J Biosci (2023)48:8
DOI: 10.1007/s12038-023-00326-6

Review

Appearing and disappearing acts of cilia

Shashank Arora†, Mausam Rana†, Ananya Sachdev and Jacinta S D'Souza*

School of Biological Sciences, UM-DAE Centre for Excellence in Basic Sciences, Kalina Campus, Santacruz (E), Mumbai 400098, India

**Corresponding author (Email, jacinta@cbs.ac.in)*

†Shashank Arora and Mausam Rana contributed equally to this work.

MS received 20 July 2022; accepted 14 November 2022

The past few decades have seen a rise in research on vertebrate cilia and ciliopathy, with interesting collaborations between basic and clinical scientists. This work includes studies on ciliary architecture, composition, evolution, and organelle generation and its biological role. The human body has cells that harbour any of the following four types of cilia: 9+0 motile, 9+0 immotile, 9+2 motile, and 9+2 immotile. Depending on the type, cilia play an important role in cell/fluid movement, mating, sensory perception, and development. Defects in cilia are associated with a wide range of human diseases afflicting the brain, heart, kidneys, respiratory tract, and reproductive system. These are commonly known as ciliopathies and affect millions of people worldwide. Due to their complex genetic etiology, diagnosis and therapy have remained elusive. Although model organisms like *Chlamydomonas reinhardtii* have been a useful source for ciliary research, reports of a fascinating and rewarding translation of this research into mammalian systems, especially humans, are seen. The current review peeks into one of the complex features of this organelle, namely its birth, the common denominators across the formation of both 9+0 and 9+2 ciliary types, the molecules involved in ciliogenesis, and the steps that go towards regulating their assembly and disassembly.

Keywords. Assembly; basal body; centrioles; cilia; ciliogenesis; disassembly

Abbreviations: ACE2, angiotensin converting enzyme 2; AHI1, Abelson-helper integration site 1; ALI, air–liquid interface; BBS, Bardet–Biedl syndrome; BCC, basal cell carcinoma; C2CD3, C2 calcium-dependent domain containing 3; CaM, calmodulin; CC2D2A, coiled-coil and C2 domain-containing protein 2A; CDPKs, calcium-dependent protein kinases; CEP, centrosomal proteins; CH, calponin homology; CLS, ciliary localization signal; CSF, cerebrospinal fluid; CTSs, ciliary targeting signals; CV, ciliary vesicle; DA, distal appendages; DAVs, distal appendage vesicles; EHD, Eps15 homology domain; ER, endoplasmic reticulum; GEF, guanine exchange factor; GPCRs, G protein coupled receptors; HDAC2/6, histone deacetylases 2/6; HEF1, human enhancer of filamentation 1; IFT, intraflagellar transport; KAP3, kinesin-associated protein; KIFC1, kinesin family member C1; LRO, left–right organizer; LRRK2, leucine-rich repeat kinase2; MACF1A, microtubule actin crosslinking factor 1; MAPK15, mitogen-activated protein kinase 15; MCCs, multiciliated cells; MKS, Meckel syndrome; MTOC, microtubule-organizing centre; NEURL4, neuralized-like protein 4; NLS, nuclear localization signal; OFD1, orofaciodigital syndrome 1; PCM, pericentriolar material; PCVs, preciliary vesicles; PDGFRβ, platelet derived growth factor receptor β; Pifo, pitchfork; PLCγ, phospholipase C-gamma; PLD, phospholipase D; PLK1, polo-like kinase; RILPL1, Rab-interacting lysosomal protein-like protein 1; RSPs, radial spoke proteins; SDA, subdistal appendages; SHH, Sonic Hedgehog; TCHP, Trichoplein; TESK1, testicular protein kinase 1; TPR, tetratricopeptide repeats; TZ, transition zone; XAP5, X-chromosome associated protein 5.

http://www.ias.ac.in/jbiosci
Published online: 11 March 2023

1. Cilia: An introduction

1.1 *Cilium as an organelle*

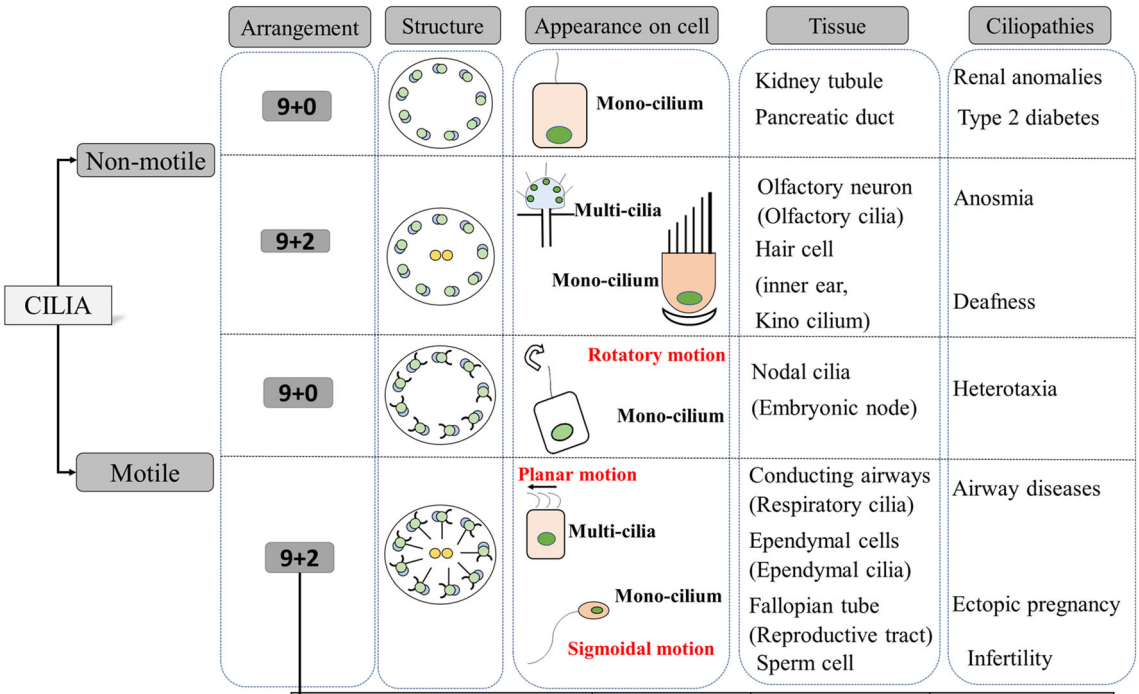
Cilia are thin, hair-like cell membrane extensions with a microtubule-based core (axoneme), specialized in structure and functions such as sensory perception and motility. Flagella, whip-like projections of the cell but longer than cilia, were considered different organelles until their internal structure was found to be identical to cilia. Ciliary/flagellar motility is the movement of cilia/flagella such that cells that harbour them can swim from one point to another or can 'sweep' fluid/particles around them. This motility is driven by axonemal dyneins situated on the tubulin-based structure. Intraciliary/intraflagellar motility is the bidirectional transport of particles along axonemal microtubules. Recently, the prevalence, utility, and importance of the cilium are being thoroughly investigated, thereby unravelling its genesis, architecture, composition as well as biological functions. Found widely in species ranging from protozoa to mammals (figure 1; Mitchison and Valente 2017), cilia display significantly varying extents of motility (figure 1). In multicellular organisms, cilia give rise to a diverse spectrum of functionality, specifically those including different tissue types and organ systems. Aberration in ciliary proteins results in a dysfunctional organelle, thereby leading to a series of ailments collectively known as ciliopathies that afflict the brain, heart, kidneys, respiratory tract, reproductive system, etc. Studies on ciliary composition and structure encompassed the use of both simple organisms such as *Tetrahymena*, *Drosophila melanogaster*, *Caenorhabditis elegans*, *Chlamydomonas reinhardtii*, and sperm of multicellular organisms such as rat and sea urchin (Afzelius 1959; Gibbons 1961). The use of complex model organisms, like *Xenopus* and zebrafish, has highlighted the subtle differences in the conserved structures of this organelle.

1.2 *Ciliary structure*

All cilia are covered by the plasma membrane (ciliary membrane) and have an axoneme at their core that extends from the centriole-derived basal bodies (figure 2). The arrangement of the microtubules and associated proteins is quite heterogeneous throughout the axoneme (Mohri *et al*. 2012). These differences attribute stability yet flexibility to the organelle. Out of the three (A, B, and C) tubules present in a centriole (discussed below), the C-tubule terminates within a structure known as the transition zone (TZ) that lies between the basal body and the axonemal shaft. TZ has Y-shaped linkers between the ciliary membrane and the microtubules, and a structure called the ciliary necklace, one that is conspicuously absent from the sperm flagellum (Reiter *et al*. 2012; reviewed by Gonçalves and Pelletier 2017). It seems to be the selective gate preventing the free entry of soluble proteins (Takao and Verhey 2016). Using a combination of cryo-electron tomography and expansion microscopy techniques, Hoek *et al*. (2022) have shown the molecular architecture of the ciliary base in the green alga *Chlamydomonas reinhardtii*. Using cryo-ET and subtomogram averaging, the group has demonstrated that membrane binding is not a prerequisite for Y-link formation. Distal to the axonemal structure, a previously unseen helical 'sleeve' was observed decorating the microtubules, which the authors predict might designate sites of axoneme severing.

Each of the A-tubules in the outer doublet is connected to nine lollipop-like structures called the radial spokes that project toward a structure called the central pair of singlet microtubule apparatus and its proteinaceous projections, which together are believed to regulate ciliary beating (figure 2; Satir *et al*. 2014; Teves *et al*. 2016). An excellent cryo-EM structure of the active central pair has recently been solved (Han *et al*. 2022). Cilia are classified based on the presence of this central pair (designated 9 + 2) and its absence (designated 9 + 0). Both 9+2- and 9+0-containing cilia are either motile or immotile, depending on the structural differences present in the axoneme (Satir and Christensen 2007). Extensive work on this ciliary architecture has revealed that the arrangement of the sub-ciliary structures like central pair, radial spokes, and dynein arms may be responsible for the observed differences among the types of motilities (figures 1 and 2; Teves *et al*. 2016; Kempeneers and Chilvers 2018; Kempeneers *et al*. 2019). This makes it important to characterize cilia in several organisms so that the scope of cross-species differences is evident.

Based on the function and axonemal format, the human body can generate four types of cilia: 9+0 motile, 9+0 non-motile, 9+2 motile, and 9+2 non-motile (figure 1). Of the four axonemal formats, the 9+2 motile cilia in vertebrates are present in the respiratory airways, the reproductive system, and the ependymal cells, whose ciliary motility renders fluidic movement (figure 1). Also known as primary cilia, 9+0 non-motile cilia are usually solitary and are



Lower eukaryotes	Cilia/Flagella number	Movement type	Function
Euglena gracilis	one	Corkscrew (euglenoid)	Motility
Chlamydomonas reinhardtii	two	Breaststroke-like	Motility, mating
Paramecium caudatum	multiple	Whiplash	Motility, mating, ingestion

Figure 1. Types of cilia in unicellular and multicellular organisms, their respective tissue types, and their functions. Note the axonemal structures for each type and the ciliary diseases that the respective cilia types cause when proteins that make these organelles are mutated leading to dysfunctional cilia.

deprived of the dynein arms, radial spokes, and central pair. These cilia are present on most differentiated cells in humans relying on the solitary cilium to monitor the environment. Recent studies on the sensory role of primary cilia have revealed their importance in development and tissue homeostasis. It has been found that some tissue-specific cell types also harbour 9+2 non-motile cilia lacking dynein arms; for example, olfactory neurons with multiple long chemoreceptive cilia, the vestibular and cochlear mechano-sensory cilia present in the inner ear of the embryo (figure 1; Satir and Christensen 2007; Kempeneers and Chilvers 2018; Kempeneers *et al*. 2019). Primary cilia present on sensory cells of mammalian olfactory and vision systems transduce extracellular signals that are chemical, physical (smell and visual), or electrical in nature (Falk *et al*. 2015). In particular, the specialized kinocilia present in the auditory hair cells of the inner ear use their sensory feature for responding to sound. Unlike non-motile cilia, the 9+2 motile cilia are usually (although not always) present as clusters (figure 1). The 9+0 motile nodal monocilia found at the embryonic node are solitary and show unique rotatory motion. They function in determining the left/right (L/R) body symmetry during early development by generating the leftward fluid flow (nodal flow) followed by the differential patterning of lateral plate mesoderm and then asymmetric organogenesis (McGrath *et al*. 2003;

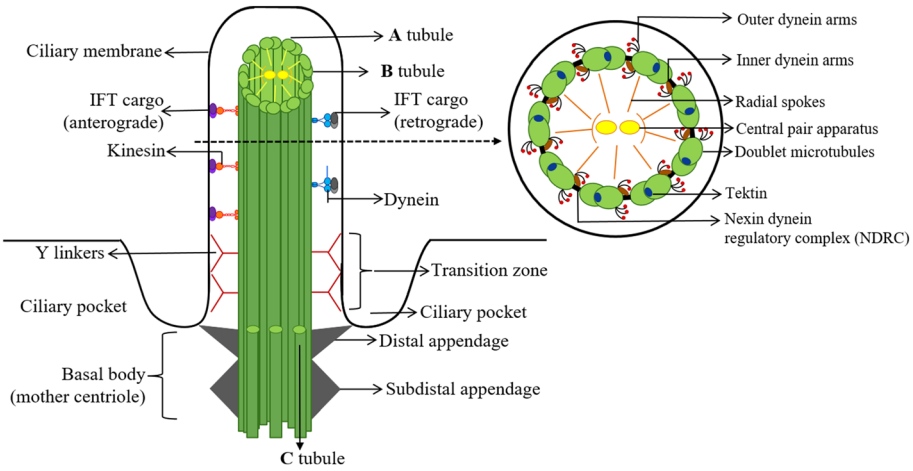


Figure 2. Overall structure of the 9+2 cilium and its cross-section. The cilium is made up of the axoneme that is formed by nine microtubule doublets surrounded by the ciliary membrane. The other substructures (see the adjacent illustration of the cross-section) include the central pair, radial spokes, and nexin–dynein regulatory complex, typical of the 9+2 axoneme. Kinesin present along the axoneme moves the IFT complex and its cargo towards the ciliary tip (anterograde transport), whereas dynein moves the IFT complex and its cargo towards the cell body (retrograde transport). The transition zone present at the base of the cilium provides selective entry of proteins into the axoneme. Y-shaped linkers are occupied in the transition zone and connect the axoneme to the base of the ciliary membrane. The cilium is anchored on to the basal body (in gray). The ciliary pocket programs ciliary-associated vesicular trafficking. Note the A, B (axonemal doublets), and C (centriolar) microtubules.

Tabin and Vogan 2003). The clockwise movement of this cilium present at the centre of the ventral node is also known as the left–right organizer (LRO). It acts to break the L/R symmetry in organisms such as zebrafish, *Xenopus*, and mouse (Nonaka *et al*. 1998; Essner *et al*. 2019; Schweickert *et al*. 2007). This cilium is different from the airway cilia that show planar beating. But, it is the 9+0 immotile sensory cilium present at the periphery of the ventral node that can sense the flow-dependent signal coming from calcium (Pennekamp *et al*. 2002; Field *et al*. 2011; Yoshiba *et al*. 2012). FOXJ1, a master regulator of the multiciliogenesis program (Yu *et al*. 2008), and NOTO, a homeodomain transcription factor expressed in the ventral node of the mouse embryo, play a crucial role in determining L/R patterning (Abdelkhalek *et al*. 2004; Didon *et al*. 2013). Microarray analysis of the NOTO targets identified about 716 candidate genes of which 59 overlap with lung candidate genes (Stauber *et al*. 2017). Mutational and rescue studies in mouse embryos showed that both function together in the localization of cilia at the posterior pole. NOTO acts upstream of FOXJ1, and mutation in FOXJ1 causes randomization of left/right body asymmetry (Beckers *et al*. 2007; Alten *et al*. 2012; Wallmeier *et al*. 2019). However, studies in urochordate and sea urchin embryos show the involvement of the 9+2 motile cilia in establishing L/R patterning (Nishide *et al*. 2012; Tisler *et al*. 2016). Further, the molecular mechanism of motility is central to understanding physiological processes carried out by the respiratory airways for clearing mucus, fertilization rendered by sperm, movement of the ovum in the fallopian tubes, and the directional flow of cerebrospinal fluid (CSF) generated by the ciliated ependymal cells (Banizs *et al*. 2005). In contrast, the 9+2 motile sperm flagellum and other multiciliated epithelial cells exhibit sigmoidal, i.e. symmetric waveform and planar waveform, respectively (figures 1 and 2; Hirokawa *et al*. 2006). Taken together, a detailed understanding of cilia will provide the foundation in determining the cause and effect of ciliopathies.

