Short- and long-term effects of chromosome mis-segregation and aneuploidy

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Abstract | Dividing cells that experience chromosome mis-segregation generate aneuploid daughter cells, which contain an incorrect number of chromosomes. Although aneuploidy interferes with the proliferation of untransformed cells, it is also, paradoxically, a hallmark of cancer, a disease defined by increased proliferative potential. These contradictory effects are also observed in mouse models of chromosome instability (CIN). CIN can inhibit and promote tumorigenesis. Recent work has provided insights into the cellular consequences of CIN and aneuploidy. Chromosome mis-segregation per se can alter the genome in many more ways than just causing the gain or loss of chromosomes. The short- and long-term effects of aneuploidy are caused by gene-specific effects and a stereotypic aneuploidy stress response. Importantly, these recent findings provide insights into the role of aneuploidy in tumorigenesis.

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The term aneuploidy was coined by Gunnar Täckholm in 1922 (REF. 1). He studied the karyotypes of meiotic cells of first filial generation (F_1) hybrids from crosses between different rose species. He noted that, in meioses of a subset of these F_1 hybrids:

> *bivalent and univalent chromosomes are not a multiple of seven* [author's note: the haploid chromosome number of the genus Rosa]. *In many instances this is also the case for their somatic karyotypes. Because it is necessary to coin a term for a chromosome number that is not a multiple of the base chromosome number, I will call this condition aneuploidy. Henceforth, aneuploidy refers to hyper and hypoploid chromosome numbers*[1].

Aneuploidy is a frequent outcome of meioses in progeny of interspecies crosses, but it rarely arises during the mitotic divisions that form the soma and during meioses of intraspecies crosses. This is because surveillance mechanisms that prevent chromosome mis-segregation, such as the spindle assembly checkpoint (SAC; also known as the mitotic checkpoint) (BOX 1), are in place[2–6]. Although these safeguard mechanisms are well characterized, the cellular consequences of their failure and what happens to cells that have become aneuploid because these safeguard mechanisms failed are only beginning to be understood.

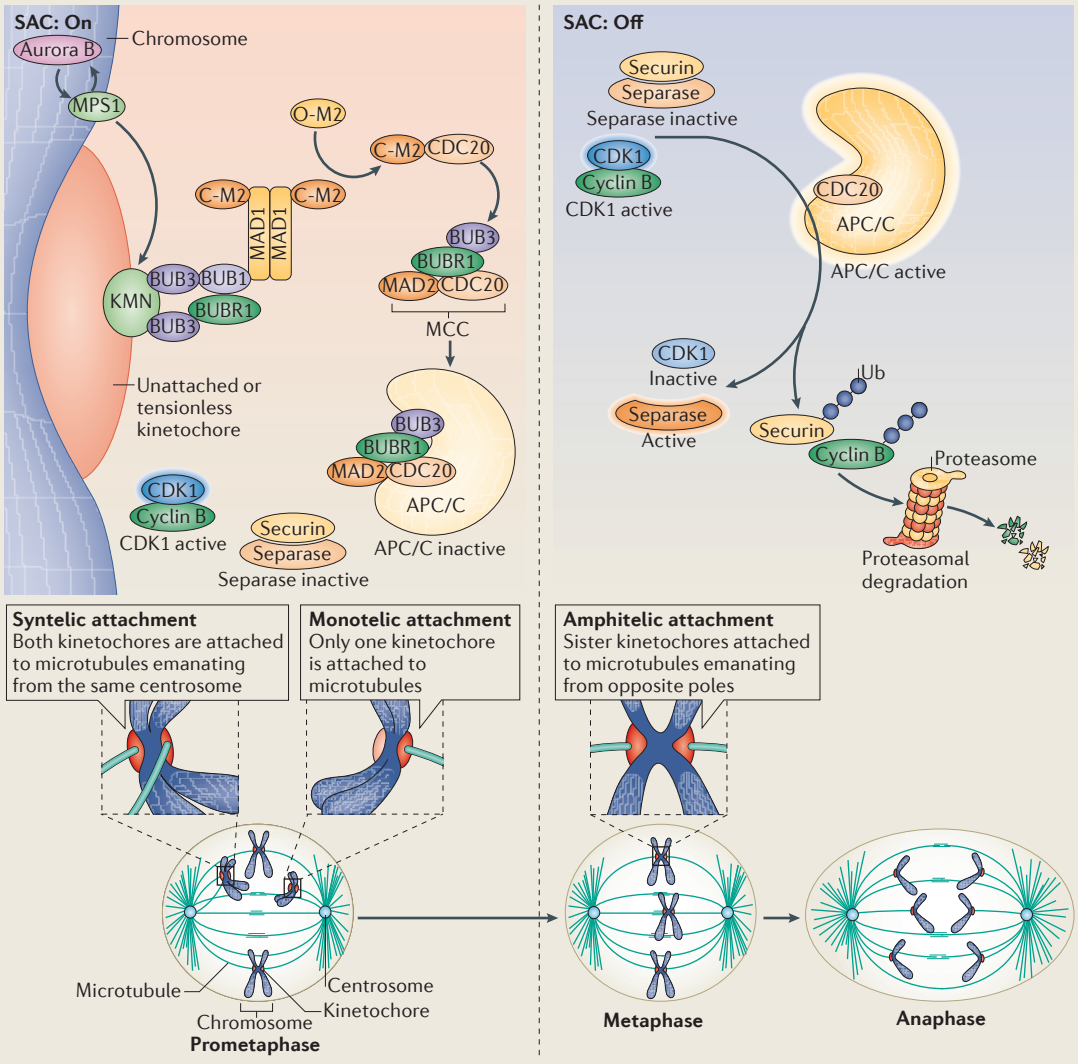
The reason we lack a detailed understanding of the consequences of chromosome mis-segregation is that studying faulty chromosome segregation and the resultant aneuploidies is difficult. Chromosome mis-segregation is a rare event and hence difficult to capture. The analysis of the products of chromosome mis-segregation, cells with aneuploid genomes, is equally tricky. Studying small changes in gene dosage — chromosome gains or losses result in a 50% change in gene expression[7–16] — is difficult. Dissecting the complex consequences of hundreds, if not thousands, of such small changes in gene expression occurring simultaneously is even more challenging. However, with the development of increasingly sophisticated live-cell imaging tools and quantitative genome-wide methods, we are beginning to make headway. We now appreciate that chromosome mis-segregation can have a dramatic effect on genome integrity, causing DNA damage and genomic rearrangements. We are also making progress towards understanding how an unbalanced karyotype affects cell and organismal physiology. It has now become clear that the phenotypes of aneuploid cells are composites of phenotypes caused by specific gene imbalances and general aneuploidy-associated traits caused by simultaneous changes in the gene dosage of many genes, which have little effects when varied individually. Advances in understanding the immediate and long-term effects of chromosome mis-segregation are

Box 1 | **Mechanisms that prevent chromosome mis-segregation**

The process of chromosome segregation is tightly controlled by the spindle assembly checkpoint (SAC), an evolutionarily conserved surveillance mechanism that prevents the mis-segregation of chromosomes[5,135] (see the figure). When the sister kinetochores attach to microtubules emanating from only one spindle pole (syntelic attachment) or when only one of the two sister kinetochores attaches to microtubules (monotelic attachment), the SAC is activated and inhibits anaphase onset. When all kinetochores have attached to microtubules emanating from opposite poles (amphitelic attachment or bi-orientation), the SAC is silenced and anaphase commences (see the figure). Syntelic and monotelic microtubule–kinetochore attachments recruit core components of the SAC — mitotic arrest deficient 1 (MAD1), MAD2, BUB3, BUB1-related 1 (BUBR1) and the checkpoint kinases aurora B, BUB1 and monopolar spindle protein 1 (MPS1). The recruitment of these proteins catalyses the inhibition of the APC/C (anaphase-promoting complex, also known as the cyclosome)–CDC20 complex (APC/C^{CDC20}), an E3 ubiquitin ligase that triggers the metaphase to anaphase transition (see the figure, left panels).

Inhibition of APC/C^{CDC20} is brought about by the incorporation of the APC/C activator CDC20 into the mitotic checkpoint complex (MCC), which is composed of the checkpoint proteins MAD2, BUBR1, BUB3 and CDC20 itself. When all kinetochores achieve amphitelic attachment, the SAC is turned off and APC/C^{CDC20} is activated. APC/C^{CDC20} then targets securin and cyclin B for degradation by the 26S proteasome. This leads to loss of sister chromatid cohesion and inactivation of cyclin-dependent kinase 1 (CDK1). These events trigger chromosome segregation and mitotic exit, respectively (see the figure, right panels).

A kinetochore that attaches to microtubules that emanate from both spindle poles is referred to as exhibiting a merotelic attachment, and this type of incorrect attachment is thought to be the major cause of aneuploidy in mammalian cells[136]. These types of kinetochore–microtubule attachments are not recognized by the SAC but instead are converted into amphitelic attachments through the action of aurora B and MPS1 kinases. The protein kinases convert merotelic attachments into amphitelic ones by destabilizing microtubule–kinetochore interactions through phosphorylation of outer kinetochore components.



C-M2, closed MAD2; KMN, KNL1–MIS12–NDC80 network; O-M2, open MAD2; Ub, ubiquitin.

Table 1 | **Frequency of aneuploidy**

Cell division	Method of detection	Incidence of aneuploidy or chromosome mis-segregation rate*	Refs
Mitotic division			
Saccharomyces cerevisiae	Plasmid and YAC loss	0.001–0.01%	19–22
Schizosaccharomyces pombe	Minichromosome loss	0.01%	137
Human tissue culture cells	FISH	~1%	23,24
Mouse keratinocytes	Single-cell sequencing	2.7%	26
Human keratinocytes	Single-cell sequencing	0%	26
Human and mouse brain	Single-cell sequencing	3–5%	26,34
Human and mouse liver	Single-cell sequencing	~5%	26
Human tissue culture cells displaying CIN	FISH	20–100%	23
Cancer	SKY	>85%	6,138
Meiotic division			
Saccharomyces cerevisiae	YAC mis-segregation	~4%	19
Schizosaccharomyces pombe	Minichromosome loss	~4%	139
Drosophila melanogaster	SKY	~0.1%	140–142
Mouse fertilized eggs	SKY	1–2%	143
Human sperm	SKY	1–4%	144,145
	FISH	1–3%	146
Human oocytes	SKY	10–35%	147,148
	FISH	20–70%	147,148
	CGH	30–75%	149,150
Zygotes (human)	FISH and SKY	5–25%	17
Spontaneous abortions (human)	SKY	35%	17,36,37
Stillbirths (human)	SKY	4%	17,36,37
Newborns (human)	SKY	0.3%	17,36,37

CGH, comparative genomic hybridization; CIN, chromosomal instability; FISH, fluorescence in situ hybridization; SKY, spectral karyotyping; YAC, yeast artificial chromosome. *Plasmid, minichromosome and YAC loss measure mis-segregation rates; SKY, FISH, CGH and single-cell sequencing measure incidence of aneuploidy.

urgently needed. Whole chromosome gains and losses dramatically affect human health. They are the leading cause of miscarriages and mental retardation in humans and a hallmark of cancer.

