Primary Cilia: Cellular Sensors for the Skeleton

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ABSTRACT

The primary cilium is a solitary, immotile cilium that is present in almost every mammalian cell type. Primary cilia are thought to function as chemosensors, mechanosensors, or both, depending on cell type, and have been linked to several developmental signaling pathways. Primary cilium malfunction has been implicated in several human diseases, the symptoms of which include vision and hearing loss, polydactyly, and polycystic kidneys (reviewed in Tobin and Beales 2007; Yoder 2007). Recently, primary cilia have also been implicated in the development (Zhang et al., 2003; Xiao et al., 2006; Haycraft et al., 2007; Koyama et al., 2007; McGlashan et al., 2007) and homeostasis (Malone et al., 2007) of the skeleton. In this review, we discuss the structure and formation of the primary cilium and some of the mechanical and chemical signals to which it could be sensitive, with a focus on skeletal biology. We also raise several unanswered questions regarding the role of primary cilia as mechanosensors and chemosensors and identify potential research avenues to address these questions.

The primary cilium is a solitary, immotile cilium that is present in almost every mammalian cell type (Wheatley et al., 1996). Multiple developmental signaling pathways have been linked to primary cilia (reviewed in Christensen et al., 2007), and they are thought to function as chemosensors, mechanosensors, or both, depending on cell type. Primary cilium malfunction has been implicated in several human diseases, the symptoms of which include vision and hearing loss, polydactyly, and polycystic kidneys (reviewed in Tobin and Beales 2007; Yoder 2007). Recently, primary cilia have also been implicated in the development (Zhang et al., 2003; Xiao et al., 2006; Haycraft et al., 2007; Koyama et al., 2007; McGlashan et al., 2007) and homeostasis (Malone et al., 2007) of the skeleton. In this review, we discuss the structure and formation of the primary cilium and some of the mechanical and chemical signals to which it could be sensitive, with a focus on skeletal biology. We also raise several unanswered questions regarding the role of primary cilia as mechanosensors and chemosensors and identify potential research avenues to address these questions.

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Fig. 1. Schematic of primary cilium structure. The primary cilium is enclosed in a specialized membrane (dark green) that is continuous with the plasma membrane (light green). Membranes are shown in cutaway view. The axoneme of the primary cilium (dark blue) extends from the mother centriole/basal body (purple), which possesses subdistal appendages (orange) that are connected to a subset of cytoplasmic microtubules (red). The mother centriole/basal body is part of the centrosome, which includes a daughter centriole (light blue) and pericentriolar material (light gray) from which other cytoplasmic microtubules (red) are nucleated. The mother centriole/basal body is connected to the plasma membrane via transition fibers (yellow) that separate the ciliary compartment from the cytoplasm. Axonemal microtubules are reduced in number and shortened for simplicity; they actually extend far beyond the boundaries of the image. Image not to scale.

**PRIMARY CILIUM GROWTH**

Primary cilium growth begins when the distal end of the mother centriole docks with a \( \sim 300 \) nm diameter vesicle of unknown origin (Sorokin 1962; Alieva and Vorobjev 2004). This docking event precedes the extension of nine doublet microtubules from the nine triplet microtubules of the centriole. Further axoneme growth is accomplished by a process called intraflagellar transport (IFT), wherein cargo is transported up and down the axoneme via complexes of IFT adaptor proteins by the motor proteins kinesin-2 and cytoplasmic dynein 1b, respectively (reviewed in Scholey and Anderson 2006). The processes by which cargo is acquired and released and the regulation of motor attachment to the axonemal microtubules are unclear, as is the mechanism that determines the final length of the axoneme, which varies from a few \( \mu \)m to over 100 \( \mu \)m, depending on cell type (unpublished observations). The membrane surrounding the axoneme then fuses with the plasma membrane, allowing the cilium to protrude into the extracellular space. In cycling cells, primary cilia appear early in G1 (Alieva and Vorobjev 2004) and are disassembled prior to mitosis by an Aurora A-dependent mechanism (Pugacheva et al., 2007).

