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# Control of Translocations between Highly Diverged Genes by Sgs1, the *Saccharomyces cerevisiae* Homolog of the Bloom's Syndrome Protein

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**Sgs1 is a RecQ family DNA helicase required for genome stability in *Saccharomyces cerevisiae* whose human homologs BLM, WRN, and RECQL4 are mutated in Bloom's, Werner, and Rothmund Thomson syndromes, respectively. Sgs1 and mismatch repair (MMR) are inhibitors of recombination between similar but divergent (homeologous) DNA sequences. Here we show that *SGS1*, but not MMR, is critical for suppressing spontaneous, recurring translocations between diverged genes in cells with mutations in the genes encoding the checkpoint proteins Mec3, Rad24, Rad9, or Rfc5, the chromatin assembly factors Cac1 or Asf1, and the DNA helicase Rrm3. The S-phase checkpoint kinase and telomere maintenance factor Tel1, a homolog of the human ataxia telangiectasia (ATM) protein, prevents these translocations, whereas the checkpoint kinase Mec1, a homolog of the human ATM-related protein, and the Rad53 checkpoint kinase are not required. The translocation structures observed suggest involvement of a dicentric intermediate and break-induced replication with multiple cycles of DNA template switching.**

RecQ-like DNA helicases play important roles in the maintenance of genome stability from bacteria to humans. The only member of the RecQ family of 3′ to 5′ DNA helicases in the yeast *Saccharomyces cerevisiae* is Sgs1. Sgs1 has been implicated in the coordination between DNA replication and recombination, in the regulation of homologous recombination (HR) and the suppression of crossover products during HR, and in S-phase checkpoint activation as well as in transcription (16, 26, 38, 49, 61, 96). As a consequence, cells that lack Sgs1 display a hyperrecombination phenotype, accumulate extrachromosomal ribosomal DNA (rDNA) circles, frequently missegregate chromosomes during mitosis and meiosis, have modestly increased rates of accumulating gross chromosomal rearrangements (GCRs), are sensitive to agents such as hydroxyurea and methyl-methanesulfonate, and show signs of premature aging (29, 58, 61, 86, 100, 101, 104).

To date, five human genes encoding RecQ-like (*RECQL*) proteins have been identified. Mutations in *RECQL2* (*WRN*) (105), *RECQL3* (*BLM*) (23), and *RECQL4* (41, 42) cause three rare, cancer-prone disorders, Werner syndrome (WS), Bloom's syndrome (BS), and a subset of Rothmund Thomson syndrome (Type II RTS) (97), respectively, while defects in *RECQL1* (73, 74) and *RECQL5* (41) have not been linked to a disease. Besides short stature, early onset of diabetes mellitus, and immunodeficiency, BS is characterized by extreme cancer risk, which has been estimated to be 150 to 300 times higher than the risk of malignancy in the unaffected population; in 168 BS patients, 100 cancers of many types had arisen at a mean age of 24.7 years, with many of the patients suffering from multiple primary cancers (31). Although WS patients share some of these symptoms, including early onset of diabetes mellitus and increased cancer susceptibility, they also show numerous other signs of accelerated aging not typical for BS. RTS patients also show a high prevalence of cancers, especially osteosarcomas, and suffer from skeletal abnormalities and skin changes (95, 98).

WS, BS, and RTS cells exhibit a wide range of chromosomal aberrations. WS cells have an increased spontaneous mutation rate, mainly due to the accumulation of large deletions (>20 kb), but translocations and insertions have also been observed (27, 28, 79). Structural and numerical chromosome instability has been described for RTS cells (22, 51, 55, 67). Chromosome aberrations in BS cells include approximately 0.29 chromatid and chromosome breaks per cell, translocations, and ring chromosomes (34). The most striking feature of BS cells, however, is the increased rate of spontaneous reciprocal exchange of genetic material between sister chromatids (sister chromatid exchange [SCE]) as well as between chromatids of two different chromosomes leading to the appearance of, mostly, symmetric quadriradial (QR) chromosomes (12). While SCEs are not mutagenic per se, such hyperactivity of recombinogenic processes may result in mutations if it leads to recombination between homologs or sister chromatids at nonhomologous sites. Moreover, exchanges between ectopic homologous regions of single chromatids of two different chromosomes, whether they occur between homologous or nonhomologous chromosomes, as suggested by the formation of asymmetric QRs in BS cells, can lead to translocations as well as the formation of dicentric and acentric chromosomes which cannot be segregated properly. QRs are approximately 100-fold more frequent in BS cells than in normal cells, where they occur at a frequency of ~1/1,000 (12, 91). The highly elevated rate of mitotic crossing over in BS cells between homologous chromosomes or regions of homology located on nonhomologous chromosomes, such as the rDNA regions in the satellite stalks

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of acrocentric chromosomes, has been shown to lead to a high degree of loss of heterozygosity (LOH) in BS cells and in BS mice, which may expose recessive mutations in tumor suppressor genes and has thus been suggested to be a source of tumorigenic chromosomal rearrangements in BS (52, 91).

Unlike most other DNA helicases, the 3′ to 5′ DNA helicases WRN, BLM, and Sgs1 have been shown to unwind a duplex DNA preferentially from an internal loop rather than from a blunt end or a 3′ overhang (6, 56). Moreover, WRN, BLM, and Sgs1 can unwind G-quadruplexes and Holliday junctions in vitro, while Sgs1 has also been shown to resolve three-way junctions (6, 37, 56). These structural substrate preferences suggest that these RecQ-like DNA helicases may be required for a variety of DNA metabolic processes during which such structures may arise, most prominently during HR and at stalled replication forks. In the absence of RecQ-like DNA helicases, recombinogenic lesions may instead be formed in an attempt to process anomalous replication forks or HR intermediates. The importance of RecQ family helicases for genome integrity is further supported by their physical interaction with proteins known to be involved in replicational and repair processes. For instance, Sgs1, BLM, and WRN interact with the single-stranded DNA binding protein RPA (11, 15, 17), Sgs1 and BLM interact with the strand exchange protein Rad51 (70, 103), RecQL4 colocalizes with Rad51 foci after induction of DNA damage (70), BLM and Sgs1 interact with topoisomerases (29, 101), and Sgs1, BLM, and WRN have been found in complexes with proteins that function in DNA damage response pathways (16, 20, 26, 47, 99). Moreover, genetic interactions have been demonstrated between *sgs1* and mutations in DNA helicase genes *RRM3* and *SRS2* and the structure-specific endonuclease genes *MUS81*, *MMS4*, *SLX1*, and *SLX4* (24, 30, 49, 59, 81, 93). Recently, additional interaction partners have been identified in large-scale genetic screens (66, 92). In contrast to Sgs1, BLM, and WRN helicases, recombinant RecQL4 purified from *Escherichia coli* cells lacks DNA helicase activity (53). Instead, Sangrithi et al. (78) identified a region at the N terminus of RecQL4 that shares homology with Sld2/Drc1, which is required for the establishment of replication forks in *S. cerevisiae*, thus suggesting a role of RecQL4 in the loading of replication factors at origins.

*S. cerevisiae* cells lacking Sgs1 have proven to be excellent model systems for some cellular phenotypes of the Bloom's and Werner syndromes, especially with respect to their hyperrecombination phenotype. Cells that lack Sgs1 display elevated rates (~10-fold) of intrachromosomal HR between direct repeats and interchromosomal HR between homologous sequences or heteroalleles (100, 104). *sgs1* mutations, including mutations that mimic two missense mutations found in BS, also cause an approximately fourfold elevated rate of SCE using an assay that measures reconstitution of a functional *ADE3* gene from two nonfunctional *ADE3* truncations containing a 305-bp overlap (40, 65). An increase in the frequency of LOH in diploid *sgs1* mutants has also been reported and is mainly the result of chromosome loss and chromosome rearrangements in the form of ectopic interchromosomal rearrangements, such as translocations and unequal crossing over (3). In addition, *sgs1* mutants exhibit an increased rate of recombination between similar, but nonidentical (homeologous), DNA sequences, leading to the conclusion that Sgs1 functions in the same pathway as the mismatch repair (MMR) proteins to suppress homeologous recombination (61, 87). Here we have evaluated the role of a wide range of DNA metabolic pathways in the suppression of spontaneous translocations between three highly diverged genes in *sgs1* mutants of *S. cerevisiae*. We observed homology-driven translocations, which are suppressed by Sgs1 but not MMR, suggesting that a function of Sgs1 other than its regulation of homeologous recombination is responsible for its role in suppressing translocations between related genes. Based on our analysis of translocation structures, we propose a model for the formation of complex translocations by a single recombinational event that may be facilitated by the extraordinary relaxation of mitotic HR in the absence of Sgs1.

## MATERIALS AND METHODS

**Strains and general genetic methods.** *S. cerevisiae* strains used for determination of mutation rates are derivatives of S288C. Gene deletions in RDKY3615 (*MAT***a** *ura3-52 trp1Δ63 his3Δ200 leu2Δ1 lys2ΔBgl hom3-10 ade2Δ1 ade8 hxt13*::*URA3*), RDKY5027 (*MATα ura3-52 trp1Δ63 his3Δ200 leu2Δ1 lys2ΔBgl hom3-10 ade2Δ1 ade8 hxt13*::*URA3*), and their diploid derivatives were generated by HR-mediated integration of PCR fragments using standard methods. All haploid strains used for determination of mutation rates were obtained by sporulation of appropriate diploid strains. To minimize the emergence of suppressors, slow-growing haploid strains containing deletions of the DNA helicase genes *SGS1* and *RRM3* were freshly obtained by sporulation of the appropriate diploid strain for every experiment. *S. cerevisiae* strains were grown at 30°C. *S. cerevisiae* strains used in this study and their complete genotypes are listed in Table 1. Media for propagating *S. cerevisiae* strains have been described previously (14).

