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REVIEW

SUBJECT COLLECTION: CILIA AND FLAGELLA

Ciliary mechanosensation – roles of polycystins and mastigonemes

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ABSTRACT

Cilia are surface-exposed organelles that provide motility and sensory functions for cells, and it is widely believed that mechanosensation can be mediated through cilia. Polycystin-1 and -2 (PC-1 and PC-2, respectively) are transmembrane proteins that can localize to cilia; however, the molecular mechanisms by which polycystins contribute to mechanosensation are still controversial. Studies detail two prevailing models for the molecular roles of polycystins on cilia; one stresses the mechanosensation capabilities and the other unveils their ligand–receptor nature. The discovery that polycystins interact with mastigonemes, the 'hair-like' protrusions of flagella, is a novel finding in identifying the interactors of polycystins in cilia. While the functions of polycystins proposed by both models may coexist in cilia, it is hoped that a precise understanding of the mechanism of action of polycystins can be achieved by uncovering their distribution and interacting factors inside cilia. This will hopefully provide a satisfying answer to the pathogenesis of autosomal dominant polycystic kidney disease (ADPKD), which is caused by mutations in PC-1 and PC-2. In this Review, we discuss the characteristics of polycystins in the context of cilia and summarize the functions of mastigonemes in unicellular ciliates. Finally, we compare flagella and molecular features of PC-2 between unicellular and multicellular organisms, with the aim of providing new insights into the ciliary roles of polycystins in general.

KEY WORDS: Polycystin, Mastigoneme, Cilia, Flagella, Mechanosensation

Introduction

Cilia and flagella are synonymous in eukaryotic cells, describing the hair-like structures that protrude from the cell surface into the surrounding space. A cilium consists of two parts: the axoneme, which is the inner microtubule that elongates from the centriole core, and the outer ciliary membrane sheath, which is continuous with the cytoplasm membrane (Emmer et al., 2010; Li et al., 2012; Reiter et al., 2012). Based on their ability to beat, cilia can be further categorized into non-motile and motile cilia. Non-motile cilia lack the ability to beat and are referred to as the primary cilia in mammalian cells. Non-motile cilia are capable of transducing signaling cascades and contribute to embryo and organ development (for reviews on this topic, see Anvarian et al., 2019; Huangfu and Anderson, 2005; Nachury, 2014; Nachury and Mick, 2019; Sreekumar and Norris, 2019). Meanwhile, motile cilia provide locomotion or coordinate beating to push liquid across the cell surface. For example, on airway epithelia, motile cilia move mucus out of the lung, and their disruption causes airway disease, classified as primary ciliary dyskinesia (PCD) (Afzelius, 1976; Legendre et al., 2021). Most motile cilia move in a whip-like beating pattern, although a small group of primary cilia that are only transiently present on the embryo node, called the nodal cilia, whirl instead of beating, breaking the bilateral symmetry in most vertebrates during embryonic development (McGrath et al., 2003).

Much recent attention has been focused on the sensory roles played by cilia, such as those involved in photoreception, chemoreception or mechanoreception (Delling et al., 2016; Shah et al., 2009). Indeed, cilia could be considered the 'ancient' organelles for receiving mechanical stimuli. Already in 1898, when describing primary cilia on kidney epithelial cells, Karl Wilhelm Zimmermann discussed a possible mechanoreceptive role for primary cilia; he speculated that "one could also imagine that this delicate flagellum […] may work as a kind of sensory organ" (Zimmermann, 1898). It took more than 100 years for subsequent researchers to demonstrate that fluid flow induces bending of cilia and activates stress-sensitive plasma membrane channels, increasing the movement of Ca^{2+} into epithelial cells (Resnick, 2010, 2015; Vilfan et al., 2010). In this Review, we discuss the mechanosensation role of cilia, as well as the prevailing working models debating the mechanosensory properties of polycystin-1 and -2 (PC-1 and PC-2, respectively), which are cilia-localized multi-span membrane proteins, mutations of which are the most common cause of autosomal dominant polycystic kidney disease (ADPKD). In particular, we focus on mastigonemes, as they have been recently identified to interact with polycystins, and we summarize the functions of mastigoneme structures in unicellular ciliates, with the aim of providing new insights into the molecular mechanisms of polycystins in cilia.

Mechanosensory cilia

A conserved function of cilia

The importance of the mechanosensory functions of cilia is exemplified by their conserved existence across organisms in a number of tissues. For example, primary cilia in endothelial cells lining blood vessels function as mechanosensors that sense blood flow and mediate angiogenesis in zebrafish (Goetz et al., 2014; Kallakuri et al., 2015). Osteocyte primary cilia react to mechanical stress to regulate osteogenic and bone-resorptive responses, as well as to regulate cartilage development (Malone et al., 2007; Xiang et al., 2019; Yuan and Yang, 2016). Motile cilia in ciliated respiratory cells always beat in a consistent way, regardless of viscous load, to propel mucus along the respiratory tract (Johnson et al., 1991). Cerebrospinal fluid-contacting neurons (S-CSF-cNs) require motile cilia to perform critical mechanosensory functions (Orts-Del'Immagine et al., 2020). In mouse brain ventricles, reorientation of basal bodies on the apical surface of cells requires

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hydrodynamic forces of flowing fluid and planar cell polarity (PCP) signaling, indicative of mechanosensation-mediated morphogenesis of motile cilia (Guirao et al., 2010). Motile cilia of the mammalian respiratory epithelium exhibit mechanosensation combined with chemosensation. Airway epithelia have been found to express bitter-taste sensory receptors, which localize on motile cilia (Shah et al., 2009). Bitter compounds increase the intracellular Ca^{2+} concentration and stimulate ciliary beat frequency to remove hazards (Shah et al., 2009). Taken together, these studies highlight that cilia represent conserved structures that are present in multiple organisms and are crucial to the healthy functioning of a number of tissues.

Debated mechanisms of ciliary mechanotransduction

Despite the numerous examples of mechanosensory cilia, the nature of the underlying mechanotransduction and the possible involvement of Ca^{2+} influx is still debated. In cultured Madin–Darby canine kidney (MDCK) cells and mouse primary inner medullary collecting duct (mIMCD) epithelial cells, the flow-induced mechanosensation and Ca^{2+} concentration change in cilia has been thoroughly studied (Delling et al., 2016; Jin et al., 2014; Praetorius and Spring, 2001). Experimental data show that the primary cilium and motile cilia function as flow sensors and mediate a large increase in intracellular Ca^{2+} concentration (Praetorius and Spring, 2001, 2003a,b; Yuan et al., 2015). The current model of mechanosensation in cilia suggests that the liquid flow (such as that which occurs in kidney tubules) bends the cilia on the apical surface of the epithelial cells, and the resulting ciliary membrane curvature activates mechanosensitive channels or rearranges the cytoskeleton network (Lee et al., 2015).

To distinguish whether the intracellular Ca^{2+} concentration change indeed originates from inside the cilia, researchers have used genetically encoded Ca^{2+} indicators (GECIs) and microscopy to directly observe the *in vivo* Ca^{2+} concentration change inside cilia by changes in fluorescence signal. In mouse fibroblasts and kidney cells, it has been reported that Ca^{2+} spiking triggered by fluid shear stress is initiated in the primary cilium and can be distinguished from the subsequent cytosolic Ca^{2+} spikes (Jin et al., 2014; Su et al., 2013). A similar observation has been made in the primary cilia of osteocytes; here, a Ca^{2+} concentration increase upon fluid flow can be detected using a fluorescence resonance energy transfer (FRET)-based biosensor fused to ARL13B, a cilia marker (Lee et al., 2015). However, one recent study has challenged this prevailing model (Delling et al., 2016). Using mIMCD epithelial cells and mouse embryonic fibroblast cells isolated from GECI-expressing transgenic mice, the Ca^{2+} concentration inside cilia has been found to not significantly change when cilia are mechanically bent by an applied liquid jet (Delling et al., 2016). The authors further argue that, due to the small volume of the cilium compared to that of the cell body, even if the Ca^{2+} concentration inside the cilium does change upon mechanical stimulation, it is unlikely that the global cytosolic Ca^{2+} concentration will be substantially altered (Delling et al., 2016). The significance of Ca^{2+} concentration change in cilia, therefore, is still under debate. As laminar fluid flow can sweep across both the cilium and apical plasma membrane, a more precise way of controlling mechanostimulation is therefore required to validate the mechanosensory role of cilia. By combining GECIs, super-resolution microscopy and optogenetics, two recent studies have successfully used optical tweezers to deflect non-motile cilia in the left–right organizer (LRO) of zebrafish and the mouse embryo node (Djenoune et al., 2023; Katoh et al., 2023). Such deflections are observed to induce detectable intraciliary Ca^{2+} transients, including a repetitive response to prolonged mechanical oscillation of the cilium, revealing that cilia are the mechanosensor that mediates Ca^{2+} signaling. An interesting point is that the cilia require repetitive mechanical bending (~30 times) to generate a Ca^{2+} spike, instead of reacting to the deflection immediately. This scenario might allow cilia to discriminate between true (repetitive) and false (random) stimuli by filtering out extraneous biomechanical noise, including non-directional cytoskeletal movements (Djenoune et al., 2023). In the future, GECIs, optogenetics and super-resolution microscopy could be applied to detect Ca^{2+} dynamics in the kidney cilia. For different GECIs and their working condition, see Nauli et al. (2016). Understanding the links between cilia and cytoplasm upon mechanical stimuli, including the trafficking of messenger molecules and the downstream signaling cascades, is therefore important for elucidating the mechanosensation circuit. Renal epithelial cells have also been shown to produce nitric oxide (NO), which can respond to fluid shear stress and regulate downstream gene expression (Cai et al., 2000; 't Hart et al., 2021). Therefore, it may also be informative to explore readouts other than Ca^{2+}, such as NO (Nauli et al., 2013).

Polycystins – the answer to cilia mechanosensation?

