# The Life Cycle of Centrioles

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Centrioles organize the centrosome and nucleate the ciliary axoneme, and the centriole life cycle has many parallels to the chromosome cycle. The centriole cycle in animals begins at fertilization with the contribution of two centrioles by the male gamete. In the ensuing cell cycles, the duplication of centrioles is controlled temporally, spatially, and numerically. As a consequence of the duplication mechanism, the two centrioles in a typical interphase cell are of different ages and have different functions. Here, we discuss how new centrioles are assembled, what mechanisms limit centriole number, and the consequences of the inherent asymmetry of centriole duplication and segregation.

Centrioles have a complex ninefold symmetry that is remarkably conserved from ciliated protists to humans. The centriole barrel in most organisms contains unique triplet microtubules. The microtubules confer polarity on the centriole; throughout this chapter, we follow the convention of referring to the end of the centriole that nucleates a cilium as the "distal end" and the other as the "proximal end." Most animal cells have two centrioles at the beginning of the cell cycle; we follow the convention of referring to the older of the two (based on their duplication cycle) as the "mother centriole" and the younger as the "daughter." The mother centriole is distinguished by its appendages. Centrioles duplicate in S phase with each new procentriole forming adjacent to an existing parental centriole (Fig. 1).

In many cell types, centrioles are surrounded by a dense protein matrix called the pericentriolar material (PCM), and this combination of centriole and PCM defines the centrosome of animal cells. In cycling cells in $G_1$ phase, the proximal end of the mother centriole is the focus of PCM; however, it is important to note that the components of PCM are often localized to other locations in differentiated cells (Luders and Stearns 2007), conferring centrosome-like microtubule-organizing activities on those other sites. The microtubule-nucleating γ-tubulin ring complex (γ-TuRC) localizes to the PCM throughout the cell cycle and both nucleates microtubules and stabilizes the minus ends of microtubules. During mitosis, centrosomes nucleate spindle microtubules at the spindle poles and are segregated with the chromosomes by virtue of their position in the spindle. A centriole that extends a cilium is called a "basal body." Many mammalian cell types extend a single nonmotile primary cilium during interphase that is nucleated by the older of the two centrioles. Before mitosis, the cilium is disassembled, and the centriole detaches from the plasma membrane.

Here, we discuss recent advances in our understanding of how new centrioles are assembled and how centriole number is regulated. Finally, we address current views of centrosome function, focusing on how the asymmetry in centriole age in interphase affects cell function.

## CENTRIOLE BIRTH AND ENGAGEMENT

The life cycle of the centriole in many animals may be said to begin at fertilization, when the sperm unites its centrioles with proteins in the egg to form a centrosome. In some species, this newly formed centrosome is essential for pronuclear migration and the first mitotic spindle (O'Connell et al. 2000, 2001; Hamill et al. 2002; Dix and Raff 2007). Before the first division of the embryo, the centrioles introduced by the sperm duplicate and take part in organizing the first mitotic division.

New centrioles assemble during S phase of the cell cycle in dividing cells. We refer to the new daughter centrioles that are adjacent to a mother centriole as "procentrioles." Until late in mitosis, the procentrioles are aligned at right angles to their mother centriole with their proximal end juxtaposed to the wall of the mother centriole. This orthogonal arrangement is termed "engagement" and is maintained until the mitosis/interphase transition, when the pair of centrioles becomes disengaged. After disengagement and cell division, the daughter centriole (as well as the mother) duplicates during S phase of the ensuing cell cycle and acquires a set of centriolar appendage proteins during $G_2$ and M of this cycle. These appendages form on the centriole in $G_1$ and are required for cilium formation (Ishikawa et al. 2005) and possibly for other functions of the centriole. Thus, the development of a fully mature centriole requires one and one-half cell cycles (Fig. 2).

