Boveri revisited: chromosomal instability, aneuploidy and tumorigenesis

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Abstract | The mitotic checkpoint is a major cell cycle control mechanism that guards against chromosome missegregation and the subsequent production of aneuploid daughter cells. Most cancer cells are aneuploid and frequently missegregate chromosomes during mitosis. Indeed, aneuploidy is a common characteristic of tumours, and, for over 100 years, it has been proposed to drive tumour progression. However, recent evidence has revealed that although aneuploidy can increase the potential for cellular transformation, it also acts to antagonize tumorigenesis in certain genetic contexts. A clearer understanding of the tumour suppressive function of aneuploidy might reveal new avenues for anticancer therapy.

Transformation
The change that a normal cell undergoes when it becomes immortalized and acquires the potential to grow in an uncontrolled manner.

Microtubule spindle
A dynamic array of microtubules that forms during mitosis and serves to partition the duplicated chromosomes into the daughter cells.

Kinetochore
A complicated protein assembly that links the chromosomes to the microtubule-based mitotic spindle.

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Every time a cell divides it must accurately duplicate its genome and faithfully partition the duplicated genome into daughter cells. If this process fails to occur accurately, the resulting daughters might inherit too many or too few chromosomes, a condition that is known as aneuploidy. Over 100 years ago, the German zoologist Theodor Boveri described the effect of aneuploidy on organism development. Studying sea urchin embryos undergoing abnormal mitotic divisions, Boveri showed that aneuploidy has a detrimental effect on cell and organism physiology[1]. Drawing on this discovery and von Hansemann's observations of abnormal mitotic figures in tumour cells[2], Boveri proposed that an abnormal chromosome constitution might promote cancer[3]. Today, it is clear that aneuploidy is a common genetic feature of solid human tumours[4]. However, whether aneuploidy is a cause or a consequence of malignant transformation remains hotly debated.

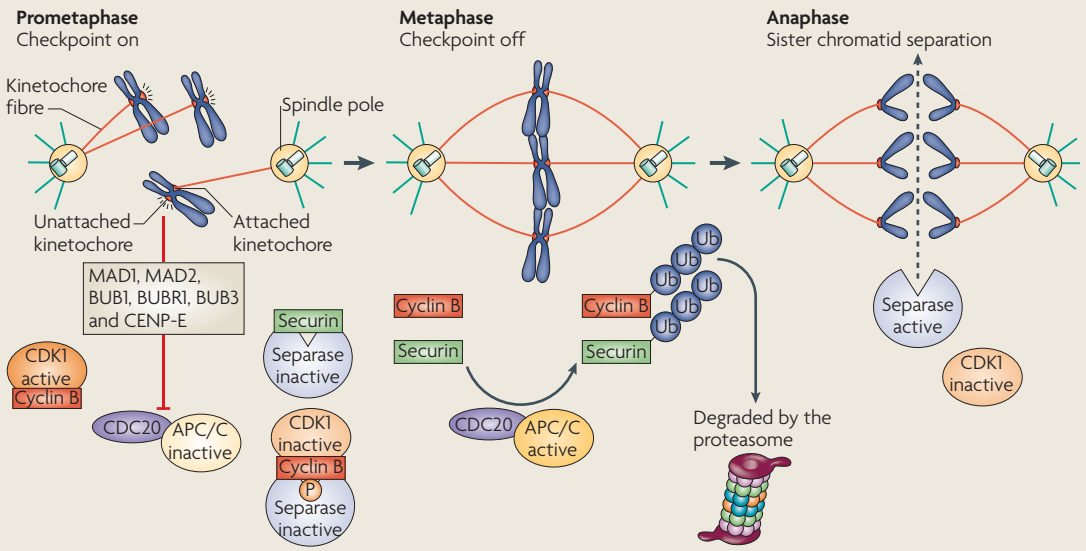
Part of the difficulty in studying the role of aneuploidy in cancer stems from the complex and diverse array of chromosomal abnormalities found among different types of tumours[5]. Indeed, coupled with numerical changes in whole chromosomes, cancer cells often display structural chromosomal alterations, including deletions, amplifications and translocations. Such structural alterations are an established cause of cancer and thus, for the purpose of this Review, we use aneuploidy to describe numerical alterations in whole chromosomes. Here, we review the pathways by which aneuploidy arises and consider the defects that allow frequent chromosome missegregation in cancer cells. We also discuss evidence that suggests a causative role for aneuploidy in the development of tumours and highlight surprising new evidence that shows aneuploidy can suppress tumorigenesis in certain genetic contexts and cell types[6].

The roads to aneuploidy

Aneuploidy is often caused by errors in chromosome partitioning during mitosis. A surveillance mechanism called the mitotic checkpoint (also known as the spindle assembly checkpoint) is the primary guard against chromosome missegregation[7,8] (BOX 1). This major cell cycle control mechanism ensures the high fidelity of chromosome segregation by delaying the onset of anaphase until all chromosomes are properly bi-oriented on the microtubule spindle. In some organisms, such as yeast and flies, the mitotic checkpoint is not essential for viability[9–11]. In others, it is essential[12–13]. In mammals, however, complete inactivation of the mitotic checkpoint leads to massive chromosome missegregation, cell death and early embryonic lethality[12–14,16].

