DOI: 10.1534/genetics.108.089250

A Genetic Screen for Increased Loss of Heterozygosity in *Saccharomyces cerevisiae*

Marguerite P. Andersen, Zara W. Nelson, Elizabeth D. Hetrick and Daniel E. Gottschling[1]

Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, Washington 98109

Manuscript received March 14, 2008
Accepted for publication April 17, 2008

ABSTRACT

Loss of heterozygosity (LOH) can be a driving force in the evolution of mitotic/somatic diploid cells, and cellular changes that increase the rate of LOH have been proposed to facilitate this process. In the yeast *Saccharomyces cerevisiae*, spontaneous LOH occurs by a number of mechanisms including chromosome loss and reciprocal and nonreciprocal recombination. We performed a screen in diploid yeast to identify mutants with increased rates of LOH using the collection of homozygous deletion alleles of nonessential genes. Increased LOH was quantified at three loci (*MET15*, *SAM2*, and *MAT*) on three different chromosomes, and the LOH events were analyzed as to whether they were reciprocal or nonreciprocal in nature. Nonreciprocal LOH was further characterized as chromosome loss or truncation, a local mutational event (gene conversion or point mutation), or break-induced replication (BIR). The 61 mutants identified could be divided into several groups, including ones that had locus-specific effects. Mutations in genes involved in DNA replication and chromatin assembly led to LOH predominantly via reciprocal recombination. In contrast, nonreciprocal LOH events with increased chromosome loss largely resulted from mutations in genes implicated in kinetochore function, sister chromatid cohesion, or relatively late steps of DNA recombination. Mutants of genes normally involved in early steps of DNA damage repair and signaling produced nonreciprocal LOH without an increased proportion of chromosome loss. Altogether, this study defines a genetic landscape for the basis of increased LOH and the processes by which it occurs.

HETEROZYGOUS alleles were first used in the early 20th century to examine mutation frequencies in somatic cells of diploid organisms such as maize and Drosophila (Emerson 1929; Demerec 1932). In the ensuing decades, these ideas were refined and heterozygosity was also used to discover and monitor mitotic recombination and chromosome segregation in somatic cells (Stern 1936; Pontecorvo *et al.* 1954; Roper and Pritchard 1955).

The inactivation of a functional allele at a heterozygous locus took on new meaning in studies of cancer, as the concept of tumor suppressor genes and their significance emerged (reviewed in Brown 1997). These ideas are encapsulated by a study in the 1980s of patients who had a predisposition to retinoblastoma (Cavenee *et al.* 1983). Patients who were heterozygous at the Rb locus, with one wild-type allele and one nonfunctional allele, had a high incidence of tumors that had lost the wild-type allele of Rb in somatic cells. There are several mechanisms by which the normal allele of Rb could become nonfunctional, but two pathways predominated to inactivate the wild-type allele: loss of part or all of the chromosome (a hemizygous state) or a recombination event that replaced the wild-type allele with the mutant allele from the homologous chromosome (a homozygous state) (reviewed in Carr and Gottschling 2008). These genetic changes became known as loss of heterozygosity (LOH) events. Other events that can inactivate a wild-type tumor suppressor gene such as point mutations, small deletions, or epigenetic changes can mimic LOH phenotypically, but are not loss of heterozygosity in the true sense.

Cancer is generally considered an age-associated disease that is driven by somatic genetic changes (Depinho 2000). By middle age it is thought that a sufficient number of changes accumulate to initiate carcinogenesis (Knudson 2001). However, on the basis of rates of spontaneous mutation observed in human cells, the steady accumulation of mutations does not account for the number of genetic changes that are present in most tumors (Lengauer *et al.* 1998; Bielas *et al.* 2006). This led to the hypothesis that one of the early steps in cancer progression is a genetic change leading to a higher than normal rate of mutation, creating a "mutator phenotype" that increases the likelihood of subsequent genetic events (Loeb 1991; Loeb *et al.* 2003). Colon cancer provides an example that supports this hypothesis; common mutations found in colon cancer cells increase genome instability (Grady 2004).

[1]*Corresponding author:* Fred Hutchinson Cancer Research Center, 1100 Fairview Ave. N., P.O. Box 19024, Seattle, WA 98109.
E-mail: dgottsch@fhcrc.org

The budding yeast, *Saccharomyces cerevisiae*, has provided more details about spontaneous LOH than has been possible in other organisms (Esposito and Bruschi 1993; Acuna *et al.* 1994; Hiraoka *et al.* 2000; McMurray and Gottschling 2003; Barbera and Petes 2006). In yeast, there are methods to identify the cell division in which an LOH event occurs and to determine the types of genomic changes that occurred in each cell. While most spontaneous LOH in diploid yeast occurs primarily through mitotic recombination (Acuna *et al.* 1994), all the same types of LOH found in tumors are also observed in yeast. In addition, an LOH event in yeast can be easily classified as either reciprocal (LOH occurs in both cells) or nonreciprocal (one cell undergoes LOH while the other remains heterozygous) (reviewed in Carr and Gottschling 2008). This has facilitated a better mechanistic understanding of LOH events.

Studies in *S. cerevisiae* have also served as a rich resource for defining the genetic basis of virtually all processes involved in genome maintenance and integrity (reviewed in Paques and Haber 1999; Aylon and Kupiec 2004; Krogh and Symington 2004; Shrivastav *et al.* 2008). However, defining the genetic determinants that lead to increased spontaneous LOH has been more limited. Most large-scale genetic screens examine mutants for changes in viability when exposed to DNA damaging agents or for synthetic genetic interactions with other mutant genes known to be involved in genome integrity (reviewed in Chang *et al.* 2006). Other screens that specifically identified mutants with increased genomic instability were also carried out in haploid cells (*e.g.*, Huang *et al.* 2003). The information gained in these haploid studies is unlikely to provide an accurate prediction of mutations that will increase LOH in diploid cells. Besides the obvious fact that there is no homologous chromosome in haploid cells, regulation of recombination and DNA repair processes between haploid and diploid yeast cells is different (Frank-Vaillant and Marcand 2001; Kegel *et al.* 2001; Ooi *et al.* 2001; Valencia *et al.* 2001). Thus, haploid genetic analyses are likely to provide only a portion of the genetic landscape that will be relevant to LOH.

A recent screen that did specifically examine LOH, focused on chromosome loss but did not characterize recombination-based LOH in diploid cells (Yuen *et al.* 2007). Furthermore, in other studies where selected mutants were analyzed for recombination-based LOH, it was mostly in cells where double-stranded breaks were induced by expressing an endonuclease; spontaneous LOH events have been examined in a limited number of mutants (*e.g.*, Esposito *et al.* 1994; Signon *et al.* 2001; Ajima *et al.* 2002; Daigaku *et al.* 2004).

We are interested in identifying genes, that when defective, can lead to a mutator phenotype specifically for LOH. Ultimately, our goal is to develop a basic understanding of how these defects lead to LOH. To this end, we performed a genomewide screen for deletion mutants with increased LOH at the *MET15* locus in *S. cerevisiae*. We analyzed the resulting candidate mutants both quantitatively and qualitatively for LOH on three chromosomes. Here we describe how the screen was performed and our findings.

MATERIALS AND METHODS

Growth media: Rich (YEP) and synthetic (YC) media for growth of *S. cerevisiae* have been described previously (Van Leeuwen and Gottschling 2002) and are available on the lab Web site: http://www.fhcrc.org/science/labs/gottschling/yeast/.

In synthetic media containing G418, clonNAT, and/or hygromycin, monosodium glutamate (MSG) was used as the nitrogen source instead of ammonium sulfate (Tong *et al.* 2001).

LOH screen: The homozygous diploid yeast deletion strains (Giaever *et al.* 2002) were screened for increased frequency of LOH at *MET15*. Two hundred microliters YEPD medium was inoculated with 2-μl deletion strains using a 96-pin replicator (VP Scientific). Strains were incubated at 30° for 3 days and then 2 μl of culture was pinned onto solid media containing 0.7 mg/ml lead nitrate (McMurray and Gottschling 2003) in omni trays (Nalge Nunc, NY) in triplicate and incubated at 30° until color developed. Each deletion strain was then scored for black sectors within the outgrowth. Strains with at least two black sectors on all three replicates were rescreened in the same manner. Deletion strains that exhibited at least two black sectors on each plate during this second round were subjected to further analysis.

Plasmid construction: pDA1 was constructed by cleaving pRG356 (gift from R. Gardner) with *Bam*HI and *Bgl*II to remove the *SAN1* gene and place GFP expression under control of the *TDH3* promoter. pDA2 was made by inserting the P_{TDH3}-*GFP* fragment from pDA1 immediately upstream of *HIS3* in pUC9-HIS3 (Gottschling *et al.* 1990). The P_{TDH3}-*GFP* fragment was PCR amplified from pDA1 using primers 3tdh3-gfp and 5tdh3gfp-2 (see supplemental Table S1 for all primer sequences). Both pUC9-HIS3 and the P_{TDH3}-*GFP* fragment were digested with *Sal*I and ligated together.

Multiple heterozygous markers strain construction: The multiple heterozygous markers (MHM) strain background was created in a series of eight steps, and all the relevant genotypes for the strains can be found in supplemental Table S2. UCC7530 was created by transforming the *TRP1* gene into the *met15Δ0* locus of BY4705 using primers met15RS3 and met15RS5 (supplemental Table S1) and template pRS304 (Sikorski and Hieter 1989). UCC7531 was created by replacing the *SAM2* gene with *ADE2* in UCC7530. The *ADE2* gene was amplified from pRS302 (Sikorski and Hieter 1989) using primers sam2ko5 and sam2ko3 (supplemental Table S1). UCC7532 was created by inserting the *NatMX* cassette into a noncoding region (1,515,634–1,515,738) on the right arm of chromosome IV in UCC7531. The *NatMX* cassette was amplified from pRS40Nat [gift from Fred van Leeuwen: *KAN* replaced with *NAT* in pRS400 (Brachmann *et al.* 1998)] using primers MarthaN/H2L and MarthaN/H2R (supplemental Table S1).

UCC780 was made by replacing the *SAM2* gene with *URA3* in UCC762, using pRS306 as a template with sam2ko5 and sam2ko3 as PCR primers (supplemental Table S1). UCC762 was made by transforming a *MET15* PCR product into BY4705a

(Dubois *et al.* 2002) to restore the wild-type allele. UCC780 and UCC762 were gifts from M. McMurray. UCC7534 was created by inserting the *HphMX* cassette into a noncoding region (1,515,634–1,515,738) on the right arm of chromosome IV in UCC780. The HphMX cassette was amplified from plasmid pRS40HYG (gift from Fred van Leeuwen: *KAN* replaced with *HYG* in pRS400) using primers MarthaN/H2L and MarthaN/H2R.

UCC7532 and UCC7534 were mated to create diploid UCC7536. UCC7537 was created by replacing one copy of *HIS4* with the *HIS3, P_{TDH3}-GFP* cassette from plasmid pDA2, using primers Martha His4R and Martha His4L (supplemental Table S1), at the *HIS4* locus of UCC7536. UCC7542 was created by replacing one copy of *LEU3* with the *LEU2* gene, amplified from pRS305 using primers MarthaLeu3R and MarthaLeu3L (supplemental Table S1), in UCC7537.

UCC7542 was sporulated to produce UCC7540 and UCC7541. All strain construction was verified by PCR and Southern blot analysis. UCC7540 and UCC7541 were mated to create the isogenic wild-type MHM strain, UCC7800.

Multiple heterozygous markers deletion mutant strain construction: Diploid MHM deletion mutants were created in a series of three steps. Individual haploid deletion mutants of each mating type were made, and then the haploids were mated to create a diploid strain that is heterozygous for the LOH markers and homozygous for the respective deletion alleles.

UCC7540 and UCC7541 are the parents of these deletion mutants. Individual haploid mutants (supplemental Table S3) were created by integrating PCR fragments containing the KanMX deletion allele into the target locus of both UCC7540 and UCC7541. The KanMX cassette along with 200–300 bp of homology was amplified from the corresponding yeast knockout collection strains (Johnston *et al.* 2002) using primers "YFG"A and "YFG"D described in supplemental Table S4 and a multiwell transformation protocol (http://www-sequence.stanford.edu/group/yeast_deletion_project/transprot.html). Deletions were verified by PCR analysis using primers YFGA and YFGB (supplemental Table S4).

Half-sector assay: The following assay was performed on each diploid MHM deletion strain, listed in supplemental Table S3, to calculate the rate of LOH. MHM deletion mutant strains were grown on selective media plates [YC(msg) −trp −his −met −ade −ura −leu +ClonNAT +hygromycin] to maintain heterozygosity. Four colonies from each deletion mutant were analyzed. Two colonies each, from two independent mutant strain isolates were picked and resuspended in PBS at ~3000 cells/ml. For color detection, 0.5 ml was plated on large plates (150 mm × 15 mm) of lead nitrate media and incubated at 30° for 3–10 days. The rate of LOH, per cell division, was determined by obtaining the frequency of half-sectored colonies, either red/white or black/white among total colonies, excluding those colonies that were completely colored (McMurray and Gottschling 2003): rate of LOH = half-sectored colonies ÷ [all colonies − (2 × fully colored colonies)]. (Fully colored colonies are no longer heterozygous and therefore cannot undergo further LOH. Fully colored colonies account for approximately one-half of the homozygous colonies in the population, so the total number of homozygous colonies was estimated by doubling the number of fully colored colonies.)

