Progress in ciliary ion channel physiology

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Mammalian cilia are ubiquitous appendages found on the apical surface of cells. Primary and motile cilia are distinct in both morphology and function. Most cells have a solitary primary cilium (9+0), which lacks the central microtubule doublet characteristic of motile cilia (9+2). The immotile primary cilia house unique signaling components and sequester several important transcription factors. In contrast, motile cilia commonly extend into the lumen of respiratory airways, fallopian tubes, and brain ventricles to move their contents and/or produce gradients. In this review, we focus on the composition of putative ion channels found in both types of cilia and in the periciliary membrane and discuss their proposed functions. Our discussion does not cover specialized cilia in photoreceptor or olfactory cells, which express many more ion channels.

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The Journal of General Physiology

A single cilium is about the size of a bacterium protruding from the surface of a cell (Fig. 1). Only 200–300 nm in diameter, cilia grow from periplasmic membrane centrioles and extend 1–15 µm into the extracellular space. Their hallmark structure is the axoneme, a rigid structure of microtubule doublets: in both primary and motile cilia, nine microtubular doublets form an internal cylindrical backbone that extends most of the length of the cilium. In motile cilia, two central doublets are also present, much like in the tail of spermatozoa (Fig. 1, A and B) or the cilia of unicellular organisms. In contrast, primary cilia lack fast motility and do not contain central doublets (Fig. 1 C). Nodal cilia are an exception to this rule, as they are motile but lack central doublets.

All eukaryotic cells have subcellular compartments, but the ciliary compartment differs from other organelles by its lack of a continuous membrane isolating it from the cytoplasm. However, this is not conceptually distinct from the nucleus, in which large nuclear pores allow ions and many proteins to exchange readily between compartments. In rough analogy to nuclear pores, cilia have a highly structured transition zone at the base of the cilia linking it, the plasma membrane, and the cytoplasmic centriole in the cytoplasm.

The fact that the primary cilium protrudes from the cell has evoked much speculation about it sensing flow force, the proximity of other cells, or chemical gradients. However, it is also noteworthy that the cilium is a semi-isolated cellular compartment with restricted protein trafficking, diffusion, and membrane lipid composition. Many cilia, such as those on adult neurons, are in tight intercellular spaces and do not experience high levels of flow. The volume of a cilium (e.g., ~0.5 fl) is 10,000-fold smaller than that of the cell and has a >1.5-fold larger surface/volume ratio. Any or all of these properties may be significant to the regulation of ciliary function.

In this review, we will discuss the various ion channels proposed to function in mammalian cilia and the experiments leading to such conclusions, as well as the potentially unique environment the cilia provides for ion channel function. The types of channels differ between primary and motile cilia. Primary cilia channels so far appear to be predominantly members of the polycystin family (e.g., PKD2, PKD2-L1), but investigators have reported other channels in or near cilia, including other transient receptor potential (TRP) channels such as TRPM4 and TRPV4, as well as the calcium-activated chloride channel ANO1. Ependymal motile cilia appear at present to be largely autonomous motors without significant regulation by the few voltage-gated calcium (Ca_V) channels present in their membranes. Voltage-gated potassium channels, TRP channels, and epithelial sodium channels (ENaCs) are also reported on motile cilia. Sperm flagella, although similar to motile cilia, have cationic channels of sperm (CatSper) channels that are unique to sperm flagella and crucial to their motion and force generation. In addition, numerous other channels and transporters function or are proposed to function in sperm flagella, including P2X2, Slo3, and sNHE in mice and KSper and Hv1 in humans (Miller et al., 2015).

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Abbreviations used: ADPKD, autosomal-dominant polycystic kidney disease; CatSper, cationic channels of sperm; Ca_V, voltage-gated calcium; GPCR, G protein–coupled receptor; Hh, Hedgehog; IMCD, inner medullary kidney collecting duct; Smo, Smoothened; TRP, transient receptor potential.

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The Rockefeller University Press $30.00
J. Gen. Physiol. 2017 Vol. 149 No. 1 37–47
https://doi.org/10.1085/jgp.201611696