**GCR rates and rearrangement analysis.** GCR rates were determined independently by fluctuation analysis of at least 15 independent cultures from three different strain isolates, and the median rate is reported (13, 61). To minimize the emergence of suppressors in slow-growing *S. cerevisiae* strains, GCR rate measurements with *rrm3 sgs1* and *sgs1 rrm3 mec3* strains were carried out immediately after genotyping of freshly obtained, individual spore clones (i.e., day 7 after plating of spores on nonselective media). All of the cells originating from an individual spore clone were inoculated into 10 ml of yeast extract/peptone/glucose (YPD) medium, and the cultures were incubated for 2 to 3 days at 30°C with vigorous shaking and then plated on YPD and selective media to isolate GCRs. Rearrangements between the *CAN1*, *ALP1*, and *LYP1* genes were identified by PCR using a primer pair that anneals to the 5′ end of *CAN1* (5′-ATG ACAAATTCAAAAGAAGACGCC-3′) and the 3′ end of *ALP1* (5′-GAAAGG ACATCCCAAACTCGTTGC-3′) or the 3′ end of *LYP1* (5′-CAGCAGCCCA GAATTTCTCC-3′). PCR conditions were as follows: 5 min at 95°C and then 30 cycles of 30 s at 95°C, 30 s at 55°C, 4 min at 68°C, and 7 min at 68°C. PCR products were analyzed by 1% agarose gel electrophoresis. Prior to sequencing, PCR products were treated with shrimp alkaline phosphatase and exonuclease 1. The predicted orientation of the translocation target with respect to the centromere was determined by comparing the DNA sequences at the breakpoint to the *Saccharomyces* Genome Database. If the translocation target was predicted to contain a centromere, the translocation chromosome was classified as dicentric (e.g., *CAN1-LYP1* translocation chromosomes). If the translocation target was not predicted to contain a centromere, then the translocation chromosome was classified as monocentric (e.g., *CAN1-ALP1* and *CAN1-LYP1-ALP1* translocation chromosomes).

## RESULTS

**Checkpoint proteins, chromatin assembly factors, and the DNA helicase Rrm3 suppress recurring translocations in the absence of Sgs1.** Cells lacking Sgs1 have a moderately increased rate of accumulating GCRs; these consist of broken chromosomes healed by de novo telomere addition (62%) and translocations with microhomology (25%) or without homology (13%) at the breakpoint (61). In contrast, *rrm3*Δ mutants do not have an increased GCR rate. *sgs1*Δ *rrm3*Δ double mu-

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

| Strain | Genotype | Reference or source |
|---|---|---|
| RDKY3615 | *MAT***a** *ura3-5*, *trp1Δ63 his3Δ200 leu2Δ1 lys2ΔBgl hom3-10 ade2Δ1 ade8 hxt13*::*URA3* | 13 |
| RDKY3719 | RDKY3615 *rad9*::*HIS3* | 61 |
| RDKY3731 | RDKY3615 *tel1*::*HIS3* | 61 |
| RDKY3735 | RDKY3615 *mec1*::*HIS3 sml1*::*TRP1* | 61 |
| RDKY3749 | RDKY3615 *sml1*::*KAN rad53*::*HIS3* | 61 |
| RDKY4561 | RDKY3615 *rfc5-1.TRP1 sgs1*::*HIS3* | 63 |
| RDKY4566 | RDKY3615 *tel1*::*HIS3 sgs1*::*TRP1* | 63 |
| RDKY4588 | RDKY3615 *sml1*::*KAN rad53*::*HIS3 sgs1*::*TRP1* | 63 |
| RDKY4753 | RDKY3615 *cac1*::*HIS3* | 64 |
| RDKY4755 | RDKY3615 *asf1*::*HIS3* | 64 |
| RDKY4765 | RDKY3615 *sgs1*::*HIS3 cac1*::*TRP1* | 64 |
| RDKY4767 | RDKY3615 *asf1*::*HIS*, *sgs1*::*TRP1* | 64 |
| RDKY5502 | RDKY3615 *tsa1*::*KAN* | 35 |
| RDKY5529 | RDKY3615 *tsa1*::*KAN sgs1*::*HIS3* | 36 |
| RDKY5556 | RDKY3615 *rrm3*::*TRP1* | This study |
| RDKY5558 | RDKY3615 *sgs1*::*TRP1* | This study |
| RDKY5564 | RDKY3615 *rad51*::*HIS3 sgs1*::*TRP1 rrm3*::*KAN* | This study |
| RDKY5569 | RDKY3615 *mec3*::*HIS3* | This study |
| RDKY5572 | RDKY3615 *mec3*::*HIS3 sgs1*::*TRP1* | This study |
| RDKY5573 | RDKY3615 *rad24*::*HIS3* | This study |
| RDKY5575 | RDKY3615 *rad24*::*HIS3 sgs1*::*TRP1* | This study |
| RDKY5577 | *MAT***a**/α *ura3-52/ura3-52*, *trp1Δ63/trp1Δ63 his3Δ200/his3Δ200 leu2Δ1/leu2Δ1 lys2ΔBgl/lys2ΔBgl hom3-10/hom3-10 ade2Δ1/ade2Δ1 ade8/ade8 hxt13*::*URA3/hxt13*::*URA3 SGS1/sgs1*::*TRP1 RRM3/rrm3*::*KAN* | This study |
| RDKY5579 | RDKY3615 *mec3*::*HIS3 sgs1*::*TRP1 rrm3*::*KAN* | This study |
| RDKY5772 | RDKY3615 *mec3*::*KAN asf1*::*HIS3 sgs1*::*TRP1* | This study |
| RDKY5773 | RDKY3615 *rad52*::*HIS3* | This study |
| RDKY5774 | RDKY3615 *rad52*::*HIS3 sgs1*::*TRP1* | This study |
| RDKY5775 | RDKY3615 *rad52*::*HIS3 sgs1*::*TRP1 mec3*::*KAN* | This study |
| RDKY5776 | RDKY3615 *sgs1*::*TRP1 rrm3*::*KAN rad51*::*HIS3 mec3*::*HIS3* | This study |
| RDKY5777 | RDKY3615 *sgs1*::*HIS3 mec1*::*TRP1 sml1*::*KAN* | This study |
| RDKY5778 | RDKY3615 *rad9*::*HIS3 sgs1*::*TRP1* | This study |
| RDKY5779 | RDKY3615 *msh2*::*KAN1 asf1*::*HIS3* | This study |
| RDKY5780 | RDKY3615 *msh2*::*KAN1 cac1*::*HIS3* | This study |
| RDKY5781 | RDKY3615 *msh2*::*KAN1 mec3*::*HIS3* | This study |
| RDKY5782 | RDKY3615 *msh2*::*KAN1 rfc5-1.TRP1* | This study |
| RDKY5783 | RDKY3615 *msh2*::*KAN1 tel1*::*HIS3* | This study |
| RDKY5784 | RDKY3615 *msh2*::*KAN1* | This study |
| KHSY1357 | RDKY3615 *sgs1*::*TRP1mec3*::*G418 rad51*::*HIS3* | This study |
| KHSY816 | *MAT***a**/α *ura3-52/ura3-52 trp1Δ63/trp1Δ63 his3Δ200/his3Δ200 leu2Δ1/leu2Δ1 lys2ΔBgl/lys2ΔBgl hom3-10/hom3-10 ade2Δ1/ade2Δ1 ade8/ade8 hxt13*::*URA3/hxt13*::*URA3*, *SGS1/sgs1*::*TRP1 RRM3/rrm3*::*KAN MEC3/mec3*::*HIS3* | This study |
| KHSY1448 | RDKY3615 *msh6*::*TRP1 rad24*::*HIS3* | This study |
| KHSY1452 | RDKY3615 *msh6*::*TRP1 mec3*::*HIS3* | This study |

tants have a severe growth defect that can be rescued by deletion of the HR genes *RAD51*, *RAD55*, and *RAD57* (66, 81, 93). During a detailed investigation of checkpoint activation and genome instability in *sgs1*Δ *rrm3*Δ double mutants, we observed that *sgs1*Δ *rrm3*Δ double mutants have a synergistic increase in GCR rates that is reduced by mutations in HR genes and is increased by defects in checkpoint genes (K. H. Schmidt and R. Kolodner, unpublished data; also see Table 2). In this study, we repeatedly found translocations between the *CAN1* gene on chromosome 5 and *LYP1* or *ALP1* on chromosome 14 in *sgs1*Δ *rrm3*Δ double mutants. *CAN1*, *LYP1*, and *ALP1* are closely related genes, expressing basic amino acid transporters; the *CAN1* gene is 64% identical to the *LYP1* and *ALP1* genes, while *ALP1* and *LYP1* show 59% sequence identity. This observation, taken together with the finding that, with the exception of a single *CAN1-ALP1* translocation in a *tlc1*Δ *rad51*Δ mutant, sequencing of more than 358 translocation breakpoints in this laboratory has never identified translocations between *CAN1* and any of its nine most closely related genes in any strain not carrying an *sgs1*Δ mutation (75), suggests that Sgs1 may be a regulator of translocations between highly diverged genes. To test this hypothesis, we combined an *sgs1*Δ mutation with mutations causing defects in cell cycle checkpoints (*mec3*Δ, *rad24*Δ, *rad9*Δ, *rfc5-1*, *rad53*Δ, *mec1*Δ, and *tel1*Δ), in HR (*rad51*Δ and *rad52*Δ), in the oxidative stress response (*tsa1*Δ), and in chromatin assembly (*cac1*Δ and *asf1*Δ), many of which are known to increase GCR rates, albeit through different defects, to determine the effect of the additional *sgs1*Δ mutation on the GCR rate and spectrum (36, 44). To facilitate this analysis, we designed a PCR assay to screen a large collection of independent GCRs isolated from these mutants for *CAN1-ALP1* and *CAN1-LYP1* translocations using two primer pairs that anneal to the 5′ end of *CAN1*, located on chromosome 5 (Fig. 1, primer F), and the 3′ end of either *LYP1* or *ALP1*, located on chromosome 14 (Fig. 1, primers R1 and R2, respectively). The resulting PCR products were char-

