

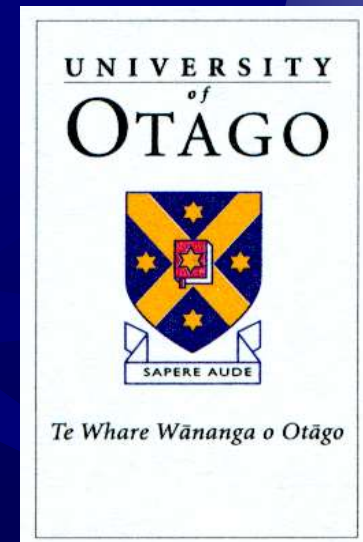
The Biology of Skeletal Tissues

By Murray C Meikle

Biological Foundations of Orthodontics
and Dentofacial Orthopaedics

Seminar 3

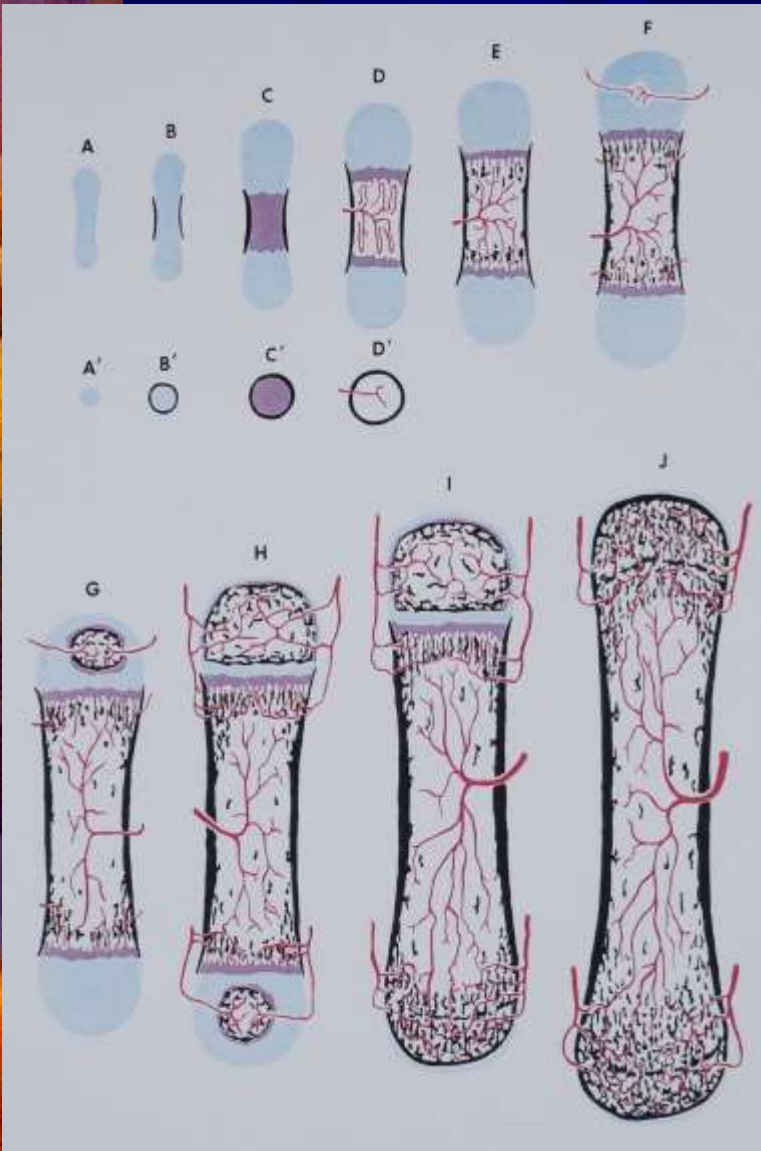
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Formation and growth of the skeleton

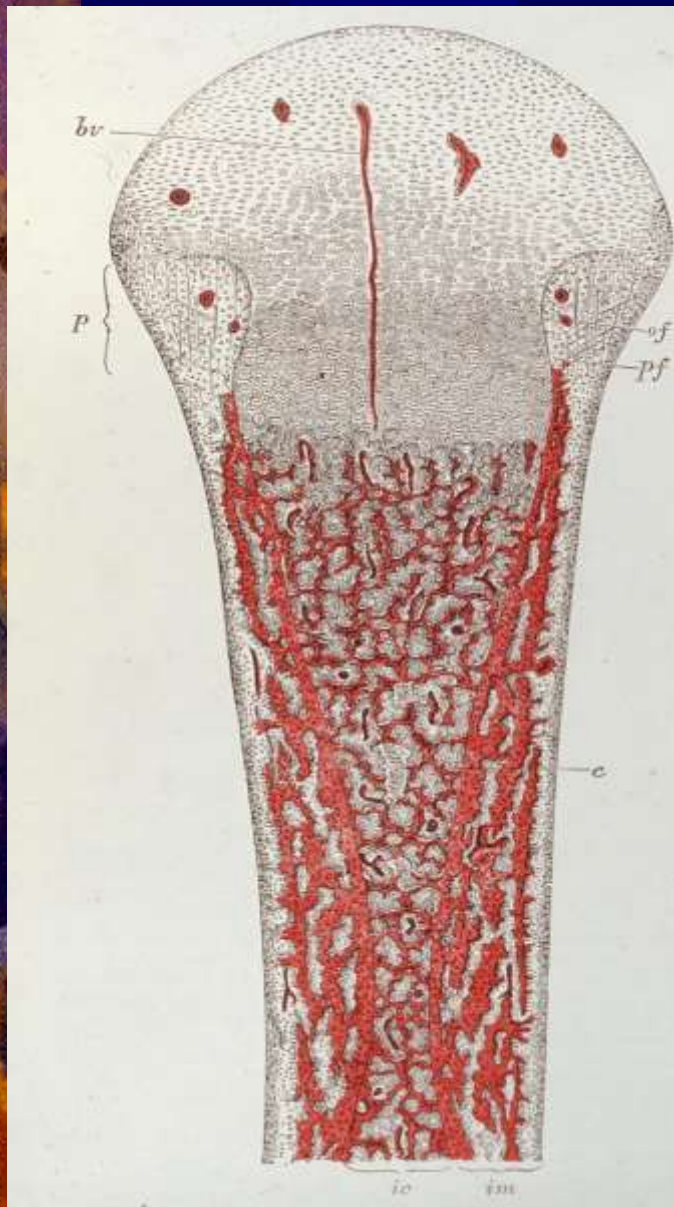
- ✿ In higher vertebrates structural support and protection for the tissues and organs of the body is provided by a mineralized skeleton composed of cartilage and bone. These highly specialized forms of connective tissue, in addition to their protective and structural roles, also support the vital functions of calcium homeostasis and haematopoiesis and act as levers to which muscles and tendons are attached to facilitate locomotion.
- ✿ Cartilage provides the models in which most of the bones develop, including those of the appendicular and axial skeleton (cartilage bones). Cartilaginous growth plates and synchondroses also constitute an important part of the growth mechanism of the skeleton.
- ✿ The bones of the skull, apart from the chondrocranium are not formed in cartilaginous precursors. They are derived phylogenetically from the dermal shield and arise directly from ossification centres that appear within the ectomesenchyme of the developing head (membrane bones).

Cartilage bones



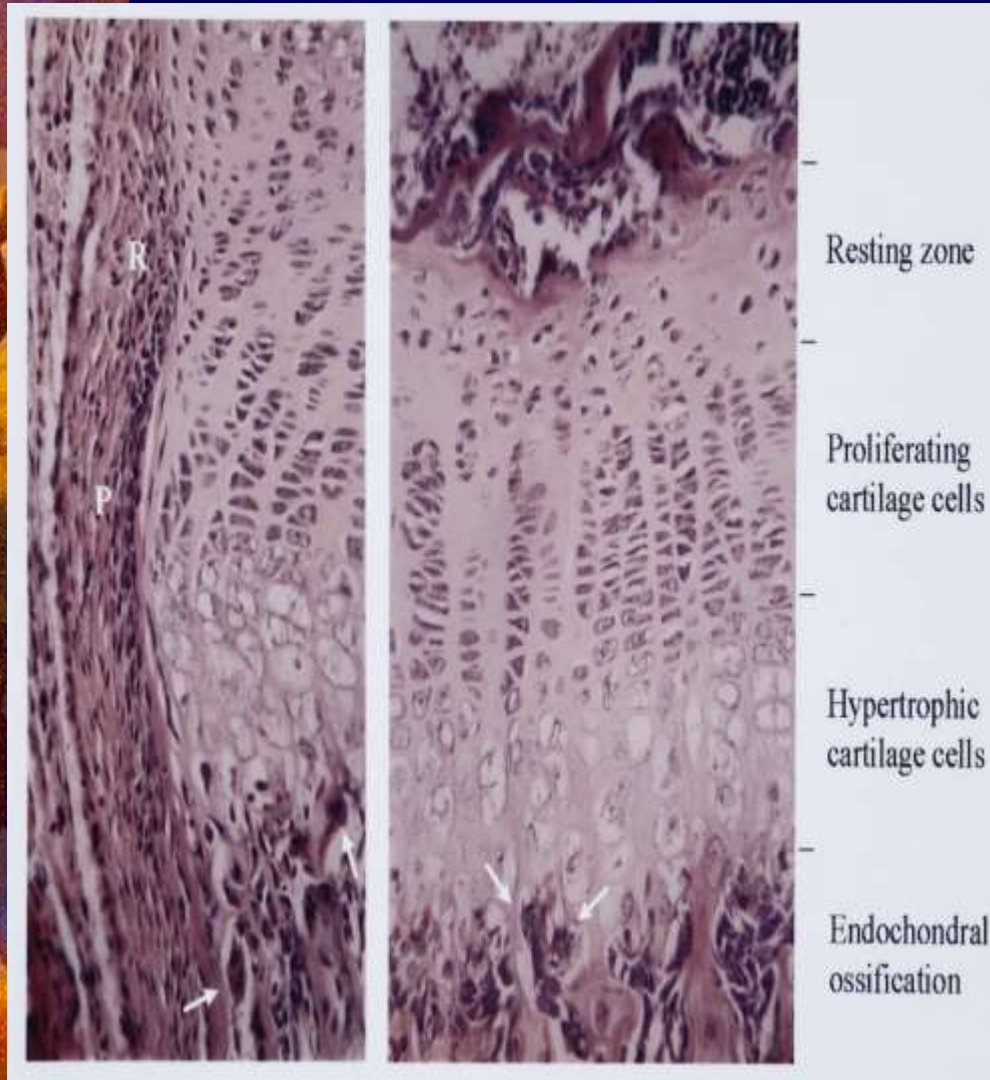
- Cartilage bones arise by endochondral ossification in which a cartilage anlagen closely resembling the final form of the bone is laid down.
- The initial step is the migration and condensation of cells and their differentiation into chondrocytes.
- The first sign of ossification is formation of a periosteal bony collar in the mid-shaft (diaphysis), followed by the hypertrophy and calcification of chondrocytes and vascular invasion.
- The extremities remain cartilaginous until secondary ossification centres appear within the epiphyses and epiphyseal growth plates are formed.
- This classic illustration of the development of a long bone, was first published in *A Textbook of Histology* (1930) by Maximov and Bloom.

