PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B

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Review

Cite this article: Chavali PL, Pütz M, Gergely F. 2014 Small organelle, big responsibility: the role of centrosomes in development and disease. *Phil. Trans. R. Soc. B* **369**: 20130468. http://dx.doi.org/10.1098/rstb.2013.0468

One contribution of 18 to a Theme Issue 'The centrosome renaissance'.

Subject Areas:
cellular biology

Keywords:
centrosome, centriole, microcephaly, dwarfism, cilia, ciliopathy

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Royal Society Publishing

Small organelle, big responsibility: the role of centrosomes in development and disease

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The centrosome, a key microtubule organizing centre, is composed of centrioles, embedded in a protein-rich matrix. Centrosomes control the internal spatial organization of somatic cells, and as such contribute to cell division, cell polarity and migration. Upon exiting the cell cycle, most cell types in the human body convert their centrioles into basal bodies, which drive the assembly of primary cilia, involved in sensing and signal transduction at the cell surface. Centrosomal genes are targeted by mutations in numerous human developmental disorders, ranging from diseases exclusively affecting brain development, through global growth failure syndromes to diverse pathologies associated with ciliary malfunction. Despite our much-improved understanding of centrosome function in cellular processes, we know remarkably little of its role in the organismal context, especially in mammals. In this review, we examine how centrosome dysfunction impacts on complex physiological processes and speculate on the challenges we face when applying knowledge generated from *in vitro* and *in vivo* model systems to human development.

1. Centrosomes and primary cilia

The vertebrate centrosome comprises a pair of centrioles embedded in a pericentriolar matrix (PCM), which is enriched in γ-tubulin complexes responsible for microtubule nucleation. The involvement of centrosomes in mitosis is well documented; they facilitate mitotic spindle assembly, improve the fidelity of chromosome segregation and orient mitotic spindles in relation to the cell cortex and extracellular cues. In addition, centrosomes have been implicated in cell migration, vesicle trafficking and cell polarity [1]. Centrosomes duplicate once per cell cycle in a semi-conservative manner, whereby a procentriole assembles at a perpendicular angle to each old centriole (figure 1) [2]. Centrosome duplication is tightly controlled: per centriole only one procentriole forms per cell cycle. The engagement between mother centriole and its procentriole persists until late mitosis, ensuring that they co-segregate as part of the same spindle pole in cytokinesis. Centrosomes mature prior to mitotic entry by recruitment of further PCM components and γ-tubulin complexes, thereby increasing microtubule nucleation capacity. In many cell types, cell cycle exit triggers the conversion of the mother centriole into a basal body, which nucleates the assembly of a primary cilium (figure 2) [3]. Primary cilia comprise a microtubule axoneme, built of nine doublet microtubules, surrounded by plasma membrane. Motile cilia contain an additional central pair of singlet microtubules. Once believed to be vestigial organelles, primary cilia have fundamental sensory and signalling functions that mediate a range of processes, from proliferation through polarity to differentiation. In particular, primary cilia have been implicated in Sonic Hedgehog (Shh) and Wnt signalling pathways [4]. Moreover, ciliary dysfunction has emerged as the root cause of many human disorders, collectively termed ciliopathies [5]. Below, we will discuss the role of centrosomes in development and pathologies.

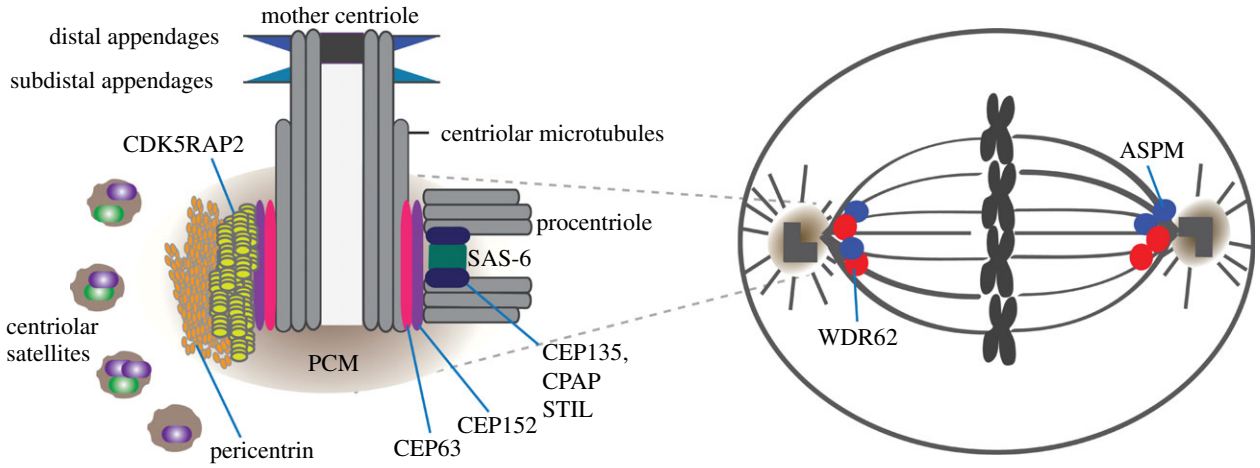

Figure 1. Schematic of the vertebrate centrosome and the mitotic spindle. Key structural features of centrosomes are indicated. Locations of disease-associated centrosomal proteins are shown. Mother centrioles acquire two sets of appendages during centriole maturation. Procentriole formation requires assembly of SAS-6 homodimers into a cartwheel-like structure. STIL interacts with CPAP and possibly SAS-6 in the procentriole. By binding SAS-6, CPAP and centriolar microtubules, CEP135 could stabilize centrioles. CDK5RAP2 and pericentrin are scaffolding proteins located in the PCM, surrounded by centriolar satellites. Centrosomes are positioned at the mitotic spindle poles where spindle microtubules focus and nucleate astral microtubules. ASPM and WDR62 localize to the mitotic spindle poles. (Online version in colour.)

