

# Heteroduplex DNA Position Defines the Roles of the Sgs1, Srs2, and Mph1 Helicases in Promoting Distinct Recombination Outcomes

Katrina Mitchel, Kevin Lehner, Sue Jinks-Robertson\*

Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, North Carolina, United States of America

## Abstract

The contributions of the Sgs1, Mph1, and Srs2 DNA helicases during mitotic double-strand break (DSB) repair in yeast were investigated using a gap-repair assay. A diverged chromosomal substrate was used as a repair template for the gapped plasmid, allowing mismatch-containing heteroduplex DNA (hDNA) formed during recombination to be monitored. Overall DSB repair efficiencies and the proportions of crossovers (COs) versus noncrossovers (NCOs) were determined in wild-type and helicase-defective strains, allowing the efficiency of CO and NCO production in each background to be calculated. In addition, the products of individual NCO events were sequenced to determine the location of hDNA. Because hDNA position is expected to differ depending on whether a NCO is produced by synthesis-dependent-strand-annealing (SDSA) or through a Holliday junction (HJ)-containing intermediate, its position allows the underlying molecular mechanism to be inferred. Results demonstrate that each helicase reduces the proportion of CO recombinants, but that each does so in a fundamentally different way. Mph1 does not affect the overall efficiency of gap repair, and its loss alters the CO-NCO by promoting SDSA at the expense of HJ-containing intermediates. By contrast, Sgs1 and Srs2 are each required for efficient gap repair, strongly promoting NCO formation and having little effect on CO efficiency. hDNA analyses suggest that all three helicases promote SDSA, and that Sgs1 and Srs2 additionally dismantle HJ-containing intermediates. The hDNA data are consistent with the proposed role of Sgs1 in the dissolution of double HJs, and we propose that Srs2 dismantles nicked HJs.

**Citation:** Mitchel K, Lehner K, Jinks-Robertson S (2013) Heteroduplex DNA Position Defines the Roles of the Sgs1, Srs2, and Mph1 Helicases in Promoting Distinct Recombination Outcomes. *PLoS Genet* 9(3): e1003340. doi:10.1371/journal.pgen.1003340

**Editor:** Lorraine S. Symington, Columbia University College of Physicians and Surgeons, United States of America

**Received:** June 18, 2012; **Accepted:** January 9, 2013; **Published:** March 14, 2013

**Copyright:** © 2013 Mitchel et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Funding:** This work was funded by NIH grant GM038464 to SJ-R (<http://www.nih.gov/>). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing Interests:** SJ-R is currently an associate editor for PLOS Genetics.

\* E-mail: Sue.Robertson@duke.edu

## Introduction

Faithful transmission of genetic information in mitotically dividing cells requires the repair of DNA damage that occurs from exogenous and endogenous sources. Damage to both strands of DNA can cause a double-strand break (DSB), as can replication of a damaged DNA template containing a nick. A single, unrepaired DSB can result in the loss of essential genes and lead to permanent cell-cycle arrest and cell death. To prevent these outcomes, DSBs are repaired by one of two pathways: error-prone nonhomologous end joining or error-free homologous recombination (HR). As the major DSB repair pathway in the yeast *Saccharomyces cerevisiae*, HR promotes high-fidelity repair through the use of an intact template DNA sequence. However, HR can also lead to loss of heterozygosity and gross chromosomal rearrangements and thus requires tight regulation.

To initiate HR, the 5' ends of the DSB are resected to yield 3' single-stranded regions of DNA (for reviews, see [1,2,3,4]). These 3' ends are coated with Rad51 to form nucleoprotein filaments that are competent to conduct a homology search and invade a donor duplex DNA molecule, promoting pairing with the complementary strand. Successful strand invasion of a homologous duplex results in the formation of a D-loop structure consisting of a region of heteroduplex DNA (hDNA) and a

displaced single strand of DNA (Figure 1). New DNA synthesis occurs using the 3' invading end as a primer, and this reaction enlarges the D-loop. Expansion of the D-loop, or its movement with the extending 3' end [5], eventually exposes sequences complementary to the other side of the break (Figure 1A). In the canonical DSB repair (DSBR) model of recombination [6], annealing between the D-loop and the non-invading end of the DSB ("2<sup>nd</sup> end capture") results in the formation of a double Holliday junction (dHJ) intermediate (Figure 1B). Alternatively, if the annealed D-loop is nicked, an intermediate with a single HJ (sHJ) will be generated [4,7]. In the gap-repair system used here, there is a strong dependence of CO events on the Rad1-Rad10 endonuclease [8], which would be consistent with D-loop nicking. HJ-containing intermediates can be resolved by cleavage (Figure 1C), and this process is generally assumed to yield either noncrossover (NCO) products that maintain the original linkages of DNA flanking the break, or crossover (CO) products in which the linkages of flanking DNA are switched. As an alternative to cleavage, a dHJ-containing intermediate can be "dissolved" to yield exclusively NCO products (Figure 1D) (reviewed in [9]). In lieu of engaging the second end of the DSB and subsequent HJ formation, the D-loop can be dismantled (Figure 1E). Annealing of the newly synthesized DNA to the non-invading 3' end of the break then provides a template for the synthesis of the other strand

## Author Summary

Chromosomal damage that occurs during normal cell division can be repaired using an intact sequence elsewhere in the genome as a template. This process, termed homologous recombination, is crucial for the repair of a particularly deleterious lesion, the DNA double-strand break. Although recombination is a repair process, it can also lead to exchanges of genetic material, generating crossovers (COs) between the involved chromosomes. Repair of the break without exchange of flanking DNA is called a noncrossover (NCO). As COs can uncover recessive mutations or result in large-scale genome rearrangements, understanding how the CO-NCO outcome is regulated is critical to issues of genome stability. The current study examines the distinctive mechanisms whereby three yeast DNA helicases—Mph1, Sgs1, and Srs2—contribute to the repair of a DNA double-strand break.

of the damaged molecule. As this Synthesis-Dependent Strand-Annealing (SDSA) pathway does not go through an HJ-containing intermediate, it yields exclusively NCO products [10].

In *S. cerevisiae*, three 3' to 5' DNA helicases - Srs2, Sgs1 and Mph1 - have been implicated in regulating the outcome of mitotic DSB repair [11], and each increases the frequency of NCO events relative to CO events [12,13]. Srs2 (suppressor of *rad6* sensitivity) was the first of the three helicases to be identified, and its gene was discovered in a screen for mutations that suppress the UV sensitivity of *rad6* strains [14]. Because suppression depends on the HR machinery, it was suggested that this helicase normally inhibits recombinational bypass of DNA lesions [15]. In spontaneous recombination assays, loss of Srs2 increases the rate of recombination, confirming that Srs2 can inhibit recombination [16,17]. The anti-recombination activity of Srs2 has been attributed to its “strippase” activity, which removes the Rad51 protein from single-stranded DNA ends and thereby prevents strand invasion [18,19]. However, when Srs2 function was examined in the context of an HO endonuclease-induced DSB, it was paradoxically found to play a pro-recombination role [20]. The loss of Srs2 not only decreased the overall level of DSB repair, it led to a proportional increase of COs among the recovered products [12], suggesting a specific role in NCO formation. Although it has been suggested that Srs2 directly dismantles D-loops to promote NCOs via SDSA [21], an alternative possibility is that its pro-recombination role reflects the removal of Rad51 from single-stranded DNA (ssDNA) ends, which would promote annealing between the 2<sup>nd</sup> end of the DSB and the newly extended strand upon D-loop collapse. Consistent with this possibility, biochemical studies have shown that Rad51 complexed with ssDNA is a potent inhibitor of Rad52-mediated annealing reactions [22].

Sgs1 (slow growth suppressor) was identified based on genetic interactions with Top3, with *sgs1* mutations suppressing the genetic instability and slow growth of *top3* strains [23]. Sgs1 is a member of the RecQ family of 3' to 5' DNA helicases and is the ortholog of the human BLM helicase [23,24]. Mutations in the *BLM* gene lead to the autosomal recessive disorder Bloom's syndrome, which is characterized by genetic instability and increased sister chromatid exchange [25]. Like Srs2, the Sgs1 helicase has multiple roles in recombination. First, Sgs1 acts with the endonuclease Dna2 to promote extensive 5' to 3' resection of the DSB ends (for a review, see [26]). Second, biochemical and *in vivo* studies suggest that Sgs1, together with Top3 and Rmi1, promotes NCO formation by dissolving dHJ-containing intermediates that could alternatively be

cleaved to yield COs [27,28]. Dissolution involves migration of the two HJs towards each other, followed by decatenation of the two linked strands. Consistent with a role in dHJ dissolution, loss of Sgs1 results in increased CO formation during repair of an HO-induced DSB [12].

