Once and only once: mechanisms of centriole duplication and their deregulation in disease

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Abstract | Centrioles are conserved microtubule-based organelles that form the core of the centrosome and act as templates for the formation of cilia and flagella. Centrioles have important roles in most microtubule-related processes, including motility, cell division and cell signalling. To coordinate these diverse cellular processes, centriole number must be tightly controlled. In cycling cells, one new centriole is formed next to each pre-existing centriole in every cell cycle. Advances in imaging, proteomics, structural biology and genome editing have revealed new insights into centriole biogenesis, how centriole numbers are controlled and how alterations in these processes contribute to diseases such as cancer and neurodevelopmental disorders. Moreover, recent work has uncovered the existence of surveillance pathways that limit the proliferation of cells with numerical centriole aberrations. Owing to this progress, we now have a better understanding of the molecular mechanisms governing centriole biogenesis, opening up new possibilities for targeting these pathways in the context of human disease.

Procentriole
A newly constructed centriole that is unable to duplicate.

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Centrosomes function in animal cells as microtubule organizing centres and thus have key roles in regulating cell shape, polarity and motility, as well as spindle formation, chromosome segregation and cytokinesis[1–4]. A typical animal cell begins the cell cycle with a single centrosome, comprising a pair of centrioles. The centrioles assemble a protein matrix known as the pericentriolar material (PCM), which harbours not only proteins important for microtubule nucleation[5] but also regulators of the cell cycle and its checkpoints[6]. Fully mature centrioles can also dock at the plasma membrane where they function as basal bodies for the formation of cilia and flagella[7], and dysfunction of the basal body–ciliary apparatus gives rise to ciliopathies[8].

In cycling cells, the two parental centrioles duplicate once in each cell cycle to form two centrosomes, which function as spindle poles in mitosis. Here, we summarize the recent progress in understanding the mechanisms underlying the regulation of centriole duplication, and we discuss how centrosome aberrations contribute to human diseases such as cancer and neurodevelopmental disorders[1,9,10]. We primarily focus on vertebrate centrosomes but incorporate data from other organisms where appropriate. To provide a guide to nomenclature, the names of prominent orthologous proteins involved in centriole biogenesis in different species are presented in TABLE 1.

Centrosome structure and assembly

Centriole duplication and centrosome assembly are complex processes that need to be tightly regulated during proliferation and development. Key components involved in these processes have recently been identified, setting the stage for mechanistic analyses of centriole biogenesis and PCM assembly.

Establishing centriole structure. Centrioles are cylindrical structures characterized by an evolutionarily conserved radial nine-fold symmetry[11,12] (FIG. 1Aa). In vertebrates, the walls of centrioles are composed of nine triplet microtubule blades that are arranged circumferentially. The wall of a fully mature centriole carries two sets of appendages: subdistal appendages, which are required for anchoring cytoskeletal microtubules, and distal appendages, which are needed for membrane docking during ciliogenesis. Several appendage markers have been identified, but much remains to be learned about the assembly and function of these structures[13,14]. The proximal part of the procentriole lumen harbours a scaffolding structure known as the cartwheel[15] (FIG. 1Aa–1Ad), onto which microtubules are added to form the centriolar wall. Cartwheel assembly represents the first step in the construction of a new procentriole during duplication. In some organisms, cartwheels are permanent features of centrioles, but in

Table 1 | **A brief guide to the nomenclature of the main centriole biogenesis proteins**

Homo sapiens	Function	*Drosophila melanogaster*	*Caenorhabditis elegans*	*Chlamydomonas reinhardtii*
PLK4	Centriole duplication	Plk4 (Sak)	ZYG-1	–
SAS6	Centriole duplication	Sas6	SAS-6	BLD12
STIL	Centriole duplication	Ana2	SAS-5	–
CPAP	Centriole assembly	Sas4	SAS-4	–
CEP135	Centriole assembly	Cep135	–	BLD10
CEP152	PLK4 recruitment	Asl	–	–
CEP192	PLK4 recruitment, PCM assembly	Spd2	SPD-2	–
CDK5RAP2	PCM assembly	Cnn	SPD-5	–
CEP295	PCM assembly	Ana1	–	–

Ana, anastral spindle; Asl, asterless; CDK5RAP2, CDK5 regulatory subunit associated protein 2; CEP, centrosome-associated protein; Cnn, centrosomin; CPAP, centrosomal P4.1-associated protein; PCM, pericentriolar material; PLK4, polo-like kinase 4; SAS6, spindle assembly abnormal protein 6 homologue; SPD, spindle defective; STIL, SCL/TAL1-interrupting locus protein; ZYG, zygote defective.

human cells, they act as transient scaffolding structures and are disassembled as the procentriole matures following exit from mitosis. At the centre of the cartwheel is a ring-shaped hub, from which nine spokes emanate to connect to the A tubules of the nine microtubule triplets. In side views, the cartwheel appears as a stack of rings that vary in height depending on species and cell cycle stage[11,16–20] (FIG. 1Ab–1Ad). Structural studies and cell-free reconstitution experiments have revealed that each cartwheel ring is composed of nine homodimers of spindle assembly abnormal protein 6 homologue (SAS6) proteins. *In vitro*, SAS6 can oligomerize into structures closely resembling the cartwheel hub, suggesting that SAS6 imparts the typical nine-fold symmetry to centrioles[21–23]. However, the assembly of stable cartwheels *in vivo* probably requires additional proteins and interactions with the microtubule wall and/or pre-existing centrioles[24,25]. The conserved centriole duplication factor SCL/TAL-interrupting locus protein (STIL) interacts with SAS6 and plays a central role in promoting SAS6 recruitment and/or assembly[26–32]. In *Chlamydomonas reinhardtii*, cartwheel formation requires the protein BLD10 (basal body protein)[19,33], which interacts with SAS6 to relieve the inhibitory action of the SAS6 carboxyl terminus on cartwheel assembly[23]. In human cells, the putative BLD10 homologue centrosome-associated protein 135 (CEP135) also interacts with SAS6 (REF. 34), but most CEP135 localizes to the parent centriole and not to the procentriole[35,36], suggesting additional roles for this protein in centriole biogenesis and PCM assembly. The exact role of CEP135 in cartwheel formation in vertebrates therefore remains unclear, and no homologue of BLD10 has been identified in *Caenorhabditis elegans*. Finally, the deposition of microtubules onto the cartwheel clearly requires centrosomal P4.1-associated protein (CPAP; also known as CENPJ)[37–39].

Centriole length control. Human centrioles display a length of 450–500 nm and a diameter of 200–250 nm (REF. 11). The dimensions of centrioles are remarkably constant in most cells of any given organism, but occasional, striking deviations can be seen in specific cell types[40]. In principle, organelle size can be governed by a variety of mechanisms, including molecular rulers or the regulation of the kinetics of subunit assembly and disassembly[41]. For centriole length, the polymerization and depolymerization of centriolar microtubules are likely to be critical. The most direct evidence for this notion stems from the demonstration that the *Drosophila melanogaster* kinesin-like protein at 10A (Klp10A) of the kinesin-13 subfamily acts as a microtubule depolymerase to control centriole length[42]. Mammalian kinesin-like protein 24 (KIF24), another member of the kinesin-13 subfamily, has similarly been shown to localize to centrioles, but although KIF24 is required for normal cilia assembly, it does not influence centriole length[43]. Interestingly, both Klp10A and KIF24 interact with centrosomal protein of 110 kDa (CP110; also known as CCP110), a protein previously implicated in centriole length control. Although the precise functions of CP110 may differ between species[44], in humans it caps the distal tips of centrioles (FIG. 1Aa), and its depletion results in overly long centriolar microtubules[36,45]. Given that the removal of CP110 is required to extend the centriolar microtubules and to form the axoneme during ciliogenesis[43,45,46], it is not surprising that CP110 levels are regulated by multiple mechanisms[47–50].

Consistent with structural studies showing that CPAP controls the speed of microtubule growth during centriole assembly[37–39], overexpression of CPAP or its interaction partners, CEP120 and spindle and centriole-associated protein 1 (SPICE1), triggers the assembly of excessively long centrioles[45,51–54]. Centriole length can also be modulated by the deregulation of proteins implicated in building the distal halves of centrioles, including the WD40 protein POC1 (proteome of centriole protein 1)[55], the centrosomal protein POC5 (REF. 56) or the microtubule binding protein CEP295 (REFS 57,58). Interestingly, depletion of CEP295 not only impairs the recruitment of POC5 and POC1

A tubules
A typical microtubule triplet is composed of A, B and C tubules, with the innermost A tubule being built from 13 protofilaments.

Molecular rulers
Molecules of defined size that can be used to set distances between other structures.

Axoneme
The nine-fold symmetrical microtubule-based structure at the centre of cilia and flagella.

