

## Defects in DNA Lesion Bypass Lead to Spontaneous Chromosomal Rearrangements and Increased Cell Death<sup>∇</sup>

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**Rev3 polymerase and Mph1 DNA helicase participate in error-prone and error-free pathways, respectively, for the bypassing of template lesions during DNA replication. Here we have investigated the role of these pathways and their genetic interaction with recombination factors, other nonreplicative DNA helicases, and DNA damage checkpoint components in the maintenance of genome stability, viability, and sensitivity to the DNA-damaging agent methyl methanesulfonate (MMS). We find that cells lacking Rev3 and Mph1 exhibit a synergistic, Srs2-dependent increase in the rate of accumulating spontaneous, gross chromosomal rearrangements, suggesting that the suppression of point mutations by deletion of *REV3* may lead to chromosomal rearrangements. While *mph1Δ* is epistatic to homologous recombination (HR) genes, both Rad51 and Rad52, but not Rad59, are required for normal growth of the *rev3Δ* mutant and are essential for survival of *rev3Δ* cells during exposure to MMS, indicating that Mph1 acts in a Rad51-dependent, Rad59-independent subpathway of HR-mediated lesion bypass. Deletion of *MPH1* helicase leads to synergistic DNA damage sensitivity increases in cells with *chl1Δ* or *rrm3Δ* helicase mutations, whereas *mph1Δ* is hypostatic to *sgs1Δ*. Previously reported slow growth of *mph1Δ srs2Δ* cells is accompanied by G<sub>2</sub>/M arrest and fully suppressed by disruption of the Mec3-dependent DNA damage checkpoint. We propose a model for replication fork rescue mediated by translesion DNA synthesis and homologous recombination that integrates the role of Mph1 in unwinding D loops and its genetic interaction with Rev3 and Srs2-regulated pathways in the suppression of spontaneous genome rearrangements and in mutation avoidance.**

Nonreplicative DNA helicases play an important role in the maintenance of genome stability from bacteria to humans, most likely by affecting the formation and/or resolution of recombination intermediates and by facilitating replication fork progression through chromosomal regions with a propensity to adopt unusual DNA structures or those bound by proteins. In *Saccharomyces cerevisiae*, this group of DNA helicases includes the 3'-to-5' helicases Sgs1 and Srs2 and the 5'-to-3' DNA helicase Rrm3. In the absence of any two of these three helicases, unresolved recombination intermediates accumulate and lead to extremely slow growth that is fully suppressed by deletion of genes encoding early homologous recombination (HR) factors (4, 6, 17, 20, 37, 46). In the absence of Sgs1, cells exhibit increased rates of mitotic recombination, frequent chromosome missegregation, accumulation of extrachromosomal ribosomal DNA (rDNA) circles, and increased rates of gross chromosomal rearrangements (GCRs) involving nonhomologous chromosomes (5, 24, 25, 38, 40, 43, 49, 50). Based on the increased crossover frequency during HO endonuclease-induced double-strand breaks (DSBs) in cells lacking Sgs1, it has also been proposed that Sgs1 may function in decatenation of Holliday junctions (HJs) to yield noncrossovers (12, 22). Like Sgs1, Srs2 acts to favor noncrossover outcomes during DSB repair but appears to act earlier than Sgs1 in regulating

recombination outcomes through its ability to dislodge Rad51 from recombinogenic 3' overhangs, thereby promoting a non-crossover synthesis-dependent single-strand annealing (SDSA) pathway (12, 33, 35). In contrast, Rrm3 has not been implicated in DNA repair but is thought to be important for avoidance of recombination substrate formation by removal of DNA protein complexes in certain chromosomal locations, such as chromosome ends and replication fork barriers at the rDNA locus, thus facilitating replication fork progression (13, 14).

In addition to Sgs1, Rrm3, and Srs2, the yeast genome encodes two other nonreplicative DNA helicases with proposed functions in DNA repair, Mph1 and Chl1. Mph1 possesses 3'-to-5' helicase activity, and its ATPase activity requires a relatively long fragment of single-stranded DNA (ssDNA) (≥40 nucleotides [nt]) for full activity *in vitro* (32). Mph1 is also necessary for resistance to the DNA damaging agents methyl methanesulfonate (MMS) and 4-nitroquinoline-1-oxide (4-NQO) and suppresses spontaneous mutations toward canavanine resistance (3, 41). The modest mutator phenotype of the *mph1Δ* mutant is enhanced by additional mutations in base excision repair (*apn1Δ* and *apn2Δ*) and is suppressed by mutations in translesion DNA synthesis (TLS) (*rev3Δ*) (36, 41). These findings, in combination with the observation of an epistatic relationship between *mph1Δ* and homologous recombination mutations, have led to the proposal that Mph1 may act in Rad52-dependent, error-free bypassing of DNA lesions (41). Like the 3'-to-5' DNA helicases Sgs1 and Srs2, Mph1 was recently shown to affect crossover frequency during repair of an HO endonuclease-induced DNA DSB, favoring noncrossovers as the outcome (33). The authors showed that Mph1 can

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unwind intermediates of homologous recombination *in vitro*, specifically D loops that are thought to form early during homologous recombination when a homoduplex is invaded by a Rad51 filament. While Srs2 has been shown to be able to disassemble Rad51 filaments *in vitro*, it does not appear to possess Mph1's ability to dissociate D loops once they have formed (19, 47).

Although Chl1 has been shown to be required for the establishment of sister chromatid cohesion, a possible role in DNA repair by homologous recombination has also been proposed (11, 28, 30, 42). While Chl1 possesses a conserved helicase domain, helicase activity has so far been shown only for its putative human homolog, hCHLR1 (10).

To further elucidate the functional interaction between non-replicative DNA helicases and DNA repair pathways, we generated a series of mutants with combinations of *mph1Δ*, *chl1Δ*, *rrm3Δ*, *srs2Δ*, and *sgs1Δ* mutations and mutations in translesion DNA synthesis (TLS), base excision repair (BER), homologous recombination (HR), and DNA damage checkpoints. In addition to synthetic fitness defects due to aberrant HR and checkpoint activation, we identified epistatic and synergistic relationships with regard to fitness, the accumulation of gross chromosomal rearrangements (GCRs), and sensitivity to DNA damage. We propose that Mph1 functions in a Rad51-dependent, Rad59-independent pathway of HR for DNA lesion bypass and interacts genetically with *REV3* in the suppression of gross chromosomal rearrangements.

#### MATERIALS AND METHODS

**Yeast strains and media.** All strains used in this study are derived from *Saccharomyces cerevisiae* strain S288C and are listed in Table 1. For GCR rate measurements, desired gene deletions were introduced into KHSY802 (*MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 lys2Bgl hom3-10 ade2Δ1 ade8 hxt13::URA3*), RDKY5027 (*MATα ura3-52 trp1Δ63 his3Δ200 leu2Δ1 lys2Bgl hom3-10 ade2Δ1 ade8 hxt13::URA3*), or RDKY6678 (*MATa ura3-52 leu2Δ1 trp1Δ63 his3Δ200 lys2ΔBgl hom3-10 ade2Δ1 ade8 can1::hisG yel072w::CAN1/URA3 iYEL072::hph*) by HR-mediated integration of PCR products by the lithium acetate method (9). All haploid strains, including single mutants, for GCR rate measurements, growth analysis, and fluorescence-activated cell sorting (FACS) were obtained by sporulating diploids heterozygous for the desired mutation(s). Spores were genotyped on selective media or by PCR. For tetrad dissection, desired mutations were introduced by HR-mediated integration of PCR fragments in the strain background RDKY2666 (*MATa ura3-52 trp1Δ63 his3Δ200*) or RDKY2664 (*MATα ura3-52 trp1Δ63 his3Δ200*).

Media for propagating strains have been previously described (2).

**GCR analysis.** GCR rates were determined exactly as previously described (39). Initially, GCR rates were derived from 10-ml cultures of two or three independent strain isolates. For mutants with low GCR rates, up to 75 cultures, ranging from 10 to 50 ml in volume, were analyzed per mutant. To determine the statistical significance of differences between median GCR rates, 95% confidence intervals ( $\alpha < 0.05$ ) for all median GCR rates were calculated according to the method of Nair (26). GCR rates were measured in the standard GCR strain background RDKY3615 and a modified GCR strain background, RDKY6678 (both strains were kindly provided by Richard Kolodner, University of California—San Diego). In RDKY3615, the *CAN1* gene is in its wild-type location on chromosome V and a *URA3* cassette was used to replace the *HXT13* gene on chromosome V (34, 39). In RDKY6678, the *CAN1* gene is deleted (*can1::hisG*) and a *URA3/CAN1* cassette is inserted into *YEL072W*, located telomeric of *HXT13* on chromosome V (34).

**Tetrad analysis.** Diploids were sporulated in 1% potassium acetate for 5 days at 30°C, washed, digested with zymolase (500  $\mu$ g/ml in 1 M sorbitol), and dissected on yeast extract-peptone-dextrose (YPD) agar plates using a micro-manipulator mounted on an Axioskop 40 microscope (Zeiss). The YPD plates were incubated for 2 days at 30°C and photographed.

