

Chapman University Chapman University Digital Commons

Pharmacy Faculty Articles and Research

School of Pharmacy

2014


Sensing a Sensor: Identifying the Mechanosensory Function of Primary Cilia

Rahul M. Prasad
University of Kentucky

Xingjian Jin
University of Toledo

Surya M. Nauli
Chapman University, nauli@chapman.edu

Follow this and additional works at: http://digitalcommons.chapman.edu/pharmacy_articles

 Part of the [Cells Commons](#), and the [Sense Organs Commons](#)

Recommended Citation

Prasad RM, Jin X, Nauli SM. Sensing a Sensor: Identifying the Mechanosensory Function of Primary Cilia. *Biosensors*, 2014, 4(1), 47-62.
DOI: 10.3390/bios4010047

This Article is brought to you for free and open access by the School of Pharmacy at Chapman University Digital Commons. It has been accepted for inclusion in Pharmacy Faculty Articles and Research by an authorized administrator of Chapman University Digital Commons. For more information, please contact laughtin@chapman.edu.

Sensing a Sensor: Identifying the Mechanosensory Function of Primary Cilia

Comments

This article was originally published in *Biosensors*, volume 4, issue 1, in 2014. DOI: [10.3390/bios4010047](https://doi.org/10.3390/bios4010047)

Creative Commons License



This work is licensed under a [Creative Commons Attribution 3.0 License](https://creativecommons.org/licenses/by/3.0/).

Review

Sensing a Sensor: Identifying the Mechanosensory Function of Primary Cilia

Rahul M. Prasad ^{1,2}, Xingjian Jin ^{1,2} and Surya M. Nauli ^{1,2,*}

¹ College of Pharmacy and Pharmaceutical Sciences, The University of Toledo, Toledo, OH 43614, USA; E-Mails: rprasad@rockets.utoledo.edu (R.M.P.); xingjian.jin@rockets.utoledo.edu (X.J.)

² College of Medicine and Life Science, The University of Toledo, Toledo, OH 43614, USA

* Author to whom correspondence should be addressed; E-Mail: surya.nauli@utoledo.edu; Tel.: +1-419-383-1910; Fax: +1-419-383-1909.

Received: 3 January 2014; in revised form: 24 February 2014 / Accepted: 7 March 2014 /

Published: 13 March 2014

Abstract: Over the past decade, primary cilia have emerged as the premier means by which cells sense and transduce mechanical stimuli. Primary cilia are sensory organelles that have been shown to be vitally involved in the mechanosensation of urine in the renal nephron, bile in the hepatic biliary system, digestive fluid in the pancreatic duct, dentin in dental pulp, lacunocanalicular fluid in bone and cartilage, and blood in vasculature. The prevalence of primary cilia among mammalian cell types is matched by the tremendously varied disease states caused by both structural and functional defects in cilia. In the process of delineating the mechanisms behind these disease states, calcium fluorimetry has been widely utilized as a means of quantifying ciliary function to both fluid flow and pharmacological agents. In this review, we will discuss the approaches used in associating calcium levels to cilia function.

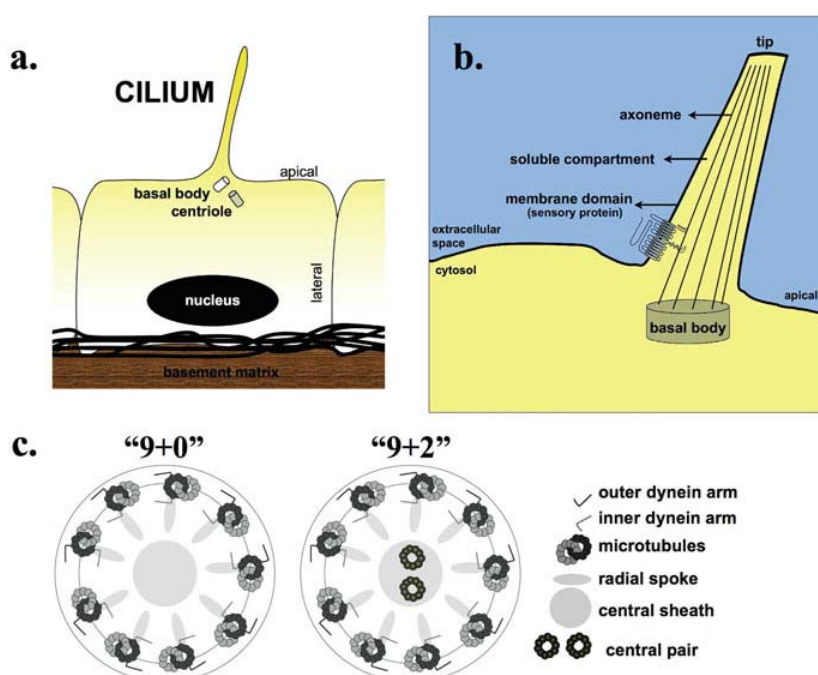
Keywords: cilia; calcium signaling; shear stress; cell culture; tissue; perfusion

1. Introduction

Once enigmatic organelles, cilia have become a growing field of biomedical research. Cilia are often described based upon the structural arrangement of the microtubules of which they are composed, with the two types of classifications being “9+0” and “9+2”. In addition to the structure of cilia, the ability for independent movement or motility is another characteristic by which cilia are

classified, with those capable of this function being termed “motile” and those incapable termed “non-motile.” Non-motile cilia with a 9+0 microtubule arrangement are often referred to as primary cilia (Figure 1).

Figure 1. Structure of cilia. (a) Cilia are cellular organelles composed of microtubules, and they project from the apical surface of numerous cell types. (b) A cilium is composed of a membrane domain, a soluble compartment or cilioplasm, an axoneme and a basal body. The membrane domain contains multiple sensory and channel proteins, several of which play a role in calcium-mediated fluid-flow mechanosensation. (c) The orientation of the microtubules in the axoneme is categorized as “9+0” or “9+2” and is anchored to the basal body. Taken and adapted from [1].



First described in 1898, primary cilia are found in a diverse group of cell types [2]. Over the past twenty years, cilia have emerged as ubiquitous organelles with a profound physiological impact. Indeed the growing list of disease states caused by ciliary defects has merited the use of the comprehensive term, “ciliopathies” (Table 1).

Paralleling our understanding of the importance of cilia, delineating the specific functions of cilia has begun to gain momentum. Through a variety of functional assays, the primary cilium has been identified as a mechanical and chemical antenna that serves to translate extracellular stimulations to intracellular signals [3]. The mechanosensory function of cilia has been shown in renal epithelia, vascular endothelia, nodal cells, hepatocytes, pancreatic epithelial cells, osteocytes, chondrocytes, and odontoblasts [4–11].

During the sensing process, calcium plays an important role as a second messenger. This central paradigm has been developed by the findings of many independent groups showing that when challenged by cilia, specific mechano- or chemo-stimulations, an influx of intracellular calcium is often observed. This intracellular calcium-level fluctuation can be abolished in cells with dysfunctional primary cilia, and ultimately giving rise to ciliopathies. It is also noteworthy that primary cilia are

loaded with a variety of calcium ion permeable channels, many of which, if mutated, could lead to abnormal intracellular calcium concentrations and subsequent cellular catastrophes. In this review, we discuss the three experimental approaches to observing and quantifying ciliary fluid-flow sensation: cell culture, single-cell, and *ex vivo* tissue perfusion assays.

