Mineral Homeostasis

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Calcium Homeostasis
Parathyroid hormone (PTH (PTHrP) and PTHR1)
Vitamin D ($1,25(OH)_2D_3$, VDR)
Calcitonin (CT and CALCR)

Phosphate Homeostasis
Fibroblast growth factor 23 (FGF23, Klotho, FGFR1c)
Vitamin D ($1,25(OH)_2D_3$, VDR)
Parathyroid hormone (PTH and PTHR1)

Regulation of Calcium

corrected calcium level (cCa^{2+}) 2.15-2.6mmol/l (adjusted for albumen)

Calcium homeostasis

Calcium is essential for

- **Normal function of muscle, nerve, bone and coagulation**

Daily requirement

- **1000mg/d normal adult**
- **1300mg/d during growth, pregnancy and lactation,**
- **1200mg/d in the elderly**

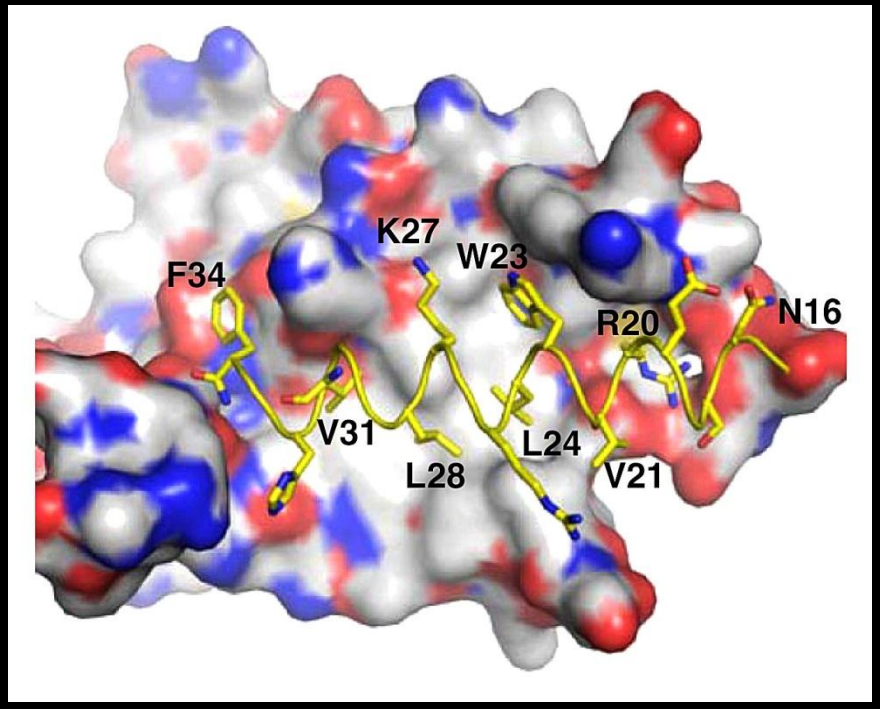
Dietary sources

- **Milk, cheese other dairy products**
- **Dark leafy greens or dried beans**

Calcium concentration is very tightly regulated (2.1-2.6 mmol/l)

- **Parathyroid hormone**
- **1,25 $(OH)_2$Vitamin D**

Parathyroid Hormone



PTH 1.6-6.8 pmol/l

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

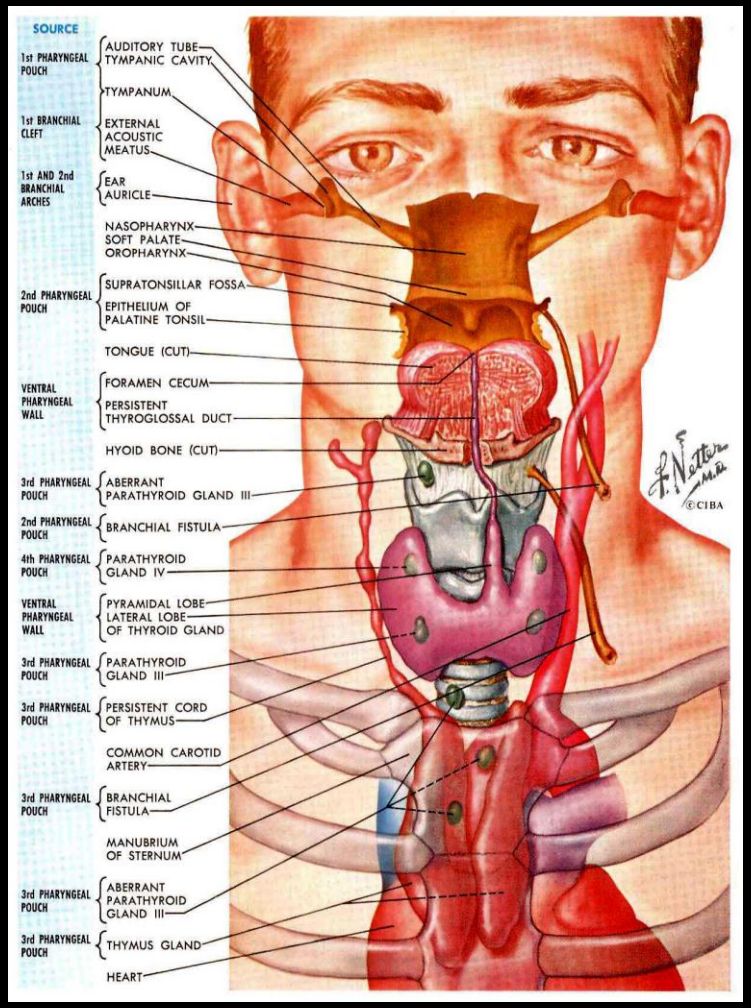
PTH regulates ionised calcium levels

- 99% of calcium in body hydroxyapatite crystals $Ca_{10}(PO_4)_6(OH)_2$ in bone
- In blood 50% protein bound and 50% free 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% of individuals have 5 parathyroids
- Thymic location is common

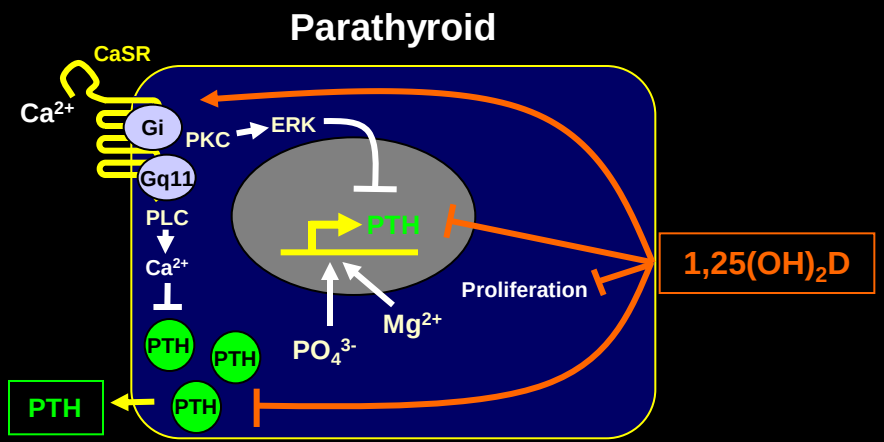
Parathyroid Hormone (PTH)

