Homologous recombination is required for recovery from oxidative DNA damage

Michio Hayashi and Keiko Umezu *
Section of Biochemistry, Fukuoka Dental College, 2-15-1 Tamura, Sawara-ku, Fukuoka, Fukuoka 814-0193, Japan

(Received 8 December 2016, accepted 27 February 2017; J-STAGE Advance published date: 3 April 2017)

We have been studying the genetic events, including chromosome loss, chromosome rearrangements and intragenic point mutations, that are responsible for the deletion of a URA3 marker in a loss of heterozygosity (LOH) assay in the yeast Saccharomyces cerevisiae. With this assay, we previously showed that homologous recombination plays an important role in genome maintenance in response to DNA lesions that occur spontaneously in normally growing cells. Here, to investigate DNA lesions capable of triggering homologous recombination, we examined the effects of oxidative stress, a prominent cause of endogenous DNA damage, on LOH events. Treatment of log-phase cells with H2O2 first caused growth arrest and then, during the subsequent recovery, chromosome loss and various chromosome rearrangements were induced more than 10-fold. Further analysis of the rearrangements showed that gene conversion was strongly induced, approximately 100 times more frequently than in untreated cells. Consistent with these results, two diploid strains deficient for homologous recombination, rad52Δ/uni0394/Δ and rad51Δ/uni0394/Δ, were sensitive to H2O2 treatment. In addition, chromosome DNA breaks were detected in H2O2-treated cells using pulsed-field gel electrophoresis. Altogether, these results suggest that oxidative stress induced recombinogenic lesions on chromosomes, which then triggered homologous recombination leading to chromosome rearrangements, and that this response contributed to the survival of cells afflicted by oxidative DNA damage. We therefore conclude that homologous recombination is required for the recovery of cells from oxidative stress.

Key words: oxidative stress, loss of heterozygosity (LOH), genome instability

INTRODUCTION

Genetic alterations are more complex in diploid cells than in haploid cells. In particular, alterations at the chromosome level that involve the loss of an essential gene, such as chromosome loss and rearrangement, can only be studied in diploid cells because they are lethal in haploids. In humans, chromosome aberrations are often observed in cancer cells as well as in normal cells of elderly individuals, and are implicated in both cancer development and the aging process (Tischfield, 1997; Lengauer et al., 1998; Rao et al., 2017). Thus, it is clearly important to understand the mechanisms and causes leading to chromosome aberrations.

To gain an overview of chromosome aberrations at the molecular level, we are utilizing the diploid yeast Saccharomyces cerevisiae (Hiraoka et al., 2000; Ajima et al., 2002; Umezu et al., 2002; Watanabe et al., 2002; Yoshida et al., 2003). As shown in Fig. 1, we have developed a loss of heterozygosity (LOH) assay method to analyze the genetic events responsible for loss of a heterozygous URA3 marker on chromosome III, which makes the cell resistant to 5-fluoroorotic acid (5-FOA). Loss of the URA3 marker is caused by several genetic alterations including chromosome loss, various chromosome rearrangements and intragenic point mutations, and, to classify these events, two additional markers on the same chromosome are utilized to monitor how chromosomes are rearranged in three different phenotypes: 5-FOA’ Leu− Ade− (Class A), 5-FOA’ Leu+ Ade− (Class B) and 5-FOA’ Leu+ Ade+ (Class C). In addition to determining the frequencies of each class, chromosome structure in LOH clones can be physically analyzed by pulsed-field gel electrophoresis (PFGE) and PCR, in which aberrant-sized chromosome III derivatives are readily detected (Hiraoka et al., 2000). We can define these events at the nucleotide level, when necessary, by determining the fusion points of aberrant-sized chromosomes using a PCR-based
M. HAYASHI and K. UMEZU

method to quantify the ploidy at a series of loci along chromosome III (Umezu et al., 2002).