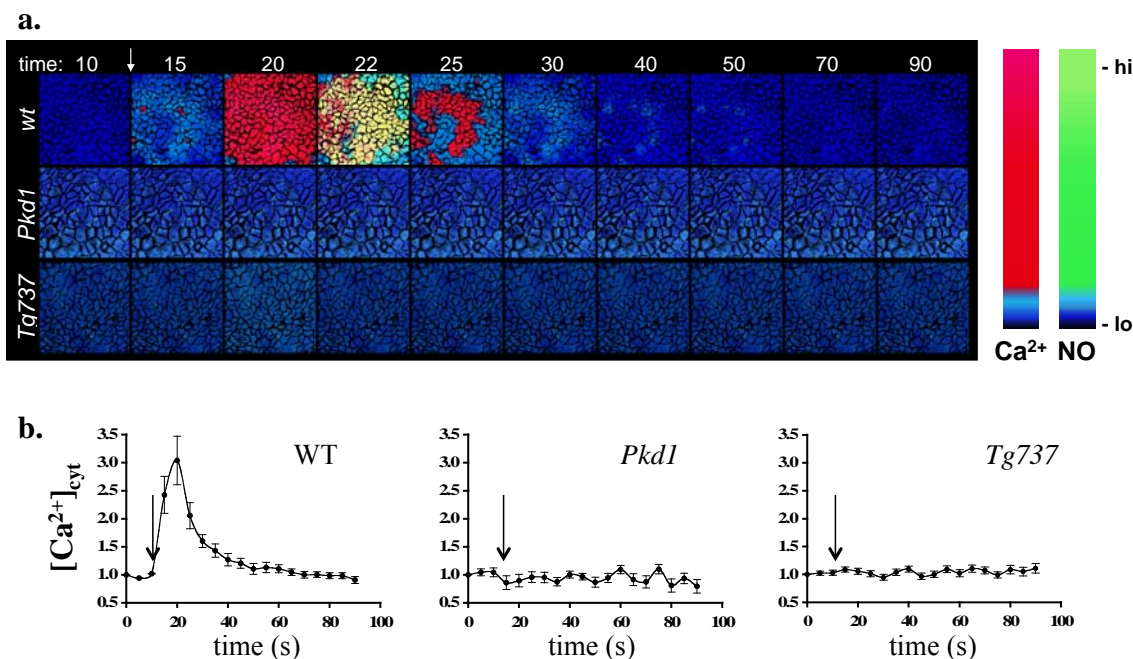
Table 1. Ciliary classification, function and disease relevance in mammals. Taken and modified with permission from [1].

Axoneme	Motility	Function	Disease Relevance	Reference
“9+0”	Motile	Generation of nodal flow	Situs inversus; Situs ambiguous; Situs isomerism	[12–14]
	Non-motile	Sensation of nodal flow	Situs inversus; Situs ambiguous; Situs isomerism	[4,15]
		Mechanosensor	Polycystic Kidney, Liver, and Pancreas Diseases	[5,16,17]
		Shear stress sensor	Hypertension; Atherosclerosis; Aneurysm formation	[6,7,18,19]
		Osmolarity sensor	Respiratory diseases; Infertility	[20,21]
		Gravitational sensor	Osteoporosis; Chondroporosis	[22–24]
		Olfaction sensor	Anosmia; Hyposmia	[25,26]
		Light sensor	Retinitis pigmentosa; Blindness	[27–29]
		Chemosensor	Nephrocystin; Diabetes; Obesity	[30–32]
		Neurotransmitter sensor	Impaired brain plasticity	[33]
		Developmental regulator	Developmental defects; Cancer	[34–36]
		Pressure sensor	Bone maintenance, development	[22,37,38]
“9+2”	Motile	Chemosensor	Chronic obstructive pulmonary disease (COPD)	[39]
		Airway remodeling	Bronchiectasis; Hyperreactive airways	[40]
		Fluid Clearance	Chronic obstructive pulmonary disease (COPD)	[41–43]
	Non-motile	Oocyte Transport	Infertility	[44,45]
		Sperm Motility	Infertility	[46–48]
		Fluid Transport	Hydrocephalus; Cell Migration	[49–51]

2. Cell Culture Assay

Due to their widespread use, *in vitro* cell culture studies have provided impressive insight into the ciliary mechanosensation of fluid flow. The approach to conducting fluid flow cell culture assays is for the most part conserved: upon growing the cell types on a cytologically conducive surface, the cultured cell layers can be treated with a parallel fluid flow. The resulting shear-stress forces induce a rise in intracellular Ca^{2+} that can either be observed through transgenic calcium markers or fluorescent calcium dyes (Figure 2). In the case of fluorescent calcium dyes, the cell cultures must be incubated with the dye prior to induction of flow.

Figure 2. Calcium response to fluid flow in cell cultures. (a) The induction of fluid flow causes a rise in calcium in cells (pseudocolored). (b) In transgenic cell lines with knocked-out *Pkd1* or *Tg737*, both of which are ciliary proteins, calcium response is lost. The cells were incubated with fluorescent calcium dyes. Blue represents a low level; red denotes a higher level of calcium. Taken and adapted from [7].



In line with this overall approach, Nauli *et al.* details an experimental setup for growing cell monolayers, delivering fluid-shear stress, and observing the subsequent responses [52]. In the procedure, the authors describe several steps that are essential to the cell culture assay. The first such step is the presence of primary cilia to be induced and verified in the cell culture monolayer. As the primary cilia are only present in differentiated cells, one approach to inducing ciliation in, for example, kidney epithelial cells, is to grow cells overnight in normal serum and then in reduced serum (0.5%) for 48 h [52]. In addition to kidney epithelial and endothelial cells, other cell types can be utilized including embryonic cells, hepatocytes, pancreatic epithelial cells, osteocytes, chondrocytes, and odontoblasts [4–11].

In cell lines lacking transgenic calcium indicators, some of which have been established specifically for the ciliary domain, incubation with a fluorescent dye allows for visualization and quantification of ciliary function [53]. While the standard dyes utilized are Fura-2AM or Fluo-2, other conventional calcium indicators that are described above can be used. Following incubation with the calcium indicator, the cell culture must be transferred to a flow-delivering apparatus that is positioned with a computer and fluorescent microscope. Through the use of imaging software, such as MetaMorph, image capture can be used to analyze changes in fluorescence before, during and after perfusion of the cell culture monolayer. The calcium response profile produced as a result of flow is distinctive. Within renal epithelial and endothelial cell types, shear stress has been shown to cause a transient calcium influx after 5 seconds [6,7,16,54]. However, the peaks of calcium in response to shear stress could extend to 10–20 s, as has been seen by independent laboratories [55–57].

3. Single Cell Assays

It has long been speculated that the primary cilium itself functions as an independent calcium signaling compartment that might initiate a series of downstream events. To test this hypothesis, it becomes critical to monitor calcium signaling in a single cell and cilium to achieve optimal resolution. However, such a signal cell assay is very difficult to conduct, owing to the submicron diameter and flexible nature of cilia. Therefore, it is only until recently that several independent research groups reported their observations about calcium signaling within an individual cilium.

In contrast to cell culture assays, which use fluorescent calcium dyes, the majority of single-cell studies have utilized genetics to construct genetically encoded calcium indicators (GECI) that are specific to the primary cilium of the cell. By linking such indicators to a guide sequence that targets the primary cilium, researchers are able to dissect the calcium signals within an individual cilium from that of cytoplasm. Su *et al.* conducted a well-designed comparison experiment wherein several different GECI are fused with different cilia targeting sequences [58]. Parameters such as targeting efficiency, calcium signal dynamic range and cellular toxicity are carefully measured (Table 2).

