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Occurrence of Cell Junctions and Microfilaments in Osteoblasts*

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Summary. Osteoblasts in the diaphysis of the tibia during endochondral ossification in young rats are attached to one another by nexus, by "adhaerens" junctions, and by simple appositions. "Adhaerens" junctions and nexus also occur between preosteoblasts and osteoblasts. Furthermore, the osteoblasts exhibit a network of microfilament bundles in the cell periphery overlying the osteoid. From this network bundles extend into the cell processes which protrude into the unmineralized matrix. The mean diameter of individual microfilaments is 5.9 ± 0.06 nm. A possible role of nexus and microfilaments in controlled bone growth and differentitation is discussed.

Key words: Cell junctions — Microfilaments — Nexus — Osteoblasts.

Introduction

In a previous electron microscopic investigation of the plasmalemma of some cell sypes (Stanka, 1975) peculiarities of osteoblasts were observed which were hitherto undescribed. These observations, because they may be of interest in developmental biology, were reexamined and are reported in the present article. Investigation was centered upon the occurrence of cell junctions and microfilaments in osteoblasts, little attention being given to the general development of bone. More general descriptions of the process of ossification were given by Scherft (1973), and by Luk *et al.* (1974a, b). The morphologically distinct cell junctions were described by Farquhar and Palade (1963), Brightman and Palay (1963), Brightman and Reese (1969). Furshpan and Potter (1968) have summarized the significance of the different junctional specializations. More recent and comprehensive reviews on intercellular junctions are given by McNutt and Weinstein (1973), and Staehelin (1974).

Material and Methods

Osteoblasts in the diaphysis of the tibia beneath the proximal epiphyseal plate of 17-day old rats (black hooded rats of the own bred strain) were studied. The specimens were excised under ether anesthesia and fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer containing 5 mM $CaCl_2$, pH 7.3–7.6, 17 h.

For rinsing after fixation 0.1 M cacodylate buffer containing $CaCl_2$ was used. The osmolarity was adjusted with sucrose to about 200 mOsm according to Bone and Denton (1971). Postfixation was done with sodium barbital-buffered 1% OsO_4 adjusted to about 200 mOsm with sucrose. After block-staining with uranyl acetate (Kellenberger *et al.*, 1958) the specimens were dehydrated in a graded series of ethanol and embedded in Epon 812 (Luft, 1961). Semi-,

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thin sections were cut on a LKB-Huxley ultra-microtome and examined by phase contrast microscopy for orientation. Thin sections were cut on an LKB Ultrotome III with a diamond knife. They were stained with uranyl acetate and lead citrate (Huxley and Zubay, 1961; Venable and Coggeshall, 1965), and examined with a Philips EM 300 electron microscope operating at 80 kV. Direct magnifications of up to 42000 times were used.

Results

For the sake of orientation, a brief description of the general organization of an osteogenic unit in the material studied is given (Fig. 1a). This osteogenic unit should be considered as an idealized model, not always to be found as such a well defined structure. The center is formed by a blood capillary. The periphery is composed of the mineralized bone trabecula with an unmineralized edge (osteoid) pointing inward. Adjacent to this unmineralized matrix are the osteoblasts. Active osteoblasts are marked by a well developed rough endoplasmic reticulum, often with dilated cisternae as seen electron microscopically (Knese and Knoop, 1958). Another sign of osteogenic activity are the calcification islands within the osteoid. The space between the capillary and the osteoblasts is occupied by loosely arranged mesenchymal cells and preosteoblasts closely adjacent to the osteoblasts.

Cell Junctions. Both between preosteoblasts and osteoblasts as well as between neighbouring osteoblasts two different attachment regions occur in addition to so-called simple appositions (Figs. 1b, 2, 3a, b). The first type of attachment consists of an approximation of the cell membranes of adjacent cells to within a distance of 15 to 25 nm. In these regions both the extra-cellular space and the cytoplasm immediately adjacent to the cell membrane show increased densities ranging from slight to marked as already reported by Ross and Greenly (1966). The density of the cytoplasm is often due to fine filaments at these sites (Fig. 3a). Densely stained material applied to both apposed membranes gives the membranes a thickened appearance. It seems that there are developmental stages between the simple apposition and the "adhaerens" junction.