In this Review, we first discuss the detrimental effects of chromosome mis-segregation and aneuploidy on cell physiology. We describe recent findings that show that the process of chromosome mis-segregation has dramatic effects on genome integrity, causing DNA damage and activation of p53. We next summarize our current understanding of how an altered karyotype affects the cell's proteome and physiological state. We end with a discussion of links between chromosome mis-segregation, aneuploidy and cancer, reviewing recent evidence suggesting a causative role for chromosome mis-segregation and aneuploidy in tumorigenesis.

Aneuploidy is rare in normal tissues

When aneuploidy is present throughout the organism, it is known as constitutional aneuploidy. Such aneuploidies are caused by chromosome segregation errors during germ cell formation, usually during meiosis (reviewed in REF. 17). Somatic aneuploidy is the result of mitotic errors and describes a condition in which only some of cells in an organism harbour an abnormal karyotype. Most constitutional aneuploidies cause embryonic lethality, the most notable exception in humans being trisomy 21 (also known as Down syndrome). The consequences of having high levels of somatic aneuploidy are also severe. Patients with the rare human syndrome mosaic variegated aneuploidy (MVA), which is caused by mutations in (among others) *BUB1B*, a gene required for accurate chromosome segregation (reviewed in REF. 5) (BOX 1), exhibit growth retardation, microcephaly and childhood cancers[18].

Given the profound adverse effects of aneuploidy on human health, it is not surprising that cells with an unbalanced karyotype are rare. In budding and fission yeast, for example, the chromosome loss rate is estimated to be between 1×10^{-5} and 1×10^{-4} per generation[19–22] (TABLE 1). Primary and non-transformed tissue culture cell lines exhibit a chromosome mis-segregation rate of approximately 0.025%[23] per chromosome. Extending this result to all chromosomes suggests a chromosome loss or gain rate of around 1%[23,24] (TABLE 1). The degree of aneuploidy observed in tissues is in agreement with

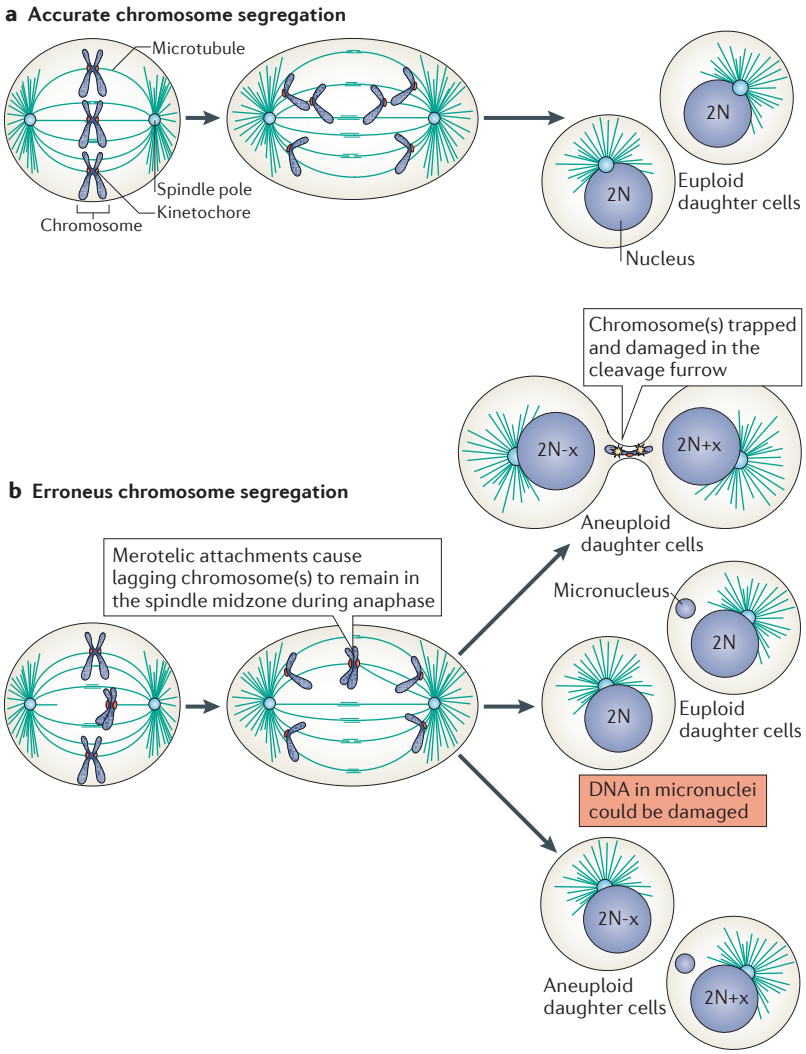


Figure 1 | **Lagging chromosomes experience DNA damage.** **a** | Accurate chromosome segregation leads to the equal partitioning of the genome and the generation of two euploid daughter cells, with a balanced, diploid karyotype (depicted as 2N in the figure). **b** | Merotelic attachment, defined as a kinetochore that attaches to microtubules emanating from both spindle poles, can cause chromosomes to lag behind in the spindle midzone during anaphase. Such lagging chromosomes can have multiple fates. They can be trapped in the cytokinetic furrow and break during cytokinesis (top). Alternatively, they can form their own micronucleus, which is either accurately segregated (middle) or mis-segregated (bottom). Irrespective of how micronuclei are segregated, their DNA is poorly replicated and experiences significant damage in the subsequent cell cycle. 2N+x and 2N-x indicate aneuploid karyotypes in which an undefined number of chromosomes has been gained (+x) or lost (-x).

these mis-segregation frequencies. Mouse lymphocytes and mouse and human keratinocytes exhibit aneuploidy frequencies of around 3%[25,26]. However, mammalian brain and liver were reported to exhibit significantly higher levels of aneuploidy. Fluorescence *in situ* hybridization (FISH) analyses suggested that as many as 50% of liver cells in humans are aneuploid[27–29], and spectral karyotyping (SKY)[30] or FISH[25,30–33] studies reported 20–33% of brain cells are aneuploid. However, subsequent single-cell sequencing analyses contradicted these previous results and revealed that the brain and liver have low levels of aneuploidy, similar to those seen in other tissues[26,34,35] (TABLE 1). The overestimation of aneuploidy by FISH and SKY is probably due to hybridization and chromosome-spreading artefacts, respectively. Furthermore, even a low frequency of artefacts for a single chromosome can lead to a gross overestimation of aneuploidy when extrapolated across all chromosomes.

Chromosome segregation defects are more frequent during meiosis, and their effects on reproductive success are significant (reviewed in REF. 17). In humans, approximately 35% of spontaneous abortions, 4% of stillbirths and as many as 25% of all zygotes are aneuploid[17,36,37] (TABLE 1).

Together, these observations indicate that constitutional and somatic aneuploidies are rare but, when they occur, their effect on health is dramatic. Below, we summarize our current understanding of how the immediate and long-term effects of chromosome mis-segregation cause decreased fitness, disease and even death.

Immediate effects of segregation errors

There are two consequences of chromosome mis-segregation: a faulty mitosis occurs and the resulting daughter cells are aneuploid. Recent studies indicate that both outcomes have a dramatic impact on cells.

Chromosome mis-segregation causes DNA damage. To understand the immediate consequences of chromosome mis-segregation, the frequency of chromosome mis-segregation events was increased by interfering with mitotic spindle function[38]. In such abnormal mitoses, mis-segregating chromosomes frequently lag behind during anaphase and can become trapped and damaged in the cleavage furrow during cytokinesis (FIG. 1). The broken chromosomes elicit a DNA-damage response. Their repair via non-homologous end-joining during the following G1 phase of the cell cycle can, but may not always, lead to translocations and deletions[38,39].

Lagging chromosomes sometimes also do not catch up with the other chromosomes in time to be incorporated into the reforming nucleus. Such chromosomes then form their own micronuclei[40–43] (FIG. 1). These micronuclei are not just miniature nuclei, as they are not fully functional[39]. DNA replication proceeds slowly in micronuclei and results in abnormal and stalled replication forks[39,43]. Given the importance of a functional nuclear envelope for efficient DNA replication[44], defects in nuclear import[39–41] or irreversible nuclear envelope collapse[45] could be responsible for this inability of micronuclei to properly replicate their DNA (FIG. 1). The consequences on DNA integrity are dramatic: levels of DNA damage are high in micronuclei, and repair of this damage leads to extensive DNA rearrangements[39,46], as elegantly demonstrated by combining live-cell-imaging and single-cell-sequencing techniques[46]. The complex chromosomal rearrangements that form in micronuclei are reminiscent of chromothripsis[47–49], which has been observed in approximately 3% of cancers and is prevalent in osteosarcomas (35%) and aggressive neuroblastomas (18%)[48,49]. Chromothripsis has also been observed in some human congenital diseases[50], in which, as in cancer, it might provide the fuel for rapid genome evolution.

Spectral karyotyping (SKY). A cytogenetic technique used to simultaneously visualize all chromosomes in a cell by using different fluorescently labelled probes for each chromosome.