ADPKD is one of the most common kidney diseases, affecting millions of people worldwide (Gallagher et al., 2010; Torres et al., 2007). Most of the mutations associated with ADPKD occur within the *PKD1* and *PKD2* genes, which encode PC-1 and PC-2, respectively. Since PC-1 and PC-2 localize to cilia (Barr and Sternberg, 1999; Huang et al., 2007; Pazour et al., 2002; Yoder et al., 2002), ADPKD is considered a ciliopathy. Evidence from patients and animal models suggests that Ca^{2+} signaling is an important factor in the pathogenesis of ADPKD (Ferreira et al., 2015). Specifically, mutations in polycystins affect intracellular Ca^{2+} homeostasis and numerous additional signaling pathways (Nagao et al., 2008; Yamaguchi et al., 2006). Furthermore, in the mouse embryo node and the LRO of zebrafish embryos, which are required for left–right asymmetry of body morphology, the Ca^{2+} transients responding to mechanical stimuli in cilia and the cytoplasm are lost in the absence of PC-2 (Djenoune et al., 2023; Katoh et al., 2023). Taken together, these observations suggest that understanding polycystin function is important in a range of biological contexts that involve cilia, from ADPKD to body morphology. In this section, we review the recent progress in polycystin research by discussing the working models of PC-1 and PC-2 on cilia.

Molecular features of polycystins

PC-1 is a megadalton-sized receptor-like protein; it has 11 transmembrane regions and an extensive extracellular N-terminal fragment (NTF) consisting of several annotated domains, including a C-type lectin (CTL) domain and the G-protein-coupled receptor autoproteolysis-inducing (GAIN) domain (Hardy and Tsiokas, 2020). PC-1 exhibits features of both a channel and a receptor, acting, for example, as a receptor for WNT ligands (Kim et al., 2016). PC-2 belongs to the transient receptor potential (TRP) channel family, which shows a conserved structure across species. PC-2 comprises six transmembrane regions. The extracellular tetragonal opening for polycystin (TOP) domain of PC-2 is situated between transmembrane regions 1 and 2, functioning as the putative 'lid' for the channel (Shen et al., 2016; Yu et al., 2009). PC-2 mediates movement of Ca^{2+} and monovalent cations (Na^+ and K^+) across membranes (Liu et al., 2018). Indeed, mutations in the TOP domain and those affecting the channel permeability are hotspots for pathogenic mutations (Grieben et al., 2017; Su et al., 2018).

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Using lipid bilayer nano-discs, it has been demonstrated that PC-2 forms a homotetramer (Shen et al., 2016). Using purified truncated polycystins from the human embryonic kidney (HEK) 293F cell line, another study has found that PC-1 and PC-2 form heterotetramers with a 1:3 stoichiometry (Su et al., 2018). A different study has shown that on cilia membranes, polycystin homo- and hetero-tetramers may both exist *in situ* (Ha et al., 2020). Moreover, PC-2 homotetramers exhibit a higher probability of forming an open conformation than the PC-1–PC-2 heterotetramers, suggesting that PC-1 negatively regulates the channel properties of PC-2 (Ha et al., 2020). This observation is consistent with reports that PC-1 regulates PC-2 channel activity after PC-1 binding to a ligand; for example, WNT proteins have been shown to bind a region of the NTF of PC-1 and induce whole-cell currents and Ca^{2+} influx that are dependent on PC-2 (Kim et al., 2016).

Polycystins are integral membrane proteins and are thought to be freely diffusible on the membrane. Indeed, PC-1 and PC-2 have a wide distribution within primary cilia (Geng et al., 2006; Liu et al., 2018; Pazour et al., 2002; Yoder et al., 2002). Unicellular organisms can show a detailed distribution of PC-2 in flagella (Liu et al., 2020) (Fig. 1). In *Chlamydomonas*, a type of algae that lacks PC-1, PC-2 exhibits a conserved ciliary distribution pattern (Huang et al., 2007). Here, the diffusion dynamics of PC-2 molecules differ in different regions of the cilium (Fig. 1A). Specifically, PC-2 is immobile in the distal two-thirds of the *Chlamydomonas* flagella; in contrast, within the proximal third of the flagellar membrane, PC-2 exhibits rapid diffusion (Liu et al., 2020) (Fig. 1A). The immobile fraction of PC-2 probably binds to the base of the mastigonemes, the extracellular fibrils on the flagella of many protists (depicted in Figs 1 and 3 and discussed in detail below). Thus, these findings suggest that the dynamics of PC-2 are a reflection of their working status in the flagella, and it is therefore appealing to hypothesize that the immobile PC-2 represents those channels that are in a 'working' state or vice versa. However, more work must be done in the future to test this hypothesis. In conclusion, although the core structures of PC-1 and PC-2 have been described, the physiological role of ciliary polycystins still needs further investigation.

Polycystin trafficking and localization to cilia

PC-1 and PC-2 are dependent on each other for their ciliary localization, and different domains of PC-1 and PC-2 have been analyzed for their roles in ciliary trafficking (for reviews see Hu and Harris, 2020; Padovano et al., 2020). The intraflagellar transport (IFT) complex and Bardet–Biedl syndrome complex (BBSome) are megadalton-sized protein complexes that have been thoroughly studied for their roles in distributing cargo molecules inside cilia (Lechtreck, 2015; Lechtreck et al., 2009; Nachury et al., 2007). PC-2 and IFT20 have been reported to form a protein complex with pericentrin around the ciliary base, suggesting that this IFT subunit has a role in delivering PC-2 to the target site before entering cilia (Jurczyk et al., 2004). TUB-like protein 3 (TULP3), which is involved in trafficking membrane proteins with IFT subunits, regulates the presence of PC-2 in the cilia of renal cells (Hwang et al., 2019). Fluorescence recovery after photobleaching (FRAP) analysis suggests that delivery of PC-2 within the *Chlamydomonas* flagella is dependent on the IFT system (Huang et al., 2007). However, IFT-dependent movement of fluorescently tagged PC-2 has been observed to occur at low frequency (~0.9 events/min/cilium for PC-2 versus ~60 events/min/cilium for the IFT complex), and the frequency is not elevated in growing cilia, which need increased delivery of PC-2 compared to the full-length cilia, suggesting that PC-2 might not be delivered by the IFT system in cilia (Liu et al., 2020). Intriguingly, the intracellular C-terminal tail of PC-1 physically interacts with Bardet–Biedl syndrome proteins, suggesting that PC-1 is a potential BBSome cargo (Su et al., 2014).

Two working models of polycystins in cilia

Polycystins have conserved ciliary localization across species. Zebrafish PC-2 localizes to cilia and intracellular membrane vesicles in kidney epithelial cells (Obara et al., 2006). Of note, zebrafish PC-2 is expressed in a variety of sensory cells that are associated with mechanotransduction, including cells of the ear, the lateral line organ, the LRO and the olfactory placodes (Goetz et al., 2014; Obara et al., 2006). In *Caenorhabditis elegans,* the homologs of PC-1 (LOV-1) and PC-2 (PKD-2) localize to the cilia of sensory

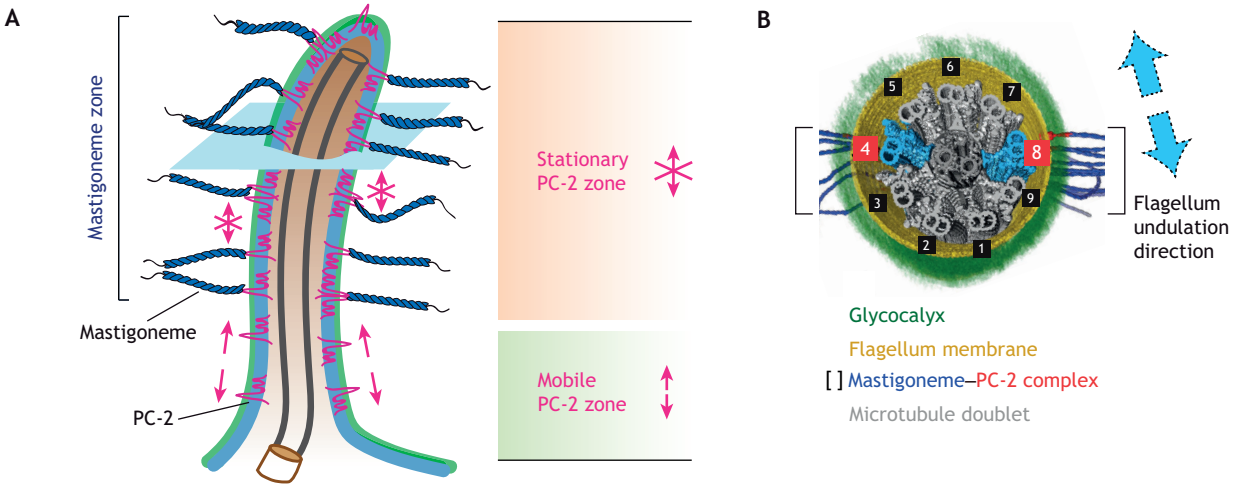

Fig. 1. The distribution of mastigonemes and PC-2 on the flagella of *Chlamydomonas*. (A) Schematic drawing of the *Chlamydomonas* flagellum. Note that the distal two-thirds of the flagellum is covered with mastigonemes, which bind to PC-2. PC-2 distributes along almost the entire flagellum, with the stationary fraction of PC-2 present in the distal two-thirds of the flagellum and mobile PC-2 found close to the flagellum base (indicated by pink arrows). The two zones containing the stationary and mobile PC-2 are separated by a narrow gap devoid of PC-2. The position of the cross section shown in B is indicated in blue. (B) Schematic depicting a cross section of a *Chlamydomonas* flagellum (adapted with permission from Liu et al., 2020). Key structures are highlighted in different colors, and the microtubule doublets are numbered. Note that the mastigoneme–PC-2 complexes locate next to doublets 4 and 8. Dotted arrows indicate the directions of flagellum undulation, which are nearly perpendicular to the mastigoneme–PC-2 plane.

