

# Centrioles, Centrosomes, and Cilia in Health and Disease

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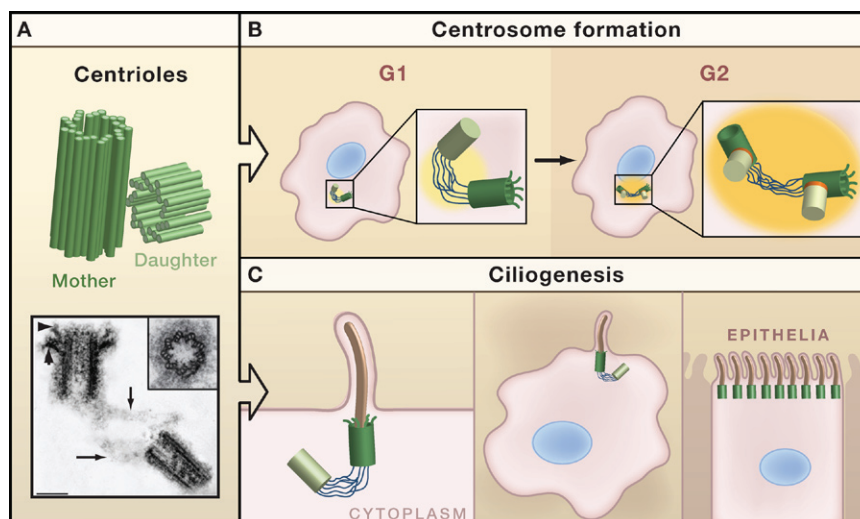
Centrioles are barrel-shaped structures that are essential for the formation of centrosomes, cilia, and flagella. Here we review recent advances in our understanding of the function and biogenesis of these organelles, and we emphasize their connection to human disease. Deregulation of centrosome numbers has long been proposed to contribute to genome instability and tumor formation, whereas mutations in centrosomal proteins have recently been genetically linked to microcephaly and dwarfism. Finally, structural or functional centriole aberrations contribute to ciliopathies, a variety of complex diseases that stem from the absence or dysfunction of cilia.

## Introduction

In the ancestral eukaryote, the early centriolar apparatus is presumed to have been structurally associated with the nucleus and functionally associated with three basic cellular tasks: sensation, motion, and cell division. Among the organisms populating our planet today, centrioles are found in all eukaryotic species that form cilia or flagella but are conspicuously absent from higher plants and yeasts (Marshall, 2009).

Centrioles are complex microtubule-based structures (Figure 1A) that form the basal bodies required for the formation of cilia and flagella (Figure 1C). In addition, a pair of centrioles—embedded in a matrix of proteins known as the pericentriolar material (PCM)—forms the core of the centrosome, the major

organizer of microtubule arrays in animal cells (Figure 1B). In proliferating cells centrioles duplicate exactly once per cell cycle, whereas in some specialized cell types hundreds of basal bodies can form nearly simultaneously (Figure 1C). How the biogenesis of centrioles and basal bodies is controlled has long been mysterious but is now beginning to be unraveled. This progress has important implications for the understanding of human pathology. A causal link between aberrations in the number of centrioles and human cancer has long been proposed (Boveri, 1914, 2008), but direct genetic support for this intriguing notion has yet to be obtained. In contrast, unequivocal genetic evidence now links a variety of other human diseases to mutations in gene coding for both



**Figure 1. Centrioles Form Cilia and Centrosomes**

(A) A centriole pair is shown schematically and as seen by electron microscopy (EM). In the EM image the older, mother, centriole can be distinguished by two types of appendages at its distal end (thick arrow points to subdistal appendages, arrowhead to distal appendages). The diagram shows a newly duplicated centriole pair (mother is dark green, daughter is light green), in which the centrioles are tightly “engaged” (engagement is indicated by an orange disc at the proximal end of the daughter in Figures 1B and 2A); they will remain in this configuration until they have passed through mitosis (see Figure 2A). The EM micrograph shows a centriole pair in G1; the centrioles are “disengaged,” although they remain linked by a fibrous network (arrows). The inset shows a cross-section through a centriole barrel.

(B) In actively proliferating cells, the centrioles organize a pericentriolar matrix (PCM—yellow) to form a centrosome. The PCM efficiently nucleates and organizes cytoplasmic microtubules (not shown), ensuring that the centrosome functions as the dominant microtubule-organizing center

(MTOC) in many cell types. In G1, the single centriole pair usually organizes relatively small amounts of PCM. As the cells prepare to enter mitosis in G2, the duplicated centrioles start to accumulate additional PCM, allowing them to organize many more microtubules during mitosis.

(C) In many cells that are not actively proliferating, the centrosome migrates to the cell surface and a cilium (brown) is assembled from the mother centriole. In certain epithelial cells, many centrioles are assembled at once leading to the formation of multiciliated cells.

The EM image was kindly provided by M. Bornens. Scale bar = 0.2  $\mu$ m.

centrosomal and centriolar proteins (Bettencourt-Dias and Glover, 2007; Bond and Woods, 2006; Gerdes et al., 2009; Quinlan et al., 2008)

Recent studies offer exciting new insight into the physiological roles of centrioles and the pathologies that result from their deregulation. When considered from a mechanistic perspective, human diseases resulting from centriole aberrations are expected to reflect defects in (1) centriole biogenesis, (2) centrosome structure, function, and positioning, or (3) the formation or maintenance of cilia and flagella. In this Review we will summarize our current understanding of the function and biogenesis of centrioles and then focus on their role in disease. Our emphasis will be on the expected impact of centrosome dysfunction on tumorigenesis, the genetic evidence linking centrosomes to the regulation of brain and body size, and the contribution of centriole aberrations to ciliopathies.

### The Functions of Centrosomes and Cilia Cell Division

In most animal cells, centrosomes are a major source of spindle microtubules, and they are absolutely essential for cell division in several early embryonic systems. Perhaps the most striking example of this is provided by studies on the newly fertilized embryo of the frog *Xenopus laevis* (Klotz et al., 1990). As in many species, centrioles are eliminated from the developing oocyte in *Xenopus*, so the centriole is normally supplied to the egg by the fertilizing sperm. Pricking a *Xenopus* egg with a needle mimics fertilization, and the egg proceeds through several rounds of the cell cycle but fails to cleave at the end of each cycle. If, however, a centriole or centrosome is coinjected into the egg as it is pricked, the egg can now divide and, in some cases, develop into a morphologically normal tadpole using only the maternal complement of chromosomes. Thus, in *Xenopus*, the centriole is essential to allow the embryo to divide. Similarly, the fertilized embryos of both the fruit fly *Drosophila* and the nematode *Caenorhabditis elegans* absolutely require functional centrosomes for the earliest stages of embryonic development (Kirkham et al., 2003; Leidel and Gonczy, 2003; O'Connell et al., 2000; Stevens et al., 2007). Conversely, the presence of extra centrosomes in many early embryonic systems leads to the formation of multipolar spindles, which in turn leads to abnormal chromosome segregation and division as the duplicated chromosomes are segregated between the multiple spindle poles. The strong conclusion from these studies is that centrosomes are the major driving force for bipolar spindle assembly and cell division in most animal cells, a view that has dominated the centrosome field for most of the last 100 years.

It is clear, however, that centrosomes are not absolutely essential for division in many cell types. When centrosomes are absent, either naturally (as in higher plants or in the female germ cells of many animal species) or due to experimental manipulation, bipolar spindles can form in the vicinity of chromosomes through a centrosome-independent pathway that involves the small GTPase Ran and the action of microtubule motors and microtubule-bundling proteins (Kalab and Heald, 2008). This pathway also presumably explains the surprising finding that *Drosophila* mutants lacking the centriole duplication protein DSas-4 appear to proceed normally through most of development, provided that a maternally supplied pool of DSas-4 is ini-

tially present to allow centrosome formation during the earliest stages of embryogenesis (Basto et al., 2006). In these mutants, centrioles and centrosomes are undetectable in adult cells, yet adults appear morphologically normal and eclose with near-normal timing at near-normal Mendelian ratios. This is in stark contrast to the rare larvae that develop to adulthood in many mutants that show dramatic defects in cell division (for critical discussion see Gonzalez, 2008). Thus, although spindle assembly is slowed in fly cells that lack centrosomes, flies appear to proceed through most of development relatively normally using only the centrosome-independent pathway of spindle assembly. Perhaps this reflects the fact that *Drosophila* cells have only four chromosomes to segregate, and organisms with larger numbers of chromosomes may depend on the greater efficiency of spindle assembly afforded by centrosomes—an intriguing notion that remains to be experimentally tested.

Interestingly, *Drosophila* mutants that lack centrioles nevertheless die soon after they eclose. Rather than reflecting the absence of centrosomes, however, this death appears to result from the lack of cilia that are essential for the function of certain mechano- and chemosensory neurons. These observations support the view that centrioles may have originally acquired the ability to form centrosomes not to increase the efficiency of cell division but rather to ensure that the centrioles associate with the spindle poles and are thereby equally partitioned between the two daughter cells (Marshall, 2009).