Table 2. Comparison of genetically engineered calcium indicators. Data from Su *et al.* [58].

Fluorescent Indicator	Targeting Efficiency	Cytosolic Signal Dynamic Range (Maximum Percent Change in Fluorescence or FRET [SEM])	Ciliary Signal Dynamic Range (Maximum Percent Change in Fluorescence or FRET [SEM])
GFP	0%	-	-
5HT ₆ -GFP	90%	-	-
5HT ₆ -GCaMP5G	0%	65.1% [7.9%]	
5HT ₆ -YC3.60	80%	58.2% [11.9%]	74.6% [8.8%]
5HT ₆ -G-GECO1.0	75%	135.1% [42.4%]	360.0% [62.1%]
IA-GECO1.0	65%	175.6% [42.3%]	443.1 [39.5%]

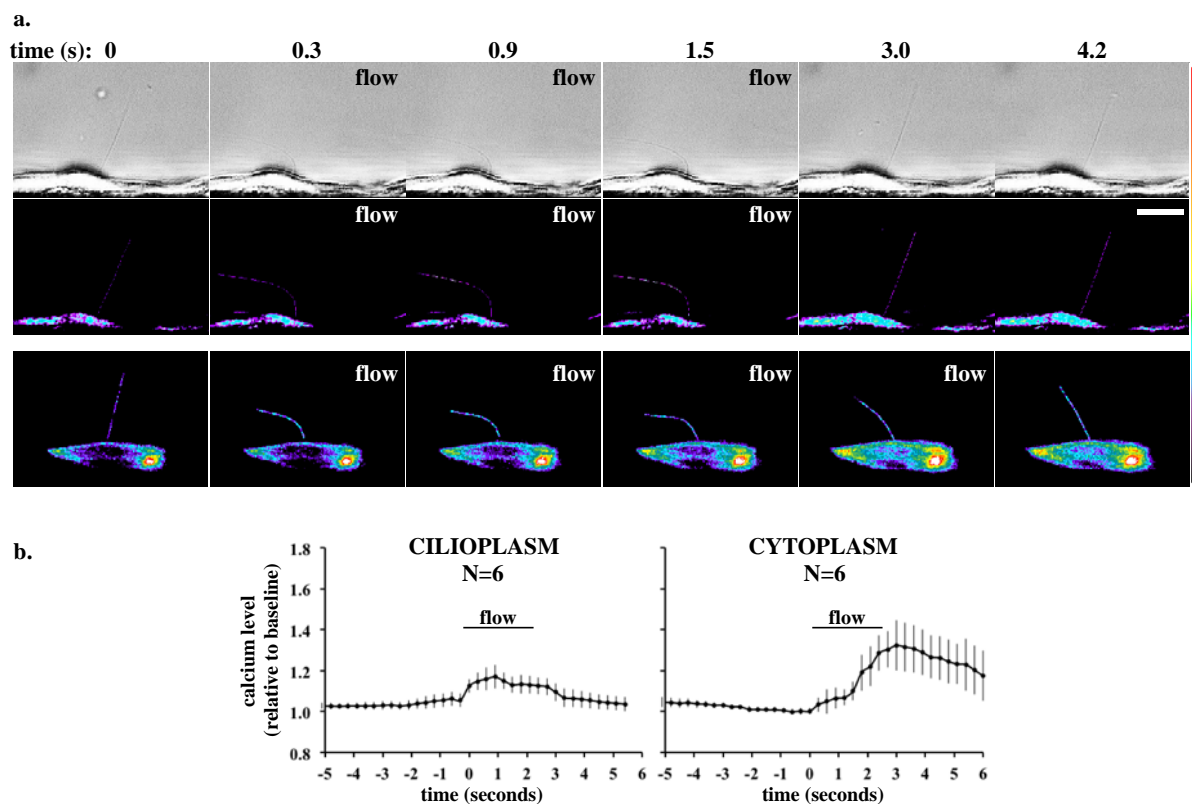
This screening allows researchers to select a combination of GECI and cilia targeting sequences that can produce the highest signal definition and minimal cilia growth interferences. Ionomycin, ATP and fluid flow were applied to cells transfected with optimal GECI construct, and the subsequent responses were monitored and recorded by a confocal microscope system. In regard to fluid flow, a series of z-stacking frames with a thickness of about 2 microns were continuously acquired along the base of a cilium. Once the cilium was bent, the whole image of a cilium could be captured. Also, the raw calcium signal readouts were normalized by an endogenous non-GECI fluorescent signal, such as mCherry, so that signal changes due to focal plane alternation could be eliminated. They reported cilia-specific calcium responses following fluid flow, echoing many previous reports that considered fluid flow as a cilia-specific stimulation.

Similar to this investigation, Markus Delling *et al.* also studied cilia-specific calcium signaling using GECI and a confocal microscope [53]. Differently, they challenged the cilia with a laser pulse that could rupture the ciliary membrane and induce calcium influx localized to cilia specifically. It is very interesting to note that the calcium fluctuation following the rupture didn't seem to be transduced to cytoplasm. Such highly localized calcium signaling was also related to the hedgehog pathway. This finding is different than some other reports that proposed a calcium-induced calcium release response

that relayed ciliary calcium signaling to that of cytoplasm. In addition, Markus Delling *et al.* investigated the identity of calcium channels in an accompanying report [59]. Their finds show that the PKD1L1-PKD2L1 heteromeric channel establishes the cilium as a unique calcium compartment that modulates established hedgehog pathways. An important point is that this study did not investigate cilia response to fluid flow, which is thought to be the most physiologically significant stimulation to primary cilia.

Xingjian Jin *et al.* utilized GECI in their study but, different from the aforementioned studies, they made use of a novel cell culture system [60]. Cells that carry GECI construct were cultured on a glass coverslip-based flexible substrate. When cells reached 100% confluence and were serum starved overnight, the substrate was peeled off the coverslip and placed diagonally on a string of microwire. This microwire coated with the substrate was then transferred to a regular fluorescent microscope equipped with cell culture chamber and special prism. By focusing on the edge of microwire, they were able to obtain a side view of cells growing on the substrate (Figure 3).

Figure 3. Ciliary calcium response to fluid flow in a single cell. (a) The calcium responses within the ciliary and cytoplasmic compartments of cells (pseudocolored) can be observed utilizing fluorescent calcium dyes or cell lines containing transgenic calcium indicators. (b) The induction of fluid flow causes bending of the primary cilium and a subsequent influx of calcium, first through the cilioplasm and then into the cell body. Color bar indicates calcium level, where black–purple and yellow–red colors represent low and high calcium levels, respectively. Bar = 4 μm. Taken and adapted from [60].



One major advantage of this method compared to the previous studies is that the whole cilium always remained in focus, with no cytoplasmic background, which made it much easier and more

accurate to differentiate calcium responses of cilium and those of cytoplasm. Using these systems, the authors showed cilia calcium responses to stimulations including fluid flow, fenoldopam and ionomycin. By applying different inhibitions, two calcium channels, Polycystin-2 (PC2) and L-type calcium channel (Cav 1.2), were found to be responsible for ciliary calcium responses. Intriguingly, calcium signaling initiated by these two channels seemed to have different fates, the former transduced to cytoplasm and the latter confined within cilium. Such differences, along with the findings made by Markus Delling *et al.*, suggest that the calcium transduction from cilium to cytoplasm could be controlled by an unknown machinery that can switch on and off the calcium transfer between cilium and cytoplasm in response to different initial stimulations [53].

