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Review

Attenuated Chromosome Oscillation as a Cause of Chromosomal Instability in Cancer Cells

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Simple Summary: Chromosomal instability (CIN), a condition in which chromosome missegregation occurs at high rates, is widely seen in cancer cells. Causes of CIN in cancer cells are not fully understood. A recent report suggests that chromosome oscillation, an iterative chromosome motion typically seen in metaphase around the spindle equator, is attenuated in cancer cells, and is associated with CIN. Chromosome oscillation promotes the correction of erroneous kinetochore-microtubule attachments through phosphorylation of Hec1, a kinetochore protein that binds to microtubules, by Aurora A kinase residing on the spindle. In this review, we focused on this unappreciated link between chromosome oscillation and CIN.

Abstract: Chromosomal instability (CIN) is commonly seen in cancer cells, and related to tumor progression and poor prognosis. Among the causes of CIN, insufficient correction of erroneous kinetochore (KT)-microtubule (MT) attachments plays pivotal roles in various situations. In this review, we focused on the previously unappreciated role of chromosome oscillation in the correction of erroneous KT-MT attachments, and its relevance to the etiology of CIN. First, we provided an overview of the error correction mechanisms for KT-MT attachments, especially the role of Aurora kinases in error correction by phosphorylating Hec1, which connects MT to KT. Next, we explained chromosome oscillation and its underlying mechanisms. Then we introduced how chromosome oscillation is involved in the error correction of KT-MT attachments, based on recent findings. Chromosome oscillation has been shown to promote Hec1 phosphorylation by Aurora A which localizes to the spindle. Finally, we discussed the link between attenuated chromosome oscillation and CIN in cancer cells. This link underscores the role of chromosome dynamics in mitotic fidelity, and the mutual relationship between defective chromosome dynamics and CIN in cancer cells that can be a target for cancer therapy.

Keywords: chromosomal instability; cancer; chromosome oscillation; kinetochore-microtubule attachment; Aurora kinase; aneuploidy; Hec1

1. Introduction

Most cancer cells have an abnormal number and structure of chromosomes [1,2]. Gain or loss of entire chromosomes is called (whole chromosome) aneuploidy, while amplification or loss of parts of chromosomes is called structural (or segmental) aneuploidy [3–5]. Aneuploidy is caused by chromosome missegregation during mitosis, which is derived not only from mitotic defects, but also from defects in interphase such as replication stress [6]. In many cases, aneuploid cancer cells exhibit increased rates of chromosome missegregation, which is called chromosomal instability (CIN) [7–9]. CIN is a cause of intratumor heterogeneity, and related to cancer progression and poor prognosis, including metastasis and drug resistance [10–13]. Various paths to CIN have been revealed, although a whole picture of the etiology of CIN in cancer has not yet been clarified [7,14]. Proper
attachment of a kinetochore (KT), a proteinous structure formed at the centromeric region on a chromosome, to spindle microtubules (MTs) is a prerequisite for faithful chromosome segregation. MTs are cylindrical assembly of protofilaments formed by heterodimers of α and β tubulin. This structure stochastically repeats elongation (by polymerization of tubulin heterodimers) and shrinkage (by depolymerization of tubulin heterodimers), a phenomenon known as dynamic instability that facilitates attachment to KTs [15]. Failure to achieve proper KT-MT attachments is involved in the generation of CIN [16,17]. Dysfunction of the mechanisms to resolve erroneous KT-MT attachments (error correction mechanisms) plays a particularly crucial role in the emergence of CIN [18,19]. Mitosis is a dynamic process where KT-MT attachments are regulated both temporally and spatially, and chromosome dynamics is closely linked to the formation of these attachments [20–24]. For example, in prometaphase, chromosome alignment at the spindle equator (congression) is required for the establishment of proper KT-MT attachments and faithful chromosome segregation [24,25]. In metaphase, chromosomes are aligned at the spindle equator, forming the so-called metaphase plate. These aligned chromosomes are not just staying at the metaphase plate, but moving around the spindle equator periodically, a phenomenon known as chromosome oscillation [26,27]. In this review, we first provide an overview of the error correction mechanisms of KT-MT attachments and the features and mechanisms of chromosome oscillation. We then introduce a previously unappreciated link between chromosome oscillation and error correction mechanism, and also describe attenuated chromosome oscillation as a novel cause of CIN in cancer cells.

2. Correction Mechanisms of Erroneous KT-MT Attachments and Its Relevance to CIN

2.1. Overview of Chromosome Dynamics in Mitosis

Chromosome segregation is carried out on the spindle, comprising two spindle poles and MTs that connect spindle poles to KTs (see Figure 1). Mitosis is divided into five phases: prophase, prometaphase, metaphase, anaphase, and telophase (see Figures 3 and 4). Following prophase, when chromatin condenses into well-defined chromosomes, prometaphase starts with nuclear envelope breakdown (NEBD). During prometaphase, spindle is formed while MTs attach to KTs. In metaphase, chromosomes align to the middle of the spindle, forming the metaphase plate. Anaphase onset is marked by the synchronous separation of all sister chromatids, and the separated chromosomes move towards the poles. In telophase, nuclear envelope reforms around the clustered chromosomes, and chromatin decondenses. These are common processes among eukaryotic cells, although there are many variations between species. In animal cells, spindle poles are defined by centrosomes that act as MT-organizing centers (MTOCs), while equivalent structures in fungi are called spindle pole bodies. Higher plant cells and the oocytes in many animal species do not have centrosomes. NEBD does not occur in many lower eukaryotes such as yeast, and they undergo a closed mitosis by forming the spindle in the nucleus. The number of MTs attaching to KTs also differ between species: one for a KT in budding yeast (Saccharomyces cerevisiae) [28,29], 2–4 for that in fission yeast (Schizosaccharomyces pombe) [30], and 20–30 in animal cells (kinetochore-fiber or K-fiber) [31,32]. On the other hand, chromosomes in C. elegans contain multiple centromeres along chromosome arms, which is called holocentric [33]. In the following sections, we mainly describe the mechanisms in mammalian cells.

2.2. Proper and Erroneous KT-MT Attachments

For equal chromosome segregation to daughter cells, a KT pair on sister chromatids has to be attached to MTs from opposite spindle poles, which is called bi-orientation, or amphitelic attachment (Figure 1A [16,34]. After NEBD, KTs initially attach to the lateral surface of MTs (lateral attachment), and are transported to the spindle surface by a minus end-directed motor dynein [35–41]. Then chromosomes are transported toward the spindle equator along spindle MTs by a process called chromosome congression, which involves the cooperative actions of two plus end-directed motor proteins, CENP-E on KTs and
Kid on chromosome arms [20,21,42–45]. Lateral attachment is then converted to end-on attachment, in which MT ends attach to KTs, forming K-fiber [22,46,47]. In the process of establishment of bi-orientation, three types of erroneous KT-MT attachments can arise (Figure 1B–D) [16]. One is monotelic attachment, a situation where only one of the sister KTs attaches to MTs. The second is syntelic attachment, in which both sister KTs attach to MTs from the same spindle pole. The third is merotelic attachment, where a single KT attaches to MTs from both spindle poles. The back-to-back geometry of sister KTs facilitates the formation of bi-orientation, by making a sister KT face one spindle pole when the other sister KT attaches to MTs from the opposite spindle pole [48–50]. Chromosome congression through lateral attachments also promotes bi-orientation establishment by placing KTs at the spindle equator, where MTs from both spindle poles exist at comparable density [22,51]. However, erroneous KT-MT attachments are still formed frequently, partly due to the incomplete separation of centrosomes at nuclear envelope breakdown, which become spindle poles in mitosis [35,51–53]. KT expansion in early mitosis [23,54,55], which increases the chance for MT attachment, also contributes to the increased risk of erroneous attachment formation. To resolve these erroneous attachments, there are mechanisms, referred to as error correction mechanisms, which ensure mitotic fidelity [17,56].

2.3. Error Correction Mechanisms of KT-MT Attachments

Error correction of KT-MT attachments works by destabilizing erroneous attachments while stabilizing correct ones. As a premise to enable the error correction, KT-MT attachments have to be adequately dynamic; not too unstable, but also not too stable [57,58]. Error correction mainly occurs in prometaphase, when MTs initially attach to KTs in a stochastic manner through the process of dynamic instability [40,59–61]. It was reported that KT-MT attachments in prometaphase are less stable than that in metaphase, making it more suitable for efficient error correction [62,63]. The stability of KT-MT attachment is defined by turnover rates of MTs on KTs and the stability of MTs forming K-fibers. Among the KT components, the KMN network is responsible for connecting KT to MT [64]. Located at the outer part of KT (outer plate), this network is comprised of the Knl1 complex, the Mis12 complex, and the Ndc80 complex. The Ndc80 complex is a heterotetramer composed of Hec1 (Ndc80), Nuf2, Spc24, and Spc25 (Figure 2A). Human Hec1 (highly expressed in cancer) was originally identified as an Rb-binding protein that is highly expressed in

![Figure 1](image-url)

**Figure 1.** Correct and erroneous KT-MT attachments: (A) amphitelic attachment (bi-orientation), (B) monotelic attachment, (C) syntelic attachment, (D) merotelic attachment. See text for details.
several cancers [65,66]. The globular calponin-homology domain formed by Hec1 and Nuf2 directly binds to MTs, playing a major role in the attachment to MTs [67,68]. The disordered N-terminal region of Hec1 (Hec1 tail) has nine phosphorylation sites for Aurora kinases, and their phosphorylation reduces its affinity to MTs, which is the main mechanism for error correction (Figure 2B) [69–71]. This Hec1 phosphorylation is counteracted by phosphatases, which stabilizes KT-MT attachment [69,72–76]. Other components of the KMN network are also phosphorylated by Aurora B and cooperatively regulate the affinity to MTs [77]. Stability of MTs forming K-fiber is regulated by various MT-binding proteins. Among them, two kinesin-13 family motor proteins that promote MT depolymerization, Kif2b, and MCAK (Kif2c), and destabilize MTs, forming K-fibers specifically in prometaphase and metaphase, respectively [62].

![Figure 2](image)

**Figure 2.** Regulation of KT-MT attachment through Hec1 tail phosphorylation by Aurora kinases. (A) On KTs in mammalian cells forming bi-orientation, multiple copies of the Ndc80 complex, composed of Hec1, Nuf2, Spc24, and Spc25, bind to the lateral surface of MTs to tether KTs to MT ends. (B) Phosphoregulation of KT-MT attachment. Phosphorylation of Hec1 tail by Aurora kinases reduces its affinity to MTs, allowing KT detachment, while dephosphorylation stabilizes the attachment. Phosphorylation sites on the Hec1 tail are shown in an inset.

Two Aurora kinases, Aurora A and B, are critical for proper chromosome alignment and maintenance of mitotic fidelity [78]. While Aurora A localizes to spindle poles and the spindle, Aurora B localizes to the inner centromere in early mitosis, spindle midzone in anaphase, and midbody in telophase and cytokinesis (Figure 3) as a component of the chromosome passenger complex, comprising Aurora B, INCENP, Survivin, and Borealin [71,79]. Aurora B is a key player for error correction that destabilizes erroneous KT-MT attachments, which is explained by the “spatial separation model” [80–82]. When bi-orientation is established, sister KTs are under tension due to MTs pulling towards opposite spindle poles, causing an increase in inter-KT distance. The resulting separation of the outer plate from inner centromere leads to the reduction of Hec1 tail phosphorylation by Aurora B, thereby increasing the affinity to MTs and stabilizes KT-MT attachments [77]. In contrast, sister KTs forming syntelic attachments are not under tension, and thus Hec1 phosphorylation continues. This facilitates KT detachment, which allows another chance for MT attachment.