2. Centrosomes in brain development

(a) Autosomal primary recessive microcephaly: a disorder of neurogenesis?

Autosomal primary recessive microcephaly (MCPH) is a genetically heterogeneous neurodevelopmental disorder caused by mutations in at least nine genes. Its main characteristics are non-progressive mental retardation and reduction in head circumference at birth primarily affecting cerebral cortex size [6].

Development of the cerebral cortex begins with the rapid expansion of neuroepithelial progenitor (NEP) cells leading to the thickening of the ectoderm and formation of the neural plate. Normal cortical development relies on neurogenesis, a process responsible for the production of neurons through coordinated neural stem cell proliferation and differentiation. NEP and NEP-derived radial glial (RG) cells reside in the ventricular and sub-ventricular zones (SVZs) and are collectively referred to as apical progenitors (APs) (figure 3). APs are polarized cells with characteristic cytoplasmic extensions: the long basal process extends to the pial surface, whereas the centrosome-containing short apical process contacts the lumen of the ventricle. Initially, most APs undergo symmetric cell divisions to expand the progenitor pool, but the balance subsequently shifts towards asymmetric neurogenic divisions yielding an AP and an immature neuron [7]. Inheritance or loss of the apical and/or basal processes seems important for cell fate decisions [8–11]. Postmitotic neurons generated by neurogenic divisions migrate radially to the pial surface, where they differentiate and form the six-layered neocortex. Therefore, in addition to balancing self-renewing and neurogenic divisions, the formation of the cortex requires neuronal migration. Although MCPH is widely believed to arise from abnormal neurogenesis, simplified gyral patterns have been reported in some cases, raising the possibility that neuronal migration defects occur in MCPH [12–16].

(b) Autosomal primary recessive microcephaly genes: the centrosome connection

Centrosomes have been implicated in multiple processes during brain development, including neurogenesis, neuronal migration and polarity, although their precise contributions are not well established [17]. To date, MCPH represents the best candidate for a *bona fide* genetic disorder of the centrosome. Of the nine genes implicated in MCPH, five encode core centrosomal components (CPAP, CEP152, CEP135, STIL and CDK5RAP2) and two code for proteins associated with mitotic spindle poles (WDR62 and ASPM) (table 1) [18]. The remaining two proteins exhibit no obvious functional overlap; CASC5/Blinkin is a centromere-associated

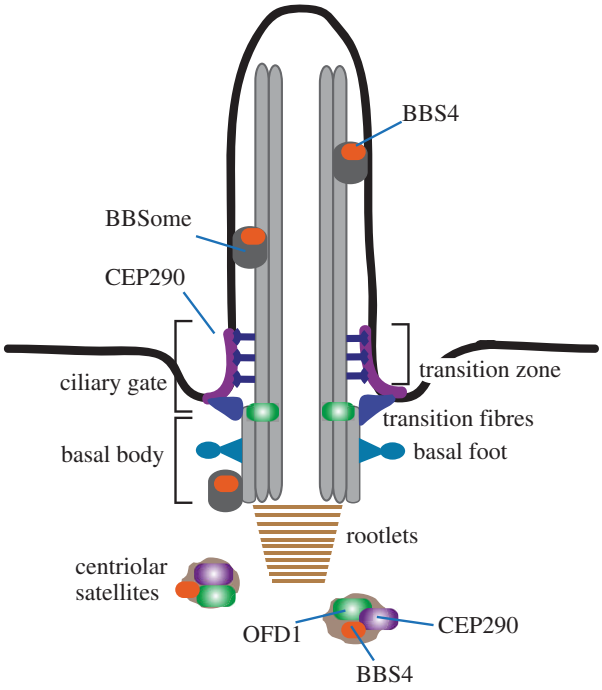

Figure 2. Schematic of the primary cilium. Key structural features of cilia are indicated. The basal body is attached to the plasma membrane via transition fibres derived from the distal appendages. Structural stability is provided by the ciliary rootlet. During ciliogenesis, centriolar satellites deliver proteins like CEP290, BBS4 and OFD1 to the cilium. CEP290 localizes to the transition zone and functions as the ciliary gate. BBS4 assembles into the BBSome at the ciliary base and is transported inside the cilium. OFD1 is localized to the distal end of the basal body. (Online version in colour.)

