Centrosome biogenesis and function: centrosomics brings new understanding

Mónica Bettencourt-Dias* and David M. Glover‡

Abstract | Centrosomes, which were first described in the late 19th century, are found in most animal cells and undergo duplication once every cell cycle so that their number remains stable, like the genetic material of a cell. However, their function and regulation have remained elusive and controversial. Only recently has some understanding of these fundamental aspects of centrosome function and biogenesis been gained through the concerted application of genomics and proteomics, which we term 'centrosomics'. The identification of new molecules has highlighted the evolutionary conservation of centrosome function and provided a conceptual framework for understanding centrosome behaviour and how it can go awry in human disease.

Centrosome
The primary microtubule-organizing centre (MTOC) in animal cells. It is comprised of two centrioles surrounded by an electron-dense matrix, the pericentriolar material (PCM).

Microtubule
A hollow tube, 25 nm in diameter, formed by the lateral association of 13 protofilaments. Each protofilament is a polymer of α- and β-tubulin subunits.

**Instituto Gulbenkian de Ciência, Cell Cycle Regulation Laboratory, Rua da Quinta Grande, 6, P-2780-156 Oeiras, Portugal.
‡Department of Genetics, Cancer Research UK Cell Cycle Genetics Group, University of Cambridge, Downing Street, Cambridge CB2 3EH, UK.
e-mails: mdias@igc.gulbenkian.pt; dmg25@hermes.cam.ac.uk*

The centrosome is the primary microtubule-organizing centre (MTOC) in animal cells, and so it regulates cell motility, adhesion and polarity in interphase, and facilitates the organization of the spindle poles during mitosis. Abnormalities in the spindle-pole-organization function occur in many cancers and can be associated with genomic instability[1] because extra and often irregular centrosomes can give rise to aberrant cell division[2]. In addition, the core centrosomal components, the centrioles, have another distinct function as basal bodies that seed the growth of cilia and flagella, which have crucial roles in physiology, development and disease (reviewed in REFS 1,3,4). Therefore, strict control of centrosome and centriole number is imperative in the healthy organism.

The importance of the centrosome was realized at the end of the 19th century by Boveri, who asked many of the key questions that still intrigue us about the regulation of centrosome number and its role in cancer. It has now been known for more than 30 years that centrosomes duplicate during S phase[5–10]. However, whereas the molecular mechanisms that restrict DNA replication to a single round (known as DNA licensing) are well understood (reviewed in REFS 11,12), little is known about the mechanisms of centriole duplication or of their control. Moreover, if destroyed or eliminated artificially or naturally, as occurs during some stages of development, centrioles can re-form de novo[13–15].

Here we discuss recent advances in our understanding of centrosome function and we dedicate the majority of our review to centrosome biogenesis. This is a rapidly expanding area of research and has broad implications for the understanding of human disease. The recent availability of complete genome sequences of several organisms, together with advances in proteomics and functional genomics, is enabling the components of centrioles and the putative regulatory molecules of their duplication cycle to be identified[16–31] (BOX 1). This has revealed a strong evolutionary conservation of the molecules that are involved in centriole biogenesis. Among these there seem to be common elements with the DNA-licensing system, which leads to a conceptual framework for understanding the mechanisms that regulate centriole number.

Centrosome structure

The cellular context is highly unfavourable for spontaneous microtubule (MT) nucleation. The centrosome is the primary MTOC in animal cells and regulates the nucleation and spatial organization of MTs. These MTs provide cytoskeletal support, and tethering of their minus ends to the centrosome directly influences cell polarity. The interphase centrosome is usually positioned near the nucleus, which allows several MT-associated organelles to occupy specific positions in the cell.

The centrosome is comprised of two centrioles that are surrounded by an electron-dense matrix, the pericentriolar material (PCM). The canonical centriole has 9 MT triplets and is ~0.5 μm in length and 0.2 μm in diameter[32,33] (FIG. 1). However, there are variations on this structure, the molecular basis for which has become clearer through molecular phylogeny (BOX 2). Centrioles are polarized along the proximo–distal axis (FIG. 1a,b). The mother centriole has subdistal and distal appendages, which dock cytoplasmic MTs and might anchor centrioles to the cell membrane where they serve as basal bodies (FIG. 1c).

Centriole
The canonical centriole is a cylinder that is comprised of nine microtubule triplets, is ~0.5 μm in length and has appendages at the distal ends upon maturation. There are variations of this structure, in which triplets are substituted by singlets or doublets and there are no appendages.

Basal body
A structure found at the base of eukaryotic cilia and flagella that organizes the assembly of the axoneme. Centrioles can give rise to basal bodies and *vice versa*. The structure of the basal body is the same as the one of the centriole; additionally, basal bodies have a transition zone at their distal end, which is contiguous with the axoneme.

Cilia
Microtubule-based membrane-surrounded cellular projections that extend up to 10 μm outwards from the cell. The majority of the cells in vertebrates have cilia. Cilia can serve as sensory organelles or, in the case of motile cilia, can move fluids around the cell. Motility is thought to depend on the structure of the axoneme, with most motile cilia displaying a 9C2 axoneme structure.

Flagella
Axoneme (9C2)-based cellular projections that help propel cells.

DNA licensing
A regulatory mechanism that divides the cell cycle into two phases: a licensed phase, during which initial steps for DNA replication are taken, but progression cannot take place; and an unlicensed state, during which the initial steps cannot be taken and progression takes place. This mechanism ensures that DNA replication occurs only once per cell cycle.

Pericentriolar material
Fibrillar material that surrounds centrioles in the centrosome that nucleates the growth of new microtubules.

Centriolar characteristics determine most properties, such as stability, capacity to reproduce, dynamicity and polarity, of the centrosome. Single centrioles[34], and even small centrioles[25,35], can recruit the PCM, but centriole loss leads in general to PCM dispersal[29,36,37]. The ability of centrioles to duplicate is therefore central to the reproductive capacity of the centrosome[38]. Centrioles are extremely stable structures, and their MTs are cold and detergent resistant. Therefore, when labelled tubulin is injected into cells, only the daughter centriole incorporates the label over a period of one cell cycle[8]. This stability might be provided by post-translational modifications, such as polyglutamylation, of centriolar tubulin[37,39]. Other structural components of the centriole, such as tektins and ribbon proteins[40,41], might also contribute to the stability. As centrioles persist through generations of cells, they could be a stable internal landmark of the centrosome for morphogenesis and polarity[42].

Centrosome function

As mentioned above, the centrosome has several different functions during interphase and mitosis.

The centrosome as a microtubule-organizing centre. The capacity of the centrosome to organize MT arrays depends on its ability to nucleate, anchor and release MTs. Here we will discuss some of the principal molecular players, including components of the PCM and certain protein kinases[43], that are involved in MT nucleation during mitosis.

The PCM is a key structure that anchors and nucleates cytoplasmic MTs during interphase and mitosis. It is a molecular coat that gets thicker as the cell prepares for division (a process known as centrosome maturation). Principal among PCM components are members of the pericentrin and AKAP450 family of proteins, coiled-coil molecules that are thought to form a lattice-like structure (reviewed in REF. 33) that docks regulatory components and molecules that mediate the nucleation of MTs, such as γ-tubulin[44]. γ-tubulin exists in the γ-tubulin small complex (γTuSC), a core tetrameric complex that comprises two γ-tubulin molecules and one molecule each of DGRP84 and DGRP91 in *Drosophila melanogaster* (GCP2 and GCP3 in mammals). A ring of these subcomplexes is held together by four or so other proteins and is known as the γ-tubulin ring complex (γTuRC). Although the depletion of any one of the γTuRC-specific molecules in *D. melanogaster* cells leads to abnormal mitotic spindles, γ-tubulin is still recruited to the centrosome. By contrast, depletion of the subunits of γTuSC blocks MT nucleation[45]. In fission yeast, γTuSC is also essential for MT nucleation, whereas γTuRC seems to only be important for regulating MT dynamics during interphase (reviewed in REF. 46).

Anchorage of MTs occurs on subdistal appendages, but probably also at the PCM, and depends on molecules including ninein, centriolin, dynactin and EB1 (reviewed in REFS 3,33). Ninein is a component of the subdistal appendages of the mother centriole. The C terminus of ninein connects the centriole, whereas its N terminus interacts with the γTuRC[47]. In the absence of ninein, the γTuRC might be insufficient to anchor MTs at the centrosome. However, this regulation seems to be most important in interphase cells. MT-severing proteins, such as katanin, release MTs into the cytoplasm, playing a role in remodelling MTs in the interphase–mitosis transition and in the differentiation of many cell types, such as epithelia[48,49].

The changes in the MT-nucleating ability of the centrosome during the cell cycle seem to be directed by a balance of factors that either restrict or promote
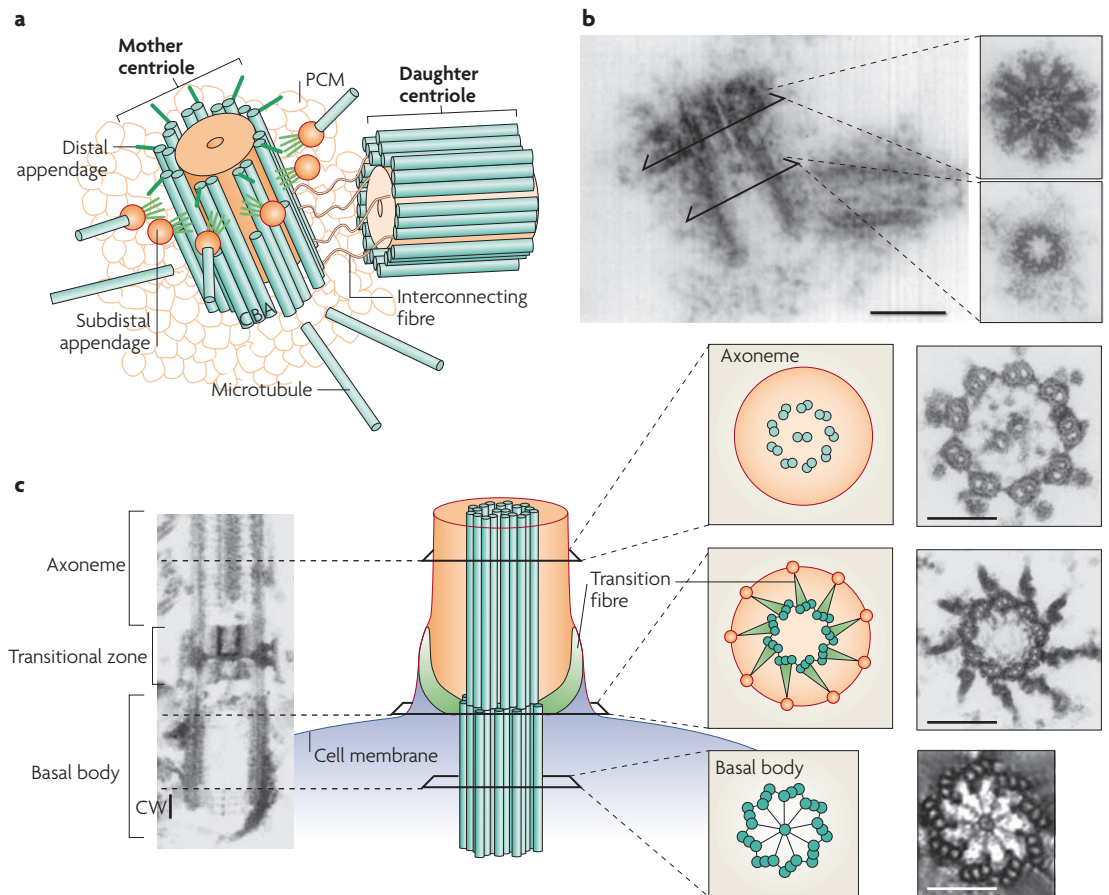
Figure 1 | **Centriole and basal body structure. a** | Schematic view of the centrosome. In each triplet, the most internal tubule is called the A-tubule; the one following it is the B-tubule; and this is followed by the most external one, the C-tubule. At its distal end, the centriole constitutes of doublets. Adapted with permission from REF. 63 © (2005) Macmillan Magazines Ltd. **b** | Electron micrograph of the centrosome. The top inset indicates a cross-section of subdistal appendages; the bottom inset indicates a cross-section of the proximal part of the centriole. Note the triplet microtubules (MTs) . Scale bar: 0.2 μm. Adapted with permission from REF. 32 © (1992) Elsevier. **c** | Electron micrographs and schematic view of the flagella of green algae. There are different types of cilia and flagella, depending on the structure of the axoneme. The axoneme is a cylindrical array of nine doublet MTs that surround either zero MTs (called structure 9C0) or the two singlet MTs (structure 9C2), represented here. The two singlet MTs are called the central pair. Differences in the structure of axonemes might be reflected in their properties: for example, whether they are motile or not (reviewed in REF. 137). The transition fibres extend from the distal end of the basal body to the cell membrane. It has been suggested that they can be part of a pore complex that controls the entry of molecules into the cilia. Scale bar: 0.25 μm. CW, cartwheel (one of the first structures to appear in a forming centriole). Adapted with permission from REF. 138 © (2002) Macmillan Magazines Ltd, and with permission from REF. 139 © (2004) Company of Biologists.

