Centrosome function and assembly in animal cells

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Abstract | It has become clear that the role of centrosomes extends well beyond that of important microtubule organizers. There is increasing evidence that they also function as coordination centres in eukaryotic cells, at which specific cytoplasmic proteins interact at high concentrations and important cell decisions are made. Accordingly, hundreds of proteins are concentrated at centrosomes, including cell cycle regulators, checkpoint proteins and signalling molecules. Nevertheless, several observations have raised the question of whether centrosomes are essential for many cell processes. Recent findings have shed light on the functions of centrosomes in animal cells and on the molecular mechanisms of centrosome assembly, in particular during mitosis. These advances should ultimately allow the *in vitro* reconstitution of functional centrosomes from their component proteins to unlock the secrets of these enigmatic organelles.

As their name implies, centrosomes have long been thought to have a central role in many aspects of cell organization[1–4]. More than a hundred years ago, it was recognized that centrosomes form the two poles of the bipolar mitotic spindle, which separates chromosomes to daughter cells during animal cell division. If embryos from certain species were induced to assemble too many centrosomes, multipolar spindles could form, leading to the mis-segregation of chromosomes and usually embryo death. However, some embryos survived for long enough to develop into abnormal 'monsters', prompting Boveri to famously speculate that the disturbance to homeostasis resulting from such chromosome imbalance might predispose cells to malignant transformation[5].

We now know that centrosomes contain a pair of centrioles at their core, each having a ninefold symmetric structure (BOX 1). In animal cells, the centrioles exhibit a complex behaviour during the cell cycle, often forming a cilium in quiescent cells and a centrosome in proliferating cells (FIG. 1). Cilia have many important functions in cells, and their dysfunction has been linked to many human pathologies; this subject has been reviewed extensively elsewhere[6–8]. Centrosomes are formed when centrioles assemble and organize a matrix of pericentriolar material (PCM) around themselves (FIG. 1). The PCM contains hundreds of proteins[9,10], including important cell cycle regulators and signalling molecules[4], and many proteins that help to organize and nucleate microtubules, explaining why centrosomes function as the dominant microtubule-organizing centres (MTOCs) in many cell types. Through the microtubules that they organize and the proteins that they recruit, centrosomes seem to play an important part in many cell processes[2,4,11,12].

In this Review, we first discuss recent findings that address the role of centrosomes in animal cells. These studies show that centrosomes are not essential for cell division in most animal cells, but that centrosome loss triggers a p53-dependent block to proliferation in human and mouse cells, but not in fly cells. We then discuss the potential contribution of centrosome defects to human disease, focusing on cancer and several diseases that affect organ and body size. Finally, we review new data that shed light on how centrosomes are formed, with particular attention to centrosome maturation during mitosis.

Centrosome function in animal cells

Like DNA, centrosomes duplicate once, and only once, per cell cycle[13,14] (FIG. 1). Centrosome duplication occurs during the S phase of the cell cycle, and by the time a cell enters mitosis, it contains two centrosomes, which will form the poles of the bipolar mitotic spindle. Classic experiments demonstrated that cells usually divide halfway between the two spindle poles, thus ensuring that each new daughter cell inherits one complete set of chromosomes and a single centrosome[15]. According to this classical view of mitosis, the presence of two centrosomes ensures spindle bipolarity, emphasizing the importance of precisely regulated centrosome duplication.

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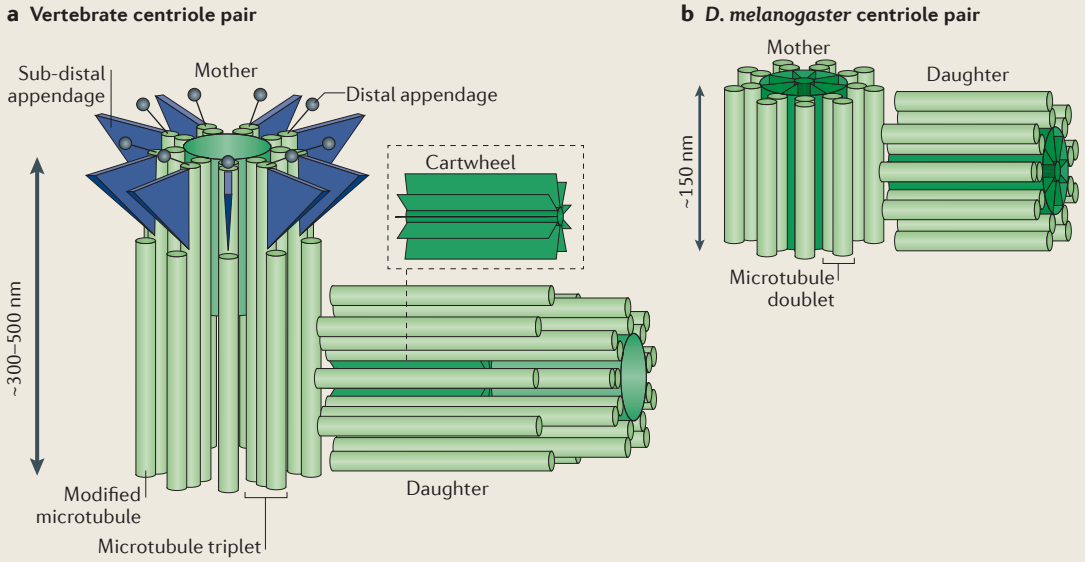
Box 1 | **A schematic illustration of centriole structure**



In most vertebrate cells (see the figure, part **a**), new centrioles (called 'daughter centrioles') are assembled around a central cartwheel structure (shown in dark green), which sets the ninefold symmetrical arrangement of the microtubules (light green). The older centriole of the pair (the 'mother') usually loses its central cartwheel and is often decorated with distal (grey) and sub-distal (blue) appendages. In vertebrate centrioles, the microtubules are usually arranged as triplets, which often become doublets towards the distal end. The new daughter centriole is formed at right angles to the mother during the S phase of the cell cycle, and the centrioles remain in this tightly apposed 'engaged' configuration until the late stages of mitosis, when they disengage, allowing the daughter centriole to mature into a mother that is now competent to form its own daughter and to organize its own pericentriolar material. In some species, such as worms and flies, centrioles are simpler in structure. A typical *Drosophila melanogaster* centriole pair is illustrated here (see the figure, part **b**): the centrioles are usually shorter than vertebrate centrioles and are usually composed of microtubule doublets rather than triplets; the mother centriole lacks appendages; and the cartwheel is present in both mother and daughter centrioles.

It has long been known, however, that higher plant cells and the oocytes of many animals assemble bipolar mitotic and meiotic spindles, respectively, without centrosomes[16,17]. Nevertheless, it was not until the discovery that bipolar spindles could assemble around chromatin-coated beads in *Xenopus laevis* egg extracts lacking centrosomes that it became generally accepted that centrosomes are not the only drivers of spindle assembly and bipolarity, even in systems that normally have centrosomes[18,19]. We now know that there are several non-centrosomal pathways that can nucleate and stabilize microtubules during mitosis, and that multiple molecular motors can work together to organize these microtubules into a bipolar spindle around the mitotic chromatin: in the chromatin-mediated pathway, microtubules are nucleated and stabilized in the vicinity of mitotic chromatin[20–22]; in the augmin complex-mediated pathway, new microtubules grow out from the sides of pre-existing microtubules[23–26]; in the acentriolar MTOC pathway, some PCM components self-organize into foci without centrioles, and these structures can nucleate and organize microtubules[27–30]. These pathways can make important contributions to spindle assembly, even when centrosomes are present[20,22,31]. Although it is clear that centrosomes are not essential for spindle assembly, in systems that have centrosomes, they clearly contribute to the efficiency of spindle assembly[32–35] and are thought to have important functions in many other cell processes[2,4,11,12].

