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Scientific Section

REVIEW SERIES

"This is the first in a series of articles where advances in basic sciences relevant to clinical orthodontics, are presented by clinicians with a research interest. This first article on bone modelling by Peter Hill, sets a high standard for successive articles. Further articles on muscle biology, regulation of tissue turnover, craniofacial development, cell signalling and cytokines will appear in future journal issues. It is hoped that these articles will be of interest, not only to postgraduate students but also established clinicians".

J. Sandy, Sub Editor

Bone Remodelling

P. A. HILL, B.D.S., F.D.S., M.ORTH., B.SC, M.SC., PH.D.

Department of Orthodontics and Paediatric Dentistry, UMDS of Guy's and St.Thomas' Hospitals, London Bridge, London SE1 9RT.

Introduction

The remarkable increase in research in bone biology during the last two decades has both enhanced our understanding of the regulation of bone remodelling and enabled us to define some of the major unanswered questions. Bone is a dynamic tissue that constantly undergoes remodelling even once growth and modelling of the skeleton have been completed. Bone remodelling is a coupled process in which there is localized removal of old bone (resorption) and replacement with newly formed bone. The process is complex, requiring interaction between different cell phenotypes, that are regulated by a variety of biochemical and mechanical factors. It is likely that the major reason for remodelling is to enable the bones to respond, and adapt to mechanical stresses as occurs as a result of physical exercise and during mechanical loading as occurs during orthodontic tooth movement. Abnormalities in bone remodelling occur in some of the most common diseases that affect humans such as osteoporosis, periodontitis, arthritis, and tumour-induced osteolysis. Although these disorders are common and cause considerable suffering, in most cases little is known of the mechanisms responsible for the dysfunctional bone remodelling that characterizes them. This is not unexpected, since at present we do not understand the mechanisms responsible for the control of normal bone remodelling or how it is so highly co-ordinated and balanced. However, novel techniques for studying bone function at the cellular level (bone cell and organ culture), the availability of recombinant molecules and complementary DNA probes, the new understanding revealed by gene 'knockout' and transgenic experiments, as well as new techniques for studying bone function at the clinical level, should clarify the control mechanisms for the cellular events in normal bone remodelling, and seem certain to lead ultimately to new information and treatment measures to inhibit or prevent these disorders. Furthermore, an understanding of the biochemical and molecular mechanisms that enable bone cells to adapt to changes in their mechanical environment is essential for the practice of clinical orthodontics and for its future development as an academic discipline. This review outlines our current understanding of bone remodelling and its regulation as a basis to addressing the unanswered questions.

Bone Structure and Remodelling

Bone is a specialized connective tissue composed of both mineral and organic phases that is exquisitely designed for its role as the load-bearing structure of the body. To accomplish this task, it is formed from a combination of dense compact bone and cancellous (trabecular) bone that is re-inforced at points of stress. The mineral phase of the skeleton contributes about two-thirds of its weight, the remaining one-third is organic matrix, consisting primarily of type I collagen and small amounts of non-collagenous proteins. Two principle cell types are found in bone, the osteoclast, and the osteoblast, which are the major effectors in the turnover of bone matrix (Fig. 1). The osteoblast produces the matrix which becomes mineralized in a well regulated manner. This mineralized matrix can be removed by the activity of the osteoclast when activated.

Bone is constantly undergoing bone remodelling which is a complex process involving the resorption of bone on a particular surface, followed by a phase of bone formation. In normal adults, there is a balance between the amount of bone resorbed by osteoclasts and the amount of bone formed by osteoblasts (Frost, 1964). Bone remodelling occurs in small packets of cells called basic multicellular units (BMUs), which turn bone over in multiple bone surfaces (Frost, 1991); at any one time, ~20% of the cancellous bone surface is undergoing remodelling. Each BMU is geographically and chronologically separated from other packets of remodelling. This suggests that activation of the sequence of cellular events responsible for remodelling is locally controlled, perhaps by autocrine or paracrine factors generated in the bone micro-environment. The current concept of bone remodelling is based on the hypothesis that osteoclastic precursors become activated and differentiate into osteoclasts, and

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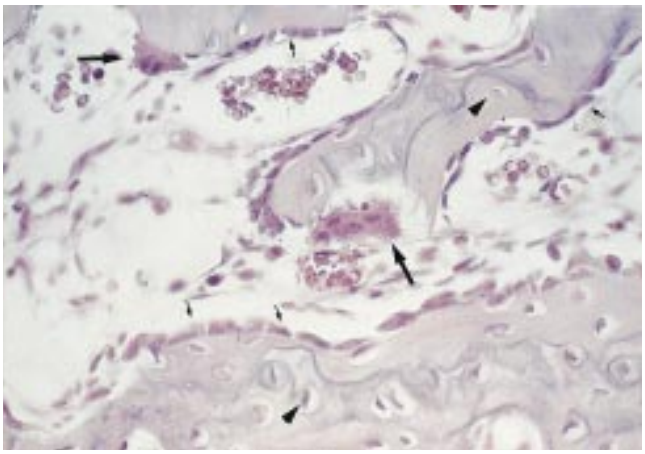

FIG. 1 Light micrograph of trabecular bone. Multinucleate osteoclasts (large arrows) are resorbing calcified bone in a Howship's resorption lacuna, while osteoblasts (small arrows) are laying down matrix on the surface of osteoid. Osteocytes (arrowheads) are found within the mineralized matrix. (Haematoxylin and Eosin, x80, enlarged to 250% on reproduction).

this begins the process of bone resorption. This step is followed by a bone formation phase. The number of sites entering the bone formation phase, called the activation frequency, together with the individual rates of the two processes, determines the rate of tissue turnover (Charles *et al*., 1987; Ericksen *et al*., 1986).

