Trends in Genetics

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REVIEWS

Review

Consequences of Genomic Diversification Induced by Segregation Errors

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Chromosome segregation errors are an important source of genomic diversification that promote tumor heterogeneity and evolution. However, the aneuploidy induced by chromosome missegregations causes cellular stress at many levels, raising the question of how segregation errors can be tolerated in cancer. Additionally, we now know that chromosome segregation errors can lead to activation of the innate immune system, producing yet another challenge for chromosomally unstable cells. These observations imply that several liabilities are encountered during tumor evolution, which could potentially be exploited for cancer therapies. Here, we provide an overview of the different causes of segregation errors, their impact on cellular and genomic homeostasis, and discuss recent studies that help to understand how tolerance towards imbalanced karyotypes can be obtained.

Highlights

Chromosome segregation errors lead to aneuploidy, resulting in a number of stresses such as replication stress, proteotoxic stress, and oxidative stress.

There are important differences between the effects of specific karyotypes (i.e., between whole aneuploidies, segmental aneuploidies, and micronuclei).

Segregation errors may directly or indirectly lead to DNA damage, and this damage can be a source for chromoanagenesis and chromosomal instability.

Besides p53, there is mounting evidence of p53-independent factors that play an important role in tolerance to abnormal karyotypes.

Segregation errors can trigger an immune response but their exact role in affecting tumor development or promoting resistance remains unclear.

Chromosome Segregation Errors

During each round of cell division, the genome is duplicated and segregated into two newly formed daughter cells. Proper segregation of the genome is of crucial importance for cell viability and many error-correction mechanisms are at play to ensure high fidelity of the chromosome segregation process. Despite the presence of these surveillance mechanisms, mistakes can occur, which can result in imbalances of the genome, a state that is referred to as **aneuploidy** (see Glossary). Genomic imbalances that solely involve whole chromosome deviations are called **numerical aneuploidies**, while segregation errors that lead to the inheritance of parts of a chromosome are referred to as **segmental aneuploidy**.

Segregation errors and associated chromosomal aberrations occur at a very low frequency in healthy tissues [1,2], but they are a common trait in cancer [3]. Tumors displaying a high rate of segregation errors are classified as chromosome unstable [a trait also known as **chromosomal instability (CIN)**]. Ongoing segregation errors and associated karyotypic diversification can drive genetic heterogeneity and evolutionary selection in cancer. CIN is often confused with aneuploidy, but it is important to note that CIN refers to ongoing karyotype diversification, while aneuploidy solely describes the state of a cell harboring an imbalanced karyotype. CIN tumors are associated with poor prognosis and display enhanced therapy resistance [4–6]. Thus, CIN appears to provide a driving force in tumorigenesis, which is remarkable considering all of the challenges that a cell is presented with when its genome is drastically altered. In this review we discuss how segregation errors arise, how they affect genomic evolution, and how tumor cells adapt to such drastic changes in their genome.

Types of Segregation Errors

Accurate segregation of chromosomes relies on a highly orchestrated cell division process, also known as mitosis (defined in Box 1), as well as on the integrity of the chromosomes themselves. Errors related to any of these two aspects represent the main causes for incorrect

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Box 1. Mitosis

The aim of mitosis is to ensure proper segregation of two identical sets of intact chromosomes into two newly formed daughter cells. To accomplish this, eukaryotic cells evolved a number of interconnected mechanisms that are tightly orchestrated, altogether referred to as the mitotic machinery. In brief, at the start of mitosis, the centrosomes separate and form the basis of the mitotic spindle, a microtubule-based structure that is critical for the partitioning of the chromosome complement into two identical parts. Upon nuclear envelope breakdown, microtubules start to interact with chromosomes at kinetochores, structures that are formed at the centromeric regions of chromosomes. A safeguard mechanism that is at play during mitosis, also referred to as the spindle assembly checkpoint (SAC), monitors the quality of microtubule-kinetochore attachments and halts mitotic progression until the equal segregation of all chromosomes can be ensured. The correct attachment of all chromosomes will silence the SAC, which will allow sister chromatids to separate, by cleavage of the cohesin molecules that keep sister chromatids together. Once the sister chromatids are cleaved from each other, correct segregation is led by pulling forces from the opposite spindle poles towards each daughter cell. All of the described steps together will result in the faithful and balanced segregation of the genome (a more comprehensive description of the different steps of mitosis is described in [112]).

chromosome segregation. Below, we will provide a short summary of the different sources that can underlie segregation errors.

