Journal of Cellular Biochemistry 55:287–299 (1994)

Function of Osteocytes in Bone

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Abstract Although the structural design of cellular bone (i.e., bone containing osteocytes that are regularly spaced throughout the bone matrix) dates back to the first occurrence of bone as a tissue in evolution, and although osteocytes represent the most abundant cell type of bone, we know as yet little about the role of the osteocyte in bone metabolism. Osteocytes descend from osteoblasts. They are formed by the incorporation of osteoblasts into the bone matrix. Osteocytes remain in contact with each other and with cells on the bone surface via gap junction–coupled cell processes passing through the matrix via small channels, the canaliculi, that connect the cell body–containing lacunae with each other and with the outside world. During differentiation from osteoblast to mature osteocyte the cells lose a large part of their cell organelles. Their cell processes are packed with microfilaments. In this review we discuss the various theories on osteocyte function that have taken in consideration these special features of osteocytes. These are 1) osteocytes are actively involved in bone turnover; 2) the osteocyte network is through its large cell-matrix contact surface involved in ion exchange; and 3) osteocytes are the mechanosensory cells of bone and play a pivotal role in functional adaptation of bone. In our opinion, especially the last theory offers an exciting concept for which some biomechanical, biochemical, and cell biological evidence is already available and which fully warrants further investigations.

Key words: osteocyte, gap junction, cytoskeleton, extracellular matrix, osteocytic osteolysis, bone membrane, functional adaptation, mechanical loading, strain

Bone is a specialized connective tissue. Like all connective tissues, it consists of various types of cells, a complex extracellular matrix, and extracellular fluid. The uniqueness of bone, which it shares with calcified cartilage, is that the extracellular matrix is calcified. This property determines the functions of bone. Bone provides support for the body and sites for muscle attachment. Vital organs (brain) and tissues (bone marrow) are protected by bone. Bone acts as a reservoir for ions such as calcium, phosphate, and magnesium and helps to maintain the homeostasis of these ions in the blood.

Bone is a living, continuously self-renewing tissue. At first sight, the most important cell types involved in the formation, modeling, and remodeling of bone are the osteoblasts or bone forming cells and the osteoclasts, the bone resorbing cells. The most abundant cell type in mature bone is, however, the osteocyte. There are approximately ten times as many osteocytes as osteoblasts in normal human bone [Parfitt, 1977]. Osteocytes have a particular location in bone. During bone formation some osteoblasts are left behind while the bone formation front moves on together with the other, retracting osteoblasts. The encapsulated osteoblasts differentiate into osteocytes. They lose a large part of their cell organelles but gain long, slender cell processes by which the cells remain in contact with earlier incorporated osteocytes and with osteoblasts lining the bone surface. This structural design of bone with its complex cellular (osteocytic) organization within the bone matrix is very old. Its first occurrence in evolution dates back to the jawless, armoured fish called Ostracoderms that lived 400 to 250 million years ago [Hancox, 1972; Hall, 1992]. The tissues of the dermal plates of some representatives of the ostracoderms were similar or even identical to modern bone. Stellate shaped osteocytes are also found in dinosaur bone of some 80 million years old, arranged in a complex pattern of concentric

Received February 10, 1994; accepted February 14, 1994.

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layers around a Haversian channel containing a blood vessel, remarkably similar to the pattern in larger vertebrates of the present day (Fig. 1a,b).

These phenomenological considerations (i.e., the relative large numbers, the morphology, the location of osteocytes, and the evolutionary highly conserved osteocyte network) suggest a crucial, specific role for osteocytes in bone. In this review we will concern ourselves with the question what this role may be.

OSTEOCYTE FORMATION AND FATE

The mature osteocyte is a stellate shaped or dendritic cell, enclosed within the lacunar-canalicular network of bone. The lacuna contains the cell body, the canaliculi the cytoplasmic processes of the osteocyte. Osteocytes originate from osteoblasts. During the process of bone formation some of the osteoblasts, involved in the production of bone matrix, become embedded in that matrix as a result of their own activity and that of neighbouring osteoblasts. The matrix around the newly incorporated cell is at first not yet calcified (osteoid) but gradually calcifies as the formation front moves away because of continuing osteoblastic activity. A cell only recently incorporated in osteoid or sparsely calcified matrix still possesses many features of the original osteoblast: an abundance of granular endoplasmic reticulum (ER), numerous free ribosomes, many mitochondria, and a well-developed juxtanuclear Golgi apparatus. In older, more deeply embedded osteocytes the number of organelles is reduced and the cytoplasmic volume decreased. The cellular body volume of the osteoid osteocyte is reduced by 30% and that of the mature osteocyte by even 70% compared to the volume of the original osteoblast [Palumbo, 1986]. Detailed descriptions of the change from osteoblast to osteocyte have been reported by Palumbo et al. [1990a,b] and Nefussi et al. [1991]. Both groups roughly come to the same conclusions. A signal, perhaps emitted by a recently matrix-embedded osteocyte [Palumbo et al., 1990a], stimulates an osteoblast to become committed to osteocyte differentiation. At the same time a preosteoblast in the direct neighbourhood of this committed osteoblast (COB) starts to differentiate into a mature osteoblast, which will eventually occupy the place of the COB in the layer of osteoblasts. Matrix production of the COB diminishes [Nefussi et al., 1991] or remains at the same level but is spread over a larger area because of the flattening of the COB over the osteoid surface (reduction of the rate of the linear appositioned matrix production) [Palumbo et al., 1990a,b]. As the osteoblasts around the COB keep up their linear appositional matrix production rate, the matrix formation front slowly catches up with the COB. This stage is called the preosteocyte type 1 stage by Palumbo et al. [1990a,b]. In the next stage, the preosteocyte type 2 [Palumbo et al., 1990a,b] or osteoblastic osteocyte [Nefussi et al., 1991] stage, the cell has completely left the layer of osteoblasts but is still lying directly against this layer and is connected to the osteoblasts by many cell-cell contacts. No matrix has as yet been formed between the preosteocyte 2 and the osteoblast layer. Then, as matrix formation continues, the preosteocyte becomes more and more separated from the osteoblastic layer by a growing layer of osteoid (stage "preosteocyte type 3" or "osteoid osteocyte"). Finally the calcification front catches up with the preosteocyte that then becomes a ma-

