November 29, 2016

PATHOPHYSIOLOGICAL ASPECTS OF CALCIUM AND PHOSPHATES HOMEOSTASIS. BONE PATHOPHYSIOLOGY

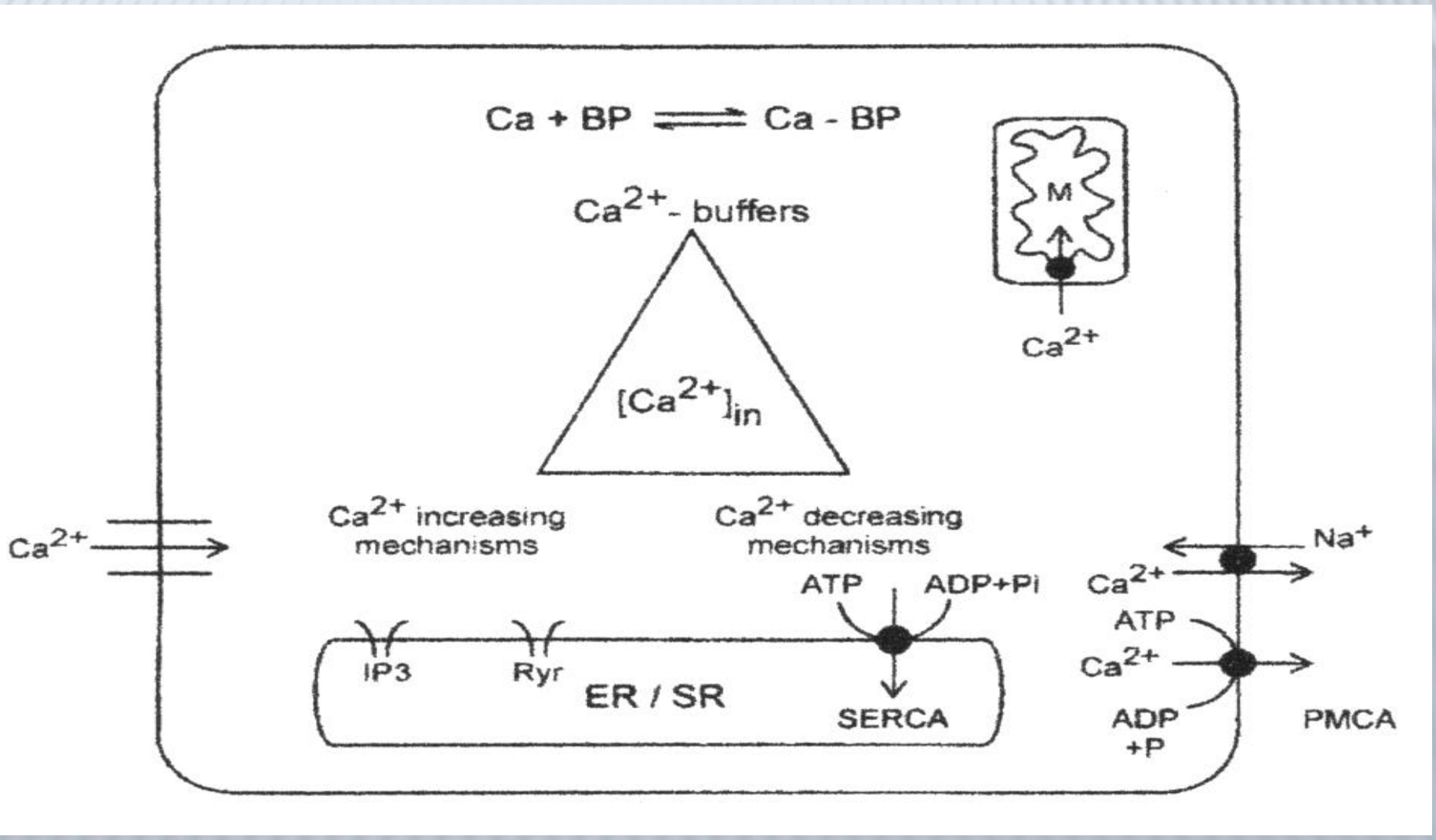
CALCIUM

- In all eukaryotic cells, the cytosolic concentration of calcium ions ([Ca2+]c) is tightly controlled by complex interactions among transporters, pumps, channels and binding proteins. Finely tuned changes in [Ca2+]c modulate a variety of intracellular functions, and disruption of Ca2+ handling leads to cell death.
- Despite the importance of Ca2+ in physiology and pathology, the number of known genetic diseases that can be attributed to defects in proteins directly involved in Ca2+ homeostasis is limited to few examples. This paucity in contrast with the wide molecular repertoire may depend on the extreme severity of the phenotype (leading to death *in utero*) or, conversely, on functional compensation due to redundancy. In the latter case, it stands to reason that other genetic defects in calcium signaling have yet to be identified owing to their subtle phenotype.

CALCIUM HOMEOSTASIS

- Intracellular
- Extracellular

Intracelullar calcium homeostasis



ALTERED CELLULAR CALCIUM HOMEOSTASIS

Bazal $[Ca^{++}]_i$	Response of $[Ca^{++}]_i$ on stimulation	Example	Type
Gradually increasing	=/↓	MI, toxin-induced cell death, acute pancreatitis	Acute
Increased, stabile	↑	Hypertension	Chronic
	↓	Idiopathic heart failure	Chronic
Normal, stabile	↑	Alzheimer´s disease	Chronic
	↓	Chronic inflammatory diseases (M. Crohn, RA)	Chronic

SYSTEMIC CALCIUM HOMEOSTASIS

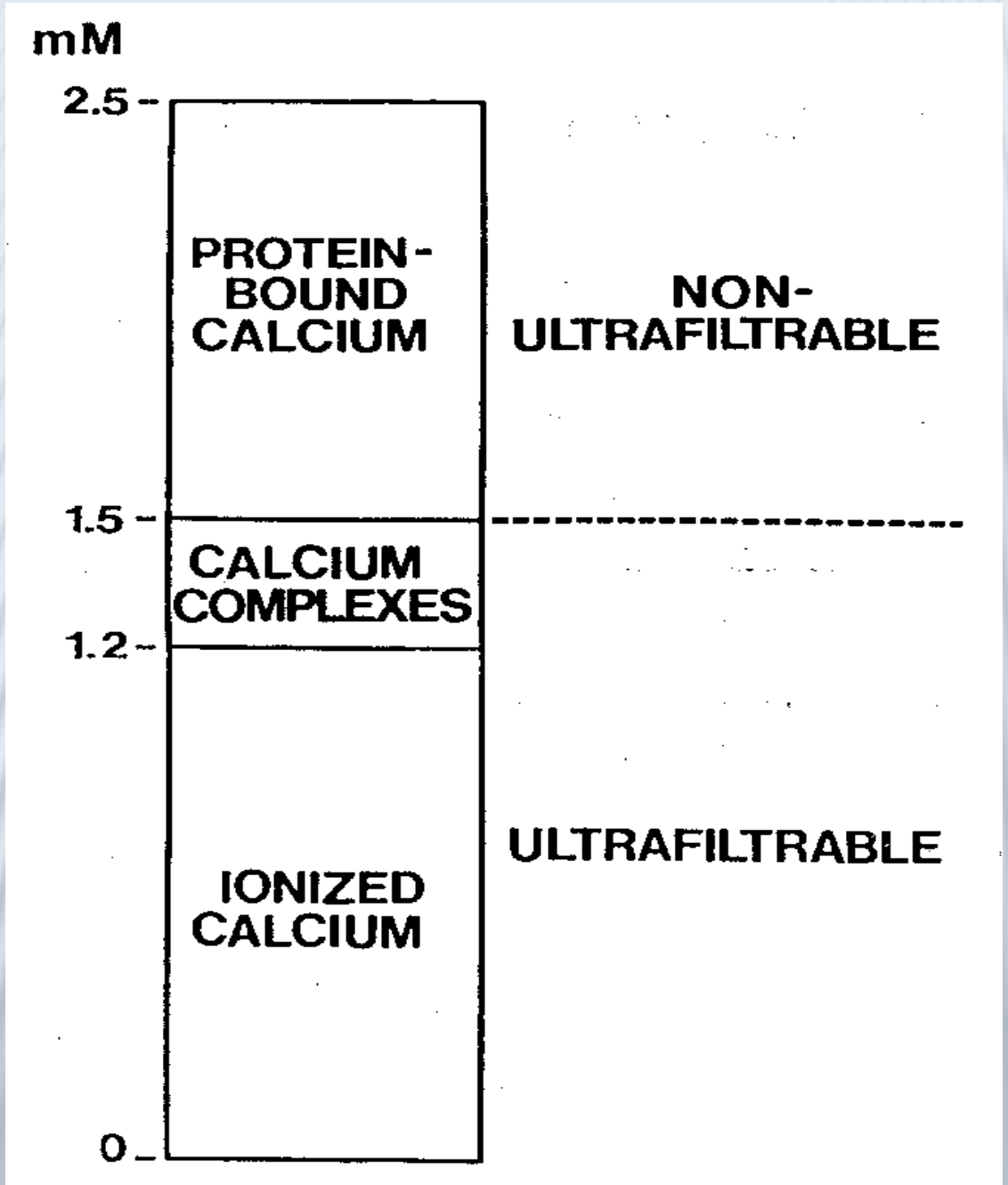
- Systemic calcium homeostasis is critical to the survival of multicellular organisms, and complex, inter-dependent regulatory systems have evolved to maintain Ca^{2+} in the extracellular fluid within a narrow range (1.1–1.4 mM Ca^{2+} for humans).
- The calcium sensing receptor, CaR, is exquisitely sensitive to small changes in Ca^{2+} . In parathyroid chief cells, this permits sensing of minute fluctuations in Ca^{2+} (±200 μM) with increases in Ca^{2+} causing decreases in parathyroid hormone (PTH) secretion.
- PTH has effects on the kidney to increase Ca^{2+} reabsorption from the filtrate and synthesis of vitamin D, 1,25(OH)2D (which enhances intestinal absorption of Ca^{2+}), and on bone to increase release of Ca^{2+} and phosphate by demineralization. Recent reviews detail the mechanisms involved in systemic calcium homeostasis and the pathologies resulting from their dysregulation).
- CaR is also expressed in many cell types which are not directly involved in systemic calcium homeostasis, including neurons and glia, endocrine and exocrine glands, epithelia, cells of hematopoietic origin, and keratinocytes.