**Doubling time measurement.** Overnight cultures of independent isolates were diluted in 5 ml of YPD to an optical density at 600 nm ( $OD_{600}$ ) of 0.1 to 0.2, and

the  $OD_{600}$  was measured in 60-min or 120-min intervals for 6 to 8 h. Doubling times are reported in minutes and are presented as the average doubling time of two or three independent strains for each genotype, with error bars showing the standard deviations.

**DNA content analysis.** Cells were grown overnight at 30°C in YPD medium. Cultures were diluted in YPD to an  $OD_{600}$  of 0.2, and incubation was continued until cultures reached an  $OD_{600}$  of 0.6 to 0.8. Cells were then fixed in 70% ethanol for 1 h at room temperature and sonicated in 50 mM sodium citrate (pH 7). The cells were washed once in 50 mM sodium citrate (pH 7), and RNase A was added to a final concentration of 250  $\mu$ g/ml. After overnight incubation at 37°C, the cells were washed twice in 50 mM sodium citrate. To stain the DNA, Sytox green (Molecular Probes) was added to a final concentration of 1  $\mu$ M and the cells were incubated in the dark at room temperature for 1 h immediately prior to fluorescence-activated cell sorting (FACS) on a BD LSR II analyzer. The distribution of cells throughout the cell cycle phases was quantified with the FlowJo v8.3.3 software program. The mean obtained from measurements of at least three cultures and standard deviation are reported for every strain.

**MMS sensitivity.** Overnight cultures were diluted in YPD to an  $OD_{600}$  of 0.2 and grown at 30°C to an  $OD_{600}$  of 0.6. A series of 10-fold dilutions was prepared for every yeast culture, and 5  $\mu$ l was spotted on YPD and on YPD containing the appropriate levels of MMS. Colony growth was recorded after 24 h, 48 h, and 72 h of incubation at 30°C. The 48-h time point is shown.

## RESULTS

**Translesion DNA synthesis suppresses GCR accumulation in *mph1Δ* cells.** Deletion of *MPH1* has been shown to cause an increase in spontaneous base substitutions at *CAN1*, which can be suppressed by disrupting error-prone translesion DNA synthesis (41). To determine how spontaneous DNA lesions are processed if they cannot be bypassed by Mph1 or TLS, we deleted *MPH1* and *REV3* in a yeast strain that has been modified to allow the detection of gross chromosomal rearrangements, such as translocations, large deletions, and de novo telomere additions (18, 39). We found that the *rev3Δ mph1Δ* mutant showed a statistically significant increase in the GCR rate over that of the single mutants, as indicated by the non-overlapping 95% confidence intervals ( $\alpha < 0.05$ ) for the median GCR rates (Table 2). This may indicate that the avoidance of point mutations by deletion of the error-prone DNA polymerase Rev3 occurs at the expense of increased formation of chromosomal rearrangements, suggesting that as long as Rev3 is present, spontaneous DNA lesions in the *mph1Δ* mutant are preferentially taken care of by TLS, whereas an alternative repair pathway preferentially utilized in the *rev3Δ mph1Δ* mutant is prone to GCR formation. To test the possibility that Srs2, a DNA helicase that has been shown to regulate homologous recombination by disrupting recombinogenic Rad51-filaments (19, 47), may channel DNA lesions into this alternative DNA repair pathway, we determined the effect of an *srs2Δ* mutation on GCR formation in the *rev3Δ mph1Δ* mutant and found that GCR formation was eliminated (Table 2). That the viability of the *rev3Δ mph1Δ* mutant was significantly reduced upon introduction of the *srs2Δ* mutation (Fig. 1A) is consistent with previous reports of reduced fitness for the *mph1Δ srs2Δ* mutant (33, 45) and suggests that spontaneous DNA lesions in the *rev3Δ mph1Δ* mutant may become substrates for homologous recombination pathways that need to be regulated by Srs2 to prevent cell death. In contrast to the *mph1Δ* mutant, the *rev3Δ* mutant does not require Srs2 for normal growth (Fig. 1A).

To test whether slow growth of the *mph1Δ srs2Δ* and *rev3Δ mph1Δ srs2Δ* mutants was due to increased  $G_2/M$  arrest or to slowed progression through S phase resulting from impaired

TABLE 1. *Saccharomyces cerevisiae* strains used in this study

Strain	Genotype
RDKY3615 <sup>a</sup>	<i>MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 lys2ΔBgl hom3-10 ade2Δ1 ade8 hxt13::URA3</i>
RDKY2666 <sup>a</sup>	<i>MATa ura3-52 trp1Δ63 his3Δ200</i>
RDKY2664 <sup>a</sup>	<i>MATα ura3-52 trp1Δ63 his3Δ200</i>
RDKY6678 <sup>a</sup>	<i>MATa ura3-52 leu2Δ1 trp1Δ63 his3Δ200 lys2ΔBgl hom3-10 ade2Δ1 ade8 can1::hisG yel072w::CAN1/URA3 iYEL072::hph</i>
RDKY6795 <sup>a</sup>	<i>MATa ura3-52 leu2Δ1 trp1Δ63 his3Δ200 lys2ΔBgl hom3-10 ade2Δ1 ade8 can1::hisG yel072w::CAN1/URA3 iYEL072::hph mph1::HIS3</i>
KHSY883	<i>MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 lys2ΔBgl hom3-10 ade2Δ1 ade8 hxt13::URA3 rad51::HIS3</i>
KHSY1258	<i>MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 lys2ΔBgl hom3-10 ade2Δ1 ade8 hxt13::URA3 rad52::HIS3</i>
KHSY1399	<i>MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 lys2ΔBgl hom3-10 ade2Δ1 ade8 hxt13::URA3 rrm3::kanMX6</i>
KHSY1557	<i>MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 lys2ΔBgl hom3-10 ade2Δ1 ade8 hxt13::URA3 mph1::HIS3</i>
KHSY1561	<i>MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 lys2ΔBgl hom3-10 ade2Δ1 ade8 hxt13::URA3 chl1::HIS3</i>
KHSY1598	<i>MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 lys2ΔBgl hom3-10 ade2Δ1 ade8 hxt13::URA3 rrm3::TRP1 mph1::HIS3</i>
KHSY1600	<i>MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 lys2ΔBgl hom3-10 ade2Δ1 ade8 hxt13::URA3 sgs1::TRP1 mph1::HIS3</i>
KHSY1630	<i>MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 lys2ΔBgl hom3-10 ade2Δ1 ade8 hxt13::URA3 sgs1::TRP1</i>
KHSY1702	<i>MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 lys2ΔBgl hom3-10 ade2Δ1 ade8 hxt13::URA3 srs2::kanMX6 mph1::HIS3</i>
KHSY1713	<i>MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 lys2ΔBgl hom3-10 ade2Δ1 ade8 hxt13::URA3 rrm3::TRP1 mph1::HIS3</i>
KHSY1725	<i>MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 lys2ΔBgl hom3-10 ade2Δ1 ade8 hxt13::URA3 chl1::HIS3 mph1::HIS3</i>
KHSY1872	<i>MATa/α ura3-52/ura3-52 trp1Δ63/trp1Δ63 his3Δ200/his3Δ200 MPH1/mph1::HIS3 SRS2/srs2::TRP1</i>
KHSY1878	<i>MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 lys2ΔBgl hom3-10 ade2Δ1 ade8 hxt13::URA3 mec3::kanMX6 mph1::HIS3</i>
KHSY1889	<i>MATa/α ura3-52/ura3-52 trp1Δ63/trp1Δ63 his3Δ200/his3Δ200 MPH1/mph1::HIS3 MRE11/mre11::URA3</i>
KHSY1894	<i>MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 lys2ΔBgl hom3-10 ade2Δ1 ade8 hxt13::URA3 mph1::HIS3 mec1::HIS3 sml1::TRP1</i>
KHSY1932	<i>MATa/α ura3-52/ura3-52 trp1Δ63/trp1Δ63 his3Δ200/his3Δ200 MPH1/mph1::URA3 SRS2/srs2::HIS3 MEC3/mec3::TRP1</i>
KHSY1935	<i>MATa/α ura3-52/ura3-52 trp1Δ63/trp1Δ63 his3Δ200/his3Δ200 MPH1/mph1::HIS3 SRS2/srs2::TRP1 RAD51/rad51::kanMX6</i>
KHSY1954	<i>MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 lys2ΔBgl hom3-10 ade2Δ1 ade8 hxt13::URA3 rev3::TRP1</i>
KHSY1957	<i>MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 lys2ΔBgl hom3-10 ade2Δ1 ade8 hxt13::URA3 apn1::TRP1</i>
KHSY1970	<i>MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 lys2ΔBgl hom3-10 ade2Δ1 ade8 hxt13::URA3 apn1::TRP1 mph1::HIS3</i>
KHSY1976	<i>MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 lys2ΔBgl hom3-10 ade2Δ1 ade8 hxt13::URA3 rev3::TRP1 mph1::HIS3</i>
KHSY2020	<i>MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 lys2ΔBgl hom3-10 ade2Δ1 ade8 hxt13::URA3 rad51::HIS3 srs2::kanMX6 mph1::HIS3</i>
KHSY2038	<i>MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 lys2ΔBgl hom3-10 ade2Δ1 ade8 hxt13::URA3 mec3::HIS3 srs2::kanMX6 mph1::TRP1</i>
KHSY2226	<i>MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 lys2ΔBgl hom3-10 ade2Δ1 ade8 hxt13::URA3 rev3::TRP1 srs2::kanMX6 mph1::HIS3</i>
KHSY2416	<i>MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 lys2ΔBgl hom3-10 ade2Δ1 ade8 hxt13::URA3 rev3::TRP1 rad52::HIS3 mph1::HIS3</i>
KHSY2420	<i>MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 lys2ΔBgl hom3-10 ade2Δ1 ade8 hxt13::URA3 rev3::TRP1 rad52::HIS3</i>
KHSY2492	<i>MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 lys2ΔBgl hom3-10 ade2Δ1 ade8 hxt13::URA3 mph1::HIS3 rad51::HIS3</i>
KHSY3042	<i>MATa ura3-52 leu2Δ1 trp1Δ63 his3Δ200 lys2ΔBgl hom3-10 ade2Δ1 ade8 can1::hisG yel072w::CAN1/URA3 iYEL072::hph mec3::TRP1 mph1::HIS3</i>
KHSY3056	<i>MATa ura3-52 leu2Δ1 trp1Δ63 his3Δ200 lys2ΔBgl hom3-10 ade2Δ1 ade8 can1::hisG yel072w::CAN1/URA3 iYEL072::hph rev3::TRP1 mph1::HIS3</i>
KHSY3065	<i>MATa ura3-52 leu2Δ1 trp1Δ63 his3Δ200 lys2ΔBgl hom3-10 ade2Δ1 ade8 can1::hisG yel072w::CAN1/URA3 iYEL072::hph mec3::TRP1</i>
KHSY3067	<i>MATa ura3-52 leu2Δ1 trp1Δ63 his3Δ200 lys2ΔBgl hom3-10 ade2Δ1 ade8 can1::hisG yel072w::CAN1/URA3 iYEL072::hph srs2::HIS3</i>
KHSY3101	<i>MATa ura3-52 leu2Δ1 trp1Δ63 his3Δ200 lys2ΔBgl hom3-10 ade2Δ1 ade8 can1::hisG yel072w::CAN1/URA3 iYEL072::hph srs2::HIS3 mph1::HIS3</i>
KHSY3123	<i>MATa ura3-52 leu2Δ1 trp1Δ63 his3Δ200 lys2ΔBgl hom3-10 ade2Δ1 ade8 can1::hisG yel072w::CAN1/URA3 iYEL072::hph srs2::HIS3 mph1::HIS3 rev3::TRP1</i>
KHSY3126	<i>MATa ura3-52 leu2Δ1 trp1Δ63 his3Δ200 lys2ΔBgl hom3-10 ade2Δ1 ade8 can1::hisG yel072w::CAN1/URA3 iYEL072::hph srs2::HIS3 mph1::HIS3 mec3::TRP1</i>