Nucleus-associated body
The microtubule-organizing centre of *D. discoideum* is a nucleus-associated body that consists of a multilayered, box-shaped core embedded in an amorphous corona from which the microtubules emerge.

Coiled-coil
A region of low complexity formed by a number of α-helices wound around each other, which is common among structural and motor proteins. Coiled-coil domains are also involved in protein interactions.

the recruitment of MT-organizing molecules during interphase and mitosis. It has long been clear that protein phosphorylation has an important role in this: among other protein kinases, Polo-like kinase-1 and Aurora A promote MT nucleation, and this is opposed by protein phosphatase-1 (PP1), PP4 and other protein phosphatases (reviewed in REFS 43,50). A model for the coordinate functions of Polo and Aurora A in centrosome maturation has been proposed in which Polo promotes the recruitment of the MT-organizing γTuRC[51]. Polo also activates the abnormal spindle protein (Asp), a conserved PCM-associated protein that itself has MT-organizing properties. Aurora A also promotes MT growth from centrosomes, and this seems to be achieved through the phosphorylation of a conserved centrosome protein, TACC, and its recruitment to the centrosome in *D. melanogaster*[52–55]. TACC in complex with the MT-associated protein minispindles (MSPS; XMAP215 in *Xenopus laevis*) modulates astral MT behaviour[52–55].

A surprising recent finding has been that ubiquitylation might also play a role in maturation. The ubiquitin-ligase activity of the tumour suppressor protein BRCA1 has been found to modify *in vitro* γ-tubulin on residues Lys48 and Lys344, which leads to the inhibition of MT-nucleating activity by the centrosome[56]. Two other proteins, spindle-defective protein-2 (SPD-2) and SPD-5, are essential for centrosome maturation in *Caenorhabditis elegans*[27,57,58]; however, their homologues have not yet been studied in other organisms.

Molecular phylogeny implies that the centriole is an ancient structure that first appeared in association with a flagellum in early eukaryotes[134]. Centrioles evolved differently in different groups of species and were lost from several eukaryotic phyla over the course of evolution[134]. For example, yeasts do not assemble cilia or flagella, and have developed a structure that is known as the spindle pole body (SPB). The SPB does not have centrioles but, like the animal centrosome, it has a role at the spindle poles during cell division. Despite their differences in structure, some of the core proteins of the centrosome and the SPB are the same (reviewed in REF. 77). The common origin of these structures is highlighted by the similarities between the human and *Chlamydomonas reinhardtii* centriole. It has been argued that for cell types that do not need to form cilia or flagella the selective pressure to maintain a centriole is smaller[42], hence yeast developed distinct SPBs. In addition, species that only have few cell types with cilia at a particular stage of their life cycle display centrioles with a simpler structure at all other stages. This is the case for both *Drosophila melanogaster*[135] and *Caenorhabditis elegans*[57], in which the embryos have centrioles with nine doublet or nine singlet microtubules (MTs), respectively. On the other hand, cells in several of the adult tissues of *D. melanogaster*, such as the male germ line, have triplet centriolar MTs (reviewed in REF. 136). Despite the structural differences between cilia and flagella from different species, new proteomics studies have highlighted that they share a similar molecular composition. Homologues of human ciliary-disease genes, such as *OFD1*, *qilin* and *reptin*, have indeed been found in *C. reinhardtii*[17–20].

Axoneme
The microtubule-based structure of cilia and flagella that gives them rigidity and the ability to move. It is a cylindrical structure comprised of nine pairs of doublet microtubules, arranged around a central pair of single microtubules (9C2). The central microtubules can be absent in non-motile cilia (9C0).

Spindle pole body
The microtubule-organizing centre of yeast and diatoms. It is a plaque-like structure that is embedded in the nuclear membrane. It nucleates microtubules both on the cytoplasmic and nuclear side.

Centriole disengagement
(also known as centriole disorientation). Both centrioles in the centrosome lose their orthogonal orientation towards each other at the end of mitosis and beginning of G1 phase. This event precedes new centriole formation.

Organization of cilia and flagella. Cilia and flagella are projections from cells that can either enable movement of the cell itself, or facilitate the movement or sensing of substances around cells. There is growing evidence for their indispensable role in various cellular and developmental processes: motility, propagation of morphogenetic signals in embryogenesis and sensory perception (reviewed in REFS 1,3).

Little is known about the early stages of cilia formation, such as the centriole to basal body conversion, when the centriole acquires a transitional zone at the distal end, which is contiguous with the axoneme[59] (FIG. 1). Conversely, basal bodies of interphase cilia or flagella can also be transformed into centrioles, and so centrosomes, in ciliated cells of vertebrates[60] and in *Chlamydomonas reinhardtii*[59].

In vertebrate cells, only the mature mother centriole can nucleate primary cilia[10]. The appendages that are present on the mother centriole seem important; deletion of ODF2 (an appendage marker) in mouse cells resulted in centrioles that lacked appendages and that could not form cilia[61]. The characterization of molecular components of basal bodies, flagella, cilia and, in particular, the mother centriole is likely to provide us with a better understanding of the biogenesis of these structures (BOX 1).

Centrioles in cell-cycle regulation. It has been suggested that centrosomes and spindle pole bodies (SPBs), which have a structure that is analogous to the centrosome in yeasts, are signalling platforms because many regulatory complexes, including tumour suppressors and checkpoint proteins, localize in them[62,63]. The centrosome might have a role in cell-cycle regulation (reviewed in REFS 63,64) as they have been proposed to regulate cytokinesis and the G1–S transition. In certain human cell lines, the mother centriole moves towards the midbody at the end of telophase, and this movement coincides with abscission, the event that separates the two cells[65,66].

When the centrosome was removed, either by laser removal or by microsurgery, a significant proportion of the cells could form a furrow but could not complete cytokinesis[66–68]. This might be a conserved property given that the SPB sequesters regulatory molecules of the mitotic-exit network (MEN; budding yeast) and of the septation-initiation network (SIN; fission yeast) to control mitotic exit and the onset of cytokinesis, respectively (reviewed in REFS 69,70). Ablation of both SPBs in fission yeast led to problems in cytokinesis[71].

Both transformed and non-transformed human cells can progress through the G1 phase in the absence of centrioles after centrosomes are ablated by microsurgery or laser treatment[72]. This study demonstrates that the centrosomes themselves are not necessary for the G1–S transition, as already shown before in *D. melanogaster*[29,36]. However, the silencing of several centrosome-associated proteins, as well as centriole ablation followed by stronger exposure to light, led to G1 arrest (reviewed in REFS 63,64). These studies suggest that centrosome loss is a stress signal that can function additively with other stresses to arrest cells in the G1 phase. This arrest is dependent on p38 and p53 (REFS 13,73). Further studies are needed to understand the reported stress response.

Centrioles and centrosomes: why are they important? Despite the presence of centrosomes at spindle poles in most animal cells, they are generally absent during female meiosis. Moreover, there are no centrosomes in higher plant cells. The long-standing suggestion that the centrosome is not essential to form a spindle has been reinforced by the demonstration that spindles can be nucleated by chromosomes, both *in vivo* and *in vitro*, and that the minus ends of MTs can be focused, presumably through the concerted action of minus-end-directed motors and MT-associated proteins (reviewed in REF. 74). The existence of organisms that bear mutations in genes that are necessary for centriole biogenesis enabled us to obtain acentriolar cells and to query the role of centrioles in cell physiology (Supplementary information S1 (table)). These studies highlight a diversity of cell-cycle requirements for centrioles, both among various species and among different tissues of the same organism. In certain species, centrioles seem to be important in assuring mitotic fidelity[24,29,75], and they might also contribute to spindle orientation. Common to all studied species, centrioles are essential in their duality as basal bodies that form cilia and flagella (Supplementary information S1 (table)). As such, responses to centrosome insults, such as the G1–S arrest described above, could have evolved to prevent cells from becoming aneuploid or from developing cellular defects that lead to ciliary disease. It will be important in the future to study this response in the context of animal models, in which the centrosome number can be manipulated.

The centrosome cycle

The main phases of the centrosome cycle have been defined through electron microscopy[5,6,9,10,76]. Four consecutive steps have been described[5]: centriole disengagement, nucleation of the daughter centrioles

a Centriole disengagement

Duplication and elongation

Maturation and separation

Mitotic exit–early G1 phase   S phase   G2 phase   Mitosis

b

SAS-5
SAS-6   SAS-4

ZYG-1

CDK-2/SPD-2
Oocyte fertilization
(meiosis)

Pronuclear
appearance
(S phase)

Pronuclear
migration
(prophase)
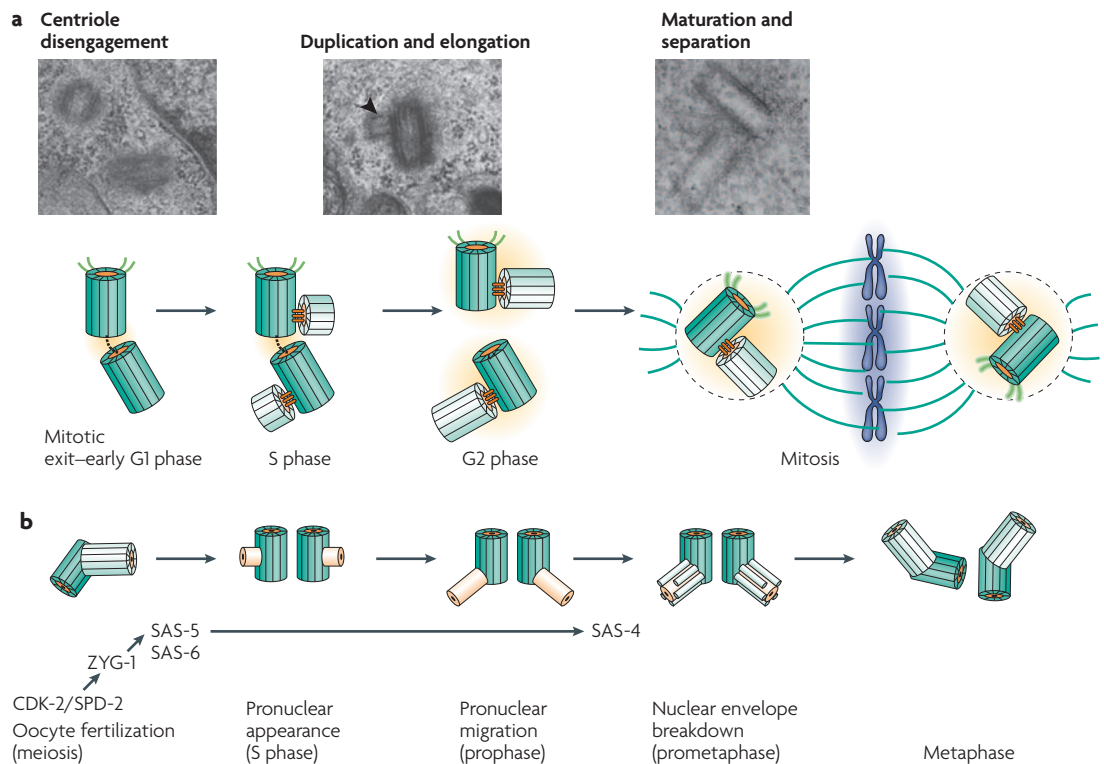
Nuclear envelope
breakdown
(prometaphase)