Mitotic Defects Underlying Segregation Errors

In order to undergo faithful segregation of sister chromatids into two daughter nuclei, proper **kinetochore–microtubule (KT–MT) attachments** are needed. Incorrect microtubule attachments and altered microtubule dynamics can lead to chromosome segregation errors [7–9]. Erroneous attachments can be provoked by an abnormal formation of the mitotic spindle. While the geometry of a bipolar spindle promotes the formation of correct, **amphitellic attachments**, monopolar or multipolar spindles promote the formation of abnormal connections, such as **monotelic**, **syntelic**, and **merotelic attachments** [10,11]. To avoid erroneous attachments and associated segregation errors, cells have several surveillance mechanisms [12]. The **spindle assembly checkpoint (SAC)** halts division until all kinetochores are properly attached to the mitotic spindle. Indeed, segregation errors occur very frequently in SAC-impaired cells [13–18]. These types of missegregation mostly involve whole chromosomes, although chromosomes may also be broken during cytokinesis, leading to segmental aneuploidy [19]. It is important to note that SAC genes are rarely found mutated in cancer, indicating that very high rates of segregation errors are not compatible with tumorigenesis. In contrast, SAC genes are in fact often found overexpressed in tumors (reviewed in [20]). This paradoxical observation can be explained by the fact that overexpression of SAC proteins, such as Mad2, results in a less severe phenotype as compared with the complete loss of Mad2 function. Specifically, Mad2 overexpression leads to delayed mitotic exit and delayed degradation of Cyclin B1 and Securin, thereby inducing tetraploidy and moderate levels of aneuploidy [21,22]. Besides, SAC-independent functions in, for example, KT–MT attachment [23], DNA damage [24,25], apoptosis [26], and RNA splicing [27] can be disturbed when SAC genes are overexpressed, which can also contribute to the transformation process. Besides acting as drivers of tumorigenesis, the high expression of SAC genes in tumors might actually protect the cells from severe CIN by hyperactivating the SAC [28].

In addition, chromosome segregation can also be perturbed by defects in chromosome cohesion. Both premature and delayed cleavage of cohesin, the protein complex that keeps sister chromatids together, affect chromosome alignment and can cause segregation errors, including **lagging chromosomes** and **anaphase bridges** [29–32].

Anomalous DNA Structures That Promote Segregation Errors

Besides dysfunctionalities in the mitotic machinery, anomalous DNA structures that are not resolved before a cell enters mitosis can impede flawless segregation of chromosomes. Such

Glossary

Amphitellic attachment: correct connection between sister kinetochores and microtubules pulling from opposite poles, resulting in a bioriented chromosome.
Anaphase bridge: type of segregation error that involves a stretched chromosome over the two new sister nuclei.
Aneuploidy: the presence of an abnormal number of chromosomes.
cGAS–STING pathway: part of the innate immune system. It is stimulated upon the detection of DNA in the cytoplasm, upon which it induces the expression of type I interferons, resulting in an inflammatory response.
Chromoanagenesis: literally means 'rebirth of a chromosome'. This refers to chromosome rearrangements that may involve translocations, inversions, amplifications, and losses of pieces of DNA, leading to aberrant chromosomes. This term was first introduced by Holland and Cleveland in 2012 [113].
Chromosomal instability (CIN): ongoing rate of segregation errors at every round of division, leading to continuous karyotype diversification.
Chromothripsis: phenomenon by which hundreds of chromosomal rearrangements occur in a single event, usually limited to one or few chromosomes in a cell.
Double stranded breaks (DSBs): cytotoxic lesion on a chromosome involving the breakage of both DNA strands.
Kinetochore–microtubule (KT–MT) attachments: attachments that are formed between kinetochores and microtubules during cell division. Proper attachment to each sister chromatid from microtubules emanating from each pole ensures a correct segregation of each chromosome into two identical daughter nuclei.
Lagging chromosome: type of segregation error that involves a chromosome that lags behind in the spindle midzone during anaphase.
Merotelic attachment: erroneous KT–MT attachment where a single kinetochore attaches to microtubules from both poles.
Micronucleus: small nucleus that harbors one or few chromosomes or

abnormal structures are formed in interphase and are mainly produced during DNA replication or by factors that induce DNA damage. For example, if the cell enters mitosis before replication is completed, sister chromatids remain intertwined. As the cell progresses to form two daughter cells, the entangled DNA structure will likely break, resulting in broken chromosomes [33,34]. **Double stranded breaks** (DSBs) may in turn result in abnormal fusions, leading to CIN (see section on DNA damage as a consequence of segregation errors). In addition, if a cell enters mitosis with a broken chromosome, this can also perturb normal chromosome segregation. Chromosome fragments that lack centromeres will be unable to properly attach to the spindle and are therefore likely to missegregate [35,36]. Taken together, the common outcome of anomalous DNA structures that persist into mitosis is segmental aneuploidy, imbalances of a piece of chromosome rather than whole chromosomes.

Consequences of Segregation Errors

Depending on the specific cause, a missegregation event can either lead to whole chromosome aneuploidy or segmental aneuploidy. Moreover, entire chromosomes or chromosome fragments can form a separate entity in the cell following a missegregation event. These structures are referred to as micronuclei. In this section we will first focus on the direct and indirect consequences associated with segregation errors that affect the main nucleus. Then, we will separately discuss the consequences of the exclusion of a chromosome or a piece of chromosome in a **micronucleus**.