The LOH rates of all four colonies for each MHM mutant were analyzed by chi-square contingency-table analysis (Prism software) to determine whether the rates were not different from one another with 95% confidence. Isolates that appeared to be different from the others were excluded. In some cases, plating was repeated or a third mutant isolate was tested. The final data for each deletion was pooled to determine the rate of LOH at each locus. Fisher's exact test (Prism software) was used to determine the probability (*P*-value) that the rate of LOH was different between each mutant and the isogenic wild-type strain, UCC7800.

Phenotypic analysis of half-sectored colonies: Cells from each portion of a half-sectored colony were picked and plated onto color detection media to isolate individual colonies. Single colonies representing each half of the original half-sectored colony were then patched onto rich YEPD media and subsequently replicated onto selective media for phenotypic analysis. These data were used to determine whether events were reciprocal or nonreciprocal and whether LOH events were local or occurred over >50 kbp. In some cases, cells from analyzed patches were also used to create genomic DNA for quantitative Southern analysis.

Phenotypic analysis of LOH at the MAT locus: This assay was adapted from the quantitative mating assay (Sprague 1991) and performed on diploid wild-type and deletion mutants. Heterozygous deletion mutants were grown on selective media to maintain heterozygosity. Two isolated colonies of each mutant were suspended in YC −his liquid media and incubated overnight at 30°. The PT-1 (*MAT***a** *ilv1 can1*) (Dorer *et al.* 1995) (gift from F. Cross) mating tester strain was grown in liquid YEPD overnight at 30°. Cells (2×10^6) of the diploid MHM deletion mutant and 1×10^8 PT-1 cells were mixed and collected on a filter (Osmonics MAGNA nylon 1.2 μm 47 mm, material no.1213797). Filters were transferred (cell side up) to YEPD plates and incubated for 4 hr at 30° to allow cells to mate. Mutant cells that have lost the *MAT***a** locus become competent to mate with the *MAT***a** PT-1 cells. Cells from the filter were then resuspended in PBS, plated at several dilutions onto Ymin, which selects for deletion mutants that mated with PT-1 cells, and Ymin +His +Lys, which is permissive for growth of deletion mutants but not PT-1 cells, and incubated at 30° until colonies were large enough to count. Total colonies on both types of media were counted to determine the frequency of LOH at *MAT*. Colonies on Ymin plates were then analyzed for GFP expression using a GFP colony illuminator to determine the frequency of chromosome loss at *MAT* (Cronin and Hampton 1999). The frequencies of both LOH and chromosome loss at the *MAT* locus were compared with wild-type frequencies using a Mann–Whitney test (Prism). Median frequencies were determined and displayed in Figure 8.

Quantitative Southern analysis: By analyzing both halves of a half-sectored colony, the copy number of genes from LOH events was examined. In diploid cells, the *NDC1* gene must be maintained in precisely two copies for cell viability, which allows it to be used as a reference for copy number in quantitative Southern blotting (Chial *et al.* 1999). In addition to NDC1, probes were made for both opposing heterozygous markers on the right arm, as well as a gene on the left arm (chromosome XII: *MET15*, *TRP1*, and *ISA1*; chromosome IV: *ADE*2, *URA3*, and *SHR3*). Analysis of band intensity gives accurate information regarding copy number and allowed us to distinguish between BIR, chromosome loss/nondisjunction, and truncation.

Quantitative Southern analysis was performed on DNA samples from 24 deletion mutants for chromosome XII and 23 deletion mutants for chromosome IV. For each mutant, both halves of at least 8 half-sectored colonies were examined for each chromosome. Genomic DNA was prepared using standard techniques (Adams *et al.* 1998).

For chromosome XII analysis, DNA from nonreciprocal half-sectored colonies was digested using *Eco*RI and *Xmn*I. Probes for *TRP1*, *MET15*, *ISA1*, and *NDC1* were made by PCR with genomic DNA as a template and using the following oligos: trp1shortprobefwd, trp1shortproberev, met15short-

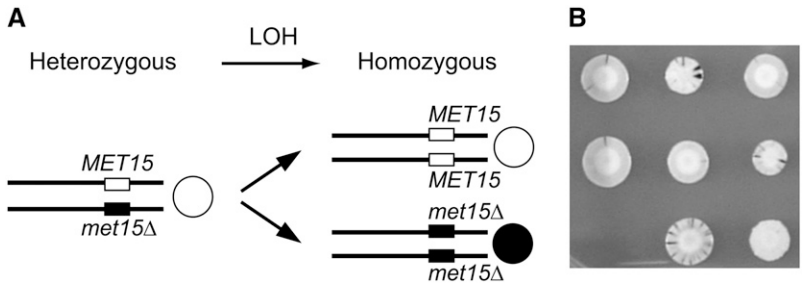


FIGURE 1.—Loss of *MET15* function serves as a robust assay for LOH. (A) In the presence of Pb^{+2} ion, colonies formed by cells with a functional copy of *MET15* appear normal cream colored, while cells lacking a functional *MET15* allele are black. LOH events in heterozygous cells (*MET15/met15*Δ) that become homozygous (*met15/met15*), result in a black colony. (B) A portion of a 96-well plate from the LOH screen is shown. Liquid cultures of *MET15/met15*Δ deletion mutants were applied in a 96-well format on Pb^{+2}-containing agar medium. Upon growth and color development, LOH events at *MET15* appeared as black sectors.

probefwd, met15shortproberev, isa1probefwd, isa1proberev, ndc1probefwd, and ndc1proberev (supplemental Table S1). Similarly, chromosome IV was analyzed using *Eco*RI and *Ssp*I digests, and probes for *URA3*, *ADE2*, *SHR3*, and *NDC1* were made with the following oligos: ura3shortprobefwd, ura3shortproberev, ade2shortprobefwd, ade2shortproberev, shr3shortprobefwd, shr3shortproberev, ndc1probefwd, and ndc1proberev (supplemental Table S1).

DNA samples were separated on 1% agarose gels and transferred to a nylon membrane (Osmonics MAGNA) using standard techniques. Probes were made using 10 rounds of PCR in buffer [68 mM Tris pH 8.5, 16mM $(NH_4)_2SO_4$, 2.5 mM $MgAc_2$, 0.01% Triton X-100, 5% glycerol, 0.2 mM dNTPs] with [α-^{32}P]dCTP (Perkin Elmer, 3000 Ci/mmol), using gel-isolated PCR product as template. Probes were combined and then hybridized to the blots using standard techniques. Multiple exposures of the blot were scanned using a Typhoon phosphoimager and analyzed with Image Quant software (GE Healthcare Life Sciences).

RESULTS AND DISCUSSION

A genetic screen for increased loss of heterozygosity: To identify loss-of-function mutations that would increase the incidence of LOH, a diploid set of strains with homozygous deletions of each nonessential gene was screened for mutants that produced increased levels of LOH at the *MET15* locus. This collection of strains is heterozygous at the *MET15* locus (*MET15/met15*Δ) (GIAEVER *et al.* 2002). Loss of Met15 function in yeast colonies is easily detected. When placed on media containing Pb^{+2}, cells lacking functional Met15 produce colonies that are black, whereas cells that are heterozygous or homozygous wild type for *MET15* yield colonies that are creamy white in appearance (Figure 1A) (COST and BOEKE 1996). We took advantage of this phenotype in screening the set of homozygous deletion strains. If *MET15/met15*Δ cells carry a deletion allele that results in elevated rates of LOH compared to wild type, then *met15*Δ*/met15*Δ cells are expected to arise at a higher frequency as cells form a colony. When grown on Pb^{+2}-containing media, such mutant hyper-LOH cells will produce many more black sectors in a colony compared to wild-type cells.

We screened the collection of ~4800 nonessential homozygous deletion mutants for increased sectoring. A cell suspension of each strain was applied in triplicate onto plates of Pb^{+2}-containing media in 96-well format and grown to detect colored sectors. Out of the entire collection, 132 deletion mutants were judged to have higher rates of sectoring on all three plates (see MATERIALS AND METHODS and Figure 1B) and were selected for further analysis.

A strain for monitoring LOH on three chromosomes: To verify that the candidate mutants did indeed have elevated rates of LOH, and to characterize them further, a diploid strain with MHM was created (Figure 2). In the MHM strain, three chromosomes are each marked at two loci to permit quantitative and qualitative analysis of LOH (Figure 2).

Spontaneous LOH in diploid yeast occurs primarily through mitotic recombination which is initiated by DNA damage along the chromosome and is often accompanied by LOH at all centromere-distal loci (PAQUES and HABER 1999; AGUILERA *et al.* 2000). Previous work in our lab confirmed that most spontaneous LOH on chromosomes IV and XII occurs by recombination and that distal markers are more likely to undergo spontaneous LOH than centromere-linked loci (MCMURRAY and GOTTSCHLING 2003). To maximize the chance of observing LOH events we marked distal loci on the two longest chromosome arms in *S. cerevisiae*. The *MET15* locus on the right arm of chromosome XII and the *SAM2* locus on the right arm of chromosome IV are ≥1.5 Mb and ~1 Mb from their respective centromeres (CHERRY *et al.* 1997). The relatively high rates of LOH at these loci permitted use of half-sector analysis to assess rates of LOH in each of the mutants (MCMURRAY and GOTTSCHLING 2003). In addition, markers distal to these two loci were inserted, so that LOH events that occurred at *MET15* or *SAM2* could be assessed as to whether they were local (*i.e.*, point mutation, short gene conversion, and gene deletion) or part of a larger change to the chromosome (*i.e.*, recombination, truncation, and chromosome loss) (Figure 3). One copy of *MET15* at its endogenous locus on the right arm of chromosome XII was replaced with *TRP1* (*MET15/*met15Δ::*TRP1*). For a distal marker, LEU3, which lies ~300 kb from *MET15*, was used. By inserting the *LEU2* gene at the *LEU3* locus on one homolog, selection for heterozygosity can be main-

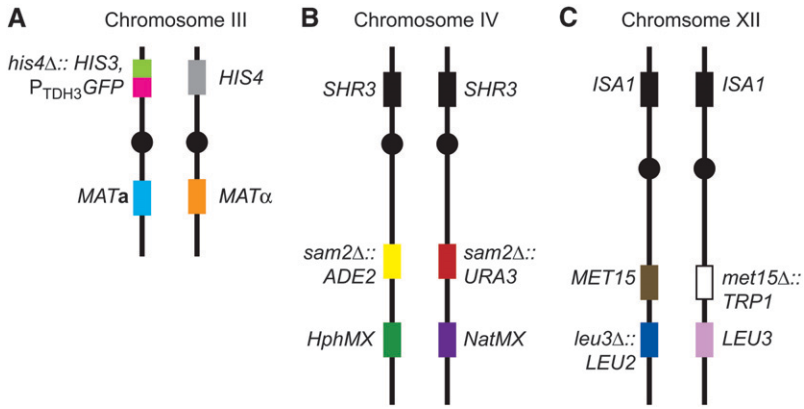


FIGURE 2.—The multiple heterozygous markers (MHM) strain enables quantitative and qualitative study of LOH on three chromosomes. All chromosomes are shown with the left arm up. Chromosomes III, IV, and XII were each marked with two pairs of selectable heterozygous markers. One locus (on the right arm of each chromosome) is marked for quantitative measurement of LOH and a second locus is marked to facilitate qualitative analysis of the LOH events. (A) The naturally heterozygous MAT locus on the right arm of chromosome III was used for quantitative measurement of LOH. To enable detection of chromosome loss, one homolog of chromosome III was marked with *HIS3* and *GFP* at the *HIS4* locus on the left arm. (B) Chromosome IV contains heterozygous markers on the right arm only. The *ADE2* marker, at the *SAM2* locus of one homolog, was used for quantitative measurement of LOH. The *URA3* marker inserted at the *SAM2* locus of the homologous chromosome was used to distinguish between reciprocal and nonreciprocal events. *HphMX* and *NatMX* are located distal to *SAM2* in a noncoding region. These markers were used to distinguish between single and multilocus LOH events. (C) *MET15*, on the right arm of chromosome XII, was used for quantitative measurement of LOH. The opposite homolog is marked with *TRP1* to distinguish between reciprocal and nonreciprocal LOH. The *LEU3* locus contains the *LEU2* marker on one homolog to distinguish between single and multilocus LOH events.