Weakening mitotic checkpoint signalling. Under normal circumstances, the mitotic checkpoint delays mitotic progression in response to a single unattached kinetochore[17]. However, if checkpoint signalling is weakened, cells can initiate anaphase before all of the chromosomes have established their proper spindle attachments, leading to chromosome missegregation and subsequent aneuploidy (FIG. 1a). An extensive search has uncovered altered expression or mutation of

Box 1 | **The mitotic checkpoint: a safeguard to protect against aneuploidy**



The microtubule-organizing centre of the cell, the centrosome, is duplicated during S phase and separates at the beginning of mitosis. Microtubules nucleated by the centrosomes overlap to form a bilaterally symmetrical mitotic spindle, with each of the spindle poles organized around a single centrosome. Chromosomes attach to spindle microtubules at specialized proteinaceous structures known as kinetochores, which are assembled on centromeric chromatin early in mitosis (see the figure). To ensure that microtubules pull sister chromatids to opposite sides of the cell, kinetochores of duplicated chromosomes must attach to microtubules emanating from opposite spindle poles, a state known as bi-orientation. Errors in this process lead to the missegregation of chromosomes and the production of aneuploid daughter cells. To guard against chromosome missegregation, cells have evolved a surveillance mechanism called the mitotic checkpoint (also known as the spindle assembly checkpoint), which delays the onset of anaphase until all chromosomes are properly attached and bi-oriented on the microtubule spindle[7,8]. Core components of the mammalian mitotic checkpoint machinery include MAD1, MAD2, BUB1, BUBR1, BUB3 and centromere protein E (CENP-E). These proteins localize to unattached or malorientated kinetochores, which in turn catalytically generate a diffusible signal[90] that inhibits cell division cycle 20 (CDC20)-mediated activation of an E3 ubiquitin ligase, the anaphase promoting complex/cyclosome (APC/C). Separase, the protease that cleaves the cohesins that hold sister chromatids together, is inhibited by at least two mechanisms. The first mechanism involves the binding of the chaperone securin, whereas the second involves the phosphorylation-dependent binding of cyclin B associated with cyclin-dependent kinase 1 (CDK1)[91]. The binding of CDK1–cyclin B inhibits the activity of both separase and CDK1 (REF. 91). Following attachment and alignment of all the chromosomes at metaphase, the checkpoint signal is silenced and the APC/C ubiquitylates and targets securin and cyclin B for proteasome-mediated destruction, thereby initiating anaphase. At the same time, the degradation of cyclin B inactivates CDK1, thereby promoting exit from mitosis.

mitotic checkpoint components in a subset of aneuploid human cancers, including types of leukaemia, and breast, colorectal, ovarian and lung cancer[4]. In addition, germline mutations in the mitotic checkpoint component BUBR1 (also known as BUB1B) have been identified in patients with the rare genetic disorder mosaic variegated aneuploidy (MVA), in which as many as 25% of cells in multiple tissues are aneuploid[18,19]. Nevertheless, at present, mutated or altered expression of mitotic checkpoint genes can account for only a minor proportion of the aneuploidy that is observed in human tumours.

Defects in chromosome cohesion or attachment. To identify other mechanisms that lead to aneuploidy in cells, genes that have putative functions in guarding against chromosome missegregation were systematically sequenced in a panel of aneuploid colorectal cancers[20]. Surprisingly, 10 of the 11 mutations identified were in genes that directly contribute to sister chromatid cohesion, indicating that defects in the machinery that controls sister chromatid cohesion might promote aneuploidy (FIG. 1b). Consistently, overexpression of separase or securin (also known as pituitary tumour transforming gene 1 (*PTTG1*)), two key regulators that control the loss of chromatid cohesion, promotes aneuploidy and cellular transformation[21–24]. Chromosome missegregation might also arise from the improper attachment of kinetochores to spindle microtubules. This can occur when a single kinetochore attaches to microtubules that emanate from both poles of the spindle, a situation known as merotelic attachment[25] (FIG. 1c). Because merotelically orientated kinetochores are attached and under tension, their presence does not activate mitotic checkpoint signalling. Merotelic attachments are usually corrected before entry into anaphase[26], but if they persist, both sister chromatids might be missegregated towards the same pole or lagging chromosomes might be left in the spindle midzone and excluded from both daughter nuclei[27,28].

Separase
A Cys protease that triggers anaphase by cleaving the cohesin complex that holds sister chromatids together.

Securin
A chaperone that binds and inhibits the catalytic activity of separase.