Growth of long bones



- Growth in the length of a long bone is the result of endochondral ossification at the epiphyseal growth plate.
- The subperiosteal layer (P), however, makes a significant contribution to the growth in width of the epiphyseal cartilage.
- At the bottom, *ic* is the part of the bone that was ossified in cartilage and *im* the part ossified in membrane – the osteoblastic layer of the periosteum. Although classified as cartilaginous, much of the osseous tissue will have been produced by perichondrial and periosteal ossification, *i.e.* by an intramembranous process.
- From Schäfer (1878), *Quarterly Journal of Microscopical Science*.

Endochondral ossification



- Longitudinal section through the epiphyseal cartilage. Note relationship of the periosteum (P) and groove of Ranvier (R) to the cartilage, where periosteal chondrogenesis contributes to the lateral growth of cartilage.
- The growth plate provides a good example of the maturational changes in the life cycle of cartilage cells from the resting zone, through to the proliferation and hypertrophy of the cartilage cells, mineralization of the cartilage matrix and its eventual resorption and replacement by bone.
- From Meikle (2002), *Craniofacial Development, Growth and Evolution*.

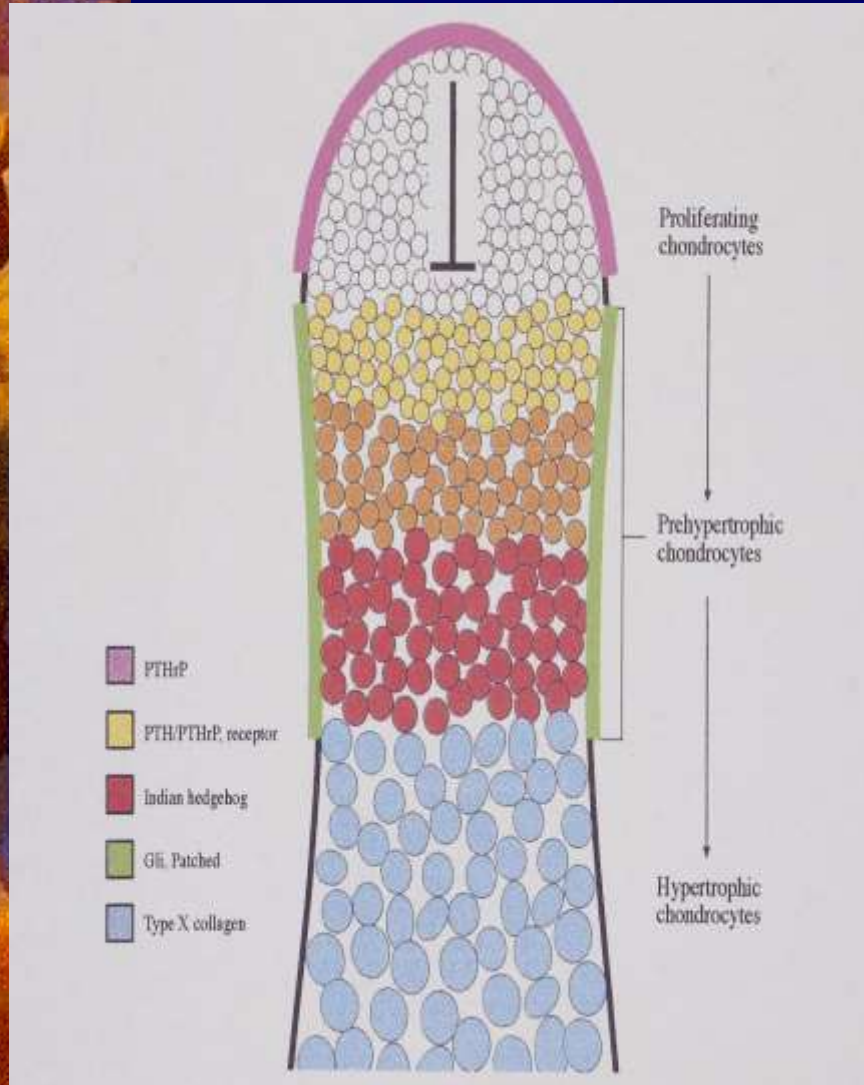
Molecular regulation of chondrocyte differentiation

- Proliferating chondrocytes (white) differentiate into chondrocytes expressing the PTH/ PTHrP receptor (yellow) and Ihh (red), but both domains of expression overlap (orange). Ihh (Indian hedgehog) level reflects the number of cells committed to the hypertrophic pathway.

- The direct target of Ihh signalling is (1) the perichondrium (green) which responds by producing Gli and Patched, and (2) the articular perichondrium (purple) to produce PTHrP.

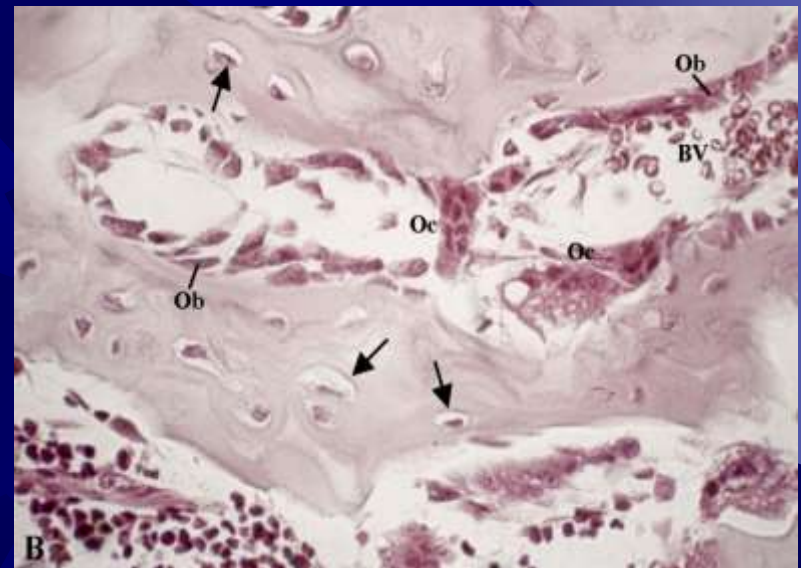
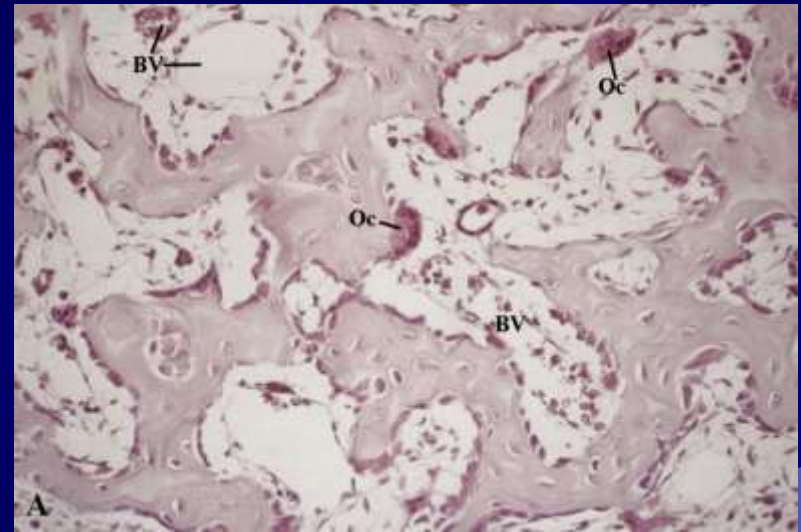
- PTHrP prevents additional chondrocytes from moving down the differentiation pathway and expressing Ihh, and shows that PTHrP mediates the effect of Ihh via a negative feedback loop.

- Redrawn from Vortkamp *et al.*(1996), *Science*.



Intramembranous ossification

- Sections from the developing rabbit mandible showing the characteristic appearance of woven bone in the embryo, young children, and sites of rapid growth in adults (*i.e.* fracture callus). Original magnification x80. H&E
- The bone surfaces are lined by osteoblasts (Ob) laying down matrix and osteocytes (arrows) embedded within. x160.
- The presence of several multinucleate osteoclasts indicates that the bone is being rapidly remodelled. BV, blood vessel; Oc, osteoclast; Ob, osteoblast.
- Courtesy of JJW Breckon.



Cells of the osseous skeleton

- Bone is composed of four different cell types; bone lining cells, osteoblasts, osteocytes and osteoclasts. Bone lining cells are flat, elongated cells covering resting bone surfaces and constitute a quiescent osteoblastic cell population awaiting activation.
- At one time osteoblasts and osteoclasts were thought to arise by modulation from a common precursor cell, a concept based on ^3H -thymidine pulse-labelling studies of bone, and now known to be incorrect.
- Osteoblasts are derived from mesenchymal stem cells (also called stromal stem cells), whereas osteoclasts are cells of haematopoietic origin derived from cells that comprise the monocyte–macrophage lineage.

Regulation of osteoblast differentiation

- A key regulator of osteoblast differentiation is core-binding factor alpha-1 (Cbfa-1), also known as Runx2, the vertebrate homologue of the *Drosophila* pair-rule gene *runt*.
- Left. Cbfa-1-deficient mouse (*Cbfa-1*^{-/-}); the skeleton shows a complete absence of bone due to maturational arrest of osteoblasts; cartilage is unaffected.
- Right. Heterozygous mice (*Cbfa-1*^{+/-}) develop skeletal abnormalities characteristic of cleidocranial dysplasia.
- Stained with alizarin red (bone) and alcian blue (cartilage). From Komori *et al.* (1997), *Cell*.



Cleidocranial dysplasia

- Human cleidocranial dysplasia has been shown to be due to mutations in the *CBFA1/RUNX2* gene.
- It is characterized by a combination of aplasia or hypoplasia of one or both clavicles, brachycephaly, delayed ossification of the fontanelles, short stature, and multiple unerupted and supernumerary teeth.
- Transmission is autosomal dominant, with 20–40% of cases due to a spontaneous mutation; linkage studies have mapped the gene to chromosome 6p21.