WD40 protein
A structural motif of approximately 40 amino acids, often terminating in a tryptophan-aspartic acid (WD) dipeptide.

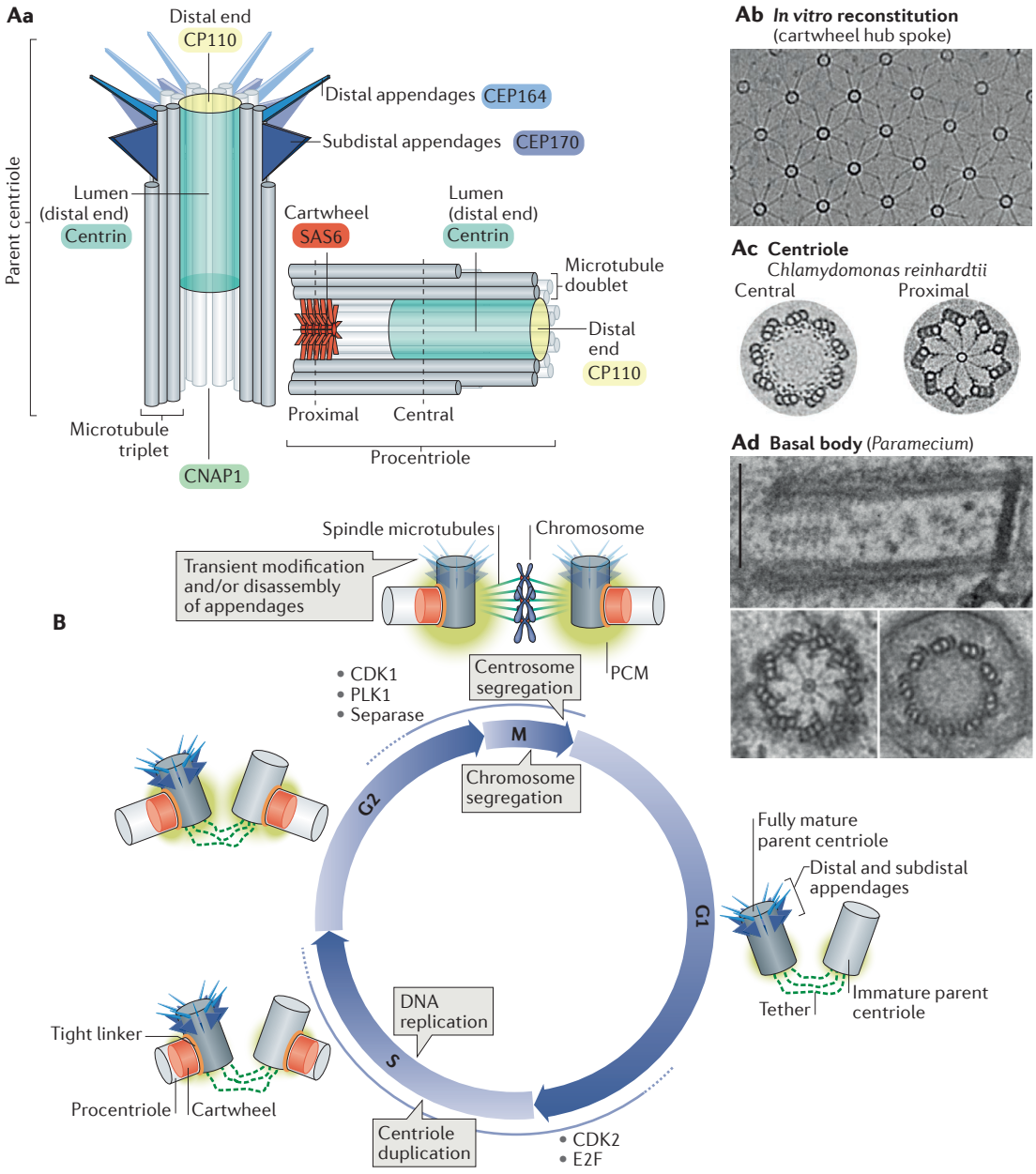


Figure 1 | **Centriole architecture and the centrosome duplication–segregation cycle. A** | Structure of a centriole. **Aa** | Schematic showing fully mature parent centriole and a tightly associated procentriole. Prominent markers representative for the different sub-structures are indicated. **Ab** | Micrograph showing lattice of *in vitro* reconstituted cartwheel hub and spoke structures visualized by cryo-electron microscopy. **Ac** | Image derived from cryotomogram sections of a *Chlamydomonas reinhardtii* procentriole emphasizes cartwheel and triplet microtubules. **Ad** | Transmission electron microscopy showing a longitudinal section (top) and cross sections at proximal (lower left) and distal parts (lower right) of the *Paramecium* basal body. **B** | Shared pathways ensure coordination of centrosome duplication–segregation and chromosome replication–segregation cycles. At the G1/S transition, both centriole duplication and DNA replication depend on cyclin-dependent kinase 2 (CDK2) as well as phosphorylation of retinoblastoma protein and liberation of E2F transcription factors[204]. Similarly, overlapping sets of enzymes, including the kinases CDK1, polo-like kinase 1 (PLK1) and the protease separase govern entry into mitosis, chromosome segregation and licensing of DNA and centrioles for a new round of duplication. Finally, several proteins with well-established functions in DNA transactions have been implicated in the centrosome cycle, but indirect effects on centrosomes remain difficult to exclude[205]. Centrioles are depicted in different shades of grey to indicate different states of maturity. A procentriole (light grey) is a newly created centriole that is not yet duplication competent. A procentriole converts into an immature parent centriole (darker grey) following disengagement in mitosis. An immature parent centriole becomes a mature parent centriole (dark grey) following the acquisition of appendages. Appendage structures undergo a transient modification and disassembly during mitosis. CEP, centrosome-associated protein; CP110, centriolar coiled-coil protein of 110 kDa; PCM, pericentriolar material; SAS6, spindle assembly abnormal protein 6 homologue. Part **Ab** is adapted from REF. 23, Macmillan Publishers Limited. Part **Ac** is reproduced with permission from REF. 19, Elsevier. Images in part **Ad** courtesy of Anne-Marie Tassin.

but also blocks the acetylation and glutamylation of centriolar microtubules[57]. In vertebrates, these tubulin modifications accumulate on centrioles as well as cilia, and polyglutamylation is required for long-term stability of centriolar microtubules[59]. It may be rewarding to explore whether enzymes implicated in post-translational microtubule modifications contribute to centriole length control[60].

Pericentriolar material assembly. Human centrosomes comprise ~200–300 proteins, many of which harbour coiled-coil domains[61,62]. However, centrosome composition is not static, and some PCM components are rapidly exchanged through trafficking on microtubules that are anchored within the centrosome[63]. Other PCM proteins assemble into centrosomes through transient incorporation into highly dynamic cytoplasmic granules termed centriolar satellites[13,64]. Satellites have been implicated in the delivery of proteins for centrosome assembly as well as ciliogenesis, and they form and dissolve rapidly in response to a variety of internal and external cues. Although numerous satellite components have recently been identified, centriolar satellites do not seem to be present in all cell types, and their exact physiological roles remain to be fully understood. Centrosomes are not surrounded by membranes, raising the questions of how the PCM assembles and how its boundaries are defined. Early electron microscopy led to the perception of PCM as an amorphous structure, but super-resolution microscopy has revealed that individual proteins occupy distinct radial layers within the PCM[35,65–67]. Large PCM proteins may self-assemble into micron-scale structures through multimerization[68,69], and this view is strongly supported by recent structural work on the formation of a PCM scaffolding structure by centrosomin (Cnn) in *D. melanogaster*[70]. An alternative model is centred on the role of phase separation as a driving force for the formation of non-membrane-bounded organelles[71,72]. Recent work was focused on *C. elegans* spindle defective 5 (SPD-5), a core PCM component and putative functional homologue of *D. melanogaster* Cnn[73]. Recombinant SPD-5 was shown to assemble *in vitro* into spherical condensates that concentrate tubulin and other proteins required for microtubule polymerization and stabilization[74]. In the future, it will be interesting to determine the extent to which *in vivo* PCM assembly occurs through a liquid-to-condensate phase transition, as opposed to high-affinity, well-ordered interactions between complementary surfaces on large proteins. The two mechanisms are not necessarily mutually exclusive, as PCM could form by an initial phase separation that concentrates components, which subsequently harden into a gel-like or solid structure with ordered protein–protein interactions.

Control of centriole number

Similar to DNA replication, centriole — and as a result, centrosome — duplication is tightly regulated to ensure that centriole duplication occurs once and only once per cell cycle (cell cycle control) and that only one new centriole is produced per pre-existing centriole (copy number control)[75]. Furthermore, duplication and segregation of centrosomes must be coordinated with the chromosome duplication–segregation cycle, and these processes are co-regulated (FIG. 1B). The following discussion focuses on the three main stages of the centrosome duplication cycle. First, we describe the processes that occur around the time of mitosis to endow the procentrioles with competence for duplication and allow reduplication of the parent centrioles (FIG. 2a). Second, we summarize the salient features that underpin the biogenesis of new procentrioles at the G1/S transition (FIG. 2b). Third, we discuss the final steps that result in full maturation of both centrioles and centrosomes at the G2/M transition (FIG. 2c).