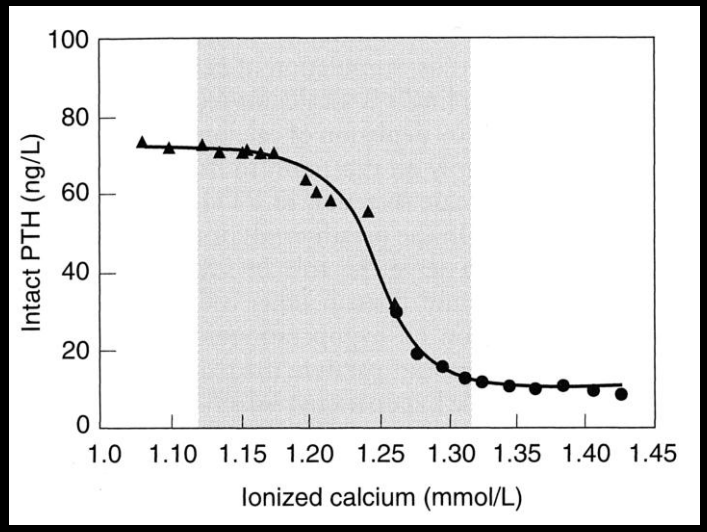
- PTH gene encodes PreproPTH
- Pre leader sequence cleaver in ER
- Pro sequence cleaved in Golgi
- 84 amino acid mature peptide secreted
- First 34 amino acids required to bind PTHR1

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

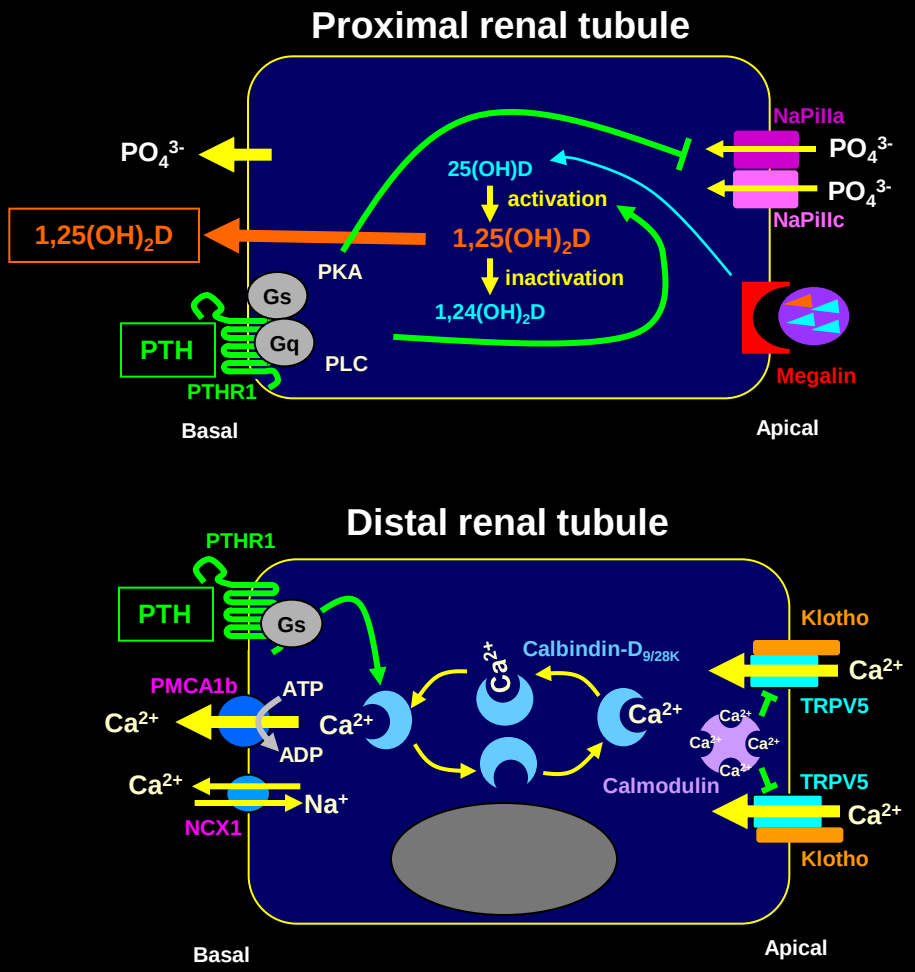
$1,25(OH)_2D$/VDR
- Increases CaSR expression
- Inhibits PTH gene transcription
- Inhibits PTH secretion
- Inhibits parathyroid cell proliferation

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

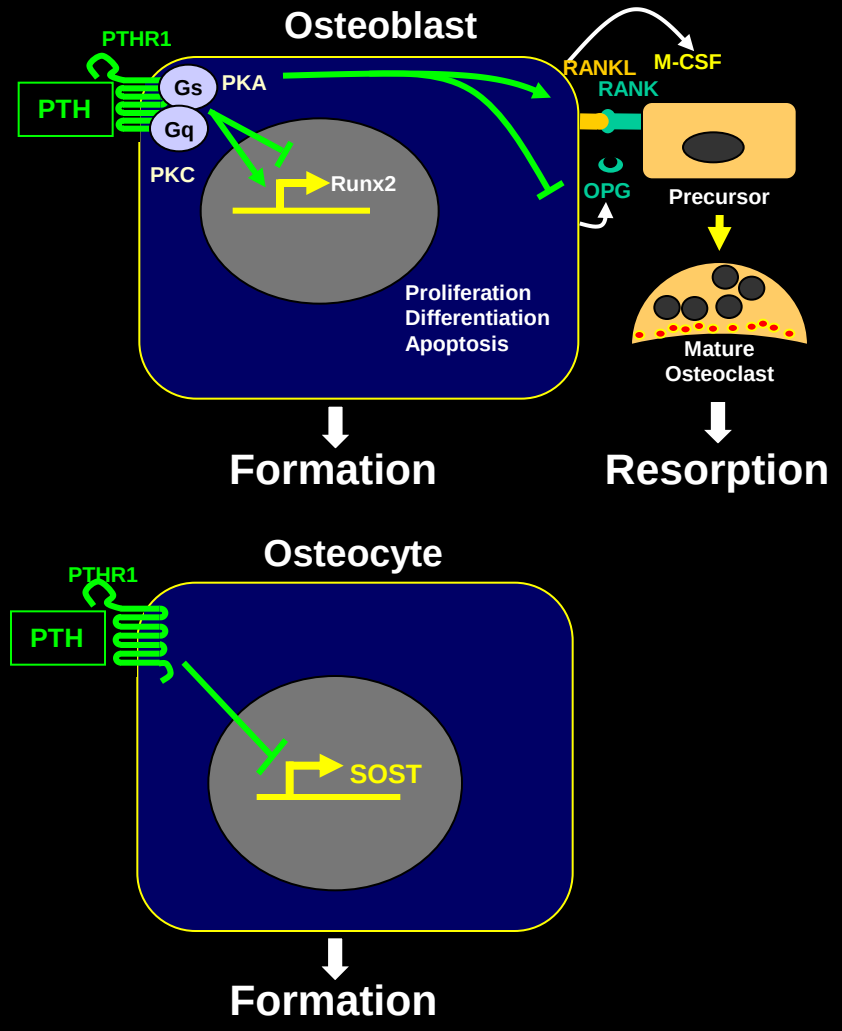
Distal renal tubule

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

PTH regulates calcium via PTHR1 in bone

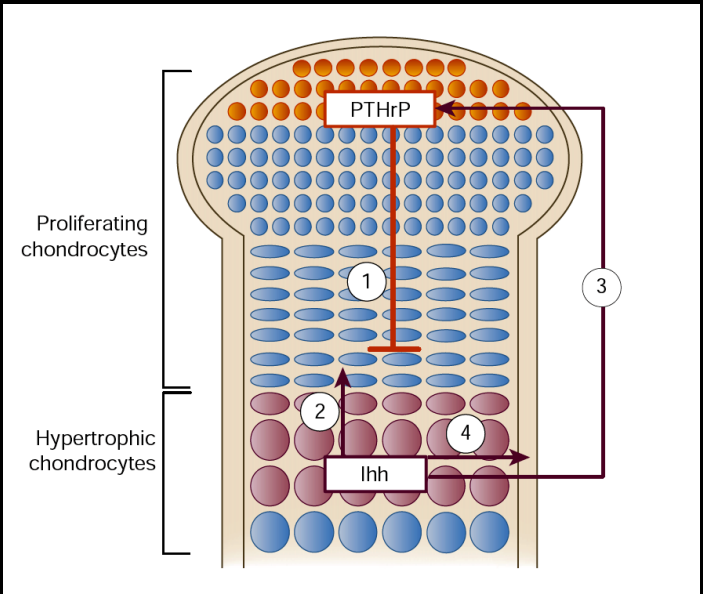
PTHR1 expressed in osteoblasts and osteocytes but not osteoclasts

