Bone Development and Metabolism

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Skeletal physiology
   Bone structure
   Bone development
     Chondrocytes
     Osteoblasts
   Bone remodelling
     Osteocytes
     Osteoclasts
     Osteoblasts

Skeletal pathology
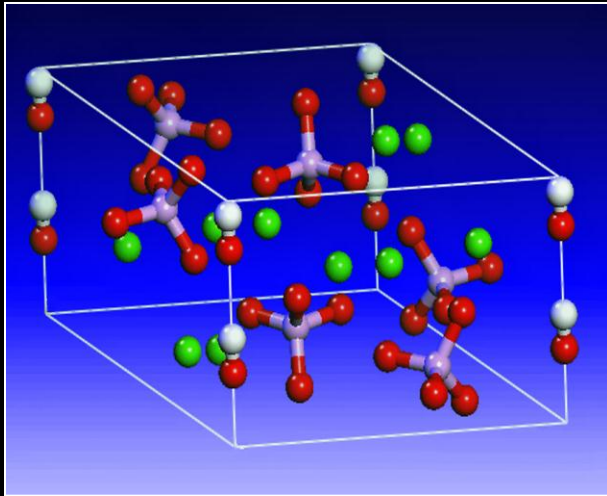   **Osteoporosis**
   Paget's disease of bone

Skeletal Physiology

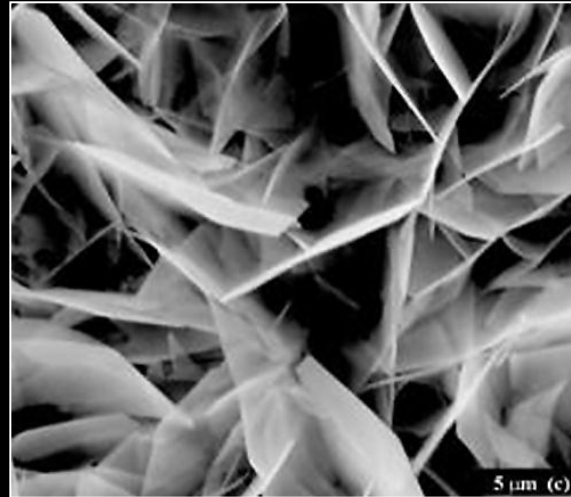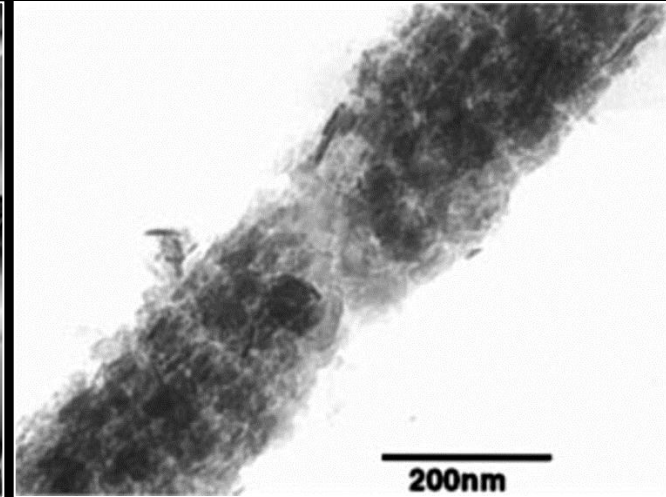# Bone Structure

Bone must be stiff yet flexible and light yet strong

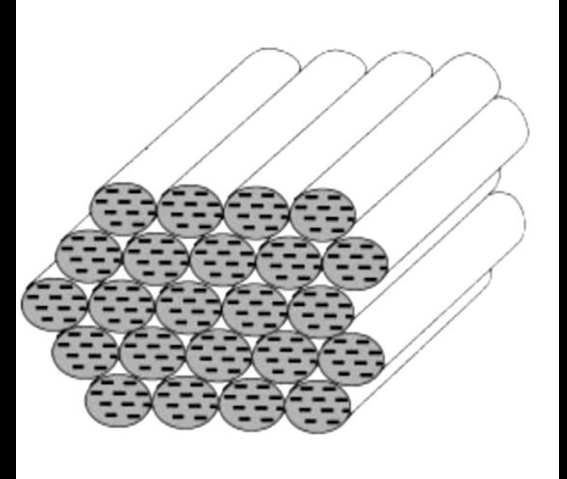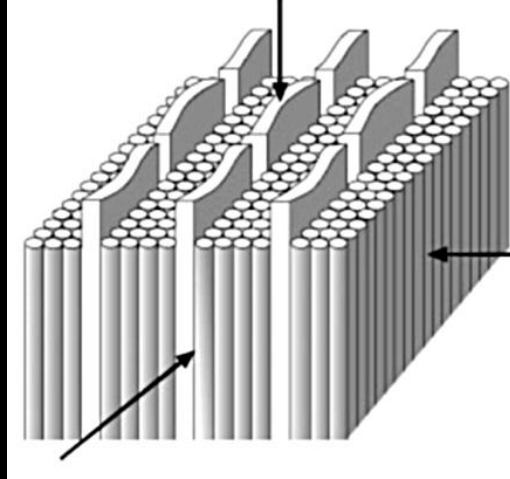# Bone mineral

Hydroxyapatite
$Ca_{10}(PO_4)_6(OH)_2$

Roof tile like crystals
(4 x 50 x 25nm)

Crystals pack into
Type 1 collegen

Bone matrix

Matrix component
- **Type I collegen rich Osteoid**
- **Type I collagen molecule (1.5nm in diameter)**
    - **Triple helicle collagen molecule 300nm long**
- **Collagen fibril (100nm in diameter)**
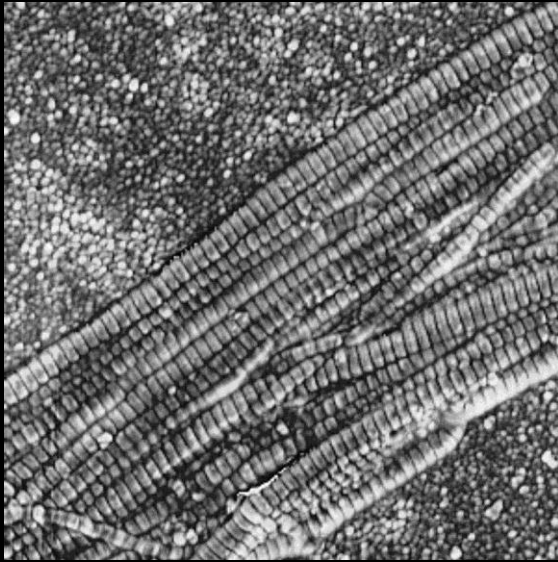   - **Collagen packs in an array with mineral crystals**
- **200 non-collagenous proteins**
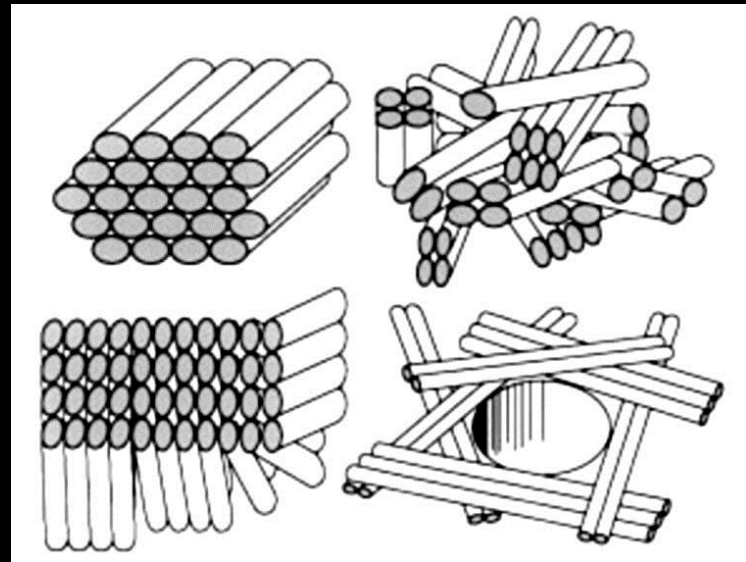   - **<10% of total protein**

Human bone is 60% mineralised
- **Increased mineralisation increases stiffness but reduces flexability**

Bone structure

Collagen fibrils

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

Macro and microstructure of cortical bone

Cortical bone

Trabecular bone
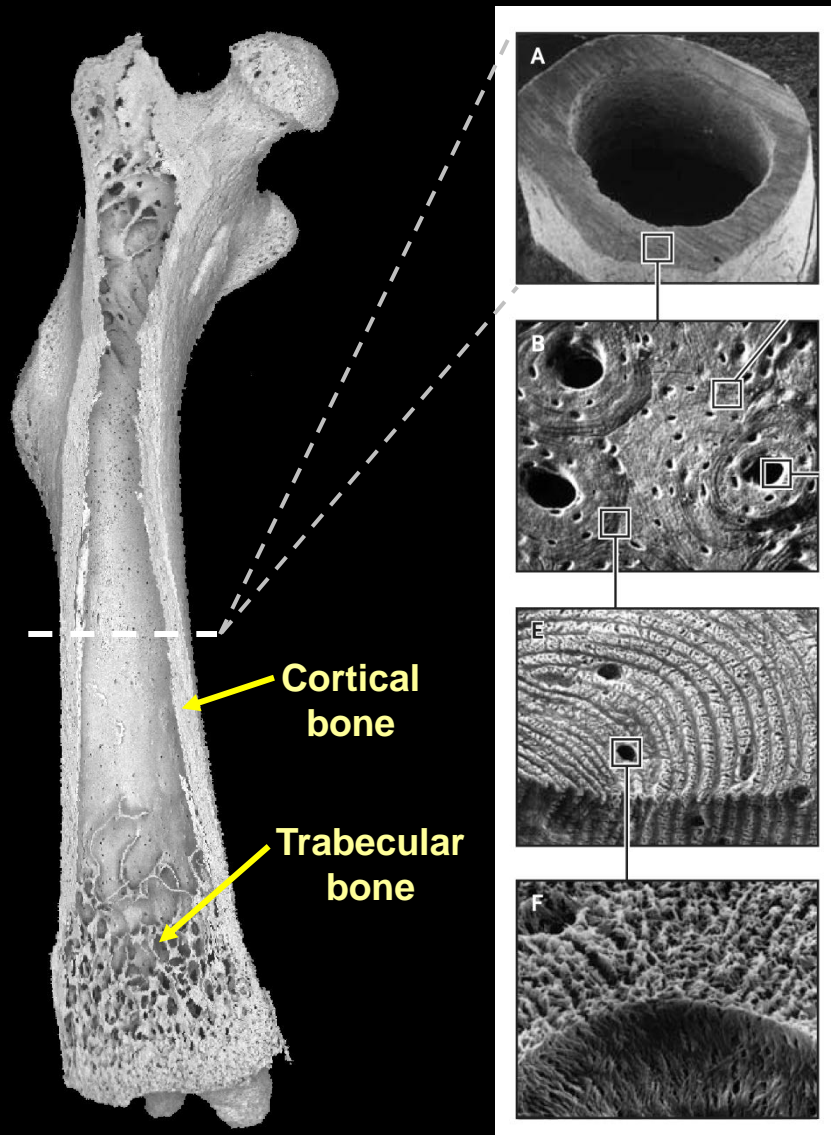
Ovelapping parallel osteon structure
    Result of completed remodelling cycles

