The Transient Inactivation of the Master Cell Cycle Phosphatase Cdc14 Causes Genomic Instability in Diploid Cells of *Saccharomyces cerevisiae*

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ABSTRACT Genomic instability is a common feature found in cancer cells. Accordingly, many tumor suppressor genes identified in familiar cancer syndromes are involved in the maintenance of the stability of the genome during every cell division and are commonly referred to as caretakers. Inactivating mutations and epigenetic silencing of caretakers are thought to be the most important mechanisms that explain cancer-related genome instability. However, little is known of whether transient inactivation of caretaker proteins could trigger genome instability and, if so, what types of instability would occur. In this work, we show that a brief and reversible inactivation, during just one cell cycle, of the key phosphatase Cdc14 in the model organism *Saccharomyces cerevisiae* is enough to result in diploid cells with multiple gross chromosomal rearrangements and changes in ploidy. Interestingly, we observed that such transient loss yields a characteristic fingerprint whereby trisomies are often found in small-sized chromosomes, and gross chromosome rearrangements, often associated with concomitant loss of heterozygosity, are detected mainly on the ribosomal DNA-bearing chromosome XII. Taking into account the key role of Cdc14 in preventing anaphase bridges, resetting replication origins, and controlling spindle dynamics in a well-defined window within anaphase, we speculate that the transient loss of Cdc14 activity causes cells to go through a single mitotic catastrophe with irreversible consequences for the genome stability of the progeny.

KEYWORDS Cdc14; *Saccharomyces cerevisiae*; gross chromosomal rearrangements; aneuploidy; caretaker genes

GENOMIC instability is one of the hallmarks of cancer cells. Many genomic alterations result in gene dose variation including deletions/duplication, gross chromosomal rearrangements (GCRs), and aneuploidy; loss of heterozygosity (LOH) associated with mitotic recombination is also observed (Hanahan and Weinberg 2011). In wild-type cells, specialized mechanisms exist, such as cell cycle checkpoints and DNA repair activities, which suppress genome instability. Genes encoding for proteins that protect cells

from cancer-related genomic instability are referred to as caretakers. Many of the tumor suppressor genes identified in familiar cancer-prone syndromes are caretakers. Although caretakers are not directly responsible for the decision of a cell to divide, their loss leads cells to rapidly accumulate genomic aberrations and mutations that potentially result in disorganization of cell division and their subsequent transformation into cancer cells (Kinzler and Vogelstein 1997; van Heemst *et al.* 2007). For example, mutations in genes involved in DNA repair pathways such as nonhomologous end joining, homologous recombination, mismatch repair, base excision repair, and nucleotide excision repair have been shown to lead to the accumulation of mutations within the genome that significantly increase the risk of carcinogenesis (Negrini *et al.* 2010; Aguilera and García-Muse 2013).

Whereas the effects of the permanent loss of caretakers have been extensively studied, less is known about the consequences that a transient loss of function of a caretaker might have on the cell fate. One study, however, reported that the transient loss of the helicase Bloom syndrome

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protein (BLM) activity leads to a significant increase in LOH events throughout the genome in mouse cell lines, pointing out a role of the BLM dysfunction in the early steps of tumorigenesis (Yamanishi *et al.* 2013). In addition, it has been also shown that the transient inactivation of the BRCA2- and CDKN1A(p21)-interacting protein BCCIP is sufficient to promote tumorigenesis. Permanent loss of BCCIP does not result in tumorigenesis. This apparent discrepancy is consistent with the observation that the activity of BCCIP is required for the later steps of tumor progression (Huang *et al.* 2013).

The yeast *Saccharomyces cerevisiae* has been used as a model organism to understand the molecular functions of caretakers. Since yeast cells can tolerate most loss-of-function caretaker genes, most studies of the effects of these genes on genome stability have been carried out using strains with permanent inactivating mutations. Nevertheless, it is presumed from the molecular function that many essential genes related to the cell cycle progression must be caretakers as well. Although their essential role for cell division precludes the analysis of strains with null mutations, transient inactivation of the protein function might still be possible and might have destabilizing effects on the genome. One clear candidate for such a gene in yeast is the cell cycle phosphatase *CDC14*, which is essential for the mitosis-to-G₁ transition (Stegmeier and Amon 2004), and whose transient loss in haploids leads to irresolvable anaphase bridges (Machín *et al.* 2006; Quevedo *et al.* 2012). *CDC14* was first identified by Culotti and Hartwell (1971) in their screening for genes that regulate the cell cycle in the yeast *S. cerevisiae*. This protein belongs to a superfamily of dual-specific phosphatases highly conserved during evolution, from yeasts to humans, that preferentially dephosphorylate cyclin-dependent kinase (CDK) targets (Mocciaro and Schiebel 2010).

The clearest molecular role for *Cdc14* in the maintenance of the yeast genome is related to its function in resolving sister-chromatid linkages that preclude their segregation during anaphase. Indeed, timely *Cdc14* activity is essential to ensure the proper segregation of the ribosomal DNA array (rDNA) (D'Amours *et al.* 2004; Stegmeier and Amon 2004; Sullivan *et al.* 2004; Torres-Rosell *et al.* 2005; Machín *et al.* 2005, 2006). The rDNA is a highly repetitive region of ~150 copies of 9-kb repeats located on the right arm of the chromosome XII (Petes 1979). In the past decade, it has been shown that the rDNA requires specialized mechanisms to ensure its proper segregation during mitosis. Thus, its heterochromatin-like structure, which is silenced for the RNA polymerase II-dependent transcription (Bryk *et al.* 1997; Smith and Boeke 1997), and its high transcription rate by the RNA polymerases I and III generate linkages that must be removed before segregation (Machín *et al.* 2006; Clemente-Blanco *et al.* 2009). To ensure the efficient removal of these linkages, *Cdc14* switches off the rDNA transcription by dephosphorylating the RNA polymerase I regulatory subunit *Rpa43*, which in turn allows the loading of condensin and *Top2* onto

the rDNA. These steps are required for the condensation of the rDNA and the removal of catenations and other segregation constraints within this locus (D'Amours *et al.* 2004; Sullivan *et al.* 2004; Wang *et al.* 2004; Machín *et al.* 2005, 2006; Tomson *et al.* 2006; Clemente-Blanco *et al.* 2009; Dulev *et al.* 2009). In addition, it has also been shown that *Cdc14* plays a role in the segregation of telomeres containing Y' elements through inhibition of the RNA polymerase II-dependent transcription (Clemente-Blanco *et al.* 2011).

Two *Cdc14* proteins with several isoforms, *Cdc14A* and *Cdc14B*, have been identified in mammals (Li *et al.* 1997). In addition, a third gene closely related to *CDC14B*, named *CDC14C*, has been identified in hominoids (Rosso *et al.* 2008). Apart from their conservation in sequence, both human *CDC14A* and *CDC14B* phosphatases (hereafter, referred to as *hCDC14A* and *hCDC14B*, respectively) have been shown to rescue mutants in orthologous genes in budding and fission yeast (*CDC14* and *flp1/clp1*, respectively) (Li *et al.* 1997; Vázquez-Novelle *et al.* 2005). The *hCdc14A* phosphatase is localized mainly on the centrosome during interphase and in the cytoplasm during mitosis (Kaiser *et al.* 2002; Mailand *et al.* 2002) and plays a role in counteracting CDK-mediated phosphorylation at the G₂/M transition (Vázquez-Novelle *et al.* 2010; Ovejero *et al.* 2012). Its overexpression leads to a premature centrosome splitting and the formation of a supernumerary mitotic spindle (Kaiser *et al.* 2002; Mailand *et al.* 2002), whereas its downregulation causes an impaired centrosome separation and nonproductive cytokinesis (Mailand *et al.* 2002). Thus, deregulation of *hCdc14A* leads to centrosome malfunction and defects in cytokinesis, aberrant situations expected to compromise genome stability. In addition, it has also been reported that cells lacking *hCdc14A* activity have inefficient DNA damage repair, even in the absence of DNA-damaging agents (Mocciaro *et al.* 2010), which is also consistent with a role of *hCdc14A* in preserving genomic integrity. On the other hand, *hCdc14B* is located at the nucleolus during interphase and is released during mitosis (Kaiser *et al.* 2002; Mailand *et al.* 2002). Several studies assigned several different roles for *hCDC14B*, including assembly and stabilization of the mitotic spindle (Cho *et al.* 2005), the activation of the G₂ DNA damage checkpoint (Bassermann *et al.* 2008), efficient DNA damage repair (Mocciaro *et al.* 2010; Wei *et al.* 2011), nuclear organization (Nalepa and Harper 2004), centriole duplication (Wu *et al.* 2008), mitotic exit (Dryden *et al.* 2003), regulation of the M-to-G₁ progression (Rodier *et al.* 2008; Tumurbaatar *et al.* 2011), and the control of the transcription of cell cycle regulators (Guillamot *et al.* 2011).