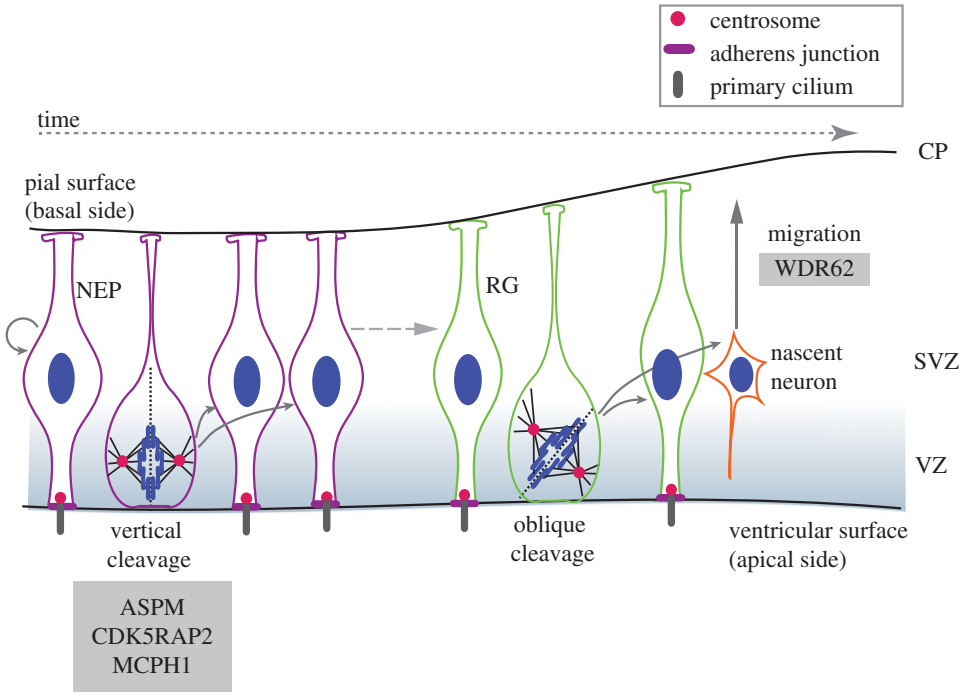

Figure 3. Neurogenesis in the rodent brain. NEPs and RG are collectively termed APs that reside in the ventricular zone (VZ). NEPs first expand through rapid symmetric divisions, but after the onset of neurogenesis they give rise to RG cells, which can divide symmetrically into two RGs or asymmetrically into an RG and a nascent neuron. APs undergoing symmetric proliferative divisions display a vertical cleavage plane relative to the apical surface, whereas those undergoing neurogenic divisions show oblique or horizontal cleavage planes. In the case of symmetric divisions, the cleavage furrow bisects the apical membrane and adjacent adherens junctions. In asymmetric divisions, the RG inherits the apical plasma membrane, whereas the newborn neuron migrates through the SVZ towards the cortical plate (CP) where it matures. Loss-of-function of CDK5RAP2 and ASPM in mouse models compromises symmetric AP divisions, similarly to CDK5RAP2-deficient human brain organoids. Individuals with WDR62 mutations exhibit cortical malformations and neuronal migration defects. (Online version in colour.)

protein involved in the spindle assembly checkpoint [19,20], whereas MCPH1, a nuclear and centrosomal protein, controls cell cycle checkpoint transitions, DNA damage response and repair [21,22].

Despite not being core centrosomal components, both ASPM and WDR62 localize to the mitotic spindle poles and are involved in mitotic spindle assembly and normal mitotic progression [23–25]. Centriole biogenesis requires a small core set of proteins: PLK4, SAS-6, STIL/Ana2/SAS-5, CPAP/CENPJ/SAS-4, CEP135/Bld10 and CEP152/Asl [2]. Strikingly, four of these carry MCPH-linked mutations [26–28]. CPAP/MCPH6 appears to be a key node of the MCPH protein network, because it binds STIL/MCPH7, CEP152/MCPH9 and CEP135/MCPH8, although probably not simultaneously [29–32]. A missense MCPH mutation weakens the ability of CPAP to bind STIL and impairs centriole production [32–35]. By contrast, MCPH-causing mutations in STIL do not interfere with centriole formation, but prevent cell cycle-dependent degradation of the protein, thereby triggering centriole amplification [36]. Thus, MCPH mutations seem to target essential components of the centriole biogenesis pathway. By perturbing the precise control of centriole duplication, these mutations are able to deregulate centrosome numbers in cells. Indeed, although not required for centriole formation *per se*, CDK5RAP2 has also been implicated in controlling centrosome numbers, in addition to roles in centrosomal microtubule nucleation and organization, mitotic spindle orientation and ciliogenesis [37].

Thus far, there is little evidence to suggest that centriole biogenesis is impaired in MCPH patient-derived cells, although ultrastructural studies will be needed to confirm if this is indeed the case [38]. Conversely, the presence of normal centrosomes in patient-derived adult somatic cells does not necessarily rule out defective centriole biogenesis during development, because cell division cycles in the embryo must be subject to stricter temporal control. Centrioles are involved in recruiting and organizing the PCM, and centriole length correlates with the amount of PCM in the centrosome [39–41]. CPAP, an important node in the MCPH protein network, is a key regulator of centriolar microtubule elongation, and thus centriole length may be impaired by MCPH-linked mutations in CPAP and its interactors [41–43]. We speculate that if centrioles are too short in MCPH cells, this could impair PCM formation and centrosome maturation, which in turn may interfere with centriole duplication [44]. Moreover, short centrioles may not be able to acquire appendages, structures implicated in centriole maturation as well as basal body function and ciliogenesis.

