Metabolism of calcium and phosphates

Laboratory diagnostics (Ca, phosphates, PTH, PTHrP, vitamin D, paraproteins)

Calcium metabolism

- Body of adult human (young) contains 1000 – 1100 g of calcium
  - skeleton (98 – 99 %)
  - 1 – 2 % extraosseous, particularly extracellularly
- Very small amounts of calcium intracellularly
  - 55 % ER, the rest namely in mitochondria
  - The cytoplasmic concentration level 10^{-7} $mol.L^{-1}$ versus blood plasma level 10^{-3} $mol.L^{-1}$ (normal concentration around 2.5 $mmol.L^{-1}$)
  - The necessity of strict regulation - signaling role of calcium ions
    - Muscle contraction, neurotransmission, secretion mechanisms, cell cycle and proliferation, cell death, coagulation, etc.
    - Ligand-gated or voltage-gated channels (type T – transient/type L – long lasting), eventually channels activated mechanically
    - Ca^{2+}/H^{+} ATPase
    - Antiport driven by Na^{+} gradient

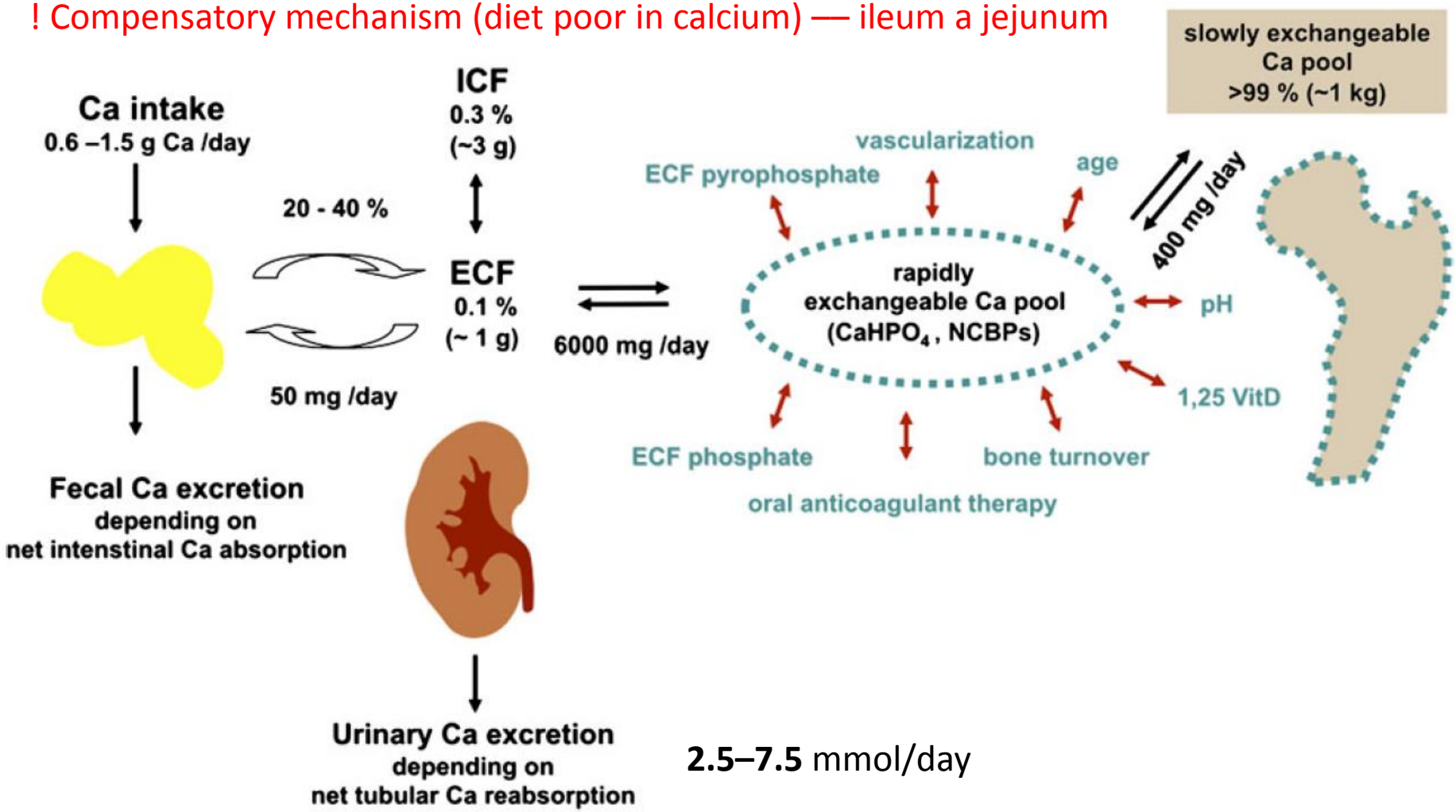
Calcium intake

- Daily intake is about 1.0 g per day and increases during pregnancy, lactation, growth, etc. (up to 1.5 g)
- Under physiological conditions it absorbed about 25 to 40% of received calcium (duodenum - 15%, jejunum - 20%, ileum - 65%)
- Paracelullular/transcelular transport
  - Paracelullar transport – claudin 2 and claudin 12
- Role of 1,25-dihydroxycholecalciferol!
  - Decreased levels of plasma Ca^{2+} increases the synthesis of 1,25-dihydroxycholecalciferol and vice versa

Calcium homeostasis and factors influencing the exchangeable calcium pool

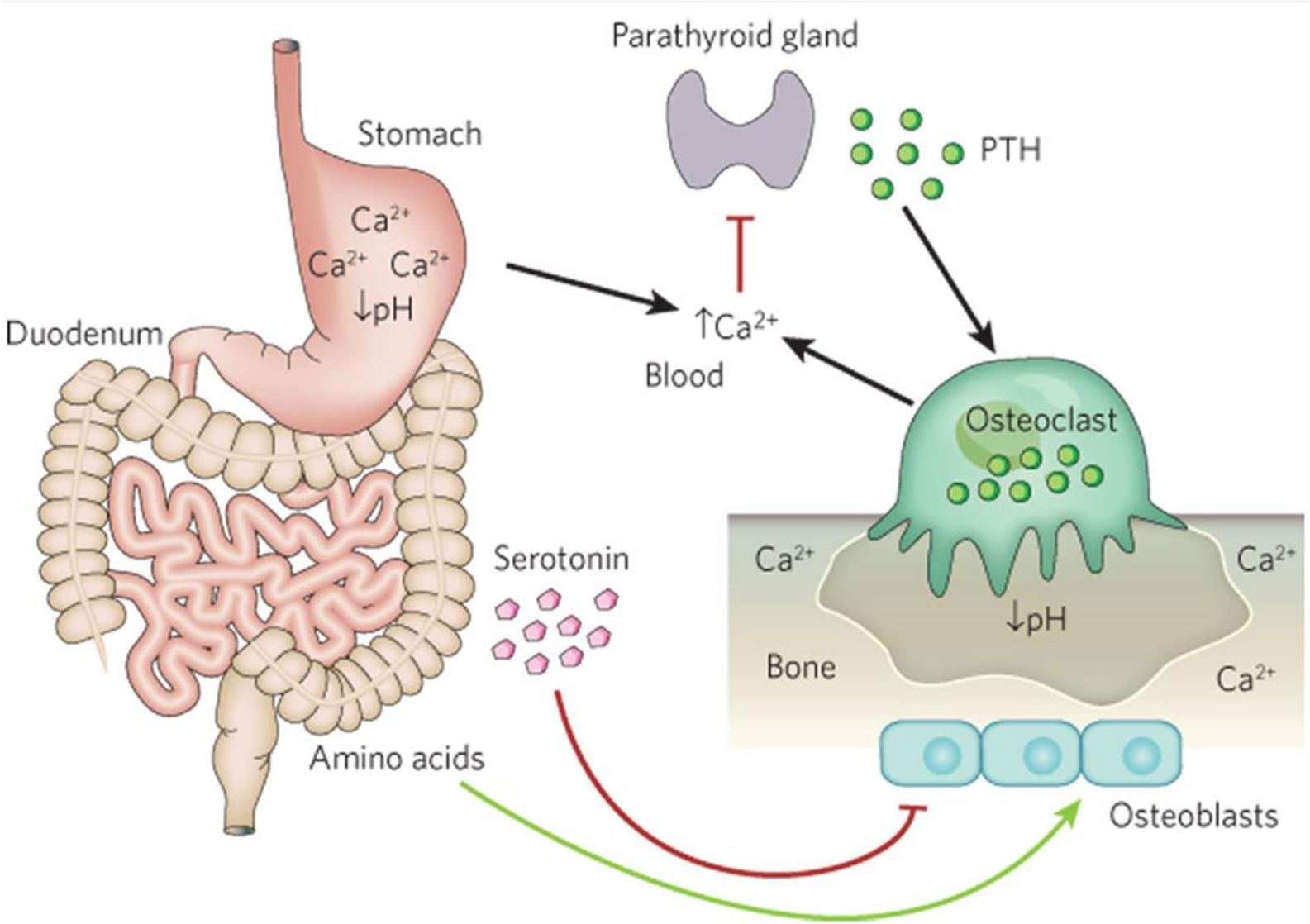
Duodenum + jejunum – 90 %

! Compensatory mechanism (diet poor in calcium) — ileum a jejunum



2.5–7.5 mmol/day

Fig. 1. Calcium homeostasis in a healthy adult individual with emphasis on the ECP in the bone and factors associated with CKD that may affect its size and/or accessibility. ICF, intracellular fluid volume; ECF, extracellular fluid volume; NCBPs, non collagenous bone proteins; CaHPO4, brushite.



Stomach acidity (low pH) is required for the proper absorption of calcium (Ca^{2+}) and is, therefore, essential to maintain normal levels of serum calcium. Serum calcium, in turn, negatively regulates secretion from the parathyroid gland of PTH, a hormone that stimulates osteoclast differentiation and bone resorption. Bone resorption by osteoclasts also occurs at low pH and contributes to the maintenance of serum calcium. Peripheral serotonin is produced by the duodenum and inhibits bone formation by osteoblasts, whereas dietary intake of amino acids (proteins) favours collagen synthesis by osteoblasts.

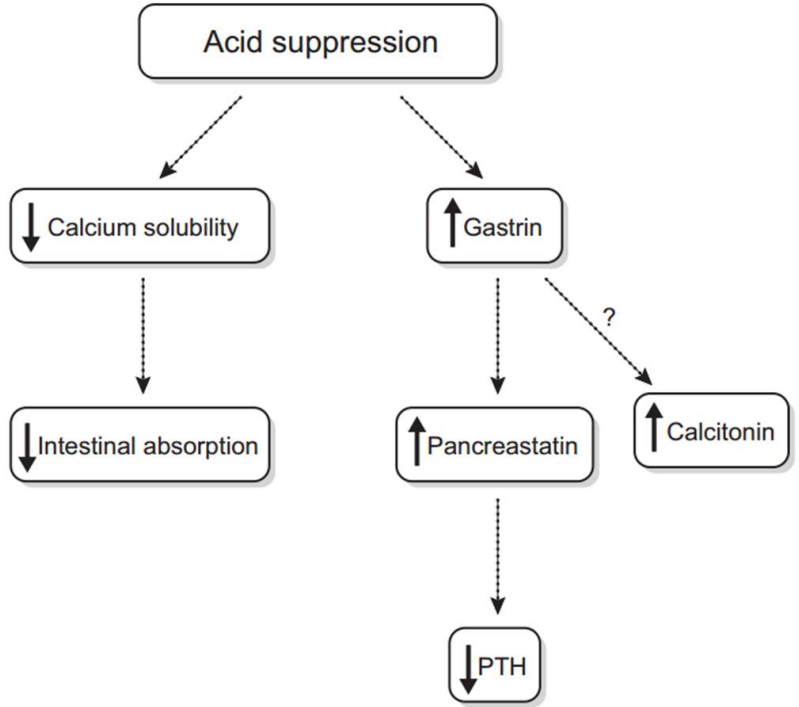


FIGURE 9. Model summarizing the potential impact of acid suppression on calcium homeostasis.

Kopic, S., and J.P. Geibel. 2013. GASTRIC ACID, CALCIUM ABSORPTION, AND THEIR IMPACT ON BONE HEALTH. Physiological Reviews 93:189-268.

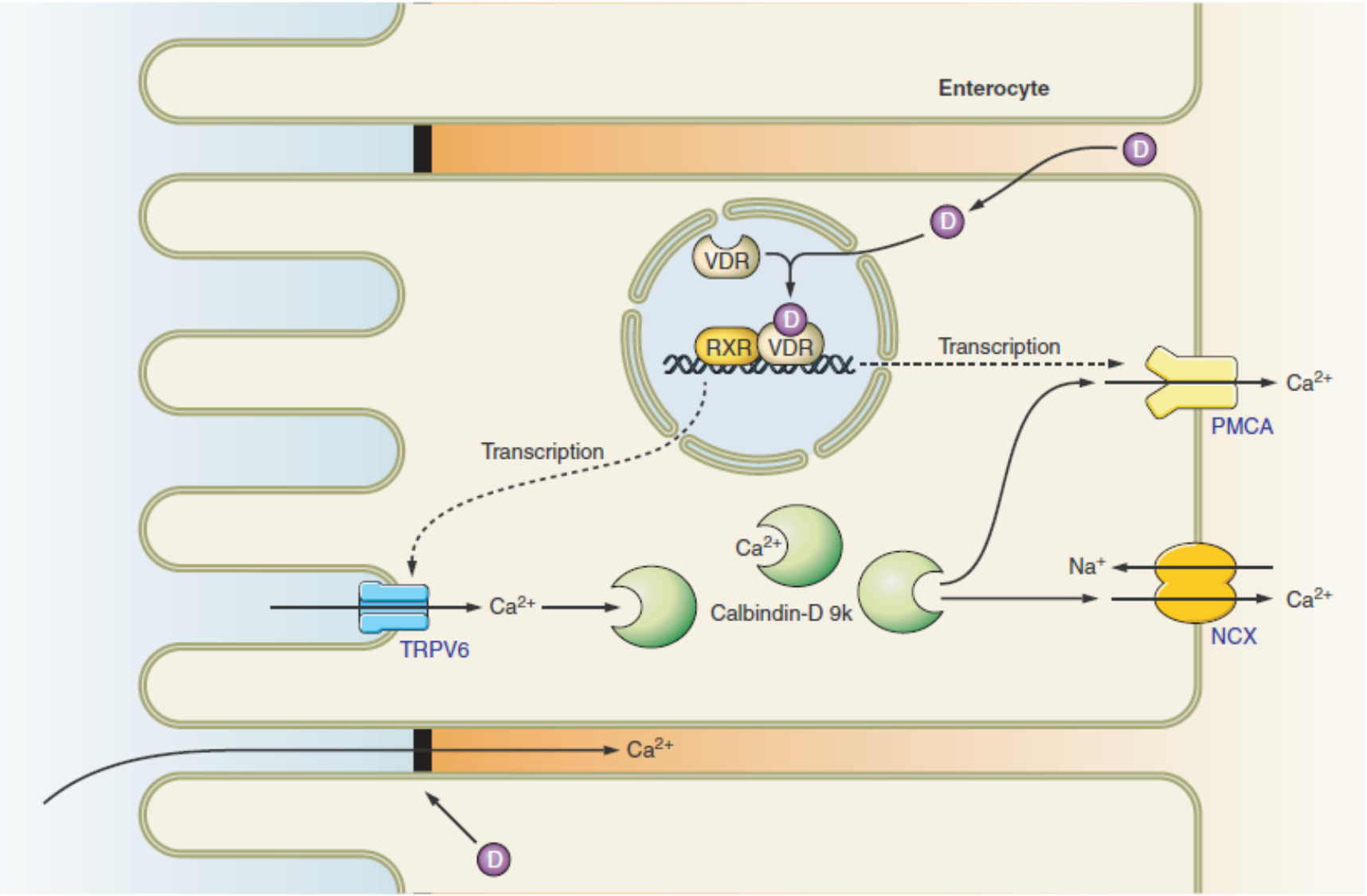


FIGURE 3. Transcellular and paracellular calcium absorption in the intestine. The transcellular intestinal absorption of calcium relies on apical calcium entry through TRPV6, intracellular calcium transport by calbindin-D9k, and basolateral calcium extrusion via either NCX or PMCA. 1,25(OH)$_2$-vitamin D regulates most of these ion transport proteins on a transcriptional level. 1,25(OH)$_2$-vitamin D passes the plasma membrane of the enterocyte and binds to its receptor (VDR), which then heterodimerizes with RXR to initiate transcription. Evidence also suggests that 1,25(OH)$_2$-vitamin D regulates the permeability of tight junctions, which gate the paracellular absorption of calcium. D, 1,25(OH)$_2$-vitamin D.

NCX - sodium-calcium exchanger

PMCA - plasma membrane calcium ATPase

TRPV6 - transient receptor potential cation channel subfamily V member 6

Kopic, S., and J.P. Geibel. 2013. GASTRIC ACID, CALCIUM ABSORPTION, AND THEIR IMPACT ON BONE HEALTH. Physiological Reviews 93:189-268.

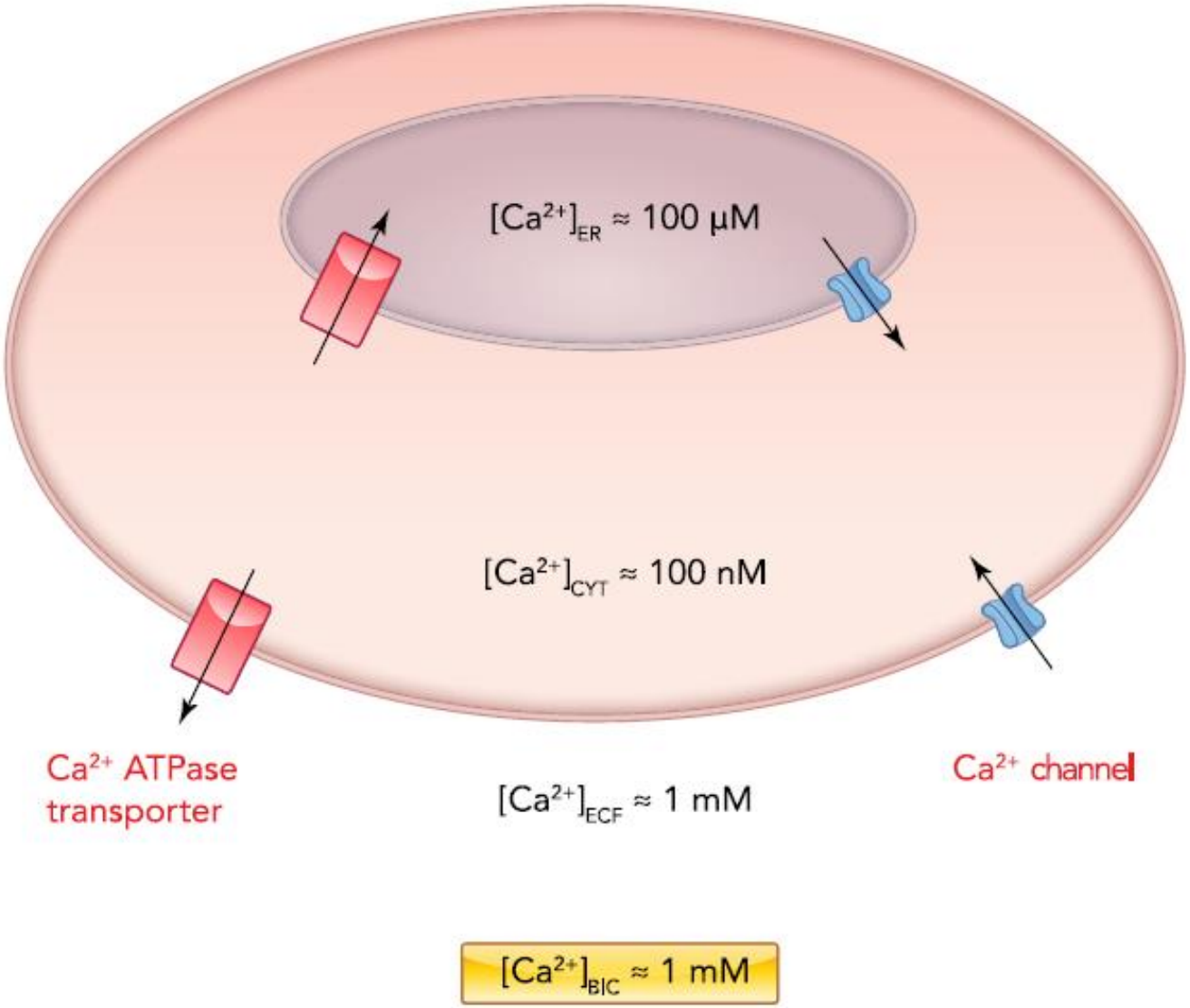


FIGURE 2. **Cellular regulation of calcium concentrations**
Approximate calcium concentrations in a typical, nonexcitable cell's endoplasmic reticulum (ER) and cytosol (CYT), in the extracellular fluid (ECF) and in the blood as ionized calcium (BIC). Calcium flows down its concentration gradient via Ca^{2+} channels and against its gradient via Ca^{2+} ATPase transporters.

Doherty, A.H., C.K. Ghalambor, and S.W. Donahue. 2015. Evolutionary Physiology of Bone: Bone Metabolism in Changing Environments. *Physiology 30:17-29.*

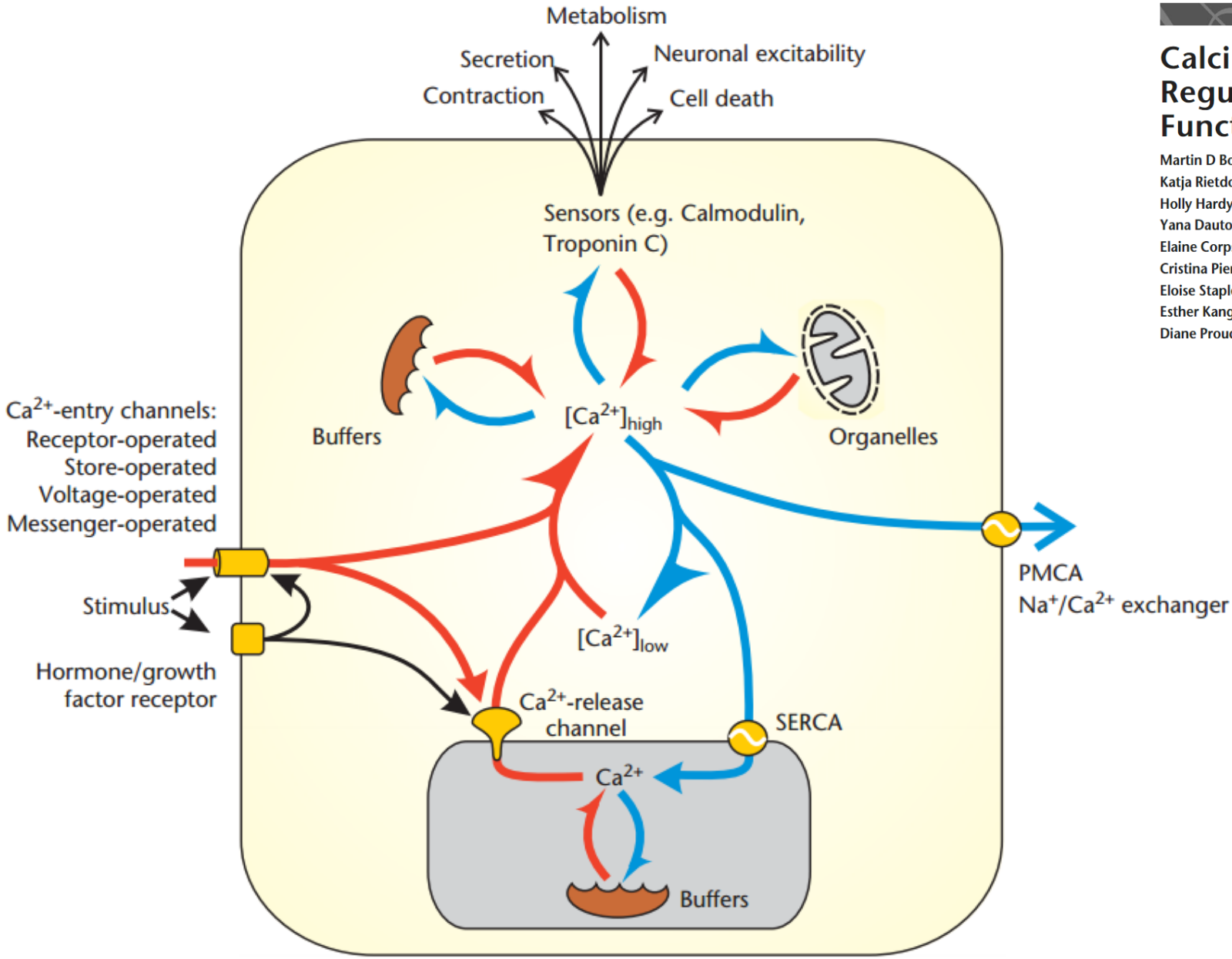


Figure 2 Ca^{2+} 'on' and 'off' mechanisms. This figure represents a summary of processes that modulate cytoplasmic Ca^{2+} levels. The Ca^{2+} 'on' mechanisms responsible for increasing cytosolic Ca^{2+} are marked by red arrows, and the Ca^{2+} 'off' mechanisms are shown in blue. A dynamic interplay of these processes determines the spatio-temporal characteristics of a Ca^{2+} signal. See the text for details. Plasma membrane Ca^{2+}ATPase, PMCA; sarcoplamic/endoplasmic reticulum Ca^{2+}ATPase, SERCA.

Calcium Signalling and Regulation of Cell Function

Martin D Bootman, *The Open University, Walton Hall, Milton Keynes, UK*
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Advanced article

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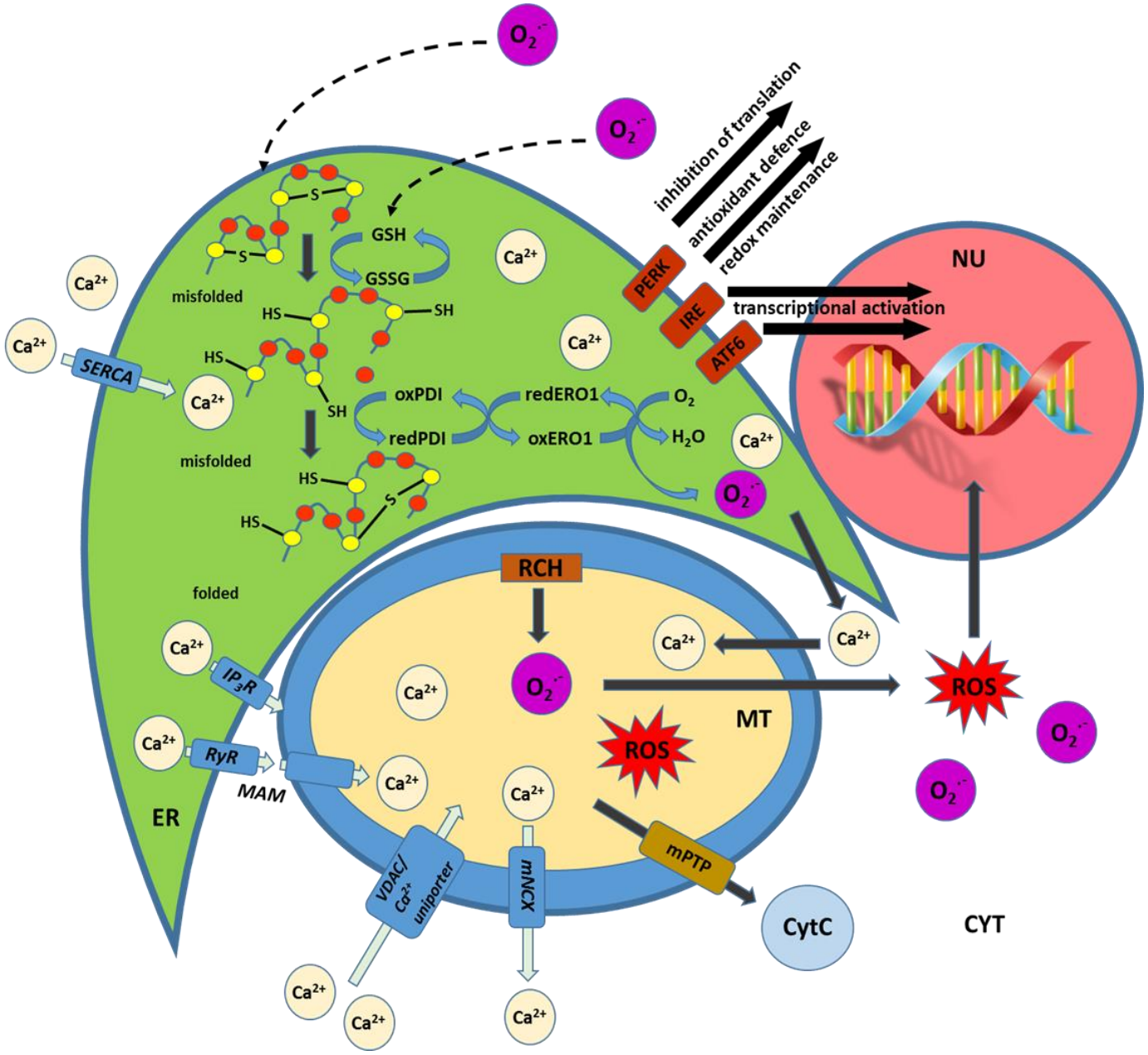


An interplay between mitochondria, endoplasmic reticulum and cytoplasm in handling ROS and calcium ions. Briefly, endoplasmic reticulum is the crucial and major site for calcium storage in cell. Sarco-/endoplasmic reticulum Ca2+-ATPase represents the most important transport mechanism for influx of calcium ions. On the other hand, mitochondria represent the second most important calcium store in the cell. However, these two organelles are closely connected in calcium handling, mainly in response to ROS. Increase in ROS levels in the mitochondria, where the respiratory chain (RCH) represents the major site for creation of ROS, triggers the ER to release calcium and sensitizes a calcium-releasing channel in the ER membrane, sending a feedback signal. On the other hand, process of folding proteins contributes significantly to creation ROS directly in ER. When incorrect disulfide bonds form, they need to be reduced by GSH, resulting in a further decrease of GSH/GSSG ratio, altering the redox state within the ER. Alternatively, misfolded proteins can be directed to degradation through ER-associated degradation machinery. Accumulation of misfolded proteins in the ER initiates the unfolded protein response, which includes involvement of protein kinase RNA-like endoplasmic reticulum kinase (PERK), inositol-requiring enzyme (IRE), and activating transcription factor 6 (ATF6). All these proteins influence cellular responses at different levels (transcription, translation, antioxidant defence). Calcium ions released from ER during these processes (inositol 1,4,5-trisphosphate receptors (IP3R) and ryanodine receptors (RyR) – accumulated in membranes with close connection with mitochondria - in mitochondrial associated membranes (MAMs) trigger mitochondrial ROS stimulation via stimulation of the tricarboxylic acid cycle. Mitochondria release calcium ions via mitochondrial sodium/calcium exchanger (mNCX), influx of calcium ions from cytoplasm in provided by voltage-dependent anion channel (VDAC) and calcium uniporter. Increased load of mitochondria with calcium ions stimulate release of cytochrome c and pro-apoptic factors via mitochondrial permeability transition pore (mPTP).

