

Published in final edited form as:

*Trends Cell Biol.* 2015 February ; 25(2): 65–73. doi:10.1016/j.tcb.2014.10.002.

## Centrosome dynamics as a source of chromosomal instability

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### Abstract

Accurate segregation of duplicated chromosomes between two daughter cells depends on bi-polar spindle formation, a metaphase state in which sister kinetochores are attached to microtubules emanating from opposite spindle poles. To ensure bi-orientation, cells possess surveillance systems that safeguard against microtubule-kinetochore attachment defects, including the spindle assembly checkpoint and the error correction machinery. However, recent developments have identified centrosome dynamics – that is, centrosome disjunction and poleward movement of duplicated centrosomes – as a central target for deregulation of bi-orientation in cancer cells. Here we review novel insights into the mechanisms that underlie centrosome dynamics and discuss how these mechanisms are perturbed in cancer cells to drive chromosome missegregation and advance neoplastic transformation.

### Keywords

Centrosome disjunction; centrosome movement; centrosome dynamics; chromosomal instability; cancer

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## A CLINICAL PERSPECTIVE ON CENTROSOME DYNAMICS

Whole chromosome instability (W-CIN), the inability to faithfully segregate duplicated chromosomes between two daughter cells, can result in cells with abnormal numbers of chromosomes, a condition referred to as aneuploidy [1]. Although a causal relationship between aneuploidy and cancer remains a topic of debate, aneuploidy is a common feature of human cancers and one of several mechanisms by which aneuploidy is thought to drive tumorigenesis is through loss of whole chromosomes that contain key tumor suppressor genes [1, 2]. Nonetheless, the molecular and genetic bases of W-CIN in human cancer cells remain poorly understood. While much attention has been focused on the role of mitotic checkpoint signaling, attachment error correction, and centrosome duplication, mutations in key regulators of these mitotic processes are rarely observed in human cancer [3]. Recently,

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improper timing of centrosome separation has been appreciated as a new and potentially frequent source of aneuploidization in human cancers [4–7]. In this review, we highlight novel insights into normal centrosome biology and explain how abnormal centrosome dynamics are thought to drive chromosome segregation errors and promote cancer.

## CENTROSOME STRUCTURE

In animal cells, the centrosome is the primary microtubule-organizing center [8, 9]. In non-dividing cells, centrosomes are located in close proximity to the nucleus where they organize microtubules to help establish cell shape, polarity and proper positioning of subcellular organelles [10]. In dividing cells, centrosomes duplicate to form a bipolar mitotic spindle that separates chromosomal content evenly between two daughter cells. [9]. One centrosome consists of two orthogonally positioned cylindrical organelles, called centrioles that are joined by fibers connecting their proximal ends. Centrioles are surrounded by an unstructured mass of proteins referred to as the pericentriolar material (PCM) [11]. Each centriole consists of nine sets of microtubule triplets assembled into a cartwheel structure. Paired centrioles differ from each other in that one is the mother centriole with appendages at its distal ends and the other is the daughter centriole, which lacks these structures. The PCM contains  $\gamma$ -tubulin ring complexes ( $\gamma$ -TuRCs) as well as several large coiled-coil proteins including pericentrin, ninein and Cep135, which together function in nucleating, anchoring, and positioning microtubules [12].

## THE CENTROSOME CYCLE

The centrosome is duplicated and separated once per cell cycle and proceeds in a timely fashion that is coordinated with the cell cycle. For the purposes of this review, we will divide the centrosome cycle into two parts: centrosome duplication and centrosome dynamics, which encompasses centrosome splitting (disjunction) and movement. Centrosome duplication takes place between late G1 and early G2 phase and is characterized by four stages: centriole disengagement, centriole duplication, centriole elongation, and centrosome maturation. The key events and molecular drivers of each of the four stages are summarized in Box 1. Additional background on centrosome duplication can be found in several recent in-depth reviews [13–16]. Part two of the centrosome cycle, centrosome dynamics, takes place between late G2 and early M phase and will be reviewed in detail below.

## CENTROSOME DYNAMICS

Centrosome dynamics can be subdivided into two stages: centrosome disjunction in late G2, and microtubule motor protein-mediated centrosome movement toward opposite poles in prophase or prometaphase.

### Centrosome disjunction

Until late G2, centrosomes are joined by centrosomal cohesin complexes, which include C-Nap1 and rootletin [49]. C-Nap1 is a large coiled-coil protein that localizes rootletin to the proximal end of centrioles, which physically links the two centrioles via fibrous polymers

[49]. Late in G2, C-Nap1 and rootletin are phosphorylated by the NIMA-related S/T kinase Nek2A, causing centrosome disjunction (Figure 1A) [50, 51]. Accumulation of Nek2A to centrosomes is regulated by two components of the Hippo pathway, hSav1 and Mst2, the latter of which activates Nek2A through phosphorylation (Figure 1A) [52]. In turn, Plk1 activates Mst2 through phosphorylation. This particular post-translational modification also promotes centrosome disjunction by preventing Nek2A binding to PP1 $\gamma$  a phosphatase, which antagonizes Nek2A (Figure 1A).

Recently, it was discovered that cyclin B2/Cdk1 is an upstream regulator of the Nek2-dependent centrosome disjunction pathway [5]. Cyclin B2 is a member of the B-type cyclin family and activates Cdk1 through direct binding [53]. Prior to this study, little was known about cyclin B2 other than it localized to centriolar satellites in somatic cells and had an undefined role in spindle formation in *Xenopus* oocytes [54, 55]. In experiments using gain- and loss-of-function models, cyclin B2 was found to play a critical role in centrosome separation during G2 by promoting Aurora A activity at centrosomes (Figure 1A). Aurora-A is required for the activation of Plk1 [56], which, as mentioned, regulates centrosome separation by Nek2A-dependent phosphorylation of the centrosome linker proteins C-Nap1 and rootletin [13, 57]. Overexpression of cyclin B2 triggers hyperactivation of this pathway, resulting in premature centrosome separation. Conversely, depletion of cyclin B2 reduced centrosomal p-Aurora-A and p-Plk1 and inhibited centrosome disjunction in mouse and human primary cells. Control of centrosome separation by cyclin B2 is dependent on p53, which negatively regulates Aurora-A both transcriptionally and post-transcriptionally [58]. Loss of p53 increases Aurora-A expression and phosphorylation, resulting in premature centrosome separation. This finding indicates that cyclin B2 and p53 antagonistically coordinate centrosome disjunction and demonstrate that cyclin B2/Cdk1 is the upstream regulator of the Nek2A-dependent centrosome disjunction pathway.

