Osteocyte Dendrogenesis in Static and Dynamic Bone Formation: An Ultrastructural Study

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ABSTRACT
The present ultrastructural investigation into osteocyte dendrogenesis represents a continuation of a previous study (Ferretti et al., Anat. Embryol., 2002; 206:21–29), in which we pointed out that, during intramembranous ossification, the well-known dynamic bone formation (DBF), performed by migrating osteoblast laminae, is preceded by static bone formation (SBF), in which cords of stationary osteoblasts transform into osteocytes in the same site where they differentiated. The research was carried out on the perichondral center of ossification surrounding the mid shaft level of various long bones of chick embryos and newborn rabbits. Transmission electron microscope observations showed that the formation of osteocyte dendrites is quite different in the two types of osteogenesis, mainly depending on whether or not osteoblast movement occurs. In DBF, osteoblasts transform into small ovoidal/ellipsoidal osteocytes and their dentrites form in an asynchronous and asymmetrical manner in concomitance with, and depending on, the advancing mineralizing surface and the receding osteogenic laminae. In SBF, stationary osteoblasts give rise to big globous osteocytes, located inside confluent lacunae, with short and symmetrical dendrites that can radiate simultaneously all around their cell body because they are completely surrounded by unmineralized matrix. Contacts and gap junctions were observed between all osteocytes (both SBF- and DBF-derived) and between osteocytes and osteoblasts. Finally, a continuous osteocyte network extends throughout the bone, regardless of its static or dynamic origin. This network has the characteristic of a functional syncytium, potentially capable of modulating, by wiring transmission, the cells of the osteogenic lineage covering the bone surfaces. Anat Rec Part A 278A:474–480, 2004. © 2004 Wiley-Liss, Inc.

Key words: osteocyte; dendrogenesis; intramembranous ossification; static bone formation; dynamic bone formation

It is a well-established fact that osteocytes derive from plump osteoblasts through conspicuous morphological and ultrastructural changes (Dudley and Spiro, 1961; Hancox and Boothroyd, 1965; Cooper et al., 1966; Cameron, 1972; Rasmussen and Bordier, 1974; Nijweide et al., 1981), resulting in the formation of dendritic cells. For many years, osteocyte morphology was indirectly desumed from the shape of the lacunocanalicular network in which they are enclosed. In fact, the stains commonly used in histological sections for light microscopy (LM) perfuse osteocyte cavities rather than staining their protoplasm. In these samples, osteocyte lacunae display different shapes according to the type of bone tissue: they appear to be globous in woven bone, ovoidal in parallel-fibered bone, and ellipsoidal in lamellar bone (Marotti et al., 1990). Subsequent ultrastructural studies fully confirmed such differences in shape of the osteocyte cell body in the three types of bone tissue, but they failed to define the length of osteocyte dendrites inside the canaliculi. Thus, the tacit agreement among bone researchers was that osteocyte dendrites should radiate more or less symmetrically all around the cell body, since, looking at the canalicular network, the first impression is that the cytoplasmic processes end

