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Motile ciliogenesis and the mitotic prism

Adel Al Jord*¹ , Nathalie Spassky†‡§ and Alice Meunier†‡§¹ 

*Center for Interdisciplinary Research in Biology (CIRB), Collège de France, CNRS 7241 INSERM U1050, PSL Research University, Paris 75005, France, †Institut de Biologie de l'École Normale Supérieure (IBENS), Paris Sciences et Lettres (PSL) Research University, Paris F-75005, France, ‡CNRS, UMR 8197, Paris F-75005, France, and §INSERM, U1024, Paris F-75005, France

Motile cilia of epithelial multiciliated cells transport vital fluids along organ lumens to promote essential respiratory, reproductive and brain functions. Progenitors of multiciliated cells undergo massive and coordinated organelle remodelling during their differentiation for subsequent motile ciliogenesis. Defects in multiciliated cell differentiation lead to severe cilia-related diseases by perturbing cilia-based flows. Recent work designated the machinery of mitosis as the orchestrator of the orderly progression of differentiation associated with multiple motile cilia formation. By examining the events leading to motile ciliogenesis with a methodological prism of mitosis, we contextualise and discuss the recent findings to broaden the spectrum of questions related to the differentiation of mammalian multiciliated cells.

Introduction

Multiple motile cilia form patches of hair-like protrusions that emanate from the apex of specialised epithelial cells, commonly referred to as multiciliated cells. Studies in mammals predominantly describe multiciliated cells of the reproductive, respiratory and central nervous systems. The coordinated and polarised beating of the motile cilia bundles generate directional streams of particle-rich fluids with diverse yet essential contributions. Multiciliated cell functions range from ovum transport in fallopian tubes to pathogen elimination from airways and cerebrospinal fluid circulation along brain cavities. Defects in multiciliated cell differentiation or function cause cilia-related pathologies, known as motile ciliopathies, with severe impacts on reproductive, respiratory and brain functions [Spassky and Meunier 2017; Reiter and Leroux 2017].

Multiciliated cells generate 30 to 300 motile cilia depending on the tissue of localisation. Each motile

cilium of the ciliary tuft stems from a centriole-based root known as a basal body. Centrioles are cylindrical organelles that pair up to form the centrosome, a complex structure known for its microtubule organising capacity in animal cells [Nigg and Holland 2018]. A strict control of centriole number is ensured by symmetric centriole duplication that parallels DNA replication during cell cycle progression (Figure 1A). The formation of supernumerary centrioles in dividing cells is linked to tissue degeneration and tumorigenesis [Nigg and Holland 2018]. In contrast to proliferative contexts, post-mitotic progenitors of multiciliated cells containing a single centrosome evade centriole tally control as their number expands up to several hundred to subsequently nucleate an identical number of motile cilia. Centriole multiplication in differentiating progenitors is characterised by three sequential phases: the amplification phase (A-phase), during which procentrioles form around centrosome-derived deuterosome platforms; the growth phase (G-phase), during which all procentrioles grow synchronously; the disengagement phase (D-phase), during which maturing centrioles detach from their growing platforms to then migrate apically and nucleate multiple motile cilia at the plasma membrane [Spassky and Meunier 2017].

Recent studies exploring multiciliated cell differentiation implicated regulators of mitosis in the

¹To whom correspondence should be addressed (email:

alice.meunier@ens.fr;