The signal that initiates bone remodelling has not been identified, but evidence shows that mechanical stress can alter local bone architecture. The requirement for mechanical tension in the formation of bony tubercles at sites of tendon insertions is elegantly demonstrated in mice in which both the *myf-5* and *myoD* genes are inactivated (Rudnicki *et al*., 1993). These mice lack bony tubercles presumably as a result of impaired muscle development and, therefore, reduced mechanical tension at tendon insertion sites. While prostaglandins have been implicated, how tension is sensed by resident bone cells and how such signals contribute to the cellular and molecular control of bone remodelling are major unresolved issues in skeletal biology. More recently, it has been shown that mechanical stress can be sensed by osteocytes and that these cells

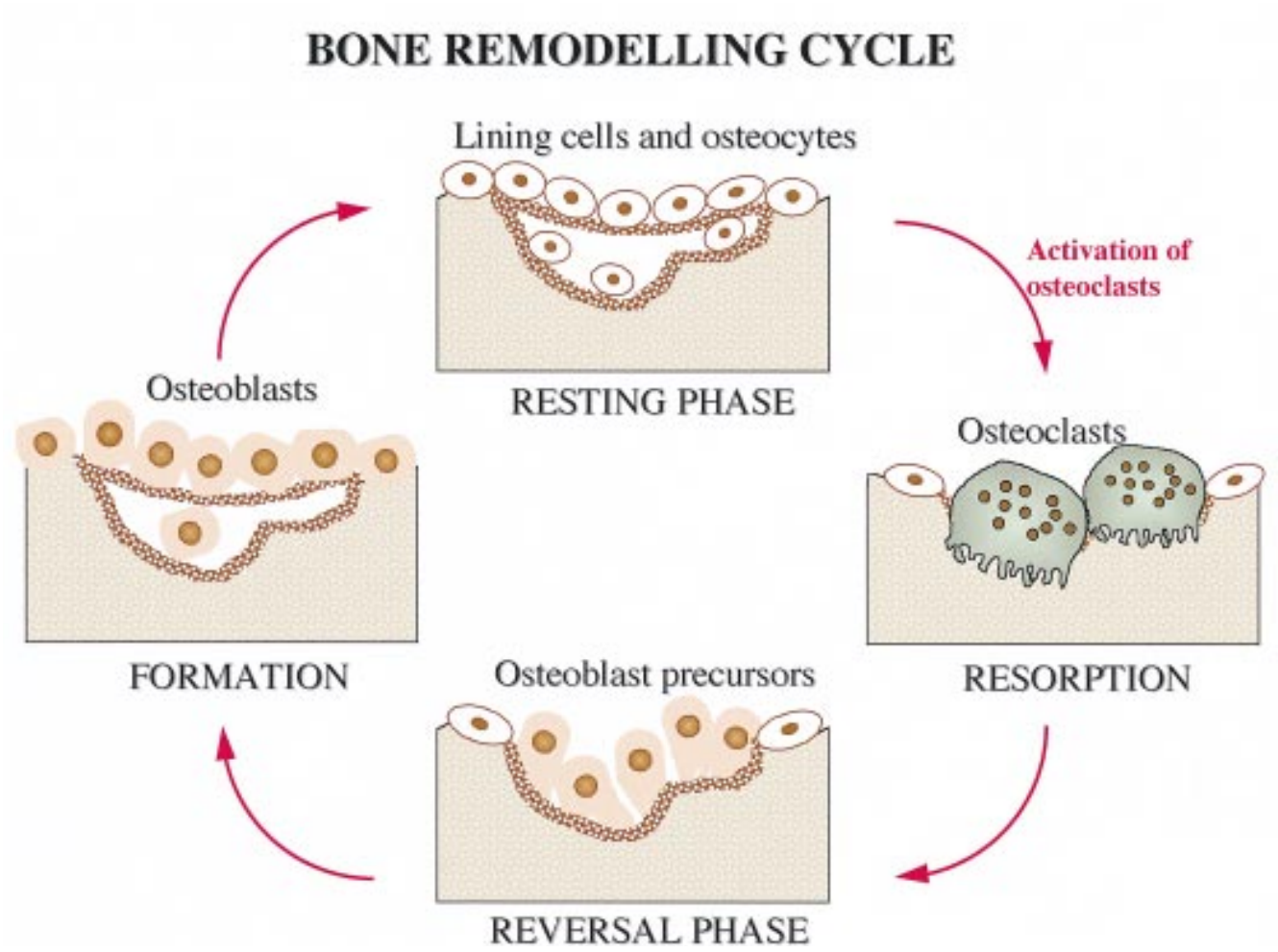

FIG. 2. Stages of bone remodelling. Resorptive phase: activated multinucleated osteoclasts derived from bone marrow monocytes resorb a discrete area of mineralized bone matrix. Reversal phase: subsequently osteoprogenitor (osteoblast precursor) cells, which can locally proliferate and differentiate into osteoblasts, migrate into the resorption lacuna and disclose the former osteoclastic activity. Formative phase: the osteoblasts deposit new bone matrix, which is initially unmineralized and called osteoid, and in this way fill the resorption lacuna. Resting phase: once embedded in osteoid, the osteoblasts mature into terminally differentiated osteocytes. The osteoblasts lying on the surface of the newly formed bone packet are quiescent lining cells until activated.

secrete paracrine factors such as insulin-like growth factor (IGF)-I in response to mechanical forces (Lean *et al.*, 1996). Although IGF-I may act as a coupling factor in the bone remodelling cycle (see below), the signal that initiates the cycle remains elusive. The sequence of events in the normal remodelling cycle are always the same, osteoclastic bone resorption, a reversal phase, followed by osteoblastic bone formation to repair the defect (Fig. 2).