Metaphase

Figure 2 | **The centriole duplication cycle. a** | Electron microscopy micrographs from HeLa cells showing distinct steps in centriole duplication (also shown diagrammatically). The mother centriole is represented in dark green showing appendages. Daughter centrioles are shown in light green. At mitotic exit–early G1 phase, centrioles in a centrosome loose their orthogonal configuration[5,9,65]. There might be an intercentriole link at this stage[32,65]. Next, duplication starts in late G1–S phase with the nucleation of daughter centrioles (see electron micrograph; the arrowhead shows a procentriole). Note that the axis of the daughter intercepts the parent[76]. The procentrioles elongate fully by late G2 phase or by the beginning of G1 phase of the next cell cycle. Last, maturation and separation of the two centrosomes occur at the G2–M transition by the acquisition of maturation markers[140], the recruitment of pericentriolar material (PCM; yellow) and an increase in microtubule-organizing centre (MTOC) activity[76]. Micrographs reproduced with permission from REF. 6 © (1968) Rockefeller University Press. **b** | The centrioles in *Caenorhabditis elegans* are smaller and simpler than the human ones, showing a central tubule surrounded by 9 singlet microtubules (MTs)[80]. Despite differences in the structure, the centriole cycle seems to be regulated in a similar way. Nucleation of daughter centrioles (yellow) also happens in S phase. These structures are mainly composed of short central tubules that elongate during G2 phase and mitosis. In addition, the centrioles acquire the external tubules (light green). Cyclin-dependent kinase-2 (CDK-2) has been shown to be important for recruiting spindle-defective protein-2 (SPD-2) to the mother centriole. SPD-2 is necessary for the recruitment of ZYG-1. ZYG-1 is important for the recruitment of the complex that is formed by SAS-5 and SAS-6, which is necessary for formation of the inner centriole tube. At a later step, the formation of this tube is essential for the binding of SAS-4, with consequent production of the surrounding MTs. Adapted with permission from REF. 80 © (2006) Macmillan Magazines Ltd.

Half-bridge
When a spindle pole body (SPB) is formed, it has a lateral structure, known as half-bridge. The half-bridge, like the SPB, is embedded in the nuclear envelope. This structure is the 'seed' for SPB duplication because the first step in duplication involves its elongation. Elongation leads to the deposition of satellite material that will expand into a duplication plaque, leading to the formation of a new SPB.

Cartwheel structure
A basal body precursor and one of the first structures to appear during basal body formation. It consists of a central hub and nine spokes, on top of which microtubules are added.

Kinetochore
A protein structure that assembles on the centromere during cell division and that links the chromosome to microtubules from the spindle.

(also called procentrioles before they acquire full centriolar length), elongation of the procentrioles and separation of the centrosomes (FIG. 2a).

Incorporation of labelled tubulin into only the daughter centriole shows that centriole formation is conservative[8]. So, is there a seed on the side of the mother centriole that nucleates the formation of the daughter analogously to the half-bridge that is seen when yeast SPBs duplicate in budding yeast (reviewed in REF. 77)? Ultrastructural observations of basal body duplication in *Paramecium aurelia* suggest that an amorphous ring-like structure appears by the side of the mother and a basal body precursor, a cartwheel structure that consists of a central hub and nine spokes, is formed[78]. Whether a similar seed exists in other species is not clear. It is also not clear how centriole elongation proceeds.

Two different models have been put forward regarding the elongation of basal bodies[59]. The first suggests that the most internal A-tubule (FIG. 1) is the first to be added on top of the cartwheel structure, with the B- and C-tubules being added to the pre-existing A-tubules, followed by elongation to the mature length[78,79]. The fact that two proteins in *C. reinhardtii* are found both at probasal bodies and at the distal ends has led to the alternative suggestion that the elongation of centrioles can proceed with a capping structure, analogously to the growth of kinetochore MTs. In this model, proteins that are destined for fibres at the distal end might travel at the tips of elongating MTs[59]. Tomography in *C. elegans* has shown that daughter-centriole assembly begins with the formation and elongation of a central tube followed by the peripheral assembly of the nine singlet MTs.

In some respects, this fits the first model best. However, singlet MT assembly did not seem to occur preferentially at proximal extremities, as would be expected from that model (REF. 80; FIG. 2b). The localization of recently identified centriolar proteins on these structures, together with new mutants in centriole assembly in different species, should provide a route towards understanding intermediate steps in centriole formation.

A conserved centriole-assembly protein module. Centriole duplication has been studied both during the normal cell cycle and in the context of cells that are arrested in S phase and reduplicate their centrosomes[81]. Despite the difficulty in identifying orthologues of many centriolar molecules and the diversity in centriolar structures (BOX 2), a unified picture of centriole-duplication mechanisms is emerging.

The ease of screening gene function by RNA interference (RNAi) in *C. elegans* has proven to be powerful for the identification of molecules that are involved in centriole duplication. Failure to duplicate centrioles in the early *C. elegans* embryo leads to the formation of monopolar spindles and consequent embryonic arrest. Additionally, the recruitment of proteins to the mother centriole and the nascent daughter centriole can be followed in different mutant contexts when eggs that express a fluorescently tagged protein of interest are fertilized with wild-type sperm that provide two non-labelled centrioles. This distinguishes *de novo* centriolar recruitment from the prior presence of the protein in question at centrioles[80,82]. Last, approaches that couple the live imaging of molecules to the immediate fixation of embryos for electron microscopy have proven to be particularly powerful[80].

These studies have identified a group of proteins, which includes ZYG-1, SPD-2, SAS-5 in complex with SAS-6, and SAS-4, as part of a conserved centriole-assembly protein module[23]. SPD-2 is also involved in centrosome maturation, but all of the other molecules seem to be exclusively required for centriole duplication. Following the entry of sperm, SPD-2 recruitment to the centrioles seems to be mediated by cyclin-dependent kinase-2 (CDK-2)[83]. SPD-2 is necessary for the recruitment of the kinase ZYG-1 prior to the splitting of the two mother centrioles that are provided by the sperm. ZYG-1 recruits a complex of SAS-6 and SAS-5, two coiled-coil molecules that are necessary for the formation and elongation of the central tube (FIG. 2b). The assembly of the singlet MTs onto the central tube is dependent on SAS-4, another coiled-coil centriolar protein. Despite the localization of SAS-5 and SAS-6 in the centriolar tube, it is not known whether they are necessary for the recruitment, assembly and/or stabilization of structural components[80]. The localization of SAS-4, and the fact that centrosomes in *sas-4* RNAi embryos recruit γ-tubulin in proportion to their residual levels of SAS-4, suggests that SAS-4 plays an important part in recruiting the PCM[35]. It is not clear whether it also has a structural role.

Recent studies in other organisms, such as *D. melanogaster* and humans, have highlighted the importance of counterparts of these molecules in centriole duplication, with the exception of SAS-5, for which a counterpart has yet to be found. This has also enabled a better understanding of the regulation of the centriole cycle in somatic cells as well as of basal body formation. SAK (also known as PLK4) kinase, a homologue of ZYG-1, and SAS6 are master regulators of centriole duplication. Their absence leads to a lack of centriole duplication, whereas their overexpression leads to an increase in the number of MTOCs[24,29,30]. Existing work suggests that the function of SAK/PLK4 is dependent on SAS6 (REF. 30). SAS4 is also essential in centriole duplication in *D. melanogaster*, where both SAK/PLK4 and SAS4 mutants show a reduced number of basal bodies[29,36].

Other molecules and mechanisms. The relationship between the protein module described above and other molecules proposed to have a role in centriole duplication has not yet been determined, and so it is difficult to place them into a universal mechanism for centriole duplication. Several molecules, such as CDK2 and CAMKII, have been suggested to play a part in centriole disengagement[84]. Degradation of a potential 'glue' that holds mother and daughter centrioles together might involve the SCF complex (reviewed in REF. 84), the anaphase promoting complex/cyclosome (APC/C)–CDC20 (REF. 85) and separase[86], a protease that is active at the metaphase–anaphase transition to trigger the separation of sister chromatids.

The process of site selection for the nucleation of new centriolar MTs is poorly understood but could be important in limiting the number of centrioles in the cell. Downregulation of CDK1 in somatic *D. melanogaster* cells led to the appearance of complexes that consist of one mother and two daughter centrioles, which suggests that centrioles have more than one potential site for the assembly of daughter centrioles[87]. The study of mutants in *C. reinhardtii*, *Saccharomyces cerevisiae* and *P. tetraurelia* suggests that centrin might have an important role in linking mother and daughter centrioles, and perhaps in determining the site of new centriole formation. Centrins are members of a conserved subgroup of the EF-hand superfamily of Ca^{2+}-binding proteins. Centrin mutants also fail to form the half-bridge structure in budding yeast and so fail to duplicate their SPBs[88,89]. Depletion of one of the centrins (Cen2ap) in *P. tetraurelia* led to the mislocalization of the newly formed basal bodies, whereas depletion of the second centrin (Cen3ap) led to a failure of mother and daughter centrioles to separate[90]. Centrin has also been shown to be necessary for centriole formation in human cells[91]. A better understanding of the evolution of centrins and their binding proteins will be important to clarify their role in this process[42]. As the *C. reinhardtii* protein BLD10 localizes to the cartwheel, the base from which the centriole elongates, it has been suggested that BLD10 is essential in the early stages of basal body assembly[75]. The counterpart of BLD10 in other organisms is not known (TABLE 1).

Rare tubulin isoforms are involved in the elongation of centrioles, and in particular in making doublet and triplet MTs (TABLE 1; reviewed in REF. 92). Some of

---

SCF complex
A multisubunit ubiquitin ligase that targets proteins for degradation. It comprises SKP1, a member of the cullin family (CUL1), a RING-finger-containing protein (ROC1/RBX1) and an F-box-containing protein, which specifically recognizes certain substrates. Substrate recognition is enhanced following phosphorylation.

