# Mineral Homeostasis

*Duncan Bassett*
*Molecular Endocrinology Group*

**Bone structure and formation**

**Maintenance of adult bone**

**Parathyroid hormone (PTH, PTHrP and PTHR1)**

**Vitamin D (1,25$(OH)_2D_3$, VDR)**

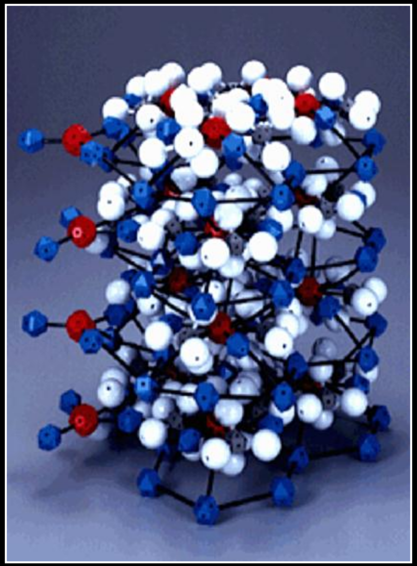
**Fibroblast growth factor 23 (FGF23, Klotho, FGFR1c)**

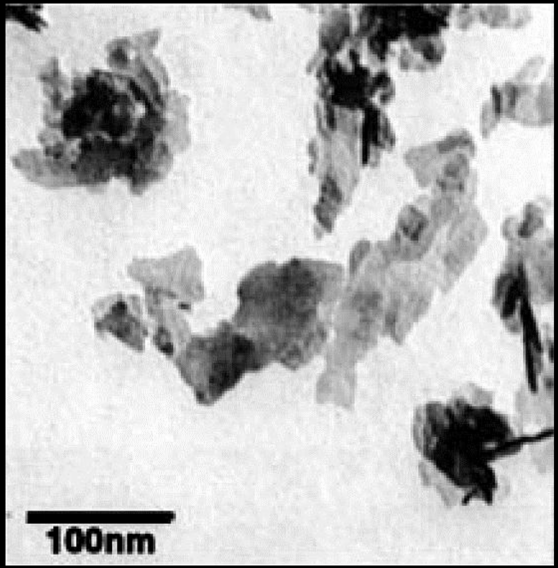
**Calcitonin**

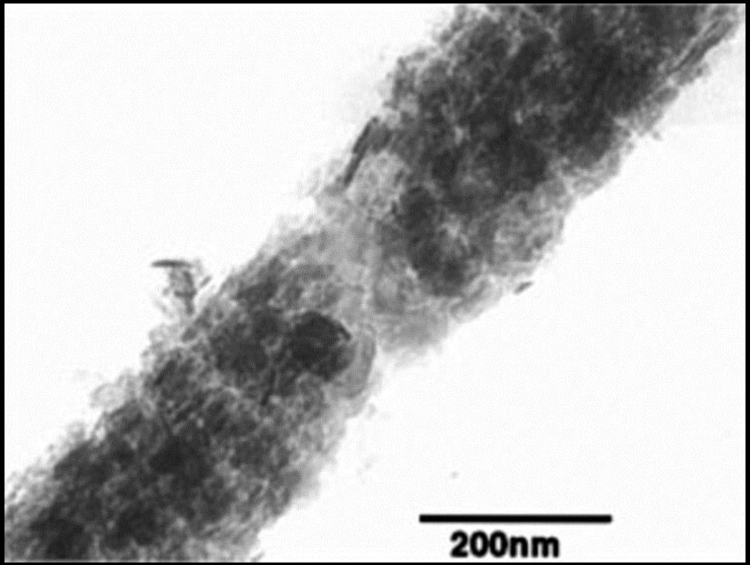
# Bone Structure

**Bone must be stiff yet flexible and light yet strong**

# Bone mineral





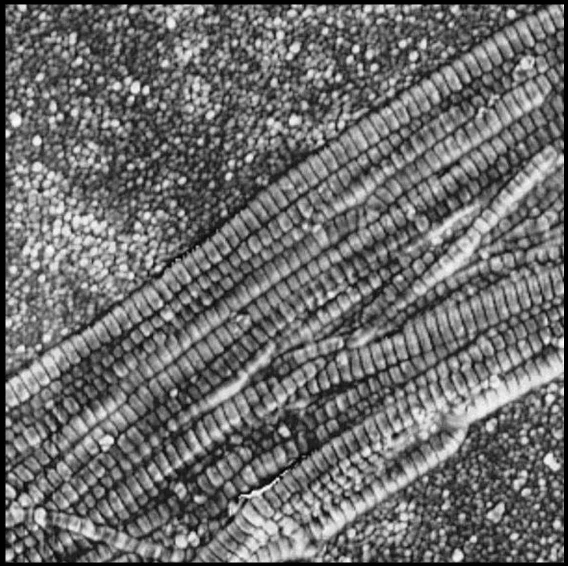

**Mineral component**

- Roof tile shaped crystals 4 x 50 x 25nm
- Hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$

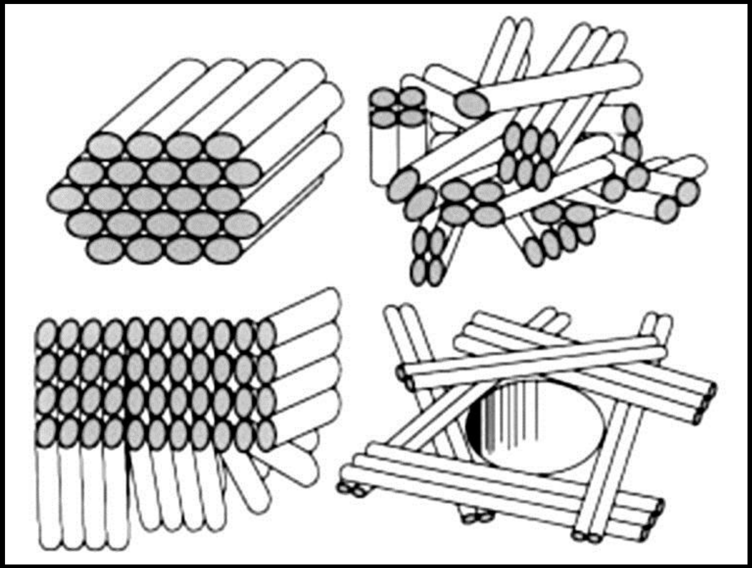
**Bone contains**

- 99% of the body's calcium
- 85% of the phosphate
- 50% of the magnesium

# Bone matrix

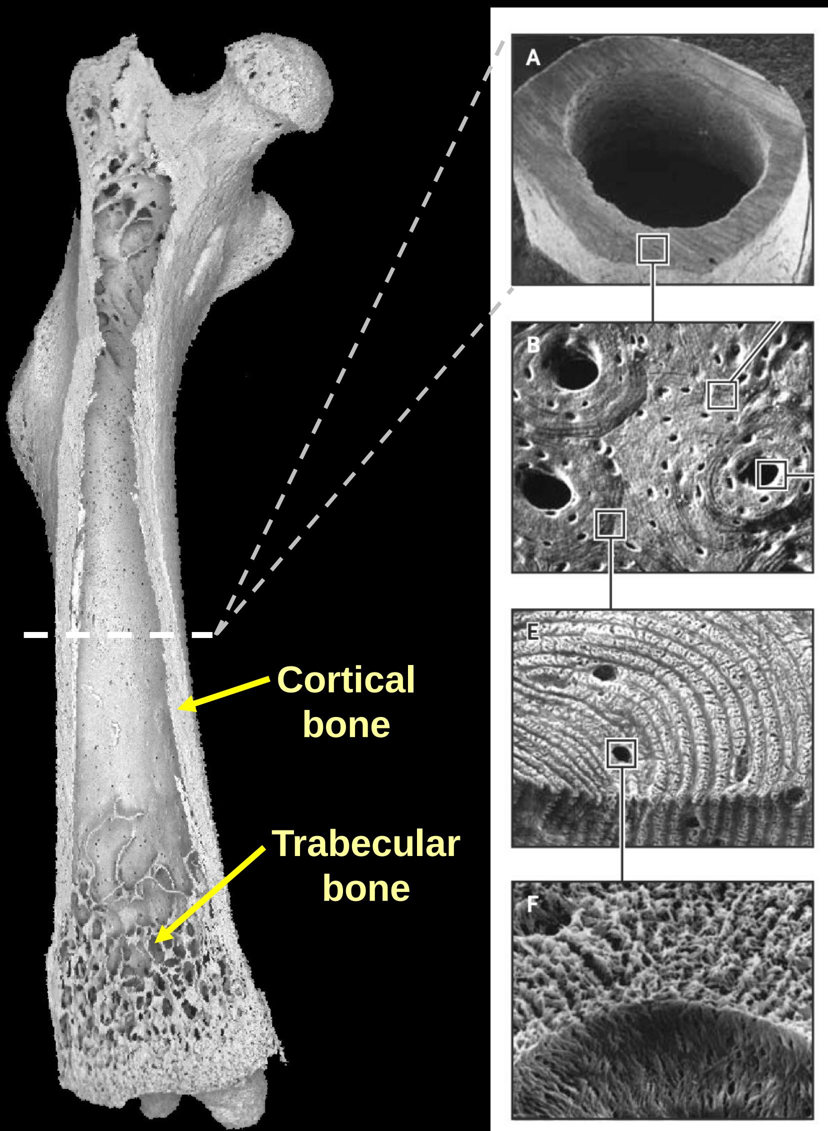


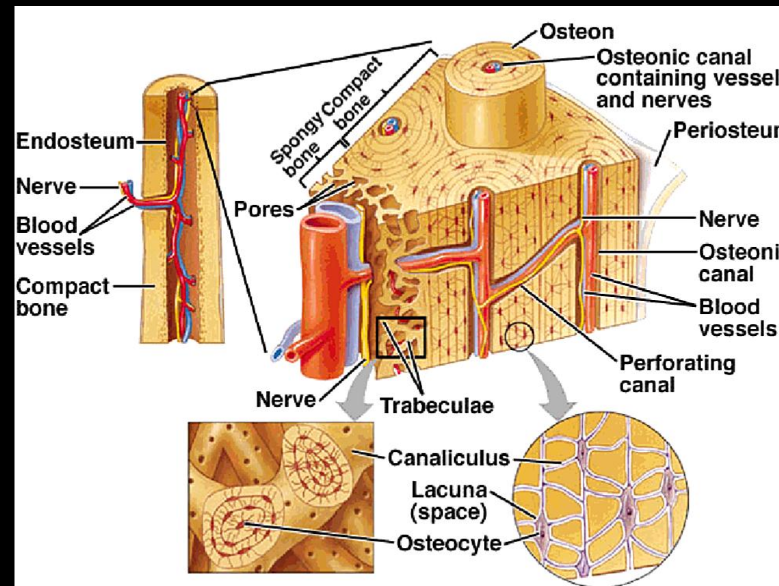
Collagen fibrils
Matrix component



Orientation of collagen fibrils
- Parallel (tendons)
- Woven bone
- Lamella bone
- Radial array (dentine)

# Macro and microstructure of cortical bone




Ovelapping parallel osteon structure
- Result of completed remodelling cycles

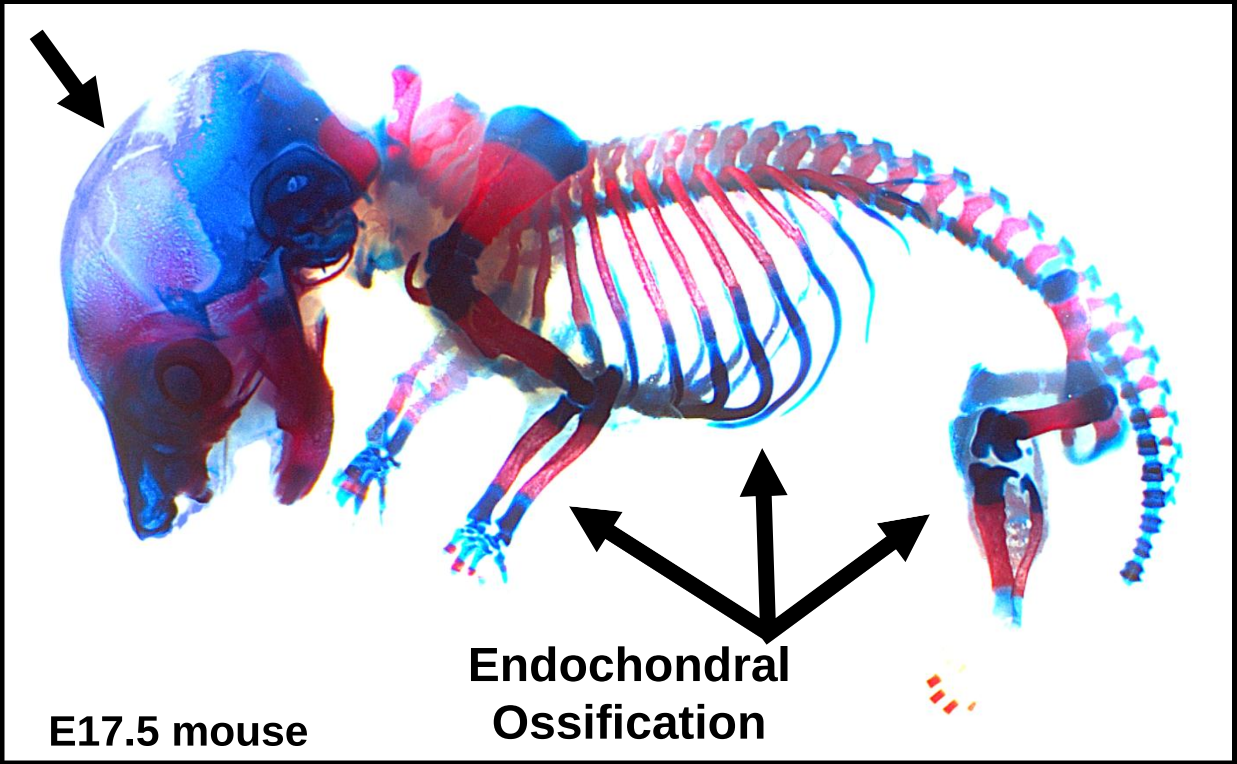
Osteon structure limits fracture propagation
- Concentric lamellae
- Alternately loose and dense packing
- Collagen orientated in various directions

