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Webster S.S. Jee

The past, present, and future of bone morphometry: its contribution to an improved understanding of bone biology

Abstract It was not until the 1950s that a better paradigm for bone biology evolved, which led to the birth of bone histomorphometry. Two clinicians, Harold Frost (1958–1964) and Lent Johnson (1964), were responsible for the paradigm stating that the primary function of bone is mechanical load bearing with subsidiary function to participate in plasma calcium homeostasis to support hematopoesis. Dynamic bone histomorphometry was born when Milch et al. (1958) discovered bone localization of tetracycline and Frost generated the methodology to study tetracycline-based dynamic histological analysis of cortical bone remodeling (1961–1965). Dynamic bone histomorphometry did not blossom until Frost, while a Sun Valley Workshop participant, developed it to address trabecular bone dynamics. The combination of Arnold (1948) producing thin sections of plastic-embedded undecalcified bone and Frost's (1977–1983) modification of dynamic cortical bone histology for cancellous bone made it possible to study tetracycline-based dynamic histomorphometry of cancellous bone. It led to the better understanding of basic metabolic unit (BMU) remodelling and to Frost's mechanostat hypothesis, and characterized the rat model to accelerate the development of several drugs in the treatment of bone diseases. Currently, dynamic bone histomorphometry has contributed to studies in bone's mechanical usage windows, mechanical usage setpoint hypothesis, muscle–bone relations, marrow–bone relations, the Utah paradigm of musculoskeletal physiology, apoptosis, genetics (transgenic mice) and bone structure, bone quality, the lacunocanalicular network and bone modelling, and remodeling hypothesis, osteocyte role as mechanosensory, chemosensory, and regulatory in bone maintenance, targeted and untargeted remodeling, the role of permissive agents, etc., items in bone biology expounded briefly by Lent Johnson (1965) and continuously by Harold Frost at the Sun Valley Workshop (1965–2003). Finally, "What's next?" covers how to improve and perpetuate the employing of qualitative histomorphometry in research opportunities in hard tissue research.

Key words Dynamic histomorphometry · Skeletal adaptation to mechanical usage · Mechanostat · Utah paradigm of skeletal physiology · What's next?

W.S.S. Jee (✉)
Radiobiology Division, University of Utah School of Medicine, 729 Arapeen Drive, Suite 2338, Salt Lake City, UT 84108-1218, USA
Tel. +1-801-581-6366; Fax +1-801-581-7008
e-mail: webster.jee@hsc.utah.edu

Introduction

It was not until the 1950s that a better paradigm evolved for bone biology that led to the birth of a new paradigm of skeletal physiology. The paradigm stated that the primary function of bone is mechanical load bearing with subsidiary function to participate in plasma calcium homeostasis and to support hematopoesis. The new paradigm emerged from studies on the morphology and dynamics of bone cells and tissue in growth, modeling, and remodeling, the osteocyte as mechanoreceptor, fatigue and microdamage repair, biomechanical influence on bone adaptation, maintenance and turnover, transient and steady-state conditions, muscle–marrow–bone relations, osteonal bone remodeling, and tissue changes in aging and select bone diseases by two clinicians. One is Lent Johnson, a pathologist, and the other is Harold Frost, an orthopedic surgeon (Fig. 1).

These two individuals were different Lent Johnson published fewer than half a dozen, but lengthy, bone review articles [1–5]. His main contribution was his Henry Ford symposium article entitled "Morphologic analysis in pathology: the kinetics of diseases and general biology of bone" [2].

After the articles, Lent published a few review articles on bone cancer and was known as the world expert on bone tumors. Harold was different. During the 1950s and 1960s, his extensive and innovative publications mainly appeared in the Henry Ford Hospital Bulletins [6–13] because most scientific editors viewed his findings as unreliable and/or controversial. Harold went on to publish nearly 500 articles

Fig. 1. Harold Frost is seated on the front row, fourth from the left (*oval outline*) and Lent Johnson is standing on the left on the back row (*circle*). The others seated are Talmage, Belanger, Jee and Gruelich

and 16 books that made contributions to skeletal biology and metabolic bone diseases which had a profound impact on the field. He also was a mentor to countless clinicians and basic scientists.

From the few non-peer-review articles by Lent Johnson, it was obvious he had a tremendous grasp of bone biology. He viewed the skeleton as "a record of past events and an oracle of future behavior." Table 1 lists the aspects of bone biology on which Lent expounded in his 1964 article [2], topics that are under active research currently. In my opinion, his greatest contribution was his attempt at the theoretical biology of the skeleton to tie together what he viewed through the microscope. His bone biology encompassed every bit of the cycled removal and renewal, explains how mechanical, circulation, and metabolic stress signals the adaptive modification of bone that accounts for the cell modulations which are activated in response to the signal under renewal cycle, rate of activity, continuity of activity, mechanical, circulatory, and metabolic signals, collaborating, metabolic, and general principles, cell modulation and cell, and field and constitutional interplay. Needless to say, his bone biodynamic article should be required reading for bone researchers.

Table 2 lists Frost's bone findings culminating in what he called "connecting the dots" or what I call "solving the puzzle or mystery" that led to the "mechanostat hypothesis" and the ever-evolving Utah paradigm of skeletal physiology of load-bearing bones [6–49]. In my opinion, the best of Harold was his astute contribution to our understanding of activation, resorption, and formation (ARF) of the basic multicellular unit (BMU) concept of bone remodeling, the development of dynamic histomorphometry of cancellous and cortical bones, the "mechanostat hypothesis," and the Utah paradigm of skeletal physiology and microdamage and repair. A brief history and the current status of these concepts follow.