The Wnt signaling effector  $\beta$ -catenin has also been implicated in regulating centrosome disjunction [59].  $\beta$ -catenin was found to be a substrate of Nek2A and localize to proximal and distal regions of centrioles [60]. Depletion of  $\beta$ -catenins lead to monopolar spindles, underscoring its role in centrosome dynamics [59]. Depletion of either rootletin or C-Nap1 causes precocious centrosome disjunction, indicating that  $\beta$ -catenin operates in a functionally distinct manner.

In addition to C-Nap1 and rootletin, Cep68 and Cep215 were identified as centrosomal linker proteins in an siRNA-based screening approach for centrosome cohesion [61]. Both Cep68 and Cep215 are found at centrosomes, but differ in their precise localization. While Cep68 contributes to a dynamic linker structure, Cep215 interacts with and is thought to function downstream of pericentrin [61]. The loss of Cep68 from centrosomes upon mitotic entry of cells and its reliance upon the presence of other linker proteins for recruitment to centrosomes is consistent with Cep68 being a target of Nek2A. The differing behavior and localization of Cep215 favors an alternative mode of regulation, perhaps directly through Plk1.

## Centrosome movement

After centrosome disjunction (at G2/M transition), the two untethered centrosomes begin to move toward their respective anchoring sites. Centrosome movement is a complex, multifaceted process that remains incompletely understood at the molecular level [13, 62]. Eg5 plays a central role in centrosome movement. This plus-end directed microtubule motor protein accumulates at centrosomes and astral microtubules, where it generates an outward “pushing” force by sliding antiparallel microtubules between centrosome pairs in opposite directions (Figure 2). Nek6 and 7, and presumably cyclin-Cdk1, facilitate Eg5 targeting to centrosomes through phosphorylation (Figure 1B) [63]. Activation of Nek6 and 7 is mediated by Nek9, which in turn is activated by Plk1 kinase activity [63–66]. While the importance of Eg5 in poleward movement of centrosomes has been firmly established, recent studies have demonstrated that additional proteins are also required in order to generate the necessary forces for centrosome movement [62, 67]. Dynein/dynactin motor complexes at the cell cortex and the nuclear envelope generate a “pulling” force to properly position individual centrosomes (Figure 2) [68, 69]. Additionally, kinetochores are implicated in centrosome movement through the formation of K-fibers [70]. Moreover, the actin cytoskeleton was found to be involved in centrosome movement [62]. However, how actin generates the necessary forces for centrosome movement remains largely unclear.

## ABNORMAL CENTROSOME DYNAMICS

The relationship between centrosome separation and chromosome missegregation has been historically overlooked for several reasons. First, bipolar spindle formation can be achieved in some experimental settings when centrosomes are removed via laser ablation or microsurgery [71, 72], leading some to question their role in the process. Second, initiation of centrosome separation within individual cells of a culture has been reported to occur over a range of time spanning prophase to prometaphase, implying flexibility between the synchronization of centrosome separation and mitotic progression [70, 73]. Furthermore, centrosomes that are immobilized prior to NEBD can compensate for this delay by separating their centrosomes in prometaphase without overtly compromising chromosome segregation [74, 75]. Third, *Drosophila melanogaster* lacking centrosomes can develop without any conspicuous abnormalities [76]. Therefore, irregularities in the control of centrosome splitting and movement prior to NEBD have been largely neglected as a potential source of aneuploidization. However, following several recent advances, it is becoming increasingly apparent that centrosome separation is a highly orchestrated process that needs to unfold in a timely and controlled fashion in order to create a bipolar spindle that accurately segregates duplicated chromosomes between two daughter cells.

### Abnormal centrosome dynamics and aneuploidization

As aforementioned, recent work from several laboratories now points to a critical role of proper centrosome dynamics in ensuring accurate segregation of sister chromatids, with both delayed and accelerated centrosome separation increasing rates of spindle geometry defects in metaphase and mitotic errors [4, 5, 7, 77] (Figure 3). Furthermore, evidence is mounting that centrosome dynamics are tightly regulated to limit the risk of neoplastic transformation, which will be discussed in greater detail in the next section. However, we will first review

the recent advances made in understanding how delayed centrosome separation leads to W-CIN and discuss potential models for how accelerated centrosome separation might also promote mitotic errors.

Pioneering work in understanding how delayed centrosome separation facilitates chromosome missegregation has come from the work of Silkworth and Cimini [77]. Using a subclone of Ptk1 cells with slow separating centrosomes, they uncovered that microtubules emanating from spindle poles in close proximity to each other promote merotelic attachments, a type of attachment error in which one kinetochore is attached to microtubules originating from both spindle poles (Figure 3). These merotelic attachments can form either directly or indirectly through a syntelic intermediate, where both sister kinetochores are attached to microtubules from a single centrosome. Merotelic attachments are particularly dangerous for dividing cells because they are not recognized by the spindle assembly checkpoint and can result in chromosome lagging if left uncorrected by an overwhelmed error correction machinery (Figure 3) [78]. This principle was later illustrated in *Usp44* knockout mouse embryonic fibroblasts (MEFs), where delayed centrosome splitting led to abnormal spindle positioning in metaphase and lagging chromosomes [4].

