Haploidization in *Saccharomyces cerevisiae* Induced by a Deficiency in Homologous Recombination

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ABSTRACT Diploid *Saccharomyes cerevisae* strains lacking the *RAD52* gene required for homologous recombination have a very high rate of chromosome loss. Two of four isolates subcultured ~20 times (~500 cell divisions) became haploid. These strains were capable of mating with wild-type haploids to produce diploid progeny capable of undergoing meiosis to produce four viable spores.

In previous studies (Mortimer *et al.* 1981; Yoshida *et al.* 2003), it was shown that diploid *Saccharomyces cerevisiae* strains that lacked Rad52p had substantially elevated frequencies of chromosome loss relative to wild-type strains. In X-ray-treated *rad52* mutants, chromosome loss rates were further elevated (Mortimer *et al.* 1981). Since *rad52* strains are unable to efficiently repair double-stranded DNA breaks (DSBs) by homologous recombination (Krogh and Symington 2004) and since nonhomologous recombination is suppressed in diploid cells (Shrivastav *et al.* 2008), these high frequencies of chromosome loss likely reflect the lack of repair of DSBs generated spontaneously or induced by X rays. Previous studies of chromosome loss in *rad52* strains involved genetic approaches that were restricted to specific chromosomes. In the study below, we used DNA microarrays, which allowed us to examine all chromosomes. This approach revealed that two of four subcultured *rad52* diploids underwent rapid chromosome loss eventually resulting in haploidy.

Results

We constructed a diploid (WS82, Table 1 legend) homozygous for the *rad52* mutation; the haploid strains used in the construction (WS30-3 and WS53) differed by >25,000 single-nucleotide polymorphisms (SNPs). Four independent isolates of this diploid were subcultured on plates from a single cell to a colony at least 18 times, representing ~450 cell divisions. Samples were taken for analysis from the strain before subculturing and after various numbers of subculturing events. DNA was isolated from each isolate, and the chromosome compositions were examined by comparative genome hybridization (CGH) microarrays. For two of the four isolates, we observed progressive chromosome loss, culminating in haploidization for two of these isolates (Table 1). For example, in WS82-1, although the starting strain was a normal diploid (Figure 1A), by the fifth subcloning (SC5), the isolate had lost chromosomes IV, V, X, XII, and XIII (Figure 1B). Continued subcloning resulted in further chromosome loss (Figure 1, C and D).

By the 18th subcloning, WS82-1 had the same gene dosage for all 16 chromosomes (Figure 1E). This hybridization pattern, by itself, cannot distinguish between haploids and diploids. To determine whether the strain was a haploid or a diploid, we crossed WS82-1 from SC18 with a *RAD52 MAT***a** haploid strain (EAS18). The resulting strain would be a diploid or a triploid, depending on whether the strain shown in Figure 1E was a haploid or a diploid, respectively. When induced to undergo meiosis, diploid strains have good spore viability (>80%) whereas triploids have poor spore viability (<50%) (St. Charles *et al.* 2010). We found that the strain produced by the cross had excellent spore viability (143 viable spores of 160 total, or 89%), indicating that the subcultured derivative of WS82-1 shown in Figure 1E was a haploid rather than a diploid. Similarly, by the same criteria described above, WS82-2 underwent haploidization. The WS82-3 and WS82-4 isolates will be described further below.

In addition to detecting changes in gene dosage, oligonucleotide-containing microarrays can also be used to

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Table 1 Number of each homolog (I–XVI) per cell in two derivatives of the *rad52/rad52* diploid WS82 (WS82-1 and WS82-2) that show progressive chromosome loss during subculturing

Strain	SC	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI
WS82-1	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
	5	2	2	2	1	1	2	2	2	2	1	2	1	1	2	2	2
	10	2	2	2	1	1	1	2	1	1	1	2	1	1	2	1	1
	14	2	1	2	1	1	1	1	1	1	1	2	1	1	1	1	1
	18	**1**	**1**	**1**	**1**	**1**	*1*	*1*	**1**	*1*	*1*	*1*	**1**	**1**	**1**	*1*	*1*
WS82-2	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
	5	2	2	2	1	2	2	2	2	2	1	1	2	1	2	2	1
	10	2	1	1	1	1	1	2	1	2	1	2	1	1	2	1	1
	14	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	18	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	22	*1*	**1**	*1*	**1**	*1*	**1**	**1**	*1*	**1**	**1**	**1**	**1**	**1**	*1*	**1**	**1**