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

Bone development

Skeletal development

Intramembranous Ossification

Endochondral Ossification

E17.5 mouse
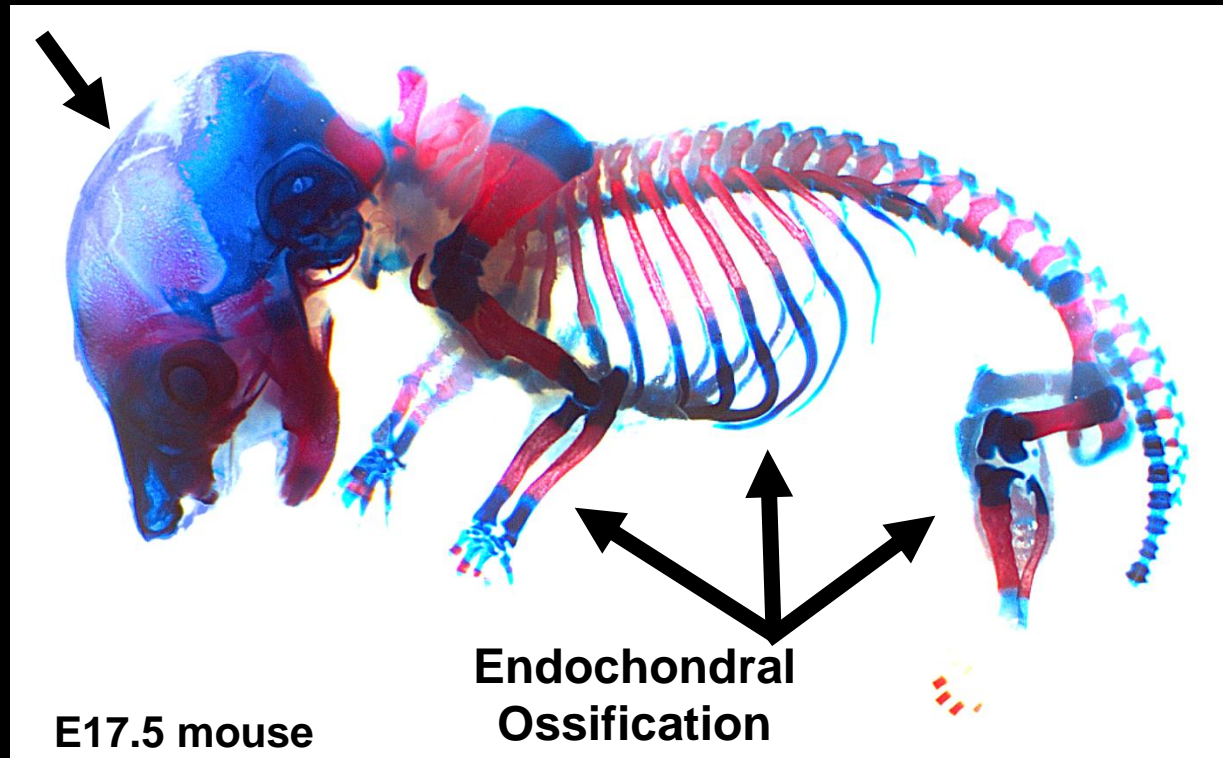
Long bone form by endochondral ossification

Craniofacial bones by intramembranous ossification

Chondrocytes
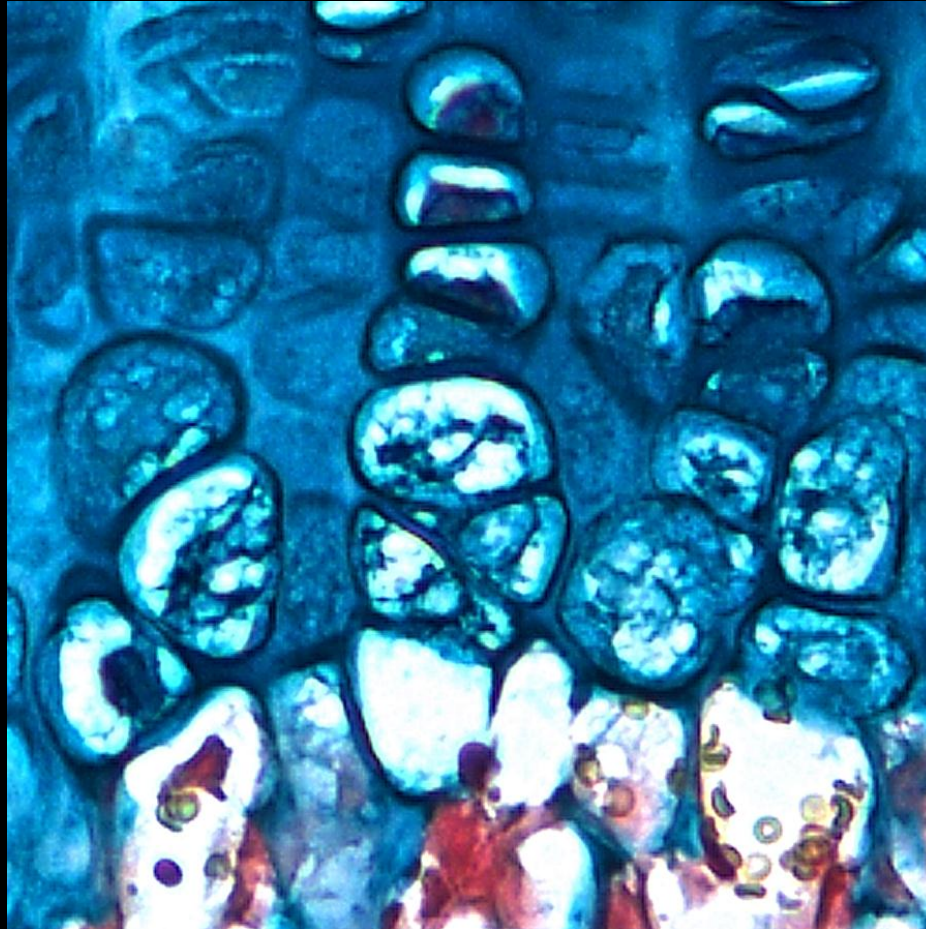
Chondrocytes and endochondral ossification

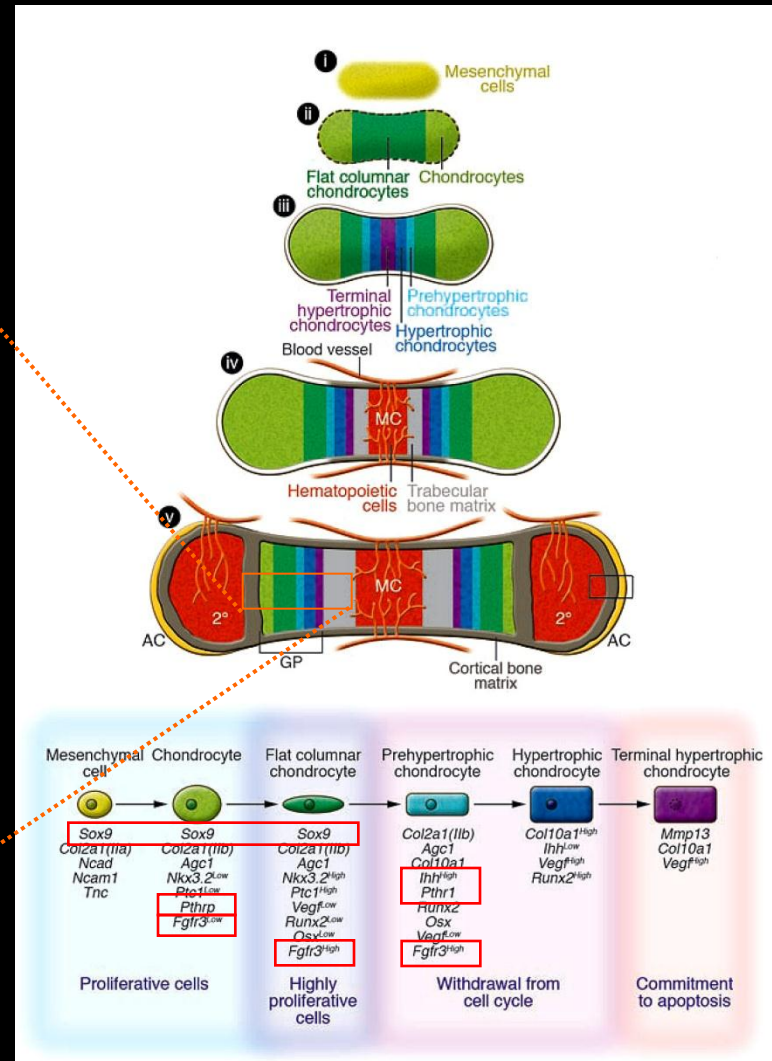## Mesenchymal cells differentiate into chondrocytes
Long bones form on a cartilage scaffold

Reserve

Proliferative
(Collagen II)

Pre-hypertrophic

Hypertrophic
(Collagen X)

(Zuscik MJ et al 2008 J. Clin. Invest. 118:429–438)
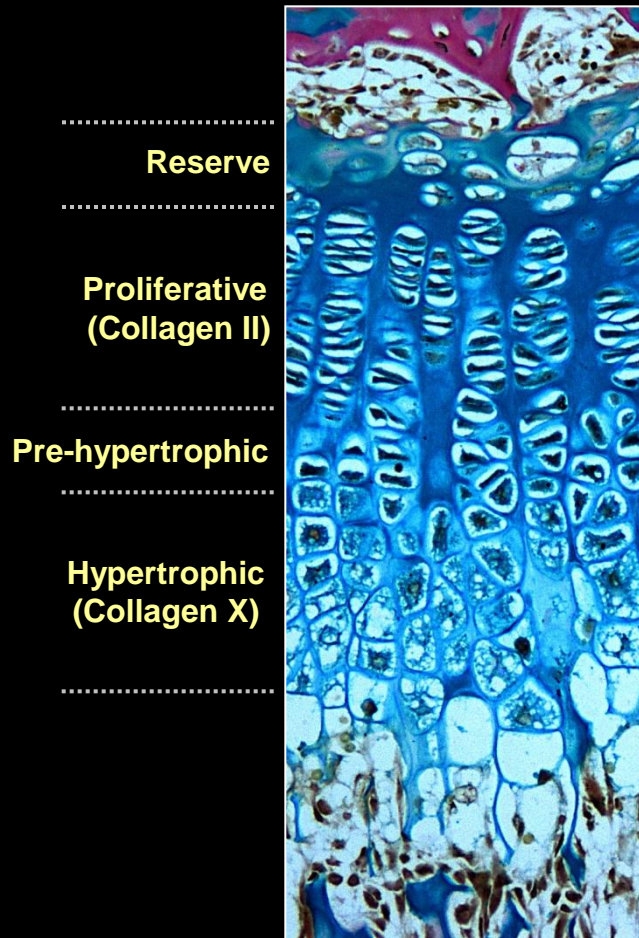
Endochondral ossification
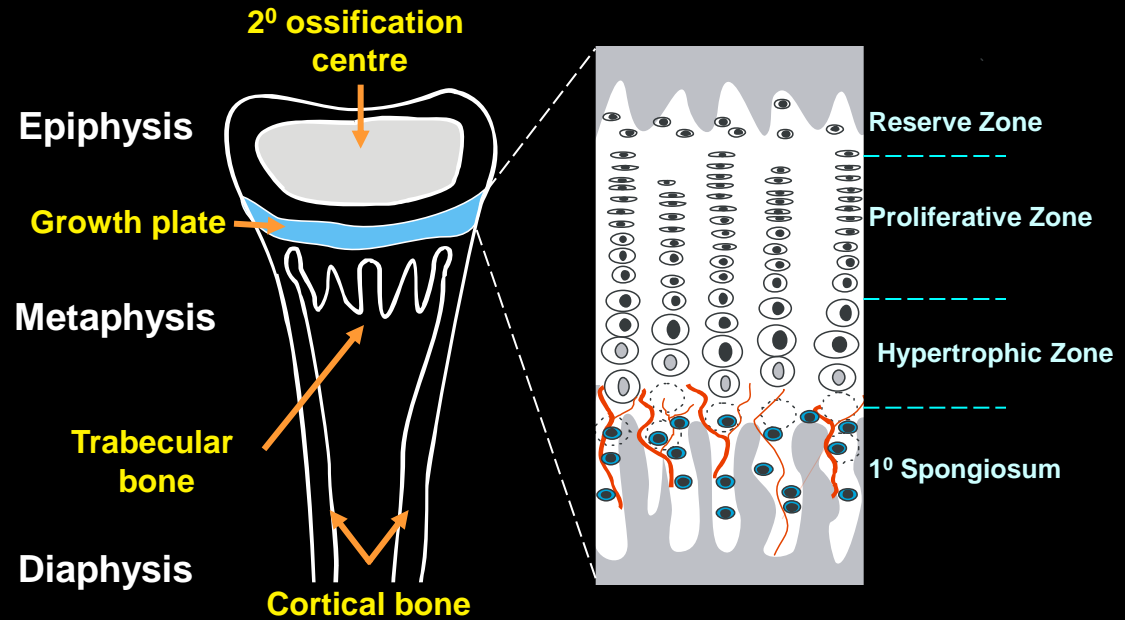
Sox9   Master transcriptional regulator in chondrocyte

