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Cellular Stress Associated with Aneuploidy

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Summary

Aneuploidy, chromosome stoichiometry that deviates from exact multiples of the haploid compliment of an organism, exists in eukaryotic microbes, several normal human tissues and the majority of solid tumors. Here, we review the current understanding about the cellular stress states that may result from aneuploidy. The topics of aneuploid-induced proteotoxic, metabolic, replication, and mitotic stress are assessed in the context of the gene dosage imbalance observed in aneuploid cells. We also highlight emerging findings related to the downstream effects of aneuploidy-induced cellular stress on the immune surveillance against aneuploid cells.

Introduction

Aneuploidy is a type of chromosomal aberration in which the chromosome number is abnormal (Figure 1A). For organisms whose genomes are carried on multiple chromosomes, aneuploidy encompasses thousands to billions of possible numerical combinations of chromosome numbers. As such, aneuploidy is not a single genetic state but rather a large repertoire of diverse states. The most frequent cause of aneuploidy is chromosome missegregation during meiosis or mitosis. Errors in chromosome segregation are often seen in human meiosis, which result in aneuploid gametes, leading to embryos or offspring with aneuploid cells throughout the body (constitutional aneuploidy) (Nagaoka et al., 2012). Sporadic mitotic chromosome missegregation occurs during normal human development and may lead to aneuploidy in fractions of the cells in the body (mosaic aneuploidy). Recent studies suggested that chromosomal mosaicism could be common in human preimplantation embryos (van Echten-Arends et al., 2011). Mitotic error rate is lower at later developmental stages, and therefore most of the cells in adult tissues are euploid (Mantikou et al., 2012). Still, aneuploid cells can be found in certain healthy tissues, although the prevalence of aneuploidy seems to vary with methods used, with spectral karyotyping and fluorescence in situ hybridization (FISH) giving higher estimates than single-cell sequencing. Various studies estimated ~1% to 33% of human neurons to be aneuploid (Cai et al., 2014b; Knouse et al., 2014; Rehen et al., 2001; van den Bos et al., 2016; Vitak et al., 2017), and ~4% to 50% of the normal human primary hepatocytes were aneuploid (Duncan et al., 2012; Knouse

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et al., 2014). The most frequent aneuploidy occurs in cancer cells. Over 90% of solid tumors and 75% of hematopoietic cancers are aneuploid (Weaver and Cleveland, 2006) as a result of chromosomal instability (CIN), which has been extensively reviewed (Funk et al., 2016; Herbert et al., 2015; Thompson et al., 2010).

The past decade witnessed an increasing effort to understand the effect of aneuploidy on cellular physiology. In this review, we aim to summarize the recent progress in understanding the consequences of aneuploidy, with a focus on the various types of cellular stress likely to be elicited by aneuploidy. We first summarize chromosome-specific and genome-wide transcriptome and proteome alterations caused by aneuploidy; we then discuss the effects of these changes on cellular physiology and stress states. We will explore in depth four major types of stress caused by aneuploidy-induced gene expression changes.

Physiological consequences of aneuploidy

Because the cell is a chemical system, normal cell physiology depends on a balanced dosage of gene products. Aneuploidy alters the relative dosage of genes on the affected chromosomes. In animals, constitutional autosomal aneuploidy is highly detrimental. For example, Down syndrome is the only human autosomal aneuploidy that allows survival to adulthood. Down syndrome is caused by the gain of an extra copy of chromosome 21 (trisomy 21) and accounts for ~1/800 live births (de Graaf et al., 2015). Affected individuals display varying levels of physical and mental disability and usually have reduced life expectancy (Carfi et al., 2014; Roper and Reeves, 2006). All the other autosomal aneuploidy results in death in utero or during childhood (trisomy 13, trisomy 18) (Brewer et al., 2002). In laboratory mice, only three trisomies could survive until or beyond birth (Gropp et al., 1983). No live birth for autosomal monosomy has been reported in mammals, suggesting that missing one copy of a chromosome is even more deleterious. The fruit fly *Drosophila melanogaster* can tolerate the loss of a copy of chromosome 4, the smallest chromosome (Bridges, 1921), and monosomy 4 flies display decreased body size compared to diploid flies (Li, 1927). Interestingly, plants tend to be more tolerant of aneuploidy than animals. This is best exemplified by the viability of all 12 possible trisomies in jimsonweed (*Datura stramonium*) (Blakeslee and Belling, 1924). Each of the trisomies shows a distinctive phenotype and grows slower than the diploid plant.

Unicellular organisms exhibit even more varied levels of tolerance to aneuploidy. Extensive aneuploidy was documented in amphibian-killing chytrid fungus *Batrachochytrium dendrobatidis* (Rosenblum et al., 2013), the human pathogen *Giardia intestinalis* (Tumova et al., 2016), and the genus of parasitic protozoa *Leishmania* (Downing et al., 2011; Rogers et al., 2011). It appears that these micro-organisms naturally prefer the aneuploid state over a euploid one. A recent study in *Leishmania donovani* suggested that karyotype fluctuation could allow for selection of beneficial haplotypes under strong selection of the host environment (Prieto Barja et al., 2017). For organisms whose cells exist mostly in the euploid state, recent studies revealed that aneuploidy is generally detrimental to cellular fitness. The proliferation defect of aneuploid cells was noted over 40 years ago when the growth rate of early passage skin fibroblasts from Down syndrome patients were compared with age-matched diploid cells in several tissue culture media (Segal and McCoy, 1974).

More recently, a systematic study, using a collection of disomic budding yeast (*Saccharomyces cerevisiae*) strains, showed reduced growth rates relative to euploid strains under standard culture conditions (Torres et al., 2007). Mouse embryonic fibroblasts (MEFs) trisomic for chromosome 1, 13, 16, or 19 were also found to have proliferation defects (Williams et al., 2008). Similar growth impairment was observed in human colorectal carcinoma HCT116 cells carrying extra copies of chromosome 3 or 5 (Stingele et al., 2012). However, specific aneuploidy can confer growth advantages under specific conditions. The spontaneously generated trisomy 8 mouse embryonic stem (ES) cells outgrew diploid ES cells during passaging in culture (Liu et al., 1997). Similarly, trisomy 12 was found to enhance the proliferation of human pluripotent stem cells (hPSCs) over diploid controls (Ben-David et al., 2014). In the colorectal adenocarcinoma cell line DLD-1, trisomy 13 cells proliferated better than the parental diploid cells in serum-free or fluorouracil-containing media (Rutledge et al., 2016).

