Journal of Cellular Biochemistry 89:233–237 (2003)

PROSPECT

Primary Cilium—Is It an Osteocyte's Strain-Sensing Flowmeter?

James F. Whitfield*

Institute for Biological Sciences, National Research Council of Canada, Ottawa, Ontario, Canada

Abstract With few exceptions, the non-cycling cells in a vast range of animals including humans have a non-motile primary cilium that extends from the mother centriole of the pair of centrioles in their centrosomes located between their Golgi apparatuses and nuclei. It has very recently been shown that the primary cilium of a dog or a mouse embryonic kidney cell is a fluid flowmeter studded with heterodimeric complexes of mechanoreceptors linked to Ca^{2+}-permeable cation channels that when the cilium is bent can send Ca^{2+} signals into the cell and beyond to neighboring cells through gap junctions. More than 30 years ago, osteocytes were reported also to have primary cilia, but this was promptly ignored or forgotten. Osteocytes are the bones' strain sensors, which measure skeletal activity from the effects of currents of extracellular fluid caused by their bones being bent and squeezed during various activities such as walking and running. Since bending a kidney cell's primary cilium can send a Ca^{2+} wave surging through itself and its neighbors, the bending of an osteocyte's primary cilium by sloshing extracellular fluid is likely to do the same thing and thus be involved in measuring and responding to bone strain. J. Cell. Biochem. 89: 233–237, 2003.

Key words: bone strain; Ca^{2+}signaling; kidney cells; osteocytes; primary cilia bending; primary cilia functions; stretch-activated Ca^{2+}channels

Osteocytes are the bones' strain sensors [e.g., Martin et al., 1998]. They are programmed to signal in certain ways when the strain pulses are above, below, or within the locally expected range [Turner et al., 2002]. They are retired osteoblasts, which, instead of committing apoptotic suicide at the end of their bone-making lives, were buried in the new bone where they retooled themselves for a new career of strain-sensing and responding to requests to draw Ca^{2+} from the bone stores to top up the circulating Ca^{2+} concentration when necessary. They are connected to each other and to bone-lining cells by gap junctions as well as to blood vessels and nerves to form an extensive 3-D "osteointernet" [Martin et al., 1998]. They live in tiny cubicles or lacunae and extend processes through narrow canals known as canaliculi to plug into other members of the network. While the cubicles, canaliculi, and their extracellular fluids make up only about 1% of the total bone volume, their total surface area in an adult male skeleton has been estimated as 1,200 m^2 while the total surface areas of the Haversian plus Volkmann canals and the trabeculae are only 3 and 9 m^2, respectively [Johnson, 1966; Martin et al., 1998]. Clearly the osteocyte network with its extensive contact with various cells systems at the bone surface is a powerful device for regulating Ca^{2+} traffic between bone and blood and monitoring and responding to strain.

STRAIN TRANSMISSION

The normal cycles of tension and compression in the hip and leg bones of a walking, running, or jumping human cause the fluid in the osteocyte network to slosh back and forth generating ionic streaming potentials and piezoelectric currents and inflicting membrane-deforming shear forces on the osteocyte network [Kufahl and Saha, 1990; Martin et al., 1998; Burr et al., 2002; Ehrlich and Lanyon, 2002]. According to Knoth

*Correspondence to: James F. Whitfield, Institute for Biological Sciences, Bldg. M-54, National Research Council of Canada, Montreal Road Campus, Ottawa, Ontario, Canada K1A 0R6. E-mail: pthosteo@rogers.com

Received 27 January 2003; Accepted 30 January 2003

DOI 10.1002/jcb.10509

Tate et al. [1998, 2000] and Smit et al. [2002], strain pulses in long bones such as femur and tibia from heel strikes during the walking cycle, for example, cause fluid to surge through the 3-D canalicular network to the bone surface and back again. The back-and-forth sloshing of the fluid in the osteocyte network tells the osteocytes about the level of bone activity and to send an appropriate stream of signals to its fellows in the osteointernet [Burr et al., 2002; Ehrlich and Lanyon, 2002].

