Insights into the Consequences of Chromosome Gains and Losses in *S. cerevisiae*

by

Rebecca Ruth Beach

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**ABSTRACT**

When cells divide, they must properly duplicate and segregate their genome to generate two identical daughter cells with faithful transmission of the genomic content. Errors in chromosome segregation lead to aneuploidy, a state of chromosomal imbalance where the karyotype is not an exact multiple of the haploid complement. At an organismal level, aneuploidy is the leading cause of miscarriage and developmental defects, while at a cellular level, aneuploidy causes proliferative defects and is detrimental to cell fitness. However, aneuploidy is also associated with cancer, a disease of uncontrolled proliferation. Understanding the effects of aneuploidy on cellular physiology has broad implications for many areas of human health.

Here, I have characterized the G1 delay in aneuploid *S. cerevisiae*. Aneuploid yeast exhibited a growth defect in G1. Additionally, aneuploid cells displayed a cell cycle entry delay due to delayed accumulation of G1 cyclins. Like other cellular stresses, aneuploidy interferes with the cell’s ability to grow and to enter the cell cycle. I also developed a novel method to systematically examine the immediate consequences of gaining and—for the first time—losing single or multiple chromosomes. Using this system, I found that phenotypes wide-spread among aneuploid cells develop immediately following chromosome mis-segregation. Also, phenotypes common to chromosome gains are also wide-spread among cells that lost chromosomes, indicating that chromosome gains and losses fundamentally affect cells in similar ways. Finally, cell cycle analyses following chromosome mis-segregation revealed a surprising phenotypic variability among cells harboring the same aneuploidies. Together, these results provide insights into the mechanisms for the observed consequences of chromosome gains and losses on cellular physiology.

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This thesis is dedicated to Lawrence Vanaria, who instilled in me a love for biology.
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Chapter 1: Introduction
When cells divide, they must properly duplicate and segregate their genome to generate two identical daughter cells with faithful transmission of the genomic content. Although many molecular mechanisms exist to ensure that this process happens correctly, chromosome missegregation occurs once in every $5 \times 10^4$ cell divisions in yeast (L. H. Hartwell et al., 1982) and once every $10^4$ or $10^5$ cell divisions in mammalian cells (Rosenstraus and Chasin, 1978). In multicellular organisms, cell division is a continual process in many tissues, with innumerable divisions occurring during development, leading to many opportunities for errors in cell division to occur. In single celled organisms, faithful duplication and segregation of the genome is vital for the organism’s survival, as a single error can result in organismal death.

Errors in chromosome segregation lead to a condition known as aneuploidy. Aneuploidy is a state of chromosomal imbalance where the number of chromosomes is not an exact multiple of the haploid complement. At an organismal level, aneuploidy can cause developmental defects (reviewed in Siegel and Amon, 2012; Torres et al., 2008), while at a cellular level aneuploidy causes proliferative defects (Stingele et al., 2012; Torres et al., 2007; Williams et al., 2008), proteotoxic stress (Oromendia et al., 2012; Santaguida et al., 2015; Stingele et al., 2012; Tang et al., 2011; Torres et al., 2007), and genomic instability (Sheltzer et al., 2011; Zhu et al., 2012). However, aneuploidy is also associated with cancer, a disease of uncontrolled proliferation. Understanding the effects of aneuploidy on cellular physiology will help us better understand the paradox of how aneuploidy is detrimental to cell fitness in many contexts, yet is a hallmark of a disease of rapid proliferation.
In this introduction, I will first discuss the consequences of aneuploidy and current models of study. Current models of aneuploidy either utilize cells with chronic aneuploidies present for many divisions, or models induce chromosome missegregation of random chromosomes, generating heterogeneous populations. The current models of aneuploidy are lacking in a model that allows for induction of chromosome missegregation in a defined manner. In Chapter 3, I introduce and characterize a novel model of aneuploidy in budding yeast in which missegregation of specific chromosomes can be induced by a controlled mechanism. I will next give an overview of the G1/S transition in budding yeast, as many aneuploid cells exhibit delays in G1. In Chapter 2, I investigate the cause of the G1 delay in chronic aneuploid cells, and in Chapter 3 I characterize cell cycle progression of aneuploid cells following chromosome missegregation. Finally, I provide an overview of the protein quality control network, as many aneuploid cells exhibit proteotoxic stress. In Chapter 3, I investigate the onset of proteotoxic stress following chromosome missegregation. Finally, I will conclude this introduction with a brief outline of the work I will present in this thesis.
CONSEQUENCES OF ANEUPLOIDY AND MODELS OF STUDY

Aneuploidy impairs organismal fitness

At an organismal level, aneuploidy is detrimental to fitness in all organisms studied to date (reviewed in Siegel and Amon, 2012; Torres et al., 2008). Whole-organism aneuploidy is the leading cause of miscarriage in humans; only three autosomal trisomies can survive to birth, and all monosomies are embryonic lethal. Individuals with Edwards syndrome (Trisomy 18) and Patau syndrome (Trisomy 13) rarely survive beyond the first year of life (Torres et al., 2008). Individuals with Edwards syndrome often have severe cardiovascular and brain defects (Lin et al., 2006). Patau syndrome causes many severe developmental abnormalities at birth, including neurological, muscular, and heart defects (Hsu and Hou, 2007). Down Syndrome is the most common trisomy, and humans with this disorder are able to survive into adulthood, although most experience reduced life expectancy (Glasson et al., 2002; Hassold and Jacobs, 1984). Individuals with Down Syndrome often have mental retardation and heart defects, although the degree of severity varies across individuals (Roizen and Patterson, 2003).

Whole-organism aneuploidy has detrimental effects in other organisms as well (Figure 1). In mice, only Trisomy 19 animals survive a few weeks postpartum, while all other aneuploidies are embryonic lethal (Lorke, 1994). Aneuploid nematodes, flies, Arabidopsis, and maize also exhibit developmental defects and lethality (Siegel and Amon, 2012).
Figure 1: Aneuploid organisms exhibit growth defects (adapted from Siegel and Amon, 2012).

(A) Aneuploid *Arabidopsis thaliana* (right, trisomic for chromosomes 3 and 5) exhibit smaller, thinner leaves than diploid plants (left) (images from Henry et al., 2010). (B) Mouse embryos trisomic for chromosome 16 (right) are smaller than euploid littermates (left) and exhibit developmental defects including nuchal edema (images from Williams et al., 2008). Abbreviations: WT, wild-type; Ts, trisomy.

Random aneuploidies can also be present in an organism with heterogeneous karyotypes across cells. In humans, a rare genetic disorder known as Mosaic Variegated Aneuploidy (MVA) is caused by mutations in the *BUB1B* gene, which encodes a mitotic spindle assembly checkpoint protein (Hanks et al., 2004), or in the *CEP57* gene, which encodes a centrosomal protein important in microtubule stabilization and proper chromosome segregation (Snape et al., 2011). Individuals with MVA exhibit developmental delays and rarely survive past childhood (Callier et al., 2005; García-Castillo et al., 2008). A mouse model containing a hypomorphic allele of *BUB1B*, encoding the BubR1 protein, has been used to study the effects of increased aneuploidy at
an organismal and a cellular level (Baker et al., 2004). BubR1 hypomorphic mice exhibit progeria and increased infertility. Mouse embryonic fibroblasts derived from BubR1 hypomorphic mice exhibit increased aneuploidy and increased cellular senescence.

**Aneuploidy and cancer**

While aneuploidy is associated with impaired organismal fitness, aneuploidy also is a hallmark of cancer, a disease of uncontrolled proliferation (Hanahan and Weinberg, 2000). Approximately 90% of all solid tumors and 75% of all hematopoietic tumors contain aneuploid cells, and many of these cells have complex karyotypes with chromosome numbers deviating far from euploid (Weaver and Cleveland, 2006). Over 100 years ago, Theodore Boveri proposed that aneuploidy was involved in tumorigenesis (Boveri, 2008). However, given that aneuploidy in primary cells and whole organisms is detrimental to fitness, we must reconcile these data with the fact that aneuploid tumors exhibit unabated proliferation (reviewed in Pfau and Amon, 2012). One hypothesis is that aneuploidy exists in tumor cells to increase copy number of oncogenes or decrease copy number of tumor suppressors. For instance, many types of cancer including Ewing’s sarcoma, Wilm’s tumor, and several leukemias frequently gain chromosome 8, which contains the oncogenic MYC locus (Kibbelaar et al., 1991; Maurici et al., 1998; Oudat et al., 2001; Peres et al., 2004). In other cancers, the MYC locus is focally amplified to increase copy number (La Farina et al., 2008; Jennings and Mills, 1998). Although aneuploidy is a hallmark of cancer, it remains to be seen whether aneuploidy is a cause or a consequence of tumorigenesis. Mitotic errors may lead to aneuploidy, which then leads
to tumorigenesis; alternately, cancer may arise by other mechanisms such as mutations, which then promote genomic instability and thus generate aneuploidy.

**Benefits of aneuploidy for microorganisms**

Aneuploidy generally causes severe impairment of an organism; however, there are situations in which aneuploidy is beneficial under stringent conditions, particularly for single-celled organisms. In general, aneuploidy appears to be beneficial when changes in gene copy number of a small number of genes is beneficial for the organism, particularly when dealing with a certain stress. *C. albicans* acquires aneuploidies when grown in the presence of fluconazole—an anti-fungal agent—in clinical and laboratory settings. In this setting, aneuploidy serves to amplify the genes *ERG11* (the target of fluconazole) and *TAC1* (a transcriptional regulator of drug efflux pumps), causing increased azole resistance (Coste et al., 2007; Selmecki et al., 2006, 2008). In *S. cerevisiae*, cells grown under nutrient-limiting conditions often gain extra copies of the transporter used to uptake the limited nutrient (Dunham et al., 2002; Gresham et al., 2008). Also in *S. cerevisiae*, chromosomal duplications were observed as a first response to stressful conditions, however the aneuploidy was lost over time under the stressful condition, and particular genomic alterations at the gene level were gained instead to cope with the stress (Yona et al., 2012). The loss of aneuploidy and replacement with alterations at the gene level suggests that while aneuploidy may be beneficial under certain conditions due to changing copy number of one or a few genes, in general, aneuploidy causes a burden on the cell due to the imbalance caused by gene changes across an entire chromosome. In fact, we see that under normal growth conditions, the
phenotypes associated with chromosome-gain aneuploidy are caused by changes in many genes, and are not due to the effects of one or a few dosage-sensitive genes on the particular chromosome gained (Bonney et al., 2015).

**Consequences of aneuploidy at the cellular level**

Since aneuploidy has a profound effect on human health, it is important to elucidate the mechanisms by which aneuploidy impairs cellular fitness. Current cellular models of aneuploidy can be divided into two categories: chronic defined aneuploidies and acute random aneuploidies. All cellular models studied to date show reduced proliferation in aneuploid cells under normal growth conditions.

*Chronic defined aneuploidies*

Aneuploid cells were initially studied using fibroblasts from individuals with Down Syndrome (Segal and McCoy, 1974). These studies showed that aneuploid cells grow and proliferate more slowly than age-matched euploid controls, in line with the developmental defects observed in Down Syndrome individuals. Fibroblasts from Down Syndrome individuals also contained more protein per cell than the euploid controls.

Microcell mediated chromosome transfer has also been employed to generate aneuploid human cells with defined karyotypes (Stingele et al., 2012). This method transfers extra chromosomes to an existing euploid line to generate stable aneuploid cells. These cells exhibited reduced proliferative capacity, metabolic alterations, and activation of autophagy. In addition, the extra chromosome(s) was transcribed and translated in these cells, although downregulation of subunits of protein complexes and protein kinases
was observed. These cells also exhibited a transcriptional signature associated with stress and slow growth.

In our lab, we have generated trisomic mouse embryonic fibroblasts using a breeding scheme of Robertsonian chromosome fusions that allows for collection of sibling-matched trisomic cells and euploid controls. As nearly all trisomies are embryonic lethal in mice, initial studies were done by deriving mouse embryonic fibroblast (MEF) cell lines from the collected embryos (Williams et al., 2008). Characterization of trisomic MEFs showed decreased proliferation and altered metabolism. In addition, genes on the extra chromosome were transcribed in proportion to gene copy number.

We have also generated a set of haploid *S. cerevisiae* that contain an extra copy of one chromosome, referred to as disomes (Torres et al., 2007). The two copies of the disomic chromosome are marked with selectable markers to maintain the presence of both copies of the chromosome throughout strain generation and manipulation. The extra chromosome in disomic strains is transcribed and translated (Figure 2), resulting in a 2-fold increase of nearly all proteins on the duplicated chromosome, although subunits of multi-protein complexes are often downregulated (Dephoure et al., 2014; Torres et al., 2007, 2010). These disomic strains exhibit impaired proliferation (Figure 2) and delayed cell cycle progression, metabolic alterations, and increased sensitivity to chemicals inhibiting protein synthesis and folding (Torres et al., 2007). Subsequent studies have shown that proteotoxic stress is a feature of disomic yeast as well as those generated by triploid meiosis, and that these cells have increased protein aggregate burdens and impaired proteasome function (Oromendia et al., 2012). Further studies in the lab have shown that disomic yeast cells have increased genomic instability (Sheltzer et al., 2011)
and have increased DNA damage during DNA replication, which often goes unrepaired before mitosis and leads to cell death (Blank et al., 2015).

Figure 2: Disomic yeast transcribe and translate the extra chromosome and exhibit proliferation defects (adapted from Siegel and Amon, 2012).

(A) DNA content analysis from microarray, gene expression data from microarray, and protein quantification via SILAC for *S. cerevisiae* Disome V (haploid with an extra copy of chromosome V) show nearly a two-fold increase in DNA, RNA, and protein levels for genes encoded on chromosome V compared to a euploid (data from Torres et al., 2007, 2010). (B) Nearly all disomic budding yeast display proliferation defects (data from Torres et al., 2007). Abbreviations: Dis, disome; WT, wild-type; OD, optical density.

**Acute random aneuploidies**

Genetic defects in the spindle assembly checkpoint have been used to generate cells with high levels of chromosomal instability (CIN) (Babu et al., 2003; Kops et al., 2004; Li et al., 2010). These cells exhibit acute random aneuploidies, and they have been shown to proliferate more slowly than euploid cells. Chemical disruptions of the spindle have also been employed to induce acute random aneuploidies (Thompson and Compton, 2008, 2010). These studies transiently disrupt the spindle to generate random
aneuploidies. These cells also exhibit proliferative defects in addition to other phenotypes, such as decreased autophagic flux (Santaguida et al., 2015). Meiosis of triploid and pentaploid yeast strains in *S. cerevisiae* (Pavelka et al., 2010) and *S. pombe* (Niwa and Yanagida, 1985; Niwa et al., 2006) have also been utilized to generate heterogeneous populations of random aneuploidies which show reduced proliferative capacity.

**Need for an acute, defined aneuploidy model**

Current cellular models of aneuploidy are either chronic defined aneuploidies or acute random aneuploidies. As cancerous cells could potentially arise from an improper cell division producing an aneuploid cell, it is important to understand the physiological changes that occur in the cell following chromosome missegregation. Additionally, all models of defined aneuploidies are of chromosome gains, as chromosome losses are difficult to maintain (St Charles et al., 2010). Models of acute random aneuploidies generate both chromosome gains and losses, but these occur in a heterogeneous manner. Currently, a model of aneuploidy that produces acute, defined aneuploidies of both chromosome gains and losses is needed to better understand acute aneuploidies in a systematic way. In Chapter 3, I describe and characterize a novel model that generates acute, defined aneuploidies in *S. cerevisiae*. 
COMMITMENT TO CELL CYCLE ENTRY IN *S. CEREVISIAE*

The G1/S Transition

In budding yeast, entry into the cell cycle begins in early G1 when the cyclin-dependent kinase (CDK) Cdc28 is activated by the Cln3 cyclin subunit, initiating a cascade of events culminating in cell cycle entry, known as START (Figure 3) (reviewed in Johnson and Skotheim, 2013). Activation of Cdc28 by Cln3 is coordinated with growth rate and extracellular signals such as nutrient levels and the presence of mating pheromone (reviewed in Turner et al., 2012). Cln3-CDK regulates the transcriptional inhibitor Whi5. In early G1, Whi5 is localized to the nucleus where it inhibits the G1/S transcription factor complexes SBF (composed of Swi4 and Swi6) and MBF (composed of Mbp1 and Swi6) (de Bruin et al., 2004; Costanzo et al., 2004). SBF and MBF activate over 100 genes responsible for progression through START, including the transcriptional program responsible for DNA replication and bud formation and two key targets: the cyclins *CLN1* and *CLN2* which promote S-phase and budding (Sidorova and Breeden, 1993).
Figure 3: The G1/S transition in *S. cerevisiae*.

In early G1, Whi5 is bound to SBF, inhibiting transcription of genes required to enter the cell cycle. The transition from G1 to S phase begins when Cln3-CDK phosphorylates Whi5, causing nuclear exit of Whi5. This allows for transcription of genes required to pass through START, including *CLN1*, *CLN2*, and genes required for budding and DNA replication. Cln1 and Cln2 can then associate with CDK to further inactivate Whi5 via phosphorylation, promoting cell cycle entry via a positive feedback loop.

Upon Cln3-CDK activation, Cln3-CDK phosphorylates Whi5, causing its exit from the nucleus (de Bruin et al., 2004; Costanzo et al., 2004). Nuclear export of Whi5 allows for SBF and MBF to activate their gene targets, leading to the accumulation of Cln1 and Cln2. This sets off a positive feedback loop, as Cln1 and Cln2 associate with CDK and further phosphorylate Whi5, promoting its nuclear exit. When Cln1 and Cln2
have accumulated to sufficient levels, Cln-CDKs phosphorylate proteins necessary for bud formation and initiation of DNA replication, driving entry into the cell cycle. Cell cycle entry is also promoted by pathways parallel to CLN3. BCK2, a gene of unknown function, promotes cell cycle entry by inducing transcription of CLN1 and CLN2, potentially through SBF and CLN2, although its exact roles in the onset of S phase are unknown (Di Como et al., 1995). Deletion of BCK2 causes a G1 delay and an increase in critical size (Di Como et al., 1995).

**Regulation of cell size**

Coordination of growth and division is necessary to maintain proper cell size. Adjusting cell size in unicellular organisms provides a mechanism for cells to control their surface-to-volume ratio as a way to adapt to their external environment under changing conditions. Cell volume also correlates with DNA content over a wide range of organisms. In yeast, diploid cells contain twice the amount of DNA and are twice the volume of their haploid counterparts. Some multicellular organisms manipulate DNA content via endoreplication in particular cell types as a means for increasing cell volume and metabolic production, such as follicle and nurse cells in *Drosophila* and endosperm cells in plants (Lee et al., 2009).

Cln3-CDK activation is coordinated with growth rate and extracellular cues including nutrients and mating pheromone. Cell growth rate alters cell cycle progression by controlling critical size. The critical size is the volume at which a cell commits to START (Jagadish et al., 1977; Johnston et al., 1977). Generally, slow-growing cells enter the cell cycle at smaller volumes than faster-growing cells (Johnston et al., 1979). For
instance, yeast cells lacking Sfp1, a transcriptional regulator required for ribosome biogenesis (Fingerman et al., 2003), accumulate volume at ~50% of the rate of a wild-type cell (Hoose et al., 2012; Jorgensen et al., 2002). $sfp1\Delta$ cells spend much longer in G1 and enter the cell cycle at a volume about half the volume of a wild-type cell (Hoose et al., 2012; Jorgensen et al., 2002).

Nutrient sources also affect cellular growth rate. Yeast cells grown in glucose have a larger critical size and a faster doubling time than cells grown in raffinose as the carbon source, although cells grown in raffinose spend a longer fraction of their cell cycle in G1 before commitment to START (Johnston et al., 1979). Varying the nitrogen source also affects growth rate, and cells enter the cell cycle at volumes proportional to their growth rate (Johnston et al., 1979). The cell is able to react rapidly to carbon source changes to alter its critical size based on available nutrients. When cells are shifted from a poorer carbon source (where their growth rate is low) to a richer carbon source, cell growth rate increases and cells in G1 delay transiently until their cell size has increased to an appropriate new volume to pass START (Johnston et al., 1979).

For growth rate to influence critical size it must somehow affect activation of the Cln3-CDK cascade; however, regulation of G1 progression by growth rate is poorly understood. A potential mechanism for coupling cell cycle progression to growth rate would involve the presence of a ‘sizer’ protein that is synthesized proportionally to the overall rate of protein production. Cln3 is a promising candidate for a sizer protein, as Cln3 levels are affected by metabolic state and growth rate. Acetyl-CoA, a central metabolite in glycolysis, promotes $CLN3$ transcription by promoting acetylation of histones in the regulatory region of $CLN3$ (Shi and Tu, 2013).
Cln3 is targeted for degradation by the proteasome by two SCF ubiquitin ligases, SCF\textsuperscript{Cdc4} and SCF\textsuperscript{Grr1} (Landry et al., 2012). Cln3 is redundantly targeted by the F-box proteins Cdc4 and Grr1 \textit{in vivo} (Landry et al., 2012), but it is not known whether the degradation of Cln3 is cell cycle dependent. Grr1 is involved in the glucose repression pathway (Flick and Johnston, 1991), making it an interesting candidate to link the cycling of Cln3 to availability of nutrients.

Currently only one mechanism of Cln3 regulation by macromolecular biosynthesis rate has been described. Cln3 is translationally regulated by the presence of a uORF in the 5’UTR (Polymenis and Schmidt, 1997). Under low growth rate conditions, translation rarely proceed through the uORF to the Cln3 ORF; under optimal growth conditions, the protein synthesis rate is increased, allowing translation to proceed through the uORF to the Cln3 ORF, resulting in accumulation of Cln3. However, the presence of the uORF cannot fully explain Cln3 regulation, as interfering with the uORF produces only subtle effects (Polymenis and Schmidt, 1997). Yet, the Cln-CDK pathway must be the target of yeast size control, as genetic alteration of the \textit{CLN1}, \textit{CLN2}, or \textit{CLN3} alters critical size. Deletion of any of these genes causes an increase in critical size, while overexpression of any of these genes causes a decrease in critical size (Tyers et al., 1992, 1993).

Varying growth rates in the cell can also affect Cln1/2 levels; however, thresholds for the amount of Cln1/2 required to progress through START also vary with growth rate (Schneider et al., 2004). As Cln1 and Cln2 are highly unstable proteins, a single threshold would not be possible for all cells, as cells with a lower growth rate would never be able to accumulate enough Cln protein to progress through START. Large cells also produce
more Cln protein per \textit{CLN} transcript; therefore, posttranscriptional mechanisms may also exist to control cell size (Schneider et al., 2004).

There are two models for how Cln3 acts as a sizer. The first model proposes that Cln3 is titrated against the fixed number of SCB sites in the genome, where Whi5/SBF sit. As Cln3 levels rise, Cln3 activity increases at this fixed number of sites, allowing inactivation of Whi5 and progression through START (Wang et al., 2009). However, although Cln3 protein levels rise during G1, the concentration of Cln3—a nuclear protein—remains relatively constant during G1 as nuclear size increases with increasing cell volume (Jorgensen et al., 2007; Landry et al., 2012; Tyers et al., 1993). Therefore, Cln3 activity is unlikely to increase at SCB sites because the concentration of Cln3 remains relatively constant during G1.

The second model proposes that Cln3 is sequestered at the endoplasmic reticulum in early G1 and released in late G1 in a cell-cycle dependent manner. As the cell grows in G1, levels of the chaperone Ydj1 increase in a growth-dependent manner. Ydj1 is limiting for Cln3 release from the endoplasmic reticulum, thus connecting cell growth with cell cycle entry. Upon sufficient accumulation of Ydj1, Cln3 is released and can initiate the cascade promoting entry into the cell cycle (Vergès et al., 2007; Yahya et al., 2014).

Recent work has identified Whi5 as a potential sizer in the G1/S transition (Schmoller et al., 2015) and is in contrast to the two models presenting Cln3 as a sizer. In this model, all cells are born with a similar amount of Whi5, as Whi5 is synthesized during S/G2/M at a rate independent of cell size. The dilution of Whi5 by cell growth decreases Whi5 activity, thus coupling cell volume with cell cycle entry. When daughter
cells are born, the concentration of Whi5 is higher in smaller daughter cells and lower in larger daughter cells; therefore, smaller daughter cells must spend longer in G1 than larger daughter cells to grow to a volume at which Whi5 is dilute enough to allow passage through START. In this model, conditions that modulate Cln3 concentration are still able to modulate cell size, as altering the Cln3 concentration affects the Whi5 threshold necessary for progression through START. Therefore, the studies showing regulation of Cln3 transcription (Shi and Tu, 2013), translation (Polymenis and Schmidt, 1997), and stability (Menoyo et al., 2013) are in harmony with the Whi5 sizer model.

**Aneuploidy and the cell cycle**

Although aneuploidy is beneficial in a few, select contexts, aneuploidy is detrimental to cellular fitness in the majority of situations. In all primary cells studied under normal growth conditions, aneuploidy decreases growth and proliferation rates. Aneuploid cells have been shown to have a G1 delay (Stingele et al., 2012; Torres et al., 2007), yet the cause of the delay is not known. In Chapter 2, I address this outstanding question by elucidating which steps in the G1 pathway are delayed in disomic yeast cells. Understanding the mechanisms by which aneuploid cells delay passage through G1 is necessary for our understanding of how aneuploidy alters growth and proliferation. In Chapter 3, I characterize cell cycle progression in aneuploid cells following the onset of aneuploidy via chromosome missegregation. Understanding the effects of aneuploidy on growth and proliferation may facilitate future insight into how cancerous aneuploid cells are able to evade the detrimental effects of aneuploidy and proliferate rapidly.
THE PROTEIN QUALITY CONTROL NETWORK

Protein folding is an integral part of the cell’s functions. Proteins are the main players by which the cellular machinery operates. For proteins to function properly, they must adopt the correct conformation, which can be difficult in the crowded cellular environment. Cellular mechanisms exist to promote proper protein folding. If proteins are not folded properly, they have the propensity to aggregate (reviewed in Tyedmers et al., 2010). However, protein aggregation is not a haphazard event, but is instead a regulated process in controlling an imbalance in protein homeostasis. Protein aggregates are deposited at specific sites in the cell where they are refolded or degraded by components of the protein quality control network. Protein aggregation is also associated with aging and disease (reviewed in David, 2012).

Protein folding begins concurrently with protein synthesis, as proteins being synthesized are coming off of the ribosome. Ideally, proteins will adopt their native, or correct, fold; however, misfolded states are often quite close in energetics to the native conformation, making non-native conformations a danger both to folding proteins and native proteins (Dobson, 2003; Jahn and Radford, 2005). Misfolded proteins usually expose hydrophobic residues that are prone to aggregation. In a properly folded protein, these residues are buried inside the three-dimensional structure. The cell has a protein quality control system that acts as a defense mechanism against a wide range of protein stresses. When the protein quality control system is overwhelmed by a large number of misfolded proteins or the protein quality control system is impaired, protein aggregates accumulate in the cell.
**Molecular chaperones**

The cell’s first line of defense in combating misfolded proteins and preventing aggregation is for proteins called molecular chaperones to assist the folding of newly synthesized proteins or to mediate the refolding of misfolded proteins (reviewed in Richter et al., 2010). Molecular chaperones are divided into classes based on their mechanism of action, the clients they fold, and the co-chaperones they require. Many classes of chaperones exist in a single cell, often with different members in each cellular compartment to ensure folding of a wide variety of clients throughout the cell. In fact, a single polypeptide can sequentially interact with multiple chaperones throughout its lifetime, from initial folding during translation to complex assembly.

**Protein degradation**

If proteins cannot be refolded properly, they must be degraded. Proteins can be degraded in the nucleus or cytoplasm by cellular machinery such as the 26S proteasome (Goldberg, 2003; Wójcik and DeMartino, 2003), or they can be transported into the lysosome where they are degraded by acidic hydrolases (Nakatogawa et al., 2009). The 26S proteasome is a complex cellular machine that degrades soluble, misfolded proteins. Misfolded proteins are recognized and targeted for degradation by specific E3 ubiquitin ligases, which conjugate ubiquitin to the polypeptide targeted for degradation. Polyubiquitylated proteins are recognized for degradation by the proteasome which deubiquitinates the substrate and cleaves the substrate into short polypeptides for recycling of amino acids (Goldberg, 2003).
**Protein aggregation**

When the level of misfolded proteins overwhelms the cell’s capacity to refold or degrade these species, protein aggregation occurs. Proteins that are neither properly folded nor degraded accumulate in protein aggregates. Protein aggregation is thought to be a cytoprotective function, as it seems that soluble, misfolded proteins are the toxic species, instead of aggregates (Arrasate et al., 2004; Chiti and Dobson, 2006; Cohen et al., 2006; Douglas et al., 2008; Saudou et al., 1998; Tanaka et al., 2004). The causes of protein aggregation can be categorized into four main classes (reviewed in Tyedmers et al., 2010). Protein aggregation may occur from a single, severe condition that exceeds the cell’s protein quality control capacity, or it may arise from the combination of multiple, moderate conditions.

First, proteins may aggregate due to mutations that cause an increased propensity for proteins to misfold and aggregate. Mutations can be present in client proteins, leading to diseases such as in Huntington’s disease or type II diabetes (Chiti and Dobson, 2006), or, mutations may occur in members of the protein quality control network. For example, mutations in the chaperone \(\alpha\)-crystallin lead to cataract formation (Andley, 2006).

Second, defects in protein biogenesis such as translation errors or defects in protein complex assembly can lead to aggregation, as members of protein complexes that are not properly assembled are prone to aggregate (Drummond and Wilke, 2008). Third, environmental stresses such as increased temperature or oxidative stress can lead to aggregation. Increased heat causes unfolding of proteins that may be reversible (Parsell et al., 1994); however, oxidative damage often leads to irreversible protein modifications that induce misfolding and aggregation (Stadtman and Levine, 2000). Fourth, protein...
aggregation increases in cells during aging due to exhaustion of the protein quality control network over time (David, 2012).