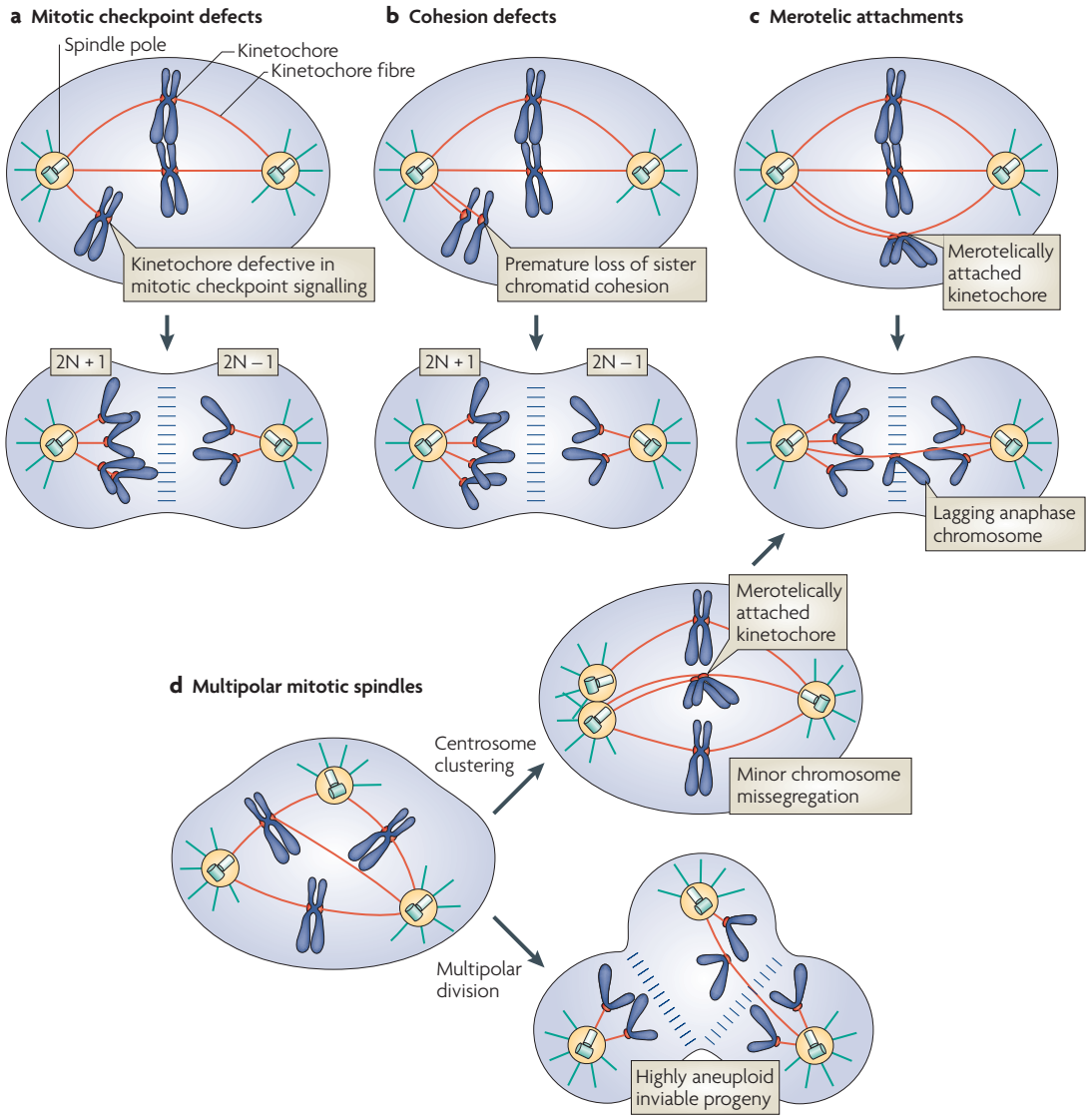


Figure 1 | **Pathways to the generation of aneuploidy.** There are several pathways by which cells might gain or lose chromosomes during mitosis. **a** | Defects in mitotic checkpoint signalling. A weakened mitotic checkpoint might allow cells to enter anaphase in the presence of unattached or misaligned chromosomes. As a consequence, both copies of one chromosome might be deposited into a single daughter cell. **b** | Cohesion defects. If sister chromatid cohesion is lost prematurely or persists during anaphase, chromosomes can be missegregated. **c** | Merotelic attachment. One kinetochore can attach to microtubules from both poles of the spindle. If these attachments persist into anaphase then lagging chromatid pairs might be missegregated or excluded from both daughter cells during cytokinesis. **d** | Multipolar mitotic divisions. Cells that possess more than two centrosomes might form multiple spindle poles during mitosis. If this defect is not corrected then a multipolar division might occur, resulting in the production of highly aneuploid and often inviable daughter cells. Often, however, centrosomes in multipolar spindles cluster into two groups to allow cells to divide in a bipolar fashion. Centrosome clustering will increase the frequency of incorrect kinetochore microtubule attachments (such as merotelic attachments). Extra centrosomes are therefore capable of driving chromosome missegregation through a mechanism that is independent of multipolar divisions. The monoploid number of chromosomes is represented by N (23 in the case of human cells).

Assembly of multipolar mitotic spindles. A final source of aneuploidy arises when a cell that contains more than two centrosomes enters mitosis (FIG. 2a,b). Extra centrosomes are frequently found in human cancer cells and their presence often correlates with aneuploidy[29,30] (BOX 2). The centrosome forms the poles of the mitotic spindle and cells that possess more than two centrosomes might form multipolar spindles (FIG. 1d). If these spindle defects are not corrected, a multipolar anaphase can occur, producing three or more highly aneuploid daughter cells. Time-lapse imaging has revealed that the progeny of multipolar divisions are typically inviable[101] (FIG. 1d). However, multipolar mitotic divisions are rare because, in most cases, extra centrosomes are clustered into two groups, thereby allowing bipolar spindles to form[29,30] (BOX 2). High-resolution microscopy has shown that cells that pass through a

Centrosome
The major microtubule-organizing centre of animal cells that forms the poles of the mitotic spindle.

