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COMMENTARY

The role of mitotic kinases in coupling the centrosome cycle with the assembly of the mitotic spindle

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ABSTRACT

The centrosome acts as the major microtubule-organizing center (MTOC) for cytoskeleton maintenance in interphase and mitotic spindle assembly in vertebrate cells. It duplicates only once per cell cycle in a highly spatiotemporally regulated manner. When the cell undergoes mitosis, the duplicated centrosomes separate to define spindle poles and monitor the assembly of the bipolar mitotic spindle for accurate chromosome separation and the maintenance of genomic stability. However, centrosome abnormalities occur frequently and often lead to monopolar or multipolar spindle formation, which results in chromosome instability and possibly tumorigenesis. A number of studies have begun to dissect the role of mitotic kinases, including NIMA-related kinases (Neks), cyclin-dependent kinases (CDKs), Polo-like kinases (Plks) and Aurora kinases, in regulating centrosome duplication, separation and maturation and subsequent mitotic spindle assembly during cell cycle progression. In this Commentary, we review the recent research progress on how these mitotic kinases are coordinated to couple the centrosome cycle with the cell cycle, thus ensuring bipolar mitotic spindle fidelity. Understanding this process will help to delineate the relationship between centrosomal abnormalities and spindle defects.

KEY WORDS: Cell cycle, Centrosome cycle, Centriole, Pericentriolar material, Mitotic kinase, Spindle assembly

Introduction

The centrosome is the main microtubule-organizing center (MTOC) in animal cells. Under certain circumstances, the centrosome also acts as the basal body of the primary cilium or flagellum, which is crucial for signal transduction, movement and development. A centrosome consists of two centrioles surrounded by the pericentriolar material (PCM); centrioles are conserved organelles comprising nine microtubule triplets that are organized in a symmetric 'cartwheel' (Bettencourt-Dias and Glover, 2007). Conventionally, the PCM is described as an amorphous mass of electron-dense granules. However, using super-resolution microscopy, several groups have found recently that the PCM is actually a highly ordered hierarchical structure, the components of which occupy separable spatial domains around centrioles by adopting a concentric toroidal distribution of a discrete diameter (Fu and Glover, 2012; Lawo et al., 2012; Mennella et al., 2012). Centrosome duplication is coordinated with cell-cycle progression, occurring during S phase, and the duplicated centrosomes are linked together until late G2 phase when they separate to define the opposite spindle poles for mitotic spindle assembly (Fig. 1). At the onset of mitosis, the PCM surrounding the centrioles dramatically increases in size and, remarkably, acquires the capacity for microtubule nucleation and anchoring. This process, termed centrosome maturation, depends on the orchestrated activity of multiple mitotic kinases, including NIMA-related kinases (Neks), cyclin-dependent kinases (CDKs), Polo-like kinases (Plks) and Aurora kinases (Fig. 1).

Mitotic spindle assembly, a complicated but precisely regulated process that is coupled to the centrosome cycle, is required for faithful chromosome separation during cell division in all centrosome-containing cells. Owing to the crucial links between centrosomal functions, mitotic spindle assembly and cell division, defects in the structural dynamics and functions of the centrosome often lead to abnormal mitotic spindle formation, chromosome instability and even tumorigenesis (Basto et al., 2008; Fukasawa, 2007; Holland and Cleveland, 2009). Therefore, characterizing the key factors that participate in the regulation of the functions of the centrosome is extremely important. Using genomics and proteomics analysis, different biological processes that are involved in centrosome dynamics and functions have been identified, including centriole biogenesis, centriole engagement and disengagement, procentriole formation and elongation, and centrosome segregation and maturation (Avidor-Reiss and Gopalakrishnan, 2013; Bettencourt-Dias and Glover, 2007; Mardin and Schiebel, 2012; Tanenbaum and Medema, 2010) (Fig. 1). In this Commentary, we focus on recent advances that illustrate the role of mitotic kinases in orchestrating the regulation of the centrosome cycle and bipolar mitotic spindle assembly, before providing a framework for understanding how abnormal centrosome functions induce unfaithful spindle formation and, possibly, aneuploidy.

The role of mitotic kinases in centriole biogenesis

The centrosome duplicates only once per cell cycle in order to avoid an uncontrolled centriole number, thereby ensuring mitotic spindle fidelity. New insights into centriole biogenesis will greatly enhance our understanding of the orchestration of the centrosome cycle and of mitotic spindle formation. As a number of excellent reviews on centriole biogenesis have been published recently (Avidor-Reiss and Gopalakrishnan, 2013; Gönczy, 2012; Jana et al., 2014), here, we mainly discuss how mitotic kinases function in centrosome duplication and summarize the general aspects of this process (see Table 1).

Functions of mitotic kinases in centriole disengagement

The orthogonal arrangement of the paired centrioles is established during the duplication of the daughter from the mother and maintained until the end of mitosis (Tsou and Stearns, 2006). It has been postulated that a cohesin ring complex plays an

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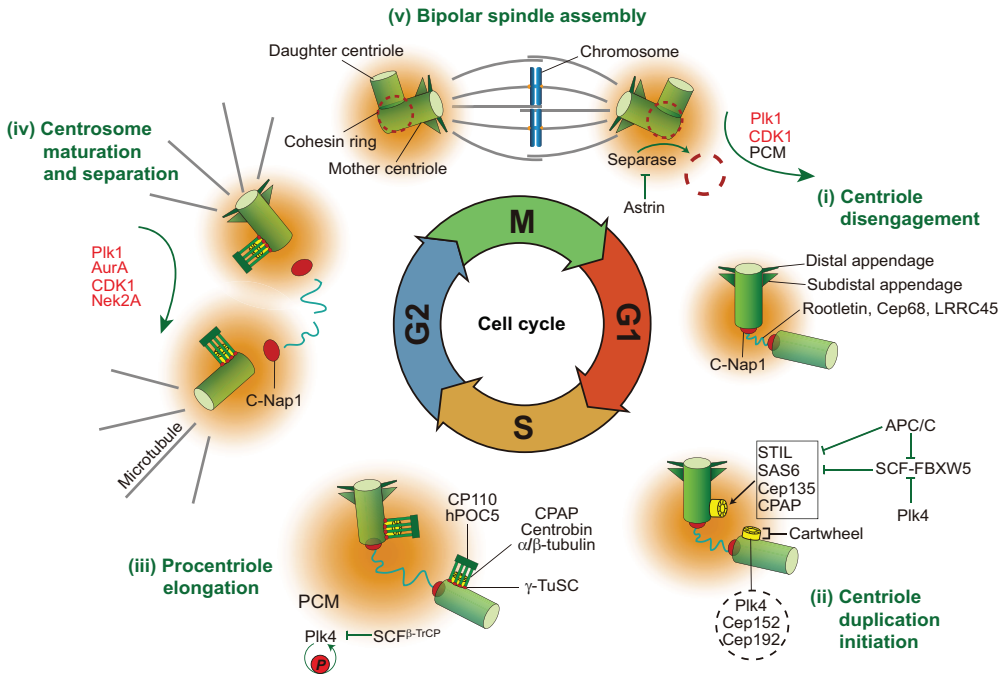