Below we describe the cellular pathways most susceptible to centrosome dysfunction and their potential contribution to neurogenesis.

(c) Linking cellular phenotypes of autosomal primary recessive microcephaly mutations to neurogenesis

(i) Cell cycle progression, centrosome duplication and mitosis

The cell cycle machinery faces an immense challenge during development; in addition to its crucial task of maintaining genome integrity, it needs to ensure that cell division occurs in a temporally controlled fashion. Changes in cell cycle length have been reported during the development of

Table 1. Summary of centrosomal genes implicated in genetic disorders. (NP, neural progenitors.)

gene	disease locus (disease)	major clinical features	protein localization	protein function	phenotype in mouse models
ASPM	MCPH5	microcephaly, mild to severe cognitive impairment, seizures and short stature	mitotic spindle pole and midbody	mitotic spindle orientation and organization, mitotic progression, interkinetic nuclear migration and cytokinesis	microcephaly, reduced proliferation of NPs, neuronal migration defects, abnormal cortical layering, germ cell defects and abnormal Wnt signalling
CDK5RAP2	MCPH3	microcephaly and short stature	centrosome	centrosome organization and maturation, microtubule organization, centrosome cohesion, centriole engagement and centrosome-spindle pole connection	microcephaly, reduced proliferation of NPs, mitotic progression defects and apoptosis in NPs, supernumerary centrosomes and mild macrocytic anaemia
CEP63	SCKL6 (Seckel)	microcephaly and short stature	centrosome	centriole duplication	
CEP135	MCPH8	microcephaly	centrosome	centriole biogenesis, centrosome cohesion and microtubule organization	
CEP152	MCPH9	microcephaly, moderate cognitive impairment and simplified gyri	centrosome	centriole biogenesis	
	SCKL5 (Seckel)	proportionate short stature, severe microcephaly, mental retardation and simplified gyri			
CPAP/CENPJ	MCPH6	microcephaly, mild to severe cognitive impairment and facial dysmorphism	centrosome	centriole biogenesis and scaffolding PCM assembly	dwarf, microcephaly, increased apoptosis, abnormal centrosome numbers, aneuploidy, memory impairment and skeletal anomalies
	SCKL4 (Seckel)	Proportionate short stature, microcephaly, skeletal anomalies			
MCPH1	MCPH1	microcephaly, short stature, premature chromosome condensation and mild to severe cognitive impairment	centrosome and nucleus	DNA damage response and repair and cell cycle checkpoints	growth retardation, microcephaly, reduced proliferation of NPs and radiation sensitivity
ORC1	MGS1 (Meier-Gorlin)	proportionate short stature, microcephaly, knee and ear abnormalities and skeletal abnormalities	centrosome and nucleus	DNA replication, centrosome cycle and centriole copy number regulation	
PCNT	MOPD2 (microcephalic osteodysplastic PD II)	extreme but proportionate short stature, bone abnormalities, microcephaly and near normal intelligence	centrosome	centrosome maturation and mitotic spindle organization	perinatal lethality, microcephaly, reduced olfactory bulb, defective olfactory cilia and neuronal migration defects

(*Continued.*)

Table 1. (*Continued.*)

gene	disease locus (disease)	major clinical features	protein localization	protein function	phenotype in mouse models
STIL	MCPH7	microcephaly, ataxia and short stature	centrosome	centriole biogenesis, mitotic entry	embryonic lethality, defects in neural tube closure and heart development
WDR62	MCPH2	microcephaly, pachygyria, hypoplesia of corpus callosum, cortical thickening, lissencephaly, polymicrogyria and facial dysmorphism	mitotic spindle pole	mitotic spindle assembly and orientation, and centrosome-spindle pole connection	premature cell cycle exit and reduced proliferation of NPs (*in utero* siRNA only)
BBS4	BBS4 (Bardet–Biedl)	retinal dystrophy, obesity, polydactyly, renal malformation, hypogenitalism and male infertiliy	centriolar satellites, centrosome and cilium	centriolar satellite function, microtubule anchorage and cilium formation	partial embryonic lethality, low birth weight and small size, obesity, defects in airway cilia function, retinal degeneration and sperm lacks flagellum
CEP290	BBS14 (Bardet–Biedl)	retinal dystrophy, obesity, polydactyly, cognitive impairments, hypogenitalism and renal malformation	centriolar satellites, centrosome, cilia (transition zone) and nucleus	microtubule organization, cilium assembly and ciliary gatekeeper	retinal degeneration, olfactory dysfunction and defective cerebellar midline fusion
	JBTS5 (Joubert)	cerebellar malfunction, ataxia, retinal degeneration and nephronepthisis			
	MKS4 (Meckel)	occipital encephalocele (neural tube defect), polydactyly and dysplastic kidneys			
	SLSN6 (Senior–Loken)	retinal dystrophy and nephronepthisis			
OFD1	OFD type I	male embryonic lethality; abnormalities of face, oral cavity and digits, polycystic kidney disease, mental retardation and macrocephaly	centriolar satellites and centrosome	regulation of centriole length, control of distal appendage formation and cilium assembly	male mice: early gestation lethal and neural tube closure defects; female mice: die at birth, left-right axis randomization, facial and oral abnormalities, and polydactyly
	Joubert syndrome 10	mental retardation, recurrent infections, developmental delay, cerebellar abnormalities and retinitis pigmentosa			
	SGBS2	perinatal male lethality, macrocephaly, craniofacial anomalies and respiratory problems			

the mammalian cortex. In particular, G1 phase lengthens and S phase shortens as cells transition from proliferative to neurogenic divisions [45–47]. Thus, cell cycle duration and cell fate determination may be linked in the neuroepithelium. Loss of centrosome integrity in cultured cells has been reported to induce senescence or p38 mitogen-activated protein kinase-dependent G1 arrest but whether these occur in cells with MCPH mutations remains to be seen [48–50].

