)



Minimal secretion



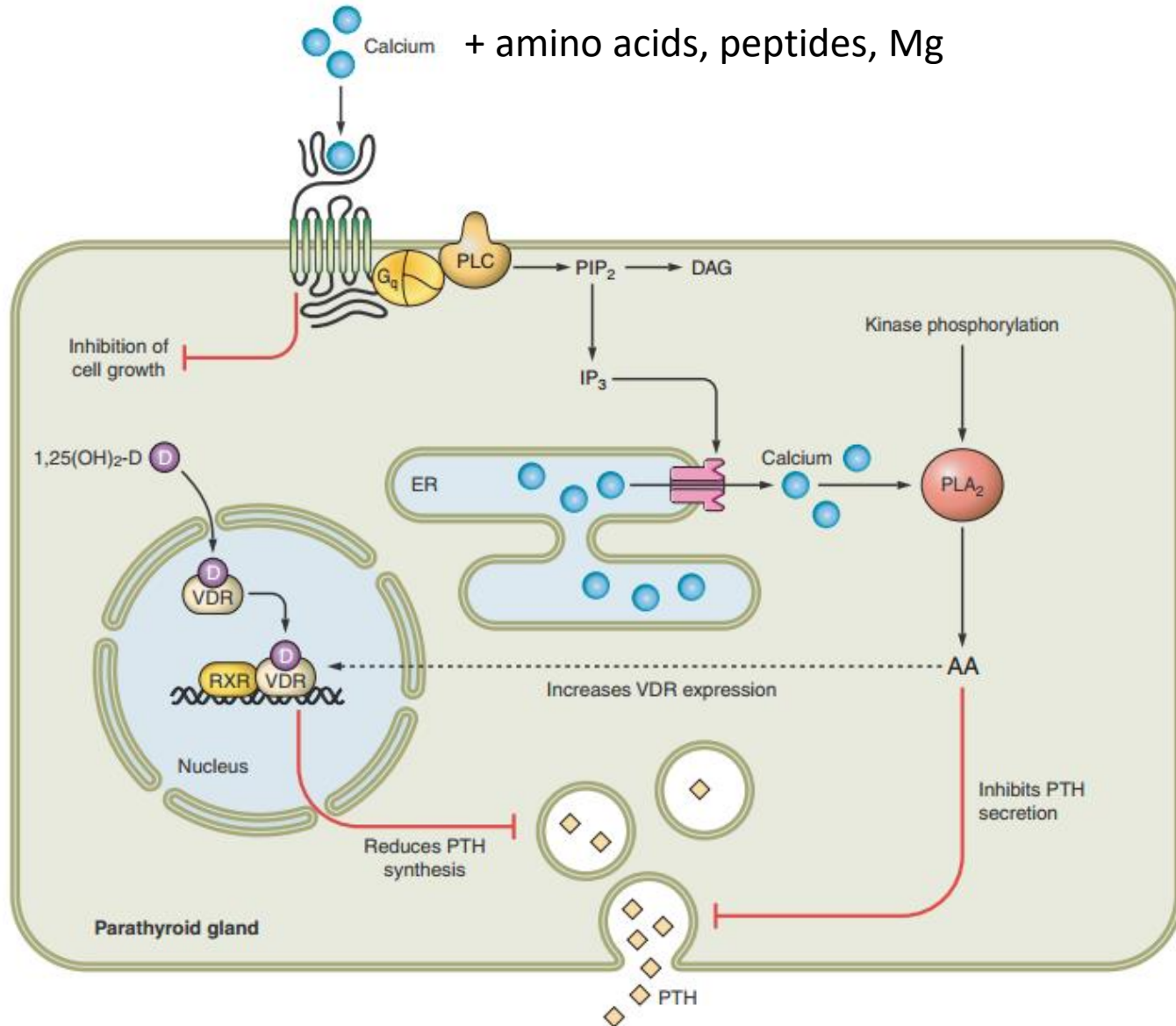
**Level of ionized calcium in blood is a key parameter for PTH secretion.**

**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 –  $\text{Ca}^{2+}$  inhibition
- Calbindin-D28K
- NCX1 and PMCA

(+) phosphate excretion

- PT and DT
- Inhibition of resorption
- NaPi cotransporters – internalization, degradation

