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Review

Gene Copy-Number Alterations: A Cost-Benefit Analysis

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Changes in DNA copy number, whether confined to specific genes or affecting whole chromosomes, have been identified as causes of diseases and developmental abnormalities and as sources of adaptive potential. Here, we discuss the costs and benefits of DNA copy-number alterations. Changes in DNA copy number are largely detrimental. Amplifications or deletions of specific genes can elicit discrete defects. Large-scale changes in DNA copy number can also cause detrimental phenotypes that are due to the cumulative effects of copy-number alterations of many genes simultaneously. On the other hand, studies in microorganisms show that DNA copy-number alterations can be beneficial, increasing survival under selective pressure. As DNA copy-number alterations underlie many human diseases, we will end with a discussion of gene copy-number changes as therapeutic targets.

Introduction

Every species is defined by its karyotype. Maintaining this karyotype is essential for species survival, a truth perhaps best illustrated by the fact that numerous mechanisms have evolved to ensure that the species-specific karyotype is maintained. Changes in the copy number of specific genes, entire chromosomes, or parts thereof can have a dramatic impact on the fitness and reproductive abilities of an organism. Trisomy 21 in humans illustrates this point. Individuals with trisomy 21, also known as Down syndrome, exhibit a number of developmental disabilities and have a significantly reduced life span (Yang et al., 2002). The fact that changes in copy number of small regions of the genome can have a significant impact on fitness is emphasized by genome-wide association studies of genetically complex human diseases. Small-scale often submicroscopic changes in DNA copy number are estimated to be responsible for at least 15% of human neurodevelopmental defects and are associated with psychiatric disorders and kidney and heart defects (reviewed in Girirajan et al., 2011).

Much progress has been made in our ability to identify DNA copy-number alterations and to determine their biological impact. Depending on their size, DNA copy-number alterations are referred to by different terms (Figure 1). Multiples of the entire genetic complement are referred to as polyploidies. Whole-chromosome (34 ~230 Mbp) gains or losses are known as aneuploidies (Figures 1A and 1C). Changes in copy number of subchromosomal regions that are visible by light microscopy are referred to as partial or segmental aneuploidies. Submicroscopic DNA copy-number alterations that are between 1 kbp and 1 Mbp in length are referred to as copy-number variations (CNVs; Figures 1A and 1B). Smaller changes in DNA copy number—ranging 1 bp to 1 kbp in size—are called deletions or insertions depending on whether sequences are deleted or amplified, respectively (Figure 1A) (Feuk et al., 2006; Lupski, 2007). The mechanisms leading to small- and large-scale DNA copy-number changes have been investigated in detail. We refer the reader to several excellent recent reviews that summarize our current understanding of these mechanisms (Alkan et al., 2011; Holland and Cleveland, 2012; Malhotra and Sebat, 2012; Zhang et al., 2009).

Here we discuss the costs and benefits of small- and large-scale DNA copy-number alterations. We will describe examples that illustrate how copy-number alterations of individual genes can result in cellular and organismal abnormalities. We will further discuss findings that show that large-scale DNA copy-number changes, such as whole-chromosome gains and losses, cause general, detrimental phenotypes that result from the cumulative effects of changes in copy number of a large number of genes. The potential benefits of gene copy-number alterations will also be examined, summarizing studies that show that gene copy-number alterations can improve survival of microorganisms under selective pressure. Finally we will consider the possibility of targeting gene copy-number changes in the treatment of human diseases.

The Impact of Copy-Number Changes on Gene Expression

Before considering the biological impact of changes in gene copy number, it is important to address the question of whether such changes are translated into corresponding changes in gene expression or whether mechanisms are in place that ensure wild-type levels of expression irrespective of gene copy number. Such mechanisms, collectively called dosage-compensation mechanisms, exist for sex chromosomes, which naturally vary in copy number between sexes (reviewed in Nguyen and Disteche, 2006). With the exception of *Drosophila* and some