In the case of merotelic attachment, KTs are supposed to be elongated towards the inner centromere through MT pulling force to the opposite spindle pole. It is suggested that Aurora B phosphorylates Hec1 on the elongated KT portions, thus destabilizing merotelic attachment [83–86]. This spatial separation model, which is based on Aurora B localization at the inner centromere, can explain tension-dependent error correction. However, recent
reports suggest the possibility that MT binding of Aurora B through INCENP is critical for error correction by selective destabilization of erroneous attachments [87,88]. A recent report that exploited optogenetics to manipulate Aurora B at individual KTs showed that Aurora B rather promotes MT release under high tension while depolymerizes MT under low tension [89]. Other Aurora B substrates, such as MCAK [90,91], CENP-E [92], the SKA complex [93], and HURP [94,95], also contribute to the establishment and stabilization of bi-orientation.

Recently, it was reported that Aurora A also plays a role in the correction of erroneous KT-MT attachments [96,97]. Aurora A, which plays a role in spindle assembly, mainly localizes to spindle poles and the spindle near spindle poles (Figure 3). Aurora A and B share many substrates, and their specificity is mainly determined by the proximity of the kinases to the respective substrates [98]. In prometaphase when chromosomes are near spindle poles, Hec1 on the KTs is phosphorylated and KT-MT attachments are destabilized. This resolves the erroneous attachments formed in early mitosis, especially syntelic attachment that tends to be formed when chromosomes are closest to the spindle pole.

Mps1 is a kinase that plays a crucial role in the spindle assembly checkpoint (SAC) and also functions in error correction through its interplay with Aurora B [99–104]. It was reported that Chk1, which phosphorylates and activates Aurora B [105], is also involved in error correction together with Mps1 [106]. A recent report suggests that Cdk1, a main kinase important for mitotic progression, phosphorylates the Hec1 tail, which may be involved in error correction (bioRxiv doi:10.1101/2021.02.16.431549). As another mechanism of error correction, ch-TOG (Stu2 in budding yeast) is involved in the correction of erroneous attachments by stabilizing KT-MT attachment when tension is exerted [107,108].

Figure 3. Localization of Aurora A and Aurora B during mitosis. Left: Immunofluorescence imaging of HeLa cells in different mitotic phases. HeLa cells were fixed with methanol and stained with Aurora A (red; Abcam, ab12875, 1:2000) and Aurora B (green; BD Bioscience, 611082, 1:2000) antibodies. DNA was stained with DAPI (blue). Scale bar: 5 μm. Right: Schematic diagrams showing localization of Aurora A (red) and Aurora B (green) in different mitotic phases.
2.4. CIN Caused by Insufficient Correction of Erroneous KT-MT Attachments

Erroneous KT-MT attachments are corrected by various mechanisms in a context-dependent manner, as mentioned in the previous section. For example, an unattached KT in a monotelically-attached sister KT pair is sensed by the SAC, which halts anaphase onset until both sister KTs attach to MTs [109]. Syntelically-attached KTs, which are under low tension, are also sensed by the SAC [110,111]. In contrast, merotelic attachment is not sensed by the SAC, because merotelically-attached KTs are attached to MTs and are under tension [25]. It is thus considered that merotelic attachment is a major cause of CIN, as chromosome segregation can occur in the presence of merotelic attachments if they are left uncorrected [86,112]. Even when uncorrected, merotelic attachments are resolved during spindle elongation in most cases [113]. However, remaining merotelic attachments are liable to form lagging chromosomes in anaphase, which are left behind around the spindle equator while other chromosomes are segregated to the respective spindle poles. Lagging chromosome is one of the typical patterns of chromosome missegregation. This can cause aneuploidization when they are ultimately segregated to the wrong side, or micronuclei formation when they are excluded from the main nuclei [114]. It is known that replication and repair of chromosomes in micronuclei are often defective [115–118], which results in structural chromosomal abnormalities including chromothripsis, a mutational phenomenon characterized by extensive genomic rearrangements of one or a few chromosome(s) [119,120]. Lagging chromosomes are also sometimes stuck in the cleavage furrow, resulting in structural chromosomal abnormalities due to DNA damage and cytokinesis failure through furrow regression [121]. Collectively, merotelic attachment is causative of CIN by generating both numerical and structural chromosomal abnormalities [122].

Merotelic attachments are increased either by increased formation or insufficient correction. A cause of increased formation of merotelic attachments is centrosome amplification, which is often observed in cancer cells [123,124]. To avoid multipolar spindle formation that results in catastrophic multipolar division [125], cells form pseudo-bipolar spindles by clustering excessive centrosomes (centrosome clustering) [126–129]. However, the process of centrosome clustering increases the chance of merotelic attachment formation [128,129]. A cause of insufficient correction of merotelic attachments is MT hyperstabilization, which hampers the destabilization of these attachments [57,62,130]. Another cause of inefficient error correction is reduced Aurora B activity. It was reported that Aurora B is enriched at misaligned centromeres in non-transformed cells, but not in aneuploid tumor cells [131]. A recent report suggests that heterochromatin protein 1 (HP1) binds to INCENP and augments Aurora B activity. In cancer cells, the HP1 binding to INCENP is reduced, which results in insufficient error correction due to reduced Aurora B activity [132].

3. Chromosome Oscillation

3.1. The Features of Chromosome Oscillation

On the metaphase plate, chromosomes, especially KTs, wobble around the spindle equator, which is called chromosome (or KT) oscillation (Figure 4) [26,27]. Chromosome oscillation is widely seen from yeast to human cells in mitosis and meiosis, but is not observed in some cases, such as in mitosis of insect cells, Xenopus egg extracts, and plant cells [133–138]. The main driving force of chromosome oscillation is the MT pulling force exerted by K-fibers. Although individual MTs within K-fiber behave independently, their dynamics is coordinated by MT-binding proteins, and collectively drive iterative chromosome motion, which is also known as directional instability [139–141]. This directional instability is seen not only in metaphase, but throughout mitosis, and also seen on monopolar spindles [142]. Chromosome oscillation declines as cells progress toward anaphase [143,144], reflecting the stabilization of KT-MT attachments for chromosome segregation.
Figure 4. Chromosome oscillation. Left: Live cell imaging of an RPE-1 cell expressing EGFP-α-tubulin and EGFP-CENP-A, which visualize MTs (green) and KTs (green), respectively. Chromosomes (red) were visualized using SiR-DNA (Cytoskeleton, Inc., Denver, CO, USA, 200 nM). Images were collected every minute by a DeltaVision microscope (Cytiva). Representative images of different mitotic phases are shown. Times from nuclear envelope breakdown (NEBD) are indicated. Scale bar: 5 μm. Middle: Trajectories of a pair of sister KTs in an RPE-1 cell from metaphase to telophase plotted as the distance from the spindle equator. Right: A kymograph of the sister KTs in metaphase visualized by EGFP-CENP-A. Images were collected every 2 s by a DeltaVision microscope. Horizontal scale bar: 1 μm, vertical scale bar: 10 s.

3.2. The Mechanisms of Chromosome Oscillation

In RPE-1 cells, a non-transformed cell line, the duration of metaphase is around 10 min, while one round of chromosome oscillation spans 1 to 2 min [145–147]. Therefore, a pair of sister chromatids oscillates ~10 cycles during metaphase before segregation. Symmetrical chromosome motion during oscillation is derived from the tug of war of K-fibers attaching to sister KTs. In metaphase, directional instability of chromosomes changes the distance between a KT and a spindle pole, the distance between the centroid of sister KTs and the spindle equator, and the distance between sister KTs [148,149]. When one of the sister KTs is pulled toward a spindle pole by a shrinking K-fiber (called leading KT), it causes a stretching of the elastic inter-KT region, which is comprised of the inner centromere and cohesin bond [150]. Another sister KT (called trailing KT) follows the leading KT,
pulled by the cohesin bond. Pulling force on the leading KT is increasingly counteracted by other forces (see below) as it approaches a spindle pole. Consequently, directional instability is triggered, which switches the role of the KT from leading to trailing. During the process, inter-KT distance reduces until the new leading KT stretches the inter-KT region. Therefore, the inter-KT stretch occurs twice the frequency of the oscillation of the centroid of sister KTs [151]. An important molecule that regulates chromosome oscillation is a motor protein called Kif18A [152,153]. Kif18A is a kinesin-8 family of plus end-directed motor protein, and accumulates at plus ends of MTs in K-fibers. At plus ends, Kif18A suppresses the MT polymerization, thus restricting chromosome oscillation [152]. Longer MTs accumulate more Kif18A at the plus ends, as the protein moves along MTs [154]. Therefore, polymerization is suppressed preferentially on longer MTs, limiting the range of chromosome oscillation. Kid and Kif4A, collectively called chromokinesins, are other motor proteins involved in chromosome oscillation, which localize to chromosome arms [153,155]. Kid is a plus end-directed motor belonging to the kinesin-10 family, which pushes chromosomes to the spindle equator along spindle MTs, known as polar ejection force or polar wind [156,157]. As the density of spindle MTs is higher in the vicinity of spindle poles, polar ejection force increase as chromosomes are closer to spindle poles, restricting the amplitude of chromosome oscillation [153,158]. Polar ejection force also contributes to chromosome oscillation on monopolar spindles as an opposing force against the pulling force by K-fibers, although the oscillatory motion is not symmetric as the one in metaphase [159]. A MT-associating protein NuSAP was reported to play a role in chromosome oscillation by regulating Kid-generated polar ejection force [160]. Kif4A is a kinesin-4 family plus end-directed motor protein that also plays a role in chromosome oscillation by regulating MT dynamics. At spindle poles, a kinesin-13 motor called Kif2A engages in the depolymerization of MT minus ends in K-fibers, called MT flux, which facilitates the pulling of chromosomes to spindle poles [161,162]. Recently, it was proposed that MT flux is driven by Kif4A on chromosome arms in coordination with Eg5, a kinesin-5 family, and Kif15, a kinesins-12 family motor protein, which slide antiparallel MTs [163]. Collectively, this induces Kif2A-dependent MT depolymerization. At KTs, proteins other than the Ndc80 complex, such as CENP-H, ch-TOG, and SKAP, are also involved in chromosome oscillation [146,164,165]. It was recently reported that K-fibers on sister KTs are connected by MTs that overlaps in the middle of the spindle, referred to as bridging MTs [166]. Bridging MTs contribute to the generation of tension between sister KTs and also chromosome congression and oscillation [167]. Bridging MTs branch from K-fibers via the augmin complex (bioRxiv doi:10.1101/2020.09.10.291740), and opposing bridging MTs are connected with PRC1 at the overlapping region [168]. It was suggested that bridging MTs associate with K-fibers, and the range of chromosome oscillation is determined by the length of the antiparallel overlaps of bridging MTs, which is regulated by Kif4A and Kif18A [167,169].