Bone morphogenetic proteins

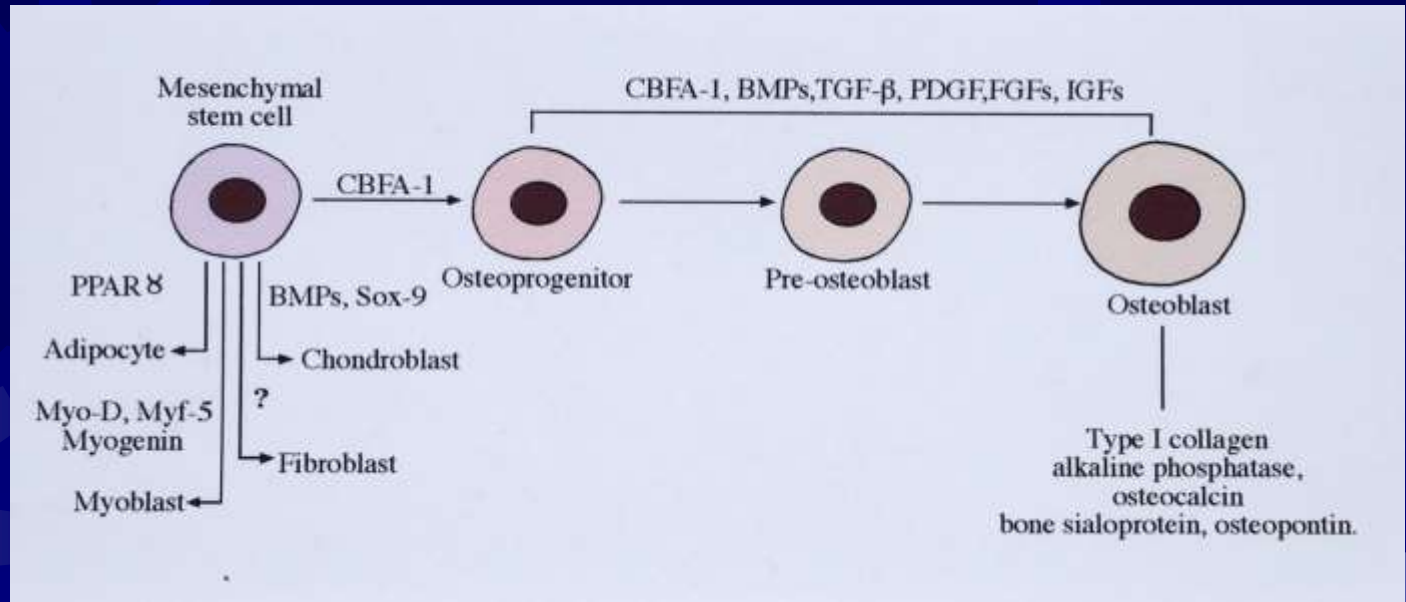
- BMPs are members of the TGF- β gene superfamily of secreted signalling molecules. Originally discovered by their ability to induce ectopic cartilage and bone formation in rats (Urist, 1965), purification of BMP activity from bovine bone eventually led to the identification of seven novel human proteins designated BMP-1 to BMP-7.
- The family name BMP turned out to be something of a misnomer; sequence data showed that BMP-1 was a proteinase related to the crayfish enzyme astacin, BMP-2 and BMP-3 were related to TGF- β and BMP-4 was the vertebrate homologue of the *Drosophila decapentaplegic (dpp)* gene that specifies dorsoventral patterning.
- In addition to their roles in morphogenesis, BMPs are also expressed at sites of skeletogenesis, where their function is to induce the formation of cartilage, bone and connective tissues associated with the skeleton. All BMPs first induce chondrogenesis; they are therefore also 'cartilage morphogenetic proteins'.

Bone morphogenetic proteins



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Differentiation of mesenchymal stem cells



- ☀ CBFA-1 is an osteoblast-specific transcription factor that regulates the expression of multiple genes (*osteocalcin*, *type I collagen*, *bone sialoprotein*, *osteopontin*). BMPs are important determinants of osteoblast/ chondroblast cell lineages
- ☀ The growth factors TGF- β , FGFs, PDGFs and IGFs influence differentiation of committed osteoblast precursors, but cannot induce differentiation from uncommitted precursor cells.
- ☀ Osteoprogenitor cells are proliferative; preosteoblasts show limited proliferation; osteoblasts are post-mitotic.

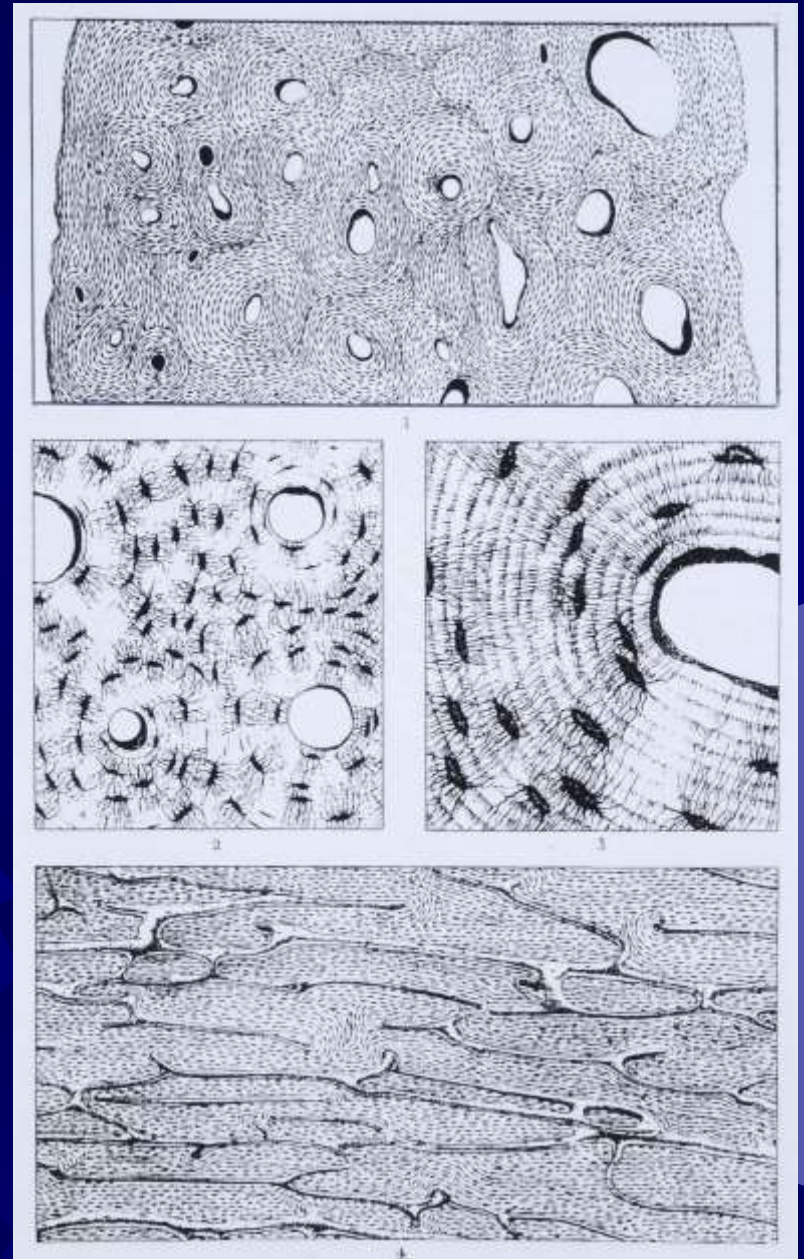
Function of osteoblasts

- Osteoblasts (1) synthesize the extracellular matrix of bone. This includes type I collagen (more than 90% of the organic matrix), numerous proteoglycans, noncollagenous proteins and cell adhesion molecules.
- (2) Promote mineralization of the matrix by budding off matrix vesicles (top figure); these contain ALP, ATPase, inorganic pyrophosphatase and plasminogen activator and act as seeding sites for the growth of hydroxyapatite crystals.
- (3) Osteoblasts also play a key role in regulating bone resorption.

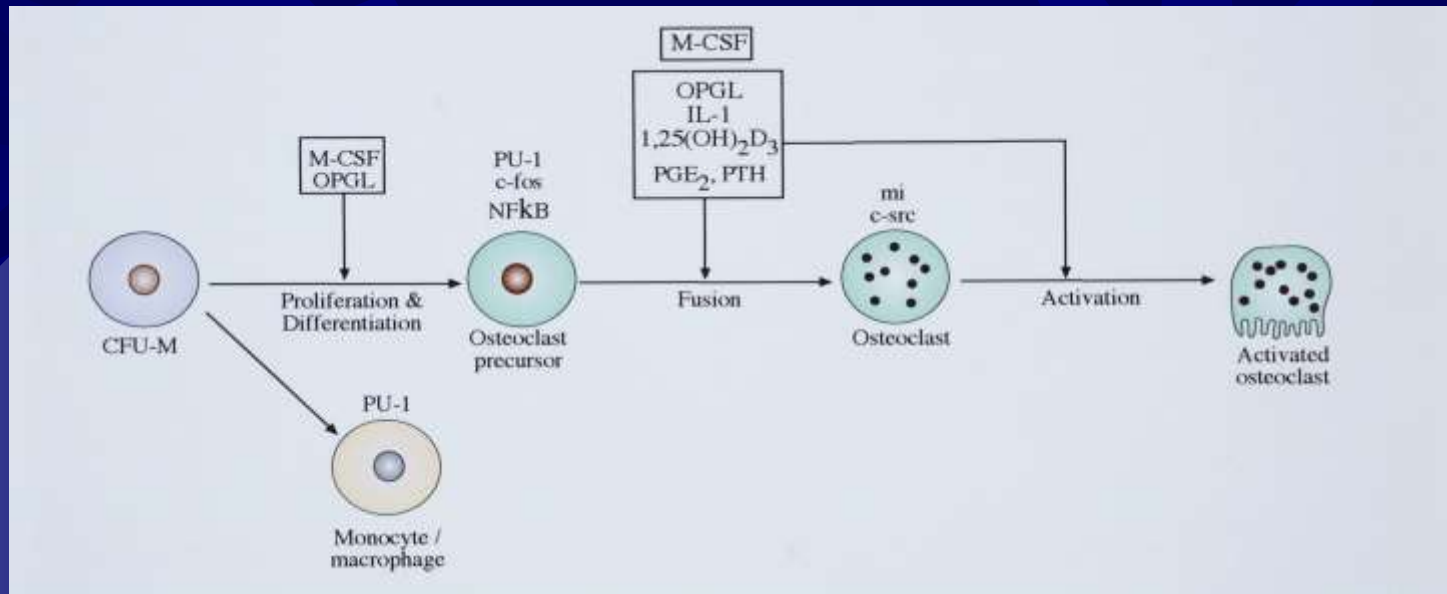


Osteocytes

- Osteocytes represent the most mature differentiated state of the osteoblast lineage, and are the most abundant cells in bone.
- They communicate through a network of canaliculi; osteocyte vitality is maintained from the vascular Haversian canals, via a bone fluid that circulates through the canalicular system; osteocyte death is synonymous with bone death.
- Osteocytes are sensitive to mechanical deformation and act as mechanoreceptors in bone.
- From Hassall (1849), *The Microscopic Anatomy of the Human Body in Health and Disease*.

