

# Molecular and cellular biology of alveolar bone

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Alveolar bone is a specialized part of the mandibular and maxillary bones that forms the primary support structure for teeth. Although fundamentally comparable to other bone tissues in the body, alveolar bone is subjected to continual and rapid remodeling associated with tooth eruption and subsequently the functional demands of mastication. The ability of alveolar bone to undergo rapid remodeling is also important for positional adaptation of the teeth but may be detrimental to the progression of periodontal disease. The anatomical structure of alveolar bone, which is quite complex, has recently been described in detail (200). Alveolar bone is composed of bundle bone (209), which is formed in layers in a parallel orientation to the coronal-apical direction of the tooth. Sharpey's fibers extend obliquely from the thin lamella of bone that lines the socket wall and are continuous with fibers of the periodontal ligament. A thicker outer layer of bone formed of cortical plates extends from the jaw bone and forms the lingual and labial surfaces of the alveolar process and is largely made up of spongy cancellous bone. Within the cancellous bone are numerous marrow spaces, with smaller endosteal spaces present in the cortical bone. Some of the small endosteal spaces extend into, and are contiguous with, the periodontal ligament.

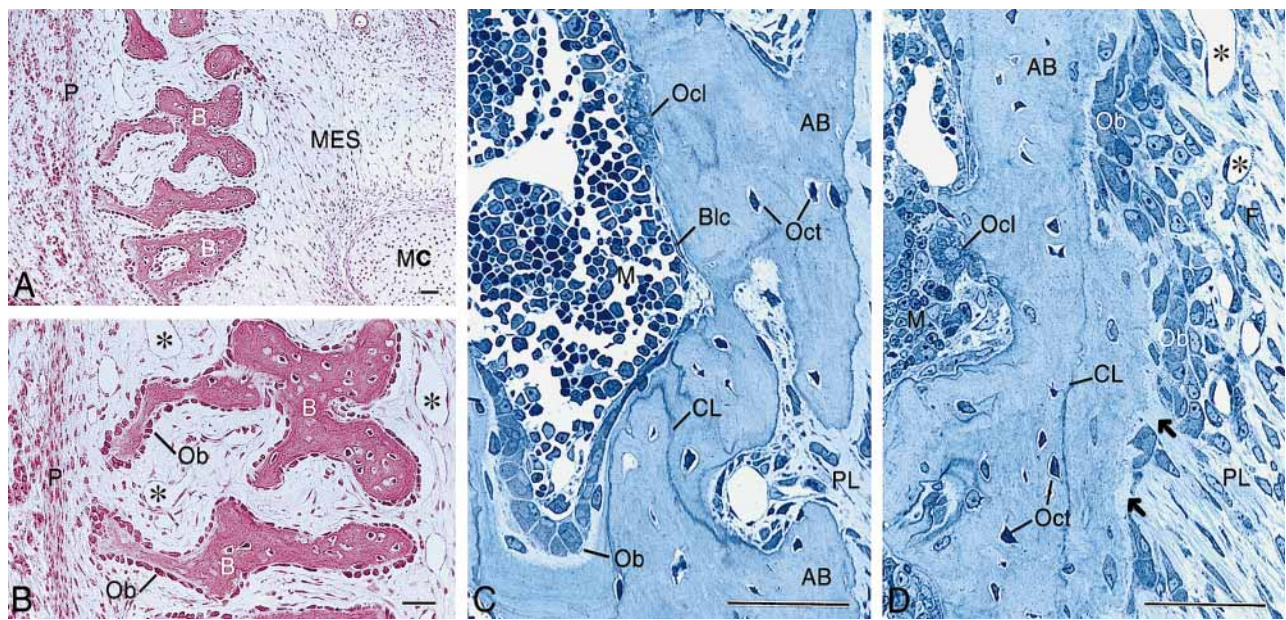
Because of the small size and anatomical complexity of alveolar bone, relatively few studies on the cellular, and particularly the molecular, aspects of alveolar bone structure and metabolism have been performed using alveolar bone itself. However, the ability of bone cells derived from adult rabbit alveolar bone to form mineralized tissue nodules with the characteristics of bone has been described (142, 224). A procedure has also recently been developed for the isolation of adult human alveolar bone cells, from 2-week-old osteogenic tissue recovered from dental implant surgery, that form bone tissue in culture (174). While these systems provide a means of investigating specialized aspects of the molecular

and cellular biology of alveolar bone in a controlled environment, much of the current information on alveolar bone must be extrapolated from studies of other bone tissues.

## Cellular components

### Osteogenic cells

**Osteoblasts.** During embryonic development, intramembranous bone of the maxilla and mandible initially forms from osteoblasts arising from condensing mesenchyme in the facial region (Fig. 1) that creates bony alveoli that house the individual teeth of the developing dentition. The most active secretory cells in bone, the osteoblasts, are generally cuboidal or slightly elongated cells that line a large percentage (depending on age and anatomical site) of bone surfaces and are primarily responsible for the production of the organic matrix of bone (94). At the innermost surface of the tooth alveolus, the positional arrangement of alveolar bone osteoblasts must accommodate the interdigitating portions of the periodontal ligament collagen fibers known as Sharpey's fibers that insert into the bone (115). Thus, in three dimensions, these cells form an extensively perforated sheet of otherwise contiguous osteoblasts which, in addition to producing alveolar bone matrix proper, must additionally embed continuously remodeling periodontal ligament fibers in a rather precise manner (124). The concerted cellular actions by which this occurs appears complex and have not been well studied. Also, the precise contributions of nearby periodontal ligament fibroblasts and the alveolar osteoblasts remains to be determined for this particular soft tissue–hard tissue interface. Occurring simultaneously with these processes whereby periodontal ligament fibers are attached to the bone surface, continuous bone remodelling at the alveolar surface and within the alveolar bone causes temporary detachment of small portions of the periodontal



**Fig. 1.** Light micrographs of early and late stages of alveolar bone development, organization and structure. **A, B.** In embryonic development, mesenchyme (MES) in the facial region condenses to form nodules of differentiated osteoblasts (Ob) that produce intramembranous bone (B) within a space defined by the periosteum (P). In the mandible, this process occurs in the vicinity of a transitory cartilaginous rod known as Meckel's cartilage (MC). At this early stage, all intramembranous bone formation occurs in close relation to capillaries (asterisks). As osteogenesis progresses, all bone surfaces are lined by a contiguous layer of osteoblasts that continue to produce bone in order to enlarge the dimensions of the trabeculae. Concomitant with the growth of this established bone, new mesenchymal condensations arise in neighboring areas to produce new osteoblasts and new trabeculae, all of which will collectively form the fetal maxilla and mandible. **C, D.** Post-natally, the macroscopic merging of individual trabeculae to form the maxilla and mandible leaves

defined spaces that house bone marrow (M) and vascular channels, and where the bone outlines the wall of the tooth alveolus, it becomes the attachment point (arrows) for collagen fibers of the periodontal ligament (PL). In histological sections of remodeling alveolar bone (AB), the four major cell types of bone can be readily observed (Ob, osteoblast; Oct, osteocyte; Ocl, osteoclast; Blc, bone-lining cell). Cement lines (CL), indicative of remodelling activity, are also prominent features within the bone extracellular matrix. Osteoblasts embed the extremities of periodontal ligament collagen fibers as Sharpey's fibers into the bone on the innermost wall of the alveolus. The periodontal ligament resides as a dense connective tissue rich in fibroblasts (F), and highly vascularized with capillaries (asterisks), linking the tooth to the alveolar bone. **A, B:** paraffin sections of embryonic pig mandible stained with hematoxylin and eosin. **C, D:** Epon sections of post-natal mouse mandibular alveolar bone stained with toluidine blue. Bars equal 50  $\mu\text{m}$ .

ligament from the alveolus. Soon after, however, new synthesis of periodontal ligament by fibroblasts together with new alveolar bone production by osteoblasts collectively allow for re-attachment at these remodelled sites. Asynchronous remodeling of the alveolar bone allows for maintenance of tooth bio-mechanical function.

Osteoblasts are of mesenchymal origin, and when fully differentiated and actively secreting bone matrix are considered to be post-mitotic cells containing a cytoplasm rich in synthetic and secretory organelles such as rough endoplasmic reticulum, Golgi apparatus, secretory granules and microtubules (Fig. 2, 3) (155, 207). Osteoblasts also contain a variety of other organelles normally associated with cell metabolism such as mitochondria and endosomal/lys-

osomal elements, and an extensive cytoskeleton. The organic matrix produced by osteoblasts consists predominantly of type I collagen and various other non-collagenous bone proteins and plasma proteins (47, 70, 88, 267), described below. Regarding the production of collagen by these and other cells (144), the stages of secretory granule formation and maturation have been well documented for the major collagen-producing cells – namely, osteoblasts (254), fibroblasts (41, 143, 241) and odontoblasts (68, 253). A particularly intriguing question that remains unanswered, however, concerns the packaging site and exocytotic route taken by the noncollagenous proteins known to be secreted by osteoblasts. Indeed, it is still not clear whether these proteins are packaged into the same secretory granules as collagen and se-

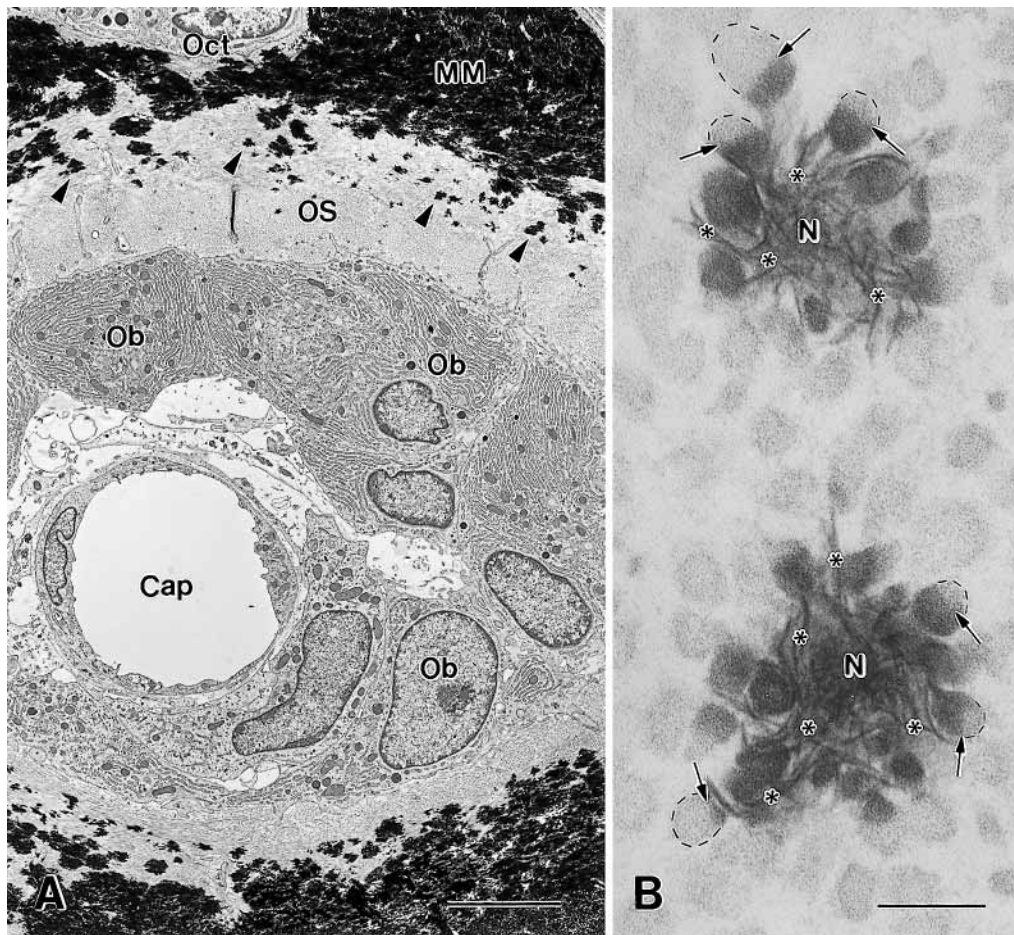
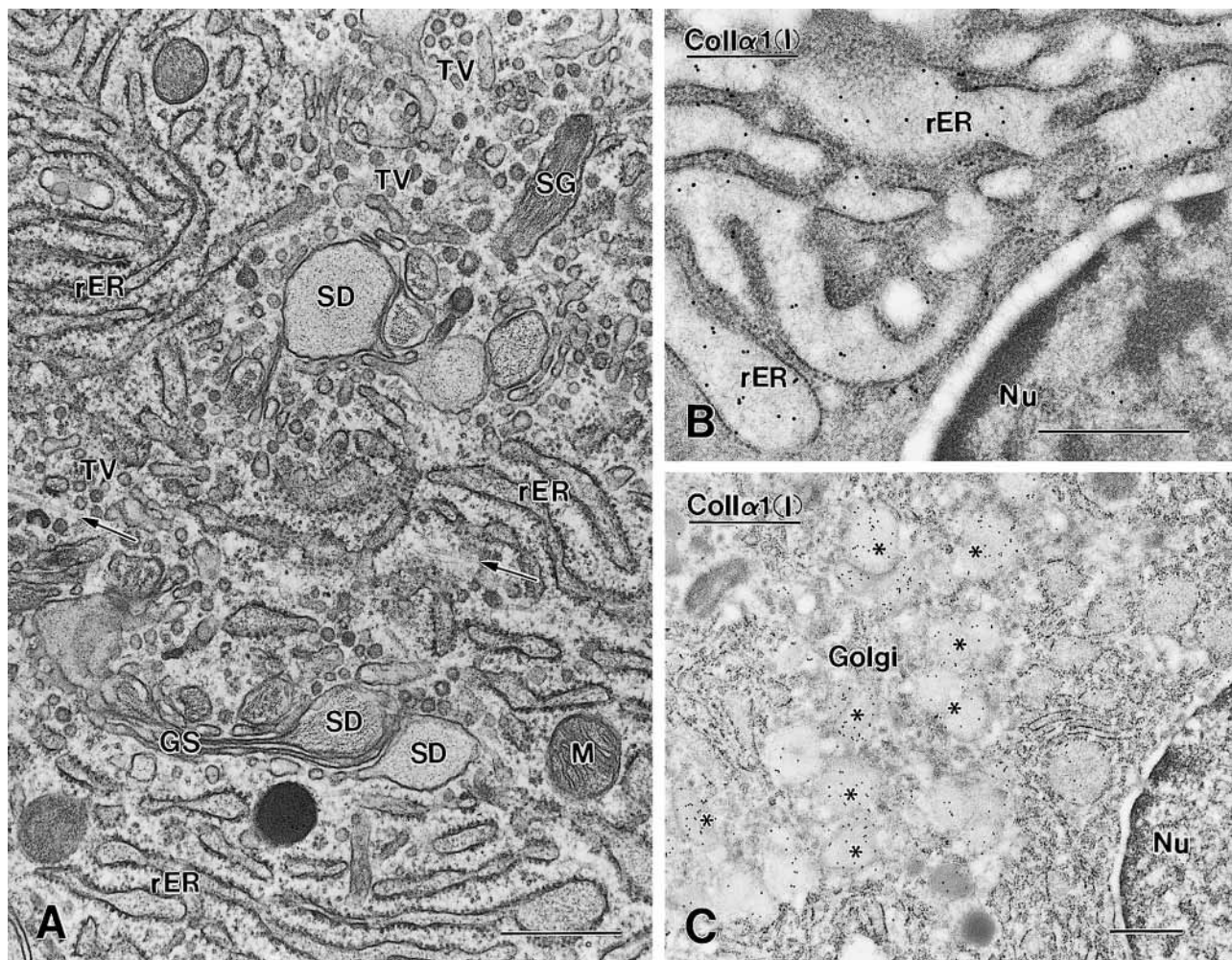


Fig. 2. Transmission electron micrographs of cells, extracellular matrix compartments and mineral of undecalcified alveolar bone. **A.** Within vascular channels of alveolar bone, active bone deposition can be observed where osteoblasts (Ob), in relation to a centrally located capillary (Cap), initially lay down a generally unmineralized osteoid (OS) layer composed predominantly of type I collagen fibrils associated with some small foci of calcification (arrowheads). Osteocytes (Oct) derive from osteoblasts that become encased in mineralized matrix (MM). The irregular, planar interface between generally unmineralized osteoid and the mineralized matrix proper is referred to as the mineralization front. **B.** At higher magnification, details of the initial mineralization pattern can be observed within the extracellular matrix. Electron-dense mineral (carbonate-substituted hydroxyapatite) is found in two locations, namely, between and within the collagen fibrils. Mineralization sites often occur as a centrally located ni-

du (N) of relatively large crystals (asterisks) that appear to be generally between the collagen fibrils, and as a very finely textured mineral having nanometer crystal dimensions (apparent at only much higher magnifications) within the collagen fibrils, here shown in cross-section. From a morphological perspective, an intimate relationship exists between these two mineral deposition sites in that mineral (or apparent mineral contact) appears continuous across the inter- and intrafibrillar space, with collagen fibrils commonly showing mineral incorporation into the portion of the fibril closest to the larger crystals of the central nidus. Thus, collagen fibrils cut in cross-section (hashed lines) are consistently only partially replete (arrows) with mineral on the side facing the larger crystals. With time, intrafibrillar mineral fills the collagen fibrils across their entire diameter. Epon sections of post-natal rat alveolar bone stained with uranyl acetate and lead citrate. Bars equal (A) 5  $\mu$ m, (B) 100 nm.

creted in tandem via the same pathway or whether a separate population of secretory granules and a separate exocytotic pathway exist specifically for these proteins. Here, it should be emphasized that the often misquoted early radioautographic studies by Weinstock et al. (251, 252, 255) on bone and teeth demonstrated phosphoprotein and/or glycoprotein deposition into prebone (osteoid) and preentin, fol-

lowed by its subsequent accumulation at the mineralization front in these tissues. Thus, as shown for collagen, the release of noncollagenous proteins appears to occur primarily at the base of osteoblast (and odontoblast) cell processes and from the cell body itself. While some secreted proteins associate with the collagen to form new matrix, some of the acidic proteins appear to diffuse through the osteoid



**Fig. 3.** Electron micrographs and immunocytochemical preparations showing osteoblast morphology and the synthetic pathway for type I collagen. **A.** Osteoblasts are highly secretory cells producing abundant extracellular matrix. As for other collagen-producing connective tissue cells, osteoblasts contain abundant rough endoplasmic reticulum (rER), and a well-developed Golgi apparatus consisting of Golgi saccules (GS) and associated peripheral, spherical distensions destined to become collagen-containing secretory granules (SG). Transport vesicles (TV) are abundant in the cytoplasm in close proximity to

the Golgi apparatus, as are microtubules (arrows) and mitochondria (M). **B, C.** Using post-embedding, colloidal-gold immunocytochemistry and antibodies raised against mouse *N*-terminus collagen  $\alpha 1(I)$  to examine intracellular pathways for the production of type I collagen, gold particle labeling indicates the presence of this protein in the rough endoplasmic reticulum (rER) of osteoblasts and in the various compartments of the Golgi apparatus (asterisks). Nu: nucleus. Epon (A) and LR White (B, C) sections of postnatal mouse alveolar bone stained with uranyl acetate and lead citrate. Bars equal 0.5  $\mu\text{m}$ .

and, due to their affinity for hydroxyapatite, accumulate at the mineralization front. The possibility that matrix proteins may also be secreted deeper in the matrix is indicated by the presence of secretory granules in some osteoblast cell processes.