Chromosome oscillation is a complex process driven by various forces acting on chromosomes, and its underlying mechanism has been addressed by numerical models [26,141,170–176]. In these models, forces acting on a chromosome are considered in equilibrium at a given time (force balance model) [171,176]. Viscous resistance from cytoplasm is included as well as pulling force by K-fiber through Hec1, polar ejection force, and pulling force from sister KT through cohesin bonds (Figure 5). To enable oscillatory motion, several assumptions are integrated depending on the models, including force-dependent detachment kinetics of the Ndc80 complex, length and polymerization rate-dependent MT catastrophe frequency, position-dependent polar ejection force, and position-dependent activity gradient that weakens affinity of KT to MT [174,177,178]. These models help us to understand the relative contribution of each force on chromosome oscillation.
4. Chromosome Oscillation Plays a Role in Correction of Erroneous KT-MT Attachments

4.1. Hec1 Phosphorylation by Aurora A in Metaphase

As described above, Hec1 phosphorylation by Aurora kinases is reduced when bi-orientation is established and sister KTs are under tension. However, when observed carefully, it was found that in RPE-1 cells, serine 55 on Hec1 (Hec1-S55) is phosphorylated in a fraction of KTs in metaphase, preferentially at the periphery of the metaphase plate [147]. In contrast, Hec1-S55 phosphorylation in metaphase was barely detectable in HeLa cells, a cervical cancer-derived cell line. This Hec1 phosphorylation in metaphase was dependent on Aurora A, but not Aurora B. This was determined by specific inhibitors for the respective kinases, and metaphase-specific depletion of either kinase with the auxin-inducible protein degradation method [179–181]. Aurora A-dependent Hec1 phosphorylation in metaphase was also reported for serine 69 of Hec1 (Hec1-S69), which was observed on KTs throughout the metaphase plate, and attributed to a fraction of Aurora A in the inner centromere [182]. In contrast, Hec1-S55 phosphorylation was dependent on Aurora A localizing to the spindle, which was shown by reduction of the phosphorylation when spindle localization of Aurora A was inhibited [147]. This was achieved by replacing endogenous TPX2, a MT-binding protein that recruits Aurora A to the spindle, with TPX2 mutants unable to bind to Aurora A.

As described, Hec1 phosphorylation in prometaphase by Aurora A localizing around spindle poles was previously reported when KTs were closest to spindle poles [96,97]. The Hec1 phosphorylation on KTs near spindle poles resolve monotelic or syntelic attachments, which otherwise are stabilized by the tension exerted by KT pulling force via end-on-attached MTs and polar ejection force on chromosome arms [183]. However, it was not known whether Aurora A localizing near spindle poles plays a role in error correction in metaphase, when chromosomes are distant from spindle poles. In metaphase, Aurora A localization on the spindle increases upon mature K-fiber formation [147], which may extend the phosphorylation activity gradient closer to the metaphase plate.

4.2. Chromosome Oscillation Promotes Hec1 Phosphorylation by Aurora A

Amplitude of chromosome oscillation in HeLa cells was significantly smaller compared with that in RPE-1 cells [147]. This amplitude was increased when Kif18A was depleted by siRNA or treatment with a highly specific small molecule inhibitor. In this situation, the Hec1-S55 phosphorylation by Aurora A in HeLa cells was increased. On the other hand, when chromosome oscillation was suppressed in RPE-1 cells by reducing MT dynamics through treatment with taxol, a MT stabilizing agent, Hec1-S55 phosphorylation by Aurora A was reduced. These data suggest that chromosome oscillation expedites Hec1 phosphorylation by Aurora A. Considering that Aurora A distribution on the spindle is higher near spindle poles and KTs at the periphery of the metaphase plate are preferentially
phosphorylated at Hec1-S55, chromosome oscillation facilitates Hec1-S55 phosphorylation by moving KTs closer to the area of higher Aurora A activity near spindle poles (Figure 6).

**Figure 6.** Chromosome oscillation facilitates Hec1 phosphorylation and error correction. In non-transformed cells, chromosome oscillation facilitates the Hec1-S55 phosphorylation by Aurora A, promoting the correction of erroneous KT–MT attachments. In cancer cells, chromosome oscillation is attenuated, which leads to reduced Hec1-S55 phosphorylation by Aurora A, resulting in inefficient error correction and increase in chromosome missegregation, such as the appearance of lagging chromosomes. P: phosphorylation.

It is known that chromosome oscillation is significantly suppressed in cells expressing a Hec1 mutant in which all nine Aurora kinase-dependent phosphorylation sites were mutated to alanine [184]. This is probably due to suppression of K-fiber dynamics by hyperstabilized KT-MT attachments. It was also shown that chromosome oscillation is suppressed by inhibiting Aurora A, but not Aurora B [147, 182]. These data indicate that Hec1 phosphorylation by Aurora A is required for robust chromosome oscillation. Analyzing Hec1 constructs in which respective phosphorylation sites were mutated, it was found that phosphorylation of Hec1-S55 and S69 cooperatively promotes chromosome oscillation [147, 182]. Collectively, chromosome oscillation and Hec1 phosphorylation by Aurora A mutually promote each other.

### 4.3. Chromosome Oscillation Is Attenuated in CIN Cancer Cell Lines

Chromosome oscillation observed in cancer cell lines were significantly attenuated compared to non-transformed cell lines (Figure 6) [147]. Compared to CIN cancer cell lines (e.g., U2OS, HeLa, A549, DU145, and MCF-7 cells) which show high rates of chromosome missegregation, other cancer cell lines (e.g., HCT116, HCT-15, and DLD-1 cells, so-called “non-CIN” cell lines) show lower rates of chromosome missegregation. Interestingly, these non-CIN cell lines exhibit milder attenuation of chromosome oscillation compared with CIN cell lines, showing that the amplitude of chromosome oscillation is inversely correlated with the level of CIN.

The cause of attenuated chromosome oscillation in CIN cancer cell lines has not been specified yet. The amount of Aurora A on the spindle did not differ significantly depending on the CIN levels, excluding the possibility that difference in the spindle localization of Aurora A causes attenuation of chromosome oscillation in CIN cells [147]. It is known that KT-MT attachment stability is higher in CIN cells than those in non-transformed cells [62, 130]. When activity of MCAK, a kinesin-13 family motor protein that destabilize MTs, was upregulated in HeLa cells, the amplitude of chromosome oscillation increased, suggesting that MT hyperstabilization is related to attenuated chromosome oscillation in CIN cancer cells. Another possibility is that the balance between opposing motor protein activities is altered in cancer cells. Expression of most of the mitotic motor proteins are upregulated in the majority of cancers (Figure 7). In particular, multiple types of cancer display elevated levels of Kif18A [185–187], which suppresses the amplitude of chromosome oscillation [152]. Recently, three papers reported that CIN or aneuploid cancer cells are vulnerable to Kif18A depletion, which causes spindle defects such as multipolar spindle formation [188–190]. It was suggested that increased rates of spindle MT polymerization in CIN cells confer an enhanced dependence on the role of Kif18A to
limit MT growth [190,191]. One plausible idea is that Kif18A upregulation enables tumor cell survival through spindle assembly in prometaphase at the expense of CIN caused by attenuated chromosome oscillation in metaphase.

Figure 7. Expression of motor proteins in cancer. Expression datasets of kinesin superfamily for normal and cancer tissues were obtained from microarray gene expression data using GPL570 platform (HG-U133_Plus_2) in GENT2. GENT2: a platform for exploring Gene Expression patterns across Normal and Tumor tissues. Available online: http://gent2.appex.kr/gent2/ (accessed on 28 July 2021). The expression value of each kinesin gene in each tissue was averaged and the expression ratio of the cancer tissue to the normal tissue was calculated. Expression ratios were converted to binary logarithms and used for heatmapping and clustering, which were drawn by the heatmap.2 function of the gplots package in R. gplots: Various R Programming Tools for Plotting Data. Available online: https://CRAN.R-project.org/package=gplots (accessed on 4 August 2021). The R Project for Statistical Computing. Available online: https://www.R-project.org/ (accessed on 4 August 2021).

4.4. Chromosome Oscillation Facilitates Correction of Erroneous KT-MT Attachments

The amplitude of chromosome oscillation was inversely correlated not only with the CIN level, but also with the level of Hec1-S55 phosphorylation; cancer cell lines exhibit reduced Hec1-S55 phosphorylation depending on the CIN level [147]. This implies that Hec1 phosphorylation by Aurora A in metaphase, which is facilitated by chromosome oscillation, plays a role in the error correction of KT-MT attachments. Its dysfunction is related to CIN in cancer cell lines (Figure 6). This idea is supported by the fact that Aurora A depletion or inhibition in RPE-1 cells in metaphase led to increased chromosome missegregation. Chromosome oscillation enhancement via Kif18A inhibition also led to reduced chromosome missegregation, further supporting the idea that dysfunctions in chromo-
some oscillation is related to CIN. It was reported that Hec1 phosphorylation specifically affects KT attachments to polymerizing MTs [192]. It is postulated that when a leading KT approaches a spindle pole, Hec1 on the KT is phosphorylated by Aurora A on the spindle, which leads to the release of merotely-attached, polymerizing MTs (Figure 6). Even when both correct and erroneous attachments are destabilized on the leading KT, the higher density of MT from the closer spindle pole will facilitate the formation of correct attachments. In contrast to Hec1-S55, the level of Hec1-S69 phosphorylation in metaphase is not related to the level of CIN. However, Hec1-S69 phosphorylation is also important for suppressing formation of lagging chromosomes [182], corroborating the relationship between chromosome oscillation, Hec1 phosphorylation, and error correction. How different phosphorylation sites on Hec1 tail are differentially regulated is currently unknown.

When the Aurora A activity gradient was integrated in a numerical model of chromosome oscillation, reduction of erroneous KT-MT attachments was reproduced [178]. In this model, an Aurora A-like activity gradient was considered in a force-balance model describing chromosome kinetics. The gradient peaks at spindle poles and declines toward the spindle equator, and the activity reduces Hec1 affinity to MT. In the simulation, the number of merotelic attachments sharply declined in the first few rounds of oscillatory KT motion. The numerical model also reproduced KT oscillatory motion, which is promoted by the Aurora A activity gradient that reduces KT-MT affinity when KTs approach spindle poles. Importantly, both the amplitude of chromosome oscillation and the efficiency of error correction are reduced not only when the Aurora A activity was suppressed, but also when it was upregulated. This is because high Aurora A activity close to the equator confines the range of KT motion that hampers selective destabilization of erroneous attachments. These simulation results may be relevant to the finding that Aurora A is generally upregulated in cancer [193].

5. Conclusions and Outlook

Regarding physiological roles of chromosome oscillation, several possibilities have been proposed, such as checking the correct balance of force across KTs as a “self-test” for error-free anaphase and prevention of entanglement or damage of chromosomes [158,176]. A recent report suggests another possibility that chromosome oscillation contributes to the correction of erroneous KT-MT attachments [147].

Error correction largely occurs in prometaphase, when MTs and KTs encounter stochastically, mainly through Aurora B-mediated Hec1 phosphorylation on KTs not under tension. However, it was shown that KT reorientation, which reflects the KT detachment and reattachment to MTs, occurs even in metaphase [51]. The previously-unappreciated role of chromosome oscillation in the correction of erroneous KT-MT attachments may ensure the establishment of bi-orientation as a final check before anaphase onset (Figure 8). Error correction by chromosome oscillation was also reported in yeast meiosis [194,195]. Even when merotelic attachments remain at anaphase onset and result in the formation of lagging chromosomes, several mechanisms work to resolve erroneous attachments during this phase ([113], bioRxiv doi:10.1101/2021.03.30.436326), ensuring mitotic fidelity.

Defective regulation of KT-MT attachments is manifested as abnormal chromosome dynamics, such as lagging chromosomes and chromosome misalignment. On the other hand, abnormal chromosome dynamics can cause defects in KT-MT attachment regulation, exemplified by the finding that attenuated chromosome oscillation reduces the efficiency of error correction of KT-MT attachments [147]. Another example is that delayed chromosome alignment increases the rate of chromosome missegregation, due to an increase in erroneous KT-MT attachment formation during delayed chromosome alignment and/or insufficient error correction during relatively shortened metaphase [196]. These relationships between chromosome dynamics and KT-MT attachment regulation were identified by direct observation of living cells, but not through genomic analysis or gene expression profiles, warranting microscopic study for investigating CIN.
Defects in chromosome dynamics specifically seen in cancer cells can be a target for cancer therapy. Inhibitors for various mitotic motor proteins that alter chromosome dynamics, such as Eg5, CENP-E, and Kif18A, are now under investigation for the efficacy against cancer cells [203–205]. Aurora A and B are often dysregulated in cancer cells, and various Aurora kinase inhibitors are in clinical trials [193,206,207]. Hec1 is also a promising target for cancer therapy, and several inhibitors have been developed [208]. Further study on the relationship between chromosome dynamics and mitotic fidelity will pave the way for development of novel anti-cancer drugs.

