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REVIEW

SUBJECT COLLECTION: CILIA AND FLAGELLA

The cilium as a force sensor–myth versus reality

Rita R. Ferreira[1,2,3,4], Hajime Fukui[1,2,3,4], Renee Chow[1,2,3,4], Andrej Vilfan[5,6,*] and Julien Vermot[1,2,3,4,*]

ABSTRACT

Cells need to sense their mechanical environment during the growth of developing tissues and maintenance of adult tissues. The concept of force-sensing mechanisms that act through cell–cell and cell–matrix adhesions is now well established and accepted. Additionally, it is widely believed that force sensing can be mediated through cilia. Yet, this hypothesis is still debated. By using primary cilia sensing as a paradigm, we describe the physical requirements for cilium-mediated mechanical sensing and discuss the different hypotheses of how this could work. We review the different mechanosensitive channels within the cilium, their potential mode of action and their biological implications. In addition, we describe the biological contexts in which cilia are acting – in particular, the left–right organizer – and discuss the challenges to discriminate between cilium-mediated chemosensitivity and mechanosensitivity. Throughout, we provide perspectives on how quantitative analysis and physics-based arguments might help to better understand the biological mechanisms by which cells use cilia to probe their mechanical environment.

KEY WORDS: Low Reynolds number, Mechanotransduction, Transient receptor potential channel, TRP channels

Introduction

Cells can sense and respond to their physical environment by converting mechanical stimuli into biochemical signals, a process called mechanotransduction (Iskratsch et al., 2014; Vogel, 2018). A variety of force-sensing mechanisms act in cells. Interaction of integrin-mediated adhesions with specific extracellular matrix (ECM) proteins allows cells to sense the rigidity of their environment (Seetharaman and Etienne-Manneville, 2018). Other force-sensing mechanisms involve stretch-sensitive channels that drive many physiological processes, such as touch, hearing and the sensation of pain, as well as the regulation of blood pressure (Murthy et al., 2017; Ranade et al., 2014). Cilia have been proposed to act as efficient mechanosensing organelles owing to their structural geometry (Song et al., 2017).

Cilia are structures that are several microns long and comprise a microtubule-based core; they protrude from many types of cell. Cilia can be divided into two classes – primary cilia and motile cilia – depending on their internal molecular arrangement and their ability to move. Most mammalian cells possess a solitary, non-motile cilium, i.e. the primary cilium, which projects from the apical surface of differentiated cells into the internal lumen of tissue. Because of their unique shape, localization and role in signaling, cilia are often called 'the antenna of the cells' (Malicki and Johnson, 2017). Given their deflection upon mechanical stimuli, properties and location at the cell surface of tissues, it has been proposed that cilia act as a sensor of the mechanical environment. This hypothesis proposes that through bending, a cilium initiates downstream mechanotransduction signaling cascades, including ionic fluxes within the cilium (Spasic and Jacobs, 2017). Cilium bending can be triggered by different mechanical stimuli, such as (i) shear stress generated by flow in blood vessels and the kidneys (Goetz et al., 2014; Nauli et al., 2008), (ii) distortion of the ECM in bones and cartilage (McGlashan et al., 2006), together with compression forces that activate interstitial flows or strain and, (iii) the biological flows generated by motile cilia in the brain and spinal cavities.

There has been a long tradition of quantification and modeling of the mechanical properties of cilia. Careful analysis of ciliary deformation *in vivo* combined with mathematical modeling has been helpful in furthering our understanding of how the mechanical properties of cilia enable them to decode mechanical signals (Battle et al., 2015; Boselli et al., 2015; Rydholm et al., 2010). However, the molecular mechanism of ciliary mechanotransduction is still debated and, as a result, even the existence of mechanosensitivity in cilia is often questioned. Direct ionic flux measurements in cilia have recently revealed that, when isolated from cells, cilia are unresponsive to mechanical deformation but, instead, respond to the Hedgehog pathway (Delling et al., 2013, 2016). It is possible that flow sensing is not directly mediated by the bending of a cilium but, rather, through shear stress or by sensing pressure directly at the cell surface. In addition, cilia function can be assessed in genetic studies through knockout and/or knockdown of potential factors involved in ciliogenesis. However, genetic studies might be hampered when these factors have additional functions. For example, the intraflagellar transport protein 88 homolog (IFT88) is involved in ciliogenesis but has well-described functions outside the cilium (Delaval et al., 2011; Bizet et al., 2015; Taulet et al., 2017).

In this Review, we focus our attention on describing the physics of primary cilia and the proposed process of vertebrate mechanotransduction in both flow-sensing cilia and ECM-embedded cilia. We discuss the evidence for mechanosensation in cilia, present different hypotheses on how this could work and outline experimental approaches to test them.

Physics of primary cilia under flow

The location of cilia at the cell surface and their deformability in response to external forces have suggested that they act as force sensors (Box 1). A key point of this hypothesis is that cilium bending leads to the activation of a mechanosensitive channel that will initiate a mechanosensitive signaling cascade (Box 1). As the molecular mechanism of mechanosensation in cilia is still elusive, possible hypotheses can be narrowed down by examining the physics of a cilium under flow and considering the sensitivity limits of the different potential mechanisms. The detection

[1]Institut de Génétique et de Biologie Moléculaire et Cellulaire, 67404 Illkirch, France. [2]Centre National de la Recherche Scientifique, UMR7104, 67404 Illkirch, France. [3]Institut National de la Santé et de la Recherche Médicale, U1258, 67404 Illkirch, France. [4]Université de Strasbourg, 67404 Illkirch, France. [5]Max Planck Institute for Dynamics and Self-Organization (MPIDS), Department of Living Matter Physics, 37077 Göttingen, Germany. [6]J. Stefan Institute, 1000 Ljubljana, Slovenia.

*Author for correspondence (andrej.vilfan@ijs.si, julien@igbmc.fr)

R.R.F., 0000-0001-7291-9495; A.V., 0000-0001-8985-6072; J.V., 0000-0002-8924-732X

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Box 1. Current hypothesis for cilium-mediated mechanotransduction

Cilia deflect in response to external stimuli and the deflection angle correlates with the response of the cell to mechanical forces (Fig. 1A). This observation has led to the proposal that cilium mechanosensitivity is active in numerous biological settings:

- *Left–right organizer* (LRO): immotile cilia located in the periphery of the mouse LRO are essential for flow sensing. PKD2 is located within cilia, and modulates the intraciliary and cytoplasmic Ca^{2+} in the LRO cells. This Ca^{2+} rise (McGrath et al., 2003; Yuan et al., 2015a) is believed to initiate an asymmetric genetic cascade that is responsible for the left–right patterning of embryos.
- *Kidney cells*: cilia are present in developing and mature kidney cells. The presence of primary cilia and PKD2 is crucial for the increase of Ca^{2+} (Nauli et al., 2003, 2008).
- *Endothelial cells*: cilia in endothelial cells have a unique ultrastructure, which makes them particularly flexible. Alterations in shear stress, ciliogenesis or expression of the Ca^{2+} channel PKD2 impair endothelial Ca^{2+} levels and perturb vascular morphogenesis.

threshold, i.e. the minimum shear rates needed to stimulate a signal (see Table 1 for examples), provides a good way to test the plausibility of the different hypothetical mechanosensing mechanisms. In renal cilia, the threshold has been measured to be 30 s^{-1} (Rydholm et al., 2010). In the following, we will look at a deflected cilium within such a flow and calculate the quantities that are relevant for the proposed detection mechanisms.