**PRIMARY CILIA AS MECHANOSENSORS IN BONE**

The skeleton is primarily a mechanical organ whose function is to support the body against gravitational force and aid in locomotion. The skeleton adapts to mechanical stimuli: increased loading results in greater bone formation, whereas reduced loading results in bone loss. The ability of bone to sense and respond to mechanical stimuli depends on bone cells, including osteocytes, osteoblasts, and osteoclasts. Osteocytes have been proposed as candidate mechanosensors because of their location within the bone matrix. Osteocytes are housed in small pockets, or lacunae, which are connected by a network of channels, or canaliculi (Lanyon 1993). Osteocytes extend projections throughout this canalicular network and communicate with one another and with preosteoblasts on bone surfaces through gap junctions on these projections.

Osteocytes respond to mechanical stimuli both *in vivo* (Robling et al., 2006) and *in vitro* (Klein-Nulend et al., 1995). Osteoblasts and mesenchymal stem cells (MSCs), the main source of osteoblast progenitors, have also been shown to respond to mechanical stimuli and appear to play an important role in bone mechanotransduction (Reich and Frangos 1993; Meinel, Karageorgiou et al. 2004). However, the relative importance of different types of local, mechanical stimuli to which bone cells respond is unclear. One such mechanical stimulus is oscillatory fluid flow. During dynamic loading of the skeleton, bending moments are created, causing bone matrix deformation and intramedullary pressure gradients. These pressure gradients result in fluid flow through the canalicular network from regions of high pressure to regions of low pressure (Knothe Tate et al., 1998). Flow-induced osteocyte surface shear stress is estimated to be in the range of 8–30 dynes/cm^2 under normal loading conditions (Weinbaum et al., 1994). Fluid flow may also cause drag force on cell processes resulting in a hoop strain of >0.5% (Han et al., 2004); however, this model assumes that osteocyte processes are tethered to the lacunar wall, which has not been clearly established. Osteocytes may also experience cytoskeletal strain or de-
formation as the lacunar spaces in which they reside are deformed.

The mechanism by which bone cells sense and respond to these mechanical stimuli is not known, but recent evidence (Malone et al., 2007) demonstrates that primary cilia are mechanically sensitive in bone cells. Primary cilia are known to act as flow sensors in renal epithelial cells (Liu et al., 2005), in which bending of the cilium results in an increase of intracellular calcium (Schwartz et al., 1997). They have also been observed in osteocytes and osteoblasts (Matthews and Martin, 1971; Tonna and Lampen, 1972; Xiao et al., 2006) and suggested as potential bone mechanical sensors (Whitfield, 2003; Xiao et al., 2006). Primary cilia projecting from osteocytes might experience fluid flow shear stress, mechanical deformation, or pressure gradients depending on their size and orientation. Mechanical stimuli that could affect primary cilia on preosteoblasts are less well defined, as these cells reside on bone surfaces rather than within the bone matrix. Determining the size, orientation and properties of primary cilia on different bone cell types in situ will be important for understanding their possible roles in vivo. Interestingly, chondrocytes in cartilage also have primary cilia, which possess integrins in their membranes and contact the extracellular matrix (Jensen et al., 2004; McGlashan et al., 2006). Since osteocytes likely experience fluid flow in the confined matrix-filled spaces between the cell surface and the lacunar wall, cilium-matrix interactions might also play a role in mechanosensation in bone.

**PRIMARY CILIA AS CHEMOSENSORS IN BONE**

Primary cilia may also function as specialized regions of the cell membrane that are equipped with receptors that transmit chemical or molecular signals from the environment into the cell. One of the best known of these molecular signaling pathways is mediated by secreted growth factors in the Hedgehog family. Hedgehog signaling is critical throughout embryonic development and adulthood: disruption in Hedgehog signaling has been associated with multiple birth defects and human cancers (reviewed in Helms et al., 2007). Much of our current knowledge of Hedgehog signaling comes from studies conducted in the fruit fly, *Drosophila*. Collectively, these studies indicate that the Hedgehog receptor complex is composed of two trans-membrane proteins, Smoothened and Patched. In the absence of Hedgehog, Patched represses pathway activity by inhibiting the function of Smoothened, but when Hedgehog is present and binds to Patched, Smoothened is relieved from this inhibition and activates target gene transcription. It is thought that a similar mechanism is responsible for Hedgehog signaling in mammalian cells, where Shh is one of the Hedgehog family ligands.