Cells with abnormal centrosome numbers take longer to form a bipolar spindle and consequently show a mitotic delay [51–55]. In fact, mitotic delay is the major phenotype seen after depletion of WDR62, ASPM and STIL in developing zebrafish retinal neuroepithelium [56]. Mutations in CDK5RAP2 also cause mitotic delay in the mouse brain [57]. A recent study suggests that cells sense the duration of mitosis; in particular, prolonged prometaphase in the mother cell causes daughter cells to exit the cell cycle, even if mitosis is completed normally [58]. Therefore, mitotic delay triggered by aberrant centrosomes could obliterate long-term proliferative capacity of progenitors leading to MCPH. In support of this scenario, reduced proliferation is the shared phenotype of CDK5RAP2 mutation and WDR62- or ASPM-depletion in the mouse neocortex [24,57,59]. Premature entry into mitosis has also been implicated in MCPH [60].

Cells containing too few or too many centrosomes exhibit transient anastral, mono- or multipolar spindles and frequent chromosome missegregation [53,61,62]. Chromosomal instability generates aneuploidy, which has a strong anti-proliferative effect owing to imbalanced gene expression [63]. Remarkably, centrosome amplification induced by PLK4 overexpression in the mouse brain causes mitotic delay, aneuploidy and apoptosis in neural progenitors, collectively leading to microcephaly [64]. Of these, mitotic delay and aneuploidy are more likely to account for the proliferative impairment than apoptosis, because p53 disruption does not restore normal brain size in PLK4-overexpressing mice, and elevated apoptosis is not consistently observed in mouse models of MCPH. Multipolar spindles are also seen in CDK5RAP2 mutant mouse neuroepithelium, but a link to aneuploidy is yet to be established in this system [57].

An example for the physiological effects of aneuploidy is provided by the rare genetic disease, mosaic variegated aneuploidy syndrome [65–67]. Whole chromosome aneuploidy occurs in greater than 25% of cells of affected individuals. Consistent with a deleterious effect of aneuploidy on brain development, microcephaly is frequent in this syndrome, and additional structural brain defects and seizures are also seen in some patients [66]. The severity of these defects may depend on the extent of aneuploidy acquired during prenatal brain development. We hypothesize that the aneuploidy triggered by centrosome dysfunction in MCPH is not as profound as in mosaic variegated aneuploidy, thus explaining the overall milder brain phenotype of MCPH.

(ii) Mitotic spindle orientation

Balancing the number of progenitors and differentiated cells is crucial for normal brain development. Cell fate decisions frequently depend on the orientation of the cell division axis, which controls partitioning of fate determinants into daughter cells [7]. APs undergoing symmetric proliferative divisions display vertical cleavage plane (i.e. perpendicular to the apical surface), whereas those undergoing neurogenic divisions show oblique or horizontal cleavage planes (figure 3) [7]. Only if the plane is vertical to the apical surface, do both daughter cells inherit a region of the apical membrane, important for maintenance of stem cell fate [68,69]. Mitotic spindle orientation is governed by centrosome-nucleated astral microtubules that are captured by cortically tethered microtubule motor complexes [70]. For centrosomes to orient the mitotic spindle, they must nucleate astral microtubules and maintain stable association with the mitotic spindle poles, processes impaired in CDK5RAP2- and WDR62-deficient cells [24,37,71,72]. Mitotic spindle orientation defects have been described in cells depleted of WDR62, CPAP and STIL, and a premature shift to oblique orientation occurs in developing mouse brains depleted of ASPM, MCPH1 and CDK5RAP2 [24,57,73–75]. Correct positioning of the cleavage furrow and accurate execution of cytokinesis are also crucial for regulating the cell division plane. So far, ASPM is the only MCPH protein directly implicated in cytokinesis [76–78]. Although spindle orientation defects are likely to contribute to the aetiology of MCPH, they probably account only for part of the cell loss, because randomized spindle orientation in the mouse neocortex does not preclude normal neuron production rates [8].

(iii) Centrosome asymmetry and ciliogenesis

By nature of the centrosome duplication cycle, the two centrosomes contain differentially aged centrioles: a fully mature mother centriole and an immature daughter centriole produced in the preceding cell cycle. In the mouse neocortex, inheritance of the mother centriole maintains stemness; interfering with this process causes premature depletion of progenitors from the proliferative zone [79]. Why is this so? Recent work suggests that centrosome asymmetry determines the speed of cilia assembly in daughter cells following mitosis. The cell in possession of the mother centriole assembles a primary cilium faster [80]. Cilia transmit Shh and Wnt signals, both involved in neurogenesis, and rapid access to these could impact on cell fate decisions [81–83]. If MCPH mutations impaired centriole maturation, this could impede speed of cilium formation, causing abnormal signalling and premature neural differentiation.