By exploiting these molecular genetic methods, we have determined the factors involved in spontaneous genetic alterations arising in normally growing cells. The total LOH frequency of wild-type cells was $1 - 2 \times 10^{-4}$ and the analysis of LOH events revealed that homologous recombination is involved in processes leading to LOH in multiple ways, including allelic recombination, chromosome size alteration and a particular kind of chromosome loss (Hiraoka et al., 2000). Analysis of the fusion points of more than 80 aberrant chromosomes revealed that all of them had breakpoints within repetitive sequences scattered over the genome, such as Ty1, indicating that homologous recombination is a leading process in chromosome size alteration (Umezu et al., 2002). In recombination-deficient mutants, on the other hand, LOH events were significantly increased up to frequencies of $3 - 5 \times 10^{-3}$, and mostly involved chromosome loss, indicating that homologous recombination also plays an important role in proper chromosome maintenance (Yoshida et al., 2003). Taken together, these results strongly suggest that spontaneous DNA lesions capable of triggering homologous recombination occur at a strikingly high frequency throughout the genome during normal cell growth; the majority of lesions are repaired through sister chromatid recombination, but occasionally this process leads to LOH events.

To characterize DNA lesions triggering homologous recombination, here we studied the effects of oxidative stress on LOH events. Although previous studies by others have shown that radical oxygen species are involved in chromosome rearrangements in yeast, the assays used can detect a narrower range of rearrangements than our LOH assay. One group analyzed events called gross chromosomal rearrangements in haploid cells (Chen et al., 1998; Ragu et al., 2007), and the other identified a specific deletion between a tandem repeat on a chromosome in diploids (Brennan et al., 1994). We therefore anticipated that our LOH assay would yield an overview of the mutagenic effects of oxidative stress. Yeast cells were treated with H$_2$O$_2$, which in the cells generates highly reactive hydroxyl radicals that have direct effects on most cellular constituents including DNA (Dawes, 2006). The effects of oxidative damage on DNA and nucleotides are well defined and cause specific point mutations (Nakatsu and Sekiguchi, 2006; Rasmussen, 2006). We demonstrate that chromosome alterations as well as point mutations were induced by the oxidative treatment, and that gene conversion-type rearrangements, the least erroneous rearrangements among LOH events, were especially increased.

**MATERIALS AND METHODS**

*Media* Media for yeast strains included complex glucose (YPD), synthetic complete (SC) and various drop-out media, and were prepared as previously described (Rose et al., 1990). 5-FOA plates were prepared as described (Rose et al., 1990) and depleted of leucine and/or adenine sulfate where indicated.

*Strains* All yeast strains used in this study are derivatives of YKU23 (MATa lys2::KAN ura3-52 his3::HIS4 ade2::hisG) and YKU34 (MATa lys2::KAN ura3-52 trp1::LYS3 ade2::hisG III-205::URA3 III-314::ADE2) with the S288c background (Hiraoka et al., 2000). III-205::URA3 signifies that the URA3 fragment was inserted at a locus 205 kb from the left end of chromosome III. Similarly, III-
Homologous recombination and oxidative DNA damage

314::ADE2 denotes that the ADE2 fragment was inserted at 314 kb. Nucleotide coordinates are as given in the Saccharomyces Genome Database (http://www.yeastgenome.org). Derivatives of rad52Δ and rad51Δ mutations from YKU23 are YMO2 and YMO6, respectively, and those from YKU34 are YMO9 and YMO8, respectively (Yoshida et al., 2003). Diploid cells were constructed by mating between haploid a cells and α cells, and selection on SC medium depleted of uracil, leucine, adenine, histidine and tryptophan just before use. Diploid strains are designated RD301 (wild-type), RD304 (rad52Δ/uni0394) and RD305 (rad51Δ/uni0394) (Hiraoka et al., 2000; Yoshida et al., 2003).