The impact of aneuploidy on growth under diverse conditions was investigated in a comprehensive analysis of 38 aneuploid budding yeast strains derived from triploid or pentaploid meiosis (Pavelka et al., 2010). It was found that although under stress-free conditions most, though not all, aneuploids grew more poorly than euploid controls, some aneuploid strains grew significantly better than euploids under suboptimal conditions. Furthermore, different karyotypes appear to confer growth advantages under different conditions. Other studies demonstrated that unicellular organisms, such as budding yeast or the pathogenic fungus *Candida albicans*, although maintaining a euploid state under stress-free conditions or for sexual reproduction, use aneuploidy as a readily available source of heritable variation for evolutionary adaptation to stressful environments (Chen et al., 2012; Kaya et al., 2015; Liu et al., 2015; Pavelka et al., 2010; Ryu et al., 2016; Selmecki et al., 2006; Sunshine et al., 2015; Yona et al., 2012). Accumulating evidence also suggests that specific aneuploid chromosome patterns may be selected during the evolution of cancer (Davoli et al., 2013; Graham et al., 2017).

Effects of aneuploidy on gene expression

Effects of aneuploidy on the transcriptome

A key to understanding how aneuploidy affects cell behavior is the concept of gene dosage effects, which holds that the concentration of the primary gene product is proportional to the copy number of the gene (Epstein, 1986). Indeed, recent studies in different species indicated that aneuploid autosomes are transcribed at a level largely proportionally to their copy numbers (Figure 1B). As a result of the strong correlation between RNA expression level and chromosome copy number, transcriptome analysis revealed that approximately 8% of the yeast haploid gene deletion mutants became aneuploid for various chromosomes (Hughes et al., 2000). Subsequent studies confirmed this correlation in diverse aneuploid yeast strains without gene mutations (Pavelka et al., 2010; Torres et al., 2007). In aneuploid mouse embryonic fibroblast cell lines, the RNA expression from the gained chromosomes was also found to be overall proportional to chromosome copy numbers (Williams et al., 2008). Similar observations were also made in human monosomy 7 myeloid malignancies in a recent single-cell RNA sequencing analysis (Zhao et al., 2017).

Besides the direct dosage effect on the expression of genes located on aneuploid chromosomes, it was shown in yeast that aneuploidy also brings indirect effects to the expression of genes on other chromosomes (Figure 1D and1E) (Pavelka et al., 2010; Rancati et al., 2008). It was found that 43%-78% of these genes were downstream targets of transcription factors encoded on aneuploid chromosomes. A similar indirect effect was also seen in *Arabidopsis* plants with trisomy 5 (Huettel et al., 2008). In a recent study comparing the transcriptome of fetal skin primary fibroblasts from a pair of monozygotic twins discordant for trisomy 21 after normalization to chromosome stoichiometry (Letourneau et al., 2014), 182 genes were found to be significantly differentially expressed but only 6 genes were on chromosome 21. Of these differentially expressed genes not present on chromosome 21, 53 were up-regulated and 123 were down-regulated. In a recent study, 3% of random aneuploid karyotypes were found to disrupt heterochromatin assembly in yeast, leading to expression of genes that are normally silenced and consequently the destabilization of cell identity (Mulla et al., 2017). This perhaps highlights a more dramatic effect of chromosome dosage alteration on gene expression (Figure 1E).

The expression of genes on some aneuploid chromosomes can be subjected to dosage compensation, as exemplified in the triple X syndrome (47, XXX). Unlike most other aneuploidy-associated syndromes, women carrying three copies of the X chromosome usually have normal life expectancy and appear indistinguishable from the rest of the female population (Bittles et al., 2007; Tartaglia et al., 2010). This is due to the silencing of supernumerary X chromosomes mediated by the dosage compensation mechanism of X-chromosome inactivation (XCI) (Payer and Lee, 2008). XCI works through the noncoding RNA, *XIST*, inactivating one of the two copies of the X chromosome in diploid females. In triple X females, two of the three X chromosomes are silenced, although some X-linked genes escape the silencing and their increased expression leads to the mild phenotype of the triple X syndrome. The effect of this incomplete silencing is also seen in Klinefelter syndrome patients (47, XXY) (Brown et al., 1991). Aneuploidy of autosomes could also be dosage-compensated in some species. A recent study in *Drosophila* S2 cells, which contain segmental aneuploidy, found that dosage compensation not only occurs for the X chromosomes but also for aneuploid autosomes, although this compensation was imperfect and resulted in a sublinear relationship between copy number and gene expression (Zhang et al., 2010). Autosomal dosage compensation was also noted in maize and the common wheat *Triticum aestivum* L. (Makarevitch et al., 2008; Zhang et al., 2017). In the ten studied aneuploid wheat strains, 50-90% of the expressed genes on a given aneuploid chromosome were found be subjected to dosage compensation. Gene Ontology (GO) analysis revealed that genes showing dosage effects or dosage compensation were enriched for distinct terms, suggesting that dosage compensation is more likely to be due to functional adaptation rather than a structural mechanism. There is also a recent debate on whether dosage compensation happens in wild strains of aneuploid yeast (Gasch et al., 2016; Hose et al., 2015; Torres et al., 2016). Differences in how dosage compensation was assessed may account for the discrepancy. Gasch et al. used a gene specific approach that may account for gene-to-gene variability, while Torres et al. took a chromosome level distribution-based approach. Thus, these two studies may be focused on two sides of the same coin: while there may be no system-level dosage compensation, the expression change of specific genes and pathways

may deviate from that predicted by gene dosage change either due to additional adaptive mechanisms or simply as a downstream consequence of other changes in the complex gene regulatory network.