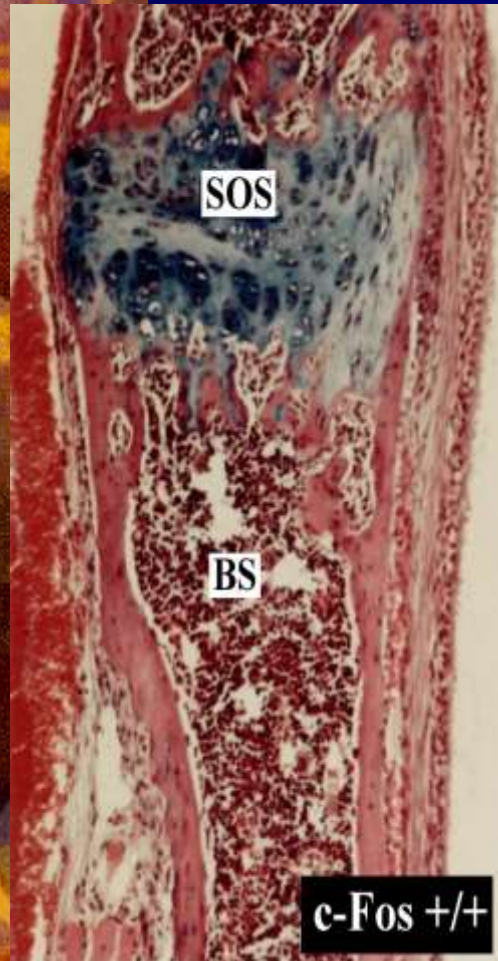


Osteoclast differentiation



- Osteoclasts are derived from mononuclear precursor cells that migrate from the bone marrow to skeletal sites via the vasculature.
- Expression of PU-1, an ETS-domain transcription factor is required for osteoclast and macrophage differentiation. PU-1 knockout mice lack osteoclasts and develop osteopetrosis.
- C-fos and NFκB are required to form multinucleate osteoclasts from precursor cells. *Microphthalmic (mi)* and *c-src*-deficient mice form osteoclasts but they are nonfunctional and fail to resorb bone.

c-Fos mutant mice



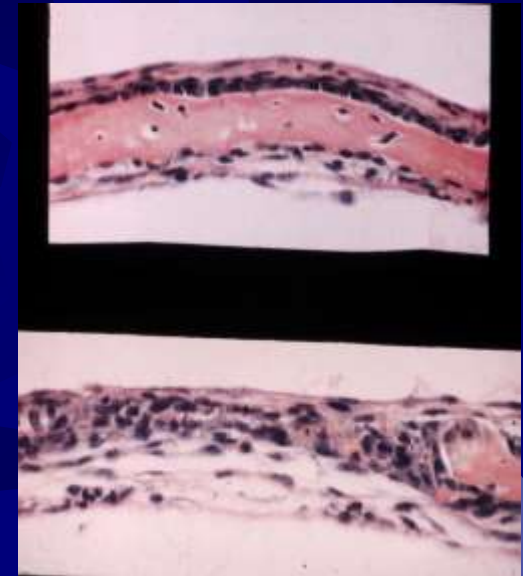
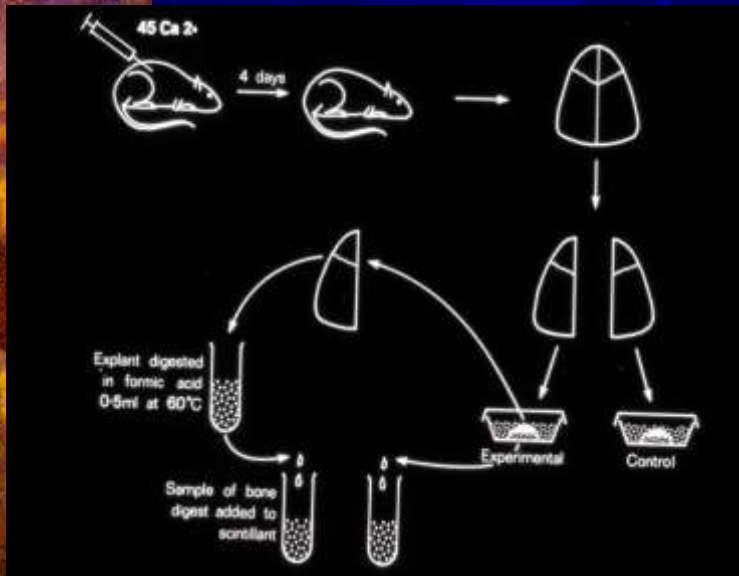
- Histological analysis of the basisphenoid (BS) and the sphenoid-occipital synchondrosis (SOS) from normal or wild-type (+/+) mice on the left and c-Fos (-/-) mutant mice.
- Absence of osteoclasts results in unresorbed bone and cartilage, the absence of marrow spaces (*) and development of osteopetrosis.
- Arrowheads indicate hypertrophic chondrocytes which may also indicate aberrant chondrocyte differentiation.

Courtesy of AE Grigoriadis.

Bone resorption

- ✦ Bone resorption is a complex multistep process that involves removal of both the mineral and organic constituents of bone matrix. Osteoclasts are the cells responsible for this process which occurs in the subosteoclastic resorption zone, a specialized extracellular compartment bounded by the ruffled border of the cell and the mineralized matrix.
- ✦ Osteoclasts acidify the subosteoclastic resorption zone leading to dissolution of mineral, while the organic matrix is degraded by proteolytic enzymes, especially the matrix metalloproteinases (MMPs) and cysteine proteinases (CPs).
- ✦ Although it is not clear how resorption sites are determined, in addition to their role in directly regulating osteoclast formation and function, osteoblastic cells also carry out an accessory role. They are involved, first, by retracting to expose the bone surface and, second, by releasing MMPs that degrade the surface layer of nonmineralized osteoid facilitating access of osteoclasts to the underlying mineralized matrix.

Bone resorption *in vitro*



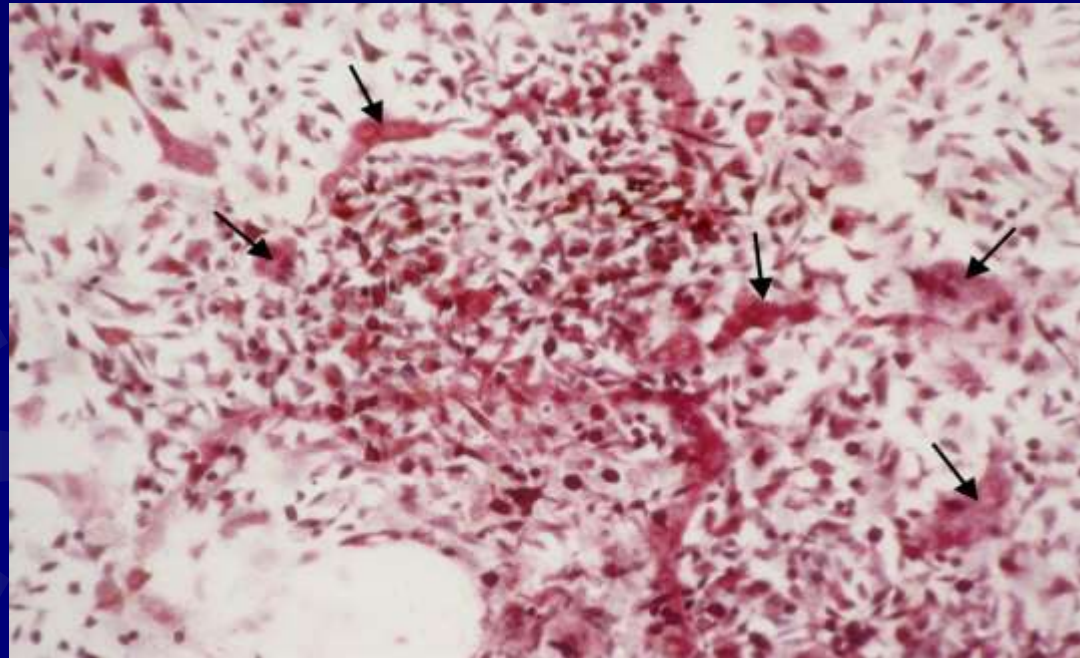
- *In vitro* models have been widely used to study the cellular and molecular mechanisms involved in bone resorption.
- The mouse calvarial assay measures $^{45}\text{Ca}^{2+}$ release from bone explants into the culture medium and used to establish whether a substance can stimulate or inhibit bone resorption.
- However, bone explants contain several differentiated cell types. Cell isolation methods have been developed to study the response of individual cell populations. Courtesy of J J Reynolds.

Osteoclast resorption pit assay



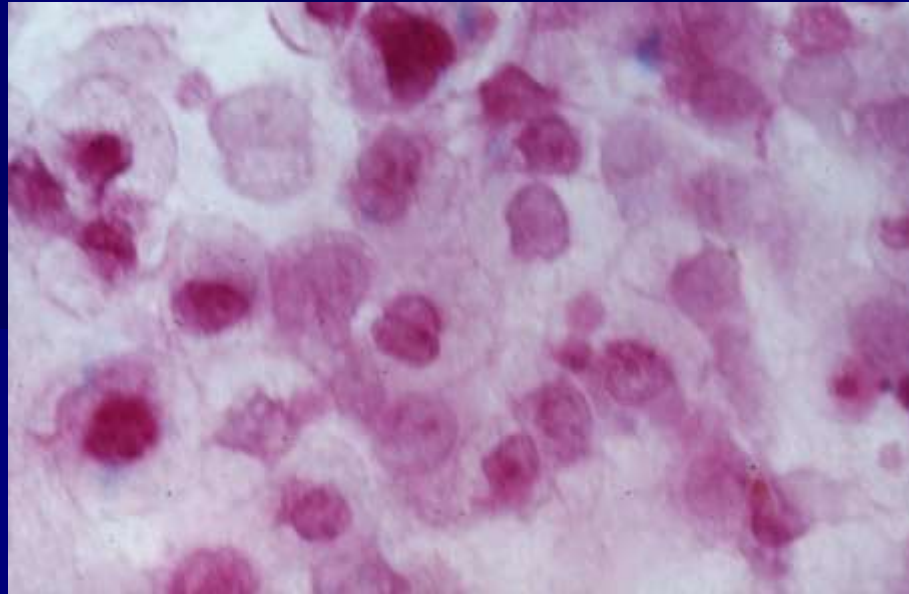
- ☀ Left: SEM of osteoclasts on an ivory slice. This assay is based on the ability of isolated osteoclasts to resorb bone, dentine or ivory slices *in vitro* (Boyde *et al.*, 1984). Ivory slices make an ideal substrate being free of vascular channels and pre-existing resorption surfaces.
- ☀ Right: In this experiment bone marrow cells were allowed to settle for 25 min at 37°C, washed free of nonadherent cells and incubated in the presence of interleukin-11 for 24–48 h; the number and/or volume of the resorption pits can be quantified by image analysis. Courtesy of T C Chambers (left) and A M Tumber (right).

Osteoclast differentiation *in vitro*



- ✦ The bone marrow assay was important in showing that osteoclast formation from haematopoietic precursors in bone marrow cultures, does not occur unless cells of the osteoblast/stromal cell lineage are present.
- ✦ In this experiment cells from the tibiae of 5–6-week-old mice have been cultured at a density of 2×10^6 /well in 0.5 ml of culture medium containing fetal calf serum and $1,25(\text{OH})_2\text{D}_3$. Arrows indicate the formation of TRAP-positive multinucleate cells (three or more nuclei) after 8 days. Courtesy of A M Tumber.