The termination of bone resorption and the initiation of bone formation in the resorption lacunae occurs through a coupling mechanism (Parfitt, 1982). The coupling process ensures that an equivalent amount of bone is laid down following the previous resorption phase. The detailed nature of the activation and coupling mechanism is still unknown, although some growth factors and proteinases, such as transforming growth factor (TGF)-β, IGFs I and II, and plasminogen activators have been proposed (Martin and Ng, 1994; Mundy, 1994). Whether the activation of osteoblasts begins simultaneously with osteoclast recruitment or at some later stage during lacunar development is still unsettled. A model illustrating this 'coupling' process is presented in Fig. 3. During resorption, the osteoclasts release local factors from the bone, which have two effects: inhibition of osteoclast function and stimulation of osteoblast activity. Moreover, osteoclasts themselves produce and release factors that have a negative regulatory effect on their activity, and enhance osteoblast function. Finally, when the osteoclasts complete the resorptive cycle, they secrete proteins that later serve as a substrate for osteoblast attachment (McKee *et al.*, 1993).

Bone remodelling is regulated by systemic hormones and by local factors, which affect cells of both the osteoclast and osteoblast lineages and exert their effects on (i) the replication of undifferentiated cells, (ii) the recruitment of cells, and (iii) the differentiated function of cells (see Tables 1 and 2; Canalis, 1983). The end product of remodelling is the maintenance of a mineralized bone matrix and the major organic component of this matrix is type I collagen. The local factors are synthesized by skeletal cells and include growth factors, cytokines, and