Although many somatic cells can clearly divide without centrosomes, there is compelling evidence that centrosomes are required for the efficient division of cells that split asymmetrically to produce two daughter cells of different fates. Well-studied examples are the early *C. elegans* embryo, *Drosophila* male germline stem cells (GSCs), and *Drosophila* neuroblasts (the stem cell-like progenitors of the *Drosophila* nervous system) (Gonczy, 2008). In these cells, the astral microtubules generated by centrosomes play an important part in aligning the spindle relative to cortical fate determinants, thus ensuring the accurate segregation of the determinants between the two daughter cells. In flies that lack functional centrosomes, ~15% of neuroblast divisions appear to be symmetric (Basto et al., 2006), whereas the asymmetric divisions of male GSCs are also compromised when centrosome function is perturbed (Yamashita et al., 2003). Thus, centrosomes have important, although not essential, roles in these asymmetric divisions.

Remarkably, centrosomes themselves often behave asymmetrically in many instances. In *Drosophila* neuroblasts and male GSCs, for example, only one of the newly separated centrosomes initially nucleates a robust aster of microtubules, which allows this centrosome to maintain a stable position at the apical side of the cell (Rebollo et al., 2007; Rusan and Peifer, 2007; Yamashita et al., 2007). As the cell enters mitosis, the other centrosome starts to nucleate microtubules, but the pre-positioning of the first centrosome ensures that the spindle forms in correct alignment with the cortical determinants. Interestingly, in male GSCs, the oldest centrosome (that contains the older, “mother,” centriole) is always retained within the stem cell (Yamashita et al., 2007). This observation raises the possibility that an “immortal centrosome,” always retained in the stem cell, could help determine stem cell fate

(Morrison and Spradling, 2008). Moreover, a gradual decline in the ability of the male GSCs to maintain proper centrosome orientation as they age is correlated with a gradual loss of their proliferative capacity, as GSCs appear unable to enter mitosis with improperly aligned centrosomes (Cheng et al., 2008). On the other hand, centrosomes do not exhibit an obvious asymmetric behavior during the asymmetric division of *Drosophila* female GSCs, and, unlike neuroblasts and male GSCs, the asymmetric division of female GSCs is not detectably perturbed in the absence of centrosomes (Stevens et al., 2007), arguing that an immortal centrosome is unlikely to be a ubiquitous mechanism for ensuring the maintenance of stem cell fate. A potentially interesting twist to the role of centrioles in determining cell behavior comes from the observation that the relative age of the mother centrioles can determine the timing of primary cilium formation in the two daughter cells formed after cell division (Anderson and Stearns, 2009). This intriguing discovery implies that differences in centriole age can confer an asymmetry to every animal cell division, which in turn may have far-reaching consequences for cell differentiation in vivo.

#### **Centrosomes and the Cell Cycle**

Although centrosomes are not essential for cell division in all cell types, there is evidence that they contribute to efficient cell-cycle progression at both the G1/S and the G2/M transitions. In response to experimentally induced perturbation of centrosomes, some vertebrate cells undergo a G1 arrest, prompting speculation that a specific checkpoint might monitor the functional integrity of the centrosome (Mikule et al., 2007). However, considering that this G1 arrest depends on a pathway involving p38 kinase and p53, it most likely reflects a stress response rather than a specific novel checkpoint (Uetake et al., 2007; Srsen et al., 2006). During S phase, a subpopulation of cyclin E associates with centrosomes and possibly contributes to the regulation of S phase entry (Matsumoto and Maller, 2004), and several cell-cycle regulatory proteins are concentrated at centrosomes and spindle poles during mitosis (Fry and Hames, 2004). These observations have led to the proposal that centrosomes might function as “scaffolds” to promote interactions between various regulatory components during the cell cycle (Doxsey et al., 2005). At the G2/M transition, the key mitotic kinases Cdk1/Cyclin B, Aurora-A, and Polo family members all accumulate at centrosomes, and the mitotic activation of Cdk1 is first detected at centrosomes (Jackman et al., 2003). Furthermore, centrosomal Aurora-A has been implicated in the timing of mitotic entry in *C. elegans* (Hachet et al., 2007; Portier et al., 2007), and, in *Drosophila* embryos, the destruction of Cyclin B at the end of mitosis appears to be initiated at centrosomes (Wakefield et al., 2000).

There are also several reports indicating that components of the DNA-damage checkpoint are concentrated at centrosomes (Doxsey et al., 2005). Although the significance of these observations remains to be fully understood, a strong case can be made for a functionally important link between the DNA-damage response protein Chk2 and centrosomes in *Drosophila* syncytial embryos. In this case, the Chk2-dependent inactivation of centrosomes in response to DNA damage causes the damaged nuclei to fall into the interior yolk region of the embryo, thereby preventing their incorporation into the

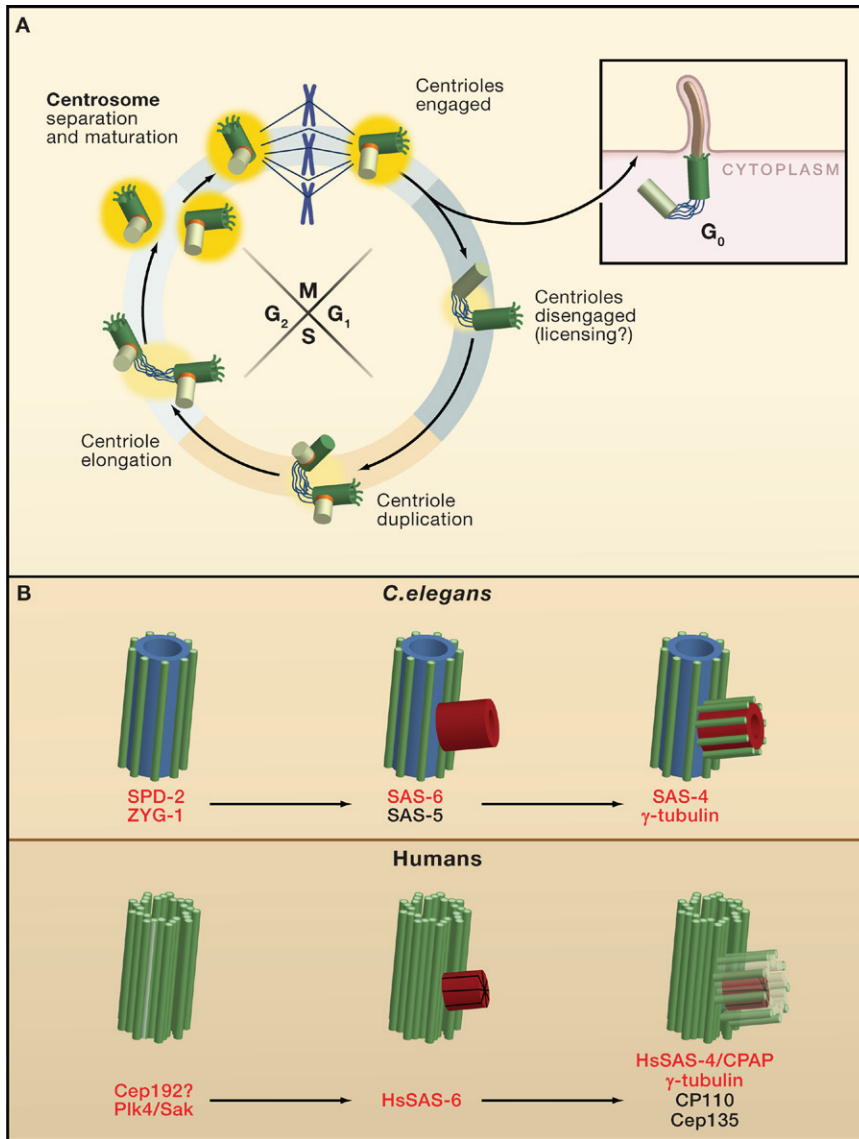
developing embryonic tissues (Takada et al., 2003). Although this exact mechanism is not expected to operate in vertebrate somatic cells, connections between impaired DNA integrity and centrosomes have been observed in mammalian cells (Dodson et al., 2004; Hut et al., 2003). Furthermore, the protein kinase Chk1, a major mediator of the DNA-damage response checkpoint, associates with centrosomes and contributes to the regulation of the G2/M transition even in unperturbed cells (Kramer et al., 2004).

#### **Centriole and Centrosome Function in Differentiated Cells**

The traditional emphasis on the role of centrosomes in cell division should not detract from the fact that these organelles contribute to the organization of microtubule arrays throughout interphase of the cell cycle as well as in postmitotic, differentiated cells. In turn, microtubule arrays are important for determining the shape, polarity, and motility of cells and organisms. For example, studies in *Chlamydomonas* have suggested that the proper positioning of the centriole is required for establishing the overall geometry of the interphase cell (Feldman et al., 2007). Moreover, centrosomes adopt specific positions within many different types of polarized cells, such as migrating fibroblasts, epithelial cells, and neuronal cells (Gundersen, 2002; Higginbotham and Gleeson, 2007; Ueda et al., 1997). Centrosomes are generally thought to be positioned within the cell via centrosome-associated microtubules that are preferentially stabilized at specific cortical sites. In the case of cytotoxic T lymphocytes (CTLs), for example, the centrosome migrates to the site of contact between the CTL and its target cell, where it helps deliver the lytic granules that will ultimately kill the target cell (Stinchcombe et al., 2006).

