

THE PRIMARY CILIUM: A WELL-DESIGNED FLUID FLOW SENSOR

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The primary cilium is a highly specialized surface projection which extends from the apical surface of almost every vertebrate cell. After its initial discovery over 100 years ago, primary cilia were long overlooked and even purported by some to be extraneous genetic remnants from our evolutionary past. However, in the past decade, a wealth of evidence has begun to accumulate, indicating that cilia in various cell types act not only as mechanical and chemical sensors, but also play important roles in intracellular signaling and cell division¹. Some have even suggested that cilia-related dysfunction may have an important role in modern human epidemics such as obesity, hypertension and diabetes². One such link between cilia-related dysfunction and human disease that has been explored extensively involves the role of the primary cilia of renal epithelial cells as flow sensors. It is believed that a dysfunction in these cilia results in polycystic kidney disease (PKD), the most common inherited disease in the United States, with an estimated 600,000 current cases³. Numerous models have been proposed to explain the mechanotransduction mechanism which allows the primary cilia of renal epithelial cells to detect fluid flow, but many questions remain. Understanding the transduction mechanism and the features of the primary cilium which make it an ideal flow sensor will not only answer many interesting questions in biology and biomechanics, but could aid in the treatment of PKD and other diseases which are caused by cilia-related dysfunction.

INTRODUCTION TO THE PRIMARY CILIUM

The primary cilium is a long, cylindrical, microtubule-based structure which extends from the apical surface of most vertebrate cells, as shown in Figure 1. In general, cells only have a single primary cilium. Referred to as the axoneme, the main structural element of the primary cilium is a collection of nine circumferentially-arranged doublet microtubules encased by membrane continuous with the cell membrane¹. These doublet microtubules extend from a structure known as a basal body within the cell, which links the base of the primary cilium to the cytoskeleton. The basal body consists of nine triplet microtubules, and two of the microtubules of each triplet form the axoneme of the primary cilium¹. Further structural support is provided by the transitional fibers (alar shields), which add stability to the complex via attachment to the cell membrane⁴. In conjunction with a terminal plate at the end of the basal body, these transitional fibers also act as a protein filter, only allowing certain proteins to enter and exit the cilium⁵. At the far end of the cilium, the axoneme becomes more variable, but is typically composed of nine single microtubules¹. Although cilia are not isolated from the cell by a membrane, it seems reasonable to consider them to be organelles due to their unique structure, their extreme location past the cell periphery, and the selectivity to protein movement across their boundaries resulting from the transitional fibers and the terminal plate.

Depending on the species, primary cilia of renal epithelial cells typically vary between 2-20 μm in length *in vivo*⁶. However, lengths up to 30 μm have been observed *in vitro*⁴. In addition, studies involving mice renal epithelial cells measured primary cilia 2-3 μm long and 0.2 μm in diameter on average⁷. Since microtubules have an outer diameter of ~ 30 nm, this relatively small diameter indicates that nearly half the volume of a primary cilium is occupied by the microtubules alone⁸.

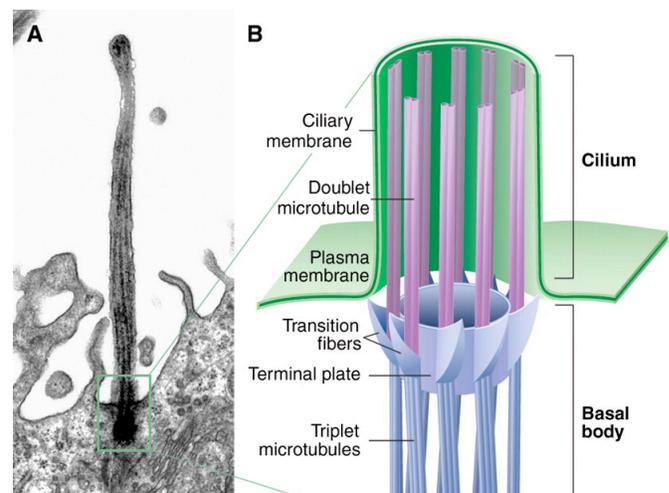


Fig. 1: Primary cilium structure. (A) Electron micrograph of the primary cilium of a canary brain radial glia. (B) Schematic showing structure of the basal body and primary cilium. Adapted from Singla *et al.* (2006)⁵.