Recent evidence from a number of laboratories demonstrates that Shh signaling occurs at the primary cilium (Huangfu et al., 2003; Rohatgi et al., 2007). These groups showed that in the absence of Shh, Patched occupies the primary cilium, which may exclude Smoothened from this structure. Because Patched functions as a Shh receptor, its presence makes the primary cilium a receptacle for any secreted Shh in the vicinity. When Shh binds to its receptor, Patched leaves the cilium and Smoothened instead accumulates in the ciliary membrane (Rohatgi et al., 2007). Mutations that affect formation of the primary cilium thus lead to disruption in Shh signaling.

The connection between Shh signaling and primary cilia in bone was first hinted at by the finding that mice with mutations in IFT components have skeletal patterning defects that are similar to Shh mutants (Huangfu et al., 2003; Zhang et al., 2003). Null mutation of IFT components results in embryonic lethality (Marszalek et al., 1999; Murcia et al., 2000), precluding studies of complete skeletogenesis in these mutants, but tissue-specific mutation of these same IFT components causes a more restricted range of embryonic defects. For example, mice lacking the kinesin-2 subunit Kif3a in retinal photoreceptors display abnormal accumulation of opsin in the inner segment, causing photoreceptor death (Marszalek et al., 1999), and elimination of the IFT component Ift88 (also known as polaris) in the central nervous system of mice causes cerebellar disruptions (Chizhikov et al., 2007). Likewise, loss of Ift88/polaris or Kif3a in the developing skeleton leads to a plethora of skeletal malformations (Haycraft et al., 2007; Koyama et al., 2007) for reasons that are not yet clear. Analysis of additional tissue- and cell type-specific knockouts in mice and other experimental animals should provide insight into how primary cilia function as molecular detectors in bone. It will be important to compare skeletal structure and growth dynamics in conditional mutants lacking components required for primary cilium structure (e.g., Kif3a) against those lacking specific ciliary signaling pathway components (e.g., Patched) to determine the contribution of each signaling pathway to overall primary cilium-mediated signaling during bone development and homeostasis.

**UNANSWERED QUESTIONS**

**How are Mechanical Signals Sensed by Primary cilia?**

As observed in time-lapse movies of cultured kidney tubule cells, primary cilia exposed to unidirectional fluid flow bend in the direction of flow. However, it is not known whether mechanosensitive proteins are distributed asymmetrically around the cilium, which would maximize the sensitivity to this bending. Although the primary cilium has been modeled as a cantilevered beam (Schwartz et al., 1997), the minimum degree to which it must be deflected in order to elicit a response is not known. Furthermore, it is not known whether the cilium functions as a switch or a rheostat: is there a threshold level of mechanostimulation that toggles an on–off signal from the cilium, or is the ciliary signaling output graded in proportion to the degree of mechanical stimulation? If the signaling activity is switch-like, there might also be a recalcitrant period immediately after activation during which the cilium cannot re-respond to stimulation. Combining high-resolution imaging of primary cilia and a signaling output (e.g. calcium flux) with careful control of the timing and strength of an experimental mechanical stimulus should allow definition of some of these parameters. In the case of bone cells, combining current models of fluid flow in bone with
these parameters would provide useful constraints on models for how primary cilia function in vivo.