<sup>a</sup> Obtained from Richard Kolodner (University of California—San Diego).

DNA lesion bypass, cell cycle profiles were obtained (Fig. 2A) and the fraction of cells in each cell cycle phase from three independent cultures was quantified (Fig. 2B). We found that the *mph1Δ srs2Δ* and *rev3Δ mph1Δ srs2Δ* mutants showed increased arrest in G<sub>2</sub>/M compared to the corresponding single and double mutants, but the mutations lacked any discernible affect on S phase. That the fraction of cells in S phase was largely unaffected indicates that impairment of DNA lesion bypass does not hinder the timely completion of genome replication

but may instead cause the formation of DNA structures that later in the cell cycle impair progress through mitosis.

Deletion of *RAD51*, which had previously been shown to improve colony growth of the *mph1Δ srs2Δ* mutant and other DNA helicase double mutants (4, 6, 17, 20, 33, 37, 46), abolished the G<sub>2</sub>/M arrest of *mph1Δ srs2Δ* cells and allowed them to progress through the cell cycle as did the *srs2Δ* single mutant (Fig. 3A). In addition to disrupting homologous recombination, we found that disruption of the DNA damage checkpoint

TABLE 2. Effect of defects in DNA lesion bypass, homologous recombination, DNA helicases, and the DNA damage checkpoint on accumulation of gross chromosomal rearrangements in the standard GCR strain background RDKY3615

Relevant genotype	Strain	GCR rate (Can <sup>+</sup> 5-FOA <sup>r</sup> ) ( $\times 10^{-10}$ ) <sup>a</sup>	95% CI <sup>b</sup> (Can <sup>+</sup> 5-FOA <sup>r</sup> ) ( $\times 10^{-10}$ )	Fold increase over wild- type level
Wild type	RDKY3615	3.5 <sup>c</sup>		1
<i>mph1</i>	KHSY1557	20	5–34	6
<i>rev3</i>	KHSY1954	10	5–21	3
<i>rev3 mph1</i>	KHSY1976	56	44–71	16
<i>rev3 mph1 srs2</i>	KHSY2226	<14	<11–18	<4
<i>srs2</i>	RDKY5557	2 <sup>d</sup>	<2–11	0.6
<i>mph1 srs2</i>	KHSY1702	1.2	<2–6	0.3
<i>mph1 mec3</i>	KHSY1878	55	24–73	16
<i>mph1 mec3 srs2</i>	KHSY2038	56	30–68	16
<i>mec1 sml1</i>	KHSY895	471	209–859	135
<i>mec1 sml1 mph1</i>	KHSY1894	290	154–467	83
<i>apn1</i>	KHSY1957	19	14–41	5
<i>apn1 mph1</i>	KHSY1970	15	<15–51	4
<i>rad52</i>	KHSY1258	435	317–520	124
<i>rad52 mph1</i>	KHSY1598	275	131–467	79
<i>sgs1</i>	KHSY1630	220	144–276	64
<i>sgs1 mph1</i>	KHSY1600	239	162–528	68
<i>chl1</i>	KHSY1561	14	<14–94	4
<i>chl1 mph1</i>	KHSY1725	40	<10–202	11
<i>rrm3</i>	KHSY1399	14 <sup>d</sup>	5–28	4
<i>rrm3 mph1</i>	KHSY1713	21	<17–48	6

<sup>a</sup> 5-FOA, 5-fluoroorotic acid.

<sup>b</sup> Ninety-five percent confidence intervals (CIs) were calculated according to the method of Nair (26), with nonoverlapping confidence intervals indicating statistically significant differences ( $\alpha < 0.05$ ) between median GCR rates.

<sup>c</sup> GCR rate from reference 2.

<sup>d</sup> GCR rate from reference 38.

clamp by *MEC3* deletion also improved growth of the *mph1* $\Delta$  *srs2* $\Delta$  mutant (Fig. 1A) and had the same effect on viability and cell cycle progression as the deletion of *RAD51* (Fig. 3). In contrast, introduction of a *rev3* $\Delta$  mutation into the *mph1* $\Delta$  *srs2* $\Delta$  mutant did not affect growth (Fig. 1A) but led to a small increase in the fraction of G<sub>2</sub>/M-arrested cells (Fig. 2). Similarly, sensitivity of the *mph1* $\Delta$  *srs2* $\Delta$  mutant to MMS was aggravated further by a *rev3* $\Delta$  mutation but was alleviated by a *rad51* $\Delta$  mutation (Fig. 4). In the presence of MMS, *rev3* $\Delta$  *mph1* $\Delta$  *srs2* $\Delta$  cells emerged only after incubation for >72 h. This strong synergistic increase in MMS sensitivity of the triple mutant compared to that of any of the double mutants suggests that all three genes mediate independent pathways for survival in the presence of DNA damage. Taken together, these findings suggest that deleting the error-prone DNA polymerase Rev3 in *mph1* $\Delta$  cells while effectively avoiding points mutations causes the appearance of a different mutation type, i.e., gross chromosomal rearrangements, which activates the DNA damage checkpoint in G<sub>2</sub>/M and causes cell death if Srs2 is not present to regulate HR-dependent DNA lesion bypass.

**Suppression of genome rearrangements by *srs2* $\Delta$  depends on functional DNA damage checkpoint.** Genome instability in cells lacking Sgs1 helicase is suppressed by the DNA damage checkpoint, as demonstrated by synergistic GCR rate increases upon introduction of the *mec3* $\Delta$ , *rad24* $\Delta$ , *mec1* $\Delta$ , *rad53* $\Delta$  or *rad9* $\Delta$  mutation into the *sgs1* $\Delta$  mutant (40). As demonstrated by overlapping 95% confidence intervals, no statistically significant changes in the GCR rate of the *mph1* $\Delta$  mutant were observed upon introduction of checkpoint mutations (*mec3* $\Delta$  and *mec1* $\Delta$ ) (Table 2), suggesting that the DNA damage checkpoint is not required for the suppression of GCRs in the

*mph1* $\Delta$  mutant. However, when we introduced the *mec3* $\Delta$  mutation into the *mph1* $\Delta$  *srs2* $\Delta$  mutant, which itself exhibited wild-type levels of GCRs, the GCR rate increased to that of the *mph1* $\Delta$  *mec3* $\Delta$  mutant (Table 2), thus suggesting that, in contrast to the case with the *rev3* $\Delta$  *mph1* $\Delta$  and *mph1* $\Delta$  mutants, GCR formation in checkpoint-deficient mutants is not dependent on Srs2. Similarly, the GCR rate of the *mec3* $\Delta$  mutant did not change upon introduction of an *srs2* $\Delta$  mutation. This ability of the *srs2* $\Delta$  mutation to suppress GCR formation in checkpoint-proficient cells but not in checkpoint-deficient cells suggests that the Mec3 checkpoint detects the aberrant HR intermediates that form in the absence of Srs2, leading to G<sub>2</sub>/M arrest and avoidance of GCRs, whereas in the absence of the checkpoint, these aberrant HR intermediates go on to form GCRs.