Centrosomes are also considered relevant to interkinetic nuclear migration (INM), a process characteristic of APs. INM involves the movement of nuclei between the apical and basal surfaces in phase with the cell cycle [84]. Although its precise role in neurogenesis is not clear, INM exposes nuclei to signalling gradients, thereby potentially influencing cell fate decisions [85]. It will be important to establish whether INM is affected in MCPH mouse models, especially since ASPM in *Drosophila* contributes to this process [86].

(d) *In vivo* autosomal primary recessive microcephaly models: rodent or human?

Our current understanding of MCPH, and much of neurogenesis, is based on studies of murine brain. However, the human cortex is increased in size compared with most other species, partly owing to the protracted proliferative activity of progenitors in the outer SVZ, a zone unique to gyrencephalic brains. Unlike APs in the rodent cortex, these progenitors display basal but not apical processes, yet undergo self-renewing and neurogenic divisions [9–11]. Thus, gyrencephalic cortical

development requires cell behaviour atypical of the rodent brain. A recent breakthrough in brain research has been the generation of human cerebral organoids from induced pluripotent stem cells [16]. These recapitulate many of the complex features of embryonic brain development and could prove invaluable in deciphering disease mechanisms of MCPH. Remarkably, organoids derived from CDK5RAP2-deficient MCPH patient cells display premature neural differentiation and spindle orientation defects. Premature differentiation could reduce the number of early progenitors (i.e. founder cells), causing an overall collapse in neuron numbers.

3. Centrosomes and body size

(a) Primordial dwarfism syndromes

Microcephalic primordial dwarfism (PD) defines a group of autosomal recessive human genetic disorders that show pre- and postnatal growth failure accompanied by microcephaly [87]. Of these, the most prominent diseases are Seckel syndrome, microcephalic osteodysplastic PD (MOPD) types I and II, and Meier-Gorlin syndrome. At least 14 different genes have been implicated in PD so far. These encode proteins involved in DNA replication such as subunits of the origin recognition complex (ORC), in DNA damage response like ATR kinase, ATR-interacting protein (ATRIP) [88] and CtIP [89], or in centrosome function like CEP152, CPAP, pericentrin (*PCNT*) and CEP63 [90–94] (table 1). In line with the consensus that PD represents a type of hypocellular dwarfism, PD proteins are all implicated in the cell cycle. Below, we will discuss how centrosome-related pathways could contribute to the profound cell loss during fetal development in PD.

(b) Cellular pathways disrupted by mutations in centrosomal primordial dwarfism genes and their putative link to organismal growth

(i) DNA damage response and replicative stress

The first Seckel allele identified was a mutation in ATR (ATM- and RAD3-related), a kinase that orchestrates cellular response to single-stranded DNA resulting from stalled replication forks or DNA repair intermediates [95,96]. Subsequent discoveries of Seckel mutations in proteins implicated in ATR activation, ATRIP and CtIP, further strengthen the case for impaired ATR signalling being the cause of Seckel syndrome [88,89]. ATR, and its substrate, the checkpoint kinase CHK1, coordinate the response to replicative stress, accumulation of which can disrupt development and tissue homoeostasis [97]. Mutations in the ORC proteins, involved in DNA replication, cause Meier-Gorlin syndrome [90,92]. Cells derived from MOPDII patients with *PCNT* mutations exhibit defective ATR signalling and a marked reduction in the centrosomal pool of CHK1 [60,93]. Although the precise role of this pool remains controversial, reduced centrosomal CHK1 levels correlate with premature activation of cyclin B-CDK1 in pericentrin-deficient cells [60,98,99]. Thus, anti-proliferative effects of replicative stress and premature CDK1 activation could contribute to the global growth failure seen in PD patients. However, impaired ATR signalling may not be universal across all PD complementation groups. CHK1 kinase function is normal in CEP152-Seckel fibroblasts, and instead these show activation of ataxia telangiectasia-mutated (ATM) kinase, which recognizes double-stranded DNA breaks [100]. By contrast, DNA damage response is intact in a mouse model of CPAP-Seckel, which recapitulates features of Seckel syndrome including intrauterine growth retardation and small brain size [101,102]. Therefore, dwarfism caused by mutations in centrosomal genes may not be directly linked with their capacity to activate DNA damage response or DNA repair.

(ii) Centrosome amplification and aneuploidy

Centrosome amplification has been observed in fibroblasts with disease-associated mutations in pericentrin, CEP152, CPAP and ORC1 [91,96,100]. It is thus possible that centrosomal PD proteins prevent centriole overduplication, for instance by promoting engagement between the mother centriole and its procentriole [38,103,104]. Recent studies suggest that ORC1 exercises centrosome copy number control by suppressing CDK2-cyclin E-dependent reduplication of centrioles, a function specifically disrupted by Meier-Gorlin mutations in *ORC1* [91]. Cells with centrosome amplification must inactivate excess centrosomes or cluster these into two poles [105]. In the absence of such mechanisms, centrosome amplification results in multipolar cytokinesis and cell death [106]. If inactivation or clustering is efficient, cells can survive but at a cost; centrosome clustering causes not only mitotic delay, but also chromosomal instability [55,61]. Importantly, genome instability has been documented in CPAP-Seckel mouse and in Seckel patient-derived cells; CEP152-Seckel lymphocytes exhibit aneuploidy in addition to abnormal mitotic spindle morphologies [100,101].

