Most solid human tumours are aneuploid, that is, they contain an abnormal number of chromosomes. Paradoxically, however, aneuploidy has been reported to induce a stress response that suppresses cellular proliferation in vitro. Here, we review the progress in our understanding of the causes and effects of aneuploidy in cancer and discuss how, in specific contexts, aneuploidy can provide a growth advantage and facilitate cellular transformation. We also explore the emerging possibilities for targeting the cause or consequences of aneuploidy therapeutically.

Keywords: aneuploidy; cancer; mitosis; centrosome; tumour

Introduction
Numerical and structural alterations in chromosomes are a defining characteristic of the cancer cell genome. Structural chromosomal rearrangements have received considerable attention for their role in tumorigenesis, whereas the role of numerical chromosomal changes in cancer is less clearly understood. Aneuploidy refers to an aberrant chromosome number that deviates from a multiple of the haploid set and was first associated with cancer more than a century ago [1]. As cancer cells are rife with defects in many cellular processes, some have considered aneuploidy to be a benign side effect that accompanies cellular transformation. An alternative view, however, is that aneuploidy is a core element that contributes to the growth, development and adaptability of tumours [2]. In this review, we highlight the pathways by which tumour cells acquire abnormal karyotypes, discuss the evidence for the role of aneuploidy in cellular transformation and highlight how the cause and consequences of aneuploidy might be targeted therapeutically.

Abnormal karyotypes in cancer
Aneuploidy is a remarkably common feature of human cancer, present in ~90% of solid human tumours and >50% of haematopoietic cancers [3]. Although the degree and spectrum of aneuploidy varies considerably among tumour types, many show recurrent whole-chromosome aneuploidies. For example, gain of chromosome 8 is found in ~10–20% of cases of acute myeloid leukaemia [3,4].

In addition to changes in the number of chromosomes, tumour cells also frequently have structural alterations of chromosomes, including deletions, duplications, inversions, translocations and double minute chromosomes—small circular fragments of extrachromosomal DNA lacking centromeres and telomeres. Recurrent balanced translocations are observed in specific types of leukaemia and lymphomas and are known to drive tumorigenicity [5,6]. The most famous example is the ‘Philadelphia chromosome’, a translocation between chromosomes 9 and 22 that creates the oncogenic chimeric fusion protein BCR–ABL, an important driver of chronic myeloid leukaemia [7].

Routes to aneuploidy
Each time a cell divides, it must duplicate the entire genome and distribute one copy of each chromosome into each daughter nucleus. Aneuploidy arises as a result of errors in chromosome partitioning during mitosis. Millions of cell divisions occur every minute in the adult human and therefore the maintenance of a diploid chromosome content requires that each chromosome is segregated with high fidelity during every division. A surveillance pathway known as the mitotic checkpoint—also known as the spindle assembly checkpoint—has evolved to fulfil this purpose. The mitotic checkpoint is a complex signalling network that consists of several proteins, including MAD1, MAD2, BUB1, BUBR1, BUB3 and CENP-E. The most important features of mitosis and this checkpoint are described in Fig 1.

Defects in the mitotic checkpoint. During mitosis, chromosomes attach to the microtubule spindle at proteinaceous structures known as kinetochores that assemble onto centromeric chromatin. The mitotic checkpoint delays the irreversible transition to anaphase until the kinetochore on each replicated sister chromatid has correctly
attached to spindle microtubules, thereby ensuring accurate chromosome segregation (Fig 1; [8]). A single unattached kinetochore is sufficient to delay progression to anaphase [9]. In mammals, complete inactivation of the mitotic checkpoint results in rampant chromosome missegregation and early embryonic lethality [10–14]. However, impairment of checkpoint signalling allows premature mitotic exit before complete kinetochore attachment and thus, significantly increases the probability of chromosome missegregation (Fig 2A). Mosaic variegated aneuploidy (MVA) is a rare disorder characterized by high levels of mosaic aneuploidy and a significantly increased risk of cancer [15,16]. Germline mutations in the mitotic checkpoint component \textit{BUBR1} and the centrosomal protein \textit{CEP57} have been identified in MVA patients, providing strong evidence that mitotic checkpoint defects can cause aneuploidy in humans [15–17]. Nevertheless, mutations in mitotic checkpoint genes are rare in human cancer. Altered expression of checkpoint genes might, in fact, be more common—including, for example, increases in the mitotic checkpoint component MAD2 [18–25]—but in many cases the significance of the reported differences is unclear, as the expression of many mitotic checkpoint genes is regulated during the cell cycle and the proliferative index of tumours is invariably higher than that of the surrounding normal tissue used as a reference.

\textit{Chromosomal instability}. In contrast to simple aneuploidy caused by rare missegregation of one or a few chromosomes, many tumour cells acquire chromosomal instability (CIN), a condition characterized by...
high rates of chromosome gain and loss during division [26]. CIN is recognized as a general property of many aneuploid cancer cells and drives continually evolving karyotypes and tumour heterogeneity [26–28]. Furthermore, aneuploidy and CIN have been associated with poor prognosis and resistance to therapy ([29–34]; see also review by Swanton and colleagues in this issue of EMBO reports). It is important to recognize that aneuploidy and CIN are not synonymous: aneuploidy defines the ‘state’ of having abnormal chromosome content, whereas CIN defines the ‘rate’ of karyotypic change. Therefore, although CIN inevitably leads to aneuploidy, some tumour cells are stably aneuploid without continuing CIN.