In previous studies, we and others showed that loss of *Cdc14* in budding yeast haploid cells by means of temperature-sensitive alleles such as *cdc14-1* leads to a cell cycle block at the anaphase-to-telophase transition (Culotti and Hartwell 1971), with most of the genome segregated except for the rDNA array. The rDNA array remains uncondensed and unresolved, forming a bridge between the daughter cells (Torres-Rosell *et al.* 2004; Machín *et al.* 2005). We reported

that lowering the temperature back to permissive conditions (25°) provides enough *Cdc14* activity to exit from mitosis and enter a new cell cycle. However, this re-entry into the cell cycle occurs even in the 50% of the cells that fail to properly resolve the rDNA-associated bridge. This bridge eventually breaks, giving rise to two daughter cells with different genomes (Machín *et al.* 2006; Quevedo *et al.* 2012). Importantly, one of the daughter cells often ends up losing essential genomic information and, therefore, is unviable. The above-mentioned effects of *Cdc14* on the integrity of the chromosome XII (cXII) in haploid cells prompted us to further study the consequences that the transient loss of this phosphatase has on the genomic integrity using diploid yeast cells. One rationale for this approach is that diploids would be more tolerant of deletions and/or chromosome loss than haploids. In addition, as described below, using diploids created by mating sequenced diverged haploids, we could detect LOH events generated by interhomolog recombination. Here, we show that a transient loss of *Cdc14* in diploids leads to a frequency of inviability that is higher than that observed in haploids. The surviving daughter cells have both elevated levels of GCRs and aneuploidy. Although these events affected most chromosomes, the rDNA-bearing cXII was most frequently affected. These observations suggest a role of *Cdc14* in preserving genomic stability similar to that of a caretaker gene.

Materials and Methods

Yeast strains and culture conditions

FM1860 (S288C genetic background) is the diploid strain resulting from the FM322 × FM1275 mating. The haploid strain FM322 [genotype: *MATa bar1Δ leu2-3,112 ura3-52 his3-Δ200 trp1-Δ63 ade2-1 lys2-801 pep4 cXII(coordinate 1061 Kb)::tetOx224 TetR-YFP cdc14-1 NET1-CFP*] has been described before (Quevedo *et al.* 2012). FM1275 is a *MATα* haploid isogenic to FM322 except for the *tetO* and *TetR-YFP* insertions and the *NET1-CFP* tagging. We examined the effects of the *cdc14-1* mutation on genome stability in a diploid strain (FM1468) that was heterozygous for ~55,000 single-nucleotide polymorphisms (SNPs). For the construction of this diploid, FM1142 [YJM789 genetic background (Wei *et al.* 2007)] and FM1184 (W303a genetic background) strains were made by introducing the *cdc14-1* allele into the haploid strains JSC21 [*MATα ade2-1 ura3 gal2 ho::hisG can1Δ::natMX4 cIV(coordinate 1510386 bps)::SUP4-o*] and JSC12 [*MATa leu2-3,112 his3-11,15 ura3-1 ade2-1 trp1-1 can1Δ::natMX4 RAD5 cIV(coordinate 1510386 bps)::kanMX4/can1-100*], respectively (St. Charles and Petes 2013). Strains FM1142 and FM1184 were mated to make the diploid FM1468, which was used in the SNP microarray experiments. The *CDC14/CDC14* diploid with the W303a × YJM789 hybrid background (FM2013) was made by mating JSC12 with JSC21. We also constructed *CDC14/CDC14* and *cdc14-1/cdc14-1* homozygous diploids that had either the W303a

background or the YJM789 background. For this purpose, we performed mating switches of the *CDC14* haploids JSC12 and JSC21 and the *cdc14-1* haploids FM1142 and FM1184. Mating-type switching was performed by transforming each strain with a centromeric plasmid carrying the *HO* endonuclease under the control of the *GAL1* promoter. The mating-type switch was induced by briefly incubating the transformed cells in galactose as previously reported (Herskowitz and Jensen 1991). We then mated the isogenic strains of opposite mating types. Diploid colonies were selected and tested for the presence of both *MATa* and *MATα* by PCR as well as for their sporulation capability. FM2026 and FM2030 are *CDC14/CDC14* and *cdc14-1/cdc14-1* diploids of the W303a × W303a background, respectively. FM2027 and FM2028 are *CDC14/CDC14* and *cdc14-1/cdc14-1* diploids of the YJM789 × YJM789 background, respectively.

All strains were grown on YPD at 25° with moderate shaking (200 × g) until the culture reached early log phase (OD₆₆₀ ~0.3). Cells were incubated at 37° for 3 hr to inactivate the protein Cdc14-1. To restore *Cdc14* activity, we shifted the temperature back to 25°. Since *Cdc14* inactivation for 3 hr causes all cells to block at telophase, and its subsequent reactivation allows entry into a new synchronous G₁, we termed this transient temperature shift to 37° a “*cdc14* block-and-release experiment.” To determine cell survival in the *cdc14-1* block-and-release experiments, we spread a known number of cells (counted by a hemocytometer) on YPD plates just before and 2 hr after the *cdc14-1* block-and-release. The plates were then incubated at 25°. Survival rates were calculated as the ratio between the number of colony-forming units (CFU) and the number of plated cells. To follow the long-term fate of daughter cells after the *cdc14-1* block-and-release, we harvested individual dumbbell (*cdc14*-blocked) cells with a Singer Sporeplay tetrad microdissector. Cell micro-manipulation was carried out at the time of the *cdc14-1* block (3 hr at 37°), and dumbbells were placed at defined positions on a YPD plate, which was then incubated at 25° for 3 days. To follow up the short-term fate of these dumbbells, we photographed them at several time points after the shift from 37° to 25°.

Fluorescence microscopy

In FM1860, the location of the right end of one cXII homolog within cells could be visualized by binding of the yellow fluorescent protein (YFP)-fused TetR to *tet* operator sequences integrated near the *Saccharomyces* Genome Database (SGD) coordinate 1061 kb. The fluorescent TetR-YFP protein was visualized using a Leica DMI6000 fluorescence microscope as described previously (Quevedo *et al.* 2012). Cells were treated with 1 μg/ml 4',6-diamidino-2-phenylindole in 1% Triton X-100 to visualize the nuclear masses. A series of 20 z-focal plane images were collected using a ×63/1.30 immersion objective and an ultrasensitive DFC 350 digital camera to better see the tetO fluorescent dot. Images were processed with the AF6000 software (Leica).