Although primary cilia sometimes appear cylindrical, their tips often exhibit swelling consisting of an $\sim 0.5 \mu\text{m}$ spherical infolding of excess cell membrane⁷. As will be discussed, this unique structural feature actually serves a useful purpose.

Since primary cilia cannot produce the proteins needed for cilium assembly, maintenance, and function, the necessary proteins must be transported outwards from within the cell. This occurs via a transport system known as the intraciliary or IFT system, which has been described as macromolecular rafts traversing up and down the microtubules on the outside of the axoneme⁴. Molecular motors have been identified which carry proteins in anterograde motion towards the tip of the axoneme and in retrograde motion towards the base².

THE KIDNEY, RENAL EPITHELIAL CELLS, AND PKD

The kidneys play a crucial role in humans and many other organisms. Each about the size of a fist, they filter waste and extra fluids from the blood to form urine and also regulate a variety of vital substances in the body⁹. Approximately one million tiny nephrons, which intertwine with tiny blood vessel capillaries and urine-collecting tubules, remove waste from the blood and direct waste products and excess fluids into tiny collecting duct tubules which drain to the bladder⁹. As Figure 2 shows, each collecting duct tubule is lined by renal epithelial cells, most of which have a single primary cilium projecting into the flow. Due to the relatively low flow rates and small dimensions of the ducts, the Reynold's number associated with flow through the kidney tubules is extremely low ($Re < 2 \times 10^{-5}$), so laminar flow profiles are expected⁶.

Polycystic kidney disease (PKD) is a genetic disorder characterized by the growth of many fluid-filled cysts that grow over time and replace healthy kidney tissue⁹. The cysts reduce kidney function and can eventually lead to kidney failure, which is fatal without dialysis or kidney transplantation. All cases of the more common form of PKD, autosomal dominant PKD (ADPKD), are known to be caused by mutations in the genes PKD1 and PKD2, which encode the integral membrane proteins polycystin-1 (PC1) and polycystin-2 (PC2), respectively¹⁰.

Most current models of the genesis of PKD suggest that the inability of the epithelial cells to sense flow leads to a disruption of the Ca^{2+} metabolism, alterations to the



Fig. 2: Renal epithelial cells line the collecting duct tubules of the kidney. Each extends a single primary cilium into the duct. Adapted from Pan *et al.* (2005)³.

cellular responses to second messengers such as cAMP, and an imbalance between apoptosis and proliferation which leads to cyst formation³. Although not completely proven, it is believed that primary cilia are crucial to sensing flow in the kidney and that a cilia-related dysfunction is directly linked to the onset of PKD.

PRIMARY CILIUM MECHANICS

As discussed, the primary cilium consists of nine circumferentially-arranged doublet microtubules. Individual microtubules have been previously measured to have a bending stiffness of approximately $2.2 \times 10^{-23} \text{ Nm}^2$ on average⁸. Thus, one would expect a primary cilium, which consists of nine doublet microtubules spread from the central axis, to have a much higher bending stiffness. However, several studies have shown that this is not the case.

In a pioneering set of experiments by Schwartz *et al.*, primary cilia were subjected to a range of flow conditions commonly experienced within the kidney. An example of