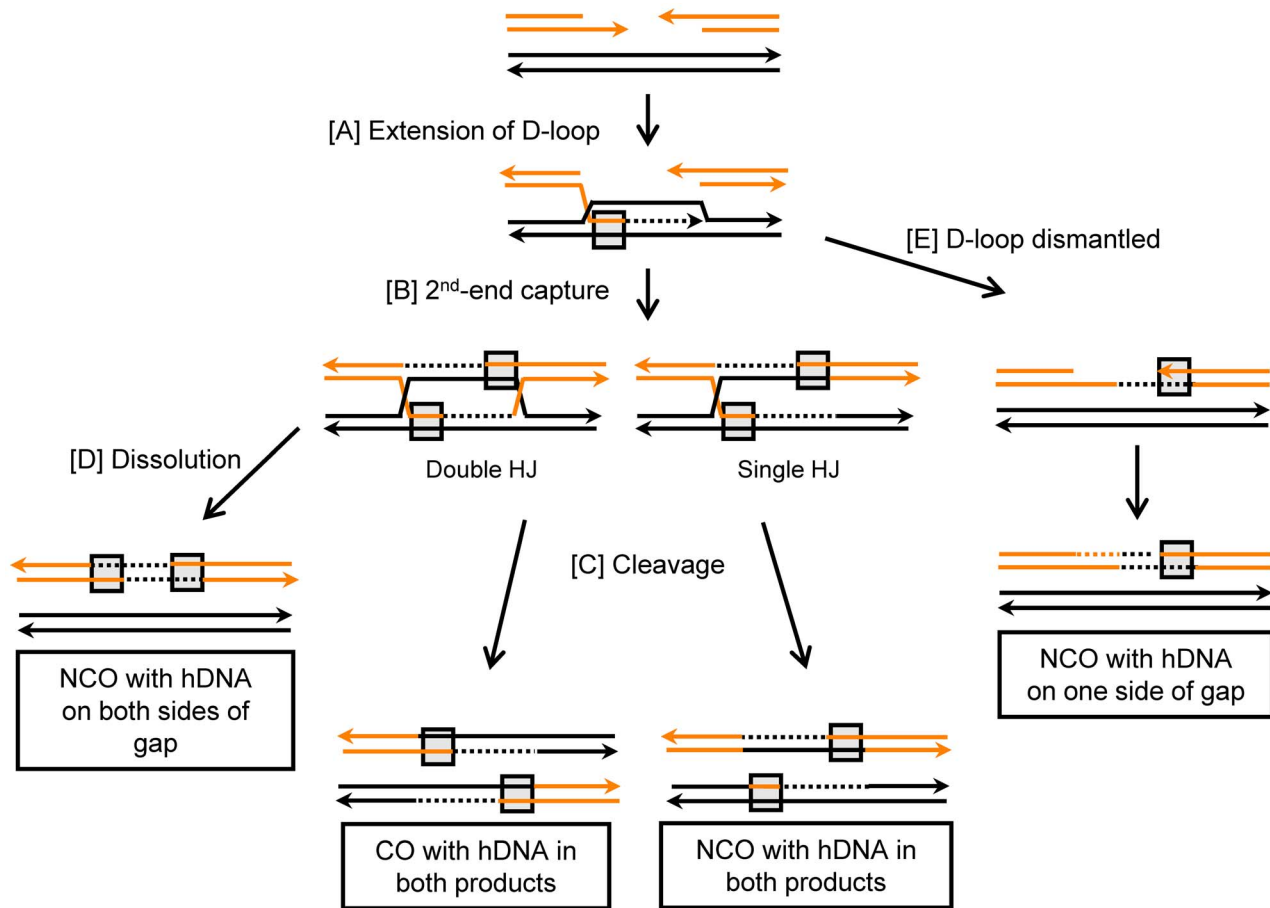
*MPH1* was identified in a screen for mutants exhibiting a mutator phenotype [29], and the encoded protein is the ortholog of the human Fanconi Anemia protein FANCM [30,31]. The participation of Mph1 in HR was initially inferred from epistasis analysis [32]. In its absence, the frequency of HO-induced COs was found to be elevated, but overall levels of repair were not affected [13]. The increase in COs was suggested to specifically reflect a loss of SDSA events, and consistent with this, Mph1 efficiently dismantles D-loops *in vitro* [33]. In a plasmid-based gap-repair assay, COs were similarly found to be elevated in the absence of Mph1, a function that may be partially dependent on the mismatch repair (MMR) complex MutS $\alpha$  [34].

Although biochemical studies have suggested specific roles for Srs2, Sgs1 and Mph1 in promoting either SDSA or dHJ dissolution, corresponding *in vivo* evidence has been lacking. In particular, prior studies have not been able to distinguish whether a given NCO product was produced by HJ cleavage, HJ dissolution or SDSA. To more rigorously assess the specific functions of the Mph1, Srs2 and Sgs1 helicases in NCO formation, gapped plasmids were transformed into wild-type and mutant strains that were defective for the MMR protein Mlh1 and contained a diverged chromosomal template for repair. We measured gap-repair efficiency, determined the CO-NCO distribution among repair events and sequenced both products of individual NCO repair events to detect regions of hDNA. As the location of hDNA can be used to infer the underlying molecular mechanism of NCO formation, the data provide novel insight into how recombination intermediates are processed by these helicases. These molecular analyses are consistent the presumed roles of these helicases and suggest additional functions as well.

## Results

To analyze the roles of helicases in mitotic DSB repair, we used a transformation-based gap-repair system described previously (Figure 2A; [35]). Briefly, the introduced plasmid contained an 800 bp *HIS3* gene within which a centrally located, 8-bp gap was created by restriction digest. As a repair template, a *his3* allele missing the C-terminal 11 amino acids and containing 19 single nucleotide polymorphisms (SNPs) was inserted on chromosome V. The nearest SNPs are located 18 bp from each side of the gap. Because the donor sequence and plasmid are not identical, regions of hDNA formed during HR will contain mismatches. If not repaired, such mismatches segregate at the next round of replication, giving rise to a sectored colony with respect to the SNPs. To allow detection of hDNA, all experiments were conducted in an *mlh1* $\Delta$ , MMR-defective background. This background was chosen because our prior studies demonstrated that loss of Mlh1 affects neither the efficiency of gap repair nor the CO-NCO outcome [8]. The single-mutant, *mlh1* $\Delta$  parental strain thus served as the reference “wild-type” (WT), and all helicase-defective strains will be referred to hereafter by only their relevant helicase genotype.

The unique aspect of analyses done here is the tracking of hDNA in NCO products, as distinct patterns are predicted by SDSA, HJ dissolution and HJ cleavage. As illustrated in Figure 1, each of the NCO products generated by HJ cleavage is expected to contain a single region of hDNA. One region should be present on one side of the break in the repaired plasmid allele, and the other



**Figure 1. Gap-repair pathways.** Single strands of DNA are represented by orange and black lines, and arrowheads indicate 3' ends. Regions of hDNA are boxed, and newly synthesized DNA is depicted as dashed lines in the same color as the template allele. Additional detail is provided in the text.

doi:10.1371/journal.pgen.1003340.g001

on the opposite side of the break in the chromosomal, donor allele. In contrast to NCO products generated by HJ cleavage, no change is expected to the donor duplex following HJ dissolution. Two regions of hDNA are predicted to be in the repaired allele, one on either side of the break. Finally, NCOs produced by SDSA are expected to contain a single region of hDNA in the repaired allele located specifically on the annealing side of the gap. As with HJ dissolution, no change to the donor allele is expected.

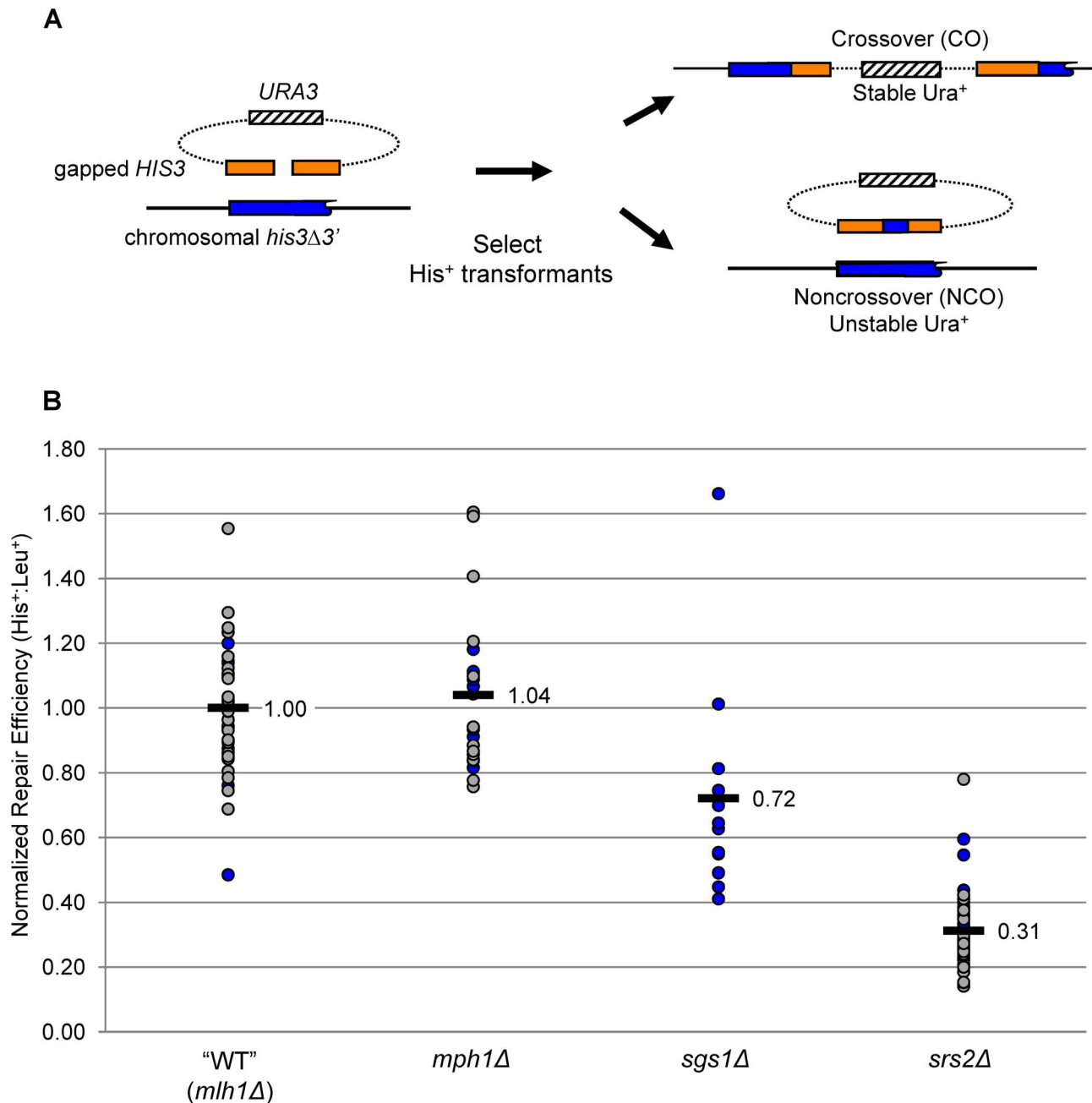
### Helicases and their effects on gap-repair efficiency