1.3 *Ciliary base*

The ciliary base comprises two major components: the basal body, which nucleates axonemal microtubules, and the periciliary membrane, which serves as a diffusion barrier and separates the ciliary membrane from the plasma membrane. Formation of the basal body and the ciliary membrane are the two key steps towards the initiation of ciliogenesis. The ciliary base is also an important site for sorting the cargo destined for the ciliary compartment. All proteins required for ciliary growth are first transported to the ciliary base, sorted

further, and then sent to different destinations of the axoneme (Sorokin 1962; Rieder *et al*. 1979).

2. Ciliogenesis: An introduction

The birth of a cilium is known as ciliogenesis. Even though most information on ciliogenesis was acquired using vast studies on *Chlamydomonas* flagella, the focus has recently shifted to mammalian models such as mice and zebrafish (Bangs and Anderson 2017; Fu *et al*. 2019; Haider *et al*. 2019). Most of this information comes from the identification of genes whose mutations have resulted in the disruption of ciliary functions including birth and assembly; some of these have been associated with human ciliopathies (Waters and Beales 2011). Genetic screens generated in *Chlamydomonas*, zebrafish, and *Drosophila* have helped enormously in identifying molecules whose gene mutations result in the disruption of the major steps of ciliogenesis (Satir and Christensen 2007). The primary cilium, being solitary, only needs a single basal body as the nucleation site, as opposed to multicilia, where the centriole needs to multiply several times to generate enough basal bodies. Studies are being conducted using primary cell cultures of ependymal brain cells, respiratory airway epithelial cells, and fallopian tube epithelial cells, right from tracing the events during the vigorous multiplication of centrioles to the formation of mature tufts of cilia. Although the complete molecular mechanism remains elusive, extensive efforts are ongoing to deduce the molecular players responsible for this transition. One important term is ciliary compartmentalization, which suggests an important role for sub-ciliary regions in regulating the structure and/or function of cilia. For the convenience of studying ciliogenesis, cilia could be viewed as consisting of three main sub-compartments: ciliary base, transition zone (TZ), and distal ciliary compartment (ciliary tip). Ciliogenesis and ciliary compartmentalization happen simultaneously. Taken together, the entire process involves the synthesis of precursors that contribute towards the making of the centriole, transportation and axoneme elongation, and a regulatory network. It is emphasized that the regulation of ciliary growth dynamics forms an important aspect of this process and is required for accurate cilia formation. Right from the development of progenitor cells and their differentiation into fully mature cilia, various pathways intersect to give rise to the developed multiciliated tissue. The following is a detailed description of the steps in the ciliogenesis process of 9+2 and 9+0 cilia.

3. Process of ciliogenesis

3.1 *Centriole structure*

Cilia are derived from centrioles, structures that are evolutionarily conserved and play a role in organizing microtubules and generating spindle fibres during cell division. Understandably, therefore, ciliogenesis and cell division are coupled events. A centriole is a barrel-shaped microtubular organelle composed of α- and β-tubulin heterodimers, ∼150 different proteins with dimensions of about 400–500 nm length and 250 nm diameter (Andersen *et al*. 2003; Keller *et al*. 2005; Winey and O'Toole 2014). Each barrel is a cylindrical structure consisting of nine sets of microtubular triplets that display the 9-fold radial (cartwheel) symmetry (figure 3; Marshall and Rosenbaum 1999). The two centrioles (mother and daughter) are present at a perpendicular position to each other with a surrounding pericentriolar material (PCM), which is a fibrous meshwork and can bind to several proteins (figure 3; Schnackenberg and Palazzo 1999) – all this together forms the centrosome. The proximal and distal ends of each centriole perform distinct roles, the former is responsible for PCM recruitment (Woodruff *et al*. 2014), whereas the latter functions in the accurate

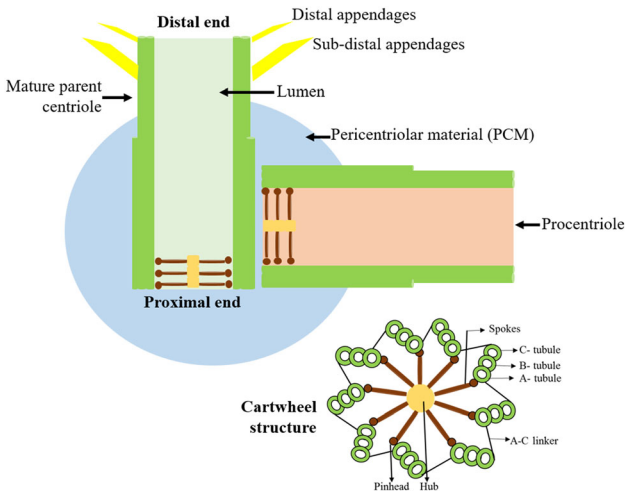


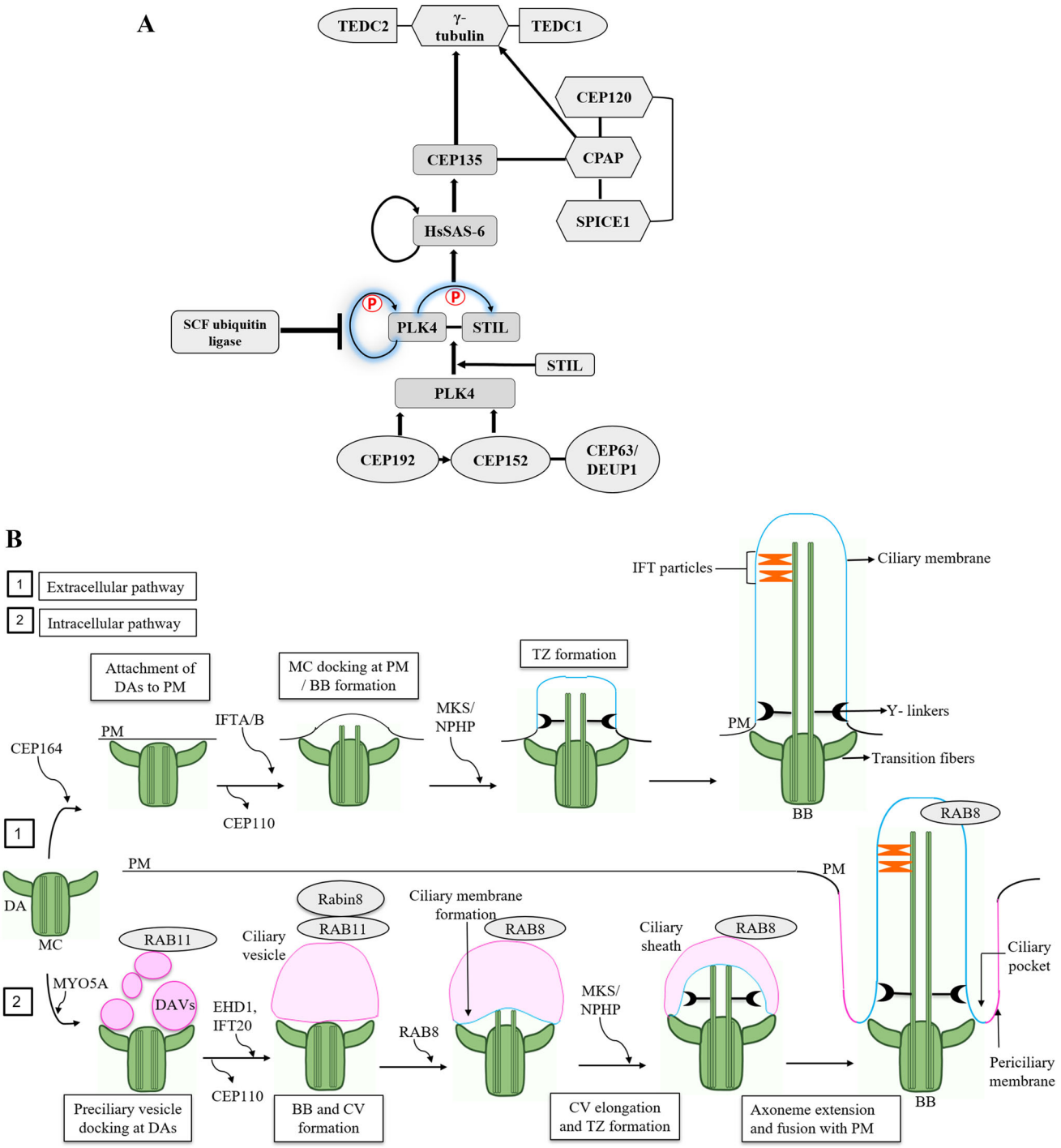
Figure 3. The structure of a centriole. Centrioles are the paired barrel-shaped structures. The procentriole is arranged at the proximal end of the mature parent centriole. At the distal end of the mature parent centriole are attached the distal and subdistal appendages. The A, B, and C microtubules are shaped like a cartwheel.

docking of centrioles (figure 3; Tanos *et al*. 2013). Docking accurately positions both the centrioles in its cellular *niche*. While the mother centriole consists of distal and subdistal appendages (DA and SDA, respectively), the daughter centriole does not have any such structures (Paintrand *et al*. 1992). The PCM also acts as the centrosomal microtubule-organizing centre (MTOC). It can nucleate the microtubular array formed during the interphase of cell division and the spindle microtubules during mitosis (Euteneuer and McIntosh 1981). Electron microscopy studies have revealed the presence of small aggregates of electron-dense non-membrane-bound proteinaceous particles around the centrosome termed as centriolar satellites in some vertebrates (Lopes *et al*. 2011). These help in the recruitment of PCM material and proteins required for ciliogenesis and are especially known for the presence of the scaffolding protein, PCM1. The absence of PCM1 abrogates ciliogenesis in some cells, indicating an important role in cilium assembly (Lopes *et al*. 2011; Kodani *et al*. 2015).

3.2 *Centriole production (centriole genesis, or centriologenesis) and duplication (copy number)*

Cell division requires centriole duplication that begins during the G1–S phase transition in cycling cells. This duplication is marked by the appearance of a new procentriole (a short daughter centriole) at the proximal end of each existing centriole in an orthogonal orientation. Polo-like kinase (PLK4) is known as the earliest marker that depicts the site for procentriole emergence (figure 4A; Bettencourt-Dias *et al*. 2005; Habedanck *et al*. 2005; Eckerdt *et al*. 2011). The recruitment of PLK4 is mediated by CEP152 and CEP192, which co-operate in promoting this process (Kim *et al*. 2013). During the G1–S phase transition, PLK4 further interacts with SCL/TAL1 interrupting locus (STIL, where SCL is stem cell leukaemia and TAL is T-cell acute lymphoblastic leukaemia), which initiates trans-autophosphorylation and activation of PLK4. This active PLK4 phosphorylates STIL which in turn triggers the recruitment of spindle assembly abnormal-6 (SAS-6; Arquint *et al*. 2015), leading to the formation of the cartwheel structure serving as a building block for the procentriole (Dzhindzhev *et al*. 2014). Another protein centrosomal P4.1-associated protein (CPAP), with its interacting partners CEP135, CEP120, and spindle and centriole-associated protein 1 (SPICE1), further elongates the procentriole by forming microtubules (figure 4A; Tang *et al*. 2009). The procentriole elongates during the S and G2 phases, finally detaching from the mother centriole in the late G2 phase. After mitosis, each cell inherits a pair consisting of the mother centriole and a newly duplicated daughter centriole. At this stage, the mother centriole acquires its unique fibrous DAs and SDAs. This basal body finally serves as a nucleation centre for a cilium.

The spatial, numerical, and temporal regulation of this process serves importantly to ensure copy number control where one mother centriole can only give rise to a single new procentriole. Studies in human somatic cells have shown that experimental depletion of existing centrioles triggers *de novo* centriole genesis (Uetake *et al*. 2007). An example of *de novo* centriole production is seen in terminally differentiated multiciliated cells (MCCs). These have completely different molecular dynamics as compared with monociliated cells. Being multiciliated, and to ensure multiciliogenesis, MCCs require several centrioles to be docked at the plasma membrane after exiting the cell cycle (at a point where normal cells suppress centriole duplication). It was in 1971 that Anderson and Brenner distinguished two pathways for centriologenesis in MCCs, namely, centriolar and acentriolar (major pathway) (figure 4B; Anderson and Brenner 1971). In the centriolar pathway, an existing centriole acts as a scaffold to produce multiple new procentrioles around it. The origin of the fundamental centriole that produces these new procentrioles is yet to be elucidated. In the acentriolar pathway, clusters of dense granules (intermediary structures) that may or may not be associated with the existing centriole spontaneously arise in the cytoplasm. New procentrioles are produced around these rather than the existing centriole. These dense granules further condense and give rise to novel structures called deuterosomes triggered by the expression of the Deup1 protein. Deup1 is a paralog (37% identical) of CEP63 (a human microcephaly protein) that regulates mother centriole-dependent centriole duplication (Zhao *et al*. 2013). Another protein, multicilin (encoded by the gene *Mcidas*), is largely expressed during multiciliated cell differentiation and positively regulates the expression of *Deup1*, *Plk4*, *Cep152*, *Stil*, and *Sas-6* (figure 4A; Kim *et al*. 2018). High expression of *Deup1* initiates deuterosome formation, thus making a scaffold for supporting multiple *de novo* centriole duplication. Deup1 binds to the CEP152 protein which in turn activates PLK4 resulting in the initiation of the process of centriolar assembly. These centrioles further mature to form basal bodies. Most of the key regulators (*Plk4, Cep152, Stil,* and *Sas-6*) are similar in both centriolar and acentriolar duplication pathways (figure 4B).


A
TEDC2
γ-tubulin
TEDC1
CEP120
CEP135
CPAP
SPICE1
HsSAS-6
SCF ubiquitin ligase
PLK4
STIL
STIL
PLK4
CEP192
CEP152
CEP63/ DEUP1
B
1 Extracellular pathway
2 Intracellular pathway
Attachment of DAs to PM
MC docking at PM / BB formation
TZ formation
IFT particles
Ciliary membrane
PM
IFTA/B
MKS/ NPHP
Y- linkers
Transition fibers
CEP164
CEP110
BB
1
PM
PM
RAB8
DA
MC
Rabin8
RAB11
Ciliary membrane formation
RAB8
Ciliary sheath
RAB8
Ciliary pocket
RAB11
Ciliary vesicle
MYO5A
DAVs
EHD1, IFT20
RAB8
MKS/ NPHP
Periciliary membrane
2
CEP110
BB
Preciliary vesicle docking at DAs
BB and CV formation
CV elongation and TZ formation
Axoneme extension and fusion with PM

◀**Figure 4.** (**A**) Molecules important for centriole duplication and assembly in humans. The flow diagram shows the molecular pathway responsible for centriole production. The solid lines represent a physical association between the molecules and the arrow heads indicate the recruitment/regulation of the next molecule in the pathway. The pathways can be separated in three major parts. First, the PLK4-STIL association is required for the initiation of centriolar duplication. CEP192/CEP152/CEP63 is required for the recruitment of PLK4 to the parent centriole. This association results in PLK4 activation, which further phosphorylates STIL and itself, as represented by blue arrows. Second, the association with HsSAS-6 is required for the initiation of cartwheel assembly in the centriole. The black arrow indicates self-polymerization of HsSAS-6 after its activation. The third step includes the recruitment of CEP135 which further recruits a set of molecules required for centriole elongation. CPAP, in collaboration with CEP135, CEP120, and SPICE1, facilitates the formation of the procentriole's microtubule wall. γ-tubulin helps in the microtubule stabilization. (**B**) Pathways for centriole migration and docking at plasma membrane (formation of basal body and cilium assembly). The mature mother centriole serves as a precursor for the basal body formation and cilium assembly. There are two main pathways known in mammals for centriole migration and/or docking at the plasma membrane: (1) In the extracellular pathway (top), the parent centriole migrates towards the plasma membrane and the axonemal elongation starts only after it has docked at the plasma membrane on the cell surface. Loss of CP110 from distal end of the parent centriole marks the initiation of cilium assembly. At similar time points, IFT particles are also recruited to the centriole for initiating the formation of the TZ. The TZ specific complexes are then localized to TZ. (2) The intracellular pathway (bottom) starts with the recruitment of pre-ciliary vesicles to the distal appendages of the mature centriole with the help of MYO5A. These are called distal appendage vesicles (DAVs), and they contain Rab-11 GTPase associated with Rabin8 that helps in the recruitment of Rab8. Protein EHD1 helps in fusion of these DAVs to form the ciliary vesicle (CV), and Rab8 is responsible for the growth of CV. The loss of CP110 happens at the same time marking the initiation of TZ formation. This results in maturation of ciliary vesicles forming the distinct domains of ciliary sheath and ciliary membrane. Axonemal extension takes place inside the ciliary sheath until the fusion of ciliary sheath with plasma membrane exposes the axoneme to the external environment.