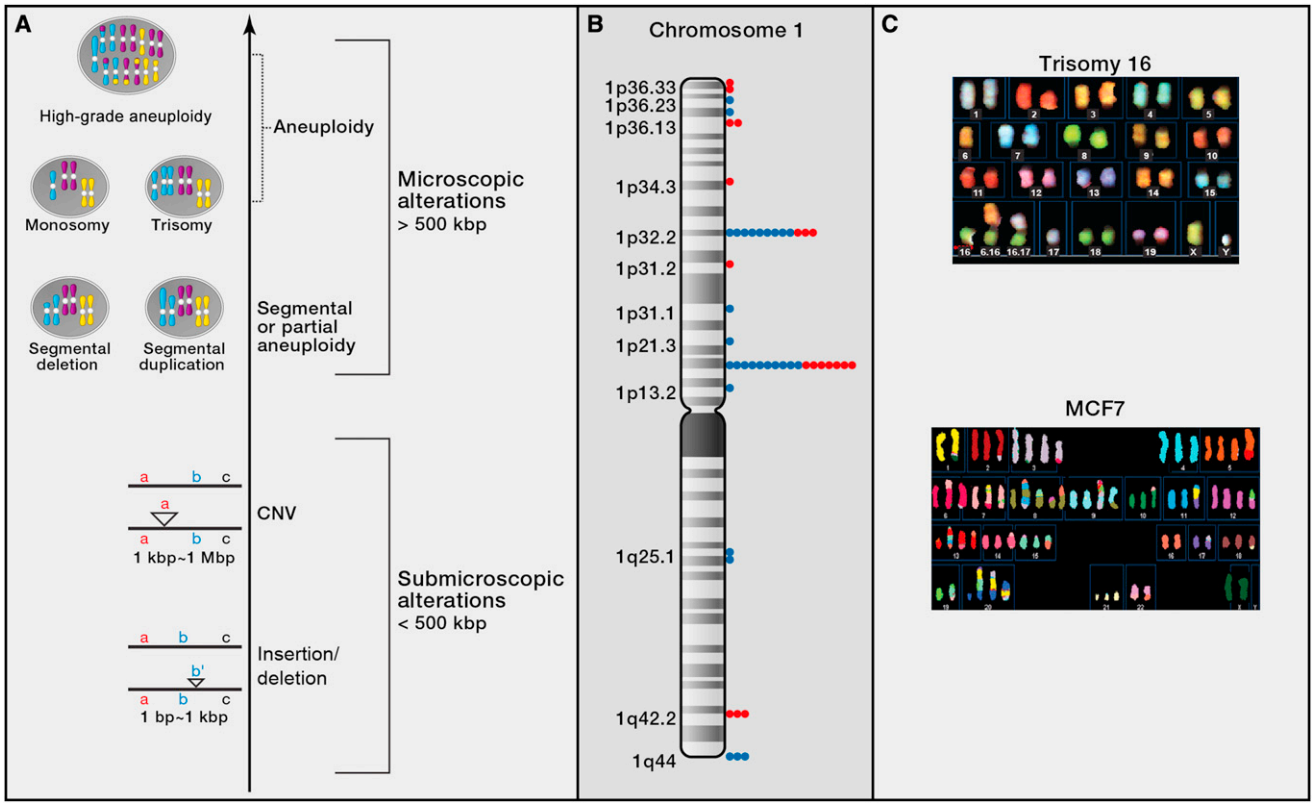

Figure 1. Defining DNA Copy-Number Alterations

(A) DNA copy-number alterations can be categorized into submicroscopic variations, which are smaller than 500 kbp, and microscopic alterations, which are greater than 500 kbp. DNA copy-number changes between 1 bp and 1 kbp in size are called insertions or deletions, depending on whether DNA is gained or lost, respectively. Copy-number variations (CNVs) vary between 1 kbp and 1 Mbp in size. Examples for CNVs are shown for duplication. Microscopically visible karyotype changes are called segmental or partial aneuploidies, when parts of chromosomes are amplified or deleted. Whole-chromosome losses or gains are called aneuploidies.

(B) CNV distribution on human chromosome 1. Dots show the number of individuals with copy gains (blue) or losses (red) among 39 unrelated, healthy control individuals (data from Iafrate et al., 2004).

(C) Spectral karyotyping (SKY) analysis of a trisomy 16 MEF cell line (data from Williams et al., 2008) and a MCF7 breast cancer cell line (data from http://www.path.cam.ac.uk/~pawefish/BreastCellLineDescriptions/mcf7.htm).

plant species (Guo and Birchler, 1994; Kim et al., 2011; Larsson et al., 2001; Miclaus et al., 2011; Stenberg and Larsson, 2011), dosage-compensation mechanisms do not exist for autosomes. Gene copy-number proportional expression of whole chromosomal or segmental aneuploidies has been observed in fission yeast, budding yeast, *Arabidopsis*, trisomic mouse embryonic fibroblasts (MEFs), partially trisomic mouse tissues, and human trisomies (Chikashige et al., 2007; Huettel et al., 2008; Kahlem et al., 2004; Lyle et al., 2004; Pavelka et al., 2010; Torres et al., 2007; Upender et al., 2004; Vacík et al., 2005; Williams et al., 2008; Stingele et al., 2012) (Figure 2). CNVs also typically result in a corresponding change in gene expression. In humans and mice, 85%–95% of CNVs are associated with changes in expression of the affected genes (Henrichsen et al., 2009; Stranger et al., 2007).

In organisms where this has been studied, increases in RNA levels result in increased protein production. Quantitative proteomic analyses in aneuploid budding yeast and human cells showed that changes in gene copy number result in changes in protein levels in the majority of cases (Pavelka et al., 2010; Stingele et al., 2012; Torres et al., 2010). The proteins that do not show this coordinate increase in protein levels with gene copy number are predominantly components of large protein complexes (Stingele et al., 2012; Torres et al., 2010), presumably because unassembled subunits of protein complexes are often unstable. Thus, gene copy-number changes generally translate into changes in gene expression.

The Biological Impact of DNA Copy-Number Alterations

Changes in DNA copy number generally have adverse effects on fitness. The degree of adversity scales with the size of the alteration. Whole-chromosome and segmental aneuploidies invariably lead to severe developmental abnormalities or death of the organism in all species analyzed to date (reviewed in Torres et al., 2008). CNVs are, for the most part, also detrimental. Sequencing of human populations revealed that CNVs are rare, occurring at a frequency of less than 1% (Itsara et al., 2009), and that they are under strong negative selection (Itsara et al., 2010). Insertions and deletions on the other hand are widespread in the human genome, indicating that their impact on fitness is

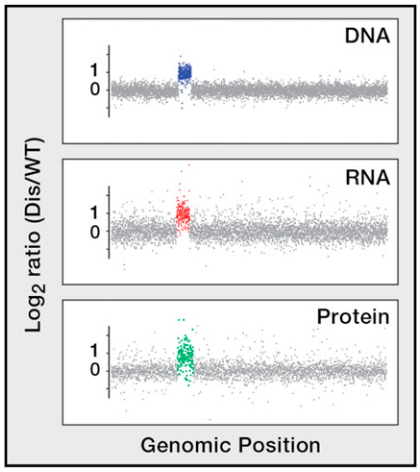

Figure 2. Aneuploid Chromosomes Are Active in Budding Yeast
DNA, RNA, and protein content of a budding yeast strain carrying an additional copy of chromosome 5 (data from Torres et al., 2010).

likely to be minor (Girirajan et al., 2011; Iafrate et al., 2004; Sebat et al., 2004).

How do changes in gene copy number lead to phenotypes? Changes in the copy number of individual genes can cause phenotypes. Phenotypes can also be driven by the cumulative effect of changes in copy number of a large number of genes, which individually have little or no effect. In this section we will discuss the gene-specific and general effects of changes in DNA copy number.

Gene-Specific Effects

It has long been known that changes in the copy number of specific genes can have a dramatic impact on organismal and cellular fitness. For example, budding yeast cells harboring an extra copy of the β-tubulin-encoding gene are inviable (Katz et al., 1990). Studies in humans suggest that as many as 15% of neurodevelopmental disorders and other diseases are due to rare, large CNVs resulting in the imbalance of a handful of genes (for a recent review, see Malhotra and Sebat, 2012). For example, duplication of PMP22 leads to Charcot-Marie-Tooth 1A neuropathy (reviewed in Hanemann and Müller, 1998). Duplication of *SNCA* is associated with Parkinson's disease (Singleton et al., 2003), duplication of *GSK3b* with bipolar disorder (Lachman et al., 2007), and low-copy amplification of the *C4* gene with lupus (Yang et al., 2007). Finally, amplifications and deletions of individual genes are major drivers of tumorigenesis. Amplification of the oncogene Myc, for example, is thought to be a driving factor in human acute myeloid leukemia (AML) (Jones et al., 2010).