The activation-resorption-formation and basic multicellular unit in bone remodeling dogmas

The activation, resorption, and formation and basic multicellular unit concepts in adult bone remodeling came about in 1957 when Frost reported a method to generate thin undecalcified cortical bone specimens rapidly. He discovered that with some waterproof sandpaper he could make rib cross sections in 10 min. In his material he was able to observe cement lines of which 95% were scalloped, a record of osteoclastic activity. From his findings, he

Table 1. Lent Johnson's findings

"The skeleton is a record of past events and an oracle of future behaviors"

- Mass and surface relation of cancellous and cortical bone
- Periosteal bone growth – a renewed apposition in aging
- Changes with aging in the character of adult cortex – cancellation next to marrow
- Osteon and cortical remodeling
- Osteoblasts cycle
- Interplay of muscle, marrow, and bone
- Lipogenic skeletogenic relationship
- Hematopoetic and fatty marrow relationship
- Circulation in bone: positive-osteoblastic; passive-osteoblastic
- Glomoid system – adapts circulation to bone needs
- Lipogenic skeletogenic relationship
- Mechanics and bone (disuse, stress, fracture)
- Transient and steady state responses
- Osteocyte as mechanosensor
- Osteocyte sequences
- Fatigue damage
- Articular remodeling and osteoarthritis
- Bone remodeling and osteoporosis
- Muscle and hip dysplasia
- Genetics and growth
- Theoretical biology of the skeleton that encompasses every bit of the cycled removal and renewal, explains how mechanical circulation and metabolic stress signals the adaptive modification of bone, which accounts for the cell modulations that are activated in response to the signal:
  - Renewal cycle
  - Rate of activity
  - Continuity of activity
  - Mechanical, circulating, and metabolic signals
  - Collaborating, metabolic and general principles
  - Cell modulation
  - Cell, field, conditional interplay

Source: From Johnson L (1964) Morphologic analysis in pathology: the kinetics of diseases and general biology of bone. In: Frost HM (ed) Bone Biodynamics. Little Brown, Boston, pp. 543–644, [2]

Table 2. Harold M. Frost's findings

• Preparation of thin undecalcified sections	1958
• Measurement of scalloped cement lines	1959
• Measurement of osteocyte and canaliculae per unit volume in man	1960
• Joint biomechanics	1960
• Presence of microscopic cracks in bone	1960
• Osteoid seams: the existence of resting state	1960
• Observations of fibrous and lamellar bone	1960
• Halo volume	1960
• Bone formation in a 57-year-old man by means of tetracycline	1960
• A new bone affection: feathering	1960
• Micropetrosis	1960
• Human Haversian system measurements	1961
• Multiband tetracycline measurement of lamellar osteoblastic activity	1961
• Postmenopausal osteoporosis: a disturbance in osteoclasia	1961
• Introduction to biomechanics	1963
• Lamellar bone remodeling	1964
• Drift in osteon in human rib cortex	1965
• Correlation of patterns of bone resorption and formation in loaded bone	1965
• Remodeling at periosteal, Haversian canal, cortical endosteal and trabecular surfaces	1965
• Cortical bone loss in human rib with aging	1965
• Periosteal bone growth from 2 to 70 years in humans	1966
• Bone dynamics in osteoporosis and osteomalacia	1966
• Bone dynamics in metabolic disease	1966
• Bone dynamics in metabolic disease	1966
• Age- and sex-related change in the amount of cortex in human ribs	1966
• Bone resorption rate in osteoporoses	1967
• Bone remodeling hypoparathyroid states in humans	1968
• Haversian bone formation rates in diabetes mellitus, osteogenesis imperfecta, and osteoporosis	1969
• Bone remodeling in osteopetrosis	1969
• Tetracycline-based histological analysis of cortical bone remodeling	1969
• Origin and nature of transients in bone remodeling	1973

Table 2. *Continued*

• Bone modeling and remodeling errors	1973
• Histomorphometric analysis of trabecular bone in renal dialysis patients treated with 25-$(OH)_2D_3$	1977, 1981
• A method of analysis of trabecular bone dynamics	1977
• Treatment of osteoporosis by coherent bone cells	1979, 1981
• Chondral modeling	1979
• Lamellar bone modeling	1979
• Theory of lamellar bone modeling	1980
• Mechanical determinants of bone modeling	1980
• Mechanical microdamage, bone remodelling, and osteoporosis	1981
• Labeling escape errors	1983
• The skeletal intermediary organization	1983, 1986
• Minimum effective strain	1983
• Pathomechanics of osteoporosis	1985
• Regional acceleratory phenomenon	1983
• The mechanostat	1987, 1996, 1998, 1999, 2003
• Mean bone tissue age – osteon population	1987
• Skeletal adaptations to mechanical usage	1988
• Mechanical usage, bone mass, bone fragility	1988
• Transient–steady state in microdamage physiology	1989
• Biology of fracture healing	1989
• General mediator mechanism properties	1989
• Bone balance and ΔBMU (basic metabolic unit)	1989
• Skeletal adaptations to mechanical usage – bone modeling	1990
• Skeletal adaptations to mechanical usage – bone remodeling	1990
• Skeletal adaptations to mechanical usage – hyaline cartilage modeling	1990
• Skeletal adaptations to mechanical usage – influences on fibrous tissue	1990
• The ABCs of skeletal pathophysiology: the growth modeling, remodeling distinction	1991
• Tissue mechanisms controlling bone mass	1991
• Skeletal adaptations to mechanical usage – during growth	1992
• Changing mechanical usage setpoints	1992
• Bone's mechanical usage windows	1992
• Vital biomechanical model of arthroses	1994
• Biomechanical model of endochondral ossification	1994
• On a paradigm shift in skeletal science	1995
• The Utah paradigm of bone physiology – muscle, (osteoporosis and other clinical application, animal models), Wolff's law, distraction osteogenesis	1995, 1996, 1997, 1998, 1999, 2000, 2001, 2002, 2003, 2004
• A general model of the mechanostat	1995, 1996, 1997, 1998, 1999, 2000, 2001, 2002, 2003
• Biomechanical explanation: why do marathon runner have less bone than weight lifters	1997
• Obesity and bone strength and mass	1997
• Increased fracture during growth spurt	1997
• On rho, a marrow mediator, and estrogen on bone strength	1998
• Biomechanical effects of growth hormone	1998
• Age-related bone loss and muscle strength	1998
• Estrogen and bone-muscle strength and mass relationship	1998
• Fatigue damage (microdamage) in bone	1998
• Biomechanical effects of growth hormone	1998
• Aging adults become unresponsive to vigorous physical activities	1999
• Muscle strength–bone strength relationship in human	1999
• New approach to estimating bone and joint loads and muscle strength in living subjects and skeletal remains	1999
• Does bone design minimize fracture failures?	2000
• "Muscle-bone unit" in children	2000
• Muscle, neuromuscular physiology, bone nephron equivalents	2000
• Longitudinal bone growth – insights about cartilage physiology from Utah paradigm	2001

Source: Summarized from nearly 500 articles: a partial listing is found in the reference section [6–49]

deduced that in adult bones, bone resorption preceded bone formation and that the two activities were coupled. This finding led to the addition of activation, resorption, and formation (ARF) and basic multicellular unit (BMU) to his concepts of bone remodeling [13,48]. Frost's BMU concept first appeared in one of his books [13], and the concept began to be widely accepted, but not until the late 1970s. Parfitt stated in the landmark article in the *Journal of NIH Research*, as holds true even today: "There are still a lot of people who pay lip service to it because it has now become the current dogma but who do not incorporate it into their thinking. You see this particularly in people who study the skeleton by measuring bone density—they rarely make an effort to think how their observations relate to what is actually going on inside the skeleton" [50].