*(Seeman E et al 2008 NEJM 354:2250–2261)*

# Bone development

# Skeletal development

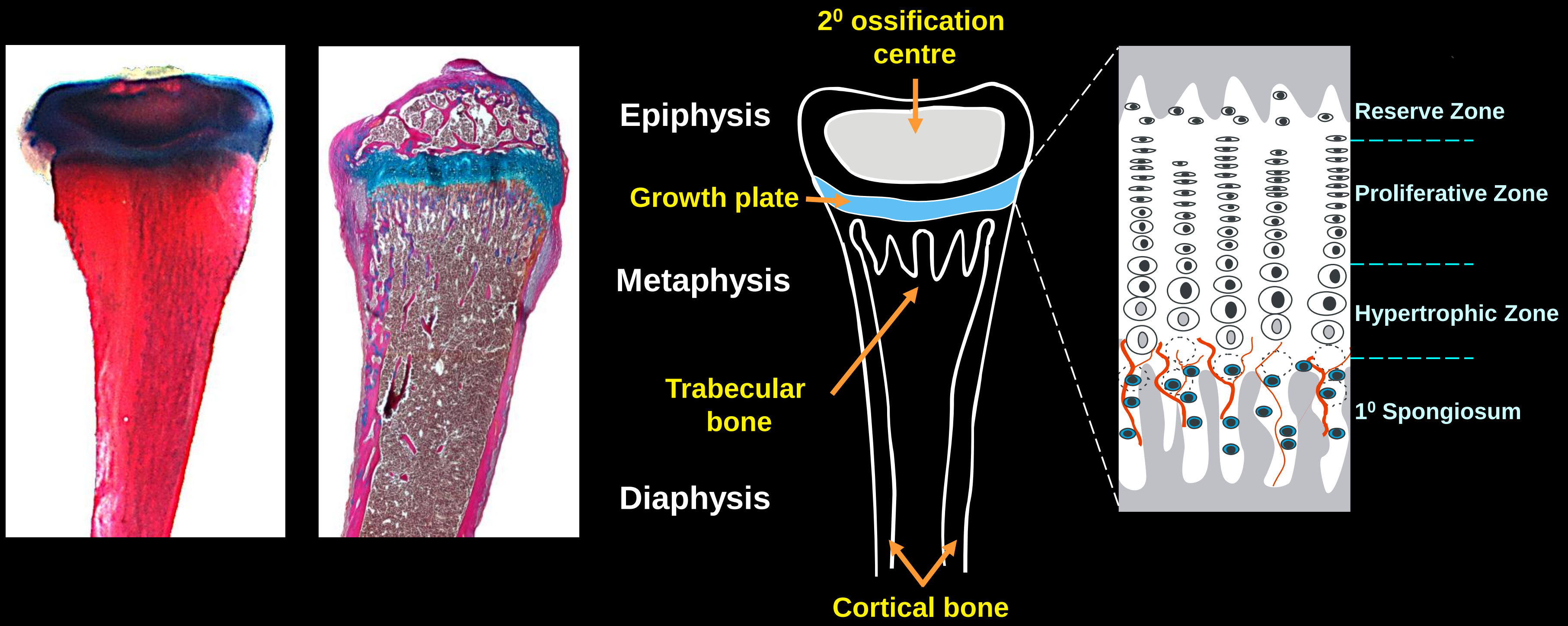
Intramembranous
Ossification



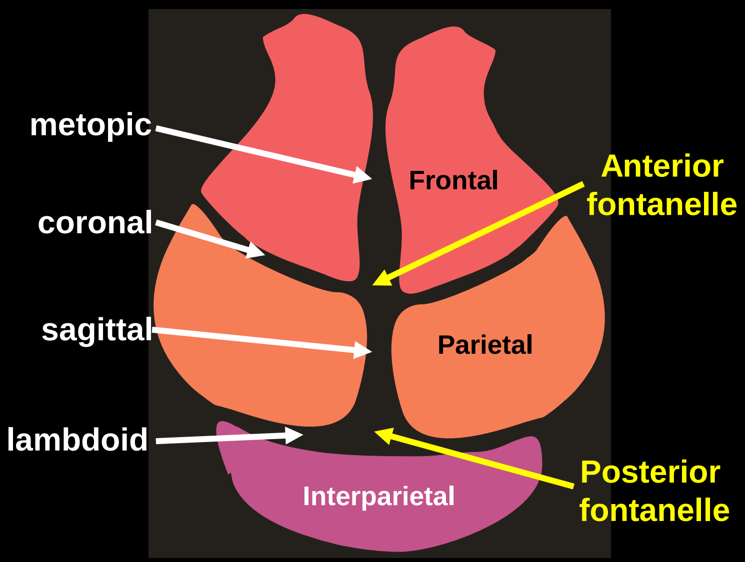

**Long bone form by endochondral ossification**

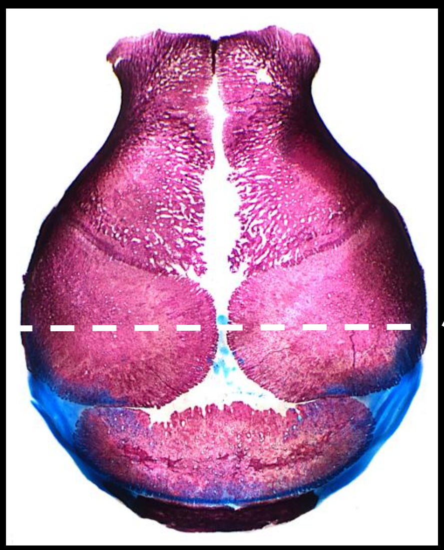
**Craniofacial bones by intramembranous ossification**

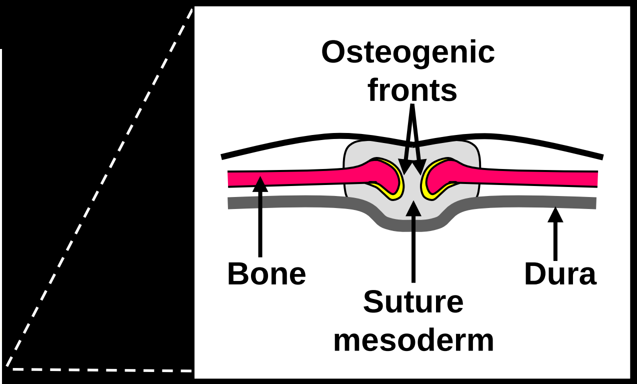
# Endochondral ossification

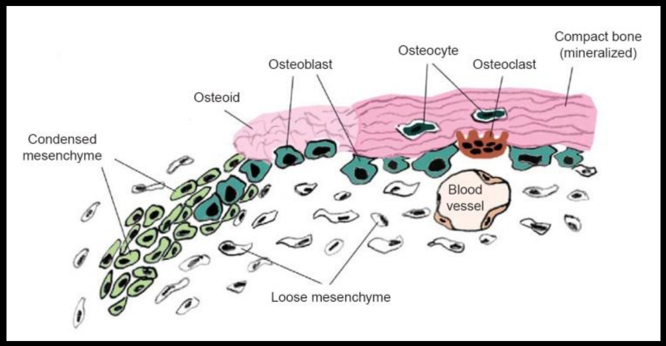


# Intramembranous ossification





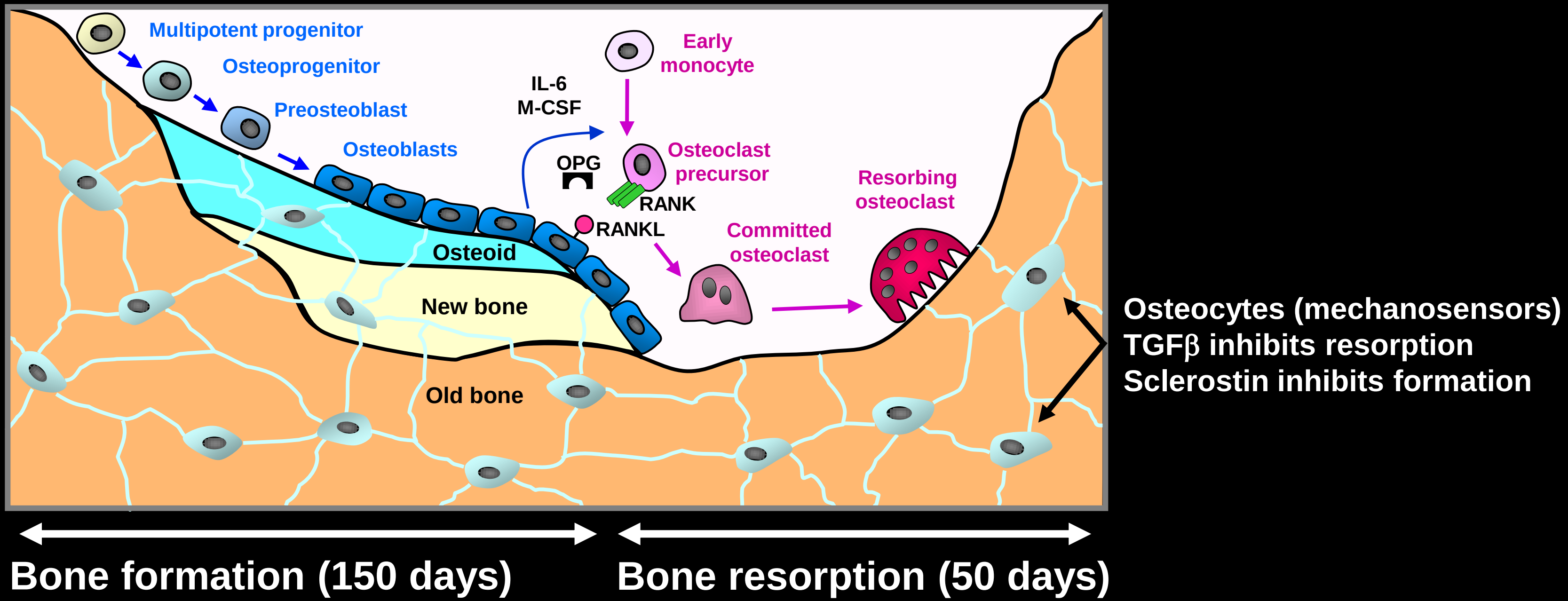




**Craniofacial skeleton forms by intramembranous ossification**
**Mesenchymal cells differentiate into osteoblasts**
**Bone is formed directly without a cartilage scaffold**