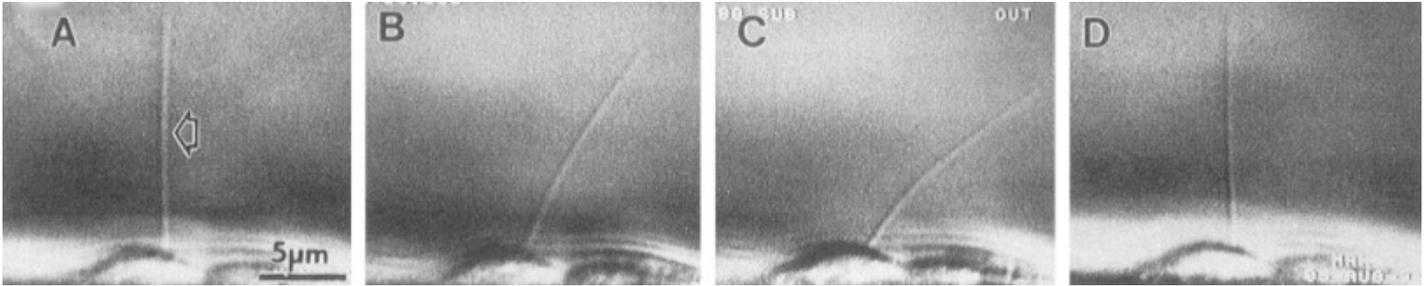


Fig. 3: Bending of a renal primary cilium in response to fluid flow. (A) Initial shape. (B) Low flow rate. (C) High flow rate. (D) After the flow is stopped. Adapted from Schwartz *et al.* (1997)⁶.

a renal primary cilium bending in response to fluid shear in their experiments is shown in Figure 3. The primary cilium was modeled as a uniform cylindrical cantilevered beam subjected to a unidirectional distributed load, which bends according to the beam bending equation

$$\frac{d^2\psi}{ds^2} + k^2 \cos(\psi) = 0$$

where ψ is the tangent angle at a distance s along the cilium and k is constant that depends on the shape and material properties of the cilium and the flow conditions⁶. In general, the cosine term cannot be approximated as 1 as in simple beam bending theory because primary cilia often exhibit large deflections of 15° or more.

Since the Reynold's number associated with flow within the kidney tubules is so small, the fluid drag per unit length of cilium, w , can be calculated from a standard formula for laminar flow around a cylindrical object as

$$w = \frac{4\pi\rho v^2 d}{\text{Re}[2.002 - \ln(\text{Re})]}$$

where d is the diameter of the cilium, v is the fluid velocity, and ρ is the fluid density⁶. Since the velocity increases with distance from the cell surface, as the length of a cilium increases, the total drag force, and resulting bending and associated stresses increase dramatically.

Several mathematical models were analyzed, but the "heavy elastica" theory, which solves for the tangent angle ψ by utilizing a Maclaurin series expansion, was found to model primary cilium bending in shear flow most accurately. Using this model, the bending stiffness of the primary cilium was calculated to be $1.4 - 1.6 \times 10^{-23} \text{ Nm}^2$

on average⁶. This is extremely surprising because it indicates that the primary cilium has a bending stiffness on the same order as that of a single microtubule. Some have suggested that if these values are both correct, then doublet microtubules may be more flexible than single microtubules, or cilia may have an active mechanism involving microtubule motors which gives the nine doublet microtubule structure more flexibility¹¹. This result also indicates that the primary cilium has likely adapted to exhibit large deflections, and hence large stresses and strains, over the range of flow conditions experienced in kidney tubules.

In addition to this surprisingly low stiffness, several other features also suggest that primary cilia were, in some sense, designed to be flow sensors. As discussed previously, the tip of the primary cilium consists of a spherical infolding of cell membrane approximately $0.5 \mu\text{m}$ in diameter. A recent study conducted by Resnick *et al.* modeled the primary cilium as a cylinder with a hemispherical cap and determined that more than 50% of the total drag force is exerted on the cap¹². Thus, bending of primary cilia in fluid flow is greatly increased by the presence of the relatively large spherical cap, which significantly increases the total drag without increasing the already low bending stiffness. By subjecting renal epithelial cells to orbital shaking, they also observed that primary cilia respond to forces as small as 5.2 fN ¹².

Since the total drag force on a primary cilium is approximately proportional to the square of the cilium length and the thermal noise is proportional to the inverse of the length, the signal to noise ratio is proportional to the length cubed¹². In other words, doubling the length of the primary cilium results in an 8-fold increase in signal to noise ratio. Therefore, the spherical cap, in conjunction with the long slender body, result in a primary cilium structure which is extremely sensitive to fluid flow.