Licensing centrioles for a new round of duplication. Like DNA replication, which depends on licensing of DNA replication origins, centrioles only acquire the competence for duplication after cells pass through mitosis. In molecular terms, the licensing of centrioles is now recognized to depend on two main processes: centriole disengagement, which permits the reduplication of the parent centriole, and centriole-to-centrosome conversion, which is required for the procentriole to acquire competence for duplication.

Centriole engagement, the tight, near-orthogonal connection between each parent centriole and its procentriole, has long been shown to block the reduplication of the parent centriole[76–78]. Polo-like kinase 1 (PLK1) and the protease separase have been implicated in promoting the loss of this tight connection, a process termed disengagement, prompting searches for the substrates of these enzymes[77] (FIG. 2a). One likely substrate of separase is the PCM component pericentrin (PCNT), which is released from centrosomes following cleavage by separase in late mitosis[79,80]. Moreover, cleavage of PCNT is positively regulated by PLK1 (REF. 81), and expression of a non-cleavable PCNT mutant suppressed centriole disengagement[79,80]. Centriole-associated cohesin has also been reported as a separase substrate[82]. However, cohesin cleavage is not sufficient for centriole disengagement in *D. melanogaster* embryos; thus, further experiments are needed to clarify the role of cohesin in centriole engagement[83].

Early electron microscopy showed that a loss of the orthogonal orientation between the parent centriole and procentriole occurs in late M/early G1 (REF. 84). More recently, correlative live and electron microscopy revealed that the activity of PLK1 drives the distancing of procentrioles during early prophase, thereby conferring parent centrioles with competence for reduplication even if the procentriole remains orthogonal to the parent[85] (FIG. 2a). The PCM is likely to maintain the close association of the centriole pair during mitosis, with the action of separase contributing to PCM remodelling and the loss of this orthogonal orientation at mitotic exit. Although the activity of PLK1 is essential for conferring competence for reduplication, separase is likely to have a supporting role that ensures disengagement occurs soon after mitotic exit[77]. Finally, removal of the cartwheel from the procentriole

is mediated by cyclin-dependent kinase 1 (CDK1)[86] and has been shown to be important for relieving the block to reduplication of the parental centriole[87] (FIG. 2a).

For procentrioles, competence for duplication additionally requires the acquisition of PCM, a process termed centriole-to-centrosome conversion[88,89], which is also governed by CDK1 and PLK1 (REFS 90,91) (FIG. 2a). This process is best described in *D. melanogaster*, where Plk1 is first recruited to Sas4 (fly homologue of CPAP) through cyclin-dependent kinase 1 (Cdk1)-dependent phosphorylation of a single docking site[91]. In both *D. melanogaster* and mammalian cells, centrosome-associated PLK1 triggers the sequential assembly of CEP135, anastral spindle 1 (Ana1; CEP295 orthologue) and asterless (Asl; CEP152 orthologue) followed by downstream PCM formation[58,88,89]. Importantly, in *D. melanogaster*, embryonic recruitment of Asl only occurs after disengagement, indicating that these licensing processes occur sequentially[92]. In mammalian cells, CEP295 directly binds to CEP192 and contributes to the stabilization of centrioles after the loss of the cartwheel upon mitotic exit[88,93]. Considering that CEP152 and CEP192 form scaffolds for the recruitment of PLK4 (REFS 94–98), the kinase essential for centriole duplication (see below), these results explain why PCM assembly after mitosis is required to confer duplication competence to procentrioles[90]. Although *C. elegans* lacks an obvious CEP295 homologue, spindle assembly abnormal (SAS-7) may function analogously to CEP295 in this organism as it interacts with *C. elegans* CEP192 orthologue spindle defective 2 (SPD-2) and is required for procentrioles to acquire competence to duplicate[99].

The birth of a new centriole. While cell-cycle-coupled mechanisms of centriole licensing ensure that centriole duplication occurs only once per cell cycle, it remains to be explained how cells limit the building of procentrioles to one per pre-existing parent centriole. Whereas PLK1 has a key role in cell cycle control of centriole duplication, PLK4 takes centre stage as the linchpin for copy number control[100,101]. As indicated by morphological studies, at the G1/S transition one single procentriole begins to assemble perpendicularly to the parent centriole, and this newly formed procentriole then remains closely linked to its parent centriole while it elongates throughout G2 (FIGS 1,2b). Consistent with a central role in controlling centriole biogenesis, the levels and activity of PLK4 are tightly regulated. PLK4 exists as a homodimer, and low steady-state levels arise from PLK4 *trans*-autophosphorylation within the dimer, which triggers SCF–βTrCP-mediated proteolytic degradation[102–106]. Upon binding to STIL, PLK4 undergoes a conformational change and is activated through *trans*-autophosphorylation within the activation segment[28,107,108]. Activated PLK4 then phosphorylates STIL within the so-called STAN motif, triggering the centriolar recruitment of SAS6 and cartwheel formation[26–29] (FIG. 2b). However, in *C. elegans*, recruitment of the SAS-5–SAS-6 complex was shown to require a direct interaction with the PLK4-related kinase zygote defective: embryonic lethal (ZYG-1), independent of its catalytic activity[109]. Further downstream events in centriole biogenesis remain to be fully elucidated, but there is evidence that CEP135 serves to connect SAS-6 to CPAP and to outer microtubules of the microtubule triplets[34]. During centriole elongation, CPAP then regulates the growth of centriolar microtubules[37–39,52], which are inserted underneath a cap of CP110 (REF. 36). Interestingly, CPAP also interacts with STIL, and it will be important to understand how CPAP and STIL modulate each other's activities[32,110–112].

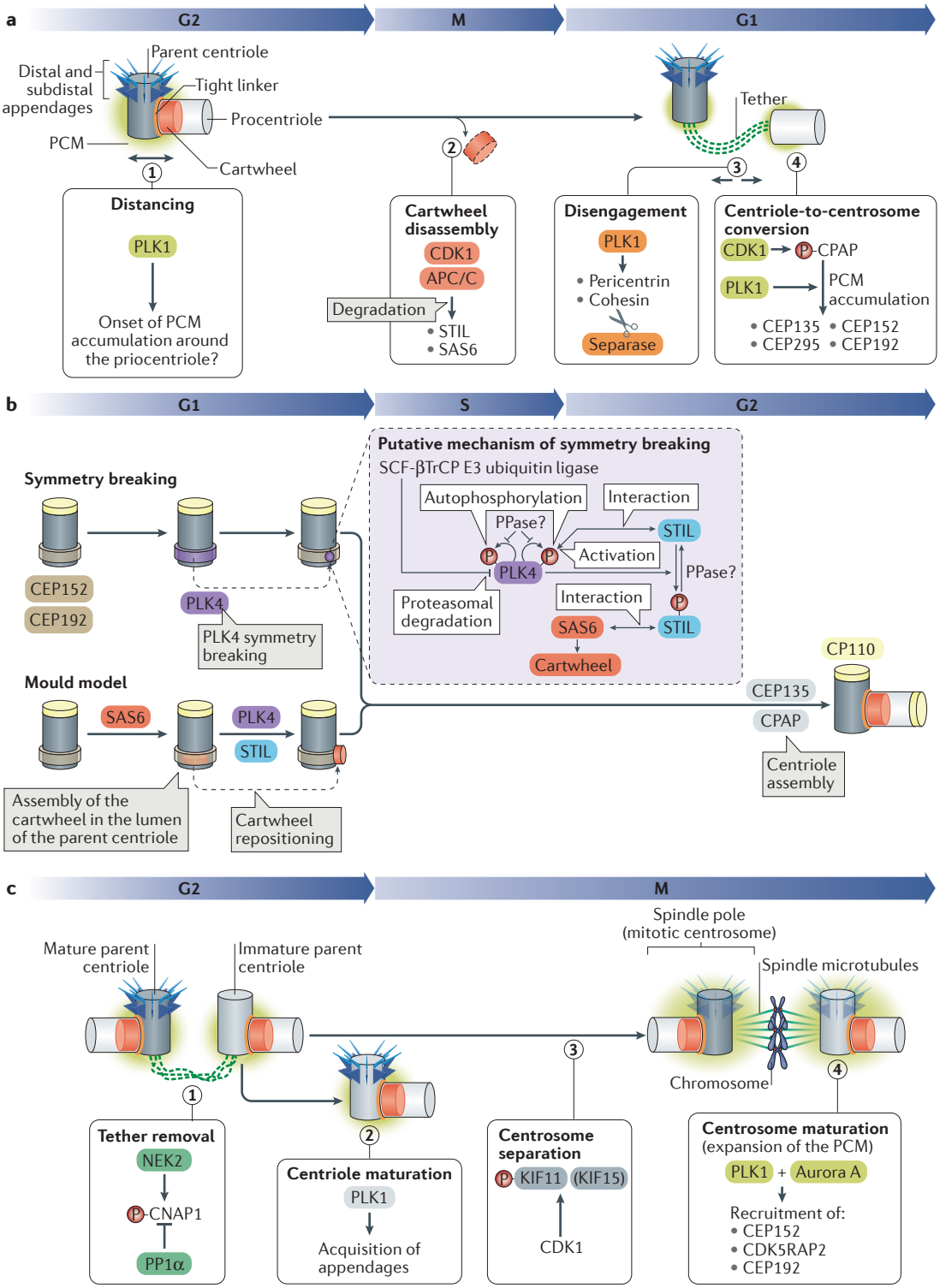
One major question that remains to be answered is how the construction site for a new procentriole is chosen on the circumference of the parent centriole (FIG. 2b). In mammalian cells, PLK4 is recruited to centrioles through binding to two distinct scaffolding proteins, CEP152 and CEP192 (REFS 94–98). Super-resolution microscopy shows that both CEP152 and CEP192 form rings around parent centrioles and, accordingly, PLK4 can also be seen to form rings in G1 phase. However, PLK4, STIL and SAS6 then coalesce to a precise region on the circumference of the parent centriole (a dot on the CEP152–CEP195 ring) that marks the site of procentriole assembly[26,35,96]. *A priori*, there is no structural limitation to impose the formation of a single procentriole around the circumference of the parental cylinder, as indicated by the near-simultaneous formation of multiple procentrioles at numerous sites in response to overexpression of PLK4 (REFS 36,100). So, what mechanisms ensure copy number control? One plausible view invokes a symmetry-breaking event that leads to the stochastic choice of a building site and the suppression of all other potential sites (FIG. 2b). In one attractive model, STIL is proposed to stabilize PLK4 at the site of procentriole assembly, allowing the remaining PLK4 within the ring to be turned over by self-catalysed degradation[26,108]. Such a process would be controlled by both PLK4 kinase activity and counteracting phosphatases and would probably involve multiple feedback loops, as suggested by theoretical modelling of the role of GTPases for symmetry-breaking during yeast cell polarization[113]. If correct, this symmetry-breaking model raises the challenge of understanding how PLK4 is regulated in time and space.