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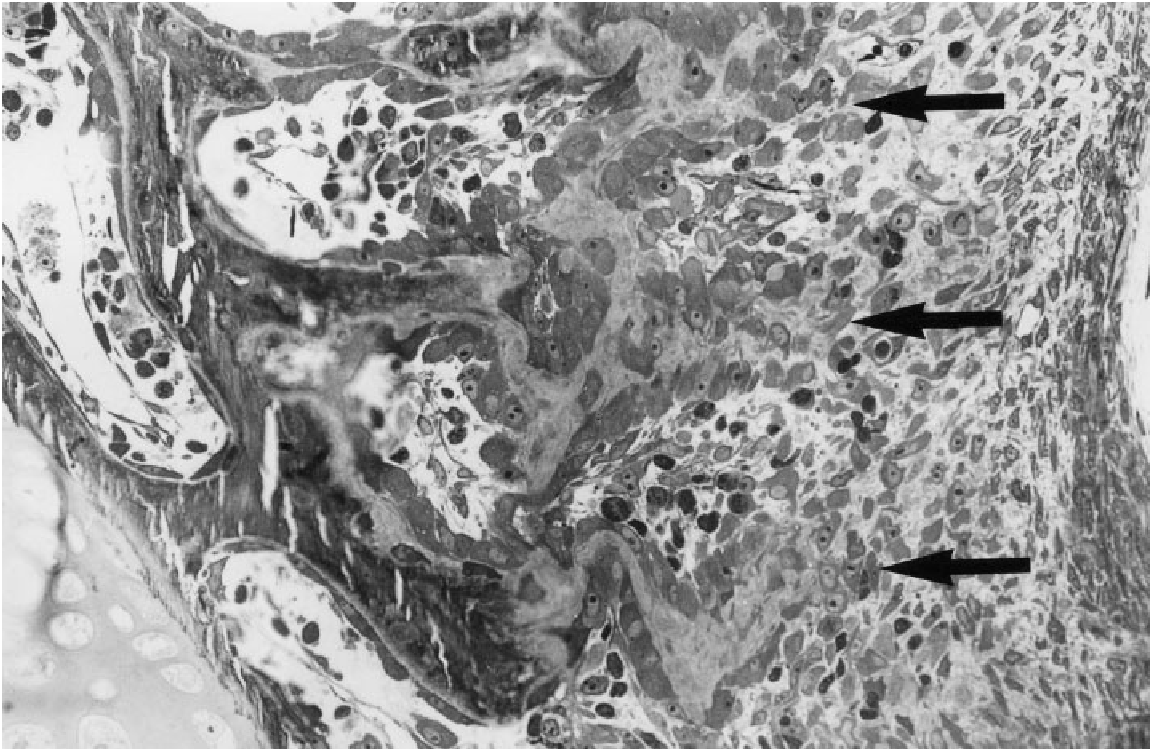

Fig. 1. LM micrograph (×440) of a cross-sectioned (mid shaft level) intramembranous perichondral center of ossification around the cartilagineous bud of the tibia in a 15-day-old chick embryo. Note the cords of stationary osteoblasts (arrows) differentiating around the blood capillaries in the inner layer of periosteum.

roughly midway between adjacent lacunae. It was only in a study on the three-dimensional reconstruction of the osteoblast-transforming osteocyte from ultrathin serial sections that, for the first time, it was shown that osteocyte dendritic arborization is quite asymmetric and forms in an asynchronous manner: preosteocytes first radiate short and thick cytoplasmic processes inside the osteoid seam (mineral dendrites) to remain in contact with the vascular dendrites of preexisting mature osteocytes; only when the mineralization surface reaches them do they radiate long and slender vascular dendrites to remain in contact with the receding osteoblast lamina (Palumbo, 1986; Palumbo et al., 1990a, 1990b).

It is to be pointed out that this asynchronous and asymmetric dendrogenesis was found to take place in an osteoblast-transforming osteocyte that detaches from typical movable osteogenic laminae carpeting a preexisting bone surface [dynamic osteogenesis, according to our terminology (Ferretti et al., 2002)]. The question arises as to whether this type of osteocyte dendrogenesis also occurs in the static osteogenesis we observed at the onset of intramembranous ossification centers, where no bone preexists and osteoblasts are arranged in stationary cords and transform into osteocytes in the same site where they differentiated (Ferretti et al., 2002).

MATERIALS AND METHODS

The observations were performed on the intramembranous perichondral centers of ossification surrounding the mid shaft level of various long bones, particularly the tibiae, of six White Leghorn chick embryos aged 8–16 days [stages 34–42 according to Hamburger and Hamilton (1951)] and five newborn rabbits. All specimens were fixed for 2 hr with 4% paraformaldehyde in 0.13 M phosphate buffer, pH 7.4, postfixed for 1 hr with 1% osmium tetroxide in 0.13 M phosphate buffer, pH 7.4, dehydrated in graded ethanol and embedded in epoxy resin (Durcupan ACM), and sectioned with a diamond knife mounted in an Ultracut-Reichert Microtome. The perichondral centers of ossification were cross-sectioned perpendicular to the longitudinal axis of the shaft. Ultrathin sections (70–80 nm) were mounted on Formvar- and carbon-coated copper grids, stained with 1% uranyl acetate and lead citrate, and examined by Zeiss EM109 transmission electron microscope (TEM).

RESULTS

It should be pointed out that no morphological differences were observed between the ossification centers of chick embryos and those of newborn rabbits. The following description therefore applies to both animals.