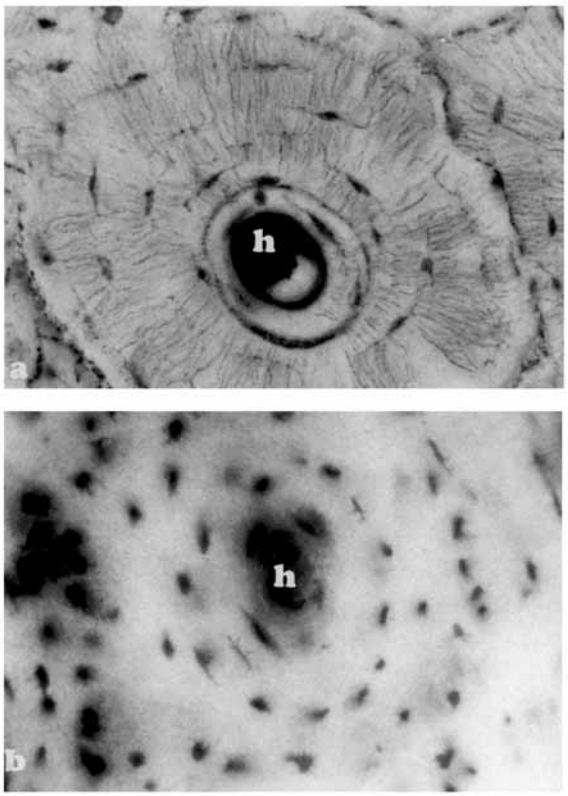

Fig. 1. Osteons with osteocytes. **a:** An osteon in human compact bone is shown. Osteocytes are stained with Schmorl. Fine canaliculi can be seen that radiate from the lacunae. **b:** Osteocyte lacunae within *Brontosaurus* bone are shown. The osteocytes in this dinosaur bone are arranged similar to "modern" (human) bone. h, Haversian channel. ×150.

ture osteocyte. During this process of differentiation from cuboidal osteoblast to ellipsoid osteocyte the cell gradually loses cell organelles and its capacity to produce extracellular matrix. The nucleus to cytoplasm ratio increases.

As to the total life span of the osteocyte, four phases have been described [reviewed by Bonucci, 1990]: a formative phase, which comprises the preosteocyte stages described above and the young mature osteocyte stage (during this phase the osteocyte is supposed to have still bone forming potential); a steady-state phase, during which the cell is metabolically more or less inactive; a resorptive phase, during which the cell is supposed to be capable of bone resorption (osteocytic osteolysis [Bélanger, 1969]); and a degenerative phase followed by cell death. The existence of a resorptive phase in the life cycle of the osteocyte is now seriously questioned, as will be discussed later. Cell death following the degenerative phase may not be the only possible, fatal end of an osteocyte. There is some evidence that osteoclasts may engulf and destroy osteocytes by intracellular degradation when they encounter an osteocyte during bone resorption [Elmardi et al., 1990]. It has also been suggested that osteocytes when liberated from their lacunae by osteoclastic activity, dedifferentiate into fibroblasts- (osteoblast-) like cells [Jones and Boyde, 1977].

MORPHOLOGY AND PROPERTIES OF THE OSTEOCYTE

Characteristic of and essential to osteocytes are the cytoplasmic processes which couple osteocytes to each other and to the cells on the bone surface. They and the canaliculi are the only possible communication routes to the outside world. Deeply embedded osteocytes depend on their cytoplasmic processes and the canaliculi for the transport of nutrients and waste products to and from the blood circulation. Although the cell processes radiate in all directions, their distribution is not symmetrical [Palumbo et al., 1990b]. More processes are directed to the deeper lying osteocytes than to the bone surface (the vascular side). According to Palumbo et al. [1990b] the formation of cytoplasmic processes during the transition of osteoblast to osteocyte is an asynchronous and asymmetrical phenomenon. Osteoid osteocytes are polarized towards the mineralization front, and the formation of cell processes on this side precedes the formation on the vascular side.

The typical stellate shape of the osteocyte is not imposed on the cell by the matrix that surrounds it in vivo. When osteocytes are isolated and seeded onto glass or plastic supports, the cells regain their typical stellate morphology [van der Plas and Nijweide, 1992]. Within 24 h after adherence the (when suspensed) globular cells, form long, dendritic cell processes. Neighbouring cells make many contacts with each other via these cell processes, thereby creating a complex network in the plane of the support, strongly reminiscent to the in vivo situation (Fig. 2a,b).

The intercellular attachment sites between osteocytes or between osteocytes and osteoblasts are of various shapes: invaginated-finger-like, side-to-side, and end-to-end [Palumbo et al., 1990a; Weinger and Holtrop, 1974]. These differences may reflect differences in function of these contacts. The cell junctions in the cell-cell contacts were first described as tight or occluding junctions, characterized by fusion of the outer leaflets of the opposing cell membranes [Holtrop and Weinger, 1972], although the possibility that they were gap junctions was kept open [Weinger and Holtrop, 1974]. Later, a 2–4 nm interspace between the opposing cell membranes was discovered, and the junctions were defined as gap junctions [Doty, 1981]. Gap junctions were found on the cell body of the osteocytes as well as on their cytoplasmic processes [Jones et al., 1993]. Functional proof of cellular coupling via gap junctions has been obtained for osteoblasts by injecting dye into an osteoblast [Jeansonne et al., 1979]. The dye spread rapidly over a number of surrounding cells, thereby demonstrating coupling. A similar result is expected for osteocytes, although the actual experiment has not yet been reported.