**Protein disaggregation**

Once protein aggregation has occurred, the cell must sequester aggregates and then work to clear them. To clear protein aggregates, misfolded proteins can either be re-solublized and refolded or degraded. Refolding proteins is a less costly process for the cell compared to proteolysis and re-synthesis of the protein. In yeast, a two-component system composed of the Hsp70 system and the AAA+ chaperone Hsp104 is primarily responsible for reversing protein aggregation (Glover and Lindquist, 1998). Hsp70 and a co-chaperone of the Hsp40 system (a J protein) bind to aggregates, restricting access of the aggregate to the proteasome and thus favoring the refolding pathway over the degradation pathway. Hsp70/40 then transfer the aggregated polypeptide to the processing core of Hsp104 for refolding (Hasberger et al., 2007, 2008). Hsp104 uses ATP to thread the polypeptide from the aggregate into the Hsp104 channel. ATP-driven conformational changes in Hsp104 pull the substrate through the Hsp104 channel, extracting the polypeptide from the aggregate and allowing for refolding (Lum et al., 2004; Weibezahn et al., 2004). As Hsp104 is often found at protein aggregates, protein aggregates can be visualized *in vivo* using fluorescently labeled Hsp104.

**Protein aggregation and aneuploidy**

Proteotoxicity has been reported in multiple aneuploid systems, including yeast and mouse and human cell lines (Oromendia et al., 2012; Santaguida et al., 2015;
Aneuploid cells transcribe and translate the majority of the genes on the extra chromosome(s), leading to a two-fold increase for most proteins on the extra chromosome(s) and causing protein imbalances in the cell (Dephoure et al., 2014; Pavelka et al., 2010; Stingele et al., 2012; Torres et al., 2010). Interestingly, many proteins on the extra chromosome(s) that are members of complexes are downregulated (Dephoure et al., 2014; Stingele et al., 2012; Torres et al., 2007, 2010). Therefore, aneuploid cells are required both to properly fold all of the excess proteins from the additional chromosome(s) and to deal with excess protein complex subunits, either through degradation or sequestration (Figure 4) (Oromendia and Amon, 2014). Without their binding partners, many protein complex subunits are unstable and are bound by chaperones (Boulon et al., 2010).
Figure 4: Aneuploidy increases the burden on the protein quality control network (adapted from Oromendia and Amon, 2014).

(A) In euploid cells, subunits of complexes are produced in roughly the proper stoichiometric ratios, resulting in little burden on the protein quality control (QC) network. (B) In aneuploid cells, a chromosome present in an extra copy causes subunits of complexes found on that chromosome to be synthesized in excess, resulting in an increased burden on the protein quality control network.

Chaperones are upregulated as part of a cellular stress response signature found in aneuploid cells across a wide variety of species (Sheltzer et al., 2012; Stingele et al., 2012; Torres et al., 2007). In addition, many aneuploid cells are sensitive to inhibitors of the protein quality control network (Tang et al., 2011; Torres et al., 2007). Aneuploid cells rely on the protein quality control network to cope with their protein imbalances, which makes aneuploid cells more susceptible than euploid cells to impairment of the
protein quality control network (Figure 4). In addition, both the Hsp90 chaperone family and the proteasome are compromised in many aneuploid yeast strains (Oromendia et al., 2012).

Aneuploid cells harbor an increased load of protein aggregates, as measured by Hsp104-eGFP foci (Oromendia et al., 2012), suggesting that the protein quality control network is overwhelmed in aneuploid cells. In Chapter 3, I investigate the induction of proteotoxic stress in budding yeast following the onset of aneuploidy. I examine the kinetics of protein aggregate formation following chromosome missegregation using fluorescently-tagged Hsp104 as a marker of protein aggregates.
SUMMARY OF WORK PRESENTED

Here, I investigate the effects of aneuploidy on cellular physiology, both in the context of cells that have been aneuploid for many generations (chronic defined aneuploidy) and in newly aneuploid cells immediately following chromosome missegregation (acute defined aneuploidy). I focus on the effects of aneuploidy on cellular proliferation, particularly on delays in cell cycle progression arising from aneuploidy.

In Chapter 2, I focus on the G1 delay in disomic yeast cells. Previous work in the lab showed that disomic yeast exhibit a delay in G1 (Torres et al., 2007). We show that 10 of 14 aneuploid yeast strains have a growth defect in G1. 10 of 14 aneuploid yeast strains also exhibit a delay in cell cycle entry that correlates with the size of the extra chromosome. We find that disomic yeast are delayed in cell cycle entry at the most upstream known component of the G1 pathway, Cln3 protein accumulation. Delayed G1 cyclin accumulation in aneuploid yeast can be overcome by overexpression of a G1 cyclin required for entry into the cell cycle. Aneuploidy, like other cellular stresses, interferes with the cell’s ability to enter the cell cycle.

In Chapter 3, I look at the immediate cellular response to aneuploidy following chromosome missegregation. I describe a novel set of yeast strains that can be induced to become aneuploid with a defined karyotype. I use these strains to characterize the onset of aneuploidy, particularly looking at alterations in cell cycle progression and accumulation of proteotoxic stress. While I showed in Chapter 2 that aneuploidy, like many other stresses, elicits a G1 delay, in Chapter 3 I address whether the G1 delay and other cell cycle delays are an immediate response to aneuploidy, or whether the cell cycle
delays accumulate over time after the onset of aneuploidy. Here, I see that the cell cycle delays occur immediately following chromosome missegregation, with nearly all aneuploid strains exhibiting cell cycle delays in the second division following chromosome missegregation. Also, I report that aneuploid populations of a homogeneous karyotype exhibit increased variance compared to euploid populations.

In Chapter 3 I also look at monosomy. Monosomy has not been systematically studied, in part due to the fact that it is hard to select for chromosome loss and endoreduplication can often restore monosomies to euploidy (Alvaro et al., 2006; Anders et al., 2009; St Charles et al., 2010). I report a systematic characterization of monosomic cells and demonstrate that monosomic cells generally have decreased cellular fitness compared to disomes and trisomes, suggesting that chromosome loss is more detrimental to the cell than chromosome gain.

I also report in Chapter 3 that proteotoxic stress accumulates immediately following chromosome missegregation. Disomes, trisomes, and monosomes all accumulate Hsp104 foci following chromosome missegregation, suggesting that proteotoxic stress accumulates in aneuploid cells due to protein imbalances, and not simply due to accumulation of excess protein from an extra chromosome, as monosomes accumulate Hsp104 aggregates despite having lost genomic content. Interestingly, we see that cell cycle progression is not required for the accumulation of Hsp104 foci, as cells that are held in G1 immediately following chromosome missegregation accumulate proteotoxic stress.

In Chapter 4, I summarize important results from my work and discuss future directions of study.
REFERENCES


Chapter 2: Aneuploid yeast strains exhibit defects in cell growth and passage through START

Reprinted from Molecular Biology of the Cell:


The experiments in Figures 1B-D,F,I,K-N; 2B-D,F,I,K-N; 5A-D,G,H; 6B,C,E,H,I; 4; 8; 12; and 13F-I were performed by CG.

The experiments in Figure 9B-D were performed by CG and GAB, with data analysis assistance from NTI.

The experiments in Figure 10A,B were performed by CG and TMC.

The experiments in Figure 7 were performed by SC.

The experiments in Figure 17 were performed by AA.

All other experiments were performed by RRT (RRB).
**ABSTRACT**

Aneuploidy, a chromosome content that is not a multiple of the haploid karyotype, is associated with reduced fitness in all organisms analyzed to date. In budding yeast aneuploidy causes cell proliferation defects, with many different aneuploid strains exhibiting a delay in G1, a cell cycle stage governed by extracellular cues, growth rate and cell cycle events. Here, we characterize this G1 delay. We show that 10 of 14 aneuploid yeast strains exhibit a growth defect during G1. Furthermore, 10 of 14 aneuploid strains display a cell cycle entry delay that correlates with the size of the additional chromosome. This cell cycle entry delay is due to a delayed accumulation of G1 cyclins that can be suppressed by supplying cells with high levels of a G1 cyclin. Our results indicate that aneuploidy frequently interferes with the ability of cells to grow and, as many other cellular stresses, entry into the cell cycle.
INTRODUCTION

In most eukaryotes, the decision of whether or not to enter the cell cycle is made in G1 and governed by extracellular and intracellular cues (reviewed in Turner et al., 2012). Identifying and characterizing the pathways that regulate this decision is critical not only for understanding normal cell division, but also the abnormal cell divisions that are seen in cancer. The molecular events governing entry into the cell cycle – known as START – are well characterized in budding yeast (Jorgensen and Tyers, 2004). During early G1, the cyclin-dependent kinase (CDK) Cdc28 is activated by the Cln3 cyclin subunit in a manner that is intimately coordinated with growth rate and extracellular signals such as nutrient availability and the presence of mating pheromone (reviewed in Turner et al., 2012). In turn, Cln3-CDKs regulate the transcriptional inhibitor Whi5. During G1, Whi5 localizes to the nucleus where it inhibits the SBF transcription factor complex (de Bruin et al., 2004; Costanzo et al., 2004). This complex (composed of Swi4 and Swi6) and a similar complex MBF (composed of Mbp1 and Swi6), activate the transcriptional program essential for DNA replication and bud formation (Futcher, 2002). Key among SBF’s targets are the two G1 cyclin-encoding genes CLN1 and CLN2 (Cross et al., 1994; Stuart and Wittenberg, 1994). Cln1/2-CDKs trigger S phase and budding.

Cln3-CDKs phosphorylate Whi5 (de Bruin et al., 2004; Costanzo et al., 2004), triggering nuclear export of this transcriptional inhibitor and allowing SBF-dependent transcription to commence and Cln1/2-CDKs to accumulate. This sets in motion a positive feedback loop where Cln1/2 CDKs further phosphorylate Whi5, promoting its nuclear export and hence SBF-meditated transcription of CLN1 and CLN2 (Skotheim et al., 2008). Once cells have accumulated enough Cln-CDK activity, proteins critical for
bud formation and initiation of DNA replication are phosphorylated, driving entry into the cell cycle (Bloom and Cross, 2007). Pathways parallel to CLN3 also promote entry into the cell cycle. BCK2 promotes passage through START in parallel to CLN3 by inducing CLN1/2 transcription by an unknown mechanism (Di Como et al., 1995; Epstein and Cross, 1994).

Activation of the Cln3-CDK cascade is controlled by macromolecule biosynthesis rates (reviewed in Turner et al., 2012). Cell growth controls cell cycle progression by influencing the critical size, which is the volume at which cells enter the cell cycle (Rupes, 2002). In general, slow-growing cells enter the cell cycle at smaller volumes than cells growing at faster rates (Ferrezuelo et al., 2012; Jorgensen and Tyers, 2004). In other words, a higher growth rate leads to an increase in critical cell size. How growth rate controls the G1 cell cycle machinery is poorly understood. So far, only one mechanism, translational regulation of Cln3, has been described whereby growth rate affects the G1 cell cycle machinery (Polymenis and Schmidt, 1997). However, additional mechanisms must exist that link growth rate to cell cycle entry, as the consequences of interfering with this CLN3 regulatory mechanism are subtle (Polymenis and Schmidt, 1997). Whatever these additional mechanisms are, it is clear that Cln-CDKs must be the target, as modulating Cln-CDK activity affects the critical cell size. For example, overexpression of any of the G1 cyclins (CLN1, CLN2, or CLN3) leads to a “small cell” phenotype, while their deletion causes a “large cell” phenotype (Tyers et al., 1992, 1993).

We previously constructed a series of haploid budding yeast strains that are disomic for one or two chromosomes (henceforth disomes; Torres et al., 2007). All strains were found to exhibit proliferation defects compared to a wild-type strain.
Analysis of cell cycle progression of aneuploid yeast strains released from a pheromone-induced G1 arrest showed that most disomes exhibit a delay in CLN2 expression and cell cycle entry (Torres et al., 2007). Here, we further characterize the defects underlying the G1 delay in these strains. We find that most of the disomes (10 of 14) exhibit a growth defect during G1. Entry into the cell cycle was also delayed in 10 of 14 strains, resulting in an increase in critical cell size that correlated with the degree of aneuploidy. Our analyses further show that a defect in G1 cyclin accumulation underlies the G1 delay of disomic strains that can be suppressed by supplying cells with high levels of CLN2. Our results indicate that aneuploidy causes a G1 delay in most strains analyzed by interfering with cell growth and/or the activation of the Cln3-CDK cascade.

RESULTS

G1 is extended in most disomic yeast strains

The 20 disomic budding yeast strains we previously generated (Torres et al., 2007) share a number of phenotypes, prominent among them a delay in entry into S phase after release from an α-factor pheromone-induced G1 arrest. 16 of 20 aneuploid yeast strains exhibited a delay in bud formation and initiation of DNA replication, ranging from 10-20 minutes (Torres et al., 2007). Treatment of cells with pheromone inhibits cell cycle progression but cell growth continues, disrupting the co-ordination of cell growth and division. Thus, pheromone block-release experiments do not allow one to determine whether cell growth or cell cycle entry (or both) is impaired in aneuploid yeast cells.

To characterize the effects of aneuploidy on cell growth and cell cycle entry, we isolated small, unbudded cells using centrifugal elutriation and examined their growth
and cell cycle entry properties. We chose two aneuploid yeast strains for this analysis that did not exhibit a G1 delay upon pheromone release (strains disomic for chromosome II and V) and 12 strains that showed cell cycle entry delays after release from an α-factor arrest (strains disomic for chromosome IV, VIII, X, XI, XII, XIII, XIV, XV, XVI, VIII+XIV, XI+XV, and XI+XVI; Figure 1). Consistent with our previous studies (Torres et al., 2007), strains disomic for chromosome II and V did not exhibit a cell cycle entry delay as judged by the time to bud formation following isolation of isovolumetric populations of wild-type and disomic cells (Figure 1A, C). Cell cycle entry was delayed in all other disomic yeast strains tested, with delays ranging from ~5 min for disome XIII to ~110 min for disome IV. We conclude that most of the disomic yeast strains tested are delayed in cell cycle entry.
Figure 1: Cells carrying an extra chromosome delay budding.

Wild-type ([A-N], A11311, filled circles) and disomes (open circles) II ([A], A6865), IV ([B], A12687), V ([C], A14479), VIII ([D], A13628), X ([E], A21986), XI ([F], A13771), XII ([G], A12694), XIII ([H], A21987), XIV ([I], A13979), XV ([J], A12697), XVI ([K],
A12700), VIII+XIV ([L], A15615), XI+XV ([M], A12691), and XI+XVI ([N], A12699) were grown at 30°C in selective medium supplemented with 2% raffinose. Small unbudded cells were isolated by centrifugal elutriation and released into the cell cycle at 30°C in YEPD. Time points were taken every 15 minutes to determine the percentage of budded cells.

Critical cell size is increased in many disomic strains

A delay in cell cycle entry can be caused by a cell cycle defect that culminates in a delay in the accumulation of Cln-CDK activity, slowed growth, or a combination of both. Cell cycle entry delays caused by defects in the cell cycle machinery promoting the G1 – S transition result in an increase in critical cell size (Rupes, 2002). We found that 10 of 14 aneuploid strains analyzed showed an increase in critical cell size, defined by the modal cell volume corresponding to half-maximal budding (Figure 2A-N; Table 1). This increase in critical size ranged from 3 fL for disome VIII+XIV to 22 fL for disome XI+XV. Interestingly, the increase in critical size correlates with the size of the additional chromosome (Figure 2O), suggesting that some aspect of aneuploidy proportional to the amount of additional genetic material contributes to the increase in critical size.
Figure 2: Cells carrying an extra chromosome delay entry into the cell cycle by increasing the critical size for budding.

Wild-type ([A-N], A11311, filled circles) and disomes (open circles) II ([A], A6865), IV ([B], A12687), V ([C], A14479), VIII ([D], A13628), X ([E], A21986), XI ([F], A13771), XII ([G], A12694), XIII ([H], A21987), XIV ([I], A13979), XV ([J], A12697), XVI ([K], A12700), XI+XV ([L], A12691), XI+XVI ([M], A12699), and VIII+XIV ([N], A15615).
were grown at 30°C in selective medium supplemented with 2% raffinose. Small, unbudded cells were isolated by centrifugal elutriation and released into the cell cycle at 30°C in YEPD. Time points were taken every 15 minutes to measure cell volume and the percentage of budded cells. (O) Correlation between the critical size for budding and the size of the extra chromosome in the disome. The critical size (shown as percent of wild-type critical size for each experiment) is positively correlated with the size of the extra chromosome ($r^2 = 0.41$, $p = 0.019$). Disome XII was excluded from the correlation analysis due to variations in rDNA copy number, which preclude determination of the exact chromosome size. The data shown are the same as in Figure 1 but here budding is plotted as a function of cell volume instead of time.

### Table 1: Critical sizes and growth constants of disomic cells

<table>
<thead>
<tr>
<th>Strain</th>
<th>Critical size (FL)</th>
<th>Critical size, percentage of wild type</th>
<th>Exponential growth constant, $k$ (min$^{-1}$)$^*$</th>
<th>Exponential growth constant, percentage of wild type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dis II</td>
<td>35 (38)</td>
<td>92</td>
<td>0.006144 (0.005882)</td>
<td>104</td>
</tr>
<tr>
<td>Dis IV</td>
<td>53 (39)</td>
<td>136</td>
<td>0.004667 (0.006939)</td>
<td>67</td>
</tr>
<tr>
<td>Dis V</td>
<td>33 (40)</td>
<td>83</td>
<td>0.006101 (0.007391)</td>
<td>83</td>
</tr>
<tr>
<td>Dis VIII</td>
<td>46 (38)</td>
<td>121</td>
<td>0.006339 (0.007433)</td>
<td>85</td>
</tr>
<tr>
<td>Dis X</td>
<td>40 (35)</td>
<td>114</td>
<td>0.006894 (0.006931)</td>
<td>99</td>
</tr>
<tr>
<td>Dis XI</td>
<td>55 (40)</td>
<td>138</td>
<td>0.005967 (0.006944)</td>
<td>86</td>
</tr>
<tr>
<td>Dis XII</td>
<td>53 (38)</td>
<td>139</td>
<td>0.004160 (0.005239)</td>
<td>79</td>
</tr>
<tr>
<td>Dis XIII</td>
<td>40 (40)</td>
<td>100</td>
<td>0.004738 (0.005839)</td>
<td>81</td>
</tr>
<tr>
<td>Dis XIV</td>
<td>41 (36)</td>
<td>114</td>
<td>0.006343 (0.007126)</td>
<td>89</td>
</tr>
<tr>
<td>Dis XV</td>
<td>52 (39)</td>
<td>133</td>
<td>0.005213 (0.005098)</td>
<td>102</td>
</tr>
<tr>
<td>Dis XVI</td>
<td>36 (38)</td>
<td>95</td>
<td>0.005558 (0.007879)</td>
<td>71</td>
</tr>
<tr>
<td>Dis XI + XV</td>
<td>60 (38)</td>
<td>158</td>
<td>0.006480 (0.007471)</td>
<td>87</td>
</tr>
<tr>
<td>Dis XI + XVI</td>
<td>54 (37)</td>
<td>146</td>
<td>0.005835 (0.008001)</td>
<td>73</td>
</tr>
<tr>
<td>Dis VIII + XIV</td>
<td>42 (39)</td>
<td>108</td>
<td>0.006196 (0.006460)</td>
<td>96</td>
</tr>
<tr>
<td>Diploid</td>
<td>70 (38)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

$^*$ Values in parentheses are the haploid wild-type results for each experiment

To determine whether the delay in cell cycle entry was proportional to the amount of additional DNA or proportional to other factors dependent on chromosome size such as the amount of yeast gene products produced from the additional chromosomes, we
examined the critical sizes of yeast strains containing human DNA carried by yeast artificial chromosomes (YACs). We do not know whether transcription occurs from the human DNA; however, due to the fundamentally different splicing mechanisms in yeast and mammals, any mRNA produced from the YAC is likely to produce few, if any, functional gene products. This allowed us to examine the impact of excess chromosomal DNA on G1 – S phase progression, without excess gene products.

Introduction of an 850 kb, 670 kb or 620 kb YAC did not delay cell cycle entry as judged by budding (Figure 3). We conclude that additional functional yeast chromosomes, but not chromosome-sized amounts of DNA that do not produce yeast gene products, cause an increase in critical size. This increase in critical size is proportional to the size of the additional chromosome and thus the amount of additional genes, as chromosomes in yeast are densely packed with genes.

![Figure 3: Yeast artificial chromosomes carrying human DNA do not cause an increase in critical size.](image)

Wild-type (A11311, filled circles), YAC-2 (A17392, 850 kb, open triangles), YAC-3 (A17393, 670 kb, open circles), and YAC-4 (A17394, 620 kb, open squares) cells were grown at 30°C in selective medium supplemented with 2% raffinose. Small unbudded cells were isolated by centrifugal elutriation and released into the cell cycle at 30°C in YEPD. Time points were taken every 15 minutes to measure cell volume and the percentage of budded cells.
Cell volume accumulation in G1 is slowed in aneuploid yeast strains

Following elutriated cells as they progress through G1 also allowed us to assess
the effects of disomy on cell growth during G1, monitored by cell volume accumulation.
This was of interest because slow growth can delay entry into the cell cycle. For example,
cells lacking Sfp1, a transcription factor required for ribosome biogenesis (Fingerman et
al., 2003), exhibit a severe G1 delay (Hoose et al., 2012; Jorgensen et al., 2002). \textit{sfp1Δ}
cells obtained by centrifugal elutriation take a longer time to enter the cell cycle than
wild-type cells as judged by bud formation (Figure 4A; Hoose et al., 2012; Jorgensen and
Tyers, 2004). Cell volume measurements during G1 show that \textit{sfp1Δ} cells have a cell
volume accumulation rate $\sim$50% of wild-type when G1 time points ($<20\%$ budded) were
fit to an exponential function, and a critical size smaller than wild-type, consistent with
other reports (Figure 4B-D; Hoose et al., 2012; Jorgensen et al., 2002).
Figure 4: Deletion of *SFP1* delays entry into the cell cycle by decreasing the rate of volume accumulation.

Wild-type (A11311, black) and sfp1Δ (A3009, red) cells were grown at 30°C in selective medium supplemented with 2% raffinose. Small unbudded cells were isolated by centrifugal elutriation and released into the cell cycle at 30°C in YEPD. Time points were taken every 15 minutes to determine the percentage of budded cells (A), G1 growth rate (B), cell size distributions for elutriated cells at the same volume and after 1 hour of growth (C), and the percentage of budded cells as a function of cell volume (D).

To determine whether a growth defect contributes to the G1 delay observed in aneuploid cells, we examined rates of volume increase of aneuploid and euploid cells during G1. We isolated small, unbudded cells using centrifugal elutriation and monitored
cell volume as a function of time. Of the 14 aneuploid strains analyzed, we found 10 exhibited a defect in volume accumulation (Figure 5; Figure 6). This is easily seen when size distributions of wild-type and aneuploid cells of the same volume are compared with size distributions after growth in rich medium for 60 minutes (Figure 5B, D, F, H).
Figure 5: Cells carrying an extra chromosome show a decrease in growth rate during G1.

(A-H) Wild-type ([A-H], A11311, black), disome IV ([A,B], A12687, red), disome XIV ([C,D], A13979, red), disome XV ([E,F], A12697, red), and disome XVI ([G,H], A12700, red) cells were grown at 30°C in selective medium supplemented with 2% raffinose. Small unbudded cells were isolated by centrifugal elutriation and released into
the cell cycle at 30°C in YEPD. Time points were taken every 15 minutes to monitor cell volume. G1 time points (less than 20% budded cells in the population) were fitted to an exponential function to determine growth parameters. (B, D, F, H) Cell size distributions for elutriated wild-type (black) and disomic cells (red) of the same volume and after 1 hour of growth in YEPD medium.

(I) Distribution of growth constants for disomes II (A6865), IV (A12687), V (A14479), VIII (A13628), X (A21986), XI (A13771), XII (A12694), XIII (A21987), XIV (A13979), XV (A12697), XVI (A12700), XI+XV (A12691), XI+XVI (A12699), and VIII+XIV (A15615), are shown as the percent of the wild-type (A11311) growth constant for each experiment. Disomes IV, XVI, and XI+XVI (red) show growth rates <75% of wild-type; disomes V, VIII, XI, XII, XIII, XIV, and XI+XV (yellow) show growth rates 75-90% of wild-type; disomes II, X, XV, and VIII+XIV (green) show growth rates 90-110% of wild-type.
Figure 6: Growth rate is decreased in disomic cells during G1.

Wild-type ([A-J], A11311, black), and disomes (red) II ([A], A6865), V ([B], A14479), VIII ([C], A13628), X ([D], A21986), XI ([E], A13771), XII ([F], A12694), XIII ([G], A21987), XI+XV ([H], A12691), XI+XVI ([I], A12699), and VIII+XIV ([J], A15615) cells were grown at 30°C in selective medium supplemented with 2% raffinose. Small unbudded cells were isolated by centrifugal elutriation and released into the cell cycle at 30°C in YEPD. Time points were taken every 15 minutes to measure cell volume. G1 time points (less than 20% budded cells in the population) were fitted to an exponential
To determine the growth rate during G1, we fit the plots of volume increase vs. time to exponential functions, restricting this analysis to time points in which less than 20% of the cells in the population were budded. Growth rates were reduced in 10 of 14 aneuploid strains; this reduction is best observed when the growth rate is shown as a percentage of the wild-type control (Figure 5I, Table 1). Three strains (disomes IV, XVI and XI+XVI) showed growth rates of less than 75% of wild-type (Figure 5I, red); 7 strains (disomes V, VIII, XI, XII, XIII, XIV, and XI+XV) exhibited growth rates between 75-90% of wild-type (Figure 5I, yellow); four strains (disomes II, X, XV, and VIII+XIV) grew as well as wild-type cells (Figure 5I, green). The decreases in growth rates of aneuploid strains were significant (p=0.0007, paired Student’s t-test); however, the extent of the growth defect did not correlate with the size of the additional chromosome (Figure 6K).

The growth properties of disome XVI cells are particularly notable, as the delay in S phase entry observed in this strain is entirely due to a defect in cell volume accumulation. Critical cell size was not affected in disome XVI, yet budding was delayed for almost 40 minutes (Table 1; Figure 1K; Figure 2K). Cell volume measurements showed that growth was impaired in disome XVI cells (Figure 5G, H), providing an explanation for the delay in bud formation. It is possible that the additional copy of CLN2 located on chromosome XVI masks any cell cycle defect, as G1 cyclin levels are rate-limiting for cell cycle entry (Futcher, 1996). In summary, our results indicate that most aneuploid strains analyzed show a reduced growth rate in G1. In contrast to the increased
critical size observed in aneuploid cells, the severity of the cell volume accumulation
defect is not correlated with the amount of additional DNA (Figure 6K). These findings
suggest that gene-specific effects, and not general features of aneuploidy, are responsible
for the cell volume accumulation defect seen in the disomic strains.

**Decreased growth rates in aneuploid cells are not due to gross amino acid
biosynthesis defects**

Our data show that aneuploid yeast strains exhibit both growth defects and cell
cycle entry delays. We decided to first characterize the growth defect in more detail. To
determine whether the G1 growth defect was due to a lack of amino acids, we measured
pools of free intracellular amino acids in aneuploid cells. *sfp1Δ* cells were analyzed for
comparison as this mutant is able to synthesize amino acids but grows slowly due to
defects in ribosome biogenesis (Fingerman et al., 2003).

To measure intracellular amino acid pools, we grew cells in medium
supplemented with the essential amino acids for these strains (lysine, leucine, tryptophan,
and methionine). Levels of the amino acids alanine, asparagine, aspartate, glycine,
histidine, isoleucine, phenylalanine, proline, serine, threonine, tyrosine and valine as well
the intermediates of the TCA cycle were quantified using time-of-flight mass
spectrometry. Intracellular amino acid pools were not lower in disomic strains IV, VIII,
XI, XV, or XVI (Figure 7), indicating that amino acid abundance (at least of the amino
acids measured) was not affected in aneuploid strains. In fact, disome IV showed
consistently higher levels of most amino acids (except aspartate and isoleucine) and TCA
cycle intermediates. Increased amino acid levels in disome IV cells may be related to the
significant proliferation defect of this disome as this phenotype is also seen in the sfp1Δ strain, which proliferates slowly (Figure 7R).

<table>
<thead>
<tr>
<th>Figure 7</th>
<th>Amino acids and organic acid levels are not altered in aneuploid cells.</th>
</tr>
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<tbody>
<tr>
<td>(A-R)</td>
<td>Metabolomic analysis of wild-type (A11311), sfp1Δ (A3009), and disomes IV (A12687), VIII (A13628), XI (A13771), XV (A12697), and XVI (A12700). Cells were grown in synthetic medium supplemented with the necessary nutrients for growth (see Materials and Methods). Metabolites were extracted, separated by gas chromatography and quantified by time-of-flight mass spectrometry. Error bars represent standard error of the mean (n≥5 for metabolites [A-Q]; n=4 for doubling time [R]).</td>
</tr>
<tr>
<td>(S)</td>
<td>Overview of TCA cycle.</td>
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Consistent with the conclusion that free amino acids are not limiting in aneuploid cells is the observation that aneuploid cells do not exhibit a starvation response (Figure 8). *GCN4* encodes a transcription factor that controls the expression of 30 amino acid biosynthetic genes (Hinnebusch, 2005). Its abundance is translationally regulated; upon amino acid starvation, *GCN4* translation is increased (Hinnebusch, 2005). We monitored a *GCN4*-LacZ reporter construct (Hinnebusch, 1985) by LacZ activity in the absence or presence of amino acid starvation induced by the addition of 3-amino-1,2,4-triazole (3-AT), a competitive inhibitor of an intermediate step in histidine synthesis. In the absence of 3-AT, all disomes tested (IV, VIII, XI, XV, and XVI) showed similar levels of LacZ activity to the euploid control (Figure 8, gray bars). In the presence of 3-AT, disomic cells exhibited an increase in LacZ activity due to *GCN4*-LacZ translational up-regulation consistent with the euploid control (Figure 8, white bars). Therefore, we conclude that the disomes analyzed do not exhibit a starvation response under normal growth conditions and are not defective in eliciting a starvation response. Thus, the slower growth rate seen in aneuploid cells is not the result of limiting amounts of amino acids, but is likely due to decreased rates of biomass production.
Figure 8: Starvation response is normal in disomes IV, VIII, XI, XV and XVI.