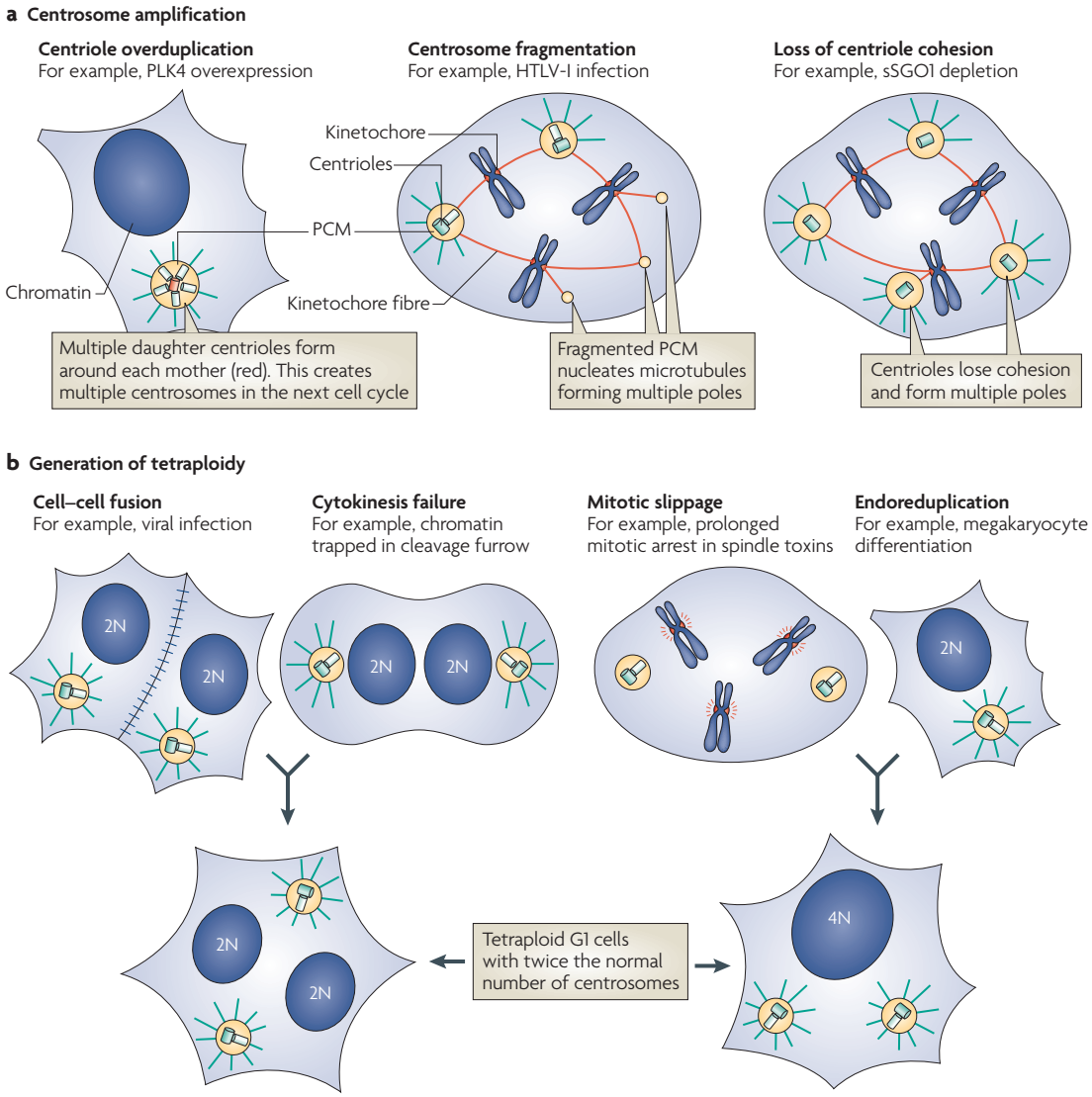


Figure 2 | **Pathways to the acquisition of extra centrosomes.** The centrosome consists of a pair of centrioles that are surrounded by the pericentriolar material (PCM). There are two major mechanisms by which cells can gain extra centrosomes. **a** | Centrosome amplification. Defects in the processes that control centriole replication can lead to centriole overduplication, which results in multiple centrosomes in the next cell cycle. This process can occur when Polo-like kinase 4 (PLK4), a regulator of centriole biogenesis, is overexpressed[92,98]. Impairment of centrosome structure can cause fragmentation of the pericentriolar material. The acentriolar fragments can then nucleate microtubules and create multipolar spindles. This has been found to occur following cellular infection with the human T cell lymphotrophic virus type 1 (HTLV-I)[99]. Finally, defects in centriole cohesion can lead to the separation of paired centrioles before the completion of chromosome segregation, creating multiple microtubule-nucleating foci. Cells with reduced levels of the short isoform of shugoshin1 (sSGO1) have been shown to lose centriole cohesion prematurely[100]. **b** | Cells become tetraploid. This can occur following cell–cell fusion or after cytokinesis failure. Alternatively, cells might skip mitosis altogether and endoreduplicate, or 'slip', out of mitosis and progress into the next cell cycle without undergoing anaphase or cytokinesis. In all of these situations, G1 tetraploid cells are created with two centrosomes that are duplicated during the next cell cycle. The monoploid number of chromosomes is represented by N (23 in the case of human cells).

multipolar intermediate before centrosomal clustering display an increased frequency of merotelic attachments and lagging anaphase chromosomes (FIG. 1d) (REF. 101; W. Silkworth and D. Cimini, personal communication). In this manner, initially multipolar spindles, coupled with subsequent centrosome clustering, can promote minor chromosome missegregation through a mechanism that is independent of multipolar divisions[25].

Centriole
A short, barrel-shaped array of microtubules localized in the centrosome.

Aneuploidy and chromosomal instability

Some tumour cells are stably aneuploid, reflecting a transient chromosome missegregation event at some point in the development of the tumour that leads to a stably propagated and inherited abnormal karyotype[31]. More often, however, aneuploidy is a result of an underlying chromosomal instability (CIN) that is characterized by an increase in the rate of gain or loss of whole chromosomes during

Box 2 | **Centrosome amplification in cancer**

In addition to numerical alterations in chromosomes, cancer cells frequently have an amplified centrosome number[30]. Extra centrosomes can lead to the formation of multiple spindle poles during mitosis, resulting in the unequal distribution of chromosomes and the production of aneuploid daughter cells. This led to the proposal that centrosome amplification might drive genomic instability and tumorigenesis[3]. A direct test of the role of centrosome amplification in cancer was recently carried out in the fly[92]. Remarkably, flies that possessed extra centrosomes in ~60% of somatic cells were overtly normal. However, larval brain cells with extra centrosomes generated metastatic tumours when transplanted into the abdomen of host flies, demonstrating that centrosome amplification can initiate tumorigenesis[92]. The tumour-promoting activity of supernumerary centrosomes occurred despite only a modest elevation in the aneuploidy of the transplanted cells, indicating that cancer might not be caused by elevated aneuploidy in this instance. An alternative interpretation is that the tumorigenic activity of extra centrosomes arises as a result of defects in the asymmetric division of larval brain neural stem cells[93].

The observation that cells from flies and human cancers proliferate nearly normally in the presence of extra centrosomes is consistent with previous studies that indicate that cells have evolved pathways to minimize the damaging effect of centrosome amplification[29]. At least three mechanisms are known to exist. First, centrosomes can be clustered into two groups to allow division to occur in a bipolar fashion[94–96]. Second, centrosomes are inactivated such that they no longer nucleate microtubules and participate in spindle formation[92]. Last, the mitotic checkpoint is activated by the unstable or incorrect microtubule attachments that are formed in multipolar mitotic spindles[92,94,97]. This delays cells in mitosis to provide additional time to cluster and inactivate centrosomes, enabling a bipolar spindle to form. Recently, a genome-wide RNA interference screen was used to identify the processes that suppress the formation of multipolar spindles in *Drosophila melanogaster* S2 cells[97]. This led to the identification of non-essential genes that are required to suppress the formation of multipolar spindles. One such gene is *HSET*, which encodes a minus-end-directed microtubule-dependent motor protein. Importantly, reduced levels of HSET selectively killed cells with amplified centrosomes, providing a possible therapeutic avenue for the treatment of cancer cells with supernumerary centrosomes[97].

cell division[32]. It is important to note that aneuploidy and CIN are not synonymous: whereas aneuploidy describes the state of having an abnormal chromosome number, CIN refers to an elevated rate of chromosome gain or loss. Abnormal chromosome number is exemplified in Down's syndrome, a condition that is associated with widespread aneuploidy but not CIN.