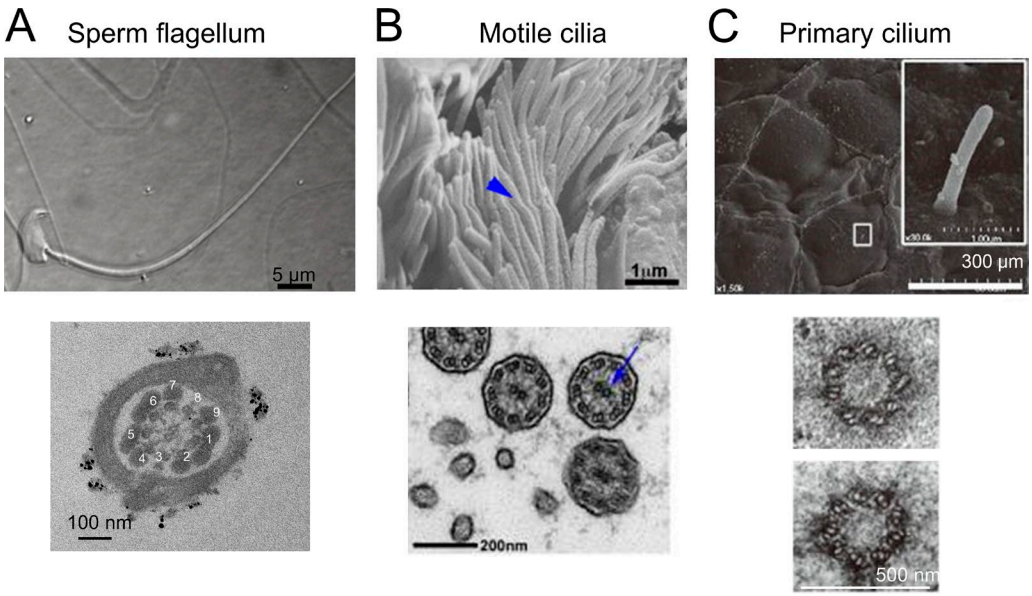

Figure 1. **Longitudinal (top) and cross-sectional (bottom) images.** (A) Mouse sperm with nine microtubule doublets surrounding a central pair (reproduced from Chung et al. [2014] with permission from Elsevier). (B) Ciliated (motile) cells (blue arrowhead) in a mouse respiratory tract with the characteristic nine doublets and central pair of microtubules (blue arrow; reproduced from Voronina et al. [2009] with permission from The Rockefeller University Press). (C) Primary cilium from a cell on a mouse limb bud. Note the absence of a central microtubule pair (reproduced from Moon et al. [2014] with permission from the National Academy of Sciences).

Ciliary structure and function

Scientists once considered primary cilia in mammalian cells to be vestigial with no clear function. The discovery that the absence or mutation of proteins that reside in cilia or are required for their assembly causes genetic disorders has dramatically altered this view (Novarino et al., 2011). To date, there are more than 50 known diseases, called ciliopathies, affecting primary or motile cilia. Because some of these proteins are present in both cilia and cytoplasm, or change in localization during cell division, this definition has some ambiguity.

Microtubule-based cilia and flagella were present in the ancestor of animals, the Urmetazoa, and their closest living relatives, the Choanoflagellates, more than 600 million years ago (Richter and King, 2013). Choanoflagellates are typically single-celled organisms that can form multicellular colonies and provide a template for understanding the development of multicellularity in animals. In these organisms as well as in the choanocyte cells of sponges, which are highly similar, the flagella are used to drive bacterial prey toward feeding collars for ingestion. Molecular phylogeny shows that the evolution of multicellular forms happened during a relatively short period during which signaling receptors and ligands (Wnt, Frizzled, Notch, Hedgehog [Hh], tyrosine kinases, and G protein–coupled receptors [GPCRs]) as well as adhesion (lectins and cadherins) and extracellular matrix receptors (integrins) greatly expanded (Richter and King, 2013). Because many of these transmembrane proteins and signaling pathways are found in cilia in modern-day animals, it is conceivable that this arrangement increased Urmetazoan fitness by acting as a chemosensory apparatus or trapping bacteria prey for ingestion. The fact that mammalian primary cilia retain many of these molecules and pathways might thus indicate that they preserve some essential functions required for formation of multicellular organisms.

Cilia play crucial roles even at the earliest stages of life. During embryogenesis, vertebrates establish a left–right axis that determines the development of organs such as the heart (Basu and Brueckner, 2008). Central to this early change from bilateral symmetry is a transient structure called the embryonic node, which contains several primary cilia. Although primary cilia are typically considered nonmotile, nodal cilia are known to be motile and beat in a stereotypic motion (Okada et al., 2005). This induces a leftward (viewed dorsally) fluid flow that is critical to the establishment of left–right asymmetry through poorly understood mechanisms (Nonaka et al., 1998; Buceta et al., 2005; Lee and Anderson, 2008). That the flow itself is essential has been proven by experiments in which reversal of the fluid flow leads to reversals of organ patterning (Nonaka et al., 2002). Popular hypotheses are that flow either leads to asymmetric distribution of secreted signaling components or that mechanosensors sense the flow and open a Ca^{2+}-permeant pathway (Tanaka et al., 2005; Yoshiba et al., 2012). In either case, this eventually leads to asymmetric gene expression.

The Hh pathway is the clearest illustration of the intimate coupling between cilia and cell signaling. Cru-

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cially, vertebrate Hh signaling requires a primary cilium (Huangfu et al., 2003; Huangfu and Anderson, 2005; May et al., 2005; Goetz et al., 2009). In the absence of a ligand, the receptor (and potential transporter), Patched (Ptch), is concentrated in the cilium (Rohatgi et al., 2007). Upon activation with an Hh ligand, Ptch exits the cilia to be replaced by the GPCR-like receptor, Smoothened (Smo; Corbit et al., 2005). Smo activity then leads to release of Gli transcription factors from the cilium that can be processed and translocated to the nucleus to activate target genes (Haycraft et al., 2005). Ciliary membranes house other GPCRs such as SSTR3 and GRP161, as well as growth factor receptors (Hilgendorf et al., 2016). The ion channels present in the primary cilium that may also effect signal transduction are just beginning to be elucidated.