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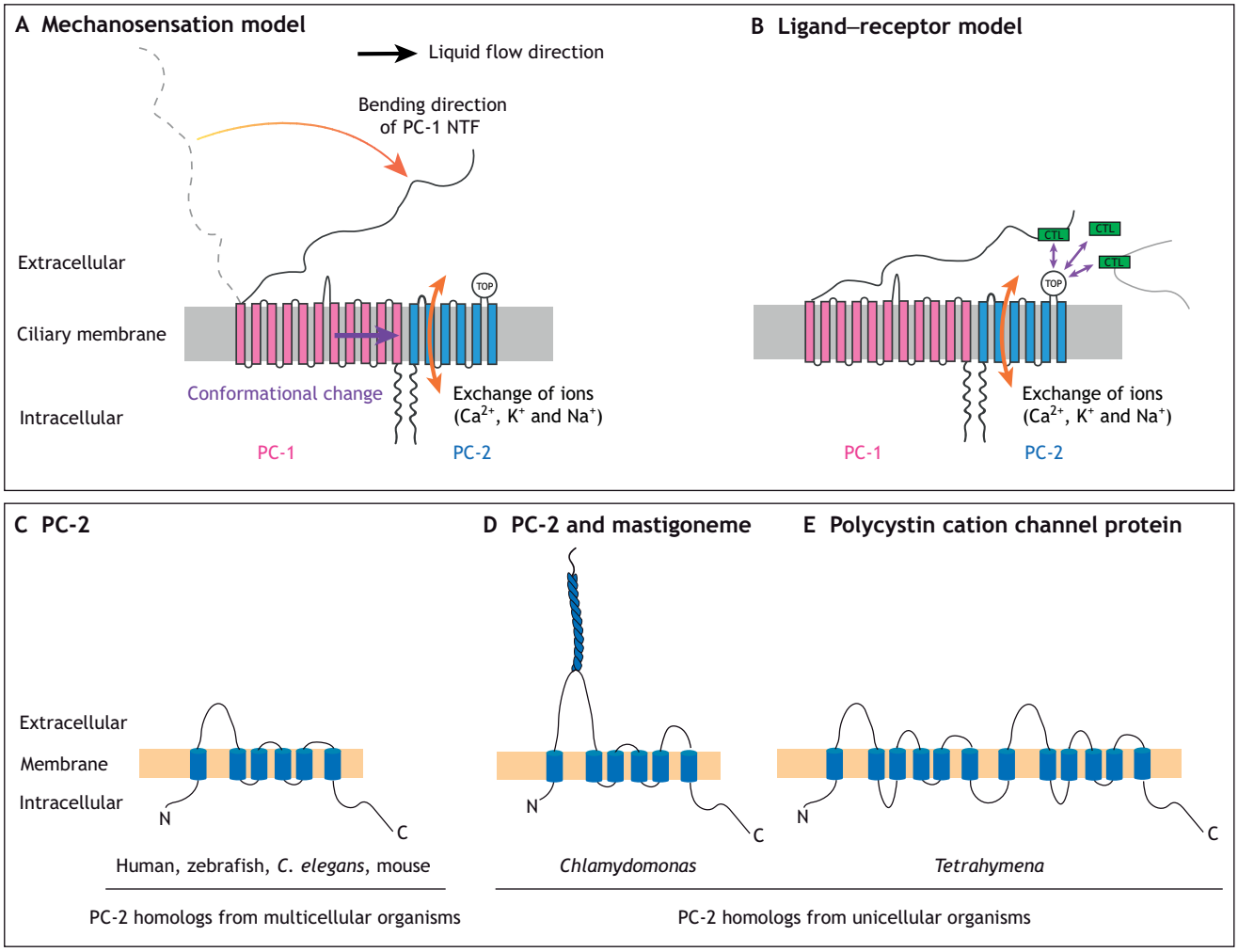

Fig. 2. Two working models of polycystins, and PC-2 homologs on the ciliary membrane. (A,B) Schematics depicting both working models of PC-1 and PC-2 on the ciliary membrane. (A) In the mechanosensation model, flowing liquid applies a mechanical shear force to the NTF of PC-1, causing deflection of the NTF. This process is believed to cause conformational changes in PC-2 (purple arrow), leading to the opening of the PC-2 channel for ion exchange, including an influx of Ca^{2+} that is thought to transduce the mechanical force into an intracellular signal. (B) In the ligand–receptor model, the CTL domain of the PC-1 NTF is proposed to function as a ligand that binds to the TOP domain of PC-2 as a receptor. Note that a single CTL domain alone can activate the opening of the PC-2 channel. (C–E) Schematic presentations of PC-2 homologs from multicellular and unicellular organisms. In general, PC-2 homologs are similar across species, although PC-2 homologs from multicellular organisms share a higher degree of similarity compared to homologs from unicellular organisms. (C) *In silico* modeling yields similar 3D molecular structures for human, zebrafish, *C. elegans* and mouse PC-2 homologs. However, PC-2 homologs from unicellular organisms show unique features. (D) The *Chlamydomonas* PC-2 homolog has a large extracellular loop – corresponding to the TOP domain of human PC-2 – that is the putative site of mastigoneme binding. (E) The *Tetrahymena* PC-2 homolog is a large channel that appears to have 12 transmembrane domains.

neurons (Barr and Sternberg, 1999). PC-2 is reported to mediate mating in nematodes (Wang et al., 2014). Here, mechanical stimulation triggers the release of PC-2-carrying extracellular vesicles (EVs) from cilia tips and periciliary membranes during mating (Wang et al., 2021). The release of PC-2-containing EVs appears to positively correlate with the mechanical stimuli applied to the male worms (Wang et al., 2020). This evidence from model organisms appears to reveal the mechanosensory properties of polycystins, a feature that has been more thoroughly explored in mammalian cell lines. Applying fluid flow or pipette suction acting on renal cell cilia has been shown to elevate intracellular free Ca^{2+} (Praetorius and Spring, 2001). The intracellular Ca^{2+} concentration change depends on the presence of functional PC-1 and PC-2 in cilia (Nauli et al., 2003; Praetorius and Spring, 2003a). A mechanosensation model has thus been proposed to explain the molecular mechanism of the polycystin complex, in which the force applied on the flexible extracellular domains of PC-1 generates conformational changes in the polycystin complex, thereby regulating PC-2 channel activity (Nauli and Zhou, 2004) (Fig. 2A).

More recently, a ligand–receptor model has provided an alternative explanation for the molecular function of polycystins on cilia (Ha et al., 2020). In this model, a portion of the N terminus of PC-1, namely the CTL domain, either attached or after cleavage to form a soluble peptide, functions as a ligand that is necessary and sufficient for activating the PC-2 channel (Ha et al., 2020) (Fig. 2B). Furthermore, the ligand derived from PC-1 is able to activate PC-2 channels on the plasma membrane outside cilia, ruling out the necessity of the presence of cilia membrane (Ha et al., 2020). In this scenario, the flow from the surrounding liquid only functions to bring together the ligand and receptor – the PC-1 CTL domain and PC-2 TOP domain, respectively. However, both models have limitations in explaining the mechanisms of action of polycystins. In

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the mechanosensation model, the functions of the extracellular domains of polycystins are underappreciated. It has been shown that multiple domains exist on the extracellular portions of polycystins, and these domains might exert potential functional roles other than just acting as flexible peptides in the jet flow (Hardy and Tsiokas, 2020). Also, testing such a model will be challenging, due to the difficulty of quantifying extracellular force characteristics (direction, intensity) and their effect on the 3D conformational change of the channels. In contrast, the ligand–receptor model is a novel explanation for channel activity. However, it might be difficult to explain why no significant change in ciliary Ca^{2+} concentration is observed during an applied jet flow, given that the ligand and receptor are both present (Delling et al., 2016; Ha and Delling, 2021). Furthermore, the ligand–receptor model may not take into account that PC-1 negatively regulates the opening of the PC-2 channel. Thus, the working mechanisms of polycystins are still enigmatic, and further work is clearly needed to improve and enrich the current models of polycystin action in kidney cilia; for example, how could these models explain the origins of ADPKD?

Mastigonemes

Mastigoneme morphology

Mastigonemes are protein polymers that protrude from the surface of one or both flagella of some unicellular ciliates (Bouck, 1969, 1971; Brooker, 1965; Deflandre, 1934; Fischer, 1894; Tran et al., 2022). Light microscopy observations of mastigonemes date back to the 19th century (Bouck, 1969; Deflandre, 1934; Fischer, 1894), and they can be divided into two groups based on their morphology: tubular and non-tubular (Fig. 3). Tubular mastigonemes can be found on the anterior flagellum of heterokont organisms, which have two flagella of unequal length. Tubular mastigonemes are stiff and straight, and are ∼1 µm in length (Fig. 3). Protists usually use the flagellum with tubular mastigonemes as the driving flagellum to locomote (Bouck, 1971). Tubular mastigonemes can be further divided into two categories: bipartite and tripartite. Bipartite mastigonemes consist of two distinct morphological components, a tubular shaft and several fine terminal filaments. For example, flagella in *Acronema sippewissettensis* have bipartite mastigonemes, which are ∼1 µm in length (Teal et al., 1998). Tripartite mastigonemes have a morphology similar to that of bipartite mastigonemes, with the addition of a basal region (Fig. 3). Such tripartite mastigonemes can be observed in *Paraphysomonas butcheri* (Pennick and Clarke, 1972). Functional differences between bipartite and tripartite mastigonemes are yet to be reported, but it is reasonable to assume that the architecture and rigidity determine their roles in flagella hydrodynamics. Cryo-electron microscopy and *in silico* hydrodynamic modeling could be feasible ways to further explore the function of tubular mastigonemes.