Taken together, the above studies suggest that centrosomes contribute to the proper spatial organization of many nondividing cells. Importantly, though, in many cases the specific spatial organization of microtubules may depend primarily on the localization of specific PCM components, rather than on structurally intact centrosomes. In particular, although microtubule nucleation and anchoring are often associated with centrosomes, this is not necessarily the case in all cell types. Indeed, many differentiated cells do not contain the “textbook” arrangement of radial microtubule arrays emanating from a centrally located centrosome. Instead, microtubules may be running parallel along the cell axis, as in many epithelial cells, or nucleate from the nuclear envelope, as in differentiated muscle cells (Mogensen, 2004). Moreover, at least some interphase cells that have had their centrosomes removed experimentally can re-establish relatively normal polarized microtubule arrays that appear to lack centrosomes (Wadsworth and Khodjakov, 2004). How these noncentrosomal microtubule arrays are organized remains to be fully understood, but it seems that certain PCM components can dissociate from centrioles and become concentrated at different organizing centers such as the plasma membrane or the nuclear envelope.

Remarkably, there is also evidence that centrosomes have functions that are independent of their ability to organize microtubules. For example, the efficient breakdown of the nuclear envelope in *C. elegans* embryos requires the activity of centrosome-associated Aurora-A in a way that is apparently independent of microtubules (Hachet et al., 2007; Portier et al., 2007).



**Figure 2. The Centriole Duplication Cycle**

(A) A schematic representation of centriole behavior during the cell cycle. At the end of mitosis each new daughter cell inherits a single pair of “disengaged” centrioles. Cells then progress into G1 or enter a quiescent state (G0), during which many cell types will form a cilium. In cycling cells the centrioles duplicate in S phase, with newly born procentrioles (light green) remaining tightly engaged with their mother centrioles (dark green) and gradually elongating throughout S and G2. At the G2/M transition, the centrioles accumulate more pericentriolar material (PCM, yellow) and the two centrosomes start to separate from one another, eventually forming the poles of the spindle in mitosis.

(B) The top illustration depicts centriole duplication in *C. elegans* embryos. SPD-2 recruits the protein kinase ZYG-1 to mother centrioles, which then recruits a complex of SAS-6 and SAS-5. This promotes the formation of a central tube (red) at right angles to the mother centriole. SAS-6 and SAS-5 then recruit SAS-4, which allows the centriolar microtubules (green) to associate with the central tube, thus forming the procentriole. The protein  $\gamma$ -tubulin is also required at about this time. The proteins highlighted in red all have functional orthologs implicated in centriole duplication in other species. The bottom illustration depicts the early events of centriole duplication in human cells. Cep192 is the human homolog of SPD-2, but this protein does not appear to be essential for centriole duplication in flies (Dix and Raff, 2007; Giansanti et al., 2008), and there is conflicting data as to whether it is essential in humans (Gomez-Ferrera and Sharp, 2008; Zhu et al., 2008). The protein kinase Plk4 (Sak in flies) is only distantly related to ZYG-1, but it appears to play an analogous role to ZYG-1 in recruiting human SAS-6 (HsSAS-6) to centrioles, and both Plk4 and HsSAS-6 are essential for centriole duplication. HsSAS-6 seems to be required for the formation of a central “cartwheel” structure (rather than a central tube) (red) and human SAS-4 (HsSAS-4, also called CPAP) and  $\gamma$ -tubulin appear to be required to convert this structure to a procentriole. Several additional proteins, such as CP110 and Cep135/Bld10, have also been implicated in centriole duplication (Dobbelaere et al., 2008; Kleylein-Sohn et al., 2007). This notwithstanding, it is surprising that the duplication of a structure as elaborate as the centriole appears to rely on such a small number of key proteins.

**The Functions of Cilia**

In many nondividing cells the centrioles migrate to the cell surface where the mother centriole forms a basal body that organizes the formation of a cilium or flagellum. The functional identity between centrioles and basal bodies is exemplified beautifully in the green algae *Chlamydomonas*, where the exact same microtubule barrels function as centrioles within centrosomes at the spindle poles during cell division and as basal bodies for the formation of flagella during interphase (Dutcher, 2003). Broadly speaking, there are two types of cilia: motile cilia that have a central pair of microtubules (known as the 9+2 organization) and nonmotile cilia that lack the central pair of microtubules (the 9+0 organization). The importance of motile cilia in several epithelial tissues such as the trachea and bronchial tubes (where the beating cilia function to clear debris) has long been appreciated (Afzelius, 1976). In contrast, although most vertebrate cells can form a single nonmotile primary cilium, it is only recently that the functions of primary cilia have become more widely recognized. An important clue to the

function of primary cilia came from the realization that targeted disruption of the KIF3B kinesin in mice led to defects in left-right asymmetry (*situs inversus*) that were correlated with the loss of primary cilia in the embryonic node (Nonaka et al., 1998). Surprisingly, these 9+0 primary cilia were actually motile, although they exhibit an unusual twirling motion that is quite distinct from the beating of 9+2 motile cilia. The movement of these primary cilia appears to set up a “nodal flow” within the extracellular milieu surrounding the node, and this leads to the asymmetric activation of signaling pathways within the embryo. Intriguingly, *KIF3B* knockout mice also had a variety of other defects in neural, heart, and kidney development that could not readily be explained by the loss of cilia in the node cells, suggesting that primary cilia could have other important functions during development.

The groundwork for understanding the origins of these additional defects came from pioneering studies in *Chlamydomonas* of a process termed intraflagellar transport (IFT) (Pedersen and Rosenbaum, 2008). IFT is the process by which various



cargos move along the ciliary microtubules from the cytoplasm to the tip of the cilia/flagella and then back down to the cell body—a process that is essential for establishing and maintaining cilia/flagella organization. Indeed, defects in IFT are now known to be the cause of the ciliary defects in the *KIF3B* mutant mice. The cloning of one of the genes required for IFT in *Chlamydomonas* revealed that it was related to a gene that caused polycystic kidney disease when mutated in mice (Pazour et al., 2000). In contrast to the node cells, the cilia in kidney cells do not seem to generate liquid flow, but rather to sense it, and this is essential for normal kidney development (Jonassen et al., 2008).

Subsequently, several mouse mutations initially identified on the basis of a failure in sonic hedgehog (*shh*) signaling were also found to reside in genes encoding proteins required for IFT (Corbit et al., 2005; Huangfu et al., 2003). The *shh* signaling pathway is highly conserved and is used in many tissues throughout development. It is also important in adult tissues, as it can promote the proliferation of adult stem cells (Ahn and Joyner, 2005) and is one of the most frequently upregulated signaling pathways in human cancers (Jiang and Hui, 2008). Remarkably, there is now strong evidence that cilia are essential for most, if not all, *shh* signaling in mice, and that several proteins in the *shh* pathway are concentrated in cilia (Singla and Reiter, 2006). Moreover, there is increasing evidence that cilia can modulate several other signaling pathways such as those activated by the Wnt and PDGF $\alpha$  ligands. Unlike their role in the *shh* pathway, however, it seems that cilia are not essential for signaling via these other pathways; instead, they exert a more indirect effect that modulates the strength of signaling (Singla and Reiter, 2006). Studies in frogs and fish have confirmed the importance of cilia in influencing several of these developmental pathways, although the molecular details vary between species (Wessely and Obara, 2008). It remains to be understood why cilia are essential for *shh* signaling in mice but not in *Drosophila*, where *hedgehog* signaling functions in many tissues that lack cilia.

### Biogenesis of Centrioles and Cilia Centriole Formation

Most vertebrate cells contain either two centrioles (one centrosome) or two pairs of centrioles (two centrosomes), depending on whether they are in G1 or G2 of the cell cycle (Figure 1). During S phase, the two centrioles duplicate through the formation, at a near-orthogonal angle, of exactly one new centriole close to their proximal ends (Figure 2). Although centriole numbers are under tight cell-cycle control in most proliferating cells, there are notable exceptions. For example, cells lining the epithelia of the respiratory and reproductive tracts form hundreds of centrioles in order to provide the basal bodies for the formation of beating cilia. These centrioles are generated nearly simultaneously from fibrous aggregates termed deuterosomes. Until recently, centriole formation was therefore thought to occur via one of two distinct pathways, a “centriolar” and an “acentriolar” pathway.

Implicit in this distinction was the assumption that the centriolar pathway is dependent on a “templating” function exerted by the pre-existing centrioles. It has now been recognized, however, that even cells that normally form centrioles through

the centriolar pathway are competent to form new centrioles *de novo*, provided that the resident centrioles are removed beforehand (Khodjakov et al., 2002; La Terra et al., 2005; Marshall et al., 2001). Furthermore, Polo-like kinase 4 (Plk4; also known as Sak), a distant member of the Polo kinase family, was found to be a key regulator of centriole biogenesis in both the centriolar (Bettencourt-Dias et al., 2005; Habedanck et al., 2005) and acentriolar pathways (Peel et al., 2007; Rodrigues-Martins et al., 2007). Thus, the emerging view is that the two pathways share key regulatory elements and that pre-existing centrioles may function primarily as platforms for centriole biogenesis rather than as genuine “templates.” Compared to the *de novo* assembly of centrioles in the cytoplasm, a pre-existing centriole may offer a kinetic advantage to centriole formation by providing a surface that favors the assembly reaction (Loncarek and Khodjakov, 2009). One attractive possibility is that the PCM “cloud” surrounding each centriole may favor centriole assembly by ensuring a higher local concentration of critical assembly factors (Dammermann et al., 2004, 2008; Kirkham et al., 2003; Loncarek et al., 2008). Just as the PCM is important for centriole formation, the centrioles are in turn important for PCM assembly (Bobinnec et al., 1998), suggesting the development of a symbiotic relationship between the centrioles and the PCM during evolution.