As we described in our previous study (Ferretti et al., 2002), static osteogenesis occurs at the onset of intramembranous ossification and is characterized by cords of 2–3 layers of plum-shaped stationary osteoblasts all transforming into osteocytes in the same site where they differentiated (Fig. 1). Unlike DBF osteocytes (Dynamic Bone Formation-derived osteocytes), which are derived from typical movable osteoblasts and whose cell bodies are greatly reduced in size as dendrite formation takes place,

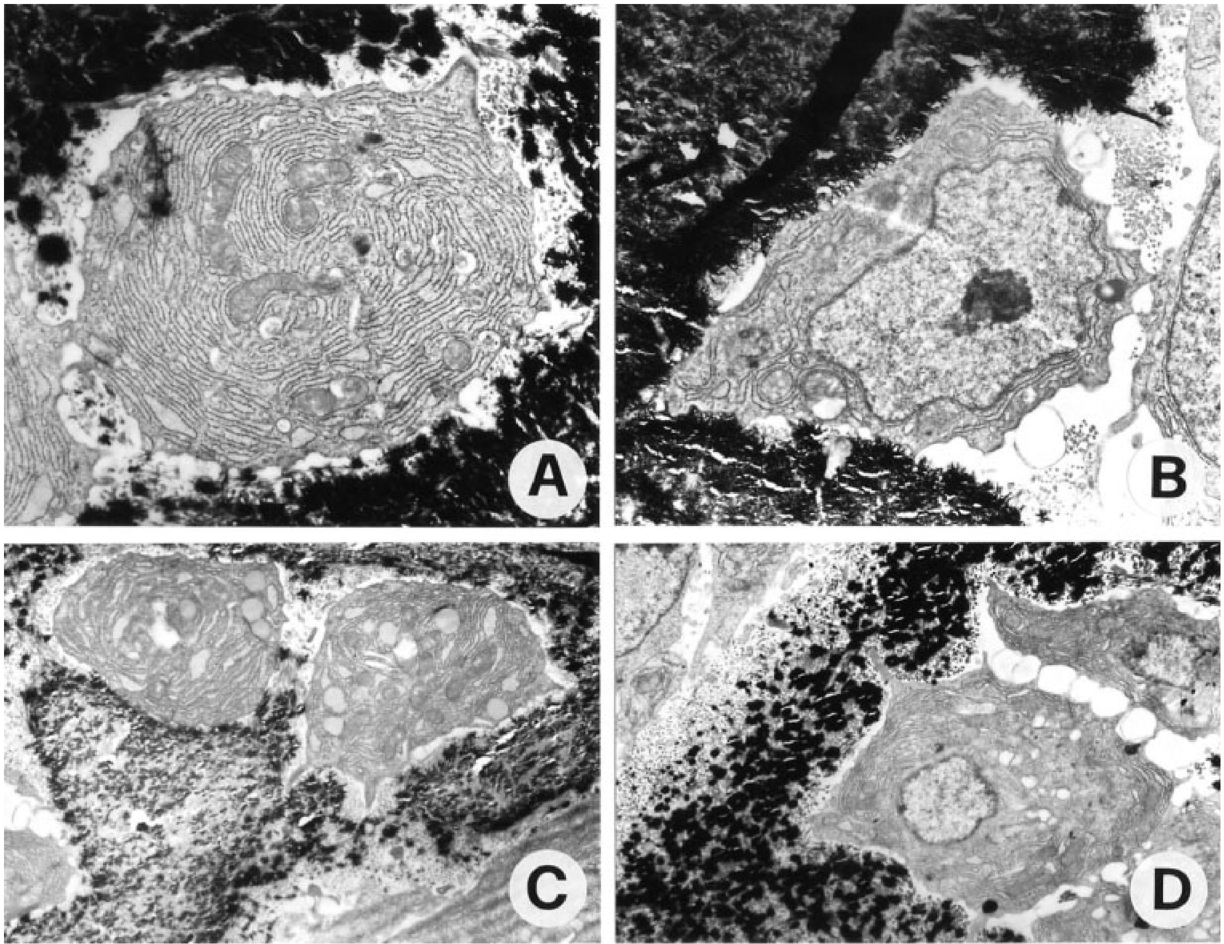

Fig. 2. TEM micrographs (**A** and **B**, ×14,000; **C** and **D**, ×6,000) of SBF osteocytes. Note their globous shape, spinous aspect, and location within confluent lacunae. In A, B, and D, osteocyte cytoplasmic spines appear to be connected by simple contacts.