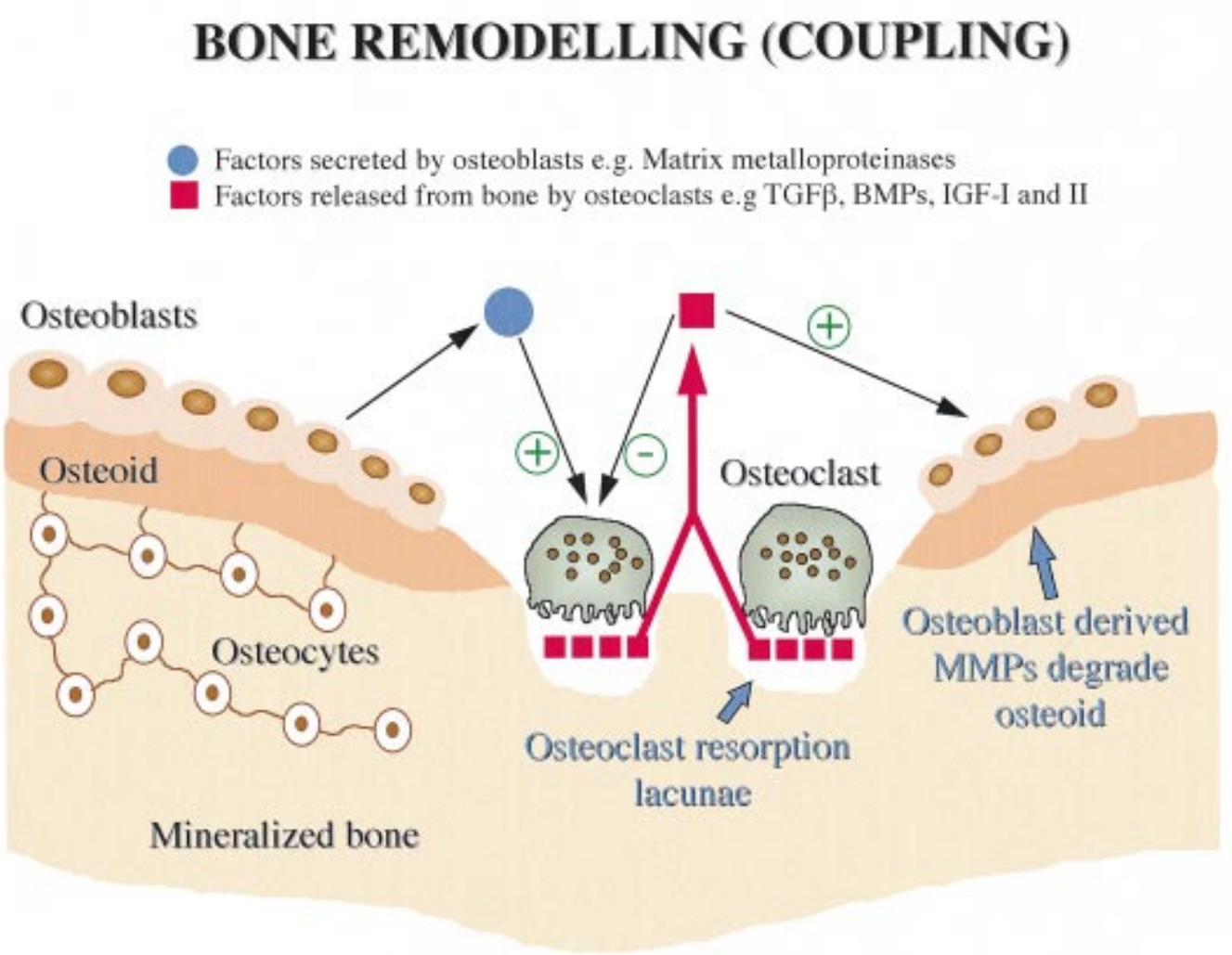

FIG. 3. Bone remodelling (coupling). Diagrammatic representation of the coupling of osteoclastic bone resoprtion followed by osteoblastic bone formation. The initial event involves the synthesis and release of matrix metalloproteinases (MMPs) by osteoblasts which are responsible for degrading the osteoid, exposing the mineralized matrix which maybe chemotactic to the osteoclast. The osteoblast also directly stimulates osteoclast activity. During the resorption process growth factors are released from the matrix which then activate osteoprogenitor cells. The osteoprogenitor cells mature into osteoblasts and ultimately replace the resorbed bone. The mechanism by which osteoblasts are directed to form bone only in the resorption lacunae may be due to the presence of molecules such as TGF-β and BMPs which are left behind during osteoclastic activity. Osteocytes communicate with one another via intercellular processes.

TABLE 1 *Hormones that regulate bone remodelling*

Polypeptide hormones
Parathyroid hormone
Calcitonin
Insulin
Growth hormone
Steroid hormones
1,25-Dihydroxyvitamin D3
Glucocoticoids
Sex steroids
Thyroid hormones

TABLE 2. *Growth factors that regulate bone remodelling*

Insulin-like growth factors (IGF) I and II
Transforming growth factor-b (TGF-b) superfamily, including the bone morphogenetic proteins (BMPs).
Fibroblast growth factors (FGF)
Platelet-derived growth factors (PDGF)
Selected cytokines of the interleukin (IL), tumour necroisis factor (TNF), and colony-stimulating factor (CSF) families.

prostaglandins. Growth factors are polypeptides that regulate the replication and differentiated function of cells. Growth factors have effects on cells of the same class (autocrine factors) or on cells of another class within the tissue (paracrine factors). The presence of local factors is not unique to the skeletal system, because non-skeletal tissues also synthesize, and respond to autocrine and paracrine factors. Growth factors are also present in the circulation and may act as systemic regulators of skeletal metabolism, but the locally produced factors have more direct and important functions in cell growth. Growth factors may play a critical role in the coupling of bone formation to bone resorption, and possibly in the pathophysiology of bone disorders.