The molecular mechanisms that underlie CIN have remained unclear. Cells with CIN were originally reported to have impaired ability to sustain mitotic arrest in response to spindle toxins[33], leading to widespread acceptance of the proposal that an attenuated mitotic checkpoint could be the primary cause of CIN[7]. This view is probably wrong. Direct measurements using live-cell imaging to visualize mitosis have revealed that, in response to spindle toxins, the duration of mitosis in CIN cells is at least as long as in chromosomally stable diploid cells[34]. Moreover, it was found that CIN cells do not enter anaphase in the presence of misaligned chromosomes, thereby demonstrating that, at least in these cells, mitotic checkpoint dysfunction is not a primary cause of CIN[34,35]. Although CIN cells do not enter anaphase precociously, they exhibit an increase in the incidence of lagging anaphase chromosomes, which is caused at least in part by unresolved merotelic attachments. This indicates that frequent and persistent merotelic attachment is an important driving force for the CIN and aneuploidy that is found in human tumour cells.

The underlying cause of increased malorientations in CIN cells has not been determined, but might arise from errors in spindle assembly. For example, kinetochore malorientations are enriched when multipolar spindles collapse into bipolarity after centrosome clustering (BOX 2; FIG. 1d). Alternatively, merotelic attachments might be enriched because of an acquired defect in resolving these attachments before anaphase. Indeed, it was recently shown that reductions in the turnover of kinetochore microtubules in early mitosis increase the frequency of kinetochore malorientations and chromosome missegregation[36]. Remarkably, a modest increase in the expression of either of a pair of centromere- or kinetochore-bound microtubule-depolymerizing enzymes — mitotic centromere-associated kinesin (also known as KIF2C) or KIF2B — increased microtubule turnover at the kinetochore and substantially reduced the incidence of chromosome missegregation in CIN cells. This suggests that diminished dynamics of kinetochore microtubules can inhibit the correction of kinetochore malorientations, thereby predisposing cells to chromosome missegregation and CIN[36].

Aneuploidy facilitates tumour formation

The role of aneuploidy in tumorigenesis has been extensively studied in mouse models of mitotic checkpoint dysfunction. So far, conventional gene knockouts have been constructed for almost all known mitotic checkpoint genes, including those encoding MAD1 (also known as MAD1L1), MAD2 (also known as MAD2L1), BUB1, BUB3, BUBR1 and centromere protein E (CENP-E)[12,14,37–41]. In addition, hypomorphic alleles that express dramatically reduced levels of BUB1 and BUBR1 have also been generated[42,43]. Whereas complete loss of these gene products results in early embryonic lethality, heterozygous and hypomorphic mice are viable and fertile. In all cases, mice with genetically reduced levels of mitotic checkpoint components have an increased level of aneuploidy and CIN in mouse embryonic fibroblasts (MEFs) and tissues[12,14,37,38,40,42–45]. However, the degree of aneuploidy, including the proportion of aneuploid cells and the range of chromosome losses and gains, varies depending on the gene product and to what level it has been reduced (TABLE 1).

Down's syndrome
A chromosomal disorder caused by trisomy of chromosome 21.

Centromere
A specialized chromatin structure on which the kinetochore assembles. This occurs at the constricted point at which the two chromatids that form the chromosome are joined together.

Hypomorphic
A mutant that produces less than the normal amount of a gene product.

Table 1 | **Context-dependent roles of aneuploidy in tumorigenesis**

Mouse genotype	Aneuploidy in MEFs	Aneuploidy in spleen cells	Prevalence of spontaneous tumorigenesis	Refs
Aneuploidy promotes an increase in spontaneous tumorigenesis				
Mad1$^{+/-}$	ND	ND	24% develop tumours	37
Mad2$^{+/-}$	57% (16% control)	ND	28% develop lung tumours	12
Bub1$^{H/H}$	35% (7% control)	35% (1% control)	48% develop lethal tumours	43
CenpE$^{+/-}$	36% (18% control)	35% (10% control)	20% develop spleen and lung tumours, but 50% decrease in liver tumours*	46
BubR1$^{+/-}$*Apc*$^{Min/+}$	65% (14% control)	ND	Tenfold increase in colon tumours relative to *Apc*$^{Min/+}$	45
BubR1$^{H/H}$*p16*$^{-/-}$	ND	ND	Increased lung tumours relative to *p16*$^{-/-}$	52
Aneuploidy promotes an increase in carcinogen-induced tumours				
Bub1$^{+/-}$	14% (7% control)	16% (1% control)	None‡	43
Bub3$^{+/-}$	19% (9% control)	9% (0% control)	None‡	38,47
	42% (35% control)	ND	None	38,47
Rae1$^{+/-}$	19% (9% control)	9% (0% control)	None‡	38
Bub3$^{+/-}$*Rae1*$^{+/-}$§	41% (9% control)	37% (0% control)	None‡	38,48
Rae1$^{+/-}$*Nup98*$^{+/-}$	37% (9% control)	32% (0% control)	None‡	50,51
BubR1$^{+/-}$	14% (9% control)	0% (0% control)	None	42,45,49
	61% (14% control)	ND	ND‖	42,45,49
BubR1$^{H/H}$§	36% (9% control)	15% (0% control)	None	42,48
Aneuploid mouse models with overexpressed mitotic components				
Mad2¶	53% (5% control)	ND	50% develop spontaneous tumours	58
Hec1	31% (16% control)	ND	40% develop spontaneous tumours	57
Aurora A#	ND	ND	40% develop mammary tumours	76
Aneuploidy suppresses tumorigenesis				
CenpE$^{+/-}$ *p19*$^{Arf-/-}$	ND	ND	93 day increase in survival relative to *p19*$^{ARF-/-}$	46
BubR1$^{+/-}$*Apc*$^{Min/+}$	65% (14% control)	ND	50% decrease in small intestine tumours relative to *Apc*$^{Min/+}$	45
Ts65Dn**$Apc^{Min/+}$	100%	100%	44% decrease in small intestine tumours relative to *Apc*$^{Min/+}$	81
Securin$^{-/-}$*Rb*$^{+/-}$	ND	ND	56% decrease in pituitary tumours relative to *Rb*$^{+/-}$	80