Collagen fibril formation in bone is initiated when collagen filaments released by osteoblasts assemble extracellularly into striated fibrils to form the osteoid, a light microscopic term for the seam of unmineralized bone matrix closest to the osteoblast, although numerous small foci of mineralization can also be observed within the osteoid layer by electron

microscopy (Fig. 2) (157). Ultrastructurally, certain noncollagenous and plasma proteins co-localize with these early foci, although the vast majority appear to accumulate at the mineralization front and throughout the mineralized bone matrix (18, 36, 99, 153, 154, 156, 196, 214). Morphological indications for the synthesis, post-translational modification and packaging of proteins for secretion by osteoblasts can be readily appreciated by examining the prominence and extent of rough endoplasmic reticulum and the Golgi apparatus by routine transmission electron microscopy, and by radioauto-

graphic, cytochemical and immunocytochemical studies that trace the flow of proteins through these various intracellular compartments (Fig. 3, 4).

**Osteocytes.** Following maturation, osteoblasts may undergo apoptosis, become encased in matrix as osteocytes or remain on the bone surface as bone-lining cells. Osteoblasts that become osteocytes occupy spaces (lacunae) in bone and are defined as cells surrounded by bone matrix (20, 148), whether mineralized or still part of the osteoid seam. Although a sub-classification of osteocyte development has been proposed based on their progressive encasement first into the osteoid, and then into mineralized matrix (20, 107, 264), the formation of osteocytes should be viewed as a continuum involving a change in the surrounding extracellular environment with accompanying cellular metabolic changes. Osteocytes have a decreased quantity of synthetic and secretory organelles, and indeed are smaller cells than osteoblasts (149), with the nucleus occupying a significantly larger proportion of the cell. Although they are diminished in size, these cells have the full complement of organelles capable of effecting protein secretion (14, 238), a feature perhaps reflected by the variable appearance of the perilacunar matrix frequently observed surrounding these cells (20, 108, 106). Although controversial (24, 25, 116, 148, 182), it is believed by some that the formative and/or resorptive activity of these cells may vary under certain metabolic requirements, resulting in the concept of "osteocytic osteolysis" (12, 13, 15, 16, 239). Finally, a major feature of osteocytes is the presence of numerous and extensive cell processes that ramify throughout the bone in canaliculi and make contact, frequently via gap junctions, with processes from other osteocytes or with similar processes extending from osteoblasts or bone-lining cells at the surface of the bone (180, 181). Thus, considerable potential exists for communication among these cells, a factor of great importance considering the spatial isolation of osteocytes within such a dense, rigid mineralized matrix.

**Bone-lining cells.** Bone-lining cells cover most, but not all (42), quiescent bone surfaces in the adult skeleton (136, 151, 164, 166). The transition from osteoblast to bone-lining cell clearly involves a series of gradual morphological and functional changes (162, 164) that culminate in decreased protein secretion. The relative paucity of organelles in these cells indicate that they are less involved, if at all, in classi-

cal protein secretion of bone matrix, although not precluding the ability of these cells to produce local regulatory substances and to modify the composition of the underlying lamina limitans. Transformation of osteoblasts into bone-lining cells may represent the final phenotype of the osteoblast lineage prior to activation of the bone remodelling sequence at sites occupied by these cells (185). The ultimate fate of bone-lining cells is presumably death by apoptosis (113). Although in mammals there is little evidence that bone-lining cells can regain a capacity to produce lamellar bone matrix, in adult birds, bone-lining cells appear to retain osteogenic potential, proliferating in response to estrogen and contributing to the formation of woven medullary bone (22). A large percentage of bone surfaces is covered by a contiguous layer of bone-lining cells with an extended, flattened morphology which potentially bestows an important homeostatic role on the lining cells in compartmentalizing the bone matrix and influencing calcium and phosphate metabolism, substance exchange and/or the initiation of events leading to activation of the bone remodeling sequence (151, 165, 166, 185). Together with osteocytes, bone-lining cells and their connecting cell processes appear to form an extensive homeostatic network of cells capable of regulating plasma calcium concentration through mechanisms partly independent of those related to the bone remodeling system (145, 183, 230). Indeed, quiescent surfaces are known to be a primary site of mineral ion exchange between blood and adult bone (184).

### Osteoclastic cells

Of central importance in the ability of bone to respond to biological regulatory factors and functional forces is the capacity of the large, multinucleated osteoclasts to resorb bone. Indeed, the coupling of bone resorption with bone formation constitutes one of the fundamental principles by which bone is necessarily remodeled throughout life (87, 190, 243). Apart from its multinucleation, the most striking feature of the osteoclast is the presence of an actin-, vinculin- and talin-containing clear (sealing) zone (5, 126, 235) in the peripheral cytoplasm of this cell that delineates a more central region of membrane infoldings (plates) and finger-like processes termed the ruffled border (10, 52). Resorption of bone occurs in an acidified extracellular matrix compartment as a result of the combined actions of a variety of ruffled border membrane-associated enzymes including a tartrate-resistant, vanadate-sensitive acid adenosine

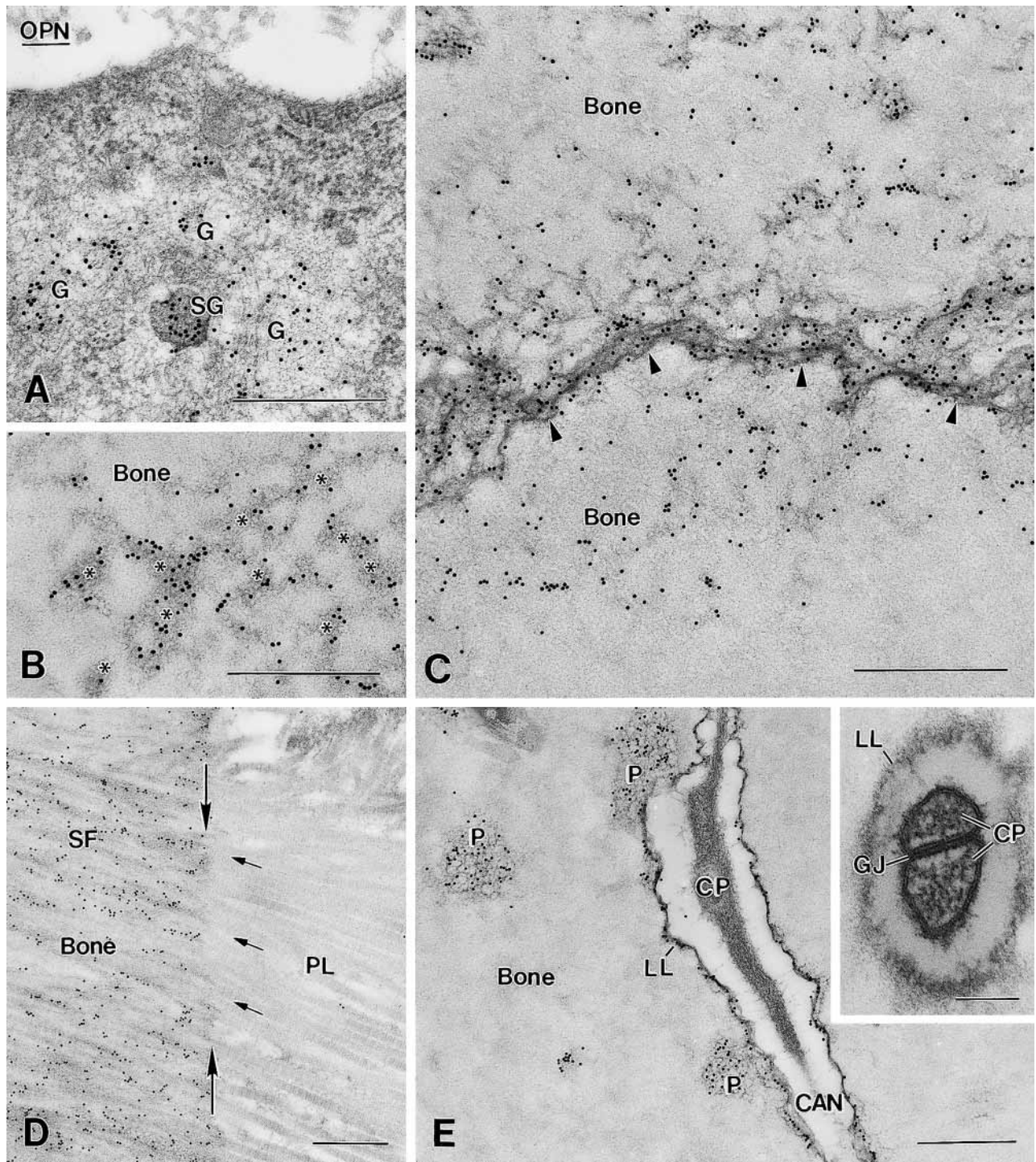


Fig. 4. Colloidal-gold immunocytochemical preparations of osteoblasts and alveolar bone matrix. The gold particle immunolabeling pattern for the noncollagenous protein osteopontin (OPN) is shown. **A.** The Golgi apparatus (G), and secretory granules (SG), of alveolar bone osteoblasts show intense labeling for osteopontin. **B, C.** In decalcified sections of the otherwise mineralized bone matrix, osteopontin is predominantly dispersed within electron-dense aggregates (asterisks) among the collagen fibrils of the bone. Cement lines (arrowheads), typically found as reversal lines at bone remodeling sites, also contain abundant osteopontin. **D.** Osteopontin is typically found at high

levels where collagen fibrils (small arrows) of the periodontal ligament (PL) insert into alveolar bone (large arrows) as Sharpey's fibers (SF). **E.** Coursing throughout the extracellular matrix of bone are numerous cell processes (CP) contained in canaliculi (CAN) lined by an osteopontin-rich lamina limitans (LL), the latter presumably used in mediating cell membrane attachment to the bone matrix and in limiting calcification across this interface. Discrete patches (P) of matrix rich in osteopontin are dispersed throughout the bone. Inset illustrates a gap junction (GJ) between two adjoining cell processes from different cells contained within a canaliculus lined by a

triphosphatase (3), carbonic anhydrase isozyme II (69, 146, 245) and proton-pumping adenosine triphosphatases (1, 9, 244). Although numerous states of osteoclast differentiation can be observed and are reflected by different osteoclast morphologies (66), it is thought that the presence of small and large cathepsin-containing (82, 179, 204) cytoplasmic vacuoles/vesicles (135), often in the immediate vicinity of the ruffled border, are indicative of resorptive activity by these cells. Among these membrane-bound structures, there exists a population of small, spherical vesicles having a single indentation of the membrane at one site and appear to contain lysosomal (155) and plasma membrane (66) enzymes, and thus may participate in certain degradative activities performed by the osteoclast and/or plasma membrane recycling.

## Matrix components

Although alveolar bone and the alveolar process have specialized features relating to their functional properties, the composition of the extracellular matrix of alveolar bone appears to be similar to other bone tissues as indicated largely by immunohistochemical analyses. Consequently, the following general description of bone matrix components is given with references to alveolar bone made where appropriate. The bone matrix is formed from a scaffold of interwoven collagen fibers within and between which small, uniform, plate-like crystals of carbonated hydroxyapatite ( $\text{Ca}_{10}[\text{PO}_4]_6[\text{OH}]_2$ ) are deposited. Other proteins, including proteoglycans, acidic glycosylated and non-glycosylated proteins associate with and regulate the formation of collagen fibrils and mineral crystals, or provide continuity between matrix components and between the matrix and cellular components. In addition, small amounts of carbohydrate and lipid contribute to the organic matrix, which comprises approximately one-third of the matrix while the inorganic components account for the remaining two-thirds. Calcium and phosphate in the form of poorly crystalline, carbonated apatite, also described as dahllite, predominates the inorganic phase, largely replacing the water component of the soft, dense connective tissues that include the periodontal ligament and gingiva.

**lamina limitans.** LR White sections of post-natal rat alveolar bone stained with uranyl acetate and lead citrate. Bars equal 0.5  $\mu\text{m}$ ; inset, 0.1  $\mu\text{m}$ .

## Collagen

Collagen comprises the major (~80–90%) organic component in mineralized bone tissues. Type I collagen (>95%) is the principal collagen in mineralized bone and, together with type V (<5%) collagen, the type I collagen forms heterotypic fiber bundles that provide the basic structural integrity of connective tissues. In addition to the presence of type I (191) and V (27) collagens in alveolar bone, both type III and XII collagens are also present (119, 137, 249). The type III collagen is present as mixed fibers with type I collagen (97) in Sharpey's fibers that insert from the periodontal ligament into the lamellar bone lining the alveolus to provide a stable connection with the tooth. The expression of type XII collagen is related to mechanical strain (40) and the alignment of collagen fibers, as demonstrated in the maturation of the periodontal ligament (119). While the type I, V and XII collagens are expressed by osteoblasts, the type III and some of the type XII collagen fibers appear to be produced by fibroblasts during the formation of the periodontal ligament. The suggestion that type III collagen may prevent the mineralization of Sharpey's fibers in alveolar bone and cementum (249) has not been verified experimentally.

The collagen fibrils in bone are stabilized by intermolecular cross-linking (59) involving lysines and modified lysines that form pyridinium ring structures (pyridinolines). These cross-links are primarily responsible for the high tensile strength of collagen fibers, which are formed from fibrils as higher order structures laid down in a specific orientation by the bone-forming osteoblasts. In rapidly forming (woven) bone that is produced during early development and in repair sites, the fibers are extensively interwoven, leaving a substantial volume of interfibrillar space that is largely occupied by mineral crystals and associated acidic proteins. In mature (lamellar) bone, the collagen fibers form highly organized sheets in which successive layers of fibers are oriented perpendicular to each other with little interfibrillar space. In both woven and lamellar bone the mineral crystals within the collagen fibrils are believed to form initially within the gap region between successive collagen molecules such that their c-axes are aligned with the long axis of the collagen fibril (250). Additional formation of crystals and crystal growth occurs in the channels formed by the gap regions and in the spaces that exist between the collagen molecules, which have a characteristic intermolecular spacing (261).

## Noncollagenous proteins

Using dissociative extraction procedures, most of the major noncollagenous proteins from mineralized bone have been isolated and characterized (62, 64, 233). Although age-related differences in the relative amounts of these proteins have been reported together with differences in various types of bone and in bones of different species, the same proteins are always present. Some of these proteins, typically osteocalcin and bone sialoprotein, are essentially unique to mineralized tissues, whereas others, such as osteonectin/SPARC (secreted protein, acidic, rich in cysteine) and osteopontin have a more general distribution (Fig. 5). These proteins are released from bone by demineralization, reflecting the predominant association with the mineral phase. Other proteins are present in bone in specifically modified forms. Thus small proteoglycans, primarily chondroitin sulfate proteoglycans, are present in bone (62), whereas their dermatan sulfate counterparts are characteristically found in soft connective tissues (Fig. 6). The proteoglycans are generally associated with the collagenous matrix, although interaction with mineral crystals also occurs through the acidic glycosaminoglycan side chains. In addition to those proteins produced by bone-forming cells, certain proteins derived from blood and tissue fluids are concentrated in bone, largely due to their affinity for the mineral crystals. These include albumin,  $\alpha$ 2HS-glycoprotein, immunoglobulins and matrix gla protein (47).

Osteocalcin, also known as bone gla protein, represents <15% of the noncollagenous proteins and was the first noncollagenous bone protein to be characterized. Its presence in alveolar bone has been demonstrated immunohistochemically (26, 29). It is a small, highly conserved, 5.8-kDa acidic protein that is characteristically modified by vitamin K-dependent carboxylating enzymes that convert two to three glutamic acids into  $\gamma$ -carboxyglutamic acids (gla groups), linking this protein with a family of blood coagulation factors (Fig. 5). The human osteocalcin gene comprising 4 exons is located on chromosome 1 and codes for a 125-amino-acid pre-pro-osteocalcin that includes a 26-amino-acid signal peptide. The promoter of the osteocalcin gene has been extensively characterized for its transcriptional regulation (133) and tissue-specific expression in which the runt domain transcription factor, osteoblast-specific transcription factor 2/core binding factor  $\alpha$ 1 (see below), is directly implicated (7, 54). The gla groups formed on the pro-osteocalcin prior to secretion bind calcium ions strongly ( $K_d \sim 1$  mM) and

increase the affinity of osteocalcin for bone mineral ( $K_d \sim 10^{-7}$  M). A predicted spacing of the gla groups in an  $\alpha$ -helical conformation corresponds to the 0.545 nm spacing of the calcium ions in the 001 plane of the hydroxyapatite crystal (86). Despite extensive studies, the role for osteocalcin in bone formation and remodeling is not entirely clear. Abrogation of carboxylating activity by treatment with the vitamin K antagonist warfarin reduces osteocalcin levels in bone, which becomes hypermineralized (188). A similar effect is observed in osteocalcin knockout mice (53), indicating that osteocalcin regulates mineral maturation. However, the regulation of osteocalcin by osteotropic hormones, such as 1,25-dihydroxyvitamin D<sub>3</sub> (vitamin D<sub>3</sub>) and parathyroid hormone (187), together with the ability of a carboxy-terminal segment to act as a chemoattractant to osteoclast precursors (170), also suggests a role in bone resorption (76).

Osteopontin and bone sialoprotein, originally characterized as bone sialoproteins I and II (64), are expressed in alveolar bone (39) and have been localized using immunohistochemistry (35, 89) and immunogold labeling (160). These proteins share a number of biochemical and biophysical properties that have been detailed in recent reviews (67, 215). Thus, the genes for human osteopontin and bone sialoprotein comprise 7 exons, spanning  $\sim 11.1$  kb and  $\sim 15$  kb respectively (120, 268), and are located within 340 kb on the long arm of chromosome 4 (4q21–23), which also includes the genes for dentin matrix protein 1 and dentin sialophosphoprotein (139, 140). The transcribed messenger RNA code for  $\sim 34$ -kDa proteins that include a 16-amino-acid signal peptide. Both proteins are heavily glycosylated and phosphorylated, with high levels of acidic amino acids; glutamic acid is predominant in bone sialoprotein and aspartate predominant in osteopontin. A stretch of aspartate residues in osteopontin and two to three stretches of glutamate in bone sialoprotein are implicated in hydroxyapatite binding. Both proteins also have an RGD sequence that recognizes the vitronectin receptor  $\alpha_v\beta_3$  through which these proteins can mediate cell attachment and activate cell signaling pathways. Notably, the RGD sequence in bone sialoprotein is located in the carboxy-terminal region and is flanked by several sulfated tyrosines (Fig. 5).

Despite the structural similarities (Fig. 5), these proteins have clearly different functional roles. Whereas bone sialoprotein is essentially restricted to mineralizing tissues, osteopontin has a more general distribution that reflects a broader biological role.