Fluorescence-activated cell sorting analysis

Fluorescence-activated cell sorting analysis (FACS) analysis was used to follow single-cell DNA content. Samples were treated and analyzed using a BD FACScalibur machine as previously described (Quevedo *et al.* 2012; García-Luis and Machín 2014). Briefly, 300 μ l of culture was taken and mixed with 900 μ l of 100% ethanol. Cells were then pelleted and resuspended in $1\times$ saline-sodium citrate (SSC) buffer with 0.01 mg/ml of RNaseA and incubated overnight at 37°. After that, 50 μ l of $1\times$ SSC with 1.2 mg/ml of proteinase K was added and incubated at 50° for 1 hr. Finally, 500 μ l of $1\times$ SSC with propidium iodide at 3 μ g/ml was added and incubated at room temperature for 1 hr.

Pulsed-field gel electrophoresis and Southern-blot analysis

Agarose plugs containing DNA samples were prepared as described previously (Quevedo *et al.* 2012). Pulsed-field gel electrophoresis (PFGE) was performed using a CHEF (contour-clamped homogenous electric field) DR-III system (Bio-Rad) in a 0.8% agarose gel in $0.5\times$ Tris-Borate-EDTA buffer. Gels were run at 12° for 40 hr at 6 V/cm, with an initial switching time of 80 sec, a final switching time of 150 sec, and an angle of 120°. Chromosomes were visualized by staining with 0.5 μ g/ml ethidium bromide. The separated chromosomal DNA molecules were transferred to a nylon membrane and hybridized with digoxigenin-labeled 1.2- to 1.5-kb-long probes. Probes were labeled by polymerase chain reaction (PCR) using the PCR DIG Probe Synthesis Kit (Roche Applied Science). For cXII, an rDNA-specific probe was used; other probes were used to detect chromosome X (near SGD coordinate 300 kb) and chromosome I (near SGD coordinate 175 kb). The primers used for the rDNA-specific probe were 5'-CTGGTAGATATGCGCGCAACC-3' and 5'-CTTGTCTTCAACTGCTTTCGC-3'; 5'-CGAAAGACGC AAAGACCTTGCGGAGAGAGCTTCAGAGCTGG-3' and 5'-GCTC TGATTCTGACTCTAACTCCAGTTCGGA CTCCGTATCGG-3' were used to detect the cX; and 5'-AAACACTGAAAAG CACATGC-3' and 5'-TCTTGAGCACGA ACTCGAAGCGTTTC-3' were used to detect the cI. Digoxigenin detection and stripping were done using the DIG Luminescent Detection Kit (Roche Applied Science). Quantifications of stained gels and Southern blots were performed using ImageJ (<http://imagej.nih.gov/ij/>).

SNP microarray analyses

As discussed in the text, in the diploid FM1468, mitotic crossovers on chromosome IV can be detected by the formation of red/white sector colonies. Pink/red and pink/white sector colonies were also analyzed. Purified colonies were derived from each sector by restreaking. DNA was then isolated from single-colored colonies to perform the SNP microarray analyses as described previously (St. Charles *et al.* 2012). In brief, cells derived from each sector colony were grown to stationary phase. Cells were embedded in low-melting-point agarose blocks for

the DNA extraction. After this step, DNA derived from the sectors was labeled with Cy5-dUTP, whereas the control DNA sample (extracted from the corresponding strain grown at the permissive temperature) was labeled with Cy3-dUTP. The two labeled DNA samples were then competitively hybridized to Agilent microarrays containing SNP-specific oligonucleotides. The level of hybridization to each labeled sample was determined by scanning the microarray at wavelengths of 635 and 532 nm using a GenePix scanner and GenePix Pro software (for a detailed description of the procedure, see St. Charles *et al.* 2012). This procedure allowed us to detect genomic regions of FM1468 that had undergone LOH, deletion, or duplication. Microarray data are available at the Gene Expression Omnibus (<http://www.ncbi.nlm.nih.gov/geo/>) with the accession no. GSE68530.

Results

After a transient loss of Cdc14, the frequency of survival and the segregation of chromosome XII are worse in diploids than in haploids

We previously showed that in haploid cells that had a transient loss of *Cdc14*, the structural integrity of cXII was compromised (Quevedo *et al.* 2012). In this report, we examine the effects of transient loss of *Cdc14* on genome integrity in a diploid strain. We employed an isogenic diploid strain (FM1860) in which one cXII homolog includes the *tetO* array near the right arm telomere. The *tetO* array is bound by a TetR-YFP fusion protein, allowing us to monitor the location of the right end of cXII by fluorescence microscopy. Similar to its haploid counterpart, the diploid strain also was fully blocked at telophase at 37° and resumed the cell cycle after being shifted back to 25° (*cdc14* block-and-release experiment) (Quevedo *et al.* 2012). Thus, >95% of *cdc14-1* diploid cells were “dumbbells” (single-budded cells where the size of the bud equaled that of the mother) after 3 hr at 37°. We filmed 105 dumbbells on YPD plates after shifting back the temperature to 25° and found that in 97% of them both daughter cells budded again (Figure 1A). Importantly and as previously observed in haploids, most daughter cells remained together after rebudding, becoming “foursomes,” which greatly facilitated cytological analysis. To our surprise, we observed correct cXII segregation for the labeled homolog in ~25% of the telophase blocks (3 hr at 37°, the “arrest”) and in ~30% of the foursomes 2 hr after the shift back to 25° (the “release”). Assuming that the homolog without the *tetO* array has the same segregation properties as the labeled homolog, we estimate that both homologs were properly segregated in <10% of cells 2 hr after the release (0.3×0.3) (Figure 1B). Previously, we studied the segregation in the parental haploid strain and reported that the segregation of the cXII was successful in ~40% of cells 3 hr after the release (Quevedo *et al.* 2012). Therefore, we conclude that the segregation of the cXII in diploids is significantly worse in diploids than in haploids.

Figure 1 Cell survival and segregation of cXII in *cdc14-1* strains is worse in diploids than in haploids. (A) Representative pictures of cells (*cdc14-1* homozygous diploid FM1860, S288C background) at various times after release from the 37° temperature block ($t = 0'$ is after 3 hr at 37°; the other time points are after shifting back to 25°). (B) Estimated segregation of chromosome XII homologs at the time of the *cdc14-1* block (left bar) and 2 hr after the block-and-release procedure (right bar) in FM1860. (C) Survival frequency estimated as number of CFU vs. number of cells plated 2 hr after the release (note that both dumbbells and foursomes were counted as a single entity under the hemocytometer). Error bars represent the SEM of three independent experiments. (D) Representative pictures of colonies plated before (top) and 2 hr after the *cdc14-1* block-and-release (bottom). (E) Representative picture of a microcolony observed 3 days after the *cdc14-1* block-and-release.

Next, we determined long-term survival after passing a *cdc14* block-and-release. As in our experiments with haploids, attempts to separate either dumbbells at the *cdc14-1* block or foursomes 2 hr after the release by micromanipulation led to a technical loss of viability (Quevedo *et al.* 2012). Thus, we plated the cells without separating them and looked for their ability to form colonies. Each colony, therefore, indicates that at least one of the daughter cells was viable. We found that <50% of these diploid daughter pairs gave rise to a colony (Figure 1C). In contrast, ~75% of

the plated haploid pairs formed a visible colony. In addition, the colony size was highly heterogeneous for diploid survivors after the *cdc14* block-and-release (Figure 1D), with irregular borders observed in at least 5% of the colonies (data not shown).

The high frequency of very small diploid colonies after the *cdc14* block-and-release suggested the possibility that some of the daughter pairs might form microcolonies that never developed into visible colonies. To test this scenario, we micromanipulated dumbbells at the time of the *cdc14* block to defined positions on plates and examined these cells with a microscope after 3 days. As expected, ~50% of the plated cells formed a visible colony. Of the 50% that did not form a visible colony, around half of them formed a microcolony (Figure 1E).