Cilia are typically located within a fluid of low Reynolds number (i.e. the ratio of inertial to viscous force of a fluid). Not only is the flow laminar but inertial effects are completely negligible and, in the simplest case, the flow is governed by the Stokes equation

$$\eta\Delta\vec{v} - \nabla p = 0 \tag{1}$$

together with the incompressibility condition $\nabla \cdot \vec{v} = 0$. Here p denotes the pressure, v the fluid velocity and η the viscosity of the fluid. In some cases, the fluid can be viscoelastic, such as the mucus enveloping the respiratory cilia (Smith et al., 2007), but the following derivations assume the case of purely viscous fluids. Both the surface of the cilium and the epithelium impose a no-slip boundary condition on the fluid, i.e., velocity v=0 on the surface.

We illustrate the relationship between the global flow and the forces acting on a cilium in Fig. 1. It shows two typical geometries: a flat channel, frequently used in microfluidic experiments *in vitro* (Fig. 1A), and a tube with a circular cross section, representing a blood vessel or a renal tubule (Fig. 1B). The figure also lists the equations that describe how the quantities that affect the mechanics of a cilium, i.e. shear rate ($\dot{\gamma}$) and shear stress (σ), are related to the experimentally or physiologically relevant parameters, i.e. pressure difference (Δp), volume flow rate (Q) and maximum velocity (v_{max}). Close to the epithelial surface, the flow has a uniform shear rate, and a velocity that is parallel to the surface and grows linearly with the distance $\vec{v} = \dot{\gamma} z\, \hat{e}_x$ (Fig. 1C). $\dot{\gamma}$ is the shear rate, related to the shear stress $\sigma = \eta\dot{\gamma}$. Because of the slender shape of the cilium, we make a second simplification and calculate the force on the cilium according to resistive force theory (Gray and Hancock, 1955). The cilium is surrounded by fluid, which would move with velocity v if the cilium were absent (we assume v is orthogonal to the cilium); acting on it is the local force density, given by

$$f = C_N \dot{\gamma} z, \tag{2}$$

where C_N is the transverse drag coefficient. It has a weak dependence on the cilium thickness and the value of $C_N \approx 1.2\pi\eta$ for a typical cilium (Vilfan, 2012). Finally, we describe the cilium as an elastic beam, whose local curvature is proportional to the local bending moment

$$\kappa = \tau / EI \tag{3}$$

with a bending modulus of about $EI = 3\times10^{-23}\,\mathrm{Nm}^2$ (Battle et al., 2015). For a small deflection, the bending moment as a function of height in a shear flow is

$$\tau = C_N \dot{\gamma}\left(\frac{L^3}{3} - \frac{L^2 z}{2} + \frac{z^3}{6}\right). \tag{4}$$

Table 1. Observed cilium-dependent sensitivities of cells or tissues to shear flows

System	Cilium length (μm)	Shear stress (mPa)	Shear stress (dyn/cm^2)	References
Madin-Darby canine kidney (MDCK) cells	3.5–11.6 (*in vitro*) 8 (modeled)	44	0.44	Rydholm et al., 2010
Mouse inner medulllary collecting duct (mIMCD) cells*	4–6	11	0.11	Delling et al., 2016
Mouse embryonic endothelial cell	~2–	720	7.2	Nauli et al., 2008
Ciliated chicken heart endothelial cell (CHEC), non-ciliated chicken artery endothelial cell (CAEC)	1–3	50–2500	0.5–2.5	Hierck et al., 2008; Van der Heiden et al., 2006
Mouse long-bone osteocyte Y4 (MLO-Y4) cells	2–4	1000	10	Xiao et al., 2006; Xu et al., 2014
MLO-A5 postosteoblasts/preosteocytes	<8	41–51	0.41–0.51	Delaine-Smith et al., 2012
Mouse calvaria MC3T3-E1 preosteoblasts	4–9	36	0.36	Malone et al., 2007
Zebrafish endothelial cells	4–6	10–50	0.1–0.5	Goetz et al., 2014; Samsa et al., 2015; Vermot et al., 2009
Zebrafish endocardial cells	4–6	200	2	Goetz et al., 2014; Samsa et al., 2015; Vermot et al., 2009
Zebrafish Kupffer's vesicle	6‡	1‡	0.01‡	Ferreira et al., 2017; Supatto et al., 2008
Zebrafish vestibular system (kinocilium with hair bundle)	~14	1	0.01	Kindt et al., 2012; Windsor and McHenry, 2009

*, cilia deflection but no Ca^{2+} signal.
‡, values calculated on basis of simulations that recapitulate endogenous flow profiles measured *in vivo*.

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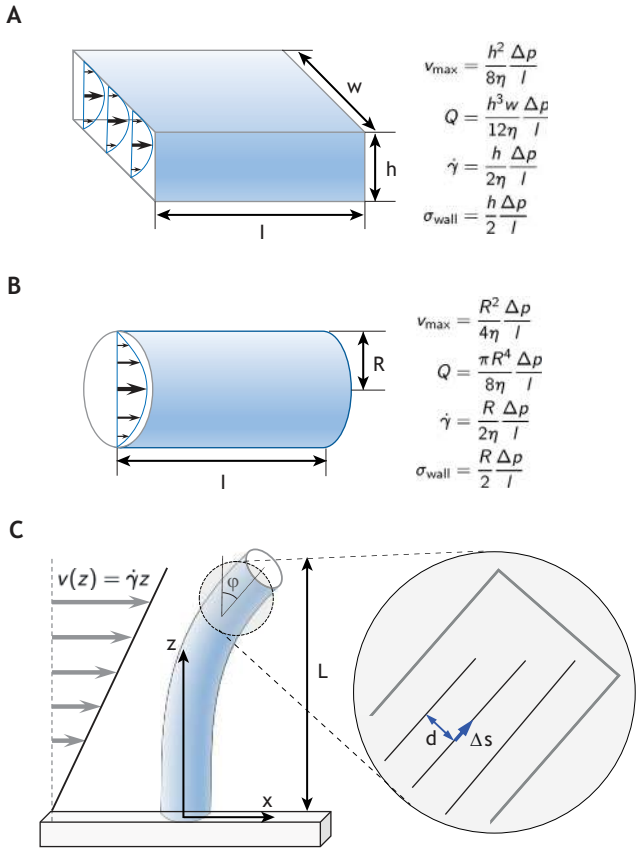

Fig. 1. Properties of a low Reynolds number flow and its effect on a cilium. (A) Flow in a thin channel, frequently used in microfluidic experiments. The channel width is *w* and its height *h*. A pressure difference (Δp) across the length (l) induces a volume flow (Q), i.e. a fluid volume that passes the channel per unit time, and velocity (v_{max}) at the center of the channel. The shear rate near the upper and lower wall is $\dot{\gamma}$ and the shear stress on these two walls σ_{wall}. (B) Flow in a cylindrical tube with the radius *R* (also called *Poiseuile* flow). The tubular geometry describes, for instance, blood vessels or renal tubules, both containing primary cilia on the inside. (C) Schematic illustration of the deflection of a cilium under shear flow. Here, we assume that the cilium is much shorter than the height of the channel ($L<<h$) or the radius of the tube ($L<<R$) and, therefore, senses a uniform shear flow. The flow velocity is parallel to the surface and increases linearly with the distance ($v = \dot{\gamma} z$). The deflected cilium assumes a shape described by the function $x(z)$. This results in microtubule doublets that are separated by a distance (d) to slide relative to each other by distance ($\Delta s = \varphi d$; see inset). Flow is represented by black horizontal arrows in A and B, and grey arrows in C.