Consequences of Imbalanced Karyotypes

The fact that only few specific chromosomal abnormalities are compatible with human life suggests that karyotype variations are poorly tolerated [37]. Indeed, aneuploidy leads to growth impairment and developmental problems in nearly all organisms studied thus far (reviewed in [38]). Even in transformed settings, extra copies of individual chromosomes result in a proliferative disadvantage and a reduced capacity to form tumors *in vivo* [39]. The leading view on the poor tolerance to (whole) chromosome imbalances involves the stresses associated with the imbalances in gene expression. The majority of organisms, including yeast, mouse, and human, display a strong correlation between the altered copy number and mRNA expression levels of the genes on the aneuploid chromosome [40–44] (Figure 1). However, some studies suggest that a subset of genes display a nonlinear correlation between their copy number and mRNA expression level in these organisms [45,46]. This phenomenon, referred to as 'dosage compensation', has also been observed in *Drosophila melanogaster* to occur on the transcriptional level [47,48]. It needs to be noted that the extent of transcriptional dosage compensation in other organisms, such as yeast, varies by the use of different analysis methods and thus it is unclear whether such effects are relevant or whether they reflect analysis artefacts [49]. Besides transcriptome alterations, it has been well established that aneuploidy also affects the proteome landscape in most organisms. While the abundance of most proteins increases according to their transcript levels, there is a subset of proteins that behave differently. An estimated 10%–25% of proteins displays extensive dosage compensation on the protein level, as their relative abundance is unchanged, despite the genomic and transcriptional imbalance (Figure 1) [43,50]. Dosage-compensated proteins are mainly those that are part of multisubunit complexes and critical signaling regulators, such as kinases [43,50]. The reduced abundance of such proteins is established by enhanced protein degradation [51], which explains the enhanced **proteotoxic stress** observed in aneuploid cells (see below).

Besides altered expression of genes on the involved chromosomes, aneuploid cells also display differential expression of genes that are located on other chromosomes. This can, in part, be explained by the presence of transcription regulators such as transcription factors and microRNAs (miRNAs) on the aneuploid chromosome. The altered transcription of such regulators will,

pieces of chromosomes. They generally arise from DNA that did not reach the newly formed daughter nuclei in time during mitotic exit. These small structures are known to be defective for most of the main physiological nuclear functions.
Monotelic attachment: erroneous KT–MT attachment where only one sister chromatid is attached to a pole and the other is not attached.
Numerical aneuploidy: an imbalanced karyotype due to gains and or losses of whole chromosomes.
Proteotoxic stress: impairment of the cellular homeostasis caused by accumulation of unfolded or misfolded proteins.
Reactive oxygen species (ROS): chemically reactive molecules containing oxygen. High levels of ROS are known to be produced as a cellular response to certain stresses.
Segmental aneuploidy: an imbalance of a large fragment of a chromosome.
Spindle assembly checkpoint (SAC): molecular machinery that acts to halt progression through mitosis until all chromosomes are properly attached to microtubules, ensuring a faithful segregation.
Syntelic attachment: erroneous KT–MT attachment where both sister chromatids attach to the same pole.
Type I interferon: subgroup of signaling proteins that help to regulate the activity of the immune system. Type I interferons activate INFNAR receptors.
Warburg effect: observation that most cancer cells show an increase in the rate of glucose uptake and preferential production of lactate, even in the presence of oxygen.

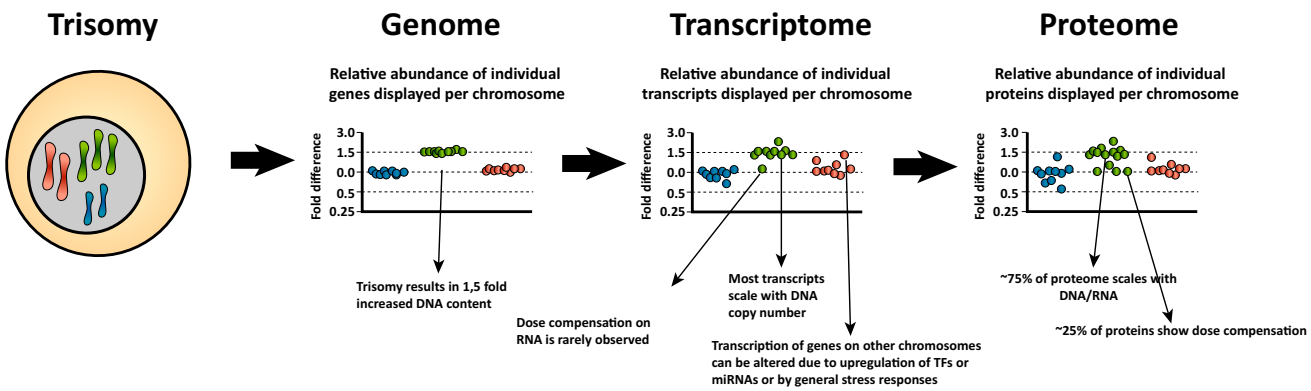

Figure 1. Transcriptome and Proteome Changes Induced by Genome Imbalances. An extra copy of a somatic chromosome has been shown to result in a linear correlation between DNA copy number and gene expression levels (this is true for most studied organisms, with the exception of *Drosophila*). For instance, in the case of a trisomy (note the extra copy of the green chromosome), there will be 1.5-fold increase of the DNA copy number of the genes located on this specific chromosome, and an equivalent increase for the majority of the transcripts. There is some evidence that suggests that few genes undergo dosage compensation effects on the transcriptome level. Besides transcriptional changes of the genes located on the trisomic chromosome, genes on other chromosomes can also be affected. Those transcriptional changes can occur when the imbalanced chromosome encodes transcription factors or miRNAs that regulate genes on other chromosomes. Nevertheless, general transcriptome changes are largely determined by general stress responses. For the proteome, although most of the proteins display a linear correlation with RNA abundance, ~10%–25% of the proteins are expressed at normal levels, involving mostly proteins that are part of multi-subunit complexes or critical signaling regulators.