The dynamic bone histomorphometry of cancellous and cortical bone and the tissue-level response

In 1957, Milch, Rall, and Tobie [51] reported that tetracycline antibiotics will deposit in vivo in sites of bone formation and subsequently can be studied in undecalcified sections by fluorescent microscopy. This led Frost [14,15] and others, Amprino and Marotti [52] and Hass et al. [53], to develop a number of tetracycline-based approaches to the histological studies of bone remodeling. Frost was the first to exploit the analysis of tetracycline-labeled bone in a systematic manner from his human rib biopsy specimens. His double-labeled rib specimens allowed him to develop data measurements at the microscope into physiologically meaningful formation and resorption rates and into tissue-level parameters such as activation frequencies, remodeling, formation and resorption periods, and bone turnover of compacta [6,14–16]. There was no wide acceptance of Harold's approach to his study of cortical bone histomorpometry because not many investigators harvested human rib bone biopsy specimens that were sequentially in vivo labeled as Frost performed in mass production.

The technology of dynamic bone histomorphometry did not blossom until Frost extended his dynamic cortical bone histomorphometry to cancellous bone. It was the combination of the feasibility of a Frenchman named Phillip Bordier and the popularity of obtaining transilial bone biopsies [54,55], plus a development in the late 1940s by a radiobiologist named James Arnold producing reliable thin plastic-embedded cancellous bone samples for histological sectioning and its application to radioautoradiography [56,57], that made it possible to readily analyze cancellous bone tetracycline-based dynamic histomorphometry, which Frost also developed [17–21,43]. James Arnold and coworkers used his plastic-embedded undecalcified thin bone sections to perform autoradiographs for the study of bone-seeking radionuclides [56–58]. The methodology produced excellent histology sections, exceeding decalcified paraffin-embedded and ground undecalcified bone sections in quality as well as retaining the mineral in which radionuclides and fluorescent antibiotics localized. By 1964, Frost had perfected his analysis of cortical bone histomorphometry [14], but there was a need to modify his cortical bone histomorphometry for cancellous bone to analyze the cancellous bone-dominated human transilial biopsy. The stimulus for the modification came about from the interest of the Upjohn Company to evaluate the effect of 25-dihydroxyvitamin D_3 in the treatment of renal dialysis patients [43]. The experimental design called for the analysis of cancellous bone histomorphometry in sequential transilial biopsies. Frost accomplished this task in record time [17–20]. The technology gained wide acceptance by clinicians and basic scientists as an essential tool to assess the tissue-level organization of bone, the dynamic aspects of osteoblastic and osteoclastic functions, the pathophysiology of osteopenias and osteoporoses, the efficiency of treatment, and the safety and mechanisms of action of new therapuetic agents, etc. [59–61]. Furthermore, the technology improved our understanding of basic multicellular unit (BMU) remodeling, skeletal adaptation to mechanical usage, the "mechanostat hypothesis" and the Utah paradigm of skeletal physiology in preclinical bone studies.

The mechanostat hypothesis

The mechanostat hypothesis is based on the idea that bone senses its mechanical environment and can adapt to it. The first demonstration of bone responses to its mechanical environment was by Koch in 1917 [62]. The first suggestion that strain might be the causative stimulus was Thompson in 1917 [63], while much later Lanyon and Smith [64] pioneered experiments measuring bone strains in living animals. In the 1960s, Frost was one of a few orthopedic surgeons interested in bone and joint mechanics when he published one article and a book on joint-bone biomechanics [11,26]. During that period, Johnson in his Bone Biodynamic opus reported his impressions of bone mechanics and its adaptive responses to disuse, stress, and fracture in addition to stating the osteocyte as a logical mechanosensor [2].

Frost first heard the idea of a "mechanostat" applied to bone at a Gordon Conference about 1957. There originators were W.D. Armstrong, F.C. McLean, A. Reifenstein, and I. Snapper (Fig. 2), all long deceased, so the idea died and was buried. By 1987 Frost "dug up the "mechanostat" coffin, exhumed and published its contents and admitted he undeservedly received most of the credit for it" [65]. The mechanostat hypothesis is based on the idea that minimum effective strains must be exceeded to excite a positive adaptive response to mechanical overloading. He suggested there are strain thresholds or windows that will evoke no response, "E"; strains above this E threshold will evoke a positive adaptive response (i.e., modeling-dependent bone gain) and strains below the no-response threshold will cause a negative adaptive response (i.e., disuse mode remodeling-dependent bone loss) [24,25]. Similar adaptive thresholds or window values were suggested by Carter et al. in 1981 [66] and Cowin in 1984 [67]. In addition, there are pathological strain thresholds (i.e., for microdamage) and threshold for fractures (Fig. 3). In total, one can visualize Frost's "general biomechanical relation" thresholds of fracture incidence (Fx) > minimum effective strain pathological (MESp) > minimum effective strain for modeling (MESm) > E, the normally adaptive or the response threshold > minimum effective strain for disuse mode remodeling. The mechanostat also contains the "baseline conditions" created at birth that include the basic bone anatomy and anatomical relationships, neurological, muscular, and circulatory physiology, the minimum effective strain setpoint, and the biological machinery that adapts bones to mechanical usage. The biologic machinery includes (1) modeling by formation and resorption drift that can increase bone strength (modeling-dependent bone gain) "mass" and strength; (2) disuse-mode BMU-based remodeling that removes bone next to marrow (remodeling-dependent bone loss in "mass" and strength); and (3) the

Fig. 2. F.C. McLean (*2*), A.C. Reifenstein (*3*), and W.D. Armstrong (*5*) seen at the Fifth Conference on Metabolic Interrelations, New York City, January 5–6, 1953; I. Snapper was not in attendance. *Source*: from Metabolic Interelationships with special reference to Reifenstein EC (ed) (1954) Calcium. Josiah Macy Jr. Foundation, New York, p. 8