Table 1 | **Proteins involved in centriole duplication**

Molecule	Organism	Assay	Phenotype	Refs
SAK/PLK4/ ZYG-1	Hs, Dm, Ce	RNAi; mutations	No duplication; no reduplication	29,30,80,82, 141,152,153
		Overexpression	Amplification	
SPD-2	Ce	RNAi; mutations	No duplication; no recruitment of PCM	27,58
SAS-6–SAS-5	Hs (only SAS6), Ce	RNAi; mutations	No duplication; no reduplication	24,28
		Overexpression	Amplification	
SAS4	Dm, Ce	RNAi; mutations	No duplication	25,35,36
CDK2	Hs, Mm, Xl, Ce	Inhibition (dominant-negative, chemical); RNAi	Duplication can occur in its absence, no reduplication; defective SPD-2 localization	83,98–100
Centrin/CDC31/ VFL2	Hs, Sp, Sc, Cr, Pt	RNAi; mutations	No duplication (Hs, Sp, Sc); segregation of centrioles affected (Cr); geometry of duplication affected (Pt)	88–91,142
SFI1	Hs, Sc	RNAi; mutations	No duplication	143,144
CP110	Hs	RNAi	No reduplication	145
Nucleophosmin	Hs	RNAi; inhibition of release from centrosome	Amplification; no duplication	110
γ- tubulin	Hs, Dm, Ce, Pt, Tt	RNAi; mutations	No duplication (Ce, Hs, Tt); problems in centriolar structure, elongation and separation (Pt, Dm)	28,59, 146–148
Δ-tubulin	Mm, Cr, Pt	Mutations	Doublets are formed (less cells with C-tubules)	92
ε-tubulin	Xl, Cr, Pt	Mutations; immunodepletion	Shorter centrioles, only singlets, no subsequent duplication; no duplication	92,149
BLD10	Cr	Mutations	No duplication	75
CEP135	Hs	Inhibition, RNAi	Disorganization of microtubules	150
		Overexpression	Accumulation of particles	
CAMKII	Xl	Inhibition	Blocks early steps in duplication	7
SKP1, SKP2, CUL1, Slimb (SCF complex)	Mm, Xl, Sc, Dm	Mutations; inhibition	Blocks separation of M–D pairs; blocks reduplication; increase in centrosome number	7,105,106 126,151
p53	Hs	Mutations	Amplification	112,113
Separase	Xl	Inhibition	Blocks centriole disengagement	86

This table focuses only on the components described in the main text. The term inhibition is used here for different forms of inhibiting the function of a protein: dominant-negative, chemical compounds and antibodies. Reduplication refers to centrosome amplification in the context of cells that were arrested during S phase. Please refer to the references for more details on observed phenotypes. Ce, *Caenorhabditis elegans*; Cr, *Chlamydomonas reinhardtii*; D, daughter centriole; Dm, *Drosophila melanogaster*; Hs, *Homo sapiens*; M, mother centriole; Mm, *Mus musculus*; Pt, *Paramecium tetraurelia*; PCM, pericentriolar material; RNAi, RNA interference; Sc, *Saccharomyces cerevisiae*; Sp, *Schizosaccharomyces pombe*; Tt, *Tetrahymena thermophila*; Xl, *Xenopus laevis*.

these isoforms seem to be absent from the genomes of *D. melanogaster* and *C. elegans*. Interestingly, in the presence of low CDK1 activity, *D. melanogaster* wing imaginal discs had cells in which the daughter centriole was longer than the mother centriole[87]. This indicates that there is no absolute limit to centriole size and that the levels of CDKs are likely to regulate centriole nucleation and elongation.

Specific mechanisms have been proposed to regulate the separation of the two centrosomes at the G2–M transition (reviewed in REF. 7). c-Nap, a molecule that is recruited to the proximal end of both centrioles, binds rootletin, a fibre-forming molecule. This might provide a dynamic link between both mother centrioles[93,94]. The human kinase Nek2 phosphorylates c-Nap1, releasing this link. Downregulation of rootletin and c-Nap1, as well as the overexpression of Nek2, leads to premature centrosome disjunction[93,94]. The activity of Nek2 is counteracted by PP1 during interphase (REF. 95, reviewed in REF. 7). However, the inactivation of PP1 in late G2

phase would allow the phosphorylation of c-Nap1 to increase, causing it to be released from the centrosome and causing the separation of the two centrosomes at mitotic entry.

Coordinating the two cycles

For daughter cells to inherit one centrosome, this structure duplicates once and only once per cell cycle. As this is also the case for chromosomal DNA, the cell-division cycle can be viewed as two cycles in parallel (FIG. 3). The two cycles might be coordinated so that when the chromosome cycle is delayed, the centrosome cycle stops, thereby avoiding the generation of extra centrosomes. Problems in such coordination are commonly seen in many cancers[1,96]. Whether changes in centrosome numbers are a cause or consequence of oncogenic transformation has been a long-standing argument (reviewed in REFS 1,97), but in certain cancers there is growing evidence that centrosomal changes might appear very early in tumorigenesis (reviewed in REF. 97).

Box 3 | Licensing in DNA replication

To ensure that re-replication cannot occur in the same cell-division cycle, replicated DNA has to be marked as different from unreplicated DNA (reviewed in REF. 11). The DNA-licensing mechanism divides the cell cycle into two phases: a licensed state (FIG. 3b1), during which the initial steps for DNA replication are taken but progression cannot occur; and an unlicensed state, during which the initial steps cannot be taken and progression occurs (FIG. 3c1). Replication can only occur once the DNA is licensed, after mitosis. It was postulated that once this happens, proteins known as licensing factors can bind the DNA at the end of mitosis and beginning of G1 phase and 'license' it for the next round of DNA replication (FIG. 3b1).

For the origins of replication to fire and start DNA replication, the DNA needs to be unwound. The loading of the minichromosome maintenance (MCM) helicase proteins is the licensing event that permits the DNA to unwind (FIG. 3b1). At the end of mitosis, the MCM complex is loaded onto chromatin with the aid of the origin-recognition complex (ORC), CDC6 and CDT1 (FIG. 3b1). Because the DNA is licensed, the replication machinery can bind to the DNA during S phase and synthesize new DNA strands. Replication of the DNA helix starts at specific origins in S phase. Cyclin-dependent kinases (CDKs), together with the CDC7 kinase, trigger the initiation of replication, recruiting the DNA-replicating enzymes to the sites of replication and activating the MCM proteins. It then moves along with the replication fork and the origins are converted to an unlicensed state (FIG. 3c1).

Licensing is regulated by inactivation, degradation or relocalization of the licensing factors (FIG. 3c1). The increase in CDK activity, as referred to above, is crucial in this respect. Although the precise mechanism varies from species to species, it generally leads to the degradation of CDC6 and CDT1 mediated by the SCF E3 ubiquitin ligase (FIG. 3c1,d1). In yeast, the MCMs become excluded from the nucleus, whereas in vertebrates, CDT1 is excluded. Geminin mediates the inhibition of CDC6 and CDT1 in metazoans and is degraded by the anaphase-promoting complex/cyclosome (APC/C) during mitosis (FIG. 3a1), allowing licensing in subsequent stages.

In the absence of licensing factors, cells cannot replicate their DNA, and when these factors are ectopically expressed they can induce re-replication of DNA. Hence, the control of expression and activity or stability of licensing factors is crucial to avoid re-replication.

It was proposed that CDK2 couples both cycles as it promotes centriole and DNA replication in S phase in different species[98,99]. However, this enzyme is not essential because both cycles progress in *CDK2*-null cells[100] (possibly because CDK1 takes over its function[101]). Molecules such as SAK/PLK4 and SAS6 could, in principle, drive centriole formation (TABLE 1); however, their activity would have to be coordinated with cell-cycle progression.

Once a new centriole is made, what limits the cell from making more? To address this question, Wong and Stearns[102] carried out similar experiments to the classical study of Rao and Johnson[103] by fusing cells in different cell-cycle stages. They found that centrosome duplication is controlled extrinsically, as S-phase cytoplasm advances the duplication of a G1 centrosome. However, there is a centrosome-intrinsic block to reduplication, as the centrosome of a G2 cell does not duplicate in S-phase cytoplasm. This is not due to an inhibitory effect from the cytoplasm, as when G2 cells were fused to G1 cells the G1 centrosomes duplicated. So, once centrioles have duplicated in S phase, they cannot duplicate again until the next S phase[102].

The mechanism that prevents re-replication of DNA in any cell cycle (reviewed in REFS 11,12) might provide insights into the control of centriole biogenesis. The DNA-licensing mechanism divides the cell cycle into two phases (BOX 3): a licensed state (FIG. 3b1) in which initial steps for DNA replication are taken but progression cannot occur;

and an unlicensed state in which the initial steps cannot be taken and progression occurs. Perturbations that interfere with the DNA-licensing system, such as low CDK activity, the absence of geminin and interference with protein degradation by the SCF ubiquitin-ligase system, lead to reduplication of both DNA and centrosomes (BOX 3; REFS 11,12,87,104–106), which implies a similar mechanism of regulation for DNA and centrosomes.

The licensing event for DNA replication involves the loading of the minichromosome maintenance (MCM) DNA helicases to the origins of replication, presumably allowing the unwinding of the DNA strands. Could there be a similar licensing event in centrosome duplication? If so, this event should also occur at the end of mitosis, when there is low CDK activity. Moreover, the overexpression of licensing factors should be able to drive these processes outside the licensing window[11,107,108]. One prerequisite for the growth of daughter centrioles is centriole disengagement (reviewed in REF. 7). Tsou and Stearns[86] have suggested that separase triggers disengagement, licensing the centrioles for a new round of duplication, perhaps by relieving a pre-existing block to duplication or by exposing the site from which new centrioles can bud (FIG. 3). They found that in the context of *X. laevis* extracts, CDK2 was only needed afterwards, for centriole elongation, therefore uncoupling the events of licensing from centriole growth[86]. However, it remains to be seen whether the overexpression of separase induces ectopic centriole duplication. The generality of this mechanism also needs to be tested.

Interestingly, the overexpression of SAK/PLK4 and SAS6 leads to centrosome amplification, which suggests that these proteins could have a licensing function. How SAS6 is regulated is not yet known. In human cells, *SAK/PLK4* mRNA levels peak at mitosis and are at a minimum in G1 (REF. 109). The level of ZYG-1, the *C. elegans* homologue of SAK/PLK4, is maximal at mitosis in anaphase[82], as is expected for a licensing factor. Exactly how SAK/PLK4 would be regulated to license centrioles only at the end of mitosis is not clear; perhaps separase could unveil a site where SAK/PLK4 binds to form a new centriole (FIG. 3a2). If there are too many of these molecules, they might bind ectopically, generating more centrioles. The activity of SAK/PLK4 would have to be well controlled and low during the S and G2 phases (perhaps through protein degradation) to avoid ectopic centriole formation (FIG. 3c2,d2).

Other molecules might regulate the number of centrioles. The nucleolar protein nucleophosmin shuttles between the nucleus and the centrosome, and is released from the centrosome during mitosis. Overexpression of a non-phosphorylatable form of nucleophosmin, which is bound to the centrosome, inhibits disengagement, whereas depletion of nucleophosmin results in centrosome amplification (reviewed in REF. 110). Understanding the temporal and spatial regulation of the proteins involved in centriole biogenesis will be crucial for achieving a better understanding of their activity. The roles of geminin, CDK activity and the SCF complex in inhibiting reduplication of the centrioles must also fit into a licensing model.
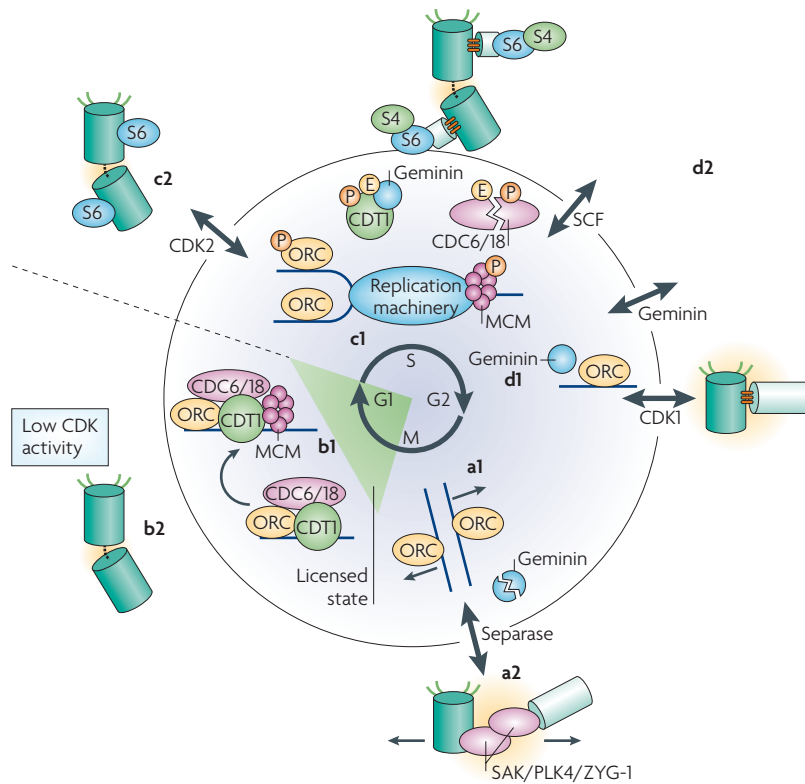
such as SAK/PLK4 (REFS 29,30) and perhaps SAS6 (REFS 24,28), for which loss of function leads to the absence of centrosome duplication and overexpression leads to over-duplication; and third, identical activities that control the licensing of both DNA and centriole replication, such as geminin, CDK activity and SCF-mediated protein degradation, to facilitate the coordination of both cycles (FIG. 3). Understanding how the activity of the different molecules discussed here is regulated along the cell cycle is crucial for understanding the control of the regulation of the number of centrioles and whether there is indeed a licensing system.