Cilia present an interesting context for ion channel function. If we assume a 7-µm-long cylindrical cilium with a diameter of 300 nm (Delling et al., 2013), these dimensions would yield ~6.8-μm^2 surface area and a 0.5-fl volume. Compared with the cell body, this constitutes a larger surface area to volume ratio, conceivably exposing a larger proportion of ion channels to the same spatially limited extracellular stimulus (e.g., secreted ligand). As is true for other subcellular organelles, the same density of ion channels or transporters could alter the state of the small cilioplasmic volume much more rapidly than in the cell. Indeed, in primary cilium, resting concentrations of calcium ($[Ca^{2+}]$) are approximately sevenfold greater than in the cytoplasm (~700 vs. ~100 nM; Delling et al., 2013). Ca^{2+} changes in the cytoplasm propagate into the cilia, but the inverse is not true (Delling et al., 2013): the large cytoplasmic volume buffers and dissipates the number of free calcium ions diffusing from the small cilioplasmic volume.

To drive home the small size of a cilium, consider that 100 nM $[Ca^{2+}]$ corresponds to ~30 free Ca^{2+} ions in the 0.5-fl volume of a cilium. This is an approximation, as the details of geometry, buffers, and transporters in the cilium will affect this estimate. Because there is no membrane to delay the Ca^{2+} diffusion (Fawcett, 1954), it appears that large ciliary $[Ca^{2+}]$ influx into the limited volume of the cilia is a sufficient mechanism to maintain a $[Ca^{2+}]$ gradient. Thus, the cilium is a distinct compartment from the cytoplasm. An analogy is that of a freshwater outlet into the ocean; the ocean's waves and concentrations of saline affect the cove more profoundly than the cove's waters can affect the ocean. Different ionic concentrations in the cilia would have consequences for cellular signaling (especially in the case of $[Ca^{2+}]$) as well as for the electrochemical gradients that determine ion flux through ciliary ion channels. This is not the case for all cilia however; motile cilia have similarly small dimensions, but ciliary $[Ca^{2+}]$ is only slightly elevated over cytoplasmic $[Ca^{2+}]$ (Doerner et al., 2015). The difference may lie in the ion channels present in these different cilia.

The small dimensions of the cilium also introduce a considerable electrical resistance between cilioplasm and cytoplasm, thus creating another condition of nonuniformity. In another such case, the sharp geometric transition from the neuronal soma to the axon initial segment (AIS) is critical to the function of ion channels in the AIS membrane, which initiate action potentials (Baranauskas et al., 2013). In that situation, isolation of Na^+ channels from the capacitive load of the cell body allows the channels to effectively change the membrane potential of the axon. Cilia are fivefold narrower than axons and thus could provide even higher resistance (assuming similar internal resistance). Currently, we do not know whether this is relevant to the physiology of ion channels in ciliary membranes. In the section on primary cilia, we discuss an interesting case in a unique population of neurons.

Cilia have unique protein and lipid compositions (Verhey and Yang, 2016). As with any other protein class, the local ionic environment, binding proteins, and lipids all affect ion channels. Proteins are shuttled in and out of cilia by the intraflagellar transport (IFT) system (Mourão et al., 2016; Taschner and Lorentzen, 2016) but may also migrate into the cilia membrane from the plasma membrane (Milenkovic et al., 2009) or exchange via simple diffusion in solution. Ciliary ion channels would most likely be subject to sorting, trafficking, and endocytosis (Pedersen et al., 2016) similar to other ciliary transmembrane proteins. The motors, adaptors, and fellow travelers in this system may regulate ciliary ion channels spatially and temporally.

Finally, the lipid content of cilia is also distinct from that of the plasma membrane. $PI(4,5)P_2$, is a known modulator of many ion channel and transporter functions (Hille et al., 2015), but phosphatidylinositol 4-phosphate (PI4P) predominates over $PI(4,5)P_2$ in cilia (as it does in Golgi). One hypothesis is that cilia could also contain significant $PI(4,5)P_2$ at times (by diffusion from plasma membrane), which is switched to PI(4)P by Tubby-like protein (TULP3)–mediated trafficking of InPP5E (phosphoinositide 5-phosphatase) into cilia. Thus, this switch in the PIP state of the cilia may favor certain GPCRs or channels within cilia (Chávez et al., 2015; Garcia-Gonzalo et al., 2015). Lipid remodeling is a crucial part of sperm physiology, and cholesterol efflux is a marker of sperm capacitation (Cohen et al., 2016; Stival et al., 2016). Except for sperm flagella, the relative abundance of other lipids in the cilium's membrane, such as cholesterol, is not well known.