Centrosome function in invertebrates. It was a surprise, therefore, to discover that the fruit fly *Drosophila melanogaster* could proceed through most of its development without centrosomes[36] (FIG. 2a). Although centrosomes are essential for the extremely rapid nuclear divisions in early syncytial fly embryos[37,38], embryos that are homozygously mutant for the *Spindle assembly abnormal 4 orthologue* (*Sas4*) gene, which is essential for centriole (and therefore centrosome) assembly, can proceed through these early stages of embryogenesis using Sas4 proteins that are transferred to the egg by their heterozygous mothers. Once past these early stages of development, centrioles and centrosomes are rapidly lost as maternal Sas4 proteins are diluted, but morphologically normal flies lacking detectable centrosomes are born a few days later, with only a slight delay in their development (FIG. 2a). Lacking detectable centrioles, these flies cannot form cilia (FIG. 1), which in adult flies are essential for mechano- and chemosensing; as a consequence, they are uncoordinated, cannot feed and die shortly after eclosion. Interestingly, and in contrast to vertebrate cells (see below), the lack of centrosomes per se does not seem to activate cell stress pathways in flies, at least at the transcriptional level[39].

Molecular motors
Proteins containing a 'motor' domain that allows them to move along microtubules or actin filaments.

Syncytial fly embryos
The initial form of fly embryos, consisting of a giant single cell (a syncytium) in which the nuclei rapidly and synchronously divide.

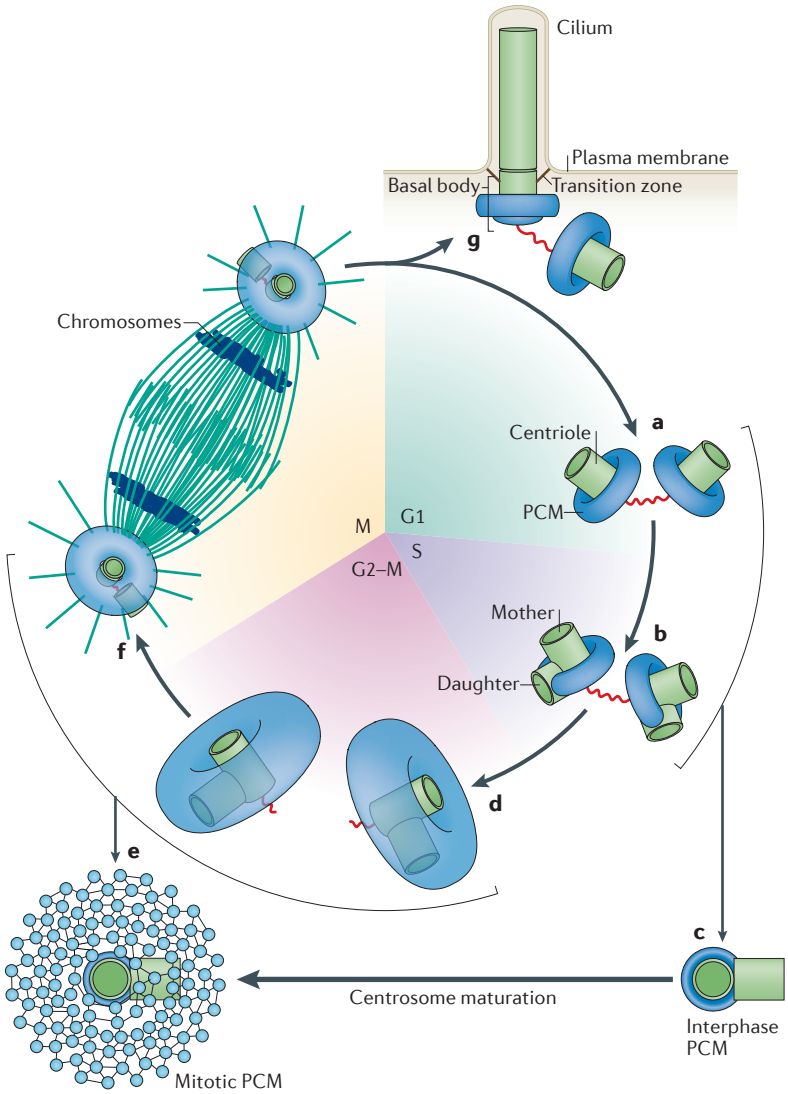


Figure 1 | **Centriole, centrosome and cilium behaviour during the cell cycle.** **a** | A 'newborn' cell in the G1 phase of the cell cycle usually contains two centrioles (shown in light green) that are often joined together by a flexible linker (red)[200]. The centrioles can form centrosomes by organizing pericentriolar material (PCM; light blue) around themselves. **b** | The centrioles duplicate in the S phase, each forming a daughter centriole that is tightly apposed at right angles to the original mother centriole in an 'engaged' configuration[13,201]. Although the centrioles in most cells in G1–S organize very little PCM, this PCM is highly organized around the mother centriole (shown in part **c**), with several PCM proteins forming a single layer of molecules around the mother centriole, with either their amino- or carboxy-terminal region found close to the centriole and the other terminal region extending outwards (see, for example, the distribution of pericentrin-like protein (Plp) in FIG. 4a). **d** | As cells enter mitosis (G2–M), the two pairs of centrioles start to move apart as the linkage between them is broken. The mother centrioles start to recruit much larger amounts of PCM, and this is thought to be organized by a 'scaffold' structure that assembles around the mother centrioles — indicated by the interconnected matrix in the end-on view of the mother centriole shown in part **e**. **f** | The enlarged PCM allows the centrosomes to nucleate and organize many more microtubules, which then play an important part in assembling and positioning the mitotic spindle (dark green). As cells exit mitosis, the chromosomes (dark blue) segregate on the mitotic spindle and the mother and daughter centrioles disengage, losing their tight orthogonal arrangement — although they often remain loosely associated through the flexible linker structure. The mechanisms of PCM assembly are discussed in the second part of this Review. **g** | In many animal cells that have exited the cell cycle, the centriole pair migrates to the cell surface, and the mother centriole forms a basal body from which a cilium extends. The cilium is known to have many important functions in cells, and cilium dysfunction is associated with many human pathologies.

An equally surprising observation was that the planarian flatworm *Schmidtea mediterranea* does not require centrosomes to accomplish its astonishing feats of regeneration, in which a whole organism, including a head, gut and tail, can be regenerated from just a small piece of tissue[40]. This worm assembles many centrioles to form the thousands of cilia that are required for its gliding motility; remarkably, however, it never uses these centrioles to form centrosomes. Thus, the rapidly dividing neoblasts, which are the stem cells responsible for regeneration, accomplish this complex developmental task without centrosomes. Interestingly, the closely related regenerating flatworm *Macrostomum lignano* does form centrosomes during mitosis. One possible explanation for this difference is that early *M. lignano* embryos rely on a stereotypical pattern of oriented cell divisions, whereas early *S. mediterranea* embryos do not. Centrosomes often play an important part in ensuring proper spindle orientation in such patterned cell divisions[1,41,42], so evolutionary pressure may have maintained centrosome assembly in *M. lignano* but not in *S. mediterranea*[40].

Although these findings demonstrate that centrosomes are not essential for many of the complex cell and developmental processes that are required to produce an adult fly or to regenerate a flatworm, they do not exclude the possibility that centrosomes have many important functions in invertebrate cells. Indeed, spindle assembly is invariably slowed in fly cells that lack centrosomes, and mutant fly neural progenitor cells (known as neuroblasts) and larval wing disc epithelial cells that lack centrosomes have clear defects in spindle orientation[36,43]. Moreover, *D. melanogaster* and *S. mediterranea* only have four pairs of chromosomes; a lack of centrosomes in organisms that have to segregate larger numbers of chromosomes during mitosis might be expected to result in more severe mitotic defects. In support of this expectation, centrosome loss perturbs chromosome segregation and leads to a rapid loss of viability in several vertebrate[35,44–46], but not fly[32,47,48], cultured cells.