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The concept that attenuated chromosome oscillation is a cause of CIN has to be corroborated by specifying the underlying cause of attenuated chromosome oscillation in CIN cancer cells. It is also important to reveal how chromosome oscillation is dampened in the process of oncogenic transformation. Currently, mechanisms ensuring mitotic fidelity are mainly studied in cultured cancer cell lines, which have different properties from primary cancer cells growing in three-dimensional microenvironment [197,198]. Therefore, chromosome oscillation has to be observed in primary cancer cells under conditions similar to physiological circumstances, e.g., organoid culture [199,200]. Regarding Kif18A, the relationship between its roles in spindle assembly and chromosome oscillation needs to be clarified. It has been suggested that Kif18A activity must be kept in a proper range, because not only Kif18A depletion, but also Kif18A overexpression causes multipolar spindle formation [201]. Chromosome oscillation must also be kept in a proper range, as its hyperenhancement by Kif18A depletion was reported to cause KT detachment and micronuclei formation [189,202]. This is of clinical relevance, because Kif18A depletion specifically compromises survival of CIN cancer cells [188–190]. Whether lowering CIN level by Kif18A depletion through enhancing chromosome oscillation acts synergistically with spindle disruption for cancer therapy is an interesting possibility to be examined.

Figure 8. Error correction by Aurora B and Aurora A. During prometaphase, Hec1 on KTs is phosphorylated by Aurora B (green), which resides at the inner centromere, as well as by Aurora A (red), which localizes around spindle poles, facilitating correction of erroneous KT–MT attachments. In metaphase, Hec1 phosphorylation by Aurora B is reduced, while Aurora A on the spindle phosphorylates Hec1-S55 when KTs approach spindle poles through chromosome oscillation, thereby correcting any remaining erroneous attachments. P: phosphorylation.
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**References**

1. Weaver, B.A.; Cleveland, D.W. Does aneuploidy cause cancer? *Curr. Opin. Cell Biol.* 2006, 18, 658–667. [CrossRef]
2. Taylor, A.M.; Shi, J.; Ha, G.; Gao, G.F.; Zhang, X.; Berger, A.C.; Schumacher, S.E.; Wang, C.; Hu, H.; Liu, J.; et al. Genomic and functional approaches to understanding cancer aneuploidy. *Cancer Cell* 2018, 33, 676–689.e3. [CrossRef]
3. Pfau, S.J.; Amon, A. Chromosomal instability and aneuploidy in cancer: From yeast to man. *EMBO Rep.* 2012, 13, 515–527. [CrossRef] [PubMed]
4. Orr, B.; Godek, K.M.; Compton, D. Aneuploidy. *Curr. Biol.* 2015, 25, R538–R542. [CrossRef]
5. Sansregret, L.; Swanton, C. The role of aneuploidy in cancer evolution. *Cold Spring Harb. Perspect. Med.* 2017, 7, a028373. [CrossRef]
6. Burrell, R.A.; McClelland, S.E.; Endesfelder, D.; Groth, P.; Weller, M.C.; Shaikh, N.; Domingo, E.; Kanu, N.; Dewhurst, S.M.; Gronroos, E.; et al. Replication stress links structural and numerical cancer chromosomal instability. *Nature* 2013, 494, 492–496. [CrossRef]
7. Tanaka, K.; Hirota, T. Chromosomal instability: A common feature and a therapeutic target of cancer. *Biochim. Biophys. Acta* 2016, 1866, 64–75. [CrossRef]
8. Jo, M.; Kusano, Y.; Hirota, T. Unraveling pathologies underlying chromosomal instability in cancers. *Cancer Sci.* 2021, 112, 2975. [CrossRef]
9. Bakhour, S.F.; Compton, D.A. Chromosomal instability and cancer: A complex relationship with therapeutic potential. *J. Clin. Investig.* 2012, 122, 1138–1143. [CrossRef]
10. McGranahan, N.; Burrell, R.A.; Endesfelder, D.; Novelli, M.R.; Swanton, C. Cancer chromosomal instability: Therapeutic and diagnostic challenges. *EMBO Rep.* 2012, 13, 528–538. [CrossRef]
11. Bakhour, S.F.; Landau, D.A. Chromosomal instability as a driver of tumor heterogeneity and evolution. *Cold Spring Harb. Perspect. Med.* 2017, 7, a029611. [CrossRef] [PubMed]
12. McGranahan, N.; Swanton, C. Clonal heterogeneity and tumor evolution: Past, present, and the future. *Cell* 2017, 168, 613–628. [CrossRef] [PubMed]
13. Zasadil, L.M.; Britigan, E.M.; Weaver, B.A. 2n or not 2n: Aneuploidy, polyploidy and chromosomal instability in primary and tumor cells. *Semin. Cell Dev. Biol.* 2013, 24, 370–379. [CrossRef] [PubMed]
14. Holland, A.J.; Cleveland, D.W. Boveri revisited: Chromosomal instability, aneuploidy and tumorigenesis. *Nat. Rev. Mol. Cell Biol.* 2009, 10, 478–487. [CrossRef]
15. Desai, A.; Mitchison, T.J. Microtubule polymerization dynamics. *Annu. Rev. Cell Dev. Biol.* 1997, 13, 83–117. [CrossRef]
16. Tanaka, T.U.; Stark, M.J.; Tanaka, K. Kinetochore capture and bi-orientation on the mitotic spindle. *Nat. Rev. Mol. Cell Biol.* 2015, 16, 57–64. [CrossRef]
17. Godek, K.M.; Kabeche, L.; Compton, D.A. Regulation of kinetochore-microtubule attachments through homeostatic control during mitosis. *Nat. Rev. Mol. Cell Biol.* 2016, 17, 52–61. [CrossRef]
18. Tanaka, K.; Hirota, T. Chromosome segregation machinery and cancer. *Cancer Sci.* 2009, 100, 1158–1165. [CrossRef]
19. Ricke, R.M.; van Deursen, J.M. Correction of microtubule-kinetochore attachment errors: Mechanisms and role in tumor suppression. *Semin. Cell Dev. Biol.* 2011, 22, 559–565. [CrossRef]
20. Maiato, H.; Gomes, A.M.; Sousa, F.; Barisic, M. Mechanisms of chromosome congression during mitosis. *Biology* 2017, 6, 13. [CrossRef]
21. Auckland, P.; McAlinsh, A.D. Building an integrated model of chromosome congression. *J. Cell Sci.* 2015, 128, 3363–3374. [CrossRef]
22. Tanaka, K. Dynamic regulation of kinetochore-microtubule interaction during mitosis. *J. Biochem.* 2012, 152, 415–424. [CrossRef] [PubMed]
23. Renda, F.; Khodjakov, A. Role of spatial patterns and kinetochore architecture in spindle morphogenesis. *Semin. Cell Dev. Biol.* 2021, 117, 75–85. [CrossRef] [PubMed]
24. Ferreira, L.T.; Maiato, H. Prometaphase. *Semin. Cell Dev. Biol.* 2021, 117, 52–61. [CrossRef]
25. Cimini, D. Merotetic kinetochore orientation, aneuploidy, and cancer. *Biochim. Biophys. Acta* 2008, 1786, 32–40. [CrossRef] [PubMed]
26. Civelekoglu-Scholey, G.; Cimini, D. Modelling chromosome dynamics in mitosis: A historical perspective on models of metaphase and anaphase in eukaryotic cells. *Interface Focus* 2014, 4, 20130073. [CrossRef] [PubMed]
27. Bissonette, S.; Stumpf, J. Quantifying mitotic chromosome dynamics and positioning. *J. Cell Physiol.* 2014, 229, 1301–1305. [CrossRef] [PubMed]
28. Winey, M.; Mamay, C.L.; O’Toole, E.T.; Mastronarde, D.N.; Giddings, T.H., Jr.; McDonald, K.L.; McIntosh, J.R. Three-dimensional ultrastructural analysis of the Saccharomyces cerevisiae mitotic spindle. *J. Cell Biol.* 1995, 129, 1601–1615. [CrossRef]
Cancers 2021, 13, 4531

29. O’Toole, E.T.; Winey, M.; McIntosh, J.R. High-voltage electron tomography of spindle pole bodies and early mitotic spindles in the yeast Saccharomyces cerevisiae. Mol. Biol. Cell 1999, 10, 2017–2031. [CrossRef]

30. Ding, R.; McDonald, K.L.; McIntosh, J.R. Three-dimensional reconstruction and analysis of mitotic spindles from the yeast, Schizosaccharomyces pombe. J. Cell Biol. 1993, 120, 141–151. [CrossRef][PubMed]

31. Wendell, K.L.; Wilson, L.; Jordan, M.A. Mitotic block in HeLa cells by vinblastine: Ultrastructural changes in kinetochore-microtubule attachment and in centrosomes. J. Cell Sci. 1976, 104 Pt 2, 261–274. [CrossRef]

32. McEwen, B.F.; Heagle, A.B.; Cassels, G.O.; Buttle, K.F.; Rieder, C.L. Kinetochore fiber maturation in PtK1 cells and its implications for the mechanisms of chromosome congression and anaphase onset. J. Cell Biol. 1997, 137, 1567–1580. [CrossRef]

33. Rieder, C.L. The formation, structure, and composition of the mammalian kinetochore and kinetochore fiber. Int. Rev. Cytol. 1982, 79, 1–58.

34. Tanaka, T.U. Kinetochore-microtubule interactions: Steps towards bi-orientation. EMBO J. 2010, 29, 4070–4082. [CrossRef]

35. Itoh, G.; Ikeda, M.; Lemer, K.; Amin, M.A.; Kuriyama, S.; Tanaka, M.; Mizuno, N.; Osakada, H.; Haraguchi, T.; Tanaka, K. Lateral attachment of kinetochores to microtubules is enriched in prometaphase rosette and facilitates chromosome alignment and bi-orientation establishment. Sci. Rep. 2018, 8, 3888. [CrossRef]

36. Tanaka, K. Regulatory mechanisms of kinetochore-microtubule interaction in mitosis. Cell Mol. Life Sci. 2013, 70, 559–579. [CrossRef]

37. Alexander, S.P.; Rieder, C.L. Chromosome motion during attachment to the vertebrate spindle: Initial saltatory-like behavior of chromosomes and quantitative analysis of force production by nascent kinetochore fibers. J. Cell Biol. 1991, 113, 805–815. [CrossRef]

38. Yang, Z.; Tulu, U.S.; Wadsworth, P.; Rieder, C.L. Kinetochore dynein is required for chromosome motion and congression independent of the spindle checkpoint. Curr. Biol. 2007, 17, 973–980. [CrossRef][PubMed]

39. Tanaka, K.; Muka, N.; Dewar, H.; van Breugel, M.; James, E.K.; Prescott, A.R.; Antony, C.; Tanaka, T.U. Molecular mechanisms of kinetochore capture by spindle microtubules. Nature 2005, 434, 987–994. [CrossRef][PubMed]

40. Rieder, C.L.; Alexander, S.P. Kinetochores are transported poleward along a single astral microtubule during chromosome attachment to the spindle in newt lung cells. J. Cell Biol. 1990, 110, 81–95. [CrossRef][PubMed]