WS82 was constructed by a cross of WS30-3 (*MAT***a** *ade5-1 leu2-3 trp1-289 ura3-52 his7-2 LEU2*::*XII rad52Δ*::*NAT*) and WS53 (*MATα ho*::*hisG lys5 rad52Δ*::*NAT*). WS30-3 was constructed by transformation of EAS18 (*MAT***a** *ade5-1 leu2-3 trp1-289 ura3-52 his7-2 LEU2*::*XII*) (Casper *et al.* 2008) with a PCR fragment generated by amplifying the plasmid pAG25 (Goldstein and McCusker 1999) with primers WS5 (5′ GGAGGTTGCCAAGAACTGCTGAAGGTTCTGGTGGCTTTGGTGTGTTGTTGCGTACGCTGCAGGTCGAC) and WS6 (5′ AGTAATAAATAATGATGCAAATTTTTTATTTGTTTCGGCCAGGAAGCGTTATCGATGAATTCGAGCTCG). The same fragment was used to derive WS53 from YJM849 (*MATα ho*::*hisG lys5* Gal$^+$), a strain obtained from J. McCusker (Duke University) that is isogenic with YJM789 (*MATα ho*::*hisG lys2 gal2*) (Wei *et al.* 2007), except for alterations introduced by transformation. WSMD58-2, a diploid generated by crossing MS71 (a *MATα* strain otherwise isogenic with EAS18) with YJM850 (a *MAT***a** strain otherwise isogenic with YJM849), was used as a control in the CGH experiments. We used CGH microarrays to determine the number of chromosomes per cell for all 16 chromosomes in four isolates of WS82 before subculturing (SC 0) and after various numbers of subcultures (single cell to colony for each subculture). Genomic DNA of the two subcultured strains that had undergone haploidization was examined by SNP arrays. In the rows showing the 18th subculturing of WS82-1 and the 22nd subculturing of WS82-2, the boldface numbers indicate that the retained chromosome was derived from the WS53/YJM789 parent, and italics show that the retained chromosome was derived from the WS30-3/MS71 parent.

determine whether a diploid strain is heterozygous or homozygous for a SNP (Gresham *et al.* 2008). We used SNP arrays to confirm haploidy in the subcultured derivatives of WS82-1 and WS82-2 and to determine whether the chromosomes were preferentially lost from one of the two haploid parental strains (WS30-3 and WS53). Figure 2A illustrates that genomic DNA isolated from subculture 0 of WS82-1 hybridized equally well to WS30-3-specific and WS53-specific oligonucleotides; although all chromosomes were examined, only the data for chromosome VII are shown in Figure 2A. In contrast, genomic DNA isolated from SC18 of WS82-1 (the presumptive haploid strain) preferentially hybridized to the WS30-3-specific oligonucleotides for chromosome VII (Figure 2B) and to the WS53-specific

