© 2017. Published by The Company of Biologists Ltd | Journal of Cell Science (2017) 130, 3789-3800 doi:10.1242/jcs.203505

REVIEW

Maintaining centrosomes and cilia

Sascha Werner*, Ana Pimenta-Marques and Mónica Bettencourt-Dias*

ABSTRACT

Centrosomes and cilia are present in organisms from all branches of the eukaryotic tree of life. These structures are composed of microtubules and various other proteins, and are required for a plethora of cell processes such as structuring the cytoskeleton, sensing the environment, and motility. Deregulation of centrosome and cilium components leads to a wide range of diseases, some of which are incompatible with life. Centrosomes and cilia are thought to be very stable and can persist over long periods of time. However, these structures can disappear in certain developmental stages and diseases. Moreover, some centrosome and cilia components are quite dynamic. While a large body of knowledge has been produced regarding the biogenesis of these structures, little is known about how they are maintained. In this Review, we propose the existence of specific centrosome and cilia maintenance programs, which are regulated during development and homeostasis, and when deregulated can lead to disease.

KEY WORDS: Centrosomes, Cilia, Ciliopathies, Maintenance

Introduction

Centrioles, first described in the 1880s by Edouard Van Beneden and Theodor Boveri (see Scheer, 2014), are microtubule (MT)-based cylindrical structures with nine-fold symmetry (Fig. 1A). They are present in most eukaryotes, but have been lost in higher plants, higher fungi and amoeba (Azimzadeh, 2014; Carvalho-Santos et al., 2011; Marshall, 2009). Centrioles can form both centrosomes and cilia. The centrosome is the primary microtubule-organizing center (MTOC) in most animal cells, regulating cell shape, polarity and spindle pole organization. Centrosomes are composed of two centrioles surrounded by a proteinaceous non-membrane-bound compartment called pericentriolar material (PCM, Fig. 1A). Centrioles also form basal bodies in motile and immotile cilia and flagella, which are critical for signaling functions (most cilia), for extracellular fluid flow (e.g. cilia of tracheal cells that move mucus) and for cellular motility (e.g. flagella of sperm cells, Fig. 1B) (Jain et al., 2012; Shah et al., 2009).

Research on these structures took off with the advent of electron microscopy in the 1950s and more recently super-resolution microscopy, genomics, proteomics, RNAi screens and identification of disease-causing mutations (Andersen et al., 2003; Balestra et al., 2013; Dobbelaere et al., 2008; Gönczy et al., 2000; Goshima et al., 2007; Jakobsen et al., 2011; Kamath et al., 2003; Lawo et al., 2012; Lukinavičius et al., 2013; Mennella et al., 2012; Sonnen et al., 2012; Sönnichsen et al., 2005). Hundreds of centrosome and cilia components are now identified, with current efforts focusing on understanding their role in centrosome and cilia biogenesis and function. However, due to experimental limitations, little is known about their maintenance.

In this Review, we briefly summarize centrosomes and cilia assembly and function (for longer reviews, refer to: Brito et al., 2012; Conduit et al., 2015; Garcia-Gonzalo and Reiter, 2017; Ishikawa, 2017; Loreng and Smith, 2017; Reiter et al., 2012; Zhu et al., 2017). We provide a generalized definition of maintenance (Box 1) and use it to explore the evidence for specific centrosomes and cilia maintenance programs during development, homeostasis and disease.

Centrosomes and cilia biogenesis

Centrosome structure and biogenesis

Centrioles are cylinders, normally composed of a proximal part with a cartwheel, which defines their nine-fold symmetry (Fig. 1A). At their distal ends, depending on species and maturity stage, centrioles can have subdistal and distal appendages (Fig. 1A). Upon cilia formation, these are also called transition fibers (Fig. 1B). These appendages play important roles in MT anchoring and in docking basal bodies to the membrane during ciliogenesis, respectively (Vertii, Hehnly, and Doxsey, 2016). On the outside of the centriole barrel, depending on the species, the centriole is composed of singlet, doublet or triplet MTs (A,B and C tubules, Fig. 1A). Recent cryoEM studies have unveiled highly elaborate electron-dense links within the cartwheel, the MTs and between both structures (Guichard et al., 2013; Li et al., 2012). While the function of these links is not known, their localization suggests importance for maintaining the stability of the structure.

In cycling cells, centrosomes are assembled once per cell cycle and their number is tightly controlled (Fig. 1C). However, there are exceptions, such as *de novo* centriole biogenesis observed in the germ line in lower plants or certain insects, or the massive amplification events in multiciliated cells (Meunier and Spassky, 2016). In cycling cells, only one centriole (daughter) is formed orthogonally to each existing one (mother) in S phase, and then elongates throughout S and G2 phases (see Fig. 1 for more details). Each daughter matures in a process called centriole-to-centrosome conversion, which involves cartwheel loss in most studied species and recruitment of PCM during G2 phase. This process is dependent on the centriole component CEP295 (*Drosophila* Ana1) and the mitotic kinase Polo-like kinase 1 (PLK1; Polo in *Drosophila*, see Figs 1 and 2) (Fu et al., 2016; Izquierdo et al., 2014; Wang et al., 2011). At the beginning of the next G1 phase, the mother centriole, now called a 'grandmother', gains distal and subdistal appendages (Kong et al., 2014).

At the G2–M transition, centrosomes recruit more PCM, thereby increasing their MTOC activity. This process is regulated by three mitotic kinases [PLK1, cyclin-dependent kinase 1 (CDK1) and Aurora A (AURKA)], which phosphorylate several components of the centrosome and mitotic apparatus, and also regulate each other (Conduit et al., 2015; Haren et al., 2009). MT nucleation requires high tubulin levels, which is achieved either by increasing the

Cell Cycle Regulation Lab, Instituto Gulbenkian de Ciência, Rua da Quinta Grande, 6, 2780-156 Oeiras, Portugal.

*Authors for correspondence (swerner@igc.gulbenkian.pt; mdias@igc.gulbenkian.pt)

S.W., 0000-0001-8350-2417; A.P., 0000-0001-6318-3922; M.B., 0000-0003-1987-5598

Journal of Cell Science (2017) 130, 3789-3800 doi:10.1242/jcs.203505

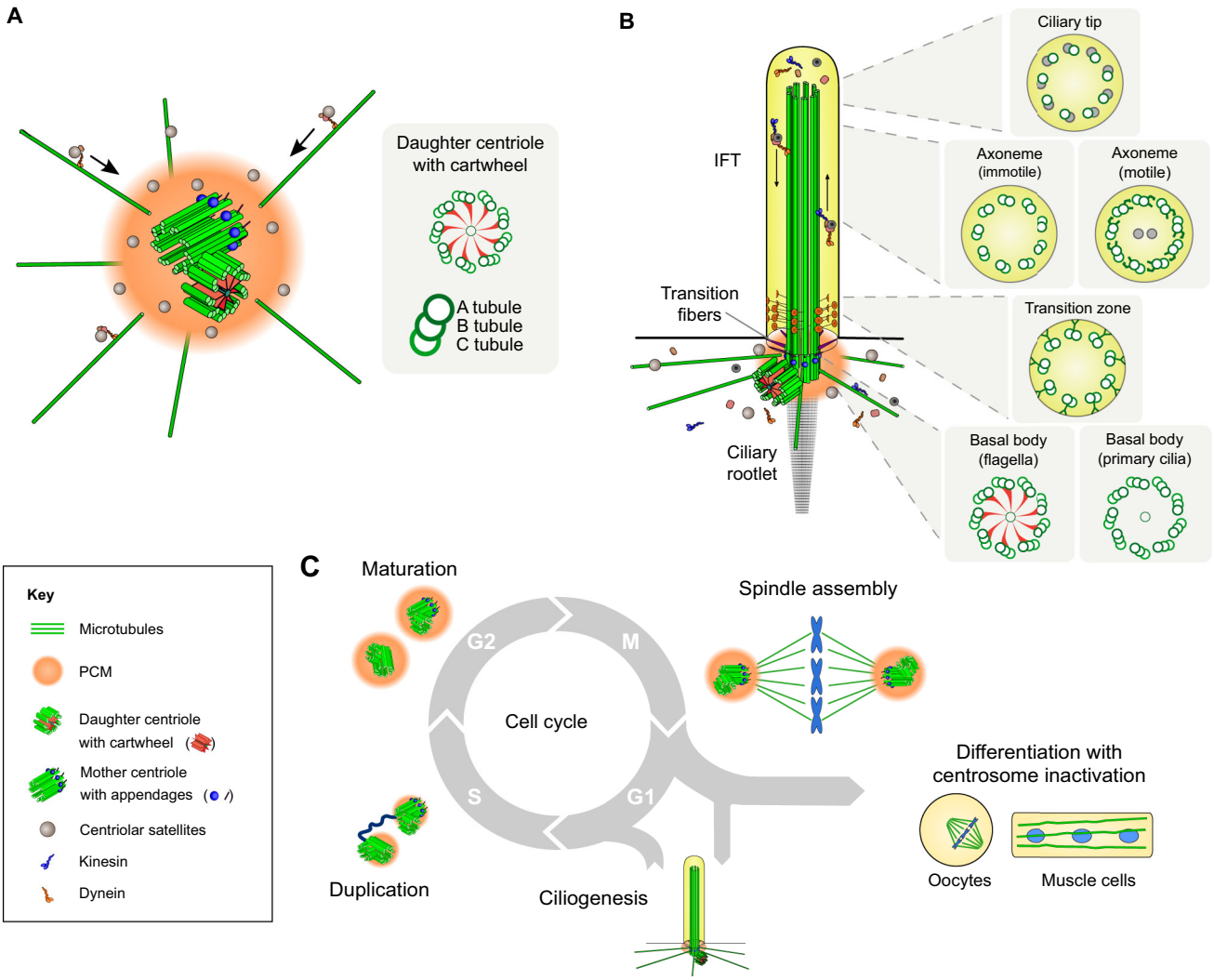

Fig. 1. General architecture of centrosomes and cilia and their interconnectedness throughout the cell cycle. (A) The centrosome is composed of two barrel-shaped microtubule-based centrioles, which are surrounded by a matrix of proteins called the pericentriolar material (PCM). The PCM is required for microtubule (MT) nucleation. Centrioles are duplicated once per cell cycle. The mother centriole is generally distinguished by the presence of subdistal and distal appendages. The daughter centriole contains the cartwheel at its proximal end from which the centriole elongates. The cartwheel is also involved in establishing the ninefold symmetry of the centrioles (cross-section shown in gray box). Small electron-dense spherical granules, called centriolar satellites (CSs), play an important role in the replenishment of centrosomal and/or ciliary components from the cytoplasm to the centrosome. They are focused at the centrosomes through the action of MT-based motor proteins. (B) Cilia generally consist of four subcompartments, which are shown in cross-sections (gray boxes, some variable components are shown in gray). A cilium is formed from a fully mature mother centriole (now called a basal body), which docks to the cell membrane and is embedded in PCM. The axoneme extends from the basal body in the form of doublet MTs. At its base, it contains fibers that link the MTs to the membrane (in the shape of a 'Y' in cross-section). The most distal part of the cilium is called the ciliary tip. Many cilia have a fibrous structure called a rootlet, mostly composed of rootletin, extending from the basal body into the cell. Cilia also carry out intraflagellar transport (IFT), which moves proteins into and within the cilia. Cilia can be divided based on their ability to move. Motile cilia have dynein arms connecting neighboring MT doublets along the axoneme. Often, they also have a central MT pair (in cross-section). (C) Simplified centrosome biogenesis cycle and its coordination with the cell cycle. Centriole duplication occurs in S phase, with the formation of the two daughter centrioles orthogonally to the already existing centrioles. In late G2, the daughter centrioles reach final length and maturation with the recruitment of several molecules to the PCM. Upon mitotic entry, the two centrosomes separate and nucleate MTs leading to the assembly of a bipolar spindle. When the cell exits mitosis, both daughter cells have inherited one centrosome. If the cell goes through a new cell cycle, the centrioles will again duplicate in S phase. Alternatively, cells may not enter a new cell cycle and centrosome inactivation or ciliogenesis might take place as indicated.

concentration of tubulin and MT stabilizers at the centrosome, and/or by increasing the concentration of γ-tubulin, which nucleates MTs and stabilizes them (Woodruff et al., 2017). Other centrosomal proteins are also crucial in localizing γ-tubulin to the PCM, such as neural precursor cell expressed developmentally downregulated protein 1 (NEDD1), pericentrin and CDK5 regulatory subunit-associated protein 2 (CDK5RAP2) (Choi et al., 2010; Fong et al., 2008; Haren et al., 2006; Lüders et al., 2006). After their nucleation, MTs can be anchored to centrosomes by subdistal appendages and/or PCM (Chrétien et al., 1997; Delgehyr et al., 2005; Guo et al., 2006; Ibi et al., 2011; Ishikawa et al., 2005; Mogensen et al., 2000). Alternatively, they can be released and anchored somewhere else, forming a differently shaped cytoskeleton (Ahmad et al., 1999; Muroyama and Lechler, 2017; Sharp and Ross, 2012; Yu et al., 1993).