Another example where the copy number of centrioles deviates is seen in the sexual reproduction of mammals wherein the oocytes lose the centrioles and the centrosome of the zygote comes entirely from sperm cells (Pimenta-Marques *et al*. 2016) (table 1).

3.3 *Centrioles in flagella* (Chlamydomonas reinhardtii)

Centrioles in *Chlamydomonas* play the role of basal bodies after attachment of DAs and ciliary vesicles docking to it. Here too, the basal bodies serve as the main nucleation centre for flagellar assembly. The 9+2 flagella are anchored onto the basal bodies and are formed in three cycles of semi-conservative division. Out of the three generations of basal bodies present during the interphase, a flagellum is extended from the first- (mother) and the second-generation (daughter) basal body, while that of the third generation – the pro-basal body – is associated with each of the two at the proximal end. The two pairs of basal bodies duplicate during the late prophase/early metaphase that follows and form spindle poles (Wingfield *et al*. 2018). A class of mutants in *C. reinhardtii* called uniflagellate have a mutation in the *uni3* gene that encodes for δ-tubulin (makes the C-tubules). These cells preferentially assemble the flagella on the mother basal body; this means that more than one cycle of division is required for a basal body to mature for flagellar transformation (Beech *et al*. 1991). Another mutant called bld2 shows no presence of flagella. The gene *BLD2* codes for ε-tubulin, which is required for forming the incomplete B-tubules (Dutcher and Trabuco 1998). Mutation in this gene results in the defective formation of the whole cartwheel structure of the basal body. The normal formation of the ring structure is controlled by the SAS-6 complex encoded by the *bld12* gene. The Sas-6 spokes harbour CEP135 at their tips, which is encoded by the gene *bld10*. Bld12 mutants show defective ring structure in basal bodies, but bld10 mutants completely lack basal bodies (Hiraki *et al*. 2007). This shows that these two proteins are of utmost importance for the normal formation of the 9-fold symmetry of the basic cartwheel structure. These proteins are recruited to their location by CEP70, a protein present in the ring structure in pro-basal bodies. An amorphous ring structure serves as a precursor for the cartwheel formation and harbours CEP70. This protein is lost during the maturation of basal bodies (Shiratsuchi *et al*. 2011). Along with proper formation, the correct positioning of these basal bodies is also very important for flagellar assembly. The attached distal and proximal fibres with the basal body ensure that docking happens at the correct site. Distal fibres contain a protein called centrin that provides elastic properties to these fibres, ensuring correct spatial positioning of basal bodies. A mutant with a mutation in the *VLF2* gene that encodes for centrin has defective basal body positioning with

Table 1. Protein molecules involved in centriole production and duplication, and important for ciliogenesis in humans and their respective homologs in other model organisms

Events	Molecules involved				Function	References
	Chlamydomonas	*C. elegans*	*Drosophila*	Humans		
Centriole production and duplication	–	Zyg-1 (zygote defective-1)	SAK (serine/ threonine protein kinase)	PLK4 (polo-like kinase-4)	Initiation of centriole duplication	Bettencourt-Dias *et al.* (2005), Eckerdt *et al.* (2011), Shimanovskaya *et al.* (2014)
	–	SPD-2 (spindle defective protein)	spd-2	CEP192 (centrosomal protein 192)		
	–	–	Asterless (asl)	CEP152		
	–	SAS-5	Ana2 (anastral spindle 2)	STIL		
	crSAS-6 (spindle assembly abnormal protein-6)	ceSAS-6	drSas-6	SASS-6	Cartwheel assembly	Hilbert *et al.* (2013)
	Bld10 (basal body protein 10)	Bld10	Cep135	CEP135		
	–	SAS-4	Sas-4	CPAP (centrosomal P4.1-associated protein)	Centriole length elongation	
	–	SAS-7	Ana1 (anastral spindle 1)	CEP295		

'-' means protein molecules not identified yet.

varied numbers of flagella (Wright *et al*. 1985; Silflow and Lefebvre 2001). The basal bodies also harbour proteins required for the formation of the TZ. The two major protein components of TZ are NPHP4 and CEP290. In *Chlamydomonas*, mutants for these two proteins show altered protein content in the axoneme resulting in defective biochemical and structural composition of the whole axoneme (Craige *et al*. 2010; Awata *et al*. 2014). The proteins destined for the axoneme are first recruited to the basal body and then allowed to enter the axoneme through the TZ. These proteins are carried towards the axoneme through the IFT system (discussed in section 3.5) (table 1).

3.4 *Centriole migration and acquisition*

After the entire centriole is formed, it is positioned correctly and is ready to function, a process known as centriolar maturation. This sets the stage for the initiation of cilium assembly. Sorokin (1962) described two different pathways for cilium assembly. One of the pathways elucidated in the fibroblast and smooth muscle cells includes small vesicles most likely derived from the Golgi bodies directly binding to the DAs of the mother centriole thereby forming a large membranous ciliary vesicle at the distal end. These small (pre-ciliary) vesicles are directed to the centriole by the coordinated action of dynein and myosin-Va (Wu *et al*. 2018). Later, it was demonstrated that one of the DA proteins, CEP164, is responsible for the docking of vesicles at the centriole (figure 4; Schmidt *et al*. 2012). With the help of the Eps15 homology domain (EHD) family of proteins, these pre-ciliary vesicles (PCVs) then fuse together to form the ciliary vesicles (CVs; Lu *et al*. 2015). In turn, these vesicles act as a protective sheath for the growth of nascent cilium inside the cell. Following the growth of this nascent cilium, CVs fuse with the plasma membrane, exposing the cilium for the very first time to the extracellular environment. This fusion results in the formation of the ciliary membrane and the adjacent ciliary pocket (a specific membrane domain found at the base of cilia). The other pathway has been reported in epithelial cells, where the distal appendage protein CEP164 facilitates the attachment of DAs to cell membrane without the involvement of CVs (Graser *et al*. 2007; Schmidt *et al*. 2012). This migration is facilitated by microtubule and actin cytoskeleton (Pitaval *et al*. 2010, 2017). It has been reported that since actin inhibitors can promote ciliogenesis, actin is absent from the site of docking (Kim *et al*. 2010; Francis *et al*. 2011). After migration, the fully mature mother centriole that docks at the plasma membrane and nucleates the axoneme serves as basal bodies for ciliary elongation. The basal body acquires additional structures like transition fibres and basal feet (like DAs and SDAs, respectively), as well as ciliary rootlets for support (Anderson and Brenner 1971). The DAs play a role in the docking of the centriole to the apical plasma membrane, while the SDAs help in microtubule anchoring (Tanos *et al*. 2013; Mazo *et al*. 2016; Breslow and Holland 2019).

How a cell decides which pathway to undertake remains elusive. It can be dependent on the molecular requirements for different pathways or on the functional aspects of the cilia that are formed. The cells harbouring the motile cilia are mostly seen to undergo the extracellular pathway where the cilium extends beyond the cell surface. This helps sensory cilia sense and regulate the extracellular fluid flow (Sorokin 1968), extending very little of the cilium beyond the apical surface. These cilia are found emerging near the nucleus and Golgi body deep inside the cell (Sorokin 1962). Experimental alteration in the positioning of these cilia can result in the dysregulation of their function. A study wherein the submerged cilia were converted to apical cilia although showed poor sensory functions, did promote the regulation of fluid flow (Mazo *et al*. 2016). This implies that the cell type specificity for cilium positioning is correlated with the functional properties of the cell.

3.5 *Transportation and compartmentalization of ciliary components to the site of assembly (protein trafficking)*

Several proteins are required to build the cilium. Since cilia do not contain ribosomes, and therefore lack the protein synthesis machinery, proteins required for the elongation of cilia have to be imported from the cytoplasm (Rosenbaum and Child 1967; Long and Huang 2020). For the continuous assembly of cilia, these proteins must be transported to their site of assembly. This transport can either happen by diffusion or by a special translocation system called the intraflagellar transport (IFT), the pathways that contribute towards their formation being highly conserved across ciliated organisms (Kozminski *et al*. 1993; Rosenbaum and Witman 2002; Lechtreck 2015). Interestingly, soluble proteins smaller than 50 kDa and certain transmembrane proteins can freely diffuse into cilia (Kee *et al*. 2012; Breslow *et al*. 2013; Lin *et al*. 2013). Several studies have demonstrated the presence of a

gate that serves as a barrier with a lower limit of the Stokes radius (~4.5 nm) for free diffusion into the cilia (Lin *et al*. 2013). For proteins with higher molecular weights such as outer dynein arms (~2 MDa), the TZ acts as a molecular barrier, therefore making IFT an important part of cilium assembly. It has been reported that for ciliary transmembrane-associated proteins, a targeting sequence known as ciliary localization signal (CLS) is required for the transport of these proteins into cilia (Berbari *et al*. 2008). However, in contrast to the nuclear localization signal (NLS), no unique consensus sequence is found for the transport of ciliary proteins, suggesting that other pathways are involved in the transport of these proteins. A rare exception was found in *Plasmodium falciparum* where there is no requirement of IFT for ciliogenesis as the axoneme assembles in the cytoplasm and directly transports proteins from cytoplasm to the tip of the growing cilium (Sinden *et al*. 1976; Briggs *et al*. 2004; Absalon *et al*. 2008). Another exception was found in *Drosophila* sperm flagella where necessary proteins for the formation of cilia are independent of the IFT (Han *et al*. 2003; Hao and Scholey 2009). First observed in the green alga *C. reinhardtii*, IFT provides the bidirectional movement of multimolecular protein complexes formed by strings of many IFT particles encompassing molecular motor proteins (Kozminski *et al*. 1993; Hao and Scholey 2009; Thomas *et al*. 2010). To be precise, its IFT system consists of 22 IFT protein particles and IFT motors (kinesin-2 for anterograde and dynein for retrograde movements) that come together to make the multisubunit long filamentous chains/strings called IFT trains for the transport of cargo to and fro from the axonemal tip. Particles that constitute the IFT are essential for flagellar assembly and maintenance. Jordan and Pigino (2019) used of *in situ* cryo-electron tomography (cryo-ET) and subtomogram averaging to investigate the structure and mechanism of the IFT depicting the movement of IFT trains along the microtubule doublets while transporting ciliary building blocks between the cell body and the ciliary tip. A comparison of the spatial arrangement of the various IFT components revealed that trains are assembled in a sequential manner (Hoek *et al*. 2022). The trains or strings that were complete were adjacent to the TZ at one end. The other end extended into the cytosol, and as it extends into the cytosol, the densities of dynein-1b and IFT-A keep reducing in a sequential order. This suggests that the IFT-B backbone is built first, followed by IFT-A and dynein-2 recruitment. Using cryo-ET and expansion microscopy techniques, the relatively small and flexible kinesin-2 was identified as the final component that is loaded onto the IFT trains close to the TZ (Hoek *et al*. 2022). These studies also indicated drastic differences between assembling and mature IFT trains. Assembling IFT trains were found to be more flexible than their mature axonemal counterparts and contained an extra density on IFT-B near the kinesin-2 binding site of unknown identity (Hoek *et al*. 2022). The importance of this process is evident wherein mutations in the motors DHC1B (a member of dynein superfamily) and kinesin-II cause defective or no flagella assembly, respectively (Silflow and Lefebvre 2001). The IFT protein subunits are formed in the cell body and then recruited by the basal body, where they start the construction of the IFT particles (Wingfield *et al*. 2017).

Further, the BBSome, a complex composed of eight proteins, works as an adapter in association with the IFT trains, thereby providing stabilization and maintenance. Mutation in these proteins causes Bardet-Biedl syndrome, a multi-organ disease specified by the defects in the sensory function of cilia (Wei *et al*. 2012; Williams *et al*. 2014). The axonemal assembly is normal in these mutants and so suggests the main role for the BBSome in ciliary signalling mechanisms (Lechtreck *et al*. 2009; Weihbrecht *et al*. 2017). Certain ciliary signalling proteins including G protein-coupled receptors (GPCRs), protein kinases, and ion channels have been found to transmit signals from the extracellular environment to the cell body. The BBS3 protein is thought to have a role in the interaction of ciliary signalling cargoes like phospholipase D (PLD). It has been shown that the BBSome relies on the BBS3 protein to enter the basal body via direct interaction with the BBS1 subunit of the BBSome in mammalian cells (Jin *et al*. 2010; Zhang *et al*. 2012; Mourão *et al*. 2014). However, in *C. reinhardtii*, GTPases ARL6/BBS3 recruit BBsomes from the cell body to the basal body through IFT22/RABL5 (Xue *et al*. 2020; Liu *et al*. 2021). Further, the BBSome splits from BBS3 and loads on to the anterograde IFT train and enters the cilium (Xue *et al*. 2020). The GTP-bound BBS3 then binds to the cell membrane, allowing it to laterally diffuse into the cilium (Liu and Lechtreck 2018). Through BBSome/IFT train remodelling, the BBSome is liberated from anterograde IFT trains at the ciliary tip. BBS3 binds to GTP and attracts the BBSome to the ciliary membrane, where it interacts with PLD. PLD-laden BBSomes are subsequently loaded onto retrograde IFT trains for ciliary escape by connecting with IFT-B1 (Liu and Lechtreck 2018; Liu *et al*. 2021; Xue *et al*. 2020). The entire protein transportation by the IFT/BBSome complex is divided into (i) IFT binding to

cargo, (ii) anterograde movement, and (iii) retrograde movement (sections 3.5.1 to 3.5.3) (table 2).

3.5.1 *IFT binding to cargo:* In *Chlamydomonas*, the IFT trains pass through the TZ and then move towards the axonemal tip, dropping off the cargo in between at its exact locations. The flagellar assembly and disassembly are facilitated by the IFT along with a few other proteins. The X-chromosome-associated protein 5 (XAP5) has been identified as the transcription factor in *C. reinhardtii* for regulating the transcription of flagellar assembly genes by recruiting RNA polymerase II (Pol II) for transcriptional activation (Li *et al*. 2018). FAP256, a homolog of the mammalian protein CEP104, is a flagellar-tip-associated protein and seems to have a function in the regular assembly of the flagella (Tammana *et al*. 2013). Other proteins like katanin and aurora kinases participate in flagellar disassembly during cell division. Katanin is a microtubule-sequestering protein that does not allow further microtubule elongation beyond the transition zone (Lohret *et al*. 1998). Having served as the pioneer model system for investigations on the IFT and motors, most orthologs have been found in higher organisms (tables 2 and 3).

