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Primary cilia: Cell and molecular mechanosensors directing whole tissue function

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Abstract

Primary cilia are immotile, microtubule-based organelles extending from the surface of nearly every mammalian cell. Mechanical stimulation causes deflection of the primary cilium, initiating downstream signaling cascades to the rest of the cell. The cilium forms a unique subcellular microdomain, and defects in ciliary protein composition or physical structure have been associated with a myriad of human pathologies. In this review, we discuss the importance of ciliary mechanotransduction at the cell and tissue level, and how furthering our molecular understanding of primary cilia mechanobiology may lead to therapeutic strategies to treat human diseases.

Keywords

Mechanotransduction; primary cilia; mechanosensor; microdomain; ciliopathy

II. Introduction

The primary cilium is a single immotile organelle protruding from the apical surface of nearly all mammalian cells [1]. Primary cilia share similar structural characteristics with motile cilia, both consisting of 9 microtubule doublets forming the ciliary axoneme. Motile cilia, however, also contain a central microtubule doublet, radial spokes, and a nexin ring, allowing for propulsion of the cell not experienced in primary cilia. While primary cilia were identified in mammalian cells over 100 years ago, they were long considered vestigial structures. Recently improved molecular and biochemical techniques have enabled keen insights into this once forgotten organelle. Today, primary cilia are understood to play a critical role in the transduction of chemical and mechanical cues.

Impairment of primary cilia function and ciliary associated proteins has been identified as the cause of numerous different conditions known as ciliopathies. Polycystic kidney disease can result from genetic mutations in *Pkd1* and *Pkd2*, coding for the PC1/2 mechanosensitive

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ion channel complex [2]. Bardet-Biedl Syndrome (BBS) is characterized by renal failure, polydactyly, and retinitis pigmentosa, and is caused by mutations in ciliary associated BBS proteins [3]. Retinal and skeletal conditions, such as Joubert and Meckel-Grouber Syndromes, are also attributed to cilia dysfunction [1,4]. The growing list of ciliopathies has stimulated further research into the vast array of roles of the primary cilium. A mouse model was also developed with a hypomorphic mutation to cause a partial loss of IFT88 function – an intraflagellar transport protein required for cilia formation [5]. The mice survive birth and develop to maturity, but with several noticeable traits, primarily a cystic renal phenotype, but also severe growth retardation, polydactyly, and diminished olfaction. Using a cell therapy strategy, it was demonstrated that adenoviral-mediated expression of IFT88 rescues both formation and sensory function of primary cilia [6]. This work suggests that rescuing cilia form and function may be a viable strategy to treat numerous ciliopathies.

While primary cilia do serve as chemosensors, this review focuses on their mechanosensory capabilities. The prevailing theory is that mechanical stimulation, such as fluid flow or tissue deformation, causes primary cilia deflection to initiate downstream mechanotransduction signaling cascades. As will be discussed, the cilium forms a unique microdomain to which specific proteins localize to mediate the mechanotransduction response. Primary cilia play a critical role in mechanotransduction in a myriad of cell types, dictating tissue function. Furthering our understanding of the molecular and biophysical basis of primary cilia mechanobiology will allow development of therapeutic treatment strategies to manipulate primary cilia-mediated mechanosensing and direct whole tissue adaptation.

III. Primary cilia mediate cell mechanosensing and tissue function

The primary cilium functions as a mechanosensor in a wide range of cell and tissue types. These various tissues all require mechanical stimulation for proper function and homeostasis and rely on primary cilia-mediated mechanotransduction.

Kidney

Much of the early work describing primary cilia mechanotransduction was conducted in kidney cells [7,8]. Proper kidney function is dependent on regulated fluid flow through the nephrons and collecting ducts to control glomerular filtration rate, and this flow is sensed by kidney epithelial cell primary cilia [9,10]. Deflection of the primary cilium, by both fluid flow and micropipette manipulation, induces an increase in intracellular calcium, in a primary cilia-dependent manner [8,10]. This calcium influx is also mediated by the PC1/2 ion channel complex [10]. PC1 and PC2 both localize to the primary cilium, where PC1 is a mechanosensitive membrane protein regulating the opening of the PC2 channel [11]. In renal cells, functional defects of either portion of this ion channel complex, along with complete disruption of cilia formation, result in polycystic kidney disease [12].

Endothelia

Endothelial cells sense blood flow and regulate vessel diameter to maintain blood pressure [13]. The shear stress stimulates endothelial nitric oxide production, an important vasodilator, and endothelial primary cilia have been implicated as important mechanosensors

that regulate nitric oxide production [14,15]. Furthermore, PC1/2 has been identified as a critical component for this mechanotransduction response to occur, with cells lacking this ion channel complex exhibiting an attenuated response to fluid flow [14]. Mice lacking PC1 display decreased serum nitric oxide leading to a hypertensive phenotype [16]. Similarly to kidney epithelial cells, chemical removal chloral hydrate of primary cilia, using chloral hydrate, in endothelial cells abrogates the response to flow [17]. Interestingly, *in vivo*, endothelial cilia are only present at areas of low shear stress, potentially as a means to sensitize cells to lower amounts of mechanical stimulation [17,18]. Furthermore, flow sensing primary cilia appear to be more prevalent at atherosclerotic predilection sites where shear stresses are low and disturbed [19]. Removal of endothelial primary cilia impairs nitric oxide signaling resulting in increased atherosclerotic plaque formation [20].

Liver

Similarly to endothelial and renal epithelial cells, cholangiocyte primary cilia detect fluid flow in the bile duct [21]. Fluid flow through isolated intrahepatic bile duct units results in a similar calcium influx as in renal cells, as well as a decrease in intracellular cAMP – responses which are cilium dependent [8,21]. Cilia-localized PC1 and PC2 are necessary for this flow induced calcium increase and cAMP decrease. Furthermore, adenylyl cyclase 6 (AC6), which converts ATP into the second messenger cAMP, is calcium inhibited and localizes to cholangiocyte primary cilia [21]. Mutations to these proteins can impair mechanotransduction, resulting in several cholangio-ciliopathies such as cystic and fibrotic liver disease [22]. It has also been identified that ciliogenesis is heterogenous between larger and smaller bile ducts; however it is not yet clear if this is due to spatial limitations or to optimize primary cilia-mediated mechanosensing to varying fluid flow conditions [22].

Bone

Osteocytes are considered the primary mechanosensing cells within bone, detecting fluid flow through the lacunar-canalicular network [23]. Within these cells, the primary cilium has been identified as a critical mechanosensor, both *in vitro* and *in vivo* [24,25]. Mice with an osteoblast and osteocyte specific deletion of primary cilia, *Kif3a*^{-/-} (a motor protein subunit required for cilia formation), exhibit a reduction in load-induced bone formation [25]. It has also been revealed that PC1 plays a significant role in osteocyte mechanosensing. Xiao *et al.* (2011) utilized a Dmp1-driven Cre mouse model to conditionally disrupt *Pkd1* in osteocytes [26]. When subjected to mechanical loading, the *Pkd1* deficient mice displayed reduced load-induced bone formation, suggesting that PC1 plays an important role in bone cell mechanotransduction. Mutations to another cilia-localized mechanosensitive ion channel, TRPV4, results in a wide range of skeletal dysplasias [27]. As in cholangiocytes, AC6 is also found in the osteocyte primary cilium, and fluid flow induces an increase in intracellular calcium and a decrease in cAMP, in a process mediated by AC6 [28]. *In vivo*, AC6 knockout mice display no phenotypic difference in bone microarchitecture compared to wildtype mice, but when subjected to mechanical load, the *AC6*^{-/-} mice have an attenuated bone formation response [29]. The osteocyte primary cilium also directs mechanically-induced soluble paracrine signaling to promote osteogenic differentiation of mesenchymal stem cells, by encouraging epigenetic changes to induce osteogenic lineage commitment [30,31]. Primary cilia-mediated mechanotransduction has even been implicated in healing

around a bone implant, with mice lacking primary cilia within bone displaying impaired cell mechanosensitivity and osteogenesis in the peri-implant space [32].