Apart from their characteristic morphology and location in the bone matrix, we have virtually no evidence for specific metabolic activities of osteocytes that may give a clue to their function. Several lytic enzymes such as collagenase [Woods and Nichols, 1965], acid phosphatase [Doty et al., 1968], and aminopeptidase [Lipp, 1959] were reported to be present in osteocytes and were tentatively correlated to an osteolytic function. As the concept of osteocytic osteolysis [Bélanger, 1969] is now seriously questioned [Boyde, 1980; Marotti et al., 1990] (see Osteocyte Function: Osteocytic Osteolysis) and the presence or level of activity of these enzymes is not specific, these observations do not help us to

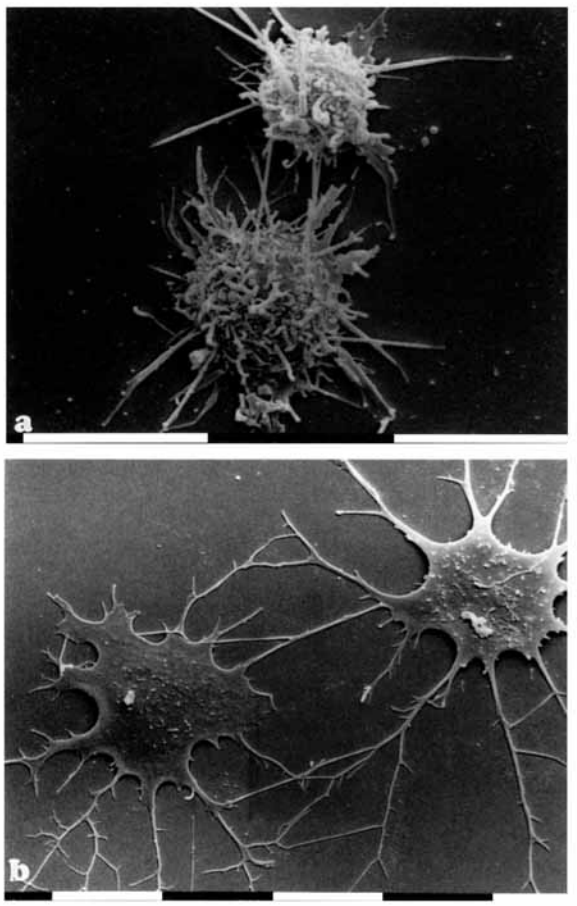

Fig. 2. Isolated osteocytes in culture. Bone cell populations were isolated from 18-day-old fetal chickens. These populations contained apart from osteoblastic and fibroblastic cells, also osteocytes. The osteocytes were isolated from the mixed bone cell populations by an immunodissection procedure [van der Plas et al., 1992]. The isolated osteocytes were seeded on a glass support, cultured for 10 min (**a**) or 24 h (**b**), and studied with a scanning electron microscope. Note that the osteocytes have formed cytoplasmic extrusions in all directions immidiately after attachment (a). After 24 h the osteocytes have flattened, the cell processes perpendicular to the support have disappeared, and the processes in the plane of the support branched and elongated. Cell processes from neighbouring cells have formed apparantly smooth contacts with each other. Bar = 10 μm.

envisage a specific role for the osteocyte. Alkaline phosphatase activitydecreases parallel to the differentiation from osteoblast to osteocyte, as was shown both enzyme-cytochemically [Doty, 1992] and immunocytochemically [Bruder and Caplan, 1990a; Turksen and Aubin, 1991]. Again, this reflects the loss of a specific cellular activity, as alkaline phosphatase is correlated to matrix calcification, rather than helping us to understand the role of the osteocyte in bone. Nevertheless, at least two important considerations imply an involvement of the osteocyte with the production or adaptation of its immediate adjacent matrix. The first is that, to survive, the cell is obliged to maintain an unmineralized area immediately around the cell body and the cell processes, to allow the diffusion of nutrients and waste products in and out of the tissue. In other words, osteocytes have to take care that mineralization does not progress up to their cell membranes. The second is that the formation, the morphology, and the possible function of the osteocyte as mechanosensory cell (see Osteocyte Function: Osteocytes as Mechanosensory Cells) predict an important role for the interaction between the osteocyte and its surrounding matrix (e.g., for specific cell-matrix adherence structures).

The important question therefore arises whether osteocytes produce the same matrix components in the same proportion as their parental osteoblasts but in a diminishing degree parallel to their maturation, or whether they produce the same components but in different proportions to each other. It is even possible that osteocytes produce specific matrix constituents, related to the specific properties of the matrix immediately around them. In the older literature, electron microscopical studies have shown that the osteocyte and its cell processes are surrounded by a thin layer of unmineralized matrix of a different composition than the mineralized interlacunar matrix [Jande, 1971]. The material was found to be PAS-positive and therefore was supposed to contain, apart from a few randomly oriented collagen fibres, mucopolysaccharides, now called proteoglycans. These results were corroborated by more recent studies. The concentration of proteoglycans in the pericellular area was found to be enhanced compared to the interlacunar matrix [Sauren et al., 1992]. Interestingly the proteoglycans were also larger than those in the interlacunar matrix. This may indicate that the proteoglycans were excreted by the osteocytes [Sauren et al., 1992].