Binding of the IFT complex to the cargo is the foremost and necessary step for the transportation of ciliary proteins into the growing cilium. Broadly, IFT trains are composed of two large complexes, labelled as IFT-A and IFT-B (Ishikawa and Marshall 2011; Taschner *et al*. 2012; Picariello *et al*. 2019). These multimeric protein complexes have distinct yet complementary roles and move together into the cilium. IFT-B consists of 16 subunits that transport proteins from the base to the tip of the cilium (anterograde movement), while IFT-A consists of 6 subunits that transport proteins from the tip to the cell body (retrograde movement) (Cole *et al*. 1998). IFT-B further assembles into IFT-B1 and IFT-B2 sub-complexes composed of at least 10 and 6 distinct subunits, respectively (Boldt *et al*. 2016; Katoh *et al*. 2016; reviewed by Taschner and Lorentzen 2016). They are arranged within the basal body region in the vicinity of transition fibres (part of the transition zone that connects the distal basal body to the base of the ciliary membrane). The IFT complexes are first gathered into IFT particles, and the latter connect to IFT motors forming IFT trains (Lechtreck 2015; Ishikawa and Marshall 2017; Prevo *et al*. 2017). Following this, binding of the BBSome protein to IFT trains occurs. However, the recruitment of the BBSome to the basal body for binding to IFT trains remains elusive. IFT complexes contain cargo-binding sites that bind to a variety of cargo molecules, mainly axonemal proteins such as ciliary tubulins and motor proteins (Wren *et al*. 2013; Craft *et al*. 2015). *In silico* analysis has shown several protein–protein interaction motifs present on the IFT molecule including tetratricopeptide repeats (TPR), WD40, and a coiled–coiled domain which helps in the binding of these IFTs to the cargo (Smith *et al*. 1999; Burkhard *et al*. 2001). For example, structural studies have shown that a calponin homology (CH) domain is present in the N-terminal region of IFT81 (part of IFT-B1) that harbours recognition sites for tubulin (Bhogaraju *et al*. 2013). Interaction of tubulin with IFT81 is enhanced by an IFT74 that has a high-affinity binding site at its C-terminal tail. Knockdown of IFT81 with point mutation and its rescue experiments indicate the combined role of these IFTs in tubulin-binding revealing its importance for ciliogenesis (Bhogaraju *et al*. 2013). Additionally, similar binding mechanisms are present in the IFT-B2 complex suggesting the transport of two tubulins per IFT-B complex (Taschner and Lorentzen 2016). The mode of transport of ciliary transmembrane proteins requires their synthesis in the endoplasmic reticulum (ER) followed by modification and sorting in the Golgi apparatus. After processing from the Golgi apparatus, proteins are carried to the ciliary base via a transport vesicle that moves into the cilium with the help of the IFT-B subunit and KIF17 motor protein (Long and Huang 2020). Axonemal proteins, such as radial spoke proteins (RSPs), enter into cilia as a multiprotein complex requiring pre-assembly at the ciliary base. Of the 23 known RSPs, the 12S radial spoke complex (component of 11 RSPs) is first assembled at the ciliary base and then transported to the tip of the cilia via IFTs, where they combine with the remaining RSPs to form the 20S radial spokes complex (Diener *et al*. 2011). Likewise, the dynein arms are transported by the same mechanism (described above) (Fowkes and Mitchell 1998).

3.5.2 *Delivering of cargo proteins (anterograde movement) and regulation of IFT transport:* Once the IFT complexes, IFTB/A (designated so because in the complex, IFT-B is present on the top and IFT-A at the bottom) assemble into IFT trains, the movement of cargo proteins is initiated and mediated by the motor protein kinesin-2 family (Cole *et al*. 1993; Kozminski *et al*. 1995; Engelke *et al*. 2019). The kinesin-2 motor family is known to be present in the heterotrimeric and homodimeric forms, both of which contribute to the anterograde IFT movement. The heterotrimeric kinesin-2 consists of two motor subunits of the kinesin family member, KIF3A and KIF3B, and an accessory

Table 2. Protein molecules from the IFT complexes important for ciliogenesis in humans and their respective homologs in other model organism

Events	Molecules involved				Function	References
	Chlamydomonas	*C. elegans*	*Drosophila*	Humans		
IFT (Complex A)	IFT144 (intraflagellar transport)	DYF-2 (abnormal DYe filling)	OSEG-6 (outer segment 6)	WDR19 (WD repeat-containing protein 19)	Retrograde transport	Rosenbaum and Witman (2002), Ishikawa and Marshall (2011), Balmer *et al*. (2015), *https://flybase.org/*
	IFT140	CHE-11 (abnormal CHEmotaxis)	RempA (reduced mechanoreceptor potential A)/OSEG3	WDTC2/IFT140		
	IFT139	ZK328.7 (tetratricopeptide repeat protein 21)	–	THM1/TTC218 (tetratricopeptide repeat protein 21B)		
	IFT122/FAP80 (flagellar-associated protein 80)	DAF-10 (abnormal DAuer formation)	OSEG-1	IFT122/WDR10		
	IFT121	IFTA-1	OSEG-4	WDR35		
	IFT43	–	CG5780	IFT43/C14orf179		
IFT (Complex B)	IFT172	OSM-1 (OSMotic avoidance abnormal-1)	Oseg2/CG13809	IFT172	Anterograde transport	
	IFT88	OSM-5	NOMPB (no mechanoreceptor potential B)	IFT88		
	IFT81	IFT81	–	Polaris/Tg737/IFT81		
	IFT80	CHE-2	OSEG5	IFT80/WDR56		
	IFT74/72	IFT74	–	IFT74/IFT72		
	IFT57	CHE-13	CG8853	IFT57/HIPPI		
	IFT52/BLD1	OSM-6	CG9595	IFT52/NGD5		
	IFT46	DYF6	CG15161	IFT46/C11orf60		
	IFT27	–	–	IFT27/RABL4 (RAS oncogene family-like 5)		
	IFT20	Y110A7A.20	CG30441	IFT20		
	IFT70/FAP259	DYF-1	CG5142	TTC30A/B		
	IFT56/DYF13	DYF13	–	TTC26		
	IFT25/FAP232	–	–	IFT25/HSPB11 (heat shock protein family B (small) member 11)		
	IFT22/FAP9	IFTA-2	–	RABL5		
	IFT38/FAP22	DYF-3	CG3259	CLUAP1 (clusterin associated protein 1)		

'-' means protein molecules not identified.

Table 3. Motor protein molecules important for ciliogenesis in humans and their respective homologs in other model organisms

	Molecules involved					
Events	*Chlamydomonas*	*C. elegans*	*Drosophila*	Humans	Function	References
Motor protein kinesin 2 (heterotrimeric)	FLA8 (flagellar assembly 8)	KLP-11 (kinesin like protein)	Klp68D	KIF3B (kinesin family 3B)	Movement of IFT particles and proteins essential for cilia	Rosenbaum and Witman (2002), *https://flybase.org/*
	FLA10	KLP-20	Klp64D	KIF3A		
	FLA3	KAP-1 (kinesin associated protein)	Kap3	KAP3/KIFAP3 (kinesin family associated protein)		
Motor protein kinesin 2 (homodimeric)	–	OSM-3/KLP-2	Kif3C	KIF17		
Motor protein dynein 2	DHC1B (dynein heavy chain 1b)	CHE-3	Btv (Beethoven locus)	DYNC2H1		
	FAP133	DYCI-1	CG13074	WDR34		
	DIBLIC (dynein 1b light intermediate chain)	D2LIC/XBX-1 (dynein 2 light intermediate chain)	CG3769	DYNC2LI1		
	LC8/FLA14 (light chain 8)	DLC-1	Ctp (cut up protein)	DYNLL1 (dynein light chain 1, cytoplasmic)		

'-' means protein molecules not identified.

subunit kinesin-associated protein (KAP3). It is the KIF3B subunit that modulates ciliary transportation by binding directly to IFT-B (Yamazaki *et al*. 1996; Mueller *et al*. 2005; Scholey 2013). The transport of IFT machinery is highly regulated at the base and inside the cilia, especially at the ciliary tip. Phosphorylation events serve as a molecular switch between the binding and unloading of the cargo proteins (Craige and Witman 2014; Liang *et al*. 2014). For example, dephosphorylation of the KIF3B subunit is required for binding to IFT-B at the ciliary base. As soon as it is organized, IFT trains release the cargo proteins into the cilium. Inside the cilium, phosphorylation by numerous local protein kinases induces a conformational change that leads to the disruption of the binding between IFT particles, motor protein, and cargo, thereby unloading the cargo at the ciliary tip. An instance of phosphorylation has been reported in *Chlamydomonas* wherein the FLA8 subunit (flagella assembly 8, homologue of KIF3B) of the kinesin-2 family serves as a substrate for calcium-dependent protein kinases (CDPKs) that are enriched at the flagellar tip during flagellar assembly. This disrupts the interaction between IFT-B and kinesin-2 such that the cargo does not bind again (Liang *et al*. 2014). On the other hand, in animals such as *C. elegans* and vertebrates, different subunits of kinesin-2 (OSM-3 in *C. elegans* and kif17 in vertebrates) help in building the cilium (Taschner *et al*. 2012). Full-length KIF17 and a construct of KIF17 without its motor domain accumulate at the ciliary tip (Jiang *et al*. 2015), suggesting that KIF17 can function as cargo as well. Further, a co-immunoprecipitation of KIF17 pulls down the IFT46-IFT56 dimer of the IFT-B complex. Knockout of IFT56 in the human RPE1 cell line (Funabashi *et al*. 2017) restricts the entry of KIF17 into cilia. Nevertheless, KIF17-deficient mice do not affect IFT-B localization and cells show normal ciliogenesis, indicating an ambiguous role for this protein (Yin *et al*. 2011). Mutational analysis in the subunits of the motor protein shows that inactivation of kinesin-2 blocks cilia assembly and IFT transport in various organisms, including *Chlamydomonas, Drosophila, C. elegans*, and mice (Kozminski *et al*. 1995; Marszalek *et al*. 1999; Snow *et al*. 2004; Chaya *et al*. 2014; Engelke *et al*. 2019).

3.5.3 *Returning of IFT trains to the cell body (retrograde movement):* After the cargo is released into the cilium, the designated proteins diffuse to their docking site, and anterograde IFT trains immediately disassemble (by the molecular switch discussed earlier) at the ciliary tip to rearrange into retrograde trains. The retrograde movement of the IFT is mediated by the dynein motor protein, a multiprotein complex such as cytoplasmic dynein 2 that contributes to the returning of IFT trains from the ciliary tip to the cell body (Pazour *et al*. 1998; Porter *et al*. 1999; Perrone *et al*. 2003; Engel *et al*. 2012). Defects in the retrograde motor protein show the accumulation of IFT-B proteins on the tip of the cilium suggesting the important role of IFT-A in returning IFT-B to the cell body (Efimenko *et al*. 2006; Iomini *et al*. 2009). Overall, these IFT particles provide the assembly of cilia in a very coordinated manner and take part in the maintenance of the organelles in most ciliated organisms.

3.6 *Regulatory network*

Right from the first step of centriole duplication, the entire process of ciliogenesis is strictly regulated. Its understanding comes chiefly from the loss of specific proteins such as CEP120 and neuralized-like protein 4 (NEURL4) from the daughter centriole that marks the beginning of mother centriole formation (Zou *et al*. 2005; Mahjoub *et al*. 2010; Li *et al*. 2012). The recruitment of DA proteins is regulated by C2 calcium-dependent domain containing 3 (C2CD3), oro-faciodigital syndrome 1 (OFD1), and mitogen-activated protein kinase 15 (MAPK15; Kazatskaya *et al*. 2017), whereas the recruitment of SDA proteins is regulated by a different set of proteins consisting of trichoplein (TCHP) and coiled-coil and C2 domain-containing protein 2A (CC2D2A; Veleri *et al*. 2014). While the role of these proteins is known in ciliogenesis, their interaction with other regulatory proteins and how they work as a group to ensure synchronous maturation of the mother centriole with cell cycle is unclear. The PCVs arising from either the Golgi or endosomes are recruited to the mother centriole appendages with the help of kinesins and dyneins. Specific proteins such as kinesin family member C1 (KIFC1) and dynein-1 heavy chain (DYNC1H1) regulate the trafficking of these vesicles to the mother centriole (Lee *et al*. 2017; Wu *et al*. 2018). Along with these motor proteins, the actin network formed with the help of the actin-nucleator complex containing actin-related proteins 2/3 (ARP2/3) and the nucleation-promoting complex WASH (Wiskott Aldrich syndrome protein and scar homolog) results in the actin assembly with the centrosome acting as the actin-organizing centre (Antoniades *et al*. 2014). This observation is also supported by the localization of specific actin cytoskeleton-associated proteins (LIM-kinase 2, LIMK2 wherein LIM stands for LIN-11, Isl-1 and

MEC-3; testicular protein kinase 1, TESK1) near the centriole (Kim *et al*. 2015a, b). Specific regulators (microtubule actin crosslinking factor 1, MACF1A) that maintain the actin cytoskeleton network further help in vesicle trafficking (May-Simera *et al*. 2016). After the formation of CVs by the fusion of numerous PCVs, the axoneme elongation process starts. The formation of CVs is triggered by the recruitment of Rab11-Rabin8-Rab8 signalling pathways. Proteins including CEP164, Chibby (CBY1), Abelson-helper integration site 1 (AHI1), and Talpid3 (KIAA0586) (Burke *et al*. 2014) serve as substrate and facilitate the interaction of Rab8 with the DAs on the mother centriole.

3.6.1 *Regulators present at the ciliary base:* The mother centriole-associated DAs fuse with the recycling endosomes containing the signalling molecule Rab-11 GTPase, which are then called distal appendage vesicles (DAVs). Rab-11 helps in the recruitment of Rab8, a protein that is important for the progression of ciliogenesis (Westlake *et al*. 2011). This activation is mediated through the recruitment of Rabin8, a guanine exchange factor (GEF) that is responsible for the final activation of Rab8. DAVs also contain some membrane-shaping proteins delivered by Rab-11 endosomes. These proteins are called EHD family proteins; EHD1 and EHD3 proteins work together to bring the DAVs proximal to each other. This results in the vesicle fusion mediated by SNAP29 (a member of the SNARE protein family). This fusion results in the formation of the CVs associated with the mother centriole (Lu *et al*. 2015). After this, the CVs extend because of Rab8 activation (figure 4B). It is also hypothesized that the EHD proteins help in the removal of a centriolar protein (CP110, a negative regulator of ciliogenesis) from the M-centriole that leads it to become a basal body. CP110 forms a complex with CEP97, CEP290, and KIF24, and promotes centriolar duplication during cell cycle stages ensuring that abnormal cilia are not formed. This complex is stabilized by a centrosomal deubiquitinase, ubiquitin carboxyl-terminal hydrolase 33 (USP33), that de-ubiquitinylates CP110. CP110 is degraded by abundant ubiquitylation with the help of the skp1-cul1-F-box-protein ($SCF^{CyclinF}$) complex that is a positive regulator of ciliogenesis. This equilibrium between ubiquitylation and de-ubiquitylation regulates the level of centrosomal proteins and therefore ciliogenesis (Li *et al*. 2013). Another protein, tau tubulin kinase 2 (TTBK2; a serine-threonine kinase), has been shown to displace CP110 from the centriole to initiate basal body formation and hence ciliogenesis. It removes CP110 by phosphorylating one of the members of the complex CP110/CEP97/CEP290/KIF24 and further helps in the recruitment of IFTs for axoneme extension (figure 4B). Hence, TTBK2 also serves as an important player in the initiation and progression of ciliogenesis (Goetz *et al*. 2012). Another regulator is leucine-rich repeat kinase2 (LRRK2) that functions in commanding the microtubule dynamics through proper and direct interaction with microtubule components like *TUBB* (one of the ten β-tubulin-encoding genes present in the human genome). This also suggests the potential role of axonemal tubulin acetylation in ciliary biogenesis (Gillardon 2009; Kett *et al*. 2020; Law *et al*. 2014). Besides this, LRRK2 also helps in removing CP110 and recruiting TTBK2 at the mother centriole (Sobu *et al*. 2021). It is speculated that the EHD proteins along with the pre-ciliary membrane proteins help in the recruitment of the ubiquitination-related enzymes to the ciliary base. Further, these EHD proteins are also responsible for the transport of proteins of the TZ and IFT complex (specifically IFT20) to the ciliary base where further sorting of proteins that are destined to become the axoneme occurs (Lu *et al*. 2015). The ciliary base is an important site for ciliogenesis initiation as well as cargo sorting for ciliary proteins. All the proteins required for ciliary growth are first transported to the ciliary base and then sorted and sent to different destinations of the axoneme. This protein trafficking happens in two parts, viz., pre-ciliary and intraciliary transport. All the proteins (cytoplasmic and transmembrane) that are meant to be sent to the ciliary base are sorted at the trans-Golgi network based on specific ciliary targeting signals (CTSs) (Mazelova *et al*. 2009). All the ciliary-destined proteins are carried to the ciliary base by four different pathways based on their initial localization and abundance. For example, plasma membrane-based proteins do not require the formation of separate vesicles for transport. These proteins directly transfer to the ciliary base through lateral diffusion to the periciliary membrane.