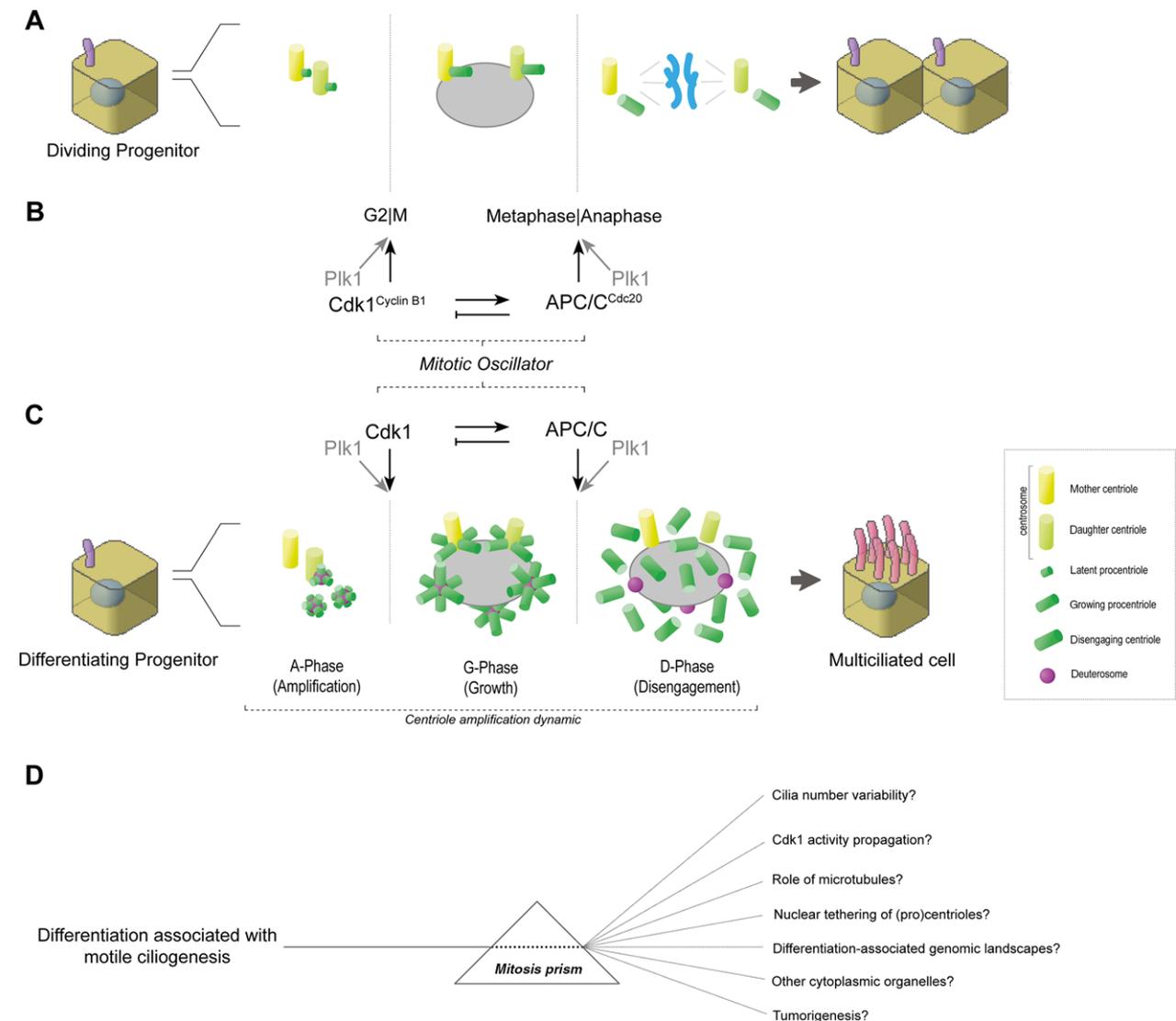
adel.aljord@college-de-france.fr)

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Abbreviations: Cdk1, Cyclin-dependent kinase 1; Cdk2, Cyclin-dependent kinase 2; APC/C, Anaphase Promoting Complex/Cyclosome; Plk1, Polo-like kinase 1; ATR, Ataxia Telangiectasia and Rad3-related protein; FOXM1, Forkhead Box protein M1.

Figure 1 | The mitosis machinery in dividing and differentiating cells

(A and B) Simplified schematics of the regulatory Cdk1–APC/C axis controlling the two key mitosis transitions in cycling cells. Activation of Cdk1^{Cyclin B1} initiates mitotic entry via Cdk1-mediated phosphorylations that prepare the cell for centrosome and chromosome separation by coordinating parallel dynamics of duplicated centrosomes, the cytoskeleton and replicated DNA. Increasing levels of Cdk1 activity promote APC/C^{Cdc20} activation which, together with Plk1, trigger the metaphase/anaphase transition. APC/C^{Cdc20} promotes centriole disengagement and chromosome separation. APC/C^{Cdc20} also triggers the degradation of Cyclin B1, thus inactivating Cdk1 and allowing mitotic exit. (C) Illustration depicting the differentiation associated with motile ciliogenesis. Multiciliated cell differentiation is marked by the amplification (A-phase), growth (G-phase) and disengagement (D-phase) of cilia-nucleating centrioles. The orderly progression of these differentiation steps is orchestrated by the machinery of mitosis, centred on the conserved Cdk1-APC/C mitotic oscillator. (D) Selection of questions discussed in this review relative to the differentiation of mammalian multiciliated cells and viewed through a mitosis prism.



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control of centriole amplification that subsequently conditions motile ciliogenesis [Al Jord et al. 2017; Revinski et al. 2018]. Mitosis regulators coordinate cell division in space and time around two key events: mitosis entry at the G₂|M transition followed by mitosis exit after the metaphase|anaphase transition. The core mitotic oscillator driving the two events involves a negative feedback loop between the protein kinase Cdk1 (Cyclin-dependent kinase 1) and the E3 ubiquitin ligase APC/C (Anaphase Promoting Complex/Cyclosome) [Wieser and Pines 2015] (Figure 1B). This same conserved mitotic oscillator was found to spatiotemporally coordinate the phases of centriole biogenesis in post-mitotic progenitors of multiciliated cells (Figure 1C) [Al Jord et al. 2017; Revinski et al. 2018]. The oscillator's activity, however, is calibrated beneath the mitotic commitment threshold to drive the orderly progression of organelle remodelling associated with motile ciliogenesis while avoiding mitosis [Al Jord et al. 2017]. A selection of nascent questions arising from these findings are highlighted and discussed below, with an emphasis on mammalian multiciliated cells (Figure 1D).