According to an alternative model, the lumen of the parent centriole acts as a mould for the assembly of a cartwheel that is subsequently released and used to direct formation of a procentriole[114] (FIG. 2b). In this case, future work would have to explain how cells limit the use of the mould to once per centriole and cell cycle and how the cartwheel is transferred from the lumen onto the wall of the parent centriole.

Overall, it will be important to better define when and where different complexes involving the centriole duplication factors PLK4, STIL and SAS6 are formed and stabilized[115]. Another attractive area ripe for investigation relates to the role of phosphatases in the spatiotemporal control of centriole duplication[116].

Maturation of centrioles and centrosomes. In a proliferating human cell, both centrioles and centrosomes undergo final maturation during the G2 and

M phases (FIG. 2c). In late G2, each of the two duplicated centrosomes comprises one parental centriole associated with PCM and one procentriole that lacks the ability to recruit PCM. The two parent centrioles are connected by a tether containing rootletin and other proteins, anchored to CNAP1 (REFS 117,118). Concomitantly, each procentriole is closely associated with the proximal end of the parent cylinder through a linkage that remains to be characterized[119]. Importantly, only one of the two parental centrioles is fully mature and competent to function as a basal body for ciliogenesis, a feature indicated by the presence of subdistal and distal appendages. Acquisition of appendages by the younger parental centriole requires PLK1 (REF. 120). Of note, mitotic progression is accompanied by transient modification and/or disassembly of appendage structures (FIGS 1B,2c).



Planarians
Flatworms used as a model system to study regeneration.

At the G2/M transition, the PCM expands considerably in preparation for mitotic spindle formation (FIG. 2c). This process, termed centrosome maturation[5], has long been known to be governed by PLK1 (REFS 121,122), and a contribution of Aurora A is also well documented[123]. More recent work, carried out largely in *D. melanogaster* and *C. elegans* embryos, has yielded additional insight into the mechanisms underlying PCM expansion[3]. The emerging view is that PLK1 triggers the ordered assembly of an initial set of core scaffolding proteins that subsequently recruit all other PCM components. In *D. melanogaster*, these core proteins are Asl, Cnn and defective spindle 2 (DSpd-2), corresponding to CEP152, CDK5RAP2 (also known as CEP215) and CEP192, respectively, in mammalian cells[69]. According to one model, phosphorylation of Cnn by Plk1 promotes the continuous recruitment of Cnn around the centrioles, from where the Cnn scaffold then gradually spreads outward. One attractive feature of this model is that the activity of Plk1 could be used to control the rate of Cnn incorporation into the PCM, offering a plausible mechanism for calibrating the size of PCM associated with the centrosome during mitosis[3]. However, it is not immediately clear how to reconcile this flux model with data from *C. elegans*, where incorporation of SPD-5 into PCM was found to occur isotropically throughout the entire PCM[124].

Sensing centriole number

Although centriole number is normally tightly maintained at two or four copies per cell in cycling cells, there are several instances where centriole number is altered as part of a normal developmental programme. One striking example is in multiciliated epithelial cells that line the airways, ventricles and oviducts of vertebrates. These specialized cells form hundreds of centrioles that serve as basal bodies for the formation of multiple cilia[125]. However, as we describe in the following sections aberrations to centriole number are not well tolerated in cycling cells and can contribute to pathologies. The mechanisms by which cells survey centriole number are now starting to emerge.

Centriole loss and the mitotic surveillance pathway. While centrosomes are a major source of spindle microtubules during mitosis, it is clear that chromatin and microtubule-mediated nucleation pathways can support spindle assembly in the absence of centrosomes[63]. A striking example of the dispensability of centrosomes for cell division are planarians, where cell divisions and regeneration occur in the absence of centrosomes, and centrioles are only assembled in terminally differentiated multiciliated cells to allow the formation of cilia used in locomotion[126]. In *D. melanogaster*, centrosomes are required during rapid, syncytial cell divisions in the early stages of embryogenesis but are dispensable thereafter[127]. Importantly, flies lacking centrioles from the later stages of development grow to a normal size and are morphologically normal but perish soon after hatching because of a lack of sensory cilia. These examples support the view that the ancestral role of centrioles was to direct the formation of cilia and flagella and that their association with the poles of the mitotic spindle acted to ensure their equal segregation into the daughter cells[128].

Although cell division can proceed in the absence of centrosomes in some circumstances (as described above)[129–131], centrosomes are generally required for the sustained proliferation of mammalian cells. Mouse embryos lacking centrioles undergo widespread p53-dependent apoptosis at an earlier developmental stage than mutants that lack cilia[132]. In cultured mammalian cells, centrosome loss resulted in a robust cell cycle arrest within a few divisions[133,134]. This arrest could be overcome by the removal of p53, explaining why cancer cells often fail to respond to centrosome loss. In contrast to planarians and flies, mammalian cells possess mechanisms to sense centrosome loss and to prevent their continued cell proliferation in the absence of centrosomes.

Insights into how centrosome depletion can activate p53-dependent pathways came from genome-wide knockout screens that led to the identification of a USP28–53BP1–p53–p21 signalling axis (USP28, ubiquitin carboxyl-terminal hydrolase 28; 53BP1, TP53 binding protein 1; p21, also known as CDKN1A) referred to as the mitotic surveillance pathway[135–137]. Deletion of any component of this pathway allowed the continued proliferation of cells in the absence of centrosomes. Functionally, 53BP1 interacts with p53 and is a pivotal regulator of DNA double-strand break repair, and USP28 is a deubiquitinase that interacts with 53BP1 and has a minor function in DNA damage response signalling[138–140]. Notably, the role of 53BP1 in responding to centrosome loss is distinct from its established role in DNA damage repair[135–137,141]. Although much remains to be learned

