The Roles of the *Saccharomyces cerevisiae* RecQ Helicase SGS1 in Meiotic Genome Surveillance

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**Abstract**

**Background:** The *Saccharomyces cerevisiae* RecQ helicase Sgs1 is essential for mitotic and meiotic genome stability. The stage at which Sgs1 acts during meiosis is subject to debate. Cytological experiments showed that a deletion of *SGS1* leads to an increase in synapsis initiation complexes and axial associations leading to the proposal that it has an early role in unwinding surplus strand invasion events. Physical studies of recombination intermediates implicate it in the dissolution of double Holliday junctions between sister chromatids.

**Methodology/Principal Findings:** In this work, we observed an increase in meiotic recombination between diverged sequences (homeologous recombination) and an increase in unequal sister chromatid events when *SGS1* is deleted. The first of these observations is most consistent with an early role of Sgs1 in unwinding inappropriate strand invasion events while the second is consistent with unwinding or dissolution of recombination intermediates in an Mlh1- and Top3-dependent manner. We also provide data that suggest that Sgs1 is involved in the rejection of ‘second strand capture’ when sequence divergence is present. Finally, we have identified a novel class of tetrads where non-sister spores (pairs of spores where each contains a centromere marker from a different parent) are inviable. We propose a model for this unusual pattern of viability based on the inability of sgs1 mutants to untangle intertwined chromosomes. Our data suggest that this role of Sgs1 is not dependent on its interaction with Top3. We propose that in the absence of *SGS1* chromosomes may sometimes remain entangled at the end of pre-meiotic replication. This, combined with reciprocal crossing over, could lead to physical destruction of the recombined and entangled chromosomes. We hypothesise that Sgs1, acting in concert with the topoisomerase Top2, resolves these structures.

**Conclusions:** This work provides evidence that Sgs1 interacts with various partner proteins to maintain genome stability throughout meiosis.

**Introduction**

Meiotic Recombination

During meiosis, the process of homologous recombination is critical for ensuring accurate chromosome segregation and in generating genetic diversity. Homologous recombination is initiated by a double-strand break (DSB) catalysed by Spo11 [1]. Following the formation of the DSB, 3’ strand resection generates 3’ single-stranded overhangs that are then able to invade the homolog [2]. Strand invasion is facilitated by the strand-exchange proteins Rad51 and Dmc1 [3] and leads to the formation of a Single End Invasion (SEI) structure [4].

When breaks are repaired via the crossover pathway, the second single-stranded end is captured by the D-loop following invasion and DNA synthesis. Finally, ligation leads to formation of a double Holliday junction (dHJ) [4, 5]. Resolution of the dHJ yields a crossover [6]. This pathway is promoted by the ZMM proteins (Mer3, Msh4, Msh5, Zip1, Zip2, Zip3 and Zip4), as well as Exo1, Mlh1 and Mlh3 (reviewed in [7]). Non-crossover products arise from the Synthesis Dependent Strand Annealing (SDSA) pathway [8]. The invading strand is not captured as in the crossover pathway, but is instead displaced. This is followed by strand annealing to complementary sequences on the second DSB end, and DNA synthesis, culminating in the formation of a non-crossover product.

Crossovers lead to the establishment of chiasmata, which provide the physical connections during meiotic prophase that promote the accurate segregation of homologous chromosomes (Figure 1A). Therefore, the absence of crossovers leads to the mis-segregation defect known as meiosis I non-disjunction (Figure 1B). Another class of segregation defect, known as precocious separation of sister chromatids (PSSC), is thought to arise from hyper-recombination at centromeres [10].

Homeologous recombination and its suppression

The process of homologous recombination allows the transfer of genetic information between nearly identical stretches of DNA. In contrast, homeologous recombination is the transfer of genetic information between sequences that are diverged. In order to maintain the integrity of chromosomes, and ultimately the...
genome, crossing over between diverged sequences must be regulated. Interactions between diverged tandem and/or inter-spersed repeat sequences, such as the abundant Alu family found in primates, must be suppressed in order to prevent translocations, deletions or inversions [11–14]. While chromosomal rearrangements of this type may be important for driving evolution, adaptation and speciation, they are also responsible for causing disease [15,16]. Thus despite potential evolutionary advantages, preventing recombination between diverged repeats is important [17].