Diploid *cdc14-1* cells are unable to complete the replication of the genome after the release

Haploid and diploid *cdc14-1* cells become foursomes after the block-and-release procedure (Figure 1) (Quevedo *et al.* 2012). To further characterize the transition between dumbbells and foursomes, we decided to study how diploid cells progress through the first cell cycle after the transient *Cdc14* loss, focusing on completion and synchrony of the most relevant cell cycle events. For this purpose, we repeated the *cdc14* block-and-release experiment in liquid cultures, collecting samples every 30 min to monitor (i) the budding pattern (Figure 2A), (ii) the number of nuclei (Figure 2B), (iii) the DNA content (Figure 2C), and the genomic integrity (Figure 2D). To monitor the budding pattern, we counted single cells, classifying them according to the following categories (Figure 2A): unbudded cells (0), single-budded cells (1), dumbbells (2), threesomes (3), foursomes (4), and chains of cells (>4). Since daughter cells remain together after the *cdc14* block-and-release, we defined the three-some and foursome categories, depending on whether just one or both daughter cells initiated a new budding event, respectively.

As mentioned above, most diploid cells are dumbbells after 3 hr at 37°. Upon reactivation of the *cdc14-1* gene, the percentage of dumbbells dropped (Figure 2A, red line). Concomitant with the disappearance of the dumbbell category, we observed an increase in the frequency of foursomes (~60% of cells by 3 hr; Figure 2A, blue line). The transition of diploids to foursomes occurs more slowly in diploids than in haploids. Although the transition begins at about the same time in haploids and in diploids (between 60 and 90 min after the release), in haploids, most cells were foursomes 120 min after the release (figure 2A in Quevedo *et al.* 2012). In contrast, the accumulation of foursomes in diploids was observed from 120 to 150 min onward after the release (Figure 2A). This difference could indicate a delay in the initiation of the replication in diploid compared with haploid cells. Interestingly, the percentage of dumbbells plateaued at ~20% from 150 min onward. Taking into account the kinetics of the other categories and the data shown in

Figure 2 Diploid cells are severely delayed in completing DNA replication and nuclear division after the transient loss of Cdc14. (A) Budding pattern observed after the release (time 0 indicates the switch from 37° to 25°). (Right) Photographs that show cell morphology for the various budding patterns. (B) Nuclei number per foursome observed during the release. (C) Flow cytometry analysis after the release; the 2N and 4N peaks are indicated. (D) PFGE of DNA samples during a *cdc14-1* block-and-release: ethidium bromide staining (leftmost picture) and Southern-blot analysis with three different chromosome-specific hybridization probes (as labeled at the bottom of each picture). The lane labeled "as." contains DNA isolated from asynchronous cultures of the *cdc14-1* strain before exposure to the restrictive temperature.

Figure 1A, the most likely interpretation of this plateau is that a small percentage of foursomes managed to eventually split apart into two dumbbells.

Notably, foursomes accumulated with just two nuclei and did not segregate the nucleus during the first 5 hr after the release (Figure 2B). This result suggests that daughter cells are largely delayed in their ability to enter a new anaphase, presumably because they have not completed an important cell cycle event. Indeed, we observed that most diploid cells

struggled to complete DNA replication after the transient *Cdc14* loss, contrary to what we reported for haploid cells (figure 2D in Quevedo *et al.* 2012). We examined DNA replication at the population level in two ways: by measuring the DNA content of the cells after the release by FACS analysis (Figure 2C) and by monitoring the structure of the chromosomes using PFGE (Figure 2D and [Supporting Information, Figure S1](#)). Thus, although >80% of cells initiated the replication of the genome, as judged by their

ability to begin budding, most of them were unable to complete it as indicated by the absence of a clear shift toward an 8N content, expected if the genome is duplicated (Figure 2C); note that most daughter cells remain together and, therefore, a G₁ pair of diploid daughters yields a 4N peak, whereas an 8N peak is expected if both daughter cells complete replication.

PFGE, followed by Southern analysis, can be used to determine whether yeast chromosomes are branched as a result of replication or recombination (Hennessy *et al.* 1991). When we visualized the separated chromosomes by ethidium bromide staining following release from the *cdc14-1* block, we found that all bands became fainter (leftmost part of Figure 2D); note that bands should have become stronger throughout the time course if chromosomes had been fully replicated. In addition, the larger chromosomes appear to have a larger drop in band intensity than the smaller chromosomes. To examine the effects on individual chromosomes, the intensities of the stained bands representing single chromosomes (IV, IX, XI, and XII) or small groups of similarly sized chromosomes (VII/XV, XVI/XIII, VIII/V, and XIV/II/X) were compared to the sum of the intensities of the smallest chromosomes (I, VI, and III). In addition, we normalized the band intensities to that observed at time 0 for each band. After this double normalization, we confirmed that the larger chromosomes had a stronger loss of intensity than the smaller chromosomes (Figure S1A). This effect was especially striking for the rDNA-bearing cXII.

To further examine the behavior of individual chromosomes, we performed a Southern-blot analysis using three different probes, one against the largest chromosome (cXII), one against the mid-sized chromosome X (cX), and one against the smallest chromosome I (cI). When hybridized to an rDNA-specific probe (Figure 2D, second image from left), the in-gel band signal for the cXII was almost completely gone at the 90 min time point, concomitant with an increase in the signal in the gel well (Figure 2D, Figure S1B, and Figure S2). By the 120-min time point, the in-gel band signal reappeared, but its intensity was much fainter than for the earlier time points (<10% relative to 0 and 30 min). A similar observation was made using a cX-specific probe, although the effect was more subtle (Figure 2D, third image from left; Figure S1B). In contrast, when we rehybridized the membrane against the smallest chromosome (cI), we did not observe a significant loss or gain in the in-gel signal (Figure 2D, rightmost image; Figure S1B). These observations suggest that chromosome replication, if initiated, was seriously compromised and that none of the chromosomes, including the smallest ones, were duplicated by 5 hr.

Gross chromosomal rearrangements and ploidy changes in the genomes of survivors

The colonies formed by survivors after the *cdc14-1* block-and-release were very variable in size. In addition, some of the colonies had scalloped edges, a property observed in some colonies derived from aneuploid strains (Jung *et al.*

2011; Tan *et al.* 2013). The possibility of aneuploidy is also suggested by the high frequency of nondisjunction observed for cXII (Figure 1B). As described above, the loss of in-gel chromosomal DNA is consistent with DNA breaks and the repair of these breaks by recombination during the block and release. We previously suggested that chromosome breaks on cXII would likely be generated in telophase in haploid *cdc14-1* cells as a consequence of the cXII anaphase bridge between sister chromatids. Such double-stranded DNA breaks (DSBs) would be expected to be one-ended. These DSBs could be repaired by break-induced replication (BIR) using the sister chromatid as a template or, in diploid strains, by recombination with the homolog (Heyer *et al.* 2010). In addition, if the break occurs near or within a dispersed repeat (for example, a Ty retrotransposon), homologous recombination could generate a translocation (McCulley and Petes 2010). In summary, we anticipated that the diploid cells that survive the block-and-release protocol were likely to have high levels of aneuploidy and high frequencies of mitotic recombination. Although we expected that cXII would be particularly unstable, since loss of *Cdc14* results in delayed segregation of telomeric regions on other chromosomes (Clemente-Blanco *et al.* 2011), we analyzed genetic instability throughout the genome.