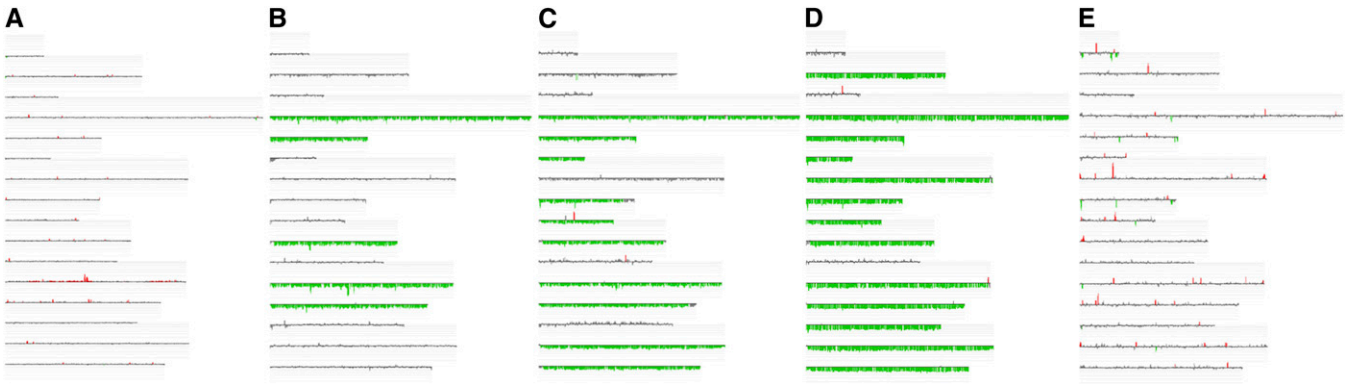

Figure 1 CGH microarray analysis of aneuploidy in the subcultured *rad52/rad52* diploid strain WS82. To examine the effects of the *rad52* mutation on chromosome loss, we subcultured independent isolates of WS82 18–22 times. Each subculturing involved growth from a single cell to a colony in plates incubated at 30° for 4 days. The first 10 subcultures were done on YPR-LG (rich growth medium with 0.005% galactose and 2% raffinose) plates, and the subsequent subcultures were done on plates containing YPD (Lemoine *et al.* 2005). DNA was isolated from subcultured samples and analyzed by CGH microarrays as described previously (Lemoine *et al.* 2005; McCulley and Petes 2010). In brief, subcultured DNA samples were labeled with Cy5-dUTP and hybridized in competition with control samples labeled with Cy3-dUTP to microarrays containing PCR fragments with ORFs and intergenic regions. The $\log_2$ Cy5/Cy3 ratio for each ORF or intergenic region was divided by median $\log_2$ Cy5/Cy3 ratio for all elements present on the array. Representative microarrays from isolate WS82-1 are shown. The data are depicted with CGH-Miner software. Each of the horizontal lines depicts one of the 16 yeast chromosomes, shown in order from chromosome I at the top to XVI at the bottom. A gray line indicates that the chromosome is euploid, whereas a green line shows that the chromosome is under-represented. Most of the short red segments represent "noise" in the analysis, although the red segment on chromosome XII is a region of the ribosomal DNA that is often amplified. (A–E) The CGH analysis for the diploid before subculturing and after 5, 10, 14, and 18 rounds of subculturing, respectively. SC5, SC10, and SC14 are monosomic for 5, 10, and 13 of the 16 chromosomes, respectively. Note that both the starting strain (A) and SC18 (E) are euploid, but a genetic test (described in *Results*) demonstrates that SC18 is haploid rather than diploid.

