The arithmetic of centrosome biogenesis

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Summary

How do cells regulate centrosome number? A canonical duplication cycle generates two centrosomes from one in most proliferating cells. Centrioles are key to this process, and molecules such as centrins, SAS-4 and ZYG-1 govern daughter centriole formation. Cdk2 activity probably couples centrosome duplication with the S phase, and a licensing mechanism appears to limit centrosome duplication to once per cell cycle. However, such mechanisms must be altered in some cells – for example, spermatocytes – in which centrosome duplication and DNA replication are uncoupled. There are also alternative pathways of centrosome biogenesis. For example, one centrosome is reconstituted from two gametes at fertilization; in this case, the most common strategy involves differential contributions of centrioles and pericentriolar material (PCM) from each gamete. Furthermore, centrioles can sometimes form de novo from no apparent template. This occurs, for instance, in the early mouse embryo and in parthenogenetic species and might rely on a pre-existing seed that resides within PCM but is not visible by ultrastructural analysis.

Key words: Centrosome, Centriole, Duplication, De novo formation

Introduction

The centrosome has fascinated cell biologists for over a century since the pioneering studies of Boveri put this organelle in the spotlight (Fig. 1). Centrosomes, which are the major microtubule-organizing centres (MTOCs) of animal cells, comprise a pair of centrioles surrounded by pericentriolar material (PCM), electron-dense material that nucleates most microtubules of the cell. Because centrosomes are the main focus of microtubule nucleation, their number must be carefully regulated: a single centrosome is present early in the cell cycle, but two are present during mitosis to direct bipolar spindle assembly and ensure faithful chromosome segregation. In most cases, this doubling of centrosomes is coupled to DNA replication.

Here, we review the mechanisms that govern centrosome biogenesis and control centrosome number. We focus strictly on the question of organelle generation and do not cover related topics such as centrosome maturation or microtubule nucleation, which have been reviewed elsewhere (Bornens, 2002; Gunawardane et al., 2000). We also do not discuss the basal bodies that derive from centrioles or the spindle pole bodies (SPBs) of yeast cells (reviewed by Dutcher, 2003; Francis and Davis, 2000; Helfant, 2002), except when appropriate to illustrate analogies with centrosome duplication.

First, we discuss the canonical centrosome duplication cycle that generates two centrosomes from one in most proliferating cells, emphasizing recent work that has identified important molecular players. We also mention how coupling between centrosome duplication and DNA replication can be altered – for example, when centrosome duplication naturally takes place prior to meiosis II during spermatogenesis or occurs in an uncontrolled manner in cancer cells. Second, we discuss instances in which centrosome biogenesis occurs outside the canonical duplication cycle. For example, in most species, the centrosome of the one-cell stage embryo is reconstituted from paternally contributed centrioles and maternally contributed PCM, thus generating one complete centrosome from two partial entities. Elsewhere, including in some parthenogenetic species and in the mouse embryo, centrioles appear de novo, the centrosome forming from no apparent preexisting structure. In the last part of the Commentary, we discuss future research directions that are likely to help unravel a question that has intrigued Boveri and many others since: how do cells regulate centrosome number?

From one to two: the canonical centrosome duplication cycle

Centrioles: at the heart of the centrosome duplication cycle

Extensive ultrastructural analysis revealed the remarkable structure of the core constituents of the centrosome – a pair of centrioles (Chretien et al., 1997; Kuriyama and Borisy, 1981; Paintrand et al., 1992; Vorobjev and Chentsov Yu, 1982). Centrioles are open cylinders ~100-150 nm in diameter and 100-400 nm in length that consist of a radial array of nine microtubule triplets, doublets or singlets, depending on the cell type (Fig. 2). Electron microscopy also revealed the features of the duplication cycle that enables the pair of centrioles and surrounding PCM to be reproduced in proliferating cells. First, the two centrioles lose their orthogonal arrangement and split slightly while remaining linked by a flexible connection (Fig. 3A). Second, a new centriole forms perpendicular to each parental centriole (Fig. 3B) and elongates until it reaches the same size (Fig. 3C). Third, the two parental centrioles disconnect fully (Fig. 3D) and the PCM separates to give rise to two independent structures akin to the original one – a pair of centrioles surrounded by PCM (Fig. 3E). Although this series of steps can be considered typical, the exact stage of the cell cycle at which each step occurs can vary between cell types (reviewed

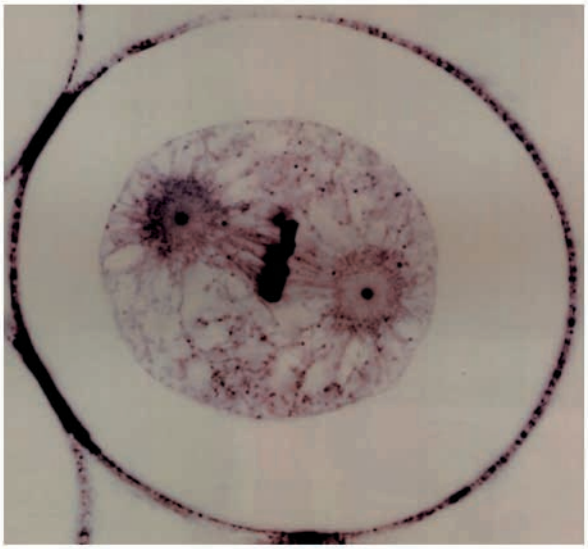

Fig. 1. Boveri's discovery. One of Boveri's slides showing a one-cell stage metaphase embryo of the parasitic nematode *Parascaris equorum*, coloured with Heidenhain's iron haematoxylin method. Reproduced with permission from The American Society for Cell Biology (Gall, 1996). Centrioles appear as prominent dark structures in the centre of each spindle pole. The centrosome is said to have been first spotted by Fleming in 1875 and Van Beneden in 1876, but was extensively studied and named by Boveri a few years thereafter (referenced in Schatten et al., 2000).

by Hinchcliffe and Sluder, 2001). Moreover, the exact sequence of steps can differ. For example, the PCM separates before new daughter centrioles are formed in *Drosophila melanogaster* embryos (Callaini and Riparbelli, 1990).

Importantly, microinjection of biotinylated tubulin into proliferating cells established that labeled tubulin is incorporated solely into the newly formed centriole during the duplication cycle (Kochanski and Borisy, 1990). Therefore, centriole formation is conservative, but distribution of daughter centrioles to the two resulting centrosomes is semi-conservative. As a result, each newly formed centrosome contains a mother centriole and a daughter centriole. Because one of the two mother centrioles is in fact a grandmother, each centrosome has a unique generational pedigree.