For this purpose, we introduced the *cdc14-1* allele into two haploids with different genetic backgrounds, strains JSC12 (W303-1A background) and JSC21 (YJM789 background) (St. Charles and Petes 2013), and then mated them to obtain the hybrid diploid. The resulting diploid (FM1468) is heterozygous for ~55,000 SNPs distributed throughout the genome (Lee *et al.* 2009; Lee and Petes 2010) and can be used to study genomic instability events by SNP microarrays (St Charles and Petes 2013). The SNP microarrays contain 25-base oligonucleotides that have sequences identical to either the W303-1A allele or the YJM789 allele for ~13,000 SNPs distributed throughout the genome. By measuring the level of hybridization to each SNP (details in *Materials and Methods*), it is possible to determine whether the diploid strain is heterozygous for the SNPs or homozygous for the W303-1A-derived or the YJM789-derived allele of the SNP. Thus, the microarrays allow the identification of LOH regions produced by mitotic recombination between homologs. The SNP microarrays also allow us to determine whether the strain has duplicated or deleted a segment of a chromosome and/or lost or gained whole chromosomes.

The diploid also has markers on chromosome IV that allow detection of LOH events by the color of the colony. At a subtelomeric region of chromosome IV (SGD coordinate 1510386), the W303-1A-derived homolog has an insertion of a cassette with the *kanMX4/can1-100* genes, and the YJM789-derived homolog has an insertion of the ochre-suppressor *SUP4-o*. The diploid is homozygous for *ade2-1*, an ochre mutation. The diploid FM1468 forms pink colonies because a single copy of *SUP4-o* partially suppresses the red colony color associated with *ade2* mutations. Derivatives of FM1468 that have no copies or two copies of *SUP4-o* form

red or white colonies, respectively (Barbera and Petes 2006; Andersen and Petes 2012; St. Charles and Petes 2013). A reciprocal mitotic crossover would be expected to result in a red/white sectored colony (Figure 3A), and BIR events could result in pink/red (Figure 3B) or pink/white sectored colonies. Chromosome loss events could also produce pink/red colonies (Figure 3C). Whether a sectored colony is produced is also determined by whether both daughter cells survive. Although the color of a colony in wild-type genetic background is primarily a function of the number of *SUP4-o* genes, the color can also be affected by other factors such as genetic alterations that affect growth rate. It is important to note that cIV is the second most likely chromosome (after cXII) to form anaphase bridges in *cdc14-1* strains (Clemente-Blanco *et al.* 2011; Quevedo *et al.* 2012).

As with the diploid FM1860, we plated FM1468 before and after the *cdc14-1* block-and-release. The viability of both parental haploid strains was ~70–75% (Figure S3), similar to what we observed for S288C *cdc14-1* haploids (Quevedo *et al.* 2012). Strikingly, the drop of viability for the *cdc14-1/cdc14-1* hybrid W303/YJM789 diploid was greater than that of the *cdc14-1/cdc14-1* diploid that was homozygous for the S288C genetic background (compare Figure S3 and Figure 1C; ~20 vs. ~50% viability after the block-and-release, respectively). To address whether this worsened viability was a consequence of the presence of the *cdc14-1* alleles in this particular hybrid background, we constructed *cdc14-1/cdc14-1* diploids homozygous for either the W303a or the YJM789 parental backgrounds. Both diploids had a viability of just 30% after the block-and-release (Figure S4). Therefore, we concluded that the extra loss of viability compared to the S288C-derived diploids is a consequence of these other genetic backgrounds rather than the heterozygosity of the W303/YJM789 hybrid strain. Finally, we also ruled out any contribution of the temperature-shift procedures that we used for the *cdc14-1* block-and-release experiment in this loss of viability by analyzing the *CDC14/CDC14* counterparts of all these homozygous and hybrid diploids (Figure S4).

Colonies derived from W303/YJM789 *cdc14-1/cdc14-1* diploid cells grown only at the permissive temperature were almost always pink (97% pink, 3% white; >99% pink for the W303/YJM789 *CDC14/CDC14* diploid). In contrast, of the colonies plated after the *cdc14-1* block-and-release procedure, only 76% were pink; for the comparable wild-type diploid, >99% of the colonies were pink following the temperature shift. The other classes in the W303/YJM789 *cdc14-1/cdc14-1* strain following the temperature shift were red (2.9%), white (14.3%), sectored pink/white (5.5%), sectored pink/red (1.1%), and sectored red/white (0.4%). For 13 sectored colonies of various types, we restreaked the whole sectored colony and analyzed individual colonies of different colors by SNP microarrays. Most of the sectored colonies contained only two colors, but two had three different colors. Surprisingly, in ~15% of the colonies that were restreaked, we observed additional sectored colonies,

a result that suggests on-going genetic instability in some survivors.

As expected, these survivors had a high level of genetic instability (Table 1). Of 13 sectored colonies examined, all except one had detectable genetic alterations in at least one of the sectors; in Table 2, we summarize the number of alterations for each individual chromosome. In Figure 4, we show examples of various genomic changes as revealed by the microarray analysis. The most common alteration was trisomy, representing about half of the total events (Figure 4A). About one-third of the trisomic events were associated with terminal LOH (Figure 4B). Terminal LOH events in euploid chromosomes were also found especially on cXII (Figure 4C). In addition, there were smaller numbers of terminal or interstitial duplications and deletions (Table 2), one example of which is shown in Figure 4D. The likely source of these alterations will be described in the Discussion.

There are several general features of the microarray analysis of sectors that should be noted. First, although colonies with more than one color were common, red/white sectored colonies were infrequent (~5% of the sectored colonies). Consistent with this finding, the microarray analysis detected only a single reciprocal event, a crossover on cIV in colony 13 (Table 1). Second, although we expected that most of the changes in colony color would reflect loss or duplication of the *SUP4-o* gene located on chromosome IV, only three terminal LOH events on cIV were detected. It is clear, therefore, that other genetic changes such as ploidy alteration can substantially affect colony color in this assay. Third, sectors derived from single colonies often shared the same genetic alterations. For example, both the red and white sectors of colony 6 were trisomic for chromosomes I, II, VIII, and XI, although the white sector had two additional events (monosomy for cXII and trisomy for XIV). These shared events suggest that both sectors were derived from one survivor that had the alterations, although other possibilities exist. Finally, it should be pointed out that the *cdc14*-surviving colonies had a higher frequency of genomic alterations detectable by microarrays than wild-type cells. Of 13 sectored colonies derived from an isogenic wild-type diploid, only one unselected LOH event was observed (St. Charles *et al.* 2012).

In summary, the transient loss of *Cdc14* leads to a high frequency of errors in chromosome disjunction and elevated frequencies of mitotic recombination and other types of alterations (deletions and duplications). As expected from previous results, the chromosome with the most alterations was the rDNA-bearing chromosome XII.

Discussion

The inactivation of caretaker genes is known to increase genetic instability and to play an important role in tumor development (Kinzler and Vogelstein 1997; Vogelstein and Kinzler 2004; van Heemst *et al.* 2007; Yamanishi *et al.*

Figure 3 System for detecting recombination events and chromosome nondisjunction by alterations in colony color. As described in the text, in the diploid FM1468, near the right telomere of cIV, we inserted a *SUP4-o* gene on one homolog and the drug-resistance gene *kanMX4* at an allelic position on the other. The strain is also homozygous for the ochre-suppressible *ade2-1* allele. The starting strain is geneticin-resistant and forms pink colonies. (A) Crossover resulting in a red/white sectored colony. (B) Break-induced replication. A break on one of the black chromatids, followed by BIR, results in duplication of sequences derived from the red chromatid distal to the break. Segregation of the resulting chromosomes would result in a pink/red sectored colony. A break occurring on the red chromatid would result in a pink/white sectored colony by the same mechanism. (C) As the result of an unrepaired break on a black chromatid, a pink/red sectored colony would form.

