Review

Emerging principles of primary cilia dynamics in controlling tissue organization and function

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Abstract

Primary cilia project from the surface of most vertebrate cells and are key in sensing extracellular signals and locally transducing this information into a cellular response. Recent findings show that primary cilia are not merely static organelles with a distinct lipid and protein composition. Instead, the function of primary cilia relies on the dynamic composition of molecules within the cilium, the context-dependent sensing and processing of extracellular stimuli, and cycles of assembly and disassembly in a cell- and tissue-specific manner. Thereby, primary cilia dynamically integrate different cellular inputs and control cell fate and function during tissue development. Here, we review the recently emerging concept of primary cilia dynamics in tissue development, organization, remodeling, and function.

Keywords cilia dynamics; ciliary signaling; cilium disassembly; primary cilia; tissue organization

Subject Categories Cell Adhesion, Polarity & Cytoskeleton; Development; Signal Transduction

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Introduction

Almost all cells, from embryonic development until adulthood, form a hair-like membrane protrusion with a microtubule-based core, called the primary cilium. Primary cilia are immotile and have sensory and signaling functions (Malicki & Johnson, 2017; Anvarian *et al*, 2019; Nachury & Mick, 2019; Wachten & Mick, 2021). The primary cilium originates from a modified centriole called the basal body (Fig 1A). The basal body forms a nucleation site for the ciliary axoneme, consisting of parallel microtubule doublets in a characteristic '9 + 0' cylindrical arrangement, which, however, may change along the axoneme depending on the cell type (Webber & Lee, 1975; Gluenz *et al*, 2010; Sun *et al*, 2019; Kiesel *et al*, 2020). Along the axoneme, the intraflagellar transport (IFT) machinery, consisting of IFT-A and IFT-B protein complexes, transports molecules anterogradely towards the ciliary tip or retrogradely towards the ciliary base via kinesin or dynein motors, respectively (Goetz & Anderson, 2010) (Fig 1A). The IFT machinery is supported by the BBSome protein complexes, which act as adaptors for the retrograde transport of ciliary membrane proteins via the IFT machinery and, thereby, determine the ciliary protein composition (Garcia-Gonzalo & Reiter, 2017; Garcia *et al*, 2018; Nachury, 2018). The BBSome is a multi-subunit protein complex, consisting of BBS proteins, named after the syndromic ciliopathy, the Bardet-Biedl syndrome (BBS) (Nachury *et al*, 2007; Forsythe & Beales, 2013). In addition, the

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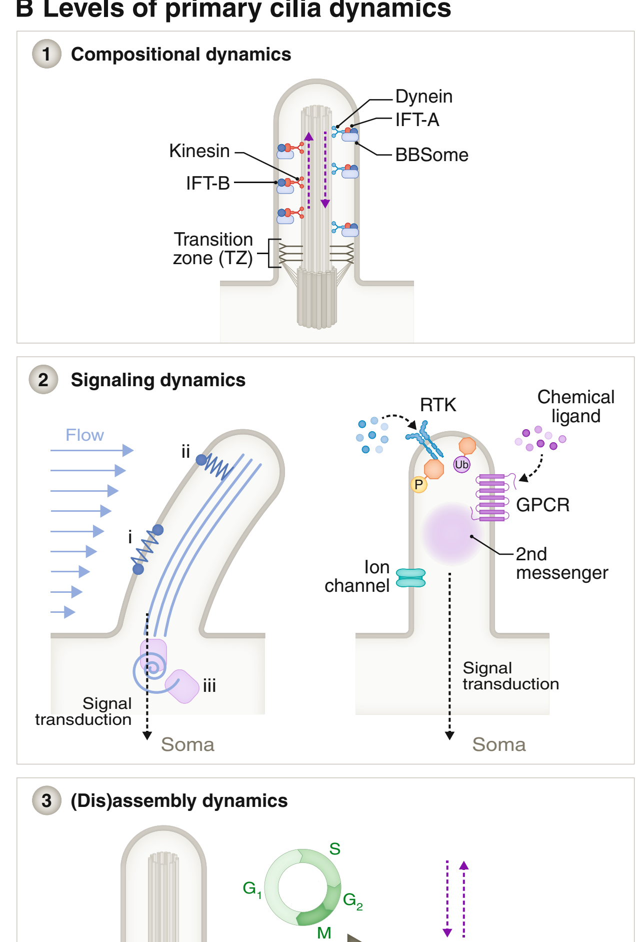

Figure 1. Primary cilia: structure and dynamics.

(A) The core structure of the primary cilium is the microtubule-based axoneme. The primary cilium originates from a modified centriole called the basal body. Protein transport in and out of the cilium is passively controlled by the transition zone (TZ) and the transition fibers, and actively by the intraflagellar transport (IFT) machinery, which transports proteins anterogradely in a kinesin-2-dependent manner using IFT-B trains and retrogradely in a dynein-2-dependend manner using IFT-A trains. The BBSome is assembled with the help of the BBS chaperonin complex and functions as a cargo adaptor complex for the retrograde IFT transport of transmembrane proteins, in particular G protein-coupled receptors (GPCRs). (B) Primary cilia dynamics can be observed on three different levels: 1. Compositional dynamics, determined by the active and passive gating structures that determine the compartmentalization of the primary cilium; 2. Signaling dynamics, referring to the signal-dependent fine tuning to adapt the distinct molecular ciliary makeup appropriate to the signal status across time, space, and cellular state; 3. Assembly/disassembly dynamics, which are tightly coupled to the cell cycle. Figure has been created using Biorender.

transition zone (TZ) at the ciliary base acts as a diffusion barrier, controlling the lateral diffusion of membrane proteins between the cell body and the cilium (Park & Leroux, 2022). In combination, the IFT machinery, the TZ, and the BBSome determine the unique protein and lipid composition that controls primary cilia signaling and function (Fig 1A).

The primary cilium acts as a sensory organelle for different environmental stimuli. These cues can be ligand-based (chemosensation), mechanical (mechanosensation, e.g., upon fluid flow), or even photons. The latter are sensed by the light-sensitive GPCR rhodoposin in specialized ciliated cells in the eye, the photoreceptors, which do not possess prototypic primary cilia. Thus, we will not cover this aspect here, but rather refer to another recent review (Barnes *et al*, 2021).

In vertebrates, canonical Hedgehog (Hh) signaling is the prime example of a chemosensory ciliary signaling pathway (Rohatgi *et al*, 2007; Bangs & Anderson, 2017; Ingham, 2022). The Hh receptor Patched1 (PTCH1) is activated upon ligand binding and exits the cilium, while Smoothened (SMO) enters the cilium. SMO then triggers pathway activation through GLI transcription factors, which

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constantly cycle through the cilium to sense Hh signaling status and control gene expression in the nucleus. This tightly controlled sequence of events, consisting of ciliary protein export and import, is key for Hh signaling and, in turn, tissue development, homeostasis, and regeneration (Rohatgi *et al*, 2007; Mukhopadhyay & Rohatgi, 2014; Bangs & Anderson, 2017; Kopinke *et al*, 2021; Ingham, 2022). But not only Hh signaling, also Receptor Tyrosine Kinase (RTK) (Christensen *et al*, 2017), WNT (May-Simera & Kelley, 2012), as well as G protein-coupled receptor (GPCR) (Hilgendorf *et al*, 2016; Mykytyn & Askwith, 2017; Wachten & Mick, 2021) signaling pathways have been implicated in primary cilia signaling.

Fluid flow has been described as the main mechanical stimulus sensed by primary cilia, e.g., fluid flow in the renal tubules or in the embryonic left–right organizer (LRO) during development. Luminal fluid flow in renal tubules determines the direction and bending of the cilium, which, in turn, evokes a ciliary signaling response (Basten & Giles, 2013; Praetorius, 2015). Polycystins, polycystin-like proteins, and calcium signaling have been associated with ciliary mechanosensation, although the precise molecular mechanisms have been debated (Delling *et al*, 2016; Wachten & Mill, 2023). Recently, two studies added new experimental evidence that primary cilia function as calcium-mediated mechanosensors to determine left–right asymmetry (Djenoune *et al*, 2023; Katoh *et al*, 2023), answering some but also opening up new questions (Wachten & Mill, 2023). In summary, not only changes in the chemical environment but also in the mechanical forces can lead to dynamic cilia signaling responses, which we will describe later in further detail.

Primary cilia dysfunction caused by structural or signaling defects leads to severe diseases, commonly referred to as ciliopathies (Fliegauf *et al*, 2007; Hildebrandt *et al*, 2011; Reiter & Leroux, 2017). Since the first molecular mechanisms connecting primary cilia dysfunction and ciliopathy development were described (Pazour *et al*, 2000), many studies revealed molecular insights into how primary cilia dysfunction impairs tissue development and homeostasis and, in turn, causes the clinical manifestations of ciliopathies, as reviewed extensively by others (Badano *et al*, 2006; Fliegauf *et al*, 2007; Marshall, 2008; Quinlan *et al*, 2008; Gerdes *et al*, 2009; Hildebrandt *et al*, 2011; Waters & Beales, 2011; Oh & Katsanis, 2012; Mitchison & Valente, 2017; Reiter & Leroux, 2017; Elliott & Brugmann, 2019; Suciu & Caspary, 2021).

Recent investigations have revealed that the primary cilium is not a static, sensory organelle, which is simply turned on and off by ciliogenesis and cilium disassembly, respectively. Instead, primary cilia are highly dynamic, and ciliary dynamics appear to be vital in controlling tissue development. Thus, in this review, we will focus on primary cilia dynamics and how they control tissue development and function.

Primary cilia dynamics can be observed on three levels (Fig 1B): (i) Compositional dynamics, (ii) Signaling dynamics, (iii) Assembly/disassembly dynamics. The compositional dynamics are controlled by the active and passive gating structures that determine the compartmentalization of the primary cilium. The signaling dynamics refer to the signal-dependent fine-tuning to adapt the distinct molecular ciliary make-up in response to the signal status across time, space, and cellular state. Finally, the assembly and disassembly dynamics are tightly coupled to the cell cycle. Indeed, cells assemble primary cilia when exiting mitosis (M to G0/G1 phase), they start to disassemble the primary cilium when re-entering the cell cycle (G1-S transition), and fully disassemble the primary cilium before entering mitosis (G2 to M phase) (Ishikawa & Marshall, 2017; Wang & Dynlacht, 2018). However, the timing of the dynamics depends on the *in vivo* context (Ford *et al*, 2018).

Although the dynamic ciliary processes in primary cilia can be assigned to the aforementioned three levels, they are all closely connected and interdependent—not only on a single cell level but even within an entire tissue. Primary cilia coordinate cellular functions in a tissue context to control tissue organization and function (Anvarian *et al*, 2019). The close connection between ciliation and tissue development is illustrated by the fact that the complete loss of primary cilia causes embryonic death in all animals examined to date (Nonaka *et al*, 1998; Huangfu *et al*, 2003). Even the cell type-specific loss of primary cilia leads to severe tissue developmental defects (Pazour *et al*, 2000; Jonassen *et al*, 2008; May-Simera *et al*, 2015; De-Castro *et al*, 2021; Chinipardaz *et al*, 2022).

Defects in cilium disassembly and ciliogenesis have been associated with ciliopathy development, e.g., with autosomal dominant polycystic kidney disease (ADPKD) (Gerakopoulos *et al*, 2020), microcephaly displaying defects during brain development (Rocha & Prinos, 2022), or tumor development (Wang & Dynlacht, 2018). This demonstrates the importance of assembling and disassembling a primary cilium at the right time and in a given cell type to drive tissue development and organization.

Trying to dissect primary cilia dynamics is challenging on a single-cell level, let alone in a tissue-wide perspective. A holistic approach must be pursued to fully understand the function of the primary cilium as a dynamic organelle. This requires analyzing cilia dynamics at all levels from the compositional to the signaling and assembly/disassembly dynamics (Fig 1B), and is a significant challenge that needs to be tackled in primary cilia research in the coming years.

Here, we review current literature regarding the three different levels of primary cilia dynamics, how they can be analyzed using state-of-the-art technologies, and highlight primary cilia dynamics within the context of tissue organization and function.

The different levels of primary cilia dynamics

Compositional dynamics

To function as a signaling hub and maintain a unique protein and lipid composition, the cilium utilizes the coordinated action of active and passive gating structures. This includes the transition zone (TZ), the IFT machinery, and the BBSome (Fig 1A and B). In addition, proteins need to contain a ciliary targeting sequence. However, the molecular mechanisms underlying the recognition of ciliary proteins are still not fully understood. A summary of the respective findings can be found here (Malicki & Avidor-Reiss, 2014; Schou *et al*, 2015; Mukhopadhyay *et al*, 2017).

To enter the primary cilium, proteins need to cross the TZ, which acts as a passive, size-selective diffusion barrier for cytoplasmic and membrane proteins, maintaining the unique molecular makeup of the cilium (Park & Leroux, 2022). Molecularly, the TZ contains functionally different protein modules, the NPHP (nephrocystin), the MKS (Meckel Gruber syndrome), and the core scaffolding module, whose components are hierarchically organized (Park & Leroux, 2022). Of note, the regulation of the TZ seems to be cell and tissue

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type-specific (Wiegering *et al*, 2018; Lewis *et al*, 2019), with interesting implications for the molecular etiologies of ciliopathies.