PTH has catabolic and anabolic actions



- PTH can stimulate bone resorption or formation
  - Intermittent PTH (net trabecular formation)
  - Continuous PTH (net cortical resorption)
- 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**

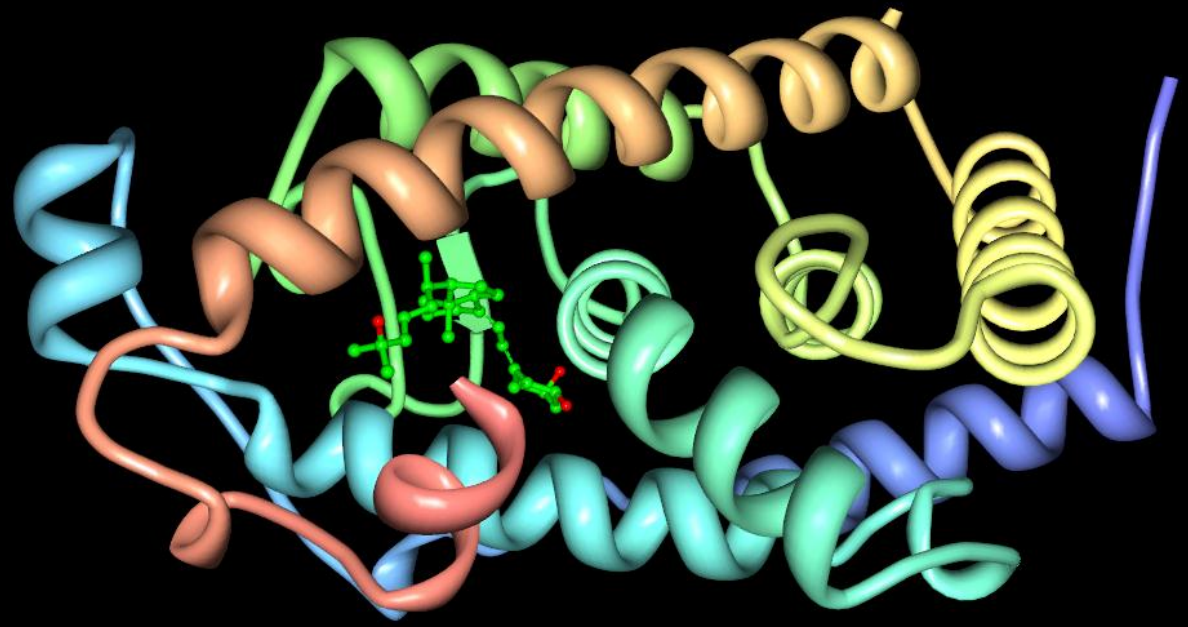
PTHrP is required for

- **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

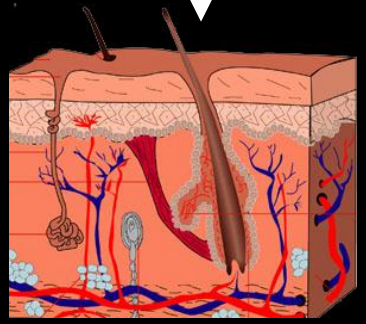
Vitamin D is not a vitamin it is a hormone



25-OH-VitaminD 70-150 nmol/l?