To control for variations in transformation efficiency, a linearized plasmid containing a *LEU2* marker was co-transformed with the gapped *HIS3* plasmid. His<sup>+</sup> and Leu<sup>+</sup> colonies were selected separately during each transformation, with the His<sup>+</sup>:Leu<sup>+</sup> ratio providing a measure of gap-repair efficiency. At least 12 independent transformations were done with the WT and each single-helicase mutant. To facilitate comparisons, the His<sup>+</sup>:Leu<sup>+</sup> ratio obtained in each individual transformation was divided by the mean His<sup>+</sup>:Leu<sup>+</sup> ratio obtained in the WT strain (see Materials and Methods). The normalized ratios are presented in Figure 2B, where the mean transformation efficiency of WT is 1.00. Relative to WT, the mean His<sup>+</sup>:Leu<sup>+</sup> ratio in the *mph1Δ* strain was 1.04, indicating that loss of Mph1 does not affect the overall efficiency of gap repair ( $p=0.54$  using a two-tailed Student's *t*-test). By contrast, the mean His<sup>+</sup>:Leu<sup>+</sup> ratio following transformation of

the *sgs1Δ* strain was reduced 30% and that in the *srp2Δ* strain was reduced 3-fold relative to WT ( $p=0.018$  and  $p<0.0001$ , respectively). These data confirm that loss of either Sgs1 or Srs2 leads to decreased gap repair [8], and additionally demonstrate that loss of Mph1 has no effect in this system.

### Effects of individual helicase deficiencies on CO and NCO production

The gapped plasmid used in the transformation experiments contained an autonomously replicating sequence (*ARS*) but no centromere (*CEN*) sequence, allowing the repaired plasmid either to integrate into the chromosome with the repair template (a CO event) or to remain autonomous (a NCO event). These two outcomes were distinguished by examining the stability of the plasmid-encoded *URA3* marker, allowing His<sup>+</sup> products to be partitioned into NCO and CO events (Figure 2A; [8]). Simply comparing the proportions of COs and NCOs in different genetic backgrounds (Figure S1) can be misleading, however, as it does not take into account changes in overall gap-repair efficiency. For example, an elevation in the proportion of COs could reflect either a specific gain in CO events with no effect on NCOs, a channeling of potential NCO products into the CO pathway or a specific loss of NCO products with no effect on COs. The efficiency of CO (or NCO) repair was thus calculated by multiplying the mean gap-repair efficiency by the proportion of CO (or NCO) events



**Figure 2. Gap-repair system and gap-repair efficiency in the absence of Mph1, Sgs1, or Srs2.** A. Schematic of the gap-repair system, which detects both CO and NCO events. B. The efficiency of gap repair in WT and helicase-deficient strains, all of which are MMR-defective (*mlh1Δ*). Normalized ratios of His<sup>+</sup> to Leu<sup>+</sup> transformants in individual transformations are depicted by filled circles; ratios obtained with the first transformation mix are labeled in blue, and grey indicates ratios obtained with the second transformation mix. The mean is indicated with a black bar. doi:10.1371/journal.pgen.1003340.g002

(Table 1). To allow statistical comparisons, the normalized His<sup>+</sup>:Leu<sup>+</sup> ratios in individual transformation experiments were multiplied by the proportion of COs or NCOs among gap-repair products, yielding a distribution of CO-type or NCO-type His<sup>+</sup>:Leu<sup>+</sup> ratios, respectively. The distributions in different strains were then compared using a two-tailed Student's t-test.

In the WT yeast strain, only 9% of repair events were COs; the mean CO and NCO efficiencies were thus 0.09 and 0.91, respectively (Table 1). Although the overall efficiency of gap repair in the *mph1Δ* strain was indistinguishable from that in WT, the

proportion of COs increased to 19%. The mean CO efficiency in the *mph1Δ* strain was thus 0.20, a change that was highly significant when compared to WT ( $p < 0.0001$ ). Even though there was a roughly compensatory decrease in proportion of NCOs in the *mlh1Δ* (from 0.91 in WT to 0.84), this did not translate into a significant change in mean NCO efficiency when compared to WT ( $p = 0.13$ ). In the *sgs1Δ* strain, the proportion of COs among the repaired products increased to 15%. When the accompanying decrease in mean gap-repair efficiency in the *sgs1Δ* background was considered, however, there was no significant change in CO

**Table 1.** Gap-repair efficiency and CO production in WT and helicase-deficient strains.

| Relevant genotype | Plasmid repair efficiency <sup>1</sup> | CO events      |                         | NCO events     |                         |
|-------------------|--|----------------|-------------------------|----------------|-------------------------|
|                   |  | Proportion (%) | Efficiency <sup>2</sup> | Proportion (%) | Efficiency <sup>2</sup> |
| WT                | 1.00 (N = 36)                          | 41/453 (9%)    | 0.09                    | 412/453 (91%)  | 0.91                    |
| <i>mph1Δ</i>      | 1.04 (N = 24)                          | 89/462 (19%)   | 0.20*                   | 373/462 (81%)  | 0.84                    |
| <i>sgs1Δ</i>      | 0.72* (N = 12)                         | 26/176 (15%)   | 0.11                    | 150/176 (85%)  | 0.62*                   |
| <i>srs2Δ</i>      | 0.31* (N = 40)                         | 86/343 (25%)   | 0.08*                   | 257/343 (75%)  | 0.23*                   |

All strains were MMR-defective (*mlh1Δ*). Asterisks indicate a significant difference when compared to WT using a Student's t-test ( $p < 0.05$ ).

<sup>1</sup>Plasmid repair efficiency reflects the mean His<sup>+</sup>:Leu<sup>+</sup> ratio normalized to that obtained in the WT strain. N is number of independent transformations used to determine the mean.

<sup>2</sup>Mean CO and NCO efficiencies were determined by multiplying the repair efficiency by the proportion of transformants that were categorized as CO and NCO events, respectively, based on plasmid stability.

doi:10.1371/journal.pgen.1003340.t001

production relative to WT (0.09 and 0.11, respectively;  $p = 0.30$ ). By contrast, the mean efficiency of NCOs decreased from 0.91 in the WT to 0.62 in the *sgs1Δ* strain, a change that was highly significant ( $p = 0.005$ ). Finally, an even greater proportional increase in COs was observed in the *srs2Δ* strain: from 9% in WT to 25% of total repair events in the mutant. Taking into account the 3-fold decrease in overall gap-repair efficiency, however, the mean efficiency of CO formation was only very slightly reduced in the *srs2Δ* strain (from 0.09 to 0.08;  $p = 0.039$ ). In contrast to the marginal effect on COs, the mean NCO efficiency decreased from 0.91 in WT to only 0.23 in the *srs2Δ* mutant ( $p < 0.0001$ ). Thus, with either an Sgs1 or Srs2 deficiency, the reduction in overall gap-repair efficiency reflects a strong reduction in NCO formation with little, if any, compensatory gain in COs. Upon loss of Mph1, however, there was a re-distribution of products types without a change in overall gap-repair efficiency.

### Differentiating NCO mechanisms based on the position of hDNA

Alterations in CO or NCO production could reflect an effect on the NCO-specific SDSA pathway, a change in the efficiency of forming HJ-containing intermediates and/or a change in how HJ-containing intermediates are resolved. To differentiate between these possibilities, a *HIS3*-containing *CEN* plasmid, which generates only viable NCO products, was used in transformation experiments. Both the plasmid and chromosomal alleles involved in individual gap-repair events were sequenced (Table S1). Of 249 NCO products sequenced from the WT strain, regions of hDNA were detected on the plasmid allele in 159 (Table 2). In 18 of these, hDNA was present on both sides of the gap (bidirectional hDNA), consistent with the hDNA pattern predicted by formation of an HJ-containing intermediate; the remaining 141 had hDNA on only one side of the gap (unidirectional hDNA), consistent with production via the SDSA mechanism (Figure 3). It should be noted the distribution of hDNA observed here when using the NCO-only plasmid is very similar to that previously reported using an *ARS*-containing plasmid [35], and confirms that ~90% of NCOs are likely derived from SDSA. Although the corresponding chromosomal alleles were sequenced in each NCO event analyzed, none had the hDNA pattern predicted by HJ cleavage, confirming that HJ cleavage does not contribute significantly to NCO formation in this system (Table S1; [35]). To estimate the efficiencies with which HJ dissolution and SDSA occurred, the proportions of bidirectional and unidirectional hDNA tracts, respectively, among NCOs was multiplied by the mean efficiency of NCO production. In WT, the mean NCO efficiency of 0.91 was

thus broken down into a bidirectional and unidirectional hDNA values of 0.10 and 0.81, respectively (Table 2).