The IFT machinery was first identified in *Chlamydomonas* (Kozminski *et al*, 1993) and is essential for moving proteins in and out of the cilium. Kinesin motors move IFT complexes anterogradely from the cell body to the ciliary tip, whereas dynein motors carry out retrograde protein transport of IFT complexes from the tip to the cell body (Fig 1A). The IFT complexes, IFT-A and -B, consist of numerous IFT protein subunits. Similarly, the retrograde dynein-2 and the canonical, anterograde kinesin-2 motor complex consist of multiple subunits (Mill *et al*, 2023). The IFT machinery is essential for ciliary protein transport as well as for primary cilia formation and maintenance. In turn, primary cilia loss, as in *Ift88* mutants, results in cystic kidney disease (Pazour *et al*, 2000). The components of the IFT machinery, the molecular motors, and how they function has been extensively described in several reviews (Lechtreck, 2015; Taschner & Lorentzen, 2016; Ishikawa & Marshall, 2017; Lechtreck *et al*, 2017; Prevo *et al*, 2017; Morthorst *et al*, 2018; Nakayama & Katoh, 2018, 2020; Webb *et al*, 2020; Jordan & Pigino, 2021; Mul *et al*, 2022; Mill *et al*, 2023). Thus, we will not focus on these aspects here.

Besides the passive gating function of the TZ and the active transport function of the IFT, the third component determining the dynamics of the ciliary membrane protein composition is the BBSome protein complex (Nachury, 2018; Wingfield *et al*, 2018). The BBSome acts as an adaptor for the retrograde transport of ciliary membrane proteins via the IFT machinery (Fig 1A). The BBSome consists of eight BBS protein subunits, whose assembly is regulated by three additional, chaperonin-like BBS proteins (Seo *et al*, 2010; Nachury, 2018). Genetic loss of or mutations in one of the 26 *BBS* genes results in the Bardet-Biedl Syndrome, the archetypical ciliopathy, which presents predominantly with obesity, polydactyly, retinal degeneration, and kidney cysts (Beales *et al*, 1999; Forsythe & Beales, 2013). Generally, the BBSome picks up cargo, e.g., activated G protein-coupled receptors (GPCRs), for transport out of the cilium (Wingfield *et al*, 2018; Ye *et al*, 2018; Shinde *et al*, 2020). In turn, the loss of BBSome function results in defects of the stimulus-dependent control of GPCR localization in the cilium, whereby downstream GPCR signaling is severely affected (Schou *et al*, 2015; Wingfield *et al*, 2018; Anvarian *et al*, 2019). This has been shown for ciliary GPCRs, such as the somatostatin 3 receptor (SSTR3), the melanin-hormone concentrating receptor 1 (MCHR1) (Berbari *et al*, 2008; Nager *et al*, 2017; Eintracht *et al*, 2021), the neuropeptide Y receptor 2 (NPY2R) (Loktev & Jackson, 2013), and the dopamine receptor 1 (DR1) (Stubbs *et al*, 2022).

Mechanistically, the BBSome and the receptors accumulate at the ciliary tip, where retrograde cargo is loaded (Ye *et al*, 2018). Subsequently, the cargo is moved towards the ciliary base and passes it through the barrier at the TZ. If this mechanism is impaired, GPCRs accumulate at the ciliary tip and are removed by shedding extracellular vesicles (see below) (Nager *et al*, 2017; Phua *et al*, 2017).

Not only the ciliary proteome but also the ciliary lipidome is distinct from the rest of the cell (Garcia *et al*, 2018; Conduit *et al*, 2021). Here, phosphoinositides (PI) play a central role, and their content in the cilium is mainly regulated by the inositol polyphosphate 5-phosphatase INPP5E, which produces PI(4)P and PI(3,4)P_2 from PI(4,5)P_2 and PI(3,4,5)P_3, respectively. Different PI species bind different pleckstrin-homology (PH) domain-containing effectors and determine their subcellular localization, e.g., in the cilium (Chavez *et al*, 2015; Garcia-Gonzalo *et al*, 2015). Furthermore, other lipids, e.g., sterols, have been shown to fine tune ciliary signaling, such as Hh signaling, as reviewed by others (Nguyen *et al*, 2022).

In summary, the interaction of the IFT machinery with the BBSome and TZ components is key to dynamically regulate the molecular make-up of the primary cilium, e.g., the ciliary protein and lipid composition.

Signaling dynamics

The primary cilium acts as a "cellular antenna" that responds to extra-cellular signals, and adapts its molecular content, relying on the gating structures described above (Fig 1B). This section reviews signal-dependent regulation of ciliary signaling and compositional dynamics, with a particular focus on mechanosensation, and discusses a novel function of the primary cilium as a signal emitter releasing extracellular vesicles (EVs).

Mechanosensation

Primary cilia have been proposed to act as dynamic flow sensors (Fig 1B). Ciliary flow sensing has been discussed in the kidney (Schwartz *et al*, 1997; Praetorius & Spring, 2003), in blood vessels (Nauli *et al*, 2008; Goetz *et al*, 2014), in the developing heart (Hierck *et al*, 2008), in bone (Malone *et al*, 2007; Xiao *et al*, 2011), and the left–right organizer (LRO) during embryonic development (McGrath *et al*, 2003; Yoshiba *et al*, 2012; Djenoune *et al*, 2023; Katoh *et al*, 2023); for an in-depth overview, see reviews by others (Spasic & Jacobs, 2017; Ferreira *et al*, 2019).

The working model of cilia-based mechanosensation suggests that mechanical forces bend the cilium, which generates membrane tension and opens tension-activated channels, leading to calcium influx and downstream signaling. However, the processes involved in mechanosensing appear more complicated, and the role of the primary cilia in flow sensing remains controversial (Delling *et al*, 2016; Ferreira *et al*, 2017).

A key challenge is the vastly different time-scales involved: from sub-second detection of mechanical forces to signaling cascades lasting tens of minutes. Two recent publications provide additional evidence for mechanosensation by cilia. In these studies, oscillatory mechanical forces using optical tweezers were directly applied to primary cilia in the left–right organizer of mice and zebrafish, thereby establishing a causal link between mechanical manipulations and left–right patterning (Djenoune *et al*, 2023; Katoh *et al*, 2023; Wachten & Mill, 2023).

Even though external flow has been shown to bend cilia (Praetorius & Spring, 2001) and trigger signaling cascades (Nauli *et al*, 2003; Praetorius & Spring, 2003; Liu *et al*, 2005b), it remains debated whether primary cilia act as bona fide flow sensors.

A few calculations might help to shed light on whether primary cilia act as flow sensors (Box 1). The dynamics of mechanical stimuli, i.e., detecting steady versus oscillatory flow, may be important and may partially account for different sensitivity thresholds observed in different systems. We note that several authors report flow as volume flow rates or maximal flow speed in a channel (Young *et al*, 2012; Khayyeri *et al*, 2015). To make quantitative comparisons across reports easier, it may be useful if future reports state flow in comparable ways using physical conversion formulas (Ferreira *et al*, 2019).

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Box 1. Flow sensing via primary cilia-calculations.

The sensitivity threshold for cilia flow sensing seems to be a wall shear stress of ~20 mPa, corresponding to a flow velocity of v ~ 200 μm/s at the tip of a 10-μm long cilium (Ferreira *et al*, 2017). For flow above a planar surface, a wall shear stress of σ = 100 mPa (= 1 dyn/cm^2) corresponds to a shear rate $\alpha = \sigma/\eta \sim 100$ s^{-1} and hence a speed $\alpha h \sim 1$ mm/s at a distance h = 10 μm from the surface (where η is the dynamic viscosity of the fluid, here taken as $\eta = 10^{-3}$ Pa for water at 20°C). Estimated wall shear stresses range from 1 to 1,000 mPa in systems with proposed cilia flow sensing (Ferreira *et al*, 2017), with 200 mPa for renal flow (Kang *et al*, 2006) (computed from glomerular filtration rate 32.4 nl/min), but only 1 mPa for the left–right organizer of zebrafish (Kupffer's vesicle) (Supatto *et al*, 2008; Ferreira *et al*, 2017). Based on these numbers, cilia flow sensing in the kidney seems plausible, while flow speeds may be below the detection limit of cilia flow sensing in the left–right organizer (Ferreira *et al*, 2017). Yet, Djenoune and colleagues reported that forces of 0.6 pN are sufficient to elicit calcium transients in the immotile cilia of the Kupffer's vesicle (Djenoune *et al*, 2023).

Next, to be able to gauge whether a given flow is sufficient to deform a cilium and, thus, induce a mechanoresponse, it is key to know the bending stiffness (or flexural rigidity) of a cilium. So far, it has not been possible to measure this bending stiffness directly, but all estimates rely on fitting computational models to experimental data. The recent estimate EI = 30 pN μm^2 (= 3 *10^{-23} N m^2) (Battle *et al*, 2015) is in agreement with previous measurements in the range 10–50 pN μm^2 (Nag & Resnick, 2017), and implies that primary cilia are substantially softer than motile cilia. Confusingly, direct deformation of immotile nodal cilia using optical traps resulted in a 10-fold higher stiffness 300–500 pN μm^2 (Katoh *et al*, 2023), which would imply a smaller mechanoresponse.

Even though flow sensing in some tissues appears plausible, it is not clear by which physical mechanism cilia would sense flow. Additionally, the identity and location of the mechanosensory remains unknown (Ferreira *et al*, 2019) (Fig 1B):

1 Mechano-sensitive channels inside the cilia membrane could sense membrane curvature or membrane tension. However, the estimated membrane tension is low (~0.1 mN/m for α = 100 1/s), which is below the detection threshold of known, tension-gated ion channels (~ 3–10 mN/m) (Ferreira *et al*, 2019). Moreover, because membranes are fluid, membrane tension should relax < 1 s after the onset of external flow, thereby limiting sensing to rapidly changing flows.
2 This issue could be rectified if mechano-sensitive channels were mechanically connected to the axoneme, nearby microvilli, or extracellular polymer chains. This is for instance the case for Polycystin-2 (PC2), which targets and anchors polyglycan-based cilia extensions known as mastigonemes in swimming *Chlamydomonas* (Liu *et al*, 2020). Arthropods express mechanosensory TRP channels, e.g., NOMPC (Yan *et al*, 2013), which localizes to distal cilium of *Drosophila* campaniform and chordotonal receptor cells (Liang *et al*, 2011) and seems to require mechanical linkage to the cytoskeleton for their mechano-sensory function (Zhang *et al*, 2015).
3 Lastly, external flow will not only induce local shear forces and bending moments on a cilium, but also exert a torque at its base (Young *et al*, 2012; Battle *et al*, 2015). Hence, mechanosensation could also be measured by torque sensors at the basal body. Generally, physics predicts that molecular mechano-sensors can be more sensitive when coupled to a rigid support, yet such processes remain to be identified in the context of cilia-mediated mechanosensation.

Polycystins (Polycystin-1 and -2, PC1 and PC2, encoded by *PKD1* and *PKD2*) and polycystin-like proteins (e.g., PKD1L1) have been suggested as putative channel complexes for flow sensing in the cilium for multiple reasons. First, polycystin and polycystin-like mutants cause cyst formation in the kidney and defects in left–right patterning, reminiscent of ciliary defects and potentially disturbed flow sensing (Pazour *et al*, 2002; Nauli *et al*, 2003; Field *et al*, 2011; Kamura *et al*, 2011; Yoshiba *et al*, 2012; Grimes *et al*, 2016; Vetrini *et al*, 2016; Ma *et al*, 2017; Liu *et al*, 2018b; Walker *et al*, 2019; Djenoune *et al*, 2023; Katoh *et al*, 2023). Second, polycystins localize to primary cilia (Pazour *et al*, 2002; Nauli *et al*, 2003; Field *et al*, 2011; Grimes *et al*, 2016), and their ciliary localization is important for preventing kidney cystogenesis (Xu *et al*, 2007; Walker *et al*, 2019). Third, loss of PC2 in either the zebrafish or mouse left–right organizer seems to abolish ciliary signaling upon oscillatory mechanical stimulation of cilia by optical tweezers (Djenoune *et al*, 2023; Katoh *et al*, 2023).

However, the mechanisms by which polycystins sense fluid flow remain elusive. PC2, as a TRP channel, lacks common motifs identified in known mechanosensitive channels such as NOMPC (Zhang *et al*, 2015) or the Piezo ion channel family (Kefauver *et al*, 2020). PC1 and PKD1L1 are transmembrane proteins with a large extracellular N terminal domain (Su *et al*, 2018; Ta *et al*, 2020), which are prone to respond to external stimuli. It is, however, important to note that PC1/2 channels have also been shown to have chemosensory properties (Kim *et al*, 2016; Ha *et al*, 2020), indicating that PC1/2 in the cilium could serve both chemo- and mechanosensory function.

Lastly, which signaling cascades are involved in mechanosensation remains poorly understood. Calcium signals were observed upon application of external forces in kidney cells in a cilia-dependent manner (Nauli *et al*, 2003; Praetorius & Spring, 2003; Liu *et al*, 2005b) and in the left–right organizer of mice and zebrafish upon manipulation with optical tweezers (Djenoune *et al*, 2023; Katoh *et al*, 2023; Wachten & Mill, 2023). Reducing ciliary calcium activity using a genetically encoded calcium-binding protein interfered with left–right asymmetry breaking in the zebrafish (Yuan *et al*, 2015), suggesting a causal relationship between ciliary calcium activity and left–right patterning. Since calcium activity was shown to be abolished in polycystins or polycystin-like mutants during mechanosensation (Nauli *et al*, 2003; Grimes *et al*, 2016; Djenoune *et al*, 2023; Katoh *et al*, 2023), it was hypothesized that polycystins could be mechanosensory elements regulating calcium influx to the cilium. However, calcium signaling is comparatively slow, rendering it improbable that calcium enters the cilium directly upon mechanical deflection, in line with the observation that primary cilia are not calcium-responsive mechanosensors in the kidney and the left–right organizer (Delling *et al*, 2016). Taken together, even though there is increasing evidence showing that calcium is implicated in mechanosensation, the precise dynamics of calcium signaling, its key players, and physiological importance remain to be characterized in further detail.