◀ Figure 2 | **Key aspects of the centrosome duplication cycle. a** | Four major events (see numbered steps) during the progression from late G2 through M and into early G1 that are considered necessary to license a new round of centriole duplication. Although these events are conceptually distinct, they are expected to be integrated at a molecular and structural level. **b** | The birth of a new centriole. The master regulator of procentriole formation polo-like kinase 4 (PLK4) is initially recruited to a ring of centrosomal protein of 152 kDa (CEP152) and CEP192 at the proximal end of the parent centriole. According to one model (top), a symmetry-breaking event leads to the accumulation of active PLK4 at a single, dot-like spot. A putative mechanism underlying symmetry breaking is shown in the inset. An alternative model (bottom) attributes an important role to the lumen of the parent centriole in serving as a mould and assisting spindle assembly abnormal protein 6 homologue (SAS6) self-assembly into a cartwheel structure. PLK4 and SCL-interrupting locus protein (STIL) would subsequently cooperate to release the mould and allow its repositioning laterally on the parent centriole. **c** | A G2 cell typically comprises two centrosomes, each harbouring a pair of centrioles that are connected by a loose tether. Before mitotic entry, this tether is removed by a shift in the balance of activities of the protein kinase NEK2 and the phosphatase PP1 acting on centrosome-associated protein CEP250 and other substrates[117,118,206]. In G2, only one parent centriole is fully mature (that is, carries appendages); the second parent centriole acquires appendages during G2 and/or M phase in a process triggered by PLK1 (REF. 120) (step 2). On mitotic entry, the two centrosomes are separated by kinesin-like protein KIF11 and the partially redundant KIF15 (REF. 207), with KIF11 being recruited to centrosomes in response to cyclin-dependent kinase 1 (CDK1) phosphorylation[208]. Finally, mitotic spindle formation requires expansion of the pericentriolar material (PCM; centrosome maturation). APC/C, anaphase-promoting complex/cyclosome; βTrCP, β-transducin repeats-containing proteins; CP110, centriolar coiled-coil protein of 110 kDa; CPAP, centrosomal P4.1-associated protein; PPase, unknown phosphatase; SCF, SKP1–CUL1–F-box protein.

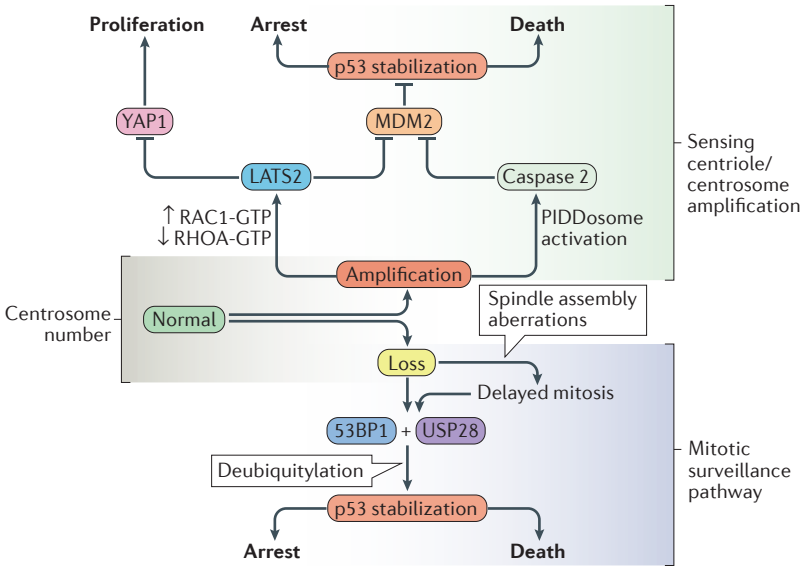


Figure 3 | **Responding to centrosome defects.** Pathways activated by centrosome loss (bottom) and centrosome amplification (top). Centrosome loss leads to P53-binding protein 1 (53BP1) and ubiquitin carboxyl-terminal hydrolase 28 (USP28)-dependent stabilization of p53. Active p53 promotes either cell death or cell cycle arrest[132,135–137]. An increased duration of mitosis (mitotic delay) also activates p53 through the same pathway, suggesting that centrosome loss is sensed via a mitotic delay. This failsafe mechanism has been termed the mitotic surveillance pathway[145]. Centrosome amplification leads to hyperactivation of RAC1 and a corresponding decline in RHOA. Downregulation of RHOA results in activation of Hippo serine/threonine-protein kinase LATS2. LATS2 then inhibits E3 ubiquitin-protein ligase MDM2 that targets p53 for degradation, thereby resulting in p53 stabilization and cell death or cell cycle arrest. In addition, LATS2 phosphorylates and inactivates the transcriptional coactivator YAP1 to inhibit proliferation[147]. In an alternative pathway, supernumerary centrosomes promote activation of the PIDDosome, which leads to activation of caspase 2 (REF. 148). Active caspase 2 cleaves MDM2 and thereby stabilizes p53 (REF. 209).

about how the mitotic surveillance pathway functions to survey centrosome number, a plausible model is that in response to centrosome loss, 53BP1 binds to USP28 and p53 to facilitate USP28-dependent deubiquitylation and activation of p53, leading to cell cycle arrest or cell death[136,141] (FIG. 3).

None of the components of the mitotic surveillance pathway show robust localization to the centrosomes, making it unlikely that they directly monitor centrosome number. How, then, is centrosome loss sensed? In the absence of centrosomes, spindle assembly is less efficient, and cell division time is consistently increased[134–137]. Remarkably, increasing the duration of mitosis past a specific threshold elicits a durable *TP53*-dependent G1 arrest in human epithelial cells[142]. This raises the possibility that centrosome loss triggers a cell cycle arrest by delaying mitosis (FIG. 3). Consistent with this view, all the components of the mitotic surveillance pathway were found to be required to arrest the cell cycle following a prolonged mitosis[135–137]. Moreover, activation of p53 in mouse embryos lacking centrioles was associated with an increase in the duration of mitosis[132]. Additional evidence comes from the identification of the E3 ubiquitin-protein ligase TRIM37 as a hit in genome-wide screens for knockouts that allow proliferation without centrosomes[136,137]. While TRIM37 is required to arrest the cell cycle after centrosome loss, it is not required to prevent cell proliferation following a delayed mitosis. Loss of TRIM37 enables the formation of extra-centrosomal microtubule organizing centres that speed up spindle assembly in cells lacking centrosomes. TRIM37 deletion may thus bypass the arrest caused by centrosome loss by reducing the duration of mitosis in cells lacking centrosomes[137].

Surprisingly, USP28 knockout mice are viable and have no clear phenotypes, suggesting that, in unchallenged conditions, activation of the mitotic surveillance pathway is a rare event[143,144]. Nevertheless, there is evidence to suggest that activation of the mitotic surveillance pathway underlies the growth defects observed in autosomal recessive primary microcephaly (MCPH) (see below). Future work will be required to elucidate cell-specific and tissue-specific differences in signalling through the mitotic surveillance pathway as well as the impact of the activation of this pathway in normal physiology and disease[145].

Suppression of cell proliferation following centrosome amplification. Like centrosome loss, increases in centrosome number (centrosome amplification) also suppress the proliferation of cells in culture[106,146]. This defect can be overcome by the removal of p53, but does not depend on USP28 and 53BP1 (REF. 135), suggesting that distinct pathways activate p53 in response to an increase or decrease in centrosome number. Initial insight into how centrosome amplification suppresses cell proliferation came from the discovery that tetraploid cells, which contain twice the normal number of centrosomes, stabilize p53 through the Hippo pathway serine/threonine-protein kinase LATS2 (REF. 147) (FIG. 3). Inducing extra centrosomes by an alternative means also led to LATS2-dependent p53 stabilization, suggesting that extra centrosomes are, at least in part, responsible for the activation of LATS2.

Recently, an additional pathway controlled by the PIDDosome was found to be important in preventing the proliferation of cells with extra centrosomes[148]. The PIDDosome controls the proximity-induced activation of caspase 2 (REF. 149) and is required to stabilize p53 after cytokinesis failure (FIG. 3). Importantly, some PIDDosome components localize to the older parent centriole, suggesting that PIDDosome activation may be controlled by the presence of additional mature centrioles[148]. Consistent with this idea, depletion of the appendage outer dense fibre protein 2 (ODF2) reduced caspase 2 activation and p53 stabilization in tetraploid cells with supernumerary centrosomes[148]. While counting mature parent centrioles offers a method to detect centriole amplification, it remains unclear how excess mature parent centrioles would be detected and, in turn, how they would promote the activation of the PIDDosome. It will be interesting to test whether driving premature maturation of the younger parent centriole with constitutively active PLK1 can promote PIDDosome activation in the absence of centriole amplification[120].

Hippo pathway
A signalling pathway that controls organ size in animals by restraining cell proliferation and promoting apoptosis.

PIDDosome
A protein complex composed of death domain-containing protein CRADD (also known as RAIDD) and p53-induced death domain-containing protein 1 (PIDD1) that is implicated in the activation of caspase 2.

Cytokinesis failure
Failure to physically separate the two daughter cells after chromosome segregation is completed.

Unlike p53 loss, LATS2 or caspase 2 knockout does not allow the continued proliferation of cells with extra centrosomes[135,148]. It is therefore likely that additional pathways feed into p53 activation in response to centrosome amplification. Because many tumour cells possess supernumerary centrosomes (see below), overcoming the inhibitory effect of extra centrosomes on cell proliferation seems to be a key step to allow cells with extra centrosomes to acquire the necessary oncogenic mutations required for tumour development.