The resulting shape of the cilium can be given by

$$x(z) = \frac{C_N \dot{\gamma}}{EI}\left(\frac{1}{6}L^3 z^2 - \frac{1}{12}L^2 z^3 + \frac{1}{120}z^5\right). \quad (5)$$

A solution for larger deflections can be found in Boselli et al., 2015; Young et al., 2012.

As the microtubule doublets approximately keep a constant distance, the bending of a cilium causes a shearing motion between them. For adjacent doublets that are separated by distance d ($d \approx 50$ nm), the shearing displacement is $\Delta s = d \cdot \varphi$, where φ is the angle between the cilium tangent and surface normal (Fig. 1C). For small deflections, its value at the tip is

$$\Delta s(L) = \frac{C_N \dot{\gamma} d L^4}{8\, EI}. \quad (6)$$

In renal cilia that are deflected by a stimulus at the detection threshold, we obtain $\Delta s \approx 50$ nm, a distance that is generally sufficient to open a channel.

If the flow changes abruptly, the shape of the cilium adapts with the relaxation time (Battle et al., 2015)

$$t_{relax} = \frac{C_N L^4}{EI k_1^4} \quad (7)$$

with $k_1 = 1.88$ denoting the coefficient of the slowest relaxation mode of an elastic beam. A cilium with a length (L) =10 µm, therefore, has a relaxation time (t_{relax}) of 0.1 s in water. Stimuli significantly shorter than this time window do not get transmitted to the base of the cilium.

It is also instructive to look at the total bending energy of the cilium, which can be calculated as

$$U_{elastic} = \int_0^L \frac{1}{2} EI\, \kappa(z)^2 dz = \frac{11 C_N^2 \dot{\gamma}^2 L^7}{840 EI}. \quad (8)$$

For a typical renal cilium ($L = 10$ µm) and a weak detectable stimulus ($\sigma = 20$ mPa), the result is about 6000 $k_B T$. Mechanosensitive channels typically work close to thermal equilibrium, meaning that the work needed to open them is of the order of a few $k_B T$ (Liang and Howard, 2018). A small fraction of the bending energy is, therefore, sufficient to open an ion channel, provided that the channel is mechanically connected in a way that transfers a sufficient fraction of the bending energy to it (see next section).

An interesting and usually overlooked aspect of flow around primary cilia is that the shear stress on the surface of the cilium largely exceeds that on the epithelial wall. The stress can be estimated from the force density, which we calculated from resistive force theory. The force on a cylinder consists of shear stress and pressure contributions of equal magnitude (Oseen, 1927). The maximum shear stress on the ciliary boundary is, therefore, related to the force density as

$$\sigma_{cilium} = \frac{f}{2\pi a} \approx \frac{0.6L}{a}\sigma \quad (9)$$

where $a \approx 100$ nm is the radius of the cilium. Compared to an unciliated epithelium, the cilium amplifies the shear stress on its surface by a factor of 0.6 $L/a \approx 60$. A renal cilium at its detection threshold can have a local shear stress of $\sigma_{cilium} \approx 1$ Pa, which is sufficient to affect the conformation of many proteins (Bekard et al., 2011).

The membrane tension on a cilium is more difficult to estimate. A recent computational result, assuming an elastic membrane, estimates that a shear rate of $\dot{\gamma} = 1\,\mathrm{s}^{-1}$ leads to a membrane tension of 1 µN/m (Omori et al., 2018). However, this result only holds for short time scales (sub-second). On time scales of seconds or longer, the membrane becomes fluid and redistributes itself, relaxing any tension that results from stretching of the membrane. Another way to estimate the membrane tension is from force balance: as the flow deflects the cilium, an increasing tangential component of the shear stress is counterbalanced by the membrane tension:

$$2\pi a\, \tau = \int_0^L \sin[\varphi(z)] f(z) dz \approx \frac{C_N \dot{\gamma} L^2 \varphi_{eff}}{2}. \quad (10)$$

With data for a renal cilium at the sensitivity threshold, the result is of the order of µN/m.

To summarize, the bending moment (torque) and the curvature of a cilium are maximal in the proximal region. The bending moment at the

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base of a cilium depends strongly on the length of the cilium – it scales with L^3. The local shear stress on the surface of a cilium is highest in the distal region and surpasses the shear stress on the epithelial wall by two orders of magnitude. The line tension of the membrane due to flow is very modest, i.e. several orders of magnitude below the sensitivity threshold of known tension gated channels. These considerations suggest that the torque at the base, the internal shear and external shear stress are the most plausible mechanisms for ciliary mechanosensation. Thus, cilia mechanosensation is theoretically possible with shear stresses in the mPa range. However, to prove the existence of cilia mechanosensation, coupling of cilia with mechanosensors needs to be demonstrated. In the next section, we discuss possible mechanisms of coupling.

Possible mechanosensory coupling mechanisms

To date, there is no clear mechanism on how cilium bending is coupled with its mechanosensor. The mapping of ciliary deformations onto cellular signals is tightly connected with the question of how the mechanosensor is attached to the ciliary structure. Given the lack of experimental information about ciliary mechanosensors, we start with a general discussion of possible coupling mechanisms that are summarized in Fig. 2. One hypothesis