in turn, modulate the transcription of genes located elsewhere on the genome [52,53]. However, the majority of changes are induced by the general activation of stress-related signaling pathways (reviewed in [54]). In fact, there is a general transcriptional response associated with aneuploidy both in yeast (environmental stress response [55]) and in mammalian cells [56]. This response can be partially explained by a slow growth phenotype and involves the upregulation of factors related to protein processing, metabolism, and immune activity, and downregulation of proteins involved in transcription, replication, and splicing [56–59]. The upregulation of genes involved in protein processing is in place to aid the higher demand aneuploid cells put on protein folding and turnover pathways to maintain proteostasis [60] (Figure 2, Key Figure). Aneuploid cells have increased levels of misfolded proteins and display enhanced autophagy [61]. Additionally, it has become evident that cells bearing abnormal karyotypes rewire their metabolic pathways. The increase of aerobic glycolysis in aneuploid cells might contribute to the **Warburg effect** often observed in cancer [42]. Moreover, aneuploid cells deregulate sphingolipid biosynthesis [62] and display altered expression of DNA and RNA metabolism-related genes [43]. Importantly, all of these stress responses and metabolic alterations form a potential liability that might be exploited to specifically eradicate aneuploid cells [61].

DNA Damage as a Consequence of Segregation Errors

Another main consequence of segregation errors is the induction of DNA damage. Damage after segregation errors has been shown to be a direct consequence of segregation errors *per se*. Chromosome breakage can occur on lagging chromosomes that are trapped in the cleavage furrow, where breakage can be induced by physical forces [19]. Moreover, it has been shown that cytoplasmic nucleases cleave and thereby resolve DNA bridges [63]. Thus, chromosome missegregations themselves can directly result in DSB formation.

However, it has been well established that DNA damage can also be inflicted indirectly by the metabolic stress displayed by aneuploidy. Imbalanced karyotypes lead to increased levels of

Key Figure

Physiological Consequences of Segregation Errors

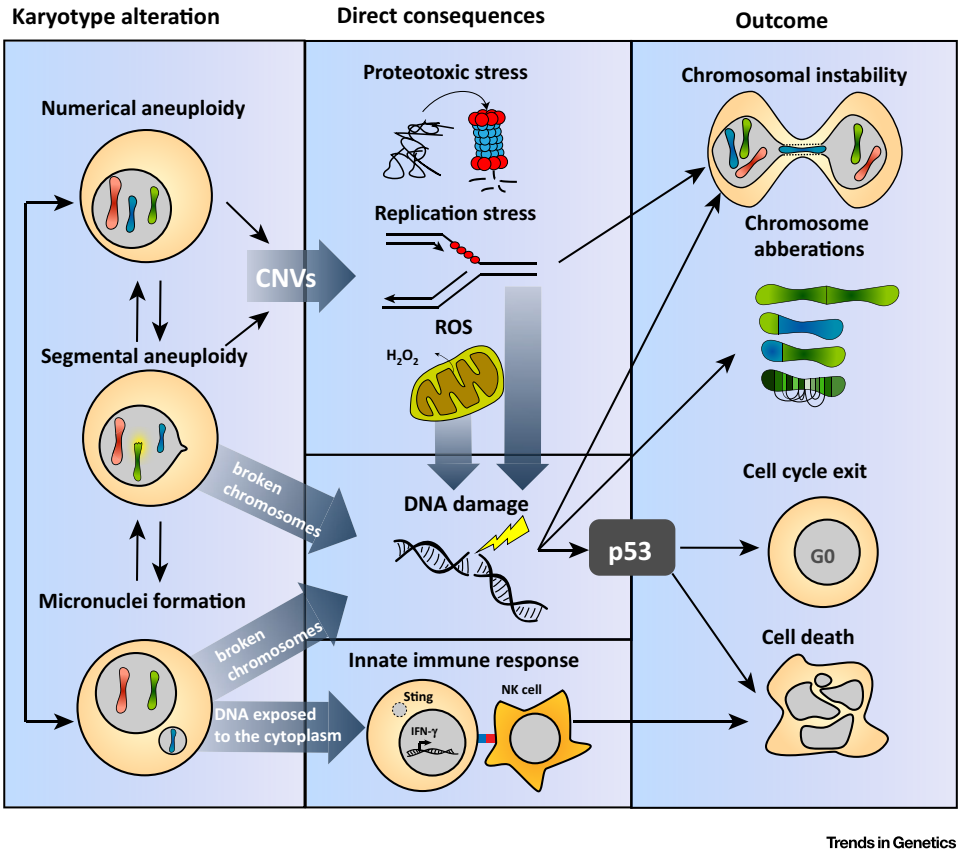

Figure 2. Overview of the many direct and indirect consequences of aberrant karyotypes, involving either numerical or segmental aneuploidy or the presence of micronuclei. An unbalanced genome can induce physiological stresses involving proteotoxic stress, replication stress, and increased levels of reactive oxygen species (ROS). Those stresses or the presence of broken chromosomes could lead to chromosomal instability (CIN). A central node in the response to aneuploidy is p53, which can be activated by DNA damage induced directly by broken chromosomes or by indirect DNA damage associated to ROS and replication stress. p53 activation can result in cell cycle exit or cell death. A cell that harbors a micronucleus could also be exposed to DNA damage, with its respective consequences, including CIN and the formation of abnormally structured chromosomes. Besides, micronuclei often have a dysfunctional nuclear envelope, resulting in the exposure of the micronuclear DNA to the cytoplasm. This, in turn, can induce a STING-dependent innate immune response. CNV, copy number variation.

reactive oxygen species (ROS) that, in turn, damage DNA [50,64]. Additionally, many studies have linked imbalanced karyotypes to problems in replication and consequent DNA breaks [60,65–68]. Interestingly, it has been suggested that aneuploidy-induced replication stress is triggered by the downregulation of DNA replication proteins such as the MCM helicases [68]. Thus, in addition to a direct cause of DNA damage, segregation errors may also indirectly inflict chromosome breakage.