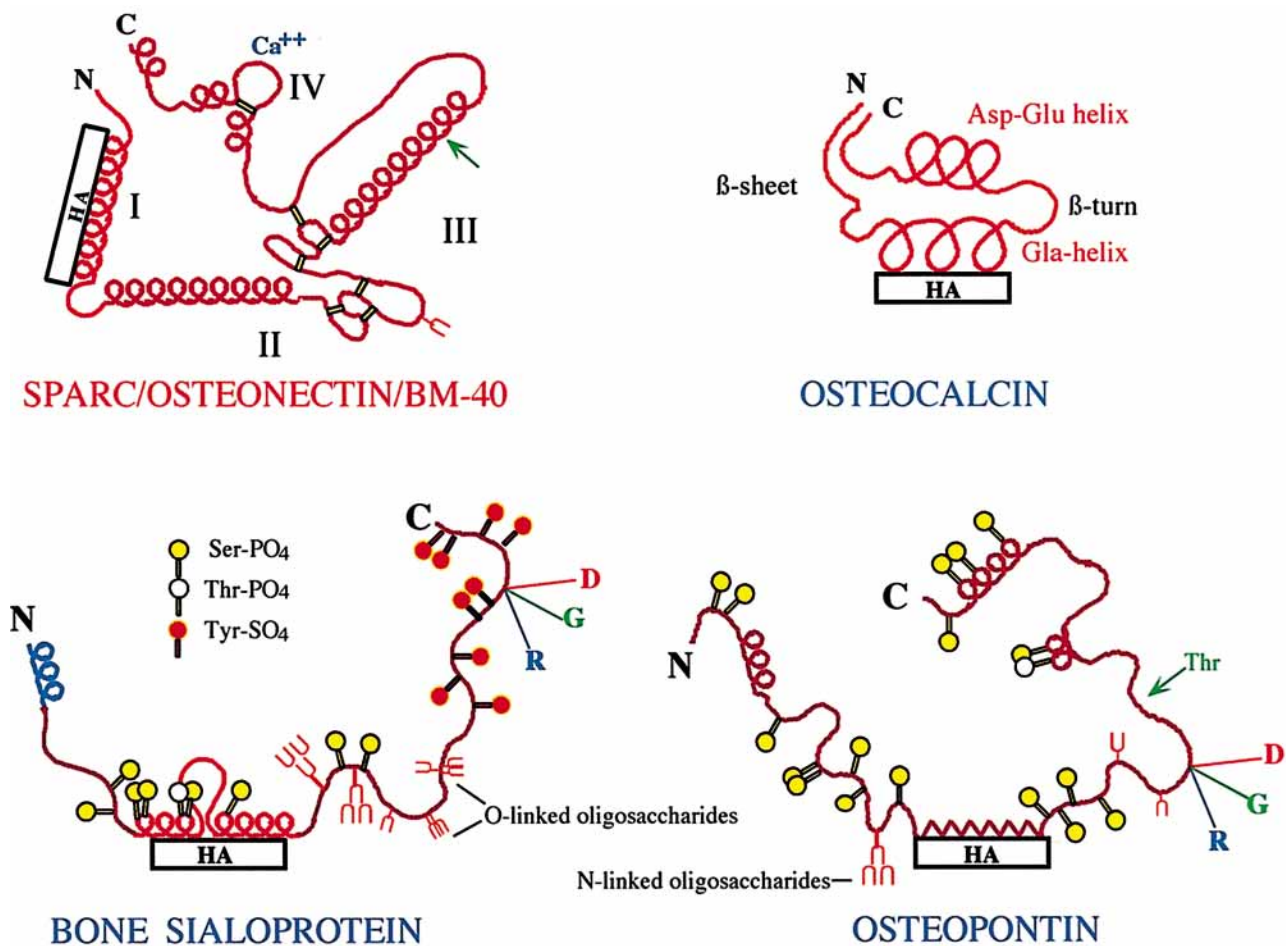


Fig. 5. Noncollagenous bone proteins. Structural diagrams of four proteins that are relatively abundant in bone are shown. Secreted protein, rich in cysteine (SPARC) protein has a compact structure stabilized with cystine bridges (yellow) and containing several regions of  $\alpha$ -helical structure (coils). Four domains have been identified: the  $\alpha$ -helical domain I binds hydroxyapatite (HA); domain II is cysteine-rich and has homology to follistatin and ovomucoid; domain III is susceptible to proteolysis (green arrow) and has an EF-hand high-affinity calcium-binding site in a

cystine loop structure. The small osteocalcin molecule forms two  $\alpha$ -helical sections; the gla helix containing the  $\gamma$ -carboxyglutamyl groups interacts with mineral crystals (HA). Bone sialoprotein and osteopontin are chemically similar molecules with an open flexible structure. Both are highly glycosylated and phosphorylated, with mineral (HA) and cell-binding (RGD) sites. In bone sialoprotein, a number of sulfated tyrosines surround the RGD while osteopontin has a thrombin (Thr) sensitive site (arrow) near the RGD.

Similar to blood clotting factors, osteopontin is also susceptible to thrombin, indicative of an origin in the blood or blood-forming organs. Thrombin digestion occurs close to the RGD sequence and generates two large fragments with altered biological activities. Since bone sialoprotein is expressed coincident with the first appearance of mineral crystals in cementum and bone (37, 39, 141) and is also able to nucleate hydroxyapatite crystal formation *in vitro* through the polyglutamate sequence (101), it is thought to function in the initiation of mineral crystal formation *in vivo*. However, since no definitive effects on mineralization have been reported in bone sialoprotein knockout mice (4), the function(s) of this protein

have yet to be clearly defined. In contrast, osteopontin is a potent inhibitor of hydroxyapatite crystal growth (78) and is enriched at all cell-matrix interfaces (159) where it can mediate the attachment of bone cells, including osteoclasts. Although there is no obvious effect on bone in osteopontin knockout mice (134, 194), recent studies have shown impaired osteoclast development and activity (266) that is compensated for in normal animals by an increase in osteoclast numbers. Human gene promoters for bone sialoprotein and osteopontin have been cloned and the major sites of transcriptional regulation determined (90, 120). Similar to osteocalcin, osteopontin transcription is strongly up-regulated by vitamin

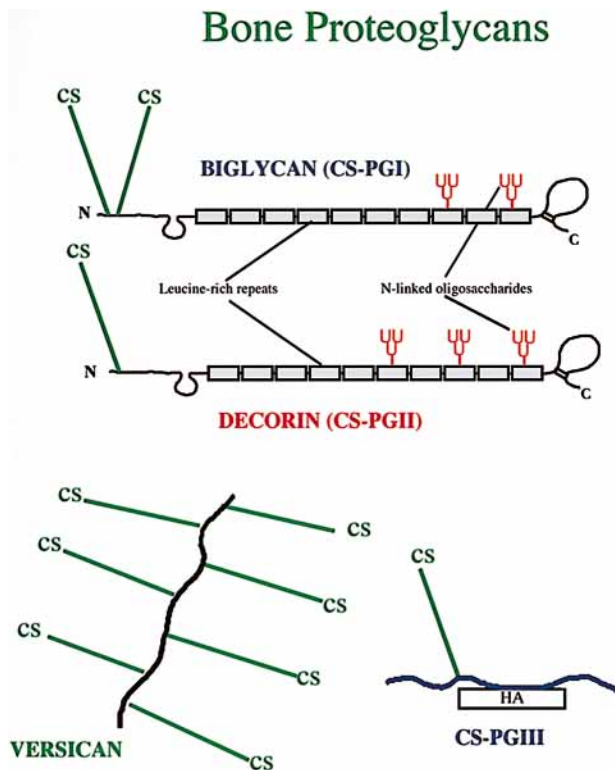


Fig. 6. Bone proteoglycans. Diagrammatic representation of the major proteoglycans in bone matrix are shown. All are characteristically chondroitin sulfate proteoglycans (CS-PGs) with one or more chondroitin sulfate glycosaminoglycan side chain (green) attached to a single protein core. Biglycan and decorin belong to a family of small leucine-rich proteins and have similar structure; the leucine-rich segments shown as boxes. At the C-terminus are N-linked polysaccharides and a cystine bridge (yellow) linking a terminal loop. The protein core of chondroitin sulfate proteoglycan III is acidic and binds to bone mineral crystals (HA), but its structure is unknown. The large versican-like molecule is present in the unmineralized bone matrix.

D<sub>3</sub> (189) and core binding factor a1 (205), whereas bone sialoprotein transcription is suppressed by vitamin D<sub>3</sub>. The expression of both proteins is stimulated by factors that stimulate bone formation, such as transforming growth factor- $\beta$  family members and glucocorticoids, consistent with the role of bone sialoprotein in bone formation and the dual role of osteopontin in bone formation and resorption (214).

SPARC/osteonectin, a 40-kDa glycoprotein that is predominantly bound to hydroxyapatite, was one of the first proteins to be isolated from bone by combined dissociative extraction and demineralization (234). While SPARC can comprise as much as 25% of the non-collagen proteins, levels of SPARC in rodent mineralized tissues are low, questioning the biological significance of this protein in the bone matrix

(271). SPARC, which has also been characterized in basement membranes as BM40, is a secreted calcium-binding glycoprotein (Fig. 5) that interacts with a range of extracellular matrix molecules (214, 129). It is widely expressed during embryogenesis, and *in vitro* studies have suggested roles in the regulation of cell adhesion and proliferation, and in the modulation of cytokine activity. SPARC can bind thrombospondin 1 and collagen, although the collagen-binding site is cryptic, lying between two  $\alpha$ -helices and exposed by proteolysis (203). SPARC has both a high-affinity EF-hand calcium-binding site and a number of low-affinity calcium-binding sites concentrated towards the amino-terminus. The low-affinity calcium binding involves glutamate and aspartate groups and produces a conformational change promoting an  $\alpha$ -helical structure (57). This region (domain I), which is the least conserved, also appears to be responsible for mineral binding, resulting in its presence in mineralized tissues, and an ability of SPARC to inhibit hydroxyapatite crystal growth (197). The second domain (domain II) of SPARC is rich in cysteines, which stabilize the protein structure through disulfide bridges, and has homology to ovomucoid and follistatin (129). Domain III is  $\alpha$ -helical and is susceptible to proteolysis, while domain IV contains the EF-hand high-affinity calcium binding site (57). Although the function of SPARC has not yet been determined, its ubiquitous expression and association with rapidly remodeling tissues (201) is indicative of a fundamental biological role (129); a view supported by its conserved structure and expression in lower organisms including *C. elegans* (210). SPARC has been characterized as a counteradhesive protein that modulates interactions of cells with the extracellular matrix (172). Recent studies have indicated a role for SPARC in early development and that it has a signaling function, which may involve effects on the nuclear matrix following endocytosis and transportation to the nucleus (80). Human SPARC, which is on chromosome 5, has 10 exons that code for a  $\sim$ 300-amino-acid nascent protein that includes a 17-amino-acid signal sequence. The mammalian gene promoter lacks TATA and CAAT boxes, but contains overlapping SP1 sequences and proximal promoter purine-rich GGA-box repeats (GGA-box 1 and 2) that are required for maximal expression. These are separated by a pyrimidine-rich spacer element that acts as a repressor (84). Mice deficient for SPARC develop normally without obvious bone defects, but show severe age-onset cataract formation and disruption of the lens (74).

The major proteoglycans in bone, including alveolar bone (11, 248), are characteristically expressed with chondroitin sulfate side chains, reflecting the lack of an epimerase activity in osteoblastic cells that converts glucuronic acid into iduronic acid found in dermatan sulfate. Notably, dermatan sulfate forms of proteoglycan are expressed by undifferentiated bone cells (17), indicating that the epimerase activity can be used as a differentiation marker for osteogenesis. Biochemical studies of rabbit alveolar bone have shown that mineral-binding proteoglycans contain chondroitin 4-sulfate, chondroitin 6-sulfate and dermatan sulfate, whereas collagenous matrix-associated proteoglycans showed a predominance of dermatan sulfate with a trace of chondroitin 4-sulfate and no detectable chondroitin 6-sulfate or unsulfated chondroitin (225).

A large 1000-kDa chondroitin sulfate proteoglycan, that is similar to versican, has been extracted from the non-mineralized bone matrix, while two small proteoglycans, biglycan (chondroitin sulfate proteoglycan I) and decorin (chondroitin sulfate proteoglycan II), are found predominantly in ethylenediaminetetraacetic acid-extracts of bone (60), while a third small proteoglycan (chondroitin sulfate proteoglycan) is entirely associated with the mineral crystals (Fig. 6). Decorin is a member of an expanding group of widely distributed small leucine-rich proteoglycans that are expected to have important functions in tissue assembly. Biglycan and decorin, which exist as dermatan sulfate proteoglycans in soft connective tissues, belong to a group of small leucine-rich repeat proteins and have significant structural homology that is present within four domains (104). Thus, both proteoglycans have glycosaminoglycans attached at the amino terminus (domain I) with conserved disulfide bridges at each end of the molecule (domains II and IV). In domain III are 10 homologous 25-amino-acid leucine-rich repeats through which these small leucine-rich repeat proteins can interact with collagen and growth factors. Together, decorin and biglycan can comprise <10% of the non-collagen proteins in bone, but this decreases with maturation of the bone.

Biglycan ( $M_r$  ~350 kDa) has a 46-kDa protein core with two chondroitin sulfate chains of ~150 kDa each, attached near the amino terminus. Biglycan is more prominent in developing bone and has been localized to pericellular areas. Although mice with a targeted disruption of the biglycan (*Bgn*) gene appear normal at birth, they develop a phenotype characterized by a reduced growth rate and decreased bone mass (260). While the precise function

of biglycan is unknown, similar to decorin, it can bind transforming growth factor- $\beta$  and extracellular matrix macromolecules including collagen and thereby regulate fibrillogenesis. The human biglycan gene, which is localized to the end of the long arm of the X chromosome, has a promoter that lacks both a CAAT and TATA box but is rich in guanosine + cytosine content and has many Sp1 sites (61).

Decorin ( $M_r$  ~120–200 kDa) also has a protein core of approximately 46 kDa with a single chondroitin sulfate chain of variable size attached at the amino terminus. Decorin is known to bind within the gap region of collagen fibrils (211) and, as suggested by its name, decorates the fibril surface. Mice harboring a targeted disruption of the decorin gene are viable but have fragile skin with markedly reduced tensile strength due to abnormal collagen fibers that form with uncontrolled lateral fusion, thereby demonstrating a fundamental role for decorin in regulating collagen fiber formation (45). Of note, the primary calcification in bones is reported to follow removal of decorin and the fusion of collagen fibrils (96). Decorin has also been shown to bind strongly to the cytokine transforming growth factor- $\beta$  and regulate its activity. Human decorin, which is located on chromosome 12 (247) has a high-expression promoter that contains a CAAT and two operative TATA boxes that are in close proximity to the transcription start site (202). Increased transcription is observed in association with an upstream region that contains sites for transforming growth factor- $\beta$  and nuclear factor  $\kappa$ B regulation.

The mineral-bound chondroitin sulfate proteoglycan (molecular weight ~110 kDa) has a protein core of ~35 kDa that is stained with "Stains-All", but not with Coomassie blue, a characteristic of several of the acidic bone proteins (77). Although the protein appears to be bone-specific, to date it has only been identified in porcine bone. Since the sequence of the 12-amino terminal amino acids is identical with BAG-75, a sialoprotein identified in rat bone (81) and dentin matrix protein-1, an acidic glycoprotein isolated from dentin (71), it is possible that chondroitin sulfate proteoglycan may be a proteoglycan form of these proteins (Goldberg & Sodek, unpublished observations). Recent studies have also identified a bone-specific 85-kDa keratan-sulfate proteoglycan with a 46-kDa protein core in bovine bone called osteoadherin that displays both mineral- and cell-binding properties (218, 256). Osteoadherin also has characteristics of small leucine-rich repeat proteins with expression and distribution patterns similar to bone sialoprotein.

Lysyl oxidase and tyrosine-rich acidic matrix protein are prominent components of the demineralized bone (50) and dentin (49) matrix. While lysyl oxidase is known to be a critical enzyme for collagen cross-linking, tyrosine-rich acidic matrix protein (TRAMP) is a recently discovered 22-kDa (183 amino acids with a 18-amino-acid signal sequence) extracellular matrix protein with proteoglycan and cell-binding properties (63) that is located on human chromosome 1. Tyrosine-rich acidic matrix protein, which is also known as dermatopontin (175, 223), binds decorin and transforming growth factor- $\beta$ , and together these proteins can regulate the cellular response to transforming growth factor- $\beta$ .

Other proteins that are found in bone include procollagen peptides (79), thrombospondin, fibronectin and vitronectin, proteins that modulate cell attachment and the enzyme alkaline phosphatase, which is important for mineralization to occur (47). Of the proteins that are not produced by osteoblasts but accumulate in bone, matrix gla protein and  $\alpha$ 2HS-glycoprotein (bovine fetuin) are of particular interest with respect to regulation of mineralization. Although a clear bone phenotype was not evident in either MGP-null (138) or  $\alpha$ 2HS-glycoprotein-null (105) mice, definitive effects on ectopic mineralization were evident, indicative of a regulatory role in bone similar to that observed with osteocalcin. Matrix gla protein is a mineral-binding extracellular matrix protein synthesized by vascular smooth-muscle cells and chondrocytes that prevents mineralization in vascular tissues and cartilage as indicated in matrix gla protein-deficient mice. The absence of  $\alpha$ 2HS-glycoprotein, which is produced by the liver, compromises the inhibition of apatite formation by serum.

## Ultrastructural organization

The organic matrix of bone serves a major biomechanical function in housing the solid, inorganic calcium-phosphate mineral phase of bone. A detailed discussion of collagen-mineral relationships in calcified tissues has recently been described (127) in which the mineral forms thin, plate-like crystallites having an ordered orientation within the collagen fibrils. The structural organization and functional contributions of mineralized collagen fibrils have been well studied (75, 128, 240, 246). Other matrix-mineral relationships exist between the collagen fi-

brils, and these are discussed below. The overall organization of alveolar bone is far more complex than the more extensively studied diaphysis of long bones whose properties are based primarily on the ordered, longitudinal arrangement of Haversian systems (osteons). The geometrical complexity of multi-rooted teeth within the alveolar bone, and the response of these tissues to mastication and other unique forces within the oral cavity, results in an intricate pattern of bone remodeling that does not involve classic Haversian remodeling. Continuous accommodation of remodeling Sharpey's fibers further complicates the remodeling pattern (124).

Extracellular matrix in bone can be arbitrarily and broadly divided into several spatially distinct compartments that conceptually allow for a useful description of the sequence of events comprising the synthesis, secretion, accumulation and mineralization of bone matrix (Fig. 2) (155). Following synthesis and release of organic molecules by the cellular compartment (osteoblasts), a collagenous stroma called the osteoid is formed that ultimately acts as a scaffolding for apatite mineral deposition and the accumulation of noncollagenous and plasma proteins and proteoglycans. These latter events occur predominantly at what is known as the mineralization front – a site where mineralization propagates extensively throughout and between the collagen fibrils. Although the first mineral to appear may be found at small, discrete foci within the osteoid seam, the precise nucleation sites of this, and the subsequent deposition of more confluent mineral at the mineralization front of the mineralized bone matrix proper (the mineralized bone compartment), remain controversial (43).

Regarding the precise location and distribution of noncollagenous proteins and proteoglycans in bone, it has long been known that, at the light microscopic level in decalcified sections of this tissue, certain general protein stains (such as toluidine blue) show a particularly strong affinity for bone matrix commencing at the mineralization front and extending throughout the mineralized bone matrix compartment. This contrasts with a relatively light staining of the adjacent osteoid. Similarly, using heavy metal stains such as uranyl acetate in conjunction with transmission electron microscopy, there is an increased electron density associated with originally mineralized portions of the matrix in decalcified sections (153). Notably, a similar phenomenon can be observed as pre-dentin is converted to dentin in the developing tooth (252). This is currently interpreted as representing

the accumulation of various noncollagenous proteins at these mineralized sites. An additional level of molecular organization may also occur through the aggregation of noncollagenous proteins, some of which can be cross-linked through the action of tissue transglutaminase (117, 118).