Recent studies in cyclin B2 overexpressing cells have revealed that accelerated centrosome separation can also culminate in abnormal spindle positioning in metaphase, the formation of merotelic attachments, and lagging chromosomes [5]. While this study indicates that centrosome movement and microtubule-kinetochore attachment need to be tightly coordinated to ensure faithful chromosome segregation, the mechanistic basis for increased merotelically upon accelerated centrosome splitting remains currently unknown.

One possibility is that early in prometaphase, the immature kinetochores of cells with completely (or almost completely) separated centrosomes are less susceptible to capturing laterally-approaching microtubules and are therefore prone to forming merotelic attachments (Figure 3). After NEBD, kinetochore assembly is still ongoing and cytoplasmic kinetochore proteins that are important for proper microtubule-kinetochore attachment, like BubR1, are being actively recruited to kinetochores. Mature kinetochores are massive, multi-protein structures that may promote proper microtubule attachments and prevent improper attachments in part due to steric hindrance. When a microtubule emanating from a spindle pole is laterally approaching an immature kinetochore, the immature kinetochore may be insufficiently developed to direct the microtubule to its optimal position at the unformed outer kinetochore and, therefore, the microtubule may be just as likely to bind improperly to the opposite kinetochore and form a merotelic attachment.

Another possibility may be that elevated rates of merotelically are a consequence of the spindle geometry defects resulting from early centrosome disengagement (Figure 3). Perpendicular spindle geometry is defined as a roughly 90° angle between the metaphase plate and the imaginary line connecting the two spindle poles positioned on opposite sides of the metaphase plate (Figure 4). Spindle poles that are not positioned perpendicular to the metaphase plate may promote merotelic attachments that could effectively overwhelm the error correction machinery and reduce its ability to correct merotelic attachments prior to anaphase.

Alternately, the Aurora B-driven error correction machinery may function less efficiently in correcting merotelically in the presence of aberrant spindle forces generated at inner centromeres of asymmetric spindles (Figure 3). Inner centromeric Aurora B destabilizes faulty kinetochore-microtubule attachments, including merotelic attachments, by phosphorylating its substrates located on outer kinetochores. Recent studies suggest that Aurora B kinase activity is dependent on its proximity to these substrates [79–81]. Because intrakinetochore stretch appears to function as an error correction switch [82, 83] – low stretch in the absence of tension will promote destabilization whereas high stretch in the presence of tension will promote stable attachments – the tension generated under asymmetric spindle conditions may inappropriately silence Aurora B kinase activity by physically dissociating it from its substrates, leaving merotelically attached kinetochores unresolved.

As mentioned previously, centrosome dynamics encompass both disengagement and poleward movement. It is conceivable that altered centrosome movement, either too slow or too rapid, also acts to promote merotelic attachment and chromosome lagging. The molecular mechanisms driving centrosome movement remain poorly understood, which is a key barrier to the thorough testing of this idea. Currently, Eg5 is perhaps the most obvious candidate for a molecular target that would slow centrosome movement and impair establishment of an orthogonal bipolar spindle. In addition to Eg5-specific mutations that might limit its motor potential or impair its microtubule binding ability, defective centrosomal targeting of Eg5 might also delay poleward movement and result in abnormal spindle geometry. Similarly, overexpression of Eg5 may also negatively impact centrosome dynamics and promote chromosome segregation errors. In line with this idea, MEFs that overexpress Eg5 were found to be genomically unstable and were unable to form proper bipolar spindles in mitosis [84]. Furthermore, Eg5 transgenic mice were highly prone to spontaneous tumor formation, further linking centrosome separation defects, W-CIN, and cancer.

Intriguingly, both delayed and accelerated centrosome separation promote formation of non-symmetrical mitotic spindles with improperly oriented poles [4, 5]. One possible explanation could be that improper timing of centrosome movements causes deregulation of molecular events that mediate spindle pole anchoring at the cell cortex (Figure 4). Much of the current mechanistic knowledge about cortical anchoring of spindle poles comes from stem cells, which exploit anchoring to specific cortical regions to mediate unequal separation of proteins and organelles during cell division, allowing one daughter cell to retain self-renewal capacity and the other to engage in cell differentiation [85–87]. Cortical anchoring sites are created by specific multi-protein complexes. The G protein G $\alpha$ i accumulates at designated anchoring sites and mobilizes LGN, a large scaffold protein. LGN in turn is responsible for recruitment of the microtubule binding protein NuMA and the motor complex dynein/dynactin, which provides plus end directed microtubule-pulling forces (Figure 4) [85, 88]. Core components of the spindle pole anchoring machinery are also present in cells, where they may play a central role in spindle orientation and anchoring as well [88, 89]. It will therefore be interesting to explore whether these components are subject to deregulation in cells with spindle geometry defects due to abnormal spindle pole anchoring. Furthermore, signals from the extracellular matrix are known to contribute to spindle orientation, for instance by regulating cortical actin structure and dynamics [90–92], and should also be

further interrogated with regards to spindle geometry defects associated with untimely centrosome separation.

### Abnormal centrosome dynamics and cancer

The physiological relevance of incomplete centrosome separation was recently demonstrated in mice lacking the Cdc20 deubiquitinase Usp44 [4]. *Usp44* knockout mice were prone to aneuploidization and highly susceptible to spontaneous tumor formation. Usp44 loss caused chromosome lagging and Usp44 was subsequently found to be required for proper centrosome disjunction and perpendicular spindle formation in metaphase (Figure 5). Conversely, accelerated centrosome separation in G2 phase seems to be a key tumor-promoting event in mice that overexpress cyclin B2 [5]. High levels of cyclin B2 were found to promote centrosomal aurora A and Plk1 hyperactivity, which resulted in abnormal spindle geometry and W-CIN characterized predominantly by lagging chromosomes (Figure 5). Moreover, knockdown of cyclin B2 in non-transformed primary cells led to reduced activity of aurora A and Plk1, incomplete centrosome separation, asymmetric metaphase spindle geometry, and chromosome lagging.