Cilia structure and biogenesis

Cilia can be highly diverse depending on the tissue and its function (Choksi et al., 2014), and are found in proliferating, quiescent and

Journal of Cell Science (2017) 130, 3789-3800 doi:10.1242/jcs.203505

Box 1. Towards a maintenance concept in cell and molecular biology

The word maintenance is frequently used in cell and molecular biology at several levels of organization over different time scales. Here, we refer to maintenance as all processes in a cell that prevent a given property from deteriorating, and which can occur at any time during the life of the organism. Given that any property can be perturbed either through dilution or damage of components, we envision two different but not exclusive types of maintenance mechanisms. (1) Homeostatic maintenance returns properties to a set point after the physiological dilution of a property, with no insult. For example, the physiological loss of material (e.g. vesicle formation with local membrane composition being altered). (2) Reparative maintenance is activated upon damage, which can arise through external insults or time. This requires a recognition process that identifies impaired subunits and to replace them (e.g. DNA repair after ionizing radiation). It is also possible to avoid the accumulation of damage through turnover. If components are replaced faster than the rate at which damage occurs, then damage might not accumulate to dangerous levels. This might be a common form of maintenance, such as that suggested for synaptic vesicle proteins. Their turnover is regulated in an activity-dependent manner to prevent accumulation of damaged proteins, which could lead to synaptic dysfunction and neuron degeneration (Sheehan et al., 2016). Under some circumstances, a cell might require the loss of a property that would be otherwise maintained. In this case, the maintenance machinery will have to be disabled.

differentiated cells. Whether the presence of cilia and cell cycle progression regulate each other depends on the species and the tissue; however, formation of cilia in cultured cells is often enhanced by serum starvation and entry into quiescence. In these cells, cilia are often resorbed upon cell cycle re-entry (Kim et al., 2011; Li et al., 2011). Differentiating cells form diverse cilia types after their exit from the cell cycle and are induced to form cilia by the expression of specific transcription factors, such as FOXJ1 in the case of motile cilia (Choksi et al., 2014). Cilia are sometimes reabsorbed during differentiation, as observed in muscle cells, lymphocytes and hepatocytes (Fu et al., 2014; Stinchcombe et al., 2015; Wheatley et al., 1996).

Once ciliogenesis is triggered, the mother centriole recruits RAB11-positive vesicles that transfer membrane material from the Golgi to the distal appendages. This membrane forms a cap structure, called the ciliary vesicle, at the distal end of the centriole. This process has been termed centriole-to-basal-body conversion (Kobayashi and Dynlacht, 2011). Subsequently, basal body MTs (A and B tubules) start elongating to form the axoneme, the ciliary 'skeleton' (Fig. 1B). The ciliary vesicle and the cell membrane fuse, giving rise to the cilium. In some cell types, however, the axoneme only starts elongating once the basal body docks at the membrane (Garcia-Gonzalo and Reiter, 2012). The proximal part of the ciliary axoneme, called the transition zone (TZ), shows an electron-dense structure in the shape of a 'Y' (so-called Y-links) by EM. The transition fibers and the TZ together form the 'ciliary gate', a protein-dense 'molecular sieve' that restricts protein entry into the ciliary cytoplasm by limiting diffusion (Garcia-Gonzalo and Reiter, 2017; Takao and Verhey, 2016). Many causal mutations reported in human patients with ciliopathies are found in TZ proteins (Braun and Hildebrandt, 2017). Nuclear pore proteins and several membrane-associated septins localize to the ciliary base, where they may create a diffusion barrier between both cytoplasm and cilioplasm, and the ciliary and plasma membranes, respectively (Hu et al., 2010; Palander et al., 2017; Takao et al., 2014; Takao and Verhey, 2016). These barriers control the enrichment of membrane and cytoplasmic proteins at the cilium, making it an ideal compartment to initiate signaling cascades through amplification of cues from the environment (Takao and Kamimura, 2017). Cilia potentially also integrate stimuli from different signaling pathways (Christensen et al., 2007; Hu et al., 2010; Lancaster and Gleeson, 2009). The full complexity of ciliary transport is still under investigation (Takao and Verhey, 2016) as nuclear proteins were found to localize too far away from the TZ to form a diffusion barrier (Del Viso et al., 2016). It was thus suggested that they only have a scaffold function at the ciliary base (Del Viso et al., 2016). Furthermore, membrane proteins and soluble proteins use distinct mechanisms for ciliary entry (Takao and Verhey, 2016).

The diffusion barrier poses a problem for cilium assembly and function as proteins are not translated and are probably also not degraded inside cilia. Intraflagellar transport (IFT; refer also to Box 3) via a multiprotein complex was initially described within the flagella of the green algae *Chlamydomonas* (Kozminski et al., 1995, 1993), but transport of molecules in and out of cilia also occurs in almost all other cilia types. IFT is required in most eukaryotes to form cilia and to regulate their length (Marshall et al., 2005; Marshall and Rosenbaum, 2001; Pazour et al., 2000); different combinations of subunits form the complex in different species (van Dam et al., 2013), as reviewed extensively elsewhere (Ishikawa and Marshall, 2011; Lechtreck, 2015; Mourão, et al., 2016; Prevo et al., 2017; Taschner and Lorentzen, 2016). IFT is mediated by two subcomplexes that bind either kinesin II or cytoplasmic dynein and move anterogradely or retrogradely, respectively, along the axoneme. Many cargo proteins, including tubulin, have been identified (Bhogaraju et al., 2013; Taschner and Lorentzen, 2016). Recently, IFT-independent transport processes have also been described in *Chlamydomonas* cilia (Harris et al., 2016) and IFT-independent assembly of cilia in the cytoplasm was shown in *Drosophila* spermatogenesis (Han, et al., 2003; Sarpal et al., 2003). The individual contributions of these different transport modes for cilia assembly and function are not fully understood. Cilia also form additional structures such as the ciliary rootlet, which is not required for ciliogenesis, but contributes to ciliary maintenance (Chen et al., 2015; Mohan et al., 2013; Yang et al., 2002), as discussed below.

Evidence for the existence of active maintenance programs for centrosomes and cilia

Any given biological structure, from organelles to cells and whole organisms has to be assembled and maintained. The word 'maintenance' has been used in different ways (Box 1). We define maintenance as all processes that prevent the structural and functional properties of centrosomes and cilia from deteriorating after their full assembly. Most of the examples we describe here relate to homeostatic maintenance through turnover (see Box 1).

Mechanistic research on maintenance has been scarce, as germline mutations or ubiquitous RNA interference (RNAi) lead to assembly defects, precluding further studies on maintenance. Recent work on protein life span showed that structures such as the nuclear pore complex are maintained over the lifetime of a cell and active exchange of constituent subcomplexes takes place (Toyama et al., 2013). This indicates that even very long-lived protein complexes are likely to require both homeostatic and reparative maintenance (see Box 1 for concepts). Although it is formally possible that centrosomes and cilia are very stable and do not require any maintenance, this should limit the plasticity of these structures, precluding their adaption to different environmental challenges and their disassembly under physiological conditions. However, there is clear evidence for their inactivation and/or disassembly at different stages in development. Here, we review such evidence and advocate for specific maintenance programs.

Journal of Cell Science (2017) 130, 3789-3800 doi:10.1242/jcs.203505

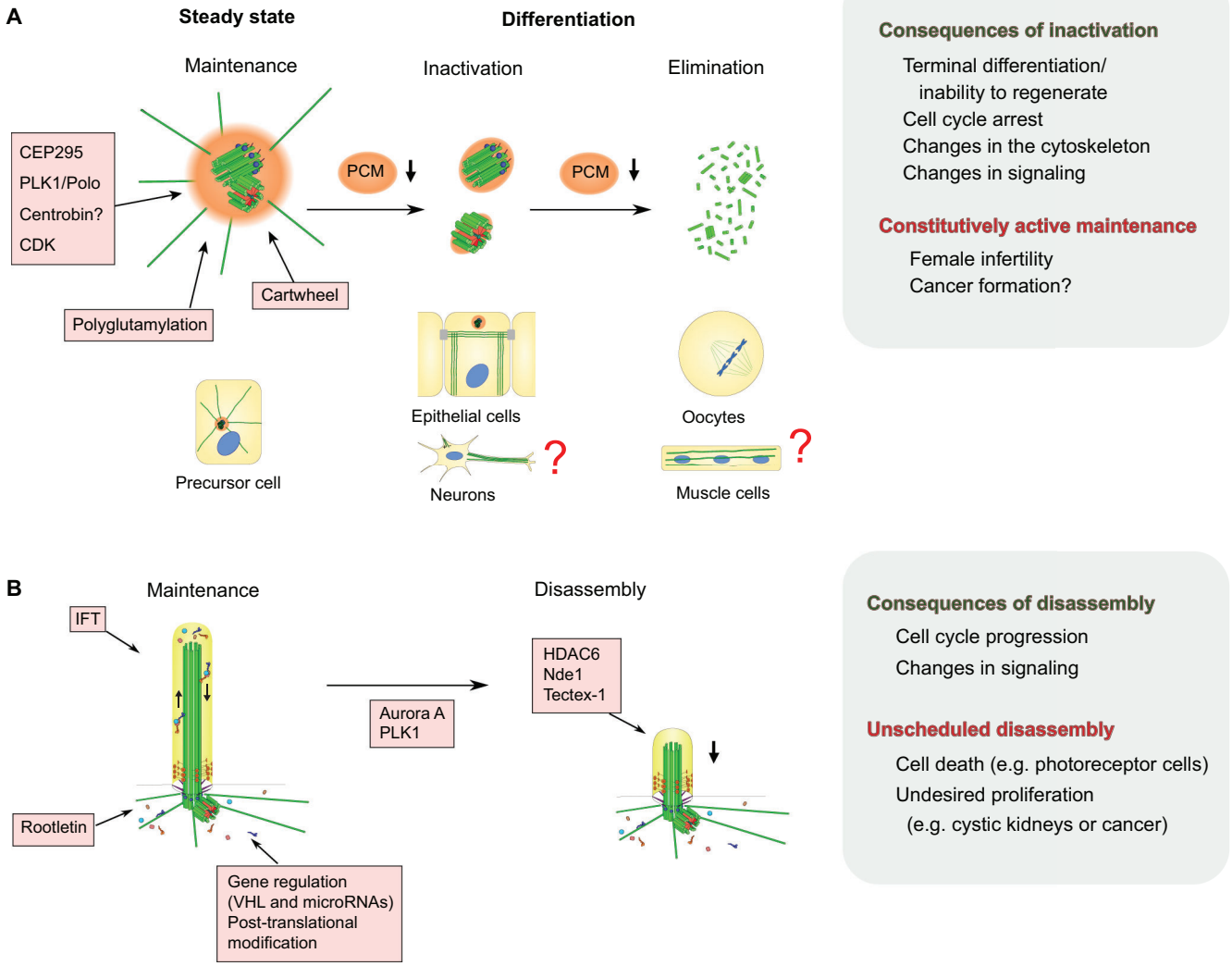

Fig. 2. Molecular factors involved in the regulation of centrosome and cilia maintenance programs. (A) Known factors in centrosome maintenance. Both the pericentriolar material (PCM, orange) and the cartwheel (red) protect the integrity of the centrioles. PLK1 (Polo in *Drosophila*) and cyclin-dependent kinase (CDK) are involved in activating the centrosomal microtubule-organizing center (MTOC). Additionally, Polo prevents loss of PCM from the centrioles. The centriolar protein CEP295 protects centriole disassembly by anchoring PCM during the process of centrosome maturation. Post-translational modifications (PTMs), such as polyglutamylation of the tubulin at centriole walls confer additional stability to the centriolar structure. Centrobin, another centrosomal protein, which binds to tubulin, also has a role in centriole integrity, although the mechanism is not known. Upon differentiation, different cell types, such as oocytes, epithelial, muscle and neuronal cells, naturally inactivate and/or eliminate centrosomes. The programmed inactivation of centrosomes is illustrated as a two-step process, i.e. loss of PCM which leads to loss of MTOC activity, which, in some cell types, is followed by loss of the centriole. It is not yet clear whether in neurons, muscle and some epithelial cell types, centrosomes only lose their MTOC activity or also disappear entirely. The significance of centrosome inactivation and/or loss in the differentiation of these cell types is poorly understood (question marks). Potential consequences of centrosome inactivation or of constitutive maintenance (failed centrosome inactivation) for a cell are highlighted on the right. It is still not clear whether centrosomal MTOC activity in cancer cells with supernumerary centrosomes is modulated in order to allow the viability of these cells. (B) Ciliary functions at steady state. Different factors are involved in cilia maintenance. Intraflagellar transport (IFT) maintains cilia integrity through the transport of cargoes into the cilia and is hence essential for turnover of ciliary proteins. Rootletin, the main component of the ciliary rootlet, is involved in the functional and structural maintenance of cilia, but the underlying mechanism is not yet clear. As for centrosomes, PTMs of cilia microtubules (MTs) also appear to be important for cilia integrity. Gene regulation has also been implicated in maintaining cilia. MicroRNAs and the tumor suppressor gene (VHL) were found to be required for maintenance of cilia in photoreceptor cells and kidney, respectively. However, in certain scenarios (including re-entry into the cell cycle or changing responsiveness to signaling molecules), the cilium needs to be disassembled. Both Nde1 (mother centriolar protein) and Tectex-1 (recruited to the cilia transition zone) inhibit ciliogenesis, allowing cilia resorption and progression into S-phase. Aurora A and PLK1 kinases also mediate cilia reabsorption through the activation of HDAC6. Potential consequences of cilia maintenance failure are highlighted on the right.