*(Hartmann C (2006) Trends in Cell Biol 16:151-8)*

# Maintenance of adult bone

# The bone remodelling cycle




Ostoclastic bone resorption then ostoblastic bone formation

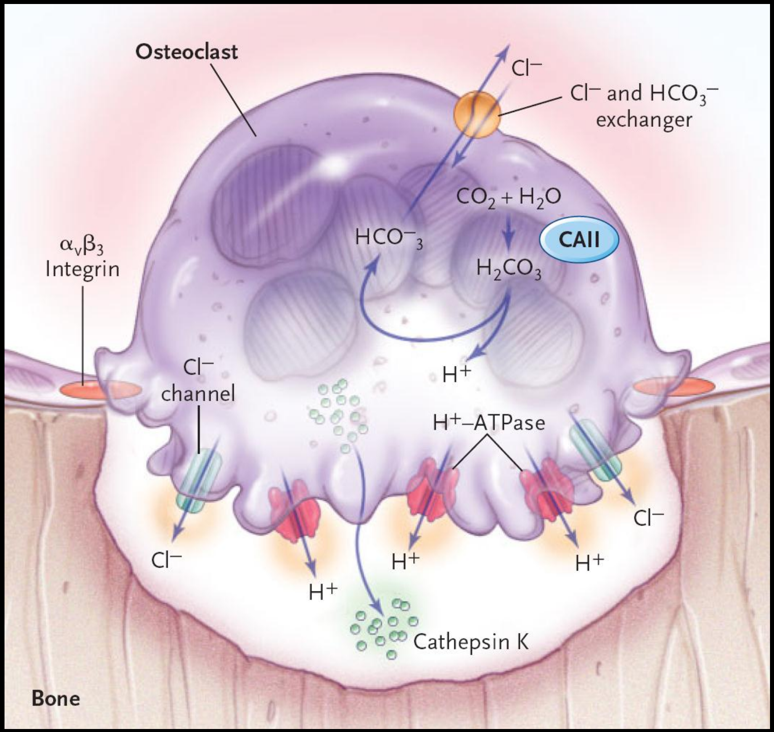
- Maintain homeostasis of $Ca^{2+}$ and $PO_4^{3-}$
- Repair damaged matrix and micro-fracture
- Adapt to mechanical stress and strain

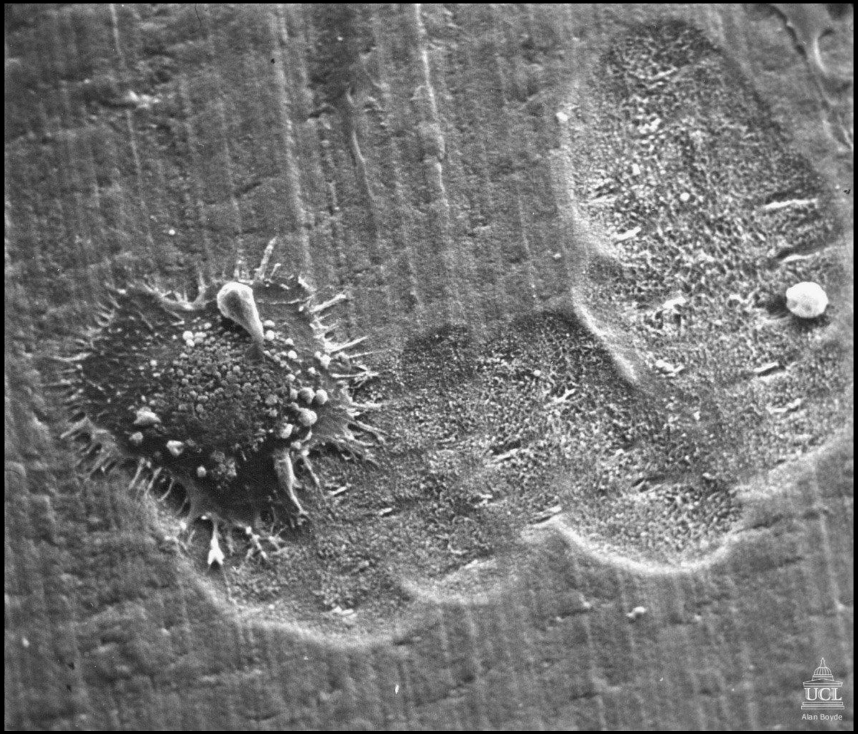
Resorption and formation are coupled temporally and spatially

Uncoupling of bone formation from resorption can lead to

- Osteoporosis or Osteopetrosis

# Osteoclast resorption

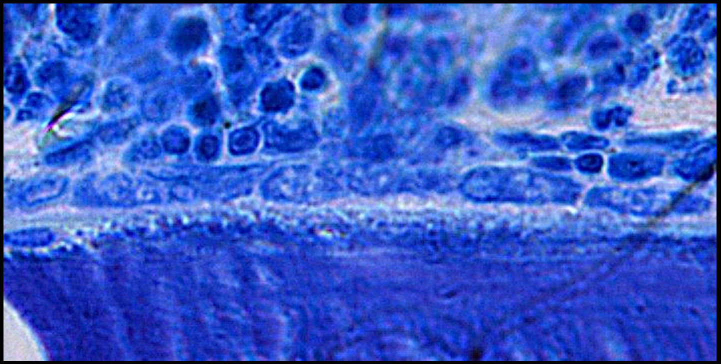


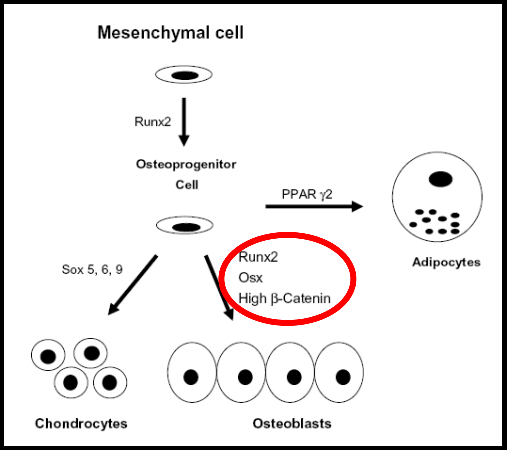



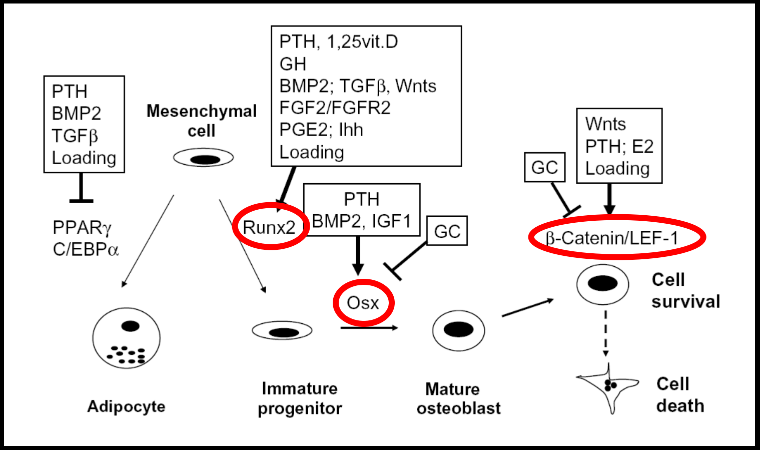

Osteoclasts attach to the bone surface

Secrete hydrogen ions that dissolve bone mineral and MMPs and Cathepsin K degrade the collagen matrix

# Osteoblastic bone fomation



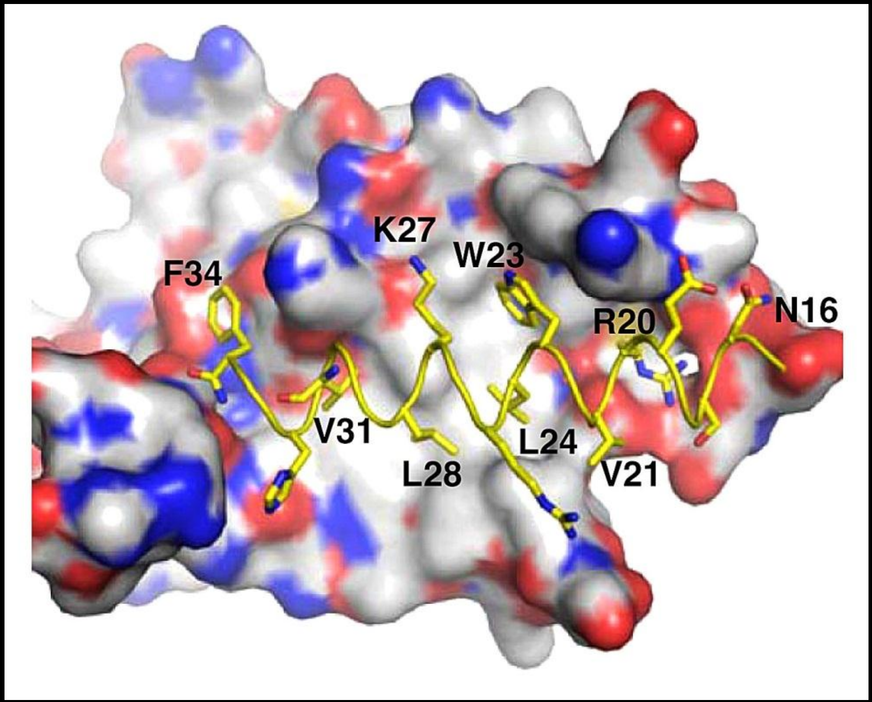




Osteoblastogenesis

- Osteoblast, chondrocytes and adipocytes derive from mesenchym
- Regulated by transcription factors Runx2, osterix and β-catenin
- Key regulators, Wnt, BMPs, FGFs, GH/IGF1, GCs, E2, PTH, 1,25(OH)$_2$D,T3

# Regulation of Calcium

# Parathyroid Hormone



# Parathyroid hormone (PTH) and the PTH receptor (PTHR1)

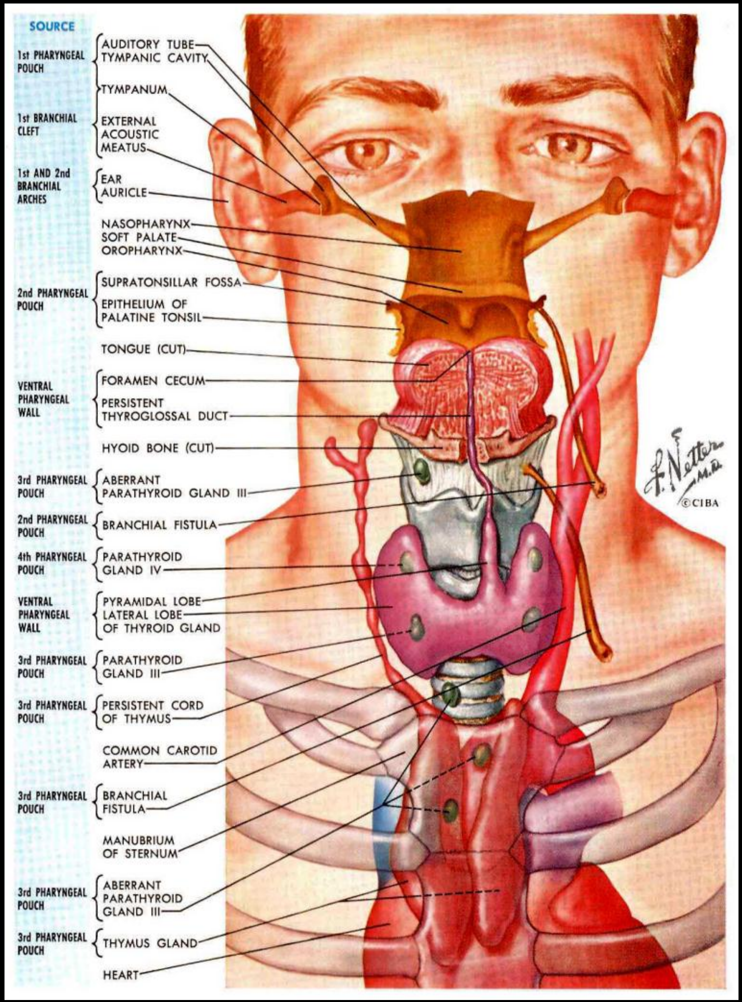
PTH regulates ionised calcium levels

- 99% of calcium in body hydroxyapatite crystals in bone
- In blood 50% protein bound and 50% ionized calcium
- Extracellular calcium is 10,000x greater than intracellular calcium

Calcium

- Regulates neuromuscular excitability
- Release of neurotransmitters and hormones
  - (excitation-secretion coupling)
- Intracellular messenger and muscular contraction
- Blood clotting factor (factor IV)
- Intracellular co-enzyme activity

# Development of the parathyroids




**Parathyroid gland**

- Secreted by 4 glands adjacent to thyroid
- Superior pair from 4rd branchial pouch
- Inferior pair from 3rd branchial pouch
- Exact location and number is variable
- 15% have 5 parathyroids
- Thymic location is common