The second type of junctional structure is the so-called nexus, also referred to as gap junction (Brightman and Reese, 1969). The gap cannot be seen in this material. Nexus are observed between osteoblasts, between preosteoblasts and osteoblasts, and occasionally between mesenchymal cells in the close vicinity of a capillary. Fig. 3b shows a nexus with a septate appearance. The center-to-center spacing of the "septa" measures about 9 nm.

Microfilaments. Bundles of roughly parallel oriented microfilaments are found in a 70 to 250 nm thick zone subjacent to the cell membrane of osteoblasts. The bundles are plentiful in the area of the cell periphery adjacent to the osteoid. Longitudinally sectioned bundles show that they run along the cell surface (Fig. 3d) Cross sectioned bundles show that they are mostly flat and of different calibers (Fig. 4a). Sometimes the bundles appear to join or intersect with one another. Bundles crossing over one another can also be observed (Fig. 3d). Thus, a network of filament-bundles is formed in the subplasmalemmal cytoplasmic zone (Fig. 4b). In the cell periphery opposite to the bone trabecula such a distinct network of filament-bundles cannot be found. Only individual small bundles of loosely arranged filaments are present beneath the plasmalemma.

Bundles of microfilaments originating from the described network extend into the cell processes, which protrude into the unmineralized matrix (Figs. 3c, 5a).