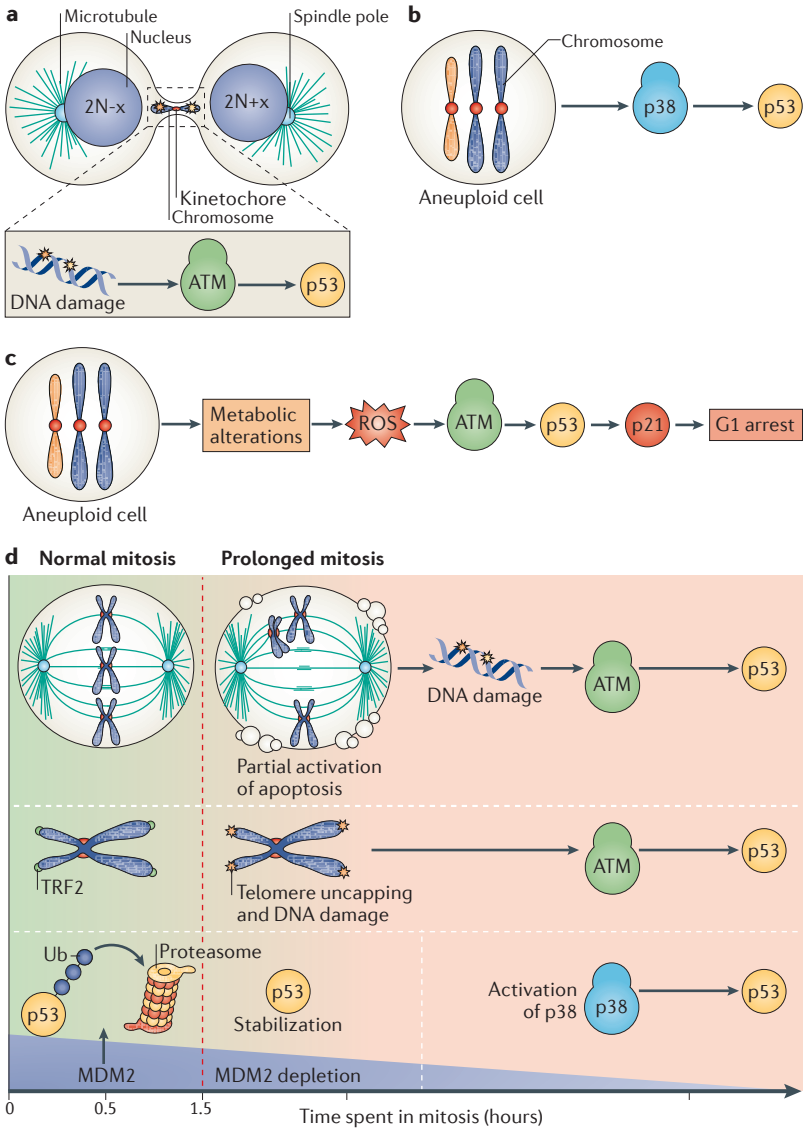


Figure 2 | **Multiple mechanisms could be responsible for p53 activation following chromosome mis-segregation.** **a** | Chromosomes trapped in the cytokinetic furrow are damaged and cause activation of the DNA damage checkpoint pathway and hence p53 activation[38]. 2N+x and 2N-x indicate aneuploid karyotypes in which an undefined number of chromosomes has been gained (+x) or lost (-x). **b** | Aneuploidy per se causes activation of p53 through p38 by an unknown mechanism[51]. **c** | Aneuploidy causes metabolic changes that lead to an increase in reactive oxygen species (ROS). ROS activate the DNA damage checkpoint kinase ataxia-telangiectasia mutated (ATM), which in turn activates p53 (REF. 54). **d** | Prolonged mitotic arrest causes p53 activation. When cells are arrested in prometaphase for more than 1.5 hours, cells activate p53 on release from the prometaphase block[55]. How p53 activation occurs is not understood but could result from partial activation of apoptosis, telomere uncapping (through the loss of telomere-capping protein telomeric repeat-binding factor 2 (TRF2)), p38 activation or double minute 2 (MDM2) downregulation that occur during prolonged mitotic arrest[57]. Ub, ubiquitin.

Chromothripsis
A process in which entire chromosomes become fragmented and then are repaired in a seemingly random manner, leading to dozens (sometimes even hundreds) of rearrangements within a single chromosome.

Chromosome mis-segregation causes p53 activation. Errors in chromosome segregation result in p53 (also known as TP53) activation. Increased levels of p53 and expression of p53-responsive genes were detected following chromosome mis-segregation that resulted in G1 arrest[51]. Consistent with a role of p53 in causing the arrest, G1 arrest was alleviated by p53 inactivation. Moreover, p53 limits the proliferation of cells experiencing high levels of chromosome mis-segregation in the embryo. Mutant mice lacking the gene encoding the SAC component mitotic arrest deficient 2 (MAD2) die at embryonic day 6.5 (REF. 52). Similarly, *Mad2*$^{-/-}$ mouse blastocysts die in culture within 5 days, but when p53 is deleted blastocysts remain viable for many weeks[53]. Thus, p53 plays a central part in the prevention of cell cycle progression following chromosome mis-segregation.

Which aspect of chromosome mis-segregation causes p53 activation remains a key unanswered question, for which consensus has not yet been reached. One study[38] suggested that DNA damage during cytokinesis causes p53 activation (FIG. 2a). However, in another study[51], DNA damage following chromosome mis-segregation was not detected, and it was proposed that aneuploidy per se activates p53 (FIG. 2b). Similarly, another group[54] observed p53 activation but not DNA damage in SAC-deficient mouse embryonic fibroblasts (MEFs). Instead, this group reported that reactive oxygen species (ROS) were elevated following chromosome mis-segregation. High levels of ROS caused activation of the DNA damage checkpoint kinase ataxia-telangiectasia mutated (ATM) and p53 (FIG. 2c). It is noteworthy that increased levels of ROS have also been observed in aneuploid budding yeast strains[11].

What could be the reason for these different results? DNA damage occurring during chromosome mis-segregation is likely to be transient and could have been missed in some studies. Differences in experimental procedures could also determine whether p53 is activated. Some approaches used to generate aneuploid cells involve arresting cells in prometaphase for prolonged periods of time. Arresting cells in prometaphase for more than 90 minutes causes a p53-dependent G1 arrest when cells are released from the cell cycle block, irrespective of whether chromosomes have been mis-segregated[55]. The mechanisms whereby prometaphase length causes p53 activation are not understood, but multiple events could contribute (FIG. 2d). Prolonged prometaphase arrest causes apoptosis and hence a DNA-damage response[56], telomere uncapping[57], p38 activation[55] and a decrease in double minute 2 (MDM2) levels, which targets p53 for degradation[58–60]. All these events could lead to p53 activation.

In summary, which aspects of chromosome mis-segregation — there could be multiple — activate p53 remains to be clearly defined. Interestingly, p53 activation has not been observed in cells with constitutional aneuploidies[61], which suggests that p53 activation is an immediate consequence of chromosome mis-segregation and is attenuated in cells with constitutional aneuploidies or is only elicited by specific aneuploid karyotypes.

Long-term effects of having an altered karyotype

Changes in chromosome composition cause numerous phenotypes and have long-term effects. The reason is that changes in the copy number of genes located on autosomes largely, although not universally, result in a corresponding change in gene expression. A systematic

analysis of budding yeast strains carrying single additional chromosomes showed that approximately 80% of genes that are present in an additional copy are expressed at an accordingly increased level[11]. The genes that do not show increased expression predominantly encode proteins that function in multi-protein complexes, such as the ribosome[11]. Similar observations were made in fission yeast, *Arabidopsis thaliana* and mammalian cells[10,12,13,15,16,62–66], but the principle that gene copy number determines the abundance of gene product may not be universal. Mechanisms of dosage compensation, such as those described for sex chromosomes, may exist for autosomes in *Drosophila melanogaster* and in some plants[67–71].

Which aspects of gaining and losing whole chromosomes cause the phenotypes observed in aneuploid cells and organisms? Although studies in budding yeast have shown that the presence of five additional centromeric plasmids interferes with microtubule–kinetochore attachment[72], gaining or losing DNA per se is generally not considered the major cause for phenotypic changes associated with aneuploidy. This was demonstrated by introducing large amounts of mammalian DNA into budding yeast cells. Few or no proteins are synthesized in budding yeast from this foreign DNA because, even if the mammalian genes were transcribed, the yeast splicing machinery cannot splice mammalian mRNAs. Introducing mammalian DNA in the form of yeast artificial chromosomes (YACs) as large as approximately 13% of the yeast genome (1.6 Mb) has little effect on the fitness of yeast strains[14]. This observation, together with the fact that autosomal dosage-compensation mechanisms are not in place, indicates that the phenotypes that are observed in aneuploid yeast cells are caused by changes in the expression of genes located on the aneuploid chromosomes.

The finding that in budding yeast all aneuploidy-associated phenotypes analysed to date are attenuated by increased ploidy[14,73] further indicates that it is relative levels of gene dosage that are mainly responsible for the phenotypes associated with an altered karyotype. Phenotypes caused by the gain of single chromosomes are drastically attenuated in diploid yeast cells compared to haploid yeast cells[14,73]. Thus, polyploidy represents an aneuploidy-tolerating condition. This is illustrated by the observation that tetraploid yeast strains exhibit a 200-fold increase in chromosome loss compared to diploid yeast strains, but proliferation is only mildly impaired[22]. In cancers too, an increase in genome-wide ploidy (many cancers are tetraploid) probably protects cancer cells from the adverse effects of aneuploidy, allowing them to take advantage of potential beneficial traits conferred by altered dosage of specific oncogenic drivers.

Below, we provide examples of how changes in the copy number of specific genes (gene-specific effects) interfere with development and cause diseases. We then describe how simultaneously changing the copy number of many genes that on their own have little effect on cellular functions causes a generic set of phenotypes known as aneuploidy-associated stresses.

Dosage compensation
Alteration of mRNA or protein expression to compensate for variation in DNA copy number.

Gene-specific effects of aneuploidy

Changes in gene copy number have been linked to many diseases (reviewed in REFS 74,75). For example, duplication of the *APP* gene (encoding amyloid-β precursor protein) has been implicated in early onset Alzheimer disease[76], and deletion of one copy of *PMP22* (the gene encoding peripheral myelin protein 22) is the cause of Charcot–Marie–Tooth 1A neuropathy[77]. Although examples of changes in gene copy number causing developmental abnormalities and diseases are numerous, dramatic effects of gaining or losing single genes on cellular fitness are less common. The best-known example is the β-tubulin gene in *Saccharomyces cerevisiae*: having an additional copy of this gene is lethal[78]. Only a handful of other genes have been shown to reduce fitness when present in an additional copy under standard growth conditions[79]. Similarly, only few genes were found to reduce fitness when present in one copy instead of two. In budding yeast, only 184 genes (3% of the yeast genome) are haploinsufficient for growth under optimal growth conditions[80]. This number is higher in fission yeast[81] (455 genes), most likely because fission yeast predominantly propagates as a haploid, whereas budding yeast propagates as diploid, which presumably causes haploinsufficiency to be under strong negative selection[81].

Understanding how changes in the gene dosage of individual genes affect development and organismal functions will be important for developing strategies to improve the lives of individuals with Down syndrome. Two recent studies suggest that some of the defects caused by an additional copy of chromosome 21 are reversible. It was found that the cognitive deficiencies in mouse models of Down syndrome can be ameliorated by a Hedgehog-agonist therapy[82]. Silencing one copy of chromosome 21 by targeting the X chromosome-inactivating long non-coding RNA X-inactive specific transcript (*Xist*) to one of the three copies of this chromosome greatly improved proliferation and neural rosette formation in pluripotent Down syndrome stem cells[83]. These findings open potential new avenues for the development of therapies for the treatment of Down syndrome.

Aneuploidy-associated stresses

A 50% change in expression of the majority of genes individually has little, if any, effect on cellular fitness. By contrast, the same change in dosage of many such genes simultaneously contributes to the decrease in fitness of cells with unbalanced karyotypes and is responsible for traits shared by cells with different aneuploidies[84] (FIG. 3). We refer to these general traits as aneuploidy-associated stresses. Thus far, they have been studied only in cellular models of aneuploidy, but they probably contribute to the numerous phenotypes observed in aneuploid organisms.