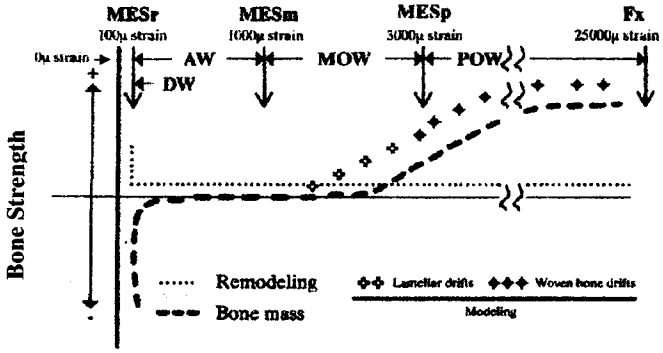

Fig. 3. A diagram illustrating the relationship of stains and adaptive responses, a key component of the "mechanostat: minimum effective strain (*MES*) for remodelling (*r*), modelling (*m*), pathology (*p*), and fracture (*Fx*); windows or threshold for disuse (*DW*), adapted (*Aw*), mildly overloaded (*MOW*), and pathological overload (*POW*)

error-driven negative mechanical feedback loop, aided by signaling systems that detect and process signals (i.e., mechanotransduction) for threshold ranges of MESm for modeling MESr for disuse-mode remodeling to help switch the two functions on and off. In addition, Frost factored in the mechanostat, the influence of muscle function, and nonmechanical agents [24,25,31,37,40,68]. The nonmechanical agents (drugs, diet, hormones, genetics, diseases, etc.) can change the mechanostat's setpoints of its various biological activities and adaptation. Thus, after the minimum effective strain threshold or setpoint decides if an adaptation is necessary, it causes the appropriate mediator mechanisms (i.e., modeling, remodeling, microdamage, and fracture repair) to achieve it, usually under the error-driven negative mechanical feedback loops (Fig. 4).

Frost's hypothesis is based on the idea of a minimum effective strain that must be exceeded to excite a positive adaptive response (i.e., increase bone "mass" and strength) to mechanical overloading [23–25,30,31,36,37]. In contrast, a minimum effective strain below the no-response or adapted threshold excites a negative adaptive response (i.e., decreases bone mass and strength) that is similar to a room that is too cold, which activates the thermostat to turn on the furnace to warm the room (i.e., increase bone mass and strength); when a room is too warm, another thermostat will be turned on to kick in the air conditioner to cool the room (i.e., decrease bone "mass" and strength). This analogy to the room thermostat persuaded Harold to name the bone biological machinery involved in adaptation to mechanical environment the mechanostat for load-bearing bones.

The osteocyte, microdamage, and repair revisited

Currently it has become clear that (1) physiological strains continually produce fatigue damage in bone; (2) this damage weakens bone and is associated with both osteocyte apoptosis and the activation of remodeling; and (3) remodeling is the only means by which this damage can be removed and repaired [69–76]. The highly significant nature of these events has intrigued the author to revisit osteocyte function and death and microdamage physiology.

Harold Frost was the first to demonstrate microscopic cracks (i.e., microdamage) in human cortical bone, interpreted as fatigue cracks detected by bulk staining with basic fuschin, and suggested the osteocyte as detector of microdamage and somehow stimulated BMU creation to

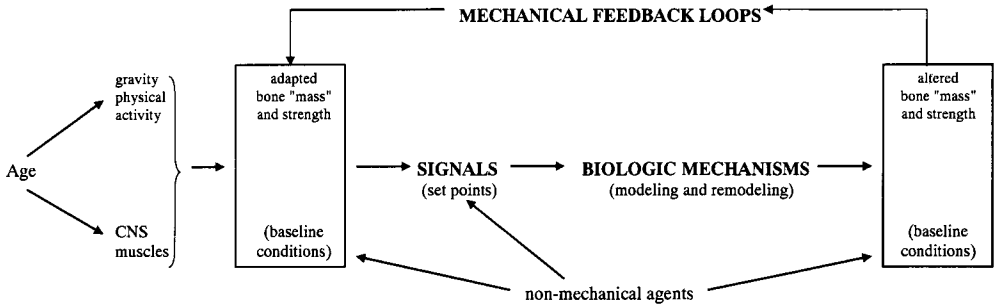

Fig. 4. The mechanostat (*in bold letters*) with its mechanical feedback loops. The nonmechanical agents can act in a permissive manner or directly to modulate or attenuate the mechanical loading response

- Base line conditions: bony anatomy and relationship, basic neuromuscular anatomy and physiology; biologic "machinery" for bone adaptation; and genetically determined threshold (set point) range for modeling and remodeling.

repair them [10,22]. He further indicated that disruption of canalicular connections could provide the stimulus for activating remodeling. At the same time Johnson reported overuse may lead to bone resorption as observed in stress fracture [2]. Soon after, Jim Arnold was the first to demonstrate cancellous bone microfracture repair in human vertebral body deformation and crush fracture observed as trabecular microcallus [77,78]. Thus, these three pioneers were the ones who initiated the current interest in microdamage that Burr, Martin, Schaffler, and coworkers revisited with experimental evidence that there was bone remodeling in response to in vivo fatigue microdamage [70–74,79–86]. Furthermore, both Harold Frost and Lent Johnson earlier observed osteocyte death. Harold saw dead "micropetrotic" bone in elderly adults when the osteocytes died, and most of their lacunae and canaliculae became filled with mineral. The dead micropetrotic bone accumulated microdamage [9]. Lent Johnson described the aging osteocyte sequence involving ossification of canaliculi, pyknosis, and disintegration with or without ossification of lacunae or oncosis with lacunar and canalicular enlargement and filling with lamellar bone [2]. In addition, they both postulated the osteocyte as the logical choice as the mechanosensor to mechanical usage. Currently, osteocyte death (apoptosis) is associated with aging [87,88], estrogen deficiency [83,89], excessive glucocorticoid [90,91], disuse state [75,92,93], and microdamage [76,94] that all led to a reduction in bone strength.

The hypothesis of the osteocyte three-dimensional cellular network as the mechanosensory organ of bone has generated several concepts that explain adaptive modeling and remodeling at the level of cells, osteons, and trabeculae. These models are generally in agreement with Frost's mechanostat hypothesis. There is growing support for the formation of canalicular interstitial fluid as the likely stress-derived factor that informs the osteocyte about the level of bone loading; thus, the canaliculi are the bone porosity of interest and the osteocytes, the mechanosensor cells [82,95–101].