Transport and metabolism

UVB
290-315nM

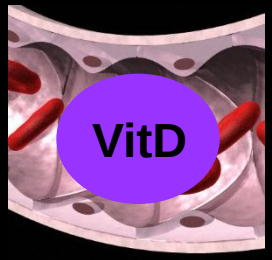


$VitD_3$

VitD

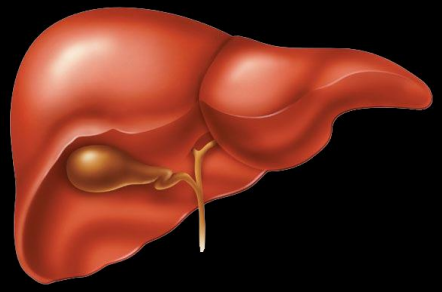
Vitamin D binding protein

VitD



VitD

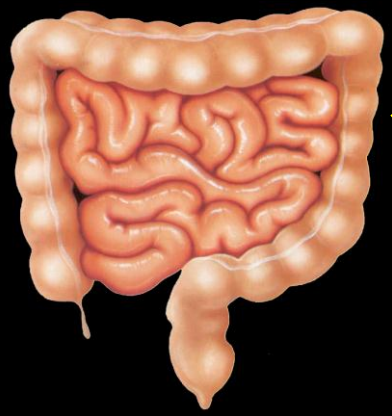
25(OH)D



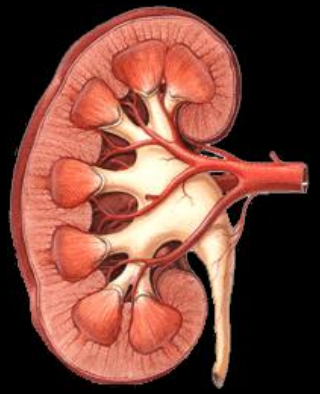
Vitamin D-25-hydroxylase
CYP2R1

25(OH)D

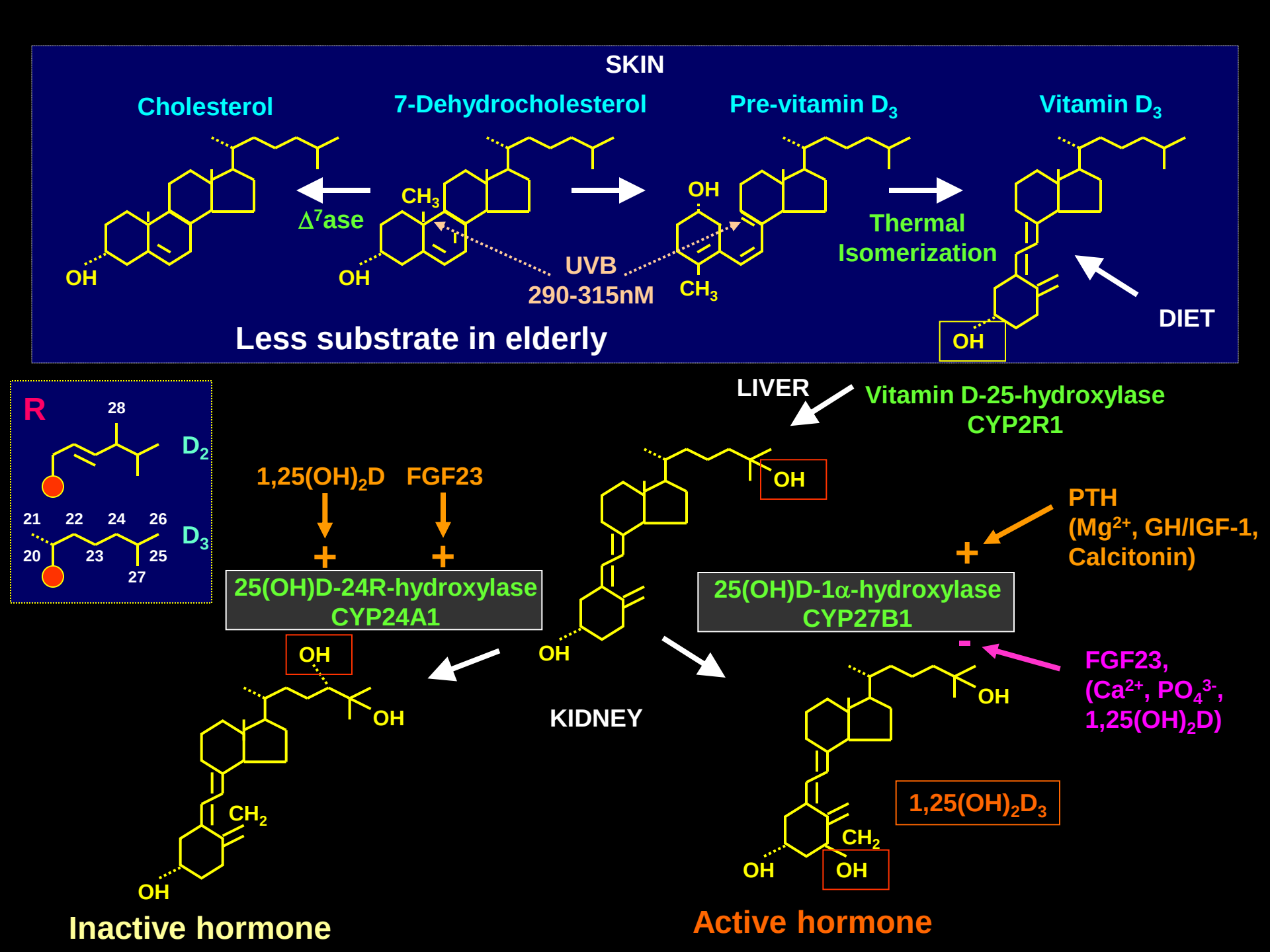
Megalin resorption of DBP in PCT



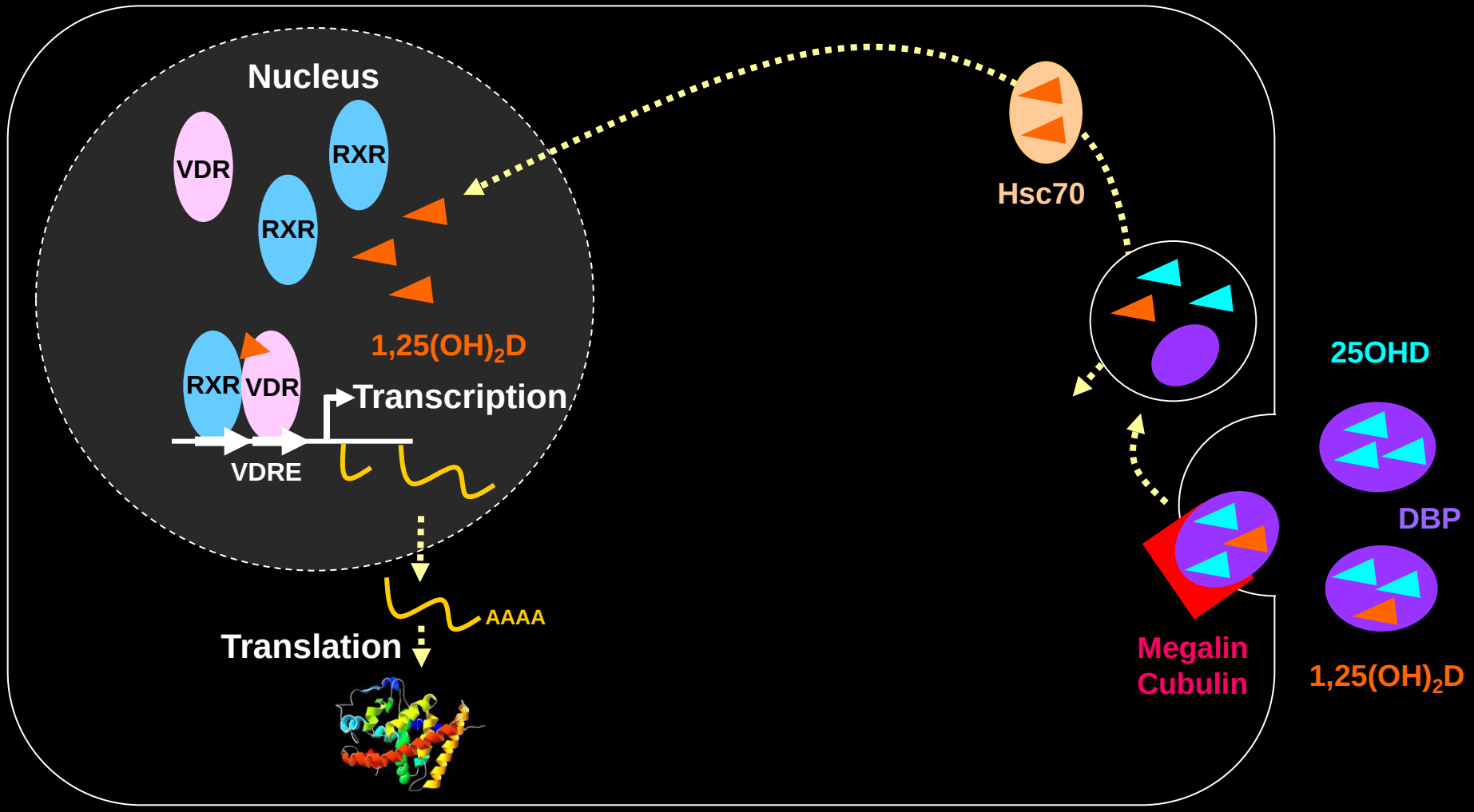
$1,25(OH)_2D$



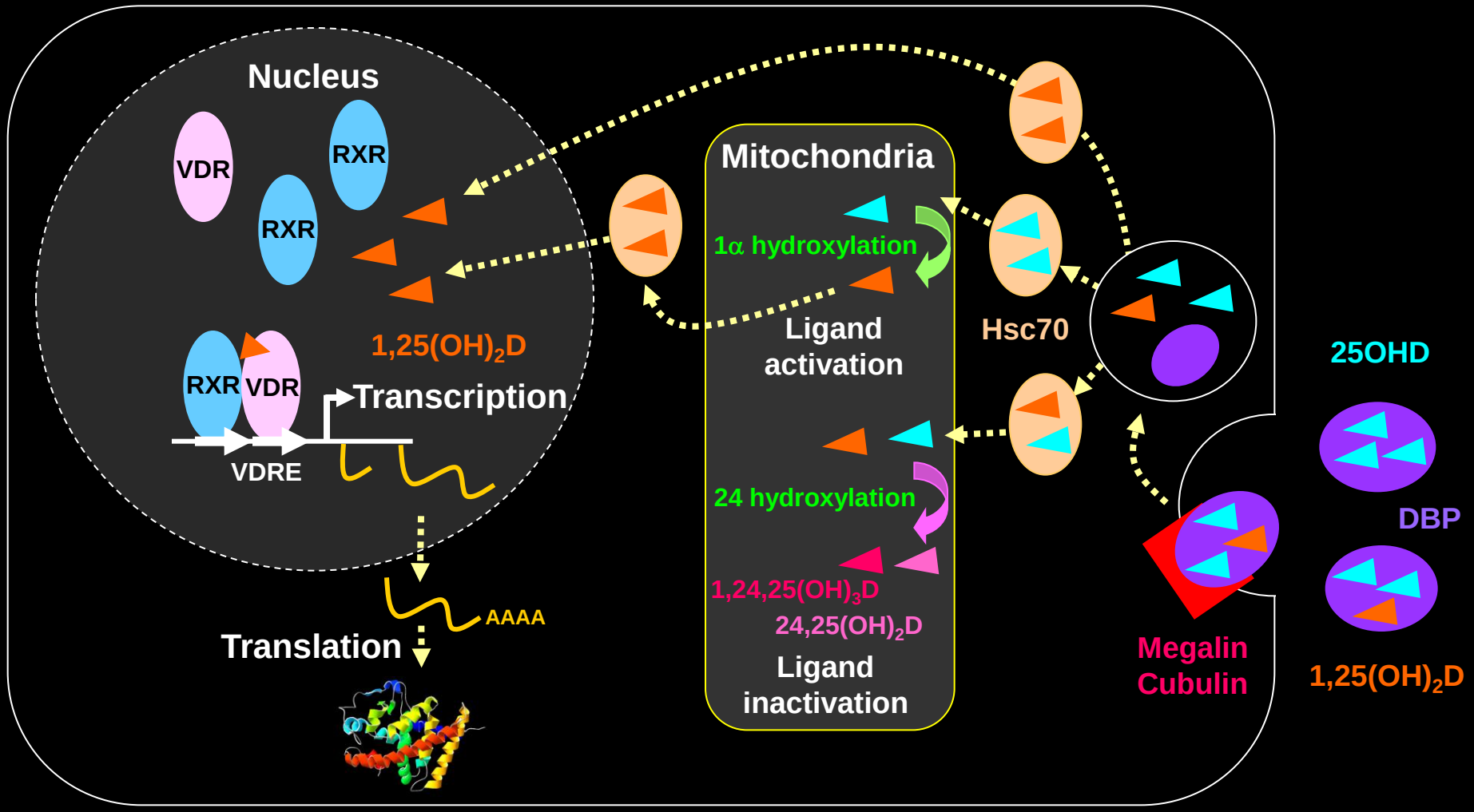
25(OH)D-1α-hydroxylase
CYP27B1


SKIN
Cholesterol
7-Dehydrocholesterol
Pre-vitamin D3
Vitamin D3
Δ7ase
UVB
290-315nM
Thermal
Isomerization
DIET
Less substrate in elderly
R
28
D2
21
22
24
26
20
23
25
27
D3
LIVER
Vitamin D-25-hydroxylase