Gene-dosage changes of individual genes also appear to be responsible for some of the phenotypes observed in syndromes caused by chromosome-size DNA copy-number changes. For example, chromosome 21-located *APP*, which encodes a protein that when cleaved forms the main component of amyloid-β plaques, has been found to be duplicated in familial forms of early onset Alzheimer's disease. This observation suggests that early onset Alzheimer's disease, which is observed in virtually all individuals with Down syndrome, is due to a second copy of *APP* (reviewed in Kingsbury et al., 2006). Many more examples have been described where imbalances in the copy number of one or a few genes elicit a discrete phenotype, and we refer the reader to recent reviews that provide a comprehensive overview of the contribution of gene copy-number changes to human diseases (Girirajan et al., 2011; Zhong et al., 2009).

LARGE-SCALE GENE COPY-NUMBER CHANGES

Gene-specific effects	B	F	M	A
Changes in gene dosage of a specific gene affect a cellular function i.e., α–β tubulin ratios	X	X	X	
Local and genome-wide genomic instability	X	X	X	

General effects	B	F	M	A
Changes in gene dosage of many genes impact a cellular function–aneuploidy-associated stresses				
Cell proliferation	X	X	X	
Metabolic changes	X		X	
Increased burden on protein quality-control systems	X		X	
Protein aggregate formation	X			
Aneuploidy-associated stress response				
p53 activation			X	
G1 delay	X	X	X	
Induction of protein quality-control pathways	X		X	
ESR-like transcriptional signature	X	X	X	X
DNA-damage response as a result of chromosome mis-segregation			X	

B= Budding yeast M= Mammals
F= Fission yeast A= *Arabidopsis*

Figure 3. Gene-Specific and General Effects of Aneuploidy on Fitness
X means observed, empty box means that this has not been examined. See text for details.

General Non-Gene-Specific Effects of Large-Scale DNA Copy-Number Alterations

Changes in the copy number of large regions of the genome can also cause phenotypes that are the result of cumulative effects of copy-number changes in many genes, which on their own have little phenotypic consequences. Studies on whole-chromosome gains in yeast and mammalian cells revealed phenotypes that are seen in many different aneuploidies (summarized in Figure 3). The severity of these phenotypes tends to scale with the degree of deviation from the euploid karyotype, and thus they likely also exist in cells harboring large segmental aneuploidies, but not in cells with CNV-size DNA copy-number alterations. In the following section, we will describe the general phenotypes associated with large-scale DNA copy-number alterations.

(A) Whole-Chromosome Copy-Number Alterations Interfere with Cell Proliferation

Studies of *S. pombe* and *S. cerevisiae* aneuploid strains (Niwa et al., 2006; Pavelka et al., 2010; Torres et al., 2007) showed that aneuploidy impairs proliferation under standard growth conditions. Similar slowed growth was observed in mammalian cells. Trisomic MEFs exhibit proliferation defects, as do cells harboring random aneuploidies caused by mutations of BubR1 or Cdc20 that lead to increased chromosome missegregation (Baker et al., 2004; Li et al., 2009; Thompson and Compton, 2008; Williams et al., 2008). These adverse effects on cell proliferation have severe consequences for the animal. Mice expressing mutations leading to high levels of chromosome missegregation die in utero or exhibit premature aging phenotypes (Baker et al., 2004; Li et al., 2009). In humans, hypomorphic mutations in BubR1 lead to mosaic-variegated aneuploidy, which is associated with growth deficiency and short stature, mental retardation, developmental defects, as well as a predisposition to cancer (Hanks et al., 2004). Several mouse models of chromosome instability (CIN) do not exhibit dramatic defects in cell proliferation, and animals show little if any decline in overall fitness (reviewed in Pfau and Amon, 2012), indicating that some level of aneuploidy can be tolerated and perhaps compensated for by cells especially in the context of the organism, where proliferative potential is frequently not rate limiting.

How large-scale copy-number changes such as chromosomal gains or losses interfere with cell proliferation is not understood. In budding yeast, fission yeast, and human cells, chromosome gains cause a G1 delay (Niwa et al., 2006; Torres et al., 2007; Stingele et al., 2012). Many stresses cause a transient G1 delay, by impacting the cell-cycle machinery governing the G1–S phase transition. Gross copy-number changes elicit phenotypes that are reminiscent of a stress response (see below). The observed G1 delay could thus be part of such a stress response. It is, however, also possible that a different genetic imbalance underlies each G1 delay in the different aneuploid strains. In mammalian cells, chromosome missegregation and the ensuing aneuploidy cause activation of p53, which results in G1 arrest or apoptosis (Li et al., 2010; Thompson and Compton, 2010). The origins of this p53 response are controversial but could be due to various aneuploidy-associated stresses, which are discussed in the following section.

(B) Whole-Chromosome Copy-Number Alterations Cause Several Cellular Stresses, Collectively Called the "Aneuploidy-Associated Stresses"

Studies in aneuploid yeast and MEFs indicate that aneuploid cells experience metabolic stress. Yeast cells and MEFs with unbalanced karyotypes produce less biomass (Li et al., 2010; Torres et al., 2007; Williams et al., 2008). Trisomic MEFs and MEFs containing the aneuploidy-inducing *CDC20*^{AAA} mutation were also found to take up more glutamine and to exhibit sensitivity to the energy stress-inducing compound AICAR (Li et al., 2010; Tang et al., 2011; Williams et al., 2008). Aneuploid cells generated by the *CDC20*^{AAA} mutation also display increased glucose uptake and lactate production (Li et al., 2010). In all, these findings point toward increased energy needs of cells with large-scale karyotypic changes. Perhaps, cells waste energy by producing and then dealing with the excess proteins produced from the additional genes.