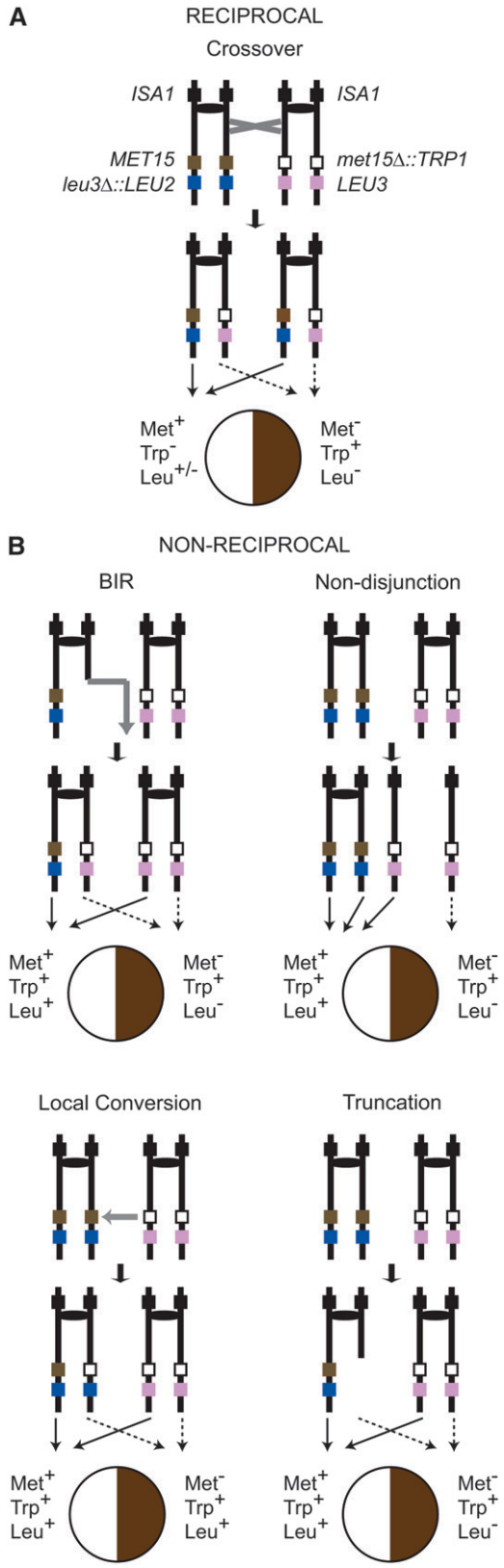
tained in this strain where the normal locus of *LEU2* was deleted on both homologs. Cells that are heterozygous (*LEU3*/leu3Δ::*LEU2*) grow normally in the absence of leucine. Chromosome IV was arranged similarly, using the color marker *ADE2* at the *SAM2* locus with *URA3* in opposition (*sam2*Δ::*ADE2*/*sam2*Δ::*URA3*). Drug resistance genes (*HphMX* and *NatMX*) were inserted on opposite homologs into a noncoding region 61 kb distal to the *SAM2* locus (GOLDSTEIN and MCCUSKER 1999).

Chromosome III was marked to monitor LOH and to identify events that resulted from chromosome loss. In a normal diploid *S. cerevisiae* cell the mating type locus, which is ~85 kbp from the centromere on the right arm of chromosome III, is heterozygous (*MAT***a**/*MAT*α). Such a diploid cell cannot mate with haploid **a** or α-cells. However, if an LOH event occurs at the *MAT* locus, it is easily detected, because the diploid cell gains the ability to mate with an appropriate haploid cell. On the left arm of chromosome III, heterozogosity was created by replacing the *HIS4* gene with *HIS3* and *GFP* on one homolog (*HIS4*/*his4*Δ::*HIS3*, P_{TDH3}*GFP*). In the MHM strain, LOH is detected when diploids mate as α-cells. When there is concomitant loss of GFP expression it indicates that LOH is due to loss of the entire chromosome. In total, this strain allows for an independent test of whether LOH at *MET15* has increased, detection of LOH on three different chromosomes, and distinction between different mechanisms of LOH.

In the primary screen to identify mutants with increased LOH, colonies with multiple colored sectors were used to screen for candidates. The amount of hypersectoring within a colony provided a semiquantitative level of LOH produced in a mutant strain. With the MHM strain, we can use colony color changes to calculate a rate of LOH at the *MET15* or *SAM2* locus, using half-sectored colony analysis (JOHNSTON 1971; ZIMMERMANN 1973). A half-sectored colony is assumed to represent each daughter cell produced from the first division of a cell that was plated onto solid medium. As the cells continue to divide to form a colony, each half represents the fate of the early daughter cells. Canonically, an LOH event is thought to initiate in the G_2 phase of the progenitor cell and the phenotypic outcome of the process is manifested in the two daughter cells (outlined in Figure 3). This is certainly true when LOH occurs via reciprocal recombination. However, for nonreciprocal events, a half-sector colony could also arise as the result of a process that occurred after the first cell division in the G_1 phase of one of the resulting daughter cells but not in the other (WILDENBERG 1970). This alternative explanation for producing a half-sector means that if all LOH events occurred in G_1 of one daughter cell rather than in G_2 of the progenitor cell, then an extra cell division must be considered in calculating the rate. Regardless of this potential caveat, a twofold or greater increase in the number of half-sectored colonies indicates there is a change in the normal maintenance of genome integrity.

Rate of LOH at *MET15* on chromosome XII: To determine whether the deletion mutations identified in the primary screen did indeed cause increased LOH at *MET15*, each mutant allele was introduced as a homozygous mutation into the MHM strain and the rate of LOH at the *MET15* locus was determined by half-sector analysis (described in MATERIALS AND METHODS). Of the 132 deletion mutants identified in the primary screen, 114 homozygous deletion strains were successfully created in the MHM strain background (supplemental Table S5). Of these, 61 had a statistically significant increase ($P \leq 0.01$) of twofold or more in the rate of LOH at *MET15* compared to wild-type cells (Figure 4 and supplemental Table S6).

Rate of LOH at *SAM2* on chromosome IV: The 61 deletion mutants with confirmed hyper-LOH at the *MET15* locus were examined in the MHM strain for LOH at the *SAM2* locus (*sam2Δ::ADE2/sam2Δ::URA3*) on chromosome IV. Half-sector analysis was also used at *SAM2*, this time taking advantage of the *ADE2* phenotype; colonies are white when the gene is present, but red when functional *ADE2* is absent. Using the same criteria as described above, 38 of the 61 *MET15* hyper-LOH deletion mutants also had a *SAM2* hyper-LOH phenotype (Figure 4 and supplemental Table S6).



Identification of reciprocal and nonreciprocal LOH events on chromosomes IV and XII: The MHM strain was designed to distinguish between several types of LOH. Recombination via crossing over results in a reciprocal exchange, while other mechanisms of LOH result in a nonreciprocal exchange (Figure 3). When LOH occurs by crossing over, the result is two cells that are both homozygous at the affected locus, but the cells are distinguished in that they are homozygous for the opposing alleles (Figure 3A). By contrast, a nonreciprocal LOH event produces one cell that retains heterozygosity while the other cell becomes homozygous (Figure 3B).

Further phenotypic analysis of half-sectored colonies permitted us to determine whether an LOH event occurred by a reciprocal or nonreciprocal mechanism. The heterozygosity created at the *MET15* locus on chromosome XII is *MET15/met15Δ::TRP1*. When both halves of a black/white half-sectored colony were analyzed, the black half was Met^- and Trp^+, as expected (Figure 3). The white half of the colony was either Met^+ and Trp^-, indicating that LOH was reciprocal (Figure 3A), or Met^+ and Trp^+, indicating LOH was nonreciprocal (see Figure 3B). Consistent with previous studies (McMurray and Gottschling 2003), 73% of LOH events at *MET15* were reciprocal in the wild-type MHM strain, suggesting that most DNA lesions are repaired by a mechanism that includes a crossover event. The hyper-LOH mutants ranged from 0 to 88% reciprocal LOH at the *MET15* locus (Figure 5 and supplemental Table S6). In 55% of the mutants, the fraction of reciprocal events was significantly decreased relative to wild type ($P \leq 0.01$), suggesting that in these mutants an alternative mechanism of LOH was favored.

Figure 3.—Distinguishing between types of chromosome XII LOH events in the MHM strain. Phenotypes were determined for both halves of half-sectored colonies. Analysis of the *TRP1* marker in the white half of the colony was used to determine whether *MET15* LOH events were reciprocal (A) or nonreciprocal (B). The dark half of the colony was always Met^- and Trp^+. (A) Following a reciprocal LOH event (due to a crossover), the white half of the colony is Trp^-. (B) The white half of the colony resulting from a nonreciprocal LOH event is Trp^+. Analysis of distal markers, by growth phenotype on media lacking leucine, was used to distinguish between local LOH events (local gene conversion) and multilocus LOH events (BIR, nondisjunction or chromosome truncation). The black half of a colony resulting from a local gene conversion LOH event is Leu^+, whereas in any other nonreciprocal LOH it is Leu^-. [Note: in the absence of leucine, a *leu3Δ* allele yields slow growth (+/−) while *leu2Δ* does not grow (−).]

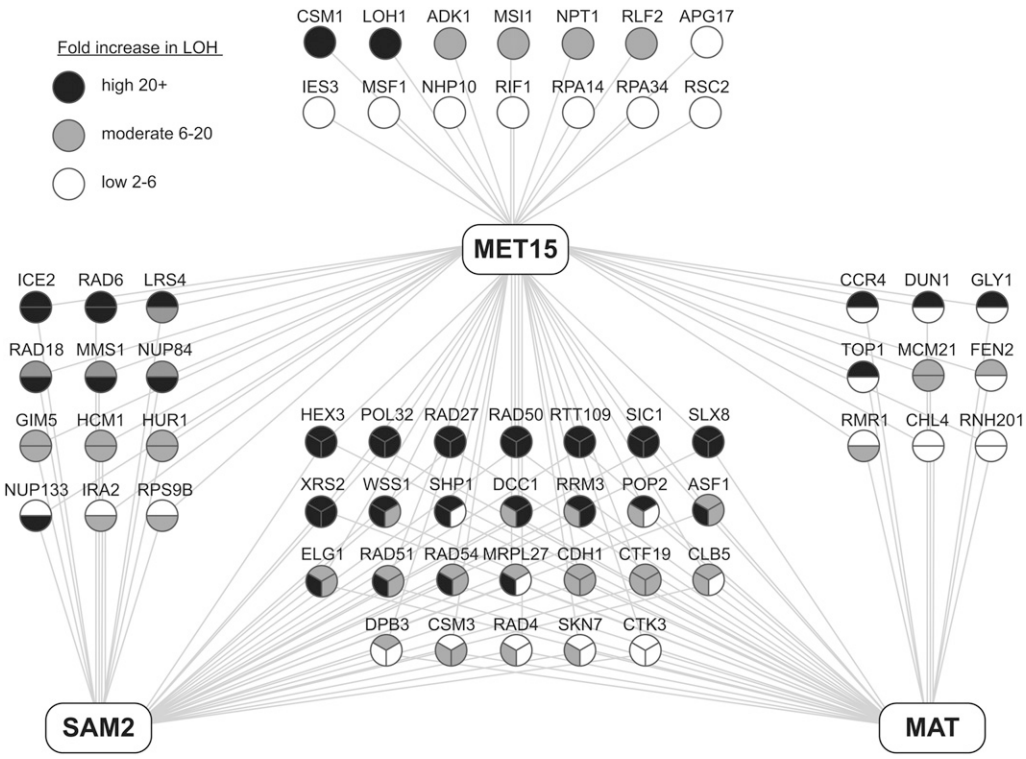


FIGURE 4.—Deletion alleles can have locus-specific effects on LOH. Each circular node corresponds to a mutant with increased LOH at *MET15*. Connecting lines are drawn between nodes (deletion mutants) and loci (*MET15*, *SAM2*, and *MAT*) indicating which deletion mutants have increased LOH at these loci. For the *MET15* and *SAM2* loci, the criteria for increased LOH was a twofold or greater increase in the rate of LOH relative to wild type and $P \leq 0.01$ (Fisher's exact test). The criteria for increased LOH at the MAT locus was a twofold or greater increase in the frequency of LOH relative to wild type and $P \leq 0.05$ (Mann–Whitney test). Nodes are shaded to represent the level of increased LOH as indicated. Where appropriate, nodes are divided into multiple parts to indicate different levels of increased LOH for each locus.