With recent developments in the molecular characterization of individual bone proteins, and with the production of specific antibodies to these purified molecules, it has been possible to localize these proteins with high resolution *in situ* using ultrastructural immunocytochemical techniques (Figs. 3, 4). Thus, immunocytochemical studies have been able to identify components of the organic material described above and to reveal its ultrastructural association with sites of mineralization throughout the tissue (18, 29, 36, 157, 158, 214). What has been more difficult to achieve, however, is a determination of the spatio-temporal sequence by which these proteins accumulate and associate with the mineral phase; that is, whether the proteins accumulate prior to, concomitant with or after mineral nucleation. Nevertheless, the co-localization of certain noncollagenous proteins with mineral is a necessary prerequisite for determining their participation in the mineralization process. Conversely, proteins not found at these sites can be considered as not having a direct effect on mineralization. Noncollagenous proteins typically found at sites of mineralization include bone sialoprotein and osteopontin (Fig. 4). The temporal expression of bone sialoprotein and osteopontin with respect to mineralization (214, 262) and their ability to respectively nucleate and inhibit the growth of hydroxyapatite crystals *in vitro* (21, 101, 102) is indicative of their regulatory roles on calcification. Other noncollagenous proteins secreted by bone cells and plasma-derived proteins such as albumin and fetuin are also distributed throughout the bone matrix and may compete and/or participate in mineralization processes (155, 208). Notably, osteopontin is a prominent protein at cement lines in alveolar bone and elsewhere (159), and here most likely plays a role in cell adhesion and the various extracellular matrix events that integrate newer bone with older bone during the remodeling cycle (158). Osteopontin also lines the lacunae of alveolar bone osteocytes and the canalicular walls, and here most likely functions in cell-matrix adhesion (159). Similarly, ultrastructural immunocytochemical studies on human alveolar bone proteoglycans have localized chondroitin sulfate-rich proteoglycans to osteocyte lacunae and canaliculi (213).

## Formation, maintenance and regeneration of alveolar bone

Both the mandibular and maxillary jaw bones develop from the first branchial arch under the direction of homeobox genes that are expressed in a temporo-spatial manner and have a central role in skeletal pattern formation (58). Alveolar bone comprises the alveolar process, which is an extension of the basal bone of the jaws. The alveolar bone forms in relation to the teeth (121) but structurally it is similar to, and continuous with, the basal bone. Paracrine factors, including cytokines, chemokines and growth factors, which have been implicated in the local control of mesenchymal condensations that occur at the onset of organogenesis, are likely to have a prominent role in the development of the alveolar processes. While the growth and development of the jaw bones determines the position of the teeth, a certain degree of re-positioning of teeth can be accomplished through occlusal forces and in response to orthodontic procedures that rely on the adaptability of the alveolar bone and associated periodontal tissues. Consequently, understanding the molecular events that regulate the formation and remodeling of bony tissues is of fundamental importance in the development of rational treatment modalities to circumvent or correct structural and functional anomalies.

## Physiological remodeling of alveolar bone

Complete remodeling of the alveolar bone occurs when the primary dentition is replaced by succedaneous teeth. The alveolar bone associated with the primary tooth is completely resorbed together with the roots of the tooth while new alveolar bone is formed to support the newly erupted tooth. Significant remodeling of the alveolar process also occurs as part of this process. The ability of the alveolar bone to remodel rapidly (217) also facilitates positional adaptation of teeth in response to functional forces and in the physiological drift of teeth that occurs with the development of jaw bones. From a clinical perspective, the rapid remodeling of the alveolar bone facilitates movement of teeth within the jaw bone by the application of orthodontic forces. However, the application of force on bone tissues can also influence the remodeling rate. Formation of alveolar bone is a prerequisite for the regeneration of tissues lost through periodontal disease and for

osseointegration of implants used in restorative dentistry. Bone remodeling involves the co-ordination of activities of cells from two distinct lineages, the osteoblasts and the osteoclasts, which form and resorb the mineralized connective tissues of bone, respectively.

Regulation of bone remodeling is a complex process involving hormones and local factors acting in an autocrine and/or paracrine manner on the generation and activity of differentiated bone cells. While there is considerable knowledge of the kinetics of bone turnover at the cellular level, the regulation of bone remodeling at the molecular level is poorly understood. Specific factors are believed to regulate each step in the remodeling process and to integrate the development of osteoblast and osteoclasts and their activities as well as modulate control that is exerted through the endocrine system. Notably, the cellular and molecular events involved in bone remodeling have a strong similarity to many aspects of inflammation and repair, and the relationships between matrix molecules, such as osteopontin, bone sialoprotein, SPARC and osteocalcin, and blood clotting and wound healing are clearly evident. The associations between bone formation and remodeling and inflammatory response systems are further emphasized by the recent identification of "master genes" involved in the generation of osteoblasts and osteoclasts that belong to families of transcription factors with prominent roles in the development of immune responses. The regulated remodeling of alveolar bone is anticipated to follow the general principles of bone formation and resorption described below.

## Bone formation

Formation of bone, which appears to be linked with bone resorption to maintain bone mass, involves the proliferation and differentiation of stromal stem cells along an osteogenic pathway that leads to the formation of osteoblasts (Fig. 7). The process of cellular differentiation is controlled by a cascade of events that involves a combination of genetic programming and gene regulation by various hormones, cytokines and growth factors. While an understanding of the complexities of the differentiation process is still at an elementary stage, significant advances have been made in recent years in identifying regulatory genes and molecular markers that define specific stages of osteogenic cell development. Notably, matrix macromolecules have, to date, proven to be the best devel-

opmental markers, particularly for the later stages of differentiation (132, 262). Although stromal stem cells have yet to be isolated from osteogenic tissues, a population of small, agranular cells has been isolated from fetal rat calvaria and shown to be enriched with stem and progenitor cells (269). Following plating and attachment, these cells begin expressing collagens I, II and III as well as alkaline phosphatase and osteopontin. The alkaline phosphatase and collagen I expression are characteristic of the osteogenic lineage and their synthesis continues to increase while the expression of type II collagen is lost and type III progressively diminishes. At early stages of differentiation, the potential for entering alternate pathways of differentiation is retained, as depicted for periodontal cell differentiation (Fig. 7).

The formation of a collagen substratum appears to trigger the differentiation of pre-osteoblastic cells into osteoblasts through interactions with the  $\alpha_2\beta_1$  receptor (259). *In vivo*, this stage likely follows the condensation of mesenchymal cells. The subsequent emergence of osteoblasts is indicated by the induced expression of bone sialoprotein, which correlates with the initiation of mineralization *in vitro* (262) and *in vivo* (37). In comparison, the expression of osteopontin is variable and is complicated by its presence in migrating fibroblastic cells (270) and its production by cement-forming cells (262). Nevertheless, osteopontin generally declines prior to osteoblastic differentiation and together with osteocalcin is expressed at high levels after mineralization has been initiated, with high levels being maintained as osteoblasts differentiate further into osteocytes and lining cells.

Expression of developmentally regulated genes and transcription factors that regulate the expression of differentiation-associated genes appear to be the most useful for defining the early stages of osteodifferentiation. Many of the developmental genes, including homeobox genes such as *hoxa-2*, *hoxd-13* and *hoxa-13*, *dlx5*, *msx-1* and *msx-2*, are common to various forms of organogenesis. Similarly, different classes of transcription factors involved in osteogenesis have broad targets of regulation. However, recent studies have identified a runt domain-related gene core binding factor a1/PEBP2 $\alpha$ A/AML-3 as a bone-restricted transcription factor (54, 163) that has been described as a potential "master gene" for osteogenic differentiation (195). Expression in developing odontoblasts, cementoblasts and ameloblasts indicate that core binding factor a1 may also have a functional role in the differentiation of all mineralizing tissue cells (111). Deletion of the core binding

## Cellular Differentiation in Periodontal Tissues

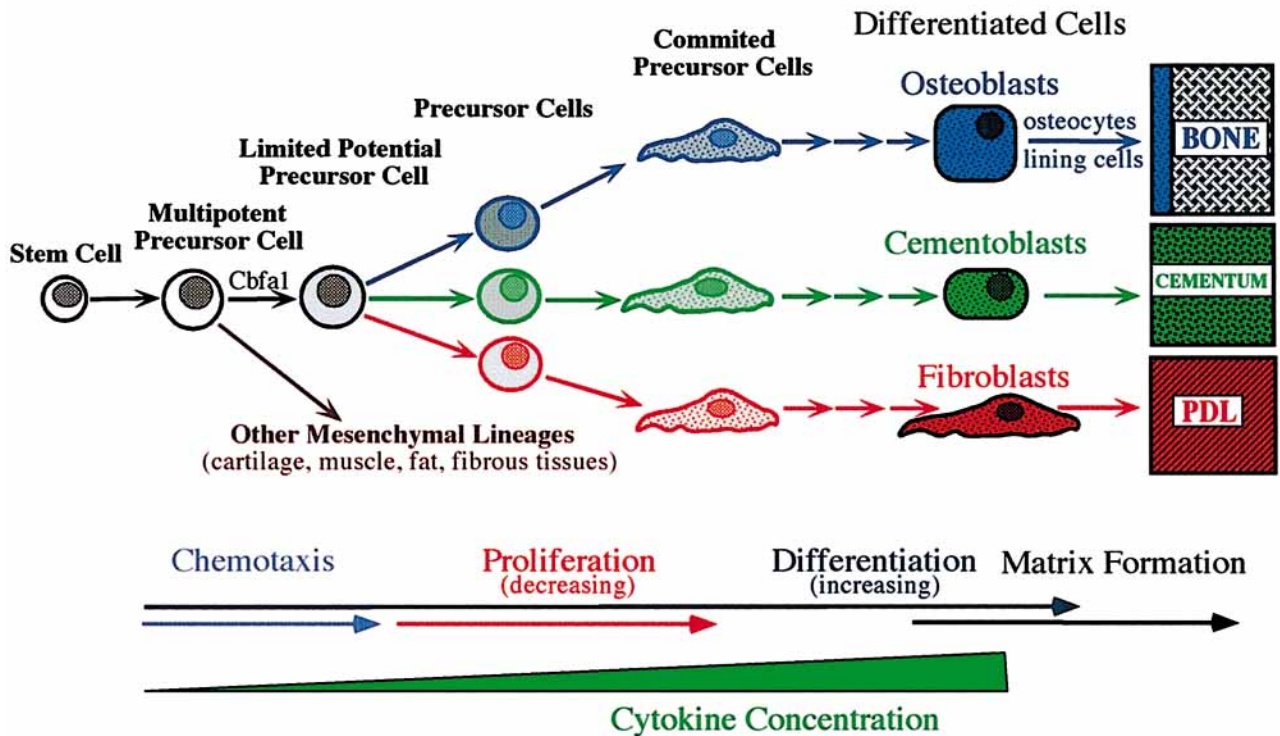


Fig. 7. Osteogenic differentiation. A schematic model of the events believed to be involved in osteogenic differentiation is shown in the context of a multipotent stem cells in the periodontium. Several lineages can potentially be generated including the osteogenic lineage. In each case a decrease in proliferation potential occurs as differentiation progresses. Expression of the “osteogenic master

gene” core binding factor a1 (cbfa1) is likely a key event that directs multipotent cells into the osteoblast, cementoblast and periodontal ligament fibroblast pathways. The physical environment as well as growth factors and cytokines can influence the progression along each lineage until the fully differentiated cell is generated. PDL: periodontal ligament.

factor a1 gene results in the complete absence of ossified tissues (122) and heterozygous mutations of the gene in humans (171) and mice (176) cause cleidocranial dysplasia syndrome, in which the development of supernumerary teeth in humans is a characteristic. Studies *in vitro* have shown that bone morphogenetic proteins act upstream of core binding factor a1 (54). Thus, bone morphogenetic protein-2 treatment of the myogenic cell line C2C12 transiently upregulates core binding factor a1 and Msx-2 leading to osteogenic differentiation. Notably, while transforming growth factor- $\beta$  can also increase core binding factor a1 and suppresses myogenic differentiation in these cells, osteogenic differentiation does not occur, indicating that other factors induced by bone morphogenetic proteins are necessary for complete expression of the osteoblastic phenotype

(130). A potential factor is the homeobox-containing gene *dlx5* which regulates osteoblast differentiation and has been shown to be induced by bone morphogenetic protein-4 (167). Treatment of undifferentiated cells with bone morphogenetic protein-7/OP-1, which has been shown to signal through Smad 5 (231), has also identified (229) a non-translated RNA, bone morphogenetic protein/OP-1 responsive gene, and a novel zinc finger transcription factor, AJ-18 (110), as immediate targets of bone morphogenetic protein-7, in osteogenic systems. However, the functional attributes of these target genes have yet to be determined. Of note, recent studies indicate that the transcriptional activities of Smads and core binding factor a1 may be linked by the formation of regulatory complexes which may include other transcription factors (85).

## Regulation of bone formation

Bone formation is regulated by factors that affect either the production of osteoblastic cells or their activity. Many of these factors also effect bone resorption either directly or indirectly. Thus, parathyroid hormone, which regulates serum calcium levels by stimulating bone resorption, can also have anabolic effects *in vivo* that appear to be mediated through transforming growth factor- $\beta$  and insulin-like growth factor-I (31). Such opposing effects of parathyroid hormone are consistent with the apparent coupling of bone formation and remodelling. The seco-steroid vitamin D<sub>3</sub> also has paradoxical effects in bone remodeling (48). While stimulating bone resorption, it is essential for normal bone growth and mineralization and has a primary function in calcium absorption from the intestine. Vitamin D<sub>3</sub> also strongly stimulates the synthesis of osteocalcin and osteopontin by osteoblastic cells while suppressing collagen production. In contrast, insulin and growth hormone have anabolic effects on bone. Insulin targets osteoblasts directly, stimulating bone matrix formation and mineralization, and indirectly affects bone formation through a stimulation of insulin-like growth factor-I produced in the liver. Growth hormone is required for attaining normal bone mass, the anabolic effects apparently being mediated through the local production of insulin-like growth factor-I (32). As with other hormones, the effects of glucocorticoids are complicated by secondary effects initiated in response to the primary effects (46). Thus, the ability of glucocorticoids to promote differentiation of osteoblastic cells and to stimulate bone matrix formation has been well established *in vitro*. However, prolonged treatment with glucocorticoids *in vivo* results in bone loss, which can be attributed to increased parathyroid hormone production in response to the inhibitory effects of glucocorticoids on calcium absorption and perhaps also to the depletion of osteogenic precursor cells. Thyroid hormone (8) and the sex steroids (112) are also necessary for normal growth and development of bones, but they appear to act indirectly and the mechanisms are poorly defined. Thus, thyroid hormone affects endochondral bone formation through its action on cartilage formation, while the manner whereby estrogens aid in maintaining bone mass through anabolic effects on bone has yet to be established.

Of the many growth and differentiation factors that influence bone formation, the bone morphogenetic proteins have the most profound effect on

bone formation (257). These cytokines, which belong to the transforming growth factor- $\beta$  family, can induce chondrogenic and osteogenic differentiation in undifferentiated mesenchymal cells, their prolonged presence being required to generate endochondral bone in ectopic sites (192). However, bone morphogenetic proteins do not have marked effects on bone matrix formation (131). In contrast, transforming growth factor- $\beta$  can act as a potent inhibitor of osteogenic induction by bone morphogenetic protein (34) while strongly stimulating expression of matrix proteins by osteoblastic cells (258). The anabolic effects of transforming growth factor- $\beta$  are augmented by a suppression of matrix degradative activity through the inhibition of matrix metalloproteinase expression and the enhanced expression of tissue inhibitor of matrix metalloproteinases (177). The insulin-like growth factors (I and II) are also potent anabolic agents in bone, having effects similar to transforming growth factor- $\beta$  on matrix proteins and matrix metalloproteinases, but insulin-like growth factors also stimulate proliferation of osteoblast precursors (93, 152). The acidic, and particularly the basic, fibroblast growth factors, which are characteristically expressed early in skeletal development, exert their effects on bone formation primarily through increased proliferation of osteoprogenitors and promotion of osteogenic differentiation (30). Platelet-derived growth factor has similar effects to fibroblast growth factors in promoting osteogenesis, but also influences the expression of other cytokines as part of a more generalized role that platelet-derived growth factor has in wound and fracture healing (92).

## Bone resorption

Resorption of mineralized tissues requires the recruitment of a specialized cell, the osteoclast, which is produced by the monocyte/macrophage lineage of hematopoietic cells that are derived from bone marrow (10). The stages in the life cycle of the osteoclast are summarized in Fig. 8. Osteoclasts develop from a pluripotential mononuclear precursor (colony-forming unit-granulocyte/macrophage) which is stimulated to proliferate and differentiate under the influence of monocyte-macrophage colony-stimulating factor. A variety of soluble and membrane-bound factors play a critical role in regulating osteoclast formation, including growth factors, systemic hormones, and cells in the marrow microenvironment, such as osteoblasts and marrow stromal cells.

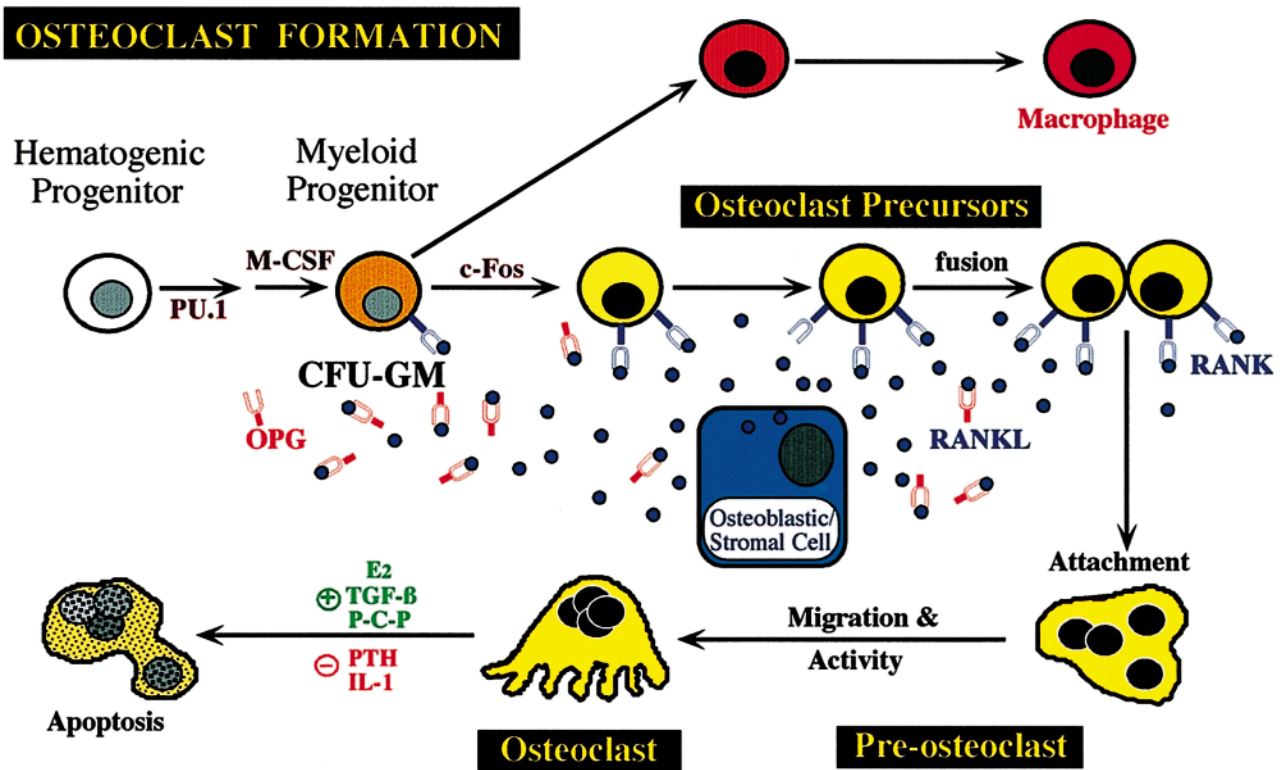


Fig. 8. Osteoclastic differentiation. Osteoclasts are formed from a haematogenic precursor cell which generates a progenitor of the granulocyte/macrophage (CFU-GM) lineage under the influence of the PU.1 gene and M-colony-stimulating factor. The myeloid progenitor requires a functional *c-Fos* gene to differentiate along the osteoclast pathway which is regulated by the receptor activator of nuclear factor  $\kappa$ B (RANK)/receptor activator of nuclear factor  $\kappa$ B ligand (RANKL) receptor/ligand system. Receptor activator of nuclear factor  $\kappa$ B ligand, also known as osteoprotegerin ligand produced by stromal bone cells binds to receptor activator of nuclear factor  $\kappa$ B receptors on the pre-osteoclasts and promotes osteoclast differentiation

while the truncated, soluble form of receptor activator of nuclear factor  $\kappa$ B, osteoprotegerin (OPG), can bind receptor activator of nuclear factor  $\kappa$ B ligand and block signaling, thereby preventing osteoclast differentiation. Pre-osteoclasts form by fusion of precursors and following attachment to the bone surface become active osteoclasts. The survival and activity of the osteoclast is dependent upon factors such as estrogen (E), transforming growth factor- $\beta$  (TGF- $\beta$ ) and bis-phosphonates (P-C-P) which promote osteoclast apoptosis, while parathyroid hormone (PTH) and interleukin-1 (IL-1) block osteoclast apoptosis.