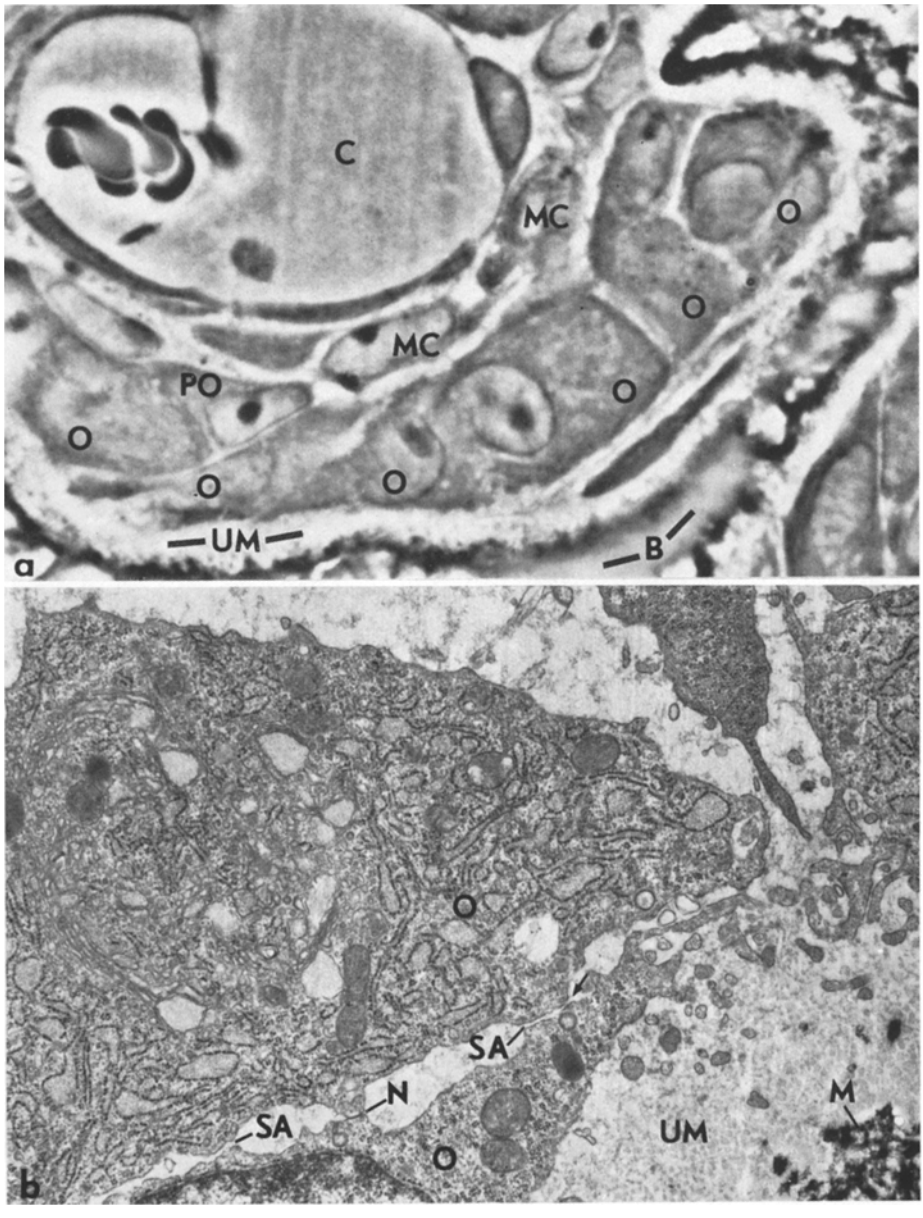

Fig. 1. (a) Phase contrast enhanced light micrograph of an osteogenic unit during endochondral ossification in the diaphysis of the tibia of a young rat. *B* Bone trabecula with an edge of unmineralized matrix (*UM*), *O* osteoblast, *PO* preosteoblast, *MC* mesenchymal cell, *C* blood capillary. ×2000. (b) Low-magnification electron micrograph showing adjacent osteoblasts (*O*) which make contact at a nexus (*N*) and simple appositions (*SA*). The *arrow* points to a possible developing "ahdaerens" junction. *M* mineralized matrix, *UM* unmineralized matrix. ×13500