Centrosome loss activates p53 in vertebrate cells. Recent evidence suggests, however, that the response of vertebrate cells to centrosome loss *in vivo* may not be so simple. Mouse embryos that lack centrosomes, such as *Sas4*$^{-/-}$ mutants, are very small and arrest development at approximately embryonic day 9, showing a dramatic increase in apoptosis[49,50] (FIG. 2b). Apoptosis seems to be induced by a p53-dependent pathway that is gradually activated over several cell cycles as a result of delayed spindle assembly in the absence of centrosomes[49]. Consistent with p53 having a pro-apoptotic role, in *Sas4*$^{-/-}$*p53*$^{-/-}$ mouse embryos, apoptosis is largely suppressed, and the embryos develop relatively normally until they die, apparently as a result of defects caused by the lack of cilia. Remarkably, although their cells contain 20 pairs of chromosomes, these embryos show no detectable increase in chromosome segregation errors, aneuploidy or DNA damage. A similar result was obtained when centrosomes were specifically removed

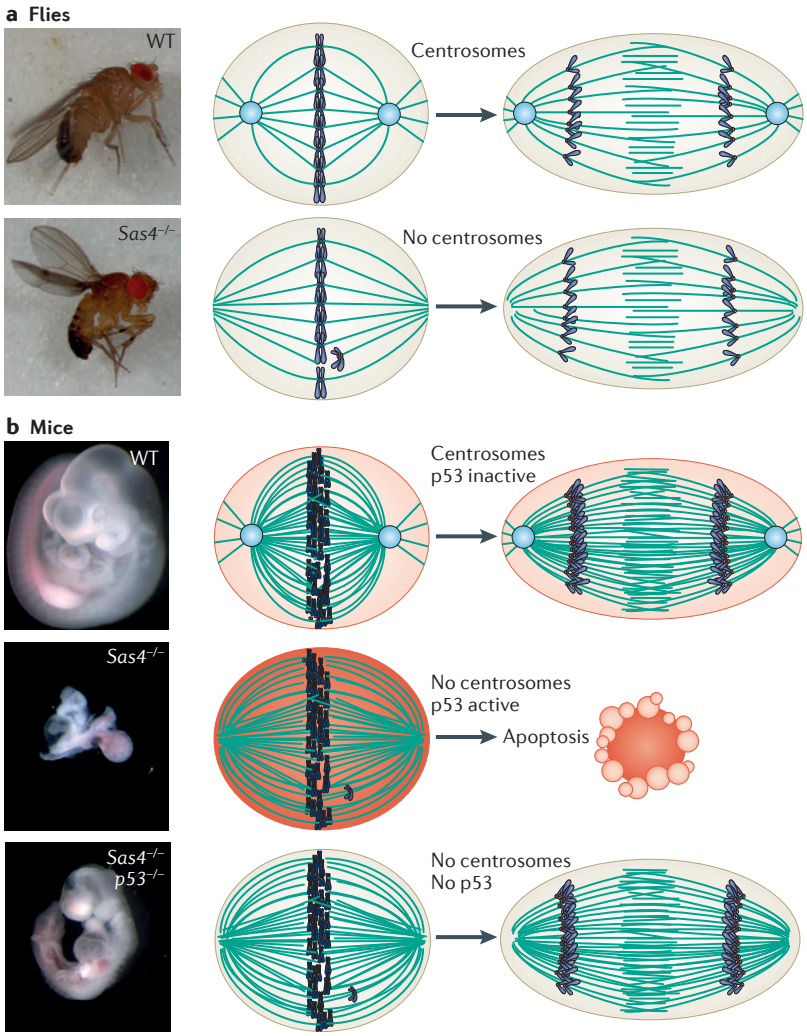


Figure 2 | **Consequences of a lack of centrosomes on cell division and proliferation in flies and in mice.** **a** | Adult *Drosophila melanogaster Spindle assembly abnormal 4 orthologue* (*Sas4*)$^{-/-}$ mutant flies lack detectable centrioles, centrosomes and cilia, yet they are largely morphologically normal when compared to wild-type (WT) flies. Because the mutants lack cilia, they are deficient in mechano- and chemosensing (hence the held-up wings and abnormal posture of the mutant fly shown here); as a result, they cannot feed and die soon after eclosion. Although the mutant cells are slowed in mitotic spindle assembly (indicated by the unaligned chromosome in the schematic of the metaphase cell), the vast majority of cells ultimately segregate their chromosomes normally. **b** | *Sas4*$^{-/-}$ mouse embryos also lack centrioles, centrosomes and cilia, and mitotic spindle assembly is also slowed (indicated by the unaligned chromosome in the schematic of the metaphase cell). Unlike in the mutant fly cells, however, the delay in spindle assembly activates a p53-dependent apoptosis programme, triggering large-scale cell death. The embryos survive until mid-gestation, with a basic body plan, a heart and a neural tube, but they are deformed and are much smaller than normal. If p53 is also inactivated, apoptosis is suppressed and the cells can proliferate surprisingly well: these embryos survive until at least embryonic day 9.5, and have fewer than 20 somites and complete embryonic turning, but they have the randomized left–right situs and abnormal brain phenotype typical of mutants that lack cilia. Remarkably, the cells in these embryos show no detectable increase in the rates of lagging chromosomes, aneuploidy or DNA damage. A lack of centrosomes in cultured human cells also triggers a strong p53-dependent apoptotic response, but these cells can also proliferate surprisingly well if p53 activity is suppressed — although, unlike the mouse cells *in vivo*, the human cultured cells have elevated levels of chromosome mis-segregation and aneuploidy. It is unclear whether this difference in mitotic fidelity reflects intrinsic differences between acentrosomal vertebrate cell behaviour *in vivo* and *in vitro*, or differences in how efficiently p53 has been inactivated in the different experiments. Images of mouse embryos are courtesy of Hisham Bazzi and Kathryn Anderson, Memorial Sloan Kettering Cancer Center, USA.

from the developing mouse brain[51]. Thus, mouse cells *in vivo* can segregate their chromosomes and proliferate well without centrosomes, as long as p53 is inactivated (FIG. 2b).

Recent results suggest that centrosome loss also gradually activates p53 in vertebrate cultured cells[52–54]. Polo-like kinase 4 (PLK4) is an important driver of centrosome duplication, and the recent development of a specific PLK4 inhibitor, centrinone, has provided a powerful new tool with which to probe centrosome function[53]. Cancer cells lacking a functional p53 pathway can continue to proliferate indefinitely if centrosomes are depleted by centrinone treatment, although such transformed cells grow more slowly and, unlike mouse cells *in vivo*, they have increased levels of chromosome mis-segregation. Centrosome loss in non-transformed cells, however, triggers a p53-dependent G1 arrest, but only after a few cell cycles. Similar results were reported when PLK4 was rapidly inactivated using an auxin-inducible degron system[54]. The reason that centrosome loss activates p53 in these cultured cells is unclear, but it does not seem to be owing to the delay in mitosis that activates p53 in mouse cells *in vivo* or by the p38 stress-induced pathway; moreover, p53 is activated even in cells that segregate their chromosomes normally, suggesting that aneuploidy is not the trigger.

Thus, a consensus seems to be emerging that most vertebrate cells can segregate their chromosomes surprisingly well in the absence of centrosomes, but that a specific p53-dependent pathway, which is presumably not active in fly cells, eventually detects centrosome loss, triggering either apoptosis or cell cycle arrest. Clearly, it will be important to determine how and why this p53-dependent pathway is activated by centrosome loss in vertebrate cells. An intriguing possibility is that this pathway normally serves to 'lock' differentiated cells — in which centrosomes are often inactivated (see below) — into a permanently non-proliferative state[53].

Centrosomes in human disease

The studies described above have revealed previously unknown aspects of the response of vertebrate cells to centrosome loss. Several recent studies have highlighted some unexpected links between centrosome defects and human disease.