41. Sharp, D.J.; Rogers, G.C.; Scholey, J.M. Cytoplasmic dynein is required for poleward chromosome movement during mitosis in Drosophila embryos. Nat. Cell Biol. 2000, 2, 922–930. [CrossRef]

42. Iemura, K.; Tanaka, K. Chromokinesin Kid and kinetochore kinesin CENP-E differentially support chromosome congression without end-on attachment to microtubules. Nat. Commun. 2015, 6, 6447. [CrossRef][PubMed]

43. Kapoor, T.M.; Lampson, M.A.; Hergert, P.; Cameron, L.; Cimini, D.; Salmon, E.D.; McEwen, B.F.; Khodjakov, A. Chromosomes can congress to the metaphase plate before biorientation. Science 2006, 311, 388–391. [CrossRef]

44. Wood, K.W.; Sakowicz, R.; Goldstein, L.S.; Cleveland, D.W. CENP-E is a plus end-directed kinetochore motor required for metaphase chromosome alignment. Cell 1997, 91, 357–366. [CrossRef]

45. Kops, G.J.; Saurin, A.T.; Meraldi, P. Finding the middle ground: How kinetochores power chromosome congression. Cell Mol. Life Sci. 2010, 67, 2145–2161. [CrossRef][PubMed]

46. Tanaka, K.; Kitamura, E.; Kitamura, Y.; Tanaka, T.U. Molecular mechanisms of microtubule-dependent kinetochore transport toward spindle poles. J. Cell Biol. 2007, 178, 269–281. [CrossRef]

47. Shrestha, R.L.; Draviam, V.M. Lateral to end-on conversion of chromosome-microtubule attachment requires kinesins CENP-E and MCAK. Curr. Biol. 2013, 23, 1514–1526. [CrossRef]

48. Loncarek, J.; Kisurina-Evgenieva, O.; Vinogradova, T.; Hergert, P.; La Terra, S.; Kapoor, T.M.; Khodjakov, A. The centromere geometry essential for keeping mitosis error free is controlled by spindle forces. Nature 2007, 450, 745–749. [CrossRef][PubMed]

49. Indjeian, V.B.; Murray, A.W. Budding yeast mitotic chromosomes have an intrinsic bias to biorient on the spindle. Curr. Biol. 2007, 17, 1837–1846. [CrossRef]

50. Edelmaier, C.; Lamson, A.R.; Gergely, Z.R.; Ansari, S.; Blackwell, R.; McIntosh, J.R.; Glaser, M.A.; Betterton, M.D. Mechanisms of chromosome biorientation and spindle assembly analysed by computational modeling. eLife 2020, 9, e48787. [CrossRef][PubMed]

51. Magidson, V.; O’Connell, C.B.; Loncarek, J.; Paul, R.; Mogilner, A.; Khodjakov, A. The spatial arrangement of chromosomes during prometaphase facilitates spindle assembly. Cell 2011, 146, 555–567. [CrossRef]

52. Silkworth, W.T.; Nardi, I.K.; Paul, R.; Mogilner, A.; Cimini, D. Timing of centrosome separation is important for accurate chromosome segregation. Mol. Biol. Cell 2012, 23, 401–411. [CrossRef][PubMed]

53. Kaseda, K.; McInish, A.D.; Cross, R.A. Dual pathway spindle assembly increases both the speed and the fidelity of mitosis. Biol. Open 2012, 1, 12–18. [CrossRef][PubMed]

54. Sacristan, C.; Ahmad, M.U.D.; Keller, J.; Fernie, J.; Groeneveld, V.; Toner, E.; Fish, A.; Melero, R.; Carazo, J.M.; Klumperman, J.; et al. Dynamic kinetochore size regulation promotes microtubule capture and chromosome biorientation in mitosis. Nat. Cell Biol. 2018, 20, 800–810. [CrossRef]

55. Magidson, V.; Paul, R.; Yang, N.; Ault, J.G.; O’Connell, C.B.; Tikhonenko, I.; McEwen, B.F.; Mogilner, A.; Khodjakov, A. Adaptive changes in the kinetochore architecture facilitate proper spindle assembly. Nat. Cell Biol. 2015, 17, 1134–1144. [CrossRef]

56. Lampson, M.A.; Grishchuk, E.L. Mechanisms to avoid and correct erroneous kinetochore-microtubule attachments. Biology 2017, 6, 1. [CrossRef]
57. Bakhoum, S.E.; Compton, D.A. Kinetochores and disease: Keeping microtubule dynamics in check! Curr. Opin. Cell Biol. 2012, 24, 64–70. [CrossRef] [PubMed]
58. Zaytsev, A.V.; Grishchuk, E.L. Basic mechanism for biorientation of mitotic chromosomes is provided by the kinetochore geometry and indiscriminate turnover of kinetochore microtubules. Mol. Biol. Cell 2015, 26, 3985–3998. [CrossRef] [PubMed]
59. Mitchison, T.; Kirschner, M. Dynamic instability of microtubule growth. Nature 1984, 312, 237–242. [CrossRef] [PubMed]
60. Kirschner, M.; Mitchison, T. Beyond self-assembly: From microtubules to morphogenesis. Cell 1986, 45, 329–342. [CrossRef] [PubMed]
61. Hayden, J.H.; Bowser, S.S.; Rieder, C.L. Kinetochores capture astral microtubules during chromosome attachment to the mitotic spindle: Direct visualization in live newt lung cells. J. Cell Biol. 1990, 111, 1039–1045. [CrossRef]
62. Bakhoum, S.F.; Thompson, S.L.; Manning, A.L.; Compton, D.A. Genome stability is ensured by temporal control of kinetochore-microtubule dynamics. Nat. Cell Biol. 2009, 11, 27–35. [CrossRef]
63. Kabeche, L.; Compton, D.A. Cyclin A regulates kinetochore microtubules to promote faithful chromosome segregation. Nature 2013, 502, 110–113. [CrossRef] [PubMed]
64. Varma, D.; Salmon, E.D. The KMN protein network—chief conductors of the kinetochore orchestra. J. Cell Sci. 2012, 125, 5927–5936. [CrossRef]
65. Durfee, T.; Becherer, K.; Chen, P.L.; Yeh, S.H.; Yang, Y.; Kilburn, A.E.; Lee, W.H.; Elledge, S.J. The retinoblastoma protein associates with the protein phosphatase type 1 catalytic subunit. Genes Dev. 1993, 7, 555–569. [CrossRef] [PubMed]
66. Chen, Y.; Riley, D.J.; Chen, P.L.; Lee, W.H. HEC, a novel nuclear protein rich in leucine heptad repeats specifically involved in mitosis. Mol. Cell. Biol. 1997, 17, 6049–6056. [CrossRef]
67. Tooley, J.; Stukenberg, P.T. The Ndc80 complex: Integrating the kinetochore’s many movements. Curr. Biol. 2012, 22, 164–70. [CrossRef] [PubMed]
68. Joglekar, A.P.; DeLuca, J.G. Chromosome segregation: Ndc80 can carry the load. Curr. Biol. 2009, 19, R404–R407. [CrossRef]
69. Wimbish, R.T.; DeLuca, J.G. Hec1/Ndc80 tail domain function at the kinetochore-microtubule interface. Front. Cell Dev. Biol. 2020, 8, 43. [CrossRef] [PubMed]
70. DeLuca, J.G.; Gall, W.E.; Ciferri, C.; Cimini, D.; Musacchio, A.; Salmon, E.D. Kinetochore microtubule dynamics and attachment stability are regulated by Hec1. Cell 2006, 127, 969–982. [CrossRef] [PubMed]
71. Krenn, V.; Musacchio, A. The Aurora B Kinase in chromosome Bi-orientation and spindle checkpoint signaling. Front. Oncol. 2015, 5, 225. [CrossRef]
72. Liu, D.; Vleugel, M.; Backer, C.B.; Hori, T.; Fukagawa, T.; Cheeseman, I.M.; Lampson, M.A. Regulated targeting of protein phosphatase 1 to the outer kinetochore by KNL1 opposes Aurora B kinase. J. Cell Biol. 2010, 188, 809–820. [CrossRef] [PubMed]
73. Suikerbuijk, S.J.; Vleugel, M.; Teixeira, A.; Kops, G.J. Integration of kinase and phosphatase activities by BUBR1 ensures formation of stable kinetochore-microtubule attachments. Dev. Cell 2012, 23, 745–755. [CrossRef] [PubMed]
74. Kruse, T.; Zhang, G.; Larsen, M.S.; Lischetti, T.; Streicher, W.; Kragh Nielsen, T.; Bjorn, S.P.; Nilsson, J. Direct binding between BubR1 and B56-PP2A phosphatase complexes regulate mitotic progression. J. Cell Sci. 2013, 126, 1086–1092. [CrossRef] [PubMed]
75. Xu, P.; Raetz, E.A.; Kitagawa, M.; Virshup, D.M.; Lee, S.H. BUBR1 recruits PP2A via the B56 family of targeting subunits to promote chromosome congression. Biol. Open 2013, 2, 479–486. [CrossRef] [PubMed]
76. Smith, R.J.; Cordeiro, M.H.; Davey, N.E.; Vallardi, G.; Ciliberto, A.; Gross, F.; Saurin, A.T. PP1 and PP2A use opposite phospho-dependencies to control distinct processes at the kinetochore. Cell Rep. 2019, 28, 2206–2219.e8. [CrossRef] [PubMed]
77. Welburn, J.P.; Vleugel, M.; Liu, D.; Yates, J.R., III; Lampson, M.A.; Fukagawa, T.; Cheeseman, I.M. Aurora B phosphorylates spatially distinct targets to differentially regulate the kinetochore-microtubule interface. Mol. Cell 2010, 38, 383–392. [CrossRef] [PubMed]
78. Keen, N.; Taylor, S. Aurora-kinase inhibitors as anticancer agents. Nat. Rev. Cancer 2004, 4, 927–936. [CrossRef] [PubMed]
79. Carmen, M.; Wheelock, M.; Funabiki, H.; Earnshaw, W.C. The chromosomal passenger complex (CPC): From easy rider to the godfather of mitosis. Nat. Rev. Mol. Cell Biol. 2010, 11, 179–190. [CrossRef] [PubMed]
80. Liu, D.; Vader, G.; Vromans, M.J.; Lampson, M.A.; Lens, S.M. Sensing chromosome bi-orientation by spatial separation of aurora B kinase from kinetochore substrates. Science 2009, 323, 1350–1353. [CrossRef] [PubMed]
81. Tanaka, T.U.; Rachidi, N.; Janke, C.; Pereira, G.; Galova, M.; Schiebel, E.; Stark, M.J.; Nasmyth, K. Evidence that the Ipl1-Sli15 (Aurora kinase-INCENP) complex promotes chromosome bi-orientation by altering kinetochore-spindle pole connections. Cell 2002, 108, 317–329. [CrossRef] [PubMed]
82. Sarangapani, K.K.; Ashbury, C.L. Catch and release: How do kinetochores hook the right microtubules during mitosis? Trends Genet. 2014, 30, 150–159. [CrossRef] [PubMed]
83. Cimini, D.; Lan, W.; Hirel, C.B.; Salmon, E.D. Aurora kinase promotes turnover of kinetochore microtubules to reduce chromosome segregation errors. Curr. Biol. 2006, 16, 1711–1718. [CrossRef] [PubMed]
84. Knowlton, A.L.; Lan, W.; Stukenberg, P.T. Aurora B is enriched at merotelic attachment sites, where it regulates MCAK. Curr. Biol. 2006, 16, 1705–1710. [CrossRef] [PubMed]
85. Cimini, D.; Moree, B.; Canman, J.C.; Salmon, E.D. Merotelic kinetochore orientation occurs frequently during early mitosis in mammalian tissue cells and error correction is achieved by two different mechanisms. J. Cell Sci. 2003, 116, 4213–4225. [CrossRef] [PubMed]
86. Gregan, J.; Polakova, S.; Zhang, L.; Tolic-Norrelykke, I.M.; Cimini, D. Merotelic kinetochore attachment: Causes and effects. Trends Cell Biol. 2011, 21, 374–381. [CrossRef] [PubMed]
87. Funabiki, H. Correcting aberrant kinetochore microtubule attachments: A hidden regulation of Aurora B on microtubules. *Curr. Opin. Cell Biol.* 2019, 58, 34–41. [CrossRef] [PubMed]