What is the relationship between centrioles and the PCM? Perturbations of PCM components can affect centriole reproduction. For example, expression of a dominant-negative form of the PCM component AKAP450 results in displacement of the endogenous protein and impairs centriole duplication (Keryer et al., 2003). Conversely, disruption of centrioles in vertebrate cells following injection of antibodies directed against centriole-specific polyglutamylated tubulin is accompanied by dissolution of the PCM (Bobinnec et al., 1998). Similarly, PCM recruitment is compromised in *Caenorhabditis elegans* embryos that have partially formed centrioles (Kirkham et al., 2003). Interestingly, centrioles purified from *Xenopus laevis* can organize surrounding electron-dense material containing characteristic PCM components such as γ-tubulin (Félix et al., 1994). Therefore, centrioles play a crucial role in PCM assembly and, consequently, in determining MTOC number. In addition, centrioles play a key role in centrosome duplication. For instance, in sea urchin embryos from the genus *Lytechinus*, centrosomes stripped of centrioles using a microneedle can no longer duplicate (Sluder et al., 1989b). Most revealing are cases where centrosomes have a single centriole instead of the usual pair. When sea urchin embryos are held in mitosis, the two centrioles in each spindle pole disconnect and the PCM separates, giving rise to four MTOCs, each containing one centriole (Sluder and Rieder, 1985). If the block to cell-cycle progression is relieved, daughter centriole formation ensues (Sluder and Rieder, 1985). Importantly, each centrosome duplicates only one cell cycle later, when a pair of centrioles is present at the onset of the duplication cycle. A similar situation is encountered when wildtype *C. elegans* embryos are fertilized by *zyg-1* mutant sperm that have a single centriole instead of the usual two (O'Connell et al., 2001). Whereas daughter centriole formation occurs during the first cell cycle, the centrosome duplicates only one cell cycle later in this case as well. Such observations indicate that the reproductive capacity of the centrosome correlates with the presence of a centriole pair.

How does the mother centriole ensure assembly of the daughter centriole? In the case of DNA replication, the double-helical nature of the genetic material constitutes the heart of the templating mechanism. Is there an equivalent structural principle for daughter centrioles? Daughter centrioles are not built in continuity with the mother centrioles, nor do they arise from a halving process followed by elongation to the original size – two scenarios that would have been plausible considering the structure at hand. Instead, they always form perpendicular to the proximal end of mother centrioles. This suggests either that this end acts as a seed for assembly or that the distal end prevents budding of daughter centrioles. It is interesting to note that, in *Saccharomyces cerevisiae*, a structure called the half-bridge that defines the site of SPB formation at the next cell cycle is already detected on one side of newly formed SPBs (reviewed by Francis and Davis, 2000). Whereas an analogous structure serving as a seed for daughter centriole formation has not been identified, a functional seed not detectable by electron microscopy might also mark mother centrioles in a polar manner (reviewed by Sluder and Rieder, 1996).

Regardless of whether a functional seed exists, it is unclear from structural features alone why elongation should stop after the daughter centriole reaches a size equivalent to that of the mother centriole. Similarly, structural features alone might not explain why a single daughter centriole should bud next to the proximal end of each mother centriole. Interestingly, when *D. melanogaster* wing imaginal disc cells are maintained for a prolonged period in S phase by a temperature-sensitive allele of Cdk1, unusual centriole configurations are observed (Vidwans et al., 2003). In some cases, the daughter centriole is longer than the mother centriole, indicating that there is no absolute limit to centriole size. In other cases, groups of one mother and two daughter centrioles appear, which suggests that mother centrioles can have more than one assembly site. Such findings raise the possibility that regulatory events, rather than rigid structural constraints, contribute to uniqueness and length control in daughter centriole formation.