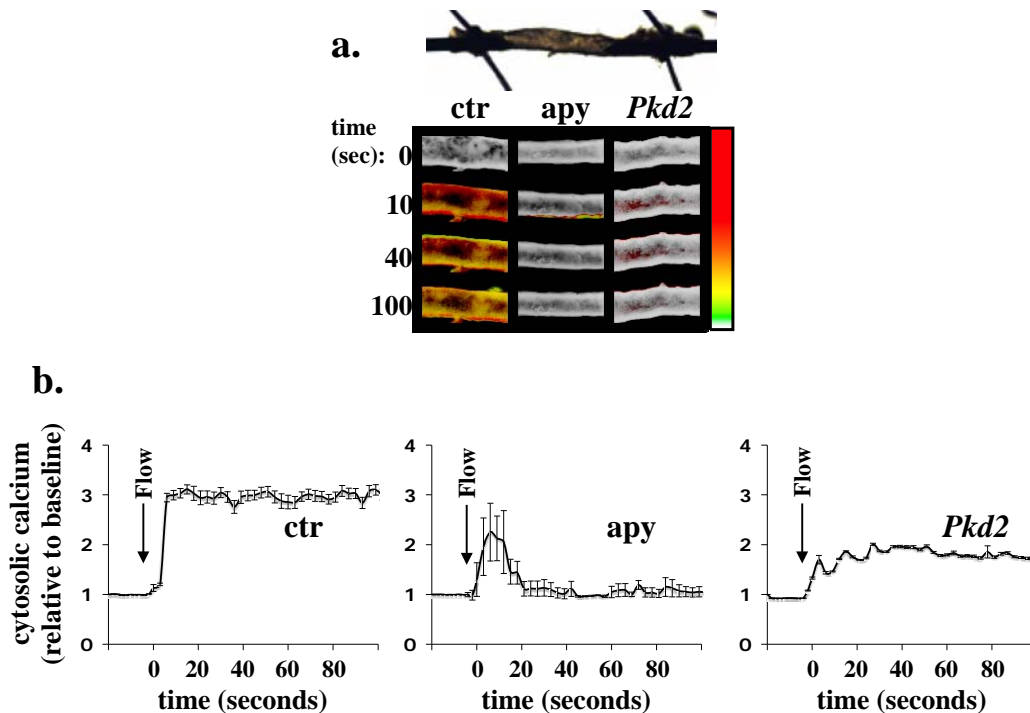
Although a major breakthrough in single cilium calcium assay has been made the past year, there are still technical issues remaining to be solved. Firstly, all the GECIs utilized in the above studies were not ratiometric or semi-ratiometric. They are more comparable to Fluo-4 than Fura-2. Therefore, inaccuracies caused by random and uneven distribution of GECIs are inevitable. Secondly, the optimal expression levels of GECIs were not quantified in these studies, which might make it hard to reproduce such assays under other conditions. Thirdly, as for cell types that have very short primary cilia, such as osteoblasts and endothelial cells, it is still extremely difficult to visualize calcium signaling in their cilia. Considering how important ciliary calcium signaling is to these cell types, it becomes very urgent to develop a GECI construct that appears brighter at the baseline level combining with an imaging system that enables higher resolution. It is worth mentioning that the most direct way to study cilia as mechanosensory organelles is arguably by direct bending with a micropipette, an original technique introduced by Praetorius and Spring [61]. Despite the technical obstacles that remain to be solved, new methods that have enabled researchers to explore the significant and novel role of primary cilia as independent calcium signaling compartments are still exciting. Nevertheless, the discussed progress should serve as the significant first step to revealing more important roles of primary cilia in calcium signaling and related human diseases.

4. *Ex Vivo* Assays

Analyzing tissue responses *ex vivo* is a powerful tool in overcoming the limitations of the cell culture approach to describing ciliary mechanosensation. Cell culture studies have provided numerous advancements in delineating the signaling pathways and mechanisms of *ciliary* fluid flow sensation. However, the nature of *in vitro* cell culture prevents a direct application of novel findings to physiologic models. *Ex vivo* studies provide an important bridge between applying cell culture findings in a more controlled, consistent, and efficient system than is possible with *in vivo* studies. The result is an approach that can be best utilized for confirming cell culture findings in a more physiological setting as well as for conducting high-throughput screens for pharmacological agents (Figure 4).

Observing ciliary mechanosensation *ex vivo* follows three experimental phases: tissue collection, preparation of tissue and flow system, and flow induction. While a variety of biological buffers have been utilized in perfusion systems, the selected buffer must be of constant pH, electrolyte, and O₂/CO₂ concentrations in order to ensure tissue viability. The fluid-flow assays have been developed for skeletal, renal, and cardiovascular tissues [62,63].

Figure 4. Calcium response to fluid flow in *ex vivo* arteries. (a) Within *ex vivo* arteries, fluid flow causes an influx of calcium. (b) In tissues with knocked-down *Pkd2*, a ciliary calcium channel, or that are treated with apyrase, the response profile is changed. Tissues were incubated with a fluorescent calcium dye prior to perfusion. Change in cytosolic calcium was pseudocolored; white/green represents a low level of cytosolic calcium, and yellow/red denotes a higher level. Taken and adapted from [6].



Ishihara *et al.* describe a method for observing shear stress-induced calcium within bone tissue. Utilizing embryonic chick tissue, they prepared bone fragments by first stripping away the periosteal layer and then trimming samples into 2×2 mm pieces, with a thickness between 60 to 80 μm . As with cell culture assays involving cells that lack a transgenic calcium marker, the tissue samples were incubated with fluorescent calcium dyes, such as fluo-8 and fura-2, in order to visualize intracellular calcium responses. The flow system utilized by Ishihara *et al.* uses capillary diffusion to apply fluid flow over the tissue sample [64]. The tissue is first placed on a glass slide that is held in place using adhesive grease, and the fluid flow is induced by adding a drop of solution to one side of the slide and suctioning the fluid from the opposite side using a piece of filter paper. As with all fluid flow calcium fluorimetry methods, a baseline is acquired prior to induction of flow. The osteocytes investigated by the group were identified within the tissue utilizing a three-dimensional analysis of the tissue layers [65]. Although Ishihara *et al.* do not specifically investigate primary cilia, presence and function of cilia have been identified in osteocytes [22,37].

The role of cilia in the pathogenesis of renal disease, namely polycystic kidney disease, emphasizes the need for applying cell culture findings to physiological systems in order to realize the potential for drug development. Liu *et al.* utilized *ex vivo* tubules from cortical collecting ducts in rabbits to characterize the function of cilia and, in the process, further consolidated paradigms of cilia as fluid flow sensors in the renal system [55]. Freehand isolation of the tubules was conducted, followed by microperfusion. The perfusion system involved cannulation of the isolated tubule, positioning of the

tubule on the Cell-Tak painted coverslip within a specimen chamber filled with incubation media and, finally, perfusion of media via syringe pump [66]. As with previously mentioned methods, fluorescent calcium dyes were utilized to observe the intracellular responses. In contrast to incubating the tissue prior to flow system setup, as in Ishihara *et al.*, the calcium dye was added to the incubating media, followed by an incubation period prior to flow induction. In addition to perfusion of intact tubules, Liu *et al.* perfused tubules that had been cut longitudinally, thereby exposing the lumen, as well as intact tubules with occluded lumens. Their findings showed that cell types within the opened tissue had a different calcium response to intact tubules or occluded lumens, explained by the lack of circumferential stretch and subsequent signaling response when the lumen is opened and exposed [55]. The effect of circumferential stretch is an example of a nuance within the physiological system that is neglected in cell culture experiments. Worth mentioning is that fluid perfusion in renal tubules can also regulate acid–base balance in the kidney [67]. In addition, the primary cilia in macula densa cells within the cortical thick ascending limb can detect tubular fluid-flow rate, which can alter the glomerular filtration rate via the tubuloglomerular feedback pathway [68].