Effects of aneuploidy on the proteome

Do the chromosome-dosage–induced transcriptomic changes also translate into the protein level? In budding yeast, changes in protein abundance of genes on aneuploid chromosomes generally scale with changes in DNA copy numbers (Figure 1C). This conclusion was derived from analysis of aneuploid strains with different chromosome stoichiometry using either the multidimensional protein identification technology (MudPIT)-based (Pavelka et al., 2010) or the stable isotope labeling with amino acids in cell culture (SILAC)-based mass spectrometry (Torres et al., 2010). In human cell lines, the levels of protein expression of genes on gained aneuploid chromosomes were also found to increase with chromosome copy number. However, it was less pronounced when compared with RNA expression. For instance, SILAC analysis of HCT116 cells tetrasomic for chromosome 5 in comparison with a diploid cell line found the median ratio of protein levels to be 1.6-fold for genes encoded on chromosome 5 (Stingele et al., 2012). A similar observation was made in a stable near-haploid leukemia cell line disomic for chromosome 8 (Burckstummer et al., 2013). This suggests that some genes on the aneuploid chromosome may not be translated efficiently or the protein products may be unstable. To this end, Dephoure *et al.* used 12 disomic yeast strains to investigate the impact of aneuploidy on the rate of translation (Dephoure et al., 2014). They found ~20% of proteins to be expressed at a lower level than expected based on copy number changes, and the majority of these proteins were components of multi-subunit complexes. Ribosomal footprint analysis did not reveal decreases in translation efficiency, whereas protein degradation seemed to be involved in the observed dosage compensation. In a recent study analyzing a group of evolutionarily conserved non-exponentially degraded (NED) proteins, it was found that NED proteins are degraded more quickly immediately after translation and are more stable later (McShane et al., 2016). Many NED proteins are core components of heteromeric protein complexes but are produced in super-stoichiometric amounts relative to their exponentially degraded (ED) counterparts. If NED proteins are encoded on a gained aneuploid chromosome, only a fraction of the extra NED proteins would be stabilized by complex formation while the rest degraded rapidly. As such, these proteins would not express as highly as predicted based on gene copy number.

Aneuploidy-associated stress

The reduced fitness of most aneuploid cells in organisms that are normally euploid led to the hypothesis that aneuploidy introduces certain stress, likely due to the unbalanced global gene expression as discussed in the above sections. While the copy-number effects predict that gene expression changes in aneuploid cells should be largely dependent on the identity of aneuploid chromosome(s), studies have looked for common gene expression signatures in multiple aneuploid models. For example, transcriptome analysis of a set of disomic budding yeast strains revealed that many, particularly disomy 4, 8, 15 and 16, exhibited gene expression signatures similar to those encompassed within the yeast environmental stress response (ESR) (Torres et al., 2007). Yeast ESR denotes the global expression programs in

response to a diverse stress conditions, such as oxidative stress, heat shock and osmotic shock (Gasch et al., 2000). 615 of the ESR genes showed RNA-level changes in many disomic strains. A follow-up meta-analysis of transcriptional changes in aneuploid cells from diverse organisms, including *Arabidopsis thaliana* (trisomy 5), MEFs (trisomy 1, 13, 16, and 19) and human cells (trisomy 13, 18, and 21) (Sheltzer et al., 2012), found that the ESR-like signature in aneuploids could be partially attributed to the growth defect, which is consistent with a recent finding that ESR signature can largely be explained by a redistribution of cells across different cell cycle phases (O'Duibhir et al., 2014). Another study analyzed the transcriptional profiles of aneuploid human cell lines including 11 trisomic and tetrasomic cell lines and two cell lines with complex aneuploid karyotypes (Durrbaum et al., 2014). A unique aneuploidy response pattern (ARP) was identified, characterized by upregulation of genes involved in diverse functions such as the ER, Golgi and lysosome-related pathways, MHC complex and antigen processing, and metabolic pathways. These findings led to the notion that aneuploidy, regardless of specific karyotype, results in some general stress state (Figure 2 and Table 1), but the mechanism and generality of the stress identified in various studies using specific aneuploid strains or cell lines remain to be further elucidated. Furthermore, the extent of the stress could also depend on the karyotype, ploidy level, and cell type or tissue origin. Below we review the current evidence for the association of several different types of cellular stress with aneuploidy.

Proteotoxic stress

Because of unbalanced gene expression from alterations in chromosome stoichiometry in aneuploidy, it is natural to speculate that aneuploid cells may experience proteotoxic stress, broadly referring to the overburdening of cellular systems that maintain proper protein folding and homeostasis (Deshaies, 2014; Morimoto, 2008). Multi-subunit protein complexes, either structural or enzymatic, require well-defined stoichiometry to function properly, which is thought to be accomplished by tightly regulated and balanced expression of complex components (Kaizu et al., 2010). A consequence of unbalanced production of proteins could be impairment of specific cellular functions associated with the affected protein complexes. For example, gain of chromosome 6, carrying the gene encoding β-tubulin, causes lethality in yeast. However, this lethality can be rescued by additional gain of chromosome 13, carrying α-tubulin genes, thus restoring the stoichiometry of α/β-tubulin dimers (Anders et al., 2009). Another consequence of unbalanced gene expression could be a general loss of proteostasis (Dai and Sampson; Kaushik and Cuervo, 2015). It is thought that aneuploid cells, carrying extra copies of one or several chromosomes, would exhibit an overproduction of proteins relative to the chaperone systems needed to fold nascent polypeptides or the degradation systems that remove misfolded or damaged proteins (Donnelly and Storchová, 2015). This could result in accumulation of misfolded proteins as well as titration of chaperones or protein degradation machineries away from the cellular functions that depend on those activities.

Up-regulation of the ubiquitin-mediated proteasome pathway has been observed in neuronal cells from Down syndrome patients (Engidawork and Lubec, 2001), suggestive of an increased burden in protein turnover. Consistently, these cells were hypersensitive to heat or ER stressors. Unlike control euploid cells, in which expression of heat shock proteins (HSP)

increased in response to heat shock stress, HSP90 and HSP70 expression showed no changes under a moderate heat shock in aneuploid cells (Aivazidis et al., 2017). This is likely due to a pre-existing stress state in the aneuploid cells that compromises the induction of HSP expression. Indeed, in other trisomic human cell lines, overexpression of the transcription factor heat shock factor 1 (HSF1) could attenuate the negative effects of the extra chromosome on protein folding (Donnelly et al., 2014). Phenotypes suggestive of proteotoxic stress were also observed in certain aneuploid yeast strains. For example, yeast strains carrying chromosome 4, 12, 13, 14 or 16 disomy were hypersensitive to the proteasome inhibitor MG132 (Torres et al., 2007). Most of yeast disomic strains also showed moderate proliferative defects in the presence of the Hsp90 inhibitor, geldanamycin, or when exposed to a temperature slightly higher than the optimal yeast growth conditions. By monitoring Hsp104-associated protein aggregates in the same series of disomic yeast strains, an increased load of protein aggregation and altered kinetics of heat adaptation were observed in 11 of 13 disomic strains.