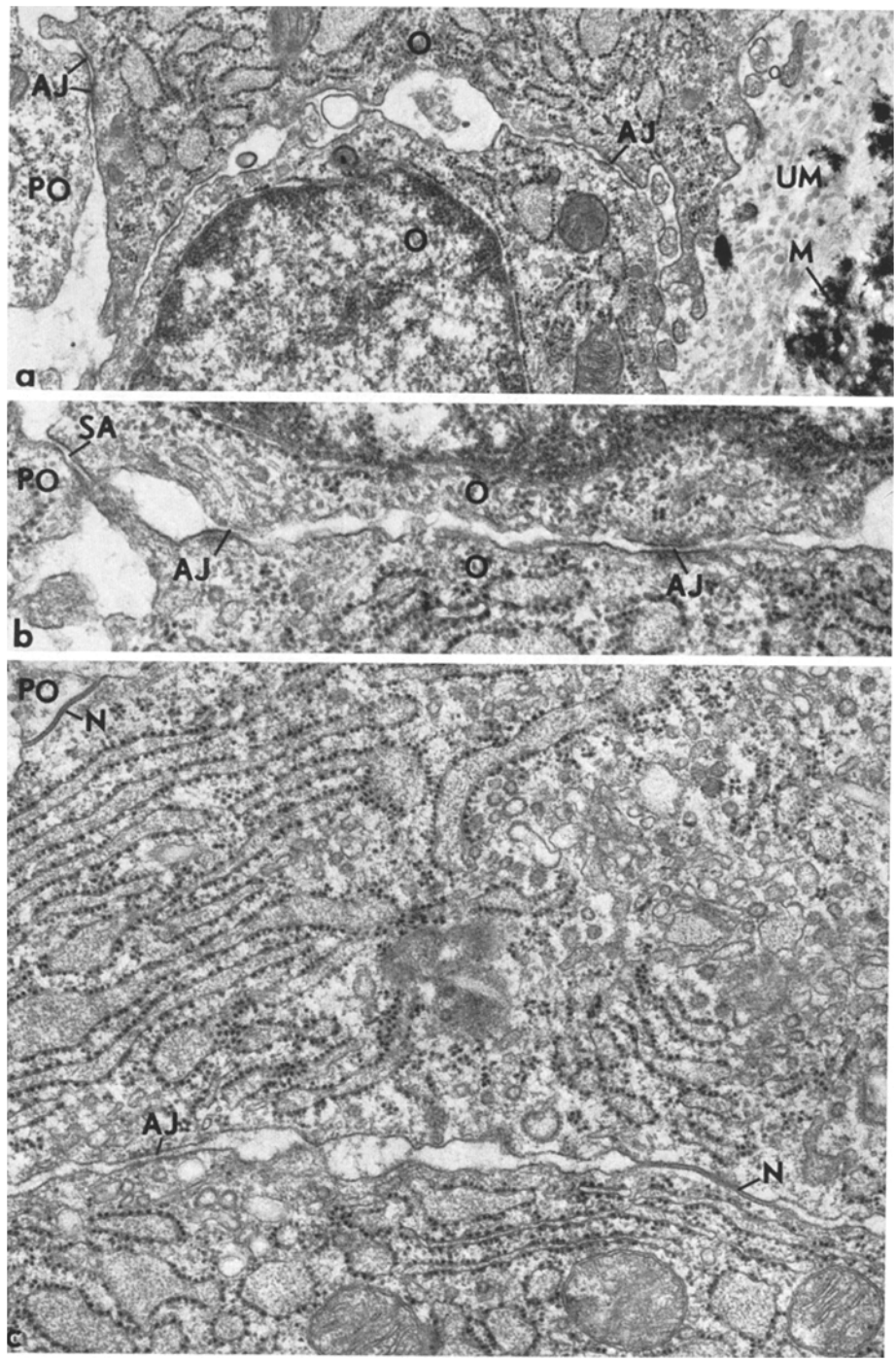

Fig. 2a—c. Junctional structures of osteoblasts (*O*) and preosteoblasts (*PO*). *AJ* "Adhaerens" junction, *N* nexus, *SA* simple apposition; *UM* unmineralized matrix, *M* mineralized matrix of the developing bone. (a) ×20400, (b) ×30000, (c) ×30000