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In conclusion, a growing number of experimental studies suggest that primary cilia may sense steady or unsteady flow and promote cellular signaling, which instructs tissue re-organization. However, the precise molecular and biophysical mechanisms are not yet well understood, and flow speeds may be too low in some systems to be sensed by primary cilia. Future work is needed to characterize the dynamic stimulus–response relationship of mechanosensation on both fast timescales of seconds and longer timescales of minutes and hours.

Chemosensation

The view that the primary cilium acts as the cell's "chemical antenna" stems from its chemosensory function in olfactory sensory neurons (Kaupp, 2010). Chemosensation is often thought to be the main function of primary cilia. The general concept of how primary cilia respond to ligand-based cues has been extensively reviewed (Mukhopadhyay & Rohatgi, 2014; Schou *et al*, 2015; Hilgendorf *et al*, 2016; Bangs & Anderson, 2017; Mykytyn & Askwith, 2017; Garcia *et al*, 2018; Anvarian *et al*, 2019; Wachten & Mick, 2021; Ingham, 2022; Mill *et al*, 2023). Ciliary Hh signaling is the prime example of a chemosensory ciliary signaling pathway and showcases how ciliary proteins are dynamically localized along the cilium to fine-tune ciliary signaling upon ligand sensing. In brief, in the absence of a Hh ligand, PTCH1 and the orphan GPCR GPR161 are localized to the primary cilium, which represses the expression of Hh target genes by promoting proteolytic cleavage of the transcription factors GLI2/3, turning them into their repressor forms. Upon ligand binding, PTCH1 and GPR161 leave the cilium, whereas SMO accumulates in the cilium. The localization of the constitutively active GPR161 inside and outside of the cilium is key to regulate ciliary cAMP levels and, thereby, promote Hh signaling. Furthermore, ciliary removal of PTCH1 and GPR161 converts GLI2/3 into their active form, promoting the expression of Hh target genes (Rohatgi *et al*, 2007; Mukhopadhyay & Rohatgi, 2014; Bangs & Anderson, 2017; Kopinke *et al*, 2021; Ingham, 2022).

While the sequence of events of signal-dependent fine-tuning of ciliary signaling dynamics has been well described for Hh signaling, the detailed understanding of other chemosensory signaling pathways in the cilium is relatively sparse. Receptor Tyrosine Kinase (RTK) (Christensen *et al*, 2017), the Frizzled receptor binding WNT ligands (May-Simera & Kelley, 2012), and G protein-coupled receptor (GPCRs) (Hilgendorf *et al*, 2016; Mykytyn & Askwith, 2017; Wachten & Mick, 2021) signaling have been described in primary cilia, yet, mechanistic insight about their dynamics is rather limited.

To highlight the advances in the field, we will focus on the recent findings regarding second messenger dynamics downstream of receptor engagement in the primary cilium, and highlight how posttranslational modifications control signaling dynamics in the primary cilium (see 2.3).

Dynamics of ciliary second messengers and beyond

On a molecular level, upon activation of receptors in the cilium, second messengers, such as calcium and cAMP, are utilized to either directly modify downstream signal mediators or evoke signaling through posttranslational modifications (PTMs) (May *et al*, 2021b) (Fig 1B). This concept mainly holds true for GPCRs (Schou *et al*, 2015; Hilgendorf *et al*, 2016; Mykytyn & Askwith, 2017; Garcia *et al*, 2018; Wachten & Mick, 2021; Scamfer *et al*, 2022). Other ciliary chemoreceptors, such as Receptor Tyrosine Kinases (RTKs) and Transforming Growth Factor beta (TGFβ) receptors, directly phosphorylate their targets to change ciliary signaling dynamics and evoke a downstream cellular response (Christensen *et al*, 2017).

While the modulation of ciliary calcium levels upon GPCR and Gq protein activation remains not well understood, activation or inhibition of ciliary adenylyl cyclases via GPCR/Gs proteins to modulate cAMP signaling has been described in more detail (Schou *et al*, 2015; Mykytyn & Askwith, 2017; Anvarian *et al*, 2019; Nachury & Mick, 2019; Barbeito *et al*, 2021; Wachten & Mick, 2021; Brewer *et al*, 2022; Jin & Zhong, 2022). Several studies have started to dissect ciliary cAMP dynamics upon ligand-dependent GPCR activation in different cell types using genetically encoded biosensors (see below). This allowed to follow cAMP dynamics during Hh signaling (Moore *et al*, 2016; Jiang *et al*, 2019; Truong *et al*, 2021) as well as the sensing of omega-3 fatty acids via the Free Fatty-Acid Receptor 4 (FFAR4) in preadipocytes (Hilgendorf *et al*, 2019) or prostaglandin E2 in renal epithelial cells via the GPCR Prostaglandin E4 receptor (EP4) during cyst formation (Hansen *et al*, 2022).

Downstream of the known effector proteins for cAMP, mainly cAMP-dependent protein kinases (PKA) and exchange proteins directly activated by cAMP (EPAC) have been studied in the context of the primary cilium. EPAC activation seems to induce transcriptional changes via chromatin remodeling (Hilgendorf *et al*, 2019), but the molecular mechanisms have not been fully revealed yet. In contrast, signaling dynamics downstream of PKA have been well described for Hh signaling, where the cAMP signal is converted into PKA-dependent PTMs on the GLI2/3 transcription factors, which in turn changes gene expression (Tuson *et al*, 2011; Niewiadomski *et al*, 2014; Tschaikner *et al*, 2020).

Besides the ciliary GPCRs that indirectly evoke PTMs through activation of PKA via cAMP, ciliary RTKs and TGFβ receptors can directly modify downstream effectors by phosphorylation. Upon ligand binding, RTKs are auto-phosphorylated, forming a signaling platform where other ciliary proteins assemble, are activated, and, in turn, control downstream signaling. This principle has been described for the Platelet-Derived Growth Factor alpha (PDGFR-α) (Schneider *et al*, 2005, 2010; Clement *et al*, 2013; Gerhardt *et al*, 2013; Nielsen *et al*, 2015; Umberger & Caspary, 2015; Goranci-Buzhala *et al*, 2021) and the Insulin-Growth Factor 1 Receptor (IGF-1R) (Zhu *et al*, 2009; Dalbay *et al*, 2015), and has been extensively reviewed by others (Christensen *et al*, 2012, 2017).

Activation of PDGFR-α engages downstream signaling via the MAPK (Mitogen-Activated Protein Kinase), PI3K-AKT (Phosphoinositide 3 Kinase, AKT = Protein kinase B), and PLCγ (Phospho-lipase C delta) pathways, whereas activation of IGF-1R involves IRS-1 (Insulin Receptor Substrate 1) and PI3K-AKT signaling (Christensen *et al*, 2017). However, how the cilium achieves specificity for these signaling dynamics and how they are interpreted differently from the cell body remains poorly understood.

Elegant studies have dissected the role of PTMs in the regulation of the compositional (intraciliary protein) dynamics in cilia. For instance, the signal-induced removal of GPCRs from cilia is initiated by phosphorylation (Pal *et al*, 2016) and precedes ubiquitination by E3 ubiquitin ligases, such as Wwp1 (WW Domain Containing E3 Ubiquitin Protein Ligase 1), which has been shown to ubiquitinate SMO (Smoothened) (Lv *et al*, 2021). Ubiquitinated GPCRs are then

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recognized by ubiquitin adaptors (Shinde *et al*, 2023) that bridge the interaction to the BBSome for IFT-dependent removal (Desai *et al*, 2020; Shinde *et al*, 2020). Similarly, a complex interplay between phosphorylation and ubiquitination regulates cilium biogenesis (Wang & Dynlacht, 2018; Breslow & Holland, 2019).

While these findings demonstrate that ciliary signaling follows the same general principles of cellular signaling, it also points to the requirement of cilium-specific components to create a distinct signaling environment. Of note, calculations of the ciliary volume and absolute protein abundance in sperm flagella revealed that signaling proteins in the flagellum exist in a concentration of up to 400 μM (Trötschel *et al*, 2020). As primary cilia are much smaller than flagella and display a distinct geometry (Truong *et al*, 2021), the protein concentrations and the efficacy of the signaling kinetics might be higher.

The role of ciliary extracellular vesicles (EVs) in controlling ciliary signaling dynamics

Recent studies revealed that cilia not only function as signal receivers but are also able to transmit signals by releasing bioactive extracellular vesicles (EVs) containing proteins and mRNAs (Wang & Barr, 2018; Luxmi & King, 2022; Ojeda Naharros & Nachury, 2022; Vinay & Belleannee, 2022). This has mainly been studied in *Chlamydomonas* (Wood *et al*, 2013) or in *C. elegans* (Wang *et al*, 2015, 2021; Wang & Barr, 2018), where ciliary EVs are involved in mating and behavior. However, a few studies have already shown bioactivity of mammalian ciliary EVs involved in regulation of cellular signaling pathways during development and disease conditions (Ding *et al*, 2021; Volz *et al*, 2021).

EVs are a heterogenous group of small membranous vesicles that can shuttle cargo from one cell to another and are, therefore, important for intercellular communication and physiological processes (Simons & Raposo, 2009; Mathivanan *et al*, 2010; Cocucci & Meldolesi, 2015). These include cell proliferation, survival, and transformation through autocrine and paracrine interactions (Maas *et al*, 2017; van Niel *et al*, 2018). Disruption of EV-regulated signaling can lead to severe pathophysiological conditions, including cancer, neurodegeneration, and kidney disease (Hill, 2019; Li *et al*, 2021; Grange & Bussolati, 2022). EVs were initially seen as an evolutionarily conserved mechanism to dispose cellular waste (Deatherage & Cookson, 2012). However, it is now well-accepted that EV secretion is a highly orchestrated process to regulate membrane composition and signal transduction.

EVs can generally be divided into two main classes according to their size and biogenesis (Thery *et al*, 2018). Large EVs (lgEVs) (150–350 nm; also referred to as microvesicles or ectosomes) are formed through outward budding directly at the plasma membrane, whereas small EVs (smEVs, 50–150 nm) comprise microvesicles that are shed from the plasma membrane, or exosomes of endocytic origin, which are released by exocytosis of multivesicular bodies (MVB).

Cilia are considered evolutionarily conserved sites of EV production. However, studies have shown distinct processes for ciliary EV biogenesis and shedding in different species (Wood *et al*, 2013; Wang *et al*, 2014; Salinas *et al*, 2017; Akella *et al*, 2020). Due to size-selectivity at the ciliary transition zone, the ciliary axoneme is devoid of MVBs; hence ciliary membrane-derived EVs are most likely to be large EVs (lgEVs) or small EVs (smEVs), formed via directly budding from the ciliary membrane. In mammalian cells, the most common site of EV budding from the cilium is the ciliary tip (Hogan *et al*, 2009; Nager *et al*, 2017; Phua *et al*, 2017), whereas the ciliary base and potentially the ciliary pocket seem to be a source of ciliary smEVs (Hogan *et al*, 2009; Chacon-Heszele *et al*, 2014; Wang *et al*, 2014; Volz *et al*, 2021). Occasionally, EVs are also observed along the length of the cilium. However, in the absence of data on ciliary EV dynamics, it is unclear whether these EVs are being released or absorbed by the cilium (Cao *et al*, 2015; Huang *et al*, 2016).

A large fragment of the ciliary axoneme can also be shed at once, referred to as ciliary decapitation or excision, which often precedes cilia disassembly (Phua *et al*, 2017; Wang *et al*, 2019; Ojeda Naharros & Nachury, 2022). Related to this phenomenon is a process referred to as ciliary amputation or autotomy, by which the entire cilium is instantly removed distally to the transition zone (Quarmby, 2004; Das & Storey, 2014; Ford *et al*, 2018; Mirvis *et al*, 2019; Toro-Tapia & Das, 2020).

Several functions besides promoting intercellular signaling (Wang *et al*, 2014; Cao *et al*, 2015; Volz *et al*, 2021) have been proposed for ciliary EVs, including waste disposal (Nager *et al*, 2017). This leads to the removal of ciliary proteins via EVs, which, in turn, could also regulate ciliary signaling. This could also be viewed as a safety valve mechanism to prevent protein overload or excessive ciliary elongation, which has been observed in a multitude of different organisms (Nager *et al*, 2017; Akella *et al*, 2020; Razzauti & Laurent, 2021). EV biogenesis also controls cilia length (Long *et al*, 2016; Phua *et al*, 2017), and modulates ciliation via initiating cell cycle reentry into mitosis (Phua *et al*, 2017; Wang *et al*, 2019), as covered by other recent reviews (Wang & Barr, 2018; Ikegami & Ijaz, 2021; Ojeda Naharros & Nachury, 2022; Vinay & Belleannee, 2022). Since EVs are important in intercellular communication in many tissues, it is tempting to speculate that the dynamic generation and shedding of ciliary EVs control tissue organization and function.

In summary, ciliary EV biogenesis is a critical process in regulating ciliary signaling dynamics, which seems to be organism, cell-, and tissue-type specific.

Assembly and disassembly dynamics

The primary cilium is not a static organelle; it dynamically assembles and disassembles in coherence with the cell cycle (Pan & Snell, 2007). Generally, in cycling cells, the cilium is assembled as the cell exits mitosis to G0/G1 and is disassembled when the cell reenters into the cell cycle, beginning from the G1-S transition. Two phases of cilium disassembly have been observed in cultured mammalian cells: The first phase starts at the G0/G1-S transition after growth factor stimulation, and the second phase follows before the mitotic onset (Munger, 1958; Tucker *et al*, 1979; Pan & Snell, 2007; Pugacheva *et al*, 2007; Sanchez & Dynlacht, 2016; Wang & Dynlacht, 2018) (Fig 2). As the presence of a cilium is incompatible with mitosis, cilia-dependent signaling and cell division are mutually exclusive. Consequently, defects in cilium assembly prevent ciliary signaling and promote cell proliferation. In contrast, defects in cilium disassembly have been proposed to prolong ciliary signaling and suppress re-entry into the cell cycle (Gerakopoulos *et al*, 2020).