Several studies have shown that the prokaryotic and eukaryotic mismatch repair (MMR) systems (reviewed in [18]) are involved in the regulation of homologous recombination. One of the earliest of these studies [19] demonstrated that mutation of mutS, mutL, mutH or mutU leads to an increase in recombination in conjugational crosses between E. coli and S. typhimurium of up to 1000-fold. This led Rayssiguier et al. [19] to suggest that the MMR system enforces a barrier to inter-species recombination.

The eukaryotic MMR proteins Msh2, Msh3, Msh6, Mlh1 and Pms1 also enforce a barrier to both mitotic and meiotic homologous recombination in yeasts. Several studies in S. cerevisiae have shown that mutations of mismatch repair genes lead to an increase in mitotic homologous recombination [20–22]. Hunter et al. [23] showed that mismatch repair proteins also play an important role in preventing meiotic homologous recombination. They demonstrated that while an inter-specific hybrid between the sibling species S. cerevisiae and S. paradoxus was able to perfectly grow mitotically, it had severe meiotic defects. Only 1% of the spore colonies were viable and they exhibited a slow growth phenotype due to aneuploidy [23]. This aneuploidy was attributed to increased meiosis I non-disjunction as a consequence of a decrease in the rates of recombination [23]. However, mutation of PMS1 or MSH2 leads to significant increase in the rate of recombination, which is accompanied by a decrease in the rates of meiosis I non-disjunction and an improvement in overall spore viability [23]. These observations led them to propose that the MMR system is involved in the assessment of the degree of divergence when heteroduplex DNA is formed.

These data were confirmed and extended by Chambers et al. [24], utilizing a partial hybrid strain in which the chromosome III from S. cerevisiae was replaced by chromosome III from S. paradoxus. This system facilitates the study of the effects of sequence divergence as it does not cause massive aneuploidy. The improved viability allowed Chambers et al. [24] to notice an increase in the number of three viable spore tetrads in the partial hybrid strain. By inferring the genotype of the dead spore using the 1st Law of Mendel...
Sgs1 acts in the same pathway as Top2, both the acidic regions (ARs), and the helicase domain of Sgs1 with amino acids 4, 5 and 9 of Sgs1 being most important for this. The N-terminus of Sgs1 has been shown to interact with Top3, leading to both chromosome loss and disomy. Watt et al. [51] also noted aneuploidy during meiosis which they attributed to a similar failure to decatenate sister chromosomes.

Sgs1 also negatively regulates crossovers during meiosis. Deletion of SGS1 leads to an increase in closely spaced crossovers without an apparent increase in the number of gene conversions or non-crossovers [54–56]. The data therefore implicate Sgs1 as an anti-crossover factor, whose actions are opposed by the pro-crossover activities of the ZMM proteins [54–56]. Sgs1 acts to specifically inhibit the formation of closely spaced inter-sister and multi-chromatid crossovers [55,57]. Failure to carry out this inhibition may be detrimental to the cell, as their presence may perturb chromosome segregation [58].

Sgs1 acts in the suppression of mitotic homeologous recombination

As discussed above, several studies implicated heteroduplex rejection by MMR proteins in the suppression of mitotic homeologous recombination [19–22,59,60] and suggested that heteroduplex rejection might require a helicase [59–61]. Consistent with this, SGS1 mutations were found in screens for elevated mitotic homeologous recombination where it was shown to act in the same pathway to suppress mitotic homeologous recombination as the MMR genes MSH2 and MLH1 [14,62]. An increase in the rate of mitotic homeologous recombination was also seen for a truncation mutation that deleted the C-terminal 200 amino acids of Sgs1, which contains the Mlh1-interacting domain [63,64], suggesting that the interaction with Mlh1 may be important for the suppression of mitotic homeologous recombination [62]. Studies by Myung et al. [65] and Putnam et al. [66] also implicate the topoisomerase Top3 in suppressing rearrangements between ectopic copies of diverged sequences. These data are consistent with models in which Sgs1 unwinds homeologous intermediates and acts to dissolve crossovers between inappropriate substrates. Recently, a second helicase, Mph1, has been shown to be partially redundant to Sgs1 in the suppression of mitotic homeologous recombination [67].

The aim of this study was to further elucidate the mechanism by which homeologous recombination is suppressed in meiosis. Preliminary data obtained from a screen to identify genes whose
mutation leads to increased homeologous recombination suggested Sgs1 might be involved in this process [60]. The data presented here indicate that Sgs1 acts to suppress recombination between diverged sequences at both the single-end invasion stage and at the strand capture stage. Also, in some SGS1 mutant strains, we find an elevated frequency of an unusual class of two viable spore tetrads containing non-sister spores (as shown in Figure 1C). We present a model showing how these might arise due to a failure to decatenate sister chromatids after pre-meiotic DNA replication. This work therefore highlights the importance of Sgs1 during a number of different stages of meiosis.