Fig. 1. Interplay between the centrosome cycle and the cell cycle. The centrosome cycle consists of several steps that are linked to the cell cycle. (i) Centriole disengagement. Centriole disengagement is accompanied by the displacement of the cohesin ring from the centrosome, occurs from the end of mitosis to early G1 phase and is regulated by Plk1, CDK1, PCM, separase and astrin. After centriole disengagement, a proteinaceous linker, mainly consisting of C-Nap1, rootletin, Cep68 and LRRC45, is established. (ii) Initiation of centriole duplication. Cep152 and Cep192 are responsible for the centrosomal recruitment of Plk4 to initiate centriole duplication (as shown within the dotted circle). Then, Cep135, SAS6, STIL and CPAP form the structural basis of a cartwheel, which is tightly regulated by APC/C, SCF-FBXW5 and Plk4. (iii) Procentriole elongation. Centrobin stabilizes the α/β-tubulin dimers that nucleate at γ-TuSC to promote procentriole elongation. CP110 and CPAP antagonistically determine procentriole length, whereas hPOC5 is responsible for the assembly of the distal portion of the centriole. Autophosphorylation of Plk4 induces its degradation (mediated by $SCF^{\beta\text{-}TrCP}$) to prevent centriole reduplication. (iv) Maturation and separation. After centriole duplication, the newly formed mother centriole assembles distal and subdistal appendages. Both centrosomes recruit additional PCM (orange) and separate to form two mature centrosomes. This process is tightly regulated by AurA, Plk1, CDK1 and Nek2A. (v) Bipolar spindle assembly. The separated centrosomes act as robust MTOCs to assemble the bipolar mitotic spindle. The inner circle shows the different phases of the cell cycle and mitotic kinases are shown in red.

important role in maintaining this orthogonal architecture, although this is still controversial (Schöckel et al., 2011; Tsou et al., 2009). Furthermore, Aki1 and astrin (also known as CC2D1A and SPAG5, respectively) function in preventing premature centrosome splitting by recruiting the cohesin ring subunit Scc1 (also known as Rad21) to the centrosome and by inactivating separase (also known as separin; a cysteine protease that promotes cleavage of Scc1), respectively (Matsuo et al., 2012; Nakamura et al., 2009; Thein et al., 2007) (Fig. 1; Table 1). The disengagement of the paired centrioles is a prerequisite for centrosome duplication, and is tightly regulated by Plk1, CDK1 and the PCM (Cabral et al., 2013; Oliveira and Nasmyth, 2013; Tsou et al., 2009).

Upon mitotic exit, the centrosomal cohesin ring is broken down and removed with the help of separase (Schöckel et al., 2011; Tsou and Stearns, 2006). During this process, Plk1 probably functions by phosphorylating Scc1, which enhances its cleavage by separase (Hauf et al., 2005; Schöckel et al., 2011). Consistently, centriole overduplication during an artificially prolonged interphase requires the kinase activity of Plk1 (Lončarek et al., 2010), and inhibition of Plk1 with a chemical inhibitor or by RNA interference dramatically suppresses both precocious centriole disengagement and centrosome duplication (Schöckel et al., 2011; Tsou et al., 2009). Otherwise, Plk1 phosphorylates sSgo1 (a splice variant of SGO1, which is also known as SGOL1) and promotes its localization on the centrosome to protect cohesin from premature cleavage (Wang et al., 2008). Overexpression of sSgo1 dominant-negative mutants, especially of a mutant that cannot be phosphorylated by Plk1, induces the separation of centrioles and spindle poles, suggesting that shutdown of Plk1 kinase activity upon mitotic exit might also contribute to centriole disengagement in order to license centrosome duplication.

However, other reports have argued that cooperation of the PCM with cytoskeleton forces or a reduction in CDK1 kinase activity, but not cohesin cleavage, is sufficient for centriole disengagement in *Caenorhabditis elegans* mitosis or *Drosophila* embryos, respectively (Cabral et al., 2013; Oliveira and Nasmyth, 2013). Accordingly, the paired centrioles are held together by the PCM until cortical microtubule pulling forces coupled with PCM disassembly 'tear' the centrioles apart in anaphase. In agreement with this, cleavage of pericentrin (a PCM component) efficiently induces centriole separation (Matsuo et al., 2012). As CDK1 is required for centrosome maturation (discussed below), reduced CDK1 kinase activity perhaps indirectly induces centriole disengagement by promoting the dissociation of the PCM from the centrosome. Alternatively, CDK1 functions to phosphorylate separase and binds to phosphorylated separase through its cyclin B1 subunit – this stable complex formation disrupts the protease activity of separase (Simizu and Osada, 2000). At anaphase onset, cyclin B1 degradation that is mediated by anaphase-promoting complex/cyclosome (APC/C) (an E3 ubiquitin ligase) would

Table 1. Factors involved in centriole biogenesis

Steps of centriole biogenesis	Factors	Properties	References
Maintaining orthogonal arrangement of paired centrioles; preventing premature centriole splitting	Cohesin complex	Maintains the orthogonal architecture of paired centrioles	Kitajima et al., 2004; Schöckel et al., 2011; Wang et al., 2008
	sSgo1	Protects the cohesin ring from untimely dissociation	Kitajima et al., 2004; Wang et al., 2008
	Astrin	Negatively regulates separase activity	Thein et al., 2007
	Aki1	Responsible for centrosomal recruitment of Scc1	Nakamura et al., 2009
	Pericentrin	Substrate of separase that maintains centriole engagement	Matsuo et al., 2012
Promoting centriole disengagement before centrosome duplication	CDK1	Multiple roles; see the main text	Oliveira and Nasmyth, 2013; Simizu and Osada, 2000
	Plk1	Multiple roles; see the main text	Mardin and Schiebel, 2012; Schöckel et al., 2011; Tsou et al., 2009; Wang et al., 2008
	Separase	Cleaves the cohesin ring and pericentrin	Lee and Rhee, 2012; Nakamura et al., 2009; Tsou et al., 2009; Zhang et al., 2008
	PCM and cytoskeleton	Cooperatively promote separation of paired centrioles	Cabral et al., 2013
Establishing the cartwheel structure; initiating procentriole formation	SAS6	Basis of the ninefold symmetry of the cartwheel structure	Gudi et al., 2011; Kitagawa et al., 2011b; van Breugel et al., 2011
	Cep152	Recruits Plk4 and SAS6 for the initiation of centriole duplication	Cizmecioglu et al., 2010; Hatch et al., 2010; Kim et al., 2013
	Cep192	Cooperates with Cep152 to recruit Plk4 to the centrosome	Kim et al., 2013; Sonnen et al., 2013
	Plk4	Multiple roles; see the main text	Bettencourt-Dias et al., 2005; Habedanck et al., 2005; Kleylein-Sohn et al., 2007; Puklowski et al., 2011; Rogers et al., 2009; Swallow et al., 2005
	Cep135	Stabilizes the cartwheel structure by interacting with SAS6 and CPAP	Cottee et al., 2013; Hatzopoulos et al., 2013; Lin et al., 2013a; Roque et al., 2012
	STIL	Establishes the linkage between centriolar scaffold and microtubules	Arquint and Nigg, 2014; Kitagawa et al., 2011a; Lin et al., 2013b
Promoting procentriole elongation and determining centriole length	γ-TuSC	Provides the template for microtubule nucleation	Kollman et al., 2010
	CPAP	Connects the cartwheel with microtubules and promotes procentriole elongation	Cottee et al., 2013; Hatzopoulos et al., 2013; Lin et al., 2013b; Schmidt et al., 2009; Tang et al., 2009
	Cep120	Promotes procentriole elongation	Comartin et al., 2013; Lin et al., 2013b
	Centrobin	Stabilizes centriolar microtubules	Gudi et al., 2011
	CP110	Determines the length of newly formed centrioles	Chen et al., 2002; Li et al., 2013; Schmidt et al., 2009
	hPOC5	Ensures centriolar integrity	Azimzadeh et al., 2009
Ensuring centriole duplication initiation and preventing centriole re-duplication	APC/C	Degradation of FBXW5 and STIL	Arquint and Nigg, 2014; Puklowski et al., 2011
	SCF	Degradation of SAS6 and Plk4	D'Angiolella et al., 2010; Holland et al., 2010; Puklowski et al., 2011; Rogers et al., 2009