During cilium disassembly, ciliary length progressively decreases, the axoneme is deacetylated, the membrane composition is remodeled, and the basal body is released to participate in spindle assembly. These

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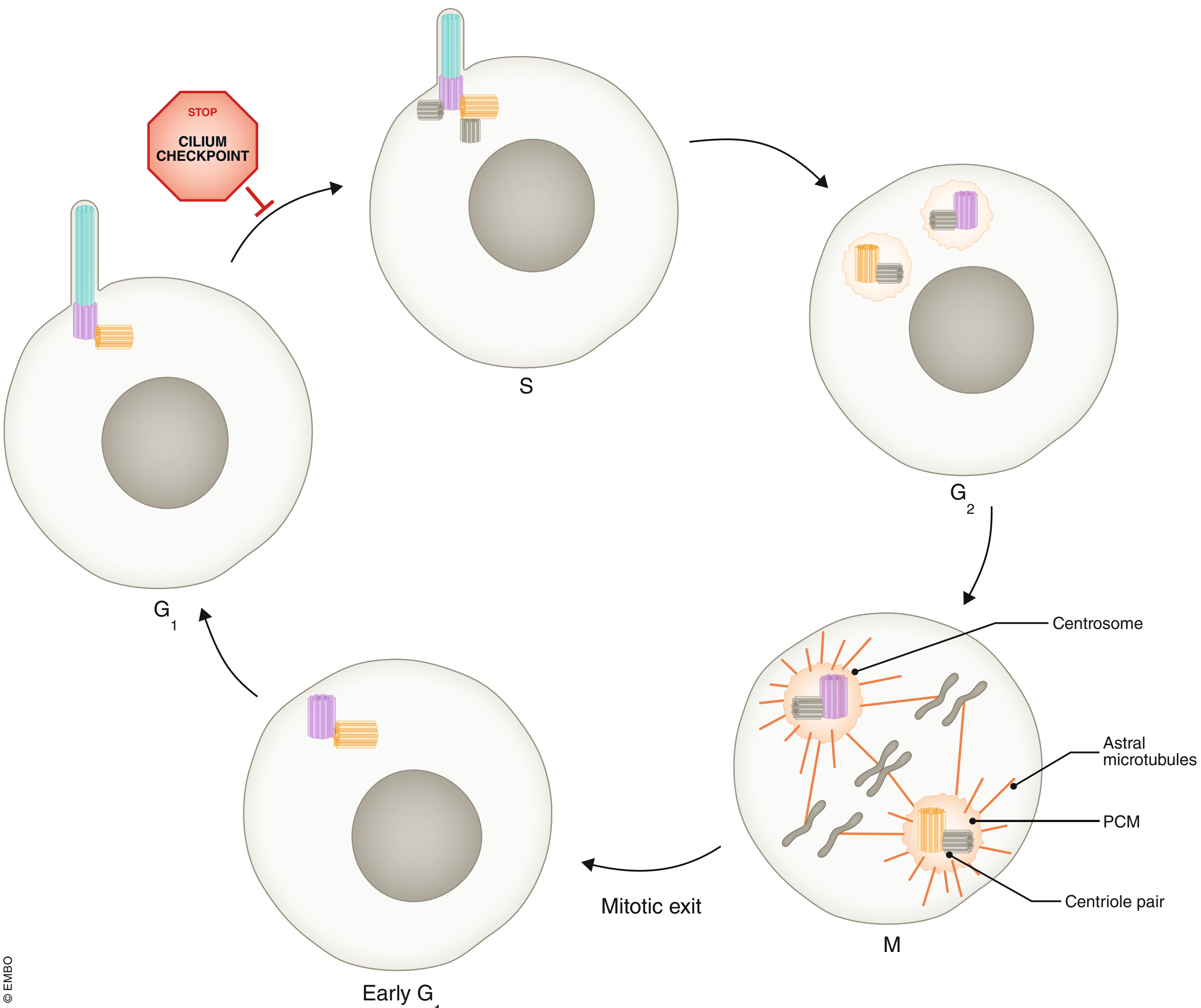

Figure 2. Primary cilium assembly/disassembly dynamics in a cycling cell *in vitro*.

The mature mother centriole (purple) templates the axoneme for cilium formation when the cell exits mitosis to the G1 phase. The daughter centriole at this stage is indicated in yellow. The cilium begins to disassemble at the onset of the S-phase. Failure of or delay in cilium disassembly acts as a brake ("cilium checkpoint") in cell cycle progression (stop sign). Cilium disassembly at G2 triggers cells to continue with mitotic progression.

dynamic processes are orchestrated with cell cycle progression in a highly regulated manner to ensure that the centrioles are freed and can be used for cell division. Delayed cilium disassembly can impose a brake on cell cycle progression, also referred to as "cilium checkpoint", which must be released before the cell proceeds through the cell cycle (Jackson, 2011; Kim *et al*, 2011; Li *et al*, 2011) (Fig 2).

Here, we briefly summarize how timely cilium disassembly is accomplished by mechanisms occurring in the cytoplasm, the cilium, and ciliary membrane. Excellent reviews provided detailed summaries of the mechanisms underlying cilium disassembly (Pan & Snell, 2007; Sanchez & Dynlacht, 2016; Patel & Tsiokas, 2021). Of note, these principles predominantly apply to cultured cells, whereas the correlation between primary cilia disassembly and cell cycle progression is less well understood *in situ* and *in vivo*.

In the final part of this section, we describe the consequences of defective cilium disassembly on cell fate and tissue organization and highlight findings on ciliated states in a tissue context-dependent manner.

Aurora kinase A (AURKA) and polo-like kinase 1 (PLK1) initiate the first phase of cilium disassembly, when cells re-enter the cell cycle from G0/G1 (Pan *et al*, 2004; Pugacheva *et al*, 2007; Lee *et al*, 2012; Wang *et al*, 2013). AURKA activation promotes cilium disassembly, which is tightly regulated. For example, calcium/calmodulin (CaM)-dependent formation of the human enhancer of

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filamentation 1 (HEF1)-AURKA complex (Pugacheva *et al*, 2007; Plotnikova *et al*, 2012) as well as binding of lysophosphatidic acid (LPA) to the LPA receptor 1 (LPAR1), triggering YAP/TAZ and calcium/CaM signaling, regulates AURKA activation (Hu *et al*, 2021). AURKA and PLK1 together activate histone deacetylase 6 (HDAC6) (Pugacheva *et al*, 2007; Maisonneuve *et al*, 2009), which functions as an α-tubulin deacetylase, facilitating deacetylation of ciliary microtubules during ciliary disassembly (Ran *et al*, 2015).

The predominant mechanism for the second phase of cilium disassembly is microtubule (MT) depolymerization at the ciliary base. Here, the NIMA-related kinase 2 (NEK2) activates KIF24, a kinesin that possesses MT depolymerization activity and prevents axoneme re-assembly (Spalluto *et al*, 2012; Kim *et al*, 2015). The NEK2-KIF24 axis ensures that the cilium is completely disassembled before the G2-M transition (Kim *et al*, 2015; Sanchez & Dynlacht, 2016). In addition, KIF2B, another MT depolymerase, is activated by PLK1 (Miyamoto *et al*, 2015). Finally, KIF24 interacts with centriolar coiled-coiled protein 110 (CP110) and the centrosomal protein 97 (CEP97) and recruits them to the mother centriole (Kobayashi *et al*, 2011). CP110 and CEP97 have centriole-capping functions, ensuring that the centriole cannot assemble the cilium in cycling cells (Spektor *et al*, 2007; Kobayashi *et al*, 2011).

Altogether, the components that regulate cilium disassembly are recruited at the ciliary base at the onset of cilium disassembly to form the cilium disassembly complex (CDC). Besides the essential kinases (AURKA, NEK2, PLK1) (Gabriel *et al*, 2016; Goranci-Buzhala *et al*, 2017, 2021), the CDC contains dynein binding-, centriolar satellite, and centrosomal proteins, such as NudE neurodevelopment protein 1 (NDE1), oral-facial-digital syndrome 1 protein (OFD1), and centrosomal-P4.1 associated protein (CPAP) (Tang *et al*, 2013; Gabriel *et al*, 2016; Monda & Cheeseman, 2018). CPAP has already been shown to play a central role in centrosomal duplication as well as centriolar and cilia length control (Kohlmaier *et al*, 2009; Tang *et al*, 2009; Zheng *et al*, 2016). Additionally, studies analyzing a *CPAP* mutation revealed that CPAP also provides a scaffold for CDC recruitment at the base of the cilium. However, its role in cilium disassembly independent from its centrosome duplication functions remains unknown (Gabriel *et al*, 2016).

Another aspect of cilium disassembly is remodeling of the membrane in the ciliary pocket and removing receptors from the cilium. The rearrangement of the ciliary pocket is controlled by the activation of TCTEX-1, a light-chain dynein subunit (Li *et al*, 2021). Activated TCTEX-1 interacts with annexin A2, which mediates the remodeling of the actin network-rich ciliary pocket (Saito *et al*, 2017). TCTEX-1-mediated remodeling precedes cilium disassembly at the onset of the S-phase (Li *et al*, 2011).

Cilium assembly and disassembly dynamics and their consequences for cell signaling seem to be context-dependent in different tissues. For example, abnormal or primary cilia loss has been observed in different types of cancer, including renal cell carcinoma, pancreatic ductal adenocarcinoma, prostate cancer, cholangiocarcinoma, glioblastoma, thyroid cancer, breast cancer, ovarian cancer, and melanoma (Eguether & Hahne, 2018; Liu *et al*, 2018a; Wang & Dynlacht, 2018; Higgins *et al*, 2019). However, in some cancer types, e.g., in medulloblastoma, cilia are present and cilia-dependent signaling can promote tumorigenesis (Goranci-Buzhala *et al*, 2017, 2021; Marino, 2022; Paul *et al*, 2022), e.g., by altering Hh signaling (Wu *et al*, 2017; Jeng *et al*, 2020) but also through WNT and ERK/MAPK signaling (Higgins *et al*, 2019).

Not only cell proliferation but also cell fate decisions are controlled by the cilium assembly/disassembly dynamics. Microcephaly, a neurodevelopmental disorder, is typically associated with defective centrosome function, including errors in centriole duplications (An *et al*, 2022). Importantly, a microcephaly causing mutation in CPAP(Lin *et al*, 2020), a centrosome duplication factor, exhibits a delayed cilium disassembly without affecting centrosome structure or function (Gabriel *et al*, 2016). A similar delay in cilium disassembly was also observed when the spindle pole-associated scaffold protein WDR62 was genetically deleted (Zhang *et al*, 2019). Notably, mutations in *WDR62* are the second most common genetic cause of primary microcephaly (Bilguvar *et al*, 2010; Nicholas *et al*, 2010). These findings indicate that defects in ciliary dynamics underlie the development of microcephaly and, thereby, underline a role for primary cilia assembly/disassembly modulation in regulating the fate of neural progenitor cells.

These studies have just started to reveal the importance of timely cilium assembly/disassembly in determining cell fate establishment and tissue organization. So far, we mainly have a snapshot view of the primary cilium in various cell culture models and some tissues. Developing tools that allow analyzing cilia assembly/disassembly dynamics at various cell cycle stages in living cells and *in vivo* within their tissue context will be important. To this end, the development of a tri-cistronic cilia and cell cycle biosensor (ARL13B-Cerulean combined with the cell cycle senor Fucci2a) allows identifying the ciliation state of cycling cells at high resolution *in vitro* and *in vivo* (Ford *et al*, 2018). Strikingly, these analyses revealed that cilia persist even beyond the G1/S transition into the S/G2/M-phase, which seems to be a general property in different cell types and in tissues of the developing mouse embryo (Ford *et al*, 2018). This novel tool paves the way to dissect the impact of cilia assembly and disassembly dynamics in tissue organization *in vivo* (Mill *et al*, 2023). This is underlined by findings in chicken and mouse epithelial cells of the neural tube and the developing cortex, respectively, which disassemble the cilium in G2 (Spear & Erickson, 2012). Combining *in vivo* tools with recent advances in analyzing the ciliary proteome and signaling states (see below) will allow to disentangle the interaction between cilia-mediated signaling and cilia status in specific cell types during tissue organization.

Analyzing primary cilia dynamics with state-of-the-art methods

Within the last few years, major technical advances have opened new frontiers and helped to overcome previous limitations in investigating primary cilia function. In the following sections, we will highlight some of the recent approaches that led to discoveries in cilia biology.

Spatial proteomic approaches in primary cilia

Over a decade ago, advances in mass spectrometry allowed in-depth analyses of the flagellar and ciliary protein composition in an unbiased fashion (Pazour *et al*, 2005; Liu *et al*, 2007; Mayer *et al*, 2008, 2009; Ishikawa *et al*, 2012). Comprehensive comparisons of the flagellar proteome, obtained from isolated flagella, were successfully

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performed in the green alga *Chlamydomonas reinhardtii* by classic biochemical methods (Pazour *et al*, 2005; Lechtreck *et al*, 2009; Craige *et al*, 2010). However, such approaches using isolated organelles were not feasible for mammalian primary cilia due to limitations in biological material quantity, sample purity, and the complexity of fractionation protocols used for analysis (Mayer *et al*, 2008; Ishikawa *et al*, 2012). These limitations were overcome by introducing proximity labeling technologies (Roux *et al*, 2012; Rhee *et al*, 2013).

