REVIEW ARTICLE

# Recent insights into the causes and consequences of chromosome mis-segregation

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Mitotic cells face the challenging task of ensuring accurate and equal segregation of their duplicated, condensed chromosomes between the nascent daughter cells. Errors in the process result in chromosome missegregation, a significant consequence of which is the emergence of aneuploidy—characterized by an imbalance in chromosome number—and the associated phenomenon of chromosome instability (CIN). Aneuploidy and CIN are common features of cancer, which leverages them to promote genome heterogeneity and plasticity, thereby facilitating rapid tumor evolution. Recent research has provided insights into how mitotic errors shape cancer genomes by inducing both numerical and structural chromosomal changes that drive tumor initiation and progression. In this review, we survey recent findings regarding the mitotic causes and consequences of aneuploidy. We discuss new findings into the types of chromosome segregation errors that lead to aneuploidy and novel pathways that protect genome integrity during mitosis. Finally, we describe new developments in our understanding of the immediate consequences of chromosome mis-segregation on the genome stability of daughter cells.

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## INTRODUCTION

Aneuploidy is an abnormal state in which the number of chromosomes in a cell or organism deviates from a multiple of the haploid number [1]. First observed over 100 years ago by the German zoologist Theodor Boveri while studying sea urchin embryo divisions [2], it has fascinated generations of scientists since, not least of all because of its association with cancer. Current studies estimate that aneuploidy is pervasive in solid tumors [3], including approximately 30% of prostate tumors [4], 60% of non-small cell lung cancers [5], 70% of colorectal tumors [6], and 60–80% of breast tumors [7]. The degree and spectrum of aneuploidy varies among cancer types—many display recurrent specific chromosomal abnormalities, while others harbor more complex combinations and permutations with no clear specificity [8–11]. Massive gains and losses of chromosomes, which are primarily caused by chromosome segregation errors during cell division, tend to be deleterious to the cell; therefore, they are limited in human cancer cell lines and tumors [12, 13]. Segmental or structural aneuploidy, in which chromosomal segments are rearranged and often amplified or deleted can also occur, and are often caused by faulty DNA replication or repair [14]. A distinct form of structural aneuploidy called chromothripsis has been more recently recognized as a downstream consequence of mis-segregated chromosomes that become isolated in extranuclear structures known as micronuclei. Chromothripsis is characterized by extensive genomic rearrangements and an oscillating pattern of DNA copy numbers, often restricted to one or a few chromosomes [15]. Whole and segmental chromosomal alterations are not mutually exclusive, and cancer cells can display both, resulting in composite aneuploidy [16]. Distinguishing between whole chromosome and segmental aneuploidy is essential to identifying and understanding their origins.

Aneuploidy is also often associated with chromosomal instability (CIN), a more complex phenotype characterized by a higher rate of chromosome gains and losses during cell division, and hence a greater propensity for karyotypic change. [2, 17–20] CIN positive cells create progeny with variable aneuploid karyotypes [2, 17]. Significant evidence now exists demonstrating that aneuploidy promotes CIN under certain conditions and vice versa [21–23]. Genomic instability is a long-established hallmark of cancer, and compound aneuploidy and CIN are central to the heterogeneity observed within tumors [24]. These defects drive adaptation that results in tumor development, progression, and chemotherapeutic resistance [10, 11].

In this review, we provide an overview of recent developments in the understanding of the intimate relationship between mitotic machinery dysfunction and aneuploidy. We highlight novel insights into the molecular mechanisms that rectify errors caused by these dysfunctions, focusing our discussion on animal cells. Additionally, we explore recent studies detailing the immediate consequences of uncorrected errors on genomic stability when the mitotic error correction mechanisms fail and underscore the relevance of these to cancer.

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## THE CENTRAL EVENTS OF MITOSIS

The central events of mitosis are sister chromatid separation from one another and their segregation to opposite ends of the dividing cell, and eventually into the nascent daughter cells. These events occur in a tightly coordinated manner that can be conceptually divided into distinct stages. The first is prophase during which chromosomes, rearranged into condensed rod-like structures, begin to partially lose the cohesion complexes that hold them together (known as cohesins), and to resolve the intertwined strands of DNA between the linked sister-chromatids (known as catenations). In cell types and organisms that harbor centrosomes, their separation starts during mid to late prophase. These structure act as spindle poles, providing hubs for the nucleation and organization of spindle microtubules, an event that continues into the second stage, prometaphase [25–27]. Spindle formation, attachment of the dividing sister chromatids to spindle microtubules and their eventual congression towards the equatorial plane of the dividing cell are the major events of prometaphase. This is facilitated by kinetochores, large macromolecular machines that assemble on each sister chromatid during mitosis and form not only the major site of interaction between spindle microtubules and chromatids, but also serve as major signaling hubs during mitosis [28, 29]. Prometaphase culminates in chromosome alignment at the spindle equator with each kinetochore attached to an opposing pole in a bioriented manner. Metaphase, the third stage, can be defined as the period of time between sister chromatid alignment and separation, and is dependent on the attenuation of the Spindle Assembly Checkpoint (SAC), a major quality control pathway that slows the release of cells from metaphase into the next step, anaphase, until all chromosomes are attached and bioriented [30–32]. The metaphase-anaphase transition is generally irreversible and marks the beginning of the end for mitosis. It is characterized by the dissolution of sister chromatid cohesion, inactivation of the SAC and of cyclin-dependent kinase 1, the major mitotic kinase. The now separated sister chromatids begin to move from each other to opposite poles of the spindle before the poles themselves begin to move apart, completing chromosome segregation and heralding the physical division of the cytoplasmic contents (cytokinesis) into nascent daughter cells.