Especially during the last decade many different noncollagenous matrix (glyco)proteins have been isolated and characterized. Several of these bone proteins that have been found in the osteoblasts and in the matrix laid down by the osteoblasts, have also been demonstrated in or around the osteocytes. Bone gamma carboxyglutamic acid containing protein (BGP) (osteocalcin) has been identified in the ER and the Golgi cisternae of osteocytes, although less dominantly than in young osteoblasts [Ohta et al., 1989; Boivin et al., 1990]. The perilacunar areas surrounding

the osteocytes did not show more osteocalcin than the interlacunar areas [Groot et al., 1986]. Osteonectin was demonstrated immunocytochemically in active osteoblasts and osteoprogenitor cells, as well as in osteoid osteocytes [Jundt et al., 1987] and osteocytes of alveolar bone [Chen et al., 1993]. However, no osteonectin immunoreactivity was found in human iliac crest osteocytes, which did stain for osteocalcin [Hinrichs et al., 1993]. Bone sialoprotein (BSP) (originally called BSP II) production in osteocytes seems to vary with species. In porcine alveolar bone, BSP was more abundant in osteocytes than in osteoblasts, while no BSP was found in rat osteocytes [Sodek et al., 1992]. Osteopontin (44 kD phosphoprotein; originally called BSP I) has been found in the bone matrix and in a higher concentration at the mineralization front [Hultenby et al., 1991], as well as in osteocytes [Mark et al., 1987]. In alveolar bone sections of rat, osteopontin was demonstrated in osteoblasts (Golgi apparatus), in the lamina limitans at the bone surface, and in cement lines, but also around cytoplasmic processes and osteocyte lacunae [McKee et al., 1993]. In the osteocytes the amount of osteopontin was intermediate to BSP and osteonectin [Chen et al., 1993].

In sum, no enzyme, cell- or matrix-protein specific for the osteocyte has been described. Still, the osteocyte plasma membrane appears to possess antigenic determinants specific for that cell. Two monoclonal antibodies (MAbs), OB7.3 [Nijweide and Mulder, 1986] (Fig. 3a,b) and SB5 [Bruder and Caplan, 1990b], apparently specific for osteocytes, have been described. Their antigens have, however, not yet been identified.

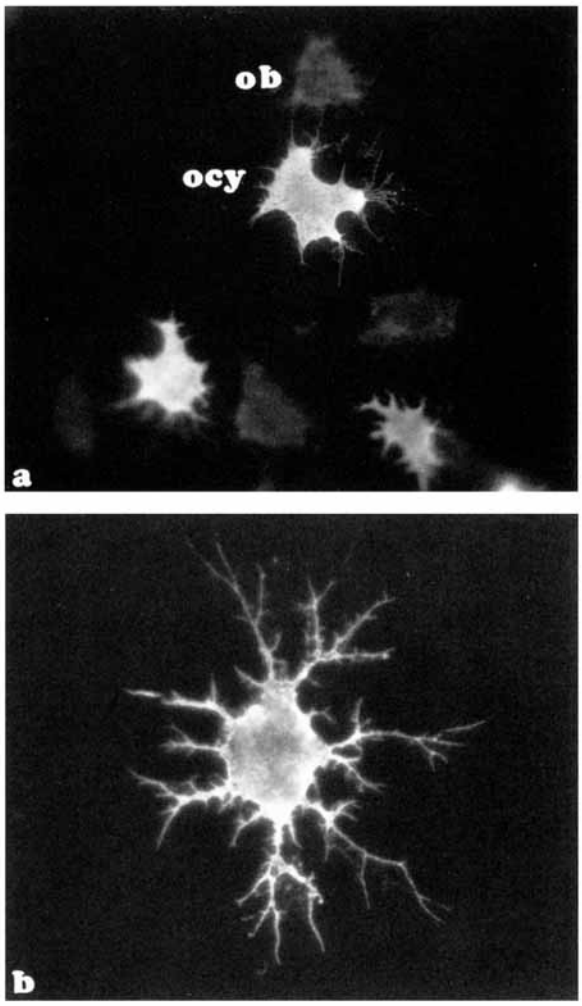

Fig. 3. Staining of osteocytes with MAb OB7.3. A bone cell population was isolated from calvariae of 18-day-old fetal chickens and cultured for 3 days. Subsequently, the population was successively incubated with MAb OB7.3, biotin-labeled horse-antimouse IgG and FITC-conjugated Extravidine. **a:** Osteocytes show bright fluorescence on their cell body as well as their cytoplasmic processes, while osteoblastic cells also present in the culture are negative. ocy, osteocyte, ob, osteoblastic cell; ×365. **b:** A higher magnification of a single osteocyte is shown, demonstrating that even the very fine cell processes are positive for the MAb. ×910.

THE CYTOSKELETON IN OSTEOCYTES

The cytoskeleton is important in all cells. It determines cell shape, organizes the cytoplasm by repositioning and moving cell organelles, and coordinates cell movement. The complex networks of the cytoskeletal filaments (microtubules, microfilaments, intermediate filaments) together with their associated proteins serve as the "bone and muscle" of the cell.

Considering the stellate shape of the osteocyte and its position in the bone matrix, the cytoskeleton may have a particularly important role to play in osteocytes. Microtubules are found in the cell body but not in the cell processes. They are probably, among other things, involved in the secretion of matrix proteins, such as collagen. The most striking feature of the cytoskeletal arrangement in the osteocyte is, however, the bundles of microfilaments [Weinger and Holtrop, 1974; King and Holtrop, 1975] that run parallel to the cell surface in the cell body and almost completely fill the cell processes. The function of these microfilaments is not known. They may play a role in the transport of (small) molecules from cell body to process tip and vice versa [King and Holtrop, 1975]. The gap junctions would then allow these molecules to pass from one cell to another. As microfilaments have contractile properties, it is also possible that osteocytes facilitate diffusion of

waste products and nutrients in the extracellular space of the canaliculi by repeated contraction and relaxation of their cell processes. The rapid passage of intravenously injected horseradish peroxidase (MW 40,000) through the lacunar-canalicular system [Doty and Schofield, 1972; Dillaman et al., 1991; Ayasaka et al., 1992] may be an expression of this process of facilitated diffusion. Thirdly, it is possible that the microfilaments play a role in the detection of mechanical strain by osteocytes (see Osteocyte Function: Osteocytes as Mechanosensory Cells).

