REVIEW

The Osteoblast: A Sophisticated Fibroblast under Central Surveillance

Patricia Ducy, Thorsten Schinke, Gerard Karsenty*

The study of the biology of osteoblasts, or bone-forming cells, illustrates how mammalian genetics has profoundly modified our understanding of cell differentiation and physiologic processes. Indeed, genetic-based studies over the past 5 years have revealed how osteoblast differentiation is controlled through growth and transcription factors. Likewise, the recent identification, using mutant mouse models, of a central component in the regulation of bone formation expands our understanding of the control of bone remodeling. This regulatory loop, which involves the hormone leptin, may help to explain the protective effect of obesity on bone mass in humans. In addition, it provides a novel physiologic concept that may shed light on the etiology of osteoporosis and help to identify new therapeutic targets.

Bone is a mineralized tissue that confers multiple mechanical and metabolic functions to the skeleton. Bone contains two distinct cell types, the osteoblasts, or bone-forming cells, and the osteoclasts, or bone-resorbing cells. Given the variety and the importance of the biological processes in which these two cell types participate during development and in postnatal life, there is great interest in understanding their differentiation and function. Here, we review our current knowledge of the molecular mechanisms regulating the differentiation and function of the osteoblast. In a companion review (*1*), Teitelbaum discusses recent advances in osteoclast biology.

The term "bone formation" is sometimes used to describe osteoblast differentiation during embryonic development, i.e., skeletogenesis. For the sake of clarity, we will restrict our use of this term to osteoblast function, namely the synthesis and deposition of the bone extracellular matrix (Fig. 1). Bone formation is implicated directly or indirectly in longitudinal bone growth, bone mineralization, and bone remodeling, all functions that are not easily studied in vitro. Thus, our understanding of the molecular control of osteoblast function has been greatly enhanced by the emergence of gene deletion technology.

The functions of osteoblasts and osteoclasts are intimately linked. During skeletal development and throughout life, cells from the osteoblast lineage synthesize and secrete molecules that in turn initiate and control osteoclast differentiation [reviewed in (*1*)]. This is a direct and crucial interaction that has been well established in vivo. Once osteoblasts and osteoclasts are fully differentiated, there is a less direct relationship. As will be detailed below, bone is constantly destroyed or resorbed by the osteoclasts and then replaced by the osteoblasts in a physiologic process called bone remodeling. Multiple genetic models such as an osteoblast ablation model and three models of increased bone formation indicate that the osteoblasts do not influence the activity of the osteoclasts in any overt way in vivo (*2–5*). Nevertheless, bone remodeling is tightly regulated by local (i.e., autocrine and/or paracrine) and endocrine factors. The endocrine regulation of bone resorption has been well known for many years, but it is only recently that bone formation has been shown to be under endocrine control. We review the emerging evidence for this in the second half of this article.

Osteoblast Differentiation

The osteoblast is of mesenchymal origin. In cell culture, osteoblasts are nearly indistinguishable from fibroblasts. The only morphological feature specific to osteoblasts is located outside the cell, in the form of a mineralized extracellular matrix. However, there is no evidence to date that bone matrix mineralization is orchestrated by genes selectively expressed in osteoblasts. All the genes expressed in fibroblasts are also expressed in osteoblasts, and, conversely, only two osteoblast-specific transcripts have been identified: one encoding Cbfa1, a transcription factor (*6*), and the other encoding Osteocalcin, a secreted molecule that inhibits osteoblast function (*7*). Thus, genetically, the osteoblast can be viewed as a sophisticated fibroblast.

During embryonic development, osteoblast differentiation can occur through two distinct pathways. For the entire future skeleton, except the clavicles, the mandibles and certain bones of the skull, a cartilage template surrounded by a bone collar prefigures each future bone. Upon vascular invasion of the cartilage template, the chondrocytes (cartilage cells) die through apoptosis and are replaced by osteoblasts brought in from the bone collar. This process is called endochondral ossification (*8*). In contrast, in the condensations prefiguring the clavicles, the mandibles, and certain bones of the skull, the mesenchymal progenitor cells differentiate directly into osteoblasts. This process, which does not include any cartilaginous templates, is called intramembranous ossification.