Bone Resorption

The bone resorption cascade involves a series of steps directed towards the removal of both the mineral and organic constituents of bone matrix by osteoclasts, aided by osteoblasts. The role of the osteoclast as a major resorbing cell, and its structure and biochemical properties have been well characterized (Roodman, 1996). The first stage involves the recruitment and dissemination of osteoclast progenitors to bone. The osteoclast precursors are clearly of haemopoietic origin (Walker, 1973) and related to the monocyte-macrophage lineage, but the point of divergence from that lineage is not established (Hattersley *et al*., 1991). The progenitor cells are recruited from the haemopoietic tissues such as bone marrow and slenic tissues to bone via the circulating blood stream. They proliferate and differentiate into osteoclasts through a mechanism involving cell-to-cell interaction with osteoblast stromal cells (Suda *et al.*, 1996). It seems likely that a subpopulation of marrow and circulating monocytes are, in fact, determined pre-osteoclasts. The next step involves the prepartion of the bone surface by removal of the unmineralized osteoid layer by the lining osteoblasts, which produce a variety of proteolytic enzymes, in particular the matrix metalloproteinases (MMPs), collagenase and gelatinase (Meikle *et al*., 1992). This facilitates access of the osteoclasts to the underlying mineralized bone. The next step involves the recognition of extracellular bone matrix proteins via members of the integrin superfamily of adhesion receptors, in particular the $\alpha v\beta 3$ vitronectin receptor (Lakkakorpi *et al*., 1991). The vitronectin receptor binds to the extracellular matrix proteins, such as osteopontin, at a tripeptide arginine-glycine-aspartic acid (RGD) recognition site and appears essential for inducing osteoclast polarization. The latter process involves the formation of ruffled borders and clear zones, two of the most characteristic features of osteoclasts (Roodman, 1996; Suda *et al*., 1996). The clear zone is an organelle-free region of the cytoplasm that is rich in F-actin filaments (actin rings). These actin rings in concert with the integrin receptors and RGD containing-extracellular proteins form focal adhesions or podosomes. The focal adhesions are responsible for the tight cell-to-substratum interaction and seal the external space beneath the cell where the ruffled border spreads and bone matrix dissolution occurs. This extracellular space is called the 'resorbing compartment' or 'resorption lacuna'. The third stage involves osteoclast activation at the surface of the mineralized bone. This is probably initiated by the effects of local factors on cells of the osteoblast lineage rather than direct activation of osteoclasts and their precursors (Martin and Ng, 1994). In addition to the classical concept that osteoblasts release an osteoclast activating factor(s), recent findings have proposed a new concept that osteoblasts may activate osteoclasts through a mechanism involving cell-to-cell contact (Fuller *et al*., 1991). The next step involves the activated osteoclast resorbing the bone by the production of hydrogen ions (dissolution of mineral) and proteolytic enzymes (degradation of organic matrix) in the localized environment (hemivacuole) under the ruffled border of the cell. Hydrogen ions are generated within the cell by the enzyme carbonic anhydrase II (Laitala and Vaananen, 1994) which is located in the cytoplasm close to the ruffled border (Gay and Mueller, 1974). The critical importance of carbonic anhydrase II in the osteoclast has been shown by studies in patients with a congenital absence of this enzyme and osteopetrosis (Sly *et al.*, 1985). The protons are extruded across the ruffled border into the resorptive micro-environment by a polarized vacuolar proton pump (Blair *et al*., 1989). Degradation of the collagenous organic matrix follows dissolution of the mineralized matrix and involves two major classes of enzymes, lysosomal cysteine proteinases such as cathepsin B, L and K (Hill *et al*., 1994a; Drake *et al*., 1996), and MMPs including collagenase and gelatinase B (Hill *et al*., 1993;1994b;1995).

Osteoclasts ultimately undergo apoptosis or programmed cell death that is characterized by nuclear and cytoplasmic condensation, and fragmentation of nuclear DNA into nucleosomal-sized units. TGF-β, which blocks bone resorption can induce apoptosis in osteoclasts, while osteoclast-stimulatory factors, such as parathyroid hormone, PTH, and 1,25-dihydroxyvitamin D_3, inhibit osteoclast apoptosis *in vitro* (Roodman, 1996). These data suggest that regulation of osteoclast life span plays an important role in the normal bone remodelling process to either enhance or inhibit osteoclastic bone resorption. Cytokines that enhance osteoclast activity do so in part by

increasing osteoclast life span and factors that inhibit osteoclast activity appear to induce osteoclast apoptosis, in addition to blocking osteoclast formation and bone resorption. Since osteoclasts have a limited life span ~12·5 days, the progression of bone remodelling requires the continual addition of osteoclasts precursors to maintain an existing team. Mononuclear osteoclast precursors need to be directed to their destination by a specific 'homing' signal. It has been suggested that the targeting of pre-osteoclasts for the initiation of remodelling is carried out by lining cells under instruction from osteocytes, but that the targeting for progression is carried out by osteoclasts themselves. Evidence suggests that osteoclasts may release paracrine factors, in particular interleukins (IL)-1, IL-6, and annexin-II that are concerned with osteoclast recruitment (Roodman, 1996).

Reversal

After the maximum eroded depth has been achieved by the osteoclasts, there is a reversal phase that lasts ~9 days. The regulatory mechanisms that arrest osteoclastic activity are poorly understood, but there are several possibilities. First, since the osteoclast has a limited life span, the cell probably undegoes apoptosis following an extensive episode of resorptive activity. Secondly, it has been demonstrated that the accumulation of calcium at high concentrations in the resorption lacunae directly controls osteoclast activity causing both rapid cell retraction and in the longer term, an inhibiton of enzyme release and bone resorption (Zaidi *et al*., 1990). A third possibility is that the release of TGF-β or related peptides from the matrix during the resorption process inactivates osteoclasts and attracts osteoblasts (Pfeilschifter and Mundy, 1987; Pfeilschifter *et al.*, 1990a,b). During the reversal phase, osteoclasts disappear and macrophage-like cells are seen on the bone surface. These latter cells could release factors that inhibit osteoclasts and stimulate osteoblasts. Macrophages may also remove residual matrix since they are richer in collagenase than the osteoclast.