Crucial insight into the molecular mechanisms underlying centriole duplication first emerged through a combination of genetic and RNA interference-based approaches with high-resolution light and electron microscopy/tomography applied to centriole biogenesis in *C. elegans* early embryos (Dammermann et al., 2004; Delattre et al., 2004; Kemp et al., 2004; Kirkham et al., 2003; Leidel et al., 2005; Leidel and Gonczy, 2003; O’Connell et al., 2001). These pioneering studies revealed an ordered assembly pathway that involves the products of just five essential genes, termed *zyg-1*, *spd-2*, *sas-4*, *sas-5*, and *sas-6* (Delattre et al., 2006; Pelletier et al., 2006) (Figure 2B). Whereas SAS-4, SAS-5, SAS-6, and SPD-2 are coiled-coil proteins, ZYG-1 is a protein kinase. Shortly after fertilization of the egg, SPD-2 is recruited to the paternal centrioles, which then allows the centriolar recruitment of ZYG-1. Next, a complex comprising SAS-5 and SAS-6 is recruited, which leads to the formation of a “central tube” that is closely associated with the original centriole. Finally, SAS-4 facilitates the assembly of microtubules onto the periphery of this tube, resulting in the formation of a procentriole (Pelletier et al., 2006). Importantly, the significance of these findings is not limited to nematodes. Although an ortholog of SAS-5 awaits definitive identification, SPD-2, SAS-4, and SAS-6 clearly have orthologs in human cells termed Cep192 (Andersen et al., 2003), CPAP/HsSAS-4 (Hung et al., 2000), and HsSAS-6 (Leidel et al., 2005), respectively. Curiously, ZYG-1 does not have obvious structural orthologs outside of nematodes, but the available evidence suggests that Plk4/Sak plays a functionally analogous role in *Drosophila* and human cells (Bettencourt-Dias et al., 2005; Habedanck et al., 2005).

The picture that emerges is that basic mechanisms underlying centriole biogenesis have been conserved during evolution (Figure 2B). Although proteomic studies have revealed a surprisingly large number of centrosome-associated proteins

(Andersen et al., 2003; Keller et al., 2005; Kilburn et al., 2007), it is striking that relatively few core proteins, notably the homologs of ZYG-1, SAS-4, and SAS-6, have been found to be essential for centriole biogenesis in all species examined. SPD-2, on the other hand, is essential in *C. elegans* (Pelletier et al., 2004), but not in *Drosophila* (Dix and Raff, 2007; Giansanti et al., 2008). Thus, different organisms may show distinct requirements for additional proteins in centriole duplication (Dobbelaere et al., 2008; Kleylein-Sohn et al., 2007) (Figure 2B). During centriole biogenesis in human cells, the first proteins to assemble at the site of procentriole formation include HsSAS-6, CPAP/HsSAS-4, Cep135, and  $\gamma$ -tubulin (Kleylein-Sohn et al., 2007). Clearly, these proteins constitute attractive candidate substrates for the centriole duplication kinase Plk4/Sak. Once a procentriolar seed has formed, the protein CP110 then associates with the distal tip of the nascent procentriole, before  $\alpha$ - $\beta$ -tubulin dimers are apparently inserted underneath a CP110 cap during centriole elongation (Kleylein-Sohn et al., 2007).

Not surprisingly, some differences in centriole biogenesis have been noticed between cell types and organisms. For instance, the early stage of procentriole formation in *Chlamydomonas*, *Tetrahymena*, and vertebrate cells is characterized by the appearance of a complex symmetrical structure termed the cartwheel (Culver et al., 2009; Loncarek and Khodjakov, 2009; Matsuura et al., 2004; Nakazawa et al., 2007) that is positioned at the base of the newly born centriole, whereas in *C. elegans* a hollow tube structure initially forms, onto which microtubules are then deposited (Pelletier et al., 2006). Considering that centriole duplication is an ancient process with key regulators conserved during evolution, the observed differences (cartwheel versus tube, for example) most likely represent variations on a common theme, perhaps reflecting differences in the half-lives of intermediate structures, rather than fundamentally distinct mechanisms. In strong support of this view, the protein SAS-6 is required for tube formation in *C. elegans* (Pelletier et al., 2006), whereas its homolog in *Chlamydomonas* and *Tetrahymena* clearly localizes to the cartwheel (Culver et al., 2009; Nakazawa et al., 2007).

Once formed during early S phase, each new centriole elongates throughout the remainder of S and G2 phases. How the final length of the centriole is determined remains to be understood, but recent studies point to antagonistic roles for CP110 and CPAP/HsSAS-4. Whereas CP110 appears to limit centriolar microtubule extension, overexpression of CPAP/HsSAS-4 results in the formation of long microtubule-based structures that extend beyond the normal length of the centrioles (Kohlmaier et al., 2009; Schmidt et al., 2009; Tang et al., 2009). An additional protein recently implicated in centriole duplication and length control is the WD40 domain protein POC1 (Keller et al., 2009).

After cell division, the new centriole is able to function as a parental centriole during S phase, before it reaches full maturity during late G2 or early M phase. This final maturation step is reflected by the acquisition of distal and subdistal appendages (Figure 2A). Thus, it takes more than one complete cell cycle for a newly produced centriole to achieve complete structural maturity. As a result, the two centrioles present within a given human centrosome can readily be distinguished by the fact that only the older (mature) one carries appendages. This structural

difference is functionally important for at least two reasons: first, at least in some cell types, it is the mature centriole that anchors most of the microtubules to the centrosome (Piel et al., 2000) and, second, it is also the mature centriole that is uniquely competent to function as a basal body during ciliogenesis (Ishikawa et al., 2005) (Figure 1). Thus, it would be of considerable interest to identify and functionally characterize centriolar proteins that mark the mother centriole for the addition of appendages. One such candidate is the human POC5 protein, which appears to be specifically required to properly assemble the distal portion of the centriole (Azimzadeh et al., 2009). Intriguingly centrioles can behave asymmetrically in fly stem cells—even though the mother centrioles in most *Drosophila* cells lack recognizable appendages (Callaini and Riparbelli, 1990). This suggests that mother and daughter centrioles can be distinguished even when they both lack appendages.

### The Control of Centriole Number

By analogy to the situation with DNA replication, the maintenance of constant centriole numbers in proliferating cells is likely to require two types of control. A “cell-cycle control” is expected to enforce the rule that centriole duplication occurs exactly once in every cell cycle, whereas a “copy-number control” must ensure that only one new centriole is assembled next to each pre-existing centriole (Nigg, 2007). With regard to cell-cycle control, it has long been established that centriole duplication requires the activity of cyclin-dependent kinases and passage of cells beyond a stage in G1 where the retinoblastoma protein is phosphorylated and E2F transcription factors become active (Nigg, 2002). Additional kinases, notably Plk2 (Warnke et al., 2004), Mps1 (Fisk et al., 2003; see, however, Stucke et al., 2002), and Ndr1 (Hergovich et al., 2007), as well as the DNA replication proteins Orc1 (Hemerly et al., 2009) and geminin (Tachibana et al., 2005) have also been reported to play a role.

Most importantly, elegant cell fusion studies have revealed that there is a centrosome-intrinsic block to reduplication during S and G2 phases, ensuring that duplicated centrioles do not normally duplicate again until they have passed through M phase (Wong and Stearns, 2003). Newly assembled centrioles display a tight, near-orthogonal association with the parental centriole, and this engagement persists from the time of centriole formation during S phase until late mitosis (Figure 2A). The separation of the newly built centrosome from its parent, a process now termed disengagement (formerly disorientation), appears to constitute an essential prerequisite for a new round of duplication (Tsou and Stearns, 2006; Wong and Stearns, 2003), potentially explaining why newly duplicated centrioles must pass through mitosis before they can duplicate again. Although the detailed mechanism underlying centriole disengagement is unclear, the cysteine protease Separase as well as Plk1 have recently been implicated in the process (Tsou and Stearns, 2006; Tsou et al., 2009). The dependency of centriole disengagement on Separase and Plk1 activity provides a plausible and attractive mechanism for ensuring that a license for centriole duplication is issued only as cells pass through mitosis.