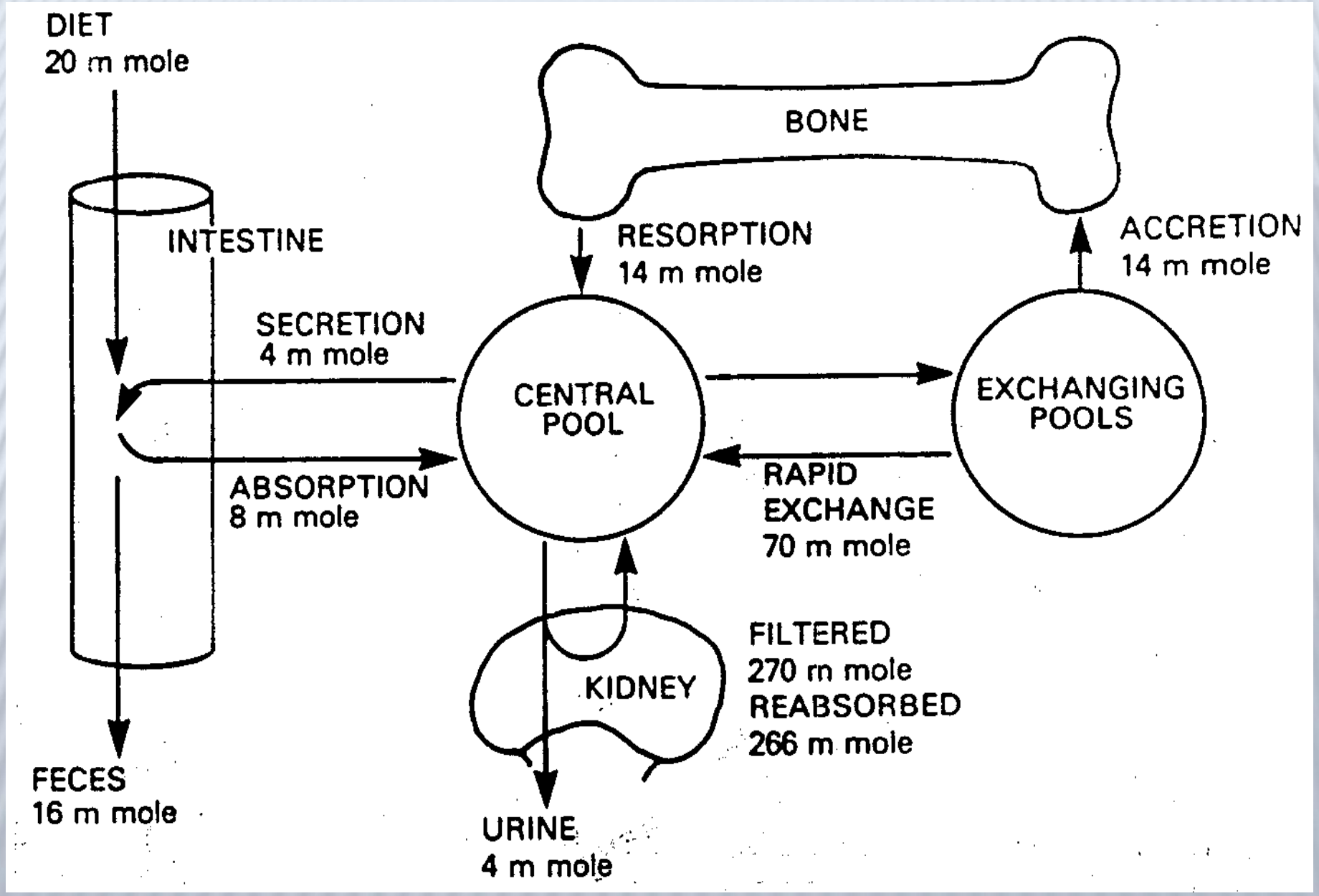
BODY DISTRIBUTION OF CALCIUM AND PHOSPHATE

- There are three major pools of calcium in the body:
- *Intracellular calcium*: A large majority of calcium within cells is sequestered in mitochondria and endoplasmic reticulum. Intracellular free calcium concentrations fluctuate greatly, from roughly 100 nM to greater than 1 μM, due to release from cellular stores or influx from extracellular fluid. These fluctuations are integral to calcium's role in intracellular signaling, enzyme activation and muscle contractions.

BODY DISTRIBUTION OF CALCIUM AND PHOSPHATE

- Calcium in *blood and extracellular fluid:* Roughly half of the calcium in blood is bound to proteins. The concentration of ionized calcium in this compartment is normally almost invariant at approximately 1 mM, or 10,000 times the basal concentration of free calcium within cells. Also, the concentration of phosphorus in blood is essentially identical to that of calcium.
- *Bone calcium*: A vast majority of body calcium is in bone. Within bone, 99% of the calcium is tied up in the mineral phase, but the remaining 1% is in a pool that can rapidly exchange with extracellular calcium. As with calcium, the majority of body phosphate (approximately 85%) is present in the mineral phase of bone. The remainder of body phosphate is present in a variety of inorganic and organic compounds distributed within both intracellular and extracellular compartments.


mM
2.5
1.5
1.2
0
PROTEIN-BOUND CALCIUM
CALCIUM COMPLEXES
IONIZED CALCIUM
NON-ULTRAFILTRABLE
ULTRAFILTRABLE


DIET
20 m mole
BONE
INTESTINE
RESORPTION
14 m mole
ACCRETION
14 m mole
SECRETION
4 m mole
CENTRAL
POOL
EXCHANGING
POOLS
ABSORPTION
8 m mole
RAPID
EXCHANGE
70 m mole
KIDNEY
FILTERED
270 m mole
REABSORBED
266 m mole
FECES
16 m mole
URINE
4 m mole

THE MOLECULAR ACTORS OF CALCIUM SIGNALING

- Calcium ions are the most common second messengers of eukaryotic cells, decoding the information conveyed by a variety of extracellular molecules that do not cross the plasma membrane (hormones, neurotransmitters, growth factors) into widely diverse intracellular effects (for example, muscle contraction, oocyte fertilization, endocrine or exocrine secretion, cell proliferation or death).
- For this purpose, all cells finely tune Ca2+ signals using a ubiquitous, broad group of gene products: **pumps, channels, transporters and binding proteins.**
- The Ca2+ signal then impinges on a number of (directly or indirectly) **Ca2+-sensitive enzymes** that convert changes in Ca2+ concentration into defined cell actions.
- Given the steep electrochemical gradient between the outside world (or the intracellular stores) and the cytoplasm, increases in [Ca2+]c can be elicited by both Ca2+ release from intracellular stores and Ca2+ influx through plasma membrane channels
- The relative importance of Ca2+ influx versus Ca2+ mobilization depends on the stimulus and the cell type.

MOBILIZATION OF CA2+

- Mobilization of Ca2+ from intracellular organelles is also a ubiquitous mechanism for increasing [Ca2+]c. This process is highly specialized in striated (cardiac and skeletal) muscle.
- In other cells, including neurons, the primary route for Ca2+ mobilization is most often that initiated by the stimulation of G protein– or phosphotyrosine-coupled receptors of the plasma membrane, which activate different isoforms of phospholipase C. In turn, this enzyme hydrolyzes the plasma membrane lipid phosphatidylinositol 4,5-bisphosphate into inositol 1,4,5-trisphosphate (IP3) and diacylglycerol. IP3 interacts with Ca2+ channels localized in the ER1,2 and Golgi apparatus4 (the IP3 receptors (IP3Rs) occur in three isoforms), causing them to open and release Ca2+ into the cytosol. In many cells, the IP3R then synergizes with other intracellular channels operated by different second messengers; for example, nicotinamide adenine dinucleotide acts on an unknown target, and cyclic ADP ribose acts on all three isoforms of ryanodine receptors (RyRs), albeit at higher concentrations on type 1. Ca2+ release through RyRs can be directly triggered by Ca2+ itself (as well established incardiac cells), or IP3 can be produced by more unusual routes, such as through cAMP-activated phospholipase Cε5.
- Ca2+ release from intracellular stores is most often accompanied by Ca2+ influx through plasma membrane channels, and the mechanism by which these open is still debated.
- The Ca2+ signal is terminated by the combined activities of the Ca2+ extrusion mechanisms (the plasma membrane Ca2+ ATPase (PMCA) and the Na+/Ca2+ exchanger (NCX)) or the sarcoendoplasmic reticulum Ca2+ ATPase (SERCA) that reaccumulates the cation in the ER lumen.

CA2+ SIGNALING

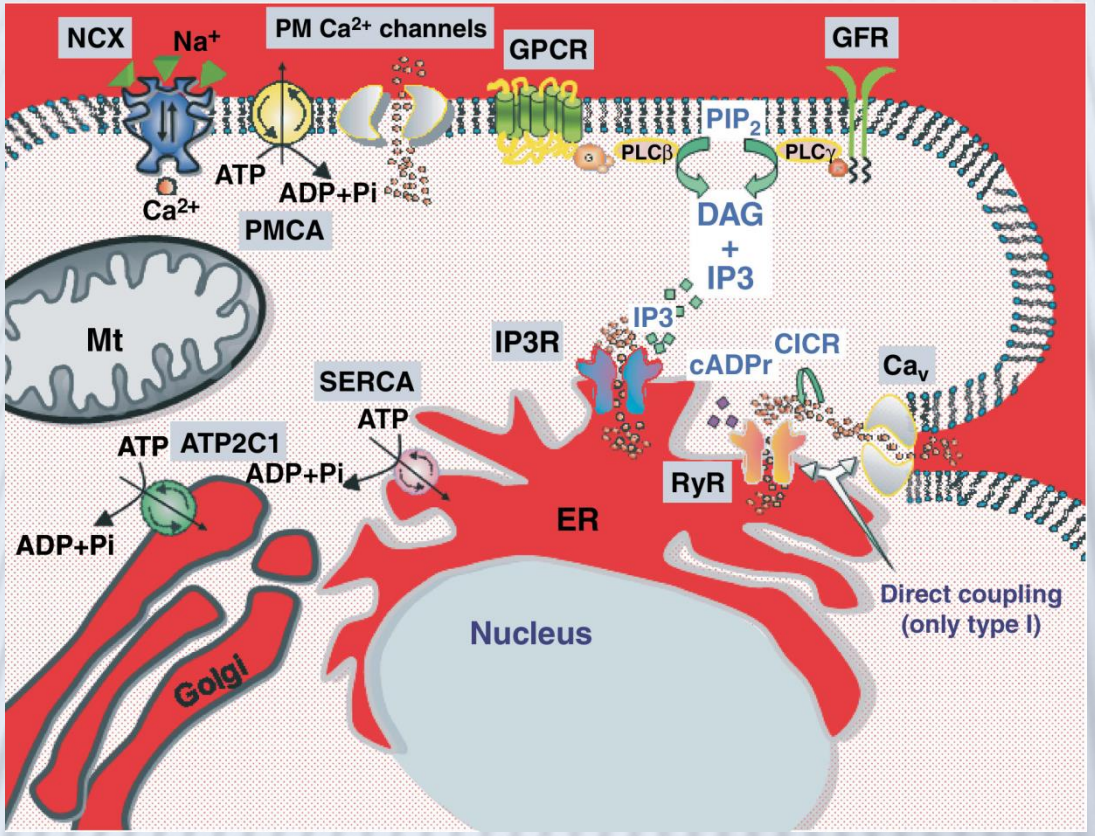
- But Ca2+ signaling is frequently implicated in the pathophysiological process of common multifactorial diseases with a genetic basis (for example, neurodegenerative diseases, including Alzheimer dementia and Huntington disease, ischemia, demyelinating and autoimmune disorders, cardiac hypertrophy and others).
- Of particular interest, presenilin-1 and -2, mutated in early-onset familial Alzheimer disease, can modulate in neurons and heterologous expression systems the kinetics of ER Ca2+ release through the IP3R and the ensuing process of capacitative Ca2+ entry. The molecular basis of this alteration is unclear.
- Moreover, Ca2+ signals are involved in the modulation of the apoptotic process, and Ca2+ dysregulation is also associated with death by necrosis. Ca2+ signals regulate both the metabolic activity of mitochondria and their release of pro-apoptotic factors.

THE CA2+-PERMEABLE CHANNELS

- The Ca2+-permeable channels of the plasma membrane are traditionally grouped into three different families:
- (1) voltage-operated Ca2+ channels (Cav), whose opening probability is increased by decreases in membrane potential (the traditional nomenclature of the currents, L, N, T, P/Q and R subtypes) is based on their functional properties, pharmacological sensitivity or cellular expression and reflects the different nature of the pore-forming subunit.

THE CA2+-PERMEABLE CHANNELS

- (2) receptor-operated Ca2+ channels, also referred to as ligand-gated channels, whose opening probability is increased by binding of a ligand to the channels themselves;
- (3) second messenger–operated channels, whose opening probability is controlled by the binding of a second messenger on the inner surface of the membrane.
- The TRP **(transient receptor potential**) family includes the channels responsible for the Ca2+ entry triggered by the *agonist-dependent release of Ca2+ from the endoplasmic reticulum (ER)*. The various members of the TRP family have distinct gating mechanisms (second messengers, extracellular ligands, osmotic or mechanical stress, depletion of intracellular stores and, in a few cases, unknown). These ion channels, like many others, are homo- or heteromultimer protein complexes in which one subunit is principally responsible for forming the channel pore and the other subunits have a modulatory role. They are polymodal sensory ion channels as they integrate multiple physical and chemical stimuli including heat, pH, and lipids.