This table lists only the components discussed in the main text.

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release active separase to promote the cleavage of centrosomal cohesin. Overall, it is conceivable that both cleavage of cohesin and the dissociation of the PCM, regulated by Plk1 and CDK1, are required for licensing centrosome duplication by inducing centriole disengagement (Fig. 1; Table 1).

Plk4 functions in initiating centrosome duplication

Plk4 is a master regulator of centrosome duplication (Bettencourt-Dias et al., 2005; Kleylein-Sohn et al., 2007) (Fig. 1). After centriole disengagement, the centrosomal protein Cep152 collaborates with Cep192 to recruit Plk4 to the centrosome (Cizmecioglu et al., 2010; Dzhindzhev et al., 2010; Kim et al., 2013; Sonnen et al., 2013); here, Plk4 phosphorylates Cep152, GCP6 (also known as TUBGCP6; γ-tubulin complex protein 6, a component of γ-TuRC), and possibly other currently unidentified factors, in order to initiate the centriole biogenesis machinery (Bahtz et al., 2012; Hatch et al., 2010). Then, SAS6, which constitutes the structural basis of the cartwheel, is loaded onto the sides of pre-existing centrioles towards the proximal ends and, together with Cep135, CPAP (also known as CENPJ) and STIL, forms the new cartwheel required for procentriole formation. γ-tubulin small complex (γ-TuSC) initiates the nucleation of the nine triplet microtubules for elongation of this procentriole, and centrobin stabilizes the newly formed centriolar microtubules by directly interacting with their tubulin subunits. CPAP acts antagonistically with CP110 to determine procentriole length; CPAP – together with Cep120 – mainly promotes centriolar elongation, whereas CP110 acts as a sensor to determine the final centriole length. Moreover, hPOC5 ensures centriolar integrity by assembling the distal part of the newly formed daughter centriole (Fig. 1; Table 1).

During mitosis, the activity of Plk4 is autoregulated and mediated by the E3 ubiquitin ligase complex $SCF^{Slimb/β\text{-}TrCP}$ to limit centrosome amplification (Cunha-Ferreira et al., 2009; Holland et al., 2010; Rogers et al., 2009). The SCF complex consists of Skp1, Cullin1 and Rbx1, as well as a variable F-box protein that is responsible for targeting specific substrates for degradation (Nakayama and Nakayama, 2006). Plk4 autophosphorylates its amino acid residue Ser293, thereby generating a phosphodegron to promote its interaction with the F-box protein β-TrCP (the vertebrate homolog of *Drosophila* Slimb), and thus the SCF complex, for degradation (Cunha-Ferreira et al., 2013; Meraldi and Nigg, 2002). Furthermore, both SCF (in this case employing the F-box protein FBXW5) and APC/C can contribute to inhibiting the reduplication of the centrosome by ubiquitylating SAS6 and STIL, respectively (Arquint and Nigg, 2014; Puklowski et al., 2011). However, to alleviate this, Plk4 phosphorylates FBXW5, which suppresses its ability to ubiquitylate SAS6. Furthermore, APC/C targets FBXW5 for degradation during mitosis and G1 phase to prevent the premature degradation of SAS6, thereby ensuring correct centrosome duplication (Puklowski et al., 2011). Collectively, Plk4, together with E3 ubiquitin ligases, functions in regulating different stages of centriole biogenesis to guarantee the initiation of centrosome duplication and to prevent reduplication.

Role of mitotic kinases in centrosome separation and maturation and bipolar spindle assembly

The accurate distribution of replicated chromosomal DNA to daughter cells depends on accurate bipolar spindle formation. Before mitotic entry, the duplicated centrosomes separate and move in opposite directions to serve as spindle poles (Fig. 1). During this process, the fibrous proteinaceous linker [consisting of C-Nap1 (also known as CEP250), rootletin, Cep68, LRRC45, β-catenin and perhaps other thus far undefined components], which joins duplicated centrosomes together after centriole disengagement, needs to be removed to allow complete centrosome separation (Bahe et al., 2005; Bahmanyar et al., 2008; Fry et al., 1998; Graser et al., 2007; He et al., 2013) (Figs 1 and 2). Among the linker components, C-Nap1 serves as the docking site for the other factors by binding to the proximal ends of parental centrioles, and dissociation of C-Nap1 from the centrosome efficiently induces centrosome separation (Yang et al., 2006). Simultaneously, from G2 to M phase, PCM is incorporated into the centrosomes, and the distal/sub-distal appendages assemble on the newly formed mother centriole to facilitate centrosome maturation by enhancing microtubule nucleation and anchoring. The roles of several mitotic kinases, including Neks, CDK1, Plk1 and Aurora A (AurA), in orchestrating centrosome separation and maturation, as well as in subsequent mitotic spindle assembly, have been described and are discussed below.

Nek2A, together with Plk1, CDK1 and AurA, regulates centrosome separation

Neks form a conserved serine/threonine kinase family, which, in mammalian cells, consists of 11 members, Nek1 to Nek11. These kinases play roles in regulating cell cycle progression (Fry et al., 2012; Quarmby and Mahjoub, 2005). Nek9 activates Nek6 and Nek7 by phosphorylation to maintain spindle structure and function, whereas Nek2A is required for centrosome disjunction and mitotic fidelity (O'Regan et al., 2007; O'Regan and Fry, 2009). Overexpression of active Nek2A results in centriole separation in interphase, whereas expression of kinase-dead mutant of Nek2A causes defective centrosome separation and leads to the formation of a monopolar spindle (Bahe et al., 2005; Faragher and Fry, 2003; Fry et al., 1998). Based on these data, a model has been proposed whereby, at the onset of mitosis, Nek2A phosphorylates some of the centrosomal linker proteins, including C-Nap1, rootletin, LRRC45 and β-catenin, which displaces them from the inter-region of parental centrioles to promote premitotic centrosome separation (Bahmanyar et al., 2008; Faragher and Fry, 2003; Fry et al., 1998; He et al., 2013) (Fig. 2). Consistently, Fry and colleagues recently reported that Nek2A phosphorylates multiple sites in the C-terminal domain of C-Nap1, which disturbs both its oligomerization and its interaction with the centriolar protein Cep135, thereby triggering the centrosomal dissociation of C-Nap1 and subsequent centrosome disjunction (Hardy et al., 2014).