Osteoblasts in culture

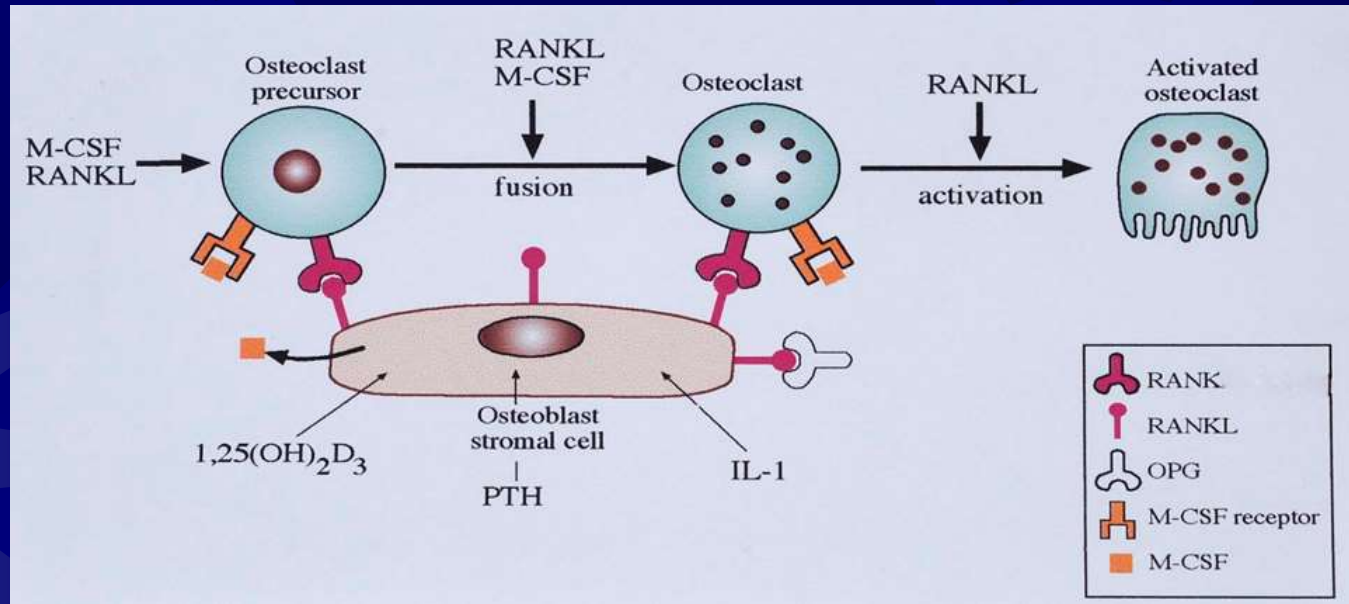


- Osteoblasts in monolayer culture have been used to study the response of the cells to systemic and local hormones and to mechanically-induced strain.
- Osteoblasts are prepared from neonatal mouse calvaria by sequential enzymatic digestion (Heath *et al.*, 1984) and then grown either on tissue culture plastic or collagen films. These are mouse osteoblasts that have been grown on a type I collagen substrate and stained for alkaline phosphatase, an osteoblast marker.

Cytokine signalling in bone resorption

- ✱ Cytokines are small molecular weight polypeptides (MW<25 kDa) produced by cells that regulate or modify the action of other cells. They act as autocrine (acting on the cell of origin) and paracrine (acting on adjacent cells) regulators of cellular activity. The definition includes the interleukins, tumour necrosis factors, interferons, growth factors and colony stimulating factors.
- ✱ As discussed earlier, the bone marrow assay showed that osteoclast formation and function *in vitro*, was dependent upon the presence of osteoblasts/stromal cells, which suggested that osteoblasts produced short range soluble mediators or cytokines that were involved in cell–cell signalling.
- ✱ This led to the discovery of the RANK (receptor activator of nuclear factor κ B) signalling pathway in osteoclasts, which has transformed our understanding of the mechanisms regulating osteoclast formation and bone resorption (see next slide). The ligand for the membrane receptor RANK is called RANKL (also referred to as OPGL).

RANKL, RANK, OPG triad and M-CSF



- ★ RANKL stimulates bone resorption by increasing osteoclast formation; OPG (osteoprotegerin) another cytokine produced by osteoblasts acts as an inhibitor of osteoclast formation by competing with RANKL. In other words, it acts as a decoy receptor.
- ★ M-CSF (CSF-1) acts directly on osteoclast precursor cells to regulate their proliferation and differentiation; hormones and cytokines such as $1,25(\text{OH})_2\text{D}_3$, PTH and IL-1 stimulate bone resorption by increasing the expression of RANKL by osteoblasts/stromal cells.

The role of proteolytic enzymes

Proteinases degrading CTs

- **Matrix metalloproteinases**
- **Serine Proteinases**
 - ◆ plasmin, plasminogen activators (t-PA, u-PA)
 - ◆ neutrophil elastase, cathepsin G (PMNs)
- **Cysteine Proteinases (active at acid pH)**
 - ◆ cathepsins B, L, S
- **Aspartic Proteinases**
 - ◆ cathepsin D (lysosomal)

- ★ Tissue resorption is tightly regulated by cell–cell and cell–matrix interactions involving the production of enzymes, activators, inhibitors and regulatory molecules such as cytokines and growth factors.
- ★ The proteolytic enzymes involved in extracellular matrix degradation are grouped into four major classes (metallo, serine, cysteine and aspartic active site residues).
- ★ Although enzymes from each of these classes are involved in the resorptive cascade, current evidence suggests that cysteine proteinases and particularly matrix metalloproteinases (MMPs), also known as matrixins, are the most important.

Matrix metalloproteinases

Matrix Metalloproteinases

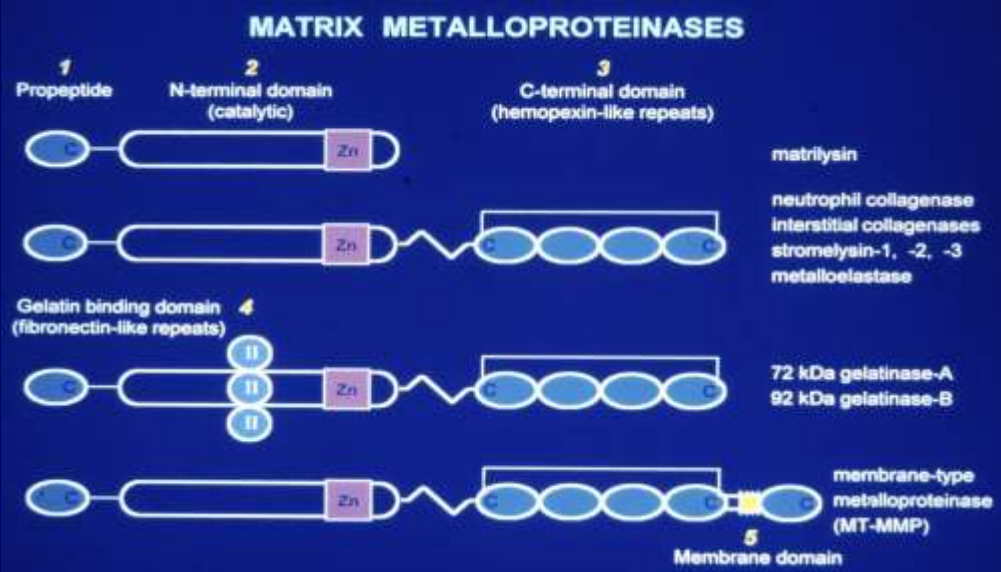
- Zinc dependent, require calcium ions
- Active at neutral pH
- Secreted in latent proform
- Inhibited by TIMP
 - ◆ Collagenases (interstitial collagenases)
 - ◆ Gelatinases (type IV collagenases)
 - ◆ Stromelysins (proteoglycanases)

Matrix Metalloproteinases

- Collagenases (1-3)
 - ◆ degrade interstitial collagens I, II and III
- Stromelysins (1-3)
 - ◆ proteoglycan, laminin, fibronectin
- Gelatinases (-A, 72 kDa; -B, 92 kDa)
 - ◆ degrade gelatin,
 - ◆ collagen types IV, V and VII
 - ◆ elastin

- ☀ MMPs function at neutral pH and can digest all the structural molecules in bone and other connectives. They are divided into three major classes depending upon substrate specificity.
- ☀ Collagenases cleave interstitial collagens.
- ☀ Gelatinases degrade collagen types IV, V, VII and X and as the name indicates denatured collagens (gelatins).
- ☀ Stromelysin has a broader substrate specificity and can degrade basement membrane collagens, proteoglycans and other matrix glycoproteins.

Matrix metalloproteinases



- All MMPs are secreted in latent proforms and activation involves loss of a propeptide of about 80 residues. The best known MMP is collagenase originally discovered in tadpole tails.
- There are 3 collagenases: MMP-1 (fibroblast or interstitial, CL-1), MMP-8 (neutrophil, CL-2) and MMP-13 (CL-3) the collagenase present in mice (and humans).
- There are two gelatinases: MMP-2 (gelatinase-A, 72 kDa) and MMP-9 (gelatinase-B, 92 kDa).
- And 3 stromelysins: MMP-3 (transin, SL-1); MMP-10 (SL-2); MMP-11 (SL-3).

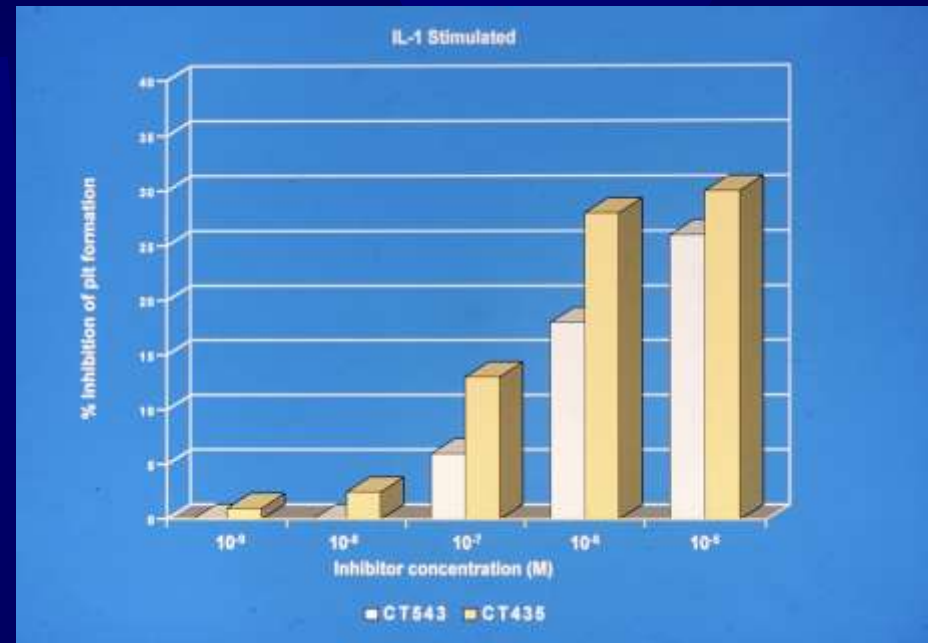
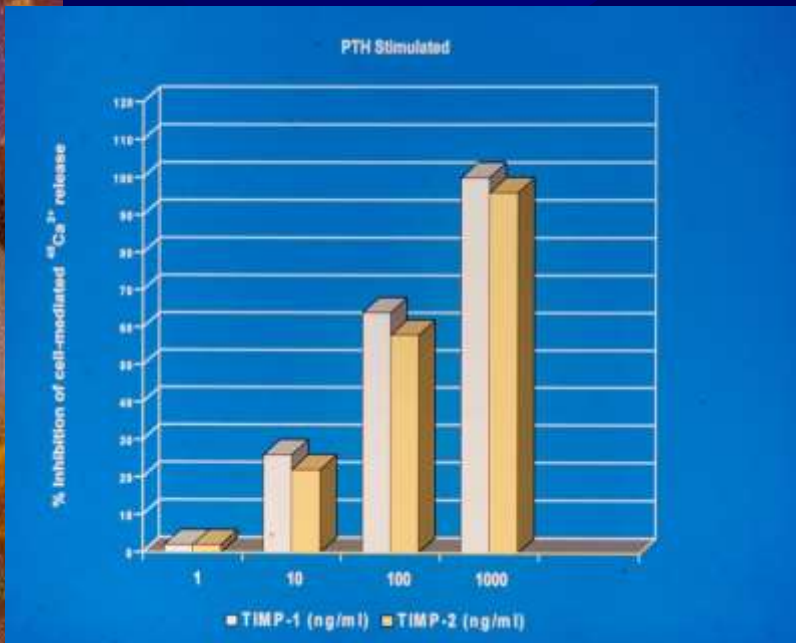
TIMPs