Primary cilia ion channels

In several primary cilia measured to date, basal levels of $[Ca^{2+}]$ are higher (Delling et al., 2013; Yuan et al., 2015),

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and membrane potential is ~30 mV depolarized relative to the cell body (Delling et al., 2013). Because most cells contain a nonmotile primary cilium, this may represent an important signaling environment within the cell. An interesting but unproven hypothesis is that ciliary calcium concentrations affect calcium-dependent adenylyl cyclases to regulate the known potent PKA-dependent inhibition of the Hh pathway (Tuson et al., 2011). Current experiments are only beginning to address links between ciliary calcium and cAMP levels (Moore et al., 2016). Other signaling pathways within primary cilia are present, and thus, many other possibilities exist.

Several proteins (e.g., TRPM4, TRPV4, TRPC1, PKD1, and PKD2; Tsiokas et al., 1999; Pazour et al., 2002; Yoder et al., 2002; Gradilone et al., 2007; Köttgen et al., 2008; Flannery et al., 2015; Kleene and Kleene, 2016) have been proposed as ciliary ion channels. With the exception of polycystin-1 (PKD1), these proteins are members of the TRP family of ion channels (Ramsey et al., 2006). PKD1s are a family of putative 11-transmembrane–spanning domain proteins of unknown function (The International Polycystic Kidney Disease Consortium, 1995; Hughes et al., 1995; Yuasa et al., 2002). PKD1 has been proposed to be a GPCR (Parnell et al., 1998, 2002), an integral component of a channel complex via its last six transmembrane domains (Ponting et al., 1999; Hanaoka et al., 2000), and a novel receptor (Kim et al., 2016). The PKD1 family member PKD1-L1 (L1 for Like-1) differs from PKD1 primarily in its N-terminal domain. Within this 1,748–amino acid N terminus are multiple extracellular recognition domains and an autocleavage domain known as the GAIN domain (Yuasa et al., 2002; Araç et al., 2012).

In contrast to the mysterious function of PKD1s, PKD2 family members have primary sequence and putative structure similar to TRP channels. PKD2-L1 was identified by homology to PKD2; mice with PKD2-L1 deletions have kidney and eye deficits (Nomura et al., 1998). PKD2-L1 has also been implicated in the detection of sour taste (Horio et al., 2011). Direct patch clamp of primary cilia from retina pigmented epithelium and embryonic fibroblasts (Fig. 2 A) showed that genetically ablating PKD1-L1 or PKD2-L1 reduces the constitutively active ciliary current (DeCaen et al., 2013). However, given the huge variety of cells that have cilia, other channels are likely to be present. Indeed, PKD2 has recently been identified as a subunit of the ciliary ion channel in the inner medullary kidney collecting duct (IMCD3) cell line (Kleene and Kleene, 2016), with a conductance similar to PKD1-L1/2-L1 heteromers but with distinct cation selectivity. The ciliary PKD2-dependent conductance prefers potassium over calcium and sodium ions, with relative permeabilities $P_K/P_{Ca}/P_{Na}$ of 1:0.6:0.1 (Kleene and Kleene, 2016). PKD2's preference for potassium over sodium is supported by the recent report of the PKD2 single partial cryo-electron microscopy structure (Shen et al., 2016). Here, grafting the pore helices of PKD2 onto the PKD2-L1 channel suggests that PKD2 is more permeant to K^+ and Na^+ than Ca^{2+}, with $P_K/P_{Ca}/P_{Na}$ of 2:0.5:1. In contrast, PKD1-L1/2-L1 channels were more selective for calcium with $P_K/P_{Ca}/P_{Na}$ of 1:6:1 (DeCaen et al., 2013). These important developments confirm that PKD2 is a ciliary ion channel and challenges the view that PKD2 is a calcium-selective channel. Future work will determine which channels are present in the cilia of native epithelial cells within the lumen of the kidney-collecting duct. Interestingly, although heterologous expression of PKD2-L1, but not PKD1-L1, is capable of supporting currents in the plasma membrane, the full complement of ciliary ion channel features depends on their coexpression in cilia (DeCaen et al., 2013). PKD2-L1 coimmunoprecipitates PKD1-L1 and mice lacking PKD2-L1 lack the constitutively active current (Fig. 2 A; DeCaen et al., 2013). The exact composition/stoichiometry of a putative PKD1-L1/PKD2-L1 ciliary ion channel is unknown.

Extracellular uridine and adenosine phosphates (Fig. 2 B), as well as the calmodulin blocker calmidazolium, potentiate the PKD2-L1 channel in recordings from cilia (DeCaen et al., 2013). Whole ciliary currents are more calcium selective and outwardly rectify, reminiscent of other TRP currents (Fig. 2 C). Estimates place channel density in the cilium at 30 channels/μm^2, which is comparable with other specialized ion channel signaling domains in intracellular organelles such as lysosomes and mitochondria (DeCaen et al., 2013). As discussed previously, $[Ca^{2+}]$ permeability may explain how the cilia maintains $[Ca^{2+}]$ higher than in the cytoplasm (Delling et al., 2013). Intriguingly a mechanism for negative feedback may exist in the PKD2-L1 protein itself. Patch clamp experiments of heterologously expressed PKD2-L1 showed that at high internal $[Ca^{2+}]$ levels, the channel inactivates (DeCaen et al., 2016). Thus, this channel may help to ensure a desired range of ciliary $[Ca^{2+}]$ by allowing Ca^{2+} into the cilium when levels are low and by inactivating to prevent further Ca^{2+} entry when levels are high.