Bone Formation

Bone formation results from a complex cascade of events that involves proliferation of primitive mesenchymal cells, differentiation into osteoblast precursor cells (osteoprogenitor, pre-osteoblast), maturation of osteoblasts, formation of matrix, and finally mineralization. Osteoblasts converge at the bottom of the resorption cavity and form osteoid which begins to mineralize after 13 days at an initial rate of ~1μm/day. The osteoblasts continue to form and mineralize osteoid until the cavity is filled. The time to fill in the cavity at any given point on the surface is 124–168 days in normal individuals (Ericksen *et al.*, 1984).

At the bottom of the cavity osteoblasts are plump and vigorous, they have tall nuclei, and they make a thick layer of osteoid. The cells gradually flatten and become quiescent lining cells. Some of the osteoblasts differentiate into osteocytes and become embedded in the matrix.

The initial event must be the chemotactic attraction of osteoblasts or their precursors to sites of the resorption defect. This is likely to be mediated by local factors produced during the resorption process. Resorbing bone has been shown to produce chemotactic factors for cells with osteoblast characteristics *in vitro* (Mundy *et al*., 1982). One mediator that may be responsible for this effect is TGF-β, since active TGF-β is released by resorbing bone cultures (Pfeilschifter and Mundy, 1987) and TGF-β is chemotactic for bone cells (Pfeilschifter *et al*., 1990a,b). Structural proteins such as collagen could also be involved, since type I collagen and its fragments cause the same effect (Mundy *et al*., 1982).

The second event involved in the formation phase of the coupling phenomenon is proliferation of osteoblast precursors. This is likely to be mediated by osteoblast-derived growth factors and those growth factors released from bone during the resorption process. There are several leading candidates which represent autocrine and paracrine factors. These include members of the TGF-β superfamily and several other growth factors that are sequestrated in bone matrix and stimulate osteoblast proliferation, including IGF- I and II, fibroblast growth factors (FGFs) and platelet-derived growth factor (PDGF). Interestingly, these growth factors may have a more subtle role to play in bone formation as they have recently been shown to prevent osteoblast apoptosis *in vitro* (Hill *et al*., 1997).

The third event of the formation phase is the differentiation of the osteoblast precursor into the mature cell. Several of the bone-derived growth factors can cause the appearance of markers of the differentiated osteoblast phenotype, including expression of alkaline phosphatase activity, type I collagen, and osteocalcin.. Most prominent of these are IGF-I and bone morphogenetic protein (BMP)-2, the latter is a member of the TGF-β superfamily of polypeptides. The resorption lacunae are usually repaired completely, although it is not known how this is achieved. The cessation of osteoblast activity may be due to negative feedback inhibiton or the induction of osteoblast apoptosis by tumour necrosis factor released from neighbouring marrow cells (Hill *et al.*, 1997).

Local regulation of bone remodelling

Bone is a rich source of growth factors with important actions in the regulation of bone formation and bone resorption (Tables 1 and 2). Frequently, these local factors are synthesized by skeletal cells, although some cytokines are secreted by stromal cells and by cells of the immune or haematological system, and as such they are present in the bone microenvironment (Manologas and Jilka, 1995). These factors are likely to be released locally from bone as it resorbs or by bone cells activated as a consequence of the resorption process. They may then act in a sequential manner to regulate all of the cellular events required for the formation of bone.

The TGF-β superfamily may be particularly important in the coupling that links bone formation to prior bone resorption (Fig. 3). It has been proposed that the following sequence of events occur during normal bone remodelling.

Bone resorption leads to the release of active TGF-β from bone (Pfeilschifter and Mundy, 1987) and exposure of osteoblast precursors to active TGF-β causes pro-

liferation. However this exposure to TGFβ is transient and, as a consequence, the proliferating cells undergo differentiation and express BMPs. The latter are responsible for an autostimulatory effect on osteoblasts and the formation of mineralized nodules. Of course, it is unlikely that the TGF-β superfamily members are acting alone. Other growth factors such as IGFs, FGFs, and PDGF are also likely to be having effects on osteoblast proliferation and differentiation. These factors are all bone growth stimulants. There is much evidence to suggest that there are synergistic, as well as inhibitory interactions between the growth factors that act on osteoblasts. For example, TGF-β, FGFs, PDGF, BMPs, IGFs-I, and II may all influence osteoblasts directly, but also may modulate osteoblast responsiveness to these other growth factors (Massague, 1985; Roberts *et al*., 1985). The potential interactions between these factors are complex, but it will be essential to unravel them to understand the local control of bone formation. It is likely that the complicated interactions between these factors released locally in active form as a consequence of the resorption process are responsible for the carefully co-ordinated formation of new bone that occurs at these sites.

