Cilia-like structure, primary cilium and mechano-transduction in the osteocyte

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The centrosome represents the real core of microtubule organization in cells. The centrosome is involved in organelles such as cilia and flagella. The osteocyte is the bone cell in charge of mechano-reception and mechano-transduction in the skeleton [1, 2, 3, 4]

Primary cilia are present on most eukaryotic cells, and are considered to play a role in mechanosensing, particularly in the translation from mechano-reception to biochemical signals [5, 6, 7]. This role has been largely studied in the renal tubule epithelium; in hepatocytes, myocytes, red blood cells [6, 8, 9].

The primary cilium is a part of the cytoskeleton, it is characterized by a microtubule organization [10], under dependence of the centrosome. The centrosome is an organelle responsible of the microtubule organizing centers. It is involved in the mitotic spindle generation of the cell division, and in the function of primary cilium.

It is constituted of two centrioles: the mother and the daughter centrioles. Its molecular structure is mainly acetylated tubulin [11] with a cylinder shape of nine triplets in the mother centriole.

Our experiment [12] was developed on male Wistar rats, 26 weeks old, specifically on the upper part of the tibia, to study the osteocytes of cortical bone, with a particular care to the cell orientation. The study involved microscopy immunostaining (acetylated alpha-tubulin coupled to confocal microscopy), and TEM (transmission electron microscopy).

How many osteocytes possess such a structure? From 236 cells we observed, 222 were positively immunostained (94 %) [12]. It remains to be defined if the 6 % without staining of the centrosome could be young osteocytes or osteocytes entering apoptosis, but this percentage is prone to correspond to such a nature.

How many cilia-like structures per osteocyte ? In all our experiments, there was one and only one such a structure [12]. This is in accordance with the current knowledge on osteocytes [13, 14].

What size and ultrastructure for the centrosome? There were two centrioles: the mother centriole connected to the cell membrane, and daughter centriole never connected to the cell membrane. The size of the mother centriole average 482±71 nm while the daughter centriole was smaller (351±38 nm). The two centrioles were connected by striated rootlets in a large percent of cells, the mean distance between mother and daughter centrioles averaging 148±74 nm.

What orientation for the centrosome?

In our experiment, the mother centriole connected to the cell membrane was oriented close to a parallel position to the section plane, which means an orientation perpendicular to the long axis of tibial bone.

This preferential orientation suggests a role in mechanosensing of this structure.

Morphology of the area connecting the mother centriole to the cell membrane

There was an electron-dense material in this area, with in some cells short cilium rods.

In one case, we observed a "cilium membrane rame" [12] between the cell body and the bone tissue. In cell-culture of osteocytes, such structures in extra cellular location have been described [15, 16, 17]. We have suggested that the specific bone environment could limit cilium growth [12]. The distal appendages are also located in this region, nine per mother centriole, one per triplet of the mother centriole.

We have also underlined [12] that the osteocyte differentiation was associated to a variation of the respective orientation of mother and daughter centriole.

The role of this ciliary structure in mechano-transduction has been underlined by several experiments: the loss of ciliary structure is characterized by the disappearance of prostaglandin expression in response to mechanical stress [16].

Another experiment has shown that the deletion of the PKD1 protein (part of the primary cilium structure) leads to a low bone mass, low anabolic processes, and bone defects [19]. Other studies confirm the evidence of these primary cilium structures' role in mechano-reception, conditioning bone formation and bone mass [19, 20]. Furthermore, the role of the ciliary structures in Wnt-signaling induced by mechanosensing has been demonstrated by hypogravity experiments

[21]. The cilia had previously been implicated in the regulation of Wnt-signaling [22, 23, 24].

In other organs, the primary cilium structures appear to be related to the calcium signaling [25, 26] while it does not seem to be the case in bone cells [27]. A direct role of the primary cilium structure on the PGE2 signaling has been suggested [27, 28, 29, 16].

In conclusion, the osteocyte primary cilium structure has been largely imaged by our recent study, and appears to play a major role in the mechano-sensing process in bone, considered as an early step in the bone formation process.