A bottom-up approach to investigating the stress state associated with aneuploidy was to identify mutations that alter the fitness of aneuploid cells. In one study, 13 disomic strains were evolved for improved growth, and mutations associated with the adaptation were analyzed (Torres et al., 2010). A loss-of-function mutation in *UBP6*, encoding a deubiquitinating enzyme regulating proteasome-mediated degradation and ubiquitin recycling, rescued the growth defect in 4/11 disomic strains. Importantly, deletion of *UBP6* allowed for attenuation of protein overexpression in disomy 5 and disomy 14 strains (Dephoure et al., 2014), possibly through enhancing the ubiquintin-proteosome system (UPS). In another study, Dodgson et al. performed a genome-wide screen to find synthetic interaction between single-gene deletion and chromosome disomy in yeast (Dodgson et al., 2016). This screen identified genes enriched for the GO term “vesicle-mediated transport”. Consistently, protein trafficking pathways were found to be crucial for the growth of certain disomic strains. Later, a mutation in the deubiquitinase *UBP3* gene was found to impair the fitness of 6/10 disomic strains by enhancing proteotoxic stress (Oromendia et al., 2012). Taken together, indications of proteotoxic stress were observed in many but not all yeast disomic strains. In line with the observation with yeast aneuploid strains, studies in mammalian cells revealed that enhancing HSP90-dependent protein folding reduced the proliferation defects of trisomic and tetrasomic human cell lines (Donnelly et al., 2014), whereas inhibition of HSP90 exacerbated the growth defect of several trisomy mouse fibroblast cell lines (Tang et al., 2011).

Other than UPS, activation of lysosomal-mediated autophagy was also detected upon aneuploidy induction in human HCT116 and retinal pigment epithelial RPE-1 cell lines (Stingele et al., 2012). Increased p62/sequestosome expression, generally triggered by oxidative stress to sequester misfolded protein into aggregates, was found in aneuploid cells. Notably, an increase in the active autophagy marker, LC3, was observed in trisomic cell lines, suggesting that p62-dependent autophagy was activated in aneuploid cells to modulate protein homeostasis. Another study found that lysosomal stress responses were activated following chromosome missegregation and this response was important for the survival of aneuploid cells (Santaguida et al., 2015). p62 accumulation and activation of unfolded

protein response were also observed in HeLa and mammary gland epithelial cell lines induced to undergo chromosome missegregation (Ohashi et al., 2015).

In sum, evidence for the presence of proteotoxic stress has been reported in studies using a variety of approaches and in model systems ranging from yeast to human cells. The results corroborate the idea that over-expression of genes associated with aneuploid chromosomes leads to disrupted proteostasis. However, there are also reasons for caution when drawing a general conclusion. Many of the studies use specific aneuploid strains or cell lines that were limited to a small number of karyotypes, compared to the vast number of possible chromosome number combinations. In fact, in many aneuploid yeast strains with simple or complex chromosome stoichiometry (Pavelka et al., 2010), a propensity for protein aggregation was not evident (our unpublished observations). Studies that involved acute induction of aneuploidy in mammalian cell lines may be complicated by the possible presence of genotoxic or metabolic stress in these cells, which are also known to induce autophagy (Balaburski et al., 2010; Santaguida et al., 2017; Santaguida et al., 2015; Soto et al., 2017; White, 2016; Xiao et al., 2015). Finally, the extent to which an aneuploid genome can tolerate extra protein expression may vary greatly from karyotype to karyotype. For example, gaining chromosomes that carry major chaperones or activators of UPS may render cells more tolerant to proteotoxic stressors (Chen et al., 2012; Kalapis et al., 2015).

Metabolic stress

The metabolic homeostasis of cells depends on a precise coordination of metabolic pathways, which depends on the stoichiometry of specified sets of enzymes and regulators. As such, it is unsurprising that metabolic alterations have been linked to aneuploid-induced stress. Disomic yeast strains exhibited changes in nucleotide and carbohydrate metabolism as well as an increased glucose uptake when compared to euploids, which may be due to upregulation of some genes encoding glucose transporters (Torres et al., 2007). However, the same study also showed that biomass production is decreased within these aneuploid cells. Amino acid levels (with the exception of aspartate and isoleucine) and many TCA cycle intermediates were found to be increased in a yeast disomy 4 (Thorburn et al., 2013). However, these changes were not observed in other disomies examined, which suggests that metabolic defects may be as difficult to generalize as the proteotoxic stress discussed above.

Altered metabolism was also observed for aneuploid mammalian cells. In MEFs trisomic for one of four specific chromosomes, each trisomy displayed alterations in glutamine use and the production of ammonium and lactate, and a subset of karyotypes exhibited increased glucose uptake (Williams et al., 2008). Trisomic and tetrasomic HCT116 cell lines generated through microcell-mediated chromosome transfer (MMCT) showed specific downregulation in proteins involved in DNA, RNA, and carbohydrate metabolism, whereas other pathways such as mitochondrial metabolism were upregulated (Stingele et al., 2012). The observed alteration of DNA metabolic pathways is consistent with DNA replication defects that have also been noted for aneuploid cells (see following section). Aneuploid cells from mice bearing a conditional knockout of *Mps1*, encoding a mitotic checkpoint kinase, showed a set of significantly overexpressed genes involved in cell metabolism (Foijer et al., 2014). More recently, highly aneuploid colorectal cells were found to be sensitive to an antagonist of

ceramide glucosyltransferase due to overabundance of intracellular ceramide, possibly as a result of dysregulated sphingolipid metabolism (Tang et al., 2017). It was also shown that ceramide levels were increased in some aneuploid budding yeast strains and further increasing ceramide levels either genetically or pharmacologically could slow down their proliferation (Hwang et al., 2017).

There are indications that the metabolic defects associated with aneuploidy are accompanied by an increase in reactive oxygen species (ROS) levels. ROS are free radicals derived from molecular oxygen, usually as a result of metabolic output (Apel and Hirt, 2004). In yeast disomic strains, proteomic analyses revealed upregulation of genes involved in oxidative stress response pathways (such as thioredoxins and oxidoreductases) and these cells harbored higher levels of ROS (Dephoure et al., 2014). Aneuploid MEFs also contained more ROS than control diploid cells (Li et al., 2010). It remains to be understood how metabolic changes could lead to altered redox regulation in aneuploid cells. A consequence of increased ROS is oxidative DNA damage. ROS produced in aneuploid MEFs were partially responsible for activating the DNA damage checkpoint (Li et al., 2010). ROS can also lead to proteomic damage and thus potentially contributes to the proteotoxic stress discussed earlier. Interestingly, in a *Drosophila* model of aneuploidy, ROS play a role in activating a JNK-dependent apoptotic clearance response (Clemente-Ruiz et al., 2016). These observations point to potential interplay between different types of stress experienced by aneuploid cells and suggest that molecular signals generated by these stress states may help tissues eliminate aneuploid cells.