Non-tubular mastigonemes are thin and flexible filaments, thinner than tubular mastigonemes and not nearly as stiff (Fig. 3). One of the best described examples are the mastigonemes found on the surface of both of the *Chlamydomonas* flagella (Witman et al., 1972). They are assembled from square-shaped subunits, which are aligned end to end to form mastigonemes with a total length of ∼0.9 µm (Witman et al., 1972). The subunits appear to mostly

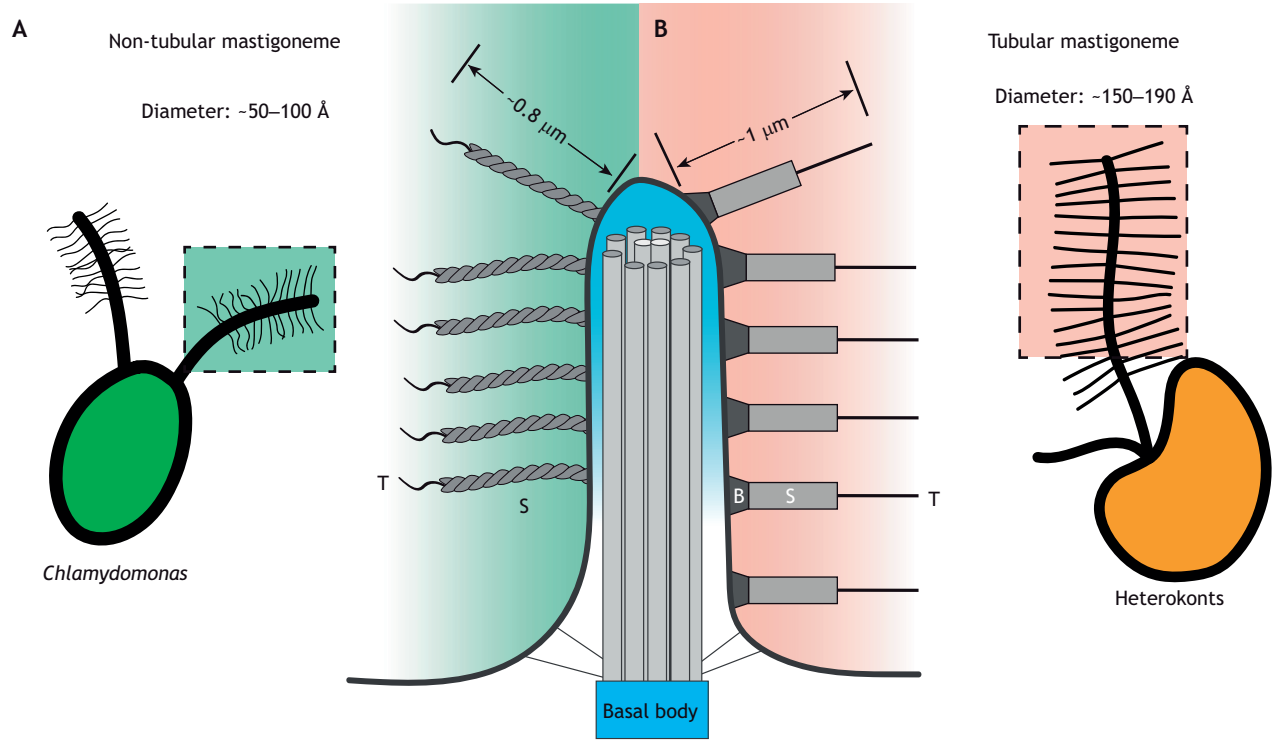

Fig. 3. Tubular and non-tubular mastigonemes in protists. (A,B) Schematic illustrations comparing non-tubular and tubular mastigonemes in *Chlamydomonas* and heterokonts, respectively. The flagella have a '9+2'-patterned axoneme, depicted as cylindrical shapes attached to the basal body in the middle of the flagellum shown in the enlarged central view. (A) Non-tubular mastigonemes are present on the distal two-thirds of the flagella, as indicated in the green dashed box, with a diameter of ∼50–100 Å and a length of ∼0.8 µm. *Chlamydomonas* non-tubular mastigonemes have two morphological parts: the shaft (S) and the terminal tip (T) (see enlarged central view). (B) Heterokont tubular mastigonemes are tripartite, comprising a base region (B), a tubular shaft (S) and one or more terminal filaments (T), with a diameter of ∼150–190 Å and a length of ∼1 µm (see enlarged central view). Interestingly, only one of the two heterokont flagella, the driving flagellum, is covered with tubular mastigonemes along its full length (orange dashed box); the other flagellum is relatively smooth.

consist of mastigoneme 1 (MST1, also known as mastigoneme-like protein 1) proteins, which contain four cysteine-rich epidermal growth factor (EGF)-like domains (Blackman et al., 2011; Liu et al., 2020). MST1 interacts with the *Chlamydomonas* PC-2 homolog, with the latter anchoring mastigonemes to the axoneme by interacting with their bases (Liu et al., 2020). The base of the mastigoneme is buried in a loose carbohydrate coat around the flagellum called the glycocalyx (Fig. 1B), which is required for flagella-dependent whole-cell gliding motility (Bloodgood et al., 2019). Additionally, the distal region of non-tubular mastigonemes terminates with a much thinner filament, suggesting a delicate tip structure (Fig. 3).

Mastigoneme function

The function of mastigonemes has long been a mystery, in part because they are only found on some protists and zoospores, which can be challenging experimental systems (Faktorová et al., 2020). The proposed function of mastigonemes is to increase the effective surface of the flagellum (Brennen, 1976; Holwill and Peters, 1974; Jahn et al., 1964; Namdeo et al., 2011). Emerging data, however, suggest that mastigonemes perform functions much more complicated than that.

Tubular mastigonemes play a major role in controlling the direction of cell motion (Brennen, 1976; Namdeo et al., 2011). Unlike the situation in most motile spermatozoa, which have a flagellum tail to push the cell body, in many protists, the driving flagellum is held in a forward position, pulling rather than pushing the cell (Holwill and Sleigh, 1967). The ability to move the cell body forward in the direction of wave propagation probably depends on mastigonemes (Brennen, 1976; Namdeo et al., 2011). Based on high-speed microscopy imaging and mathematical calculations, flagella with tubular mastigonemes differ from smooth flagella in their hydrodynamic properties (Namdeo et al., 2011). For example, a smooth flagellum swims in a direction opposite to flagella wave propagation, whereas a flagellum bearing mastigonemes swims in the direction of flagella wave propagation (Namdeo et al., 2011). This forward movement, however, requires the rigidity of tubular mastigonemes. Mathematical modeling provides further evidence that mastigoneme length and rigidity determine the propulsive ability of the flagella, even contributing to reversal of cell swimming direction (Namdeo et al., 2011, 2013). The density of mastigonemes on the flagella is also important for propulsion. Using computational fluid dynamics, hydrodynamic interactions between mastigonemes have been shown to be key to thrust generation and reversal in hairy flagellates (Asadzadeh et al., 2022). However, much remains unknown; for instance, the distribution pattern of mastigonemes on flagella is unclear. Some mastigonemes may be spirally arranged on the flagellum surface, whereas in some protist flagella, both tubular and non-tubular mastigonemes may be present, adding another layer of complexity to the roles of mastigonemes in hydrodynamics (Bouck et al., 1978).

Some studies have also suggested a role for rigid mastigonemes in flagellar mechanosensation (Christensen-Dalsgaard and Fenchel, 2004; Thomazo et al., 2021). *Paraphysomonas vestita*, a golden alga, has rigid mastigonemes that change their flagellar bending pattern after the mastigonemes make contact with food particles, resulting in the particles being moved towards the ingestion area (Christensen-Dalsgaard and Fenchel, 2004). This might be an indication that mastigonemes can be both propulsive and key for flagellar mechanosensation, although the underlying molecular mechanisms are currently unclear. A recent study has used anchored elastic fibers submerged in a granular suspension to mimic cilia and the surrounding particles (Thomazo et al., 2021). The authors demonstrate that bending of the artificial cilia changes and can detect particles as small as their own diameter after making contact under shear force (Thomazo et al., 2021). Assuming that the mastigonemes act like multiple cilia on the flagellum, the findings described above suggest that either mastigonemes or cilia are mechanosensitive due to their hydrodynamical properties.

Conversely, the functions of non-tubular mastigonemes remain enigmatic. Whilst non-tubular mastigonemes are known to contribute to whole-cell gliding on solid surfaces in *Peranema trichophorum* (Saito et al., 2003), their roles in swimming are under debate. Due to their flexible nature, non-tubular mastigonemes appear to be easily shed from the flagellum. Indeed, scattered mastigonemes of *Chlamydomonas* flagella are easily observed in whole-mount electron microscopy images (Liu et al., 2020). When antibodies are introduced to induce shedding-mediated loss of the mastigonemes from live cells, this mastigoneme shedding affects the swimming velocity of *Chlamydomonas* (~20% reduction) (Nakamura et al., 1996). Accordingly, null-mutant *mst1* *Chlamydomonas* (from the *Chlamydomonas* Library Project, CLiP; https://www.chlamylibrary.org/index), which completely lacks mastigonemes, exhibits a swimming velocity that is 17% slower than that of the wild type (Liu et al., 2020). In contrast, a study using another *mst1* mutant allele (also from CLiP) has reported that the average and maximum swimming velocities are unaffected by the absence of mastigonemes in *Chlamydomonas*, raising the question of the contribution of non-tubular mastigonemes to cell motility (Amador et al., 2020). *In silico* analysis has found that flexible mastigonemes contribute less to the propulsion compared to the contribution of rigid mastigonemes (Namdeo et al., 2011). From this perspective, non-tubular mastigonemes appear to have additional roles other than being the major determinant of swimming velocity. Consistently, in a recent study on the flagella of zoospores in *Phytophthora parasitica*, the anterior flagellum, which is covered with rigid tubular mastigonemes, appears to be the main motor of zoospores, whereas the posterior flagellum, which is covered with flimsy mastigonemes, might play a role in chemical or electrical sensing and provide an anchor-like role for turning of swimming cells (Tran et al., 2022). However, whether the flexible mastigonemes on the posterior flagellum are involved in this sensing and anchoring is unknown.