Conceptually, biogenesis and maintenance could use either completely independent programs or overlapping programs with differential regulation at different time points. Within that framework, three groups of molecules might exist, those required for biogenesis, those necessary for maintenance and those involved in both processes (Fig. 3). Loss of biogenesis molecules leads to impaired formation of a given structure. In contrast, inactivation of molecules involved in maintenance specifically, will allow biogenesis to proceed to a functional end product. However, the function of the structure will eventually deteriorate and the structure may even be lost entirely (Fig. 3). Can deregulation of maintenance arise if the ‘maintenance machinery’ is also involved in biogenesis?

Journal of Cell Science (2017) 130, 3789-3800 doi:10.1242/jcs.203505

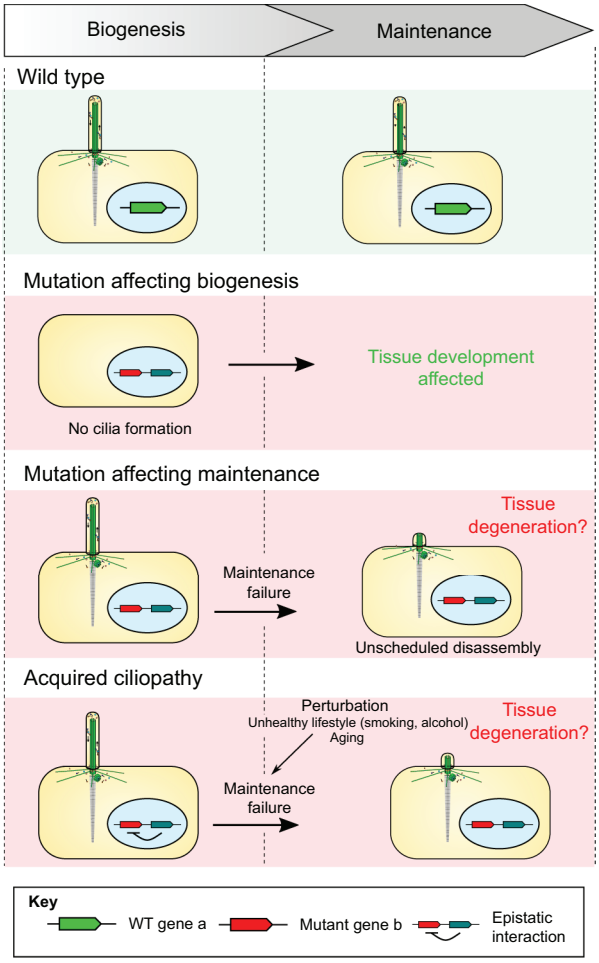

Fig. 3. Different scenarios that can give rise to maintenance defects. Cilia are chosen here for illustration purposes, but the different scenarios shown here should also apply to other structures in the cell. Cilia play an integral role in the development of many cell lineages and/or tissues. Maintenance of the cilium is important to maintain the particular cellular properties (e.g. responsiveness to signaling molecules). For simplicity, we represent distinguishable scenarios for biogenesis and maintenance defects. However, it is conceivable that biogenesis and maintenance can be affected simultaneously, giving rise to a similar onset and strength of phenotypes. Mutations affecting cilia biogenesis (second row) are known to interfere with cell differentiation and therefore with proper tissue function. It is becoming more and more apparent, however, that the effect of certain mutations can be compensated for or buffered by epistatic interactions through modifier genes. These modifiers can determine the onset and strength of phenotypes as well as the tissue that is affected. Mutations specific to cilia maintenance (third row) will allow cell lineage or tissue differentiation to occur normally (i.e. give rise to fully functional cells initially), but will lead to compromised function later on. An acquired ciliopathy (bottom row) may arise from an external perturbation, which might interfere directly with cilia maintenance. These perturbations might disrupt epistatic interactions, hence uncovering gene variants that on their own are insufficient to maintain cilia function.

One possibility is that mutations have different thresholds for their phenotypic manifestation in biogenesis and maintenance (Fig. 3). Alternatively, the biogenesis phenotype of a certain mutation might be compensated by epistasis (i.e. suppression of the effect of a particular mutation in one gene through the interaction with a different gene; Fig. 3). Such compensation might be compromised by a variety of scenarios. For example, in *Caenorhabditis elegans*, hypomorphic IFT mutants can recover from cilia phenotypes arising early in development in a chaperone-dependent manner. This compensation, however, fails with aging (Cornils et al., 2016). Other perturbations, including increased demand on tissue activity, age, stress, additional acquired mutations and environmental perturbations (e.g. smoking, alcohol consumption), are also likely to interfere with compensatory mechanisms and thus lead to phenotypic manifestation of mutations after biogenesis. Epistasis can thus explain the presence of disease-causing mutations in apparently healthy individuals (Novarino et al., 2011). In fact, the genetic background strongly influences phenotype onset in mouse models of cystic kidneys and in human patients (Lehman et al., 2008; Davis et al., 2011; Khanna et al., 2009), suggesting the existence of modifier gene variants.

Centrosome maintenance

Historically, centrioles have been regarded as exceptionally stable structures. They are resistant to drug- and cold-induced MT depolymerization (Kochanski and Borisy, 1990), to forces and MT instability in mitosis (Belmont et al., 1990). Furthermore, fluorescence recovery after photobleaching (FRAP) of the basal body α-tubulin in *Tetrahymena* shows little turnover (Pearson et al., 2009). An elegant experiment in *C. elegans* demonstrated that, upon fertilization with sperm containing a single paternally contributed centriole that has been labeled with SAS4 tagged to GFP to mark the centriolar walls, could be detected up to the ∼350-cell stage after fertilization (Balestra et al., 2015), suggesting centrioles are stably inherited through many divisions.

However, centrosomes are lost from oocytes of most metazoan species (Delattre and Gönczy, 2004; Manandhar et al., 2005) and are known to be inactivated (i.e. loss of their MTOC capacity) in some cell types that undergo differentiation, such as neuronal, muscle and epithelial cells (Sanchez and Feldman, 2017). Upon neuronal differentiation in mammals and *Drosophila*, centrosomes lose PCM proteins and, consequently, their MTOC capacity (Stiess et al., 2010; Nguyen et al., 2011). Axon extension can occur in the absence of active centrosomes in mammals and in *Drosophila* (Tassin et al., 1985; Stiess et al., 2010; Nguyen et al., 2011). In myocyte differentiation, centrosomes lose PCM proteins and were described as absent from muscle fibers (Srsen et al., 2009; Przybylski, 1971). At the same time, PCM proteins accumulate at the nuclear periphery from which MTs are nucleated (Srsen et al., 2009; Przybylski, 1971). Several epithelial cell types also inactivate MT nucleation and/or abolish their anchoring from centrosomes and so generate MTs along the apical–basal axis (Sanchez and Feldman, 2017; Muroyama and Lechler, 2017). These lines of evidence suggest that the centrosome is under a homeostatic maintenance program that can be regulated, thereby giving rise to different MT arrays.

Moreover, several centriole components are dynamic, such as centrin in the lumen of the centriole (Bahmanyar et al., 2010), spindle assembly abnormal protein 6 (SAS6) in the cartwheel (Keller et al., 2014) and centrosomal protein 120 (CEP120) at the centriolar wall (Mahjoub et al., 2010). Therefore, a picture is emerging whereby a general homeostatic maintenance program (Box 1) exists for centrosomes that might underlie both their stability in cycling cells (Izquierdo et al., 2014) and their instability to a certain extent in some tissues, such as oocytes, neurons and epithelial cells (Pimenta-Marques et al., 2016; Yonezawa et al., 2015; Muroyama et al., 2016).

The centrosome homeostatic maintenance program depends on critical aspects of its structure, such as the PCM, the centriole walls and the centriole cartwheel, and is under the control of cell cycle regulators, such as CDKs and PLKs (Yang and Feldman, 2015; Muroyama et al., 2016). These kinases are known to regulate

Journal of Cell Science (2017) 130, 3789-3800 doi:10.1242/jcs.203505

centrosome biogenesis, maturation and function; they are degraded or inactivated, respectively, by the anaphase-promoting complex, also known as the cyclosome (APC/C) at the end of mitosis (Ferris et al., 1998; Glotzer et al., 1991; Lindon and Pines, 2004; Murray, 1989). A high activity of CDKs and PLKs is needed for an active mitotic centrosome, whereas a lower activity of those kinases is often associated with centrosomes of cells in interphase or those that have exited the cell cycle. Finally, no activity of these kinases is observed when the centrosome has been fully inactivated, which is often associated with centriole loss (Fig. 2A).

We next discuss in more detail different components of the centrosome maintenance program.

In human cultured cells, newly formed centrioles that have been blocked from maturing into centrosomes by removal of CEP295 disassemble at the end of the cycle upon cartwheel loss (Izquierdo et al., 2014). Inhibition of PLK1 retained the cartwheel (Wang et al., 2011) and rescued the loss of non-matured centrioles (Izquierdo et al., 2014). Matured centrioles, however, even though they normally lose the cartwheel at the end of the cell cycle, do not disassemble because they have PCM. This suggested that both the PCM and cartwheel are redundant in conferring stability to centrioles and compensate for each other in centrosome protection. Such a redundancy might not exist in all species and/or tissues. In S-phase-arrested *Drosophila* cells, depletion of four major PCM proteins (SPD-2, CNN, Asl and D-PLP) or of Polo (the ortholog of PLK1), was sufficient to lead to a reduction in centriole number, demonstrating that centrosomes are maintained homeostatically through the renewal of their components (Pimenta-Marques et al., 2016).

Centriole wall components and their post-translational modifications are also important for centriole stability. Injection or electroporation with an antibody against tubulin glutamylation (α-GT335) resulted in the disappearance of centrioles and centrosomes (Bobinnec et al., 1998). In this case, centrioles and discrete centrosomes ultimately reappeared in the cell population; however, some centrioles exhibited loss of the MT triplets (Bobinnec et al., 1998), which are characteristic of normal centrioles (Fig. 1A). It is possible that glutamylation itself stabilizes the centriolar MT structure or promotes the binding of stabilizers. On that note, it was shown that downregulation of ATF5, which interacts with both glutamylated tubulin and pericentrin, thereby linking the centriole to the PCM, blocks the accumulation of PCM at the centrosome and causes the fragmentation of centrioles (Madarampalli et al., 2015). Centrobin, another factor that binds to centriolar tubulin and is normally associated with daughter centrioles (Gudi et al., 2011; Zou et al., 2005), promotes centriole elongation and prevents PCM recruitment in cultured cycling cells. Expression of dominant-negative centrobin led to an increase in the number of cells without centrioles (Gudi et al., 2011). Finally, in certain species, δ- and ε-tubulins found on centrioles contribute to the formation and/or stability of the triplet MTs (reviewed in Winey and O'Toole, 2014). All together, these studies suggest that there is an interplay between the cartwheel, the centriole walls (including the post-translational modifications of tubulin and centrobin function) and the PCM in supporting centriole stability.

Recent studies show that inactivation of the PCM leads to scheduled centrosome inactivation (e.g. in neurons, muscle and epithelial cells) or even their entire disappearance (e.g. in oocytes, Fig. 2A). Originally observed by Huettner in 1933, it is now known that oocytes of multiple species lose their centrosomes during meiosis (Huettner and Rabinowitz, 1933; Manandhar et al., 2005), which is achieved in different ways in different organisms (see Box 2). In *Drosophila*, during early oogenesis, a cyst of 16 interconnected cells is formed; of these, one becomes the oocyte and inherits all centrioles by intercellular centriole migration, which results in a large MTOC consisting of 64 centrioles. Polo and some PCM components such as SPD-2 are transcriptionally downregulated before centrioles disappear (Jambor et al., 2015). This is correlated with loss of Polo and PCM proteins from the oocyte MTOC, followed by centriole disappearance before the egg divides (Pimenta-Marques et al., 2016). Forced localization of Polo to centrioles in oogenesis resulted in PCM maintenance and, consequently, persistence of the centrioles (Pimenta-Marques et al., 2016) (Fig. 2A). These findings in oocytes point to the importance of the active recruitment of newly synthesized Polo and PCM components to the centrosome, further supporting the existence of a regulated homeostatic maintenance program that can be switched off.