Because gains and losses of genetic information impact gene expression, large-scale changes in gene copy number have a profound impact on the cell's protein composition. Overproduced proteins and proteins lacking their appropriate binding partners eventually misfold and lead to a condition known as proteotoxic stress. Thus, not surprisingly, cells with whole-chromosome gains are under proteotoxic stress. Aneuploid *S. cerevisiae* cells are more sensitive to conditions that elicit proteotoxic stress such as elevated temperature or treatment with the protein-synthesis inhibitors cycloheximide or hygromycin and the Hsp90 inhibitor geldanamycin (Pavelka et al., 2010; Torres et al., 2007). Furthermore, aneuploid yeast cells form protein aggregates and appear challenged in their ability to fold proteins (Oromendia et al., 2012). In addition, inactivation of the proteasome antagonist Ubp6 improved the fitness of several different yeast strains carrying an additional chromosome and partially attenuated the proteomic changes elicited by the aneuploid karyotype (Torres et al., 2010). Evidence for proteotoxic stress also exists in mammalian cells harboring additional chromosomes. Trisomic MEFs and human cells show increased basal levels of autophagy and the chaperone Hsp72 and are more sensitive to the Hsp90 chaperone inhibitor 17-AAG than euploid cells (Stingele et al., 2012; Tang et al., 2011). In summary, changes in gene copy number of many genes simultaneously, as occurs when entire chromosomes are lost or gained, profoundly alter the cell's protein composition. This aneuploidy-induced change in the cell's proteome places a burden on the cell's protein quality-control pathways and hence impacts fitness.

(C) Large-Scale Changes in DNA Copy Number May Elicit a Stress Response

Whole-chromosomal aneuploidies have been shown to lead to a transcriptional response in all organisms where this has been analyzed. A gene-expression signature similar to the environmental stress response (ESR) in budding yeast has been found to exist not only in aneuploid budding yeast strains (Torres et al., 2007) but also in fission yeast, *Arabidopsis*, mouse, and human cells with whole-chromosome gains (Sheltzer et al., 2012). The ESR was first defined in budding yeast. The ESR gene-expression signature is characterized by genes involved in the response to stress being upregulated and genes associated with cell growth and proliferation being downregulated (Gasch, 2007). The ESR is elicited by a large number of stress conditions and is associated with slow growth (Brauer et al., 2008; Gasch et al., 2000; Regenberg et al., 2006). The finding that an ESR-like gene-expression pattern exists in many different whole-chromosome aneuploidies in different organisms raises the interesting possibility that aneuploidy affects similar cellular pathways and causes an antiproliferative response in most if not all organisms.

In addition to an ESR-like transcriptional response, several studies indicate that in mammalian cells, p53 is activated in response to chromosome missegregation and/or aneuploidy, though the proposed mechanisms differ. The first study to implicate p53 in antagonizing the proliferation of cells that missegregate chromosomes or that are aneuploid examined mouse

embryos lacking the spindle assembly checkpoint component *MAD2*. Such embryos die by embryonic day (E) 7.5 due to massive chromosome missegregation, but deletion of p53 permits such embryos to survive until E10.5 (Burds et al., 2005; Dobles et al., 2000). In 2010, Thompson and Compton showed that the process of missegregating chromosomes leads to activation of p53 through the stress kinase p38 (Thompson and Compton, 2010). Li et al. (2010) also observed p53 activation upon chromosome missegregation but suggested that the accumulation of reactive oxygen species (ROS) was responsible for p53 activation (Li et al., 2010). ROS, generated by increased metabolism in aneuploid cells, activate the DNA-damage checkpoint kinase ATM in a DNA-damage-independent manner (Li et al., 2010). ATM in turn activates p53. Interestingly, the authors of this study also found that p53 activity is correlated with aneuploidy level. A low degree of aneuploidy caused a less pronounced p53 response than high levels. This graded response to aneuploidy may explain why p53 activity was not found to be increased in trisomic MEFs, in which the degree of aneuploidy is low, and cells have had time to adapt to the aneuploid state prior to analysis (Tang et al., 2011).

The studies that observed a p53 response in aneuploid cells did not examine cells with constitutive aneuploidies but cells that actively missegregate chromosomes, a condition known as CIN. p53 activation may thus not be a consequence of aneuploidy per se but could also be caused by events associated with chromosome missegregation. This is the conclusion that Janssen et al. (2011) arrived at. Their studies suggest that the process of missegregating a chromosome leads to lagging chromosomes, which suffer DNA damage during cytokinesis. This cytokinesis-inflicted DNA damage in turn causes p53 activation through the canonical DNA-damage pathway (Janssen et al., 2011). It should further be noted that cytokinesis failure even in the absence of DNA damage can cause p53 activation (de Stanchina et al., 1998; Serrano et al., 1997). Consistent with the idea that p53 activation may be a consequence of chromosome missegregation rather than aneuploidy per se is the observation that in cells harboring whole-chromosome gains without accompanying CIN, a p53 response has not been detected. Trisomic MEFs do not mount a p53 response (Tang et al., 2011). Thus it remains to be determined whether p53 activation is a response to chromosome missegregation-induced DNA damage or cytokinesis defects, to the aneuploid state, or to all of the above. Although the exact function of p53 in CIN and aneuploidy remains to be determined, it is clear that mechanisms other than p53 activation also limit the proliferation of cells harboring aneuploidies or other large-scale gene copy-number changes. Trisomic MEFs proliferate poorly without mounting a p53 response (Tang et al., 2011).

(D) Aneuploidy and CIN Cause Genome Instability

It has recently become clear that the presence of additional chromosomes as well as the process of gaining or losing whole chromosomes have a dramatic effect on genome stability. Budding yeast strains harboring additional chromosomes exhibit increased rates of chromosome missegregation, mitotic recombination, mutation, and DNA damage, as well as increased sensitivity to genotoxic agents (Sheltzer et al., 2011; Zhu et al., 2012). Aneuploid fission yeast cells are also sensitive to DNA-damaging agents and harbor an increased number of DNA-damage foci (Sheltzer et al., 2011). The origins of these genome instability-inducing effects of whole-chromosome aneuploidies are not yet known but have been suggested to be due to changes in the copy numbers of specific genes. For example, changes in the dosage of the gene encoding the kinesin Kip3 impact mitotic spindle dynamics and cause increased chromosome missegregation in budding yeast (Su et al., 2011). Changes in gene copy number also impact the stability of their immediate environment. Repeated DNA sequences are prone to engage in nonallelic homologous recombination especially during meiosis, which can lead to genomic rearrangements.

Two recent studies in mammalian cells showed that the process of changing the copy number of whole chromosomes, chromosome missegregation, also induces genomic instability, especially DNA damage. Merotelically attached chromosomes—these are chromosomes that attach to microtubules emanating from both centrosomes—remain in the center of the cell during anaphase (Janssen et al., 2011; Thompson and Compton, 2010). These lagging chromosomes are then broken during cytokinesis, which leads to translocations and other genomic rearrangements (Janssen et al., 2011).