Non-tubular mastigonemes have also been demonstrated to determine the flagellar distribution of other proteins, for example, PC-2 in *Chlamydomonas*. The enrichment of PC-2 in flagella is significantly reduced without mastigonemes, leading to a reduction in swimming speed (Liu et al., 2020). It is appealing to suggest that non-tubular mastigonemes may function to provide a mechanosensory role or function in a ligand–receptor manner for binding extracellular ligands. Further functional studies of the domains of non-tubular mastigonemes will help to clarify their roles and identify their homologs in multicellular organisms.

Structural feature analysis of flagella and polycystins across species

Structural conservation of cilia between unicellular and multicellular ciliated organisms provides an important advantage of investigating with unicellular protists. Eukaryotic cilia can be categorized into two groups, ‘9+2’ and ‘9+0’, based on the arrangement of their axonemal microtubules inside the cilia. Axonemes of cilia in the ‘9+2’ group have nine doublet microtubules (each consisting of conjoined A and B tubules)

arranged in a ring around a central pair of singlet microtubules. The '9+2' axoneme architecture is the principal feature of most motile cilia, whereas '9+0' is the typical axoneme structure of immotile cilia. In '9+0' axonemes, the central pair of microtubules is absent, as are the decorated dynein motors and other complexes on A tubules that are important for ciliary motility (Amos and Klug, 1974; Petriman and Lorentzen, 2020; Satir and Christensen, 2007). However, a recent study has shown that the microtubules of '9+0' axonemes are neither arranged in a ring nor are they in a typical '9+0' arrangement in the shank of primary cilia (Kiesel et al., 2020). This structural flexibility, however, explains why primary cilia can elastically withstand liquid flow for mechanosensation (Sun et al., 2019). Most protist flagella have a conserved '9+2' type, reflecting their motile nature (Ginger et al., 2008). The conservation of axonemal structures between protist flagella and the motile cilia of multicellular organisms has led to some protists becoming classical models for the study of cilia (e.g. *Chlamydomonas* and *Tetrahymena*) (Liu and Lechtreck, 2018; Louka et al., 2018; Stepanek and Pigino, 2016).

Whilst mostly sharing conserved features, some unicellular organisms do have unique features on their flagella, named extra-axonemal structures (Moran et al., 2014). Indeed, mastigonemes are extra-axonemal structures of the flagella of some protist species (Bouck et al., 1978; Bouck, 1969, 1971). Some euglenozoan protists and parasites, such as trypanosomatids, have a paraflagellar rod (PFR), which is a complex lattice of filamentous structures attached to and running alongside the axoneme inside the flagellar membrane (Portman and Gull, 2010). The PFR facilitates motility and maintains morphogenesis for the flagellum and cell; in the absence of the PFR, *Trypanosoma brucei* cells (in the bloodstream form) start to become distorted (Broadhead et al., 2006; Griffiths et al., 2007). To our knowledge, prominent appendages similar to mastigonemes and the PFR have not been reported in motile and primary cilia in mammals. Choanoflagellates, which are the closest living unicellular relatives of metazoans, are uniquely positioned within the opisthokonts. The structure of flagella in the choanoflagellate species *Salpingoeca rosetta* has recently been revealed by cryo-electron tomography (Pinskey et al., 2022). The axonemal structure of *S. rosetta* closely resembles those of metazoan flagella, but unique features of their flagella are also apparent, such as the structure of the choanoflagellate vane, which is a fine mesh of intertwined filaments extending bilaterally from the flagella membrane. As a flagellar appendage, the choanoflagellate vane differs from mastigonemes in regard to size, arrangement and connections to the axonemes. Choanoflagellate vane filaments have a diameter of ∼3.5 nm and are arranged as wispy hairs or meshed networks, whereas mastigonemes are independent filaments with an overall diameter ranging from ∼5–19 nm (Fig. 3). In addition, unlike mastigonemes, which appear to anchor to axonemal microtubules, connections between vane filaments and axonemal microtubules have not been detected (Bouck, 1969; Pinskey et al., 2022; Liu et al., 2020). Homologs of the mastigoneme protein MST1 and PC-2 are not detected in the *S. rosetta* genome via BLAST search (Pinskey et al., 2022). Therefore, it is unlikely that the vane filaments interact with polycystins in choanoflagellates (Pinskey et al., 2022).

Metazoan spermatozoa can be considered morphologically close to unicellular organisms. Mammalian spermatozoa have an outer dense fiber paired with each outer doublet microtubule along the length of the axoneme, and a fibrous sheath surrounds the axoneme along the principal piece of the sperm tail (Leung et al., 2021). Intriguingly, Ca^{2+} channel proteins, such as CatSper channels and PC-2, have been shown to form a specialized distribution pattern on motile and non-motile cilia. CatSper channels distribute into four linear patterns that run along each side of the longitudinal columns of the mammalian sperm flagella, forming the linear calcium domains (Chung et al., 2014). PC-2–mastigoneme complexes preferentially distribute into two linear patterns on each side of *Chlamydomonas* flagella, close to microtubule doublets 4 and 8 (Fig. 1B) (Liu et al., 2020). In the mouse embryo node, PC-2 channels are preferentially localized to one side of the non-motile cilia, forming a polarized distribution on the surface (Katoh et al., 2023). The organization of channels into special distribution patterns exerts significant influence on channel functions; in motile cilia, one could speculate that Ca^{2+} transients mediated by the channels are likely to fine-tune the outer dynein arms on the axoneme and therefore regulate the beating pattern, whereas in non-motile cilia, the specialized distribution pattern might serve to maximize reception of mechanical stimuli or chemotactic signals.

Polycystin homologs in unicellular organisms receive much less attention than they deserve. Compared to PC-1 homologs, which have a large molecular mass and multiple domains, the structures of PC-2 homologs are more conserved across species. As discussed above, human PC-2 has six transmembrane regions and four distinct domains, including one extracellular TOP domain, which is conserved among the polycystins (Douguet et al., 2019). Although PC-2 structures are conserved, *in silico* 3D-structure prediction shows that unicellular PC-2 homologs have unique features. For instance, the PC-2 homolog in *Chlamydomonas* has an extended TOP domain, forming an extraordinary extracellular loop (Fig. 2D). The TOP domain might thus be the putative site for the interaction between PC-2 and mastigonemes (Fig. 2D) (Liu et al., 2020). *Tetrahymena thermophila* polycystin cation channel PC-2 appears to be two channels in tandem order (Fig. 2E). The closely related ciliates of the genus *Paramecium,* such as *Paramecium tetraurelia,* have PC-2 homologs with structures similar to that in *Tetrahymena*. Whether the PC-2 in these organisms undergoes proteolysis *in vivo* as in *Chlamydomonas* flagella, however, is unclear (Huang et al.,2007; Liu et al., 2020). These *in silico* models of PC-2 reveal that polycystins in unicellular organisms act as putative channels, and in some unicellular organisms, PC-2 structure might represent a premature model of mammalian polycystin complexes (Fig. 2). Based on this, it is appealing to hypothesize that the polycystins in different organisms might represent various evolutionary states. However, this hypothesis needs further supporting evidence. Additionally, analyzing the relationship between flagella undulation patterns and the structural features of polycystins present on flagella in unicellular organisms could be useful for exploring the mechanosensation (or other) properties of polycystins and provide new insights into the role of their mammalian counterparts.

Conclusions and future directions

In this Review, we have examined the current state of the literature that suggests a collaborative role for cilia and polycystins. In particular, the recent discovery of an interaction between PC-2 and mastigonemes on *Chlamydomonas* flagella suggests a previously unappreciated role for PC-2 in cilia and flagella (Liu et al., 2020). However, whether and how this interaction regulates the channel status of PC-2 remains to be determined. *Chlamydomonas* flagella are unusual in the sense that they have flexible mastigonemes and contain PC-2, but not PC-1, making it problematic to determine whether the potential paradigm found in *Chlamydomonas* flagella also extends to motile or primary cilia on metazoan cells. Much

remains to be understood about the roles that polycystins have in primary cilia and flagella; however, the discoveries made in unicellular organisms will better inform us of these functions of polycystins and their interacting partners. Further work will hopefully elucidate the roles played by these molecules in the sensory and motor properties of cilia and flagella, which might be the next key step in understanding the origins of ADPKD.