The fact that nucleoside phosphates and calmodulin antagonists potentiate the ciliary current (DeCaen et al., 2013) may be useful in dissecting out the function of the PKD2-L1 complex. The primary question is the functional significance of PKD2-L1 activation and subsequent alteration of primary cilia $[Ca^{2+}]$, $[Na^+]$, and the ciliary membrane potential. Although the most common hypothesis is that cilia are mechanical sensors that lead to Ca^{2+} influx (Praetorius and Spring, 2001; Nauli et al., 2003; Kindt et al., 2012; Lee et al., 2015; Grimes et al., 2016), no such changes in ciliary Ca^{2+} are detectable in primary cilia of various tissues over a wide range of mechanical force (Delling et al., 2016). In con-

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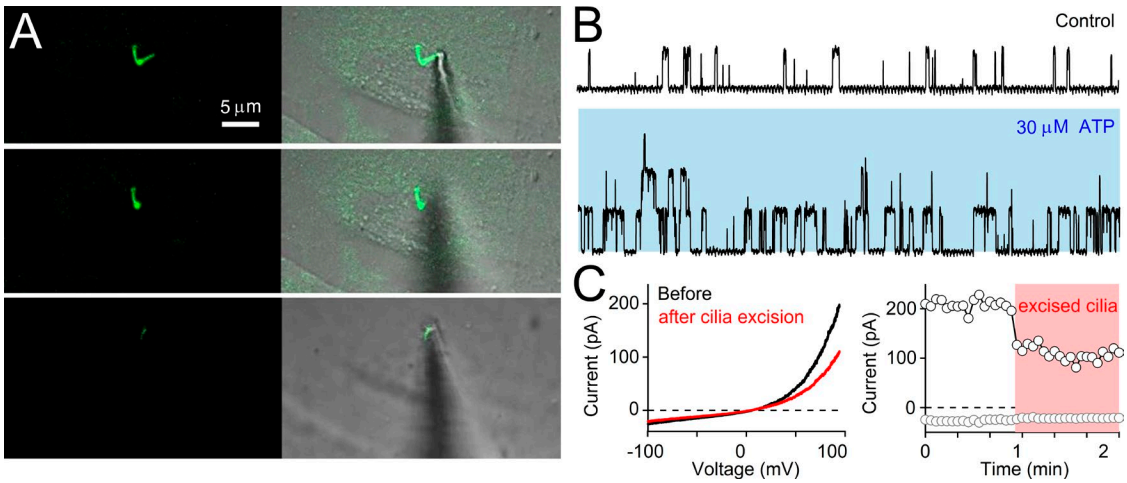

Figure 2. **Patch clamp of primary cilia.** (A, top) A patch pipette sealed to the membrane of a primary cilium (identified by GFP-tagged *Smo*) from RPE cells. (middle) The pipette is raised in order to pull the cilium from the plasma membrane. (bottom) The excised cilium remains attached to the pipette but detached from the cell. (B) Single channel openings in the ciliary membrane are potentiated by application of ATP in the on-cilia configuration. (C) Whole ciliary currents before and after cilia excision show that the majority of the current is conducted by channels in the ciliary membrane and not indirectly via channels in the plasma membrane. The current outwardly rectifies similar to other TRP currents. Modified from DeCaen et al. (2013) with permission from Nature Publishing Group.

trast, stereocilia (actually actin-based microvilli despite the name) exhibit a threefold increase in local Ca^{2+} upon physical deflection (Fig. 3; Delling et al., 2016). Unlike the higher density of Ca^{2+} permeant channels in primary cilia (DeCaen et al., 2013), the truly mechanosensitive stereocilia contain only a handful of mechanosensitive channels per stereocilium (Denk et al., 1995; Beurg et al., 2009). PKD2-L1 openings were not very sensitive to changes in membrane pressure (DeCaen et al., 2013), and thus, this channel is unlikely to be a physiologically relevant mechanosensor.

A recent study of cerebrospinal fluid–contacting neurons (CSF-cNs) contains a possibly important consequence of PKD2-L1's large conductance. These neurons line the central canal of the nervous system with primary cilia on their cell bodies (Orts-Del'Immagine et al., 2014). Mathematical estimates indicate that a single PKD2-L1 opening in the primary cilium may be sufficient to trigger an action potential (Orts-Del'Immagine et al., 2016). Because PKD2-L1 is present throughout CSF-cNs, whether the cilium is the primary site for PKD2-L1 function in these neurons remains an open question.

Based on immunocytochemistry and colocalization analyses with PKD2, TRPV4 (Köttgen et al., 2008) and TRPC1 (Bai et al., 2008) have been proposed to be ciliary ion channels. Taken as further evidence are reports that both TRPV4 (Köttgen et al., 2008; Stewart et al., 2010) and TRPC1 (Bai et al., 2008; Kobori et al., 2009; Zhang et al., 2009) form heteromeric channel complexes with PKD2 that give rise to unique ion channel properties. But as we discuss, it is not clear that PKD2 channel current has been correctly identified. Most important, there are no direct ciliary measurements of TRPV4 and TRPC1 activity. One study in renal collecting duct cells indicates that TRPV4's function may be more important in cells that do not have cilia (Zhang et al., 2013).