Chromosome breaks on lagging chromosomes not only occur because of breakage of the chromosome during cytokinesis, they also form because of their inefficient replication in the subsequent cell cycle (Crasta et al., 2012). Lagging chromosomes can form micronuclei if they fail to reach the bulk of the segregated DNA prior to nuclear-envelope reformation. These micronuclei then undergo defective and asynchronous DNA replication, which results in DNA damage and fragmentation and rearrangement of the chromosome in the micronucleus. This phenomenon could explain a recently discovered feature of some cancers, called chromothripsis, where individual chromosomes undergo massive breakage and rearrangement (Stephens et al., 2011). Amplifications, deletions, and whole-chromosome gains or losses are a hallmark of cancer. The genome-instability-inducing effects of chromosome missegregation and the resulting whole-chromosomal aneuploidies could fuel the dramatic karyotype changes characteristic of tumorigenesis.

(E) Are Some Developmental Defects Seen in Aneuploid Organisms due to the General Effects Caused by Large-Scale Gene Copy-Number Alterations?

In cells changes in chromosome copy number elicit a set of phenotypes that appears to be independent of the identity of the genes whose copy numbers are altered. Are such general phenotypes also evident at the organismal level? Most organisms with whole-chromosome aneuploidies or large segmental aneuploidies are growth retarded (reviewed in Torres et al., 2008), which is most likely due to the impact of large-scale gene copy-number alterations on cell proliferation (see above). Beyond the stunted growth, few commonalities are observed among different aneuploids at the organismal level. This finding indicates that most developmental aberrations observed in organisms with large-scale DNA copy-number alterations are due to copy-number changes of specific genes. However, it is intriguing to note that in mammals, some developmental phenotypes are observed in many different trisomies. Mice and

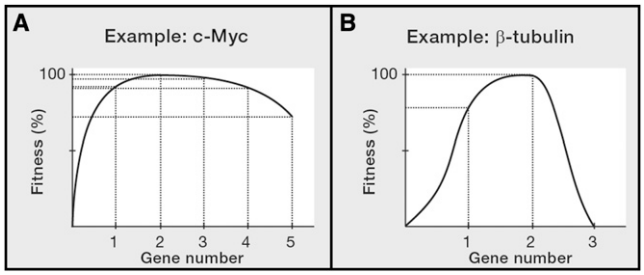

Figure 4. Effects of Increasing and Decreasing Gene Copy Number
(A) Increasing or decreasing the levels of most genes by 50% has a minimal impact on fitness, but decreases or increases beyond that can affect fitness (based on Kacser and Burns, 1981).
(B) Changing the copy number of structural genes can have dramatic effects. The example of the β-tubulin-encoding gene is shown (Katz et al., 1990).

humans trisomic for any chromosome exhibit craniofacial defects such as cleft palate, microcephaly, cardiac defects, and nuchal edema (Gropp et al., 1975; Hassold and Jacobs, 1984; Krushinskiĭ et al., 1986). It may simply require a large number of dosage-sensitive genes to build the organs whose development is affected in many different aneuploidies, but it is also possible that some general aspects of large-scale gene copy-number changes affect pathways used in the development of these organs.

In summary, the phenotypic analyses of small- and large-scale changes in DNA copy number lead to two general conclusions. First, changes in the copy number of single or a small number of genes can lead to specific phenotypes, as illustrated by human diseases such as Charcot-Marie-Tooth 1A neuropathy or APP-driven early onset Alzheimer's disease. Second, when the copy number of large genomic regions is altered, such as in whole-chromosome aneuploidies, the origins of the phenotypes are more complex. Some phenotypes are the result of cumulative effects of changes in copy number of a large number of genes, whereas others are caused by changes in the dosage of single genes.

Why Are Gene Copy-Number Alterations Detrimental?

Although it is possible that in some circumstances, changes in specific DNA sequences (i.e., centromeres or telomeres) can have adverse effects (Futcher and Carbon, 1986; Runge and Zakian, 1989), the most likely explanation for the majority of detrimental phenotypes caused by changes in gene copy number is the *gene dosage hypothesis*: gains or losses of gene copies change the expression levels of the affected gene. This altered dosage impacts fitness. The comparison of the phenotypes caused by changing the copy number of autosomes and sex chromosomes illustrates this point. The consequences of losing or gaining autosomes, for which dosage compensation does not occur, are severe. Alterations in sex chromosome copy number, for which dosage compensation occurs, are mild (reviewed in Berletch et al., 2011; Prestel et al., 2010).

We can envision several ways in which an altered gene dosage can negatively impact cellular or organismal fitness:

(1) Dramatic over- or underexpression of a specific gene can cause phenotypic changes (Figures 4A and 5A). The recurrent amplifications of c-Myc or of receptor tyrosine kinases in various tumors are examples of such effects.

(2) Changing the levels of individual genes encoding structural proteins or proteins that function in protein complexes by 50% can affect fitness (Kacser and Burns, 1981; Veitia, 2002) (Figures 4B and 5B).

(3) Overexpression of individual genes that encode proteins that engage in promiscuous low-affinity interactions can result in off-target interactions, thereby impairing fitness (Vavouri et al., 2009) (Figure 5C).

(4) The detrimental phenotypes associated with gene copy-number changes can also result from synergistic effects of large-scale DNA copy-number alterations. Overexpression of many proteins at once can impact basic cellular functions such as energy homeostasis and protein quality-control mechanisms (Figure 5D). In the absence of sufficient chaperone capacity to accommodate overexpressed proteins, other chaperone clients that fail to fold in a timely manner will be degraded or deposited as aggregates (Olzscha et al., 2011) (Figure 5D). Protein stoichiometry imbalances caused by gene copy-number changes can also contribute to proteotoxicity. Many subunits of protein complexes only acquire a stable conformation by binding to other subunits of the complex (Kaizu et al., 2010; Vavouri et al., 2009). Thus, changes in dosage of genes encoding polypeptides that normally have binding partners will produce excess proteins that require the continuous assistance of chaperones, preventing chaperones from assisting other folding reactions. This could reduce the general folding capacity of the cell and thus interfere with their essential function of mediating folding of essential proteins (Olzscha et al., 2011). Indeed, the production of a misfolded protein representing 0.1% of the total yeast proteome leads to a significant reduction in fitness and elicits a cytoplasmic unfolded protein response (Geiler-Samerotte et al., 2011). Excess proteins also impact proteasomal degradation, as excess proteins are frequently cleared by ubiquitin-proteasomal degradation (Figure 5D). Energy stress may also exist in cells with large-scale DNA copy-number alterations. The production of additional gene products and the shielding of the cell from the effects cost energy. This increased energy demand may negatively impact fitness.