Building a daughter centriole

Among the handful of molecules known to be required

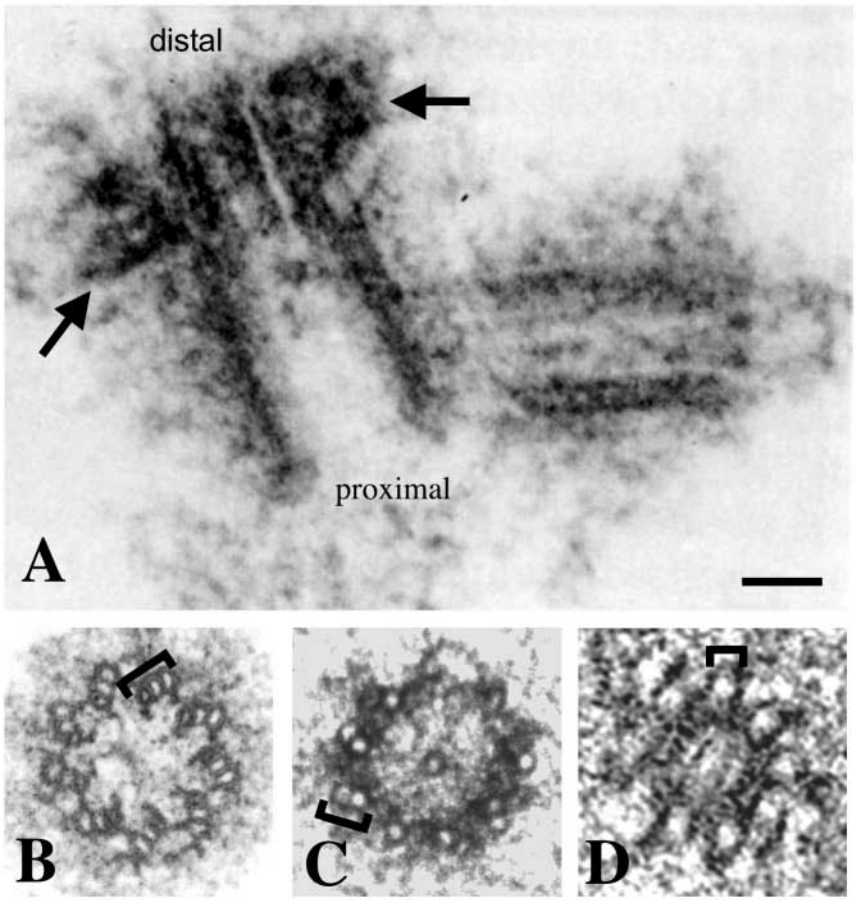

Fig. 2. Centriole structure. Structural features of centrioles in vertebrates [A: side view, B: cross-section; reproduced with permission from Elsevier (Paintrand et al., 1992)], *D. melanogaster* (C: cross-section; courtesy of Patrick O'Farell) and *C. elegans* (D: cross-section; courtesy of Thomas Müller-Reichert). Bar, 100 nm. (A,B) In vertebrates, centrioles have nine sets of triplet microtubules (B, bracket) and are ~150 nm in diameter and ~400 nm long. Note that the long axis of the daughter centriole bisects the mother centriole. Note also that the mother centriole bears elaborate appendages on its distal end (A, arrows; only sub-distal appendages are visible in this picture). In many cells, the mother centriole serves as a basal body for the primary cilium, with the appendages anchoring the basal body to the plasma membrane (reviewed by Preble et al., 2000). However, appendages are also present in cells that do not grow primary cilia (Paintrand et al., 1992), where they may have a distinct anchoring function (Piel et al., 2000). Some proteins, including cenexin (Lange and Gull, 1995), ninein (Ou et al., 2002), OFD-2 (Nakagawa et al., 2001), CEP110 (Ou et al., 2002) or ε-tubulin (Chang et al., 2003) are specific of appendages, and can thus serve to distinguish mother and daughter centrioles using immunofluorescence or GFP fusion proteins. (C,D) Centrioles with nine sets of doublet microtubules in *D. melanogaster* embryos (C, bracket) and nine sets of singlet microtubles in early *C. elegans* embryos (D, bracket). In both species, centrioles are ~100×100 nm in size (Moritz et al., 1995; Vidwans et al., 1999; Wolf et al., 1978). *D. melanogaster* centrioles have singlet microtubules in the early embryo (Moritz et al., 1995), doublet microtubules during later embryogenesis (Vidwans et al., 1999) and doublet or triplet microtubules in sperm cells (discussed by Callaini et al., 1999). *C. elegans* centrioles have singlet microtubules both in the early embryo and in sperm cells (Wolf et al., 1978). Although sub-distal appendages have not been described in *C. elegans*, they are absent from *D. melanogaster* embryonic cells, but present in somatic cells (Rothwell and Sullivan, 2000). The apparent absence of appendages in embryos of both species indicates that they are not essential for centrosome duplication.

for daughter centriole formation, centrins are particularly interesting to consider. Members of this conserved protein family localize to the MTOC and are required for its duplication across eukaryotic evolution (D'Assoro et al., 2001; Paoletti et al., 1996; Spang et al., 1993). Mutations in the centrin CDC31 block SPB duplication in *S. cerevisiae* and *Schizosaccharomyces pombe* (Byers, 1981; Paoletti et al., 2003), whereas RNAi-mediated inactivation of one of the human centrins, centrin-2, prevents daughter centriole formation in HeLa cells (Salisbury et al., 2002). Centrins are small Ca^{2+}-binding proteins of the calmodulin superfamily thought to confer Ca^{2+}-independent elasticity and Ca^{2+}-dependent contractility to fibrous structures (reviewed by Adams and Kilmartin, 2000). How could these mechanical properties explain a requirement for MTOC duplication? Sfi1p, a protein that was identified in budding yeast because of its ability to bind Cdc31p, might provide part of the answer (Kilmartin, 2003). Sfi1p binds multiple copies of Cdc31p through a series of internal repeats, localizes to the SPB and is essential for its duplication. A working hypothesis posits that the Sfi1p-Cdc31p complex forms filaments that confer elasticity and contractility to replicating SPBs. Bioinformatic analysis revealed the existence of distant Sfi1p homologues in other eukaryotes, including centrosome-bearing organisms. Sfi1p homologues in *S. pombe* and humans also bind centrin molecules and localize to the MTOC (Kilmartin, 2003), raising the possibility that they are similarly required for organelle reproduction.

Two other proteins essential for daughter centriole formation are *C. elegans* SAS-4 and ZYG-1. SAS-4 is a coiled-coil protein that localizes to centrioles throughout the cell cycle (Kirkham et al., 2003; Leidel and Gönczy, 2003) (Fig. 4). FRAP experiments established that green fluorescence protein (GFP)-SAS-4 is recruited to centrioles once per cell cycle, coincident with the onset of the duplication cycle (Leidel and Gönczy, 2003). Additional experiments demonstrated that SAS-4 is incorporated strictly in newly forming daughter centrioles, and is stable at that location thereafter, much like tubulin dimers (Kirkham et al., 2003; Leidel and Gönczy, 2003). By contrast, ZYG-1 is a kinase that localizes to centrioles transiently just before daughter centriole formation (O'Connell et al., 2001). The targets of ZYG-1 are not known, but it will be interesting to test whether SAS-4 is one of them.

A divergent tubulin isoform is also involved in daughter centriole formation. ε-tubulin localizes to the sub-distal appendages of the mother centriole in vertebrate cells (Chang

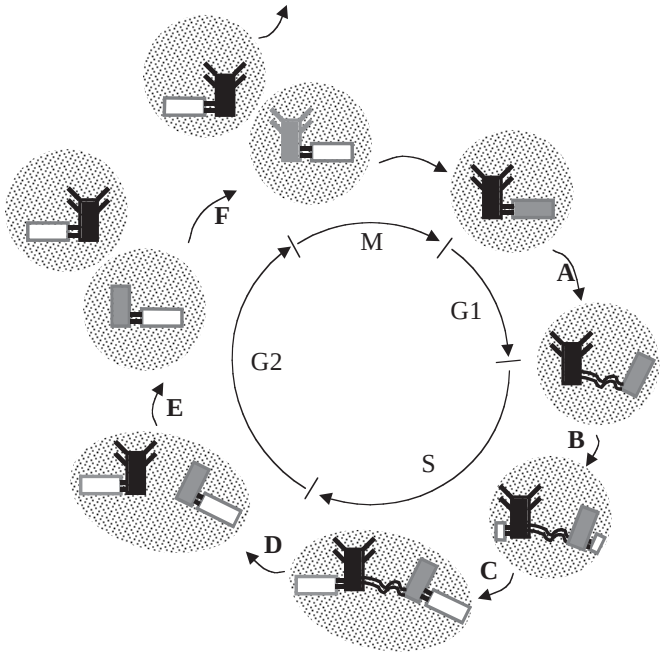

Fig. 3. The canonical centrosome duplication cycle. Cells in early G1 phase have a single centrosome, which comprises a pair of perpendicular centrioles (dark rectangle: mother centriole; grey rectangle: daughter centriole) and surrounding PCM (shaded disk). Usually at the G1-to-S transition, the two parental centrioles loose their arrangement and split slightly from each other (A). During S phase, a new daughter centriole (white rectangle) forms perpendicular to the proximal side of each parental centriole (B) and elongates (C). The two newly formed centriole pairs then disconnect fully (D), as does the PCM (E). Steps D and E involves the Nek2 kinase and its substrate C-Nap1. A working model posits that C-Nap1 connects centrioles within a pair during the bulk of the cell cycle and is phosphorylated by Nek2 in G2 phase, resulting in disconnection of parental centrioles and generation of two distinct centrosomes (Fry, 2002). Full acquisition of appendages on daughter centrioles is achieved by the end of the subsequent cell cycle (F, shown for daughter centriole from previous duplication round).