Centriole and motile cilia tally

Mammalian progenitors of multiciliated cells from nervous, respiratory and reproductive systems share common cell fate determinants that initiate differentiation [Spassky and Meunier 2017] and implement a comparable dynamic of centriole amplification [Vladar and Stearns 2007; Dehring et al. 2013; Zhao et al. 2013; Al Jord et al. 2014, 2017; Revinski et al. 2018]. This suggests that mammalian multiciliated cells in different tissues deploy a conserved regulatory mechanism to drive centriole amplification and motile ciliogenesis. However, the number of motile cilia nucleated by a multiciliated cell varies significantly when comparing different regions of a single tissue [Mirzadeh et al. 2010], or multiple tissues of a single organism (e.g. 30–70 in the mouse brain compared to up to 300 in mouse airways; Figure 2A) [Spassky and Meunier 2017]. The reasons behind the mentioned variabilities are still obscure.

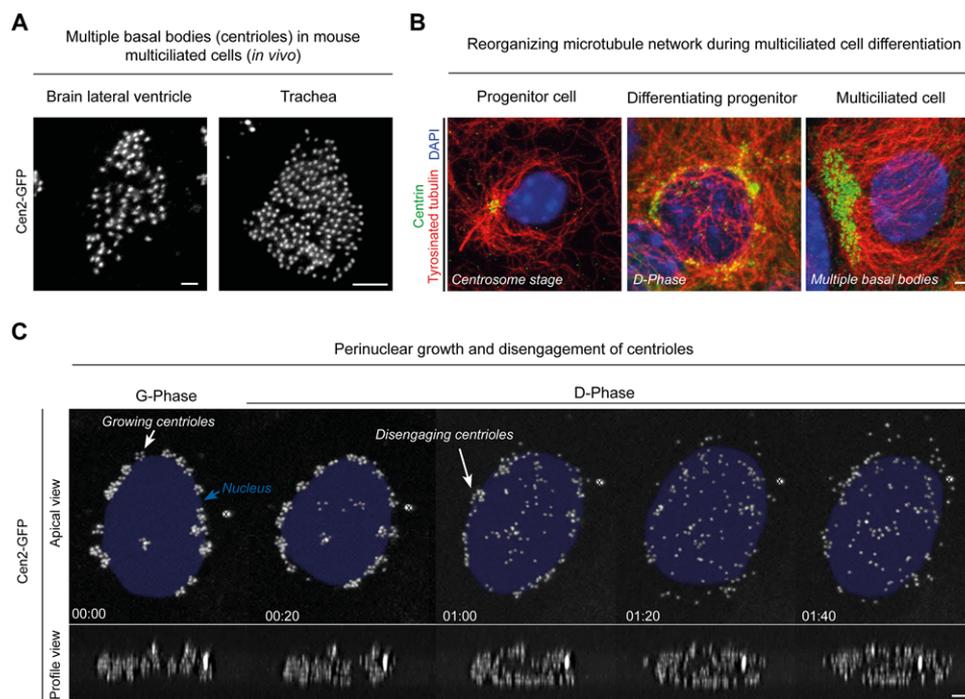
The amount of procentrioles produced during the A-phase seems to dictate final motile cilia number. The initiation of procentriole amplification in progenitors of multiciliated cells was recently shown to

depend on Cdk2 (Cyclin-dependent kinase 2) [Vladar et al. 2018]. Cdk2 is a major cell cycle regulator that initiates both centrosome and DNA duplication at the G₁|S phase transition in dividing mammalian cells [Meraldi et al. 1999]. Despite absence of DNA replication during multiciliated cell differentiation, Cdk2's necessity for procentriole multiplication is suggestive of a G₁|S-like transition during early stages of multiciliated cell differentiation. Moreover, it raises the question of whether differentiating progenitors of multiciliated cells dampen Cdk2 activity, as with Cdk1, to promote procentriole amplification (A-phase) in the cytoplasm while evading DNA replication in the nucleus. Another model of a minimal single Cdk control network, based on two distinct Cdk activity thresholds, was previously shown to be sufficient in driving the yeast cell cycle [Coudreuse and Nurse 2010]. Whether, in differentiating multiciliated progenitors, the two Cdk activities, or their respective activity thresholds, are functionally linked or redundant relative to the centriole amplification-drive is unclear.

Despite the potential links between Cdk2 and Cdk1 in multiciliated cells, the procentriole-generating A-phase remains the time-limiting variable of the motile cilia-associated differentiation process [Al Jord et al. 2017]. Subsequent (pro)centriole growth- and disengagement-phase (G- and D-phase) time spans are relatively constant suggesting that they are temporally insulated, as proposed for mitosis duration in dividing cells [Araujo et al. 2016] (compare panels A and C in Figure 1). The A|G transition is marked by a halt in procentriole production and correlates with procentriole growth and the appearance of Cdk1-mediated phosphorylations. Modulating the timing of the Cdk1-network activity modifies A-phase length and thus final centriole and subsequent cilia number. However, the precise causal link between the procentriole amplification arrest and subsequent (pro)centriole growth that occurs at the A|G transition remains unknown. The link may lie in mechanisms similar to the ones piloting the centriole duplication cycle in dividing cells, and which are governed by key mitosis regulators. Indeed, Cdk1^{Cyclin B1} was implicated in preventing procentriole biogenesis during mitosis, whereas Plk1 (Polo-like Kinase 1) activity was shown to be necessary for the timely structural maturation of procentrioles [Zitouni et al. 2015; Kong et al. 2014].