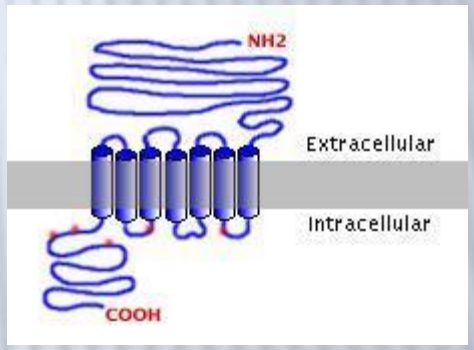
Scheme of an idealized mammalian cell with the localization of the main players of Ca2+ homeostasis. PM Ca2+ channels, generic plasma membrane Ca2+ channels (voltage-, ligand- or second messenger–operated); GPCR, G protein–coupled receptor; PLC, phospholipase C; PIP2, phosphatidylinositol 4,5 bisphosphate; DAG, diacylglycerol; GFR, growth factor receptor; ATP2C1, Golgi-resident Ca2+ ATPase; cADPR, cyclic ADP ribose; CICR, Ca2+ induced Ca2+ release; Mt, mitochondrion.

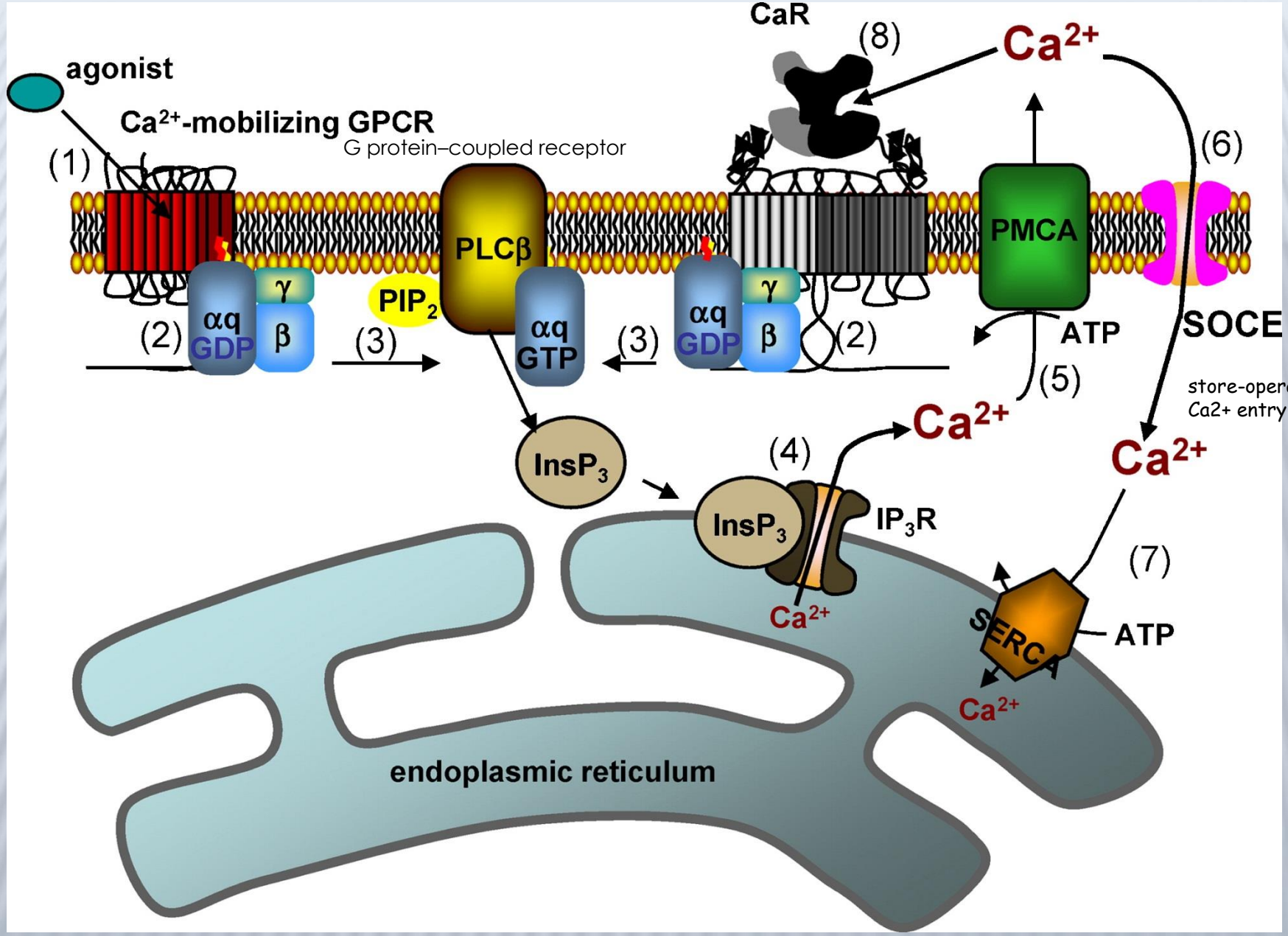
CALCIUM SENSOR

- The calcium sensor is expressed in a broad range of cells, including **parathyroid cells** and **C cells** in the **thyroid gland**, indicating its involvement in controlling the synthesis and secretion of parathyroid hormone and calcitonin. The calcium sensor directly affects secretion of these two hormones.
- The calcium sensor is also expressed in several cell types **in the kidney, osteoblasts**, a variety of **hematopoietic cells** in bone marrow, and in the **gastrointestinal mucosa.**
- Such a broad distribution of expression supports that concept that calcium, acting as a hormone, has direct effects on the function of many cell types.

THE EXTRACELLULAR CALCIUM-SENSING RECEPTOR (CaR)

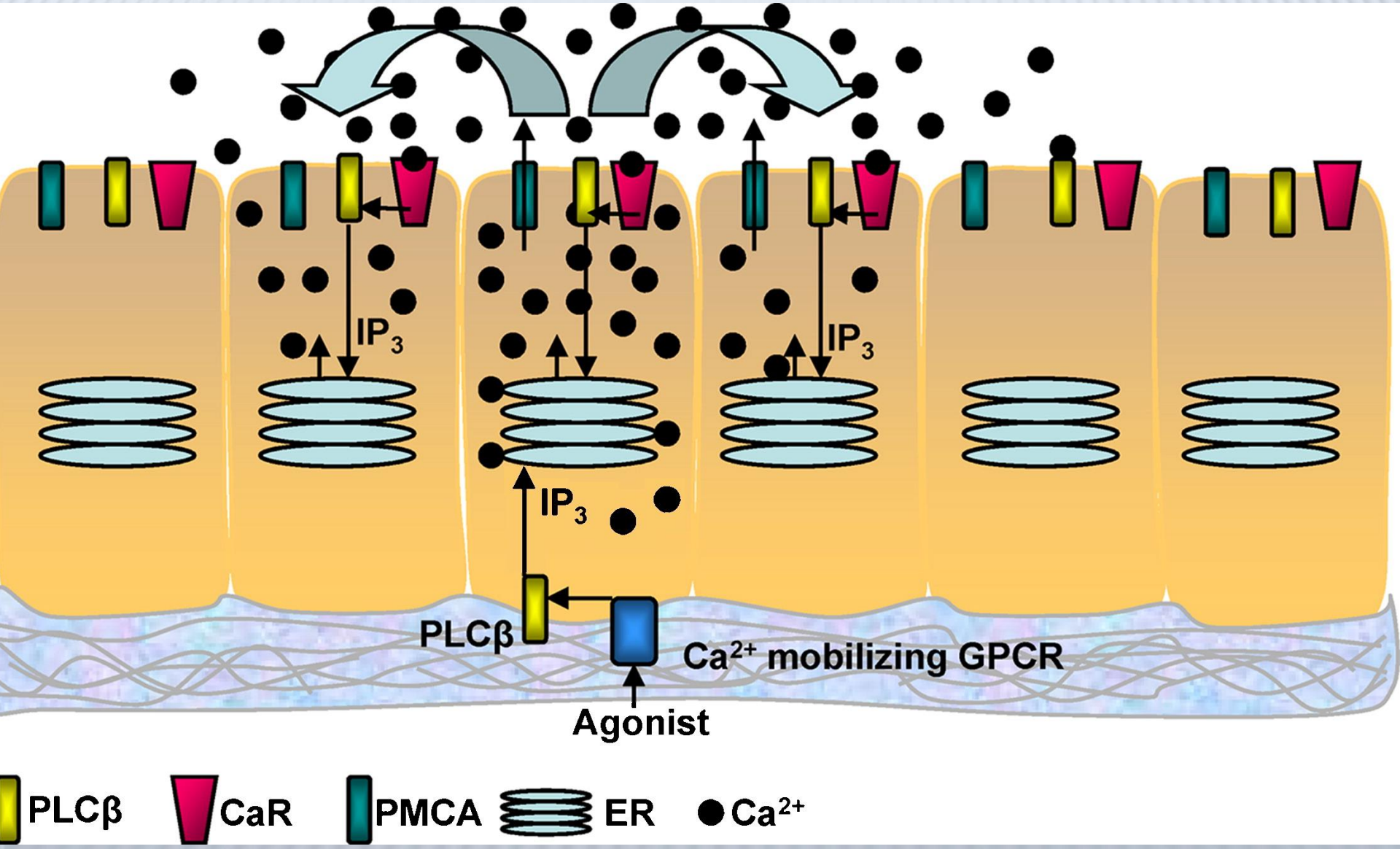
- The calcium-sensing receptor is a member of the G protein-coupled receptor family. Like other family members, it contains seven hydrophobic helices that anchor it in the plasma membrane.
- The large (~600 amino acids) extracellular domain is known to be critical to interactions with extracellular calcium. The receptor also has a rather large (~200 amino acids) cytosolic tail. These features are depicted in the figure to the right; the red highlights on the intracellular domain correspond to potential protein kinase phosphorylation sites.




agonist
Ca2+-mobilizing GPCR
G protein–coupled receptor
(1)
(2)
αq GDP
γ
β
(3)
PIP2
PLCβ
αq GTP
(3)
αq GDP
γ
β
(2)
CaR
(8)
Ca2+
PMCA
(6)
SOCE
ATP
(5)
store-operated Ca2+ entry channels
Ca2+
InsP3
(4)
InsP3
IP3R
Ca2+
Ca2+
(7)
SERCA
ATP
Ca2+
endoplasmic reticulum

TO THE PREVIOUS PICTURE

- Molecular players in CaR-mediated autocrine/paracrine integration of Ca2+-mediated signaling. Agonist activation of a Ca2+-mobilizing GPCR (1) activates heterotrimeric G protein Gq (2), leading to activation of phospholipase Cβ (PLCβ) (3), and generation of inositol 1,4,5-trisphosphate (InsP3), which binds to the endoplasmic reticulum-localized inositol trisphosphate receptor (IP3R) (4), inducing release of Ca2+ into the cytosol. Most of the Ca2+ released from the endoplasmic reticulum is pumped out of the cell by the plasma membrane-localized Ca2+ ATPase (PMCA) (5). Restitution of endoplasmic reticulum Ca2+ content occurs via store-operated Ca2+ entry channels (SOCE) (6) and the sarcoendoplasmic reticulum Ca2+ ATPase (SERCA) (7). If the activated cell also expresses the calcium sensing receptor (CaR), potentiation of the response is possible, as PMCA pumps Ca2+ out of the cell into a restricted diffusion space, significantly increasing the [Ca2+] extracellulary, leading to CaR activation **(8)**.


IP3
IP3
IP3
PLCβ
Ca2+ mobilizing GPCR
Agonist
PLCβ
CaR
PMCA
ER
Ca2+

TO THE PREVIOUS PICTURE

- Potential mechanism for CaR-mediated integration of Ca2+ signaling. When a single cell (or a few cells) in a multicellular network is (are) activated by an agonist for a Ca2+-mobilizing GPCR, Cai2+ increases, and is pumped out of the cell via PMCA.
- The local increase in [Ca2+] in the restricted diffusion space surrounding the cells potentiates the activity in the agonist-activated cell (**autocrine activation**), and also activates CaR on adjacent cells (**paracrine activation**).
- CaR activation increases intracellular Ca2+, leading to PMCA-mediated Ca2+ efflux in adjacent cells, propagating the Ca2+ signaling response through the tissue. In this example, CaR is present in proximity to PMCA, which has been observed in epithelial tissue.