To prevent premature centrosome separation, the kinase activity of Nek2A is tightly regulated. Recent studies have revealed that Hippo pathway components [Mst2 (also known as STK3) and hSav1] and Plk1 participate in regulating centrosome separation upstream of Nek2A (Mardin et al., 2011). Mst2 and hSav1 interact with Nek2A and mediate its centrosomal localization. Subsequently, Mst2 phosphorylates Nek2A to promote its centrosomal recruitment, which allows Nek2A to phosphorylate C-Nap1 and rootletin to stimulate centrosome disjunction (Mardin et al., 2010). Moreover, Plk1 functions upstream of the Mst2–Nek2A centrosome disjunction pathway by selectively phosphorylating Mst2 (Mardin et al., 2011). In the absence of Plk1 phosphorylation, Mst2 preferentially assembles into a complex with Nek2A and the protein phosphatase 1 gamma

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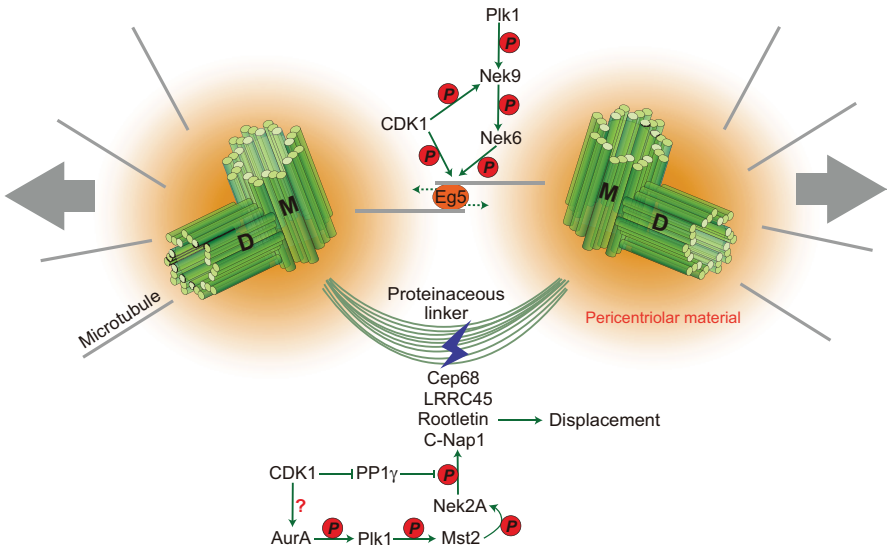

Fig. 2. Coordinated activity of mitotic kinases to regulate centrosome disjunction and separation. Centrosome disjunction is tightly regulated by the CDK1–AurA–Plk1–Nek2A signaling cascade. CDK1 triggers the phosphorylation of AurA, which then activates Plk1 by phosphorylation. Subsequently, Plk1 phosphorylates Mst2 to promote its interaction with Nek2A, thereby promoting the centrosomal recruitment of Nek2A. The centrosome-associated Nek2A phosphorylates C-Nap1, rootletin, Cep68 and LRRC45, resulting in their displacement from the centrosome and inducing centrosome disjunction (as shown by the blue lightning bolt symbol). Moreover, CDK1 contributes to centrosome disjunction by inactivating PP1γ, as PP1γ can interact with unphosphorylated Mst2 to form the Mst2–PP1γ–Nek2A complex that counteracts Nek2A-mediated phosphorylation of C-Nap1. The centrosome separation pathway might be directly regulated by Eg5 and other motor proteins that are regulated by CDK1 and the Plk1–Nek9–Nek6 cascade. Active Eg5 moves towards the plus-end along the antiparallel microtubules and generates a pushing force to propel the duplicated centrosomes away from each other, forming the spindle poles. The dashed arrows indicate the direction of Eg5 motility, and the large gray arrows show the direction of centrosome movement. M, mother centriole; D, daughter centriole.

(PP1γ); here, PP1γ counteracts Nek2A kinase activity by dephosphorylating C-Nap1 to prevent the premature splitting of centrosomes. By contrast, phosphorylation of Mst2 by Plk1 decreases its ability to bind to PP1γ, thereby preventing the formation of the Mst2–PP1γ–Nek2A complex and allowing Nek2A to phosphorylate C-Nap1 and induce centrosome disjunction (Mardin et al., 2011).

Plk1 also contributes to centrosome separation by regulating the kinase activity of Nek9 (Bertran et al., 2011; Rapley et al., 2008; Sdelci et al., 2011). In this pathway, CDK1 acts as a priming kinase by phosphorylating Nek9 at Ser869, which promotes it being bound by Plk1 and activated by phosphorylation. Following this, activated Nek9 phosphorylates and activates Nek6, which allows Nek6 to phosphorylate the mitotic kinesin-5 (Eg5, also known as KIF11) at Ser1033 to promote its centrosomal accumulation. The movement of phosphorylated Eg5 [which is modified at position Thr926 by CDK1 (discussed below) and at Ser1033 by Nek6], together with that of other motors, such as dynein and myosin, along the microtubule generates pushing and pulling forces that propel the centrosomes apart, which is essential for centrosome separation and the maintenance of mitotic spindle bipolarity (Fig. 2) (see also reviews by Mardin and Schiebel, 2012; Tanenbaum and Medema, 2010).

In addition, CDK1 and AurA also contribute to centrosome separation. Activated CDK1 promotes centrosome separation in three ways: firstly, it phosphorylates and inactivates PP1γ to allow Nek2A to phosphorylate and displace C-Nap1 and rootletin from the parental centrioles (Dohadwala et al., 1994; Mardin et al., 2011); secondly, CDK1 phosphorylates Eg5 at Thr926, which facilitates its binding to antiparallel microtubules and its movement towards the microtubule plus-end to ensure centrosome separation and spindle bipolarity (Slangy et al., 1995; Sawin and Mitchison, 1995; Smith et al., 2011); finally, CDK1–cyclin-B2 triggers activation of AurA through an undefined mechanism, before AurA phosphorylates and activates centrosome-associated Plk1 (discussed below) to permit centrosome separation (discussed above) (Nam and van Deursen, 2014). Taken together, it is conceivable that the CDK1–AurA–Plk1–Nek2A signaling cascade is highly regulated to ensure appropriate centrosome separation before mitosis (Fig. 2).

Plk1 functions in centrosome maturation and spindle formation

The incorporation of the PCM, especially of γ-TuRC, is a crucial step for centrosome maturation that is required to increase the capacity of the centrosome to organize microtubules, and it is tightly regulated by Plk1. Consistently, the ability of the centrosome to nucleate and anchor microtubules oscillates during the cell cycle and is highest during mitosis, when the kinase activity of Plk1 is also at its maximum. Over the past decades, increasing evidence has made it possible to dissect the functions of Plk1 in normal mitotic progression (Fig. 3).