Conceptually, proximity labeling technologies are based on genetically engineered enzymatic activities that biotinylate nearby proteins, which are subsequently affinity purified and subjected to mass spectrometric analysis. Ultimately, the purification and identification of biotinylated proteins do not require lengthy protocols. Initially, an engineered ascorbate peroxidase (APEX) fused to a peptide targeted to mitochondria was used to comprehensively identify mitochondrial proteins (Rhee *et al*, 2013). Inspired by this approach, this technique has now also been applied to cilia by either fusing APEX to the first 200 amino acids of the ciliary protein nephrocystin-3 (NPHP3, cilia-APEX) (Mick *et al*, 2015) or to the C-terminus of the ciliary 5-hydroxytryptamine (serotonin) receptor 6 (Htr6/5HT6), sitting in the ciliary membrane (cm-APEX) (Kohli *et al*, 2017). Proximity labeling in cilia, including recently emerged approaches, such as BioID, to study protein spatial interactions, has been comprehensively reviewed (Arslanhan *et al*, 2020; Chen *et al*, 2022).

Apart from proteomic profiling of primary cilia to investigate the consequences of mutations in cilia genes (Mick *et al*, 2015), the genetically optimized, 'high speed' biotin ligases TurboID or MiniTurbo (Branon *et al*, 2018) and APEX2 (Lam *et al*, 2015) will have a significant impact on our future understanding of ciliary dynamics due to their high temporal resolution. High enzymatic activities and short labeling times make them ideal tools to investigate temporal dynamics and, combined with targeting to subcellular compartments, provides the spatial resolution. Cilia-APEX has already been used to study the dynamics of ciliary Hh signaling *in vitro*, identifying a new component that dynamically localizes to the primary cilium and revealing a rapid removal of PKA from primary cilia after pathway induction (May *et al*, 2021a). Moreover, APEX labeling in the cilium also determined the role of actin depolarization on the ciliary proteome. Depolarization of branched F-actin with cytochalasin D increased ciliary length and the abundance of actin-binding proteins within the cilium (Kohli *et al*, 2017). In summary, ciliary-targeted APEX and TurboID are in a prime position and have already started to improve the study of ciliary dynamics on a proteomic level by providing high temporal and spatial resolution in a context-dependent manner.

Imaging primary cilia dynamics using markers and biosensors

To understand cilia dynamics, live imaging of molecules is imperative, whereby imaging of primary cilia usually requires cilia-localized fluorescent markers. So far, these approaches rely on the expression of a fusion protein between a fluorophore and a ciliary targeting sequence or a full-length ciliary protein, e.g., ARL13B or somatostatin receptor 3 (SSTR3) (Borovina *et al*, 2010; Nakata *et al*, 2012; Guadiana *et al*, 2013; O'Connor *et al*, 2013; Bangs *et al*, 2015; Mick *et al*, 2015). Alternatives are cilia-targeted nanobodies that recruit ciliary cargoes by binding and avoid changes in protein activity or interactions upon fusion with large protein domains (Hansen *et al*, 2020). Even though the identification of various cilia targeting sequences has improved our understanding of cilia trafficking over the last decades (Nachury *et al*, 2010; Barbeito *et al*, 2021), such motifs can be difficult to use and may not apply to all ciliated cell types and protein types (Hansen *et al*, 2020). Importantly, overexpression of such constructs may lead to altered cilia signaling and function. Careful titration of expression levels is crucial to minimize artifacts while maintaining high fluorescence levels, which are required for a fast image acquisition (Guadiana *et al*, 2013; Hansen *et al*, 2020, 2021).

Fusion proteins and nanobodies have allowed ciliary targeting of biosensors to detect a wide range of signaling molecules, such as calcium or cAMP, in the cilium, providing insight into the signaling dynamics (Delling *et al*, 2013, 2016; Yuan *et al*, 2015; Moore *et al*, 2016; Mukherjee *et al*, 2016; Hansen *et al*, 2020; Mizuno *et al*, 2020). Since levels of second messengers may differ in the cilium *versus* the cell soma or other organelles, it is crucial to use biosensors with the right detection range for ciliary concentrations of second messengers (Delling *et al*, 2013). Moreover, even though primary cilia are not motile, they are not rigid and can also move on the cell surface during imaging (Hansen *et al*, 2020). Hence, to overcome motion artifacts, ratiometric approaches have been well implemented with the use of a second fluorophore whose fluorescence is insensitive to the second messenger under investigation (Delling *et al*, 2013, 2016; Yuan *et al*, 2015; Moore *et al*, 2016; Mizuno *et al*, 2020; Djenoune *et al*, 2023; Katoh *et al*, 2023). Alternatively, FRET-based sensors naturally overcome the challenges of (i) recording motion artifacts and (ii) different expression levels of the biosensor between individual cells by normalization, i.e., normalizing the FRET signal to the donor and acceptor signal (Mukherjee *et al*, 2016).

For unbiased, quantitative image analysis of cilia properties, such as length, signaling state, cilia position, and its relation to cellular and organ physiology, the first step is the detection and segmentation of structures from 3D (x,y,z) or 4D (x,y,z,t) datasets. Various tools have been developed to identify and track cilia on either open-source or commercial platforms (Ruhnow *et al*, 2011; Dummer *et al*, 2016; Ferreira *et al*, 2017; Hansen *et al*, 2018, 2021; Lauring *et al*, 2019; Geyer *et al*, 2022; Djenoune *et al*, 2023).

While second messenger dynamics are usually investigated at high temporal resolution, monitoring cilia dynamics during the cell cycle requires prolonged live imaging. Using a cilia and cell cycle biosensor consisting of ARL13B-Cerulean and the Fucci2a system (Ford *et al*, 2018) revealed a surprising persistence of primary cilia after the G1/S transition both *in vitro* and *in vivo* (Ford *et al*, 2018).

The increasing quality and availability of such tools will be instrumental in increasing our understanding of how primary cilia dynamics relate to the physiological output of cells and control tissue development and organization.

Optogenetic and chemical tools to manipulate ciliary signaling

Optogenetic techniques of altering biochemical functions or localization of molecules have emerged as powerful tools to study biological processes with spatiotemporal control. The photo-activated adenylyl cyclase (bPAC) and the light-activated phosphodiesterase (LAPD) have been used to increase or decrease cAMP levels in cilia, respectively (Jansen *et al*, 2015; Guo *et al*, 2019; Raju *et al*, 2019; Hansen

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et al, 2020, 2022; Truong *et al*, 2021). Targeting these light-regulated enzymes to cilia or the cytoplasm allowed disentangling the contribution of ciliary versus cytoplasmic cAMP levels to cell signaling and cilia function (Hansen *et al*, 2020, 2022; Truong *et al*, 2021). Using this approach, it was shown that (i) elevation of ciliary cAMP, but not cytoplasmic cAMP, inhibits Hh signaling (Truong *et al*, 2021), (ii) an increase in ciliary cAMP lengthens the cilium, whereas an increase in cytoplasmic cAMP levels shortens it (Hansen *et al*, 2020), and that (iii) a chronic increase in ciliary cAMP levels promotes cyst formation in a 3D kidney epithelial cell model (Hansen *et al*, 2022).

Optogenetic systems have also been used to recruit proteins of interest, such as enzymes, to primary cilia by light-dependent dimerization of protein domains fused to the protein of interest and a cilia-localized partner protein. Such systems have been used to study cell signaling (e.g., Akt and PI3K), to induce F-actin formation and ciliogenesis, and to disrupt microtubules (Guo *et al*, 2019; Liu *et al*, 2022; Yasunaga *et al*, 2022). The blue-light or red-/far-red-light inducible protein dimerizers CIBN/GRY2 or PIF/PHYB, respectively, were fused to the N-terminus of NPHP3 or the small GTPase ARL13B for ciliary targeting. Furthermore, they were combined with the binding domains of the Microtubule-Associated Protein 4 (MAP4m) or the actin-binding domain of Ezrin for recruitment to microtubules or F-actin, respectively (Guo *et al*, 2019; Hansen *et al*, 2020; Liu *et al*, 2022; Yasunaga *et al*, 2022). As described above, nanobody-dependent protein recruitment into cilia has proven successful and has also been applied to optogenetic tools (Hansen *et al*, 2020). Such an approach was combined with photoswitchable nanobodies that alter protein binding-ability upon blue light illumination to achieve temporal control over protein localization, e.g., targeting to actin (Gil *et al*, 2020). These tools employ the light-oxygen-voltage domain 2 (LOV2), which reversibly changes conformation in a light-dependent manner (He *et al*, 2021).

Not only optogenetics but also chemogenetics have been used to localize proteins to primary cilia. Here, protein recruitment is induced by protein dimerization upon addition of small molecules. Two hallmark studies used the rapamycin-inducible dimerization of FKBP-FRB (FK506 Binding Protein, FKBP Rapamycin Binding-Domain) to characterize the ciliary diffusion barrier that maintains cilium integrity (Breslow *et al*, 2013; Lin *et al*, 2013). The FKBP-FRB system has also been applied to study protein trafficking into primary cilia via IFT complexes. In this case, induced dimerization has been used to deplete IFT proteins from cilia and trap them at mitochondria, also referred to as "knocksideways" (Eguether *et al*, 2018). The outcome of these experiments suggested that IFT is dispensable for SMO transport to primary cilia, as previously suggested (Milenkovic *et al*, 2009). Such methods for inducible inactivation will not only allow the investigation of ciliary components that lead to a loss of cilia, such as IFT complexes, but will also allow discriminating primary from secondary effects that classic genetic approaches may miss.

Chemogenetics were also used to manipulate second messenger dynamics in primary cilia by DREADDs (Designer Receptors Exclusively Activated by Designer Drugs), which are engineered GPCRs activated by otherwise inert small molecules. Applying DREADDs in cilia revealed that (i) neuronal primary cilia length is controlled by ciliary cAMP signaling, (ii) ciliary cAMP shapes the development of interneuronal connectivity, (iii) ciliary Ca^{2+} and cAMP signaling alters axonal behavior, and (iv) ciliary cAMP controls Hh signaling (Truong *et al*, 2021; Alhassen *et al*, 2022). Altogether, these studies highlight that both light- and chemically inducible approaches allow specific manipulation of ciliary processes, whereby the consequences of protein perturbation for cilia can be temporally dissected.

How do primary cilia control tissue organization and function?

Primary cilia are key determinants of tissue organization and function. In the following section, we highlight findings that demonstrate the importance of the different levels of primary cilia dynamics in controlling tissue organization during embryonic development and show how dysfunction of primary cilia dynamics leads to diseases. We focus on tissue types in which cilia dynamics at the defined levels (compositional, signaling, and assembly/disassembly) have been characterized in sufficient detail, and which has not been extensively described in recent reviews.

Primary cilia dynamics during development and disease

Primary cilia are required for tissue and organ formation, where they are dynamically assembled and disassembled in developing tissues during morphogenesis (Fig 3A). As such, loss of primary cilia causes severe developmental defects in humans and in animal models. Many ciliopathies are either embryonic lethal or characterized by morphological malformations and impaired organ formation, arising from misguided developmental processes (Goetz & Anderson, 2010; Reiter & Leroux, 2017; Anvarian *et al*, 2019).

Primary cilia dynamics in the left–right organizer

A classic example of cilia function in vertebrate development is the specification of the left–right (LR) body axis, which allows the development of asymmetric organs, including the heart, lungs, and intestine (Hamada, 2020; Little & Norris, 2021). During late gastrulation and neurulation, most vertebrate embryos form a transient, ciliated structure that acts as a left–right organizer (LRO), called the node in mammals, the gastrocoel roof plate in amphibians, and the Kupffer's vesicle in teleost fish (Blum *et al*, 2014). To break the initial bilateral symmetry, medially located cells of the LRO form motile monocilia with a '9 + 0', '9 + 2', or '9 + 4' MT arrangement (Feistel & Blum, 2006; Odate *et al*, 2016), which are posteriorly tilted while they are growing (Schweickert *et al*, 2007) and which rotate clockwise (Nonaka *et al*, 1998). This generates a cilia-driven, leftward fluid flow, a dynamic physical process which, in consequence, induces differential gene expression. Laterally located cells of the LRO harbor primary cilia that sense fluid flow, which leads to de-repression of the TGFβ family signaling molecule Nodal on the left side of the embryo (Schweickert *et al*, 2010; Maerker *et al*, 2021). Nodal induces its own expression and the expression of downstream factors in the left lateral plate mesoderm and heart anlage, which instructs asymmetric organ morphogenesis (Schweickert *et al*, 2017). Defective motile or primary cilia formation and function lead to abnormal asymmetric organ placement, called *situs inversus*, when a complete mirror-image orientation develops, and *situs ambiguus*, when left and right sides are duplicated (left or right isomerism) or when individual organs are inverted (heterotaxia) (Fliegauf *et al*, 2007).