2013). In this work, we have focused on the consequences for the genetic stability of *S. cerevisiae* of a transient temperature shift in diploid cells carrying the thermosensitive allele *cdc14-1*; *CDC14* is an essential gene that encodes for the master cell cycle phosphatase required for exiting from mitosis. We previously showed by tagging *cdc14-1* with the green fluorescent protein-coding gene that most of this phosphatase is likely degraded shortly after incubating the cells at 37° and quickly comes back after shifting the temperature down to 25° (probably from a newly synthesized pool) (see figure S1 in Torres-Rosell *et al.* 2004 and figure S4C in Machín *et al.* 2006). We later showed that temporary loss of *Cdc14* resulted in breakage of cXII near or within the highly repetitive rDNA array (Quevedo *et al.* 2012). In the current analysis, we showed that transient inactivation/degradation of *Cdc14* reduced the viability of diploids more than haploids. Furthermore, the frequencies of trisomic and monosomic chromosomes were greatly elevated following the transient loss of *Cdc14*. Finally, transient loss of *Cdc14* elevated the frequency of mitotic recombination and deletions/duplications with the largest effects on cXII. Each of these results will be discussed further below.

Since diploids would be expected to be more tolerant of chromosome loss and/or deletions than haploids, it is

counterintuitive that *cdc14-1* diploids would have poorer survival of a *Cdc14* block-and-release than haploids. Since we estimated that only 10% of the diploid cells correctly segregated both copies of cXII vs. 40% correct segregation of the single cXII in haploid strains, one explanation of this result is that incorrect segregation of even one copy of cXII usually leads to cell death. It might also happen that diploid cells are more dose-dependent on *Cdc14* than haploids. Thus, *Cdc14* levels upon release could be insufficient to accomplish sensitive processes other than sister-chromatid unlinkage during mitotic exit such as spindle dynamics or replication origin licensing (see below). An alternative possibility of the different survival of haploid and diploid strains is related to recombination. It is possible that mitotic recombination between homologs during telophase sometimes results in unresolvable recombination intermediates; this type of recombination would be restricted to diploids. It has been noted previously that certain genotypes in yeast are viable as haploids but not as diploids. For example, *src2 rdh54* haploids are viable, whereas *src2 rdh54* diploids are inviable (Klein 1997). This difference is likely to be a consequence of unresolvable recombination intermediates formed between homologs in the diploid since *src2 rdh54* strains that have an additional mutation in *rad51*

Table 1 Genotype for each sector in 13 analyzed survivors that developed as sectorized colonies (white/pink, pink/red, red/white, or white/pink/red)

Survivor ^a	Sector color	Genetic alterations ^b	Breakpoints of LOH event (SGD coordinates in kb) ^c
1A	White	None	
1B	Pink	Trisomy XII (W)	
2A	Pink	Trisomy I (Y); trisomy VI ^d (W); trisomy X (Y); trisomy XI (Y); terminal LOH XIII (W)	cXIII (532–541)
2B	Red	Trisomy VI ^d (W); monosomy XII (W); interstitial duplication on XIII (Y); terminal duplication on XIV (W)	cXIII (B1, 372–380 ; B2, 499–505) cXIV (561–568)
3A	White	Terminal LOH XII (Y)	cXII (rDNA, 447–490)
3B	Pink	Terminal LOH XII (Y)	cXII (rDNA, 447–490)
4A	White	Trisomy+terminal LOH III; monosomy XII (W)	cIII (161–165)
4B	Pink	Trisomy I (Y); UPD VI ^e ; terminal LOH XII (Y)	cXII (rDNA, 447–490)
5A	White	Interstitial deletion IV (W)	cIV (B1, 512–521 ; B2, 866–889)
5B	Red	Monosomy III (W); terminal duplication XII ^f (W); terminal deletion XIV ^f (Y); interstitial deletion XV (W)	cXII (941–947) cXIV (631–634) cXV (B1, 594–601 ; B2, 703–717)
6A	White	Trisomy I (Y); trisomy II (Y); trisomy+terminal LOH VIII; trisomy+terminal LOH XI; monosomy XII (Y); trisomy XIV (Y)	cVIII (254–264); cXI (137–146)
6B	Pink	Trisomy I (Y); trisomy II (Y); trisomy+terminal LOH VIII; trisomy+terminal LOH XI	cVIII (254–264); cXI (134–137)
7A	White	Terminal LOH XII (Y)	cXII (rDNA, 447–490)
7B	Pink	Terminal LOH XII ^g (Y)	cXII (368–382)
8A	Pink	Trisomy X (W); monosomy XII (W)	
8B	Red	Trisomy X (W); trisomy XII (Y)+ terminal LOH	cXII (rDNA, 447–490)
9A	White	Monosomy III (W); trisomy VI (Y); terminal deletion XII (W)	cXII (rDNA, 447–490)
9B	Pink	Monosomy III (W); trisomy VI (Y); terminal LOH XII (Y)	cXII (rDNA, 447–490)
10A	White	Tris III (Y); interstitial duplication III (W) ^h ; trisomy X (Y); interstitial LOH event X (Y)	cIII (BP1, 82–95 ; BP2, 167–175) cX (BP1, 143–146; BP2, 167–170)
10B	Pink	Terminal LOH XII (Y)	cXII (rDNA, 418–491) ^j
10C	Red	Terminal LOH IV (W); terminal LOH XII (Y)	cIV (1334–1337); cXII (rDNA, 447–490)
11A	White	Terminal LOH XII (W)	cXII (rDNA, 447–490)
11B	Pink	None	
11C	Red	Terminal duplication XII ^g (W)	cXII (215–221) ^j
12A	White	None	
12B	Red	Trisomy+terminal LOH V (Y); trisomy VI (W); trisomy X (W)	cV (547–551)
13A	White	Terminal LOH IV (Y)	cIV (447–483)
13B	Red	Terminal LOH IV (W)	cIV (450–453)

^a Each sectorized colony is given a different number. A, B, and C indicate different sectors from the same survivor colony.

^b For trisomy and monosomy events, the letters W (W303-1A) and Y (YJM789) indicate the homolog that is duplicated or lost; similarly, for deletions/duplications, W and Y indicate the homolog from which sequences were duplicated or lost. “Terminal LOH” indicates loss of heterozygosity extending to the telomere. These events have a single breakpoint whereas interstitial LOH events have two breakpoints. For LOH events, letters in parentheses show the homolog that is the source of the homozygous SNPs.

^c SGD coordinates of breakpoints between heterozygous and homozygous SNPs for various types of LOH events. Boldface indicates that there is a Ty or δ/α -element located between the indicated coordinates.

^d For these chromosomes, the hybridization ratio suggests that the DNA sample was derived from a mixture of two subpopulations of cells, one with the alteration and one without the alteration.

^e UPD indicates uniparental disomy (Andersen and Petes 2012). In this strain, there were two copies of the YJM789-derived homolog and none of the W303-1A-derived homolog.

^f As discussed in the text, the deletion on chromosome XIV may be related to the duplication on chromosome XII.

^g The breakpoint of this event on XII is not associated with the rDNA.

^h This duplication includes the centromere of III.

ⁱ The breakpoints in this rearrangement were unclear but occurred near the rDNA.

^j This region has a δ -element in S288c, but in some strains has a Ty (Argueso *et al.* 2008). The duplication contains *CEN12*.