Centrosome defects and cancer

Over a century ago, the German cytologist Theodor Boveri postulated that centrosome aberrations could contribute to human cancer. Indeed, centrosome defects are present in a broad array of both solid and haematopoietic human cancers, and in some tumour types, centrosome abnormalities have been observed early in disease development and correlate with advanced tumour grade and poor clinical outcome[10,150]. Centrosome anomalies can be subdivided into either numerical or structural alterations[150]. While structural alterations are likely to originate from alterations in the levels or activity of centrosome proteins[151], numerical alterations reflect increases in centrosome copy number and arise owing to the acquisition of an excessive number of centrioles. Although structural and numerical centrosome aberrations are conceptually distinct, they often coexist in tumours.

The role of supernumerary centrosomes in tumorigenesis. To test the role of extra centrosomes in cancer, many studies have exploited PLK4 overexpression to increase centrosome number. Pioneering work in flies showed that while centrosome amplification does not promote the development of spontaneous tumours, neuroblasts and epithelial cells with extra centrosomes can initiate tumorigenesis when transplanted into host flies[152,153]. However, how centrosome amplification affects tumour development in mammals is complex.

In the mouse brain, extra centrosomes do not promote tumorigenesis[154]. Similarly, centrosome amplification in the epidermis resulted in spindle orientation defects and aneuploidy, but these abnormalities were not able to initiate spontaneous tumorigenesis or enhance the development of carcinogen-induced skin tumours[155]. By contrast, centrosome amplification did accelerate tumorigenesis in a p53-deficient epidermis[156]. Moreover, global PLK4 overexpression also accelerated the onset of lymphomas and sarcomas in *p53*-null mice and promoted hyperproliferation in the skin and pancreas[157]. Taken together, these studies validated a central role of p53 in restricting the continued proliferation of cells with centrosome amplification in mammals[106].

While initial studies failed to observe the development of spontaneous tumours in animals with widespread PLK4 overexpression[155,157,158], a more modest increase in PLK4 levels was shown to promote a persistent centrosome amplification that promoted the development of spontaneous tumours[146]. Importantly, these tumours exhibited dramatic numerical and structural chromosomal alterations, mirroring the complex karyotype changes frequently observed in human tumours with extra centrosomes[146]. Some impairment of the p53 pathway is to be expected in tumours that form spontaneously in response to centrosome amplification. Accordingly, spontaneous lymphomas that develop in mice with centrosome amplification show downregulation of p53 target genes[146]. Thus, centrosome amplification can clearly promote tumour development, but the exact mechanisms of tumour promotion remain to be clarified.

The origin of centrosome defects in tumour cells. Cancer cell lines show wide variation in the penetrance and extent of centrosome amplification. Reversible depletion of centrosomes using a PLK4 kinase inhibitor has shown that tumour cell lines reach an equilibrium of centrosome number distribution that is determined by the rate at which extra centrosomes are accumulated and the rate at which cells harbouring them are selected against[133]. One pathway leading to the acquisition of extra centrosomes is dysregulation of the centriole duplication cycle. Whereas genes encoding centrosome proteins are rarely mutated in human cancers, increased or decreased expression of centrosome proteins is more common[1,10,150] (TABLE 2). In addition, perturbation of cell cycle progression can lead to defects in centriole biogenesis. The clearest example is that of a prolonged arrest in G2 phase, which leads to PLK1 activation, centriole disengagement and premature centriole reduplication[159]. Accordingly, DNA damage can induce centrosome amplification by increasing the time cells spend in G2 phase[160,161]. A final pathway to generate extra centrosomes is through failed cell division. In addition to the doubling of centrosomes, failed division provides the benefit of doubling the genome to buffer against deleterious mutations or chromosome segregation errors. These properties allow tetraploid cells to sample novel karyotypes, eventually landing upon a rare combination that provides a growth advantage[162]. Consistent with a pro-tumorigenic property of tetraploid cells, a growing body of evidence suggests that a large fraction of human tumours arise from a tetraploid intermediate[163]. Although the uncontrolled proliferation of tetraploid cells can drive tumorigenesis[164], extra centrosomes in tetraploid cells initially trigger a p53-dependent cell cycle arrest[147] (FIG. 3). As a consequence, repeated cytokinesis failure does not result in the long-term establishment of centrosome amplification in cell culture[165]. This suggests that further genetic alterations, such as loss of LATS2, caspase 2 or p53, are required to bypass this fitness disadvantage and generate long-term increases in centrosome number following cytokinesis failure. In the future, it will be interesting to test whether a deficiency of LATS2 or PIDDosome components could accelerate the development of tumours driven by centrosome amplification.

Deregulation of oncogenes or tumour suppressor genes has been shown to lead to the formation of supernumerary centrosomes. For example, KLF14 is a transcriptional repressor of PLK4, and knockout of KLF14

Neuroblasts
Dividing neuronal precursor cells.

Aneuploidy
The presence of an abnormal chromosome number that is not a multiple of the haploid chromosome complement.

Table 2 | **Proteins involved in centriole number control, their functions and links to disease**

Gene symbol	Function	Links to disease
Centriole genes linked to tumorigenesis		
PLK4	Centriole duplication	Overexpressed in breast cancer, controls invasion through regulation of the actin cytoskeleton, *Plk4*$^{+/-}$ mice are predisposed to liver and lung cancer
STIL	Centriole duplication	Promoter fused to TAL1 in T cell acute lymphoblastic leukaemia
NLP	Microtubule nucleation	Overexpressed in multiple cancers
Genes linked to MCPH		
PLK4	Centriole duplication	Mutations reduce the levels of PLK4
STIL	Centriole duplication	Mutations inhibit the cell cycle-controlled degradation of STIL
CPAP	Controls centriole length and centriole duplication	Mutations weaken binding of CPAP to STIL
CEP135	Centriole duplication	Recessive mutations (effects not known)
CEP152	Centriole duplication	Recessive mutations (effects not known)
CEP63	Centriole duplication	Recessive mutations (effects not known)
CDK5RAP2	Centriole duplication	Recessive mutations (effects not known)
WDR62	Spindle pole organization and centriole duplication	Recessive mutations (effects not known)
ASPM	Spindle pole organization and centriole duplication	Recessive mutations (effects not known)
TUBGCP6	Centriole duplication, component of the γ-tubulin ring complex	Recessive mutations (effects not known)
TUBGCP4	Component of the γ-tubulin ring complex	Recessive mutations (effects not known)
CDK6	Centrosome-associated in mitosis	Mutations mislocalize CDK6
Centriole genes linked to primordial dwarfism		
PLK4	Centriole duplication	Mutations reduce the levels of PLK4
CPAP	Centriole duplication	Recessive mutations (effects not known)
CEP152	Centriole duplication	Recessive mutations (effects not known)
CEP63	Centriole duplication	Recessive mutations (effects not known)
PCNT	Component of the PCM	Mutations mislocalize PCNT from the centrosome
Centriole genes linked to other disorders		
ALMS1	Functions in ciliogenesis	Alström syndrome (retinitis pigmentosa, deafness, obesity and diabetes)
OFD1, *C2CD3*	Centriole length control	Orofaciodigital syndrome (malformations of the face, oral cavity and digits)

ALSM1, centrosome and basal body associated protein; *ASPM*, abnormal spindle microtubule assembly; *C2CD3*, C2 calcium-dependent domain-containing 3; *CDK6*, cyclin-dependent kinase 6; *CDK5RAP2*, CDK5 regulatory subunit associated protein 2; *CEP*, centrosomal protein; *CPAP*, centrosomal P4.1-associated protein; MCPH, autosomal recessive primary microcephaly; *NLP*, ninein-like protein; *OFD1*, centriole and centriolar satellite protein; PCM, pericentriolar material; *PCNT*, pericentrin; *PLK4*, polo-like kinase 4; *STIL*, SCL/TAL1-interrupting locus; *TAL1*, T cell acute lymphocytic leukaemia protein 1 homologue; *TUBGCP*, tubulin γ-complex associated protein; *WDR62*, WD repeat domain 62.

leads to PLK4-induced centrosome amplification and tumour formation in mice[166]. PLK4 is also transcriptionally repressed by the p53 tumour suppressor[157,167]. Nevertheless, p53 knockout is insufficient to induce centrosome amplification in human cell lines and in tissues of mice[134,135,146,154,155,157]. Rather than having a direct role in controlling centrosome number as originally proposed[168], loss of p53 is likely to offer a permissive environment for the continued proliferation of cells with centrosome abnormalities, as it allows cells to bypass centrosome number surveillance pathways[106,155–157] (FIG. 3).