## NEW DEVELOPMENTS IN THE SOURCES AND SOLUTIONS OF MITOTIC ERRORS

High temporal and high resolution imaging of mitotic cells has recently indicated that the number of chromosomes at risk of mis-segregation in non-transformed human cells is considerably higher than previously thought with one study putting mis-segregation estimates at 18% in control cells that increases to 44% with nocodazole treatment [33]. Aberrant functioning of mitotic structures and pathways is a major source of chromosome mis-segregation and thus aneuploidy. For example, centrosome amplification can lead to divisions with multipolar spindles which increases the rate of chromosome mis-segregation [34, 35]; altered kinetochore-spindle dynamics result in mis-attachments that lead to mis-segregation [36]; sister chromatid cohesion defects can lead to premature segregation [37], as can aberrant SAC signaling [38]. All of these are associated with aneuploidy and cancer and have been expertly reviewed elsewhere [1, 39, 40]. We also refer the reader to Table 1 and the references within, for a more detailed description of the drivers of CIN. Below, we describe very recent studies that shed new light on the sources of chromosome segregation errors that lead to aneuploidy in cancer, and that describe new pathways active during cell division that control the fidelity of the genome through correcting errors in chromosome copies as well as errors in their safe delivery to the daughter cells.

**Table 1.** Causes of aneuploidy and CIN.

| CIN-Type | Driver | Defect | Mechanism | References |
|---|---|---|---|---|
| W-CIN | Impaired chromosome segregation | Sister-chromatid cohesion defects | Premature sister chromatid segregation | 173, 174 |
| | Incorrect centrosome or centriole numbers | Centrosome over or under amplification | Incorrect numbers of centrosomes resulting in an aberrant number of spindle poles and thus unequal divisions (mono- or multipolar) | 175–177 |
| | | Lack of clustering of overamplified centrioles | Multipolar spindles that cannot transition to biopolar spindles | 178, 179 |
| | | Improper timing of centrosome disengagement | Delayed or accelerated centrosome separation. Monopolar spindle formation. | 67, 180 |
| | Microtubule kinetochore attachment error. | Accumulation of merotelic attachments that remain uncorrected | Loss of dynamic turnover of kinetochore-microtubule interactions. Hyperstable attachments often observed. | 36, 68 |
| | SAC defects | Weakening of the SAC (suboptimal activity) | Premature separation of sister chromatids and chromosome mis-segregation | 181–186 |
| | | SAC hyperactivation | Delays the onset of anaphase and prolongs mitosis leading to cohesion fatigue | 187–189 |
| | | Premature SAC inactivation | Premature separation of sister chromatids and chromosome mis- segregation | 190, 191 |
| Whole genome duplication | Polyploidy | Cytokinesis failure | Mitotic slippage, errors in contractile ring function in cytokinesis. | 184, 190, 192 |
| | | Endoreduplication | Reduced centrosome clustering and multipolar divisions | 193–195 |
| | | Cell fusion | Increase in ploidy and centrosome numbers | 196 |
| Structural-CIN | Replication stress and defective DNA repair. | Abnormal replication licensing, replication stress, stalled replication forks | Altered microtubule dynamics, premature centriole disengagement, | 14, 197–199 |

## Lagging and misaligned chromosomes as sources of aneuploidy

A hallmark of metazoan mitosis is the formation of a metaphase plate at the spindle equator, where sister chromatid pairs align and form connections with microtubules emanating from opposite spindle poles (termed bioriented or amphitelic attachment, Fig. 1A). This back-to-back geometry is the only one to ensure correct segregation of chromatids towards their respective poles. Other conformations including monotelic/mono-oriented (where only a single sister-kinetochore is connected to microtubules emerging from one pole, Fig. 1B), syntelic (where both sister kinetochores attach to the same pole, Fig. 1C) both activate the SAC and are corrected before anaphase onset. In addition to these, lateral attachments (where a kinetochore is attached to the lateral surface of a microtubule) are thought to form as an intermediate step before conversion into end-on attachments between kinetochores and microtubule ends [41]. When these unproductive attachments are detected, the so-called error correction pathway destabilizes them. Mechanistically, this pathway requires the activity of the centromere and kinetochore kinase Aurora B (AURKB), the catalytic subunit of the chromosomal passenger complex (CPC). AURKB is a central organizer of centromere structure and kinetochore function through spatially restricted phosphorylation of key substrates involved in maintaining the stability of kinetochore-microtubule attachments. This includes most notably the NDC80 homolog (NDC80 or HEC1) subunit of the major microtubule binding interface at the kinetochore, the KNL1-MIS12-NDC80 network, and the microtubule-depolymerizing kinesin mitotic centromere-associated kinesin (MCAK) [42, 43]. The unstructured N-terminal tail of HEC1 is overall positively charged [44] enabling direct interaction with negatively charged microtubules, thereby stabilizing their polymerizing ends [45]. AURKB phosphorylation reduces the tail's positive charge, decreasing its affinity for microtubules, causing release of the attachment [44, 46–48] as well as stimulating microtubule depolymerization [45]. AURKB-phosphorylation of MCAK changes the kinesin's conformation, reducing its microtubule affinity and depolymerization activity [49–51]. More recently, it was shown that AURKB phosphorylated MCAK in its microtubule binding region resulting in allosteric control and graded microtubule depolymerase activity [49]. In this manner, AURKB activity through phosphorylation of MCAK, NDC80 and additional substrates [52, 53], enables the kinetochore to release and reattach until the correct orientation is achieved. In line with this, alterations in AURKB activity have been repeatedly shown to result in an increase in improper attachments [54, 55].

Particularly detrimental to mitotic fidelity are merotelic attachments, in which a single or both sister-kinetochores bind microtubules from both spindle poles [56] (Fig. 1D). Although mostly corrected before anaphase by AURKB-mediated error correction, merotelic attachments are not efficiently detected by the SAC and a proportion of merotelic chromosomes remain stably tethered forming lagging chromosomes that fail to segregate during anaphase [57, 58]. Estimates suggest that between 0.1% and 10% of human primary, non-transformed, and chromosomally stable cancer cells progress into anaphase with one or few chromosomes lagging behind due to merotelic attachments [59–62]. Lagging chromosomes are thought to be a major cause of CIN in non-transformed cells [57, 60, 63] and in a subset of CIN-positive cancer cell lines [12, 64] as they can ultimately segregate to the incorrect daughter cell. This failure to segregate before the nuclear envelope reassembly (NER) can result in micronuclei ("Micronuclei") that are prone to chromothripsis ("Chromothripsis"), or may initiate anaphase bridges that are eventually broken, initiating cycles of chromosome breakage and fusion known as "breakage fusion bridge" (BFB, "The chromosome breakage-fusion-bridge cycle").