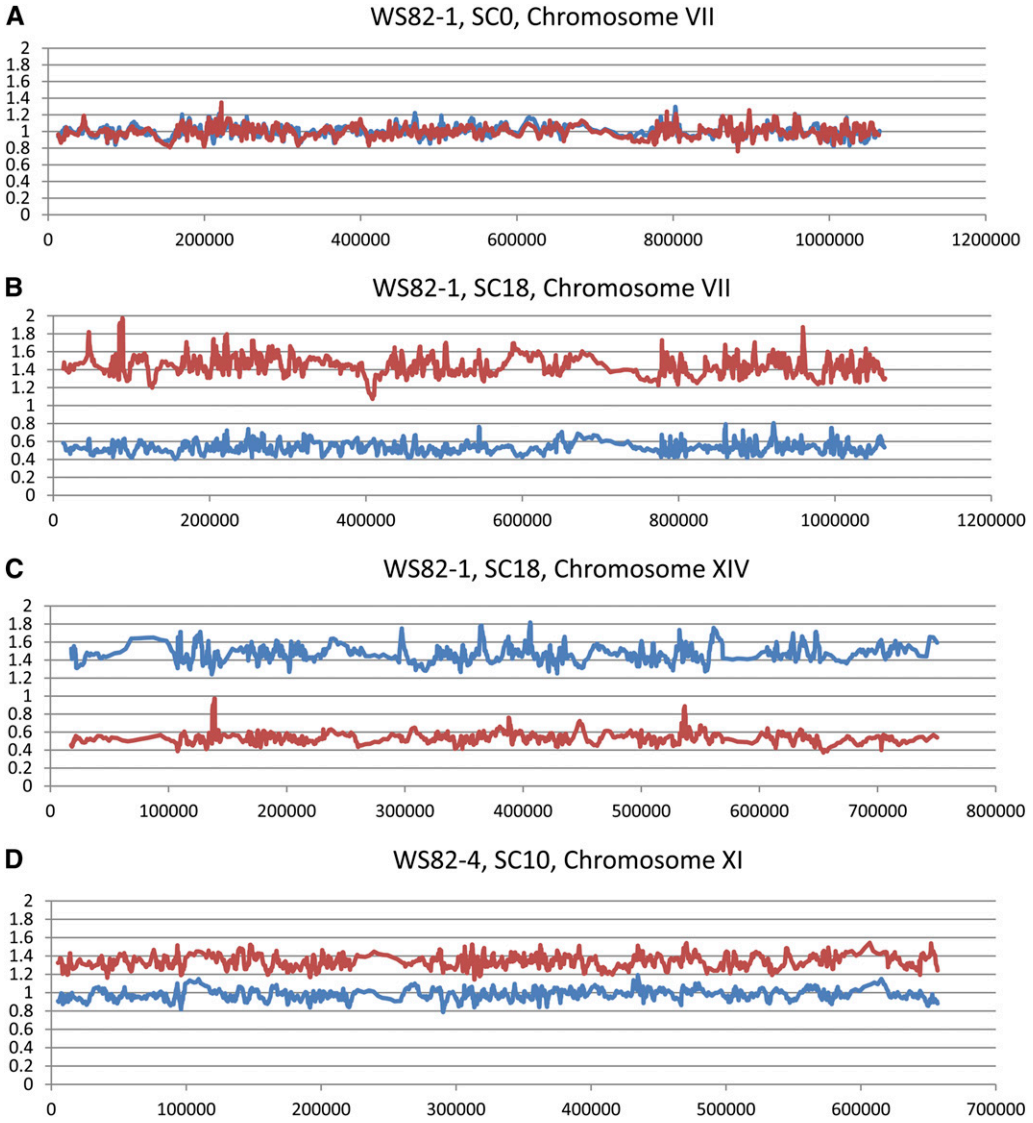

Figure 2 Analysis of chromosome loss using SNP microarrays. WS82 was derived from a cross of the haploids WS30-3 (closely related to S288c, sequence in Saccharomyces Genome Database) and WS53 (closely related to YJM789, sequenced by Wei *et al.* 2007). Four 25-base oligonucleotides were designed for each of 13,000 SNPs distinguishing S288c and YJM789 (St. Charles *et al.* 2012); for each SNP, two of the oligonucleotides had the sequence of the S288c form (Watson and Crick) and two had the sequence of the YJM789 form (Watson and Crick). These oligonucleotides were incorporated in Agilent microarrays. Genomic DNA isolated from experimental strains labeled with Cy5-dUTP was hybridized to these arrays in competition with a control heterozygous strain that was labeled with Cy3-dUTP (McCulley and Petes 2010). For each oligonucleotide, we determined the ratio of hybridization (R_M) of Cy5/Cy3. These values were centered to a value of 1 by dividing each oligonucleotide R_M by the average of all oligonucleotide R_M values of the microarray. Loss of heterozygosity for a particular SNP results in an increased hybridization signal for one pair of strain-specific oligonucleotides and a decrease in the signal for the other pair of strain-specific oligonucleotides (Gresham *et al.* 2008). Since WS30-3 is not isogenic to S288c, only oligonucleotides that distinguished WS30-3 SNPs from WS53 SNPs were used in the analysis. In A–D, we show the ratio of hybridization of the experimental strain to the control strain (*y*-axis) *vs.* the position of the probe in the Saccharomyces Genome Database coordinates (*x*-axis). Hybridization to S288c/WS30-3 oligonucleotides is shown in red and hybridization to YJM789/WS53 oligonucleotides is shown in blue. (A) Chromosome VII, WS82-1, before subculturing. In this strain, the ratios of hybridization to both types of oligonucleotides were ~1, indicating that WS82-1, before subculturing, had one copy each of the WS30-3- and WS53-derived chromosomes. All chromosomes in this strain had the same pattern. (B) Chromosome VII, WS82-1, and SC18. After SC18, the strain had lost the WS53-derived chromosome VII and retained the WS30-3-derived chromosome VII. In these experiments, the retained chromosome had a hybridization ratio of ~1.5, and the lost chromosome had a ratio of ~0.5. The difference in hybridization ratios is not greater because there is some degree of cross-hybridization of genomic DNA from the different strains to the strain-specific oligonucleotides. (C) Chromosome XIV, WS82-1, and SC18. After SC18, this isolate had lost the WS30-3-derived chromosome XIV and retained the WS53-derived copy. (D) Chromosome XI, WS82-4, and SC10. After SC10, genomic DNA was isolated and hybridized to the SNP arrays. Although the hybridization ratios were higher for the WS30-3-specific oligonucleotides, the hybridization ratios for the WS53 oligonucleotides were higher than expected if the experimental strain lacked the WS53-derived chromosome. The simplest explanation of this pattern is that the strain had three copies of chromosome XI, two derived from WS30-3 and one derived from WS53.

oligonucleotides for chromosome XIV (Figure 2C). As shown in Table 1 (SC18 for WS82-1 and SC22 for WS82-2), of 32 chromosome losses, 12 were losses of the WS30-3-derived chromosomes and 20 were losses of the WS53-derived chromosomes; this difference is not statistically significant. These results confirm that WS82-1 and WS82-2 are haploid strains and further show that, as expected, none of the retained chromosomes had undergone mitotic recombination.