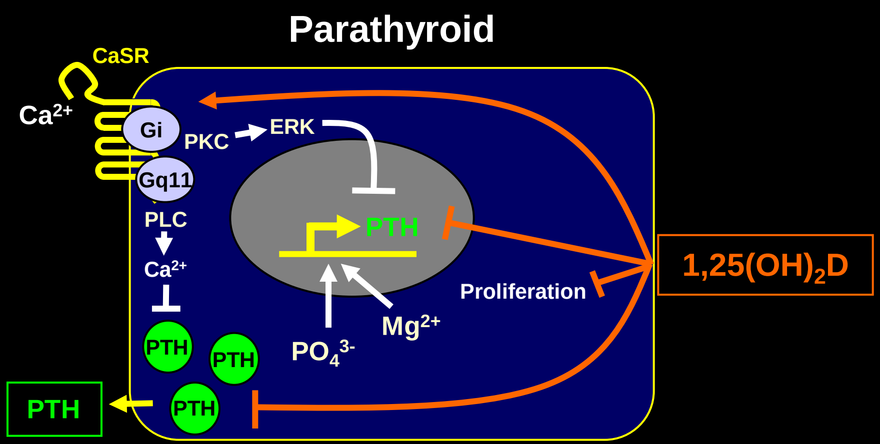
**Parathyroid Hormone (PTH)**

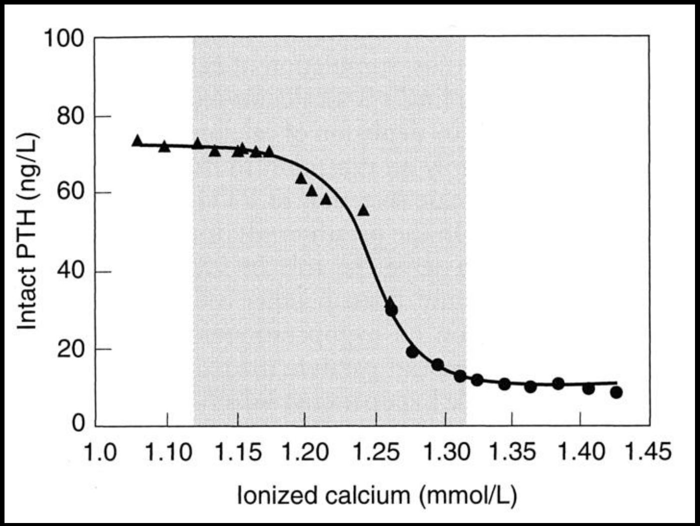
- PTH gene encodes PreproPTH
- Pre leader sequence cleaver in ER
- Pro sequence cleaved in Golgi
- 84 amino acid mature peptide secreted
- 1-34 required to bind PTHR1

**PTH metabolism**

- Cleared by liver and kidney
- Half life 4 minutes

# Regulation of PTH synthesis and secretion




Extracellular $Ca^{2+}$

Via calcium sensing receptor CaSR
Inhibit transcription of PTH

Inhibits secretion of PTH

$1,25(OH)_2D$/VDR

Increases CaSR expression

Inhibits PTH gene transcription

Inhibits PTH secretion

Inhibits parathyroid cell proliferation

Magnesium

Hypermagnesemia or prolonged hypomagnesemia inhibits PTH release

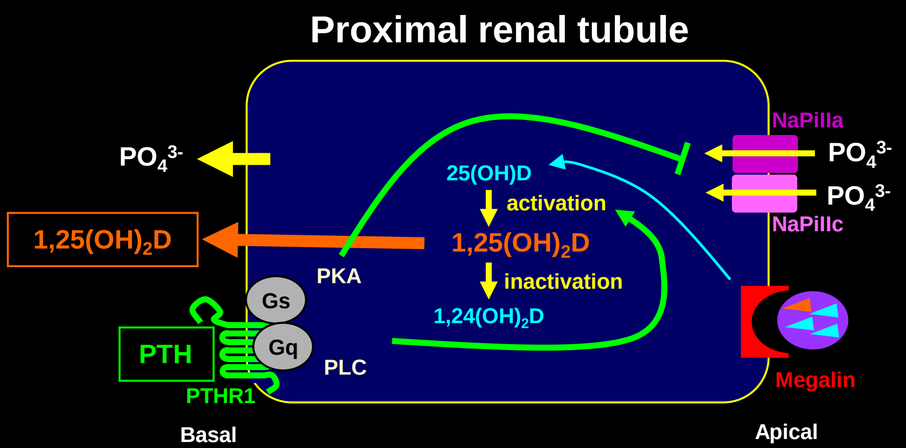
Catecholamines

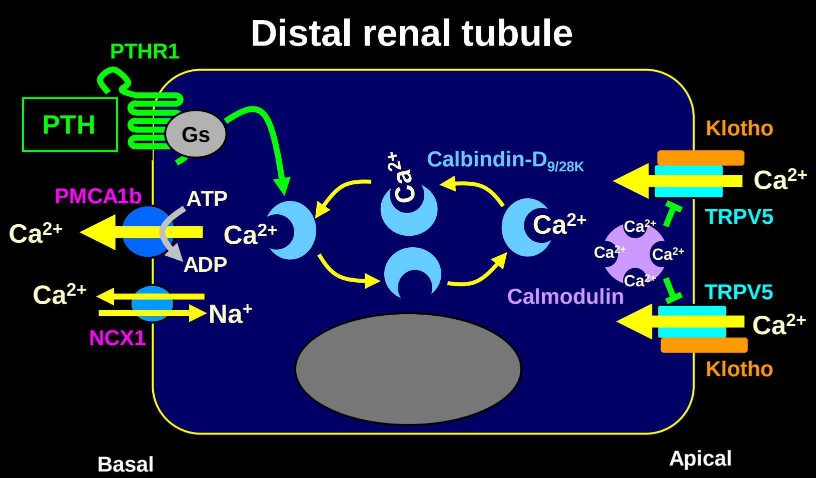
Stimulate PTH secretion

Hyperphosphatemia

Stimulates PTH synthesis

# PTH regulates ionised calcium via PTHR1 (Kidney)




**Proximal renal tubule**

**Inhibits phosphate resorption (Gs)**

**Stimulates synthesis of $1,25(OH)_2D$**

- **Increased $Ca^{2+}/PO_4^{3-}$ gut absorption**
- **Increases $Ca^{2+}$ absorption in DCT**
- **Increases CaSR in DCT**

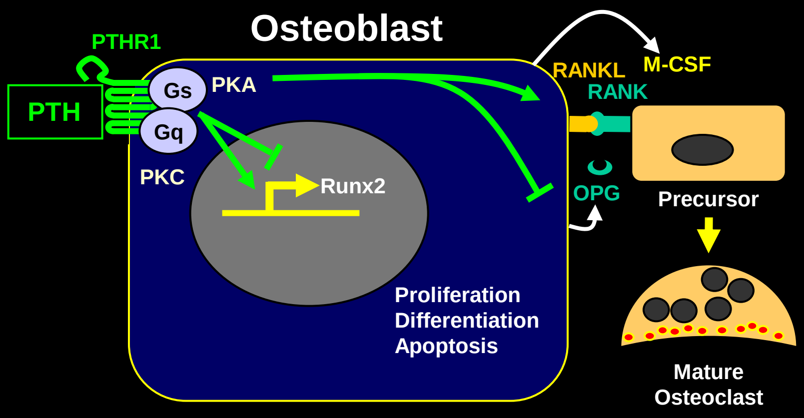
**Distal renal tubule**

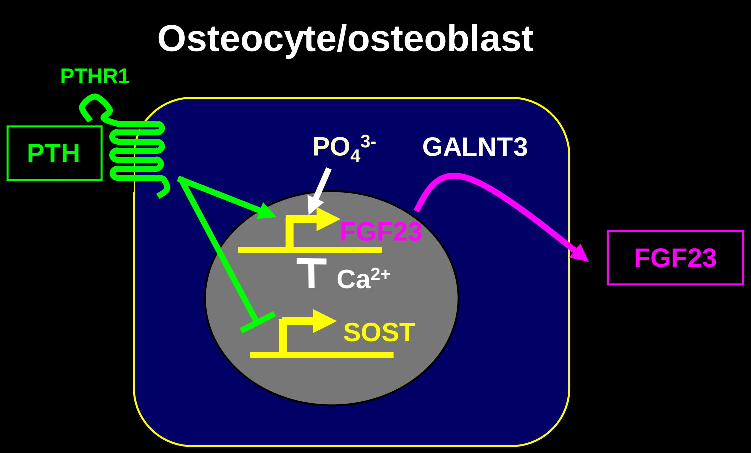
- **Increases expression of Calbindin and $Ca^{2+}$ resorption**

# PTH regulates calcium via PTHR1 in bone

## PTHR1 expressed in osteoblasts and osteocytes but not osteoclasts

## PTH has catabolic and anabolic actions



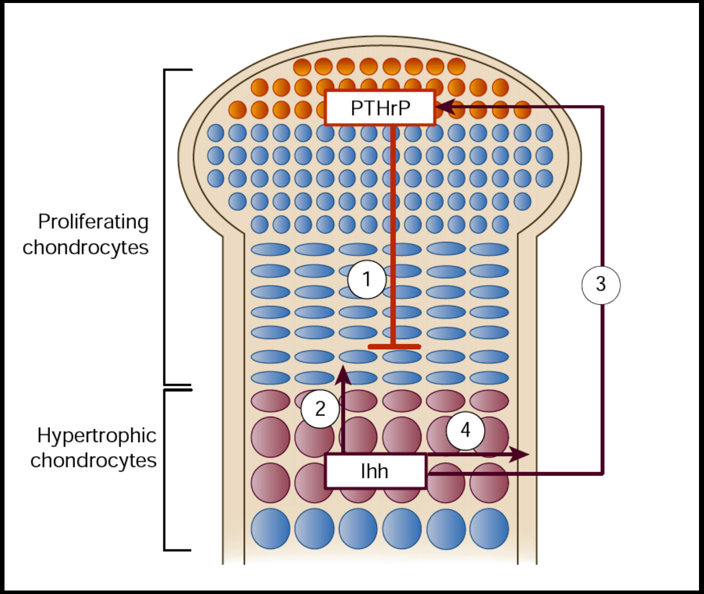

**PTH stimulates resorption and formation**
- Continuous PTH (net cortical resorption)
- Intermittent PTH (net trabecular formation)

**PTH increases osteoclast differentiation indirectly by action in osteoblasts**
- Increased expression of M-CSF/RANKL
- Reduced expression of OPG

**PTH regulates maturation of preosteoblasts**
- Continuous PTH represses Runx2
- Intermittent PTH increases Runx2

**PTH also increases bone formation by paracrine mechanisms**
- Increased IGF-1 and FGF release
- Increasing Wnt signalling
  - Reduced dickkopf and SOST

# PTH related peptide




PTH related peptide (PTHRP) is an alternative ligand for PTHR1

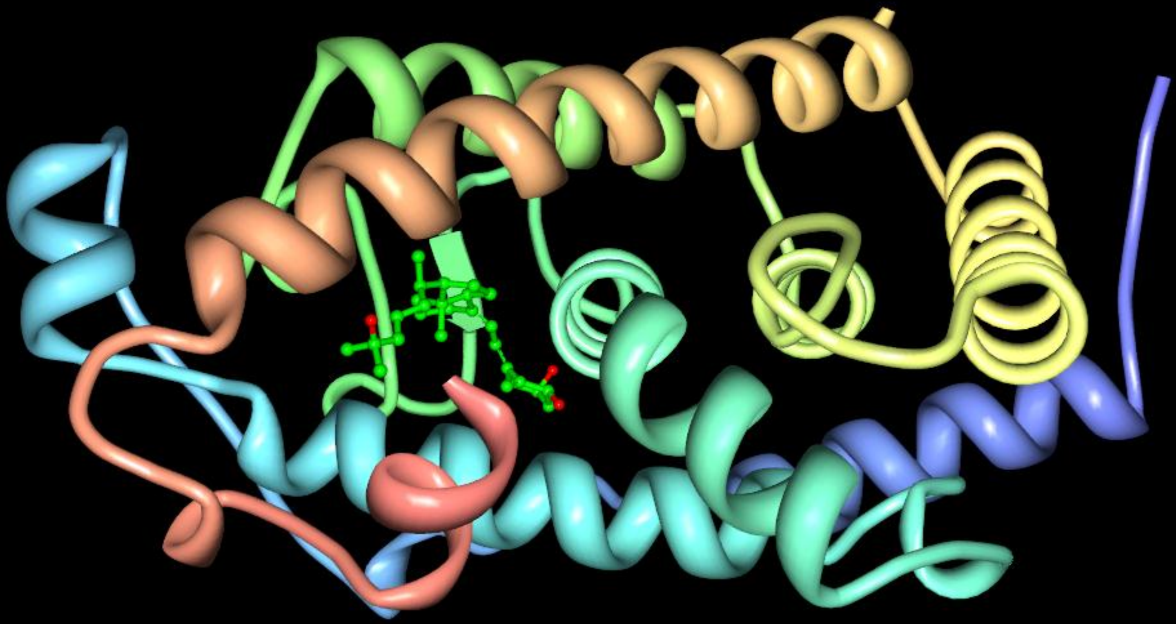
- PTHrP is a paracrine rather than endocrine factor