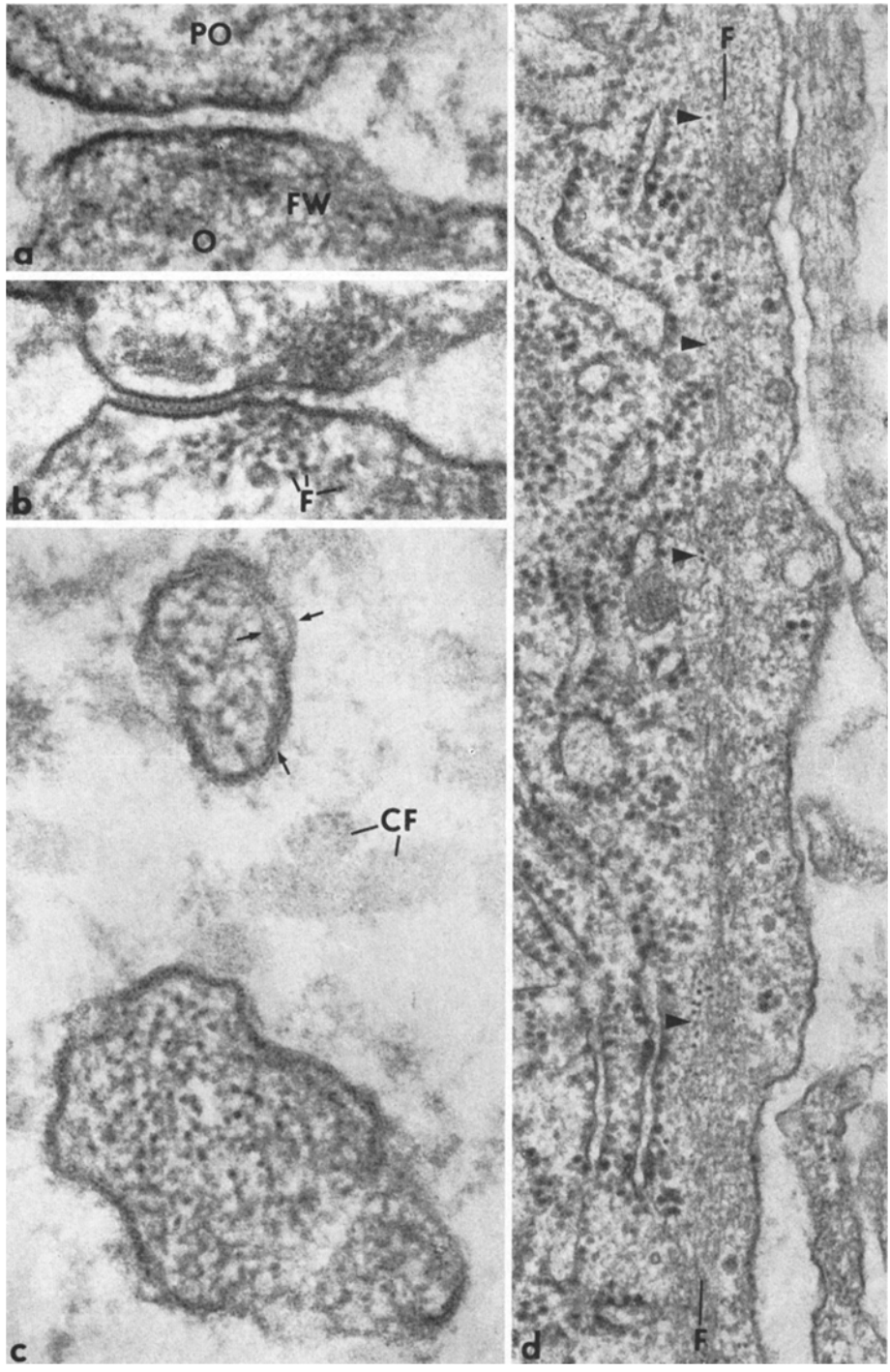

Fig. 3. (a) An "adhaerens" junction between an osteoblast (*O*) and a preosteoblast (*PO*); *FW* filamentous web. ×126000. (b) A nexus with a 9 nm periodicity between two osteoblasts; *F* microfilaments. ×126000. (c) Cross-section of two osteoblast processes filled with microfilaments. The appearance of a twisted and doubled plasmalemma (*arrows*) is caused by tilting and superimposition within the section (Stanka, 1973, 1974a). *CF* Collagen fibrils of the unmineralized matrix. ×160000. (d) Longitudinally sectioned filament-bundle (*F-F*) in the subplasmalemmal zone of an osteoblast. *Arrow-heads* mark crossing filaments. ×60000