Marotti et al. [102] were the first to quantitate the association of bone resorption activity of osteocytes and experimental disuse osteoporosis. This work was followed by their hypothesis that an inhibitory signal traveling through canalicular processes from osteocytes to osteoblasts initiates recruitment of selected osteoblasts into osteoblast lineage – the mechanism of conversion of osteoblast to osteocytes [103–106]. Martin [107] further extended this theory, assuming that osteocytes display a general inhibitory effect on function. He theorized osteocytes send an inhibitory signal in response to mechanical loading to inhibit bone lining cells from activating remodeling. The theory proposes that a single kind of signal transmitted through cellular synctium of osteocytes, osteoblasts, and lining cells controls remodeling in response to mechanical loading and in the presence of events such as osteocyte death and hormonal fluctuations. Thus, remodeling would increase in a disuse state, as predicted by the mechanostat hypothesis and experimentally observed because strain-generated inhibitory signals would be diminished. Remodeling would be activated by mechanical damage (microcracks) along with osteocyte apoptosis, which interferes with both signal generation and transmission [73].

There is mounting evidence of Martin's theory that osteocytes display general inhibitory signals: (1) Maejuma-Ikeda et al. [108] reported a chick-derived protein that inhibits bone resorption; (2) Gowen et al. [109] showed that matrix extracellular phosphoglycoprotein (MEPE)/osteoblast/osteocyte factor 45 (OF-45) inhibits bone formation; (3) Lowik et al. [110] reported sclerostin is an osteocyte-derived negative regulator of bone formation; and (4) Gluhak-Heinrich et al. [111] showed a differential effect of mechanical loading in bone formation and resorption by osteocyte-derived dentin matrix protein 1 (i.e., the inhibition of resorption and transient inhibition of bone formation). Nevertheless, further studies are needed.

What's next?

The aim of this section is to improve the performance of current and future bone histomorphometrists and perpetuate the technology of bone histomorphometry as well as point the way for needed bone studies that contribute to a better understanding of bone biology and disorders.

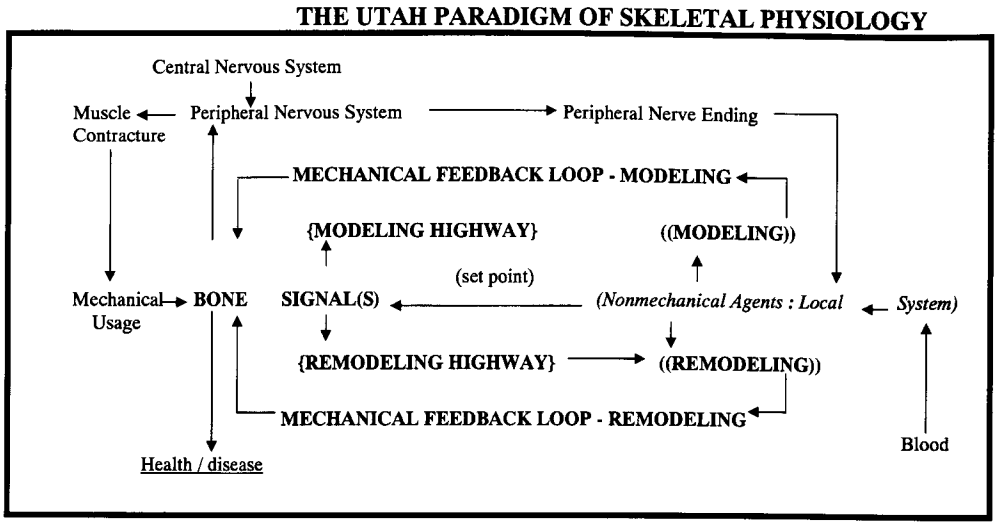

Fig. 5. The ever-evolving Utah paradigm of skeletal physiology with the mechanostat (*in bold letters*) as its key component. The paradigm incorporates the influence of the central and peripheral nervous system, musculature, and nonmechanical agents. There is the need to factor the circulatory system, etc., in the paradigm

There are numerous bone biology and disorders problems needing attention, but here are a few of my suggestions: (1) persuade bone morphometry societies to sponsor a repository for books on bone morphometry data across species (i.e., on maps of age-related bone formation, resorption, remodelling, and turnover rates, etc.) to aid investigators to study more relevant bone sites than the one of convenience; (2) require histomorphometrists to be more global by including more multiple bone sites (e.g., hematopoetic versus fatty, loaded versus nonloaded, active and passive circulation, etc.) in their studies; (3) lobby human-use committees to allow for more harvesting of human autopsy specimens that generated studies such as those by Arnold [77,78] and Fazzalari et al. [112,113]; (4) stimulate the development of a "super" animal model for osteoarthrosis and glucocorticoid-induced osteopenia like the rat ovariectomy model for estrogen deficiency-induced osteopenias; (5) initiate experimental studies of the forgotten osteoblast lineage cell – the bone lining cell (BLC) (e.g., provide more experimental evidence for Rodan and Martin's hypothesis that BLC are remodeling activators [114], Evert's hypothesis that BLC fills the role in coupling bone resorption to formation [115], and Martin's hypothesis that BLCs activate remodeling unless inhibited by an inhibitory signal [73,107]); (6) initiate studies on the marrow and periosteal mediator (e.g., Horiuchi et al. [116]) periostin; and (7) characterize the osteocyte-derived inhibitory factors of bone resorption (e.g., Heino et al. [117]: the osteocyte inhibits osteoclastic activity through transforming growth factor (TGF)-beta enhancement by estrogen). Finally (8), I had asked Harold Frost what he sees for the future: "Wide acceptance of the mechanostat hypothesis and suitable modification of dynamic bone histomorphometry to study and learn how to control all the body's mechanics and molecular histomorphometry studies to support remodeling BMUs and modeling drift in the context to explain tissue level phenomena." Last but not least (9), further modifications of the ever-evolving Frost's Utah paradigm of skeletal physiology (i.e., involving the circulatory system, etc.) (Fig. 5).