STRAIN-INDUCED CELL SIGNALING

The back-and-forth flowing of the extracellular fluid in a bone being bent and squeezed during a walking or running cycle stretches the cell membrane and tugs on cadherins and signal-generating integrins in the focal adhesion plaques that respectively attach osteocytes and their processes to each other and to the matrix-lined walls of their lacunae and canaliculi [Pavalko et al., 2003]. Among the results of this fluid pumping and shearing is the clustering of integrins with growth factor receptors such as those for FGF and the ligand-independent activation of the clustered receptors [Yamada and Even-Ram, 2002]. Stretch-activated Ca^{2+} channels are opened to let Ca^{2+} flow into the cell and stretch-activated cation channels are also opened through which Na^{+} flows to depolarize the cell and thus open other, voltage-sensitive, L-type Ca^{2+} channels [Duncan and Misler, 1989; Kizer et al., 1997; Ryder and Duncan, 2001; Pavalko et al., 2003]. The stream of signals generated by the pushed and pulled cadherins, ion channels, and integrin-growth-factor receptor clusters set off a cascade of events starting, for example, with a burst of c-Src protein tyrosine kinase activity and the generation and release of phospho-$p130^{cas}$•NmP4 complexes from actin-associated complexes clustered against the cell membrane and integrins as well as β-catenin from the transmembrane cadherins [Pavalko et al., 2003]. These messengers leave the surface and travel from the cell membrane into the nucleus where they stimulate the expression of various bone-specific genes [Yamada and Even-Ram, 2002; Pavalko et al., 2003]. These shear-triggered events also include the stimulation of NO synthase and glutamate release by the Ca^{2+} surges and prostaglandin [PGI_2 and PGE_2] syntheses by cycloxygenase-2 (COX-2), which add more things to the cascade of events [Pavalko et al., 1998, 2003; Skerry, 1999].

But to date, all models of strain sensing by osteocytes are missing something—the primary cilium—which would be bent by currents of lacunar fluid [Pazour and Witman, 2003]. The osteocye's primary cilium was first mentioned, but only in passing, by Cameron [1971] and Matthews and Martin [1971], but was described in some detail by Tonna and Lampen [1972]. But then the cilium was promptly forgotten or ignored. After all, it is difficult to see and furthermore why would an osteocyte need a cilium to swim around its lacuna? But we are finally beginning to learn how important this organelle can be.

PRIMARY CILIUM

The primary (or solitary) cilium appears to have been first reported by the Swiss anatomist K. Zimmermann [1898] in an extensive study of the cells of various tissues except bone. Since then it has been found with few exceptions in almost every type of cell in a vast range of animals including humans [see Wheatley [1982] as well as the online Primary Cilium Resource Page].

The primary cilium is a cell's chemo-, mechano-, and/or photo-sensing organelle, which grows from the mature mother centriole of the mother–daughter pair of centrioles in the cell's centrosome located between nucleus and the Golgi apparatus [Albrecht-Buehler, 1977; Albrecht-Buehler and Bushnell, 1980; Poole et al., 1985, 2001; Sloboda, 2002; Pazour and Witman, 2003]. It is non-motile. Motile cilia have 9 peripheral microtubule doublets with 2 central singlet microtubules and are known as $9+2$ cilia, but primary cilia have no central tubules and hence are known as $9+0$ cilia [Barnes, 1961; Sorokin, 1962; Tonna and Lampen, 1972; Albrecht-Buehler, 1977; Albrecht-Buehler and Bushnell, 1980; Wheatley, 1982; Poole et al., 1985, 1997, 2001; Wheatley et al., 1996; Alvarez-Buylla et al., 2001; Praetorius and Spring, 2001; Calvert, 2002; Rosenbaum and Witman, 2002; Nauli et al., 2003; Pazour and Witman, 2003]. Though not motile, the primary cilium is humming with kinesin and dynein motors carrying tubulin and many other cargos back and forth beneath the cilium's membrane [Rosenbaum and Witman, 2002; Sloboda, 2002].

STRAIN SENSING WITH THE PRIMARY CILIUM

The kidney cell's primary cilium is a fluid flowmeter that suggests how osteocytes might measure and respond appropriately to bone strain [Praetorius and Spring, 2001; Nauli et al., 2003] (Fig. 1). Each kidney epithelial cell has a long, stiff primary cilium sticking out of its apical surface and into the passing urine stream [Praetorius and Spring, 2001; Nauli et al., 2003]. The kidney cilia look remarkably like car aerials (see the striking picture of one in the online Primary Cilium Resource Page) and have now been thrust into the limelight because genetically disabling them causes autosomal dominant polycystic kidney diseases [Calvert, 2002; Rosenbaum and Witman, 2002; Yoder et al., 2002; Nauli et al., 2003; Pazour and Witman, 2003].