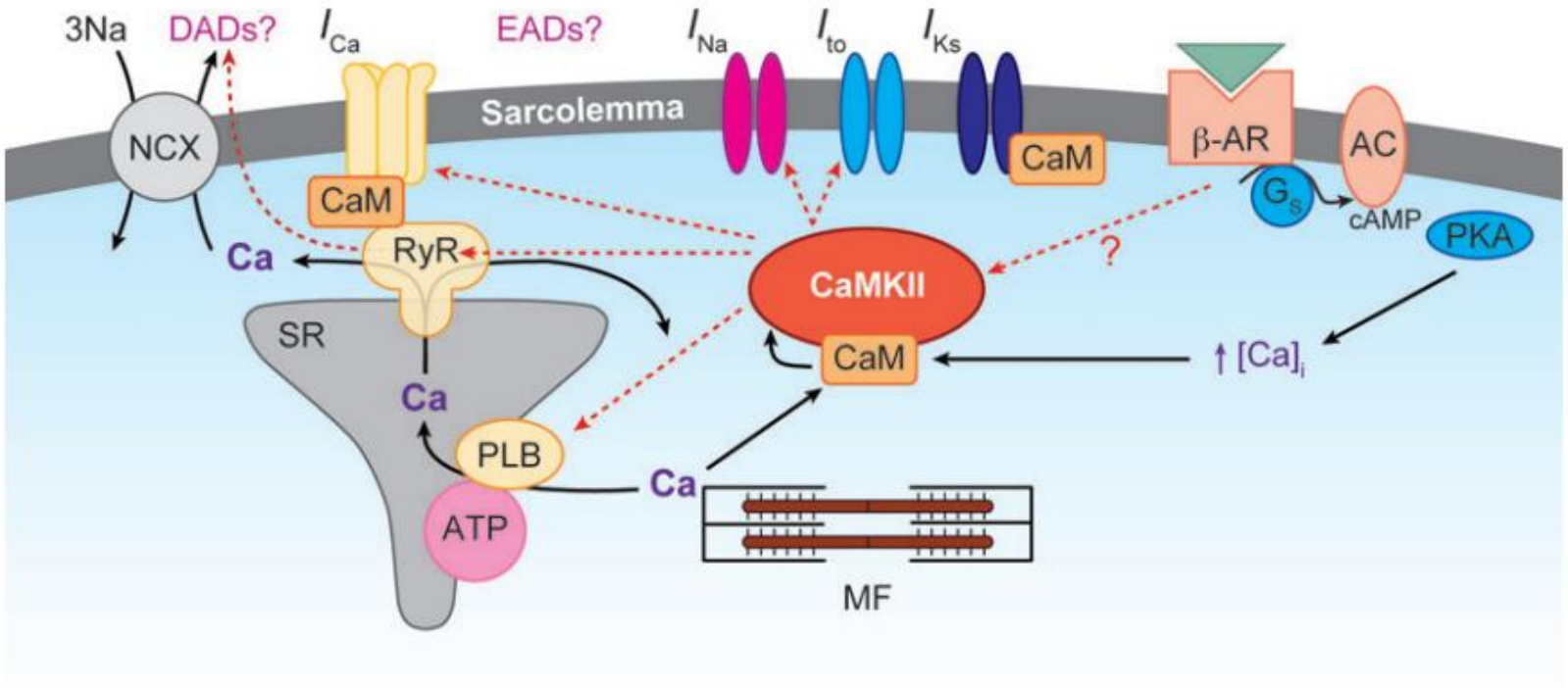
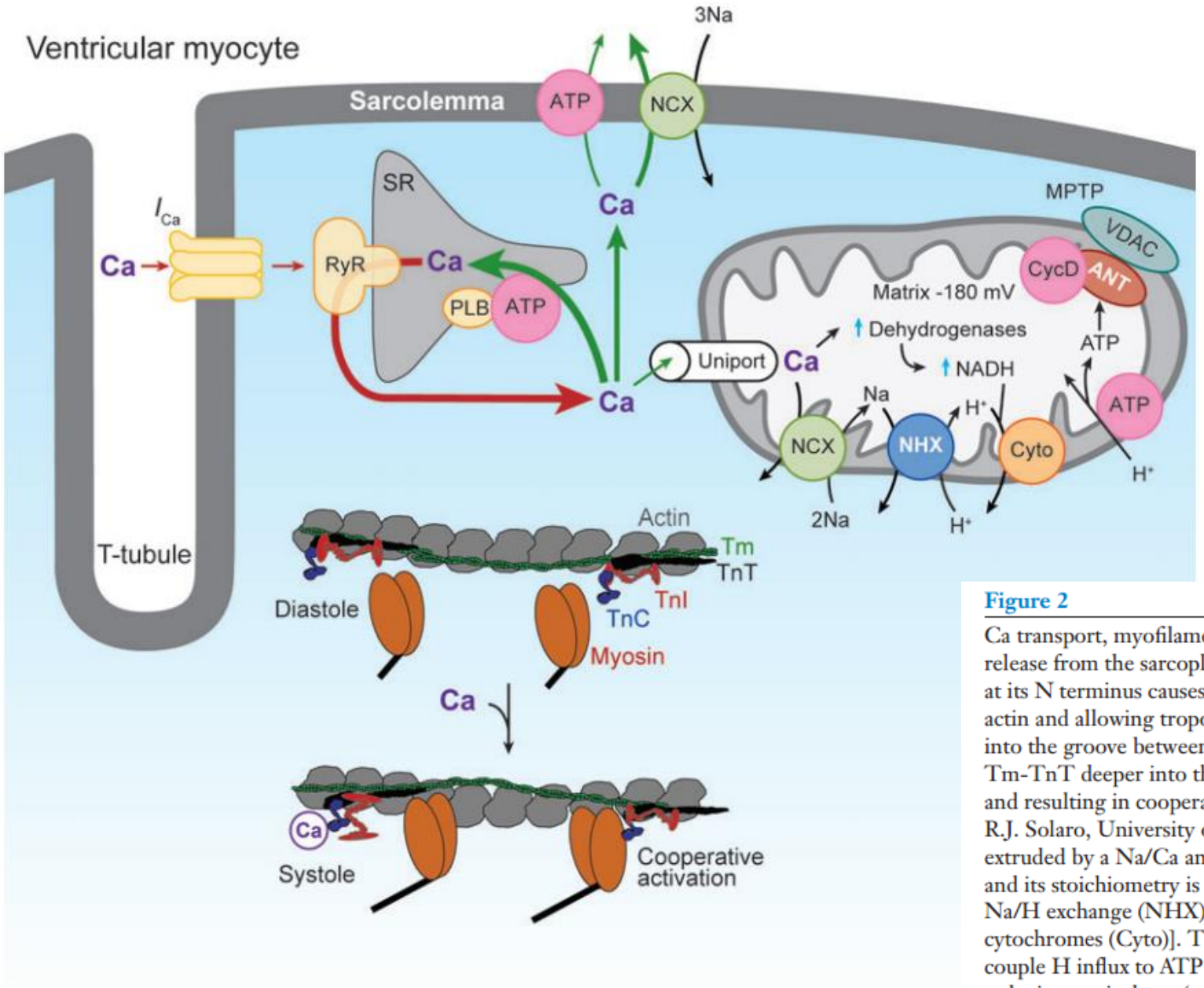


Figure 1

Ca-dependent signaling to cardiac myocyte ion channels. Ca entry via I_{Ca} activates sarcoplasmic reticulum (SR) Ca release via the ryanodine receptor (RyR), resulting in the activation of contraction. SR Ca uptake via the SR Ca-ATPase (ATP) and extrusion via Na/Ca exchange (NCX) allow relaxation. Calcium-calmodulin-dependent protein kinase (CaMKII) can phosphorylate phospholamban (PLB), causing enhanced SR Ca uptake, and also the RyR, enhancing spontaneous diastolic SR Ca release. That Ca release activates inward NCX current and arrhythmogenic delayed afterdepolarizations (DADs). CaMKII can also phosphorylate Ca and Na channel subunits, thereby altering I_{Ca} and I_{Na} gating, thereby prolonging APD and increasing the propensity for early afterdepolarizations (EADs). CaMKII can also modulate I_{to}, whereas calmodulin (CaM) itself can modulate RyR, I_{Ca}, and I_{Ks} gating. Activation of β-adrenergic receptors (β-AR) activates adenylate cyclase (AC) to produce cyclic AMP (cAMP) and activate PKA. PKA phosphorylates PLB and regulates SR Ca uptake, I_{Ca}, I_{Ks}, and RyR, with a net increase in Ca transient amplitude. This is accepted to contribute to CaM and CaMKII activation, but there may also be a more direct, Ca-independent pathway by which β-AR can activate CaMKII. MF, myofilaments.

Bers, D.M. 2008. Calcium cycling and signaling in cardiac myocytes. In Annual Review of Physiology. Annual Reviews, Palo Alto. 23-49.



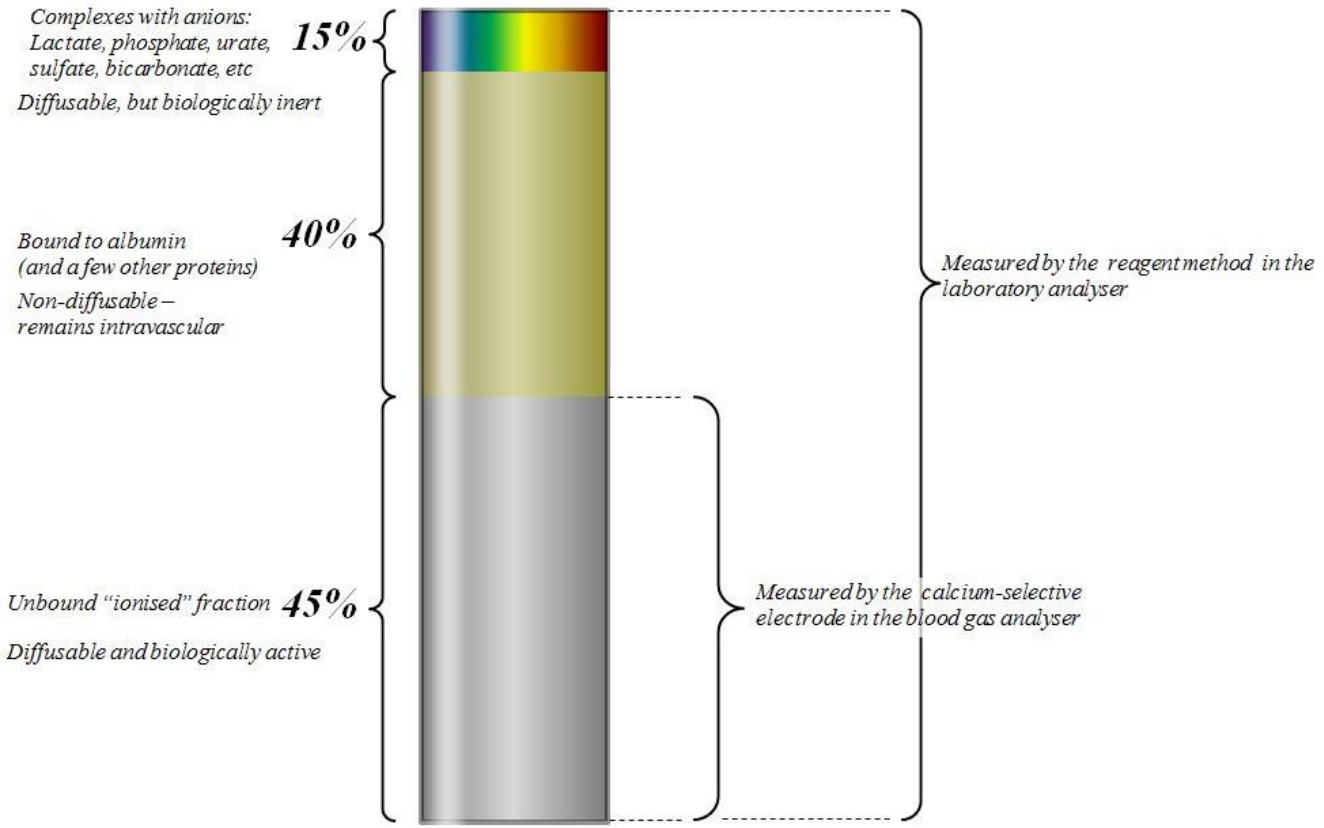
Bers, D.M. 2008. Calcium cycling and signaling in cardiac myocytes. In Annual Review of Physiology. Annual Reviews, Palo Alto. 23-49.

Figure 2

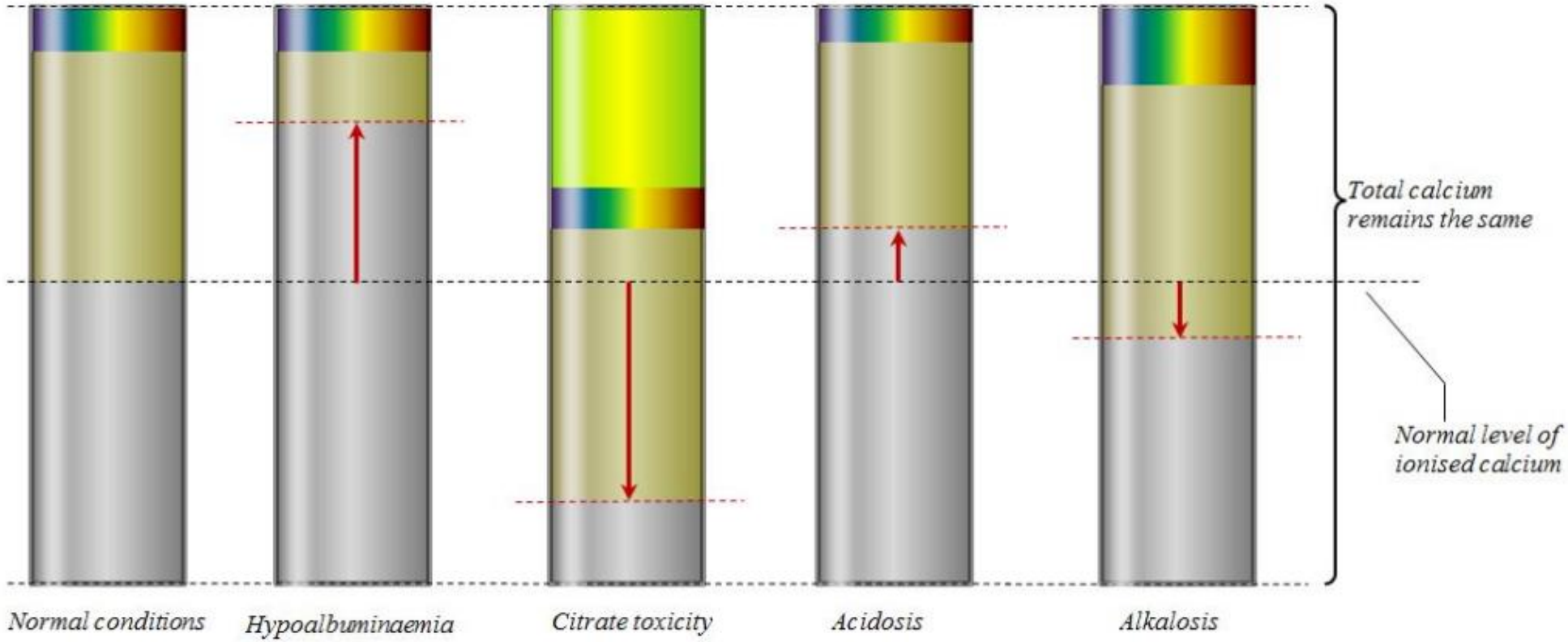
Ca transport, myofilament Ca activation, and mitochondrial Ca handling. Ca influx and Ca-induced Ca release from the sarcoplasmic reticulum (SR) activate the myofilaments. Ca binding to troponin C (TnC) at its N terminus causes TnC to bind to the C terminus of troponin I (TnI), pulling TnI off its site on actin and allowing tropomyosin (Tm) and the third part of the troponin complex (TnT) to roll deeper into the groove between actin monomers. This allows myosin to bind to actin, and this further shifts Tm-TnT deeper into the groove, enhancing Ca binding and crossbridge formation at neighboring sites and resulting in cooperativity (myofilament depiction is modified from a version kindly supplied by Dr. R.J. Solaro, University of Illinois, Chicago). Ca can also enter mitochondria via a Ca uniporter and is extruded by a Na/Ca antiporter (NCX). This mitochondrial NCX is different from sarcolemmal NCX, and its stoichiometry is controversial (2–3Na:1Ca). Na is extruded from mitochondria by electroneutral Na/H exchange (NHX), and protons (H) are extruded by the electron transport chain [including cytochromes (Cyto)]. The mitochondrial F_0F_1-ATPase uses the energy in the inward H gradient to couple H influx to ATP synthesis. Increases in mitochondrial [Ca] activate dehydrogenases that supply reducing equivalents (as NADH) to stimulate ATP synthesis. The mitochondrial permeability transition pore (MPTP) is thought to be composed of the voltage-dependent anion channel (VDAC), adenine nucleotide translocator (ANT), and cyclophilin D (CycD). PLB, phospholamban; RyR, ryanodine receptor.

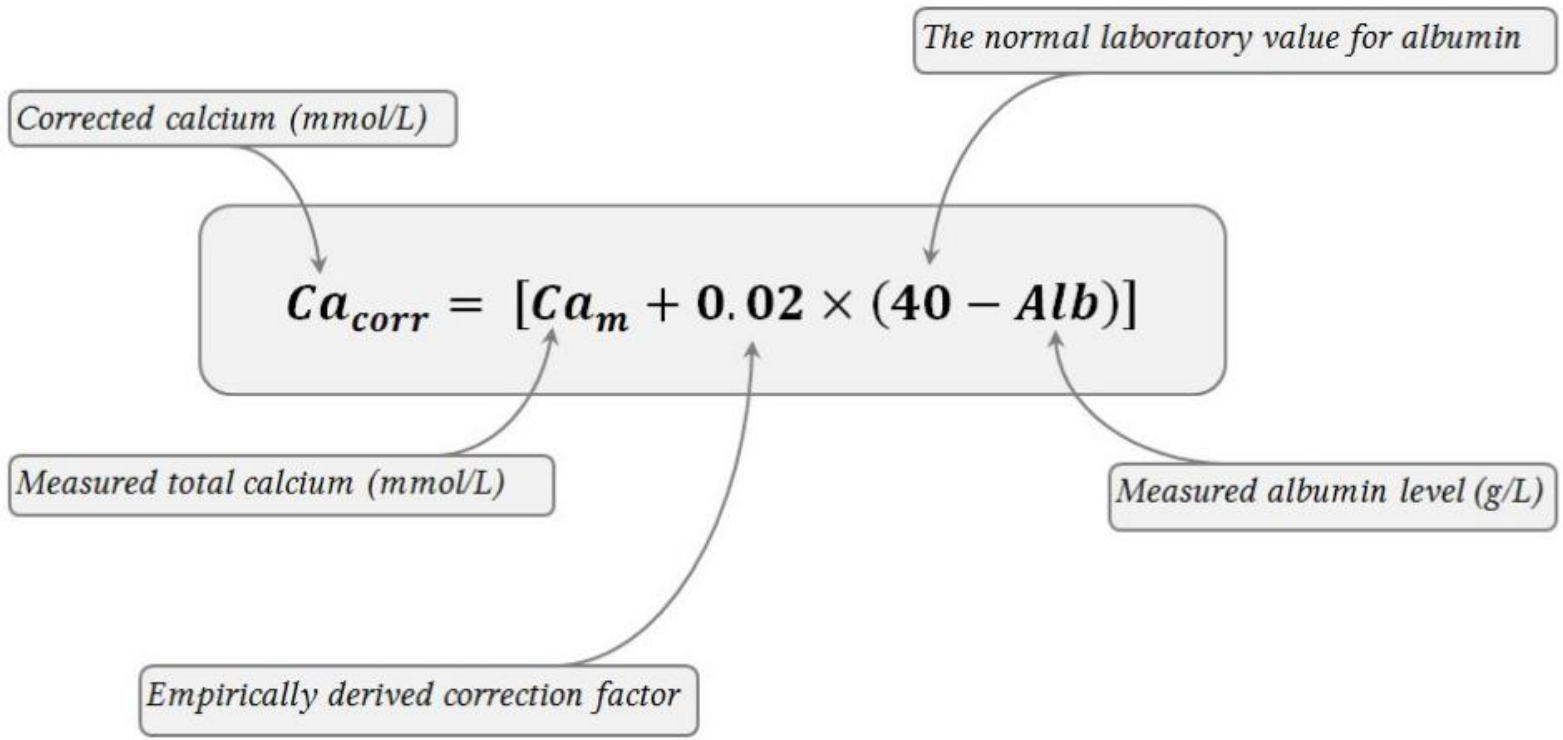
Serum calcium

- Concentration 2.5 $mmol.L^{-1}$, resp. 2.2 – 2.6 $mmol.L^{-1}$ (100 $mg.L^{-1}$)
- The upper limit compatible with life 4 – 5 $mmol.L^{-1}$
- The lower limit 1 $mmol.L^{-1}$
- About 60 % in diffusible form:
  - filtered by the kidneys
  - 50 % ionized – free (Ca^{2+}) = biologically active form (1.1 – 1.3 $mmol.L^{-1}$)
  - 10 % in low-molecular complexes (citrates, phosphates, hydrogen carbonates)
- About 40 % in nondiffusible form:
  - Calcium ions bound to proteins
  - Albumin 90 %, globulin 10 %
  - Cannot be filtered
  - Biologically inactive form, BUT may be released at hypocalcemia
  - hypoalbuminemia – fraction bound to albumin decreases (decrease for 10 $g.L^{-1}$ causes no changes in the concentration of ionized calcium)
  - hyperproteinemia (multiple myeloma) – increase in total calcemia, again without changing in the concentration of free calcium
- pH:



Factors which Influence the Concentration of Ionised Calcium	
Albumin	increased albumin = decreased ionised calcium
pH	increased pH = decreased ionised calcium
Lactate	increased lactate = decreased ionised calcium
Phosphate	increased phosphate = decreased ionised calcium
Bicarbonate	increased bicarbonate = decreased ionised calcium
Citrate	increased citrate = decreased ionised calcium
Heparin	Presence of heparin in the sample = decreased ionised calcium
Free fatty acids	Increase in free fatty acids = decreased ionised calcium


Total calcium remains the same
Normal level of ionised calcium
Normal conditions
Hypoalbuminaemia
Citrate toxicity
Acidosis
Alkalosis


The normal laboratory value for albumin
Corrected calcium (mmol/L)
$Ca_{corr} = [Ca_m + 0.02 \times (40 - Alb)]$
Measured total calcium (mmol/L)
Measured albumin level (g/L)
Empirically derived correction factor

CASR

Kopic, S., and J.P. Geibel. 2013. GASTRIC ACID, CALCIUM ABSORPTION, AND THEIR IMPACT ON BONE HEALTH. Physiological Reviews 93:189-268.

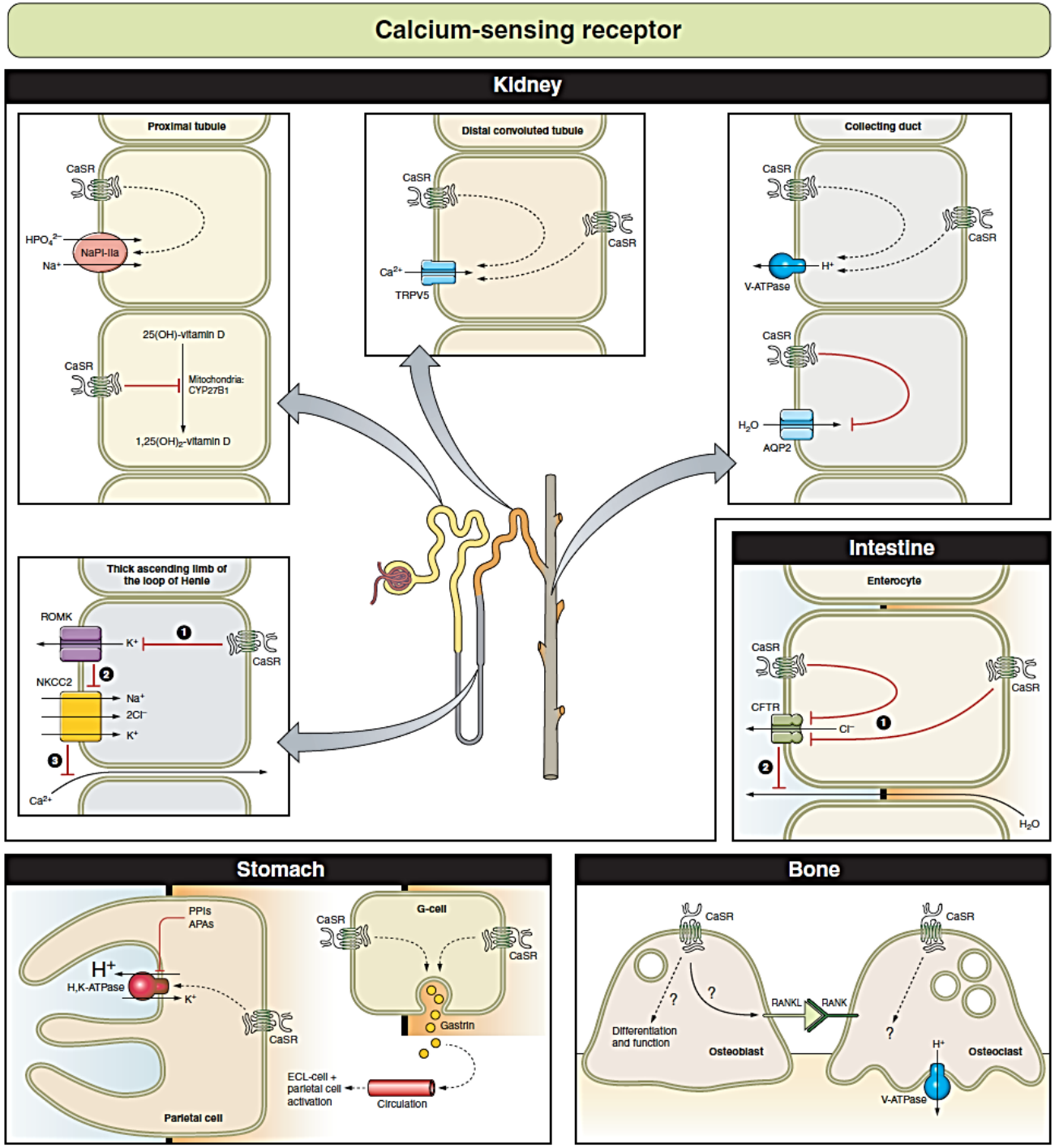


FIGURE 8. CaSR in the gastrointestinal tract, kidney, and bone. *Kidney*: the effects of CaSR activation on ion transport in various nephron segments are shown. In the proximal tubule, CaSR stimulates phosphate absorption and $1,25(OH)_2$-vitamin D synthesis. In the thick ascending limb of the loop of Henle, CaSR inhibits apical potassium channels (ROMK), thereby inhibiting NKCC2 (potassium recycling). The resulting changes in the lumen-positive potential inhibit paracellular calcium uptake. In the distal convoluted tubule, CaSR presumably stimulates apical calcium entry through TRPV5. In the collecting duct, CaSR stimulates proton extrusion through the V-type ATPase and inhibits urine concentration through AQP2. *Stomach*: in the parietal cell, CaSR induces acid secretion by activating H^+-K^+-ATPase. In the G-cell, CaSR activation results in gastrin secretion. Of note, CaSR serves as a luminal nutrient and calcium sensor on the G-cell. *Bone*: CaSR on osteoblasts presumably regulates their differentiation and RANKL expression. *Intestine*: in the intestine, CaSR activation reduces water secretion by inhibiting chloride secretion through CFTR.

Bone tissue and three types of cells

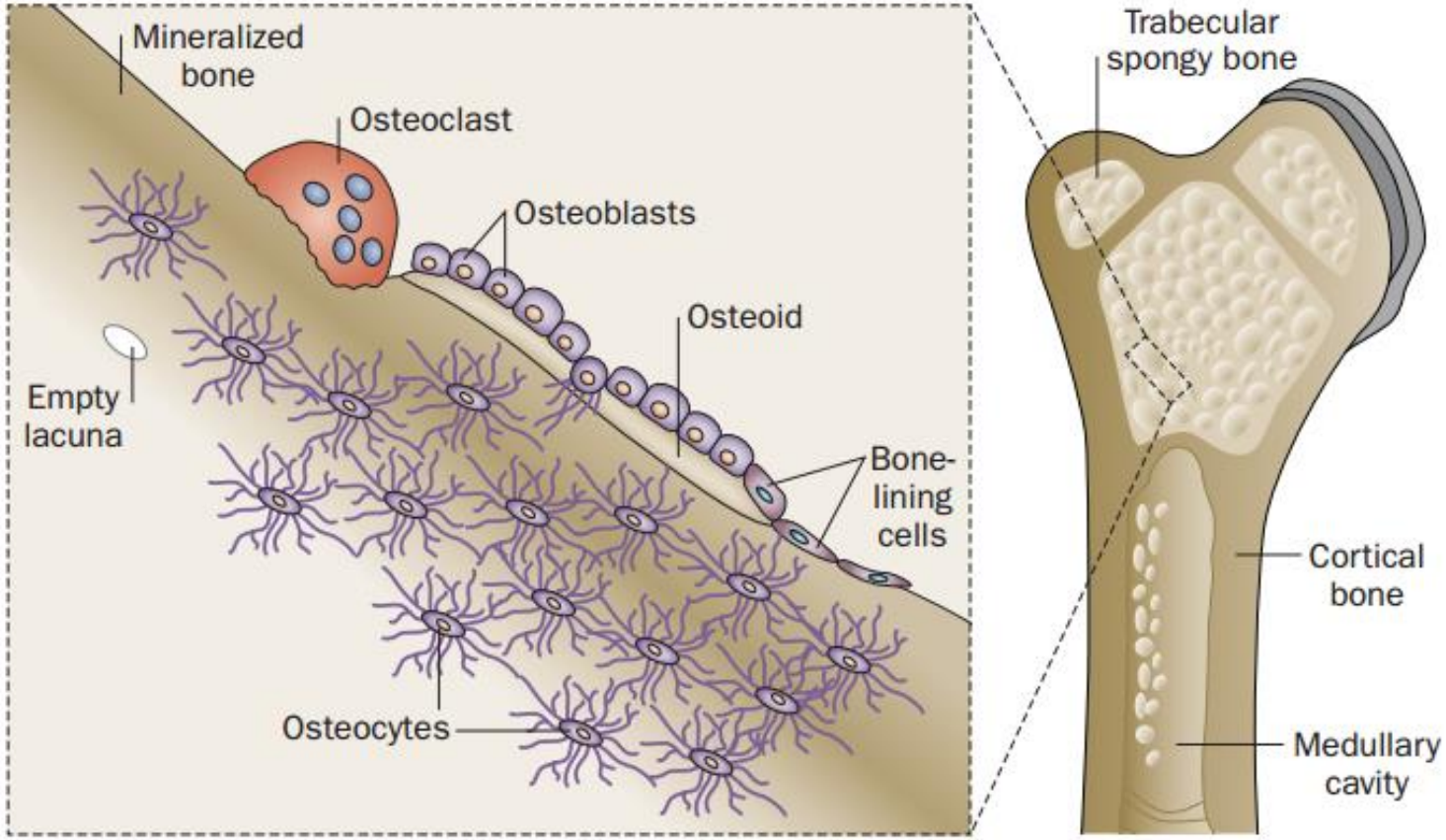
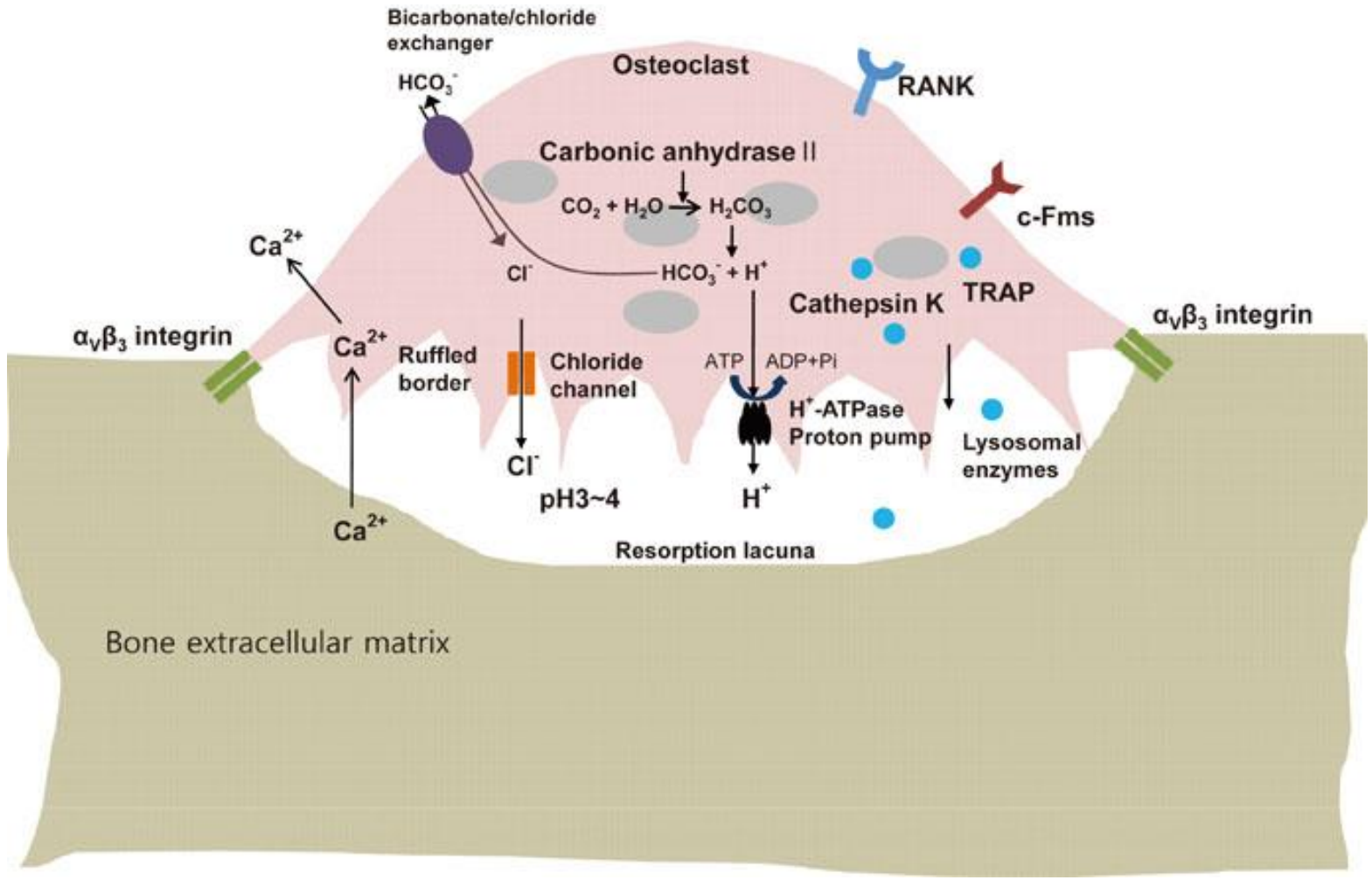


Figure 1 | Microstructure and macrostructure of mammalian bone. Microstructure (left) of an actively remodelling trabecular bone surface. The osteoclast initiates the remodelling cycle by resorbing an area of bone matrix, immediately followed by osteoblast differentiation and osteoid (unmineralized bone matrix) production to replace the resorbed bone. During this process, a small fraction of osteoblasts differentiate further to become osteocytes, encasing themselves within the mineralizing bone matrix and joining the osteocyte network. Mature bone surfaces are populated with bone-lining cells, whose origin and function remain unclear. Macrostructure (right) of the proximal femur illustrating the dense cortical shell and inner trabecular, or 'spongy', bone.