FGF/FGFRs   Inhibit chondrocyte proliferation and differentiation

Indian hedgehog (Ihh)   Promotes chondrocyte proliferation and induced PTHrP

PTHrP/PTHR1   Inhibit chondrocyte differentiation

Indian hedgehog regulates growth plate chondrocyte proliferation and differentiation

(Kronenberg HM (2003) Nature 423:332-336; Mak KK et al (2008) Development 135:1947-1956)

Achondroplasia

FGF/FGFR3 signalling inhibits chondrocyte proliferation and differentiation
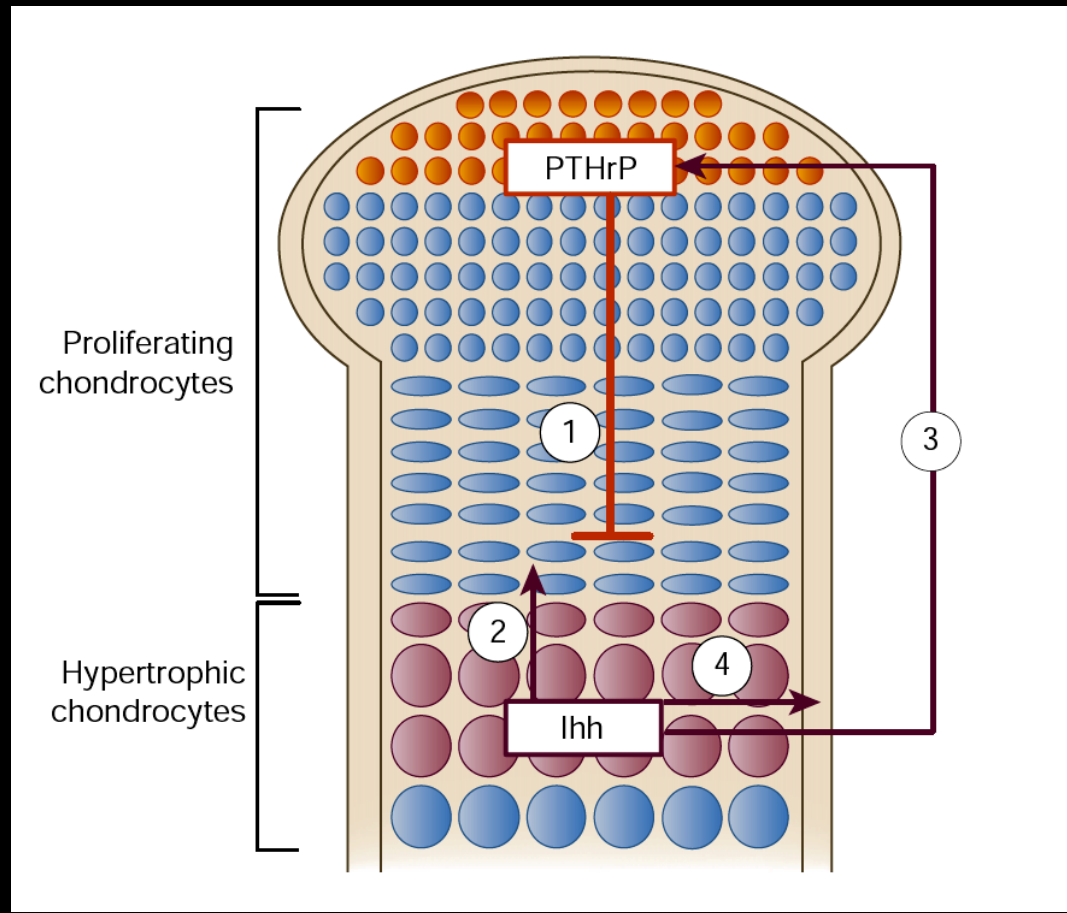

Most common form of dwarfism (1:250 000)
Gain of function mutation FGFR3 (Gly380Arg)
  95% have the same point mutation
  80% of these are new mutations
  Macrocephaly, frontal bossing, midface hypoplasia, small chest, rhizomelia

FGF/FGFR3 action in chondrocytes

(Day TF et al (2008) J Bone Joint Surg 90:19-24; Horton WA et al (2007) Lancet.370:162-72)
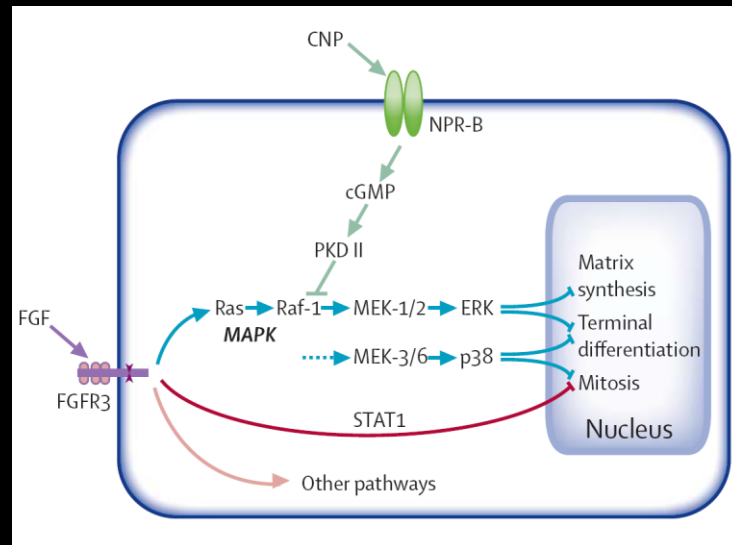
Osteoblasts

Intramembranous ossification

Craniofacial skeleton forms by intramembranous ossification
Mesenchymal cells differentiate into osteoblasts
Bone is formed directly without a cartilage scaffold
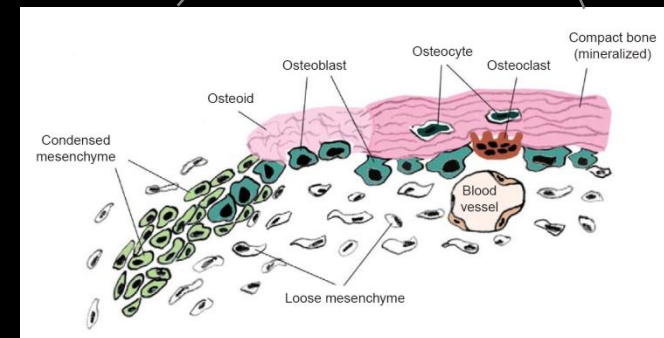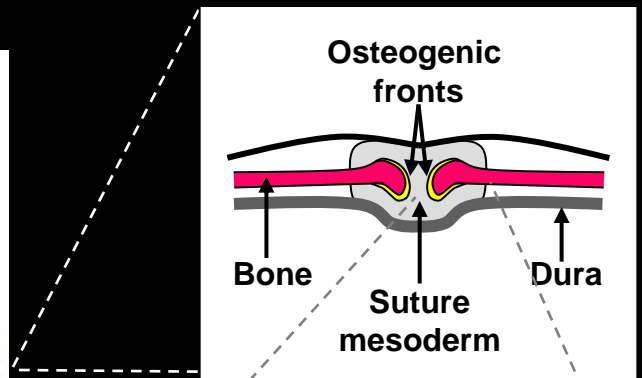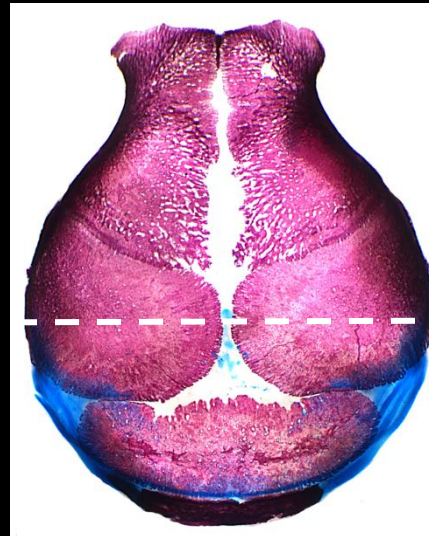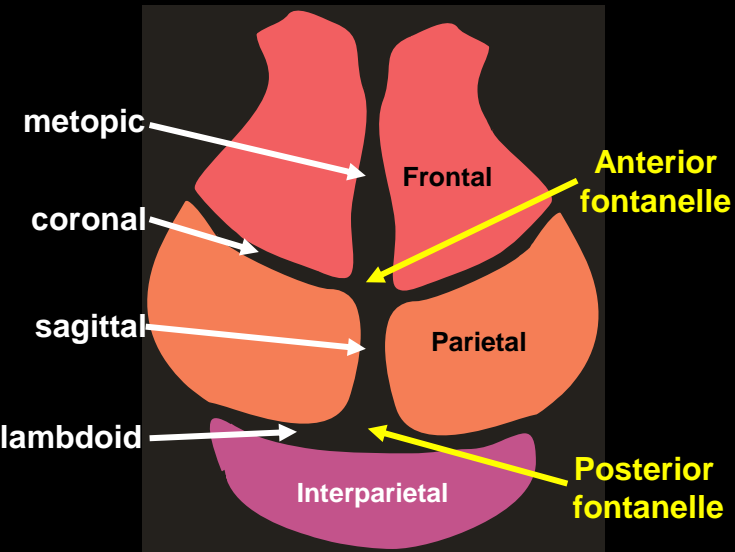
Osteoblastic bone formation
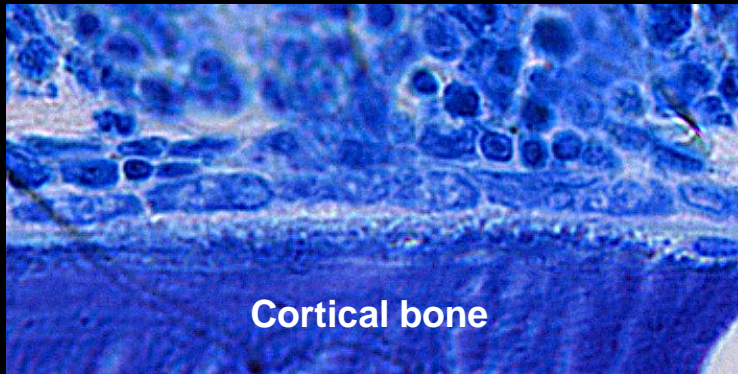
Osteoblasts
Osteoid
Cortical bone
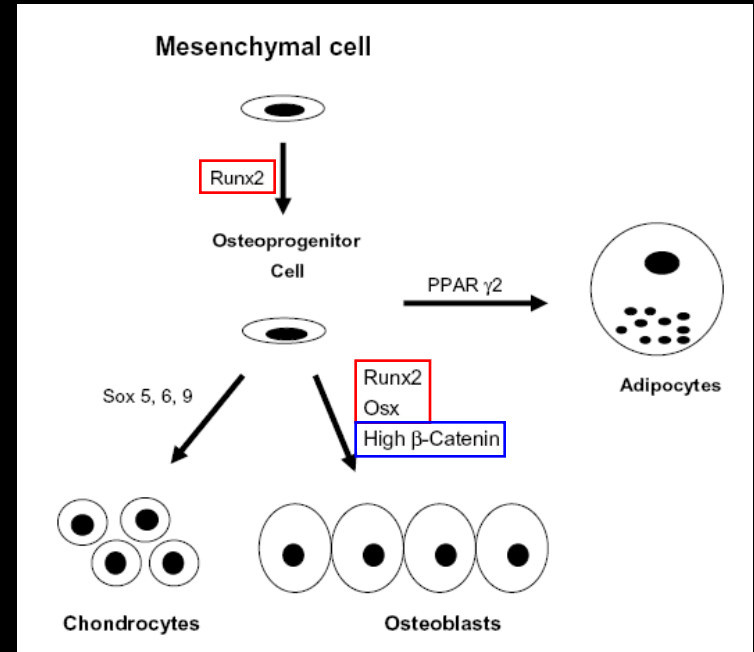
Osteoblastogenesis

Osteoblasts, chondrocytes and adipocytes all derive from mesenchymal cells

Key transcription regulators: **Runx2, Osterix and β-Catenin**
Paracrine factors: **Wnt, BMPs and FGFs**
Systemic hormones: **GH/IGF1, GCs, E2, PTH and 1,25(OH)$_2$D**