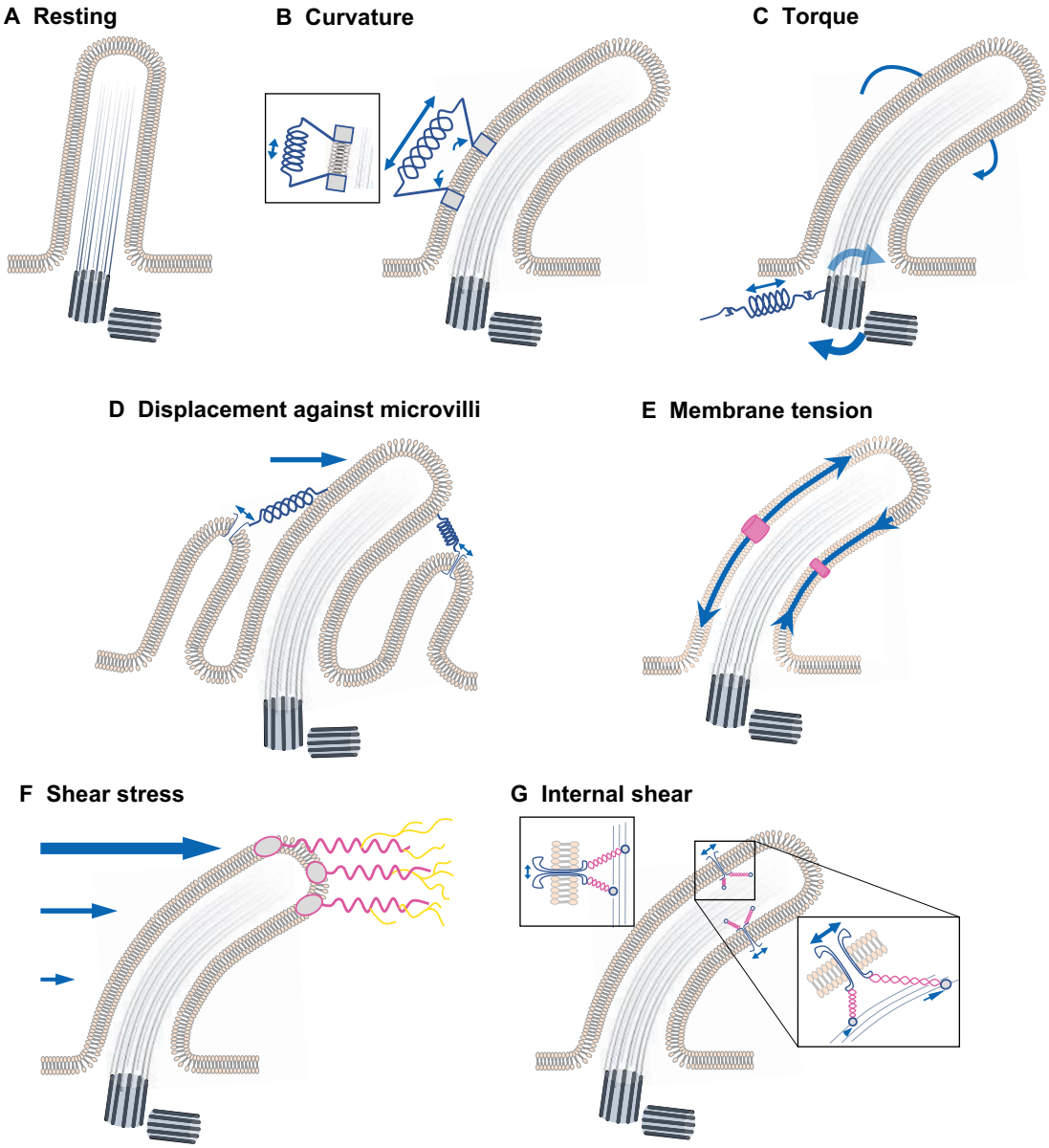

Fig. 2. Possible mechanisms of mechanosensation in primary cilia. (A) Illustration of a resting cilium in the absence of flow. (B) Curvature-gated mechanosensitive channel. Bending of the cilium causes tension in an elastic element on the surface (inset). This, in turn, opens a stretch-sensitive channel (blue) connected to each end of the elastic element. A curvature-sensitive channel achieves the highest sensitivity when located close to the base of the cilium. (C) Strain-sensing elements (blue) connected to the basal body sense the bending moment (torque) on the cilium. (D) Stretch-sensitive channels located in microvilli that are connected to a cilium through tip links elements (blue). The flow deflects a cilium, which causes a shearing motion between the cilium and the microvilli. The resulting tension in the tip links opens the channels. (E) Stretch-sensitive channels (pink) that sense increased membrane tension on the outer side of the bent cilium compared to the inner side. (F) The shear stress close to the tip of a cilium is significantly larger than that acting on an epithelial surface without cilia. This could lead to tension in extracellular polymer chains (pink) located at the cilium tip, thereby pulling on mechanosensitive channels. (G) The channel (left inset) is connected to two different microtubule doublets in the axoneme. Deflection of a cilium leads to internal shear between adjacent microtubule doublets (right inset), which could trigger the opening of a channel that is linked to them. The shearing motion and, therefore, the sensitivity is highest close to the tip of the cilium.

is that the mechanosensitive channel senses the curvature of the cilium. In this case, the channel needs to be gated by the distance between two anchoring points that are themselves attached to the axoneme at some radial distance (Fig. 2B). Curvature will cause tension in the linkage, in a similar way as the deflection of a hair bundle causes tension in the tip link and opens the mechanotransduction channel. Because the curvature of a deflected cilium has its maximum at the base, this is where one expects a curvature-based sensor to be located. A curvature sensor can be directionally selective if attached to a specific microtubule doublet in the axoneme. Alternatively, there could be a torque sensor inside the cell that senses stress on the basal body caused by flow-induced torque on the cilium (Fig. 2C). Most probably, such a sensor would not be a transmembrane channel but, rather, a bridging protein that binds to a constituent of the cilium and the cytoskeleton of the cells. Potential candidates include many microtubule- and/or actin-binding proteins. Such a mechanosensor could work as a mechanochemical switch similar to talin (Goult et al., 2018). In this scenario, Ca^{2+} channels would act downstream of the actual force sensor. Another possibility is that displacement against microvilli in response to flow will open a channel located in the microvilli (Fig. 2D).

A different concept has been proposed for nodal cilia; it involves a stretch-sensitive channel in the membrane of a cilium (Omori et al., 2018) (Fig. 2E). However, the changes in membrane tension are estimated to be in the range of μN/m, which is more than three orders of magnitude below the known detection thresholds of other tension-gated channels, such as the Piezo-type mechanosensitive ion channel component 1 (Piezo1) (3 mN/m; Lewis and Grandl, 2015), and the bacterial large- and small-conductance mechanosensitive channels MscL (12 mN/m; Sukharev et al., 1999; Nomura et al., 2012) and MscS (6 mN/m; Nomura et al., 2012), respectively. Besides that, as discussed above, membrane fluidity quickly compensates any stresses (Shi et al., 2018). This has important consequences for the coupling between cilia and ion channels: even though the membrane is flowing slowly above the cortex, it would relax within ∼1 s on the thin cilium. If we postulate the likely scenario that the membrane of the cilium does not have many anchoring points, membrane fluidity would be high and quickly compensate for any stresses, thus making the detection of membrane tension almost impossible. Another argument against the detection of membrane tension comes from Delling et al., (2016), who applied supraphysiological shear rates (1000/s) but did not show any Ca^{2+} signal in the cilium until the point when the membrane ruptured under tension.

An interesting, and largely unexplored, possibility is that the cilium detects shear stresses on its surface, for example through protruding chains made of glycosylated protein that are subject to pulling forces in a shear flow (Fig. 2F). By analogy, similar mechanisms exist in endothelial cells that express a glycocalyx at their cell surface, providing them mechanosensitivity (Zeng et al., 2018) but, as we have demonstrated above, the shear stress on the surface of a cilium close to its tip is ∼60 times larger than the stress on a flat surface in the same flow. In this scenario, the function of the cilium is to amplify local shear rates due to the geometry of the cilium itself and to hold the sensor at a distance from the cell surface. The deflection, then, has no role in the mechanosensitive mechanism. Such structures have never been reported for sensory cilia but a number of different types of protrusion (mastigoneme) are known in motile flagella (Moestrup, 1982). Ciliary tips are now the focus of special attention, as it becomes clear that intense vesicle trafficking takes place in this region (Nager et al., 2017; Wang et al., 2014). Furthermore, recent progress in electron microscopy and high-pressure freezing fixation is now beginning to unravel extraordinary heterogeneities in ciliary distal tips, indicating that the ciliary tip may fulfill specific needs for sensorial signaling, such as increasing the surface area of receptor organelles (Croft et al., 2018). Interestingly, it has been shown that the ultrastructure of endothelial cilia is changing along its proximal–distal axis (Goetz et al., 2014), suggesting once again that the cilium tip is special.