*Aneuploidy inhibits tumorigenesis in mice treated with the carcinogen 7,12-dimethylbenz[α]anthracene (DMBA). ‡Aneuploidy promotes tumorigenesis in mice treated with DMBA. §Mice exhibit premature ageing. ‖Aneuploidy promotes tumorigenesis in mice treated with the carcinogen azoxymethane. ¶Cells from these mice have an increase in structural chromosomal alterations and the proportion of tetraploid cells. #Aurora A is overexpressed specifically in the mammary gland. **Ts65Dn mice are trisomic for ~50% of the orthologous genes on human chromosome 21. *Apc*, adenomatous polyposis coli; *CenpE*, centromere protein E; H, hypomophic allele; *Hec1*, highly expressed in cancer 1; MEF, mouse embryonic fibroblast; Min, multiple intestinal neoplasia; ND, not determined; *Nup98*, nucleoporin 98; *Rae1*, RNA export 1; *Rb*, retinoblastoma.

Downregulation of mitotic checkpoint components. In some instances, reduced expression of mitotic checkpoint components is associated with an increase in spontaneous cancer (TABLE 1). Specifically, mice that are heterozygous for *Mad1* and *Mad2* develop benign lung tumours, whereas *CenpE* heterozygous animals show an increased incidence of benign lung tumours and splenic lymphomas[12,37,46]. The cancers formed in these animals occur late in life (>18 months), demonstrating that transformation is a rare event that requires many consecutive generations of chromosome missegregation. By contrast, *Bub1* hypomorphic mice develop a wide array of lethal cancers, including lymphomas, lung tumours and liver tumours[43]. Nevertheless, in all situations in which aneuploidy has been found to promote spontaneous tumorigenesis, tumours form in only a fraction of animals that are aneuploid (TABLE 1), which suggests that the transformation of aneuploid cells relies on the chance acquisition of additional, cooperating mutations in key regulatory genes.

The increase in spontaneous tumorigenesis in some aneuploid mice supports the hypothesis that aneuploidy increases the probability of neoplastic transformation. However, several mitotic checkpoint-deficient mice display a significantly elevated level of aneuploidy without an increase in spontaneous tumorigenesis, demonstrating that cancer is not an inescapable fate of aneuploidy[38,42,43,47–50]. Surprisingly, there is no direct correlation between the level of aneuploidy and the incidence of spontaneous tumour development. For example, mice that are heterozygous for *Bub3* and RNA export 1 (*Rae1*) or *Rae1* and nucleoporin 98 (*Nup98*) possess similar levels of aneuploidy to *Bub1* hypomorphic mice. However, unlike *Bub1* hypomorphs, neither *Bub3*;*Rae1* or *Rae1*;*Nup98* compound heterozygotes show an increase in spontaneous tumour development[43,48,50,51] (TABLE 1).

Benign
A tumour that does not grow in an uncontrolled manner, invade surrounding tissues or metastasize to other parts of the body.

RAE1
A protein initially charcterized as an mRNA export factor that shares sequence and structural similarity with BUB3.

NUP98
A nuclear pore complex component that interacts with RAE1.

It remains unclear why the degree of aneuploidy is not an accurate predictor of tumour susceptibility in mice. One possibility is that, in addition to guarding against aneuploidy, the gene products that are reduced in these mice also have other tumour suppressive roles. For example, BUB1 has recently been proposed to have a role in eliminating aneuploid cells from the population, which might explain the high tumour susceptibility of *Bub1* hypomorphic mice[43]. Alternatively, loss of different gene products might give rise to distinct types of aneuploidy that could have different effects on tumorigenesis. For instance, aneuploid splenocytes from mice with reduced levels of BUB1, BUBR1, BUB3 and RAE1 show both gains and losses of whole chromosomes[38,42,43], whereas *CenpE* heterozygous animals show almost exclusive chromosome loss[46].

Although aneuploid animals with reduced levels of BUB1, BUBR1, BUB3, RAE1 or both RAE1 and NUP98 fail to display an increase in spontaneous tumorigenesis, these mice are prone to carcinogen-induced tumours[38,43,49,51] (TABLE 1). This suggests that aneuploidy does not initiate cancer in these mouse models, but rather drives tumour formation in cases in which mutations at oncogenic or tumour suppressor loci have already increased the potential for cellular transformation. Consistently, mutations in some tumour suppressor genes cooperate with aneuploidy to promote tumour progression. For example, reduced levels of BUBR1 promote an increase in lung tumours in mice that lack the p16 tumour suppressor[52] and a tenfold increase in colon tumours in mice that carry a heterozygous truncating mutation in the adenomatous polyposis coli (*Apc*) tumour suppressor gene (in mice, this mutation is denoted Apc^{Min})[45]. Together, these data suggest that the mutations that cooperate with aneuploidy to promote tumour formation do not occur at a significant frequency during the lifetime of laboratory mice. Nevertheless, we would emphasize that the potential of aneuploidy to contribute towards tumour progression might be more substantial in humans, which have longer lifespans and greater exposure to environmental carcinogens.

Upregulation of mitotic checkpoint components. Paradoxically, whereas inactivating mutations in mitotic checkpoint genes are rarely observed in human cancer, abnormally high expression of their gene products is much more frequent[4]. Indeed, overexpression of MAD2 and the kinetochore component HEC1 (highly expressed in cancer 1; also known as NDC80) is common in human tumours, and elevated levels of these proteins are often associated with a poor prognosis[53–56]. Increased expression of HEC1 drives aneuploidy and an elevation in spontaneous lung and liver tumours in mice[57]. In addition, conditional overexpression of MAD2 predisposes animals to a wide range of early onset, lethal tumours[58]. Continued tumour growth does not remain dependent on expression of the *Mad2* transgene, suggesting that, once neoplastic transformation has occurred, excessive MAD2 is not required for tumour maintenance. Surprisingly, MAD2-overexpressing mice are considerably more prone to tumours than mice that have reduced levels of MAD2 (REFS 12,58). However, in addition to rampant aneuploidy, cells derived from mice that overexpress MAD2 also show large-scale structural defects, including chromosomal breaks, fusions, amplifications and interstitial deletions. Thus, it remains unclear whether it is aneuploidy or structural defects that are the primary cause of tumorigenesis in these animals.