The complex link between centrosomes and cancer. A link between centrosomes and cancer has long been postulated[55–57], and centrosome defects have been shown in transplantation experiments to predispose fly cells to form tumours[58,59]. Centrosome abnormalities are thought to encourage tumorigenesis in humans, largely by promoting chromosomal instability (CIN), and both phenomena are common and often correlated in human cancer[60–63]. However, the relationship between CIN and cancer in mouse models is complex, with high levels of CIN potentially suppressing cancer and low levels potentially promoting cancer[64,65]. It now seems clear that the original idea that centrosome amplification might lead to large-scale CIN and thereby promote cancer development is not correct: somatic cells

can efficiently cluster or inactivate extra centrosomes, enabling the cells to form bipolar spindles and segregate chromosomes almost normally during mitosis[58,66,67]. Nevertheless, multipolar intermediates often form during spindle assembly in somatic cells with extra centrosomes and can increase the rate of chromosome mis-segregation by promoting merotelic chromosome attachments[68,69]. Thus, although centrosome amplification does not lead to large-scale CIN in most somatic cells, it could contribute to an insidious, low-level CIN, which may promote cancer more effectively.

Further complicating the link between centrosomes and cancer is the close relationship between centrosome defects and p53. As described above, centrosome loss triggers a p53-dependent response in many vertebrate cells, and the same is true of centrosome amplification[70,71], although these centrosome abnormalities may activate p53 in different ways. For example, centrosome amplification in tetraploid cells activates p53 (at least in part) through activation of the Hippo pathway[72], whereas this pathway is not required for p53 activation in cells depleted of centrosomes[53]. Moreover, a proportion of p53 localizes to centrosomes (as is the case for several other tumour suppressor proteins, such as breast cancer type 1 susceptibility protein (BRCA1) and BRCA2), and p53 loss often leads to centrosome amplification[73].

Given that centrosome defects often activate p53 and thereby inhibit the proliferation of normal vertebrate cells, it is puzzling why they are such a common feature of human cancer cells[55,56]. One possibility is that inactivation of p53 simply allows centrosome defects to accumulate (as cells with these defects are no longer eliminated efficiently); in this scenario, centrosome defects would be more a consequence than a cause of cancer development. Alternatively, centrosome defects might actively promote tumour progression in some way, perhaps by generating low levels of CIN or, as a recent study suggests, by promoting metastasis[74]. This study showed that cells in which the assembly of too many centrosomes was induced show increased motility and invasive-like behaviour in 3D cell culture assays; the increase in centrosome numbers led to an increase in centrosomal microtubule nucleation, which in turn activated the small GTPase RAC1, disrupting cell–cell adhesion and increasing cell motility[74]. Reducing the amount of the PCM-assembly centrosomal protein CEP192 (which is the human homologue of spindle defective 2; SPD-2 in worms and Spd2 in flies; see below) ameliorated these abnormalities, suggesting that the increased PCM and microtubule nucleation were responsible for the invasive behaviour. It remains unclear how increased microtubule nucleation activates RAC1, although a correlation between increased microtubule-nucleating capacity of centrosomes and increased cancer grade has been reported previously[75]. It will be important to determine whether this effect of centrosome amplification and increased microtubule nucleation can be replicated in *in vivo* tumour models; if so, drugs that inhibit centrosome amplification or PCM assembly might be useful anticancer agents.

Centrosomes, microcephaly and primordial dwarfism. Although a strong genetic link between centrosomes and human cancer is still lacking, compelling genetic evidence continues to accumulate that links centrosome defects to primary autosomal recessive microcephaly (MCPH, in which individuals are born with small, but relatively normally formed, brains) and primordial dwarfism (in which individuals are born with small brains and small stature, including Seckel syndrome, Meier–Gorlin syndrome and microcephalic osteodysplastic primordial dwarfism)[76–79]. Although centrioles and centrosomes are composed of hundreds of proteins, only a small number of these are essential for centrosome assembly. Moreover, remarkably, many patients with MCPH or primordial dwarfism have mutations in a gene that encodes one of these essential proteins (see FIG. 3; proteins highlighted with yellow in the *Homo sapiens* centriole and centrosome assembly pathway). These observations strongly suggest that centrosomes have an important role in human development, but it remains a mystery why centrosome defects lead to such specific pathologies, in which the size of the body and the brain, or just the brain, is dramatically reduced, but organization and function are largely unperturbed[79].

Perhaps the simplest explanation for the specific size defects is that during human development, centrosome abnormalities specifically induce progenitor cells to undergo apoptosis or premature differentiation and/or cell cycle arrest — or some combination of these — resulting in reduced cell numbers. Indeed, premature cell cycle withdrawal and differentiation of brain progenitor cells has been observed in developing human cerebral organoid cultures derived from patients with MCPH with mutations in the gene encoding CDK5 regulatory subunit-associated protein 2 (CDK5RAP2; also known as CEP215), which is a protein involved in several aspects of centrosome function in vertebrates and is the homologue of fly Centrosomin (Cnn; see below). Crucially, these mutant human cells form abnormally small organoids[80]. A similar decrease in neural progenitor proliferation and increase in premature differentiation can be observed when CDK5RAP2 is depleted in developing mouse brains[81]. MCPH can also be induced in mouse models by the induction of centrosome amplification[70] or centrosome loss[51] in neural progenitor cells. As discussed above, these defects induce a p53-dependent anti-proliferative response, either directly through the delay in mitosis triggered by centrosome loss[51] or indirectly through the induction of aneuploidy by centrosome amplification[70].

Why do some centrosome defects cause MCPH, whereas others cause primordial dwarfism? An attractive possibility is that subtle centrosome defects specifically perturb neural progenitor cell proliferation, leading to MCPH, whereas stronger centrosome defects perturb progenitor cell proliferation more widely, leading to primordial dwarfism. One reason that brain progenitors may be especially sensitive to centrosome defects is that proper spindle alignment may be particularly important for the complex pattern of asymmetric progenitor cell divisions that occur during vertebrate brain

Wing disc epithelial cells
Epithelial cells in the imaginal disc tissues of fly larvae that will ultimately form most of the adult fly structures (such as the wing and leg) when the larvae pupate.

Transformed cells
Cells that have lost some of their normal growth-control mechanisms (such as cancer cells).

Auxin-inducible degron system
A system that allows appropriately tagged proteins to be rapidly degraded in cultured cells when the plant auxin protein is added to the media.

p38 stress-induced pathway
A pathway by which p38 protein is often activated in cells in response to various forms of cellular stress; it induces responses that protect cells from the stress.

Chromosomal instability
(CIN). A property of many cancer cells, in which the genome of the cell is constantly being rearranged as whole chromosomes or parts of chromosomes are lost or gained during the process of cell division.

Organoid
Populations of cells — formed by division and differentiation when some cell types are grown in culture under specific conditions — that can self-organize to resemble the tissue (for example, brain, liver or gut) from which they were derived.

development[82,83]. Indeed, during the development of the much simpler fly brain, the loss of centrosomes perturbs the asymmetric divisions of neuroblasts but has little effect on the division of several other cell types, including male and female germline stem cells[36,37,84]. In developing mouse brains, however, the situation seems to be more complicated. Here, the loss or gain of centrosomes leads to the induction of apoptosis and