Finally, there is the possibility that a mechanosensitive channel senses the shearing motion between microtubule doublets in the axoneme (Fig. 2G). This would require the channel to be linked between two microtubule doublets. Unlike curvature-sensitive channels, shear-sensitive ones would have to be located close to the tip of the cilium.

These general considerations significantly narrow down the possible structures and locations of the mechanoreceptors. Of all the hypothetical mechanisms we propose here, the scenarios where the mechanosensor is either located at the basal body or between microtubules provide an efficient and plausible mechanism of mechanosensory coupling.

Mechanosensitive channels in primary cilia

The best-established ion channels active in cilia are probably those belonging to the family of transient receptor potential (TRP) channels. TRP channels are non-selective cation channels that serve as cellular sensors for a wide spectrum of physical and chemical stimuli, including temperature, redox status and environmental stress (Kaneko and Szallasi, 2014). Since they are often located within cilia, TRPs are good candidates of mechanosensitive channels that are active in cilia mechanosensation. Nevertheless, many TRP channels are also activated by the well-known second messenger Ca^{2+}. The specific mechanism of TRP channel activation is an intense focus of study, especially in cilia.

A number of TRP proteins, such as TRPM4 (Flannery et al., 2015), TRPV4 (Kottgen et al., 2008), TRPC1 (Bai et al., 2008), PKD2 (also known as TRPP2) (Liu et al., 2018; Ramsey et al., 2006; Venkatachalam and Montell, 2007), PKD2L1 (DeCaen et al., 2013, 2016) and TRPM3 (Siroky et al., 2017) have been proposed to form ciliary ion channels. They usually act as heterodimers and TRPV4 (Kottgen et al., 2008; Stewart et al., 2010), TRPC1 (Bai et al., 2008) and TRPM4 (Flannery et al., 2015) all form complexes with PKD2. Since TRPV4 can sense changes in osmolarity, an attractive hypothesis is that presence of TRPV4 in primary cilia is required to sense tonicity in the microenvironment (Gradilone et al., 2007). To date, the mechanism of activation of TRPV4 by mechanical forces remains elusive. This is complicated by the fact that the PKD2–TRPV4 complex has distinct pharmacological, biophysical and regulatory readouts compared to either PDK2 or TRPV4 channels on their own (Zhang et al., 2013). TRPC1 is a stretch-sensitive channel (Maroto et al., 2005) and acts as a heteromultimer with PKD2 at the base of cilia (Bai et al., 2008). Here, both PKD2 and TRPC1 assemble into a new G-protein-coupled receptor (GPCR)-activated channel with biophysical properties distinct from those of PKD2 and TRPC1 alone. The proposed physiological role of this new channel is to mediate GPCR-induced conductance in native kidney epithelial cells, indicating that GPCR-dependent signaling might underlie the PKD2-mediated mechanotransduction (through fluid-shear stress or cilium bending) at the cilia (Bai et al., 2008). However, the mechanism of activation of this GPCR by mechanical forces remains to be established.

On its own, PKD2 can make a homotetrameric channel (Shen et al., 2016). In addition, PKD2 interacts with another polycystin,

PKD1, to form a functional ion channel. Indeed, PKD2 is a large subunit of ciliary ion channels in immortalized mouse inner medullary collecting duct (mIMCD-3) cells (Kleene and Kleene, 2017), that are thought to act in a complex with PKD1 in the primary cilium and endoplasmic reticulum (Ong and Wheatley, 2003; Yoder et al., 2002). The PKD1 family members are classified as large proteins (~1700–4300 amino acids), and composed of 11 putative transmembrane segments and a large N-terminal extracellular domain, whose function is unclear (The International Polycystic Kidney Disease Consortium, 1995; Hughes et al., 1995; Yuasa et al., 2002). Still, PKD1 has been proposed to be a GPCR (Parnell et al., 1998, 2002). The cryo-electron microscopy structure of truncated human PKD1–PKD2 reveals that the complex is assembled in a 1:3 ratio (Su et al., 2018a). However, it has recently been shown that PKD2 forms a functional ion channel in the primary cilia of primary IMCD cells and that PKD1 is not essential for its basal activity, thus, suggesting that PKD2 – but not PKD1 – is the required subunit for the ion channel in the primary cilium (Liu et al., 2018). A confounding aspect of the activity profile of the PKD1–PKD2 heterodimer is that Wnt ligands can activate the Ca^{2+} conductance in kidney cells by physically binding to PKD1 (Kim et al., 2016). This could be important during embryonic development, when the Wnt ligand and PKD2 were shown to modulate heart valve and blood vessel development (Goddard et al., 2017; Goetz et al., 2014; Heckel et al., 2015; Samsa et al., 2015). Thus, Ca^{2+} conductance within cells might be triggered by the PKD1–PKD2 complex without the need of cilium mechanosensitivity.

Other TRP members are becoming more characterized and their mechanosensitivity in cilia are tested. PDK2L1 has been identified by homology to PKD2 (Nomura et al., 1998; Wu et al., 1998), and interacts with PKD1L1 in the retinal pigmented epithelium and embryonic fibroblasts (DeCaen et al., 2013). In contrast to PKD2 (Liu et al., 2018; Arif Pavel et al., 2016), PKD2L1 can form a constitutively active ion channel in both the plasma membrane and primary cilia (DeCaen et al., 2013, 2016). PKD2L1 forms a functional channel as a homotetramer that has many structural similarities to that of the PKD2 homotetramer. However, some subtle structural differences between the two ion channels (Hulse et al., 2018) potentially explain their different mechanisms of mechanosensitive activation. Indeed, differences in pore diameter and electrostatic fields between PKD2L1 and PKD2 (Hulse et al., 2018) might explain why the first conducts Ca^{2+}, whereas the latter does not (DeCaen et al., 2013; Kleene and Kleene, 2017; Liu et al., 2018). These subtle changes in relative permeation of the TRP channels might function in order to titrate ion levels within very small sub-cellular compartments, such as a cilium (Hulse et al., 2018). PKD2L1 opening is not sensitive to changes in membrane pressure (0–60 mm Hg) within the primary cilia of immortalized retinal pigmented epithelial (mRPE) cells (DeCaen et al., 2013). Also, there is no evidence of activity of the PKD1L1–PKD2L1 channel, suggesting that PKD2L1 lacks the sensitivity observed in other mechanosensitive channels (Coste et al., 2012; Sukharev et al., 1994); it is, thus, unlikely to function as a mechanosensor under physiological conditions.