CYP2R1
1,25(OH)2D
FGF23
PTH
(Mg2+, GH/IGF-1,
Calcitonin)
25(OH)D-24R-hydroxylase
CYP24A1
25(OH)D-1α-hydroxylase
CYP27B1
FGF23,
(Ca2+, PO43-,
1,25(OH)2D)
KIDNEY
1,25(OH)2D3
Inactive hormone
Active hormone

Vitamin D action in target cells



Vitamin D action in target cells



$1,25(OH)_2D$ 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 of calcified structures (lamprey)

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

The VDR is expressed widely
(not only in tissues associated with calcium and phosphate metabolism)

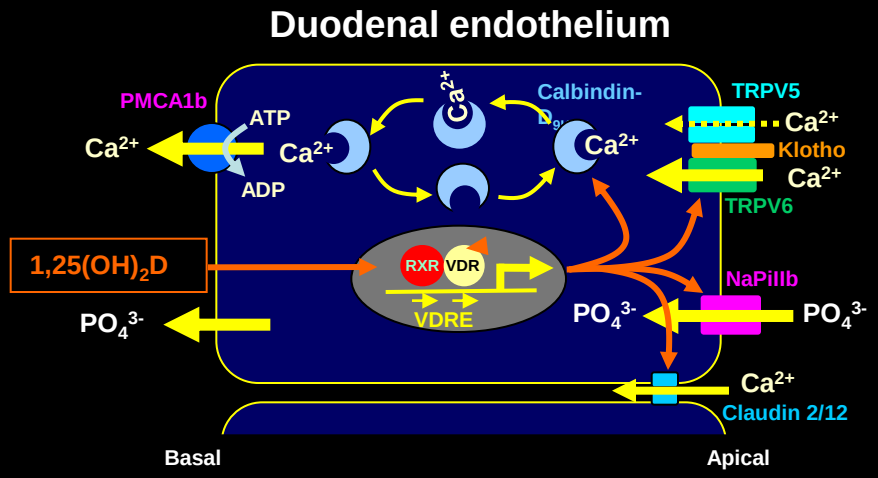
The activating 1α-hydroxylase enzyme is expressed in multiple tissues

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 effects on mineral homeostasis