Figure 2 | Centriole number variability and centriole dynamics in multiciliated cells

(A) Images showing *in vivo* inter-tissue variability of centriole (Cen2-GFP⁺) number in typical mature multiciliated cells observed along brain lateral ventricles extracted from P4 mice (left panel) or along tracheal epithelia extracted from E18 mice (right panel). (B) Immunostainings showing the reorganisation of microtubules in differentiating progenitors *in vitro* at the centrosome stage (left panel), the D-phase (central panel) and the mature multiple basal body stage (right panel). Note centriole colocalisation with the tubulin network. Centrioles (20H5 antibody) are in green, microtubules (YL1/2 antibody) in red and DNA (DAPI) in blue. (C) Apical and profile views of centriole dynamics in Cen2-GFP⁺ mouse brain differentiating progenitors from the G- to D-phase showing a wave of perinuclear disengagement of 136 centrioles; nucleus is shaded in blue; 'x' marks GFP aggregates. Scale bars, 2 μ m. Panels B and C are adapted from Al Jord et al. (2017).



As previously stated, the physiological intra- and inter-tissue variability of centriole and cilia number in ciliated epithelia remains unaddressed. Whether tissue- or cell-dependent restriction points of cilia tally are implemented is therefore unclear. Hypothetically, an active differential regulation of the Cdk1 network at the transcriptional or post-translational level [Fu et al. 2008; Bristow et al. 2014; Wieser and Pines 2015; Rahi et al. 2016; Banyai et al. 2016] could participate in the observed centriole/cilia variabilities. Another perspective was discussed by two studies mentioning a potential link between final centriole number and the cell surface size of mature multiciliated cells [Vladar and Stearns 2007; Dehring et al. 2013]. Regulators of mitosis are known to support the dividing cell shape dynamism [Ramkumar and Baum 2016] and are linked to cell surface growth

in proliferating yeast [McCusker et al. 2007; Moseley et al. 2009; Martin and Berthelot-Grosjean 2009; Anastasia et al. 2012]. This raises the question of whether the mitotic network of regulators could integrate morphological changes of multiciliated cells with centriole phase progression or vice versa. Experimental studies testing the existence of a multiciliated cell 'size gate' regulating cilia number have yet to be undertaken.

Cdk1 activation and propagation

In differentiating progenitors of multiciliated cells, the mitosis machinery coordinates the large-scale centriole dynamic in space and in time (Figures 1B and 1C) [Al Jord et al. 2017, Revinski et al., 2018]. However, the precise spatiotemporal profile of Cdk1

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activation, or the nature of the propagation of the calibrated mitotic state throughout the differentiating progenitor, is unknown. At mitosis onset in proliferating yeast and human cells, Cdk1^{Cyclin B1} activity is proposed to first appear at the centrosome [Bailly et al. 1989; Jackman et al. 2003; Krämer et al. 2004; Chan et al. 2017] and to increase via feedback loop mechanisms [Wieser and Pines 2015]. Cytoplasmic and nuclear reorganisation [Gavet and Pines 2010b, 2010a] are subsequently orchestrated by massive phosphorylation of a myriad of substrates [Dephoure et al. 2008]. The rapid spread of active Cdk1^{Cyclin B1} complexes by random walk diffusion is suggested to be sufficient to spatially coordinate the near synchronicity of mitotic events within an average-sized dividing cell [Chang and Ferrell Jr 2013]. Contrasting mitosis of a typical cycling cell, the timescale of Cdk1-dependent activity in differentiating multiciliated cell progenitors and thus, of a mitosis-like state, differs significantly. Mitosis duration values in various human or mouse cell lines revolve around the 1 hour mark with minor variability [Araujo et al. 2016]. In the context of multiciliated cell differentiation, the delay between visible Cdk1 activation and inactivation (G-phase initiation and D-phase termination, respectively) seemingly averages at around 6 h, as judged by downstream readouts [Al Jord et al. 2017]. Nevertheless, Cdk1-dependent phosphorylations of APC3, an APC/C subunit [Fujimitsu, Grimaldi, and Yamano 2016], are first observed in proximity with the centrosome as of the G-phase. The resorption of the primary cilium, a signalling antenna nucleated by the centrosome and resorbed via APC/C activity in cycling contexts [Wang, Wu, and Kirschner 2014], timely correlates with a wave of centriole disengagement. This raises the possibility that activation of Cdk1 may also originate from the pre-existing centrosome before propagating throughout the cell. Although the enrichment profile of Cdk1^{Cyclin B1} at subcellular compartments remains unaddressed in differentiating progenitors, A-phase procentrioles/deuterosomes present an enrichment of Plk1 [Al Jord et al., 2017, Revinski et al., 2018], an upstream regulator of Cdk1^{Cyclin B1} [Toyoshima-Morimoto et al. 2001; Gheghiani et al. 2017], questioning their role in spatiotemporal regulation of Cdk1 activity. If amplified procentrioles could act as centrosome-independent hubs that participate in the propagation of Cdk1 activation is unknown.