BMU - basic multicellular unit
90 – 130 days

DiGirolamo, D.J., T.L. Clemens, and S. Kousteni. 2012. The skeleton as an endocrine organ. *Nature Reviews Rheumatology 8:674-683.*


Bicarbonate/chloride exchanger
HCO_3^-
Osteoclast
RANK
Carbonic anhydrase II
$CO_2 + H_2O \rightarrow H_2CO_3$
c-Fms
Ca^{2+}
Cl^-
$HCO_3^- + H^+$
Cathepsin K
TRAP
$\alpha_V\beta_3$ integrin
$\alpha_V\beta_3$ integrin
Ca^{2+}
Ruffled border
Chloride channel
ATP
ADP+Pi
H^+-ATPase Proton pump
Lysosomal enzymes
Cl^-
pH3~4
H^+
Ca^{2+}
Resorption lacuna
Bone extracellular matrix

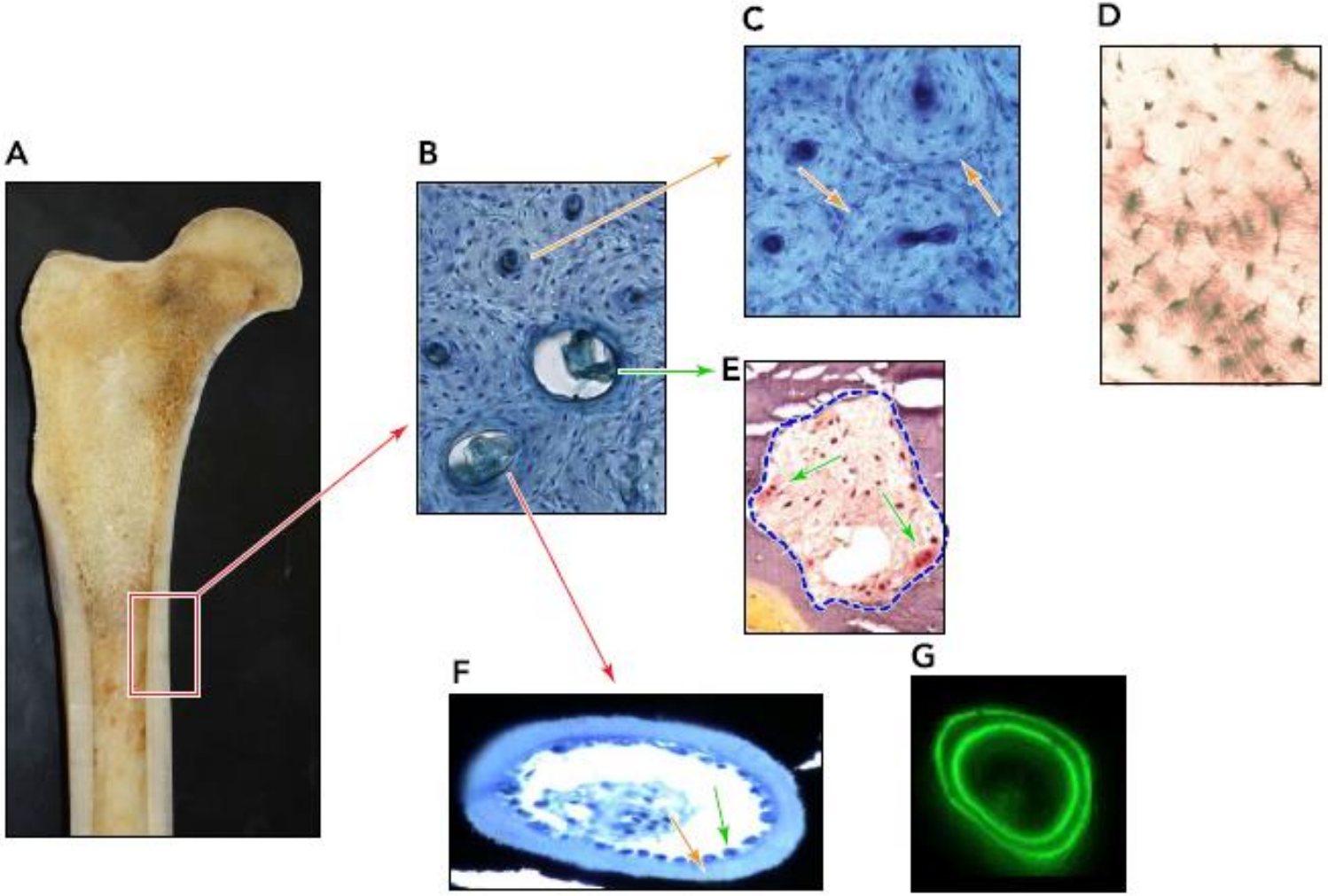
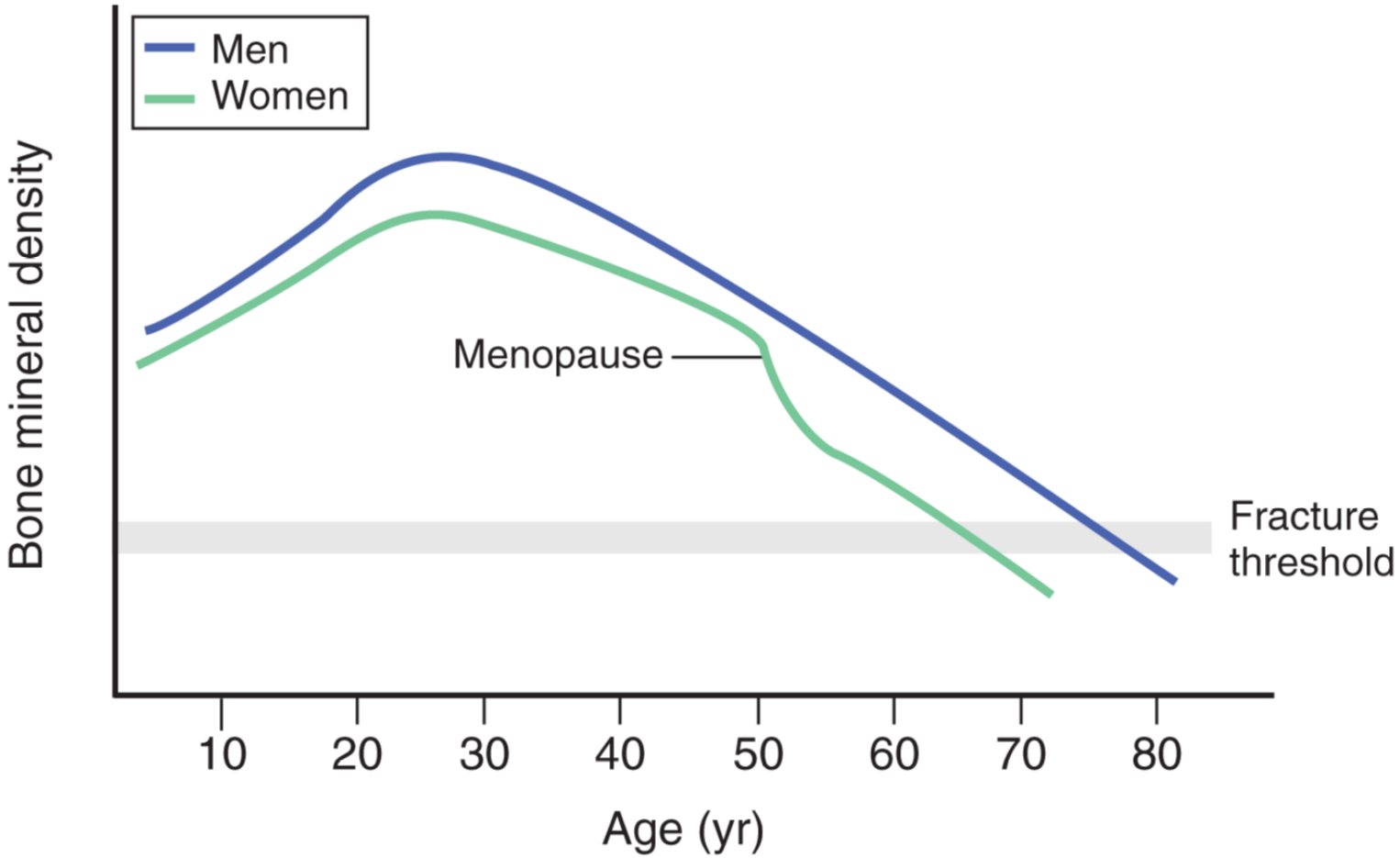


FIGURE 1. **The bone remodeling process**
A: bone remodeling occurs on the surfaces of trabecular bone found in epiphyses and metaphyses of long bones, as shown in the proximal femur, and within cortical bone (red box). *B*: a cross section through the diaphysis shows the secondary osteonal structure of cortical bone: completed secondary osteon (orange arrow), large remodeling cavity (green arrow), and partially refilled remodeling cavity (red arrow). *C*: the periphery of secondary osteons are defined by the cement line (arrows). *D*: extensive canalicular network between osteocyte lacunae. *E*: osteoclasts (arrows) excavating a resorption cavity (dashed line). *F*: osteoblasts (green arrow) refilling a remodeling cavity by producing osteoid (orange arrow). *G*: calcein-labeled mineralizing osteon; measurement of the distance between labels can be used to calculate the mineral apposition rate.

Doherty, A.H., C.K. Ghalambor, and S.W. Donahue. 2015. Evolutionary Physiology of Bone: Bone Metabolism in Changing Environments. *Physiology 30:17-29.*

Factors affecting bone remodeling

- Genetic factors
  - 60-80% of the amount of bone tissue is genetically determined
  - The differences between the races (most Negroes, Asians least)
- Mechanical factors
  - Remodeling of bone structure according to the mechanical requirements
  - Physical activity is essential for proper bone development
- Vascular / neural factors
  - Vascularization necessary for proper bone development, especially for ossification
  - Innervation - neuropeptides and their receptors
- Nutritional factors
  - calcium intake
  - Addictions - coffee, smoking, alcohol, excess salt - risk factors for osteopenia
- The function of the endocrine system
- Besides the above mentioned also:
    - Androgens - anabolic effect, stimulation of osteoblasts, bone density modification
    - Estrogens - estrogen receptors found in osteoblasts, osteocytes, and osteoclasts, dual effect - stimulation of of osteoblasts, increased levels of osteoprotegerin, reduction of bone resorption
    - Progesterone - anabolic effect on bone, osteoblasts - receptors - direct / indirect effect (competes with glucocorticoids)
    - Insulin - stimulating the creation of matrix
    - Glucocorticoids - differentiation during development, but postnatally inhibit bone formation, inhibition of of IGF-1 / BMP-2, which are important for osteoblastogenesis
    - Growth hormone - direct effect (stimulation of osteoblasts), the indirect effect via increasing IGF-1/2 = stimulation of osteoblast proliferation and differentiation



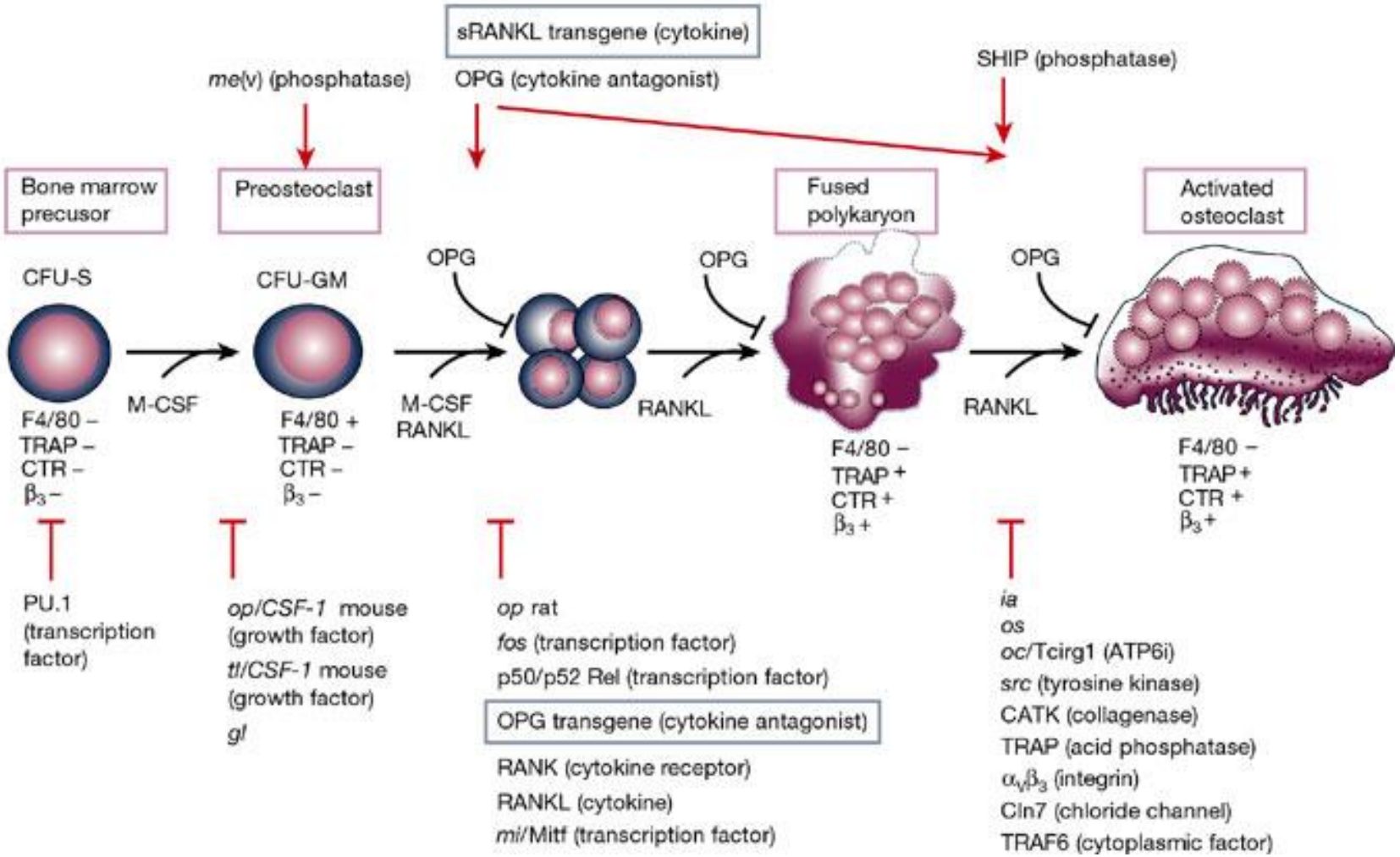
- Diet poor in calcium
- Diet poor in vitamin D
- Exposition to sun!

Local remodeling of bone tissue

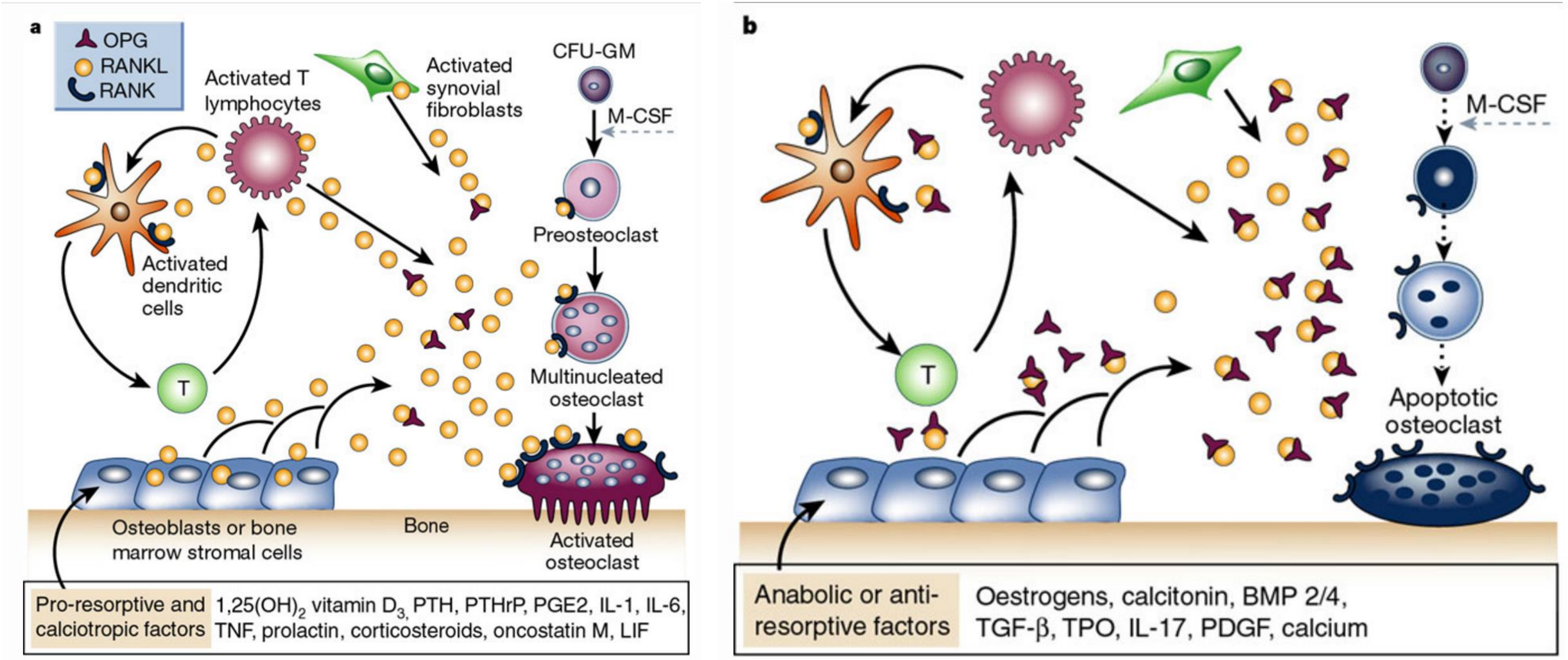
- Especially growth factors and cytokines
- Growth factors:
  - Polypeptides produced by bone tissue or extraosseously
  - Modulation of growth, proliferation, differentiation
  - IGF-1/2
    - Liver / osteoblasts
    - In high concentration in the bone matrix
    - Stimulation of collagen synthesis
    - Regulation of interactions between osteoblasts and osteoclasts
    - IGF-2 – embryogenesis
  - TGF-β (Transforming growth factor - β)
    - Inhibition of bone resorption by inhibiting osteoclast differentiation and apoptosisStimulace tvorby kostní tkáně, indukce diferenciace a proliferace osteoblastů
    - Inhibition of the synthesis of matrix protease (MMP)
  - BMP
  - PDGF (Platelet - derived growth factor)
    - stimulation of protein synthesis
    - Fibroblast proliferation, neovascularization, collagen synthesis
  - FGF (Fibroblastic growth factor) – mitogenic effect on osteoblasts, mutations in receptors - e.g. Apert syndrome (premature closure of sutures, syndactyly, extension of cranium - turricephaly)
  - EGF (Epidermal growth factor)
  - VEGF (Vascular endothelial growth factor) – stimulation of angiogenesis and proliferation of endothelium, important especially in the early stages of regeneration (fracture)
  - GM-CSF (Granulocyte / macrophage - colony stimulating factor) – osteoclastogenesis, osteopetrosis
  - M-CSF (Macrophage - colony stimulating factor) – osteoclastogenesis first phase, without any direct effect on osteoclasts
  - TNF (Tumor necrosis factor) – stimulation of bone resorption

Local remodeling of bone tissue

- Cytokines – immune cells, a number of functions (immune response, inflammation, hematopoiesis, autocrine / paracrine effect, pleiotropic effect)
  - Osteoprotegerin!
- Interleukin 1
  - Direct stimulation of osteoclastic bone resorption
  - Stimulation of proliferation and differentiation of preosteoblasts
  - Inhibition of apoptosis of osteoclast
- Interleukin 6
  - Stimulation of bone resorption
  - Role in the Paget's disease
  - The initial phase of osteoclastogenesis
- Interleukin 11
  - Bone marrow, stimulation of osteoclastogenesis
- Prostaglandins
  - Especially PGE2
  - Stimulation of bone resorption
  - Experimentally – inhibition of COX2 = inhibition of bone formation in the dependence on the mechanical stress
- Leukotrienes
  - Role in the bone remodeling



Development schema of haematopoietic precursor cell differentiation into mature osteoclasts, which are fused polykaryons arising from multiple (10–20) individual cells. Maturation occurs on bone from peripheral blood-borne mononuclear cells with traits of the macrophage lineage shown below. M-CSF (CSF-1) and RANKL are essential for osteoclastogenesis, and their action during lineage allocation and maturation is shown. OPG can bind and neutralize RANKL, and can negatively regulate both osteoclastogenesis and activation of mature osteoclasts. Shown below are the single-gene mutations that block osteoclastogenesis and activation. Those indicated in italic font are naturally occurring mutations in rodents and humans, whereas the others are the result of targeted mutagenesis to generate null alleles. Shown above are the single-gene mutant alleles that increase osteoclastogenesis and/or activation and survival and result in osteoporosis. Note that all of these mutants represent null mutations with the exception of the OPG and sRANKL transgenic mouse overexpression models (in blue-outlined boxes).



Schematic representation of the mechanism of action of **a**, pro-resorptive and calcitropic factors; and **b**, anabolic and anti-osteoclastic factors. RANKL expression is induced in osteoblasts, activated T cells, synovial fibroblasts and bone marrow stromal cells, and subsequently binds to its specific membrane-bound receptor RANK, thereby triggering a network of TRAF-mediated kinase cascades that promote osteoclast differentiation, activation and survival. Conversely, OPG expression is induced by factors that block bone catabolism and promote anabolic effects. OPG binds and neutralizes RANKL, leading to a block in osteoclastogenesis and decreased survival of pre-existing osteoclasts.

Endocrine regulation of bone metabolism

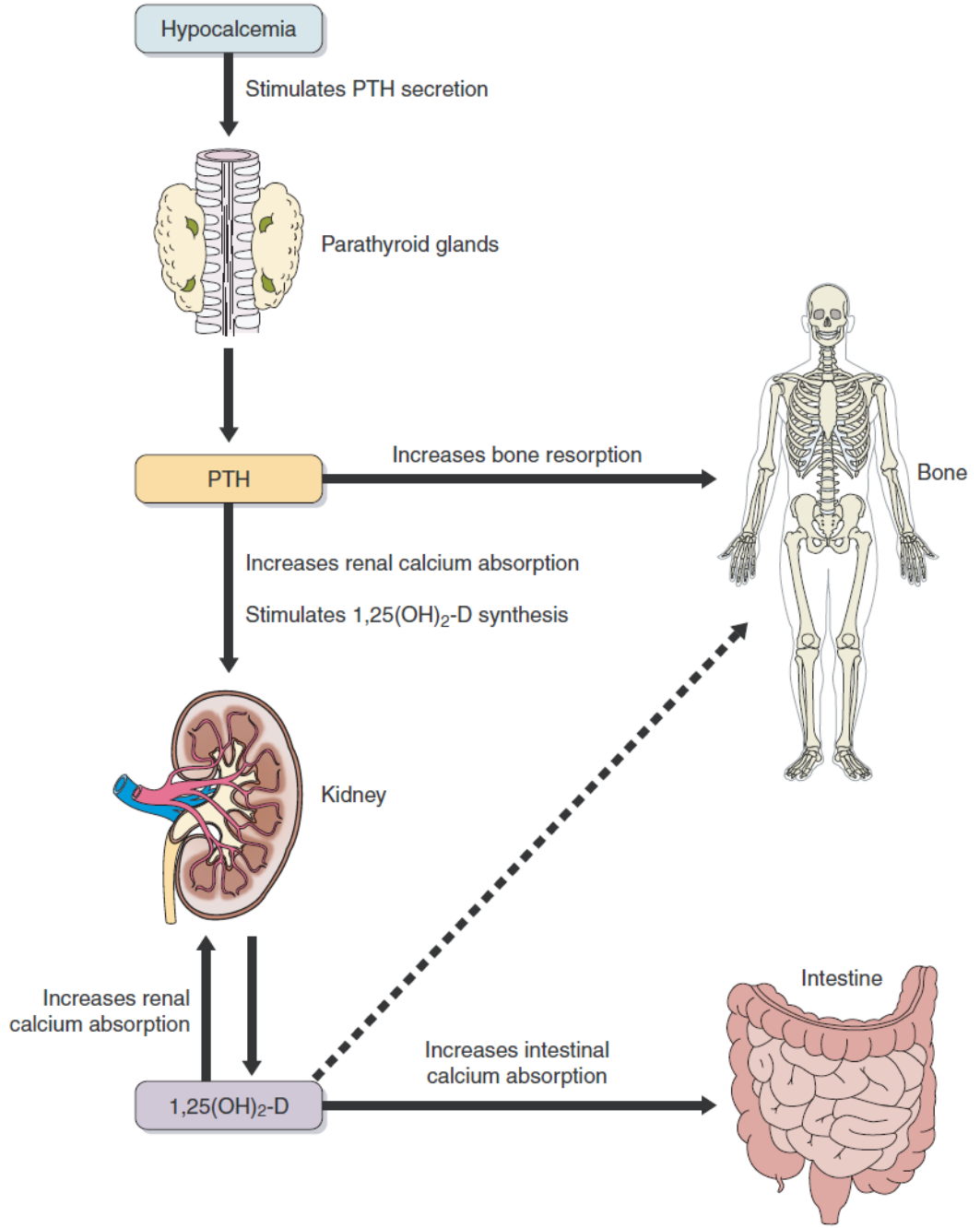
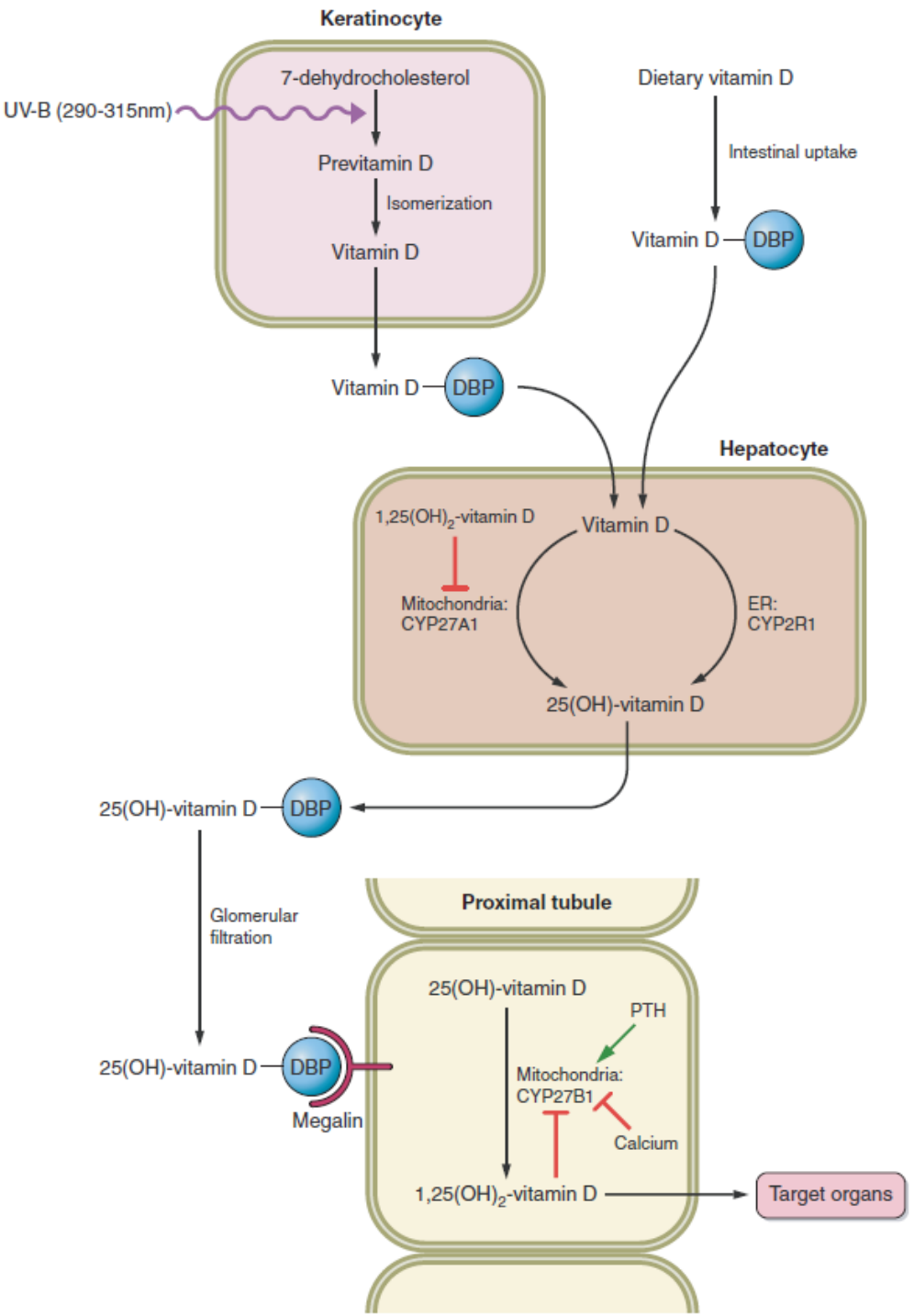
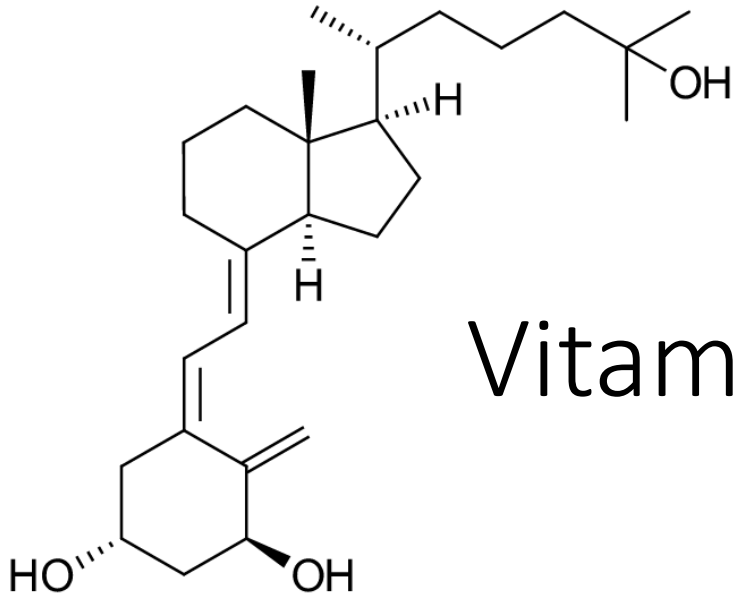


FIGURE 4. Endocrine regulation of serum calcium levels. Calcium homeostasis is mainly regulated by PTH and $1,25(OH)_2$-vitamin D. Both hormones act at their respective target organs to increase serum calcium levels.

Kopic, S., and J.P. Geibel. 2013. GASTRIC ACID, CALCIUM ABSORPTION, AND THEIR IMPACT ON BONE HEALTH. Physiological Reviews 93:189-268.



Kopic, S., and J.P. Geibel. 2013. GASTRIC ACID, CALCIUM ABSORPTION, AND THEIR IMPACT ON BONE HEALTH. Physiological Reviews 93:189-268.



Vitamin D

Přímý efekt na kostní tkáň? (osteoblasty)

FIGURE 5. Vitamin D metabolism. Vitamin D can either be synthesized in the skin or absorbed from our diet. It is then transported to the liver where it undergoes 25-hydroxylation by one of two hepatic enzymes (CYP27A1 or CYP2R1). During transport through the circulation, vitamin D is bound to a carrier protein (DBP). The 25(OH)-vitamin-D-DBP complex passes the glomerular filter and is scavenged from the primary urine by the apical megalin receptor of the proximal tubule. Here, 25(OH)-vitamin D is converted to the active vitamin D metabolite $1,25(OH)_2$-vitamin D. DBP, vitamin D binding protein.