Bending the primary cilium on a MDCK dog kidney cell or a mouse embryonic kidney cell in flowing medium opens Ca^{2+} channels through which Ca^{2+} flows into the cell from the external medium. The size of the Ca^{2+} signal depends on how much the cilium is bent by the flowing medium [Praetorius and Spring, 2001]. Bending activates polycystin-1•polycystin-2 (PC-1•PC-2) heterodimers anchored along the ciliary membrane [Nauli et al., 2003]. PC-1

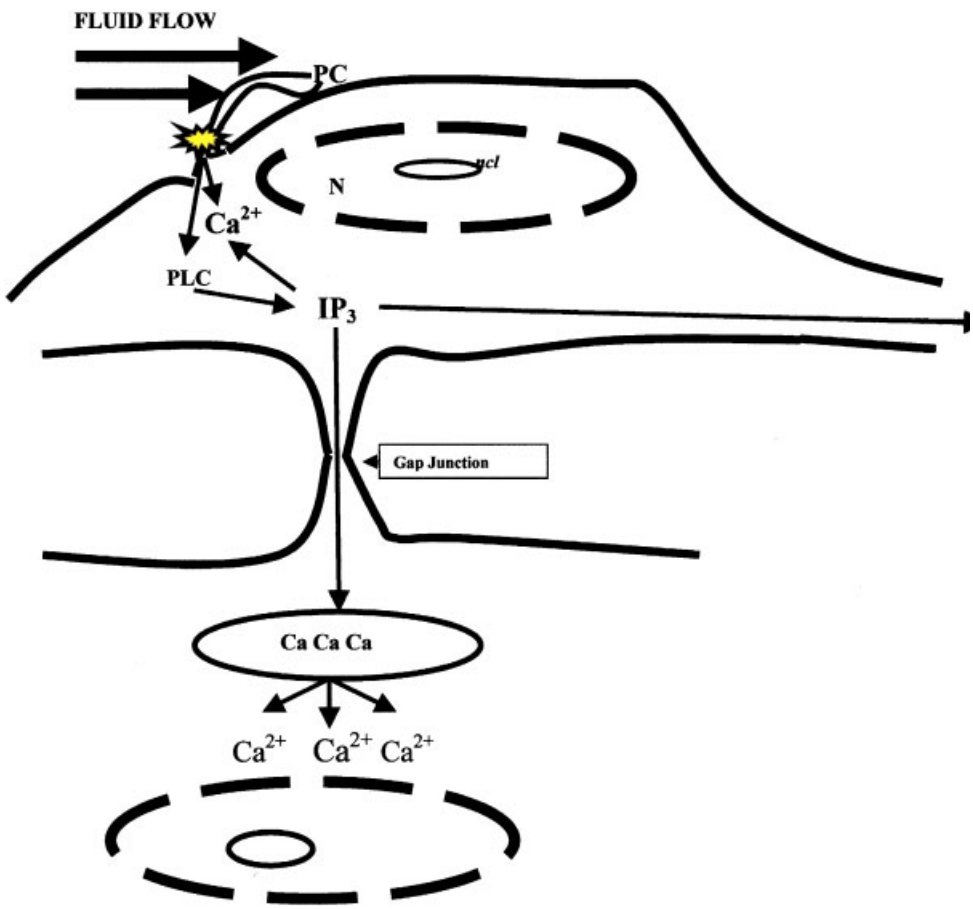

Fig. 1. How an osteocyte might use its primary cilium to measure and initiate responses to bone strains from lacunar and canalicular fluid currents caused by the bending and squeezing of bones during walking or running as suggested by the examples of the primary cilia (PC) on Madin Darby canine kidney (MDCK) and mouse embryo kidney cells. Bending a cilium opens PC-2 Ca^{2+}-permeable cation channels linked to PC-1 mechanoreceptors in heterodimeric complexes arrayed along the ciliary membrane [Nauli et al., 2003]. In MDCK cells, the result is a Ca^{2+} surge, which triggers a cascade of events in the nucleus (N) and cytoplasm. This cascade includes the activation of phospholipase-C which, in turn, chops membrane phosphatidylinositol bisphosphate into diacylglycerols and IP_3, which releases Ca^{2+} from internal stores and passes through gap junctions into neighboring cells where it triggers the release of Ca^{2+} from the neighbors' stores to start a spreading wave of Ca^{2+} signaling and cascades of Ca^{2+}-induced events [Praetorius and Spring, 2001]. The same thing happens when a mouse embryo kidney cell's primary cilium is tweaked, but IP_3 does not mediate the transmission of the signal to neighboring cells [Nauli et al., 2003].