3.6.2 *Regulators of cilium disassembly:* In contrast to the knowledge gathered on cilium assembly, ciliary disassembly/resorption remains less understood. Before mitosis, the centriole pair present on the apical membrane of the cell is disengaged from the cortex and moves to the spindle poles. The main rationale behind the resorption of cilia is the formation of mitotic spindles that ensure the proper organization and position of chromosomes during the cell cycle. The detachment of the centriole from the surface involves mainly two proteins, mitotic kinase Aurora A and histone deacetylases (HDAC2/6). Aurora A kinase is a

key regulator of cell proliferation and participates in primary cilia resorption by phosphorylating and stimulating HDAC; the latter destabilizes and depolymerizes the tubulins of the axonemal microtubule, thereby promoting cilium disassembly (Pugacheva *et al*. 2007; Plotnikova *et al*. 2012; Ran *et al*. 2015). The activation of Aurora A is controlled by a complex signalling pathway initiated by the extracellular signal platelet-derived growth factor (PDGFRβ) that further activates the downstream enzyme, phospholipase C-gamma (PLCγ). Activation of PLCγ induces the release of intracellular Ca^{2+}, whose influx activates the Ca^{2+}-calmodulin complex (CaM) that binds to Aurora A and its binding partner, a scaffolding protein, human enhancer of filamentation 1 (HEF1) (Plotnikova *et al*. 2012). This multiprotein complex harbours the active Aurora kinase enzyme that causes phosphorylation of HDAC6. Phosphorylated HDAC6 destabilizes the axonemal microtubule by removing the acetylated tubulins present in cilia. Another protein pitchfork (Pifo) also promotes cilium disassembly by activation of Aurora A kinase. Experimental analysis shows that Pifo accumulates at the basal body and physically interacts with Aurora A kinase, culminating in the destabilization of the ciliary microtubules (Kinzel *et al*. 2010). Mutation in pitchfork R80K blocks Aurora A activation. However, the exact interacting site involved in the binding of pitchfork to Aurora A kinase is poorly understood. Apart from the two proteins discussed above, two kinesin superfamily proteins have also been implicated in disassembly of primary cilia before mitosis, KIF2A and KIF24. Depolymerization of microtubules by KIF2A is activated by a mitotic kinase PLK1 (polo-like kinase). PLK1 phosphorylates KIF2A at the T554 position which in turn activates the KIF2A and promotes the disassembly of ciliary microtubules in a growth signal-dependent manner (Miyamoto *et al*. 2015). Similarly, depolymerization of microtubules by KIF24 is enhanced by the activation of NEK-2, a protein kinase that is generally expressed in the S- and G2-phase and phosphorylates KIF24, ensuring the blockage of reciliation throughout the S/G2/M-phases of the cell cycle (Kim *et al*. 2015a, b).

The loss of the cilium has been investigated in *Chlamydomonas* and in mammalian cells. When Mirvis *et al*. (2019) used live-cell imaging to characterize the individual events during loss of the cilium, they found rapid deciliation wherein the membrane and axoneme of the cilium were shed from the cell. While rapid deciliation was the most prominent, some gradual resorption was also observed. In some cells, a period of gradual resorption was followed by rapid deciliation. Deciliation or disassembly as seen in *Chlamydomonas* results in the intact cilia being shed in the medium that could be recovered. Such cilia (in both cases) contain both membrane and axoneme proteins. Interestingly, when the levels of katanin are modulated, the intracellular calcium rises and deciliation occurs. It is also known that excess katanin can alone promote cilia loss by deciliation, independently of calcium. Together, these results suggest that mammalian ciliary loss involves a tuneable decision between deciliation and resorption (Lohret *et al*. 1998; Mirvis *et al*. 2019).

4. Role of cilia in human health and diseases

4.1 *Involvement of cilia in non-ciliopathic diseases*

Primary cilia have a diverse array of receptors present on the ciliary membrane which efficiently coordinate through the various cellular signalling pathways for the proper functioning of the cell, which also makes them prone to diseases, commonly called ‘ciliopathies’. However, accumulating evidence shows the involvement of cilia in non-ciliopathic diseases like anosmia. Recently, infections by a novel coronavirus (SARS-COV2), which is the causative agent of COVID-19, reported patients with symptoms that included loss of smell (anosmia). Ciliary membrane receptors seem to be points of entry for the virus. Upon binding, the dysfunctional cilia render the patient with a loss of smell. TEM studies on influenza and the Sendai virus clearly depict the attachment of the virus particles to the ciliary membrane. As soon as the virus enters, the nucleocapsid of the virus partially unwinds and escalates the entry into the cilia (Dourmashkin and Tyrrell 1970). Also, research on human nasal epithelial cells infected with a variant of coronavirus (hCov) shows cilia dysfunction (Chilvers *et al*. 2001), thereby causing a defect in axonemal structure, and subsequently damaging the respiratory epithelium (Chilvers *et al*. 2003). ACE2 localization in the ciliated epithelial cell of respiratory, pulmonary, and urinary systems is probably seen as a host receptor for the entry of virus into the cells (Lukassen *et al*. 2020; Sungnak *et al*. 2020). Investigation using human airway epithelial cell culture revealed that the virus in the form of virions is trapped at the base of the cell (near the ciliary pocket) which marks the endocytosis pathway of virus entry to the cells (Sims *et al*. 2005). The ciliary pocket has abundant clathrin-coated pits, making cilia a potential target for viruses to enter the cell (Otsuka *et al*. 2015;

Verhey and Yang 2016). Studies have indicated that despite the virus deciliating the organelle upon infection, with some delay, the cilia are known to reassemble and return to normal functioning post recovery (Afzelius 1994). Moreover, the loss of several cytokines in COVID-19 infection impacts ciliary function mainly due to the loss of regulation in cilia length (Dummer et al. 2018). Greater understanding is needed to find out the molecular link between the virus and cilium biology to strengthen knowledge on the impact of cilia on human health and diseases.

4.2 *Role of Sonic Hedgehog signalling in primary cilia*

Sonic Hedgehog (SHH) signalling is the major pathway that regulates crucial events during the development process. It is noted that abnormal activation of this process has a significant association with glioma, basal cell carcinoma (BCC), prostate, and lung cancer (Goetz *et al*. 2009; Hassounah *et al*. 2012). The presence of the canonical SHH receptor has therefore a connection with the primary cilia. Almost 90% of BCC and 30% of medulloblastoma have a mutation in the genes involved in SHH signalling (Hassounah *et al*. 2012; Pugh *et al*. 2012). When the ligand SHH binds to the receptor patched (PTCH1) present on the plasma membrane, there is an accumulation of SMO (smoothened, a seven-pass transmembrane protein) and SUFU (suppressor of fused) on the primary ciliary membrane. Out of the three Gli transcription factors (Gli1, Gli2, and Gli3), Gli2 and Gli3 are converted by proteolytic cleavage to perform roles of transcriptional activation or repression. Active forms of SMO and SUFU recruit Gli1 to form a complex that together targets Gli2 and Gli3 to act as transcriptional activators upon translocation to the nucleus and mediate SHH signalling. In the absence of SHH, Gli2 and Gli3 act as transcriptional repressors resulting in the suppression of SHH signalling genes (Rohatgi *et al*. 2007; Wong *et al*. 2009). Depending on the initiating carcinogenic factor, SHH signalling has a dual role in promoting and preventing carcinoma (Han and Alvarez-Buylla 2010; Archer *et al*. 2012; Hassounah *et al*. 2012; Gerhardt *et al*. 2016). This was observed in BCC (a skin cancer) wherein cells with an activated SMO possess primary cilia, and removal of primary cilia inhibits the progression of BCC (Wong *et al*. 2009; Athar *et al*. 2014; Basset-Seguin *et al*. 2015). In contrast, however, cells with the active form of Gli2 possess primary cilia and the removal of primary cilia accelerates the progression of BCC (Wong *et al*. 2009). Similarly, in the case of medulloblastoma (malignant brain tumour), cilia are present when there is a loss of function in PTCH1 and SUFU. On the other hand, a gain of function in SMO and Gli causes an enhanced activity of SHH, thereby promoting medulloblastoma. In non-ciliated cells, amplification/mutation of Gli transcription factors without the activation of PTCH1 and SMO causes an activation of Gli2-R which acts as an antagonist of Gli2-A, causing the suppression of SHH medulloblastoma (Han and Alvarez-Buylla 2010).

Apart from the primary cilium, motile cilia also contain some components of SHH signalling, but in a non-canonical manner (Mao *et al*. 2018). Studies on human airway epithelia showed that SHH (Cigna *et al*. 2012; Peng *et al*. 2015; Henkin *et al*. 2017) is present in the basolateral side of epithelia. During early development, the primary cilium present on the airway cell produces SHH for its normal functioning. After completion of development, i.e., in mature airway epithelia, the primary cilium disappears and numerous motile cilia appear on the apical portion of cells (Sorokin 1968; Jain *et al*. 2010). Several efforts have been carried out to identify the components of SHH signalling based on arrayed siRNA libraries (Roosing *et al*. 2015). Recently, a whole-genome-wide CRISPR screen technology was used to identify the factors required for ciliary signalling, revealing the relation of certain disorders caused by ciliary signalling (Breslow *et al*. 2018; Pusapati *et al*. 2018). The novel ciliary components (FAM92A, TTC23, TXNDC15, and TEDC proteins) that were candidate ciliopathy genes came to light after the CRISPR-based screening. For FAM92A, a mutant cell pool was generated to identify the precise role in ciliogenesis, and it has been shown that the formation of cilia is drastically reduced in Fam92A-knockout pools (Breslow *et al*. 2018). Further, localization studies using FAM92A antibodies found it to be present in the TZ (Breslow *et al*. 2018). FAM92A was found to co-localize with its partners, viz., CBY1 and DZIP1L (Glazer *et al*. 2010; Burke *et al*. 2014; Li *et al*. 2021). TTC23, on the other hand, has been found to be associated with IQCE (IQ domain-containing protein E) and EFCAB7 (EF-hand calcium-binding domain-containing protein 7) previously shown to localize to the Ellis-van Creveld (EvC) zone (proximal region of cilium; Ruiz-Perez and Goodship 2009; Dorn *et al*. 2012; Pusapati *et al*. 2014). Although knockout of TTC23 does not show any defect in the cilium assembly, it certainly reduces the localization of IQCE and EFCAB7 proteins to the EvC zone (Breslow *et al*. 2018). Thioredoxin-domain containing 15 (TXNDC15) has been shown to be associated with

the Meckel syndrome (Shaheen *et al*. 2016). Knockout studies of TXNDC15 have shown decreased levels of the ciliary GTPase ARL13B, indicating a clear defect in SHH signalling (Breslow *et al*. 2018). Tubulin ε and δ complex 1 and 2 (TEDC1 and 2) proteins have been found to associate with ε-tubulin, δ-tubulin, α/β-tubulin, and CENPJ (centriolar regulator of microtubule dynamics). Mutation in the *cenpj* gene disrupts the triplet microtubules present in the flagella of *Drosophila* spermatocytes (Zheng *et al*. 2016). Knockout of both TedC1 and TedC2 showed complete loss of the centrioles. These mutants lack cilia and show strong defects in the SHH signalling (Breslow *et al*. 2018). Thus, the CRISPR screening platform has led to the identification of genes involved in ciliary signalling with direct relevance to human health and diseases.

4.3 *Role of cilia in neurodegenerative diseases*

LRRK2 (leucine-rich repeat kinase 2), a large multifunctional kinase, is responsible for neurodegeneration such as autosomal-dominant Parkinson's disease. It has been shown that in cultured cells, the pathogenic form of LRRK2 phosphorylates the switch II domain of Rab GTPases which are considered master regulators of intracellular vesicle trafficking (Steger *et al*. 2017). Rab-interacting lysosomal protein-like proteins 1 and 2 (RILPL1 and 2) localize in both primary cilium and centrosome (mother centriole) and are known to regulate the protein concentration of the primary ciliary membrane. Depletion of these two proteins causes an imbalance in the concentration of signalling molecules in the ciliary membrane and prevents the proper organization of epithelial cells in 3D (ALI) cultures (Schaub and Stearns 2013). RILPL1 and RILPL2 are the primary ciliogenesis regulators that help in the formation of cilia; the phosphorylated form of Rab GTPases interacts with RILPL1 and RILPL2 and thus blocks the formation of primary cilia (Schaub and Stearns 2013). This was the first study to show the link between Parkinson's disease and cilia. Experimental analysis has shown that phosphorylation of Rab GTPase proteins and the dephosphorylation by a phosphatase, viz., protein phosphatase Mg^{2+}/Mn^{2+}-dependent1H (PPM1H) is crucial for ciliary functioning. Precisely, PPM1H, a family of phosphatase enzymes, has been shown to counter this process by specifically dephosphorylation of the subset of Rab proteins, namely, Rab8A, Rab8B, Rab10, and Rab35 (Steger *et al*. 2017; Dhekne *et al*. 2018; Schmidt *et al*. 2022). In conclusion, cells expressing hyperactive LRRK2 kinase mutants cause depletion of PPM1H and subsequently block the formation of primary cilia. Hence, this needs to be further ascertained with greater insight into these processes so that one can apply these as therapeutics to treat Parkinson's disease (Berndsen *et al*. 2019).

4.4 *Role of cilia in skeletal dysplasia*

As mentioned earlier, the IFT machinery is a highly conserved mechanism for the motor-driven transport of cargo within cilia. WDR35, also known as IFT121, is an IFT machinery protein (Quidwai *et al*. 2021). It is a component of the IFT-A complex best known for its role in ciliary retrograde transport. Mutation in the *Wdr35* gene causes mutant cilia but fails to enrich diverse classes of ciliary membrane proteins, resulting in severe disorders in both humans and mice including a range of potentially lethal skeletal dysplasia. The absence of this protein not only disrupts the IFT, but also stops certain ciliary proteins and their associated membranes from entering cilia. It also causes the accumulation of transport intermediates from its cellular manufacturing site to the cilia (Quidwai *et al*. 2021). Analysis of the structure of WDR35 and other IFT proteins has shown that they are very similar to a protein complex called COPI, which is involved in transporting membrane proteins around the cell. The role of COPI is to coat the vesicles (small lipid bubbles) used by cells to store newly synthesized proteins until they are destined for their sites. This process has been referred to as vesicular transport wherein COPI has a major role to play. Quidwai *et al*. (2021) found a similar role for WDR35 and other IFT proteins. For example, these proteins can bind to specific types of lipid molecules, suggesting a role in vesicular transport. Likewise, when mouse cells were mutated to stop producing WDR35, coatless vesicles accumulated around the base of the cilia. Adding back normal WDR35 production to these mutant cells rescued these defects giving rise to functional cilia (Quidwai *et al*. 2021). These results indicate a COPI-like complex system to deliver proteins to growing cilia. Therapy on these lines could prove useful in human skeletal dysplasia.