TABLE 2. Effect of defects in cell cycle checkpoints, chromatin assembly, homologous recombination, oxidative stress response, and DNA helicases on GCRs in *sgs1* and mismatch repair mutants

| Relevant genotype | Total rate of GCRs ($10^{10}$) | Total *CAN1/LYP1/ALP1* translocations | | Rates of individual *CAN1/LYP1/ALP1* translocation classes ($10^{10}$)[b] | | | |
|---|---|---|---|---|---|---|---|
| | | Rate ($10^{10}$)[a] | Frequency (%)[b] | *CAN1-LYP1* | *CAN1-ALP1* | *CAN1-LYP1-ALP1* | *CAN1-LYP1-ALP1-LYP1-ALP1* |
| Wild type[c,d] | 3.5 | ND (<0.2) | ND | ND | ND | ND | ND |
| *sgs1* | 77 | <2.6 | <0.03 (0/30) | <3 (0/30) | <2.6 (0/30) | <2.6 (0/30) | <2.6 (0/30) |
| *cac1*[e] | 216 | <7.4 | <0.03 (0/29) | <7.4 (0/29) | <7.4 (0/29) | <7.4 (0/29) | <7.4 (0/29) |
| *cac1 sgs1* | 355 | 60 | 17 (5/30) | <12 (0/30) | 24 (2/30) | 36 (3/30) | <12 (0/30) |
| *asf1*[e] | 250 | ND (<23) | ND | ND | ND | ND | ND |
| *asf1 sgs1* | 318 | 64 | 23 (6/30) | <11 (0/30) | 21 (2/30) | 32 (3/30) | 11 (1/30) |
| *rad24* | 23 | <0.8 | <0.03 (0/30) | <0.8 (0/30) | <0.8 (0/30) | <0.8 (0/30) | <0.8 (0/30) |
| *rad24 sgs1* | 136 | 68 | 50 (15/30) | 4.5 (1/30) | 18 (4/30) | 41 (9/30) | 4.5 (1/30) |
| *mec3*[c] | 17 | <0.6 | <0.03 (0/30) | <0.6 (0/30) | <0.6 (0/30) | <0.6 (0/30) | <0.6 (0/30) |
| *mec3 sgs1* | 339 | 79 | 23 (7/30) | 11 (1/30) | 45 (4/30) | 23 (2/30) | <11 (0/30) |
| *mec3 sgs1 rad51* | 1,491 | 198 | 13 (4/30) | ND | ND | ND | ND |
| *mec3 sgs1 asf1* | 2,588 | 431 | 17 (5/30) | 172 (2/30) | <86 (0/30) | 259 (3/30) | <86 (0/30) |
| *rad52*[f] | 161 | ND (<16) | ND | ND | ND | ND | ND |
| *rad52 sgs1* | 125 | <4.3 | <0.03 (0/29) | <4.3 (0/29) | <4.3 (0/29) | <4.3 (0/29) | <4.3 (0/29) |
| *rad52 sgs1 mec3* | 3,168 | <23 | <0.007 (0/136) | <40 (0/79) | <40 (0/79) | <40 (0/79) | <40 (0/79) |
| *rad51* | <8 | ND | ND | ND | ND | ND | ND |
| *rrm3* | 14 | <0.6 | <0.04 (0/24) | <0.6 (0/24) | <0.6 (0/24) | <0.6 (0/24) | <0.6 (0/24) |
| *rrm3 sgs1* | 656 | 81 | 12 (7/57) | <12 (0/57) | 35 (3/57) | 46 (4/57) | <12 (0/57) |
| *rrm3 sgs1 mec3* | 1,942 | 349 | 18 (7/39) | <50 (0/39) | 100 (2/39) | 249 (5/39) | <50 (0/39) |
| *rrm3 sgs1 rad51* | 15 | 2.4 | 16 (3/19) | <0.8 (0/19) | <0.8 (0/19) | 2.4 (3/19) | <0.8 (0/19) |
| *rrm3 sgs1 rad51 mec3* | 1,640 | 66 | 4 (2/50) | <33 (0/30) | 33 (1/30) | 33 (1/30) | <33 (0/30) |
| *mec1 sml1*[c] | 460 | ND (<51) | ND | ND | ND | ND | ND |
| *mec1sml1 sgs1* | 1,930 | <10 | <0.005 (0/190) | <10 (0/190) | <10 (0/190) | <10 (0/190) | <10 (0/190) |
| *tel1*[c] | 2 | ND (<0.3) | ND | ND | ND | ND | ND |
| *tel1 sgs1* | 126 | 21 | 17 (5/30) | <4 (0/30) | <4 (0/30) | 21 (5/30) | <4 (0/30) |
| *rad53 sml1* | 95 | ND | ND | ND | ND | ND | ND |
| *rad53 sml1 sgs1* | 879 | <29 | <0.03 (0/30) | <29 (0/30) | <29 (0/30) | <29 (0/30) | <29 (0/30) |
| *rad9*[g] | 20 | ND (<2) | ND | ND | ND | ND | ND |
| *rad9 sgs1* | 748 | 25 | 3 (1/30) | <25 (0/30) | <25 (0/30) | 25 (1/30) | <25 (0/30) |
| *rfc5-1*[c] | 660 | ND (<66) | ND | ND | ND | ND | ND |
| *rfc5-1 sgs1* | 631 | 42 | 7 (2/30) | <21 (0/30) | <21 (0/30) | 42 (2/30) | <21 (0/30) |
| *tsa1*[h] | 173 | ND (<17) | ND | ND | ND | ND | ND |
| *tsa1 sgs1*[i] | 1,139 | <38 | <0.03 (0/30) | <38 (0/30) | <38 (0/30) | <38 (0/30) | <38 (0/30) |
| *msh2*[j] | 5 | <0.3 | <0.07 (0/15) | <0.3 (0/15) | <0.3 (0/15) | <0.3 (0/15) | <0.3 (0/15) |
| *msh2 asf1* | 100 | <3.7 | <0.04 (0/27) | <3.7 (0/27) | <3.7 (0/27) | <3.7 (0/27) | <3.7 (0/27) |
| *msh2 cac1* | 296 | <11 | <0.04 (0/28) | <11 (0/28) | <11 (0/28) | <11 (0/28) | <11 (0/28) |
| *msh2 mec3* | 32 | <1.5 | <0.05 (0/22) | <1.5 (0/22) | <1.5 (0/22) | <1.5 (0/22) | <1.5 (0/22) |
| *msh2 rfc5-1* | 193 | <6.7 | <0.03 (0/29) | <6.7 (0/29) | <6.7 (0/29) | <6.7 (0/29) | <6.7 (0/29) |
| *msh2 tel1* | 21 | <0.9 | <0.04 (0/23) | <0.9 (0/23) | <0.9 (0/23) | <0.9 (0/23) | <0.9 (0/23) |
| *msh6 mec3* | 19 | <0.7 | <0.04 (0/27) | <0.7 (0/27) | <0.7 (0/27) | <0.7 (0/27) | <0.7 (0/27) |
| *msh6 rad24* | 16 | <0.8 | <0.05 (0/20) | <0.8 (0/20) | <0.8 (0/20) | <0.8 (0/20) | <0.8 (0/20) |

[a] ND, not determined; numbers in parentheses indicate an estimate of the *CAN1/LYP1/ALP1* rate based on previous GCR breakpoint analyses as listed in footnotes *c* to *h*, in which no *CAN1/LYP1/ALP1* rearrangements were found (75).

[b] Numbers in parentheses indicate the number of *CAN1/LYP1/ALP1* rearrangements detected among all GCR clones tested.

[c] GCR rates and breakpoint analyses from reference 62. Breakpoint analysis results were the following: for *rad53*Δ *sml1*Δ, 5 telomere additions, 4 translocations with microhomology; *rfc5-1*, 10 telomere additions; *mec1*Δ *sml1*Δ, 9 telomere additions; *tel1*Δ, 6 translocations with microhomology; *mec3*Δ, 8 telomere additions, 2 translocations (0 nonhomology/2 microhomology/0 *CAN1/ALP1/LYP1*).

[d] Breakpoint analysis for the wild type from references 61 and 69: 14 telomere additions, 3 translocation (1 nonhomology/2 microhomology/0 *CAN1/ALP1/LYP1*).

[e] GCR rates and breakpoint analyses from reference 64. Breakpoint analysis results were the following: *cac1*Δ, 7 telomere additions, 3 translocations (2 nonhomology/1 microhomology/0 *CAN1/ALP1/LYP1*); *asf1*Δ, 9 telomere additions, 2 translocations (0 nonhomology/2 microhomology/0 *CAN1/ALP1/LYP1*).

[f] Breakpoint analysis for *rad52*Δ from reference 60: 3 telomere additions, 7 translocations with microhomology.

[g] GCR rate from reference 60. Breakpoint analysis for *rad9*Δ from reference 63: 8 telomere additions, 2 translocations with microhomology.

[h] GCR rate and breakpoint analysis from reference 35. *tsa1*Δ, 8 telomere additions, 1 large deletion, 1 translocation (0 nonhomology/1 microhomology/0 *CAN1/ALP1/LYP1*).

[i] GCR rate from reference 36.

[j] Breakpoint analysis for *msh2*Δ from reference 61: 4 telomere additions, 2 independent base substitution mutations, 3 translocation (2 nonhomology/1 microhomology/0 *CAN1/ALP1/LYP1*).

acterized by DNA sequencing, and the rate of each type of rearrangement was calculated.