Cell-to-cell interactions are important in both the formation and activity of the osteoclast. Recent molecular biological studies have identified transcription factors, such as *c-Fos* and PU.1, that are required for osteoclast differentiation (23). In the *op/op* mouse, in which there is impaired production of colony-stimulating factor-1 (CSF-1), osteoclasts fail to form leading to osteopetrosis (265). Similarly, an osteopetrotic phenotype resulting from ablation of *c-Fos* (83), both the p50 and p52 sub-units of nuclear factor  $\kappa$ B (65), and PU.1 (237) has revealed the importance of these genes in the early development of osteoclasts. The expression of PU.1, a myeloid and B cell-specific transcription factor, is progressively increased as marrow macrophages differentiate into osteoclasts and also with the induction of osteoclastogenesis by 1,25-dihydroxyvitamin D<sub>3</sub>. Since ab-

lation of the PU.1 gene blocks the formation of both macrophages and osteoclasts, it is believed to act early in the differentiation pathway. Similarly, colony-stimulating factor is required for myeloid precursor development while the formation of macrophages in *Fos*-null mice indicates that this transcription factor acts early in the osteoclast pathway (Fig. 8). Mutation of another transcription factor, the microphthalmia-associated transcription factor, in *mi/mi* mice has revealed its association with *c-Fos* and PU.1 in the regulation of osteoclast development (206). While the mutated microphthalmia-associated transcription factor can still bind to *c-Fos* and PU.1, it blocks their nuclear translocation, causing osteopetrosis.

The identification of a novel receptor, termed osteoprotegerin (OPG) (212, 264), has recently un-

covered a key regulatory mechanism in osteoclast differentiation and activity (Fig. 8). The osteoprotegerin ligand (OPGL), which has been identified (125) as the putative osteoclast differentiation factor that is expressed on the surface of stromal/bone cells (221), has been shown to signal osteoclast differentiation through a tumor necrosis factor- $\alpha$ -related receptor known as either tumor necrosis factor receptor (TNFR), osteoclast differentiation and activation receptor (ODAR) or receptor activator of nuclear factor  $\kappa$ B (RANK). Osteoprotegerin, itself, acts as a decoy receptor that binds ligand but is incapable of signaling. Thus, while ablation of the osteoprotegerin gene in transgenic mice results in excessive production of osteoclasts and osteoporosis (28, 169), overexpression of osteoprotegerin leads to impaired osteoclast formation and osteopetrosis (212). A similar impairment in osteoclast development is observed in osteoprotegerin ligand knock-out mice (123) and in nuclear factor  $\kappa$ B knockout mice (65, 103). From these observations it is evident that signaling by receptor activator of nuclear factor  $\kappa$ B-ligand through the receptor activator of nuclear factor  $\kappa$ B receptor, which is regulated by colony-stimulating factor-1, is a crucial step in osteoclast formation. Moreover, the involvement of the osteoprotegerin-osteoprotegerin ligand regulatory pathway in the immune system (2) and the calcification of arteries in osteoprotegerin-deficient mice (28) provides further links between the hematopoietic system and bone and provides a connection between vascular calcification and osteoporosis (6).

Typically, formation of osteoclasts involves fusion of monocytic precursors which occurs at the site of bone resorption. The hyaluronan receptor CD44 is upregulated during this process and is required for fusion to take place (220). Together with CD44, the  $\alpha_v\beta_3$  integrin is highly expressed in osteoclasts and osteoclast precursors; both receptors being a primary target for the bone matrix protein osteopontin in signaling, cell attachment and also possibly for osteoclast chemotaxis (haptotaxis) and migration (215). On the bone surface, osteoclasts become polarized and form a ruffled border beneath which bone resorption takes place (19). While the molecular mechanisms involved are not known, cell attachment and recruitment of Src appear to be crucial events, as evidenced by an osteopetrotic phenotype in Src knockout mice (219). Demineralization of the bone matrix, which is a prerequisite for matrix degradation, is achieved through the acidification of a protected environment beneath the ruffled border. A specific type of electrogenic adenosine triphos-

phatase (33) pumps protons, generated by type II carbonic anhydrase activity, into the resorption bay, which also receives lysosomal enzymes and thereby acts as a functional secondary lysosome. Following the dissolution of the mineral phase in the acidic environment, the lysosomal enzymes can degrade matrix macromolecules, including collagen, in a manner similar to that described for the phagocytic degradation of matrix. Matrix metalloproteinases, which can be activated under the acidic conditions, have also been observed in resorption lacunae and could contribute to matrix degradation (216). Following resorption, osteoclasts may undergo apoptosis, which provides a mechanism for limiting resorptive activity (98). While factors such as transforming growth factor- $\beta$ , estrogen and bis-phosphonates promote apoptosis, parathyroid hormone and interleukin-1 act as suppressors, prolonging osteoclast activity. Thus, the formation, activity and survival of osteoclasts are all potential targets for regulation of osteoclast-mediated bone-resorptive activity (Fig. 8).

## Regulation of osteoclast activity

The primary factors that stimulate bone resorption through osteoclasts include parathyroid hormone, vitamin D<sub>3</sub>, interleukin-1, interleukin-6, tumor necrosis factor  $\alpha$ , and transforming growth factor- $\alpha$ , whereas calcitonin, transforming growth factor- $\beta$ , estrogen and interferon- $\gamma$  inhibit osteoclastic bone resorption. While osteoclasts have receptors for calcitonin and estrogen, as well as for most cytokines, vitamin D<sub>3</sub> (198, 242) and parathyroid hormone (161) affect osteoclasts indirectly through receptors on pre-osteoblasts, osteoblasts and lining cells. Although several cytokines have been identified as mediators of vitamin D<sub>3</sub> and parathyroid hormone, the mechanism of regulation has been poorly understood. Parathyroid hormone, parathyroid hormone-related protein, vitamin D<sub>3</sub>, transforming growth factor- $\alpha$  and pro-inflammatory cytokines, such as interleukin-1 and tumor necrosis factor  $\alpha$ , all promote differentiation of osteoclasts (193). The pro-inflammatory cytokines can act through the OPG/OPGL/RANK regulatory pathway (65, 150) which may be a key target of factors that affect osteoclast generation and activity. Similarly, parathyroid hormone and vitamin D<sub>3</sub> have recently been shown to regulate osteoclast development through the osteoprotegerin/osteoprotegerin ligand/receptor activator of nuclear factor  $\kappa$ B pathway (95). Interleukin-6 is

also produced by osteoblastic cells in response to parathyroid hormone and vitamin D<sub>3</sub> and is a prominent cytokine produced by osteoclasts. Although interleukin-6 has important effects on bone remodeling and has been implicated in bone resorption associated with estrogen deficiency, it is a much less potent stimulator of osteoclast generation than interleukin-1 and tumor necrosis factor  $\alpha$  (112). Arachidonic metabolites are also important modulators of bone cell function. In particular, prostaglandins of the E-series can act as powerful mediators of bone resorption and can also influence bone formation (44). The prostaglandins exert a local effect on osteoclasts and their precursors, often mediating the effects of growth factors and cytokines such as epidermal growth factor and transforming growth factor- $\beta$ . Prostaglandins can also stimulate bone formation when administered systemically and the local infusion of prostaglandin E<sub>2</sub> has been used to stimulate alveolar bone formation *in vivo* (147).

Estrogen is believed to suppress the production of bone-resorbing cytokines, including interleukin-1 (178) and interleukin-6 (112), while transforming growth factor- $\beta$  (186) and interferon- $\gamma$  (226) inhibit proliferation and differentiation of committed precursors into mature osteoclasts. Calcitonin is a particularly potent inhibitor of osteoclast activity, but its effects are transient, likely due to the downregulation of calcitonin receptors on osteoclasts in the sustained presence of hormone (227). Calcitonin inhibits proliferation and differentiation of osteoclast precursors and causes cytoplasmic contraction of the cell membrane in mature osteoclasts and their dissociation into monocytic cells.

In addition to their effects on osteoclast development, interleukin-1, tumor necrosis factor  $\alpha$  and the functionally related, lymphotoxin, also stimulate osteoclastic activity (236). Although the mechanism is possibly indirect, involving cells of the osteoblastic lineage, which also mediate the effects of parathyroid hormone and vitamin D<sub>3</sub> on osteoclast activity, it is also possible that regulation of osteoclast activity by these cytokines and osteotropic hormones may also involve the OPG/OPGL/RANK pathway. Notably, the effects of vitamin D<sub>3</sub> can also be mediated by the bone matrix protein osteopontin, which is strongly upregulated by the seco-steroid. In *Opn*<sup>-/-</sup> mice, osteoclast formation and activity are impaired (194), and these mice are resistant to the enhanced osteoclastogenesis that typically follows ovariectomy (266) or treatment with interleukin-1 (228). Thus, ligation of osteopontin to the  $\alpha_v\beta_3$  receptor in pre-osteoclasts and osteoclasts can modu-

late osteoclast development and activity through the OPG/OPGL/RANK pathway (123). Another bone matrix protein, bone sialoprotein, can also regulate osteoclast activity through the  $\alpha_v\beta_3$  integrin (168, 199) which was originally characterized as a vitronectin receptor. Binding of ligand to the  $\beta$  component of the  $\alpha_v\beta_3$  integrin activates the focal adhesion kinase-related PYK2 (protein tyrosine kinase2) through c-Src, which binds PYK2 through the SH2 domains. Upon activation, PYK2 translocates to the Triton X-100 insoluble cytoskeletal compartment and together with p130<sup>cas</sup> is found in the sealing zone required for osteoclastic bone resorption (55, 56).

## Clinical implications

Alveolar bone is dependent upon the presence of teeth for its preservation. Consequently, the alveolar bone ridge is progressively reduced following the extraction of teeth. Maintenance of alveolar bone is also compromised following trauma and inflammatory episodes associated with periodontal disease. Preventing, or minimizing, alveolar bone loss is a major clinical objective in dentistry, and restoration of alveolar bone mass after losses have been incurred is extremely difficult to attain. The interdependence of teeth and alveolar bone make the restoration of alveolar bone more difficult than simply enhancing osteogenesis. Moreover, regeneration of bone lost through periodontal disease requires the simultaneous and coordinated regeneration of the associated periodontal tissues in an environment that is subject to ongoing inflammatory activity. While the formation of alveolar bone is more readily achieved within a sterile environment, such as that utilized for the placement of two-stage implants in prosthodontics, integration of the newly formed bone to the surface of the implant introduces an additional challenge that relates to physicochemical characteristics of the implant surface.

The ability of the alveolar bone to undergo rapid remodelling is of fundamental importance for physiological movement of teeth in the jaw that occurs in response to occlusive forces and orthodontic forces used for the clinical re-positioning of teeth. Orthodontic tooth movement is believed to occur as a consequence of site-specific bone remodeling in the absence of inflammation. It is well recognized that tensional forces will stimulate the formation and activity of osteoblastic cells while compressive forces promote osteoclastic activity. However, the

molecular mechanisms that transduce the signals generated by forces are only beginning to be identified. At sites of alveolar bone compression, osteoclasts proliferate and an initial resorption of the superficial bone is believed to take place. The initial response may involve osteoblasts, which can produce collagenolytic enzymes to remove a portion of the unmineralized extracellular matrix, thereby facilitating access of osteoclast precursors to the bone surface. Osteoblastic cells also produce chemokines and cytokines, which can attract monocyte precursors and promote osteoclast differentiation and activity. At quiescent bone surfaces covered by bone-lining cells, it is likely that retraction or apoptotic death of these cells will expose the mineralized bone surface to osteoclasts. In contrast, at sites of tension osteoblasts are activated to produce an osteoid layer that subsequently mineralizes to form new bone. The formation of new bone during orthodontic movement has been studied by *in situ* hybridization using collagen type I (173) and bone sialoprotein (51) as markers of bone formation.

## Pathologies affecting alveolar bone

Diseases that affect bone mass and the normal metabolism of bone can impact on tooth formation and the maintenance of tooth support through the alveolar bone. Thus, defects in genes regulating osteoclast formation and activity often lead to impaired tooth eruption, as observed in *op/op* mice (265) and core binding factor  $\alpha 1$ -null mice (122) and in osteopetrotic mutations found in rats (222), such as incisors-absent (*ia*), osteopetrosis (*op*), toothless (*tl*), and microphthalmia (*mib*). Similarly, defects in tooth development would be expected in osteoprotegerin ligand (123) and nuclear factor  $\kappa B$  (65) knockout mice and mice over-expressing osteoprotegerin (212). Systemic and congenital diseases, such as osteoporosis and osteogenesis imperfecta, also effect the quality and quantity of bone and can impact on the ability of alveolar bone to perform its normal functions. Moreover, the ability of the bone to resist superimposed secondary diseases, such as periodontal infections, is further compromised. Thus, while the consequences of osteoporotic bone loss on alveolar bone and its susceptibility to periodontal disease has been controversial, the weight of evidence supports the concept that the risk of alveolar bone loss and the progression of bone loss is increased in conditions of impaired bone quality (91, 109). Such conditions also include the effects of type

2 diabetes (232), as well as estrogen deficiency-induced osteoporosis (73, 114). The destruction of alveolar bone is also crucial to the progression of periodontal disease, and the associated release of bone matrix components into the crevicular fluid can provide valuable markers for the detection and diagnosis of disease activity (72).

## Conclusion

Alveolar bone, which has an interdependence with the dentition, has a specialized function in the support of teeth. While there are architectural specifications for alveolar bone that relate to its functional role, the basic cellular and matrix components are consistent with other bone tissues. Similarly, the cellular activities involved in the formation and remodeling of alveolar bone and the factors that influence these cellular processes are common to bone tissues generally. However, specific features, such as the rate of bone remodeling, may be unique to alveolar bone and may be important for its adaptability. Recent studies have identified key genes, core binding factor  $\alpha 1$  and osteoprotegerin, that have a central role in the formation and activity of bone-forming and bone-resorbing cells. Many of the factors that regulate bone remodeling appear to exert their effects either directly or indirectly through these genes, which have become important targets for developing pharmacological and clinical strategies to regulate the rate of bone formation and resorption that will be important for maintenance of a healthy periodontium.

## References

1. Aisaka T, Gay CV. Ultracytochemical evidence for a proton-pump adenosine triphosphatase in chick osteoclasts. *Cell Tissue Res* 1986; **245**: 507–512.
2. Anderson DM, Maraskovsky E, Billingsley WL, Dougall WC, Tometsko ME, Roux ER, Teepe MC, DuBose RF, Cosman D, Galibert L. A homologue of the TNF receptor and its ligand enhance T-cell growth and dendritic-cell function. *Nature* 1997; **390**: 175–179.
3. Andersson G, Ek-Rylander B. The tartrate-resistant purple acid phosphatase of bone osteoclasts – a protein phosphatase with multivalent substrate specificity and regulation. *Acta Orthop Scand* 1995; **266**(suppl): 189–193.
4. Aubin JE, Gupta AK, Zirngibl R, Rossant J. Knockout mice lacking bone sialoprotein expression have bone abnormalities. *J Bone Miner Res* 1996; **11**: S102.
5. Aubin JE. Osteoclast adhesion and resorption: The role of podosomes. *J Bone Miner Res* 1992; **7**: 365–368.