Cartilage

Chondrocytes actively respond to cartilage compression and deformation, and moderate loading has been shown to slow the progression of osteoarthritis [33]. Primary cilia on chondrocytes, however, may have a distinct structure from previously mentioned flow sensors. Extracellular matrix binding proteins and integrins both localize to chondrocyte cilia, suggesting that these cilia may not be flow sensors, but rather directly sense deformations of the surrounding tissue [34]. Mechanical stimulation of chondrocytes initiates an up-regulation of proteoglycan and aggrecan synthesis, but this response is abrogated in cells lacking primary cilia [35]. In addition, chondrocytes display a significant calcium influx in response to hypoosmotic loading, a response that is lost with chemical removal of primary cilia by chloral hydrate treatment [36]. Compressive loading increases chondrocyte matrix deposition, in a response that is mimicked with treatment with an agonist (GSK1016790A) of the ciliary ion channel, TRPV4 [37]. These works demonstrate that chondrocyte cilia are not exclusively flow sensors, but may also respond to compressive and osmotic loading conditions. Chondrocyte primary cilia may also play a role in organizing cell polarity. In the growth plate, chondrocytes organize in columnar stacks that become completely disorganized with primary cilia disruption by Kif3a knockout [38]. This cell and cilia organization may optimize the cells to sense mechanical stimulation, in a manner similar to tenocytes within tendon [39].

Cancer

Recently, primary cilia mechanosensing has been associated with tumor progression. Increased interstitial pressure and fluid flow have been described in a number of cancers, but how these biophysical effects are sensed and transduced to regulate tumor progression are incompletely understood [40]. Primary cilia facilitate Hedgehog (Hh) and Wnt signaling pathways which help direct cell proliferation [1]. Mutations or irregular stimulation of Hh and Wnt signaling are instrumental in the progression of numerous cancers [41,42]. Furthermore, both Hh and Wnt signaling can be modulated by the presence or absence of fluid flow mechanical stimulation [43]. Breast, prostate, and liver cancer cells experience a decrease in primary cilia incidence through tumor progression, and recovery of cilia incidence by HDAC6 inhibition has been shown to slow progression of cholangiocarcinoma [44,45]. In contrast, basal cell carcinomas are actually frequently ciliated and removal of primary cilia can inhibit tumor formation, demonstrating multiple roles of cilia in cancer progression [46]. While it is incompletely understood how ciliogenesis dictates tumor mechanosensing and growth, evidence is mounting that targeting primary cilia-mediated signal transduction may be a therapeutic strategy to slow tumorigenesis.

IV. Primary cilia as a molecular mechanosensing nexus

Primary cilia-mediated mechanotransduction is dependent on specific proteins localizing to the cilium where a distinct subcellular microdomain is formed to sensitize the cilium to mechanical stimulation. These proteins need to be specifically trafficked to the primary

cilium, and aberrant trafficking can disrupt mechanotransduction and downstream signaling events.

Protein localization

Numerous proteins, such as PC1, PC2, TRPV4, and AC6, localize to the primary cilium and have been identified as critical for mechanotransduction. The localization of stretch-activated ion channels such as PC2 and TRPV4 suggest that their activation in response to fluid shear induces changes in ion concentrations within the cilium that initiate mechanotransduction signaling cascades. This signaling cascade is continued through proteins converting these ion changes into second messengers, with one hypothesis being that AC6 is inhibited by calcium increases to decrease cAMP levels, altering downstream PKA signaling and CREB-mediated transcriptional regulation [21,28].

Interestingly, not all proteins localize to the cilium to the same degree. Some proteins such as somatostatin receptor 3 (SSTR3) are present solely in the primary cilium [47], while other proteins such as AC6 and PC2 are enriched in the cilium but also expressed throughout the cell [28,48]. On the other hand, some proteins such as Piezo1 are present within the primary cilium, but do not appear to be enriched in any particular subcellular location [49]. This is distinct from proteins which do not localize to the primary cilium at all, such as non-ciliary AC isoforms [28]. The cilium comprises approximately 1/10 000th of the whole cell volume, with limited space for soluble proteins. As such, ciliary protein composition plays a critical role in potentiating molecular mechanosensing.

Ciliary Microdomain

The localization of only specific proteins to the primary cilium suggests that the cilium is a distinct microdomain within the cell. Not only do certain proteins localize to the primary cilium, but others are excluded from entering this microdomain. Hu *et al.* (2010) utilized fluorescence recovery after photobleaching (FRAP) to examine mobility of proteins which localize to the primary cilium [50]. Four proteins were examined – serotonin receptor 6, SSTR3, fibrocystin, and smoothened – all of which contained a fluorescent tag. By photobleaching only a portion of the cilium, the authors noticed that all of the proteins display high mobility within the cilium. This mobility, however, is confined to the cilium, and appears to halt at the ciliary base. Septins are a class of GTPases which bind to microtubules, and Septin 2 (SEPT2) localizes to the base of the primary cilium [50,51]. When SEPT2 is depleted by small hairpin RNA interference, proteins are able to more freely traffic in and out of the cilium [50]. This suggests that a septin-based diffusion barrier exists, allowing specific entry into the cilium, and also retaining proteins within this microdomain.

The ciliary membrane also contains a pore complex for size-based exclusion. Kee *et al.* (2012) utilized FITC-labeled dextrans of varying molecular weights to determine a size-based limit (30 kDa) to diffusion across the membrane at the base of the cilium in retinal cells [52]. This finding was later contradicted with a report that species up to 80kDa could freely diffuse into the cilium [53]. Breslow *et al.* (2013) expanded these studies to examine the ciliary diffusion membrane in kidney epithelial cells [54]. GTP-binding proteins of varying sizes allowed for the determination of a 9 nm (~100 kDa) size limit to ciliary

diffusion. Furthermore, the authors utilized a mass transport model to verify their findings that species larger than 100 kDa require active transport to enter the primary cilium microdomain. This can be simply demonstrated with AC6 (130 kDa) localizing to the cilium, while non-ciliary AC isoforms of similar size are excluded [28].

The purpose of this ciliary diffusion barrier goes beyond solely compartmentalizing the primary cilium, as it also acts as a means to regulate signal transduction. For example, the ciliary diffusion barrier may regulate activation (or inhibition) of specific proteins. cAMP is involved in numerous signaling pathways, not all of which have the same functional outcome. Utilizing a cAMP biosensor, Agarwal *et al.* (2014) demonstrated that compartmentalization of cAMP to specified locations within the cell results in varied responses to cAMP stimulation and inhibition [55]. This suggests that by restricting molecular entry and exit from the primary cilia microdomain, signal transduction can be constrained to defined pathways.

Trafficking to the primary cilium

Recently, distinct ciliary targeting sequences have been identified which are responsible for protein localization to the cilium. Geng *et al.* (2006) found that PC2 localizes to the primary cilium utilizing an RV×P amino acid motif [56]. Interestingly, rhodopsin was found to also utilize this V×P motif to target the cilium in retinal cells [57]. SSTR3, however, utilizes a completely different ciliary targeting sequence, A×[S/A]×Q, located at the third intracellular loop [47]. While ciliary targeting sequences may be conserved between several proteins, numerous distinct targeting sequences exist. The review by Nachury *et al.* (2010) details several other ciliary targeting sequences utilized by a range of proteins [58].