H2O2 or methyl methanesulfonate (MMS) treatment H2O2 or MMS treatment was performed as previously described (Ragu et al., 2007) with minor modifications. Haploid (YKU34) or diploid cells were grown to mid-logarithmic phase at 30 °C in SC medium depleted of uracil, leucine and adenine, and about 1 × 107 cells were then collected, washed twice with distilled water and suspended in distilled water at 1 × 107 cells/ml, after which H2O2 or MMS was added to the indicated concentration. After being incubated for 60 min at 30 °C, the cells were washed twice with distilled water and analyzed. Survival of the H2O2-treated cells was determined on YPD plates after incubation for 4 days at 30 °C.

Analysis of cell growth and 5-FOA+ conversion after H2O2 treatment H2O2-treated cells were inoculated in YPD medium and incubated at 30 °C with vigorous shaking. At the indicated time points, aliquots of cells were removed, diluted, and spread on YPD and 5-FOA plates. Colonies were counted after incubation for 4 days at 30 °C. At least three independent experiments were performed to determine the frequencies of 5-FOA+ clones.

LOH assay LOH assay was performed basically as previously described (Hiraoka et al., 2000; Yoshida et al., 2003) with minor modification. H2O2-treated cells were inoculated in YPD medium and incubated at 30 °C with vigorous shaking for 22 h. After appropriate dilution, the cells were spread on YPD, 5-FOA, 5-FOA leucine-depleted, and 5-FOA leucine- and adenine-depleted plates, and incubated at 30 °C for 3–5 days. At least three independent experiments were performed to determine the frequencies of LOH. The frequency of FOA+ Leu+ clones was determined by subtracting the mean frequency of FOA+ Leu+ clones from that of FOA- clones. Similarly, the frequency of FOA+ Leu- Ade+ clones was determined by subtracting the mean frequency of FOA+ Leu- Ade+ clones from that of FOA+ Leu- cells.

PCR procedures to classify Class C events Yeast genomic DNA was purified using InstaGene Matrix (Bio-Rad, Hercules, CA, USA) according to the manufacturer’s instructions. Two microliters of the DNA preparation was used in a 20-μl PCR reaction. PCR was performed under standard conditions with Ex Taq DNA polymerase (Takara, Tokyo, Japan) to determine genetic events of Class C clones as previously described (Hiraoka et al., 2000). Briefly, intrachromosomal deletion between the MAT and HMR loci was detected by PCR with primers encompassing these loci. Semi-quantitative PCR using primers encompassing the URA3-inserted locus (III-205) was performed to distinguish intragenic point mutation and gene conversion-type rearrangements. Genomic DNA of the former generated PCR products from both intact III-205 and URA3-inserted III-205 loci, while that of the latter generated a product only from the intact III-205 locus.

PFGE PFGE analysis of chromosomes was performed as previously described (Hiraoka et al., 2000). Agarose plugs of chromosomal DNA were prepared using a CHEF yeast genomic DNA plug kit (Bio-Rad) according to the manufacturer’s instructions. The plugs were made using cells from equal volumes of culture. Electrophoresis was carried out with 1% PFGE-certified agarose (Bio-Rad) in 0.5 × TBE buffer at 14 °C, using a CHEF Mapper XA pulsed-field electrophoresis system (Bio-Rad). Chromosomes were separated at 6 V/cm of pulses angled at 120°. Switch time was linearly increased from 24.03 sec to 1 min 33.69 sec for 29 h 57 min for standard analysis (Fig. 6B). Higher resolution around chromosome XII was obtained by altering the switch time linearly from 24.03 sec to 3 min 48.48 sec for 28 h 59 min (Fig. 6A). After electrophoresis, the gel was stained with ethidium bromide and destained with water. Gel images were acquired with the CCD camera of a Dolphin-View image system (Wealtec, Sparks, NV, USA).