How lagging chromosomes form is unclear, but spindle geometry in early prometaphase [65–67] and hyperstabilization of kinetochore-microtubule interactions and dampened turnover dynamics [36, 68] have both been shown to increase incidences of merotely. Recently, elegant super resolution microscopy experiments demonstrated that the vertebrate centromere and kinetochore forms a bipartite structure resulting in two subdomains that independently associate with the spindle microtubules [69]. In amphitelic kinetochores, the subdomains are oriented in the same direction; however, merotelic kinetochores have bioriented subdomains, resulting in lagging chromosomes with highly stretched kinetochores. Importantly, merotelic attachments resulting from the biorientation of kinetochore subdomains occurred relatively frequently during cancer cell mitoses, indicating that they play a significant role in promoting CIN [69].

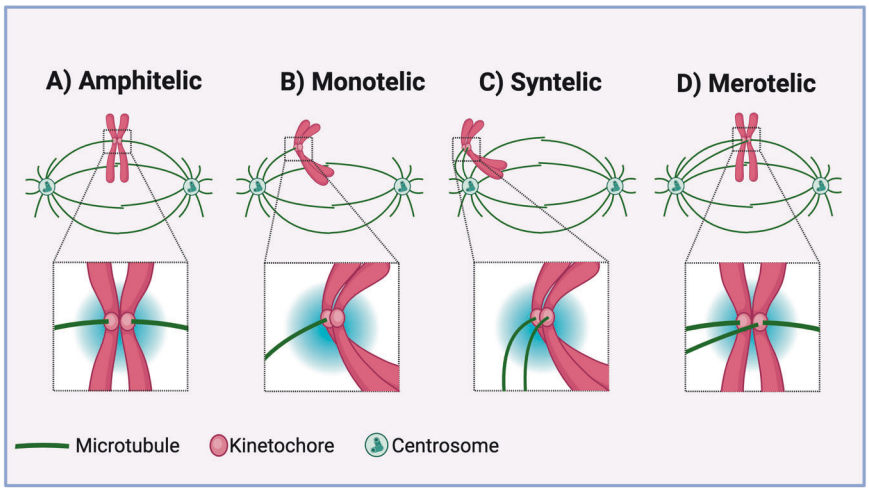


**Fig. 1 The geometries of kinetochore-microtubule attachments.** Error-free chromosome segregation depends on the correct attachment of the sister kinetochores to microtubules associated with opposite spindle poles in an amphotelic (or bi-oriented) manner (**A**). Three major types of incorrect attachments have been observed: (**B**) Monotelic attachment occurs when only one of the sister-kinetochores is attached and to spindle microtubules, while the other remains unattached; (**C**) Syntelic attachments occur when both sister kinetochores attach to microtubules from a single pole; and (**D**) Merotelic attachments typically involve a single kinetochore attached to microtubules emerging from both poles. Figure generated with biorender.

The proper biorientation and alignment of chromosomes at metaphase is generally monitored by the SAC; this infers that under normal physiological states, cells only transition to anaphase once all chromosomes align and biorient. However, recent evidence suggests that misaligned chromosomes that failed to completely align at metaphase before anaphase onset contribute to segregation errors and aneuploidy more frequently than previously thought. To explore mitotic and chromosome segregation defects that contribute to CIN in tumors, Tucker et al [70]. studied a panel of breast cancers and breast cancer cell lines. These authors demonstrated that misaligned chromosomes were the most predominant defects in primary and metastatic breast cancers (including primary-metastatic matched pairs) and that their presence correlated with the increase in CIN between primary and metastatic tumors. These observations were corroborated in patient-derived organoids and multiple human cancer cell lines [70, 71]. In a complementary study, Gomes et al [71]. systematically depleted 125 proteins involved in kinetochore-microtubule attachment including proteins that regulate attachment stability, attachment turnover and microtubule cross-linking, and investigated how human cells respond to the resulting chromosome segregation defects using high-content live-cell imaging. The authors found that, regardless of the underlying molecular defect, cells frequently entered anaphase with misaligned chromosomes that often subsequently mis-segregated, despite apparent satisfaction of the SAC [71]. In addition, these misaligned chromosomes were found to be a strong predictor of micronuclei formation. Consistent with the detrimental effects of widespread chromosome mis-segregation on viability, in both studies, the loss or gain of single chromosomes was observed at a significantly higher frequency than more widespread changes in cancer cell models [70, 71]. This could indicate that in these cells, SAC activation is not robust enough to prevent the metaphase-anaphase transition with single (or very few) unattached kinetochores [72, 73]. Alternatively, these results could reflect inappropriate silencing and inactivation of the SAC, and it will be important to distinguish between these two scenarios in the future. Moreover, although depletion of one of many proteins involved in maintaining correct kinetochore-microtubule attachment can result in mis-aligned chromosomes in cancer cells, the underlying defects at the site of attachment, including the attachment geometry, the conformation of the kinetochore subdomains, and the presence of merotelic attachments, remain to be explored.

**AURKB-mediated in error correction during anaphase**

As noted above, the error correction pathway mediated by AURKB is a major mechanism of rectifying aberrant (in particular merotelic and syntelic) attachments between spindle microtubules and kinetochores in early mitosis [54, 55, 74, 75]. The error correction activity of AURKB was thought to be limited to prometaphase. However, discrepancies between the rate of anaphase lagging chromosomes (approximately 5%) [12, 36, 61, 68] and the rate of chromosome mis-segregation in non-transformed human cells (approximately 1%) [76] hinted at the existence of an anaphase-specific mechanism to limit chromosome mis-segregation (Fig. 2A).

At the metaphase to anaphase transition, the CPC including AURKB relocates to the spindle midzone in a manner dependent on the kinesin protein Mitotic Kinesin-Like Protein 2 (MKLP2) [77], where it generates a phosphorylation gradient starting at the midzone that becomes progressively weaker towards the poles thus providing spatial information for events in anaphase and cytokinesis [78]. Initial work has suggested that this phospho-gradient delays chromosome decondensation and NER in response to incomplete chromosome segregation during anaphase [79]. More recently, three independent groups have collectively demonstrated additional roles for this gradient: maintenance of anaphase kinetochore structure over distance and time, and anaphase error correction of merotelic attachments.