Parathormon

PTH1R versus PTH2R

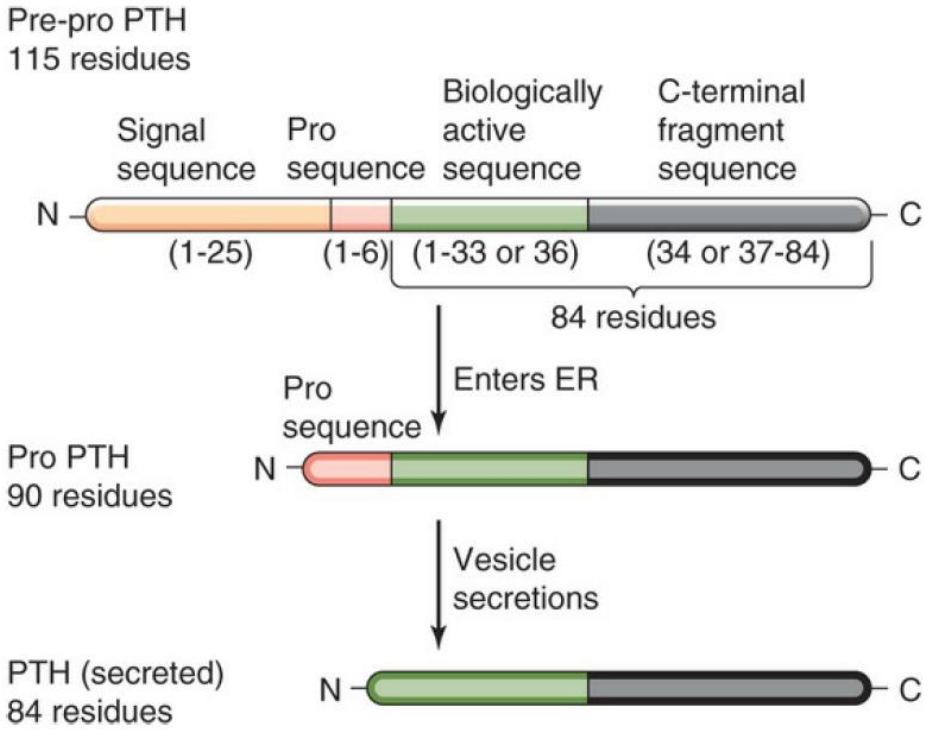
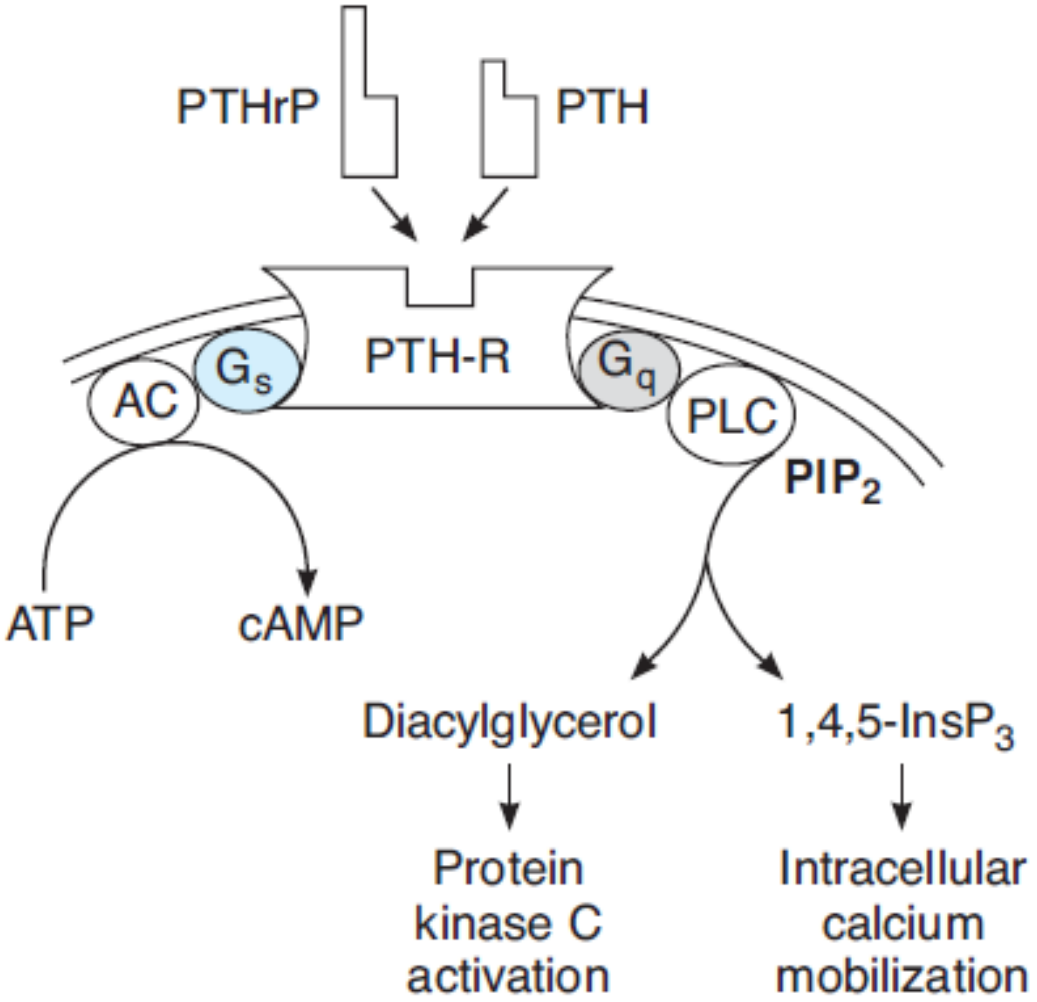
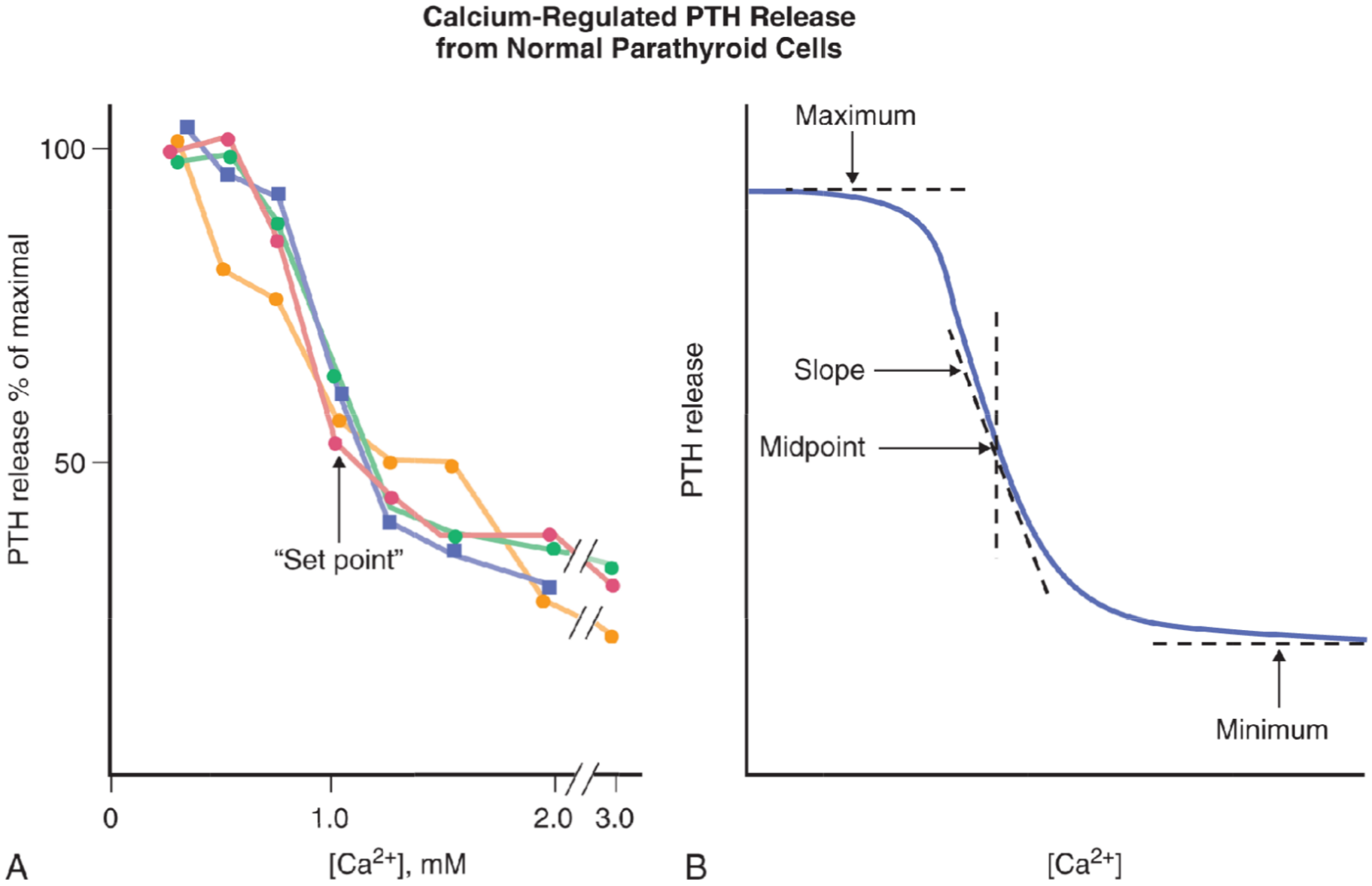


Figure 52-6 PTH synthesis. The synthesis of PTH begins with the production of pre-pro-PTH (115 amino acids) in the rough endoplasmic reticulum (ER). Cleavage of the signal sequence in the ER lumen yields pro-PTH (90 amino acids). During transit through the vesicular pathway, enzymes in the Golgi cleave the "pro" sequence, thus yielding the mature or "intact" PTH (84 amino acids), which is stored in secretory granules. Beginning in the secretory granule, enzymes cleave PTH into two fragments. The N-terminal fragment is either 33 or 36 amino acids in length and contains all the biological activity.



Barret, K.E., Boitano, S., Barman, S.M., Brooks, H.L. Ganong´s Review of Medical Physiology. 23rd Ed. McGraw-Hill Companies 2010



A, PTH secretion by dispersed normal human parathyroid cells in culture in response to varying concentrations of extracellular calcium. B, The four-parameter model describing the inverse sigmoidal relationship between extracellular calcium and PTH secretion. Parameter 1 is the maximal secretory rate, parameter 2 is the slope of the curve at the midpoint, parameter 3 is the set point, and parameter 4 is the minimum secretory rate.

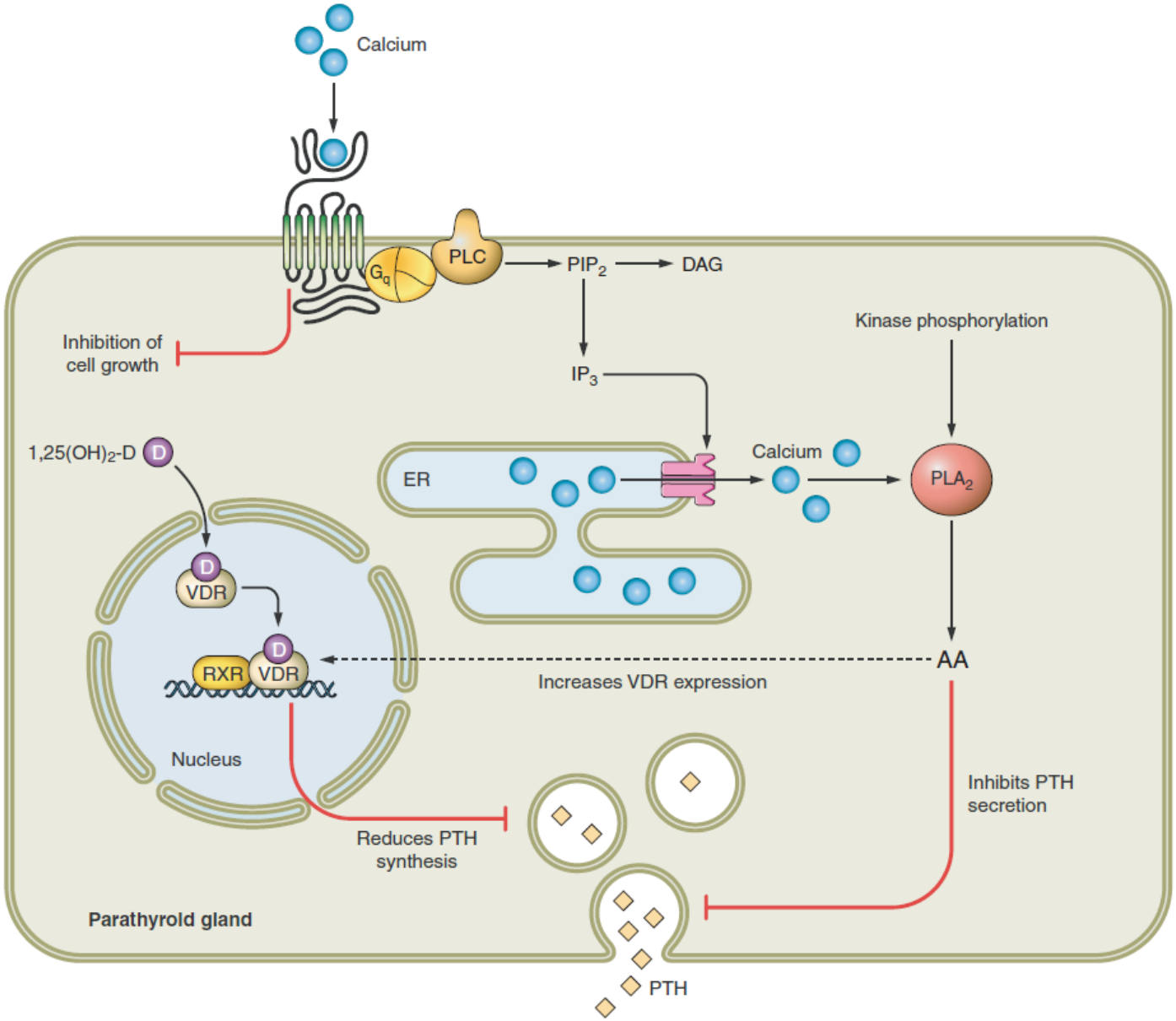
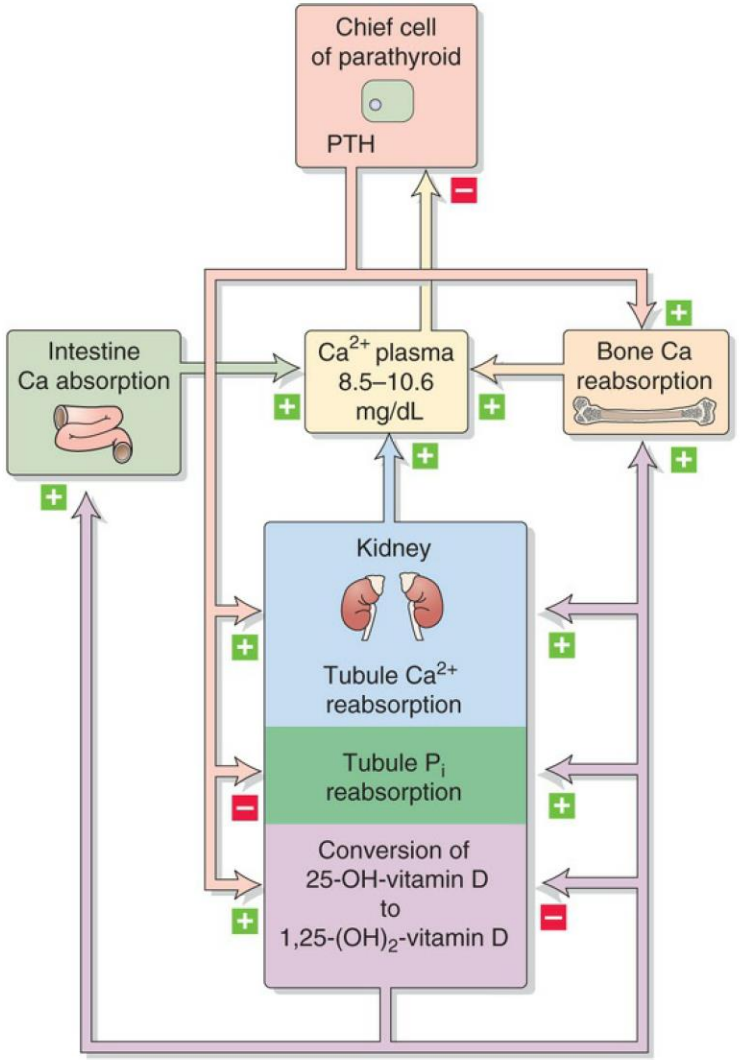


FIGURE 6. CaSR signaling in the parathyroid gland. Increased serum calcium levels lead to an inhibition of PTH secretion. Serum calcium levels are measured by the CaSR receptor. Activation of CaSR causes generation of arachidonic acid (AA) metabolites, which inhibit the release of PTH and increase the expression of VDR, thereby increasing the cell's sensitivity to the negative feedback exerted by $1,25(OH)_2$-vitamin D. $1,25(OH)_2$-vitamin D suppresses the synthesis of PTH. Furthermore, CaSR activation inhibits parathyroid gland growth.



Kopic, S., and J.P. Geibel. 2013. GASTRIC ACID, CALCIUM ABSORPTION, AND THEIR IMPACT ON BONE HEALTH. Physiological Reviews 93:189-268.

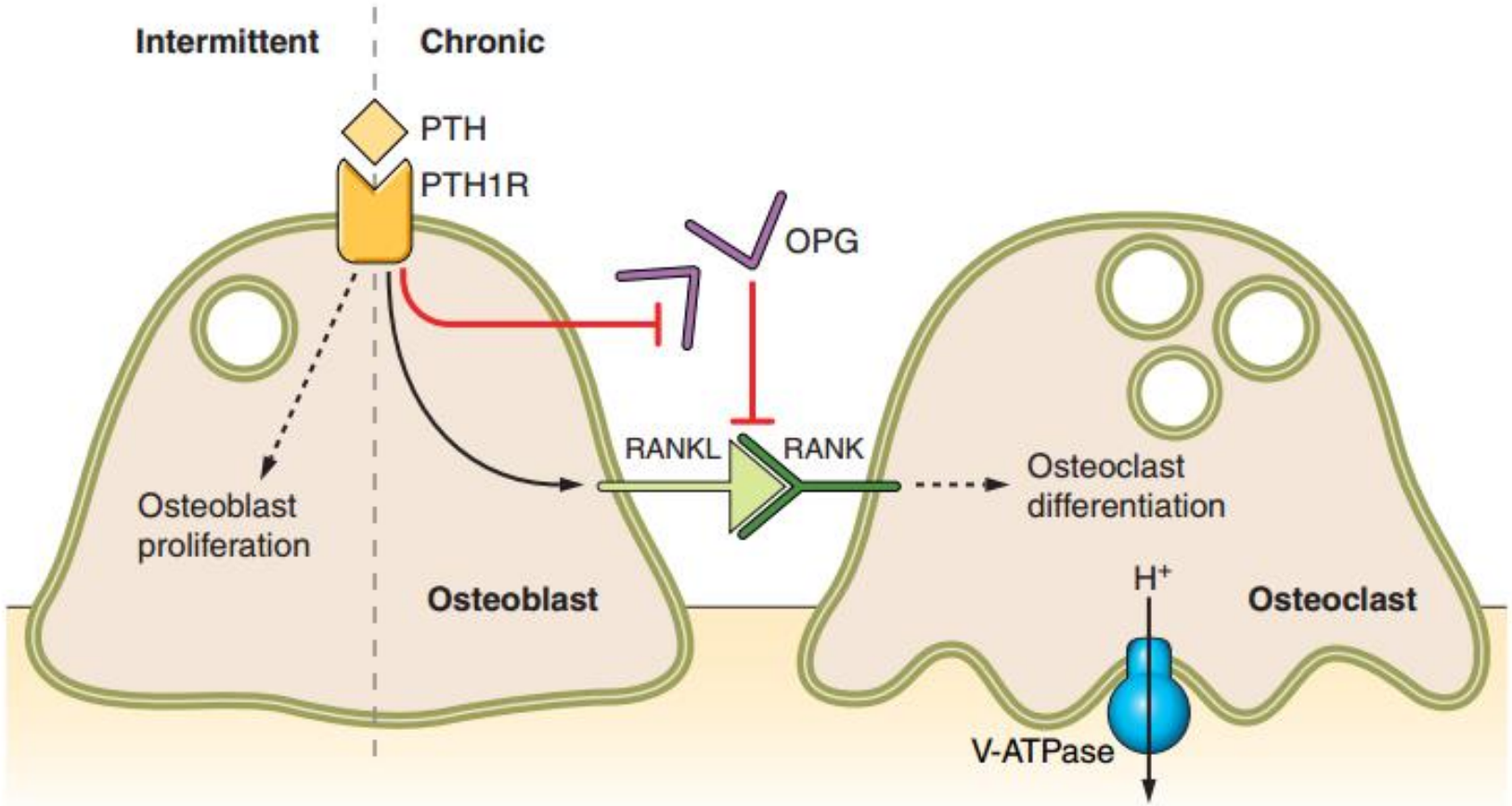
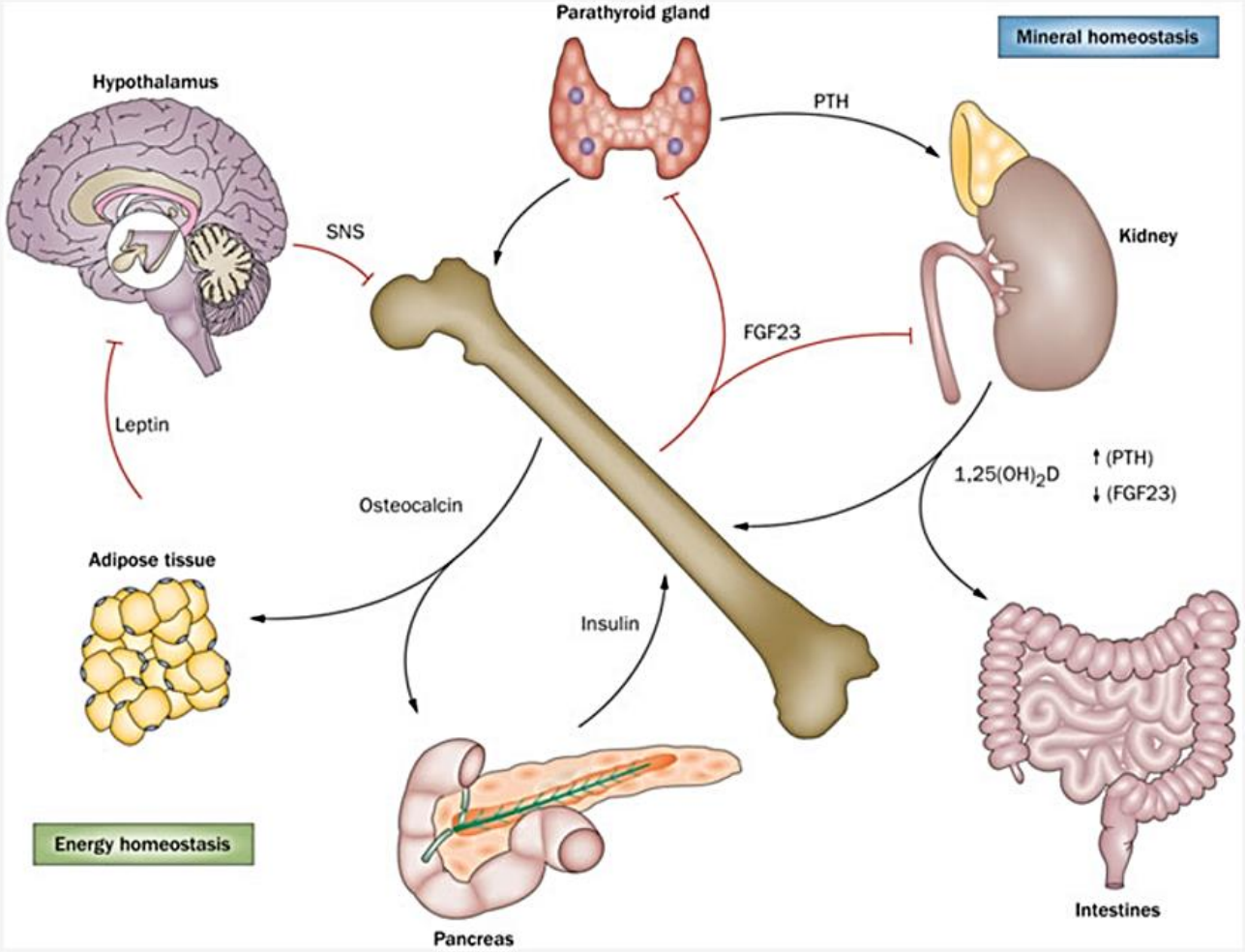


FIGURE 7. The effects of PTH on bone. PTH has a dual effect on bone. Intermittent PTH exposure causes osteoblast proliferation, leading to an increase in bone mass. Continuous PTH exposure results in RANKL upregulation and concomitant OPG suppression (OPG serves as a decoy receptor for RANKL and prevents its interaction with osteoclast RANK). The stimulated RANKL-RANK interaction leads to osteoclast proliferation and increased bone turnover.

OPG – osteoprotegrin; RANK - receptor activator of nuclear factor κB

Kopic S, Geibel JP: **GASTRIC ACID, CALCIUM ABSORPTION, AND THEIR IMPACT ON BONE HEALTH.** ***Physiol Rev 2013, 93(1):189-268.***



In mineral homeostasis, a decrease in circulating calcium stimulates the parathyroid gland to release PTH, which then causes an increase in blood calcium levels by stimulating osteoclastic bone resorption, renal calcium reabsorption and renal production of $1,25(OH)_2D$ to increase intestinal calcium absorption. Increased serum phosphate and $1,25(OH)_2D$ stimulate FGF23 production in bone, which subsequently inhibits PTH production from the parathyroid gland, inhibits $1,25(OH)_2D$ production in the kidney (thereby inhibiting intestinal absorption) and promotes renal phosphate excretion. Endocrine regulation of energy homeostasis by the skeleton is comprised of two mini loops: a negative bone–hypothalamic loop and a positive bone–pancreas loop. Leptin inhibits bone formation and the homeostatic function of the skeleton indirectly through the hypothalamus by suppressing SNS tone. However, SNS signalling also increases the production of osteocalcin from bone, which feeds into the positive loop. Osteocalcin acts on pancreatic β-cells to increase insulin production, which feeds positively back to bone, stimulating osteoblasts and driving further production of osteocalcin. Osteocalcin also acts on fat to increase the production of adiponectin, an insulin-sensitizing hormone. Abbreviations: $1,25(OH)_2D$, active vitamin D; 25(OH)D, 25-hydroxyvitamin D; FGF23, fibroblast growth factor 23; PTH, parathyroid hormone; SNS, sympathetic nervous system.

DiGirolamo, D.J., T.L. Clemens, and S. Kousteni. 2012. The skeleton as an endocrine organ. *Nature Reviews Rheumatology 8:674-683.*

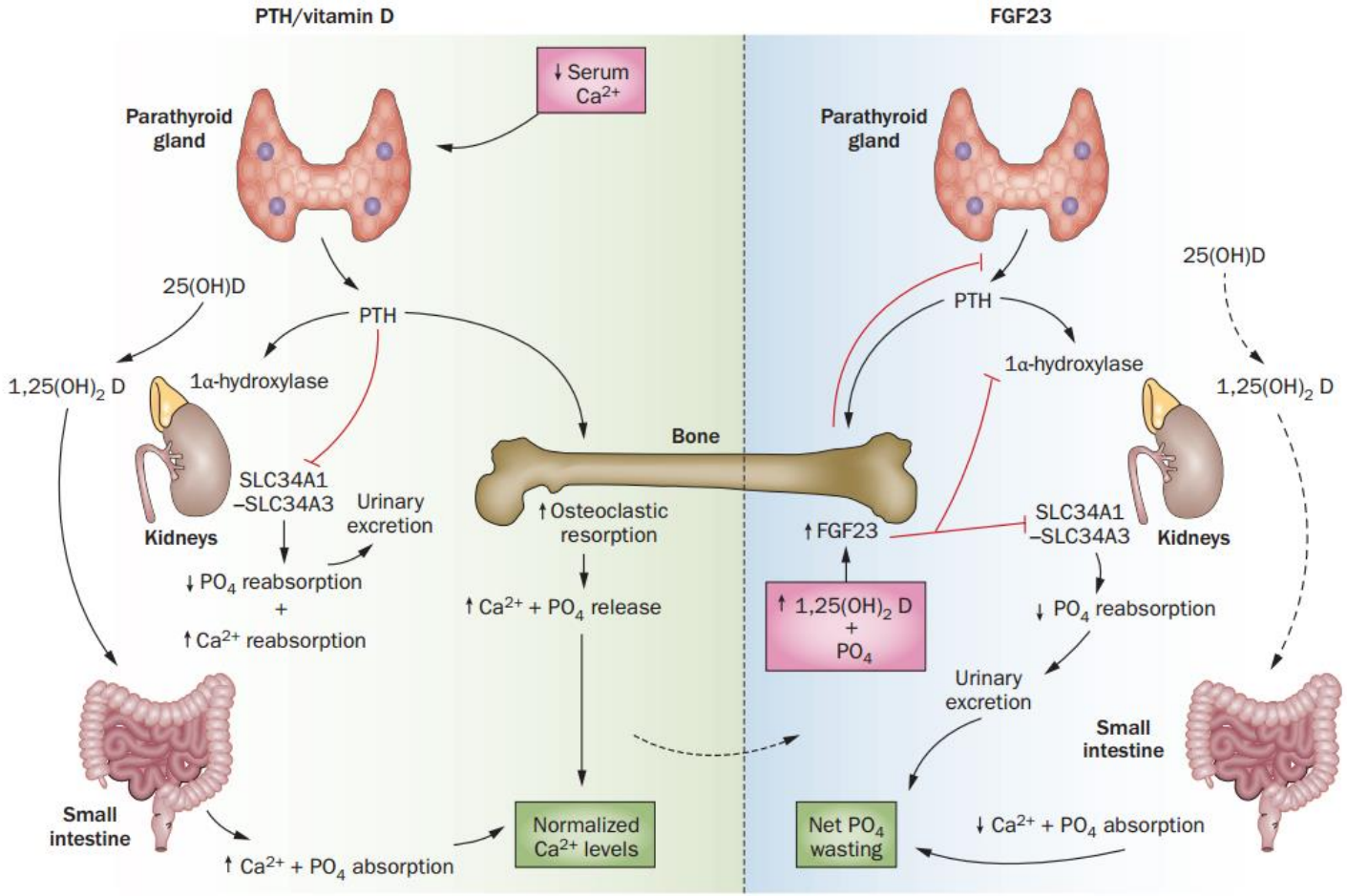


Figure 3 | The regulation of calcium and phosphate homeostasis by PTH, vitamin D and FGF23. The parathyroid gland detects changes in the level of calcium in blood by means of the calcium-sensing receptor, which then modulates the secretion of PTH. A decrease in circulating calcium stimulates the parathyroid gland to produce and release PTH. Circulating PTH then works in a rapid, pleiotropic fashion to increase blood calcium levels by stimulating osteoclastic bone resorption to release calcium and phosphate, calcium reabsorption and phosphate excretion in the renal distal convoluted tubule by downregulating the sodium–phosphate co-transporters SLC34A1–SLC34A3, and production of $1,25(OH)_2D$ by 1α-hydroxylase in the kidney, which, in turn, increases intestinal calcium and phosphate absorption. The kidney is the principle physiological target, where FGF23 signalling acts to promote phosphate excretion by downregulating SLC34A1–SLC34A3 and inhibiting $1,25(OH)_2D$ production, thus preventing vitamin-D-mediated phosphate absorption in the gut. Serum levels of FGF23 increase in response to increased serum phosphate and $1,25(OH)_2D$, and furthermore, FGF23 inhibits the production of PTH. Boxes in pink show input into the system, boxes in green show the output. Abbreviations: $1,25(OH)_2D$, active vitamin D; 25(OH)D, 25-hydroxyvitamin D; Ca^{2+}, calcium; FGF23, fibroblast growth factor 23; FGFR, fibroblast growth factor receptor; PO_4, phosphate; PTH, parathyroid hormone; SLC34A1, sodium-dependent phosphate transport protein 2A (also known as NPT2a); SLC34A3, sodium-dependent phosphate transport protein 2C (also known as NPT2c).

DiGirolamo, D.J., T.L. Clemens, and S. Kousteni. 2012. The skeleton as an endocrine organ. *Nature Reviews Rheumatology 8:674-683.*

Parathyroid hormone-related peptide (PTHrP) and hypercalcemia

- “ectopic” production by cancers of peptide hormones (ACTH, PTH?)
- PTH? – hypercalcemia, hypophosphataemia (bone metastases, renal cancer, lung cancer, some neuroendocrine tumors)
- Radioimmunoassays – PTHrP (↓ PTH versus ↑ PTHrP)
- Physiological functions of PTHrP?
  - **auto-/para-**/endocrine
  - affecting endochondral bone formation - blocks maturation of chondrocytes
  - growth and differentiation of mammary gland, skin and pancreatic islets
  - smooth muscle relaxation
  - transepithelial transport of calcium (placenta)

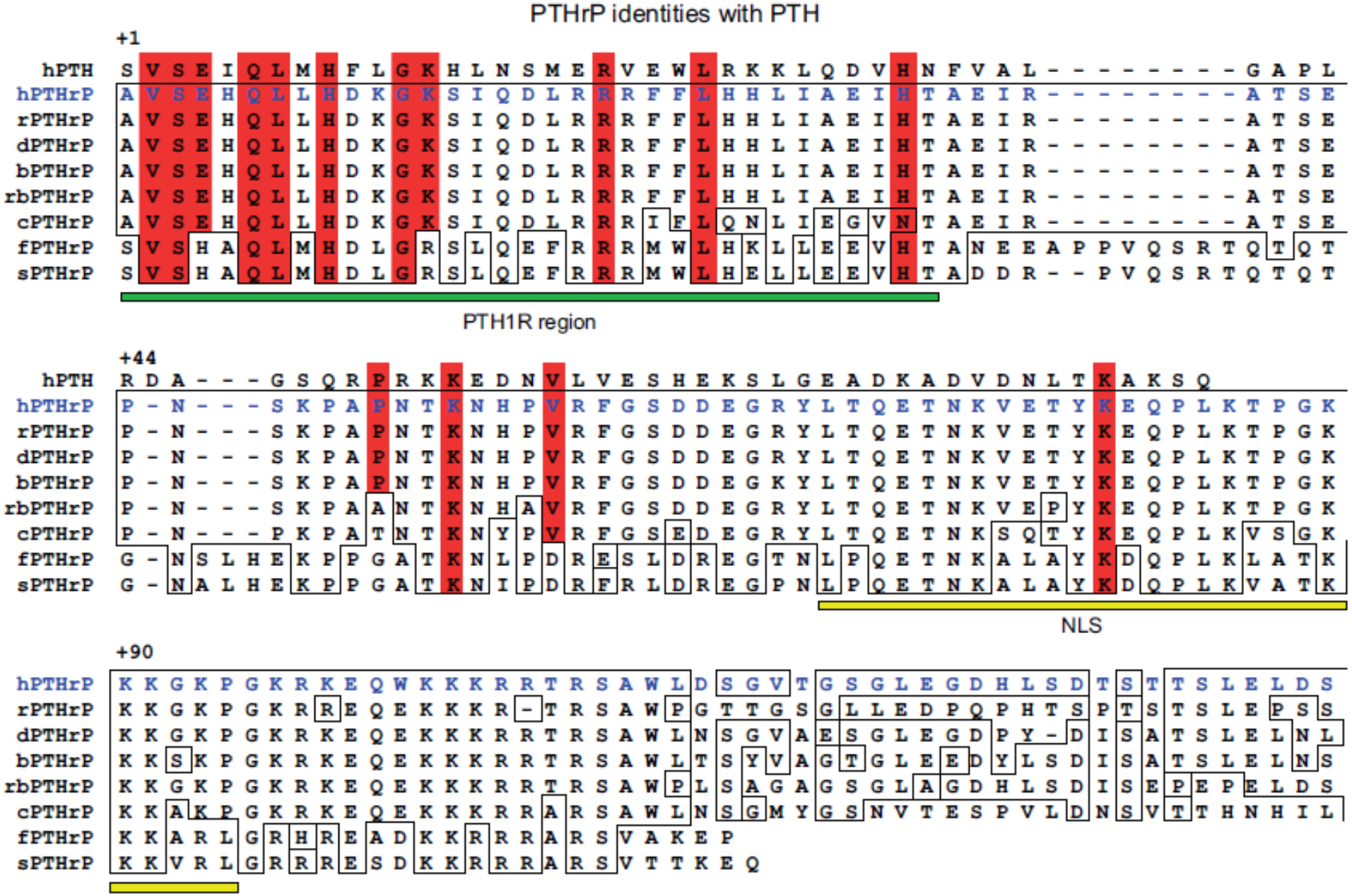


FIGURE 1. Comparison with human PTH, predicted primary structures of PTHrP in several species, based on cDNA sequences: human (332), dog (291), bovine (380), rat (159), mouse (157), chicken (297), fugu (93), seabream (276). Residues in red indicate those conserved across all species.

Martin, T.J. 2016. PARATHYROID HORMONE-RELATED PROTEIN, ITS REGULATION OF CARTILAGE AND BONE DEVELOPMENT, AND ROLE IN TREATING BONE DISEASES. *Physiological Reviews 96:831-871.*

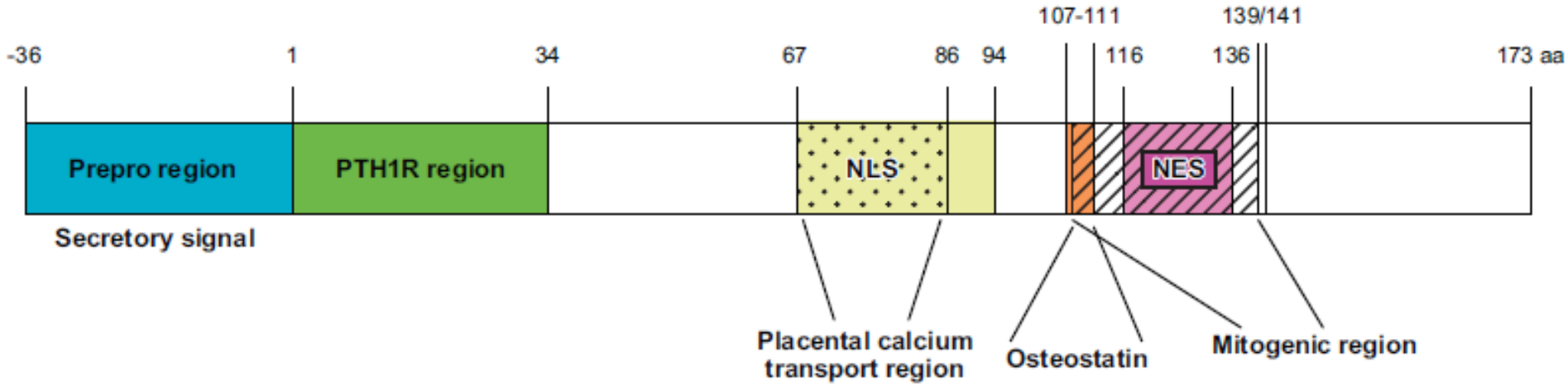


FIGURE 3. Functional domains of PTHrP. The 3 isoforms resulting from alternative splicing terminate at 139, 141, and 173 amino acids (aa). The prepro region (blue) includes the signal sequence (−36-1 aa). The PTH-like PTH1R region (green) binds to the PTH1R receptor (1–34 aa). The region responsible for placental calcium transport is stippled (67–86 aa). The nuclear localizing sequence (NLS) (yellow) is (67–94 aa), and the nuclear export sequence (NES) (pink) is (116–136 aa). The osteostatin region (orange) is (107–111 aa). The region that is mitogenic in osteoblast and vascular smooth muscle cells (striped) is (108–139 aa).