RESULTS

Diploid cells are more resistant to oxidative stress than haploid cells To examine whether oxidative DNA damage could be a cause of the spontaneous homologous recombination that we detected previously with our LOH analysis (Hiraoka et al., 2000; Umezu et al., 2002), yeast cells were first exposed to oxidative stress using H2O2, which is known to be produced endogenously during normal cellular metabolism (Friedberg et al., 2006). Within the cells, H2O2 is converted in the Fenton reaction to the hydroxyl radical, which is one of the most reactive oxygen radicals and causes serious damage to most cellular constituents including DNA (Dawes, 2006; Nakatsu and Sekiguchi, 2006). In Fig. 2, the survival curves against H2O2 are compared for diploid and haploid cells. The results showed that diploid cells were approximately 10-fold more resistant to H2O2 than haploids, suggest-
ing that some recovery processes available only in diploid cells contributes to cell survival. Another oxidant called menadione, which also generates hydroxyl radicals in the cells (Nutter et al., 1992), yielded similar survival curves to those against H$_2$O$_2$, with diploids again clearly more resistant than haploids (data not shown), suggesting that the difference between diploid and haploid cells was associated with their recovery from damage caused by hydroxyl radicals. Since one repair mechanism that operates only in diploids is recombination between homologous chromosomes, we next tested whether chromosome rearrangements were indeed induced in these cells during their recovery from H$_2$O$_2$ treatment.

**Oxidative stress causes cell growth arrest and increases LOH emergence** During the above experiments, we noticed that H$_2$O$_2$ treatment arrested cell growth, presumably by activating DNA damage checkpoints. Figure 3A shows the growth curves of yeast cells after treatment with various concentrations of H$_2$O$_2$. The duration of cell growth arrest was prolonged in a dose-dependent manner: treatment with 32 mM H$_2$O$_2$ delayed cell growth for several hours, whereas the delay with 8 mM H$_2$O$_2$ was about one hour.

We therefore next examined if and when LOH convertants emerged in the populations after H$_2$O$_2$ treatment (Fig. 3B). The total LOH frequency in untreated cells, providing a baseline of spontaneous LOH in normally growing cells, increased depending on cell growth up to nearly $10^{-4}$, consistent with our previous results (Hiraoka et al., 2000). LOH frequency in H$_2$O$_2$-treated cells increased in a dose-dependent manner; when the cells were treated with 32 mM H$_2$O$_2$, the emergence of LOH convertants was more than 10-fold higher than that in untreated cells. A comparison of the induction of LOH (Fig. 3B) with the population growth curves (Fig. 3A) indicated that LOH clones began to emerge above the basal level of the untreated cells after cells started to grow again. When cells were treated with 32 mM H$_2$O$_2$, it took several hours after the treatment until LOH clones emerged more frequently than the spontaneous level, and afterward the frequency of LOH increased depending on cell growth, suggesting that the recovery process from oxidative damage was accompanied by genetic alterations observed as LOH.

In Table 1 and Fig. 4, LOH clones induced by various concentrations of H$_2$O$_2$ were further classified into three groups of genetic events (Classes A, B and C) according to their phenotypes (Fig. 1). For untreated cells, half of the events were Class B interchromosomal rearrangements, and most of the others were Class A chromosome loss, in agreement with our previous findings for spon-
Homologous recombination and oxidative DNA damage

For LOH events induced by \( H_2O_2 \), although the frequencies of all three classes increased, the degree of induction and the dose dependency of frequencies varied among classes. Class C was most highly induced among the three and the frequency increased in a dose-dependent manner. While Class C events that occurred spontaneously without \( H_2O_2 \) treatment accounted for about 10% of total LOH, treatment with 32 mM \( H_2O_2 \) increased their proportion to around 25%, and the frequency was 30-fold higher than that in untreated cells. The induction of Class B interchromosomal recombination events was also dose-dependent. When the cells were treated with 32 mM \( H_2O_2 \), the frequency of Class B was 11-fold higher than that in untreated cells, about one-third of the induction level of Class C. On the other hand, Class A chromosome loss events were induced 6–9-fold by \( H_2O_2 \) and the dose dependency of their induction was unclear. These results led us to consider whether one specific genetic event within Class C might be particularly induced by \( H_2O_2 \).