Taken together, these studies imply a critical role for proper timing of centrosome separation in tumor suppression. Both incomplete and accelerated centrosome separation are tightly associated with tumorigenesis *in vivo*, raising the question of how common abnormal centrosome separation is in human cancers without centrosome amplification. Because centrosome dynamics are so sensitive to perturbations – that is, both premature and delayed separation result in improperly positioned spindles and W-CIN – one can envision centrosome separation defects being a common hallmark of genetically unstable cancer cells. The observation that lagging chromosomes represent the main segregation defect in cancerous cells [78] supports this notion. Indeed, p53 was discovered to govern proper timing of centrosome separation and three-dimensional spindle positioning by regulating centrosomal aurora A activity [5, 58]. The implication of this discovery is that tumors characterized by p53 loss-of-function may adopt a mutator phenotype in part due to whole chromosome loss or gain resulting from early centrosome disjunction and formation of asymmetric mitotic spindles (Figure 5). In this way, p53 is a prototypical example of a commonly altered cancer-critical gene that can also drive CIN. In fact, several tumor suppressors and oncogenes have been reported to induce CIN [3]. It will be important to determine which kinds of p53 mutations found in human cancers perturb aurora A control in order to better understand the prevalence of abnormal centrosome dynamics in human cancers. Likewise, it will be important to discern which other commonly altered tumor suppressor proteins and/or oncogenes drive CIN by altering normal centrosome dynamics.

### CONCLUDING REMARKS

The centrosome organizes microtubules within the cell and helps establish cell shape, polarity, subcellular localization of organelles, and bipolar spindle formation. The centrosome is duplicated, disjoined, and translocated once per cell cycle in distinct phases that are governed by a subset of master regulators that include Plk1, Plk4/SAS-6, aurora A, Eg5/dynein, cyclin B2, and p53. Recent advances in the field have implicated normal

centrosome dynamics as a critical process to ensure accurate segregation of duplicated chromosomes between daughter cells. Furthermore, studies aimed to decipher the physiological relevance of centrosome dynamics in mice have suggested that abnormal timing of centrosome separation may be a common and potent CIN-inducing event tightly associated with cancer. These studies have shown that proper centrosome splitting may be a commonly deregulated process in human cancers by virtue of the fact that the most frequently mutated tumor suppressor, p53, plays a central role in governing centrosome disjunction by controlling aurora A activity. This observation raises the possibility that other frequently mutated cancer-critical tumor suppressor genes and oncogenes may promote W-CIN by disrupting the timing and/or movement of centrosomes as the cell attempts to form a bipolar spindle. The frequency of centrosome separation defects in cancer cells and the molecular pathways implicated therein will be important to identify in order to better understand the stepwise process of cellular transformation, pathogenesis of cancer, and to develop novel therapeutic approaches for patients with malignant neoplasms.

## Acknowledgements

We thank Paul Galardy, Darren Baker, Janine van Ree and Arun Kanakkanthara for critical reading of the manuscript and providing helpful comments and discussion. R.M.N. (F30 CA189339-01 and T32 GM 65841) and J.M.v.D. (CA126828) were supported by grants from the National Institutes of Health.

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### Highlights

- The relationship between centrosome separation and chromosome missegregation has historically been overlooked
- Gene mutations that delay or accelerate centrosome separation cause spindle malformation, merotely, aneuploidy and cancer
- Cyclin B2 and p53 play a central role in centrosome separation
- Centrosome separation defects may be a frequent source of chromosomal instability in human cancers

## BOX 1. The four stages of centrosome duplication

### Centriole disengagement

During late mitosis and early G1 phase, the perpendicular orientation of centriole pairs is lost as the daughter centriole begins to dissociate and remains attached to the mother centriole merely through flexible fibers (Figure I). This process, termed centriole disengagement, is essential for limiting centriole duplication to one round per cell cycle and for recruiting PCM [17]. Separase and polo-like kinase 1 (Plk1) have been identified as key regulators of centriole disengagement [18]. Separase is thought to drive disengagement by removing cohesin rings that hold mother and daughter centrioles together through proteolytic cleavage of Scc1 [19]. However, subsequent publications have argued against a need for cleavage of centriole-associated cohesin in disengagement [20, 21]. Plk1 regulates centriole disengagement through its interaction with a centrosomal splice variant of Sgo1 known as shorter shugoshin 1 (sSgo1). sSgo1 protects centriolar cohesin from untimely separase-mediated cleavage through a direct interaction with Plk1 [22]. Astrin and Akt kinase interacting protein 1 (Aki1 kinase) also regulate centromeric and centriolar cohesin dissolution through a largely unresolved mechanism [23, 24].

### Centriole duplication

Following disengagement, the cell contains one centrosome comprised of two structurally and functionally dissimilar centrioles. The mother centriole, which originates from the mother cell, nucleates and organizes microtubules, whereas the daughter centriole, which is replicated from the mother centriole as a procentriole, only nucleates microtubules and is relatively mobile [25]. Synthesis of the daughter centriole from a pre-existing mother centriole, termed centriole duplication, takes place in early S phase. Centriole duplication is incompletely understood, but involves at least five key proteins: CEP192, SAS-4 (CPAP), SAS-5 (STIL), SAS-6 and Plk4 [26]. The latter two are particularly important as their overexpression is associated with increased centriole synthesis, centrosome amplification, and chromosomal instability [27–35]