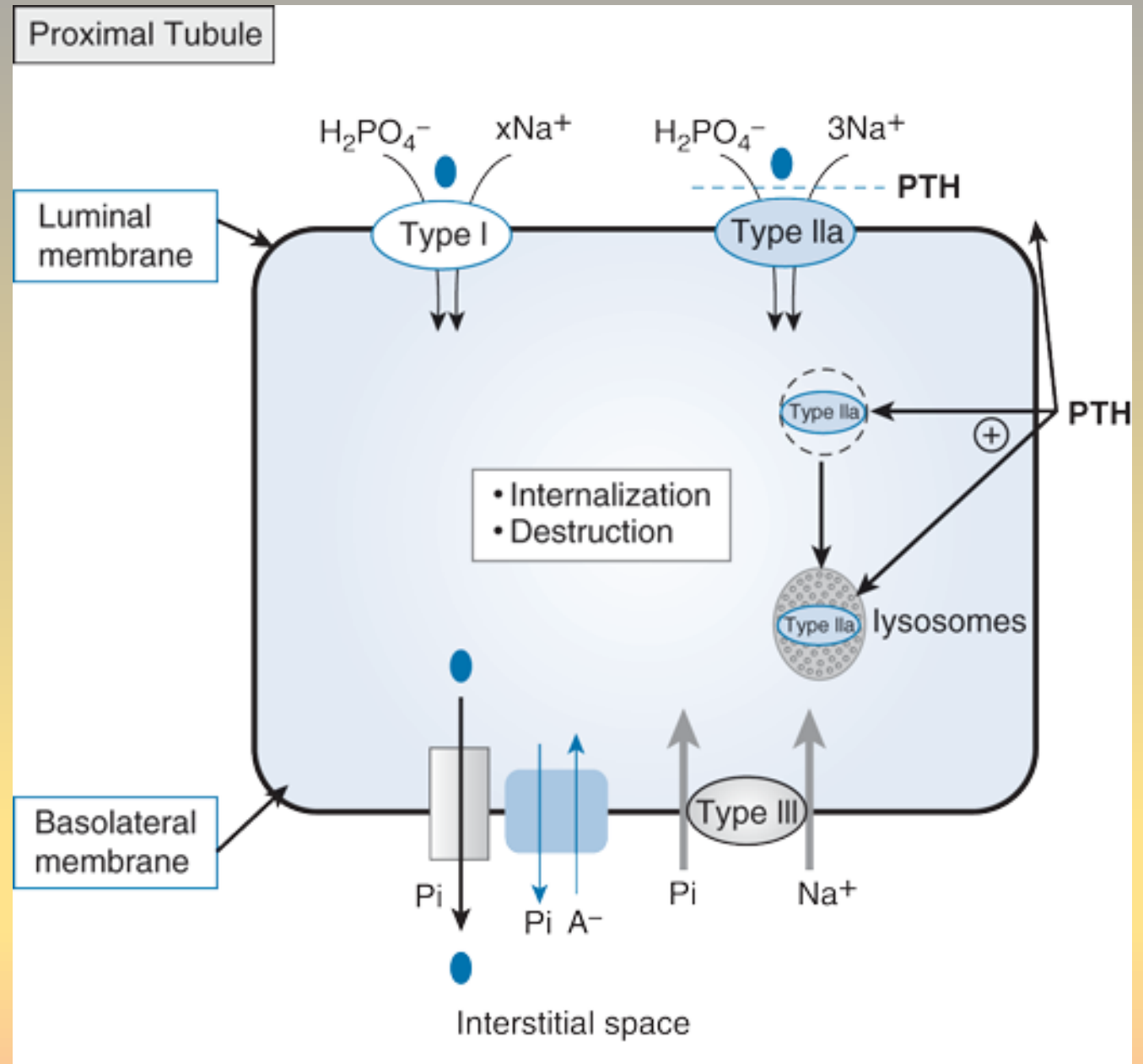
(+) activity of  $1\alpha$ -hydroxylase - PT