Despite all these studies, the origin of the Ca^{2+} involved in secondary messaging within the cilium is still debated. In fact, by performing slow imaging, Delling and colleagues revealed that, in cells containing primary cilia, the Ca^{2+} wave is initiated in the cytoplasm before propagating into the primary cilium, and not vice versa. This suggests that lack of temporal resolution is the reason that the origin of Ca^{2+} influxes in cells is within the cilium. (DeCaen et al., 2013). Given its small size (7 µm long with a diameter of 300 nm), a cilium has a larger surface-to-volume ratio compared with the cell body. It, therefore, exposes a higher proportion of ion channels to the extracellular space, with an estimated channel density of 30 channels/µm^2 (Delling et al., 2013). Patch-clamp measurements revealed that Ca^{2+} currents in primary cilia from distinct cell lines have shown that the Ca^{2+} concentration [Ca^{2+}] at resting state is much greater inside the cilium than in the cell cytoplasm (Delling et al., 2013; Yuan et al., 2015a). In addition, changes in cytoplasmic Ca^{2+} spread to the cilium but, given the larger volume of the cytoplasm, any Ca^{2+} changes that occur in the cilium are then buffered in the cytoplasm (Delling et al., 2013). If primary cilia are not Ca^{2+}-responsive mechanosensors themselves, the mechanically-induced Ca^{2+} waves have to be initiated elsewhere to raise the ciliary [Ca^{2+}] (Delling et al., 2016). A consequence of increasing cytoplasmic free [Ca^{2+}], by mechanical or other stimuli, is an increase in cilioplasmic [Ca^{2+}], which indirectly potentiates the activity of the ciliary PKD2 channel. If TRP channels are, indeed, not involved in ciliary mechanosensitivity, we might have to consider other proteins that can sense changes in geometry and force without eliciting changes in ciliary [Ca^{2+}]. Probable candidates are GPCR proteins, as a number of them are found in the ciliome (Sigg et al., 2017) and some of these display mechanosensitive properties (Xu et al., 2018).

Tissues with cilium-dependent mechanosensitivity

Primary cilia have been proposed to act as a mechanosensor in response to fluid-flow shear stress in different vertebrate tissues. For example, the primary cilium might be able to sense flow-derived shear stress in blood vessels, central nervous system and kidneys. In bone, primary cilia have been proposed to act as a mechanosensor that responds to fluid flows caused by deformations due to loading (Malone et al., 2007). According to one hypothesis, mechanosensitive cilia also detect flows in the vertebrate left–right organizer (LRO) (Shinohara and Hamada, 2017). In many of these systems, the evidence for cilium-mediated mechanosensing is challenged by the presence of different confounding factors. In the sections below, we discuss evidence provided by work already performed on ciliary mechanosensation, as well as outline the future work needed to clarify the role of cilium-mediated mechanosensing in these vertebrate systems.

Blood vessels

Blood flow is well known to influence the shape and growth of vascular networks. The mechanism by which endothelial cells detect and interpret flow-derived signals to control their behavior remains unclear. Endothelial cells are ciliated, and several studies have shown that flow sensing could be involved (Fig. 3A). Work in zebrafish has shown that primary cilia deflect in response to subtle changes in flow forces (Goetz et al., 2014), and cilia seem key to modulate remodeling of the vascular network where the flow is low, such as in the vascular plexus (Goetz et al., 2014). Furthermore, work in the mouse retina reinforces the idea that primary cilia stabilize vessel connections in specific parts of the remodeling vascular plexus that experiences low and intermediate shear stress (Vion et al., 2018). However, proving cilium-mediated mechanosensitivity is difficult as cilia have additional functions in the developing vascular system. For example, mutants that lack cilia also display brain hemorrhages that seem related to defects in Hedgehog signaling (Kallakuri et al., 2015). This makes definite conclusions about cilium mechanosensitivity in endothelial cells difficult, as the impact of the Hedgehog pathway on the function of endothelial cells has not been systematically assessed. A good indicator of cell mechanosensitivity is the fact that flow activates

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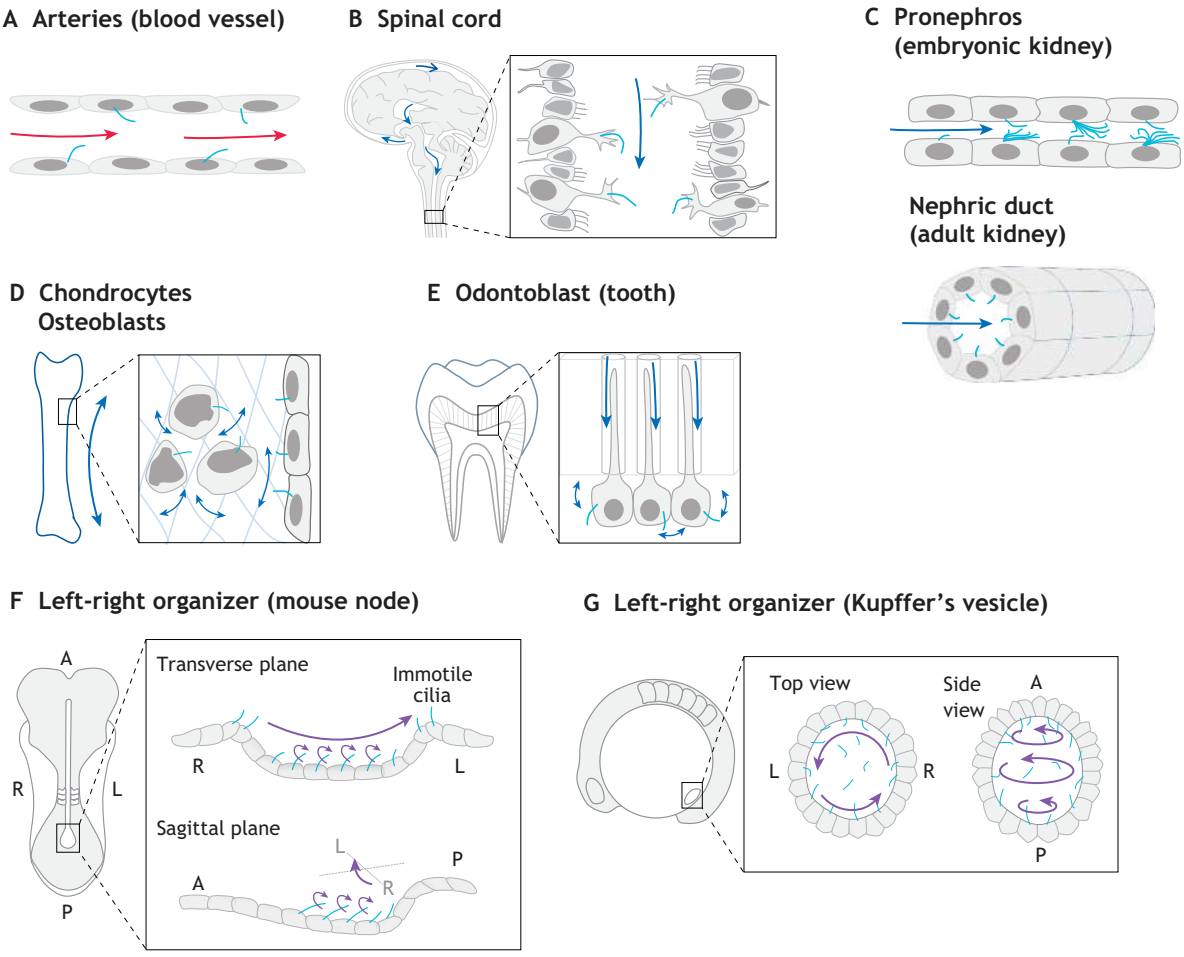