The crucial role of Plk1 in centrosome maturation was identified in the 1990s (Lane and Nigg, 1996). Both the depletion of Plk1 by microinjecting Plk1 antibodies or by RNA interference and the inhibition of Plk1 kinase activity with a chemical inhibitor lead to impaired centrosome separation and maturation, and monopolar spindle formation (Lane and Nigg, 1996; Steegmaier et al., 2007; Zhang et al., 2009). Phosphorylation of pericentrin at Ser1235 and Ser1241 by Plk1 initiates centrosome maturation, which is required for the centrosomal accumulation of Cep192, NEDD1, γ-tubulin, AurA and Plk1 itself (Lee and Rhee, 2011). Interestingly, depletion of Cep192 disrupts the mitotic centrosomal localization of pericentrin, NEDD1 and γ-tubulin, whereas depletion of NEDD1 or γ-tubulin only interferes with the distribution but

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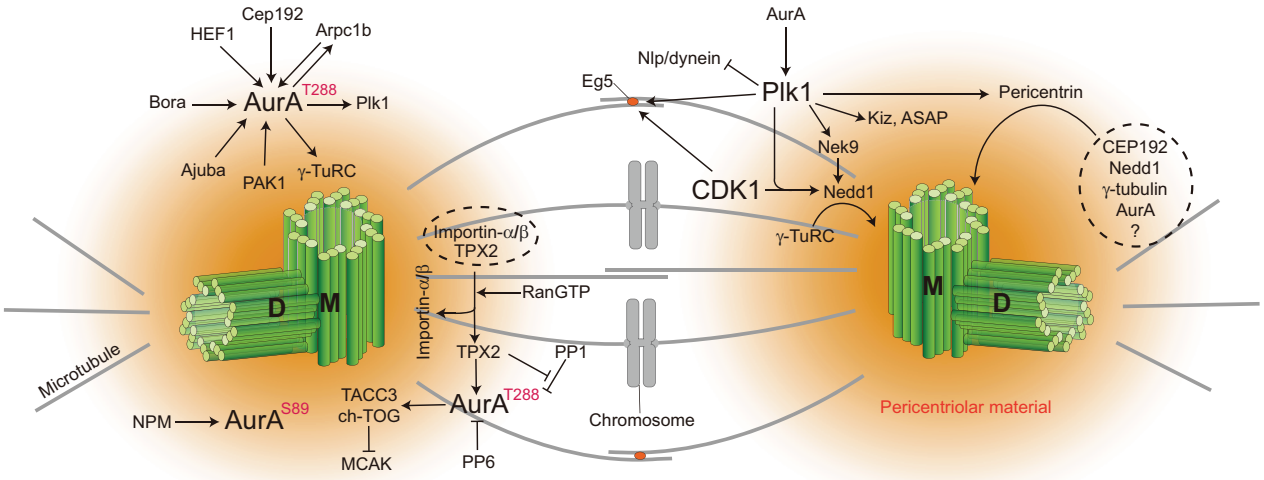

Fig. 3. Mitotic kinases regulate centrosome maturation and mitotic spindle assembly. Before mitotic entry, RanGTP releases TPX2 from inhibition mediated by importin-α/β (shown within the dotted oval), thereby allowing TPX2 to interact with and activate AurA. Furthermore, the kinase activity of AurA is also tightly regulated by other cofactors, including Ajuba, HEF1, Bora, Arpc1b, Cep192, PAK1 and NPM, that act through distinct pathways (see the main text). Activated AurA then phosphorylates and activates Plk1, and together they function in centrosome maturation. AurA promotes the centrosome recruitment of γ-tubulin and TACC3 to the centrosome; here, TACC3 forms a complex with ch-TOG that stabilizes centrosomal microtubules by opposing the activity of MCAK. Plk1 phosphorylates multiple substrates, such as pericentrin, Nedd1, Nlp and Nek9 to facilitate centrosome maturation and microtubule nucleation. Furthermore, Plk1 phosphorylates Kiz and ASAP to ensure mitotic centrosomal integrity. Both Plk1 and CDK1 are required for Eg5-mediated centrosome separation and the establishment of spindle bipolarity. M, mother centriole; D, daughter centriole.

not total amount of mitotic centrosome-associated Cep192 or pericentrin. These data suggest that the localization of Cep192 and pericentrin to the centrosome is mutually dependent and that both are required for recruitment of NEDD1 and γ-tubulin, and subsequent centrosome maturation (Haren et al., 2006; Zhu et al., 2008). Furthermore, CDK1 and Plk1 sequentially phosphorylate NEDD1 to promote its interaction with γ-tubulin and to target γ-TuRC to the centrosome (Zhang et al., 2009). During this process, Nek9 acts downstream of Plk1 and upstream of NEDD1 by phosphorylating NEDD1 at Ser377, which is essential for the centrosomal recruitment of NEDD1 and γ-tubulin to promote centrosome maturation (Sdelci et al., 2012; Zhu et al., 2008).

Intriguingly, not only recruitment of the PCM to the centrosome as mentioned above, but also displacement of specific proteins, such as Nlp (ninein-like protein), from the centrosome is required for centrosome maturation before mitotic entry (Casenghi et al., 2005; Casenghi et al., 2003). Nlp is a γ-tubulin-binding protein (GTBP) that functions in centrosomal microtubule nucleation specifically in interphase, whereas it is removed from the mitotic centrosome by Plk1. Plk1 phosphorylates and displaces Nlp from the centrosome by preventing its interaction with γ-TuRC or the dynein–dynactin complex. Consequently, the dissociation of Nlp might be a prerequisite for the recruitment of other distinct, currently unidentified mitotic GTBPs that might promote centrosome maturation and microtubule anchoring (Casenghi et al., 2005; Casenghi et al., 2003). Therefore, the orchestrated recruitment and dissociation of centrosomal components guarantees that centrosomes can act as robust MTOCs for spindle assembly.

In addition to initiating centrosome maturation, Plk1 also plays a crucial role in stabilizing the link between the PCM and centrioles by phosphorylating Cnn (the *D. melanogaster* homolog of mammalian CDK5RAP2) (Conduit et al., 2014). Raff and colleagues recently showed that Plk1 phosphorylates Cnn at its phosphoregulated-multimerization (PReM) domain, which allows Cnn to multimerize and assemble a scaffold around centrioles. Because multimerized Cnn is localized in very close proximity to the centrioles and diffuses from there throughout the entire PCM, this Cnn-mediated scaffold helps to anchor the PCM and thus facilitates centrosome maturation (Conduit et al., 2014). Consistently, the lack of Cnn induces mitotic defects owing to an unstable linkage between centrioles and PCM (Conduit et al., 2010; Lucas and Raff, 2007).

Centrosomal functions must be precisely restricted to a specific stage of the cell cycle, where the activity of centrosome-associated Plk1 might act as a limiting factor, thus fulfilling a crucial role in coupling the centrosome cycle to cell cycle progression. Consistent with this hypothesis, the centrosomal localization of Plk1 is highly dynamic from interphase to mitosis, and Plk1 is recruited to the PCM by PCM1 just before mitotic entry (Petronczki et al., 2008; Wang et al., 2013). Artificial and persistent tethering of Plk1 to the centrosome induces a delay in G2, prometaphase arrest and defective spindle formation (Kishi et al., 2009). Moreover, inhibition of the kinase activity of Plk1 results in structural defects of the centrosome even after its maturation, which can be rescued by restoring Plk1 activity (Mahen et al., 2011). Intriguingly, only the centrosome that has been phosphorylated by Plk1 has the capacity to duplicate and incorporate PCM, as well as to anchor microtubules (Wang et al., 2011). All this evidence prompts the notion that the continuous but dynamic kinase activity of Plk1 is essential for the appropriate organization of the PCM lattice and centrosome function in normal mitotic spindle formation; however, the underlying mechanisms are still far from being fully elucidated.