### Centriole elongation

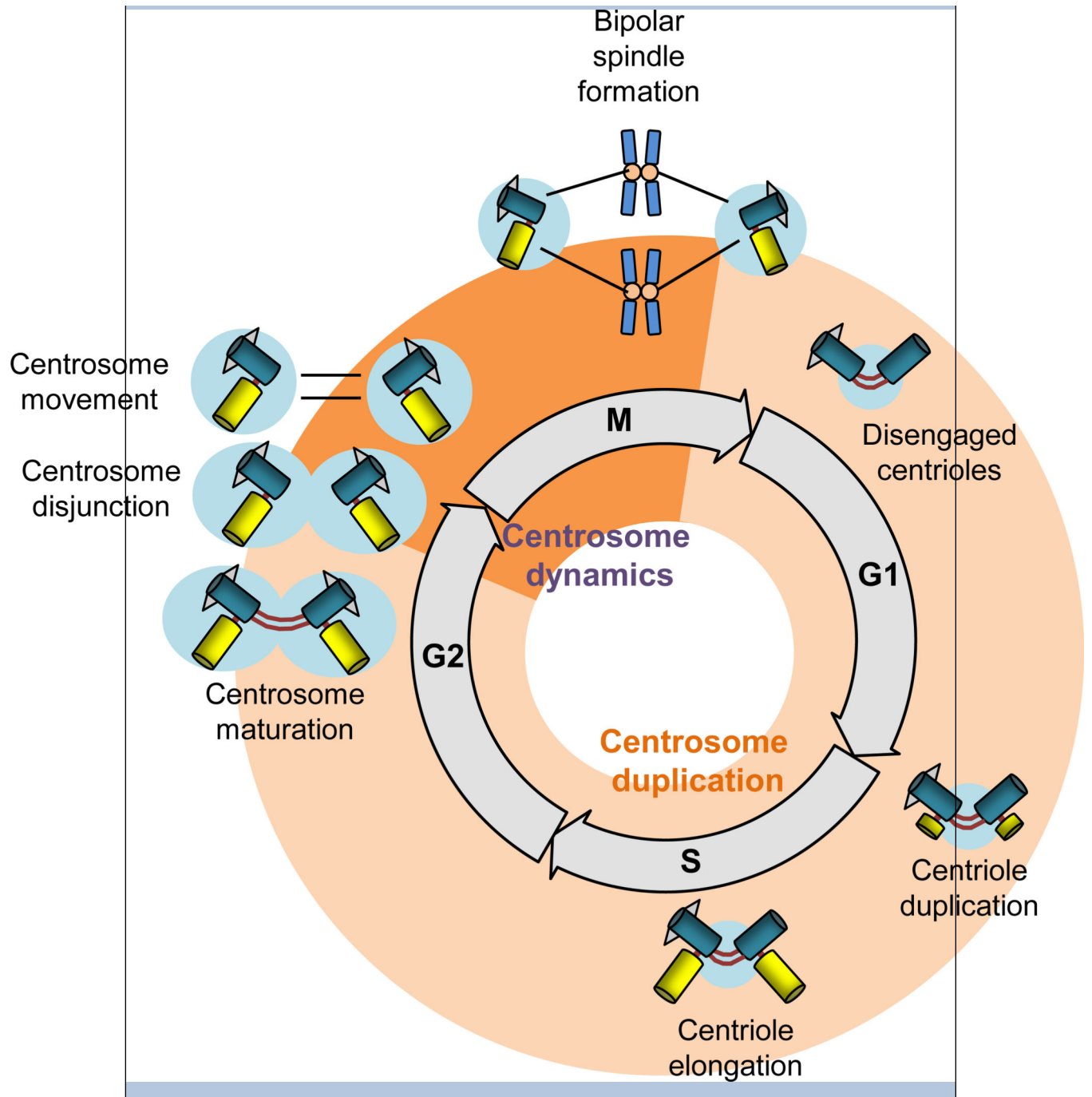
Newly generated daughter centrioles (also called procentrioles) that emerge near the proximal ends of mature centrioles during early S phase elongate and reach full length by late G2 or early M phase (Figure I). Elongation requires several proteins, including POC5, OFD1, SAS-4, and CP110 [14], with the latter two being best understood at a molecular level [36–38].

### Centrosome maturation

In late G2, the PCM dramatically increases  $\gamma$ -TuRCs and its associated proteins, a process referred to as centrosome maturation (Figure I) [39]. Both Plk1 and Aurora-A are recruited to the centrosomes in G2 to mediate maturation [40, 41]. Recruitment of Aurora-A to centrosomes is dependent on Plk1 activity, while activation of centrosome-associated Plk1 is dependent on Aurora-A kinase activity [42]. The microtubule-binding protein TPX2 is also involved in centrosome maturation, presumably by interacting with,

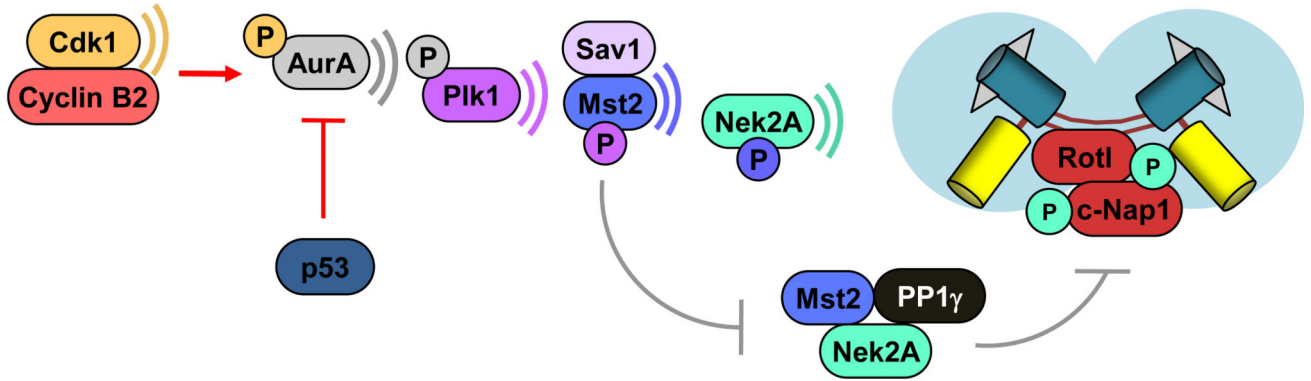
and activating Aurora-A [43–45]. Other Plk1 substrates implicated in maturation are pericentrin, NEDD1, CEP192, and CEP215 [46]. Centrosome maturation is initiated when Plk1 phosphorylates pericentrin and NEDD1, which properly targets  $\gamma$ -TuRC to the PCM [47, 48]. Aurora-A then localizes to the centrosome and is activated by Plk1 and the LIM kinase protein Ajuba to complete centrosome maturation by recruiting LATS2, NDEL1 and TACC3 to the centrosome via phosphorylation [42].