How copy-number control operates to ensure the production of only a single centriole next to each pre-existing centriole remains to be understood, but important first clues have emerged. The observation that centriole disengagement is required for centriole

duplication might suggest that there is only a single potential site of assembly on the pre-existing centriole and that this site must be liberated before the next round of duplication. However, there clearly is no fundamental structural limitation to the simultaneous growth of multiple centrioles around the wall of a single “parent” centriole (Kleylein-Sohn et al., 2007). Remarkably, excess Plk4 kinase activity can lead to the simultaneous formation of multiple centrioles around a single parent, suggesting that Plk4 overexpression creates additional sites on “duplication-competent” parental centrioles (Habedanck et al., 2005; Kleylein-Sohn et al., 2007). Intriguingly, the overexpression of the centriole duplication protein SAS-6 also induces the formation of extra centrioles (Leidel et al., 2005; Peel et al., 2007; Rodrigues-Martins et al., 2007; Strnad et al., 2007). Thus, the number of centrioles produced during each S phase is likely dictated by limiting amounts of active Plk4 that in turn recruit limiting amounts of SAS-6 to the parental centriole: excessive production of either protein can lead to the formation of extra daughter centrioles around a single parent within a single cell cycle.

The near-simultaneous formation of hundreds of basal bodies in ciliated epithelial cells has been extensively studied by both light and electron microscopy, but little is known about the molecular aspects of this process, let alone its regulation. With the realization that proteins like Plk4/Sak and SAS-6 underlie not only the canonical (centriolar) pathway of centriole biogenesis but almost certainly also the *de novo* pathway of basal body biogenesis (Kuriyama, 2009; Peel et al., 2007; Rodrigues-Martins et al., 2007), this situation is already changing.

### **The Biogenesis of Cilia**

In cells that form primary cilia, the centrosome inherited during the last round of division migrates to the cortex of the cell where a Golgi-derived vesicle will encapsulate the distal end of the mature centriole, thus initiating cilium extension (Satir and Christensen, 2007). The centriolar appendages are thought to be required to anchor the mature centriole to the plasma membrane, but their precise role remains unknown. Likewise, it remains to be understood how the transition from centriole to basal body is regulated. Of interest in this context, recent experiments suggest that the centriolar proteins Cep97 and CP110 need to be removed from the distal end of the mature centriole to allow the formation of a cilium (Schmidt et al., 2009; Spektor et al., 2007).

The building and maintenance of a cilium crucially depends on the trafficking of membrane vesicles, and this requires the small GTPase Rab8 (Yoshimura et al., 2007), as well as several proteins encoded by genes mutated in Bardet-Biedl syndrome (BBS) (Blacque and Leroux, 2006). Interestingly, seven BBS proteins (BBS1, 2, 4, 5, 7, 8, and 9) have been shown to form a complex of ~450 kDa (termed the BBSome) and to associate with Rabin8, a GTP exchange factor specific for Rab8 (Nachury et al., 2007). These findings confirm and extend the notion that several small GTPases are important for cilia formation and/or function. The assembly and maintenance of cilia also depend on IFT particles as well as microtubule-dependent motor proteins (kinesin II and dynein for anterograde and retrograde transport, respectively) for the delivery of cargo to the growing tip of the structure (Pedersen and Rosenbaum, 2008). How cilia are resorbed upon cell-cycle re-entry is an important question that is only beginning to be addressed at a molecular level,

but recent reports attribute a key role in this process to the Aurora-A family of mitotic kinases (Pan et al., 2004; Pugacheva et al., 2007) as well as the ubiquitin conjugation system (Huang et al., 2009).

### **Centrosomes and Cilia in Human Disease Centrosome Anomalies and Cancer**

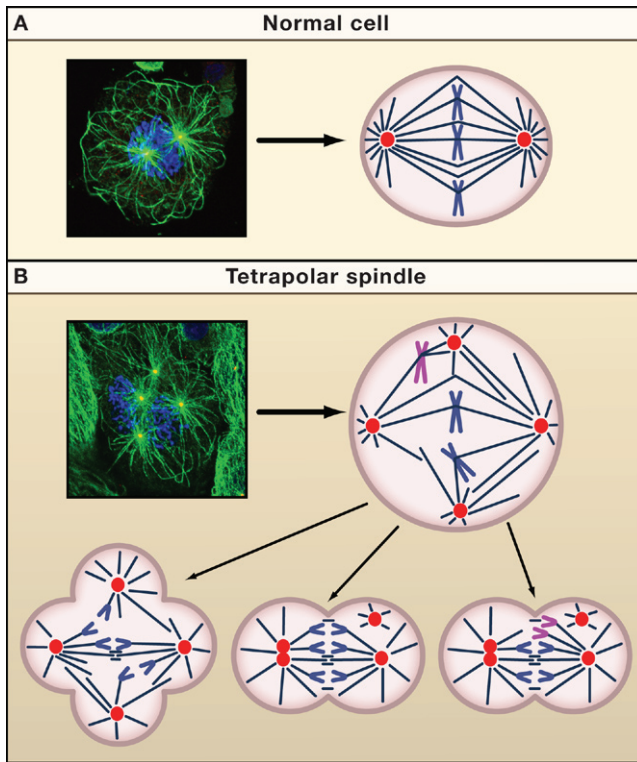
The existence of an important connection between centrosome abnormalities and cancer has long been proposed (Boveri, 1914). In particular, Boveri advanced the hypothesis that cancer frequently arises from chromosome segregation errors during cell division and that numerical centrosome aberrations constitute a plausible source of such errors. Over the past decade, interest in Boveri’s hypothesis has been revitalized and several fundamental questions concerning centrosome abnormalities are beginning to be addressed. First and foremost stands the question of whether centrosome abnormalities do indeed contribute to tumor development, and, if so, through what mechanisms. Second, it is of obvious interest to understand how centrosome abnormalities arise in the first place, how cells respond to them, and what selective advantage they might confer to tumor cells. Finally, the question arises as to whether centrosome abnormalities could be exploited for new diagnostic, prognostic, or therapeutic approaches.

### **Centrosome Anomalies in Tumors**

Centrosome aberrations are commonly observed in many different cancers and are sometimes already present in early, premalignant lesions (Lingle et al., 2002; Pihan et al., 2003). Furthermore, they are often accompanied by extensive chromosome aberrations (D’Assoro et al., 2002; Pihan et al., 2003), a phenotype which in turn correlates with poor clinical outcome (Gisselsson, 2003). Yet, a causal relationship between centrosome abnormalities and cancer has been difficult to establish. The deregulation of several oncogenes and tumor suppressor genes is well known to affect the number of centrosomes (Fukasawa, 2007; Nigg, 2002), but there is a conspicuous absence of direct genetic evidence linking centrosomal proteins to human carcinogenesis. Considering the large number of proteins involved in the assembly of a centrosome (Andersen et al., 2003), one could argue that many genes coding for centrosomal proteins are occasionally mutated in cancers, but that the frequency of mutations in any individual gene is low. Alternatively, it is plausible that alterations in the expression levels of centrosomal proteins may foster centrosome abnormalities.

Although conclusive genetic evidence implicating specific centrosomal proteins in human cancer is currently lacking, recent work has provided a compelling link between centrosomal abnormalities and tumorigenesis in flies. Viable and fertile flies harboring extra centrosomes in ~60% of their somatic cells could be generated by constitutive overexpression of the centriole duplication kinase Plk4/Sak (Basto et al., 2008). Tumorigenesis is not observed in adults, but this is perhaps not surprising, as adult flies do not normally grow tumors, presumably because they have a short life span and there is relatively little cell division in most adult fly tissues. Strikingly, however, when larval brain cells with extra centrosomes were transplanted into the abdomen of normal hosts, these cells could overproliferate and form disseminating tumors that killed the host within weeks, a phe-





**Figure 3. Centrosome Aberrations and Chromosomal Instability**

(A) A normal cell entering mitosis with two centrosomes (red) establishes a bipolar spindle with the chromosomes (blue) aligned at the metaphase plate. (B) A cell entering mitosis with four centrosomes can form a tetrapolar spindle; when the cell divides, the chromosomes may segregate unevenly (left outcome). This occurs in many early embryos with extra centrosomes, providing the basis for Boveri's hypothesis that centrosome amplification can drive chromosomal instability. Many somatic cells with extra centrosomes, however, ultimately divide in a bipolar fashion due to the clustering of centrosomes and the partial inactivation of centrosomes that fail to cluster (middle outcome). Although this type of division does not appear to generate large-scale chromosomal instability, it can generate low-level chromosomal instability. In particular, it has recently been shown that the presence of extra centrosomes leads to an increase in merotelic chromosomal attachments (where both sister kinetochores attach to microtubules from the same spindle pole—as shown here with the purple chromosome). This can lead not only to chromosome missegregation but also to a failure in cytokinesis, as chromosomes left behind at the spindle equator can block the final stages of cytokinesis (right outcome). Micrographs were kindly provided by F. Gergely.

notype that was never seen when normal brain cells were transplanted. In an independent study using the same transplantation assay, mutations causing various centrosomal defects (including the complete loss of centrosomes) were also shown to cause tumors (Castellanos et al., 2008) (Figure 4A).

In addition to providing direct evidence in support of a causal link between centrosome aberrations and cancer, the above *Drosophila* studies also begin to elucidate the underlying mechanism. According to the prevailing model, well rooted in Boveri's original hypothesis, centrosome aberrations are proposed to promote carcinogenesis by fostering aberrant spindle formation during cell division, which in turn leads to chromosomal instability and aneuploidy. Surprisingly, however, although most *Drosophila* cells with extra centrosomes initially form multipolar spindles, they ultimately divide in a bipolar fashion, due to

the clustering of the extra centrosomes at just two dominant spindle poles, or to the progressive "inactivation" of any of the extra centrosomes that failed to cluster at one of the two poles (Figure 3) (Basto et al., 2008). As a consequence, centrosome amplification led to only a very modest increase in chromosomal instability, in line with the viability of these flies.