Results and Discussion

To investigate the role of Sgs1 in the suppression of meiotic homeologous recombination and sister chromatid exchange, we created a variety of SGS1 mutations in the partial hybrid strain in which chromosome III from S. cerevisiae was replaced with chromosome III from S. paradoxus [24] (see Figure 2 and Methods and Materials for description, Table S1 for haploid strain list and Table S2 for diploid strain list). We also replaced the endogenous promoter of SGS1 with the promoter of the CLB2 gene (pCLB2-SGS1) to create a meiotic null of Sgs1 [54,57,58,69] (as described in Methods and Materials). In order to measure unequal sister chromatid exchange we inserted a reporter construct on S. cerevisiae chromosome III, as described in Methods and Materials.

Sgs1 acts in the suppression of meiotic homeologous recombination

To investigate the involvement of Sgs1 in the suppression of homeologous recombination, we assessed the levels of crossing over in three genetic intervals along chromosome III: HML-ADE1-HIS4, HIS4-LEU2 and LEU2-MAT (Figure 3 and Tables S3 and S4) in various mutant strains. In addition we assessed the levels of meiosis I non-disjunction for the partial hybrid diploids. As shown in Figure 1, failure to cross over in a single chromosome pair leads to meiosis I non-disjunction and inviability of the two nullisomic spores. The two remaining viable spores are disomic, and because the mating-type cassettes are located on chromosome III, these will be non-maters. We measured the proportion of the two viable spore class of tetrads that were caused by non-disjunction of the homeologous chromosome IIIIs by checking the mating status of the viable spore colonies (Figure 4 and Table S5).

As seen previously, the presence of sequence divergence significantly decreases the levels of wild-type recombination (ACD 94) 132-fold, 196-fold and 14-fold in HML-HIS4, HIS4-LEU2 and LEU2-MAT respectively, when compared to the homologous cross (ACD 97) (Figure 3 and Tables S3 and S4) [24]. The greatest decrease is seen in the HIS4-LEU2 interval. Consistent with the decrease in crossing over observed for the homologous cross (Figure 3B), we saw a high level of meiosis I non-disjunction (11.5%) similar to that seen previously for the wild type hybrid diploid [24] (Figure 4 and Table S5 – ACD 94).

Previously, it has been shown that a deletion of SGS1 causes a modest yet significant increase in homologous recombination [10,54,56]. We failed to reproduce these observations in two of the three intervals studied (Figure 3A – ACD 95). This may be due to the different strain backgrounds and sporulation conditions used in these studies [70]. However, when sequence divergence was present, we observed a significant increase in recombination for sg11A/sg11A (ACD 96), sg1-AC795/sg11A (ADA 6) and pCLB2-SGS1/sg11A (ADA 2) in two of the three intervals analysed. Furthermore, we also observed a significant decrease in the levels of meiosis I non-disjunction when SGS1 is deleted in the partial hybrid (4.6% for pCLB2-SGS1/sg11A; p = 2.1 × 10−6 – ADA 2) (Figure 4 and Table S5). These data suggest that the absence of Sgs1 facilitates recombination between diverged sequences and, in doing so, decreases the likelihood of chromosome mis-segregation.

The failure to observe a significant effect on crossing over in the HIS4-LEU2 interval between the homeologous chromosomes might be due to fewer successful strand invasions as a consequence of a higher degree of sequence divergence present in this interval. In order to determine whether the variation in the map distances amongst the three intervals was related to different levels of sequence divergence we calculated the sequence identity for each interval as described in the Methods and Materials. The sequence identity for HML-HIS4, HIS4-LEU2 and LEU2-MAT was 88.4%, 85.6% and 88.5%, respectively. While the degree of sequence divergence between the intervals appears to be small, it may still be sufficient to account for the variation in fold reduction in crossing over, as large effects due to small changes in divergence have been noted before [60]. Additionally, despite not seeing any significant difference in the HIS4-LEU2 interval, the data shown in Figure 3B and Table S4 suggests that deletion of SGS1 in the HIS4-LEU2 interval does cause an increase in map distance. Thus, a second possible explanation as to why we do not observe any significant difference may be due to the number of tetrads analysed in this study. In order to increase the size of the data set, we tested the data obtained for the different mutations of SGS1 (sg11A/sg11A, sg1-AC795/sg11A and pCLB2-SGS1/sg11A) for homogeneity by comparing the distribution of PDs, NPDs and TTs using the G-test. The data from these crosses were not significantly different from each other for either the homologous or homeologous diploids. This allowed us to pool the data for all three SGS1 mutant strains and to reanalyse the effect of deleting SGS1 on homeologous recombination collectively (Table S4). We observed a significant increase in recombination for the combined SGS1 mutant data when compared to wild type in all three intervals. This suggests that crossovers between diverged sequences are rescued in the absence of SGS1 in the HIS4-LEU2 interval as well as the other two intervals.