Consequences of centrosome defects. Irrespective of how they arise, extra centrosomes are capable of nucleating microtubules, which may lead to the formation of multipolar mitotic spindles. If not corrected, this results in a multipolar division leading to extensive chromosome missegregation and inviable progeny[169] (FIG. 4a). The primary mechanism by which tumour cells suppress multipolar divisions is through the coalescence of centrosomes into two groups to form a pseudo-bipolar spindle[170]. The efficiency of the clustering process is likely to be an important parameter in determining the ability of cells

Merotelic attachments
Spindle microtubule–chromosome attachments in which one kinetochore binds microtubules emanating from two centrosomes located on opposite sides of the mitotic spindle.

to tolerate centrosome amplification[171,172]. Importantly, however, centrosome clustering increases the frequency of incorrect, merotelic attachments of chromosomes to the mitotic spindle, which leads to low rates of chromosome segregation errors. These defects can be compatible with cell viability[152,169,173], while at the same time leading to losses or gains of genetic material (FIG. 4a), which provides an explanation for the tight correlation of centrosome amplification and aneuploidy in human cancer[10,150].

An additional source of mitotic errors emerges from the improper timing of centrosome separation before cell division. Both accelerating and delaying centrosome separation increase the frequency of chromosome mis-attachments to the mitotic spindle, leading to chromosome segregation errors[174–177]. It will be interesting to investigate whether structural or numerical alterations in centrosomes can contribute to defects in the timing of centrosome separation.

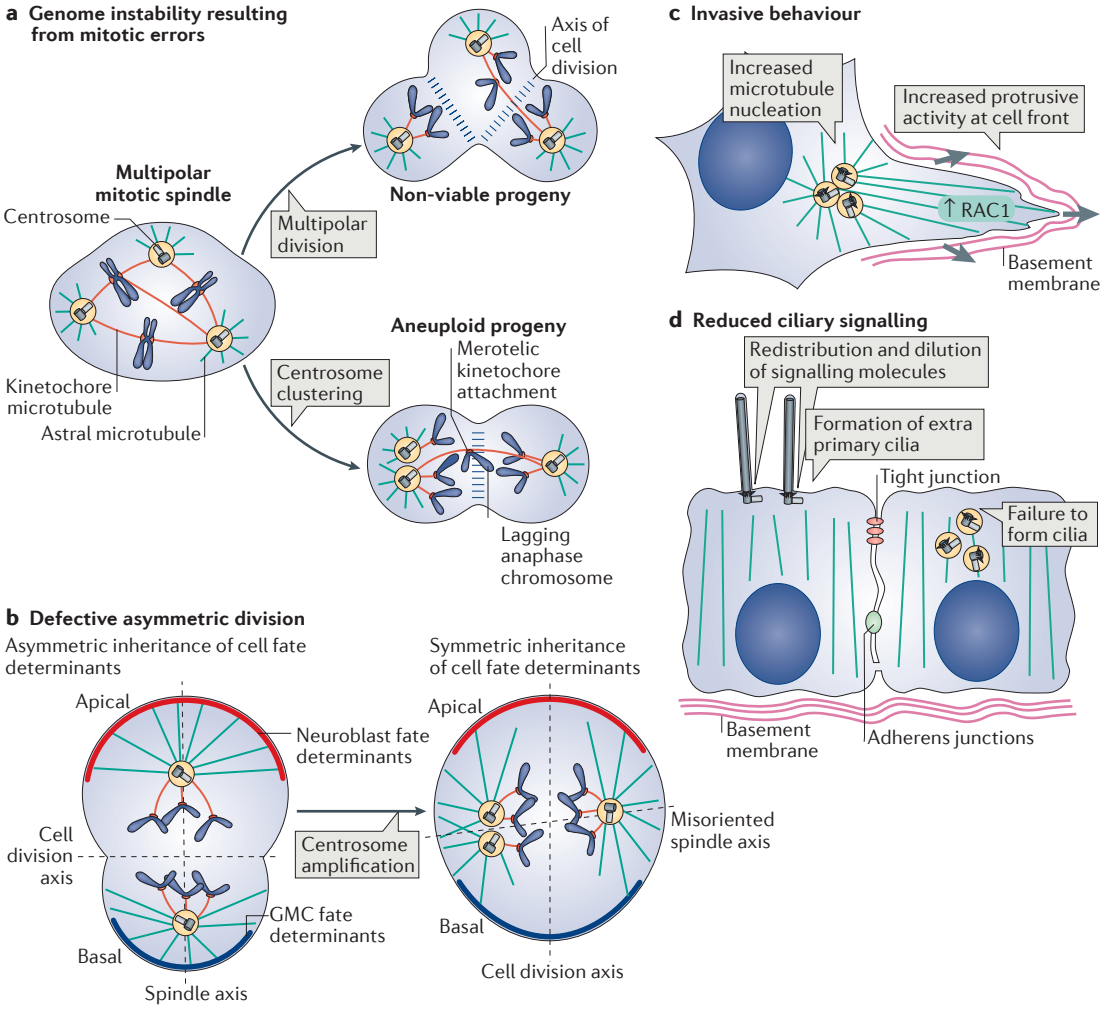


Figure 4 | **Mechanisms through which centrosome amplification can contribute to tumorigenesis. a** | Genome instability. Cells with supernumerary centrosomes form multipolar mitotic spindles. Multipolar divisions lead to the production of highly aneuploid daughter cells that are typically non-viable. To avoid multipolar divisions, cells cluster their centrosomes before anaphase. Centrosome clustering enriches for incorrect merotelic attachments of chromosomes to the mitotic spindle, resulting in chromosome segregation errors (aneuploidy)[152,169,173]. In addition to creating whole chromosome aneuploidy, mitotic errors caused by extra centrosomes can promote the acquisition of DNA double-strand breaks that result in chromosomal rearrangements[178–180]. **b** | Defective asymmetric divisions. *Drosophila melanogaster* neuroblasts undergo asymmetric cell division to self-renew and produce a differentiated ganglion mother cell (GMC). Centrosome amplification can lead to a failure to correctly align the spindle, resulting in the equal partitioning of cell fate determinants into the daughter cells. This leads to an expansion of the stem cell pool and tissue overgrowth[152]. Of note, centrosome amplification does not seem to produce similar spindle orientation defects in mouse neuronal progenitors. Instead, extra centrosomes promote multipolar divisions and apoptosis, leading to the depletion of progenitor cells[154]. These findings indicate that the effects of centrosome amplification are likely to be species and/or cell-type specific. **c** | Invasive behaviour. Increased microtubule nucleation resulting from the presence of increased number of centrosomes has been shown to induce RAC1 hyperactivation that drives invasive behaviour[183]. **d** | Reduced ciliary signalling. Signalling by primary cilia can be disrupted in response to centrosome amplification by either dilution of cilia signalling components owing to their distribution into multiple cilia or a failure to form cilia[157,184].

Along with whole-chromosome aneuploidy, mitotic errors driven by supernumerary centrosomes also promote the formation of DNA double-strand breaks that lead to chromosomal rearrangements. Extra centrosomes increase the frequency of chromosomes that lag in the middle of the spindle during anaphase, and these chromosomes can be damaged by constriction in the cleavage furrow during cytokinesis[178]. Moreover, lagging chromosomes are often partitioned into micronuclei, which accumulate high levels of DNA damage that promote chromosomal rearrangements[179,180]. Supernumerary centrosomes can therefore facilitate karyotype evolution by acting as a source of both numerical and structural chromosomal alterations.

While centrosome amplification provides a source of genetic instability, extra centrosomes could also contribute to tumorigenesis through additional mechanisms. In *D. melanogaster*, neuroblasts or epithelial cells with extra centrosomes are capable of initiating tumorigenesis when transplanted into host flies[152,153]. While aneuploidy was observed in transplanted epithelial cells with extra centrosomes, supernumerary centrosomes generated only a modest increase in aneuploidy in transplanted neuroblasts, suggesting that genomic instability is unlikely to be the cause of the uncontrolled proliferation of the transplanted brain cells. Instead, neuroblasts with extra centrosomes have spindle alignment defects that result in an increase in symmetric over asymmetric cell divisions[152] (FIG. 4b). This impairment in asymmetric divisions has been proposed to lead to amplification of the neuroblast stem cell pool and subsequent tissue overgrowth[181]. Examining whether defects in asymmetric cell division contribute to tumorigenesis in vertebrates is an exciting area of future work.

In addition to perturbing cell divisions, numerical and structural centrosome aberrations can also alter the architecture of the interphase microtubule cytoskeleton[151,182]. Centrosome amplification promotes the formation of invasive protrusions in non-transformed mammary cells grown in a three-dimensional culture system[183]. Importantly, this invasive behaviour was not caused by aneuploidy. Instead, cells with extra centrosomes exhibited increased microtubule nucleation that activated the small GTPase RAC1 (FIG. 4c). This provides a possible explanation for the association of centrosome amplification and advanced tumour grade. Further work will be needed to define the impact of centrosome aberrations on cellular invasion and metastasis *in vivo*.