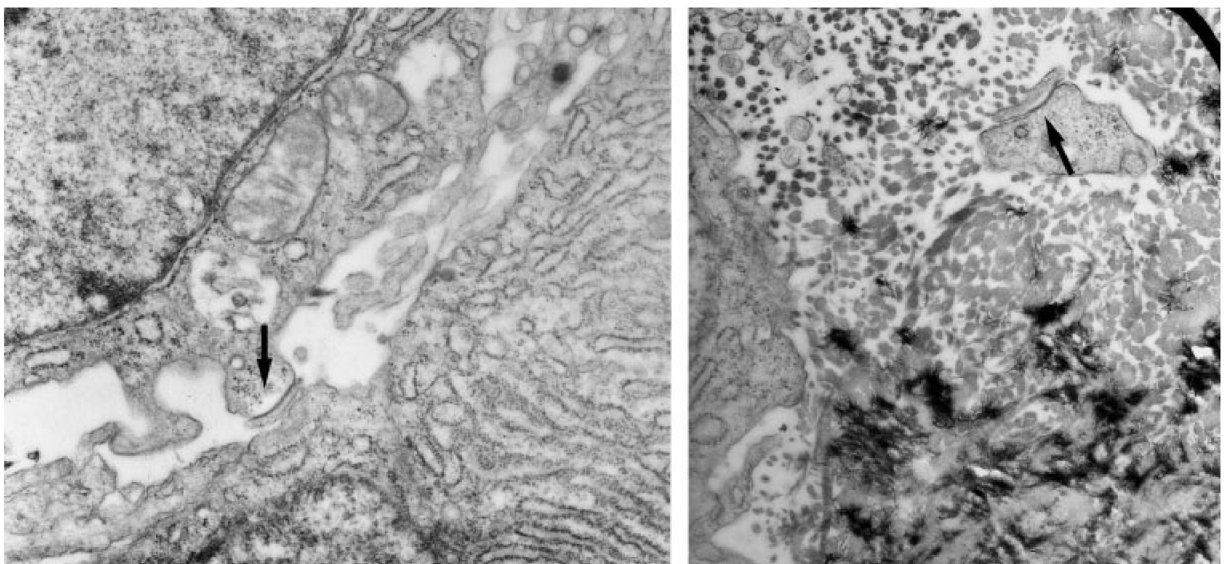

Fig. 3. TEM micrographs (×24,000) showing gap junctions (arrows) between SBF osteocytes.