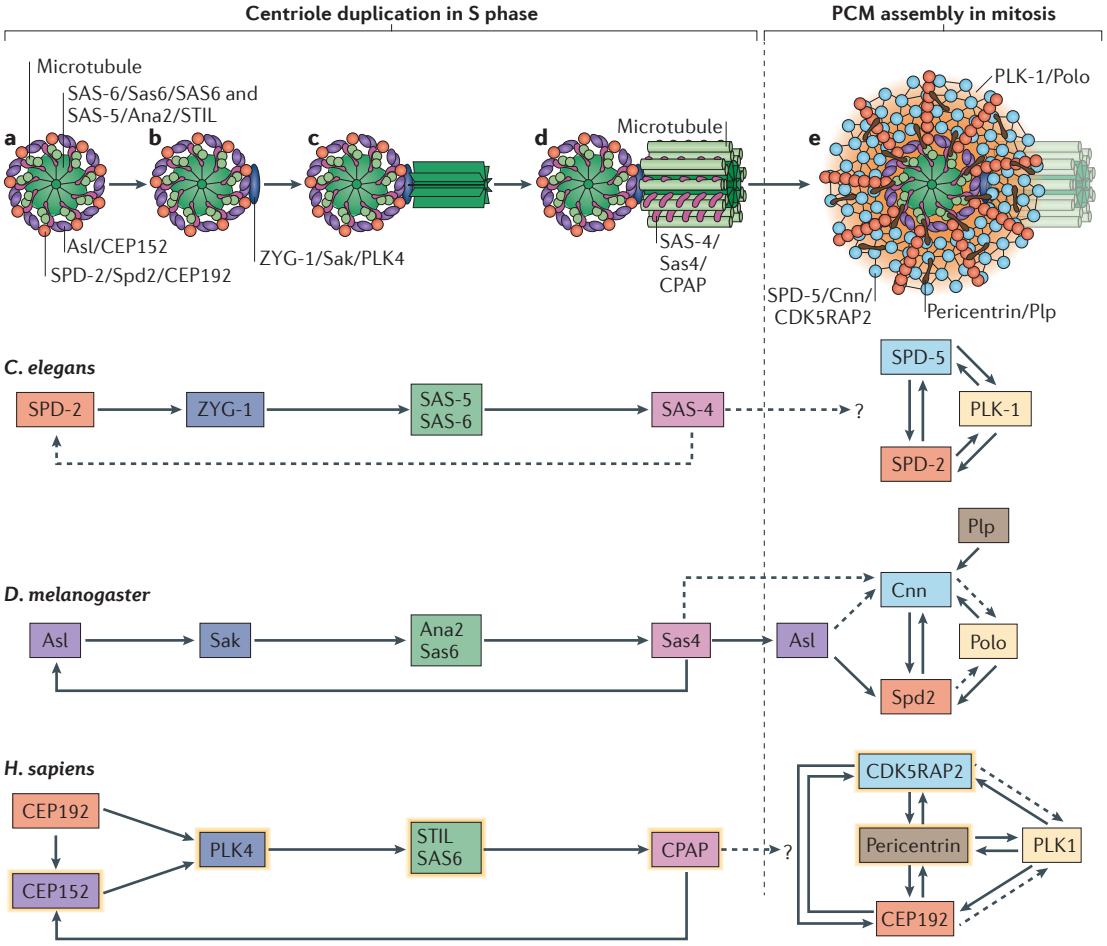


Figure 3 | **A model of the molecular pathways of centriole and mitotic centrosome assembly in worms, flies and humans.** The schematics illustrate how a mother centriole assembles a daughter centriole during the S phase (shown in parts **a**–**d**), and how the mother centriole recruits pericentriolar material (PCM) during mitosis (part **e**). The putative molecular pathways in worms, flies and humans are illustrated below the schematics: putative functional homologues are boxed in the same colour, and human proteins that have been implicated in primary autosomal recessive microcephaly and/or primordial dwarfism have a yellow highlight in the *H. sapiens* pathway. Solid arrows indicate that one protein is required for the proper localization of another, dashed arrows indicate localization relationships that can be inferred and question marks indicate localization relationships that are unknown. Many of these localization relationships depend on direct protein–protein interactions, although in several cases these remain unproven. **a**,**b** | The protein kinase zygote defective 1 (ZYG-1) in worms, Sak in flies or Polo-like kinase 4 (PLK4) in humans is recruited to a single patch on the mother centriole by spindle defective 2 (SPD-2) in worms, by Asterless (Asl) in flies, and by a combination of their respective homologues, CEP192 and CEP152, in humans. **c**,**d** | The protein kinase recruits SAS-6 and SAS-5 (Ana2 in flies; STIL in humans), which form either a central cartwheel (shown here) or, in worms, a central tube structure. SAS-4 (worms), Sas4 (flies) or CPAP (humans) is then recruited; this helps to recruit the centriole microtubules, and it can also interact directly with Asl or CEP152 in flies and in humans, respectively. In flies, Sas4 helps to recruit Asl to new centrioles after they disengage from their mothers; this completes the duplication cycle and allows daughter centrioles to mature into mothers that are competent for centriole duplication[118]. **e** | In fly embryos, Asl also plays an important part in allowing mother centrioles to recruit the mitotic PCM by helping to recruit Spd2 and Cnn to mother centrioles[166]. In flies, Spd2 and Polo cooperate with Cnn to drive the assembly of a mitotic PCM scaffold (see also FIG. 4), whereas in worms, SPD-2 and PLK-1 cooperate with SPD-5 to drive the assembly of a mitotic PCM scaffold. In flies, pericentrin-like protein (Plp) seems to strengthen this scaffold. Interestingly, no Asl or pericentrin-like proteins have been identified in *C. elegans*, although worm SPD-2 seems to combine some of the functions of Asl and Spd2, and worm SPD-5 seems to combine some of the functions of Cnn and Plp. In humans, a similar core module of CDK5 regulatory subunit-associated protein 2 (CDK5RAP2), CEP192 and PLK1 is likely to exist, with pericentrin also having a central role in mitotic PCM assembly. *C. elegans*, *Caenorhabditis elegans*; *D. melanogaster*; *Drosophila melanogaster*; *H. sapiens*, *Homo sapiens*.

to microcephaly. In the case of centrosome loss, inactivating p53 suppresses apoptosis and rescues the microcephaly defect, but not the defects in spindle orientation, suggesting that spindle orientation defects alone cannot explain the microcephaly[51]. In the case of centrosome amplification, inactivating p53 substantially suppresses apoptosis but does not rescue the microcephaly[70]. Thus, centrosome loss and amplification seem to induce microcephaly in different ways in the mouse brain, and any link between microcephaly and spindle orientation defects remains to be definitively established.

A further potential complication is that primordial dwarfism in humans has been linked to the DNA damage repair (DDR) and replicative stress pathways, and several centrosomal proteins function in these pathways[85]. The DDR pathway is, however, not detectably perturbed in chicken DT40 cells or in NIH/3T3 or HeLa cells that lack centrosomes[35,53], indicating that centrosomes are not essential for this pathway in these cells. Thus, the potential interplay between centrosomes and the DDR and replicative stress pathways, and its relationship to primordial dwarfism, remain to be fully explored.

Centrosome assembly and maturation

Until recently, the mechanisms and regulation of centrosome assembly and maturation had remained largely unknown. In this section, we briefly discuss the conserved protein pathway that regulates centriole assembly and consider how recent studies in worms and in flies are beginning to elucidate a common pathway that is responsible for mitotic PCM assembly.

Centriole assembly. Centrioles are essential for initiating efficient PCM assembly in most cells[36,86]. To understand centrosome assembly, it is therefore important to understand centriole assembly. Our understanding of this process has progressed rapidly in the past decade, with ground-breaking discoveries in *Caenorhabditis elegans* leading the way in defining a 'core' set of proteins that seem to be essential for centriole assembly in all eukaryotic species[87,88] (FIG. 3). This work is discussed extensively in several recent reviews[89–92], and so we only briefly describe centriole assembly here.