Martin, T.J. 2016. PARATHYROID HORMONE-RELATED PROTEIN, ITS REGULATION OF CARTILAGE AND BONE DEVELOPMENT, AND ROLE IN TREATING BONE DISEASES. *Physiological Reviews 96:831-871.*

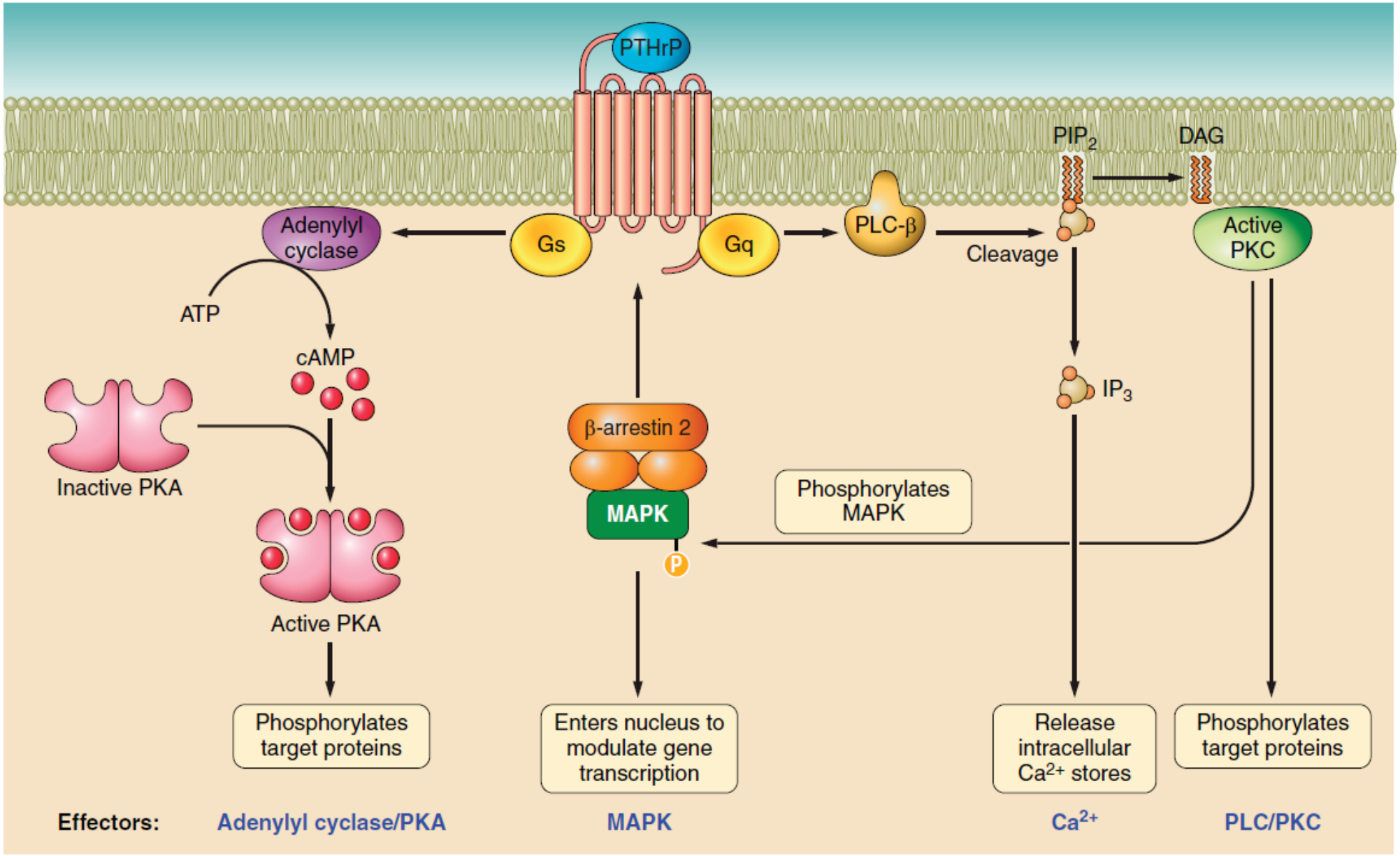
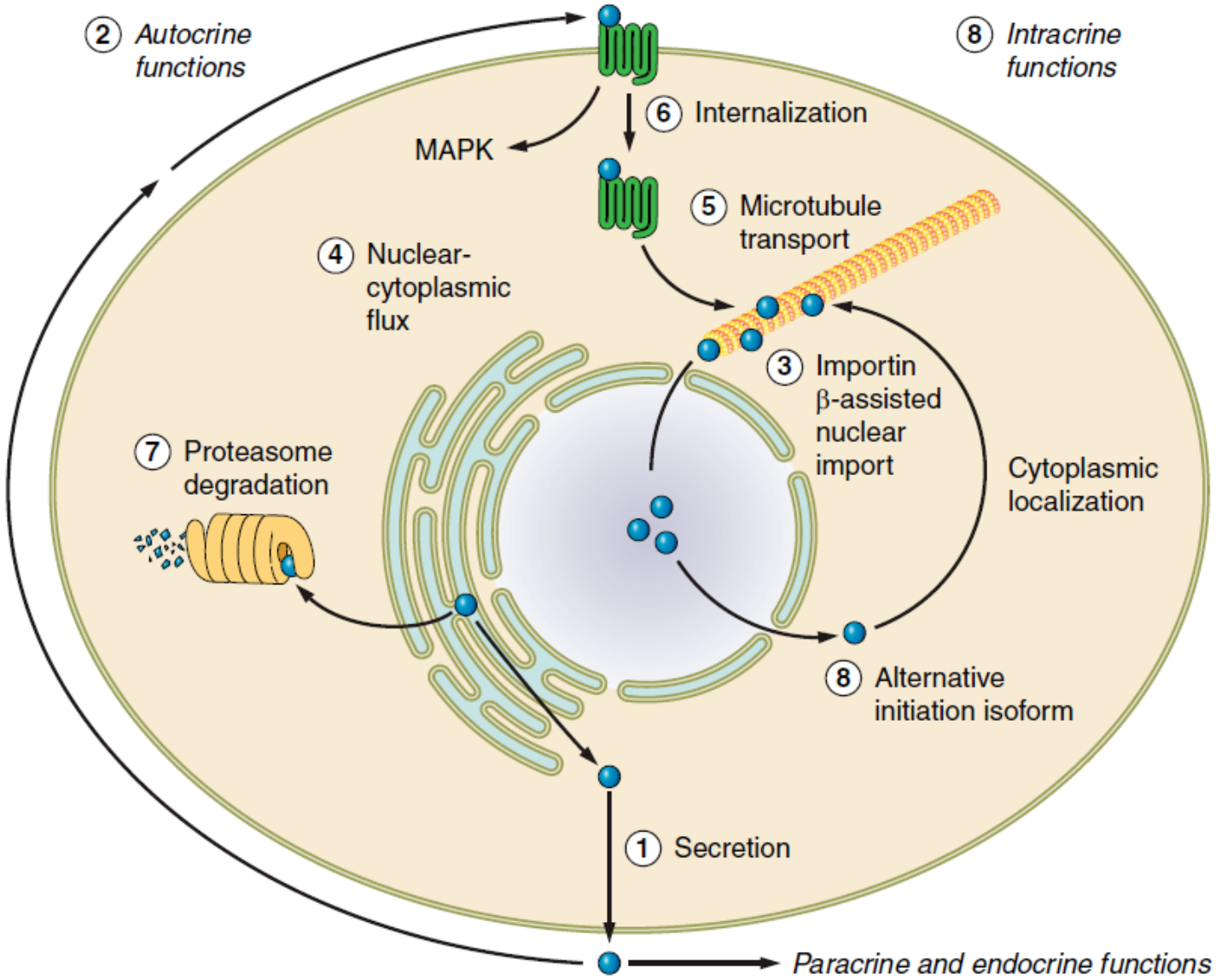


FIGURE 5. Signal transduction pathways in ligand-induced activation of PTHR1. Ligand binding leads to association with Gs α subunit and adenylyl cyclase activation, or with Gq α that activates phospholipase C-β (PLC-β). MAPK can be involved through interaction of PTHR1 with the MAPK scaffolding protein β-arrestin 2. (Figure drawn by L. Conlan.)

Martin, T.J. 2016. PARATHYROID HORMONE-RELATED PROTEIN, ITS REGULATION OF CARTILAGE AND BONE DEVELOPMENT, AND ROLE IN TREATING BONE DISEASES. *Physiological Reviews 96:831-871.*



Intrakrinní funkce – regulace buněčné proliferace a apoptózy?

FIGURE 7. Trafficking pathways of PTHrP. After synthesis as a prepromolecule, PTHrP is targetted to the ER before secretion (*1*), or can be subject to proteasomal degradation (*7*). Secreted PTHrP acts in a paracrine or autocrine manner by binding to PTHR1 (*2*), activating signaling, and can be internalized (*6*) and escape degradation to localize in the nucleus (*4*). PTHrP can remain intracellular to act in an intracrine manner, sometimes as a result of translation from an alternative start codon (*8*), and then transported to the nucleus by importin β (*3*, *5*). See text for details.

Martin, T.J. 2016. PARATHYROID HORMONE-RELATED PROTEIN, ITS REGULATION OF CARTILAGE AND BONE DEVELOPMENT, AND ROLE IN TREATING BONE DISEASES. *Physiological Reviews 96:831-871.*

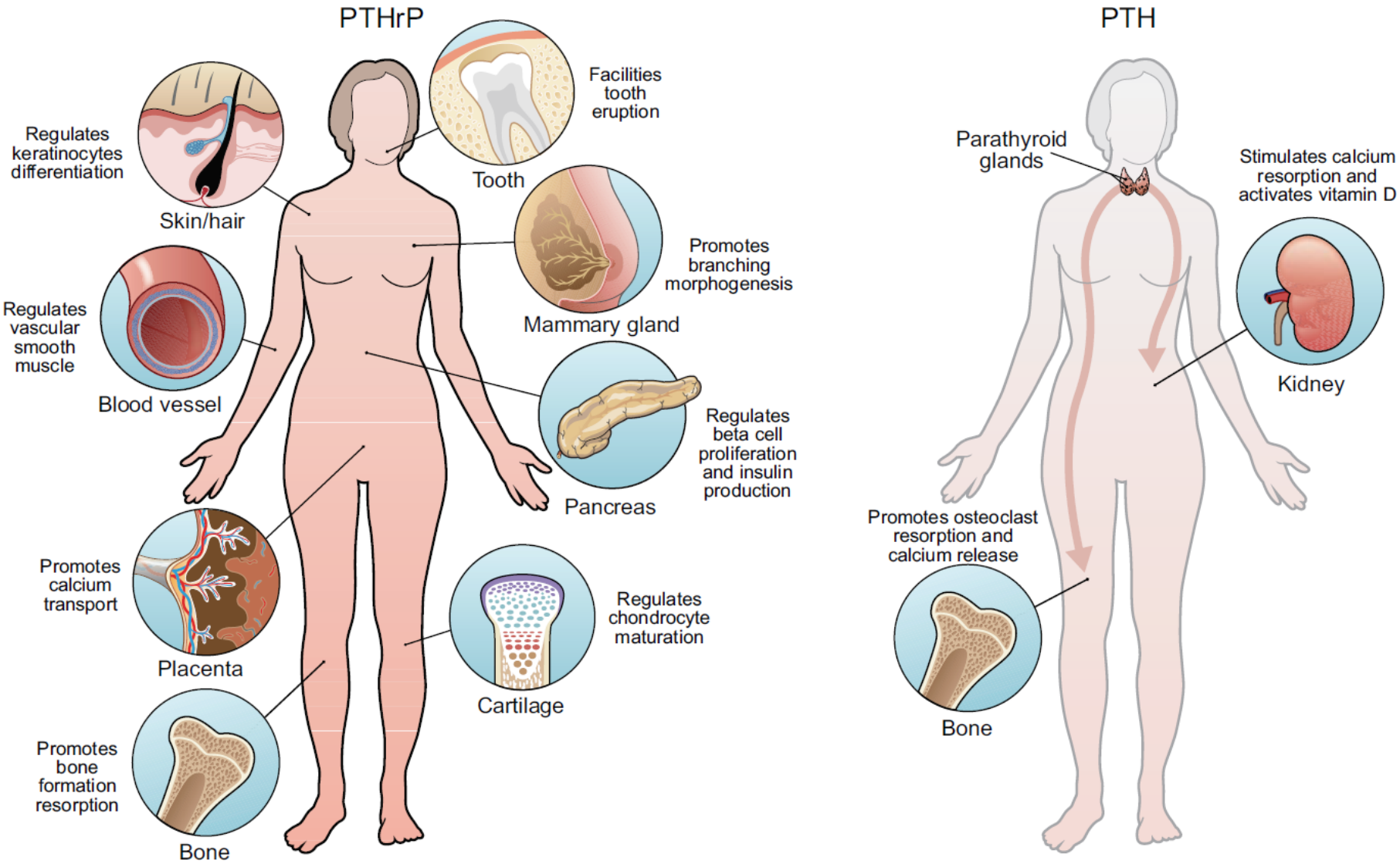


FIGURE 8. Paracrine actions of PTHrP and endocrine actions of PTH. PTHrP has paracrine actions in physiological homeostasis in many tissues, including keratinocytes/hair follicles, cartilage, vascular smooth muscle, bone, mammary gland development, tooth eruption, and pancreas, whereas PTH has relatively fewer physiological actions through its role as a circulating hormone. The summary diagram omits important details such as the role of PTHrP in lactation (see text for details). [From McCauley and Martin (231), with permission from Wiley.]

Martin, T.J. 2016. PARATHYROID HORMONE-RELATED PROTEIN, ITS REGULATION OF CARTILAGE AND BONE DEVELOPMENT, AND ROLE IN TREATING BONE DISEASES. *Physiological Reviews 96:831-871.*

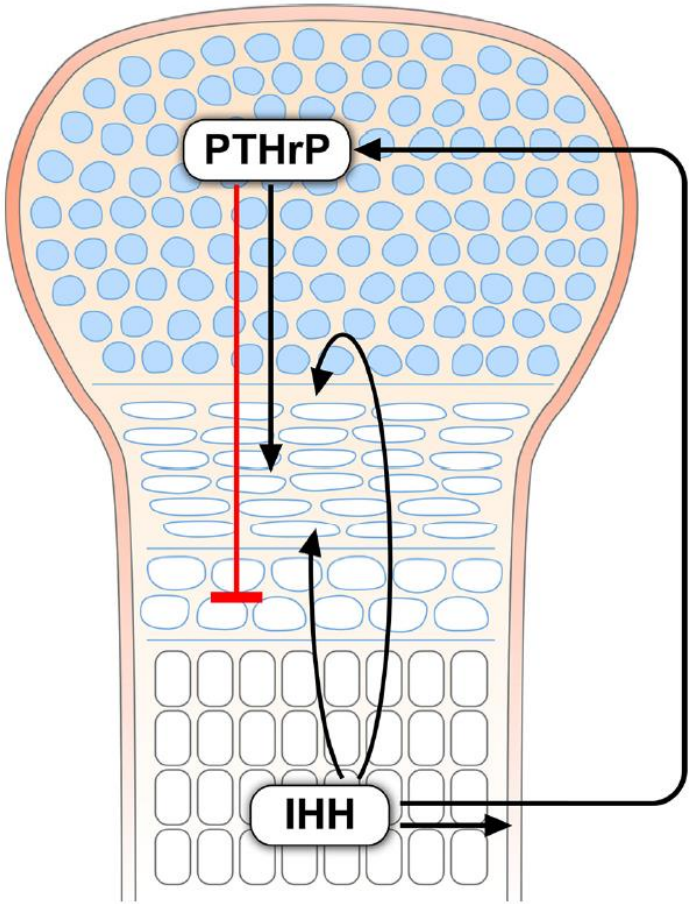


FIGURE 9. Growth plate interactions of PTHrP and Ihh. PTHrP is produced by chondrocytes at the end of long bones. It stimulates proliferation of adjacent chondrocytes and delays them from further differentiating into prehypertophic and then hypertrophic chondrocytes. Synthesis of Ihh by hypertrophic chondrocytes begins when the signal of PTHrP is no longer able to reach those cells. Ihh increases proliferation and accelerates differentiation into prehypertrophic chondrocytes, promotes the formation of osteoblasts from adjacent perichondrial cell, and completes a feedback control system by promoting PTHrP production at the articular end (see text for further details). [Modified from Maes and Kronenberg (207), with permission from Elsevier.]

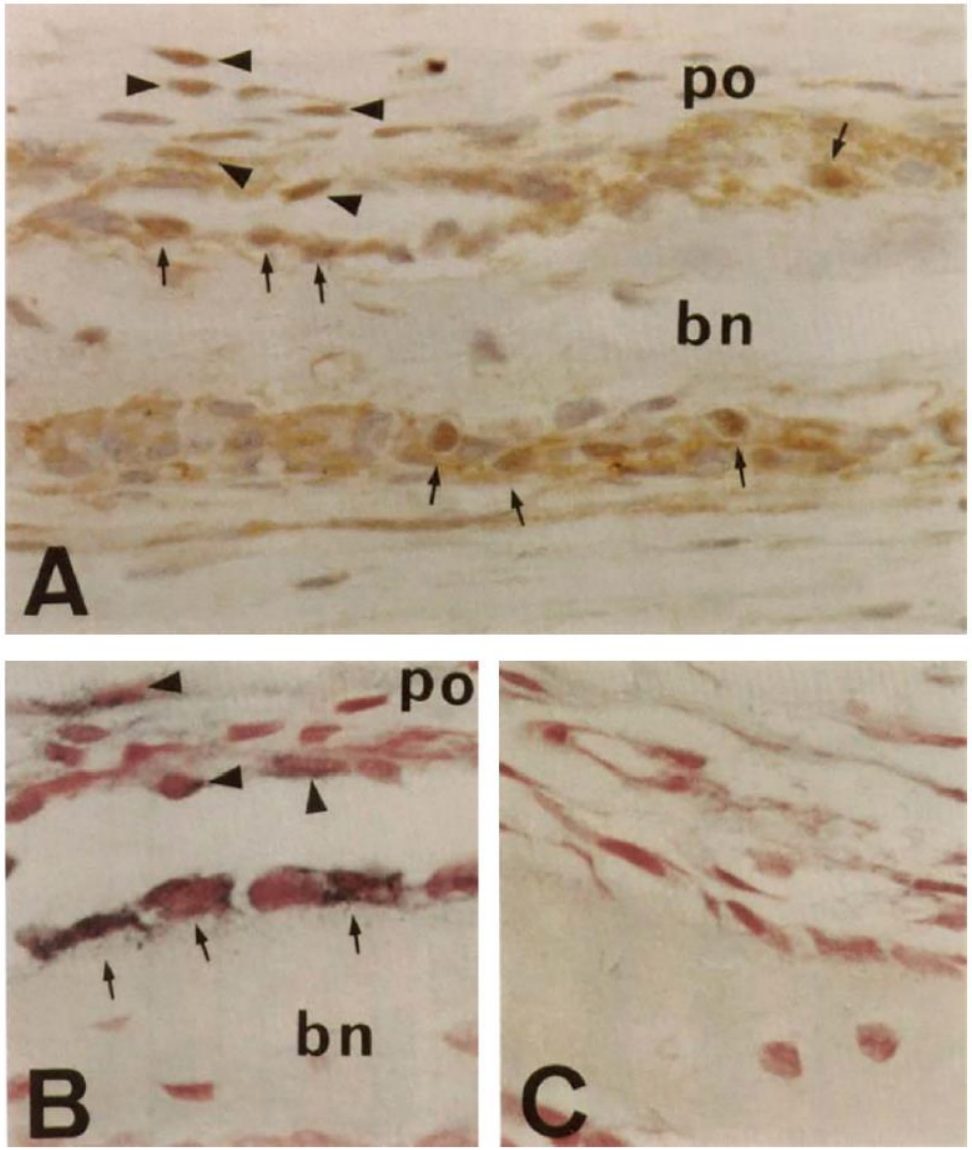


FIGURE 10. Immunohistochemistry and in situ hybridization in newborn rat calvarial sections. PTHrP localized in cells (arrows) in periosteum and in osteoblasts adjacent to cortical bone. ISH with antisense (*A*) and sense (*B*) riboprobes shows PTHrP mRNA in the same cells. [From Suda et al. (328).]

Martin, T.J. 2016. PARATHYROID HORMONE-RELATED PROTEIN, ITS REGULATION OF CARTILAGE AND BONE DEVELOPMENT, AND ROLE IN TREATING BONE DISEASES. *Physiological Reviews 96:831-871.*

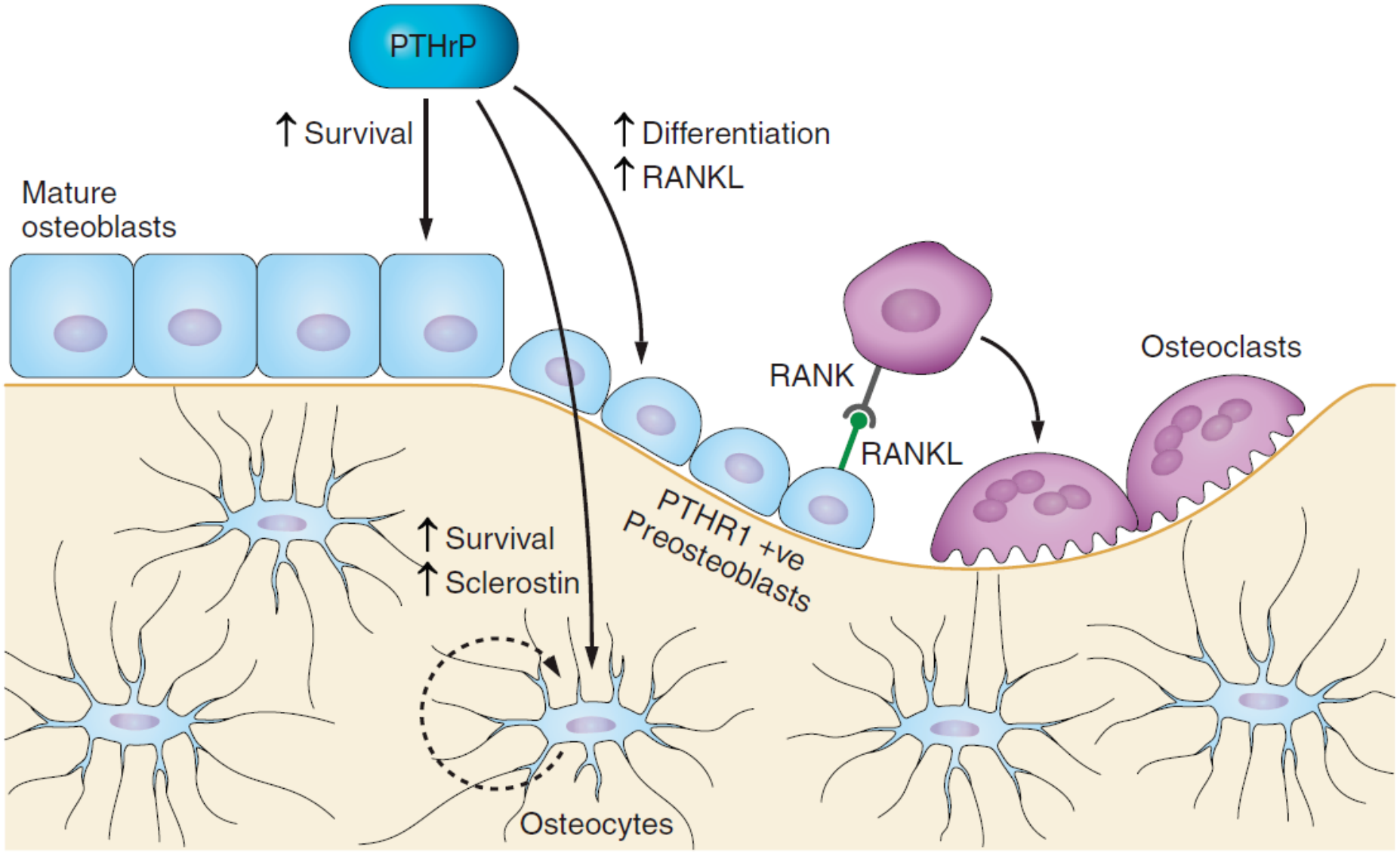


FIGURE 12. Paracrine actions of PTHrP in bone remodeling. PTHrP produced by cells early in the osteoblast lineage acts on cells of the lineage that have differentiated to the stage of possessing the PTH1R, promoting their differentiation and therefore bone formation, as well as increasing production of RANKL and osteoclast formation. PTHrP also inhibits apoptosis of mature osteoblasts, of earlier cells, and of osteocytes (see text for details).

Martin, T.J. 2016. PARATHYROID HORMONE-RELATED PROTEIN, ITS REGULATION OF CARTILAGE AND BONE DEVELOPMENT, AND ROLE IN TREATING BONE DISEASES. *Physiological Reviews 96:831-871.*

cAMP response element binding (CREB) protein

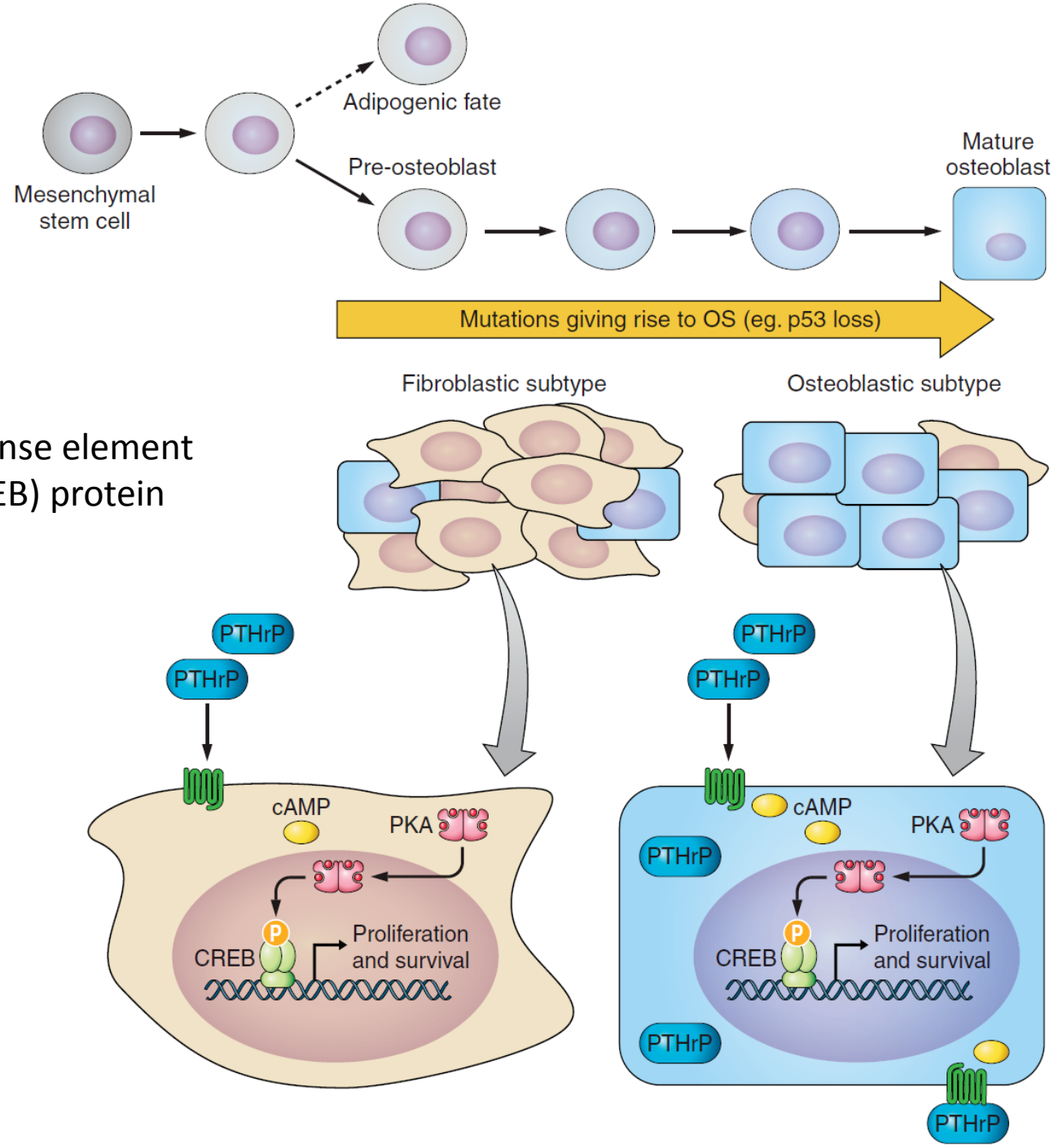


FIGURE 13. Fibroblastic and osteoblastic osteosarcoma subtypes; origins and regulation by PTHrP-PTHR1-CREB axis. Subtypes of osteosarcoma arise from unique cells in the lineage from skeletal stem cell through to osteoblast. PTHrP, PTHR1, and CREB activities are greater in osteoblastic than in fibroblastic subtype, and intracrine PTHrP may contribute (131, 247) (see text for details). (Figure drawn by C. Walkley.)

Martin, T.J. 2016. PARATHYROID HORMONE-RELATED PROTEIN, ITS REGULATION OF CARTILAGE AND BONE DEVELOPMENT, AND ROLE IN TREATING BONE DISEASES. *Physiological Reviews 96:831-871.*

Calcitonin

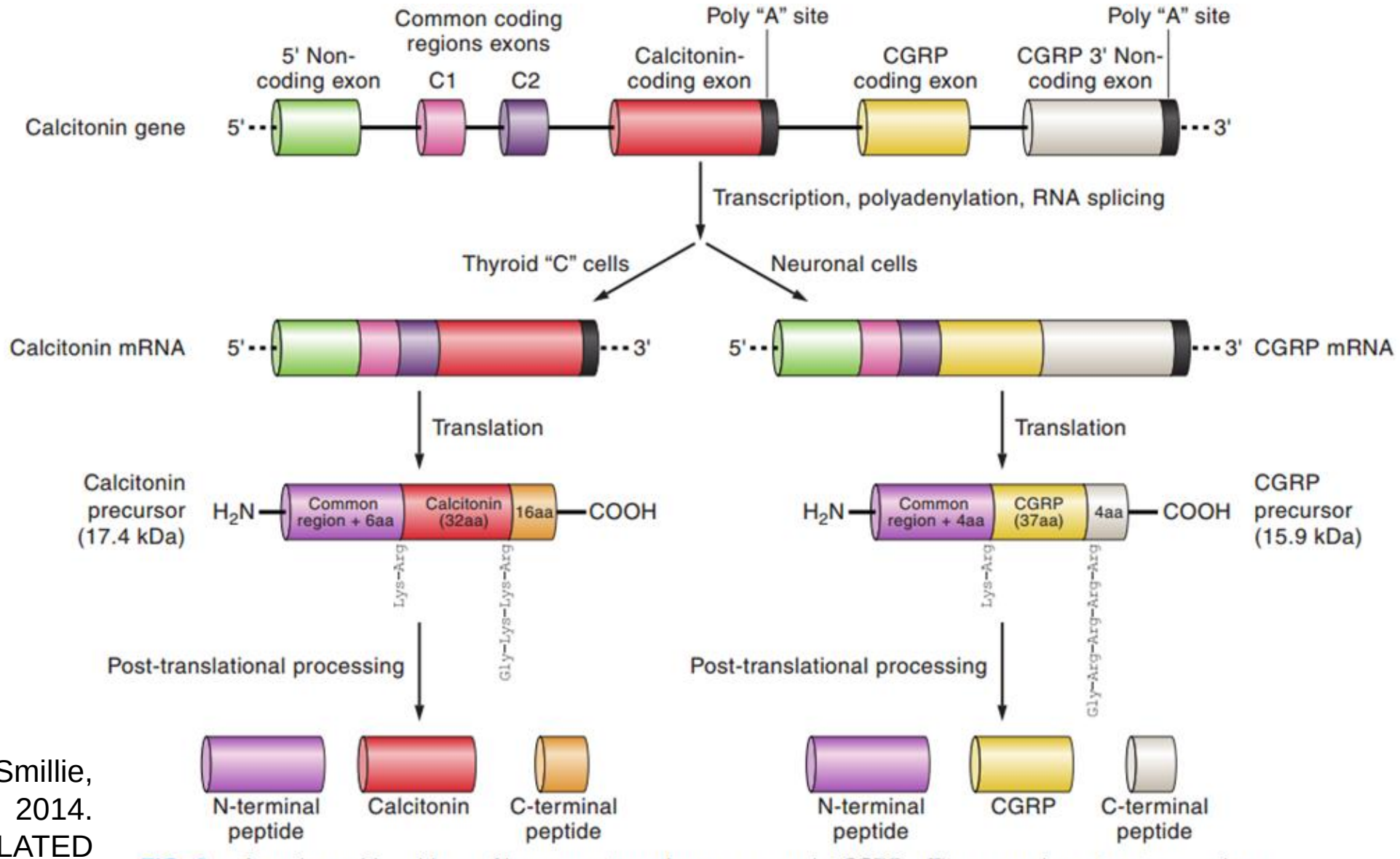
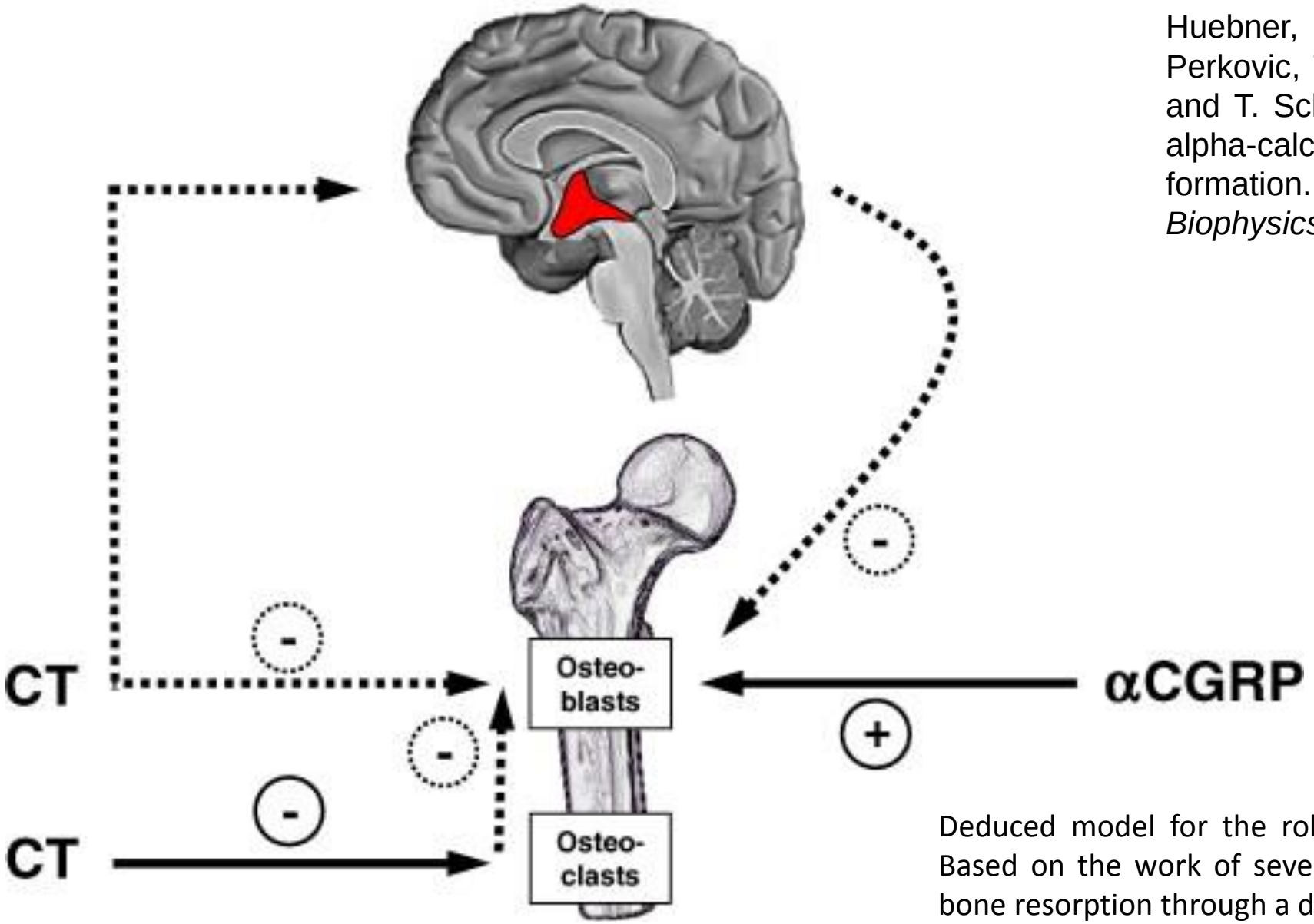


FIG. 1. *A*: amino acid residues of human, rat, and mouse α- and β-CGRPs. The secondary structure regions and disulfide bonds are indicated. The residues in bold are nonidentical homologs to the human-α-CGRP. In italics are the residues that are nonidentical homologs between the α- and β-CGRP of the same species. *B*: processing of the calcitonin CALC I gene leading to either primarily calcitonin in the thyroid or α-CGRP in sensory neurons.