Sgs1 is known to interact with the MMR protein Mlh1 [63,64]. We hypothesised that Mlh1 might act as a ‘molecular matchmaker’ between the MMR complex and Sgs1 to facilitate the unwinding of homeologous recombination intermediates. To test this, we analysed the effects of a mutation that disrupts the ability of Sgs1 to interact with Mlh1 (sg1-mlh1-id) [64]. We saw a significant decrease in the levels of meiosis I non-disjunction for sg1-mlh1-id/sg11A (ADA 5; p = 0.0003) when compared to wild type, equivalent to that seen for pCLB2-SGS1/sg11A (ADA 2) (Figure 4). These data indirectly suggest that the interaction between Sgs1 and Mlh1 is important for the ability of Sgs1 to suppress meiotic homeologous recombination. However, there is no obvious effect on crossing over between the homeologous chromosomes in the sg1-mlh1-id/sg11A (ADA 5) strain (Figure 3B).

Does Sgs1 aid in the completion of reciprocal homeologous recombination?

As described in the Introduction, Chambers et al. [24] proposed that the MMR proteins Mlh2 and Pms1 act to suppress meiotic homeologous recombination at the strand capture stage. They based this proposal on the observation that recombination was six-fold higher in tetrads with only three viable spores than in the four viable spore tetrads and that the dead spore was preferentially recombinant. This phenotype was abolished in MMR defective strains. To assess the potential role of Sgs1 in this phenomenon, we determined if the increase in the rate of recombination for the
three viable spore class of tetrads compared to the four viable spore class of tetrads was dependent on \( \text{SGS1} \) (Table 1). As seen previously [24], there was a significant increase in recombination for the three viable spore class of tetrads in the wild type (\( \text{ACD 94} \)). However, when Sgs1 was repressed during meiosis (\( \text{pCLB2-SGS1/sgs1Δ} \)), this increase was no longer seen, suggesting that Sgs1 aids in rejecting strand capture of the reciprocal product in the presence of mismatches. This activity does not seem to be dependent on the interaction between Sgs1 and Mlh1 as crossing over was still significantly enriched in the three viable spore class of tetrads from the \( \text{sgs1-mlh1-id/sgs1Δ} \) cross (\( \text{ADA 5} \)) (Table 1). This is consistent with the observation that abolishing the interaction with Mlh1 did not have any effect on increasing recombination in the four viable spore class of tetrads discussed above.

Figure 3. Map distances on chromosome III. (A) Homologous recombination. B) Homeologous recombination. Map distances were calculated using the Perkins formula [85]. The distribution of PDs, NPDs and TTs for homologous diploids was compared using the G-test. After correcting for multiple comparisons using the Benjamini-Hochberg correction [86], p-values <0.05 were considered significant. The standard error of the map distances was calculated using Stahl Online Tools (http://molbio.uoregon.edu/~fstahl/compare2.php). * - significantly different from WT/WT; # - significantly different from \( \text{sgs1Δ/sgs1Δ} \); † - significantly different from \( \text{sgs1ΔC795/sgs1Δ} \); ± - significantly different from \( \text{pCLB2-SGS1/sgs1Δ} \).

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This role in meiosis, we designed a reporter construct on chromosome III that detects unequal intra-chromosomal recombination events (described in Methods and Materials and Figure 5).