Replication stress

Similar to the intricate pathways involved in maintaining metabolic homeostasis, accurate eukaryotic DNA replication requires the precise choreography and proper stoichiometric balance of many proteins and protein complexes. DNA replication starts from various chromosomal sites, termed replication origins, in a two-step process involving origin licensing followed by origin firing (Masai et al., 2010). Replication licensing relies on an origin recognition complex that works with CDC6 and CDT1 to recruit the replicative helicase, MCM2-7. Several more protein complexes are then loaded to form the pre-initiation complex. Not all licensed origins will fire, and dormant origins are passively replicated or can be activated during times of replication stress to ensure faithful genome replication (Alver et al., 2014). At the start of S-phase, replication forks proceed bidirectionally from an origin forming a replication bubble. Since aneuploidy imparts changes in protein stoichiometry, one consequence of aneuploidy is impaired functionality of DNA replication complexes.

Replication stress refers to the slowing or stalling of replication fork progression (Mazouzi et al., 2014). Stalled replication forks can either be repaired following cell cycle checkpoint activation or further break down resulting in DNA double-strand breaks (DSBs). Replication stress leads to delay or complete arrest of the cell cycle. Indeed, several recent studies highlight replication stress caused by aneuploidy. For example, induction of simple aneuploidy in RPE-1 cells with an MPS1 inhibitor, which was defined by cells with genome imbalances that contained less than 5% of their genome, was shown to lead to a significant

reduction in fork rate and increased replication fork stalling when compared to untreated euploid cells (Santaguida et al., 2017). Slower replication rates were also observed in trisomic and tetrasomic cells derived from both RPE-1 and HCT116 cell lines (Passerini et al., 2016). The replication defects observed were in part related to an imbalance in the production of the six subunits of the MCM2-7 helicase. This finding was consistent with a previous dataset from the same group using similar aneuploid cell lines that found a general downregulation of proteins involved in DNA replication (Stingele et al., 2012).

In addition to general replication defects such as reduced fork progression rate, some chromosome elements have also been shown to contribute to aneuploidy-associated replication stress. For example, induction of aneuploidy in fibroblasts through shRNAs knockdown of genes required for mitotic fidelity led to reduced proliferation potential and telomere-related replication stress, and this stress was rescued by expression of telomerase (Meena et al., 2015). In aneuploid cancer cells, the overall replication timing profiles appear to differ from euploid non-transformed cells. Notably, replication of usually synchronous loci became asynchronous in cells of breast cancer patients that had a higher occurrence of chromosome 17 aneuploidy (Grinberg-Rashi et al., 2010). Similarly, non-alcoholic fatty liver disease and cryptogenic cirrhosis tissues displaying high prevalence of aneuploidy showed a more asynchronous replication of two loci compared to controls where the loci replicate synchronously (Laish et al., 2016). A significant increase in centromeric replication asynchrony accompanied by a high frequency of aneuploidy in lymphocytes of hepatocellular carcinoma patients compared with those of liver cirrhosis patients and healthy control participants was also reported (Hanna et al., 2012). These studies suggest that aneuploidy can cause a disruption of the normal replication timing. However, more precise testing such as a replication timing analysis between isogenic euploids and aneuploids should be conducted to further identify differences outside of the complex cancer genome. In general, the current data has revealed various abnormalities in DNA replication associated with aneuploidy but a clear shared pattern has yet to emerge.

What are consequences of the increased levels of replication defects in aneuploid cells? Increased DNA damage, especially in the form of DSBs or increased mutational load, has been shown to accompany replication stress in aneuploid cells. DSB accumulation could be due to aberrantly exposed single-stranded DNA because of slowed or stalled replication forks. Budding yeast disomic strains contained elevated levels of DNA damage, evidenced by the accumulation of 53BP1 foci during S-phase (Blank et al., 2015). Further experiments revealed increased mutational rates for two assayed loci when compared to euploid yeast (Sheltzer et al., 2011). The mutations observed were reminiscent of mutations caused by a translesional DNA polymerase (Pol ζ). When the catalytic subunit of Pol ζ was deleted, the mutational rate of the aneuploids decreased. This study revealed that aneuploid cells may replicate some of their DNA with different polymerases than euploid cells. Consistent with results in budding yeast, DNA damage markers such as 53BP1 foci accumulated in aneuploid human RPE-1 cells (Santaguida et al., 2017). The accumulation of break point junction patterns suggestive of replication defects was observed in specific trisomic and tetrasomic human cells (Passerini et al., 2016).

In addition to increased DNA damage, aneuploid cells experiencing replication stress (including those harboring DNA damage) are also subject to several other fates, such as cell cycle delays, DNA condensation defect, inappropriate mitotic entry, senescence, and even immunological recognition and destruction (Andriani et al., 2016; Blank et al., 2015; Burrell et al., 2013; Lamm et al., 2016; Meena et al., 2015; Santaguida et al., 2017; Soto et al., 2017). Cell cycle delays associated with replication stress may be a cause of the perturbation in cell cycle/proliferative dynamics observed for aneuploid cells in the past (Segal and McCoy, 1974; Stingele et al., 2012; Williams et al., 2008).