The initial step in centriole assembly is thought to be the recruitment of the protein kinase zygote defective 1 (ZYG-1) in worms — or its functional homologues Sak and PLK4 in flies and humans, respectively — to the side of a pre-existing mother centriole[46,47,93,94]. This step is mediated by SPD-2 in worms[87,88,95,96], by Asterless (Asl) in flies[97,98] and by a combination of CEP152 and CEP192 (which are the human homologues of Asl and SPD-2, respectively)[99–102] in humans. The protein kinases then recruit SAS-6 (REF. 103) and SAS-5 (Anastral spindle 2 (Ana2) and SCL-interrupting locus protein (STIL) are the functional homologues of SAS-5 in flies and humans, respectively)[104–106] that together form a central cartwheel structure that establishes the highly conserved ninefold symmetry of the centriole[107,108]. These two proteins then recruit SAS-4 (often called CPAP or CENPJ in humans) to the outer region of the cartwheel, where it helps to assemble the surrounding centriolar microtubules[87,109,110]. In flies and in humans, this is probably with the help of CEP135 (also known as BLD10)[111–113], although CEP135 does not seem to be essential for centriole assembly in flies or in chicken DT40 cells[114–117], and no homologue has been identified in worms. In flies, Sas4 is required to recruit Asl to centrioles[98,118], thereby allowing new centrioles to start the cycle again (FIG. 3), but this only occurs when the newly formed daughter centriole has disengaged from its mother at the end of mitosis[118].

For centriole assembly, the challenge now is to understand how these core centriole proteins interact with one another and how these interactions are regulated to ensure that centrioles are only assembled in the right place and at the right time (FIG. 3a–d). Substantial progress is being made in understanding these molecular mechanisms on the basis of structural studies. The crystal structures of parts of the SAS-6 protein from several species have been solved, showing that SAS-6 self-oligomerizes to form the backbone of the cartwheel structure[107,108,119–121], and essential self-oligomerizing domains in SAS-5 and Ana2 have also recently been structurally characterized[121,122]. The crystal structures of the interaction interfaces between ZYG-1 and SPD-2, and between PLK4 and CEP152 or CEP192, have been solved or modelled[123,124], as has the interface between Sas-4 (CPAP in humans) and Ana2 (STIL in humans)[125,126]. Importantly, recent studies suggest that Ana2 (flies) or STIL (humans) interacts with and is an *in vivo* substrate of Sak or PLK4; the kinase can phosphorylate Ana2 or STIL at several sites within the conserved STIL–Ana2 (STAN) domain, allowing Ana2 or STIL to interact more efficiently with SAS-6, thereby promoting SAS-6 recruitment and centriole assembly[104–106]. The interaction interface between STIL and PLK4 has recently been solved[127], and exciting new data show that STIL binding activates PLK4 kinase activity, although it is unclear how this occurs[128]. Although SAS-6 is probably not a ZYG-1 substrate in worms[103], PLK4 can phosphorylate other centriole and centrosome assembly proteins *in vitro*, but the *in vivo* significance of these findings remains to be determined[99,129,130]. Elucidating how the ZYG-1, Sak or PLK4 protein kinase is concentrated at only a single site on the mother centriole (FIG. 3b) is likely to be the key to understanding why a mother centriole forms only one daughter during centriole duplication.

PCM assembly in interphase. Once centrioles are formed, how do they recruit and organize their PCM? The answers seem to be different depending on whether the cells are in interphase or mitosis. Interphase centrioles recruit less PCM than do mitotic centrioles (FIG. 4), although the relative levels seem to vary between cell types. In many post-mitotic cells[131], including epithelial[132–135], muscle[136,137] and nerve cells[138], centrioles organize very little PCM and few microtubules, and so do not function as dominant MTOCs. By contrast, in proliferating HeLa cells, interphase centrioles can organize ~15–40% of the amount of PCM that is organized by mitotic centrioles, depending on the PCM protein analysed[139,140]. Moreover, proteins such as ninein-like protein (NLP) and centrobin function as interphase-specific