Wild-type (A25870), disome IV (A25872), disome VIII (A25871), disome XI (A25874), disome XV (A25873), and disome XVI (A25875) carrying a GCN4-LacZ reporter were untreated (gray bars) or treated with 100 mM 3-amino-1,2,4-triazole (3-AT; white bars) for 5 hours. Cells were harvested, lysed, and β-galactosidase activity measured. Error bars represent standard deviation (n=3).

Effects of disomy XVI on translation

Next we examined whether defects in translation are responsible for the growth defects observed in the disomic yeast strains. For this analysis we chose disome XVI as this strain exhibits one of the most dramatic growth defects of the disomes, yet does not show a cell cycle entry delay as judged by critical cell size measurements. \(^{35}\)S-methionine incorporation studies did not reveal any significant differences between wild-type and disome XVI cells (data not shown), yet we were able to detect defects in \(^{35}\)S-methionine incorporation in sfp1Δ cells (data not shown), which exhibit a severe growth defect (Hoose et al., 2012; Jorgensen et al., 2002; Figure 4). Although a decrease in growth rate of disome XVI was detected by cell volume measurements (Figure 5; Table 1), changes were not evident when translation was examined using polysome profiling (Figure 9A). These results indicate that this disomic strain does not suffer from dramatic translation defects.
Figure 9: Translational efficiency is not altered in disome XVI.

(A) Polysome profiles of wild-type (A22361) and disome XVI (A12700) cells.

(B-D) Analysis of translation in wild-type (A11311) and disome XVI (A12700) cells.

(B) The log$_2$ ratio of disome XVI to wild-type mRNA RPKM values is shown with the x-axis representing genes in rank order by chromosomal location. Genes included have 128 raw counts between the wild-type and disomic strains.

(C) The log$_2$ ratio of disome XVI to wild-type ribosome footprint RPKM values is shown with the x-axis representing genes in rank order by chromosomal location. Genes included have 128 raw counts between the wild-type and disomic strains.

(D) Translation efficiencies [TE = log$_2$ (mRNA RPKM/footprint RPKM)] were calculated for each gene with at least 128 summed raw counts between mRNA and footprints in both the disomic and wild-type strains. These TE values are represented as a histogram.

Since disome XVI cells did not show major translation defects by polysome profiling or $^{35}$S-methionine incorporation, we measured translation efficiency in this
strain to determine whether translation efficiencies of individual genes were altered. Consistent with previous studies (Torres et al., 2007, 2010), we found that disome XVI cells have twice the amount of mRNA of genes located on chromosome XVI as wild-type cells (Figure 9B), and these additional mRNAs are translated as judged by their association with ribosomes (Figure 9C). Importantly, we observed no major shifts in relative translation efficiency between wild-type and disome XVI cells (Figure 9D). In summary, our data indicate that many disomic yeast strains exhibit a subtle growth defect that is detected only by cell volume measurements. As all chromosomes are efficiently translated in disome XVI cells, we conclude that the decreased growth rate of this disome is not due to major translational defects or changes in translation efficiencies of individual genes.

Aneuploid cells exhibit delays in the transcriptional program governing the G1 – S transition

Most disomic yeast strains are defective in cell cycle entry, having a cell volume larger than that of wild-type at the time of budding (Figure 2; Table 1). We next wanted to determine the basis for this defect. The genetic program governing entry into the cell cycle culminates in the expression of the G1 cyclins CLN1 and CLN2 (Dirick et al., 1995). To determine whether the increased cell volume of disomic cells at the time of budding was due to a defect in cyclin expression, we examined CLN2 mRNA levels in disomes IV, XI and XV, which have larger critical sizes than wild-type cells (Figure 2B, F, J). Small, unbudded cells were isolated by centrifugal elutriation and CLN2 mRNA levels were measured as a function of cell volume. These analyses showed that
transcription of \textit{CLN2} occurred at a larger cell volume in the three disomic strains tested than in wild-type cells (Figure 10A, B, D). Maximal \textit{CLN2} transcription occurred at 42 fL, 48-50 fL and 43 fL in disome IV, XI and XV cells, respectively, whereas \textit{CLN2} levels were maximal in wild-type cells at a size of 26-35 fL. We conclude that \textit{CLN2} transcription occurs at a larger cell volume in disome IV, XI and XV strains. This finding is consistent with our previous studies of \textit{CLN2} expression in disomic strains released from a pheromone-induced G1 arrest, which also revealed delays in \textit{CLN2} transcription (Torres et al., 2007).
Figure 10: Transcription of *CLN2* and other SBF and MBF targets is delayed in disomes IV, XI and XV.

Disomic and wild-type cells were grown at 30°C in selective medium supplemented with 2% raffinose to mid-log phase. Small unbudded cells were isolated by centrifugal elutriation and released into the cell cycle at 30°C in YEPD. Samples were taken to measure RNA levels and cell volume.

(A, B) *CLN2* RNA levels were examined in wild-type (A11311), disome IV (A12687),
and disome XI (A13771) cells. ACT1 was used as a loading control. (B) Quantification of
CLN2 levels in (A) normalized by ACT1.
(C-H) RNA levels of the SBF targets CLN1 (C), CLN2 (D), and PCL1 (E) and of the
MBF targets POL1 (F) RNR1 (G), and CLB5 (H) were determined by real-time RT-PCR
in wild-type ([C-H], A11311, filled circles), disome XI ([C-H], A28266, open circles),
and disome XV ([C-H], A27930, open triangles). Transcript levels were normalized to
ACT1 levels. Dashed lines indicate the critical sizes (modal volume at half-maximum of
budding) for each strain.

SBF and MBF, two transcription factor complexes with overlapping specificities,
control transcription of approximately 200 genes important for events in late G1 and S
phase (Ferrezuelo et al., 2010). SBF and MBF targets are cell cycle regulated, peaking at
the G1 – S transition (Futcher, 2002). To determine whether the entire G1 – S
transcriptional program was delayed in aneuploid cells with a G1 delay, we analyzed the
expression of three SBF targets (CLN1, CLN2, and PCL1) and three MBF targets (POL1,
CLB5, and RNR1) in disomes XI and XV as a function of cell volume. Peak expression of
all SBF and MBF targets tested occurred at a larger cell volume in both disomic strains
(Figure 10C-H), suggesting that SBF and MBF activation is delayed in these aneuploid
strains.

Xbp1 is a stress-induced transcriptional repressor that can inhibit transcription of
G1 cyclins, delaying cell cycle entry under stressful conditions (Mai and Breeden, 1997,
2000). Deletion of XBPI had no significant effect on the critical size of wild-type or
disome VIII and XI cells (Figure 11; Table 2), suggesting that inhibition of cyclin
expression by Xbp1 is not causing the G1 delay seen in the disomes. We conclude that
the SBF and MBF transcription program is delayed in disomic yeast cells, and this delay
is not caused by Xbp1-mediated inhibition of G1 cyclin expression.

**Figure 11: Deletion of XBPI does not suppress the critical size increase in disomes VIII and XI.**

Wild-type ([A], A31522, open circles), disome VIII ([B], A31523, open circles), and
disome XI ([C], A31524, open circles) cells lacking XBP1 and wild-type ([A], A11311,
filled circles), disome VIII ([B], A13628, filled circles), and disome XI ([C], A13771,
filled circles) containing XBP1 were grown at 30°C in selective medium supplemented
with 2% raffinose. Small unbudded cells were isolated by centrifugal elutriation and
released into the cell cycle at 30°C in YEPD. Time points were taken every 15 minutes to
determine the percentage of budded cells as a function of cell volume. Experiments for
strains containing XBP1 are the same experiments as in Figure 1 and are shown here for
comparison; strains were not run in parallel with the xbp1Δ strains.
Table 2: Critical sizes of strains used in this study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Critical size (fL)</th>
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<tbody>
<tr>
<td>GAL-CLN2</td>
<td>22 (29)</td>
</tr>
<tr>
<td>Dis IV GAL-CLN2</td>
<td>19 (34)*</td>
</tr>
<tr>
<td>Dis VIII GAL-CLN2</td>
<td>20 (44)*</td>
</tr>
<tr>
<td>Dis XI GAL-CLN2</td>
<td>25 (50)*</td>
</tr>
<tr>
<td>Whi5-GFP</td>
<td>38 (36)†</td>
</tr>
<tr>
<td>Dis IV Whi5-GFP</td>
<td>48 (46)†</td>
</tr>
<tr>
<td>Dis XI Whi5-GFP</td>
<td>54 (50)†</td>
</tr>
<tr>
<td>Dis XV Whi5-GFP</td>
<td>56 (50)†</td>
</tr>
<tr>
<td>Dis XVI Whi5-GFP</td>
<td>39 (34)†</td>
</tr>
<tr>
<td>whi5Δ</td>
<td>30 (39)‡ (-20%)§</td>
</tr>
<tr>
<td>Dis IV whi5Δ</td>
<td>44 (50)‡ (-12%)§</td>
</tr>
<tr>
<td>Dis VIII whi5Δ</td>
<td>33 (46)‡ (-28%)§</td>
</tr>
<tr>
<td>Dis XI whi5Δ</td>
<td>52 (60)‡ (-13%)§</td>
</tr>
<tr>
<td>bck2Δ</td>
<td>42 (11%)§</td>
</tr>
<tr>
<td>Dis VIII bck2Δ</td>
<td>67 (46%)§</td>
</tr>
<tr>
<td>Dis XI bck2Δ</td>
<td>74 (35%)§</td>
</tr>
<tr>
<td>Dis XV bck2Δ</td>
<td>67 (29%)§</td>
</tr>
<tr>
<td>Dis XVI bck2Δ</td>
<td>52 (44%)§</td>
</tr>
<tr>
<td>xbp1Δ</td>
<td>34</td>
</tr>
<tr>
<td>Dis VIII xbp1Δ</td>
<td>48</td>
</tr>
<tr>
<td>Dis XI xbp1Δ</td>
<td>47</td>
</tr>
<tr>
<td>Cln3-13Myc</td>
<td>31</td>
</tr>
<tr>
<td>Dis VIII Cln3-13Myc</td>
<td>47</td>
</tr>
<tr>
<td>Dis XI Cln3-13Myc</td>
<td>38</td>
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</tbody>
</table>

* Value in parentheses is the critical size of the strain lacking the GAL-CLN2 construct
† Value in parentheses is the volume at the half-maximal percent of cells with cytoplasmic Whi5
‡ Value in parentheses is the critical size of the strain containing WHI5
§ Value in parentheses is the percent change in critical size compared to the strain lacking the gene deletion

The SBF and MBF transcriptional program is delayed, suggesting aneuploidy interferes with the G1 – S transition upstream of CLN2 expression; however, this does not exclude the possibility of aneuploidy also interfering with entry into S phase at a step following CLN1/2 expression. To address this possibility, we examined whether overexpression of CLN2, which is known to accelerate entry into the cell cycle (reviewed in Turner et al., 2012), suppresses the increase in critical size of cells disomic for...
chromosome IV, VIII or XI. CLN2 was overexpressed from the galactose-inducible GAL1-10 promoter for 1 hour and then small, unbudded cells were isolated by centrifugal elutriation. Because cells were grown in medium supplemented with raffinose and galactose as the carbon source, euploid cells expressing wild-type levels of CLN2 budded at a smaller critical size of 29 fL (Figure 12A). As expected, overexpression of CLN2 reduced the critical size of cells: wild-type cells overexpressing CLN2 exhibited a critical size of 22 fL (Figure 12B; Table 2). Overexpression of CLN2 suppressed the cell cycle entry defect of disomes IV, VIII, and XI (Figure 12B), with critical sizes of 19, 20, and 25 fL compared to critical sizes of 34, 44, and 50 fL, respectively, for those not overexpressing CLN2. We conclude that the increase in critical size in aneuploid cells is due to a delay in CLN2 expression.

Figure 12: Overexpression of CLN2 suppresses the critical size increase in disomes IV, VIII and XI.

Wild-type ([B], A25475, filled circles), disome IV ([B], A25477, open circles), disome VIII ([B], A25476, open triangles), and disome XI ([B], A25474, open squares) cells carrying a GAL-CLN2 construct and wild-type ([A], A11311, filled circles), disome IV ([A], A12687, open circles), disome VIII ([A], A13628, open triangles), and disome XI ([A], A13771, open squares) cells lacking the construct were grown to mid-log phase in selective medium supplemented with 2% raffinose and induced for one hour with 2%
galactose. Small, unbudded cells were isolated by centrifugal elutriation and released at 30°C in YEP medium containing 2% galactose + 2% raffinose. Samples were taken every 15 minutes to determine cell volume and the percentage of budded cells.

**Whi5 nuclear exit is delayed in aneuploid cells**

SBF, the transcription factor complex that promotes *CLN1/2* expression, is composed of Swi4 and Swi6 and is negatively regulated by the transcriptional inhibitor Whi5. Whi5 binds to Swi4/6 at the promoters of genes required for the G1 – S transition, inhibiting transcription (de Bruin et al., 2004; Costanzo et al., 2004). In G1, Cln3-CDKs phosphorylate Whi5, promoting its export from the nucleus (de Bruin et al., 2004; Costanzo et al., 2004). In wild-type cells, Whi5-GFP exits the nucleus just before budding. In three disomes tested (IV, XI, and XV), Whi5-GFP protein levels were not dramatically altered and Whi5-GFP nuclear exit occurred, as in wild-type, just before budding (Figure 13). However, both budding and Whi5-GFP nuclear exit were delayed compared to the euploid control (Figure 13; Table 2). A fourth aneuploid strain, disome XVI, exhibits a growth defect but not a critical size increase (Figure 1K; Figure 2K; Table 1); as expected, Whi5 nuclear exit also occurred just before budding in this strain, and Whi5-GFP levels were not dramatically altered. We conclude that the relationship between Whi5 nuclear exit and budding is not altered in aneuploid cells; however, the entire program of Whi5 nuclear exit, SBF/MBF activation, and budding is delayed in the disomic strains analyzed.
Figure 13: Nuclear exit of Whi5 is delayed in disomes IV, XI, XV and XVI, but deletion of \textit{WHI5} does not suppress the S phase entry delay of disomes IV, VIII and XI.

(A-E) Wild-type ([A], A25877), disome IV ([B], A25879), disome XI ([C], A31515), disome XV ([D], A31516), and disome XVI ([E], A25880) were grown at 30°C in...
selective medium supplemented with 2% raffinose. Small unbudded cells were isolated by centrifugal elutriation and released into the cell cycle at 30°C in YEPD. Time points were taken every 15 minutes to monitor budding, Whi5-GFP localization and cell volume. Bud = budding; Cyt = cytoplasmic Whi5.

(F-I) Wild-type ([F], A25479, open circles), disome IV ([G], A25481, open circles), disome VIII ([H], A25480, open circles), and disome XI ([I], A25482, open circles) with \( WHI5 \) deleted and wild-type ([F], A11311, filled circles), disome IV ([G], A12687, filled circles), disome VIII ([H], A13628, filled circles), and disome XI ([I], A13771, filled circles) cells with functional \( WHI5 \) were grown in selective medium supplemented with 2% raffinose. Small unbudded cells were isolated by centrifugal elutriation and released at 30°C in YEPD. Time points were taken every 15 minutes to determine cell volume and the percentage of budded cells.

(J) Wild-type (A25877), disome IV (A25879), disome XI (A31515), disome XV (A31516), and disome XVI (A25880) cells were grown at 30°C in YEPD and Whi5-GFP protein levels were detected by Western blot analysis. Disome XV (A31516) contains one copy of Whi5-GFP and one untagged copy of Whi5, as \( WHI5 \) is located on chromosome XV. Wild-type cycling cells (A2587) were used as a no-tag control.

To further investigate the mis-regulation of START control in disomic cells, we examined the consequences of \( WHI5 \) deletion in disomes IV, VIII and XI. The critical size of wild-type cells is 39 fL, but when \( WHI5 \) is deleted the critical size decreases to 30 fL (Figure 13F). Deletion of \( WHI5 \) did not suppress the critical size increase in aneuploid cells; it affected wild-type and disomic cells similarly, decreasing the critical size by 6-13 fL (Figure 13F-I; Table 2). Together, our data indicate that the timing of Whi5 nuclear exit is delayed in the disomic strains analyzed, but time of budding is also perturbed through parallel pathways. Perhaps disomy IV, VIII and XI interfere with the positive feedback loop regulating Whi5 nuclear export, alter SBF/MBF activity, or affect parallel pathways.
Accumulation of Cln3 is delayed in disome VIII and XI cells.

Whi5 export from the nucleus is promoted by Cln3-CDKs. To determine whether lower Cln3 protein levels could be responsible for the delayed exit of Whi5 from the nucleus in the disomic yeast strains, we measured Cln3-13Myc levels in elutriated cells. Cln3 protein levels fluctuated significantly during G1 in wild-type cells. Cln3 protein levels were low in small G1 cells, accumulated as cells reached a size of 21 fL, and declined shortly before cells entered the cell cycle (Figure 14A). This fluctuation in protein levels was not due to changes in CLN3 RNA levels (Figure 14A), indicating that posttranscriptional mechanisms are responsible for Cln3 protein fluctuations. The CLN3-13MYC allele is hypermorphic, causing a decrease in critical size from 38 fL to 31 fL in wild-type cells (Figure 14A). This is most likely because the C-terminal tag stabilizes the protein by interfering with degradation of Cln3, which is mediated by the PEST sequences located in the C-terminus of the protein (Tyers et al., 1992). Thus, the fluctuations in untagged Cln3 are likely to be, if anything, more dramatic.
Figure 14: Cln3 accumulation is delayed in disome XI.

Wild-type ([A,C], A33692) and disome XI ([B,C], A33694) cells were grown at 30°C in selective medium supplemented with 2% raffinose. Small unbudded cells were isolated by centrifugal elutriation and released into the cell cycle at 30°C in YEPD. Time points
were taken every 15 minutes to determine the percentage of budded cells, cell volume and Cln3 protein levels. In separate experiments, wild-type (A) and disome XI (B) were elutriated as described above and samples were taken every 15 minutes to determine the percentage of budded cells, cell volume and CLN3 RNA levels by RT-PCR. CLN3 protein and RNA levels are presented as fold-change from the first time point and are plotted as a function of cell volume to demonstrate that Cln3 protein but not CLN3 RNA levels fluctuate significantly during G1. Cln3 protein levels were normalized to Pgk1 and CLN3 RNA levels were normalized to ACT1. Wild-type cycling cells (A2587) were used as a no-tag control for the Western blots. The arrow underneath the blot marks the critical size (cs).

(C) Wild-type and disome XI Cln3 protein levels from the experiments in (A) and (B) were quantified and normalized to Pgk1 levels. The Western blot used for quantification is not shown. This analysis shows that overall Cln3 protein levels are comparable between wild-type and disome XI strains.

Overall Cln3 levels were comparable between wild-type and disomic strains, but Cln3 protein levels peaked at a larger volume in both disome VIII and XI cells as compared to wild-type (Figure 14, Figure 15). This finding indicates that Cln3 translation and/or stability is affected in these disomic strains. The effect of the CLN3-13MYC allele on the critical sizes of the two disomic yeast strains was variable. The basis of this variability is presently unclear (Figure 14B; Figure 15A; Table 2). We conclude that the delay of START in disome VIII and XI cells is at least in part due to a delay in the accumulation of Cln3 protein.
**Figure 15: Cln3 protein accumulation is delayed in disome VIII cells.**

(A) Disome VIII (A33693) cells were grown at 30°C in selective medium supplemented with 2% raffinose. Small unbudded cells were isolated by centrifugal elutriation and released into the cell cycle at 30°C in YEPD. Time points were taken every 15 minutes to measure the percentage of budded cells, cell volume and Cln3 protein levels. Quantification of the Western blot is plotted with budding index as a function of volume to demonstrate that Cln3 protein levels fluctuate in G1. Cln3 protein levels were normalized to Pgk1. Wild-type cycling cells (A2587) were used as a no-tag control for the Western blot. The arrow underneath the blot marks the critical size (cs).

(B) Cln3 protein levels from wild-type cells from the experiment shown in Figure 9A and from disome VIII cells from the experiment shown in Supplemental Figure 6A were quantified and normalized to Pgk1 levels. The Western blot used for quantification is not shown. This analysis shows that overall Cln3 protein levels are comparable between wild-type and disome VIII cells.
Aneuploidy impairs cell cycle entry in parallel to BCK2

Cln3-CDK activity is not essential for cell cycle entry, indicating that parallel pathways exist that activate Cln1/2-CDKs (Cross, 1988; Nash et al., 1988). Bck2 is one such factor; cells lacking BCK2 are large and exhibit a delay in CLN1 and CLN2 transcription (Di Como et al., 1995). In addition, deletion of BCK2 causes lethality or severe growth retardation in cells lacking CLN3 (Di Como et al., 1995; Epstein and Cross, 1994). To determine whether aneuploidy interferes with cell cycle entry through BCK2, we examined the critical size of disomic cells lacking BCK2. If aneuploidy inhibits cell cycle entry through mechanisms that function in parallel to BCK2, we expect disomes lacking this gene to exhibit an increased critical size. If aneuploidy delays G1 by disrupting BCK2 function, the effects of combining aneuploidy and a BCK2 deletion should not be additive.

Small, unbudded cells were isolated by centrifugal elutriation and budding was measured as a function of volume. In wild-type cells, deletion of BCK2 caused an increase in critical size of about 11% (Figure 16A; Table 2). Deletion of BCK2 had a greater effect on the disomic strains than the wild-type strain, causing increases in critical size ranging from 29%-46% in the disomes tested (Figure 16B-E; Table 2). A greater increase in critical size in the disomes upon deletion of BCK2 suggests that disomic strains depend more on the BCK2 pathway for cell cycle entry than do wild-type cells. This conclusion is consistent with our data suggesting that the Cln3-CDK/Whi5/SBF pathway is impaired in disomic cells. Together, our results indicate that aneuploidy affects entry into the cell cycle in at least two ways: aneuploidy elicits a growth defect and causes a cell cycle entry delay that is due to a delay in Cln3 accumulation.
Figure 16: Deletion of BCK2 exacerbates the G1 delay in disomic yeast cells.

Wild-type ([A], A31519, open circles), disome VIII ([B], A26163, open circles), disome XI ([C], A26162, open circles), disome XV ([D], A31520, open circles), and disome XVI ([E], A26161, open circles) lacking BCK2 and wild-type ([A], A11311, filled circles), disome VIII ([B], A13628, filled circles), disome XI ([C], A13771, filled circles), disome XV ([D], A12697, filled circles), and disome XVI ([E], A12700, filled circles) with functional BCK2 were grown at 30°C in selective medium supplemented with 2% raffinose. Small unbudded cells were isolated by centrifugal elutriation and released into the cell cycle at 30°C in YEPD. Time points were taken every 15 minutes to determine cell volume and the percentage of budded cells. Experiments for strains containing BCK2 are the same experiments as in Figure 1 and are shown here for comparison; strains were not run in parallel with the bck2Δ strains.
Many progeny of triploid meioses exhibit a G1 delay

The strategy we employed to create the disomic yeast strains is limited in that only low complexity aneuploidies (gains of one or two chromosomes) can be obtained (Torres et al., 2007). To determine whether yeast strains with more complex aneuploidies also exhibit a G1 delay, we induced triploid strains to undergo meiosis. In such meioses progeny are generated with a range of karyotypes: haploid, diploid, and aneuploidies ranging from 1N+X to 2N-X. Nearly all aneuploid strains created in this manner are highly unstable (Pavelka et al., 2010; Sheltzer et al., 2011; St Charles et al., 2010), but, we reasoned, could be cultured for brief periods of time to determine whether they also exhibit a G1 delay.

The viability of spores obtained from triploid meioses was very low, as expected (Parry and Cox, 1970; St Charles et al., 2010). The few colonies that did grow up exhibited a wide range of proliferation rates. Based on our previous studies, we assume that progeny obtained from these meioses with wild-type or near wild-type proliferation rates are either euploid or harbor aneuploidies of chromosomes with little impact on cell proliferation, such as chromosome I (Torres et al., 2007), whereas slow-growing progeny harbor aneuploidies of (presumably multiple) chromosomes that cause significant growth impairment.

DNA content analysis revealed that many of the progeny of triploid meioses exhibit a G1 delay (Figure 17). This G1 delay correlated remarkably well with proliferation rates as observed by growth on solid medium (Figure 17). Spores 1-1, 1-2 and 1-3 obtained from the triploid parent 1 grew very well and exhibited a DNA content profile very similar to that of the parent (Figure 17B-E). Spores 1-4, 1-5, 1-6 and 1-7
grew poorly and showed an increase in G1 cells (Figure 17F-I). Similar results were obtained with progeny from triploid parent 2. The percentage of cells in G1 was very low in exponentially growing cultures of this strain, the reason for which is currently unknown. It was nevertheless evident that in the aneuploid progeny, the G1 delay correlated with growth ability. Spore 2-1 grew well and exhibited a cell cycle distribution close to that of the parent (Figure 17K); cultures of spores that grew poorly (spores 2-2, 2-3, 2-4, 2-5 and 2-6) showed an increase in G1 cells (Figure 17L-P). We conclude that many aneuploid yeast strains obtained as offspring of triploid meioses also exhibit a G1 delay.
Figure 17: Progeny of triploid meioses exhibit a G1 delay.

MATa/α ([B], triploid parent 1, A6424) and MATa/α ([J], triploid parent 2, A6425) cells were induced to sporulate and tetrads were dissected. Individual colonies were picked after 1 week and grown to exponential phase in YEPD medium to determine DNA content ([C-I], A6424 progeny; [K-P], A6425 progeny). FL2-H detection thresholds were adjusted for each strain so that the cell population was within the linear detection range of the instrument. Plus signs indicate proliferation rates on solid medium, with +++ representing proliferation rates close or equal to wild-type controls, and fewer plus signs indicating poorer proliferation rates. A haploid strain (A2587) is shown in (A).
DISCUSSION

Here, we investigate the basis of the G1 delay observed in many disomic yeast strains to understand how aneuploidy alters the regulatory networks controlling the G1 – S phase transition. Two aspects of G1 regulation appear to be affected: cell growth and entry into the cell cycle. Thus, the disomic yeast strains are unique in that they grow slowly, yet, unlike most slow-growing mutants, they must reach a larger critical size to pass through START.

Most of the disomic strains show a growth defect during G1 as measured by cell volume accumulation (Figure 5; Figure 6). This growth defect is not nearly as dramatic as that seen in mutants impaired in ribosome biogenesis or protein synthesis (Figure 4; Hoose et al., 2012; Jorgensen et al., 2002), but nevertheless, the defect is readily observed by cell volume measurements. However, it should be noted that we did not detect any defects in global protein synthesis as judged by polysome profiles or $^{35}$S-methionine incorporation (Figure 9A; C.G. unpublished observations). These observations raise the possibility that the disomic cells are defective in cell volume accumulation but not protein synthesis, which would imply that these aneuploid cells must be denser than euploid cells. We favor the idea that the defect in cell volume accumulation reflects an overall defect in macromolecule biosynthesis, but the defect is too subtle to be detected by methods such as $^{35}$S-methionine incorporation and polysome profiling.

Our investigation of the basis for the cell volume accumulation defect of the disomic yeast strains indicates that it does not stem from diminished amino acid pools (Figure 7) or a constitutively active amino acid starvation response (Figure 8), nor from shifts in translation efficiency (Figure 9A). Rather, we propose that macromolecule
biosynthesis and/or other aspects of cell growth are hampered in aneuploid cells. One explanation for this growth defect is that particular genes present on the additional chromosome(s) in aneuploid cells may be causing slow growth. For instance, additional copies of key regulators of growth processes such as macromolecule biosynthesis, vesicle fusion, protein sorting and/or energy production could lead to a slowing of growth. In this scenario, the reason for the growth defect is different in each aneuploid strain. It is also possible that slow growth is caused by a feature common to all aneuploidies, such as an increase in the number of gene products due to the extra chromosome(s). Prior studies have shown the additional chromosomes to be actively transcribed and translated in the disomic strains (Torres et al., 2007, 2010); therefore, it is possible that an increased burden on the transcription and translation machinery hampers cell growth. This scenario predicts that the growth defect correlates with the amount of additional translation that occurs in the disomes. This is not what we observe, as the growth defect in the disomes does not correlate with the size of the additional chromosome (Figure 6K). Therefore, we favor the idea that imbalances in individual proteins critical for cell growth hamper cell volume accumulation in the disomic strains.

The delay in cell cycle entry (as judged by a delay in bud formation) observed in the disomes was more pronounced than the growth defect, which led us to investigate this phenotype in more detail. Of the 14 aneuploid strains studied, 10 showed increased critical size. The following observations points towards a common origin of the cell cycle entry defects observed in the disomes:

1. The cell cycle entry delay correlated with the size of the additional chromosome (Figure 2; Table 1).
2. All disomes analyzed, namely disomes IV, XI and XV, exhibit a delay in \textit{CLN2} mRNA accumulation (Figure 10A, B, D);

3. The increase in critical size of all disomes analyzed (disomes IV, VIII and XI) is suppressed by high levels of \textit{CLN2} (Figure 12).

4. Whi5 exit from the nucleus was delayed in all disomes studied (disomes IV, XI, and XV; Figure 13).

5. The accumulation of Cln3 is delayed in all disomes analyzed (disomes VIII and XI; Figure 14 and Figure 15).

If the detailed characterization of a subset of disomic strains is representative of all disomic strains exhibiting a cell cycle entry defect, our data point towards Cln3 accumulation being affected by aneuploidy.