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

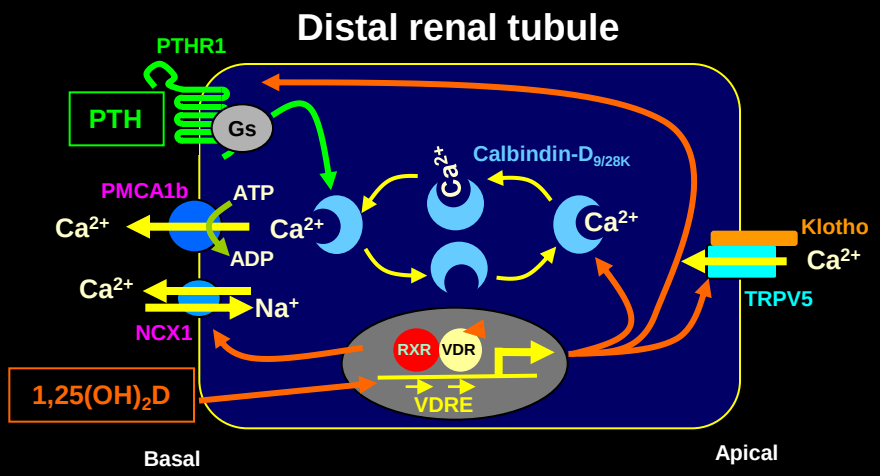


$1,25(OH)_2D$ increases expression of

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

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

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

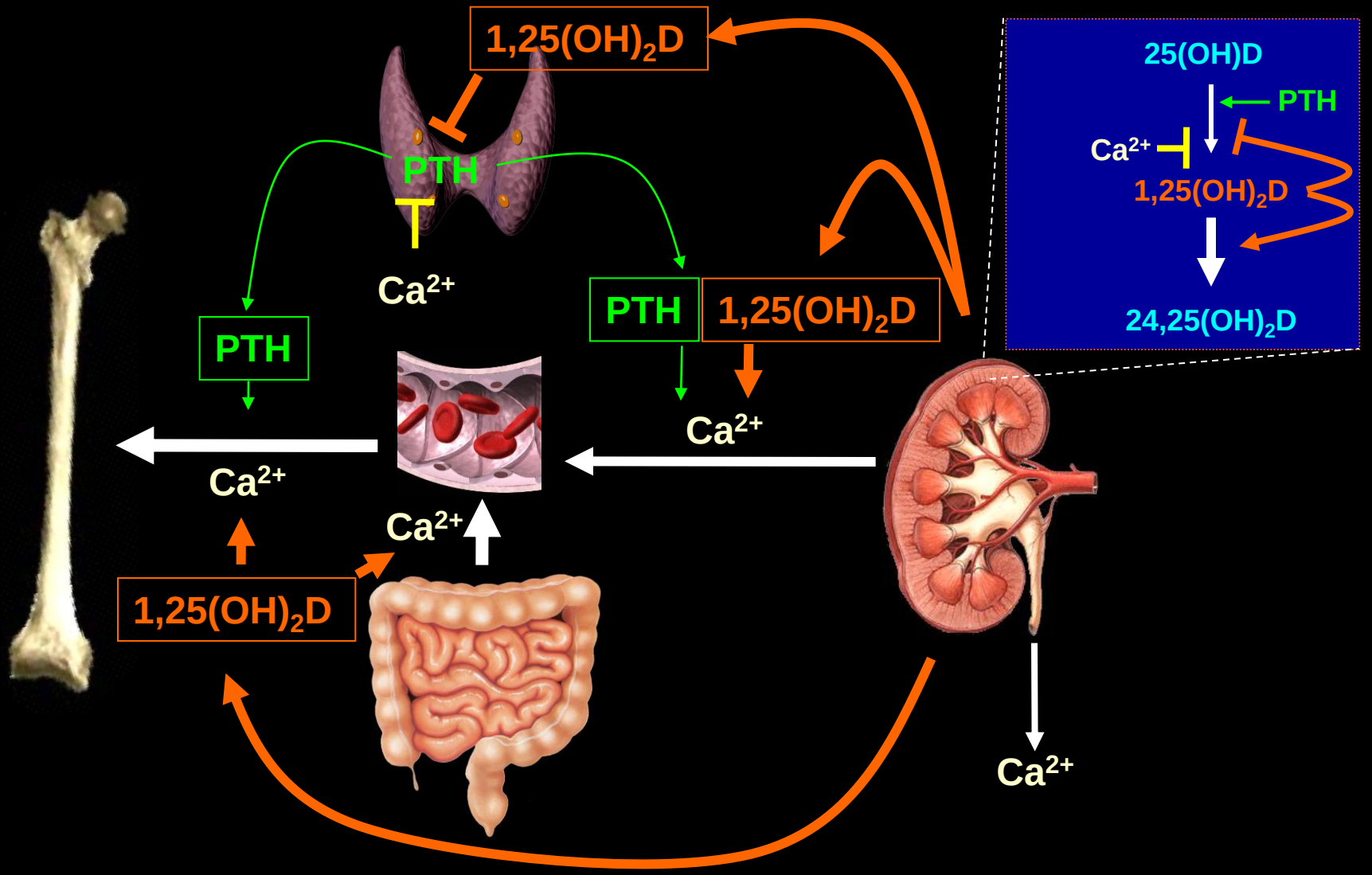


$1,25(OH)_2D$ increases expression of

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

$1,25(OH)_2D$ also increases sensitivity to PTH by increasing PTHR1 expression

Replete vitamin D and calcium



$1,25(OH)_2D$ stimulates Ca^{2+} absorption from gut and reabsorption from kidney

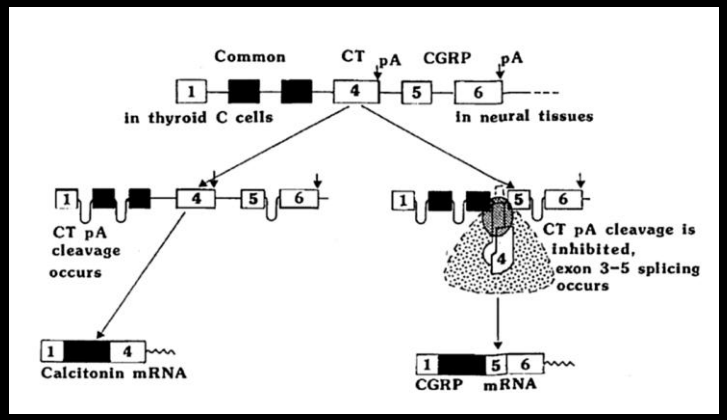
Negative feed back: $1,25(OH)_2D$ inhibits PTH synthesis/release and its own synthesis

Calcitonin

Serum Calcitonin level (CT) 0.8-7.6 pmol/L

Calcitonin

Calcitonin is not physiologically important for mineral homeostasis in humans



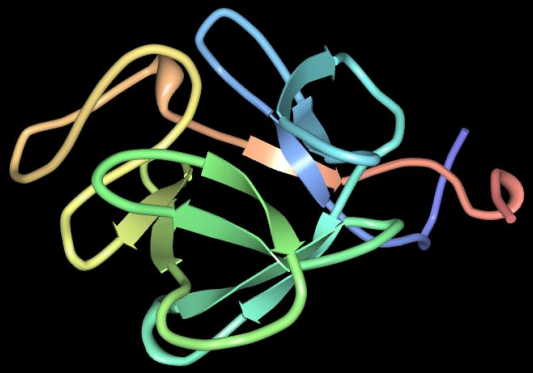
- Thyroid parafollicular cells express calcitonin
  - CALCA gene encodes a 141 amino acid protein
  - Proteolytically cleaved to yield a 32 amino acid peptide
  - Release stimulated by Ca^{2+}, Gastrin and Pentagastrin
- CaSR is expressed by C-cells
  - Calcium stimulates calcitonin synthesis and release
- Calcitonin receptor *(CALCR)*
  - G-protein coupled receptor (osteoclasts, intestine and renal PCT)
- Calcitonin
  - Inhibits Ca^{2+} absorption from intestine
  - Rapidly inhibits osteoclast resorption (rapid fall in calcium)
  - Inhibits renal calcium and phosphate resorption

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

Regulation of Phosphate

Phosphate level (PO_4^{3-}) 0.8-1.4 mmol/l

FGF23

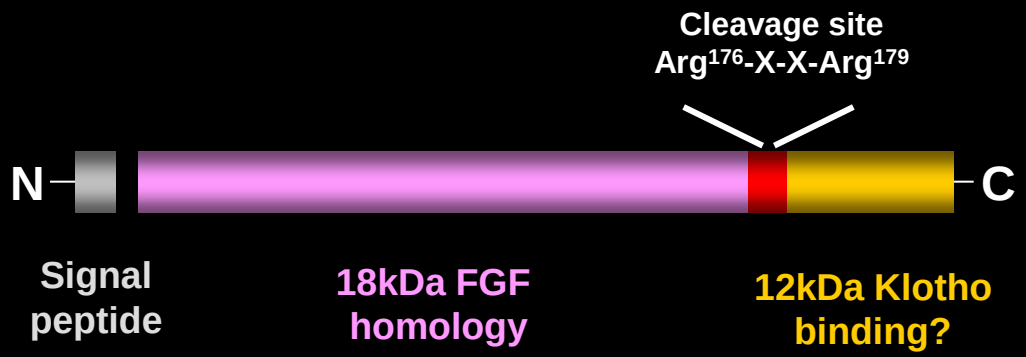


FGFs are secreted proteins that act as paracrine factors

Regulation of cell proliferation, differentiation and function

FGF23 identified in 2000 as the protein mutated in ADHR
(Autosomal dominant hypophosphataemic rickets)