(iii) Ciliogenesis

Recent studies implicate cilia dysfunction in the aetiology of PD. Both ORC1 and pericentrin mutant cells show defective ciliary recruitment of the Shh receptor, Smoothened and consequently abnormal Shh signalling with potentially wide-ranging effects on development [107]. As the primary cilium assembles on a basal body, a structure derived from the mother centriole, CEP152 and CPAP, along with the MCPH proteins, STIL and CEP135, are required not only for centriole biogenesis but also for ciliogenesis [108–112]. Given the link between centriole biogenesis and primary cilia assembly, it is puzzling that patients carrying MCPH- or PD-associated mutations in these essential regulators do not exhibit clinical signs of defective ciliary function. We speculate that in the context of a tissue, cells with numerical or structural centrosome abnormalities might be eliminated and/or prevented from assembling aberrant cilia [64].

4. Ciliopathies

(a) Primary cilia formation and centriolar satellites

Most human cell types are ciliated and therefore defects in ciliary function or structure cause pleiotropic genetic disorders, termed ciliopathies. These share many clinical features, with kidney, eye, liver and brain being the most affected organs [113]. Over the last decade, cilia have emerged as vital cellular compartments for transducing mechanical and extracellular signals, regulating organogenesis, planar polarity, proliferation, DNA damage response and autophagy [4,114,115]. Indeed, impaired signalling is considered a key underlying factor in ciliopathies.

Mutations in over 30 proteins have been identified in ciliopathies (see [113] for extensive review). Despite many of these localizing to centrosomes and basal bodies, only a handful have known functions both in centrosomes and ciliogenesis, and these include CEP290 and the oral-facial-digital syndrome 1 (OFD1) protein. CEP290, OFD1 and a third ciliopathy protein, Bardet–Biedl syndrome (BBS4), also associate with centriolar satellites, electrondense granules concentrated around the PCM [116,117]. Through a detailed discussion of these factors, we will highlight key themes of ciliary research and aim to provide insight into the complex nature of ciliopathies.

The first step in ciliogenesis is the transformation of the mother centriole into a basal body, which then docks at the plasma membrane and nucleates the assembly of axonemal microtubules (figure 2). Golgi-derived vesicles fuse with the plasma membrane to extend the membrane protrusion around the growing axoneme, a process dependent on the GTPase, Rab8a, and its guanine nucleotide exchange factor, Rabin8. Cilium assembly and maintenance require a selective transport system for ciliary proteins called intraflagellar transport (IFT) [3]. Centriolar satellites have been implicated in centrosomal protein transport and ciliogenesis [118]. In addition to providing a platform for pro-ciliogenesis proteins, satellites suppress untimely cilia formation by sequestering key ciliogenesis factors [115,119–122]. Thus, certain ciliopathy phenotypes could arise from ill-timed ciliogenesis and signalling.

In cycling cells, CEP290 mediates interphase microtubule organization [123]. In ciliogenesis, CEP290 promotes vesicle trafficking to the ciliary membrane and ciliary outgrowth; it facilitates ciliary targeting of Rab8a, but it also promotes ciliary translocation of BBS4, thereby completing the assembly of the BBSome, a multiprotein complex that binds Rabin8 and triggers Rab8a activation [122–124]. CEP290 may also act as a ciliary gatekeeper; in *Chlamydomonas reinhardtii*, CEP290 is found at the transition zone, a region that restricts entry of soluble proteins into the ciliary compartment [125]. By contrast, OFD1 acts at the distal ends of centrioles to control distal appendage formation and centriole length [126]. It is involved in targeting the IFT protein, IFT88, to the distal end of the centriole and possibly to the basal body [127]. Through recruitment of CEP164, a distal appendage protein mutated in cystic kidney disease, OFD1 also promotes basal body docking to the plasma membrane [114,128].

(b) Phenotypic spectra of ciliopathies caused by mutations in centriolar satellite components

Animal models underscore the importance of OFD1, CEP290 and BBS4 in ciliogenesis. Zebrafish depleted of *Ofd1* show typical signs of defective cilia: body curvature and hydrocephalus. Laterality defects are also present owing to shorter motile primary cilia in the Kupffer vesicle, an embryonic organ required for left–right asymmetry [129]. Such defects also occur in CEP290- and BBS4-depleted zebrafish, with the former exhibiting additional retinal anomalies [130,131].

Ofd1 is located on the X chromosome both in mice and humans. Loss-of-function studies in mice show a gender-dependent effect of Ofd1 inactivation; hemizygous males die during gestation probably as a result of neural tube closure failure, whereas heterozygous females die at birth exhibiting abnormalities in oral and facial structures (table 1) [132]. On the cellular level, ciliary axoneme elongation is defective in the developing forebrain of mutant females despite basal bodies being able to dock normally to the plasma membrane [133]. These models recapitulate many of the phenotypes of human oral-facial-digital syndrome type 1, which also exhibits an X-linked dominant male-lethal trait [134]. Male lethality occurs in early pregnancy, whereas affected females exhibit polycystic kidney disease and neurodevelopmental abnormalities. There is considerable phenotypic variability even within the same family, most likely owing to cellular mosaicism created by X-inactivation. Mutations in *OFD1* have also been uncovered in other ciliopathies such as Joubert syndrome and Simpsons–Golabi–Behmel syndrome type 2 (SGBS2) [135]. While mental retardation is common to both, Joubert syndrome involves cerebellar malfunction and ataxia, whereas SGBS2 is characterized by macrocephaly.