The molecular defects underlying CIN have been studied intensively for more than a decade. CIN cancer cell lines were originally reported to have an impaired ability to sustain a mitotic arrest in the presence of spindle poisons, suggesting that an attenuated mitotic checkpoint could be responsible for the aneuploidy found in human tumours [35]. However, although mitotic checkpoint defects can indeed cause CIN, it has become increasingly accepted that the overwhelming majority of CIN tumour cells have an intact checkpoint [36–38]. Thus, mitotic checkpoint defects are unlikely to be a primary cause of CIN in the majority of human tumours.

**Cohesion defects.** Sister chromatid cohesion controls the separation of duplicated sister chromatids during mitosis (Fig 1; [39]). In an effort to identify additional pathways that induce CIN, the human homologues of budding yeast CIN genes—genes which when disrupted lead to CIN—were sequenced in aneuploid colorectal cancers [40]. Ten of the eleven mutations identified occurred in four genes that are involved in sister chromatid cohesion. The functional consequences of these mutations on chromosome segregation have yet to be elucidated, but this work suggests that defects in the machinery that controls sister chromatid cohesion might contribute to CIN (Fig 2B). A recent study that identified deletions or inactivating mutations in the STAG2 gene in a diverse range of aneuploid primary tumours and human cancer cell lines further supported this idea [41]. The STAG2 gene encodes a subunit of the cohesion complex and is carried on the X chromosome, requiring only a single mutational event for its inactivation in men. Furthermore, targeted inactivation of STAG2 in cells with an otherwise stable karyotype leads to chromatid cohesion defects and aneuploidy [41]. Notably, the cohesion complex has been implicated in several cellular roles in addition to regulating mitotic chromosome separation; therefore, further work is needed to define through which pathway(s) cohesion defects contribute to aneuploidy [42].

**Merotelic attachments.** Direct live-cell analysis has revealed that although CIN cells do not show evidence of a mitotic checkpoint defect, they do show an increase in lagging anaphase chromosomes caused by unresolved merotelic attachments [36,37]. Merotelic attachments occur when a single kinetochore becomes attached to microtubules anchored at both spindle poles (Fig 2C, D). These attachments are possible because each kinetochore has the capacity to bind ~20–25 microtubules in human cells. As the overall microtubule occupancy of merotelically attached kinetochores is similar to that of aligned bi-oriented kinetochores, merotelic attachments are not detected as aberrant by the mitotic checkpoint and anaphase ensues despite their presence. Despite this, most merotelically attached chromosomes segregate correctly during anaphase, as the smaller bundle of microtubules—which orients to the wrong spindle pole—is detached (Fig 3A, B; [43]). However, a proportion of merotelically attached chromosomes remain stably tethered to both poles and fail to move towards the spindle poles during anaphase (Fig 3C; [43–46]). These lagging chromosomes can be missegregated, thereby producing two aneuploid daughter cells (Fig 3D). In addition, lagging chromosomes that fail to reach the main chromosome masses near the two poles undergo nuclear envelope reassembly and form a micronucleus (Fig 3E, F; [43,47]).

Merotelic attachments are probably a leading cause of CIN and aneuploidy observed in human tumours; two important causes of these attachment errors—hyperstable kinetochore–microtubule interactions and centrosome amplification—are discussed below.

**Hyperstabilized kinetochore–microtubule interactions.** There is evidence to suggest that CIN cells are less efficient at resolving merotelic attachment errors before anaphase [48]. The efficient correction of kinetochore–microtubule attachments requires the release of incorrectly attached microtubules. Consequently, reducing the overall turnover rate of kinetochore-bound microtubules leads to an increased frequency of kinetochore mal-orientations and predisposes cells to CIN [49]. Conversely, elevated levels of the ATP-dependent microtubule depolymerases MCAK and KIF2B increase microtubule turnover at kinetochores and reduce the incidence of chromosome missegregation in CIN cells [49]. Importantly, kinetochore–microtubule attachments are more stable in various CIN cancer cells than in a diploid, non-tumour cell line. Thus, diminished kinetochore–microtubule dynamics are probably one factor that predisposes CIN cells to kinetochore mal-orientations and chromosome segregation errors (Fig 2D; [50]). Overexpression of MAD2 hyperstabilizes kinetochore–microtubule attachments independently of the mitotic checkpoint, explaining how increased levels of MAD2 can cause CIN in tumours [18–25,51,52]. Nevertheless, the molecular defect(s) that contribute to the increased stability of kinetochore–microtubule attachments in most CIN cells has not been established.

**Centrosome amplification.** Centrosomes are the main microtubule-organizing centres of animal cells and organize the poles of the
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Mitotic checkpoint defects