Recent work on centriole duplication has identified several procentriole components and determined their function in the centriole assembly pathway. The group of core procentriole assembly proteins includes Sas6, CPAP/Sas4, Cep135/Bld10, and Sas-5/Ana2 (Strnad and Gonczy 2008; Mottier-Pavie and Megraw 2009; Carvalho-Santos et al. 2010; Stevens et al. 2010). These proteins have con-

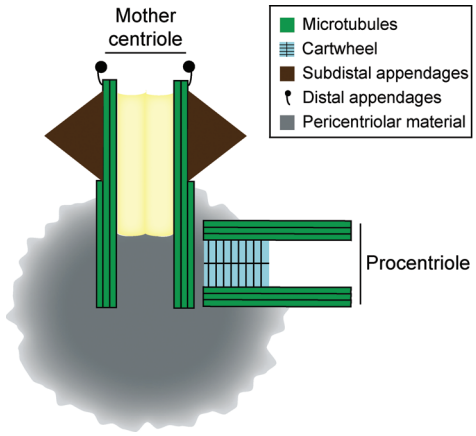


**Figure 1.** Vertebrate centrosome structure. Depicted is a longitudinal section of a $G_2$-phase mammalian centrosome. The immature procentriole is attached to its mother centriole and has an internal cartwheel structure in its proximal half. The fully mature mother centriole has two types of appendages, distal and subdistal, and lacks the cartwheel structure. Mature centriole cylinders are ~150 nm in diameter and ~400 nm long. The base of the mother centriole is embedded in the pericentriolar material, which appears in electron micrographs as darkly staining material around the centrioles.

served centriole assembly functions in a wide range of ciliated organisms (Carvalho-Santos et al. 2010; Hodges et al. 2010). Sas-6 and Cep135 localize to the cartwheel, a ninefold symmetric structure in the proximal procentriole lumen that is important for setting the ninefold symmetry of the centriole (Matsuura et al. 2004; Hiraki et al. 2007; Nakazawa et al. 2007; Rodrigues-Martins et al. 2007a; Jerka-Dziadosz et al. 2010). CPAP/Sas-4 and Sas-5/Ana2 also localize to the procentriole (Leidel and Gonczy 2003; Delattre et al. 2004; Stevens et al. 2010) before assembly of the centriolar microtubules (Leidel and Gonczy 2005; Delattre et al. 2006; Kleylein-Sohn et al. 2007). Sas-4/CPAP may regulate centriole length (Kohlmaier et al. 2009; Schmidt et al. 2009; Tang et al. 2009). Sas-5 has a human ortholog, STIL, which localizes to the centrosome and is associated with microcephaly (Kumar et al. 2009), but it is unknown if this protein also functions in centriole formation.

Several lines of evidence suggest that procentriole formation is a multistep process. First, the stable incorporation of Sas-4 into the procentriole depends on triplet microtubule formation (Pelletier et al. 2006; Dammermann et al. 2008). Second, cells depleted of CP110 local-