(-) resorption of Na, water and bicarbonate – PT

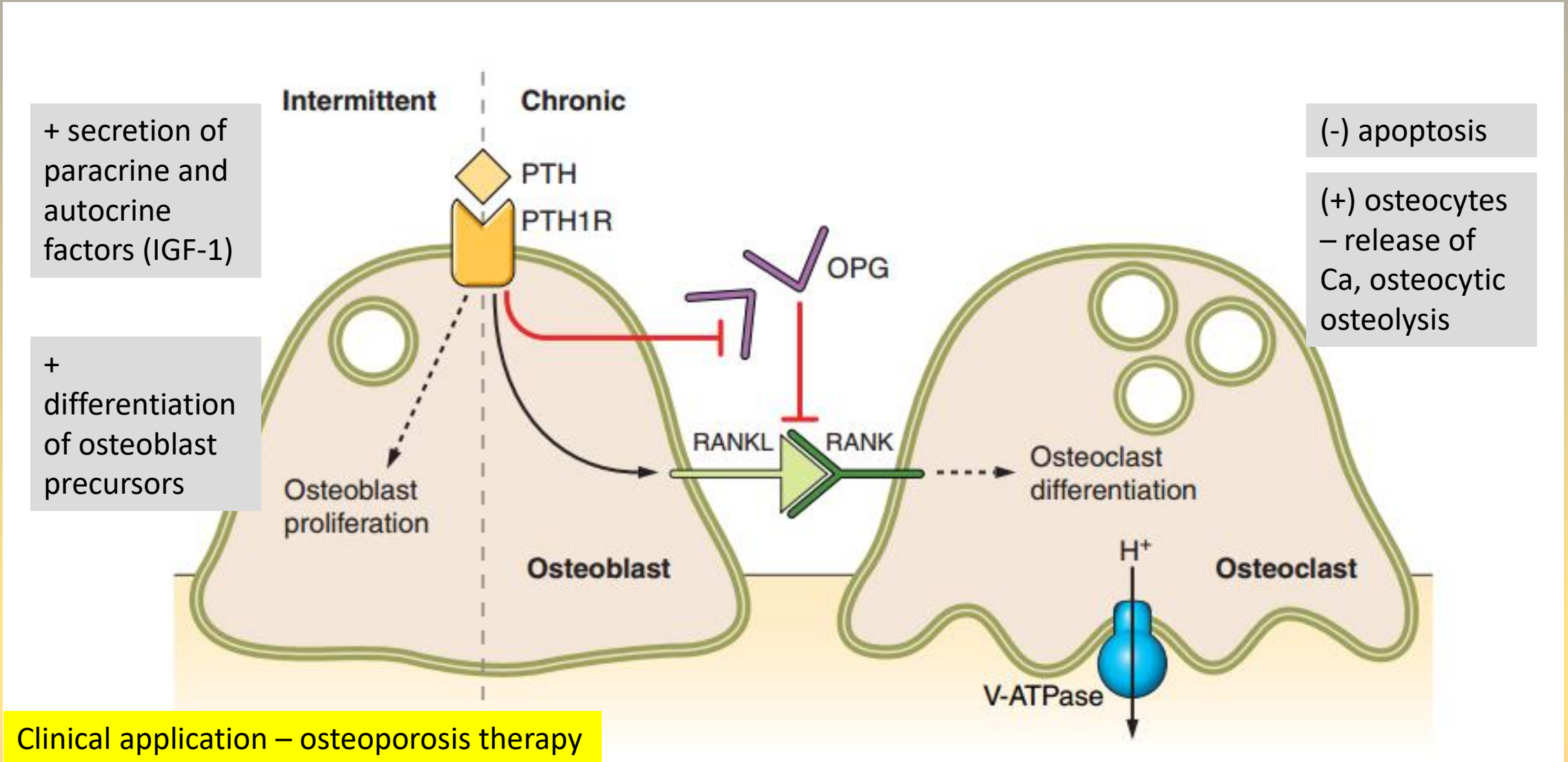
(-)  $\text{Na}^+/\text{K}^+$ -ATPase (basolateral membrane)