Cohesion defect

Centrosome amplification

Hyperstabilized kinetochore–microtubule interactions

Fig 2 | Pathways to aneuploidy. There are several pathways by which a cell might gain or lose chromosomes during mitosis. (A) Defects in mitotic checkpoint signalling. A compromised checkpoint allows onset of anaphase with unattached kinetochores, resulting in both copies of one chromosome being partitioned into the same daughter cell. (B) Chromosome cohesion defects. Chromosomes might be missegregated if sister chromatid cohesion is lost prematurely or persists during anaphase. (C) Multipolar mitotic spindle. Cells with extra centrosomes form multipolar mitotic spindles. In most instances, centrosomes cluster into two groups before anaphase. Centrosome clustering increases the frequency of merotelic attachments, in which a single kinetochore attaches to microtubules arising from both sides of the spindle. Merotelic attachments are sufficient to silence the mitotic checkpoint and, if not corrected before anaphase, merotelically attached chromosomes lag in the spindle midzone. Lagging chromosomes are either missegregated or excluded from both daughter nuclei forming a micronucleus (see Fig 3). (D) Hyperstable kinetochore–microtubule attachments. Correction of kinetochore–microtubule attachment errors requires the release of incorrectly attached microtubules. The slow turnover of kinetochore microtubules in CIN cells reduces their ability to correct spontaneous attachment errors, thereby increasing the frequency of merotelic attachments. CIN, chromosomal instability.
bipolar microtubule spindle apparatus on which chromosomes are segregated. Centrosome amplification occurs when a cell acquires more than two centrosomes, and can arise from several types of defect, including cell division failure, cell fusion and centrosome overduplication [53]. Almost one hundred years ago, Theodor Boveri proposed that centrosome amplification can contribute to tumorigenesis [2]. Since then supernumerary centrosomes have been reported in various tumour cells in vitro and in vivo and are a consistent feature of aneuploid tumours [54–58]. Moreover, centrosome amplification is found early in the development of some haematological cancers and solid tumours, and has been shown to correlate with tumour grade, proliferative index and level of genomic instability [59–65].

Extra centrosomes can induce the formation of a multipolar mitotic spindle [66–68]. Multipolar divisions lead to catastrophic chromosome missegregation, and the progeny of such divisions are almost invariably inviable [67]. To overcome this problem, cancer cells adopt mechanisms to suppress multipolar divisions, of which the best characterized is the clustering of centrosomes into two spindle poles [69–71]. However, the passage through a multipolar intermediate before centrosome clustering inadvertently enriches for merotelic attachments, leading to chromosome missegregation (Fig 2C; [66,67]). This provides a mechanistic explanation for the longstanding link between centrosome amplification and aneuploidy and suggests that extra centrosomes might facilitate the evolution of malignant phenotypes by promoting CIN. It will be important to validate the proposed causes of CIN in vivo in the context of human tumours, although this will undoubtedly pose a significant technical challenge.

**Tetraploidy.** Tetraploid cells have twice the normal diploid chromosome content. This could arise as a result of failed cytokinesis, mitotic slippage—escape from mitosis without cytokinesis—cell fusion or two rounds of DNA replication without an intervening mitosis, known as endoreduplication. Telomere dysfunction has also been linked to the generation of tetraploid cells through two distinct pathways [72]. The continued proliferation of somatic cells in the absence of telomerase activity leads to progressive telomere shortening and eventually to the exposure of uncapped chromosome ends. Two unprotected telomere ends have been proposed to fuse together to create a dicentric chromosome with two kinetochores. If the two centromeres of the dicentric chromosomes are pulled towards opposite poles during mitosis the resulting lagging chromosome might cause a failure of cytokinesis. In addition, unprotected telomere ends create a persistent DNA damage signal that can support endoreduplication in p53-deficient cells [73]. As short telomeres are frequent in cancers before telomerase reactivation, transient telomere dysfunction might be an important cause of tetraploidization in human tumours.

In addition to a doubling of the chromosome number, tetraploid mammalian cells also have twice the normal number of centrosomes and, consequently, a CIN phenotype (Fig 2C; [67]). Hence, tetraploidy has been proposed to be an unstable intermediate that precedes the development of aneuploid human tumours with a near tetraploid karyotype [74]. Indeed, in Barrett's oesophagus and cervical cancer, tetraploidy had been found to precede aneuploidy and cellular transformation [75,76].

In many cases, experimentally created tetraploid cells undergo a p53-dependent cell cycle arrest [77,78]. Although the mechanism underlying this p53-dependent arrest is not understood, it does not seem to be caused by tetraploidy or the presence of extra centrosomes per se [79–82]. There is strong evidence that the unscheduled proliferation of tetraploid cells can initiate tumorigenesis: p53-deficient tetraploid mouse cells form tumours in immunocompromised mice, whereas isogenic diploid cells do not [83]. The tetraploid-derived tumours show structural and numerical chromosomal aberrations, indicating that tetraploidy can act as a catalyst to promote further genomic instability. Viral infection can promote cell fusion and tetraploidization, which subsequently facilitates transformation in vitro [84,85]. In this latter case, transformation is again coupled with massive genetic instability including both numerical and structural chromosomal abnormalities.

Finally, increased expression of oncogenes and loss-of-function of tumour suppressor genes has also been shown to induce tetraploidization. For example, overexpression of the Aurora A kinase leads to cytokinesis failure in vitro [78], and elevated levels of Aurora A in the murine mammary gland induce tetraploidization, CIN and the formation of mammary tumours [86,87]. Moreover, mutations in the APC tumour suppressor lead to cytokinesis failure and tetraploidization in mice [88].

**Consequences of aneuploidy**

**Impairment of organism development and cellular growth.** Aneuploidy in the germline presents a significant barrier towards successful organisinal development. In humans, aneuploidy is the leading cause of miscarriage and mental retardation [89,90]. Most congenital aneuploidies arise from errors in chromosome segregation in maternal meiosis I (see also review by Jessberger in this issue of *EMBO reports*; [91]). All human autosomal monosomies are lethal and only three autosomal trisomies are viable: trisomy of chromosome 13, 18 and 21, which are the smallest human chromosomes regarding the number of genes they encode. Of these viable trisomies, only Down syndrome patients—who are trisomic for chromosome 21—survive until adulthood. Aneuploidies of human sex chromosomes are much better tolerated than abnormal numbers of autosomal chromosomes, probably because the Y chromosome encodes few genes for nonsexual traits and only one X chromosome is active in diploid adult cells, regardless of how many copies are present.