In exploring the mechanisms of kinetochore stability in early anaphase, a critical timepoint in the transport of chromosomes, Papini and colleagues found that phosphorylation of the kinetochore substrate DSN1 Component Of MIS12 Kinetochore Complex (DSN1) was sensitive to its distance from midzone AURKB [80]. Specifically, midzone AURKB-mediated phosphorylation of S100/S109 of DSN1 reduced the rate at which DSN1 was lost from kinetochores as anaphase progressed, suggesting that the AURKB gradient may prolong kinetochore structure and microtubule attachment stability, specifically in anaphase. Because phosphorylation of S100/S109 of DSN1 in prometaphase destabilizes kinetochore-microtubule interactions, the level of AURKB gradient activity experienced by kinetochores in early anaphase could be sufficient to maintain DSN1 phosphorylation to stabilize kinetochores but may be insufficient to globally destabilize kinetochore-microtubule interactions as observed in pre-anaphase cells [81, 82].

In a complementary study Orr et al. found that while both CIN-, non-transformed (RPEI) and CIN+ transformed (U2OS) cells displayed transient lagging chromosomes during anaphase, a much smaller proportion of these events resulted in micronuclei formation in both cellular contexts, again suggesting that most lagging chromosomes are corrected during anaphase [83]. In investigating the role of AURKB in this error correction, the authors found that inhibition of AURKB catalytic activity or its delocalization from the central spindle by MKLP2 depletion or chemical inhibition abolished the formation of a phosphorylation gradient on both segregating and lagging chromosomes while significantly increasing the frequency of anaphase cells with lagging chromosomes and micronuclei [83]. Building on the role of AURKB in activating the pleiotropic mitotic kinase polo-like kinase 1 (PLK1) at kinetochores in early mitosis [84, 85], the authors found that active PLK1, a regulator of kinetochore-microtubule interactions in prometaphase [86], was specifically enriched at kinetochores of lagging chromosomes. This suggests that PLK1 is anaphase target of AURKB. Interestingly, correction of anaphase lagging chromosomes and their subsequent re-integration into the main nuclei were greatly compromised by partial RNAi-mediated depletion of additional key kinetochore proteins involved in the formation or regulation of microtubule attachments suggesting that AURKB may target additional proteins at anaphase kinetochores in conjunction with DSN1 and PLK1 to regulate stability of kinetochore-microtubule interactions. Overall, these two studies suggest that, although centromeric AURKB promotes microtubule detachment from kinetochores under low tension to correct errors in early mitosis, midzone-associated AURKB is required for the local stabilization of kinetochore-microtubule attachments necessary for efficient mechanical transduction of spindle forces involved in error correction during anaphase.

High resolution and high temporal imaging enabled the tracking of chromosomes in anaphase in the aforementioned studies. However, distinguishing truly lagging chromosomes from those that are late segregating constitutes a significant challenge in cell division research, as these classes of dividing chromosomes will likely have distinct fates. To address this issue, recent work employing a combination of lattice light-sheet live-cell imaging and computational analysis of chromosome segregation errors enabled a quantitative measure of chromosome lag, termed “laziness” that reflects the behavior of a single kinetochore throughout anaphase. Using this approach, the stretched presumably merotelic population of lazy kinetochores were found to persist in anaphase more frequently than the unstretched population, with incidences of 50% versus 14%, respectively [33]. These findings demonstrate how merotely is linked to lazy behavior and implied the presence of an error correction mechanism in anaphase capable of resolving such improper