TIMPs

- **T**issue **I**nhibitor of **M**etallo**p**roteinases
- Inactivate 'active' MMPs
- Three forms
 - ◆ TIMP-1 22 kDa
 - ‡ TIMP-1 also binds progelatinase-B
 - ◆ TIMP-2 27 kDa
 - ‡ TIMP-2 also binds progelatinase-A
 - ◆ TIMP-3 27 kDa

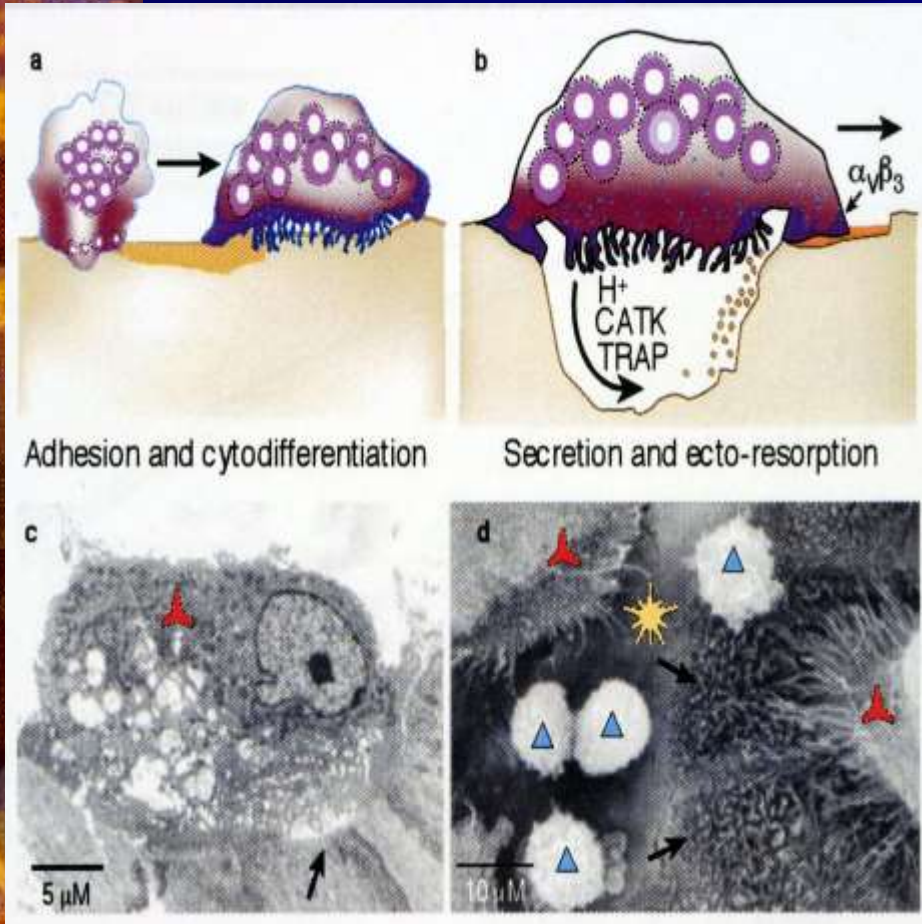
- ★ TIMPs are important in controlling the extracellular actions of MMPs. The major inhibitor is TIMP-1; it is synthesized and secreted by connective tissue cells and macrophages and can be detected in most body fluids.
- ★ The amino acid sequence of TIMP-1 is largely conserved across species; it forms high affinity, essentially irreversible non-covalent complexes with the active forms of MMPs.
- ★ Physiological turnover of connective tissues is largely regulated by MMPs and TIMPs; many studies suggest that pathological resorption of the extracellular matrix is correlated with an imbalance of inhibitor and proteinase expression.

TIMPs and synthetic MMP inhibitors



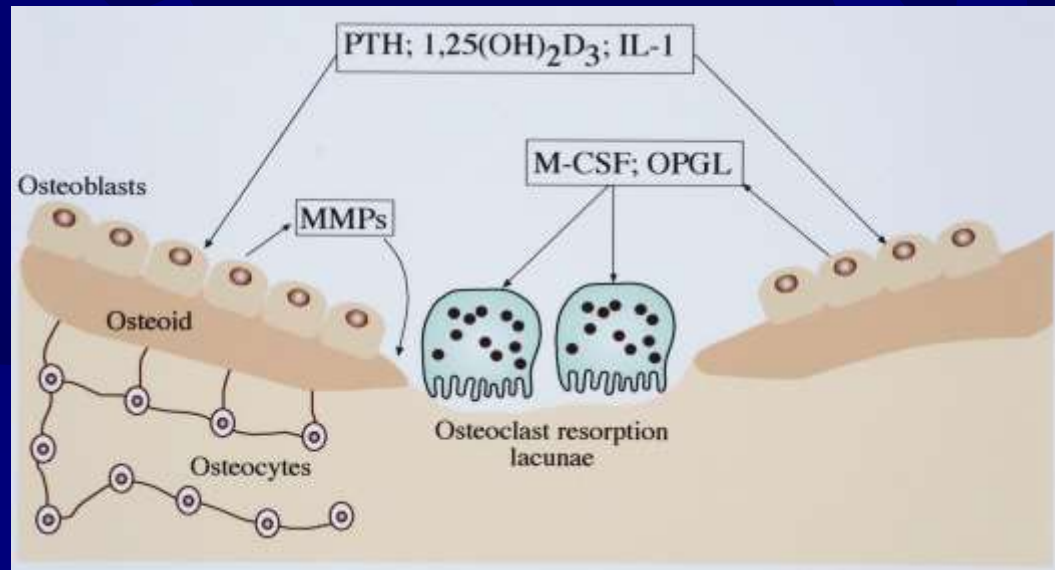
- ☀ TIMPs are effective inhibitors of bone resorption *in vitro* using the mouse calvarial assay. Left. Inhibition of PTH-stimulated $^{45}\text{Ca}^{2+}$ release by human recombinant TIMP-1 and TIMP-2.
- ☀ Right . Inhibitory effect of a general MMP inhibitor (CT435) and a specific gelatinase inhibitor (CT543) on IL-1-stimulated resorption in an isolated osteoclast pit assay. Synthetic MMP inhibitors have been developed for clinical use in the treatment of arthritic diseases and to prevent tumour metastasis. Courtesy of P A Hill.

Osteoclast differentiation and function



- a. Recruitment of precursors and formation of osteoclasts by action of RANKL and M-CSF.
- b. Adhesion to bone matrix via the $\alpha_v\beta_3$ integrin receptor (vitronectin receptor) to form the osteoclast sealing zone. Solubilisation of bone mineral is facilitated by acidification of the sub-osteoclastic resorption lacuna by H⁺ ions and activation of TRAP and cathepsin K.
- c. TEM of osteoclast with ruffled border (arrow) in a resorption lacuna.
- d. SEM showing 2 osteoclasts (Y) and 4 mononuclear precursor cells.
- From Boyle *et al.* (2003), *Nature*.

Regulation of bone resorption

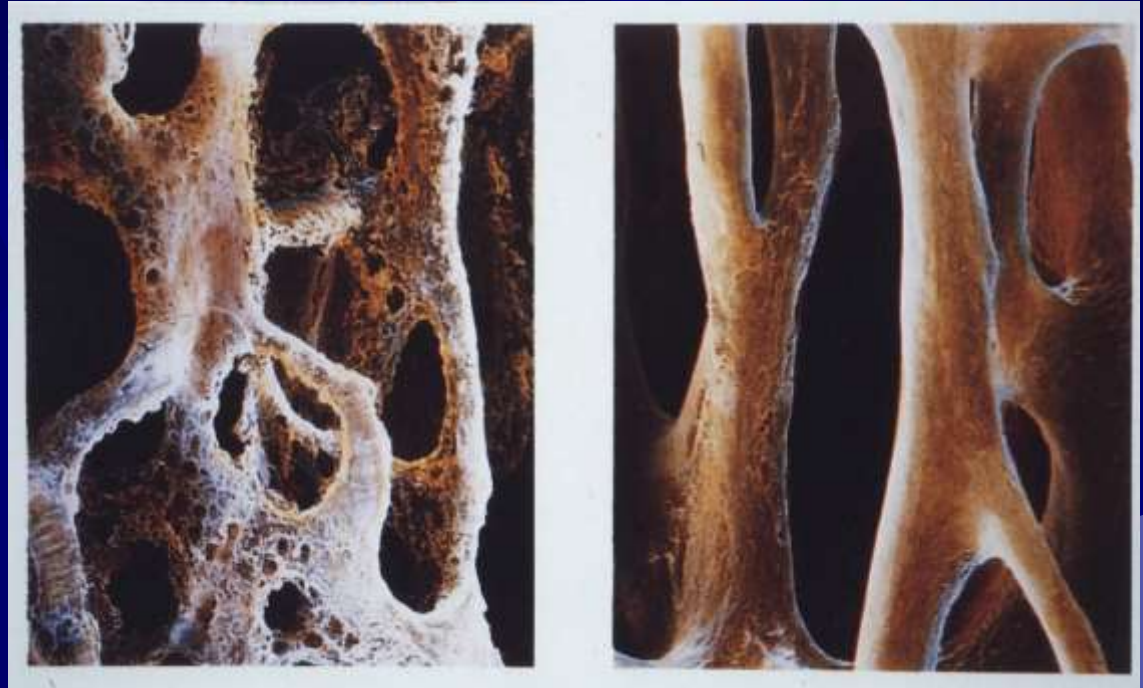


- ✿ The initial event in the resorption of a trabecular bone surface, is the degradation of the nonmineralized surface osteoid layer by MMPs produced by osteoblasts in response to hormones PTH , $1,25(\text{OH})_2\text{D}_3$ and cytokines such as IL-1 . This exposes the mineralized matrix which may be chemotactic for osteoclasts.
- ✿ Osteoblasts also release M-CSF and RANKL (OPGL) which stimulate osteoclast formation and function. As a result, growth factors are released from the bone matrix; these activate cells of the osteoblast lineage and convert the resorptive phase to one of deposition.
- ✿ From Meikle (2002), *Craniofacial Development, Growth and Evolution*.