The frequencies and distributions of unequal recombination events are given in Tables 2 and 3. The frequency of gene conversion of the HIG-CHY2 insert in the SGS1 mutations does not significantly differ from wild type (ACD 94) (Table 2). However, mutation of SGS1 (ACD 116) leads to an increase in the rates of unequal recombination when compared to wild type (Table 3), as evaluated using the G-test for homogeneity (p = 0.0005). This is in agreement with previous genetic studies that showed an elevation in sister-chromatid recombination during mitosis [29], and with physical studies showing increases in inter-sister joint molecules during meiosis [55,57] when SGS1 is deleted. The sgs1-top3-id/sgs1Δ cross (ACD 12) and the sgs1-mlh1-id/sgs1Δ cross (ACD 4) also show a significant increase in the number of unequal recombination events (both USCE and deletions) compared to wild type (p = 0.0267 and p = 0.0079, respectively). The data therefore suggest that the suppression of unequal recombination by Sgs1 is dependent on its interactions with Top3, presumably by recruiting Top3 to dissolve dHJs. Previous studies have suggested that Msh4, Msh5, Mlh3 (and Mlh1 by inference) act to protect inter-homolog recombination intermediates from dissolution by Sgs1 [54,55]. In addition, Mlh3 (and presumably Mlh1), Sgs1 and Top3 have been shown to form a complex during meiosis [71] which is inconsistent with the sole function of Mlh1 and Mlh3 being to block dissolution. Our observations suggest that the interaction of Sgs1 with Mlh1 is important for Sgs1 to carry out its anti-recombination activity, at least as it relates to preventing inter-sister recombination. We propose that the role of Mlh1 may be to recruit Sgs1 to unwind/dissolve inappropriate inter-sister recombination events [71], perhaps mediated by structures recognized by Msh2/Msh3 [73,74]. Thus, in the absence of interactions between Sgs1 and Mlh1/Top3, Top3-mediated dissolution of these unequal recombination intermediates cannot be carried out.

Deletion of SGS1 leads to an increase in the number of non-sister spores in the two viable spore class of tetrads

Using the centromere marker TRP1, we were able to assess the frequency of sister and non-sister spores in the two viable spore class of tetrads (Figure 1C and Table 4). Sister spores contain the same centromere allele and therefore both will either be prototrophic or auxotrophic for growth on tryptophan. Both classes arise with equal frequencies if spore death is due to random causes. However, a significant bias toward the recovery of sister spores is a hallmark of meiosis I non-disjunction. When analysing spore viability in homologous SGS1 mutant strains, we noted a significant increase in the number of two viable spore tetrads for sgs1-1Δ/sgs1Δ (27.08%) and for pCLB2-SGS1/sgs1Δ (19.1%) compared to wild type (0.9%). These could not be attributed to meiosis I non-disjunction as they were significantly enriched for non-sister spores (see below). An increase in non-sister spores has not been reported previously but could be predicted based on the known activities of Sgs1 (Figure 6). As shown in Figure 6A, Sgs1 and Top3 are proposed to act in the dissolution of dHJ structures [37–41]. When SGS1 is deleted, these structures cannot be dissolved. Thus, one possibility is that the failure to dissolve the interacting homologues leads to breakage of the entangled chromosomes and death of the spores containing them [57]. The two surviving spores will be non-sisters. Alternatively, Sgs1 is known to interact with Top2 [50,51] and this interaction may be required for decatenating sister chromatids after pre-meiotic replication (Figure 6B). If this is the case, when there is a crossover between the entangled
region and the centromere, failure to decatenate would lead to broken chromosomes and the death of two spores. The two remaining spores would be non-sisters.

To test the model shown in Figure 6A, we assessed whether the sgs1-top3-id mutation also results in an elevation of non-sister spores. There is no significant difference between the frequency of sister and non-sister spores in the sgs1-top3-id/sgs1Δ cross (ADA Y55) when compared to wild type (Table 4). This suggests that the preferential death of non-sister spores in the absence of Sgs1 does not result from a Top3-dependent inability to dissolve dHJs. Support for this idea comes from the analysis of the sgs1-top3-id/sgs1Δ cross (ADA Y55) which also shows an increase in the number of non-sister spores compared to sister spores (Table 4) despite Sgs1 retaining the ability to interact with Top3 [75]. However, the Top2-interacting domain of Sgs1 has been disrupted in this mutant [50,51]. Therefore, it is possible that the inability of Sgs1 to interact with Top2 in both the sgs1Δ/sgs1Δ and sgs1Δ-Top2 mutant strains leads to the increase in non-sister spores due to the failure to decatenate sister chromatids after replication, as suggested in Figure 6B.

Summary

The data presented in this study highlight the importance of Sgs1 in the early stages of meiosis. Firstly, based on the discovery of an unusual type of tetrads, we propose a role for Sgs1 and Top2 in the decatenation of entangled chromosomes prior to entry into meiosis. Secondly, we propose that Sgs1 unwinds recombination events between homologous chromosomes at the strand invasion stage and the strand capture stage. Finally, the data presented here are most consistent with the role of Sgs1 in the prevention of unequal sister chromatid exchange being carried out via Top3-mediated dissolution aided, in an unknown fashion, by an interaction between Sgs1 and Mlh1.