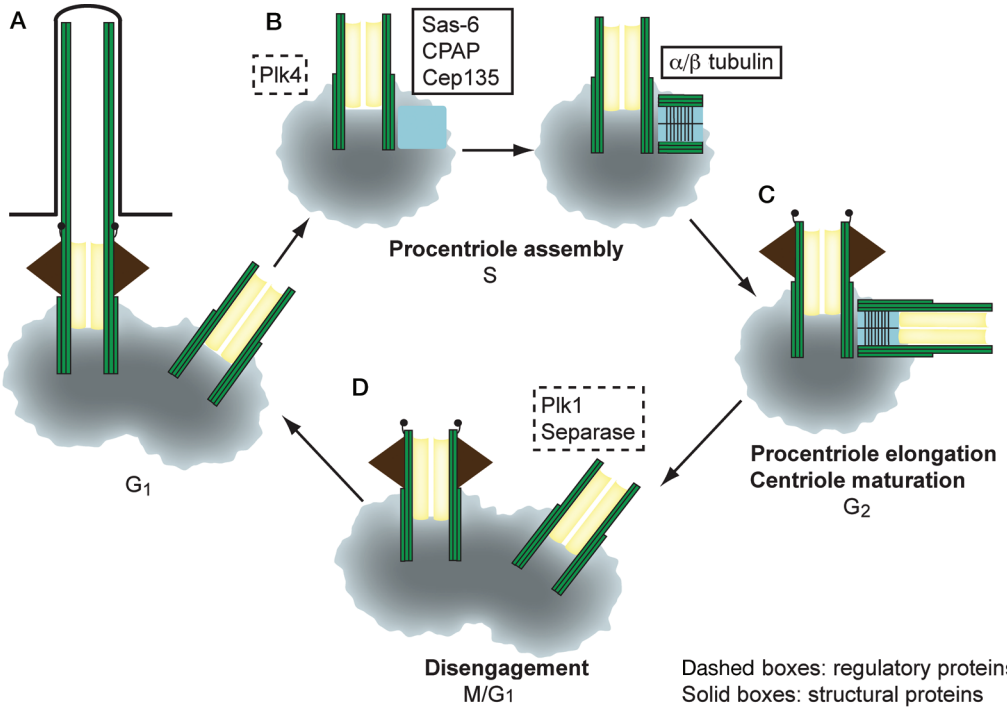


**Figure 2.** Vertebrate centriole duplication cycle following only the daughter centriole. Cell cycle stages are indicated under each image. Important regulators and structural components are indicated. (*A*) In $G_1$, the daughter centriole can recruit pericentriolar material. Primary cilia are often nucleated by the mother centriole at this stage. (*B*) Procentriole assembly occurs during S phase, orthogonal to the base of the parental centriole, and requires Plk4 kinase activity. Structural proteins are recruited to the site of assembly before assembly of the centriolar microtubule triplets. The internal cartwheel structure appears during this stage. (*C*) During $G_2$ and M, the procentriole elongates, and the newer mother centriole (shown) acquires appendage proteins so that by mitosis, each centrosome contains one fully mature centriole and one procentriole. Note that appendages themselves have not been observed on mitotic centrioles; however, the appendage proteins remain associated with the centrioles during mitosis. (*D*) The mother centriole and the procentriole disengage at anaphase and become the mother and daughter centrioles. Disengagement, mediated by separase and Plk1, is a prerequisite for centriole duplication in the next cell cycle. In addition, the cartwheel structure is lost from the daughter centriole at this time.

ize CPAP, Sas-6, and Cep135 to the nascent procentriole but fail to make the centriole microtubule barrel (Kleylein-Sohn et al. 2007). Consistent with this, in *Caenorhabditis elegans*, the central tube in the lumen of the centriole forms before the centriole microtubule barrel, by electron microscopy (Pelletier et al. 2006).

## REGULATION OF CENTRIOLE NUMBER

There are several situations, both normal and pathological, in which the number of centrioles per cell is greater or less than that described for canonical cell division above. To understand the purpose, or consequences, of such situations, it is useful first to consider which processes require centrioles, which are made more efficient by their presence, and which do not require centrioles (Fig. 3). In most cases, this has been assessed in flies and/or worms, in which it is possible to manipulate the embryonic and maternal genotypes and determine the phenotype of the organism. Centrioles are required for cilium formation (Fig. 3A), and organisms that cannot make cilia because of mutation die before birth in mouse (Murcia et al. 2000) or after eclosure in *Drosophila* (Basto et al. 2006). The requirement for cilia in mammalian development is due to the involvement of the primary cilium in Hedgehog signaling (Huangfu et al. 2003). As discussed above, centrosomes increase the efficiency of pronuclear migration in the zygote. In addition, centrioles are required to establish polarity in *C. elegans* embryos (Cowan and Hyman 2004) and to effect the accurate segregation of cell-fate determinants in asymmetric cell divisions (Fig. 3B) (Basto et al. 2006; Castellanos et al. 2008).