Russell, F.A., R. King, S.J. Smillie, X. Kodji, and S.D. Brain. 2014. CALCITONIN GENE-RELATED PEPTIDE: PHYSIOLOGY AND PATHOPHYSIOLOGY. *Physiological Reviews 94:1099-1142.*



Huebner, A.K., J. Keller, P. Catala-Lehnen, S. Perkovic, T. Streichert, R.B. Emeson, M. Amling, and T. Schinke. 2008. The role of calcitonin and alpha-calcitonin gene-related peptide in bone formation. *Archives of Biochemistry and Biophysics 473:210-217.*

Deduced model for the roles of CT and α-CGRP in bone formation. Based on the work of several investigators it is likely that CT inhibits bone resorption through a direct effect on osteoclasts, and that α-CGRP activates bone formation through a direct effect on osteoblasts (solid lines). The negative influence of CT on bone formation however, may be indirectly mediated by the hypothalamus or by osteoclasts (dashed lines).

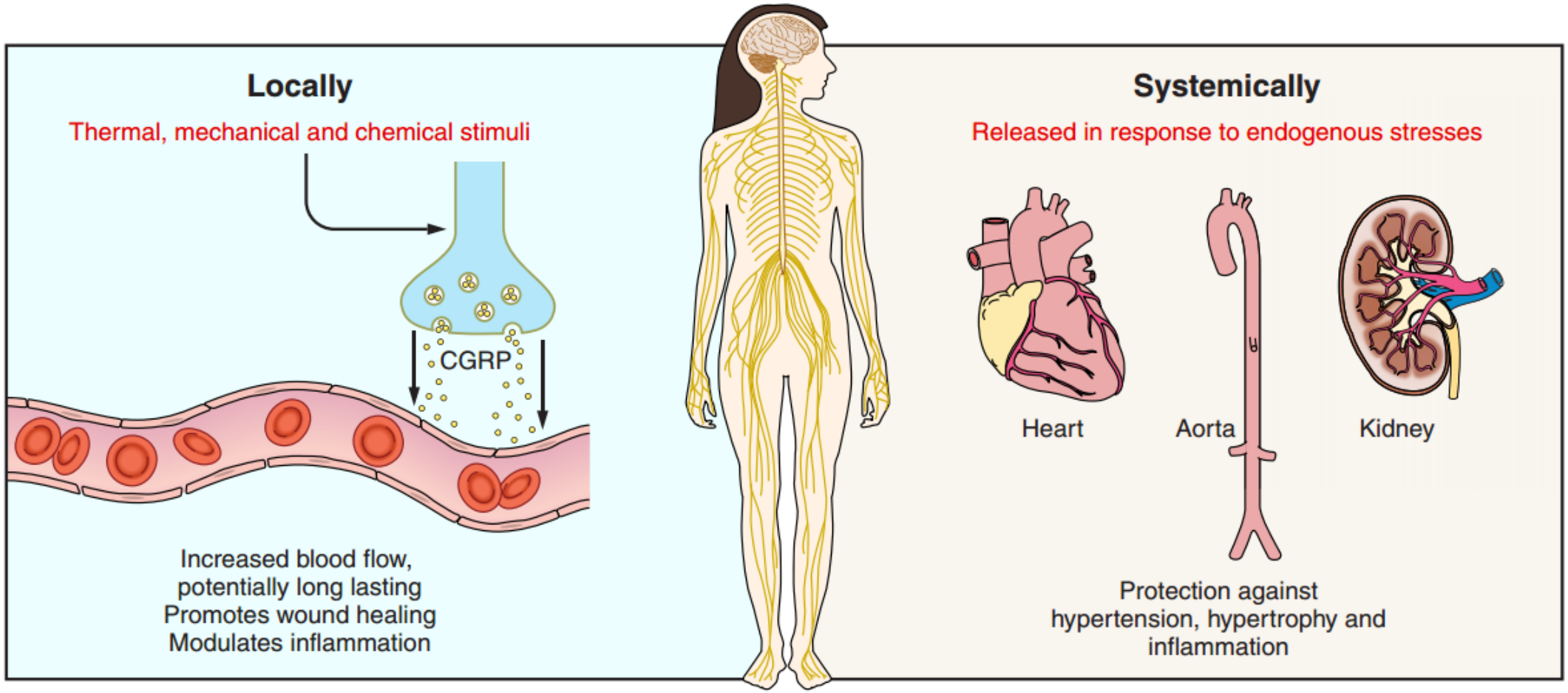
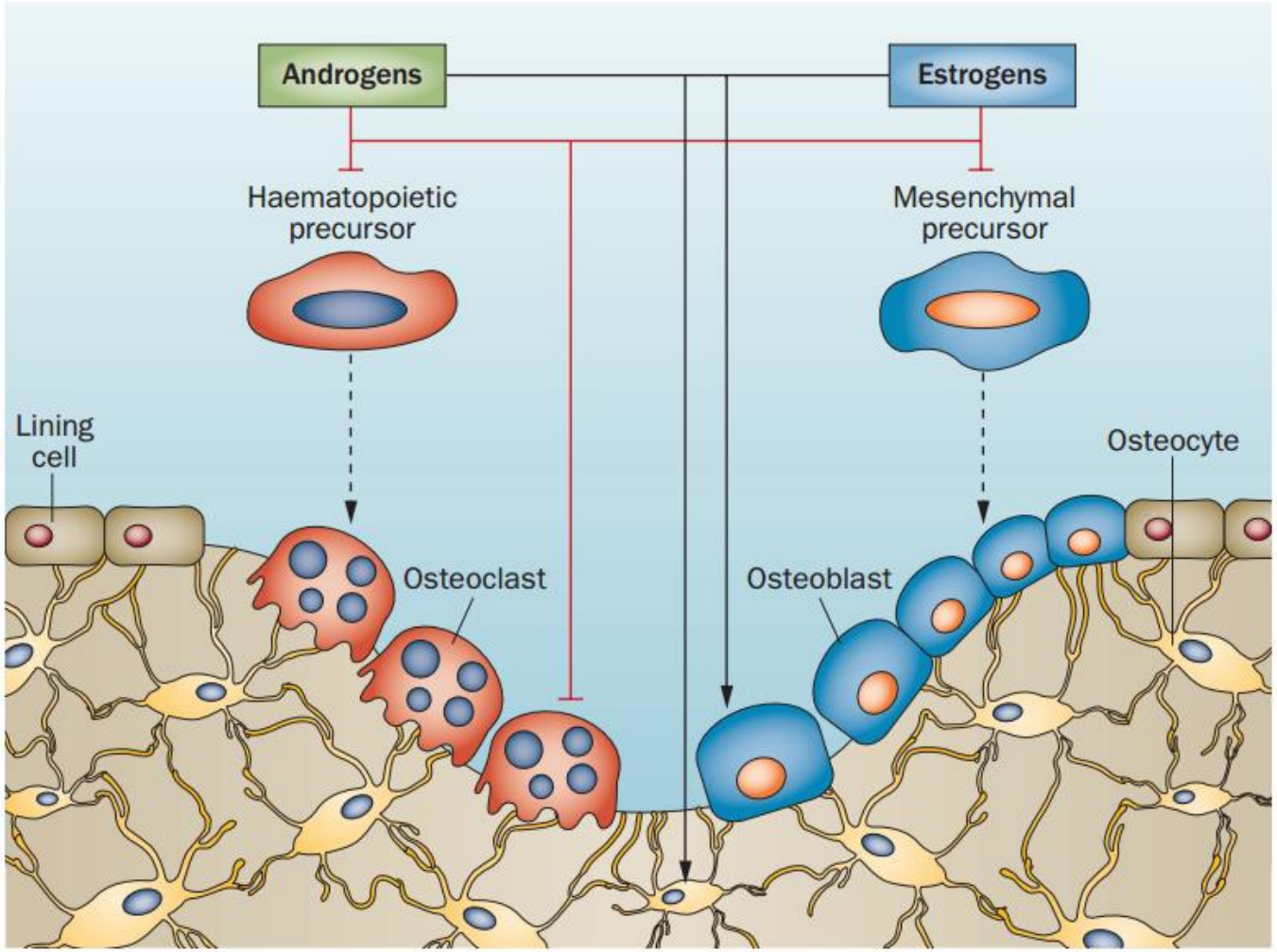
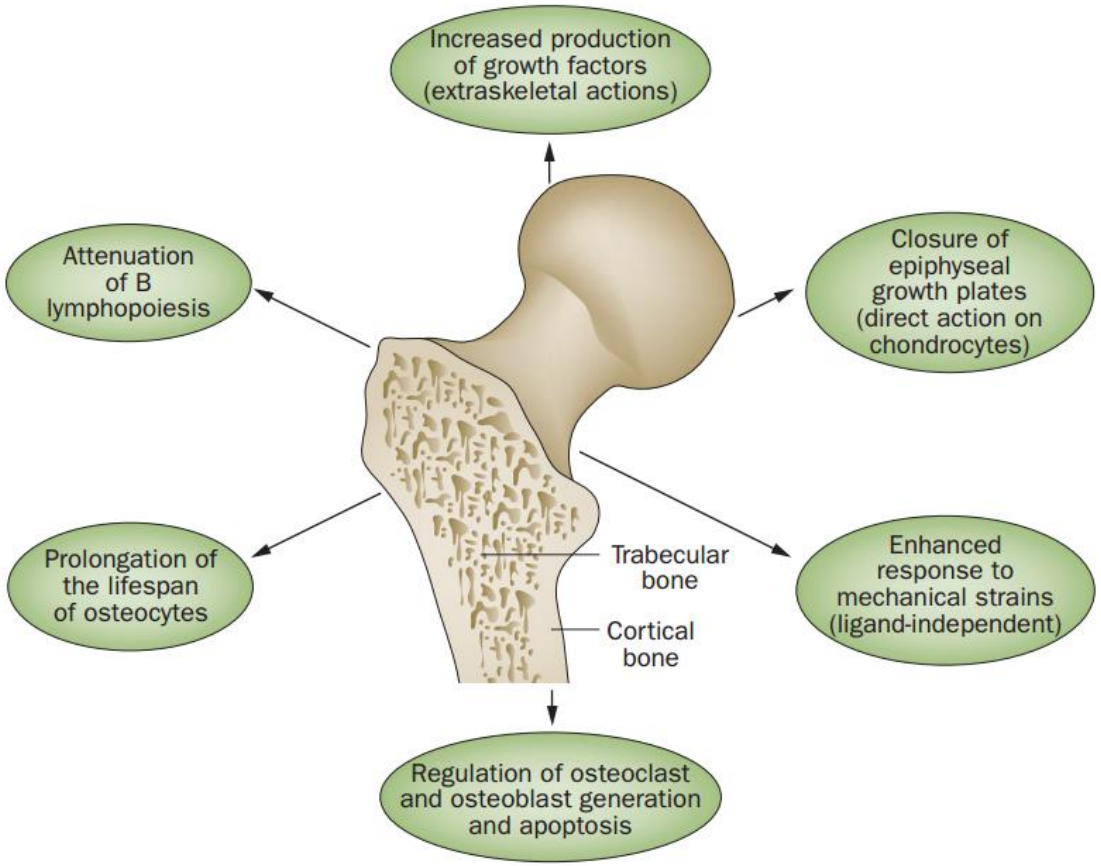


FIG. 4. Local and systemic mechanisms involving CGRP in cardiovascular regulation. Locally (e.g., in skin), CGRP is released from the peripheral sensory nerve endings (*left*). CGRP acts to increase blood flow, in a long-acting manner, which can lead to involvement in neurogenic inflammation and as a regulatory factor in inflammation. These effects can contribute to enhanced wound healing. Systemically, CGRP is not considered to have a major role in the normal individual. However, animal studies imply that CGRP may delay or protect against cardiovascular disease (*right*). This leads to protection against hypertension, hypertrophy, and inflammation and may be via direct mechanisms, or indirectly as a consequence of vasodilator activity.

Russell, F.A., R. King, S.J. Smillie, X. Kodji, and S.D. Brain. 2014. CALCITONIN GENE-RELATED PEPTIDE: PHYSIOLOGY AND PATHOPHYSIOLOGY. *Physiological Reviews 94:1099-1142.*

Estrogens/androgens





Manolagas, S.C., C.A. O'Brien, and M. Almeida. 2013. The role of estrogen and androgen receptors in bone health and disease. *Nat. Rev. Endocrinol. 9:699-712.*

Glucocorticoids

Weinstein, R.S. 2011. Glucocorticoid-Induced Bone Disease. *New England Journal of Medicine 365:62-70.*

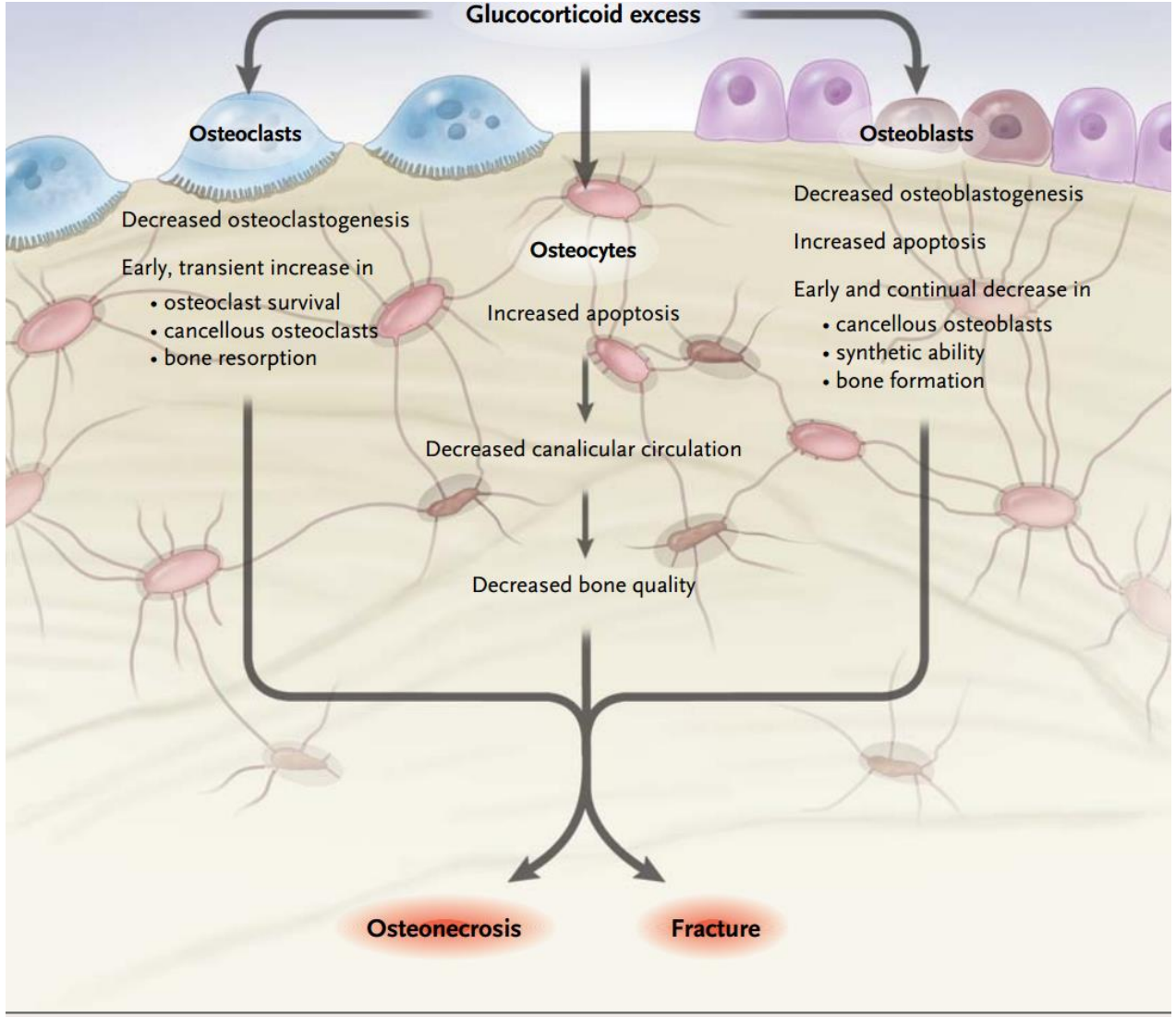


Figure 1. Direct Effects of Glucocorticoids on Bone Cells.
Shown are the adverse skeletal changes that result from an excess of glucocorticoids and lead to osteoporosis and osteonecrosis. The brown, condensed cells are apoptotic osteoblasts and osteocytes. Apoptotic osteocytes disrupt the osteocyte–lacunar–canalicular network.

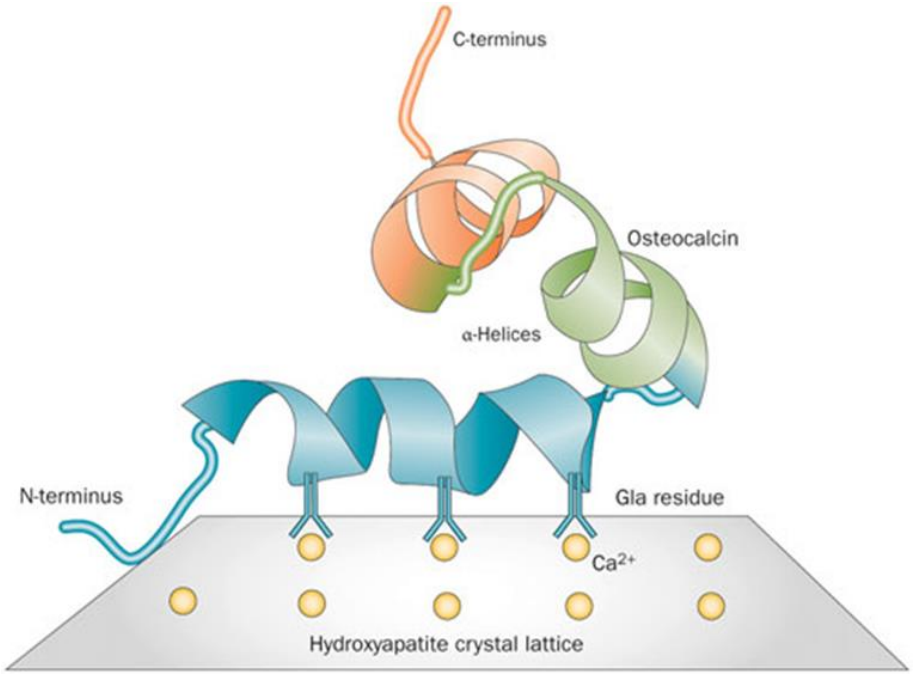
Table 1. Risk Factors for Glucocorticoid-Induced Osteoporosis.*

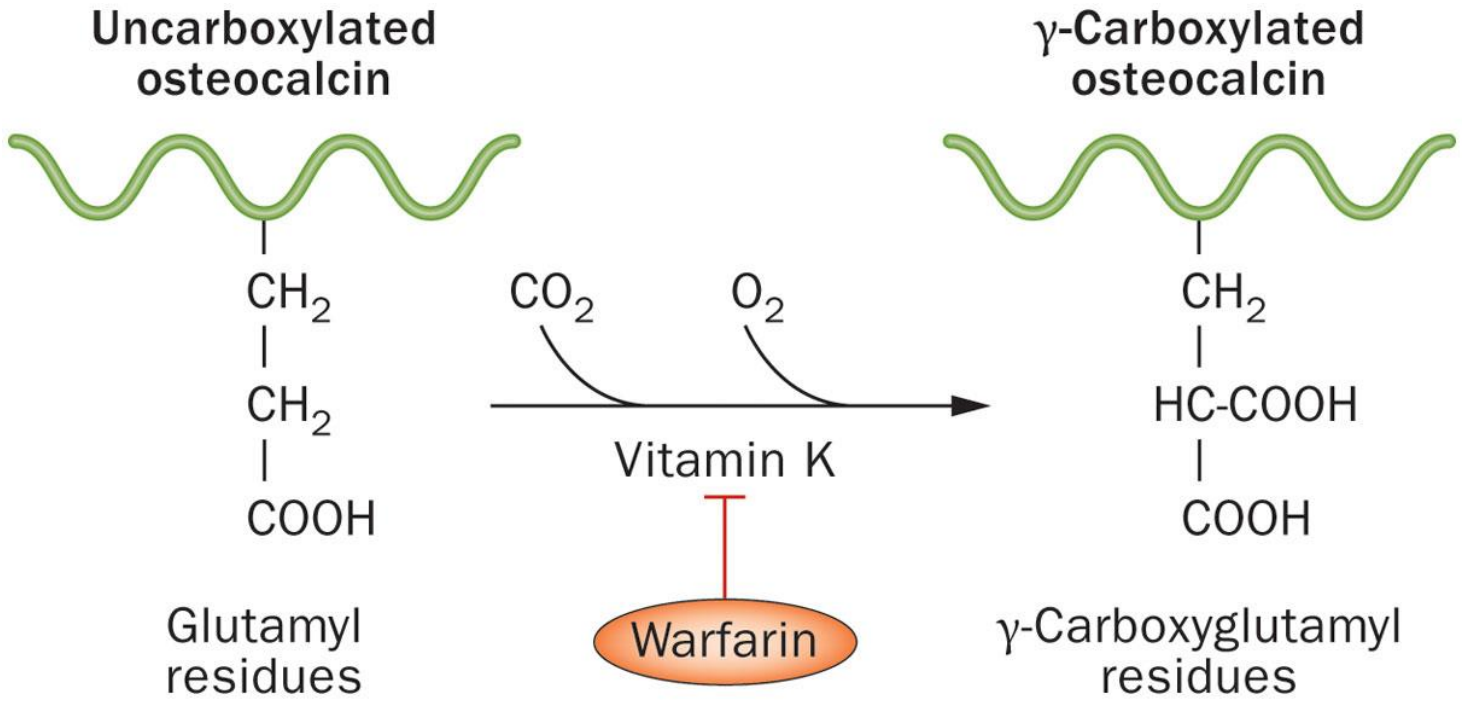
Risk Factor	Evidence of a Contribution
Advanced age	Patients 60 to 80 years of age receiving glucocorticoid therapy, as compared with patients 18 to 31 years of age, had a relative risk of vertebral fracture of 26 and a shorter interval between initiation of treatment and the occurrence of fracture[8]
Low body-mass index (<24)†	Low body-mass index is a risk factor for glucocorticoid-induced osteoporosis and probably fractures as well[9]
Underlying disease	Rheumatoid arthritis, polymyalgia rheumatica, inflammatory bowel disease, chronic pulmonary disease, and transplantation are independent risk factors[4]
Prevalent fractures, smoking, excessive alcohol consumption, frequent falls, family history of hip fracture	All are independent risk factors for osteoporosis but have not been extensively studied in patients receiving glucocorticoids
Glucocorticoid receptor genotype	Individual glucocorticoid sensitivity may be regulated by polymorphisms in the glucocorticoid receptor gene[10]
Increased 11β-HSD1 expression	11β-HSD1 expression increases with the age of the patient and with glucocorticoid administration[11]
High glucocorticoid dose (high current or cumulative dose; long duration of therapy)	Risk of fracture escalates with increased doses and duration of therapy; alternate-day or inhaled therapies also confer risks of glucocorticoid-induced osteoporosis[4,12]
Low bone mineral density	Glucocorticoid-induced fractures occur independently of a decline in bone mass, but patients with very low bone mineral density may be at higher risk[4,8]

* 11β-HSD1 denotes 11β-hydroxysteroid dehydrogenase 1.
† The body-mass index is the weight in kilograms divided by the square of the height in meters.

- GnRH ! (androgens/estrogens)
- IGF-1
- Reduced resoprtion of calcium

Osteocalcin





Booth, S. L. *et al.* (2012) The role of osteocalcin in human glucose metabolism: marker or mediator? *Nat. Rev. Endocrinol.* doi:10.1038/nrendo.2012.201

Vitamin K is required for the formation of γ-carboxyglutamic acid

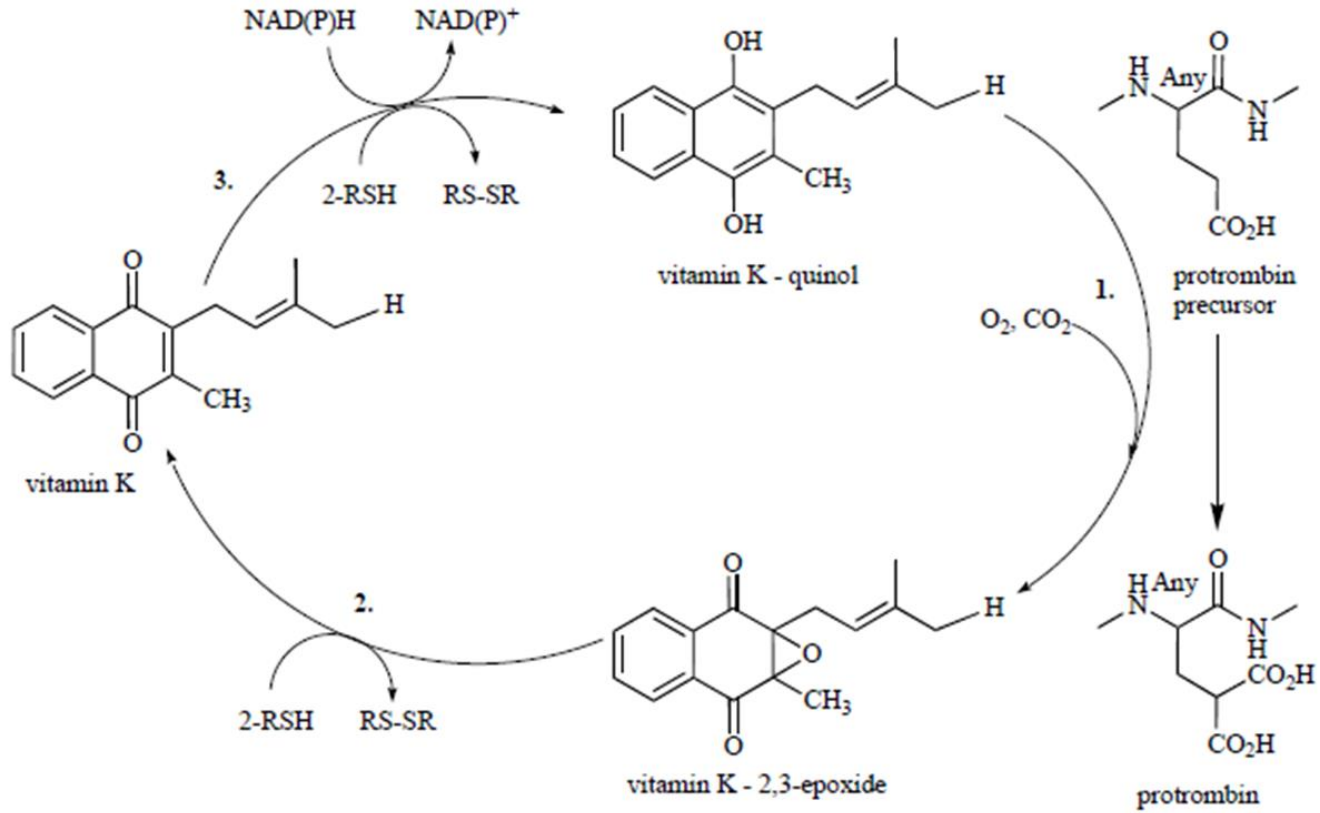
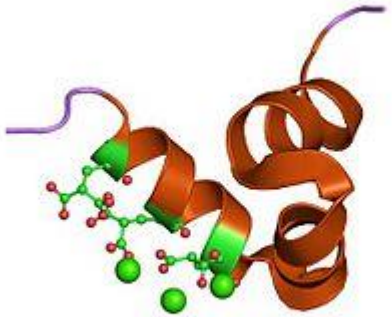
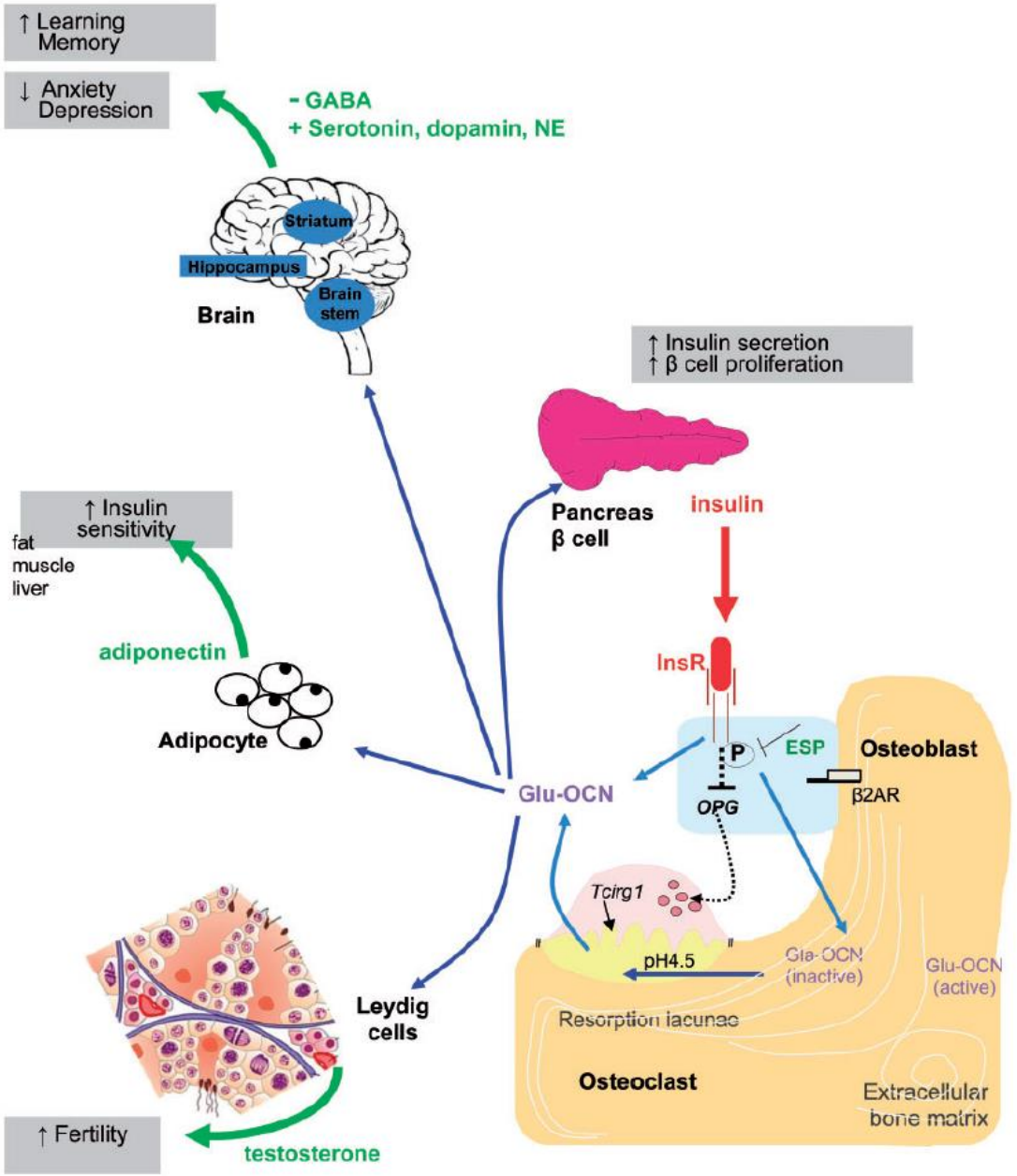


Fig. (15). The role of vitamin K in coagulation.