**Lack of Rev3 and Mph1 causes synergistic GCR rate increase in new GCR strain susceptible to duplication-mediated rearrangements.** Putnam et al. (34) recently showed that the rate of GCR accumulation depends significantly on chromosomal features in the breakpoint region. For example, while

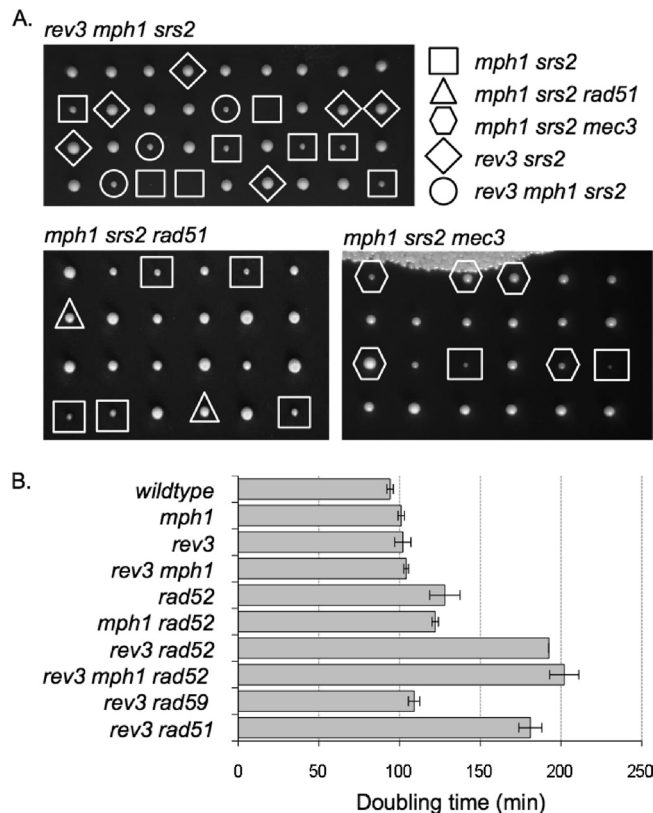


FIG. 1. Genetic interactions between *rev3* $\Delta$ , *mph1* $\Delta$ , *srs2* $\Delta$ , and HR mutations were assessed by testing the fitness of mutants. (A) Tetrads from diploids heterozygous for *rev3* $\Delta$ , *mph1* $\Delta$ , and *srs2* $\Delta$ ; *mph1* $\Delta$ , *srs2* $\Delta$ , and *rad51* $\Delta$ ; or *mph1* $\Delta$ , *srs2* $\Delta$ , and *mec3* $\Delta$  were dissected on rich medium and genotyped by spotting on selective medium or by PCR to determine the presence of gene deletions. In contrast to the *mph1* $\Delta$  mutant, the *rev3* $\Delta$  mutant does not require *SRS2* for normal growth. Deletion of *MEC3* or disruption of HR rescues the slow growth of the *mph1* $\Delta$  *srs2* $\Delta$  mutant. (B) Doubling times of mutant strains and appropriate controls in rich medium (YPD) are shown with standard deviations. Cells lacking Rev3 require Rad51 and Rad52 but not Rad59 for normal growth, and these growth defects are unaffected by Mph1.

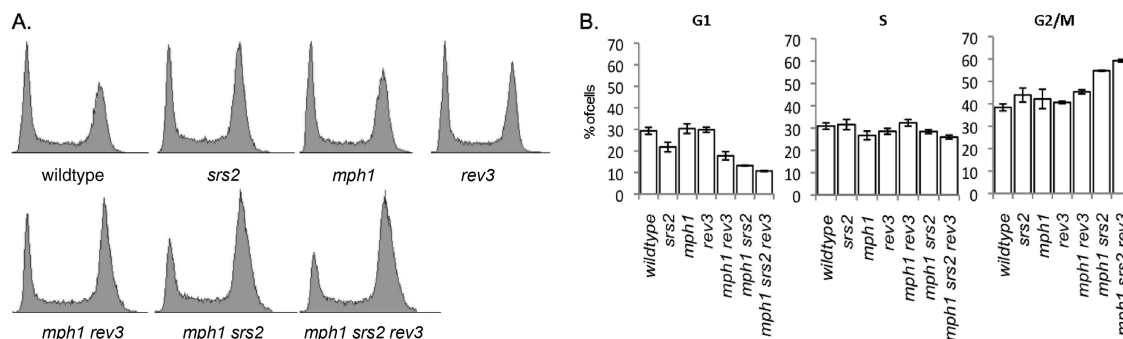


FIG. 2. Effect of *rev3* $\Delta$  and *srs2* $\Delta$  mutations on cell cycle progression of cells lacking Mph1. Asynchronous cultures were grown to mid-log phase, fixed, and stained with Sytox green to measure DNA content by FACS. (A) Cell cycle profiles reveal that *mph1* $\Delta$  *srs2* $\Delta$  cells accumulate in G<sub>2</sub>/M phase, which is enhanced further by a *rev3* $\Delta$  mutation. (B) Quantification of cell fractions in G<sub>1</sub>, S, and G<sub>2</sub>/M phases, determined by FACS analysis of three cell cultures for each strain, using FlowJo v8.3.3. Error bars indicate the standard deviations.

GCRs in the standard GCR strain background (RDKY3615) are due largely to single-copy-sequence-mediated rearrangements, GCRs in a newly designed strain (RDKY6678) are duplication mediated due to the presence of imperfect homology between the *HXT13-DSF1* sequence in the breakpoint region on chromosome V and sequences on chromosomes IV, X, and XIV (34). This new GCR strain accumulates chromosomal rearrangements at an increased rate compared to that for the standard GCR strain, with wild-type cells having a 56-fold-higher GCR rate than the standard strain (34). To assess the effect of DNA lesion bypass defects on GCR formation in this new strain, *rev3* $\Delta$ , *mph1* $\Delta$ , *srs2* $\Delta$ , and *mec3* $\Delta$  mutations were introduced into RDKY6678 (Table 3). Consistent with our observations with the standard GCR strain background (Table 2), the *rev3* $\Delta$  *mph1* $\Delta$  double mutant exhibited a synergistic GCR rate increase compared to results for the *mph1* $\Delta$  and *rev3* $\Delta$  single mutants. Interestingly, the significantly greater synergistic effect of combining the *rev3* $\Delta$  and *mph1* $\Delta$  mutations in the new GCR strain background (Table 3; 167-fold increase over rates for the RDKY6678 wild type) compared to results with the standard GCR strain background (Table 2, 16-fold increase over rates for the RDKY3615 wild type) indicates that alternative pathways utilized for DNA lesion bypass in the *rev3* $\Delta$  *mph1* $\Delta$  mutant may be more prone to duplication-mediated than to single-copy-sequence mediated

genome rearrangements. As in the standard GCR strain (Table 2), deletion of *SRS2* in the new GCR strain led to a significant decrease in the GCR rate of the *rev3* $\Delta$  *mph1* $\Delta$  mutant to the level of the *srs2* $\Delta$  mutant, suggesting that viable GCR formation depends on the antirecombinase Srs2 despite the different breakpoint regions in the two GCR strain backgrounds and the different GCR types that are likely to arise from them. The fact that deletion of *SRS2* did not cause a GCR rate increase in the *mph1* $\Delta$  *mec3* $\Delta$  mutant in the standard GCR background (Table 2) but led to a significant GCR rate increase in the new GCR background (Table 3) is likely due to the greater requirement of Srs2 for GCR suppression in the new GCR strain background (Table 3, *srs2* $\Delta$ : 26-fold increase over wild-type level) than in the standard GCR strain (Table 2, *srs2* $\Delta$ : 0.6-fold increase over wild-type level).