BBS4 null mice display partial embryonic lethality. Newborns show low body weight before weaning, yet subsequently develop obesity and retinal degeneration [136]. By contrast, CEP290 mutant mice are viable. Hypomorphic alleles cause retinal degeneration and impaired olfaction [137,138], whereas null animals exhibit a defective cerebellar midline fusion [139]. In humans, *BBS4* mutations cause BBS characterized by retinal dystrophy, obesity, cognitive impairments and renal malformation [140]. *BBS4* mutations are also present in the embryonically lethal Meckel syndrome [141]. Likewise, *CEP290* mutations have been linked to both BBS and Meckel, in addition to Senior–Loken and Joubert syndromes [142].

How can mutations in a single gene lead to so many disease phenotypes and disorders? First, evidence suggests that hypomorphic mutations target specific aspects of protein function [126]. Second, CEP290 and OFD1 participate in multiple protein complexes during ciliogenesis, which could be differentially affected by mutations [122,123,126,143]. Last, patients often carry mutations in multiple ciliary genes [116]. As normal ciliary function is implicated in a growing number of fundamental signalling pathways, mutations causing suboptimal function will have wide-ranging effects in development and homoeostasis. Further work will be necessary to shed light on how disease-linked perturbations of cilia function and signalling pathways generate these complex organ-specific phenotypes.

5. Perspectives

Remarkably, CPAP and CEP152 are mutated in both MCPH and Seckel syndrome, suggesting that the two disorders might represent different ends of a disease continuum [26,100, 102,144]. Indeed, recent reports uncovered mutations in the Seckel genes *ATR* and *CtIP* that cause primary microcephaly without PD, whereas short stature has been noted in some individuals with mutations in the MCPH gene, *CDK5RAP2* [16,89,145]. Thus, MCPH and Seckel must also share common cellular mechanisms. We speculate that mitotic defects, especially mitotic delay and chromosome segregation errors, are probable common causes of MCPH and PD (figure 4). Over 30 billion neurons are produced during human fetal brain development with little proliferation later in life, rendering the brain particularly susceptible to mitotic defects and aneuploidy. Indeed, microcephaly is frequent in mice and humans with trisomies. Moreover, CEP63 and CEP152 form a complex with CEP57, a protein mutated in mosaic variegated aneuploidy syndrome, a genetic disorder associated with severe

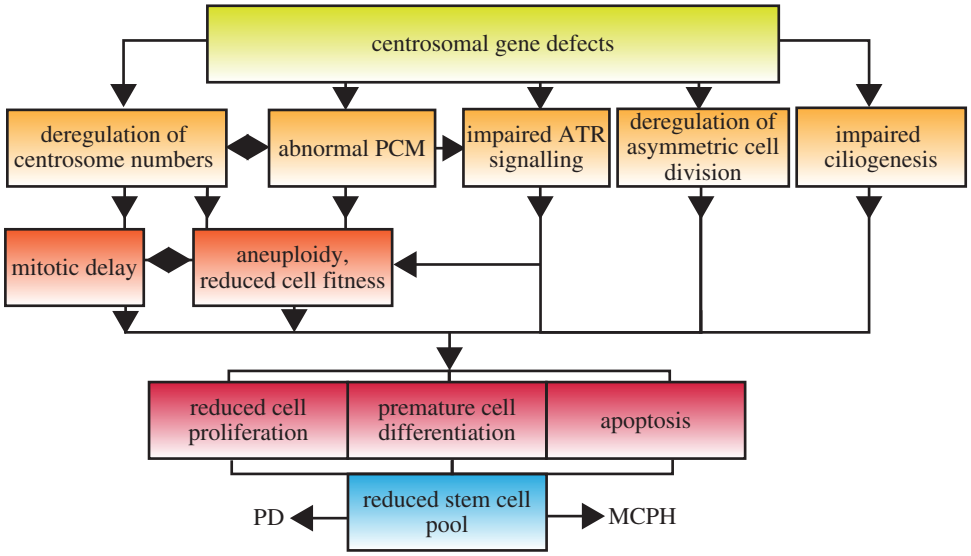

Figure 4. Putative cellular mechanisms implicated in MCPH and PD. (Online version in colour.)

microcephaly and short stature [67,146]. Genome instability disorders such as Fanconi anaemia, Nijmegen breakage or Bloom syndromes also manifest with microcephaly and short stature, arguing for a vital role of genome integrity in neurogenesis and normal body size [147]. A striking difference between these disorders and MCPH or PD is the lack of cancer predisposition in the latter, indicative of a robust anti-proliferative effect of abnormal centrosomes. Validated *in vivo* models that mimic disease-associated mutations will be needed to tease apart the cellular mechanisms and address the physiological consequences of centrosome dysfunction.

Acknowledgements. We thank members of the Gergely group and the reviewers for helpful comments.

Funding statement. We thank Cancer Research UK for the generous core funding of our laboratory.

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