(encoded by the murine *Pkd1* gene) is a G-protein-coupled mechanosensor, which when stretched by the ciliary bending opens the linked PC-2 Ca^{2+}-permeable cation channel (encoded by the murine *Pkd2* gene) [Nauli et al., 2003]. The signal from the bending cilium triggers the emptying of the cell's internal Ca^{2+} stores which, in MDCK cells by producing inositol(1,3,5)-*tris* phosphate (IP_3) that passes through gap junctions into neighboring cells where it transmits the ciliary signal by stimulating the release of Ca^{2+} from the neighbors' internal stores [Praetorius and Spring, 2001] (Fig. 1). But IP_3 does not mediate the passage of the ciliary Ca^{2+} signal from cell to cell in mouse embryonic kidney cultures. The Ca^{2+} signal coming from the bent cilium in these mouse cells releases internal Ca^{2+} stores by activating ryanodine receptors instead of IP_3 receptors [Nauli et al., 2003]. Nevertheless, bending just one cilium on a dog or embryonic mouse kidney cell sends a wave of Ca^{2+} signaling though the cellular network as might happen when the osteocyte's primary cilium is tweaked by the sloshing of intralacunar fluid during a walking cycle (Fig. 1).

SUMMARY AND FUTURE DIRECTIONS

The so far largely ignored non-motile $9+0$ primary cilium is a kind of antenna which a wide range of cells use to measure and respond to various features of their surroundings. Thus, there are the cilia that brain's subventricular astrocytes probably use to monitor the cerebrospinal fluid [Alvarez-Buylla et al., 2001], the connecting cilium of retinal photoreceptor cells [Rosenbaum and Witman, 2002], the cilium that 3T3 cells may use to guide their migration [Albrecht-Buehler, 1977; Albrecht-Buehler and Bushnell, 1980], and the ciliary flowmeter that kidney tubule cells use to measure urine flow rate [Praetorius and Spring, 2001; Nauli et al., 2003].

The osteocyte's primary cilium was discovered more than 30 years ago and then promptly forgotten or ignored. However, the elegant experiments of Nauli et al. [2003] and Praetorius and Spring [2001] who showed that bending just one kidney cell's primary cilium sends a wave of Ca^{2+} signaling first through the cilium's cell and then through gap junctions into neighboring cells suggests that the osteocyte cilium might do the same thing when bent back and forth by pulses of extracellular fluid during walking or running cycles. To test this possibility, osteocytes must be isolated, the presence of primary cilia with PC-1•PC-2 complexes determined as Nauli et al. [2003] have done with mouse kidney cells, and then the cilia must be tweaked to see whether they respond like kidney cells to their primary cilia. Pavalko et al. [2003] have said that bending bones bend genes and perhaps it can soon be said that bending an osteocyte's primary cilium can bend its and its neighbors' bone-specifc genes.

REFERENCES

Albrecht-Buehler G. 1977. Phagokinetic tracks of 3T3 cells: Parallels between the orientation of track segments and of cellular structures which contain actin or tubulin. Cell 12:333–339.

Albrecht-Buehler G, Bushnell A. 1980. The ultrastrcture of primary cilia in quiescent 3T3 cells. Exp Cell Res 126: 427–437.

Alvarez-Buylla A, Garcia-Verdugo J, Tramontin AD. 2001. A unified hypothesis on the lineage of neural stem cells. Nat Rev Neurosci 2:287–293.

Barnes BG. 1961. Ciliated secretory cells in the pars distalis of the mouse hypophysis. J Ultrastruct Res 5: 453–467.

Burr DB, Robling AG, Turner CH. 2002. Effects of biomechanical stress on bone in animals. Bone 30:781–786.

Calvert JP. 2002. Cilia in PKD—Letting it all hang out. J Am Soc Nephrol 13:2614–2616.

Cameron DA. 1971. The ultrastructure of bone. In: Bourne GH, editor. The ultrastructure of bone. New York: Academic Press. p 191–236.

Duncan R, Misler S. 1989. Voltage-activated and stretch-activated Ba^{2+}-conducting channels in an osteoblast-like cell line (UMR 106). FEBS Lett 251:17–21.

Ehrlich PJ, Lanyon LE. 2002. Mechanical strain and bone cell function: A review. Osteoporos Int 13:688–700.

Johnson LC. 1966. The kinetics of skeletal remodeling. Birth Defects Orig Artic Ser 2:66–142.

Kizer N, Guo KL, Hruska K. 1997. Reconstitution of stretch-activated cation channels by expression of the epithelial sodium channel cloned from osteoblasts. Proc Natl Acad Sci USA 94:1013–1018.

Knoth Tate ML, Knotke U, Niederer P. 1998. Experimental elucidation of mechanical load-induced fluid flow and its potential role in bone metabolism and functional adaptation. Am J Med 315:189–195.

Knoth Tate ML, Steck R, Forwood MR, Niederer P. 2000. In vivo demonstration of load-induced fluid flow in the rat tibia and its potential implications for processes associated with functional adaptation. J Exp Biol 203:2737–2745.