Localized proteins do not gain entry to the cilium simply by containing a ciliary targeting sequence, but rather, these sequences allow proteins to be selectively packaged and transported into the cilium. By studying the trafficking of the Kif17 motor protein in and out of the cilium, Dishinger *et al.* (2010) found that the Kif17 ciliary targeting sequence interacts with the import protein importin-β2 to facilitate entry into the cilium [59]. The authors suggest that binding of protein cargo to such motor proteins may induce a conformational change enabling entry to the cilium. It has also been demonstrated that the Arf and Rab families of GTPases create ciliary targeting complexes which direct trafficking of proteins that contain specific ciliary targeting sequences [57,58]. For example, the BBS proteins assemble a polymerized coat which recognizes SSTR3, creating a complex which can enter the primary cilium [60].

Interestingly, disruption of primary cilia formation does not affect trafficking towards the cilium. PC1 localizes to the cilium of wildtype endothelial cells, but when IFT88 is disrupted, PC1 appears concentrated at the basal body [14]. In this case, the ciliary targeting machinery is still intact, even without the presence of functional cilia. When these cilia are exposed to fluid flow, the response to mechanical stimulation is lost. The presence of polycystins at the base of the cilium is not enough to transduce this mechanical signal. Mechanotransduction relies on an intact cilium with a proper proteome to potentiate mechanosensing.

Targeting sequences can also be used as molecular tools to induce protein trafficking to the cilium. The RV×P ciliary targeting motif identified on PC2 was used to direct trafficking of the non-ciliary human transferrin receptor (hTFR) to the cilium [56]. Targeting sequences and whole ciliary proteins, such as SSTR3, Arl13b, and 5HT6 have been used to induce ciliary trafficking [61–63]. These ciliary proteins and their targeting sequences have also been used as molecular tools to label cilia and their proteins. For example, cilia-targeted biosensors are currently used to analyze mechanically induced signaling dynamics specifically within the primary cilia microdomain.

Intraciliary calcium signaling

Utilizing ciliary targeting sequences to direct fluorescent calcium indicators to the cilium has enabled examination of calcium flux within the cilium, distinct from the cytoplasm. The first calcium indicators were based on circularly permuted GFP located between M13 (light chain kinase) and CaM (calmodulin) domains. As calcium binds CaM, a conformational change allows for increased GFP fluorescence. Work by Su *et al.* (2013) demonstrated that fusing the 5HT6 cilia-targeted protein to this type of genetically encoded calcium indicator allows for examination of calcium transients within the ciliary microdomain [63]. This indicator was co-transfected with a 5HT6-mCherry-GFP fusion protein, which localizes to the cilium but is not calcium responsive, to allow for ratiometric measurements of calcium flux. Indeed, when IMCD kidney epithelial cells are exposed to fluid flow mechanical stimulation, there is a significant influx of calcium into the cilium, reaching a peak within 15 seconds. The authors, however, did not distinguish whether ciliary calcium flux preceded cytoplasmic calcium increases. Similarly, work by Jin *et al.* (2014) leveraged the previously identified targeting sequence of the cytoplasmic tail of fibrocystin to direct a circularly permuted GFP genetically encoded calcium indicator specifically to the cilium [64]. In addition, the authors utilized a non-ciliary targeted version of the calcium indicator to simultaneously record cytoplasmic calcium. Kidney epithelial cells exposed to fluid flow, display a ciliary calcium flux that appears to precede cytoplasmic changes by 2 seconds, with ciliary influx occurring within 1 second, and cytoplasmic calcium flux 3 seconds, after the initiation of fluid flow. Furthermore, when calcium is removed from the media, or PC2 is inhibited by siRNA-mediated knockdown, no change in ciliary calcium occurs. Together this suggests that extracellular calcium enters through stretch-activated ion channels to initiate signaling cascades in the cilium that propagate to the rest of the cell.

A FRET-based calcium biosensor was developed to enable ratiometric measurements with a single probe [65]. This sensor was then fused to Arl13b to traffic to the cilium in both osteocytes and kidney epithelial cells [49]. Fluid flow induced calcium mobilization in both the cilium and cytoplasm with ciliary calcium influx typically preceding cytoplasmic changes, but both reaching a peak approximately 8 seconds after initiation of mechanical stimulation. The roles of several mechanosensitive ion channels were then examined by siRNA-mediated knockdown of TRPV4, PC2, and Piezo1. Knockdown of each channel has no effect on the peak amplitude of flow-induced cytosolic calcium, and only knockdown of TRPV4 abrogates the peak calcium flux in the cilium (in an immortalized osteocyte cell line, MLO-Y4). This work suggests that the ciliary localized stretch-activated ion channel, TRPV4, mediates mechanically induced calcium entry into the cilium.

Most recently, a ratiometric genetically encoded calcium indicator was developed by Delling *et al.* (2016) that localizes to the cilium via Arl13b, and was used to challenge the current paradigm and claim that primary cilia are not calcium responsive mechanosensors [66]. Several cell types, including osteocyte-like MLO-Y4 cells, were transfected with this biosensor and a micropipette was used to initiate fluid flow over a single cell. No change in ciliary calcium was examined within the 2 seconds of applied fluid flow. The authors comment that the lower temporal resolution of previous works could lead to imaging artifacts resulting in other groups improperly examining ciliary calcium mobilization. However, Delling and co-authors did not subject cells to flow for the longer durations that previous groups required to examine peak calcium flux.

Current findings have left questions as to the origin of mechanically induced ciliary calcium, and if calcium even plays a role within the ciliary microdomain to initiate mechanotransduction. These works all used distinct means of quantifying ciliary calcium flux with varying responses. Clearly, interest in ciliary signaling mechanisms has been growing over the past several years, and these contrasting results further demonstrate the need to uncover this complex signal transduction pathway.

V. Primary cilia structure dictates mechanosensing

Understanding the physical properties of primary cilia will help elucidate ways to manipulate its mechanosensing function. Several computational models have been developed to investigate how cilium structure may dictate the physical response to mechanical stimulation. Recent work now utilizes this input to demonstrate that the cilium actively responds to mechanical forces, and that its structure can be modulated to dictate cellular function. This presents the cilium as a pharmacologic target to direct cell mechanotransduction.

Structure influences bending

Through the use of computational models, it can be inferred that manipulating primary cilia structure impacts bending in response to mechanical stimulation. Longer cilia will experience increased membrane tension, but modulating bending stiffness will also directly influence the ability of a cilium to act as an antenna transducing mechanical inputs into molecular signals. The first computational models of cilia bending in response to fluid flow estimate a bending stiffness on the order of $10^{-23} \text{ N}\cdot\text{m}^2$, two orders of magnitude lower than motile cilia, and demonstrate that longer cilia experience greater deflection and thus membrane strain [7,67]. A more recent computational model incorporates fluid drag and allows for rotation at the base of the cilium, claiming a flexural rigidity an order of magnitude greater ($10^{-22} \text{ N}\cdot\text{m}^2$) than previous models [68]. This difference is likely due to the use of different cell lines and different fluid flow conditions between each study, and also suggests that the cilium may not have a rigid attachment to the cell body and experiences some degree of basal rotation in response to fluid flow. The diversity of cilium axoneme stiffness indicates that primary cilia may be adaptable and possibly cell/environment-specific mechanosensors.

Cilia are adaptable mechanosensors

Not only do primary cilia function as mechanosensors, but they actively respond to mechanical stimulation. Fluid flow causes an increase in the number of microtubules surrounding the base of the cilium, likely as a mechanism to withstand the applied shear stress [61]. In addition, flow causes a rapid increase in microtubule acetylation and ciliary bending stiffness [69]. Microtubule acetylation occurs on the lysine-40 residue of α -tubulin on the lumen of the microtubule causing an increase in bending stiffness of the cilium [70]. Acetylation also helps to stabilize microtubules to prevent disassembly, yet it is unknown what percentage of α -tubulin in primary cilia is acetylated; furthermore, this acetylation is likely not constant along the length of the cilium. Early electron micrographs of cilia structure actually suggest that cilia structure may not be constant along their length, and that cilia may lose microtubule symmetry and number towards the distal tip [71,72]. In fact, two of the described bending models also comment that cilia do not always appear to deflect with a constant curvature, potentially due to this lack of symmetry along the length of the cilium [67,68].