Mosaic aneuploidy is remarkably common in early human embryos, with only ~10% of embryos diploid in all blastomeres [92]. As successful development of these embryos occurs at a higher frequency than 10%, selection could occur at the cellular level and aneuploid blastomeres outcompeted during development to give rise to chromosomally normal fetuses [93,94]. Several studies have shown that aneuploidy is deleterious for cellular growth in vitro. An array of aneuploid yeast strains carrying one or more additional chromosomes all proliferated more slowly than euploid strains under normal growth conditions, showing a G1 cell cycle delay and increased glucose uptake [95–97]. In mice, naturally occurring Robertsonian translocations—caused by end-to-end fusions of two acrocentric chromosomes—were exploited to create mouse embryonic fibroblasts (MEFs) trisomic for chromosomes 1, 13, 16 or 19 [98]. Consistent with the yeast results, all four trisomic MEF cell lines have impaired proliferation and altered metabolic properties, suggesting that aneuploidy could be partly responsible for the altered metabolism of tumour cells. Fibroblasts derived from human patients with Down syndrome proliferate more slowly than non-isogenic diploid control cells [99]. In addition, if aneuploidy is introduced into an otherwise diploid cell line, aneuploidy cells are
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**Fig 3** | The fate of merotelically attached chromosomes. (A) Merotelic kinetochore–microtubule interactions arise when a single kinetochore is attached to microtubules emanating from both poles of the mitotic spindle. There are several possible fates for merotelically attached chromosomes. (B) Normal chromosome segregation. The merotelic kinetochore has only a few microtubules oriented to the wrong spindle pole and therefore the chromosome is segregated correctly. (C) The chromosome is trapped in the cleavage furrow. The merotelic kinetochore has approximately equal numbers of microtubules oriented towards the correct and incorrect pole, resulting in even pulling forces from both sides of the cell. The chromosome therefore remains in the centre of the cell and can be constricted by the cytokinetic furrow resulting in DNA damage that might cause subsequent chromosome rearrangements. The electron micrograph shows a chromosome lagging in the midzone of the spindle. (D) Chromosome missegregation. The merotelic kinetochore has many microtubules oriented to the wrong spindle pole and therefore the chromosome is segregated into the wrong daughter nucleus. (E) Chromosome missegregation into a micronucleus. The merotelic kinetochore lags in the middle of the spindle and is missegregated. In addition, the chromosome fails to incorporate into the daughter nucleus and forms a micronucleus. Chromosomes trapped in micronuclei accumulate high levels of DNA breaks in the subsequent cell cycle as a result of aberrant DNA replication or the cell entering mitosis while the micronuclei is still undergoing DNA replication. (F) Chromosome segregation into a micronucleus. The merotelic kinetochore lags in the middle of the spindle and is correctly segregated, but forms a micronucleus. As in (E), the micronucleus accumulates DNA damage in the next cell cycle that might lead to subsequent chromosome rearrangements.
outcompeted by diploid cells; supporting the idea that aneuploidy is deleterious to rapid cell cycling in culture [36].

*Balancing the proteome.* Gains and losses of whole chromosomes simultaneously alter the copy number of hundreds of genes. Yeast strains harbouring artificial chromosomes with large amounts of mouse or human DNA, which presumably undergo little if any transcription, proliferate at normal rates, demonstrating that the detrimental effects of aneuploidy are not caused by increases in the DNA content per se [95]. Consistently, the severity of the growth inhibitory effects observed in aneuploid yeast and MEFs correlates with the size of the aneuploid chromosome and the number of genes it encodes [95,98]. As most genes on aneuploid chromosomes are transcribed and translated, the production of additional proteins is responsible for the inhibitory effect of aneuploidy on cellular growth in vitro [95,96,98,100]. This might be caused by an indirect effect of increased protein production—such as depletion of the tRNA pool—or a direct effect resulting from the creation of protein imbalances. As many proteins exist as part of complexes, aneuploidy can result in the production of excess free protein subunits or partly assembled protein complexes. In some cases, the accumulation of the uncomplexed gene products might be toxic—such as β-tubulin in budding yeast [101]. In addition, Amon and colleagues have proposed the occurrence of ‘dosage compensation’, in which cells activate protein folding and proteolytic pathways in an attempt to normalize protein stoichiometries ([100,102]; see also review by Pflau & Amon in this issue of *EMBO reports*). The increased load on protein quality control pathways in cells with abnormal chromosome numbers would create a ‘proteotoxic stress' that could explain why aneuploid yeast and MEFs, and some aneuploid cancer cell lines, are more sensitive to drugs that interfere with protein synthesis, folding and destruction [95,103]. The increased synthesis and destruction of proteins in aneuploid cells would also place an increased energy burden on cells. However, the degree to which aneuploid cells undergo dosage compensation to correct protein stoichiometry imbalances remains controversial, as others have argued that protein expression levels correlate largely with chromosome copy number [96].

The ‘aneuploid paradox’. The observation that aneuploid yeast cells and MEFs have reduced fitness for rapid cycling in vitro has brought to light an ‘aneuploid paradox’: despite the association of aneuploidy with tumours, an abnormal chromosome content provides a growth disadvantage in vitro as a result of slower cell cycling (Sidebar A; [104]). The simple resolution to this paradox is that yeast and mammalian cells in culture are selected for the fastest doubling time, whereas tumour cells must acquire the capacity for continued growth in changing intracellular and extracellular environments. Indeed, tumours might trade a reduced proliferation rate for an increased capacity to adapt and evolve. Interestingly, a slow proliferation rate in human colorectal cancers has been linked to increased tumour aggressiveness and ability to metastasize [105], and aneuploidy is linked to poor patient outcome in those cancers [106].