Whole cilia recordings have proposed that TRPM4, a Ca^{2+}-activated, voltage-dependent channel, is active in cilia as well as in the apical membrane (Flannery et al., 2015). However, these whole cilia recordings encompassed the entire, intact cilia with the pipette forming a seal at the ciliary base, similar to methods used to measure from olfactory cilia (Kleene and Gesteland, 1991). Because these recordings were not confined to the cilia tip, it is possible that TRPM4 is located at the regions of the cilia near or in the plasma membrane. Further work should settle this issue.

Thus far, we have focused on channels present in the ciliary membrane. Interestingly, the ANO1 (TMEM16A) calcium-activated chloride channel is found on the apical membrane of cells before the formation of cilia. Loss of ANO1 function interferes with ciliogenesis (Ruppersburg and Hartzell, 2014), suggesting that this channel may regulate vesicle fusion at the site of future cilia formation.

Polycystic kidney disease proteins

Autosomal-dominant polycystic kidney disease (ADPKD) is caused by mutations in the *Pkd1* (~85%) or *Pkd2* (~15%) genes (Wu and Somlo, 2000). Morbidity is associated with primarily renal, but also liver and pancreatic cysts, and vascular abnormalities such as intracranial aneurysms and aortic artery defects. ADPKD is a common monogenetic disorder for which there are no effective

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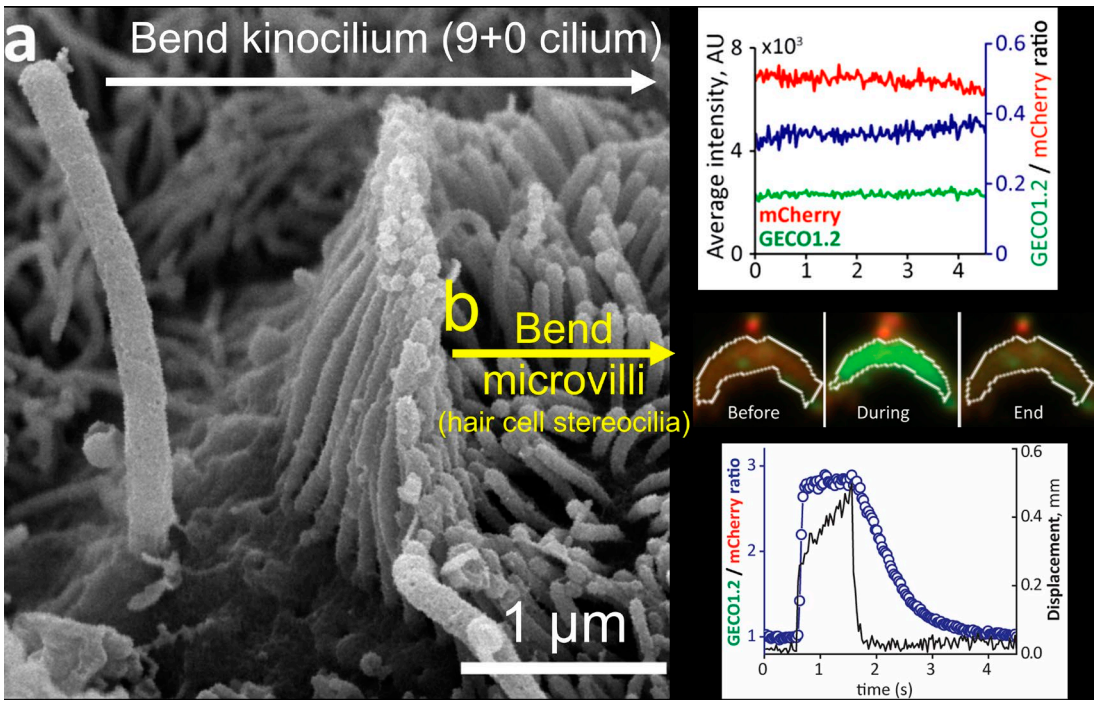

Figure 3. **Primary cilia are not calcium-responsive mechanosensors.** (a) The sensory epithelium of the inner ear contains hair cells that are required for hearing. The hair cells have chevron-shaped rows of actin-based microvilli, called stereocilia (by definition, not true cilia). At the tip of each chevron is a single cilium, called a kinocilium. Because a known mechanosensor (stereocilium) is adjacent to a true cilium, the Ca^{2+}-responsive mechanosensitivity of the true cilium can be objectively tested. When flow bends both structures, [Ca^{2+}] remains constant in the adjacent kinocilium. (b) In contrast, the established mechanosensitive stereocilia display robust Ca^{2+} increases in response to bending. Modified from Delling et al. (2016) with permission from Nature Publishing Group.