**Genetic interactions between MPH1 and other DNA helicases.** Negative genetic interactions between any two of the DNA helicases Sgs1, Srs2, and Rrm3 have previously been shown to be caused by the accumulation of aberrant intermediates of homologous recombination (6, 29, 37, 46). Since such a negative, HR-dependent genetic interaction has now also been established between *mph1* $\Delta$  and *srs2* $\Delta$ , we tested *mph1* $\Delta$  mutants with deletions of other confirmed or putative DNA helicase genes (*sgs1* $\Delta$ , *rrm3* $\Delta$ , and *chl1* $\Delta$ ) for growth defects, GCR accumulation, and sensitivity to MMS. We observed that

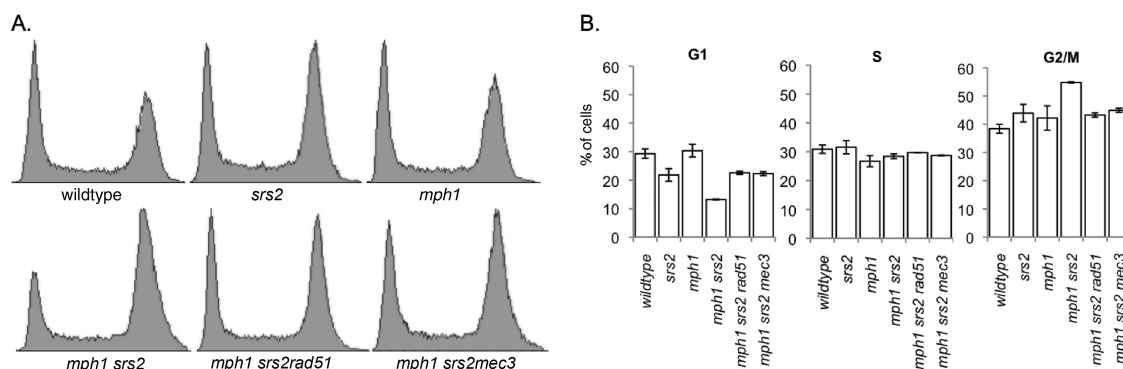


FIG. 3. The G<sub>2</sub>/M arrest of *mph1* $\Delta$  *srs2* $\Delta$  cells is suppressed by disrupting homologous recombination or the DNA damage checkpoint. Cell cycle profiles (A) and quantification (B) of the fractions of cells in G<sub>1</sub>, S, and G<sub>2</sub>/M phases show that the *rad51* $\Delta$  and *mec3* $\Delta$  mutations are equally effective at decreasing cell accumulation in G<sub>2</sub>/M, showing an increase in the fraction of cells in G<sub>1</sub>. Neither mutation affects the fraction of cells in S phase. The DNA content of Sytox green-stained cells from at least three mid-log-phase cultures of every strain was analyzed by FACS.

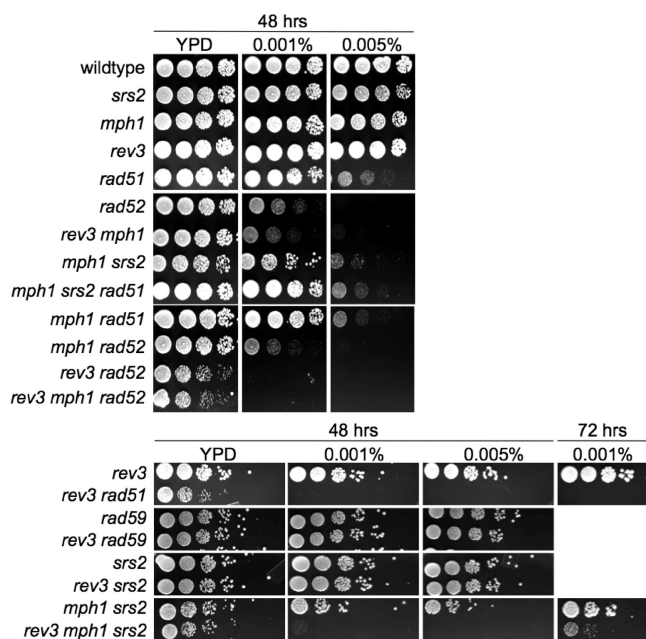


FIG. 4. Effect of mutations affecting translesion DNA synthesis and homologous recombination on sensitivity to MMS. Tenfold dilutions of exponentially growing cultures were spotted on YPD (viable cell count) or YPD containing 0.001% or 0.005% MMS. Colony growth after 48 h (and 72 h for selected mutants) of incubation at 30°C is shown.

unlike the case with the *mph1Δ srs2Δ* mutant, the meiotic products of diploids heterozygous for the *mph1Δ* mutation and either the *sgs1Δ*, *rrm3Δ*, or *chl1Δ* mutation grew normally. In addition to a synergistic increase in sensitivity to MMS for the *mph1Δ srs2Δ* mutant (1), we also identified synergistic increases in sensitivity for the *mph1Δ rrm3Δ* and *mph1Δ chl1Δ* mutants but not for the *mph1Δ sgs1Δ* mutant, which appeared as sensitive as the *sgs1Δ* single mutant (Fig. 5). This indicates

TABLE 3. Effects of *mph1*, *rev3*, *srs2*, and *mec3* deletions on the accumulation of chromosomal rearrangements in a new GCR strain background (RDKY6678) that is prone to duplication-mediated rearrangements<sup>a</sup>

Relevant genotype	Strain	GCR rate (Can <sup>+</sup> 5-FOA <sup>a</sup> ) ( $\times 10^{-8}$ ) <sup>b</sup>	95% CI <sup>c</sup> (Can <sup>+</sup> 5-FOA <sup>a</sup> ) ( $\times 10^{-8}$ )	Fold increase over wild-type level
Wild type	RDKY6678	2 <sup>d</sup>		1
<i>mph1</i>	RDKY6795	18	10–31	9
<i>rev3</i>	KHSY3110	13	8–20	7
<i>rev3 mph1</i>	KHSY3056	334	237–389	167
<i>rev3 mph1 srs2</i>	KHSY3123	57	41–67	29
<i>srs2</i>	KHSY3067	31	21–54	16
<i>mph1 srs2</i>	KHSY3101	40	32–61	20
<i>mec3</i>	KHSY3065	44	30–59	22
<i>mph1 mec3</i>	KHSY3042	43	30–98	22
<i>mph1 mec3 srs2</i>	KHSY3126	142	116–191	71

<sup>a</sup> See reference 34.

<sup>b</sup> 5-FOA, 5-fluoroorotic acid.

<sup>c</sup> Ninety-five percent confidence intervals (CIs) were calculated according to the method of Nair (26), with nonoverlapping confidence intervals indicating statistically significant differences ( $\alpha < 0.05$ ) between median GCR rates.

<sup>d</sup> GCR rate from reference 34.

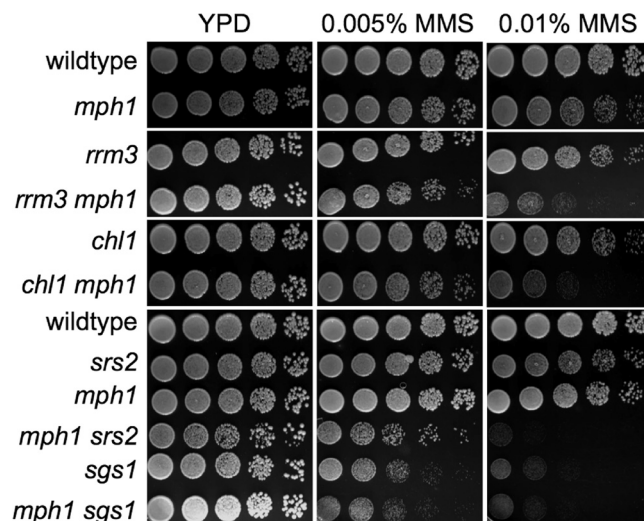


FIG. 5. Effect of an *mph1Δ* mutation on MMS sensitivity of mutants lacking various other confirmed (*Sgs1*, *Rrm3*, and *Srs2*) or putative (*Chl1*) DNA helicases. Tenfold dilutions of exponentially growing cultures were spotted on YPD or YPD containing 0.01% or 0.005% MMS. Colony growth after 48 h of incubation at 30°C is shown.

that *Mph1*, *Chl1*, *Rrm3*, and *Srs2* contribute independently to survival during exposure to MMS, while *Mph1* appears to be hypostatic to *Sgs1*. Since Schurer et al. (41) reported a synergistic increase in mitotic homologous recombination at three markers for the *mph1Δ sgs1Δ* mutant and therefore suggested that *Mph1* may play an antirecombinogenic role in the *sgs1Δ* mutant, we tested whether *Mph1* also interacted with *Sgs1* or other DNA helicases in the suppression of GCRs. However, we found that the *mph1Δ sgs1Δ* mutant accumulates GCRs at the same rate as the *sgs1Δ* mutant, indicating no genetic interaction between *MPH1* and *SGS1* in the suppression of chromosomal rearrangements (Table 2). Deletion of *MPH1* also failed to induce significant changes in the accumulation of GCRs in *srs2Δ*, *chl1Δ*, and *rrm3Δ* mutants, as indicated by the overlap between 95% confidence intervals (Table 2).