We therefore further defined Class C clones and classified them into the following three genetic events by PCR-based analysis: a) an intrachromosomal 94-kb deletion between the \( MAT^a \) and \( HMR \) loci encompassing the \( URA3 \) marker, known as Hawthorne deletion (Hawthorne, 1963); b) allelic interchromosomal rearrangements in which only \( URA3 \) among the three markers was lost (gene conversion-type); and c) point mutations within the \( URA3 \) marker (Fig. 1). Among spontaneous Class C LOH events analyzed in our previous work, \( MAT^a - HMR \) deletion was the most common, followed by gene conversion-type rearrangements, while point mutations were rare (Hiraoka et al., 2000). \( H_2O_2 \) treatment significantly changed the distribution of these events within Class C. Table 2 shows the classification of Class C clones obtained from untreated and 32 mM \( H_2O_2 \)-treated cells. Although all three events were induced by \( H_2O_2 \) treatment, the contributions of individual events differed from those in spontaneous LOH. The vast majority of the events induced by \( H_2O_2 \) were gene conversion-type rearrangements, while point mutations were rare (Hiraoka et al., 2000). \( H_2O_2 \) treatment significantly changed the distribution of these events within Class C. Table 2 shows the classification of Class C clones obtained from untreated and 32 mM \( H_2O_2 \)-treated cells. Although all three events were induced by \( H_2O_2 \) treatment, the contributions of individual events differed from those in spontaneous LOH.

![Fig. 4. Oxidative stress induces all three classes of LOH events, but especially Class C. The cumulative bar graphs plot the results shown in Table 1. Frequencies of LOH Classes A–C in cells treated with the indicated concentration of \( H_2O_2 \) are incorporated into each bar, whose height consequently indicates the total LOH frequency.](image)

| \( H_2O_2 \) (mM) | Class A 5-FOA' frequency (× 10^{-5}) (fold increase) | Class B 5-FOA' frequency (× 10^{-5}) (fold increase) | Class C 5-FOA' frequency (× 10^{-5}) (fold increase) | Total 5-FOA' frequency (× 10^{-5}) (fold increase) |
|-----------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|
| 0               | 3.9 ± 0.7 (×1.0)                                | 5.4 ± 1.1 (×1.0)                                | 1.1 ± 0.2 (×1.0)                                | 10.4 ± 0.9 (×1.0)                                 |
| 0.5             | 24.5 ± 6.0 (×6.3)                               | 27.9 ± 3.2 (×5.2)                               | 10.2 ± 1.6 (×9.3)                               | 62.6 ± 10.0 (×6.0)                                |
| 2               | 28.4 ± 7.5 (×7.3)                               | 33.3 ± 3.5 (×6.2)                               | 12.9 ± 1.4 (×11.7)                              | 74.6 ± 12.3 (×7.2)                                |
| 8               | 29.5 ± 5.7 (×7.6)                               | 39.8 ± 3.1 (×7.4)                               | 17.4 ± 1.2 (×15.8)                              | 86.8 ± 8.7 (×8.3)                                 |
| 16              | 33.9 ± 13.6 (×8.7)                              | 44.5 ± 11.1 (×8.2)                              | 24.1 ± 4.0 (×21.9)                              | 102.6 ± 9.2 (×9.7)                                |
| 32              | 29.8 ± 3.3 (×7.6)                               | 58.6 ± 17.1 (×10.8)                             | 32.3 ± 7.6 (×29.4)                              | 120.7 ± 16.4 (×11.6)                              |

LOH clones were classified into the indicated three classes according to their phenotypes as shown in Fig. 1. Indicated are the mean frequencies with standard deviations determined from at least three independent experiments. In parenthesis, fold increase relative to untreated cells is indicated.
Mutant strains defective in homologous recombination are more sensitive to oxidative stress

Next, we examined the effects of the recombination genes RAD51 and RAD52 on survival after H2O2 treatment (Fig. 5). rad51Δ/rad51Δ and rad52Δ/rad52Δ mutant cells, known to be totally deficient in homologous recombination (Symington, 2002; Mehta and Haber, 2014), were sensitive to H2O2, displaying 30–40% survival relative to wild-type cells. These strains were also treated with menadione and showed patterns of sensitivity similar to those obtained with H2O2 (data not shown). These results suggest that homologous recombination plays an important part in the recovery process and consequent survival of cells damaged by hydroxyl radicals.