Interestingly, the mitotic clustering of extra centrosomes has also been observed in vertebrate cells in culture (Kwon et al., 2008; Quintyne et al., 2005). Recent studies, however, reveal that centrosome amplification can lead to an increase in chromosome missegregation even when the centrosomes cluster, due to increased rates of merotelic chromosomal attachments to the spindle (where a single sister kinetochore becomes simultaneously attached to two spindle poles—Figure 3B) (Ganem et al., 2009; Silkworth et al., 2009). These studies provide a direct mechanistic link between centrosome amplification and chromosomal instability and suggest that extra centrosomes may initially facilitate the evolution of malignant phenotypes by promoting relatively low levels of chromosome missegregation. Considering the prevalence of centrosome amplification in human cancers, these aberrations may constitute a major cause of the chromosomal instability that is typical of tumor cells.

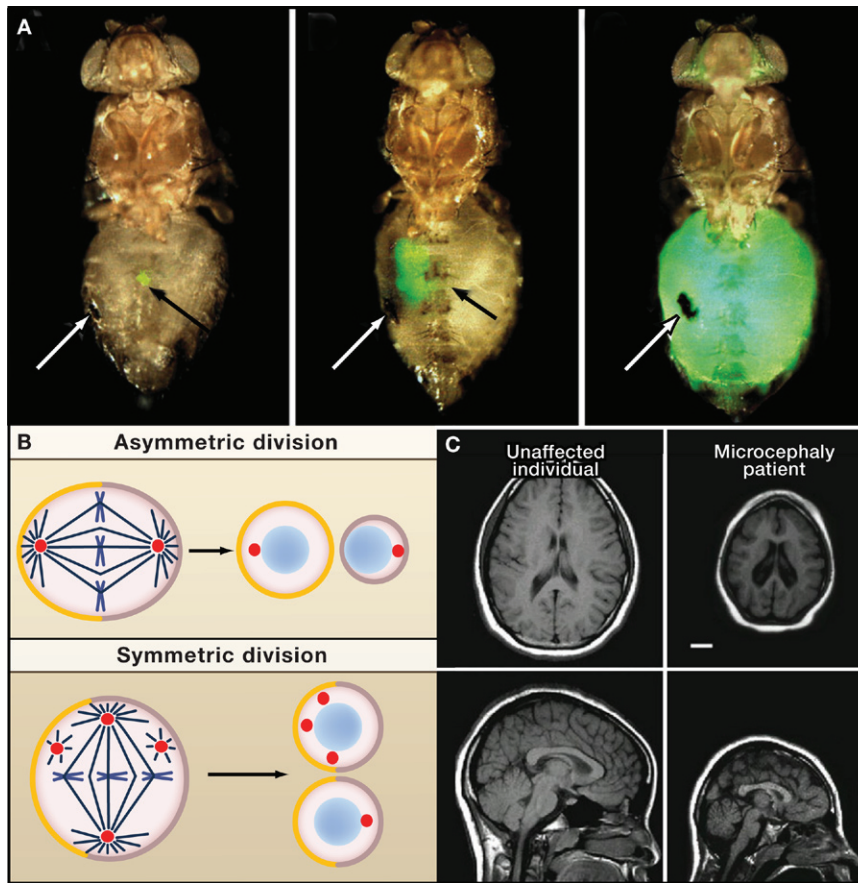
Intriguingly, the studies in flies have also revealed a new potentially important role for aberrant centrosomes in tumorigenesis (Figure 4B). In previous abdominal transplantation studies, a striking correlation had been observed between the ability of injected mutant brain cells to form tumors and defects in the asymmetric divisions of the corresponding mutant larval neuroblasts (Caussinus and Gonzalez, 2005). Defects in the asymmetric divisions of these cells can result in the expansion of the neuroblast population, which ultimately can lead to overproliferation (Betschinger et al., 2006; Lee et al., 2006). As described above, centrosomes are essential for the efficient asymmetric division of neuroblasts, and this process is often perturbed in neuroblasts with amplified or defective centrosomes (Basto et al., 2006, 2008). Thus, centrosome abnormalities could cause tumors through interfering with the asymmetric divisions of neuroblasts, rather than merely through the promotion of chromosomal instability (Figure 4B). Indeed, there is currently much interest in the possibility that alterations in stem cell biology could be central to the generation of cancer, and the abdominal transplantation assay may provide a powerful system with which to address this hypothesis (Gonzalez, 2007).

It is also important to bear in mind that centrosome abnormalities could impact on tumor biology through processes that are unrelated to cell division altogether. In particular, it might be rewarding to explore the possibility that centrosome abnormalities could contribute to tumor progression through microtubule-mediated effects on the shape, polarity, or motility of interphase cells. Centrosome-dependent alterations in these cellular properties might determine the architecture of the tumor tissue as well as the tumor's propensity to metastasize (Nigg, 2002).

#### **Generating Centrosomal Anomalies**

The demonstration that extra centrosomes can lead to tumor formation, at least in flies, begs the question of how these anomalies might arise in the first place. In principle, centrosome amplification can arise through several distinct pathways. First,





**Figure 4. Centrosome Aberrations and Disease**

(A) Studies in flies reveal that centrosomal aberrations can lead to tumor formation. Green fluorescent protein (GFP)-labeled larval brain tissue is transplanted into the abdomen of normal flies (white arrows highlight the scar tissue from the operation, black arrows the transplanted tissue). If normal brain tissue is transplanted (left), the tissue maintains homeostasis and does not grow. If the brain tissue harbors centrosome defects (middle, right), the transplanted tissue can overproliferate (middle, the example shown here is from a *Sak/Plk4* mutant that has reduced numbers of centrosomes) or form aggressive tumors that ultimately kill the host (right, the example shown here is from a *DSas-4* mutant that completely lacks centrosomes).

(B) Centrosome aberrations may contribute to tumorigenesis through interference with the asymmetric division of stem cells. This panel shows *Drosophila* neuroblasts dividing asymmetrically (top cell)—the centrosomal microtubules ensure that the spindle is correctly oriented relative to cortical cell-fate determinants (yellow and purple). In neuroblasts with extra centrosomes (bottom cell) or that have no centrosomes (not shown), some neuroblasts appear to divide symmetrically.

(C) Magnetic resonance imaging (MRI) scans from a microcephaly patient harboring a mutation in *ASPM/MCPH5* (right panels) and an unaffected individual (left panels).

Micrographs were kindly provided by E. Castellanos and C. Gonzalez (A) (reproduced from Castellanos et al., 2008) and G. Woods (C). Reprinted by permission from MacMillan Publishers Ltd: *Nature Genetics*, Bond et al., 2002, copyright (2002).

it may reflect genuine deregulation of the centrosome cycle, due either to centriole overduplication (resulting from successive rounds of duplication in the same S phase) or excessive centriole multiplication (resulting from the near-simultaneous formation of multiple centrioles around the pre-existing centriole pair). Second, it may reflect a failure of cell division, thereby generating a tetraploid cell with four centrosomes. Third, centrosome amplification can arise through cell fusion, for instance under the influence of fusogenic viruses. Finally, the formation of extra spindle poles may occasionally be triggered by the fragmentation of the pericentriolar material, although this mechanism does not represent a genuine amplification of centrosomes. The evidence available so far indicates that deregulation of centrosome duplication and cell division failure represent common causes of centrosome amplification. One major and potentially important difference between these two mechanisms is that only division failure will induce tetraploidization, which under *in vivo* conditions may favor the long-term survival of progeny from multipolar divisions (Nigg, 2002; Storchova and Pellman, 2004).

#### **Centrosome Anomalies as Potential Therapeutic Targets**

Considering that many tumors harbor centrosome abnormalities, the question arises as to whether these abnormalities could be exploited for the clinical management of tumors. One attractive approach stems from the realization that excess centrosomes put an extra burden on the cell division machinery, and cells with extra centrosomes clearly have requirements for the success-

ful completion of mitosis that exceed those of normal cells. For instance, the minus-end-directed kinesin-related motor HSET (Ncd in *Drosophila*) is not essential for mitosis in normal cells but is essential for efficient centrosome clustering in cells with extra centrosomes (Basto et al., 2008; Kwon et al., 2008). Furthermore, although many cells with extra centrosomes ultimately divide in a bipolar fashion, the spindle assembly checkpoint (SAC, the system that monitors whether chromosomes are properly attached to the mitotic spindle before allowing cells to exit mitosis) is needed to provide the time necessary for centrosome clustering, so these cells are more dependent on a functional SAC than normal cells (Basto et al., 2008; Kwon et al., 2008; Yang et al., 2008). A striking visualization of this dependency is provided by studies in *Drosophila*. In contrast to vertebrates, the SAC is not essential during unperturbed cell divisions in flies, so it is dispensable for fly survival (Buffin et al., 2007). In flies with extra centrosomes, however, the SAC is essential for survival as cells require extra time to form a bipolar spindle; in the absence of the SAC, flies with extra centrosomes die during development due to an accumulation of mitotic defects.