A potentially fruitful new approach to the problem of understanding the role of these growth factors in bone remodelling is to establish appropriate transgenic mouse models to study the many paracrine growth and differentiation factors that, based upon cell culture studies, have been implicated in bone remodelling (see Tables 1 and 2). The initial success of this approach can be seen with demonstration that transgenic mice over-expressing interleukin-4 develop osteoporosis due to impaired osteoblastic function (Lewis *et al*., 1993).

Conclusions

Bone remodelling is a complex process involving a number of cellular functions directed toward the co-ordinated resorption and formation of new bone. Bone remodelling is regulated by systemic hormones and by local factors. Hormones regulate the synthesis, activation, and effects of the local factors that have a direct action on cellular metabolism, and they modify the replication and differentiated function of cells of the osteoclast or osteoblast lineage. It is possible that the role of the hormones is to provide tissue specificity for a given growth factor, because most of these factors are synthesized by a variety of skeletal and non-skeletal cells.

The rapidly accumulating new knowledge about the multiple possible regulatory mechanisms within bone should aid the understanding of physiological bone remodelling and also offer potential explanations for the changes in bone turnover seen in a variety of disease states. This knowledge will be important in devising new therapeutic strategies to control bone formation and resorption based upon these novel regulatory mechanisms.

References

Blair, H., Teitelbaum, S., Ghiselli, R. and Gluck, S. (1989)
Osteoclastic bone resorption by a polarised vacuolar proton pump,
Science, **245**, 855–857.

Canalis, E. (1983)
The hormonal and local control of bone formation,
Endocrine Reviews, **4,** 62–77.

Charles, P., Ericksen, E.F., Mosekilde, L., Melsen, F. and Jensen, F.T. (1987)
Bone turnover and balance evaluated by a combined calcium balance and 47calcium kinetic study and dynamic histomorphometry,
Metabolism: Clinical and Experimental, **36**, 1118–1124.

Drake, F.H., Robert, A.D., James, I.E., Conner, J.R., Debouck, C.C., Richardson, S., Lee-Rykaczewski, E., Coleman, L., Rieman, D., Barthlow, R., Hastings, G. and Gowen, M. (1996)
Cathepsin K, but not cathepsins B, L, or S, is abundantly expressed in human osteoclasts,
Journal of Biological Chemistry, **269,** 15006–15009.

Ericksen, E., Mosekilde, L. and Melsen, F. (1986)
Trabecular bone remodelling and balance in primary hyperparathyroidism,
Bone, **7,** 213–221.

Fuller, K., Gallagher, A. C. and Chambers, T. J. (1991)
Osteoclast resorption-stimulating activity is associated with osteoblast surface and/or extra cellular matrix,
Biochemical and Biophysical Research Communications, **181**, 67–73.

Frost, H.M. (1964)
Dynamics of bone remodelling,
In: *Bone Biodynamics,* Frost H. M. (ed.), pp.315–333,
Little Brown, Boston.

Frost, H. (1991)
A new direction for osteoporosis research: a review and proposal,
Bone, **12,** 429–437.

Gay, C. and Mueller, W. (1974)
Carbonic anhydrase and osteoclasts: localization by labelled autoradiography,
Science, **183,** 432–434.

Hattersley, G., Kerby, J. and Chambers, T. (1991)
Generation of osteoclast precursors in multilineage hemopoietic colonies,
Endocrinology, **128**, 259–262.

Hill, P. A., Reynolds, J. J. and Meikle, M. C. (1993)
Inhibition of stimulated bone resoprtion *in vitro* by TIMP-1 and TIMP-2,
Biochemica et Biophysica Acta, **1177,** 71–74.

Hill, P. A., Buttle, D., Jones, S., Boyde, A., Murata, M., Reynolds, J. J. and Meikle, M. C. (1994a)
Inhibition of bone resorption by selective inactivators of cysteine proteinases,
Journal of Cellular Biochemistry, **56**, 118–130.

Hill, P. A., Murphy, J., Docherty, A., Hembry, R., Millican, T., Reynolds, J. J. and Meikle, M. C. (1994b)
The effects of selective inhibitors of matrix metalloproteinases (MMPs) on bone resoprtion and the identification of MMPs and TIMP-1 in isolated osteoclasts,
Journal of Cell Science, **107**, 3055–3064.

Hill, P. A., Docherty, A., Bottomley K., O'Connell, J. P., Morphy, J. R. Reynolds, J. and Meikle, M. C. (1995)
Inhibition of bone resorption in vitro by selective inhibitors of gelatinase and collagenase,
Biochemical Journal, **308,** 167–175.

Hill, P. A., Tumber, A. and Meikle, M. C. (1997)
Multiple extracellular signals promote osteoblast survival and apoptosis,
Endocrinology, **138**, 3849–3858.

Laitala, T. and Vaananen, H. K. (1994)
Inhibition of bone resorption *in vitro* by antisense RNA and DNA molecules targeted against carbonic anhydrase II or two subunits of vacuolar H(+)-ATPase,
Journal of Clinical Investigation, **93**, 2311–2318.