Studies on elicited calcium responses have been conducted not only in tubular renal tissues but also in vascular tissues. Abou-Alawi *et al.* conducted studies on *ex vivo* mouse aortae, simulating the effects of blood flow [6]. Similar to the studies conducted by Liu *et al.*, the dissected tissue was cannulated in a bath of media and then perfused with media. In contrast to other studies, the tissue was incubated in a solution containing the fluorescent calcium dye before cannulation and perfusion. Although the calcium response profiles in the *ex vivo* tissues were not identical to cell culture studies, reflecting the complexity of physiologic factors that limit cell culture studies, the knockdown of the ciliary calcium channel *Pkd2* did inhibit the response profile as it did in cell culture studies (Figure 4). The ability to reinforce novel cell culture findings using the *ex vivo* system, coupled with defined responses through calcium fluorimetry, is an integral step in the process of contributing to physiological models and developing clinical treatments for disease states.

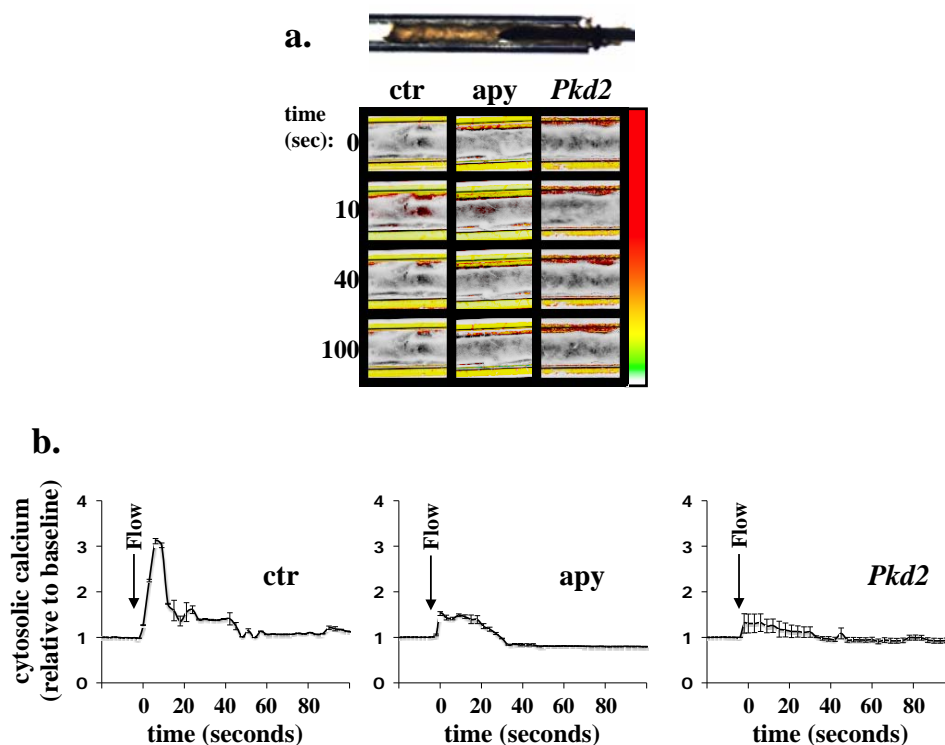
As in the renal tubule, fluid flow can cause vessel distension, and this may not reflect a function of the primary cilia. Furthermore, the calcium profile is substantially different between the control vessel and the vessel treated with apyrase which is an enzyme that hydrolyzes any nucleoside triphosphates or diphosphates (such as ATP and ADP). It is very possible that the vessel distention induces secretion of ATP or ADP. In particular, it has been shown that ATP can increase calcium in the cilioplasm [58]. Thus, the vessel distention and the presence of ATP could undermine the strategy to study cilia function.

To avoid these issues with vessel distention and ATP, AbouAlaiwi *et al.* have developed a technique to insert a vessel into a glass capillary tube (Figure 5). The basis of the approach is that the vessel inside the capillary tube would have no or very limited room for distention or expanding due to the perfusate pressure [6]. The calcium profiles between freely placed and capillary-enclosed vessels are indeed very different. Because the calcium profile of the capillary-enclosed artery represents a much closer resemblance to the profile of a cell culture, we believe the capillary-enclosed approach should be used to study mechanosensory cilia function.

Another exciting *ex vivo* assay to study cilia function is the embryonic nodal system [4,69,70]. Primitive streak and pit are formed during embryogenesis, where the upper layer of embryonic cells invaginates. The *Hensen's node* located at the end of the primitive streak is most likely the earliest role of primary cilia that is currently known. This *ex vivo* model has allowed us to study different functions

of primary cilia in sensing fluid shear stress and detecting chemical gradient. Responding to the nodal flow or chemical gradient, only the left-side margin of the node would show an increase in intracellular calcium, an early contributing factor to determining left–right body asymmetry. Thus, regardless of the mechano- and/or chemo-sensory roles of primary cilia, cytosolic calcium is an important indicator to validate cilia function within the *Hensen's node*.

Figure 5. Calcium response to fluid flow in capillary-enclosed arteries *ex vivo*. (a) Within the capillary-enclosed arteries *ex vivo*, fluid flow causes an influx of calcium. (b) In tissues with knocked-down *Pkd2* or in tissues treated with apyrase, the response profile is changed. Tissues were incubated with a fluorescent calcium dye prior to perfusion. Change in cytosolic calcium was pseudocolored; white/green represents a low level, and yellow/red denotes a higher level of cytosolic calcium. Taken and adapted from [6].



Despite the fact that calcium has been shown to play an essential role as a second messenger to transmit the extracellular signal into an intracellular biochemical reaction, it is also important to mention that some studies have indicated that calcium might not be involved in cellular mechanosensory transduction [22,71,72]. There is no doubt that the discrepancy in this area requires more research, through a better, more sensitive and innovative technology. However, the idea of primary cilia as sensory organelles seems to be generally accepted. Perhaps an *in vivo* system would help to provide a better understanding of the mechanosensory function of primary cilia in the near future.

Acknowledgements

This work was supported by the National Institute of Health DK080640 and the Department of Defense office of the Congressionally Directed Medical Research Programs PR130153. Authors thank Charisse Montgomery for her editing assistance.

Author Contributions

RMP drafted and wrote the manuscript; XJ provided advice on experimental techniques; SMN drafted and revised the manuscript.

Conflicts of Interest

The authors claim no competing financial interests.