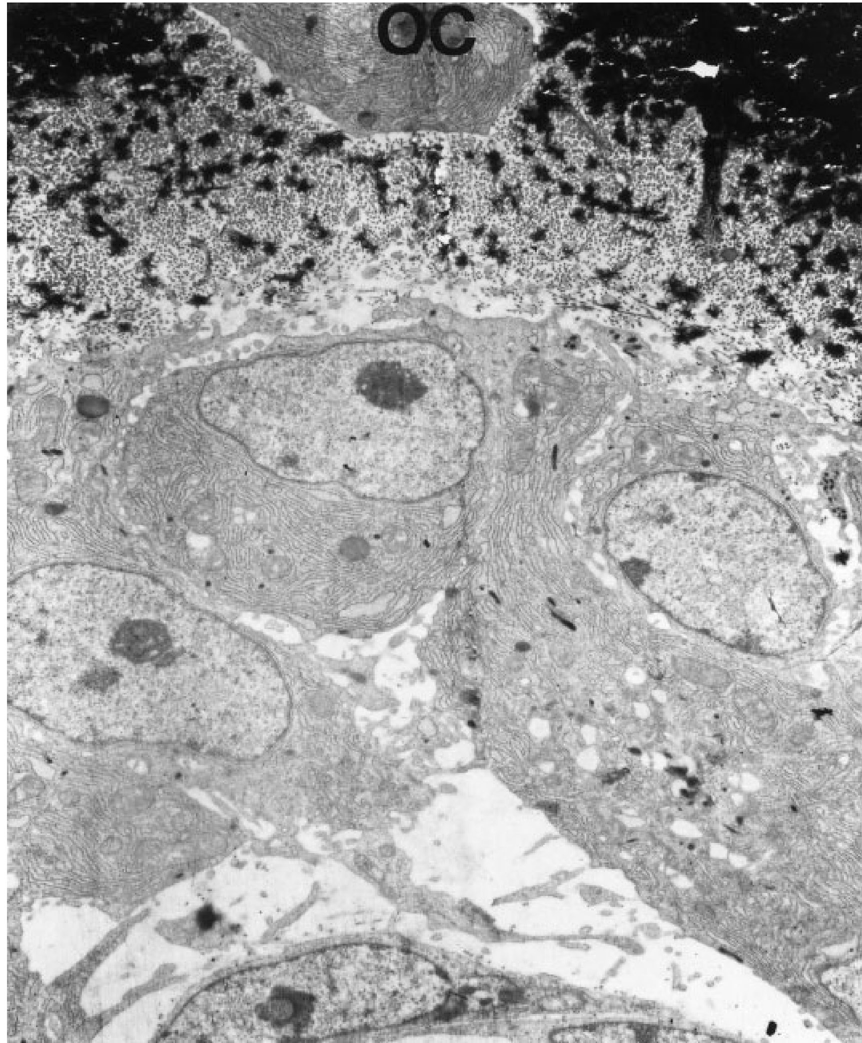

Fig. 4. TEM micrograph (×6,000) of a typical lamina of movable osteoblasts carpeting a mineralized bony trabecula laid down by SBF and containing an SBF osteocyte (OC).

SBF osteocytes (Static Bone Formation-derived osteocytes) approximately retain the same globous shape and ultrastructure of the parental stationary osteoblasts and do not reduce significantly in size; also, their dendrites are much shorter than those of DBF osteocytes and radiate simultaneously all around the cell body (Fig. 2). At first, the dendrites are so short as to look like spines; afterward, some of them elongate, but only marginally, because their parental osteoblasts are practically stationary, withdrawing from one another by just a few micra. Gap junctions or simple contacts were observed between stationary osteoblasts and between the spines and dendrites of SBF osteocytes (Fig. 3).

When static osteogenesis ends, which, as previously described, occurs after the cords of stationary osteoblasts have turned into bony trabeculae containing 2–3 rows of osteocytes inside confluent lacunae, typical laminae of movable osteoblasts (Fig. 4) lay down layers of bone aimed at increasing the thickness of the trabeculae and consequently leading to bone compaction (Fig. 5). The bone tissue secreted by movable osteoblasts contains DBF osteocytes, whose dendrogenesis occurs in an asynchronous and asymmetrical manner, as described above (Fig. 6).

It should be noted that, unlike the SBF osteocytes in the core of SBF trabecula, the SBF osteocytes more externally located display an asymmetrical dendrite arborization, with short dendrites (or spines) connecting them with the SBF osteocytes in the trabecular core and longer dendrites coming into contact with the movable osteoblastic lamina or DBF osteocytes (Fig. 7). Gap junctions and simple contacts were also observed between SBF and DBF osteocytes.

DISCUSSION

The ultrastructural observations reported here clearly demonstrate that osteocyte differentiation in static osteogenesis occurs in a quite different manner from that in dynamic osteogenesis. In the former, osteocytes approximately retain the same shape, size, and ultrastructure of the parental stationary osteoblasts,