The analysis of GCRs described above in many cases yielded the expected PCR products for the *CAN1-ALP1* translocations. Surprisingly, however, translocations that had been determined by other breakpoint mapping and sequencing methods (14) to contain *CAN1-LYP1* breakpoints yielded *CAN1-ALP1* PCR products instead of the expected *CAN1-LYP1* PCR products. Sequencing of these PCR products revealed that these GCR breakpoint regions all contained a

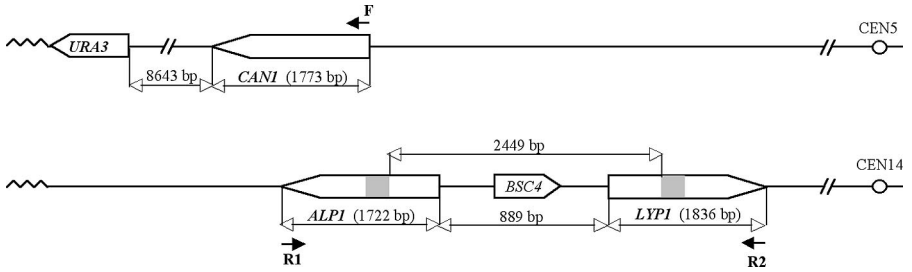


FIG. 1. Location of the *CAN1*, *LYP1*, and *ALP1* genes in the yeast genome. *CAN1* is located on chromosome 5, while *ALP1* and *LYP1* are located on the same arm of chromosome 14 in opposite orientations, separated by 889 bp that include a single 396-bp gene, *BSC4*. *CAN1* and *ALP1* are in the same orientation with respect to their centromeres, whereas *LYP1* is in the opposite orientation. The lengths of the genes and distances between them are indicated in base pairs. The 174-bp regions of 93% sequence identity present in *ALP1* and *LYP1*, indicated by a gray box, are 2,449 bp apart. The locations of the primers used to screen GCR clones for *CAN1/LYP1/ALP1* rearrangements are indicated by F, R1, and R2 (see the text for details).

secondary *LYP1-ALP1* breakpoint only a few hundred base pairs downstream of the identified primary *CAN1-LYP1* breakpoint. Thus, these rearrangements were tripartite *CAN1-LYP1-ALP1* translocations in which the region between *LYP1* and *ALP1* was deleted and both *ALP1* and *LYP1* sequences were now in the same orientation relative to each other, compared to being in inverted orientations on the normal chromosome 14. In total, PCR analysis of 880 chromosomal rearrangements isolated from 19 different *sgs1* mutants revealed 41 tripartite *CAN1-LYP1-ALP1* translocations, 18 *CAN1-ALP1* translocations, 4 *CAN1-LYP1* translocations without a secondary *ALP1* breakpoint, and 2 *CAN1* translocations with three breakpoints within *LYP1* and *ALP1*, resulting in *CAN1-LYP1-ALP1-LYP1-ALP1* rearrangements (Table 2).

None of these classes of translocations was identified in any single mutant analyzed, including the *sgs1*Δ single mutant. They were observed, however, if an *sgs1*Δ mutation was combined with *rad24*Δ, *mec3*Δ, *asf1*Δ, *cac1*Δ, or *tel1*Δ mutation and, at lower frequencies, with *rad9*Δ or *rfc5-1* mutations. The synergistic increase in translocation rates in *sgs1*Δ *mec3*Δ and *sgs1*Δ *asf1*Δ double mutants and further increase in the translocation rate in the *sgs1*Δ *asf1*Δ *mec3*Δ triple mutant indicates that the formation of this class of translocations in *sgs1*Δ mutants is normally inhibited independently by DNA damage checkpoint sensors Mec3 and Rad24 and the chromatin assembly factors Cac1 and Asf1 (Table 2). A synergistic GCR rate increase was also observed when *rrm3*Δ and *sgs1*Δ mutations were combined, and introduction of a *mec3*Δ mutation into the *sgs1*Δ *rrm3*Δ double mutant led to a further GCR rate increase (Table 2). However, the doubling times of the *rrm3*Δ *sgs1*Δ and *sgs1*Δ *rrm*Δ*3 mec3*Δ strains are longer than those of HR-proficient *sgs1*Δ mutants with a functional Rrm3 helicase (e.g., Δ*rrm3* Δ*sgs1*, 286 ± 32 min; *sgs1*Δ, 109 ± 3 min; *rrm3*Δ, 98 ± 1 min; wild type, 94 ± 2 min) (81); therefore, it is possible that their GCR rates may not be directly comparable to that of normally growing *sgs1*Δ mutants, even though all cultures were grown to the same final cell density during the fluctuation analysis. The critical role of the DNA damage checkpoint sensors Mec3 and Rad24 in suppressing these recurring translocations is further emphasized by the exceptionally high frequency of *CAN-LYP1-ALP1* translocations, which make up 23% and 50% of all GCRs that were isolated from *sgs1*Δ *mec3*Δ and *sgs1*Δ *rad24*Δ double mutants, respectively. The weaker synergistic interactions and lower frequencies of *CAN-LYP1-ALP1* translocations seen when *rfc5-1* or *rad9*Δ mutation was combined with an *sgs1*Δ mutation indicate that the Rfc5 and Rad9 checkpoint proteins play a significant but lesser role in suppressing these translocations in *sgs1*Δ mutants than other checkpoint proteins. In contrast, we found no *CAN1-LYP1-ALP1* translocations if the *sgs1*Δ mutation was combined with mutation in *MEC1* or *RAD53*. This observation was surprising considering that Rad53, a central checkpoint kinase of the DNA damage checkpoint, is believed to act downstream of Rad24 and Mec3, while Mec1 has been implicated in virtually all DNA damage checkpoints in *S. cerevisiae*. Mec1 is known to phosphorylate Rad53, Rad9, and Ddc1, the latter of which is a subunit of the PCNA-like DNA damage-sensing complex that also contains Mec3 and interacts with Rad24, both of which we find are critical for the suppression of *CAN1/LYP1/ALP1* translocations. In addition to its function in telomere maintenance, Tel1 forms a complex with Mre11 to establish a DNA damage checkpoint for double-strand breaks (19, 94). However, synthetic lethality between *sgs1*Δ and *mre11*Δ mutations prevented the further exploration of the role of *MRE11* in the suppression of complex translocations in the absence of Sgs1. Altogether, these findings suggest that Rad24/Mec3-dependent processes effectively suppress translocations between related genes in the *sgs1* mutant and that this suppression also requires the checkpoint kinase Tel1 but not the Mec1 kinase. Mutations that eliminated the HR pathway (*rad52*Δ) or the oxidative damage response system (*tsa1*Δ) did not lead to *CAN1-LYP1-ALP1* translocations when combined with an *sgs1*Δ mutation; in fact, introduction of a *rad52*Δ mutation into the *sgs1*Δ *mec3*Δ double mutant eliminated the *CAN1/LYP1/ALP1* translocations, demonstrating that HR is essential for the formation of these recurring translocations. However, introduction of a *rad51*Δ mutation, which causes a 4-fold reduction in mitotic recombination as opposed to the 3,000-fold reduction reported for the *rad52*Δ mutation (76), into the *sgs1*Δ *mec3*Δ double mutant did not reduce the rate of *CAN1/LYP1/ALP1* translocations, suggesting that these translocations are formed by a Rad52-dependent, Rad51-independent recombination process.

**Suppression of translocations between highly diverged genes depends on Sgs1 but not on Msh2 or Msh6.** MMR proteins have been shown to suppress recombination between

homeologous DNA sequences (21, 83, 84, 88). To determine if the suppression of translocations between the highly diverged *CAN1*, *LYP1*, and *ALP1* genes by Sgs1 is due to its role in the suppression of homeologous recombination, we analyzed GCRs isolated from MMR-defective mutants. We constructed *msh2*Δ mutants with an additional mutation in genes, such as *CAC1*, *ASF1*, *MEC3*, and *TEL1*, which had led to increased rates of *CAN1*/*LYP1*/*ALP1* translocations in cells lacking Sgs1. All of these *msh2*Δ double mutants had GCR rates similar to those of the single mutants, and no rearrangements between *CAN1* and *ALP1* or *LYP1* were detected (Table 2). However, in addition to its role in heteroduplex rejection, Msh2, together with the MMR protein Msh3, is also required for the removal of nonhomology from the ends of recombination intermediates (89), raising the possibility that the *msh2*Δ mutation may, in addition to inhibiting heteroduplex rejection, inhibit other early recombination steps that may prevent the formation of *CAN1*/*LYP1*/*ALP1* translocations. We therefore combined an *msh6* mutation, which inhibits heteroduplex rejection but not the removal of nonhomologous 3′ ends (89), with *mec3*Δ and *rad24*Δ mutations, which yield high frequencies of *CAN1*/*LYP1*/*ALP1* translocations when combined with an *sgs1*Δ mutation (23% and 50% of total GCRs, respectively). We found that an *msh6*Δ mutation, like an *msh2*Δ mutation, did not lead to the formation of *CAN1*/*LYP1*/*ALP1* translocations in the permissive *mec3*Δ and *rad24*Δ mutants, suggesting that relaxation of homeologous recombination is not sufficient for the formation of the complex translocations seen in *sgs1*Δ mutants, but that an Sgs1-specific function other than suppression of homeologous recombination normally prevents these rearrangements.

**Structure of translocations between *CAN1*, *ALP1*, and *LYP1*.** Sequence alignments between *CAN1* and *LYP1* as well as between *CAN1* and *ALP1* show 64% sequence identity, while *ALP1* and *LYP1* show 60% sequence identity. All of the 65 translocation breakpoints identified in this study were found within blocks of identical bases ranging from 1 to 17 bp in the *CAN1-LYP1* alignment, from 5 to 20 bp in the *CAN1-ALP1* alignment, and from 5 to 47 bp in the *LYP1-ALP1* alignment (Fig. 2). Analysis of the frequency of breakpoints at specific sites revealed that longer homology blocks were utilized up to 31-fold more often than expected by chance, suggesting that *CAN1*/*LYP1*/*ALP1* translocations are facilitated by the increasing length of homologous regions (Fig. 3). For instance, the 46 *CAN1-LYP1* breakpoints were restricted to 26 homology blocks distributed over 717 bp near the 5′ end of *CAN1* and *LYP1* in a region of 74% sequence identity (Fig. 2A, breakpoints 1 to 26). The frequency of breakpoints in homology blocks of ≥9 bp was 2- to 10-fold higher than expected by chance, while those in homology blocks of <5 bp were underrepresented (Table 3; Fig. 3). The utilization of shorter than average homology blocks in the formation of *CAN1-LYP1* translocations in mutants with *rad51*Δ or *tel1*Δ mutation (4.8 bp or 6 bp, respectively, versus the average of 8.6 bp) suggests that usage of long homology blocks for interchromosomal translocations between *CAN1* and *LYP1* depends on HR and may also depend on Tel1 (Table 4).