(blocking homologous recombination) are viable (Klein 1997). In this regard, it is interesting that our PFGE analysis of the *cdc14-1/cdc14-1* block-and-release showed that the in-gel cXII band completely disappeared twice during the time course, and twice came back (Figure 2D), although the new reappearance was restricted to <10% of the dumbbells/four-somes during the release (Figure S1B). Loss of the in-gel cXII band could occur for a variety of reasons: replication forks, branched recombination intermediates, or broken linear molecules. The first two structures would be expected to cause accumulation of DNA within the gel wells, whereas

broken DNA molecules would be expected to produce DNA fragments with faster electrophoretic mobility. Of course, it is possible that the loss of the in-gel cXII reflects more than one mechanism. Based on the detection of frequent cXII recombination events (Table 1 and Table 2), we suggest that loss of the in-gel cXII is at least partly a consequence of chromosome breaks, followed by the formation of branched recombining molecules (which are therefore trapped in the well). We previously suggested that breakage and recombination took place in haploid cells at the G₁-to-S transition (Quevedo *et al.* 2012). Further supporting this notion, the

Table 2 Number of genetic alterations per chromosome detected by SNP microarrays (30 samples analyzed)

	Chromosome no.																Sum of events for all chromosomes
	XII	IV	XV	VII	XVI	XIII	II	XIV	X	XI	V	VIII	IX	III	VI	I	
Chromosome length (kb)	2443	1532	1091	1091	948	924	813	784	746	667	576	563	440	317	270	230	
Trisomies (NCO)	1						2	1	4	1					5	4	18
Trisomies (CO) ^a	1								1 ^b	2	1	2		2 ^c			9
Monosomies	4													3			7
LOH events (euploid chromosomes) ^d	9(T)	3(T)				1(T)											13
Deletions	1(T)	1 (I)	1 (I)					1(T)									4
Duplications	2(T)					1(I)		1(T)									4
UPD ^e															1		1
Total events/chromosome	18	4	1	0	0	2	2	3	5	3	1	2	0	5	6	4	56

The letters "T" and "I" indicate terminal and interstitial alterations, respectively. CO and NCO refers to crossover and non-crossover, respectively.

^a Unless otherwise indicated, these events have the microarray pattern expected for a crossover or BIR event.

^b The trisomy was associated with an interstitial deletion.

^c One trisomy was associated with a crossover/BIR event and another with an interstitial LOH event.

^d For purposes of this table, LOH events are defined as "those rearrangements in which SNPs from one homolog are duplicated and those from the other homolog are lost." Deletions are considered in a separate category.

^e UPD indicates uniparental disomy, a condition in which one homolog is lost and the other duplicated.

second wave of cXII in-gel disappearance ($t = 210' - 240'$) coincides with the split of part of the foursomes toward dumbbells and a shift of the DNA content toward $<4N$ (and even $<2N$) (Figure 2, C and D). In addition to this, the amount of total cX dropped to around half at about the same time (Figure 2D and Figure S1B). All these data suggest that new chromosome breakages might take place at the foursome stage, perhaps associated with bulk DNA degradation and cell death for a subpopulation of diploid cells.

Of the 56 genetic alterations observed in our study, 34 were changes in the number of chromosomes (Table 2), with trisomy being particularly common (27 of the 34 events). In the 30 genomic samples examined, we found trisomies for 10 of the 16 chromosomes. All of the trisomies (except for two cXII events) involved chromosomes that were <900 kb in length. In contrast, only cXII and cIII were detected as monosomic chromosomes. Trisomies were significantly ($P < 0.002$ by chi-square analysis) more frequent than monosomies. Although simple nondisjunction events would be expected to produce equal frequencies of trisomies and monosomies, the relative growth rates of trisomic and monosomic strains might skew the ratio of these events. It is unlikely that this factor is completely responsible for our observations, since we previously showed that, in yeast strains with low levels of DNA polymerase α , monosomic strains were five times more frequent than trisomic strains (Song *et al.* 2014).

There are several mechanisms that could result in high frequencies of trisomy or monosomy in *cdc14-1* strains. One possibility is that the lack of *Cdc14* directly affects the chromosome segregation apparatus. Trisomies are found at high frequency in diploid yeast cells lacking *BUB1*, which are defective in both the spindle assembly checkpoint and kinetochore function (McCulley and Petes 2010). *Cdc14* could contribute to full chromosome disjunction through a related

mechanism involving the correct localization of the Aurora B-like kinase *Ipl1/Sli15* to the spindle midzone to ensure the stabilization of the mitotic spindle (Zeng *et al.* 1999; Buvelot *et al.* 2003; Pereira and Schiebel 2003; Winey and Bloom 2012). Alternatively, the high frequency of trisomy could be a consequence of chromosome entanglements or breakage. Since *Cdc14* has a role in resolving sister-chromatid linkages, particularly in the rDNA (D'Amours *et al.* 2004; Stegmeier and Amon 2004; Sullivan *et al.* 2004; Torres-Rosell *et al.* 2005; Machín *et al.* 2005, 2006), it is possible that the entangled two sister chromatids are segregated without breakage into the same daughter cell, resulting in trisomy in one daughter cell and monosomy in the other daughter (Figure 5A). A related possibility is that sister chromatids are segregated into the same daughter cell as a consequence of incomplete replication, a defect that has been reported for *cdc14* conditional alleles (Dulev *et al.* 2009). Since trisomic strains are much more common than the monosomic strains in our experiments, the model shown in Figure 5A requires the additional assumption that monosomic strains are less likely to survive the block-and-release protocol than trisomic strains.

An alternative possibility is that the trisomy is a consequence of DNA lesions that occur during the block-and-release protocol. If the centromeres of an entangled pair of sister chromatids are segregated into the two daughter cells, a break might occur in one of the sister chromatids (Figure 5B). If the broken acentric chromatid is repaired by a BIR event with the other homolog, trisomy associated with LOH would be observed. If the repair event involved the intact sister chromatid, trisomy unassociated with LOH would occur (Figure 5B). This model is consistent with the nonrandom association of LOH and trisomy. About one-third of the trisomic chromosomes have an associated LOH event (Table 2). However, production of an extra chromosome by this

Survivor 5A. This strain has a large deletion on the W303-1A-derived cIV. The breakpoints occur at Ty elements, *YDRCTy2-1* near coordinate 515 kb, and an inverted pair of Ty elements (*YDRWTy2-2* and *YDRCTy1-2*) near coordinate 880 kb.

Figure 4 Microarray analysis of ploidy alterations, mitotic recombination events, and insertion/deletions in FM1468. As described in the text, genomic DNA was isolated from sectorized colonies that survived a *cdc14-1* block-and-release procedure and was examined by SNP microarrays. The y-axis shows normalized hybridization ratios to SNP-specific oligonucleotides; a ratio of 1 indicates that the strain is heterozygous. The x-axis indicates SGD coordinates in base pairs. Examples of genomic alterations are shown in A–D. The analysis of all chromosomes in all strains is summarized in Table 1. (A) Survivor 2A. This pattern indicates that the strain is trisomic for cX, with two copies of the YJM789-derived homolog and one copy of the W303-1A-derived homolog. (B) Survivor 6A. This strain is trisomic for cVIII and has the hybridization expected if one copy of VIII was identical to the YJM789-derived homolog, one copy was identical to the W303-1A-derived homolog, and one copy was a recombinant between the two homologs (left end derived from YJM789 and right end derived from W303-1A). The location of the recombination event is near SGD coordinate 250 kb. (C) Survivor 3B. This strain has a terminal LOH region on cXII with a breakpoint in the rRNA gene cluster. The terminal LOH event could reflect either a crossover or a BIR event. (D)

mechanism requires that the BIR event extend through the centromere, an inefficient process (Morrow *et al.* 1997). The third possibility is that the centromere-containing broken chromatid is segregated into the same daughter cell as the intact chromatid (Figure 5C). In this scenario, the trisomy is a consequence of a BIR event that is not required to extend through the centromere.

In a previous study, Chua and Jinks-Robertson (1991) showed that mitotic recombination in wild-type yeast cells was associated with trisomy ~5% of the time. They suggested that either the cells had a general problem with DNA metabolism that resulted in DNA damage and chromosome nondisjunction or that recombination interfered with some aspect of the chromosome segregation apparatus. Our observation that LOH is much more common in chromosomes that have become trisomic argues for a direct linkage between mitotic recombination and defective chromosome segregation. In this regard, it has been recently shown that persistent recombination intermediates form anaphase bridges (García-Luis and Machín 2014).