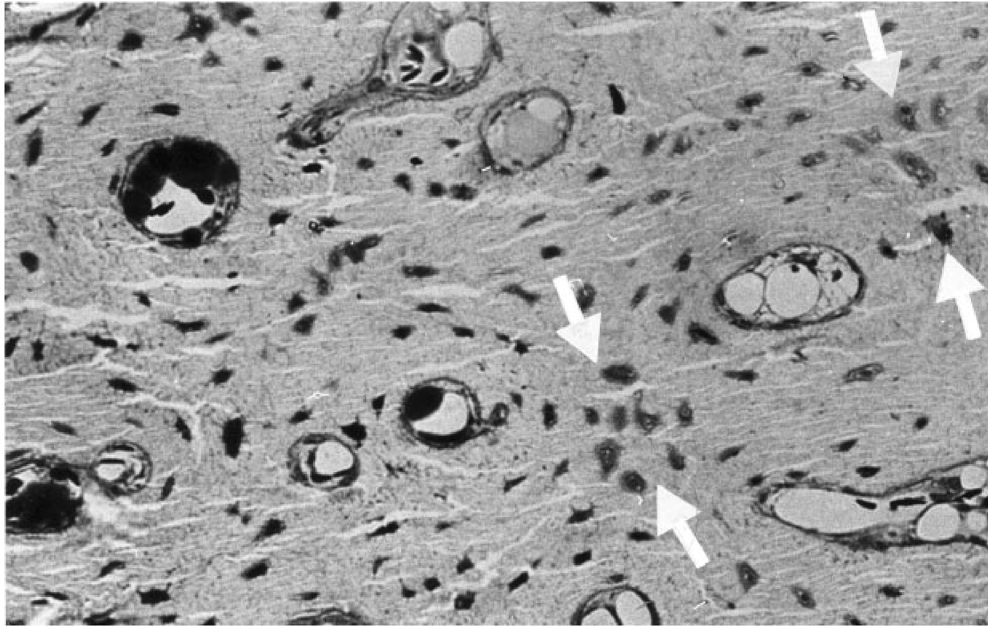

Fig. 5. LM micrograph (×440) showing a cross-section of a rabbit shaft tibia at the end of bone compaction. Note that in such primary compact bone it is possible to distinguish SBF osteocytes (between arrows) from DBF-derived ones. The former, with a larger and roundish shape, are located in between primary osteons, namely, where the SBF primary trabeculae form. The latter, having a smaller and elliptical size, are arranged concentrically in the wall of the primary osteons.

and their dendrogenesis is characterized by short cytoplasmic processes radiating simultaneously all around the cell body. DBF osteocytes, on the other hand, are reduced in body size during asynchronous and asymmetrical dendrogenesis. Thus, it seems that the reduction of the osteocyte cell body, during its differentiation from parental osteoblasts, occurs in parallel with the formation of dendrite arborization.

SBF osteocytes display short dendrites that form simultaneously and are of similar length around their cell body because their parental stationary osteoblasts are arranged in cords, inside which they are polarized in different directions, and because preosteocytes are completely surrounded by unmineralized matrix, which does not prevent their formation in any direction.

DBF osteocyte dendrogenesis is asynchronous and asymmetrical because their parental osteoblasts are arranged in laminae, inside which they are all polarized in the same direction, i.e., toward the mineralizing surface, and because the mineralizing surface contacts the preosteocyte cellular body first on its mineral side and then on its vascular side. In fact, while mineral dendrites cannot elongate since preosteocyte cell bodies soon come into contact with the mineralizing surface, vascular dendrites are able to grow over a longer period since they radiate inside the soft tissue of the osteoid seam. Thus, it appears that matrix mineralization precludes dendrite growth. Theoretically, dendrites might elongate inside the bone matrix if their tips possessed osteolytic activity, which, however, does not seem to be the case. However, the asymmetric arborization we found in preosteocytes and young osteocytes could be partially lost over time owing to a possible secondary elongation of mineral dendrites inside the canaliculi of preexisting osteocytes. This assumption is substantiated by the fact that two and sometimes three dendrites have been observed inside one canaliculus, suggesting that mature osteocytes probably need to increase their contact and to retain the capability of regenerating damaged dendrites (Marotti et al., 1990; Marotti, 1996).

In summary, this study indicates that osteoblast movement appears to be the main determinant of the type of osteocyte dendrogenesis: stationary osteoblasts, tightly packed in cords, can only give rise to big globous SBF osteocytes with short and symmetrical dendrites; movable osteoblasts, on the other hand, may give rise to DBF osteocytes, whose longer vascular dendrites continue to elongate in order to remain in contact with the osteoblastic laminae.

In conclusion, as shown in Figure 8, the most remarkable finding of the present investigation is that, regardless of the type of osteogenesis (static or dynamic), the bone contains, from its very inception, a continuous cytoplasmic network made up of osteocytes, which, regardless of their shape and origin (SBF or DBF), are all joined by gap junctions; whether or not they are actually active, these gap junctions undoubtedly represent the morphological substrate potentially acting as electric synapses that unite osteocytes in a functional syncytium.