Transcriptional and post-transcriptional responses. Studies of aneuploid budding yeast, fission yeast and plants, as well as of aneuploid primary, untransformed mouse and human cells, have revealed a conserved gene expression response to the aneuploid state[14,85–87] (FIG. 3). Transcripts associated with cell growth, proliferation and nucleic-acid metabolism are downregulated, while

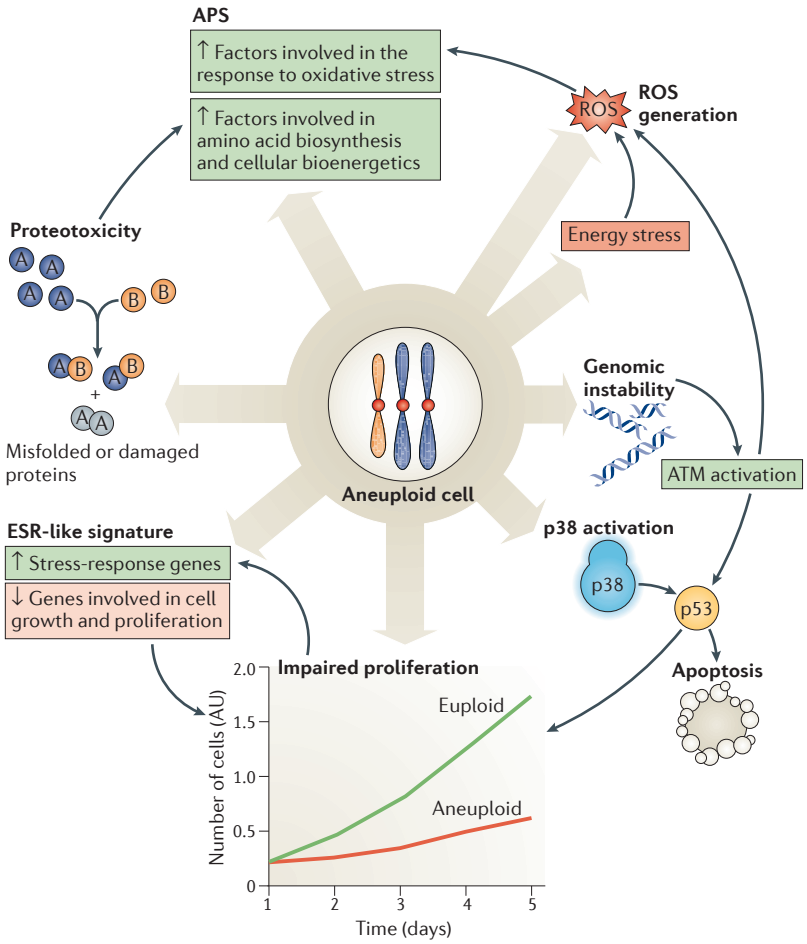


Figure 3 | **Aneuploidy-associated stresses.** The aneuploid state elicits several cellular responses. Proteotoxic and energy stress have been proposed to cause activation of the aneuploidy-associated protein signature (APS), which involves the upregulation of proteins required for oxidative-stress response and energy homeostasis. Aneuploidy also leads to slowed proliferation and an associated environmental-stress response (ESR)-like response, in which stress-response genes are upregulated and cell proliferation genes are downregulated. Additionally, aneuploid cells activate p53, leading to impaired proliferation or apoptosis. This could be the result of genomic instability and activation of the DNA damage kinase ataxia-telangiectasia mutated (ATM), activation of the stress kinase p38, prolonged cell cycle arrest, altered energy homeostasis or a combination thereof. AU, arbitrary unit; ROS, reactive oxygen species.

transcripts associated with stress and membrane functions are upregulated. These transcriptional alterations are reminiscent of the environmental-stress response (ESR), which was first described in budding yeast[88,89] (FIG. 3). The ESR is triggered by several stresses and/or slowed growth, and the gene expression pattern commonly observed in aneuploid cells is probably the result of cellular stress as well as a reflection of the sluggish proliferation of aneuploid cells[85,87].

In cancers, some transcripts have been found to be more abundant in cancers with a high degree of aneuploidy[90]. A gene expression signature derived from this study (known as CIN70) had been proposed to function as a marker of chromosomal instability (CIN) in cancer. However, subsequent research has demonstrated that CIN70 more accurately reflects the proliferation rate of tumours, rather than intrinsic CIN[91,92]. A comparison of high-CIN and low-CIN cancer cell lines identified a set of transcriptional changes distinct from CIN70 but similar to the ESR observed in primary aneuploid cells. Interestingly, an ESR was not observed when comparing the transcriptomes of highly aneuploid breast tumours with those of near-diploid breast tumours. These findings led to the proposal that two types of aneuploidy exist in cancers: 1) continuously changing karyotypes that, like aneuploidies in primary cells, have a negative impact on cellular fitness; and 2) selected, stable aneuploid karyotypes that have evolved to support maximal proliferation and in which the stresses caused by aneuploid karyotypes are suppressed. It is important to note that the gene expression changes caused by CIN in cancer and by aneuploidy in primary cells are related but not identical. For example, genes annotated to the mitotic cell cycle are very strongly downregulated in trisomic fibroblasts, whereas in high-CIN cancer cell lines, cell cycle genes are moderately downregulated but RNA metabolism genes are strongly suppressed. The cause of these differences is unknown at present.

Recently, an additional gene expression signature shared among aneuploid budding yeast strains was identified[11]. This signature, named the aneuploidy-associated protein signature (APS), is characterized by the upregulation of proteins but not transcripts of genes involved in the oxidative-stress response. The strength of the APS correlates with the degree of aneuploidy, suggesting that a higher degree of karyotype imbalance causes an increase in ROS. Which aspect of the aneuploid condition is responsible for elevated ROS remains to be determined. However, hyper-activation of the proteasome, through inactivation of the proteasome-associated deubiquitylating enzyme ubiquitin carboxyl-terminal hydrolase 6 (UBP6) suppresses the APS[11], raising the interesting possibility that proteotoxicity, a hallmark of the aneuploid state (discussed below), contributes to the APS (FIG. 3).

Aneuploidy causes proteotoxic stress. In healthy cells, a complex regulatory network maintains cellular protein homeostasis (proteostasis) by ensuring that proteins are present only in their fully active form and at appropriate levels[93,94]. Chaperone-mediated folding pathways facilitate the folding of proteins; protein degradation pathways — autophagy and the ubiquitin proteasome system (BOX 2) — ensure that misfolded proteins are eliminated (reviewed in REFS 95–99). When these systems become limiting or are impaired, unfolded and misfolded proteins accumulate, resulting in proteotoxic stress (FIG. 4). This stress is met by a multi-pronged response aimed at increasing the protein quality control capacity of the cell.

Aneuploidy affects all protein quality control pathways in the cell. Analysis of budding yeast strains carrying single additional chromosomes (disomic strains) showed that at least one chaperone, Hsp90 (reviewed in REF. 100), is limiting in several different disomic yeast strains[73]. Aneuploid immortalized and tumorigenic human cells[101] are also defective in HSP90-mediated protein folding. Reduced protein folding capacity was suggested to be caused by a reduced ability to activate a heat

Chromosomal instability (CIN). A condition in which the rate of chromosome mis-segregation is elevated.

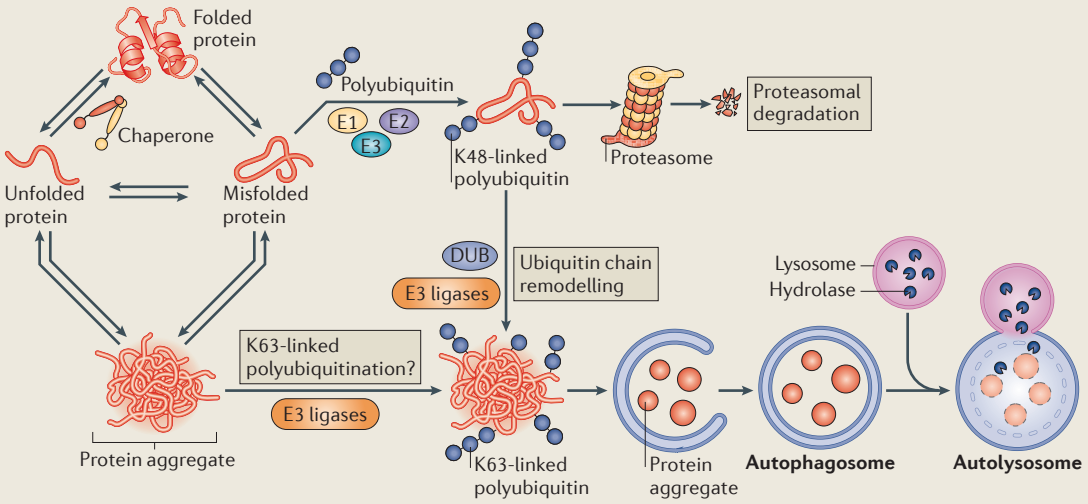
Proteotoxic stress
A cellular stress elicited by unfolded and/or misfolded proteins.

Box 2 | **Cellular protein quality control**

Proteins must adopt a defined three-dimensional structure to be functional. A complex network of chaperone systems ensures that polypeptides reach their functional conformation. However, even after adopting the folded conformation, proteins are at risk of unfolding because the energy barrier between folded and unfolded or misfolded conformations is not insurmountable. Stress conditions or intrinsic instability can further contribute to protein misfolding.

Misfolded proteins either re-engage chaperones to reattempt correct folding or are degraded. Degradation is mediated by ubiquitin-mediated proteasomal degradation. The concerted functions of E1, E2 and E3 enzymes leads to ubiquitylation of the misfolded protein, thereby targeting the misfolded protein for degradation by the proteasome[98,99] (see the figure, top).

When folding load exceeds chaperone capacity and/or when the ubiquitin–proteasome system is compromised, misfolded or unfolded proteins can form aggregates. Such aggregates are cleared by autophagy. Deubiquitylating enzymes (DUBs) and E3 ubiquitin ligases remodel ubiquitin chains (which can involve either the Lys48 or the Lys63 residue of ubiquitin), which are then able to bind ubiquitin receptors, such as sequestosome 1 (also known as p62) and NBR1 (REF. 98). Autophagosomal membranes then form around the ubiquitylated aggregates[98]. When encapsulated into autophagosomes, protein aggregates are delivered to lysosomes, where they are degraded (see the figure, bottom).



shock factor 1 (HSF1)-induced heat shock response[101]. Conversely, it was found that basal levels of expression of the HSF1 target HSP72 (also known as HSPA2) were increased in aneuploid MEFs[61]. Primary mouse cells may respond differently to folding stress than immortalized and cancerous human cell lines. Despite these differences, all types of mammalian cell lines carrying one or two additional chromosomes analysed to date are more sensitive than euploid cells to the HSP90 inhibitor 17-*N*-allylamino-17-demethoxygeldanamycin (17-AAG)[61,101], indicating that HSP90 is limiting in several aneuploid cells.

Aberrant karyotypes also affect protein degradation pathways, including ubiquitin-mediated proteasomal degradation of short-lived proteins and autophagy-mediated removal of protein aggregates. Some disomic budding yeast strains exhibit sensitivity to the proteasome inhibitor MG132 (REF. 14). Furthermore, hyperactivation of proteasomal degradation by deletion of the proteasome-associated deubiquitylating enzyme *UBP6* attenuates the aneuploidy-induced changes in cellular protein composition and improves cell fitness[11,63]. Unlike some disomic budding yeast strains, mammalian aneuploid cells do not exhibit increases sensitivity to proteasome inhibitors[61]. Recent studies indicate that proteasome-mediated degradation is increased in aneuploid mammalian cells[101], suggesting that proteasome activity is upregulated in cells with abnormal karyotypes[101]. Conversely, autophagy seems to be insufficient in aneuploid mammalian cells. Trisomic MEFs and aneuploid human cells exhibit increased sensitivity to the lysosome inhibitor chloroquine[10,61]. Furthermore, the gene expression signature of aneuploid human cells is similar to that of cells in which lysosomal degradation is inhibited[87]. Our research indicates that autophagosomes accumulate within lysosomes without evidence of lysosome malfunction (S.S. and A.A., unpublished observations). It thus seems that autophagy is a major route of clearance of misfolded proteins in aneuploid mammalian cells.