FLUXES OF CALCIUM AND PHOSPHATE

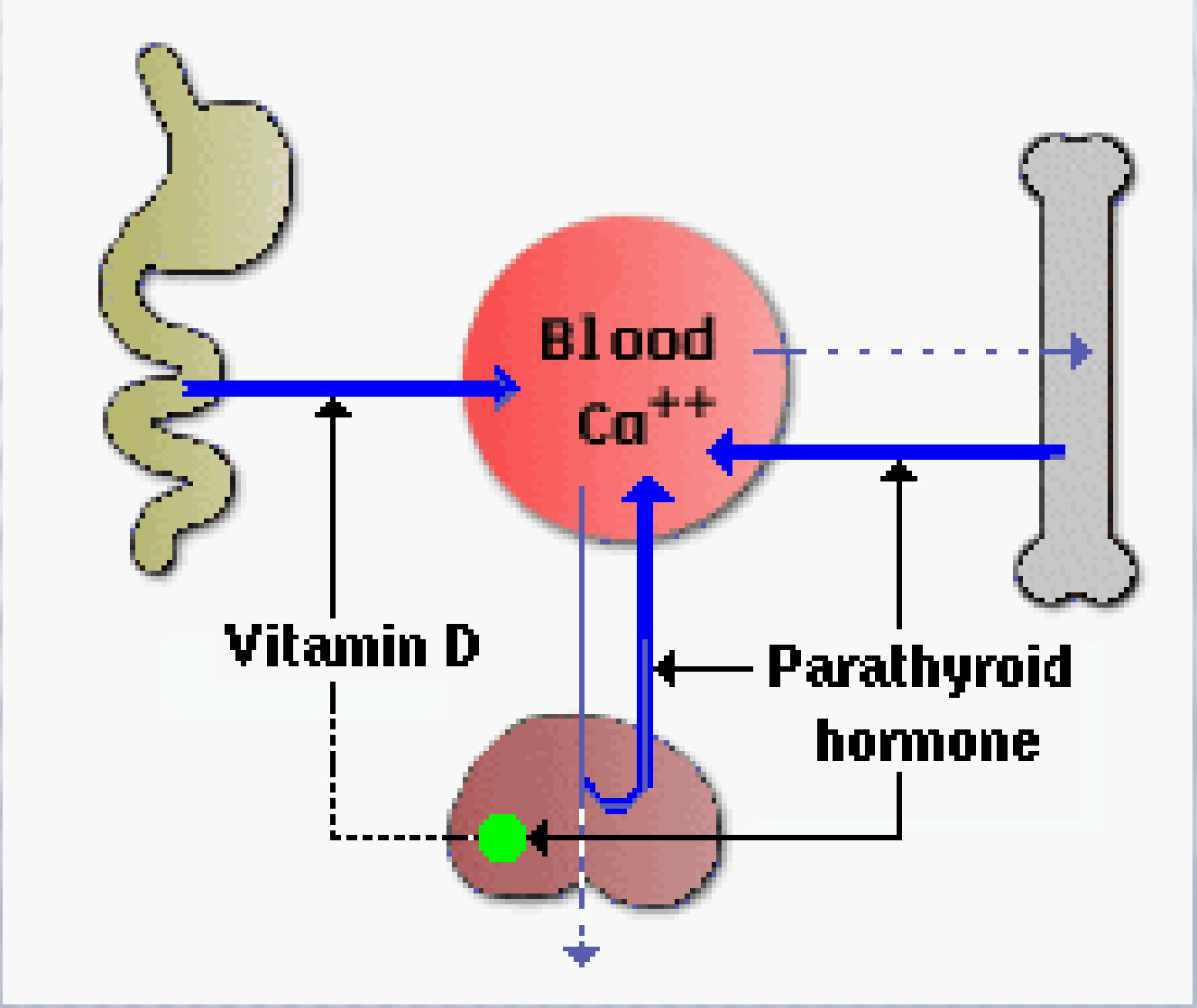
Two main tasks for regulation:

- Ca x HPO_4^- product in blood = constant (!!!)
- Normal levels of calcium and phosphates at the same time are necessary

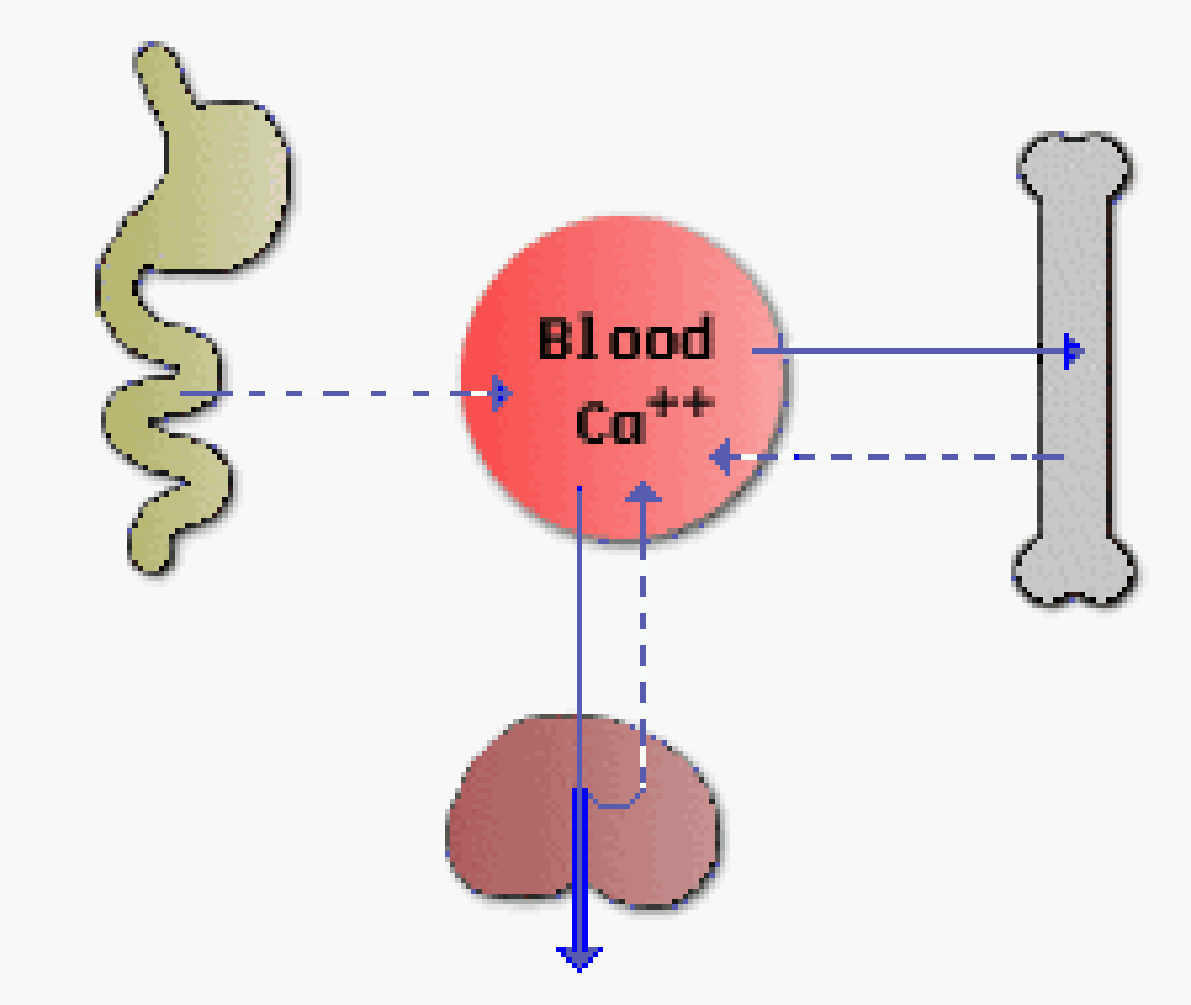
FLUXES OF CALCIUM AND PHOSPHATE

- Maintaining constant concentrations of calcium in blood requires frequent adjustments, which can be described as fluxes of calcium between blood and other body compartments.
- **Three organs participate in supplying calcium to blood and removing it from blood when necessary:**
- The **small intestine** is the site where dietary calcium is absorbed. Importantly, efficient absorption of calcium in the small intestine is dependent on expression of a calcium-binding protein in epithelial cells.
- **Bone** serves as a vast reservoir of calcium. Stimulating net resorption of bone mineral releases calcium and phosphate into blood, and suppressing this effect allows calcium to be deposited in bone.
- The **kidney** is critically important in calcium homeostasis. Under normal blood calcium concentrations, almost all of the calcium that enters glomerular filtrate is reabsorbed from the tubular system back into blood, which preserves blood calcium levels. If tubular reabsorption of calcium decreases, calcium is lost by excretion into urine.

CALCIUM DEFICIENCY IN BLOOD



CALCIUM OVERLOAD IN BLOOD



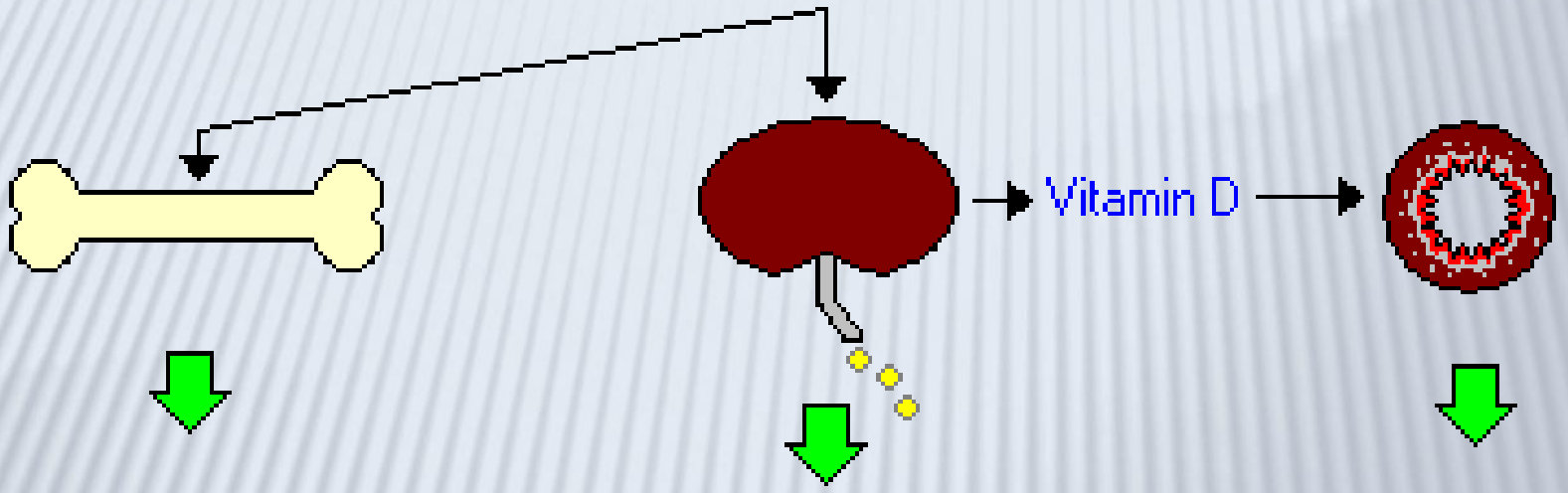
	Calcium Deprivation	Calcium Loading
Parathyroid hormone	Secretion stimulated	Secretion inhibited
Vitamin D	Production stimulated by increased parathyroid hormone secretion	Synthesis suppressed due to low parathyroid hormone secretion
Calcitonin	Very low level secretion	Secretion stimulated by high blood calcium
Intestinal absorption of calcium	Enhanced due to activity of vitamin D on intestinal epithelial cells	Low basal uptake
Release of calcium and phosphate from bone	Stimulated by increased parathyroid hormone and vitamin D	Decreased due to low parathyroid hormone and vitamin D
Renal excretion of calcium	Decreased due to enhanced tubular reabsorption stimulated by elevated parathyroid hormone and vitamin D; hypocalcemia also activates calcium sensors in loop of Henle to directly facilitate calcium reabsorption	Elevated due to decreased parathyroid hormone-stimulated reabsorption.
Renal excretion of phosphate	Strongly stimulated by parathyroid hormone; this phosphaturic activity prevents adverse effects of elevated phosphate from bone resorption	Decreased due to hypoparathyroidism
General Response	Typically see near normal serum concentrations of calcium and phosphate due to compensatory mechanisms. Long term	Low intestinal absorption and enhanced renal excretion guard against development of hypercalcemia.