**Rad52/Rad51, but not Rad59, are essential for DNA damage tolerance and normal growth in the absence of translesion DNA synthesis.** Although the *rev3Δ mph1Δ* mutant exhibits a synergistic increase in the GCR rate and in sensitivity to MMS, it grows unimpaired in the absence of MMS, with a doubling time indistinguishable from that of the single mutants (Fig. 1B). However, sporulation of diploids heterozygous for the *rev3Δ* and *rad52Δ* mutations revealed slower growth for the *rev3Δ rad52Δ* mutant that was unaffected by deletion of *MPH1* (Fig. 1B). That the *rev3Δ rad52Δ* mutant does grow, albeit slowly, could mean that spontaneous DNA lesions needing to be bypassed during DNA replication are rare and/or that alternative, yet minor, pathways for lesion bypass exist in addition to HR and TLS. To distinguish between these possibilities, the ability of the HR-deficient *rev3Δ* mutant to grow in the presence of MMS was assessed (Fig. 4). While the *rev3Δ* mutant was no more sensitive than wild-type cells, consistent with previous findings (41), the *rev3Δ rad52Δ* mutant was significantly more sensitive than the *rad52Δ* mutant. In fact, not a single colony emerged in repeated experiments, even after a

>72-h incubation time on 0.001% MMS, for strains lacking both *REV3* and *RAD52*, lending support to the proposal that besides HR and TLS, no other pathways exist in yeast for the bypassing of induced DNA lesions. To determine whether Rad51- or Rad59-dependent branches of homologous recombination are essential for *rev3Δ* survival, the viability of spores obtained from diploids heterozygous for *rev3Δ* and either the *rad51Δ* or *rad59Δ* mutation was assessed, revealing normal growth for the *rev3Δ rad59Δ* mutant while the *rev3Δ rad51Δ* mutant grew slowly (Fig. 1B). Moreover, the *rev3Δ rad59Δ* mutant was no more sensitive than the single mutants, whereas the *rev3Δ rad51Δ* mutant could not form any colonies in the presence of MMS (Fig. 4), similar to the case with the *rev3Δ rad52Δ* mutant. Thus, although *rev3Δ* exhibits synergistic increases in sensitivity to MMS when combined with *mph1Δ*, *mph1Δ srs2Δ*, *rad52Δ*, or *rad51Δ*, the normal growth of the *rev3Δ mph1Δ* mutant as opposed to the impaired growth of the *rev3Δ rad51Δ* and *rev3Δ rad52Δ* mutants suggests that in addition to Mph1-dependent HR, other, Mph1-independent, Rad51-dependent HR pathways exist for DNA lesion bypass.

## DISCUSSION

We have investigated genetic interactions between genes involved in DNA lesion bypass (*MPH1* and *REV3*), homologous recombination (*RAD52*, *RAD51*, *RAD59*, and *SRS2*), and the DNA damage checkpoint (*MEC3* and *MEC1*) with regard to fitness, MMS sensitivity, and suppression of genome instability. We find that suppression of point mutations that arise in an *mph1Δ* mutant as a result of the error-prone Rev3 polymerase replicating across a template lesion results in the appearance of GCRs. This finding may suggest that mutations are not actually avoided but are simply shifted toward a different mutation type. Synergistic GCR rate increases in two strain backgrounds, each designed to accumulate different GCR spectra (34), demonstrate that *REV3* and *MPH1* interact genetically to suppress various types of spontaneous GCRs but are especially effective at suppressing GCRs in the newly designed GCR strain background. For this new GCR strain, Putnam et al. (34) determined that GCRs accumulate largely as a result of nonallelic homologous recombination (NAHR) between DNA sequences in the breakpoint region on chromosome V and similar regions on chromosomes IV, X, and XIV. Hence, the greater synergistic GCR rate increase identified here in this new GCR background compared to that for the standard GCR strain suggests greater roles for *MPH1* and *REV3* in the suppression of such NAHR-mediated GCRs than in the suppression of single-copy-sequence-mediated rearrangements. The requirement of Srs2, which regulates the outcomes of HR by antagonizing strand invasion, for the formation of viable chromosomal rearrangements further supports a prominent role of HR in the formation of GCRs when Mph1 and Rev3 are absent for DNA lesion bypass. We further show that the negative genetic interaction between the *mph1Δ* and *srs2Δ* mutations, coupled with accumulation of cells in  $G_2/M$  and further exacerbation of the  $G_2/M$  arrest by disruption of *REV3*, is suppressed by disrupting the DNA damage checkpoint. Synergism in MMS sensitivity was observed for *mph1Δ* mutants lacking *CHL1*, *RRM3*, *SRS2*, or *REV3*, whereas epistasis was observed for *mph1Δ* mutants lacking *RAD52*,

*RAD51*, or *SGS1*. Combined with our observation that the *rev3Δ* mutant required *RAD51* and *RAD52* but not *RAD59* or *MPH1* for normal growth, this suggests that Rev3 (TLS) and *RAD51* (HR) are the two pathways for bypass of spontaneous DNA lesions, with Mph1 defining only one *Rad51* subpathway. While the *rad51Δ* mutation appeared to suppress MMS sensitivity of the *mph1Δ srs2Δ* mutant to the level exhibited by a *rad51Δ* single mutant, the *rev3Δ* mutation led to a further synergistic increase, suggesting that Mph1, Srs2, and Rev3 contribute to bypass and/or repair of induced DNA lesions independently.

Our observation of suppression of the  $G_2/M$  arrest of the *mph1Δ srs2Δ* mutant by *mec3Δ*, in addition to the recently reported suppression by *rad51Δ* (33), suggests that cells lacking Mph1 and Srs2 are overwhelmed with HR intermediates that do not impair S phase but activate the DNA damage checkpoint prior to mitosis. That Srs2 is essential for normal growth in the absence of Mph1 could mean that DNA lesions, normally bypassed by the Mph1 pathway, will enter another HR pathway that is potentially lethal if it is not regulated by Srs2. According to recent findings by Prakash et al. (33), Srs2, Mph1, and Sgs1 independently promote noncrossover pathways during mitotic DSB repair. They suggest that Srs2 diverts DNA lesions away from crossover events that can result from double Holliday junction (dHJ) resolution into the noncrossover SDSA pathway by preventing second-strand invasion. Accumulation of dHJs due to the absence of Srs2 could overwhelm resolution pathways, especially when alternative pathways for DNA lesion bypass, such as TLS, are absent. In addition to its ability to inhibit crossover formation during repair of an HO-induced DSB, Mph1 has also been reported to unwind D loops *in vitro* (33). It has therefore been proposed that Mph1 promotes SDSA and may reverse strand invasion events before they can form dHJs. Thus, the overall burden of lesions that are committed to HR pathways and could potentially go on to form dHJs would be expected to increase in the absence of Mph1 and even further when Rev3 is also absent.

Recent findings suggest how FANCM, a human homolog of Mph1, could perform a role in error-free bypass of DNA lesions. FANCM is part of the eight-component Fanconi anemia core complex, which is involved in the repair of intrastrand cross-links and is associated with Fanconi anemia (15, 16, 23, 27, 48). FANCM can branch migrate three- and four-way junctions and, like Mph1, unwind D loops (7, 8). Combining these two activities, it has been proposed that FANCM may stall and remodel replication forks to promote repair of an approaching DNA lesion, thus preventing the fork from encountering the lesion and collapsing (7). Without FANCM, forks would collapse, leading to broken chromatids and increased gross chromosomal rearrangements, both hallmarks of Fanconi cells (44).

The recruitment of Srs2 to the replisome when PCNA becomes sumoylated in the presence of DNA damage (31) and the ability of the human Mph1 homolog FANCM to remodel replication forks *in vitro* lead us to propose a model in which Mph1 and Srs2 perform their roles in recombination directly at the fork to restart replication after encountering a DNA lesion (Fig. 6). Based on the slow growth of *Rad52/Rad51*-deficient *rev3Δ* cells and the inability of the *rev3Δ rad52Δ* and *rev3Δ rad51Δ* mutants to form any colonies in the presence of MMS,

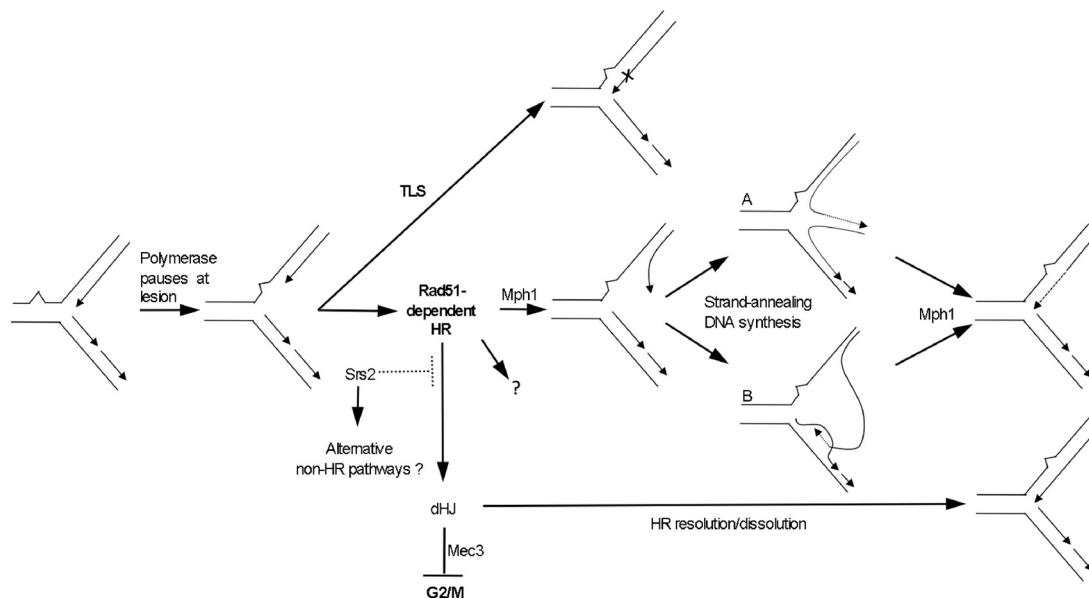


FIG. 6. Model for the role of Mph1 in the maintenance of genome stability. DNA lesions arise spontaneously during DNA replication and are bypassed by an error-free, Mph1-mediated, noncrossover pathway of homologous recombination (HR). Mph1 may unwind the leading strand from its template, suggested to resemble a D loop (7), followed by Rad51/52-mediated chicken-foot formation (A) and then resolution by reverse branch migration. A D-loop structure could also form when the leading strand switches template (B), and Mph1 could dissolve this D loop by reverse branch migration. In the absence of Mph1, lesions are bypassed by error-prone, Rev3-mediated TLS or they are channeled by Srs2 into alternative bypass pathways that can result in GCRs. If TLS is disrupted in the *mph1* $\Delta$  mutant, point mutations from TLS are avoided, but GCRs arise as a consequence of aberrant repair, most likely nonallelic HR. In the absence of both Mph1 and Srs2, cells accumulate at G<sub>2</sub>/M and lose viability due to Rad51-mediated accumulation of dHJs and Mec3-mediated checkpoint activation. In the absence of the checkpoint, cells continue through the cell cycle in the presence of DNA lesions. The dotted line emerging from Srs2 indicates that Srs2 tightly regulates the levels of dHJ formation at paused replication forks by inhibiting Rad51-mediated strand invasion.