Aneuploid tumour cells might also accumulate mutations that allow them to alleviate proteomic imbalances and restore a more normal proliferative potential [102]. Indeed, a genetic screen identified that mutations in the deubiquitinating enzyme UBP6 improved the proliferation rate of some aneuploid yeast strains [100]. This finding highlights the importance of proteasome degradation pathways in suppressing the growth of aneuploid cells and provides evidence for the existence of mutations that suppress the adverse effects of aneuploidy. Structural alterations in chromosomes often coexist with aneuploidy in solid tumours and, thus, there might be a selective pressure to acquire mutations that allow cells to tolerate both types of chromosomal aberration.

Tetraploidy results in an increase in ploidy and consequently does not produce the imbalanced synthesis of gene products observed in other forms of aneuploidy. Importantly, the genetic imbalance caused by an additional chromosome is reduced as gene copy number increases, explaining why diploid yeast strains with an extra chromosome have milder phenotypes than iso- genic haploid strains carrying the same extra chromosome [95]. Tetraploidization might therefore buffer the detrimental imbalances caused by additional aneuploidy for individual chromosomes, and help to protect cells against the deleterious effects of mutations in essential and haploinsufficient genes.

Another explanation for the aneuploid paradox is that many of the beneficial effects of aneuploidy might be masked in cell culture, which scores rapid cycling as the primary characteristic of fitness, and in which nutrients, growth factors and oxygen are in abundance and the selective pressures of the tumour microenvironment are absent. Aneuploidy changes the copy number and expression of many genes simultaneously, increasing the probability that large adaptive leaps can be achieved. In most cases, such alterations might be expected to reduce fitness and increase cell cycling time, explaining why aneuploidy often proliferate more slowly in optimal growth conditions than diploid cells. However, in rare instances, karyotypic alterations might create new chromosome content that can provide a selective advantage in a specific environmental setting. Indeed, aneuploid yeast strains with multiple chromosomal aneuploidies proliferated poorly in non-selective conditions, but some aneuploid strains grew significantly better than euploid controls under severe genetic or environmental pressures [96]. CIN induced by stress conditions in yeast can also facilitate the acquisition of new karyotypes and the emergence of drug resistance [107]. In addition, long-term culture of human embryonic stem cells often leads to the generation of aneuploid cells that acquire a growth advantage and take over the population. For example, trisomy for chromosome 12 is recurrent in ~40% of aneuploid human embryonic stem cell lines [108,109]. This demonstrates that aneuploidy does not inevitably suppress cellular proliferation, but rather the impact of aneuploidy depends on the particular karyotype and the environmental conditions. As the intracellular and extracellular environments of tumours are continually evolving, distinct aneuploid karyotypes could be advantageous at different points during the initiation and development of tumours.

In addition, although aneuploidy often impairs cellular proliferation in vitro, somatic aneuploidy seems to be well tolerated in
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Aneuploidy promotes genomic instability. As discussed above, the karyotype of tumour cells is marked by both aneuploidy and structural alterations in chromosomes. Aneuploidy is caused by chromosome segregation errors in mitosis, whereas structural chromosomal alterations are produced by inappropriate repair of DNA double strand breaks [119]. These two distinct types of chromosomal aberration have often been thought to arise through largely different pathways [120], although it is clear that they can be mechanistically linked, with chromosome missegregation promoting additional genomic instability through at least three pathways; two of these pathways are considered below and a third in the following section.

As a first path to genomic instability, aneuploidy creates imbalances in the levels of proteins required for DNA replication, repair or mitosis, which increases the DNA mutation rate. This can lead to a ‘mutator phenotype’ that facilitates the development of genetic alterations that drive cellular growth and transformation [121–123]. Indeed, many aneuploid yeast strains also have increased rates of whole-chromosome missegregation, and initial chromosome missegregation has also been shown to induce CIN in p53-deficient mammalian cells. This demonstrates that aneuploidy can induce CIN and therefore act as a self-propagating form of instability [124,125]. In addition, single-chromosomal aneuploidy in yeast produces a modest, but significant, elevation in the rates of point mutations and mitotic recombination [124]. If aneuploidy of even a single whole chromosome is sufficient to induce genome instability, more complex aneuploidies involving changes in the copy number of several chromosomes might be expected to show even higher degrees of genomic instability.

Second, aneuploidy-driven genomic instability could arise from chromosome missegregation errors in mammalian cells, which lead to double strand breaks as a result of lagging anaphase chromosomes trapped in the cleavage furrow during cytokinesis (Fig 3C; [126]). In addition to damage caused directly by the mitotic machinery, lagging chromosomes, including those that are not missegregated, often form micronuclei, which also accumulate high levels of DNA damage (see below; Fig 3E,F; [43]). Taken together, these studies illustrate that errors in mitotic chromosome segregation can directly and indirectly lead to both numerical and structural chromosomal alterations, explaining why these two types of karyotypic abnormality often coexist.

Chromosome segregation errors can promote chromosome shattering. A new pathway for generating genetic instability in cancer cells—termed ‘chromothripsis’—has been recently discovered [127,128]; thripsis is Greek for ‘shattering into pieces’. Chromothripsis is a genomic change, characterized by the presence of tens to hundreds of DNA rearrangements that scramble blocks of sequences within a spatially localized genomic region, often involving only a limited subset of chromosomes or occurring on a single chromosome or chromosome arm [127]. Remarkably, chromosome shattering seems to occur in a single event and chromosome fragments are then haphazardly joined back together. Chromothripsis is a widespread phenomenon: it occurs in ~2–3% of all cancers, with frequencies of up to ~25% in some bone cancers [127–131].