### Mph1 alters the distribution of uni- versus bidirectional hDNA among NCO products

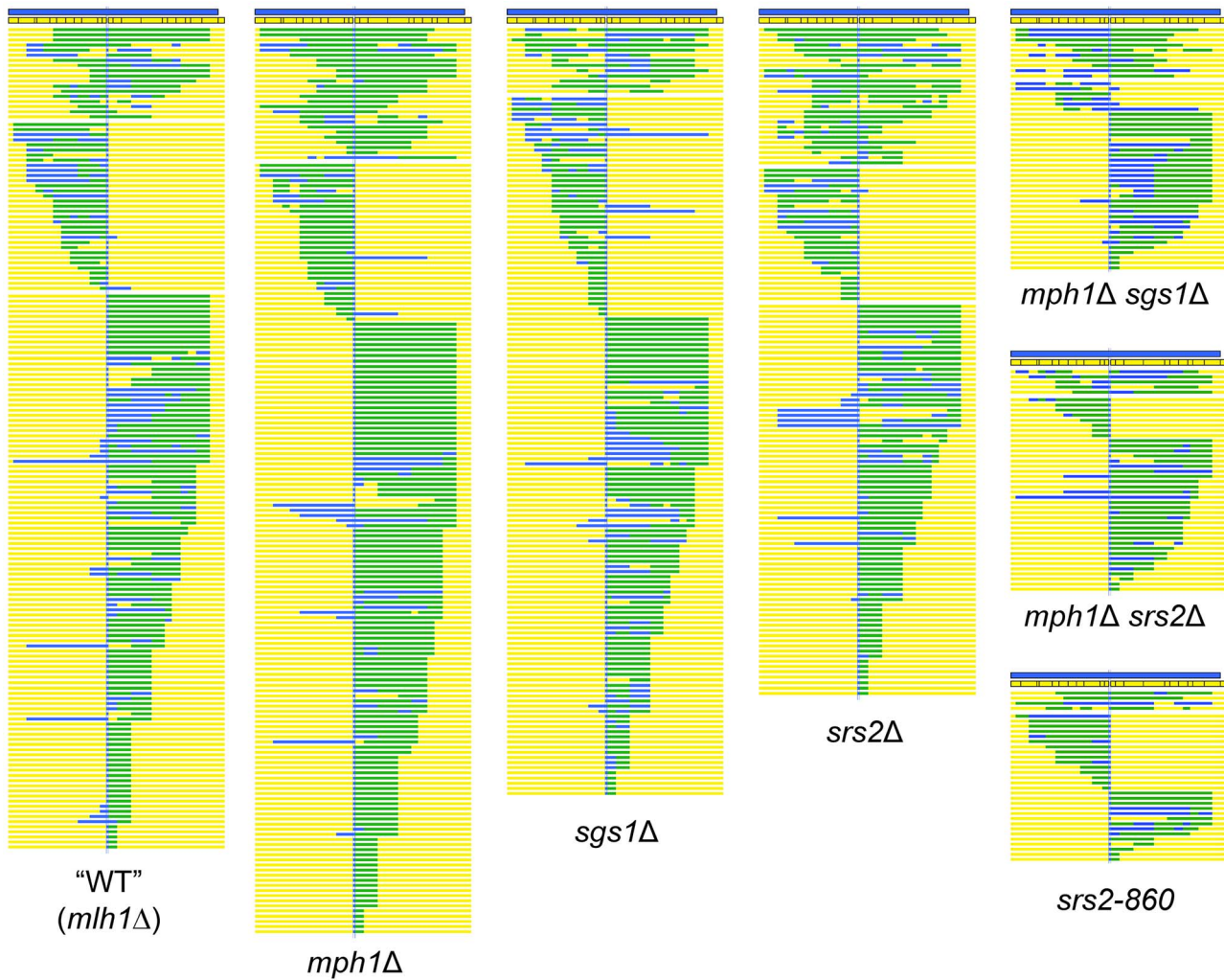
The sequences of 242 NCO products isolated from the *mph1Δ* strain were analyzed and 176 hDNA tracts were detected. Of these tracts, 15% were bidirectional, and 85% were unidirectional (Figure 3; Table 2). When these proportions were multiplied by the mean NCO efficiency in the *mph1Δ* background, there was a small but significant increase in NCOs with bidirectional hDNA (from 0.10 in WT to 0.12 in *mph1Δ*;  $p = 0.002$ ) and a corresponding reduction in NCOs with unidirectional hDNA (from 0.81 in WT to 0.71 in *mph1Δ*;  $p = 0.027$ ). Thus, even though there was no significant decrease in total NCOs in the absence of Mph1, there was a shift from unidirectional to bidirectional NCO products.

### Sgs1 promotes NCOs with either unidirectional or bidirectional hDNA

In the absence of Sgs1, the overall gap-repair efficiency dropped to approximately 70% of the WT level, and this reflected a selective loss of NCO events with no compensatory gain in COs. The products of 285 NCOs isolated from the *sgs1Δ* strain were sequenced, 149 of which had detectable hDNA on the plasmid. Thirteen hDNA tracts were bidirectional, and 136 were unidirectional (Figure 3; Table 2). Taking into account the reduction in overall gap-repair efficiency in the *sgs1Δ* strain, NCOs with bidirectional hDNA decreased from 0.10 in the presence of Sgs1 to 0.05 in its absence ( $p < 0.0001$ ). NCOs with unidirectional hDNA also dropped significantly in the *sgs1Δ* strain (from 0.81 in WT to 0.56;  $p = 0.009$ ). These data are consistent with the presumed role for Sgs1 in promoting NCO formation via dHJ dissolution and additionally indicate that Sgs1 promotes SDSA.

### Gap repair in the absence of both Sgs1 and Mph1

To examine the relationship between Sgs1 and Mph1 during gap repair, we constructed an *mph1Δ sgs1Δ* double-mutant strain. The mean gap-repair efficiency decreased significantly from ~1.00 in the WT and *mph1Δ* strains to 0.87 in the double mutant (Table 2;  $p = 0.039$  and  $p = 0.032$ , respectively), a decrease that was similar to that observed in the *sgs1Δ* single mutant ( $p = 0.21$ ). The mean efficiency of NCO production in the *mph1Δ sgs1Δ* strain also was similar to that obtained in the *sgs1Δ* strain ( $p = 0.52$ ) and significantly less than that in either the WT or *mph1Δ* background ( $p = 0.0001$  and  $p = 0.012$ , respectively). Though the overall



**Figure 3. Position of hDNA in NCO products of WT and helicase-deficient strains.** Each line represents the plasmid allele of a single NCO event; plasmid sequence is depicted in yellow, chromosomal sequence in blue and hDNA in green. The positions of the SNPs are indicated to scale within the chromosomal allele. Only those NCOs with hDNA detected on the plasmid allele are shown, as they were the samples used for statistical analysis. Samples are grouped by strain background (all strains were *mlh1Δ*) and arranged based on the position of hDNA. doi:10.1371/journal.pgen.1003340.g003

efficiencies of NCOs with unidirectional hDNA were similar in the *mph1Δ sgs1Δ* and *sgs1Δ* backgrounds ( $p = 0.6$ ), the efficiency of the minority, bidirectional events in the double mutant was greatly

elevated ( $p < 0.0001$ ), and only slightly different from that observed in the *mph1Δ* single mutant (0.14 and 0.12; respectively;  $p = 0.049$ ). Altogether, the data suggest (1) that Sgs1 is more important than

**Table 2. Efficiency of NCO events with unidirectional versus bidirectional hDNA in the repaired plasmid allele.**

| Relevant genotype  | Plasmid repair efficiency | CO efficiency  | NCO efficiency  | Position of hDNA in repaired plasmid allele |            |                |            |
|--------------------|---------------------------|----------------|-----------------|---|------------|----------------|------------|
|                    |                           |                |                 | Unidirectional                              |            | Bidirectional  |            |
|                    |                           |                |                 | Proportion (%)                              | Efficiency | Proportion (%) | Efficiency |
| WT                 | 1.00                      | 0.09           | 0.91            | 141/159 (89%)                               | 0.81       | 18/159 (11%)   | 0.10       |
| <i>mph1Δ</i>       | 1.04                      | 0.20*          | 0.84            | 150/176 (85%)                               | 0.71*      | 26/176 (15%)   | 0.12*      |
| <i>sgs1Δ</i>       | 0.72*                     | 0.11           | 0.62*           | 136/149 (91%)                               | 0.56*      | 13/149 (9%)    | 0.05*      |
| <i>mph1Δ sgs1Δ</i> | 0.87* (N = 12)            | 0.19* (41/186) | 0.67* (145/186) | 36/47 (77%)                                 | 0.52*      | 10/47 (21%)    | 0.14*      |

All strains are *mlh1Δ*. Mean plasmid-repair, CO and NCO efficiencies were determined as in Table 1. The position of hDNA was determined by sequencing individual NCO events; proportions of unidirectional and bidirectional hDNA were derived using only those transformants in which hDNA was detected. One NCO event with the pattern of hDNA consistent with HJ cleavage was detected in the *mph1Δ sgs1Δ* background. Asterisks indicate a significant difference when compared to WT ( $p < 0.05$ ). doi:10.1371/journal.pgen.1003340.t002

Mph1 for the production of NCO events with unidirectional hDNA and (2) that Sgs1 does not remove bidirectional hDNA-containing NCO intermediates that arise in the absence of Mph1. As will be elaborated further in the Discussion, we speculate that the elevated bidirectional hDNA in the *mph1Δ* background may reflect nicked HJ-containing intermediates, which are not expected to be substrates for the Sgs1-Top3-Rmi1 dissolvase.

### Srs2 promotes unidirectional and bidirectional hDNA classes of NCOs

In the absence of Srs2, the overall gap-repair efficiency decreased ~3 fold and, as in the *sgs1Δ* strain, this reflected a specific reduction in NCO events (Table 1). Among 254 NCOs sequenced from the *srs2Δ* strain, hDNA was detected in 129. Bidirectional hDNA was present in 27 of these and unidirectional hDNA in the remaining 102 (Figure 3 and Table 3). As expected, the decrease in the efficiency of NCOs with unidirectional hDNA (from 0.81 in WT to 0.19 in *srs2Δ*;  $p < 0.0001$ ) was similar to the overall reduction in NCO events. There also, however, was a significant decrease in NCOs with bidirectional hDNA (from 0.10 in WT to 0.05 in the *srs2Δ* mutant;  $p < 0.0001$ ). To further explore the unexpected role of Srs2 in promoting the formation of bidirectional hDNA-containing NCO products, we examined gap repair in *mph1Δ srs2Δ* double- and *srs2-860* single-mutant backgrounds.