According to the data reported in the literature, the osteocyte syncytium seems to play two different roles: it determines the manner of osteocyte recruitment, and it transducts mechanical strains into biological signals (Rubinacci et al., 1998, 2002) capable of modulating the other cells of the osteogenic lineage (osteoblasts, bone lining cells) (Marotti et al., 1990; Marotti, 1996; Palazzini et al., 1998).

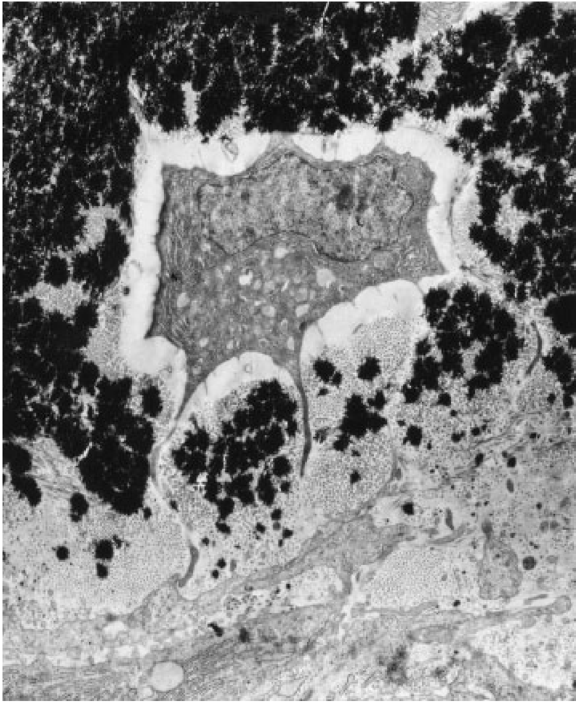

Fig. 6. TEM micrograph (×14,000) showing the typical shape of a DBF osteocyte. Note its asymmetrical arborization, with short mineral dendrites radiating toward the preexisting bone (top) and long vascular dendrites radiating toward the osteoblastic lamina (bottom).