Osteocalcin in bone metabolism

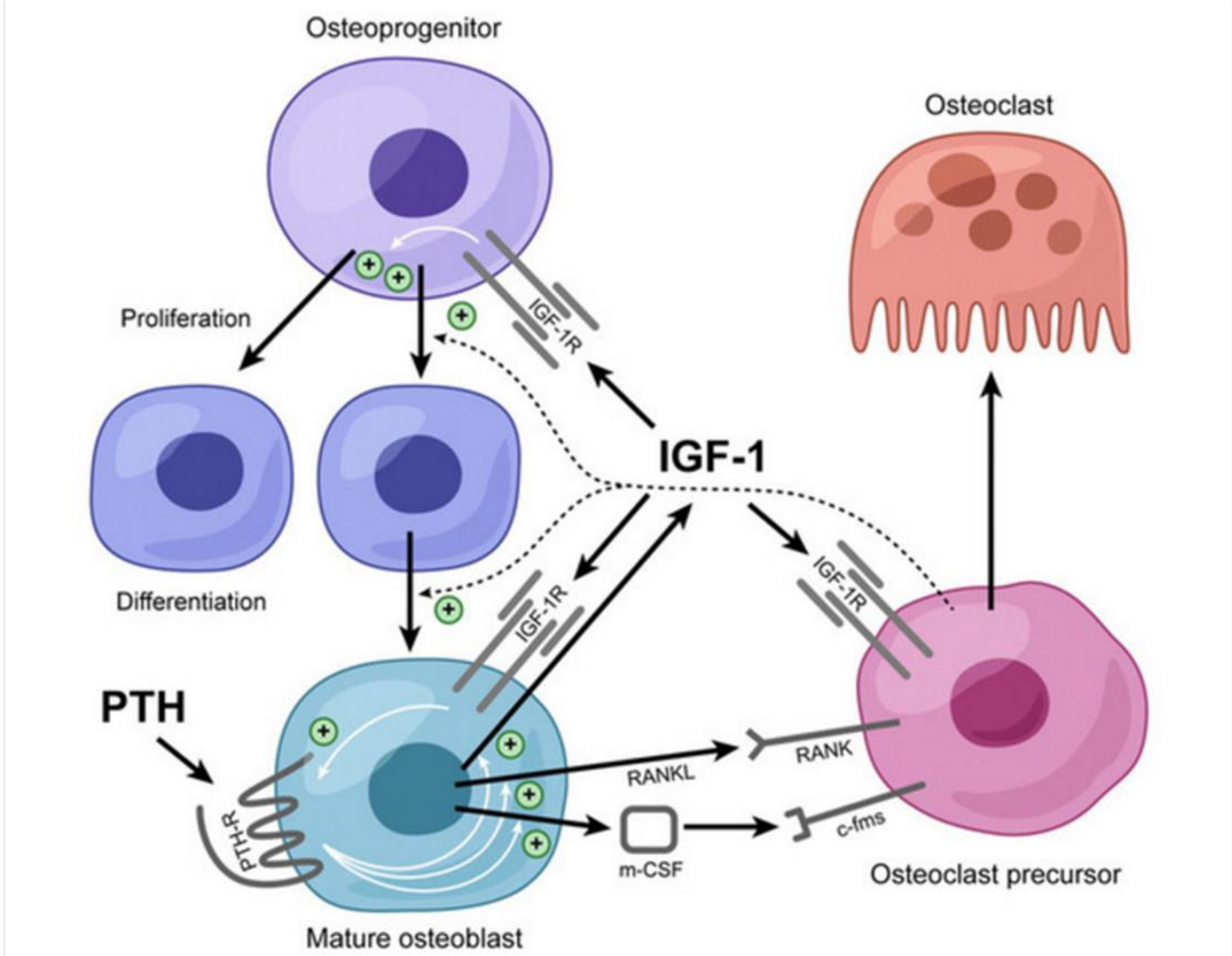




Osteocalcin favours glucose handling, promotes fertility and enhances cognitive performances. β2AR: β2-adrenergic receptor; ESP: tyrosine-phosphatase; GABA: γ-aminobutyric acid; GLA-OCN: carboxylated osteocalcin; GLU-OCN: uncarboxylated osteocalcin; InsR: insulin receptor; NE: norepinephrine; OPG: osteoprotegerin; TCIRG1: V-type proton ATPase. Modified from Confavreux CB, Karsenty G. Pathologie phosphocalcique et osseuse de l'enfant, Chapter 12 p. 61. Paris: Doin Ed., 2015 (with permission from Doin).

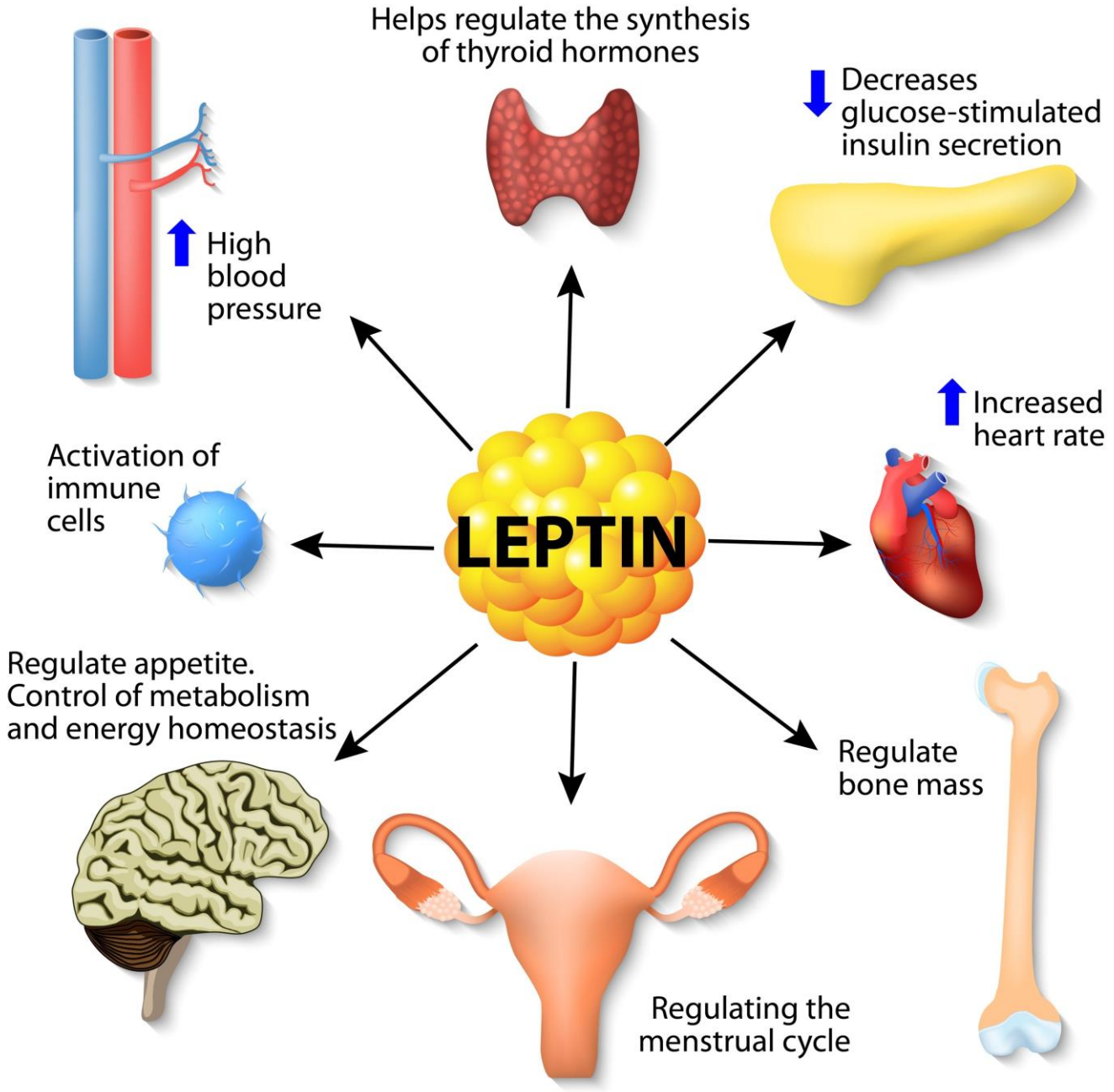
Chapurlat, R.D., and C.B. Confavreux. 2016. Novel biological markers of bone: from bone metabolism to bone physiology. Rheumatology 55:1714-1725.

IGF-1 and bone metabolism



The role of IGF-I in the actions of PTH in bone. We propose that in bone, the mature osteoblast is the major responder to PTH and producer of IGF-I. The IGF-I induced in the mature osteoblast by PTH stimulates the proliferation and differentiation of osteoprogenitors. IGF-I thus produced also feeds back on the mature osteoblast to enable PTH to induce RANKL and m-CSF that, along with IGF-I, promote osteoclastogenesis.

Leptin and bone metabolism



Leptin and bone metabolism

Karsenty, G., and F. Oury. 2012. Biology Without Walls: The Novel Endocrinology of Bone. In Annual Review of Physiology, Vol 74. D. Julius, and D.E. Clapham, editors. 87-105.

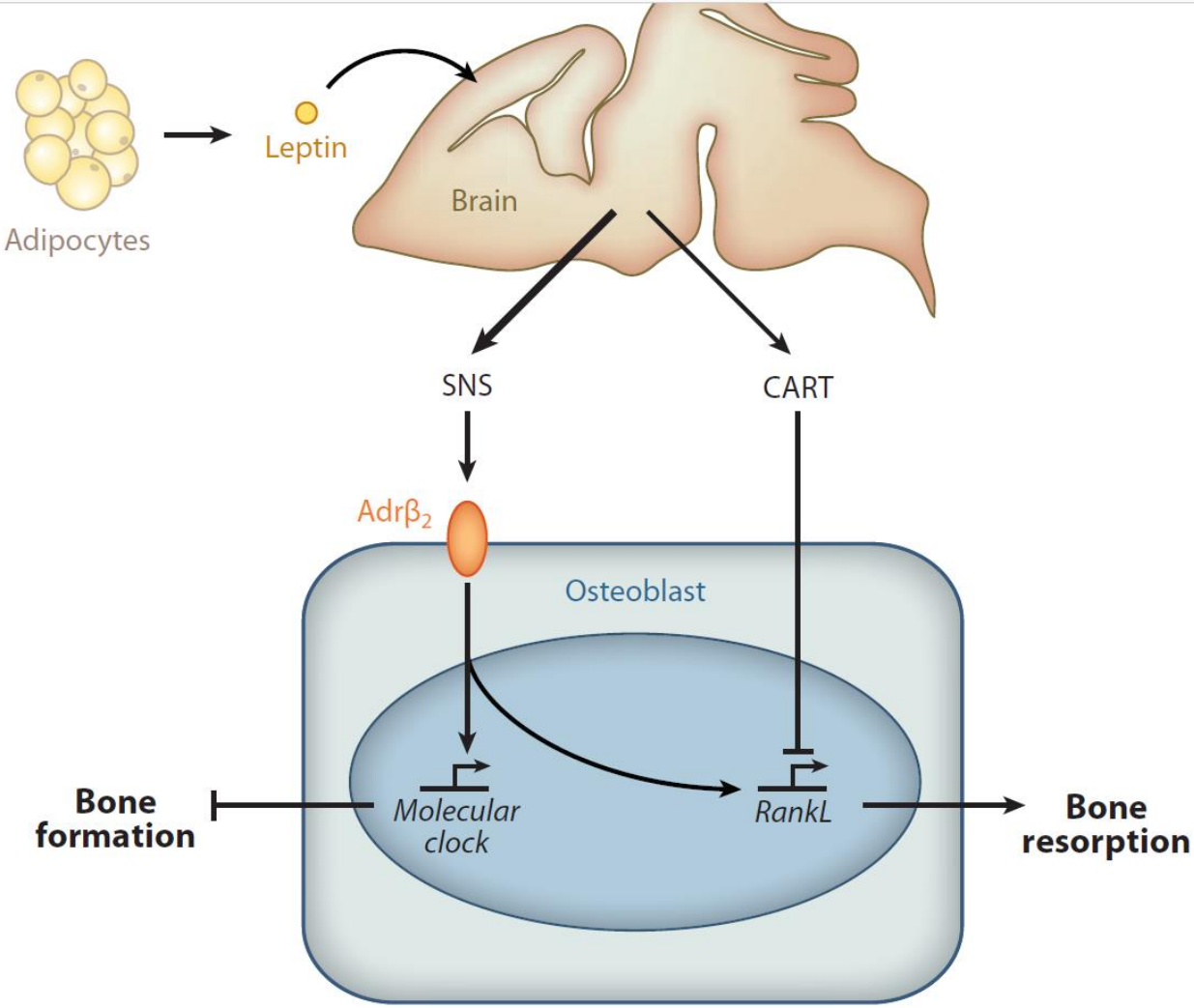


Figure 1

The sympathetic nervous system (SNS) and CART (cocaine amphetamine regulated transcript) mediate leptin signaling in the brain to the osteoblasts. The SNS inhibits bone formation and favors bone resorption. Following β_2-adrenergic receptor (Adrβ_2) activation in osteoblasts, the sympathetic tone favors expression of *RankL*, the most powerful osteoclast differentiation factor, and recruits several transcriptional components of the molecular clock, inhibiting bone formation. CART, the second mediator of the leptin regulation of bone mass accrual, also acts on osteoblasts, but by inhibiting *RankL* expression and bone resorption.

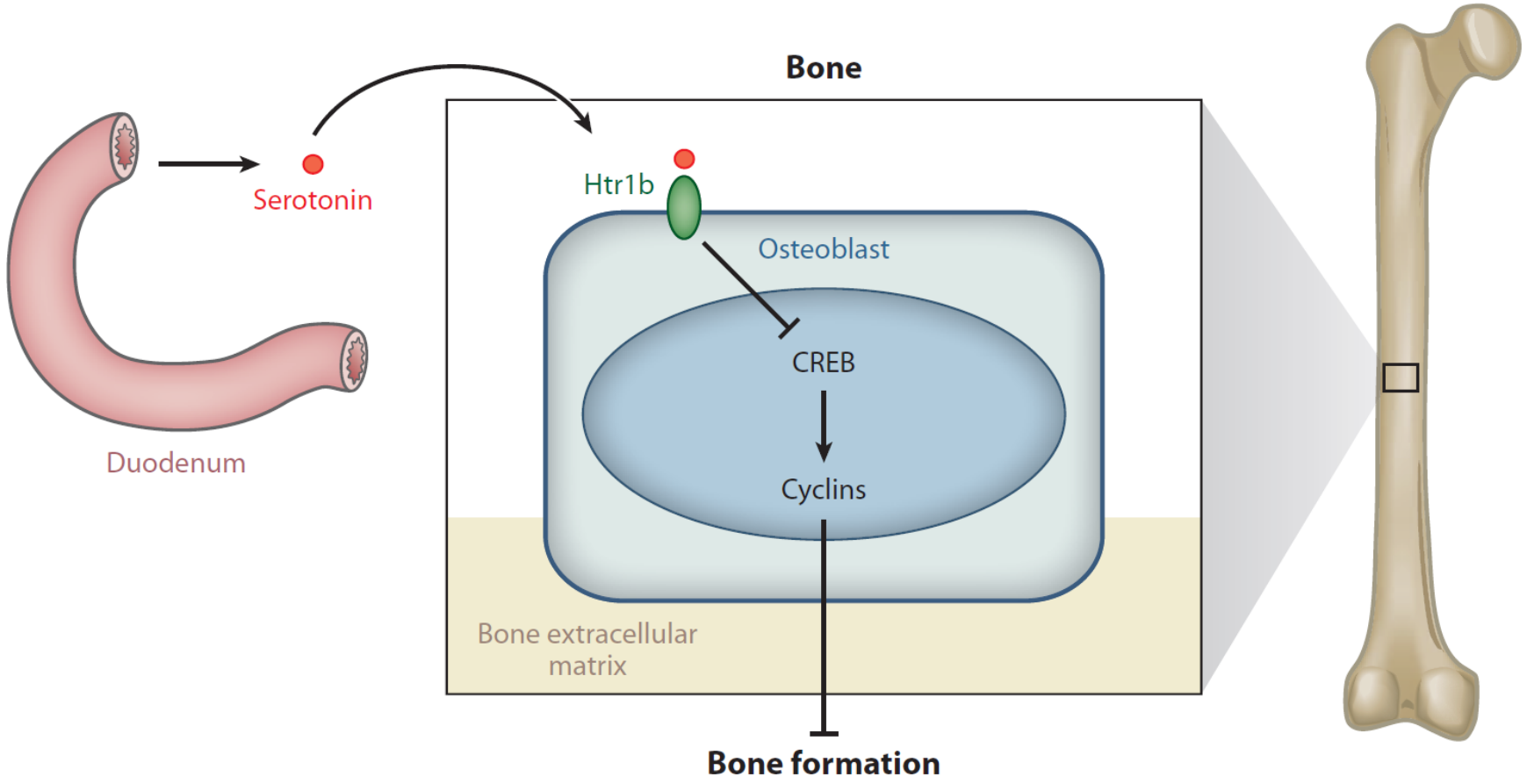


Figure 3

Gut-derived serotonin regulation of bone mass accrual. Gut-derived serotonin is synthesized in enterochromaffin cells of the duodenum and acts on osteoblasts through its receptor, Htr1b, and CREB to inhibit osteoblast proliferation.

Karsenty, G., and F. Oury. 2012. Biology Without Walls: The Novel Endocrinology of Bone. In Annual Review of Physiology, Vol 74. D. Julius, and D.E. Clapham, editors. 87-105.

Karsenty, G., and F. Oury. 2012. Biology Without Walls: The Novel Endocrinology of Bone. In Annual Review of Physiology, Vol 74. D. Julius, and D.E. Clapham, editors. 87-105.

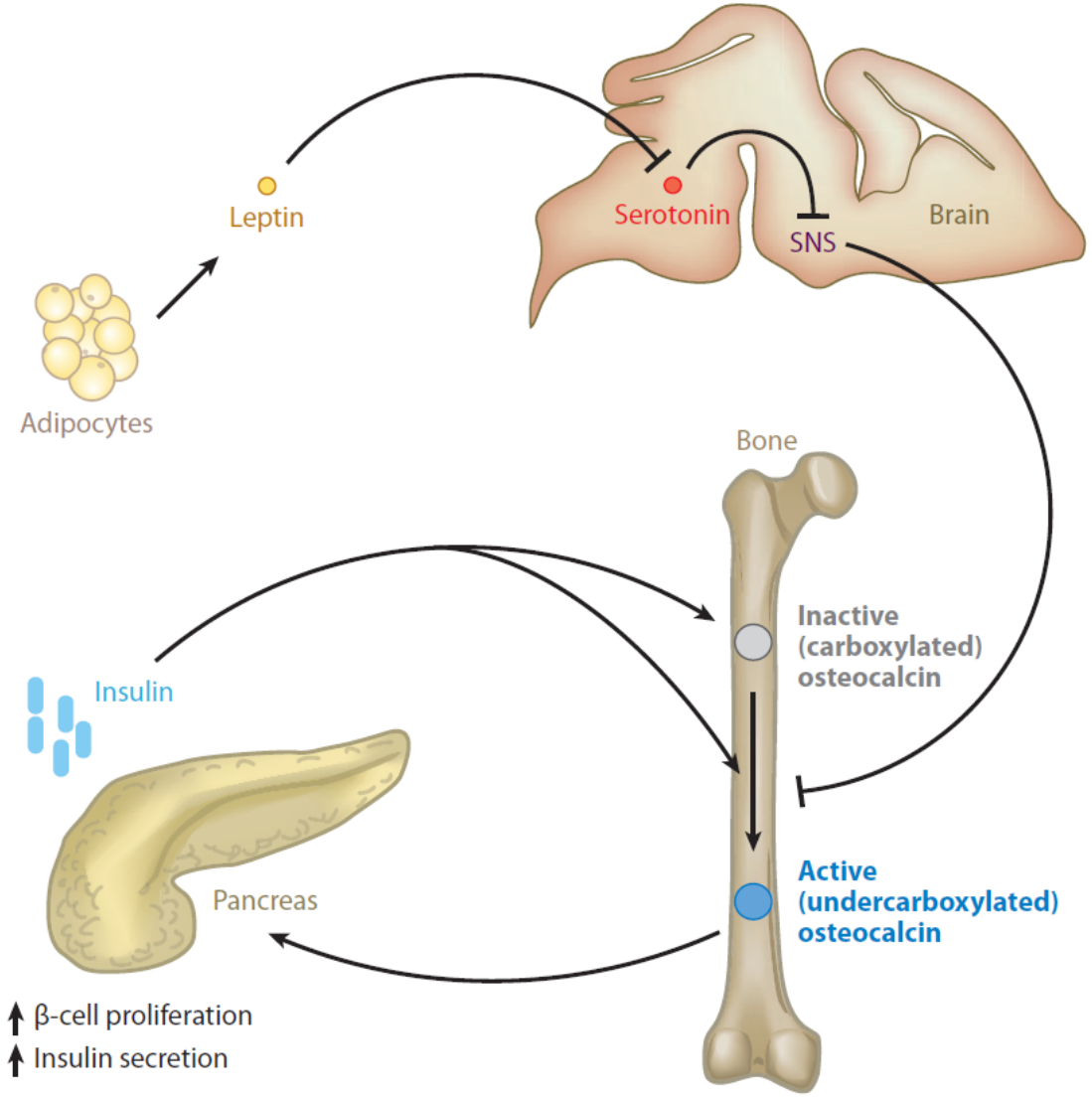


Figure 4

Endocrine regulation of energy metabolism by bone. Bone mediates such regulation by an osteoblast-specific secreted molecule, osteocalcin, that when undercarboxylated acts as a hormone favoring β-cell proliferation and insulin secretion in the pancreas. The mechanism by which osteocalcin may be activated is regulated in osteoblasts by insulin signaling, which favors osteocalcin bioavailability by promoting its undercarboxylation. In contrast, the sympathetic tone, which is regulated centrally by leptin, decreases osteocalcin bioactivation. SNS denotes sympathetic nervous system.

Karsenty, G., and F. Oury. 2012. Biology Without Walls: The Novel Endocrinology of Bone. In Annual Review of Physiology, Vol 74. D. Julius, and D.E. Clapham, editors. 87-105.

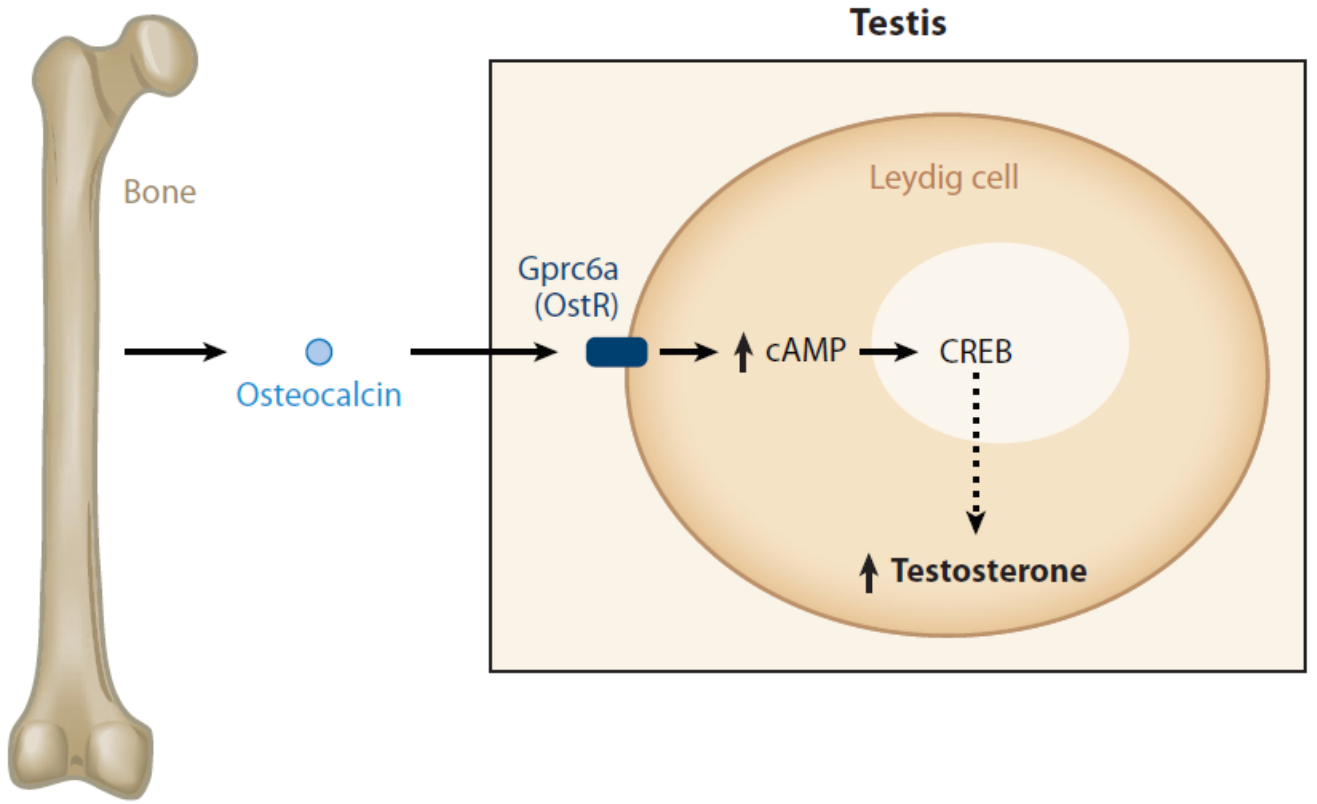


Figure 5

Endocrine regulation of male fertility by bone. Osteocalcin favors male fertility, increasing testosterone production by Leydig cells of the testes. By binding to a G protein–coupled receptor expressed in the Leydig cells of the testes, osteocalcin, an osteoblast-derived hormone, promotes testosterone production by the testes in a cAMP response element binding (CREB) protein–dependent manner. The dashed arrow indicates that regulation is not a primary signal (but direct); there may be other molecules in between.

Oxytocin and bone metabolism

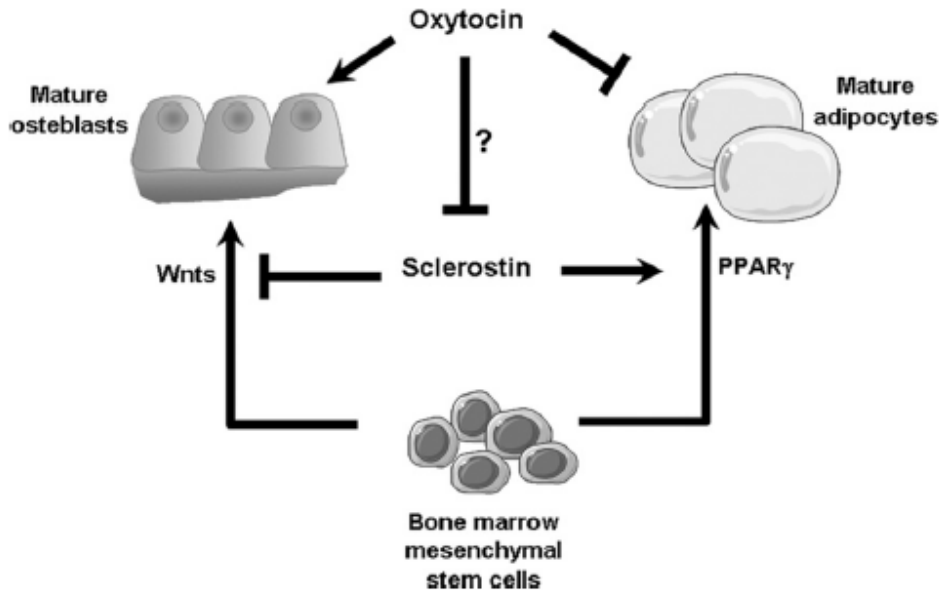


Fig. 1. Osteoblasts and adipocytes are derived from a common mesenchymal stem cell precursor, and their balance is regulated by molecules that lead to osteoblastogenesis and inhibit adipogenesis or vice versa. Activation of Wnt signaling pathway inhibits adipogenesis, while supporting osteogenesis. In contrast, PPARγ promotes the differentiation of mesenchymal stem cells into adipocytes over osteoblasts. Besides these proteins, the Wnt inhibitor molecules are also necessary to control the balance between osteogenesis and adipogenesis. The interplay of these molecular regulators could be crucial in the pathogenesis of obesity, as it was found to be essential in osteoporosis. Future studies could reveal a putative role of oxytocin in controlling the balance in favor of osteogenesis at the expense of adipogenesis through downregulation of sclerostin synthesis.

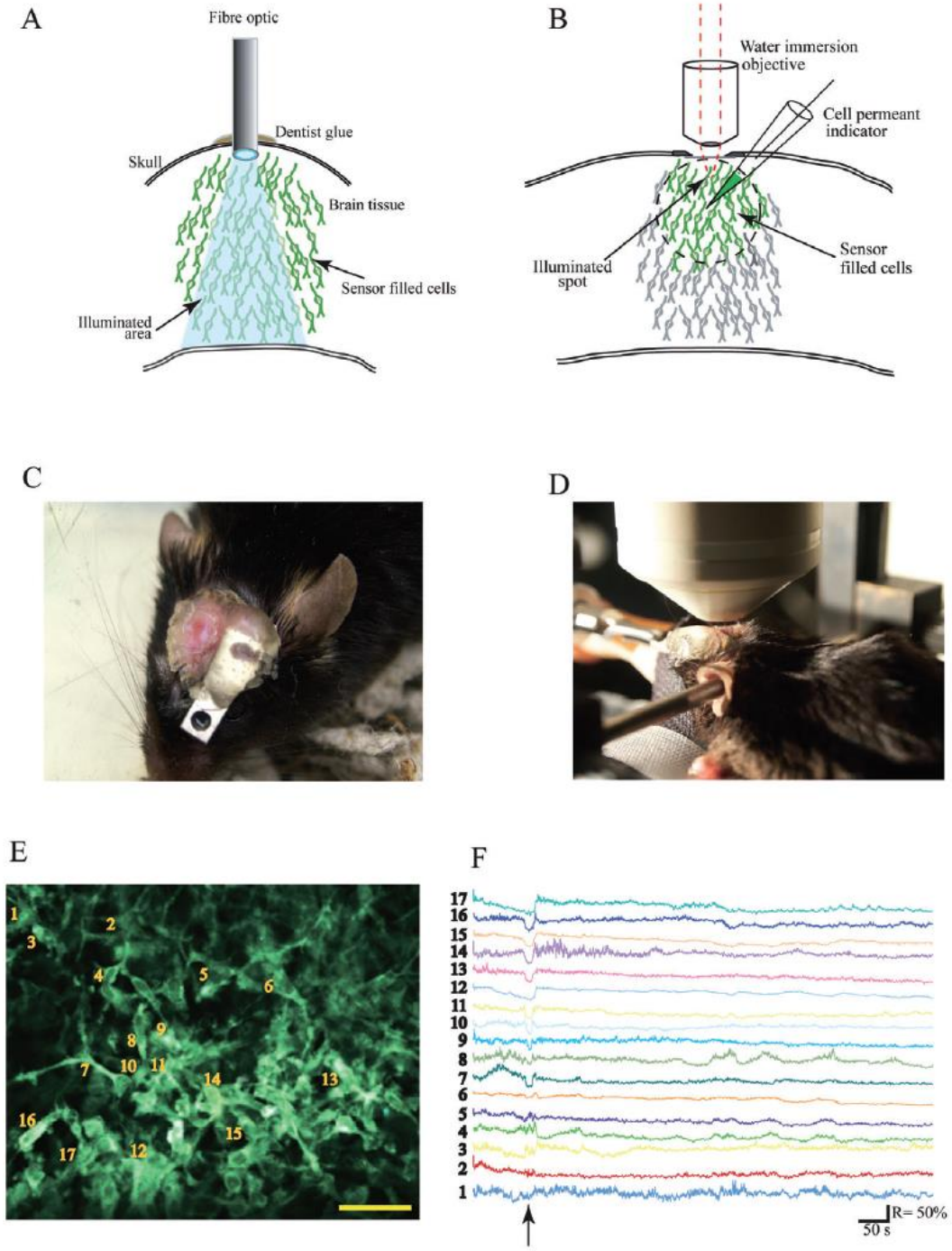
Colaianni, G., L. Sun, M. Zaidi, and A. Zallone. 2014. Oxytocin and bone. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology 307:R970-R977.*

In vivo calcium imaging

Indicator	Species	Application/tissue studied	Labelling technique	Imaging technique	Spatial resolution	Response amplitude	References
Organic synthetic fluorescent dyes							
Fura-2	Blowfly	Visual system	Cell microinjection	Epifluorescence/CCD camera	Cellular/ subcellular	10–20%	Borst and Egelhaaf, 1992
Fura-2	Cricket	Auditory system	Cell microinjection	Epifluorescence/CCD camera	Cellular	6–20%	Sobel and Tank, 1994
Fluo-3	*C. elegans*	Apoptosis	Bulk microinjection	Confocal microscopy	Cellular	15%	Jain *et al.*, 1993
Fluo-3	*Drosophila*	Motor nerve terminals	Bulk loading	Confocal microscopy	Cellular	100–200%	Karunanithi *et al.*, 1997
Fura-2, Fluo-4, Indo-1	Mouse	Neocortex	Bulk microinjection	Two-photon microscopy	Cellular	20–50%	Stosiek *et al.*, 2003
Calcium green-1	Honeybee	Olfactory system	Bulk microinjection	Epifluorescence/CCD camera	Glomerular	2–5%	Galizia *et al.*, 1999
Calcium green-1	Zebrafish	Olfactory bulb	Bulk microinjection	Epifluorescence/CCD camera	Glomerular	5–10%	Friedrich and Korsching, 1997
Calcium green-1	Turtle	Olfactory system	Bulk loading	Epifluorescence/CCD camera	Cellular	5–20%	Wachowiak *et al.*, 2002
Oregon green BAPTA	Mouse	Neocortex	Cell microinjection	Two-photon microscopy	Cellular/ subcellular	40–200%	Helmchen *et al.*, 1999; Svoboda *et al.*, 1999
Oregon green BAPTA	Cat	Visual cortex	Bulk microinjection	Two-photon microscopy	Cellular	40–200%	Ohki *et al.*, 2006
Oregon green BAPTA	Ferrett	Visual cortex	Bulk microinjection	Two-photon microscopy	Cellular	10–30%	Shummers *et al.*, 2008
Rhod-2	Mouse	Cortical astrocytes	Bulk microinjection	Two-photon microscopy	Cellular	10–100%	Takano *et al.*, 2007
Aequorin-based luminescence calcium indicators							
Aequorin	Tobacco	Whole plant	Transgenic	Luminescence detection	Bulk tissue	NA	Knight *et al.*, 1991
Aequorin	*Arabidopsis*	Whole plant	Transgenic	Luminescence detection	Bulk tissue	~10-fold	Knight *et al.*, 1996; Liu *et al.*, 2006
Aequorin	Zebrafish	Development	mRNA injection/transgenic	Luminescence detection	Tissue/cellular	5- to 10-fold	Creton *et al.*, 1997; Cheung *et al.*, 2006
Aequorin	*Drosophila*	Brain/mushroom bodies	Transgenic	Luminescence/photon counting	Bulk tissue	>100%	Martin *et al.*, 2007
Fluorescent protein-based calcium indicators							
DsRed/inverse pericam	*C. elegans*	Pharyngeal muscles	Transgenic	Epifluorescence/CCD camera	Bulk tissue	20–30%	Shimozono *et al.*, 2004
YC 2.1, YC 3.1	*C. elegans*	Brain/sensory neurons	Transgenic	Epifluorescence/CCD camera	Bulk tissue	50–60%	Kerr *et al.*, 2000
G-CaMP	*Drosophila*	Olfactory system	Transgenic	Two-photon microscopy	Bulk cellular	100%	Wang *et al.*, 2003
YC 3.1	*Drosophila*	Flight muscle	Transgenic	Confocal microscopy	Bulk cellular	12%	Gordon and Dickinson, 2006
G-CaMP2, synapcam, YC 2.3, TN-L15	*Drosophila*	Motor neurons	Transgenic	Epifluorescence/CCD camera	Cellular	30–700%	Guerrero *et al.*, 2005; Mank *et al.*, 2006; Reiff *et al.*, 2005
YC 2.1	Zebrafish	Spinal cord/neurons	Transgenic	Confocal microscopy	Cellular	15%	Higashijima *et al.*, 2003
YC 2.12	Zebrafish	Development	Transgenic	Epifluorescence/CCD camera	Bulk cellular	NA	Tsuruwaka *et al.*, 2007
Camgaroo, inverse pericam	Mouse	Olfactory bulb/neurons	Transgenic	Epifluorescence/CCD camera	Bulk tissue	1–3%	Hasan *et al.*, 2004
G-CaMP2	Mouse	Cerebellum/neurons	Transgenic	Epifluorescence/CCD camera, two-photon microscopy	Bulk tissue	50%	Diez-Garcia *et al.*, 2007
G-CaMP2	Mouse	Heart	Transgenic	Two-photon microscopy	Bulk tissue	60–70%	Tallini *et al.*, 2006
YC 3.12	Mouse	Brain/neurons	Transgenic	Two-photon microscopy	Cellular	10–30%	Hasan *et al.*, 2004
Cer TN-L15	Mouse	Cortex/neurons	Transgenic	Two-photon microscopy	Cellular	5–10%	Heim *et al.*, 2007
Cer TN-L15	Mouse	Brain/astrocytes	Transgenic	Two-photon microscopy	Cellular	10–20%	Atkin *et al.*, 2009
YC 3.60, Cer TN -L15, G-CaMP3	*C. elegans*, mouse	Cortex/neurons	Transgenic	Two-photon microscopy	Cellular	30–500%	Tian *et al.*, 2009

C. elegans, *Caenorhabditis elegans*; CCD, charge coupled device; NA, not available.

Russell, J.T. 2011. Imaging calcium signals in vivo: a powerful tool in physiology and pharmacology. *British Journal of Pharmacology 163:1605-1625.*



Russell, J.T. 2011. Imaging calcium signals in vivo: a powerful tool in physiology and pharmacology. *British Journal of Pharmacology 163:1605-1625.*

Figure 1

Different modes of transcranial imaging cortical cells using two-photon microscopy of a living mouse. A, Drawing of a fibre-optic endoscopic design for two-photon microscopy. The fibre is implanted and imaging is done in an awake, behaving mouse to image cortical neurons or glial cells. B, Imaging cortical cells through an acutely or chronically implanted cranial window. A glass coverslip is glued over a hole drilled through the skull using dental glue. In both cases, cells under the viewing port are labelled either by local injection of acetoxymethyl ester form of an organic dye or virus packaged fluorescent sensor, or transgenically expressed fluorescent Ca^{2+} sensor. C, Photograph of a mouse with an implanted cranial window. D, Photograph of transcranial two-photon imaging of an anaesthetized mouse with cranial window. These transgenic mice express the YC 3.60 cameleon in astrocytes under the control of the S-100b promoter. E, A field of astrocytes in the somatosensory cortex of the transgenic mouse imaged through the cranial window as in panel D. The image is an overlay of CFP and YFP channels. Images were acquired at 3 Hz. Scale = 50 µm. F, Spontaneous Ca^{2+} transients occurring in astrocytes numbered in panel E. Traces represent YFP/CFP ratios of intensities of pixels in regions of interest drawn around each cell, plotted against time. Data are from the author's laboratory.