Why is proteotoxicity a universal feature of aneuploid cells? The comparison of haploid yeast strains carrying an additional chromosome (disomic strains) with diploid yeast strains carrying an extra copy of the same chromosome (trisomic strains) provided insight into this question. All phenotypes indicative of proteotoxic stress are greatly reduced in trisomes compared to in disomes, suggesting that changes in the relative ratio of proteins are a major source of proteotoxicity in aneuploid cells. In haploid cells, an extra copy of a gene leads to a doubling of gene expression. In diploid cells, the relative increase or decrease in expression is only 50%. Although this difference may be of little consequence for proteins

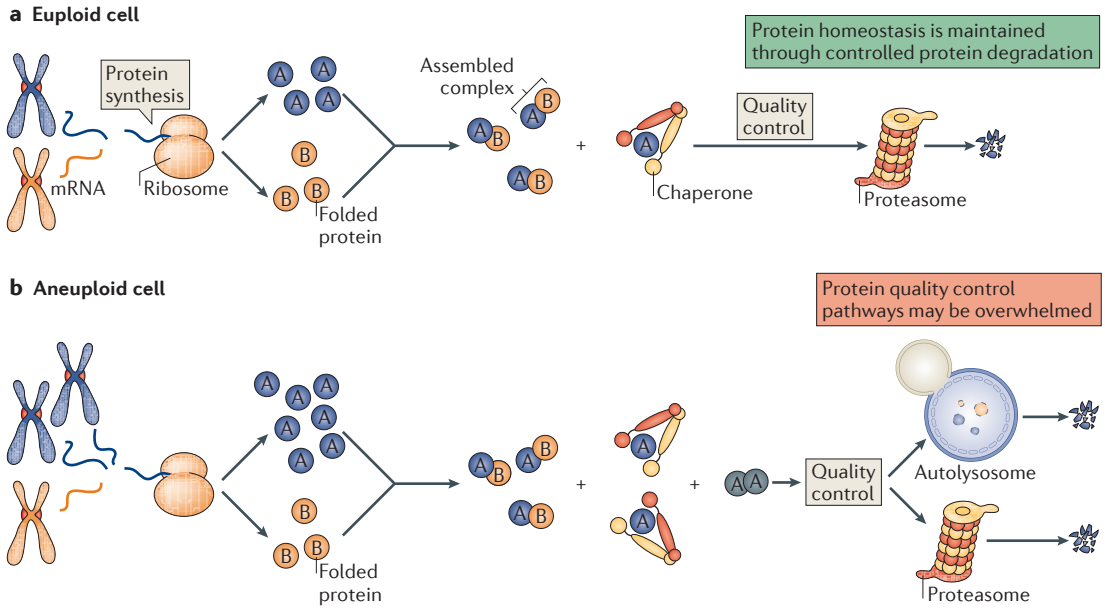


Figure 4 | **Protein quality control is limiting in aneuploid cells. a** | In euploid cells, protein quality control and feedback mechanisms ensure that equal amounts of protein complex subunits are produced. Chaperones promote protein folding and maintain complex subunits that lack a binding partner in a soluble state. Eventually, excess and misfolded subunits are degraded by the proteasome. **b** | In aneuploid cells, protein stoichiometries of protein complex subunits are altered. Every subunit encoded by an unbalanced chromosome that functions in a protein complex lacks its binding partner (or partners) and must rely on cellular chaperones to remain soluble and, if no binding partner is found, on cellular proteases for its eventual degradation. This can lead to an increased burden on the cell's protein quality control systems.

that fold spontaneously, it has profound consequences for proteins that require chaperones to reach their native conformation. Many protein complex subunits are unstable unless bound to their partners and will often bind to chaperones to remain soluble until they associate with their binding partners[102]. Thus, in aneuploid cells, every single subunit produced from the additional gene copy will require the continuous engagement of chaperones to remain in a soluble state and will eventually need to be degraded when a binding partner cannot be found. This is illustrated by the analysis of the proteome of aneuploid cells. In disomic budding yeast strains, approximately 20% of proteins do not exhibit increased expression when gene dosage is doubled, even though transcript levels are upregulated according to gene copy number[13,63]. The vast majority of proteins whose expression does not scale with gene number are subunits of multi-protein complexes[10,11,14,63]. The ribosome is especially worth mentioning in this context. Increasing the copy number of ribosomal genes does not lead to a corresponding increase in protein levels[11]. Given that ribosomal proteins constitute approximately 20% of the total protein content in yeast eliminating ribosomal subunits produced from excess gene copies alone could place a burden on the cell's protein quality control pathways.

At first glance, it may seem surprising that changing the expression of genes by 50% challenges the cell's protein quality control pathways. Granted, gaining or losing whole chromosomes causes changes in the expression of hundreds (sometimes thousands) of genes, but why does the cell not simply continuously increase protein quality control activity, as occurs during heat shock? The inability of cells to adapt to the aneuploid state could be explained by a recently discovered feature of gene expression control: the expression of subunits that assemble into a complex is coordinated[103]. This indicates that, rather than maintaining a large protein quality control reservoir to keep a large pool of unassembled protein complex subunits in a soluble state, cells have evolved to minimize the need for protein quality control pathways to assemble complexes. When subunits of complexes are continuously produced in the incorrect stoichiometries, as occurs in aneuploid cells, the protein quality control pathways of the cells are challenged, and proteotoxic stress ensues.

Aneuploidy inhibits cell proliferation. Decreased proliferation is another characteristic of aneuploid cells[10,13,14,16,51,54,104–107]. Aneuploid fission yeast strains derived as progeny from triploid meioses delay in G1 (REF. 104). Haploid budding yeast strains disomic for one or two chromosomes or harbouring complex aneuploidies proliferate slowly, and many such strains also show a G1 delay[14,106].

Chromosome mis-segregation and aneuploidy also interfere with proliferation in mammalian cells. MEFs harbouring hypomorphic mutations in the SAC gene BUB1-related 1 (*BUBR1*)[107], mutations in the SAC target CDC20 that render it insensitive to checkpoint regulation[54], or mutations that interfere with the chromosome segregation process (through depletion of the kinesin MCAK or on monastrol washout) exhibit proliferation defects. Some mutations that increase chromosome mis-segregation (for example, cells heterozygous for a

deletion in centromere-associated protein E (CENPE)[108], mRNA export factor RAE1 (REF. 109) or BUB3 (REF. 109)) have not been reported to decrease cell proliferation. This could be due to the fact that only a small fraction of cells in the population mis-segregate chromosomes, causing the ensuing proliferation defect to be subtle and thus missed. Proliferation defects are also observed in cells harbouring constitutive aneuploidies. Trisomy 21 human fibroblasts and MEFs trisomic for chromosome 1, 13, 16 or 19 divide more slowly than normal cells, although a specific cell cycle delay could not be identified[16]. However, other trisomic human cells show a G1 delay[10], indicating that, as in yeast, G1 delay is a common occurrence in aneuploid mammalian cells.

An important question regarding the proliferation defects of aneuploid cells is whether they are the consequence of copy number changes of a few especially harmful genes or of many genes that cause no growth defect when varied individually. As with most, if not all, aneuploidy-associated phenotypes, the answer is likely to be that both contribute. For example, having a single additional copy of chromosome 6 causes lethality in haploid budding yeast cells because having a single additional copy of the β-tubulin-encoding gene *TUB2* is lethal[78]. However, such cases are rare, at least in budding yeast. A genome-wide study in budding yeast determined the upper copy number limit of every gene in the budding yeast genome and identified 55 genes that are not tolerated at more than 5 copies per haploid genome[110]. However, changes in the copy number of these most dosage-sensitive genes are insufficient to drive the proliferation defects of aneuploid cells[84]. Introducing an additional copy of these dosage-sensitive genes into yeast strains did not recapitulate the growth defects of yeast strains carrying an additional copy of the chromosome the genes are located on. This finding indicates that the proliferation defects of aneuploid budding yeast cells are largely caused by simultaneous gene copy number changes that independently are benign, at least under standard growth conditions.

So, if it is not only individual genes that at altered dosage impair proliferation in aneuploid cells, which other aspects of the aneuploid condition also affect proliferation? Aneuploidy-induced proteotoxicity seems to contribute to the proliferation defect of aneuploid cells. In budding yeast, increasing protein quality control improves not only protein homeostasis but also cellular fitness. Deleting *UBP6* improves proliferation in 11 out of 13 disomic yeast strains under conditions of heat stress (growth at 37 °C)[11]. In mammalian cells, increased chaperone expression also improves fitness. Overexpression of the heat shock transcription factor HSF1 rescues not only the folding defect of human aneuploid cells but also their proliferation defect[101]. Although other aspects of HSF1 biology that are beyond its role in protein folding might contribute to improved proliferation of aneuploid cells on HSF1 overexpression, this remarkable finding points to a link between aneuploidy-induced proteotoxicity and proliferation defects[101]. Whether other aneuploidy-associated stresses contribute to the reduced proliferative abilities of aneuploid cells remains to be determined.

Aneuploidy in cancer

90% of solid tumours and 50% of blood cancers are aneuploid[111,112]. Whether and how aneuploidy promotes tumorigenesis has been an active area of research and discussion. The realization that mutations in genes regulating chromosome segregation are rare in cancers[113–115], together with the observation that aneuploidy inhibits proliferation, suggests that aneuploidy is a by-product of tumorigenesis that interferes with the process rather than causes it. Indeed, loss of tumour suppressors has been shown to cause CIN. RB inactivation not only deregulates the G1–S phase transition but also compromises centromere function, which leads to CIN and hence aneuploidy[116,117]. Loss-of-function mutations in adenomatous polyposis coli (APC) cause deregulation of the WNT pathway and decrease chromosome segregation fidelity[118].

Although aneuploidy can be a by-product of oncogenic transformation, there is mounting evidence that aneuploidy can promote tumorigenesis. Analysis of cancer genomes indicates that loss of tumour-suppressor genes and gain of oncogenes drives karyotype changes, such as whole or partial chromosome gains and losses, creating the clonal aneuploid karyotypes characteristic for a specific cancer[119]. However, the analysis of specific aneuploid karyotypes and mouse models of CIN revealed that aneuploidy can both promote and inhibit tumorigenesis. Mice trisomic for part of chromosome 16 are resistant to APC multiple intestinal neoplasia (Apc^{Min}) mutation-induced colon cancer[120]. Individuals trisomic for chromosome 21 are less likely to develop solid tumours than the euploid population[121]. This tumour-protective function of trisomy 21 has been attributed to the triplication of the Down syndrome critical region 1 (*DSCR1*) gene[122]. Conversely, trisomy 8 seems to promote haematopoietic malignancies. 25% of chronic myeloid leukaemias (CMLs), 10–15% of acute myeloid leukaemias (AMLs) and 5% of acute lymphoblastic leukaemias (ALLs) harbour an additional copy of chromosome 8 (REF. 123). MYC, a key driver of haematopoietic malignancies, is located on chromosome 8 and could be the reason for the prevalence of additional copies of chromosome 8 in blood cancers[124].