Materials and Methods

Strains

All of the strains used in this study are in a Y55 background and are listed in Table S1. Diploid strains are listed in Table S2. Deletion strains were made by deleting the coding region of the relevant gene with a KANMX4 cassette [76]. sgs1-ΔC795 [75] was made by replacing the sequences downstream from amino acid 652 with a NATMX4 cassette [77]. As an sgs1 null leads to defects during both mitosis and meiosis, in addition to analysing a complete deletion of SGS1 (ACT 2), we also investigated the effects of eliminating meiotic transcription of SGS1 by replacing the native promoter of SGS1 with the promoter of the CLB2 gene (Y55 3565) [54,57,58,69]. sgs1-top3-id was made using site directed mutagenesis (using the pJET cloning kit by Fermentas) to change the amino acids at positions 4, 5 and 9 into alanine residues [49]. sgs1-mlh1-id was made by changing the amino acids at positions 1383, 1385 and 1386 to alanine residues [64]. Mutations were introduced into yeast by transformation [78] followed by selection on 5-FOA [79]. The oligonucleotides used for the construction of these strains are listed in Table S6.

In order to determine whether the mutant alleles made for this study (Figure 2) were functional in mitosis, we assayed growth on YEPD plates supplemented with 0.02% methyl methanesulfonate (MMS) [Figure S1]. As previously shown, deletion of SGS1 was sensitive to MMS [28,49,75]. In both the homologous (Y55 3567) and homeologous (Y55 3565) strains expressing pCLB2-SSGS1, resistance to MMS was normal. Thus, pCLB2-SSGS1 expresses sufficient amounts of the Sgs1 protein to fulfill its mitotic roles. Jessop et al. [54] have demonstrated that expression of the Sgs1 protein in a pCLB2-SSGS1 strain is repressed approximately 2 hours after the onset of sporulation, with no noticeable traces of the protein after 4 hours. Other groups have

| Homeologous Diploid         | Interval | Tetrad Class | PD  | NPD | TT  | Recombination (cM) | p-value |
|-----------------------------|----------|--------------|-----|-----|-----|--------------------|---------|
| WT/WT (ACD 94)              | HML-HIS4 | 4 Viable     | 626 | 0   | 4   | 0.317              | 2.87×10⁻⁶ * |
|                             |          | 3 Viable     | 147 | 0   | 13  | 4.06               |         |
| HIS4-LEU2                   |          | 4 Viable     | 630 | 0   | 1   | 0.079              | 0.218   |
|                             |          | 3 Viable     | 158 | 0   | 2  | 0.625              |         |
| LEU2-MAT                    |          | 4 Viable     | 611 | 1   | 19  | 1.981              | 4.97×10⁻⁸ * |
|                             |          | 3 Viable     | 126 | 2   | 24  | 11.84              |         |
| pCLB2-SGS1/sgs1Δ (ADA 2)    | HML-HIS4 | 4 Viable     | 194 | 0   | 11  | 2.7                | 0.736   |
|                             |          | 3 Viable     | 151 | 0   | 12  | 3.7                |         |
| HIS4-LEU2                   |          | 4 Viable     | 202 | 0   | 3   | 0.7                | 0.25    |
|                             |          | 3 Viable     | 156 | 0   | 7  | 2.1                |         |
| LEU2-MAT                    |          | 4 Viable     | 179 | 2   | 24  | 8.8                | 0.86    |
|                             |          | 3 Viable     | 140 | 2   | 22  | 10.4               |         |
| sgs1-mlh1-id/sgs1Δ (ADA 5)  | HML-HIS4 | 4 Viable     | 744 | 0   | 13  | 0.9                | 0.0002 * |
|                             |          | 3 Viable     | 143 | 0   | 14  | 4.5                |         |
| HIS4-LEU2                   |          | 4 Viable     | 756 | 0   | 2  | 0.1                | 0.0065 * |
|                             |          | 3 Viable     | 153 | 0   | 5  | 1.6                |         |
| LEU2-MAT                    |          | 4 Viable     | 728 | 1   | 31  | 2.4                | 4.27×10⁻⁸ * |
|                             |          | 3 Viable     | 129 | 0   | 29  | 9.2                |         |

The distribution of PDs, NPDs and TTs for the three viable spore class of tetrads were compared to the four viable spore class of tetrads. p-values <0.05 were considered significant using the G-test (denoted by *).