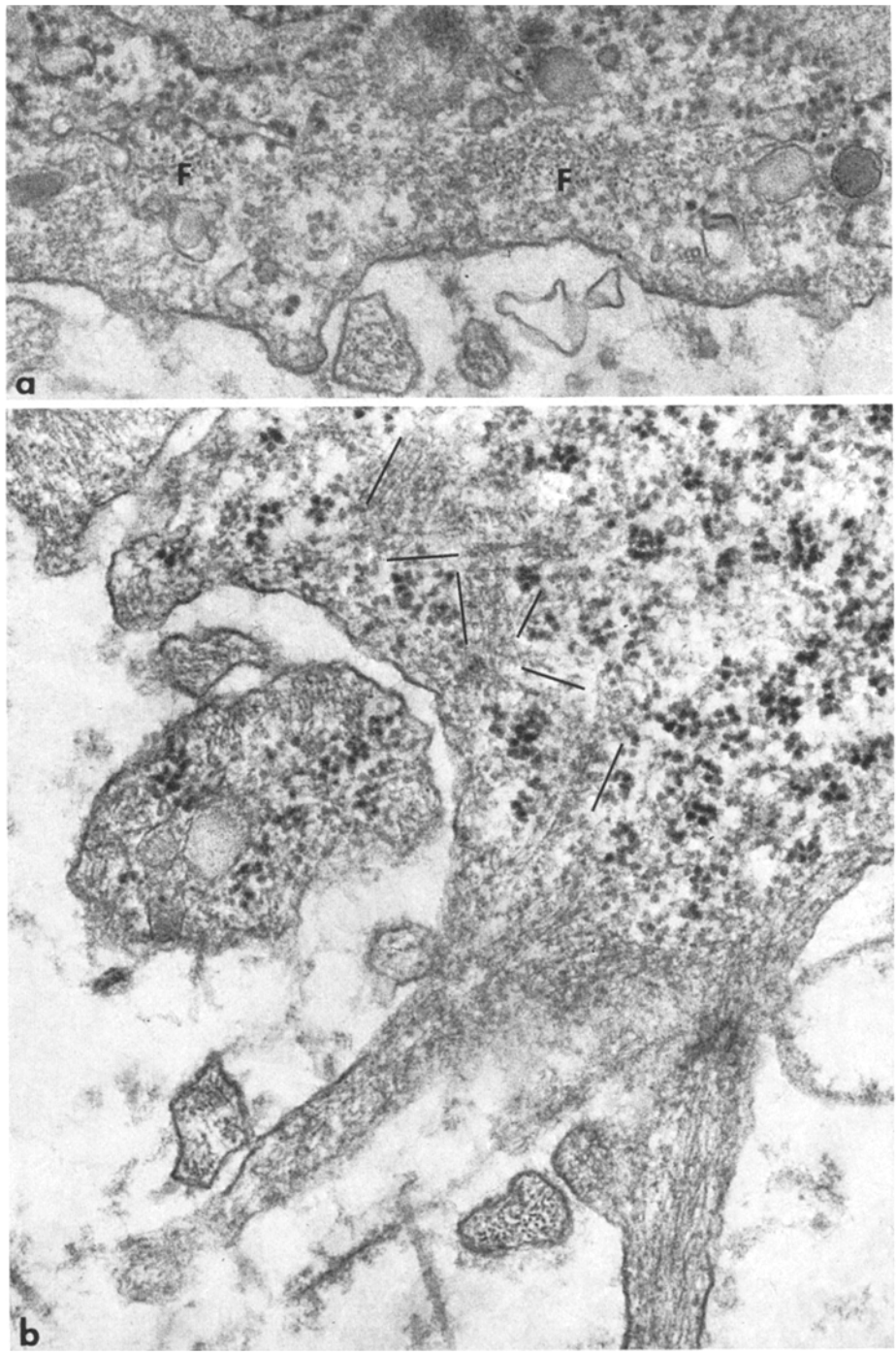

Fig. 4. (a) Cross-sectioned flat filament-bundles (*F*) in the peripheral, subplasmalemmal zone of an osteoblast. ×60000. (b) An obliquely sectioned part of an osteoblast displays filament-bundles in a zigzag pattern, due to intersectioning of the bundles. Bars mark the running directions. ×60000