Chromoanagenesis

The presence of DNA damage will activate a signaling cascade to facilitate repair [69,70]. Importantly, the activation of DNA repair pathways, as well as their faithful accomplishment, play key roles in preventing CIN. Erroneous DNA repair may lead to loss of chromosome fragments but also to the formation of aberrant chromosomes, also referred to as '**chromoanagenesis**' (Figure 3). For example, two unrelated free DNA ends originating from distant DSBs could find each other and fuse, leading to the formation of an abnormal

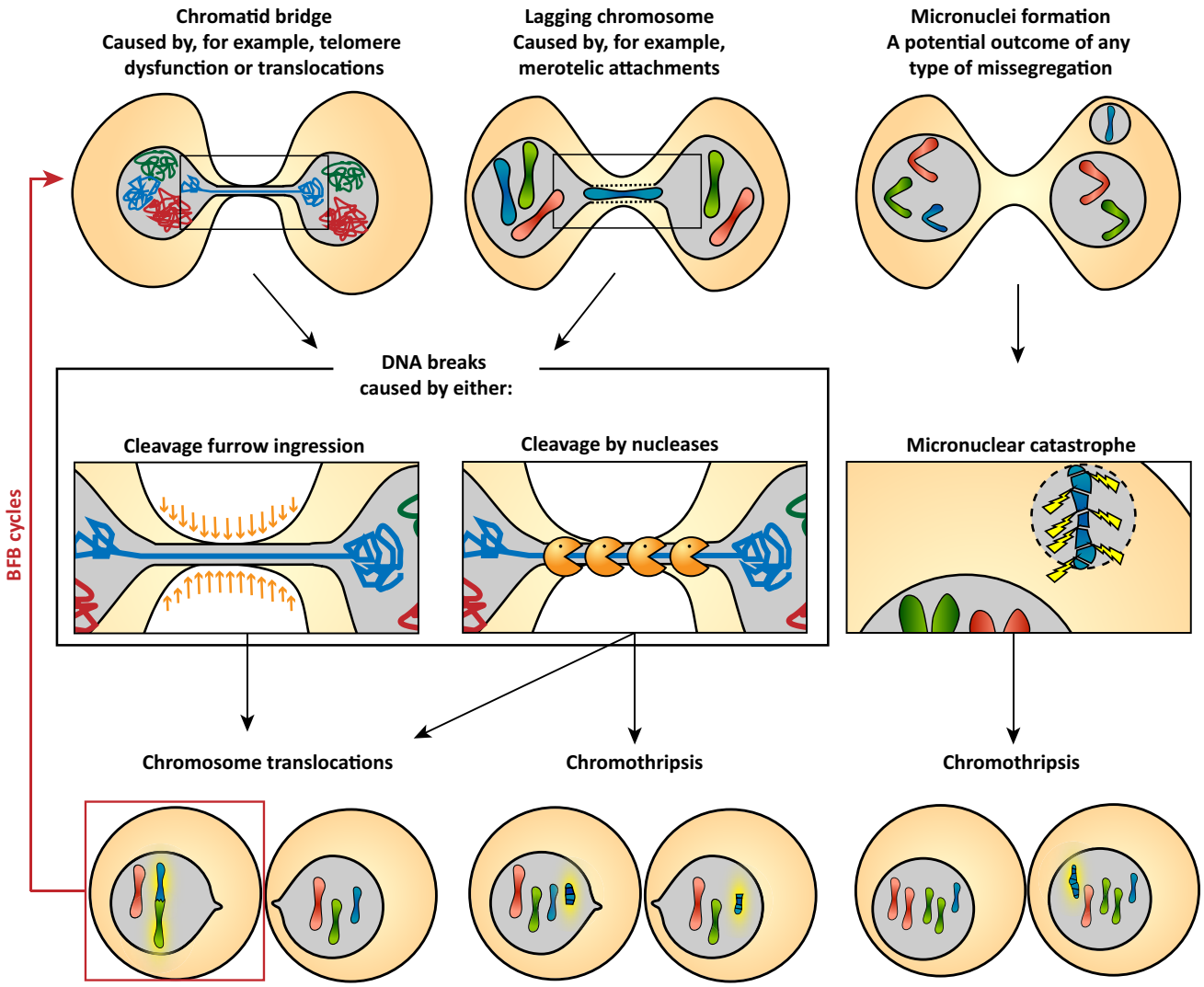

Figure 3. The Formation of Abnormal Chromosomal Structures (Chromoanagenesis). Chromosome segregation errors are an important source for the formation of abnormally structured chromosomes. Chromosome bridges or lagging chromosomes may lead to DNA damage in mitosis. This can be caused by physical forces exerted by ingression of the cleavage furrow or by direct cleavage by cytoplasmic nucleases. The broken DNA may in turn lead to chromosome translocations or to chromothripsis. Chromosome translocations may result in the formation of chromosomes harboring two centromeres (dicentric chromosomes), which are likely to form a chromatid bridge again in the subsequent mitosis. This reflects a breakage-fusion-bridge (BFB) cycle. As shown in more detail in Figure 4, micronuclei may undergo catastrophe in interphase leading to massive DNA damage and impairment of main physiological functions. Once the micronucleus is reincorporated in a daughter nucleus, the fragmented DNA can re-ligate and give rise to a chromothriptic chromosome.