PTHrP is required for

- Linear growth
  - Regulated chondrocyte proliferation and differentiation
- Calcium transport across the placenta
- Growth and differentiation
  - breast epithelia, pancreatic islets and skin

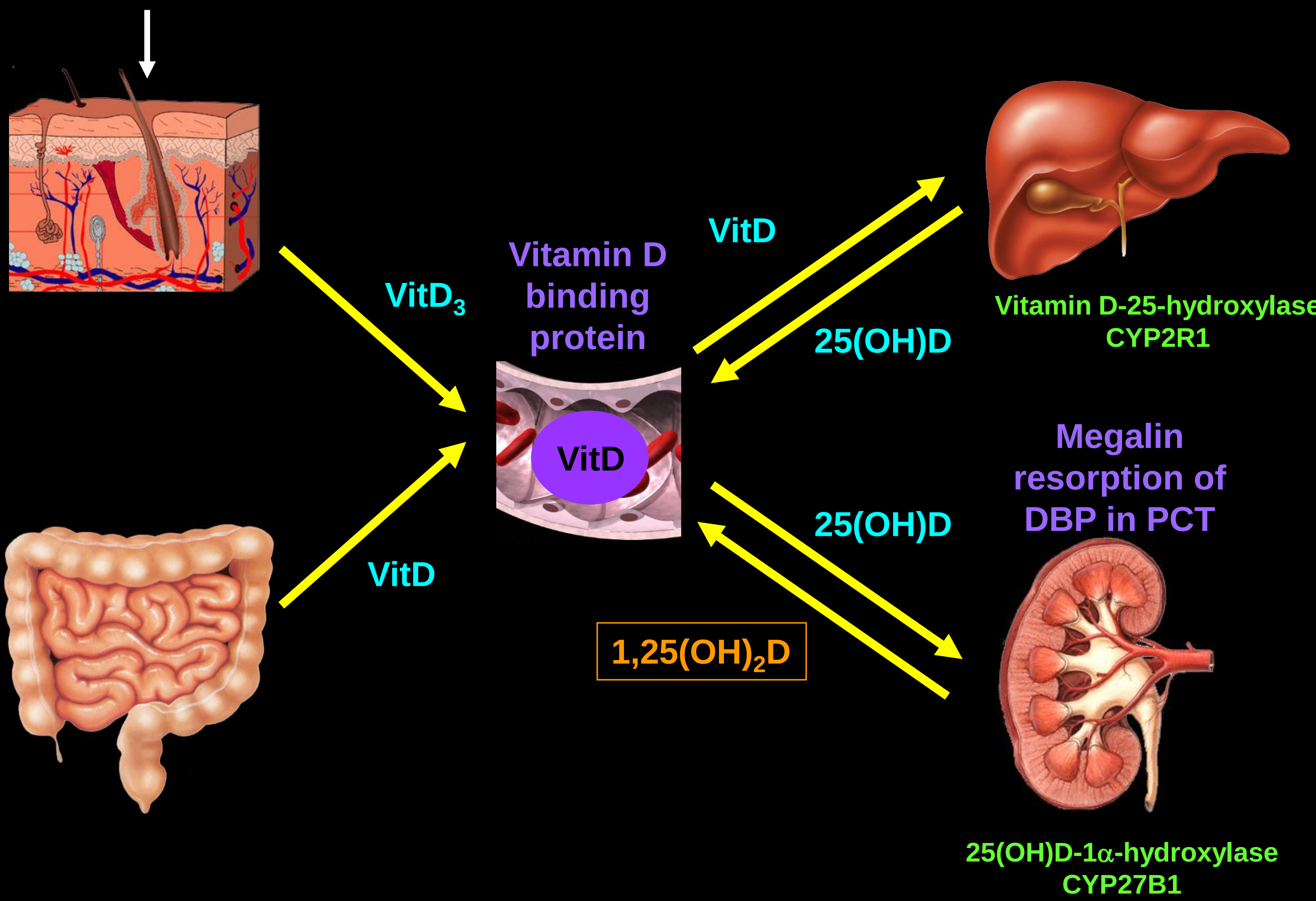
*(Kronenberg HM 2003 Nature 423:332-336)*

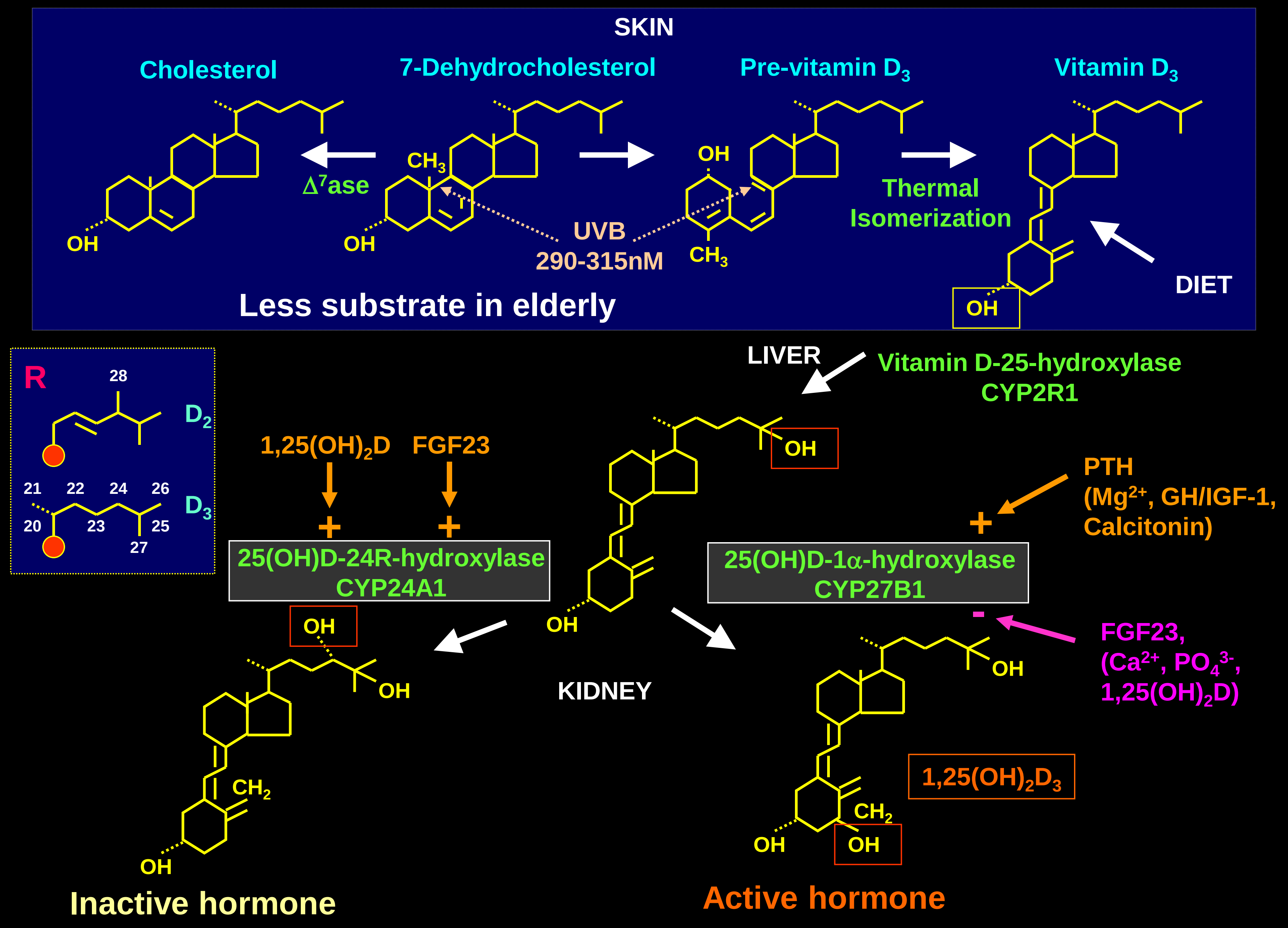
# Vitamin D



**Vitamin D is not a vitamin it is a hormone**

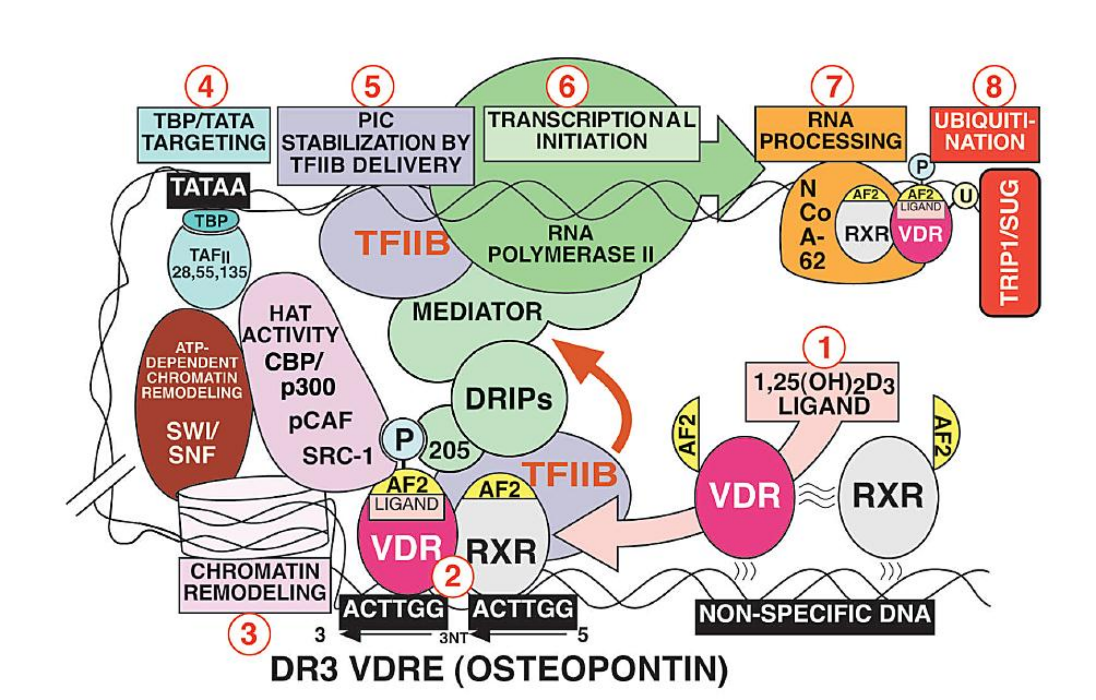
# Transport and metabolism





SKIN
Cholesterol
7-Dehydrocholesterol
Pre-vitamin $D_3$
Vitamin $D_3$
$\Delta^7$ase
$CH_3$
OH
UVB
290-315nM
Thermal
Isomerization
DIET
Less substrate in elderly
R
28
$D_2$
21 22 24 26
20 23 25
27
$D_3$
LIVER
Vitamin D-25-hydroxylase
CYP2R1
$1,25(OH)_2D$
FGF23
+
25(OH)D-24R-hydroxylase
CYP24A1
PTH
($Mg^{2+}$, GH/IGF-1,
Calcitonin)
25(OH)D-1α-hydroxylase
CYP27B1
-
FGF23,
($Ca^{2+}$, $PO_4^{3-}$,
$1,25(OH)_2D$)
KIDNEY
$CH_2$
$1,25(OH)_2D_3$
Inactive hormone
Active hormone