Similar analysis was also carried out at the *SAM2* locus (*sam2*Δ::*ADE2*/*sam2*Δ::*URA3*) on chromosome IV. The red half of the red/white half-sectored colony was always Ade$^-$ and Ura$^-$, as expected. Phenotypic analysis of the white half of the colony identified whether the LOH event was reciprocal or nonreciprocal. When the white half was Ade$^+$ and Ura$^-$, the event was reciprocal, and when it was Ade$^+$ and Ura$^+$ the event was nonreciprocal LOH. At *SAM2*, 36% of LOH events were reciprocal in the wild-type strain (Figure 6 and supplemental Table S6). In contrast to *MET15*, most of the LOH at *SAM2* normally occurs by a

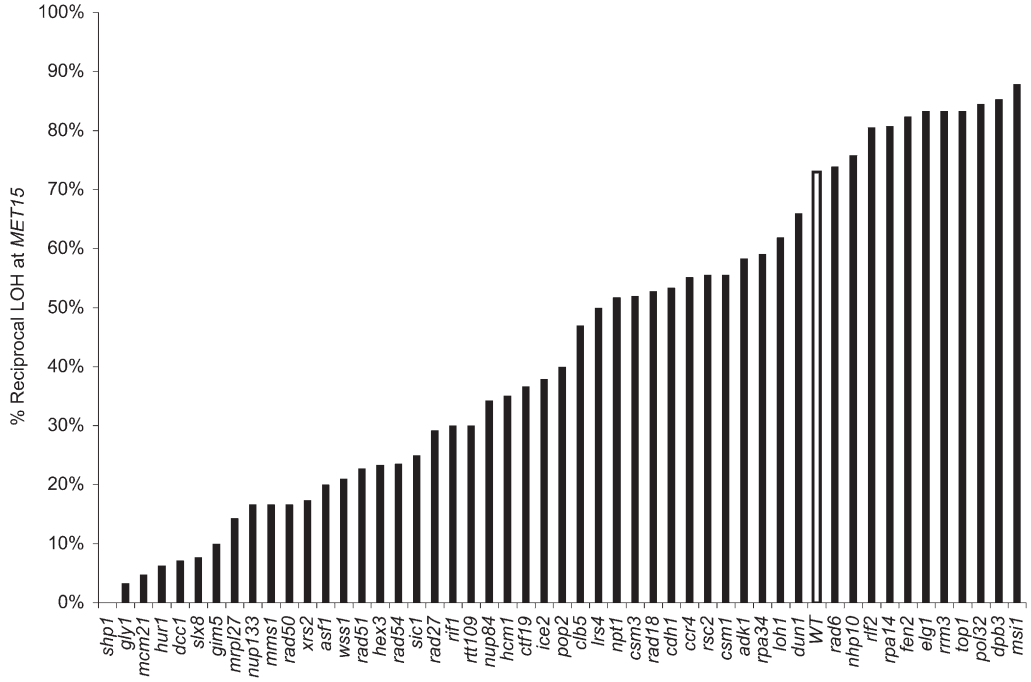


FIGURE 5.—Deletion alleles can affect the proportion of reciprocal LOH events at the *MET15* locus. The percentages of reciprocal LOH events at *MET15* in mutants with at least a fivefold increase in LOH at *MET15* are shown. Phenotypic analysis was used to determine whether LOH events were reciprocal or nonreciprocal as described in Figure 3.

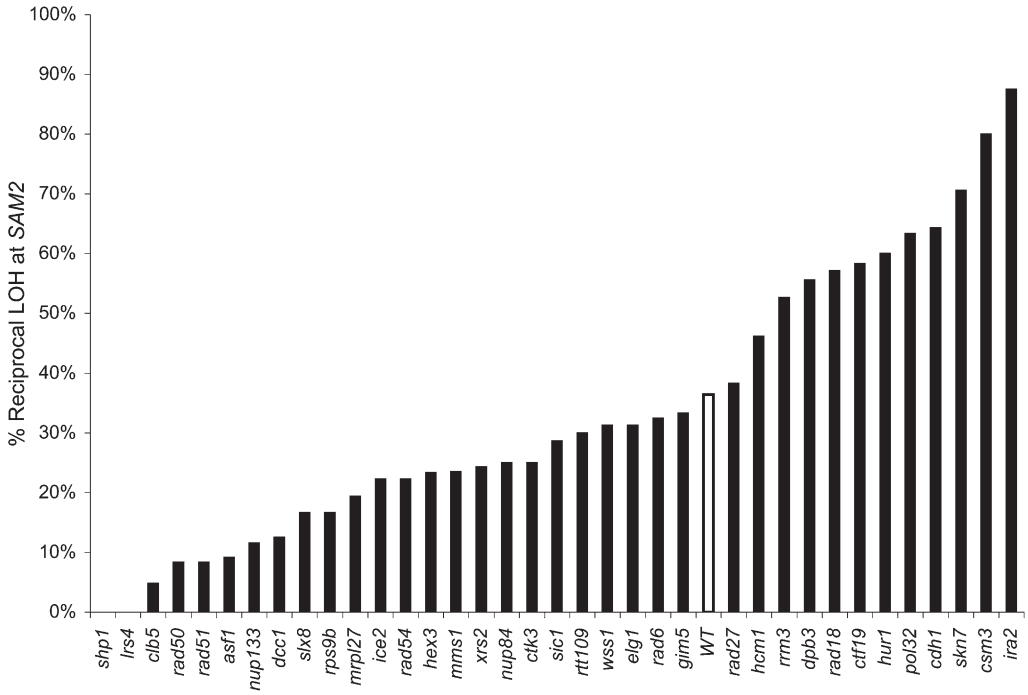


FIGURE 6.—Deletion alleles can affect the proportion of reciprocal LOH events at the *SAM2* locus. The percentages of reciprocal LOH events at *SAM2* in mutants with at least a fivefold increase in LOH at *SAM2* are shown. The analysis was carried out in the same manner as for Figure 5 and is outlined in supplemental Figure 1.

nonreciprocal mechanism. The hyper-LOH mutants ranged from 0 to 88% reciprocal recombination at *SAM2*. Due to the relatively low rate of LOH at *SAM2* (compared to *MET15*), it was difficult to determine for most of the mutants whether they differed significantly in the amount of reciprocal recombination compared to wild type. At best, we could assign only eight mutants as being different from wild type at *SAM2* ($P \leq 0.05$).

Most LOH events on chromosomes IV and XII affect large regions of the chromosome in wild-type and mutant strains: The markers distal to *MET15* (*leu3*Δ*::LEU2/LEU3*) and *SAM2* (*NatMX* and *HphMX*) made it possible to distinguish between local events, such as point mutation, gene conversion or local deletion, and long distance LOH events, such as BIR, chromosome loss, or truncation. For instance, if the distal markers remained heterozygous when markers at *SAM2* were homozygous, then it suggested a local LOH event had occurred at *SAM2*. For chromosome XII, few of the observed LOH events appeared to be local. In wild-type cells a single local event was observed out of 96 events analyzed, and in only three mutants (*rad18*Δ, *rtt109*Δ, and Δ*skn7*) did the number of local events elevate to account for ~6% of total LOH events. At the *SAM2* locus no local LOH events were observed in wild-type cells and only two local LOH events were seen in the mutants, accounting for ≤5% of LOH in *elg1*Δ and *gim5*Δ. Thus the mutants identified in this screen primarily have increased rates of LOH that affect large stretches of the chromosome arm, rather than causing increased LOH by point mutation, small deletion, or local gene conversion.

LOH on chromosome III: The markers on chromosome IV and XII facilitated the analysis of LOH events that occurred via recombination, however they did not lend themselves to identifying LOH events that occur by chromosome loss, typically as a result of nondisjunction. Neither chromosome IV nor XII appears to be able to be lost and produce a stable monosomic chromosome in a diploid cell (ALVARO *et al.* 2006). By contrast, one copy of chromosome III can readily be lost from a diploid cell and yield a stable cell line with a monosomic III (LIRAS *et al.* 1978; WAGHMARE and BRUSCHI 2005). As noted above, the MHM strain is heterozygous on the right arm of chromosome III at the *MAT* (*MAT***a**/*MAT*α) locus and on the left arm at *HIS4* (*HIS4*/*his4*Δ::*HIS3* P_{TDH3}*GFP*) (Figure 2A).

A quantitative mating assay was used to measure the frequency of LOH at the *MAT* locus (SPRAGUE 1991). MHM cells that were capable of mating were examined for GFP expression. Colonies that retained GFP expression arose from cells that had undergone LOH, but had not lost the *MAT***a**-containing chromosome III, while colonies without GFP expression arose from cells that had apparently lost the entire *MAT***a** chromosome (Figure 7). Of the 61 deletion mutants with hyper-LOH at *MET15*, 35 had a frequency of LOH at *MAT* that was statistically different from and at least twofold greater than the wild-type strain (Figure 4 and supplemental Table S6). When tested for GFP expression, 28 of those mutants (80%) had increased frequencies of chromosome III loss relative to wild type ($P \leq 0.05$, fold increase ≥2). This indicates that chromosome nondisjunction contributes to the increased *MAT* LOH in these mutants (Figure 8 and supplemental Table S6).

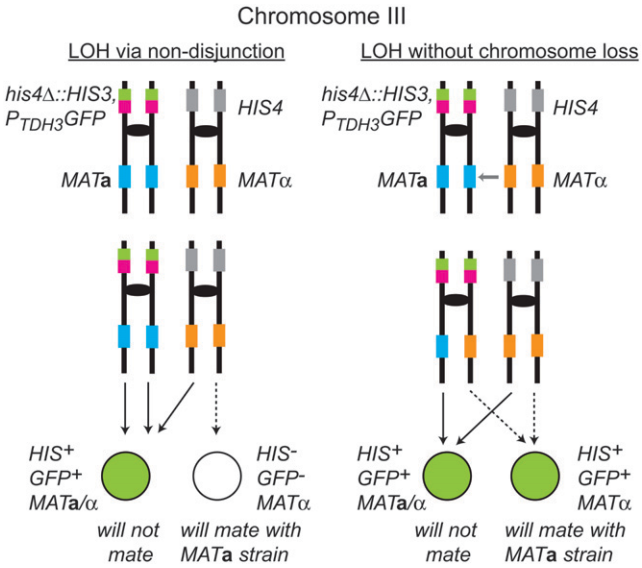


FIGURE 7.—The LOH and chromosome loss assay for chromosome III. Heterozygous markers on both arms of chromosome III were used to distinguish between loss of a chromosome III homolog and other types of LOH. A quantitative mating assay was used to measure LOH at the *MAT* locus on the right arm. Diploid cells that lose the *MAT***a** allele, an LOH event, are able to mate with a *MAT***a** haploid tester strain. Those cells that can mate are screened for GFP expression. When the entire chromosome is lost in the LOH event at *MAT*, colonies do not express GFP. When LOH affects only the right arm of the chromosome, such as in a recombination event, colonies are GFP^+.

The majority of nonreciprocal LOH events on chromosomes IV and XII appear to be due to BIR: Essentially all of the nonreciprocal LOH events on chromosomes IV and XII extended over large regions of the chromosome (data not shown), indicating that LOH may have occurred by BIR, truncation, or chromosome nondisjunction. We explored which of these pathways led to LOH in 23 mutants that proceeded predominantly (>60%) by nonreciprocal LOH on chromosome XII, were significantly elevated for LOH on both chromosome IV and XII, and had an increased LOH rate of at least 10-fold relative to wild type on either chromosome.

Quantitative Southern blot analysis was used to distinguish between the possible nonreciprocal LOH mechanisms (Figure 3B). The copy number of each of the marker alleles used on chromosomes IV and XII and a wild-type gene on the left arm of each chromosome was determined. By analyzing both halves of a half-sectored colony in this way, it was possible to establish the ultimate fate of chromosomes following an LOH event. Examining both halves of the colony was particularly informative in identifying nondisjunction events. Because chromosome IV and XII are not stably maintained as monosomes and undergo endoreduplication, it was not possible to distinguish between chromosome loss and BIR in a colony half that demonstrated nonreciprocal LOH (ALVARO *et al.* 2006). However, if cells in the other half of the colony, which do not display LOH in a nonreciprocal event, are trisomic with an extra copy of the homolog that is lost from the LOH cell, then it indicates that chromosome nondisjunction occurred. If these cells contain a normal complement of two chromosomes, then the event likely occurred by BIR. Truncations are easily distinguished from BIR and chromosome loss. Following truncation, there is only one copy of the marker that underwent LOH (in BIR there are two copies), while the gene on the other arm of the chromosome is maintained at two copies (there is only one copy following chromosome loss).