Fig. 3. Animal organs and tissues proposed to have mechanosensitive primary cilia. (A) The endothelial cells of the zebrafish and mouse arteries are lined by primary cilia. Their number and location vary according to the flow profile, and primary cilia are absent in areas of high shear stress. (B) Epithelial cells and the cerebrospinal fluid-contacting neurons that line the brain ventricles have been shown to be ciliated. (C) Embryonic kidney cells display primary cilia, potentially involved in flow sensing. (D) Chondrocytes and osteoblasts have primary cilia; here, flow sensing has been proposed in areas of interstitial flow. (E) Odontoblastic cells populating the tooth are ciliated and could use their primary cilia to generate the flow in the dentine tubules. (F) The mammalian left–right organizer (LRO), called the node, consists of a membrane-covered cavity, lined with motile primary cilia in the center and immotile cilia at the periphery. According to the mechanosensing hypothesis, immotile cilia mechanically sense the direction of the directional flow. (G) The zebrafish LRO, i.e. the Kupffer's vesicle, comprises a sphere-like structure containing both motile and immotile cilia. Cilia are represented as thin pale blue lines; blood flow and cilium-mediated flow directions are indicated by red and purple arrows, respectively; general flow direction is represented as dark blue arrows. L, left; R, right; A, anterior; P, posterior.

Ca^{2+} fluxes in the cells via a PKD2-dependent mechanism (Goetz et al., 2014; Nauli et al., 2008). Nevertheless, Ca^{2+} transients have been also shown to fluctuate in response to activation of the Notch signaling pathway (Yokota et al., 2015), a mechanically activated pathway in endothelial cells (Mack et al., 2017). This leaves the possibility that cilium-mediated mechanosensitivity and Notch/Hedgehog signaling are interconnected. Importantly, cilia on endothelial cells seem to be dispensable for endothelial development (Dinsmore and Reiter, 2016), since the conditional IFT88 knockout does not display an obvious phenotype and the conditional endothelial PKD2 knockout has a 50% lethality rate. This suggests that PKD2 also has an extra ciliary function that is independent of the cilium. Here, the evidence for mechanosensitivity is largely based on genetics, and a direct demonstration of cilium bending leading to cytoplasmic Ca^{2+} activation is still lacking.

Central nervous system

Throughout vertebrates, the brain and spinal cord are subjected to cerebrospinal fluid flows during embryonic development and in the adult (Orts-Del'Immagine and Wyart, 2017). The cerebrospinal fluid-contacting neurons (CSF-cNs) are ciliated cells that line the central canal in the ventral spinal cord (Fig. 3B). Recently, it has been shown that CSF-cNs modulate locomotion by directly projecting onto the locomotor central pattern generators and that CSF-cNs form an intraspinal mechanosensory organ that detects spinal bending. CSF-cNs regulate locomotion by relaying mechanical stimuli to spinal circuits (Böhm et al., 2016). The cellular basis of CSF-cN mechanosensation remains unclear but involves a motile kinocilium and multiple microvilli. Importantly, CSF-cNs specifically require the TRP channel PKD2L1 to respond to pressure (Sternberg et al., 2018). The unambiguous demonstration that cilia act as mechanosensors would require showing that the bending of CSF-cN cilia leads to cell response. In fact, whether CSF-cNs are the direct mechanosensory cells is still debated as other unidentified mechanosensitive cells might be at work. It is also possible that CSF-cNs respond to chemical cues released in the central canal as a response to mechanical stimuli (Orts-Del'Immagine and Wyart, 2017).

Kidney

Epithelial cells (EpCs) lining the proximal tubule of each kidney are continuously exposed to variations in flow rates of the glomerular ultrafiltrate and are responsible for reabsorbing up to 70% of the ions, glucose, and water from this flow. In most regions of the renal tubules, EpCs possess a primary cilium on their apical surface, and the current hypothesis is that these cilia can sense glomerular flow (Praetorius and Spring, 2003a,b) and embryonic flows generated in the pronephros (Fig. 3C). Renal EpCs in culture have been used to uncover the role of the primary cilium in response to laminar flow variations in renal tubules and most of the evidence of cilia as mechanosensors hinges on the study of Ca^{2+} rise in the cilium upon mechanical stimulation. Work from the Spring group has shown that the mechanical bending of the primary cilium, either by applied flow or micropipette manipulation, results in an intracellular rise of Ca^{2+}, and also, that cilium removal impairs the flow-sensing response (Praetorius and Spring, 2001, 2003a,b). Furthermore, studies in cells report a robust cilium-dependent intracellular Ca^{2+} mechanism in response to subtle changes in laminar flow (Praetorius, 2015). This Ca^{2+} influx was proposed to be mediated by mechanosensitive channels located at the cilium or its base, such as the PKD1–PKD2 ion channel complex (Praetorius and Spring, 2003b). Mouse embryonic kidney cells were used to uncover the role of PKD1 and PKD2 in flow sensing: unlike wild-type and control cells, cells from mutant PKD1 or non-functional PKD2 do not increase their intracellular Ca^{2+} in response to urine flow (Nauli et al., 2006). Immunohistochemistry shows that both PKD1 and PKD2 are localized within kidney cell cilia (Yoder et al., 2002). These results suggest that both PKD1 and PKD2 participate in the mechanosensitive flow-sensing response. However, more recent work has challenged this model and shown that no changes in ciliary Ca^{2+} are detectable in primary cilia of cultured kidney EpCs in response to flow (Delling et al., 2016). As a consequence, direct evidence that intraciliary secondary messenger is generated as a response of cilium bending is lacking.

Bone

The primary cilium has also been suggested to act as a mechanosensor in osteocytes and chondrocytes, connective tissue cells involved in the maintenance of bone and cartilage, respectively (Yuan et al., 2015b).

Osteocytes reside in lacunae and create a network throughout the bone tissue by extending their processes through the canaliculi (Whitfield, 2003) (Fig. 3D), where fluid moves as a result of strain pulses along the bones (Knothe Tate et al., 1998, 2000; Smit et al., 2002). Interestingly, odontoblastic cells that populate the tooth and are responsible for dentine formation have also been reported to be ciliated (Fig. 3E) (Magloire et al., 2004; Hampl et al., 2017). Osteocytes are considered to be mechanosensing cells that, through their primary cilia, can detect flow variations inside the lacunar-canalicular network (Tatsumi et al., 2007) and, thereby, transmit signals for bone remodeling (Whitfield, 2003). Here, primary cilia are thought to sense extracellular flow (Leucht et al., 2013; Malone et al., 2007), i.e. through PKD1 and TRPV4 channels (Corrigan et al., 2018; Masuyama et al., 2008; Xiao et al., 2011). Nevertheless, the bending stimulus and the link between TRP channels and mechanosensitivity are yet to be established in these cells.