OSTEOCYTE FUNCTION

Despite their relative abundance in bone tissue, no particular function has as yet unequivocally been demonstrated for osteocytes. Their location in bone and organization in a syncytium with two extensive communication systems, one intracellular (osteocyte–gap junction–osteocyte) and another extracellular (lacuna-canaliculus-lacuna), suggest at least three possible ways by which the presence of osteocytes in bone may be exploited by the tissue: 1) to ensure communication between sites deep in the bone and the extraosseous world; 2) to create an enormous increase in mineral surface exposed to extracellular fluid and cellular activity; and 3) to provide matrix repair capacity deep inside the bone, far from the (re)modeling system of osteoblasts and osteoclasts on the surface of bone.

These considerations have led to the formulation of several theories about the function of the osteocyte.

Osteocytic Osteolysis

According to Johnson [1966], the total surface area of the Haversian and Volkman canals in an adult man is 3.2 m^2, the total cancellous bone surface 9 m^2, the total lacunar surface 90 m^2, and the total canalicular surface 1,200 m^2. In other words, the bone surface available to the osteocytes is 100× larger than that available to osteoblasts and osteoclasts. Even if these estimates are exaggerated [Boyde, 1980], it is no wonder that the hypothesis of osteocytic bone remodeling or osteocytic osteolysis [reviewed by Bélanger, 1969] originally was met with much approval. A large number of studies in the 1960s and 1970s concluded that osteocytes were capable of and actively involved in bone resorption and that this process was regulated by calcium regulating hormones such as parathyroid hormone (PTH) and 1,25-dihydroxy vitamin D_3 ($1,25\text{-}(OH)_2D_3$). Some of the evidence came from studies of pathological situations, others from experimental studies in which hormones were administered to animals.

However, in later years, the existence of a process of osteocytic osteolysis has been attacked as strongly as it was earlier advocated [Boyde, 1980; Marotti et al., 1990]. The opponents to the osteocytic osteolysis theory have claimed that there is no hard evidence for such a process and that the described phenomena (e.g., enlarged lacunae) may be otherwise explained. They showed that the apparent size of the lacuna of any osteocyte is very much dependent on the type of bone, the orientation of the specimen during histological sectioning, and the size of the parental osteoblast. Depending on the activity and size of the parental osteoblast and the type of bone formed, a new osteocyte may have smaller or larger dimensions than mature osteocytes, already embedded in the mineralized matrix. As osteocytes and their lacunae are elongated, oriented with their long axis parallel to the collagen fibres around them, the orientation of the tissue section in relation to the orientation of the osteocyte/lacuna determines the size and shape of its cross-sectional image. The conclusion was that no change of existing osteocyte lacunae had been demonstrated but that during the experimental treatment or pathological situation the size and shape of newly formed osteocytes/lacunae were altered.

In an attempt to reconcile the two views, Bonucci [1990] has suggested that osteocytes are capable of removing crystalline or amorphous calcium phosphate present on the lacunar wall surface or in the matrix between osteocyte membrane and lacunar wall. The osteocyte would be able to do so without the need of matrix digestion and without overtly changing the size of the lacuna. Here lays the main "bone of contention" between the supporters and opponents of osteocytic osteolysis. The osteocyte-facilitated release of calcium as a factor in blood calcium homeostasis is, however, neither proved nor disproved.

The question remains whether osteocytes are sensitive to calcium regulating hormones. Virtually all evidence demonstrating effects of hormones such as PTH, $1,25\text{-}(OH)_2D_3$, and calcitonin on the metabolism of osteocytes is circumstantial. Direct evidence (e.g., coming from binding studies) is scarce and not unanimous. Rouleau et al. [1988, 1990] do not men-

tion the osteocyte as one of the cells that bind PTH. Silve et al. [1982] found no receptors for PTH on osteocytes. They do, however, describe binding to osteoblasts surrounded by collagen, which we have defined here as osteoid osteocytes. This would agree with the results of Nijweide et al. [1988], who reported binding of PTH on isolated (osteoid) osteocytes. Finally, the presence of 1.25-$(OH)_2D_3$ receptors in the nucleus and cytoplasm of both osteoblasts and osteocytes has been shown immunocytochemically by Boivin et al. [1987].

The Osteocyte as a Part of the "Bone Membrane"

Although the lack of tight junctions between osteoblasts, lining cells, and osteocytes suggests otherwise, many investigators have for a long time hypothesized that the bone mineral phase is functionally separated from the extracellular fluid and blood by a "bone membrane" [for a review see Ramp, 1975]. The bone membrane was thought to consist of the syncytium of osteoblasts, lining cells, and osteocytes. This assumption was mainly based on two considerations: the K^+ concentration of the fluid bathing the mineral is much higher than that of extracellular fluid and blood; secondly, blood and extracellular fluid are supersaturated for calcium and phosphate with respect to the bone mineral.

Although the "potassium problem" is still not satisfactory elucidated, the question of supersaturation was later solved by Neuman [1982], one of the earliest and originally strongest advocates of the bone membrane theory. The supersaturation does not exist; blood and extracellular fluid are in equilibrium with the bone mineral.