In contrast to the above processes, centrosomes are not required for general cell division, cell migration, or axon extension. These points were all shown convincingly in *Drosophila* by Basto et al. (2006), who used mutant alleles of the *Drosophila* ortholog of Sas-4 to create flies lacking centrioles. Remarkably, these flies develop into morphologically normal adult flies; however, these centriole-less flies die soon after birth because of signaling defects resulting from the absence of neuronal cilia. It is important to note that although DSas-4 homozygous mutant flies developed to adulthood, they required the maternal contribution of DSas-4 in the embryo for viability. This requirement presumably reflects the need to maintain centrioles through the embryonic cell divisions, which are much more rapid than somatic cell divisions and require centrosomes to occur efficiently (Klotz et al. 1990; O'Connell et al. 2001; Kirkham et al. 2003; Leidel and Gonczy 2003; Stevens et al. 2007).

Having too many centrioles can be as detrimental to centrosome-dependent functions as having too few. Both embryonic cell division and asymmetric segregation of cell-fate determinants are less efficient when extra centrosomes are present at mitosis (Bornens et al. 1987; Kim and Roy 2006; Basto et al. 2008). Amplification of centrosome number has been known for more than 100 years to be associated with cancer cells and, by extension, thought to be responsible at least in part for the genome instability typical of those cells (for review, see Hardy and Zacharias 2005). The original notion was that extra centrosomes in mitosis resulted in multipolar divisions, ultimately leading to aneuploidy. More recent examination of mitosis with extra centrosomes showed that most such mammalian cells ultimately complete a bipolar mitosis, with the extra centrosomes clustered at the poles (Ring et al. 1982). However, extra centrosomes can contribute to genome instability by increasing the frequency of merotelic chromosome attachments (Ganem et al. 2009; Silkworth et al. 2009), possibly explaining the selective advantage provided to cancer cells by extra centrosomes

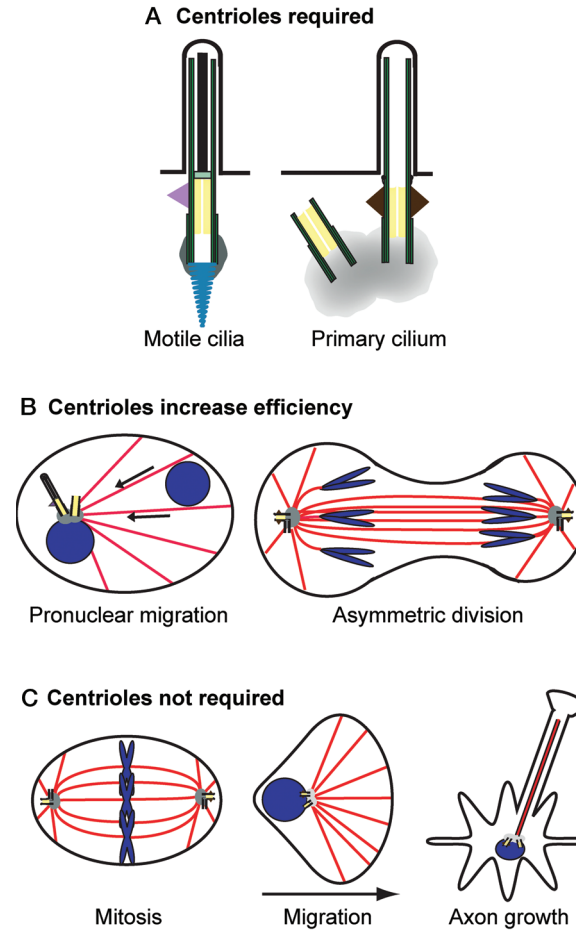


**Figure 3.** Centriole functions. (*A*) Centrioles are required for the formation of both motile and nonmotile cilia and are often referred to as "basal bodies" in these contexts. The two types of cilia differ in their structure and functions (Dawe et al. 2007). (*B*) Centrioles increase the efficiency of asymmetric cell divisions and pronuclear migration. Asymmetric cell division is often mediated by the interaction of spindle microtubules with the cell cortex. In the absence of microtubule nucleation from the centrosome, cells more frequently divide symmetrically, leading to the mis-segregation of cell-fate determinants. After fertilization, the female pronucleus moves along microtubules nucleated by the sperm centrosome to meet the male pronucleus. In the absence of centrosomes, this meeting is delayed or does not occur before the first mitotic division. (*C*) Centrioles are not required for general mitosis, cell migration, and axon growth. Some of these processes require pericentriolar material proteins, but the organization of these proteins around a centriole is not necessary for their function.