Oxidative stress causes strand breaks on chromosomal DNA

Finally, we directly analyzed chromosomal DNA within H2O2-treated cells by PFGE to examine if visible DNA lesions were induced by the treatment (Fig. 6). As a control agent, cells were also treated with MMS, which is known to cause strand breaks in DNA (Schwartz, 1989). As shown in Fig. 6A, when the cells were treated with H2O2, chromosome bands became smeared in a dose-dependent manner and the intact bands of longer chromosomes became faint at the higher concentrations. These results indicate that DNA double-strand breaks were introduced by the treatment. In the time-course experiments shown in Fig. 6B, smeared chromosome bands were observed up to six hours after H2O2 treatment, corresponding to the growth-arrested cell population (Fig. 3A). The extent of degradation of chromosomal DNA proceeded up to four hours after treatment, presumably reflecting the occurrence of double-strand breaks within cells entering S phase (see Discussion). On the other hand, within the cells surviving growth arrest that were sampled at 22 h after treatment, chromosomal DNA appeared to have become intact again. DNA double-strand breaks are

### Table 2. Determination of genetic events giving rise to Class C LOH clones

| Class C event | 0 mM H2O2 | 32 mM H2O2 | Fold increase in frequency |
|---------------|-----------|------------|---------------------------|
| Ratio Frequency | 6.6 | 0.07 | 6.7 | 2.2 | 31 |
| Ratio Frequency | 26.7 | 0.29 | 86.6 | 27.9 | 97 |
| Deletion | 66.7 | 0.74 | 6.7 | 2.2 | 3 |
| Total | 100 | 1.1 | 100 | 32.3 | 29 |

LOH clones of Class C obtained from untreated and 32 mM H2O2-treated cells were analyzed by PCR-based methods as described in Materials and Methods to determine genetic causes of LOH. Thirty clones were analyzed for H2O2-treated cells and 15 for untreated cells.
well-known recombinogenic lesions (Symington, 2002; Mehta and Haber, 2014), and the breaks detected here may represent the trigger events for the observed chromosome rearrangements induced by H$_2$O$_2$ treatment.

**DISCUSSION**

In living cells, genomic DNA is continuously assaulted, with various DNA lesions occurring endogenously even in normal growth, and the efficient repair of these lesions is essential to protect genome integrity (Friedberg et al., 2006; Rasmussen, 2006). In this context, homologous recombination serves as an important pathway, as shown by the fact that its inactivation results in loss of chromosomes at a high frequency (Yoshida et al., 2003). Although the majority of recombination is precise and accompanied by no genetic changes, it is occasionally inaccurate, leading to chromosome rearrangements (Kuzminov, 1999; Mehta and Haber, 2014). These observations indicate that DNA lesions triggering recombination occur at a high frequency during mitotic growth. Several lines of evidence suggest that, in our assay, most LOH events occur in S phase, implying that recombinogenic lesions arise during DNA replication (Watanabe et al., 2002; Yoshida et al., 2003). When the replication machinery encounters DNA damage in regions lacking coding information, the machinery collapses or stalls, leaving replication-induced lesions on both strands, namely two-stranded lesions (Kuzminov, 1999; Symington, 2002; Mehta and Haber, 2014). These lesions can be repaired using an undamaged sister chromatid as a donor for homologous recombination, and this kind of role for recombination in replication restart is widely accepted (Kuzminov, 1999; Symington, 2002; Mehta and Haber, 2014). However, the nature of the original DNA damage that collapses or stalls the replication machinery has not yet been clarified. Here, we focused on oxidative stress as a trigger of homologous recombination, as it is one of the leading causes of DNA damage in normally growing cells (Friedberg et al., 2006).