The underlying cause of chromothripsis was initially perplexing, but recent work from Pellman and colleagues has identified a specific type of chromosome missegregation event as a potential cause for these highly localized chromosomal rearrangements [132]. Lagging chromosomes that do not join the main chromosome mass by the time of nuclear envelope reassembly are encapsulated into a micronucleus. Surprisingly, such micronuclei acquire a reduced density of nuclear pore complexes, leading to defective and delayed DNA replication that often continues even when the main nucleus reaches G2 phase. Consequently, a chromosome trapped in a micronucleus accumulates high levels of DNA breaks resulting in chromosome fragmentation [132]. A plausible pathway for this extensive fragmentation is the entry into mitosis before the micronucleus has completed DNA replication.

Furthermore, the disassembly of the micronuclear envelope often fails during mitosis through an unknown mechanism. Correspondingly, micronuclei can persist for more than one cell cycle, providing an opportunity to use non-homologous end joining to stitch back together the broken chromosomal fragments in a subsequent cell cycle. Therefore, the identification of DNA damage in chromosomes spatially isolated in micronuclei provides an attractive explanation for how highly localized DNA breaks and rearrangements are generated during chromothripsis.

The role of aneuploidy in tumorigenesis

Mouse models of CIN. The most extensive evaluation of the role of aneuploidy in tumour formation stems from the analysis of mouse models with conditional or hypomorphic mutations in mitotic checkpoint genes [10,12,14,111,112,133–136]. Complete inactivation of the checkpoint early in embryogenesis leads to embryonic lethality, underscoring the essential role of the checkpoint in organism development. However, genetically engineered mice with an attenuated mitotic checkpoint are viable and display CIN and increased levels of aneuploidy in cells and tissues [10,12,14,111,112,133,136–139]. Notably, as these animal models induce aneuploidy through continued CIN, the effect of aneuploidy in tumour development independently of CIN cannot be assessed. Several of these mice have increased spontaneous tumorigenesis, strongly supporting that CIN increases the probability of tumour formation [10,110,133,139]; for extensive reviews of the types and spectrum of tumours formed in these animals, see [53,140]). Nevertheless, it is important to recognize that spontaneous tumours form late in life, ~18 months, and with incomplete penetrance. Moreover, several genetically engineered mouse models of CIN have significantly elevated aneuploidy without an increase in spontaneous tumour formation [111,112,136,141–144], but with elevation of carcinogen-induced tumour formation [111,112,143,145].

Impaired mitotic checkpoint function is rare in human cancers, whereas increased accumulation of mitotic checkpoint components might be more common [146,147]. Elevated levels of MAD2 and the kinetochore component HEC1 are found in some
human tumours and their increased expression is often associated with aneuploidy and a poor prognosis [18–25,148]. Conditional overexpression of MAD2 in cells hyperstabilizes kinetochore–microtubule attachments, leading to CIN and large-scale structural defects [51,52]. High levels of MAD2 are sufficient to promote tumour formation in many mouse tissues [51]. Interestingly, excessive MAD2 is not required for the maintenance of these tumours, suggesting that once a transformed karyotype has been acquired, it can be maintained in the absence of the initiating CIN. Importantly, ~40% of MAD2-overexpressing cells are near-tetraploid, which could explain the high tumour susceptibility of these animals. Overexpression of HEC1 also drives aneuploidy and an increase in lung and liver tumours in mice, but how elevated levels of HEC1 lead to aneuploidy remains unclear [149].

Current evidence shows that the degree of aneuploidy is not an accurate predictor of tumour susceptibility in mice [53]. One possible explanation is that many of the proteins that are reduced in these animal models—such as BUB1, BUBR1 and MAD2—have functions outside the mitotic checkpoint that confound the interpretation of their impact on tumorigenic potential from aneuploidy alone. Importantly, reduced levels of different proteins might cause differences in the range of chromosome loss and gain, or in the acquisition of structural chromosomal aberrations. Indeed, the pathway by which chromosomes are missegregated is probably important when considering the final impact on tumour potential (Fig 3). For example, reduced levels of CENP-E allow the onset of anaphase with polar chromosones—the purest whole-chromosome missegregation phenotype [14,137]—whereas overexpression of MAD2 increases the frequency of lagging anaphase chromosomes and chromosome bridges [51]. As discussed above, lagging anaphase chromosomes are sometimes trapped in the cleavage furrow or incorporated into micronuclei, resulting in increased levels of DNA double strand breaks (Fig 3). The resulting breaks in these missegregated chromosomes could explain the existence of structural chromosomal alterations and tumour development in some animal models with increased rates of chromosome segregation errors [51,83,150].

Aneuploidy in promoting tumour formation. Cancer cells with CIN missegregate one chromosome every 1–5 divisions in vitro [26,36]. As a result, CIN drives a continually evolving karyotype that leads to phenotypic diversity in the tumour cell population. This heterogeneity provides new genetic avenues for tumour cells to explore in response to changing selection pressures and, as such, CIN probably has an important role in determining the response to anticancer therapies. In mice, KRAS-driven lung tumours remain dependent on KRAS for tumour maintenance and growth [151]. CIN induced by MAD2 overexpression did not affect the regression of KRAS-driven lung tumours after KRAS withdrawal. However, tumours that experienced MAD2-driven CIN relapsed with a much higher frequency after the removal of the KRAs oncogene, suggesting that the genetic instability imparted by CIN facilitated the evolution of resistant karyotypes [152]. Therefore, genetically engineered mice that model CIN and recapitulate the karyotypic diversity found in human cancers will form powerful platforms for testing the efficacy of, and resistance to, future clinical drug candidates.