What remains unquestioned is the fact that the bone mineral surface is bordered by osteocytes, lining cells, and osteoblasts and that these cells are connected via gap junctions (see Morphology and Properties of the Osteocyte). Of these three cell types, the osteocytes occupy by far the largest part of the bone mineral surface. Could this cellular organization in bone have a function in the transport of ions to and from the mineral, even though there is ample room between the individual cells for ion diffusion? The recent reports on the presence of ion pumps, ion exchangers, and ion channels in the plasma membranes of osteoblasts are supportive of such a view. Osteoblast plasma membrane calcium pump activity has been observed by Shen et al. [1988] and Akisaka et al. [1988]. The calcium pump was immunocytochemically demonstrated in human osteoblast-like cells [Borke et al., 1988], and its cDNA nucleotide sequence was published [Kumar et al., 1993]. Also, ion exchangers such as the sodium/calcium exchanger, have been demonstrated in the osteoblast [Krieger, 1992]. Finally, a large number of K^+, Cl^-, and Ca^{2+} ion channels and conductances were described in bone cells of several animal species [for a review see Ypey et al., 1992].

Almost all of these pumps, exchangers, and conductances were demonstrated in osteoblast-like cells. Osteocytes are descendants of the osteoblasts and therefore may still possess the same ion transport mechanisms. Indeed, similar ion conductances have been found in isolated osteocytes [Ravesloot et al., 1990, 1991]. When we take into consideration the presence of these extensive and varied ion transport systems in the osteoblast and possibly in the osteocyte, a role for the osteoblast-osteocyte syncytium in ion transport to and from the bone mineral seems likely. Obviously the role of the osteoblasts would be to increase calcium and phosphate concentrations near the calcification front, perhaps in concert with the osteoid-osteocytes. Mature osteocytes, embedded in the bone mineral, on the other hand, could be involved in cell-mediated exchange of calcium and phosphate through uptake or release and intracellular transport of these ions via the cellular network of osteocytes and osteoblasts. Even if the bulk transport of calcium and phosphate into or out of the bone tissue is taken care of by osteoclasts and osteoblasts, the osteocytes may still be involved in the fine regulation of blood calcium homeostasis.

Osteocytes as Mechanosensory Cells

The primary function of the skeleton is to carry mechanical loads, which is why it has a hard, mineralized extracellular matrix. Mineralization of the organic bone matrix considerably enhances its stiffness and, consequently, the load bearing capacity of the tissue, but also increases its specific gravity. It pays, therefore, in terms of survival of both predatory and prey animals, to combine maximal bone strength with minimal bone mass. It has long been recognized that the severity of mechanical loading to which a piece of bone is exposed and the geometry and mass of that bone are related. Living bone is continually undergoing processes of remodeling,

which allows a continuous fine tuning of the amount and spatial organization of the tissue, to provide maximal strength with a minimum of bone mass. This process is called functional adaptation and was originally described as Wolff's law about 100 years ago.

Although it is generally considered that functional adaptation is achieved by the concerted action of osteoblasts and osteoclasts, the mechanism whereby these cells are instructed for such a task remains obscure. To obtain a meaningful change of the existing bone tissue, osteoblasts and osteoclasts must be informed about local needs of tissue increase or tissue reduction, dependent of a situation of, respectively, mechanical overuse or underuse. Both osteoblasts and osteoclasts act on the surface of the tissue, while mechanical loads produce displacements, or strains, throughout that tissue. Thus, detection of (aberrant) strain is best performed by living elements dispersed throughout the matrix, and osteocytes are the only cells that fulfil this demand in bone. Sensor cells that can detect loading deviations need not also be the actor cells that accomplish the adaptation, as long as sensors and actors can communicate with each other. In this respect the cellular network organization of bone tissue, where osteocytes embedded in the matrix are connected via cell processes and gap junctions to osteoblasts on the surface of that matrix, becomes significant. If osteocytes are the sensors of (aberrant) mechanical load, they may indeed instruct osteoblasts to change their metabolic activity, either via intracellular signals such as cAMP and/or ionic fluxes or via extracellular signal molecules such as prostaglandins. As osteoblasts are able to regulate the activity of osteoclasts, and thereby also modulate local bone resorption, all the cellular elements for such a scenario are indeed present. The question is, then, how osteocytes are informed about their mechanical environment and how they instruct osteoblasts.

Before addressing this issue, the experimental evidence that osteocytes are indeed sensitive to mechanical loading should be considered. Several reports from the group of Lanyon have implicated osteocytes as load-sensitive cells. In vivo experiments using the functionally isolated turkey ulna have shown that immediately following a 6 min period of intermittent (1 Hz) loading, the number of osteocytes expressing glucose-6-phosphate-dehydrogenase (G6PD) activity was increased in relation to local strain magnitude [Pead et al., 1988; Skerry et al., 1989]. Strain magnitude varied between 0.0005 and 0.002 (500–2,000 microstrain). In vivo, the magnitude of local strain in bone as a result of physiological load is of the order of 0.001–0.003 (1,000–3,000 microstrain) [Lanyon, 1984; Rubin and Lanyon, 1984, 1987]. In addition, osteocytes of loaded cores of adult dog cancellous bone showed a loading-related rise in ^{3}H-Uridine uptake 6 h after applying a bulk strain across the core of 5,000 microstrain [El Haj et al., 1990]. In organ cultures of embryonic chicken tibia-tarsi, loading increased the G6PD activity in osteocytes as well as osteoblasts [Dallas et al., 1993]. These studies convincingly show that intermittent loading at physiological strain magnitude produces rapid strain-related changes of metabolic activity in osteocytes and suggest that osteocytes may indeed function as mechanosensors of local strain in bone.