Among the reactive oxygen species produced in normal cellular metabolism, hydroxyl radicals are the most prominent and active, and are known to directly react with DNA and nucleotides, which could lead to recombinogenic lesions on chromosomal DNA (Friedberg et al., 2006; Nakatsu and Sekiguchi, 2006; Rasmussen, 2006). To generate hydroxyl radicals in yeast cells, we utilized H$_2$O$_2$ and also, in some cases, menadione. H$_2$O$_2$ treatment of cells caused growth arrest, presumably by activating DNA damage checkpoints, and the process of recovery from the arrest was accompanied by genetic alterations which were isolated as LOH events. Point mutations were induced by H$_2$O$_2$ treatment about 30-fold more frequently than in untreated cells (Table 2), indicating that H$_2$O$_2$ treatment directly caused DNA and/or nucleotide damage within the treated cells. In addition, physical analysis of chromosomal DNA within H$_2$O$_2$-treated cells revealed that many double-strand breaks were generated in the arrested cells, as previously shown by others (Ribeiro et al., 2006; Azevedo et al., 2011). These double-strand breaks could either have been caused directly by hydroxyl radicals or have resulted from replication-induced strand breaks triggered by oxidative DNA damage generated by the hydroxyl radicals. In either case, the resulting DNA double-strand breaks are well known to induce homologous recombination reactions (Symington, 2002; Mehta and Haber, 2014).

Consistent with the idea that double-strand breaks are recombinogenic lesions, various chromosome rearrangements were observed at high frequency within H$_2$O$_2$-treated cells (Table 1 and 2), among which gene conversion-type rearrangements were most highly increased, to about 100-fold their frequency in untreated cells. As the gene conversion-type rearrangements of our LOH assay could result from several mechanisms of homologous recombination, the reason for this particular induction of gene conversion is, as yet, unclear. However, it cannot be explained simply by two crossing-over events across the URA3 marker on the observed chromosome, which may be triggered when multiple DNA lesions are generated by H$_2$O$_2$ treatment, because allelic interchromosomal recombination of Class B (crossing-over type) was not increased to a level comparable to that of the gene conversion type. It is noteworthy that, in our previous studies of spontaneous LOH events, gene conversion-type rearrangements were absolutely dependent on both RAD51 and RAD52 genes (Yoshida et al., 2003). The requirement of RAD51 strongly suggests that gene conversion-type rearrangements in our LOH assay, at least spontaneous ones, are produced by a conventional type of homologous recombination reaction involving homology search and strand exchange by Rad51 protein. In the case of gene conversion-type rearrangements, the resulting rearranged chromosome maintains its overall original structure, other than in the region around the URA3 marker, and hence may be defined as the least erroneous rearrangement within LOH events, compared both with more drastic rearrangements such as translocations and also with allelic crossing over. Cells may possess a regulatory mechanism to control homologous recombination in such a way as to suppress genome instability if possible, even when responding to a massive dose of recombinogenic DNA lesions, as in the case of the H$_2$O$_2$ treatment in this study. In the absence of such a mechanism, the same repair process might result in more extensive genetic alterations, such as aberrant chromosomes and chromosome loss, and then lead to devastating outcomes including cancer and various genetic diseases.

This work was supported by the MEXT-Supported Program
for the Strategic Research Foundation at Private Universities, 2008–2012 and 2011–2016. We thank Dr. Mutsuo Sekiguchi and Dr. Hiroaki Nakayama for discussions and advice.

REFERENCES

Ajima, J., Umezu, K., and Maki, H. (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 rearrangements, and the simultaneous incidence of both events during mitotic growth. Mutat. Res. 504, 157–172.

Azevedo, F., Marques, F., Fokt, H., Oliveira, R., and Johansson, B. (2011) Measuring oxidative DNA damage and DNA repair using the yeast comet assay. Yeast 28, 55–61.

Brennan, R. J., Swoboda, B. E. P., and Schiestl, R. H. (1994) Oxidative mutagens induce intrachromosomal recombination in yeast. Mutat. Res. 308, 159–167.