How does aneuploidy interfere with Cln3 accumulation and/or other aspects of cell cycle entry? Are these G1 processes affected by changes in copy number of specific proteins or features common to all disomies? Consistent with the former idea are the critical size increases observed in disomes VIII, XIV and VIII+XIV. Whereas the single disomes exhibit increases in critical size of 8 fL (disome VIII) and 5 fL (disome XIV), the double disome shows only a 3 fL increase in critical size compared to wild-type cells (compare Figure 2D, I, N). This finding suggests that the cell cycle entry delay in the individual disomes is caused by a defined number of imbalanced gene products that can be suppressed by providing a surplus of gene products located on another chromosome. In this model, the correlation between increased critical size and size of the additional chromosome would be explained by genes affecting cell cycle entry being distributed
more or less evenly across the yeast genome. Moriya et al. (2006) have identified all yeast genes whose increased dosage affects cell proliferation. It will be interesting to determine, which of these when present at two copies affect cell cycle entry.

Although single gene imbalances could contribute to the delay in cell cycle entry, our data also indicate that a response to a common feature of disomy is responsible for the observed cell cycle entry delay observed in many disomic strains. The critical size increase in the disomes correlates with the size of the additional chromosome (Figure 2O) and all disomes analyzed appear to show a similar defect in the molecular pathway governing START (see earlier discussion). An increase in the amount of DNA is not responsible for the G1 delay in the disomes as YACs containing human DNA do not cause an increase in critical size. A previous study suggested that increasing the number of SBF binding sites delays cell cycle entry because they titrate Cln3 molecules (Wang et al., 2009). We analyzed the distribution of SBF binding sites in the yeast genome as published in Ferrezuelo et al. (2010) and found them to be evenly distributed across the genome (data not shown). It is thus possible that Cln3 titration by SBF binding sites contributes to the G1 delay observed in the disomes. While an increase in SBF binding sites could contribute to the G1 delay in the disomes, our analyses point towards delayed Cln3 protein accumulation as the major defect in at least disomes VIII and XI. We do not yet know whether CLN3 translation and/or Cln3 stability is affected in the aneuploid strains. Protein synthesis rates affect CLN3 translation (Polymenis and Schmidt, 1997) and so aneuploidy could, via its effects on macromolecule biosynthesis, affect Cln3 synthesis. Aneuploidy could also affect Cln3 stability. Recently the two ubiquitin ligases SCF-Cdc4 and SCF-Grr1 have been identified as being responsible for regulating Cln3...
stability (Landry et al., 2012). It will be interesting to determine whether aneuploidy affects Cln3 ubiquitination by these ligases.

Many stresses cause a G1 arrest or delay (Herrero et al., 2003). Aneuploidy has been suggested to cause proteotoxic and energy stress in yeast and mouse cells (Tang et al., 2011; Torres et al., 2007, 2010). These or other stresses elicited by aneuploidy could elicit a transient G1 delay by interfering with Cln3 accumulation. Indeed, proteotoxic stress induced by heat shock causes a G1 delay (Johnston and Singer, 1980), which, like the G1 delay seen in the disomic yeast strains, can be suppressed by CLN2 overexpression (Rowley et al., 1993). Whether and how aneuploidy and other cellular stresses antagonize Cln3 accumulation is an important question that remains to be addressed.

Is the G1 delay that we observe in many of the disomes also seen in other models of aneuploidy? Our study of the progeny of triploid meioses suggests that many of the poorly growing and thus presumably highly aneuploid yeast strains show a significant G1 delay that is detectable in exponentially growing cells. A study of aneuploid fission yeast cells created by triploid meioses showed that these cells also exhibit a delay in G1 during germination (Niwa et al., 2006), but the cause of the G1 delay is not known. Mouse embryonic fibroblasts trisomic for either chromosome 1, 13, 16 or 19 (Williams et al., 2008) and trisomy 21 human fibroblasts (Segal and McCoy, 1974) exhibit cell proliferation defects, but the basis of this proliferation defect has not been explored. Thus, whether a conserved effect on cell cycle entry exists in aneuploid cells of different species remains to be established. However, a common transcriptional response reminiscent of the budding yeast environmental stress response (indicative of cellular
stress and slowed proliferation) is observed in aneuploid yeast, *Arabidopsis*, mouse and human cells (Sheltzer et al., 2012). It is tempting to speculate that aneuploidy, like many other cellular stresses, impacts cell proliferation by delaying cells in G1. Determining the molecular mechanisms underlying the effects of aneuploidy on progression through G1 will be an important aspect of understanding how aneuploidy impacts cell physiology.
MATERIALS AND METHODS

Yeast strains, plasmids, and growth conditions

All yeast strains are derivatives of W303 and are described in Table 3. Yeast strains were generated and manipulated as described previously (Guthrie and Fink, 1991). The GCN4-LacZ construct (Hinnebusch, 1985) was integrated at the URA3 locus. Cells were grown at 30°C in YEP or in synthetic medium containing G418 at 0.2 mg/ml, supplemented with 2% raffinose, 2% raffinose + 2% galactose, or 2% glucose (YEPD).

Elutriation

Cells were grown in 1-2 L of synthetic medium supplemented with 2% raffinose at 30°C. Cells were collected by centrifugation and resuspended in 30 ml YEP without sugar. Cells were sonicated to break clumps and kept at 4°C for the duration of the elutriation. A Beckman elutriation rotor JE 5.0 (Beckman Coulter, Brea, CA) was chilled to 4°C and equilibrated with YEP (no sugar) at 2400 rpm. Cells were loaded into the rotor at a pump speed of approximately 14 ml/min and allowed to equilibrate for 15-20 minutes at a rotor speed of 2400 rpm and a pump speed of approximately 14 ml/min. The pump speed was increased until small, unbudded cells exited the elutriation chamber. One liter was collected at that pump speed (about 20 ml/min for most strains). Pump speed settings varied slightly among experiments as the disomic strains have different cell volumes. Elutriated cells were concentrated using a filtration apparatus and resuspended in YEP supplemented with 2% glucose at 30°C.
**Cell volume determination**

Cell volume was measured using a Beckman Coulter Multisizer 3 (Beckman Coulter). 40-100 µl of culture was diluted into Isoton II solution (Beckman Coulter) to give <10% saturation. 50,000 particles were sized for each sample. Particles smaller than 3 µm were excluded to decrease background signal and decrease the chances of counting dead cells. Cell volume distributions were smoothed with a rolling window of 3 bins. Modal population values were used for the plots. The critical size of a strain is defined as the modal cell volume at half-maximal budding.

**Ribosome profiling and mRNA-seq**

Ribosome profiling was performed as described (Ingolia et al., 2009), with subtraction of three prominent ribosomal rRNA species performed as in Brar et al. (2012). In short, cells were treated with 100 µg/ml cycloheximide to freeze ribosomes, then flash-frozen, and subjected to mixermill lysis. Clarified extract was treated with RNase I and subjected to sucrose gradient fractionation. The 80S/monosome fraction was harvested and RNA was extracted and size-selected for ribosome footprints (28-30 nt). These footprints were converted to a sequencing library, which was sequenced using an Illumina GAI machine (Illumina, San Diego, CA). Sequences were aligned to the W303 genome and quantified as described (Ingolia et al., 2009). For translation efficiency (TE) analyses, calculations were only done on genes with 128 summed counts among mRNA and footprint samples, with TE = (ribosome footprint RPKM)/(mRNA RPKM). RPKM = reads per kilobase million, a normalizing unit for gene length and total sequencing counts per lane. mRNA-seq was also performed as described (Ingolia et al., 2009). In short,
flash-frozen cells were lysed in hot acid phenol and total RNA was extracted. polyA selection was performed to isolate mRNA, which was fragmented to 40-60 nt species, which were converted to a sequencing library, sequenced and analyzed in parallel with ribosome footprint samples.

**Polyribosome profile analysis**

Polysomes were prepared as described (Clarkson et al., 2010). Briefly, 250 ml cultures were grown in YEPD at 30°C to an OD$_{600}$ = 0.5. Cycloheximide was added to a final concentration of 0.1 mg/ml for 3 minutes. Cells were pelleted by centrifugation and lysed by vortexing with zirconia/silica beads in 1x PLB (20 mM HEPES-KOH [pH 7.4], 2 mM magnesium acetate, 100 mM potassium acetate, 0.1 mg/ml cycloheximide, 3 mM DTT) and treated with RNasin Plus RNase inhibitor (Promega, Fitchburg, WI). Lysates were clarified by centrifugation and 25 A$_{260}$ units were resolved on 11 ml linear 10%-50% sucrose gradients in 1x PLB by centrifugation in a Beckman SW41 rotor (Beckman Coulter) for 3 h at 35,000 rpm.

**Metabolomic analysis**

All strains were grown in shake flasks at 30°C and 250 rpm in defined minimal medium containing per liter (adapted from Verduyn et al., 1992): 5 g KH$_2$PO$_4$, 0.5 g MgSO$_4$*7H$_2$O, 2 g monosodium glutamate, 1.5 mg EDTA, 4.5 mg ZnSO$_4$*7H$_2$O, 0.3 mg CoCl$_2$, 1 mg MnCl$_2$*4H$_2$O, 0.3 mg CuSO$_4$*5H$_2$O, 4.5 mg CaCl$_2$*2H$_2$O, 3 mg FeSO$_4$*7H$_2$O, 0.4 mg NaMoO$_4$*2H$_2$O, 1 mg H$_3$BO$_3$, 0.1 mg KI. Filter-sterilized vitamins were added separately to a final concentration per liter of: 0.005 mg biotin, 0.1 mg Ca-
pantothenate, 0.1 mg nicotinic acid, 2.5 mg inositol, 0.1 mg pyridoxine, 0.02 mg p-aminobenzoic acid, 0.1 mg thiamine. The medium was buffered with 100 mM KH-phtalate at a pH of 5. Due to the genetic markers in the strains, the medium was supplemented with the following compounds per liter: 0.031 g lysine, 0.02 g uracil, 0.24 g leucine, 0.08 g adenine, 0.02 g tryptophan, 0.021 g methionine, 0.2 g geneticin (G418).

Cells were grown to an OD_{600} between 0.7 and 1.5 when metabolism of 1 ml culture was arrested by quenching in -40°C methanol + 10 mM ammonium acetate. After centrifugation at -9°C (3 min, 5000 rpm) the samples were stored at -80°C. Intracellular metabolites were extracted by incubation in 75% ethanol + 10 mM ammonium acetate for 3 min at 80°C. The supernatant was dried using a vacuum centrifuge. The dried extracts were derivatized with TBDMS (N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide) for quantification. The metabolites were separated by gas chromatography and injected to a time-of-flight mass spectrometer as described in Ewald et al. (2009).

**β-galactosidase activity assay**

Cell extracts and activity measurements were performed as described (Dever, 1997). Cells were grown in synthetic medium lacking histidine and supplemented with 2% glucose. Amino acid starvation was induced by the addition of 3-amino-1,2,4-triazole (3-AT), a competitive inhibitor of an intermediate step in histidine synthesis, to a final concentration of 100 mM.
Northern blot analysis

Cells were grown at 30°C in synthetic medium supplemented with 2% raffinose, elutriated as described and released into the cell cycle. Time points were taken until after the critical size had been reached. Total RNA was isolated as described (Cross and Tinkelenberg, 1991) and Northern blots were performed as described (Hochwagen et al., 2005). Blots were probed for CLN2 and the loading control ACT1.

Real-time RT-PCR

Approximately 2-4 OD$_{600}$ units of culture were pelleted by centrifugation at 4°C (3000 rpm, 2 min). The pellet was resuspended in 1 ml cold DEPC-treated water, transferred to a 2 ml microfuge tube and repelleted by centrifugation at 4°C (3000 rpm, 2 min). The pellet was flash-frozen in liquid nitrogen and stored at -80°C. To extract total RNA, ~200 µl of glass beads, 400 µl TES buffer (10 mM Tris [pH 7.5], 10 mM EDTA, 0.5% SDS) and 400 µl acid phenol:chloroform (pH 4.5) were added to the cell pellet and the tubes were vortexed for 45 minutes at 65°C. The phases were separated by centrifugation, and the top phase was transferred to a new tube containing 1 ml of 120 mM sodium acetate in ethanol to precipitate RNA at 4°C. Precipitates were collected by centrifugation and resuspended in 100 µl DEPC-treated water. Total RNA was further purified using the RNeasy Mini kit (Qiagen, Hilden, Germany), including DNase treatment, according to the manufacturer’s instructions. cDNA was synthesized from 750 ng total RNA using the SuperScript III First-Strand Synthesis SuperMix kit (Invitrogen, Grand Island, NY) with random hexamer primers according to the manufacturer’s instructions. Real-time qPCR reactions were run using the SYBR Premix Ex Taq Perfect Real Time kit (TaKaRa Bio,
Otsu, Japan) and a Roche LightCycler 480 (Roche, Penzberg, Germany) according to manufacturer’s instructions. qPCR primers are listed in Table 4.

**Western blot analysis**

Approximately 2-4 OD<sub>600</sub> units of culture were pelleted by centrifugation at 4°C (3000 rpm, 2 min). Cells were incubated at 4°C in 5% trichloroacetic acid for at least 10 minutes. Cell pellets were washed once with acetone and dried. Cells were lysed with glass beads in 100 µL of lysis buffer (50 mM Tris-Cl at pH 7.5, 1 mM EDTA, 2.75 mM dithiothreitol [DTT], complete protease inhibitor cocktail [Roche, Mannheim, Germany]) with a bead mill and samples were boiled in 1X SDS loading buffer. Following sodium dodecyl sulfate-polyacrilamide gel electrophoresis (SDS-PAGE) and transfer of proteins to a nitrocellulose membrane, proteins were detected with the following antibodies. Whi5-GFP was detected using a mouse anti-GFP antibody (JL-8 epitope, Clontech, Mountain View, CA) at a dilution of 1:1000. Cln3-13Myc was detected using a mouse anti-Myc antibody (9e10 epitope; Covance, Princeton, NJ) at a dilution of 1:600. Pgk1 was detected using a mouse anti-Pgk1 antibody (Invitrogen, Carlsbad, CA) at a 1:10,000 dilution. Kar2 was detected using a rabbit anti-Kar2 antibody (kindly provided by Mark Rose) at a dilution of 1:400,000. Quantification was performed using ImageQuant software.

**Fluorescence microscopy**

For Whi5-GFP imaging, cells were fixed in 4% paraformaldehyde/3.4% sucrose for 15 minutes and then resuspended in 0.1 M potassium phosphate (KPi pH 6.4)/1.2 M
sorbitol/1% Triton. Cells were then resuspended in 0.05 µg/ml DAPI in KPi/sorbitol and imaged using a Zeiss Axioplan 2 microscope (Carl Zeiss, Göttingen, Germany) and a Hamamatsu OCRA-ER digital camera (Hamamatsu, Hamamatsu, Japan).

**Flow cytometric analysis**

Analysis of DNA content by flow cytometry was performed as previously described (Hochwagen et al., 2005).
Table 3: Yeast strains used in this study (all derivatives of the W303 background).

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ACKNOWLEDGMENTS

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REFERENCES


Chapter 3: A system to analyze the immediate effects of chromosome gain and loss on cell physiology reveals immediate onset of aneuploidy-associated phenotypes and increased cellular variance.
ABSTRACT

Aneuploidy, a genomic state defined by chromosome gains and losses, is the leading cause of miscarriages and mental retardation in humans and a hallmark of cancer. Understanding how the condition affects cellular physiology is thus essential if we want to understand these diseases. Here, we employ a novel method to systematically examine the immediate consequences of gaining and—for the first time—losing single or multiple chromosomes. Our studies led to three important conclusions. First, phenotypes wide-spread among aneuploid cells manifest themselves immediately following chromosome mis-segregation. Second, phenotypes previously described as prevalent among cells harboring chromosome gains are also wide-spread among cells that lost chromosomes, indicating that chromosome gains and losses fundamentally affect cells in similar ways. Finally, our cell cycle analyses revealed a surprising variability among cells harboring the same aneuploidies, suggesting that chromosome gains and losses severely impact the robustness of biological networks.
INTRODUCTION

Gains or losses of genes encoded on autosomes generally leads to a corresponding change in gene expression (reviewed in Henrichsen et al., 2009). It is thus not surprising that the consequences of mis-segregating entire chromosomes are severe. Aneuploid cells, the products of a mitosis in which a chromosome has been mis-segregated, exhibit proliferation defects, a gene expression signature similar to the environmental stress response (ESR), form protein aggregates at an elevated level and are genomically unstable (reviewed in Oromendia and Amon, 2014; Siegel and Amon, 2012). Collectively, these phenotypes are called the aneuploidy-associated stresses.

In addition to the traits observed in a broad range of aneuploidies, aneuploid cells exhibit gene specific phenotypes where changes in dosage of specific genes cause a specific phenotype. The best-known example for this category of phenotypes is the lethality caused by an additional copy of the gene encoding β-tubulin in budding yeast (Katz et al., 1990). How quickly aneuploidy-wide and gene-specific phenotypes develop following chromosome mis-segregation and when the cell mounts adaptive responses to the dramatic genomic imbalances that ensue is not known. Models to assess the immediate consequences of chromosome mis-segregation have been developed, but they rely on mutations that decrease chromosome segregation fidelity. Thus, upon chromosome mis-segregation, populations of cells are generated with heterogeneous karyotypes due to random chromosome mis-segregation. This makes the analysis of a specific phenotype difficult. A system that allows one to induce aneuploidy of one or perhaps multiple chromosomes would eliminate this short-coming, enabling one to address how quickly specific aneuploidy-associated phenotypes are established.
Not only do we lack an understanding of the kinetics with which aneuploidy-induced phenotypes develop, but also our studies of aneuploidy to date have largely been confined to cell lines that have gained chromosomes. Budding yeast and mammalian cell lines have been established carrying an additional chromosome (Stingele et al., 2012; Torres et al., 2007; Williams et al., 2008). These “chronic” aneuploid cell lines are maintained by continuous selection for the aneuploid karyotype which can cause the development of genetic alterations allowing cells to adapt to the aneuploid state (Torres et al., 2010). Cell lines harboring monosomies are difficult to maintain. Monosomic cells quickly revert to the euploid state owing to the lack of effective methods to select for monosomy, as observed previously (Alvaro et al., 2006; Anders et al., 2009). Thus, it is not known how chromosome loss impacts cell physiology nor whether cells lacking chromosomes exhibit some or all of the aneuploidy-associated stresses that are so widespread among cells with chromosome gains. However, the fact that all autosomal monosomies cause lethality very early in development, whereas many trisomies die later during embryonic development or even survive into adulthood (Torres et al., 2008), indicates that chromosome losses have more dramatic effects on organismal fitness than chromosome gains. Haploinsufficiency is likely to be a major contributor to this difference between chromosome gains and losses. Whether the aneuploidy associated stresses observed in cells with chromosome gains contribute to the monosomic phenotypes is not known.

Here, we report the development of an aneuploidy system that allowed us to examine the immediate consequences of defined aneuploidies and to assess the effects of monosomy on cellular physiology in budding yeast. We adapted the conditional
centromere system developed by Hill and Bloom (1987) and Anders et al. (2009) to induce mis-segregation of specific chromosomes. This system, combined with a method to track the chromosome mis-segregation event, allowed us to examine the immediate consequences of specific chromosome gains and losses on the cell’s proteome, protein aggregate formation and cell cycle progression. Our analyses revealed that the cell’s proteome rapidly adapts to changes in chromosome copy number and that the adverse effects of aneuploidy on protein homeostasis and cell cycle progression are immediate. Our analyses further revealed high cell-to-cell variability in cell cycle progression among cells harboring the same aneuploidies. Importantly, these phenotypes are observed in both cells that have gained or lost chromosomes, indicating that the general aneuploidy associated phenotypes are characteristic of both chromosome gains and losses.

RESULTS

A system to examine the immediate consequences of chromosome mis-segregation

In budding yeast, when chromosomes cannot attach to the mitotic spindle they are retained in the mother cell (Hill and Bloom, 1987; Murray and Szostak, 1983). Thus, when a centromere fails to assemble a kinetochore, the chromosome on which that centromere is located will be retained in the mother cell. We adapted a system developed by Hill and Bloom (1987) to prevent kinetochore assembly, thereby inducing chromosome mis-segregation. To prevent kinetochore assembly, we placed the galactose inducible/glucose repressible GAL1 promoter adjacent to the point centromere on each chromosome, generating a set of strains with inducible chromosomes (Figure 1A). Cells grown in medium containing glucose repress the GAL1 promoter, allowing for proper
chromosome segregation. Cells grown in the presence of galactose activate the \textit{GAL1} promoter, which leads to transcription through the centromere, thus preventing kinetochore assembly (Hill and Bloom, 1987; Figure 1A). In a haploid strain, chromosome mis-segregation generates nullisomes (N-1), which are inviable, and disomes (N+1). In a diploid strain, chromosome mis-segregation generates monosomes (2N-1) and trisomes (2N+1). Using this system, we found that upon addition of galactose to the medium, 67 - 93 percent of cells retained both sister chromatids harboring the conditional centromere in the mother cell (Table 1).

To follow chromosome mis-segregation, we introduced a GFP dot onto the chromosome harboring the conditional centromere (Figure 1A). This chromosome marking system involves the integration of lac operator (\textit{E. coli}, \textit{lacO}) array sequences near the conditional centromere. When a GFP-LacI fusion protein is expressed in these cells, it binds to the \textit{lacO} sequences and forms a GFP dot at \textit{lacO} array sites visible by fluorescence microscopy (Straight et al., 1996). All of our cytological analyses were conducted in strains harboring GFP dots on the chromosome with the conditional centromere to ensure that we only analyzed cells that had indeed gained or lost a chromosome. GFP-LacI is integrated on chromosome XV. As we were not able to generate cells with a conditional centromere on chromosome XV (see below), GFP-LacI was always present in cells following chromosome mis-segregation.
Figure 1: The proteome rapidly adjusts to changes in gene dosage following chromosome mis-segregation.

(A) Schematic of the inducible aneuploidy system. The GAL1 promoter is located upstream of the CEN sequence on a particular chromosome. In medium containing glucose, the GAL1 promoter is repressed and chromosome segregation occurs properly. In medium containing galactose, the GAL1 promoter is activated, and strong transcription through the CEN sequence inactivates centromere function, leading to chromosome mis-segregation. (B) Plots show log₂ ratios of the relative protein abundance of chronic disome IV (A24367) grown in YEP-D at 30°C compared to mother wild-type cells (I363).
5 hours following transfer to YEP-D (5 hour time point; see schematic in panel C) (C)
Schematic of the experimental setup used for experiment shown in (D,E). Cells were
grown to mid-log in YEP-R medium at 30°C and then arrested in YEP-R with α-factor at
30°C to arrest cells in G1. Arrested cells were labeled with biotin and then released into
YEP-RG medium at 30°C and grown for 2 hours (~1 cell division) to induce
chromosome mis-segregation. Cells were then switched into YEP-D medium at 30°C to
stop further chromosome mis-segregation and grown for 1 hour. Mother cells were
isolated by sorting for biotin-labeled cells. A 1-hour sample was taken following sorting.
Remaining cells were grown in YEP-D at 30°C for 4 hours, after which a 5-hour sample
was taken. (D,E) Plots show log2 ratios of the relative protein abundance of inducible
disome IV (I777) mother cells compared to wild-type (I363) mother cells 1 hour (D) and
5 hours (E) following chromosome mis-segregation. (B,D,E) Proteins are ordered by
chromosomal location. Proteins from chromosome IV are shown in red. Abbreviations:
WT, wild-type; Dis, disome; Chr, chromosome; SD, standard deviation.

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*mis-segregation rates were calculated using cells from the colony assay in Figure 9B
We replaced the endogenous centromere with the conditional centromere construct (the *GAL1* promoter adjacent to the *CEN3* sequence) for every single chromosome (I-XVI) except for chromosome XV in haploid and diploid cells. Replacement of *CEN15* with the conditional centromere led to proliferation defects in cells prior to induction of chromosome mis-segregation (data not shown). All other strains containing the conditional centromere construct exhibited doubling times equal to that of wild-type cells when grown in glucose prior to induction of chromosome mis-segregation (data not shown). We also generated strains harboring multiple conditional centromeres. This allowed us to examine the consequences of mis-segregating multiple (up to 8; data not shown) chromosomes. Importantly, this system also allowed us—for the first time—to systematically analyze the consequences of chromosome losses on yeast cell physiology.

**Chromosome mis-segregation leads to a rapid change in cellular protein composition**

Quantitative proteomic SILAC analyses of budding yeast cells harboring an additional chromosome showed that approximately 80 percent of proteins encoded on the disomic chromosome are present at a two-fold higher level in cells (Dephoure et al., 2014; Torres et al., 2010). Protein quantification of cells chronically harboring an extra copy of chromosome IV using the TMT proteome quantification method also showed that levels of proteins encoded on the disomic chromosome were increased. However, the increase was only 1.4-fold instead of two fold (Figure 1B). This less than two-fold increase in protein levels is likely the result of the ‘ratio compression’ effect, a well-
known artifact occurring in isobaric labeling analyses due to protein quantification interference that results in systematic underestimation of quantitative ratios (reviewed in Christoforou and Lilley, 2012). The ratio compression effect can be nearly eliminated by using an MS3 method (Ting et al., 2011); however, an MS2 method was used for this quantification.

To determine how quickly protein levels change upon gain of an additional chromosome we measured the protein content of cells that had gained a copy of chromosome IV (disome IV) 1 hour and 5 hours (1-2 cell divisions) following chromosome mis-segregation. To specifically isolate cells that harbored an additional copy of chromosome IV we first arrested haploid cells in G1 using α-factor pheromone (Figure 1C). The G1 arrested cells were then labeled with biotin as described previously (Smeal et al., 1996; see Materials and Methods). Biotin forms permanent amide bonds with primary amines on cell surface proteins, thus labeling the cell wall of the G1 arrested cells. As the cell wall is built de novo during budding, release from the G1 block in the absence of biotin will result in the mother cell being labeled with biotin while the bud is not labeled. This allows for the purification of mother cells using magnetic sorting (Smeal et al., 1996).

Upon release from the pheromone-induced G1 arrest, cells were switched to medium containing galactose to inactivate CEN4, thereby inducing chromosome mis-segregation and retention of both sister chromatids of chromosome IV in the mother cell. Cells were switched to medium containing glucose two hours following release from the G1 arrest, when most cells had completed cell division, to prevent further chromosome mis-segregation. Biotin-positive cells were then isolated to separate cells disomic for
chromosome IV (mother cells) from nullisomic (N-1) daughter cells (Figure 1C; see Materials and Methods).

While mis-segregation occurs in many of the cells in the population, the process is not fully efficient. 1 hour following chromosome mis-segregation, the culture contained 67% cells disomic for chromosome IV, 20% euploid cells, and 13% cells lacking chromosome IV. 5 hours following chromosome mis-segregation the population was comprised of 61% disomes, 34% euploid cells, and 5% nullisomes. The slight decrease in the proportion of disomic cells at the 5 hour time point compared to the 1 hour time point is likely due to the reduced proliferation rate of disome IV cells compared to euploid cells.

One hour following chromosome mis-segregation, proteins encoded on chromosome IV were elevated compared to proteins encoded by the euploid chromosomes, with a mean log₂ protein abundance ratio of 0.21 for proteins on chromosome IV, compared to a mean of 0 for proteins on all other chromosomes (Figure 1D). Levels of chromosome IV encoded proteins increased further to a mean log₂ protein abundance ratio of 0.32 five hours following chromosome mis-segregation (Figure 1E).

One hour following chromosome mis-segregation, the ratio expected for full adjustment of the proteome to the disomic state is 54% (67% disomes – 13% nullisomes) of the value obtained from the chronic disome, which is a log₂ protein abundance ratio of 0.27, based on the distribution of disomes, nullisomes and euploid cells in the population at 1 hour following chromosome mis-segregation. However, the log₂ protein abundance ratio observed was only 0.21, which is below the expected log₂ protein abundance ratio of 0.27 for a 54% population of cells constitutively disomic for chromosome IV. This raises the
possibility that the protein composition had not quite adjusted to the genome composition of the disome IV cells within one hour of chromosome mis-segregation. Five hours following chromosome mis-segregation, the ratio expected for full adjustment of the proteome to the disomic state ought to be 57% (61% disomes – 5% nullisomes) of the value obtained from the chronic disome, which is a log₂ protein abundance ratio of 0.285. This number is in good agreement with the experimentally observed log₂ protein abundance ratio of 0.32. We conclude that chromosome mis-segregation leads to a rapid remodeling of the cell’s proteome to reflect the cell’s genetic make-up.

**Chromosome mis-segregation causes protein aggregate accumulation**

The aneuploidy-induced alterations in cellular protein composition lead to the accumulation of protein aggregates (Oromendia et al., 2012; Santaguida et al., 2015). Given that chromosome mis-segregation results in the rapid adjustment of the cell’s proteome to the new aneuploid karyotype (Figure 1D – E), we next investigated how quickly protein aggregates form following chromosome mis-segregation. To monitor protein aggregate formation, we examined the localization of the disaggregase Hsp104, which localizes to protein aggregates (Liu et al., 2010) and forms visible foci at aggregates in cells when using an Hsp104-mCherry fusion protein.