In addition to the LOH events associated with trisomy, we observed 13 terminal LOH events involving euploid chromosomes. Nine of these 13 events occurred on cXII, and 8 of these 9 had a breakpoint within the rRNA gene cluster. This observation is consistent with our cytological analysis and the PFGE results indicating that the rRNA gene cluster is a specific target for breakage in cells depleted for *Cdc14* (figures 1 and 2 and figure S1 in Quevedo *et al.* 2012). Although we again analyzed all sectors for these 13 colonies, two sectors, in general, did not contain reciprocal recombination

products, likely because of the poor viability of the diploid progeny. Consequently, we cannot determine whether the terminal LOH events observed in our study represent crossovers or BIR events.

There were four other chromosome alterations involving cXII. Survivors 5B and 11C had terminal duplications with breakpoints located near Ty elements. Although we have not investigated the structure of cXII in these strains, in studies of other genetically unstable strains (McCulley and Petes 2010) and of γ -ray-treated strains (Argueso *et al.* 2008), such duplications usually reflect the formation of translocations generated by recombination between Ty elements on nonhomologous chromosomes. We also found a strain in which trisomy of cXII was associated with an LOH event with an rDNA breakpoint. Finally, we detected a terminal deletion of cXII in which the breakpoint was in the rDNA (survivor 9A). It is possible that this structure results from a chromosome that had a break in the rDNA that was “capped” by addition of telomeric sequences. Chromosomes of this type have been detected previously in *tel1 mec1 sml1* diploids (J. McCulley and T. Petes, unpublished observations).

In previous studies of chromosome rearrangements in yeast strains with low levels of DNA polymerase α (Song *et al.* 2014), we found deletions and duplications in addition to other types of changes. Most of these events were a consequence of homologous recombination between Ty elements or other dispersed repeats. Although we have not investigated the structures of the four deletions and four duplications in the present study in detail, most have Ty

Figure 5 Mechanisms for generating trisomy and monosomy in diploid cells after the *cdc14-1* block-and-release. The W303-1A- and the YJM789-derived homologs are indicated by red and blue lines, respectively. Horizontal arrows show the direction of chromosome segregation, and boxes contain the chromosomes of daughter cells (DC1 and DC2). (A) The sister chromatids of one homolog are entangled and segregate together, resulting in one trisomic daughter cell and one monosomic daughter cell. (B) One of the entangled sister chromatids undergoes a DNA break (indicated by the lightning sign). The intact blue chromatid and the distal portion of the broken blue chromatid segregate into one cell, with the centromere-containing broken blue chromatid segregating into the other daughter cell. A BIR event through the centromere (shown as dashed arrows) in the left daughter cell results in trisomy either with or without a concomitant terminal LOH event for one of the chromosomes. This event depends on whether BIR takes place using the intact homolog (hc-BIR) or the intact sister (sc-BIR). A BIR event in the other daughter cell would result in a terminal LOH event with retention of the euploid chromosome number. (C) As in B, a break occurs in one of the sister chromatids. In C, how-

ever, the centromere-containing broken fragment cosegregates with the intact blue chromosome. A BIR event involving the red chromosome would produce trisomy with one recombinant chromosome. In the other daughter cell, if the acentric fragment cannot perform BIR through the centromere, this daughter would become monosomic. Note that alternative outcomes may be possible for daughter cells in B and C but are not depicted for the sake of simplicity (e.g., in B, DC2 may become monosomic as in C; in C, DC1 may become trisomic without LOH as in B).

elements or other repetitive elements at the breakpoints (Table 1) and are likely to represent similar events. For example, a homologous recombination event between two directly oriented Ty repeats located 365 kb apart on chromosome IV is likely responsible for the large heterozygous deletion shown in Figure 4D.

The LOH events and deletions/duplications are likely all initiated by DSBs. There were a total of 30 of these events in 30 strains analyzed. Since only one such event was observed in 13 unselected wild-type strains, it is clear that transient loss of *Cdc14* results in elevated levels of DSBs. It is possible that there are multiple sources of DSBs: breaks resulting from attempts to segregate catenated chromosomes, scission of DNA “bridges” (particularly rDNA) during nuclear division, and cellular nucleases acting on incompletely condensed chromosomes. In addition, *CDC14* has a role in the licensing of replication origins, and the transient inactivation of *Cdc14* interferes with the normal timing of the replication (Dulev *et al.* 2009; Zhai *et al.* 2010); a similar delay is apparent in our study (Figure 2C). These disruptions of the normal pattern of replication may create recombinogenic DNA damage.

In conclusion, transient loss of the caretaker *Cdc14* (the master regulator of anaphase progression and mitotic exit in budding yeast) leads to both an increase in genome instability and cell death. The genome instability was manifested by very high frequencies of trisomy and elevated levels of monosomy for most of the smaller yeast chromosomes. In addition, as expected from our previous study, the rDNA of

cXII was a hotspot for recombination events. Our data highlight the critical importance of perfectly regulating the transition from anaphase onset to cytokinesis. The genetic instability in strains with transiently low levels of *Cdc14* occurs despite two checkpoints that delay cytokinesis if anaphase bridges are present (Yang *et al.* 1997; Mendoza *et al.* 2009). Our results support the possibility that brief inactivation of a single caretaker gene can lead to dramatic genomic instability that may predispose a cell toward tumor formation.

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Supporting Information

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The Transient Inactivation of the Master Cell Cycle Phosphatase Cdc14 Causes Genomic Instability in Diploid Cells of *Saccharomyces cerevisiae*

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Figure S1 Quantitative analysis of the relative amounts of intact chromosomes in the homozygous S288C diploid strain analyzed by PFGE after the *cdc14-1* block-and-release. (A) The intensities of each ethidium-bromide-stained band in Fig. 2D and two more independent experiments were normalized to the sum of the band signals corresponding to chromosomes I, VI, and III, and then to the intensities at time 0 (time of the *cdc14* block). Error bars represent s.e.m (N=3). (B) In-gel and in-well signals of unsaturated exposures of the Southern blots shown in Fig. 2D were quantified and normalized to that of time 0' (*cdc14*-block). Overall is the sum of both in-gel and in-well signals.

Figure S2 Southern analysis with a cXII hybridization probe of DNA isolated from the homozygous diploid *cdc14-1* strain during the block-and-release time course. This figure shows a less exposed film of the same blot shown in Fig. 2D (central-left image) in order to better visualize the amount of cXII that remains in the well during the time course. The lane labeled “as.” contains DNA isolated from asynchronous cultures of the *cdc14-1* strain before exposure to the restrictive temperature.

Figure S3 Viability analysis of haploid and diploid strains used for the SNP microarrays following the *cdc14-1* block-and-release protocol. FM1142 and FM1184 strains are the *cdc14-1* haploids that were mated to generate FM1468 (the diploid used to perform the SNP microarray analysis). Error bars represent s.e.m (N=3).

Figure S4 Viability analysis following the temperature shift procedure used for the block-and-release protocol of *CDC14* and *cdc14-1* diploid strains homozygous and heterozygous, respectively, for the ~55,000 SNPs. In order to obtain the corresponding diploid strains, reference W303 (W) and YJM789 (Y) haploids carrying either the wild type *CDC14* or the temperature sensitive *cdc14-1* alleles were mated with themselves (Y/Y or W/W) or between them (W/Y). Each diploid strain was grown at 25°C in YPD and then an aliquot was directly plated on YPD and incubated at 25°C for three days ("before", blue bars), whereas another aliquot was taken after incubating the culture at 37°C for 3 h ("after", red bars). For each aliquot, plated cells were estimated under a haemocytometer and viability represents CFU versus plated cells. Error bars represent s.e.m (N=3).