Studies of mouse models of CIN (Supplementary information S1 (table)), the condition that spawns aneuploid karyotypes, also show that, akin to other forms of genomic instability, such as reduction of telomerase activity[125,126], CIN can promote and inhibit tumorigenesis. A prime example for this dual role of CIN in tumorigenesis is the motor protein CENPE[108]. Animals heterozygous for a *CENPE* deletion harbour increased levels of aneuploidy[108] and are significantly less likely to develop spontaneous liver tumours and 7,12-dimethylbenz(a)anthracene (DMBA)-induced tumours[108] (see also Supplementary information S1 (table)). However, the same animals exhibit an increase in the incidence of spleen lymphomas and lung adenomas[108]. Many other mouse models of CIN have been described to promote or inhibit tumorigenesis in a manner that depends on the cell type and genetic background

in which the abnormal karyotype arises (summarized in Supplementary information S1 (table)).

Recent studies revealed a perhaps general principle whereby aneuploidy can promote tumorigenesis[127]. In a *KRAS*-driven model of lung cancer, continuous *KRAS* expression is required for tumour maintenance. On repression of *KRAS*, tumours regress. When chromosome mis-segregation is induced in KRAS-driven tumours through the overexpression of the SAC factor MAD2, the disease relapses quickly. This finding suggests that the karyotype heterogeneity created by increased CIN facilitates the emergence of *KRAS*-independent tumours[127].

Although increased karyotypic instability can facilitate the evolution of advantageous karyotypes, it of course is much more likely to generate disadvantageous ones. A recent study[128] illustrates this double-edged nature of CIN. Mice lacking one copy of *CENPE* exhibit an increase incidence of spleen and lung tumours. Increasing chromosome mis-segregation in these mice by interfering with SAC function decreased tumour formation by increasing cell death[128]. These findings indicate that low rates of chromosome mis-segregation can promote tumorigenesis by increasing the likelihood of generating a tumour-promoting karyotype. However, when chromosome mis-segregation rates become too high, tumour cells cannot 'hold on' to such tumorigenesis-promoting karyotypes. Instead, cells with inviable karyotypes are continuously generated, leading to cell death and hence tumour suppression.

Conclusions and future directions

Research over the past 5 years has provided significant insights into the immediate and long-term consequences of chromosome mis-segregation and has provided concrete hypotheses for how CIN and aneuploidy could promote tumorigenesis. Chromosome mis-segregation can lead to structural alterations of chromosomes. These alterations and the genomic instability that arises from the aneuploid state per se[129,130] are probably drivers of tumour evolution. We now also understand that aneuploid karyotypes negatively affect cellular fitness but rare favourable variants can provide a survival advantage, as has been seen in microbial evolution studies[131–133].

Understanding how certain karyotypes promote specific aspects of tumorigenesis will be an important next step in understanding the role of aneuploidy in tumorigenesis. Whether the gene-specific phenotypes and general stresses caused by the aneuploid state can be exploited in cancer therapy should also be determined. Synthetic negative interactions between proteotoxic and energy-stress inducing compounds and aneuploidy have been described[61]. Strategies that first select for a specific karyotype to then eliminate it have been reported recently in inhibiting the growth of aneuploid fungal pathogens and several central nervous system cancer cell lines[134]. Aneuploidy is a hallmark of cancer yet is rare in normal tissues. Compounds that target the aneuploid state therefore have ideal therapeutic properties: broad-spectrum efficacy and high specificity. A large-scale effort is now required to identify such compounds.

1. Täckholm, G. Zytologische Studien über die Gattung Rosa. *Acta Hort. Berg.* **7**, 97–381 (in German) (1922).
2. Holland, A. J. & Cleveland, D. W. Boveri revisited: chromosomal instability, aneuploidy and tumorigenesis. *Nat. Rev. Mol. Cell Biol.* **10**, 478–487 (2009).
3. Holland, A. J. & Cleveland, D. W. Losing balance: the origin and impact of aneuploidy in cancer. *EMBO Rep.* **13**, 501–514 (2012).
4. Pfau, S. J. & Amon, A. Chromosomal instability and aneuploidy in cancer: from yeast to man. *EMBO Rep.* **13**, 515–527 (2012).
5. Musacchio, A. & Salmon, E. D. The spindle-assembly checkpoint in space and time. *Nat. Rev. Mol. Cell Biol.* **8**, 379–393 (2007).
6. Gordon, D. J., Resio, B. & Pellman, D. Causes and consequences of aneuploidy in cancer. *Nat. Rev. Genet.* **13**, 189–203 (2012).
7. Upender, M. B. *et al.* Chromosome transfer induced aneuploidy results in complex dysregulation of the cellular transcriptome in immortalized and cancer cells. *Cancer Res.* **64**, 6941–6949 (2004).
8. Lyle, R., Gehrig, C., Neergaard-Henrichsen, C., Deutsch, S. & Antonarakis, S. E. Gene expression from the aneuploid chromosome in a trisomy mouse model of Down syndrome. *Genome Res.* **14**, 1268–1274 (2004).
9. Huettel, B., Kreil, D. P., Matzke, M. & Matzke, A. J. M. Effects of aneuploidy on genome structure, expression, and interphase organization in *Arabidopsis thaliana*. *PLoS Genet.* **4**, e1000226 (2008).
10. Stingele, S. *et al.* Global analysis of genome, transcriptome and proteome reveals the response to aneuploidy in human cells. *Mol. Syst. Biol.* **8**, 1–12 (2012).
11. Dephoure, N. *et al.* Quantitative proteomic analysis reveals posttranslational responses to aneuploidy in yeast. *eLife* **3**, e03023 (2014).
This paper reports that changes in gene copy number lead to corresponding changes in protein expression in 80% of budding yeast genes. It also reports the identification of a new gene expression signature that is characterized by the upregulation of proteins involved in the oxidative-stress response.
12. Kahlem, P. *et al.* Transcript level alterations reflect gene dosage effects across multiple tissues in a mouse model of Down syndrome. *Genome Res.* **14**, 1258–1267 (2004).
13. Pavelka, N. *et al.* Aneuploidy confers quantitative proteome changes and phenotypic variation in budding yeast. *Nature* **468**, 321–325 (2010).
14. Torres, E. M. *et al.* Effects of aneuploidy on cellular physiology and cell division in haploid yeast. *Science* **317**, 916–924 (2007).
15. Vacík, T. *et al.* Segmental trisomy of chromosome 17: a mouse model of human aneuploidy syndromes. *Proc. Natl Acad. Sci. USA* **102**, 4500–4505 (2005).
16. Williams, B. R. *et al.* Aneuploidy affects proliferation and spontaneous immortalization in mammalian cells. *Science* **322**, 703–709 (2008).
17. Hassold, T. & Hunt, P. To err (meiotically) is human: the genesis of human aneuploidy. *Nat. Rev. Genet.* **2**, 280–291 (2001).
18. Hanks, S. *et al.* Constitutional aneuploidy and cancer predisposition caused by biallelic mutations in *BUB1B*. *Nat. Genet.* **36**, 1159–1161 (2004).
19. Sears, D. D., Hegemann, J. H. & Hieter, P. Meiotic recombination and segregation of human-derived artificial chromosomes in *Saccharomyces cerevisiae*. *Proc. Natl Acad. Sci. USA* **89**, 5296–5300 (1992).
20. Hartwell, L. H. & Smith, D. Altered fidelity of mitotic chromosome transmission in cell cycle mutants of *S. cerevisiae*. *Genetics* **110**, 381–395 (1985).
21. Brown, M. *et al.* Fidelity of mitotic chromosome transmission. *Cold Spring Harb. Symp. Quant. Biol.* **56**, 359–365 (1991).
22. Storchova, Z. *et al.* Genome-wide genetic analysis of polyploidy in yeast. *Nature* **443**, 541–547 (2006).
23. Thompson, S. L. & Compton, D. A. Examining the link between chromosomal instability and aneuploidy in human cells. *J. Cell Biol.* **180**, 665–672 (2008).
24. Cimini, D., Tanzarella, C. & Degrassi, F. Differences in malsegregation rates obtained by scoring ana-telophases or binucleate cells. *Mutagenesis* **14**, 563–568 (1999).
25. Rehen, S. K. *et al.* Constitutional aneuploidy in the normal human brain. *J. Neurosci.* **25**, 2176–2180 (2005).
26. Knouse, K. A., Wu, J., Whittaker, C. A. & Amon, A. Single cell sequencing reveals low levels of aneuploidy across mammalian tissues. *Proc. Natl Acad. Sci.* **111**, 13409–13414 (2014).
This analysis shows that aneuploid karyotypes are rare in normal tissues.
27. Duncan, A. W. *et al.* Aneuploidy as a mechanism for stress-induced liver adaptation. *J. Clin. Invest.* **122**, 3307–3315 (2012).
28. Duncan, A. W. *et al.* Frequent aneuploidy among normal human hepatocytes. *Gastroenterology* **142**, 25–28 (2012).
29. Duncan, A. W. *et al.* The ploidy conveyor of mature hepatocytes as a source of genetic variation. *Nature* **467**, 707–710 (2010).
30. Rehen, S. K. *et al.* Chromosomal variation in neurons of the developing and adult mammalian nervous system. *Proc. Natl Acad. Sci. USA* **98**, 13361–13366 (2001).
31. Pack, S. D. *et al.* Individual adult human neurons display aneuploidy: detection by fluorescence *in situ* hybridization and single neuron PCR. *Cell Cycle* **4**, 1758–1760 (2005).
32. Yurov, Y. B. *et al.* Aneuploidy and confined chromosomal mosaicism in the developing human brain. *PLoS ONE* **2**, e558 (2007).
33. Faggioli, F., Wang, T., Vijg, J. & Montagna, C. Chromosome-specific accumulation of aneuploidy in the aging mouse brain. *Hum. Mol. Genet.* **21**, 5246–5253 (2012).
34. McConnell, M. J. *et al.* Mosaic copy number variation in human neurons. *Science* **342**, 632–637 (2013).
35. Cai, X. *et al.* Single-cell, genome-wide sequencing identifies clonal somatic copy-number variation in the human brain. *Cell Rep.* **8**, 1280–1289 (2014).
36. Hassold, T. *et al.* Human aneuploidy: incidence, origin, and etiology. *Environ. Mol. Mutagen.* **28**, 167–175 (1996).
37. Hartl, D. L. & Jones, E. W. *Genetics: Analysis of Genes and Genomes* (Jones & Bartlett Learning, 2005).