In the human body, there are several other examples of cells, in which centrosomes are either partially or completely inactivated. In some of these cases centrioles persist, while in others there is no conclusive evidence. For example, during differentiation of skeletal muscle, centrioles are inactivated upon fusion of myoblasts to give rise to the syncytial myotubes. Here, proteins such as γ-tubulin, pericentrin and ninein are captured by nesprin at the nuclear envelope, from which MTs are nucleated and extend, resulting in the formation of longitudinal MT bundles along the long axis of the cell (Tassin et al., 1985; Espigat-Georger et al., 2016). In differentiating hippocampal neurons, centrosome inactivation is associated with loss of γ-tubulin, pericentrin and centrin from the centrosome (Stiess et al., 2010). MTs are generated by augmin- and γTuRC-dependent nucleation from existing MTs. This ensures a uniform plus-end-out MT polarity in axons (Sánchez-Huertas et al., 2016).

Box 2. Centrosome elimination is essential in oogenesis of most metazoan species

Centrosomes are eliminated from the oocytes of the majority of metazoan species (Manandhar et al., 2005), allowing for a correct number of centrosomes to be attained after fertilization. In fruit flies, worms and humans, centrioles are eliminated prior to meiotic division, one of the few acentriolar divisions in those species (Delattre and Gönczy, 2004; Manandhar et al., 2005; Cunha-Ferreira et al., 2009; Mikeladze-Dvali et al., 2012). In *Drosophila*, a centrosome maintenance program was elucidated that depends on Polo and PCM, which is turned off during oogenesis (Pimenta-Marques et al., 2016; see main text for discussion). In some species, centrioles are eliminated together with DNA through their extrusion inside the polar bodies during meiotic divisions, such as in snail oocytes, which only contain a single pair of centrioles (Krioutchkova et al., 1994). Interestingly, echinoderms (sea urchin, starfish and sea-cucumber) use both strategies to eliminate centrioles: extrusion and elimination. These species enter meiosis with two pairs of centrioles, one at each pole of meiosis I spindle. One pair is extruded through the polar body I (PBI). Subsequently, single centrioles are present on the spindle poles of meiosis II; of these, the mother, which has MT nucleation capacity, is extruded with PBII (Borrego-Pinto et al., 2016), leaving a single centriole in the mature egg (Kato et al., 1990; Miyazaki et al., 2005; Nakashima and Kato, 2001). The remaining centriole is eventually eliminated, but it is not known exactly how this is achieved. If centrioles are artificially retained, they cannot be inactivated, resulting in multipolar zygotic spindles (Borrego-Pinto et al., 2016). The retained daughter centriole does not nucleate MT, perhaps because of insufficient levels of PCM, which may cause centriole destabilization. Perhaps mother and daughter centrioles have to be eliminated in different ways, because these cells have no mechanism to actively remove PCM. Future studies are needed to understand whether different species use similar mechanisms to inactivate centrosome maintenance programs and achieve centrosome elimination.

Journal of Cell Science (2017) 130, 3789-3800 doi:10.1242/jcs.203505

However, in both muscles and neurons, is not clear whether centrioles eventually disappear and what would happen if centrosome activity was maintained.

During epithelial differentiation, centrosomes often cease to be the major MTOC in the cell when acentrosomal MTOCs are established. Loss of CDK1 activity appears to be a major trigger for this change (Muroyama et al., 2016). An interesting example is proliferative basal cells of the mammalian epidermis; here, MTs are recruited to the cell cortex upon differentiation. Loss of CDK1 activity upon exit from the cell cycle results in several changes at centrosomes and in the cell. MTs continue to be nucleated by CDK5RAP2–γ-TuRC complexes at the centrosome, whereas Nedd1–γ-TuRC complexes, which are required for MT anchoring in this system, rapidly delocalize from centrosomes, leading to a loss of astral MT configuration (Fig. 2A) (Muroyama, et al., 2016). This study suggests that different populations of γ-TuRCs have distinct functions and are regulated differently. Loss of centrosomal MTOC activity in these cells is associated with loss of pericentrin and γ-tubulin from centrosomes, but centrioles are not completely eliminated (Fig. 2A) (Muroyama et al., 2016). Similarly, during cell differentiation of *C. elegans* embryonic intestinal cells, MTOC function is reassigned to the apical membrane after downregulation of CDK-1. Interestingly, in this case, cells can divide after differentiation. Reactivation of the centrosomal MTOC is dependent on the conserved centrosome protein spindle-defective protein 2 (SPD-2; CEP192 in humans) and mitotic CDK activity (Yang and Feldman, 2015).

Taken together, it is possible that kinases, such as PLK1 and CDK1, function both as regulators of centrosome activity and maintenance, which makes it difficult in some cases to establish clear boundaries between both processes (Muroyama et al., 2016). Upregulation of PLK1 and CDK1 in mitosis leads to centrosome maturation and increased MT nucleation. However, their presence in interphase and in many differentiated cells is necessary for centrosome maintenance and even nucleation, with their absence leading to centrosome inactivation and disappearance in *Drosophila* (Fig. 2A). Future work will hopefully unravel how this program is regulated at the transcriptional and post-transcriptional level and how dynamic the PCM and centriole proteins actually are.

Cilia maintenance

Different organisms and cell types are likely to differently regulate the structural and functional maintenance of cilia. Although certain long-lived differentiated cells harbor stable cilia such as photoreceptors, cilia are known to assemble and disassemble in cycling cells (Fig. 2B). The photoreceptor outer segment (a modified cilium) is completely replaced in a matter of days and hence requires continuous maintenance (Besharse and Hollyfield, 1979; Hsu et al., 2017). The integrity of the cilium, both as a structural and signaling compartment, is critical for its function (Fig. 2B). It is likely that modifications that make the structure more robust, as well as the synthesis of ciliary components and their transport into the cilia, are all important factors for their maintenance (see also Box 3). As for centrioles, post-translational modifications of tubulin play a role in stabilizing axonemal MTs in cilia. Mutation in the tubulin deglutamylase CCPP-1 in *C. elegans* leads to a progressive degeneration phenotype of the axonemal MTs (O'Hagan et al., 2011). These worms appear to form normal cilia in early larval stages, but cilia defects arise over time (O'Hagan et al., 2011). Tubulin glycylation is not required for retina development in the mouse, but the photoreceptors degenerate with age if tubulin glutamylation and glycylation are not properly balanced (Bosch Grau et al., 2017) (Fig. 2B). The authors propose that this phenotype is linked to the inability of the photoreceptor to appropriately adapt to mechanical load (Bosch Grau et al., 2017).

Box 3. *Chlamydomonas* – what a green alga taught us about cilia maintenance

Important insights into homeostatic cilia maintenance came from the bi-flagellated green alga *Chlamydomonas reinhardtii*. *Chlamydomonas* flagella (hereafter referred to as cilia) can be biochemically isolated (Witman et al., 1972) and since the late 1970s, a collection of temperature-sensitive mutants has been available that allow to acutely disassemble cilia (Huang et al., 1977). These tools led to the identification of genes and mechanisms involved in cilia maintenance. In particular, it was shown that a previously described intra-ciliary 'motility' (Kozminski et al., 1993) is dependent on kinesin-driven motor movement (Kozminski et al., 1995). This motility is now called intraflagellar transport (IFT) and is known to consist of a protein complex of 22 subunits (Piperno et al., 1998; Taschner and Lorentzen, 2016; Vashishtha et al., 1996). Acute removal of IFT leads to cilia resorption, which demonstrated that this complex is important for cilia maintenance. Live-imaging and modeling revealed that cilia length is dynamically controlled through tubulin turnover (Marshall and Rosenbaum, 2001; Marshall et al., 2005). Blocking IFT prevents the addition of new tubulin at the tip and causes cilia shortening (Marshall and Rosenbaum, 2001). However, only 12 years later, it was demonstrated that tubulin is in fact a bona fide IFT cargo in several species (Bhogaraju et al., 2013). Core IFT components are conserved in other species, and when mutated, can cause phenotypes similar to those seen in human pathologies (Pazour et al., 2000, 2002). Maintenance of protein composition in cilia, however, goes beyond tubulin transport: 20% of all axonemal and membrane proteins turn over within 6 hours in *Chlamydomonas* (Song and Dentler, 2001). At least some of these could be IFT cargoes, suggesting that IFT maintains additional ciliary properties apart from length. More recently, however, it was demonstrated that not all *Chlamydomonas* proteins depend on IFT for their ciliary localization (Harris et al., 2016). This opens the possibility that ciliary properties are maintained through both IFT-dependent and IFT-independent mechanisms.

The maintenance of cilia, as for centrioles, is also likely to require continuous transcription and translation of ciliary components. Evidence for this comes from a study on the role of miRNAs in photoreceptor maintenance in mice; here, animals in which miRNA182 and miRNA183 were deleted formed fully functional photoreceptors in the first weeks after birth; however, they exhibited specific defects in the maintenance of those cells (Busskamp et al., 2014). In the same study, a number of gene targets of miRNA182 and miRNA183 were annotated as associated with cilia and/or centrosomes, and could thus be relevant for maintenance of photoreceptors, and more generally, cilia (Fig. 2B). Additionally, the nuclear activity of the tumor suppressor gene VHL was linked to cilia maintenance in the kidney (Thoma et al., 2007). Here, loss of VHL and the subsequent reabsorption of cilia allows cells to re-enter the cell cycle and leads to cyst formation (Fig. 2B). However, it is unclear through which genes VHL controls cilia maintenance.

IFT is likely to have important roles in the maintenance of both the ciliary structure and the integrity of the cilium as a signaling compartment (Box 3). IFT occurs constantly, even in fully formed cilia, as shown by FRAP experiments and other live-cell imaging techniques (Hu et al., 2010; Milenkovic et al., 2015; Trivedi et al., 2012; Ye et al., 2013). In green algae, inhibition of IFT led to shortening of cilia that initially functioned normally (Marshall and Rosenbaum, 2001; Marshall et al., 2005). This raised the notion that ciliary proteins need to be replenished constantly in order to maintain flagella length (Marshall et al., 2005). Indeed, removal of IFT by gene deletion specifically in the retina of adult mice leads to photoreceptor degeneration (Jiang et al., 2015). Amongst the IFT

cargoes, tubulin, the main component of the ciliary axoneme, was identified to be transported by the IFT complex proteins IFT81 and IFT74 in different species (Bhogaraju et al., 2013), and directly by kinesin-2 in *Drosophila*, which does not have these IFT subunits (Girotra et al., 2017; van Dam et al., 2013). However, not all systems may depend equally on IFT for cilia maintenance, as fully formed flagella of *Trypanosoma* show IFT-mediated motility, but, in this case, the IFT complex is only required to maintain flagellar function and not their structural integrity (Fort et al., 2016). In addition, *Drosophila* sperm does not need IFT either for biogenesis or for maintenance (Han et al., 2003; Sarpal et al., 2003) and IFT is absent from mature mouse sperm and hence is also not required for its maintenance (San Agustin et al., 2015). Further insights into how IFT regulates the maintenance cilia function can be gained from hedgehog signaling. Like many other signaling pathways, hedgehog signaling requires cilia (Bangs and Anderson, 2017). The G-protein-coupled receptor smoothened initiates hedgehog signaling when it localises to the ciliary membrane. In mice, IFT27 was shown to be required to keep unstimulated cilia in an off state by exporting smoothened out of the cilium (Eguether et al., 2014); however, IFT27 is frequently lost from the genomes of ciliated species (van Dam et al., 2013). Since IFT27 is required for hedgehog function (Eguether et al., 2014), it is unlikely that IFT maintains hedgehog signaling capacity in those systems. In fact, in *Drosophila*, hedgehog signaling is mostly independent of cilia (Han et al., 2003; Sarpal et al., 2003), with the exception of its role in olfaction (Kuzhandaivel et al., 2014; Sanchez et al., 2016). This suggests that the properties that are maintained by IFT depend on the species and perhaps also vary among the cilia within one organism.

The role of IFT in ciliary maintenance may also be regulated by the ciliary rootlet. In *C. elegans* rootletin mutants, which do not form rootlets but assemble cilia and IFT complexes (Mohan et al., 2013), IFT particles move at a lower speed and the cilia eventually degenerate. In *Drosophila*, no structural defects were found in rootletin mutants, but ciliary dysfunction was observed as well (Chen et al., 2015). These findings indicate that the ciliary rootlet is required for homeostatic maintenance of cilia function, but not for its structural integrity. In the future, it will be important to understand which IFT cargoes are important for maintaining cilia structure compared with its signaling capacities, as well as how transport is regulated by the rootlet.