### Gap repair in an *mph1Δ srs2Δ* double mutant

The mean gap-repair efficiency in the *mph1Δ srs2Δ* double mutant was 0.55, a value 2-fold less than that in the *mph1Δ* single (1.04;  $p < 0.0001$ ) but significantly greater than that obtained in the *srs2Δ* single mutant (0.31;  $p = 0.009$ ). This suggests that in the absence of Mph1, the need for the pro-recombination activity of Srs2 is relaxed. A similar, intermediate value for mean NCO efficiency was observed in the double mutant (0.39) relative to the *mph1Δ* (0.84;  $p < 0.0001$ ) or *srs2Δ* (0.23;  $p = 0.015$ ) single mutant. When NCOs obtained in double mutant were partitioned into those containing unidirectional or bidirectional hDNA (43 of 85 NCOs sequenced contained hDNA), the unidirectional class again had an intermediate value relative to the two single mutants. By contrast, the efficiency of producing the bidirectional hDNA class of NCOs in the double mutant was indistinguishable from that in the *srs2Δ* single mutant ( $p = 0.56$ ) and significantly less than that in the *mph1Δ* single mutant (0.5 and 0.12, respectively;  $p < 0.0001$ ).

Thus, in the absence of Mph1, Srs2 remains important for generating the bidirectional hDNA class of NCOs, while Sgs1 is dispensable (see above).

### Gap repair in an *srs2-860* mutant

The pro-recombination role of Srs2 in the gap-repair assay, which is specific for NCO events, could reflect its ability to unwind duplex DNA and/or its ability to remove Rad51 from nucleoprotein filaments; we will refer to these as its helicase and strippase activities, respectively. To examine the relevance of each activity to NCO production, we used the strippase-deficient *srs2-860* allele, which truncates the protein and eliminates the Rad51-interaction domain [36]. If only the helicase activity of Srs2 is important, then the efficiency of NCOs in the *srs2-860* strain is expected to be the same as in the WT background. If the strippase activity of Srs2 is relevant, however, then the efficiency of NCOs should be reduced in the *srs2-860* strain. The mean His<sup>+</sup>:Leu<sup>+</sup> ratio decreased 20% in the *srs2-860* strain relative to WT ( $p = 0.0051$ ), but was nevertheless much greater than in the *srs2Δ* strain (0.80 and 0.31, respectively;  $p = 0.001$ ). The *srs2-860* allele had a similar, intermediate effect on mean NCO production when compared to WT (0.39 and 0.91, respectively;  $p = 0.0006$ ) or *srs2Δ* (0.39 and 0.23, respectively;  $p < 0.0001$ ). This intermediate effect extended to both the unidirectional ( $p = 0.004$  and  $p < 0.0001$  when compared to WT and *srs2Δ*, respectively) and bidirectional hDNA classes of NCOs ( $p = 0.006$  and  $p < 0.0001$  when compared to WT and *srs2Δ*, respectively). By contrast, the mean level of COs was elevated to 0.12 in the *srs2-860* strain, which was significantly higher than COs in either WT (0.09;  $p = 0.022$ ) or *srs2Δ* (0.08;  $p < 0.0001$ ). This suggests that the strippase activity of Srs2 may promote NCOs at the expense of COs during gap repair.

## Discussion

Most DSB repair studies have measured relative levels of CO and NCO products through physical analysis of large populations of repaired molecules [12,13]. A distinguishing feature of the current study is the sequencing of both products of individual NCO events produced in a gap-repair assay, which allows inferences to be made about underlying molecular mechanisms and provides a unique tool for assessing specific structures acted on by candidate helicases. Specifically, the position of hDNA relative to the initiating gap indicates whether a given NCO event was

**Table 3.** Efficiency of NCO events with unidirectional versus bidirectional hDNA in the repaired plasmid allele.

| Genotype           | Plasmid repair efficiency (normalized His <sup>+</sup> :Leu <sup>+</sup> ) | CO efficiency  | NCO efficiency  | Type of NCO event   |            |                    |            |
|--------------------|--|----------------|-----------------|---------------------|------------|--------------------|------------|
|                    |  |                |                 | Unidirectional hDNA |            | Bidirectional hDNA |            |
|                    |  |                |                 | Proportion (%)      | Efficiency | Proportion (%)     | Efficiency |
| WT                 | 1.00   | 0.09           | 0.91            | 141/159 (89%)       | 0.81       | 18/159 (11%)       | 0.10       |
| <i>mph1Δ</i>       | 1.04   | 0.20*          | 0.84            | 150/176 (85%)       | 0.71*      | 26/176 (14%)       | 0.12*      |
| <i>srs2Δ</i>       | 0.31*  | 0.08*          | 0.23*           | 102/129 (79%)       | 0.19*      | 27/129 (21%)       | 0.05*      |
| <i>mph1Δ srs2Δ</i> | 0.55* (N = 11)   | 0.16* (52/179) | 0.39* (127/179) | 38/43 (88%)         | 0.34*      | 5/43 (12%)         | 0.05*      |
| <i>srs2-860</i>    | 0.80* (N = 12)   | 0.12* (26/178) | 0.68* (152/178) | 29/33 (88%)         | 0.60*      | 4/33 (12%)         | 0.08*      |

All strains are *mlh1Δ*. Mean plasmid-repair, CO and NCO efficiencies were determined as in Table 1. The position of hDNA was determined by sequencing individual NCO events; proportions of unidirectional and bidirectional hDNA were derived using only those transformants in which hDNA was detected. Asterisks indicate a significant difference when compared to WT ( $p < 0.05$ ).

doi:10.1371/journal.pgen.1003340.t003

generated by SDSA (“unidirectional” hDNA on only one side of the repaired gap), HJ cleavage (hDNA on one side of the gap in the repaired plasmid and on the other side of the gap in the chromosomal donor allele) or HJ dissolution (“bidirectional” hDNA on both sides of the gap in the repaired plasmid; see Figure 1). The data obtained in these analyses are summarized in Figure 4; panel A presents changes in the efficiencies of CO and NCO formation in various mutant backgrounds relative to WT, while panel B breaks down NCO events into those with unidirectional versus bidirectional hDNA. The major conclusions are as follows. First, as we reported previously [35], NCO production via HJ cleavage is extremely rare in a plasmid-based gap-repair assay; the hDNA pattern in ~90% of events in WT was consistent with SDSA, and the remainder was consistent with HJ dissolution. Half of the bidirectional hDNA events were dependent on Sgs1 and the other half on Srs2, suggesting a difference in the “HJ” intermediate that is removed by each. Second, only Mph1 was dispensable for gap repair; in its absence, SDSA intermediates were diverted into an alternative pathway that yielded primarily CO events. By contrast, there was a decrease in overall gap-repair efficiency in either an *sgs1Δ* or *srs2Δ* background that primarily reflected loss of SDSA-derived NCOs. This suggests that there are distinct, pro-recombination roles for Sgs1 and Srs2 during SDSA. Third, loss of Sgs1 was associated with a reduction in bidirectional hDNA, providing novel molecular evidence for the presumptive role of this helicase in the dissolution of dHJs. Unexpectedly, there also was a decrease in efficiency of bidirectional hDNA products in an *srs2Δ* background. Finally, double-mutant analyses suggest that Mph1 acts prior to Srs2 during gap repair, as loss of Mph1 partially suppressed the gap-repair defect of an *srs2Δ* mutant. Below, we discuss the roles of Sgs1, Mph1 and Srs2 deduced here and incorporate these data into a model of how each helicase functions during NCO formation (Figure 5).

### Mph1 promotes SDSA at the expense of CO formation

Though an *mph1Δ*, *srs2Δ* or *sgs1Δ* mutant accumulates proportionally more COs during HO-induced DSB repair, an important distinction is that NCOs are diverted into COs only in an *mph1Δ* background [12]. The *mph1Δ* results reported here are consistent with the HO data, with the 2-fold increase in CO events being accompanied by a coordinate decrease in SDSA-type NCOs. While there are no data from higher eukaryotes that address CO-NCO distribution in the absence of Mph1, *Schizosaccharomyces pombe* mutants defective in the ortholog Fml1 similarly have normal efficiencies of gap repair accompanied by a strong proportional increase in COs [37]. These data place Mph1 at a key decision point during mitotic DSB repair, with its activity largely determining whether an intermediate has the potential to become a CO event. It indeed has been argued that Mph1 is the only one of the three helicases examined here that can unwind a mobile D-loop created by Rad51 [13,38]. Though this suggests that Mph1 is the primary activity that dismantles D-loops in WT cells, NCO products with unidirectional hDNA are nevertheless produced efficiently in its absence. In the *mph1Δ* background, D-loop collapse could involve Srs2 and/or Sgs1, or some other helicase (Figure 5).

### Sgs1 is important for producing NCOs with unidirectional or bidirectional hDNA

As we reported previously using a similar gap-repair assay [8], there was a reduction in total gap-repair efficiency upon loss of Sgs1. The 2-fold increase in the proportion of COs observed in an *sgs1Δ* background reflected a specific loss of NCOs, with no corresponding gain in COs. The proportional gain in COs among

recombination products is consistent with previous studies of yeast spontaneous and DSB-induced recombination [8,12,34,39], as well as with studies in mammalian cells [40] and *Drosophila* [41].