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References

Afzelius, B. A. (1976). A human syndrome caused by immotile cilia. *Science* **193**, 317-319. doi:10.1126/science.1084576
Amador, G. J., Wei, D., Tam, D. and Aubin-Tam, M.-E. (2020). Fibrous flagellar hairs of Chlamydomonas reinhardtii do not enhance swimming. *Biophys. J.* **118**, 2914-2925. doi:10.1016/j.bpj.2020.05.003
Amos, L. and Klug, A. (1974). Arrangement of subunits in flagellar microtubules. *J. Cell Sci.* **14**, 523-549. doi:10.1242/jcs.14.3.523
Anvarian, Z., Mykytyn, K., Mukhopadhyay, S., Pedersen, L. B. and Christensen, S. T. (2019). Cellular signalling by primary cilia in development, organ function and disease. *Nat. Rev. Nephrol.* **15**, 199-219. doi:10.1038/s41581-019-0116-9
Asadzadeh, S. S., Walther, J. H., Andersen, A. and Kiørboe, T. (2022). Hydrodynamic interactions are key in thrust-generation of hairy flagella. *Phys. Rev. Fluids* **7**, 073101. doi:10.1103/PhysRevFluids.7.073101
Barr, M. M. and Sternberg, P. W. (1999). A polycystic kidney-disease gene homologue required for male mating behaviour in C. elegans. *Nature* **401**, 386-389. doi:10.1038/43913
Blackman, L. M., Arikawa, M., Yamada, S., Suzaki, T. and Hardham, A. R. (2011). Identification of a mastigoneme protein from Phytophthora nicotianae. *Protist* **162**, 100-114. doi:10.1016/j.protis.2010.01.005
Bloodgood, R. A., Tetreault, J. and Sloboda, R. D. (2019). The Chlamydomonas flagellar membrane glycoprotein FMG-1B is necessary for expression of force at the flagellar surface. *J. Cell Sci.* **132**, jcs233429. doi:10.1242/jcs.233429
Bouck, G. B. (1969). Extracellular microtubules: the origin, structure, and attachment of flagellar hairs in Fucus and Ascophyllum antherozoids. *J. Cell Biol.* **40**, 446-460. doi:10.1083/jcb.40.2.446
Bouck, G. B. (1971). The structure, origin, isolation, and composition of the tubular mastigonemes of the Ochromonas flagellum. *J. Cell Biol.* **50**, 362-384. doi:10.1083/jcb.50.2.362
Bouck, G. B., Rogalski, A. and Valaitis, A. (1978). Surface organization and composition of Euglena. II. Flagellar mastigonemes. *J. Cell Biol.* **77**, 805-826. doi:10.1083/jcb.77.3.805
Brennen, C. (1976). Locomotion of flagellates with mastigonemes. *J. Mechanochem. Cell Motil.* **3**, 207-217.
Broadhead, R., Dawe, H. R., Farr, H., Griffiths, S., Hart, S. R., Portman, N., Shaw, M. K., Ginger, M. L., Gaskell, S. J., McKean, P. G. et al. (2006). Flagellar motility is required for the viability of the bloodstream trypanosome. *Nature* **440**, 224-227. doi:10.1038/nature04541
Brooker, B. E. (1965). Mastigonemes in a bodonid flagellate. *Exp. Cell Res.* **37**, 300-305. doi:10.1016/0014-4827(65)90178-3
Cai, Z., Xin, J., Pollock, D. M. and Pollock, J. S. (2000). Shear stress-mediated NO production in inner medullary collecting duct cells. *Am. J. Physiol. Renal. Physiol.* **279**, F270-F274. doi:10.1152/ajprenal.2000.279.2.F270
Christensen-Dalsgaard, K. K. and Fenchel, T. (2004). Complex flagellar motions and swimming patterns of the flagellates Paraphysomonas vestita and Pteridomonas danica. *Protist* **155**, 79-87. doi:10.1078/1434461000166
Chung, J.-J., Shim, S.-H., Everley, R. A., Gygi, S. P., Zhuang, X. and Clapham, D. E. (2014). Structurally distinct Ca2+ signaling domains of sperm flagella orchestrate tyrosine phosphorylation and motility. *Cell* **157**, 808-822. doi:10.1016/j.cell.2014.02.056
Deflandre, G. (1934). Existence, sur les flagelles, de filaments latéraux ou terminaux (mastigonèmes). *CR Acad. Sci.(Paris)* **198**, 497.
Delling, M., Indzhykulian, A. A., Liu, X., Li, Y., Xie, T., Corey, D. P. and Clapham, D. E. (2016). Primary cilia are not calcium-responsive mechanosensors. *Nature* **531**, 656-660. doi:10.1038/nature17426
Djenoune, L., Mahamdeh, M., Truong, T. V., Nguyen, C. T., Fraser, S. E., Brueckner, M., Howard, J. and Yuan, S. (2023). Cilia function as calcium-mediated mechanosensors that instruct left-right asymmetry. *Science* **379**, 71-78. doi:10.1126/science.abq7317
Douguet, D., Patel, A. and Honoré, E. (2019). Structure and function of polycystins: insights into polycystic kidney disease. *Nature Reviews Nephrology* **15**, 412-422. doi:10.1038/s41581-019-0143-6
Emmer, B. T., Maric, D. and Engman, D. M. (2010). Molecular mechanisms of protein and lipid targeting to ciliary membranes. *J. Cell Sci.* **123**, 529-536. doi:10.1242/jcs.062968
Faktorová, D., Nisbet, R. E. R., Fernández Robledo, J. A., Casacuberta, E., Sudek, L., Allen, A. E., Ares, M., Jr, Aresté, C., Balestreri, C., Barbrook, A. C. et al. (2020). Genetic tool development in marine protists: emerging model organisms for experimental cell biology. *Nat. Methods* **17**, 481-494. doi:10.1038/s41592-020-0796-x
Ferreira, F. M., Watanabe, E. H. and Onuchic, L. F. (2015). Polycystins and molecular basis of autosomal dominant polycystic kidney disease. In *Polycystic Kidney Disease* (ed. X. Li). Brisbane: Codon Publications. doi:10.15586/codon.pkd.2015.ch7
Fischer, A. (1894). Ueber die Geißeln einiger Flagellaten. *Pringsh. Jahrb. Wiss. Bot.* **26**, 187-235.
Gallagher, A. R., Germino, G. G. and Somlo, S. (2010). Molecular advances in autosomal dominant polycystic kidney disease. *Adv. Chronic Kidney Dis.* **17**, 118-130. doi:10.1053/j.ackd.2010.01.002
Geng, L., Okuhara, D., Yu, Z., Tian, X., Cai, Y., Shibazaki, S. and Somlo, S. (2006). Polycystin-2 traffics to cilia independently of polycystin-1 by using an N-terminal RVxP motif. *J. Cell Sci.* **119**, 1383-1395. doi:10.1242/jcs.02818
Ginger, M. L., Portman, N. and McKean, P. G. (2008). Swimming with protists: perception, motility and flagellum assembly. *Nat. Rev. Microbiol.* **6**, 838-850. doi:10.1038/nrmicro2009
Goetz, J. G., Steed, E., Ferreira, R. R., Roth, S., Ramspacher, C., Boselli, F., Charvin, G., Liebling, M., Wyart, C., Schwab, Y. et al. (2014). Endothelial cilia mediate low flow sensing during zebrafish vascular development. *Cell reports* **6**, 799-808. doi:10.1016/j.celrep.2014.01.032
Grieben, M., Pike, A. C. W., Shintre, C. A., Venturi, E., El-Ajouz, S., Tessitore, A., Shrestha, L., Mukhopadhyay, S., Mahajan, P., Chalk, R. et al. (2017). Structure of the polycystic kidney disease TRP channel Polycystin-2 (PC2). *Nat. Struct. Mol. Biol.* **24**, 114-122. doi:10.1038/nsmb.3343
Griffiths, S., Portman, N., Taylor, P. R., Gordon, S., Ginger, M. L. and Gull, K. (2007). RNA interference mutant induction in vivo demonstrates the essential nature of trypanosome flagellar function during mammalian infection. *Eukaryot. Cell* **6**, 1248-1250. doi:10.1128/EC.00110-07
Guirao, B., Meunier, A., Mortaud, S., Aguilar, A., Corsi, J.-M., Strehl, L., Hirota, Y., Desoeuvre, A., Boutin, C., Han, Y.-G. et al. (2010). Coupling between hydrodynamic forces and planar cell polarity orients mammalian motile cilia. *Nat. Cell Biol.* **12**, 341-350. doi:10.1038/ncb2040
Ha, K. and Delling, M. (2021). Electrophysiological recordings of the polycystin complex in the primary cilium of cultured mouse IMCD-3 cell line. *Bio-protocol* **11**, e4196. doi:10.21769/BioProtoc.4196
Ha, K., Nobuhara, M., Wang, Q., Walker, R. V., Qian, F., Schartner, C., Cao, E. and Delling, M. (2020). The heteromeric PC-1/PC-2 polycystin complex is activated by the PC-1 N-terminus. *eLife* **9**, e60684. doi:10.7554/eLife.60684
Hardy, E. and Tsiokas, L. (2020). Polycystins as components of large multiprotein complexes of polycystin interactors. *Cell. Signal.* **72**, 109640. doi:10.1016/j.cellsig.2020.109640
Holwill, M. E. J. and Peters, P. D. (1974). Dynamics of the hispid flagellum of Ochromonas danica: the role of mastigonemes. *J. Cell Biol.* **62**, 322-328. doi:10.1083/jcb.62.2.322
Holwill, M. E. J. and Sleigh, M. A. (1967). Propulsion by hispid flagella. *J. Exp. Biol.* **47**, 267-276. doi:10.1242/jeb.47.2.267
Hu, J. and Harris, P. C. (2020). Regulation of polycystin expression, maturation and trafficking. *Cell. Signal.* **72**, 109630. doi:10.1016/j.cellsig.2020.109630
Huang, K., Diener, D. R., Mitchell, A., Pazour, G. J., Witman, G. B. and Rosenbaum, J. L. (2007). Function and dynamics of PKD2 in Chlamydomonas reinhardtii flagella. *J. Cell Biol.* **179**, 501-514. doi:10.1083/jcb.200704069
Huangfu, D. and Anderson, K. V. (2005). Cilia and Hedgehog responsiveness in the mouse. *Proc. Natl. Acad. Sci. USA* **102**, 11325-11330. doi:10.1073/pnas.0505328102
Hwang, S.-H., Somatilaka, B. N., Badgandi, H., Palicharla, V. R., Walker, R., Shelton, J. M., Qian, F. and Mukhopadhyay, S. (2019). Tulp3 regulates renal cystogenesis by trafficking of cystoproteins to cilia. *Curr. Biol.* **29**, 790-802.e5. doi:10.1016/j.cub.2019.01.047
Jahn, T. L., Lanoman, M. D. and Fonseca, J. R. (1964). The mechanism of locomotion of flagellates. II. Function of the mastigonemes of Ochromonas. *J. Protozool.* **11**, 291-296. doi:10.1111/j.1550-7408.1964.tb01756.x
Jin, X., Mohieldin, A. M., Muntean, B. S., Green, J. A., Shah, J. V., Mykytyn, K. and Nauli, S. M. (2014). Cilioplasm is a cellular compartment for calcium signaling

in response to mechanical and chemical stimuli. *Cell. Mol. Life Sci.* **71**, 2165-2178. doi:10.1007/s00018-013-1483-1

Johnson, N. T., Villalon, M., Royce, F. H., Hard, R. and Verdugo, P. (1991). Autoregulation of beat frequency in respiratory ciliated cells: demonstration by viscous loading. *Am. Rev. Respir. Dis.* **144**, 1091-1094. doi:10.1164/ajrccm/144.5.1091