88. Trivedi, P.; Zaytsev, A.V.; Godzi, M.; Attaullahkhanov, F.I.; Grishchuk, E.L.; Stukenberg, P.T. The binding of Borealin to microtubules underlies a tension independent kinetochore-microtubule error correction pathway. *Nat. Commun.* 2019, 10, 682. [CrossRef] [PubMed]

89. Chen, G.Y.; Renda, F.; Zhang, H.; Gokden, A.; Wu, D.Z.; Chenoweth, D.M.; Khodjakov, A.; Lampson, M.A. Tension promotes kinetochore-microtubule release by Aurora B kinase. *J. Cell Biol.* 2021, 220, e202007030. [CrossRef] [PubMed]

90. Andrews, P.D.; Ovechkina, Y.; Morrice, N.; Wagenbach, M.; Duncan, K.; Wordeman, L.; Swedlow, J.R. Aurora B regulates MCAK at the mitotic centromere. *Dev. Cell* 2004, 6, 253–268. [CrossRef]

91. Lan, W.; Zhang, X.; Kline-Smith, S.L.; Rosasco, S.E.; Barrett-Wilt, G.A.; Shabanowitz, J.; Hunt, D.F.; Walczak, C.E.; Stukenberg, P.T. Aurora B phosphorylates centromeric MCAK and regulates its localization and microtubule depolymerization activity. *Curr. Biol.* 2004, 14, 273–286. [CrossRef] [PubMed]

92. Kim, Y.; Holland, A.J.; Lan, W.; Cleveland, D.W. Aurora kinases and protein phosphatase 1 mediate chromosome congression through regulation of CENP-E. *Cell* 2010, 142, 444–455. [CrossRef]

93. Schmidt, J.C.; Arthanari, H.; Boeszoermenyi, A.; Dashkevich, N.M.; Wilson-Kubalek, E.M.; Monnier, N.; Markus, M.; Oberer, M.; Milligan, R.A.; Bathe, M.; et al. The kinetochore-bound Skal complex tracks depolymerizing microtubules and binds to curved protofilaments. *Dev. Cell* 2012, 23, 968–980. [CrossRef]

94. Koffa, M.D.; Casanova, C.M.; Santarella, R.; Kocher, T.; Wilm, M.; Mattaj, I.W. HURP is part of a Ran-dependent complex involved in spindle formation. *Curr. Biol.* 2006, 16, 743–754. [CrossRef]

95. Wong, J.; Fang, G. HURP controls spindle dynamics to promote proper interkinetochore tension and efficient kinetochore capture. *J. Cell Biol.* 2006, 173, 879–891. [CrossRef] [PubMed]

96. Chmatal, L.; Yang, K.; Schultz, R.M.; Lampson, M.A. Spatial regulation of kinetochore microtubule attachments by destabilization at spindle poles in Meiosis I. *Curr. Biol.* 2015, 25, 1835–1841. [CrossRef]

97. Ye, A.A.; Deretic, J.; Hoel, C.M.; Hinman, A.W.; Cimini, D.; Welburn, J.P.; Maresca, T.J. Aurora A Kinase contributes to a pole-based error correction pathway. *Curr. Biol.* 2015, 25, 1842–1851. [CrossRef]

98. Hochegger, H.; Hegarat, N.; Pereira-Leal, J.B. Aurora at the pole and equator: Overlapping functions of Aurora kinases in the mitotic spindle. *Open Biol.* 2013, 3, 120185. [CrossRef] [PubMed]

99. Maure, J.F.; Kitamura, E.; Tanaka, T.U. Mps1 kinase promotes sister-kinetochore bi-orientation by a tension-dependent mechanism. *Curr. Biol.* 2007, 17, 2175–2182. [CrossRef]

100. Jellumna, N.; Brenkman, A.B.; van den Broek, N.J.; Cruijssen, C.W.; van Osch, M.H.; Lens, S.M.; Medema, R.H.; Kops, G.J. Mps1 phosphorylates Borealin to control Aurora B activity and chromosome alignment. *Cell* 2008, 132, 233–246. [CrossRef]

101. Santaguida, S.; Tighe, A.; D’Alise, A.M.; Taylor, S.S.; Musacchio, A. Dissecting the role of MPS1 in chromosome biorientation and the spindle checkpoint through the small molecule inhibitor reversine. *J. Cell Biol.* 2010, 190, 73–87. [CrossRef] [PubMed]

102. Maciejowska, J.; Drechsel, H.; Grundner-Culemann, K.; Ballister, E.R.; Rodriguez-Rodriguez, J.A.; Rodriguez-Bravo, V.; Jones, M.J.K.; Foley, E.; Lampson, M.A.; Daub, H.; et al. Mps1 regulates kinetochore-microtubule attachment stability via the ska complex to ensure error-free chromosome segregation. *Dev. Cell* 2017, 41, 143–156.e6. [CrossRef]

103. Hayward, D.; Bancroft, J.; Mangat, D.; Alfonso-Perez, T.; Dugdale, S.; McCarthy, J.; Barr, F.A.; Gruneberg, U. Checkpoint signaling and error correction require regulation of the MPS1 T-loop by PP2A-B56. *J. Cell Biol.* 2019, 218, 3188–3199. [CrossRef]

104. Benzi, G.; Camaleses, A.; Atsumori, Y.; Katou, Y.; Shirahige, K.; Piatti, S. A common molecular mechanism underlies the role of Mps1 in chromosome biorientation and the spindle assembly checkpoint. *EMBO Rep.* 2020, 21, e50257. [CrossRef] [PubMed]

105. Petsalaki, E.; Akoumianaki, T.; Black, E.J.; Gillespie, D.A.; Zachos, G. Phosphorylation at serine 331 is required for Aurora B activation. *J. Cell Biol.* 2011, 195, 449–466. [CrossRef]

106. Petsalaki, E.; Zachos, G. Chk1 and Mps1 jointly regulate correction of merotelic kinetochore attachments. *J. Cell Sci.* 2013, 126, 1235–1246. [CrossRef]

107. Miller, M.P.; Asbury, C.L.; Higgins, S. A TOG protein confers tension sensitivity to kinetochore-microtubule attachments. *Cell* 2016, 165, 1428–1439. [CrossRef] [PubMed]

108. Herman, J.A.; Miller, M.P.; Higgins, S. chTOG is a conserved mitotic error correction factor. *Curr. Opin. Cell Biol.* 2020, 51, 682. [CrossRef]

109. Herrman, J.A.; Miller, M.P.; Higgins, S. chTOG is a conserved mitotic error correction factor. *eLife* 2020, 9, e61773. [CrossRef]

110. Musacchio, A. The molecular biology of spindle assembly checkpoint signaling dynamics. *Curr. Biol.* 2015, 25, R1002–R1018. [CrossRef] [PubMed]

111. Pinskyy, B.A.; Higgins, S. The spindle checkpoint: Tension versus attachment. *Trends Cell Biol.* 2005, 15, 486–493. [CrossRef] [PubMed]

112. Nezi, L.; Musacchio, A. Sister chromatid tension and the spindle assembly checkpoint. *Curr. Opin. Cell Biol.* 2009, 21, 785–795. [CrossRef] [PubMed]

113. Nezi, L.; Musacchio, A. Sister chromatid tension and the spindle assembly checkpoint. *Curr. Opin. Cell Biol.* 2009, 21, 785–795. [CrossRef] [PubMed]

114. Thompson, S.L.; Compton, D.A. Chromosome missegregation in human cells arises through specific types of kinetochore-microtubule attachment errors. *Proc. Natl. Acad. Sci. USA* 2011, 108, 17974–17978. [CrossRef]
115. Terradas, M.; Martin, M.; Tusell, L.; Genesca, A. DNA lesions sequestered in micronuclei induce a local defective-damage response. *DNA Repair* 2009, 8, 1223–1234. [CrossRef]

116. Terradas, M.; Martin, M.; Tusell, L.; Genesca, A. Genetic activities in micronuclei: Is the DNA entrapped in micronuclei lost for the cell? *Mutat. Res.* 2010, 705, 60–67. [CrossRef]

117. Terradas, M.; Martin, M.; Hernandez, L.; Tusell, L.; Genesca, A. Nuclear envelope defects impede a proper response to micronuclear DNA lesions. *Mutat. Res.* 2012, 729, 35–40. [CrossRef] [PubMed]

118. Crasta, K.; Ganem, N.J.; Dagher, R.; Lantermann, A.B.; Ivanova, E.V.; Pan, Y.F.; Nezi, L.; Protopopov, A.; Chowdhury, D.; Pellman, D. DNA breaks and chromosome pulverization from errors in mitosis. *Nature* 2012, 482, 53–58. [CrossRef] [PubMed]

119. Stephens, P.J.; Greenman, C.D.; Fu, B.; Yang, F.; Bignell, G.R.; Mudie, L.J.; Pleasance, E.D.; Lau, K.W.; Beare, D.; Stebbings, L.A.; et al. Massive genomic rearrangement acquired in a single catastrophic event during cancer development. *Cell* 2011, 144, 27–40. [CrossRef] [PubMed]

120. Zhang, C.Z.; Spektor, A.; Cornils, H.; Francis, J.M.; Jackson, E.K.; Liu, S.; Meyerson, M.; Pellman, D. Chromothripsis from DNA damage in micronuclei. *Nature* 2015, 522, 179–184. [CrossRef]

121. Janssen, A.; van der Burg, M.; Szuhai, K.; Kops, G.J.; Medema, R.H. Chromosome segregation errors as a cause of DNA damage and structural chromosome aberrations. *Science* 2013, 333, 1895–1898. [CrossRef] [PubMed]

122. Soto, M.; Raaijmakers, J.A.; Medema, R.H. Consequences of genomic diversification induced by segregation errors. *Trends Genet.* 2019, 35, 279–291. [CrossRef] [PubMed]

123. Pihan, G.A.; Purohit, A.; Wallace, J.; Knecht, H.; Woda, B.; Quesenberry, P.; Doxsey, S.J. Centrosome defects and genetic instability in malignant tumors. *Cancer Res.* 1998, 58, 3974–3985.