(Nigg 2006). In addition, mis-segregation of cell-fate determinants in *Drosophila* larval neuroblasts, as a consequence of having too many or too few centrosomes, also results in tumorigenesis (Basto et al. 2008; Castellanos et al. 2008). Thus, controlling centriole number is critical to proper cell division.

Correct centriole number is maintained by three levels of control: (1) numerical: during each round of duplication only two new centrioles are made; (2) temporal: centriole duplication is restricted to occurring once per cell cycle; and (3) spatial: procentriole formation is restricted to a site adjacent to an existing centriole.

## Number Control

Centriole number control is linked to the activity of the Polo-like kinase Plk4. Although several kinases affect centriole duplication (Strnad and Gonczy 2008), only Plk4 has a dosage-dependent effect on the number of procentrioles nucleated at each parental centriole. Procentriole assembly does not occur in cells lacking Plk4 (Bettencourt-Dias et al. 2005; Habedanck et al. 2005), and overexpression of Plk4 causes multiple procentrioles to develop simultaneously around each mother centriole (Habedanck et al. 2005). In addition, Plk4 overexpression causes *de novo* centriole formation in permissive systems (Peel et al. 2007; Rodrigues-Martins et al. 2007b). Thus, Plk4 can be considered to be a "master regulator" of procentriole assembly.

What prevents Plk4 from accumulating and driving centriole amplification in a normal cell cycle? In mammalian and *Drosophila* cells, Plk4 levels are self-regulating. Plk4 interacts with the ubiquitin ligase complex $SCF^{Slimb\beta\text{-}TrCP}$ (Cunha-Ferreira et al. 2009; Rogers et al. 2009; Holland et al. 2010; Sillibourne et al. 2010) and is subsequently degraded by the proteosome (Duensing et al. 2007). In addition, Plk4 autophosphorylation increases its affinity for Slimb/β-TrCP and thus its degradation rate (Holland et al. 2010; Sillibourne et al. 2010). It is unknown how localization to the centrosome affects Plk4 stability.

Similar to Plk4, overexpression of Sas-6, CPAP/Sas-4, or Sas-5/Ana2 also causes centriole amplification (Peel et al. 2007; Strnad et al. 2007; Kohlmaier et al. 2009; Stevens et al. 2010). The levels of Sas-6 and CPAP protein are kept low in cells starting at the end of mitosis and are low during $G_1$. This regulation is likely mediated by interactions with $APC^{Cdh1}$, another ubiquitin ligase complex (Strnad et al. 2007; Tang et al. 2009). Thus, preventing the accumulation of several centriole assembly proteins may serve as a supplemental block to centriole reduplication.

How does Plk4 initiate centriole formation at the right time and place? Some clues come from three recent papers that identify the centrosome protein Cep152 (Asterless in *Drosophila*) as a binding partner of Plk4 and possibly as a substrate of its kinase activity (Cizmecioglu et al. 2010; Dzhindzhev et al. 2010; Hatch et al. 2010). Remarkably, these three groups reached similar results starting from three quite different experimental systems: *Drosophila*, *Xenopus* eggs, and mammalian cells. Although there are some differences in the details, they each showed that Cep152 interacts with Plk4 and that Cep152, like Plk4, is required for centriole duplication. A simple model then would be that Cep152 mediates the effect of Plk4 on centriole formation, through phosphorylation of Cep152 and subsequent downstream action involving the centriole assembly proteins.