In addition to alterations in bending stiffness, repeated mechanical stimulation causes basal cilia rotation [69]. It has been suggested that the actin cytoskeleton surrounding the basal body of the cilium may also generate force to adjust ciliary positioning to tune and calibrate sensory functions [73]. In fact, treatment with the actin destabilizer, cytochalasin D, induces ciliogenesis, though it has not been clearly defined how this impacts primary cilia-mediated mechanotransduction [74]. Fluid flow mechanical stimulation has also been demonstrated to cause a decrease in primary cilia length, in a process mediated by PC2 [75]. In a model of chondrocytes embedded in a 3D matrix, compressive strain on chondrocyte cilia causes a decrease in cilia incidence and length, which are recovered after mechanical stimulation is ceased [76]. In both of these models, this shortening would minimize cilia deflection in response to mechanical stimulation, likely abrogating the response to mechanical stimuli. Together, these findings demonstrate that the cilium actively responds to mechanical stimulation by adapting its structure, typically in a manner to tune the bending response to flow and resulting mechanosensitivity.

Targeting primary cilia to direct mechanosensitivity

Manipulation of cilia structure is now being used as a strategy to direct cellular mechanosensing. We recently showed that treating osteocytes with fenoldopam or lithium, two small molecules with distinct mechanisms of action, increases primary cilia length [77]. Moreover, osteocytes with longer cilia are more mechanosensitive, displaying increased osteogenesis in response to fluid flow. It was previously demonstrated that fenoldopam also increases primary cilia length in endothelial cells *in vitro* and *in vivo* [16]. When cilia length is increased, mechanically stimulated endothelial cells produce significantly more nitric oxide, suggesting enhanced mechanosensitivity. These works demonstrate that primary cilia structure is a promising and viable pharmacologic target to direct cell mechanosensitivity and tissue function.

The correlation of cilia length and mechanosensitivity, however, is complicated by microtubule stiffness. A specific histone deacetylase isoform (HDAC6) plays a key role in

regulating microtubule bending stiffness. Disrupting HDAC6 by either siRNA-mediated knockdown or chemical inhibition significantly increases primary cilia bending stiffness, and decreases mechanosensitivity [69]. However, HDAC6 inhibition has also been shown to increase primary ciliogenesis in tumorigenic cells with low cilia incidence [45]. The increase in microtubule acetylation due to HDAC6 inhibition plays a secondary role of preventing cilia disassembly, resulting in increased cilia length. It is possible that there is a specific structure-function relationship between cilia length and stiffness, and that a threshold exists where either length or stiffness dictates cilia mechanosensitivity.

Length and stiffness are not the only factors dictating the cilium's response to mechanical stimulation, with protein composition also playing a critical role. Longer cilia may allow for a greater number of cilia-specific proteins and signaling molecules within the microdomain to potentiate mechanosensing [52,54]. Cells treated with the cilium lengthening agent, fenoldopam, also display significant increases in production of AC6, key to primary cilia-mediated mechanotransduction [77,78].

Increasing primary cilia length, however, may not always be feasible. As cilia continue to elongate and experience greater deflection in response to mechanical stimulation, they may be sheared off. In fact, fluid shear has been used as a method to cleave and isolate primary cilia for proteomic analysis [79]. *In vivo*, primary cilia are only present on endothelial cells in regions of low shear stress, potentially because they are cleaved off in areas of higher shear [19]. In a study of idiopathic scoliosis (IS), osteoblasts were isolated from patients with IS and found to have significantly longer primary cilia compared to control samples [80]. Interestingly, these IS cells with longer cilia actually display a decreased response to mechanical stimulation, suggesting that cilia length may play different roles in directing mechanosensitivity in different cell types and disease states.

In the case of osteocytes embedded within bone, it may not even be possible to increase primary cilia length *in vivo*. The lacunar space in which osteocytes reside is quite small ($< 1 \mu\text{m}$ pericellular space), making it difficult to conceive how cilia can significantly alter length [81]. A fluid-structure interaction model suggests that short cilia ($< 0.5 \mu\text{m}$) are not capable of functioning as free-standing fluid flow sensors, but cilia spanning the full pericellular space or even correctly positioned within the lacuna may be able to experience enough membrane tension to be mechanosensors [82]. The authors also postulate that a cilium anchored to the lacunar wall by integrin attachments may also be capable of sufficient membrane tension to function as a mechanosensor. This second notion is intriguing as it would suggest that osteocyte cilia may function more similarly to chondrocyte cilia than kidney epithelial or endothelial cilia.

VI: Future Directions

The work described in this review highlights key aspects of primary cilia-mediated mechanosensing, and the potential of therapeutically targeting this apparatus to dictate cell mechanotransduction. Future work will improve computational models to inform our understanding of cilia mechanobiology, as well as identify novel proteins and molecular targets to treat a myriad of ciliopathies.

Models informing mechanobiology

The interplay of cilia length, structure, and protein composition all dictate primary cilia-mediated mechanotransduction. Accounting for each of these components will improve the biological accuracy of computational models of ciliary mechanosensing, enabling additional insights into cilia biology to motivate future work. Improved models may utilize molecular dynamics to better incorporate the differences in microtubule structure and acetylation to improve our understanding of how these aspects of cilia biology dictate bending and mechanosensitivity. Additionally, models of membrane tension opening stretch-activated ion channels have been developed to examine channel-membrane interactions in response to fluid shear stress [83,84]. Such models could also be incorporated to estimate the contribution of mechanosensitive ion channels in cilia mechanotransduction. Further understanding of the critical aspects of cilia bending and mechanotransduction will unlock the potential of targeting primary cilia to direct cell mechanosensitivity as a therapeutic strategy for numerous human diseases.

Primary cilia proteomics

Recent advances in molecular technologies has enabled proteomic analysis of the ciliary microdomain. Work by Mick *et al.* (2015) utilized a proximity labeling technique to biotinylate proteins which are trafficked into the cilium [62]. The ascorbate peroxidase, APEX, was fused to Arl13b to allow trafficking to the cilium and biotinylation of proteins within a short (< 20 nm) radius for identification of cilia-specific proteins. Labeling confirms known ciliary proteins such as AC6 and PC2, and also identifies potentially new ciliary targets, informing cilia mechanobiology research. For example, primary cilia are distinct from motile cilia which contain actin filaments to drive motion, and it was believed that primary cilia lacked this actin connectivity. However, APEX labeling uncovered that actin and actin-binding proteins are actually trafficked to the primary cilium as well, potentially supporting the hypothesis that actin forces tune cilia mechanosensing [73].

Cell-specific ciliary microdomains

Regardless if mechanical stimulation initiates calcium entry into the cilium through ciliary ion channels or from cytoplasmic stores, it is believed that mechanically-induced calcium flux initiates downstream signaling cascades via adenylyl cyclases (ACs) which convert ATP to the second messenger cAMP [21]. As previously discussed, one adenylyl cyclase isoform, AC6, localizes to primary cilia in several cell types including bone, liver, and kidney, as assessed by both immunocytochemistry and proteomic analyses [21, 28,62]. Interestingly, several other isoforms have been demonstrated to localize to primary cilia in neural cells, including AC3 and AC5 [85]. A ciliary targeting sequence was also identified on AC5 (xWRx amino acid motif) that is not present on AC6. This distinct localization pattern may suggest cell-specific differences in ciliary proteome, and potentially mechanosensing function.