From a developmental tissue-wide perspective, motile ciliogenesis follows remarkable temporal and spatial gradients [Spassky et al. 2005; Toskala et al. 2005]. In the developing mouse brain for instance, a patterned wave of multiciliated cell maturation occurs during early post-natal days along lateral ventricles and follows a caudorostral and ventrodorsal gradient [Spassky et al. 2005]. It is tempting to speculate that the activation of the Cdk1 network could participate in coordinating the progression of these maturation 'waves' in multiciliated epithelia, by analogy with Cdk1-dependent waves of long-distance temporal coordination in other model systems of mitotic cell division [Chang and Ferrell Jr 2013; Deneke et al. 2016; Vergassola, Deneke, and Di Talia 2018]. The implication of Cdk1 activity in tissue-wide coordination of multiciliated cell maturation remains to be probed.

Centriole dynamics

Numerous unknowns endure relative to the mechanical contributors that support (pro)centriole movements all along the differentiation process. During the A-phase, centrosomal centrioles remain in proximity and deuterosome platforms carrying newly formed procentrioles stay within a close centrosomal radius. As differentiating progenitors progress through the G-phase, the mother and daughter centrioles of the pre-existing centrosome separate and the multiple procentriole-bearing deuterosomes slide along the nucleus in opposite directions. This patterned separation at the A|G-transition is impeded by pharmacological inhibition of Cdk1 and Plk1 [Al Jord et al. 2017]. Tubulin immunostainings at the subsequent G|D transition show that all centrioles localise with a drastically reorganising microtubule network when compared to early progenitors or mature multiciliated cells (Figure 2B) [Al Jord et al. 2017]. The microtubule network reshaping may contribute to pre-existing centrosomal centriole and deuterosome dispersion around the nucleus after Cdk1 and Plk1 activation, similar to prophase centrosome separation in cycling cells [Blangy et al. 1995; Smith et al. 2011] that primarily depends on the kinesin-5 motor Eg5 for force generation [Whitehead and Rattner 1998; Tanenbaum et al. 2008]. An Eg5-dependent mechanism may also be in play during the differentiation of multiciliated cells, yet it raises the

question of whether the newly produced centrioles on deuterosomes are capacitated to nucleate microtubules and generate opposing forces. Pericentriolar material (PCM) components, important for microtubule nucleation [Woodruff, Wueseke, and Hyman 2014], localise to immature and maturing centrioles in differentiating progenitors [Zhao et al. 2013; Al Jord et al. 2014, Revinski et al., 2018]. This suggests that the new population of centrioles could also be contributing entities to microtubule network dynamism, instead of just assuming the role of passive passengers riding the microtubule network. Such a scenario, of perinuclear platforms of microtubule nucleation, would be reminiscent of multiple microtubule nucleating foci that surround the nucleus in developing oocytes [Łuksza et al. 2013; Clift and Schuh 2015; Bennabi, Terret, and Verlhac 2016].

During G-phase, amplified procentrioles migrate to surround the nucleus. A tight proximity between the amplified centrioles and the nuclear envelope appears to be maintained until their disengagement (Figure 2C) [Al Jord et al. 2017], suggesting a potential attachment of procentrioles to components of the nuclear envelope and arguing for a non-negligible role of the nuclear envelope in centriole movements. The nuclear envelope is perforated by transport facilitating protein assemblies referred to as nuclear pores [Knockenbauer and Schwartz 2016]. In proliferating cells, nuclear pore complex proteins are involved in nuclear tethering of the centrosomes at mitosis onset [Vaughan and Dawe 2011], a process coordinated by Cdk1-mediated phosphorylation [Baffet, Hu, and Vallee 2015] and important for optimal centrosome positioning during spindle pole formation [Bolhy et al. 2011]. As for differentiating progenitors of multiciliated cells, it is unclear whether mediators of centriole-nucleus tethering, such as dynein [Splinter et al. 2010; Raaijmakers et al. 2012; Firestone et al. 2012], Nup133 [Bolhy et al. 2011] and nesprins [X. Zhang et al. 2009], have explicit regulatory roles in centriole movements. Another unknown is whether the positioning of (pro)centrioles around the nuclear membrane before centriole disengagement is a pre-requisite for the disengagement itself or for an optimal polarised migration towards the cell apex. A recent study proposed that multiple isoforms of Nesprin1 are conserved components of basal body-associated ciliary rootlets in mature brain and airway multiciliated cells [Potter et al. 2017]. The authors

did not address the origin or the potential trajectories of nuclear envelope nesprins that, in mature multiciliated cells, localise to the base of motile cilia bundles. If procentriole-nucleus proximity during the differentiation process mediates the creation of a subsequent link between the motile cilia and the nucleus is unclear.