Osteoblast differentiation
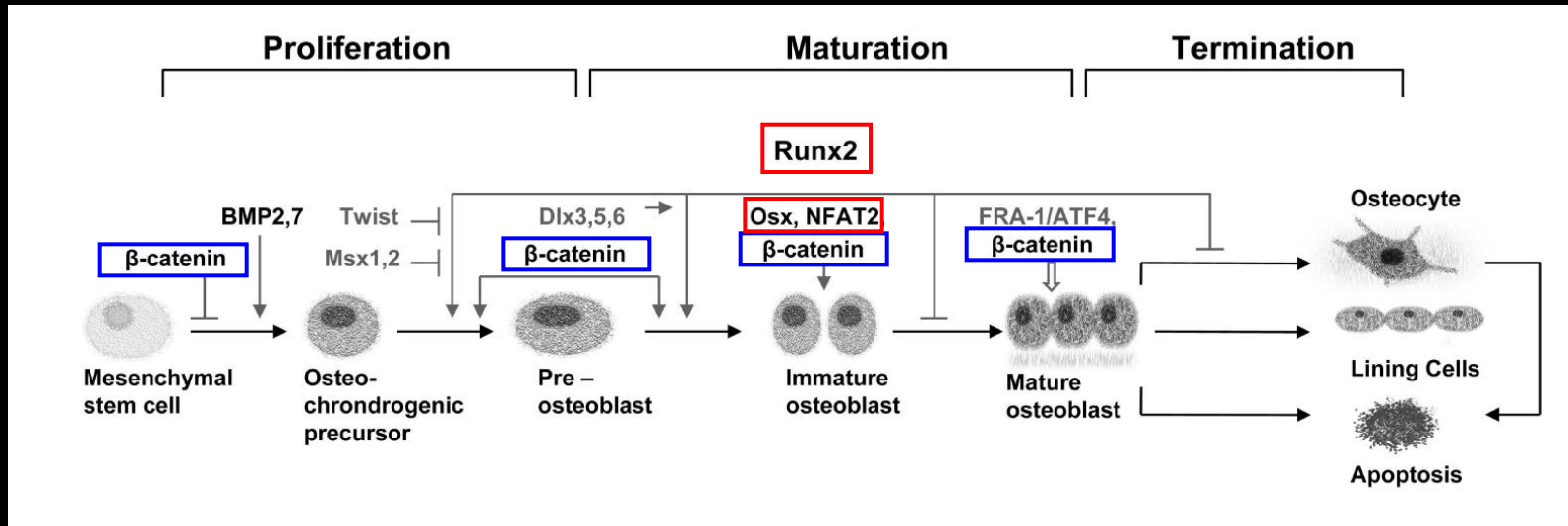
Runx2 is the master transcription factor in osteoblast differentiation
    Runx2 null mice have no osteoblasts and no mineralised bone
        Runx2 mutation cause cleidocranial dysplasia (CCD)
    Runx2 directly regulates expression of transcription factor Osterix
        Osx null mice also lack osteoblasts
    Osterix and transcription factor NFAT2 cooperatively regulate key genes
        Collagen I, osteopontin, osteocalcin, osteonectin

Wnt/β-catenin is a key regulator of osteoblast differentiation and function

Key role of Wnt signalling
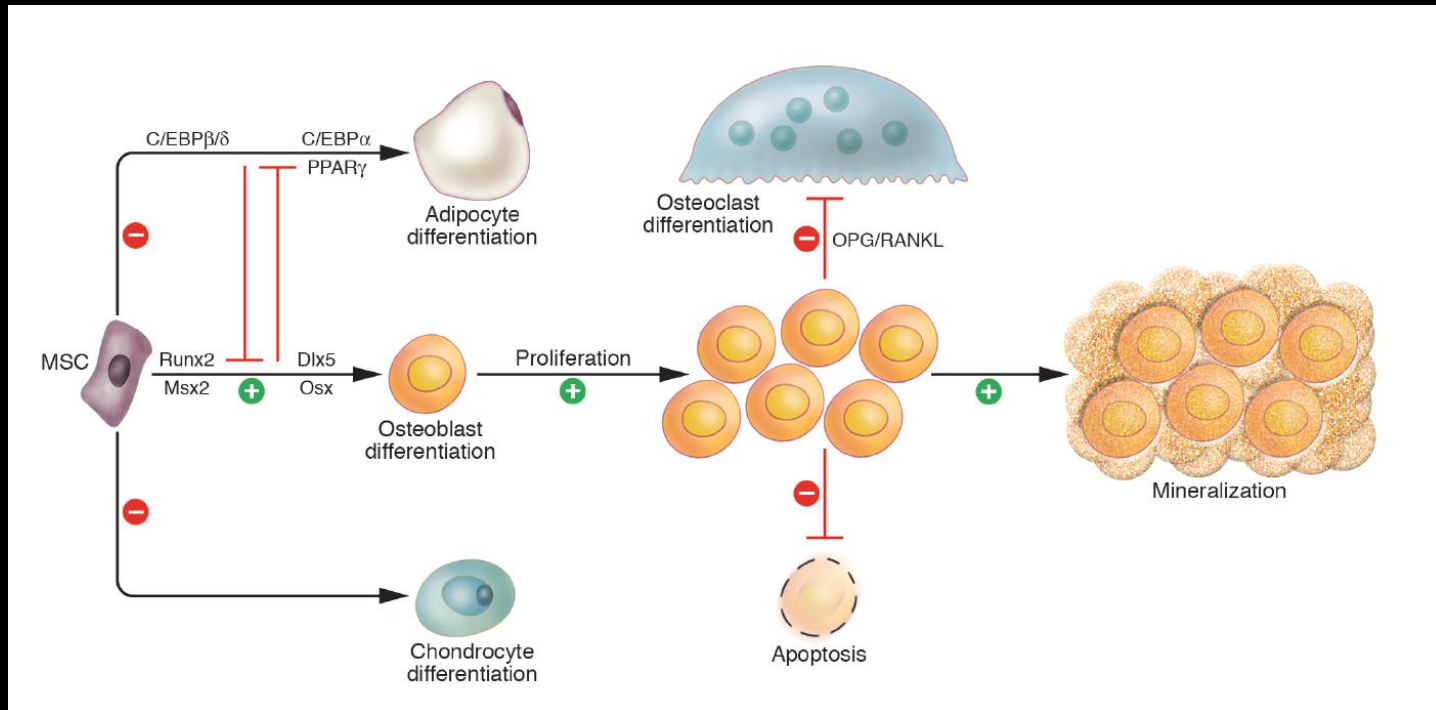
Wnts
    Repress alternative mesenchymal differentiation pathways
    Promote osteoblast differentiation, proliferation, and mineralisation
    Inhibits osteoblast apoptosis
    Represses osteoclastogenesis by increasing OPG expression

Wnt signalling regulates bone mass
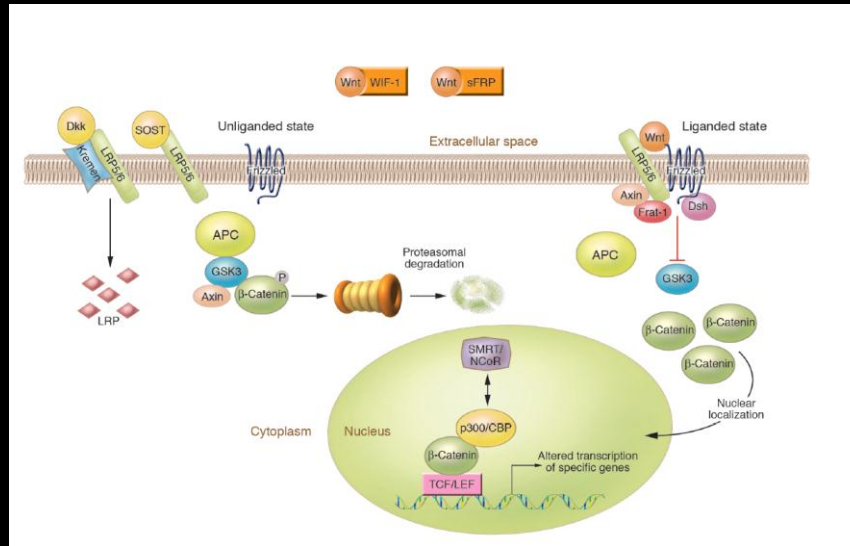
Wnt binding to co-receptors Frizzled and LRP5/6 inhibits GSK3
   GSK3, APC and Axin targets β-catenin for degradation by phosphorylation
   Wnt stabilises β-catenin preventing its degradation
   β-catenin enters nucleus and binds TCF/LEF regulating target genes
Negative regulation of Wnt signalling
   Wnt binding (WIF-1 and sFRP)
   LRP5/6 degradation (Sclerostin (SOST) and Dickkopf (Dkk))
LRP5 gain of function mutations (Gly171Val)
   Reduces affinity for Dkk and causes high bone mass
LRP5 loss of function mutations
   Osteoporosis pseudoglioma syndrome
SOST loss of function mutations cause Sclerostosis with high bone mass

(Krishnan V eta I (2006) J. Clin. Invest. 116:1202–1209)

Endocrine regulation of osteoblasts
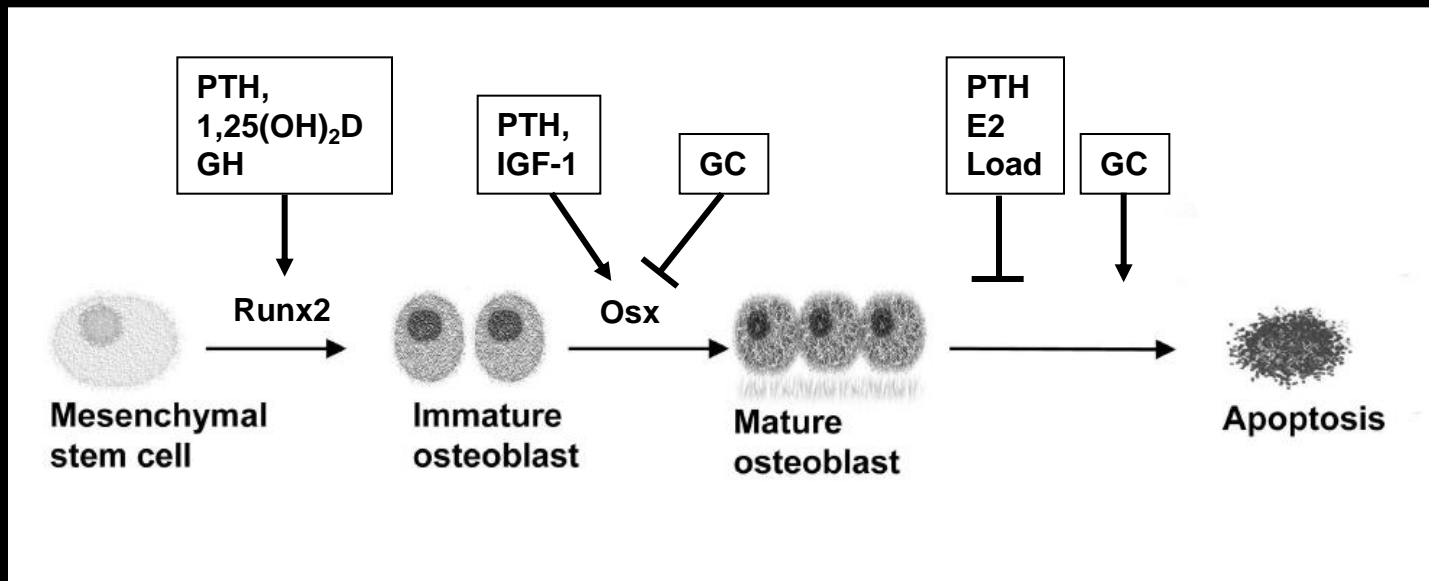
PTH has anabolic and catabolic actions