The roles of stretch-activated ion channels in distinct cells types has also been examined. TRPV4 and PC2 both localize to primary cilia in osteocytes and kidney epithelial cells, but it has been suggested that TRPV4 plays a more significant role in osteocyte mechanosensing and ciliary calcium flux, while PC2 is dominant in kidney [49]. Together these suggest

potential differences in signal transduction pathways in primary cilia-mediated mechanotransduction between distinct cell types. Further proteomic analysis will need to be performed to discern if the ciliary microdomain varies between cells and tissues, but this suggests potential for cell-specific primary cilia-targeted therapeutic strategies.

Conclusion

While primary cilia were previously regarded as vestigial structures, recent advances have uncovered the critical importance of this unique organelle. Scientific advances are enabling proteomic and signal transduction analysis to elucidate novel ciliary protein and signaling pathways, and new developments are challenging conventional paradigms. Primary cilia mechanobiology is an exciting and evolving field with room for innumerable discoveries. Furthering our understanding of ciliary mechanosensing, in a variety of cell types, also has the potential for us to discern unique signal transduction pathways between cells, ultimately leading to cell-specific cilia-targeted therapeutics. Mechanobiology is critical to tissue function, and primary cilia may be the key to unlocking the therapeutic potential of targeting cell mechanotransduction.

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References

1. Nguyen AM, Jacobs CR. Emerging role of primary cilia as mechanosensors in osteocytes. *Bone*. 2013; 54:196–204. [PubMed: 23201223]
2. Hanaoka K, Qian F, Boletta a, Bhunia aK, Piontek K, Tsiokas L, Sukhatme VP, Guggino WB, Germino GG. Co-assembly of polycystin-1 and -2 produces unique cation-permeable currents. *Nature*. 2000; 408:990–994. [PubMed: 11140688]
3. Zaghoul, Na, Katsanis, N. Mechanistic insights into Bardet-Biedl syndrome, a model ciliopathy. *J Clin Invest*. 2009; 119:428–37. [PubMed: 19252258]
4. Badano JL, Mitsuma N, Beales PL, Katsanis N. The ciliopathies: an emerging class of human genetic disorders. *Annu Rev Genomics Hum Genet*. 2006; 7:125–148. [PubMed: 16722803]
5. Lehman JM, Michaud EJ, Schoeb TR, Aydin-Son Y, Miller M, Yoder BK. The Oak Ridge Polycystic Kidney mouse: Modeling ciliopathies of mice and men. *Dev Dyn*. 2008; 237:1960–1971. [PubMed: 18366137]
6. McIntyre JC, Davis EE, Joiner A, Williams CL, Tsai IC, Jenkins PM, McEwen DP, Zhang L, Escobado J, Thomas S, Szymanska K, Johnson Ca, Beales PL, Green ED, Mullikin JC, Sabo A, Muzny DM, Gibbs Ra, Attié-Bitach T, Yoder BK, Reed RR, Katsanis N, Martens JR. Gene therapy rescues cilia defects and restores olfactory function in a mammalian ciliopathy model. *Nat Med*. 2012; 18:1423–8. [PubMed: 22941275]
7. Schwartz EA, Leonard ML, Bizios R, Bowser SS. Analysis and modeling of the primary cilium bending response to fluid shear. *Am J Physiol*. 1997; 272:F132, 8. <http://www.ncbi.nlm.nih.gov/pubmed/9039059>. [PubMed: 9039059]
8. Praetorius HA, Spring KR. Bending the MDCK cell primary cilium increases intracellular calcium. *J Membr Biol*. 2001; 184:71–79. [PubMed: 11687880]
9. Leyssac PP. Changes in single nephron renin release are mediated by tubular fluid flow rate. *Kidney Int*. 1986; 30:332–339. [PubMed: 3537444]
10. Praetorius HA, Spring KR. The renal cell primary cilium functions as a flow sensor. *Curr Opin Nephrol Hypertens*. 2003; 12:517–520. [PubMed: 12920399]

11. Piperi C. Polycystins and mechanotransduction: From physiology to disease. *World J Exp Med*. 2015; 5:200. [PubMed: 26618106]
12. Yoder BK, Hou X, Guay-Woodford LM. The polycystic kidney disease proteins, polycystin-1, polycystin-2, polaris, and cystin, are co-localized in renal cilia. *J Am Soc Nephrol*. 2002; 13:2508–2516. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=12239239. [PubMed: 12239239]
13. Zaragoza C, Márquez S, Saura M. Endothelial mechanosensors of shear stress as regulators of atherogenesis. *Curr Opin Lipidol*. 2012; 23:446–452. [PubMed: 22964993]
14. Nauli SM, Kawanabe Y, Kaminski JJ, Pearce WJ, Ingber DE, Zhou J. Endothelial cilia are fluid shear sensors that regulate calcium signaling and nitric oxide production through polycystin-1. *Circulation*. 2008; 117:1161–1171. [PubMed: 18285569]
15. Boo YC, Jo H. Flow-dependent regulation of endothelial nitric oxide synthase: role of protein kinases. *Am J Physiol Cell Physiol*. 2003; 285:C499–C508. [PubMed: 12900384]
16. Kathem SH, Mohieldin AM, Abdul-Majeed S, Ismail SH, Altaei QH, Alshimmari IK, Alsaidi MM, Khammas H, Nauli AM, Joe B, Nauli SM. Ciliotherapy: a novel intervention in polycystic kidney disease. *J Geriatr Cardiol*. 2014; 11:63–73. [PubMed: 24748884]
17. Hierck BP, Van der Heiden K, Alkemade FE, Van de Pas S, Van Thienen JV, Groenendijk BC, Bax WH, Van der Laarse A, Deruiter MC, Horrevoets AJ, Poelmann RE. Primary cilia sensitize endothelial cells for fluid shear stress. *Dev Dyn*. 2008; 237:725–735. [PubMed: 18297727]
18. Egorova AD, Khedoe PPSJ, Goumans MJTH, Yoder BK, Nauli SM, Ten Dijke P, Poelmann RE, Hierck BP. Lack of primary cilia primes shear-induced endothelial-to-mesenchymal transition. *Circ Res*. 2011; 108:1093–1101. [PubMed: 21393577]
19. Van der Heiden K, Hierck BP, Krams R, de Crom R, Cheng C, Baiker M, Pourquie MJBM, Alkemade FE, DeRuiter MC, Gittenberger-de Groot AC, Poelmann RE. Endothelial primary cilia in areas of disturbed flow are at the base of atherosclerosis. *Atherosclerosis*. 2008; 196:542–50. [PubMed: 17631294]
20. Dinsmore C, Reiter JF. Endothelial primary cilia inhibit atherosclerosis. *EMBO Rep*. 2016; 17:e201541019.
21. Masyuk AI, Masyuk TV, Splinter PL, Huang BQ, Stroope AJ, LaRusso NF. Cholangiocyte cilia detect changes in luminal fluid flow and transmit them into intracellular Ca²⁺ and cAMP signaling. *Gastroenterology*. 2006; 131:911–20. [PubMed: 16952559]
22. Masyuk AI, Masyuk TV, LaRusso NF. Cholangiocyte primary cilia in liver health and disease. *Dev Dyn*. 2008; 237:2007–2012. [PubMed: 18407555]
23. Tatsumi S, Ishii K, Amizuka N, Li M, Kobayashi T, Kohno K, Ito M, Takeshita S, Ikeda K. Targeted ablation of osteocytes induces osteoporosis with defective mechanotransduction. *Cell Metab*. 2007; 5:464–475. [PubMed: 17550781]
24. Malone AMD, Anderson CT, Tummala P, Kwon RY, Johnston TR, Stearns T, Jacobs CR. Primary cilia mediate mechanosensing in bone cells by a calcium-independent mechanism. *Proc Natl Acad Sci*. 2007; 104:13325–30. [PubMed: 17673554]
25. Temiyasathit S, Tang WJ, Leucht P, Anderson CT, Monica SD, Castillo AB, Helms JA, Stearns T, Jacobs CR. Mechanosensing by the primary cilium: Deletion of Kif3a reduces bone formation due to loading. *PLoS One*. 2012; 7
26. Xiao Z, Dallas M, Qiu N, Nicolella D, Cao L, Johnson M, Bonewald L, Quarles LD. Conditional deletion of Pkd1 in osteocytes disrupts skeletal mechanosensing in mice. *FASEB J*. 2011; 25:2418–2432. [PubMed: 21454365]
27. Nishimura G, Lausch E, Savarirayan R, Shiba M, Spranger J, Zabel B, Ikegawa S, Superti-Furga A, Unger S. TRPV4-associated skeletal dysplasias. *Am J Med Genet Part C Semin Med Genet*. 2012; 160 C:190–204.
28. Kwon RY, Temiyasathit S, Tummala P, Quah CC, Jacobs CR. Primary cilium-dependent mechanosensing is mediated by adenylyl cyclase 6 and cyclic AMP in bone cells. *FASEB J*. 2010; 24:2859–68. [PubMed: 20371630]
29. Lee KL, Hoey DA, Spasic M, Tang T, Hammond HK, Jacobs CR. Adenylyl cyclase 6 mediates loading-induced bone adaptation in vivo. *FASEB J*. 2014; 28:1157–1165. [PubMed: 24277577]