HORMONAL CALCIUM CONTROL SYSTEMS

- Maintaining normal blood calcium and phosphorus concentrations is managed through the concerted action of three hormones that control fluxes of calcium in and out of blood and extracellular fluid:
- *Parathyroid hormone serves to increase blood concentrations of calcium.*
- Mechanistically, parathyroid hormone preserves blood calcium by several major effects:
  - Stimulates production of the biologically-active form of vitamin D within the kidney.
  - Facilitates mobilization of calcium and phosphate from bone. To prevent detrimental increases in phosphate, parathyroid hormone also has a potent effect on the kidney to eliminate phosphate (phosphaturic effect).
  - Maximizes tubular reabsorption of calcium within the kidney. This activity results in minimal losses of calcium in urine.

Low concentration of calcium in blood

Release of parathyroid hormone



Efflux of calcium from bone

Decreased loss of calcium in urine

Enhanced absorption of calcium from intestine

Increased concentration of calcium in blood

CONTROL OF PARATHYROID HORMONE SECRETION

Parathyroid hormone is released in response to low extracellular concentrations of free calcium.
Changes in blood phosphate concentration can be associated with changes in parathyroid hormone secretion, but this appears to be an indirect effect and phosphate per se is not a significant regulator of this hormone.
When calcium concentrations fall below the normal range, there is a steep increase in secretion of parathyroid hormone. Low levels of the hormone are secreted even when blood calcium levels are high. The figure to the right depicts parathyroid hormone release from cells cultured in vitro in differing concentrations of calcium.
The parathyroid cell monitors extracellular free calcium concentration via an integral membrane protein that functions as a calcium-sensing receptor.

METABOLIC BONE DISEASES

- Osteoporosis
- Osteodystrophy
- Osteomalacia (rickets in childhood)

OSTEOPOROSIS

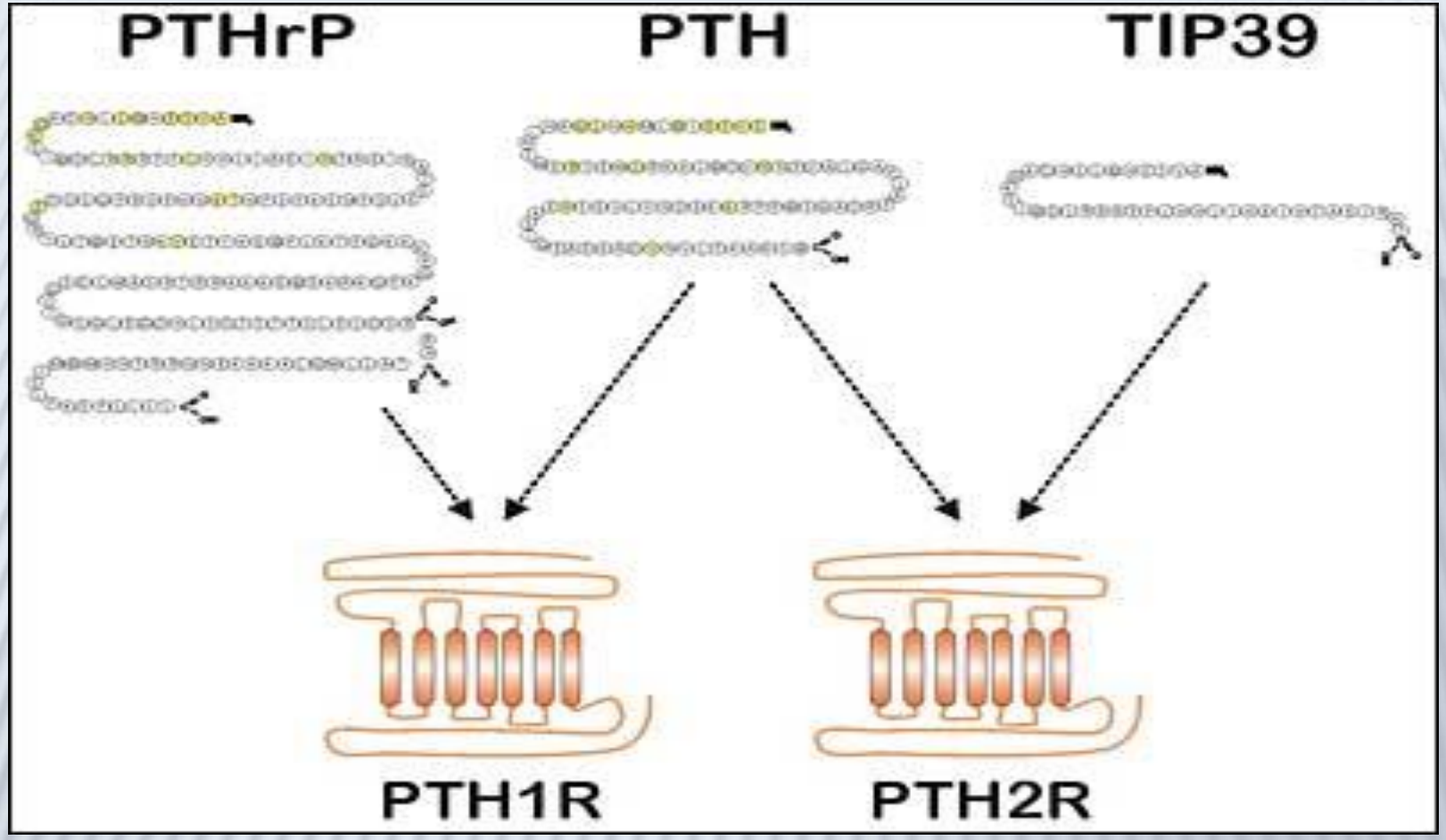
- Insufficiency of estrogenes
- Age (both men and women)
- Immobilisation
- Increased levels of glucocorticoids
- Decreased levels of vitamin K2

DISEASE STATES DUE TO HYPERATHYROIDISM- OSTEODYSTROPHIES

- (Both increased and decreased secretion of parathyroid hormone are recognized as causes of serious disease in man).
- **Excessive secretion of parathyroid hormone is seen in two forms:**
- *Primary hyperparathyroidism* is the result of parathyroid gland disease, most commonly due to a parathyroid tumor (adenoma) which secretes the hormone without proper regulation. Common manifestations of this disorder are chronic elevations of blood calcium concentration (hypercalcemia), kidney stones and remodelation of bone.
- *Secondary hyperparathyroidism* is the situation where disease outside of the parathyroid gland leads to excessive secretion of parathyroid hormone. A common cause of this disorder is kidney disease - if the kidneys are unable to reabsorb calcium, blood calcium levels will fall, stimulating continual secretion of parathyroid hormone to maintain normal calcium levels in blood. Secondary hyperparathyroidism can also result from inadequate nutrition - for example, diets that are deficient in calcium or vitamin D, or which contain excessive phosphorus (e.g. all meat diets for carnivores). A prominent effect of secondary hyperparathyroidism is remodelation of bone, leading to pathologic fractures or "rubber bones".

DISEASE STATES DUE TO HYPOPARATHYROIDISM

- Inadequate production of parathyroid hormone - *hypoparathyroidism* - typically results in decreased concentrations of calcium and increased concentrations of phosphorus in blood.
- Common causes of this disorder include surgical removal of the parathyroid glands and disease processes that lead to destruction of parathyroid glands. The resulting **hypocalcemia** often leads to **tetany and convulsions**, and can be acutely life-threatening. Treatment focuses on restoring normal blood calcium concentrations by calcium infusions, oral calcium supplements and vitamin D therapy.

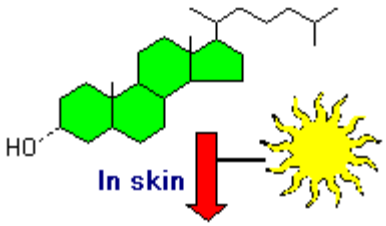


Gene families PTH a PTHrP: PTHrP, PTH and TIP39 may be members of one gene family. Their receptors PTH1R and PTH2R are 7 transmembrane G protein-coupled receptors.

VITAMIN D (CALCITRIOL)

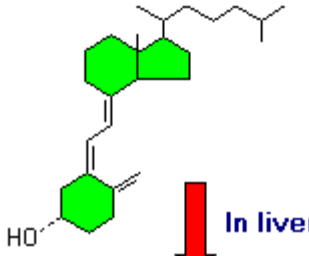
- Bioactive vitamin D or calcitriol is a steroid hormone that has long been known for its important role in regulating body levels of calcium and phosphorus, and in mineralization of bone.
- This hormone has biologic effects which extend far beyond control of mineral metabolism.

7-dehydrocholesterol



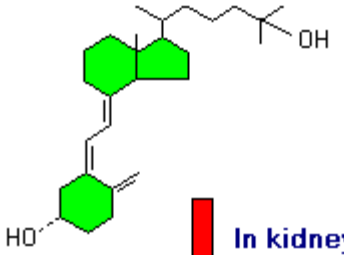
In skin

cholecalciferol (vitamin D_3)



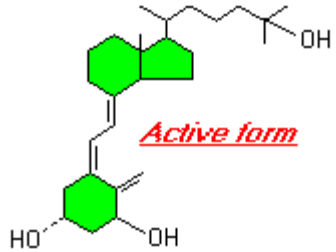
In liver

25-hydroxycholecalciferol
(25-hydroxy vitamin D_3)



In kidney

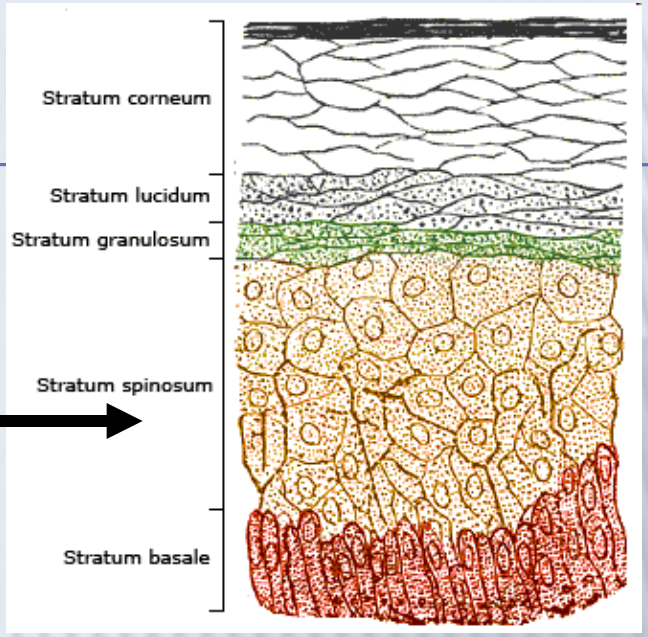
1,25-dihydroxycholecalciferol
(1,25-dihydroxy vitamin D_3)