Bone remodelling

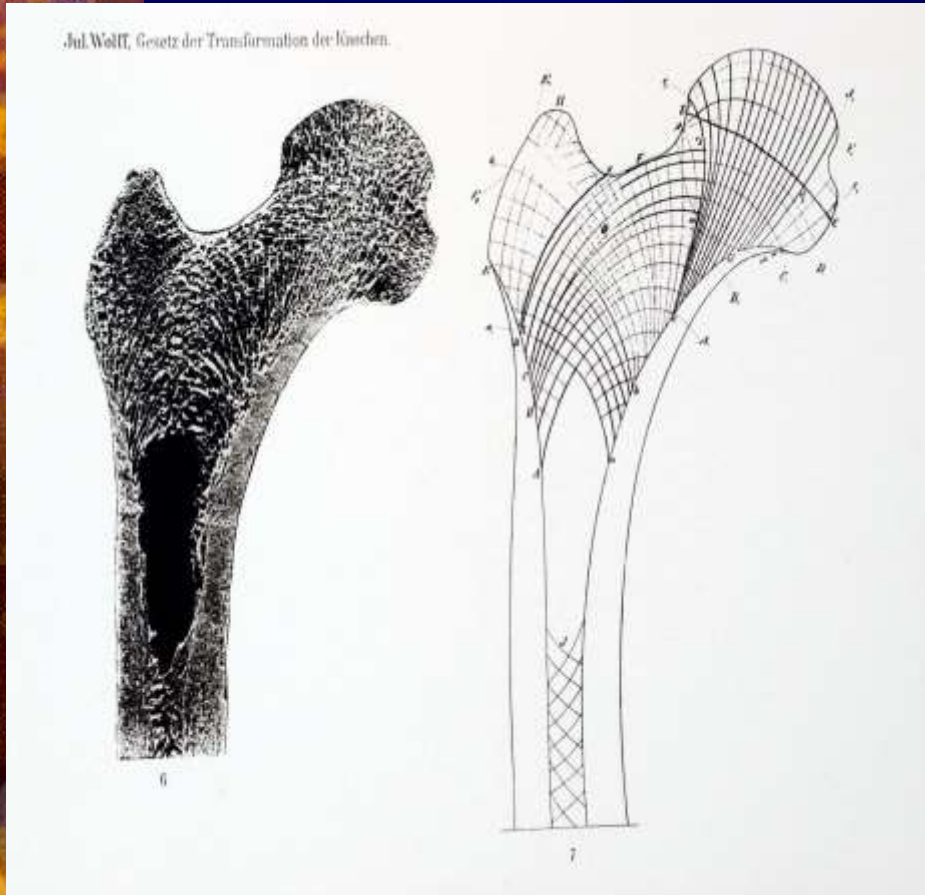
- ✱ All connective tissues undergo continual synthesis and degradation of their extracellular matrices, a process referred to as remodelling.
- ✱ The skeleton is no exception and is continuously remodelled throughout life by the resorption of old bone by osteoclasts and formation of new bone by osteoblasts. In contrast to nonmineralized connective tissues, evidence of past turnover remains visible microscopically via reversal lines.
- ✱ Internal remodelling limits the resorption and formation of bone to one location without changing its geometrical form. This distinguishes it from growth remodelling. Growth remodelling is limited to the periosteal and endosteal surfaces and serves to maintain the shape and proportions of a bone as it increases in size.

Bone resorption and bone formation are coupled

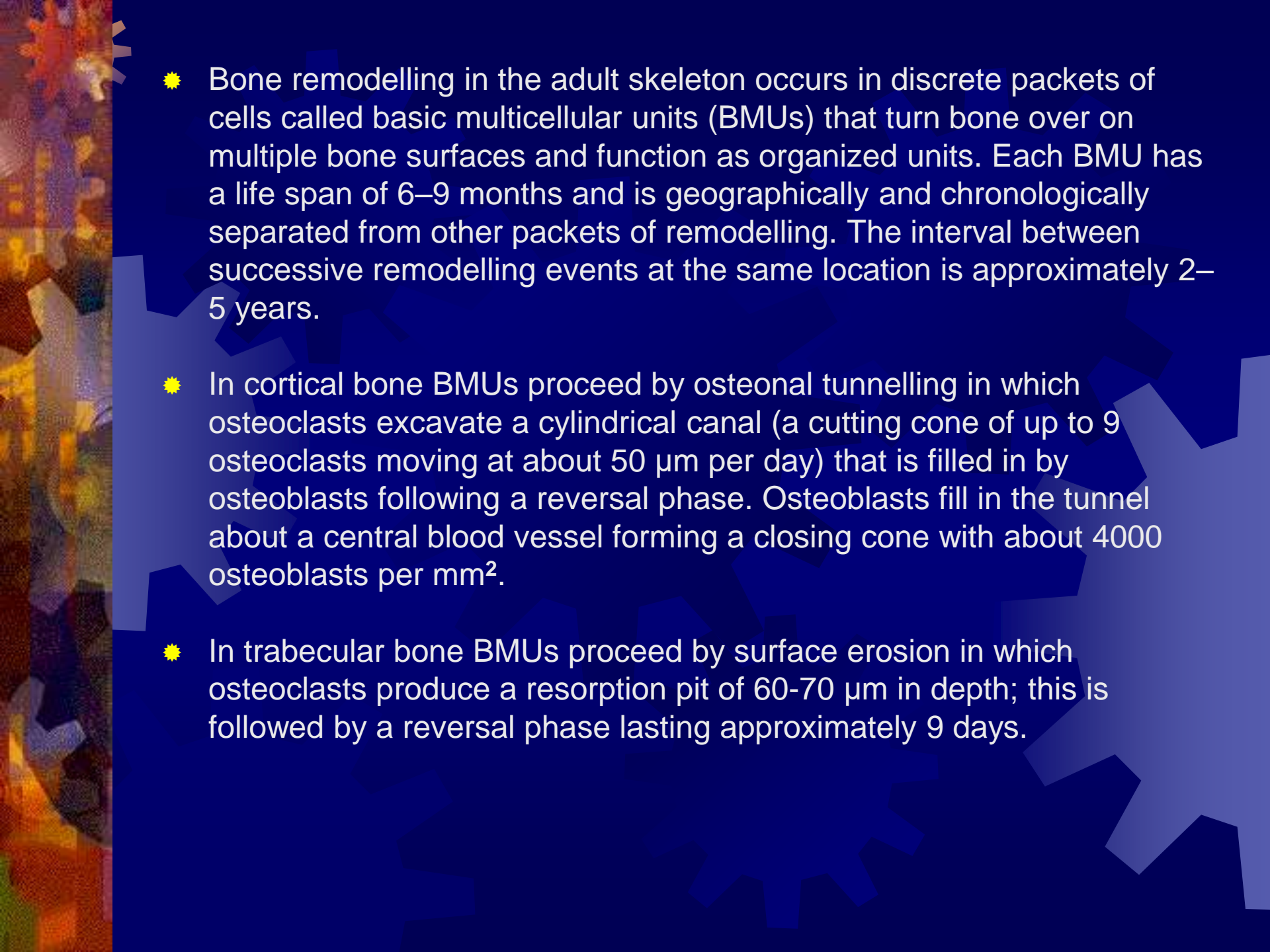


- During adult life there is a balance between the amount of bone resorbed by osteoclasts and the amount formed by osteoblasts to maintain a constant bone mass; *i.e.* they are said to be coupled, a process of renewing the skeleton while maintaining its structural integrity.
- Uncoupling of this balance leads to the reduced bone mass seen in osteoporosis (above left), or more rarely the accumulation of bone leading to osteopetrosis.
- Courtesy of P Motta/Science Photo Library.

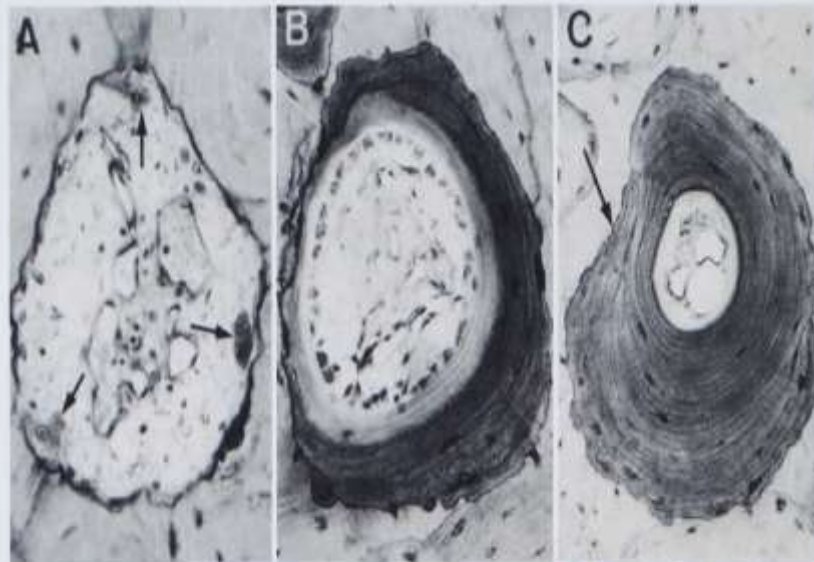
Bone structure and remodelling



- Bones are superb examples of design and engineering and consist of an outer cortex of compact or cortical bone and an inner trabecular network of cancellous bone.
- This famous illustration shows the structural arrangement of the trabeculae at the proximal end of the human femur. It has been used in numerous publications most famously by von Meyer (1967) and Wolff (1892) to show that the trabeculae in cancellous bone are laid down along the lines of maximal compressive and tensile stress.
- These stress lines are known in anatomical theory as 'trajectories'.
- From Wolff (1892), *Das Gesetz der Transformation der Knochen*

- 
- Bone remodelling in the adult skeleton occurs in discrete packets of cells called basic multicellular units (BMUs) that turn bone over on multiple bone surfaces and function as organized units. Each BMU has a life span of 6–9 months and is geographically and chronologically separated from other packets of remodelling. The interval between successive remodelling events at the same location is approximately 2–5 years.
 - In cortical bone BMUs proceed by osteonal tunnelling in which osteoclasts excavate a cylindrical canal (a cutting cone of up to 9 osteoclasts moving at about 50 μm per day) that is filled in by osteoblasts following a reversal phase. Osteoblasts fill in the tunnel about a central blood vessel forming a closing cone with about 4000 osteoblasts per mm^2 .
 - In trabecular bone BMUs proceed by surface erosion in which osteoclasts produce a resorption pit of 60-70 μm in depth; this is followed by a reversal phase lasting approximately 9 days.

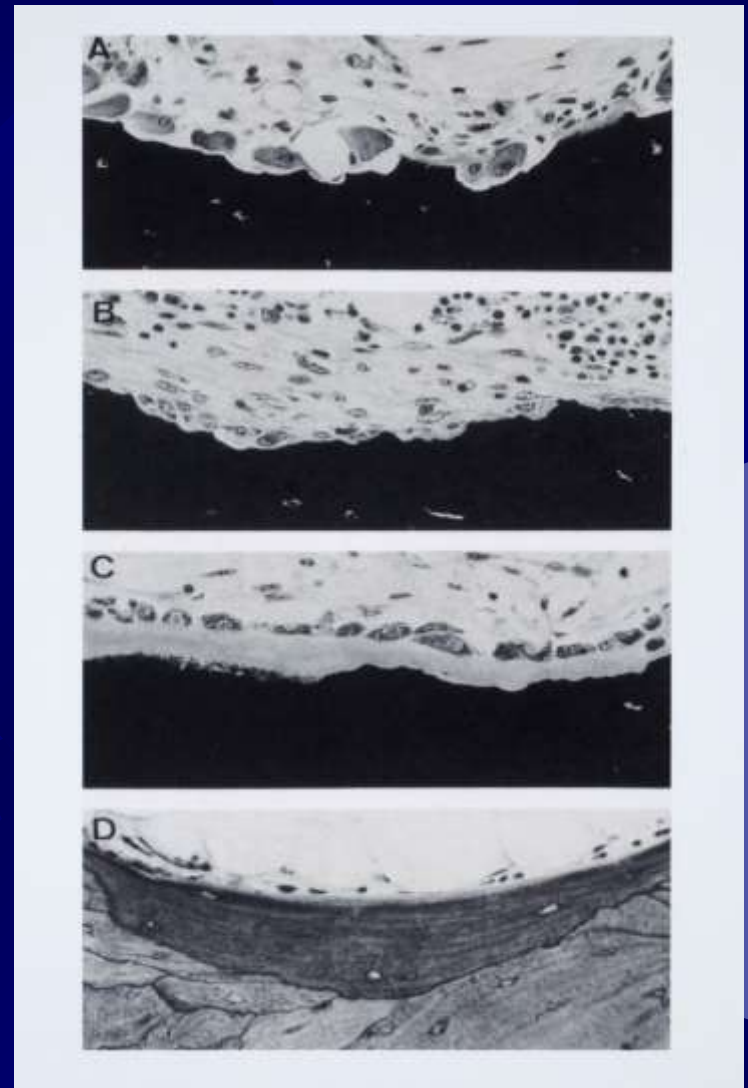
Remodelling of cortical bone



- In cortical bone BMUs proceed by osteonal tunnelling in which osteoclasts excavate a canal in the old bone that is refilled by new bone; these Haversian systems (secondary osteons) can be up to 200 μm wide and 10 mm in length.
- A-C. Transverse sections through evolving secondary osteons. Magnification x220.
- D. Longitudinal section through a secondary osteon. At the leading edge 3 osteoclasts are visible; osteoid (red) is lined by osteoblasts with a central capillary blood vessel.
- From Schenk *et al.* (1993), *Connective Tissue and Its Heritable Disorders*.