Gene Copy-Number Alterations as a Source of Adaptive Potential and Their Function during Normal Development

Gene copy-number changes are not always detrimental. Experimental evolution studies demonstrate that under selection, gene copy-number changes endow the organism with increased survival under the imposed selection. Perhaps the best example of such beneficial effects is the tissue-specific amplification of genes as part of normal development.

DNA Copy-Number Changes as a Source of Adaptive Potential

In 1981, Edlund and Normark reported that amplification of the *ampC* locus promotes antibiotic resistance in *E. coli* (Edlund and Normark, 1981). Many subsequent studies have shown that gene amplifications frequently arise during adaptive evolution experiments. For example, in budding yeast, growth under glucose limitation selects for cells in which the genes

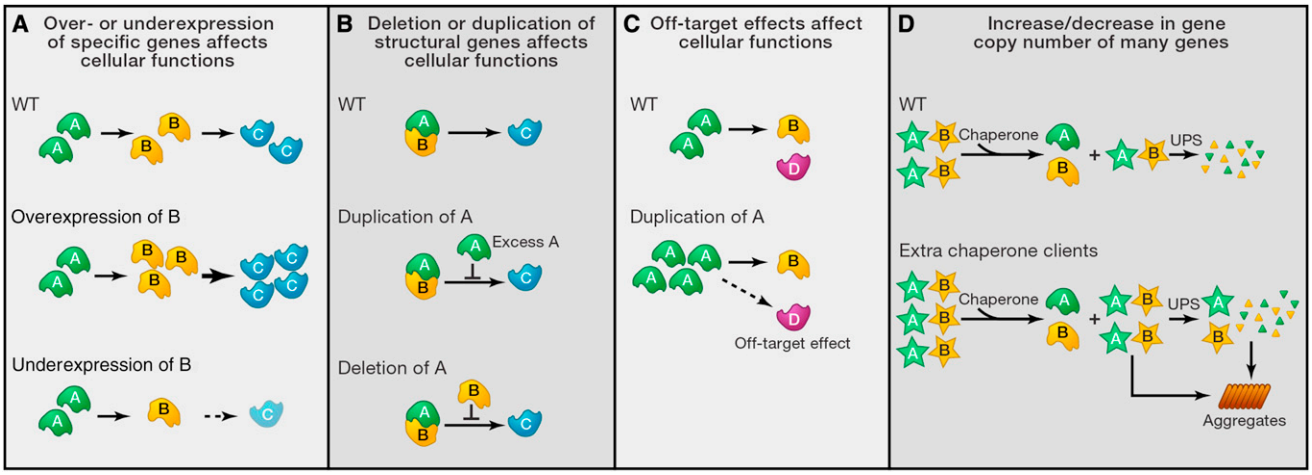

Figure 5. Consequences of Changes in Gene Copy Number

(A) An increased dosage of a single gene, such as a rate-limiting enzyme B, can increase the output or function of a cellular pathway. Conversely, reduction of enzyme B will diminish the production of C, thus decreasing pathway activity.

(B) Altered gene dosage can interfere with the function of stoichiometry-sensitive complexes, with excess of protein A or protein B inhibiting the function of C and therefore decreasing pathway activity.

(C) Overexpression of a regulatory enzyme can lead to off-target effects. For example, overexpression of a protein kinase or protein phosphatase can cause deregulation of pathways that proteins usually do not function in.

(D) Changes in the copy number of many genes simultaneously can impact protein quality-control mechanisms such as molecular chaperones and the ubiquitin-proteasome system (UPS). Misfolded proteins can eventually form aggregates.

encoding high-affinity glucose transporters are amplified (Brown et al., 1998; Dunham et al., 2002; Kao and Sherlock, 2008) (Figure 6A). Studies employing other nutrient-deprivation selections revealed similar principles; genes that facilitate the uptake and/or metabolism of the limiting factor are upregulated often through gene amplification (Gresham et al., 2008, 2010). This principle is recapitulated during tumorigenesis. Amplifications of oncogenes and losses of tumor-suppressor genes are an integral feature of the disease (Gordon et al., 2012).

Whole-chromosome and segmental aneuploidies are also obtained in experimental evolution experiments because a gene in that genomic region provides a selective advantage. For example, the pathogenic fungus *C. albicans* can develop resistance to the antifungal drug fluconazole through duplication of the left half of chromosome 5 (Selmecki et al., 2006). This region of chromosome 5 harbors *ERG11*, which encodes the biosynthetic enzyme targeted by fluconazole, as well as *TAC1,* a transcription factor that upregulates expression of (ABC) transporter genes (Selmecki et al., 2006). Their duplication is an important contributor to fluconazole resistance in strains harboring the segmental aneuploidy of chromosome 5. In budding yeast, whole-chromosome aneuploidies have been obtained in the selection for suppressors of cytokinesis defects (Rancati et al., 2008). These aneuploidies occurred in the context of an increase in base ploidy. Polyploid cells exhibit increased CIN (Storchová et al., 2006), indicating that polyploidization predisposes to aneuploidy. Polyploidy not only may facilitate the generation of aneuploidy (Storchová et al., 2006), it also attenuates the phenotypes associated with the condition (Torres et al., 2007) and may thus buffer against the adverse effects of aneuploidy, allowing cells to take full advantage of potential beneficial effects associated with certain gene copy-number alterations. This could explain why cancer cells, which are highly aneuploid, often show increased base ploidy.