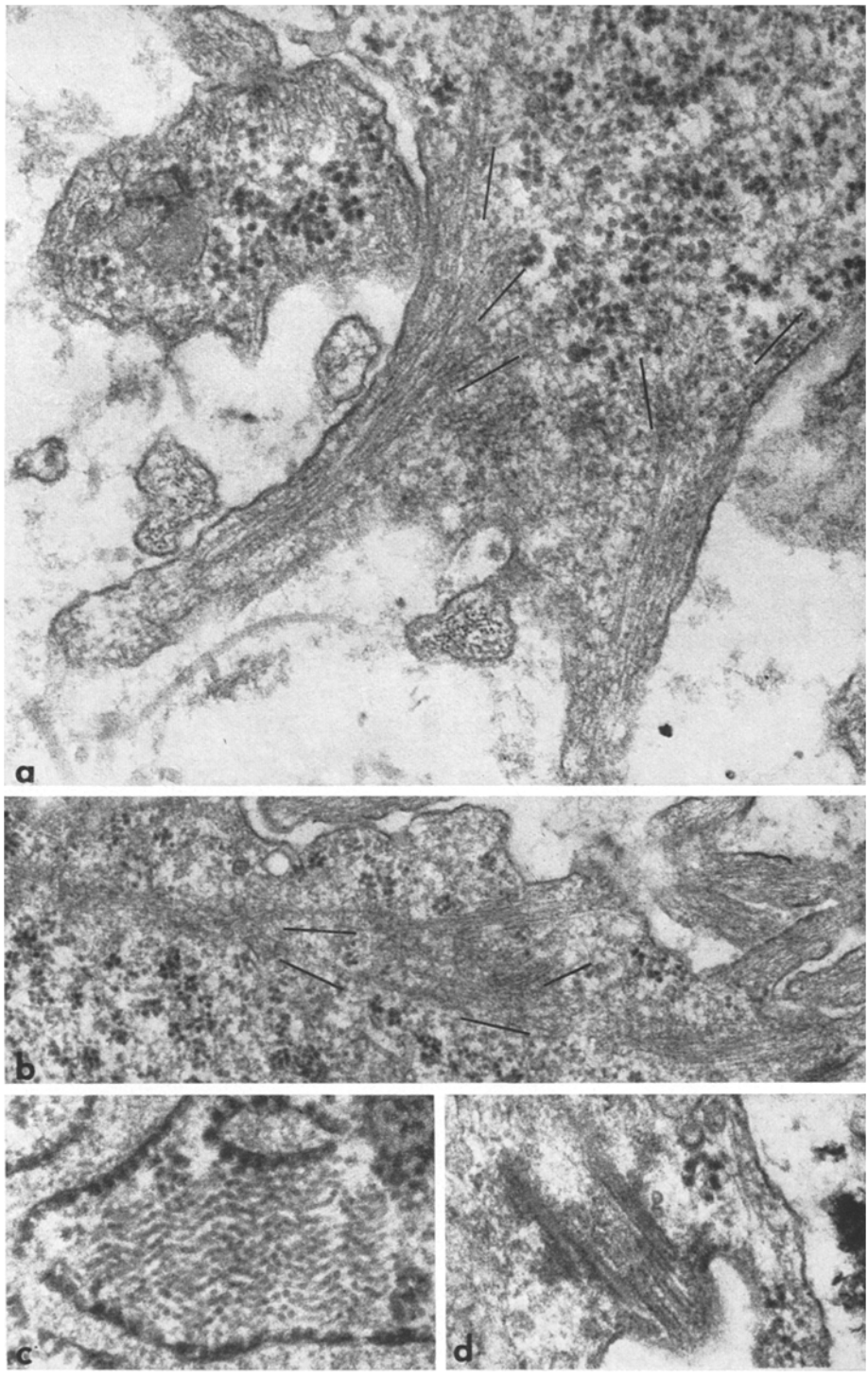

Fig. 5. (a) The same part of the osteoblast in Fig. 4b in a neighbouring section. Converging filament-bundles (marked by bars) run into cell processes. ×60000. (b) A filament-bundle divides and radiates into different cell processes. Bars mark the running directions. ×36000. (c) A bundle of thicker (10 to 15 nm) filaments between cisternae of the rough endoplasmic reticulum. ×76000. (d) A basal body of a cilia within an osteoblast. ×60000

Such bundles sometimes converge and intermingle from different directions. Fig. 5b shows a bundle that divides and radiates into different processes. In the cell processes the microfilaments are arranged parallel to the long axis. Individual filaments measure about 4 to 8 nm in diameter (mean value 5.9 ± 0.06 nm; 305 measurements of cross sections) and are of indeterminable length. Single thicker profiles (8 to 10 nm) which rarely occur among the thin filaments are not included in the calculation, because their appearance can also be due to superimposition of thinner elements within the section.