we propose that in wild-type cells, DNA lesions may be bypassed by either Rev3-mediated TLS or Rad51-dependent HR, with Mph1 being involved in only one subpathway of Rad51-dependent HR. Rev3-mediated TLS is prone to errors but not GCR formation, while properly regulated HR pathways, including the Mph1 pathway, are error free. Mph1 may act at a stalled replication fork by unwinding the leading strand from its template, a scenario which has been suggested to resemble unwinding of a D loop (7). Mediated by HR proteins, the leading strand may then anneal with the lagging strand, forming a chicken foot (Fig. 6, structure A), or invade the sister chromatid, forming a D loop (Fig. 6, structure B), followed by DNA synthesis at the 3' end. Based on the ability of Mph1 to reverse D loops *in vitro*, it also seems possible that Mph1 acts to resolve these HR intermediates. For example, Mph1 could unwind the D loop formed by HR proteins after limited DNA synthesis or dissolve the chicken-foot structure by reverse branch migration. Reannealing of the daughter strands with their templates would then result in error-free bypass of the DNA lesion in the template strand and resumption of replication. While FANCM has been shown to migrate three- and four-way junctions *in vitro* (7, 8), as proposed in this model, this remains to be determined for Mph1. The recent report of a physical interaction between Mph1 and RPA (1) could suggest that Mph1 is recruited to stalled replication forks via RPA-bound regions of ssDNA that are generated when the replication machinery stalls at a lesion in the template. While Mph1 can unwind 40 bp by itself, it requires RPA to unwind duplexes that are 100 bp and fails on those that are 500 bp (32). This

rather modest helicase activity could ensure that Mph1 does not expose unnecessarily large regions of ssDNA at stalled forks while at the same time being sufficiently strong to unwind the leading strand from its template needed for D-loop/chicken-foot formation and/or to reverse HR-mediated invasion of the sister chromatid. Moreover, the ATPase activity of Mph1 requires a relatively long stretch of ssDNA ( $\geq 40$  nt) for full activation *in vitro* (32), which could help to ensure that Mph1 is active only on replication forks that have stalled because they are likely to contain longer regions of ssDNA than unperturbed forks. In our model, Srs2 is recruited to damaged replication forks to suppress dHJ formation, thereby promoting Mph1-mediated fork rescue. Such a role for Srs2 at the replication fork is consistent with recent findings (21, 31). Hence, in the absence of Srs2, an increasing number of forks would enter dHJ pathways for rescue, overwhelming dHJ resolution pathways and leading to aberrant and/or unresolved intermediates and eventually G<sub>2</sub>/M arrest. This accumulation of *srs2* $\Delta$  cells in G<sub>2</sub>/M accelerates as more DNA lesions become substrates for dHJ pathways upon elimination of Mph1 and Rev3. Unresolved or aberrant DNA structures may not be the only cause for Mec3-dependent cell cycle arrest of *mph1* $\Delta$  *srs2* $\Delta$  cells. According to findings by Prakash et al. (33), HR intermediates during DSB repair are increasingly resolved as crossovers when Srs2 and Mph1 are absent, possibly due to increased HR and impairment of single-strand annealing pathways, such as SDSA. Thus, not only is increased dHJ formation likely to overwhelm dHJ resolution pathways, it is also likely to increase the number of crossovers, which could be dangerous for hap-



loid mitotic cells and contribute to diminished cell proliferation. Although formation and unwinding of D-loop-like structures could be envisaged at replication forks and the recently proposed role of Mph1 in SDSA repair of DSBs could be likened to reversing chicken-foot/D-loop structures at stalled forks, it remains to be tested whether Mph1 can branch migrate three- or four-way junctions to reverse these HR intermediates and does indeed function at the replication fork.

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We declare that there are no conflicts of interest.