et al., 2003) (Fig. 2A) and to an analogous location in *Chlamydomonas reinhardtii* (Dutcher et al., 2002). In this green alga, centrioles of cells lacking ε-tubulin are much shorter than normal and are made of nine singlets of microtubules instead of triplets (Dutcher et al., 2002). Moreover, these aberrant centrioles cannot template subsequent duplication events. In vertebrates, immunodepletion of ε-tubulin from *X. laevis* egg extracts prevents daughter centriole formation (Chang et al., 2003). Intriguingly, ε-tubulin is absent from the genomes of *D. melanogaster* and *C. elegans* (Chang et al., 2003) – organisms whose centrioles have microtubule doublets and singlets, respectively – perhaps because the requirement for ε-tubulin function is restricted to centrioles with triplet microtubules.

Coupling DNA replication and onset of centrosome duplication

Although it has been known for a long time that centrosome

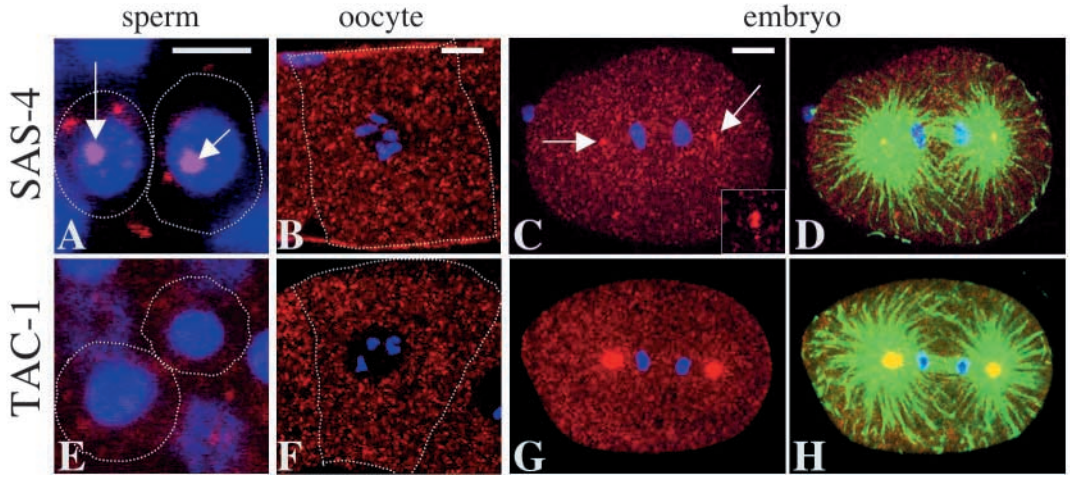

Fig. 4. *C. elegans* centrioles and PCM. SAS-4 or TAC-1 staining in sperm (A,E), oocyte (B,F) and one-cell stage telophase embryo (C and D, G and H). SAS-4 and TAC-1 are shown in red, DNA in blue and microtubules in green (shown only in D,H). The outline of sperm cells and oocytes is indicated. Bars, 10 μm. (A) In sperm, the centrosome is reduced to a pair of centrioles during sperm maturation (A, arrow). Owing to their small size, the two units of the pair cannot be distinguished. Although γ-tubulin has been reported to localize to sperm centrioles (Kirkham et al., 2003), our staining conditions did not allow us to detect a focus of TAC-1 nor other PCM components in mature sperm. Regardless, PCM material potentially provided by the sperm would probably be negligible in comparison to the contribution of the oocyte. (B,F) In the oocyte, centrioles are lacking, but PCM and centriolar components are present diffusely in the cytoplasm. Note the somatic sheath cell nucleus on the top-left in B. (C,D,G,H) After fertilization, a centrosome is reconstituted from a paternally contributed pair of centrioles and maternally contributed PCM components. This reconstituted centrosome then enters the canonical duplication cycle. Splitting of centrioles occurs already during mitosis in embryonic systems where cells oscillate between M and S phase (Callaini and Riparbelli, 1990; Kirkham et al., 2003; Leidel and Gönczy, 2003). Therefore, centrioles of the posterior (right-most centrosome) are sufficiently distant from one another in telophase embryos to be recognized separately (C, right arrow and inset). Note that the two centrioles of the anterior centrosome are not yet distinguishable as individual units at this stage (C, left arrow) (see also Leidel and Gönczy, 2003; O'Connell et al., 2001).

duplication is coupled with DNA replication, the molecular details of the coupling mechanism have begun to be unravelled only recently. Whereas the first signs of daughter centriole formation can occur in G1 phase, initiation of the duplication cycle in most cells coincides with the onset of S phase (reviewed by Sluder and Hinchcliffe, 1998). Cyclin E bound to Cdk2 (Cdk2-E) is thought to govern entry into S phase, and experiments in *X. laevis* egg extracts have established that this kinase is also required for initiating centrosome duplication (Hinchcliffe et al., 1999). Moreover, overexpression of cyclin E leads to premature onset of the duplication cycle in somatic cells (Mussman et al., 2000). Other studies show that cyclin A bound to Cdk2 is required for centrosome reduplication in CHO cells held in S phase (Matsumoto et al., 1999; Meraldi et al., 1999). Although the extent to which different cyclins are required in different organisms and cell types might vary, activation of Cdk2 appears to be a common step for coupling centrosome duplication with DNA replication (reviewed by Sluder and Hinchcliffe, 2000). However, the recent finding that both Cdk2 and cyclin E are dispensable for cell proliferation in the mouse challenges this simple model and suggests that additional components must be able to govern entry into S phase and centrosome duplication (Berthet et al., 2003; Geng et al., 2003; Ortega et al., 2003).