For most mutants, BIR was the sole mechanism of nonreciprocal LOH on chromosome IV and XII, just as it was in the wild-type cells (supplemental Table S7). A fraction of the LOH events in five mutants (*hcm1*Δ,

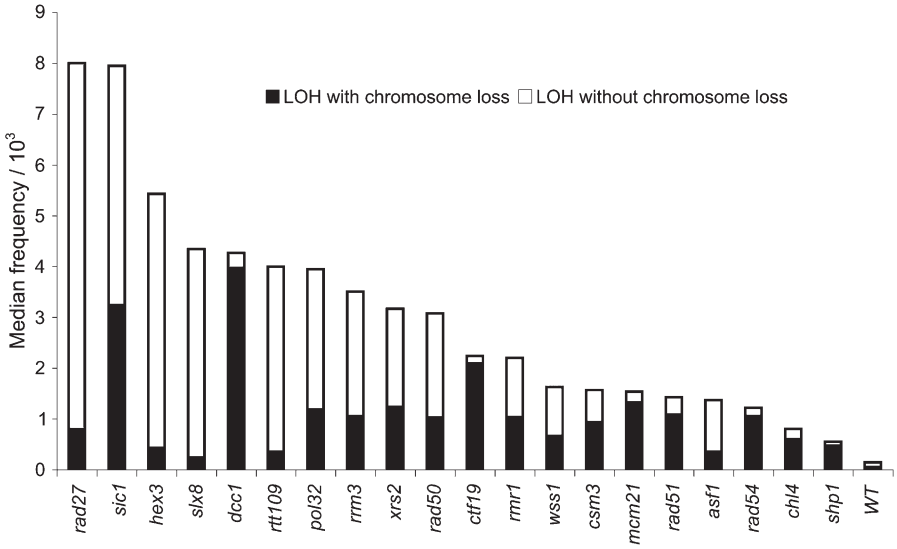


FIGURE 8.—Deletion alleles differentially affect the type of LOH event that occurs at the *MAT* locus. Mutants with at least a fourfold increase in the frequency of LOH at the MAT locus are shown. The median frequency of LOH at *MAT* is represented by the total height of the bars (both open and solid). The solid portion of the bar represents the median frequency of LOH due to chromosome III loss. Phenotypic analysis used to determine LOH and loss events is outlined in Figure 7.

*rps9b*Δ, *rtt109*Δ, *shp1*Δ, and *slx8*Δ) appeared to have chromosome loss/nondisjunction with endoreduplication (supplemental Table S7). Only two mutants, *asf1*Δ and *rad51*Δ, produced chromosome truncations, and these were observed only on chromosome IV (supplemental Table S7). Interestingly, just over half of the mutants displayed some fraction of complex rearrangements that were not easily interpreted as BIR, truncation, or chromosome loss (supplemental Table S7). These were likely the result of multiple genomic rearrangements that occurred during culture growth in this collection of strains with relatively unstable genomes.

Classifying mutants with increased LOH: By taking advantage of the *MET15/met15*Δ heterozygosity in the collection of diploid nonessential gene deletion mutant strains, we successfully performed a genomewide screen for mutants that increase the frequency of LOH events. By further characterizing LOH events on three different chromosomes, we have developed a genetic context for understanding how LOH can occur, and in the process have found that some mutations yield chromosome-specific LOH events.

We carried out phenotypic characterization on all of the mutants and organized them into groups with similar properties. On the basis of the robustness of the various assays performed and the statistical significance associated with the data, we were most confident using the values corresponding to changes in amounts of LOH at all three loci, the reciprocal character of LOH on chromosome XII, and the fraction of chromosome loss involved in LOH at chromosome III, as criteria for classifying the mutants. While there are a number of ways to define the groups, we present several groupings that we found particularly noteworthy.

The first classification includes mutants that had an exceptional increase in LOH predominantly at one of the three marked loci (summarized in Figure 4). A number of mutants had a greater effect at either the *MET15* or *SAM2* locus, while at the *MAT* locus there was a rather modest bias with a single allele (*rmr1*Δ). Such biases may be explained if the mutant gene product normally has a primary function of maintaining genome integrity at a locus (or loci) that resides in proximity to the affected marker. With regard to *MET15*, there is an obvious candidate locus—the rDNA array.

Deletion mutants that predominantly affect LOH on chromosome XII: The rDNA array consists of 100–200 copies of a 9.1-kb tandem repeat of ribosomal RNA genes (Petes and Botstein 1977) on the right arm of chromosome XII. The repetitive nature of the rDNA makes it inherently prone to recombination, however, mechanisms are in place that normally prevent this recombination (Burkhalter and Sogo 2004; Kobayashi *et al.* 2004; Huang *et al.* 2006; Johzuka *et al.* 2006; Li *et al.* 2006). It is easy to imagine that mutations that affect recombination at the rDNA array may result in increased LOH at distal loci on chromosome XII, including *MET15*, but would not have an effect upon loci on other chromosomes.

One of the well-established means of reducing rDNA recombination is via Sir2-dependent silencing. When *SIR2* is deleted, there is a 20-fold increase in LOH at *MET15* (McMurray and Gottschling 2003). About a quarter of the mutants with a *MET15* LOH bias showed a modest elevation of LOH (5- to 11-fold): *rpa34*Δ, *rpa14*, *rif1*Δ, *rlf2*Δ, *npt1*Δ, and *msi1*Δ. Each of these mutants had been previously shown to either reduce recruitment of Sir2 to the rDNA or reduce Sir2 enzymatic activity (Smith *et al.* 1999; Buck *et al.* 2002; Sandmeier *et al.* 2002).

The Lrs4 and Csm1 proteins act additively with Sir2 in rDNA silencing (Smith *et al.* 1999) and suppression of rDNA recombination (Huang and Moazed 2006). They appear to prevent unequal sister chromatid recombination by helping load cohesins onto the rDNA and maintain sister chromatids in proximity. In *lrs4*Δ, *csm1*Δ, or *sir2*Δ strains, unequal exchange increases by 20- to 30-fold, while in *lrs4*Δ *sir2*Δ or *csm1*Δ *sir2*Δ strains the increase was ~50-fold (Huang and Moazed 2006). We found that *lrs4*Δ and *csm1*Δ strains had extremely high rates of *MET15* LOH (272-fold and 117-fold above wild type, respectively), with modest or no increase in LOH at *SAM2* and *MAT* (Figure 4 and supplemental Table S6). It seems likely that the enormous increase in recombination between homologous chromosomes in these mutants is facilitated by the loss of cohesion between sister chromatids—in essence permitting the homologs to find each other more readily for recombination. Interestingly, in our analysis *top1*Δ strains had a very similar phenotypic profile to *lrs4*Δ and *csm1*Δ strains, with a 115-fold increase in *MET15* LOH relative to wild type. Like Lrs4 and Csm1, Top1 (topoisomerase I) is required for suppression of rDNA recombination (Christman *et al.* 1988) and rDNA silencing (Smith *et al.* 1999). While Top1 acts throughout the genome, it is highly enriched in the nucleolus where the rDNA resides (Edwards *et al.* 2000). The nucleolar enrichment of Top1 is mediated in part by interactions with Tof2 (Park and Sternglanz 1999) and Fob1, both of which are localized to the rDNA and are required for Lrs4- and Csm1-mediated rDNA silencing (Huang *et al.* 2006). Taken together these data suggest that Top1 mediates suppression of rDNA recombination in a similar manner as Lrs4 and Csm1.

Another possible explanation for a bias of LOH at *MET15* is that a locus on chromosome XII, such as the rDNA array, is exquisitely sensitive to perturbations in general genome maintenance. Indeed a subgroup of mutants with high rates of *MET15*-biased LOH (*dun1*Δ, *ccr4*Δ, *pop2*Δ, *adk1*Δ, and *gly1*Δ) have no obvious rDNA-specific interactions. However, they share the common property of regulating nucleotide pools. Ribonucleotide reductase (RNR) activity, which converts ribonu-

cleotides into deoxyribonucleotides, is upregulated during replication stress. Full induction of RNR activity requires Dun1, and in a parallel pathway, Pop2 and Ccr4 (components of the Ccr4-Not complex) (Huang *et al.* 1998; Zhao and Rothstein 2002; Mulder *et al.* 2005; Woolstencroft *et al.* 2006). In addition, there are several links between glycine metabolism and *de novo* purine biosynthesis (Subramanian *et al.* 2005; Christensen and Mackenzie 2006). Consistent with this, *gly1*Δ mutants are HU sensitive and have reduced dNTP pools (Hartman 2007). Finally, Adk1 is important in the last few steps of dATP biosynthesis; it catalyzes the conversion of AMP to ADP and dAMP to dADP (Rebora *et al.* 2001; Kanehisa *et al.* 2007). Thus, the *adk1*Δ mutation is also likely to alter deoxynucleotide pools of the cell. We suggest that the five genes identified in this subgroup indicate that yeast cells maintain deoxynucleotide pools very close to the threshold necessary for ensuring complete genome integrity. We speculate that perturbations in nucleotide levels result in increased LOH at *MET15* because some aspect of rDNA structure makes it more sensitive to deoxynucleotide levels than the rest of the genome. Consistent with the rDNA array being a genomic locus that could be particularly sensitive to replication stress, it was recently shown that cells can proceed into anaphase with unreplicated rDNA and without activating the DNA damage checkpoint response (Torres-Rosell *et al.* 2007).

While there are several aspects of the rDNA that may make it "special," the DNA replication fork barrier in each rDNA repeat is known to be a hotspot for recombination, apparently by generating transient double-strand breaks (DSBs) (Kobayashi and Horiuchi 1996; Burkhalter and Sogo 2004; Kobayashi *et al.* 2004). The fork-barrier activity and enhanced recombination are dependent upon DNA binding by the Fob1 protein; in the absence of Fob1, there is no increased level of recombination in *sir2* cells. Hence it will be interesting to determine if the preferential increase in *MET15* LOH for the other mutant genes identified in our screen are dependent upon Fob1 or some other aspect of the rDNA array.

Deletion mutants that predominantly affect LOH on chromosome IV: While increased LOH at *MET15* was the primary criteria for identifying mutants in this screen, there were a few mutants in which a preferential increase in LOH was observed on chromosome IV. The two with the greatest differential effect (>7:1 ratio compared to that of chromosome III or XII) were *nup84*Δ and *nup133*Δ with 142- and 87-fold increases in *SAM2* LOH compared to wild type.

Nup84 and Nup133 are components of the nuclear pore that biochemically copurify (Cook *et al.* 2007). As such, mutants of these genes have a pleiotropic effect on the cell, including defects in DSB repair (Loeillet *et al.* 2005). It is possible that the observed preference may reflect that *SAM2* is closest to the telomere of the three loci that were screened for LOH. DSBs at telomere-proximal loci are repaired less efficiently in haploid cells than more internal DSBs in a *nup84*Δ or *nup133*Δ mutant, although the reason for this observation is unclear (Therizols *et al.* 2006). It has recently been reported that the increased DNA damage sensitivity seen in these mutants is linked to mislocalization of the SUMO-protease Ulp1 (Li and Hochstrasser 2003; Palancade *et al.* 2007). While the substrates regulated by Ulp1 are numerous and not completely identified, several of the mutants identified in our screen with a modest 2:1 to 3:1 greater fold increase of LOH at *SAM2* relative to *MAT* or *MET15*, have gene products that are sumoylated (*elg1*Δ and *wss1*Δ) or have increased activity when their substrate is sumoylated (*slx8*Δ; activity of the E3 ubiquitin ligase complex, Hex3·Slx8, is enhanced when its substrates are sumoylated) (Hannich *et al.* 2005; Xie *et al.* 2007). Ultimately, identifying the relevant substrates and how they preferentially affect chromosome IV LOH may help identify a discrete locus on IV that sensitizes it to this set of mutants.

Mutants of the homologous recombination proteins Rad51 and Rad54 also had a bias toward increased LOH at *SAM2* of 4:1 or 6:1 compared to *MET15* or *MAT*. Again, it is not clear why these mutants would display such a chromosome preference, but it is likely that they manifest their effect differently than do the nuclear pore mutants. For instance, it is unlikely that the nuclear pore mutants are simply preventing Rad51 or Rad54 from entering the nucleus. The *rad51*Δ and *rad54*Δ strains have a very high level of chromosome III loss compared to the *nup84*Δ and *nup133*Δ strains (supplemental Table S6), and both *rad51*Δ and *rad54*Δ are synthetically lethal with members of the Nup84 complex (Loeillet *et al.* 2005).

Deletion mutations of genes involved in DNA replication and chromatin assembly produce predominantly reciprocal LOH at *MET15*: The phenotypic characterization of half sectors permitted us to determine whether LOH events were reciprocal or nonreciprocal in nature. Mutants with moderate to high LOH at *MET15* that was predominantly reciprocal (>60%) are enriched for deletions of genes involved in DNA replication and/or replication-associated chromatin assembly. Seven of the 11 mutants fall into these categories (Figure 9): two subunits of DNA polymerases (*POL32* and *DPB3*), two subunits of chromatin assembly factor I (*MSI1* and *RLF2*), a replication helicase (*RRM3*), a subunit of an alternative replication factor C complex (*ELG1*), and DNA topoisomerase I (*TOP1*) (Thrash *et al.* 1985; Araki *et al.* 1991; Kaufman *et al.* 1997; Gerik *et al.* 1998; Ivessa *et al.* 2002; Kanellis *et al.* 2003). An additional member of this group is *RAD6*, an ubiquitin-conjugating enzyme that has both PCNA (a replication protein) and histone H2B as substrates (Robzyk *et al.* 2000; Hoege *et al.* 2002). In wild-type cells, 73% of *MET15* LOH events are reciprocal.