Mitotic stress

Variation in chromosome copy number may lead to altered stoichiometry of proteins involved in the chromosome segregation machinery or spindle assembly checkpoint. Thus aneuploidy could potentially lead to mitotic stress and promote continuous generation of karyotype diversity. Multiple aneuploid budding yeast strains, generated via triploid meiosis, were found to experience repeated, nonrandom mitotic chromosome loss, suggesting aneuploidy could interfere with the fidelity of chromosome segregation (Campbell et al., 1981). This phenomenon was further explored more recently in aneuploid budding yeast. For example, using a yeast artificial chromosome (YAC) containing human DNA, 9/13 disomic strains were found to have a higher rate of YAC missegregation relative to the haploid control (Sheltzer et al., 2011). Another study systematically investigated the association of CIN with aneuploidy in diverse aneuploid progeny from triploid meiosis (Zhu et al., 2012). Evidence for genome-level and chromosome-specific determinants of CIN were revealed through tracking of karyotype changes in these aneuploid populations. For instance, aneuploid strains with equal copy of chromosomes 7 and 10 were karyotypically more stable than when the copy numbers of these two chromosomes were unequal. This effect may reflect a requirement for balanced dosage of spindle assembly checkpoint genes *MAD1* (on chromosome 7) and *MAD2* (on chromosome 10) for proper checkpoint function. On the genome level, the mitotic error rate appears to be positively correlated with the difference in total chromosome number of an aneuploid cell from the lower one of the closest euploid number. For example, karyotypes of 1N plus one or a few extra chromosomes tend to be more stable than those of 2N minus one or a few chromosomes. It was speculated that this was due to a growing deficit between the functional capacity of the mitotic system and an increasing chromosome segregation load.

In mammalian cells, the effect of aneuploidy on mitotic fidelity has been less clear. It was first reported that in human colon cancer cells the deviation of ploidy level from euploid was correlated with the level of CIN (Duesberg et al., 1998). When lymphocytes from Turner's syndrome (monosomy X) patients were triggered to divide, higher levels of aneuploidy for three randomly selected chromosomes were observed relative to diploid lymphocytes (Reish et al., 2006). This was also confirmed in lymphocytes from constitutional autosomal trisomies (trisomy 13, 18 or 21) (Reish et al., 2011). A later study, however, did not report elevated rates of chromosomal mosaicism in fibroblast cells from trisomy 13, 18 and 21 patients (Valind et al., 2013). The discrepancy could be attributed to differences in cell types or in the detection methods used. A recent study directly analyzed the frequency of anaphase lagging chromosomes in aneuploid and diploid cells (Nicholson et al., 2015). In the

colorectal cancer cell line DLD-1, trisomy 7 or 13 cells were found to display higher frequencies of anaphase lagging chromosomes compared to the parental diploid cells. Increased frequencies of anaphase lagging chromosomes were also observed in amniotic fibroblasts from trisomy 13 fetuses. However, another study in aneuploid cells constructed from HCT116 or RPE-1 did not find a significant increase in the frequency of lagging chromosomes (Passerini et al., 2016). Differences in cell lines or karyotype could account for the discrepancy.

Is chromosome missegregation karyotype-specific or a general effect of aneuploidy in human cells? In the above examples, human aneuploid cells were either isolated from patients or engineered using MMCT. As such, these studies were limited to the analysis of simple karyotypes. Santaguida *et al.* treated RPE-1 cells with MPS1 inhibitor to generate a diverse aneuploid cell population (Santaguida et al., 2017). Followed by the washout of MPS1 inhibitor and thus in the presence of a functional SAC, 80% of the aneuploid daughter cells continued to divide and their mitotic fidelity was followed through live cell microscopy. These cells were found to exhibit a high frequency of mitotic aberrations during mitosis, lagging chromosomes during anaphase, and micronuclei in the subsequent G1. These results argue that aneuploidy could generate mitotic stress and drive chromosome instability. In addition to the two mechanisms observed in yeast, another possibility is that aneuploidy induced replication stress could impair proper mitotic chromosome segregation. For instance, unresolved replication intermediates could persists into anaphase, leading to nondisjunction of the interconnected sister chromatids (Kawabata et al., 2011). Also replication stress-associated DNA damage could cause structurally abnormal chromosomes that missegregate during mitosis, fueling a vicious cycle of genomic instability (Burrell et al., 2013).

Emerging topics

An increasing body of experimental data supports the notion that aneuploidy-associated stresses could potentiate the immunogenicity of aneuploid cells. RNA-seq analysis on fibroblasts from 12 age- and gender-matched euploid and Down syndrome individuals revealed consistent genome-wide gene expression changes in aneuploid fibroblasts that can be attributed to a transcriptional response to interferon (IFN), and this finding was confirmed in blood samples from Down syndrome individuals (Sullivan et al., 2016). Hyper-activation of the IFN pathway could be due to the fact that four of the six interferon receptors are encoded on chromosome 21 in human, and this could explain the shared phenotypes between Down syndrome and hyperactive IFN signaling disorders (“interferonopathies”) (Crow and Manel, 2015). Intriguingly, an “Interferon alpha/beta signaling” gene set was also found to be overexpressed in RPE-1 cells that became aneuploid due to chromosome missegregation. The cGAS-cGAMP-STING (stimulator of interferon genes) pathway (Cai et al., 2014a), the main mechanism for sensing cytoplasmic DNA, was also activated (Santaguida et al., 2017). In addition, these aneuploid cells also suffered from the DNA damage-induced proinflammatory senescence-associated secretory phenotype (SASP), which was also observed in aneuploid human primary fibroblasts (Andriani et al., 2016). These observations suggest that the aneuploid state could be proinflammatory.

Coincidentally, two recent studies reported that the activation of cGAS-cGAMP-STING pathway could be attributed to the formation of micronuclei after missegregation of a chromosome or chromosome fragment (Harding et al., 2017; Mackenzie et al., 2017). Micronuclei cause DNA replication defects that alter the structure of chromosomes encapsulated within them, leading to increased DNA damage and in extreme cases chromothripsis (Crasta et al., 2012; Zhang et al., 2015). Defects of the nuclear envelope around the micronuclei could expose chromosomal DNA and activate the cGAS-cGAMP-STING pathway (Harding et al., 2017; Hatch et al., 2013; Mackenzie et al., 2017). Interestingly, aneuploidy in cancer cells was found to correlate with a reduced number of infiltrating cytotoxic T cells and an increased number of M2 (immune suppressive) macrophages (Davoli et al., 2017). A new study suggested that chromosomally unstable aneuploid cancer cells, though activating STING, suppressed the downstream canonical NF-κB or IFN signaling (Bakhoum et al., 2018). Instead, these cells upregulated the non-canonical NF-κB pathway, thereby engaging immune mimicry and promoting metastasis.