References

1. Johnson LC (1962) Joint remodeling as the basis for osteoarthritis. J Am Vet Med Assoc 141:1237
2. Johnson LC (1964) Morphologic analysis in pathology: the kinetics of disease and general biology of bone. In: Frost HM (ed) Bone Biodynamics. Little-Brown, Boston, pp 543–654
3. Johnson LC (1964) Bone density and the relation of structure to function. In: Proceedings, Conference on Aseptic Necrosis of the Femoral Head. Surgery Study Section, NIH, Bethesda, p 25
4. Johnson LC (1966) Kinetics of skeletal remodeling. In: Birth Defects. Original Article Series 2:66–142
5. Johnson LC (1971) Genetics and growth. In: Moyers RE, Krogman WM (eds) Craniofacial Growth in Man. Pergamon, New York, pp 258–283
6. Frost HM (1958) Preparation of thin undecalcified bone sections by rapid method. Stain Technol 33:273–277
7. Frost HM (1960) A new bone affection: feathering. J Bone Joint Surg 42A:447–456
8. Frost HM (1960) *In vivo* osteocyte death. J Bone Joint Surg [Am] 42:138–143
9. Frost HM (1960) Micropetrosis. J Bone Joint Surg 42A:144–150
10. Frost HM (1960) Presence of microscopic cracks in vivo in bone. Henry Ford Hosp Med Bull 8:25–35
11. Frost HM (1963) Introduction to Biomechanics. Thomas, Springield
12. Frost HM (1964) The Laws of Bone Structure. Thomas, Springfield
13. Frost HM (1964) Mathematical Elements of Lamellar Bone Remodeling. Thomas, Springfield
14. Frost HM (1969) Tetracycline-based histological analysis of bone remodeling. Calcif Tissue Res 3:211–237
15. Frost HM (1969) Measurement of human bone formation by means of tetracycline labeling. Can J Biochem Physiol 41:331–342
16. Frost HM (1973) The origin and nature of transients in human bone remodeling dynamics. In: Duncan H, Frame B, Parfitt AM (eds) Clinical Aspects of Metabolic Bone Disease. Excerpta Medica, Amsterdam, pp 124–137
17. Frost HM (1977) A method of analysis of trabecular bone dynamics. In: Meunier PJ (ed) Bone Histomorphometry. Armour-Montagu, Paris, pp 445–475

18. Frost HM (1977) Bone histomorphometry: empirical correction of appositional rate measurements in trabecular bone. In: Meunier P (ed) Second International Workshop on Bone Morphometry. University of Claude Bernard, Lyon, p 371
19. Frost HM (1977) Bone histomorphometry: a method of analysis of trabecular bone dynamics. In: Meunier P (ed) Second International Workshop on Bone Morphometry. University of Claude Bernard, Lyon, p 445
20. Frost HM (1983) Bone histomorphometry: analysis of trabecular bone dynamics. In: Recker RR (ed) Bone Histomorphometry, Techniques and Interpretations. CRC, Boca Raton, pp 109–142
21. Frost HM (1983) Analysis of trabecular bone dynamics. In: Recker RR (ed) Bone Histomorphometry: Techniques and Interpretation. CRC, Boca Raton, pp 143–224
22. Frost HM (1985) Bone microdamage: factors that impair its repair. In: Uhthoff HK (ed) Current Concepts in Bone Fragility. Springer, Berlin, pp 123–148
23. Frost HM (1986) Intermediary Organization of the Skeleton, vols I and II. CRC, Boca Raton
24. Frost HM (1987) The mechanostat: a proposed pathogenetic mechanism of osteoporoses and the bone mass effects of mechanical and nonmechanical agents. Bone Miner 2:73–85
25. Frost HM (1987) The mechanostat. Anat Rec 219:1–9
26. Frost HM (1960) Introductions to joint biomechanics. Henry Ford Hosp Med Bull 8:415–432
27. Frost HM (1987) Mechanical determinants of bone architecture. I: Bone modeling. In: Albright JA, Brand RA (eds) The Scientific Basis of Orthopaedics, 2nd edn. Appleton-Century-Crofts, New York, pp 241–266
28. Frost HM (1987) Osteogenesis imperfecta. The setpoint proposal. Clin Orthop Relat Res 216:280–297
29. Frost HM (1989) Transient-steady state phenomena in microdamage physiology: a proposed algorithm for lamellar bone. Calcif Tissue Int 44:367–381
30. Frost HM (1990) Structural adaptations to mechanical usage (SATMU): 1. Redefining Wolff's law: the bone modeling problem. Anat Rec 226:403–413
31. Frost HM (1992) Perspectives: Bone's mechanical usage windows. Bone Miner 19:257–271
32. Frost HM (1996) Perspectives: A proposed general model for the mechanostat (suggestions from a new paradigm). Anat Rec 244:139–147
33. Frost HM (1998) A brief review for orthopedic surgeons: fatigue damage (microdamage in bone – its determinants and clinical implications). J Orthop Sci 3:272–281
34. Frost HM (1998) On rho, a marrow mediator and estrogen: their roles in bone strength amd "mass" in human females, osteopenias and osteoporoses (insights from a new paradigm). J Bone Miner Metab 16:113–123
35. Frost HM (1998) Osteoporoses: new concepts and some implications for future diagnosis, treatment and research (based on insights from the Utah paradigm). Ernst Schering Research Foundation AG, Berlin, pp 7–57