One substrate of Cdk2-E that has been reported to mediate initiation of the duplication cycle in vertebrate cells is nucleophosmin (Okuda et al., 2000; Tokuyama et al., 2001). Nucleophosmin localizes to centrosomes throughout the cell cycle, except when duplication takes place, and expression of non-phosphorylatable nucleophosmin prevents splitting of centrioles and results in monopolar spindle assembly (Okuda et al., 2000). These findings suggested that phosphorylation of nucleophosmin by Cdk2-E leads to its removal from centrosomes, thus enabling centriole splitting and onset of the duplication cycle. Though attractive, the requirement of nucleophosmin has been challenged by recent results that failed to confirm its centrosomal localization or the inhibitory effect on centrosome duplication of two nucleophosmin mutants defective for Cdk2-E phosphorylation (A. Rousselet and M. Bornens, personal communication).

Another substrate of Cdk2-E proposed to play an important role is the coiled-coil protein CP110, whose inactivation by RNAi blocks centrosome reduplication in U2OS cells held in S phase (Chen et al., 2002). A further component that might be regulated by Cdk2-E in vertebrate cells is the Mps1 kinase whose homologue in *S. cerevisiae* is required for duplication of the SPB (Winey et al., 1991). Mouse Mps1 localizes to centrosomes throughout the cell cycle in a Cdk2-E-dependent manner (Fisk and Winey, 2001). Overexpression of wild-type Mps1 drives additional rounds of duplication in S-phase-arrested cells, whereas kinase-dead Mps1 prevents centrosome duplication (Fisk and Winey, 2001). Although human Mps1 also localizes to centrosomes (Liu et al., 2003), the function of vertebrate Mps1 has been questioned following studies using antibody microinjection or RNAi in human cells that failed to find a requirement for it during centrosome duplication (Stucke et al., 2002).

Changes in Ca^{2+} levels at the G1-to-S transition might also contribute to coupling of centrosome duplication with cell-cycle progression. Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) is required for initiating centrosome duplication, since inhibition of intracellular Ca^{2+} or of CaMKII in S-phase-arrested *X. laevis* egg extracts prevents an early step of the duplication cycle, perhaps centriole splitting (Matsumoto and Maller, 2002). Regulated proteolysis is also crucial at an early step, because general inactivation of SCF (Skp1-cullin-F box) E3 ligases by Skp1 or Cul1 antibodies prevents centriole splitting in *X. laevis* egg extracts (Freed et al., 1999). Moreover, mutation of the $Cdc20^{Fizzy}$ moiety of another E3 ligase, APC (anaphase-promoting complex), delays splitting in *D. melanogaster* embryos (Vidwans et al., 1999). These observations underscore the fact that E3 ligase substrates that remain to be identified must be polyubiquitylated and degraded for centriole splitting to occur.

License to duplicate: once per cell cycle

What ensures that centrosome duplication takes place only once per cell cycle? Because multiple duplication cycles proceed in *X. laevis* or sea urchin embryos in which protein synthesis is prevented (Gard et al., 1990; Sluder et al., 1990), centrosome duplication does not necessarily require a given protein to be synthesized at each cell cycle, even though protein synthesis is required for duplication in somatic cells (Phillips and Rattner, 1976). Since centrosome duplication is coupled to DNA replication, S phase might be permissive for duplication and, by extension, centrosome duplication might take place once per cell cycle merely because there is a single S phase. Indeed, centrosome over-duplication occurs not only in *D. melanogaster* Cdk1-mutant wing discs (Vidwans et al., 2003) but also in several cell types held in S phase by hydroxyurea or aphidicolin, including in *D. melanogaster* and sea urchin embryos (Hinchcliffe et al., 1998; Raff and Glover, 1988) as well as CHO or U2OS cells in culture (Balczon et al., 1995; Meraldi et al., 1999). The same is true for *X. laevis* egg extracts, which undergo up to four rounds of centrosome duplication, all but the last one of which presumably correspond to complete cycles because the resulting centrosomes can undergo further duplication (Hinchcliffe et al., 1999).

Whereas such findings are compatible with the view that S phase is permissive for centrosome duplication, the following considerations indicate that the situation is more complex. First, centrosome reduplication is not observed in some cells held in S phase, such as NIH 3T3 cells (Fisk and Winey, 2001). Second, if S phase is merely permissive for duplication, it is difficult to envisage why there is normally a single duplication event irrespective of the duration of S phase, which can vary for instance in *C. elegans* from ~10 minutes in the early embryo to ~60 minutes in larval vulva precursor cells (Edgar and McGhee, 1988; Euling and Ambros, 1996). Third, anomalous centriole configurations can be generated in the course of reduplication when cells are held in S phase, as in Cdk1-mutant wing discs (Vidwans et al., 2003), indicating that this cell-cycle phase cannot always sustain faithful daughter centriole formation. Moreover, analysis of the number and arrangement of centrioles generated when CHO cells are held in S phase suggests that the same mother centriole promotes formation of one daughter centriole per cell-cycle equivalent, which is not able to duplicate further (Sluder and Rieder, 1996). These observations indicate that over-duplication of centrosomes in cells held in S phase recapitulates only in part the events observed during the canonical duplication cycle.

An alternative explanation for the unique occurrence of centrosome duplication arises by analogy with the mechanisms restricting DNA replication to once per cell cycle (reviewed by Nishitani and Lygerou, 2002). In that case, chromatin is licensed for replication prior to S phase through the formation of pre-replication complexes at origins of replication. During S phase, origins are converted into post-replicative complexes that cannot undergo replication until mitosis has been completed. Do similar principles apply in the case of centrosome duplication? Cell fusion experiments conducted in mammalian somatic cells suggest they do (Wong and Stearns, 2003). Analysis of centrosome number following fusion of a cell in G2 with one in S phase indicates that G2 centrosomes are unable to undergo reduplication. This block is intrinsic to the centrosomes and is not imparted by the cytoplasm, because fusion of a G2 cell with a G1 cell does not prevent duplication of the G1 centrosome, only that of the G2 centrosomes. These experiments are compatible with earlier findings in CHO cells held in G2 phase by treatment with the topoisomerase inhibitor etoposide (Balczon et al., 1995) and in *D. melanogaster* embryos held in G2 phase by a mutation in $Cdc25^{string}$ (Vidwans et al., 1999); in both cases, cells blocked in G2 phase do not have more than two centrosomes. A centrosome-intrinsic block to reduplication thus appears to be acquired towards the transition between the S and G2 phases of the cell cycle. Perhaps this block is acquired at slightly different times depending on the cell type, which would explain why only some cells undergo reduplication when held in S phase. One possibility is that this block is due to a duplication inhibitor on G2 centrosomes. Alternatively, lack of duplication might be a default state that is converted after G2 phase by a licensing factor into a state permissive for duplication.