The fact that cells with extra centrosomes are dependent for their survival on certain proteins or pathways that are less critical in normal cells implies that inhibiting these pathways could selectively kill cancer cells with extra centrosomes while leaving cells with a normal complement of centrosomes unscathed. In support of this view, perturbing HSET function in human tumor cell lines effectively killed those lines with high

levels of centrosome amplification, but not lines with a largely normal complement of centrosomes (Kwon et al., 2008). A particularly exciting aspect of this approach is that centrosomal anomalies usually increase as tumors develop an increasingly aggressive behavior; thus, the more aggressive the cancer, the more susceptible it might be to this therapeutic strategy.

It is also worth considering the possibility that centrosome abnormalities might be exploited for diagnostic or prognostic applications. In particular, many cancers display not only numerical but also structural centrosome aberrations, most commonly enlarged centrosomes and extracentriolar assemblies of PCM components (Lingle et al., 2002; Pihan et al., 1998). These structures probably result from the marked propensity of many centrosomal coiled-coil proteins to self-assemble and presumably reflect the deregulated expression or modification of these proteins (Nigg, 2002). Although the enlarged centrosomes and PCM assemblies may appear superficially similar, their protein composition and functional properties are expected to differ as a function of the specific alterations in gene expression that characterize each tumor. So, depending on the identity of the centrosomal proteins that are deregulated in a particular tumor, microtubule nucleation may be enhanced or suppressed, and these alterations in turn are expected to influence the shape, polarity, adhesion, or motility of the corresponding cells. Thus, it is conceivable that future profiling studies will reveal correlations between specific patterns of structural centrosome aberrations and certain clinical properties of tumor cells, such as a predisposition to metastasize.

#### **Centrosome Anomalies and Development**

Although a direct causative link between centrosomal anomalies and human cancer is lacking, there are now clear genetic links between several centrosomal proteins and other human disease conditions. Primary autosomal recessive microcephaly is a rare condition in which individuals are born with brains that are much smaller than normal, although the overall organization of the brain is usually unaffected and individuals often suffer only a relatively mild mental retardation (Woods et al., 2005) (Figure 4C). Positional cloning has identified five genes that are mutated in this condition, and, most surprisingly, four of these genes have been shown to encode centrosome-associated proteins (CPAP/HsSAS-4, Cep215/Cdk5Rap2, ASPM, and STIL/SIL) (Bond et al., 2002, 2005; Kumar et al., 2009), whereas the fifth (MCPH1) encodes a transcription factor linked to DNA-damage repair—and even this protein appears to associate with centrosomes (Brunk et al., 2007; Jeffers et al., 2008). CPAP/HsSAS-4 is essential for centriole duplication (Figure 2) whereas Cep215/Cdk5Rap2, ASPM, and SIL/STIL are all members of protein families implicated in centrosome and microtubule organization.

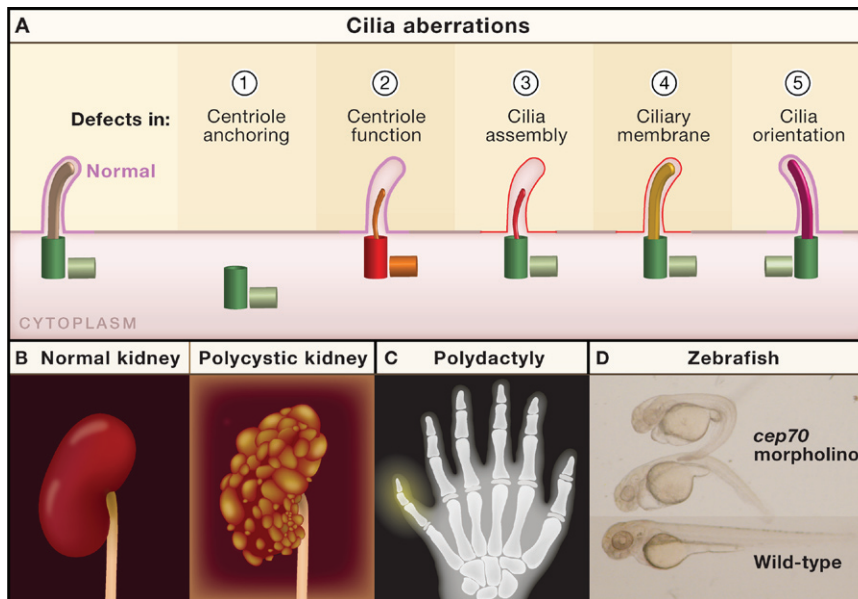
It is difficult to rationalize why mutations in genes encoding centrosomal proteins should produce such a specific defect in human brain development. One possibility is that human neuronal progenitors may be particularly sensitive to centrosome defects because they have to divide asymmetrically during normal brain development (Bond and Woods, 2006), and very recent evidence suggests that this is indeed the case (Wang et al., 2009). As discussed above, centrosome defects do not dramatically perturb mitosis in most somatic fly cells, but the asymmetric division of larval neuroblasts is more noticeably disrupted. Mutations in

DSas-4 (the fly homolog of CPAP/HsSAS-4) or Centrosomin (the fly homolog of Cep215/Cdk5Rap2) both lead to partially defective neuroblast divisions but not to a decrease in brain size. This indicates that flies can compensate for defective neuroblast divisions during development and thereby generate a brain that is largely of normal size (Basto et al., 2006; Lucas and Raff, 2007). Perhaps human brains cannot compensate for defective neuronal divisions as flies do, leading to the development of a small brain in affected individuals. Moreover, not only are *DSas-4* mutant brains not smaller than normal, but they tend to overproliferate and form tumors in abdominal transplantation assays (Castellanos et al., 2008). No predisposition to tumors has been reported in humans with primary autosomal microcephaly, but the significance of this is hard to assess as the numbers of patients analyzed is small.

Although the notion that defects in the asymmetric division of neuronal precursors may lead to microcephaly is attractive, brain development is much more complicated in humans than in flies. Although there is evidence that neural progenitors can divide asymmetrically in mammalian systems, this is controversial, and the process is much less well understood than in flies (Huttner and Kosodo, 2005; see also Wang et al., 2009). Moreover, during brain development in mammals, centrosomes exhibit a series of complex behaviors not only during cell division but also during neuronal migration (Higginbotham and Gleeson, 2007). In this context it is interesting that defects in a different set of centrosome- and microtubule-associated proteins, exemplified by Lissencephaly-1 (Lis-1), appear to specifically perturb centrosome and nuclear behavior during neuronal migration (Tsai et al., 2007). This then leads to lissencephaly (literally “smooth brain,” in which brains lack cortical furrowing) rather than microcephaly.

Recently mutations in another well-studied centrosomal protein, Pericentrin, were identified as a cause of Seckel syndrome (Griffith et al., 2008) and Majewski osteodysplastic primordial dwarfism type II (MOPD II) (Rauch et al., 2008). Intriguingly, both of these disorders cause severe microcephaly but are also associated with a severe reduction in body size as well as several other distinctive features. Why mutations in Pericentrin cause these additional problems when compared to mutations in CPAP/CenpJ, Cep215/Cdk5Rap2, ASPM, and SIL/STIL is unclear, but Seckel syndrome is usually associated with defects in the DNA-damage checkpoint, and cells from at least some of the Seckel syndrome patients carrying mutations in Pericentrin are defective for this checkpoint (Griffith et al., 2008). Thus, Pericentrin may have additional functions when compared to other centrosomal proteins. Moreover, in both flies and human cells, Pericentrin is essential for the assembly of functional cilia (Jurczyk et al., 2004; Martinez-Campos et al., 2004), raising the possibility that ciliary defects could contribute to the more pleiotropic phenotypes observed in individuals carrying mutations in Pericentrin.

A problem in interpreting all of these data is that the mutations being studied are very likely not null alleles. *DSas-4* mutant flies, for example, completely lack centrioles, centrosomes, and cilia and it seems impossible that humans lacking these structures would be phenotypically normal apart from the small size of their brains. Clearly it will be important to make mouse knock-outs of all these proteins to assess the null phenotype and then



**Figure 5. Cilia Aberrations and Disease**

(A) For normal cilia the mother centriole (dark green) organizes a cilium (brown) that is surrounded by a specialized membrane (purple). Depicted are several potential cilia defects. (1) Centrioles are normal, but they fail to migrate, or are not properly anchored, to the cortex. This appears to be the primary defect when Meckelin or MKS1, two proteins associated with Meckel-Gruber syndrome, are depleted from cells (Dawe et al., 2007). This syndrome is usually associated with renal cystic dysplasia and defects in the development of the central nervous system and liver. (2) Centrioles may be structurally defective and so unable to initiate proper cilia formation. This appears to be the case when Pericentrin is mutated in flies or its function perturbed in cultured cells (Jurczyk et al., 2004; Martinez-Campos et al., 2004). Defects in Pericentrin function are associated with Seckel syndrome and primordial dwarfism (Griffith et al., 2008; Rauch et al., 2008); it is unclear, however, whether these traits are due to centrosome defects, cilia defects, or both. (3) Cilia assembly may be defective. This appears to be the primary defect when intraflagellar transport is disrupted, or when ciliary membrane assembly is inhibited by mutations in Bardet-Biedl proteins (Nachury et al., 2007; Pazour et al., 2000). Bardet-Biedl syndrome

is associated with a plethora of clinical features including retinopathy, obesity, polydactyly, and cardiomyopathy (Blacque and Leroux, 2006). (4) Proteins normally present in the specialized membrane surrounding the cilium may be defective. The polycystins, for example, are membrane-spanning receptors/ion channels that are specifically localized to the ciliary membrane and are often mutated in patients with autosomal-dominant polycystic kidney disease (PKD) (Nauli et al., 2003), a common nephropathy that affects 1:1000 people worldwide. (5) Cilia may be positioned in the wrong orientation. It is clear that many cilia, such as motile cilia and the cilia that generate liquid flow, must be displayed in a specific orientation to function properly (Nonaka et al., 2005).