Biochemical markers of bone turnover

TABLE 1 The classical biochemical markers of bone turnover

	Origin	
	Formation (osteoblasts)	**Resorption (osteoclasts)**
Important		
	Osteocalcin (serum)	C-telopeptide of type 1 collagen (serum and urine)
	Bone Alkaline Phosphatase (serum)	N-telopeptide of type 1 collagen (urine)
	Procollagen type 1 N-terminal propeptide (serum)	
Other		
	Procollagen type 1 C-terminal propeptide (serum)	Free Crosslinks: pyridinoline (urine) and deoxypyridinoline (urine)
		Plasma tartrate resistant acid phosphatase 5b (serum)
		Telopeptide of type 1 collagen (serum)

Chapurlat, R.D., and C.B. Confavreux. 2016. Novel biological markers of bone: from bone metabolism to bone physiology. Rheumatology 55:1714-1725.

Marker	Tissue origin	Analytical sample	Analytical method
Hydroxyproline, total and dialyzable (OH-Pro, OHP); specific for all fibrilar collagens and a part of collagen proteins, including Ciq and elastin; present in newly synthesized and mature collagen	bone, skin, cartilage, soft tissues	urine	colorimetry, HPLC
Pyridinoline (PYD, Pyr); high concentrations in cartilage and bone collagen: not present in skin; present only in mature collagen	bone, tendon, cartilage	urine	HPLC, ELISA
Deoxypyrindoline (DPD, d-Pyr); high concentrations only in bone collagen: not present in cartilage or in skin; present only in mature collagen	bone, dentine	urine	HPLC, ELISA
Cross-linked C-terminal telopeptide of type I collagen (ICTP); high proportion from bone collagen in type I collagen; can partly originate from newly synthesized collagen	bone, skin	serum	RIA
Cross-linked C-terminal telopeptide of type I collagen (fragments alpha-CTX, beta-CTX); in type I collagen; probably high proportion from bone collagen	all tissue containing type I collagen	urine, serum	ELISA, RIA, ECLIA
Cross-linked N-terminal telopeptide of type I collagen (fragments NTX); in type I collagen; big proportion from bone	all tissue containing type I collagen	urine (alpha/beta), serum (only beta)	ELISA, RIA, ICMA
Hydroxylysine-glycosides (Hyl-Glyc); collagens and collagen proteins; glucogalactosyl- hydroxilysine is highly represented in soft tissue collagens and C1q; galactosil-OHLys is highly represented in bone collagen	bone, skin, soft tissue, serum complement	urine	HPLC, ELISA
Bone sialoprotein (BSP); synthesized by active osteoblasts and lay in extracellular bone matrix; it seems to express osteoclast activity	bone, dentine, hypertrophic catrilage	serum	RIA, ELISA
Tartarat-resistant acid phosphatase (TR-ACP); osteoclasts, thrombocytes, erythrocytes	bone, blood	plasma/serum	colorimetry, RIA, ELISA
Free gamma carboxyglutamin acid (GLA); resulted from bone proteins (e.g. osteocalcin, matrix Gla protein) and from coagulation factor	blood, bone	serum/urine	HPLC

HPLC – high performance liquid chromatography; ELISA – enzyme-linked immunosorbent assay; RIA – radio immuno assay; ECLIA – electrochemiluminiscence immunoassay; ICMA – immunochemiluminometric assay

„New“ markers of bone metabolism

Markers of bone metabolism	Hormones	miRNAs
Periostin	FGF-23	miR-148a
RANK-L	klotho	miR125b
Cathepsin K	Osteocalcin	
Sclerostin		
Dkk-1		
Sphingosine-1-phosphate		

Chapurlat, R.D., and C.B. Confavreux. 2016. Novel biological markers of bone: from bone metabolism to bone physiology. Rheumatology 55:1714-1725.

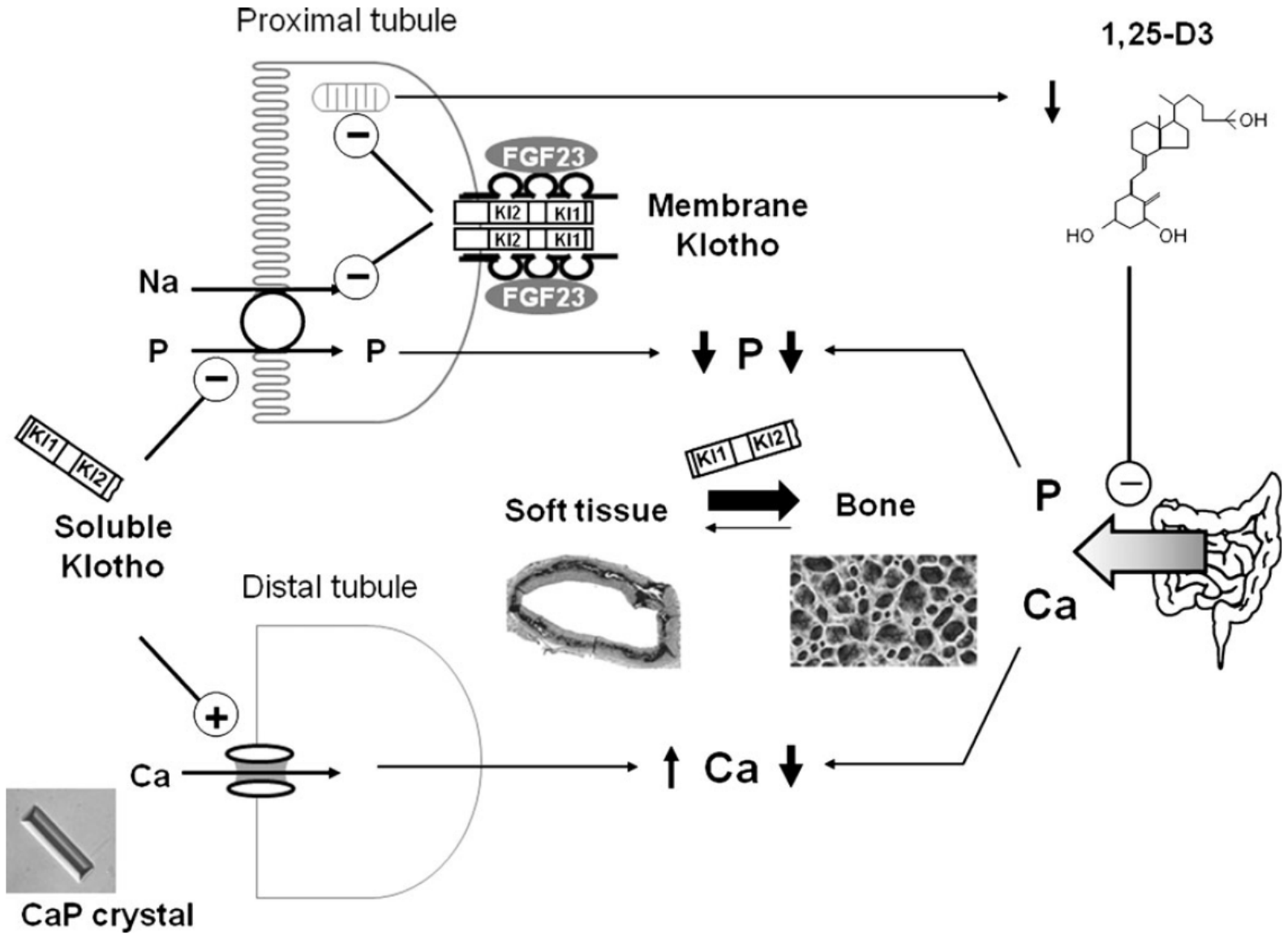


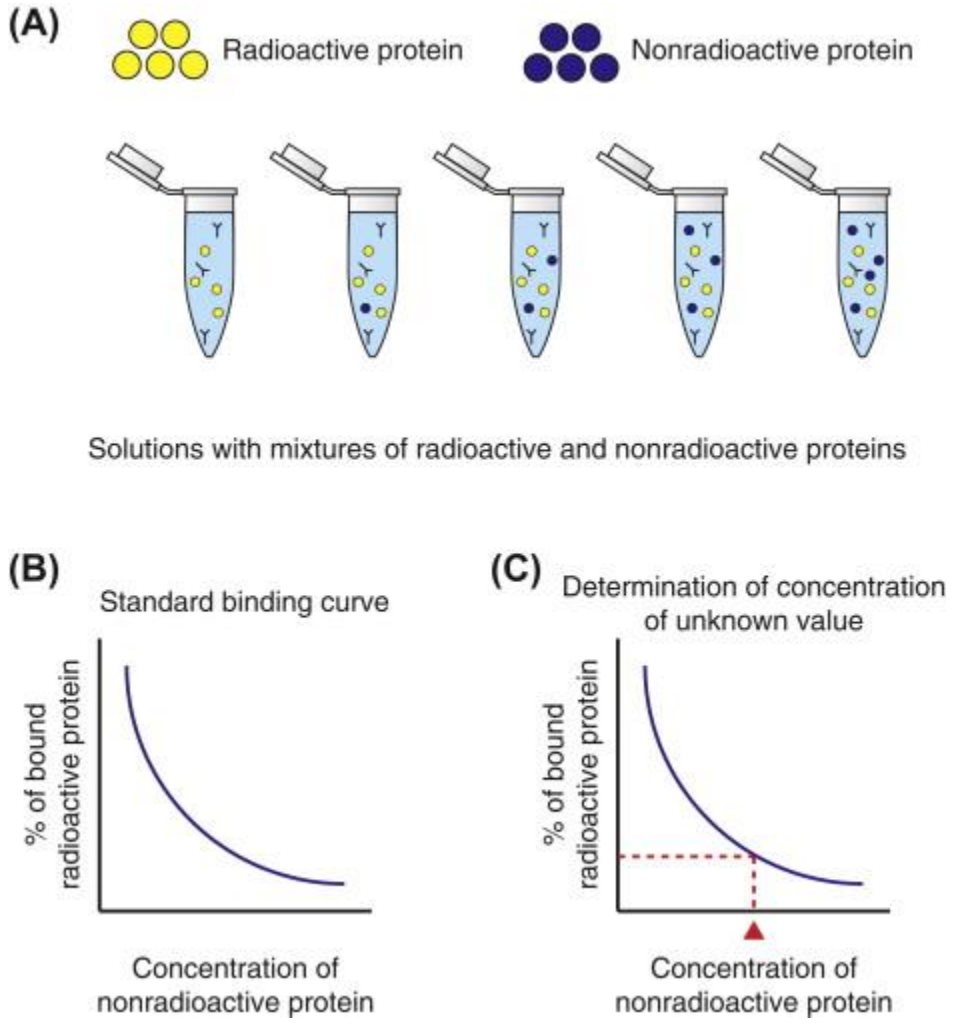
Fig. 5 Proposed model of coordinated regulation of calcium phosphate transport. Both membrane and soluble Klotho contribute to phosphaturia by inhibiting proximal phosphate transport. Suppression of $1,25(OH)_2$ vitamin D also reduce intestinal phosphate absorption. In concert, the renal and intestinal effects enhance negative external phosphate balance. Internally, soluble Klotho also promotes phosphate entry into bone and inhibits uptake by soft tissue. Klotho reduces intestinal calcium uptake via inhibition of $1,25(OH)_2$ vitamin D synthesis. This is partially offset by the calcium-retaining effects of urinary Klotho in the distal tubule which mitigates the negative calcium balance. Reduction of luminal calcium in the distal nephron can also contribute to the prevention of formation of insoluble calcium phosphate complexes during soluble Klotho-induced phosphaturia

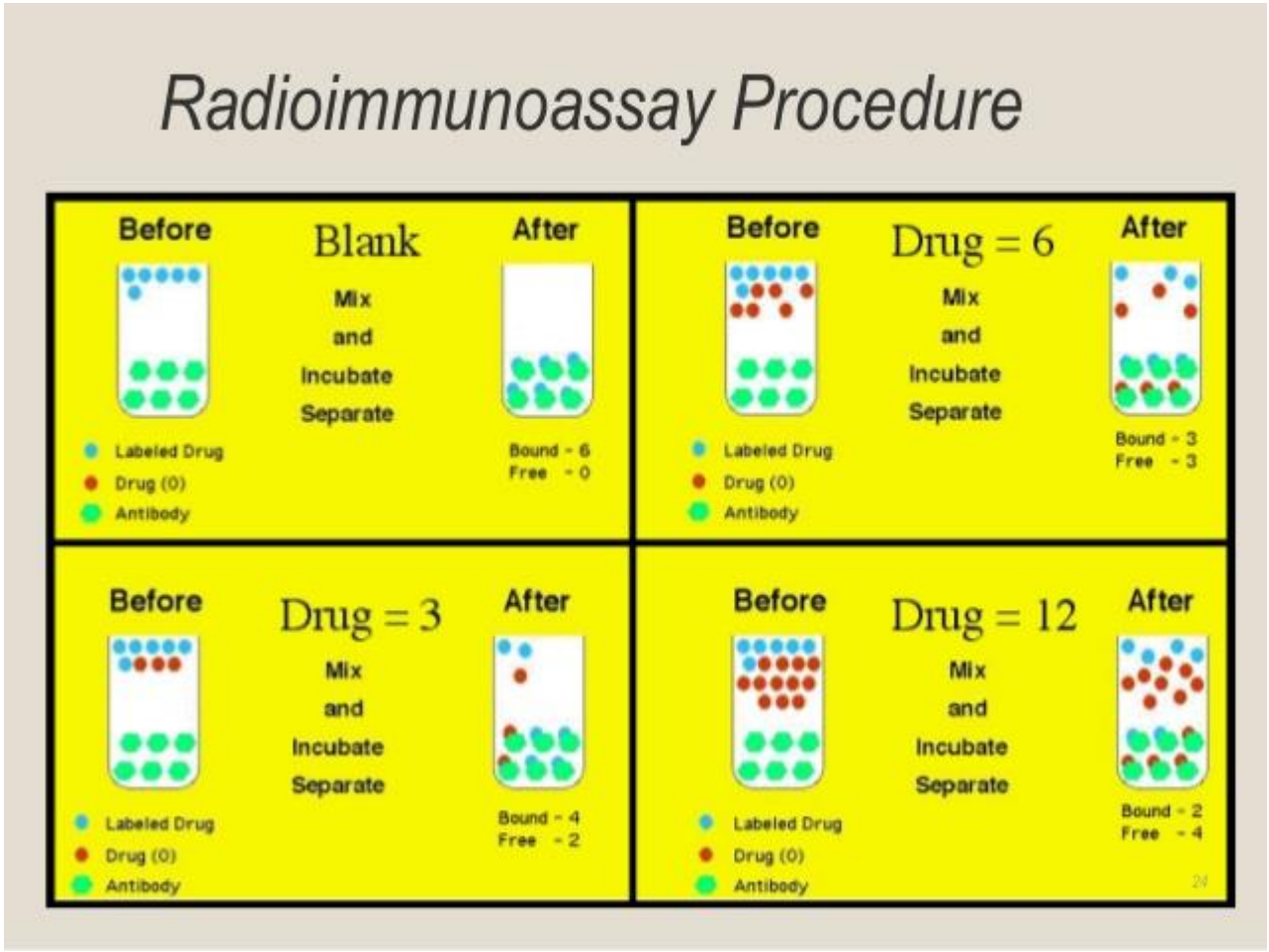
Klotho:

- β-glukuronidáza
- Stárnutí
- Kostní metabolismus
- Abusus alkoholu
- Ateroskleróza

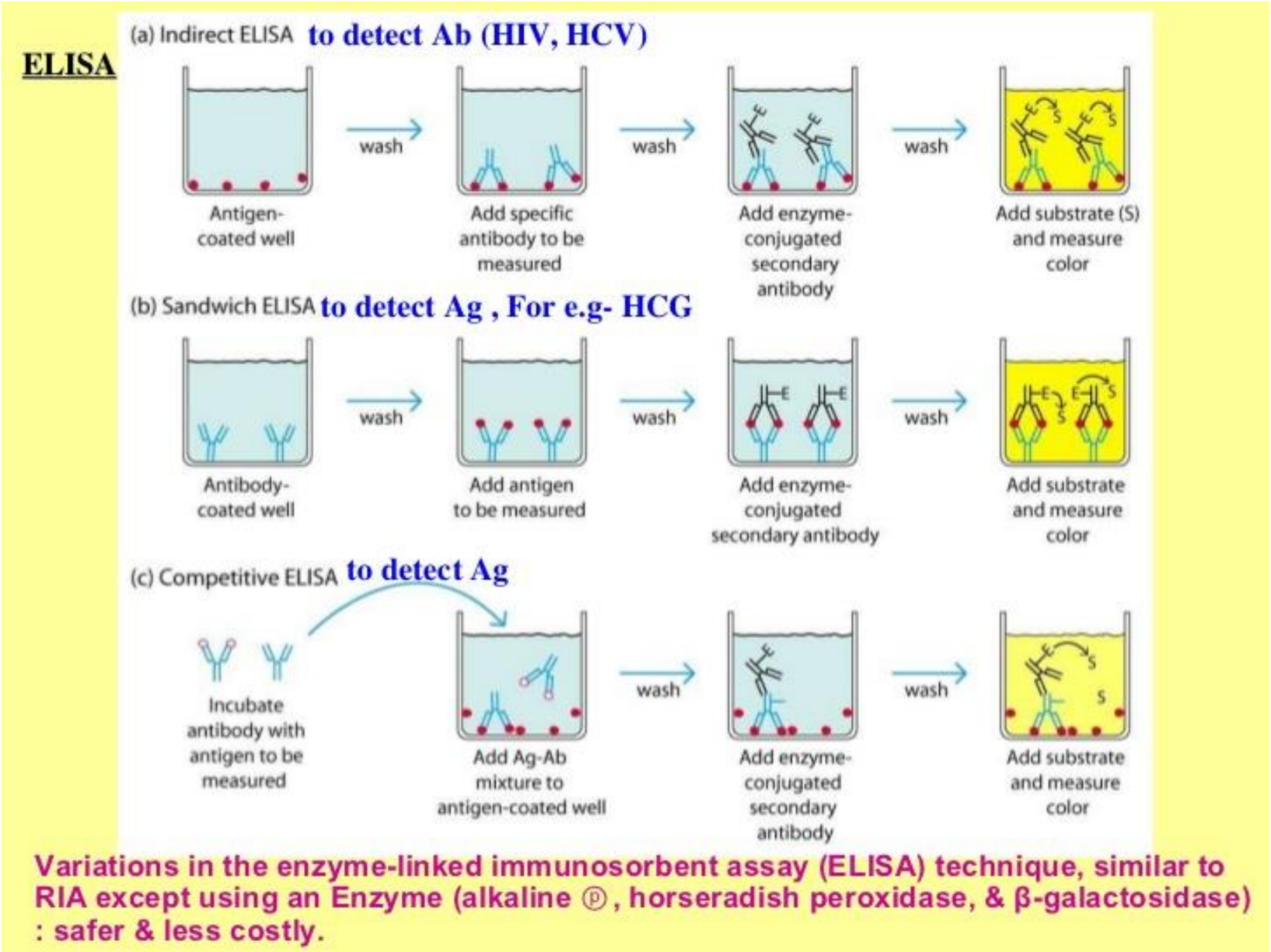
Huang, C.L., and O.W. Moe. 2011. Klotho: a novel regulator of calcium and phosphorus homeostasis. *Pflugers Archiv-European Journal of Physiology 462:185-193.*

RIA

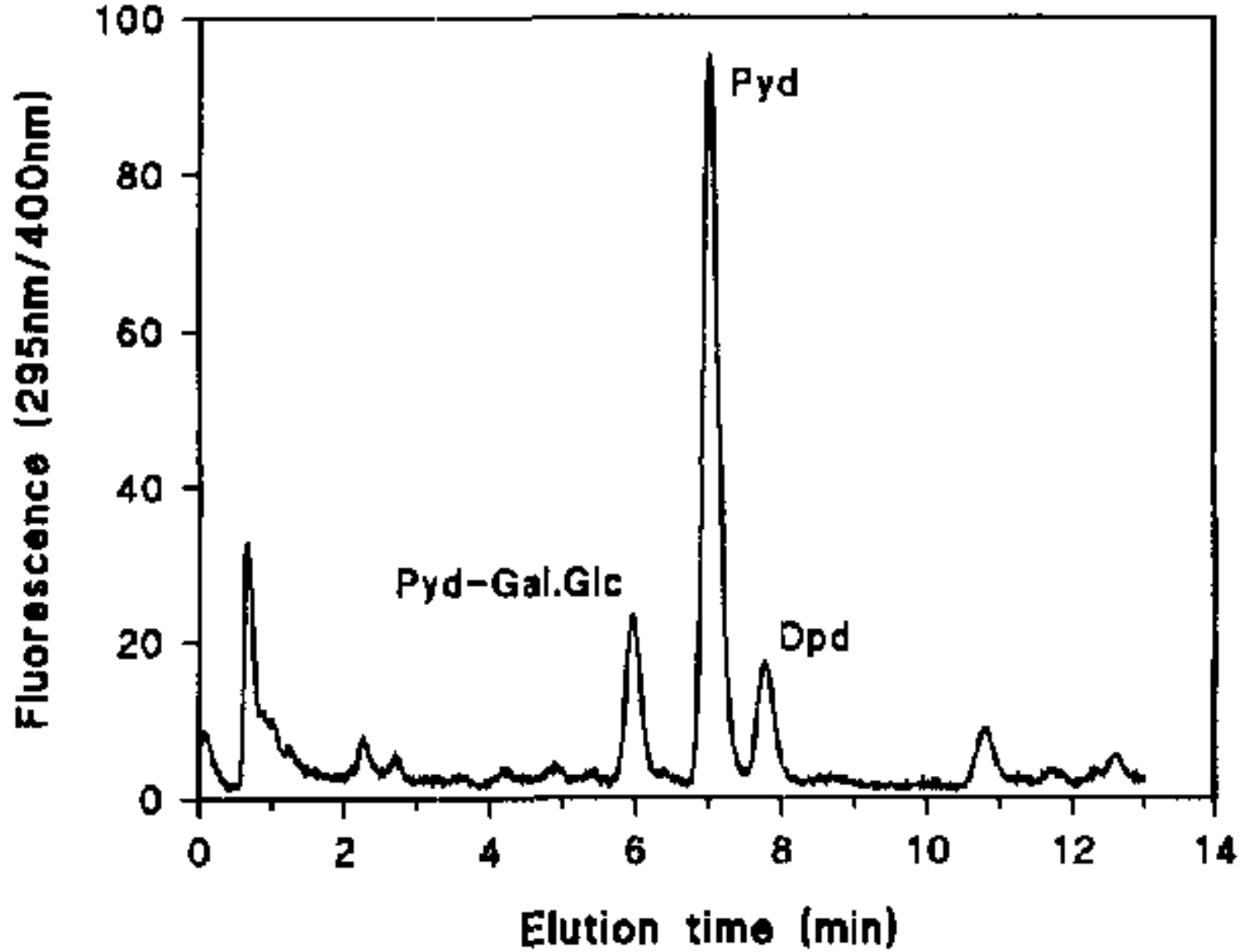

(A)
Radioactive protein
Nonradioactive protein
Solutions with mixtures of radioactive and nonradioactive proteins
(B)
Standard binding curve
% of bound radioactive protein
Concentration of nonradioactive protein
(C)
Determination of concentration of unknown value
% of bound radioactive protein
Concentration of nonradioactive protein


Radioimmunoassay Procedure
Before
Blank
After
Mix
and
Incubate
Separate
Bound = 6
Free = 0
Labeled Drug
Drug (0)
Antibody
Before
Drug = 6
After
Mix
and
Incubate
Separate
Bound = 3
Free = 3
Labeled Drug
Drug (0)
Antibody
Before
Drug = 3
After
Mix
and
Incubate
Separate
Bound = 4
Free = 2
Labeled Drug
Drug (0)
Antibody
Before
Drug = 12
After
Mix
and
Incubate
Separate
Bound = 2
Free = 4
Labeled Drug
Drug (0)
Antibody

ELISA

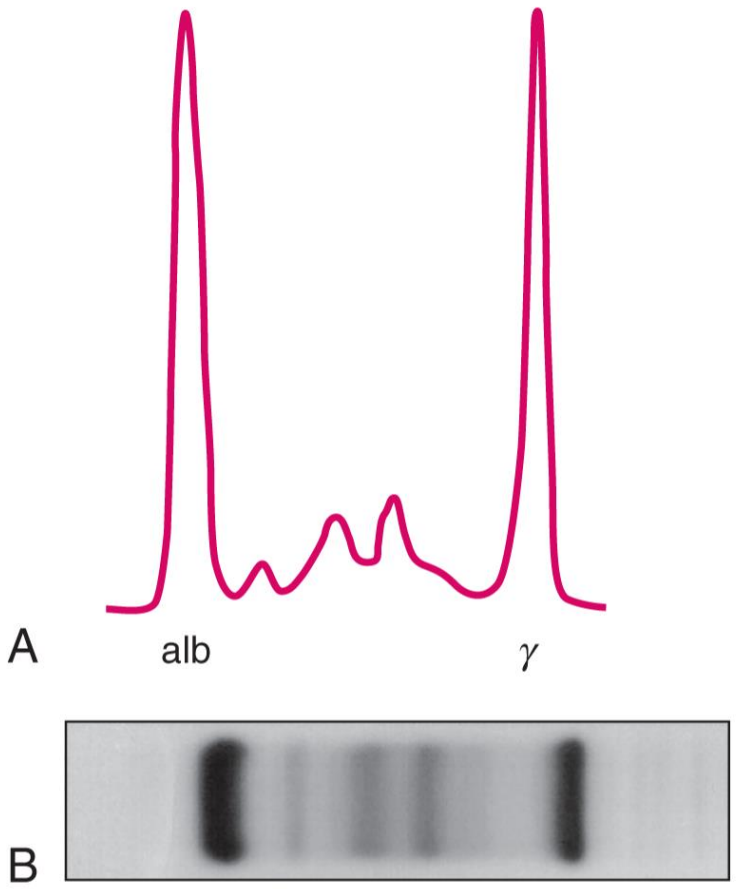

ELISA
(a) Indirect ELISA to detect Ab (HIV, HCV)
Antigen-coated well
wash
Add specific antibody to be measured
wash
Add enzyme-conjugated secondary antibody
wash
Add substrate (S) and measure color
(b) Sandwich ELISA to detect Ag , For e.g- HCG
Antibody-coated well
wash
Add antigen to be measured
wash
Add enzyme-conjugated secondary antibody
wash
Add substrate and measure color
(c) Competitive ELISA to detect Ag
Incubate antibody with antigen to be measured
Add Ag-Ab mixture to antigen-coated well
wash
Add enzyme-conjugated secondary antibody
wash
Add substrate and measure color
Variations in the enzyme-linked immunosorbent assay (ELISA) technique, similar to RIA except using an Enzyme (alkaline ⓟ, horseradish peroxidase, & β-galactosidase) : safer & less costly.

HPLC

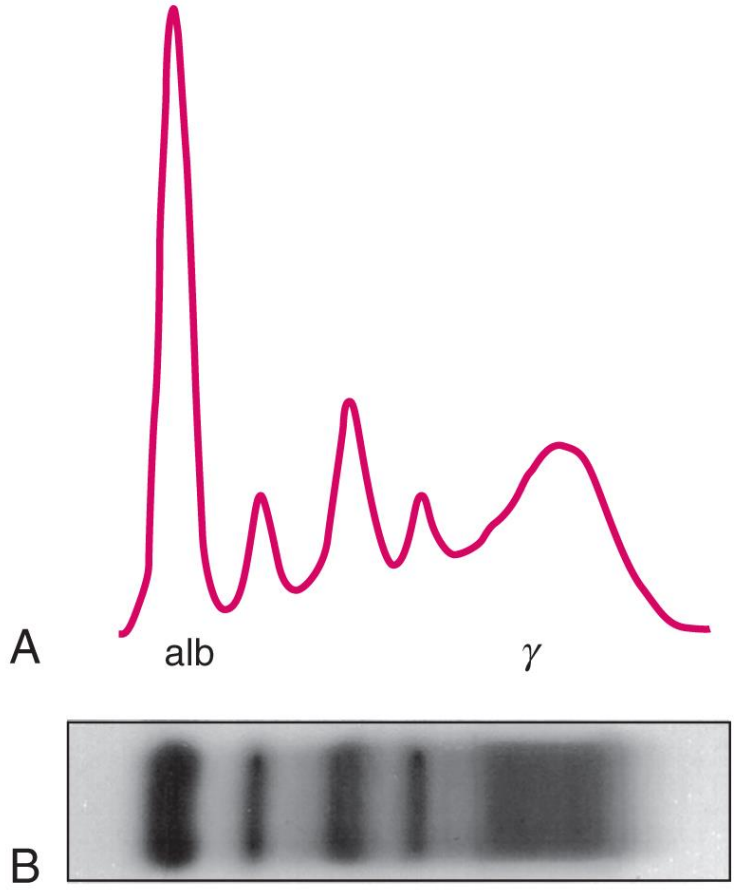

Fluorescence (295nm/400nm)
100
80
60
40
20
0
Pyd
Pyd-Gal.Glc
Dpd
0
2
4
6
8
10
12
14
Elution time (min)

Paraproteins

- M proteins
- Immunoglobulin or its fragment resulting produced by lymphoid-clone of plasma cells without a distinct antibody function
- IgG, IgA, IgM, light / heavy chain(s)
- Hematologic malignancies, blood diseases



A, Monoclonal pattern of serum protein as traced by a densitometer after electrophoresis on agarose gel: tall, narrow-based peak of γ mobility. B, Monoclonal pattern from electrophoresis of serum on agarose gel (anode on the left): dense, localized band representing monoclonal protein of γ mobility.



A, Polyclonal pattern from a densitometer tracing of agarose gel: broad-based peak of γ mobility. B, Polyclonal pattern from electrophoresis of agarose gel (anode on the left). The band at the right is broad and extends throughout the γ area.