**Text Box 1; Figure I. The centrosome cycle.** The centrosome cycle is divided into two parts: centrosome duplication, which encompasses centriole disengagement, duplication, elongation, and maturation, and centrosome dynamics, which includes centrosome disjunction and movement. In late mitosis, the daughter centriole disengages from the mother centriole and the two centrioles lose their orthogonal configuration (centriole disengagement). At the G1/S transition, one new daughter centriole is synthesized per mother centriole to form a new centrosome (centriole duplication and elongation). In late G2, both centrosomes recruit PCM components in order to prepare for mitosis (centrosome maturation). At the G2/M transition, centrosomal cohesion is gradually lost (centrosome disjunction) and centrosomes move toward opposite poles to form a bipolar spindle (centrosome movement) [9–11].

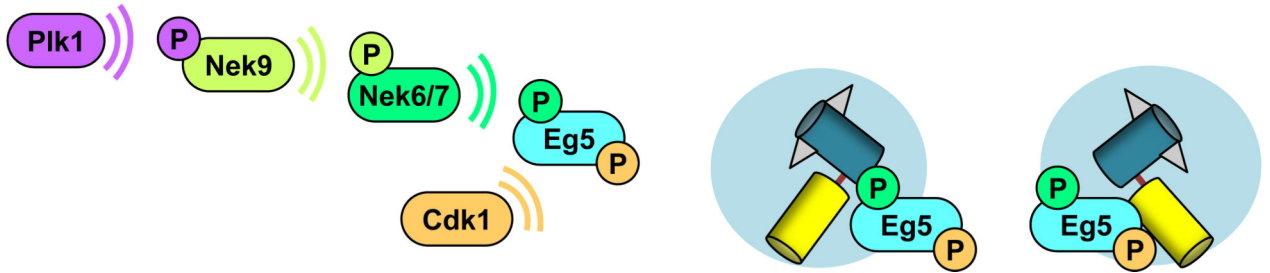




**A. Centrosome disjunction**

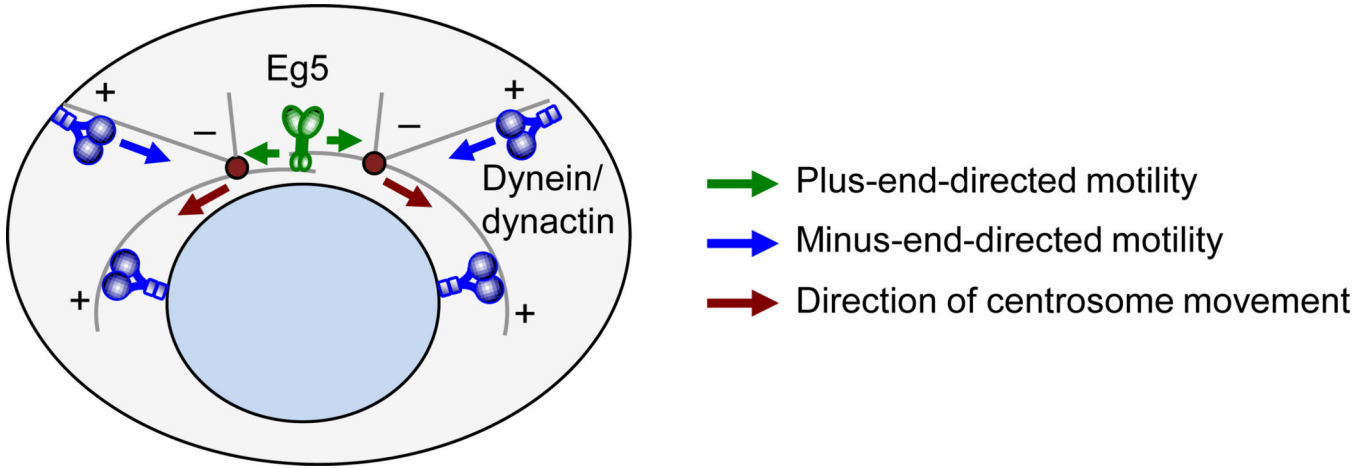


**B. Centrosome movement**

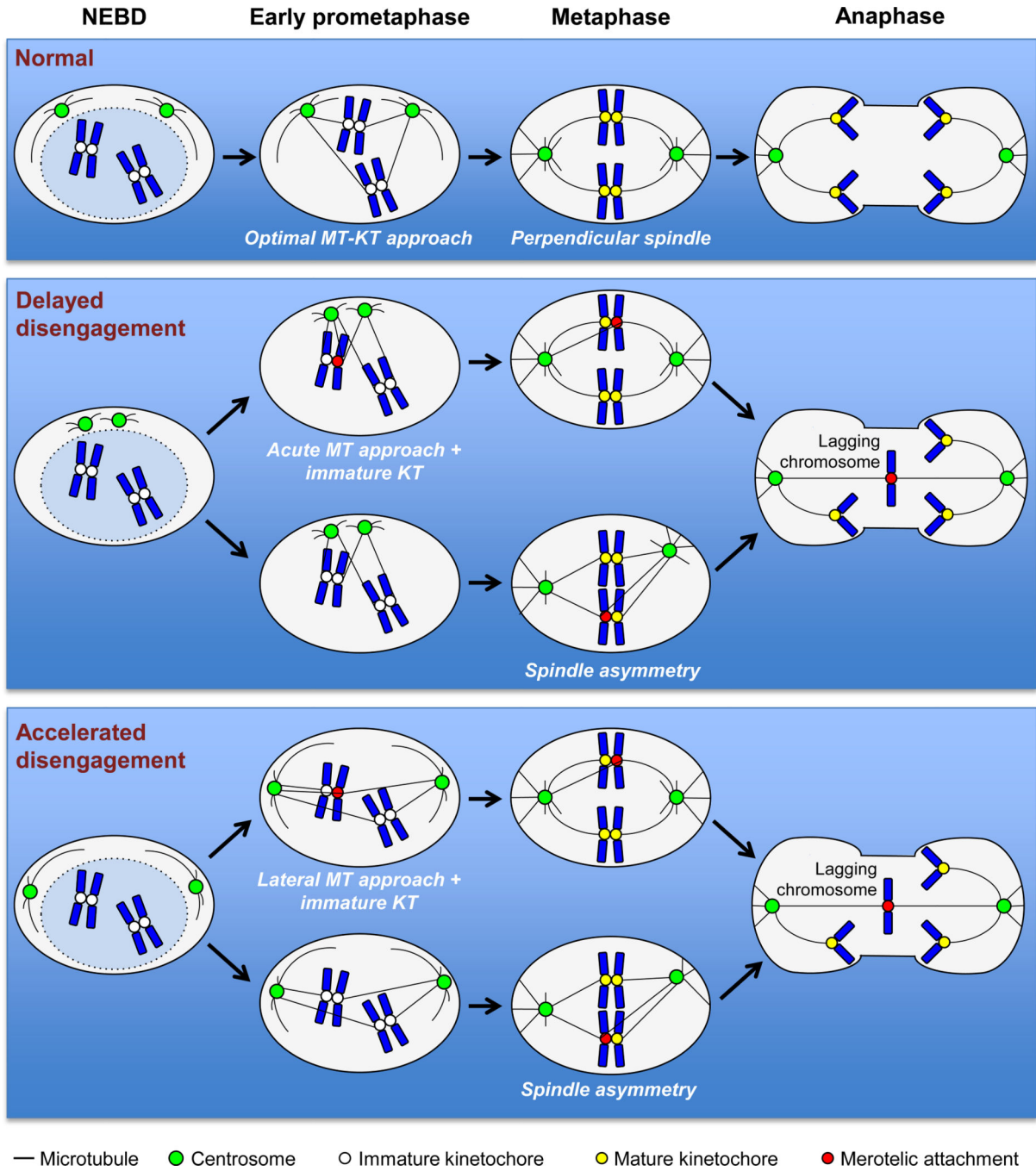