Predictions arising from comparing both cycles. Cellular checkpoint mechanisms ensure normal cell-cycle progression and the ability to respond to stress. Checkpoint pathways have a role in preventing re-replication of DNA (reviewed in REFS 12,108). Could they have a role in preventing the reduplication of centrosomes?

DNA checkpoints can be p53 dependent as the overexpression of the licensing factor CDT1 induces detectable re-replication only in cells that lack p53 (REF. 107). p53 becomes stabilized, leading to the upregulation of p21, which is known to suppress CDK2 activity. Interestingly, p53 prevents reduplication of centrosomes when cells are arrested in S phase[102,111,112], which suggests the existence of such a checkpoint. Two distinct p53 activities might regulate its function in the centrosome cycle: one is dependent on its transactivation function and the other depends on its ability to bind centrosomes, possibly sequestering a potential positive regulator of centrosome duplication[113]. CDK2 activity is necessary for the reduplication of centrosomes in the context of S-phase arrest[7,100]. Perhaps in this context, p53 is active, leading to an increase in p21 and consequent CDK2 inhibition. Alternatively, p53 might downregulate SAK/PLK4 (REF. 114), thereby controlling centrosome number. Centrosome inactivation and centrosome amplification, due to centriole reduplication and/or PCM fragmentation, also occur after DNA damage (reviewed in REF. 115). A better understanding of these checkpoints will be crucial for a better understanding of centrosome amplification in cancer.

Too many, too little and starting from nothing

In the majority of multicellular eukaryotes, most cells have either one or two centrosomes. However, some cells have many, such as megakaryocytes, and others, for example, oocytes, have none.

Centriolar loss has been described in various cell types. Centrioles disappear during oogenesis in several species (reviewed in REF. 116). They are also lost during sperm maturation in the mouse (but not in most other mammals) and during muscle differentiation when myoblasts give rise to syncytial myotubes[117]. Here, MTs are nucleated from sites that are associated with the nuclear envelope. Non-centrosomal MT arrays are found in certain epithelia, such as in the kidney, where apico–basal MTs are essential for the proper sorting of vesicles (reviewed in REFS 48,118). In several cases, the establishment of those arrays involves centriole loss, such

Figure 3 | **Coordinating the two cycles: parallel mechanisms or shared controls?** DNA replication (inside the circle: **a1**–**d1**), centrosome duplication (outside the circle: **a2**–**d2**) and common elements to both cycles (at the top of the circle); only the recruitment of new molecules to the centrioles is shown here. We have only shown SAS-4, SAS-6 and ZYG-1 (or its homologue, SAK/PLK4) at stages at which where there is strong evidence that they are localized and/or have a role in those structures. See BOX 3 for a more comprehensive description of the DNA-licensing system. **a** | At the metaphase–anaphase transition, separase is activated, which leads to the separation of sister chromatids (**a1**) and to the disengagement of the centrioles (**a2**)[86], suggesting that it might degrade an as yet unknown link between both centrioles. Disengagement of centrioles might allow the recruitment or activation of additional molecules, such as SAK/PLK4/ZYG-1 (REFS 29,30,82,141), or the modification of sites in the centrioles, licensing them for duplication. It is not known what its substrates would be. At the same time, geminin, an inhibitor of the licensing complex, is degraded. **b** | The end of mitosis and the beginning of the G1 phase are characterized by low cyclin-dependent kinase (CDK) activity, allowing the licensing complex to bind the origins of replication (**b1**). **c** | DNA replication takes place, which displaces the minichromosome maintenance (MCM) complex, leaving the DNA unlicensed (**c1**; see also BOX 3). Centriole formation takes place. Given that SAS-6 recruitment to the centriole depends on ZYG-1 (REFS 80,82), it is possible that ZYG-1 phosphorylates SAS-6 at this stage or that ZYG-1 modified sites earlier in the centriole that allow SAS-6 recruitment. SAS-6 recruits SAS-4 (REFS 80,82). CDK2 activity is important for DNA replication and centriole duplication in the context of overduplication[100]. The procentriole is engaged with the mother centriole, perhaps unlicensing the mother centriole for a further round of duplication. **d** | Several molecules contribute to keeping the DNA-licensing complex inactive: SCF-mediated degradation of CDC6 and CDT1 (after phosphorylation by CDKs); exclusion of the MCMs and/or CDT1 from the nucleus; and geminin-mediated inhibition of CDC6 and CDT1 in metazoans. Depletion or downregulation of geminin, the SCF–Slimb complex or CDK1 leads to overduplication of centrosomes and to re-replication of DNA, highlighting the crosstalk between both pathways (see main text for references). It is not clear whether geminin also sequesters any factors that are involved in the centriole cycle[104,111]. E, export; ORC, origin-recognition complex; P, phosphate; S4, SAS-4; S6, SAS-6.

In summary, the control of centrosome duplication might require a three-component system that is similar to the DNA-licensing system: first, a centrosome-duplication machinery; second, candidate licensing molecules,
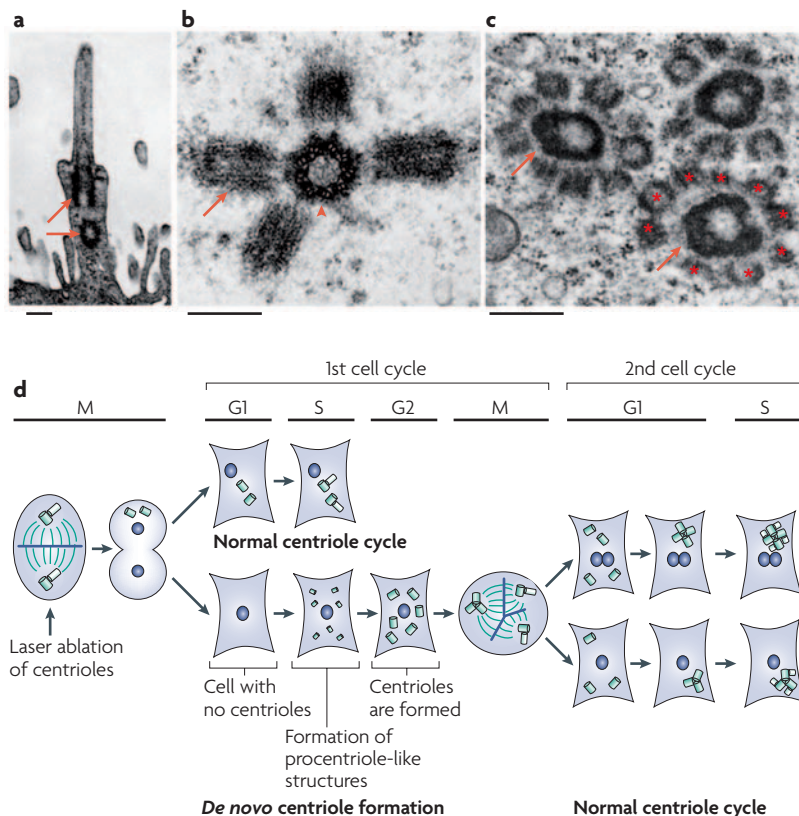
Figure 4 | **Centriole biogenesis outside the centriole cycle. a** | The formation of cilia in a monkey oviduct. Note the two basal bodies at the base of the cilia (red arrows). Scale bar: 0.25 μm. **b** | Nearly mature basal bodies (arrow) associate with a centriole (arrowhead) from a monkey oviduct. Note how a single centriole gives rise to several centrioles. Scale bar: 0.25 μm. Panels **a** and **b** are reproduced with permission from REF. 124 © (1971) Rockefeller University Press. **c** | Three generative complexes (deuterosome) with several nascent centrioles. Note how the deuterosome (red arrow) can give rise to several centrioles (asterisks). Scale bar: 0.25 μm. Reproduced with permission from REF. 122 © (1971) Rockefeller University Press. **d** | Pedigree of a cell born without a centrosome. In cells labelled with centrin–GFP, centrioles in one of the poles of a mitotic spindle were ablated with a laser. The dividing cell can give rise to one cell with normal centriole numbers and another that lacks centrioles. Both continue to progress through the cell cycle with normal kinetics. When the cell without centrioles enters S phase, multiple aggregates of centrin (procentriole-like structures; small green cylinders) form. These precentrioles transform into morphologically complete centrioles (large green cylinders) by the time the cell enters its first mitosis. However, de novo formed centrioles do not mature centrosomes until the ensuing G1 phase in the second cell cycle. As cell enters the S phase of the second cell cycle, de novo formed centrioles duplicate and normal centriolar cycles resume. GFP, green fluorescent protein. Adapted with permission from REF. 13 © (2005) Rockefeller University Press.

as found in the polarized epithelia of *D. melanogaster*. As centriolar MTs are very stable, it is possible that there is an active pathway for centriolar loss similar to the regulated disassembly of flagella in *C. reinhardtii* (reviewed in REF. 119). Proper regulation of a cyclin E–CDK complex by CDK inhibitor-2 (CKI-2) might be required for centrosome elimination in oogenesis in *C. elegans*[120].

Many ciliated cells, such as those in vertebrate respiratory and reproductive tracts, can have 200–300 cilia per cell (FIG. 4a–c). This requires the generation of multiple centrioles during ciliogenesis[121,122] (reviewed in REF. 123). Here, centrioles are generated by two pathways, a centriolar

Deuterosome
A large electron-dense cytoplasmic organelle (75–400 nm diameter) that has a role in the formation of ciliary basal bodies.

and an acentriolar mechanism. In the first one, a parent centriole usually produces one daughter at a time; however, in certain cases several centrioles have been observed to develop simultaneously around one parent centriole (FIG. 4b), with the daughter centrioles being released into the cytoplasm to mature. The acentriolar pathway is the major pathway for basal body production. In this pathway, fibrous granules[124] that are 70–100 nm in diameter first appear in the cytoplasm and subsequently move to the apical cytoplasm. Deuterosomes appear within the area. It is not clear whether deuterosomes result from the fusion of those fibrous organelles as there are only a few studies that have characterized the molecular composition of these structures. Recently, *X. laevis* PCM1 was shown to localize to the fibrous organelles but not to the deuterosomes[125]. Further studies of deuterosomes and the fibrous organelles, including imaging experiments, will be essential to clarify their origins and function. Multiple procentrioles can grow out from deuterosomes, and mature daughter centrioles travel towards the apical region where they form ciliary basal bodies (FIG. 4c). In both pathways a cartwheel intermediate has been reported[124].

In certain circumstances, centrioles can be formed *de novo*, without pre-existing template centrioles[126]. This occurs in some parthenogenic species such as insects[15] and during mouse development. The widespread nature of this process and the fact that it can contribute to aberrations of centrosome number became apparent only recently[126,127]. *De novo* centrosome formation occurs in HeLa cells in which centrosomes have been removed by laser treatment or microsurgery[13] (FIG. 4d). Here, *de novo* centriole assembly begins with the formation of 2–10 small aggregates of centrin–green fluorescent protein (GFP), which appear during S phase and that achieve a centrin intensity and an ultrastructure that are the same as those of a canonical centriole only when the cell reaches mitosis. *De novo* assembled centrioles can organize MTs and duplicate in the next cell cycle. *De novo* centriole formation has also been described in *C. reinhardtii* mutants and *D. melanogaster* in which some cells are born without centrioles[14,152,153]. In both *C. reinhardtii* and vertebrate cells, the presence of a single centriole is sufficient to inhibit *de novo* formation, and passage through S phase is required for *de novo* centriole formation[13,14]. The regulation therefore seems similar to the canonical cycle, albeit slower and unable to control the total number of generated centrioles.