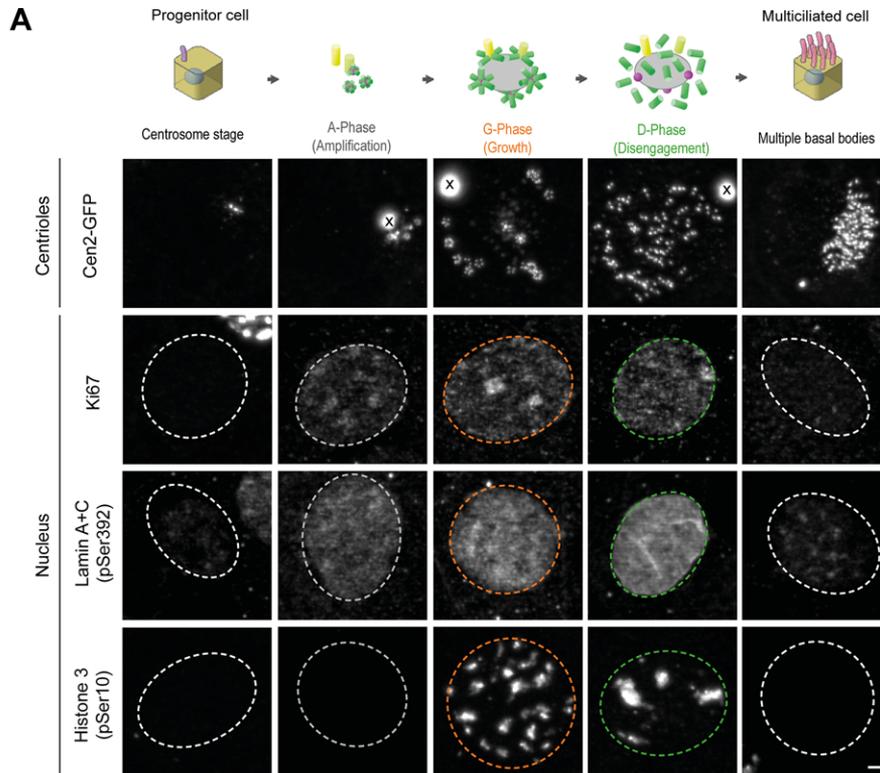
The existence of an S|G₂ checkpoint was recently revealed in cycling cells [Saldivar et al. 2018]. The authors proposed that ATR (Ataxia Telangiectasia and Rad3-related protein) enforces this checkpoint by linking S-phase termination with mitosis entry via an inhibitory action on the pro-mitotic interplay between Cdk1-dependent phospho-activity and FOXM1 (Forkhead Box protein M1). Thus, ATR prevents premature mitotic entry via inhibition of a Cdk1-dependent mitosis switch. If a similar molecular interplay between ATR and Cdk1 occurs prior to the A|G transition in differentiating multiciliated cell progenitors remains to be explored. The potential activation of an S|G₂-like molecular network in differentiating progenitors could, however, provide some insights into whether the centriole-related events occurring promptly after the transition into G-phase may be split into two sub-categories. In other words, could the growth of amplified centrioles in differentiating progenitors be uncoupled from their migratory trajectories around the nucleus?

Nucleus

Nucleus and chromosome remodelling driven by the mitotic oscillator are probably the most striking events observed during cell division. Although differentiating multiciliated cell progenitors uncouple centriole biogenesis from apparent mitotic nuclear events, the nucleus does not truly remain unaffected. The common proliferation marker, Ki-67 [Whitfield et al. 2006], and restrained mitotic phosphorylations of lamins and histones are observed in nuclear compartments during differentiation (Figure 3A) [Al Jord et al. 2017]. In the context of cell division, Cdk1-dependent phosphorylation of lamins disassembles and mobilises these intranuclear intermediate filaments [Ward and Kirschner 1990; Peter et al. 1990], whereas mitotic Histone 3 phosphorylation on Serine 10 promotes chromosome condensation [Hirota et al. 2005; Wilkins et al. 2014]. During terminal differentiation in various cell types, nuclear lamina are essential for the tethering of

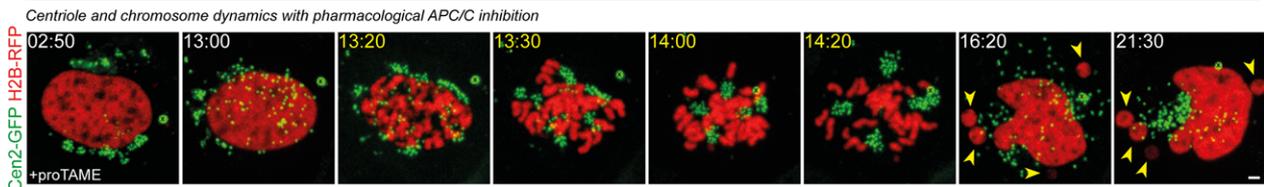
Figure 3 | Nucleus and mitotic capacity of differentiating brain multiciliated cells

(A) Immunostainings of nuclear Ki67 and mitotic phosphorylations of Histone 3 and Lamins A+C relative to phases of the centriole (Cen2-GFP⁺) dynamic in mouse brain differentiating multiciliated cell progenitors *in vitro*; nucleus is outlined and colour-coded. (B) Representative time-lapse images of centriole (Cen2-GFP⁺) and nucleus (histone H2B-RFP⁺) dynamics during proTAME-induced mitosis in mouse brain differentiating progenitors starting from G-phase; mitosis entry (prophase-like nucleus) occurs during D-phase (13:20) followed by metaphase-like (13:30–14:00) and anaphase-like (14:20) states; the small molecule inhibitor proTAME inhibits APC/C, thus indirectly hyperactivating Cdk1; arrowheads indicate micronuclei after mitotic exit (16:20). Time in hh:mm; 'x' marks GFP aggregates. Scale bars, 2 μ m. Panels A and B are adapted from Al Jord et al. (2017).