30. Hoey DA, Kelly DJ, Jacobs CR. A role for the primary cilium in paracrine signaling between mechanically stimulated osteocytes and mesenchymal stem cells. *Biochem Biophys Res Commun.* 2011; 412:182–187. [PubMed: 21810408]
31. Chen JC, Chua M, Bellon RB, Jacobs CR. Epigenetic changes during mechanically induced osteogenic lineage commitment. *J Biomech Eng.* 2015; 137:20902.
32. Leucht P, Monica SD, Temiyasathit S, Lenton K, Manu A, Longaker MT, Jacobs CR, Spilker RL, Guo H, Brunski JB, Helms JA. Primary cilia act as mechanosensors during bone healing around an implant. *Med Eng Phys.* 2013; 35:392–402. [PubMed: 22784673]
33. Leong DJ, Hardin JA, Cobelli NJ, Sun HB. Mechanotransduction and cartilage integrity. *Ann N Y Acad Sci.* 2011; 1240:32–37. [PubMed: 22172037]
34. McGlashan SR, Jensen CG, Poole CA. Localization of extracellular matrix receptors on the chondrocyte primary cilium. *J Histochem Cytochem.* 2006; 54:1005–1014. [PubMed: 16651393]
35. Wann AKT, Zuo N, Haycraft CJ, Jensen CG, Poole CA, McGlashan SR, Knight MM. Primary cilia mediate mechanotransduction through control of ATP-induced Ca²⁺ signaling in compressed chondrocytes. *FASEB J.* 2012; 26:1663–71. [PubMed: 22223751]
36. Phan MN, Leddy Ha, Votta BJ, Kumar S, Levy DS, Lipshutz DB, Suk HL, Liedtke W, Guilak F. Functional characterization of TRPV4 as an osmotically sensitive ion channel in porcine articular chondrocytes. *Arthritis Rheum.* 2009; 60:3028–3037. [PubMed: 19790068]
37. O'Connor CJ, Leddy HA, Benefield HC, Liedtke WB, Guilak F. TRPV4-mediated mechanotransduction regulates the metabolic response of chondrocytes to dynamic loading. *Proc Natl Acad Sci.* 2014; 111:1316–21. [PubMed: 24474754]
38. Song B, Haycraft CJ, Seo H, Yoder BK, Serra R. Development of the post-natal growth plate requires intraflagellar transport proteins. *Dev Biol.* 2007; 305:202–216. [PubMed: 17359961]
39. Donnelly E, Ascenzi MG, Farnum C. Primary cilia are highly oriented with respect to collagen direction and long axis of extensor tendon. *J Orthop Res.* 2010; 28:77–82. [PubMed: 19603516]
40. Munson JM, Shieh AC. Interstitial fluid flow in cancer: implications for disease progression and treatment. *Cancer Manag Res.* 2014; 6:317–28. [PubMed: 25170280]
41. Hassounah NB, Bunch TA, McDermott KM. Molecular pathways: the role of primary cilia in cancer progression and therapeutics with a focus on Hedgehog signaling. *Clin Cancer Res.* 2012; 18:2429–35. [PubMed: 22415315]
42. Polakis P. Wnt signaling in cancer. *Cold Spring Harb Perspect Biol.* 2012; 4:9.
43. Mans DA, Voest EE, Giles RH. All along the watchtower: Is the cilium a tumor suppressor organelle? *Biochim Biophys Acta - Rev Cancer.* 2008; 1786:114–125.
44. Menzl I, Lebeau L, Pandey R, Hassounah NB, Li FW, Nagle R, Weihs K, McDermott KM. Loss of primary cilia occurs early in breast cancer development. *Cilia.* 2014; 3:7. [PubMed: 24987519]
45. Gradilone SA, Radtke BN, Bogert PS, Huang BQ, Gajdos GB, LaRusso NF. HDAC6 inhibition restores ciliary expression and decreases tumor growth. *Cancer Res.* 2013; 73:2259–2270. [PubMed: 23370327]
46. Wong SY, Seol AD, So PL, Ermilov AN, Bichakjian CK, Epstein EH, Dlugosz Aa, Reiter JF. Primary cilia can both mediate and suppress Hedgehog pathway-dependent tumorigenesis. *Nat Med.* 2009; 15:1055–1061. [PubMed: 19701205]
47. Berbari NF, Johnson AD, Lewis JS, Askwith CC, Mykityn K. Identification of ciliary localization sequences within the third intracellular loop of G protein-coupled receptors. *Mol Biol Cell.* 2008; 19:1540–1547. [PubMed: 18256283]
48. Gradilone SA, Masyuk AI, Splinter PL, Banales JM, Huang BQ, Tietz PS, Masyuk TV, Larusso NF. Cholangiocyte cilia express TRPV4 and detect changes in luminal tonicity inducing bicarbonate secretion. *Proc Natl Acad Sci.* 2007; 104:19138–19143. [PubMed: 18024594]
49. Lee KL, Guevarra MD, Nguyen AM, Chua MC, Wang Y, Jacobs CR. The primary cilium functions as a mechanical and calcium signaling nexus. *Cilia.* 2015; 4:7. [PubMed: 26029358]
50. Hu Q, Milenkovic L, Jin H, Scott MP, Nachury MV, Spiliotis ET, Nelson WJ. A septin diffusion barrier at the base of the primary cilium maintains ciliary membrane protein distribution. *Science.* 2010; 329:436–439. [PubMed: 20558667]