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A: Reciprocal crossover

B: Intra-chromatid recombination

C: Inter-chromatid recombination

D: Gene conversion
Figure 5. Unequal Recombination. A strain containing the HYG-CYH HYG cassette (in red) was mated to a strain that does not (in green) to assess unequal recombination events. The genetic (drug resistance/sensitivity) phenotype and physical karyotype of all four spores from each type of recombination event are illustrated. A: A reciprocal crossover event that occurs between HIS4 and LEU2, leading to 3 HygR: 1 HygS and 2 CyhR: 2 CyhS. B: Intra-chromatid events (or Deletion events). Crossing over between the hygromycin cassettes on the same sister strand lead to a deletion event. This is seen as 2 HygR: 2 HygS and 1 CyhR: 3 CyhS segregation patterns. Four lanes of a CHEF Gel are shown, each of which represents one spore of a four viable spore tetrad. Chromosome III is indicated with an arrow (→). Due to the deletion event, approximately 27.5kb DNA will have been lost. This results in the absence of a band where expected and a band of double intensity below, as chromosome III now migrates with chromosome VI. When probed with URA3 and CYH2 sequences, the URA3 containing chromosome V (top band) and the CYH2 containing chromosome VII (middle band) are labelled. Chromosome III (bottom band) is labelled when it retains the LEU2 gene. C: Inter-chromatid events (or Unequal Sister Chromatid Exchange events). When a reciprocal crossover occurs between one hygromycin cassette on one sister strand and the other hygromycin cassette on the second sister strand, a triplication event and a deletion event are seen as 2 HygR: 2 HygS and 1 CyhR: 3 CyhS colonies. The triplication event results in chromosome III migrating more slowly, while the deletion event migrates faster (as discussed in B). Southern blot analysis is used as physical confirmation of the genetic diagnosis, as discussed above. D: Gene Conversion events. Tetrads that are 2 HygR: 2 HygS and 1 CyhR: 3 CyhS can also arise by gene conversion of the HYG-CYH region. Because a gene conversion event does not result in a major size change, the CHEF karyotype is normal. However, Southern blotting indicates that one copy of the CYH2 gene has been replaced with wild-type sequences.

Table 2. Gene conversion events for homologous diploids.

| Homologous Diploid | Number of gene conversion events | Tetrads that did not exhibit a gene conversion | Total Number of tetrads | Percentage of gene conversion events |
|--------------------|---------------------------------|-----------------------------------------------|-------------------------|-------------------------------------|
| WT/WT (ACD 97)     | 11                              | 244                                           | 255                     | 4.3                                 |
| sgs1Δ/sgs1Δ (ACD 116) | 5                              | 173                                           | 178                     | 2.8                                 |
| sgs1Δ–C795/sgs1Δ (ADA 3) | 4                              | 173                                           | 177                     | 2.6                                 |
| sgs1-mhl1-id/sgs1Δ (ADA 4) | 8                              | 413                                           | 421                     | 1.9                                 |
| sgs1-top3-id/sgs1Δ (ADA 12) | 4                              | 221                                           | 225                     | 1.8                                 |

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Table 3. Unequal recombination events for homologous diploids.

| Homologous Diploid | USCE Events | Deletion Events | Tetrads that did not exhibit an unequal recombination event | Total Number of tetrads | Percentage of unequal recombination events |
|--------------------|-------------|-----------------|-----------------------------------------------------------|------------------------|---------------------------------------------|
| WT/WT (ACD 97)     | 6           | 0               | 249                                                       | 255                    | 2.4                                         |
| sgs1Δ/sgs1Δ (ACD 116) | 13         | 5               | 160                                                       | 178                    | 10.1 *                                      |
| sgs1-ΔC795/sgs1Δ (ADA 3) | 4          | 8               | 165                                                       | 177                    | 6.8 *                                       |
| sgs1-mlh1-id/sgs1Δ (ADA 4) | 8          | 10              | 403                                                       | 421                    | 4.3 *#                                      |
| sgs1-top3-id/sgs1Δ (ADA 12) | 9          | 4               | 212                                                       | 225                    | 5.78 *                                      |

The distribution of classes of events amongst wild type and SGS1 mutant strains were compared using the G-test. p-values < 0.05 were considered significant.