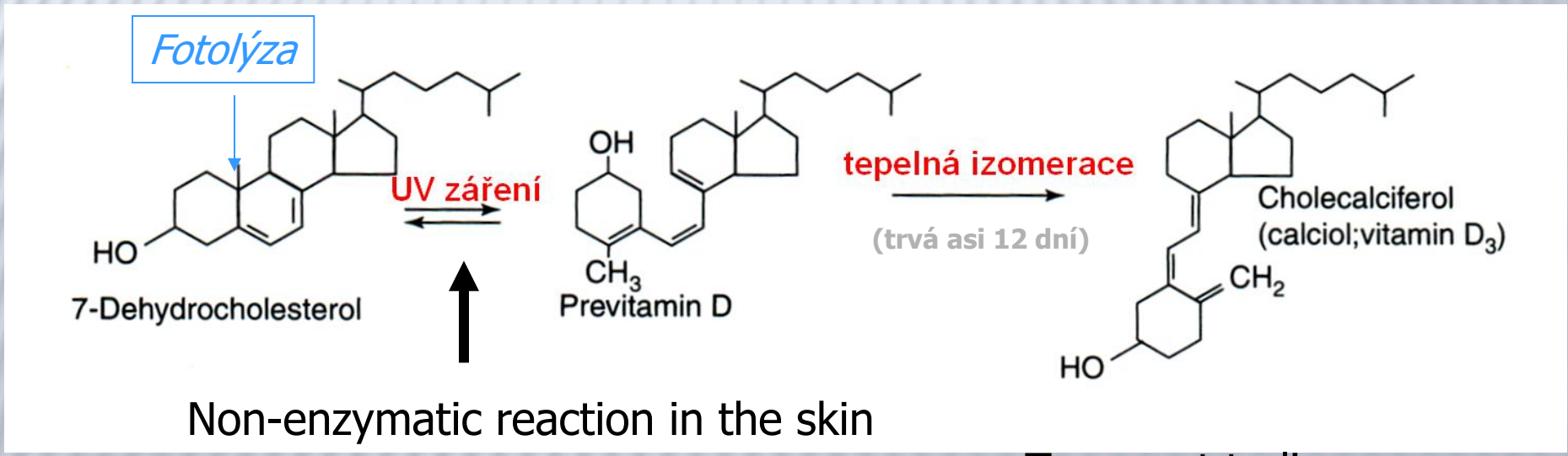


Active form

Vitamin D- synthesis

UV radiation 270 – 300 nm



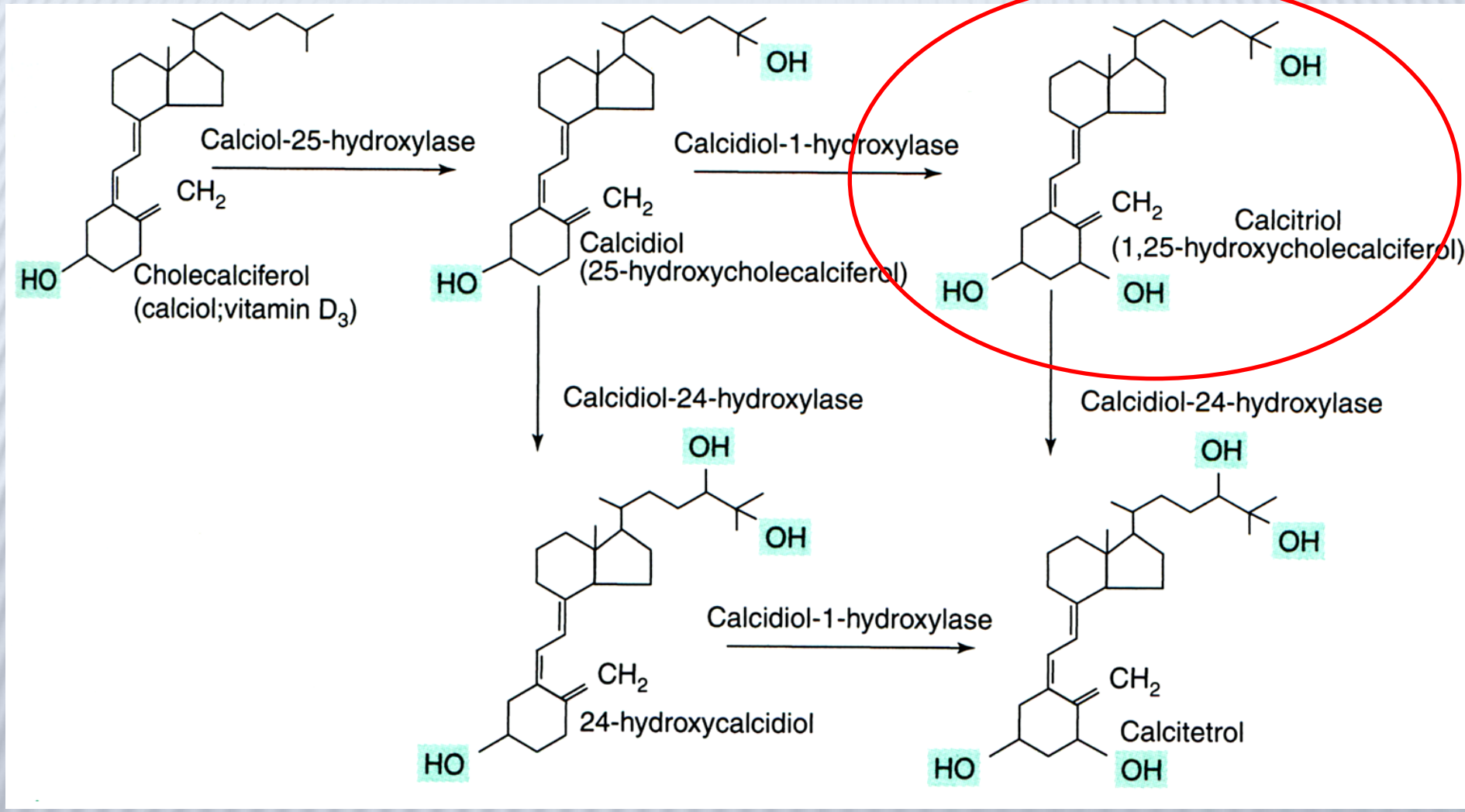


Non-enzymatic reaction in the skin

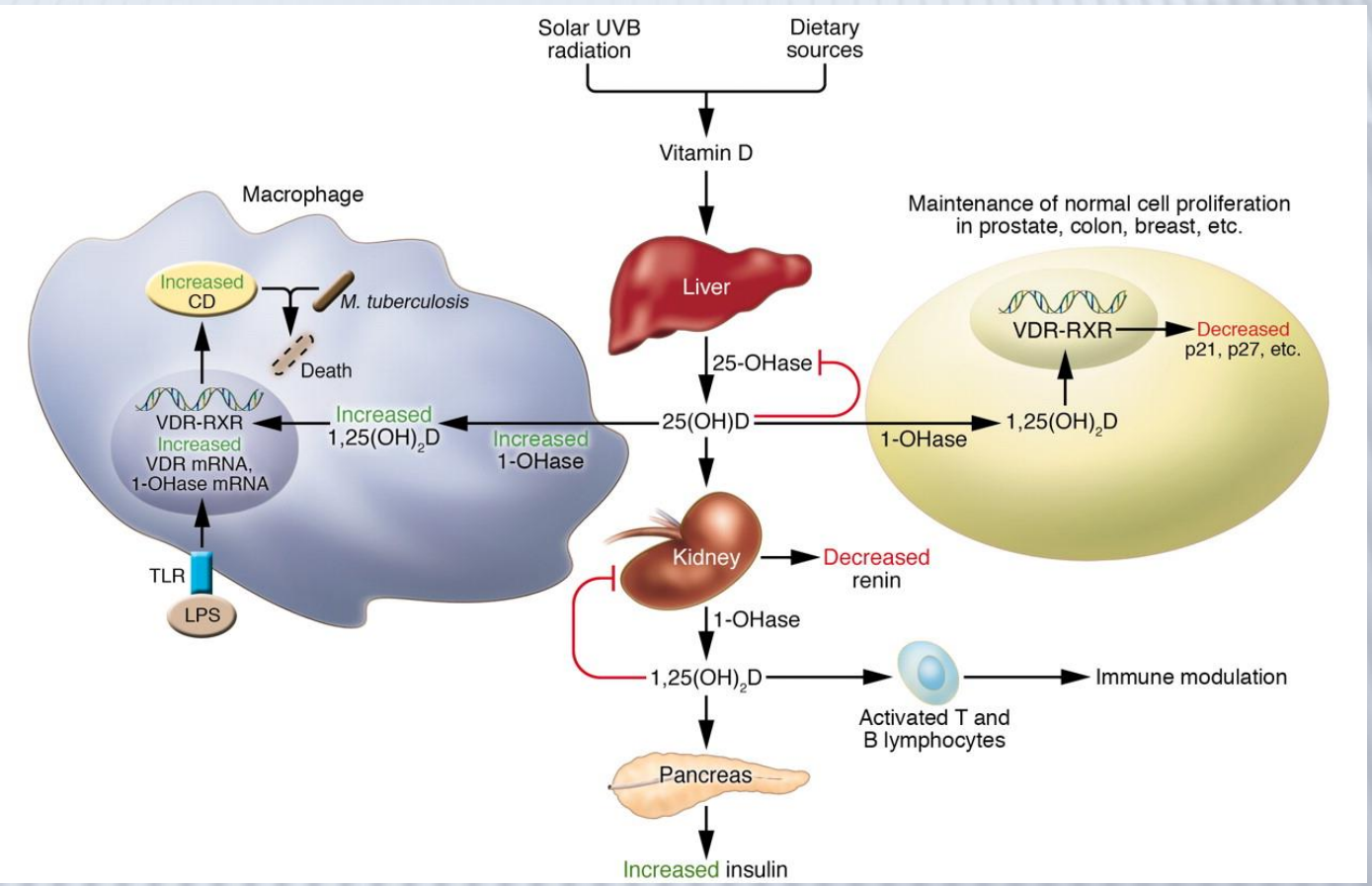
Transport to liver

Liver

Kidneys



Inactive form

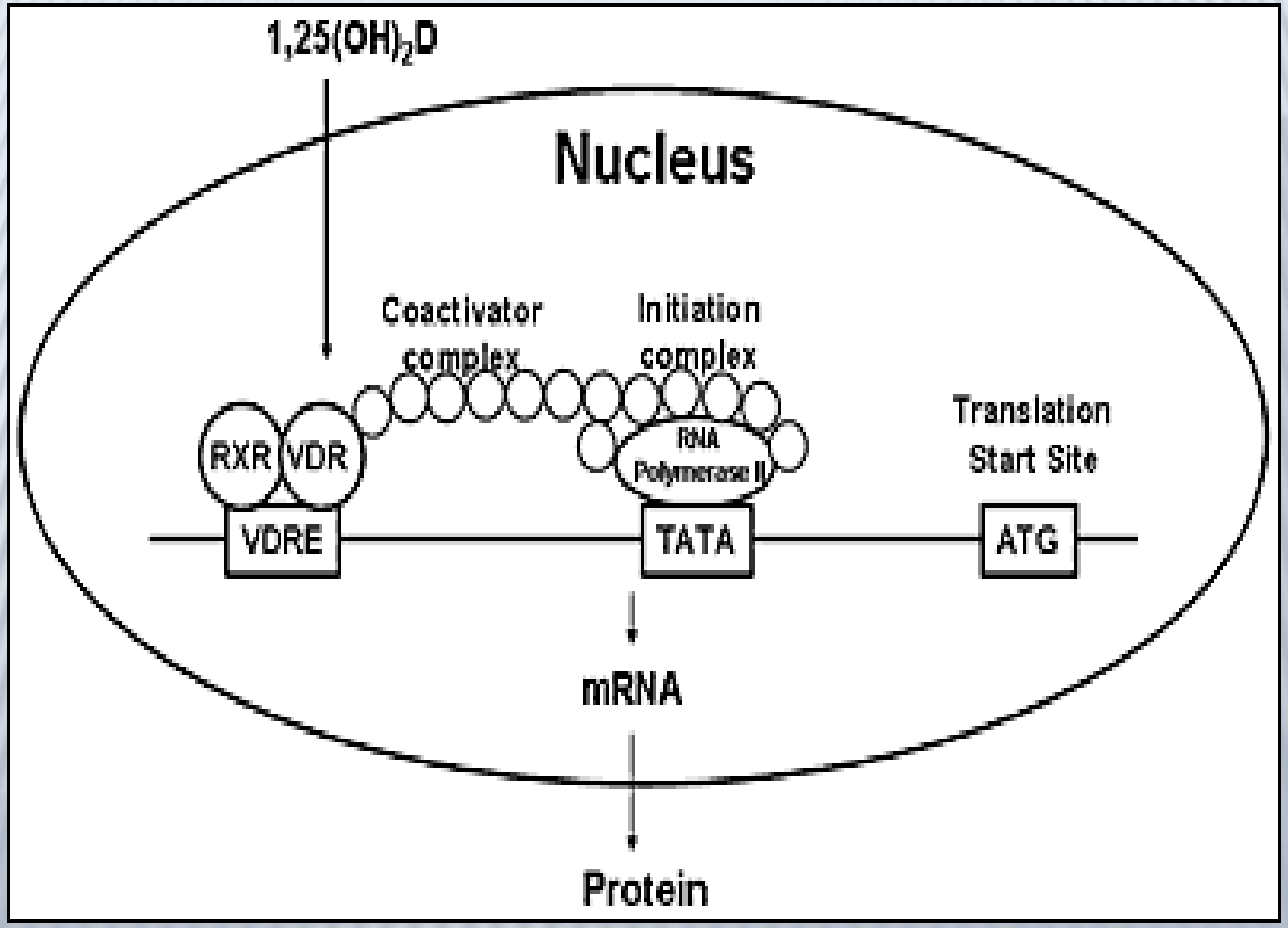


Holick, M. F. J. Clin. Invest. 2006;116:2062-2072

VITAMIN D RECEPTOR BINDING AND INTERACTIONS WITH DNA

- Being lipids, steroid hormones enter the cell by simple diffusion across the plasma membrane. The receptors exist either in the cytoplasm or nucleus, which is where they meet the hormone. When hormone binds to receptor, a characteristic series of events occurs:
- *Receptor activation* is the term used to describe conformational changes in the receptor induced by binding hormone. The major consequence of activation is that the receptor becomes competent to bind DNA.

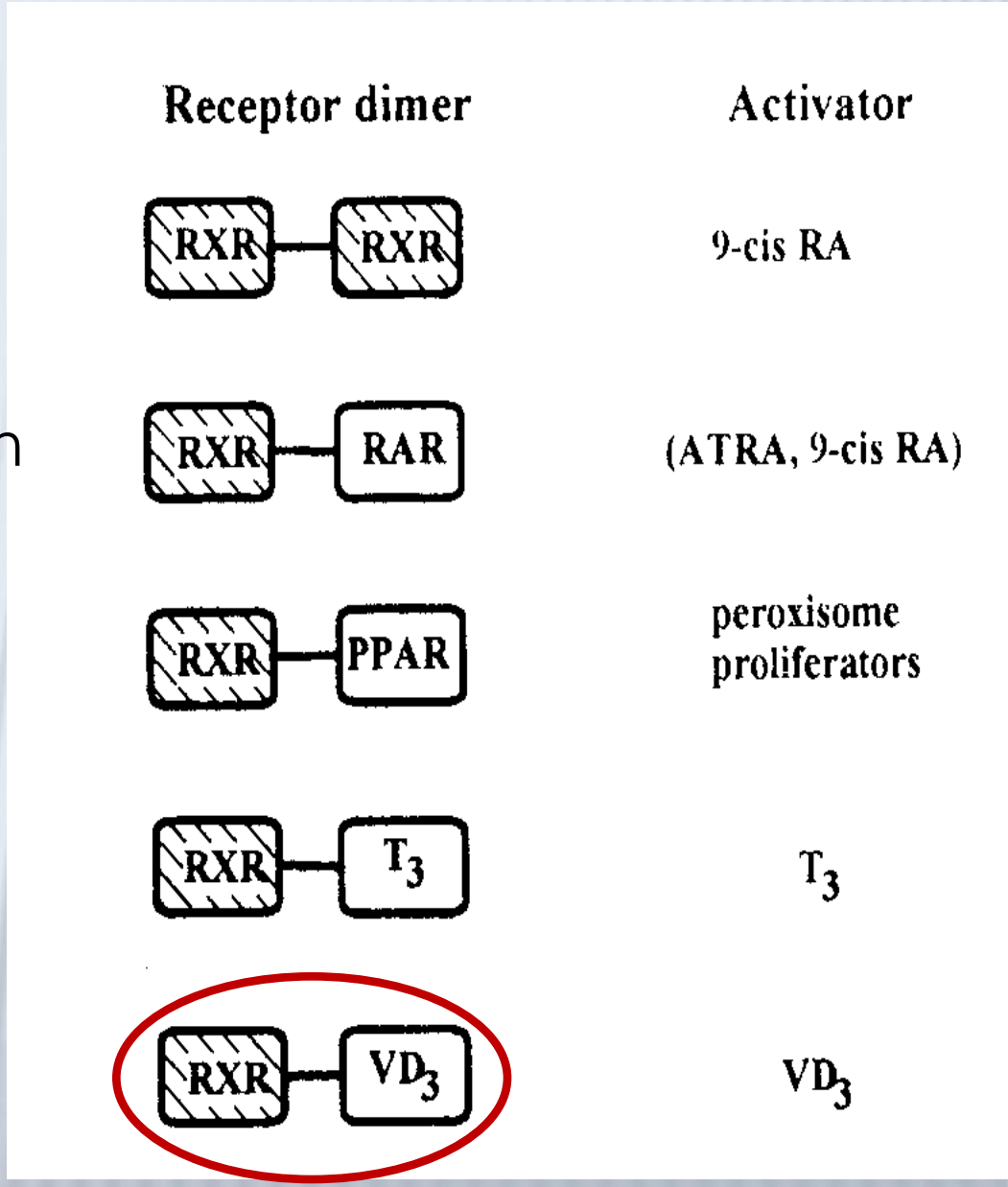
Vitamin D at the level of DNA



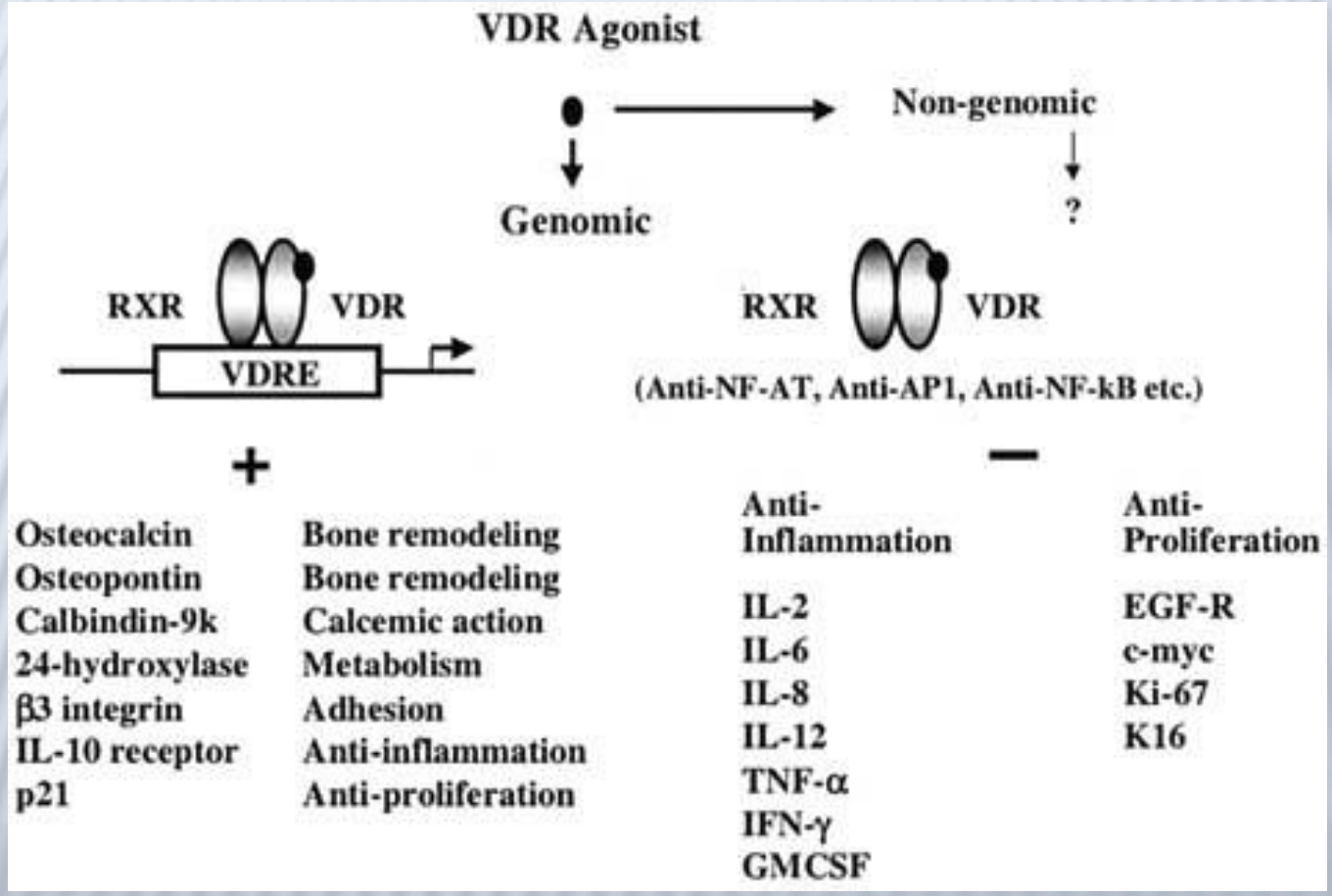
VITAMIN D RECEPTOR BINDING AND INTERACTIONS WITH DNA

- *Activated receptors bind to "hormone response elements*", which are short specific sequences of DNA which are located in promoters of hormone-responsive genes. In most cases, hormone-receptor complexes bind DNA in pairs, as shown in the figure below.
- *Transcription from those genes to which the receptor is bound is affected.* Most commonly, receptor binding stimulates tor inhibits transcription of different genes. The hormone-receptor complex thus functions as a transcription factor.

Nuclear receptor function



Regulation of gene expression by VDR



THE VITAMIN D RECEPTOR AND MECHANISM OF ACTION

- The vitamin D receptor forms a complex with another intracellular receptor, the retinoid-X receptor, and that heterodimer is what binds to DNA. In most cases studied, the effect is to activate transcription, but situations are also known in which vitamin D suppresses transcription.