In contrast to the progressive chromosome loss observed in WS82-1 and WS82-2, WS82-3 and WS82-4 underwent a different process. From the CGH analysis (samples labeled with "C" in Table 2) by SC22, WS82-3 appeared to have lost 13 of 16 chromosomes (retaining two copies of III, VIII, and IX), and WS82-4 appeared to have lost one complete set of chromosomes by SC18. At SC5, by CGH arrays, WS82-4 had lost chromosomes VIII, X, and XIII. After SC10, however, genomic DNA isolated from WS82-4 had a pattern of

Table 2 Number of each homolog (I–XVI) per cell in two derivatives of the *rad52/rad52* diploid WS82 (WS82-3 and WS82-4) that underwent genome duplications during subculturing

Strain	SC[a]	I[b]	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI
WS82-3	0C	2	2	2	2	2	2	2	2	2	2	2	2-3	2	2	2	2
	0S	**1**,*1*	**1**,*1*	**1**,*1*	**1-2**	**1**,*1*	**1**,*1*	**1**,*1*	**1**,*1*	**1**,*1*	*1*	**1**,*1*	**1-2**,*0-1*	**1**,*1*	**1**,*1*	**1**,*1*	**1**,*1*
	5C	2	1	2	1	1	1	2	2	2	1	2	2	2	2	1	1
	5S	**2**,*2*	**2**	**2**,*2*	**2**	*2*	**2**	**2**,*1*	**1-2**,*1-2*	**2**,*2*	*2*	**2**,*2*	**2**	**1-2**,*0-1*	**2**,*2*	**2**	*2*
	10C	2	1	2	1	1	1	1	2	2	1	2	1	1	1	1	1
	10S	**2**,*2*	**2**	**2**,*2*	**2**	*2*	**2**	**2**	**1**,*2*	**2**,*1*	*2*	**2**,*1*	**2**	*2*	*2*	**2**	*2*
	14C	2	1	2	1	1	1	1	2	2	1	1	1	1	1	1	1
	14S	**2**,*2*	**2**	**2**,*2*	**2**	*2*	**2**	**2**	**1**,*2*	**2**,*1*	*2*	**1**,*1*	**2**	*2*	*2*	**2**	*2*
	18C	1	1	2	1	1	1	1	2	2	1	1	1	1	1	1	1
	18S	**2**	**2**	**1**,*2*	**2**	*2*	**2**	**2**	**1**,*2*	**2**,*1*	*2*	**1**,*1*	**2**	*2*	*2*	**2**	*2*
	22C	1	1	2	1	1	1	1	2	2	1	1	1	1	1	1	1
	22S	**1**	**2**	**1**,*2*	**2**	*2*	**2**	**2**	**1**,*2*	**2**,*1*	*2*	**1**,*1*	**2**	*2*	*2*	**2**	*2*
WS82-4	0C	2	2	2	2	2	2	2	2	2	2	2	2-3	2	2	2	2
	0S	**1**,*1*	**1**,*1*	**1**,*1*	**1**,*1*	**1**,*1*	**1**,*1*	**1**,*1*	**1**,*1*	**1**,*1*	**1**,*1*	**1**,*1*	**1**,*1-2*	**1**,*1*	**1**,*1*	**1**,*1*	**1**,*1*
	5C	2	2	2	2	2	2	2	1	2	1	2	2	1	2	2	2
	5S	**1**,*1*	**1**,*1*	**1**,*1*	**1**,*1*	**1**,*1*	**1**,*1*	**1**,*1*	*1*	**1-2**,*1*	*1*	**1**,*0-1*	**1-2**,*1*	*1*	**1**,*1*	**1**,*1*	**1**,*1*
	10C	2	2	2	1	2	2	2	1	2	1	2	1	1	2	2	1
	10S	**2**,*2*	**2**,*1*	**2**,*1*	**1**,*1*	**2**,*1*	**2**,*1*	**1-2**,*1-2*	*2*	**2**,*1*	*2*	**1**,*2*	**2**	*2*	**1-2**,*1-2*	**2**,*1*	*2*
	14C	2	1	2	1	2	2	2	1	1	1	1	1	1	1	2	1
	14S	**1**,*2*	**2**	**2**,*1*	**1**,*1*	**2**,*1*	**2**,*1*	**1**,*2*	*2*	**2**	*2*	**1**,*1*	**2**	*2*	*2*	**2**,*1*	*2*
	18C	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	18S	**1**,*2*	**2**	**1**,*1*	*2*	**2**	**1**,*1*	**1**,*2*	*2*	**2**	*2*	**1**,*1*	**2**	*2*	*2*	**1**,*1*	*2*