Lakkakorpi, P., Horton,M., Helfrich, M., Karhukorp, E. and Vaananen, H. K. (1991)
Vitronectin receptor has a role in bone resorption but does not mediate tight sealing zone attachment of osteoclasts to the bone surface,
Journal of Cell Biology, **115**, 1179–1186.

Lean, J. M., Mackay, A., Chow, J. and Chambers, T. (1996)
Osteocytic expression of mRNA for c-fos and IGF-I; an immediate early gene response to an osteogenic stimulus,
American Journal of Physiology, **270,** 937–945.

Lewis, D. B., Liggitt, H. D., Effmann, E. L., motley, S. T., Teitelbaum, S. L., Jepsen, K. J., Goldstein, S. A., Bonadio, J., Carpenter, J. and Permutter, R. M. (1993)
Osteporosis induced in mice by overproduction of interleukin-4,
Proceedings of the National Academy of Science U.S.A., **90**, 11618–11622.

Manolagas, S. C. and Jilka, R. (1995)
Bone marrow, cytokines, and bone remodelling. Emerging insights into the pathophysiology of osteoporosis,
New England Journal of Medicine, **232**, 305–311.

Martin, T. J. and Ng, K. (1994)
Mechanisms by which cells of the osteoblast lineage control osteoclast formation and function,
Journal of Cellular Biochemistry, **56**, 357–366.

Massague, J. (1985)
Transforming growth factor beta modulates the high affinity receptors of epidermal growth factor alpha,
Journal of Cell Biology, **100**, 1508–1514.

McKee, M. D., Farach-Carson, M., Butler, W., Hauschka, P. and Nanci, A. (1993)
Ultrastructural immunolocalization of non-collagenous (osteopontin and osteocalcin) palsma (albumin) proteins in rat bone,
Journal of Bone and Mineral Research, **8,** 485–496.

Meikle, M. C., Bord, S., Hembry, R. M., Compston, J., Croucher, P. and Reynolds, J. J. (1992)
Human osteoblasts in culture synthesize collagenase and other matrix metalloproteinases in response to osteotropic hormones and cytokines,
Journal of Cell Science, **103**, 1093–1099

Mundy, G. (1994)
Peptides and growth regulatory factors in bone,
Rheumatic Disease Clinics of North America, **20,** 577–588.

Mundy, G., Rodan, S. B, Majeska, R. J., DeMartino, S., Trimmer, C. Martin, T. Rodan, G. A. (1982)
Unidirectional migration of osteosarcoma cells with osteoblast characteristics in response to products of bone resorption,
Calcified Tissue International, **34**, 542–546.

Parfitt, A. M. (1982)
The coupling of bone formation to bone resorption: a critical analysis of the concept and of its relevance to the pathogenesis of osteoporosis,
Metabolic Bone Disease Related Research, **4**, 1–6.

Pfeilschifter, J. and Mundy, G. R. (1987)
TGFβ stimulates osteoblast activity and is released during the bone resoprtion process,
Calcium Regulation and Bone Metabolism: Basic and Clinical Aspects, **9,** 450–454.

Pfeilschifter, J., Bonewald, L. and Mundy, G. R. (1990a)
Characterization of latent transforming beta complex in bone,
Journal of Bone and Mineral Research, **5**, 49–58.

Pfeilschifter, J., Wolf, O., Naumann, A., Minne,H. W., Mundy, G. R. and Ziegler, R. (1990)
Chemotatic response of osteoblast-like cells to transforming growth factor beta,
Journal of Bone and Mineral Research, **5,** 825–830.

Roberts, A. B., Anzano, M. A. and Wakefield, L. M. (1985)
Type beta transforming growth factor: a bifunctional regulator of cellular growth,
Proceedings of the National Academy of Science U.S.A., **82,** 119–123.

Roodman, G. D. (1996)
Advances in bone biology: the osteoclast,
Endocrine Reviews, **17**, 308–332

Rudnick, M. A., Schnegelsberg, P. N., Stead, R. H., Braun, T., Arnold, H. H. and Jaenisch, R. (1993)
Myo D or Myf-5 is required for the formation of skeletal muscle,
Cell, **75,** 1351–1359.

Sly, W. S., Whyte, M., Sundaram, V., Tashian, R., Hewett, D., Guibaud,P., Vainsel, M., Baluarte, H., Gruskin, A., Al-Mosawi, M., Sakati, N. and Ohlsson, A. (1985)
Carbonic anhydrase II deficiency in 12 families with autosomal recessive syndrome of osteoporosis with renal tubular acidosis and cerebral calcification,
New England Journal of Medicine, **313**, 139–145.

Suda, T., Udagawa, N. and Takahashi, N. (1996)
Cells of bone: osteoclast generation.
In: *Principles of Bone Biology*, Bilezikian, J. P., Raisz, L. G. and Rodan, G.A. (eds), pp.87–102,
Academic Press, San Diego.

Walker, D. (1973)
Osteoporosis cured by temporary parabiosis,
Science, **180**, 875–880.

Zaidi, M. (1990)
'Calcium receptors' on eukaryotic cells with special reference to the osteoclast,
Bioscience Reports, **10,** 493–507.