# VDR and target gene transcription

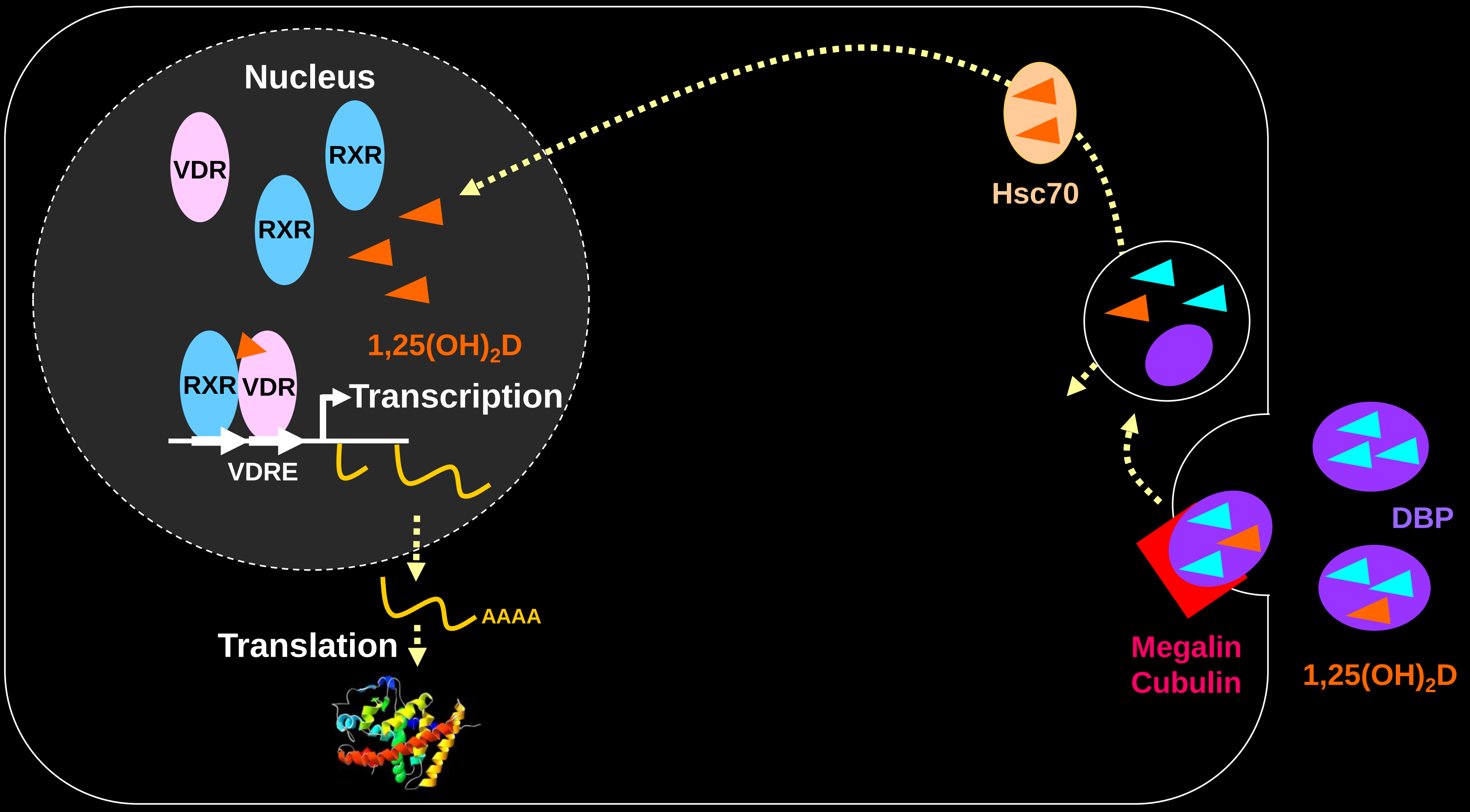



**Stage 1**
VDR binds 1,25$(OH)_2$D and heterodimerizes with RXR
**Stage 2**
VDR/RXR heterodimer binds VDRE
**Stage 3**
Chromatin remodelling by histone acetylation (SRC-1,CBP/p300 etc)
**Step 4**
Binding to TATA associated factors (TRAFs)
**Step 5**
Association with basal transcription factors (TF11B)
**Step 6**
VDR interacting proteins (DRIPs) couple to C-terminal of RNA polymerase
**Step 7**
NCoA-62 couples to spicing machinery
**Step 8**
Association with TRIP1 results in ubiqutination and degradation

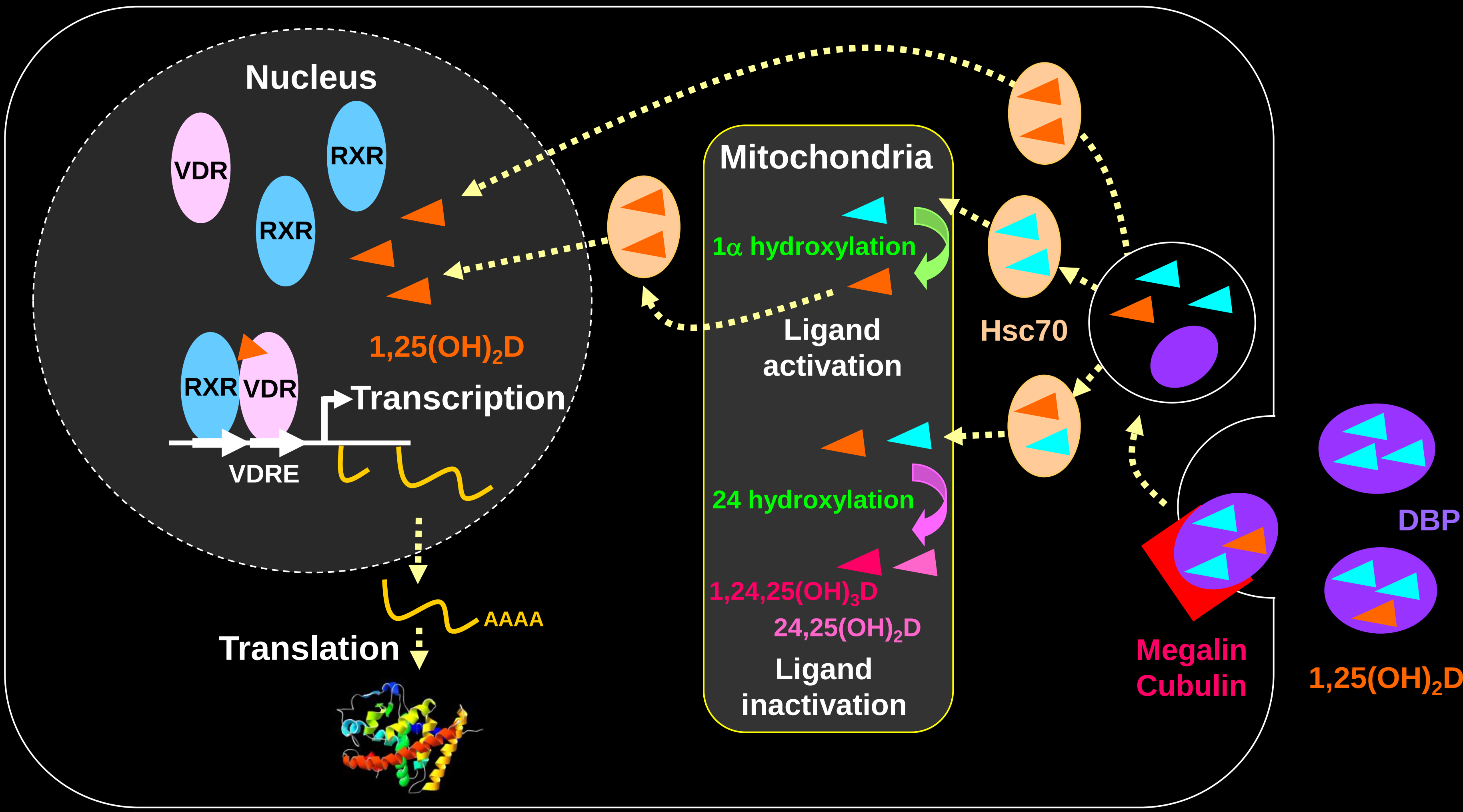
**Actively shuttled between cytoplasm and nucleus**
- Phosphorylated on binding 1,25$(OH)_2D_3$
- Ligand inducible transcription factor
- Forms a heterodimer with RXR
- Binds VDREs in promoter regions of response genes
- 1,25$(OH)_2$D/VDR directly or indirectly regulates 5% of genes

*(Jurutka PW et al. 2007 JBMR 22:V2-10)*

# Vitamin D action



# Vitamin D action




**$1,25(OH)_2D$ supply depends on expression of the activating enzyme 1α-hydroxylase and its catabolic counterpart 24-hydroxylase**

# Physiological role of $1,25(OH)_2D$/VDR signalling

$1,25(OH)_2D$/VDR signalling evolved before the development of calcified structures (lamprey)

$1,25(OH)_2D$ directly or indirectly regulates 5% of genes.
The majority are not involved in calcium and phosphate homeostasis

The VDR is expressed widely and not just in tissues associated with calcium and phosphate metabolism

The activating 1α-hydroxylase enzyme is expressed in multiple tissues

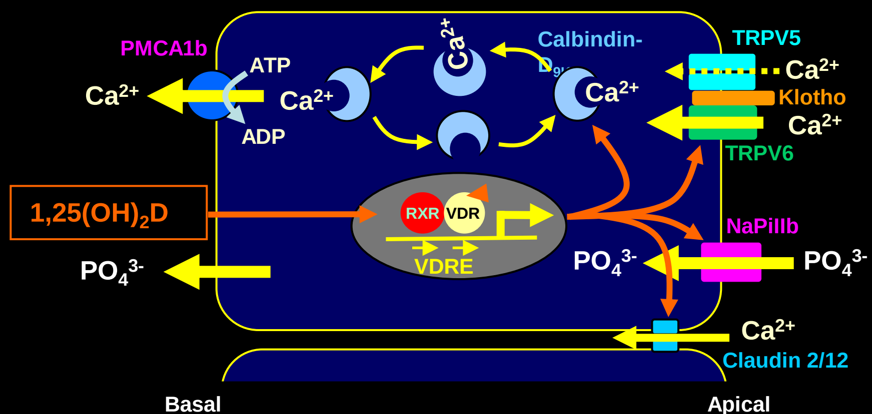
The inactivating 24-hydroxylase enzyme is expressed in multiple tissues

**$1,25(OH)_2D$/VDR signalling is likely to have physiological roles other than calcium phosphate homeostasis**

**Currently only good clinical data for its effect on mineral homeostasis**

# $1,25(OH)_2$ vitamin D increases calcium and phosphate absorption from the gut

Duodenal endothelium

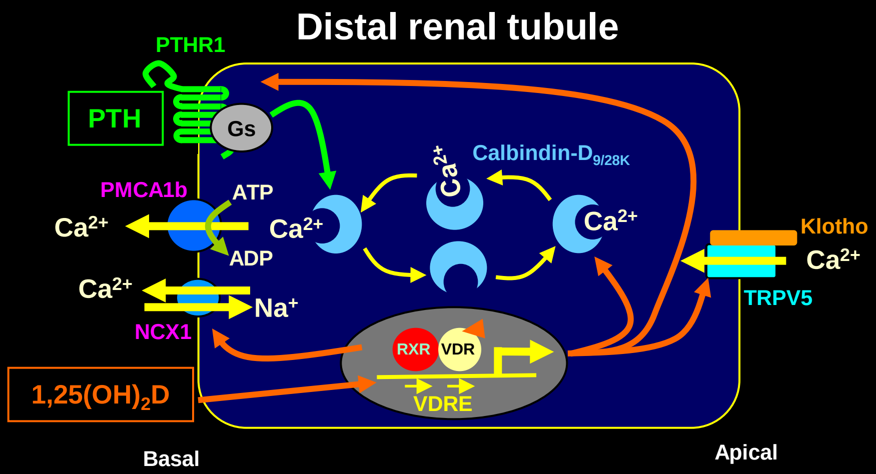



$1,25(OH)_2D$ increases expression of

- Calbindin-D9K
- Calcium transporters TRPV5/6
- Calcium channel Claudin 2/12
- Phosphate transporter NaPi2b

Regulation of calcium absorption by $1,25(OH)_2D$ is essential to maintain normal serum calcium and skeletal mineralisation.
However $1,25(OH)_2D$ also acts directly in bone