5. Concluding remarks

This review is an attempt to describe the complexity with which the cilium appears and disappears in cells. From precursor generation and their migration inside the cell to ciliary elongation and maintenance at the

membrane, ciliary programs are highly regulated. There are divergent ciliogenesis pathways and patterns in various cell types, despite conserved ciliary structures and components. This begs for further explorations showing the extent of conservation of these ciliary processes among different species (vertebrates, invertebrates, protozoans, *C. reinhardtii*), tissues (hair cells, ependymal cells, sperm, kidney) and cilia types (9+0, 9+2, motile, immotile). Since the cilia progress from centrioles, a significant direction of study would be showing the interdependence of the two organelles on each other and the level of decision-making by the cell for maintaining the location, number, structure, length, and duration of cilia for itself.

A unique question that comes up in this direction is: Is the architecture, proteome, and regulation of the basal bodies the same or different for the 9+0 and 9+2 cilia? It is interesting to note that there are differences in the cilium basal bodies of 9+0 and 9+2 cilia. The basal body plays a very important role in the making of a cilium and provides a much-needed direction to the microtubule in forming the ciliary axoneme. Although the basic structure and function of all basal bodies do not differ significantly, could there be differences between species? Could there be differences between basal bodies in various tissues, especially ones in cells that make the 9+0 cilium versus those that make the 9+2 cilium types? Such an understanding across species and ciliary types within the same organism are sparse. For example, analysis of the accessory structure of the murine basal body reveals that in most of the 9+0 ciliated cells, the basal body is connected to the proximal end of the daughter centriole via a linkage that involves the rootlet and rootletin (Bahe *et al*. 2005). Rootlets are a thick striated bunch of filaments that extend close to the nucleus. Rootletin (also known as ciliary rootlet coiled-coiled or crocc protein) is a component of the ciliary rootlet (Yang *et al*. 2002). Both rootlet and rootletin are known to be associated with basal bodies (Klotz *et al*. 1986) in which rootletin in connection with centrosomal Nek2-associated protein 1 (C-Nap1, wherein Nek stands for NIMA-related kinase) forms fibres that connect the mother and daughter centriole and help in centrosome cohesion (Yang *et al*. 2006). This mother centriole now matures after the ciliary vesicle docks to the distal part and onto the plasma membrane to form a cilium. However, in contrast to 9+2 multiciliated cells, the basal body parts are not connected with the daughter centriole, which itself is a drastic difference between the two types (Jord *et al*. 2014). In addition, the proteomes of the basal bodies of 9+0 and 9+2 are similar but not identical between the two types of cilia of the same organism.

Incidentally, the TZs are short or long depending on cell types. For example, TZ is short in fibroblast cells and long in photoreceptor cells (Garcia and Reiter 2016). On the other hand, it may be emphasized that the central pair is not in continuation with microtubules of the basal body. Although it is unknown if the basal body components help in stabilizing the central pair, in an invertebrate like *Drosophila*, one such basal body component, viz. BLD10/CEP135, stabilizes the central pair microtubule of the ciliary axoneme (Carvalho-Santos *et al*. 2012). Future implications for research on basal bodies of distinct types of cilia will provide better insight on ciliopathy diseases that are particularly linked with basal body components.

Funding

The authors are grateful for the funding received from the Department of Atomic Energy, India.

Declarations

Conflict of Interest The authors have no conflict of interest to declare.

References

Abdelkhalek HB, Beckers A, Schuster-Gossler K, *et al*. 2004 The mouse homeobox gene Not is required for caudal notochord development and affected by the truncate mutation. *Genes Dev.* **18** 1725–1736

Absalon S, Blisnick T, Kohl L, *et al*. 2008 Intraflagellar transport and functional analysis of genes required for flagellum formation in trypanosomes. *Mol. Biol. Cell.* **19** 929–944

Afzelius B 1959 Electron microscopy of the sperm tail results obtained with a new fixative. *J. Biophys. Biochem. Cytol.* **5** 269–278

Afzelius BA 1994 Ultrastructure of human nasal epithelium during an episode of coronavirus infection. *Virchows Arch.* **424** 295–300

Alten L, Schuster-Gossler K, Beckers A, *et al*. 2012 Differential regulation of node formation, nodal ciliogenesis and cilia positioning by Noto and Foxj1. *Development* **139** 1276–1284

Andersen JS, Wilkinson CJ, Mayor T, *et al*. 2003 Proteomic characterization of the human centrosome by protein correlation profiling. *Nature* **426** 570–574

Anderson RG and Brenner RM 1971 The formation of basal bodies (centrioles) in the Rhesus monkey oviduct. *J. Cell. Biol.* **50** 10–34

Antoniades I, Stylianou P and Skourides PA 2014 Making the connection: ciliary adhesion complexes anchor basal bodies to the actin cytoskeleton. *Dev. Cell.* **28** 70–80

Archer TC, Weeraratne SD and Pomeroy SL 2012 Hedgehog-GLI pathway in medulloblastoma. *J. Clin. Oncol.* **30** 2154–2156

Arquint C, Gabryanczyk AM, Imseng S, *et al*. 2015 STIL binding to Polo-box 3 of PLK4 regulates centriole duplication. *eLife* **4** e07888

Athar M, Li C, Kim AL, *et al*. 2014 Sonic hedgehog signaling in Basal cell nevus syndrome. *Cancer Res.* **74** 4967–4975

Awata J, Takada S, Standley C, *et al*. 2014 NPHP4 controls ciliary trafficking of membrane proteins and large soluble proteins at the transition zone. *J. Cell Sci.* **127** 4714–4727

Bahe S, Stierhof YD, Wilkinson CJ, *et al*. 2005 Rootletin forms centriole-associated filaments and functions in centrosome cohesion. *J. Cell Biol.* **171** 27–33

Balmer S, Dussert A, Collu GM, *et al*. 2015 Components of intraflagellar transport complex A function independently of the cilium to regulate canonical Wnt signaling in *Drosophila*. *Dev. Cell* **34** 705–718

Bangs F and Anderson KV 2017 Primary cilia and mammalian hedgehog signaling. *Cold Spring Harb. Perspect. Biol.* **9** a028175

Banizs B, Pike MM, Millican CL, *et al*. 2005 Dysfunctional cilia lead to altered ependyma and choroid plexus function, and result in the formation of hydrocephalus. *Development* **132** 5329–5339

Basset-Seguin N, Sharpe HJ and de Sauvage FJ 2015 Efficacy of Hedgehog pathway inhibitors in Basal cell carcinoma. *Mol. Cancer Ther.* **14** 633–641

Beckers A, Alten L, Viebahn C, *et al*. 2007 The mouse homeobox gene Noto regulates node morphogenesis, notochordal ciliogenesis, and left right patterning. *Proc. Natl. Acad. Sci. USA* **104** 15765–21570

Berbari NF, Johnson AD, Lewis JS, *et al*. 2008 Identification of ciliary localization sequences within the third intracellular loop of G protein-coupled receptors. *Mol. Biol. Cell.* **19** 1540–1547

Berndsen K, Lis P, Yeshaw WM, *et al*. 2019 PPM1H phosphatase counteracts LRRK2 signaling by selectively dephosphorylating Rab proteins. *eLife* **8** e50416

Bettencourt-Dias M, Rodrigues-Martins A, Carpenter L, *et al*. 2005 SAK/PLK4 is required for centriole duplication and flagella development. *Curr. Biol.* **15** 2199–2207

Bhogaraju S, Cajanek L, Fort C, *et al*. 2013 Molecular basis of tubulin transport within the cilium by IFT74 and IFT81. *Science* **341** 1009–1012

Boldt K, van Reeuwijk J, Lu Q, *et al*. 2016 An organelle-specific protein landscape identifies novel diseases and molecular mechanisms. *Nat. Commun.* **7** 11491

Breslow DK and Holland AJ 2019 Mechanism and regulation of centriole and cilium biogenesis. *Annu. Rev. Biochem.* **88** 691–724

Breslow DK, Koslover EF, Seydel F, *et al*. 2013 An *in vitro* assay for entry into cilia reveals unique properties of the soluble diffusion barrier. *J. Cell Biol.* **203** 129–147

Breslow DK, Hoogendoorn S, Kopp AR, *et al*. 2018 CRISPR-based screen for Hedgehog signaling provides insights into ciliary function and ciliopathies. *Nat Genet.* **50** 460–471

Briggs LJ, Davidge JA, Wickstead B, *et al*. 2004 More than one way to build a flagellum: comparative genomics of parasitic protozoa. *Curr. Biol.* **14** R611–R612

Burke MC, Li FQ, Cyge B, *et al*. 2014 Chibby promotes ciliary vesicle formation and basal body docking during airway cell differentiation. *J. Cell Biol.* **207** 123–137

Burkhard P, Stetefeld J and Strelkov SV 2001 Coiled coils: a highly versatile protein folding motif. *Trends Cell Biol.* **11** 82–88

Carvalho-Santos Z, Machado P, Alvarez-Martins I, *et al*. 2012 BLD10/CEP 135 is a microtubule-associated protein that controls the formation of the flagellum central microtubule pair. *Dev. Cell.* **23** 412–424

Chaya T, Omori Y, Kuwahara R, *et al*. 2014 ICK is essential for cell type-specific ciliogenesis and the regulation of ciliary transport. *EMBO J.* **33** 1227–1242

Chilvers MA, McKean M, Rutman A, *et al*. 2001 The effects of coronavirus on human nasal ciliated respiratory epithelium. *Eur. Respir. J.* **18** 965–970

Chilvers MA, Rutman A and O'Callaghan C 2003 Ciliary beat pattern is associated with specific ultrastructural defects in primary ciliary dyskinesia. *J. Allergy Clin. Immunol.* **112** 518–524

Cigna N, FarrokhiMoshai E, Brayer S, *et al*. 2012 The hedgehog system machinery controls transforming growth factor-β-dependent myofibroblastic differentiation in humans: involvement in idiopathic pulmonary fibrosis. *Am. J. Pathol.* **181** 2126–2137

Cole DG, Chinn SW, Wedaman KP, *et al*. 1993 Novel heterotrimeric kinesin-related protein purified from sea urchin eggs. *Nature* **366** 268–270

Cole DG, Diener DR, Himelblau AL, *et al*. 1998 *Chlamydomonas* kinesin-II-dependent intraflagellar transport (IFT): IFT particles contain proteins required for ciliary assembly in *Caenorhabditis elegans* sensory neurons. *J. Cell Biol.* **141** 993–1008

Craft JM, Harris JA, Hyman S, *et al*. 2015 Tubulin transport by IFT is upregulated during ciliary growth by a cilium-autonomous mechanism. *J. Cell Biol.* **208** 223–237

Craige B and Witman GB 2014 Flipping a phosphate switch on kinesin-II to turn IFT around. *Dev. Cell.* **30** 492–493

Craige B, Tsao CC, Diener DR, *et al.* 2010 CEP290 tethers flagellar transition zone microtubules to the membrane and regulates flagellar protein content. *J. Cell Biol.* **190** 927–940

Dhekne HS, Yanatori I, Gomez RC, *et al*. 2018 A pathway for Parkinson's Disease LRRK2 kinase to block primary cilia and Sonic hedgehog signaling in the brain. *eLife* **7** e40202

Didon L, Zwick RK, Chao IW, *et al*. 2013 RFX3 modulation of FOXJ1 regulation of cilia genes in the human airway epithelium. *Respir. Res.* **14** 70

Diener DR, Yang P, Geimer S, *et al*. 2011 Sequential assembly of flagellar radial spokes. *Cytoskeleton* **68** 389–400

Dorn KV, Hughes CE and Rohatgi R 2012 A Smoothened-Evc2 complex transduces the Hedgehog signal at primary cilia. *Dev. Cell.* **23** 823–835

Dourmashkin RR and Tyrrell DA 1970 Attachment of two myxoviruses to ciliated epithelial cells. *J. Gen. Virol.* **9** 77–88

Dummer A, Rol N, Szulcek R, *et al*. 2018 Endothelial dysfunction in pulmonary arterial hypertension: loss of cilia length regulation upon cytokine stimulation. *Pulm. Circ.* **8** 2045894018764629

Dutcher SK and Trabuco EC 1998 The UNI3 gene is required for assembly of basal bodies of *Chlamydomonas* and encodes δ-tubulin, a new member of the tubulin superfamily. *Mol. Biol. Cell.* **9** 1293–1308

Dzhindzhev NS, Tzolovsky G, Lipinszki Z, *et al*. 2014 Plk4 phosphorylates Ana2 to trigger Sas6 recruitment and procentriole formation. *Curr. Biol.* **24** 2526–2532

Eckerdt F, Yamamoto TM, Lewellyn AL, *et al*. 2011 Identification of a polo-like kinase 4-dependent pathway for *de novo* centriole formation. *Curr. Biol.* **21** 428–432

Efimenko E, Blacque OE, Ou G, *et al*. 2006 *Caenorhabditis elegans* DYF-2, an orthologue of human WDR19, is a component of the intraflagellar transport machinery in sensory cilia. *Mol. Biol. Cell.* **17** 4801–4811

Engel BD, Ishikawa H, Wemmer KA, *et al*. 2012 The role of retrograde intraflagellar transport in flagellar assembly, maintenance, and function. *J. Cell Biol.* **199** 151–167

Engelke MF, Waas B, Kearns SE, *et al*. 2019 Acute inhibition of heterotrimeric kinesin-2 function reveals mechanisms of intraflagellar transport in mammalian cilia. *Curr. Biol.* **29** 1137–1148

Essner JJ, Amack JD, Nyholm MK, *et al*. 2019 Kupffer's vesicle is a ciliated organ of asymmetry in the zebrafish embryo that initiates left-right development of the brain, heart and gut. *Development* **132** 1247–1260

Euteneuer U and McIntosh JR 1981 Structural polarity of kinetochore microtubules in PtK1 cells. *J. Cell Biol.* **89** 338–345

Falk N, Losl M, Schroder N, *et al*. 2015 Specialized Cilia in Mammalian Sensory Systems. *Cells* **4** 500–519

Field S, Riley KL, Grimes DT, *et al*. 2011 Pkd111 establishes left-right asymmetry and physically interacts with Pkd2. *Development* **138** 1131–1142

Fowkes ME and Mitchell DR 1998 The role of preassembled cytoplasmic complexes in assembly of flagellar dynein subunits. *Mol. Biol. Cell* **9** 2337–2347

Francis SS, Sfakianos J, Lo B, *et al*. 2011 A hierarchy of signals regulates entry of membrane proteins into the ciliary membrane domain in epithelial cells. *J. Cell Biol.* **193** 219–233

Fu Z, Gailey CD, Wang EJ, *et al*. 2019 Ciliogenesis associated kinase 1: targets and functions in various organ systems. *FEBS Lett.* **593** 2990–3002

Funabashi T, Katoh Y, Michisaka S, *et al*. 2017 Ciliary entry of KIF17 is dependent on its binding to the IFT-B complex via IFT46-IFT56 as well as on its nuclear localization signal. *Mol. Biol. Cell.* **28** 624–633

Garcia G and Reiter JF 2016 A primer on the mouse basal body. *Cilia* **5** 17

Gerhardt C, Leu T, Lier JM, *et al*. 2016 The cilia-regulated proteasome and its role in the development of ciliopathies and cancer. *Cilia* **5** 14

Gibbons IR 1961 Structural asymmetry in cilia and flagella. *Nature* **190** 1128–1129

Gillardon F 2009 Leucine-rich repeat kinase 2 phosphorylates brain tubulin-beta isoforms and modulates microtubule stability – a point of convergence in parkinsonian neurodegeneration. *J. Neurochem.* **110** 1514–1522

Glazer AM, Wilkinson AW, Backer CB, *et al*. 2010 The Zn finger protein Iguana impacts Hedgehog signaling by promoting ciliogenesis. *Dev. Biol.* **337** 148–156