CIN has been widely proposed to promote tumour formation by allowing loss of heterozygosity of a chromosome that contains a remaining intact copy of a tumour suppressor gene, as was originally shown to occur at the retinoblastoma tumour suppressor locus [153]. Consistently, CIN caused by haploinsufficiency of MAD2, or both MAD1 and MAD2, has been shown to increase both the frequency and number of tumours in mice heterozygous for the p53 tumour suppressor gene [154]. Moreover, aneuploidy-prone BUB1 hypomorphic animals form more tumours in mice that are heterozygous for p53 or have a heterozygous truncating mutation in the APC tumour suppressor gene (APC<sup>Min/+</sup>) [155]. As expected, the tumours that develop in these animals have loss of heterozygosity of the chromosome carrying the wild-type copy of the tumour suppressor gene, but surprisingly also have an extra copy of the chromosome bearing the mutated tumour suppressor [155,156]. Thus, at least in this context, whole-chromosome aneuploidy was selected against during the evolution of these tumours. This raises the possibility that duplication of a chromosome containing an inactive tumour suppressor gene might be a common pathway to counteract the haploinsufficiency that would arise from loss of heterozygosity of a chromosome carrying the intact copy of the same tumour suppressor [155,156].

The loss of tumour suppressor genes has been linked to the development of CIN and aneuploidy. Loss of the retinoblastoma tumour suppressor (Rb) pathway results in a modest upregulation of the levels of MAD2, which has been proposed to contribute to the CIN observed after inactivation of this pathway [157]. However, RB loss also causes defects in mitotic chromatin condensation and sister chromatid cohesion as well as abnormal centromere structure and an accumulation of DNA damage, and thus RB deficiency probably induces CIN and aneuploidy through several mechanisms [158–160]. Truncating mutations in the APC gene also disrupt the fidelity of chromosome segregation [161–165]. After induction of chromosome missegregation, caused by washout of monastrol, a reversible inhibitor of the mitotic kinesin EG5, diploid colon cancer cell lines arrest with high levels of the tumour suppressor proteins p53 and P21, whereas deletion of p53 allowed the proliferation of aneuploid cells [125]. Cells with a weakened mitotic checkpoint also show aneuploidy-induced stabilization of p53, which is dependent on ATM activation and increased levels of reactive oxygen species in the aneuploid cells [166]. These studies demonstrate a role of the p53 pathway in inhibiting the proliferation of aneuploid cells. However, suppressing p53 activation is unlikely to be an obligate requirement for the acquisition of an aneuploid karyotype, as aneuploid cells exist in several in vivo contexts in humans and mice in the presence of presumably wild-type p53 [120]. Moreover, many aneuploid tumour cell lines apparently have an intact p53 gene; however, these tumour cells might have inactivated regulators upstream or downstream from p53, or alternatively have mutations in other pathways that limit the growth of aneuploid cells.

Aneuploidy in suppressing tumour formation. Aneuploidy was proposed to promote tumour formation nearly 100 years ago [2]. However, it is becoming increasingly clear that the consequences of aneuploidy are context-dependent and, in certain circumstances, aneuploidy can suppress tumour development. This is clearly illustrated in individuals with Down syndrome, who have a significant increase in haematological cancers, but a reduced incidence of solid tumours [167–170]. Context-dependent effects of aneuploidy have also been observed in several mouse models. CIN caused by heterozygosity of CENP-E induced a modest, ~10%, increase in spontaneous lymphomas and lung tumours, but reduced the incidence of carcinogen-induced tumours and extended the survival
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of mice lacking the P19<sup>ARF</sup> tumour suppressor [110]. BUB1 insufficiency elevated tumour formation in p53<sup>+/–</sup> and APC<sup>Min/+</sup> mice, but suppressed the incidence of neoplasia formation in mice heterozygous for tumour suppressor PTEN [155]. Additionally, haploinsufficiency of BUBR1 increased the incidence of colon tumours in the APC<sup>Min/–</sup> mouse model, but reduced the incidence of small intestinal tumours by 50%, with a corresponding increase in apoptosis in these tumours [138].

One explanation for these observations is that low rates of chromosome missegregation can promote tumour development, whereas higher levels might promote cell death and suppress tumorigenesis. Consistent with this view, intermediate levels of CIN have been associated with a poor outcome in ER-negative breast cancer, whereas high levels of CIN are correlated with improved long-term survival [171,172]. In addition, in several of the genetic contexts in which increased CIN and aneuploidy have been found to suppress tumour development in mice, it did so by increasing the level of pre-existing aneuploidy: carcinogen-treated MEFs and animals lacking the tumour suppressor P19<sup>ARF</sup> have exacerbated levels of aneuploidy if there is CENP-E haploinsufficiency [110], and PTEN<sup>Min/+</sup> mice have higher levels of splenic aneuploidy than p53<sup>+/–</sup> and APC<sup>Min/–</sup> mice [155]. These observations support the view that the effect of aneuploidy on tumour development is dependent on the interaction of an abnormal karyotype with the particular genetic context and microenvironment of the tissue [53]. Defining the effect of aneuploidy in various cell types and tissues will therefore be an important area of future research (Sidebar A).