6. Banks LM, Lees B, MacSweeney JE, Stevenson JC. Effect of degenerative spinal and aortic calcification on bone density measurements in post-menopausal women: links between osteoporosis and cardiovascular disease? *Eur J Clin Invest* 1994; **24**: 813–817.
7. Bannerjee C, McCabe LR, Choi J-Y, Hiebert SW, Stein JL, Stein GS, Lian JB. Runt homology domain proteins in osteoblast differentiation: AML-3/CBFA1 is a major component of a bone specific complex. *J Cell Biochem* 1997; **66**: 1–8.
8. Baran DT, Braverman LE. Thyroid hormones and bone mass (editorial). *J Clin Endocrinol Metab* 1991; **72**: 1182–1183.
9. Baron R, Neff L, Louvard D, Courtoy PJ. Cell-mediated extracellular acidification and bone resorption: evidence for a low pH in resorbing lacunae and localization of a 100-kD lysosomal membrane protein at the osteoclast ruffled border. *J Cell Biol* 1985; **101**: 2210–2222.
10. Baron R, Ravesloot J-H, Neff L, Chakraborty M, Chatterjee D, Lomri A, Horne W. Cellular and molecular biology of the osteoclast. In: Noda M, ed. *Cellular and molecular biology of bone*. San Diego: Academic Press, 1993: 445–495.
11. Bartold PM. A biochemical and immunohistochemical study of the proteoglycans of alveolar bone. *J Dent Res* 1990; **69**: 7–19.
12. Baud CA. Submicroscopic structure and functional aspects of the osteocyte. *Clin Orthop* 1968; **5666**: 227–236.
13. Baylink DJ, Bernstein DS. The effects of fluoride therapy on metabolic bone disease. A histologic study. *Clin Orthop* 1967; **55**: 51–85.
14. Baylink DJ, Wergedal JE. Bone formation by osteocytes. *Am J Physiol* 1971; **221**: 669–678.
15. Bélanger LF. Osteocytic osteolysis. *Calcif Tissue Res* 1969; **4**: 1–12.
16. Bélanger LF. Osteocytic resorption. In: Bourne GH, ed. *The biochemistry and physiology of bone* (Vol. 3). New York: Academic Press, 1971: 239–270.
17. Beresford JN, Fedarko NS, Fisher LW, Midura RJ, Yanagishita M, Termine JD, Gehron-Robey P. Analysis of the proteoglycans synthesized by human bone cells *in vitro*. *J Biol Chem* 1987; **262**: 17164–17172.
18. Bianco P, Riminucci M, Silvestrini G, Bonucci E, Termine JD, Fisher LW, Robey PG. Localization of bone sialoprotein (BSP) to Golgi and post-Golgi secretory structures in osteoblasts and to discrete sites in early bone matrix. *J Histochem Cytochem* 1993; **41**: 193–203.
19. Blair HC. How the osteoclast degrades bone. *Bioessays* 1998; **20**: 837–846.
20. Bonucci E. The ultrastructure of the osteocyte. In: Bonucci E, Motta PM, ed. *Ultrastructure of skeletal tissues: bone and cartilage in health and disease*. Boston: Kluwer Academic Publishers, 1990: 223–252.
21. Boskey AL, Maresca M, Ullrich W, Doty SB, Butler WT, Prince CW. Osteopontin-hydroxyapatite interactions *in vitro*: inhibition of hydroxyapatite formation and growth in a gelatin-gel. *Bone Miner* 1993; **22**: 147–159.
22. Bowman BM, Miller SC. The proliferation and differentiation of the bone-lining cell in estrogen-induced osteogenesis. *Bone* 1986; **7**: 351–357.
23. Boyce BF, Hughes DE, Wright KR, Xing L, Dai A. Recent advances in bone biology provide insight into the pathogenesis of bone diseases. *Lab Invest* 1999; **79**: 83–94.
24. Boyde A, Jones SJ, Ashford J. Scanning electron microscope observations and the question of possible osteocytic bone mini-(re)modelling. In: Silberman M, Slavkin H, ed. *Current advances in skeletogenesis*. Amsterdam: Excerpta Medica, 1982: 305–314.
25. Boyde A. Scanning electron microscope studies of bone. In: Bourne GH, ed. *The biochemistry and physiology of bone* (Vol. 1). New York: Academic Press, 1972: 259–310.
26. Bronckers AL, Farach-Carson MC, Van Waveren E, Butler WT. Immunolocalization of osteopontin, osteocalcin, and dentin sialoprotein during dental root formation and early cementogenesis in the rat. *J Bone Miner Res* 1994; **9**: 833–841.
27. Bronckers AL, Gay S, Lyaruu DM, Gay RE, Miller EJ. Localization of type V collagen with monoclonal antibodies in developing dental and periodontal tissues of the rat and hamster. *Collagen Rel Res* 1986; **6**: 1–13.
28. Bucay N, Sarosi I, Dunstan CR, Morony S, Tarpley J, Caparelli C, Scully S, Tan HL, Xu W, Lacey DL, Boyle WJ, Simonet WS. Osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification. *Genes Dev* 1998; **12**: 1260–1268.
29. Camarda AJ, Butler WT, Finkelman RD, Nanci A. Immunocytochemical localization of gamma-carboxyglutamic acid-containing proteins (osteocalcin) in rat bone and dentin. *Calcif Tissue Int* 1987; **40**: 349–355.
30. Canalis E, Centrella M, McCarthy T. Effects of basic fibroblast growth factor on bone formation *in vitro*. *J Clin Invest* 1988; **81**: 1572–1577.
31. Canalis E, Hock JM, Raisz LG. Parathyroid hormone: Anabolic and catabolic effects on bone and interactions with growth factors. In: Bilezikian JP, Marcus R, Levine MA, ed. *The parathyroids*. New York: Raven Press, 1994: 65–82.
32. Canalis E. Growth hormone, skeletal growth factors and osteoporosis. *Endocr Prat* 1995; **1**: 39–43.
33. Chatterjee J, Chakraborty M, Leit M, Neff L, Jamsa-Kellokumpu S, Fuchs R, Bartkiewicz M, Hernando N, Baron R. The osteoclast proton pump differs in its pharmacology and catalytic subunits from other vacuolar H<sup>+</sup>-ATPases. *J Exp Biol* 1992; **172**: 193–204.
34. Cheifetz S, Li IWS, McCulloch CAG, Sampath TK, Sodek J. Influence of osteogenic protein-1 (OP-1, BMP-7) and transforming growth factor- $\beta$ 1 on bone formation *in vitro*. *Connect Tissue Res* 1996; **35**: 71–78.
35. Chen J, McCulloch CAG, Sodek J. Bone sialoprotein in developing porcine dental tissues: cellular expression and comparison of tissue localization with osteopontin and osteonectin. *Arch Oral Biol* 1993; **38**: 241–249.
36. Chen J, McKee MD, Nanci A, Sodek J. Bone sialoprotein mRNA expression and ultrastructural localization in fetal porcine calvarial bone: comparisons with osteopontin. *Histochem J* 1994; **26**: 67–78.
37. Chen J, Shapiro HS, Sodek J. Developmental expression of bone sialoprotein mRNA in rat mineralized connective tissues. *J Bone Miner Res* 1992; **7**: 987–997.
38. Chen J, Shapiro HS, Wrana JL, Reimers S, Heersche JNM, Sodek J. Localization of bone sialoprotein (BSP) expression to sites of mineralized tissue formation in fetal rat tissues by *in situ* hybridization. *Matrix* 1991; **11**: 133–143.
39. Chen J, Zhang Q, McCulloch CAG, Sodek J. Immunohistochemical localization of bone sialoprotein (BSP) in fetal porcine bone tissues: comparisons with secreted phosphoprotein I (Spp1, osteopontin) and SPARC (osteonectin). *Histochem J* 1991; **23**: 281–289.

40. Chiquet M, Matthisson M, Koch M, Tannheimer M, Chiquet-Ehrismann R. Regulation of extracellular matrix synthesis by mechanical stress. *Biochem Cell Biol* 1996; **74**: 737–744.
41. Cho MI, Garant PR. Sequential events in the formation of collagen secretion granules with special reference to the development of segment-long-spacing-like aggregates. *Anat Rec* 1981; **199**: 309–320.
42. Chole RA, Tinling SP. Incomplete coverage of mammalian bone matrix by lining cells. *Ann Otol Rhinol Laryngol* 1993; **102**: 543–550.
43. Christoffersen J, Landis WJ. A contribution with review to the description of mineralization of bone and other calcified tissues *in vivo*. *Anat Rec* 1991; **230**: 435–450.
44. Chyun YS, Raisz LG. Stimulation of bone formation by prostaglandin E<sub>2</sub>. *Prostaglandins* 1984; **27**: 97–103.
45. Danielson KG, Baribault H, Holmes DF, Graham H, Kadler KE, Iozzo RV Targeted disruption of decorin leads to abnormal collagen fibril morphology and skin fragility. *Proc Natl Acad Sci U S A* 1997; **96**: 3092–3097.
46. Delaney AM, Pash JM, Canalis E. Cellular and clinical perspectives on skeletal insulin-like growth factor. *J Cell Biochem* 1994; **55**: 1–6.
47. Delmas PD, Malaval L. In: Mundy GR, Martin TJ, ed. *Handbook of experimental pharmacology*. Vol. 107. Physiology and pharmacology of bone. Berlin: Springer-Verlag, 1993: 673–724.
48. DeLuca HF. Vitamin D revisited. *Clin Endocrinol Metab* 1980; **9**: 1–26.
49. Domenicucci C, Goldberg HA, Sodek J. Identification of lysyl oxidase and TRAMP as the major proteins associated with the demineralized collagen matrix in porcine dentine. *Connect Tissue Res* 1997; **36**: 151–163.
50. Domenicucci C. In search of a hydroxyapatite nucleator in bone. Ph.D. thesis. Toronto: University of Toronto, 1997.
51. Domon S. Observation of alveolar bone remodeling accompanied with tooth movement by *in situ* hybridization—expression of type I collagen and bone sialoprotein mRNAs. *Kokubyo Gakkai Zasshi* 1995; **62**: 94–105.
52. Domon T, Wakita M. A three-dimensional reconstruction of the ruffled border of osteoclasts. *Arch Histol Cytol* 1989; **52**: 1–13.
53. Ducy P, Desbois C, Boyce B, Pinero G, Story B, Dunstan C, Smith E, Bonadio J, Goldstein S, Gundberg C, Bradley A, Karsenty G. Increased bone formation in osteocalcin-deficient mice. *Nature* 1996; **382**: 448–452.
54. Ducy P, Zhang R, Geoffroy V, Ridall AL, Karsenty G (1997). *Osf2/Cbfa1*: a transcriptional activator of osteoblast differentiation. *Cell* 1997; **89**: 747–754.
55. Duong LT, Lakkakorpi PT, Nakamura I, Machwate M, Nagy RM, Rodan GA. *PYK2* in osteoclasts is an adhesion kinase, localized in the sealing zone, activated by ligation of  $\alpha$ -*pha(v)beta3* integrin, and phosphorylated by *src* kinase. *J Clin Invest* 1998; **102**: 881–892.
56. Duong LT, Rodan GA. The role of integrins in osteoclast function. *J Bone Miner Metab* 1999; **17**: 1–6.
57. Engel J, Taylor W, Paulsson M, Sage H, Hogan B. Calcium binding domains and calcium-induced transition of SPARC/BM-40/osteonectin, an extracellular glycoprotein expressed in mineralized and non-mineralized tissues. *Biochemistry* 1987; **26**: 6958–6965.
58. Erlebacher A, Filvaroff EH, Gitelman SE, Derynck R. Toward an understanding of skeletal development. *Cell* 1995; **80**: 371–378.
59. Eyre DR, Dickson IR, Van Ness K. Collagen cross-linking in human bone and articular cartilage. Age-related changes in the content of mature hydroxyxyridinium residues. *Biochem J* 1988; **252**: 495–500.
60. Fisher LW, Hawkins GR, Tuross N, Termine JD. Purification and partial characterization of small proteoglycans I and II, bone sialoproteins I and II, and osteonectin from the mineral compartment of developing human bone. *J Biol Chem* 1987; **262**: 9702–9708.
61. Fisher LW, Heegaard AM, Vetter U, Vogel W, Termine JD, Young MF. Human biglycan gene. Putative promoter, intron-exon junctions, and chromosomal localization. *J Biol Chem* 1991; **266**: 14371–14377.
62. Fisher LW, McBride OW, Termine JD, Young MF. Human bone sialoprotein. *J Biol Chem* 1990; **265**: 2347–2351.
63. Forbes EG, Cronshaw AD, MacBeath JR, Hulmes DJ. Tyrosine-rich acidic matrix protein (TRAMP) is a tyrosine-sulphated and widely distributed protein of the extracellular matrix. *FEBS Lett* 1994; **351**: 433–436.
64. Franzén A, Heinegård D. Isolation and characterization of two sialoproteins present only in bone calcified matrix. *Biochem J* 1985; **232**: 715–724.
65. Franzoso G, Carlson L, Xing L, Poljak L, Shores EW, Brown KD, Leonardi A, Tran T, Boyce BF, Siebenlist U. Requirement for NF-kappaB in osteoclast and B-cell development. *Genes Dev* 1997; **11**: 3482–3496.
66. Fukushima O, Bekker PJ, Gay CV. Characterization of the functional stages of osteoclasts by enzyme histochemistry and electron microscopy. *Anat Rec* 1991; **231**: 298–315.
67. Ganss B, Kim RH, Sodek J. Bone sialoprotein. *Crit Rev Oral Biol Med* 1999; **10**: 79–98.
68. Garant PR, Cho M-I. Ultrastructure of the odontoblast. In: Butler WT, ed. *The chemistry and biology of mineralized tissues*. Birmingham: EBSCO Media, 1985: 22–32.
69. Gay CV, Ito MB, Schraer H. Carbonic anhydrase activity in isolated osteoclasts. *Metab Bone Dis Rel Res* 1983; **5**: 33–39.
70. Ghebron Robey P. Bone matrix proteoglycans and glycoproteins. In: Bilezikian JP, Raisz LG, Rodan GA, ed. *Principles of bone biology*. San Diego: Academic Press, 1996: 155–165.
71. George A, Silberstein R, Veis A. *In situ* hybridization shows *Dmp1* (AG1) to be a developmentally regulated dentin-specific protein produced by mature odontoblasts. *Connect Tissue Res* 1995; **33**: 67–72.
72. Giannobile WV. Crevicular fluid biomarkers of oral bone loss. *Curr Opin Periodontol* 1997; **4**: 11–20.
73. Gilles JA, Carnes DL, Dallas MR, Holt SC, Bonewald LF. Oral bone loss is increased in ovariectomized rats. *J Endod* 1997; **23**: 419–422.
74. Gilmour DT, Lyon GJ, Carlton MB, Sanes JR, Cunningham JM, Anderson JR, Hogan BL, Evans MJ, Colledge WH. Mice deficient for the secreted glycoprotein SPARC/osteonectin/BM40 develop normally but show severe age-onset cataract formation and disruption of the lens. *EMBO J* 1998; **17**: 1860–1870.
75. Glimcher MJ, Krane SM. The organization and structure of bone and the mechanism of calcification. In: Ramachandran GN, Gould BS, ed. *Treatise on collagen*. Vol. 7B. New York: Academic Press, 1968: 68–251.
76. Glowacki J, Rey C, Cox K, Lian J. Effects of bone matrix

- components on osteoclast differentiation. *Connect Tissue Res* 1989; **21**: 121–129.
77. Goldberg HA, Domenicucci C, Pringle GA, Sodek J. Mineral binding proteoglycans of fetal porcine calvarial bone. *J Biol Chem* 1988; **263**: 12092–12101.
  78. Goldberg HA, Hunter GK. The inhibitory activity of osteopontin on hydroxyapatite formation *in vitro*. *Ann N Y Acad Sci* 1995; **760**: 305–308.
  79. Goldberg HA, Maeno M, Domenicucci C, Zhang Q, Sodek J. Identification of small collagenous proteins with properties of procollagen  $\alpha 1(I)$  pN-propeptide in fetal porcine calvarial bone. *Collagen Rel Res* 1988; **8**: 187–197.
  80. Gooden MD, Vernon RB, Bassuk JA, Sage EH. Cell cycle-dependent nuclear location of the matricellular protein SPARC: association with the nuclear matrix. *J Cell Biochem* 1999; **74**: 152–167.
  81. Gorski JP, Shimizu K. Isolation of a new phosphorylated glycoprotein from the mineralized phase of bone that exhibits limited homology to the adhesive protein osteopontin. *J Biol Chem* 1998; **263**: 15938–15945.
  82. Goto T, Tsukuba T, Ayasaka N, Yamamoto K, Tanaka T. Immunocytochemical localization of cathepsin D in the rat osteoclast. *Histochemistry* 1992; **97**: 13–18.
  83. Grigoriadis AE, Wang QZ, Cecchini MG, Hofstetter W, Felix R, Fleisch HA, Wagner EF. c-fos: a key regulator of osteoclast-macrophage lineage determination and bone remodelling. *Science* 1994; **266**: 443–448.
  84. Hafner M, Zimmermann K, Pottgiesser J, Krieg T, Nischter R. A purine-rich sequence in the human BM-40 gene promoter region is a prerequisite for maximum transcription. *Matrix Biol* 1995; **14**: 733–741.
  85. Hanai J, Chen LF, Kanno T, Ohtani-Fujita N, Kim WY, Guo WH, Imamura T, Ishidou Y, Fukuchi M, Shi MJ, Stavnezer J, Kawabata M, Miyazono K, Ito Y. Interaction and function cooperation of PEBP2/CBF with Smads. Synergistic induction of the immunoglobulin germline  $C\alpha$  promoter. *J Biol Chem* 1999; **274**: 31577–31582.
  86. Hauschka PV. Osteocalcin and its functional domains. In: Butler WT, ed. *The chemistry and biology of mineralized tissues*. Birmingham: Ebsco Media, 1985: 149–158.
  87. Hayden JM, Mohan S, Baylink DJ. The insulin-like growth factor system and the coupling of formation to resorption. *Bone* 1995; **17**(suppl): 93S–98S.
  88. Heinegård D, Hultenby K, Oldberg Å, Reinholt F, Wendel M. Macromolecules in bone matrix. *Connect Tissue Res* 1989; **21**: 3–14.
  89. Helder MN, Bronckers AL, Woltgens, JH. Dissimilar expression of osteopontin (OPN) and collagen type I in dental tissues and alveolar bone of the neonatal rat. *Matrix* 1993; **13**: 415–425.
  90. Hijiya N, Setoguchi M, Matsuura K, Higuchi Y, Akizuki S, Yamamoto S. Cloning and characterization of the human osteopontin gene and its promoter. *Biochem J* 1994; **303**: 255–262.
  91. Hildebolt CE. Osteoporosis and oral bone loss. *Dentomaxillofac Radiol* 1997; **26**: 3–15.
  92. Hock JM, Canalis E. Platelet-derived growth factor enhances bone cell replication but not differentiated function of osteoblasts. *Endocrinology* 1994; **134**: 1423–1428.
  93. Hock JM, Centrella M, Canalis E. Insulin-like growth factor I (IGF-I) has independent effects on bone matrix formation and cell replication. *Endocrinology* 1988; **122**: 254–260.
  94. Holtrop ME. Light and electron microscopic structure of bone-forming cells. In: Hall BK, ed. *Bone: the osteoblast and osteocyte*. Vol. 1. Caldwell: Telford Press, 1990: 1–39.
  95. Horwood NJ, Elliott J, Martin TJ, Gillespie MT. Osteotropic agents regulate the expression of osteoclast differentiation factor and osteoprotegerin in osteoblastic stromal cells. *Endocrinology* 1998; **139**: 4743–4746.
  96. Hoshi K, Kemmotsu S, Takeuchi Y, Amizuka N, Ozawa H. The primary calcification in bones follows removal of decorin and fusion of collagen fibrils. *J Bone Miner Res* 1999; **14**: 273–280.
  97. Huang YH, Ohsaki Y, Kurisu K. Distribution of type I and type III collagen in the developing periodontal ligament of mice. *Matrix* 1991; **11**: 25–35.
  98. Hughes DE, Wright KR, Uy HL, Sasaki A, Yoneda T, Roodman GD, Mundy GR, Boyce BF. Bisphosphonates promote apoptosis in murine osteoclasts *in vitro* and *in vivo*. *J Bone Miner Res* 1995; **10**: 1478–1487.
  99. Hultenby K, Reinholt FP, Oldberg Å, Heinegård D. Ultrastructural immunolocalization of osteopontin in metaphyseal and cortical bone. *Matrix* 1991; **11**: 206–213.
  100. Hunter GK, Goldberg HA. Modulation of crystal formation by bone sialoproteins: role of glutamic acid-rich sequences in the nucleation of hydroxyapatite by bone sialoprotein. *Biochem J* 1994; **302**: 175–179.
  101. Hunter GK, Goldberg HA. Nucleation of hydroxyapatite by bone sialoprotein. *Proc Natl Acad Sci U S A* 1993; **90**: 8562–8565.
  102. Hunter GK, Hauschka PV, Poole AR, Rosenberg LC, Goldberg HA. Nucleation and inhibition of hydroxyapatite formation by mineralized tissue proteins. *Biochem J* 1996; **317**: 59–64.
  103. Iotsova V, Caamano J, Loy J, Yang Y, Lewin A, Bravo R. Osteopetrosis in mice lacking NF- $\kappa$ B1 and NF- $\kappa$ B2. *Nat Med* 1997; **3**: 1285–1289.
  104. Iozzo RV, Murdoch AD. Proteoglycans of the extracellular environment: clues from the gene and protein side offer novel perspectives in molecular diversity and function. *FASEB J* 1996; **10**: 598–614.
  105. Jahnen-Dechent W, Schinke T, Trindl A, Muller-Esterl W, Sablitzky F, Kaiser S, Blessing M. Cloning and targeted deletion of the mouse fetuin gene. *J Biol Chem* 1997; **272**: 31496–31503.
  106. Jande SS, Belanger LF. Electron microscopy of osteocytes and the pericellular matrix in rat trabecular bone. *Calcif Tissue Res* 1971; **6**: 280–289.
  107. Jande SS, Belanger LF. The life cycle of the osteocyte. *Clin Orthop* 1973; **94**: 281–305.
  108. Jande SS. Fine structural study of osteocytes and their surrounding bone matrix with respect to their age in young chicks. *J Ultrastruct Res* 1971; **37**: 279–300.
  109. Jeffcoat MK. Osteoporosis; a possible modifying factor in oral bone loss. *Ann Periodontol* 1998; **3**: 312–321.
  110. Jheon A, Ganss B, Cheifetz S, Sodek J. Identification of a novel tissue-specific, bone morphogenic protein-7 early response gene using differential display. *J Am Acad Orthop Surg* (in press).
  111. Jiang H, Sodek J, Karsenty G, Thomas H, Ranly D, Chen J. Expression of core binding factor *Osf2/Cbfa-1* and bone sialoprotein in tooth development. *Mech Dev* 1999; **81**: 169–173.
  112. Jilka RL, Hangoc G, Girasole G, Passeri G, Williams DC, Abrams JS, Boyce B, Broxmeyer H, Manolagas SC. Incre-