As discussed in *Results*, in the WS82-3 and WS82-4 isolates, during subculturing the strain underwent genome duplication. In WS82-3, this duplication event occurred between SC0 and SC5, and in WS82-4, the duplication occurred between SC5 and SC10.

[a] SC shows the subculture number. C and S indicate results obtained with CGH and SNP microarray analysis, respectively. Analysis of chromosome number by CGH arrays was performed as described in the Table 1 legend.

[b] As in Table 1, the number in boldface in the "S" row is the number of WS53/YJM789-derived chromosomes, and the number in italics is the number of WS30-3/MS71-derived chromosomes. A range of numbers indicates that the sample of cells was heterogeneous. For example, in WS82-4 at SC5, chromosome IX has the numbers "**1-2,** *1*," indicating that all of the cells in the culture have one copy of chromosome IX derived from WS30-3/MS71, some of the cells in the culture have two copies of IX from WS53/YJM789, and others have one copy. As discussed in *Results*, the number of chromosomes based on CGH microarrays is often smaller than that based on SNP microarrays after SC0 for WS82-3 and SC5 of WS82-4 as a consequence of a genome duplication.

hybridization by SNP arrays, indicating that it was trisomic for many chromosomes. For example, in Figure 2D, the pattern of hybridization at SC10 indicated that the strain had three copies of chromosome XI: two derived from the WS30-3 parent and one derived from the WS53 parent. Similarly, for WS82-3, by SC5, the SNP array indicated that most of the homologs were present in more than two copies (Table 2). The discrepancy between the number of chromosomes in these strains as determined by CGH and SNP microarrays reflects what is measured by the two different methods. The CGH analysis can detect only deviations in copy number from the average copy number of the experimental strain (see Figure 1 legend); although twofold differences are usually clear, smaller differences are not. In contrast, with the SNP arrays, the relative hybridization levels of the experimental strain for each homolog are measured independently (see Figure 2 legend). In this type of array, by examining the hybridization values to the SNP-specific oligonucleotides, it is simple to determine both copy number and whether the homologs are identical. Thus, for WS82-4 (SC10), it is clear that there is one copy of chromosome XI derived from WS53 because the normalized hybridization ratio is 1 and two copies of XI derived from WS30-3 because the normalized hybridization ratio is ~1.4. In summary, where there is a discrepancy between the number of chromosomes as determined with CGH and SNP arrays, the SNP arrays are more accurate. We point out that no discrepancies for the two types of arrays were observed for WS82-1 and WS82-2.

There are two explanations of the apparent genome duplications observed in isolates WS82-3 and WS82-4. First, it is possible that, during subculturing within each of these isolates, two derivatives arose: one that had lost the *MAT***a**-containing copy of chromosome III and one that had lost the *MAT*α-containing copy of III. Mating between these derivatives would result in a strain with two, three, or four copies of each homolog, consistent with the SNP array data. An alternative possibility is that, during subculturing, WS82-3 and WS82-4 undergo whole-genome duplication. We favor the second possibility for two reasons. First, in the strains observed immediately after the postulated genome duplication (SC5 for WS82-3 and SC10 for WS82-4), WS82-3 had two copies of both the *MAT***a**- and *MAT*α-containing chromosomes, and WS82-4 had two copies of the *MAT*α- and one copy of the *MAT***a**-containing chromosomes. If the diploidization reflected mating, we would expect that the resulting strain would have only two copies of chromosome III, one with each mating type. Second, we and others (J. McCusker, personal communication) have observed that haploid strains of the YJM789 genetic background spontaneously diploidize; consequently, as the WS82 diploid loses chromosomes derived from the other genetic background, the diploidization phenotype characteristic of the WS53/YJM789 haploid parent may emerge.