References

1. Nauli, S.M.; Haymour, H.S.; Aboualaiwi, W.A.; Lo, S.T.; Nauli, A.M. Primary cilia are mechanosensory organelles in vestibular tissues. In *Mechanosensitivity and Mechanotransduction*; Kamkin, A., Kiseleva, I., Eds.; Springer Science+Buisness Media: New York, NY, USA, 2011; pp. 317–350.
2. Wheatley, D.N. Landmarks in the first hundred years of primary (9+0) cilium research. *Cell Biol. Int.* **2005**, *29*, 333–339.
3. Muhammad, H.; Rais, Y.; Miosge, N.; Ornan, E.M. The primary cilium as a dual sensor of mechanochemical signals in chondrocytes. *Cell. Mol. Life Sci.* **2012**, *69*, 2101–2107.
4. McGrath, J.; Somlo, S.; Makova, S.; Tian, X.; Brueckner, M. Two populations of node monocilia initiate left-right asymmetry in the mouse. *Cell* **2003**, *114*, 61–73.
5. Masyuk, A.I.; Masyuk, T.V.; Splinter, P.L.; Huang, B.Q.; Stroope, A.J.; LaRusso, N.F. Cholangiocyte cilia detect changes in luminal fluid flow and transmit them into intracellular Ca²⁺ and camp signaling. *Gastroenterology* **2006**, *131*, 911–920.
6. AbouAlaiwi, W.A.; Takahashi, M.; Mell, B.R.; Jones, T.J.; Ratnam, S.; Kolb, R.J.; Nauli, S.M. Ciliary polycystin-2 is a mechanosensitive calcium channel involved in nitric oxide signaling cascades. *Circ. Res.* **2009**, *104*, 860–869.
7. Nauli, S.M.; Kawanabe, Y.; Kaminski, J.J.; Pearce, W.J.; Ingber, D.E.; Zhou, J. Endothelial cilia are fluid shear sensors that regulate calcium signaling and nitric oxide production through polycystin-1. *Circulation* **2008**, *117*, 1161–1171.
8. Lim, K.T.; Kim, J.; Seonwoo, H.; Chang, J.U.; Choi, H.; Hexiu, J.; Cho, W.J.; Choung, P.H.; Chung, J.H. Enhanced osteogenesis of human alveolar bone-derived mesenchymal stem cells for tooth tissue engineering using fluid shear stress in a rocking culture method. *Tissue Eng. C Meth.* **2013**, *19*, 128–145.
9. Nauli, S.M.; Rossetti, S.; Kolb, R.J.; Alenghat, F.J.; Consugar, M.B.; Harris, P.C.; Ingber, D.E.; Loghman-Adham, M.; Zhou, J. Loss of polycystin-1 in human cyst-lining epithelia leads to ciliary dysfunction. *J. Am. Soc. Nephrol.* **2006**, *17*, 1015–1025.
10. Riddle, R.C.; Taylor, A.F.; Genetos, D.C.; Donahue, H.J. Map kinase and calcium signaling mediate fluid flow-induced human mesenchymal stem cell proliferation. *Am. J. Physiol. Cell Physiol.* **2006**, *290*, C776–C784.
11. Sankar, K.S.; Green, B.J.; Crocker, A.R.; Verity, J.E.; Altamentova, S.M.; Rocheleau, J.V. Culturing pancreatic islets in microfluidic flow enhances morphology of the associated endothelial cells. *PLoS One* **2011**, *6*, doi:10.1371/journal.pone.0024904.

12. Essner, J.J.; Vogan, K.J.; Wagner, M.K.; Tabin, C.J.; Yost, H.J.; Brueckner, M. Conserved function for embryonic nodal cilia. *Nature* **2002**, *418*, 37–38.
13. Nonaka, S.; Shiratori, H.; Saijoh, Y.; Hamada, H. Determination of left-right patterning of the mouse embryo by artificial nodal flow. *Nature* **2002**, *418*, 96–99.
14. Nonaka, S.; Tanaka, Y.; Okada, Y.; Takeda, S.; Harada, A.; Kanai, Y.; Kido, M.; Hirokawa, N. Randomization of left-right asymmetry due to loss of nodal cilia generating leftward flow of extraembryonic fluid in mice lacking kif3b motor protein. *Cell* **1998**, *95*, 829–837.
15. Karcher, C.; Fischer, A.; Schweickert, A.; Bitzer, E.; Horie, S.; Witzgall, R.; Blum, M. Lack of a laterality phenotype in pkd1 knock-out embryos correlates with absence of polycystin-1 in nodal cilia. *Differentiation* **2005**, *73*, 425–432.
16. Nauli, S.M.; Alenghat, F.J.; Luo, Y.; Williams, E.; Vassilev, P.; Li, X.; Elia, A.E.; Lu, W.; Brown, E.M.; Quinn, S.J.; *et al.* Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells. *Nat. Genet.* **2003**, *33*, 129–137.
17. Cano, D.A.; Sekine, S.; Hebrok, M. Primary cilia deletion in pancreatic epithelial cells results in cyst formation and pancreatitis. *Gastroenterology* **2006**, *131*, 1856–1869.
18. Van der Heiden, K.; Hierck, B.P.; Krams, R.; de Crom, R.; Cheng, C.; Baiker, M.; Pourquie, M.J.; Alkemade, F.E.; DeRuiter, M.C.; Gittenberger-de Groot, A.C.; *et al.* Endothelial primary cilia in areas of disturbed flow are at the base of atherosclerosis. *Atherosclerosis* **2008**, *196*, 542–550.
19. Aboualaiwi, W.A.; Muntean, B.S.; Ratnam, S.; Joe, B.; Liu, L.; Booth, R.L.; Rodriguez, I.; Herbert, B.S.; Bacallao, R.L.; Fruttiger, M.; *et al.* Survivin-induced abnormal ploidy contributes to cystic kidney and aneurysm formation. *Circulation* **2014**, *129*, 660–672.
20. Andrade, Y.N.; Fernandes, J.; Vazquez, E.; Fernandez-Fernandez, J.M.; Arniges, M.; Sanchez, T.M.; Villalon, M.; Valverde, M.A. Trpv4 channel is involved in the coupling of fluid viscosity changes to epithelial ciliary activity. *J. Cell Biol.* **2005**, *168*, 869–874.
21. Teilmann, S.C.; Christensen, S.T. Localization of the angiotensin receptors tie-1 and tie-2 on the primary cilia in the female reproductive organs. *Cell Biol. Int.* **2005**, *29*, 340–346.
22. Malone, A.M.; Anderson, C.T.; Tummala, P.; Kwon, R.Y.; Johnston, T.R.; Stearns, T.; Jacobs, C.R. Primary cilia mediate mechanosensing in bone cells by a calcium-independent mechanism. *Proc. Nat. Acad. Sci. USA* **2007**, *104*, 13325–13330.
23. McGlashan, S.R.; Haycraft, C.J.; Jensen, C.G.; Yoder, B.K.; Poole, C.A. Articular cartilage and growth plate defects are associated with chondrocyte cytoskeletal abnormalities in tg737orpk mice lacking the primary cilia protein polaris. *Matrix Biol.* **2007**, *26*, 234–246.
24. Moorman, S.J.; Shorr, A.Z. The primary cilium as a gravitational force transducer and a regulator of transcriptional noise. *Dev. Dynam.* **2008**, *237*, 1955–1959.
25. Kulaga, H.M.; Leitch, C.C.; Eichers, E.R.; Badano, J.L.; Lesemann, A.; Hoskins, B.E.; Lupski, J.R.; Beales, P.L.; Reed, R.R.; Katsanis, N. Loss of bbs proteins causes anosmia in humans and defects in olfactory cilia structure and function in the mouse. *Nat. Genet.* **2004**, *36*, 994–998.
26. Layman, W.S.; McEwen, D.P.; Beyer, L.A.; Lalani, S.R.; Fernbach, S.D.; Oh, E.; Swaroop, A.; Hegg, C.C.; Raphael, Y.; Martens, J.R.; *et al.* Defects in neural stem cell proliferation and olfaction in chd7 deficient mice indicate a mechanism for hyposmia in human charge syndrome. *Hum. Mol. Genet.* **2009**, *18*, 1909–1923.