Kufahl RH, Saha S. 1990. A theoretical model for stress-generated fluid flow in the canaliculi-lacunae network in bone tissue. J Biomech 23:171–180.

Martin RB, Burr DB, Sharkey NA. 1998. Skeletal tissue mechanics. New York: Springer-Verlag.

Matthews JL, Martin JH. 1971. Intracellular transport of calcium and its relationship to homeostasis and mineralization. Am J Med 50:589–597.

Nauli SM, Alenghat FJ, Luo Y, Williams E, Vassilev P, Li X, Elia AEH, Lu W, Brown EM, Quinn SJ, Ingber DE, Zhou J. 2003. Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells. Nat Genet 33:129–137.

Pavalko FM, Chen NX, Turner CH, Burr DB, Atkinson S, Hsieh YF. 1998. Fluid shear-induced mechanical signaling in MC3T3-E1 osteoblasts requires cytoskeleton-integrin interactions. Am J Physiol 275:C1591–C1601.

Pavalko FM, Norvell SM, Burr DB, Turner CH, Duncan RL, Bidwell JP. 2003. A model for mechanotransduction in bone cells: The load-bearing mechanosomes. J Cell Biochem 88:104–112.

Pazour GJ, Witman GB. 2003. The vertebrate primary cilium is a sensory organelle. Curr Opin Cell Biol 15:1–6.

Poole CA, Flint MH, Beaumont BW. 1985. Analysis of the morphology and function of primary cilia in connective tissues: A cellular cybernetic probe. Cell Motil 5:175–193.

Poole CA, Jensen CG, Snyder JA, Gray CG, Hermanutz VL, Wheatley DN. 1997. Confocal analysis of primary cilia structure and colocalization with Golgi apparatus in chondrocytes and aortic smooth muscle cells. Cell Biol Int 21:483–494.

Poole CA, Zhang ZJ, Ross JM. 2001. The differential distribution of acetylated and detyrosinated alpha-tubulin in the microtubular cytoskeleton and primary cilia of hyaline cartilage chondrocytes. J Anat 199:393–405.

Praetorius HA, Spring KR. 2001. Bending the MDCK cell pimary cilium increases intracellular calcium. J Membrane Biol 184:71–79.

Primary Cilium Resource Page. http://www.wadsworth.org/BMS/SCBlinks/cilia.html/

Rosenbaum JL, Witman GB. 2002. Intraflagellar transport. Nat Rev Mol Cell Biol 3:813–825.

Ryder KD, Duncan RL. 2001. Parathyroid hormone enhances fluid shear-induced $[Ca^{2+}]_I$signaling in osteoblastic cells through activation of mechanosensitive and voltage-sensitive Ca^{2+} channels. J Bone Miner Res 16:240–248.

Skerry TM. 1999. Identification of novel signaling pathways during functional adaptation of the skeleton to mechanical loading: The role of glutamate as a paracrine signaling agent in the skeleton. J Bone Miner Metab 17: 66–70.

Sloboda RD. 2002. A healthy understanding of intraflagellar transport. Cell Motil Cytoskeleton 52:1–8.

Smit TH, Burger EH, Huyghe JM. 2002. A case for strain-induced fluid flow as a regulator of BMU-coupling and osteonal alignment. J Bone Miner Res 17:2021–2029.

Sorokin S. 1962. Centrioles and the formation of rudimentary cilia by fibroblasts and smooth muscle cells. J Cell Biol 15:363–377.

Tonna EA, Lampen NM. 1972. Electron microscopy of aging skeletal cells. I. Centrioles and solitary cilia. J Gerontol 27:316–324.

Turner CH, Robling AG, Duncan RL, Burr DB. 2002. Do bones behave like a neuronal network? Calcif Tissue Int 70:435–442.

Wheatley DN. 1982. The centriole: A central enigma of cell biology. Amsterdam: Elsevier Biomedical Press.

Wheatley DN, Wang AM, Strugnell GE. 1996. Expression of primary cilia in mammalian cells. Cell Biol Int 20: 73–81.

Yamada KM, Even-Ram S. 2002. Integrin regulation of growth factor receptors. Nat Cell Biol 4:E75–E76.

Yoder BK, Hou X, Guay-Woodford LM. 2002. The polycystic disease proteins, polycystin-1, polycystin-2, and cystin, are co-localized in renal cilia. J Am Soc Nephrol 13:2508–2516.

Zimmermann KW. 1898. Beiträge zur Kenntniss einiger Drüsen und Epithelien. Arch Mikr Entwicklungsmech 52:552–706.