The selective advantage gained through whole-chromosome aneuploidies comes at a price. A large-scale DNA copy-number alteration results in changes in the dosage of many genes, the adverse effects of which dampen the potential benefits that arise due to the duplication of gene(s) conferring a selective advantage. This was elegantly demonstrated by Yona et al. (2012). They showed that whole-chromosome copy-number changes are a transient occurrence in adaptive evolution experiments. They provide a quick means of adapting to a continuous selective pressure that is replaced over time by more subtle genetic changes that achieve the same goal (Yona et al., 2012). Diploid budding yeast grown continuously at an elevated temperature of 39°C developed trisomy of chromosome 3. This aneuploidy was lost once the cells were returned to growth at normal temperature (30°C). Loss of the trisomy occurred not only when the selective pressure was removed but even when cells were kept at 39°C for extended periods. After 1,000 generations, trisomy 3 was replaced by increased expression of 17–18 genes located on chromosome 3 and presumably of genes elsewhere in the genome that conveyed increased temperature tolerance. Interestingly, there was a significant overlap in upregulated genes among four repetitions. These results indicate that whole-chromosome aneuploidies can provide an effective means of quickly adapting to a selective pressure. This is not surprising given that chromosome loss rates are by three orders of magnitude higher than mutation rates (10^{-5} compared to 10^{-8}). Eventually, however, the ''crude'' whole-chromosome gain/loss solutions are replaced by ones that do not bring with them the adverse effects caused by gene-dosage changes of the other genes located on the gained/lost chromosome.

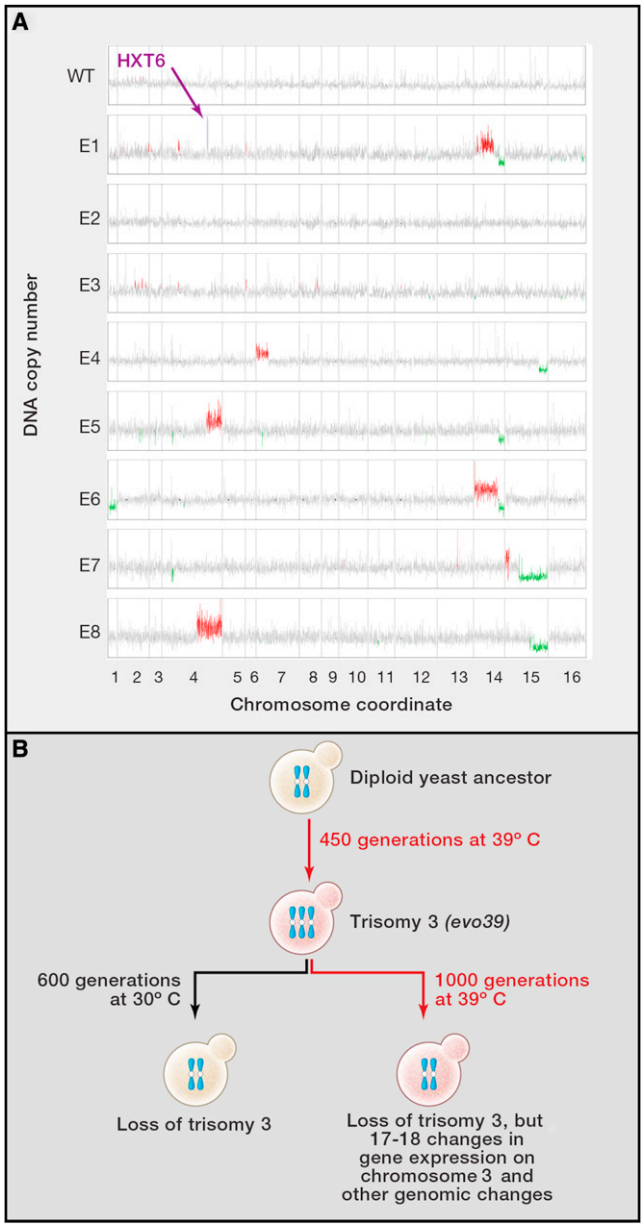

Figure 6. Small- and Large-Scale Gene Copy-Number Alterations Arise during Adaptive Evolution Experiments

(A) Eight *S. cerevisiae* strains were isolated after growth under glucose-limiting conditions for 100–500 generations, and DNA content was assessed. Red and green indicate gene copy-number amplification and reduction, respectively. *HXT6* encodes a high-affinity hexose transporter and is amplified in evolved strains E1, E5, and E8. Data from Dunham et al. (2002).

(B) Trisomy 3 is a transient intermediate during continuous growth at 39°C. Cells were grown for 450 generations at 39°C. Many isolates harbored an additional copy of chromosome 3. Upon return to the permissive temperature (30°C), the trisomy was quickly lost. The trisomy was also lost after continuous growth at 39°C (1,000 generations) and replaced by changes in the expression of genes that confer resistance to high temperature.

Recent studies in mice indicate that aneuploidy is also an effective way to adapt to selective pressure in multicellular organisms. The liver is a naturally aneuploid organ (Duncan et al., 2010; Putkey et al., 2002; Weaver et al., 2007; described in detail below), providing an opportunity to determine the importance of aneuploidy in adaptation to selective pressure. Deficiency of fumarylacetoacetate hydrolase (FAH) causes chronic liver disease that is suppressed by the loss of enzymes functioning upstream of FAH, such as homogentisic acid dioxygenase (HPD). Mice lacking FAH function and heterozygous for a deletion in HPD develop disease resistance through loss of the chromosome encoding the functional copy of HPD (Duncan et al., 2012), illustrating the power of aneuploidy in providing adaptive potential even in multicellular organisms.

Mutations that suppress the adverse effects of large-scale DNA copy-number changes may enhance the adaptive potential of chromosome gains or losses. Aneuploidy-tolerating mutations have been found in yeast (Torres et al., 2010). Loss of p53 increases survival of cells following chromosome missegregation in mammalian cells (Janssen et al., 2011; Li et al., 2010; Thompson and Compton, 2010). It will be interesting to examine the contribution of different aneuploidy-tolerating mutations to disease progression in cancer where gene copy-number alterations are a key feature. Indeed, p53 inactivation is a major contributor to tumorigenesis (Dai and Gu, 2010; Kruse and Gu, 2009; Toledo and Wahl, 2006).

Gene Copy-Number Alterations as Part of Normal Development

Differentiation into specific cell types often requires increased expression of particular genes. In some instances, this is achieved by amplification of specific loci. Ribosomal DNA (rDNA) amplification occurs in amphibian oocytes, the macronucleus of Tetrahymena, and budding yeast (Brown and Dawid, 1968; Claycomb and Orr-Weaver, 2005; Gall, 1968; Kobayashi et al., 1998; Nordman and Orr-Weaver, 2012; Oakes et al., 2006; Pesin and Orr-Weaver, 2008; Yao et al., 1974) and thus appears to be a common mechanism used to accommodate different protein-synthesis needs in different tissues and under changing environmental conditions. Although rDNA amplification is not uncommon, amplification of selective genes is rarely used to upregulate gene expression and, in the instances where this has been described, occurs in cells destined to die. In *Drosophila*, follicle cells amplify the chorion genes, which encode eggshell proteins (Spradling, 1981). This amplification allows follicle cells to make the eggshell. Once they have accomplished this task, they become part of the eggshell.