124. Chan, J.Y. A clinical overview of centrosome amplification in human cancers. *Int. J. Biol. Sci.* 2011, 7, 1122–1144. [CrossRef] [PubMed]

125. Pihan, G.A. Centrosome dysfunction contributes to chromosome instability, chromoaagenesis, and genome reprogramming in cancer. *Front. Oncol.* 2013, 3, 277. [CrossRef]

126. Quintyne, N.J.; Reing, J.E.; Hoffelder, D.R.; Gollin, S.M.; Saunders, W.S. Spindle multipolarity is prevented by centrosomal activity prevents chromosome segregation errors. *Science* 2005, 307, 127–129. [CrossRef] [PubMed]

127. Kwon, M.; Godinho, S.A.; Chandhok, N.S.; Ganem, N.J.; Azioune, A.; Thery, M.; Pellman, D. Mechanisms to suppress multipolar divisions in cancer cells with extra centrosomes. *Genes Dev.* 2008, 22, 2189–2203. [CrossRef] [PubMed]

128. Ganem, N.J.; Godinho, S.A.; Pellman, D. A mechanism linking extra centrosomes to chromosomal instability. *Nature* 2009, 460, 278–282. [CrossRef] [PubMed]

129. Silkworth, W.T.; Nardi, I.K.; Scholl, L.M.; Cimini, D. Multipolar spindle pole coalescence is a major source of kinetochore mis-attachment and chromosome mis-segregation in cancer cells. *PLoS ONE* 2009, 4, e6564. [CrossRef] [PubMed]

130. Bakhoun, S.F.; Genovese, G.; Compton, D.A. Deviant kinetochore microtubule dynamics underlie chromosomal instability. *Curr. Biol.* 2009, 19, 1937–1942. [CrossRef] [PubMed]

131. Salimian, K.J.; Ballister, E.R.; Smoak, E.M.; Wood, S.; Panchenko, T.; Lampson, M.A.; Black, B.E. Feedback control in sensing chromosome biorientation by the Aurora B kinase. *Curr. Biol.* 2011, 21, 1158–1165. [CrossRef] [PubMed]

132. Abe, Y.; Sako, K.; Takagaki, K.; Hirayama, Y.; Uchida, K.S.; Herman, J.A.; DeLuca, J.G.; Hirota, T. HP1-assisted Aurora B Kinase activity prevents chromosome segregation errors. *Dev. Cell* 2016, 36, 487–497. [CrossRef]

133. Desai, A.; Maddox, P.S.; Mitchison, T.J.; Salmon, E.D. Anaphase A chromosome movement and poleward spindle microtubule flux occur at similar rates in Xenopus extract spindles. *J. Cell Biol.* 1998, 141, 703–713. [CrossRef] [PubMed]

134. LaFountain, J.R., Jr.; Oldenbourg, R.; Cole, R.W.; Rieder, C.L. Microtubule flux mediates poleward motion of acentric chromosome fragments during meiosis in insect spermatocytes. *Mol. Biol. Cell* 2001, 12, 4054–4065. [CrossRef]

135. Brust-Mascher, I.; Scholey, J.M. Microtubule flux and sliding in mitotic spindles of Drosophila embryos. *Mol. Biol. Cell* 2002, 13, 3967–3975. [CrossRef] [PubMed]

136. Madsen, P.; Desai, A.; Oegema, K.; Mitchison, T.J.; Salmon, E.D. Poleward microtubule flux is a major component of spindle dynamics and anaphase a in mitotic Drosophila embryos. *Curr. Biol.* 2002, 12, 1670–1674. [CrossRef]

137. Maddox, P.; Straight, A.; Coughlin, P.; Mitchison, T.J.; Salmon, E.D. Direct observation of microtubule dynamics at kinetochores in Xenopus extract spindles: Principles for spindle mechanics. *J. Cell Biol.* 2003, 162, 377–382. [CrossRef]

138. de Lartigue, J.; Brust-Mascher, I.; Scholey, J.M. Anaphase B spindle dynamics in Drosophila S2 cells: Comparison with embryo spindles. *Cell Div.* 2011, 6, 8. [CrossRef]

139. Schibbens, R.V.; Skees, V.P.; Salmon, E.D. Directional instability of kinetochore motility during chromosome congression and segregation in mitotic newt lung cells: A push-pull mechanism. *J. Cell Biol.* 1993, 122, 859–875. [CrossRef]

140. Inoue, S.; Salmon, E.D. Force generation by microtubule assembly/disassembly in mitosis and related movements. *Mol. Biol. Cell* 1995, 6, 1619–1640. [CrossRef] [PubMed]

141. Banigan, E.J.; Chiou, K.K.; Ballister, E.R.; Mayo, A.M.; Lampson, M.A.; Liu, A.J. Minimal model for collective kinetochore-microtubule dynamics. *Proc. Natl. Acad. Sci. USA* 2015, 112, 12699–12704. [CrossRef] [PubMed]

142. Baijer, A.S. Functional autonomy of monopolar spindle and evidence for oscillatory movement in mitosis. *J. Cell Biol.* 1982, 93, 33–48. [CrossRef] [PubMed]

143. Hafner, J.; Mayr, M.I.; Muckel, M.M.; Mayer, T.U. Pre-anaphase chromosome oscillations are regulated by the antagonistic activities of Cdk1 and PP1 on Kif18A. *Nat. Commun.* 2014, 5, 4397. [CrossRef]
144. Su, K.C.; Barry, Z.; Schweizer, N.; Maiato, H.; Bathe, M.; Cheeseman, I.M. A regulatory switch alters chromosome motions at the metaphase-to-anaphase transition. *Cell Rep.* 2016, 17, 1728–1738. [CrossRef] [PubMed]

145. Shindo, N.; Otsuki, M.; Uchida, K.S.K.; Hirota, T. Prolonged mitosis causes separase deregulation and chromosome nondisjunction. *Cell Rep.* 2021, 34, 108652. [CrossRef]

146. Amaro, A.C.; Samora, C.P.; Holtackers, R.; Wang, E.; Kingston, I.J.; Alonso, M.; Lampson, M.; McAinsh, A.D.; Meraldi, P. Molecular control of mitochrondio-microtubule dynamics and chromosome oscillations. *Nat. Cell Biol.* 2010, 12, 319–329. [CrossRef] [PubMed]

147. Iemura, K.; Natsume, T.; Maehara, K.; Kanemaki, M.T.; Tanaka, K. Chromosome oscillation promotes Aurora A-dependent Hec1 phosphorylation and mitotic fidelity. *J. Cell Biol.* 2021, 220, e202006116. [CrossRef]

148. Wan, X.; O’Quinn, R.P.; Pierce, H.L.; Joglekar, A.P.; Gall, W.E.; DeLuca, J.G.; Carroll, C.W.; Liu, S.T.; Yen, T.J.; McEwen, B.F.; et al. Protein architecture of the human kinetochore microtubule attachment site. *Cell* 2009, 137, 672–684. [CrossRef] [PubMed]

149. Jašan, K.; King, E.M.; Amaro, A.C.; Winter, J.R.; Dorn, J.F.; Elliott, H.L.; McHedlishvili, N.; McClelland, S.E.; Porter, I.M.; Posch, M.; et al. Kinetochore alignment within the metaphase plate is regulated by centromere stiffness and microtubule depolymerases. *J. Cell Biol.* 2010, 188, 665–679. [CrossRef] [PubMed]

150. Miyazaki, W.Y.; Orr-Weaver, T.L. Sister-chromatid cohesion in mitosis and meiosis. *Annu. Rev. Genet.* 1994, 28, 167–187. [CrossRef]

151. Wan, X.; Cimini, D.; Cameron, L.A.; Salmon, E.D. The coupling between sister kinetochore directional instability and oscillations in centromere stretch in metaphase PtK1 cells. *Mol. Biol. Cell* 2012, 23, 1035–1046. [CrossRef]

152. Stumpf, J.; von Dassow, G.; Wagenbach, M.; Asbury, C.; Wordeman, L. The kinesin-8 motor Kif18A suppresses kinetochore movements to control mitotic chromosome alignment. *Dev. Cell* 2008, 14, 252–262. [CrossRef]

153. Stumpf, J.; Wagenbach, M.; Franck, A.; Asbury, C.L.; Wordeman, L. Kif18A and chromokinesins confine centromere movements via microtubule growth suppression and spatial control of kinetochore tension. *Dev. Cell* 2012, 22, 1017–1029. [CrossRef] [PubMed]

154. Stumpf, J.; Du, Y.; English, C.A.; Maliga, Z.; Wagenbach, M.; Asbury, C.L.; Ohi, R. A tethering mechanism controls the processivity and kinetochore-microtubule plus-end enrichment of the kinesin-8 Kif18A. *Mol. Cell* 2011, 43, 764–775. [CrossRef]

155. Almeida, A.C.; Maiato, H. Chromokinesins. *Curr. Opin. Cell Biol.* 2018, 48, R1131–R1135. [CrossRef]

156. Rieder, C.L.; Salmon, E.D. Motile kinetochores and polar ejection forces dictate chromosome position on the vertebrate mitotic spindle. *J. Cell Biol.* 1994, 124, 223–233. [CrossRef]

157. Wandke, C.; Barisic, M.; Sigl, R.; Rauch, V.; Wolf, F.; Amaro, A.C.; Tan, C.H.; Pereira, A.J.; Kutay, U.; Maiato, H.; et al. Human chromokinesins promote chromosome congression and spindle microtubule dynamics during mitosis. *J. Cell Biol.* 2012, 198, 847–863. [CrossRef] [PubMed]

158. Ke, K.; Cheng, J.; Hunt, A.J. The distribution of polar ejection forces determines the amplitude of chromosome directional instability. *Curr. Biol.* 2009, 19, 807–815. [CrossRef] [PubMed]

159. Rieder, C.L.; Davison, E.A.; Jensen, L.C.; Cassimeris, L.; Salmon, E.D. Oscillatory movements of monooriented chromosomes and their position relative to the spindle pole result from the ejection properties of the aster and half-spindle. *J. Cell Biol.* 1986, 103, 581–591. [CrossRef]

160. Li, C.; Xue, C.; Yang, Q.; Low, B.C.; Liou, Y.C. NuSAP governs chromosome oscillation by facilitating the Kid-generated polar ejection force. *Nat. Commun.* 2016, 7, 10597. [CrossRef] [PubMed]

161. Mitchell, T.J. Polewards microtubule flux in the mitotic spindle: Evidence from photoactivation of fluorescence. *J. Cell Biol.* 1989, 109, 637–652. [CrossRef] [PubMed]

162. Ganem, N.J.; Upton, K.; Compton, D.A. Efficient mitosis in human cells lacking poleward microtubule flux. *Curr. Biol.* 2005, 15, 1827–1832. [CrossRef] [PubMed]

163. Steblyanko, Y.; Rajendraprasad, G.; Osswald, M.; Eibes, S.; Jacome, A.; Geley, S.; Pereira, A.J.; Maiato, H.; Barisic, M. Microtubule poleward flux in human cells is driven by the coordinated action of four kinesins. *EMBO J.* 2020, 39, e105432. [CrossRef] [PubMed]

164. Cassimeris, L.; Morimoto, J. TOGp, the human homolog of XMAP215/Dis1, is required for centrosome integrity, spindle pole organization, and bipolar spindle assembly. *Mol. Biol. Cell* 2004, 15, 1580–1590. [CrossRef] [PubMed]

165. Wang, X.; Zhuang, X.; Cao, D.; Chu, Y.; Yao, P.; Liu, W.; Liu, L.; Adams, G.; Fang, G.; Dou, Z.; et al. Mitotic regulator SKAP forms a link between kinetochore core complex KMN and dynamic spindle microtubules. *J. Biol. Chem.* 2012, 287, 39380–39390. [CrossRef]

166. Kajtež, J.; Solomatina, A.; Novak, M.; Polak, B.; Vukusic, K.; Rudiger, J.; Cojoc, G.; Milas, A.; Sumanovac Sestak, I.; Risteski, P.; et al. Overlap microtubules link sister k-fibres and balance the forces on bi-oriented kinetochores. *Curr. Biol.* 2020, 30, R1131–R1135. [CrossRef]

167. Polak, B.; Risteski, P.; Lesjak, S.; Tolic, I.M. PRC1-labeled microtubule bundles and kinetochore pairs show one-to-one association in metaphase. *EMBO Rep.* 2017, 18, 217–230. [CrossRef] [PubMed]

168. Jagric, M.; Risteski, P.; Martinic, J.; Milas, A.; Tolic, I.M. Optogenetic control of PRC1 reveals its role in chromosome alignment on the spindle by overlap length-dependent forces. *eLife* 2021, 10, e61170. [CrossRef]