Although the *rad52* mutation stimulates both chromosome loss and gain in the subcultured cells in our experiments, it is likely that the main effect at the cellular level is to increase the rate of chromosome loss, and the chromosome gain observed in two isolates reflects either mating or whole-genome duplication during subculturing. A strong argument that the chromosome gains and losses in *rad52* strains are not a consequence of an elevated rate in nondisjunction is that the individual homologs in WS82-1 and WS82-2 become monosomic, rather than exhibiting a mixture of monosomic and trisomic chromosomes. It should also be pointed out that chromosome loss continued in the WS82-3 and WS82-4 isolates after mating/genome duplication. For example, the number of chromosomes in WS82-3 decreased from 44 at SC5 to 34 at SC22.

Discussion

We showed that *rad52* diploids have high rates of chromosome loss, culminating in haploidy in some subcultured isolates. Since aneuploid strains grow slowly (Torres *et al.* 2007), it is difficult to calculate an accurate rate of chromosome loss. However, after five cycles of subculturing, since the average number of chromosomes lost in WS82-1 and WS82-2 was five, we calculate a frequency of loss of ~0.04 chromosomes/cell division (five loss events/125 cell divisions). If we multiply the rate of loss of chromosome V in a wild-type diploid (2×10^{-6}/division; Klein 2001) by 16 (the number of yeast chromosomes), we estimate that the comparable frequency of chromosome loss in wild-type diploids is $\sim 3 \times 10^{-5}$, which is about three orders of magnitude less than for the *rad52* diploids.

The high rate of chromosome loss in *rad52* strains has a straightforward explanation. Yeast cells have a low level of spontaneous DNA damage that can be detected as foci of fluorescently tagged DNA repair proteins (Lisby *et al.* 2001). Since efficient repair of this damage by homologous recombination requires Rad52p, chromosomes with DSBs would be lost from the diploid. Since there is no efficient mechanism that compensates for this loss, the diploid would undergo progressive chromosome loss until the haploid state is reached. Although chromosome loss presumably continues in haploid cells, haploid cells that lose a chromosome would fail to divide since all yeast chromosomes contain essential genes.

As discussed above, strains with more than two copies of some of the homologs were observed in two *rad52* isolates, likely reflecting a genome-duplication phenotype associated with one of the haploid parental strains, although mating between aneuploid derivatives is also possible. In WS82, therefore, the cell population derived from initially diploid *rad52/rad52* isolates will have a complex composition of genotypes. The ratio of the various classes of near-diploid, near-haploid, and various other classes will presumably be dependent on the relative division rates of euploid and aneuploid strains, as well as on environmental factors. For example, haploid cells adapt more quickly than diploid cells in a variety of environments (Gerstein *et al.* 2011).

Three other studies are relevant to our observations. Alabrudzinska *et al.* (2011) showed by FACS analysis that diploid *S. cerevisiae* strains lacking Ctf18p (a protein involved in loading PCNA on DNA and interactions with the cohesion complex) have very high levels of chromosome loss, with some isolates having the DNA content of haploid or near-haploid strains by FACS analysis. In *ctf18* diploids, chromosome loss appears to involve a mechanism different from that observed in *rad52* strains, with some *ctf18* derivatives undergoing rapid reduction to near-haploidy whereas other derivatives had levels of DNA greater than the diploid level. In addition, tetraploid yeast strains undergo rapid formation of near-diploid strains in a pathway that appears to involve concerted chromosome loss (Gerstein *et al.* 2006). In *Candida albicans*, diploid strains lacking Rad52p have high rates of chromosome loss and terminal deletions (Andaluz *et al.* 2011). The loss events, however, are subsequently followed by reduplication events, and, therefore, diploidy is preserved.

Finally, our results suggest that, at least under laboratory conditions, diploid *S. cerevisae* strains can exchange information through two pathways. In wild-type strains, the traditional sexual pathway is presumably the primary mechanism for genetic interchange. However, in *rad52* diploid strains, chromosome loss results in fertile haploid strains without the necessity of undergoing meiosis. This pathway mimics some aspects of parasexual life cycles observed in *Aspergillus nidulans* and *C. albicans* (Pontecorvo 1956; Forche *et al.* 2008).

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Literature Cited

Alabrudzinska, M., M. Skoneczny, and A. Skoneczna, 2011 Diploid-specific genome stability genes of *S. cerevisiae* genomic screen reveals haploidization as an escape from persisting DNA rearrangement stress. PLoS ONE 6: e21124.