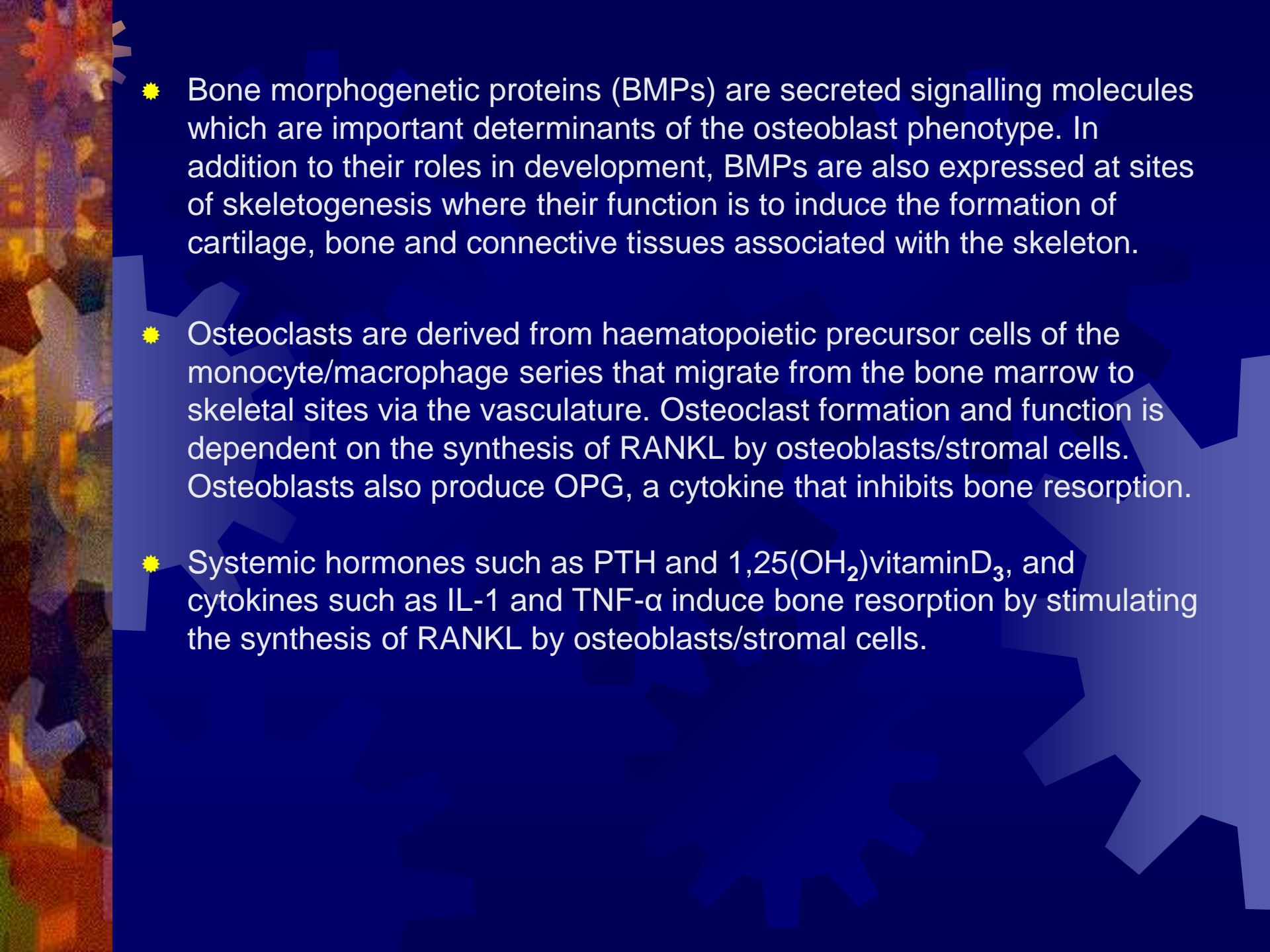
Remodelling of trabecular bone

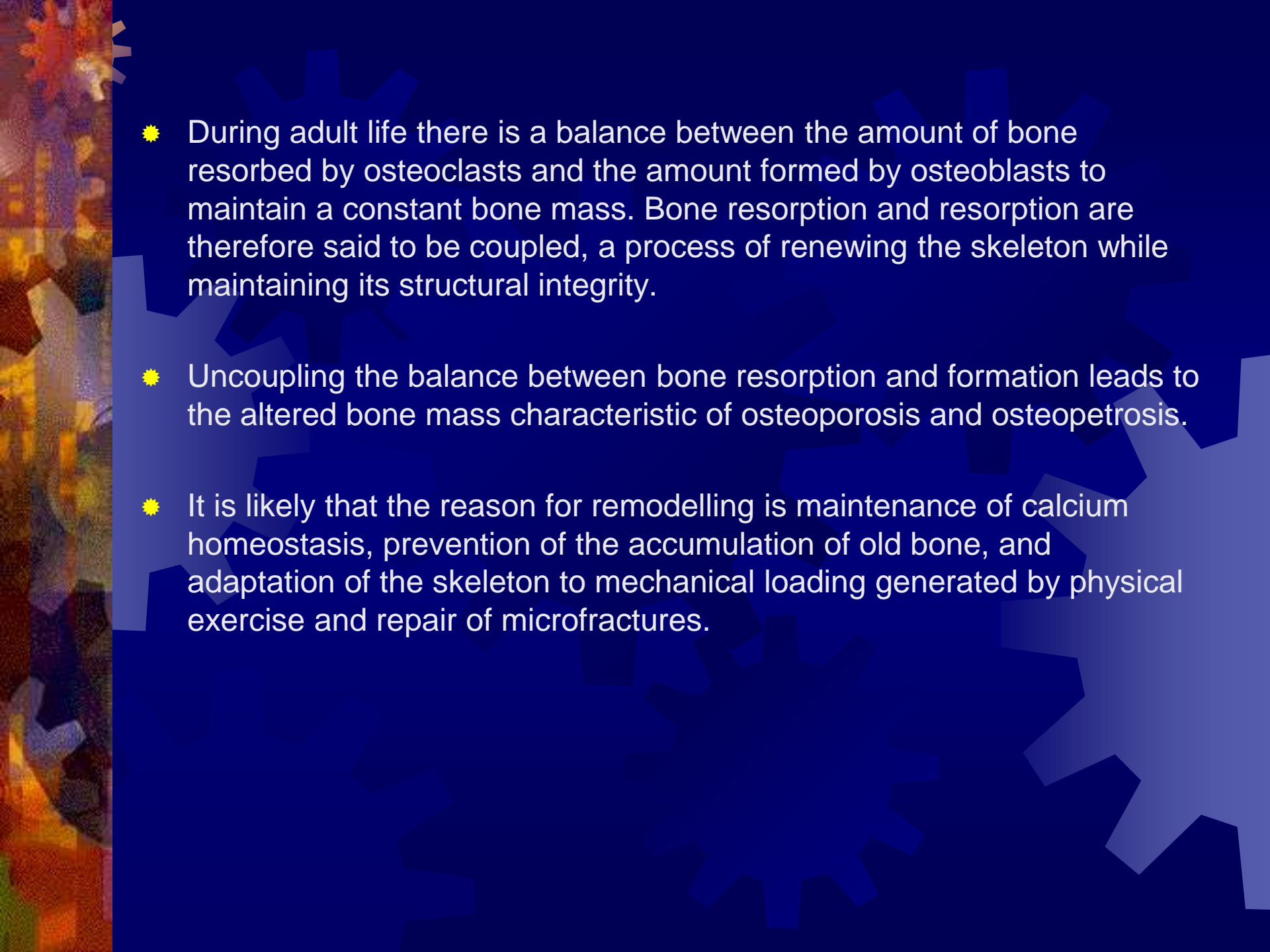
- A. Resorption phase: the surface is eroded by osteoclasts to a depth of 60–70 μm .
- B. Reversal phase: osteoblast precursor cells migrate into Howship's lacunae.
- C. Formative phase: osteoblasts deposit new bone matrix which is initially unmineralized (osteoid).
- D. Newly formed basic multicellular unit (BMU) is clearly delineated by a reversal line. Original magnification x200.
- From Schenk *et al.* (1993), *Connective Tissue and Its Heritable Disorders*.



Summary

- Whether cartilage or bone evolved first has been the subject of lengthy debate for two hundred years. However, cartilage and bone originate from a common stem cell; whether they differentiate along a cartilaginous or osteogenic pathway will be dictated by the environmental and evolutionary imperatives pertaining at the time.
- It seems probable that during evolution dentine and bone preceded cartilage in the exoskeleton, while cartilage preceded perichondrial and endochondral bone in the endoskeleton.
- Osteoblasts differentiate from mesenchymal stem cells, a precursor they share with chondroblasts, fibroblasts, adipocytes and myoblasts. A key regulator of osteoblast differentiation is the master gene *Cbfa-1*; *Cbfa-1* (-/-) mutant mice have no bone. *Cbfa-1* protein is a transcription factor which binds to and regulates the expression of multiple genes (*osteocalcin*, *type I collagen*, *bone sialoprotein*, *osteopontin*) by osteoblastic cells.

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- Bone morphogenetic proteins (BMPs) are secreted signalling molecules which are important determinants of the osteoblast phenotype. In addition to their roles in development, BMPs are also expressed at sites of skeletogenesis where their function is to induce the formation of cartilage, bone and connective tissues associated with the skeleton.
 - Osteoclasts are derived from haematopoietic precursor cells of the monocyte/macrophage series that migrate from the bone marrow to skeletal sites via the vasculature. Osteoclast formation and function is dependent on the synthesis of RANKL by osteoblasts/stromal cells. Osteoblasts also produce OPG, a cytokine that inhibits bone resorption.
 - Systemic hormones such as PTH and $1,25(\text{OH})_2\text{vitaminD}_3$, and cytokines such as IL-1 and TNF- α induce bone resorption by stimulating the synthesis of RANKL by osteoblasts/stromal cells.

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- During adult life there is a balance between the amount of bone resorbed by osteoclasts and the amount formed by osteoblasts to maintain a constant bone mass. Bone resorption and resorption are therefore said to be coupled, a process of renewing the skeleton while maintaining its structural integrity.
 - Uncoupling the balance between bone resorption and formation leads to the altered bone mass characteristic of osteoporosis and osteopetrosis.
 - It is likely that the reason for remodelling is maintenance of calcium homeostasis, prevention of the accumulation of old bone, and adaptation of the skeleton to mechanical loading generated by physical exercise and repair of microfractures.