Transcriptional control of osteoblast differentiation. Over the past 5 years, there has been an intense search for transcription factors that might act as osteoblast differentiation factors, the so-called "master" regulators of osteoblast differentiation. Two osteoblast-specific cis-acting elements (OSEs) were identified in the promoter of the Osteocalcin gene (*Bgp*): OSE1 and OSE2 (*9*). This led to the identification of Cbfa1 as the first osteoblast-specific transcription factor. Cbfa1, which binds to OSE2 (*6*), is one of the three vertebrate homologs of the *Drosophila* runt and lozenge proteins (*10*). Runt is the founding member of a small family of transcription factors that are all characterized by a unique DNA binding domain called the runt domain. These transcription factors are present in multiple species from the nematode *Caenorhabditis elegans* to human (*10*). Runt and lozenge act as differentiation factors during *Drosophila* development, controlling neurogenesis and eye and blood cell development, respectively (*11–13*). Cbfa1 has all the attributes of a differentiation factor for the osteoblast lineage. During embryonic development, Cbfa1 expression precedes osteoblast differentiation and is restricted to mesenchymal cells destined to become either chondrocytes or osteoblasts (*6*). Subsequently, *Cbfa1* expression becomes limited to the osteoblasts, with a lower level of expression in hypertrophic chondrocytes (*14*). *Cbfa1* is also expressed in odontoblasts, the dentin-synthesizing tooth homologs of the osteoblasts (*15*). Thus, *Cbfa1* is the earliest and most specific marker of osteogenesis. In cell culture, Cbfa1 acts as an activator of transcription and can induce osteoblast-specific gene expression in fibroblasts and even myoblasts (*6*). This constellation of expression and in vitro data suggested that *Cbfa1* is an important gene for osteoblast differentiation, but the true biologic importance of *Cbfa1* in osteoblast differentiation was revealed by in vivo studies of mice and humans.

Cbfa1-deficient mice develop to term with a normally patterned skeleton that is made exclusively of cartilage; osteoblast differentiation

Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA.

*To whom correspondence should be addressed. E-mail: karsenty@bcm.tmc.edu

never occurs in these mice (*16*, *17*). In addition, *Cbfa1*-deficient mice lack osteoclasts because osteoclast differentiation requires cells of the osteoblast lineage. Although the mice show defects in hypertrophic chondrocyte differentiation in certain bones (*14*, *18*), chondrocyte differentiation is largely unaffected, which explains why the mutant mice have a skeleton that is nearly normal in shape and size. Mice heterozygous for *Cbfa1* inactivation have hypoplastic clavicles and a delay in the ossification of sutures of certain cranial bones (*17*). These abnormalities are identical to those observed in a radiation-induced mouse mutant designated Ccd (*19*), which reproduces the phenotype of one of the most frequent human skeletal disorders, cleiodocranial dysplasia (CCD). *Cbfa1* and the Ccd mutation are allelic in mice, and CCD patients are heterozygous for loss-of-function mutations in *CBFA1* (*20*, *21*). Together, all the evidence demonstrates that Cbfa1 is necessary for osteoblast differentiation in both mouse and human.

The transcription factors that act upstream of *Cbfa1* to control its expression remain to be identified. The *Cbfa1* expression patterns in mouse models where various genes have been deleted suggest that *Cbfa1* expression is controlled by different transcription factors at different locations in the developing skeleton. A second important question to answer is whether any transcription factors act downstream of *Cbfa1*. This is likely to be the case because *Cbfa1* expression begins at 10.5-days post coitum (dpc), while the first osteoblasts appear only 4 days later. Transcription factors acting upstream or downstream of Cbfa1 may not be osteoblast-specific.