Taken together, mouse models have unequivocally shown that aneuploidy can increase the risk of neoplastic transformation, although a predisposed background is usually required. How aneuploidy increases this risk remains unclear. One possibility is that aneuploidy *per se* creates protein imbalances that facilitate the development of tumours by promoting additional genomic instability. In rare instances, this increased instability might allow the acquisition of transforming mutations that promote cancer. A second possibility is that aneuploidy allows for the duplication of a chromosome that contains an oncogenic allele or allows for the loss of a chromosome that possesses the remaining wild-type copy of a tumour suppressor gene, a process that is known as loss of heterozygosity (LOH). Consistent with this hypothesis, aneuploidy caused by haploinsufficiency of *Mad2*, or *Mad1* and *Mad2* together, has been shown to increase both the frequency and number of tumours in a $p53^{+/-}$ background[59]. By contrast, however, *Bub3* haploinsufficiency does not alter the rate or frequency of tumorigenesis in *p53* or retinoblastoma (Rb) heterozygous mice[47]. Although these studies seem to be contradictory, it is notable that the incidence of aneuploidy is higher in $Mad2^{+/-}$ compared with $Bub3^{+/-}$ MEFs (TABLE 1). This suggests the difference in tumour susceptibility might be a result of a higher level of LOH in *Mad2* haploinsufficient mice.

An alternative explanation for the tumour-promoting activity of aneuploidy is that additional chromosomes help to protect aneuploid cells against the effect of deleterious mutations in essential and haploinsufficient genes. Aneuploidy might therefore allow cells to survive for longer in the presence of ongoing DNA damage, allowing more time for cells to accumulate crucial growth-promoting and transforming mutations. Identifying the lesions that cooperate with aneuploidy to promote cellular transformation will be an important area for future research.

Doubling up: tetraploidy and cancer

Whereas some aneuploid human cancers have minor imbalances in chromosome numbers, a substantial number also exhibit large-scale aneuploidy, often containing a near tetraploid number of chromosomes[4]. Tetraploidy can arise through a number of mechanisms, including cell fusion, endoreduplication, cytokinesis failure and mitotic slippage, the last of which occurs when a cell exits mitosis and fails to segregate its chromosomes and undergo cytokinesis (FIG. 2b). Indeed, it has long been recognized that regression of the cytokinetic furrow and subsequent tetraploidy can arise when chromosome segregation errors result in chromatin bridges that occlude the cleavage plane[60–62]. A recent study has proposed an Aurora B kinase-dependent 'abscission checkpoint' in human cells that delays the completion

Splenocyte
A type of white blood cell that is a precursor of splenic tissue.

Apc^{Min}
A truncating mutation in the adenomatous polyposis coli tumour suppressor gene. Mice that are heterozygous for this mutation develop a large number of benign colon and intestinal tumours at an early age.

Loss of heterozygosity
Represents the loss of function of the remaining copy of a tumour suppressor gene in which the other allele has previously been inactivated.

p53
A tumour suppressor gene that is frequently mutated in human cancer. It has an important role in cell cycle regulation and apoptosis.

Retinoblastoma
A tumour suppressor gene that has an important function in the regulation of the cell cycle.

Tetraploid
Possessing four times the haploid number of chromosomes.

Endoreduplication
The duplication of the genome without subsequent cell division.

Aurora B
A member of the Aurora kinase family that localizes to the centromere during metaphase and to the spindle midzone during anaphase. Aurora B has a role in the correction of incorrect kinetochore microtubule attachments and cytokinesis.

Abscission
The separation of the two daughter cells at the end of cytokinesis.

of cytokinesis in response to chromosome bridges[63]. In this way, the abscission checkpoint guards against the generation of tetraploidy by allowing additional time for chromatin to be cleared from the midzone before cytokinesis is completed. This checkpoint is analogous to the NoCut pathway in budding yeast, which delays the completion of cytokinesis until chromosome segregation is completed[64]. Recent evidence has revealed that the NoCut pathway is triggered by an interaction between acetylated chromatin in the spindle midzone and Ipl1 (increase in ploidy 1), the budding yeast Aurora kinase[65].

There is now compelling evidence to suggest that the uncontrolled proliferation of tetraploid cells can trigger cellular transformation and tumour formation. The most direct evidence for this came from the observation that tetraploid *p53*$^{-/-}$ mouse cells initiate tumour formation when transplanted into immunocompromised mice, whereas isogenic diploid cells do not[66]. Importantly, tetraploid-derived tumours also display large-scale numerical and structural chromosomal aberrations. Further evidence that tetraploidy can promote cellular transformation has arisen from the study of viral-induced cell–cell fusion. When the cell cycle is dysregulated by the expression of an oncogene or a mutated *p53* tumour suppressor gene, tetraploids generated by fusion can proliferate and undergo transformation[67,68]. Again, transformation is coupled with massive genetic instability, including both numerical and structural chromosomal abnormalities. Interestingly, cells derived from mice that overexpress MAD2 show a substantial increase in the number of tetraploid cells, which might explain the increase in structural chromosome aberrations and high tumour susceptibility of these animals[58].

As noted above, the proliferation of tetraploid cells often gives rise to the accumulation of both numerical and structural chromosomal abnormalities, indicating that tetraploidy can act as a catalyst to promote further aneuploidy and genomic instability[66,68,69]. In addition to a doubling of the chromosome content, tetraploid cells typically contain twice the normal complement of centrosomes. Supernumerary centrosomes promote aberrant mitotic divisions and whole chromosome missegregation[66] (BOX 2; FIG. 1d). However, it remains unclear how tetraploid cells accumulate structural chromosomal alterations. This might reflect an increase in DNA damage in tetraploid cells or, alternatively, a proliferative advantage of tetraploid cells that possess broken or rearranged chromosomes[70].