Another kind of filament is present in osteoblasts: 10 to 15 nm filaments, which only seldom occur clustered between cisternae of the rough endoplasmic reticulum (Fig. 5c).

Microtubules and Cilia. Short portions of scattered microtubules can also be seen in the peripheral cytoplasmic zone, sometimes running into the interior of the cell. Both preosteoblasts and osteoblasts occasionally exhibit single cilia (Fig. 5d).

Discussion

The first type of attachment sites in the osteoblasts and preosteoblasts can be described as incomplete desmosomes corresponding to stages (a) or (b) in desmosome development according to Staehelin (1974), but associated with 4 to 8 nm filaments. This type is also similar so the fascia adhaerens or intermediate junction (Farquhar and Palade, 1963), but restricted to a macula. It must be mentioned that superimposition due to oblique sectioning may, at least in part, contribute to the increase of density adjacent to the cell membranes and within the intercellular space. This "adhaerens" junction (McNutt and Weinstein, 1973) may play a role in the maintenance of tissue architecture. However, functional interpretations based solely on morphological findings must be made cautiously.

It is only possible at this time to attempt a few speculations concerning the described nexus (gap junctions) and microfilaments. Nexus have been described between a wide variety of cell types. The occurrence of a gap between the closely apposed membranes is dependent upon the preparation method (Brightman and Reese, 1969; Goodenough and Revel, 1971). Although the gap is not revealed in this study, the septate appearance, seen in the nexus in Fig. 3b, provides evidence that this junction is a gap junction. The 9 nm periodicity is caused by a polygonal lattice, the subunits of which show a center-to-center spacing of 9 to 10 nm (Robertson, 1963; Revel and Karnovsky, 1967; Goodenough and Revel, 1970; McNutt and Weinstein, 1970).

Nexus are commonly regarded as sites of cell-to-cell communication by low resistance coupling (Dewey and Barr, 1964; Farquhar and Palade, 1965; Revel and Karnovsky, 1967; Matter, 1973; Arluk and Rhodin, 1974; Cobb, 1974). It is questionable whether they can thereby mediate phenomena such as contact inhibition (Abercrombie, 1961; Loewenstein and Kanno, 1967; Loewenstein and Penn, 1967; McNutt and Weinstein, 1973). There is, however, some information that nexus might represent permeable areas which allow movement of ions and perhaps small molecules from cell to cell, and that they thereby might "provide pathways for intercellular control of complex activities such as movement, division, or differentiation" (Furshpan and Potter, 1968).

In this context the observation of subplasmalemmal bundles of microfilaments is of interest. It is widely accepted that microfilaments of the above-described diameter represent the substrate of cellular movements (Komnick *et al.*, 1973; Reaven and Axline, 1973; Taylor *et al.*, 1973; Kapanci *et al.*, 1974; Malech and Lentz, 1974; Stanka, 1974 b).

Another possibility is that the microfilaments function in maintenance of the stability of the cells in a manner similar to tonofilaments. But the diameter of tonofilaments (7–9 nm: Odland, 1964; Cooper *et al.*, 1967; Breathnach, 1971; 10–12 nm: Kelly, 1966; 10 nm: Malech and Lentz, 1974) speaks against this assumption. It may be of interest to mention here the findings of Bereiter-Hahn (1971) of a functional change of tonofilaments in teleost epidermis: under certain conditions tonofilaments can serve cell motility.

The observed nexus and bundles of microfilaments in osteoblasts in the present study may, therefore, indicate that these cells move and work in concert, thus guaranteeing a controlled bone growth and differentiation.

It may be mentioned that the above-described "adhaerens" junction, also referred to as 70 F-macula adhaerens (McNutt and Weinstein, 1973), in developing tissues may act as "transmitters of active, internally generated forces between cells of a tissue", and it thus seems "to be involved in unidirectional morphogenetic or segregative movements" (Staehelin, 1974).

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