Several homeobox proteins affect osteoblast differentiation in vivo (Fig. 2). During mouse development two homologs of the *Drosophila distalless* gene (*Dll*) (*22*), *Dlx5* and *Dlx6*, are expressed in the cells of the skeletal condensations undergoing intramembranous ossification and in the cells of the periosteum surrounding cartilaginous templates (*23*). In addition to severe craniofacial malformations, *Dlx5*-deficient mice exhibit a delayed ossification of the membranous bones and a milder delay in bone formation in the long bones (*24*). *Cbfa1* expression is not affected in the mutant mice, indicating that *Dlx5* acts either downstream of Cbfa1 or in a separate genetic pathway. Because *Dlx5* and *Dlx6* are coexpressed in the skeleton, the relatively mild skeletal phenotype of the *Dlx5*-deficient mice could be the result of a functional redundancy between the two proteins. Inactivation of another homeobox gene, *Msx2*, one of the mouse homologs of the *Drosophila* muscle segment homeobox gene *Msh* (*25*), causes a more severe skeletal phenotype (*26*). *Msx2*-deficient mice exhibit a marked delay of ossification in bones of the skull and an overall decrease in bone volume. *Cbfa1* and *Osteocalcin* are down-regulated in these mutant mice, suggesting that Msx2 may be one regulator of *Cbfa1* expression. *Bapx1*, a mammalian homolog of the *Drosophila bagpipe* homeobox gene (*27*), may also be upstream of *Cbfa1* in the axial skeleton as *Cbfa1* expression is down-regulated in the axial skeleton of *Bapx1*-deficient mice (*28*). In contrast, another homeobox protein, Hoxa-2, known to provide the second branchial arch with regional identity (*29*), may inhibit *Cbfa1* expression. *Hoxa-2*–deficient mice exhibit ectopic bone formation associated with ectopic expression of *Cbfa1* in the second branchial arch, suggesting that *Hoxa-2* inhibits bone formation in this area by preventing *Cbfa1* expression (*30*).

Other transcription factors may control osteoblast differentiation in a Cbfa1-dependent or -independent manner. For instance, we know that OSE1, the other cell-specific regulatory element present in the *Osteocalcin* promoter, is as important as the Cbfa1 binding site for *Osteocalcin* expression (*31*). The factor binding to OSE1 has recently been biochemically characterized. Unlike Cbfa1, this binding activity is found only in nonmineralizing, i.e., poorly differentiated, osteoblasts (*9*). Identification of this factor may help to further define the genetic pathways controlling osteoblast differentiation. Lastly, it is possible that broad-based gene deletion experiments may identify new transcription factors important for osteoblast differentiation.

Growth factor control of osteoblast differentiation. Members of all the major families of growth factors have been implicated in the control of osteoblast differentiation during embryonic development. Several bone morphogenetic proteins (BMPs) can induce *Cbfa1* expression in vitro. However, this action appears to be indirect and, for now, is of unknown physiological relevance (*6*). Transforming growth factor–β (TGF-β) can control the steady-state level of osteoblast differentiation in vivo and inhibits the expression of *Cbfa1* in cultured osteoblasts. The mechanism by which it regulates *Cbfa1* expression and osteoblast differentiation is unknown. Members of the fibroblast growth factor (FGF) family seem to act earlier in skeletogenesis, when limb patterning occurs (*32*). At a later stage of skeletogenesis, they play critical roles mostly during chondrocyte differentiation (*32*). However, FGF2 may control differentiation of mesenchymal progenitor cells into mature osteoblasts (*33*).