therapies at present (Harris and Rossetti, 2010). Prior evidence suggests that ADPKD is associated with malfunction of cilia (i.e., ciliopathies) presumably caused by dysfunctional or mislocalized PKD1 and PKD2 proteins in the cilia membrane of IMCD epithelial cells (Qian et al., 1997; Grimm et al., 2003). Several studies show that PKD1 and PKD2 require each other for proper localization and function (Bertuccio et al., 2009; Chapin et al., 2010; Cai et al., 2014b; Kim et al., 2014; Gainullin et al., 2015; Trudel et al., 2016). A popular hypothesis is that PKD1 and PKD2 form a heteromeric ion channel that conducts Ca^{2+} through the ciliary or plasma membrane (Hanaoka et al., 2000). Observations of mechano- or chemically induced Ca^{2+} transients in kidney-derived Madin–Darby canine kidney (MDCK) and IMCD cells support this hypothesis (Praetorius and Spring, 2001; Nauli et al., 2003). However, these measurements do not resolve the origin of the Ca^{2+} transients, which are likely propagated from the cytoplasm (Delling et al., 2016). Indeed, cilia removal from MDCK cells did not abolish flow-induced Ca^{2+} transients, which instead were dependent on autocrine purinergic receptor activation (Hovater et al., 2008; Egorova et al., 2012; Rodat-Despoix et al., 2013). Interestingly, two groups have reported activation of cationic currents by purinergic activators when recording from the primary cilia of retinal pigmented epithelium (RPE) and IMCD cells (Kleene and Kleene, 2012; DeCaen et al., 2013). Based on single channel measurements in the primary cilia, channel modulation by ATP and ADP was shown to be indirect, possibly downstream of the activation of an unidentified GPCR.

Heterologous PKD2 ionic currents measured from the plasma membrane are controversial. Results vary with respect to PKD2 membrane localization or function in the absence of PKD1 (Hanaoka et al., 2000; Bertuccio et al., 2009) and whether a ligand is required for activity (Le et al., 2004; Ma et al., 2005; Kim et al., 2016). In some cases, PKD1 is reported to be a putative channel independent of PKD2 (Babich et al., 2004). In another study, heterologous expression of PKD1 and/or PKD2 did not enhance plasma membrane currents (DeCaen et al., 2013). Multiple studies suggested that heterologous PKD2 (or PKD1 + PKD2) produces a small ohmic current, but it is worrisome that they were not clearly distinct from endogenous channels found in host cells (Hanaoka et al., 2000; Feng et al., 2008), or indeed from leak current.

A recent study expressing a mutant form of PKD2 (F604P) in *Xenopus laevis* oocytes suggests that the WT PKD2 is not constitutively active, is cationic nonselective, and is blocked by Ca^{2+} (Arif Pavel et al., 2016). Furthermore, the recently identified PKD2 current recorded from IMCD cilia is most selective for potassium (Kleene

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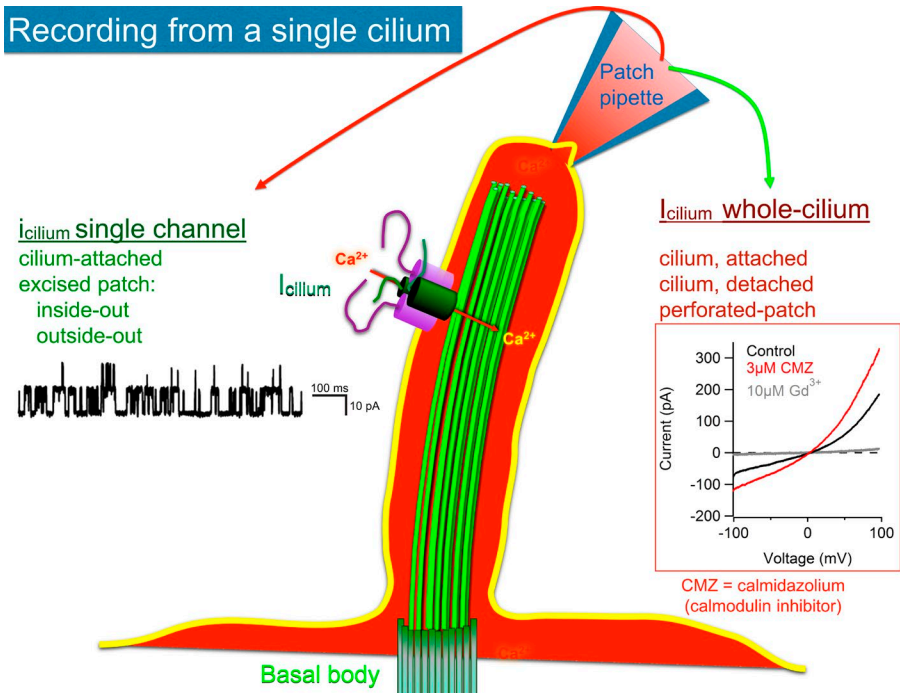

Figure 4. **Direct recording of currents in cilia using patch clamp methods.** A gigaohm seal with a patch pipette onto the ciliary membrane enables measurement of single channel activity. Alternatively, "breaking into" the cilia allows for recordings of whole ciliary currents, even when cilia are then completely detached from the cell.

and Kleene, 2016). These results contrast with reports that heterologous PKD2 (PKD1 + PKD2) is selective for calcium. The proposed location of F604 is in the fifth transmembrane helix of PKD2, and thus, the activating F604P mutation may create a bend in the activation gate, opening the pore and activating the channel (Arif Pavel et al., 2016). Given the lack of function of WT PKD2, these data suggest a significant conformational change must occur for the channel to open as supported by recent structural studies (Shen et al., 2016). In particular, the large extracellular "PKD domain" may function as a "lid" on the channel (Shen et al., 2016). It is possible that ligand binding or heteromerization with another channel subunit might surmount this energy barrier.