- The vitamin D receptor binds several forms of cholecalciferol. Its affinity for 1,25-dihydroxycholecalciferol is roughly 1000 times that for 25-hydroxycholecalciferol, which explains their relative biological potencies.

VITAMIN D DEFICIENCY

- The classical manifestations of vitamin D deficiency are **rickets**, which are seen in children and results in bony deformabilitieas including bowed long bones.
- Deficiency in adults leads to the **osteomalacia**. Both rickets and osteomalacia reflect impaired mineralization of newly synthesized bone matrix, and usually result from a combination of inadequate exposure to sunlight and decreased dietary intake of vitamin D.
- Vitamin D deficiency or insufficiency occurs in several other situations, which you might predict based on the synthetic pathway described above:
- *Genetic defects in the vitamin D receptor*: a number of different mutations have been identified in humans that lead to **hereditary vitamin D resistance**.
- *Severe skin, liver or kidney disease*: this can interfere with generation of the biologically active form of vitamin D.
- *Insufficient exposure to sunlight*: Elderly people that stay inside and have poor diets often have at least subclinical deficiency.

VITAMIN D DEFICIENCY

- Sunscreens, especially those with SPF ratings greater than 8, effectively block synthesis of vitamin D in the skin.
- **Vitamin D toxicity:** Excessive exposure to sunlight does not lead to overproduction of vitamin D. Vitamin D toxicity is inevitably the result of overdosing on vitamin D supplements. Ingestion of milligram quantities of vitamin D over periods of weeks of months can be severely toxic to humans and animals.