Of 47 *CAN1-LYP1* translocations, we found only four that did not have a secondary *LYP1-ALP1* breakpoint; these are currently being analyzed by array-based comparative genomic hybridization to detect potential secondary rearrangements. All *LYP1-ALP1* breakpoints were confined to only seven homology blocks within a 173-bp stretch of 93% sequence identity in the center of the 1,722-bp *LYP1-ALP1* alignment (Fig. 2C, homology blocks 29 to 35). Remarkably, two translocations were isolated from *sgs1*Δ *rad24*Δ and *sgs1*Δ *asf1*Δ double mutants that showed two additional breakpoints between *LYP1* and *ALP1*, resulting in even more complex *CAN1-LYP1-ALP1-LYP1-ALP1* translocations. These additional breakpoints were assigned on the basis of 1-bp discontinuities in the *LYP1* or *ALP1* alignment that corresponded to the *ALP1* or *LYP1* gene, respectively. All *LYP1-ALP1* breakpoints occurred in homology blocks of at least 5 bp, and above this threshold longer homology blocks were favored over shorter homology blocks (Fig. 3; Table 3). In fact, 60% of the 47 breakpoints were located in the same 41-bp homology block (Fig. 2C, breakpoint 29), occurring with 31-fold higher frequency than expected by chance, while another 8 (17%) were located in the same 29-bp homology block (Fig. 2C, breakpoint 35), occurring with 12-fold higher frequency than expected. A *rad51*Δ mutation did not force rearrangements into shorter *LYP1-ALP1* homology blocks or into regions without homology, as demonstrated by similar average homology block lengths in *rad51*Δ and *RAD* strains (35.8 bp and 36.7 bp, respectively). This disparity between the Rad51 independency of *CAN1*/*LYP1*/*ALP1* translocation rates, Rad51 dependency of homology block length in *CAN1-LYP1* translocations, and its lack with respect to *LYP1-ALP1* rearrangements suggests that Rad51-independent mechanisms mediate the formation of secondary *LYP1-ALP1* rearrangements (this pathway could involve Rad59, although we did not test this), whereas Rad51-dependent and Rad51-independent processes can mediate the formation of interchromosomal *CAN1-LYP1* translocations with similar effectiveness but Rad51-dependent processes are used preferentially. The Rad52 dependency of translocations between *CAN1* and *LYP1* or *ALP1*, as indicated by the absence of *CAN1*/*LYP1*/*ALP1* translocations in the *sgs1*Δ *mec3*Δ *rad52*Δ triple mutant, suggests that a Rad59-dependent HR pathway may be partially redundant with a Rad51-dependent HR pathway in promoting the initial HR event leading to the *CAN1*/*LYP1*/*ALP1* translocations.

In addition to translocations between *CAN1* and *LYP1*, translocations between *CAN1* and *ALP1* were observed in this study. All 18 *CAN1-ALP1* breakpoints were confined to a 248-bp region of 76% sequence identity (Fig. 2B, breakpoints 15 to 28). Like *CAN1-LYP1* and *LYP1-ALP1* breakpoints, *CAN1-ALP1* breakpoints occurred 3 to 20 times more frequently in larger homology blocks than expected, while no breakpoints were observed in regions with less than 4 bp of homology (Tables 3 and 4; Fig. 2B).

Note that we have excluded the possibility that *CAN1-LYP1-ALP1* translocations may have been generated by translocation of *CAN1* to a preexisting *LYP1-ALP1* gene fusion on chromosome 14 (which could have resulted from unequal sister chromatid conversion or intrachromatid crossover between the two inverted homeologous genes) by confirming by PCR that the predicted *LYP1-ALP1* rearrangements were not present in the clones containing GCRs and that intact *ALP1* and *LYP1* genes were present (data not shown). The presence of intact *LYP1* and *ALP1* genes in all GCR clones tested and the absence of

A.

B.

C.

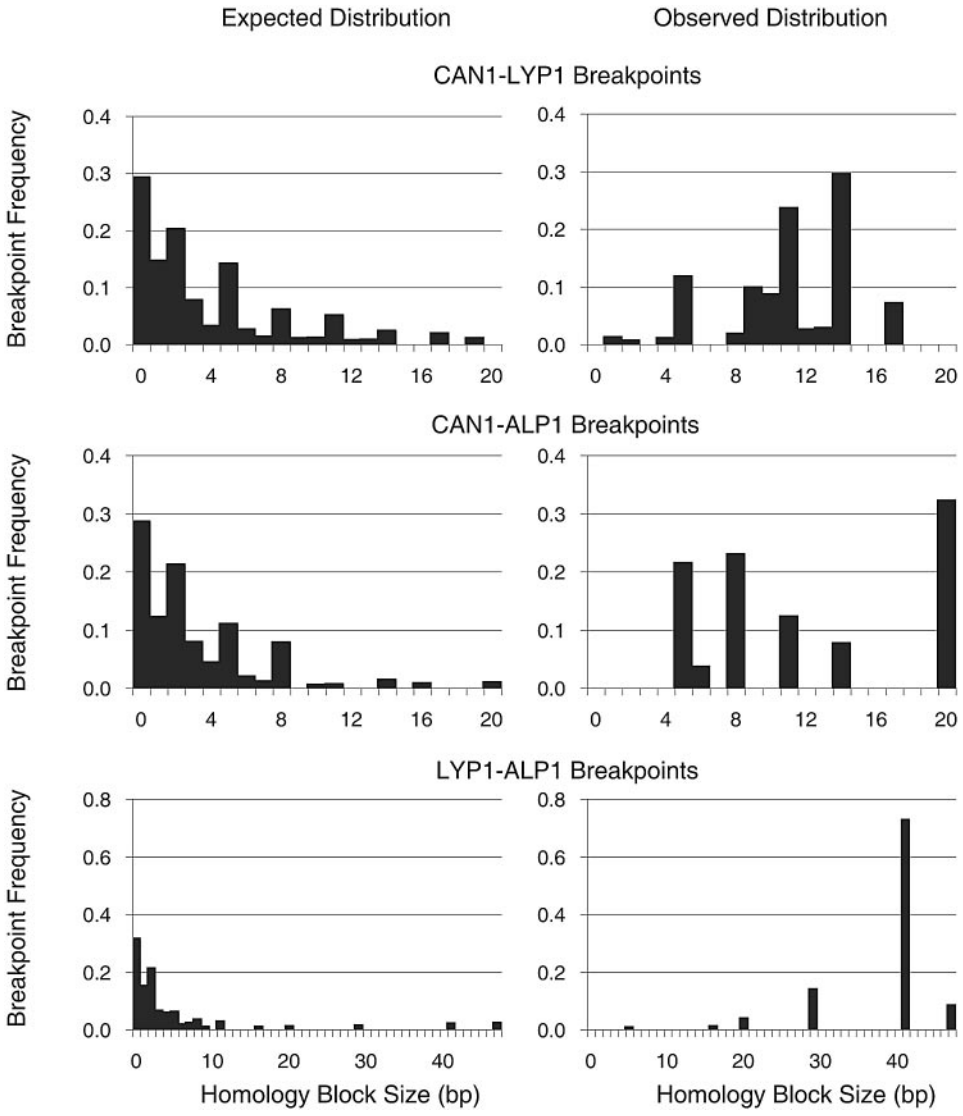


FIG. 3. Frequencies of *CAN1-LYP1*, *CAN1-ALP1*, and *LYP1-ALP1* rearrangement breakpoints in homology blocks of varying lengths. The expected distribution assumes a random distribution of breakpoints.

*ALP1-CAN1* rearrangements in all 12 tested GCR clones with *CAN1-ALP1* translocations (data not shown) indicates that *CAN1*/*LYP1*/*ALP1* rearrangements are nonreciprocal events, most likely generated by break-induced replication (BIR), rather than reciprocal events that occurred during $G_2$/M. Since *ALP1-CAN1* rearrangements are not selected against in our assay, reciprocal translocations in $G_2$/M would predict an equal association of *CAN1-ALP1* translocation chromosomes with a

FIG. 2. Location of translocation breakpoints in the *CAN1*, *ALP1*, and *LYP1* genes. (A) The open reading frame (ORF) of *CAN1* is shown. Regions of homology between the *CAN1* and *LYP1* genes that are longer than 4 bp are boxed with regions of homology that are unique to the *CAN1-LYP1* alignment in uppercase letters and regions of homology that are identical in both *CAN1-LYP1* and *CAN1-ALP1* alignments in lowercase letters. The regions of homology associated with *CAN1-LYP1* translocation breakpoints are numbered 1 to 26, with the most 5′ breakpoint having the lowest number; numbered red boxes indicate *CAN1-LYP1* breakpoints, and numbered black boxes indicate *CAN1-ALP1* breakpoints that occurred within a region of homology that is identical in *CAN1-LYP1* and *CAN1-ALP1* alignments. (B) The ORF of *CAN1* is shown. Stretches of homology between the *CAN1* and *ALP1* genes that are longer than 4 bp are boxed with regions of homology that are unique to the *CAN1-ALP1* alignment in uppercase letters and regions of homology that are identical in both *CAN1-LYP1* and *CAN1-ALP1* alignments in lowercase letters. Breakpoints are numbered 15 to 28, with the most 5′ breakpoint having the lowest number; numbered blue boxes indicate *CAN1-ALP1* breakpoints, and numbered black boxes indicate *CAN1-LYP1* breakpoints that occurred within a stretch of homology that is identical in *CAN1-LYP1* and *CAN1-ALP1* alignments. (C) The ORF of *ALP1* is shown. Regions of homology between the *ALP1* and *LYP1* genes that are longer than 4 bp are boxed. Breakpoints are numbered 29 to 35, with the most 5′ breakpoint having the lowest number; numbered green boxes indicate *LYP1-ALP1* breakpoints, and numbered dotted-line boxes indicate *ALP1-LYP1* breakpoints that were part of *CAN1-LYP1-ALP1-LYP1-ALP1* rearrangements. Breakpoint 34 was observed in *LYP1-ALP1* rearrangements as well as in *ALP1-LYP1* rearrangements.