- asd osteoclast development after estrogen loss – mediation by interleukin-6. *Science* 1992; **257**: 88–91.
113. Jilka RL, Weinstein RS, Bellido T, Parfitt AM, Manolagas SC. Osteoblast programmed cell death (apoptosis): modulation by growth factors and cytokines. *J Bone Miner Res* 1998; **13**: 793–802.
  114. Johnson RB, Gilbert JA, Cooper RC, Dai X, Newton BI, Tracy RR, West WF, DeMoss TL, Myers PJ, Streckfus CF. Alveolar bone loss one year following ovariectomy in sheep. *J Periodontol* 1997; **68**: 864–871.
  115. Johnson RB. A classification of Sharpey's fibers within the alveolar bone of the mouse: a high-voltage electron microscope study. *Anat Rec* 1987; **217**: 339–347.
  116. Jones SJ, Boyde A, Ali NN, Maconnachie E. A review of bone cell substratum interactions. *Scanning* 1985; **7**: 5–24.
  117. Kaartinen MT, Pirhonen A, Linnala-Kankkunen A, Mäenpää PH. Cross-linking of osteopontin by tissue transglutaminase increases its collagen binding properties. *J Biol Chem* 1999; **274**: 1729–1735.
  118. Kaartinen MT, Pirhonen A, Linnala-Kankkunen A, Mäenpää PH. Transglutaminase-catalyzed cross-linking of osteopontin is inhibited by osteocalcin. *J Biol Chem* 1997; **272**: 22736–22741.
  119. Karimbux NY, Rosenblum ND, Nishimura I. Site-specific expression of collagen I and XII mRNAs in the rat periodontal ligament at two developmental stages. *J Dent Res* 1992; **71**: 1355–1362.
  120. Kim RH, Shapiro HS, Li JJ, Wrana JL, Sodek J. Characterization of human bone sialoprotein (BSP) gene and its promoter sequence. *Matrix Biol* 1994; **14**: 31–40.
  121. Kjaer I, Bagheri A. Prenatal development of the alveolar bone of human deciduous incisors and canines. *J Dent Res* 1999; **78**: 667–672.
  122. Komori T, Yagi H, Nomura S, Yamaguchi A, Sasaki K, Deguchi K, Shimizu Y, Bronson RT, Gao Y-H, Inada M, Sato M, Okamoto R, Kitamura Y, Yoshiki S, Kishimoto T. Targeted disruption of *Cbfa1* results in a complete lack of bone formation owing to maturational arrest of osteoblasts. *Cell* 1997; **89**: 755–764.
  123. Kong YY, Yoshida H, Sarosi I, Tan HL, Timms E, Capparelli C, Morony S, Oliveira-dos-Santos AJ, Van G, Itie A, Khoo W, Wakeham A, Dunstan CR, Lacey DL, Mak TW, Boyle WJ, Penninger JM. OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. *Nature* 1999; **397**: 315–323.
  124. Kurihara S, Enlow DH. An electron microscopic study of attachments between periodontal fibers and bone during alveolar remodeling. *Am J Orthod* 1980; **77**: 516–531.
  125. Lacey DL, Timms E, Tan HL, Kelley MJ, Dunstan CR, Burgess T, Elliott R, Colombero A, Elliott G, Scully S, Hsu H, Sullivan J, Hawkins N, Davy E, Capparelli C, Eli A, Qian YX, Kaufman S, Sarosi I, Shalhoub V, Senaldi G, Guo J, Delaney J, Boyle WJ. Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. *Cell* 1998; **93**: 165–176.
  126. Lakkakorpi P, Tuukkanen J, Hentunen T, Järvelin K, Väänänen HK. Organization of osteoclast microfilaments during the attachment to bone surface *in vitro*. *J Bone Miner Res* 1989; **4**: 817–825.
  127. Landis WJ, Hodgens KJ, Arena J, Song MJ, McEwen BF. Structural relations between collagen and mineral in bone as determined by high voltage electron microscopic tomography. *Microsc Res Tech* 1996; **33**: 192–202.
  128. Landis WJ. The strength of a calcified tissue depends in part on the molecular structure and organization of its constituent mineral crystals in their organic matrix. *Bone* 1995; **16**: 533–544.
  129. Lane TF, Sage EH. The biology of SPARC, a protein that modulates cell-matrix interactions. *FASEB J* 1994; **8**: 163–173.
  130. Lee M-H, Javed A, Kim H-J, Shin H-I, Gutierrez S, Choi J-Y, Rosen V, Stein JL, van Wijnen AJ, Stein GS, Lian JB, Ryoo H-M. Transient upregulation of CBFA1 in response to bone morphogenetic protein-2 and transforming growth factor  $\beta$ 1 in C2C12 myogenic cells coincides with suppression of the myogenic phenotype but is not sufficient for osteoblastic differentiation. *J Cell Biochem* 1999; **73**: 114–125.
  131. Li IW, Cheifetz S, McCulloch CA, Sampath KT, Sodek J. Effects of osteogenic protein-1 (OP-1, BMP-7) on bone matrix protein expression by fetal rat calvarial cells are differentiation stage specific. *J Cell Physiol* 1996; **169**: 115–125.
  132. Lian JB, Stein GS. Concepts of osteoblast growth and differentiation: basis for modulation of bone cell development and tissue formation. *Crit Rev Oral Biol Med* 1992; **3**: 269–305.
  133. Lian JB, Stein GS, Stein JL, van Wijnen AJ. Osteocalcin gene promoter: unlocking the secrets for regulation of osteoblast growth and differentiation. *J Cell Biochem* 1998; **30–31**(suppl): 62–72.
  134. Liaw L, Birk DE, Ballas CB, Whitsitt JS, Davidson JM, Hogan BL. Altered wound healing in mice lacking a functional osteopontin gene (*spp1*). *J Clin Invest* 1998; **101**: 1468–1478.
  135. Lucht U. Cytoplasmic vacuoles and bodies of the osteoclast: an electron microscope study. *Z Zellforsch* 1972; **135**: 229–244.
  136. Luk SC, Nopajaroonsri C, Simon GT. The ultrastructure of endosteum: a topographic study in young adult rabbits. *J Ultrastruct Res* 1974; **46**: 165–183.
  137. Lukinmaa PL, Waltimo J. Immunohistochemical localization of types I, V, and VI collagen in human permanent teeth and periodontal ligament. *J Dent Res* 1992; **71**: 391–397.
  138. Luo G, Ducy P, McKee MD, Pinero GJ, Loyer E, Behringer RR, Karsenty G. Spontaneous calcification of arteries and cartilage in mice lacking matrix GLA protein. *Nature* 1997; **386**: 78–81.
  139. MacDougall M, Simmons D, Luan X, Gu TT, DuPont BR. Assignment of dentin sialophosphoprotein (DSPP) to the critical DGI2 locus on human chromosome 4 band q21.3 by *in situ* hybridization. *Cytogenet Cell Genet* 1997; **79**: 121–122.
  140. MacDougall M, Dupont BR, Leach RJ. Assignment of DMP1 to human chromosome 4 band 4q21 by *in situ* hybridization. *Cytogenet Cell Genet* 1996; **74**: 189.
  141. MacNeil RL, Sheng N, Strayhorn C, Fisher LW, Somerman MJ. Bone sialoprotein is localized to the root surface during cementogenesis. *J Bone Miner Res* 1994; **9**: 1597–1606.
  142. Maeno M, Suzuki N, Ohmori Y, Saito R, Shioji S, Sato K, Otsuka K, Suzuki K. *J Bone Miner Metab* 1992; **10**: 18–25.
  143. Marchi F, Leblond CP. Collagen biosynthesis and assembly into fibrils as shown by ultrastructural and  $^3\text{H}$ -proline radioautographic studies on the fibroblasts of the rat foot pad. *Am J Anat* 1983; **168**: 167–197.

144. Marchi F. Secretory granules in cells producing fibrillar collagen. In: Davidovitch Z, ed. *Biological mechanisms of tooth eruption and root resorption*. Birmingham: EBSCO Media, 1988: 53–59.
145. Matthews JL, Talmage R. Influence of parathyroid hormone on bone cell ultrastructure. *Clin Orthop* 1981; **156**: 27–38.
146. Marie PJ, Hott M. Histomorphometric identification of carbonic anhydrase in fetal rat bone embedded in glycomethacrylate. *J Histochem Cytochem* 1987; **35**: 245–250.
147. Marks SC Jr, Miller SC. Local delivery of prostaglandin E<sub>1</sub> induces periodontal regeneration in adult dogs. *J Periodontal Res* 1994; **29**: 103–108.
148. Marotti G, Cane V, Palazzini S, Palumbo C. Structure-function relationships in the osteocyte. *Ital J Miner Electrolyte Metab* 1990; **4**: 93–106.
149. Marotti G. Decrement in volume of osteoblasts during osteon formation and its effect on the size of the corresponding osteocytes. In: Meunier PJ, ed. *Bone histomorphometry*. Paris: Armour Montagu, 1977: 385–397.
150. Martin TJ, Romas E, Gillespie MT. Interleukins in the control of osteoclast differentiation. *Crit Rev Eukaryot Gene Expr* 1998; **8**: 107–123.
151. Matthews JL, VanderWeil C, Talmage RV. Bone lining cells and the bone fluid compartment, an ultrastructural study. *Adv Exp Med Biol* 1978; **103**: 451.
152. McCarthy TL, Centrella M, Canalis E. Regulatory effects of insulin-like growth factor I and II on bone collagen synthesis in rat calvarial cultures. *Endocrinology* 1989; **124**: 301–309.
153. McKee MD, Nanci A, Landis WJ, Gotoh Y, Gerstenfeld LC, Glimcher MJ. Developmental appearance and ultrastructural immunolocalization of a major 66 kDa phosphoprotein in embryonic and post-natal chicken bone. *Anat Rec* 1990; **228**: 77–92.
154. McKee MD, Glimcher MJ, Nanci A. High-resolution immunolocalization of osteopontin and osteocalcin in bone and cartilage during endochondral ossification in the chicken tibia. *Anat Rec* 1992; **234**: 479–492.
155. McKee MD, Nanci A. Ultrastructural, cytochemical and immunocytochemical studies on bone and its interfaces. *Cells Mater* 1993; **3**: 219–243.
156. McKee MD, Farach-Carson MC, Butler WT, Hauschka PV, Nanci A. Ultrastructural immunolocalization of noncollagenous (osteopontin and osteocalcin) and plasma (albumin and  $\alpha_2$ HS-glycoprotein) proteins in rat bone. *J Bone Miner Res* 1993; **8**: 485–496.
157. McKee MD, Nanci A. Postembedding colloidal-gold immunocytochemistry of noncollagenous extracellular matrix proteins in mineralized tissues. *Microsc Res Tech* 1995; **31**: 44–62.
158. McKee MD, Nanci A. Osteopontin and the bone remodeling sequence: Colloidal-gold immunocytochemistry of an interfacial extracellular matrix protein. *Ann N Y Acad Sci* 1995; **760**: 177–189.
159. McKee MD, Nanci A. Osteopontin at mineralized tissue interfaces in bone, teeth and osseointegrated implants: ultrastructural distribution and implications for mineralized tissue formation, turnover and repair. *Microsc Res Tech* 1996; **33**: 141–164.
160. McKee MD, Zalzal S, Nanci A. Extracellular matrix in tooth cementum and mantle dentin: localization of osteopontin and other noncollagenous proteins, plasma proteins and glycoconjugates by electron microscopy. *Anat Rec* 1996; **245**: 293–312.
161. McSheehy PMJ, Chambers TJ. Osteoblastic cells mediate osteoclastic responsiveness to parathyroid hormone. *Endocrinology* 1986; **118**: 824–828.
162. Menton DN, Simmons DJ, Change S-L, Orr BY. From bone lining cell to osteocyte – an SEM study. *Anat Rec* 1984; **209**: 29–39.
163. Merriman HL, van Wijnen AJ, Hiebert S, Bidwell JP, Fey E, Lian J, Stein J, Stein GS. The tissue-specific nuclear matrix protein, NMP-2 is a member of the AML/CBF/PEBP2/runt domain transcription factor family: interactions with the osteocalcin gene promoter. *Biochemistry* 1995; **34**: 13125–13132.
164. Miller SC, Bowman BM, Smith JM, Jee WSS. Characterization of endosteal bone-lining cells from fatty marrow bone sites in adult beagles. *Anat Rec* 1980; **198**: 163–173.
165. Miller SC, Jee WSS. The bone lining cell: a distinct phenotype? *Calcif Tissue Int* 1987; **41**: 1–5.
166. Miller SC, Jee WSS. Bone lining cells. In: Hall BK, ed. *Bone: bone metabolism and mineralization*. Vol. 4. Boca Raton, FL: CRC Press, 1992: 1–19.
167. Miyama K, Yamada G, Yamamoto TS, Tagaki C, Miyado K, Sakai M, Ueno N, Shibuya H. A BMP inducible gene, *Dlx5*, regulates osteoblast differentiation and mesoderm induction. *Dev Biol* 1999; **208**: 123–133.
168. Miyauchi A, Alvarez J, Greenfield EM, Teti A, Grano M, Colucci S, Zamboni-Zallone A, Ross FP, Teitelbaum SL, Cheresch D, Hruska KA. Recognition of osteopontin and related peptides by  $\alpha_2\beta_3$  integrin stimulates immediate cell signals in osteoclasts. *J Biol Chem* 1991; **266**: 20369–20374.
169. Mizuno A, Amizuka N, Irie K, Murakami A, Fujise N, Kanno T, Sato Y, Nakagawa N, Yasuda H, Mochizuki S, Gomibuchi T, Yano K, Shima N, Washida N, Tsuda E, Morinaga T, Higashio K, Ozawa H. Severe osteoporosis in mice lacking osteoclastogenesis inhibitory factor/osteoprotegerin. *Biochem Biophys Res Commun* 1998; **247**: 610–615.
170. Mundy GR, Poser JW. Chemotactic activity of the gamma-carboxyglutamic acid containing protein in bone. *Calcif Tissue Int* 1983; **35**: 164–168.
171. Mundlos S, Otto F, Munlos C, Mulliken JB, Aylsworth AS, Albright S, Lindhout D, Cole WG, Henn W, Knoll JHM, Owen MJ, Mertelsmann R, Zabel BU, Olsen BR. Mutations involving the transcription factor *CBFA1* cause cleidocranial dysplasia. *Cell* 1997; **89**: 773–779.
172. Murphy-Ullrich JE, Lane TF, Pallero MA, Sage EH. SPARC mediates focal adhesion disassembly in endothelial cells through a follistatin-like region and the Ca<sup>2+</sup>-binding EF-hand. *J Cell Biochem* 1995; **57**: 341–350.
173. Nakagawa M, Kukita T, Nakasima A, Kurisu K. Expression of the type I collagen gene in rat periodontal ligament during tooth movement as revealed by *in situ* hybridization. *Arch Oral Biol* 1994; **39**: 289–294.
174. Nefussi JR, Casamajor P, Serfaty R, Bolle M, Hugly C, Forrest N. Activated adult human alveolar bone cells: a new model of matrix mineralization. *Eur J Oral Sci* 1998; **106**: 424–428.
175. Okamoto O, Fujiwara S, Abe M, Sato Y. Dermatopontin interacts with transforming growth factor beta and enhances its biological activity. *Biochem J* 1999; **337**: 537–541.
176. Otto F, Thornell AP, Crompton T, Denzel A, Gilmour KC,