Previous studies showed that disomic yeast strains harbor higher levels of Hsp104 foci (Oromendia et al., 2012). We therefore initially examined the immediate consequences of chromosome gains. To this end, we grew haploid cells with a conditional centromere in medium containing raffinose (YEP-R) and arrested cells in G1 using α-factor pheromone (Figure 2A). Cells were then released from the G1 block in the
presence of galactose (YEP-RG) to inactivate the centromere. $\alpha$-factor was then readded to cells as they underwent mitosis to arrest cells in G1 following mis-segregation. Cells were then released from the G1 block in medium containing glucose (YEP-D) to repress the $GAL1$ promoter and re-activate the centromere. We then followed cell cycle progression and protein aggregate formation over time (Figure 2A).
**Figure 2: Protein aggregates accumulate in disomic cells immediately following chromosome mis-segregation in a cell cycle-independent manner.**

(A) Schematic of the experimental setup used for experiments shown in (B-D) and Figure 3. Cells were grown to mid-log in YEP-R medium and then arrested in YEP-R with α-factor at 25°C to arrest cells in G1. Arrested cells were released into YEP-RG medium at 25°C to induce chromosome mis-segregation. α-factor was added to cells after 60 min in YEP-RG to arrest cells in the next G1 to prevent further chromosome mis-segregation. Cells were then released into YEP-D medium at 25°C for 4 hours (B,C) or YEP-D medium with α-factor at 25°C for 3 hours (D, Figure 3). Cells were imaged every 30 min via microscopy to quantify Hsp104-mCherry foci as a marker of protein aggregates, to count budding as a marker of cell cycle progression, and to analyze chromosome mis-segregation by counting the number of GFP dots (on the inducible chromosome) per cell. (B,C) Percent cells with Hsp104 foci (B) and percent budded cells (C) were quantified for a haploid wild-type (I421; black), disome VIII (I746; red), disome X (I139; blue), and disome XIV (I736; green). Vertical dotted lines indicate changes in media. (D) Percent cells with Hsp104 foci were quantified for a haploid wild-type (I421; black, all panels), disome IV (I806; yellow, top left panel), disome V (I141; green, top left panel), disome VIII (I746; blue, top left panel), disome X (I139; purple, top left panel), disome II (I135; red, top right panel), disome XIV (I736; blue, top right panel), disome XI (I916; red, bottom panel), disome II+V+X (I525; yellow, bottom panel), disome II+X+XIV (I831; green, bottom panel), disome IV+V+VIII (I923; blue, bottom panel), and disome II+IV+XIV (I918; purple, bottom panel). Time shown is time in YEP-D + α-factor following chromosome mis-segregation. Budding indices for Hsp104 quantifications in (D) are shown in Supplemental Figure 4. Abbreviations: WT, wild-type; Dis, disome.

The analysis of Hsp104 localization in wild-type cells showed that the percentage of cells harboring Hsp104 foci was affected by carbon source and cell cycle stage (Figure 2B, C; Figure 3A – D). The fraction of cells harboring Hsp104 foci was low in YEP-R medium, increased upon shift to YEP-RG, and was highest in YEP-D (Figure 3C).
Furthermore, the percentage of cells with at least one Hsp104 focus was low in G1, increased as cells underwent DNA replication, and declined as cells underwent mitosis (Figure 3D). The reason for this cell cycle regulation is presently unknown; however, we speculate that it could be due to the fact that protein aggregates are preferentially retained in the mother cell during cell division (Aguilaniu et al., 2003; Liu et al., 2010), thus generating a lower percentage of cells with Hsp104 foci in G1 due to the recent birth of daughter cells. It is also possible that transient imbalances in protein complex subunits caused by differential timing of replication of the genes encoding these subunits contribute to increased aggregate formation during S phase.
Figure 3: Disomic cells accumulate protein aggregates following chromosome mis-segregation and protein aggregate formation fluctuates with the cell cycle.

(A-B) Cells were grown to mid-log in YEP-R medium and then arrested in YEP-R with α-factor at 25°C to arrest cells in G1. Arrested cells were released into YEP-RG medium at 25°C to induce chromosome mis-segregation. α-factor was added to cells after 60 min in YEP-RG to arrest cells in the next G1 to prevent further chromosome mis-segregation. Cells were then released into YEP-D medium at 25°C for 4 hours. Cells were imaged every 30 min via microscopy to quantify Hsp104-mCherry foci as a marker of protein aggregates and to analyze chromosome mis-segregation by counting the number of GFP dots (on the inducible chromosome) per cell. Vertical dotted lines indicate changes in media. (A,B) Percent cells with Hsp104 foci (A) and percent budded cells (B) were quantified for a haploid wild-type (I421; black), disome I (I178; red), disome II (I135; blue), and disome V (I141; green). (C) Haploid wild-type (I421) cycling cells were grown to mid-log in YEP-R, YEP-RG, or YEP-D medium at 23°C. Samples were taken and percent of cells with Hsp104-mCherry foci was quantified (gray bars). Cells were then shifted to 37°C for 1 hour and percent of cells with Hsp104-mCherry foci was quantified (red bars). Error bars indicate standard deviation. (D) Haploid (I421; top panel) or diploid (I810; bottom panel) wild-type cells were grown in YEP-D to mid-log and arrested with α-factor in YEP-D at 25°C. Cells were released into YEP-D at 25°C. Percent budding (open circles) and percent of cells with Hsp104-mCherry foci (filled circles) were monitored every 15 minutes. Abbreviations: WT, wild-type; Dis, disome.

Having characterized the behavior of Hsp104 foci in euploid cells, we next examined the effects of mis-segregating chromosomes I, II, V, VIII, X and XIV on Hsp104 focus formation in haploid cells. All inducible chromosomes were marked with a GFP dot, which enabled us to ensure that Hsp104 focus formation was only analyzed in cells that had acquired two copies of the mis-segregating chromosomes. We found that with the exception of the smallest chromosome (chromosome I), chromosome mis-
segregation led to an increase in the percentage of cells harboring Hsp104 foci over time (Figure 2B, C; Figure 3A, B).

We also used time-lapse microscopy to analyze Hsp104 focus formation. As cells were asynchronous in this experiment prior to inducing chromosome mis-segregation with galactose addition, some cells underwent two rounds of chromosome mis-segregation while in galactose while other cells underwent only one round of chromosome mis-segregation. The asynchrony of the population while in galactose-containing medium thus created cells harboring one or three additional copies of a particular chromosome, due to one or two rounds of chromosome mis-segregation, respectively. This analysis revealed that the number of Hsp104 foci scaled with copy number of the chromosome that is in excess. Haploid cells carrying three extra copies of chromosome II or X harbored more Hsp104 foci per cell and accumulated Hsp104 foci in a shorter amount of time than cells carrying only one copy of the chromosome (Figure 4). We conclude that protein aggregates form immediately following chromosome gain and that Hsp104 focus number per cell correlates with the degree of copy number gain immediately following chromosome mis-segregation.
Figure 4: Disomic cells accumulate protein aggregates upon aneuploidy onset and accumulate increasing numbers of aggregates per cell with increasing genomic imbalance.

Cycling cells were grown to mid-log in SC-R. Cells were then shifted to SC-RG at 25°C and loaded into a microfluidic chamber for time-lapse analysis via microscopy. Cells were shifted to SC-D at 25°C after 120 minutes and monitored for 8 hours. Chromosome mis-segregation was monitored by counting GFP dots (on the inducible chromosome) per cell and Hsp104-mCherry foci were quantified for wild-type (I421; green), disome II (I135; orange) N+II+II+II (I135; red), disome X (I139; light blue), and N+X+X+X (I139; dark blue). N+II+II+II and N+X+X+X cells were generated from two successive rounds of chromosome mis-segregation. (A) Smoothed traces of the average number of Hsp104 foci per cell for different genotypes. (B) Bar graph showing the average number of Hsp104 foci per cell at different time points. (C) Bar graph showing the time until the appearance of the first Hsp104 focus.
Protein aggregate formation is independent of cell cycle progression in disomic yeast strains

The ability to induce chromosome mis-segregation and to examine the immediate consequences of such an event also allowed us to ask whether Hsp104 focus formation in aneuploid cells required cell cycle progression. To address this question, we induced chromosome mis-segregation and then kept cells arrested in G1 following chromosome mis-segregation (Figure 2A). We analyzed seven strains in which different single chromosomes mis-segregated (chromosomes II, IV, V, VIII, X, XI, and XIV) and four strains that mis-segregated three chromosomes simultaneously. All 11 strains harbored a higher percentage of cells with Hsp104 foci than euploid control cells (Figure 2D; Figure 5). These findings indicate that protein aggregates form in aneuploid cells irrespective of whether cells divide or not. We conclude that proteomic imbalances caused by chromosome gain lead to immediate and proliferation-independent protein aggregate formation.
Figure 5: Budding indices of disomes held in α-factor arrest.

Cells were grown to mid-log in YEP-R medium and then arrested in YEP-R with α-factor at 25°C to arrest cells in G1. Arrested cells were released into YEP-RG medium at 25°C to induce chromosome mis-segregation. α-factor was added to cells after 60 min in YEP-RG to arrest cells in the next G1 to prevent further chromosome mis-segregation. Cells were then released into YEP-D medium with α-factor at 25°C for 3 hours. Cells were imaged every 30 min via microscopy to quantify Hsp104-mCherry foci as a marker of protein aggregates, to count budding as a marker of cell cycle progression, and to analyze chromosome mis-segregation by counting the number of GFP dots (on the inducible chromosome) per cell. Budding indices shown here correspond with Hsp104 quantifications shown in Figure 3. Percent budded cells was quantified for a haploid wild-type (I421; black, A-C); (A) disome IV (I806; yellow), disome V (I141; green),
disome VIII (I746; blue), disome X (I139; purple), (B) disome II (I135; red), disome XIV (I736; blue); (C) disome XI (I916; red), disome II+V+X (I525; yellow), disome II+X+XIV (I831; green), disome IV+V+VIII (I923; blue), and disome II+IV+XIV (I918; purple). Abbreviations: WT, wild-type; Dis, disome.

**Chromosome loss causes protein aggregate formation**

Is accumulation of protein aggregates specific to cells that have gained chromosomes because they are producing more proteins (Dephoure et al., 2014; Stingele et al., 2012; Torres et al., 2010), or is it also a consequence of chromosome loss? To address this question, we generated *MATα/*αΔ diploid cells that mis-segregate one or more chromosomes. The use of *MATα/*αΔ cells allowed us to synchronize diploid cells with α-factor, thus creating cell populations that synchronously mis-segregate chromosomes. We used the experimental set-up described in Figure 2A to induce chromosome loss and arrested cells in G1 following chromosome mis-segregation to monitor Hsp104 focus formation in monosomic and trisomic cells.

We noted that fewer diploid cells harbored Hsp104 foci than haploid cells. We observed that the percentage of cells harboring Hsp104 foci was directly proportional to the number of *HSP104-mCherry* or *HSP104-eGFP* gene copies in the cell. The percentage of cells harboring Hsp104 foci was lower in diploid cells carrying one copy of the Hsp104-mCherry or Hsp104-GFP fusion than in diploid cells carrying two copies of the fusion, most likely because detection of foci is increased in cells harboring two copies of fluorescently-tagged Hsp104 (Figure 6). This dosage effect could provide an explanation for the previous observation that a lower percentage of trisomic cells harbored Hsp104 foci than disomic cells (Oromendia et al., 2012). The monosomic and
trisomic strains used in this study carried only a single copy of the Hsp104-mCherry fusion, so absolute Hsp104 foci levels in trisomes and monosomes cannot be directly compared to disomic cells in this study.

Figure 6: The percent of cells harboring fluorescently labeled Hsp104-foci is in proportion to the number of fluorescently labeled copies of Hsp104.

(A) Cycling cells were grown to mid-log in YEP-D medium at 23°C. Samples were taken and percent of cells with Hsp104-mCherry foci was quantified (gray bars). Cells were then shifted to 37°C for 1 hour and percent of cells with Hsp104-mCherry foci was quantified (red bars). Haploid Hsp104-mCherry (I421), diploid Hsp104/Hsp104-mCherry (I480), diploid Hsp104-mCherry/Hsp104-mCherry (I1262). (B) Cycling cells were grown to mid-log in YEP-D medium at 23°C. Samples were taken and percent of cells with Hsp104-eGFP foci was quantified (gray bars). Haploid Hsp104-eGFP (A25654), diploid Hsp104/Hsp104-eGFP (A27287), diploid Hsp104-eGFP/Hsp104-eGFP (A25708), triploid Hsp104/Hsp104/Hsp104-eGFP (A28219), triploid Hsp104/Hsp104-
eGFP/Hsp104-eGFP (I1293), triploid Hsp104-eGFP/Hsp104-eGFP/Hsp104-eGFP (I1291). (A-B) Error bars indicate standard deviation.

Even though Hsp104 focus detection was decreased in diploid cells compared to haploid cells, it was nevertheless clear that chromosome loss caused an increase in the percentage of cells harboring Hsp104 foci. With the exception of cells monosomic for the smallest chromosome (chromosome I), every single monosome that we analyzed rapidly accumulated Hsp104 foci during a G1 arrest following chromosome mis-segregation (Figure 7A; Figure 8A-C). Mis-segregation in diploid cells also led to the formation of trisomic cells. These cells also produced Hsp104 foci rapidly during the G1 arrest following chromosome mis-segregation (Figure 7B, Figure 8D-E). We conclude that both chromosome gains and chromosome losses cause increased protein aggregation.
Figure 7: Protein aggregation occurs in chromosome losses and gains and correlates with genomic imbalance.

Cells were grown to mid-log in YEP-R medium and then arrested in YEP-R with α-factor at 25°C to arrest cells in G1. Arrested cells were released into YEP-RG medium at 25°C to induce chromosome mis-segregation. α-factor was added to cells after 60 min in YEP-
RG to arrest cells in the next G1 to prevent further chromosome mis-segregation. Cells were then released into YEP-D medium with α-factor at 25°C for 3 hours. Cells were imaged every 30 min via microscopy to quantify Hsp104-mCherry foci as a marker of protein aggregates and to analyze chromosome mis-segregation by counting the number of GFP dots (on the inducible chromosome) per cell. (A,B) Percent cells with Hsp104 foci were quantified for a diploid wild-type (I810; black, all panels), and monosomes (A) and trisomes (B) for the following chromosomes: II (I804; red, top panel), VIII (I813; yellow, top panel), X (814; green, top panel), XI (I839; blue, top panel), XIV (I805; purple, top panel), I (I811; red, middle panel), IV (I897; yellow, middle panel), V (I812; green, middle panel), IV+V+VIII (I929; blue, middle panel), II+IV+XIV (I931; purple, middle panel), II+V+X (I816; red, bottom panel), and II+X+XIV (I841; blue, bottom panel). Time shown is time in YEP-D + α-factor following chromosome mis-segregation. (C) Percent cells with Hsp104 in YEP-D + α-factor following chromosome mis-segregation was averaged across all time points in YEP-D. Average percent cells with Hsp104 foci over time 0-180 was plotted for each strain in Figure 3D (disomes, blue), Figure 4A (monosomes, red) and Figure 4B (trisomes, green) as a function of the number of open reading frames (ORFs) on the inducible chromosome(s). Lines shown are linear regressions (disomes, blue, $r^2 = 0.75$; monosomes, red, $r^2 = 0.76$; trisomes, green, $r^2 = 0.81$). Abbreviations: WT, wild-type; Mono, monosome; Tri, trisome.
**Figure 8: Budding indices of monosomes and trisomes held in α-factor arrest.**

Cells were grown to mid-log in YEP-R medium and then arrested in YEP-R with α-factor at 25°C to arrest cells in G1. Arrested cells were released into YEP-RG medium at 25°C to induce chromosome mis-segregation. α-factor was added to cells after 60 min in YEP-RG to arrest cells in the next G1 to prevent further chromosome mis-segregation. Cells were then released into YEP-D medium with α-factor at 25°C for 3 hours. Cells were imaged every 30 min via microscopy to quantify Hsp104-mCherry foci as a marker of protein aggregates, to count budding as a marker of cell cycle progression, and to analyze chromosome mis-segregation by counting the number of GFP dots (on the inducible chromosome) per cell. Budding indices shown here correspond with Hsp104 quantifications shown in Figure 4. Percent budded cells was quantified for a diploid wild-type (I810; black; A-F), and monosomes (A-C) and trisomes (D-F) for the following chromosomes: II (I804; red; monosome, A; trisome, D), VIII (I813; yellow; monosome A; trisome, D), X (814; green; monosome, A; trisome, D), XI (I839; blue; monosome, A; trisome, D), XIV (I805; purple; monosome, A; trisome, D), I (I811; red; monosome, B; trisome, E), IV (I897; yellow; monosome, B; trisome, E), V (I812; green; monosome, B; trisome, E), IV+V+VIII (I929; blue; monosome, B; trisome, E), II+IV+XIV (I931; purple; monosome, B; trisome, E), II+V+X (I816; red; monosome, C; trisome, F), and II+X+XIV (I841; blue; monosome, C; trisome, F).

**Abbreviations:** WT, wild-type; Mono, monosome; Tri, trisome.

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**Hsp104 focus formation scales with chromosome size**

Our previous studies suggested that the percentage of cells harboring Hsp104 foci did not correlate with degree of aneuploidy (Oromendia et al., 2012). This conclusion was based on the assessment of the percentage of cells with Hsp104 foci in exponentially growing chronic disomic cells. Our observation that Hsp104 focus formation fluctuated with cell cycle progression raised the possibility that a potential correlation between degree of aneuploidy and number of cells harboring Hsp104 foci was obscured by
aneuploidy-induced cell cycle delays (Thorburn et al., 2013; Torres et al., 2007). Here, we arrested cells in G1 following chromosome mis-segregation, which enabled us to examine Hsp104 focus formation in aneuploid cells without the concern that cell cycle delays affect Hsp104 focus formation. Furthermore, adaptations to the aneuploid state that may affect Hsp104 focus formation are unlikely to take place immediately following chromosome mis-segregation. Based on these considerations, we reexamined the potential correlation between degree of aneuploidy and number of cells harboring Hsp104 foci. We averaged the percentage of cells with Hsp104 foci over the time following chromosome mis-segregation (0-180 mintues) for the time courses shown in Figures 2D, 7A and 7B and observed a striking correlation between the percentage of cells harboring Hsp104 foci and degree of aneuploidy (Figure 7C). We conclude that protein aggregates (as defined by Hsp104 foci in cells) accumulate rapidly following chromosome mis-segregation and that protein aggregation scales with degree of aneuploidy under conditions of both chromosome gain and loss.

**Chromosome gains and losses cause defects in cell division**

Our previous studies showed that the continuous presence of an extra chromosome causes cell proliferation defects, especially a G1 delay (Thorburn et al., 2013; Torres et al., 2007). It has not previously been determined how quickly these cell proliferation defects arise following chromosome mis-segregation, nor how chromosome loss affects cell proliferation. To measure cell proliferation following chromosome mis-segregation, we first examined colony formation following chromosome mis-segregation. We induced chromosome mis-segregation by growing cells in the presence of galactose...
and then plating cells on solid medium containing glucose. We then separated mother-daughter pairs that had just completed a cell division by micromanipulation and allowed them to grow into colonies (Figure 9A). We measured colony size 40 – 48 hours later when colony size was still increasing exponentially. After all colonies had grown up into visible colonies, we confirmed that cells were indeed aneuploid by assaying appropriate selectable markers (see Materials and Methods). This analysis led to two observations:

First, the growth defects observed in monosomes were more severe than the growth defects observed in trisomes, despite monosomes and trisomes having the same amount of genomic imbalance. In fact, the fitness defects observed in monosomes were generally worse than the fitness defects observed in disomes (Figure 9B, Table 2). While the size of many monosomes was less than 1% of the size of colonies formed by euploid cells after ~48 hours of growth, nearly all monosomic strains eventually formed colonies after many days of growth. The only monosome that was inviable was chromosome XIII monosomy, due to monosomy of TUB1, which was previously shown to be lethal (Katz et al., 1990). The severe fitness defect of monosomic strains is likely due to the combined effects of genomic imbalances and haploinsufficiency. Disomy and trisomy were better tolerated than monosomy, with the exception of disome VI and trisome VI. These chromosomal gains are inviable due to an increase in TUB2 copy number (Katz et al., 1990).
Figure 9: Fitness of inducible aneuploids scales with chromosome size.

Cells were grown in YEP-R and then switched to YEP-RG to induce chromosome mis-segregation and grown at 30°C for 160 minutes. Cells were then plated on solid YEP-D medium. Mother-daughter pairs of cells that had just completed a cell division were chosen, separated, and placed side-by-side via micromanipulation. Cells were grown at 30°C for 40-48 hours and then colony area was measured. Euploid colonies grown on the same plate were used as controls. Disome (blue), trisome (green), and monosome (red) colony size is shown as a ratio of aneuploid to euploid colony area as a function of the number of open reading frames (ORFs) on the inducible chromosome(s). Each circle represents the mean of all colonies measured for a particular karyotype, compared to a euploid colony on the same plate. Euploid cells were either from a wild-type control strain or from cells in the experimental strain that did not mis-segregate a chromosome. Error bars indicate standard deviation. Linear regressions are shown for disomes (blue, $r^2 = 0.71$, excluding disome VI), trisomes (green, $r^2 = 0.81$, excluding trisome VI), and monosomes (red, $r^2 = 0.93$, for colonies with aneuploid to euploid ratios > 0.015). Linear
regressions exclude values for disome VI and trisome VI, as these colonies are inviable due to the presence of an extra copy of the *TUB2* gene (Katz et al., 1990). Strains used in this assay: haploid wild-type (A2587), diploid wild-type (A16629); disomes I (I261), II (I265), III (I269), IV (I273), V (I277), VI (I281), VII (I285), VIII (I289), IX (I293), X (I297), XI (I301), XII (I305), XIII (I309), XIV (I313), and XVI (I321); trisomes and monosomes I (I325), II (I327), III (I329), V (I333), VI (I335), VII (I337), VIII (I339), IX (I341), X (I343), XI (I345), XII (I347), XIII (I349), XIV (I351), and XVI (I355). Trisomes and monosomes I+II (I503), V+X (I507), II+V+X (I508), I+V+X (I509), and I+II+X (I510) were used in this assay with a different wild-type strain (I475).

**Table 2: Colony measurements for aneuploid cells shown in Figure 9B**

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</table>

* SD = standard deviation
Second, consistent with previous observations (Thorburn et al., 2013; Torres et al., 2007), colony size inversely correlated with the degree of aneuploidy (disomes $r^2 = 0.71$, excluding disome VI; trisomes $r^2 = 0.82$, excluding trisome VI; monosomes with aneuploid to colony size ratio $> 0.015$ $r^2 = 0.93$; Figure 9B). The observation that the negative fitness slope of trisomes is approximately one third of that of disomes (disome slope = -0.001289; trisome slope = -0.0004016; Figure 9B) further indicates that it is the relative ratio of aneuploid to euploid gene dosage that causes the observed fitness defects. Doubling the gene dosage relative to base ploidy of the cell, as occurs in the disomes, is proportionally more detrimental than increasing the gene dosage by 50 percent relative to the base ploidy, as occurs in the trisomes. We conclude that both chromosome gain and loss impairs cellular fitness and that changes in relative expression levels of genes are responsible for the fitness defects observed in cells with chromosome gains. The fitness defect of monosomes is likely a composite of changes in relative gene dosage and haploinsufficiency.

**Effects of chromosome loss on cell cycle progression**

The colony assays clearly showed that monosomy severely interferes with cell cycle progression. To more carefully define the cell cycle defects caused by chromosome loss, we analyzed cell cycle progression following chromosome loss by time-lapse microscopy. We used mCherry-Cdc3 and Spc42-dsRed fusion proteins along with GFP dots on the inducible chromosome(s) to follow cell cycle progression. Cdc3 encodes a component of the septin ring (Kim et al., 1991; Longtine et al., 1996) that forms a ring at the site of bud formation from the time of entry into the cell cycle until exit from mitosis.
Thus, the duration in which a cell lacks of a Cdc3 ring at the plasma membrane estimates the time a cell spends in G1 (Douglas et al., 2005). Spc42 encodes a component of the spindle pole body (SPB; Donaldson and Kilmartin, 1996). The distance between SPBs and the separation of sister chromatids as analyzed by GFP dots indicate anaphase onset (Holt et al., 2008). To ensure that we only analyzed cells that had mis-segregated a chromosome, the chromosome harboring the conditional centromere also encoded a GFP dot. For technical reasons described in Materials and Methods, we began our cell cycle measurements in either the first bud emergence to anaphase onset period or the first anaphase to cytokinesis period following chromosome mis-segregation.

Consistent with our colony size measurements, cell proliferation was delayed in all monosomic strains analyzed (Figure 10A). The degree of cell cycle delay became more severe with increasing degree of monosomy. Many cells harboring multiple monosomies ceased to divide (Figure 10A, grey points). Some monosomes (monosomes IV, X, and XI) also exhibited a progressively worsening cell proliferation defect (Figure 11), which could be due to haploinsufficiency of especially long-lived proteins or effects of gene dosage changes that take time to establish. Analysis of the duration of specific cell cycle stages further revealed that 12 of 13 monosomes analyzed (all except monosome XII) exhibit a G1 delay (Figure 10B). This G1 delay increased with degree of monosomy ($r^2 = 0.88$; Figure 10H) and increased in duration with increasing number of divisions following chromosome mis-segregation for 8 of the 13 monosomes (II, IV, X, XIV, V+X, IV+V+VIII, IV+V+VIII+XIV, and II+IV+V+VIII+X+XIV; Figure 12). Cell cycle delays in other stages of the cell cycle were also common in monosomic strains. 12 of 13 monosomes (all except monosome X) exhibited delays during S phase and/or early
mitosis/metaphase (time from bud emergence to anaphase onset delayed; Figure 10C).

These findings indicate that loss of a chromosome for most chromosomes impacts multiple cell cycle stages.

Figure 10: All inducible monosomes exhibit cell cycle delays following chromosome mis-segregation.
Cells were grown to mid-log in SC-R and then switched to SC-RG for 160 minutes to induce chromosome mis-segregation. Cells were then plated on SC-D solid medium mounted on a slide. Cells were imaged every 5 minutes for 8-10 hours via time-lapse microscopy at 25°C to monitor mCherry-Cdc3 to mark bud emergence and cytokinesis, Spc42-dsRed to monitor anaphase onset, and GFP dots to monitor chromosome mis-segregation. Division time (A), G1 duration (B), and bud emergence to anaphase onset duration (C) were calculated for disomes and normalized to euploid cells imaged during the same time-lapse. Euploid cells were either from the wild-type control strain or from cells in the experimental strain that did not mis-segregate a chromosome. Standard deviations for euploid (WT; black bars in D, E and grey points in F, G) and monosome (grey bars in D, E and blue points in F, G) populations were measured (in minutes) for G1 duration (D, F) and bud emergence to anaphase onset duration (E, G), and an F-test was used to test for equality of variance between a monosome population and the euploid population from the same experiment. Asterisks (*) in D, E indicate statistical significance between monosome and euploid population variances as calculated by an F-test (** = p < 0.01, **** = p < 0.0001). Black solid lines in A, B, C, F, and G are at the mean. Strains used in this assay: diploid wild-type (I1251); monosomes I (I1075), II (I1077), IV (I1079), V (I1081), X (I1085), XI (I1086), XII (I1093), XIV (I1018), V+X (I1243), IV+V+VIII (I1092), II+IV+XIV (I1099), IV+V+VII+X+XIV (I1247), and II+IV+V+VIII+X+XIV (I1245). The diploid wild-type (I1251) is marked with Spc42-GFP and mCherry-Cdc3.
Figure 11: Division time of monosomes by division following chromosome mis-segregation.

Cells were grown to mid-log in SC-R and then switched to SC-RG for 160 minutes to induce chromosome mis-segregation. Cells were then plated on SC-D solid medium mounted on a slide. Cells were imaged every 5 minutes for 8-10 hours via time-lapse microscopy at 25°C to monitor mCherry-Cdc3 to mark bud emergence and cytokinesis, Spc42-dsRed to monitor anaphase onset, and GFP dots to monitor chromosome mis-segregation. Division time over time was calculated for disomes and normalized to euploid cells of the same division imaged during the same time-lapse. Euploid cells were either from the wild-type control strain or from cells in the experimental strain that did not mis-segregate a chromosome. Black solid line is at mean with error bars indicating
standard deviation. Strains used in this assay: diploid wild-type (A-M; I1251); trisomes I (A; I1075), II (B; I1077), IV (C; I1079), V (D; I1081), X (E; I1085), XI (F; I1086), XII (G; I1093), XIV (H; I1018), V+X (I; I1243), IV+V+VIII (J; I1092), II+IV+XIV (K; I1099), IV+V+VIII+XIV (L; I1247), and II+IV+V+VIII+X+XIV (M; I1245). The diploid wild-type (I1251) is marked with Spc42-GFP and mCherry-Cdc3.

Figure 12: G1 duration of monosomes by division following chromosome mis-segregation.

Cells were grown to mid-log in SC-R and then switched to SC-RG for 160 minutes to induce chromosome mis-segregation. Cells were then plated on SC-D solid medium mounted on a slide. Cells were imaged every 5 minutes for 8-10 hours via time-lapse
microscopy at 25°C to monitor mCherry-Cdc3 to mark bud emergence and cytokinesis, Spc42-dsRed to monitor anaphase onset, and GFP dots to monitor chromosome mis-segregation. G1 duration over time was calculated for disomes and normalized to euploid cells of the same division imaged during the same time-lapse. Euploid cells were either from the wild-type control strain or from cells in the experimental strain that did not mis-segregate a chromosome. Filled symbols represent arrested cells (see Materials and Methods). Black solid line is at mean with error bars indicating standard deviation.

Strains used in this assay: diploid wild-type (A-M; I1251); trisomes I (A; I1075), II (B; I1077), IV (C; I1079), V (D; I1081), X (E; I1085), XI (F; I1086), XII (G; I1093), XIV (H; I1018), V+X (I; I1243), IV+V+VIII (J; I1092), II+IV+XIV (K; I1099), IV+V+VIII+XIV (L; I1247), and II+IV+V+VIII+X+XIV (M; I1245). The diploid wild-type (I1251) is marked with Spc42-GFP and mCherry-Cdc3.

Perhaps the most unexpected finding of our live-cell analysis was the observation that monosomic cells of the same karyotype exhibited great cell-to-cell variability in cell cycle duration (Figure 10A). Variability was observed in all cell cycle stages, but the increased variance was especially striking during G1 and in cells harboring multiple monosomies (Figure 10B - G). For 5 of the 13 monosomes (IV, X, XIV, IV+V+VIII+XIV, and II+IV+V+VIII+X+XIV), variance in G1 duration increased with increasing number of divisions following chromosome mis-segregation (Figure 12). To more precisely determine the origin of cell-to-cell variability outside of G1, we measured anaphase duration in monosomes. This analysis revealed that time from anaphase onset (lengthening of the spindle via Spc42 localization, and GFP separation in disomes and trisomes) to cytokinesis (loss of Cdc3 from the bud neck) varied little among cells (Figure 13A), indicating that DNA replication and early stages of mitosis are more susceptible to cell-to-cell variability than anaphase. DNA damage or difficulty in
microtubule – kinetochore attachment, which causes engagement of checkpoint arrests, could be a source of the observed variability. We conclude that monosomy interferes with multiple aspects of cell proliferation with significant cell-to-cell variability.