Consistent with a causative role for tetraploidy in cancer, tetraploidy has been identified in early stage cancers, in which it precedes the development of CIN and aneuploidy[71–73]. Furthermore, several established oncogenes and tumour suppressor genes have also been shown to induce tetraploidization. For instance, Aurora A kinase is frequently overexpressed in human cancers and increased levels have been shown to cause the failure of cytokinesis[74]. Overexpression of Aurora A in the mammary gland of mice leads to an increase in the generation of teraploidy, CIN and the formation of mammary tumours[75,76]. In addition, the tumour suppressor proteins breast cancer 2, early onset (BRCA2) and large tumour suppressor homologue 1 (LATS1) have been implicated in the normal completion of cytokinesis[77,78], whereas mutations in the tumour suppressor *Apc* cause the failure of cytokinesis and cause the generation of tetraploidy in mice[79].

Aneuploidy can act as a tumour suppressor

Although aneuploidy has long been implicated in driving cancer, aneuploidy can suppress tumorigenesis in certain cases (TABLE 1). *CenpE* haploinsufficiency reduces the incidence of carcinogen-induced tumours and greatly extends the survival of mice that lack the *p19*^{Arf} tumour suppressor by an average of 93 days[46]. Moreover, mice that are heterozygous for *BubR1* develop ~50% fewer tumours in the sensitized *Apc*$^{Min/+}$ background[45], whereas deletion of the securin gene reduces the incidence of pituitary tumours by ~50% in *Rb* heterozygous animals[80] (although, in the case of *Rb*, it remains unclear if tumour suppression results from increased levels of aneuploidy).

Tumour repression has also been observed in stably aneuploid mice that are trisomic for ~50% of the orthologue genes on human chromosome 21 (REF. 81). One explanation for these observations is that exposure to carcinogens or loss of tumour suppressor function results in low levels of genetic damage and/or chromosome missegregation that, when combined with aneuploidy, drive rates of genetic instability above a threshold compatible with cell viability[46]. Consistently, *p19*$^{Arf-/-}$ and carcinogen-treated MEFs exhibit a level of aneuploidy that is exacerbated by *CenpE* haploinsufficiency[6]. Moreover, aneuploidy and apoptosis are also increased in the intestines of *BubR1*$^{+/-}$*Apc*$^{Min/+}$ mice, thereby providing evidence that too much aneuploidy might promote cell death and inhibit tumour growth[45].

The yin and yang of aneuploidy in tumorigenesis. Unlike point mutations that only affect a small number of genes, the gain or loss of a single chromosome alters the transcription of hundreds of genes and has the capacity to disturb a large array of cellular processes[82,83]. This imbalance imparts a stress that can hamper the growth of aneuploid cells. Indeed, yeast strains that contain one or more additional chromosomes grow more slowly than their haploid counterparts[84]. Moreover, mouse cells engineered to be trisomic for specific chromosomes exhibit a proliferation delay, as do human fibroblasts derived from individuals with Down's syndrome[83,85]. Consistently, when aneuploidy is introduced into a normally diploid cancer cell line, the aneuploid cells are outcompeted by diploid cells[35]. Thus, under normal circumstances, aneuploidy might act as a barrier to suppress tumorigenesis by reducing the growth of pre-neoplastic cells.

If most of the karyotpes generated by random chromosome missegregation confer a growth disadvantage to cells or cause lethality, how can aneuploidy promote tumorigenesis in some contexts? One interesting possibility is that aneuploidy provides a selective pressure for the accumulation of additional mutations that allow cells to tolerate the adverse effects of chromosomal imbalances[86].

NoCut pathway
A signalling pathway identified in yeast that delays the completion of cytokinesis when chromatin is present in the spindle midzone.

Aurora A
A member of the Aurora kinase family that is enriched at the poles of the spindle and has a role in bipolar spindle formation. Aurora A is frequently overexpressed in human cancers.

BRCA2
(Breast cancer 2, early onset). Mutations in this protein correlate with an increased risk of breast and/or ovarian cancer.

The unbalanced gene expression caused by aneuploidy might increase the rate at which cells acquire the mutations that are necessary for their survival and proliferation. Once gained, these adaptations would unlock the oncogenic potential of aneuploidy, allowing cells to survive and continue to proliferate in the face of increased genomic instability.

Conclusions: context matters

One hundred years after Boveri initially proposed that aneuploidy drives tumorigenesis, an overriding message is now clear: aneuploidy can alter the course of tumour development. However, whether it does so in a positive or negative manner depends on the cell type and the genetic context. For example, whereas mice that are heterozygous for *CenpE* exhibit an increase in the rate of spontaneous lung and spleen tumours, these animals show a decreased incidence of liver tumours[46]. Moreover, patients with Down's syndrome that carry an extra copy of chromosome 21 have a significant increase in haematological cancers but a reduced incidence of solid tumours[87–89]. Therefore, the effect of aneuploidy might not be driven by a particular combination of chromosomes *per se,* but rather by the specific interaction of the karyotype with the various genetic contexts and microenvironments found in different tissues. This explains why some tissues, such as lung epithelial cells, seem to have a higher propensity for malignant progression in aneuploid mice (TABLE 1). A clear goal for the future is to establish the genetic contexts and cell types under which aneuploidy promotes or suppresses tumorigenesis.

Moreover, whereas current mouse modelling has predominantly focused on dysregulation of mitotic checkpoint genes as a course for driving aneuploidy *in vivo*, checkpoint dysfunction does not seem to be a primary cause of CIN in human cancers. Therefore, new models of CIN that faithfully mimic the lesions and pathways that are frequently dysregulated in aneuploid cancer cells are needed, especially models that can drive inducible or transient CIN. The use of such models might reveal novel therapeutic avenues to exploit the tumour suppressive effect of aneuploidy.

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DATABASES
Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
Apc | *Nup98* | *PTTG1* | *Rae1*
OMIM: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
Down's syndrome | MVA
UniProtKB: http://www.uniprot.org
BUB1 | BUB3 | BUBR1 | CENP-E | HEC1 | Ipl1 | KIF2C | LATS1 | MAD1 | MAD2

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