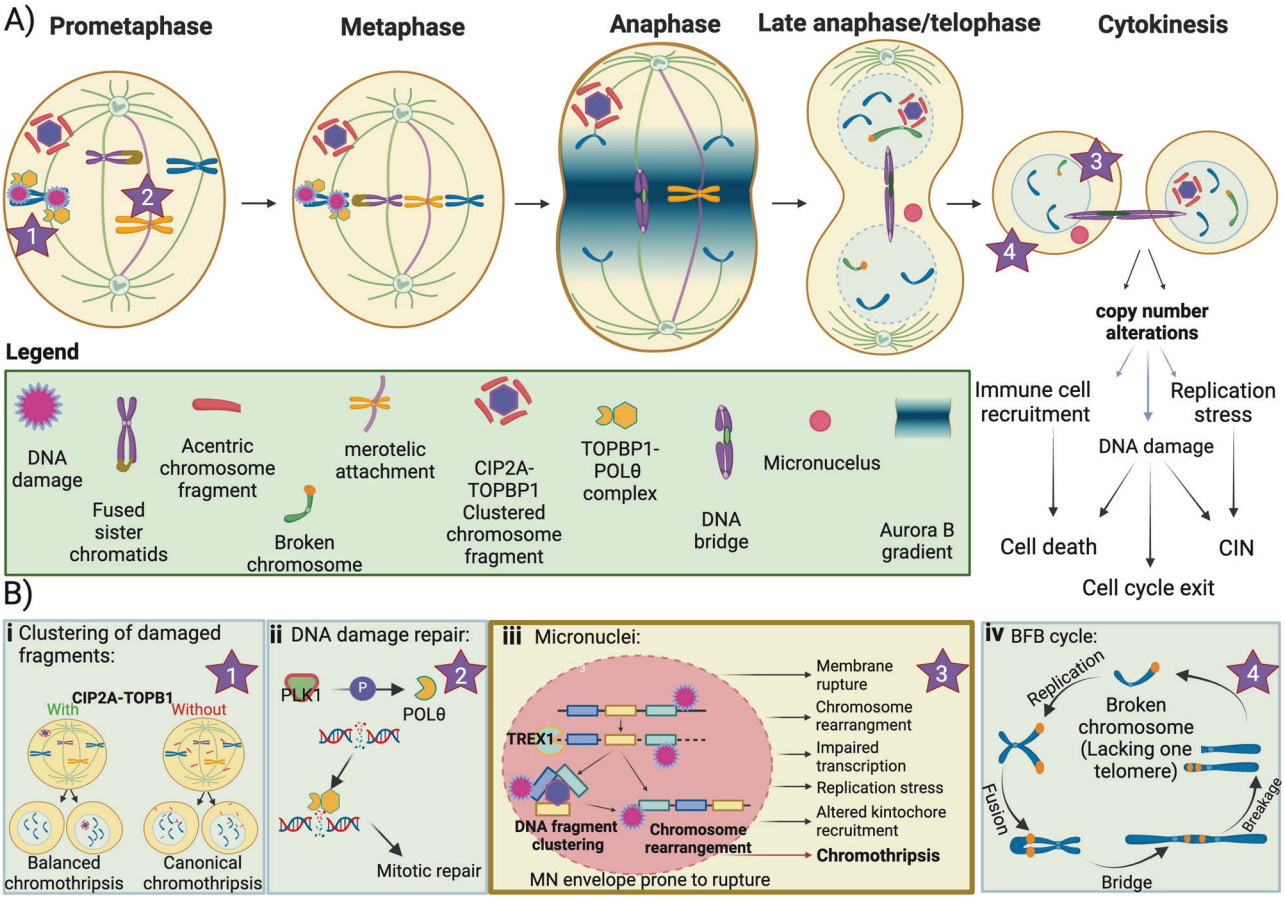


**Fig. 2 The consequences and correction of chromosome segregation errors during mitosis. A** Schematic overview of recent advances described in the present review, illustrated in association with the mitotic stages with which they are most associated. Segregation errors occur at the metaphase-anaphase transition as a result of merotelic attachment, chromosome fragmentation, DNA bridge or a weakened error correction system. The CIP2A-TOPBP1 complex act as a mitotic glue to cluster acentric chromosome fragments to limit chromosomal loss in prometaphase. During anaphase, an AURKB gradient will delay nuclear envelope reassembly around lagging chromosome to allow their reintegration into the main nuclei in anaphase. Non-resolved bridges, free acentric chromosome fragments or lagging chromosomes can induce micronuclei formation in late anaphase and telophase and leads to CIN, to an arrest of cell cycle or to cell death. **B** Schematic representation of mechanisms involved in panel A and discussed in the text: (i) Clustering of chromosome fragments and the role of the CIP2A-TOPBP1 complex in chromothripsis; (ii) Resolution of mitotic DNA damage by the TOPBP1-POLθ complex; (iii) (4) The role of micronuclei in propagating DNA damage, aneuploidy and CIN; and (iv) Representation of breakage fusion bridge cycle. Figure generated with biorender.

attachments. Building on the work of Papini et al. and Orr et al., Sen et al. observed a significant increase in laziness in cells where AURKB was inhibited after metaphase [33, 80, 83]. Lazy kinetochores failed to be corrected, and displayed severe kinetochore stretch in anaphase indicative of merotelic attachments. Ultimately inhibition of AURKB in anaphase or inhibition of its localization at the spindle midzone led to a significant increase in micronuclei formation indicating a direct link between anaphase error correction by AURKB and micronuclei formation. Overall, these studies are consistent with the idea that an AURKB gradient helps sustain kinetochore structure over the time and distance necessary for normal anaphase chromosome segregation and regulates kinetochore disassembly as cells enter telophase. This positional control of kinetochore phosphorylation may also facilitate kinetochore stability on lagging chromosomes to be maintained to promote their movement to the poles as they separate. Future work will be needed understand how these different characteristics of anaphase kinetochores are regulated by AURKB.

## DNA damage repair during mitosis

DNA double strand breaks (DSBs) are highly deleterious lesions, and maintaining genome integrity depends on their efficient repair. The cell uses distinct repair mechanisms at various stages of the cell cycle to accomplish this. Non-homologous end joining (NHEJ) is primarily active during G1 and is linked to a checkpoint at the G1/S transition that delays progression into S phase in the presence of DNA damage [87–89]. Homologous recombination (HR), which requires a DNA template, is restricted to the S and G2 phases of the cell cycle, and is associated with the G2/M "DNA damage" checkpoint [87–89]. Despite these checkpoints, cells can still enter mitosis with unresolved DSBs [90]. DSBs can also arise during mitosis, due to replication stress, anaphase bridge breakage, or under-replicated DNA regions at common fragile sites [91–95]. While the major DNA repair pathways such as HR and NHEJ are generally inactive during mitosis, some evidence suggests that mitotic cells can stabilize chromosome breaks until they can be safely repaired in the following cell cycle. Recent work has suggested that mechanistically, this may involve non-canonical functions for the cellular inhibitor of PP2A (CIP2A)-DNA topoisomerase II binding protein 1 (TOPBP1) complex initially reported to promote the segregation of acentric or damaged chromosome fragments that arises from impaired DNA synthesis [96].

In interphase, CIP2A is actively exported from the nucleus restricting its interaction with nuclear proteins [96, 97]. During

mitosis however, nuclear envelope breakdown releases nuclear proteins, including TOPBP1, allowing CIP2A-TOPBP1 complex formation and translocation to sites of DNA damage on mitotic chromosomes (Fig. 2Bi). Mechanistically, TOPBP1 can be recruited to DNA damage lesions in mitosis through direct interaction with Mediator of DNA damage checkpoint protein 1 (MDC1) [98] which, in turn, directly binds γH2AX-containing chromatin assembled at double-strand breaks [99]. CIP2A binds directly to TOPBP1 which mediates its recruitment to sites of DNA damage in mitosis [96, 97, 100]. The CIP2A-TOPBP1 complex at mitotic DSBs then stabilizes chromosomes by forming a tether between broken ends effectively preventing exposure of open DNA ends. The mechanism by which CIP2A–TOPBP1 tethers fragments is yet to be determined, but it may involve higher-order molecular interactions mediated by the extensive coiled-coil domain of CIP2A [96]. In addition, mitotic DNA lesions can recruit TOPBP1 and CIP2A in an MDC1 independent manner as well, and the role of MDC1 in mitotic tethering of broken DNA fragments remains to be clarified [98]. Regardless of the exact mechanism, these observation suggest that the CIP2A-TOPBP1 complex allows for the repair of DSBs during mitosis (described below) or at subsequent phases of the cell cycle where DNA damage can be repaired [96, 97]. In agreement with this, irradiated CIP2A-deficient mitotic cells displayed increased radiosensitivity, γH2AX foci—indicating unrepaired DNA damage—, spontaneous micronuclei formation, and DSB repair defects compared to irradiated wild-type cells. Importantly, these phenotypes were rescued by the re-expression of CIP2A [97].