Roles of Aurora A in centrosome maturation and spindle formation

The functions of AurA at centrosomes that are relevant in the context of spindle formation are mainly related to its role in regulating mitotic entry, as well as centrosomal microtubule nucleation and stabilization. At the centrosome, AurA

phosphorylates the phosphatase CDC25B at Ser353, leading to its activation. This phosphatase then activates CDK1 to partially contribute to the mitotic entry (Dutertre et al., 2004). Furthermore, AurA activates LATS2 through phosphorylation, and the latter is required for the centrosomal recruitment of γ-tubulin to promote centrosome maturation for nucleating microtubules (Abe et al., 2006; Toji et al., 2004). To stabilize the centrosomal microtubules, AurA phosphorylates the centrosomal adaptor TACC3 and, in a clathrin-dependent manner, targets it exclusively to the mitotic centrosome (Fu et al., 2010), where it interacts with the microtubule-stabilizing factor ch-TOG (also known as CKAP5). The formation of the TACC3–ch-TOG complex enhances the affinity of ch-TOG for microtubules, which, in turn functions to increase the stability of centrosomal microtubules by counteracting the activity of the microtubule-depolymerizing kinesin MCAK (also known as KIF2C) (Barros et al., 2005; Kinoshita et al., 2005; Peset et al., 2005). Taken together, these findings emphasize the importance of the centrosome in regulating aspects of cell cycle progression and spindle assembly that are controlled by AurA (Fig. 3).

Interestingly, the pleiotropic functions of AurA depend on its interactions with diverse cofactors, which are mediated through different mechanisms (Fig. 3). After nuclear envelope breakdown, TPX2 is released from importin-α/β-mediated inhibition in a RanGTP-dependent manner and then directly interacts with AurA to target it to spindle microtubules (Gruss et al., 2001; Kufer et al., 2002; Özlü et al., 2005; Tsai et al., 2003). Subsequently, TPX2 activates AurA by promoting the autophosphorylation of its T-loop Thr288 and preventing its PP1-mediated, but not PP6-mediated dephosphorylation (Bayliss et al., 2003; Eyers et al., 2003; Kufer et al., 2003; Zeng et al., 2010). Considering the fact that the RanGTP gradient that is established around the chromosome extensively contributes to spindle assembly and dynamics, it is conceivable that the formation of a secondary AurA–TPX2 gradient that contains active AurA and, consequently, its phosphorylated substrates is crucial for mitotic progression (Clarke and Zhang, 2008; Tsai et al., 2003). Indeed, RAN and its effectors regulate many aspects of centrosome and spindle function, but these are not discussed here owing to space limitations (for more information, readers can see Clarke and Zhang, 2008; Dishinger et al., 2010; Peloponese et al., 2005; Wang et al., 2005).

Although TPX2 is required for targeting AurA to the mitotic spindle, depletion of TPX2 appears to have no effect on the centrosomal localization of AurA (Kufer et al., 2002). Instead, Livingston and colleagues found that Cep192 is the AurA cofactor that is responsible for its centrosomal activity (Joukov et al., 2010). Cep192 targets AurA to mitotic centrosomes through a direct interaction, which induces the formation of AurA homodimers or oligomers that dramatically amplify the activity of centrosomal AurA. In addition, activated AurA exhibits a higher affinity for Cep192, which might facilitate the recruitment of Cep192 to the centrosome. This positive feedback enhances the centrosomal localization and activation of AurA, which, in turn, promotes centrosome maturation and drives microtubule assembly (Joukov et al., 2010). Furthermore, other AurA cofactors, such as Ajuba, Bora, HEF1 (also known as NEDD9) and Arpc1b, have also been shown to contribute to the regulation of the centrosome-associated functions of AurA. For instance, HEF1 activates AurA to promote disassembly of the primary cilium, which liberates the centrosome upon re-entry into the cell cycle (Pugacheva et al., 2007), whereas Ajuba interacts with and activates AurA in late G2 phase to accelerate the G2–M transition (Hirota et al., 2003). Furthermore, Bora cooperates with AurA to activate Plk1 – this leads to the activation of CDK1, which, in turn, contributes to centrosome maturation and mitotic entry (Hutterer et al., 2006; Macůrek et al., 2008; Seki et al., 2008b). Finally, Arpc1b is not only an activator but also a substrate of AurA, and depletion of Arpc1b inhibits the activation of AurA at the centrosome, thereby disturbing mitotic entry (Molli et al., 2010). Of note, all these cofactors share a common mechanism, in that they indirectly induce the phosphorylation of AurA at Thr288. Manser and colleagues reported that another serine/threonine kinase, PAK1 (p21-activated kinase 1), directly binds to AurA and activates it by phosphorylating it at Thr288 (Zhao et al., 2005). Inhibition of the kinase activity of PAK1 dramatically delays centrosome duplication and maturation owing to a decrease in the activation of AurA.

More recently, a novel mechanism of AurA activation by nucleophosmin (NPM, also known as B23) has been shown (Reboutier et al., 2012). NPM interacts with AurA at the centrosome and induces its autophosphorylation at Ser89 (which is necessary for its activation), but not at Thr288. Depletion of NPM has no effect on the AurA-mediated phosphorylation of Plk1 Thr210, but abolishes that of CDC25B Ser353, suggesting that different activation sites within AurA fulfill its apparently different functions by phosphorylating distinct centrosome-associated substrates. Taken together, these distinct pathways for AurA activation illustrate a highly controlled network that allows each given cofactor to induce AurA kinase activity in a spatiotemporally regulated manner for a particular AurA-mediated function during the cell cycle.

Feedback between AurA and Plk1

Among the many functions of AurA, foremost is that of an activator of Plk1, which might take place on the centrosome (Macůrek et al., 2008; Seki et al., 2008b). In this case, Bora directly interacts with Plk1 during G2 phase, which results in Thr210 of the T-loop of Plk1 being accessible for phosphorylation by AurA; this leads to the initial activation of Plk1. By contrast, the entirety of Plk1 is required for the centrosomal localization of AurA, and the phosphorylation of TPX2 by Plk1 further enhances AurA activation (De Luca et al., 2006; Eckerdt et al., 2009; Lee and Rhee, 2011). Moreover, activated Plk1 also regulates the centrosomal function of AurA by phosphorylating Bora at its DSGxxT degron to promote its interaction with and degradation by $SCF^{\beta\text{-}TrCP}$, thereby keeping the protein level of Bora below a certain threshold (Seki et al., 2008a). This might be required because excess amounts of Bora sequester AurA from the spindle poles and induce monopolar spindle formation, reminiscent of the phenotype observed when Plk1 activity is inhibited (Chan et al., 2008).