Indian hedgehog (Ihh) is the growth factor that has the greatest impact on osteoblast differentiation in vivo. In chick and in mouse, the Ihh gene is expressed in prehypertrophic chondrocytes and controls the expression of parathyroid hormone–related peptide (*PTHrP*) (*34*), a gene coding for a growth factor that inhibits chondrocyte hypertrophy (*35*). Unexpectedly, the subset of *Ihh*-deficient mice that develop to term have an osteoblast phenotype in addition to the chondrocyte abnormalities (*36*). During embryonic development, the bone collar of skeletal elements of the axial and appendicular skeleton does not form in *Ihh*-deficient mice, and chondrocyte apoptosis is not followed (as it should be) by osteoblast differentiation. Although *Cbfa1* expression is decreased, it is not known whether Ihh directly regulates the expression of any transcription factors controlling osteoblast differentiation. Remarkably, in *Ihh*-deficient mice osteoblast differentiation is unaffected in bones that form through intramembraneous ossification, suggesting that other growth factors control osteoblast differentiation in these bones.

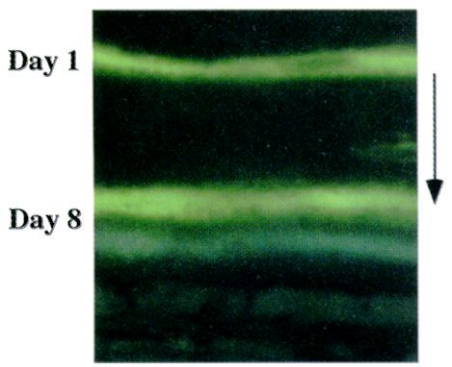

Fig 1. Visualization of osteoblast function (i.e., bone formation) by fluorescence microscopy after calcein double labeling. Two doses of calcein, a fluorochrome that incorporates at the site of newly formed bone matrix mineralization, were injected in mice 8 days apart. The distance between the two green labels represents the amount of bone formed during this period. The arrow indicates the direction of new bone formation.

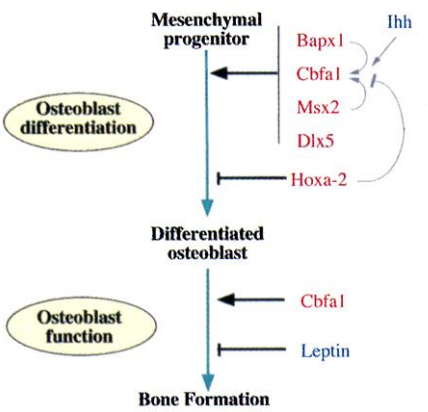

Fig 2. Schematic diagram of current knowledge of the genetic control of osteoblast differentiation and function. Transcription factors are indicated in red, secreted molecules are in blue. Black arrows indicate the stimulatory or inhibitory roles of these molecules. Gray arrows indicate possible regulatory pathways.

Osteoblast Function

In addition to providing mechanistic insights into the embryonic development of bone, genetically defined mouse models have profoundly enhanced our understanding of bone

physiology in adults. As mentioned above, bone remodeling is the dynamic physiologic process by which bone mass is maintained constant throughout adult life in vertebrates. Bone remodeling consists of two phases—bone resorption by osteoclasts followed by formation by osteoblasts—and it occurs continually and simultaneously at multiple locations in the skeleton. From a biomedical point of view, there is an urgent need to understand the regulation of bone remodeling. Indeed, the most common bone disease, osteoporosis, is a remodeling disease in which patients have a low bone mass condition with a high risk of fracture. Any pharmacological means that could be designed to increase the level of bone formation in osteoporotic patients may be of great use in preventing or treating the disease.