Targeting the aneuploid karyotype

The acquisition of an additional chromosome in yeast cells or MEFs suppresses cellular proliferation. This raises the possibility of identifying compounds that are lethal to the aneuploid state, either by exacerbating the stresses imposed on aneuploid cells or by inhibiting pathways essential for the survival of aneuploid cells (Sidebar A). Targeting aneuploid tumour cells is attractive because it has the potential to be effective against a vast array of aneuploid tumours without previous knowledge of the underlying mutations or pathways deregulated in the tumour. Recent work has begun to validate the concept of therapeutically exploiting the aneuploid state. Groups of chemical compounds have been identified that are more cytotoxic to tumour cell lines with more complex karyotypes [173,174]. MEFs trisomic for a single chromosome are more sensitive to the energy stress inducer AICR (an activator of AMPK), the proteotoxic stress-inducing compound 17-AAG (an inhibitor of heat shock protein 90) and the autophagy inhibitor chloroquine [103]. AICR and 17-AAG act synergistically in vitro and in xenograft mouse models to increase lethality in human aneuploid cancer cell lines that have CIN, compared with chromosomally stable near-diploid cell lines. Exactly how these compounds act to kill aneuploid cancer cells remains to be established. Nevertheless, this pioneering work shows that the aneuploid state can in principle be targeted therapeutically and opens the door for the development of new drugs for the treatment of aneuploid tumours (see also reviews by Pfau & Amon and Swanton & colleagues in this issue of EMBO reports).

In addition to targeting aneuploidy per se, it might also be feasible to target the molecular defects that promote the acquisition of an aneuploid karyotype. One attractive target is CIN cancer cells with extra centrosomes. Centrosome amplification occurs almost exclusively in cancer cells, raising the possibility that suppressing centrosome coalescence could selectively kill cancer cells with supernumerary centrosomes by forcing them into lethal multipolar divisions [175]. Two recent genome-wide RNAi screens identified various genes required for the clustering of centrosomes [175,176]. One gene identified was the minus-end-directed kinesin-related motor HSET, which is not essential for the division of normal cells, but is required for viability in certain cancer cells with extra centrosomes [175]. Inhibitors of the mitotic kinesin EGS have been tested for clinical use; therefore, HSET inhibitors could possibly be developed. A more complete understanding of the defects that cause the CIN observed in human cancers will probably provide additional therapeutic avenues for selectively killing aneuploid tumours (Sidebar A).

New mouse models needed

Our understanding of tumorigenesis and the development of future therapies relies largely on the ability to create animal models that faithfully recapitulate aspects of the human disease process. However, in contrast to the complex karyotypes found in human cancers, many genetically engineered mouse cancer models have relatively benign cytogenetic profiles [177–180]. This reveals a pressing need to develop animal models that more fully recapitulate the complex karyotypic alterations observed in human cancer. Optimally, such models will mimic lesions that are causative of the underlying instability found in cancer. Indeed, although great resources have been invested in developing mouse models with defects in the mitotic checkpoint, checkpoint abrogation does not seem to be a primary cause of CIN in human cancer. Given the established role of centrosome amplification in promoting CIN and the presence of extra centrosomes in premalignant and invasive tumours, it will be of considerable interest to develop animal models in which centrosome amplification can be induced in the absence of defects in other pathways. Additionally, mouse models with cohesion defects—such as loss of function mutations in the cohesion component STAG2—or with hyperstabilized kinetochore–microtubule attachments due to reduced levels of MCAK or KIF2B, will also be of interest. Clearly, a more complete understanding of the in vivo cause of aneuploidy will be paramount for the development of additional disease-relevant animal models. Furthermore, establishing new methodologies to quantify the level of aneuploidy in vivo will be important for furthering our understanding of how aneuploidy influences tumour initiation, development and resistance to therapy [181].

Exploring aneuploidy: the significance of chromosomal imbalance

This review series—published in this issue of EMBO reports—also includes:

A balancing act: focus on aneuploidy
Nonia Pariente

Chromosomal instability and aneuploidy in cancer: from yeast to man
Sarah J. Pfau and Angelika Amon

Cancer chromosomal instability: therapeutic and diagnostic challenges
Nicholas McGranaham, Rebecca A. Burrell, David Endesfelder, Marco R. Novelli and Charles Swanton

Age-related aneuploidy through cohesion exhaustion
Rolf Jessberger
Conclusions
The aneuploidy paradox—the strong association of aneuploidy with cancer despite its ability to suppress the proliferative potential of cells in vitro—is resolved by the recognition that aneuploid cells trade a reduction in proliferation rate for an increased ability to adapt and evolve. In this view, the beneficial effects of aneuploidy in enhancing cell growth will be most evident under stringent selective pressures, such as those encountered in vivo in the tumour microenvironment, rather than under conditions that stimulate minimum cell cycle time. Furthermore, aneuploidy induces a ‘mutator phenotype’ that increases DNA damage and genomic instability. The combination of changes in chromosome number and aneuploidy-induced DNA damage produces an increased cellular heterogeneity in the tumour population and provides tumours with expanded opportunities for adaptation to changing selection pressures.

A key aspect of future work will be to define the cellular response to aneuploidy and determine whether the stresses imparted on aneuploid cells can be exploited for therapeutic gain (Sidebar A). Our view is that cancer cells sit atop a three-legged pedestal: proliferation, survival and adaptability. Weakening any of these supports might dismantle the tumour, but combining ‘aneuploidy therapy’ with existing therapeutic approaches might provide our greatest hope in improved clinical outcomes.

ACKNOWLEDGEMENTS
The authors thank Quan Zhu, Benjamin Vitre and Karin Holland for helpful comments on this manuscript. We apologize to all whose work on aneuploidy was not cited because of space restrictions. This work was supported by a grant (GM29513) from the National Institutes of Health to D.W.C., who receives salary support from the Ludwig Institute for Cancer Research. A.J.H. is supported by a Leukaemia & Lymphoma Society special fellowship.

CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

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