- Rosewell IR, Stamp GWH, Beddington RSP, Mundlos S, Olsen BR, Selby PB, Owen MJ. Cbfa1, a candidate gene for cleidocranial dysplasia syndrome, is essential for osteoblast differentiation and bone development. *Cell* 1997; **89**: 765–771.
177. Overall CM, Wrana JL, Sodek J. Transforming growth factor- $\beta$  regulation of collagenase, 72-kDa-progelatinase, TIMP and PAI-1 expression in rat bone cell populations and in human fibroblasts. In: Glimcher MJ, Lian JB, ed. *Proceedings of the Third International Conference on the Chemistry and Biology of Mineralized Tissues*. Chatham, MA: Gordon and Breach Science Publishers, 1989: 289–294.
178. Pacifici R, Rifas L, McCracken R, Vered I, McMurtry C, Avioli LV, Peck WA. Ovarian steroid treatment blocks a post-menopausal increase in blood monocyte interleukin-1 release. *Proc Natl Acad Sci U S A* 1989; **86**: 2398–2402.
179. Page AE, Hayman AR, Andersson LMB, Chambers TJ, Warburton MJ. Degradation of bone matrix proteins by osteoclast cathepsins. *Int J Biochem* 1993; **25**: 545–550.
180. Palumbo C, Palazzini S, Marotti G. Morphological study of intercellular junctions during osteocyte differentiation. *Bone* 1990; **11**: 401–406.
181. Palumbo C. A three-dimensional ultrastructural study of osteoid-osteocytes in the tibia of chick embryos. *Cell Tissue Res* 1986; **246**: 125–131.
182. Parfitt AM. The cellular basis of bone turnover and bone loss. A rebuttal of the osteocytic resorption-bone flow theory. *Clin Orthop* 1977; **127**: 236–247.
183. Parfitt AM. Bone and plasma calcium homeostasis. *Bone* 1987; **8**: 51–58.
184. Parfitt AM. Plasma calcium control at quiescent bone surfaces: A new approach to the homeostatic function of bone lining cells. *Bone* 1989; **10**: 87–88.
185. Parfitt AM. Bone-forming cells in clinical conditions. In: Hall BK, ed. *Bone: the osteoblast and osteocyte*. Vol. 1. Caldwell: The Telford Press, 1990: 351–429.
186. Pfeilschifter JP, Seyedin S, Mundy GR. Transforming growth factor inhibits bone resorption in fetal rat long bone cultures. *J Clin Invest* 1988; **82**: 680–685.
187. Price PA, Baukol SA. 1,25-Dihydroxyvitamin D<sub>3</sub> increases synthesis of the vitamin-K dependent bone protein by osteosarcoma cells. *J Biol Chem* 1980; **255**: 11660–11663.
188. Price PA, Williamson MK. Effects of warfarin on bone. Studies on the vitamin K-dependent protein in rat bone. *J Biol Chem* 1981; **256**: 12754–12759.
189. Prince CW, Butler WT. 1,25-Dihydroxyvitamin D<sub>3</sub> regulates the biosynthesis of osteopontin, a bone-derived cell attachment protein, in clonal osteoblast-like osteosarcoma cells. *Collagen Rel Res* 1987; **7**: 305–313.
190. Puzas JE, Ishibe M. Osteoblast/osteoclast coupling. In: Rifkin BR, Gay CV, ed. *Biology and physiology of the osteoclast*. Boca Raton, FL: CRC Press, 1992: 337–356.
191. Rao LG, Wang HM, Kalliecharan R, Heersche JN, Sodek J. Specific immunohistochemical localization of type I collagen in porcine periodontal tissues using the peroxidase-labelled antibody technique. *Histochem J* 1979; **11**: 73–82.
192. Reddi AH. BMPs: actions in flesh and bone. *Nat Med* 1997; **3**: 837–839.
193. Reddy SV, Roodman GD. Control of osteoclast differentiation. *Crit Rev Eukaryot Gene Expr* 1998; **8**: 1–17.
194. Rittling SR, Matsumoto HN, McKee MD, Nanci A, An XR, Novick KE, Kowalski AJ, Noda M, Denhardt DT. Mice lacking osteopontin show normal development and bone structure but display altered osteoclast formation *in vitro*. *Bone Miner Res* 1998; **13**: 1101–1111.
195. Rodan GA, Harada S. The missing bone. *Cell* 1997; **89**: 677–680.
196. Romanowski R, Jundt G, Termine JD, Von der Mark K, Schulz A. Immunoelectron microscopy of osteonectin and type I collagen in osteoblasts and bone matrix. *Calcif Tissue Int* 1990; **46**: 353–360.
197. Romberg RW, Werness PG, Riggs BL, Mann KG. Inhibition of hydroxyapatite crystal growth by bone-specific and other calcium-binding proteins. *Biochemistry* 1986; **25**: 1176–1180.
198. Roodman GD, Ibbotson KJ, MacDonald BR, Kuehl TJ, Mundy GR. 1,25(OH)<sub>2</sub> Vitamin D<sub>3</sub> causes formation of multinucleated cells with osteoclast characteristics in cultures of primate marrow. *Proc Natl Acad Sci U S A* 1985; **82**: 8213–8217.
199. Ross FP, Chappel J, Alvarez JL, Sander D, Butler WT, Farach-Carson MC, Mintz KA, Gehron-Robey P, Teitelbaum SL, Cheresch DA. Interactions between the bone matrix proteins osteopontin and bone sialoprotein and the osteoclast integrin  $\alpha_v\beta_3$  potentiate bone resorption. *J Biol Chem* 1993; **268**: 9901–9907.
200. Saffar J-L, Lasfargues J-J, Cherruau M. Alveolar bone and the alveolar bone process: the socket that is never stable. *Periodontol* 2000 1997; **13**: 76–90.
201. Salonen J, Domenicucci C, Goldberg HA, Sodek J. Immunohistochemical localization of SPARC protein (osteonectin) and denatured collagen and their relationship to tissue remodelling in rat dental tissues. *Arch Oral Biol* 1990 **35**: 337–346.
202. Santra M, Danielson KG, Iozzo RV. Structural and functional characterization of the human decorin gene promoter. A homopurine-homopyrimidine S1 nuclease-sensitive region is involved in transcriptional control. *J Biol Chem* 1994; **269**: 579–587.
203. Sasaki T, Hohenester E, Gohring W, Timpl R. Crystal structure and mapping by site-directed mutagenesis of the collagen-binding epitope of an activated form of BM-40/SPARC/osteonectin. *EMBO J* 1998; **17**: 1625–1634.
204. Sasaki T, Ueno-Matsuda E. Immunocytochemical localization of cathepsins B and G in odontoclasts of human deciduous teeth. *J Dent Res* 1992; **71**: 1881–1884.
205. Sato M, Morii E, Komori T, Kawahata H, Sugimoto M, Terai K, Shimizu H, Yasui T, Ogihara H, Yasui N, Ochi T, Kitamura Y, Ito Y, Nomura S. Transcriptional regulation of osteopontin gene *in vivo* by PEBP2alphaA/CBFA1 and ETS1 in the skeletal tissues. *Oncogene* 1998; **17**: 1517–1525.
206. Sato M, Morii E, Takebayashi-Suzuki K, Yasui N, Ochi T, Kitamura Y, Nomura S. Microphthalmia-associated transcription factor interacts with PU.1 and c-Fos: determination of their subcellular localization. *Biochem Biophys Res Commun* 1999; **254**: 384–387.
207. Scherft JP, Groot CG. The electron microscopic structure of the osteoblast. In: Bonucci E, Motta PM, ed. *Ultrastructure of skeletal tissues: bone and cartilage in health and disease*. Boston: Kluwer Academic Publishers, 1990: 209–222.
208. Schinke T, Amendt C, Trindl A, Pöschke O, Müller-Esterl W, Jähnen-Dechent W. The serum protein  $\alpha_2$ -HS glyco-

- protein/fetuin inhibits apatite formation *in vitro* and in mineralizing calvaria cells – a possible role in mineralization and calcium homeostasis. *J Biol Chem* 1996; **271**: 20789–20796.
209. Schroeder HE. Biological problems of regenerative cementogenesis: synthesis and attachment of collagenous matrices on growing and established root surfaces. *Int Rev Cytol* 1992; **142**: 1–59.
  210. Schwarzbauer JE, Spencer CS. The *Caenorhabditis elegans* homologue of the extracellular calcium binding protein SPARC/osteonectin affects nematode body morphology and mobility. *Mol Biol Cell* 1993; **4**: 941–952.
  211. Scott PG, Nakano T, Dodd CM, Pringle GA, Kuc IM. Proteoglycans of the articular disc of the bovine temporomandibular joint. II. Low molecular weight dermatan sulfate proteoglycan. *Matrix* 1989; **9**: 284–292.
  212. Simonet WS, Lacey DL, Dunstan CR, Kelley M, Chang MS, Luthy R, Nguyen HQ, Wooden S, Bennett L, Boone T, Shimamoto G, DeRose M, Elliott R, Colombero A, Tan HL, Trail G, Sullivan J, Davy E, Bucay N, Renshaw-Gegg L, Hughes TM, Hill D, Pattison W, Campbell P, Boyle WJ. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. *Cell* 1997; **89**: 309–319.
  213. Smith AJ, Singhrao SK, Newman GR, Waddington RJ, Embury G. A biochemical and immuno-electron microscopic analysis of chondroitin sulfate-rich proteoglycans in human alveolar bone. *Histochem J* 1997; **29**: 1–9.
  214. Sodek J, Chen JK, Kasugai S, Nagata T, Zhang Q, McKee MD, Nanci A. Elucidating the functions of bone sialoprotein and osteopontin in bone formation. In: Slavkin H, Price P, ed. *Chemistry and biology of mineralized tissues*. Amsterdam: Elsevier Science, 1992: 297–306.
  215. Sodek J, Ganss B, McKee MD. Osteopontin. *Crit Rev Oral Biol Med* 1999; **10**: 79–98.
  216. Sodek J, Overall CM. Matrix metalloproteinases in periodontal tissue remodelling. *Matrix* 1992; **1**(suppl): 352–362.
  217. Sodek J. A comparison of the rates of synthesis and turnover of collagen and non-collagen proteins in adult rat periodontal tissues and skin using a microassay. *Arch Oral Biol* 1977; **22**: 655–665.
  218. Sommarin Y, Wendel M, Shen Z, Hellman U, Heinegård D. Osteoadherin, a cell-binding keratan sulfate proteoglycan in bone, belongs to the family of leucine-rich repeat proteins of the extracellular matrix. *J Biol Chem* 1998; **273**: 16723–16729.
  219. Soriano P, Montgomery C, Geske R, Bradley A. Targeted disruption of the c-src proto-oncogene leads to osteopetrosis in mice. *Cell* 1991; **64**: 693–702.
  220. Sterling H, Saginario C, Vignery A. CD44 occupancy prevents macrophage multinucleation. *J Cell Biol* 1998; **143**: 837–847.
  221. Suda T, Udagawa N, Nakamura I, Miyaura C, Takahashi N. Modulation of osteoclast differentiation by local factors. *Bone* 1995; **17**: 87S–91S.
  222. Sundquist KT, Väänänen HK, Marks SC Jr. Carbonic anhydrase II and H<sup>+</sup>-ATPase in osteoclasts of four osteopetrotic mutations in the rat. *Histochem Cell Biol* 1999; **111**: 55–60.
  223. Superti-Furga A, Rocchi M, Schafer BW, Gitzelmann R. Complementary DNA sequence and chromosomal mapping of a human proteoglycan-binding cell-adhesion protein (dermatopontin). *Genomics* 1993; **17**: 463–467.
  224. Suzuki N, Inukai M, Suzuki K, Nagai K, Hayashi A, Maeno M, Otsuka K. Cellular expression of alveolar bone-derived cells following mineralized nodule formation. *J Hard Tissue Biol* 1993; **2**: 28–36.
  225. Takagi M, Maeno M, Yamada T, Miyashita K, Otsuka K. Nature and distribution of chondroitin sulfate and dermatan sulfate proteoglycans in rabbit alveolar bone. *Histochem J* 1996; **28**: 341–351.
  226. Takahashi N, Mundy GR, Kuehl TJ, Roodman GD. Osteoclast like formation in fetal and newborn long term baboon marrow cultures is more sensitive to 1,25-dihydroxyvitamin D<sub>3</sub> than adult long term marrow cultures. *J Bone Miner Res* 1987; **2**: 311–317.
  227. Takahashi S, Goldring S, Katz M, Hilsenbeck S, Williams R, Roodman GD. Down regulation of calcitonin during human osteoclast-like cell differentiation. *J Clin Invest* 1995; **95**: 167–171.
  228. Takazawa Y, Rittling S, Furuya K, Yoshitake H, Yamashita T, Denhardt DT, Noda MJ. Interleukin-1-induced bone resorption does not occur in osteopontin-deficient mice. *Bone Miner Res* 1998; **23**: abstr T184, S246.
  229. Takeda K, Ichijo H, Fujii M, Mochida Y, Saitoh M, Nishitoh H, Sampath TK, Miyazono K. Identification of a novel bone morphogenetic protein-responsive gene that may function as a noncoding RNA. *J Biol Chem* 1998; **273**: 17079–85.
  230. Talmage RV. Morphological and physiological considerations in a new concept of calcium transport in bone. *Am J Anat* 1970; **129**: 467–476.
  231. Tamaki K, Souchelnytskyi S, Itoh S, Nakao A, Sampath K, Heldin CH, ten Dijke P. Intracellular signaling of osteogenic protein-1 through Smad5 activation. *J Cell Physiol* 1998; **177**: 355–363.
  232. Taylor GW, Burt BA, Becker MP, Genco RJ, Schlossman M. Glycemic control and alveolar bone loss progression in type 2 diabetes. *Ann Periodontol* 1998; **3**: 30–39.
  233. Termine JD, Belcourt AB, Conn KM, Kleinman HK. Mineral and collagen-binding proteins of fetal calf bone. *J Biol Chem* 1981; **256**: 10403–10408.
  234. Termine JD, Kleinman HK, Whitson SW, Conn KM, McGarvey ML, Martin GR. Osteonectin, a bone-specific protein linking mineral to collagen. *Cell* 1981; **26**: 99–105.
  235. Teti A, Zamboni-Zallone A. Osteoclast cytoskeleton and attachment proteins. In: Rifkin BR, Gay CV, ed. *Biology and physiology of the osteoclast*. Boca Raton, FL: CRC Press, 1992: 245–257.
  236. Thomson BM, Mundy GR, Chambers TJ. Tumor necrosis factors alpha and beta induce osteoblastic cells to stimulate osteoclastic bone resorption. *J Immunol* 1987; **138**: 775–779.
  237. Tondravi MM, McKercher SR, Anderson K, Erdmann JM, Quiroz M, Maki R, Teitelbaum SL. Osteopetrosis in mice lacking haematopoietic transcription factor PU.1. *Nature* 1997; **386**: 81–84.
  238. Tonna EA. An electron microscopic study of skeletal cell aging: the osteocyte. *Exp Gerontol* 1973; **8**: 9–16.
  239. Tonna EA. Electron microscopic evidence of alternating osteocytic osteoclastic and osteoplastic activity in the perilacunar walls of aging mice. *Z Zellforsch* 1972; **1**: 221–230.
  240. Traub W, Arad T, Weiner S. Origin of mineral crystal growth in collagen fibrils. *Matrix* 1992; **12**: 251–255.
  241. Trelstad RL, Hayashi K. Tendon collagen fibrillogenesis:

- intracellular subassemblies and cell surface changes associated with fibril growth. *Dev Biol* 1979; **71**: 228–242.
242. Tsoukas CD, Provedini DM, Manolagas SC. 1,25-Dihydroxyvitamin D<sub>3</sub>: a novel immunoregulatory hormone. *Science* 1984; **224**: 1438–1440.
243. Turner CH, Pavalko FM. Mechanotransduction and functional response of the skeleton to physical stress: the mechanisms and mechanics of bone adaptation. *J Orthop Sci* 1998; **3**: 346–355.
244. Väänänen HK, Karhukorpi E-K, Sundquist K, Wallmark B, Roininen I, Hentunen T, Tuukkanen J, Lakkakorpi P. Evidence for the presence of a proton pump of the vacuolar H<sup>+</sup>-ATPase type in the ruffled borders of osteoclasts. *J Cell Biol* 1990; **111**: 1305–1311.
245. Väänänen HK, Parvinen E-K. High active isoenzyme of carbonic anhydrase in rat calvaria osteoclasts. *Histochem* 1983; **78**: 481–485.
246. Veis A, Sabsay B. The collagen of mineralized matrices. In: Peck WA, ed. *Bone Miner Res* 1987; **5**: 1–63.
247. Vetter U, Vogel W, Just W, Young MF, Fisher LW. Human decorin gene: intron-exon junctions and chromosomal localization. *Genomics* 1993; **15**: 161–168.
248. Waddington RJ, Embery G. Structural characterization of human alveolar bone proteoglycans. *Arch Oral Biol* 1991; **36**: 859–866.
249. Wang HM, Nanda V, Rao LG, Melcher AH, Heersche JN, Sodek J. Specific immunohistochemical localization of type III collagen in porcine periodontal tissues using the peroxidase-antiperoxidase method. *J Histochem Cytochem* 1980; **28**: 1215–1223.
250. Weiner S, Traub W. Organization of hydroxyapatite crystals within collagen fibrils. *FEBS Lett* 1986; **206**: 262–266.
251. Weinstock A, Weinstock M, Leblond CP. Autoradiographic detection of <sup>3</sup>H-fucose incorporation into glycoprotein by odontoblasts and its deposition at the site of the calcification front in dentin. *Calcif Tissue Res* 1972; **8**: 181–189.
252. Weinstock M, Leblond CP. Radioautographic visualization of the deposition of a phosphoprotein at the mineralization front in the dentin of the rat incisor. *J Cell Biol* 1973; **56**: 838–845.
253. Weinstock M, Leblond CP. Synthesis, migration and release of precursor collagen by odontoblasts as visualized by radioautography after <sup>3</sup>H-proline administration. *J Cell Biol* 1974; **60**: 92–127.
254. Weinstock M. Elaboration of precursor collagen by osteoblasts as visualized by radioautography after <sup>3</sup>H-proline administration. In: Slavkin HC, Greulich RC, ed. *Extracellular matrix influences in gene expression*. New York: Academic Press, 1975: 119–128.
255. Weinstock M. Radioautographic visualization of <sup>3</sup>H-fucose incorporation into glycoprotein by osteoblasts and its deposition into bone matrix. *Calcif Tissue Int* 1979; **27**: 177–185.
256. Wendel M, Sommarin Y, Heinegård D. Bone matrix proteins: isolation and characterization of a novel cell-binding keratan sulfate proteoglycan (osteoadherin) from bovine bone. *J Cell Biol* 1998; **141**: 839–847.
257. Wozney JM. The bone morphogenetic protein family and osteogenesis. *Mol Reprod Dev* 1992; **32**: 160–167.
258. Wrana JL, Maeno M, Hawrylyshyn B, Yao K-L, Domenicucci C, Sodek J. Differential effects of transforming growth factor- $\beta$  on the synthesis of extracellular matrix proteins by normal fetal rat calvarial bone cell populations. *J Cell Biol* 1988; **106**: 915–921.
259. Xiao G, Wang D, Benson MD, Karsenty G, Franceschi RT. Role of the  $\alpha$ 2-integrin in osteoblast-specific gene expression and activation of the *Osf2* transcription factor. *J Biol Chem* 1998; **273**: 32988–32994.
260. Xu T, Bianco P, Fisher LW, Longenecker G, Smith E, Goldstein S, Bonadio J, Boskey A, Heegaard AM, Sommer B, Satomura K, Dominguez P, Zhao C, Kulkarni AB, Robey PG, Young MF. Targeted disruption of the biglycan gene leads to an osteoporosis-like phenotype in mice. *Nat Genet* 1998; **20**: 78–82.
261. Yamauchi M, Katz EP, Mechanis GL. Intermolecular cross-linking and stereospecific molecular packing in type I collagen fibrils of the periodontal ligament. *Biochemistry* 1986; **25**: 4907–4913.
262. Yao K-L, Todescan Jr. R, Sodek J. Temporal changes in matrix protein synthesis and mRNA expression during mineralized tissue formation by adult rat bone marrow cells in culture. *J Bone Miner Res* 1994; **9**: 231–240.
263. Yasuda H, Shima N, Nakagawa N, Yamaguchi K, Kinoshita M, Mochizuki S-I, Tomoyasu A, Yano K, Goto M, Murakami A, Tsuda E, Morinaga T, Higashio K, Udagawa N, Takahashi N, Suda T. Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. *Proc Natl Acad Sci U S A* 1998; **95**: 3597–3602.
264. Yeager VL, Chiemchanya S, Chaiseri P. Changes in size of lacunae during the life of osteocytes in osteons of compact bone. *J Gerontol* 1975; **30**: 9–14.
265. Yoshida H, Hayashi S, Kunisada T, Ogawa M, Nishikawa S, Okumura H, Sudo T, Shultz LD, Nishikawa S-I. The murine mutation osteopetrosis is in the coding region of the macrophage colony stimulating factor gene. *Nature* 1990; **345**: 442–444.
266. Yoshitake H, Rittling S, Denhardt DT, Noda M. Osteopontin-deficient mice are resistant to ovariectomy-induced bone resorption. *Proc Natl Acad Sci U S A* 1999; **96**: 8156–8160.
267. Young MF, Ibaraki K, Kerr JM, Heegard A-M. Molecular and cellular biology of the major noncollagenous proteins in bone. In: Noda M, ed. *Cellular and molecular biology of bone*. San Diego: Academic Press, 1993: 191–234.
268. Young MF, Kerr JM, Termine JD, Wewer UM, Wang MG, McBride OW, Fisher LW. cDNA cloning, mRNA distribution and heterogeneity, chromosomal location, and RFLP analysis of human osteopontin (OPN). *Genomics* 1990; **7**: 491–502.
269. Zohar R, Sodek J, McCulloch CAG. Characterization of stromal progenitor cells enriched by flow cytometry. *Blood* 1997; **90**: 3471–3481.
270. Zohar R, Lee W, Arora P, Cheifetz S, McCulloch C, Sodek J. Single cell analysis of intracellular osteopontin in osteogenic cultures of fetal rat calvarial cells. *Cell Physiol* 1997; **170**: 88–100.
271. Zung P, Domenicucci C, Wasi S, Kuwata F, Sodek J. Osteonectin is a minor component of mineralized connective tissues in rat. *Can J Biochem Cell Biol* 1986; **64**: 356–362.