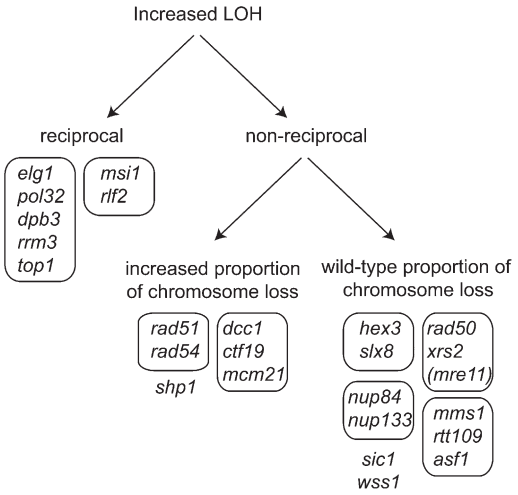


FIGURE 9.—Deletion alleles of genes involved in common processes lead to similar types of increased LOH. A schematic classification of mutants is shown. Mutants were categorized by whether they had increased LOH that occurred predominantly via reciprocal or nonreciprocal processes at the *MET15* locus. Mutants with predominantly nonreciprocal *MET15* LOH were then classified on the basis of proportions of chromosome III loss. The gene names boxed together participate in a common biochemical process or exist in a biochemically defined complex, as explained in the text.

Because the proportion of reciprocal LOH events is similar to wild type in this group of mutants, we suggest that the normal mechanisms for repair of DNA damage are able to handle the increased amount of damage created by these replication defects. Given that some of these mutants have >100-fold increase in LOH, it seems that the DNA repair machinery is very robust and capable of fixing a large load of damage. A corollary of this idea is that most LOH arises in *S. cerevisiae* as the result of DNA replication defects that sporadically occur under normal conditions.

Mutants with primarily nonreciprocal LOH at *MET15* and a low proportion of chromosome III loss: Another interesting group of mutants are those in which LOH occurred predominantly by nonreciprocal processes (<40% reciprocal) at *MET15*. The fact that LOH is predominantly nonreciprocal in these mutants suggests that an alternative mechanism/process of handling DNA lesions is occurring, compared to wild-type cells. We speculate that many or all of these mutants have the same DNA damage initiating events as in wild-type cells, but that repair is altered.

The mutants with predominantly nonreciprocal LOH at *MET15* can be divided into two general groups: those in which LOH on chromosome III is similar to what occurs in wild-type cells (33% chromosome loss) and those in which it occurs predominantly via chromosome loss (Figure 9). (Only mutants with at least a fivefold increase in LOH at *MET15* and threefold at both *SAM2* and *MAT* will be discussed.)

The mutants belonging to the first of these two groups include *rad50*Δ, *xrs2*Δ, *wss1*Δ, *mms1*Δ, *asf1*Δ, *rtt109*Δ, *sic1*Δ, and *nup133*Δ (the *nup84*Δ allele was just below the minimum criteria at *MAT*). Within this group, the roles of Rad50 and Xrs2 in DNA repair are probably best understood (KROGH and SYMINGTON 2004). These proteins, along with Mre11, are critical in early steps of normal DSB signaling and repair. As we show here, their absence results in a large increase of LOH, which indicates that they are critical in the normal process of eliminating endogenous damage and leads to an alternative pathway of repair that largely involves BIR. [At the *SAM2* locus, which occurs predominantly via nonreciprocal LOH (~36% reciprocal) in wild-type cells, the mutants were not significantly different than wild type—*i.e.*, they remained predominantly nonreciprocal.]

Interestingly, we also find that the partner proteins, Hex3 and Slx8 have an even smaller proportion of chromosome III loss (10%) than wild-type cells (33%). These proteins are reported to modulate the sumoylation of Rfa1, Rfa2, Rad52, and Rad59, all of which are involved in DNA repair (ZHANG *et al.* 2006). Our results are consistent with a recent suggestion that Hex3 and Slx8 normally prevent endogenous DNA lesions from proceeding down another "alternative" pathway of DNA repair that is distinct from the one uncovered by *rad50*Δ and *xrs2*Δ mutants (ZHANG *et al.* 2006).

Mutants with primarily nonreciprocal LOH at *MET15* and a high proportion of chromosome III loss: The last group of mutants worth noting had high levels of nonreciprocal LOH at *MET15* along with a high proportion of chromosome III loss (*shp1*Δ, *dcc1*Δ, *ctf19*Δ, *mcm21*Δ, *rad51*Δ, and *rad54*Δ) (Figure 9). Previous characterizations of some members provide an explanation for the increased chromosome loss we observed. For instance, Mcm21 and Ctf19 are components of the kinetochore subcomplex COMA, and Dcc1 is required for sister chromatid cohesion (MAYER *et al.* 2001; DE WULF *et al.* 2003). Disruption of either kinetochore structure or sister chromatid cohesion results in elevated levels of chromosome nondisjunction and we observe that there is indeed a very high incidence of chromosome III loss in these mutants. The *rad51*Δ and *rad54*Δ mutants share the same phenotypic profile in our assays, consistent with previous studies in which these mutants have parallel phenotypes in most other recombination and DNA damage assays (KROGH and SYMINGTON 2004; HEYER *et al.* 2006). Rad51 and Rad54 play critical roles at multiple steps in normal homologous recombination. Given the high rate of chromosome loss in *rad51*Δ and *rad54*Δ mutants, we speculate that the alternative pathway(s) of recombination used in their absence may occasionally generate recombination intermediates that are unresolved during mitosis, which in turn leads to chromosome nondisjunction, as one of the homologous chromosome pair is pulled to the wrong spindle pole.

The analysis of this set of mutants also suggests that the LOH rates at *MET15* and *SAM2* for this group may be higher than what we report here. Most LOH on chromosome III occurred via chromosome loss in this group, but nearly all the nonreciprocal LOH events on chromosome IV and XII appeared to occur via BIR, as indicated by quantitative Southern blot analysis (supplemental Table S7). As noted above, chromosome IV and XII are probably not stably maintained as monosomes and must undergo endoreduplication (Alvaro *et al.* 2006). Therefore we speculate that nondisjunction of chromosome IV and XII may have indeed occurred fairly often in these mutants, but if endoreduplication did not follow in a timely manner, the resulting monosomic cell would not have proliferated to produce a half-sectored colony.

Comparison of the LOH screen with other large-scale screens for genome instability: Several genetic screens have used the same set of deletion mutants that we used in the present study, to identify single alleles with increased genomic instability. One screen identified 33 genes with increased rates of mutation in the *CAN1* gene in haploid cells (Huang *et al.* 2003). Of these, six overlapped with alleles identified in our study (supplemental Table S6). In a second study, mutants with an increase of gross chromosomal rearrangements (GCRs) were screened for by selecting for loss of two adjacent genes on the left arm of chromosome XV in haploid cells (Kanellis *et al.* 2007). Of the 21 identified in this screen, 4 overlapped with our findings (supplemental Table S6). A third screen isolated chromosome instability (CIN) mutants using three different assays, one of which monitored increased LOH in diploid cells with a bias toward identifying chromosome loss events (Yuen *et al.* 2007). Of the 130 high-confidence deletion mutants identified, 28 overlapped with the mutants we reported with increased LOH (supplemental Table S6). If we consider genes that encode subunits of a known protein complex (see below), then an additional 12 mutants from this earlier study can be included as identifying the same functional complexes that we identified in our LOH study. Even with this consideration, over half of the mutant alleles we identified were not detected in the other screens (supplemental Table S6 and data not shown). This difference reflects how the spectrum of alleles identified in a genome instability screen is dependent upon the nature of the screen.

One of the important strengths of the data set we present here is that every deletion allele was recreated in the MHM strain. The independent transformants of each mutant provides a high level of confidence that each allele does indeed impart the phenotypes we report. It eliminated reporting "false positives" that are known to occur as a result of selection during growth (aneuploidy, second site mutations, etc.) or in handling of the arrays (cross-contamination or other systematic errors) (see Yuen *et al.* 2007). With this in mind, we have confidence in the identification of the *yjl038c*Δ allele, which has not been previously characterized as important in genome integrity screens. Interestingly, *YJL038C*, which we have named *LOH1*, has sequence similarity to the adjacent gene, *IRC18*; the *irc18*Δ allele was recently identified to have increased levels of Rad52-damage-repair foci (Alvaro *et al.* 2007). We suggest that this pair of genes normally plays a role in maintaining genome integrity.

In contrast, we find it difficult to make a simple mechanistic link to increased LOH with the deletion mutants of *ICE2*, a gene important in cortical endoplasmic reticulum inheritance, or *FEN2*, a gene reported to encode a plasma membrane symporter (Stolz and Sauer 1999; Estrada De Martin *et al.* 2005). While these alleles may uncover new connections between these processes and nuclear genome integrity, we consider that the *ice2*Δ and *fen2*Δ alleles may be having their effect by altering the regulation of adjacent genes: *ICE2* is immediately 5′ to *YIL091C*, which encodes a putative helicase, and *FEN2* is immediately 5′ to *RIM1*, a single-stranded DNA binding protein implicated in mitochondrial genome maintenance (Li *et al.* 1998; Saccharomyces Genome Database Project 2008). Similarly, we wonder whether the *YGL250W/RMR1* gene product is directly involved in genome integrity; it has sequence similarity only to other fungi and the deletion allele was recently identified as having reduced meiotic recombination (Jordan *et al.* 2007). It is positioned between the 5′ ends of two meiosis-specific genes, *HFM1/MER3*, which encodes a DNA helicase, and *ZIP2*, which is important in synaptonemal complex formation during meiosis (Chua and Roeder 1998; Nakagawa *et al.* 2001). We speculate that the *rmr1*Δ allele may result in misregulation of either or both of these adjacent genes and inappropriately stimulate recombination in mitotic cells.

We identified 61 deletion mutant alleles in our screen, and in many cases these mutants corresponded to more than one component of characterized biochemical complexes or processes. Examples of such partners that had a significant effect on LOH are: Hex3 and Slx8; Ctf19 and Mcm21; Mms1, Asf1, and Rtt109; Nup84 and Nup133; Ccr4 and Pop2; Rpa14 and Rpa34; Rlf2 and Msi1; Csm1 and Lrs4; Rad51 and Rad54; Rad50 and Xrs2 (Clever *et al.* 1997; Kaufman *et al.* 1997; Usui *et al.* 1998; Bai *et al.* 1999; Allen *et al.* 2001 ; McAinsh *et al.* 2003; Shpakovski *et al.* 2003; Huang *et al.* 2006; Yang *et al.* 2006; Collins *et al.* 2007). We found that each set of these partners had very similar phenotypic profiles in the quantitative and qualitative sets of assays performed in this study. As such, these profiles provide a refined biological context for information amassed in large-scale genetic interaction maps of genes involved in various aspects of chromosome biology (Pan *et al.* 2006; Collins *et al.* 2007). For instance, the genetic interaction maps were based primarily on growth phenotypes.

In the future, we can utilize the MHM strain to examine whether mutants of genes clustered together in the interaction maps also produce similar LOH phenotypes, and we can determine how deeply within a hierarchical cluster the similar phenotypes are observed. Such an analysis offers the opportunity to better understand mechanisms of action in LOH events.

In comparing the list of genes identified in our LOH screen to other genetic and biochemical characterizations of protein complexes in *S. cerevisiae*, we find that some of the expected partners of known complexes are missing. For instance, while Rad50 and Xrs2 were identified in our screen, the other member of the MRX complex, Mre11, was not (Usui *et al.* 1998). When *mre11*Δ alleles were introduced in the MHM strain, they indeed behaved very similarly to the *rad50*Δ and *xrs2*Δ alleles in each of the LOH assays (supplemental Table S6), consistent with an earlier finding that *mre11*Δ strains have increased levels of BIR and chromosome loss (Krishna *et al.* 2007). To understand why the *mre11*Δ was missed in the primary screen, we examined the *MET15* locus in the deletion collection and discovered that in our version, the locus was no longer heterozygous (*MET15/met15*Δ), but was homozygous *MET15/MET15* in the *mre11*Δ strain (data not shown). We speculate that in the initial screen some mutants with high levels of genomic instability may have produced cells that had lost heterozygosity at *MET15*. The *MET15/MET15* homozygous strains were the likely result of selective pressures created during the propagation of the strain collection. Other mutants that are missing from our analysis are those that are mating defective; the two haploid deletion mutant strains used to create each diploid homozygous deletion mutant in the collection must be mating competent. Hence the *sir2*Δ*/sir2*Δ mutant strain, which is known to have high levels of LOH at *MET15*, is missing from the strain collection because *sir2*Δ strains are sterile (McMurray and Gottschling 2003). While it is difficult to assess how many potential mutants were missed because of these limitations, we estimate that the discovery of possible mutants for increased *MET15* LOH in the deletion collection was >50%. This estimate is based on the number of partner proteins in complexes we did identify and compared to the total number of partner proteins currently known to be a part of these complexes (data not shown).