#### REFERENCES

- Banerjee, S., S. Smith, J. H. Oum, H. J. Liaw, J. Y. Hwang, N. Sikdar, A. Motegi, S. E. Lee, and K. Myung. 2008. Mph1p promotes gross chromosomal rearrangement through partial inhibition of homologous recombination. *J. Cell Biol.* **181**:1083–1093.
- Chen, C., K. Umezu, and R. D. Kolodner. 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.
- Entian, K. D., T. Schuster, J. H. Hegemann, D. Becher, H. Feldmann, U. Guldener, R. Gotz, M. Hansen, C. P. Hollenberg, G. Jansen, W. Kramer, S. Klein, P. Kotter, J. Kricke, H. Launhardt, G. Mannhaupt, A. Maierl, P. Meyer, W. Mewes, T. Munder, R. K. Niedenthal, M. Ramezani Rad, A. Rohmer, A. Romer, A. Hinne, et al. 1999. Functional analysis of 150 deletion mutants in *Saccharomyces cerevisiae* by a systematic approach. *Mol. Gen. Genet.* **262**:683–702.
- Fabre, F., A. Chan, W. D. Heyer, and S. Gangloff. 2002. Alternate pathways involving Sgs1/Top3, Mus81/ Mms4, and Srs2 prevent formation of toxic recombination intermediates from single-stranded gaps created by DNA replication. *Proc. Natl. Acad. Sci. U. S. A.* **99**:16887–16892.
- Gangloff, S., J. P. McDonald, C. Bendixen, L. Arthur, and R. Rothstein. 1994. The yeast type I topoisomerase Top3 interacts with Sgs1, a DNA helicase homolog: a potential eukaryotic reverse gyrase. *Mol. Cell. Biol.* **14**:8391–8398.
- Gangloff, S., C. Soustelle, and F. Fabre. 2000. Homologous recombination is responsible for cell death in the absence of the Sgs1 and Srs2 helicases. *Nat. Genet.* **25**:192–194.
- Gari, K., C. Decaillet, M. Delannoy, L. Wu, and A. Constantinou. 2008. Remodeling of DNA replication structures by the branch point translocase FANCM. *Proc. Natl. Acad. Sci. U. S. A.* **105**:16107–16112.
- Gari, K., C. Decaillet, A. Z. Stasiak, A. Stasiak, and A. Constantinou. 2008. The Fanconi anemia protein FANCM can promote branch migration of Holliday junctions and replication forks. *Mol. Cell* **29**:141–148.
- Gietz, R. D., and R. A. Woods. 2006. Yeast transformation by the LiAc/SS carrier DNA/PEG method. *Methods Mol. Biol.* **313**:107–120.
- Hirota, Y., and J. M. Lahti. 2000. Characterization of the enzymatic activity of hChlR1, a novel human DNA helicase. *Nucleic Acids Res.* **28**:917–924.
- Holloway, S. L. 2000. CHL1 is a nuclear protein with an essential ATP binding site that exhibits a size-dependent effect on chromosome segregation. *Nucleic Acids Res.* **28**:3056–3064.
- Ira, G., A. Malkova, G. Liberi, M. Foiani, and J. E. Haber. 2003. Srs2 and Sgs1-Top3 suppress crossovers during double-strand break repair in yeast. *Cell* **115**:401–411.
- Ivessa, A. S., B. A. Lenzeier, J. B. Bessler, L. K. Goudsouzian, S. L. Schnakenberg, and V. A. Zakian. 2003. The *Saccharomyces cerevisiae* helicase Rrm3p facilitates replication past nonhistone protein-DNA complexes. *Mol. Cell* **12**:1525–1536.
- 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.
- Joenje, H., and K. J. Patel. 2001. The emerging genetic and molecular basis of Fanconi anaemia. *Nat. Rev. Genet.* **2**:446–457.
- Kennedy, R. D., and A. D. Andrea. 2005. The Fanconi anemia/BRCA pathway: new faces in the crowd. *Genes Dev.* **19**:2925–2940.
- Klein, H. L. 2001. Mutations in recombinational repair and in checkpoint control genes suppress the lethal combination of srs2Delta with other DNA repair genes in *Saccharomyces cerevisiae*. *Genetics* **157**:557–565.
- Kolodner, R. D., C. D. Putnam, and K. Myung. 2002. Maintenance of genome stability in *Saccharomyces cerevisiae*. *Science* **297**:552–557.
- Krejci, L., S. Van Komen, Y. Li, J. Villemain, M. S. Reddy, H. Klein, T. Ellenberger, and P. Sung. 2003. DNA helicase Srs2 disrupts the Rad51 presynaptic filament. *Nature* **423**:305–309.
- Lee, S. K., R. E. Johnson, S. L. Yu, L. Prakash, and S. Prakash. 1999. Requirement of yeast Sgs1 and SRS2 genes for replication and transcription. *Science* **286**:2339–2342.
- Liberi, G., I. Chiolo, A. Pellicoli, M. Lopes, P. Plevani, M. Muzi-Falconi, and M. Foiani. 2000. Srs2 DNA helicase is involved in checkpoint response and its regulation requires a functional Mec1-dependent pathway and Cdk1 activity. *EMBO J.* **19**:5027–5038.
- Lo, Y. C., K. S. Paffett, O. Amit, J. A. Clikeman, R. Sterk, M. A. Brenneman, and J. A. Nickoloff. 2006. Sgs1 regulates gene conversion tract lengths and crossovers independently of its helicase activity. *Mol. Cell Biol.* **26**:4086–4094.
- Mathew, C. G. 2006. Fanconi anaemia genes and susceptibility to cancer. *Oncogene* **25**:5875–5884.
- Mullen, J. R., V. Kaliraman, and S. J. Brill. 2000. Bipartite structure of the Sgs1 DNA helicase in *Saccharomyces cerevisiae*. *Genetics* **154**:1101–1114.
- Myung, K., A. Datta, C. Chen, and R. D. Kolodner. 2001. Sgs1, the *Saccharomyces cerevisiae* homologue of BLM and WRN, suppresses genome instability and homeologous recombination. *Nat. Genet.* **27**:113–116.
- Nair, K. R. 1940. Table of confidence intervals for the median in samples from any continuous population. *Sankhya* **4**:551–558.
- Niedzwiedz, W., G. Mosedale, M. Johnson, C. Y. Ong, P. Pace, and K. J. Patel. 2004. The Fanconi anaemia gene FANCC promotes homologous recombination and error-prone DNA repair. *Mol. Cell* **15**:607–620.
- Ogiwara, H., A. Ui, M. S. Lai, T. Enomoto, and M. Seki. 2007. Chl1 and Ctf4 are required for damage-induced recombinations. *Biochem. Biophys. Res. Commun.* **354**:222–226.
- Ooi, S. L., D. D. Shoemaker, and J. D. Boeke. 2003. DNA helicase gene interaction network defined using synthetic lethality analyzed by microarray. *Nat. Genet.* **35**:277–286.
- Petronczki, M., B. Chwalla, M. F. Siomos, S. Yokobayashi, W. Helmhart, A. M. Deuschbauer, R. W. Davis, Y. Watanabe, and K. Nasmyth. 2004. Sister-chromatid cohesion mediated by the alternative RF-Cctf18/Dec1/Ctf8, the helicase Chl1 and the polymerase-alpha-associated protein Ctf4 is essential for chromatid disjunction during meiosis II. *J. Cell Sci.* **117**:3547–3559.
- Pfander, B., G. L. Moldovan, M. Sacher, C. Hoegge, and S. Jentsch. 2005. SUMO-modified PCNA recruits Srs2 to prevent recombination during S phase. *Nature* **436**:428–433.
- Prakash, R., L. Krejci, S. Van Komen, K. Anke Schurer, W. Kramer, and P. Sung. 2005. *Saccharomyces cerevisiae* MPH1 gene, required for homologous recombination-mediated mutation avoidance, encodes a 3' to 5' DNA helicase. *J. Biol. Chem.* **280**:7854–7860.
- Prakash, R., D. Satory, E. Dray, A. Papusha, J. Scheller, W. Kramer, L. Krejci, H. Klein, J. E. Haber, P. Sung, and G. Ira. 2009. Yeast Mph1 helicase dissociates Rad51-made D-loops: implications for crossover control in mitotic recombination. *Genes Dev.* **23**:67–79.
- Putnam, C. D., T. K. Hayes, and R. D. Kolodner. 2009. Specific pathways prevent duplication-mediated genome rearrangements. *Nature* **460**:984–989.
- Robert, T., D. Dervins, F. Fabre, and S. Gangloff. 2006. Mrc1 and Srs2 are major actors in the regulation of spontaneous crossover. *EMBO J.* **25**:2837–2846.
- Scheller, J., A. Schurer, C. Rudolph, S. Hettwer, and W. Kramer. 2000. MPH1, a yeast gene encoding a DEAH protein, plays a role in protection of the genome from spontaneous and chemically induced damage. *Genetics* **155**:1069–1081.
- Schmidt, K. H., and R. D. Kolodner. 2004. Requirement of Rrm3 helicase for repair of spontaneous DNA lesions in cells lacking Srs2 or Sgs1 helicase. *Mol. Cell Biol.* **24**:3213–3226.
- Schmidt, K. H., and R. D. Kolodner. 2006. Suppression of spontaneous genome rearrangements in yeast DNA helicase mutants. *Proc. Natl. Acad. Sci. U. S. A.* **103**:18196–18201.
- Schmidt, K. H., V. Pennaneach, C. D. Putnam, and R. D. Kolodner. 2006. Analysis of gross-chromosomal rearrangements in *Saccharomyces cerevisiae*. *Methods Enzymol.* **409**:462–476.
- Schmidt, K. H., J. Wu, and R. D. Kolodner. 2006. Control of translocations between highly diverged genes by Sgs1, the *Saccharomyces cerevisiae* homolog of the Bloom's syndrome protein. *Mol. Cell Biol.* **26**:5406–5420.
- Schurer, K. A., C. Rudolph, H. D. Ulrich, and W. Kramer. 2004. Yeast MPH1 gene functions in an error-free DNA damage bypass pathway that requires genes from homologous recombination, but not from postreplicative repair. *Genetics* **166**:1673–1686.
- Shiratori, A., T. Shibata, M. Arisawa, F. Hanaoka, Y. Murakami, and T. Eki. 1999. Systematic identification, classification, and characterization of the open reading frames which encode novel helicase-related proteins in *Saccharomyces cerevisiae* by gene disruption and Northern analysis. *Yeast* **15**:219–253.
- Sinclair, D. A., K. Mills, and L. Guarente. 1997. Accelerated aging and nucleolar fragmentation in yeast sgs1 mutants. *Science* **277**:1313–1316.
- Thompson, L. H., J. M. Hinz, N. A. Yamada, and N. J. Jones. 2005. How Fanconi anemia proteins promote the four Rs: replication, recombination, repair, and recovery. *Environ. Mol. Mutagen.* **45**:128–142.
- Tong, A. H., G. Lesage, G. D. Bader, H. Ding, H. Xu, X. Xin, J. Young, G. F.

- Berriz, R. L. Brost, M. Chang, Y. Chen, X. Cheng, G. Chua, H. Friesen, D. S. Goldberg, J. Haynes, C. Humphries, G. He, S. Hussein, L. Ke, N. Krogan, Z. Li, J. N. Levinson, H. Lu, P. Menard, C. Munyana, A. B. Parsons, O. Ryan, R. Tonikian, T. Roberts, A. M. Sdicu, J. Shapiro, B. Sheikh, B. Suter, S. L. Wong, L. V. Zhang, H. Zhu, C. G. Burd, S. Munro, C. Sander, J. Rine, J. Greenblatt, M. Peter, A. Bretscher, G. Bell, F. P. Roth, G. W. Brown, B. Andrews, H. Bussey, and C. Boone. 2004. Global mapping of the yeast genetic interaction network. *Science* **303**:808–813.
46. Torres, J. Z., S. L. Schnakenberg, and V. A. Zakian. 2004. Saccharomyces cerevisiae Rrm3p DNA helicase promotes genome integrity by preventing replication fork stalling: viability of rrm3 cells requires the intra-S-phase checkpoint and fork restart activities. *Mol. Cell. Biol.* **24**:3198–3212.
47. Veaute, X., J. Jeusset, C. Soustelle, S. C. Kowalczykowski, E. Le Cam, and F. Fabre. 2003. The Srs2 helicase prevents recombination by disrupting Rad51 nucleoprotein filaments. *Nature* **423**:309–312.
48. Wang, W. 2007. Emergence of a DNA-damage response network consisting of Fanconi anaemia and BRCA proteins. *Nat. Rev. Genet.* **8**:735–748.
49. Watt, P. M., E. J. Louis, R. H. Borts, and I. D. Hickson. 1995. Sgs1: a eukaryotic homolog of E. coli RecQ that interacts with topoisomerase II in vivo and is required for faithful chromosome segregation. *Cell* **81**:253–260.
50. Yamagata, K., J. Kato, A. Shimamoto, M. Goto, Y. Furuichi, and H. Ikeda. 1998. Bloom's and Werner's syndrome genes suppress hyperrecombination in yeast sgs1 mutant: implication for genomic instability in human diseases. *Proc. Natl. Acad. Sci. U. S. A.* **95**:8733–8738.