FIRST STEPS AND TRANSDUCTION MODELS

When Schwartz *et al.* first measured the bending of primary cilia in shear flow, they proposed that primary cilia act as flow sensors and even discussed three possible mechanotransduction mechanisms to describe how the bending of primary cilia triggers a cellular response⁶. First, they hypothesized that stretch-activated ion channels on the convex side of bending cilium would experience tensile stress and could thus be activated. Second, they suggested that bending of the cilium could induce tensile stress in the membrane upstream of the cilium, thus opening stretch-activated ion channels. Finally, they proposed that if ion channels are not involved, direct transduction via the cytoskeleton could allow cells to respond to mechanical stress induced by cilium bending. In this case, the cilium would act as a biological “lever arm,” amplifying relatively small shear forces into large stresses in the cytoskeleton. More recent work has shown not only that primary cilia can sense fluid flow, but also that the mechanotransduction mechanism involves stretch-activated ion channels on the side of the cilium itself.

The fact that primary cilia respond to flow was first demonstrated by Praetorius and Spring¹³. Using MDCK cells, a commonly utilized cell line derived from the collecting duct of a canine kidney, they bent primary cilia by micropipette suction and by increasing the fluid flow rate, and then measured the intracellular Ca^{2+} concentration with a fluorescent marker. They showed that bending the cilium results in an influx of Ca^{2+} via mechanically-sensitive ion channels on the cilium or at its base and that this small initial rise in calcium triggers a larger increase by freeing Ca^{2+} from IP_3 -sensitive stores. Further, they noticed that the calcium signal spreads to other nearby cells via diffusion of an unknown second messenger and therefore hypothesized that flow sensing is coordinated throughout the tissue rather than an isolated, cell-by-cell occurrence. In a secondary study, Praetorius and Spring incubated mature, responsive cells in chloral hydrate to remove the primary cilia by disrupting the microtubules¹⁴. Cells with no primary cilium did not respond to increased fluid flow, which indicated that the primary cilium is crucial for the flow-induced Ca^{2+} response in MDCK cells and suggested that the mechanically-sensitive channels are on the cilium itself.

When Yoder *et al.* demonstrated that polycystin-1 (PC1) and polycystin-2 (PC2) localized to renal primary cilia with an immunofluorescence approach, the details of the mechanotransduction mechanism began to unravel¹⁵.

THE MECHANOTRANSDUCTION MECHANISM

PC1 and PC2, although both members of the polycystin family, have very different structures. PC1 consists of approximately 4,300 amino acids, a large extracellular domain, 11 transmembrane domains, and a small cytoplasmic domain with a coiled-coiled structure¹⁶. On the other hand, PC2 is much smaller with only 968 amino acids and six transmembrane domains and is a member of the transient receptor protein (TRP) superfamily^{16,17}. In renal epithelial cells, these two proteins interact to form a unique cation-permeable channel complex known as the polycystin-1/2 complex¹⁶. PC2 is believed to form the actual ion channel pore, while PC1 has been suggested to act as a mechano-fluid stress sensor which activates the tightly associated PC2 ion channel when applied stresses cause it to change conformation^{10,16}. This activation then allows for an influx of Ca^{2+} into the cilium followed by a larger cytosolic release of Ca^{2+} from IP_3 -sensitive stores, as discussed previously. In support of this theory, a recent set of experiments by Qian *et al.* showed that PC1 exhibits a dynamic extensibility, whereby its length changes via folding and unfolding of its extracellular immunoglobulin domains¹⁸. Interestingly, they also discovered that the domains require a range of unfolding forces with the weakest domains requiring ~50 pN and the strongest ~250 pN¹⁸. This mechanical hierarchy could play a unique role because it might allow cells to sense not only that fluid is flowing, but also the flow rate.

Although the exact details of the regulation of PC2 by PC1 are not yet fully understood, the calcium signal which results due to primary cilium bending likely allows renal epithelial cells to respond quickly and transiently to short-term changes in flow rate¹⁹. A mechanism to sense long-term changes has also been suggested based on the finding that flow cessation results in regulated proteolytic cleavage of the C-terminal tail of PC1 followed by translocation of the cleaved domain to the nucleus²⁰. This PC1 tail interacts with a transcription factor, STAT6, and its co-activator, P100. When flow is present, the Ca^{2+} concentration is high, and proteolysis of PC1 is inhibited, isolating STAT6 and P100 to the cilium, as shown in Figure 4⁵. However, when flow ceases for an extended period, the concentration of Ca^{2+} falls, and PC1 is cleaved. STAT6 and P100, still attached to the PC1 tail, then translocate to the nucleus, where they activate transcription⁵. Thus, it is likely that ADPKD results when this pathway is inappropriately activated, leading to uncontrolled cell proliferation and cyst formation^{5,20}.