Figure 13: Anaphase duration of inducible aneuploids following chromosome mis-segregation.
Cells were grown to mid-log in SC-R and then switched to SC-RG for 160 minutes to induce chromosome mis-segregation. Cells were then plated on SC-D solid medium mounted on a slide. Cells were imaged every 5 minutes for 8-10 hours via time-lapse microscopy at 25°C to monitor mCherry-Cdc3 to mark bud emergence and cytokinesis, Spc42-dsRed to monitor anaphase onset, and GFP dots to monitor chromosome mis-segregation. Anaphase duration was calculated for monosomes (A), disomes (B), and trisomes (C) and normalized to euploid cells of the same division imaged during the same time-lapse. Euploid cells were either from the wild-type control strain or from cells in the experimental strain that did not mis-segregate a chromosome. Black solid lines are at the mean. Strains used in this assay: haploid wild-type (A; I1272) and diploid wild-type (B,C; I1251); disomes I (I1127), II (I1142), V (I1128), VIII (I1129), X (I1130), XI (I1144), XII (I1148), XIII (I1138), XIV (I1174), XVI (I1150), V+X (I1259), II+V+X (I1132), II+X+XIV (I1133), IV+V+VIII (I1139), and II+IV+XIV (I1141); monosomes I (I1075), II (I1077), IV (I1079), V (I1081), X (I1085), XI (I1086), XII (I1093), XIV (I1108), V+X (I1243), IV+V+VIII (I1092), II+IV+XIV (I1099), IV+V+VIII+XIV (I1247), and II+IV+V+VIII+X+XIV (I1245); and trisomes I (I1075), II (I1077), IV (I1079), V (I1081), X (I1085), XI (I1086), XII (I1093), XIV (I1108), and V+X (I1243). The haploid (I1272) and diploid (I1251) wild-type strains are marked with Spc42-GFP and mCherry-Cdc3.

**Cells harboring additional chromosomes exhibit cell-to-cell variability in cell cycle progression kinetics**

To determine whether the cell-to-cell variability in cell cycle duration was specific to chromosome losses, we examined cell cycle kinetics following chromosome gain in haploid and diploid cells. All disomes and trisomes exhibited cell cycle delays (Figure 14A-C, Figure 15A-C). Division times for 4 of 15 disomes (II, XVI, V+X, IV+V+VIII) increased with increasing number of cell divisions following chromosome mis-segregation (Figure 16). 11 of 15 disomes exhibited a G1 delay (V, VIII, XI, XII,
XIII, XIV, XVI, V+X, II+X+XIV, IV+V+VIII, and II+IV+XIV; Figure 14B), while all disomes showed delays during S phase and/or early mitosis/metaphase (time from bud emergence to anaphase onset delayed; Figure 14C). Furthermore, G1 duration increased with number of cell divisions following chromosome mis-segregation in 6 of 15 disomes (V, XVI, V+X, II+V+X, IV+V+VIII, and II+IV+XIV; data not shown), indicating that cumulative effects of aneuploidy contribute to the G1 delay observed in these disomes. Disomes of increasing degree of aneuploidy showed delays from anaphase onset to cytokinesis (Figure 13B).
**Figure 14: All inducible disomes exhibit cell cycle delays following chromosome mis-segregation.**

Cells were grown to mid-log in SC-R and then switched to SC-RG for 160 minutes to induce chromosome mis-segregation. Cells were then plated on SC-D solid medium mounted on a slide. Cells were imaged every 5 minutes for 8-10 hours via time-lapse microscopy at 25°C to monitor mCherry-Cdc3 to mark bud emergence and cytokinesis, Spc42-dsRed to monitor anaphase onset, and GFP dots to monitor chromosome mis-segregation. Division time (A), G1 duration (B), and bud emergence to anaphase onset duration (C) were calculated for disomes and normalized to euploid cells imaged during the same time-lapse. Euploid cells were either from the wild-type control strain or from cells in the experimental strain that did not mis-segregate a chromosome. Standard deviations for euploid (WT; black bars in D, E and grey points in F, G) and disome (grey bars in D, E and blue points in F, G) populations were measured (in minutes) for G1 duration (D, F) and bud emergence to anaphase onset duration (E, G), and an F-test was used to test for equality of variance between a disome population and the euploid population from the same experiment. Asterisks (*) in D, E indicate statistical significance between disome and euploid population variances as calculated by an F-test (* = p < 0.05, ** = p < 0.01, *** = p < 0.001, **** = p < 0.0001). Black solid lines in A, B, C, F, and G are at the mean. Strains used in this assay: haploid wild-type (I1272); disomes I (I1127), II (I1142), V (I1128), VIII (I1129), X (I1130), XI (I1144), XII (I1148), XIII (I1138), XIV (I1174), XVI (I1150), V+X (I1259), II+V+X (I1132), II+X+XIV (I1133), IV+V+VIII (I1139), and II+IV+XIV (I1141). The haploid wild-type (I1272) is marked with Spc42-GFP and mCherry-Cdc3.
Figure 15: All inducible trisomes exhibit cell cycle delays following chromosome mis-segregation.

Cells were grown to mid-log in SC-R and then switched to SC-RG for 160 minutes to induce chromosome mis-segregation. Cells were then plated on SC-D solid medium mounted on a slide. Cells were imaged every 5 minutes for 8-10 hours via time-lapse microscopy at 25°C to monitor mCherry-Cdc3 to mark bud emergence and cytokinesis, Spc42-dsRed to monitor anaphase onset, and GFP dots to monitor chromosome mis-segregation. Division time (A), G1 duration (B), and bud emergence to anaphase onset duration (C) were calculated for disomes and normalized to euploid cells imaged during
the same time-lapse. Euploid cells were either from the wild-type control strain or from cells in the experimental strain that did not mis-segregate a chromosome. Standard deviations for euploid (WT; black bars in D, E and grey points in F, G) and trisome (grey bars in D, E and blue points in F, G) populations were measured (in minutes) for G1 duration (D, F) and bud emergence to anaphase onset duration (E, G), and an F-test was used to test for equality of variance between a trisome population and the euploid population from the same experiment. Asterisks (*) in D, E indicate statistical significance between trisome and euploid population variances as calculated by an F-test (** = p < 0.01, *** = p < 0.001, **** = p < 0.0001). Black solid lines in A, B, C, F, and G are at the mean. Strains used in this assay: diploid wild-type (I1251); trisomes I (I1075), II (I1077), IV (I1079), V (I1081), X (I1085), XI (I1086), XII (I1093), XIV (I1018), and V+X (I1243). The diploid wild-type (I1251) is marked with Spc42-GFP and mCherry-Cdc3.
Figure 16: Division time of disomes by division following chromosome mis-segregation.

Cells were grown to mid-log in SC-R and then switched to SC-RG for 160 minutes to induce chromosome mis-segregation. Cells were then plated on SC-D solid medium mounted on a slide. Cells were imaged every 5 minutes for 8-10 hours via time-lapse microscopy at 25°C to monitor mCherry-Cdc3 to mark bud emergence and cytokinesis, Spc42-dsRed to monitor anaphase onset, and GFP dots to monitor chromosome mis-segregation. Division time over time was calculated for disomes and normalized to euploid cells of the same division imaged during the same time-lapse. Euploid cells were either from the wild-type control strain or from cells in the experimental strain that did
not mis-segregate a chromosome. Black solid line is at mean with error bars indicating standard deviation. Strains used in this assay: haploid wild-type (A-O; I1272); disomes I (A; I1127), II (B; I1142), V (C; I1128), VIII (D; I1129), X (E; I1130), XI (F; I1144), XII (G; I1148), XIII (H; I1138), XIV (I; I1174), XVI (J; I1150), V+X (K; I1259), II+V+X (L; I1132), II+X+XIV (M; I1133), IV+V+VIII (N; I1139), and II+IV+XIV (O; I1141). The haploid wild-type (I1272) is marked with Spc42-GFP and mCherry-Cdc3.

Generally, cell cycle delays seen in trisomes were less severe than those seen in disomes (Figure 14, Figure 15). Only 2 of 9 trisomes (XI and XII) exhibited moderate G1 delays (Figure 15B), while 7 of 9 trisomes (I, IV, V, X, XI, XIV, and V+X) showed delays from bud emergence to anaphase onset (Figure 15C). Trisomes did not show delays in anaphase duration (Figure 13C).

Haploid cells harboring one or more additional chromosomes also exhibited significant cell-to-cell variability in all cell cycle stages analyzed (Figure 14). As in monosomic strains, cell-to-cell variability was significant in the disomic strains and tended to increase with degree of aneuploidy (especially the G1 delay; Figure 14B, D, F). Variability was also observed in diploid cells harboring one or multiple additional chromosomes (Figure 15). However, the degree of variance was not as dramatic as that observed in monosomic and disomic strains (Figure 15D – G). These results suggest that increased ploidy buffers against variance brought about by the aneuploid state in the case of chromosome gain. Furthermore, our data indicate that increased cell-to-cell variability, at least with regards to cell cycle progression, is a universal feature of the aneuploid state.
DISCUSSION

Here, we employ a novel system to examine the immediate consequences of chromosome gains and losses on cell physiology in budding yeast. Our studies led to three important conclusions. First, phenotypes wide-spread among aneuploid cells—including aneuploidy-induced proteome changes, cell cycle defects, and the accumulation of protein aggregates—manifest themselves immediately following chromosome mis-segregation. Second, phenotypes previously described to be wide-spread among cells harboring chromosome gains are also wide-spread among cells harboring chromosome losses. Finally, our cell cycle analyses revealed significant cell-to-cell variability among cells harboring the same aneuploidy, suggesting that aneuploidy severely impacts the robustness of biological networks.

An experimental system to examine the acute consequences of chromosome gain and loss

Current models of aneuploidy either harbor chronic single chromosome aneuploidies maintained by selection (Stingele et al., 2012; Torres et al., 2007), or generate random, heterogeneous karyotypes by mis-segregating chromosomes during mitosis or meiosis (Babu et al., 2003; Kops et al., 2004; Li et al., 2010; Niwa and Yanagida, 1985; Niwa et al., 2006; Pavelka et al., 2010; St Charles et al., 2010; Thompson and Compton, 2008, 2010). Neither of these models allow for the systematic study of the immediate consequences of specific aneuploidies. The conditional centromere system that we adapted allowed us to do this. Additionally, the inducible aneuploidy system lets us, for the first time, study the effects of monosomy and of
complex aneuploidies in a systematic manner. Thus, this versatile system represents a unique opportunity to study aneuploidy onset in a defined manner, in a wide range of karyotypes. Here, we only investigated two aneuploidy-associated phenotypes, cell cycle delays and protein aggregate formation, although aneuploidy causes a wide range of phenotypes (reviewed in Sheltzer and Amon, 2011). Many of these phenotypes could be investigated using this system.

**Chromosome losses affect cells in a similar manner as chromosome gains**

Chromosome loss is a frequent mechanism by which cancer cells lose functional copies of tumor suppressor genes (Baker et al., 2009), yet, most studies of aneuploidy have focused on chromosome gain, in part because chromosome loss is often lethal, and selection for and maintenance of monosomy difficult (Alvaro et al., 2006; Anders et al., 2009). To our knowledge, we report here the first systematic characterization of monosomy in any organism. We focused on two phenotypes widespread among cells with chromosome gains—protein aggregate formation and cell cycle delays—and found that these phenotypes are also wide-spread among cells harboring chromosome losses. Thus, the causes underlying these phenotypes must exist in both cells with chromosome gains and losses. We propose that it is the imbalance in gene products resulting from both chromosome gains and losses that elicit these phenotypes.

Our analyses revealed that chromosome loss, like chromosome gain, causes protein aggregate formation (Figure 7). This finding indicates that protein aggregation previously observed in cells carrying additional chromosomes (Oromendia et al., 2012) is not simply due to excess protein produced from the extra chromosome(s). The fact that
cells losing chromosomes also accumulate protein aggregates indicates that stoichiometric imbalances in proteins—particularly members of protein complexes—cause proteins to mis-fold and aggregate because they lack their binding partners (Boulon et al., 2010).

Cells losing chromosomes, like cells gaining chromosomes, also exhibit fitness defects (Figure 10). Their proliferation is delayed especially in G1. This G1 delay, unlike the delay observed in other cell cycle stages, scales with the degree of monosomy ($r^2 = 0.88$; Figure 10H). Importantly, the fitness defects observed in monosomic strains were more severe than in disomic and trisomic strains (Figure 9), even though the degree of genome imbalance is the same in monosomes and trisomes. This finding indicates that for the majority of genes, excess protein is less detrimental than too little. The cell may be able to somewhat address the problems caused by a fifty percent or two-fold increase in gene dosage. Mechanisms such as protein degradation exist to cope with excess proteins, although the burden placed on these mechanisms may cause detrimental effects for the cell. In contrast, reduction in gene copy number is likely to have a more severe impact on cellular function, as mechanisms that upregulate genes on a monosomic chromosome to restore accurate gene dosage are rare (Springer et al., 2010). In summary, monosomies share the general phenotypes observed in cells with chromosome gains but are confounded by haploinsufficiency of specific loci. Our observations in yeast are in agreement with observations in mammals. In humans and mice, for example, all autosomal monosomies die very early during embryonic development. In contrast, some trisomies survive to birth and even into adulthood.
The effects of aneuploidy on cellular processes are immediate

The conditional centromere system also allowed us to ask how quickly aneuploidy-triggered phenotypes develop following chromosome mis-segregation. We found that protein levels adjust to changes in gene copy number within one hour of acquiring of an aneuploid karyotype (Figure 1). Not surprisingly, aggregate formation follows suit. Both chromosome gains and losses lead to a rapid accumulation of protein aggregates as assayed by Hsp104 focus formation (Figure 2, Figure 7). Importantly, this accumulation of protein aggregates occurs irrespectively of whether aneuploid cells divide or not. Holding cells in G1 following chromosome mis-segregation did not prevent protein aggregation, indicating that it is truly a consequence of an aneuploid karyotype rather than a secondary consequence of a mis-regulated cell cycle.

We previously reported that Hsp104 focus formation does not correlate with the degree of aneuploidy in a series of disomic yeast strains that we constructed (Oromendia et al., 2012). In the aneuploidy system developed here, we found the two parameters to be remarkably well correlated, both in cells that gained and lost chromosomes (Figure 7C). It is possible that some of the chronic disomic strains studied previously had adapted to increased protein aggregate formation, thus masking this correlation. We favor the hypothesis that changes in Hsp104 focus formation that occur naturally during the cell cycle (Figure 3D) prevented us from detecting this correlation in exponentially growing chronic disomic strains that exhibit varying cell cycle delays. We do not yet know why protein aggregate levels fluctuate during the cell cycle. Protein aggregates are preferentially retained in mother cells during cell division, resulting in very few daughter cells with Hsp104-marked foci (Aguilaniu et al., 2003; Liu et al., 2010). This is likely a
reason why fewer G1 cells harbor Hsp104 foci. The observation that Hsp104 aggregates accumulate during S phase further raises the interesting possibility that the lack of coordination in replication of genes encoding proteins of the same complex leads to transient imbalances in stoichiometries of protein complex members and hence protein mis-folding and aggregation.

Cell cycle defects were also apparent immediately following chromosome mis-segregation (Figures 10 – 16). We observed cell cycle delays as soon as the second division following chromosome mis-segregation for nearly all aneuploid strains tested. It is likely that the cell cycle defects are already present in the first cell cycle following chromosome mis-segregation, but we were not able to image cells for the entire first cell division following chromosome mis-segregation due to technical limitations of the experiment (see Materials and Methods). Consistent with previous results, G1 delays are prominent among strains with chromosome gains and losses (Thorburn et al., 2013; Torres et al., 2007). We also observe delays in S phase and/or early stages of mitosis. Aneuploid cells experience genomic instability (Blank et al., 2015; Pavelka et al., 2010; Sheltzer et al., 2011), which could engage checkpoints that cause delays in these cell cycle stages. Imbalances in key cell cycle regulators or limited protein quality control due to the need to fold and degrade proteins that mis-fold due to stoichiometric imbalances may lead to cell cycle delays as well.

Interestingly, in a number of specific aneuploidies we observed an increase in cell cycle delays over time. As the proteome rapidly adjusts to changes in gene dosage following chromosome mis-segregation (Figure 1), even for chromosome IV which has the greatest number of open reading frames of all yeast chromosomes, slow adjustment of
the proteome to the aneuploid state cannot account for this observation. In the case of monosomy, increased cell cycle delays over time could be due to eventual turnover of long-lived haploinsufficient proteins. In the case of chromosome gains, the lengthening in cell cycle duration could represent secondary effects of aneuploidy, where changes in the dosage of dosage-sensitive cell cycle regulators cause secondary cell cycle delays. It will be interesting to determine the molecular basis underlying this increase in severity of phenotypes.

**Aneuploidy causes loss of biological network robustness**

A key feature of biological processes is that they are robust. Buffering mechanisms are in place to prevent small intra- and extracellular fluctuations from having a dramatic impact on cellular functions. When these buffering mechanisms are perturbed, regulatory networks can exhibit alternate behaviors (Mileyko et al., 2008). Our live-cell analyses of single cells following chromosome mis-segregation revealed that all chromosome gains and losses that we analyzed cause a dramatic increase in variance in cell cycle duration (Figures 10, 14, and 15). In most aneuploids, variability is observed in G1 and S-phase/mitosis, indicating that aneuploidy has a broad impact on cell cycle robustness.

How does aneuploidy cause such a dramatic loss in cell cycle robustness? Our results indicate that changes in the relative ratios of genes are responsible as higher ploidy seemingly buffers against aneuploidy-induced cell-to-cell variability in the case of chromosome gain. The degree of cell cycle duration heterogeneity is substantially attenuated in diploid cells carrying an additional chromosome compared to haploid cells.
harboring chromosome gains. We do not yet know which genes decrease robustness upon doubling of gene dosage. Very few genes exist in yeast that cause substantive cell cycle defects when their gene dosage is doubled or halved (Bonney et al., 2015). However, such subtle changes in the dosage of network nodes, such as transcription factors or regulatory enzymes (i.e. protein kinases) can have wide-spread effects on the activity of the biological pathway that they control (Mileyko et al., 2008; Nikitin et al., 2014; Rancati et al., 2008), making them more susceptible to intracellular and extracellular noise. Determining the molecular mechanisms of decreased robustness upon changes in gene dosage will be an important aspect of understanding the deleterious effects of aneuploidy on cellular physiology.
MATERIALS AND METHODS

Yeast strains, plasmids, and growth conditions

All yeast strains are derivatives of W303 and are described in Table 3. Primers are listed in Table 4 and plasmids are listed in Table 5. Yeast strains were generated and manipulated as described previously (Guthrie and Fink, 1991). Cells were grown at 30°C in YEP supplemented with 2% raffinose (YEP-R), 2% raffinose + 2% galactose (YEP-RG), or 2% glucose (YEP-D), or in synthetic complete medium supplemented with 2% raffinose (SC-R), 2% raffinose + 2% galactose (SC-RG), or 2% glucose (SC-D).

Construction of conditional centromere strains

Strains with conditional centromeres were made using a PCR-based method (Anders et al., 2009; Longtine et al., 1998). Briefly, the conditional centromere construct was amplified from plasmid p1888 using primers designed to target the conditional centromere construct to a particular chromosome (Table 4). Strain A2587 was transformed with the conditional centromere constructs as previously described (Longtine et al., 1998).

Cen-LacO plasmid construction

Plasmids targeting the LacO array to various chromosomes (Cen-LacO plasmids, Table 5) were constructed by cloning a homology region to the specific target site with XhoI restriction sites into the SalI cut plasmid p1499 (pCM40). Plasmids were integrated at the target site by restriction enzyme digest using the enzymes listed in Table 5. Transformants were screened for gain of nourseothricin resistance (100 µg/ml). All
plasmids containing the LacO array were propagated in Max Efficiency Stbl2 competent cells (Life Technologies, Grand Island, NY) due to the high propensity of the LacO array to recombine. All enzymes used for cloning were obtained from New England BioLabs (Ipswich, MA).

HYGRO-LacO plasmid construction

A plasmid targeting the LacO array to the hph hygromycin B resistance gene (HYGRO-LacO; Table 5) was constructed in the same way as the Cen-LacO plasmids, using homology to the hph hygromycin B resistance gene. Integration of the HYGRO-LacO plasmid used a two-step process. First, the hph hygromycin B resistance gene was integrated into the desired site in the genome using a PCR-based method (Longtine et al., 1998). Second, the HYGRO-LacO plasmid was integrated at the hph site by EagI digest. Transformants were screened for loss of hygromycin resistance and gain of nourseothricin resistance.

GFP dot strain construction

To generate strains with GFP dots, strains containing the LacO array integration were crossed to a strain containing the GFP-LacI fusion protein. To integrate the GFP-LacI fusion protein into the genome, plasmid p1801 (Table 5) was linearized with NheI and transformed into A2587 cells as previously described (Guthrie and Fink, 1991).
Other strain construction

*HSP104*-eGFP, *HSP104*-mCherry, and *SPC42*-dsRed were constructed by PCR-based methods (Longtine et al., 1998). *mCherry-Cdc3* was constructed with an integrating plasmid.

Fluorescence microscopy

For Hsp104-eGFP, Hsp104-mCherry, and GFP dot imaging, cells were fixed in 3.7% formaldehyde for 15 minutes at room temperature and then resuspended in 0.1 M potassium phosphate (KPi pH 6.4)/1.2 M sorbitol. To visualize biotin labeling, cells were fixed in 3.7% formaldehyde for 15 minutes at room temperature and then resuspended in 0.1 M potassium phosphate (KPi pH 6.4)/1.2 M sorbitol with 1 µg/ml streptavidin conjugated to Alexa 568 fluorophore (Molecular Probes, Grand Island, NY). Cells were then washed once in 0.1 M potassium phosphate (KPi pH 6.4)/1.2 M sorbitol. Cells were imaged using a Zeiss Axioplan 2 microscope (Carl Zeiss, Göttingen, Germany) and a Hamamatsu OCRA-ER digital camera (Hamamatsu, Hamamatsu, Japan).

Differentiating aneuploid from euploid cells in Hsp104 experiments and live-cell cell cycle analysis

For Hsp104 foci analysis and cell cycle analysis, aneuploid cells were differentiated from euploid cells by counting the number of GFP dots present in a cell. In all euploid strains, 1 GFP dot per cell indicates euploidy. For disomes and trisomes of single chromosomes, the presence of 2 GFP dots in the cell indicates disomy or trisomy while 1 GFP dot indicates euploidy. For disomes and trisomes of 2 chromosomes, 4 GFP dots indicates
disomy or trisomy. For disomes and trisomes of 3 chromosomes, cells with 4, 5 or 6 GFP dots were scored as disomes and trisomes, as it was difficult to accurately count GFP dots at numbers greater than 4 dots per cell. For all monosomes (single or multiple chromosomes), 0 GFP dots indicates monosomy.

**Biotin labeling and sorting**

To purify populations of disomic cells for proteomic quantification following chromosome mis-segregation, we used a previously described method to purify mother cells (Smeal et al., 1996). Cells were grown to mid log phase in YEP-R and then arrested in G1 with the yeast mating pheromone α-factor. Following G1 arrest, cells were washed 3 times in phosphate buffered saline (PBS) pH 8.0 and labeled with EZ-Link Sulfo-NHS-LC-Biotin (1 mg biotin in PBS pH 8.0 per 1 OD_{600} unit of cells; Thermo Fisher Scientific, Grand Island, NY) at 4°C for 30 minutes. Biotin forms permanent amide bonds with primary amines on cell surface proteins. Following biotin labeling, cells were washed 4 times with PBS pH 8.0/100 mM glycine to remove excess biotin.

Cells were then transferred to YEP-RG medium to induce chromosome mis-segregation. Cells were grown for 120 minutes in YEP-RG at 30°C (one cell division). After nearly all cells had finished dividing, cells were transferred to YEP-D and grown for 1 hour. Then biotin labeled cells were purified using magnetic sorting. To purify biotin labeled cells, cells were first washed with PBS pH 7.4/0.5% bovine serum albumin (BSA). Then cells were incubated with anti-biotin magnetic beads (Miltenyi Biotec, San Diego, CA) in
PBS pH 7.4/0.5% BSA for 15 minutes at 4°C. Cells were washed once more with PBS pH 7.4/0.5% BSA and then resuspended in PBS pH 7.4/0.5% BSA and run over LS columns on a QuadroMACS separator (Miltenyi Biotec, San Diego, CA) to retain biotin-labeled cells. Biotin labeled cells were eluted from the columns with PBS pH 7.4/0.5% BSA following removal from the QuadroMACS separator. Cells were then washed once with PBS pH 7.4 and resuspended in YEP-D and grown at 30°C. Samples for analysis were taken at times indicated in Figure 1C.

**Protein quantification by mass spectrometry**

Preparation of protein extracts was adapted from Dephoure et al. (2014). Briefly, cells were lysed using a FastPrep-24 (MP Biomedicals, Santa Ana, CA) in a buffer containing 8 M urea, 75 mM NaCl, and 50 mM Tris-Cl pH 8.2 using 3-6 cycles of 45 s at 4°C separated by 5 minute incubations at 4°C. Samples were broken until ~70% of cells had lysed as confirmed by light microscopy. Lysates were cleared by centrifugation at 14,000xg for 15 minutes at 4°C. Protein concentrations were determined using the Bradford protein assay (Bio-Rad, Hercules, CA).

Further sample preparation, TMT labeling, and sample fractionation was performed as described in Dephoure et al. (2014) by the Swanson Biotechnology Center Proteomics Core at the Koch Institute for Integrative Cancer Research (Cambridge, MA). Samples were run with 3 technical replicates on a Thermo QExactive mass spectrometer (Thermo Fisher Scientific, Grand Island, NY). Database searching was done using Mascot (Matrix Science, Boston, MA).
Protein ratios were normalized to account for minor variations in sample mixing by centering the log$_2$ protein abundance ratio distribution over zero, as done in Dephoure et al. (2014). When calculating the normalization factor, proteins on the disomic chromosomes were excluded. We expect most proteins (except those on the disomic chromosome) to be present at a one-to-one ratio.

**Live-cell imaging**

*Hsp104 foci imaging*

Cells were grown in SC-R to mid log phase then transferred to SC-RG and loaded into a CellASIC ONYX microfluidic chamber attached to the CellASIC platform control system and manifold (EMD Millipore, Billerica, MA). Medium was switched to SC-D after most cells had finished one cell division. Cells were imaged at 10-minute intervals for 10 hours using a Zeiss Axio Observer-Z1 with a 63x objective (Carl Zeiss, Göttingen, Germany), equipped with a Hamamatsu ORCA-AG digital camera (Hamamatsu, Hamamatsu, Japan). 11 Z-stacks (0.5 microns apart) were acquired and maximally projected. MetaMorph software (Molecular Devices, Sunnyvale, CA) was used for image acquisition and processing.

*Cell cycle analysis image acquisition*

Cells were grown in SC-R to mid log phase then transferred to SC-RG and grown for 160 minutes to induce chromosome mis-segregation. Cells were then transferred to SC-D and layered on an agar pad (2% agarose, SC-D) affixed to a glass slide and covered with a
cover slip. Cells were imaged at 5-minute intervals for 8-10 hours using a Zeiss Axio
Observer-Z1 with a 63x objective (Carl Zeiss, Göttingen, Germany), equipped with a
Zeiss Definite Focus module (Carl Zeiss, Göttingen, Germany) and a Hamamatsu
ORCA-AG digital camera (Hamamatsu, Hamamatsu, Japan). 9 Z-stacks (0.6 microns
apart) were acquired and maximally projected. MetaMorph software (Molecular Devices,
Sunnyvale, CA) was used for image acquisition and processing.

Cell cycle analysis data processing

For cell cycle analysis of aneuploid cells, approximately 20 aneuploid cells and 10
euploid cells were scored per experiment. G1 duration, bud emergence to anaphase onset
duration, anaphase onset to cytokinesis duration, and division time (cytokinesis to
cytokinesis) were measured for each cell following chromosome mis-segregation.
Analysis of cells started either at bud emergence or at anaphase onset in the first division
following chromosome mis-segregation as cells were not able to be imaged in the first G1
following chromosome mis-segregation due to technical limitations in the time needed to
set up the cells for imaging on the microscope. Thus, division time analyses start in the
second division following chromosome mis-segregation as the first division following
chromosome mis-segregation could not be fully imaged.

At the end of the time-lapse, cells that were in G1 and had spent at least 100 minutes in
G1 were scored as arrested and included in G1 duration calculations. Cells that were in
G1 at the end of the time-lapse and had spent less than 100 minutes in G1 were not
considered arrested and excluded from analysis for that G1 duration. At the end of the
time-lapse, cells that had passed bud emergence but had not reached anaphase onset and had spent at least 250 minutes in that stage were scored as arrested and included in bud emergence to anaphase onset duration calculations. Cells that had passed bud emergence at the end of the time-lapse and had not reached anaphase onset but had spent less than 250 minutes in this stage were not considered arrested and excluded from analysis for that bud emergence to anaphase onset duration.

To calculate standard deviation of cell populations and compare variance using the F-test, raw data (in minutes) was used (Figures 10D-G, 14D-G, 15D-G). These analyses were done on the combined data from all divisions measured following chromosome mis-segregation for all cells of a given karyotype for a particular cell cycle stage (G1 or bud emergence to anaphase onset). Aneuploid cells were compared to euploid cells from the same experiment to account for minor day-to-day variability.

In Figures 10A-C, 11, 12, 13, 14A-C, 15A-C, and 16, cell cycle measurements for aneuploid cells were normalized to euploid cells from the same experiment to allow us to compare cell cycle measurements across experiments. Each aneuploid cell measurement for G1 duration, bud emergence to anaphase onset duration, anaphase to cytokinesis duration, or division time was taken as a ratio of the mean of all euploid cells for the same measurement (G1 duration, bud emergence to anaphase onset duration, anaphase to cytokinesis duration, or division time) from the same division following chromosome mis-segregation. For example, the G1 duration measurement for an aneuploid cell in its third division following chromosome mis-segregation would be normalized to the mean
of the G1 duration for all euploid cells in the third division following chromosome mis-
segregation. As euploid cells do not mis-segregate chromosomes, this is equivalent to the
third division in SC-D for both the aneuploid cell and the euploid cell. Normalization to
the euploid control accounts for day-to-day variability in experiments as well as changes
in cell cycle durations upon shift from raffinose+galactose (SC-RG) as the carbon source
to glucose (SC-D) as the carbon source.