Conclusions and future directions

In the past 10 years our views of centrosome function have changed greatly. We know that they are dominant cellular functions, as the presence of multiple centrosomes frequently leads to the formation of multipolar spindles[2]. On the other hand, in many species spindles can form without centrosomes, despite their requirement in certain species for the fidelity of cell division[29,36,128]. Other roles of this organelle have moved into the limelight. For example, as basal bodies these structures are essential for the formation of flagella and cilia. The majority of human cells have cilia and a number of diseases have

Comparative genomics
The analysis and comparison of genomes from different species to gain a better understanding of how species have evolved and to determine the function of gene products and non-coding regions in the genome.

now been associated with abnormal ciliogenesis, including cystic kidney diseases (reviewed in REF. 3). A greater understanding of the interconversion of centrioles and basal bodies might help in the design of treatments for such diseases.

Recent large-scale functional genomics and proteomics studies have highlighted the diversity of centrosome structures but have also revealed a high degree of molecular conservation. Comparative genomics studies can highlight the molecular differences and similarities among those structures, leading to a better understanding of centriole biogenesis and function. Structures that look different are formed with homologous molecules. In fact, recent studies in *C. reinhardtii* have identified the defective molecules in several human diseases with defective cilia formation[18,21,119]. In the past five years, a conserved group of proteins that are essential for centriole duplication has been brought to light. This includes the kinase SAK/PLK4 and the two proteins SAS4 and SAS6. Beautiful structural studies have shown the role of each one of these proteins in forming intermediate structures and in recruiting the others.

Recent studies have re-addressed old issues, such as *de novo* centriole formation[152,153]. These studies have highlighted the self-assembly properties of centrioles. A view has started to emerge that there might be a universal mechanism for canonical, *de novo* and ciliogenic centriole formation[152]. In all of these, procentrioles might be formed in the cytoplasm and be stabilized or catalysed by a mother centriole, or take longer to form if no centriole

is present. It is clear that the assembly of centrioles *de novo* is inhibited by the presence of a single centriole. The regulation of this process is still a mystery, and it will be interesting to identify the molecules that inhibit *de novo* centriole formation, to study the potential role of these molecules in cancer and to further compare *de novo* centriole assembly with the canonical pathway.

The regulation of centrosome number has gained additional attention owing to the observation that centrosome number is increased in tumorigenesis. A combination of recent studies highlights a group of molecules that inhibit the re-replication of the DNA and can inhibit centriole reduplication. These results suggest a licensing mechanism for the regulation of centriole duplication, which ensures that duplication occurs only at the right time. Separase, SAK/PLK4 and SAS6 have all been suggested as potential players in this mechanism. The understanding of the regulation of these molecules, and what their substrates and interactors might be, will help us to understand how the centrosome number is controlled.

Our understanding of the centrosome, its biogenesis, regulation and function is changing rapidly. The use of functional genomics has brought to light many new molecular players in centrosome biogenesis and function. Comparative genomics analyses and better imaging technologies will help us to better understand these structures as well as their role in different organisms and in different tissues in the same organism. This should keep us on track to answer the questions that Boveri first asked at the end of the 19th century.

1. Nigg, E. A. Centrosome aberrations: cause or consequence of cancer progression? *Nature Rev. Cancer* **2**, 815–825 (2002).
2. Sluder, G. & Nordberg, J. J. The good, the bad and the ugly: the practical consequences of centrosome amplification. *Curr. Opin. Cell Biol.* **16**, 49–54 (2004).
3. Badano, J. L., Teslovich, T. M. & Katsanis, N. The centrosome in human genetic disease. *Nature Rev. Genet.* **6**, 194–205 (2005).
4. Praetorius, H. A. & Spring, K. R. A physiological view of the primary cilium. *Annu. Rev. Physiol.* **67**, 515–529 (2005).
5. Kuriyama, R. & Borisy, G. G. Centriole cycle in Chinese hamster ovary cells as determined by whole-mount electron microscopy. *J. Cell Biol.* **91**, 814–821 (1981).
6. Robbins, E., Jentzsch, G. & Micali, A. The centriole cycle in synchronized HeLa cells. *J. Cell Biol.* **36**, 329–339 (1968).
7. Sluder, G. in *Centrosomes in Development and Disease* (ed. Nigg, E. A.) 167–189 (Wiley-Vch, Weinheim, 2004).
8. Kochanski, R. S. & Borisy, G. G. Mode of centriole duplication and distribution. *J. Cell Biol.* **110**, 1599–1605 (1990).
9. Alvey, P. L. An investigation of the centriole cycle using 3T3 and CHO cells. *J. Cell Sci.* **78**, 147–162 (1985).
10. Vorobjev, I. A. & Chentsov Yu, S. Centrioles in the cell cycle. I. Epithelial cells. *J. Cell Biol.* **93**, 938–949 (1982).
11. Blow, J. J. & Dutta, A. Preventing re-replication of chromosomal DNA. *Nature Rev. Mol. Cell Biol.* **6**, 476–486 (2005).
12. Machida, Y. J., Hamlin, J. L. & Dutta, A. Right place, right time, and only once: replication initiation in metazoans. *Cell* **123**, 13–24 (2005).
13. La Terra, S. *et al.* The *de novo* centriole assembly pathway in HeLa cells: cell cycle progression and centriole assembly/maturation. *J. Cell Biol.* **168**, 713–722 (2005).
    Defines the properties of *de novo* assembled centrioles in HeLa cells: they are born in S phase; they mature in the next cycle; and the presence of a single centriole inhibits the assembly of additional centrioles.
14. Marshall, W. F., Vucica, Y. & Rosenbaum, J. L. Kinetics and regulation of *de novo* centriole assembly. Implications for the mechanism of centriole duplication. *Curr. Biol.* **11**, 308–317 (2001).
    Defines the properties of *de novo* assembled centrioles in *C. reinhardtii*. The presence of a single centriole inhibits the assembly of additional centrioles, and the rate of *de novo* assembly is approximately half the rate of templated duplication.
15. Riparbelli, M. G. & Callaini, G. *Drosophila* parthenogenesis: a model for *de novo* centrosome assembly. *Dev. Biol.* **260**, 298–313 (2003).
16. Andersen, J. S. *et al.* Proteomic characterization of the human centrosome by protein correlation profiling. *Nature* **426**, 570–574 (2003).
17. Ostrowski, L. E. *et al.* A proteomic analysis of human cilia: identification of novel components. *Mol. Cell. Proteomics* **1**, 451–465 (2002).
18. Keller, L. C., Romijn, E. P., Zamora, I., Yates, J. R. 3rd & Marshall, W. F. Proteomic analysis of isolated *Chlamydomonas* centrioles reveals orthologs of ciliary-disease genes. *Curr. Biol.* **15**, 1090–1098 (2005).
19. Pazour, G. J., Agrin, N., Leszyk, J. & Witman, G. B. Proteomic analysis of a eukaryotic cilium. *J. Cell Biol.* **170**, 103–113 (2005).
20. Stolc, V., Samanta, M. P., Tongprasit, W. & Marshall, W. F. Genome-wide transcriptional analysis of flagellar regeneration in *Chlamydomonas reinhardtii* identifies orthologs of ciliary disease genes. *Proc. Natl Acad. Sci. USA* **102**, 3703–3707 (2005).
21. Li, J. B. *et al.* Comparative genomics identifies a flagellar and basal body proteome that includes the *BBS5* human disease gene. *Cell* **117**, 541–552 (2004).
    References 21 and 22 use comparative genomics to predict the ciliary and basal body proteomes, leading to a global evolutionary view and to human disease-gene candidates.
22. Avidor-Reiss, T. *et al.* Decoding cilia function: defining specialized genes required for compartmentalized cilia biogenesis. *Cell* **117**, 527–539 (2004).
23. Leidel, S. & Gonczy, P. Centrosome duplication and nematodes: recent insights from an old relationship. *Dev. Cell* **9**, 317–325 (2005).
24. Leidel, S., Delattre, M., Cerutti, L., Baumer, K. & Gonczy, P. SAS-6 defines a protein family required for centrosome duplication in *C. elegans* and in human cells. *Nature Cell Biol.* **7**, 115–125 (2005).
    Identification and characterization of SAS-6, a conserved regulator of centriole biogenesis, the overexpression of which leads to the amplification of MTOCs.
25. Leidel, S. & Gonczy, P. SAS-4 is essential for centrosome duplication in *C. elegans* and is recruited to daughter centrioles once per cell cycle. *Dev. Cell* **4**, 431–439 (2003).
26. Gonczy, P. *et al.* Functional genomic analysis of cell division in *C. elegans* using RNAi of genes on chromosome III. *Nature* **408**, 331–336 (2000).
27. Pelletier, L. *et al.* The *Caenorhabditis elegans* centrosomal protein SPD-2 is required for both pericentriolar material recruitment and centriole duplication. *Curr. Biol.* **14**, 863–873 (2004).
28. Dammermann, A. *et al.* Centriole assembly requires both centriolar and pericentriolar material proteins. *Dev. Cell* **7**, 815–829 (2004).
    Identification and characterization of SAS-6, a conserved regulator of centriole biogenesis. The results further suggest that the PCM promotes daughter centriole formation by concentrating γ-tubulin around the parent centriole.
29. Bettencourt-Dias, M. *et al.* SAK/PLK4 is required for centriole duplication and flagella development. *Curr. Biol.* **15**, 2199–2207 (2005).
    Describes the conserved role of SAK/PLK4 in centriole duplication. Both references 29 and 36 show that cells without centrioles can proliferate in the context of a whole organism. However, centrioles are needed to form basal bodies and for male meiotic divisions.
30. Habedanck, R., Stierhof, Y. D., Wilkinson, C. J. & Nigg, E. A. The Polo kinase Plk4 functions in centriole duplication. *Nature Cell Biol.* **7**, 1140–1146 (2005).
    Reports the requirement for SAK/PLK4 in centriole duplication. SAK/PLK4 might operate as a master regulator in this process, given that overexpression leads to centriole amplification.

31. Bettencourt-Dias, M. *et al.* Genome-wide survey of protein kinases required for cell cycle progression. *Nature* **432**, 980–987 (2004).

32. Paintrand, M., Moudjou, M., Delacroix, H. & Bornens, M. Centrosome organization and centriole architecture: their sensitivity to divalent cations. *J. Struct. Biol.* **108**, 107–128 (1992).

33. Bornens, M. Centrosome composition and microtubule anchoring mechanisms. *Curr. Opin. Cell Biol.* **14**, 25–34 (2002).

34. Sluder, G. & Rieder, C. L. Centriole number and the reproductive capacity of spindle poles. *J. Cell Biol.* **100**, 887–896 (1985).

35. Kirkham, M., Muller-Reichert, T., Oegema, K., Grill, S. & Hyman, A. A. SAS-4 is a *C. elegans* centriolar protein that controls centrosome size. *Cell* **112**, 575–587 (2003).

36. Basto, R. *et al.* Flies without centrioles. *Cell* **125**, 1375–1386 (2006).
   See also reference 29. The authors also show that asymmetrical cell division can occur without centrioles (although not always).

37. Bobinnec, Y. *et al.* Centriole disassembly *in vivo* and its effect on centrosome structure and function in vertebrate cells. *J. Cell Biol.* **143**, 1575–1589 (1998).

38. Sluder, G., Miller, F. J. & Rieder, C. L. Reproductive capacity of sea urchin centrosomes without centrioles. *Cell Motil. Cytoskeleton* **13**, 264–273 (1989).

39. Janke, C. *et al.* Tubulin polyglutamylase enzymes are members of the TTL domain protein family. *Science* **308**, 1758–1762 (2005).

40. Hinchcliffe, E. H. & Link, R. W. Two proteins isolated from sea urchin sperm flagella: structural components common to the stable microtubules of axonemes and centrioles. *J. Cell Sci.* **111**, 585–595 (1998).

41. Steffen, W. & Link, R. W. Evidence for tektins in centrioles and axonemal microtubules. *Proc. Natl Acad. Sci. USA* **85**, 2643–2647 (1988).

42. Azimzadeh, J. & Bornens, M. in *Centrosomes in Development and Disease* (ed. Nigg, E. A.) 93–122 (Wiley-Vch, Weinheim, 2004).

43. Blagden, S. P. & Glover, D. M. Polar expeditions — provisioning the centrosome for mitosis. *Nature Cell Biol.* **5**, 505–511 (2003).