# 1,25$(OH)_2$ vitamin D increases calcium resorption from the kidney

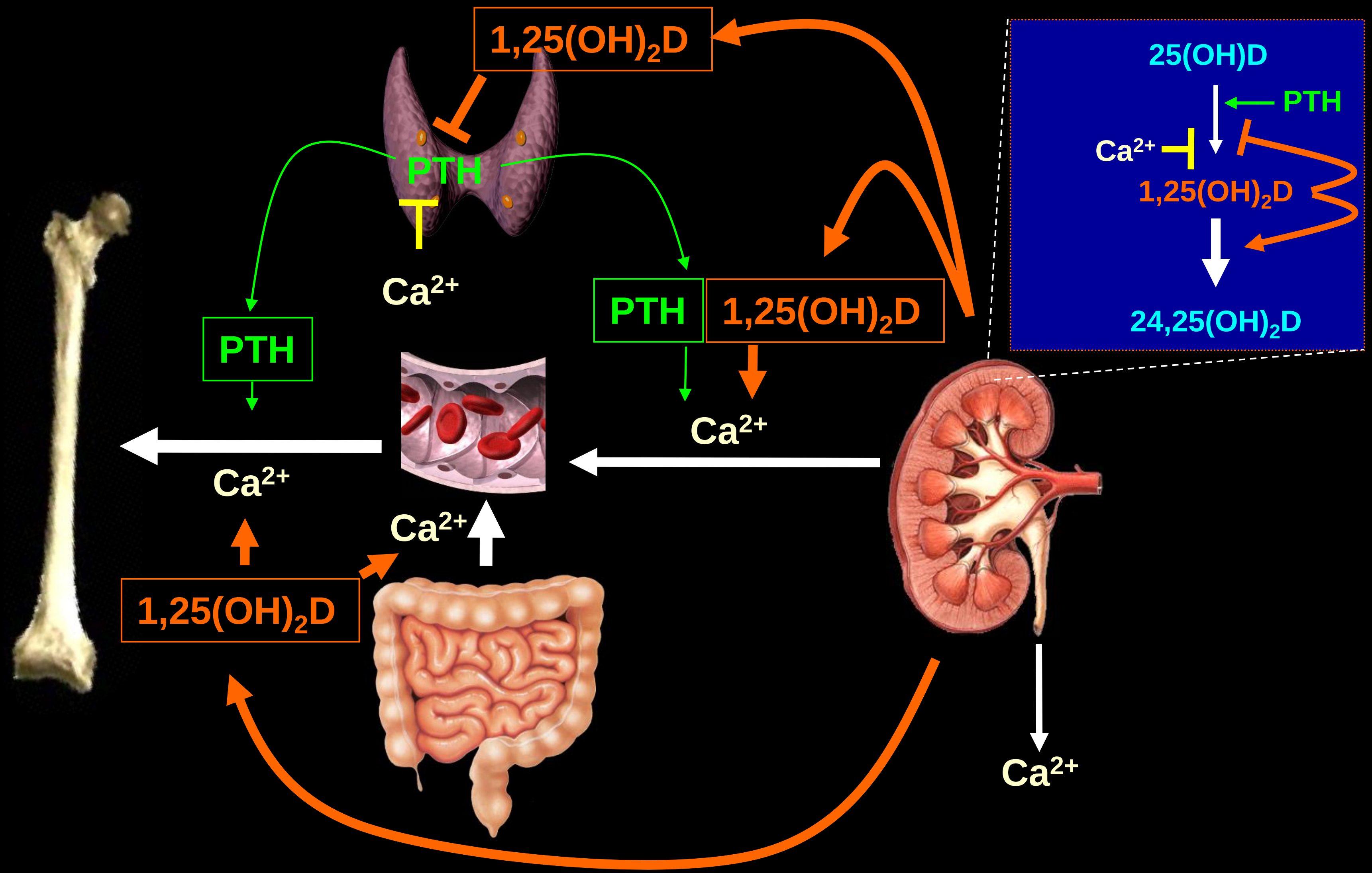



1,25$(OH)_2$D increases expression of

- Calbindin-D28K
- Calcium transporters TRPV5
- NCX1 calcium/sodium exchanger

1,25$(OH)_2$D also increases sensitivity to PTH by increasing PTHR1 expression

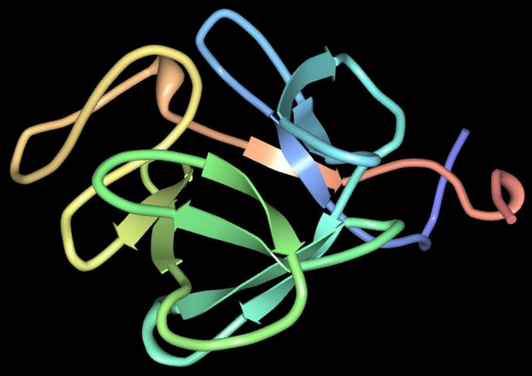
# Replete vitamin D and calcium




1,25(OH)$_2$D stimulates Ca$^{2+}$ absorption from gut and reabsorption from kidney
Negative feed back: 1,25(OH)$_2$D inhibits PTH synthesis/release and its own synthesis

# Regulation of Phosphate

# FGF23

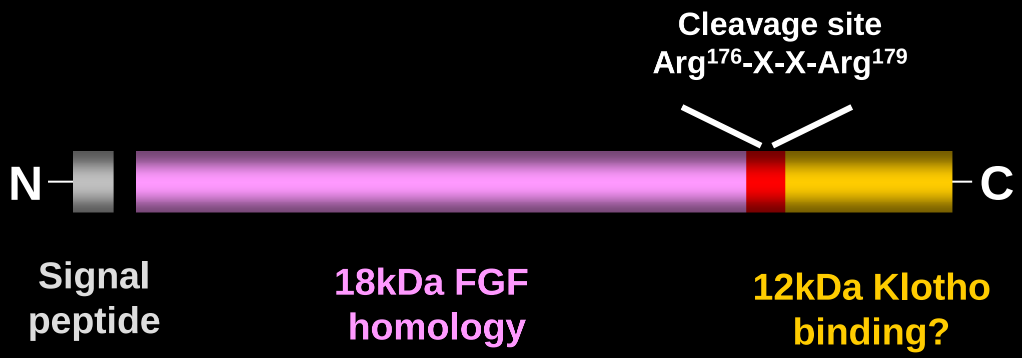


FGFs are secreted proteins that act as paracrine factors

Regulation of cell proliferation, differentiation and function

FGF23 identified in 2000 as the protein mutated in ADHR

FGF23 act as a hormone and underlies several disease with abnormal phosphate and bone metabolism




Biologically active form 251αα, 32kDa secreted protein

Inactivated by intracellular cleavage into 18kDa and 12kDa fragments

# Fibroblast growth factor 23 (FGF23)

FGF23, 1,25$(OH)_2$D and PTH regulate serum phosphate
85% of the body's phosphate is in bone
Phosphate is essential for

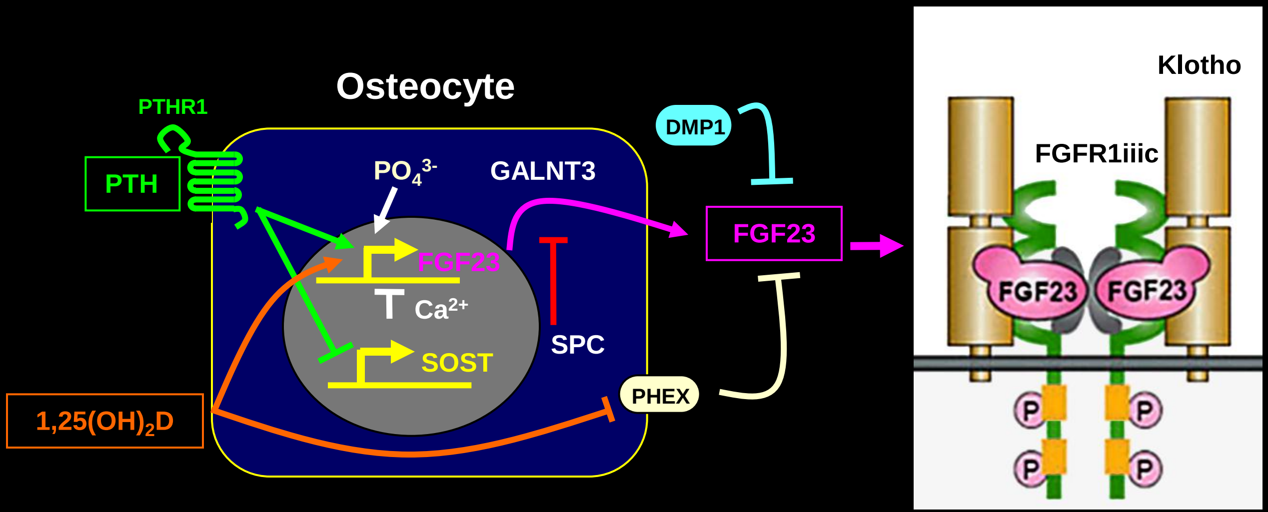
- Mineralisation of bone
- Apoptosis of hypotrophic growth plate chondrocyte

Intra and extracellular phosphate concentrations are similar

Organic phosphate is a key component of almost all classes of structural, informational and effector molecules

- Nucleic acids
- phospholipids
- complex carbohydrates
- phosphoproteins
- enzyme co-factors
- energy storage molecules
- secondary messengers (G-proteins/phosphorylation)

# FGF23 signaling




Impaired $PO_4^{3-}$ absorption

Increased renal $PO_4^{3-}$ loss

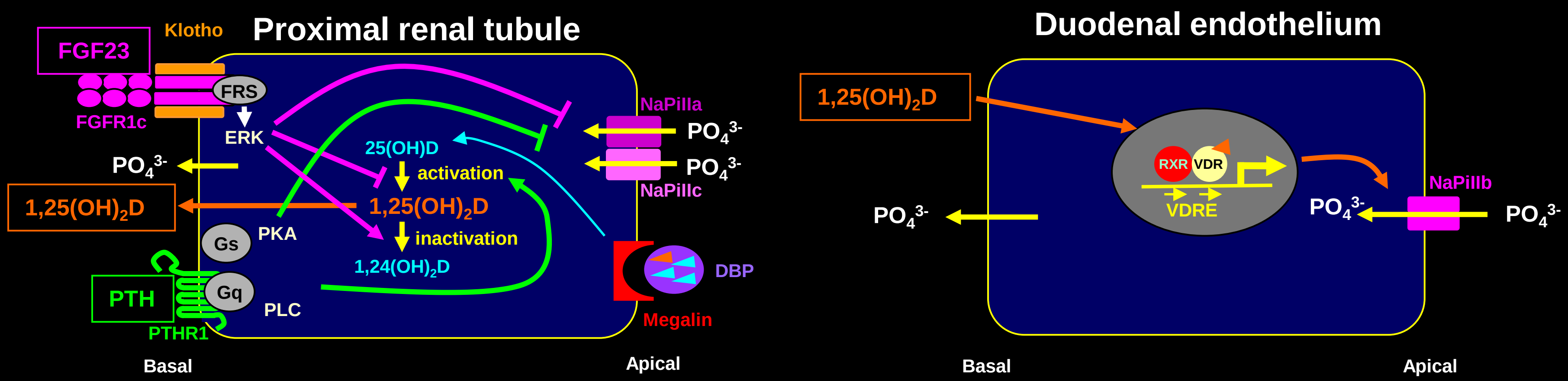
Impaired 1α-hydroxylation

$1,25(OH)_2D$, phosphate and PTH
- Induce expression FGF23 in ostoblast/osteocytes
- PHEX a metalloendopeptidase negatively regulates FGF23 signalling
- GALNT3 mediates O-glycosylation of FGF23 and prevents cleavage by Subtilisin-like proprotein convertase (SPCs) allowing secretion

FGF23 acts via
- FGFR1iiic receptor and requires the co-receptor Klotho (β-glucosidase)

*(Strom TM (2008) Current Opinion in Nephroland Hypertension 17:357–362)*

# Regulation of serum phosphate



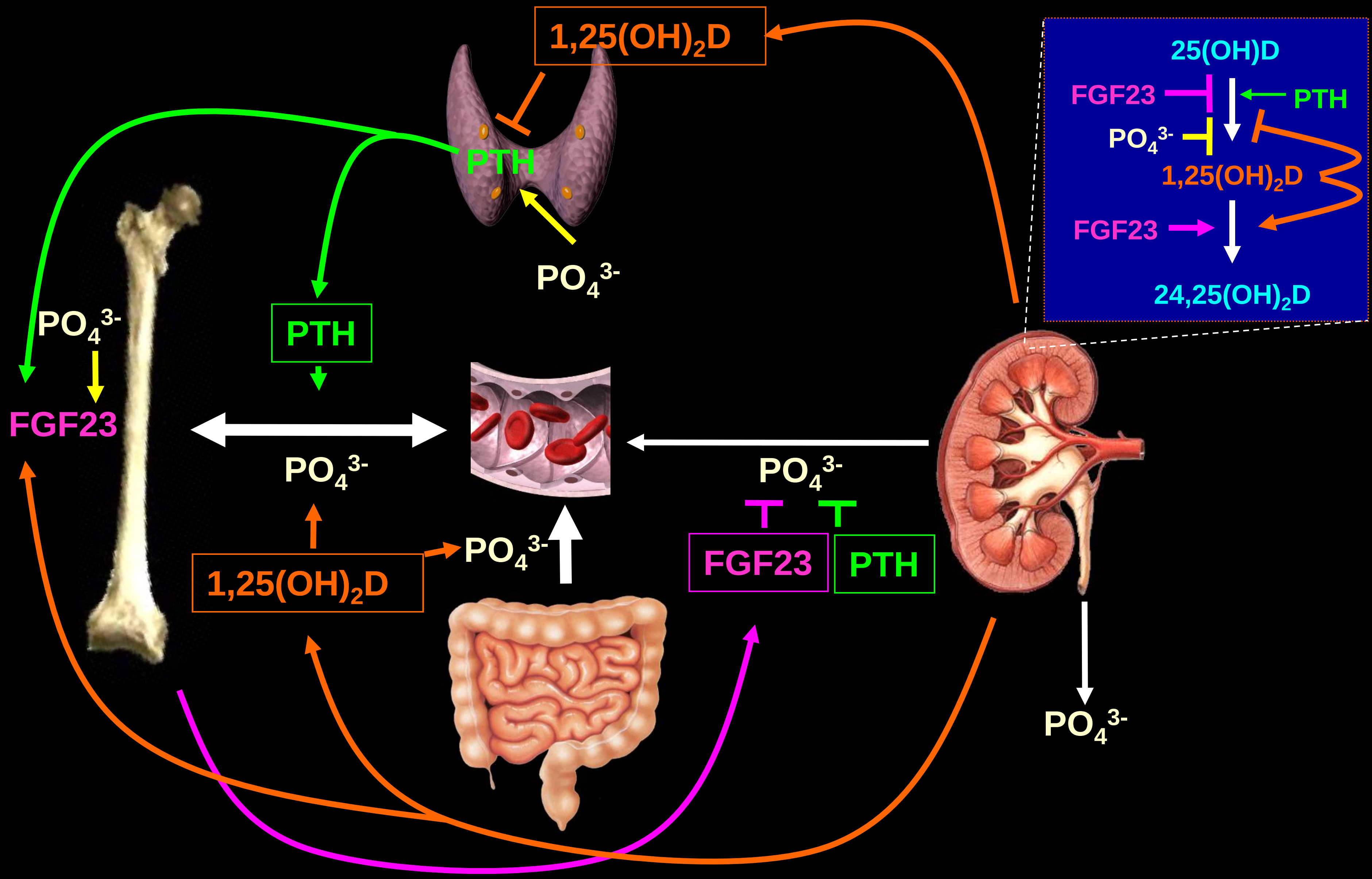

**FGF23 ensure $Ca^{2+}$ $PO_4^{3-}$ product does exceed its solubility**

- Inhibit phosphate resorption from the kidney
- Inhibit synthesis of 1,25(OH)$_2$D by 1$\alpha$-hydroxylation
- Increase 1,25(OH)$_2$D inactivation by 24-hydroxylation