The microhomology-mediated end-joining (MMEJ) pathway—previously considered to be a backup to HR and NHEJ—has emerged as a critical mechanism for mitotic DSB repair that relies on the activity of the CIP2A-TOPBP1 complex. Brambati *et al.* identified the RAD9 checkpoint clamp component A-HUS1 checkpoint clamp component-RAD1 checkpoint DNA exonuclease (RAD9A-HUS1-RAD1 or 9-1-1) complex and its interacting partner RAD9-HUS1-RAD1 interacting nuclear orphan 1 (RHINO) as key MMEJ factors. RHINO, which also functions in DNA damage sensing during replication [101], is stabilized during mitosis. The accumulation of RHINO subsequently facilitated mitotic DNA repair by binding to DNA polymerase theta (Polθ), a polymerase-helicase fusion protein that promotes MMEJ. Phosphorylation of RHINO by Polo-like kinase 1 (PLK1) during mitosis was found to be essential for its interaction with Polθ. PLK1 also directly phosphorylated and activated Polθ, which was then recruited to double-strand breaks (DSBs) to mediate the joining of broken DNA ends [102] (Fig. 2Bii). The TOPBP1-CIP2A complex plays an important role in this process; TOPBP1 interacts with and stabilizes RHINO at DSBs, promoting recruitment of Polθ to CIP2A-TOPBP1 complex-tethered DSBs [103]. In line with these observations, Polθ forms foci and filament-like structures during mitosis that colocalize with TOPBP1 foci, and TOPBP1 knockdown suppresses Polθ foci and filament structures. How TOPBP1 and Polθ interact is not clear, but one attractive hypothesis is that this may be occurring through a phospho-regulated interaction between Polθ and the BRCA1 DNA repair associated C-terminal (BRCT) domains of TOPBP1. Together, these studies establish MMEJ as a bona fide DSB repair pathway active during mitosis. Clearly, the resolution of mitotic DSBs and the tethering of acentric chromosome fragments are essential for genomic stability. Failure to resolve these breaks can result in lagging chromosome fragments, missegregation, micronuclei formation, and chromothripsis events [102, 104] as discussed below.

## THE EARLIEST CONSEQUENCES OF ANEUPLOIDY

The presence of robust mechanisms for monitoring genome fidelity in mitosis such as the AURKB error correction and MMEJ pathways described above, represent important defense mechanisms against cancer. Chromosome damage and imbalance can lead to cancer by introducing extra copies of oncogenes or deleting tumor suppressor genes. It can also result in generic stresses on the cell that are chromosome agnostic, including proteotoxic, metabolic and oxidative stress due to global increases in protein production, as summarized in numerous excellent reviews. [76, 105–108] Over the last decade, mitotic errors have been shown to drive the genesis of complex chromosomal aberrations, including the loss, gain, inversion, or translocation of chromosome fragments [109, 110]. These aberrations are common in human tumors and promote tumorigenesis by inducing further DNA damage, mutagenesis, and gene copy number changes [109]. Here, we describe recent advances in our understanding of the earliest known consequences of aberrant chromosome segregation that eventually lead to mutagenesis and the surveillance mechanisms they trigger.

### Micronuclei

Mis-segregating chromosomes (or chromosome fragments) can be excluded from the nucleus when it reforms in the daughter cell and is instead packed into a small extranuclear structure called a micronucleus that persists into interphase. DNA trapped in micronuclei can become damaged, massively rearranged, and can exhibit altered epigenetic marks [111]. Accordingly, the presence of micronuclei is associated with an increased risk of many cancers and is generally a typical characteristic of many advanced cancers [112–114]. Micronuclei have drawn exceptional attention recently because of their causal link to complex genome arrangements including chromothripsis (Fig. 2Biii).

The nuclear envelopes of micronuclei harbor fragile areas with large gaps in the nuclear lamina meshwork and fewer nuclear pores, impairing the recruitment of proteins involved in DNA transcription, replication, and repair. [104, 115–117] These fragile membranes are also more prone to rupture, which can trigger inflammatory signaling [113, 118]. In addition to the nuclear membrane defects, micronuclear chromosomes themselves lack important kinetochore assembly factors like centromere proteins A, C and T, likely due to a general import defect [119]. This results in kinetochore defects in micronuclear chromatids and to further mis-segregation in the subsequent mitosis, promoting additional aneuploidy and CIN [119].

Various scenarios have been put forward to explain how nuclear envelopes of micronuclei can be damaged during their formation in anaphase. First, the spindle midzone that forms in anaphase between the segregated chromosomes contains a high density of microtubules, which could impair nuclear envelope assembly around mis-segregated chromosomes in this region [120]. Second, as indicated above, the spindle midzone exhibits elevated activity of both AURKB and PLK1. High AURKB activity at the spindle midzone may sense lagging chromosomes and inhibit nuclear envelope assembly to allow reincorporation of laggards. In agreement, inhibition of AURKB suppresses nuclear envelope defects [79, 121, 122]. Similarly, PLK1 activity may negatively regulate nuclear pore complex protein reincorporation into nuclear membranes in anaphase; elevated midzone PLK1 activity may thus prevent efficient NPC incorporation into the nascent membranes of lagging chromosomes [121].

Once formed, micronuclei have essentially four different fates: (1) they can persist as independent cytoplasmic structures (approximately 70% of micronuclei), (2) they can be reintegrated to the main nucleus during the next mitosis, (3) they can be transported to the extracellular environment by extrusion; or (4) they can be degraded by autophagy or by an apoptosis-like process [123, 124]. None of these fates are positive: persistence results in dysregulated gene expression and further genomic instability; extrusion or degradation results in aneuploidy; and nuclear reintegration of the damaged and rearranged micronuclear DNA can further exacerbate genomic instability [104, 124].