HERITABILITY OF VITAMIN D INSUFFICIENCY

- 2 GWASy – 3 polymorphic areas
- 4p12- GC gene(rs2282679)
- 11q12- DHCR7/NADSYN1 (rs2211q12)-7-dehydrocholesterol reduktase/NAD synthetase 1
- 11p15-CYP2R1 (rs10741657)-cytochrom P450, subfamily IIR
- 1-4% of the whole variance of serum 25(OH)D3 levels

VITAMIN D AND HEALTH

Deficit and insufficincy of vitamin D = global healthy problem.

High risk for acute and chronic disease, as

- Infection diseases
- Autoimmune diseases
- DM type I and II
- High risk of atherosclerosis
- Some tumor types (colorectal carcinoma, breast and prostate cancer, ovarial cancer)
- Cognitive dysfunction
- Infertility
- Gravidity and around delivery complications

Pludowski P et al. Vitamin D effects on musculoskeletal health, immunity, autoimmunity, cardiovascular disease, cancer, fertility, pregnancy, dementia and mortality—A review of recent evidence, *Autoimmunity Reviews, Volume 12, Issue 10, August 2013, Pages 976-989*

INSUFFICIENCY OF VITAMIN D

- Can be recognised in a half of „healthy“ adults in developped countries.
- Vitamin D levels in the winter seems to be too low in latitude more than 35° without supplementation by sunshine and diet
- High heritability - to 53% - important influence of gene polymorphisms (SUNLIGHT consortium – Study of Underlying Genetic Determinants of Vitamin D and Highly Related Traits, based 2008)

CALCITONIN

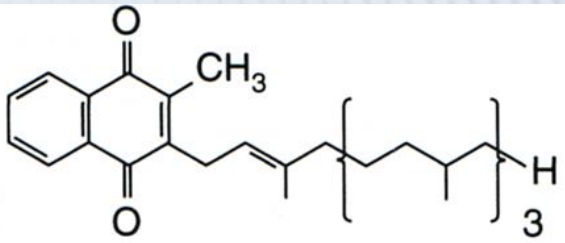
- Calcitonin is a hormone known to participate in calcium and phosphorus metabolism. It main role is to increase calcium deposition in bones.
- In mammals, **the major source of calcitonin is from the parafollicular or C cells in the thyroid gland**, but it is also synthesized in a wide variety of other tissues, including the lung and intestinal tract.
- Calcitonin is a 32 amino acid peptide cleaved from a larger prohormone. It contains a single disulfide bond, which causes the amino terminus to assume the shape of a ring.
- Alternative splicing of the calcitonin pre-mRNA can yield a mRNA encoding **calcitonin gene-related peptide**; that peptide appears to function in the nervous and vascular systems. The **calcitonin receptor** has been cloned and shown to be a member of the seven-transmembrane, G protein-coupled receptor family.

CONTROL OF CALCITONIN SECRETION

- **The most prominent factor controlling calcitonin secretion is the extracellular concentration of ionized calcium**.
- Elevated blood calcium levels strongly stimulate calcitonin secretion, and secretion is suppressed when calcium concentration falls below normal.
- A number of other hormones have been shown to stimulate calcitonin release in certain situations, and nervous controls also have been demonstrated.

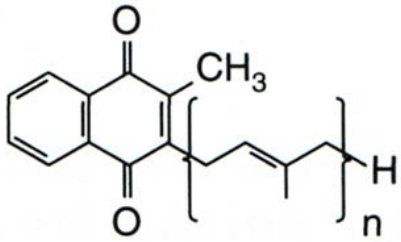
Vitamin K

- Vitamin K_1 (fylochinon) – plants origin.
- Vitamin K_2 (menachinon) – produced by intestinal microbiota
- K_1 and K_2 are used by different manner
  - K_1 – especially for blood clotting (liver)
  - K_2 – important in non- coagulation processes : cell growth and vessel wall cells metabolism.
  - Vitamin K2 is transcriptional regulator of genes specific for bone. It is functioning using SXR (steroid and xenobiotic receptor) and supports expression of osteoblastic markers. It supports bone homeostasis.



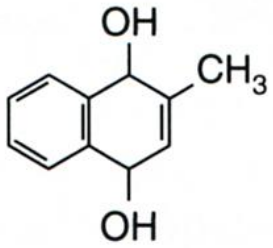
Fylochinon

Vitamin K_1

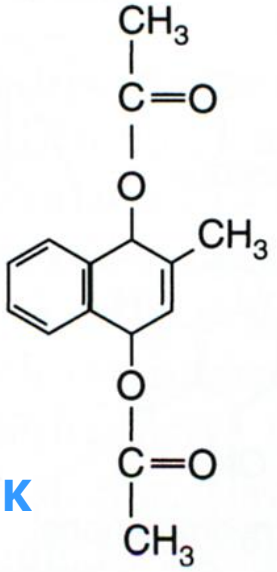


Menachinon

Vitamin K_2



Menadiol



Menadiondiacetát

Syntetické deriváty Vit.K

Vitamin K - function

- Cofactor of liver microsomal *carboxylase* → *changes* glutamates on γ-carboxyglutamates during posttranslational modification of coagulation factors II, VII, IX and X.
- This modification enables to bind vitamin K-dependent coagulation factors through Ca^{2+} ions to phospolipids of platelets.
- It forms similar binding of Ca^{2+} and other proteins – osteocalcin in bone.

Vitamin K and bones

- Vitamin K_2 is able to catalyze conversion of specific residua of glutamic acid to Gla residuals.
- Vitamin K_2 is necessary for γ-carboxylation of bone matrix proteins containing Gla, as is MGP (= matrix Gla protein) and osteocalcin.
- Incomplete γ-carboxylation of osteocalcin and MGP leads to osteoporosis and an increased risk of pathological fractures. Vitamin K_2 stimulates synthesis of osteoblastic markers and the bone deposition.
- Vitamin K_2 decreases bone resorption by osteoclast formation inhibition and by decreasing of their resorption activity.
- Vitamin K_2 treatment induces apoptosis of osteoclasts, but inhibits apoptosis of osteoblasts which leads to increased formation of bone mass.
- Vitamin K_2 supports osteocalcin expression (↑mRNA) which can be further modulated by 1,25-$(OH)_2$ vitamin D_3.

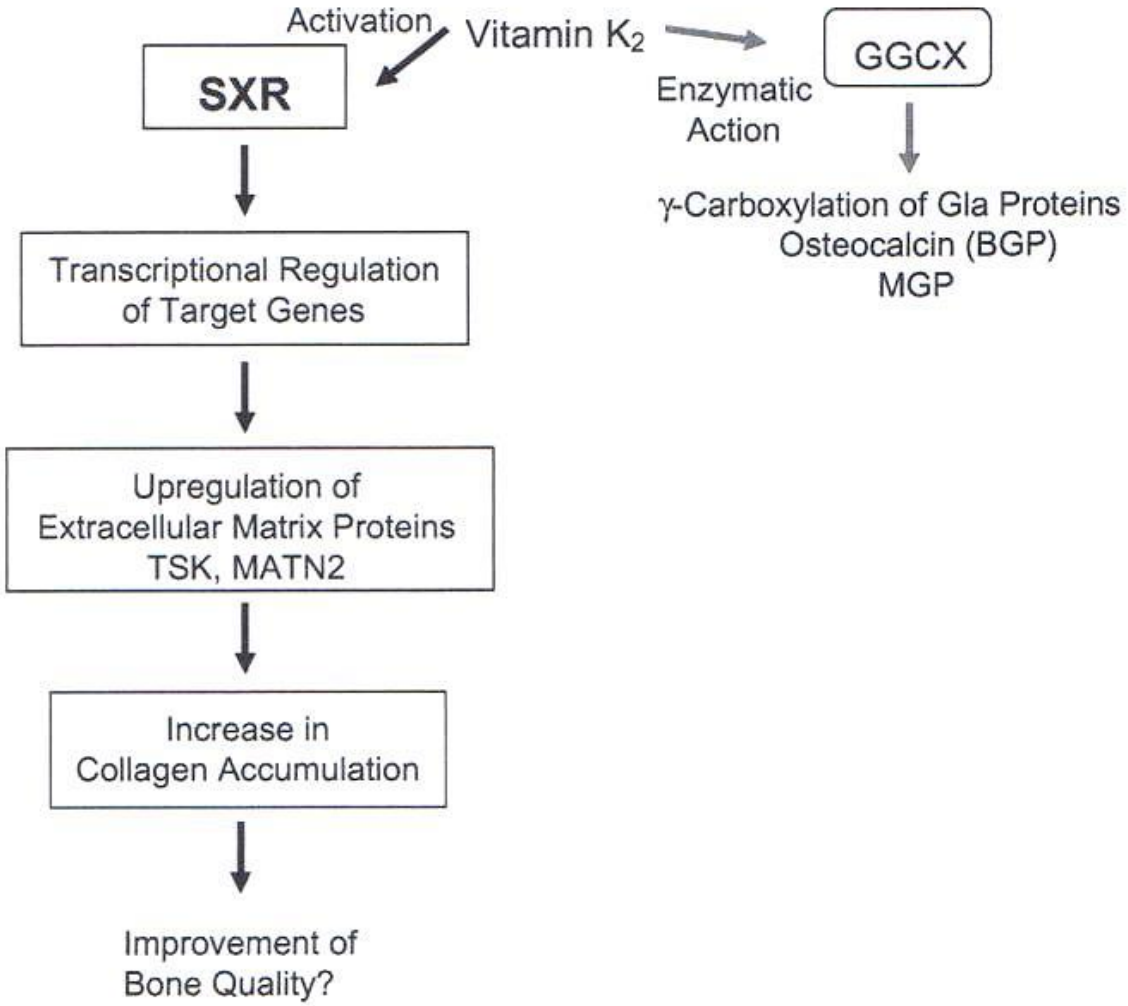


Fig. 3. SXR- and vitamin K_2-dependent regulatory mechanisms of bone metabolism in osteoblastic cells. SXR promotes collagen accumulation in osteoblastic cells by regulating the transcription of its target genes including those encode extracellular matrix proteins. Vitamin K_2 plays a role in the posttranslational modification of Gla proteins by functioning as a coenzyme of γ-glutamyl carboxylase (GGCX) and also acts as a potent SXR ligand in bone metabolism

SXR and its effects

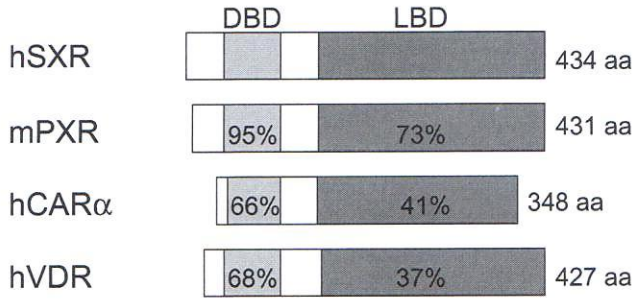


Fig. 1. Schematic comparisons among the nuclear receptor steroid and xenobiotic receptor (SXR) and its related receptors. All the receptors belong to nuclear receptor subfamily 1, group I (NR1I), and form heterodimers with their common partner retinoid X receptor (RXR). The similarity between SXR and other receptors is expressed as percent amino acid identity [1]. *DBD*, DNA-binding domain; *LBD*, ligand-binding domain; *hSXR*, human SXR; *mPXR*, mouse pregnane X receptor; *hCARα*, human constitutive androstane receptor-α; *hVDR*, human vitamin D receptor

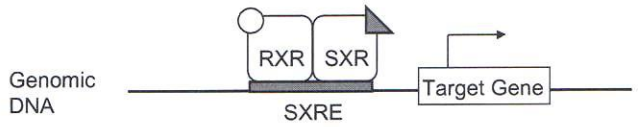


Fig. 2. Transcriptional regulatory mechanism of SXR. The ligand-activated SXR forms heterodimers with RXR and regulates the transcription of adjacent target genes by binding to SXR response elements (SXREs) in the genome

Inoue KH a Inoue S: J Bone Miner Meat (2008) 26: 9-12

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