**1,25(OH)$_2$D/VDR**

- Stimulates phosphate absorption from the gut
- Serum phosphate and 1,25(OH)$_2$D induce expression FGF23
- FGF23 inhibits 1,25(OH)$_2$D synthesis and thus mediates negative feedback

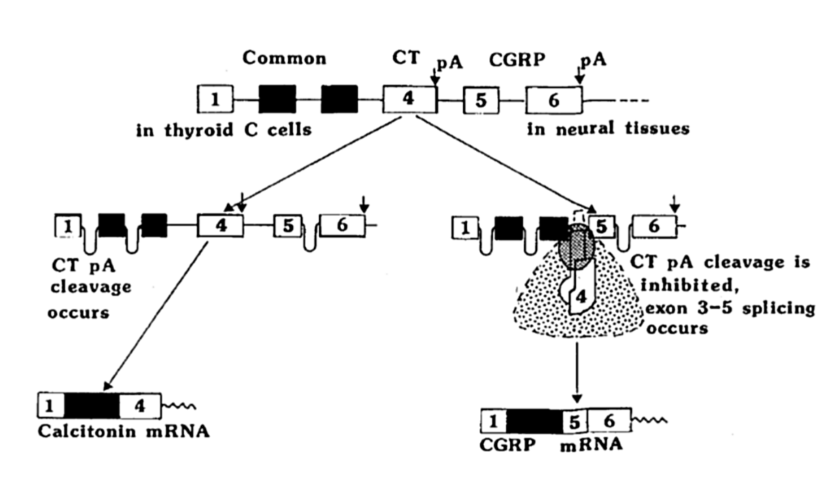
# $1,25(OH)_2D$, FGF23, PTH and Phosphate



# Calcitonin

# Calcitonin

## Calcitonin is not physiologically important for mineral homeostasis in humans




Thyroid parafollicular cells express calcitonin
- CALC-I gene encodes a 141 amino acid protein
- Proteolytically cleaved to yield a 32 amino acid peptide.

CaSR is expressed by C-cells
- Calcium stimulates calcitonin synthesis and release

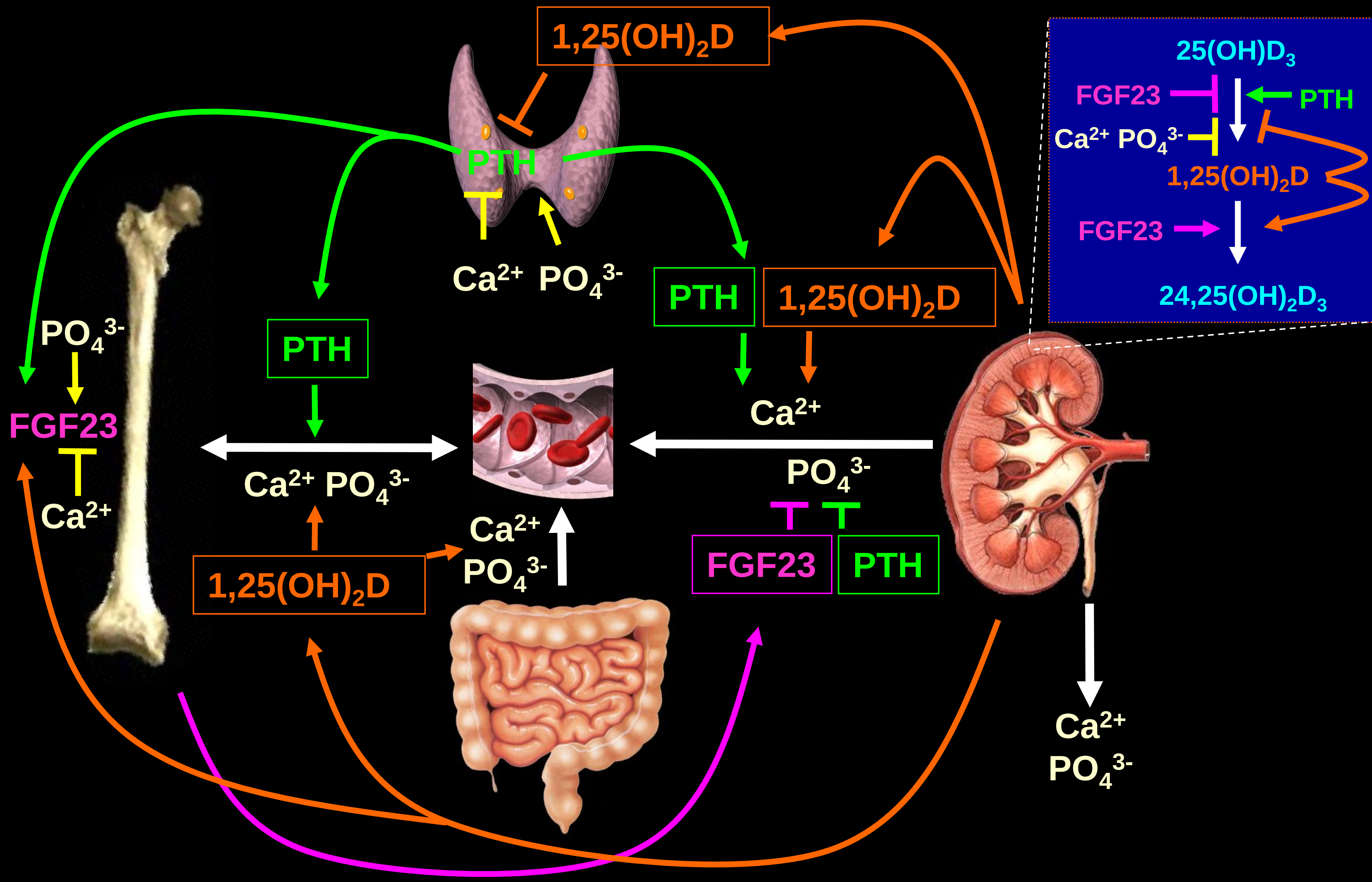
Calcitonin receptor
- G-protein coupled receptor (osteoclasts and proximal renal tubules)

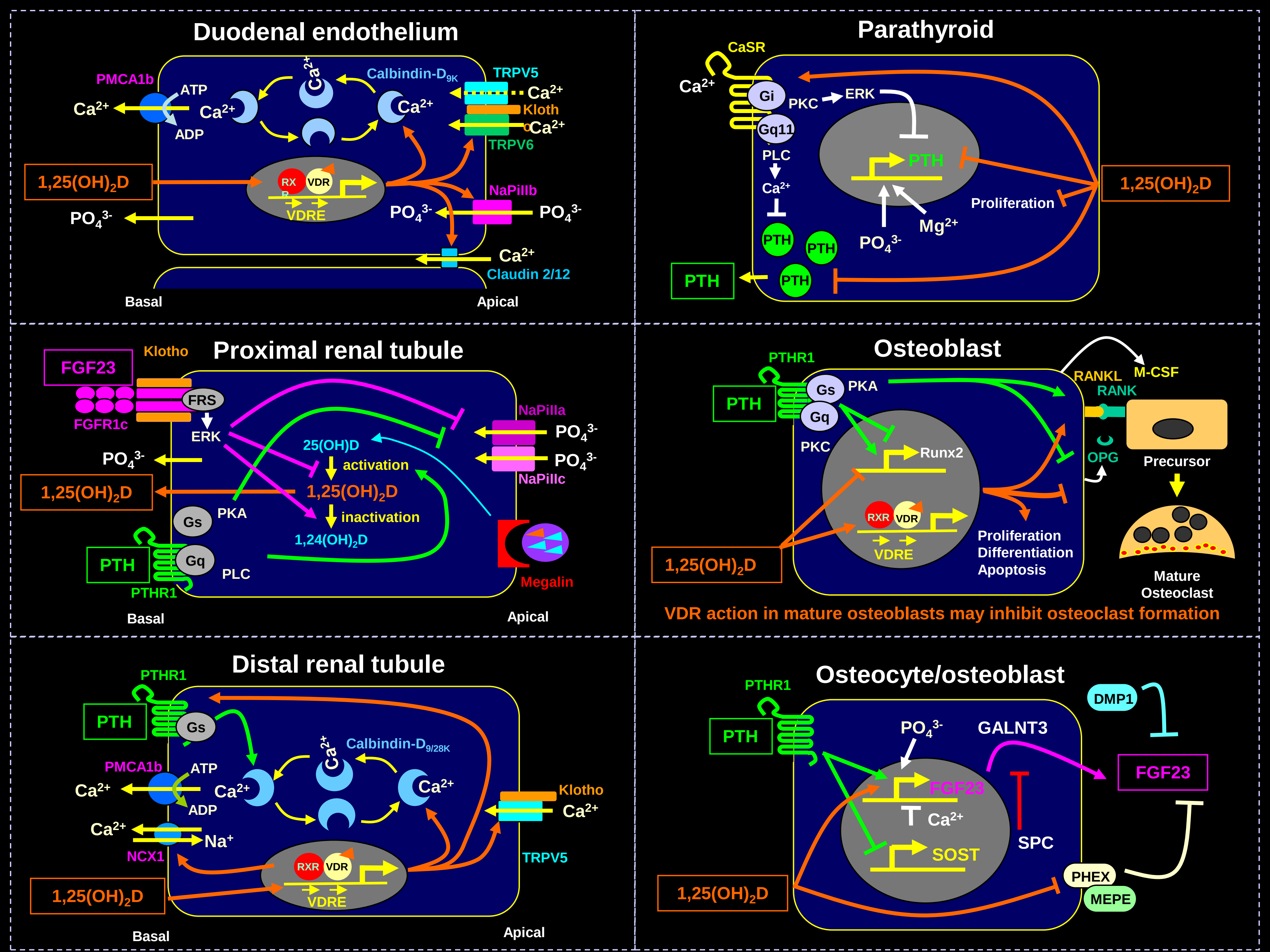
Calcitonin
- Rapidly inhibits osteoclast resorption (rapid fall in calcium)
- Inhibits renal phosphate resorption

*(Inzerillo AM (2002) Thyroid 12:791-797)*

# Summary of calcium and phosphate homeostasis

# Regulation of calcium and phosphate




Duodenal endothelium
PMCA1b
ATP
ADP
$Ca^{2+}$
Calbindin-$D_{9K}$
TRPV5
Kloth
TRPV6
$1,25(OH)_2D$
RXR
VDR
VDRE
NaPiIIb
$PO_4^{3-}$
Claudin 2/12
Basal
Apical
Parathyroid
CaSR
Gi
Gq11
PKC
ERK
PLC
PTH
$Mg^{2+}$
Proliferation
Proximal renal tubule
FGF23
Klotho
FGFR1c
FRS
ERK
25(OH)D
activation
inactivation
$1,24(OH)_2D$
NaPiIIa
NaPiIIc
Megalin
Gs
Gq
PKA
PLC
PTHR1
Osteoblast
RANKL
M-CSF
RANK
OPG
Precursor
Runx2
Proliferation
Differentiation
Apoptosis
Mature Osteoclast
VDR action in mature osteoblasts may inhibit osteoclast formation
Distal renal tubule
Calbindin-$D_{9/28K}$
NCX1
$Na^+$
Osteocyte/osteoblast
GALNT3
DMP1
SOST
SPC
PHEX
MEPE

# References

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