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



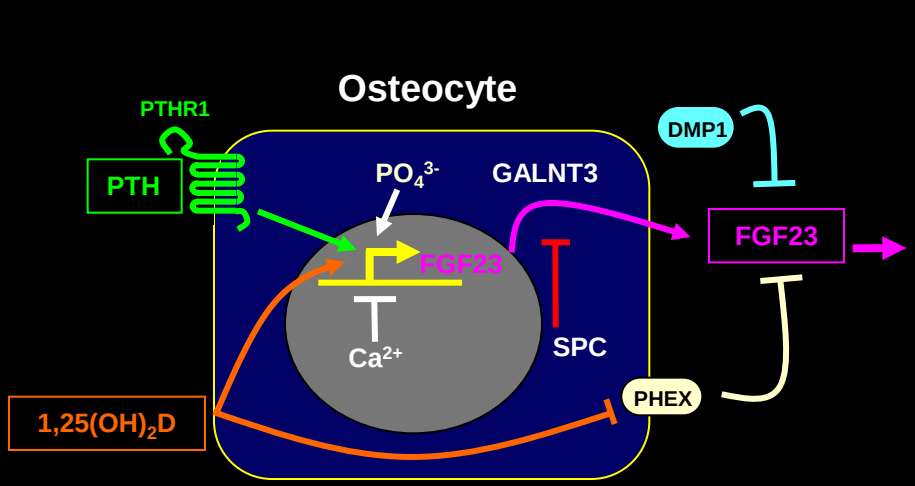
Biologically active form 251αα, 32kDa secreted protein
Inactivated by intracellular cleavage into 18kDa and 12kDa fragments

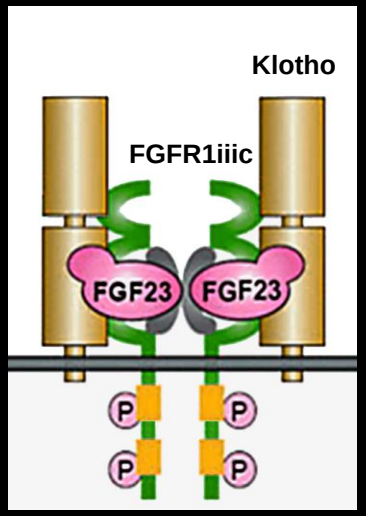
Fibroblast growth factor 23 (FGF23)

FGF23, 1,25$(OH)_2$D and PTH regulate serum phosphate
- Intra and extracellular phosphate concentrations are similar
- 85% of the body's phosphate is in bone
- In the skeleton phosphate is essential for
  - Mineralisation of bone
  - Apoptosis of hypotrophic growth plate chondrocyte

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 synthesis and signaling





Impaired PO_4^{3-} absorption

Increased renal PO_4^{3-} loss

Impaired 1α-hydroxylation

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

PHEX (metalloendopeptidase) negatively regulates FGF23 signalling

GALNT3 mediates O-glycosylation of FGF23 and thus its secretion

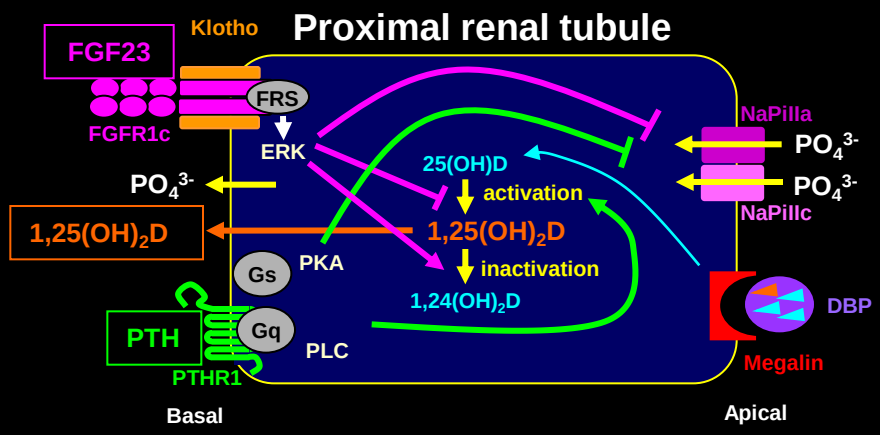
- Prevents inactivation by subtilisin-like proprotein convertase (SPCs)

FGF23 acts via FGFR1iiic receptor

- Requires the co-receptor Klotho (β-glucosidase)

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

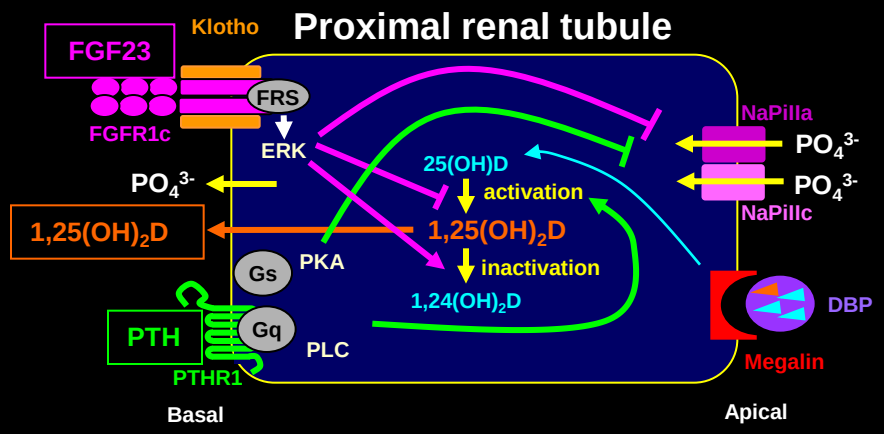
FGF23 regulation phosphate resorption in the kidney

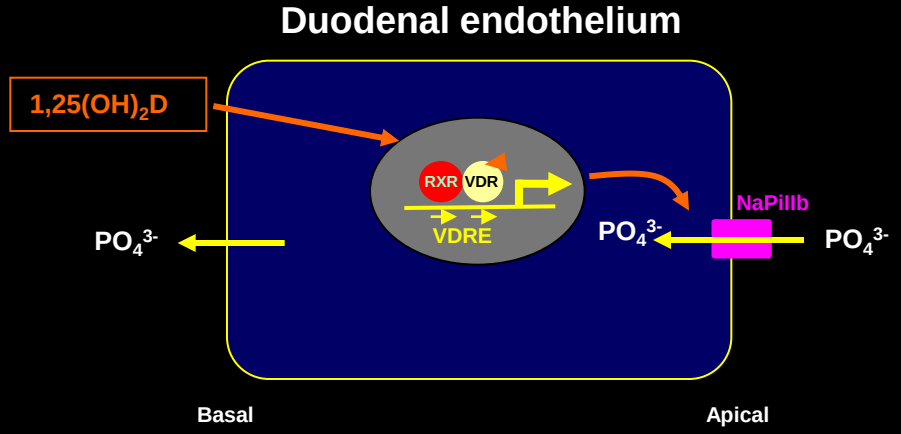


FGF23 ensure Ca^{2+} PO_4^{3-} product does exceed its solubility

- Inhibit phosphate resorption from the kidney
- Inhibit synthesis of $1,25(OH)_2D$ by 1α-hydroxylation
- Increase $1,25(OH)_2D$ inactivation by 24-hydroxylation

FGF23 inhibits phosphate resorption in the kidney and indirectly absorption in the gut