(+) gluconeogenesis – PT

(-) GFR - podocytes



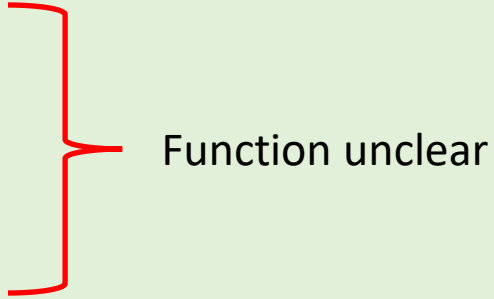
# PTH and bone tissue physiology



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
    - $\beta$ -sympatomimetics
  - Inhibition of secretion - somatostatin
- 
- Function unclear

## Functions

- Bone tissue
  - Inhibition of osteoclast motility and differentiation
  - Inhibition of osteoclast secretion
  - ATPase inhibition
- Kidneys
  - Increased excretion of Ca – inhibition of resorption (Ca<sup>2+</sup> ion channels – LS, Na<sup>+</sup>/Ca<sup>2+</sup> - 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  $1,25(\text{OH})_2\text{D}$  – cca 0,4 %

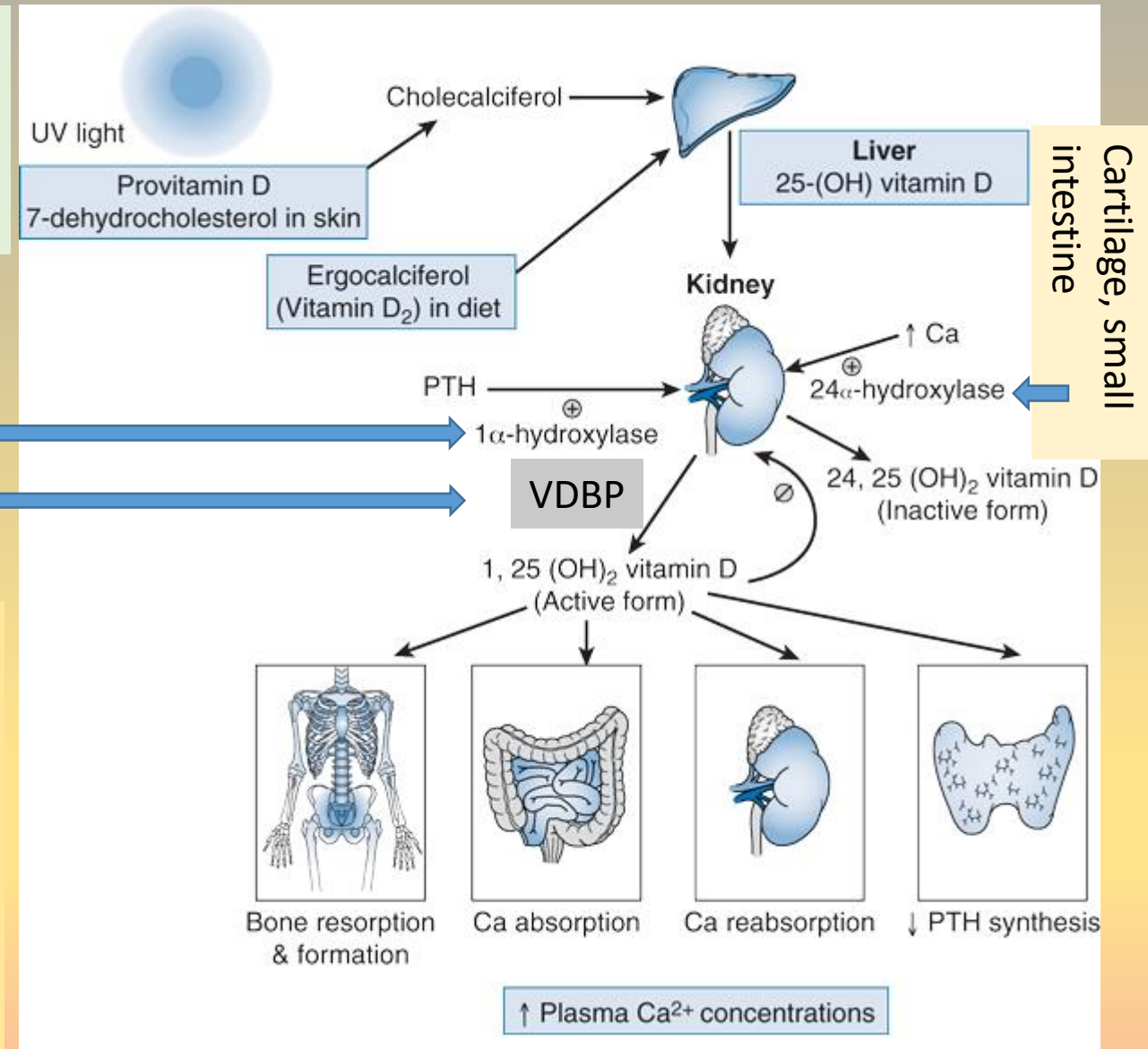
PTH, prolactin, *calcitonin*, *GH* (+)  
 T3/T4, metabolic acidosis (-)  
 Ca, phosphates,  $1,25(\text{OH})_2\text{D}$ ,  
 FGF23 (-)  
 Ketoconazole  
 Estrogens (+)