51. Spiliotis ET, Hunt SJ, Hu Q, Kinoshita M, Nelson WJ. Epithelial polarity requires septin coupling of vesicle transport to polyglutamylated microtubules. *J Cell Biol.* 2008; 180:295–303. [PubMed: 18209106]
52. Kee HL, Dishinger JF, Lynne Blasius T, Liu CJ, Margolis B, Verhey KJ. A size-exclusion permeability barrier and nucleoporins characterize a ciliary pore complex that regulates transport into cilia. *Nat Cell Biol.* 2012; 14:431–437. [PubMed: 22388888]
53. Najafi M, Maza NA, Calvert PD. Steric volume exclusion sets soluble protein concentrations in photoreceptor sensory cilia. *Proc Natl Acad Sci.* 2012; 109:203–208. [PubMed: 22184246]
54. Breslow DK, Koslover EF, Seydel F, Spakowitz AJ, Nachury MV. An in vitro assay for entry into cilia reveals unique properties of the soluble diffusion barrier. *J Cell Biol.* 2013; 203:129–47. [PubMed: 24100294]
55. Agarwal SR, Yang PCC, Rice M, Singer Ca, Nikolaev VO, Lohse MJ, Clancy CE, Harvey RD. Role of membrane microdomains in compartmentation of cAMP signaling. *PLoS One.* 2014; 9:e95835. [PubMed: 24752595]
56. Geng L, Okuhara D, Yu Z, Tian X, Cai Y, Shibasaki S, Somlo S. Polycystin-2 traffics to cilia independently of polycystin-1 by using an N-terminal RVxP motif. *J Cell Sci.* 2006; 119:1383–1395. [PubMed: 16537653]
57. Mazelova J, Astuto-Gribble L, Inoue H, Tam BM, Schonteich E, Prekeris R, Moritz OL, Randazzo PA, Deretic D. Ciliary targeting motif VxPx directs assembly of a trafficking module through Arf4. *EMBO J.* 2009; 28:183–192. [PubMed: 19153612]
58. Nachury MV, Seeley ES, Jin H. Trafficking to the ciliary membrane: how to get across the periciliary diffusion barrier? *Annu Rev Cell Dev Biol* nd. 26:59–87.
59. Dishinger JF, Kee HL, Jenkins PM, Fan S, Hurd TW, Hammond JW, Truong YNT, Margolis B, Martens JR, Verhey KJ. Ciliary entry of the kinesin-2 motor KIF17 is regulated by importin-beta2 and RanGTP. *Nat Cell Biol.* 2010; 12:703–710. [PubMed: 20526328]
60. Jin H, White SR, Shida T, Schulz S, Aguiar M, Gygi SP, Bazan JF, Nachury MV. The conserved bardet-biedl syndrome proteins assemble a coat that traffics membrane proteins to Cilia. *Cell.* 2010; 141:1208–1219. [PubMed: 20603001]
61. Espinha LC, Hoey DA, Fernandes PR, Rodrigues HC, Jacobs CR. Oscillatory fluid flow influences primary cilia and microtubule mechanics. *Cytoskeleton (Hoboken).* 2014; 71:435–45. [PubMed: 25044764]
62. Mick DU, Rodrigues RB, Leib RD, Adams CM, Chien AS, Gygi SP, Nachury MV. Proteomics of Primary Cilia by Proximity Labeling. *Dev Cell.* 2015; 35:497–512. [PubMed: 26585297]
63. Su S, Phua SC, DeRose R, Chiba S, Narita K, Kalugin PN, Katada T, Kontani K, Takeda S, Inoue T. Genetically encoded calcium indicator illuminates calcium dynamics in primary cilia. *Nat Methods.* 2013; 10:1105–7. [PubMed: 24056873]
64. Jin X, Mohieldin AM, Muntean BS, Green Ja, Shah JV, Mykityn K, Nauli SM. Cilioplasm is a cellular compartment for calcium signaling in response to mechanical and chemical stimuli. *Cell Mol Life Sci.* 2014; 71:2165–78. [PubMed: 24104765]
65. Ouyang M, Sun J, Chien S, Wang Y. Determination of hierarchical relationship of Src and Rac at subcellular locations with FRET biosensors. *Proc Natl Acad Sci.* 2008; 105:14353–14358. [PubMed: 18799748]
66. Delling M, Indzhukulian AA, Liu X, Li Y, Xie T, Corey DP, Clapham DE. Primary cilia are not calcium-responsive mechanosensors. *Nature.* 2016; 531:656–60. [PubMed: 27007841]
67. Rydholm S, Zwart G, Kowalewski JM, Kamali-Zare P, Frisk T, Brismar H. Mechanical properties of primary cilia regulate the response to fluid flow. *Am J Physiol Renal Physiol.* 2010; 298:F1096–102. [PubMed: 20089672]
68. Downs ME, Nguyen AM, Herzog Fa, Hoey Da, Jacobs CR. An experimental and computational analysis of primary cilia deflection under fluid flow. *Comput Methods Biomech Biomed Engin.* 2014; 17:2–10.
69. Nguyen AM, Young YN, Jacobs CR. The primary cilium is a self-adaptable, Integrating nexus for mechanical stimuli and cellular signaling. *Biol Open.* 2015; 4:1733–8. [PubMed: 26603473]

70. Soppina V, Herbstman JF, Skiniotis G, Verhey KJ. Luminal localization of α -tubulin K40 acetylation by cryo-EM analysis of fab-labeled microtubules. *PLoS One*. 2012; 7:e48204. [PubMed: 23110214]
71. Odor DL, Blandau RJ. Observations on the solitary cilium of rabbit oviductal epithelium: its motility and ultrastructure. *Am J Anat*. 1985; 174:437–453. [PubMed: 3909797]
72. Yamamoto M, Kataoka K. Electron microscopic observation of the primary cilium in the pancreatic islets. *Arch Histol Jpn Nippon Soshikigaku Kiroku*. 1986; 49:449–457.
73. Battle C, Ott CM, Burnette DT, Lippincott-Schwartz J, Schmidt CF. Intracellular and extracellular forces drive primary cilia movement. *Proc Natl Acad Sci*. 2015; 112:1410–5. [PubMed: 25605896]
74. Kim J, Jo H, Hong H, Kim MH, Kim JM, Lee JK, Heo Do W, Kim J. Actin remodelling factors control ciliogenesis by regulating YAP/TAZ activity and vesicle trafficking. *Nat Commun*. 2015; 6:6781. [PubMed: 25849865]
75. Besschetnova TY, Kolpakova-Hart E, Guan Y, Zhou J, Olsen BR, Shah JV. Identification of Signaling Pathways Regulating Primary Cilium Length and Flow-Mediated Adaptation. *Curr Biol*. 2010; 20:182–7. [PubMed: 20096584]
76. McGlashan SR, Knight MM, Chowdhury TT, Joshi P, Jensen CG, Kennedy S, Poole Ca. Mechanical loading modulates chondrocyte primary cilia incidence and length. *Cell Biol Int*. 2010; 34:441–6. [PubMed: 20100169]
77. Spasic M, Jacobs CR. Lengthening primary cilia enhances cellular mechanosensitivity. *Eur Cell Mater*. 2017; 33:158–168. [PubMed: 28217833]
78. Yu P, Sun M, Villar VAM, Zhang Y, Weinman EJ, Felder Ra, Jose Pa. Differential dopamine receptor subtype regulation of adenylyl cyclases in lipid rafts in human embryonic kidney and renal proximal tubule cells. *Cell Signal*. 2014; 26:2521–2529. [PubMed: 25049074]
79. Mitchell KAP. Isolation of primary cilia by shear force. *Curr Protoc Cell Biol*. 2013:1–9.
80. Oliazadeh N, Gorman KF, Eveleigh R, Bourque G, Moreau A. Identification of Elongated Primary Cilia with Impaired Mechanotransduction in Idiopathic Scoliosis Patients. *Sci Rep*. 2017; 7:44260. [PubMed: 28290481]
81. Schaffler MB, Cheung WY, Majeska R, Kennedy O. Osteocytes: Master orchestrators of bone. *Calcif Tissue Int*. 2014; 94:5–24. [PubMed: 24042263]
82. Vaughan TJ, Mullen Ca, Verbruggen SW, McNamara LM. Bone cell mechanosensation of fluid flow stimulation: a fluid-structure interaction model characterising the role integrin attachments and primary cilia. *Biomech Model Mechanobiol*. 2014; 2
83. Jeon J, Voth GA. Gating of the mechanosensitive channel protein MscL: the interplay of membrane and protein. *Biophys J*. 2008; 94:3497–511. [PubMed: 18212020]
84. Pak OS, Young YN, Marple GR, Veerapaneni S, Stone HA. Gating of a mechanosensitive channel due to cellular flows. *Proc Natl Acad Sci U S A*. 2015; 112:9822–9827. [PubMed: 26216988]
85. Vuolo L, Herrera A, Torroba B, Menendez A, Pons S. Ciliary Adenylyl Cyclases control the Hedgehog pathway. *J Cell Sci*. 2015:2928–2937. [PubMed: 26092933]

Abbreviations

PC1/2	<i>Pkd1/2</i> , polycystin-1/2
TRPV4	transient receptor potential vanilloid 4
AC6	adenylyl cyclase 6
cAMP	cyclic adenosine monophosphate
IFT88	intraflagellar transport 88
Kif3a	kinesin family member 3A
HDAC6	histone deacetylase 6

BBS	Bardet-Biedl Syndrome
HH	hedgehog
FRET	fluorescence resonance energy transfer

Figure 1.