Whole-chromosome aneuploidies have been found in various tissues in mammals. In mice and humans, ~30% of neuroblasts in the embryonic brain are aneuploid (Rehen et al., 2001). The majority of these aneuploid neurons are eliminated during development, but 10% of neurons in adult brains are estimated to be aneuploid (Rehen et al., 2001, 2005; Westra et al., 2008). The biological significance and the impact of this increased aneuploidy on neuronal physiology and fitness remain to be determined.

Mammalian hepatocytes acquire whole-chromosome gains and losses during aging and in response to toxic stresses and disease (Duncan et al., 2010; Putkey et al., 2002; Weaver et al., 2007). Aneuploidy in hepatocytes is preceded by polyploidization as a result of failed cytokinesis. Polyploid cells that harbor not only additional genome copies but also additional centrosomes then undergo a mitotic division in which all

centrosomes form a multipolar spindle to produce aneuploid progeny. Whether aneuploid liver cells are as fit as their euploid counterparts remains to be determined, but aneuploidy has been hypothesized to provide genetic variation so that the liver can adapt to nutritional and noxious challenges (Duncan et al., 2010). Determining the importance of aneuploidy in the function of tissues such as the brain and the liver and defining whether different cell types exhibit different sensitivities to gene copy-number changes will provide critical insights into how gene CNVs contribute to cell-type specification and diseases such as cancer, in which aneuploidy is the norm, yet fitness as judged by unrestricted proliferation is high.

Future Directions—Gene Copy-Number Alterations as a Therapeutic Target?

The last decade has brought to light the importance of gene copy-number changes in a wide variety of human diseases. Understanding in detail how changes in copy number of individual genes or large chromosomal regions enhance or perhaps suppress disease is a critical challenge. With an ever-more detailed view of the human genome and its variations in the normal population and in disease-prone families, we will be able to learn which copy-number changes don't matter and can focus on those that impact function.

A particularly exciting future direction is the pursuit of gene copy-number changes in developing therapies. The realization that CNVs contribute to difficult-to-treat diseases, such as neurological and psychiatric disorders, brings with it the possibility of targeting the gene products amplified/deleted in particular disorders. For example, duplication of *AUTS4*, a gene encoding the $GABA_A$ receptor subunit, has been associated with autism spectrum disorder (Zhang et al., 2009). Autistic individuals with this CNV may benefit from compounds that downregulate $GABA_A$ receptor function. CNVs causing disruptions of *MYT1L*, *CTNND2*, and *ASTN2* have been seen in patients with Schizophrenia. Crohn's disease, a bowl inflammatory disease, is linked to copy-number reduction of *HBD2*. Compounds that increase the function of the remaining copy or inhibit the function of negative regulators of the pathways that these genes function in may have a significant therapeutic index.

Cancer is the prime example in which gene amplifications and deletions have been shown to drive disease (Gordon et al., 2012). Therapies where overexpressed or amplified oncogenic drivers are targeted have been developed. The gene encoding epidermal growth factor receptor (EGFR) is amplified in non-small-cell lung cancer. Small molecules such as gefitinib or erlotinib have been applied to inhibit EGFR with success (Carling, 2004; Paez et al., 2004). *ERBB2*, which encodes the EGFR HER2, is amplified in ~30% of primary breast cancers (Slamon et al., 1987). Trastuzumab, an anti-HER2 antibody, has been used in the therapy of *HER2*-amplified breast cancers with great success (Baselga et al., 1998). These successes raise the exciting possibility that targeting amplified disease drivers provides opportunities for therapy in cancer, psychiatric disorders, and autoimmune diseases, where effective treatments are scarce.

Whether large-scale gene copy-number changes that cause Down syndrome or occur in cancer can be targeted in therapy remains to be determined. In Down syndrome, gene-dosage changes of many different genes contribute to the associated phenotypes, making the development of therapeutics a challenge. The identification of individual genes responsible for specific phenotypes could enable the development of therapeutics that target specific aspects of the condition. For example, individuals with Down syndrome could benefit from therapies that lower APP protein levels, to prevent early onset Alzheimer's disease; the development of which has, unfortunately, failed so far.

In cancer, the situation is even more complex. In this disease, the contribution of gene-dosage changes of many genes is augmented by the variability of an ever-changing genetic make-up. Many cancers do, however, harbor specific aneuploidies that could be targeted in therapy. For example, trisomy 8 is frequently observed in patients with AML and associated with poor survival when present together with other genetic aberrations (Wolman et al., 2002). Drugs that target cells with amplified chromosome 8 may aid in the treatment of AML. Genomic instability in cancers also leads to loss of many genomic regions. These genetic lesions could provide additional therapeutic targets (Nijhawan et al., 2012). The general stress phenotypes associated with aneuploidy could also be explored in cancer treatment. The advantage of such compounds is that they would show efficacy against a broad spectrum of cancers. Compounds that preferentially inhibit the proliferation of aneuploid cell lines have been shown to exist and appear to exaggerate the general stress phenotypes associated with whole-chromosome copy-number changes (Tang et al., 2011). These compounds included AICAR, an agonist of the stress-activated AMP kinase, and the Hsp90 inhibitor 17-AAG (Tang et al., 2011). Thus, targeting the general stresses associated with aneuploidy could be developed as cancer drug targets. It is worth noting that Hsp90 inhibitors such as 17-AAG display antitumor efficacy in HER2/ErbB2-positive breast cancer and are currently in phase II and III clinical trials. The proteasome inhibitor bortezomib is used in the treatment of multiple myeloma (Richardson et al., 2005). Other inhibitors of the ubiquitin-proteasome system such as highly specific inhibitors of the proteasome-associated deubiquitinating enzyme Usp14 (Lee et al., 2010) could also show efficacy against aneuploid cells and thus could be used in the treatment of aneuploid cancers. We note that, compounds that target aneuploid cells may be especially effective when combined with chemotherapeutics, such as Taxol, that increase chromosome missegregation.

Changes in gene copy number, large and small in scale, contribute to population diversity and are significant contributors to disease. Understanding their cost and benefits will provide critical insights into evolution and diseases.

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