In addition to their role at the centrosome, centrioles also serve as basal bodies required for primary cilia formation. In cultured human cells, PLK4-induced centriole amplification frequently resulted in the formation of more than one primary cilium[184]. Surprisingly, cells with additional cilia had reduced levels of ciliary signalling molecules and defective activation of the cilia-regulated Sonic Hedgehog pathway. By contrast, in the mouse epidermis and primary keratinocytes, PLK4 overexpression leads to centriole amplification and the formation of fewer primary cilia[157]. Centriole amplification can therefore disrupt ciliary signalling, owing to either dilution of ciliary signalling components or the loss of cilia (FIG. 4d). Because dysregulation of cilia-regulated signalling pathways is known to contribute to tumorigenesis, supernumerary centrioles could affect cell proliferation by perturbing normal ciliary signalling[185,186].

Centrosome anomalies and microcephaly

MCPH is a severe developmental disorder caused by reduced neuronal proliferation during embryonic development and that is characterized by small brain size and mental retardation. Curiously, the major genetic causes of MCPH are mutations in widely expressed genes coding for proteins that function at the centrosome. Currently, mutations in 12 genes encoding centrosome-localized proteins have been shown to cause MCPH, and at least 8 of these have established roles in centriole duplication[187–189] (TABLE 2). This suggests that defects in centriole biogenesis are an underlying cause of neurogenesis defects in MCPH[190]. Consistently, MCPH-associated mutations in PLK4 and CPAP have been shown to impair centriole biogenesis, and depletion of proteins required for centriole duplication reduces the brain size of mice[32,110–112,191–193]. Interestingly, MCPH-associated mutations in STIL can promote centriole amplification, and overexpression of PLK4 in the developing mouse brain resulted in centriole amplification and reduced brain size at birth[86,154]. Taken together, the evidence supports the idea that either elevated or reduced numbers of centrioles can cause MCPH.

During brain development, neural progenitors undergo symmetric proliferative divisions to self-renew. Because centrosomes have an important role in orienting the mitotic spindle, defects in the centrosome number or structure could impair symmetric divisions and lead to the premature depletion of neural progenitors[194]. In agreement with this view, spindle orientation defects have been observed in brain organoids and mice with MCPH-causing mutations in CDK5RAP2 (REFS 195,196). While this mechanism is appealing, randomizing spindle orientation in mouse neuroepithelial progenitors does not affect the rate at which neurons are produced[197], and defects in mitotic spindle orientation were not observed in the microcephalic brains of some mouse models[190].

Importantly, cells with abnormal centriole numbers exhibit delayed spindle assembly and an increased duration of mitosis[106,133,134,198]. Because a mitotic delay is observed in neural progenitors in the brains of some mouse models of microcephaly, it is plausible that this delay activates the mitotic surveillance pathway (FIG. 3) to restrict the proliferation of neural progenitors during embryogenesis, thereby producing fewer neurons than in normal brains[191,192,199]. In support of this idea, extending the duration of mitosis was shown to promote both differentiation and death of neural progenitors in the developing mouse brain[199]. Moreover, mouse models with reduced levels of centrosomal proteins exhibited microcephaly that is rescued by deletion of *Tp53* (REFS 191,192). Importantly, while deletion of *Tp53* rescued brain size, it did not correct defects in tissue architecture caused by abnormal spindle orientation and the incorrect spatial arrangement of neural progenitor cells[191]. The available

Micronuclei
Small nuclei that are separate from the cell nucleus and that contain one or a few chromosomes or chromosome fragments.

RAC1
A small GTPase member of the RAS superfamily with diverse cellular functions.

Organoids
An *in vitro* culture system that mimics the micro-anatomy of an organ.

Box 1 | **Centrosomes as therapeutic targets**

Polo-like kinase 4 (PLK4) has emerged as a therapeutic target based on its key role in controlling centrosome duplication and recent evidence that it functions to promote cancer cell migration and invasion[100,101,201]. CFI-400945 was the first described inhibitor of PLK4, which potently suppresses the growth of human xenograft tumours in mice[202]. However, CFI-400945 also inhibits the activity of other kinases, including Aurora B, making it unclear whether PLK4 is the only relevant therapeutic target of CFI-400945 (REF. 203). The recent development of the highly specific PLK4 inhibitor centrinone provides a precise means to study the effect of inhibiting centrosome biogenesis on tumour growth. Work in cultured cells showed that centrinone prevents the proliferation of non-transformed cells but does not interfere with the continued proliferation of most transformed cell lines[133]. In fact, it was shown that most cancer cell lines can proliferate *in vitro* without centrosomes, suggesting that they do not require supernumerary centrosomes to drive their pathologic proliferation[133]. Thus, inhibiting centrosome duplication alone may not be an efficacious anticancer strategy. Nevertheless, it may be possible to identify genetic alterations that are synthetically lethal with centrosome loss, and PLK4 inhibitors could offer therapeutic value in suppressing functions of PLK4 that promote invasion and metastasis[201].

An alternative therapeutic strategy is to exacerbate the challenge of divisions occurring in the presence of abnormal centrosome numbers. Because centrosome clustering is not required in cells with normal centrosome numbers but is required to ensure bipolar spindle assembly in cells with supernumerary centrosomes, one idea is to suppress centrosome clustering and force cancer cells with extra centrosomes into lethal multipolar divisions[172].

An alternative to targeting the centrosome directly is to manipulate proteins that control the response to errors in centrosome duplication. Ubiquitin carboxyl-terminal hydrolase 28 (USP28) is an enzymatic component of the mitotic surveillance pathway and in principle can be inhibited. Because USP28 knockout mice lack a clear phenotype[143,144], USP28 inhibition could be used therapeutically in conditions such as microcephaly, where the mitotic surveillance pathway may be pathologically activated.

data support a new model in which centrosome defects lead to mitotic delays that trigger activation of the mitotic surveillance pathway in the developing brain. Future work should focus on testing whether the mitotic surveillance pathway is indeed activated in neural progenitor cells with centrosome defects and whether deletion of *USP28* and/or *TP53BP1* can rescue brain size in models of MCPH. Mutations in some non-centrosomal proteins also cause MCPH, and it will be interesting to test whether these mutations also delay mitosis and activate the mitotic surveillance pathway[187–189].

A central unanswered question is why mutations in widely expressed centrosome proteins lead to specific defects in brain development. In fact, mutations in some centrosome proteins cause microcephalic primordial dwarfism, where a reduction in brain size is observed alongside a corresponding reduction in body size[187,188] (TABLE 2). Because MCPH or microcephalic primordial dwarfism can be caused by mutations in the same gene, they may represent a phenotypic spectrum with overlap in the underlying pathological mechanisms. Weak hypomorphic mutations in a gene could result in MCPH, whereas stronger hypomorphs cause global growth defects leading to microcephalic primordial dwarfism. One explanation for the increased sensitivity of the brain is that cortical development requires extensive proliferation in a brief developmental time window, while other organs might be able to catch up if there are minor delays in producing the required number of cells. An alternative possibility is that neural progenitors have a lower threshold for activation of the mitotic surveillance pathway compared with other cell types.

Hypomorphic mutations
Mutations that cause a partial loss of gene function.

Perspective

The past decade has witnessed a dramatic increase in our understanding of the molecular mechanisms that control centriole biogenesis and function. We will continue to benefit from insights provided by structural work on centriole and PCM components and ongoing research into the role of phosphorylation in controlling centriole assembly. In particular, additional substrates of kinases PLK1, PLK4 and CDK2 are likely awaiting identification. Moreover, little is currently known about the role of phosphatases in centriole biogenesis, and it will be interesting to further explore the role of other post-translational modifications of centrosome proteins.

Increased comprehension of the molecular mechanisms underlying centriole number, structure and function will have important ramifications for the understanding and treatment of diseases linked to centrosome dysfunction, and potential therapeutic approaches are now being explored (BOX 1). In this regard, the identification of pathways that restrain the cell cycle in response to abnormal centrosome numbers is particularly exciting. However, we lack a comprehensive understanding of how these pathways are triggered and how they function in the context of an organism. In the future, animal models that faithfully mimic the phenotypes produced by centrosome dysfunction will be instrumental in elucidating the mechanisms by which centrosome defects contribute to human disease. At present, studies that have examined the effect of centrosome amplification in mammals do so by increasing PLK4 expression. However, PLK4 also has a critical role in spindle assembly in the absence of centrioles in the early mouse embryo[131,200], and recent work has suggested PLK4 can control cancer cell migration and invasion through the regulation of the actin cytoskeleton[201]. It will be important, therefore, to further explore these non-canonical functions of PLK4 and to extend previous studies on centriole amplification by using alternative means to modify centriole numbers.

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