Jurczyk, A., Gromley, A., Redick, S., Agustin, J. S., Witman, G., Pazour, G. J., Peters, D. J. M. and Doxsey, S. (2004). Pericentrin forms a complex with intraflagellar transport proteins and polycystin-2 and is required for primary cilia assembly. *J. Cell Biol.* **166**, 637-643. doi:10.1083/jcb.200405023

Kallakuri, S., Yu, J. A., Li, J., Li, Y., Weinstein, B. M., Nicoli, S. and Sun, Z. (2015). Endothelial cilia are essential for developmental vascular integrity in zebrafish. *J. Am. Soc. Nephrol.* **26**, 864-875. doi:10.1681/ASN.2013121314

Katoh, T. A., Omori, T., Mizuno, K., Sai X., Minegishi, K., Ikawa, Y., Nishimura, H., Itabashi, T., Kajikawa, E., Hiver, S. et al. (2023). Immotile cilia mechanically sense the direction of fluid flow for left-right determination. *Science* **379**, 66-71. doi:10.1126/science.abq8148

Kiesel, P., Alvarez Viar, G., Tsoy, N., Maraspini, R., Gorilak, P., Varga, V., Honigmann, A. and Pigino, G. (2020). The molecular structure of mammalian primary cilia revealed by cryo-electron tomography. *Nat. Struct. Mol. Biol.* **27**, 1115-1124. doi:10.1038/s41594-020-0507-4

Kim, S., Nie, H., Nesin, V., Tran, U., Outeda, P., Bai, C.-X., Keeling, J., Maskey, D., Watnick, T., Wessely, O. et al. (2016). The polycystin complex mediates Wnt/Ca(2+) signalling. *Nat. Cell Biol.* **18**, 752-764. doi:10.1038/ncb3363

Lechtreck, K. F. (2015). IFT-cargo interactions and protein transport in cilia. *Trends Biochem. Sci.* **40**, 765-778. doi:10.1016/j.tibs.2015.09.003

Lechtreck, K.-F., Johnson, E. C., Sakai, T., Cochran, D., Ballif, B. A., Rush, J., Pazour, G. J., Ikebe, M. and Witman, G. B. (2009). The Chlamydomonas reinhardtii BBSome is an IFT cargo required for export of specific signaling proteins from flagella. *J. Cell Biol.* **187**, 1117-1132. doi:10.1083/jcb.200909183

Lee, K. L., Guevarra, M. D., Nguyen, A. M., Chua, M. C., Wang, Y. and Jacobs, C. R. (2015). The primary cilium functions as a mechanical and calcium signaling nexus. *Cilia* **4**, 7. doi:10.1186/s13630-015-0016-y

Legendre, M., Zaragosi, L.-E. and Mitchison, H. M. (2021). Motile cilia and airway disease. *Semin. Cell Dev. Biol.* **110**, 19-33. doi:10.1016/j.semcdb.2020.11.007

Leung, M. R., Roelofs, M. C., Ravi, R. T., Maitan, P., Henning, H., Zhang, M., Bromfield, E. G., Howes, S. C., Gadella, B. M., Bloomfield-Gadêlha, H. et al. (2021). The multi-scale architecture of mammalian sperm flagella and implications for ciliary motility. *EMBO J.* **40**, e107410. doi:10.15252/embj.2020107410

Li, S., Fernandez, J.-J., Marshall, W. F. and Agard, D. A. (2012). Three-dimensional structure of basal body triplet revealed by electron cryo-tomography. *EMBO J.* **31**, 552-562. doi:10.1038/emboj.2011.460

Liu, P. and Lechtreck, K. F. (2018). The Bardet-Biedl syndrome protein complex is an adapter expanding the cargo range of intraflagellar transport trains for ciliary export. *Proc. Natl Acad. Sci. USA* **115**, E934-E943. doi:10.1073/pnas.1713226115

Liu, X., Vien, T., Duan, J., Sheu, S.-H., DeCaen, P. G. and Clapham, D. E. (2018). Polycystin-2 is an essential ion channel subunit in the primary cilium of the renal collecting duct epithelium. *eLife* **7**, e33183. doi:10.7554/eLife.33183

Liu, P., Lou, X., Wingfield, J. L., Lin, J., Nicastro, D. and Lechtreck, K. (2020). Chlamydomonas PKD2 organizes mastigonemes, hair-like glycoprotein polymers on cilia. *J. Cell Biol.* **219**, e202001122. doi:10.1083/jcb.202001122

Louka, P., Vasudevan, K. K., Guha, M., Joachimiak, E., Wloga, D., Tomasi, R. F., Baroud, C. N., Dupuis-Williams, P., Galati, D. F., Pearson, C. G. et al. (2018). Proteins that control the geometry of microtubules at the ends of cilia. *J. Cell Biol.* **217**, 4298-4313. doi:10.1083/jcb.201804141

Malone, A. M. D., Anderson, C. T., Tummala, P., Kwon, R. Y., Johnston, T. R., Stearns, T. and Jacobs, C. R. (2007). Primary cilia mediate mechanosensing in bone cells by a calcium-independent mechanism. *Proc. Natl Acad. Sci. USA* **104**, 13325-13330. doi:10.1073/pnas.0700636104

McGrath, J., Somlo, S., Makova, S., Tian, X. and Brueckner, M. (2003). Two populations of node monocilia initiate left-right asymmetry in the mouse. *Cell* **114**, 61-73. doi:10.1016/S0092-8674(03)00511-7

Moran, J., McKean, P. G. and Ginger, M. L. (2014). Eukaryotic flagella: variations in form, function, and composition during evolution. *Bioscience* **64**, 1103-1114. doi:10.1093/biosci/biu175

Nachury, M. V. (2014). How do cilia organize signalling cascades? *Philos. Trans. R. Soc. B Biol. Sci.* **369**, 20130465. doi:10.1098/rstb.2013.0465

Nachury, M. V. and Mick, D. U. (2019). Establishing and regulating the composition of cilia for signal transduction. *Nat. Rev. Mol. Cell Biol.* **20**, 389-405. doi:10.1038/s41580-019-0116-4

Nachury, M. V., Loktev, A. V., Zhang, Q., Westlake, C. J., Peränen, J., Merdes, A., Slusarski, D. C., Scheller, R. H., Bazan, J. F., Sheffield, V. C. et al. (2007). A core complex of BBS proteins cooperates with the GTPase Rab8 to promote ciliary membrane biogenesis. *Cell* **129**, 1201-1213. doi:10.1016/j.cell.2007.03.053

Nagao, S., Nishii, K., Yoshihara, D., Kurahashi, H., Nagaoka, K., Yamashita, T., Takahashi, H., Yamaguchi, T., Calvet, J. P. and Wallace, D. P. (2008). Calcium channel inhibition accelerates polycystic kidney disease progression in the Cy/+ rat. *Kidney Int.* **73**, 269-277. doi:10.1038/sj.ki.5002629

Nakamura, S., Tanaka, G., Maeda, T., Kamiya, R., Matsunaga, T. and Nikaido, O. (1996). Assembly and function of Chlamydomonas flagellar mastigonemes as probed with a monoclonal antibody. *J. Cell Sci.* **109**, 57-62. doi:10.1242/jcs.109.1.57

Namdeo, S., Khaderi, S. N., den Toonder, J. M. J. and Onck, P. R. (2011). Swimming direction reversal of flagella through ciliary motion of mastigonemes. *Biomicrofluidics* **5**, 034108. doi:10.1063/1.3608240

Namdeo, S., Khaderi, S. N. and Onck, P. R. (2013). Swimming dynamics of bidirectional artificial flagella. *Phys. Rev. E* **88**, 043013. doi:10.1103/PhysRevE.88.043013

Nauli, S. M. and Zhou, J. (2004). Polycystins and mechanosensation in renal and nodal cilia. *BioEssays* **26**, 844-856. doi:10.1002/bies.20069

Nauli, S. M., Alenghat, F. J., Luo, Y., Williams, E., Vassilev, P., Li, X., Elia, A. E. H., Lu, W., Brown, E. M., Quinn, S. J. et al. (2003). Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells. *Nat. Genet.* **33**, 129-137. doi:10.1038/ng1076

Nauli, S. M., Jin, X., AbouAlaiwi, W. A., El-Jouni, W., Su, X. and Zhou, J. (2013). Non-motile primary cilia as fluid shear stress mechanosensors. *Methods Enzymol.* **525**, 1-20. doi:10.1016/B978-0-12-397944-5.00001-8

Nauli, S. M., Pala, R. and Kleene, S. J. (2016). Calcium channels in primary cilia. *Curr. Opin Nephrol. Hypertens.* **25**, 452. doi:10.1097/MNH.0000000000000251

Obara, T., Mangos, S., Liu, Y., Zhao, J., Wiessner, S., Kramer-Zucker, A. G., Olale, F., Schier, A. F. and Drummond, I. A. (2006). Polycystin-2 immunolocalization and function in zebrafish. *J. Am. Soc. Nephrol.* **17**, 2706-2718. doi:10.1681/ASN.2006040412

Orts-Del'Immagine, A., Cantaut-Belarif, Y., Thouvenin, O., Roussel, J., Baskaran, A., Langui, D., Koëth, F., Bivas, P., Lejeune, F.-X., Bardet, P.-L. et al. (2020). Sensory neurons contacting the cerebrospinal fluid require the reissner fiber to detect spinal curvature in vivo. *Curr. Biol.* **30**, 827-839.e4. doi:10.1016/j.cub.2019.12.071

Padovano, V., Mistry, K., Merrick, D., Gresko, N. and Caplan, M. J. (2020). A cut above (and below): Protein cleavage in the regulation of polycystin trafficking and signaling. *Cell. Signal.* **72**, 109634. doi:10.1016/j.cellsig.2020.109634

Pazour, G. J., San Agustin, J. T., Follit, J. A., Rosenbaum, J. L. and Witman, G. B. (2002). Polycystin-2 localizes to kidney cilia and the ciliary level is elevated in orpk mice with polycystic kidney disease. *Curr. Biol.* **12**, R378-R380. doi:10.1016/S0960-9822(02)00877-1