B

Dormant capacity for abnormal mitosis in differentiating progenitors of multiciliated cells



chromatin to the nuclear periphery and the formation of varying lamina-associated domains involved in the control of gene expression programs [Peric-Hupkes et al. 2010; Kind et al. 2013; van Steensel and Belmont 2017; Lund et al. 2013; Solovei et al. 2013]. Regarding Ki-67, this cell proliferation antigen was recently found to organise heterochromatin

and modulate gene expression [Sobecki et al. 2016; Cuylen et al. 2016]. Expression of Ki67 and curbed mitotic phosphorylations observed in differentiating progenitors may simply be silent consequences of the cell cycle-like activity necessary for centriole production in the cytoplasm. Alternatively, progenitors of multiciliated cells could potentially deploy

mitosis tools to modulate chromatin architecture and regulate differentiation-associated gene expression.

Cytoplasmic organelles

The dynamic spatiotemporal patterns of cytoplasmic organelles, other than centrioles, remain understudied in differentiating progenitors of multiciliated cells. The machinery of mitosis impacts the compartmentalisation and division of numerous organelles in dividing cells to ensure their proper inheritance between the progeny [Jongsma, Berlin, and Neefjes 2015; Champion, Linder, and Kutay 2017]. Core components of the Cdk1 network are essential for Golgi network fragmentation and mitochondrial fission as of mitotic entry to subsequently split the organelles between daughter cells [Lowe et al. 1998; Sütterlin et al. 2001, 2002; Villeneuve et al. 2013; Taguchi et al. 2007; Kashatus et al. 2011; Mishra and Chan 2014]. At mitotic entry in dividing cells, a gradual increase in Cdk1 activity was shown to coordinate a series of different events that includes centrosome separation and cell rounding, followed by nuclear envelope breakdown [Lindqvist et al. 2007; Gavet and Pines 2010a, 2010b]. The coordination of other organelles relative to Cdk1 activity levels is less clear. Yet, this raises the question of whether the calibrated Cdk1 activity associated with multiciliated cell differentiation is sufficient to induce a mitosis-like response from other cytoplasmic organelles. Electron microscopy studies noted two contrasting observations in mature multiciliated cells: a relative paucity of the Golgi apparatus, explained by the lack of secretory activity in these cells, opposed by enrichment clusters of mitochondria beneath the motile cilia patches, proposed to meet the energetic needs of motile ciliary beating [Rhodin and Dalhamn 1956; Ramos and Dym 1977]. It is unknown if progenitor cells simply transport their existing stock of mitochondria during differentiation, or multiply mitochondrial number in a mitosis-like fashion downstream of Cdk1 activity. Nevertheless, these observations may also be emphasising differential response thresholds to Cdk1 activity between Golgi and mitochondria networks.

Tumorigenesis in multiciliated epithelia

Apart from ciliopathies that disrupt brain, reproductive and respiratory functions [Reiter and Leroux

2017], epithelia lined with multiciliated cells are also concerned by tumorigenesis. Some evidence in the literature links ciliogenic programs to tumours in ciliated epithelia. Life-threatening ependymomas are tumours of the central nervous system that can arise in the walls of the brain ventricular system and that account up to 9% of all intracranial tumours. These tumours have a higher prevalence in children, as they are the third most common intracranial cancers in the paediatric population [Kleihues et al. 2002; Liberski 1996; Baloyannis and Baloyannis 2014]. Ependymomas display the ultrastructural and immunohistochemical features of normal ependymal tissue (lumen formation, junctional complexes, microvilli and cilia), suggesting that they most likely emerge from cells of the ependymal lineage [Abedalthagafi et al. 2016; Ho 1986; Kubota et al. 1986; Alfaro-Cervelló et al. 2015; Liberski 1996; Baloyannis and Baloyannis 2014]. Moreover, there is strong molecular and genetic evidence that ependymomas originate from the ependymal progenitors (radial glial cells) [Taylor et al. 2005]. In the female reproductive system, the origin of some ovarian cancers was linked to the fallopian tube [Kim et al. 2012; Perets et al. 2013; Labidi-Galy et al. 2017], and evidence of multiciliated cell differentiation in ovarian or endometrial cancers was documented [Kurman et al. 2011; Laury et al. 2011; Cochrane et al. 2017]. Several studies noted the expression of p73, an early regulator of the motile ciliogenesis program [Marshall et al. 2016], in ovarian cancers [Chen et al. 2000; Hofstetter et al. 2011; Guan et al. 2012]. The contribution of multiciliated cell progenitors to cancer in female reproductive tracts is a matter of debate [Vang, Shih, and Kurman 2013; Rohozinski, Diaz-Arrastia, and Edwards 2017]. Multiciliated cells were reported, yet poorly characterised, in some airway tumours [Bedrossian et al. 1975; Nakamura et al. 1992; Sato et al. 2010; Nair et al. 2014; Kamata et al. 2015]. It remains unclear if the motile ciliogenic program could directly contribute to tumorigenesis in ciliated epithelia.