chromosome, analogous to the fusion of uncapped telomeres [71,72]. Fusions following DNA breaks may result in reciprocal translocations that can produce dicentric chromosomes [73]. Such dicentric chromosomes create problems during mitosis because a single chromatid with two centromeres can attach to opposite poles. In this case, the chromatid will be pulled to opposite sides of the cell, thereby forming a chromatin bridge. Such an event can be the origin of a breakage-fusion-bridge (BFB) cycle, first described in the late 1930s [74]. In a BFB cycle, cells undergo constant diversification at every round of mitosis by breaking and fusing DNA [75] (Figure 3). Besides, chromatin bridges have also been related to another specific type of chromoanagenesis, as they are prone to undergo nucleolytic attack, which can be a starting point for **chromothripsis** [63] (Figure 3). Chromothripsis is a phenomenon in which one or a few chromosomes in a cell acquire dozens to hundreds of clustered rearrangements in a single catastrophic event and is linked to poor prognosis in cancer [76].

CIN Induced by Segregation Errors

Although there are some clear causes for CIN, such as increased DNA damage (see section on Chromoanagenesis) (Figures 2 and 3), the exact link between abnormal karyotypes and ongoing instability remains a controversial matter. Interestingly, the majority of the current evidence in both yeast and human cells agrees that the presence of single extra chromosomes is sufficient to initiate further instability. Enhanced whole chromosome missegregations have been observed in response to specific whole chromosome imbalances [77]. Moreover, general stress responses, including replication stress and ROS, have been shown to induce *de novo* rearrangements, thereby further enhancing instability [65,66,68]. In contrast, there are some studies in both human cells and yeast that show that certain whole chromosome imbalances may not be sufficient to induce CIN [65,78]. Such discrepancies support the idea that different types of imbalances can have different effects on instability.

Micronuclei

Aberrant chromosome segregation may also lead to the exclusion of (parts of) one or more chromosome(s) that fail to join one of the two main chromosome packs. If so, the missegregated piece of DNA recruits its own nuclear envelope and forms a so-called micronucleus. This separated entity has been shown to display reduced functionality compared with the primary nucleus in the same cell. Micronuclei display impaired membrane assembly, which compromises the integrity of their nuclear envelope [79]. Moreover, micronuclei tend to undergo membrane collapse in interphase, further affecting transport of molecules across micronuclear membranes [80]. Dysregulated transport results in a strong impairment of functions such as replication, transcription, and DNA repair in the micronucleus [80–82]. In addition, the transport defects result in a failure to build a functional kinetochore. Consequently, micronuclei are liable to undergo segregation errors, which favors the maintenance of a micronucleus over its reincorporation postmitosis (Figure 4) [83,84]. Furthermore, there is accumulating evidence showing following membrane disruption, DNA in micronuclei undergoes extensive fragmentation [81], which can also lead to chromothripsis [85] (Figures 3 and 4). Since repair is shown to be defective in micronuclei, the re-ligation of DNA breaks is thought to only occur once the micronuclear material is reincorporated into the main nucleus in mitosis, led by canonical nonhomologous end-joining [86]. Remaining small fragments of the shattered DNA have the ability to circularize and form so-called double minutes [76]. Interestingly, high amounts of double minutes that contain oncogenes are often found in cancer cells [76]. Taken together, the presence of a micronucleus can eventually result in DNA damage, leading to drastic chromosomal rearrangements that can contribute to tumorigenesis (Figure 4).