Pharmacologic intervention can increase primary cilia length. Osteocytes treated with fenoldopam (right) have elongated cilia compared to control (left). Cilia were stained with antibody against acetylated α -tubulin from a C3B9 hybridoma cell line, and visualized with Alexa Fluor 488 secondary antibody. Nuclei were stained with DAPI. Scale bar represents 20 μ m.

Figure 2.

The primary cilia mechanosensing apparatus may utilize distinct pathways in different cell types to initiate signaling cascades. One potential example is TRPV4 mediating primary cilia mechanosensing in osteocytes, while PC2 is dominant in kidney epithelial cells. Further proteomic and biosensor studies will discern cell-specific differences in cilia proteomes, enabling therapeutic interventions targeted at primary cilia of distinct cell types.

Table 1

Studies examining primary cilia-mediated mechanotransduction in various cell and tissue types. Works referenced in this review are summarized by cell type, method of mechanical stimulation, and functional readout.

Cell Type	Mechanical Stimulation	Readout
Embryonic node	Unidirectional nodal flow (110 $\mu\text{m/s}$; 14 hrs)	Left-right asymmetry with <i>Pkd2</i> and <i>Kif3a</i> knockout [12]
	Unidirectional flow in zebrafish node, <i>in vivo</i>	Left-right differentiation, and cytosolic and ciliary calcium [13]
	Unidirectional flow of mouse embryonic node (200 $\mu\text{m/s}$ for 2 sec)	Ciliary and cytosolic calcium [89]
Kidney epithelia	Unidirectional flow in MDCK cells (various flow rates, up to 50 nL/s)	Primary cilia bending stiffness [14]
	Micropipette perfusion and unidirectional flow in MDCK cells (8 $\mu\text{L/s}$ for 10 min)	Cytosolic calcium [15], [18]
	Unidirectional flow in IMCD cells (0.075 Pa for 3 hrs)	Cilium length and cAMP production [99]
	Unidirectional flow in MDCK cells (10-60 mPa, continuous and pulsed at 0.5 Hz, for 5 min)	Cytosolic calcium; cilia bending stiffness [90]
	Unidirectional flow of IMCD cells (0.25-1.5 Pa for 2.5 sec)	Cilia bending stiffness [91]
	Oscillatory fluid flow in IMCD cells (1 Pa at 1 Hz for 1-2 hrs)	Microtubule organization [85]
	Optical trap to displace MDCK cilia (Cilia displaced with a known force for calculation of bending stiffness)	Cilia bending stiffness [97]
	Unidirectional flow in IMCD cells (0.1 Pa for 10 min)	Cytosolic and ciliary calcium [86]
	Unidirectional flow in LLCPK cells (0.07 Pa for 2 sec)	Cytosolic and ciliary calcium [87]
	Unidirectional flow of IMCD cells (0.25 Pa for 2-10 min)	Cilia bending stiffness and rotation angle [92]
	Oscillatory flow of IMCD cells (1Pa at 1 Hz for 1 hr)	<i>COX2</i> mRNA expression [92]
	Unidirectional flow of IMCD cells (0.1 Pa for 5 min)	Ciliary and cytosolic calcium [66]
	Unidirectional flow of IMCD cells (0.001-0.1 Pa for 2 sec)	Ciliary and cytosolic calcium [89]
Endothelia	Unidirectional flow in mouse embryonic endothelial cells (equilibrated at 0-0.72 Pa for 10-30 minutes, followed by a step increase to 0.07-5 Pa)	Intracellular calcium and nitric oxide synthesis [25]
	Unidirectional flow in mouse endothelial cells (0.72 Pa for 90 seconds; blood pressure measured daily)	Nitric oxide synthesis and blood pressure [27]
	Unidirectional flow in primary chick endothelial cells (0.05-0.25 Pa for 5 hrs)	<i>KLF2</i> mRNA expression [28]
	Unidirectional flow in mouse embryonic endothelial cells (0.05-0.25 Pa for 2-24 hrs)	mRNA expression of endothelial and mesenchymal markers; histology [29]
Liver	Unidirectional flow in intact intrahepatic bile duct units (1-3 $\mu\text{L/s}$ for 1 min)	Cytosolic calcium, cAMP production [32]
Bone	Oscillatory flow in osteocytes and osteoblasts (1 Pa at 1 Hz for 1 hr)	Cytosolic calcium, whole cell mRNA expression [35]
	Compressive ulnar load (3.4 N at 2 Hz for 1 min)	Load-induced bone formation measured by dynamic histomorphometry [36]
	Oscillatory flow in osteocytes (1 Pa at 1 Hz for 2-30 min)	cAMP production; <i>COX2</i> mRNA expression [39]
	Compressive and tensile load applied to cortical bone implant (Force to displace implant up to 150 μm – calculated to be ~40% strain – for 1 min)	Histology for bone formation markers [41]

Cell Type	Mechanical Stimulation	Readout
	Oscillatory fluid flow in osteocytes (1 Pa at 1 Hz for 1-2 hrs)	Microtubule organization [85]
	Oscillatory flow in osteocytes (1 Pa at 1 Hz for 5 minutes)	Ciliary and cytosolic calcium; <i>COX2</i> mRNA expression [66]
	Unidirectional flow (250 μ m/s for 2 sec)	Ciliary and cytosolic calcium [89]
	Oscillatory flow in primary osteoblasts from idiopathic scoliosis patients (1 Hz for 0-16 hrs)	mRNA expression for wide range of genetic markers [107]
	Oscillatory flow in osteocytes (1 Pa at 1 Hz for 1 hr)	mRNA expression of <i>COX2</i> and <i>OPN</i> [103]
Mesenchymal progenitor	Oscillatory flow in osteocytes (0.5 Hz for 24 hrs)	mRNA expression of <i>OPN</i> , <i>COX2</i> , and <i>RUNX2</i> in mesenchymal progenitors cultured in conditioned media for 2-24 hrs [44]
	Oscillatory flow in human mesenchymal stem cells (1-2 Pa, 2 hrs, 0.5-48 hr post incubation time)	Mesenchymal progenitor osteogenic mRNA expression; proliferation [42]
	Oscillatory flow in osteocytes and osteoblasts (0.5 Hz for 24 hrs)	Mesenchymal progenitor proliferation [45]
	Compressive ulnar load (3 N at 2 Hz for 1 min)	Load-induced bone formation measured by dynamic histomorphometry [43]
Cartilage	Compressive strain of primary mouse chondrocytes (15% strain at 1 Hz for 1 hr)	Aggrecan, sGAG, cytosolic calcium, ATP, mRNA expression [48]
	Osmotic loading of porcine chondrocytes ($\pm$ 100 mOsm from iso-osmotic point for 3 min)	Cytosolic calcium [49]