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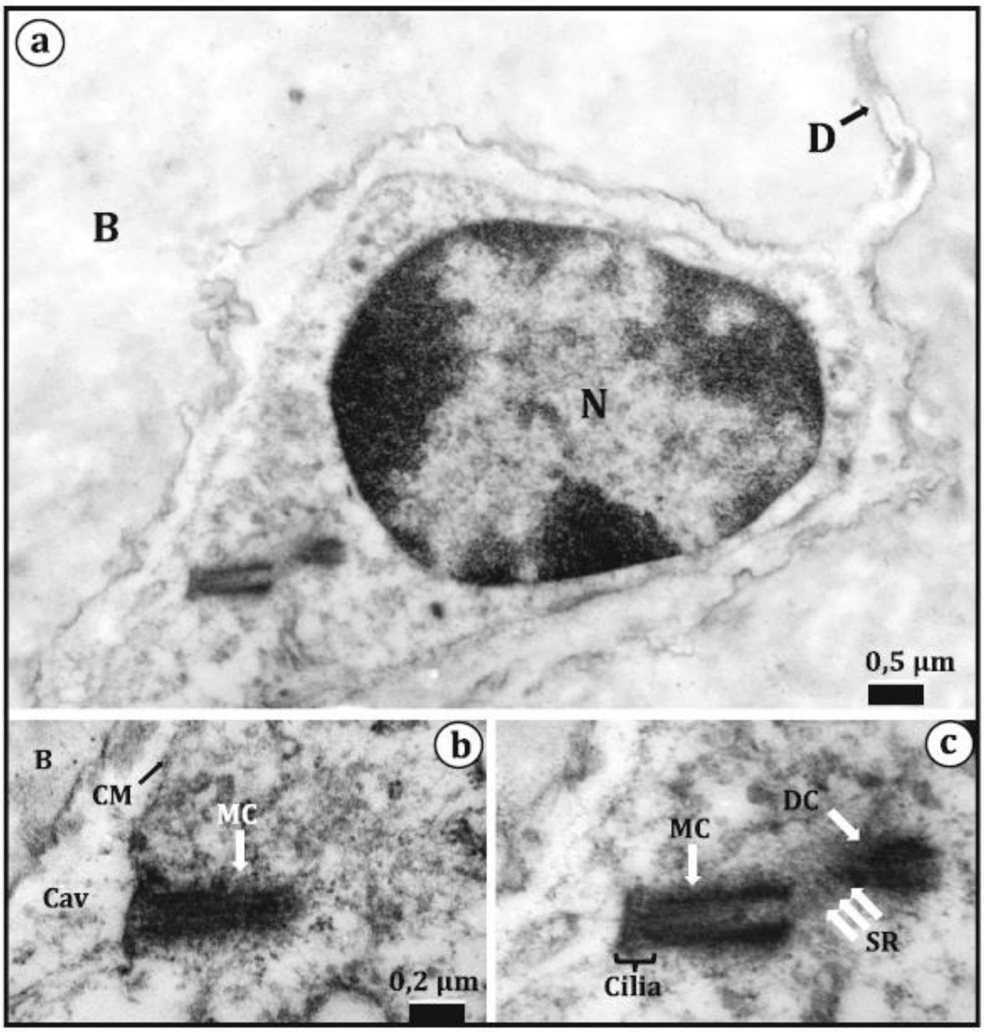

Fig. 1 The mother and daughter centriole (*MC*, *DC*) with the striated rootlet (*SR*), the extra-cellular cavity (*cav*), the dendrite (*D*), the nucleus (*N*) and the bone matrix (*B*) in an osteocyte from male Wistar rat imaged by transmission electron microscopy (TEM)