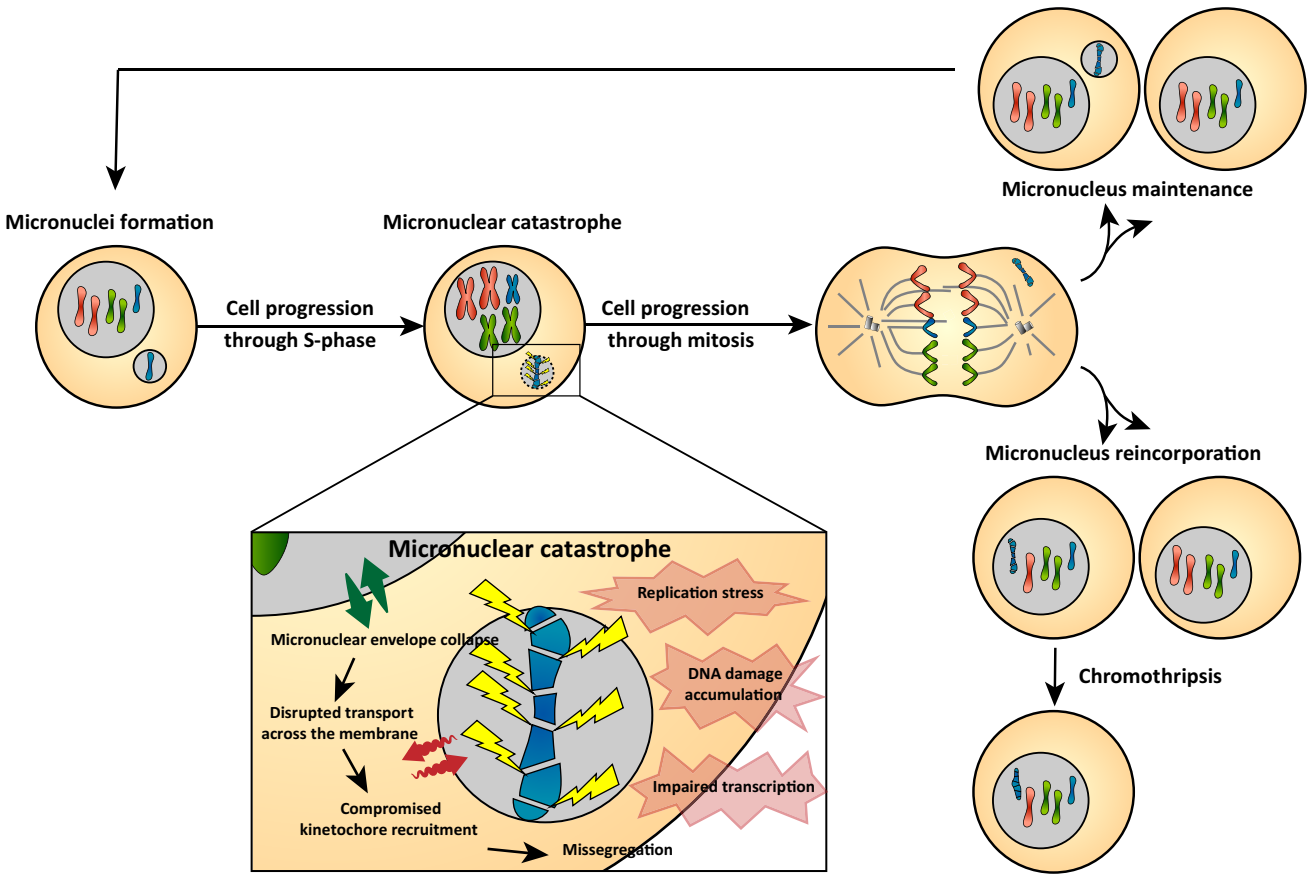

Figure 4. Fate and Consequences of Harboring a Micronucleus. Micronuclei have been shown to undergo micronuclear catastrophe when the cell progresses through S-phase. Such catastrophe starts by nuclear envelope collapse and coincides with impaired trafficking of molecules across the micronuclear envelope. This transport defect results in the impairment of main physiological functions such as replication, transcription, DNA repair, and kinetochore assembly. Micronuclei are liable to undergo massive DNA damage. When the cell enters the subsequent mitosis, the deficient kinetochore will prevent the chromatid derived from the micronucleus from participating properly in mitosis. This will favor the maintenance of the micronucleus over its reincorporation into either one of the daughter cells. Importantly, when reincorporated into the main nucleus, massive erroneous re-ligation of the broken DNA can take place, a phenomenon also known as chromothripsis.

Tolerance to Segregation Errors

Considering the extent of the detrimental effects caused by segregation errors, it is striking that the vast majority of cancers are aneuploid [87]. Importantly, karyotype diversification has been shown to promote evolution and selection under suboptimal growth conditions [41,88]. Yet, it is not completely established how the propagation of abnormal chromosome complements is tolerated. At least one clear determinant for aneuploidy tolerance is the tumor suppressor gene *TP53* (encoding p53), which is often found to be mutated in aneuploid tumors [89]. It is known that p53 can be directly activated by broken chromosomes via the activated DNA damage response [19,66,90]. Consistently, segmental aneuploidies are almost exclusively tolerated and propagated in p53-deficient cells [66,90]. However, whether there are other aspects of chromosome missegregations that can cause p53 activation remains under debate. Some studies suggested that the mere presence of an imbalanced karyotype would be sufficient to activate p53 via the activation of p38 [91]. However, the interpretation of these experiments is challenged by the fact that a prolonged mitosis, that has

been widely used as a tool to induce segregation errors, results in p53 activation, irrespective of segregation errors [92]. Importantly, it was also shown that whole chromosome imbalances are not sufficient to induce a p53-dependent cell cycle arrest, and that at least a subset of whole chromosome aneuploidies can be tolerated and propagated in p53-proficient cells [39,61,90]. These findings would support the notion that p53 cannot sense aneuploidy *per se*. Nevertheless, p53 seems to be activated when karyotypes deviate beyond a certain threshold [66,90]. This is possibly due to the increased stress levels that result from a greater increase in protein imbalance, which, in turn, can generate DNA damage [60,64,66–68] (Figure 2). There is mounting evidence that there are additional p53-independent pathways at play in the tolerance towards aneuploid karyotypes. First, yeast lack a *TP53* gene, so the poor tolerance to aneuploidy in yeast results from p53-independent responses. Furthermore, it was shown that loss of p53 does not overcome the growth impairment of aneuploid cells [39]. Importantly, aneuploidy was found to precede TP53 mutations in different cancers [93,94]. Thus, it is likely that cancer cells undergo additional modifications that allow rapid propagation of developing aneuploidies. Indeed, it has been shown that overexpression of the transcription factor HSF1 can counteract the detrimental effects of aneuploidy in protein folding in human aneuploid cells [95]. Furthermore, it was recently shown that loss of p38 might provide aneuploidy tolerance in a p53-independent manner by boosting glycolysis [96]. Additionally, Caspase-$2^{-/-}$ mice display premature aging and increased tumorigenesis with a higher tolerance to aberrant karyotypes [97–99]. Although Caspase-2 has been implicated in p53 activation [100,101], recent evidence suggests that Caspase-2 also contributes to aneuploidy tolerance in a p53-independent manner, by activation of the mitochondrial apoptotic pathway [101].