Goetz SC, Ocbina PJ and Anderson KV 2009 The primary cilium as a Hedgehog signal transduction machine. *Meth. Cell. Biol.* **94** 199–222

Goetz SC, Liem KF Jr and Anderson KV 2012 The spinocerebellar ataxia-associated gene Tau tubulin kinase 2 controls the initiation of ciliogenesis. *Cell* **151** 847–858

Gonçalves J and Pelletier L 2017 The ciliary transition zone: finding the pieces and assembling the gate. *Mol. Cells* **40** 243–253

Graser S, Stierhof YD, Lavoie SB, *et al*. 2007 Cep164, a novel centriole appendage protein required for primary cilium formation. *J. Cell Biol.* **179** 321–330

Habedanck R, Stierhof YD, Wilkinson CJ, *et al*. 2005 The Polo kinase Plk4 functions in centriole duplication. *Nat. Cell. Biol.* **7** 1140–1146

Haider S, Gamperl M, Burkard TR, *et al*. 2019 Estrogen signaling drives ciliogenesis in human endometrial organoids. *Endocrinology* **160** 2282–2297

Han YG and Alvarez-Buylla A 2010 Role of primary cilia in brain development and cancer. *Curr. Opin. Neurobiol.* **20** 58–67

Han YG, Kwok BH and Kernan MJ 2003 Intraflagellar transport is required in *Drosophila* to differentiate sensory cilia but not sperm. *Curr. Biol.* **13** 1679–1686

Han L, Rao Q, Yang R, Wang Y, *et al*. 2022 Cryo-EM structure of an active central apparatus. *Nat. Struct. Mol Biol.* **29** 472–482

Hao L and Scholey JM 2009 Intraflagellar transport at a glance. *J. Cell Sci.* **122** 889–892

Hassounah NB, Bunch TA and McDermott KM 2012 Molecular pathways: the role of primary cilia in cancer progression and therapeutics with a focus on Hedgehog signaling. *Clin. Cancer Res.* **18** 2429–2435

Henkin RI, Hosein S, Stateman WA, *et al*. 2017 Improved smell function with increased nasal mucus sonic hedgehog in hyposmic patients after treatment with oral theophylline. *Am. J. Otolaryngol.* **38** 143–147

Hilbert M, Erat MC, Hachet V, *et al*. 2013 *Caenorhabditis elegans* centriolar protein SAS-6 forms a spiral that is consistent with imparting a ninefold symmetry. *Proc. Natl. Acad. Sci. USA* **110** 11373–11378

Hiraki M, Nakazawa Y, Kamiya R, *et al*. 2007 Bld10p constitutes the cartwheel-spoke tip and stabilizes the 9-fold symmetry of the centriole. *Curr. Biol.* **17** 1778–1783

Hirokawa N, Tanaka Y, Okada Y, *et al*. 2006 Nodal flow and the generation of left-right asymmetry. *Cell* **125** 33–45

Iomini C, Li L, Esparza JM, *et al*. 2009 Retrograde intraflagellar transport mutants identify complex A proteins with multiple genetic interactions in *Chlamydomonas reinhardtii*. *Genetics* **183** 885–896

Ishikawa H and Marshall WF 2011 Ciliogenesis: building the cell's antenna. *Nat. Rev. Mol. Cell. Biol.* **12** 222–234

Ishikawa H and Marshall WF 2017 Intraflagellar transport and ciliary dynamics. *Cold Spring Harb. Perspect. Biol.* **9** a021998

Jain R, Pan J, Driscoll JA, *et al*. 2010 Temporal relationship between primary and motile ciliogenesis in airway epithelial cells. *Am. J. Respir. Cell Mol. Biol.* **43** 731–739

Jiang L, Tam BM, Ying G, *et al*. 2015 Kinesin family 17 (osmotic avoidance abnormal-3) is dispensable for photoreceptor morphology and function. *FASEB J.* **29** 4866–4880

Jin H, White SR, Shida T, *et al*. 2010 The conserved Bardet-Biedl syndrome proteins assemble a coat that traffics membrane proteins to cilia. *Cell* **141** 1208–1219

Jord AI, Lemaître AI, Delgehyr N, *et al*. 2014 Centriole amplification by mother and daughter centrioles differs in multiciliated cells. *Nature* **516** 104–107

Jordan MA and Pigino G 2019 In situ cryo-electron tomography and subtomogram averaging of intraflagellar transport trains. *Methods Cell Biol.* **152** 179–195

Katoh Y, Terada M, Nishijima Y, *et al*. 2016 Overall architecture of the Intraflagellar Transport (IFT)-B complex containing Cluap1/IFT38 as an essential component of the IFT-B peripheral subcomplex. *J. Biol. Chem.* **291** 10962–10975

Kazatskaya A, Kuhns S, Lambacher NJ, *et al*. 2017 Primary cilium formation and ciliary protein trafficking is regulated by the atypical MAPKinase MAPK15 in *Caenorhabditis elegans* and human cells. *Genetics* **207** 1423–1440

Kee HL, Dishinger JF, Blasius TL, *et al*. 2012 A size-exclusion permeability barrier and nucleoporins characterize a ciliary pore complex that regulates transport into cilia. *Nat. Cell Biol.* **14** 431–437

Keller LC, Romijn EP, Zamora I, *et al*. 2005 Proteomic analysis of isolated *Chlamydomonas* centrioles reveals orthologs of ciliary-disease genes. *Curr. Biol.* **15** 1090–1098

Kempeneers C and Chilvers MA 2018 To beat, or not to beat, that is the question! The spectrum of ciliopathies. *Pediatr. Pulmonol.* **53** 1122–1129

Kempeneers C, Seaton C, Garcia Espinosa B, *et al*. 2019 Ciliary functional analysis: Beating a path towards standardization. *Pediatr. Pulmonol.* **54** 1627–1638

Kett LR, Boassa D, Ho CC, *et al*. 2020 LRRK2 Parkinson disease mutations enhance its microtubule association. *Hum. Mol. Genet.* **21** 890–899

Kim J, Lee JE, Heynen-Genel S, *et al*. 2010 Functional genomic screen for modulators of ciliogenesis and cilium length. *Nature* **464** 1048–1051

Kim TS, Park JE, Shukla A, *et al*. 2013 Hierarchical recruitment of Plk4 and regulation of centriole biogenesis by two centrosomal scaffolds, Cep192 and Cep152. *Proc. Natl. Acad. Sci. USA* **110** E4849–E4857

Kim J, Jo H, Hong H, *et al*. 2015a Actin remodelling factors control ciliogenesis by regulating YAP/TAZ activity and vesicle trafficking. *Nat. Commun.* **6** 6781

Kim S, Lee K, Choi J-H, *et al*. 2015b Nek2 activation of Kif24 ensures cilium disassembly during the cell cycle. *Nat. Commun.* **6** 8087

Kim S, Ma L, Shokhirev MN, *et al*. 2018 Multicilin and activated E2f4 induce multiciliated cell differentiation in primary fibroblasts. *Sci. Rep.* **8** 12369

Kinzel D, Boldt K, Davis EE, *et al*. 2010 Pitchfork regulates primary cilia disassembly and left-right asymmetry. *Dev. Cell* **19** 66–77

Klotz C, Bordes N, Laine MC, *et al*. 1986 A protein of 175,000 daltons associated with striated rootlets in ciliated epithelia, as revealed by a monoclonal antibody. *Cell Motil. Cytoskeleton* **6** 56–67

Kodani A, Yu TW, Johnson JR, *et al*. 2015 Centriolar satellites assemble centrosomal microcephaly proteins to recruit CDK2 and promote centriole duplication. *eLife* **4** e07519

Kozminski KG, Johnson KA, Forscher P, *et al*. 1993 A motility in the eukaryotic flagellum unrelated to flagellar beating. *Proc. Natl. Acad. Sci. USA* **90** 5519–5523

Kozminski KG, Beech PL and Rosenbaum JL 1995 The *Chlamydomonas* kinesin-like protein FLA10 is involved in motility associated with the flagellar membrane. *J. Cell Biol.* **131** 1517–1527

Law BM, Spain VA, Leinster VH, *et al*. 2014 A direct interaction between leucine-rich repeat kinase 2 and specific β-tubulin isoforms regulates tubulin acetylation. *J. Biol. Chem.* **289** 895–908

Lechtreck KF 2015 IFT-Cargo Interactions and protein transport in cilia. *Trends Biochem. Sci.* **40** 765–778

Lechtreck KF, Johnson EC, Sakai T, *et al*. 2009 The *Chlamydomonas reinhardtii* BBSome is an IFT cargo required for export of specific signaling proteins from flagella. *J. Cell Biol.* **187** 1117–1132

Lee SH, Joo K, Jung EJ, *et al*. 2017 Export of membrane proteins from the Golgi complex to the primary cilium requires the kinesin motor, KIFC1. *FASEB J.* **32** 957–968

Li J, Kim S, Kobayashi T, *et al*. 2012 Neurl4, a novel daughter centriole protein, prevents formation of ectopic microtubule organizing centres. *EMBO Rep.* **13** 547–553

Li J, D'Angiolella V, Seeley ES, *et al*. 2013 USP33 regulates centrosome biogenesis via deubiquitination of the centriolar protein CP110. *Nature* **495** 255–259

Li L, Tian G, Peng H, *et al*. 2018 New class of transcription factors controls flagellar assembly by recruiting RNA polymerase II in *Chlamydomonas*. *Proc. Natl. Acad. Sci. USA* **115** 4435–4440

Li FQ, Chen X, Fisher C, *et al*. 2021 BAR Domain-Containing FAM92 proteins Interact with Chibby1 to facilitate Ciliogenesis. *Mol. Cell Biol.* **36** 2668–2680

Liang Y, Pang Y, Wu Q, *et al*. 2014 FLA8/KIF3B phosphorylation regulates kinesin-II interaction with IFT-B to control IFT entry and turnaround. *Dev. Cell.* **30** 585–597

Lin Y, Niewiadomski P, Lin B, *et al*. 2013 Chemically inducible diffusion trap at cilia reveals molecular sieve-like barrier. *Nat. Chem. Biol.* **9** 437–443

Liu P and Lechtreck KF 2018 The Bardet-Biedl syndrome protein complex is an adapter expanding the cargo range of intraflagellar transport trains for ciliary export. *Proc. Natl. Acad. Sci. USA* **115** E934–E943

Liu YX, Xue B, Sun WY, *et al*. 2021 Bardet-Biedl syndrome 3 protein promotes ciliary exit of the signaling protein phospholipase D via the BBSome. *eLife* **10** e59119

Lohret TA, McNally FJ and Quarmby LM 1998 A role for katanin-mediated axonemal severing during *Chlamydomonas* deflagellation. *Mol. Biol. Cell.* **9** 1195–1207

Long H and Huang K 2020 Transport of ciliary membrane proteins. *Front. Cell Dev. Biol.* **7** 381

Lopes CA, Prosser SL, Romio L, *et al*. 2011 Centriolar satellites are assembly points for proteins implicated in human ciliopathies, including oral-facial-digital syndrome 1. *J. Cell Sci.* **124** 600–612

Lu Q, Insinna C, Ott C, *et al*. 2015 Early steps in primary cilium assembly require EHD1/EHD3-dependent ciliary vesicle formation. *Nat. Cell Biol.* **17** 228–240

Lukassen S, Chua RL, Trefzer T, *et al*. 2020 SARS-CoV-2 receptor ACE2 and TMPRSS2 are primarily expressed in bronchial transient secretory cells. *EMBO J.* **39** e105114

Mahjoub MR, Xie Z and Stearns T 2010 Cep120 is asymmetrically localized to the daughter centriole and is essential for centriole assembly. *J. Cell Biol.* **191** 331–346

Mao S, Shah AS, Moninger TO, *et al*. 2018 Motile cilia of human airway epithelia contain hedgehog signaling components that mediate noncanonical hedgehog signaling. *Proc. Natl. Acad. Sci. USA* **115** 1370–1375

Marshall WF and Rosenbaum JL 1999 Cell division: The renaissance of the centriole. *Curr. Biol.* **9** R218–R220

Marszalek JR, Ruiz-Lozano P, Roberts E, *et al*. 1999 *Situs inversus* and embryonic ciliary morphogenesis defects in mouse mutants lacking the KIF3A subunit of kinesin-II. *Proc. Natl. Acad. Sci. USA* **96** 5043–5048

May-Simera HL, Gumerson JD, Gao C, *et al*. 2016 Loss of MACF1 abolishes ciliogenesis and disrupts apicobasal polarity establishment in the retina. *Cell Rep.* **17** 1399–1413

Mazelova J, Astuto-Gribble L, Inoue H, *et al*. 2009 Ciliary targeting motif VxPx directs assembly of a trafficking module through Arf4. *EMBO J.* **28** 183–192

Mazo G, Soplop N, Wang WJ, *et al*. 2016 Spatial control of primary ciliogenesis by subdistal appendages alters sensation-associated properties of cilia. *Dev. Cell.* **39** 424–437

McGrath J, Somlo S, Makova S, *et al*. 2003 Two populations of node monocilia initiate left-right asymmetry in the mouse. *Cell* **114** 61–73

Mirvis M, Siemers KA, Nelson WJ, *et al*. 2019 Primary cilium loss in mammalian cells occurs predominantly by whole-cilium shedding. *PLoS Biol.* **17** e3000381

Mitchison HM and Valente EM 2017 Motile and non-motile cilia in human pathology: from function to phenotypes. *J. Pathol.* **241** 294–309

Miyamoto T, Hosoba K, Ochiai H, *et al*. 2015 The microtubule-depolymerizing activity of a mitotic Kinesin protein KIF2A drives primary cilia disassembly coupled with cell proliferation. *Cell Rep.* **10** 664–673

Mohri H, Inaba K, Ishijima S, *et al*. 2012 Tubulin-dynein system in flagellar and ciliary movement. *Proc. Japan. Acad. Ser. B. Phys. Biol. Sci.* **88** 397–415

Mourão A, Nager AR, Nachury MV, *et al*. 2014 Structural basis for membrane targeting of the BBSome by ARL6. *Nat. Struct. Mol. Biol.* **21** 1035–1041

Mueller J, Perrone CA, Bower R, *et al*. 2005 The FLA3 KAP subunit is required for localization of kinesin-2 to the site of flagellar assembly and processive anterograde intraflagellar transport. *Mol. Biol. Cell.* **16** 1341–1354

Nishide K, Mugitani M, Kumano G, *et al*. 2012 Neurula rotation determines left-right asymmetry in ascidian tadpole larvae. *Development* **139** 1467–1475

Nonaka S, Tanaka Y, Okada Y, *et al*. 1998 Randomization of left-right asymmetry due to loss of nodal cilia generating leftward flow of extraembryonic fluid in mice lacking KIF3B motor protein. *Cell* **95** 829–837; Erratum in *Cell* **99** 117

Otsuka A, Dreier J, Cheng PF, *et al*. 2015 Hedgehog pathway inhibitors promote adaptive immune responses in basal cell carcinoma. *Clin. Cancer Res.* **21** 1289–1297

Paintrand M, Moudjou M, Delacroix H, *et al*. 1992 Centrosome organization and centriole architecture: Their sensitivity to divalent cations. *J. Struct. Biol.* **108** 107–128

Pazour GJ, Wilkerson CG and Witman GB 1998 A dynein light chain is essential for the retrograde particle

movement of intraflagellar transport (IFT). *J. Cell Biol.* **141** 979–992

Peng T, Frank DB, Kadzik RS, *et al*. 2015 Hedgehog actively maintains adult lung quiescence and regulates repair and regeneration. *Nature* **526** 578–582

Pennekamp P, Karcher C, Fischer A, *et al*. 2002 The ion channel polycystin-2 is required for left-right axis determination in mice. *Curr. Biol.* **12** 938–943

Perrone CA, Tritschler D, Taulman P, *et al*. 2003 A novel dynein light intermediate chain colocalizes with the retrograde motor for intraflagellar transport at sites of axoneme assembly in *Chlamydomonas* and mammalian cells. *Mol. Biol. Cell.* **14** 2041–2056