Andaluz, E., A. Bellido, J. Gomez-Raja, A. Selmecki, K. Bouchonville *et al.*, 2011 Rad52 function prevents chromosome loss and truncation in *Candida albicans*. Mol. Microbiol. 79: 1462–1482.

Casper, A. M., P. A. Mieczkowski, M. Gawel, and T. D. Petes, 2008 Low levels of DNA polymerase alpha induce mitotic and meiotic instability in the ribosomal DNA gene cluster of *Saccharomyces cerevisiae*. PLoS Genet. 4: e1000105.

Forche, A., K. Alby, D. Schaefer, A. D. Johnson, J. Berman *et al.*, 2008 The parasexual cycle in *Candida albicans* provides an alternative pathway to meiosis for the formation of recombinant strains. PLoS Biol. 6: 1–14.

Gerstein, A. C., H.-J. Chun, A. Grant, and S. P. Otto, 2006 Genomic convergence toward diploidy in *Saccharomyces cerevisiae*. PLoS Genet. 2: e145.

Gerstein, A. C., L. A. Cleathero, M. A. Mandegar, and S. P. Otto, 2011 Haploids adapt faster than diploids across a range of environments. J. Evol. Biol. 24: 531–540.

Goldstein, A. L., and J. H. McCusker, 1999 Three new dominant drug resistance cassettes for gene disruption in *Saccharomyces cerevisiae*. Yeast 15: 1541–1553.

Gresham, D., M. J. Dunham, and D. Botstein, 2008 Comparing whole genomes using DNA microarrays. Nat. Rev. Genet. 9: 291–302.

Klein, H. L., 2001 Spontaneous chromosome loss in *Saccharomyces cerevisiae* is suppressed by DNA damage checkpoint functions. Genetics 159: 1501–1509.

Krogh, B. O., and L. S. Symington, 2004 Recombination proteins in yeast. Annu. Rev. Genet. 38: 233–271.

Lemoine, F. J., N. P. Degtyareva, K. Lobachev, and T. D. Petes, 2005 Chromosomal translocations in yeast induced by low levels of DNA polymerase: a model for chromosome fragile sites. Cell 120: 587–598.

Lisby, M., R. Rothstein, and U. H. Mortensen, 2001 Rad52 forms DNA repair and recombination centers during S phase. Proc. Natl. Acad. Sci. USA 98: 8276–8282.

McCulley, J. L., and T. D. Petes, 2010 Chromosome rearrangements and aneuploidy in yeast strains lacking both Tel1p and Mec1p reflect deficiencies in two different mechanisms. Proc. Natl. Acad. Sci. USA 107: 11465–11470.

Mortimer, R. K., R. Contopoulou, and D. Schild, 1981 Mitotic chromosome loss in a radiation-sensitive strain of the yeast *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA 78: 5778–5782.

Pontecorvo, G., 1956 The parasexual cycle in fungi. Ann. Rev. Micro. 10: 393–400.

Shrivastav, M., L. P. De Haro, and J. A. Nickoloff, 2008 Regulation of DNA double-strand break repair pathway choice. Cell Res. 18: 134–147.

St. Charles, J., M. L. Hamilton, and T. D. Petes, 2010 Meiotic chromosome segregation in triploid strains of *Saccharomyces cerevisiae*. Genetics 186: 537–550.

St. Charles, J., E. Hazkani-Covo, Y. Yin, S. L. Andersen, F. S. Dietrich *et al.*, 2012 High-resolution genome-wide analysis of irradiated (UV and gamma rays) diploid yeast cells reveals a high frequency of genomic loss of heterozygosity (LOH) events. Genetics 190: 1267–1284.

Torres, E. M., T. Sokolsky, C. M. Tucker, L. Y. Chan, M. Boselli *et al.*, 2007 Effects of aneuploidy on cellular physiology and cell division in haploid yeast. Science 317: 916–924.

Wei, W., J. H. McCusker, R. W. Hyman, T. Jones, Y. Ning *et al.*, 2007 Genome sequencing and comparative analysis of *Saccharomyces cerevisiae* strain YJM786. Proc. Natl. Acad. Sci. USA 194: 12825–12830.

Yoshida, J., K. Umezu, and H. Maki, 2003 Positive and negative roles of homologous recombination in the maintenance of genome stability in *Saccharomyces cerevisiae*. Genetics 164: 31–46.

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