Finally, more recently, cilia were shown to produce extracellular vesicles (often referred to as exosomes) in an actin-dependent manner (Nager et al., 2017). These exosomes are biologically active and are required for hatching in *Chlamydomonas* (Wood et al., 2013) or are used in communication between individual *C. elegans* (Wang et al., 2014). Exosomes can also return cilia to an inactive state after activation (Nager et al., 2017) and might be involved in maintaining other ciliary properties.

Impact of cilia and centrosome maintenance on regeneration and disease

Given the multiple functions of centrosomes and cilia, it is likely that perturbations of their maintenance, arising either physiologically or from disease, affect the cytoskeleton and movement of a cell, as well as its signaling. Little is known about the consequences of deregulation of maintenance mechanisms. Recently, however, some ideas emerged regarding the consequences of these mechanisms for regenerative capacities, cancer and fertility, which we discuss below.

Centrosome loss in somatic cells, which is driven by the inhibition of centriole duplication, activates p53, thereby inhibiting proliferation (Fong et al., 2016; Lambrus et al., 2016; Meitinger et al., 2016). However, upon concomitant removal of p53 and inhibition of centriole duplication, human and mouse cells continue to proliferate (Bazzi and Anderson, 2014; Fong et al., 2016; Lambrus et al., 2016; McIntyre et al., 2012; Meitinger et al., 2016). It is therefore possible that this p53-dependent pathway prevents the propagation of aneuploid cells. Moreover, it may 'lock' terminally differentiated cells that did not maintain their centrosomes in a permanent non-proliferative state.

Interestingly, it was recently shown that regulation of centrosome maintenance has consequences for tissue regeneration. During fetal and postnatal development, mammalian cardiomyocytes become terminally differentiated muscle cells. In newborn rats, along with terminal differentiation and loss of the ability to proliferate, centrosomes are inactivated and lose their MTOC capacity. In this scenario, PCM proteins are removed from the centrioles and localize to the nuclear envelope from which MT nucleation takes place (Zebrowski et al., 2015). In contrast, in salamanders, the centrosome is maintained throughout differentiation, and adult heart muscle cells are therefore able to proliferate, permitting partial heart regeneration (Zebrowski et al., 2015). This suggests that centrosome inactivation in the mammalian heart restricts the proliferative capacity of muscle cells.

In oocytes, centrosome elimination is critical to ensure that the zygote has the correct centrosome number and develops normally. For instance, preventing centrosome loss, by either retaining the PCM in flies, or preventing centrosome extrusion in starfish, leads to female infertility (Borrego-Pinto et al., 2016; Pimenta-Marques et al., 2016).

Centrosome inactivation might also be important in cancer, as cancer cells often show multiple centrosomes, which may promote aneuploidy and invasion (Gönczy, 2015; Godinho, 2014; Godinho et al., 2014). Paradoxically, in extreme cases, centrosome amplification can be detrimental for the survival of a cancer cell, as each centrosome can form a spindle pole, thereby promoting a highly abnormal mitotic division that can result in cell death (Godinho et al., 2014). It has been proposed that cancer cells inactivate their supernumerary centrosomes during mitosis in order to form a bipolar spindle and survive. Indeed, fly cells with extra centrosomes inactivate them through upregulation of moesin, a regulator of the actin cytoskeleton that localizes to centrosomes during mitosis and delocalizes the PCM. This contributes to the formation of a bipolar spindle and cell survival (Sabino et al., 2015). It could be that cancer cells cope with supernumerary centrosomes by inactivating the centrosome maintenance program (Fig. 2A). However, whether this pathway is related to the p53-mediated centrosome surveillance pathway discussed above is not yet clear.

The regulation of cilia maintenance might also be important for cell cycle control. Certain quiescent ciliated cells in culture need to disassemble cilia to progress into S phase. Whether disassembly results from a failure of maintenance, or it is an active process involving a different pathway is an important question for any cellular structure. In the case of cilia, there is an active pathway that inhibits ciliogenesis mediated by Nde1 and Tectex-1, which regulate cilia length and actin dynamics (Kim et al., 2011; Li et al., 2011). Important players also involved in the initiation of cilia disassembly are Aurora A, PLK1 and HDAC6 (Inoko et al., 2012; Wang et al., 2013; Plotnikova et al., 2012; Pugacheva et al., 2007; Ran et al., 2015). HDAC6 destabilizes ciliary microtubules directly (Ran et al., 2015) and is activated by Aurora A (Pugacheva et al., 2007). It will be important in the future to dissect the extent to which interfering with the cilia maintenance program contributes to cilia disassembly during the cell cycle.

The impact of cilia on tumor progression might depend on the genetic nature of the cancer and the tissue from which the tumor originates (Han et al., 2009; Wong et al., 2009). Ciliogenesis is compromised in certain cancer types (Gradilone et al., 2013; Menzl et al., 2014; Moser et al., 2009), with inhibition of HDAC6 rescuing ciliogenesis and slowing tumor growth (Gradilone et al., 2013; Xiang et al., 2017). This led to the proposition that cilia inhibit tumor growth. However, upon deregulation of ciliary mitogenic pathways such as Hedgehog signaling, cilia can also accelerate tumor growth (Li et al., 2016). Accordingly, cilia were found to persist in a subset of patients with rhabdomyosarcoma, a cancer derived from skeletal muscle cells that normally lose cilia (Fu et al., 2014). Therefore, although a deregulation of cilia maintenance might be associated with cancer, future studies are needed to fully understand the specific conditions in which these scenarios arise.

Future directions and conclusions

In this Review, we provide evidence that centrosomes and cilia are actively maintained throughout life. We highlight the possible underlying molecular programs that maintain these structures and discuss how these could be deregulated in disease. A better understanding of these maintenance programs will help to predict the outcome of disease-causing mutations in different genetic contexts and determine how pharmacological interventions can be used to modulate their functions.

The focus of research on cilia and centrosome maintenance is still quite recent. The challenge remains in designing appropriate experimental approaches to distinguish cilia and centrosome maintenance from their biogenesis, by ensuring that, initially, these structures were fully built and functioned properly. Moreover, given that the factors involved in structural maintenance may also be important for the activity of a given structure at a given developmental stage, there is a challenge in understanding how much function regulation and maintenance programs intersect with each other. Furthermore, given that many structures are disassembled at different stages under physiological conditions, it will be important to understand whether there is an overlap between disassembly programs and the inactivation of maintenance programs. Many questions wait to be explored to fully understand the extent to which these organelles and their components are maintained and how maintenance is regulated to realize different functions. The development of new tools and experimental systems now make it possible to address questions that were previously inaccessible. It is an exciting time to turn our attention to maintenance.

Competing interests

The authors declare no competing or financial interests.

Funding

Work in the authors' lab is funded by the Fundação para a Ciência e a Tecnologia (SFRH/BPD/79680/2011 to A. P.-M.; SFRH/BD/52176/2013 to S.W; the laboratory is funded by ERC-2015-CoG - 683258_CentrioleBirthDeath.).

References

Ahmad, F. J., Yu, W., McNally, F. J. and Baas, P. W. (1999). An essential role for katanin in severing microtubules in the neuron. *J. Cell Biol.* **145**, 305-315.

Andersen, J. S., Wilkinson, C. J., Mayor, T., Mortensen, P., Nigg, E. A. and Mann, M. (2003). Proteomic characterization of the human centrosome by protein correlation profiling. *Nature* **426**, 570-574.

Azimzadeh, J. (2014). Exploring the evolutionary history of centrosomes. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **369**, 20130453.

Bahmanyar, S., Guiney, E. L., Hatch, E. M., Nelson, W. J. and Barth, A. I. M. (2010). Formation of extra centrosomal structures is dependent on beta-catenin. *J. Cell Sci.* **123**, 3125-3135.

Balestra, F. R., Strnad, P., Flückiger, I. and Gönczy, P. (2013). Discovering regulators of centriole biogenesis through siRNA-based functional genomics in human cells. *Dev. Cell* **25**, 555-571.

Balestra, F. R., von Tobel, L. and Gönczy, P. (2015). Paternally contributed centrioles exhibit exceptional persistence in C. elegans embryos. *Cell Res.* **25**, 642-644.

Bangs, F. and Anderson, K. V. (2017). Primary cilia and mammalian Hedgehog Signaling. *Cold Spring Harb. Perspect. Biol.* **9**, a028175.

Bazzi, H. and Anderson, K. V. (2014). Centrioles in the mouse: cilia and beyond. *Cell Cycle Georget. Tex.* **13**, 2809.

Belmont, L. D., Hyman, A. A., Sawin, K. E. and Mitchison, T. J. (1990). Real-time visualization of cell cycle-dependent changes in microtubule dynamics in cytoplasmic extracts. *Cell* **62**, 579-589.

Besharse, J. C. and Hollyfield, J. G. (1979). Turnover of mouse photoreceptor outer segments in constant light and darkness. *Invest. Ophthalmol. Vis. Sci.* **18**, 1019-1024.

Bhogaraju, S., Cajanek, L., Fort, C., Blisnick, T., Weber, K., Taschner, M., Mizuno, N., Lamla, S., Bastin, P., Nigg, E. A. et al. (2013). Molecular basis of tubulin transport within the cilium by IFT74 and IFT81. *Science* **341**, 1009-1012.

Bobinnec, Y., Khodjakov, A., Mir, L. M., Rieder, C. L., Eddé, B. and Bornens, M. (1998). Centriole disassembly in vivo and its effect on centrosome structure and function in vertebrate cells. *J. Cell Biol.* **143**, 1575-1589.

Borrego-Pinto, J., Somogyi, K., Karreman, M. A., König, J., Müller-Reichert, T., Bettencourt-Dias, M., Gönczy, P., Schwab, Y. and Lénárt, P. (2016). Distinct mechanisms eliminate mother and daughter centrioles in meiosis of starfish oocytes. *J. Cell Biol.* **212**, 815-827.

Bosch Grau, M., Masson, C., Gadadhar, S., Rocha, C., Tort, O., Marques Sousa, P., Vacher, S., Bieche, I. and Janke, C. (2017). Alterations in the balance of tubulin glycylation and glutamylation in photoreceptors leads to retinal degeneration. *J. Cell Sci.* **130**, 938-949.

Braun, D. A. and Hildebrandt, F. (2017). Ciliopathies. *Cold Spring Harb. Perspect. Biol.* **9**, a028191.

Brito, D. A., Gouveia, S. M. and Bettencourt-Dias, M. (2012). Deconstructing the centriole: structure and number control. *Curr. Opin. Cell Biol.* **24**, 4-13.

Busskamp, V., Krol, J., Nelidova, D., Daum, J., Szikra, T., Tsuda, B., Jüttner, J., Farrow, K., Scherf, B. G., Alvarez, C. P. P. et al. (2014). miRNAs 182 and 183 are necessary to maintain adult cone photoreceptor outer segments and visual function. *Neuron* **83**, 586-600.

Carvalho-Santos, Z., Azimzadeh, J., Pereira-Leal, J. B. and Bettencourt-Dias, M. (2011). Tracing the origins of centrioles, cilia, and flagella. *J. Cell Biol.* **194**, 165-175.

Chen, J. V., Kao, L.-R., Jana, S. C., Sivan-Loukianova, E., Mendonça, S., Cabrera, O. A., Singh, P., Cabernard, C., Eberl, D. F., Bettencourt-Dias, M. et al. (2015). Rootletin organizes the ciliary rootlet to achieve neuron sensory function in Drosophila. *J. Cell Biol.* **211**, 435-453.

Choi, Y.-K., Liu, P., Sze, S. K., Dai, C. and Qi, R. Z. (2010). CDK5RAP2 stimulates microtubule nucleation by the gamma-tubulin ring complex. *J. Cell Biol.* **191**, 1089-1095.

Choksi, S. P., Lauter, G., Swoboda, P. and Roy, S. (2014). Switching on cilia: transcriptional networks regulating ciliogenesis. *Development* **141**, 1427-1441.

Chrétien, D., Buendia, B., Fuller, S. D. and Karsenti, E. (1997). Reconstruction of the centrosome cycle from cryoelectron micrographs. *J. Struct. Biol.* **120**, 117-133.

Christensen, S. T., Pedersen, L. B., Schneider, L. and Satir, P. (2007). Sensory cilia and integration of signal transduction in human health and disease. *Traffic Cph. Den.* **8**, 97-109.

Conduit, P. T., Wainman, A. and Raff, J. W. (2015). Centrosome function and assembly in animal cells. *Nat. Rev. Mol. Cell Biol.* **16**, 611-624.