TABLE 3. Expected and observed frequencies of translocation breakpoints in homology blocks of various lengths[a]

| Homology length (bp) | Breakpoint frequency for: | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *CAN1-LYP1* | | | *LYP1-ALP1* | | | *CAN1-ALP* | | |
| | Expected frequency[b] | Observed frequency[c] | Ratio[d] | Expected frequency[b] | Observed frequency[c] | Ratio[d] | Expected frequency[b] | Observed frequency[c] | Ratio[d] |
| 1 | 0.146 | 0.064 (3) | 0.4 | 0.150 | 0 | 0 | 0.122 | 0 | 0 |
| 2 | 0.202 | 0.021 (1) | 0.1 | 0.210 | 0 | 0 | 0.211 | 0 | 0 |
| 4 | 0.031 | 0.021 (1) | 0.7 | 0.065 | 0 | 0 | 0.043 | 0 | 0 |
| 5 | 0.141 | 0.213 (10) | 1.5 | 0.061 | 0.064 (3) | 1.0 | 0.110 | 0.333 (6) | 3.0 |
| 6 | 0.026 | 0 | 0 | 0.016 | 0 | 0 | 0.019 | 0.056 (1) | 2.9 |
| 8 | 0.061 | 0.021 (1) | 0.3 | 0.033 | 0 | 0 | 0.078 | 0.278 (5) | 3.6 |
| 9 | 0.010 | 0.106 (5) | 10.2 | 0.009 | 0 | 0 | NA | NA | NA |
| 10 | 0.011 | 0.085 (4) | 7.4 | NA | NA | NA | 0.005 | 0 | 0 |
| 11 | 0.050 | 0.191 (9) | 3.8 | 0.028 | 0 | 0 | 0.005 | 0.111 | 20.2 |
| 12 | 0.007 | 0.021 (1) | 3.1 | NA | NA | NA | NA | NA | NA |
| 13 | 0.007 | 0.021 (1) | 2.9 | NA | NA | NA | NA | NA | NA |
| 14 | 0.023 | 0.191 (9) | 8.2 | NA | NA | NA | 0.014 | 0.056 | 4.0 |
| 16 | NA | NA | NA | 0.008 | 0.021 (1) | 2.7 | 0.008 | 0 | 0 |
| 17 | 0.019 | 0.043 (2) | 2.3 | NA | NA | NA | NA | NA | NA |
| 20 | NA | NA | NA | 0.010 | 0.064 (3) | 6.6 | 0.010 | 0.167 (3) | 17.3 |
| 22 | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| 29 | NA | NA | NA | 0.014 | 0.170 (8) | 12.2 | NA | NA | NA |
| 41 | NA | NA | NA | 0.019 | 0.596 (28) | 30.6 | NA | NA | NA |
| 47 | NA | NA | NA | 0.022 | 0.116 (4) | 3.8 | NA | NA | NA |

[a] NA (not available) indicates homology blocks of certain lengths that were not found in the specified gene alignment.
[b] The expected frequency of a breakpoint occurring in a homology block of a certain length was calculated by dividing the product of the homology block length and its number of occurrences within the alignment by the total length of the alignment. The total length of the alignment was defined as the sum of the products of homology block length and its number of occurrences.
[c] The observed breakpoint frequency was calculated by dividing the number of observed breakpoints within a homology block of certain length by the total number of breakpoints observed between these two genes. Numbers in parentheses indicate the total number of breakpoints observed in a homology block of this length.
[d] The ratio between the observed and expected number of breakpoints in a homology block of a certain length.

chromosome 14 that contains an intact *ALP1* gene or an *ALP1-CAN1* rearrangement, which we did not observe in this study.

The most overrepresented breakpoint location identified in this study is the 41-bp *LYP1-ALP1* homology block. The observed 31-fold-higher-than-expected frequency of breakpoints in this location supports the correlation between longer regions of sequence identity and increased HR. However, the only threefold overrepresentation of the nearby 47-bp *LYP1-ALP1* homology block shows that length of sequence identity is not the only determining factor for translocation target sites, but that structure and location of the homology block may also be important predictors of their suitability as an HR hotspot. In fact, the two most overrepresented *LYP1-ALP1* breakpoints are not the longest blocks, but they are the first and the last homology block within the 173-bp homeologous region in the *LYP1-ALP1* alignment (Fig. 2C, breakpoints 29 and 35), and they are preceded or followed by relatively long regions, 76 bp and 70 bp, respectively, that do not contain any homology blocks of at least 6 bp. This may suggest that the border between nonhomologous regions and regions of significant sequence identity may favor HR.

## DISCUSSION

We have analyzed the formation of spontaneous translocations between three related but highly diverged genes (*CAN1*, *ALP1*, and *LYP1*) in their natural locations on two different chromosomes in *S. cerevisiae* and provided evidence for a central role of the DNA helicase Sgs1 in suppressing translocations between these related sequences. An *sgs1*Δ mutation caused a modest increase in the rate of translocations but did not result in the recovery of translocations between *CAN1* and *ALP1* or *LYP1*. However, combining an *sgs1*Δ mutation with additional defects in other DNA metabolic pathways often resulted in increased rates of translocations involving the divergent gene *CAN1*, *ALP1*, or *LYP1*. This was observed when an *sgs1*Δ mutation was combined with mutations causing defects in the DNA damage checkpoint sensors Mec3 and Rad24, the DNA damage checkpoint protein Rad9, the replication checkpoint protein Rfc5, the checkpoint kinase and telomere length maintenance factor Tel1, the DNA helicase Rrm3, and the chromatin assembly factors Cac1 and Asf1, but not the checkpoint kinases Mec1 and Rad53 and the Tsa1-dependent oxidative damage response pathway. A diversity of *CAN1/LYP1/ALP1* translocations was observed, containing as many as four breakpoints that were preferentially located in regions of extended homology, suggesting these translocations were formed by HR. Where tested, these translocations were eliminated by a *rad52*Δ mutation but not by a *rad51*Δ mutation, indicating that the divergent sequence translocations were primarily formed by a Rad52-dependent HR pathway and that a Rad51-independent HR pathway could also promote translocations. An *msh2*Δ or an *msh6*Δ mutation in combination with mutations found to interact with an *sgs1*Δ mutation did not result in increased translocations between *CAN1* and *ALP1* or *LYP1*, indicating that Sgs1 plays a unique role in suppressing translocations between divergent sequences rather than simply acting in the MMR pathway that suppresses homeologous recombination.

A striking feature of our results is the observation that 66%

TABLE 4. Lengths of homology blocks with breakpoint occurrences in *sgs1* mutants with defects in cell cycle checkpoints, homologous recombination, chromatin assembly, or the DNA helicase Rrm3

| Relevant genotype[a] | Breakpoint(s) for: | | | | | |
|---|---|---|---|---|---|---|
| | *CAN1-ALP1* (monocentric chromosome) | | *CAN1-LYP1* (dicentric intermediate) | | *LYP1-ALP1* (monocentric chromosome) | |
| | Homology block length[b] | Avg length | Homology block length[b] | Avg length | Homology block length[b] | Avg length |
| *sgs1*Δ *rad9*Δ[c] | NA | NA | 5 | 5.0 | 29 | 29.0 |
| *sgs1*Δ *tel1*Δ[d] | NA | NA | 1, 1, 5, 9, 14 | 6.0 | 41, 41, 41, 41, 47 | 42.2 |
| *sgs1*Δ *rad51*Δ[e] | 8 | 8.0 | 1, 4, 5, 9 | 4.8 | 20, 41, 41, 41 | 35.8 |
| *sgs1*Δ *rrm3*Δ[f] | 5, 5, 8, 14, 20 | 10.4 | 5, 9, 9, 11, 11, 11, 14, 14, 14 | 10.9 | 16, 29, 29, 29, 29, 41, 41, 41, 41 | 33.8 |
| *sgs1*Δ *mec3*Δ[g] | 5, 5, 5, 6, 11, 20 | 8.7 | 5, 5, 5, 9, 11, 11, 11, 14, 14, 14, 14, 17 | 11.1 | 16, 29, 29, 41, 41, 41, 41, 41, 41, 41 | 36.1 |
| *sgs1*Δ *asf1*Δ[h] | 5, 8 | 6.5 | 5, 11, 14, 14, 17, 5, 10, 12, 14 | 11.3 | 41, 41, 41, 41 | 41.0 |
| *sgs1*Δ *cac1*Δ[i] | 8, 11 | 9.5 | 5, 11, 14 | 10.0 | 29, 41, 41 | 37.0 |
| *sgs1*Δ *rad24*Δ[j] | 5, 8, 20, 20 | 13.3 | 2, 5, 5, 8, 9, 10, 10, 11, 11, 11, 17 | 9.0 | 5, 5, 20, 20, 41, 41, 41, 41, 41, 47 | 30.2 |
| *sgs1*Δ *rfc5-1*[k] | NA | NA | 10, 11 | 10.5 | 41, 47 | 44.0 |
| Avg length[l] | | 9.4 | | 8.6 | | 36.6 |

[a] For additional genotype information and strain numbers, see footnotes *d* to *l*. See Table 1 for complete genotypes of these strains.
[b] The total length (in base pairs) of every homology block with an observed breakpoint is listed. NA (not available) indicates that this chromosomal rearrangement was not observed in this mutant.
[c] *CAN1/LYP1/ALP1* breakpoints were identified in the *rad9*Δ *sgs1*Δ (RDKY5778) mutant.
[d] *CAN1/LYP1/ALP1* breakpoints were identified in the *tel1*Δ *sgs1*Δ (RDKY4566) mutant.
[e] *CAN1/LYP1/ALP1* breakpoints were identified in *rrm3*Δ *sgs1*Δ *rad51*Δ (RDKY5564) and *rrm3*Δ *sgs1*Δ *rad51*Δ *mec3*Δ (RDKY5776) mutants.
[f] *CAN1/LYP1/ALP1* breakpoints were identified in *rrm3*Δ *sgs1*Δ (haploid strain freshly derived from RDKY5577) and *rm3*Δ *sgs1*Δ *mec3*Δ (RDKY5579) mutants.
[g] *CAN1/LYP1/ALP1* breakpoints listed were identified in *sgs1*Δ *mec3*Δ (RDKY5572), *asf1*Δ *sgs1*Δ *mec3*Δ (RDKY5772), and *rrm3*Δ *sgs1*Δ *mec3*Δ (RDKY5579) mutants.
[h] *CAN1/LYP1/ALP1* breakpoints were identified in *asf1*Δ *sgs1*Δ (RDKY4767) and *asf1*Δ *sgs1*Δ *mec3*Δ (RDKY5772) mutants.
[i] *CAN1/LYP1/ALP1* breakpoints were identified in the *cac1*Δ *sgs1*Δ (RDKY4765) mutant.
[j] *CAN1/LYP1/ALP1* breakpoints were identified in the *rad24*Δ *sgs1*Δ (RDKY5575) mutant.
[k] *CAN1/LYP1/ALP1* breakpoints were identified in the *rfc5-1 sgs1*Δ (RDKY4561) mutant.
[l] The average length (in base pairs) of all homology blocks with a breakpoint occurrence is shown for each of the three rearrangements.