Transcriptional control of bone formation. In addition to its critical role during osteoblast differentiation, Cbfa1 controls bone formation by differentiated osteoblasts. As noted above, Cbfa1 regulates the expression of *Osteocalcin* (*Bgp*), a gene expressed only in terminally differentiated osteoblasts. Cbfa1 binding sites are also present in the regulatory sequences of most genes required for the elaboration of a bone extracellular matrix (*6*). Experiments with transgenic mice have provided functional evidence that Cbfa1 controls the rate of bone formation by differentiated osteoblasts (*37*). The *Osteocalcin* promoter was used to drive the expression of the DNA binding domain of Cbfa1 (ΔCbfa1). This truncated Cbfa1 protein has a higher affinity for the DNA than Cbfa1 itself, but has no transcriptional ability; thus, it can act in a dominant-negative manner. Because the promoter of *Osteocalcin* is active only after birth and only in differentiated osteoblasts, the expression of this transgene was strictly postnatal and was limited to differentiated osteoblasts. As a result, the *Osteocalcin-ΔCbfa1* transgenic mice developed to term without any skeletal abnormalities. However, once the *Osteocalcin* promoter became active, the mice stopped growing because osteoblasts are required for longitudinal bone growth (*2*). The mice also developed an osteoporotic phenotype that was not due to a defect in osteoblast differentiation, but rather to a marked decline in the output of bone matrix per osteoblast. Consistent with this observation, the expression level of genes required for formation of bone extracellular matrix was reduced.

Endocrine regulation of bone formation. The fact that bone remodeling occurs simultaneously in multiple skeletal locations is generally viewed as evidence that it is controlled locally, through autocrine and/or paracrine mechanisms (*38*). However, this observation is also consistent with the possibility that bone remodeling is under endocrine control. Apart from textbook knowledge, this would not be surprising because most other homeostatic functions are known to be under endocrine, if not neuroendocrine, control. Accordingly, several hormones are already known to control bone remodeling. For instance, sexual steroid hormones such as estradiol are involved in the control of bone remodeling by affecting osteoclast differentiation and thereby bone resorption. As a result, the declining levels of sexual steroid hormones at menopause is a predisposing factor for osteoporosis (*38*, *39*). Likewise, parathyroid hormone favors bone resorption (*40*, *41*). This raises the following question: Is bone formation also under endocrine control? Without presage of which component of bone remodeling is affected, two well-known clinical observations suggest that other hormones besides sex steroids and PTH regulate bone remodeling. The first is that obesity protects against bone loss (*42*), and the second is that menopause favors bone loss. Taken together, these observations suggest that bone mass, body weight, and reproduction are regulated by the same hormone(s).

Leptin as a regulator of bone formation. Leptin is an attractive candidate for such a hormone. Leptin is synthesized by adipocytes and functions as a starvation and adiposity signal by binding to the long form of its receptor, localized primarily in the hypothalamus (*43*, *44*). Rodents and humans genetically deficient in leptin signaling are massively obese. The absence of leptin signaling also causes sterility (i.e., hypogonadism), a condition usually causing bone loss. Surprisingly, mice deficient in leptin (*ob/ob*) or its receptor (*db/db*) were found to have a two- to threefold higher bone mass than wild-type mice (*5*). To date, the *ob/ob* and *db/db* mice are the only known animal models with both hypogonadism and high bone mass, and therefore they are invaluable resources for studying the molecular basis of bone remodeling. The bone phenotype of these mutant mouse models is not caused by hyperinsulinism, because heterozygous mice also have higher bone mass and are not hyperinsulinemic.

Bone histomorphometry analysis performed before and after correction of the hypogonadism of *ob/ob* mice showed that leptin inhibits bone formation through its action on osteoblasts; it has no overt effect on osteoclast differentiation and function. Importantly, leptin action on bone formation did not involve osteoblast differentiation as *ob/ob* and *db/db* mice have a normal number of osteoblasts. This latter observation indicates that any possible local mode of action of leptin on bone must affect already differentiated osteoblasts and not their progenitors. The leptin receptor does not appear to be expressed on differentiated osteoblasts, and in cell culture studies osteoblasts from *db/db* mice behave like wild-type osteoblasts. These data indicate that leptin does not directly target osteoblasts. Moreover, intracerebroventricular (ICV) infusion of leptin in *ob/ob* mice completely rescues their bone phenotype. The fact that this occurs without any circulating leptin in the serum of these animals demonstrates that bone formation, and therefore bone remodeling, is centrally controlled, likely by the hypothalamus (Fig. 3). ICV infusion of leptin in wild-type mice causes bone loss, indicating that this regulatory loop is physiologically relevant in wild-type animals (*5*). By definition, if bone remodeling is a physiologic process with an hypothalamic or central component, then diseases of bone remodeling such as osteoporosis may also have an hypothalamic or central component.