44. Zheng, Y., Wong, M. L., Alberts, B. & Mitchison, T. Nucleation of microtubule assembly by a γ-tubulin-containing ring complex. *Nature* **378**, 578–583 (1995).

45. Verollet, C. *et al. Drosophila melanogaster* γ-TuRC is dispensable for targeting γ-tubulin to the centrosome and microtubule nucleation. *J. Cell Biol.* **172**, 517–528 (2006).

46. Sawin, K. E. & Tran, P. T. Cytoplasmic microtubule organization in fission yeast. *Yeast* **23**, 1001–1014 (2006).

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

48. Bartolini, F. & Gundersen, G. G. Generation of noncentrosomal microtubule arrays. *J. Cell Sci.* **119**, 4155–4163 (2006).

49. Doxsey, S. Re-evaluating centrosome function. *Nature Rev. Mol. Cell Biol.* **2**, 688–698 (2001).

50. Trinkle-Mulcahy, L. & Lamond, A. I. Mitotic phosphatases: no longer silent partners. *Curr. Opin. Cell Biol.* **18**, 623–631 (2006).

51. Glover, D. M. Polo kinase and progression through M phase in *Drosophila*: a perspective from the spindle poles. *Oncogene* **24**, 230–237 (2005).

52. Giet, R. *et al. Drosophila* Aurora A kinase is required to localize D-TACC to centrosomes and to regulate astral microtubules. *J. Cell Biol.* **156**, 437–451 (2002).

53. Barros, T. P., Kinoshita, K., Hyman, A. A. & Raff, J. W. Aurora A activates D-TACC–Msps complexes exclusively at centrosomes to stabilize centrosomal microtubules. *J. Cell Biol.* **170**, 1039–1046 (2005).

54. Peset, I. *et al.* Function and regulation of Maskin, a TACC family protein, in microtubule growth during mitosis. *J. Cell Biol.* **170**, 1057–1066 (2005).

55. Brittle, A. L. & Ohkura, H. Mini spindles, the XMAP215 homologue, suppresses pausing of interphase microtubules in *Drosophila*. *EMBO J.* **24**, 1387–1396 (2005).

56. Sankaran, S., Starita, L. M., Simons, A. M. & Parvin, J. D. Identification of domains of BRCA1 critical for the ubiquitin-dependent inhibition of centrosome function. *Cancer Res.* **66**, 4100–4107 (2006).

57. Pelletier, L., Muller-Reichert, T., Srayko, M., Ozlu, N., Schlaitz, A. & Hyman, A. A. in *Centrosomes in Development and Disease* (ed. Nigg, E. A.) 227–250 (Wiley-VCH, Weinheim, 2004).

58. Kemp, C. A., Kopish, K. R., Zipperlen, P., Ahringer, J. & O'Connell, K. F. Centrosome maturation and duplication in *C. elegans* require the coiled-coil protein SPD-2. *Dev. Cell* **6**, 511–523 (2004).

59. Dutcher, S. K. in *Centrosomes in Development and Disease* (ed. Nigg, E. A.) 71–92 (Wiley-VCH, Weinheim, 2004).

60. Rieder, C. L., Jensen, C. G. & Jensen, L. C. The resorption of primary cilia during mitosis in a vertebrate (PtK1) cell line. *J. Ultrastruct. Res.* **68**, 173–185 (1979).

61. Ishikawa, H., Kubo, A., Tsukita, S. & Tsukita, S. Odf2-deficient mother centrioles lack distal/subdistal appendages and the ability to generate primary cilia. *Nature Cell Biol.* **7**, 517–524 (2005).
   The authors deleted both alleles of the *Odf2* gene in mouse F9 cells and found that *Odf2* is indispensable for the formation of distal and subdistal appendages and the generation of primary cilia, providing evidence for the direct involvement of appendages in cilia formation.

62. Doxsey, S., McCollum, D. & Theurkauf, W. Centrosomes in cellular regulation. *Annu. Rev. Cell Dev. Biol.* **21**, 411–434 (2005).

63. Sluder, G. Two-way traffic: centrosomes and the cell cycle. *Nature Rev. Mol. Cell Biol.* **6**, 743–748 (2005).

64. Doxsey, S., Zimmerman, W. & Mikule, K. Centrosome control of the cell cycle. *Trends Cell Biol.* **15**, 303–311 (2005).

65. Piel, M., Meyer, P., Khodjakov, A., Rieder, C. L. & Bornens, M. The respective contributions of the mother and daughter centrioles to centrosome activity and behavior in vertebrate cells. *J. Cell Biol.* **149**, 317–330 (2000).
   Shows that only the maternal centriole retains, and presumably anchors, microtubules. Also shows that daughter centrioles are dynamic and that their movements are coordinated with those of the mother centriole, which suggests a molecular link between them.

66. Piel, M., Nordberg, J., Euteneuer, U. & Bornens, M. Centrosome-dependent exit of cytokinesis in animal cells. *Science* **291**, 1550–1553 (2001).
   References 66–68 show that somatic cells can form a spindle in the absence of centrosomes but show defects in cytokinesis and S phase progression.

67. Khodjakov, A. & Rieder, C. L. Centrosomes enhance the fidelity of cytokinesis in vertebrates and are required for cell cycle progression. *J. Cell Biol.* **153**, 237–242 (2001).

68. Hinchcliffe, E. H., Miller, F. J., Cham, M., Khodjakov, A. & Sluder, G. Requirement of a centrosomal activity for cell cycle progression through G1 into S phase. *Science* **291**, 1547–1550 (2001).

69. Krapp, A., Gulli, M. P. & Simanis, V. SIN and the art of splitting the fission yeast cell. *Curr. Biol.* **14**, R722–R730 (2004).

70. Seshan, A. & Amon, A. Linked for life: temporal and spatial coordination of late mitotic events. *Curr. Opin. Cell Biol.* **16**, 41–48 (2004).

71. Magidson, V., Chang, F. & Khodjakov, A. Regulation of cytokinesis by spindle-pole bodies. *Nature Cell Biol.* **8**, 891–893 (2006).

72. Uetake, Y. *et al.* Cell cycle progression and *de novo* centriole assembly after centrosomal removal in untransformed human cells. *J. Cell Biol.* **176**, 173–182 (2007).

73. Srsen, V., Gnadt, N., Dammermann, A. & Merdes, A. Inhibition of centrosome protein assembly leads to p53-dependent exit from the cell cycle. *J. Cell Biol.* **174**, 625–630 (2006).

74. Wadsworth, P. & Khodjakov, A. E pluribus unum: towards a universal mechanism for spindle assembly. *Trends Cell Biol.* **14**, 413–419 (2004).

75. Matsuura, K., Lefebvre, P. A., Kamiya, R. & Hirono, M. Bld10p, a novel protein essential for basal body assembly in *Chlamydomonas*: localization to the cartwheel, the first ninefold symmetrical structure appearing during assembly. *J. Cell Biol.* **165**, 663–671 (2004).

76. Rieder, C. L. *et al.* The centrosome cycle in Ptk2 cells: asymmetric distribution and structural changes in the pericentriolar material. *Biol. Cell* **44**, 117–132 (1982).

77. Adams, I. R. & Kilmartin, J. V. Spindle pole body duplication: a model for centrosome duplication? *Trends Cell Biol.* **10**, 329–335 (2000).

78. Dippell, R. V. The development of basal bodies in paramecium. *Proc. Natl Acad. Sci. USA* **61**, 461–468 (1968).

79. Cavalier-Smith, T. Basal body and flagellar development during the vegetative cell cycle and the sexual cycle of *Chlamydomonas reinhardii*. *J. Cell Sci.* **16**, 529–556 (1974).

80. Pelletier, L., O'Toole, E., Schwager, A., Hyman, A. A. & Muller-Reichert, T. Centriole assembly in *Caenorhabditis elegans*. *Nature* **30**, 619–623 (2006).
   Reports a structural and molecular pathway for the assembly of a daughter centriole using electron tomography of staged wild-type and mutant *C. elegans* one-cell embryos and centriole-recruitment assays.

81. Balczon, R. *et al.* Dissociation of centrosome replication events from cycles of DNA synthesis and mitotic division in hydroxyurea-arrested Chinese hamster ovary cells. *J. Cell Biol.* **130**, 105–115 (1995).

82. Delattre, M., Canard, C. & Gonczy, P. Sequential protein recruitment in *C. elegans* centriole formation. *Curr. Biol.* **16**, 1844–1849 (2006).

83. Cowan, C. R. & Hyman, A. A. Cyclin E–Cdk2 temporally regulates centrosome assembly and establishment of polarity in *Caenorhabditis elegans* embryos. *Nature Cell Biol.* **8**, 1441–1447 (2006).

84. Hinchcliffe, E. H. & Sluder, G. "It takes two to tango": understanding how centrosome duplication is regulated throughout the cell cycle. *Genes Dev.* **15**, 1167–1181 (2001).

85. Vidwans, S. J., Wong, M. L. & O'Farrell, P. H. Mitotic regulators govern progress through steps in the centrosome duplication cycle. *J. Cell Biol.* **147**, 1371–1378 (1999).
   Shows in *D. melanogaster* mutants that there must be coordination between the centrosome and chromosome cycles. Different cell-cycle regulators, such as CDC20, mitotic cyclins and CDC25, are important for disengagement, new centriole formation and elongation.

86. Tsou, M. F. & Stearns, T. Mechanism limiting centrosome duplication to once per cell cycle. *Nature* **442**, 947–951 (2006).

87. Vidwans, S. J., Wong, M. L. & O'Farrell, P. H. Anomalous centriole configurations are detected in *Drosophila* wing disc cells upon Cdk1 inactivation. *J. Cell Biol.* **116**, 137–143 (2003).

88. Paoletti, A. *et al.* Fission yeast cdc31p is a component of the half-bridge and controls SPB duplication. *Mol. Biol. Cell* **14**, 2793–2808 (2003).

89. Spang, A., Courtney, I., Fackler, U., Matzner, M. & Schiebel, E. The calcium-binding protein cell division cycle 31 of *Saccharomyces cerevisiae* is a component of the half bridge of the spindle pole body. *J. Cell Biol.* **123**, 405–416 (1993).

90. Ruiz, F., Garreau de Loubresse, N., Klotz, C., Beisson, J. & Koll, F. Centrin deficiency in *Paramecium* affects the geometry of basal-body duplication. *Curr. Biol.* **15**, 2097–2106 (2005).

91. Salisbury, J. L., Suino, K. M., Busby, R. & Springett, M. Centrin-2 is required for centriole duplication in mammalian cells. *Curr. Biol.* **12**, 1287–1292 (2002).

92. Dutcher, S. K. Long-lost relatives reappear: identification of new members of the tubulin superfamily. *Curr. Opin. Microbiol.* **6**, 634–640 (2003).

93. Bahe, S., Stierhof, Y. D., Wilkinson, C. J., Leiss, F. & Nigg, E. A. Rootletin forms centriole-associated filaments and functions in centrosome cohesion. *J. Cell Biol.* **171**, 27–33 (2005).

94. Yang, J., Adamian, M. & Li, T. Rootletin interacts with c-Nap1 and may function as a physical linker between the pair of centrioles/basal bodies in cells. *Mol. Biol. Cell* **17**, 1033–1040 (2006).

95. Meraldi, P. & Nigg, E. A. Centrosome cohesion is regulated by a balance of kinase and phosphatase activities. *J. Cell Sci.* **114**, 3749–3757 (2001).

96. Kramer, A., Neben, K. & Ho, A. D. Centrosome aberrations in hematological malignancies. *Cell. Biol. Int.* **29**, 376–384 (2005).

97. Kramer, A. Centrosome aberrations — hen or egg in cancer initiation and progression? *Leukemia* **9**, 1142–1144 (2005).

98. Meraldi, P., Lukas, J., Fry, A. M., Bartek, J. & Nigg, E. A. Centrosome duplication in mammalian somatic cells requires E2F and Cdk2–cyclin A. *Nature Cell Biol.* **1**, 88–93 (1999).