It appears paradoxical that Bora mediates the activation of Plk1, whereas Plk1 promotes Bora degradation. If this is indeed the case, how can the kinase activity of Plk1 be maintained during mitosis when Bora is degraded? Recently, Medema and colleagues found that although most Bora protein is degraded in mitosis, the residual Bora is responsible for AurA-dependent activation of Plk1. Consequently, it has been proposed that these minimal amounts of the AurA–Bora complex are sufficient to sustain Plk1 kinase activity during mitosis, once Plk1 has been activated (Bruinsma et al., 2014). Therefore, this mutual feedback might be sufficient to ensure centrosome maturation and correct mitotic progression by not only mediating the efficient function

of AurA in mitosis, but also by maintaining adequate kinase activity of Plk1.

Dysregulation of mitotic kinases: role in inducing spindle-assembly defects

Centrosome functions are tightly controlled, as discussed above, to ensure the bipolar mitotic spindle fidelity. However, abnormal centrosome functions that result from dysregulated mitotic kinases typically induce an abnormal centrosome cycle that is accompanied by the formation of aberrant mitotic spindles, including monopolar and multipolar spindles (Fig. 4).

Monopolar spindle formation

Before mitosis, the duplicated centrosomes are incorporated into opposite poles and act as MTOCs to ensure bipolarity of the mitotic spindle. Failure of centrosome duplication, separation and maturation, due to the inactivation of mitotic kinases, frequently induces the formation of a monopolar spindle (Faragher and Fry, 2003; Mardin et al., 2011; Steegmaier et al., 2007; Tanenbaum and Medema, 2010).

Although monopolar spindles are not that common in nature, they can be frequently observed when particular factors, especially mitotic kinases, are experimentally manipulated. For instance, the proteinaceous linker connecting parental centrioles has to move away to liberate the joint duplicated centrosomes for their timely separation at the onset of mitosis, and this process is partly dependent on the activity of Nek2A and Eg5. As the activities of both Nek2A and Eg5 are regulated by Plk1 and AurA, inactivation of Plk1 or AurA by RNA interference or chemical inhibitors is the likely cause for induced centrosome-separation defects and the consequent monopolarity of the mitotic spindle (Mardin et al., 2011; Smith et al., 2011; Steegmaier et al., 2007) (Fig. 4A). Alternatively, inhibition of Plk1 or AurA dramatically disturbs centrosome maturation and centrosomal microtubule nucleation, leading to the mislocalization of microtubule motor proteins such as Eg5 and dynein. Because the pushing and pulling forces generated by motor proteins that movie along the microtubules provide the power for initial centrosome separation and the maintenance of spindle bipolarity, the centrosomal mislocalization of motor proteins enhances the probability of monopolar spindle formation (Tanenbaum and Medema, 2010). Furthermore, similar to depletion of Plk4, depletion of the centriolar protein Cep152 or the pericentriolar component GCP6 results in failure of centrosome duplication, and the remaining solitary centrosome by some means eventually generates a single pole for the assembly of a monopolar spindle (Bahtz et al., 2012; Habedanck et al., 2005; Hatch et al., 2010). Cells containing a monopolar spindle either undergo a cell-cycle arrest and die as a consequence of spindle checkpoint activation or can get through the cell cycle with failed cytokinesis, leading to multiploidy (Fukasawa, 2007).

Multipolar spindle formation

Other than monopolarity of the mitotic spindle, functional defects of the centrosome also have the potential to lead to the assembly of multipolar spindles. It is highly likely that supernumerary centrosomes, premature centriole splitting and PCM disintegration result in spindle multipolarity (Basto et al., 2008; Fukasawa, 2007; Yang et al., 2008).

The significant upregulation of mitotic kinases, such as Plk1 and AurA, are common features of various cancer cell lines (Fukasawa, 2007). Overexpression of Plk1 does not appear to disturb the formation of a normal bipolar spindle (Mundt et al., 1997), whereas excess AurA is able to override the spindle assembly checkpoint (SAC) and results in the failure of cytokinesis, which then drives centrosome amplification (Anand et al., 2003; Meraldi et al., 2002). Furthermore, ectopic overexpression of Plk4 and of other centrosome components that are required for the initial steps of centriole biogenesis, such as Cep152, SAS6 and STIL, is sufficient to inappropriately induce centrosome reduplication (Habedanck et al., 2005; Hatch et al., 2010; Kleylein-Sohn et al., 2007; Peel et al., 2007; Sonnen et al., 2013). Although some of the cells that have supernumerary centrosomes are capable of assembling a functional bipolar spindle (Box 1), the amplified centrosomes preferentially trigger the formation of a multipolar spindle owing to their ability to initiate microtubule organization (Fig. 4B).

During mitosis, the extra centrosomes that contain only one centriole that has resulted from premature centriole splitting, as well as the fragmented PCM, can also serve as MTOCs to trigger multipolar spindle formation (Matsuo et al., 2012; Nakamura et al., 2009; Thein et al., 2007) (Fig. 4B). In order to prevent spindle multipolarity, the activity of some PCM components, such as ninein, Cep90 (the largest isoform of PIBF1) and Cep57,

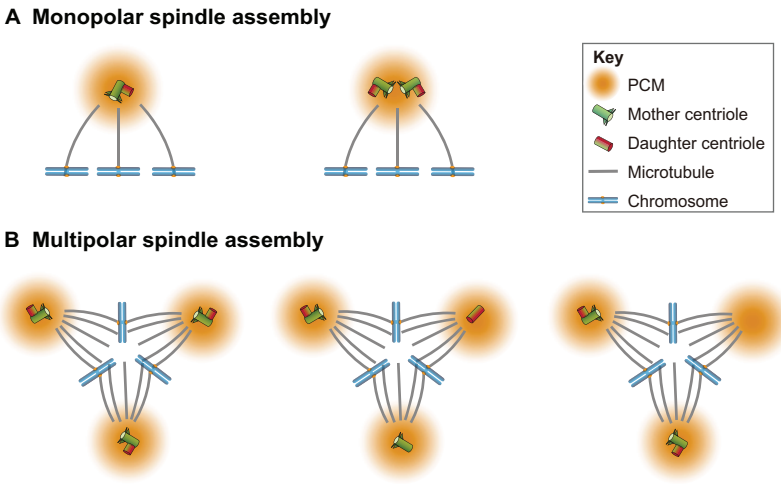

Fig. 4. Centrosomal abnormalities induce defects of spindle bipolarity. (A) Monopolar spindle assembly. Left, failure of centrosome duplication leads to the formation of a monopolar spindle, as the single centrosome forms a single spindle pole. Right, unseparated centrosomes can also serve as a single pole for assembling a monopolar spindle. (B) Multipolar spindle assembly. Overduplicated centrosomes act as extra MTOCs that can induce multipolar spindle formation (left). Aberrant premature splitting of centrioles before entry into mitosis results in the formation of supernumerary centrosomes with each of the centrosomes containing a single centriole. These deficient centrosomes can act as impaired MTOCs to assemble a multipolar spindle (middle). Furthermore, fragmented acentrosomal PCM resulting from spindle pole disintegration or newly formed acentrosomal PCM can also act as extra poles to induce multipolar spindle assembly (right).