Chen, C., Umezu, K., and Kolodner, R. D. (1998) Chromosomal rearrangements occur in S. cerevisiae rfa1 mutator mutants due to mutagenic lesions processed by double-strand-break repair. Mol. Cell 2, 9–22.

Dawes, I. W. (2006) Cellular responses to reactive oxygen species. In Oxidative Stress, Disease and Cancer. (ed: Singh, K. K.), pp. 261–308. Imperial College Press, London, UK.

Friedberg, E. C., Walker, G. C., Siede, W., Shultz, R. A., and Ellenberger, T. (2006) DNA damage. In DNA Repair and Mutagenesis, Second Edition, pp. 9–69. ASM Press, Washington D.C.

Hawthorne, D. C. (1963) A deletion in yeast and its bearing on the structure of the mating type locus. Genetics 481, 1727–1729.

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

Kuzminov, A. (1999) Recombinational repair of DNA damage in Escherichia coli and bacteriophage λ. Microbiol. Mol. Biol. Rev. 63, 751–813.

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

Mehta, A., and Haber, J. E. (2014) Sources of DNA double-strand breaks and models of recombinational DNA repair. Cold Spring Harb. Perspect. Biol. 6, a016428.

Nakatsu, Y., and Sekiguchi, M. (2006) Oxidative damage to nucleotide: consequences and preventive mechanisms. In Oxidative Stress, Disease and Cancer. (ed: Singh, K. K.), pp. 221–252. Imperial College Press, London, UK.

Nutter, L. M., Ngo, E. O., Fisher, G. R., and Gutierrez, P. L. (1992) DNA strand scission and free radical production in menadione-treated cells. Correlation with cytotoxicity and role of NADPH quinone acceptor oxidoreductase. J. Biol. Chem. 267, 2474–2479.

Ragu, S., Faye, G., Iraqui, I., Masurel-Heneman, A., Kolodner, R. D., and Huang, M. E. (2007) Oxygen metabolism and reactive oxygen species cause chromosomal rearrangements and cell death. Proc. Natl. Acad. Sci. USA 104, 9747–9752.

Rao, C. V., Asch, A. S., and Yamada, H. Y. (2017) Emerging links among Chromosome Instability (CIN), cancer, and aging. Mol. Carcinog. 56, 791–803.

Rasmussen, L. J. (2006) Oxidative damage to DNA and its repair. In Oxidative Stress, Disease and Cancer. (ed: Singh, K. K.), pp. 253–279. Imperial College Press, London, UK.

Ribeiro, G. P., Corre-Real, M., and Johansson, B. (2006) Characterization of DNA Damage in Yeast Apoptosis Induced by Hydrogen Peroxide, Acetic Acid, and Hyperosmotic Shock. Mol. Biol. Cell 17, 4584–4591.

Rose, M. D., Winston, F. M., and Hieter, P. (1990) Methods in Yeast Genetics: A Laboratory Course Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Schwartz, J. L. (1989) Monofunctional alkylating agent-induced S-phase-dependent DNA damage. Mutat. Res. 216, 111–118.

Symington, L. S. (2002) Role of RAD52 epistasis group genes in homologous recombination and double-strand break repair. Microbiol. Mol. Biol. Rev. 66, 630–670.

Tischfield, J. A. (1997) Loss of heterozygosity or: how I learned to stop worrying and love mitotic recombination. Am. J. Hum. Genet. 61, 995–999.

Umezu, K., Hiraoka, M., Mori, M., and Maki, H. (2002) Structural analysis of aberrant chromosomes that occur spontaneously in diploid Saccharomyces cerevisiae: retrotransposon Ty1 plays a crucial role in chromosomal rearrangements. Genetics 160, 97–110.

Watanabe, K., Morishita, J., Umezu, K., Shirahige, K., and Maki, H. (2002) Involvement of RAD9-dependent damage checkpoint control in arrest of cell cycle, induction of cell death, and chromosome instability caused by defects in origin recognition complex in Saccharomyces cerevisiae. Eukaryot. Cell 1, 200–212.

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