If osteocytes are mechanosensory cells, how do they sense mechanical loading? This key question is, unfortunately, still open, because it has not yet been established how loading in vivo is transduced to a cellular signal. The immediate consequence of mechanical loading (stress) is strain—that is, a (small) deformation of the calcified matrix. It is generally assumed that the bone cells react to the strain or to (one of) the consequences of strain such as flow of fluid or electrical effects, but no definite experimental data have been produced. Animal experiments have shown that the magnitude, frequency, and duration of loading influence the extent of mechanically driven bone remodeling, but such studies do not demonstrate what happens at the cell and molecular level. It is not even known what mechanical parameters are most important in modulating cellular behaviour: peak strain magnitude, strain distribution, threshold strain, or duration of the mechanical stimulus. Nevertheless, at least two transduction mechanisms of dynamic mechanical loading are feasible. First, direct straining of cells via their extracellular matrix which is connected with the cell membrane and cytoskeleton may trigger a cellular response. Second, the flow of interstitial fluid that results from loading (Fig. 4) may activate the cell [Cowin et al., 1991]. Both means of cell perturbation have been tested in vitro but not (yet) on isolated osteocytes.

Direct stress applied to isolated bone cells in vitro induces a number of responses, including release of cAMP and prostaglandins in the me-

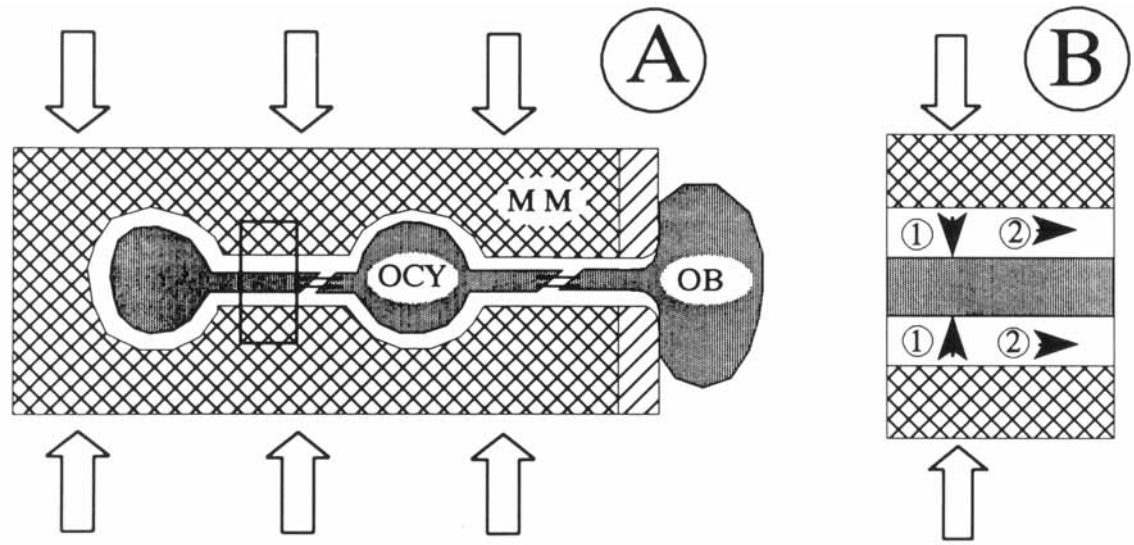

Fig. 4. Transduction mechanics of mechanical strain to osteocytes in bone. **A:** The osteocyte-osteoblast cellular network of a piece of bone tissue under stress (large arrows) is shown. **B:** The enlarged inset of A is shown. Loading (large arrows) results in 1) straining of the cellular process (vertical small arrows) as well as in 2) fluid flow in the canalicular extracellular matrix (horizontal arrows).

dium [for a review see Burger and Veldhuijzen, 1993]. Usually in these studies the cells are stretched by pulling the flexible substratum on which they were grown. Such studies have shown that osteoblasts are sensitive to stretch-induced strains of 7,000 microstrain and higher [Yeh and Rodan, 1984; Murray and Rushton, 1990], up to 100,000 microstrain. The work of Rubin and Lanyon [1984, 1987] has shown that in vivo bone deposition will occur at tissue-level strains of 3,000 microstrain or more and that bone resorption will occur for tissue-level strains less than 1,000 microstrain. This implies that the bone cells must detect strains of that level and consequently are much more sensitive to tissue-level strains than they appear when directly strained in vitro. It is possible, however, that local strains at the level of the cells are higher than the overall strains, which were measured over the entire bone cortex. For instance, the lacunar shape of the osteocyte and/or the local structural anisotropy of the matrix could amplify the tissue-level strains so that they increase by a factor of ten at the cellular level [Cowin et al., 1991], thereby entering the range of strains that were effective in vitro.

The second mechanism for strain sensing in bone uses the flow of tissue fluid that results from the strain as the physical stimulus to which the cells react, rather than the strain itself [Salzstein and Pollack, 1987; Reich et al., 1990; Cowin et al., 1991; Weinbaum et al., 1991]. When bone is loaded, the resulting compressive strain causes the fluid in the tissue to flow to regions of lower pressure. In compact bone, axial compression of an osteon causes a radial flow from the lacunae of the deepest osteocytes towards the Haversian channel, because the latter is in open connection with the surrounding soft tissue [Piekarski and Munro, 1977; Kufahl and Saha, 1990]. In trabecular bone, fluid is squeezed from the osteocytic network towards the marrow spaces which via the Haversian and Volkmann channels of the cortex are also in open connection with the periosteum. Flow of fluid as a result of loading has been linked to the stress-generated potentials that have been measured in loaded bone [Salzstein et al., 1987]. In living, fluid-filled bone these potentials are electrokinetic of origin, not piezoelectric, and fluid movement within the microporosity of bone is responsible for these "streaming potentials" [Salzstein and Pollack, 1987]. The microporosity responsible for fluid movement was originally assumed to be the fluid space in and around mineral crystals encrusting collagen fibrils [Salzstein and Pollack, 1987; Salzstein et al., 1987]. However, in a recent paper [Weinbaum et al., in press] it is argued that, because most of the water in the mineral phase of bone is bound by interaction with the ionic crystals [Neuman and Neuman, 1958], flow of fluid is more likely through the