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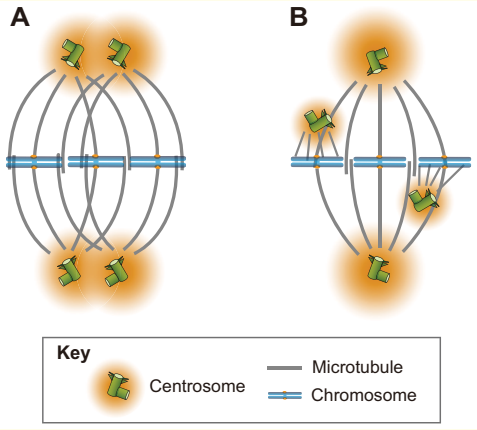
Box 1. Building a bipolar mitotic spindle with excess centrosomes

Centrosome reduplication, *de novo* extra centriole formation, failure of cytokinesis and cell fusion are thought to represent mechanisms by which supernumerary centrosomes are generated. Excess centrosomes might play dual roles in tumorigenesis; on one hand, multiple centrosomes promote the generation of aneuploidy, and on the other hand, multipolarity of the mitotic spindle that is mediated by excess centrosomes might be detrimental for cell proliferation owing to subsequent mitotic arrest and failed cytokinesis (Fukasawa, 2007; Ganem et al., 2009; Karna et al., 2011; Marthiens et al., 2013). In order to survive with supernumerary centrosomes, cancer cells use multiple mechanisms to block spindle multipolarity, and these are far from being fully characterized. One such mechanism, termed centrosomal clustering, can prevent multipolar spindle formation by grouping the supernumerary centrosomes into two sections under the regulation of microtubule integrity, motor proteins (such as dynein and kinesin) and the SAC (Basto et al., 2008; Kwon et al., 2008; Quintyne et al., 2005; Yang et al., 2008) (see panel A of the figure). One model to explain this centrosomal clustering is as follows; first, the incorrect attachment between kinetochores and microtubules that emanate from the extra centrosomes activates the SAC, which results in mitotic delay (Basto et al., 2008; Chiroli et al., 2009; Foley and Kapoor, 2013); second, during this prolonged mitotic delay, the protein motors crosslink the centrosome-anchored microtubules and move towards the microtubule minus-ends, which generates focused pushing forces to drive the extra centrosomes into two distinct groups (Kwon et al., 2008; Quintyne et al., 2005); finally, the centrosomal clustering corrects the aberrant kinetochore–microtubule attachment to inactivate the SAC and so induces spindle bipolarity and subsequent cytokinesis (Fukasawa, 2007; Marthiens et al., 2012).

Intriguingly, it was also found that the non-pole-associated extra centrosomes without robust microtubule asters are erratically dispersed within the cytoplasmic matrix (Basto et al., 2008) (see panel B). Thus, the distinct ability of accurately duplicated centrosomes to nucleate microtubules might partially contribute to spindle bipolarity in cells that also contain additional centrosomes.



is coordinated to facilitate the bundling and stabilizing of centrosomal microtubules and to withstand microtubule tensions (Kim and Rhee, 2011; Logarinho et al., 2012; Wu et al., 2012). Furthermore, both Plk1 and AurA are essential kinases for the construction of robust focused spindle poles (Fig. 3). Evidence has shown that Plk1 maintains spindle pole integrity mainly by phosphorylating different centrosomal substrates, including Kizuna (Kiz) and ASAP (Eot-Houllier et al., 2010; Oshimori et al., 2006). Phosphorylated Kiz then acts as a bond for tethering the PCM tightly to centrioles to protect mature centrosomes from collapsing under microtubule traction forces; therefore, depletion of Kiz causes fragmentation and dissociation of the PCM from the centrioles and induces multipolar spindle formation (Oshimori et al., 2006). Furthermore, Plk1 promotes the centrosome and spindle localization of ASAP, and Plk1-mediated phosphorylation of ASAP is sufficient to maintain spindle pole integrity in a microtubule-dependent manner; however, the underlying mechanism remains unclear (Eot-Houllier et al., 2010). Other than monopolar spindle formation, a fraction of the cells in which AurA has been knocked down by RNA interference or microinjection of AurA antibodies also exhibit multipolarity (De Luca et al., 2008; Marumoto et al., 2003). It has been shown that loss of AurA kinase activity dramatically stimulates the centrosomal accumulation of ch-TOG and dissociation of MCAK, which probably disturbs the centrosomal microtubule dynamics and the balance of microtubule-mediated tension, thereby inducing PCM fragmentation and multipolar spindle formation (De Luca et al., 2008). Nevertheless, the detailed molecular structural basis of how PCM components are regulated by mitotic kinases to perform roles in maintaining spindle bipolarity remains obscure.

Conclusions and future perspectives

Over the past decades, the identification and characterization of mitotic kinases, including Neks, CDKs, Plks and Aurora kinases, that are involved in centrosome duplication, separation and maturation have greatly enhanced our understanding of centrosome function in the assembly of bipolar mitotic spindles. Nevertheless, the exact mechanisms underlying kinase function in coupling the centrosome cycle to mitotic spindle formation and cell cycle progression remain largely unknown. For instance, although Plk4 is crucial for centrosome duplication, it remains unclear what factor(s) monitor Plk4 kinase activity at the onset of centriole biogenesis. Another question is whether phosphatases, such as PP1 and PP2A, have a role in preventing uncontrolled centrosome amplification, perhaps by dephosphorylating particular substrates of mitotic kinases? How is the balance between phosphorylation and dephosphorylation of centrosome-associated substrates maintained to ensure the coupling between the cell and the centrosome cycle and normal mitotic progression? Furthermore, using super-resolution microscopy, we have just been able to gain some superficial understanding of the elegant highly ordered PCM structure, but a number of questions remain; what are the entities that determine the organization of the PCM around the centrosome and how is its integrity maintained to sustain the forces that are generated from the spindle microtubules under the regulation of mitotic kinases? Moreover, it will be interesting to determine what exactly promotes the dissociation of the PCM from centrioles upon mitotic exit to restart centrosome duplication and the next cell cycle. Perhaps super-resolution microscopy combined with single-molecular imaging analysis could prove to be a powerful tool to dissect the spatiotemporal control of the relationship between centrioles and the PCM.

Despite the fact that centrosome abnormalities, especially centrosome amplification, occur frequently in cancer cells, it will be highly challenging to determine the role that defects in centrosome function have in human tumorigenesis. To date, we

still have no concrete idea whether centrosome aberrations are the cause or the consequence of cancer progression. Although Raff and colleagues reported that centrosome amplification can initiate tumorigenesis in flies (Basto et al., 2008), other studies argue that centrosome amplification alone is insufficient to induce tumor formation, but that it instead induces apoptosis and tissue degeneration and might also disturb the asymmetric division of neural stem cells, thereby leading to microcephaly during mouse brain development (Marthiens et al., 2013). This difference might at least partially stem from the ability of cells that harbor excess centrosomes to assemble functional bipolar spindles and to survive (Ganem et al., 2009; Marthiens et al., 2013). Therefore, an attractive area for future research will be to investigate the mechanisms of inhibitors that are sufficient to disturb the formation of bipolar spindle by ablating centrosome separation or clustering. Such insights might provide new avenues for cancer therapy.

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Competing interests

The authors declare no competing interests.

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