Cornils, A., Maurya, A. K., Tereshko, L., Kennedy, J., Brear, A. G., Prahlad, V., Blacque, O. E. and Sengupta, P. (2016). Structural and functional recovery of sensory cilia in C. elegans IFT mutants upon aging. *PLoS Genet.* **12**, e1006325.

Cunha-Ferreira, I., Bento, I. and Bettencourt-Dias, M. (2009). From zero to many: control of centriole number in development and disease. *Traffic Cph. Den.* **10**, 482-498.

Davis, E. E., Zhang, Q., Liu, Q., Diplas, B. H., Davey, L. M., Hartley, J., Stoetzel, C., Szymanska, K., Ramaswami, G., Logan, C. V. et al. (2011). TTC21B contributes both causal and modifying alleles across the ciliopathy spectrum. *Nat. Genet.* **43**, 189-196.

Del Viso, F., Huang, F., Myers, J., Chalfant, M., Zhang, Y., Reza, N., Bewersdorf, J., Lusk, C. P. and Khokha, M. K. (2016). Congenital heart disease genetics uncovers context-dependent organization and function of nucleoporins at Cilia. *Dev. Cell* **38**, 478-492.

Delattre, M. and Gönczy, P. (2004). The arithmetic of centrosome biogenesis. *J. Cell Sci.* **117**, 1619-1630.

Delgehyr, N., Sillibourne, J. and Bornens, M. (2005). Microtubule nucleation and anchoring at the centrosome are independent processes linked by ninein function. *J. Cell Sci.* **118**, 1565-1575.

Dobbelaere, J., Josué, F., Suijkerbuijk, S., Baum, B., Tapon, N. and Raff, J. (2008). A genome-wide RNAi screen to dissect centriole duplication and centrosome maturation in Drosophila. *PLoS Biol.* **6**, e224.

Eguether, T., San Agustin, J. T., Keady, B. T., Jonassen, J. A., Liang, Y., Francis, R., Tobita, K., Johnson, C. A., Abdelhamed, Z. A., Lo, C. W. et al. (2014). IFT27

links the BBSome to IFT for maintenance of the ciliary signaling compartment. *Dev. Cell* **31**, 279-290.
Espigat-Georger, A., Dyachuk, V., Chemin, C., Emorine, L. and Merdes, A. (2016). Nuclear alignment in myotubes requires centrosome proteins recruited by nesprin-1. *J. Cell Sci.* **129**, 4227-4237.
Ferris, D. K., Maloid, S. C. and Li, C.-C. H. (1998). Ubiquitination and proteasome mediated degradation of polo-like kinase. *Biochem. Biophys. Res. Commun.* **252**, 340-344.
Fong, K.-W., Choi, Y.-K., Rattner, J. B. and Qi, R. Z. (2008). CDK5RAP2 is a pericentriolar protein that functions in centrosomal attachment of the gamma-tubulin ring complex. *Mol. Biol. Cell* **19**, 115-125.
Fong, C. S., Mazo, G., Das, T., Goodman, J., Kim, M., O'Rourke, B. P., Izquierdo, D. and Tsou, M.-F. B. (2016). 53BP1 and USP28 mediate p53-dependent cell cycle arrest in response to centrosome loss and prolonged mitosis. *eLife* **5**, e16270.
Fort, C., Bonnefoy, S., Kohl, L. and Bastin, P. (2016). Intraflagellar transport is required for the maintenance of the trypanosome flagellum composition but not its length. *J. Cell Sci.* **129**, 3026-3041.
Fu, W., Asp, P., Canter, B. and Dynlacht, B. D. (2014). Primary cilia control hedgehog signaling during muscle differentiation and are deregulated in rhabdomyosarcoma. *Proc. Natl. Acad. Sci. USA* **111**, 9151-9156.
Fu, J., Lipinszki, Z., Rangone, H., Min, M., Mykura, C., Chao-Chu, J., Schneider, S., Dzhindzhev, N. S., Gottardo, M., Riparbelli, M. G. et al. (2016). Conserved molecular interactions in centriole-to-centrosome conversion. *Nat. Cell Biol.* **18**, 87-99.
Garcia-Gonzalo, F. R. and Reiter, J. F. (2012). Scoring a backstage pass: mechanisms of ciliogenesis and ciliary access. *J. Cell Biol.* **197**, 697-709.
Garcia-Gonzalo, F. R. and Reiter, J. F. (2017). Open sesame: how transition fibers and the transition zone control ciliary composition. *Cold Spring Harb. Perspect. Biol.* **9**, a028134.
Girotra, M., Srivastava, S., Kulkarni, A., Barbora, A., Bobra, K., Ghosal, D., Devan, P., Aher, A., Jain, A., Panda, D. et al. (2017). The C-terminal tails of heterotrimeric kinesin-2 motor subunits directly bind to α-tubulin1: possible implications for cilia-specific tubulin entry. *Traffic Cph. Den.* **18**, 123-133.
Glotzer, M., Murray, A. W. and Kirschner, M. W. (1991). Cyclin is degraded by the ubiquitin pathway. *Nature* **349**, 132-138.
Godinho, S. A. (2014). Centrosome amplification and cancer: branching out. *Mol. Cell. Oncol.* **2**, e993252.
Godinho, S. A., Picone, R., Burute, M., Dagher, R., Su, Y., Leung, C. T., Polyak, K., Brugge, J. S., Théry, M. and Pellman, D. (2014). Oncogene-like induction of cellular invasion from centrosome amplification. *Nature* **510**, 167-171.
Gönczy, P. (2015). Centrosomes and cancer: revisiting a long-standing relationship. *Nat. Rev. Cancer* **15**, 639-652.
Gönczy, P., Echeverri, C., Oegema, K., Coulson, A., Jones, S. J. M., Copley, R. R., Duperon, J., Oegema, J., Brehm, M., Cassin, E. et al. (2000). Functional genomic analysis of cell division in C. elegans using RNAi of genes on chromosome III. *Nature* **408**, 331-336.
Goshima, G., Wollman, R., Goodwin, S. S., Zhang, N., Scholey, J. M., Vale, R. D. and Stuurman, N. (2007). Genes required for mitotic spindle assembly in Drosophila S2 cells. *Science* **316**, 417-421.
Gradilone, S. A., Radtke, B. N., Bogert, P. S., Huang, B. Q., Gajdos, G. B. and LaRusso, N. F. (2013). HDAC6 inhibition restores ciliary expression and decreases tumor growth. *Cancer Res.* **73**, 2259-2270.
Gudi, R., Zou, C., Li, J. and Gao, Q. (2011). Centrobin-tubulin interaction is required for centriole elongation and stability. *J. Cell Biol.* **193**, 711-725.
Guichard, P., Hachet, V., Majubu, N., Neves, A., Demurtas, D., Olieric, N., Fluckiger, I., Yamada, A., Kihara, K., Nishida, Y. et al. (2013). Native architecture of the centriole proximal region reveals features underlying its 9-fold radial symmetry. *Curr. Biol.* **23**, 1620-1628.
Guo, J., Yang, Z., Song, W., Chen, Q., Wang, F., Zhang, Q. and Zhu, X. (2006). Nudel contributes to microtubule anchoring at the mother centriole and is involved in both dynein-dependent and -independent centrosomal protein assembly. *Mol. Biol. Cell* **17**, 680-689.
Han, Y.-G., Kwok, B. H. and Kernan, M. J. (2003). Intraflagellar transport is required in Drosophila to differentiate sensory cilia but not sperm. *Curr. Biol.* **13**, 1679-1686.
Han, Y.-G., Kim, H. J., Dlugosz, A. A., Ellison, D. W., Gilbertson, R. J. and Alvarez-Buylla, A. (2009). Dual and opposing roles of primary cilia in medulloblastoma development. *Nat. Med.* **15**, 1062-1065.
Haren, L., Remy, M.-H., Bazin, I., Callebaut, I., Wright, M. and Merdes, A. (2006). NEDD1-dependent recruitment of the gamma-tubulin ring complex to the centrosome is necessary for centriole duplication and spindle assembly. *J. Cell Biol.* **172**, 505-515.
Haren, L., Stearns, T. and Lüders, J. (2009). Plk1-Dependent Recruitment of γ-Tubulin Complexes to Mitotic Centrosomes Involves Multiple PCM Components. *PLoS ONE* **4**, e5976.
Harris, J. A., Liu, Y., Yang, P., Kner, P. and Lechtreck, K. F. (2016). Single-particle imaging reveals intraflagellar transport-independent transport and accumulation of EB1 in Chlamydomonas flagella. *Mol. Biol. Cell* **27**, 295-307.
Hsu, K.-S., Chuang, J.-Z. and Sung, C.-H. (2017). The biology of Ciliary dynamics. *Cold Spring Harb. Perspect. Biol.* **9**, a027904.
Hu, Q., Milenkovic, L., Jin, H., Scott, M. P., Nachury, M. V., Spiliotis, E. T. and Nelson, W. J. (2010). A septin diffusion barrier at the base of the primary cilium maintains ciliary membrane protein distribution. *Science* **329**, 436-439.
Huang, B., Rifkin, M. R. and Luck, D. J. (1977). Temperature-sensitive mutations affecting flagellar assembly and function in Chlamydomonas reinhardtii. *J. Cell Biol.* **72**, 67-85.
Huettner, A. F. and Rabinowitz, M. (1933). Demonstration of the central body in the living cell. *Science* **78**, 367-368.
Ibi, M., Zou, P., Inoko, A., Shiromizu, T., Matsuyama, M., Hayashi, Y., Enomoto, M., Mori, D., Hirotsune, S., Kiyono, T. et al. (2011). Trichoplein controls microtubule anchoring at the centrosome by binding to Odf2 and ninein. *J. Cell Sci.* **124**, 857-864.
Inoko, A., Matsuyama, M., Goto, H., Ohmuro-Matsuyama, Y., Hayashi, Y., Enomoto, M., Ibi, M., Urano, T., Yonemura, S., Kiyono, T. et al. (2012). Trichoplein and Aurora A block aberrant primary cilia assembly in proliferating cells. *J. Cell Biol.* **197**, 391-405.
Ishikawa, T. (2017). Axoneme structure from motile cilia. *Cold Spring Harb. Perspect. Biol.* **9**, a028076.
Ishikawa, H. and Marshall, W. F. (2011). Ciliogenesis: building the cell's antenna. *Nat. Rev. Mol. Cell Biol.* **12**, 222-234.
Ishikawa, H., Kubo, A., Tsukita, S. and Tsukita, S. (2005). Odf2-deficient mother centrioles lack distal/subdistal appendages and the ability to generate primary cilia. *Nat. Cell Biol.* **7**, 517-524.
Izquierdo, D., Wang, W.-J., Uryu, K. and Tsou, M.-F. B. (2014). Stabilization of Cartwheel-less centrioles for duplication requires CEP295-mediated centriole-to-centrosome conversion. *Cell Rep.* **8**, 957-965.
Jain, R., Javidan-Nejad, C., Alexander-Brett, J., Horani, A., Cabellon, M. C., Walter, M. J. and Brody, S. L. (2012). Sensory functions of motile cilia and implication for bronchiectasis. *Front. Biosci. Sch. Ed.* **4**, 1088-1098.
Jakobsen, L., Vanselow, K., Skogs, M., Toyoda, Y., Lundberg, E., Poser, I., Falkenby, L. G., Bennetzen, M., Westendorf, J., Nigg, E. A. et al. (2011). Novel asymmetrically localizing components of human centrosomes identified by complementary proteomics methods. *EMBO J.* **30**, 1520-1535.
Jambor, H., Surendranath, V., Kalinka, A. T., Mejstrik, P., Saalfeld, S. and Tomancak, P. (2015). Systematic imaging reveals features and changing localization of mRNAs in Drosophila development. *eLife* **4**, e05003.
Jiang, L., Wei, Y., Ronquillo, C. C., Marc, R. E., Yoder, B. K., Frederick, J. M. and Baehr, W. (2015). Heterotrimeric kinesin-2 (KIF3) mediates transition zone and axoneme formation of mouse photoreceptors. *J. Biol. Chem.* **290**, 12765-12778.
Kamath, R. S., Fraser, A. G., Dong, Y., Poulin, G., Durbin, R., Gotta, M., Kanapin, A., Le Bot, N., Moreno, S., Sohrmann, M. et al. (2003). Systematic functional analysis of the Caenorhabditis elegans genome using RNAi. *Nature* **421**, 231-237.
Kato, K. H., Washitani-Nemoto, S., Hino, A. and Nemoto, S.-I. (1990). Ultrastructural studies on the behavior of centrioles during meiosis of starfish oocytes. *Dev. Growth Differ.* **32**, 41-49.
Keller, D., Orpinell, M., Olivier, N., Wachsmuth, M., Mahen, R., Wyss, R., Hachet, V., Ellenberg, J., Manley, S. and Gönczy, P. (2014). Mechanisms of HsSAS-6 assembly promoting centriole formation in human cells. *J. Cell Biol.* **204**, 697-712.
Khanna, H., Davis, E. E., Murga-Zamalloa, C. A., Estrada-Cuzcano, A., Lopez, I., den Hollander, A. I., Zonneveld, M. N., Othman, M. I., Waseem, N., Chakarova, C. F. et al. (2009). A common allele in RPGRIP1L is a modifier of retinal degeneration in ciliopathies. *Nat. Genet.* **41**, 739-745.
Kim, S., Zaghloul, N. A., Bubenshchikova, E., Oh, E. C., Rankin, S., Katsanis, N., Obara, T. and Tsiokas, L. (2011). Nde1-mediated inhibition of ciliogenesis affects cell cycle re-entry. *Nat. Cell Biol.* **13**, 351-360.
Kobayashi, T. and Dynlacht, B. D. (2011). Regulating the transition from centriole to basal body. *J. Cell Biol.* **193**, 435-444.
Kochanski, R. S. and Borisy, G. G. (1990). Mode of centriole duplication and distribution. *J. Cell Biol.* **110**, 1599-1605.
Kong, D., Farmer, V., Shukla, A., James, J., Gruskin, R., Kiriyama, S. and Loncarek, J. (2014). Centriole maturation requires regulated Plk1 activity during two consecutive cell cycles. *J. Cell Biol.* **206**, 855-865.
Kozminski, K. G., Johnson, K. A., Forscher, P. and Rosenbaum, J. L. (1993). A motility in the eukaryotic flagellum unrelated to flagellar beating. *Proc. Natl. Acad. Sci. USA* **90**, 5519-5523.
Kozminski, K. G., Beech, P. L. and Rosenbaum, J. L. (1995). The Chlamydomonas kinesin-like protein FLA10 is involved in motility associated with the flagellar membrane. *J. Cell Biol.* **131**, 1517-1527.
Krioutchkova, M. M., Onishchenko, G. E. and Chentsov, Y. S. (1994). An ultrastructural study of the centrosome and centrioles in gametogenesis and early embryogenesis of Lymnaea stagnalis L.: II. Centrosome and centrioles in oogenesis and early embryogenesis. *J. Struct. Biol.* **112**, 59-69.
Kuzhandaivel, A., Schultz, S. W., Alkhori, L. and Alenius, M. (2014). Cilia-mediated hedgehog signaling in Drosophila. *Cell Rep.* **7**, 672-680.
Lambrus, B. G., Daggubati, V., Uetake, Y., Scott, P. M., Clutario, K. M., Sluder, G. and Holland, A. J. (2016). A USP28-53BP1-p53-p21 signaling axis arrests growth after centrosome loss or prolonged mitosis. *J. Cell Biol.* **214**, 143-153.