**Figure 1. Centrosome dynamics**

Centrosome separation occurs in two parts from late G2 through mitosis: disengagement and movement. **(A)** Hypothetical model for centrosome disjunction. Centrosome-associated cyclin B2/Cdk1 acts to initiate centrosome disjunction through phosphorylation of Aurora A, which in turn phosphorylates Plk1. Activated Plk1, phosphorylates Sav1-bound Mst2, triggering Mst2-mediated phosphorylation of Nek2A. Activated Nek2A phosphorylates the centrosome linker proteins C-Nap1 and rootletin, causing the physical separation of sister centrosomes. PPl $\gamma$  antagonizes Nek2A phosphorylation of C-Nap1 and Rootletin. [13, 52, 57]. **(B)** Model for centrosome movement. Plk1 targets Eg5 to centrosomes through sequential phosphorylation of Nek9 and Nek6/7. Cdk1 has also been proposed to be important for Eg5 activation and binding to microtubules [13, 57, 63, 93].



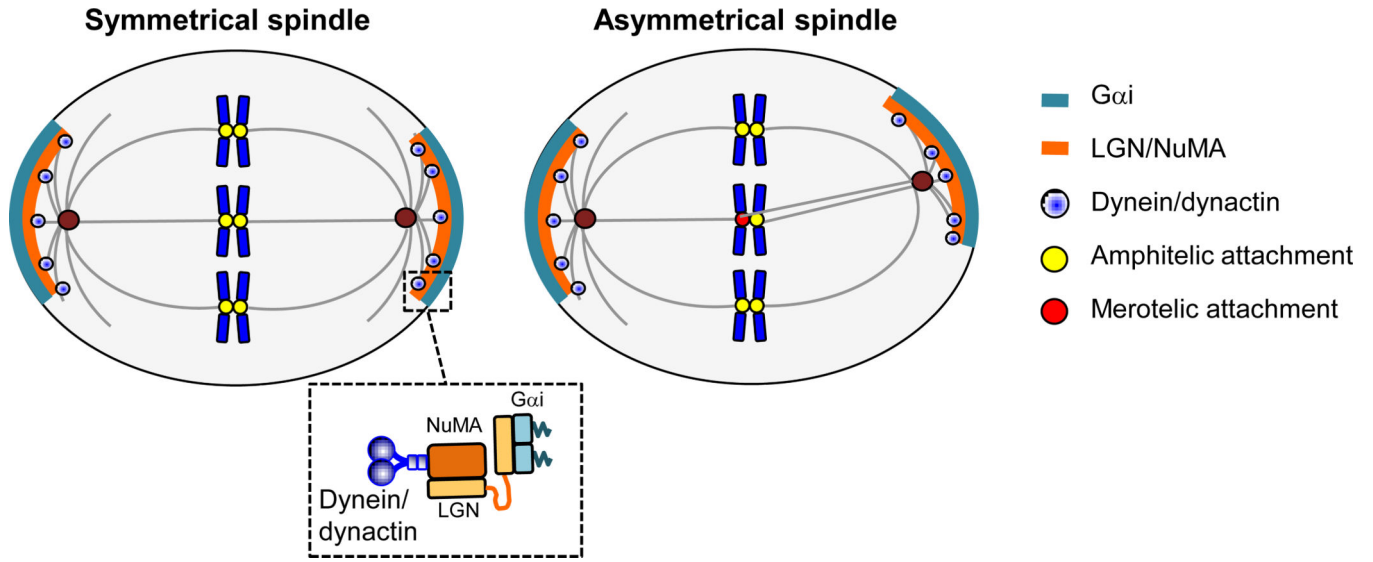
**Figure 2. Motor proteins forces drive poleward movement of duplicated centrosomes**  
 Separated centrosomes move toward their respective anchoring sites with the help of multiple motor proteins, including Eg5 and dynein. Eg5 is a plus-end-directed motor protein that generates an outward pushing force between the centrosome pair [62]. Cortical and nuclear envelope associated dynein pull centrosomes apart through minus-end-directed force [94, 95].