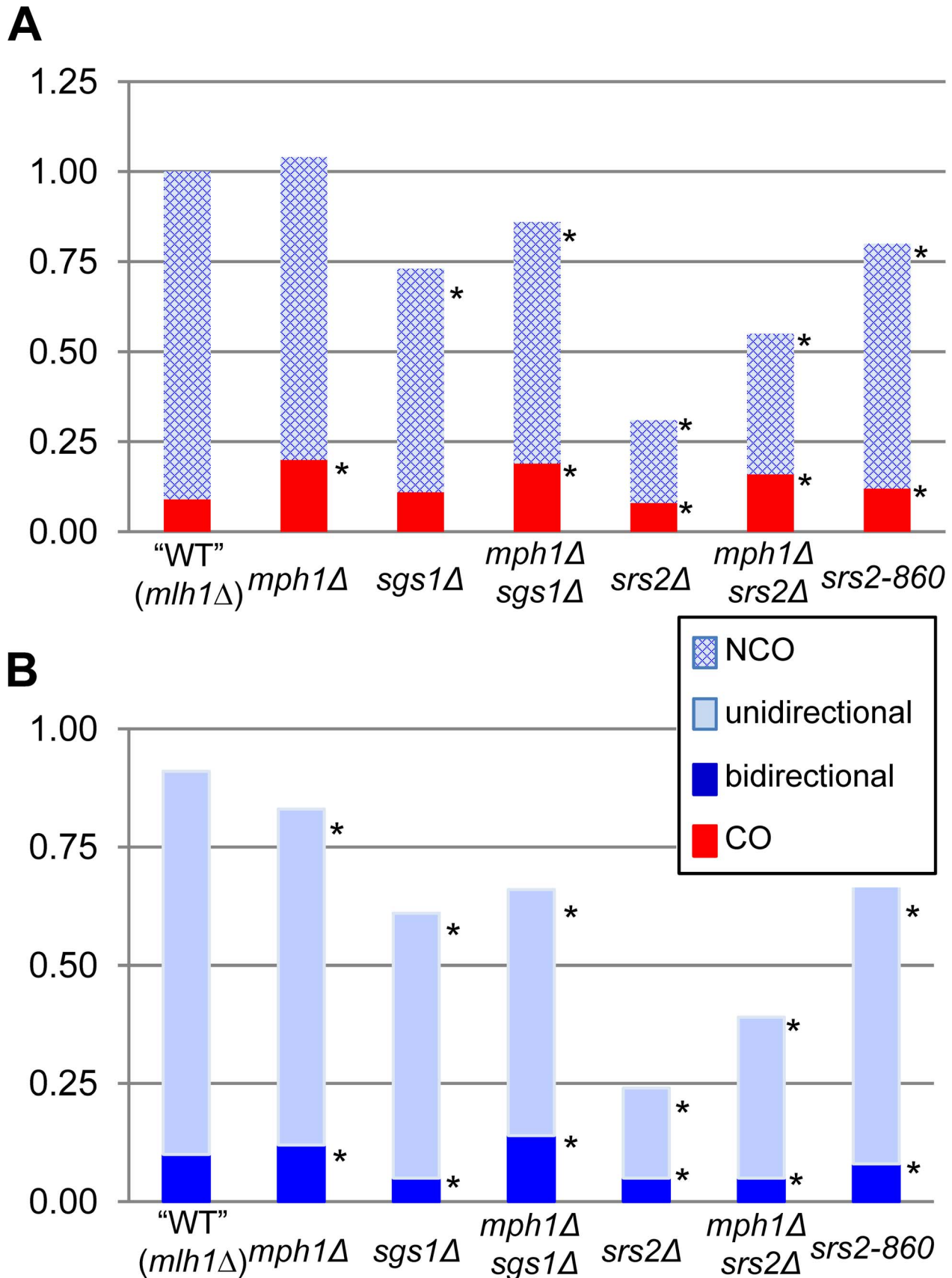
In a time-course analysis of HO-induced DSB repair in yeast, the appearance of early NCOs, which were assumed to reflect SDSA, was not affected by loss of Sgs1, leading to the suggestion that Sgs1 specifically promotes a later dHJ dissolution pathway [12]. There have been numerous *in vitro* and *in vivo* studies that support a role for Sgs1, together with Top3 and Rmi1, in dHJ dissolution. *In vitro*, for example, human and *Drosophila* BLM-TopIII $\alpha$  and yeast Sgs1-Top3 can dissolve dHJs [27,42,43]. *In vivo*, meiotic joint molecules formed in an *sgs1-AC795* mutant persist longer when cells are returned to mitotic growth, and their eventual resolution leads to proportionally more COs, consistent with dHJ dissolution by Sgs1 [44]. Furthermore, exposure of *sgs1* cells to DNA damage is associated with an accumulation of recombination-dependent X-shaped molecules [28,45]. These molecules disappear when DNA is treated with bacterial HJ resolvases, suggesting that they correspond to fully-ligated HJs that are normally dissolved by the Sgs1-Top3-Rmi1 (STR) complex [28]. We have confirmed here that loss of Sgs1 is associated with a decrease in the specific class of NCOs predicted as the product of STR-driven dHJ dissolution: NCOs with bidirectional hDNA on the repaired molecule (Figure 5).

A reduction in the bidirectional hDNA pattern predicted by dHJ dissolution was expected upon loss of Sgs1, but a similar reduction in the unidirectional hDNA diagnostic of SDSA was not (Figure 5). Studies in *Drosophila*, however, have suggested a role for BLM in dismantling D-loops formed following P element excision [46,47], and there is supporting biochemical evidence that human BLM binds and dismantles D-loops [48,49]. Recent data suggest that the role of STR in D-loop disruption might also involve migration of the back end of the D-loop and/or rewinding of the invaded duplex [49,50]. Either of these additional activities could explain why neither Mph1 nor Srs2 can fully substitute for Sgs1 during gap repair. We note that the reduction in unidirectional hDNA was no greater in the *mph1Δ sgs1Δ* double mutant than in the *sgs1Δ* single mutant, suggesting that Sgs1 and Mph1 work in the same pathway to promote SDSA. An interesting possibility is that, as the Mph1 helicase unwinds the invading strand, the catenating activity of STR is required to “rewind” the duplex that was part of the D-loop [50]. With regard to an SDSA-specific role for STR inferred here based on hDNA patterns, recent studies suggest that it is Sgs1 that promotes early SDSA-type NCOs in meiosis [51,52].

### Roles of Srs2 in promoting NCO pathways during DSB repair

A pro-recombination role for Srs2 has been demonstrated in physical studies of HO-induced mitotic recombination [12,20] and in a plasmid-based gap-repair assay similar to the one used here [8]. Furthermore, Srs2 loss has been associated with an increase in the proportion of COs produced during HO-induced DSB repair [12], during gap repair [8] and during spontaneous recombination [53]. Because only the early-appearing NCOs were lost following HO induction in an *srs2Δ* background, it was suggested that Srs2 promotes the NCO-specific SDSA [12]. In the current analyses, the efficiency of gap repair decreased 3-fold upon loss of Srs2, reflecting a specific loss of NCO events with no effect on CO events. Analyses of hDNA position in NCOs revealed a corresponding 4–5 fold reduction in SDSA-type products in the *srs2Δ* background, as well as a smaller, 2-fold decrease in bidirectional hDNA. These molecular data support that conclusion that SDSA is an early, Srs2-dependent pathway of DSB repair [12].



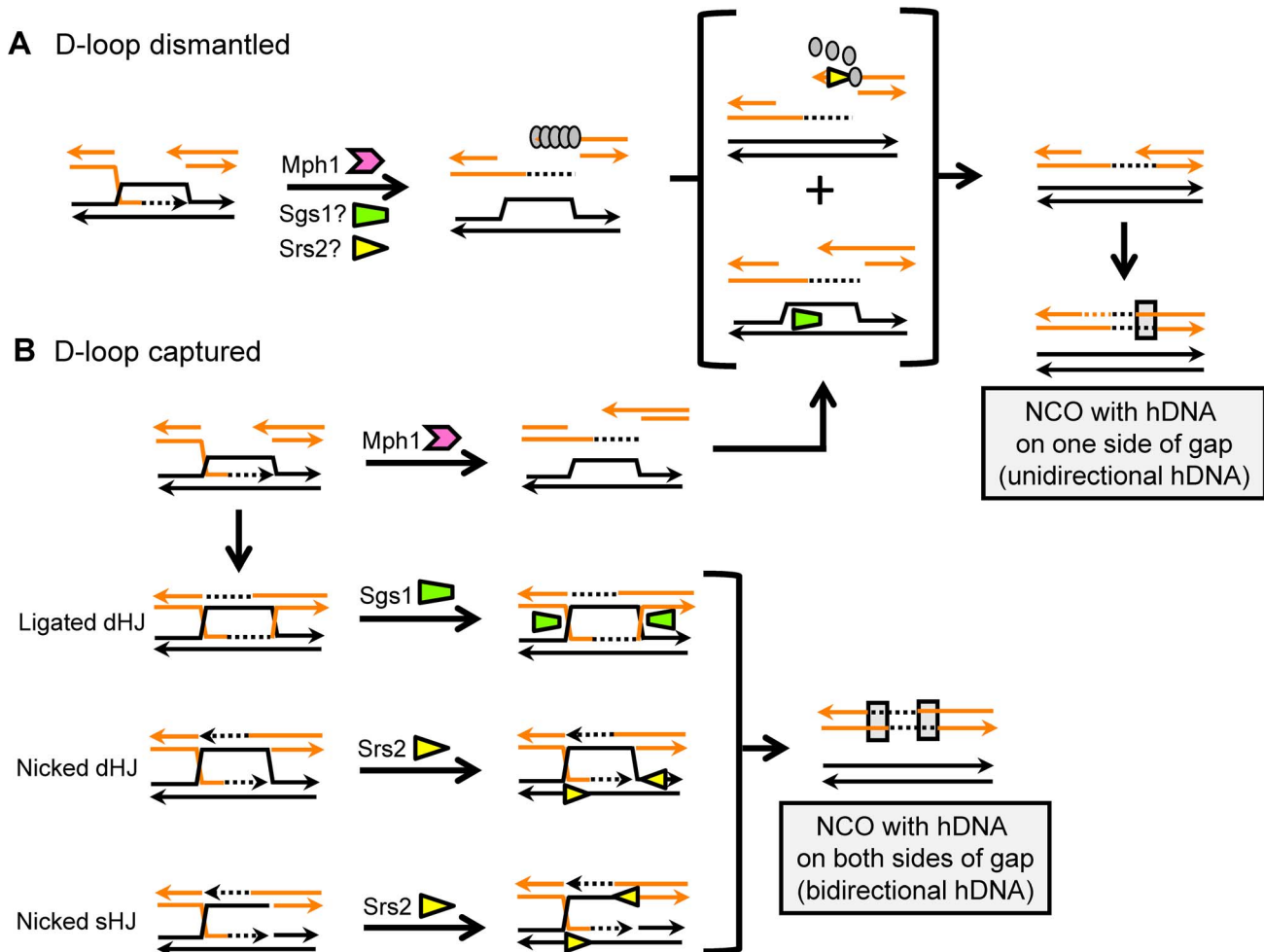


**Figure 4. Distribution of gap-repair products in WT and helicase-deficient strains.** Data presented in Table 1, Table 2, and Table 3 are summarized. A. The height of each bar indicates the normalized mean of each type of product. COs are in red and NCOs are in blue. Asterisks indicate  $p < 0.05$ . B. NCOs with bidirectional hDNA are in dark blue, and NCOs with unidirectional hDNA are in light blue. Asterisks indicate  $p < 0.05$ . doi:10.1371/journal.pgen.1003340.g004