Recent findings suggest that the block to centrosome duplication requires an evolutionarily conserved SCF. In *D. melanogaster*, mutations in *skpA*, which encodes a Skp1 homologue, or in *slimb*, which encodes an F-box protein, result in centrosome over-duplication and other cell-cycle defects (Murphy, 2003; Wojcik et al., 2000). Although cyclin E levels are elevated in *skpA* mutant cells, this does not appear to be the cause of centrosome over-duplication, because this phenotype is still apparent in *skpA*/*cycE* double mutants (Murphy, 2003). In both *X. laevis* and the mouse, the related $SCF^{\beta Trcp1/Slimb}$ is also required to prevent centrosome over-duplication, although to what extent this is independent of other alterations in cell-cycle progression remains to be clarified (Guardavaccaro et al., 2003; Margottin-Goguet et al., 2003). Overall, these findings raise the possibility that $SCF^{\beta Trcp1/Slimb}$ normally targets a protein that restricts centrosome duplication for degradation. Perhaps such a protein is a licensing factor that is normally inactivated throughout G2 to prevent illegitimate reduplication.

Changing the rule: uncoupling centrosome duplication and DNA replication

Although the centrosome duplication cycle is typically coupled with DNA replication, there are interesting exceptions. For example, during spermatogenesis in many species, centrosome duplication and DNA replication are uncoupled prior to meiosis II. Just like somatic cells, primary spermatocytes have two centrioles in each centrosome. The same is true for secondary spermatocytes, as well as for mature sperm (reviewed by Schatten, 1994). This indicates that centrosome duplication occurs prior to meiosis I and then again prior to meiosis II, despite the absence of DNA replication between the two meiotic divisions. It will be interesting to elucidate how the mechanisms imposing a block to reduplication can be altered to allow a second duplication event to take place.

A different regulatory logic is encountered during spermatogenesis in insects (reviewed by Callaini et al., 1999) and oogenesis in species of the starfish genus *Pisaster* (Sluder et al., 1989a), where centrosome duplication occurs solely prior to meiosis I. In the absence of duplication prior to meiosis II, the centriole pair disconnects and each daughter cell inherits a single centriole. In this case, coupling between centrosome duplication and DNA replication is like that in regular somatic cell cycles. However, having a single centriole in mature sperm leads to further uncoupling of centrosome duplication and DNA replication after fertilization. Indeed, because sperm cells fertilize acentriolar oocytes (see below), the single paternally contributed centriole must duplicate twice prior to mitosis to give rise to four centrioles, two in each centrosome.

Uncoupling between centrosome duplication and DNA replication is also apparent in cells undergoing endoreduplication. For example, centrosome duplication does not take place in *D. melanogaster* follicle cells that undergo repeated rounds of DNA replication without intervening mitosis (Mahowald et al., 1979). Because the *D. melanogaster* endocycle is also driven by cyclin E (Lilly and Spradling, 1996), how Cdk2-cyclin-E can initiate repeated S phases without promoting centrosome reduplication remains to be elucidated. Conversely, multiple centrioles can be generated in the course of ciliogenesis in the absence of S phase, although the underlying mechanisms are not understood (reviewed by Dirksen, 1991; Preble et al., 2000).

Cancer cells and supernumerary centrosomes

Coupling between centrosome duplication and DNA replication might also be altered during tumourigenesis. Many cancer cells exhibit increases in centriole or centrosome number, as well as aberrations in centrosome volume or phosphorylation of PCM components (reviewed by Nigg, 2002; Salisbury et al., 1999). Although, in most cases, it remains to be determined whether centrosome abnormalities are a cause or a consequence of tumourigenesis, they can be found in pre-invasive cancer cells and their frequency correlates with tumour progression at least in some instances (Lingle and Salisbury, 1999; Pihan et al., 2001).

How do supernumerary centrosomes arise in cancer cells? They could result merely from aborted cell division or cell fusion events, as is the case when the Aurora-A kinase is overexpressed in $p53^{-/-}$ cells (Meraldi et al., 2002). Alternatively, supernumerary centrosomes could result from uncoupling between centrosome duplication and DNA replication, as exemplified by experiments conducted with the oncoprotein E7 from human papillomavirus (HPV) (reviewed by Duensing and Munger, 2002). Indeed, overexpression of E7 in normal human keratinocytes generates supernumary centrosomes prior to defects in ploidy. This occurs only with E7 from the high-risk HPV-16 and not with E7 from the low-