Fortunately, as recently shown, non-transformed cells are able to avoid these consequences as a result of cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING) pathway activation. Micronuclear envelope rupture exposes the DNA directly to the cytoplasm, activating the cytosolic DNA sensor cGAS. Its activation triggers a nuclear factor-κB-dependent type I interferon response called the senescence-associated secretory phenotype (SASP) *via* the adaptor protein STING. This activates the innate immune system, triggering an inflammatory response that induces cellular senescence and stimulates the clearance of senescent and cancerous cells [125–128]. Accordingly, cells with complex karyotypes display increased secretion of SASP-related cytokines (interleukin (IL)6, IL8, C-C motif chemokine ligand 2) that both induce senescence and recruit and activate immune cells [105, 129–131]. In this manner, cells with abnormal karyotypes signal for their own removal, as a means of cancer immunosurveillance.

**Chromothripsis**

Defective DNA replication and repair processes that occur in micronuclei can also induce chromosome shattering, resulting in further chromosomal rearrangements and formation of new derivative chromosomes through chromothripsis. This can involve the complete rearrangement of loci, translocations and changes in copy number and loss of tumor suppressor genes as well as the amplification of oncogenes [116, 117, 132, 133] Damaged chromosomes in micronuclei can even be pulverized into acentromeric fragments that lack spindle attachments and that could become unequally distributed between the daughter cells during the next division [104, 116, 134]. These fragments could induce further extensive genomic rearrangements and high gene copy number variations—characteristics of chromothripsis—eventually culminating in the presence of cytoplasmic DNA fragments that could activate the cCGAS-STING pathway.

The causes of chromothripsis are not well understood; however, one potential explanation for its emergence is the disruption of micronuclear envelopes. On the one hand, rupture of the micronuclear envelope could result in exposure of the contents to cytoplasmic nucleases such as three prime repair exonuclease 1 (TREX1), leading to chromosome fragmentation. TREX1 is known to degrade cytoplasmic DNA to avoid inappropriate innate immune activation [135–137]. On the other hand, and somewhat paradoxically, degradation of exposed DNA from ruptured micronuclei by TREX1 can decrease cGAS/STING pathway activation in cancer [138, 139]. Nevertheless, cells lacking TREX1 exhibit less complex genome rearrangement after induction of chromothripsis [132, 140–144]. Moreover, TREX1 is an endoplasmic reticulum-associated enzyme and upon micronuclei envelope rupture, endoplasmic reticulum tubules have been observed invading the chromatin of ruptured micronuclei suggesting that this enzyme is at the right time and place to degrade exposed DNA [117].

Two recent studies proposed a new model to describe the fates of pulverized chromosomes from micronuclei during the next mitosis that invokes an additional role for the CIP2A-TOBPI complex described above. Using different cell lines and approaches to generate micronucleated cells and chromothripsis, Lin et al. and Trivedi et al. both observed the clustering of acentric chromosome fragments in close spatial proximity throughout mitosis that subsequently and collectively segregated asymmetrically to one of the daughter cells in a consistent manner [145, 146]. This clustering was found to be dependent on the CIP2A-TOPBP1 complex as depletion of either TOPBP1 or CIP2A resulted in an increased proportion of cells with dispersed micronuclear chromosomal fragments in mitosis. Importantly, this was a mitosis-specific function of this complex as degradation of CIP2A in mitosis was sufficient to disperse micronuclear fragments, although loss of nucleocytoplasmic compartmentalization in ruptured micronuclei in interphase may already promote cytoplasmic CIP2A and nuclear TOPBP1 association with DNA lesions [145]. Absence of fragment tethering in mitotic cells lacking the CIP2A-TOPBP1 complex after induced micronucleation and transient CIP2A depletion resulted in an increase in both deletions and inversions compared to non-depleted counterparts, as well as activation of the cGAS-STING pathway and apoptosis-related transcriptional programmes. As a result, daughter cells deficient in CIP2A were predisposed to cell death during the subsequent interphase compared to control daughter cells [145, 146]. Overall, these studies demonstrated that CIP2A-TOPBP1-regulated tethering in mitosis may be critical for bulk segregation of most chromosome fragments to one of the daughter cells, suggesting minimal loss of genomic content in the remaining daughter cell. This phenomenon was coined "balanced chromothripsis" and is generally much less deleterious than the "canonical chromothripsis" described above, which is characterized by random fragment inheritance. Supporting this finding, pan cancer tumor genome analyses revealed that CIP2A and TOPBP1 expression was elevated in cancers with genomic rearrangements, including those exhibiting chromothripsis. In contrast, their expression was comparatively lower in cancers characterized by canonical chromothripsis, where deletions were more frequently observed.

**The chromosome breakage-fusion-bridge cycle**

The chromosome breakage-fusion-bridge (BFB) cycle is a mechanism of genome instability that occurs as consequence of chromosome bridge breakage (Fig. 2Biv). Chromosome bridges are double-stranded chromatin fibres connecting the segregated chromosome masses in anaphase and can persist in the daughter nuclei in telophase and the following interphase if not adequately resolved. Chromosome bridges can be a consequence of DNA breakage, merotelic attachments, incomplete DNA replication, or incorrect resolution of chromosome catenation, and are a defining characteristic of dicentric chromosomes - chromosomes with two active centromeres [147]. In the case of dicentric centromeres, attachment of spindle microtubules to kinetochores on each of the two centromeres would result in segregation of the dicentrics towards opposite poles thereby generating chromatin bridges, breakage of which can lead to fusion of ends during the next interphase to recreate new dicentrics, engaging cells in recurrent BFB cycles. The BFB cycle can thus induce structural rearrangements such as telomere deletion and gene inversion, translocation, duplication, and loss [148, 149]. This cycle has, for example, been reported to fuel tumorigenesis by amplifying oncogenes including CDK4, cyclin E1, MDM2, EGFR, MYC, and ERBB2 [150, 151].