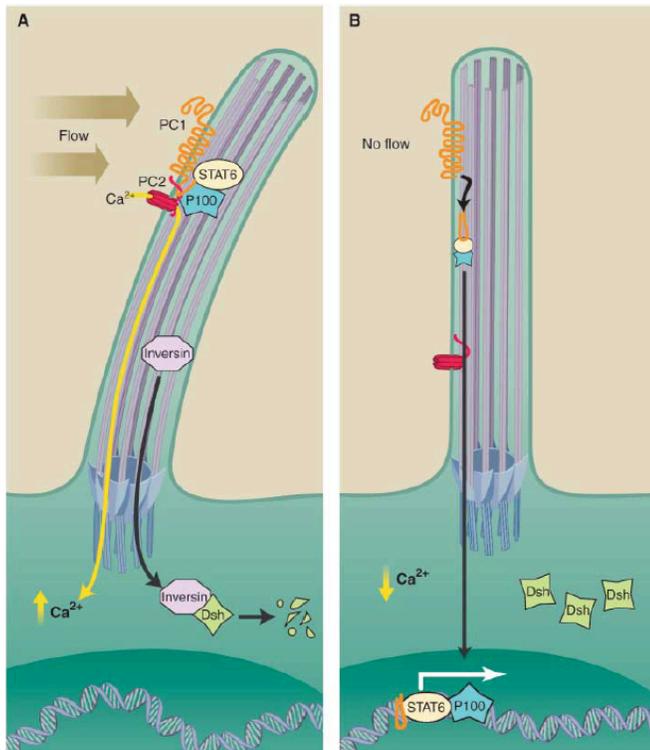


Fig. 4: Long-term sensing mechanism. (A) Regular flow results in a high Ca^{2+} concentration and inhibits proteolysis of PC1. (B) Flow cessation allows Ca^{2+} concentration to fall, resulting in proteolysis of PC1 and subsequent translocation of STAT6 and P100 to the nucleus. Adapted from Singla *et al.* (2006)⁵.

IMPORTANCE OF THE CYTOSKELETON

A recent set of interesting experiments performed by Alenghat *et al.* has shown that disturbing various cytoskeletal components can abolish the flow sensing capabilities of primary cilia²¹. They found that disordering microfilaments or microtubules, altering the cytoskeletal force balance by disrupting tension generation via actomyosin, or upsetting basal extracellular matrix adhesions prevented renal epithelial cells from exhibiting a calcium response to fluid flow. These results suggest that although the mechanism for flow sensing chiefly involves the primary cilium and the associated polycystin-1/2 complex, the cytoskeleton also plays a crucial role. Without a fully functional cytoskeleton, the primary cilium cannot properly deflect in response to fluid flow, which prevents the necessary stresses for mechanotransduction to be developed. This is likely due to the fact that the cytoskeleton anchors the cell and maintains cell structure under applied loading; without it, forces applied to the cilium result in large-scale cell deformation rather than localized cilium bending.

CONCLUSION

The primary cilium has numerous roles in many different parts of the body. In the kidneys, it protrudes from renal epithelial cells and acts as a flow sensor by bending under drag forces and allowing an influx of Ca^{2+} ions to enter the cell via a cation channel in PC2. Numerous features of the cilium, including its bending stiffness, shape, and location make it ideally suited for this purpose. Although several questions remain, it is clear that the primary cilium plays a crucial role as a flow sensor in the kidneys and most likely other organs. When it fails to function properly, health conditions such as polycystic kidney disease, among others, can result. Hopefully by gaining a complete understanding of this complex organelle, new treatment methods or artificial flow sensing devices can be developed which will aid in the treatment of PKD and other conditions which result when these tiny protrusions lose their normal function.

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