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The role of primary cilia in dynamic flow sensing and that of ciliary ion channels from the PKD family in generating dynamic ciliary calcium transients has been described above (section 2.1). Additionally, two orphan GPCRs, Gpr22 and Gpr161, the latter being a well-established Hh signaling component (Mukhopadhyay *et al*, 2013), were implicated in LRO function in the zebrafish Kupffer's vesicle using morpholino-based knock-down studies (Leung *et al*, 2008; Verleyen *et al*, 2014). Loss of Gpr22 causes abnormal axoneme formation and changes in Gpr22 levels affect cilia length, while loss of Gpr161 perturbs calcium dynamics. However, their precise signaling functions in left–right development are not understood, and the role of Gpr161 is being debated, because genetic knockouts and CRISPR-mediated mutations did not lead to laterality defects in mice and zebrafish, respectively (Mukhopadhyay *et al*, 2013; Verleyen *et al*, 2014; Tschaikner *et al*, 2021). In summary, the identity and the dynamic sensing of

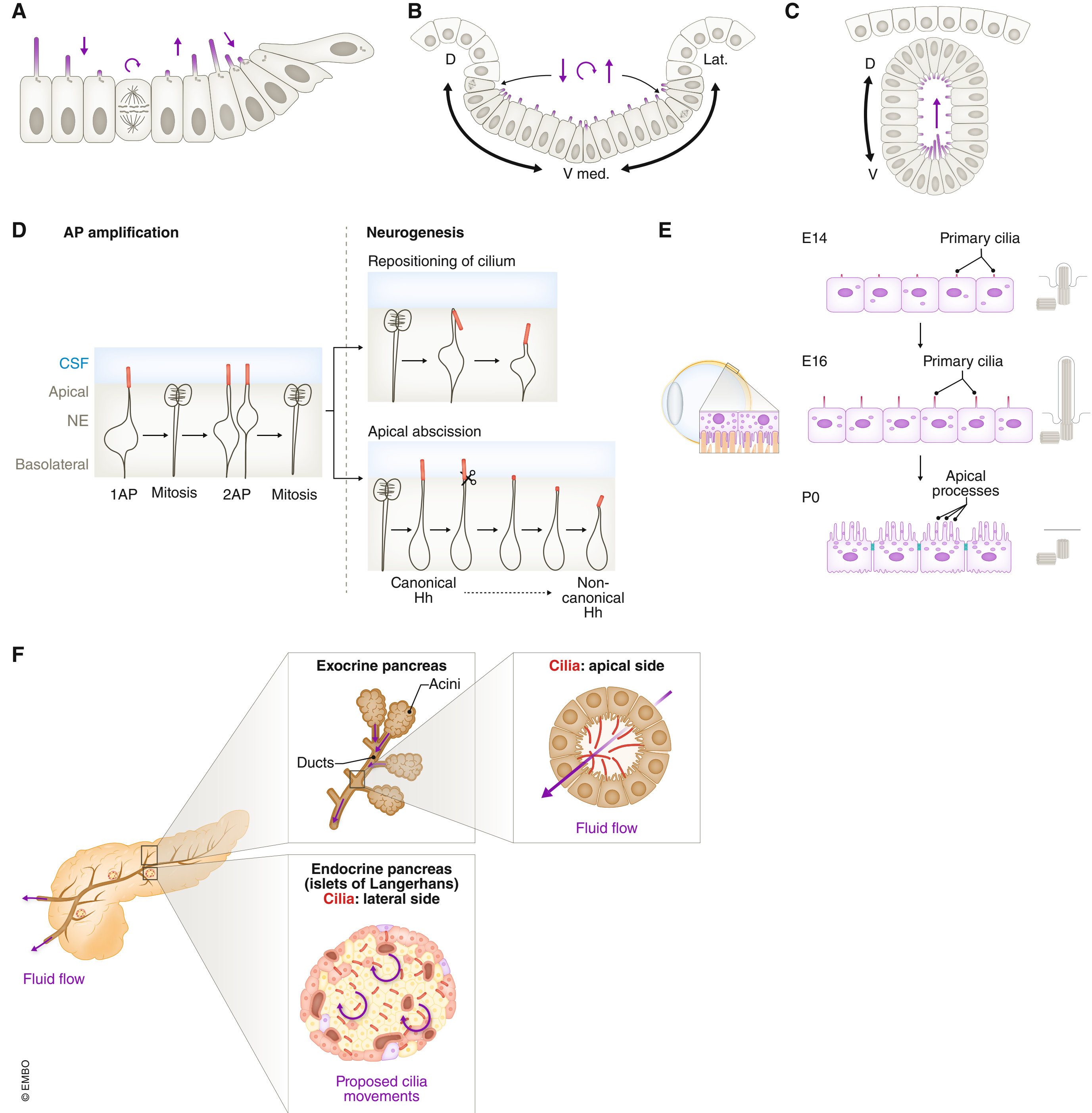

Figure 3.

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◀ **Figure 3. Primary cilia dynamics during tissue development.**

(A) Primary cilia dynamics in tissue morphogenesis. From left to right: Cilium retraction and assembly (purple arrows) during proliferation (purple curved arrow), apical constriction, and cell delamination. (B, C) Primary cilia dynamics during neural tube closure. (B) Transverse view of the neuroepithelium (dark gray cells) flanked by non-neural ectoderm (light cells); short primary cilium retraction and assembly during cell proliferation (purple and purple curved arrows) occurs differentially along medial-to-lateral (med./lat.) axis, which becomes the ventral-to-dorsal (V/D) axis during neural fold elevation. (C) Upon neural tube closure, cilia on the ventral (V) floor plate elongate dynamically, while cilia on lateral and dorsal (D) aspects of the neural tube remain short. (D) Primary cilia dynamics during brain development. Left: Apical progenitors' (AP) cilia are located at the apical surface of the neural epithelium (NE) in direct contact with embryonic cerebrospinal fluid (CSF). Right: Two different examples of ciliary dynamics upon neuronal differentiation. Top: The cilium is re-assembled within the neuronal tissue following cell division, away from embryonic CSF. Bottom: Apical abscission removes the cilium from the differentiating neuron. The cilium will eventually be re-assembled away from the apical surface of the NE. This leads to a transition from canonical to non-canonical Hh signaling. (E) Primary cilia dynamics in the retinal pigment epithelium (RPE). Left: Cross-section of the vertebrate eye. The apical processes of pigmented RPE cells (purple) engulf the outer segments of the light-sensitive photoreceptors (beige). Middle/Right: A short primary cilium (red) can be detected from E14 onwards. Primary cilia are longest and most abundant at E16, before disassembling around birth. Disassembly of the primary cilium precedes extension of actin-based apical processes. (F) Primary cilia dynamics in the pancreas. An adult mouse pancreas (left) comprises a network of ducts (brown) terminated by acini (top), forming the exocrine pancreas. A section of a large duct displayed on the right shows cilia on ductal cells (red), pointing towards the lumen. The islets of Langerhans (bottom) are located close to ducts and assemble multiple endocrine cells, which harbor a cilium on their lateral surface (red). The direction of fluid flow and the direction of cilia movement are indicated with blue arrows. Parts of the figure have been created using Biorender.

physical and chemical cues through primary cilia in the LRO is only partially understood.

Furthermore, because the LRO is a transient structure, ciliated cells have to precisely coordinate (i) proliferation to generate an appropriate number of LRO cells with (ii) cilia formation to generate and sense fluid flow, and (iii) cilium disassembly, which proceeds after left–right asymmetry is established and that is required for LRO cells to contribute to the formation of other developing tissues (e.g., the notochord and the somites).

Furthermore, how primary cilia formation in the LRO is determined also remains an open question. The specification of motile monocilia of the LRO is induced by cell signaling, i.e., canonical WNT signaling, which regulates the expression of *foxj1*, the master motile cilia inducer (Stubbs *et al*, 2008; Caron *et al*, 2012; Walentek *et al*, 2012). In the developing *Xenopus* embryo, both medial and lateral LRO cells express *foxj1* and form motile cilia. Nevertheless, differences in mechanical strain dynamics during gastrulation that medial versus the lateral LRO cells experience during cilia formation lead to differential trafficking of the motile cilia-specific protein Tektin2 only into motile cilia at the center of the LRO (Chien *et al*, 2018). Thus, the lateral LRO forms primary (flow-sensing) cilia in low mechanical strain conditions that affect ciliary protein trafficking. However, the molecular mechanism underlying this differential mode of ciliary compositional dynamics deciding between different types of cilia formation remains enigmatic.

Similarly, how the cell cycle of LRO cells is synchronized to allow for transient cilia formation throughout the entire structure remains elusive. Initial evidence from zebrafish indicates that the regulation of proliferation in LRO precursors needs to be tightly controlled to generate sufficient Kupffer's vesicle cell numbers (Gokey *et al*, 2016) and to stabilize Foxj1, indicating potential effects on the decision between motile and sensory LRO cilia (Liu *et al*, 2019). Similarly, interfering with the cilia retraction pathway disrupts left–right patterning, as demonstrated in mice haploinsufficient for Pitchfork (PIFO), which regulates cilia disassembly through AURKA activation (Kinzel *et al*, 2010). These mice have abnormal cilia and defective ciliary protein trafficking, but how that affects LRO signaling has not been investigated. Thus, while the essential role of primary cilia in vertebrate left–right axis development is widely accepted, open questions remain regarding protein trafficking, cell cycle control, and ciliary signaling dynamics during the process.

Primary cilia dynamics during neural tube development

Prominent examples of primary cilia dynamics in development are also found during all phases of nervous system formation, e.g., the morphogenetic movements leading to cranial neural tube closure or during Shh-mediated patterning of the developing brain and neural tube.

One of the first dynamic processes with regard to primary cilia is their positioning in neuroepithelial cells. During polarization and epithelialization of the neuroepithelium in zebrafish, basal bodies move from a position close to the nucleus towards the apical membrane of neuroepithelial cells (Hong *et al*, 2010). As the apical aspects of neuroepithelial cells will establish the lining of the neural tube lumen (Fig 3B and C), apical positioning of the basal body is a prerequisite step to position a primary cilium on the ventricular surface of the central nervous system.

Primary cilia signaling is already active at neural plate stages, i.e., before a ventricular surface is established by neural tube closure or neural rod cavitation. Manipulation of Shh signaling alters target gene expression already in neural plate epithelial cells (Walentek *et al*, 2015; Brooks *et al*, 2020). In the early neurula embryo, the presence and physiological function of primary cilia is clearly required to transform the neural plate epithelium into a closed neural tube (Huangfu *et al*, 2003; Caspary *et al*, 2007; Ashique *et al*, 2009; Mukhopadhyay *et al*, 2013; Manojlovic *et al*, 2014; Shimada & Mukhopadhyay, 2017). There is, however, a region-specific requirement for primary cilia function in neural tube closure. Neural tube closure defects in primary cilia mutant mice generally affect the cranial region, resulting in exencephaly, while neural tube closure in the trunk region is commonly not affected (Murdoch & Copp, 2010; Wallingford & Mitchell, 2011). Yet, not all primary cilia defects impair cranial neural tube closure. The picture has emerged that impaired signaling dynamics – specifically leading to upregulation of Shh signaling – induces exencephaly (Murdoch & Copp, 2010; Wallingford & Mitchell, 2011; Mukhopadhyay *et al*, 2013; Shimada & Mukhopadhyay, 2017; Brooks *et al*, 2020; Hwang *et al*, 2021). This is prominently exemplified by defects in the IFT-A/TULP3/GPR161 complex, a prerequisite for Hh signaling dynamics. IFT-A core proteins together with TULP3 facilitate ciliary access for GPR161, a prerequisite for the repression of Hh signaling (Mukhopadhyay *et al*, 2010, 2013). Mutations in or loss of IFT-A/TULP3/GPR161 prevent complex formation and, thus, impair ciliary

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localization of GPR161, leading to overactivation of Hh signaling and, as a result, exencephaly (Qin *et al*, 2011; Liem *et al*, 2012; Hwang & Mukhopadhyay, 2015). It has recently been shown that the spatial dynamics of Shh signaling instruct regionalized apical constriction, a cell shape change that brings about tissue deformation (Fig 3B and C). Although apical constriction occurs along the entire length of the neural plate, it is essential for cranial neural tube closure (Brooks *et al*, 2020). Although the underlying molecular mechanisms are not fully understood, calcium influx upon non-canonical Shh signaling and the subsequent calcium-mediated activation of myosin-driven actin meshwork contraction likely play a role (Belgacem & Borodinsky, 2011; Adachi *et al*, 2019).

Morphogenetic re-arrangements in the cranial region, especially (defective) neural tube closure, are readily visible readouts of primary cilia-mediated signaling dynamics in the embryo. Yet, cell-fate specification occurs "invisibly" at the same time. Dorso-ventral patterning of the emerging neural tube is already being established along the medial-to-lateral axis of the neural plate, which is transformed into a ventral-to-dorsal (or dorsoventral, DV) axis as the neural plate folds and fuses into a tube (Fig 3B and C). Here, cell-fate specification depends on opposing morphogen gradients: WNT/BMP (Bone Morphogenetic Protein), emanating from the lateral aspects of the neural plate (the future dorsal roof plate), and Shh, secreted from the midline (the future ventral floor plate). This establishes six distinct progenitor domains in the ventral part of the trunk neural tube and specifies V0-V3 interneurons, motor neurons, and the floor plate (Jessell, 2000; Ribes *et al*, 2010). All cells display a short primary cilium during patterning in the open neural plate (Fig 3B). Upon neural tube closure, primary cilia length is dynamically adjusted: while primary cilia on Shh-receiving cells remain short, cilia on Shh-expressing floor plate cells that express *foxj1* elongate, concomitant with an attenuation of Shh signaling in the floor plate (Fig 3C) (Cruz *et al*, 2010). Over time, the niche of cells with elongated cilia expands in the dorsal direction, matching the shift of Shh responsiveness (Ribes *et al*, 2010). Similarly, dynamically elongating primary cilia are found in other regions of the neural tube that express *foxj1*, i.e., the zona limitans intrathalamica (ZLI), isthmic organizer, and rhombomere boundaries (Hagenlocher *et al*, 2013). The floor plate, ZLI, isthmic organizer – and possibly also the rhombomere boundaries, which express Hh signaling components (Perron *et al*, 2003) – serve as signaling centers with crucial roles in embryonic brain patterning via Shh signaling (Kiecker & Lumsden, 2012). The dynamic regulation of cilia length in these signaling centers seems to fine-tune Shh signaling and adapt it to the spatio-temporal requirements of Shh-mediated patterning (Cruz *et al*, 2010; Ribes *et al*, 2010). Interestingly, the composition and function of elongated primary cilia in signaling centers continue to be adapted: under the influence of *foxj1*, elongated cilia will acquire motile cilia features (Yu *et al*, 2008; Cruz *et al*, 2010; Hagenlocher *et al*, 2013). It is currently unknown whether they retain their function as dynamic modulators of Shh signaling, gain mechanosensory functions, or generate extracellular fluid flow that influences Shh ligand distribution. Since motile cilia have been shown to transduce Hh signaling in sea urchin larvae (Warner *et al*, 2014), it can be envisioned that elongated motile-like cilia in the floor plate retain a sensory function for Hh and other potentially other pathways.