Concluding remarks

In this review, we have discussed the impact of aneuploidy on the cellular transcriptome and proteome, and the resulting phenotypic consequences at both organismal and cellular levels. Several cellular stress states have been observed to be associated with aneuploidy: proteotoxic, metabolic, replication, and mitotic (Table 1). Responses to these stress states may define a set of characteristics shared by at least some, if not all, aneuploid cells of diverse karyotypes. It is important to point out that the severity of these stress states may not be equally distributed across the karyotypic spectrum, and that specific aneuploid karyotypes may even help alleviate certain stress. In unicellular organisms, karyotypic diversity constitutes the substrate for evolutionary selection. CIN, caused by replication and mitotic stress associated with an unbalanced genome may enhance this diversity. On the other hand, how an aneuploid cell population explores the fitness landscape under the constraint of the fitness loss caused by the inherent stress remains to be better understood. In animals, largely uniform diploidy is likely to be important for most tissues, though exceptions exist such as the varied estimates of aneuploidy frequencies within the brain and liver. Does aneuploidy-associated cellular stress contribute to the signal that helps tissues eliminate sporadic aneuploid cells? This could occur through cell-intrinsic mechanisms such as p53 activation or via activation of immune responses. Finally, metastatic tumor cells adopt aneuploidy as a way of life, much like microbial organisms that are constitutively aneuploid. Could there be shared mechanisms that allow these populations to alleviate or cope with stress associated with the varied gene stoichiometry? Insights into these outstanding questions would provide a deeper understanding of aneuploidy in somatic evolution and tumorigenesis. Furthermore, both karyotype-specific phenotypes and the general stress associated with aneuploidy, if it exists and can be found, may be exploited for cancer therapy.

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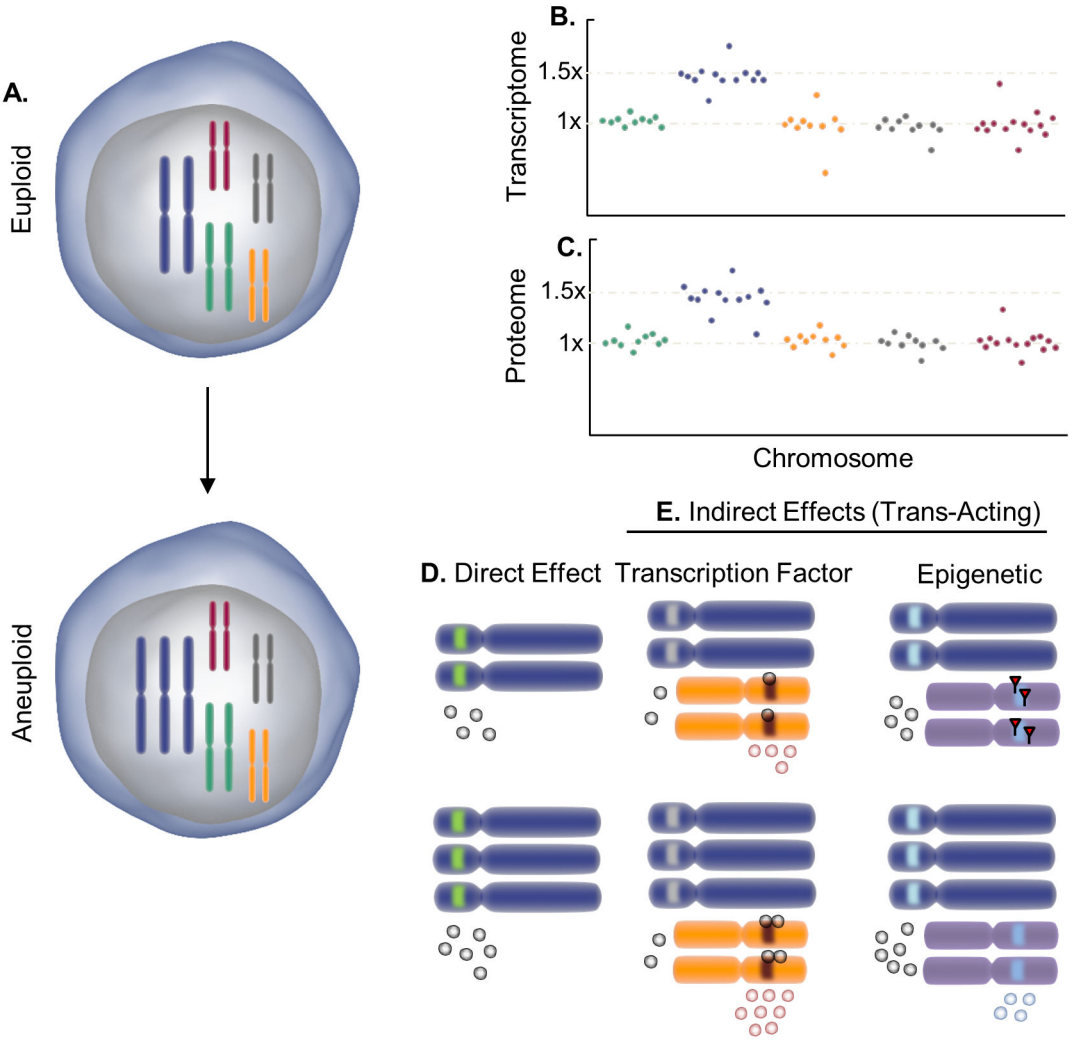

Figure 1.

Effects of aneuploidy on the transcriptome and proteome due to the alteration of gene dosage. Changes to relative chromosome dosage (A) in aneuploidy (shown here as a euploid cell becoming trisomic for the blue chromosome) results in scaled changes to the RNA (B) and protein (C) expression level of genes carried on the aneuploid chromosome as is the case for direct effects of aneuploidy (D). However, indirect effects or trans-activating effects (E) of aneuploidy can alter the expression of genes on other chromosomes by increasing transcription factor levels to promote expression or by having an epigenetic effect such as de-silencing genes. Globular circles denote gene products that scale accordingly with chromosome number.