Picariello T, Brown JM, Hou Y, *et al*. 2019 A global analysis of IFT-A function reveals specialization for transport of membrane-associated proteins into cilia. *J. Cell Sci.* **132** jcs220749

Pimenta-Marques A, Bento I, Lopes CA, *et al*. 2016 A mechanism for the elimination of the female gamete centrosome in *Drosophila melanogaster*. *Science* **353** aaf4866

Pitaval A, Tseng Q, Bornens M, *et al*. 2010 Cell shape and contractility regulate ciliogenesis in cell cycle-arrested cells. *J. Cell Biol.* **191** 303–312

Pitaval A, Senger F, Letort G, *et al*. 2017 Microtubule stabilization drives 3D centrosome migration to initiate primary ciliogenesis. *J. Cell Biol.* **216** 3713–3728

Plotnikova OV, Nikonova AS, Loskutov YV, *et al*. 2012 Calmodulin activation of Aurora-A kinase (AURKA) is required during ciliary disassembly and in mitosis. *Mol. Biol. Cell.* **23** 2658–2670

Porter ME, Bower R, Knott JA, *et al*. 1999 Cytoplasmic dynein heavy chain 1b is required for flagellar assembly in *Chlamydomonas*. *Mol. Biol. Cell.* **10** 693–712

Prevo B, Scholey JM and Peterman EJG 2017 Intraflagellar transport: mechanisms of motor action, cooperation, and cargo delivery. *FEBS J.* **284** 2905–2931

Pugacheva EN, Jablonski SA, Hartman TR, *et al*. 2007 HEF1-dependent Aurora A activation induces disassembly of the primary cilium. *Cell* **129** 1351–1363

Pugh TJ, Weeraratne SD, Archer TC, *et al*. 2012 Medulloblastoma exome sequencing uncovers subtype-specific somatic mutations. *Nature* **488** 106–110

Pusapati GV, Hughes CE, Dorn KV, *et al*. 2014 EFCAB7 and IQCE regulate hedgehog signaling by tethering the EVC-EVC2 complex to the base of primary cilia. *Dev Cell.* **28** 483–496

Pusapati GV, Kong JH, Patel BB, *et al*. 2018 CRISPR screens uncover genes that regulate target cell sensitivity to the morphogen sonic Hedgehog. *Dev Cell.* **44** 113–129

Quidwai T, Wang J, Hall EA, *et al*. 2021 WDR35-dependent coat protein complex transports ciliary membrane cargo vesicles to cilia. *eLife* **10** e69786

Ran J, Yang Y, Li D, *et al*. 2015 Deacetylation of α-tubulin and cortactin is required for HDAC6 to trigger ciliary disassembly. *Sci. Rep.* **5** 12917

Reiter JF, Blacque OE and Leroux MR 2012 The base of the cilium: roles for transition fibres and the transition zone in ciliary formation, maintenance and compartmentalization. *EMBO Rep.* **13** 608–618

Rieder CL, Jensen CG and Jensen LCW 1979 The resorption of primary cilia during mitosis in a vertebrate (PtK1) cell line. *J. Ultrastruct. Res.* **68** 173–185

Rohatgi R, Milenkovic L and Scott MP 2007 Patched1 regulates hedgehog signaling at the primary cilium. *Science* **317** 372–376

Roosing S, Hofree M, Kim S, *et al*. 2015 Functional genome-wide siRNA screen identifies KIAA0586 as mutated in Joubert syndrome. *eLife* **4** e06602

Rosenbaum JL and Child FM 1967 Flagellar regeneration in protozoan flagellates. *J. Cell Biol.* **34** 345–364

Rosenbaum JL and Witman GB 2002 Intraflagellar transport. *Nat. Rev. Mol. Cell Biol.* **3** 813–825

Ruiz-Perez VL and Goodship JA 2009 Ellis-van Creveld syndrome and Weyers acrodental dysostosis are caused by cilia-mediated diminished response to hedgehog ligands. *Am. J. Med. Genet. c. Semin. Med. Genet.* **151C** 341–351

Satir P and Christensen ST 2007 Overview of structure and function of mammalian cilia. *Ann. Rev. Physiol.* **69** 377–400

Satir P, Heuser T and Sale WS 2014 A structural basis for how motile cilia beat. *Bioscience* **64** 1073–1083

Schaub JR and Stearns T 2013 The Rilp-like proteins Rilpl1 and Rilpl2 regulate ciliary membrane content. *Mol. Biol. Cell.* **24** 453–464

Schmidt KN, Kuhns S, Neuner A, *et al*. 2012 Cep164 mediates vesicular docking to the mother centriole during early steps of ciliogenesis. *J. Cell Biol.* **199** 1083–1101

Schmidt S, Luecken MD, Trümbach D, *et al*. 2022 Primary cilia and SHH signaling impairments in human and mouse models of Parkinson's disease. *Nat. Commun.* **13** 4819

Schnackenberg BJ and Palazzo RE 1999 Identification and function of the centrosome centromatrix. *Biol. Cell.* **91** 429–438

Scholey JM 2013 Kinesin-2: a family of heterotrimeric and homodimeric motors with diverse intracellular transport functions. *Ann. Rev. Cell Dev. Biol.* **29** 443–469

Schweickert A, Weber T, Beyer T, *et al*. 2007 Cilia-driven leftward flow determines laterality in Xenopus. *Curr. Biol.* **17** 60–66

Shaheen R, Szymanska K, Basu B, *et al*. 2016 Characterizing the morbid genome of ciliopathies. *Genome Biol.* **17** 242

Shimanovskaya E, Viscardi V, Lesigang J, *et al*. 2014 Structure of the *C. elegans* ZYG-1 cryptic polo box suggests a conserved mechanism for centriolar docking of Plk4 kinases. *Structure* **22** 1090–1104

Shiratsuchi G, Kamiya R and Hirono M 2011 Scaffolding function of the *Chlamydomonas* procentriole protein CRC70, a member of the conserved Cep70 family. *J. Cell Sci.* **124** 2964–2975

Silflow CD and Lefebvre PA 2001 Assembly and motility of eukaryotic cilia and flagella. Lessons from *Chlamydomonas Reinhardtii*. *Plant Physiol.* **127** 1500–1507

Sims AC, Baric RS, Yount B, *et al*. 2005 Severe acute respiratory syndrome coronavirus infection of human ciliated airway epithelia: role of ciliated cells in viral spread in the conducting airways of the lungs. *J. Virol.* **79** 15511–15524

Sinden RE, Canning EU and Spain B 1976 Gametogenesis and fertilization in *Plasmodium yoelii* nigeriensis: a transmission electron microscope study. *Proc. R. Soc. Lond. B Biol. Sci.* **193** 55–76

Smith TF, Gaitatzes C, Saxena K, *et al*. 1999 The WD repeat: a common architecture for diverse functions. *Trends Biochem Sci.* **24** 181–185

Snow JJ, Ou G, Gunnarson AL, *et al*. 2004 Two anterograde intraflagellar transport motors cooperate to build sensory cilia on *C. elegans* neurons. *Nat. Cell Biol.* **6** 1109–1113

Sobu Y, Wawro PS, Dhekne HS, *et al*. 2021 Pathogenic LRRK2 regulates ciliation probability upstream of tau tubulin kinase 2 via Rab10 and RILPL1 proteins. *Proc. Natl. Acad. Sci. USA* **118** e2005894118

Sorokin SP 1962 Centrioles and the formation of rudimentary cilia by fibroblasts and smooth muscle cells. *J. Cell Biol.* **15** 363–377

Sorokin SP 1968 Reconstructions of centriole formation and ciliogenesis in mammalian lungs. *J. Cell Sci.* **3** 207–230

Stauber M, Boldt K, Wrede C, *et al*. 2017 1700012B09Rik, a FOXJ1 effector gene active in ciliated tissues of the mouse but not essential for motile ciliogenesis. *Dev. Biol.* **429** 186–199

Steger M, Diez F, Dhekne HS, *et al*. 2017 Systematic proteomic analysis of LRRK2-mediated Rab GTPase phosphorylation establishes a connection to ciliogenesis. *eLife* **6** e31012

Sungnak W, Huang N, Bécavin C, *et al*. 2020 SARS-CoV-2 entry factors are highly expressed in nasal epithelial cells together with innate immune genes. *Nat. Med.* **26** 681–687

Tabin CJ and Vogan KJ 2003 A two-cilia model for vertebrate left-right axis specification. *Genes Dev.* **17** 1–6

Takao D and Verhey KJ 2016 Gated entry into the ciliary compartment. *Cell Mol. Life Sci.* **73** 119–127

Tammana TVS, Tammana D, Diener DR, *et al*. 2013 Centrosomal protein CEP104 (*Chlamydomonas* FAP256) moves to the ciliary tip during ciliary assembly. *J. Cell Sci.* **126** 5018–5029

Tang CJ, Fu RH, Wu KS, *et al*. 2009 CPAP is a cell-cycle regulated protein that controls centriole length. *Nat. Cell. Biol.* **11** 825–831

Tanos BE, Yang HJ, Soni R, *et al*. 2013 Centriole distal appendages promote membrane docking, leading to cilia initiation. *Genes Dev.* **27** 163–168

Taschner M and Lorentzen E 2016 The intraflagellar transport machinery. *Cold Spring Harb. Perspect. Biol.* **8** a028092

Taschner M, Bhogaraju S and Lorentzen E 2012 Architecture and function of IFT complex proteins in ciliogenesis. *Differentiation* **83** S12–S22

Teves ME, Nagarkatti-Gude DR, Zhang Z, *et al*. 2016 Mammalian axoneme ter proteins: Broader roles revealed by gene knockout phenotypes. *Cytoskeleton* **73** 3–22

Thomas J, Morlé L, Soulavie F, *et al*. 2010 Transcriptional control of genes involved in ciliogenesis: a first step in making cilia. *Biol. Cell.* **102** 499–513

Tisler M, Wetzel F, Mantino S, *et al*. 2016 Cilia are required for asymmetric nodal induction in the sea urchin embryo. *BMC Dev. Biol.* **16** 28

Uetake Y, Loncarek J, Nordberg JJ, *et al*. 2007 Cell cycle progression and *de novo* centriole assembly after centrosomal removal in untransformed human cells. *J. Cell Biol.* **176** 173–182

van den Hoek H, Klena N, Jordan MA, *et al*. 2022 In situ architecture of the ciliary base reveals the stepwise assembly of intraflagellar transport trains. *Science* **377** 543–548

Veleri S, Manjunath SH, Fariss RN, *et al*. 2014 Ciliopathy-associated gene Cc2d2a promotes assembly of subdistal appendages on the mother centriole during cilia biogenesis. *Nat. Commun.* **5** 4207

Verhey KJ and Yang W 2016 Permeability barriers for generating a unique ciliary protein and lipid composition. *Curr. Opin. Cell Biol.* **41** 109–116

Wallmeier J, Frank D, Shoemark A, *et al*. 2019 De nmvo Mutations in FOXJ1 result in a motile ciliopathy with hydrocephalus and randomization of left/right body asymmetry. *Am. J. Hum. Genet.* **105** 1030–1039

Waters AM and Beales PL 2011 Ciliopathies: an expanding disease spectrum. *Pediatr. Nephrol.* **26** 1039–1056

Wei Q, Zhang Y, Li Y, *et al*. 2012 The BBSome controls IFT assembly and turnaround in cilia. *Nat. Cell. Biol.* **14** 950–957

Weihbrecht K, Goar WA, Pak T, *et al.* 2017 Keeping an eye on Bardet-Biedl syndrome: A comprehensive review of the role of Bardet-Biedl syndrome genes in the eye. *Med. Res Arch.* **5**. https://doi.org/10.18103/mra.v5i9.1526

Westlake CJ, Baye LM, Nachury MV, *et al*. 2011 Primary cilia membrane assembly is initiated by Rab11 and transport protein particle II (TRAPPII) complex-dependent trafficking of Rabin8 to the centrosome. *Proc. Natl. Acad. Sci. USA* **108** 2759–2764

Williams CL, McIntyre JC, Norris SR, *et al*. 2014 Direct evidence for BBSome-associated intraflagellar transport reveals distinct properties of native mammalian cilia. *Nat. Commun.* **5** 5813

Winey M and O'Toole E 2014 Centriole structure. *Philos. Trans. R. Soc. B Lond. Biol. Sci.* **369** 20130457

Wingfield JL, Mengoni I, Bomberger H, *et al*. 2017 IFT trains in different stages of assembly queue at the ciliary base for consecutive release into the cilium. *eLife* **6** e26609

Wingfield JL, Lechtreck KF and Lorentzen E 2018 Trafficking of ciliary membrane proteins by the intraflagellar transport/BBSome machinery. *Essays Biochem.* **62** 753–763

Wong SY, Seol AD, So PL, *et al*. 2009 Primary cilia can both mediate and suppress Hedgehog pathway-dependent tumorigenesis. *Nat. Med.* **15** 1055–1061

Woodruff JB, Wueseke O and Hyman AA 2014 Pericentriolar material structure and dynamics. *Phil. Trans. R. Soc. B* **369** 20130459

Wren KN, Craft JM, Tritschler D, *et al*. 2013 A differential cargo-loading model of ciliary length regulation by IFT. *Curr. Biol.* **23** 2463–2471

Wright RL, Salisbury J and Jarvik JW 1985 A nucleus-basal body connector in *Chlamydomonas reinhardtii* that may function in basal body localization or segregation. *J. Cell Biol.* **101** 1903–1912

Wu C-T, Chen H-Y and Tang TK 2018 Myosin-Va is required for pre ciliary vesicle transportation to the mother centriole during ciliogenesis. *Nat. Cell Biol.* **20** 175–185

Xue B, Liu YX, Dong B, *et al*. 2020 Intraflagellar transport protein RABL5/IFT22 recruits the BBSome to the basal body through the GTPase ARL6/BBS3. *Proc. Natl. Acad. Sci. USA* **117** 2496–2505

Yamazaki H, Nakata T, Okada Y, *et al*. 1996 Cloning and characterization of KAP3: a novel kinesin superfamily-associated protein of KIF3A/3B. *Proc. Natl. Acad. Sci. USA* **93** 8443–8448

Yang J, Liu X, Yue G, *et al*. 2002 Rootletin, a novel coiled-coil protein, is a structural component of the ciliary rootlet. *J. Cell Biol.* **159** 431–440

Yang J, Adamian M and Li T 2006 Rootletin interacts with C-Nap1 and may function as a physical linker between the pair of centrioles/basal bodies in cells. *Mol. Biol. Cell.* **17** 1033–1040

Yin X, Takei Y, Kido MA, *et al*. 2011 Molecular motor KIF17 is fundamental for memory and learning via differential support of synaptic NR2A/2B levels. *Neuron* **70** 310–325

Yoshiba S, Shiratori H, Kuo IY, *et al*. 2012 Cilia at the node of mouse embryos sense fluid flow for left-right determination via Pkd2. *Science* **338** 226–231

Yu X, Ng CP, Habacher H, *et al*. 2008 Foxj1 transcription factors are master regulators of the motile ciliogenic program. *Nat. Genet.* **40** 1445–1453

Zhang Q, Seo S, Bugge K, *et al*. 2012 BBS proteins interact genetically with the IFT pathway to influence SHH-related phenotypes. *Hum. Mol. Genet.* **21** 1945–1953

Zhao H, Zhu L, Zhu Y, *et al*. 2013 The Cep63 paralogue Deup1 enables massive de novo centriole biogenesis for vertebrate multiciliogenesis. *Nat. Cell Biol.* **15** 1434–1444

Zheng X, Ramani A, Soni K, *et al*. 2016 Molecular basis for CPAP-tubulin interaction in controlling centriolar and ciliary length. *Nat. Commun.* **7** 11874

Zou C, Li J, Bai Y, *et al*. 2005 Centrobin: a novel daughter centriole-associated protein that is required for centriole duplication. *J. Cell Biol.* **171** 437–445

Corresponding editor: Thomas John Pucadyil