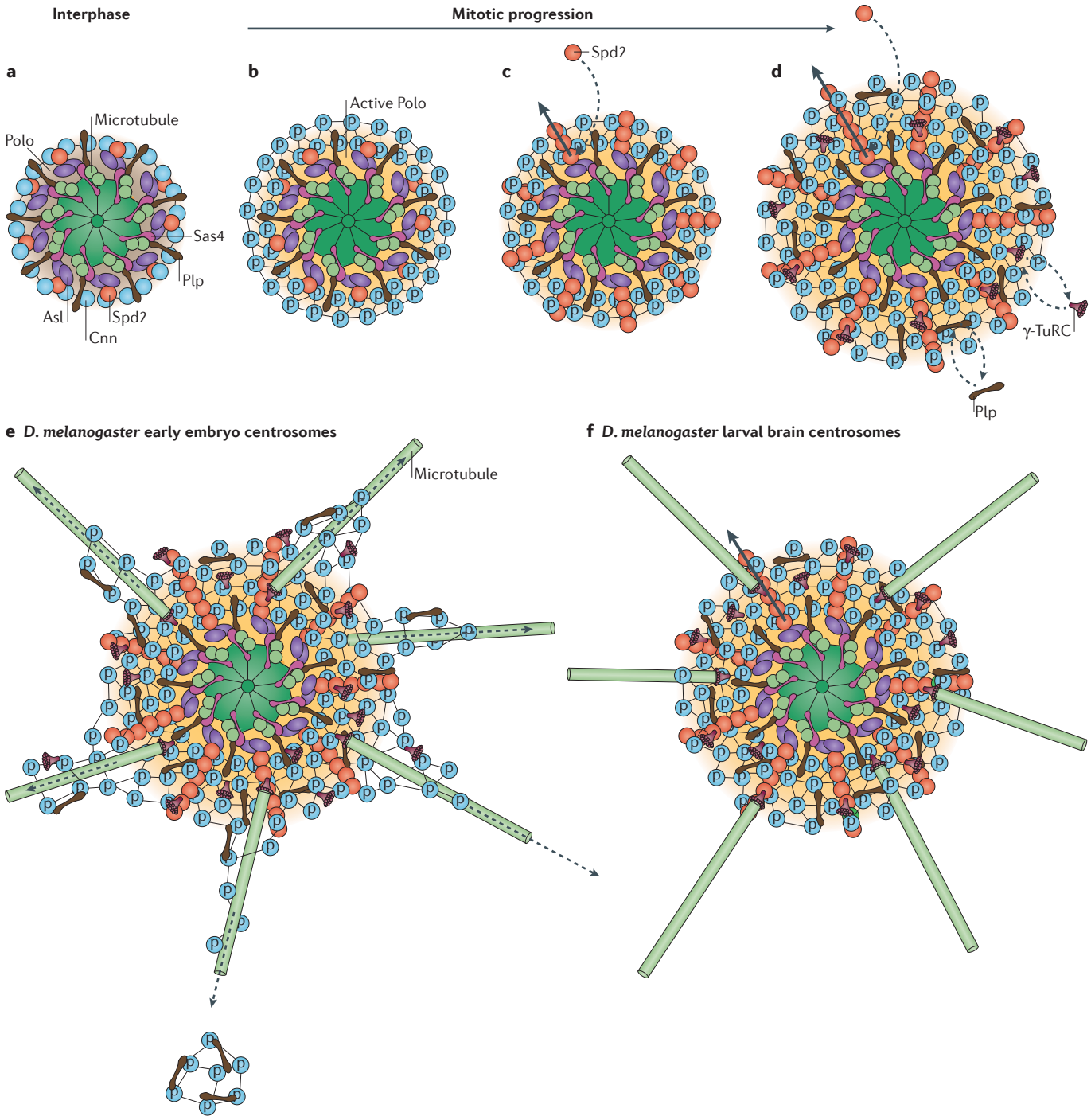


Figure 4 | **A model of mitotic pericentriolar material (PCM) assembly in flies.** **a** | In interphase, the mother centriole organizes a single layer of pericentrin-like protein (Plp) molecules (shown in brown) that have their carboxy-terminal end attached to the centriole wall and their amino terminus extending outwards. In fly cells, this layer of Plp recruits the interphase PCM. Asterless (Asl; purple) is present, but it is unclear whether it helps to recruit the PCM in interphase; Polo (brown background) is also present but is presumably inactive. **b** | As fly cells enter mitosis, active Polo (orange background) phosphorylates the Centrosomin (Cnn) molecules (blue), allowing them to assemble into an interconnected scaffold. **c** | This Cnn scaffold interacts with the Spindle defective 2 (Spd2) molecules (red), allowing them to be maintained in the PCM and to spread further away from the centrioles (solid arrow); new Spd2 molecules continue to be incorporated into the PCM around the mother centriole wall in an Asl-dependent manner (dashed arrow), replacing the molecules that have moved outwards — in this way, the Spd2 molecules flux outwards away from the mother centriole. For simplicity, the Spd2 molecules are depicted as forming a filament-like structure to illustrate this outward flux; it is not clear whether Spd2 can assemble into such structures, although filament-like structures are observed in super-resolution images. In worms and in humans, the SPD-2 or CEP192 protein interacts with Polo-like kinase 1 (PLK1), and in fly embryo centrosomes, the distributions of Spd2 and Polo are very similar (see part **g**), so we speculate that Polo binds directly to the expanding Spd2 region. In this way, the area that can recruit and phosphorylate Cnn expands around the fly mother centriole. **d** | These interactions potentially create a positive-feedback loop that drives the further expansion of the mitotic PCM scaffold: the expanding Cnn network presumably allows more Spd2 and Polo to accumulate at centrosomes, which would presumably allow more Cnn to be incorporated into the scaffold over a greater area. PCM components such as Plp and γ-tubulin ring complexes (γ-TuRCs) are recruited to the Cnn–Spd2 scaffold (dashed arrows), where they form interconnections with the scaffold and with other PCM components, further strengthening the PCM. **e** | In fly embryos, the Cnn scaffold is pulled outward on microtubules (dashed arrows; see panels 2 and 3 in part **g**), eventually forming 'flares' that break away from the bulk of the PCM[202]. Although these flares contain large amounts of Plp, they contain very little Spd2 and Polo, and they gradually disassemble in the cytoplasm. Importantly, new Cnn molecules cannot be incorporated into the outer regions of the Cnn scaffold, presumably because Spd2 and Polo are not present. **f** | In fly larval brain cells, the microtubule-independent outward movement of Spd2 still occurs (arrow), but the microtubule-dependent outward movement of Cnn does not. **g** | The panels show 3D structured-illumination microscopy super-resolution images of Asl and Spd2 (image 1), Cnn and Spd2 (image 2) and Cnn and Polo (image 3) in *Drosophila melanogaster* embryo centrosomes. Note how the Spd2 molecules have a wider distribution than the Asl molecules, and how the Cnn molecules have a wider distribution than the Spd2 and Polo molecules.

PCM assembly factors in certain cultured human cells and in *D. melanogaster* neuroblasts (in which, unusually, one centrosome remains active as an MTOC throughout interphase), respectively[141,142].

Super-resolution microscopy studies of cultured vertebrate and *D. melanogaster* cells indicate that the interphase PCM is exclusively formed around the mother centriole and is highly organized[94,143–145] (FIG. 4a). Strikingly, in human cells, the large centriole- and PCM-associated protein pericentrin (also known as kendrin) forms oriented fibrils that extend away from the mother centriole, with the carboxy-terminal pericentrin–AKAP450 centrosomal targeting (PACT) domain adjacent to the centriole wall and the amino terminus extending outward into the PCM; a similar organization of pericentrin-like protein (Plp, also known as Cp309) is observed in fly cells[143,144]. The other interphase PCM proteins are located within the area delimited by this single layer of pericentrin or Plp fibrils (FIG. 4a) and, in cultured fly cells, Plp is essential for the centrosomal localization of several of these interphase PCM components[143].

Structures termed centriolar satellites have been identified in several cell types in interphase, and proteins such as PCM1 are important for their formation and function[146]. These structures move along the centrosomal microtubules toward the centrioles and are thought to carry proteins to the interphase PCM. Intriguingly, satellites also have a role in cilia formation[147]. However, not all cell types seem to form such satellite structures.

PCM assembly in mitosis. In most animal cells, the amount of PCM that is recruited around the centrioles dramatically increases as cells prepare to enter mitosis, in a process termed centrosome maturation[148–151]. The mitotic protein kinases PLK1 and Aurora kinase A play a particularly important part in the maturation process, and several potential substrates of these kinases have been identified[140,141,152–156]. In cultured vertebrate cells, for example, PLK1 phosphorylates pericentrin to drive the centrosomal recruitment of several PCM proteins[152], and it also phosphorylates the Ser/Thr-protein kinase NEK9, which then phosphorylates NEDD1 (also known as GCP-WD) to promote the mitotic recruitment of γ-tubulin to centrosomes[157]; NEDD1 can be phosphorylated on multiple sites, and its phosphorylation is modulated by its association with the key PCM protein CEP192 (REF. 158). In budding yeast, the pericentrin-related protein spindle pole component 110 (Spc110) helps to promote microtubule nucleation by γ-tubulin complexes[159,160], and this activity is regulated by phosphorylation[161,162]. Thus, centrosome maturation allows centrosomes to organize many more microtubules during mitosis, thereby increasing the efficiency of mitotic spindle assembly and function.

The assembly of an expanded mitotic PCM presents a considerable challenge to cells, as this structure is too large to be organized by the single layer of pericentrin or Plp molecules that organize the interphase PCM in at least some cells (FIG. 4a). To overcome this challenge, it has long been thought that mitotic centrioles must assemble an underlying PCM 'scaffold' that extends around the centrioles and that is ultimately responsible for recruiting the other mitotic PCM proteins. This idea was supported by observations of mitotic centrosomes purified from the surf clam *Spisula solidissima*. These centrosomes can be extracted with high salt concentrations, which removes the majority of PCM proteins, including the γ-tubulin ring complexes (γ-TuRCs) that nucleate the centrosomal microtubules[163]. Electron microscopy studies of these salt-extracted centrosomes indicate that the centrioles are intact and are surrounded by an extensive 'centromatrix' composed of fibres 12–15 nm thick. If incubated with fresh mitotic cytoplasmic extract, the salt-extracted centrosomes recover their γ-TuRCs and their ability to nucleate microtubules, strongly suggesting that the centromatrix can recruit PCM components from the cytoplasm to form functional mitotic centrosomes. Studies in flies also revealed the presence of a similar salt-resistant centrosomal scaffold that can recruit PCM components from the cytoplasm[164,165].

Recent studies suggest that two key centrosomal proteins, Cnn in flies and SPD-5 in worms, may be crucial components of such a mitotic PCM scaffold, and that in both systems, efficient scaffold assembly is facilitated by the conserved centriole and PCM protein SPD2 and by the direct phosphorylation of Cnn or SPD-5 by Polo or PLK-1 (REFS 153,166,167). In flies, Cnn has a crucial role in mitotic PCM assembly[168,169], and a small central region — termed the phospho-regulated multimerization (PReM) domain — contains a Leu zipper and ten potential phosphorylation sites, at least some of which can be phosphorylated by recombinant Plk1 *in vitro*[153]. The PReM domain forms a dimer through its Leu zipper, but phospho-mimicking mutations allow the PReM domain to form higher-order oligomers *in vitro*. Mutant forms of Cnn that cannot be phosphorylated in their PReM domain are recruited to centrioles *in vivo* but cannot efficiently assemble a mitotic PCM scaffold; conversely, phospho-mimicking PReM-domain mutations allow Cnn to spontaneously form scaffolds in the cytoplasm that can organize microtubules in the absence of centrosomes. These findings provide strong evidence that phosphorylated Cnn can form a bona fide PCM scaffold[153]. Spd2 normally helps to recruit Cnn into the PCM, and Cnn in turn helps to maintain Spd2 within the PCM, thus potentially forming a positive-feedback loop that helps to drive the dramatic expansion of the scaffold during centrosome maturation[166] (FIG. 4b–d).