Continuous PTH results in net cortical resorption
Intermittent PTH results in net trabecular formation
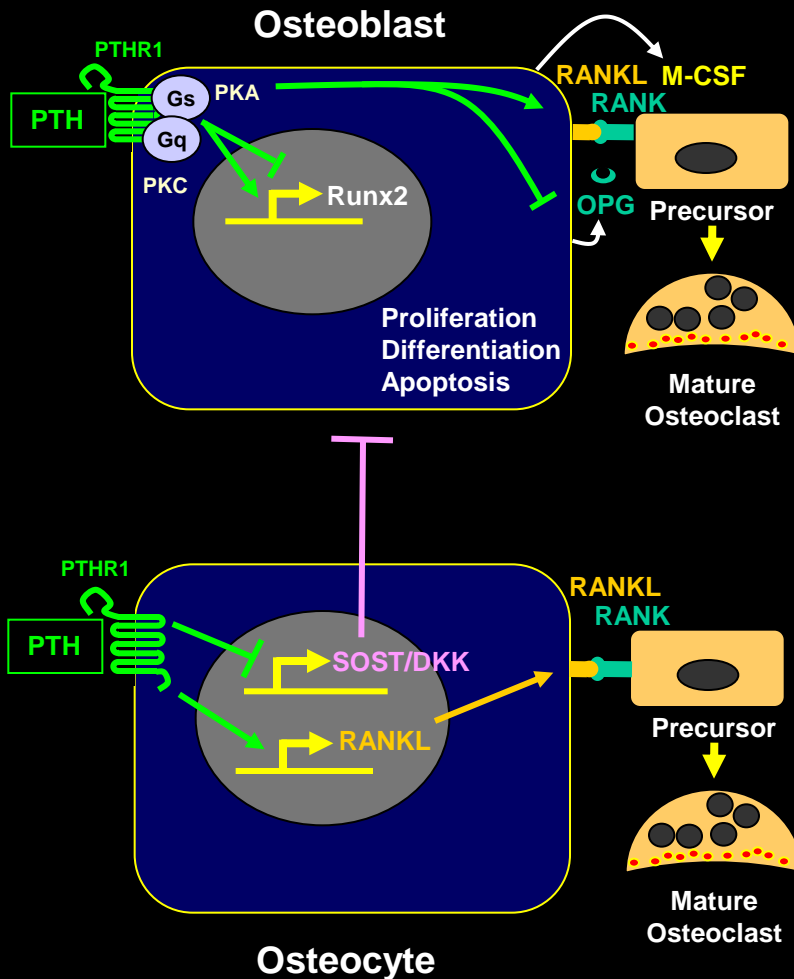
Increases osteoclast differentiation indirectly
In osteoblasts (⬆ MCSF/RANKL and ⬇ OPG)
In osteocytes (⬆ RANKL)

Regulates pre-osteoblast maturation
In pre-osteoblasts
   Continuous PTH ⬇ Runx2
   Intermittent PTH ⬆ Runx2
In osteocytes
   PTH ⬇ SOST/DKK (⬆ Wnt signalling)

Other paracrine mechanisms
   PTH ⬆ IGF-1 and FGF release

Maintenance of adult bone

The bone remodelling cycle
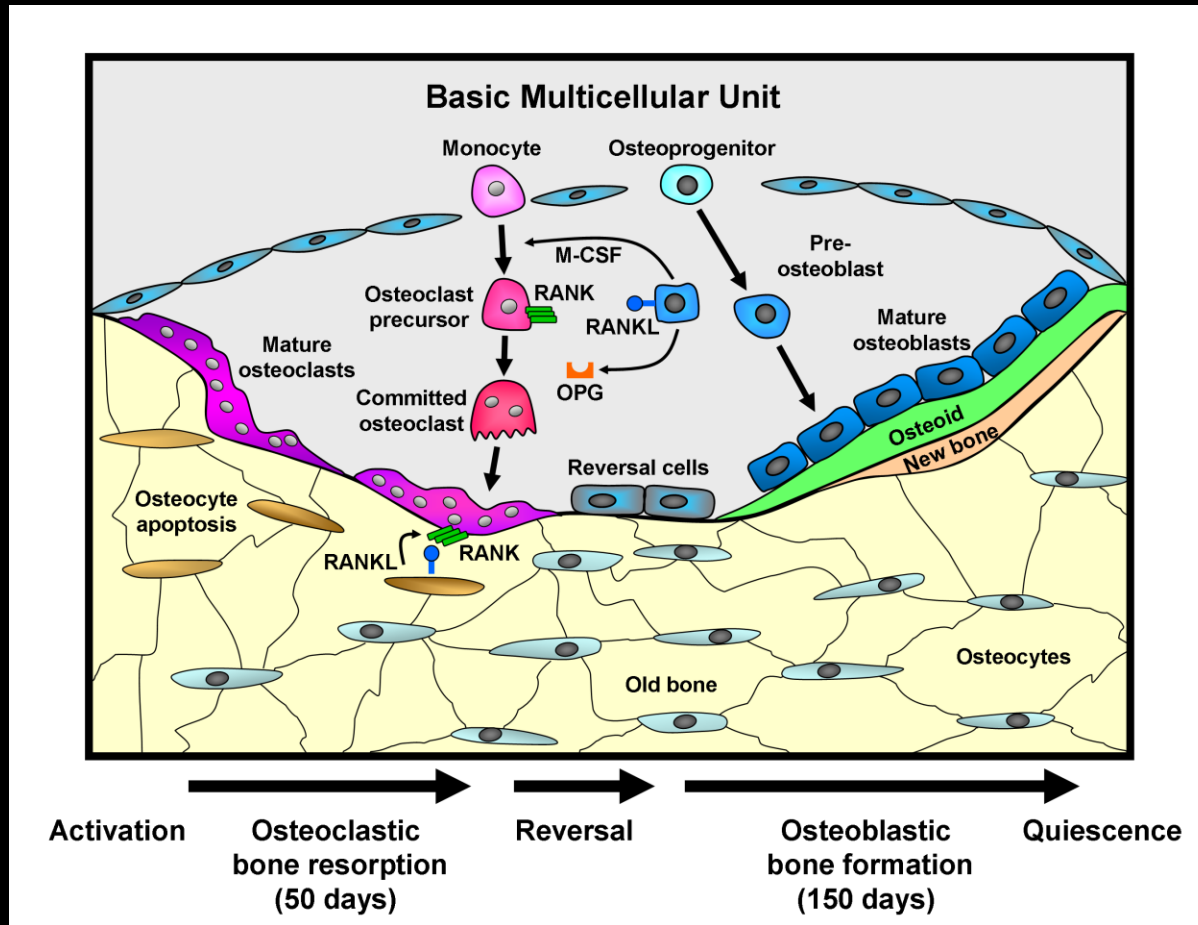
Ostoclastic bone resorption followed by ostoblastic bone formation

Maintain homeostasis of Ca $^{2+}$ and PO$_4$ $^{3-}$

Repair damaged matrix and micro-fractures

Adapt to mechanical stress and strain

Resorption and formation are coupled temporally and spatially

Uncoupling leads to osteoporosis or osteopetrosis

Osteocytes
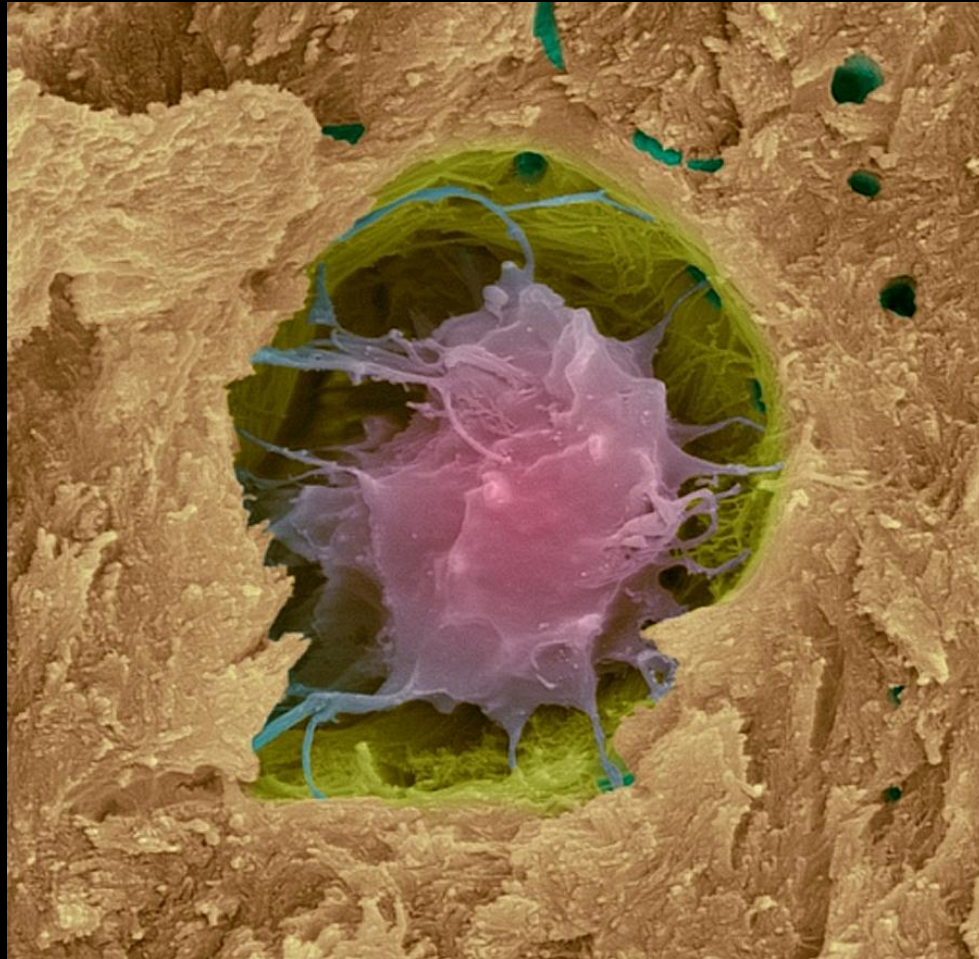
Osteocytes orchestrate bone remodeling
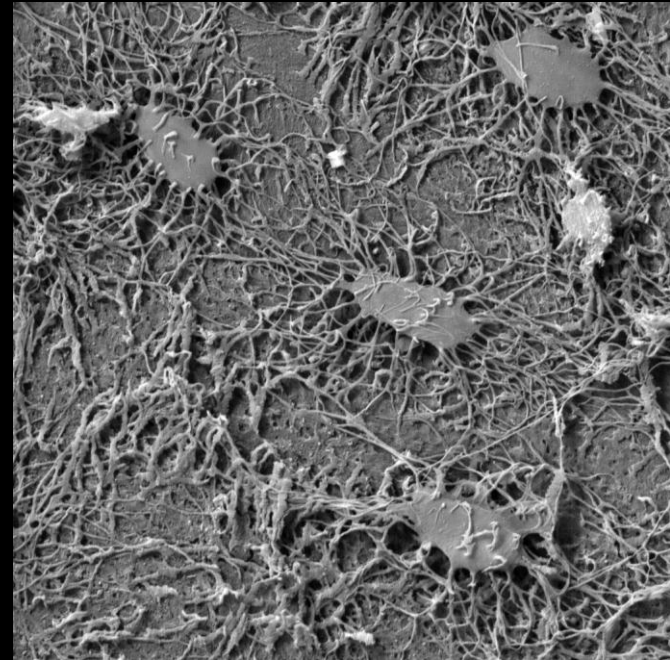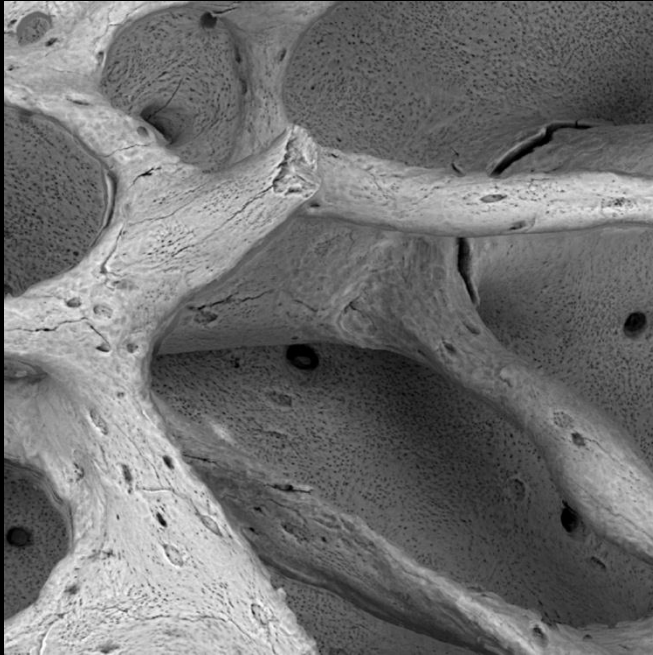
Ostrocytes make up 90-95% of all adult bone cells
Osteoblasts 5%, osteoclasts 1-2%
Osteocyte surface area >100x that of the bone itself

Osteocytes form a complex network of connected processes
Mechanical load sensors regulating bone resorption and formation
Endocrine organ regulating phosphate (FGF23)
Endocrine organ regulating metabolism (osteocalcin) ?