Primary cilia dynamics during brain development

To understand the importance of primary cilia dynamics in mammalian brain development, it is worth considering the complexity of the developing mammalian brain, which contains various progenitors that divide, differentiate, and migrate to eventually form several neocortex layers (Rakic, 1995; Goetz & Huttner, 2005; Huttner & Kosodo, 2005). During the early stage of neocortex development, the neuroepithelial cells of the anterior neural tube expand and generate radial glial stem cells (RGs), forming an apical ventricular zone (VZ) that contains apical progenitors (Rakic, 1995; Florio & Huttner, 2014). Through asymmetric division, in which the two daughter cells eventually acquire different fates, apical progenitors give rise to other progenitors, forming different germinal zones, including the inner and the outer subventricular zones, separated by an inner fiber layer. This heterogeneity of layered neural progenitor cell (NPC) populations is determined by cell cycle length, mode of cell division, and by cell polarity, and is critical for neurogenesis, thereby contributing to the massive expansion of the human neocortex. NPCs eventually differentiate and give rise to neurons, which migrate to their target destination in the developing brain. Any defect in NPC proliferation and differentiation can elicit malformation of the neocortex, leading to neurodevelopmental defects like microcephaly, macrocephaly, or heterotopia (Hill & Walsh, 2005; Jayaraman *et al*, 2018; Pinson *et al*, 2019; Gabriel *et al*, 2020).

Given the role of primary cilia in controlling cell division, cell polarity, and cellular communication, it is not surprising that primary cilia dysfunction affects NPCs and neurons and, thereby, underlies several neurodevelopmental disorders (Han & Alvarez-Buylla, 2010; Paridaen & Huttner, 2014; Liu *et al*, 2021; Suciu & Caspary, 2021; Stoufflet & Caillé, 2022; Zaidi *et al*, 2022). Notably, apical progenitors, which generate other progenitor types upon symmetrical or asymmetric cell division, exhibit a primary cilium at the apical surface, projecting into the lumen of the ventricular zone (VZ) in vertebrate brain (Wilsch-Bräuninger & Huttner, 2021).

In this review, we limit our discussions about the role of primary cilia dynamics in brain development to the timely cilium disassembly and re-assembly in NPCs and differentiating neurons, and the signals associated with these dynamic ciliary processes.

Delayed cilia disassembly in NPCs was identified in several studies modeling developmental brain defects due to mutations in the basal body or ciliary genes such as *CPAP*, *NDE1*, *WDR62*, *RRP7A*, *Tctex-1*, and *LPRA1* (Li *et al*, 2011; Gabriel *et al*, 2016; Tan *et al*, 2017; Zhang *et al*, 2019; Farooq *et al*, 2020; Hu *et al*, 2021). As cilium disassembly is required before mitosis to free the centrioles for cell division, the working model suggests that apical progenitors that exhibit a delay in cilia resorption could impact on cell proliferation. Thereby, the NPC pool is diminished, and NPC fate is changed, leading to defects in neocortex formation (Gabriel *et al*, 2016; Zhang *et al*, 2019). However, a detailed analysis of NPC fate and pool composition upon delayed ciliary disassembly is missing and will require single-cell RNA sequencing studies. Furthermore, to what extent and which of the specific ciliary signals are perturbed when cilium disassembly in NPCs is delayed and how the different signaling pathways affect brain development, is also not known.

Similar to cilium disassembly, cilium formation in NPCs also needs to be tightly controlled. Cilium formation requires first centriolar appendages to anchor the mother centriole at the plasma membrane, followed by Golgi-derived vesicle docking to the distal

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end of the mother centriole, and elongation of the microtubule-based axoneme. Alterations in any of these processes were shown to impact the proliferative capacity of the neural progenitors, as indicated hereafter. Notably, a mutation in a microtubule-associated protein, *EML1*, resulted in a shorter cilium and was sufficient to cause the mislocalization of progenitors in the developing neocortex leading to heterotopias (Uzquiano *et al*, 2019). Mutations in a distal appendage protein, *CEP83*, inhibit ciliogenesis and lead to megalocephaly, a condition with an increased abundance of neural progenitors, for which the underlying mechanisms remain unknown (Shao *et al*, 2020b). Finally, loss of the phosphatase *Inpp5e* that controls the phosphoinositide composition of the ciliary membrane resulted in shorter primary cilia with altered membrane structure, leading to neocortex defects in mice (Bielas *et al*, 2009; Jacoby *et al*, 2009).

The timing of cilium reassembly also controls NPC fate, as it has been shown that inheritance of ciliary membrane remnants after disassembly results in faster cilia re-assembly and, thereby, promotes stemness during asymmetric division of apical progenitors (Paridaen *et al*, 2013).

Dynamic ciliary assembly/disassembly processes have also been observed in early differentiating neurons. Here, we will highlight two examples and their consequences on ciliary signaling. First, in mice, it was shown that following asymmetric division, the daughter cell, which will become a neuron, rebuilds its cilium on the basolateral rather than the apical plasma membrane, which is exposed to embryonic cerebrospinal fluid (CSF) (Wilsch-Bräuninger *et al*, 2012; Paridaen *et al*, 2013) (Fig 3D). Since embryonic CSF is enriched in pro-proliferative molecules that provide a particular environment for progenitor cells (Wilsch-Bräuninger *et al*, 2012; Fame & Lehtinen, 2020), it raises the question of whether repositioning the cilium away from the ventricle potentially turns off ciliary signaling from the CSF. Second, in the chick neural tube, another process, referred as to apical abscission, was shown to cut away most of the cilia from the apical membrane of the neuron-to-be (Das & Storey, 2014; Toro-Tapia & Das, 2020) (Fig 3D). Following abscission, the cilium reassembles rapidly at the tip of the apical process during its retraction from the ventricular surface. Importantly this dynamic dis−/re-assembly process was shown to correspond to a switch from canonical to non-canonical Hh signaling in the differentiating neurons (Das & Storey, 2014; Toro-Tapia & Das, 2020). Taken together, these two examples show that the dynamic regulation of cilia assembly/disassembly can modulate the response of a differentiating neuron to its signaling environment.

Altogether, primary cilia dynamics regulate several aspects of brain development, from controlling progenitor division, neuronal differentiation, and migration to forming a fully functional brain. Understanding the principles of cilia dynamics and its regulatory roles will be instrumental for decoding mechanisms of brain development.

Primary cilia dynamics during the development of the retinal pigment epithelium

The vertebrate eye is a complex organ, consisting of multiple interconnected cell types with specialized functions. Numerous ocular cell types display a primary cilium (May-Simera *et al*, 2017). Undoubtedly, the best-studied ciliated cells in the visual system are the retinal photoreceptors, whose connecting cilium and outer segment build a 'photoreceptor connecting cilium', which has been extensively studied. Many excellent reviews have described the formation, structure, and function of this specialized cilium (Ramamurthy & Cayouette, 2009; Bachmann-Gagescu & Neuhauss, 2019; Chen *et al*, 2021). While the trafficking of molecules into and out of this cilium is highly dynamic, the structure itself is predominantly static once it is formed. However, the closely associated retinal pigment epithelium (RPE), a monolayer of highly specialized, tightly connected polarized cells, harbors a dynamic primary cilium that controls the differentiation, maturation, and function of the RPE.

During RPE development, the primary cilium is a highly dynamic structure that, in mice, can first be identified around embryonic day 14 (E14), is most abundant at E16.5, and are shorter and less abundant post birth (P0) (Nishiyama *et al*, 2002; Patnaik *et al*, 2019) (Fig 3E). Although a few cells remain ciliated, the majority of RPE cells lack primary cilia in the adult state. Similar to the *in vivo* expression in the mouse, the emergence of primary cilia on human induced pluripotent stem (hiPSC)-derived RPE cells temporally coincides with the maturation of cells and precedes the final phases of maturation (May-Simera *et al*, 2018). Intriguingly, hiPSC-derived RPE cells maintain cilia post-maturation, which do not appear to disassemble (May-Simera *et al*, 2018). However, these results raise the question whether cilium disassembly is dependent on or triggered by interactions and connections with the outer segments of adjacent photoreceptor cells or whether the differences seen are species-specific.

As in all epithelial tissues, the development and maturation of the RPE are orchestrated via a complex cascade of signaling pathways. Work from ciliopathy mutant mouse models, as well as ciliopathy patient-derived hiPSC-RPE cells has shown that the temporal and spatial modulation of WNT signaling by the primary cilium is particularly important, which is likely regulated via the dynamic presence of the cilium (May-Simera *et al*, 2018; Patnaik *et al*, 2019; Schneider *et al*, 2021). Initially, activation of β-catenin drives the expression of early transcription factors MITF, OTX2, and PAX6 (Westenskow *et al*, 2009; Bharti *et al*, 2012; Fujimura *et al*, 2015) whereas later, dampening of β-catenin activity is required for maturation and polarization of the tissue (Westenskow *et al*, 2009; Hagglund *et al*, 2013; Fujimura *et al*, 2015). Consequentially, disruption of ciliary dynamics in the RPE leads to defects in development and maturation, ultimately affecting tissue homeostasis and function. Since the RPE is intricately connected with the outer photoreceptor segments, any aberrations to ciliary dynamics in the RPE could significantly affect the neuroretinal tissue and visual function.

Primary cilia dynamics in skeletal development

Three different embryonic cell lineages define mammalian skeletal development: (i) paraxial mesoderm cells form somites and, in turn, the axial skeleton, (ii) lateral plate mesoderm cells form the limb skeleton, and (iii) neural crest cells give rise to the craniofacial skeleton (Horton, 2003). Long bone formation occurs via chondral ossification. First, a primordial skeleton forms when mesenchymal cells condensate and differentiate into chondrocytes to form hyaline cartilage. Second, transformation from cartilage to bone occurs through continuous degradation of cartilage by chondroclasts and replacement with bone by osteoblasts. Bone length is increased at the so-called epiphyseal growth plates by chondrocyte proliferation and differentiation into hypertrophic chondrocytes, which change their extracellular matrix deposition for mineralization.

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During *in-vitro* chondrocyte differentiation, cilia length is dynamically regulated with increasing cilia length in further differentiated cells (Upadhyai *et al*, 2020). In contrast, mechanical stress in form of compressive loading reversibly reduces cilia length and ciliation per se (McGlashan *et al*, 2010). Thus, it has been proposed that chondrocyte primary cilia sense peripheral tissue deformation and mechanical properties via extracellular matrix receptors, e.g., integrin alpha2-, −alpha3, and -beta1 as well as the chondroitin sulphate proteoglycan 4 (NG2), which have been localized to the ciliary membrane (McGlashan *et al*, 2006).

Primary cilia-controlled Hh signaling dynamics have been proposed to play a major role in regulating hypertrophic differentiation and proliferation (Ohba, 2020). Further, Hh signaling in the limb buds is crucial for digit patterning, and defects in Hh signaling result in poly(syn)dactyly (Litingtung *et al*, 2002). While other cell signaling pathways, such as WNT and FGF signaling, are important for growth plate patterning, their role in primary cilia and their dynamics during skeletal development is largely unknown.

Several ciliopathy phenotypes share polydactyly as a common hallmark, including the short rib thoracic dysplasia (SRTD), short rib polydactyly syndrome (SRPS) spectrum (all caused by cytoplasmic *dynein-2* or *IFT* mutations), Ellis-van Creveld (EVC) syndrome (*EVC1*/*EVC2* gene mutations), BBS (caused by *BBS* mutations), Meckel-Gruber Syndrome (MKS mutations in 13 different genes), or Oro-Facial-Digital syndrome (OFD, *OFD1* mutation) (Braun & Hildebrandt, 2017). SRTD, SRPS, and EVC are additionally characterized by shortened long bones and ribs, resulting in short stature as well as a narrow thorax causing pulmonary deficits.

The most common genetic cause of SRTD are mutations in the retrograde IFT motor complex *dynein-2*, followed by mutations in the IFT-A complex, while mutations in *IFT-B* genes or in genes encoding for proteins localized at the ciliary base, such as NEK1, are rarely identified (Schmidts, 2014; Zhang *et al*, 2018). Thus, ciliogenesis and the compositional protein dynamics controlled by the IFT machinery seem to play a key role in skeletal development.

In contrast to other ciliopathies, STRD patients usually carry partial loss-of-function (hypomorphic) *IFT-* or *dynein*-2 alleles. In mice, loss of *IFT* or *dynein-2* genes leads to early death during embryonic development (Gorivodsky *et al*, 2009; Ocbina *et al*, 2009, 2011) due to cilia loss or severe cilia shortening (Pazour *et al*, 2000; Ocbina & Anderson, 2008; Jonassen *et al*, 2012; Liem *et al*, 2012). However, these ciliary defects are not observed in patient-derived cells but instead, accumulation of IFT particles at the ciliary tip has been described (Arts *et al*, 2011; Schmidts *et al*, 2013, 2015; Doornbos *et al*, 2021). Regulation of IFT needs to be highly dynamic to enable fast adaptation to the rapidly changing cellular needs, including primary cilium assembly and disassembly, signal transduction upon extracellular stimuli as well as cell signaling receptor activation with fast-changing IFT cargo. This requires a flexible, easily changeable IFT loading and cargo binding system. However, while IFT function is required for cellular differentiation, maintenance, and survival, individual functions of the multiple IFT-complex components and their role during mammalian development have remained largely elusive due to the complete loss of cilia upon knockout of *IFT* or *dynein-2* genes, and relevant patient tissues are not available.