**Figure 3. Hypothetical mechanisms by which aberrant centrosome dynamics promote merotelically and chromosome lagging**

Normal centrosome dynamics promote amphitelic attachments and faithful chromosome segregation between daughter cells. Delayed centrosome disengagement promotes merotelic attachments in early prometaphase because kinetochores are accessible to microtubules from spindle poles in close proximity. At this stage, immature kinetochores have not yet recruited all of the proteins necessary for proper kinetochore-microtubule attachments, increasing the likelihood of merotelic attachments and/or decreasing the efficiency at which merotelic attachments are detected and resolved. Alternatively, delayed centrosome disengagement is

associated with spindle asymmetry in metaphase, which may also promote misattachments prior to anaphase. Accelerated centrosome disengagement may promote merotelic attachments in early prometaphase again through a combination of suboptimal microtubule approach angle to the kinetochore and immature kinetochores that cannot detect and/or resolve merotelic attachments. Like delayed disengagement, accelerated centrosome disengagement has been associated with spindle asymmetry in metaphase, which is likely to promote merotelic attachments just prior to anaphase onset. Unresolved merotelic attachments resolve in chromosome lagging. MT; microtubule. KT; kinetochore.



**Figure 4. Improper anchoring of astral microtubules at cell cortex as a source of spindle geometry defects**

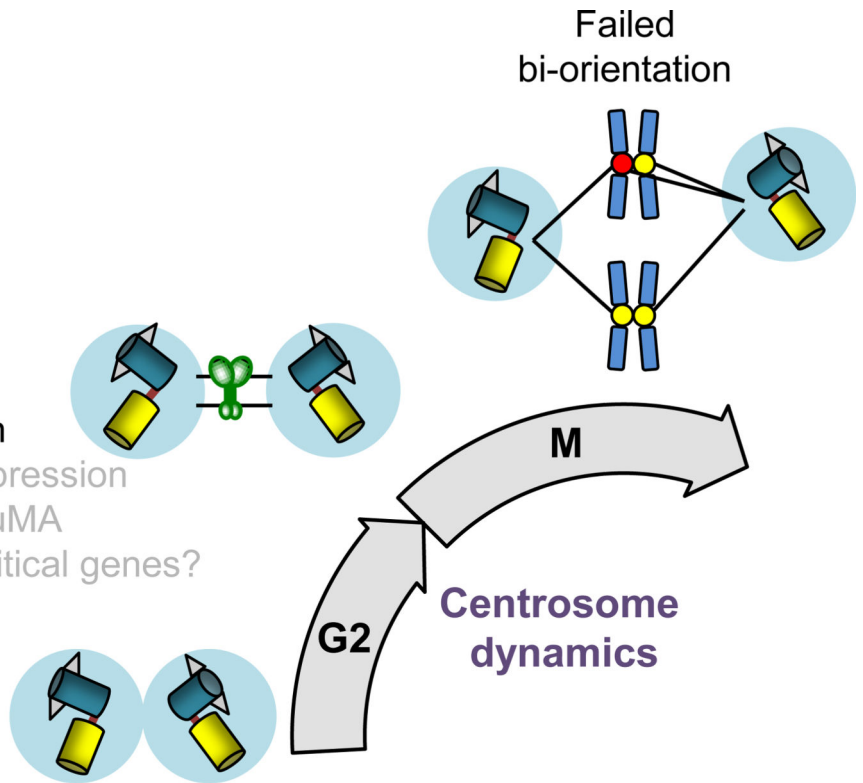
In cells with normal spindle geometry (left), dynein/dynactin interacts with the Gαi-LGN-NuMA anchor protein complex [89] and captures astral microtubules near the cortex contributing to symmetrical spindle pole orientation and proper kinetochore-microtubule attachment. In cells with abnormal spindle geometry, demarcation of cortical anchoring regions is not symmetrical, resulting in improper kinetochore-microtubule attachment (right).

**Aberrant movement:**

- Eg5 overexpression
- Others? Altered expression of Kif15, Plk1, or NuMA
- Common cancer-critical genes?

**Aberrant disengagement:**

- cyclin B2 overexpression
- Usp44 inactivation
- p53 inactivation
- Others? Aurora A, Plk1, Mst2, Nek2A overexpression or insufficiency



**Figure 5. Cancer-associated molecular drivers of abnormal centrosome dynamics**

*Usp44* knockout and cyclin B2 overexpression cause spontaneous tumors in mice and feature delayed and accelerated centrosome separation, respectively [4, 5]. p53 inactivation also causes abnormal centrosome disjunction and is the most commonly mutated tumor suppressor in human cancers. It is likely that there are many other proteins, such as the CIN70 gene *Nek2A* [96], that regulate centrosome disjunction that, when altered, promote CIN and may be associated with cancer. Likewise, abnormal centrosome movement, as is the case for Eg5 overexpression, is tightly associated with CIN and tumorigenesis. There remains a critical need to identify other cancer-associated genes that normally regulate centrosome dynamics.