Osteocytes are mechanosensors

Bone remodelling is required to repair damage and adapt load

Osteocyte Mechano-transduction → Lining Cell/Marrow cell → Lining Cell Retraction → Osteoclast Formation and Activation
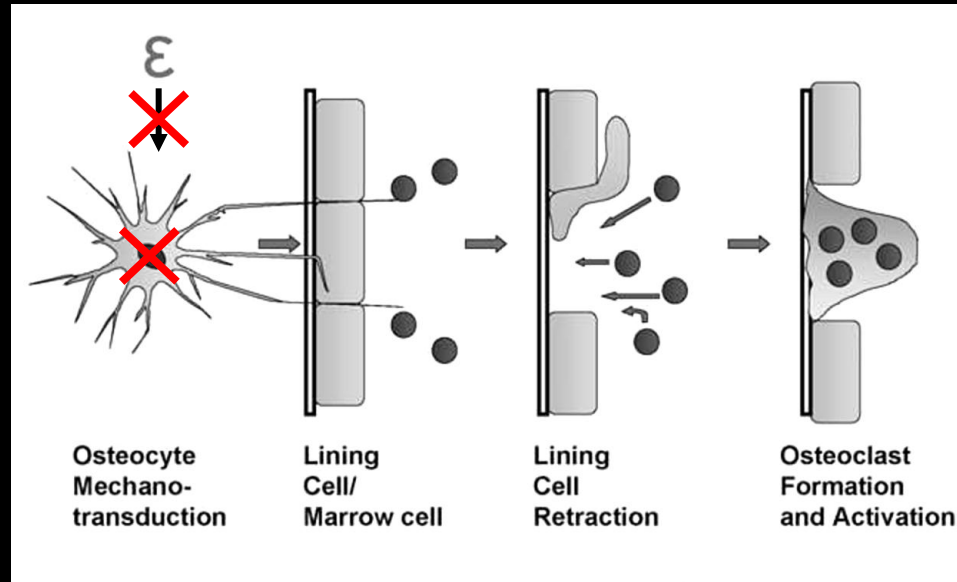
Osteocytes regulate osteoclasts resorption
   During bone loading osteocytes inhibit osteoclast resorption (⬆ TGFβ?)
   Unloading, hypoxia or osteocyte apoptotosis initiates resorption (⬆ RANKL)

Increased osteocyte apoptosis
   E2 deficiency (TNFα/IL-1), GCs

Increases osteocyte survival
   E2, SERMs, bisphosphonates

(Bonewald LF (2008) ASBMR Primer 22-27; Xiong J (2012) JBMR 27:499-505)

Osteocytes and bone formation

Osteocytes maintain balance of bone formation and mineralisation

ε

| Osteocyte Mechano-transduction | Lining Cell | Dedifferentiation or recruitment | New Bone Formation |

Sost, Dmp1, Phex, Mepe
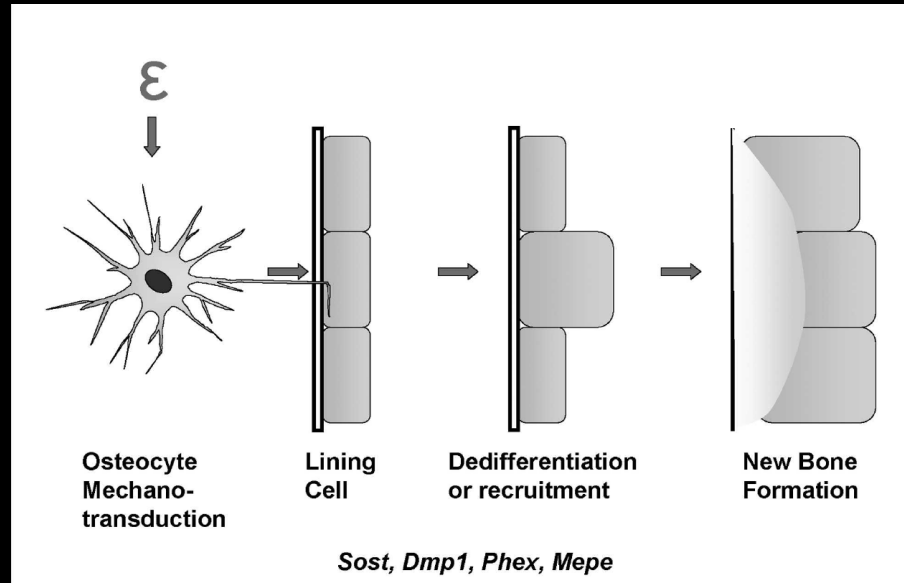
Mechanical strain is required for normal adaptive remodelling
Fluid shear stress in canaliculi is thought to regulate osteocyte gene expression

Promoters of mineralisation

Osteocyte Dmp1 increases phosphate (↓ FGF23) (ARHP)

Osteocyte Phex increases phosphate (↓ FGF23) (XLH)

Inhibitors of mineralisation/bone formation

Osteocyte Sclerostin binds LRP5 (↓ Wnt signaling) (Sclerostosis)

Osteocyte Mepe inhibits mineralisation and phosphate resorption (TIO)

Osteoclasts
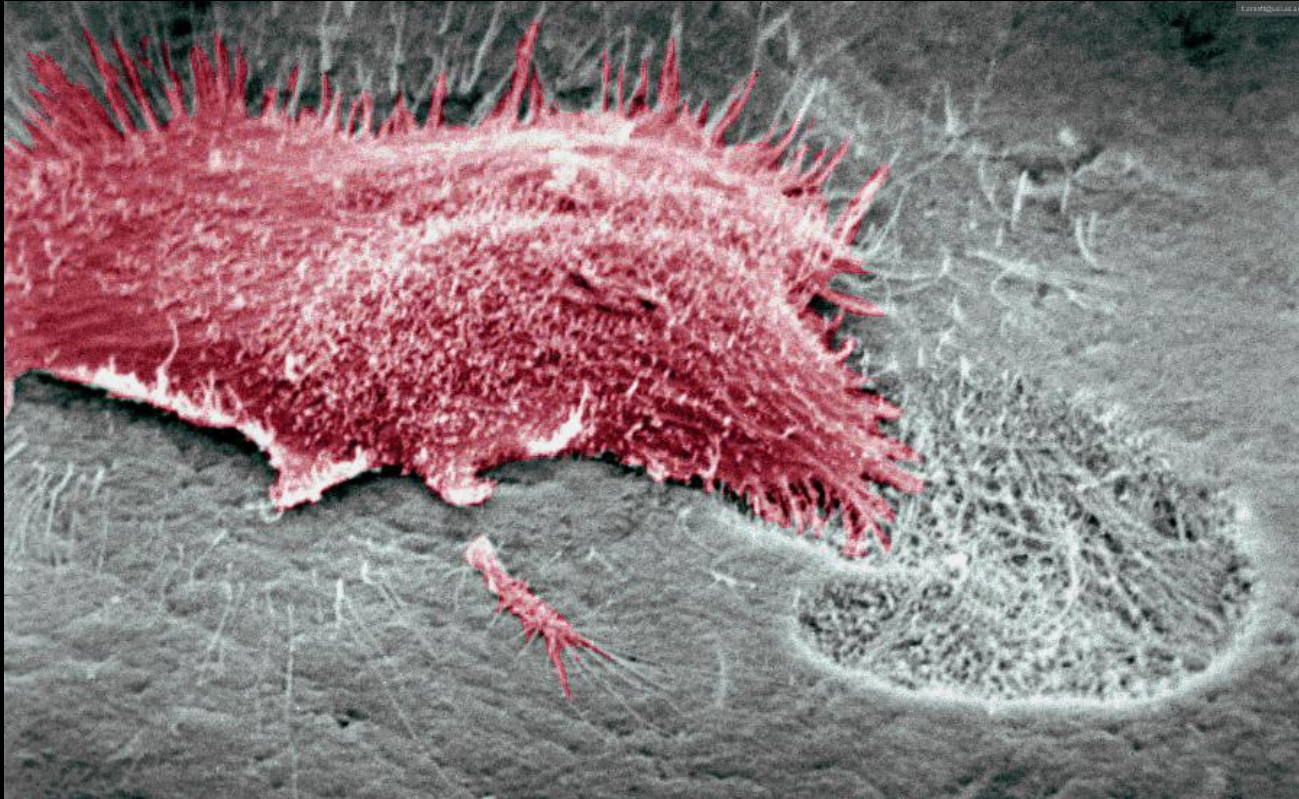
Osteoclastogenesis
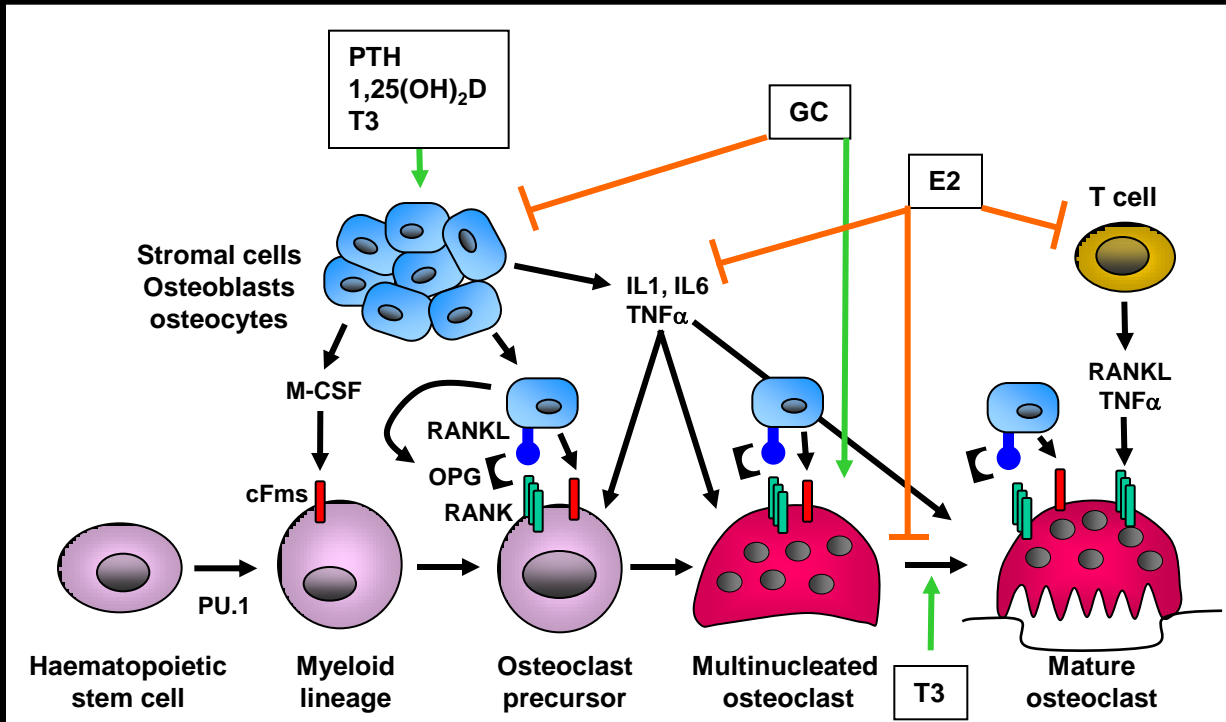
Osteoclasts derive from the myeloid lineage and are multinucleated cell
   M-CSF regulates proliferation, survival and differentiation of precursors
   RANKL is key osteoclastogenic cytokine sufficient for differentiation
   OPG is a decoy receptor (physiological inhibitor of RANKL/RANK signaling)
PTH, 1,25(OH)$_2$D and pro-inflammatory cytokines
   increase RANKL expression and suppress OPG
Loss of function mutations of OPG cause juvenile Paget's disease
Loss of function mutations of RANK cause osteopetrosis