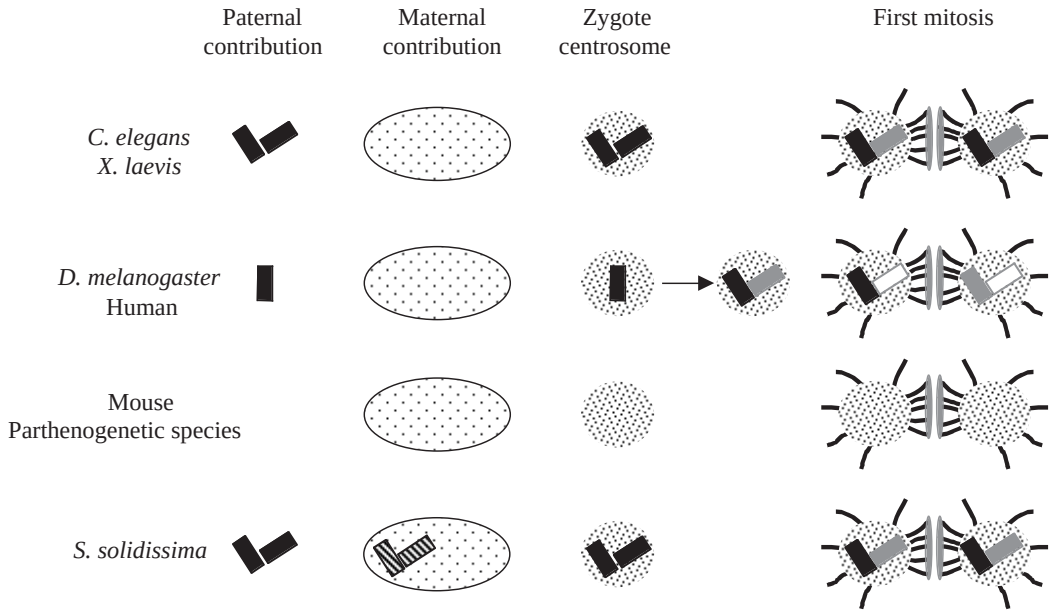

Fig. 5. Diversity of parental requirement for centrosome formation in the zygote. In all cases displayed, PCM components (shaded disk) are contributed from maternal stores. In *C. elegans* and *X. laevis*, the sperm contributes a pair of centrioles (black rectangles), which recruits PCM components from maternal stores to reconstitute one centrosome in the zygote (Karsenti et al., 1984; Wolf et al., 1978). In *D. melanogaster* and humans, the sperm contributes a single centriole (reviewed by Callaini et al., 1999; Manandhar et al., 2000). This is due to the lack of centrosome duplication prior to meiosis II in insects and to the disappearance of one centriole during sperm maturation in primates. In both cases, the single centriole must duplicate twice prior to mitosis to give rise to four centrioles, two in each centrosome. In mice and in many parthenogenetic species, no centrioles are present in the embryo at fertilization. This results from the disappearance of both centrioles during sperm maturation in the mouse (Manandhar et al., 1999) and from the absence of a male gamete altogether in parthenogenetic species (reviewed by Callaini et al., 1999). In both the mouse and parthenogenetic species, the non-centrosomal pathway of spindle assembly presumably ensures bipolarity during mitosis (reviewed by Karsenti and Vernos, 2001). In the clam *S. solidissima*, a pair of centrioles is also contributed maternally, but it is silenced in the zygote and does not nucleate microtubules or duplicate (Wu and Palazzo, 1999).

risk HPV-6, further indicating a possible causative link with tumour development.

What could be the consequences of deregulating centrosome numbers? The absence of centrosome duplication can result in monopolar spindle assembly, whereas excess centrosome duplication can lead to a multipolar spindle. The presence of a monopolar spindle should engage the spindle assembly checkpoint, and thus prevent further cell-cycle progression. However, components of the spindle assembly checkpoint are mutated in several human cancers; lack of centrosome duplication in such cases could yield tetraploid cells (reviewed by McDonald and El-Deiry, 2001). A multipolar spindle can result in the generation of aneuploid daughter cells, because the presence of supernumerary centrosomes does not engage the spindle assembly checkpoint, since kinetochores are correctly attached to spindle microtubules (Sluder et al., 1997). Although extra centrosomes can coalesce at mitosis to give rise to apparently normal bipolar figures and generate two daughter cells of correct ploidy, this is not always the case (reviewed by Brinkley, 2001). Taken together, such considerations indicate that abnormalities in centrosome number can contribute to genome instability.

Variations on a theme: alternative paths of centrosome biogenesis

Fertilization: how to reconstitute one centrosome from two gametes

There are several instances where centrosome biogenesis follows a route different from the canonical centrosome duplication cycle. One such case is fertilization. The formation of the zygote poses a special problem for centrosome number. If each gamete were to contribute one full centrosome, and if duplication of these were coupled to the S phase, there would be four centrosomes at the first mitosis. The analogous problem for DNA content is solved by meiosis generating haploid cells whose joining at fertilization restores the diploid state. What about MTOCs? In budding yeast, the two SPBs fuse after mating (Byers and Goetsch, 1975). Different strategies are used outside fungi in the face of a tough problem: how to make one from two (Fig. 5).

In the clam *Spisula solidissima* and in the brown algae *Fucus distichus*, both gametes provide apparently similar centrosomes at fertilization (Nagasato et al., 1999; Wu and Palazzo, 1999). In these cases, the maternally contributed centrosome is silenced in the zygote and does not nucleate microtubules or duplicate. A variation on this theme is encountered in the starfish *Asterina pectinifera*, where one oocyte-derived centriole is active but discarded in a polar body and the second oocyte-derived centriole is inert and remains in the zygote along with the male centrioles (Uetake et al., 2002). These examples illustrate that centrioles from distinct origins can be differentially regulated within a common cytoplasm.

The most commonly used strategy to solve the problem of centrosome number at fertilization involves differential contributions from the two gametes (reviewed by Schatten, 1994). In *C. elegans*, for example, the centrosome is reduced to a simple pair of centrioles during sperm maturation (Wolf et al., 1978). Conversely, centrioles disappear during oogenesis (Albertson and Thomson, 1993). PCM components remain present in the oocyte cytoplasm, as do centriolar components such as SAS-4 (Fig. 4B,F). At fertilization, the sperm

contributes the pair of centrioles, which recruit PCM from the oocyte, thus reconstituting one functional MTOC from two partial entities. This centrosome then enters the canonical centrosome duplication cycle. Serial-section reconstruction analysis in several species demonstrates that the characteristic centriolar structures disappear during the pachytene stage of oogenesis (reviewed by Schatten, 1994). The mechanisms underlying this disappearance are not understood. Centriole microtubules are extremely stable compared with other microtubules in the cell (Kochanski and Borisy, 1990), which makes it unlikely that centriole disappearance results merely from an overall microtubule-destabilizing environment. Centrioles are lost in other cell types upon differentiation, including when myoblasts fuse into myotubes (Connolly et al., 1986; Tassin et al., 1985) and a similar phenomenon might thus occur during oogenesis. If this were the case, a mechanism that prevents centriole destabilization from taking place during spermatogenesis has to be invoked. Alternatively, centriole disappearance during oogenesis may be an active process. In this context, it is interesting to note that vaccinia virus infection of HeLa cells induces loss of centrosomal and centriolar markers (Ploubidou et al., 2000), raising the possibility that the virus mimics or utilizes cellular components that can actively eliminate centrioles.

Centriole disappearance also occurs during spermatogenesis in some species (reviewed by Manandhar et al., 2000; Schatten, 1994). In humans, for instance, the mother centriole serves as the basal body of the flagellum but is partially destroyed and eventually disappears, whereas the daughter centriole is maintained. After fertilization, this single paternally contributed centriole must duplicate twice prior to mitosis to give rise to four centrioles, two in each centrosome, as discussed above for insects. Loss of centrioles during spermatogenesis is taken to an extreme in the mouse, where both centrioles disappear during sperm maturation (Manandhar et al., 1998). As a result, two acentriolar gametes fuse, and development of the early mouse embryo occurs without any centrioles.