36. Frost HM (2000) The Utah paradigm of skeletal physiology: an overview of its insights for bone cartilage and collagenous tissue organs. J Bone Miner Metab 18:305–316
37. Frost HM (2001) From Wolff's law to the Utah paradigm: insights about bone physiology and its clinical applications. Anat Rec 262:398–419
38. Frost HM (2001) The Utah paradigm on animal models of skeletal disorders: quo vadis? J Musculoskel Neuron Interact 1:185–191
39. Frost HM (2001) Does the anterior cruciate have a modeling threshold? A case for the affirmative. J Musculoskel Neuron Interact 2:131–136
40. Frost HM (2003) Bone's mechanostat: a 2003 update. Anat Rec 275:1081–1101
41. Frost HM (2003) On the strength-safety factor (SSF) for load-bearing skeletal organs. J Musculoskel Neuron Interact 3:136–140
42. Frost HM (2004) A 2003 update of bone physiology and Wolff's law for clinicians. Angle Orthodont 74:3–15
43. Frost HM, Griffith DL, Jee WSS, Kimmel DB, McCandliss RP, Teitelbaum S (1981) Histomorphometric analysis of trabecular bone in renal dialysis patients treated with calcifediol. J Metab Bone Dis Relat Res 2:285–295
44. Frost HM, Jee WSS (1994) Perspectives: A vital biomechanical model of the endochonral ossification mechanism. Anat Rec 240:435–446
45. Frost HM, Jee WSS (1994) Perspectives: Applications of a biomechanical model of the endochondral ossification mechanism Anat Rec 240:447–455
46. Frost HM, Schönau E (2000) The "muscle-bone unit" in children and adolescents. J Pediatr Endocrinol Metab 13:571–590
47. Frost HM, Villaneuva AR, Roth H, Stanisaolyevic S (1961) Tetracycline bone labeling. J New Drugs, p 206
48. Hattner R, Epker BN, Frost HM (1965) Suggested sequential mode of control of changes in cell behavior in adult bone remodeling. Nature (Lond) 206:489–490
49. Schiessl H, Frost HM, Jee WSS (1998) Estrogen and bone-muscle strength and mass relationships. Bone 22:1–6
50. Brown W, Haglund K (1995) Landmarks interviews. J NIH Res 7:54–59
51. Milch RA, Rall DP, Tobie JE (1957) The bone localization of the tetracycline. J Nat Cancer Inst 19:87–93
52. Amprino R, Marotti G (1964) A topographic quantitative study of bone formation and reconstruction. In: Blackwood HJJ (ed) Bone and Tooth Symposium. Macmillan, New York, p 21–33
53. Haas HG, Miller J, Schenk RK (1967) Osteomalacia: metabolic and quantitative histologic studies. Clin Orthop 53:213–222
54. Bordier P, Matrajt H, Miravet B, Hioco D (1964) Mesure histologigue de la masse in de la resorption des travees osseuse. Pathol Biol (Paris) 12:1238
55. Bordier PJ, Tun Chot S (1972) Quantitative histology of metabolic bone disease. Clin Endocrinol Metab 1:197–215
56. Arnold JS (1951) A method of embedding undecalcified bone for histologic sectioning and its application to radioautography. Science 114:178–180
57. Arnold JS, Jee WSS (1954) Embedding and sectioning undecalcified bone and its application to radioautography. Stain Technol 29:225–239
58. Arnold JS, Jee WSS (1954) Double tracer radioautographic studies of Ca^{45} and Ra^{226} bone deposition. Radiat Res 1:488
59. Parfitt AM (1983) The physiologic and clinical significance of bone histomorphometric data. In: Recker RR (ed) Bone Histomorphometry: Techniques and Interpretations. CRC, Boca Raton, pp 143–224
60. Parfitt AM, Drezner MK, Glorieux FH, Kanis JA, Malluche H, Meunier PJ, Ott SM, Recker RR (1987) Bone histomorphometry: standardization of nomenclature, symbols and units. Report of the ASBMR Histomorphometry Committee. J Bone Miner Res 2:595–610
61. Recker RR, Barges-Lux MJ (2003) Bone biopsy and histomorphometry in clinical practice. In: Favus MJ (ed) Primer on Metabolic Bone Diseases and Disorders of Mineral Metabolism. American Society for Bone and Mineral Research, Washington, DC, pp 213–219
62. Koch JC (1917) The laws of bone architecture. Am J Anat 21:12–23
63. Thompson DW (1917) On Growth and Form. Cambridge University Press, London
64. Lanyon LE, Smith RN (1969) Measurement of bone strain in walking animals. Res Vet Sci 10:83–94
65. Frost HM (1995) Introduction to a New Skeletal Physiology, vol I. Bone and Bones. Pajaro Group, Pueblo, CO
66. Carter R, Harris HW, Vaser R, Caler WE (1981) The mechanical and biological responses of cortical bone to *in vivo* strain histories. In: Cowin S (ed) Mechanical Properties of Bone. ASME, New York
67. Cowin SC (1984) Mechanical Properties of Bone. ASME, New York
68. Forwood MR, Li I, Kelly WL, Bennett MB (2001) Growth hormone is permissive for skeletal adaptation to mechanical loading. J Bone Miner Res 16:2284–2290
69. Metz LN, Martin RB, Turner AS (2003) Histomorphometric analysis of the effects of osteocyte density on osteonal morphology and remodeling. Bone 33:753–759
70. Burr DB, Martin RB, Schaffler MB, Radin EL (1985) Bone remodeling in response to in vivo fatigue microdamage. Bone 14:103–109