As is the case for the control of body weight, the role of leptin in bone formation is not merely a "mouse story." Rats deficient in leptin signaling also have a high bone mass phenotype (*45*). Patients with generalized lipodystrophy, a condition marked by a nearly complete absence of adipocytes and white fat, also exhibit osteosclerosis (increased bone formation) and accelerated bone growth (*46*). It is difficult to assess whether leptin-deficient patients have a high bone mass, because these children were treated with leptin early in their lives. Work with the *ob/ob* and *db/db* mice suggests that the high bone mass phenotype becomes more severe as the animals age. Nevertheless, the recent observation that patients with an inactivating mutation in melanocortin receptor 4 (MC-R4), a hypothalamic receptor for the melanocortin hormone, are obese and have a high bone mass (*47*) provides further support for the emerging concept of a common and central control of bone remodeling and body weight in mice and humans. Lastly—and going back to the critical observation that triggered this work—leptin's role in bone formation may help explain why obese individuals, who are often leptin resistant (*48*), are protected from bone loss.

These results also considerably expand the importance of leptin by showing that like other

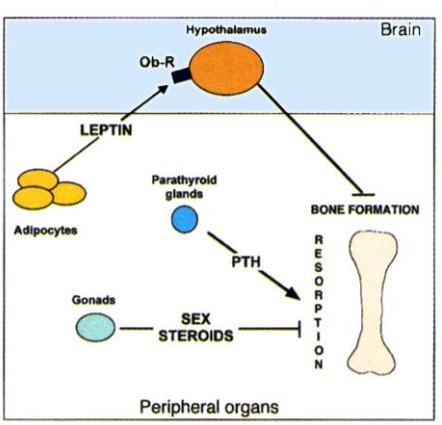

Fig 3. Hormonal control of bone remodeling. Sex steroids and PTH have been known for many years to control bone resorption. More recently, leptin was shown to regulate bone formation via a central relay.

major hormones such as thyroid hormones or cortisol, it has multiple target organs and functions (bone mass, body weight, and reproduction) without a necessary hierarchy among them. However, leptin is unlikely to be the sole central regulator of bone formation. Other yet-to-be discovered centrally acting hormones may positively or negatively regulate bone formation and possibly other aspects of bone physiology.

Prospects

In the immediate future, studies of osteoblast biology will continue to focus independently on cell differentiation and cell function. In terms of cell differentiation, molecular biology studies and the analysis of other mouse mutant strains should allow us to achieve a more precise understanding of the genetic pathways controlling osteoblast differentiation. These studies will focus on genes located upstream and downstream of *Cbfa1*, but may also reveal other pathways to achieve osteoblast differentiation. In terms of cell function, it is likely that other hormones will be shown to have a role in controlling bone formation, possibly through a central pathway. Likewise, Cbfa1 will probably not remain the only molecule to bridge the "ontogenic gap" between cell differentiation and cell function.

In the long term, it is likely that molecules affecting the function of the osteoblasts will become increasingly important in the quest for new therapies for osteoporosis. Experiments are in progress to test whether increasing Cbfa1 levels will increase bone formation and therefore be potentially useful in preventing or treating osteoporosis. Similarly, inhibitors of the leptin pathway that affect bone mass but not body weight could be beneficial for osteoporosis. The identification of other transcription factors, hormones, or secreted molecules controlling bone formation will increase the likelihood that a successful therapy will be developed for this and other degenerative bone diseases.