In animal cells, blocking cytokinesis after chromosome segregation prevents dicentric breakage [152], but the mechanisms involved in chromosome bridge breakage are unclear and a number of different hypotheses have been explored to explain this. One study suggested that stretching of chromosome bridges in the interphase following the aberrant division - now surrounded by both nuclear and plasma membrane- could result in frequent nuclear envelope ruptures and would expose the DNA to cytoplasmic nucleases such TREX1, resulting in DNA damage that promotes chromothripsis [132]. However, a separate study indicated that knockout of TREX1 did not delay bridge cleavage, suggesting the existence of TREX1-independent breakage mechanisms [153]. Instead, cellular tension across the bridge was found to be necessary to for bridge breakages and a critical role for cytoplasmic actomyosin contractile forces in inducing bridge extension and breakage was proposed. By using single-cell whole genome sequencing techniques, reciprocal chromosome segment gain and loss patterns in the daughter cells were identified after bridge breakage [153]. Not surprisingly, genome rearrangements were detected near the breakage point, a

consequence of local chromosome fragmentation and defective repair and replication of bridged DNA from previous cycles. These errors could induce a second wave of DNA damage in the mitosis following a bridge breakage, leading to further micronuclei formation and chromothripsis. Because micronuclei can harbor centromeric fragments, the stretching required to break chromosome bridges could induce the stripping of centromeric proteins resulting in compromised centromere and kinetochore functions, consistent with previous observations of stretching-induced histone ejection from DNA [132, 154]. Taken together, these studies reveal that breakage of even a single chromosome bridge can have severe consequences for genome stability and the acceleration of karyotype evolution.

### A p53-dependent mitotic surveillance mechanism

As previously discussed, mitotic defects that escape detection and repair result in misaligned and anaphase lagging chromosomes prone to micronuclei formation and further genomic insults. In normal, non-transformed cells, the presence of mitotic defects triggers a tumor protein p53 (TP53/ p53)-dependent reduction in cell proliferation [1, 12, 105, 155–159]. Striking discoveries over the last decade has revealed an additional mitotic defect to which the p53 pathway responds: prolonged mitotic timing, even in the absence of detectable defects.

P53 protein, known as the "Guardian of the genome", is a well-known protein involved in the protection of the cells from cellular damage by regulating gene expression to control DNA repair, cell division and cell death. Upon its activation in response to a range of genomic insults, p53 translocate to the nucleus to bind to the cyclin dependent kinase inhibitor 1 A (CDKN1A) promoter. This upregulates the transcription of CDKN1A (known as p21), which then binds to cyclin E/cyclin-dependent kinase (CDK)2 and cyclin D/CDK4 to arrest the cell cycle in G1 [160, 161].

Initial studies linking M phase and the p53 activation in the following cell cycle found that a prolonged prometaphase arrest longer than 90 minutes in non-transformed RPE1 cells induced a cell cycle arrest in the next G1, even in healthy, error-free cell divisions. Depletion of p53 allowed cells to proliferate, regardless of mitotic delay [162]. Building on these findings, Lambrus et al. demonstrated that depletion of polo-like kinase 4, a regulator of centriole biogenesis, triggered a p53-dependent cell cycle arrest independently of the presence of segregation errors [163]. In a CRISPR/Cas9 knockout screen, the authors identified that the cell cycle arrest caused by centrosome loss, or an extended prometaphase was dependent on the USP28–53BP1–p53–p21 pathway (also called the mitotic stopwatch complex). This complex consists of tumor protein p53 binding protein 1, which recruits p53, and ubiquitin specific peptidase 28, which stabilizes p53 *via* deubiquitination [164]. More recently, Meitinger et al. further extrapolated on these results and demonstrated that the length of mitosis is tracked by the mitotic kinase PLK1, which regulates the assembly of stopwatch complexes that are transmitted between daughter cells. The complex persists into G1, leading to increased p21 transcription, cell cycle arrest, and induction of senescence in response to a single significantly extended mitosis or successive modestly extended mitoses [165]. Collectively, these data indicated that the mitotic stopwatch complex –a biochemical memory signal - forms only when mitosis is prolonged. The accumulation of this complex progressively rises during prolonged mitosis in a PLK1-dependent manner, is passed on to daughter cells, and remains stable enough to preserve the memory of extended mitoses. Unsurprisingly, the genes encoding the three stopwatch complex subunits are classified tumor suppressors [166]. Compromised stopwatch function was associated not only with p53 mutant cancers, but also a substantial proportion of p53 wild-type human cancers. Stopwatch status also influenced the efficacy of anti-mitotic drugs that functioned by prolonging mitosis, with attenuated stopwatch function being permissive to cell proliferation after treatment. The stopwatch complex may thus function as an additional genome 'fidelity filter' that halts the proliferation of potentially deleterious cells that would otherwise pass mitotic checkpoints such as the SAC.

## CONCLUSION AND PERSPECTIVES

Over the last decade, our comprehension of how different errors in mitosis arise, the mechanisms that surveil and correct them, and the consequences to the genome should these mechanisms fail has evolved rapidly. Although the classical mitotic surveillance pathways, such as the SAC and the error correction pathway remain the best understood, recent research has revealed how lingering prometaphase errors are corrected in anaphase, how prolonged mitosis triggers a biochemical alarm that halts daughter cell proliferation, and how certain chromosome breaks are repaired in mitosis. Alternative variants and isoforms of key mitotic proteins are being identified in normal and cancer genomes and the consequences of their expression to mitotic fidelity will need to be systematically explored [167, 168]. We also have a better understanding of how segregation errors induce complex genome rearrangements that serve as continual sources of CIN (Fig. 2A), and the development of new sequencing approaches and analysis pipelines is likely to further revolutionize our understanding of the nature of the complex genome arrangements in cancer [169, 170]. Nevertheless, many questions remain: Are there any additional mechanisms that protect against chromosome mis-segregation in mitosis and its effects in the following G1? Activation of the p53 pathways is certainly a major mechanism although recent work suggested that arrest in G1 in response to mitotic errors can occur independently of p53 [171, 172]. What are the major types of chromosomes mis-segregation errors leading to micronuclei formation? Significant efforts have highlighted the contribution of merotelic attachments but the contribution of other attachments geometries remains to be further explored. Are there any additional clinically relevant consequences to chromosome segregation errors? Evolving technological approaches and innovative analysis pipelines will help answer these questions and will ultimately allow for leveraging of this information to effectively target aneuploidy and CIN in cancers.

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## AUTHOR CONTRIBUTIONS

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## COMPETING INTERESTS

The authors declare no competing interests.

## ADDITIONAL INFORMATION

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