38. Janssen, A., van der Burg, M., Szuhai, K., Kops, G. J. P. L. & Medema, R. H. Chromosome segregation errors as a cause of DNA damage and structural chromosome aberrations. *Science* **333**, 1895–1898 (2011).
This paper shows that mis-segregating chromosomes can be damaged during cytokinesis and can be the source of structural chromosome aberrations in aneuploid daughter cells.
39. Crasta, K. *et al.* DNA breaks and chromosome pulverization from errors in mitosis. *Nature* **482**, 53–58 (2012).
40. Hoffelder, D. *et al.* Resolution of anaphase bridges in cancer cells. *Chromosoma* **112**, 389–397 (2004).
41. Terradas, M., Martín, M., Tusell, L. & Genescà, A. DNA lesions sequestered in micronuclei induce a local defective-damage response. *DNA Repair* **8**, 1225–1234 (2009).
42. Terradas, M., Martín, M., Hernández, L., Tusell, L. & Genescà, A. Nuclear envelope defects impede a proper response to micronuclear DNA lesions. *Mutat. Res.* **729**, 35–40 (2012).
43. Xu, B. *et al.* Replication stress induces micronuclei comprising of aggregated DNA double-strand breaks. *PLoS ONE* **6**, e18618 (2011).
44. Newport, J. Nuclear reconstitution *in vitro*: stages of assembly around protein-free DNA. *Cell* **48**, 205–217 (1987).
45. Hatch, E. M. *et al.* Catastrophic nuclear envelope collapse in cancer cell micronuclei. *Cell* **154**, 47–60 (2013).
46. Zhang, C.-Z. *et al.* Chromothripsis from DNA damage in micronuclei. *Nature* **522**, 179–184 (2015).
Using a combination of live-cell imaging and single-cell genome sequencing, this study provides the first evidence for a causative role of micronucleation in chromothripsis.
47. Liu, P. *et al.* Chromosome catastrophes involve replication mechanisms generating complex genomic rearrangements. *Cell* **146**, 889–903 (2011).
48. Stephens, P. J. *et al.* Massive genomic rearrangement acquired in a single catastrophic event during cancer development. *Cell* **144**, 27–40 (2011).
49. Forment, J. V., Kaidi, A. & Jackson, S. P. Chromothripsis and cancer: causes and consequences of chromosome shattering. *Nat. Rev. Cancer* **12**, 663–670 (2012).
50. Zhang, C. Z., Leibowitz, M. L. & Pellman, D. Chromothripsis and beyond: rapid genome evolution from complex chromosomal rearrangements. *Genes Dev.* **27**, 2513–2530 (2013).
51. Thompson, S. L. & Compton, D. A. Proliferation of aneuploid human cells is limited by a p53-dependent mechanism. *J. Cell Biol.* **188**, 369–381 (2010).
This study shows that chromosome mis-segregation leads to p53 activation, which limits the proliferation of aneuploid cells.
52. Dobles, M., Liberal, V., Scott, M. L., Benezra, R. & Sorger, P. K. Chromosome missegregation and apoptosis in mice lacking the mitotic checkpoint protein Mad2. *Cell* **101**, 635–645 (2000).
53. Burds, A. A., Lutum, A. S. & Sorger, P. K. Generating chromosome instability through the simultaneous deletion of *Mad2* and *p53*. *Proc. Natl Acad. Sci. USA* **102**, 11296–11301 (2005).
54. Li, M. *et al.* The ATM–p53 pathway suppresses aneuploidy-induced tumorigenesis. *Proc. Natl Acad. Sci.* **107**, 14188–14193 (2010).
55. Uetake, Y. & Sluder, G. Prolonged prometaphase blocks daughter cell proliferation despite normal completion of mitosis. *Curr. Biol.* **20**, 1666–1671 (2010).
56. Orth, J. D., Loewer, A., Lahav, G. & Mitchison, T. J. Prolonged mitotic arrest triggers partial activation of apoptosis, resulting in DNA damage and p53 induction. *Mol. Biol. Cell* **23**, 567–576 (2012).
57. Hayashi, M. T., Cesare, A. J., Fitzpatrick, J. A. J., Lazzerini-Denchi, E. & Karlseder, J. A telomere-dependent DNA damage checkpoint induced by prolonged mitotic arrest. *Nat. Struct. Mol. Biol.* **19**, 387–394 (2012).
58. Blagosklonny, M. V., Demidenko, Z. N. & Fojo, T. Inhibition of transcription results in accumulation of Wt p53 followed by delayed outburst of p53-inducible proteins: p53 as a sensor of transcriptional integrity. *Cell Cycle* **1**, 67–74 (2002).
59. Blagosklonny, M. V. Prolonged mitosis versus tetraploid checkpoint: how p53 measures the duration of mitosis. *Cell Cycle* **5**, 971–975 (2006).
60. Demidenko, Z. N. *et al.* Mechanism of G1-like arrest by low concentrations of paclitaxel: next cell cycle p53-dependent arrest with sub G1 DNA content mediated by prolonged mitosis. *Oncogene* **27**, 4402–4410 (2008).
61. Tang, Y.-C., Williams, B. R., Siegel, J. J. & Amon, A. Identification of aneuploidy-selective antiproliferation compounds. *Cell* **144**, 499–512 (2011).
62. Torres, E. M., Williams, B. R. & Amon, A. Aneuploidy: cells losing their balance. *Genetics* **179**, 737–746 (2008).
63. Torres, E. M. *et al.* Identification of aneuploidy-tolerating mutations. *Cell* **143**, 71–83 (2010).
64. Chikashige, Y. *et al.* Gene expression and distribution of Swi6 in partial aneuploids of the fission yeast *Schizosaccharomyces pombe*. *Cell Struct. Funct.* **32**, 149–161 (2007).
65. Mao, R., Zielke, C. L., Ronald Zielke, H. & Pevsner, J. Global up-regulation of chromosome 21 gene expression in the developing Down syndrome brain. *Genomics* **81**, 457–467 (2003).
66. Kurnit, D. M. Down syndrome: gene dosage at the transcriptional level in skin fibroblasts. *Proc. Natl Acad. Sci. USA* **76**, 2372–2375 (1979).
67. Guo, M. & Birchler, J. A. Trans-acting dosage effects on the expression of model gene systems in maize aneuploids. *Science* **266**, 1999–2002 (1994).
68. Kim, J. C. *et al.* Integrative analysis of gene amplification in *Drosophila* follicle cells: parameters of origin activation and repression. *Genes Dev.* **25**, 1384–1398 (2011).
69. Stenberg, P. & Larsson, J. Buffering and the evolution of chromosome-wide gene regulation. *Chromosoma* **120**, 213–225 (2011).
70. Larsson, J., Chen, J. D., Rasheva, V., Rasmuson-Lestander, A. & Pirrotta, V. Painting of fourth, a chromosome-specific protein in *Drosophila*. *Proc. Natl Acad. Sci. USA* **98**, 6273–6278 (2001).
71. Miclaus, M., Xu, J.-H. & Messing, J. Differential gene expression and epiregulation of alpha zein gene copies in maize haplotypes. *PLoS Genet.* **7**, e1002131 (2011).
72. Futcher, B. & Carbon, J. Toxic effects of excess cloned centromeres. *Mol. Cell. Biol.* **6**, 2213–2222 (1986).
73. Oromendia, A. B., Dodgson, S. E. & Amon, A. Aneuploidy causes proteotoxic stress in yeast. *Genes Dev.* **26**, 2696–2708 (2012).
74. Girirajan, S., Campbell, C. D. & Eichler, E. E. Human copy number variation and complex genetic disease. *Annu. Rev. Genet.* **45**, 203–226 (2011).
75. Tang, Y.-C. & Amon, A. Gene copy-number alterations: a cost-benefit analysis. *Cell* **152**, 394–405 (2013).
76. Isacson, O., Seo, H., Lin, L., Albeck, D. & Granholm, A. C. Alzheimer's disease and Down's syndrome: roles of APP, trophic factors and ACh. *Trends Neurosci.* **25**, 79–84 (2002).
77. Hanemann, C. O. & Müller, H. W. Pathogenesis of Charcot–Marie–Tooth 1A (CMT1A) neuropathy. *Trends Neurosci.* **21**, 282–286 (1998).
78. Katz, W., Weinstein, B. & Solomon, F. Regulation of tubulin levels and microtubule assembly in *Saccharomyces cerevisiae*: consequences of altered tubulin gene copy number. *Mol. Cell. Biol.* **10**, 5286–5294 (1990).
79. Moriya, H., Makanae, K., Watanabe, K., Chino, A. & Shimizu-Yoshida, Y. Robustness analysis of cellular systems using the genetic tug-of-war method. *Mol. Biosyst.* **8**, 2513–2522 (2012).
80. Deutschbauer, A. M. *et al.* Mechanisms of haploinsufficiency revealed by genome-wide profiling in yeast. *Genetics* **169**, 1915–1925 (2005).
81. Kim, D.-U. *et al.* Analysis of a genome-wide set of gene deletions in the fission yeast *Schizosaccharomyces pombe*. *Nat. Biotechnol.* **28**, 617–623 (2010).
82. Das, I. *et al.* Hedgehog agonist therapy corrects structural and cognitive deficits in a Down syndrome mouse model. *Sci. Transl. Med.* **5**, 201ra120 (2013).
83. Jiang, J. *et al.* Translating dosage compensation to trisomy 21. *Nature* **500**, 296–300 (2013).
84. Bonney, M. E., Moriya, H. & Amon, A. Aneuploid proliferation defects in yeast are not driven by copy number changes of a few dosage sensitive genes. *Genes Dev.* **29**, 898–903 (2015).
85. Sheltzer, J. M., Torres, E. M., Dunham, M. J. & Amon, A. Transcriptional consequences of aneuploidy. *Proc. Natl Acad. Sci.* **109**, 12644–12649 (2012).
86. Foijer, F. *et al.* Chromosome instability induced by Mps1 and p53 mutation generates aggressive lymphomas exhibiting aneuploidy-induced stress. *Proc. Natl Acad. Sci.* **111**, 13427–13432 (2014).
87. Dürrbaum, M. *et al.* Unique features of the transcriptional response to model aneuploidy in human cells. *BMC Genomics* **15**, 139 (2014).
88. Gasch, A. P. Comparative genomics of the environmental stress response in ascomycete fungi. *Yeast* **24**, 961–976 (2007).
89. Gasch, A. P. *et al.* Genomic expression programs in the response of yeast cells to environmental changes. *Mol. Biol. Cell* **11**, 4241–4257 (2000).
90. Carter, S. L., Eklund, A. C., Kohane, I. S., Harris, L. N. & Szallasi, Z. A signature of chromosomal instability inferred from gene expression profiles predicts clinical outcome in multiple human cancers. *Nat. Genet.* **38**, 1043–1048 (2006).
91. Venet, D., Dumont, J. E. & Detours, V. Most random gene expression signatures are significantly associated with breast cancer outcome. *PLoS Comput. Biol.* **7**, e1002240 (2011).
92. Sheltzer, J. M. A transcriptional and metabolic signature of primary aneuploidy is present in chromosomally unstable cancer cells and informs clinical prognosis. *Cancer Res.* **73**, 6401–6412 (2013).
93. Simpson, M. V. The release of labeled amino acids from the proteins of rat liver slices. *J. Biol. Chem.* **201**, 143–154 (1953).
94. Balch, W. E., Morimoto, R. I., Dillin, A. & Kelly, J. W. Adapting proteostasis for disease intervention. *Science* **319**, 916–919 (2008).
95. Tyedmers, J., Mogk, A. & Bukau, B. Cellular strategies for controlling protein aggregation. *Nat. Rev. Mol. Cell Biol.* **11**, 777–788 (2010).
96. Dobson, C. M. Protein folding and misfolding. *Nature* **426**, 884–890 (2003).
97. Jahn, T. R. & Radford, S. E. The Yin and Yang of protein folding. *FEBS J.* **272**, 5962–5970 (2005).
98. Kirkin, V., McEwan, D. G., Novak, I. & Dikic, I. A role for ubiquitin in selective autophagy. *Mol. Cell* **34**, 259–269 (2009).
99. Ding, W.-X. & Yin, X.-M. Sorting, recognition and activation of the misfolded protein degradation pathways through macroautophagy and the proteasome. *Autophagy* **4**, 141–150 (2008).
100. Taipale, M., Jarosz, D. F. & Lindquist, S. HSP90 at the hub of protein homeostasis: emerging mechanistic insights. *Nat. Rev. Mol. Cell Biol.* **11**, 515–528 (2010).
101. Donnelly, N., Passerini, V., Dürrbaum, M., Stingele, S. & Storchova, Z. HSF1 deficiency and impaired HSP90-dependent protein folding are hallmarks of aneuploid human cells. *EMBO J.* **33**, 2374–2387 (2014).
This study shows that HSP90-mediated protein folding is reduced in aneuploid mammalian cells, providing a link between aneuploidy and proteotoxic stress.
102. Boulon, S. *et al.* HSP90 and its R2TP/prefoldin-like cochaperone are involved in the cytoplasmic assembly of RNA polymerase II. *Mol. Cell* **39**, 912–924 (2010).
103. Li, G.-W., Burkhardt, D., Gross, C. & Weissman, J. S. Quantifying absolute protein synthesis rates reveals principles underlying allocation of cellular resources. *Cell* **157**, 624–635 (2014).
104. Niwa, O., Tange, Y. & Kurabayashi, A. Growth arrest and chromosome instability in aneuploid yeast. *Yeast* **23**, 937–950 (2006).
105. Segal, D. J. & McCoy, E. E. Studies on Down's syndrome in tissue culture. I. Growth rates and protein contents of fibroblast cultures. *J. Cell. Physiol.* **83**, 85–90 (1974).
106. Thorburn, R. R. *et al.* Aneuploid yeast strains exhibit defects in cell growth and passage through START. *Mol. Biol. Cell* **24**, 1274–1289 (2013).
107. Baker, D. J. *et al.* BubR1 insufficiency causes early onset of aging-associated phenotypes and infertility in mice. *Nat. Genet.* **36**, 744–749 (2004).
108. Weaver, B. A. A., Silk, A. D., Montagna, C., Verdier-Pinard, P. & Cleveland, D. W. Aneuploidy acts both oncogenically and as a tumor suppressor. *Cancer Cell* **11**, 25–36 (2007).
109. Babu, J. R. *et al.* Rae1 is an essential mitotic checkpoint regulator that cooperates with Bub3 to prevent chromosome missegregation. *J. Cell Biol.* **160**, 341–353 (2003).
110. Makanae, K., Kintaka, R., Makino, T., Kitano, H. & Moriya, H. Identification of dosage-sensitive genes in *Saccharomyces cerevisiae* using the genetic tug-of-war method. *Genome Res.* **23**, 300–311 (2013).
111. Mitelman, F., Johansson, B. & Mertens, F. Mitelman database of chromosome aberrations and gene fusions in cancer. [online], http://cgap.nci.nih.gov/Chromosomes/Mitelman (2014).