References and Notes

1. S. Teitelbaum, *Science* **289**, 1504 (2000).
2. D. Corral *et al*, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 13835 (1998).
3. G. Sabatakos *et al*, *Nature Med.* **6**, 985 (2000).
4. W. Jochum *et al.*, *Nature Med.* **6**, 980 (2000).
5. P. Ducy *et al.*, *Cell* **100**, 197 (2000).
6. P. Ducy, R. Zhang, V. Geoffroy, A. L. Ridall, G. Karsenty, *Cell* **89**, 747 (1997).
7. P. Ducy *et al.*, *Nature* **382**, 448 (1996).
8. W. A. Horton, *Connective Tissue and Its Heritable Disorders* (Wiley-Liss, Chichester, UK, 1993), pp. 73–84.
9. P. Ducy and G. Karsenty, *Mol. Cell Biol.* **15**, 1858 (1995).
10. N. A. Speck and T. Tracy, *Crit. Rev. Eukaryot. Gene Expr.* **5**, 337 (1995).
11. M. A. Kania, A. S. Bonner, J. B. Duffy, J. P. Gergen, *Genes Dev* **4**, 1701 (1990).
12. J. B. Duffy and J. P. Gergen, *Genes Dev* **5**, 2176 (1991).
13. A. Daga, C. A. Karlovich, K. Dumstrei, U. Banerjee, *Genes Dev* **10**, 1194 (1996).
14. I. S. Kim, F. Otto, B. Zabel, S. Mundlos, *Mech. Dev.* **80**, 159 (1999).
15. R. N. D'Souza *et al.*, *Development* **126**, 2911 (1999).
16. T. Komori *et al.*, *Cell* **89**, 755 (1997).
17. F. Otto *et al.*, *Cell* **89**, 765 (1997).
18. M. Inada *et al.*, *Dev. Dyn.* **214**, 2797 (1999).
19. D. O. Sillence, H. E. Ritchie, P. B. Selby, *Am. J. Med. Gen.* **27**, 75 (1987).
20. S. Mundlos *et al.*, *Cell* **89**, 773 (1997).
21. B. Lee *et al.*, *Nature Genet.* **16**, 307 (1997).
22. S. M. Cohen *et al*, *Nature* **338**, 432 (1989).
23. A. Simeone *et al*, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 2250 (1994).
24. D. Acampora *et al.*, *Development* **126**, 3795 (1999).
25. D. R. Davidson and R. E. Hill, *Dev. Biol.* **2**, 405 (1991).
26. I. Satokata *et al.*, *Nature Genet.* **24**, 391 (2000).
27. C. Tribioli, M. Frasch, T. Lufkin, *Mech. Dev.* **65**, 145 (1997).
28. C. Tribioli and T. Lufkin, *Development* **126**, 5699 (1999).
29. M. Gendron-Maguire, M. Mallo, M. Zhang, T. Gridley, *Cell* **75**, 1317 (1993).
30. B. Kanzler, S. J. Kuschert, Y. -H. Liu, M. Mallo, *Development* **125**, 2587 (1998).
31. T. Schinke and G. Karsenty, *J. Biol. Chem.* **274**, 30182 (1999).
32. J. M. Pierre, L. Abderrahim, F. Debiais, J. Lemonnier, *Skeletal Growth Factors* (Lippincott Williams & Wilkins, Philadelphia, 2000), pp. 179–196.
33. A. Montero *et al.*, *J. Clin. Invest.* **105**, 1085 (2000).
34. A. Vortkamp *et al.*, *Science* **273**, 613 (1996).
35. A. C. Karaplis *et al.*, *Genes Dev.* **8**, 277 (1994).
36. B. St-Jacques, M. Hammerschmidt, A. P. McMahon, *Genes Dev,* **13**, 2072 (1999).
37. P. Ducy *et al.*, *Genes Dev.* **13**, 1025 (1999).
38. G. R. Mundy, B. F. Boyce, T. Yoneda, L. F. Bonewald, G. D. Roodman, *Osteoporosis* (Academic Press, San Diego, ed. 3, 1996), pp. 301–313.
39. R. Pacifici, *Endocrinology* **139**, 2659 (1998).
40. M. Chorev and M. Rosenblatt, *Principles of Bone Biology* (Academic Press, San Diego, ed. 3, 1996), pp. 305–323.
41. J. T. Potts and H. Jüppner, *Metabolic Bone Disease and Clinically Related Disorders* (Academic Press, San Diego, ed. 2, 1998), pp. 52–94.
42. F. A. Tremollieres, J. M. Pouilles, C. Ribot, *J. Clin. Endocrinol. Metab.* **77**, 683 (1993).
43. J. Friedman, *Nature* **404**, 632 (1999).
44. J. K. Elmquist, E. Maratos-Flier, C. B. Saper, J. S. Flier, *Nature Neurosci.* **1**, 445 (1998).
45. M. Amling and G. Karsenty, unpublished observation.
46. J. Westvik, *Acta Paediatr. Suppl.* **413**, 44 (1996).
47. I. S. Farooqi *et al.*, *J. Clin Invest* **106**, 271 (2000).
48. R. C. Frederick, *Nature Med.* **1**, 1311 (1995).