of the translocations involving *CAN1*, *ALP1*, and *LYP1* involved two or more apparent translocation events. Assuming that these events are mediated by HR, there are two types of models that might explain these types of translocations (Fig. 4). All of the multiply translocated chromosomes appear to involve translocation from *CAN1* to *LYP1*. Due to the opposite orientation of *CAN1* and *LYP1* relative to their respective centromeres, a translocation from *CAN1* to *LYP1* would yield a dicentric chromosome, which, as suggested by the first model, would then be predicted to break during cell division and undergo secondary rearrangements, yielding monocentric *CAN1-ALP1*, *CAN1-LYP1-ALP1*, or *CAN1-LYP1-ALP1-LYP1-ALP1* translocation (Fig. 4, model 1). The *CAN1-ALP1* translocations could also be generated by a single interchromosomal recombination event between *CAN1* and *ALP1*. A general mechanism involving multiple, independent recombination events seems unlikely for several reasons: (i) the rate of a complex event would be expected to be the product of the rates of the individual steps, and given the rate of single translocation events is low, the observation of translocations involving two or more independent events seems unlikely; (ii) the formation of *CAN1-ALP1* translocations needs to involve only one event, yet these translocations are less frequent (28%) than the *CAN1-LYP1-ALP1* translocations (72%) that require two events, which is surprising unless there is selection against the *CAN1-ALP1* translocations due to formation of active *CAN1-ALP1* fusion genes (which we did not test), or *ALP1* is a less favorable translocation target than *LYP1* despite similar sequence identity and presence of comparable homology blocks; (iii) the *CAN1-LYP1-ALP1-LYP1-ALP1* translocations, which would involve four events, would be predicted to be much more rare (square of the rate of two-event *CAN1-LYP1-ALP1* translocations) than observed; and (iv) it is not clear what would select for *CAN1-LYP1-ALP1-LYP1-ALP1* translocations, as all intermediate translocations containing *ALP1* would no longer be dicentric and would not be subject to further breakage and translocation. An alternative model suggests that translocations may have formed as the result of repairing a single DNA break on chromosome 5 in *CAN1* by BIR coupled with multiple DNA template switches (Fig. 4, model 2). This model suggests that all of the translocations are the product of a single, concerted series of events, which seems more consistent with the observed high frequency of translocation involving two or more rearrangements. A combination of factors, such as the location of *ALP1* and *LYP1* on the same chromosome arm, their close proximity (889 bp), and the presence of 173-bp regions of 93% sequence identity within *LYP1* and *ALP1* that are only separated by 2,530 bp, may have facilitated misannealing of the invading strand with the center of *ALP1* after copying the 5′ end of *LYP1* and dissociating from it, thereby facilitating the formation of complex translocations in our system. Since all *sgs1* mutants with complex translocations between *CAN1*, *LYP1*, and/or *ALP1* were mismatch repair proficient, it is possible that after the initial exchange between *LYP1* and *ALP1* occurred a *LYP1-ALP1* heteroduplex intermediate was formed, and patchy rather than continuous mismatch correction, instead of multiple chromosome breakages or template switches, yielded the two *CAN1-LYP1-ALP1-LYP1-ALP1* translocations

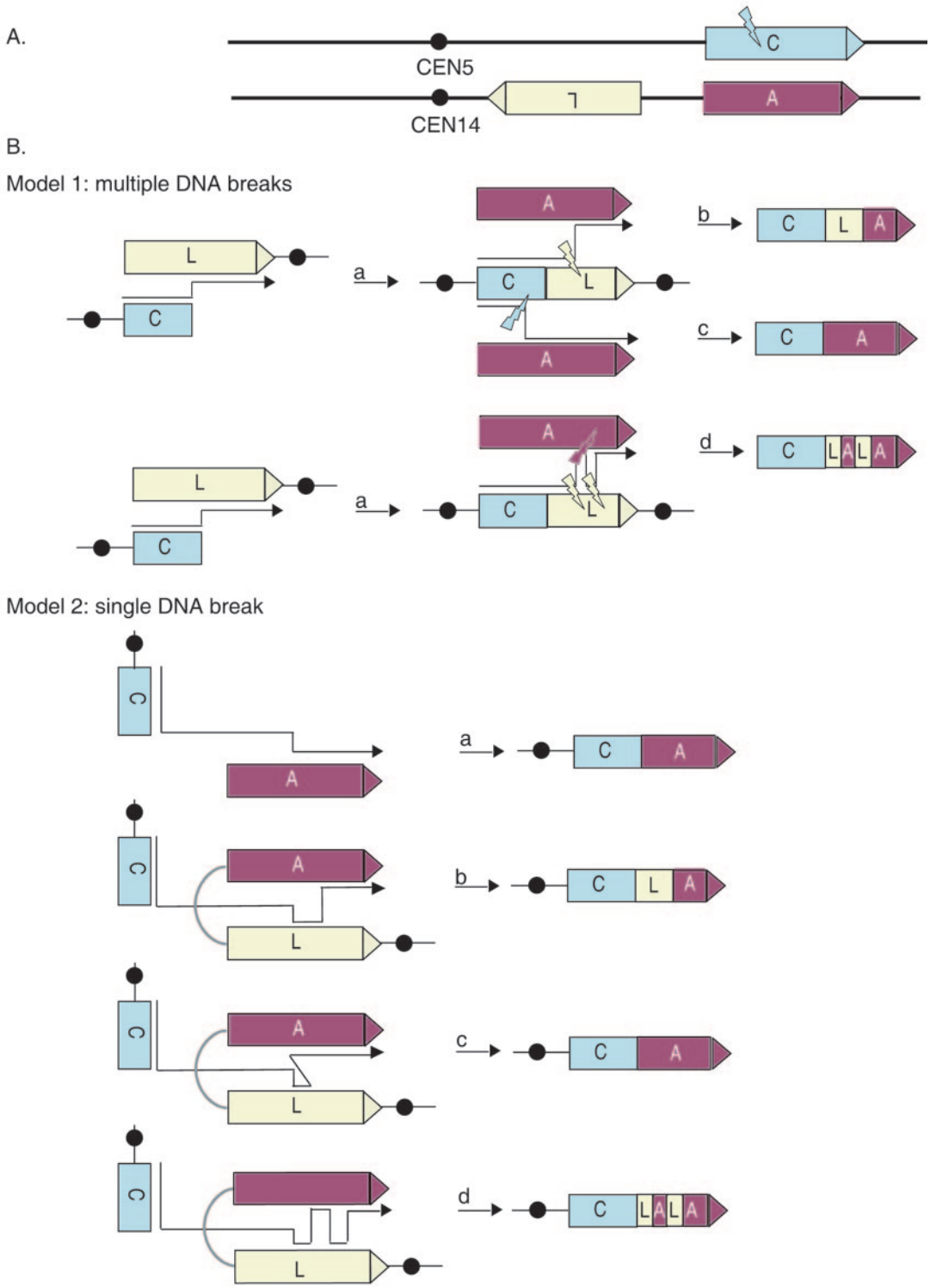


FIG. 4. Models for the formation of complex translocations between *CAN1*, *ALP1*, and *LYP1*. (*A*) Location of *CAN1* ("C") on chromosome 5 and *ALP1* ("A") and *LYP1* ("L") on the same arm of chromosome 14, facing in opposite directions. (B) Model 1 shows formation of complex translocations as a result of multiple chromosome breaks. A single DNA break in chromosome 5 leads to invasion of the related *LYP1* gene on chromosome 14, forming a D loop and initiating DNA synthesis to the end of chromosome 14 to yield a dicentric *CAN1-LYP1* chromosome (a). A second independent DNA break similarly leads to recombination between *LYP1* and *ALP1*, which is in the opposite orientation of *LYP1*, transforming the dicentric chromosome into a stable, monocentric *CAN1-LYP1-ALP1* chromosome (b). Similarly, recombination between *CAN1* and *ALP1* upstream of the original *CAN1-LYP1* breakpoint eliminates any *LYP1* sequence from the translocation chromosome and transforms the *CAN1-LYP1* chromosome into a *CAN1-ALP1* chromosome (c). *CAN1-LYP1-ALP1-LYP1-ALP1* translocations may have formed as a result of four

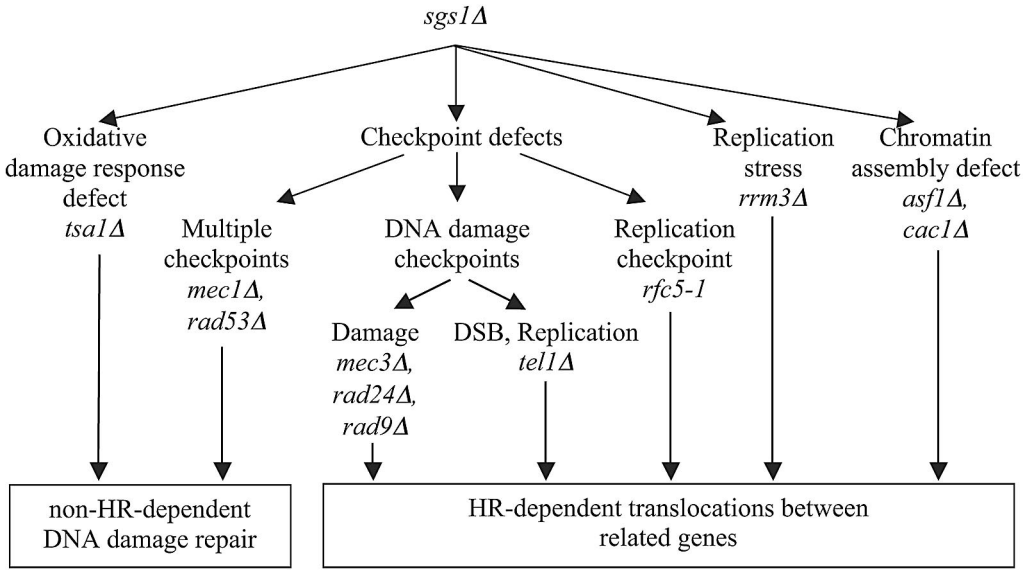


FIG. 5. Model for the suppression of homology-driven translocations in the absence of Sgs1 helicase. Replication stress as well as defects in DNA damage checkpoints, the DNA replication checkpoint, and chromatin assembly can lead to increased levels of HR-dependent translocations in *sgs1*Δ mutants, while lack of the checkpoint kinase Mec1 or Rad53 or a defective oxidative response preferentially leads to other GCR types, such as de novo telomere additions and translocations without homology at the breakpoint (see the text for details).

rather than *CAN1-LYP1-ALP1* translocations. Note that if this mechanism did occur, mismatch repair deficiency would only be expected to eliminate the *CAN1-LYP1-ALP1-LYP1-ALP1* translocations and not the *CAN1-LYP1-ALP1* translocations.