Human SRTD phenotypes differ markedly when comparing patients carrying disease-causing mutations in *IFT-A*, *IFT-B*, or *dynein-2* genes: for example, ectodermal dysplasia features affecting hair or nails are often observed for *IFT-A* but not for *IFT-B* or *dynein-2* mutations, while the childhood-onset retinal or renal disease is common in IFT-patients but not in dynein-2 patients. In contrast, individuals affected by dynein-2 dysfunction commonly present with more severe skeletal features than IFT patients (Schmidts, 2014). However, the underlying molecular mechanisms are not understood. Potentially, qualitative or quantitative differences in Hh signaling during skeletal development could play a role as well as gene-specific effects on other cell signaling pathways regulating chondrocyte differentiation and proliferation such as WNT, BMP, or FGF signaling.

Mechanistically, data from mouse models strongly suggest that Hh signaling defects during embryonic limb bud development underlie polydactyly development (Huangfu *et al*, 2003; Liu *et al*, 2005a; Ruiz-Perez & Goodship, 2009; Yin *et al*, 2009). Furthermore, primary cilia dynamics also control endochondral bone formation (Haycraft *et al*, 2007). Whether other mechanisms that regulate ciliary Hh signaling dynamics also play a role in skeletal development, is not fully understood.

More recently, the interaction between ciliary cAMP and protein kinase A (PKA) signaling has been demonstrated, and heterozygous or mosaic loss of the PKA subunits *PRKACA* or *PRKACB* in humans results in a phenotype overlapping with SRTD, including polydactyly, brachydactyly, and short stature (Abraham *et al*, 2022). However, the effect of human IFT-dynein disease alleles on ciliary cAMP dynamics and PKA signaling has not been studied to date. Besides PKA, also several other protein kinases, which, at least partially, exert their function through primary cilia, play an essential role during mammalian skeletal development and patterning (Abraham *et al*, 2022). Human disease alleles in *NEK1*, *NEK9*, *CILK1*, and *FGFR3* result in skeletal ciliopathy phenotypes, which overlap with IFT-dynein-2 phenotypes (Abraham *et al*, 2022). NEK1 has been shown to regulate cilium disassembly (Al-Jassar *et al*, 2017) and ciliary length, whereas mutations in *Dyrk2* alter ciliary length and cause accumulation of GLI2 and GLI3 at the ciliary tip, leading to loss of Hh target gene expression (Abraham *et al*, 2022).

In summary, while IFT represents the dynamic supply chain and lifeline for cilia in general, disturbances foremost result in severe skeletal developmental defects. While Hh signaling defects have been clearly linked to skeletal developmental disorders in vertebrates, putative contribution of other cell signaling defects have remained elusive. Further, clear genotype–phenotype correlations observed with hypomorphic human disease alleles strongly suggest gene-specific functions for cilia dynamics, regulating developmental cell signaling pathways at the growth plate, and, as a consequence, chondrocyte differentiation and proliferation. Loss of cilia as a consequence of *IFT-* or *dynein-2* biallelic null mutations/knockouts has hampered *in vivo* studies to investigate such gene-specific functions. The generation of hypomorphic human disease alleles *in vitro* and *in vivo* models would be one way to tackle this issue, including gene-specific effects on the regulation of Hh signaling at the growth plate and the putative contribution of other cell signaling pathways.

Ciliary dynamics in pancreas development

Similar to the RPE, the pancreatic tissue also shows dynamic presence of primary cilia in the different cell types. Cilia are known to be present in several cell types of the pancreas, notably in ductal (Boquist, 1968; Aughsteen, 2001), centroacinar, and endocrine cells

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in the adult organ (Munger, 1958; Yamamoto & Kataoka, 1986) (Fig 3F). However, the most numerous cells, the exocrine acinar cells, which synthesize and secrete digestive enzymes, do not harbor cilia (Cano *et al*, 2004). During mouse development, cilia have been reported in the pancreas as early as E12.5, and can be detected at all developmental stages on the pancreas progenitors. While their endocrine and ductal daughter cells are ciliated, their acinar daughters are not (Cano *et al*, 2004), leaving unclear whether this is caused by a primary cilia loss or different populations of progenitors. Moreover, how cilia numbers, length, and orientation changes over time is not established yet. Cilia control exocrine ductal cell development and homeostasis as well as endocrine cell function. Loss of cilia dynamics in mouse mutants that exhibit either shortened cilia, such as *Orpkd/Tg737/Ift88* knockout (KO) mice, or even complete cilia loss, such as *Kif3a* KO mice, exhibit enlarged ducts by the end of development at E18.5, and develop large cysts by P4 and subsequently acinar cell resorption. These acinar cells are replaced by fat tissue in the *Kif3a* KO, and periductal fibrosis is observed (Cano *et al*, 2004, 2006). The defect in progenitors and ductal cells, which harbor cilia, appears to be the primary defect, whereas the acinar cell defects are a secondary consequence (Cano *et al*, 2004, 2006). *Pkd2* knock-out mice, causing persistent primary cilia signaling (Gerakopoulos *et al*, 2020), develop pancreatic cysts as early as E14.5, indicating that ciliary signaling is already present in this developmental stage. Similarly, mice that lack *Hnf6*, a gene necessary to form cilia during development, display dilated ducts at E14.5 (Pierreux *et al*, 2006). Thus, the dynamics of when and where cilia are formed during embryonic development seem to be important for pancreas development.

Understanding the signaling function of primary cilia in the pancreas has proven to be difficult and so far, the prevalent hypothesis is that cilia sense flow (Fig 3F), although the dynamic response of pancreatic cilia to flow is not yet documented. In addition, chemical sensing, i.e., of Shh, has been detected (Nielsen *et al*, 2008).

Pancreatic defects have been reported in a subset of ciliopathies, whose understanding might help to shed light on how primary cilia and their compositional and signaling dynamics control pancreatic tissue organization and function (Braun & Hildebrandt, 2017; Reiter & Leroux, 2017; Srivastava *et al*, 2017; Luo & Tao, 2018). Pancreatic cysts and/or fibrosis have been reported upon mutation Nephronophtisis-causing genes that mainly encode proteins associated with the base of the cilium or IFT (Bergmann *et al*, 2008; Frank *et al*, 2013; Halbritter *et al*, 2013; Moalem *et al*, 2013; Grampa *et al*, 2016). Pancreatic fibrosis has also been observed in other ciliopathies, including the Jeune asphyxiating thoracic dystrophy, the oral-facial-digital syndrome type 1 (OFD1), and renal-hepatic-pancreatic dysplasia (Bernstein *et al*, 1987; Yerian *et al*, 2003; Chetty-John *et al*, 2010).

Diabetes is detected in syndromic ciliopathy patients with obesity, such as Bardet-Biedl or Alström syndrome (Green *et al*, 1989; Girard & Petrovsky, 2011). The reported cause of diabetes appears to be either dysfunction of beta cells, which we elaborate upon below, or insulin sensitivity defects in their target tissues. Patients with Alström syndrome carry mutations in the *ALMS1*, which encodes for ALMS1, a protein that is localized to the ciliary base and centrosomes and is highly expressed in pancreatic islets (Hearn *et al*, 2005). Accordingly, *Alms1* knock-out mice show degranulation of *β*-cells and islet cysts (Collin *et al*, 2005; Arsov *et al*, 2006). In contrast, at least in some BBS knock-out mouse models, insulin sensitivity was not impaired and they displayed normal glucose tolerance (Marion *et al*, 2012). Postnatal depletion of cilia in *Ift88* knock-out mice as well as global loss of *Bbs4* in mice progressively reduce insulin secretion (Gerdes *et al*, 2014; Volta *et al*, 2021). The underlying mechanisms are, however, still under investigation, but may include calcium signaling via GABA (Sanchez *et al*, 2023) as well as EphA3 phosphorylation (Volta *et al*, 2021). A recent study suggested that β-cell cilia oscillate and that this motion promotes insulin secretion, a surprising finding that will be worth follow-up studies (Cho *et al*, 2022). Cilia on endothelial cells are also necessary for the high-density vascularization of islets (Xiong *et al*, 2020).

In summary, primary cilia dynamics are important during pancreatic development. However, the molecular mechanisms underlying primary cilia function in the pancreas need to be revealed in the future.

Ciliary dynamics in kidney epithelium remodeling

All epithelial cells lining the renal tubules throughout the nephron carry a primary cilium on their apical surface, except for the small population of intercalated cells of the collecting duct. These cilia project into the lumen of the tubule and are in direct contact with the bypassing urine (Ma *et al*, 2017). Yet, their function during kidney development and in renal physiology is not well understood. The kidneys are among the most severely affected organs in most ciliopathy patients. Among the renal ciliopathies, autosomal-dominant polycystic kidney disease (ADPKD) is one of the most common monogenic diseases (Ma *et al*, 2017) and several rare autosomal-recessive ciliopathies (e.g., autosomal-recessive polycystic kidney disease (ARPKD) or Nephronophthisis (NPH)) are renal diseases (Hildebrandt *et al*, 2011). These renal ciliopathies typically manifest as cystic kidney disease of two significant types: ADPKD/ARPKD is characterized by massively enlarged, highly proliferative polycystic kidneys with cyst development throughout the entire organ, whereas NPH or NPH-related ciliopathies display degenerative, cystofibrotic kidneys with fewer cysts primarily limited to the cortico-medullary border. Remarkably, the kidney phenotype of PKD mouse models is ameliorated when genetically interfering with ciliogenesis (Ma *et al*, 2013; Shao *et al*, 2020a), suggesting a cilia-dependent, cyst-promoting signaling network. Consistently, delayed deciliation has been found in cystic epithelium of *Pkd1*-deficient mice (Gerakopoulos *et al*, 2020). While the impact of cilia in (poly) cystic kidney diseases is well established, the cilia-dependent remodeling processes under physiological and pathological conditions are still somewhat elusive, and it is attractive to speculate on the role of ciliary dynamics in disease pathogenesis. In recent years, it has become clear that upon kidney injury, massive proliferation occurs in the kidney to repair tubular damage (Humphreys *et al*, 2011). This is not primarily driven by progenitor cells. Instead, it has been demonstrated for the proximal tubule that repair is achieved by fully differentiated proximal tubular epithelial cells (Kusaba *et al*, 2014). Here, the dynamic process of ciliary disassembly is key, as epithelial cells need to disassemble the cilium to re-enter the cell cycle before proliferation (Kim & Tsiokas, 2011). Thus, classical ciliopathy mutations, some of which have only very subtle, minor effects on ciliary structure and function, could alter disassembly and result in inefficient repair. This hypothesis is in line with the third-hit theory in ADPKD, which predicts that – beyond

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loss-of-heterozygosity – external damaging factors, such as ischemia–reperfusion or crystal formation, drive disease progression. The continuous deterioration could be aggravated by altered susceptibility to cell death and subsequent inflammation and fibrosis (Kieckhöfer *et al*, 2022).

In summary, the requirement for timely ciliary disassembly during efficient renal repair might explain the renal phenotype developing over the years from a normal, unaffected kidney towards (poly) cystic kidney disease. Of course, it is likely that these mechanisms will also include deregulated ciliary signaling dynamics as well as alterations of the ciliary composition. Therefore, studies on ciliary dynamics will be instrumental in understanding renal ciliopathies.

Conclusion and open questions

This review summarizes the dynamic properties of primary cilia, moving away from the perception of the primary cilium as a static organelle, and towards the view that primary cilia dynamics are a key factor in regulating cell fate and tissue organization. General concepts are starting to emerge to understand primary cilia dynamics, but many open questions remain. Here, we list a few:

- What are the dynamic changes in the ciliary protein content during self-renewal, differentiation, and tissue organization?

Most studies have focused on cultured, immortalized, or terminally differentiated cells to study the ciliary protein composition. As a result, we have yet to learn about dynamic changes in intraciliary proteins in self-renewing stem cells that give rise to different cell types during tissue organization. A comparative study between a stem cell and its progeny will reveal how the ciliary protein composition and its associated signaling determine cell fate. Fortuitously, as we have described in this comprehensive review, necessary tools and technologies such as spatial proteomics as well as high-resolution and long-term imaging are in place to resolve these questions.

- How are different mechanical and chemical stimuli integrated through cilia to regulate cell fate and function, tissue organization, and consequently physiological function?

Understanding how mechanical and chemical stimuli (in time, space, and concentration) modulate ciliary functions in specific cell types to promote cell–cell communication during tissue organization and function is a challenge. However, recently emerged reductionist approaches, such as organoid cultures combined with genome editing and optogenetic tools, are in place to address these questions and transfer this knowledge *in vivo* to whole organisms.

- How is cilium disassembly coordinated *in vivo*? Does delayed cilium disassembly affect signaling competency, cell cycle progression, and cell differentiation?

Primary cilia disassembly as a cilia checkpoint for cell cycle progression is an exciting concept as it adds another checkpoint to the cell cycle. Why cells assemble and disassemble primary cilia at a particular time and space remains not fully understood. As the cilium receives extracellular signals and transduces them into the cell, the duration of signal perception has been proposed to depend on the presence of the cilium. Thus, primary cilia integrate and transduce growth factor signaling input. One hypothesis would be that cells assemble cilia in order to search and receive sufficient and specific signals before they divide.

Furthermore, the receptors and signaling components need to be removed in a timely manner when the cilium is disassembled. How this process is carried out on a molecular level is not well understood. One can hypothesize that this dynamic process is affected when cilium disassembly is delayed, whereby the fate and function of a cell and, thereby, the whole tissue could be altered.

Altogether, primary cilia dynamics are fundamental for tissue development and function. Analyzing all levels of cilia dynamics, from the compositional and signaling to the cilia assembly/disassembly dynamics in various cell and tissue types, and integrating them will allow to pinpoint general concepts of how cilia dynamics determine life and death (of an organism). With the advent of cutting-edge technologies, the coming years will uncover novel concepts in cilia biology and set the stage for translational cilia biology to diagnose and interrogate human diseases.

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The authors declare that they have no conflict of interest.

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