49. Funded by the NIH and the March of Dimes (G.K. and P.D.) and by the Arthritis Foundation (P.D. and T.S.).

REVIEW

Bone Resorption by Osteoclasts

Steven L. Teitelbaum

Osteoporosis, a disease endemic in Western society, typically reflects an imbalance in skeletal turnover so that bone resorption exceeds bone formation. Bone resorption is the unique function of the osteoclast, and anti-osteoporosis therapy to date has targeted this cell. The osteoclast is a specialized macrophage polykaryon whose differentiation is principally regulated by macrophage colony-stimulating factor, RANK ligand, and osteoprotegerin. Reflecting integrin-mediated signals, the osteoclast develops a specialized cytoskeleton that permits it to establish an isolated microenvironment between itself and bone, wherein matrix degradation occurs by a process involving proton transport. Osteopetrotic mutants have provided a wealth of information about the genes that regulate the differentiation of osteoclasts and their capacity to resorb bone.

Osteoclasts are multinucleated cells formed by the fusion of mononuclear progenitors of the monocyte/macrophage family. They are the principal, if not exclusive, resorptive cell of bone, playing a central role in the formation of the skeleton and regulation of its mass. Bone-forming cells, or osteoblasts, have an equally important role in the regulation of bone mass. This cell type is reviewed in a companion article by Ducy *et al.* (*1*). The activity of osteoclasts, relative to bone-forming osteoblasts, dictates the development of osteoporosis, a group of disorders in which skeletal mass has decreased to the point of structural instability, thereby rendering the patient susceptible to spontaneous bone fracture. Because adult osteoporosis, regardless of etiology, always represents enhanced bone resorption relative to formation, progress in understanding the pathogenesis and successful treatment of this family of diseases requires an understanding of osteoclast biology.

Department of Pathology, Washington University School of Medicine, Barnes-Jewish Hospital North, Mailstop 90-31-649, 216 South Kingshighway, St. Louis, MO 63110, USA. E-mail: teitelbs@medicine.wustl.edu

How Does a Macrophage Become an Osteoclast?

As first noted in 1990, in vitro maturation of macrophages into osteoclasts requires the presence of marrow stromal cells or their osteoblast progeny (*2*). After a decade of confusion, it is now clear that these accessory cells express the two molecules that are essential and sufficient to promote osteoclastogenesis: macrophage colony-stimulating factor (M-CSF) and receptor for activation of nuclear factor kappa B (NF-κB) (RANK) ligand (RANKL)