nonmineralized matrix of the lacunar-canalicular network. A theoretical model is proposed in which the stress-induced fluid flow through the canaliculi and lacunae of the osteocytes acts as the mechanical stimulus that activates the osteocytes, possibly via stress-generated streaming potentials [Weinbaum et al., 1991, in press]. Interestingly, the model of Weinbaum et al. predicts values for the very low fluid-induced shear stress in canaliculi (8–30 dynes/cm^2) which have been shown to induce enhanced prostaglandin E_2 production in osteoblasts, subjected to fluid flow in vitro [Reich et al., 1990]. Thus osteoblasts seem to be sensitive to the very low fluid shear stresses predicted by this model. This finding compares favourably with the unpredicted high strains that were necessary to evoke a response as a result of stretching [Murray and Rushton, 1990]. Similar studies on osteocytes are not available, but sensitivity of isolated chicken osteocytes, separated from osteoblasts as described by van der Plas and Nijweide [1992], to loading in vitro has been reported [Klein-Nulend et al., 1993].

In sum, a number of experimental and theoretical studies suggest that osteocytes are likely involved in the detection of strain in bone, possibly by means of sensitivity to strain or flow. However, experimental evidence about the means by which osteocytes sense strain and the mechanism by which they communicate their information to the osteoblasts and osteoclasts on the bone surface is still largely lacking. The report of a method to purify osteocytes from a mixture of calvarial bone cells obtained by collagenase digestion [van der Plas and Nijweide, 1992] opens up the possibility to test the responsiveness of osteocytes to strain or fluid flow or both. Such studies may provide facts in a field that up till now has been largely theoretical.

CONCLUSIONS AND PROSPECTS

The structure of cellular bone with osteocytes regularly spaced throughout the mineralized matrix and connected to each other and to cells lining the bone surface has inspired many to speculate on the role of osteocytes in bone metabolism. However, the attempts to attribute specific properties and functions to osteocytes have largely been thwarted in the past by the inaccessibility of these cells embedded as they are in the mineralized matrix.

Recently, at least three developments promise to bring a change for the better. In the first place, the finding that osteocytes do have specific properties (i.e., specific although not characterized antigenic sites on their cell surface and the preparation of monoclonal antibodies directed against these antigenic sites [Nijweide and Mulder, 1986] proved to be a major step forward. It is now possible to recognize osteocytes in mixtures of bone cells and to isolate them from these mixed populations [van der Plas and Nijweide, 1992]. Of course, at this moment the available antibody only recognizes avian osteocytes, but the preparation of antibodies directed against mammalian osteocytes is probably only a question of time. In the second place, recently developed molecular biological techniques now allow the analysis of the mRNA of small numbers of cells. This is important because, even with the immunological dissection techniques mentioned above, the number of osteocytes that can be isolated is small—so small that standard biochemical techniques are generally too insensitive to measure responses of osteocytes to hormones, local factors, or mechanical loading. In the third place, the interest in bone as a weight-bearing tissue and not as a mere sink for calcium and phosphate to be used for endocrine responses is rapidly increasing. At present, our best guess is that osteocytes are the mechanosensory cells of bone. The few hard facts concerning osteocytes that we possess implicate or at least allow the hypothesis that osteocytes are present in bone to adapt bone volume and bone mass to the physical stimuli bone is subjected to during life, as discussed earlier.

Of interest is whether hormones and local (growth) factors also have a role in the regulation of osteocyte function or whether the receptors for these humoral factors of which some were shown to be present in osteocytes [Boivin et al., 1987; Nijweide et al., 1988] are mere rudiments from an osteoblastic past. Future research will have to establish whether hormones or local factors may not sensitize osteocytes or strengthen and multiply their reaction to strain forces. In particular, estrogen is a candidate for such an interplay between hormonal and mechanical stimuli. Estrogen deficiency is strongly implicated in postmenopausal bone loss. A very attractive idea is that changes in the estrogen level may alter the set point for bone adaptation to mechanical loading [Frost, 1988; Rodan, 1991]—that is, that osteocytes may change their signalling response to certain

strain levels depending on changing estrogen levels and that the remodeling response of osteoblasts and osteoclasts may change accordingly.

An argument in favour of a role as mechanosensory cell for the osteocyte is that such a role may give spatial and temporal direction to the bone remodeling process. When a bone is subjected to mechanical loading, the strain values may not be equal throughout the bone. In some areas this will lead to bone mass increase and in other areas to bone mass loss (adaptation). The same principle may be valid for areas where (micro)stress fractures have formed. In the direct vicinity of such fractures osteocytes will perceive a change in strain level and send out signals to the most nearby cells outside the bone in order to stimulate local remodeling. The diffusion of the signal (if excreted extracellularly) and the formation of a signal gradient will direct the remodeling cells to the site where remodeling is needed.

The mechanism by which osteocytes perceive strain forces, by which mechanical stimuli are transduced in the cell into intracellular messengers that in their turn activate the cell to produce signals capable of stimulating the remodeling system, is largely unknown. However, whether it is the strain itself or the consequences of strain such as fluid flow through the lacunar-canalicular system, we expect that the anchoring of the cell to the bone matrix is important. The attachment sites of the osteocyte to specific matrix proteins may be the foci on the membrane surface where the strain forces will accumulate. If so, the cytoskeleton, coupled on the intracellular side to these attachment sites, may then be involved in the signal transduction. In the coming years, the composition of the matrix components directly around the osteocytes, the nature of the cell-matrix adhesion sites, and the role of the cytoskeleton, especially the microfilaments that are so abundant in osteocytes, must therefore be evaluated. These studies and the acquirement of a better insight into how strain is transferred from bone matrix to the osteocytes and into the nature of the signal molecules by which the osteocyte regulates the bone remodeling system are the challenges for the future.

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