In worms, SPD-5 and SPD-2 have crucial roles in mitotic PCM assembly[95,170], and it has recently been shown that recombinant SPD-5 has the ability to self-assemble into a network that can recruit purified SPD-2 and PLK-1 *in vitro*[167]. Network formation is enhanced in the presence of recombinant SPD-2, and also when SPD-5 is phosphorylated by PLK-1 at several sites in the middle of the protein. Mutating these potential phosphorylation sites in SPD-5 to non-phosphorylatable residues abolishes the ability of PLK-1 to promote SPD-5 network assembly *in vitro* and of SPD-5 to promote centrosome maturation *in vivo*. These studies with purified proteins represent the first exciting steps towards reconstituting mitotic PCM assembly *in vitro*.

Taken together, these studies suggest that in flies Polo, Spd-2 and Cnn, and in worms PLK-1, SPD-2 and SPD-5, form a conserved module that is required for the assembly of the mitotic PCM scaffold (FIGS 3e, 4). Although there is no obvious sequence similarity between Cnn and SPD-5, they are both large proteins that contain multiple predicted coiled-coil regions, and their function and interaction partners suggest that they are likely to be functional homologues (FIG. 3e). It is also clear, however, that other PCM proteins contribute to mitotic PCM assembly in flies and in worms. Fly Plp, for example, which forms the PCM-supporting fibrils that are anchored to the centriole wall in interphase, is also found distributed throughout the expanded PCM matrix in mitosis, and fly cells lacking Plp have defects in mitotic PCM assembly (although these are less severe than those caused by the loss of Cnn or Spd2)[143,171–173]. Perturbing the function of γ-tubulin complexes also perturbs PCM assembly in both flies and worms[174,175]. Thus, it seems likely that proteins such as Plp, and protein complexes such as γ-TuRC, form direct and indirect links with the underlying scaffold, with each other and with other PCM proteins, all of which strengthen the mitotic PCM and enhance its assembly (FIG. 4d).

The centriole assembly protein Sas4 (FIG. 3) has also been implicated in mitotic PCM assembly in flies[176,177]. Biochemical experiments suggest that Sas4 forms cytoplasmic complexes with important PCM components such as Asl, Cnn and Plp, and shuttles these proteins into the PCM. The importance of Sas4 for mitotic PCM assembly was demonstrated by the finding that partially assembled centrioles in fly spermatocytes lacking cytoplasmic Sas4 do not organize PCM[176]. This is controversial, however, as others have found that centrioles can still organize mitotic PCM in the absence of cytoplasmic Sas4 in flies[37,84] and after RNAi depletion of SAS-4 in worms[178]; moreover, SAS-4 does not seem to form similar cytoplasmic complexes in worm embryos[179]. It may be that Sas4 has a more indirect role in PCM recruitment: in fly embryos, for example, it helps to recruit Asl to the mother centriole[118], and Asl then plays an important part in recruiting Spd2 and Cnn into the mitotic PCM[166,180] (FIG. 3).

Despite the recent progress in understanding mitotic PCM assembly in flies and in worms, it remains unclear whether an underlying mitotic PCM scaffold exists in vertebrate cells and, if it does, whether it is formed by CDK5RAP2 and CEP192, which are the homologues of Cnn and SPD-2 (REFS 81,181), respectively (FIG. 3e). Both of these vertebrate proteins clearly have important roles in mitotic PCM recruitment[139,181–185], and it is intriguing that genes encoding SPD-2 or Cnn, or SPD-5 homologues, cannot be identified in the *S. mediterranea* genome, as these flatworms can form centrioles but not centrosomes[3]. A substantial body of evidence indicates that pericentrin also plays a particularly important part in mitotic PCM recruitment, perhaps in part through its interaction with CDK5RAP2 (REFS 81,181). The large size and predicted secondary structure of these three families of proteins (with many regions predicted to form coiled-coils) makes them strong candidates for scaffold proteins. CDK5RAP2 and pericentrin recruit γ-TuRCs into the PCM[186,187], whereas CEP192 can do this through its interaction with the adaptor protein NEDD1 (REF. 158). There is also evidence that all three protein families can interact with PLK1 and/or Aurora A kinases, and that CEP192 is an important activator of centrosomal Aurora A[140,152,183,188] and helps to coordinate the reciprocal activation of Aurora A and PLK1 (REF. 189). Thus, it seems plausible that at least one, and perhaps all three, of these proteins help to assemble an underlying mitotic PCM scaffold in vertebrate centrosomes. Interestingly, vertebrate pericentrin and CDK5RAP2 also have important roles in centriole engagement and in centrosome cohesion (which is the process that usually keeps the two centrosomes close together before mitotic entry), although it is unclear whether these functions are separate from their role in mitotic PCM assembly[190–193].

Inside-out mitotic PCM assembly in flies. Fluorescent recovery after photobleaching (FRAP) experiments have shown that the mitotic PCM is highly dynamic and that the PCM fraction of several centrosomal proteins is in constant exchange with a cytoplasmic pool of protein[194,195]. In early fly embryos, the PCM proteins γ-tubulin, Plp, Grip71 (also known as Dgp71WD), Polo and Aurora A are all incorporated throughout the PCM volume, consistent with the idea that these proteins are constantly binding to and being released from an underlying PCM scaffold[166,196]. Cnn and Spd2, however, behave differently. Newly incorporated Spd2 molecules are not incorporated throughout the volume of the PCM but are only added into the PCM around the wall of the mother centriole, in a manner that depends, at least in part, on the centriole outer-wall component Asl[166]. Once released from these initial binding sites, the Spd2 molecules gradually flux outwards, forming spoke-like projections that radiate out from the mother centriole (FIG. 4d,g); this outward flux is microtubule-independent[197]. Cnn molecules are also not incorporated throughout the volume of the PCM and initially only incorporate in the region occupied by Spd2. In embryos, the Cnn scaffold then spreads even further away from the centrioles, fluxing outward along the centrosomal microtubules[153,166] (FIG. 4e,g); this outward flux is microtubule-dependent[166,197].

The finding that key mitotic PCM scaffold proteins in flies constantly flux outwards was unexpected, but it may be important. In most cells, the two mitotic centrosomes need to be approximately the same size in order to form a symmetric bipolar spindle. If the mitotic PCM scaffold grew by continuously adding new subunits to its cytoplasmic surface, any stochastic asymmetry generated during the assembly process would be amplified, as the centrosome with the larger surface area would out-compete the smaller centrosome for new scaffold subunits, resulting in the two centrosomes being of very different sizes[198]. This problem is neatly solved if new scaffold subunits are only incorporated around the surface of the mother centrioles, which remain of equal size throughout the assembly process. Interestingly, the microtubule-independent centrosomal flux of Spd2 occurs in embryos and in somatic cells, whereas the microtubule-dependent centrosomal flux of Cnn only occurs in embryos[197] (FIG. 4e,f). Moreover, in worm embryos, SPD-5, which is the probable functional homologue of Cnn, does not detectably flux outwards[199]. Clearly, it will be important to determine whether key mitotic centrosomal scaffold proteins undergo centrosomal flux in other systems.

Conclusions and perspective

Our understanding of centrosome function and assembly has rapidly increased over the past decade. The core proteins that are involved in centriole assembly have been identified, and we are beginning to link their atomic structures to their functions; it seems likely that we will soon understand the basic mechanics of centriole assembly in atomic detail. We are also beginning to understand how the PCM assembles around the centrioles to form centrosomes in interphase and in mitosis, and the ultimate goal of reconstituting these processes *in vitro* from purified components no longer seems implausible. There remains much to learn about how the assembly of centrioles and centrosomes is regulated in space and time, and whether the key molecular interactions that drive these processes are conserved across different cell types and species. Understanding the basic molecular assembly mechanisms is likely to provide important insights into both the basic biology of eukaryotic cells and the many human disorders that have been linked to defects in the assembly and functions of centrioles, centrosomes and cilia.

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Competing interests statement
The authors declare no competing interests.