27. Ghosh, A.K.; Murga-Zamalloa, C.A.; Chan, L.; Hitchcock, P.F.; Swaroop, A.; Khanna, H. Human retinopathy-associated ciliary protein retinitis pigmentosa gtpase regulator mediates cilia-dependent vertebrate development. *Hum. Mol. Genet.* **2010**, *19*, 90–98.
28. Moore, A.; Escudier, E.; Roger, G.; Tamalet, A.; Pelosse, B.; Marlin, S.; Clement, A.; Geremek, M.; Delaisi, B.; Bridoux, A.M.; *et al.* Rpgri is mutated in patients with a complex x linked phenotype combining primary ciliary dyskinesia and retinitis pigmentosa. *J. Med. Genet.* **2006**, *43*, 326–333.
29. Nishimura, D.Y.; Fath, M.; Mullins, R.F.; Searby, C.; Andrews, M.; Davis, R.; Andorf, J.L.; Mykytyn, K.; Swiderski, R.E.; Yang, B.; *et al.* Bbs2-null mice have neurosensory deficits, a defect in social dominance, and retinopathy associated with mislocalization of rhodopsin. *Proc. Nat. Acad. Sci. USA* **2004**, *101*, 16588–16593.
30. Davenport, J.R.; Watts, A.J.; Roper, V.C.; Croyle, M.J.; van Groen, T.; Wyss, J.M.; Nagy, T.R.; Kesterson, R.A.; Yoder, B.K. Disruption of intraflagellar transport in adult mice leads to obesity and slow-onset cystic kidney disease. *Curr. Biol.* **2007**, *17*, 1586–1594.
31. Hearn, T.; Spalluto, C.; Phillips, V.J.; Renforth, G.L.; Copin, N.; Hanley, N.A.; Wilson, D.I. Subcellular localization of alms1 supports involvement of centrosome and basal body dysfunction in the pathogenesis of obesity, insulin resistance, and type 2 diabetes. *Diabetes* **2005**, *54*, 1581–1587.
32. Winkelbauer, M.E.; Schafer, J.C.; Haycraft, C.J.; Swoboda, P.; Yoder, B.K. The *C. elegans* homologs of nephrocystin-1 and nephrocystin-4 are cilia transition zone proteins involved in chemosensory perception. *J. Cell Sci.* **2005**, *118*, 5575–5587.
33. Einstein, E.B.; Patterson, C.A.; Hon, B.J.; Regan, K.A.; Reddi, J.; Melnikoff, D.E.; Mateer, M.J.; Schulz, S.; Johnson, B.N.; Tallent, M.K. Somatostatin signaling in neuronal cilia is critical for object recognition memory. *J. Neurosci.* **2010**, *30*, 4306–4314.
34. Christensen, S.T.; Pedersen, S.F.; Satir, P.; Veland, I.R.; Schneider, L. The primary cilium coordinates signaling pathways in cell cycle control and migration during development and tissue repair. *Curr. Top. Dev. Biol.* **2008**, *85*, 261–301.
35. Han, Y.G.; Kim, H.J.; Dlugosz, A.A.; Ellison, D.W.; Gilbertson, R.J.; Alvarez-Buylla, A. Dual and opposing roles of primary cilia in medulloblastoma development. *Nat. Med.* **2009**, *15*, 1062–1065.
36. Wong, S.Y.; Seol, A.D.; So, P.L.; Ermilov, A.N.; Bichakjian, C.K.; Epstein, E.H., Jr.; Dlugosz, A.A.; Reiter, J.F. Primary cilia can both mediate and suppress hedgehog pathway-dependent tumorigenesis. *Nat. Med.* **2009**, *15*, 1055–1061.
37. Xiao, Z.; Zhang, S.; Mahlios, J.; Zhou, G.; Magenheimer, B.S.; Guo, D.; Dallas, S.L.; Maser, R.; Calvet, J.P.; Bonewald, L.; *et al.* Cilia-like structures and polycystin-1 in osteoblasts/osteocytes and associated abnormalities in skeletogenesis and runx2 expression. *J. Biol. Chem.* **2006**, *281*, 30884–30895.
38. Hoey, D.A.; Tormey, S.; Ramcharan, S.; O'Brien, F.J.; Jacobs, C.R. Primary cilia-mediated mechanotransduction in human mesenchymal stem cells. *Stem Cells* **2012**, *30*, 2561–2570.
39. Shah, A.S.; Ben-Shahar, Y.; Moninger, T.O.; Kline, J.N.; Welsh, M.J. Motile cilia of human airway epithelia are chemosensory. *Science* **2009**, *325*, 1131–1134.

40. Gilley, S.K.; Stenbit, A.E.; Pasek, R.C.; Sas, K.M.; Steele, S.L.; Amria, M.; Bunni, M.A.; Estell, K.P.; Schwiebert, L.M.; Flume, P.; *et al.* Deletion of airway cilia results in non-inflammatory bronchiectasis and hyperreactive airways. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **2013**, doi:10.1152/ajplung.00095.2013.
41. Mall, M.A. Role of cilia, mucus, and airway surface liquid in mucociliary dysfunction: Lessons from mouse models. *J. Aerosol. Med. Pulm. Drug Deliv.* **2008**, *21*, 13–24.
42. Salathe, M. Regulation of mammalian ciliary beating. *Annu. Rev. Physiol.* **2007**, *69*, 401–422.
43. Zariwala, M.A.; Knowles, M.R.; Omran, H. Genetic defects in ciliary structure and function. *Annu. Rev. Physiol.* **2007**, *69*, 423–450.
44. Eddy, C.A.; Pauerstein, C.J. Anatomy and physiology of the fallopian tube. *Clin. Obstet. Gynecol.* **1980**, *23*, 1177–1193.
45. Lyons, R.A.; Saridogan, E.; Djahanbakhch, O. The reproductive significance of human fallopian tube cilia. *Hum. Reprod. Update* **2006**, *12*, 363–372.
46. Brunner, S.; Colman, D.; Travis, A.J.; Luhmann, U.F.; Shi, W.; Feil, S.; Imsand, C.; Nelson, J.; Grimm, C.; Rulicke, T.; *et al.* Overexpression of RPGR leads to male infertility in mice due to defects in flagellar assembly. *Biol. Reprod.* **2008**, *79*, 608–617.
47. Imai, H.; Hakkaku, N.; Iwamoto, R.; Suzuki, J.; Suzuki, T.; Tajima, Y.; Konishi, K.; Minami, S.; Ichinose, S.; Ishizaka, K.; *et al.* Depletion of selenoprotein gpx4 in spermatocytes causes male infertility in mice. *J. Biol. Chem.* **2009**, *284*, 32522–32532.
48. Lee, L.; Campagna, D.R.; Pinkus, J.L.; Mulhern, H.; Wyatt, T.A.; Sisson, J.H.; Pavlik, J.A.; Pinkus, G.S.; Fleming, M.D. Primary ciliary dyskinesia in mice lacking the novel ciliary protein pcdp1. *Mole. Cell. Biol.* **2008**, *28*, 949–957.
49. Ibanez-Tallon, I.; Pagenstecher, A.; Fliegauf, M.; Olbrich, H.; Kispert, A.; Ketelsen, U.P.; North, A.; Heintz, N.; Omran, H. Dysfunction of axonemal dynein heavy chain mdnah5 inhibits ependymal flow and reveals a novel mechanism for hydrocephalus formation. *Hum. Mol. Genet.* **2004**, *13*, 2133–2141.
50. Sawamoto, K.; Wichterle, H.; Gonzalez-Perez, O.; Cholfin, J.A.; Yamada, M.; Spassky, N.; Murcia, N.S.; Garcia-Verdugo, J.M.; Marin, O.; Rubenstein, J.L.; *et al.* New neurons follow the flow of cerebrospinal fluid in the adult brain. *Science* **2006**, *311*, 629–632.
51. Wodarczyk, C.; Rowe, I.; Chiaravalli, M.; Pema, M.; Qian, F.; Boletta, A. A novel mouse model reveals that polycystin-1 deficiency in ependyma and choroid plexus results in dysfunctional cilia and hydrocephalus. *PLoS One* **2009**, *4*, doi:10.1371/journal.pone.0007137.
52. Nauli, S.M.; Jin, X.; AbouAlaiwi, W.A.; El-Jouni, W.; Su, X.; Zhou, J. Non-motile primary cilia as fluid shear stress mechanosensors. *Meth. Enzymol.* **2013**, *525*, 1–20.
53. Delling, M.; DeCaen, P.G.; Doerner, J.F.; Febvay, S.; Clapham, D.E. Primary cilia are specialized calcium signalling organelles. *Nature* **2013**, *504*, 311–314.
54. Abdul-Majeed, S.; Nauli, S.M. Dopamine receptor type 5 in the primary cilia has dual chemo- and mechano-sensory roles. *Hypertension* **2011**, *58*, 325–331.
55. Liu, W.; Xu, S.; Woda, C.; Kim, P.; Weinbaum, S.; Satlin, L.M. Effect of flow and stretch on the $[Ca^{2+}]_i$ response of principal and intercalated cells in cortical collecting duct. *Am. J. Physiol. Ren. Physiol.* **2003**, *285*, F998–F1012.