(Ross FP (2008) ASBMR Primer. 16-22)

Osteoclast function
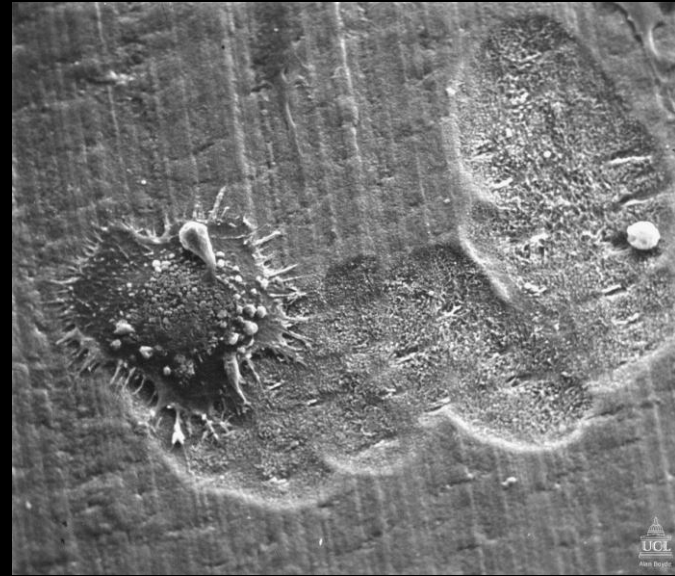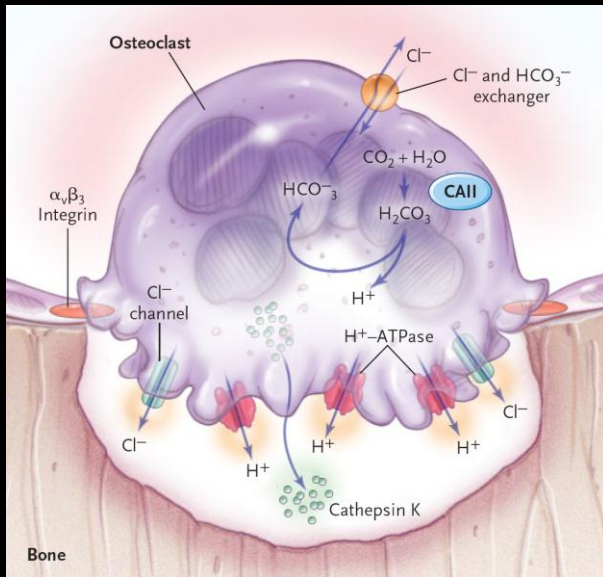
Active osteoclasts are polarised cells
   Attach to the bone surface via integrin $\alpha_v\beta_3$ (Sealing zone)
       Requires action of small GTPases (inhibited by bisphosphonates)
   Form ruffled membrane adjacent to bone surface
   Secrete hydrogen and chloride ions that dissolve bone mineral
       H^+ generated by CAII; H^+-ATPase and CLCN7 secrete H^+ and Cl^-
   MMPs and Cathepsin K degrade the collagen matrix

Loss of function mutations in CLCN7 (H^+-ATPase and CAII) cause osteopetrosis
Loss of function mutations of cathepsin K cause pyknodysostosis
   skull and facial deformity, osteosclerosis and fragility of bone

Skeletal Pathology
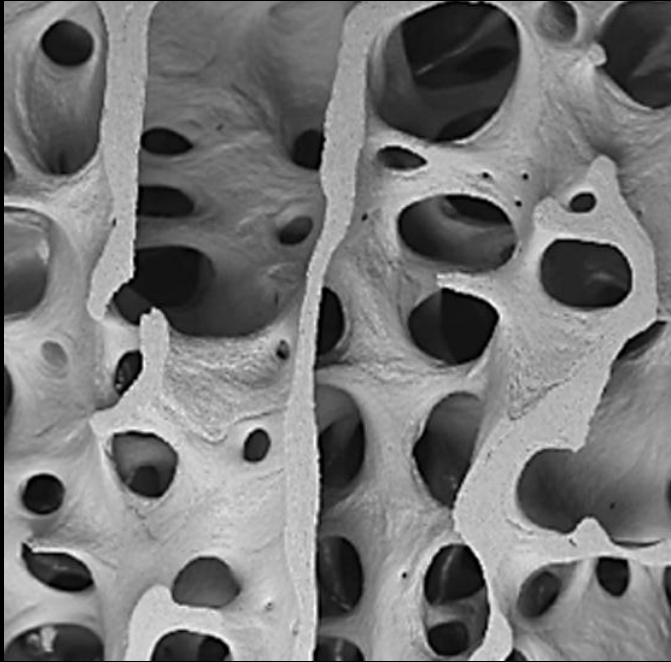
Osteoporosis

Normal bone
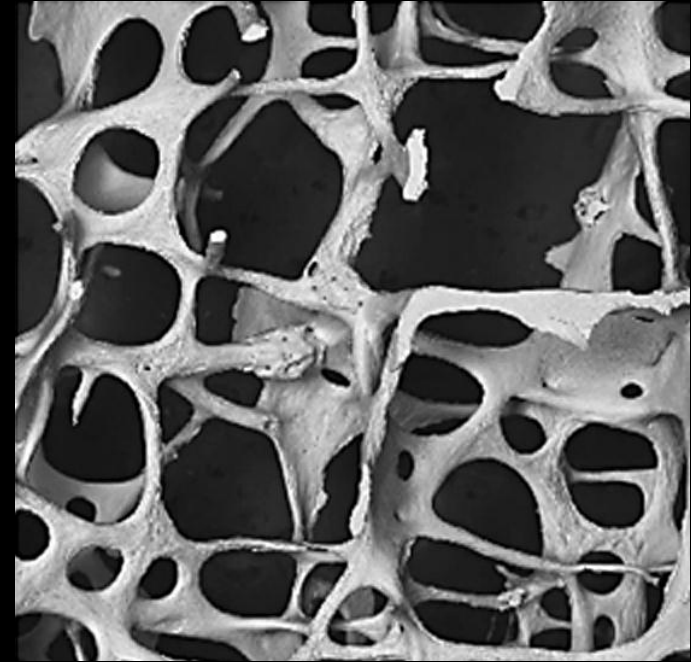
Osteoporotic bone

Low bone mass
Micro-architectural deterioration
Fragility fractures

Osteoporosis

Affects 50% of women and 1 in 5 men over 50 years old
Costs the European Community €31 billion per annum
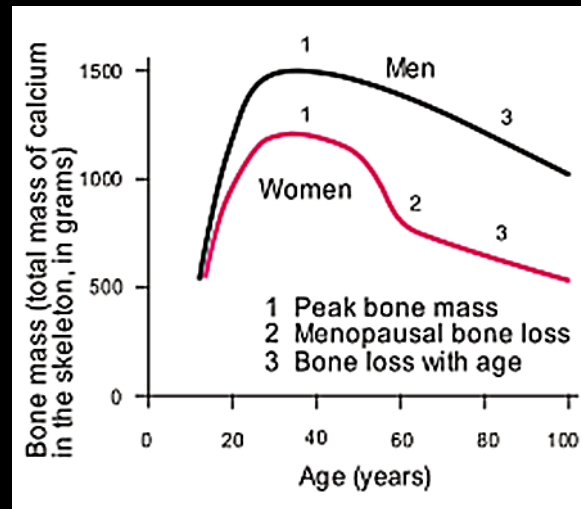
Peak bone mass
   Achieved at 20 and 30 years of age (major genetic component)
   Estrogens is critical in both male and females for peak bone mass
   Physical exercise, alcohol XS, smoking, eating disorders, systemic illness

Progressive loss of bone mass occurs from 45 years of age
   More rapid loss in women due to estrogens deficiency at menopause

Commonest fractures
   Female: Hip, vertebra and Colles'
   Male: Hip and vertebra

Age related osteoporosis
Increased bone resorption

Mechanism
    Estrogens deficiency at the menopause
        Increased expression of skeletal cytokines especially IL-6
        Reduced expression of OPG and thus increased osteoclastogenesis
    Decreased cutaneous vitamin D synthesis and 1α-hydroxylase activity
        Decreased $1,25(OH)_2D$
        Reduced intestinal Ca^{2+} absorption and increased renal losses
    Reduced calcium increases PTH
        Increases osteoclastic resorption

Risk factors for fracture
    Low BMD, advanced age, postmenopausal fracture, 1st degree relative with fracture, smoking, low BMI, vitamin D deficiency, premature menopause, alcohol excess, history of falls, institutionalisation and immobility

Diagnosis of osteoporosis
    Fragility fracture and decreased bone mineral density (BMD)

Investigation
    Ca^{2+}, Pi, ALP, Cre, PTH, 25-OH-vitD, DEXA, Urinary NTX

BMD is highly significant predictor of fracture risk
For every standard deviation below the mean fracture risk double

Secondary Osteoporosis

Endocrine

Thyrotoxicosis (increased bone turnover)
Hyperprolactinemia (reduced gonadotrophins and sex hormones)
Primary hyperparathyroidism (Increased resorption)
Hypogonadism (increased resorption)
Cushing's Syndrome (impaired bone formation)

Nutritional

Vitamin D deficiency (impaired mineralisation)
Coeliac disease (impaired mineralisation)
Chronic liver disease

Iatrogenic

High dose glucocorticoids (Glucocorticoid induced osteoporosis)
GnRH agonists (Prostate cancer)
Aromatase inhibitors (Breast cancer)
Thyroid hormone excess (Excessive replacement or Thyroid cancer)
Anticoagulants
Anticonvulsants
Immunosuppression (inhibits calcineurin and NFAT)
Thiazolidinediones (PPARγ agonists) (↓ osteoblastogenesis ↑adipogenesis)

Dual-energy X-ray absorptiometry

Lumbar spine

Right hip
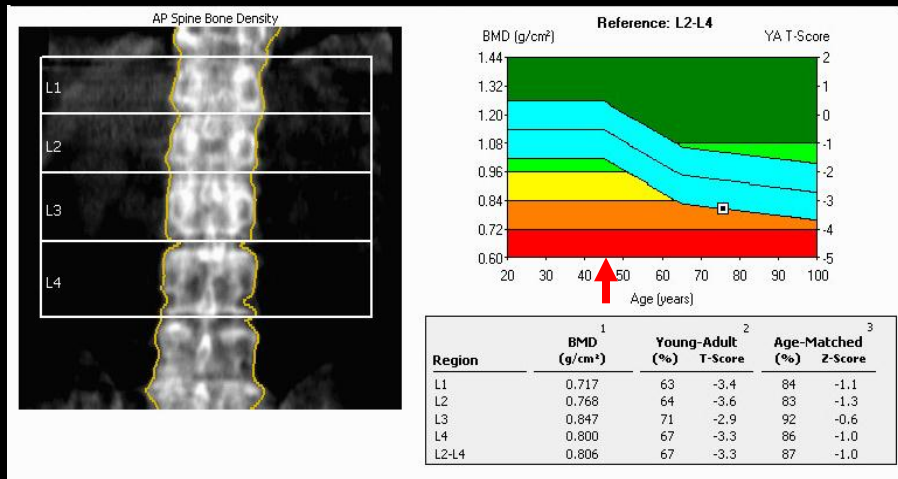
DXA results are compared to age, sex and ethnically matched data

Bone mineral density is normally distributed in the population

Results are interpreted according to the standard deviation from the mean of
a) Sex matched peak bone mass (T-score)
b) Sex and age matched BMD (Z-score)
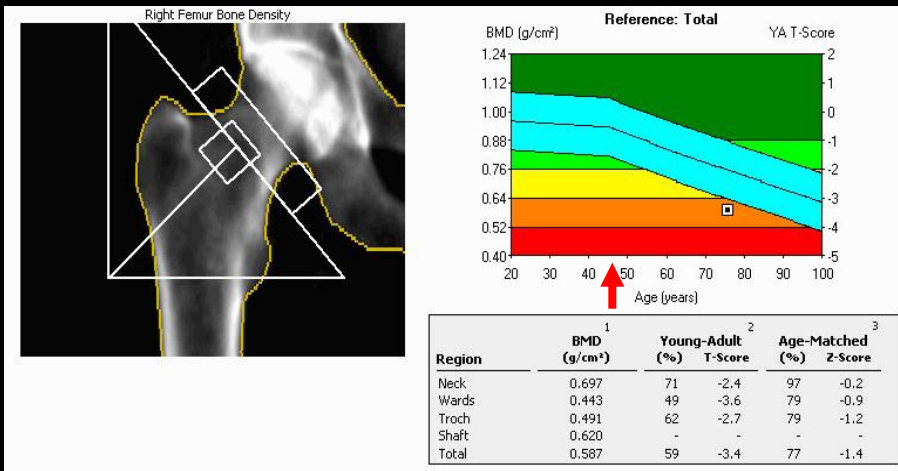
WHO diagnostic criteria
Osteoporosis
T score $\leq$ -2.5 lumbar spine, femoral neck or total hip
Osteopenia
T score $\leq$ -1.0 lumbar spine, femoral neck or total hip