As inferred in HO assays [12], the pro-SDSA role of Srs2 in our gap-repair assay was much stronger than that of Sgs1. The most straightforward way for a helicase to promote SDSA is through the dismantling of an extended D-loop (Figure 5), but whether this is the most relevant function of Srs2 *in vivo* has been the subject of debate [13,21]. The *srs2-860* allele used here encodes a protein that retains helicase activity but does not interact with Rad51, and hence is defective in the strippase activity that removes Rad51 from DNA [36]. A comparison of the overall gap-repair efficiencies in WT, *srs2Δ* and *srs2-860* strains suggests that the pro-recombination role of Srs2 likely reflects both activities. Importantly, the significant decrease in NCOs suggests that the disruption of Rad51 nucleoprotein filaments by Srs2 [18,19] helps promote SDSA. This activity of Srs2 could be relevant for removing Rad51 from the 2<sup>nd</sup> end of the resected break to allow the requisite Rad52-dependent annealing reaction and/or it could be necessary for D-loop disruption when Rad51 remains bound to duplex DNA within the D-loop (Figure 5). Either role would be consistent with the observation that overexpression of Rad51 in an

*srs2Δ* background almost completely eliminates NCOs [12]. A late role for Srs2 in promoting 2<sup>nd</sup>-end annealing, however, seems more consistent with the observation that the pro-recombination for Srs2 is relaxed in the absence of Mph1 (Table 3). It should be noted that the 2<sup>nd</sup>-end engagement required to generate a dHJ could occur either through an annealing reaction or through a second, Rad51-catalyzed strand invasion event. The latter would be expected to be more efficient in an *srs2-860* background, which could explain the small increase in COs observed in this strain (Figure 4). Finally, the observation that neither Mph1 nor Sgs1 can substitute fully for Srs2 during DSB repair is consistent with a unique activity for this helicase.

The loss of the SDSA pattern of hDNA among NCO events in the *srs2Δ* background was expected based on prior studies, but the accompanying 2-fold reduction in the bidirectional hDNA pattern assumed to be diagnostic of dHJ dissolution was not. The unwinding of a 4-way structure that mimics an HJ by yeast Srs2 has been examined, and it was concluded that it is a no better substrate for yeast Srs2 than blunt-end duplex DNA [21]. A recent



**Figure 5. Models for helicase-mediated regulation of NCO formation.** Sgs1, Srs2 and Mph1 are represented by green, yellow and pink symbols, respectively. A. Whereas all three helicases may be able to displace the invading end from the D-loop, Sgs1 and Srs2 are postulated to have additional activities during SDSA. Sgs1 may be required to rewind the invaded duplex, while the strippase activity of Srs2 may be important for 2<sup>nd</sup>-end annealing. Gray ovals represent Rad51, and its removal by Srs2 is indicated. B. If not dismantled by Mph1, the D-loop can be captured by the other end of the DSB and form an HJ-containing intermediate. A completely ligated dHJ is likely a substrate for Sgs1 complex, while a nicked sHJ or nicked dHJ may be a substrate for Srs2. The pattern of hDNA predicted by HJ cleavage is extremely rare in this system and does not contribute significantly to NCO events.

doi:10.1371/journal.pgen.1003340.g005

analysis of the putative homolog of Srs2 from *Arabidopsis thaliana*, however, reported that the helicase has significant activity against a nicked HJ [54]. We thus speculate that, in addition to a D-loop, a nicked sHJ or nicked dHJ is a cognate substrate for Srs2 *in vivo* (Figure 5). A nicked dHJ can be formed by a mechanism analogous to that assumed to occur when SDSA-mediated repair of a gapped plasmid requires that each end invade a template on a different chromosome [55,56]. In the case of the assay used here, independent invasion of the *same* repair template by each end, followed by extension and unwinding - basically two independent SDSA reactions - would produce a repaired plasmid with bidirectional hDNA. A similar mechanism was previously invoked in a study examining the effect of homology length on the CO-NCO outcome during repair of HO-induced DSBs [57]. It should be noted that the contributions of Srs2 and Sgs1 to bidirectional hDNA among NCO products appear to be independent (each is required for ~50% of these events in WT; Figure 4), which would be consistent with these helicases working on different structures: Srs2 on nicked single or double HJs, and Sgs1 only on fully ligated dHJs.

### Concluding remarks

Given the numerous roles that have been identified for the Mph1, Sgs1 and Srs2 helicases in basic DNA transactions, determining their specific activities once HR has initiated has been problematic. Although a gapped plasmid was used here to model DSB repair, it should be noted that both the efficiencies of repair and the distributions of repair products are completely consistent with those reported in HO-initiated chromosomal assays. It remains possible, however, that the roles of Mph1, Sgs1 and Srs2 inferred here may be specific to situations where the total homology between substrates is limited, a situation recently described for the Rad1-Rad10 endonuclease [58]. Through monitoring of hDNA among NCO products, the results presented here provide molecular confirmation that both Srs2 and Mph1 promote SDSA and that Sgs1 likely participates in the dissolution of dHJs. Importantly, additional roles for Sgs1 in promoting SDSA and for Srs2 in dismantling HJs have been inferred, broadening the potential range of activities of these helicases *in vivo*.

## Materials and Methods

### Media and growth conditions

Cells were grown nonselectively in YEPD (1% Bacto-yeast extract, 2% Bacto peptone, 2% dextrose) supplemented with 500 µg/mL adenine hemisulfate. Selective growth was on synthetic complete (SC) medium lacking the appropriate nutrient. Ura<sup>-</sup> segregants were identified on SC plates containing 0.1% 5-fluoroorotic acid (5-FOA). All growth was at 30°C.

### Gap-repair experiments

A complete strain list is provided in Table S2. Helicase-defective derivatives of the haploid *mlh1Δ* strain SJR2157, which contains the diverged gap-repair template [8], were constructed by targeted gene deletion. The substrate for gap repair was generated by *Bss*HII linearization of either the *ARS*-containing plasmid pSR987 [35] or the *CEN/ARS*-containing plasmid pSR1015. pSR1015 was constructed by inserting an *Xho*I/*Xba*I *HIS3* fragment from pSR987 into *Xho*I/*Xba*I-digested pRS316 [59].

The OD<sub>600</sub> of the exponentially growing cultures, each of which was derived from an independent colony, was measured to determine cell density. Six cultures with OD<sub>600</sub> values between 0.7 and 1.0 were selected for parallel transformation using the

protocol described previously [8]. Each experiment was repeated with at least six more cultures derived from independent colonies. His<sup>+</sup> and Leu<sup>+</sup> colonies were counted 5 days after selective plating. To avoid bias when partitioning recombinants into CO and NCO events, plates were divided into sections and every His<sup>+</sup> transformant within a given section was picked. His<sup>+</sup> transformants were frozen in 20% glycerol without prior purification, and an aliquot was grown nonselectively in YEPD prior to spotting an appropriate dilution on 5-FOA. Spots with full growth on 5-FOA after 3 days were scored as NCO events; those with no growth or only a few papillae were scored as CO events.

### DNA sequence analysis of recombinants

Transformations with pSR1015 and selection of His<sup>+</sup> transformants were performed as described above. An aliquot of the frozen stock of each His<sup>+</sup> colony was transferred to SC-his liquid medium and grown to saturation in 96-well microtiter plates. Following DNA extraction ([http://jinks-robertsonlab.duhs.duke.edu/protocols/yeast\\_prep.html](http://jinks-robertsonlab.duhs.duke.edu/protocols/yeast_prep.html)), the plasmid and chromosomal alleles were separately amplified with the appropriate primers (Table S3), and products were sequenced by the Duke Comprehensive Cancer Center DNA Analysis Facility. Sequence chromatograms were examined visually to detect the double peaks indicative of hDNA at a given SNP. Samples with only gene conversion or with no detectable sequence transfer were not included in further analysis because it was not possible to infer a recombination intermediate.

### Statistical analysis

Two different mixes of linear plasmids were used during the course of the transformation experiments reported here. The absolute His<sup>+</sup>:Leu<sup>+</sup> ratios obtained when using these mixes differed for the WT strain (mean ratios of 1.57 and 1.05), and depending on which mix was used for a specific mutant background, the individual transformation values were normalized to the corresponding WT mean. For the *mph1Δ* and *srs2Δ* strains, His<sup>+</sup>:Leu<sup>+</sup> ratios were generated with both mixes; for other mutant backgrounds, only a single mix was used in transformation experiments. For data analysis, normalized values of all individual transformations were pooled. The mean His<sup>+</sup>:Leu<sup>+</sup> ratios for the various events in different strain backgrounds were compared using a two-tailed Student's t-test (<http://vassarstats.net/>), and  $p < 0.05$  is considered significant.