56. Kotsis, F.; Nitschke, R.; Boehlke, C.; Bashkurov, M.; Walz, G.; Kuehn, E.W. Ciliary calcium signaling is modulated by kidney injury molecule-1 (Kim1). *Pflugers Arch.* **2007**, *453*, 819–829.
57. Siroky, B.J.; Ferguson, W.B.; Fuson, A.L.; Xie, Y.; Fintha, A.; Komlosi, P.; Yoder, B.K.; Schwiebert, E.M.; Guay-Woodford, L.M.; Bell, P.D. Loss of primary cilia results in deregulated and unabated apical calcium entry in ARPKD collecting duct cells. *Am. J. Physiol. Ren. Physiol.* **2006**, *290*, F1320–F1328.
58. Su, S.; Phua, S.C.; DeRose, R.; Chiba, S.; Narita, K.; Kalugin, P.N.; Katada, T.; Kontani, K.; Takeda, S.; Inoue, T. Genetically encoded calcium indicator illuminates calcium dynamics in primary cilia. *Nat. Meth.* **2013**, *10*, 1105–1107.
59. DeCaen, P.G.; Delling, M.; Vien, T.N.; Clapham, D.E. Direct recording and molecular identification of the calcium channel of primary cilia. *Nature* **2013**, *504*, 315–318.
60. Jin, X.; Mohieldin, A.M.; Muntean, B.S.; Green, J.A.; Shah, J.V.; Mykytyn, K.; Nauli, S.M. Cilioplasm is a cellular compartment for calcium signaling in response to mechanical and chemical stimuli. *Cell. Mol. Life Sci.* **2013**, doi:10.1007/s00018-013-1483-1.
61. Praetorius, H.A.; Spring, K.R. Bending the MDCK cell primary cilium increases intracellular calcium. *J. Membr. Biol.* **2001**, *184*, 71–79.
62. Ishihara, Y.; Sugawara, Y.; Kamioka, H.; Kawanabe, N.; Kurosaka, H.; Naruse, K.; Yamashiro, T. *In situ* imaging of the autonomous intracellular Ca^{2+} oscillations of osteoblasts and osteocytes in bone. *Bone* **2012**, *50*, 842–852.
63. Ishihara, Y.; Sugawara, Y.; Kamioka, H.; Kawanabe, N.; Hayano, S.; Balam, T.A.; Naruse, K.; Yamashiro, T. *Ex vivo* real-time observation of Ca^{2+} signaling in living bone in response to shear stress applied on the bone surface. *Bone* **2013**, *53*, 204–215.
64. Lee, D.J.; Hsu, Y.H. Fluid flow in capillary suction apparatus. *Ind. Eng. Chem. Res.* **1992**, *31*, 2379–2385.
65. Kamioka, H.; Honjo, T.; Takano-Yamamoto, T. A three-dimensional distribution of osteocyte processes revealed by the combination of confocal laser scanning microscopy and differential interference contrast microscopy. *Bone* **2001**, *28*, 145–149.
66. Woda, C.B.; Leite, M., Jr.; Rohatgi, R.; Satlin, L.M. Effects of luminal flow and nucleotides on $[\text{Ca}^{2+}]_i$ in rabbit cortical collecting duct. *Am. J. Physiol. Ren. Physiol.* **2002**, *283*, F437–F446.
67. Olteanu, D.; Liu, X.; Liu, W.; Roper, V.C.; Sharma, N.; Yoder, B.K.; Satlin, L.M.; Schwiebert, E.M.; Bevenssee, M.O. Increased Na^+/H^+ exchanger activity on the apical surface of a cilium-deficient cortical collecting duct principal cell model of polycystic kidney disease. *Am. J. Physiol. Cell Physiol.* **2012**, *302*, C1436–C1451.
68. Sipos, A.; Vargas, S.; Peti-Peterdi, J. Direct demonstration of tubular fluid flow sensing by macula densa cells. *Am. J. Physiol. Ren. Physiol.* **2010**, *299*, F1087–F1093.
69. Tanaka, Y.; Okada, Y.; Hirokawa, N. FGF-induced vesicular release of Sonic hedgehog and retinoic acid in leftward nodal flow is critical for left-right determination. *Nature* **2005**, *435*, 172–177.
70. Song, H.; Hu, J.; Chen, W.; Elliott, G.; Andre, P.; Gao, B.; Yang, Y. Planar cell polarity breaks bilateral symmetry by controlling ciliary positioning. *Nature* **2010**, *466*, 378–382.

71. Rodat-Despoix, L.; Hao, J.; Dandonneau, M.; Delmas, P. Shear stress-induced Ca²⁺ mobilization in MDCK cells is ATP dependent, no matter the primary cilium. *Cell Calcium*. **2013**, *53*, 327–337.
72. Boehlke, C.; Kotsis, F.; Patel, V.; Braeg, S.; Voelker, H.; Brecht, S.; Beyer, T.; Janusch, H.; Hamann, C.; Gödel, M.; *et al.* Primary cilia regulate mTORC1 activity and cell size through Lkb1. *Nat. Cell Biol.* **2010**, *12*, 1115–1122.

© 2014 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution license (<http://creativecommons.org/licenses/by/3.0/>).