169. Gardner, M.K.; Odde, D.J. Modeling of chromosome motility during mitosis. *Curr. Opin. Cell Biol.* 2006, 18, 639–647. [CrossRef] [PubMed]
171. Joglekar, A.P.; Hunt, A.J. A simple, mechanistic model for directional instability during mitotic chromosome movements. *Biophys. J.* 2002, 83, 42–58. [CrossRef]

172. Armond, J.W.; Harry, E.F.; McAnish, A.D.; Burroughs, N.J. Inferring the forces controlling metaphase kinetochore oscillations by reverse engineering system dynamics. *PLoS Comput. Biol.* 2015, 11, e1004607. [CrossRef]

173. Liu, J.; Desai, A.; Onuchic, J.N.; Hwa, T. An integrated mechanobiological feedback mechanism describes chromosome motility from prometaphase to anaphase in mitosis. *Proc. Natl. Acad. Sci. USA* 2008, 105, 13752–13757. [CrossRef]

174. Civelekoglu-Scholey, G.; Sharp, D.J.; Mogilner, A.; Scholey, J.M. Model of chromosome motility in Drosophila embryos: Adaptation of a general mechanism for rapid mitosis. *Biophys. J.* 2006, 90, 3966–3982. [CrossRef]

175. Gay, G.; Courtheoux, T.; Reyes, C.; Tournier, S.; Gachet, Y. A stochastic model of kinetochore-microtubule attachment accurately describes fission yeast chromosome segregation. *J. Cell Biol.* 2012, 196, 757–774. [CrossRef]

176. Vladimirou, E.; Harry, E.F.; Burroughs, N.J. Inferring the forces controlling metaphase kinetochore oscillations by reverse engineering system dynamics explain kinetochore oscillations in PtK1 cells. *J. Cell Biol.* 2013, 201, 577–593. [CrossRef]

177. Civelekoglu-Scholey, G.; He, B.; Shen, M.; Tanaka, K. A mathematical model of kinetochore-microtubule attachment regulated by Aurora A activity gradient describes chromosome oscillation and correction of erroneous attachments. *Biomed. Res. 2021*, in press.

178. Nagahara, M.; Nishida, N.; Itakura, S.; Shimizu, T.; Kanemaki, M. An auxin-based degron system for the rapid depletion of proteins in nonplant cells. *Nat. Methods* 2009, 6, 917–922. [CrossRef] [PubMed]

179. Nishimura, K.; Fukagawa, T.; Takisawa, H.; Kakimoto, T.; Kanemaki, M. An auxin-based degron system for the rapid depletion of proteins in nonplant cells. *Nat. Methods* 2009, 6, 917–922. [CrossRef] [PubMed]

180. Natsume, T.; Kiyomitsu, T.; Saga, Y.; Kanemaki, M.T. Rapid protein depletion in human cells by Auxin-inducible degron tagging with short homology donors. *Cell Rep.* 2016, 15, 210–218. [CrossRef] [PubMed]

181. Yesbolatova, A.; Natsume, T.; Hayashi, K.I.; Kanemaki, M.T. Generation of conditional auxin-inducible degron (AID) cells and tight control of degron-fused proteins using the degradation inhibitor auxin. AID: Methods 2019, 164–165, 73–80. [CrossRef]

182. DeLuca, K.F.; Meppelink, A.; Broad, A.J.; Mick, J.E.; Peersen, O.B.; Pektas, S.; Lens, S.M.A.; DeLuca, J.G. Aurora A kinase phosphorylates Hec1 to regulate metaphase kinetochore-microtubule dynamics. *J. Cell Biol.* 2018, 217, 163–177. [CrossRef]

183. Barisic, M.; Aguiar, P.; Geley, S.; Maiato, H. Kinetochore motors drive congression of peripheral polar chromosomes by overcoming random arm-ejection forces. *Nat. Cell Biol.* 2014, 16, 1249–1256. [CrossRef]

184. DeLuca, K.F.; Lens, S.M.; DeLuca, J.G. Temporal changes in Hec1 phosphorylation control kinetochore-microtubule attachment stability during mitosis. *J. Cell Sci.* 2012, 124, 622–634. [CrossRef]

185. Zhang, C.; Zhu, C.; Chen, H.; Li, L.; Guo, L.; Jiang, W.; Lu, S.H. Kif18A is involved in human breast carcinogenesis. *Carcinogenesis* 2010, 31, 1676–1684. [CrossRef] [PubMed]

186. Nagahara, M.; Nishida, N.; Iwatsuki, M.; Ishimaru, S.; Mimori, K.; Tanaka, F.; Nakagawa, T.; Sato, T.; Sugihara, K.; Hoon, D.S.; et al. Kinesin 18A expression: Clinical relevance to colorectal cancer progression. *Int. J. Cancer* 2011, 129, 2543–2552. [CrossRef] [PubMed]

187. Rath, O.; Kozielski, F. Kinesins and cancer. *Nat. Rev. Cancer* 2012, 12, 527–539. [CrossRef]

188. Cohen-Sharir, Y.; McFarland, J.M.; Abdusamad, M.; Marquis, C.; Bernhard, S.V.; Kazachkova, M.; Tang, H.; Ippolito, M.R.; Laue, K.; Zerbib, J.; et al. Aneuploidy renders cancer cells vulnerable to mitotic checkpoint inhibition. *Nature* 2021, 590, 486–491. [CrossRef] [PubMed]

189. Quinton, R.J.; DiDomizio, A.; Vittoria, M.A.; Kotynkova, K.; Ticas, C.J.; Sato, T.; Sugi, K.; Hoon, D.S.; et al. Whole-genome doubling confers unique genetic vulnerabilities on tumour cells. *Nature* 2021, 590, 492–497. [CrossRef] [PubMed]

190. Marquis, C.; Fonseca, C.L.; Queen, K.A.; Wood, L.; Vandal, S.E.; Malaby, H.L.H.; Clayton, J.E.; Stumpff, J.; Chromosomally unstable tumor cells specifically require KIF18A for proliferation. *Nat. Commun.* 2021, 12, 1213. [CrossRef] [PubMed]

191. Ertych, N.; Stolz, A.; Senezinger, A.; Weichert, W.; Kaulfuss, S.; Burfeind, P.; Aigner, A.; Wordeman, L.; Bastians, H. Increased microtubule assembly rates influence chromosomal instability in colorectal cancer cells. *Nat. Cell Biol.* 2014, 16, 779–791. [CrossRef] [PubMed]

192. Long, A.F.; O’Dea, D.B.; Dumont, S. Hec1 tail phosphorylation differentially regulates mammalian kinetochore coupling to polymerizing and depolymerizing microtubules. *Curr. Biol.* 2017, 27, 1692–1699.e3. [CrossRef] [PubMed]

193. Farag, S.S. The potential role of Aurora kinase inhibitors in haematological malignancies. *Br. J. Haematol.* 2011, 155, 561–579. [CrossRef] [PubMed]

194. Wakiya, M.; Nishi, E.; Kawai, S.; Yamada, K.; Katsumata, K.; Hitayasu, A.; Itabashi, Y.; Yamamoto, A. Chiasmata and the kinetochore component Dam1 are crucial for elimination of erroneous chromosome attachments and centromere oscillation at meiosis I. *Open Biol.* 2021, 11, 200308. [CrossRef]

195. Yamamoto, A. Shake it off: The elimination of erroneous kinetochore-microtubule attachments and chromosome oscillation. *Int. J. Mol. Sci.* 2021, 22, 3174. [CrossRef]

196. Kuniyasu, K.; Iemura, K.; Tanaka, K. Delayed chromosome alignment to the spindle equator increases the rate of chromosome missegregation in cancer cell lines. *Biomega* 2019, 9, 10. [CrossRef]

197. Orth, J.D.; Kohler, R.H.; Fojier, F.; Sorger, P.K.; Weissleder, R.; Mitchison, T.J. Analysis of mitosis and antimitotic drug responses in tumors by in vivo microscopy and single-cell pharmacodynamics. *Cancer Res.* 2011, 71, 4608–4616. [CrossRef]
198. Knouse, K.A.; Lopez, K.E.; Bachofner, M.; Amon, A. Chromosome segregation fidelity in epithelia requires tissue architecture. Cell 2018, 175, 200–211.e13. [CrossRef]

199. Bolhaqueiro, A.C.F.; Ponsioen, B.; Bakker, B.; Klaasen, S.J.; Kucukkose, E.; van Jaarsveld, R.H.; Vivie, J.; Verlaan-Klink, I.; Hami, N.; Spierings, D.C.J.; et al. Ongoing chromosomal instability and karyotype evolution in human colorectal cancer organoids. Nat. Genet. 2019, 51, 824–834. [CrossRef]

200. Nelson, L.; Tighe, A.; Golder, A.; Littler, S.; Bakker, B.; Moralli, D.; Murutu Baker, S.; Donaldson, I.J.; Spierings, D.C.J.; Wardenaar, R.; et al. A living biobank of ovarian cancer ex vivo models reveals profound mitotic heterogeneity. Nat. Commun. 2020, 11, 822. [CrossRef]

201. Du, Y.; English, C.A.; Ohi, R. The kinesin-8 Kif18A dampens microtubule plus-end dynamics. Curr. Biol. 2010, 20, 374–380. [CrossRef] [PubMed]

202. Fonseca, C.L.; Malaby, H.L.H.; Sepaniac, L.A.; Martin, W.; Byers, C.; Czechanski, A.; Messinger, D.; Tang, M.; Ohi, R.; Reinholdt, L.G.; et al. Mitotic chromosome alignment ensures mitotic fidelity by promoting interchromosomal compaction during anaphase. J. Cell Biol. 2019, 218, 1148–1163. [CrossRef] [PubMed]

203. Shi, J.; Mitchison, T.J. Cell death response to anti-mitotic drug treatment in cell culture, mouse tumor model and the clinic. Endocr. Relat. Cancer 2017, 24, T83–T96. [CrossRef]

204. Ohashi, A.; Ohori, M.; Iwai, K.; Nakayama, Y.; Nambu, T.; Morishita, D.; Kawamoto, T.; Miyamoto, M.; Hirayama, T.; Okaniwa, M.; et al. Aneuploidy generates proteotoxic stress and DNA damage concurrently with p53-mediated post-mitotic apoptosis in SAC-impaired cells. Nat. Commun. 2015, 6, 7668. [CrossRef]

205. Braun, J.; Mockel, M.M.; Strittmatter, T.; Marx, A.; Groth, U.; Mayer, T.U. Synthesis and biological evaluation of optimized inhibitors of the mitotic kinesin Kif18A. ACS Chem. Biol. 2015, 10, 554–560. [CrossRef] [PubMed]

206. Kollareddy, M.; Zheleva, D.; Dzubak, P.; Brahmkshatriya, P.S.; Lepsik, M.; Hajduch, M. Aurora kinase inhibitors: Progress towards the clinic. Investig. New Drugs 2012, 30, 2411–2432. [CrossRef] [PubMed]

207. Hilton, J.F.; Shapiro, G.I. Aurora kinase inhibition as an anticancer strategy. J. Clin. Oncol. 2014, 32, 57–59. [CrossRef]

208. Huang, L.Y.; Lee, Y.S.; Huang, J.J.; Chang, C.C.; Chang, J.M.; Chuang, S.H.; Kao, K.J.; Tsai, Y.J.; Tsai, P.Y.; Liu, C.W.; et al. Characterization of the biological activity of a potent small molecule Hec1 inhibitor TAI-1. J. Exp. Clin. Cancer Res. 2014, 33, 6. [CrossRef] [PubMed]