Finally, while most aneuploidy tolerance mechanisms identified to date seem to involve the inhibition of apoptosis, there is evidence from yeast suggesting that mutations resulting in enhanced proteasomal degradation can also promote the proliferation of aneuploid cells [40]. If such mutations also exist in mammalian cells, and whether these could contribute to aneuploidy tolerance in cancer, remains to be determined.

Segregation Errors and the Immune Response

In recent years, important data has accumulated on a novel aspect that occurs in response to abnormal karyotypes: activation of the innate immune system associated with chromosome segregation errors. This feature has attracted significant interest given the possible relevance of these findings with regards to immuno-based treatments that are rapidly advancing in cancer therapy. A recent publication showed that when co-cultured with natural killer cells, aneuploid retinal pigment epithelium-1 (RPE-1) cells with complex karyotypes were selectively cleared [66]. Coincidentally, several parallel studies showed that micronuclei trigger an inflammatory response by activating the cGAS–STING cytosolic DNA sensing pathway upon membrane rupture [102,103]. cGAS is a cyclic GMP-AMP synthase which is activated upon binding to DNA in the cytoplasm, and triggers the innate immune system by activating a STING-dependent **type I interferon** response [104]. Although the **cGAS–STING pathway** represents an important defense mechanism against a large variety of DNA containing pathogens, it also recognizes different sources of endogenous DNA when exposed to the cytoplasm [105]. Besides micronuclei, chromatin bridges are also often associated with nuclear envelope defects, which also result in exposure of DNA to the cytoplasm [63]. Since temporal and local disruption of the nuclear envelope in interphase has been shown to result in the local binding of cGAS to the nuclear DNA [106,107], it is not unlikely that chromatin bridges represent another source for cGAS/STING activation upon chromosome segregation errors [103].

These findings imply that CIN tumors promote activation of the innate immune system, raising the question of whether such activation could be used as a starting point to build up a stronger line of action against tumor progression. Indeed, a recent study in mice show a pivotal role of cGAS in intrinsic antitumor-immunity. The authors showed that an adequate antitumor immunity triggered in a cGAS-dependent manner allows for an efficient adaptive immune response, boosted by the suppression of inhibitory signals of the immune blockade [102]. In stark contrast, another study showed a negative correlation between high levels of arm/whole chromosome copy number variations and a positive response to immune response blockade in melanoma patients [108]. Moreover, others suggest that the innate immune response triggered by ongoing CIN in tumors can actually promote cellular invasion and metastasis in a STING-dependent manner [109]. Thus, the inflammatory response associated with segregation errors can act as both tumor suppressive and tumor promoting. Further research is needed to understand the exact mechanisms, not only triggering the response but also affecting tumor development and metastasis.

Concluding Remarks and Future Perspectives

Great advances have been made to understand the effects of aneuploidy on cellular homeostasis, ranging from stress-induced responses, DNA damage, chromoanagenesis, and CIN. However, causal links remain to be uncovered. For example, it is unclear if and how the different stress responses are connected to each other. Although the leading view attributes the negative effects of aneuploidy to mass changes of many genes rather than to a dominant effect of specific genes [110], there appear to be important differences between specific karyotypes. Furthermore, we lack detailed knowledge of the effects of certain imbalances due to their poor tolerance. For example, despite the fact that chromosome losses are equally prevalent in cancer as gains [111], most studies to date exploit trisomic models rather than monosomies (see Outstanding Questions). Thus, how specific stress responses are related to distinct karyotypes is still not completely understood.

A key step in the treatment of cancer would be to understand how cancer cells can tolerate all the known consequences caused by genomic diversification that are detrimental to healthy cells. Importantly, besides the direct effects caused by the imbalances themselves, other indirect factors may also have an effect on tolerance, such as mutational burden, cell type, or microenvironment (see Outstanding Questions). In line with the latter, while several groups have showed that micronuclei that accompany CIN can trigger an innate immune response by the cGAS–STING pathway, others have found a negative correlation between deviant chromosome copy numbers and response to immune checkpoint blockade [108]. Furthermore, it has been shown that the innate immune response triggered by ongoing CIN in tumors can actually promote cellular invasion and metastasis [109]. Further research is crucial to understand when and how ongoing segregation errors may trigger an immune response affecting tumor development and when they may instead promote metastasis (see Outstanding Questions). The remaining challenges ahead thus rely on precisely understanding how different pathways are affected by the presence of an abnormal karyotype (see Outstanding Questions), as well as identifying additional tolerance genes in order to translate this knowledge into clinically relevant solutions.

Outstanding Questions

What modifications in cancer explain their high tolerance to abnormal karyotypes?

What determines the threshold of aneuploidy that results in p53 activation?

Besides micronuclei, are there any additional traits of segregation errors that can trigger an immune response?

What steers the innate immune response towards affecting tumor progression or towards tumor development and metastasis?

What is the specific effect of monosomies in human cells?

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