Conclusions: The screen and characterization of deletion mutants carried out in this study provide a genetic landscape of possible defects that can drive increased rates of LOH and the means by which they occur in *S. cerevisiae*. As such, it gives us a set of potential pathways/processes that may become defective in a "diseased" state. We proposed in earlier studies that molecules involved in genome maintenance become defective over time—*i.e.*, as cells aged (McMurray and Gottschling 2004). The results presented here provide a set of gene products and processes that can be examined in testing this hypothesis.

Finally, the genetic landscape of distinct pathways to increased LOH identified in this study is consistent with the idea that multiple DNA damage repair pathways compete for the same damaged substrates. By compromising one or more of the repair pathways, the increased LOH is manifested. Given that the balance between various repair pathways differs between species and cell types, it seems likely that certain cells will be much more prone to LOH (Shrivastav *et al.* 2008). Our study serves as a starting point for exploring such a possibility.

We thank members of the Gottschling lab for their comments and critical reading of the manuscript. M.P.A. was supported by a predoctoral fellowship from the National Science Foundation. This work was supported by a pilot grant from the National Cancer Institute/National Institute on Aging CA-103728 and a National Institutes of Health grant AG-023779 awarded to D.E.G.

LITERATURE CITED

Acuna, G., F. E. Wurgler and C. Sengstag, 1994 Reciprocal mitotic recombination is the predominant mechanism for the loss of a heterozygous gene in Saccharomyces cerevisiae. Environ. Mol. Mutagen. **24:** 307–316.

Adams, A., D. E. Gottschling, C. A. Kaiser and T. Stearns, 1998 *Methods in Yeast Genetics*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Aguilera, A., S. Chavez and F. Malagon, 2000 Mitotic recombination in yeast: elements controlling its incidence. Yeast **16:** 731–754.

Ajima, J., K. Umezu and H. Maki, 2002 Elevated incidence of loss of heterozygosity (LOH) in an sgs1 mutant of Saccharomyces cerevisiae: roles of yeast RecQ helicase in suppression of aneuploidy, interchromosomal rearrangement, and the simultaneous incidence of both events during mitotic growth. Mutat. Res. **504:** 157–172.

Allen, N. P. C., L. Huang, A. Burlingame and M. Rexach, 2001 Proteomic analysis of nucleoporin interacting proteins. J. Biol. Chem. **276:** 29268–29274.

Alvaro, D., I. Sunjevaric, R. J. Reid, M. Lisby, D. J. Stillman *et al.*, 2006 Systematic hybrid LOH: a new method to reduce false positives and negatives during screening of yeast gene deletion libraries. Yeast **23:** 1097–1106.

Alvaro, D., M. Lisby and R. Rothstein, 2007 Genome-wide analysis of Rad52 foci reveals diverse mechanisms impacting recombination. PLoS Genet. **3:** e228.

Araki, H., R. K. Hamatake, A. Morrison, A. L. Johnson, L. H. Johnston *et al.*, 1991 Cloning DPB3, the gene encoding the third subunit of DNA polymerase II of Saccharomyces cerevisiae. Nucleic Acids Res. **19:** 4867–4872.

Aylon, Y., and M. Kupiec, 2004 DSB repair: the yeast paradigm. DNA Repair **3:** 797–815.

Bai, Y. L., C. Salvadore, Y. C. Chiang, M. A. Collart, H. Y. Liu *et al.*, 1999 The CCR4 and CAF1 proteins of the CCR4-NOT complex are physically and functionally separated from NOT2, NOT4, and NOT5. Mol. Cell. Biol. **19:** 6642–6651.

Barbera, M. A., and T. D. Petes, 2006 Selection and analysis of spontaneous reciprocal mitotic cross-overs in Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA **103:** 12819–12824.

Bielas, J. H., K. R. Loeb, B. P. Rubin, L. D. True and L. A. Loeb, 2006 Human cancers express a mutator phenotype. Proc. Natl. Acad. Sci. USA **103:** 18238–18242.

Brachmann, C. B., A. Davies, G. J. Cost, E. Caputo, J. Li *et al.*, 1998 Designer deletion strains derived from Saccharomyces cerevisiae S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. Yeast **14:** 115–132.

Brown, M. A., 1997 Tumor suppressor genes and human cancer. Adv. Genet. **36:** 45–135.

Buck, S. W., J. J. Sandmeier and J. S. Smith, 2002 RNA polymerase I propagates unidirectional spreading of rDNA silent chromatin. Cell **111:** 1003–1014.

Burkhalter, M. D., and J. M. Sogo, 2004 rDNA enhancer affects replication initiation and mitotic recombination: Fob1 mediates nucleolytic processing independently of replication. Mol. Cell **15:** 409–421.

Carr, L. L., and D. E. Gottschling, 2008 Does age influence loss of heterozygosity? Exp. Gerontol. **43:** 123–129.

Cavenee, W. K., T. P. Dryja, R. A. Phillips, W. F. Benedict, R. Godbout *et al.*, 1983 Expression of recessive alleles by chromosomal mechanisms in retinoblastoma. Nature **305:** 779–784.

Chang, M., A. B. Parsons, B. H. Sheikh, C. Boone and G. W. Brown, 2006 Genomic approaches for identifying DNA damage response pathways in S. cerevisiae. Methods Enzymol. **409:** 213–235.

Cherry, J. M., C. Ball, S. Weng, G. Juvik, R. Schmidt *et al.*, 1997 Genetic and physical maps of Saccharomyces cerevisiae. Nature **387:** 67–73.

Chial, H. J., T. H. Giddings, Jr., E. A. Siewert, M. A. Hoyt and M. Winey, 1999 Altered dosage of the Saccharomyces cerevisiae spindle pole body duplication gene, NDC1, leads to aneuploidy and polyploidy. Proc. Natl. Acad. Sci. USA **96:** 10200–10205.

Christensen, K. E., and R. E. MacKenzie, 2006 Mitochondrial one-carbon metabolism is adapted to the specific needs of yeast, plants and mammals. BioEssays **28:** 595–605.

Christman, M. F., F. S. Dietrich and G. R. Fink, 1988 Mitotic recombination in the rDNA of S. cerevisiae is suppressed by the combined action of DNA topoisomerases I and II. Cell **55:** 413–425.

Chua, P. R., and G. S. Roeder, 1998 Zip2, a meiosis-specific protein required for the initiation of chromosome synapsis. Cell **93:** 349–359.

Clever, B., H. Interthal, J. Schmuckli-Maurer, J. King, M. Sigrist *et al.*, 1997 Recombinational repair in yeast: functional interactions between Rad51 and Rad54 proteins. EMBO J. **16:** 2535–2544.

Collins, S. R., K. M. Miller, N. L. Maas, A. Roguev, J. Fillingham *et al.*, 2007 Functional dissection of protein complexes involved in yeast chromosome biology using a genetic interaction map. Nature **446:** 806–810.

Cook, A., F. Bono, M. Jinek and E. Conti, 2007 Structural biology of nucleocytoplasmic transport. Annu. Rev. Biochem. **76:** 647–671.

Cost, G. J., and J. D. Boeke, 1996 A useful colony colour phenotype associated with the yeast selectable/counter-selectable marker MET15. Yeast **12:** 939–941.

Cronin, S. R., and R. Y. Hampton, 1999 Measuring protein degradation with green fluorescent protein. Methods Enzymol. **302:** 58–73.

Daigaku, Y., K. Endo, E. Watanabe, T. Ono and K. Yamamoto, 2004 Loss of heterozygosity and DNA damage repair in Saccharomyces cerevisiae. Mutat. Res. **556:** 183–191.

De Wulf, P., A. D. McAinsh and P. K. Sorger, 2003 Hierarchical assembly of the budding yeast kinetochore from multiple subcomplexes. Genes Dev. **17:** 2902–2921.

Demerec, M., 1932 Rate of instability of miniature-3 gamma gene of Drosophila virilis in the males in the homozygous and in the heterozygous females. Proc. Natl. Acad. Sci. USA **18:** 656–658.

DePinho, R. A., 2000 The age of cancer. Nature **408:** 248–254.

Dorer, R., P. M. Pryciak and L. H. Hartwell, 1995 Saccharomyces cerevisiae cells execute a default pathway to select a mate in the absence of pheromone gradients. J. Cell Biol. **131:** 845–861.

DuBois, M. L., Z. W. Haimberger, M. W. McIntosh and D. E. Gottschling, 2002 A quantitative assay for telomere protection in *Saccharomyces cerevisiae*. Genetics **161:** 995–1013.

Edwards, T. K., A. Saleem, J. A. Shaman, T. Dennis, C. Gerigk *et al.*, 2000 Role for nucleolin/Nsr1 in the cellular localization of topoisomerase I. J. Biol. Chem. **275:** 36181–36188.

Emerson, R. A., 1929 The frequency of somatic mutation in variegated pericarp of maize. Genetics **14:** 488–511.

Esposito, M. S., and C. V. Bruschi, 1993 Diploid yeast cells yield homozygous spontaneous mutations. Curr. Genet. **23:** 430–434.

Esposito, M. S., R. M. Ramirez and C. V. Bruschi, 1994 Nonrandomly-associated forward mutation and mitotic recombination yield yeast diploids homozygous for recessive mutations. Curr. Genet. **26:** 302–307.

Estrada de Martin, P., Y. Du, P. Novick and S. Ferro-Novick, 2005 Ice2p is important for the distribution and structure of the cortical ER network in Saccharomyces cerevisiae. J. Cell Sci. **118:** 65–77.

Frank-Vaillant, M., and S. Marcand, 2001 NHEJ regulation by mating type is exercised through a novel protein, Lif2p, essential to the ligase IV pathway. Genes Dev. **15:** 3005–3012.

Gerik, K. J., X. Li, A. Pautz and P. M. Burgers, 1998 Characterization of the two small subunits of Saccharomyces cerevisiae DNA polymerase delta. J. Biol. Chem. **273:** 19747–19755.

Giaever, G., A. M. Chu, L. Ni, C. Connelly, L. Riles *et al.*, 2002 Functional profiling of the Saccharomyces cerevisiae genome. Nature **418:** 387–391.

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

Gottschling, D. E., O. M. Aparicio, B. L. Billington and V. A. Zakian, 1990 Position effect at S. cerevisiae telomeres: reversible repression of Pol II transcription. Cell **63:** 751–762.

Grady, W. M., 2004 Genomic instability and colon cancer. Cancer Metast. Rev. **23:** 11–27.

Hannich, J. T., A. Lewis, M. B. Kroetz, S. J. Li, H. Heide *et al.*, 2005 Defining the SUMO-modified proteome by multiple approaches in Saccharomyces cerevisiae. J. Biol. Chem. **280:** 4102–4110.

Hartman, J. L., IV, 2007 Buffering of deoxyribonucleotide pool homeostasis by threonine metabolism. Proc. Natl. Acad. Sci. USA **104:** 11700–11705.

Heyer, W. D., X. Li, M. Rolfsmeier and X. P. Zhang, 2006 Rad54: The Swiss army knife of homologous recombination? Nucleic Acids Res. **34:** 4115–4125.

Hiraoka, M., K. Watanabe, K. Umezu and H. Maki, 2000 Spontaneous loss of heterozygosity in diploid *Saccharomyces cerevisiae* cells. Genetics **156:** 1531–1548.

Hoege, C., B. Pfander, G. L. Moldovan, G. Pyrowolakis and S. Jentsch, 2002 RAD6-dependent DNA repair is linked to modification of PCNA by ubiquitin and SUMO. Nature **419:** 135–141.

Huang, J., and D. Moazed, 2006 Sister chromatid cohesion in silent chromatin: each sister to her own ring. Genes Dev. **20:** 132–137.

Huang, M., Z. Zhou and S. J. Elledge, 1998 The DNA replication and damage checkpoint pathways induce transcription by inhibition of the Crt1 repressor. Cell **94:** 595–605.

Huang, M. E., A. G. Rio, A. Nicolas and R. D. Kolodner, 2003 A genomewide screen in Saccharomyces cerevisiae for genes that suppress the accumulation of mutations. Proc. Natl. Acad. Sci. USA **100:** 11529–11534.

Huang, J., I. L. Brito, J. Villen, S. P. Gygi, A. Amon *et al.*, 2006 Inhibition of homologous recombination by a cohesin-associated clamp complex recruited to the rDNA recombination enhancer. Genes Dev. **20:** 2887–2901.

Ivessa, A. S., J. Q. Zhou, V. P. Schulz, E. K. Monson and V. A. Zakian, 2002 Saccharomyces Rrm3p, a 5′ to 3′ DNA helicase that promotes replication fork progression through telomeric and subtelomeric DNA. Genes Dev. **16:** 1383–1396.

Johnston, J. R., 1971 Genetic analysis of spontaneous half-sectored colonies of Saccharomyces cerevisiae. Genet. Res. **18:** 179–184.

Johnston, M., L. Riles and J. H. Hegemann, 2002 Gene disruption. Methods Enzymol. **350:** 290–315.