Defects in *SGS1* appear to result in multiple defects that contribute to increased genome instability. These include defects in processing replication intermediates and defects in S-phase checkpoints (10, 24, 26, 30, 38, 49, 61, 81, 82, 93, 96) as well as defects in the suppression of aberrant recombination between divergent sequences documented here. However, the increased genome instability seen in *sgs1*Δ single mutants is modest and is most strikingly revealed when *sgs1*Δ mutations are combined with defects in other pathways that are important for maintaining genome stability. Here we observed three types of genetic interactions with *sgs1*Δ mutations: cases where no genetic interaction was observed; cases resulting in a synergistic increase in the GCR rate without the occurrence of translocations involving divergent sequences; and cases resulting in small to large synergistic increases in the GCR rate along with significantly increased rates of translocations involving divergent sequences. Combined with the results of previous studies, our results suggest two distinct scenarios occur. In some cases the synergistic interaction with *sgs1*Δ results in increased damage, but the lesions may not normally be substrates for HR and hence there is no effect of *sgs1*Δ on suppression of HR between divergent sequences (Fig. 5). Examples of this include the interaction between *sgs1*Δ and the *mec1*Δ and *rad53*Δ mutations that result in checkpoint defects or a *tsa1*Δ mutation that results in increased oxidative damage to DNA. In other cases the synergistic interaction with *sgs1*Δ usually, but possibly not always, results in increased damage yielding lesions that are substrates for HR, and hence there is an effect of *sgs1*Δ on suppression of HR between divergent sequences (Fig. 5). Examples of this include the interaction

independent break-mediated recombination events of the type described for step a above sequentially, leading to generation of a dicentric *CAN1-LYP1* translocation, a *CAN1-LYP1-ALP1* monocentric translocation, a *CAN1-LYP1-ALP1-LYP1* dicentric translocation, and finally a *CAN1-LYP1-ALP1-LYP1-ALP1* monocentric translocation. A related but alternative mechanism follows the two-break mechanism described for step a above, except that after initiating repair of the second DNA break in the *CAN1-LYP1* chromosome by invading homologous sequences of *ALP1* followed by short patch DNA synthesis, what occurs is dissociation from *ALP1* and reinvasion of homologous sequences of *LYP1*, followed by another cycle of short patch DNA synthesis, dissociation, and reinvasion of homologous sequences of *ALP1*, after which DNA synthesis proceeds to the chromosome end (d). (B) Model 2 shows formation of complex translocations as a result of a single chromosome break in *CAN1*. Instead of forming a dicentric *CAN1-LYP1* chromosome first, translocations may have formed by a single event, which may or may not have involved DNA template switching during DNA synthesis. *CAN1-ALP1* translocations could be generated by repairing a DNA break in chromosome 5 through interchromosomal recombination between *CAN1* and *ALP1* (a). Discontinuous extension of the invading 3′ end could lead to the incorporation of multiple related DNA sequences: two cycles of strand invasion, DNA synthesis, and dissociation, first into *LYP1* and then into the nearby *ALP1*, would lead to *CAN1-LYP1-ALP1* translocations if dissociation and reannealing occur after *LYP1*-specific sequence has been copied (b); they would lead to *CAN1-ALP1* translocations if dissociation from *LYP1* and annealing to *ALP1* occur prior to copying of *LYP1*-specific sequence at a homology block shared by all three genes, as is the case for 94% (17/18) of *CAN1-ALP1* breakpoints (c); or four cycles of dissociation and reannealing would lead to *CAN1-LYP1-ALP1-LYP1-ALP1* translocations (d). In all of the models discussed above, recombination most likely involves BIR, because the cells lose the region of chromosome 5 between *CAN1* and the telomere but appear to retain an intact copy of chromosome 14 as evidenced by the presence of wild-type copies of *LYP1* and *ALP1*.

between *sgs1*Δ and the checkpoint-defective mutations *rad9*Δ, *rad24*Δ, *mec3*Δ, and *rfc5-1*, the checkpoint kinase-defective mutation *tel1*Δ, the *rrm3*Δ mutation that results in replication fork stalling, and the *cac1*Δ and *asf1*Δ mutations that result in defective chromatin assembly during DNA replication. Overall, these results raise the possibility that replication errors that can be processed by HR, as suggested by a number of studies (2, 8, 45, 46, 77), can be aberrantly acted on by HR to yield translocations, and that Tel1-dependent checkpoints may be critical to suppression of these replication errors or could act at later steps in the translocation suppression process (Fig. 5). The observation that a *tel1*Δ mutation did not cause as large a synergistic increase in divergent sequence translocations in combination with an *sgs1*Δ mutation as *rad24*Δ, *mec3*Δ, and *rfc5-1* mutations did raises the possibility that Mec1 might be partially redundant with Tel1 (18, 57, 62, 80), although we did not test this.

MMR proteins and Sgs1 function in the suppression of homeologous recombination in *S. cerevisiae* (61, 84, 87). That *msh2*Δ and *msh6*Δ mutations did not lead to *CAN1*/*LYP1*/*ALP1* translocations in any of the permissive genetic backgrounds tested suggests that failure to suppress homeologous recombination was not the primary cause for the increased rate of translocations between divergent DNA sequences observed in *sgs1*Δ mutants. However, the difference between *sgs1*Δ and MMR mutations seen here could reflect a key mechanistic feature of the events studied. It is possible that MMR proteins might be more effective at suppressing the intrachromosomal recombination events measured in most previous studies compared to the interchromosomal recombination events assayed here. Additionally, the DNA sequences surrounding the homeologous genes may influence the activity of MMR proteins; for example, it was proposed that recombination between homeologous sequences may not be affected by MMR proteins if recombination is initiated within regions of nonhomology surrounding the homeologous sequences, whereas MMR proteins play an important role in the suppression of recombination between homeologous sequences embedded in homology (72). It is also possible that MMR, in contrast to Sgs1, cannot act to suppress translocations resulting from HR between sequences with the high levels of divergence studied here. In human tumors, MMR defects lead to mono- and dinucleotide repeat (microsatellites) instability (MIN) (25, 43, 48, 68), but MMR-defective tumors usually do not show chromosomal instability (CIN) (33, 43, 50). However, a subset of tumor cell lines is known to show both MIN and CIN (1, 32, 90). The results presented here are consistent with the observation that MMR-defective tumors do not usually show CIN and suggest that the reason for this is that suppression of homeologous recombination by MMR proteins may not be a major mechanism for the suppression of genome rearrangements. Our results also suggest that those MMR-defective tumor cell lines showing MIN and CIN (1, 32, 90) may contain an additional genetic defect inactivating a function that normally helps prevent genome instability.

Highly elevated levels of genetic exchange between identical sequences resulting in SCEs and symmetrical QRs are a hallmark of BS. Chromosomal aberrations including translocations have also been described in BS patients (4, 39, 71, 85, 102). Furthermore, the repeated observation of partial or complete loss of chromosome 7 in bone marrow cells of BS patients with acute lymphoblastic or myeloblastic leukemia may indeed suggest that lack of BLM can increase the frequency of specific, recurring chromosomal rearrangements (4, 39, 71, 85). However, translocation breakpoints from BS cells have not yet been cloned and sequenced, so little is known about the mechanisms that produce these translocations. Previous results showing that defects in the *S. cerevisiae BLM* homolog *SGS1* result in increased recombination and altered control of crossing over and gene conversion (38, 100, 104) suggest how defects in *BLM* might result in increased SCEs and QRs. The results presented here showing that Sgs1 functions to prevent inappropriate recombination between highly diverged DNA sequences with minimal regions of identity, which would normally be excluded from HR during mitosis, suggest that such sequences may become effective target sites of chromosomal rearrangements in BS cells, leading to the translocations and other chromosome aberrations seen in BS cells. Moreover, HR between short interspersed elements, of which the 300-bp Alu element is the most abundant ($\sim 10^6$ copies/human genome), has been implicated as a major mutational mechanism in numerous common diseases with recurrent rearrangements (9). This raises the possibility that elevated short interspersed element-mediated recombination may also contribute to the increased formation of chromosomal rearrangements in BS. Similarly, elevation of nonallelic HR between region-specific, low-copy repeats, which are known mediators of recurrent rearrangements in the human genome, may contribute to chromosome rearrangements in BS. Recently the tumor suppressor protein p53 has also been implicated in the regulation of spontaneous HR (54; reviewed in reference 7), specifically in the suppression of DNA exchange between imperfectly homologous DNA sequences (5) and the suppression of HR induced by inhibition of replication (78), further highlighting the importance of stringent regulation of HR, like that mediated by Sgs1 in order to maintain stability of the repetitive human genome.

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