Start of menopausal bone loss shown by red arrow

Fracture risk prediction

65 year old woman with borderline osteoporosis (T-score -2.5)

10 year hip fracture risk is 2.8%

Treatment of age related osteoporosis

Treatment is indicated if prior fragility fracture or T-score ≤ -2.5

Simple advice
   Weight bearing exercise, smoking and alcohol

Optimise vitamin D status maintain >80nmol/l
   Calcium and vitamin D supplementation

Antiresorptive agents
   Selective estrogens receptor modulators (SERMs)
   Bisphosphonates
   Denosumab (Human monoclonal antibody to RANKL)

Anabolic agents
   Strontium ranelate
   Teriparatide (PTH 1-34)

Current antiresorptive agents

Raloxifene (od po £240/y) (Selective estrogens receptor modulators (SERMs)
    Only post menopausal women
    Estrogen receptor agonist in bone and liver
       (anti oestrogen in breast)
    Reduces incidence of vertebral fractures
       (results in menopausal symptoms)

Bisphosphonates (inhibit osteoclastic bone resorption)
    35-65% reduction in vertebral # and 25-50% reduction in hip #
    Alendronate (1/52 po £44/y) and residronate (oral once a week)
    Zolendronic acid (yearly iv £284/y)
    Monitoring: uNTX (cross-linked N-telopeptides of type I collagen)

Denosumab (6/12 s/c £366/y) (Inhibition of osteoclast formation)
    Human monoclonal antibody to RANKL (OPG like activity)
    68% reduction in vertebral # and 40% reduction in hip #

Actions of Bisphosphonates
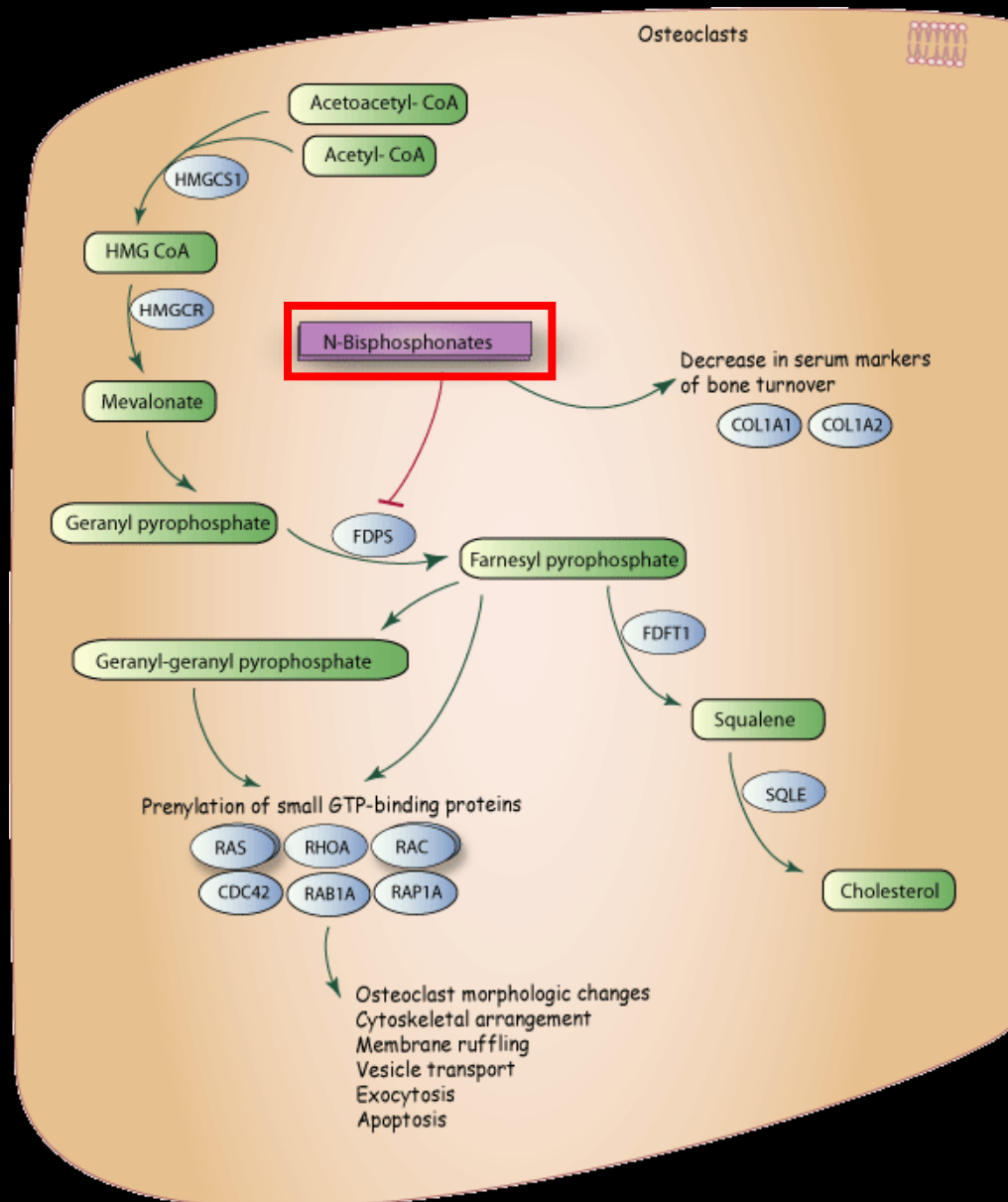
The skeletal selectivity of the bisphosphonates is due to their avid binding of hydroxyapatite

Inhibit farnesyl diphosphate synthase (FDPS)
Disrupt prenylation of small GTPase such as Ras, Rho, Rac, Rab and Cdc42

GTPases are essential for osteoclast bone resorption and survival.

Current anabolic agents

Strontium Ranelate (po od £310/y)

    Vertebral fractures reduced by 40% and non-vertebral by 20%

Mechanism of action

    Uncertain but reduces osteoclast resorption and increase bone formation
    (50% of increase in BMD is due to incorporation of strontium)

Teriparatide (PTH1-34) (od s/c £3,300/y total 18 months)

    Vertebral fractures reduced by 60% and non-vertebral by 60%

Mechanism of action

    Decreases osteblast and osteocyte apoptosis
    Increased osteoblastic bone formation
    Monitoring: P1NP (N-terminal propeptide of type I procollagen)

**Concurrent bisphosphonates reduce anabolic actions but must be
commenced after last dose of PTH to prevent rapid bone loss.**

Current indications

    Patients >65y, T score < -4, multiple fractures,
    Intolerant of bisphosphonates or fractures while on bisphosphonates

Future treatments of osteoporosis

Novel antiresorptive agents
Cathepsin K inhibitors (inhibits osteoid matrix resorption)
Small molecule RANK inhibitors
RANK receptor inhibitory peptide

Novel anabolic agents
CaSR inhibitors (Short acting calcilytic drugs increase PTH)
GSK-3β inhibitors (releases β-catenin)
DKK1-neutralising antibodies (BHQ880)
SOST-neutralizing antibodies (AMG785)

(Deal C (2009) Nat Clin Pract Rheumatol 5:20-7, Gogakos A (2009)Expert Rev Endocrinol Metab 4: 639-50)

Paget's Disease of Bone

Paget's disease of bone
(Localised disorder of bone remodelling)

Bone Scan

Tibia X-ray

Focally abnormal bone remodelling
- **Osteoclast abnormality**
  - *Increased osteoclast numbers*
- **Osteoblast abnormality**
  - *Disorganised rapid bone formation*
  - *Woven bone not lamella*

Chronic effects
- **Replacement by sclerotic bone**
- **Bone marrow cavity replaced by vascular fibrous connective tissue**
- **Increase in bone size and bone deformity**

Increased markers of formation and resorption
- **Bone alkaline phosphatase**
- **P1NP**
- **uNTX**

Paget's disease

Commonest metabolic bone disease after osteoporosis
    Single site (Monostotic); Multiple sites (Polyostotic)

Aetiology
    Predominantly unknown (Restricted geographic distribution)
    More common in women
    Family history in 15% (Sequestome-1 (SQSTM1), RANK and OPG)
    Reason for decline in frequency is unknown

Clinical features
    Bone pain, joint pain, deformity, fracture and increased temperature
    Deafness (may be conductive or sensorineural)
    Abnormal x-ray

Diagnosis
    Raised alkaline phosphatase
    X-ray (osteolysis, osteosclerosis and bone expansion)
    ^{99}Tc bone scan is far more sensitive than plain X-ray

Paget's disease

Complications
- Osteoarthritis due to deformity
- Cranial nerve palsy and spinal stenosis
- Hypercalcaema if immobilised with active disease
- Osteosarcoma (very rare 0.1% in 100 patient years)

Treatment: Bone pain is the indication for treatment
- Simple analgesia (NSAIDs)
- Physio/hydrotherapy
- Bisphosphonates: reduce pain, do not prevent #, deformity or deafness
  - Zolendronic acid 5mg iv **(Alk Phos normalises in 90%)**
  - Residronate 30mg od 2 months **(Alk Phos normalises in 60%)**
- Ensure patients are vitamin D and calcium replete
- Surgery for severe deformity or osteoarthritis

Follow up
- Alkaline phosphatase
- ^{99}Tc bone scan (if AlkP raised)

References

General
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Deal C (2009) Potential new drug targets for osteoporosis. Nat Clin Pract Rheumatol 5:20-7

Paget's Disease
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Glucocorticoid induced osteoporosis (GIO)

Commonest iatrogenic cause of osteoporosis
(Predisolone >7.5mg/d for >3/12)
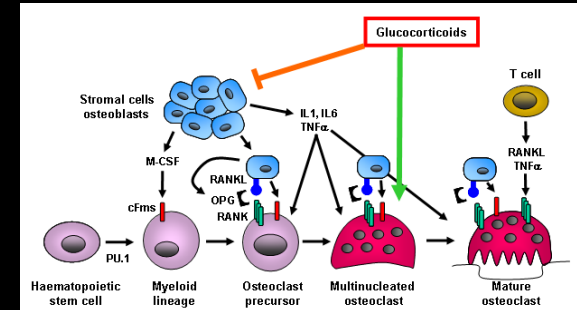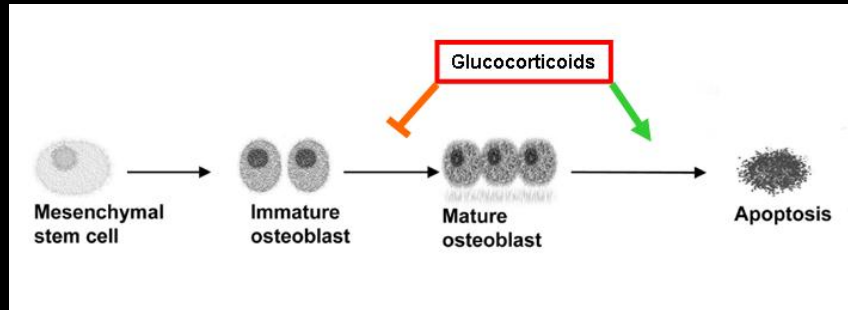
Rapid bone loss in first year slow loss thereafter
- Decreased osteoblastogenesis and increased apoptosis
- Decreased osteoclastogenesis but prolonged survival
- Increased osteocyte apoptosis

Fractures in 30-50% of chronically treated patients
- BMD correlate far less well with fracture risk in GIO

Treatment
- Bisphosphonates: Considered in if glucocorticoids required for >3months
- Teriparatide: Increases BMD more than Alendronate in GIO