## Temporal Control

Centriole duplication shares several features with DNA replication. Both processes occur at the $G_1$/S transition in cycling cells, and both have intrinsic mechanisms that block reduplication. Centriole reduplication occurs when procentrioles assemble more than once in a single cell cycle. As for DNA duplication, the logic of the centriole cycle is such that the licensing event(s) for centriole duplication is separate from the duplication itself. Procentriole assembly is restricted to S phase, and disengagement, the licensing event for centrioles, does not occur until the transition from mitosis to interphase. This logic was revealed in part by cell fusion experiments similar to those performed by Rao and Johnson to address DNA replication control (Rao and Johnson 1970). Wong and Stearns (2003) used fusion of cells in different cell cycle stages to show that previously duplicated centrioles would not reduplicate in the same cell cycle, even in a cytoplasmic environment competent for duplication.

What is the nature of this centriole-intrinsic block to reduplication? Tsou and colleagues realized that the engagement and disengagement cycle described above might provide an answer (Tsou and Stearns 2006a,b; Tsou et al. 2009). Disengagement of mother from daughter centriole is a prerequisite for centriole duplication, and disengagement requires the action of a protease, separase, that is also required for sister-chromatid separation in mitosis (Oliveira and Nasmyth 2010). Support for this model of centriole duplication licensing has come from elegant experiments showing that removal of a procentriole from the side of a mother centriole by laser ablation results in formation of a single new procentriole adjacent to the mother (Loncarek et al. 2008). In other parallels to DNA replication, disengagement of centrioles is stimulated by Plk1 (Tsou and Stearns 2006b; Tsou et al. 2009) and is inhibited by an isoform of Shugoshin, named sSgo1 (Wang et al. 2008). The proteins that engage the mother centriole and the procentriole and the substrates of separase that are relevant to control of centriole disengagement are unknown. Interestingly, engagement does not prevent the formation of additional centrioles under conditions of Plk4 overexpression (Kleylein-Sohn et al. 2007), suggesting that the temporal and numerical controls on centriole duplication work through separate pathways.

## Spatial Control

Most cell types only make two new centrioles per cell cycle, but they can be driven to make extra centrioles by overexpression of Plk4. Indeed, both animal embryos and human cells have the capacity to make centrioles de novo, in the absence of an existing centriole; however, de novo

centriole formation usually results in the initiation of multiple centrioles, losing number control (Khodjakov et al. 2002; Peel et al. 2007; Rodrigues-Martins et al. 2007b). What is it about formation of new centrioles adjacent to an existing centriole that helps to regulate duplication? Centrioles could exert spatial control over centriole duplication by sequestering and concentrating centriole assembly proteins. Because many centrioles organize a "cloud" of pericentriolar material around their proximal end and because centriole assembly proteins localize to the PCM (Strnad et al. 2007; Dammermann et al. 2008), one possible explanation is that the PCM is required to restrict centriole assembly. However, some species, including *Tetrahymena* and *Drosophila* somatic cells, do not have typical centrosomal PCM associated with centrioles (Stearns and Kirschner 1994; Raff 2004) and, yet, efficiently duplicate their centrioles, indicating that PCM is not universally required for this process. In addition, the loss of proteins required for PCM accumulation around centrioles in *Drosophila* has little effect on centriole duplication (Megraw et al. 2001).

Interestingly, a subset of PCM proteins seems to have evolved later than centriole structural proteins and is only found in vertebrates (Carvalho-Santos et al. 2010; Hodges et al. 2010). Thus, it is possible that PCM proteins could have acquired functions in centriole duplication only in this group. Consistent with this hypothesis, overexpression of PCM proteins can cause centriole amplification in mammalian cells (Loncarek et al. 2008). Another possible function for PCM recruitment is as the licensing event that makes newly disengaged daughter centrioles competent to duplicate. Acquisition of PCM by the daughter centriole appears to coincide with disengagement from the mother centriole in many cell types, but it is unknown if these two events are functionally linked.