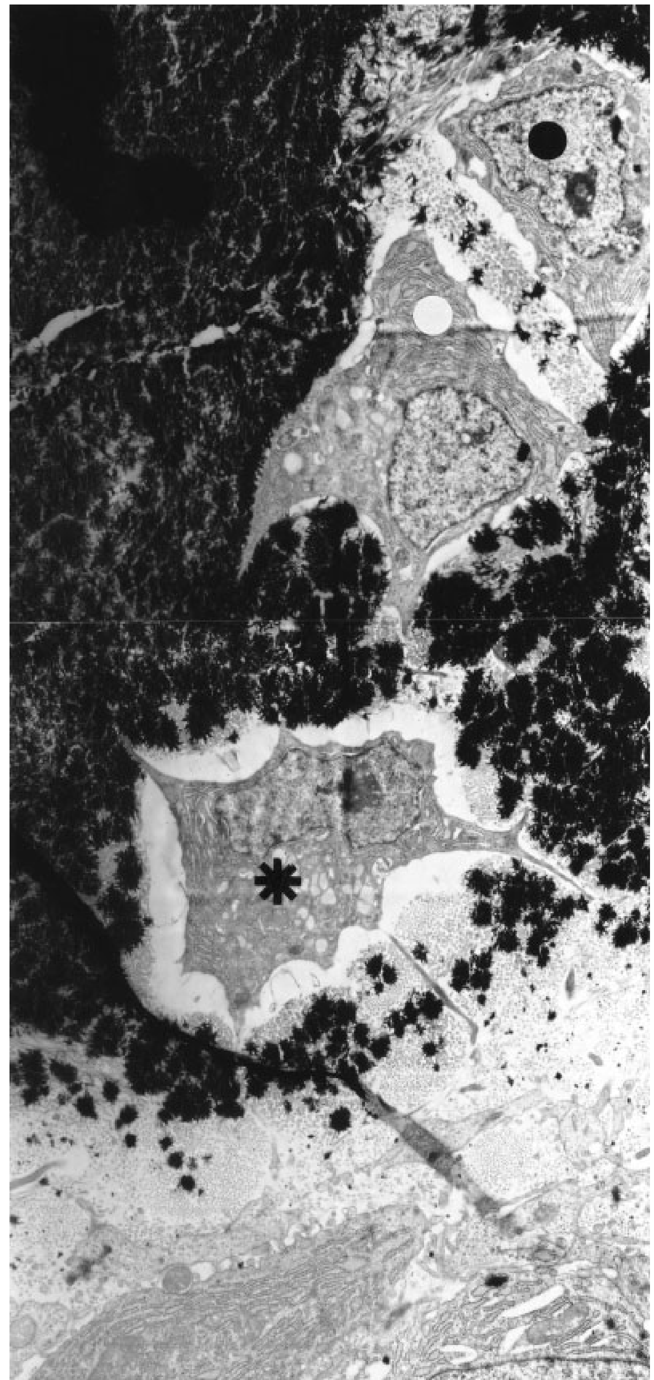

Fig. 7. TEM micrograph (×6,000) of an SBF osteocyte in the trabecular core (black spot), an SBF osteocyte in the outer part of the trabecula (white spot), and a DBF osteocyte (asterisk). Note the asymmetric arborization of the latter two osteocytes.

Regarding osteocyte recruitment, it is interesting to note that osteocytes located in the core of SBF bony trabeculae display the typical morphology of those in woven bone, whereas osteocytes more externally located have the shape of those located in bone with a more orderly arrangement of collagen fibers. This finding appears to be in close agreement with the suggestion of Marotti (1996) that without preexisting osteocytes only woven bone can form, because an orderly recruitment of osteocytes can only take place by signals issued by a preexisting osteocyte syncytium.

As regards the transmission of mechanical signals, recent literature indicate osteocytes as the main strain-sensitive cells (Frost, 1987; Pead et al., 1988; Skerry et al., 1989; El-Haj et al., 1990; Turner, 1991, 1992; Lozupone et al., 1992; Ypey et al., 1992; Burger and Veldhuijzen, 1993; Dallas et al., 1993; Dodds et al., 1993; Duncan and Turner, 1995; Marotti, 1995a). It has recently been suggested that this transmission occurs through the junctions between the dendrites of the osteocyte syncytium (wiring transmission), rather than by diffusion of soluble substances into the bone fluids (volume transmission) (Marotti et al., 1993, 1996; Marotti, 1995b). In fact, we have recently shown that shear-stress-activated osteocytes are capable of steadily increasing and maintaining the basal current produced by the ionic flux (streaming potential), which occurs inside the lacunocanalicular microcavities in response to pulsing mechanical loading (Rubinacci et al., 1998, 2002). Briefly, the fact that all osteocytes, starting from the SBF-derived ones, take part in the formation of a potential osteocyte syncytium, as shown in the present study, supports the view that mechanical signals throughout bone cells are mainly issued by wiring transmission, since volume transmission does not need cell contacts for it to occur.

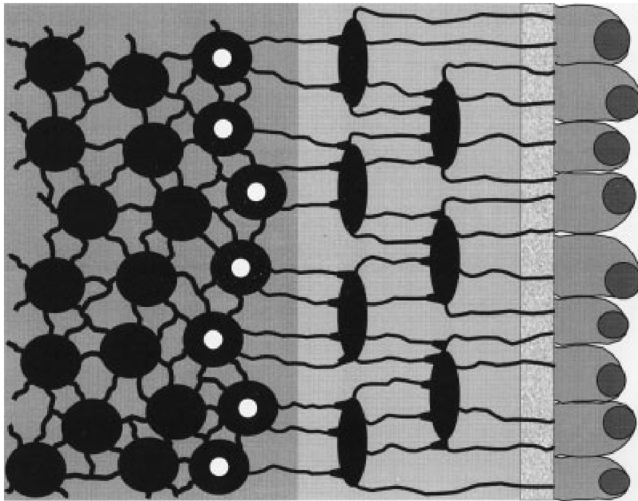

Fig. 8. Schematic drawing showing the continuous osteocytic network throughout the bone. From left to right: SBF osteocytes (circles), DBF osteocytes (ovoids), osteoid seam and osteoblasts. White spots indicate SBF osteocytes located in the outer part of the SBF trabecula; in these osteocytes, vascular dentrites are longer and thinner than mineral dendrites, as found in DBF osteocytes.

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