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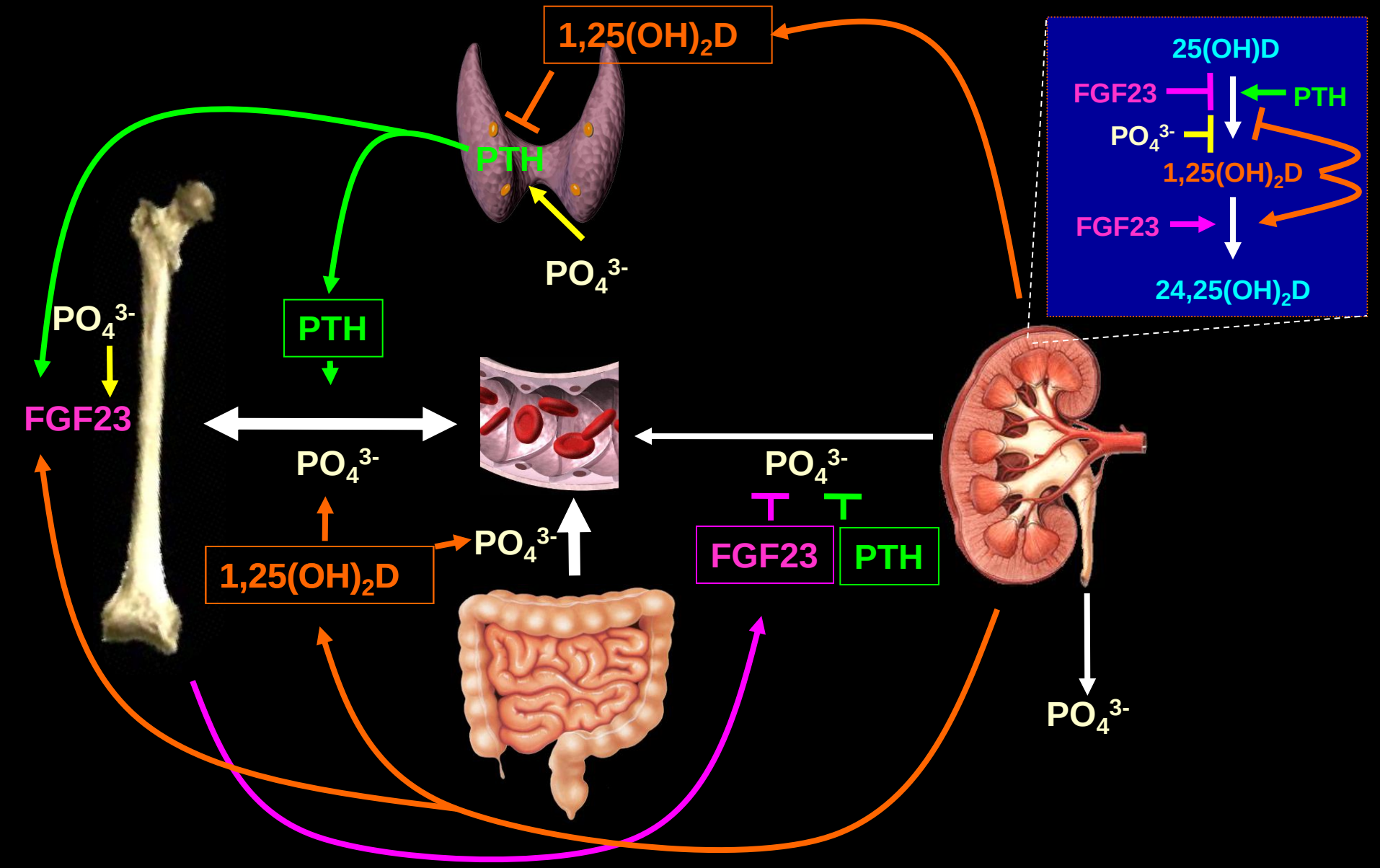
$1,25(OH)_2D$/VDR

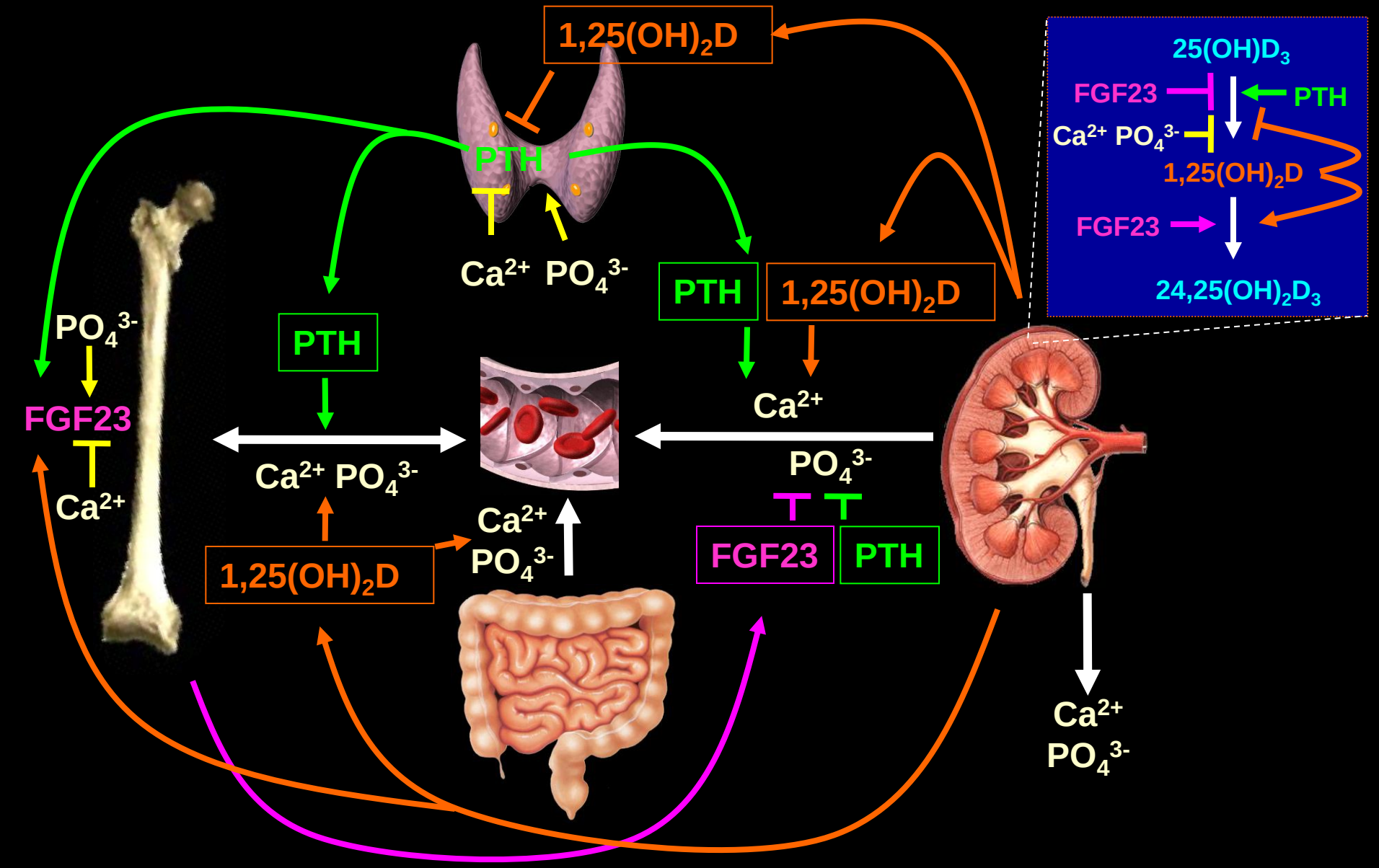
- Stimulates phosphate absorption from the gut
- Serum phosphate and $1,25(OH)_2D$ induce expression FGF23
- FGF23 inhibits $1,25(OH)_2D$ synthesis and thus negative feedback

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



Summary of calcium and phosphate homeostasis

Regulation of calcium and phosphate



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Learning objectives

1. Principal organs involved in calcium and phosphate metabolism
2. Understand the functions of calcium in the body
3. Mechanism of action of PTH
4. Regulation of PTH synthesis and secretion
5. How $1,25(OH)_2D$ is synthesized
6. Mechanism of action of $1,25(OH)_2D$
7. Regulation of vitamin D metabolism
8. Negative feedback loops involved in calcium homeostasis
9. Functions of phosphate in the body
10. Mechanism of action of FGF23
11. Negative feedback loops involved phosphate homeostasis