112. Beroukhim, R. *et al.* The landscape of somatic copy-number alteration across human cancers. *Nature* **463**, 899–905 (2010).
113. Cahill, D. P. *et al.* Characterization of *MAD2B* and other mitotic spindle checkpoint genes. *Genomics* **58**, 181–187 (1999).
114. Haruki, N. *et al.* Molecular analysis of the mitotic checkpoint genes *BUB1*, *BUBR1* and *BUB3* in human lung cancers. *Cancer Lett.* **162**, 201–205 (2001).
115. Gemma, A. *et al.* Somatic mutation of the *hBUB1* mitotic checkpoint gene in primary lung cancer. *Genes Chromosomes Cancer* **29**, 213–218 (2000).
116. Manning, A. L., Longworth, M. S. & Dyson, N. J. Loss of pRB causes centromere dysfunction and chromosomal instability. *Genes Dev.* **24**, 1364–1376 (2010).
117. Manning, A. L. & Dyson, N. J. pRB, a tumor suppressor with a stabilizing presence. *Trends Cell Biol.* **21**, 433–441 (2011).
118. Tighe, A. Truncating *APC* mutations have dominant effects on proliferation, spindle checkpoint control, survival and chromosome stability. *J. Cell Sci.* **117**, 6339–6353 (2004).
119. Davoli, T. *et al.* Cumulative haploinsufficiency and triplosensitivity drive aneuploidy patterns and shape the cancer genome. *Cell* **155**, 948–962 (2013).
By analysing genome sequence data from cancerous and normal tissues, this study provides evidence that aneuploidy drives tumorigenesis through losses of tumour-suppressor genes and gains of oncogenes.
120. Sussan, T. E., Yang, A., Li, F., Ostrowski, M. C. & Reeves, R. H. Trisomy represses ApcMin-mediated tumours in mouse models of Down's syndrome. *Nature* **451**, 73–75 (2008).
121. Hasle, H. *et al.* Risks of leukaemia and solid tumours in individuals with Down's syndrome. *Lancet* **355**, 165–169 (2001).
122. Baek, K.-H. *et al.* Down's syndrome suppression of tumour growth and the role of the calcineurin inhibitor DSCR1. *Nature* **459**, 1126–1130 (2009).
123. Paulsson, K. & Johansson, B. Trisomy 8 as the sole chromosomal aberration in acute myeloid leukemia and myelodysplastic syndromes. *Pathol. Biol.* **55**, 37–48 (2007).
124. Jones, L. *et al.* Gain of MYC underlies recurrent trisomy of the MYC chromosome in acute promyelocytic leukemia. *J. Exp. Med.* **207**, 2581–2594 (2010).
125. Greenberg, R. A. *et al.* Short dysfunctional telomeres impair tumorigenesis in the INK4a$^{\Delta 2/3}$ cancer-prone mouse. *Cell* **97**, 515–525 (1999).
126. Chin, L. *et al.* p53 deficiency rescues the adverse effects of telomere loss and cooperates with telomere dysfunction to accelerate carcinogenesis. *Cell* **97**, 527–538 (1999).
127. Sotillo, R., Schvartzman, J.-M., Socci, N. D. & Benezra, R. Mad2-induced chromosome instability leads to lung tumour relapse after oncogene withdrawal. *Nature* **464**, 436–440 (2010).
This study shows that tumour relapse after oncogene withdrawal is accelerated under conditions of increased chromosome mis-segregation in a *KRAS*-driven model of lung cancer. This finding indicates that aneuploidy can facilitate the emergence of resistant karyotypes that confer an evolutionary advantage to the tumour.
128. Silk, A. D. *et al.* Chromosome missegregation rate predicts whether aneuploidy will promote or suppress tumors. *Proc. Natl Acad. Sci.* **110**, E4134–E4141 (2013).
This article suggests that, although low levels of chromosome mis-segregation can accelerate the generation of karyotypes that promote tumorigenesis, high rates of chromosome gain and loss lead to tumour suppression and cell death.
129. Zhu, J., Pavelka, N., Bradford, W. D., Rancati, G. & Li, R. Karyotypic determinants of chromosome instability in aneuploid budding yeast. *PLoS Genet.* **8**, e1002719 (2012).
130. Sheltzer, J. M. *et al.* Aneuploidy drives genomic instability in yeast. *Science* **333**, 1026–1030 (2011).
131. Rancati, G. *et al.* Aneuploidy underlies rapid adaptive evolution of yeast cells deprived of a conserved cytokinesis motor. *Cell* **135**, 879–893 (2008).
132. Selmecki, A., Forche, A. & Berman, J. Aneuploidy and isochromosome formation in drug-resistant *Candida albicans*. *Science* **313**, 367–370 (2006).
133. Yona, A. H. *et al.* Chromosomal duplication is a transient evolutionary solution to stress. *Proc. Natl Acad. Sci.* **109**, 21010–21015 (2012).
134. Chen, G. *et al.* Targeting the adaptability of heterogeneous aneuploids. *Cell* **160**, 771–784 (2015).
135. London, N. & Biggins, S. Signalling dynamics in the spindle checkpoint response. *Nat. Rev. Mol. Cell Biol.* **15**, 736–747 (2014).
136. Cimini, D. *et al.* Merotelic kinetochore orientation is a major mechanism of aneuploidy in mitotic mammalian tissue cells. *J. Cell Biol.* **153**, 517–527 (2001).
137. Niwa, O., Matsumoto, T. & Yanagida, M. Construction of a mini-chromosome by deletion and its mitotic and meiotic behaviour in fission yeast. *Mol. Gen. Genet.* **203**, 397–405 (1986).
138. Weaver, B. A. A. & Cleveland, D. W. Does aneuploidy cause cancer? *Curr. Opin. Cell Biol.* **18**, 658–667 (2006).
139. Niwa, O., Matsumoto, T., Chikashige, Y. & Yanagida, M. Characterization of *Schizosaccharomyces pombe* minichromosome deletion derivatives and a functional allocation of their centromere. *EMBO J.* **8**, 3045–3052 (1989).
140. Zeng, Y., Li, H., Schweppe, N. M., Hawley, R. S. & Gilliland, W. D. Statistical analysis of nondisjunction assays in *Drosophila*. *Genetics* **186**, 505–513 (2010).
141. Gregg, T. G. & Day, J. W. Nondisjunction of the X chromosomes in females of *Drosophila hydei*. *Genetica* **36**, 172–182 (1965).
142. Koehler, K. E., Hawley, R. S., Sherman, S. & Hassold, T. Recombination and nondisjunction in humans and flies. *Hum. Mol. Genet.* **5**, 1495–1504 (1996).
143. Bond, D. J. & Chandley, A. C. *Aneuploidy (Oxford Monographs on Medical Genetics)* (Oxford University Press, 1983).
144. Martin, R. H., Ko, E. & Rademaker, A. Distribution of aneuploidy in human gametes: comparison between human sperm and oocytes. *Am. J. Med. Genet.* **39**, 321–331 (1991).
145. Martin, R. H. & Rademaker, A. The frequency of aneuploidy among individual chromosomes in 6,821 human sperm chromosome complements. *Cytogenet. Cell Genet.* **53**, 103–107 (1990).
146. Templado, C., Vidal, F. & Estop, A. Aneuploidy in human spermatozoa. *Cytogenet. Genome Res.* **133**, 91–99 (2011).
147. Pellestor, F., Andréo, B., Anahory, T. & Hamamah, S. The occurrence of aneuploidy in human: lessons from the cytogenetic studies of human oocytes. *Eur. J. Med. Genet.* **49**, 103–116 (2006).
148. Pacchierotti, F., Adler, I.-D., Eichenlaub-Ritter, U. & Mailhes, J. B. Gender effects on the incidence of aneuploidy in mammalian germ cells. *Environ. Res.* **104**, 46–69 (2007).
149. Obradors, A. *et al.* Whole-chromosome aneuploidy analysis in human oocytes: focus on comparative genomic hybridization. *Cytogenet. Genome Res.* **133**, 119–126 (2011).
150. Nagaoka, S. I., Hassold, T. J. & Hunt, P. A. Human aneuploidy: mechanisms and new insights into an age-old problem. *Nat. Rev. Genet.* **13**, 493–504 (2012).

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