Most studies of ciliated epithelia cancers overlook the cell cycle-like state of differentiating progenitors of multiciliated cells. During their development, S-phase regulators contribute to the initiation of the centriole amplification program [Kyrousi et al. 2015; Arbi et al. 2016; Terré et al. 2016; Tan et al. 2013; Danielian et al. 2007; Ma et al. 2014; Danielian, Hess, and Lees 2016; Vldar et al. 2018]. Progenitors

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of multiciliated cells with amplified (pro)centrioles then reactivate mitotic cell cycle regulators in a calibrated fashion to drive the course of their differentiation [Revinski et al. 2018; Al Jord et al. 2017]. As a corollary, these nondividing progenitors retain a dormant mitotic capacity. Slight modulations of the mitotic circuit's equilibrium *in vitro* can launch an abnormal mitosis in late stages of differentiation (Figure 3B) [Al Jord et al. 2017]. The abnormal mitosis is marked by anomalous chromosome condensation, segregation and fusion of decondensing chromosomes into one or more nuclei with occasional micronuclei formation (yellow arrowheads in Figure 3B), suggestive of genomic rearrangements. Differentiating progenitors end up with abnormal cytonuclear and ciliogenic phenotypes whose long-term consequences remain undetermined. Together with the aptitude for error-prone mitosis, the capacity of respiratory and brain multiciliated cells to dedifferentiate was reported both *in vitro* and *ex vivo* [Tyner et al. 2006; Park et al. 2006; Abdi et al. 2018]. Dedifferentiated brain multiciliated cells were even proposed to resume cell division and, subsequently, generate new multiciliated cells [Abdi et al. 2018]. An abnormal multiciliated cell that dedifferentiates to divide could, in theory, harbour chromosomal rearrangements responsible for tumorigenesis [Stephens et al. 2011; Crasta et al. 2012; Zhang et al. 2015; Bakhroum et al. 2018]. This hypothesis could be of concrete interest in the case of radial glial cells that, besides being progenitors of brain multiciliated cells, source ependymomas via oncogenic gene rearrangements [Taylor et al. 2005; Parker et al. 2014; Mohankumar et al. 2015; Ozawa et al. 2018].

Concluding remarks

Differentiating progenitors of multiciliated cells mimic the step-wise regulation of centriole biogenesis observed in the cycling cell to tune centriole number and timely trigger centriole growth and disengagement for subsequent motile ciliogenesis. In spite of the large number of centrioles produced for multiple motile ciliogenesis, the events outlining centriole biogenesis on the scale of a single centriole are comparable to the ones that define the centriole duplication cycle in dividing cells [Spassky and Meunier 2017; Nigg and Holland 2018]. Instead of implementing an entirely novel regulatory mechanism, the adapta-

tion of a robust network of cell division regulators for multiciliated cell differentiation may present an economy for the cell given the complexity of centriole biogenesis regulation. Interestingly, additional non canonical cell cycle-associated proteins, such as Cyclin O, Cyclin A1 and Cdc20B (cell division cycle 20B), are necessary to support the alternative regulatory landscape associated with motile ciliogenesis [Funk et al. 2015; Vladar et al. 2018; Revinski et al. 2018]. The analogy between mitotic cell division and multiciliated cell differentiation increased our understanding of multiple motile ciliogenesis. Since differentiating progenitors drive cytoplasmic events in absence of nuclear division and cytokinesis, investigating this capacity to uncouple hallmarks of mitotic cell division for terminal differentiation may, in turn, further illuminate universal attributes of mitosis.

The conserved mitotic oscillator's ability to drive differentiation instead of proliferation provides a new paradigm for future molecular dissection of other, somehow analogous, physiological or pathological developmental contexts. Components of the mitotic regulatory network were implicated in neuronal apoptosis of the developing brain [Konishi et al. 2002; Konishi and Bonni 2003] and in terminal differentiation of monociliated cochlear hair and lens fibre cells [Ezan et al. 2013; Nishimoto et al. 2003; He et al. 1998; P. Zhang et al. 1998; Chaffee et al. 2014]. Whether all major units of the mitotic oscillator are deployed in these physiological contexts is unclear. A future challenge lies in building a more complete picture of cell division regulators implicated in cellular differentiation, as redeployment of cell cycle circuits with minor modulations may plausibly be an underestimated cellular tool that sustains biological novelties for the developmental needs of a complex organism.

Conflict of interest statement

The authors have declared no conflict of interest.

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Author contributions

A.A.J. and A.M. discussed the manuscript content. N.S. commented on the manuscript. A.A.J. wrote the manuscript with edits from A.M.

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