Johzuka, K., M. Terasawa, H. Ogawa, T. Ogawa and T. Horiuchi, 2006 Condensin loaded onto the replication fork barrier site in the rRNA gene repeats during S phase in a FOB1-dependent fashion to prevent contraction of a long repetitive array in Saccharomyces cerevisiae. Mol. Cell. Biol. **26:** 2226–2236.

Jordan, P. W., F. Klein and D. R. Leach, 2007 Novel roles for selected genes in meiotic DNA processing. PLoS Genet. **3:** e222.

Kanehisa, M., M. Araki, S. Goto, M. Hattori, M. Hirakawa *et al.*, 2007 KEGG for linking genomes to life and the environment. Nucleic Acids Res. **36:** D480–D484.

Kanellis, P., R. Agyei and D. Durocher, 2003 Elg1 forms an alternative PCNA-interacting RFC complex required to maintain genome stability. Curr. Biol. **13:** 1583–1595.

Kanellis, P., M. Gagliardi, J. P. Banath, R. K. Szilard, S. Nakada *et al.*, 2007 A screen for suppressors of gross chromosomal rearrangements identifies a conserved role for PLP in preventing DNA lesions. PLoS Genet. **3:** e134.

Kaufman, P. D., R. Kobayashi and B. Stillman, 1997 Ultraviolet radiation sensitivity and reduction of telomeric silencing in Saccharomyces cerevisiae cells lacking chromatin assembly factor-I. Genes Dev. **11:** 345–357.

Kegel, A., J. O. Sjostrand and S. U. Astrom, 2001 Nej1p, a cell type-specific regulator of nonhomologous end joining in yeast. Curr. Biol. **11:** 1611–1617.

Knudson, A. G., 2001 Two genetic hits (more or less) to cancer. Nat. Rev. Cancer **1:** 157–162.

Kobayashi, T., and T. Horiuchi, 1996 A yeast gene product, Fob1 protein, required for both replication fork blocking and recombinational hotspot activities. Genes Cells **1:** 465–474.

Kobayashi, T., T. Horiuchi, P. Tongaonkar, L. Vu and M. Nomura, 2004 SIR2 regulates recombination between different rDNA repeats, but not recombination within individual rRNA genes in yeast. Cell **117:** 441–453.

Krishna, S., B. M. Wagener, H. P. Liu, Y. C. Lo, R. Sterk *et al.*, 2007 Mre11 and Ku regulation of double-strand break repair by gene conversion and break-induced replication. DNA Repair **6:** 797–808.

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

Lengauer, C., K. W. Kinzler and B. Vogelstein, 1998 Genetic instabilities in human cancers. Nature **396:** 643–649.

Li, Z., F. Ling and T. Shibata, 1998 Glucose repression on RIM1, a gene encoding a mitochondrial single-stranded DNA-binding protein, in Saccharomyces cerevisiae: a possible regulation at pre-mRNA splicing. Curr. Genet. **34:** 351–359.

Li, S. J., and M. Hochstrasser, 2003 The Ulp1 SUMO isopeptidase: distinct domains required for viability, nuclear envelope localization, and substrate specificity. J. Cell Biol. **160:** 1069–1081.

Li, C., J. E. Mueller and M. Bryk, 2006 Sir2 represses endogenous polymerase II transcription units in the ribosomal DNA nontranscribed spacer. Mol. Biol. Cell **17:** 3848–3859.

Liras, P., J. McCusker, S. Mascioli and J. E. Haber, 1978 Characterization of a mutation in yeast causing nonrandom chromosome loss during mitosis. Genetics **88:** 651–671.

Loeb, L. A., 1991 Mutator phenotype may be required for multistage carcinogenesis. Cancer Res. **51:** 3075–3079.

Loeb, L. A., K. R. Loeb and J. P. Anderson, 2003 Multiple mutations and cancer. Proc. Natl. Acad. Sci. USA **100:** 776–781.

Loeillet, S., B. Palancade, M. Cartron, A. Thierry, G. F. Richard *et al.*, 2005 Genetic network interactions among replication, repair and nuclear pore deficiencies in yeast. DNA Repair **4:** 459–468.

Mayer, M. L., S. P. Gygi, R. Aebersold and P. Hieter, 2001 Identification of RFC(Ctf18p, Ctf8p, Dcc1p): an alternative RFC complex required for sister chromatid cohesion in S. cerevisiae. Mol. Cell **7:** 959–970.

McAinsh, A. D., J. D. Tytell and P. K. Sorger, 2003 Structure, function, and regulation of budding yeast kinetochores. Annu. Rev. Cell Dev. Biol. **19:** 519–539.

McMurray, M. A., and D. E. Gottschling, 2003 An age-induced switch to a hyper-recombinational state. Science **301:** 1908–1911.

McMurray, M. A., and D. E. Gottschling, 2004 Aging and genetic instability in yeast. Curr. Opin. Microbiol. **7:** 673–679.

Mulder, K. W., G. S. Winkler and H. T. Timmers, 2005 DNA damage and replication stress induced transcription of RNR genes is dependent on the Ccr4-Not complex. Nucleic Acids Res. **33:** 6384–6392.

Nakagawa, T., H. Flores-Rozas and R. D. Kolodner, 2001 The MER3 helicase involved in meiotic crossing over is stimulated by single-stranded DNA-binding proteins and unwinds DNA in the 3′ to 5′ direction. J. Biol. Chem. **276:** 31487–31493.

Ooi, S. L., D. D. Shoemaker and J. D. Boeke, 2001 A DNA microarray-based genetic screen for nonhomologous end-joining mutants in Saccharomyces cerevisiae. Science **294:** 2552–2556.

Palancade, B., X. Liu, M. Garcia-Rubio, A. Aguilera, X. Zhao *et al.*, 2007 Nucleoporins prevent DNA damage accumulation by modulating Ulp1-dependent sumoylation processes. Mol. Biol. Cell **18:** 2912–2923.

Pan, X., P. Ye, D. S. Yuan, X. Wang, J. S. Bader *et al.*, 2006 A DNA integrity network in the yeast Saccharomyces cerevisiae. Cell **124:** 1069–1081.

Paques, F., and J. E. Haber, 1999 Multiple pathways of recombination induced by double-strand breaks in Saccharomyces cerevisiae. Microbiol. Mol. Biol. Rev. **63:** 349–404.

Park, H., and R. Sternglanz, 1999 Identification and characterization of the genes for two topoisomerase I-interacting proteins from Saccharomyces cerevisiae. Yeast **15:** 35–41.

Petes, T. D., and D. Botstein, 1977 Simple Mendelian inheritance of the reiterated ribosomal DNA of yeast. Proc. Natl. Acad. Sci. USA **74:** 5091–5095.

Pontecorvo, G., E. Tarr Gloor and E. Forbes, 1954 Analysis of mitotic recombination in Aspergillus nidulans. J. Genet. **52:** 226–237.

Rebora, K., C. Desmoucelles, F. Borne, B. Pinson and B. Daignan-Fornier, 2001 Yeast AMP pathway genes respond to adenine through regulated synthesis of a metabolic intermediate. Mol. Cell Biol. **21:** 7901–7912.

Robzyk, K., J. Recht and M. A. Osley, 2000 Rad6-dependent ubiquitination of histone H2B in yeast. Science **287:** 501–504.

Roper, J. A., and R. H. Pritchard, 1955 Recovery of the complementary products of mitotic crossing-over. Nature **175:** 639.

Sandmeier, J. J., I. Celic, J. D. Boeke and J. S. Smith, 2002 Telomeric and rDNA silencing in *Saccharomyces cerevisiae* are dependent on a nuclear NAD(+) salvage pathway. Genetics **160:** 877–889.

Saccharomyces Genome Database Project, 2008 Saccharomyces Genome Database. http://www.yeastgenome.org/.

Shpakovski, G. V., E. K. Shematorova, G. M. Proshkina, N. A. Potapenko, T. V. Ovchinnikova *et al.*, 2003 Structure-function comparison of nuclear RNA polymerases I and III of Schizosaccharomyces pombe and Saccharomyces cerevisiae. Yeast **20:** S125.

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.

Signon, L., A. Malkova, M. L. Naylor, H. Klein and J. E. Haber, 2001 Genetic requirements for RAD51- and RAD54-independent break-induced replication repair of a chromosomal double-strand break. Mol. Cell Biol. **21:** 2048–2056.

Sikorski, R. S., and P. Hieter, 1989 A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in Saccharomyces cerevisiae. Genetics **122:** 19–27.

Smith, J. S., E. Caputo and J. D. Boeke, 1999 A genetic screen for ribosomal DNA silencing defects identifies multiple DNA replication and chromatin-modulating factors. Mol. Cell Biol. **19:** 3184–3197.

Sprague, Jr., G. F., 1991 Assay of yeast mating reaction. Methods Enzymol. **194:** 77–93.

Stern, C., 1936 Somatic crossing over and segregation in *Drosophila melanogaster*. Genetics **21:** 625–730.

Stolz, J., and N. Sauer, 1999 The fenpropimorph resistance gene FEN2 from Saccharomyces cerevisiae encodes a plasma membrane H+-pantothenate symporter. J. Biol. Chem. **274:** 18747–18752.

Subramanian, M., W. B. Qiao, N. Khanam, O. Wilkins, S. D. Der *et al.*, 2005 Transcriptional regulation of the one-carbon metabolism regulon in Saccharomyces cerevisiae by Bas1p. Mol. Microbiol. **57:** 53–69.

Therizols, P., C. Fairhead, G. G. Cabal, A. Genovesio, J. C. Olivo-Marin *et al.*, 2006 Telomere tethering at the nuclear periphery is essential for efficient DNA double strand break repair in subtelomeric region. J. Cell Biol. **172:** 189–199.

Thrash, C., A. T. Bankier, B. G. Barrell and R. Sternglanz, 1985 Cloning, characterization, and sequence of the yeast DNA topoisomerase I gene. Proc. Natl. Acad. Sci. USA **82:** 4374–4378.

Tong, A. H., M. Evangelista, A. B. Parsons, H. Xu, G. D. Bader *et al.*, 2001 Systematic genetic analysis with ordered arrays of yeast deletion mutants. Science **294:** 2364–2368.

Torres-Rosell, J., G. De Piccoli, V. Cordon-Preciado, S. Farmer, A. Jarmuz *et al.*, 2007 Anaphase onset before complete DNA replication with intact checkpoint responses. Science **315:** 1411–1415.

Usui, T., T. Ohta, H. Oshiumi, J. Tomizawa, H. Ogawa *et al.*, 1998 Complex formation and functional versatility of Mre11 of budding yeast in recombination. Cell **95:** 705–716.

Valencia, M., M. Bentele, M. B. Vaze, G. Herrmann, E. Kraus *et al.*, 2001 NEJ1 controls non-homologous end joining in Saccharomyces cerevisiae. Nature **414:** 666–669.

van Leeuwen, F., and D. E. Gottschling, 2002 Assays for gene silencing in yeast. Methods Enzymol. **350:** 165–186.

Waghmare, S. K., and C. V. Bruschi, 2005 Differential chromosome control of ploidy in the yeast Saccharomyces cerevisiae. Yeast **22:** 625–639.

Wildenberg, J., 1970 The relation of mitotic recombination to DNA replication in yeast pedigrees. Genetics **66:** 291–304.

Woolstencroft, R. N., T. H. Beilharz, M. A. Cook, T. Preiss, D. Durocher *et al.*, 2006 Ccr4 contributes to tolerance of replication stress through control of CRT1 mRNA poly(A) tail length. J. Cell Sci. **119:** 5178–5192.

Xie, Y., O. Kerscher, M. B. Kroetz, H. F. McConchie, P. Sung *et al.*, 2007 The yeast Hex3.Slx8 heterodimer is a ubiquitin ligase stimulated by substrate sumoylation. J. Biol. Chem. **282:** 34176–34184.

Yang, L. T., J. R. Mullen and S. J. Brill, 2006 Purification of the yeast Slx5-Slx8 protein complex and characterization of its DNA-binding activity. Nucleic Acids Res. **34:** 5541–5551.

Yuen, K. W. Y., C. D. Warren, O. Chen, T. Kwok, P. Hieter *et al.*, 2007 Systematic genome instability screens in yeast and their potential relevance to cancer. Proc. Natl. Acad. Sci. USA **104:** 3925–3930.

Zhang, C. Y., T. M. Roberts, J. Yang, R. Desai and G. W. Brown, 2006 Suppression of genomic instability by SLX5 and SLX8 in Saccharomyces cerevisiae. DNA Repair **5:** 336–346.

Zhao, X., and R. Rothstein, 2002 The Dun1 checkpoint kinase phosphorylates and regulates the ribonucleotide reductase inhibitor Sml1. Proc. Natl. Acad. Sci. USA **99:** 3746–3751.

Zimmermann, F. K., 1973 A yeast strain for visual screening for the two reciprocal products of mitotic crossing over. Mutat. Res. **21:** 263–269.

Communicating editor: E. Alani