Pennick, N. C. and Clarke, K. J. (1972). Paraphysomonas butcheri sp. nov. a marine, colourless, scale-bearing member of the Chrysophyceae. *Br. Phycol. J.* **7**, 45-48. doi:10.1080/00071617200650051

Petriman, N.-A. and Lorentzen, E. (2020). Structural insights into the architecture and assembly of eukaryotic flagella. *Microb Cell* **7**, 289-299. doi:10.15698/mic2020.11.734

Pinskey, J. M., Lagisetty, A., Gui, L., Phan, N., Reetz, E., Tavakoli, A., Fu, G. and Nicastro, D. (2022). Three-dimensional flagella structures from animals' closest unicellular relatives, the Choanoflagellates. *Elife* **11**, e78133. doi:10.7554/eLife.78133

Portman, N. and Gull, K. (2010). The paraflagellar rod of kinetoplastid parasites: from structure to components and function. *Int. J. Parasitol.* **40**, 135-148. doi:10.1016/j.ijpara.2009.10.005

Praetorius, H. A. and Spring, K. R. (2001). Bending the MDCK cell primary cilium increases intracellular calcium. *J. Membr. Biol.* **184**, 71-79. doi:10.1007/s00232-001-0075-4

Praetorius, H. A. and Spring, K. R. (2003a). Removal of the MDCK cell primary cilium abolishes flow sensing. *J. Membr. Biol.* **191**, 69-76. doi:10.1007/s00232-002-1042-4

Praetorius, H. A. and Spring, K. R. (2003b). The renal cell primary cilium functions as a flow sensor. *Curr. Opin Nephrol. Hypertens.* **12**, 517-520. doi:10.1097/00041552-200309000-00006

Reiter, J. F., Blacque, O. E. and Leroux, M. R. (2012). The base of the cilium: roles for transition fibres and the transition zone in ciliary formation, maintenance and compartmentalization. *EMBO Rep.* **13**, 608-618. doi:10.1038/embor.2012.73

Resnick, A. (2010). Use of optical tweezers to probe epithelial mechanosensation. *J. Biomed. Opt.* **15**, 015005. doi:10.1117/1.3316378

Resnick, A. (2015). Mechanical properties of a primary cilium as measured by resonant oscillation. *Biophys. J.* **109**, 18-25. doi:10.1016/j.bpj.2015.05.031

Saito, A., Suetomo, Y., Arikawa, M., Omura, G., Mostafa Kamal Khan, S. M., Kakuta, S., Suzaki, E., Kataoka, K. and Suzaki, T. (2003). Gliding movement in Peranema trichophorum is powered by flagellar surface motility. *Cell Motil. Cytoskelet.* **55**, 244-253. doi:10.1002/cm.10127

Satir, P. and Christensen, S. T. (2007). Overview of structure and function of mammalian cilia. *Annu. Rev. Physiol.* **69**, 377-400. doi:10.1146/annurev.physiol.69.040705.141236

Shah, A. S., Ben-Shahar, Y., Moninger, T. O., Kline, J. N. and Welsh, M. J. (2009). Motile cilia of human airway epithelia are chemosensory. *Science* **325**, 1131-1134. doi:10.1126/science.1173869

Shen, P. S., Yang, X., DeCaen, P. G., Liu, X., Bulkley, D., Clapham, D. E. and Cao, E. (2016). The structure of the polycystic kidney disease channel PKD2 in lipid nanodiscs. *Cell* **167**, 763-773.e11. doi:10.1016/j.cell.2016.09.048

Sreekumar, V. and Norris, D. P. (2019). Cilia and development. *Curr. Opin. Genet. Dev.* **56**, 15-21. doi:10.1016/j.gde.2019.05.002

Stepanek, L. and Pigino, G. (2016). Microtubule doublets are double-track railways for intraflagellar transport trains. *Science* **352**, 721-724. doi:10.1126/science.aaf4594

Su, S., Phua, S. C., DeRose, R., Chiba, S., Narita, K., Kalugin, P. N., Katada, T., Kontani, K., Takeda, S. and Inoue, T. (2013). Genetically encoded calcium indicator illuminates calcium dynamics in primary cilia. *Nat. Methods* **10**, 1105-1107. doi:10.1038/nmeth.2647

Su, X., Driscoll, K., Yao, G., Raed, A., Wu, M., Beales, P. L. and Zhou, J. (2014). Bardet-Biedl syndrome proteins 1 and 3 regulate the ciliary trafficking of polycystic kidney disease 1 protein. *Hum. Mol. Genet.* **23**, 5441-5451. doi:10.1093/hmg/ddu267

Su, Q., Hu, F., Ge, X., Lei, J., Yu, S., Wang, T., Zhou, Q., Mei, C. and Shi, Y. (2018). Structure of the human PKD1-PKD2 complex. *Science* **361**, eaat9819. doi:10.1126/science.aat9819

Sun, S., Fisher, R. L., Bowser, S. S., Pentecost, B. T. and Sui, H. (2019). Three-dimensional architecture of epithelial primary cilia. *Proc. Natl Acad. Sci. USA* **116**, 9370-9379. doi:10.1073/pnas.1821064116

't Hart, D. C., van der Vlag, J. and Nijenhuis, T. (2021). Laminar flow substantially affects the morphology and functional phenotype of glomerular endothelial cells. *PLoS One* **16**, e0251129. doi:10.1371/journal.pone.0251129

Teal, T. H., Guillemette, T., Chapman, M. and Margulis, L. (1998). Acronema sippewissettensis gen. nov. sp. nov., microbial mat bicosoecid (Bicosoecales=Bicosoecida). *Eur. J. Protistol.* **34**, 402-414. doi:10.1016/S0932-4739(98)80009-6

Thomazo, J.-B., Le Révérend, B., Pontani, L.-L., Prevost, A. M. and Wandersman, E. (2021). A bending fluctuation-based mechanism for particle detection by ciliated structures. *Proc. Natl. Acad. Sci. USA* **118**, e2020402118. doi:10.1073/pnas.2020402118

Torres, V. E., Harris, P. C. and Pirson, Y. (2007). Autosomal dominant polycystic kidney disease. *Lancet* **369**, 1287-1301. doi:10.1016/S0140-6736(07)60601-1

Tran, Q. D., Galiana, E., Thomen, P., Cohen, C., Orange, F., Peruani, F. and Noblin, X. (2022). Coordination of two opposite flagella allows high-speed swimming and active turning of individual zoospores. *eLife* **11**, e71227. doi:10.7554/eLife.71227.sa2

Vilfan, M., Potočnik, A., Kavčič, B., Osterman, N., Poberaj, I., Vilfan, A. and Babič, D. (2010). Self-assembled artificial cilia. *Proc. Natl Acad. Sci. USA* **107**, 1844-1847. doi:10.1073/pnas.0906819106

Wang, J., Silva, M., Haas, L. A., Morsci, N. S., Nguyen, K. C. Q., Hall, D. H. and Barr, M. M. (2014). C. elegans ciliated sensory neurons release extracellular vesicles that function in animal communication. *Curr. Biol.* **24**, 519-525. doi:10.1016/j.cub.2014.01.002

Wang, J., Nikonorova, I. A., Gu, A., Sternberg, P. W. and Barr, M. M. (2020). Release and targeting of polycystin-2-carrying ciliary extracellular vesicles. *Curr. Biol.* **30**, R755-R756. doi:10.1016/j.cub.2020.05.079

Wang, J., Nikonorova, I. A., Silva, M., Walsh, J. D., Tilton, P. E., Gu, A., Akella, J. S. and Barr, M. M. (2021). Sensory cilia act as a specialized venue for regulated extracellular vesicle biogenesis and signaling. *Curr. Biol.* **31**, 3943-3951.e3. doi:10.1016/j.cub.2021.06.040

Witman, G. B., Carlson, K., Berliner, J. and Rosenbaum, J. L. (1972). Chlamydomonas flagella: I. Isolation and electrophoretic analysis of microtubules, matrix, membranes, and mastigonemes. *J. Cell Biol.* **54**, 507-539. doi:10.1083/jcb.54.3.507

Xiang, W., Jiang, T., Hao, X., Wang, R., Yao, X., Sun, K., Guo, F. and Xu, T. (2019). Primary cilia and autophagy interaction is involved in mechanical stress mediated cartilage development via ERK/mTOR axis. *Life Sci.* **218**, 308-313. doi:10.1016/j.lfs.2019.01.001

Yamaguchi, T., Hempson, S. J., Reif, G. A., Hedge, A.-M. and Wallace, D. P. (2006). Calcium restores a normal proliferation phenotype in human polycystic kidney disease epithelial cells. *J. Am. Soc. Nephrol.* **17**, 178-187. doi:10.1681/ASN.2005060645

Yoder, B. K., Hou, X. and Guay-Woodford, L. M. (2002). The polycystic kidney disease proteins, polycystin-1, polycystin-2, polaris, and cystin, are co-localized in renal cilia. *J. Am. Soc. Nephrol.* **13**, 2508-2516. doi:10.1097/01.ASN.0000029587.47950.25

Yu, Y., Ulbrich, M. H., Li, M.-H., Buraei, Z., Chen, X.-Z., Ong, A. C. M., Tong, L., Isacoff, E. Y. and Yang, J. (2009). Structural and molecular basis of the assembly of the TRPP2/PKD1 complex. *Proc. Natl. Acad. Sci. USA* **106**, 11558-11563. doi:10.1073/pnas.0903684106

Yuan, X. and Yang, S. (2016). Primary cilia and intraflagellar transport proteins in bone and cartilage. *J. Dent. Res.* **95**, 1341-1349. doi:10.1177/0022034516652383

Yuan, S., Zhao, L., Brueckner, M. and Sun, Z. (2015). Intraciliary calcium oscillations initiate vertebrate left-right asymmetry. *Curr. Biol.* **25**, 556-567. doi:10.1016/j.cub.2014.12.051

Zimmermann, K. W. (1898). Beiträge zur Kenntniss einiger Drüsen und Epithelien. *Arch. Mikrosk. Anat.* **52**, 552-706. doi:10.1007/BF02975837