99. Hinchcliffe, E. H., Li, C., Thompson, E. A., Maller, J. L. & Sluder, G. Requirement of Cdk2–cyclin E activity for repeated centrosome reproduction in *Xenopus* egg extracts. *Science* **283**, 851–854 (1999).

100. Duensing, A. *et al.* Cyclin-dependent kinase 2 is dispensable for normal centrosome duplication but required for oncogene-induced centrosome overduplication. *Oncogene* **25**, 2943–2949 (2006).

101. Aleem, E., Kiyokawa, H. & Kaldis, P. Cdc2–cyclin E complexes regulate the G1–S phase transition. *Nature Cell Biol.* **7**, 831–836 (2005).

102. Wong, C. & Stearns, T. Centrosome number is controlled by a centrosome-intrinsic block to reduplication. *Nature Cell Biol.* **5**, 539–544 (2003).
The authors provide evidence for a centrosome-intrinsic block to reduplication so that centrosomes that have already duplicated cannot duplicate again in the same cell cycle.

103. Rao, P. N. & Johnson, R. T. Mammalian cell fusion: studies on the regulation of DNA synthesis and mitosis. *Nature* **225**, 159–164 (1970).

104. Tachibana, K. E., Gonzalez, M. A., Guarguaglini, G., Nigg, E. A. & Laskey, R. A. Depletion of licensing inhibitor geminin causes centrosome overduplication and mitotic defects. *EMBO Rep.* **6**, 1052–1057 (2005).

105. Wojcik, E. J., Glover, D. M. & Hays, T. S. The SCF ubiquitin ligase protein slimb regulates centrosome duplication in *Drosophila*. *Curr. Biol.* **10**, 1131–1134 (2000).

106. Murphy, T. D. *Drosophila* skpA, a component of SCF ubiquitin ligases, regulates centrosome duplication independently of cyclin E accumulation. *J. Cell Sci.* **116**, 2321–2332 (2003).

107. Vaziri, C. *et al.* A p53-dependent checkpoint pathway prevents rereplication. *Mol. Cell* **11**, 997–1008 (2003).

108. Machida, Y. J. & Dutta, A. Cellular checkpoint mechanisms monitoring proper initiation of DNA replication. *J. Biol. Chem.* **280**, 6253–6256 (2005).

109. Fode, C., Binkert, C. & Dennis, J. W. Constitutive expression of murine Sak-a suppresses cell growth and induces multinucleation. *Mol. Cell Biol.* **16**, 4665–4672 (1996).

110. Budhu, A. S. & Wang, X. W. Loading and unloading: orchestrating centrosome duplication and spindle assembly by Ran/Crm1. *Cell Cycle* **4**, 1510–1514 (2005).

111. Tachibana, K. E. & Nigg, E. A. Geminin regulates multiple steps of the chromosome inheritance cycle. *Cell Cycle* **5**, 151–154 (2006).

112. Fukasawa, K., Choi, T., Kuriyama, R., Rulong, S. & Vande Woude, G. F. Abnormal centrosome amplification in the absence of p53. *Science* **271**, 1744–1747 (1996).

113. Shinmura, K., Bennett, R. A., Tarapore, P. & Fukasawa, K. Direct evidence for the role of centrosomally localized p53 in the regulation of centrosome duplication. *Oncogene* **26**, 2939–2944 (2007).

114. Li, J. *et al.* SAK, a new polo-like kinase, is transcriptionally repressed by p53 and induces apoptosis upon RNAi silencing. *Neoplasia* **7**, 312–323 (2005).

115. Sibon, O. C. Centrosomes as DNA damage regulators. *Nature Genet.* **34**, 6–7 (2003).

116. Schatten, G. The centrosome and its mode of inheritance: the reduction of the centrosome during gametogenesis and its restoration during fertilization. *Dev. Biol.* **165**, 299–335 (1994).

117. Tassin, A. M., Maro, B. & Bornens, M. Fate of microtubule-organizing centers during myogenesis *in vitro*. *J. Cell Biol.* **100**, 35–46 (1985).

118. Mogensen, M. in *Centrosomes in Development and Disease* (ed. Nigg, E. A.) 299–320 (Wiley-VCH, Weinheim, 2004).

119. Snell, W. J., Pan, J. & Wang, Q. Cilia and flagella revealed: from flagellar assembly in *Chlamydomonas* to human obesity disorders. *Cell* **117**, 693–697 (2004).

120. Kim, D. Y. & Roy, R. Cell cycle regulators control centrosome elimination during oogenesis in *Caenorhabditis elegans*. *J. Cell Biol.* **174**, 751–757 (2006).

121. Sorokin, S. P. Reconstructions of centriole formation and ciliogenesis in mammalian lungs. *J. Cell Sci.* **3**, 207–230 (1968).

122. Dirksen, E. R. Centriole morphogenesis in developing ciliated epithelium of the mouse oviduct. *J. Cell Biol.* **51**, 286–302 (1971).

123. Dirksen, E. R. Centriole and basal body formation during ciliogenesis revisited. *Biol. Cell* **72**, 31–38 (1991).

124. Anderson, R. G. & Brenner, R. M. The formation of basal bodies (centrioles) in the Rhesus monkey oviduct. *J. Cell Biol.* **50**, 10–34 (1971).

125. Kubo, A., Sasaki, H., Yuba-Kubo, A., Tsukita, S. & Shiina, N. Centriolar satellites: molecular characterization, ATP-dependent movement toward centrioles and possible involvement in ciliogenesis. *J. Cell Biol.* **147**, 969–980 (1999).

126. Delattre, M. & Gonczy, P. The arithmetic of centrosome biogenesis. *J. Cell Sci.* **117**, 1619–1630 (2004).

127. Khodjakov, A. *et al. De novo* formation of centrosomes in vertebrate cells arrested during S phase. *J. Cell Biol.* **158**, 1171–1181 (2002).

128. Cowan, C. R. & Hyman, A. A. Asymmetric cell division in *C. elegans*: cortical polarity and spindle positioning. *Annu. Rev. Cell Dev. Biol.* **20**, 427–453 (2004).

129. Beisson, J. & Wright, M. Basal body/centriole assembly and continuity. *Curr. Opin. Cell Biol.* **15**, 96–104 (2003).

130. Wigge, P. A. *et al.* Analysis of the *Saccharomyces* spindle pole by matrix-assisted laser desorption/ionization (MALDI) mass spectrometry. *J. Cell Biol.* **141**, 967–977 (1998).

131. Broadhead, R. *et al.* Flagellar motility is required for the viability of the bloodstream trypanosome. *Nature* **440**, 224–227 (2006).

132. Reinders, Y., Schulz, I., Graf, R. & Sickmann, A. Identification of novel centrosomal proteins in *Dictyostelium discoideum* by comparative proteomic approaches. *J. Proteome Res.* **5**, 589–598 (2006).

133. Wilkinson, C. J., Andersen, J. S., Mann, M. & Nigg, E. A. in *Centrosomes in Development and Disease* (ed. Nigg, E. A.) 125–142 (Wiley-Vch, Weinheim, 2004).

134. Cavalier-Smith, T. The phagotrophic origin of eukaryotes and phylogenetic classification of Protozoa. *Int. J. Syst. Evol. Microbiol.* **52**, 297–354 (2002).

135. Callaini, G., Whitfield, W. G. & Riparbelli, M. G. Centriole and centrosome dynamics during the embryonic cell cycles that follow the formation of the cellular blastoderm in *Drosophila*. *Exp. Cell Res.* **234**, 183–190 (1997).

136. Gonzalez, C., Tavosanis, G. & Mollinari, C. Centrosomes and microtubule organisation during *Drosophila* development. *J. Cell Sci.* **111**, 2697–2706 (1998).

137. Satir, P. & Christensen, S. T. Overview of structure and function of mammalian cilia. *Annu. Rev. Physiol.* **69**, 377–400 (2006).

138. Rosenbaum, J. L. & Witman, G. B. Intraflagellar transport. *Nature Rev. Mol. Cell Biol.* **3**, 813–825 (2002).

139. Geimer, S. & Melkonian, M. The ultrastructure of the *Chlamydomonas reinhardtii* basal apparatus: identification of an early marker of radial asymmetry inherent in the basal body. *J. Cell Sci.* **117**, 2663–2674 (2004).

140. Lange, B. M. & Gull, K. A molecular marker for centriole maturation in the mammalian cell cycle. *J. Cell Biol.* **130**, 919–927 (1995).

141. O'Connell, K. F. *et al.* The *C. elegans zyg-1* gene encodes a regulator of centrosome duplication with distinct maternal and paternal roles in the embryo. *Cell* **105**, 547–558 (2001).

142. Koblenz, B., Schoppmeier, J., Grunow, A. & Lechtreck, K. F. Centrin deficiency in *Chlamydomonas* causes defects in basal body replication, segregation and maturation. *J. Cell Sci.* **116**, 2635–2646 (2003).

143. Li, S. *et al.* Structural role of Sfi1p–centrin filaments in budding yeast spindle pole body duplication. *J. Cell Biol.* **173**, 867–877 (2006).

144. Kilmartin, J. V. Sfi1p has conserved centrin-binding sites and an essential function in budding yeast spindle pole body duplication. *J. Cell Biol.* **162**, 1211–1221 (2003).

145. Chen, Z., Indjeian, V. B., McManus, M., Wang, L. & Dynlacht, B. D. CP110, a cell cycle-dependent CDK substrate, regulates centrosome duplication in human cells. *Dev. Cell* **3**, 339–350 (2002).

146. Haren, L. *et al.* NEDD1-dependent recruitment of the γ-tubulin ring complex to the centrosome is necessary for centriole duplication and spindle assembly. *J. Cell Biol.* **172**, 505–515 (2006).

147. Ruiz, F., Beisson, J., Rossier, J. & Dupuis-Williams, P. Basal body duplication in *Paramecium* requires γ-tubulin. *Curr. Biol.* **9**, 43–46 (1999).

148. Raynaud-Messina, B., Mazzolini, L., Moisand, A., Cirinesi, A. M. & Wright, M. Elongation of centriolar microtubule triplets contributes to the formation of the mitotic spindle in γ-tubulin-depleted cells. *J. Cell Sci.* **117**, 5497–5507 (2004).

149. Chang, P., Giddings, T. H. Jr., Winey, M. & Stearns, T. ε-tubulin is required for centriole duplication and microtubule organization. *Nature Cell Biol.* **5**, 71–76 (2003).

150. Ohta, T. *et al.* Characterization of Cep135, a novel coiled-coil centrosomal protein involved in microtubule organization in mammalian cells. *J. Cell Biol.* **156**, 87–99 (2002).

151. Fuchs, S. Y., Spiegelman, V. S. & Kumar, K. G. The many faces of β-TrCP E3 ubiquitin ligases: reflections in the magic mirror of cancer. *Oncogene* **23**, 2028–2036 (2004).

152. Rodrigues-Martins, A., Riparbelli, M., Callaini, G., Glover, D. M. & Bettencourt-Dias, M. Revisiting the role of the mother centriole in centriole biogenesis. *Science* 26 Apr 2007 (doi:10.1126/science.1142950).

153. Peel, N., Stevens, N. R., Basto. R. & Raff, J. W. Over-expression of centriole replication proteins *in vivo* induces centriole over-replication and *de novo* centriole formation. *Curr. Biol.* 3 May 2007 (doi:10.1016/j.cub.2007.04.036).

DATABASES
The following terms in this article are linked online to:
UniProtKB: http://ca.expasy.org/sprot
CDK-2 | GCP2 | GCP3 | PLK4 | SAS-5 | SAS-6 | SPD-2 | SPD-5 | ZYG-1

FURTHER INFORMATION
Mónica Bettencourt-Dias's homepage:
http://www.igc.gulbenkian.pt/research/unit/80
David M. Glover's homepage:
http://www.gen.cam.ac.uk/Research/glover.htm
Ciliary proteome database: http://www.ciliaproteome.org
Cilia webpage: http://www.cytochemistry.net/Cell-biology/cilia.htm

SUPPLEMENTARY INFORMATION
See online article: S1 (table)
Access to this links box is available online.