Aneuploid fitness colony measurements
Cells were grown in YEP-R and then transferred to YEP-RG for 160 minutes to induce
chromosome mis-segregation. Cells were then plated on 2% agar YEP-D solid medium.
Mother-daughter pairs that had just completed a cell division were separated by
micromanipulation and allowed to grow into colonies at 30°C. Colony size was measured
40 – 48 hours later when colony size was still increasing exponentially. Colony area was
measured using ImageJ.

To calculate aneuploid to euploid colony area ratio, euploid colony area was measured
and averaged per plate, with a separate average for euploid mothers and euploid
daughters. Euploid cells on the same plate as the aneuploid cells were used for analysis to
control for plate-to-plate variability. A value for each individual aneuploid colony was
calculated as a ratio of the aneuploid colony area to the mean euploid colony area for
euploid cells. For disomes and trisomes, the mean colony area for euploid mothers was
used. For monosomes, the mean colony area for euploid daughters was used. Each circle
in Figure 9 represents the mean of all aneuploid/euploid ratios for a particular karyotype. Error bars indicate the standard deviation of the aneuploid/euploid ratios.

Chromosome mis-segregation was confirmed by checking the colonies for uracil prototrophy. The inducible chromosome is marked with *URA3* at the conditional centromere, so colony pairs in which one colony (from the daughter cell) is auxotrophic for growth on uracil and the other colony (from the mother cell) is prototrophic for growth on uracil indicates a chromosome mis-segregation event. For aneuploid strains with severe fitness defects, colonies were grown at 30°C until colonies were large enough to test for uracil prototrophy via replica-plating. Colonies were also tested for growth on glycerol and colonies that did not grow on glycerol were excluded from analysis as they are likely petite colonies. Rarely, the inducible chromosome was retained in the daughter cell instead of the mother; these mis-segregation events were excluded from analysis.

The colony mis-segregation assay was used to determine the chromosome mis-segregation rates presented in Table 1.
Table 3: Yeast strains used in this study (all derivatives of the W303 background)

<table>
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<th>Strain Number</th>
<th>Inducible Chromosome(s)</th>
<th>Genotype</th>
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<tr>
<td>A2587</td>
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<td>MATa, ade2-1, leu2-3, ura3, trp1-1, his3-11,15,15, can1-100, GAL, [phi+]. ade1::HIS3, lys2:::KanMX6</td>
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<td>A24367</td>
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\begin{align*}
\text{CEN16/cen16::p}_{\text{GAL1}}\text{-CEN3::URA3} \\

1363 & \quad - & \text{MAT}_{a}\,\text{ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL}\,\text{[\phi}^{+}\text{].} \\
& \quad & \text{LacO array::NATMX6 inserted in intergenic region (151624-152219) on chromosome I between CEN1 and NUP60, his3::p}_{\text{URA3}}\text{-GFP-LacI::HIS3;KANMX6.} \\

1421 & \quad - & \text{MAT}_{a}\,\text{ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL}\,\text{[\phi}^{+}\text{].} \\
& \quad & \text{LacO array::NATMX6 inserted in intergenic region (151624-152219) on chromosome I between CEN1 and NUP60, his3::p}_{\text{URA3}}\text{-GFP-LacI::HIS3;KANMX6.} \\

1475 & \quad - & \text{MAT}_{a/alpha}\,\text{ade2-1/ade2-1, leu2-3/leu2-3, ura3/ura3, trp1-1/trp1-1,} \\
& \quad & \text{his3-11,15/his3-11,15, can1-100/can1-100, GAL/GAL}\,\text{[\phi}^{+}\text{].} \\
& \quad & \text{+/LacO array::NATMX6 inserted in intergenic region (151624-152219) on chromosome I between CEN1 and NUP60, HIS3/his3::p}_{\text{URA3}}\text{-GFP-LacI::HIS3;KANMX6, Hsp104-mCherry::NATMX6.} \\

1480 & \quad - & \text{MAT}_{a/alpha}\,\text{ade2-1/ade2-1, leu2-3/leu2-3, ura3/ura3, trp1-1/trp1-1,} \\
& \quad & \text{his3-11,15/his3-11,15, can1-100/can1-100, GAL/GAL}\,\text{[\phi}^{+}\text{].} \\
& \quad & \text{+/LacO array::NATMX6 inserted in intergenic region (151624-152219) on chromosome I between CEN1 and NUP60, HIS3/his3::p}_{\text{URA3}}\text{-GFP-LacI::HIS3;KANMX6, Hsp104-mCherry::NATMX6.} \\

1503 & \quad I+II & \text{MAT}_{a/alpha}\,\text{ade2-1/ade2-1, leu2-3/leu2-3, ura3/ura3, trp1-1/trp1-1,} \\
& \quad & \text{his3-11,15/his3-11,15, can1-100/can1-100, GAL/GAL}\,\text{[\phi}^{+}\text{].} \\
& \quad & \text{CEN1;+/cen1::p}_{\text{GAL1}}\text{-CEN3::URA3; LacO array::NATMX6 inserted in intergenic region (151624-152219) on chromosome I between CEN1 and NUP60,} \\
& \quad & \text{CEN2;+/cen2::p}_{\text{GAL1}}\text{-CEN3::URA3; LacO array::NATMX6 inserted in intergenic region (238345-238923) on chromosome II between CEN2 and RAD16,} \\
& \quad & \text{HIS3/his3::p}_{\text{URA3}}\text{-GFP-LacI::HIS3;KANMX6,} \\
& \quad & \text{TUB1/p}_{\text{MET3}}\text{-mCherry-Tub1::URA3.} \\

1507 & \quad V+X & \text{MAT}_{a/alpha}\,\text{ade2-1/ade2-1, leu2-3/leu2-3, ura3/ura3, trp1-1/trp1-1,} \\
& \quad & \text{his3-11,15/his3-11,15, can1-100/can1-100, GAL/GAL}\,\text{[\phi}^{+}\text{].} \\
& \quad & \text{CEN5;+/cen5::p}_{\text{GAL1}}\text{-CEN3::URA3; LacO array::NATMX6 inserted in intergenic region (152351-153293) on chromosome V between CEN5 and MNNI,} \\
& \quad & \text{CEN10;+/cen10::p}_{\text{GAL1}}\text{-CEN3::URA3; LacO array::NATMX6 inserted in intergenic region (444903-445865) on chromosome X between SAG1 and APL1,} \\
& \quad & \text{HIS3/his3::p}_{\text{URA3}}\text{-GFP-LacI::HIS3;KANMX2,} \\
& \quad & \text{TUB1/p}_{\text{MET3}}\text{-mCherry-Tub1::URA3.} \\

1508 & \quad II+V+X & \text{MAT}_{a/alpha}\,\text{ade2-1/ade2-1, leu2-3/leu2-3, ura3/ura3, trp1-1/trp1-1,} \\
& \quad & \text{his3-11,15/his3-11,15, can1-100/can1-100, GAL/GAL}\,\text{[\phi}^{+}\text{].} \\
& \quad & \text{CEN2;+/cen2::p}_{\text{GAL1}}\text{-CEN3::URA3; LacO array::NATMX6 inserted in intergenic region (238345-238923) on chromosome II between CEN2 and RAD16,} \\
& \quad & \text{CEN5;+/cen5::p}_{\text{GAL1}}\text{-CEN3::URA3; LacO array::NATMX6 inserted in intergenic region (152351-153293) on chromosome V between CEN5 and MNNI,} \\
& \quad & \text{CEN10;+/cen10::p}_{\text{GAL1}}\text{-CEN3::URA3; LacO array::NATMX6 inserted in intergenic region (444903-445865) on chromosome X between SAG1 and APL1,} \\
& \quad & \text{HIS3/his3::p}_{\text{URA3}}\text{-GFP-LacI::HIS3;KANMX2,} \\
& \quad & \text{TUB1/p}_{\text{MET3}}\text{-mCherry-Tub1::URA3.} \\

1509 & \quad I+V+X & \text{MAT}_{a/alpha}\,\text{ade2-1/ade2-1, leu2-3/leu2-3, ura3/ura3, trp1-1/trp1-1,} \\
& \quad & \text{his3-11,15/his3-11,15, can1-100/can1-100, GAL/GAL}\,\text{[\phi}^{+}\text{].} \\
& \quad & \text{CEN1;+/cen1::p}_{\text{GAL1}}\text{-CEN3::URA3; LacO array::NATMX6 inserted in intergenic region (151624-152219) on chromosome I between CEN1 and}
| 1510  | I+II+X | MATα/α, ade2-1/ade2-1, leu2-3/leu2-3, ura3/ura3, trp1-1/trp1-1, his3-11,15/his3-11,15, can1-100/can1-100, GAL/GAL, [phi+], CEN1/+/cen1:PGAL1-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region (152351-153293) on chromosome V between CEN5 and MNN1, CEN10/+/cen10:PGAL1-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region (445865-445865) on chromosome X between SAG1 and APL1, HIS3/his3::pURA3-GFP-Lacl::HIS3;KANMX2, TUB1/pMET3::mCherry-Tub1::URA3 |
| 1525  | II+V+X | MATα, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, [phi+], cen2:PGAL1-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region (238345-238923) on chromosome II between CEN2 and RAD16, cen5:PGAL1-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region (152351-153293) on chromosome V between CEN5 and MNN1, cen10:PGAL1-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region (445865-445865) on chromosome X between SAG1 and APL1, his3::pURA3-GFP-Lacl::HIS3;KANMX2, TUB1/pMET3::mCherry-Tub1::URA3 |
| 1736  | XIV    | MATα, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, [phi+], cen14::PGAL1-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region (624649-624953) on chromosome XIV between HRB1 and PET8, his3::pURA3-GFP-Lacl::HIS3;KANMX2, Hsp104-mCherry::NATMX6 |
| 1746  | VIII   | MATα, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, [phi+], cen8::PGAL1-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region (105718-106030) on chromosome VIII between CEN8 and OSH7, his3::pURA3-GFP-Lacl::HIS3;KANMX2, Hsp104-mCherry::NATMX6 |
| 1777  | IV     | MATα, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, [phi+], cen4::PGAL1-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region (452513-453003) on chromosome IV between NTH1 and YRB1, his3::pURA3-GFP-Lacl::HIS3;KANMX2 |
| 1804  | II     | MATα/α, ade2-1/ade2-1, leu2-3/leu2-3, ura3/ura3, trp1-1/trp1-1, his3-11,15/his3-11,15, can1-100/can1-100, GAL/GAL, [phi+], CEN2/+/cen2:PGAL1-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region (238345-238923) on chromosome II between CEN2 and RAD16, HIS3/his3::pURA3-GFP-Lacl::HIS3;KANMX2, HSP104/Hsp104·mCherry::NATMX6, MATα1/MATα2/mata·αα1/mata·αα2·::TRP1 |
| 1805  | XIV    | MATα/α, ade2-1/ade2-1, leu2-3/leu2-3, ura3/ura3, trp1-1/trp1-1, his3-11,15/his3-11,15, can1-100/can1-100, GAL/GAL, [phi+], CEN14/+/cen14:PGAL1-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region (624649-624953) on chromosome XIV between HRB1 and PET8,
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<thead>
<tr>
<th>Chromosome</th>
<th>Region</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV</td>
<td>MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, [phi+], cen4::pgal-CEN3::URA3; LacO array::NATMX6 inserted in intergenic region (452513-453003) on chromosome IV between NTH1 and YRB1, his3::purA3-GFP-LacI::HIS3;KANMX2, HSP104/Hsp104-mCherry::NATMX6, MATA1;MATA2/matalpha1,matalpha2::TRP1</td>
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<td>I</td>
<td>MATA/alphaA, ade2-1/ade2-1, leu2-3/leu2-3, ura3/ura3, trp1-1/trp1-1, his3-11,15/11,15, can1-100/100, GAL/GAL, [phi+], CEN1;+/cen1::pgal-CEN3::URA3; LacO array::NATMX6 inserted in intergenic region (151624-152219) on chromosome I between CEN1 and NUP60, HIS3/his3::purA3-GFP-LacI::HIS3;KANMX2, HSP104/Hsp104-mCherry::NATMX6, MATA1;MATA2/matalpha1,matalpha2::TRP1</td>
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</tr>
<tr>
<td>V</td>
<td>MATA/alphaA, ade2-1/ade2-1, leu2-3/leu2-3, ura3/ura3, trp1-1/trp1-1, his3-11,15/11,15, can1-100/100, GAL/GAL, [phi+], CEN5;+/cen5::pgal-CEN3::URA3; LacO array::NATMX6 inserted in intergenic region (152351-153293) on chromosome V between CEN5 and MNN1, HIS3/his3::purA3-GFP-LacI::HIS3;KANMX2, HSP104/Hsp104-mCherry::NATMX6, MATA1;MATA2/matalpha1,matalpha2::TRP1</td>
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<tr>
<td>VIII</td>
<td>MATA/alphaA, ade2-1/ade2-1, leu2-3/leu2-3, ura3/ura3, trp1-1/trp1-1, his3-11,15/11,15, can1-100/100, GAL/GAL, [phi+], CEN8;+/cen8::pgal-CEN3::URA3; LacO array::NATMX6 inserted in intergenic region (105718-106030) on chromosome VIII between CEN8 and OSH7, HIS3/his3::purA3-GFP-LacI::HIS3;KANMX2, HSP104/Hsp104-mCherry::NATMX6, MATA1;MATA2/matalpha1,matalpha2::TRP1</td>
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</tr>
<tr>
<td>X</td>
<td>MATA/alphaA, ade2-1/ade2-1, leu2-3/leu2-3, ura3/ura3, trp1-1/trp1-1, his3-11,15/11,15, can1-100/100, GAL/GAL, [phi+], CEN10;+/cen10::pgal-CEN3::URA3; LacO array::NATMX6 inserted in intergenic region (444903-445865) on chromosome X between SAG1 and APL1, HIS3/his3::purA3-GFP-LacI::HIS3;KANMX2, HSP104/Hsp104-mCherry::NATMX6, MATA1;MATA2/matalpha1,matalpha2::TRP1</td>
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| II+V+X     | MATA/alphaA, ade2-1/ade2-1, leu2-3/leu2-3, ura3/ura3, trp1-1/trp1-1, his3-11,15/11,15, can1-100/100, GAL/GAL, [phi+], CEN2;+/cen2::pgal-CEN3::URA3; LacO array::NATMX6 inserted in intergenic region (238345-238923) on chromosome II between CEN2 and RAD16, CEN5;+/cen5::pgal-CEN3::URA3; LacO array::NATMX6 inserted in intergenic region (152351-153293) on chromosome V between CEN5 and MNN1, CEN10;+/cen10::pgal-CEN3::URA3; LacO array::NATMX6 inserted in intergenic region (444903-445865) on chromosome X between SAG1 and
<table>
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<tr>
<th>Year</th>
<th>Chromosome</th>
<th>Genomic Region and Markers</th>
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<tbody>
<tr>
<td>1831</td>
<td>II+X+XIV</td>
<td>MATa, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, [phi+]</td>
</tr>
<tr>
<td></td>
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<td>cen2::p&lt;sub&gt;GAL&lt;/sub&gt;L-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region (238345-238923) on chromosome II between CEN2 and RAD16, cen10::p&lt;sub&gt;GAL&lt;/sub&gt;L-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region (444903-445865) on chromosome X between SAG1 and APL1, cen14::p&lt;sub&gt;GAL&lt;/sub&gt;L-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region (624649-624953) on chromosome XIV between HRB1 and PET8, his3::p&lt;sub&gt;URA3&lt;/sub&gt;-GFP::LacI::HIS3;KANMX6, Hisp104-mCherry::NATMX6</td>
</tr>
<tr>
<td>1839</td>
<td>XI</td>
<td>MATa/alphaA, ade2-1/ade2-1, leu2-3/leu2-3, ura3/ura3, trp1-1/trp1-1, his3-11,15/his3-11,15, can1-100/can1-100, GAL/GAL, [phi+]</td>
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<td>CEN11::+;cen11::p&lt;sub&gt;GAL&lt;/sub&gt;L-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region (439058-439732) on chromosome XI between CEN11 and MET14, HIS3/his3::p&lt;sub&gt;URA3&lt;/sub&gt;-GFP::LacI::HIS3;KANMX6, HISp104/Hisp104-mCherry::NATMX6, MATa1;MATa2/matalpha1,matalpha2::TRP1</td>
</tr>
<tr>
<td>1841</td>
<td>II+X+XIV</td>
<td>MATa/alphaA, ade2-1/ade2-1, leu2-3/leu2-3, ura3/ura3, trp1-1/trp1-1, his3-11,15/his3-11,15, can1-100/can1-100, GAL/GAL, [phi+]</td>
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<tr>
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<td>CEN2::+;cen2::p&lt;sub&gt;GAL&lt;/sub&gt;L-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region (238345-238923) on chromosome II between CEN2 and RAD16, CEN10::+;cen10::p&lt;sub&gt;GAL&lt;/sub&gt;L-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region (444903-445865) on chromosome X between SAG1 and APL1, CEN14::+;cen14::p&lt;sub&gt;GAL&lt;/sub&gt;L-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region (624649-624953) on chromosome XIV between HRB1 and PET8, HIS3/his3::p&lt;sub&gt;URA3&lt;/sub&gt;-GFP::LacI::HIS3;KANMX6, HISp104/Hisp104-mCherry::NATMX6, MATa1;MATa2/matalpha1,matalpha2::TRP1</td>
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<tr>
<td>1897</td>
<td>IV</td>
<td>MATa/alphaA, ade2-1/ade2-1, leu2-3/leu2-3, ura3/ura3, trp1-1/trp1-1, his3-11,15/his3-11,15, can1-100/can1-100, GAL/GAL, [phi+]</td>
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<tr>
<td></td>
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<td>CEN4::+;cen4::p&lt;sub&gt;GAL&lt;/sub&gt;L-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region (452513-453003) on chromosome IV between NTH1 and YRB1, HIS3/his3::p&lt;sub&gt;URA3&lt;/sub&gt;-GFP::LacI::HIS3;KANMX6, HISp104/Hisp104-mCherry::NATMX6, MATa1;MATa2/matalpha1,matalpha2::TRP1</td>
</tr>
<tr>
<td>1916</td>
<td>XI</td>
<td>MATa, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, [phi+]</td>
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<td>cen11::p&lt;sub&gt;GAL&lt;/sub&gt;L-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region (439058-439732) on chromosome XI between CEN11 and MET14, his3::p&lt;sub&gt;URA3&lt;/sub&gt;-GFP::LacI::HIS3;KANMX6, Hsp104-mCherry::NATMX6</td>
</tr>
<tr>
<td>1918</td>
<td>II+IV+XIV</td>
<td>MATa, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, [phi+]</td>
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<tr>
<td></td>
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<td>cen2::p&lt;sub&gt;GAL&lt;/sub&gt;L-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region (238345-238923) on chromosome II between CEN2 and RAD16, cen4::p&lt;sub&gt;GAL&lt;/sub&gt;L-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region (452513-453003) on chromosome IV between NTH1 and YRB1, cen14::p&lt;sub&gt;GAL&lt;/sub&gt;L-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region (624649-624953) on chromosome XIV between HRB1 and PET8, his3::p&lt;sub&gt;URA3&lt;/sub&gt;-GFP::LacI::HIS3;KANMX6, Hsp104-mCherry::NATMX6</td>
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</table>
1923  IV+V+VIII  MATα, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, [phi+].
cen4:+pGAL1-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region
(452513-453003) on chromosome IV between NTH1 and YRB1, 
cen5:+pGAL1-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region
(152351-153293) on chromosome V between CEN5 and MNN1, 
cen8:+pGAL1-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region
(105718-106030) on chromosome VIII between CEN8 and OSH7, 
his3::pURA3-GFP-LacI::HIS3; KANMX6, 
Hsp104-mCherry::NATMX6

1929  IV+V+VIII  MATα/αADE2, ade2-1/ade2-1, leu2-3/leu2-3, ura3/ura3, trp1-1/trp1-1, 
his3-11,15/his3-11,15, can1-100/can1-100, GAL/GAL, [phi+].
CEN4:+/cen4:+pGAL1-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region
(452513-453003) on chromosome IV between NTH1 and YRB1, 
CEN5:+/cen5:+pGAL1-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region
(152351-153293) on chromosome V between CEN5 and MNN1, 
CEN8:+/cen8:+pGAL1-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region
(105718-106030) on chromosome VIII between CEN8 and OSH7, 
HIS3/his3::pURA3-GFP-LacI::HIS3; KANMX6, 
Hsp104/Hsp104-mCherry::NATMX6, 
MATα1;MATα2/matalpha1,matalpha2::TRP1

1931  II+IV+XIV  MATα/αADE2, ade2-1/ade2-1, leu2-3/leu2-3, ura3/ura3, trp1-1/trp1-1, 
his3-11,15/his3-11,15, can1-100/can1-100, GAL/GAL, [phi+].
CEN2:+/cen2:+pGAL1-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region
(238345-238923) on chromosome II between CEN2 and RAD16, 
CEN4:+/cen4:+pGAL1-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region
(452513-453003) on chromosome IV between NTH1 and YRB1, 
CEN14:+/cen14:+pGAL1-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region
(624649-624953) on chromosome XIV between HRB1 and PET8, 
HIS3/his3::pURA3-GFP-LacI::HIS3; KANMX6, 
Hsp104/Hsp104-mCherry::NATMX6, 
MATα1;MATα2/matalpha1,matalpha2::TRP1

11018  XIV  MATα, ade2-1/ade2-1, leu2-3/leu2-3, ura3/ura3, trp1-1/trp1-1, 
his3-11,15/his3-11,15, can1-100/can1-100, GAL/GAL, [phi+].
CEN14:+/cen14:+pGAL1-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region
(624649-624953) on chromosome XIV between HRB1 and PET8, 
HIS3/his3::pURA3-GFP-LacI::HIS3; KANMX6, 
URA3/ura3::mCherry-Cdc3::URA3 
SPC42/Spc42-dsRed::KANMX6

11075  I  MATα, ade2-1/ade2-1, leu2-3/leu2-3, ura3/ura3, trp1-1/trp1-1, 
his3-11,15/his3-11,15, can1-100/can1-100, GAL/GAL, [phi+].
CEN1:+/cen1:+pGAL1-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region
(151624-152219) on chromosome I between CEN1 and NUP60, 
HIS3/his3::pURA3-GFP-LacI::HIS3; KANMX6, 
URA3/ura3::mCherry-Cdc3::URA3 
SPC42/Spc42-dsRed::KANMX6

11077  II  MATα, ade2-1/ade2-1, leu2-3/leu2-3, ura3/ura3, trp1-1/trp1-1, 
his3-11,15/his3-11,15, can1-100/can1-100, GAL/GAL, [phi+].
CEN2:+/cen2:+pGAL1-CEN3:URA3; LacO array::NATMX6 inserted in
intergenic region (238345-238923) on chromosome II between CEN2 and RAD16,
HIS3/his3::URA3::GFP-LacI::HIS3::KANMX6,
URA3/ura3::mCherry-Cdc3::URA3
SPC42/Spc42::dsRed::KANMX6

11079 IV
MATα/α, ade2-1/ade2-1, leu2-3/leu2-3, ura3/ura3, trp1-1/trp1-1,
his3-11.15/his3-11.15, can1-100/can1-100, GAL/GAL, [phi+],
CEN4: +/cen4::pGAL1::CEN3::URA3; LacO array::NATMX6 inserted in
intergenic region (452513-453003) on chromosome IV between NTH1
and YRB1,
HIS3/his3::URA3::GFP-LacI::HIS3::KANMX6,
URA3/ura3::mCherry-Cdc3::URA3
SPC42/Spc42::dsRed::KANMX6

11081 V
MATα/α, ade2-1/ade2-1, leu2-3/leu2-3, ura3/ura3, trp1-1/trp1-1,
his3-11.15/his3-11.15, can1-100/can1-100, GAL/GAL, [phi+],
CEN5:: +/cen5::pGAL1::CEN3::URA3; LacO array::NATMX6 inserted in
intergenic region (152351-153293) on chromosome V between CEN5 and MNN1;
URA3
HIS3/his3::URA3::GFP-LacI::HIS3::KANMX6,
URA3/ura3::mCherry-Cdc3::URA3
SPC42/Spc42::dsRed::KANMX6

11085 X
MATα/α, ade2-1/ade2-1, leu2-3/leu2-3, ura3/ura3, trp1-1/trp1-1,
his3-11.15/his3-11.15, can1-100/can1-100, GAL/GAL, [phi+],
CEN10:: +/cen10::pGAL1::CEN3::URA3; LacO array::NATMX6 inserted in
intergenic region (444903-445865) on chromosome X between SAG1 and
APl1,
HIS3/his3::URA3::GFP-LacI::HIS3::KANMX6,
URA3/ura3::mCherry-Cdc3::URA3
SPC42/Spc42::dsRed::KANMX6

11086 XI
MATα/α, ade2-1/ade2-1, leu2-3/leu2-3, ura3/ura3, trp1-1/trp1-1,
his3-11.15/his3-11.15, can1-100/can1-100, GAL/GAL, [phi+],
CEN11:: +/cen11::pGAL1::CEN3::URA3; LacO array::NATMX6 inserted in
intergenic region (439058-439732) on chromosome XI between CEN11 and MET14;
SPC42
HIS3/his3::URA3::GFP-LacI::HIS3::KANMX6,
URA3/ura3::mCherry-Cdc3::URA3
SPC42/Spc42::dsRed::KANMX6

11092 IV+V+VIII
MATα/α, ade2-1/ade2-1, leu2-3/leu2-3, ura3/ura3, trp1-1/trp1-1,
his3-11.15/his3-11.15, can1-100/can1-100, GAL/GAL, [phi+],
CEN4:: +/cen4::pGAL1::CEN3::URA3; LacO array::NATMX6 inserted in
intergenic region (452513-453003) on chromosome IV between NTH1
and YRB1,
CEN5:: +/cen5::pGAL1::CEN3::URA3; LacO array::NATMX6 inserted in
intergenic region (152351-153293) on chromosome V between CEN5 and MNN1;
URA3
CEN8:: +/cen8::pGAL1::CEN3::URA3; LacO array::NATMX6 inserted in
intergenic region (105718-106030) on chromosome VIII between CEN8
and OSH7,
HIS3/his3::URA3::GFP-LacI::HIS3::KANMX6,
URAL/ura3::mCherry-Cdc3::URA3
SPC42/Spc42::dsRed::KANMX6

11093 XII
MATα/α, ade2-1/ade2-1, leu2-3/leu2-3, ura3/ura3, trp1-1/trp1-1,
his3-11.15/his3-11.15, can1-100/can1-100, GAL/GAL, [phi+],
CEN12:: +/cen12::pGAL1::CEN3::URA3; intergenic region (150488-150498)
on chromosome XII between CEN12 and DNM1::hphMX4::LacO
array::NATMX6
HIS3/his3::URA3::GFP-LacI::HIS3::KANMX6,
URA3/ura3::mCherry-Cdc3::URA3
SPC42/Spc42::dsRed::KANMX6
MATα/α, ade2-1/ade2-1, leu2-3/leu2-3, ura3/ura3, trp1-1/trp1-1, his3-11,15/his3-11,15, can1-100/can1-100, GAL/GAL, [ϕ+], CEN2:+pGAL1-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region (238345-238923) on chromosome II between CEN2 and RAD16, CEN4:+pGAL1-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region (452513-453003) on chromosome IV between NTH1 and YRB1, CEN14:+pGAL1-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region (624649-624953) on chromosome XIV between HRB1 and PET8, HIS3/his3::pURA3-GFP-LacI::HIS3::KANMX6, URA3/ura3::mCherry-Cdc3::URA3, SPC42/spc42::dsRed::KANMX6

MATα, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, [ϕ+], cen1::pGAL1-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region (151624-152219) on chromosome I between CEN1 and NUP60, his3::pURA3-GFP-LacI::HIS3::KANMX2, ura3::mCherry-Cdc3::URA3, Spc42::dsRed::KANMX6

MATα, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, [ϕ+], cen5::pGAL1-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region (152351-153293) on chromosome V between CEN5 and MNN1, his3::pURA3-GFP-LacI::HIS3::KANMX2, ura3::mCherry-Cdc3::URA3, Spc42::dsRed::KANMX6

MATα, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, [ϕ+], cen8::pGAL1-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region (105718-106030) on chromosome VIII between CEN8 and OSH7, his3::pURA3-GFP-LacI::HIS3::KANMX2, ura3::mCherry-Cdc3::URA3, Spc42::dsRed::KANMX6

MATα, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, [ϕ+], cen10::pGAL1-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region (444903-445865) on chromosome X between SAG1 and APL1, his3::pURA3-GFP-LacI::HIS3::KANMX2, ura3::mCherry-Cdc3::URA3, Spc42::dsRed::KANMX6

MATα, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, [ϕ+], cen2::pGAL1-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region (238345-238923) on chromosome II between CEN2 and RAD16, cen5::pGAL1-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region (152351-153293) on chromosome V between CEN5 and MNN1, cen10::pGAL1-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region (444903-445865) on chromosome X between SAG1 and APL1, his3::pURA3-GFP-LacI::HIS3::KANMX2, ura3::mCherry-Cdc3::URA3, Spc42::dsRed::KANMX6

MATα, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, [ϕ+], cen13::pGAL1-CEN3:URA3; intergenic region (272913-272923) on
chromosome XIII between MIX17 and AIM34::hphMX4::LacO array::NATMX6, his3::pURAS3-GFP-LacI:His3;KANMX2, ura3::mCherry-Cdc3::URA3, Spc42-dsRed:KANMX6

11139 IV+V+VIII
MATa, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, [phi+], cen4::pGAL1-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region (452513-453003) on chromosome IV between NTH1 and YRB1, cen5::pGAL1-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region (152351-153293) on chromosome V between CEN5 and MNN1, cen8::pGAL1-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region (105718-106030) on chromosome VIII between CEN8 and OSH7, his3::pURAS3-GFP-LacI:His3;KANMX2, ura3::mCherry-Cdc3::URA3, Spc42-dsRed:KANMX6