## CONSEQUENCES OF CENTRIOLE AGE ASYMMETRY

The mechanism of centriole duplication generates $G_1$ cells with two centrioles of different ages. The daughter centriole of the pair was made in the previous cell cycle, whereas the mother centriole is at least one generation older than the daughter. The mother centriole has associated appendages that impart unique properties relative to the somewhat inert daughter centriole. The distal appendages are involved in the interaction of the mother centriole with the plasma membrane, whereas the subdistal appendages interact with, and stabilize, a set of microtubules. In every cell division, the two mother centrioles segregated during the division differ in age—one just became a mother in that cell cycle, whereas the other was a mother at least once before. Remarkably, this age difference is manifested as a difference in the rate at which the cells project a primary cilium after division, with the cell receiving the older mother making a cilium first (Anderson and Stearns 2009). Because the primary cilium is required for several important signaling pathways, this asynchrony in growing a cilium has potential functional consequences. The basis for this difference is not known, but it might be related to the fact that the older mother has usually been associated with a cilium in a previous cell cycle; perhaps a remaining vestige of that cilium allows the older mother centriole to make a new cilium faster than the newer, cilium-naive, mother.

Centriole age is correlated with the ability of a centriole to form a cilium, but there are other correlations with centriole age that might also have an effect on phenotype. For example, in asymmetrically dividing *Drosophila* larval neuroblasts and male germline stem cells, only one centriole, the mother, accumulates PCM during interphase (Rebollo et al. 2007; Rusan and Peifer 2007; Yamashita et al. 2007). In these cells, the PCM-accumulating centriole remains close to the stem cell niche and cosegregates with the stem cell (Rebollo et al. 2007; Rusan and Peifer 2007; Yamashita et al. 2007; Januschke and Gonzalez 2010). When neither centriole nucleates microtubules or there are multiple PCM-accumulating centrosomes, cell-fate determinants are not accurately segregated (Rusan and Peifer 2007; Basto et al. 2008; Januschke and Gonzalez 2010).

Asymmetric centriole segregation is also observed in radial glial stem cells in mice. The older mother centriole cosegregates with the stem cell in this case also (Wang et al. 2009), and disruption of a centriole appendage protein in developing brain resulted in a failure of asymmetric cell divisions. It is unknown if asymmetric centriole segregation functions in brain cell differentiation explicitly, but several centrosomal proteins have now been shown to be required to maintain the radial glial stem cell population (Wang et al. 2009; Buchman et al. 2010; Ge et al. 2010).

## THE END OF THE CYCLE

If the life cycle of the centriole begins with fertilization, so too it ends in the germline. In most animals, the female germ cell, the oocyte, loses its centriole pair sometime during maturation. Presumably this is part of the mechanism to prevent parthenogenesis, ensuring that development begins with sperm-introduced centrioles. In addition, the sperm centrioles in many species also degenerate, although it is unclear why this would be beneficial (Manandhar et al. 2005). Very little is known about the mechanism of centriole elimination in oocytes. Studies in *C. elegans* identified a requirement for the Cdk inhibitor Cki-2 to eliminate centrioles during oogenesis (Kim and Roy 2006). Overexpression of known centriole assembly proteins, including Plk4, does not prevent centriole elimination (Peel et al. 2007), suggesting that this pathway is distinct from the centriole duplication mechanisms described above.

## FUTURE DIRECTIONS

Although recent studies have advanced our knowledge of the mechanism of centriole assembly and number regulation, there are still many outstanding questions about centriole formation. What "engages" mother and daughter centrioles and what prevents the formation of more procentrioles around such a pair? Electron micrographs of engaged centrioles show that the centrioles do not directly

abut one another but instead are separated by PCM-like material (Loncarek et al. 2008). Second, how is the complex, highly symmetrical (and highly conserved) centriole structure generated from the centriole assembly proteins? Finally, how does the existing centriole dominate centriole assembly, even when components for hundreds of centrioles are present in embryonic cells such as frog and *Drosophila* eggs? Studying how centriole assembly proteins interact to form a centriole and what effect the centrosomal milieu has on these interactions will help to address these remaining mysteries of the centrosome.

## ACKNOWLEDGMENTS

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