Motile cilia

The most well understood role of ion channels in vertebrate cilia is that of the CatSper Ca^{2+}-selective channels in sperm flagella (CatSpers 1–4 and additional subunits, β, γ, δ; Ren et al., 2001; Kirichok et al., 2006; Liu et al., 2007; Qi et al., 2007; Chung et al., 2011). A change in sperm environment (pH, voltage, progesterone; Ren et al., 2001; Kirichok et al., 2006; Lishko et al., 2011; Strünker et al., 2011) triggers the CatSper channels to allow Ca^{2+} influx into sperm. Calcium sensors linked to phosphorylation cascades alter the shape and frequency of flagellar motion, a process called hyperactivated motility. This process is essential for mammalian sperm to traverse the oviduct and fertilize the oocyte. Mouse sperm express CatSpers, Slo3, and P2X2 channels, whereas human sperm express CatSpers, Slo3, and Hv1 channels. Although motile cilia and sperm flagella have many structural similarities, they have diverged significantly in their regulation, perhaps because of the significant evolutionary pressure on sperm (Cai et al., 2014a). To date, none of the sperm channels appear to be present in mammalian motile or primary cilia. A thorough discussion of CatSpers and the other ion channels in mammalian sperm has been recently published (Miller et al., 2015).

Although sperm are the only flagellated mammalian cells, motile cilia are present in ependymal cells lining the brain ventricles and epithelial cells lining respiratory airways and fallopian tubes. Whereas sperm use their modified cilium to propel themselves through fluid, somatic cells use their cilia to propel fluid through luminal spaces (Enuka et al., 2012; Faubel et al., 2016). As is the case in primary cilia, members of the TRP family of ion channels (TRPV4, PKD1, and PKD2) have been reported to be present in motile cilia (Bloodgood, 2010).

Immunohistochemistry suggests that TRPV4 localizes to the cilia of tracheal cells and epithelia of reproductive organs (Teilmann et al., 2005; Lorenzo et al., 2008). Activation of TRPV4 with stimuli such as agonists, warm temperature, or increased fluid viscosity led to increased cytosolic Ca^{2+} and ciliary beat frequency (Andrade et al., 2005; Lorenzo et al., 2008). At present, there are no recordings of TRPV4 currents from these cilia. PKD1 and PKD2 have also been localized by immunohistochemistry to epithelial cells of reproductive organs, but their function there has yet to be clarified (Teilmann et al., 2005). Given the frequency of inaccurate identification of proteins by antibodies alone, we place more credence in definitive functional measurements of ion channels under voltage clamp to establish localization.

Whole-cilia patch clamping of brain ependymal cells revealed that they contain a large potassium conduc-

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tance and Ca_V channels, although the precise identities are not established (Doerner et al., 2015). Unlike sperm flagella and TRPV4 activation in tracheal cells, depolarization-induced increases in ciliary [Ca^{2+}] did not lead to any significant changes in ciliary beating (Doerner et al., 2015). Moreover, Ca_V channels do not seem to be enriched in the ciliary membrane compared with the rest of the cell membrane (Doerner et al., 2015). We suspect that the membranes of ependymal motile cilia are more similar to the cell's plasma membrane, unlike primary cilia. Motile ependymal cilia have similar resting [Ca^{2+}] levels and membrane potentials as cytoplasm. Currently, it is unknown whether similar observations will hold true for motile cilia in the respiratory tracts and fallopian tubes.

Immunohistochemical experiments have identified abundant and uniform expression of ENaCs on the motile cilia of the fallopian tubes and bronchi (Enuka et al., 2012). However, functional measurements have not yet confirmed these findings.

Future directions

Ciliary ion channels are topics of active investigation. Although several channels localize to cilia via antibody labeling, the direct measure of function is patch clamp of these channels in their native environments (Fig. 4). Correlation of phenotypes of knockout animals with cilia-specific function will further corroborate results of functional and structural studies. The large body of research on ciliopathies will greatly aid this endeavor. Much more work is required to understand how ion channels regulate these subcellular structures' functions. Thus far, different types of cilia (primary vs. motile) seem to possess different ion channels. Another area of future research will be to determine the true diversity of ion channels, or lack thereof, in cilia of different organs during development and in adults.

ACKNOWLEDGMENTS

The authors declare no competing financial interests.
Lesley C. Anson served as editor.

Submitted: 14 September 2016
Accepted: 6 December 2016

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