11141 II+IV+XIV
MATa, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, [phi+], cen4::pGAL1-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region (238345-238923) on chromosome II between CEN2 and RAD16, cen5::pGAL1-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region (452513-453003) on chromosome IV between NTH1 and YRB1, cen14::pGAL1-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region (624649-624953) on chromosome XIV between HRB1 and PET8, his3::pURAS3-GFP-LacI:His3;KANMX2, ura3::mCherry-Cdc3::URA3, Spc42-dsRed:KANMX6

11142 II
MATa, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, [phi+], cen11::pGAL1-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region (439058-439732) on chromosome XI between CEN11 and MET14, his3::pURAS3-GFP-LacI:His3;KANMX2, ura3::mCherry-Cdc3::URA3, Spc42-dsRed:KANMX6

11144 XI
MATa, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, [phi+], cen12::pGAL1-CEN3:URA3; intergenic region (150488-150498) on chromosome XII between CEN12 and DNM1::hphMX4::LacO array::NATMX6, his3::pURAS3-GFP-LacI:His3;KANMX2, ura3::mCherry-Cdc3::URA3, Spc42-dsRed:KANMX6

11148 XII
MATa, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, [phi+], cen16::pGAL1-CEN3:URA3; intergenic region (558097-558107) on chromosome XVI between CIT3 and PDH1::hphMX4::LacO array::NATMX6, his3::pURAS3-GFP-LacI:His3;KANMX2, ura3::mCherry-Cdc3::URA3, Spc42-dsRed:KANMX6

11150 XVI
MATa, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, [phi+], cen14::pGAL1-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region (624649-624953) on chromosome XIV between HRB1 and PET8, his3::pURAS3-GFP-LacI:His3;KANMX2, ura3::mCherry-Cdc3::URA3, Spc42-dsRed:KANMX6

11174 XIV
MATa, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, [phi+], cen14::pGAL1-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region (152351-153293) on chromosome V between CEN5 and MNN1, ura3::mCherry-Cdc3::URA3, Spc42-dsRed:KANMX6

11243 V+X
MATa/alpha, ade2-1/ade2-1, leu2-3/leu2-3, ura3/ura3, trp1-1/trp1-1, his3-11,15/his3-11,15, can1-100/can1-100, GAL/GAL, [phi+], CEN5::;+::mCherry-Cdc3::URA3/cen5::pGAL1-CEN3:URA3; LacO array::NATMX6 inserted in intergenic region (444903-445865) on chromosome X between SAG1 and APL1,
HIS3/his3::pURA3-GFP-LacI:HIS3;KANMX6, SPC42/Spc42-dsRed:KANMX6

11245  II+IV+VIII+X+XIV  MATα/α, ade2-1/ade2-1, leu2-3/leu2-3, ura3/ura3, trp1-1/trp1-1, his3-11,15/his3-11,15, can1-100/can1-100, GAL/GAL, [φ+], CEN2:+/cen2::pGAL1-CEN3:URA3; LacO array:NATMX6 inserted in intergenic region (238345-238923) on chromosome II between CEN2 and RAD16, CEN4:+/cen4::pGAL1-CEN3:URA3; LacO array:NATMX6 inserted in intergenic region (452513-453003) on chromosome IV between NTH1 and YRB1, CEN5:+/cen5::pGAL1-CEN3:URA3; LacO array:NATMX6 inserted in intergenic region (152351-153293) on chromosome V between CEN5 and MNN1; ura3::mCherry-Cdc3:URA3 CEN8:+/cen8::pGAL1-CEN3:URA3; LacO array:NATMX6 inserted in intergenic region (105718-106030) on chromosome VIII between CEN8 and OSH7, CEN10:+/cen10::pGAL1-CEN3:URA3; LacO array:NATMX6 inserted in intergenic region (444903-445865) on chromosome X between SAG1 and APL1, CEN14:+/cen14::pGAL1-CEN3:URA3; LacO array:NATMX6 inserted in intergenic region (624659-624953) on chromosome XIV between HRB1 and PET8, HIS3/his3::pURA3-GFP-LacI:HIS3;KANMX6, SPC42/Spc42-dsRed:KANMX6

11247  IV+V+VIII+XIV  MATα/α, ade2-1/ade2-1, leu2-3/leu2-3, ura3/ura3, trp1-1/trp1-1, his3-11,15/his3-11,15, can1-100/can1-100, GAL/GAL, [φ+], CEN4:+/cen4::pGAL1-CEN3:URA3; LacO array:NATMX6 inserted in intergenic region (452513-453003) on chromosome IV between NTH1 and YRB1, CEN5:+/cen5::pGAL1-CEN3:URA3; LacO array:NATMX6 inserted in intergenic region (152351-153293) on chromosome V between CEN5 and MNN1; ura3::mCherry-Cdc3:URA3 CEN8:+/cen8::pGAL1-CEN3:URA3; LacO array:NATMX6 inserted in intergenic region (105718-106030) on chromosome VIII between CEN8 and OSH7, CEN14:+/cen14::pGAL1-CEN3:URA3; LacO array:NATMX6 inserted in intergenic region (624649-624953) on chromosome XIV between HRB1 and PET8, HIS3/his3::pURA3-GFP-LacI:HIS3;KANMX6, SPC42/Spc42-dsRed:KANMX6

11251  -  MATα/α, ade2-1/ade2-1, leu2-3/leu2-3, ura3/ura3, trp1-1/trp1-1, his3-11,15/his3-11,15, can1-100/can1-100, GAL/GAL, [φ+], URA3/ura3::mCherry-Cdc3:URA3, SPC42/Spc42-GFP:TRP1

11259  V+X  MATα, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, [φ+], cen5::pGAL1-CEN3:URA3; LacO array:NATMX6 inserted in intergenic region (152351-153293) on chromosome V between CEN5 and MNN1, cen10::pGAL1-CEN3:URA3; LacO array:NATMX6 inserted in intergenic region (444903-445865) on chromosome X between SAG1 and APL1, his3::pURA3-GFP-LacI:HIS3;KANMX2, ura3::mCherry-Cdc3:URA3

11262  -  MATα/α, ade2-1/ade2-1, leu2-3/leu2-3, ura3/ura3, trp1-1/trp1-1, his3-11,15/his3-11,15, can1-100/can1-100, GAL/GAL, [φ+], CEN1:+/cen1::pGAL1-CEN3:URA3; LacO array:NATMX6 inserted in intergenic region (151624-152219) on chromosome I between CEN1 and NUP60, HIS3/his3::pURA3-GFP-LacI:HIS3;KANMX2,
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<tr>
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‘REPL’ in primer name indicates primer is for replacement of centromere with conditional centromere sequence.

Lowercase indicates 5’ tail that does not hybridize to original template during amplification from plasmid p1888 (pGAL-CEN-JC-13).
Table 5: Plasmids used in this study

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Targets LacO array to chromosome in intergenic region</th>
<th>Cutter to insert in middle of intergenic region</th>
<th>Selectable marker for plasmid integration</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1801 (pDB030) pURA3-GFP-LacI</td>
<td>XV: HIS3</td>
<td>Nhel to integrate at HIS3 select for KAN (has HIS3 and KANMX2 resistance)</td>
<td>KANMX2 (plasmid also has HIS3 resistance)</td>
<td>(Bressan et al., 2004)</td>
</tr>
<tr>
<td>1499 (pCM40)</td>
<td>For cloning – no targeting sequence</td>
<td>Cut with SalI to ligate in targeting insert</td>
<td>NATMX6</td>
<td>gift from Doug Koshland</td>
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<tr>
<td>Cen5-LacO</td>
<td>V: 152351-153293</td>
<td>BamHI</td>
<td>NATMX6</td>
<td>(Miller et al., 2012)</td>
</tr>
<tr>
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<tr>
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<td>PmlI or BclI</td>
<td>NATMX6</td>
<td>This study</td>
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<tr>
<td>Cen14-LacO</td>
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<td>Stul</td>
<td>NATMX6</td>
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<tr>
<td>HYGRO-LacO</td>
<td>Targets to hygromycin sequence</td>
<td>EagI (cuts twice in insert, cutting out a 165bp sequence)</td>
<td>NATMX6 (proper integration also confers loss of hygromycin B resistance)</td>
<td>This study</td>
</tr>
<tr>
<td>1888 (pGAL-CEN-JC3-13)</td>
<td>-</td>
<td>-</td>
<td>URA3</td>
<td>(Anders et al., 2009)</td>
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<tr>
<td>695 (pAG32)</td>
<td>-</td>
<td>-</td>
<td>hphMX4</td>
<td>(Goldstein and McCuskur, 1999)</td>
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ACKNOWLEDGMENTS

We thank Kirk Anders for providing plasmid pGALCEN-JC3-13 (p1888) containing the conditional centromere construct and Doug Koshland for providing plasmid pCM40 (p1499). This work was supported by the National Institutes of Health (GM056800 to A.A.). A.A. is an investigator of the Howard Hughes Medical Institute.
REFERENCES


Chapter 4: Conclusions and Future Directions
Aneuploidy research has implications for many areas of human health. Aneuploidy is a feature of developmental disorders as it is the leading cause of miscarriage and results in chromosomal disorders such as Down Syndrome, which are marked by delayed development and slow proliferation. Yet aneuploidy is also a hallmark of cancer, a disease of uncontrolled proliferation (Hanahan and Weinberg, 2000). Recently, research has given us a better understanding of the direct causes of aneuploidy and helped disentangle the consequences of aneuploidy from the effects of cancer. As aneuploidy has a great effect on human health, it is important to understand the mechanisms by which aneuploidy impairs cellular fitness.

Recent research has elucidated a wide variety of phenotypes associated with aneuploidy. In all primary cells studied to date, aneuploidy is associated with decreased proliferation (Babu et al., 2003; Kops et al., 2004; Li et al., 2010; Niwa and Yanagida, 1985; Niwa et al., 2006; Pavelka et al., 2010; Segal and McCoy, 1974; Stingele et al., 2012; Thompson and Compton, 2008, 2010; Torres et al., 2007; Williams et al., 2008). Aneuploid cells transcribe and translate the extra chromosome(s) in cases of chromosome gain, leading to a two-fold increase in protein levels for most proteins on the extra chromosome(s) (Dephoure et al., 2014; Pavelka et al., 2010; Stingele et al., 2012; Torres et al., 2010). Aneuploidy is also associated with increased proteotoxic stress (Oromendia et al., 2012; Santaguida et al., 2015; Stingele et al., 2012; Tang et al., 2011; Torres et al., 2007) and metabolic alterations (Stingele et al., 2012; Torres et al., 2007; Williams et al., 2008). Additionally, aneuploid cells exhibit increased genomic instability (Blank et al., 2015; Sheltzer et al., 2011; Zhu et al., 2012). Generally, the phenotypes observed in aneuploid cells cause decreased cellular fitness, more like the effects of aneuploidy in...
chromosomal disorders than aneuploidy and cancer, where cells have increased proliferative capacity despite a high incidence of aneuploidy (Weaver and Cleveland, 2006).

In this work, I sought to characterize the molecular mechanisms of the G1 delay observed in aneuploid cells using a set of disomic yeast strains previously generated in our lab (Torres et al., 2007). Additionally, I developed a novel system to induce targeted chromosome missegregation in budding yeast in order to study the onset of aneuploidy. Using this system, I characterized cell cycle delays and accumulation of protein aggregates resulting from chromosome missegregation. I also utilized this system to characterize monosomy in a systematic manner.

**Aneuploidy and the cell cycle**

*Aneuploidy and the G1-S transition machinery*

In Chapter 2, I characterized the molecular mechanisms responsible for the G1 delay in disomic yeast. I showed that 10 of 14 disomic yeast strains exhibited a cell cycle entry delay as measured by an increase in critical size. Defects in the cell cycle machinery promoting the G1-S transition result in an increase in critical size (Rupes, 2002).

To investigate where aneuploidy is delaying cells in the G1-S pathway, I looked at the molecular players in the G1-S pathway. The genetic program that controls cell cycle entry culminates in accumulation of *CLN1* and *CLN2* (Dirick et al., 1995). I reported that transcription of *CLN1*, *CLN2* and other SBF and MBF targets is delayed in disomic cells; however, overexpression of *CLN2* is sufficient to rescue the increase in
critical size, suggesting that the G1 delay is ultimately due to a delay in CLN2 accumulation. Whi5 inhibits SBF, the transcription factor complex that promotes CLN1/2 expression (de Bruin et al., 2004; Costanzo et al., 2004). I demonstrated that Whi5 nuclear exit, an indication of SBF de-repression, is delayed in aneuploid cells. Recent work has identified Whi5 as a potential ‘sizer’ that sets the critical size for cells, thus coordinating cell growth with cell cycle entry (Schmoller et al., 2015). Whi5 is synthesized in G2/S/M at a rate independent of cell size; therefore, all cells are born with a similar amount of Whi5. Whi5 activity decreases as Whi5 is diluted by cell growth in G1, coupling cell volume with cell cycle entry. It will be interesting to investigate absolute levels of Whi5 in aneuploid cells, as perhaps Whi5 levels in aneuploid cells are altered, potentially by altered synthesis in S/G2/M or by other mechanisms, thus perturbing the kinetics of cell cycle entry in aneuploid cells.

Ultimately, I established that aneuploidy interferes with G1 progression upstream of Cln3 protein accumulation in disomic cells, as Cln3 protein levels accumulate at a larger cell volume in disomic cells than in euploid cells. Cln3 levels are sensitive to multiple growth and nutrient cues, but the exact inputs of regulation of cell cycle entry upstream of Cln3 are poorly understood. Future work will need to elucidate the exact mechanisms controlling Cln3 accumulation in euploid cells before we can understand how aneuploidy is altering these pathways. It is possible that nutrient sensing is impaired or protein synthesis rates are decreased, leading to a G1 delay. Or, perhaps proteolysis is impaired causing cell cycle delays due to delayed proteolysis of key cell cycle regulators. Additionally, other cellular stresses are able to delay cells in G1 (Herrero et al., 2003), so
it is possible that the G1 delay is due to a secondary cellular stress response to aneuploidy. Future work is needed to address these possibilities.

A G1 delay has been seen in other models of aneuploidy, both in fission yeast (Niwa et al., 2006) and human cell lines (Stingele et al., 2012). Many other models of aneuploidy such as human fibroblasts trisomic for chromosome 21 (Segal and McCoy, 1974) and mouse embryonic fibroblasts trisomic for chromosome 1, 13, 16, or 19 (Williams et al., 2008) exhibit proliferation defects, but the cause of the proliferation defect has not yet been explored. It is possible that the G1 delay seen in other aneuploid systems is also due to a delay in G1 cyclin accumulation, as we see is the case for disomic yeast; however, future work must be done to elucidate the molecular mechanisms of cell cycle delays in other systems.

I report in Chapter 2 that the G1 delay is due to the presence of excess gene products produced by the extra chromosome, as addition of chromosome-sized amounts of DNA that do not produce functional gene products do not delay cell cycle entry. This leads us to the question of whether the proliferation defect seen in aneuploid yeast is due to a change in copy number of a few, particularly harmful, dosage-sensitive genes on the extra chromosome, or whether the proliferation defect is due to the cumulative effect of many genes on the extra chromosome that provide no proliferation defect when in excess individually. Recent work in our lab has addressed this question (Bonney et al., 2015). This work showed that the proliferation defect seen in disomic yeast is due to the presence of many genes in an extra copy, as the presence of dosage-sensitive genes in an extra copy in an otherwise euploid strain was not able to phenocopy the proliferation defect seen in the disomic yeast cells for most disomes tested. These data are in line with
the fact that the severity of the proliferation defect in disomic yeast correlates with size of the extra chromosome and thus the number of extra genes, as chromosomes are densely packed in yeast.

Aneuploidy and cell growth

In Chapter 2, I also establish that disomic yeast cells exhibit a growth defect as measured by a defect in cell volume accumulation. I demonstrate that this growth defect is not due to diminished amino acid pools, as amino acid levels in disomic cells are comparable to euploid levels. Nor is the growth defect due to major translational defects or decreased translational efficiency of particular genes, as both polysome profiling and translational efficiency measurements appeared normal in disome XVI (a disome that exhibits a growth defect yet no cell cycle defect). While this work showed that translational efficiency of specific genes was not altered, it is possible that the overall translational capacity could be decreased in disomic yeast, thus leading to an equal reduction of translation of all genes, which our assay would not have detected. Perhaps total ribosome levels are altered in aneuploid cells, leading to a decreased capacity for protein synthesis. When ribosomal components are imbalanced due to genomic imbalances, uncomplexed subunits may be degraded, leading to lower levels of functional ribosomes (Strunk and Karbstein, 2009). Ribosome subunits can also be targeted for degradation by the proteasome (Strunk and Karbstein, 2009); perhaps impairment of the protein quality control network in aneuploid cells interferes with ribosome assembly. Thus, translational capacity could be limiting in aneuploid cells due to mis-regulation of ribosome assembly, potentially causing the growth defect seen in
disomic cells. Further work is needed to investigate the possibility that absolute ribosome levels and regulation of ribosome assembly may be altered in aneuploid cells.

**Acute aneuploidy and the cell cycle**

In Chapter 3, I report that aneuploid cells generated by chromosome missegregation exhibit cell cycle delays as early as the second division following chromosome missegregation. As seen previously in Chapter 2 and in other models of aneuploidy, disomic yeast exhibit a G1 delay. We also report G1 delays for monosomic yeast cells. Additionally, we show that disomes, trisomes, and monosomes exhibit delays in other areas of the cell cycle.

Disomes, trisomes, and monosomes are delayed from bud emergence to anaphase onset, which mainly comprises S phase and metaphase in budding yeast. It is possible that DNA replication is delayed in these aneuploid cells, as recent work in our lab has demonstrated that DNA replication initiation and elongation are impaired in some aneuploid yeast (Blank et al., 2015). Aneuploid yeast cells may also be delayed in metaphase, potentially due to activation of the DNA damage checkpoint or the spindle assembly checkpoint. Aneuploid cells have been shown to have increased genomic instability (Blank et al., 2015; Sheltzer et al., 2011; Zhu et al., 2012), so it is possible that the DNA damage checkpoint is being activated in these cells, delaying the cells in metaphase. To address this possibility, it would be interesting to further investigate the delay from bud emergence to anaphase onset using more precise cell cycle markers to determine if the delay is in S phase, metaphase, or both stages. A delay could also potentially occur in G2, although G2 is not a major control point in the *S. cerevisiae* cell
cycle and G2 length is generally quite short in budding yeast. If the delay is in S phase, aneuploid cells could be sent through a round of cell division in which DNA replication is suppressed to confirm that the delay is due to slower replication of DNA. If the delay is found to be in metaphase, metaphase duration could be measured in aneuploid cells deficient for the checkpoint components \textit{RAD9} and \textit{MAD2} to determine if the delay is checkpoint-dependent. Elucidating the mechanisms by which aneuploidy delays cell cycle progression during all stages of the cell cycle is important for understanding the effects of aneuploidy on cellular physiology.

I also demonstrate in Chapter 3 that acutely aneuploid disomes, trisomes, and monosomes exhibit fitness defects via colony formation that correlate with the amount of genomic imbalance. In Chapter 2 I also report that the G1 delay observed in disomic yeast correlates with the size of the extra chromosome. Previous studies have also shown a correlation between the severity of the proliferation defect in aneuploid cells and the size of the extra chromosome (Stingele et al., 2012; Torres et al., 2007). The fitness defects observed in all aneuploid cells studied suggest that genomic imbalance affects cell fitness, whether it is gain of a chromosome or loss. Therefore, it is likely not simply the amount of extra gene products that delays cell cycle progression, as then we would expect monosomes to not exhibit cell cycle delays. Instead, cell cycle delays are likely fueled by an increasing imbalance of gene products as the genomic imbalance increases. Further work is needed to investigate exactly how imbalance of gene products results in aneuploid phenotypes such as cell cycle delays and proteotoxic stress (see below). Additionally, haploinsufficiency likely also contributes to the fitness defects observed in monosomes. Mechanisms to upregulate genes on a monosomic chromosome to restore
accurate gene dosage are rare (Springer et al., 2010), suggesting that cells have little to no recourse upon loss of a chromosome.

**Aneuploidy and proteotoxicity**

Increased proteotoxic stress is common in aneuploid cells (Oromendia et al., 2012; Santaguida et al., 2015; Stingele et al., 2012; Tang et al., 2011; Torres et al., 2007). In Chapter 3, I investigate the accumulation of protein aggregates following chromosome missegregation. I find that protein aggregates accumulate immediately following chromosome missegregation, and aggregation is not dependent on cell cycle progression. Interestingly, we demonstrate that the percentage of cells that accumulate protein aggregates correlates with the amount of genomic imbalance in aneuploid cells.

Also of note, monosomes accumulate protein aggregates at similar levels as trisomes, suggesting that protein aggregation results from exhaustion of the protein quality control network due to the increased need to sequester and degrade members of protein complexes encoded on the imbalanced chromosomes that are being produced at a stoichiometric imbalance from their binding partners. Consistent with this idea is the fact that aneuploid cells downregulate members of protein complexes (Dephoure et al., 2014; Stingele et al., 2012; Torres et al., 2007, 2010), suggesting that protein complex members are particular targets for protein degradation.

Future work will need to investigate the hypothesis that stoichiometric imbalances cause exhaustion of the protein quality control network and lead to protein aggregation in aneuploid cells. This hypothesis could be tested by overexpression of protein complex subunits in a euploid cell to generate stoichiometric imbalances. Alternatively, protein
complex members found in excess on disomic chromosomes could be returned to balance either via deletion of genes encoding protein complex members on the extra chromosome or by expression of binding partners to restore stoichiometric balance. Similar experiments could be done in monosomes to restore stoichiometric imbalance by expressing additional copies of protein complex components that were imbalanced due to chromosome loss.

**Population heterogeneity in aneuploid cells**

In Chapter 3, I report that populations of aneuploid cells exhibit increased heterogeneity in cell cycle progression compared to a euploid population. All monosomes, nearly all disomes, and nearly all trisomes demonstrate increased population heterogeneity in at least one stage of cell cycle progression compared to euploid cells, as nearly all of the aneuploid populations have variances for at least one cell cycle stage that are significantly higher than the variance seen in a euploid population. Future work is needed to further investigate population heterogeneity in aneuploid cells. It will be important to determine whether population heterogeneity is only present following chromosome missegregation, or whether it persists in aneuploid cells after many generations. To explore the persistence of the increased variance, assays to assess transcriptional noise can be performed in both acute and chronic aneuploids.

Phenotypic heterogeneity may give aneuploid cells a chance at survival in different environments. Recent work has shown that karyotypically heterogeneous populations of aneuploid cells exhibit a wide range of phenotypes, particularly while under stress, and that this range of phenotypes is beneficial in adaption of the population
to different environments (Chen et al., 2015). Additionally, another recent study showed that transient aneuploidy in *C. albicans* produces phenotypic heterogeneity that gives a karyotypically heterogeneous population a high degree of diversity and thus greater adaptability to different conditions (Hickman et al., 2015). Both of these studies report phenotypic heterogeneity in karyotypically diverse populations of aneuploid cells. To our knowledge, this thesis is the first report that a karyotypically homogeneous population of aneuploid cells may exhibit heterogeneous phenotypic variations.

**Developing a new model to study the onset of aneuploidy**

*Benefits of targeted chromosome missegregation*

Current cellular models of aneuploidy are either chronic defined aneuploidies, meaning cells have a defined karyotype and the cells have had that karyotype for many generations, or are acute random aneuploidies, where cells missegregate chromosomes randomly to generate populations of cells with heterogeneous karyotypes. In Chapter 3, I report the development and characterization of a new model of aneuploidy in budding yeast that fills the need for a model of acute defined aneuploidies. This system allows for the induction of chromosome missegregation in a targeted manner, generating populations of cells with homogeneous karyotypes. Thus, using this model I am able to study aneuploidy onset in a systematic way, with a wide range of defined karyotypes.

The development of a model of aneuploidy that uses targeted missegregation of chromosomes allows us to study karyotypes that were previously difficult to generate and maintain. First, this system allows for the generation of monosomes, which have not previously been studied in a systematic way as monosomy is often lethal and it is difficult
to select for chromosome loss to maintain monosomy. In Chapter 3, I report the first systematic study of monosomy, analyzing a wide range of karyotypes from loss of a single chromosome to loss of 6 chromosomes in budding yeast.

Second, this system can be utilized to generate strains of complex karyotypes. Aneuploidies of complex karyotypes are often difficult to generate due to the limitations of selectable markers in budding yeast and the severity of proliferation defects of complex aneuploids. In Chapter 3, I utilized this novel model of aneuploidy to generate aneuploid cells via missegregation of up to 8 chromosomes, and I characterized strains that had missegregated up to 6 chromosomes. A previous study in budding yeast attempted to generate strains of complex karyotypes via triploid meiosis (Pavelka et al., 2010), but these strains are unstable without selection for aneuploidy (Sheltzer et al., 2011). As I am only analyzing complex aneuploid cells for a few divisions following aneuploidy onset and I can monitor chromosome missegregation using GFP dots, the karyotypic stability of complex aneuploid strains does not pose a problem in this study.

**Future areas of study using the acute defined aneuploidy system**

The system of targeted chromosome missegregation described in Chapter 3 can be utilized to study a wide variety of interesting questions related to chromosome missegregation. In this section, I will raise a few interesting areas of study as examples of topics that can be addressed using this system, although certainly many more questions could be addressed.

First and foremost, the acutely aneuploid system can be utilized to study the development of other aneuploidy-associated phenotypes. In Chapter 3, I addressed the
development of cell cycle delays and accumulation of protein aggregates following chromosome missegregation; however many additional aneuploidy-associated phenotypes have yet to be explored using this system. For example, aneuploid cells have been shown to exhibit increased genomic instability (Blank et al., 2015; Sheltzer et al., 2011; Zhu et al., 2012). Future work could investigate the increase in genomic instability following chromosome missegregation. For instance, markers of DNA damage such as Rad52-GFP could be used to monitor DNA damage following the onset of aneuploidy.

The systematic study of monosomy reported in Chapter 3 raises the interesting question that genomic imbalance is responsible for many of the phenotypes seen in aneuploid cells. Previous studies of aneuploid cells focused on chromosome gain; therefore, it was difficult to determine whether phenotypes were due to genomic imbalance or simply due to the excess amount of protein produced from the additional chromosome(s), as previous studies have shown the extra chromosome(s) in aneuploid cells to be transcribed and translated (Dephoure et al., 2014; Stingele et al., 2012; Torres et al., 2007, 2010). This system can be used to address the question of how genomic imbalance causes aneuploidy-associated phenotypes as the system allows for the study of chromosome loss as well as chromosome gain. Future work can address questions of genomic imbalance by studying haploid and diploid cells with the same genomic imbalances. For example, 2N+2 cells can be generated where a diploid cell missegregates both homologous chromosomes, generating a cell with a karyotype of 2N+IV+IV, in the example of chromosome IV. A cell with the karyotype of 2N+IV+IV would have the same genomic imbalance as a cell with a karyotype of N+IV; therefore, comparison of the phenotypes of these two karyotypes—which can both easily be generated with this
system—may lead to insights into how genomic imbalance affects cellular physiology.

Generally, disomic cells exhibit greater fitness defects than trisomic cells, supposedly due to the decreased genomic imbalance in trisomy. If the phenotype of a 2N+IV+IV cell phenocopies an N+IV cell more than a 2N+IV cell, that would suggest that genomic imbalance is likely at the heart of what causes decreased cellular fitness in aneuploid cells. It will be important for future work to address the question of genomic imbalance.

Additionally, this system could be used to investigate the question as to whether the cell has a memory for ploidy following chromosome missegregation and whether the gain or loss of chromosomes alters aneuploidy phenotypes or kinetics of phenotype development. For instance, would a diploid cell that loses 8 chromosomes (half of the budding yeast chromosomes), generating a 2N-8 cell, give the same phenotype as a haploid cell that has gained the opposing 8 chromosomes, generating an N+8 cell? Would these cells exhibit the same phenotype because they have the same karyotype, or would they exhibit different karyotypes because one cell experienced chromosome gain in a haploid background while the other cell experienced chromosome loss in a diploid background? Additionally, disomic cells could be generated via the missegregation of 15 of the 16 chromosomes from a diploid background to see if these cells behave similarly to disomes. Particularly, it would be interesting to characterize the kinetics of phenotype development in a 2N-15 strain versus the corresponding N+1 strain, as the kinetics of phenotype development may be quite different for massive chromosome loss compared to a single chromosome gain. These questions were previously difficult to address without the ability to target missegregation of multiple, defined chromosomes.
In future studies, it would also be interesting to investigate the nuclear organization of chromosomes in aneuploid cells, as this may give insight into the defects we see in aneuploid cells. In euploid cells of both prokaryotes and eukaryotes, nuclear organization at both the spatial and temporal levels are key for regulation of gene expression (reviewed in Cavalli and Misteli, 2013). Recent work in fission yeast has shown cell cycle-dependent changes in nuclear organization (Grand et al., 2014). It would be interesting to investigate the organization of chromosomes in the nucleus for disomes, trisomes, and monosomes following chromosome missegregation. Altering the number of chromosomes in the nucleus—whether through chromosome gain or loss—is likely to produce drastic changes in nuclear chromosome arrangement. As chromosome arrangement is key for gene regulation, it is possible that changes in nuclear chromosome conformation are altering gene expression patterns and contributing to aneuploid phenotypes.

**Concluding remarks**

Aneuploidy alters seemingly all aspects of cellular function. In this thesis, I have investigated the cause of the G1 delay in disomic yeast to better understand how aneuploidy alters the regulation of cell cycle progression. Additionally, I have generated and characterized a novel model of aneuploidy to monitor the kinetics of phenotype development in aneuploid cells and to systematically study monosomic aneuploid cells. I hope future work will utilize this novel model of aneuploidy to investigate a wide range of open questions, particularly those of genomic imbalance, and to further our knowledge as to how aneuploidy affects cellular physiology.
REFERENCES