Supporting Information

Table S1 Haploid strain list. (DOC)
Table S2 Diploid strain list. (DOC)
Table S3 Map distance for intervals along chromosome III for homologous diploids. The distribution of PDs, NPDs and TTs for homologous diploids were compared using the G-test. After correcting for multiple comparisons using the Benjamini-Hochberg correction [86], p-values < 0.05 were considered significant. The map distances for both the homologous diploids are shown in Figure 4A. * - significantly different from WT/WT; # - significantly different from sgs1Δ/sgs1Δ; † - significantly different from sgs1-ΔC795/sgs1Δ; ± - significantly different from pCLB2-SGS1/sgs1Δ; $ = significantly different from sgs1Δ combined. (DOC)

Table S4 Map distance for intervals along chromosome III for homologous diploids. The distribution of PDs, NPDs and TTs for homologous diploids were compared using the G-test. After correcting for multiple comparisons using the Benjamini-Hochberg correction [86], p-values < 0.05 were considered significant. The map distances for both the homologous diploids are shown in Figure 4B. * - sgs1Δ combined represents the collective data from the sgs1Δ/sgs1Δ (ACD 96), sgs1-ΔC795/sgs1Δ (ADA 6) and pCLB2-SGS1/sgs1Δ (ADA 2) homologous crosses. * - significantly different from WT/WT; # - significantly different from sgs1Δ/sgs1Δ; † - significantly different from sgs1-ΔC795/sgs1Δ; ± - significantly different from pCLB2-SGS1/sgs1Δ; $ = significantly different from sgs1Δ combined. (DOC)

Table S5 Meiosis I non-disjunction events in homologous diploids. Meiosis I non-disjunction events on chromosome III were identified in homologous diploids as described in Methods and

Contour-Clamped Homogeneous Electric Field (CHEF) Gels to separate S. cerevisiae chromosomes

The CHEF DRIII system (Bio-Rad) was used to separate the chromosomes of S. cerevisiae. DNA was prepared for CHEF Gel analysis as described by Louis and Haber [87]. To obtain good separation of the smallest chromosomes gels were run for 15 hours with a 60 second switching time followed by 9 hours with a 90 second switching time. Gels were run at 14 °C in 0.5x TBE (0.045M Tris-borate, 0.045M boric acid and 0.001M EDTA) at 6 volts/cm and at an angle of 120°.

Southern Blot Analysis

Southern blotting was carried out as described in Sambrook et al. [88]. The DNA probe was prepared using the DIG-High Prime system (Roche) as described in the manufacturer’s instructions.

Table 4. Distribution of sister and non-sister spores.

| Homologous Diploids | 2 viable spore class of tetrads | p-value |
|---------------------|---------------------------------|---------|
| sgs1Δ/sgs1Δ (ACD 95) | 110 (36.54%)                   | 3 x 10^-6 * |
| sgs1-ΔC795/sgs1Δ (ADA 3) | 56 (38.62%)                    | 0.006 * |
| pCLB2-SGS1/sgs1Δ (ADA 1) | 3 (14.29%)                    | 0.001 * |
| sgs1-top3-id/sgs1Δ (ADA 12) | 47 (43.52%)                   | 0.178   |

p-values < 0.05 were considered significant using χ² test (significance denoted by *) which indicated that the ratio of sister : non-sister spores deviated significantly from 50:50.

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Materials. Frequencies of meiosis I non-disjunction events out of the total number of tetrads dissected were compared using the G-test. After correcting for multiple comparisons using the Benjamini-Hochberg correction [86], p-values <0.05 were considered significant. * - significantly different from WT/WT; #: - significantly different from sg1Δ/sg1Δ; † - significantly different from sg1Δ-AC795/sg1Δ. (DOC)

Table S6 Oligonucleotides used in this study. Bold sequences, as described by Longtine et al [89], are homologous to pA6a-KANMX6-pCLB2-3HA plasmid [69]. Underlined sequences are homologous to the pAG25 (NATMX4) and pAG32 (HYGMX4) plasmid [77]. (DOC)

Figure S1 Testing the growth of different sg1 mutants with respect to MMS resistance by spotting serial dilutions onto YEPD plates (as a control) and YEPD plates supplemented with 0.02% MMS. Failure to grow on YEPD media supplemented with 0.02% MMS is indicative of an inability to repair lesions which lead to the stalling of replication forks during mitosis. (TIFF)

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Author Contributions
Conceived and designed the experiments: RHB ADA ABHC. Performed the experiments: ADA ABHC RPM RMB. Analyzed the data: ADA RHB ABHC. Contributed reagents/materials/analysis tools: ADA ABHC RPM RMB. Wrote the paper: ADA RHB.
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