71. Burr DB, Martin RB, Schaffler MB, Radin EL (1985) Bone remodeling in response to *in vivo* fatigue microdamage. J Biomech 18:189–200
72. Bentolila V, Boyce TM, Fyhrie DP, Drumb R, Skerry TM, Schaffler MB (1998) Intracortical bone remodeling in adult rat long bones after fatigue loading. Bone 23:275–281
73. Martin RB (2003) Fatigue microdamage as an essential element of bone mechanics and biology. Calcif Tissue Int 73:101–107
74. Martin RB, Burr DB (1982) A hypothetical mechanism for the stimulation of osteonal remodeling by fatigue damage. J Biomech 15:137–139
75. Noble BS, Peet N, Stevens HY, Brabbs A, Mosley JR, Reilly GC, Reeve J, Skerry TM, Lanyon LE (2003) Mechanical loading: biphasic osteocyte survival and targeting of osteoclasts for bone destruction in rat cortical bone. Am J Physiol Cell Physiol 284: C934–C943
76. Noble B (2003) Bone microdamage and cell apoptosis. Eur Cell Mater 6:460–455; discussion 455
77. Arnold JS (1964) The quantitation of bone mineralization as an organ and tissue in osteoporosis. In: Pearson OH, Joplin GF (eds) Dynamic Studies of Metabolic Bone Disease. Blackwell, London, pp 59–79
78. Arnold JS (1968) External and trabecular morphologic changes in lumbar vertebrae in aging. In: Progress in Methods of Bone Mineral Measurement. US Government Printing Office, Washington, DC, pp 352–410
79. Burr DB, Hooser M (1995) Alterations to the en bloc basic fuschin staining protocol for the demonstration of microdamage produced in vivo. Bone 17:431–433
80. Burr DB, Stafford T (1990) Validity of the bulk-staining technique to separate artifactual from in vivo bone microdamage. Clin Orthop Relat Res 260:305–308
81. Martin RB, Burr DB (1989) Structure, Function and Adaptation of Compact Bone. Raven, New York
82. Cowin, SC (2001) Bone Mechanics Handbook, 2nd edn. CRC, Boca Raton
83. Tomkinson A, Gevers EF, Wit JM, Reeve J, Noble B (1998) The role of estrogen in the control of rat osteocyte apoptosis. J Bone Miner Res 13:1243–1250
84. Schaffler MB, Choi K, Milgrom C (1995) Aging and matrix microdamage accumulation in human compact bone. Bone 17: 521–525
85. Wenzel TE, Schaffler MB, Fyhrie DP (1996) In vivo trabecular microcracks in human vertebral bone. Bone 19:89–95
86. Mori S, Burr DB (1993) Increased intracortical remodeling following fatigue damage. Bone 14:203–209
87. Dunstan CR, Evans RA, Hills E, Wong SYP, Higgs RJED (1990) Bone death in hip fracture in the elderly. Calcif Tissue Int 47:270–275
88. Mullender MG, van der Meer DD, Huiskes R, Lips P (1996) Osteocyte density changes in aging and osteoporosis. Bone 18: 109–113
89. Tomkinson A, Reeve J, Shaw RW, Noble BS (1997) The death of osteocytes via apoptosis accompanies estrogen withdrawal in human bone. J Clin Endocrinol Metab 82:3128–3135
90. Weinstein RS, Nicholas RW, Manolagas SC (2000) Apoptosis of osteocytes in glucocorticoid-induced osteonecrosis of the hip. J Clin Endocrinol Metab 85:2907–2912
91. O'Brien CA, Jioa D, Plotkin LI, Bellido T, Powers CC, Steward SA, Manolagas SC, Weinstein RS (2004) Glucocorticoids act directly on osteoblasts and osteocytes to induce their apoptosis and reduce bone formation and strength. Endocrinology 145: 1835–1841
92. Noble BS, Stevens H, Reeve J, Loveridge N (1995) Apoptosis in normal and pathological human bone. J Bone Miner Res 10:52–64
93. Noble BS, Stevens H, Loveridge N, Reeve J (1997) Identification of apoptotic changes in osteocytes in normal and pathological human bone. Bone 20:273–282
94. Verborgt O, Givson GJ, Lundin-Cannon KD, Schaffler MB (1999) Osteocyte apoptosis occurs in association with bone fatigue, microdamage and resorption. Trans Orthop Res Soc 24:551
95. Cowin SC, Moss-Salentijn L, Moss ML (1991) Candidates for the mechanosensory system in bone. J Biomech Eng 113:191–197
96. Cowin SC, Weinbaum S, Zeng Y (1995) A case for bone canaliculi as the anatomical site of strain generated potentials. J Biomech 28:1281–1297
97. Mullender MG, Hiskes R (1997) Osteocytes and bone lining cells: which are the best candidates for mechano-sensors in cancellous bone? Bone 20:527–532
98. Burger EH, Klein-Nulend J, Van der Plas A, Nijweide PJ (1995) Function of osteocytes in bone: their role in mechanotransduction. J Nutr 125(suppl):2020S–2023S
99. Burger EH, Klein-Nulend J (1999) Mechanotransduction in bone: role of the lacuno-canalicular network. FASEB J 13(suppl):S101–S112
100. Burger EH, (2001) Experiments on cell mechanosensitivity: bone cells as mechanical engineers. In: Cowin SC (ed) Bone Mechanics Handbook, 2nd edn, vol 28. CRC, Boca Raton, 1–16
101. Klein-Nulend J, Van der Plas A, Semeins CM, Ajubi NE, Frangos JA, Nijweide PJ, Burger, EH (1995) Sensitivity of osteocytes to biomechanical stress in vitro. FASEB J 9:441–445
102. Marotti G, Delrio N, Marotti F, Fadda M (1979) Quantitative analysis of the bone destroying activity of osteocytes and osteoclasts in experimental disuse osteoporosis. Ital J Orthop Traumatol 5:225–240
103. Marotti G, Ferretti M, Muglio MA, Palumbo C, Palazzini SA (1990) A quantitative evaluation of osteoblast-osteocyte relationships on growing endosteal surfaces of rabbit tibia. Bone 13:363–368
104. Marotti G, Cane V, Palazzini S, Palumbo C (1990) Structure-function relationships in the osteocyte. Ital J Miner Electrol Metab 4:93–106
105. Marotti G, Ferritti M, Remaggi F, Palumbo C (1995) Quantitative evaluation on osteocyte canalicular density in human secondary osteons. Bone 16:125–128
106. Marotti G (1996) The structure of bone tissues and the cellular control of their deposition. Ital J Anat Embryol 101:25–79
107. Martin RB (2000) Toward a unifying theory of bone remodeling. Bone 26:1–6
108. Maejuma-Ikeda A, Aoki M, Tsuritani K, Kamioka K, Hiura K, Miyoshi T, Hara H, Takano-Yamamoto T, Kumegawa M (1997) Chick osteocyte-derived protein inhibits osteoclastic bone resorption. Biochem J 322:245–250
109. Gowen LC, Petersen DN, Mansolf Al, Qi H, Stocks JL, Tkalcevic GT, Simmons HA, Crawford DT, Chidsey-Frink KL, Ke HZ, McNeish JD, Brown TA (2003) Targeted disruption of the osteoblast/osteocyte factor 45 gene (OF45) results in increased bone formation and bone mass. J Biol Chem 278:1998–2007
110. Lowik C, TenDyke P, Van Bezooyen R (2004) Sclerostin is an osteocyte-expressed negative regulator of bone formation, but not a classical BMP antagonists. Calcif Tissue Int 74(suppl):522
111. Gluhak-Heinrich J, Ye L, Bonewald LF, Feng JQ, MacDougall M, Harris SE, Pavlin D (2003) Mechanical loading stimulates dentin matrix protein 1 ((DMP1) expression in osteocytes *in vivo*. J Bone Miner Res 18:807–817
112. Fazzalari NL, Forwood MR, Smith K, Manthey BA, Herseen P (1998) Assessment of cancellous bone quality in severe osteoarthrosis: bone mineral density, mechanics and microdamage. Bone 22:381–38
113. Fazzalari NL, Kuliwaba JS, Forwood MR (2002) Cancellous bone microdamage in the proximal femure: influence of age and osteoarthritis on damage morphology and regional distribution. Bone 31:697–702
114. Rodan GA, Martin TJ (1981) Role of osteoblasts in hormonal control of bone resorption: a hypothesis. Calcif Tissue Int 33:349–351
115. Evert V (2004) Bone lining cells and the regulation of bone resorption. Calcif Tissue Int 74:524
116. Horiuchi K, Amizuka N, Takeshita S, Takamatsu H, Katsuura M, Ozawa H, Toyama Y, Bonewald LF, Kudo A (1999) Identification and characterization of a novel protein periostin, with restricted expression to periosteum and periodontal ligament and increased expression by transforming growth factor beta. J Bone Miner Res 14:1239–1249
117. Heino TJ, Hentunen TA, Vaananen HK (2002) Ostocytes inhibit osteoclastic bone resorption through transforming growth factor-beta: enhancement by estrogen. J Cell Biochem 85:185–197