(B) A drawing of a normal (left panel) and polycystic (right panel) kidney.

(C) Polydactyly of the hand.

(D) Developmental defects in zebrafish with defective cilia (induced here with morpholinos against the centriolar protein Cep70). The two morpholino-treated embryos are noticeably shorter than the wild-type and have kinked tails. Micrographs in (D) kindly provided by C. Wilkinson and B. Harris.

make more subtle mutations based on what is known from human pathologies to see if aspects of the human conditions can be reproduced in mice. Moreover, mouse models should allow a better analysis of whether defects in well-defined centrosomal proteins lead to an increased cancer risk.

### Ciliary Defects and Human Disease

Motile and nonmotile cilia have numerous functions in the human body; their dysfunction is associated with several diseases that can be broadly classified as “ciliopathies.” Defects in motile (9+2) cilia, as occurs in Kartagener’s Syndrome for example, are often associated with chronic bronchitis and sinusitis, male sterility, and situs inversus (Afzelius, 1976). For historical reasons these diseases are usually classified as Primary Ciliary Dyskinesias (PCDs), although, confusingly, they are not usually associated with defects in primary (9+0) cilia but with mutations in genes encoding proteins essential for cilia motility, such as ciliary dynein.

As exemplified by Bardet-Biedl syndrome (BBS), diseases linked with defects in primary cilia are usually associated with a bewilderingly broad spectrum of pathologies, including polydactyly, cranio-facial abnormalities, brain malformation, situs inversus, obesity, diabetes, and polycystic kidney disease (Figure 5). Furthermore, although the kidney seems to be an organ that is particularly sensitive to perturbations in cilia function, patients with polycystic kidney disease often have less obvious additional defects in the liver, spleen, heart, and brain (Chang and Ong, 2008). Mutations that perturb primary cilia formation in mice usually lead to embryonic death due to a pleiotropic combination of developmental defects, at least some of which can

be attributed to problems in *shh* signaling (Quinlan et al., 2008). Interestingly, in conditional mouse mutants where cilia function is perturbed only in adults, the first problem to manifest itself is obesity—apparently due to a lack of cilia in the sensory neurons that signal to the mice that they have had enough to eat (Davenport et al., 2007).

At a first glance, the diversity of the phenotypes associated with ciliary dysfunction is perhaps surprising. It is unclear why, for example, BBS and Meckel-Gruber syndrome (MGS) are both usually associated with polycystic kidney disease, but only BBS is associated with obesity, whereas only MGS is associated with a shortening of the limbs. As argued recently, however, the cilium is an extremely complicated organelle, and its assembly can be disrupted in many different ways; as a consequence, individual mutations may differentially perturb different aspects of ciliary function (Marshall, 2008b) (Figure 5).

### Cilia and Cancer

Whereas a link between centrosome defects and cancer was first proposed a long time ago, the potential link between ciliary defects and cancer has received much less attention, even though features of several ciliary diseases indicate that such a link is plausible. The kidney cysts in polycystic kidney disease, for example, are associated with increased cell proliferation and often also with a loss of cell polarity (Jonassen et al., 2008), two features commonly associated with cancers.

How might defects in ciliogenesis or ciliary functions relate to cancer? One obvious possibility is that the vertebrate signaling pathways that rely on cilia (such as the *shh* pathway) or whose activity appears to be modulated by cilia (such as



the Wnt and PDGF $\alpha\alpha$  pathways) are deregulated when ciliary functions are perturbed (Singla and Reiter, 2006). It is tempting to speculate that a potential advantage of linking the activity of these pathways to cilia is that they could be rapidly and specifically regulated by changes in the length of a cilium or by changes in IFT, which in turn might affect receptor density on the cilium or signal propagation into the cell. In such a model, the primary cilium would function in a similar manner to the rheostat of an electric circuit, regulating the amount of intracellular signaling generated by a given amount of extracellular stimuli. It is easy to imagine that interfering with such a regulator could have severe consequences for a cell's ability to maintain homeostasis.

Several recent studies strongly support the conclusion that ciliary biology is highly relevant to carcinogenesis. In mouse models, oncogenic Hedgehog signaling can be induced by expression of a constitutively activated coreceptor, termed *Smoothed*, which results in basal cell carcinomas and medulloblastomas. Consistent with the notion that Hedgehog signaling depends on cilia, the disruption of primary cilia in these mouse models strongly inhibits the formation of both types of tumors (Han et al., 2009; Wong et al., 2009). Conversely, the disruption of cilia enhances tumorigenesis when the Hedgehog signaling pathway is activated downstream of cilia (by expression of an active GLI2 transcription factor). These surprising results indicate that cilia are required to balance different activities in Hedgehog signaling.

Another example of the suppression of tumor formation by primary cilia comes from studies on the von-Hippel-Lindau syndrome tumor suppressor protein (pVHL). VHL syndrome is associated with the formation of tumors in the blood, adrenal gland, and kidney. The pVHL protein is an E3 ubiquitin ligase that has a well-established role in degrading hypoxia-inducible factors (HIFs), and the upregulation of these factors in VHL patients plays an important role in tumor initiation. Unexpectedly, pVHL has recently been shown to be concentrated in cilia and to be required for the maintenance of cilia, presumably reflecting its ability to bind and orient ciliary microtubules (Hergovich et al., 2003; Schermer et al., 2006). The exact contribution of this ciliary function of pVHL to tumor suppression remains to be fully understood, but it is intriguing that a combined loss of the pVHL and PTEN tumor suppressors causes the rapid formation of cysts in mouse kidney (Frew et al., 2008). Clearly, these early observations on the role of cilia in Hedgehog signaling and VHL raise the tantalizing possibility that ciliary defects may contribute to the pathology of several human cancers.

Finally, a potentially important link between cilia and cancer comes from the recent discovery that Aurora-A kinase is required for the resorption of cilia (Pan et al., 2004; Pugacheva et al., 2007). Aurora-A is a centrosome-associated protein kinase that is required for many aspects of mitosis and several lines of evidence link Aurora-A to human cancer (Barr and Gergely, 2007). The *Aurora-A* gene is amplified in a broad range of primary tumors and tumor-derived cell lines, and overexpressing Aurora-A protein can transform certain cell lines in vitro; these lines in turn can form tumors when injected into nude mice. Moreover, the overexpression of Aurora-A leads to

centrosome amplification and chromosomal instability (Meraldi et al., 2004), and Aurora-A has been identified as a low-penetrance cancer susceptibility gene in colorectal cancers (Ewart-Toland et al., 2005). Thus, it is most intriguing that Aurora-A has recently been shown to be required for cilia resorption in mammalian cells (Pugacheva et al., 2007), echoing an earlier study implicating an Aurora-A-like kinase in this process in *Chlamydomonas* (Pan et al., 2004). In the future, it will be interesting to explore the possible contribution of this newly uncovered function of Aurora-A type kinases to oncogenesis.

### Conclusions and Prospects

Recent years have seen tremendous progress toward the identification of key regulators of centriole biogenesis, and hitherto unexpected links between centrioles, cilia, and a plethora of human diseases have been uncovered. This progress raises numerous new questions. Prominent issues to be addressed in the future concern the integration of centriole biogenesis with cell-cycle progression, the detailed molecular mechanisms underlying the production of single procentrioles next to each pre-existing centriole in proliferating cells, and the mechanisms allowing the near-simultaneous production of many basal bodies in multiciliated epithelia. Similarly, much remains to be learned about the cell-cycle cues that prompt the formation and resorption of primary cilia, the transport of centrioles to the plasma membrane, the conversion of centrioles to basal bodies, and the exact role of appendages in ciliogenesis.

From a clinical perspective, the question arises as to whether some of the discoveries linking centrioles and cilia to human pathologies can be exploited for the development of new therapeutic approaches. For example, the well-known centrosome amplification phenotype that characterizes many human cancers offers the prospect of developing strategies for specifically killing such tumor cells by the induction of spindle multipolarity through centrosome declustering. Considering the emerging links between cilia, signaling, and cell-cycle progression, we may also see the emergence of entirely new approaches to the control of cell proliferation. Clearly, however, caution will have to be taken to avoid interfering with ciliary functions in a systemic way. Likewise, the correction of the developmental defects that characterize many ciliopathies will undoubtedly be difficult, although perhaps not impossible in all cases. In particular, it appears legitimate to hope that diseases such as polycystic kidney disease might become treatable thanks to a better understanding of the underlying molecular causes. Last, but not least, it should not be overlooked that cilia and flagella are critical for the survival of many parasites, and this in turn may offer new therapeutic opportunities to combat diseases that continue to afflict many people living in less industrialized parts of the world.

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