## $1\alpha$ -hydroxylase

- Expression in various tissues
- Keratinocytes
- Placenta
- Macrophages

Different rate of  
feedback control

Different  $1\alpha$ -hydroxylase expression = local tissue homeostasis



# Physiological effects of vitamin D

## VDR

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

## Non-genomic effects

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

## Vitamin D and Ca absorption/reabsorption

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

## Parathyroid glands

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

## 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

## Muscle tissue

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

# FGF23 – fibroblast growth factor 23

## Characteristics

- New hormone?
- Overexpression = hypophosphatemia and decrease of  $1\alpha$  25(OH)D hydroxylation

## Functions

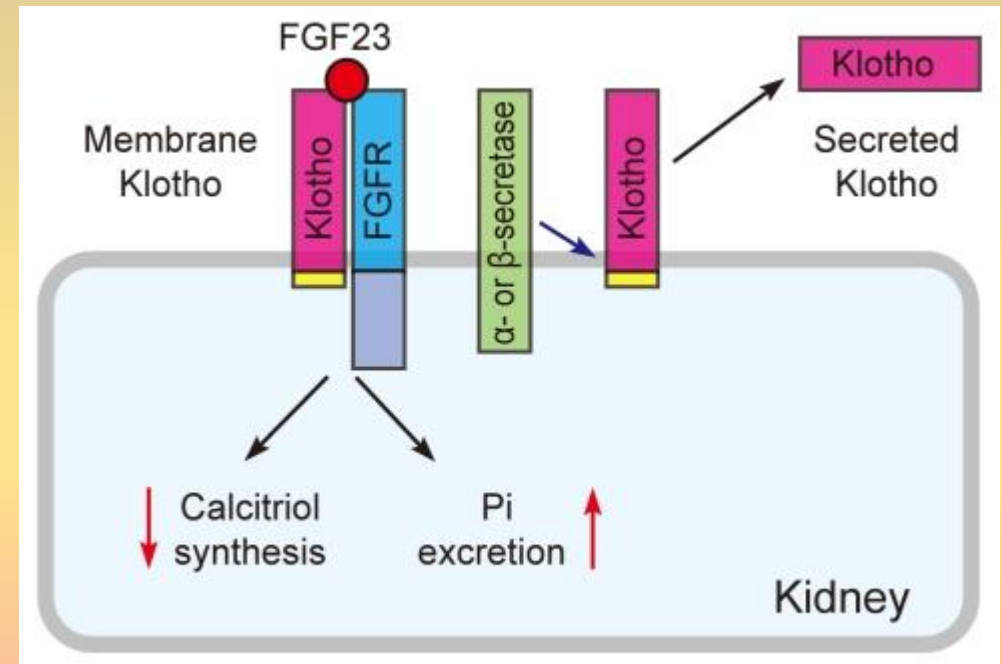
- maintaining normophosphatemia and regulation of vitamin D metabolism
- Increased expression of IIa, IIb, and IIc (NPT) – **phosphate transport**
- decreased expression of 24-hydroxylase – **inactive form**
- Klotho = co-receptor

## Regulation

- Phosphorus availability in diet (-)
- Serum phosphorus
- $1,25(\text{OH})_2\text{D}$
- 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