In contrast to EpCs and osteocytes, cilia found in chondrocytes are embedded in the extracellular matrix (ECM). Cells can sense and react to ECM composition and, consequently, trigger important signaling pathways (reviewed in Seetharaman and Etienne-Manneville, 2018). Indeed, ECM-binding proteins and adhesion-mediated integrins both localize to chondrocyte cilia (Jensen et al., 2004), suggesting that cilia cannot act as flow sensors but, instead, directly sense deformations of nearby tissue (McGlashan et al., 2006). In fact, it has been suggested that the activity of chondrocyte cilia depends on the structural organization and mechanical properties of the ECM (Seeger-Nukpezah and Golemis, 2012). Interestingly, interactions between cilia and ECM have been proposed to regulate primary cilium position and orientation in tendon cells (Donnelly et al., 2010). Work combining electron tomography and confocal imaging revealed a direct link between collagen fibers and the primary cilium, thus, demonstrating that ECM components closely associate with the cilium of chondrocyte cells (Jensen et al., 2004). Upon binding to collagen fibers, the primary cilium deflects in response to force transmitted through the ECM. Altogether, this suggests that chondrocyte primary cilia can act as mechanosensors by detecting mechanical and biochemical changes within their environment, and transducing signals to the cell, thereby controlling the chondrocytes' metabolic activity. The irrefutable demonstration that cilia act as mechanosensors in chondrocytes requires the identification of the mechanosensor and the stimulus leading to the bending of the cilia.

Left–right organizer

Perhaps the most controversial of the hypotheses related to cilium-mediated mechanosensation applies to the left–right organizer (LRO), a specialized structure that establishes body laterality in the embryo (Nonaka et al., 1998). LROs have been observed in every vertebrate, but their mechanism of action can vary from species to species (Essner et al., 2002). In most vertebrates, the LRO contains motile cilia that are needed to generate a directional flow (Fig. 3F,G). In mouse, the LRO cilia have a ring of nine outer microtubule doublets – the 9+0 axoneme structure – with dynein arms that allow cilia rotation and, thus, the production of a leftward fluid-flow (Shinohara and Hamada, 2017). The LRO of the mouse embryo is called the node and has the shape of a cavity, formed by monociliated epithelial cells and covered by a cell layer (Fig. 3F). In amphibians, the topology of the LRO is fairly similar to that of mouse (Blum et al., 2009). In zebrafish, the LRO is known as the Kupffer's vesicle, which is slightly different to the LRO in mammals, as it comprises a cavity containing mostly motile cilia (Fig. 3G). The distribution of motile cilia in the zebrafish LRO generates a flow profile that is significantly different when compared to other species. In mouse and frog, cilia are categorized into two populations, depending whether the gene encoding left-right dynein (*lrd*; officially known as *Dnah11* and *dnah11*, in mouse and frog, respectively) is expressed (McGrath et al., 2003); motile *lrd*-positive cilia reside at the center of the node, while a population of immotile *lrd*-negative cilia resides at the periphery. On the basis of this observation, two different models that explain the symmetry breaking within the LRO have been postulated: (1) establishment and detection of an asymmetric chemical gradient (Okada et al., 2005), and (2) ciliated cells that can mechanically sense the generated flow (McGrath et al., 2003; Yoshiba et al., 2012). However, for both models, the outcome is an asymmetric left-sided Ca^{2+} signal (McGrath et al., 2003; Yuan et al., 2015a) that, in turn, triggers a genetic cascade responsible for the left–right determination and biased *situs*, i.e. the usual asymmetrical position of main visceral organs (reviewed in Ferreira and Vermot,

Box 2. Evidence for and against mechanosensitive flow detection in the left–right organizer

Determination of the left–right axis in the developing embryo is crucial for the asymmetric positioning of internal organs (situs). In most vertebrate species, determination of the left–right axis is under the control of a directional flow generated by motile cilia. The center organizing this axis is called the left–right organizer (LRO) and contains both motile and immotile cilia. Mouse LROs and the Kupffer's vesicle in zebrafish constitute great model systems to study interference with the left–right symmetry because they are both genetically tractable and amenable for high-resolution live imaging. These features allow precise quantitative measurements, which can be used to test the feasibility of the mechanosensing hypothesis *in vivo*. The presence of immotile cilia in the LRO and the possible role of the ion channel PKD2 in cilia mechanosensation has led to the proposal that mechanotransduction participates in symmetry breaking (Yuan et al., 2015a). Two possible ways of mechanically sensing the directional flow have been put forward; (1) the detection of asymmetric flow velocity (Sampaio et al., 2014) and (2) the detection of the direction of the flow (McGrath et al., 2003).

Evidence for mechanosensing:

- Confirmed presence of motile cilia that generate flow and of immotile cilia that could act as flow sensors.
- PKD2 is necessary; it has been found in renal cilia, where evidence for mechanosensation is stronger.
- LRO cells that lack all cilia fail to respond to artificial flow but restoration of primary cilia in the LRO rescues its response to the flow (Yoshiba et al., 2012).

Evidence against mechanosensing:

- The calculated forces acting on cilia are below the detection threshold found for other mechanosensitive cilia.
- It is currently unclear whether the ciliary mechanosensing mechanism is able to distinguish the direction of flow.
- There are only a very few immotile cilia in zebrafish and medaka, with almost all cilia being motile at the late stages of the LRO in zebrafish.

We recently put forward an alternative mechanosensory hypothesis, whereby motile cilia are able to sense their own motion. There, an asymmetric cilia orientation might generate or amplify asymmetry (Ferreira et al., 2018). More exciting work is expected from quantitative analyses in this field.

2017; Norris, 2012; Pennekamp et al., 2015). In the mechanosensory model, the mechanical stress of the leftward flow is specifically sensed by the mechanosensitive immotile cilia on the left periphery of the node (Shinohara and Hamada, 2017). A left-sided Ca^{2+} signal is observed in LRO cells through the action of PKD2 channels that are located on the ciliary axoneme (Yoshiba et al., 2012). Importantly, PKD2 function during left–right patterning is conserved amongst vertebrates. PKD2 acts in combination with other proteins, such as PKD1L1 (Field et al., 2011) to mediate the Ca^{2+} flux in the LRO. In the Japanese rice fish (also known as medaka), both PKD2 and PKD1L1 localize on the cilia of the LRO and are involved in the establishment of the left–right axis (Field et al., 2011; Kamura et al., 2011; Schottenfeld et al., 2007; Yoshiba et al., 2012; Yuan et al., 2015a). Nevertheless, quantitative flow analyses have challenged the view that primary cilia act as mechanosensors in the LRO (see Box 2), and the demonstration that cilium bending occurs in crown cells and leads to the activation of secondary messenger within the cilia is still outstanding.

Conclusions and future directions

Elucidating the mechanisms that allow cilia to probe their mechanical environment is still at the premise stage. By summarizing recent efforts in the field, we identified the main open questions that point to important lines of research for the future. First, cilium mechanosensitivity is still debated in the biological systems we describe here and, second, the molecular basis of cilium mechanosensitivity remains unclear. We expect the use of simpler systems that are amenable to experimental approaches, such as the polychaete *Platynereis dumerilii* (Bezares-Calderon et al., 2018), as well as organoids and gastruloids (Beccari et al., 2018; Takasato et al., 2015), to have a main impact in the future on our understanding of cilia mechanotransduction. One large step forward will be the development of optogenetic and subcellular manipulation tools to assess protein functions specifically within cilia (Chow and Vermot, 2017; Harmansa and Affolter, 2018). Important questions that need to be addressed in the future to understand cilium-mediated mechanotransduction include (i) what is the ultrastructural organization of the cilium, (ii) how are forces spreading in the membrane when a cilium deflects and, (iii) what are the proteins that link the cell membrane, the cilium and the mechanosensors. Finally, quantitative biology combined with physics tools and structural biology are required to unravel the molecular principles of mechanosensation.

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Competing interests

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