Lancaster, M. A. and Gleeson, J. G. (2009). The primary cilium as a cellular signaling center: lessons from disease. *Curr. Opin. Genet. Dev.* **19**, 220-229.
Lawo, S., Hasegan, M., Gupta, G. D. and Pelletier, L. (2012). Subdiffraction imaging of centrosomes reveals higher-order organizational features of pericentriolar material. *Nat. Cell Biol.* **14**, 1148-1158.
Lechtreck, K. F. (2015). IFT-cargo interactions and protein transport in cilia. *Trends Biochem. Sci.* **40**, 765-778.
Lehman, J. M., Michaud, E. J., Schoeb, T. R., Aydin-Son, Y., Miller, M. and Yoder, B. K. (2008). The Oak ridge polycystic kidney mouse: modeling ciliopathies of mice and men. *Dev. Dyn.* **237**, 1960-1971.
Li, A., Saito, M., Chuang, J.-Z., Tseng, Y.-Y., Dedesma, C., Tomizawa, K., Kaitsuka, T. and Sung, C.-H. (2011). Ciliary transition zone activation of phosphorylated Tctex-1 controls ciliary resorption, S-phase entry and fate of neural progenitors. *Nat. Cell Biol.* **13**, 402-411.
Li, S., Fernandez, J.-J., Marshall, W. F. and Agard, D. A. (2012). Three-dimensional structure of basal body triplet revealed by electron cryo-tomography. *EMBO J.* **31**, 552-562.
Li, L., Grausam, K. B., Wang, J., Lun, M. P., Ohli, J., Lidov, H. G. W., Calicchio, M. L., Zeng, E., Salisbury, J. L., Wechsler-Reya, R. J. et al. (2016). Sonic Hedgehog promotes proliferation of Notch-dependent monociliated choroid plexus tumour cells. *Nat. Cell Biol.* **18**, 418-430.
Lindon, C. and Pines, J. (2004). Ordered proteolysis in anaphase inactivates Plk1 to contribute to proper mitotic exit in human cells. *J. Cell Biol.* **164**, 233-241.
Loreng, T. D. and Smith, E. F. (2017). The central apparatus of cilia and eukaryotic flagella. *Cold Spring Harb. Perspect. Biol.* **9**, a028118.
Lüders, J., Patel, U. K. and Stearns, T. (2006). GCP-WD is a gamma-tubulin targeting factor required for centrosomal and chromatin-mediated microtubule nucleation. *Nat. Cell Biol.* **8**, 137-147.
Lukinavičius, G., Lavogina, D., Orpinell, M., Umezawa, K., Reymond, L., Garin, N., Gönczy, P. and Johnsson, K. (2013). Selective chemical crosslinking reveals a Cep57-Cep63-Cep152 centrosomal complex. *Curr. Biol.* **23**, 265-270.
Madarampalli, B., Yuan, Y., Liu, D., Lengel, K., Xu, Y., Li, G., Yang, J., Liu, X., Lu, Z. and Liu, D. X. (2015). ATF5 connects the pericentriolar materials to the proximal end of the mother centriole. *Cell* **162**, 580-592.
Mahjoub, M. R., 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.
Manandhar, G., Schatten, H. and Sutovsky, P. (2005). Centrosome reduction during gametogenesis and its significance. *Biol. Reprod.* **72**, 2-13.
Marshall, W. F. (2009). Centriole evolution. *Curr. Opin. Cell Biol.* **21**, 14-19.
Marshall, W. F. and Rosenbaum, J. L. (2001). Intraflagellar transport balances continuous turnover of outer doublet microtubules: implications for flagellar length control. *J. Cell Biol.* **155**, 405-414.
Marshall, W. F., Qin, H., Rodrigo Brenni, M. and Rosenbaum, J. L. (2005). Flagellar length control system: testing a simple model based on intraflagellar transport and turnover. *Mol. Biol. Cell* **16**, 270-278.
McIntyre, R. E., Lakshminarasimhan Chavali, P., Ismail, O., Carragher, D. M., Sanchez-Andrade, G., Forment, J. V., Fu, B., Del Castillo Velasco-Herrera, M., Edwards, A., van der Weyden, L., Yang, F. et al. (2012). Disruption of mouse Cenpj, a regulator of centriole biogenesis, phenocopies Seckel syndrome. *PLoS Genet.* **8**, e1003022.
Meitinger, F., Anzola, J. V., Kaulich, M., Richardson, A., Stender, J. D., Benner, C., Glass, C. K., Dowdy, S. F., Desai, A., Shiau, A. K.et al. (2016). 53BP1 and USP28 mediate p53 activation and G1 arrest after centrosome loss or extended mitotic duration. *J. Cell Biol.* **214**, 155-166.
Mennella, V., Keszthelyi, B., McDonald, K. L., Chhun, B., Kan, F., Rogers, G. C., Huang, B. and Agard, D. A. (2012). Subdiffraction-resolution fluorescence microscopy reveals a domain of the centrosome critical for pericentriolar material organization. *Nat. Cell Biol.* **14**, 1159-1168.
Menzl, I., Lebeau, L., Pandey, R., Hassounah, N. B., Li, F. W., Nagle, R., Weihs, K. and McDermott, K. M. (2014). Loss of primary cilia occurs early in breast cancer development. *Cilia* **3**, 7.
Meunier, A. and Spassky, N. (2016). Centriole continuity: out with the new, in with the old. *Curr. Opin. Cell Biol.* **38**, 60-67.
Mikeladze-Dvali, T., von Tobel, L., Strnad, P., Knott, G., Leonhardt, H., Schermelleh, L. and Gönczy, P. (2012). Analysis of centriole elimination during C. elegans oogenesis. *Development* **139**, 1670-1679.
Milenkovic, L., Weiss, L. E., Yoon, J., Roth, T. L., Su, Y. R. S., Sahl, S. J., Scott, M. P. and Moerner, W. E. (2015). Single-molecule imaging of Hedgehog pathway protein Smoothened in primary cilia reveals binding events regulated by Patched1. *Proc. Natl. Acad. Sci. USA* **112**, 8320-8325.
Miyazaki, A., Kato, K. H. and Nemoto, S. (2005). Role of microtubules and centrosomes in the eccentric relocation of the germinal vesicle upon meiosis reinitiation in sea-cucumber oocytes. *Dev. Biol.* **280**, 237-247.
Mogensen, M. M., Malik, A., Piel, M., Bouckson-Castaing, V. and Bornens, M. (2000). Microtubule minus-end anchorage at centrosomal and non-centrosomal sites: the role of ninein. *J. Cell Sci.* **113**, 3013-3023.
Mohan, S., Timbers, T. A., Kennedy, J., Blacque, O. E. and Leroux, M. R. (2013). Striated rootlet and nonfilamentous forms of rootletin maintain ciliary function. *Curr. Biol.* **23**, 2016-2022.
Moser, J. J., Fritzler, M. J. and Rattner, J. B. (2009). Primary ciliogenesis defects are associated with human astrocytoma/glioblastoma cells. *BMC Cancer* **9**, 448.
Mourão, A., Christensen, S. T. and Lorentzen, E. (2016). The intraflagellar transport machinery in ciliary signaling. *Curr. Opin. Struct. Biol.* **41**, 98-108.
Muroyama, A. and Lechler, T. (2017). Microtubule organization, dynamics and functions in differentiated cells. *Development* **144**, 3012-3021.
Muroyama, A., Seldin, L. and Lechler, T. (2016). Divergent regulation of functionally distinct γ-tubulin complexes during differentiation. *J. Cell Biol.* **213**, 679-692.
Murray, A. W. (1989). Cyclin synthesis and degradation and the embryonic cell cycle. *J. Cell Sci.* **1989** Suppl. 12, 65-76.
Nager, A. R., Goldstein, J. S., Herranz-Pérez, V., Portran, D., Ye, F., Garcia-Verdugo, J. M. and Nachury, M. V. (2017). An actin network dispatches ciliary GPCRs into extracellular vesicles to modulate signaling. *Cell* **168**, 252-263.e14.
Nakashima, S. and Kato, K. H. (2001). Centriole behavior during meiosis in oocytes of the sea urchin Hemicentrotus pulcherrimus. *Dev. Growth Differ.* **43**, 437-445.
Nguyen, M. M., Stone, M. C. and Rolls, M. M. (2011). Microtubules are organized independently from the centrosome in Drosophila neurons. *Neural Dev.* **6**, 38.
Novarino, G., Akizu, N. and Gleeson, J. G. (2011). Modeling human disease in humans: the ciliopathies. *Cell* **147**, 70-79.
O'Hagan, R., Piasecki, B. P., Silva, M., Phirke, P., Nguyen, K. C. Q., Hall, D. H., Swoboda, P. and Barr, M. M. (2011). The tubulin deglutamylase CCPP-1 regulates the function and stability of sensory cilia in C. elegans. *Curr. Biol.* **21**, 1685-1694.
Palander, O., El-Zeiry, M. and Trimble, W. S. (2017). Uncovering the roles of Septins in Cilia. *Front. Cell Dev. Biol.* **5**, 36.
Pazour, G. J., Dickert, B. L., Vucica, Y., Seeley, E. S., Rosenbaum, J. L., Witman, G. B. and Cole, D. G. (2000). Chlamydomonas IFT 88 and its mouse homologue, polycystic kidney disease gene tg 737, are required for assembly of cilia and flagella. *J. Cell Biol.* **151**, 709-718.
Pazour, G. J., Baker, S. A., Deane, J. A., Cole, D. G., Dickert, B. L., Rosenbaum, J. L., Witman, G. B. and Besharse, J. C. (2002). The intraflagellar transport protein, IFT88, is essential for vertebrate photoreceptor assembly and maintenance. *J. Cell Biol.* **157**, 103-113.
Pearson, C. G., Osborn, D. P. S., Giddings, T. H., Beales, P. L. and Winey, M. (2009). Basal body stability and ciliogenesis requires the conserved component Poc1. *J. Cell Biol.* **187**, 905-920.
Pimenta-Marques, A., Bento, I., Lopes, C. A. M., Duarte, P., Jana, S. C. and Bettencourt-Dias, M. (2016). A mechanism for the elimination of the female gamete centrosome in Drosophila melanogaster. *Science* **353**, aaf4866.
Piperno, G., Siuda, E., Henderson, S., Segil, M., Vaananen, H. and Sassaroli, M. (1998). Distinct mutants of retrograde Intraflagellar Transport (IFT) share similar morphological and molecular defects. *J. Cell Biol.* **143**, 1591-1601.
Plotnikova, O. V., Nikonova, A. S., Loskutov, Y. V., Kozyulina, P. Y., Pugacheva, E. N. and Golemis, E. A. (2012). Calmodulin activation of Aurora-A kinase (AURKA) is required during ciliary disassembly and in mitosis. *Mol. Biol. Cell* **23**, 2658-2670.
Prevo, B., Scholey, J. M. and Peterman, E. J. G. (2017). Intraflagellar transport: mechanisms of motor action, cooperation, and cargo delivery. *FEBS J.* **284**, 2905-2931.
Przybylski, R. J. (1971). Occurrence of centrioles during skeletal and cardiac myogenesis. *J. Cell Biol.* **49**, 214-221.
Pugacheva, E. N., Jablonski, S. A., Hartman, T. R., Henske, E. P. and Golemis, E. A. (2007). HEF1-dependent Aurora A activation induces disassembly of the primary cilium. *Cell* **129**, 1351-1363.
Ran, J., Yang, Y., Li, D., Liu, M. and Zhou, J. (2015). Deacetylation of α-tubulin and cortactin is required for HDAC6 to trigger ciliary disassembly. *Sci. Rep.* **5**, 12917.
Reiter, J. F., Blacque, O. E. and Leroux, M. R. (2012). The base of the cilium: roles for transition fibres and the transition zone in ciliary formation, maintenance and compartmentalization. *EMBO Rep.* **13**, 608-618.
Sabino, D., Gogendeau, D., Gambarotto, D., Nano, M., Pennetier, C., Dingli, F., Arras, G., Loew, D. and Basto, R. (2015). Moesin is a major regulator of centrosome behavior in epithelial cells with extra centrosomes. *Curr. Biol.* **25**, 879-889.
San Agustin, J. T., Pazour, G. J. and Witman, G. B. (2015). Intraflagellar transport is essential for mammalian spermiogenesis but is absent in mature sperm. *Mol. Biol. Cell* **26**, 4358-4372.
Sanchez, A. D. and Feldman, J. L. (2017). Microtubule-organizing centers: from the centrosome to non-centrosomal sites. *Curr. Opin. Cell Biol.* **44**, 93-101.
Sanchez, G. M., Alkhori, L., Hatano, E., Schultz, S. W., Kuzhandaivel, A., Jafari, S., Granseth, B. and Alenius, M. (2016). Hedgehog signaling regulates the ciliary transport of odorant receptors in Drosophila. *Cell Rep.* **14**, 464-470.
Sánchez-Huertas, C., Freixo, F., Viais, R., Lacasa, C., Soriano, E. and Lüders, J. (2016). Non-centrosomal nucleation mediated by augmin organizes microtubules in post-mitotic neurons and controls axonal microtubule polarity. *Nat. Commun.* **7**, 12187.
Sarpal, R., Todi, S. V., Sivan-Loukianova, E., Shirolikar, S., Subramanian, N., Raff, E. C., Erickson, J. W., Ray, K. and Eberl, D. F. (2003). Drosophila KAP