Also unknown is the extent to which the cilium’s connection to cytoplasmic microtubules might play a role in its function as a mechanosensor. The only characterized mechanosensory mechanism in the cilium involves the polycystins, which form a stretch-sensitive membrane channel complex (Nauli et al., 2003), but as noted above, the basal body is directly connected to a subset of cytoplasmic microtubules via its appendages. These connections could serve to anchor the cilium in place and provide it with structural support, much like the roots of a tree support its trunk. However, they might also play a role in mechanical signaling; bending of the primary cilium could translate into movement of the attached microtubules, which could then lead to downstream signaling activity or changes in the transport of materials along the cytoplasmic microtubules. The cilium and the basal body could thus act as an extracellular extension of the cytoplasmic microtubule cytoskeleton and provide it with information about the extracellular environment, much as integrins link extracellular matrix proteins to the actin cytoskeleton. Because the microtubules of the primary cilium and centrioles are less sensitive to low concentrations of microtubule-destabilizing drugs, such as nocodazole, than cytoplasmic microtubules (unpublished observations), it should be possible to study the mechanosensitivity of the primary cilium in the presence or absence of cytoplasmic microtubules. Furthermore, imaging of cytoplasmic microtubules during primary cilium deformation may give insight into the strength and extent of the connections between these two microtubule systems.

Is there Feedback Between Mechanical Stimulus and Ciliary Structure?

In human umbilical cord cells, fluid flow has been shown to cause loss of primary cilia from the endothelial surface (Iomini et al., 2004). It is possible that mechanical stimuli could lead to remodeling of ciliary structure, either through changes in its length or membrane composition. As mentioned above, application of Shh to cells causes the relocalization of Smoothened to the primary cilium (Corbit et al., 2005; Rohatgi et al., 2007), indicating that trafficking of proteins to the cilium in response to external signals occurs. Analysis of primary cilium components before and after exposure to fluid flow or other chemical or molecular signals might uncover a mechanism by which bone cells remodel their primary cilia in response to mechanical or chemical stimulation.

What are the Connections Between Mechanical Stimuli and Cellular Behavior in Bone Cells?

In bone cells, primary cilia are required for cytokine release and changes in gene transcription in response to dynamic flow (Malone et al., 2007). However, the connections between mechanical stimulation of the cilium and cellular responses are not fully known. The best-studied case of a downstream signal resulting from mechanical stimulation of the primary cilium is that of calcium influx into kidney tubule cells after exposure to fluid flow, which leads to intracellular calcium release (Prætorius and Spring, 2001). Although calcium influx might also be involved in other settings, it is apparently only one of multiple mechanically activated second messenger systems in bone (Malone et al., 2007). There is growing evidence that the primary cilium can be coupled to other signaling pathways. Adenylyl cyclases have also been found in the primary cilium (Masyuk et al., 2006), suggesting that cAMP could be a second messenger in some forms of ciliary signaling. In addition, IPT could traffic activated signaling proteins from the ciliary compartment to the cytoplasm, as has been proposed for Shh signaling (May et al., 2005).

An important unanswered question is the extent to which primary cilium-mediated mechanosensation leads to changes in cellular communication and proliferation in bone. In kidney tubules, loss of primary cilia leads to overproliferation of tubule cells and the formation of cysts (Yoder et al., 2002), and primary cilia have been linked to cell cycle progression (Morgan et al., 2002; Pan and Snell, 2007). Although osteocytes themselves do not divide, it is possible that mechanical stimulation of osteocyte primary cilia could lead to cell proliferation at the bone surface, which would require cell–cell communication via the canalicular network. Conditional knockout of genes required for primary cilium in specific bone cell populations, which has already begun (Haycraft et al., 2007), should allow the dissection of primary cilium function in osteocytes, osteoblasts, and osteoclasts. An important caveat of these experiments is that primary cilia might play different signaling roles (chemosensory, mechanosensory, or both) depending on developmental stage, and the ability to control ciliogenesis temporally as well as in a tissue- or cell type-specific fashion would allow more detailed characterization of primary cilium function in bone.

The primary cilium has emerged as an important organelle for sensing environmental signals, and its function in diverse tissues is just beginning to be understood. One research goal that lies ahead is to integrate ciliary signaling with other cellular sensory systems to provide a comprehensive picture of how cells sense and respond to environmental stimuli, and bone, with its architectural complexity and plasticity, provides a challenging but exciting arena in which to accomplish it.

LITERATURE CITED

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