Out of the blue: de novo centriole formation

Mouse embryos do not stay acentriolar for long: normal-looking centrioles become detectable at the preimplantation stage (Abumuslimov et al., 1994; Calarco-Gillam et al., 1983). How can new centrioles appear in the absence of pre-existing ones? Correlative confocal and electron microscopy of mature mouse oocytes revealed the presence of multivesicular aggregates that contain the PCM component γ-tubulin, as well as 25 nm ring structures, which might serve as centriolar precursors (Calarco, 2000). Although the underlying mechanisms are unclear, there are several other instances where centrioles can form de novo that shed some light on this question.

One well-documented example occurs in plants, which normally lack centrioles. Some plant species possess flagellated sperm and therefore need centrioles to generate basal bodies during spermatogenesis (Renzaglia and Maden, 2000). Depending on the species, the number of centrioles in each sperm cell varies from two to thousands. These centrioles are structurally similar to those of animal cells and form de novo in spermatogenous cells, originating within material that resembles PCM (PCM-like material). However, the newly formed structures cannot duplicate further, indicating that they do not possess all the features of bona fide centrioles (Renzaglia and Maden, 2000). Basal bodies also form from no apparent preexisting structure in paraspermatozoa in the annelid *Tubifex tubifex* (Ferraguti et al., 2002) and when the amoeba *Naegleria gruberi* differentiates into a flagellate upon starvation (Levy et al., 1998).

De novo centriole formation is widespread among parthenogenetic species, which, by definition, cannot count on the contribution of the male gamete. These usually resort instead to de novo centriole formation in the zygote to restore centrosome number (reviewed by Callaini et al., 1999). A particularly striking example of plasticity in centrosome biogenesis is observed in the parthenogenetic hymenopteran *Muscidifurax uniraptor* (Riparbelli et al., 1998). After egg activation, many foci of microtubules appear, and their number increases to ~300 as the embryo progresses through the first cell cycle. Electron microscopy and immunofluorescence analysis demonstrate that these foci contain normal centrioles and γ-tubulin. As development proceeds, the number of asters decreases drastically until only two are left in each mitotic cell. Thus, de novo centriole formation in *M. uniraptor* involves a transient stage with excess asters, followed by a reduction to achieve the correct centrosome number. Stick insects of the *Bacillus* genus are also interesting to consider (Marescalchi et al., 2002). Here, there are sexual and parthenogenetic species, which both harbour centrioles in adult somatic cells. Mature sperm cells of the sexually reproducing species are devoid of centrioles and thus give rise to acentriolar embryos after fertilization, as in the parthenogenetic species. In both cases, de novo centriole formation occurs in the zygote. Because early development is similar between sexual and parthenogenetic species, it has been proposed that centriole disappearance during spermatogenesis in sexually reproducing clades might be a primary step towards a parthenogenetic mode of reproduction (Marescalchi et al., 2002).

Are the canonical duplication cycle and de novo centriole formation mutually exclusive pathways of centrosome biogenesis? Interestingly, de novo centriole formation has been observed when parthenogenesis is artificially activated in several species that normally use sexual reproduction, including *D. melanogaster*, sea urchin and rabbit (Kallenbach, 1983; Riparbelli and Callaini, 2003; Szollosi and Ozil, 1991). This suggests that centrioles suppress de novo formation during sexual reproduction. Consistent with this view is the observation that, although de novo formation is normally not observed in *C. reinharditii*, half of the cells lacking primary centrioles because of a mutation in a centrin gene undergo de novo centriole formation during S phase (Marshall et al., 2001). Because only half of the cells devoid of primary centrioles undergo de novo formation, this route of centrosome biogenesis appears to be less efficient than canonical duplication.

Other experiments have established that de novo centriole formation can also be triggered in vertebrate somatic cells (Khodjakov et al., 2002). In CHO, Hela or hTERT-expressing cells held in S phase whose primary centrioles are destroyed with a localized laser microbeam, new centrioles form after 24 hours from foci of PCM-like material that appear as early as 4-5 hours after laser treatment. Extensive serial-section

electron microscopy indicates that remnants of centrioles do not persist following laser treatment. As in the canonical duplication cycle, de novo centriole formation can only take place if these cells are in S phase. Because the new centrioles form 24 hours after laser treatment, it is likely that, under normal circumstances, when S phase duration is ~10 hours in these cells, de novo formation could not be initiated even if primary centrioles were absent. Note, however, that de novo formation can be much more rapid in other cell types, such as when *S. solidissima* oocytes assemble a centriole within 4 minutes of fertilization (Palazzo et al., 1992). In the laser-treated vertebrate cells, the number of newly formed centrioles ranges from 2 to 14 per cell, with some being aberrant (Khodjakov et al., 2002). This raises the possibility that the canonical duplication mechanism is favoured because it ensures a tighter control of daughter centriole number and structure. Furthermore, in the absence of a mechanism regulating excess centrioles, de novo centrosome formation can lead to the generation of multipolar spindles and aberrant cell division. Indeed, this is observed when cells that have centrioles formed de novo are induced to enter mitosis (Khodjakov et al., 2002). To summarize, both *C. reinharditii* and vertebrate cells must lack primary centrioles and be in S phase for de novo centriole formation to occur.

Could the canonical duplication cycle and de novo centriole formation be mechanistically related? It is interesting to note that new centrioles originate from preexisting PCM-like material. This is the case for instance in plant cells during spermatogenesis (Renzaglia and Maden, 2000) and in vertebrate cells after destruction of primary centrioles (Khodjakov et al., 2002). Perhaps de novo centriole formation is initiated from a preexisting seed that resides within the PCM but is not recognizable by ultrastructural analysis. If such a seed exists, it might also serve during the canonical duplication cycle, perhaps tethered to the proximal end of the mother centriole, where it would also not be recognized by electron microscopy, as discussed earlier.

Centrosome biogenesis: the second century

What does the future have in store for the second century of studies of centrosome biogenesis? Comprehensive proteomic approaches have been initiated to characterize all centrosomal components in several organisms, including *D. melanogaster* and humans (Andersen et al., 2003; Lange et al., 2000), and it will be interesting not only to identify all centrosomal proteins in each species but also to compare them between species. Genome-wide RNAi-based gene inactivation approaches have become feasible in organisms other than just *C. elegans*, which should reveal additional key players. It will be also particularly exciting to explore the diversity of mechanisms underlying centrosome biogenesis in non-model organisms. This will allow us to understand better how evolution might have played with the arithmetic of centrosome number. These are some of the many approaches that will probably be important in the years to come, and will help put together the pieces of a puzzle that Boveri defined over one hundred years ago.

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