interacts with the kinesin II motor subunit KLP64D to assemble chordotonal sensory cilia, but not sperm tails. *Curr. Biol.* **13**, 1687-1696.
Scheer, U. (2014). Historical roots of centrosome research: discovery of Boveri's microscope slides in Würzburg. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **369**, 20130469.
Shah, A. S., Ben-Shahar, Y., Moninger, T. O., Kline, J. N. and Welsh, M. J. (2009). Motile cilia of human airway epithelia are chemosensory. *Science* **325**, 1131-1134.
Sharp, D. J. and Ross, J. L. (2012). Microtubule-severing enzymes at the cutting edge. *J. Cell Sci.* **125**, 2561-2569.
Sheehan, P., Zhu, M., Beskow, A., Vollmer, C. and Waites, C. L. (2016). Activity-dependent degradation of synaptic vesicle proteins requires Rab35 and the ESCRT pathway. *J. Neurosci.* **36**, 8668-8686.
Song, L. and Dentler, W. L. (2001). Flagellar protein dynamics in Chlamydomonas. *J. Biol. Chem.* **276**, 29754-29763.
Sonnen, K. F., Schermelleh, L., Leonhardt, H. and Nigg, E. A. (2012). 3D-structured illumination microscopy provides novel insight into architecture of human centrosomes. *Biol. Open* **1**, 965-976.
Sönnichsen, B., Koski, L. B., Walsh, A., Marschall, P., Neumann, B., Brehm, M., Alleaume, A.-M., Artelt, J., Bettencourt, P., Cassin, E. et al. (2005). Full-genome RNAi profiling of early embryogenesis in Caenorhabditis elegans. *Nature* **434**, 462-469.
Srsen, V., Fant, X., Heald, R., Rabouille, C. and Merdes, A. (2009). Centrosome proteins form an insoluble perinuclear matrix during muscle cell differentiation. *BMC Cell Biol.* **10**, 28.
Stiess, M., Maghelli, N., Kapitein, L. C., Gomis-Rüth, S., Wilsch-Bräuninger, M., Hoogenraad, C. C., Tolić-Nørrelykke, I. M. and Bradke, F. (2010). Axon extension occurs independently of centrosomal microtubule nucleation. *Science* **327**, 704-707.
Stinchcombe, J. C., Randzavola, L. O., Angus, K. L., Mantell, J. M., Verkade, P. and Griffiths, G. M. (2015). Mother centriole distal appendages mediate centrosome docking at the immunological synapse and reveal mechanistic parallels with ciliogenesis. *Curr. Biol.* **25**, 3239-3244.
Takao, D. and Kamimura, S. (2017). Simulation of intra-ciliary diffusion suggests a novel role of primary cilia as a cell-signaling enhancer. *Dev. Growth Differ.* **59**, 415-422.
Takao, D. and Verhey, K. J. (2016). Gated entry into the ciliary compartment. *Cell. Mol. Life Sci.* **73**, 119-127.
Takao, D., Dishinger, J. F., Kee, H. L., Pinskey, J. M., Allen, B. L. and Verhey, K. J. (2014). An assay for clogging the ciliary pore complex distinguishes mechanisms of cytosolic and membrane protein entry. *Curr. Biol.* **24**, 2288-2294.
Taschner, M. and Lorentzen, E. (2016). The intraflagellar transport machinery. *Cold Spring Harb. Perspect. Biol.* **8**, a028092.
Tassin, A. M., Maro, B. and Bornens, M. (1985). Fate of microtubule-organizing centers during myogenesis in vitro. *J. Cell Biol.* **100**, 35-46.
Thoma, C. R., Frew, I. J., Hoerner, C. R., Montani, M., Moch, H. and Krek, W. (2007). pVHL and GSK3beta are components of a primary cilium-maintenance signalling network. *Nat. Cell Biol.* **9**, 588-595.
Toyama, B. H., Savas, J. N., Park, S. K., Harris, M. S., Ingolia, N. T., Yates, J. R. and Hetzer, M. W. (2013). Identification of long-lived proteins reveals exceptional stability of essential cellular structures. *Cell* **154**, 971-982.
Trivedi, D., Colin, E., Louie, C. M. and Williams, D. S. (2012). Live-cell imaging evidence for the ciliary transport of rod photoreceptor opsin by heterotrimeric kinesin-2. *J. Neurosci. Off. J. Soc. Neurosci.* **32**, 10587-10593.
van Dam, T. J. P., Townsend, M. J., Turk, M., Schlessinger, A., Sali, A., Field, M. C. and Huynen, M. A. (2013). Evolution of modular intraflagellar transport from a coatomer-like progenitor. *Proc. Natl. Acad. Sci. USA* **110**, 6943-6948.
Vashishtha, M., Walther, Z. and Hall, J. L. (1996). The kinesin-homologous protein encoded by the Chlamydomonas FLA10 gene is associated with basal bodies and centrioles. *J. Cell Sci.* **109**, 541-549.
Vertii, A., Hehnly, H. and Doxsey, S. (2016). The centrosome, a multitalented renaissance organelle. *Cold Spring Harb. Perspect. Biol.* **8**, a025049.
Wang, W.-J., Soni, R. K., Uryu, K. and Tsou, M.-F. B. (2011). The conversion of centrioles to centrosomes: essential coupling of duplication with segregation. *J. Cell Biol.* **193**, 727-739.
Wang, L., Piao, T., Cao, M., Qin, T., Huang, L., Deng, H., Mao, T. and Pan, J. (2013). Flagellar regeneration requires cytoplasmic microtubule depolymerization and kinesin-13. *J. Cell Sci.* **126**, 1531-1540.
Wang, J., Silva, M., Haas, L., Morsci, N., Nguyen, K. C. Q., Hall, D. H. and Barr, M. M. (2014). C. elegans ciliated sensory neurons release extracellular vesicles that function in animal communication. *Curr. Biol.* **24**, 519-525.
Wheatley, D. N., Wang, A. M. and Strugnell, G. E. (1996). Expression of primary cilia in mammalian cells. *Cell Biol. Int.* **20**, 73-81.
Winey, M. and O'Toole, E. (2014). Centriole structure. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **369**, 20130457.
Witman, G. B., Carlson, K., Berliner, J. and Rosenbaum, J. L. (1972). Chlamydomonas flagella. I. Isolation and electrophoretic analysis of microtubules, matrix, membranes, and mastigonemes. *J. Cell Biol.* **54**, 507-539.
Wong, S. Y., Seol, A. D., So, P.-L., Ermilov, A. N., Bichakjian, C. K., Epstein, E. H., Dlugosz, A. A. and Reiter, J. F. (2009). Primary cilia can both mediate and suppress Hedgehog pathway–dependent tumorigenesis. *Nat. Med.* **15**, 1055-1061.
Wood, C. R., Huang, K., Diener, D. R. and Rosenbaum, J. L. (2013). The cilium secretes bioactive ectosomes. *Curr. Biol.* **23**, 906-911.
Woodruff, J. B., Ferreira Gomes, B., Widlund, P. O., Mahamid, J., Honigmann, A. and Hyman, A. A. (2017). The centrosome is a selective condensate that nucleates microtubules by concentrating tubulin. *Cell* **169**, 1066-1077.e10.
Xiang, W., Guo, F., Cheng, W., Zhang, J., Huang, J., Wang, R., Ma, Z. and Xu, K. (2017). HDAC6 inhibition suppresses chondrosarcoma by restoring the expression of primary cilia. *Oncol. Rep.* **38**, 229-236.
Yang, R. and Feldman, J. L. (2015). SPD-2/CEP192 and CDK are limiting for microtubule-organizing center function at the centrosome. *Curr. Biol.* **25**, 1924-1931.
Yang, J., Liu, X., Yue, G., Adamian, M., Bulgakov, O. and Li, T. (2002). Rootletin, a novel coiled-coil protein, is a structural component of the ciliary rootlet. *J. Cell Biol.* **159**, 431-440.
Ye, F., Breslow, D. K., Koslover, E. F., Spakowitz, A. J., Nelson, W. J. and Nachury, M. V. (2013). Single molecule imaging reveals a major role for diffusion in the exploration of ciliary space by signaling receptors. *eLife* **2**, e00654.
Yonezawa, S., Shigematsu, M., Hirata, K. and Hayashi, K. (2015). Loss of γ-tubulin, GCP-WD/NEDD1 and CDK5RAP2 from the centrosome of neurons in developing mouse cerebral and cerebellar cortex. *Acta Histochem. Cytochem.* **48**, 145-152.
Yu, W., Centonze, V. E., Ahmad, F. J. and Baas, P. W. (1993). Microtubule nucleation and release from the neuronal centrosome. *J. Cell Biol.* **122**, 349-359.
Zebrowski, D. C., Vergarajauregui, S., Wu, C.-C., Piatkowski, T., Becker, R., Leone, M., Hirth, S., Ricciardi, F., Falk, N., Giessl, A. et al. (2015). Developmental alterations in centrosome integrity contribute to the post-mitotic state of mammalian cardiomyocytes. *eLife* **4**, e05563.
Zhu, X., Liu, Y. and Yang, P. (2017). Radial spokes-A snapshot of the motility regulation, assembly, and evolution of cilia and flagella. *Cold Spring Harb. Perspect. Biol.* **9**, a028126.
Zou, C., Li, J., Bai, Y., Gunning, W. T., Wazer, D. E., Band, V. and Gao, Q. (2005). Centrobin: a novel daughter centriole-associated protein that is required for centriole duplication. *J. Cell Biol.* **171**, 437-445.