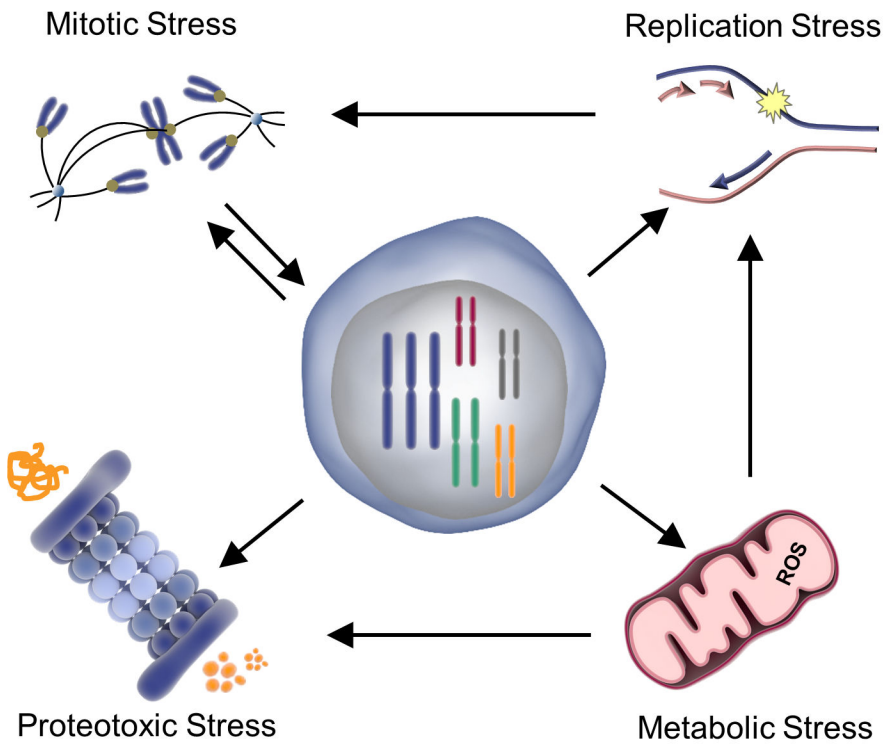

Figure 2.

The major categories of aneuploidy-associated stresses discussed in this review. As shown by the black arrows pointing to the four stresses. Aneuploidy leads to mitotic stress such as lagging chromosomes, replication stress via stalled and delayed replication fork progression, metabolic stress usually related to increased ROS levels in the mitochondria, and proteotoxic stress that overwhelms the proteasome. In addition to aneuploidy causing stress, some of the stresses can further perpetuate aneuploidization through causing different stress and increasing CIN (black arrows pointing between stressed and back toward aneuploidization). For example, aneuploid cells may produce more ROS that can damage DNA leading to replication stress and mitotic error.

Table 1.

List of published work supporting aneuploid-induced cellular stress, species, methods used to generate aneuploidy, and karyotype studied.

	Author	Year	Species/cell type	Aneuploidization technique	Karyotype
Proteotoxic Stress	Torres, E. M. et al.	2010	Budding yeast	[*kar1* × wt] mediated chromosome transfer	Disomies from Torres et al. 2007
	Stingele, S. *et al.*	2012	Human/HCT116 and RPE-1	Micronuclei-mediated chromosome transfer (MMCT)	Trisomies/tetrasomies
	Oromendia, A.B. et al.	2012	Budding yeast	[*kar1* × wt] mediated chromosome transfer	Disomies from Torres et al. 2007
	Dephoure, N. *et al.*	2014	Budding yeast	[*kar1* × wt] mediated chromosome transfer	Disomies from Torres et al. 2007
	Donnelly, N. et al.	2014	Human/HCT116 and RPE-1	MMCT	Trisomies/tetrasomies from Stingele et al. 2012
	Ohashi, A. *et al.*	2015	Human/HeLa	CENP-E inhibition in SAC-attenuated cells	Random aneuploidy
	Aivazidis, S. et al.	2017	Human/lymphoblastoid cell lines	Down syndrome patient cells	Trisomy 21
Metabolic Stress	Torres, E. M. *et al.*	2007	Budding yeast	[*kar1* × wt] mediated chromosome transfer	13 disomic strains
	Williams, B. R. *et al.*	2008	Mouse/embryonic fibroblast	Breeding scheme, Robertsonian translocations	Trisomy 1, 13, 16, or 19
	Li, M. *et al.*	2010	Mouse/embryonic fibroblast	Conditional mutation in *Mad2*	Random aneuploidy
	Thorburn, R. R. *et al.*	2013	Budding yeast	[*kar1* × wt] mediated chromosome transfer	Disomies from Torres et al. 2007
	Dephoure, N. *et al.*	2014	Budding yeast	[*kar1* × wt] mediated chromosome transfer	Disomies from Torres et al. 2007
	Clemente-Ruiz, M. *et al.*	2016	Drosophila	RNAi-mediated knockdown of SAC genes	Changes in X chromosome number
	Tang, Y. *et al.*	2017	Mouse/embryonic fibroblast	Breeding scheme, Robertsonian translocations (Williams et al. 2008).	MEFs (trisomy 13 or 16)
	Hwang, S. *et al.*	2017	Budding yeast	[*kar1* × wt] mediated chromosome transfer	Disomies from Torres et al. 2007
Replication Stress	Sheltzer, J. M. *et al.*	2011	Budding yeast	[*kar1* × wt] mediated chromosome transfer	Disomies from Torres et al. 2007
	Ohashi, A. *et al.*	2015	Human/HeLa	CENP-E inhibition in SAC-attenuated cells	Random aneuploidy
	Meena, J. K. *et al.*	2015	Human/primary fibroblasts	shRNA knockdown of GJB3, RXFP1, OSBPL3, STARD9, or MAD2L1	Random aneuploidy
	Blank, H. M. *et al.*	2015	Budding yeast	[*kar1* × wt] mediated chromosome transfer	Disomies from Torres et al. 2007
	Passerini, V. *et al.*	2016	Human/HCT116 and RPE-1	MMCT	Trisomies/tetrasomies
	Lamm, N. *et al.*	2016	Human/pluripotent stem cells	Acquired aneuploidy during passaging	Gain of chromosomes 17q or 12
	Santaguida, S. *et al.*	2017	Human/RPE-1	SAC kinase MPS1 inhibtion	Random aneuploidy
Mitotic Stress	Reish, O. et al.	2006	Human/lymphocytes	Turner's syndrome patients	Monosomy X

Author	Year	Species/cell type	Aneuploidization technique	Karyotype
Sheltzer, J. M. *et al.*	2011	Budding yeast	[*kar1* × wt] mediated chromosome transfer	Disomies from Torres et al. 2007
Reish, O. et al.	2011	Human/lymphocytes	Patient cells	Trisomy 13, 18 or 21
Zhu, J. *et al.*	2012	Budding yeast	Triploid meiosis	Random aneuploidy
Nicholson, J.M. et al.	2015	Human/DLD-1, amniotic fibroblasts	MMCT, patient cells	Trisomy 7 or 13
Santaguida, S. *et al.*	2017	Human/RPE-1	SAC kinase MPS1 inhibtion	Random aneuploidy