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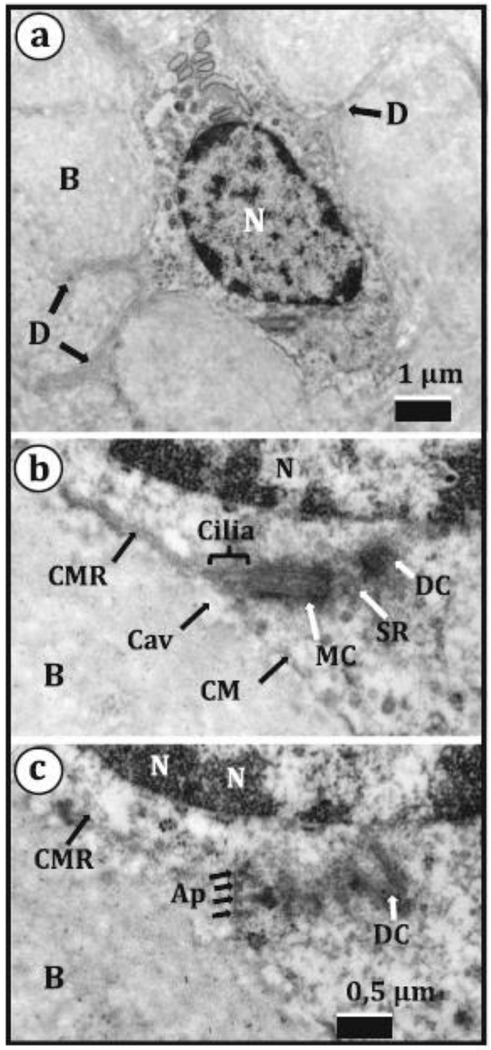
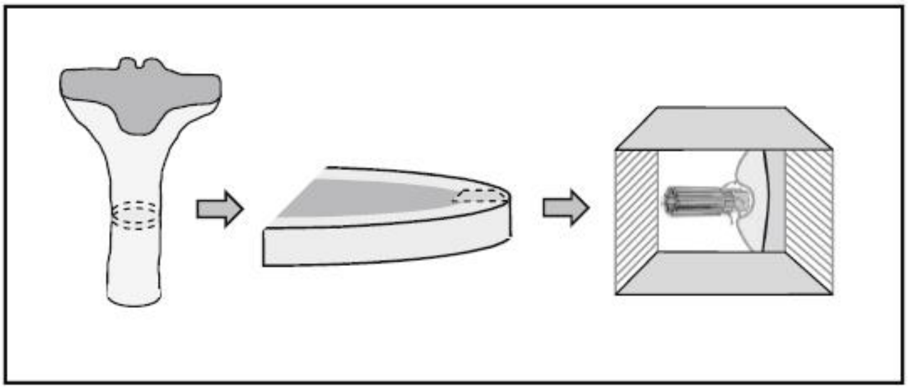


Fig. 2 Magnification of the junction between primary cilia and the extra cellular cavity with identification of the cilia membrane rame (CMR)



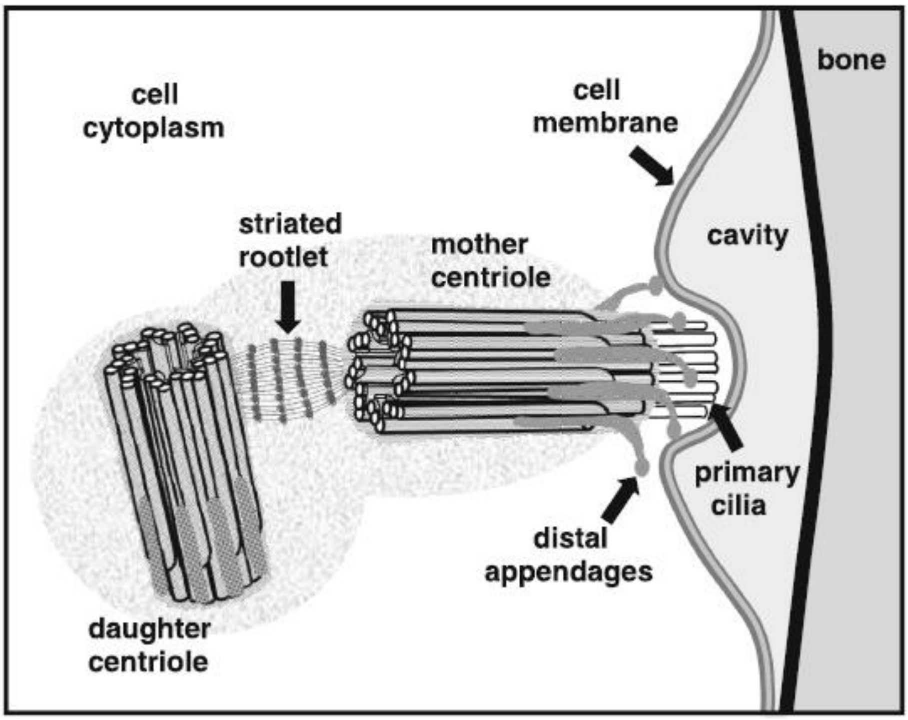


Fig. 3 Schematic representation of the centrosome with its fine microtubule constitution and its orientation relative to the rat tibia (adapted from 12)

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Osteocytes: an exquisitely sensitive mechanosensory cell

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