## Supporting Information

**Figure S1** Proportion of COs produced during gap repair in WT and helicase-deficient strains. The percentage of COs out of the total number of transformants is plotted by strain. The number of CO and NCO products were compared between WT and the helicase mutant strains, and a Fisher exact 2×2 probability test was used to calculate p-values.

(TIF)

**Table S1** Sequence changes detected in the chromosomal allele of NCO gap-repair products.

(DOC)

**Table S2** *Saccharomyces cerevisiae* strains employed in this study.

(DOC)

**Table S3** Primer sequences for PCR amplification and sequencing of gap-repair products.

(DOC)

## Acknowledgments

We thank Hannah Klein for providing a construct to introduce the *srs2-860* allele and members of the SJ-R lab for helpful discussions throughout the course of this work. We especially appreciate discussions with Xiaoge Guo and comments provided by Nayun Kim during the preparation of the manuscript.

## References

- Paques F, Haber JE (1999) Multiple pathways of recombination induced by double-strand breaks in *Saccharomyces cerevisiae*. *Microbiology and molecular biology reviews* : MMBR 63: 349–404.
- San Filippo J, Sung P, Klein H (2008) Mechanism of eukaryotic homologous recombination. *Annual review of biochemistry* 77: 229–257.
- Symington LS (2002) Role of *RAD52* epistasis group genes in homologous recombination and double-strand break repair. *Microbiology and molecular biology reviews* : MMBR 66: 630–670, table of contents.
- Symington LS, Gautier J (2011) Double-strand break end resection and repair pathway choice. *Annual review of genetics* 45: 247–271.
- Ferguson DO, Holloman WK (1996) Recombinational repair of gaps in DNA is asymmetric in *Ustilago maydis* and can be explained by a migrating D-loop model. *Proceedings of the National Academy of Sciences of the United States of America* 93: 5419–5424.
- Szostak JW, Orr-Weaver TL, Rothstein RJ, Stahl FW (1983) The double-strand-break repair model for recombination. *Cell* 33: 25–35.
- Symington LS, Kang LE, Moreau S (2000) Alteration of gene conversion tract length and associated crossing over during plasmid gap repair in nuclease-deficient strains of *Saccharomyces cerevisiae*. *Nucleic Acids Research* 28: 4649–4656.
- Welz-Voegele C, Jinks-Robertson S (2008) Sequence divergence impedes crossover more than noncrossover events during mitotic gap repair in yeast. *Genetics* 179: 1251–1262.
- Mankouri HW, Hickson ID (2007) The RecQ helicase-topoisomerase III-Rmi1 complex: a DNA structure-specific ‘dissolvosome’? *Trends in biochemical sciences* 32: 538–546.
- Nassif N, Penney J, Pal S, Engels WR, Gloor GB (1994) Efficient copying of nonhomologous sequences from ectopic sites via P-element-induced gap repair. *Molecular and cellular biology* 14: 1613–1625.
- Heyer WD, Ehmsen KT, Liu J (2010) Regulation of homologous recombination in eukaryotes. *Annual review of genetics* 44: 113–139.
- Ira G, Malkova A, Liberi G, Foiani M, Haber JE (2003) Srs2 and Sgs1-Top3 suppress crossovers during double-strand break repair in yeast. *Cell* 115: 401–411.
- Prakash R, Satory D, Dray E, Papusha A, Scheller J, et al. (2009) Yeast Mph1 helicase dissociates Rad51-made D-loops: implications for crossover control in mitotic recombination. *Genes Dev* 23: 67–79.
- Lawrence CW, Christensen RB (1979) Metabolic suppressors of trimethoprim and ultraviolet light sensitivities of *Saccharomyces cerevisiae rad6* mutants. *Journal of bacteriology* 139: 866–876.
- Schiestl RH, Prakash S, Prakash L (1990) The *SRS2* suppressor of *rad6* mutations of *Saccharomyces cerevisiae* acts by channeling DNA lesions into the *RAD52* DNA repair pathway. *Genetics* 124: 817–831.
- Rong L, Klein HL (1993) Purification and characterization of the *SRS2* DNA helicase of the yeast *Saccharomyces cerevisiae*. *The Journal of biological chemistry* 268: 1252–1259.
- Aguilera A, Klein HL (1988) Genetic control of intrachromosomal recombination in *Saccharomyces cerevisiae*. I. Isolation and genetic characterization of hyper-recombination mutations. *Genetics* 119: 779–790.
- Krejci L, Van Komen S, Li Y, Villemain J, Reddy MS, et al. (2003) DNA helicase Srs2 disrupts the Rad51 presynaptic filament. *Nature* 423: 305–309.
- Veaute X, Jeusset J, Soustelle C, Kowalczykowski SC, Le Cam E, et al. (2003) The Srs2 helicase prevents recombination by disrupting Rad51 nucleoprotein filaments. *Nature* 423: 309–312.
- Aylon Y, Liefshitz B, Bitan-Banin G, Kupiec M (2003) Molecular dissection of mitotic recombination in the yeast *Saccharomyces cerevisiae*. *Molecular and cellular biology* 23: 1403–1417.
- Dupaigne P, Le Breton C, Fabre F, Gangloff S, Le Cam E, et al. (2008) The Srs2 helicase activity is stimulated by Rad51 filaments on dsDNA: implications for crossover incidence during mitotic recombination. *Mol Cell* 29: 243–254.
- Wu Y, Kantake N, Sugiyama T, Kowalczykowski SC (2008) Rad51 protein controls Rad52-mediated DNA annealing. *The Journal of biological chemistry* 283: 14883–14892.
- Gangloff S, McDonald JP, Bendixen C, Arthur L, Rothstein R (1994) The yeast type I topoisomerase Top3 interacts with Sgs1, a DNA helicase homolog: a potential eukaryotic reverse gyrase. *Molecular and cellular biology* 14: 8391–8398.
- Cejka P, Kowalczykowski SC (2010) The full-length *Saccharomyces cerevisiae* Sgs1 protein is a vigorous DNA helicase that preferentially unwinds Holliday junctions. *The Journal of biological chemistry* 285: 8290–8301.
- Bernstein KA, Gangloff S, Rothstein R (2010) The RecQ DNA helicases in DNA repair. *Annual review of genetics* 44: 393–417.
- Mimitou EP, Symington LS (2009) DNA end resection: many nucleases make light work. *DNA Repair (Amst)* 8: 983–995.

## Author Contributions

Conceived and designed the experiments: KM SJ-R. Performed the experiments: KM KL. Analyzed the data: KM SJ-R KL. Wrote the paper: KM SJ-R.

- Cejka P, Plank JL, Bachrati CZ, Hickson ID, Kowalczykowski SC (2010) Rmi1 stimulates decatenation of double Holliday junctions during dissolution by Sgs1-Top3. *Nature structural & molecular biology* 17: 1377–1382.
- Mankouri HW, Ashton TM, Hickson ID (2011) Holliday junction-containing DNA structures persist in cells lacking Sgs1 or Top3 following exposure to DNA damage. *Proceedings of the National Academy of Sciences of the United States of America* 108: 4944–4949.
- Entian KD, Schuster T, Hegemann JH, Becher D, Feldmann H, et al. (1999) Functional analysis of 150 deletion mutants in *Saccharomyces cerevisiae* by a systematic approach. *Molecular & general genetics* : MGG 262: 683–702.
- Meetei AR, Medhurst AL, Ling C, Xue Y, Singh TR, et al. (2005) A human ortholog of archaeal DNA repair protein Hef is defective in Fanconi anemia complementation group M. *Nature genetics* 37: 958–963.
- Mosedale G, Niedzwiedz W, Alpi A, Perrina F, Pereira-Leal JB, et al. (2005) The vertebrate Hef ortholog is a component of the Fanconi anemia tumor-suppressor pathway. *Nature structural & molecular biology* 12: 763–771.
- Schurer KA, Rudolph C, Ulrich HD, Kramer W (2004) Yeast *MPH1* gene functions in an error-free DNA damage bypass pathway that requires genes from homologous recombination, but not from postreplicative repair. *Genetics* 166: 1673–1686.
- Zheng XF, Prakash R, Saro D, Longrich S, Niu H, et al. (2011) Processing of DNA structures via DNA unwinding and branch migration by the *S. cerevisiae* Mph1 protein. *DNA Repair* 10: 1034–1043.
- Tay YD, Sidebotham JM, Wu L (2010) Mph1 requires mismatch repair-independent and -dependent functions of MutSalpha to regulate crossover formation during homologous recombination repair. *Nucleic Acids Research* 38: 1889–1901.
- Mitchel K, Zhang H, Welz-Voegele C, Jinks-Robertson S (2010) Molecular structures of crossover and noncrossover intermediates during gap repair in yeast: implications for recombination. *Mol Cell* 38: 211–222.
- Colavito S, Macris-Kiss M, Seong C, Gleeson O, Greene EC, et al. (2009) Functional significance of the Rad51-Srs2 complex in Rad51 presynaptic filament disruption. *Nucleic acids research* 37: 6754–6764.
- Sun W, Nandi S, Osman F, Ahn JS, Jakovleska J, et al. (2008) The FANCM ortholog Fml1 promotes recombination at stalled replication forks and limits crossing over during DNA double-strand break repair. *Molecular cell* 32: 118–128.
- Sebesta M, Burkovics P, Haracska L, Krejci L (2011) Reconstitution of DNA repair synthesis *in vitro* and the role of polymerase and helicase activities. *DNA Repair* 10: 567–576.
- Gangloff S, Soustelle C, Fabre F (2000) Homologous recombination is responsible for cell death in the absence of the Sgs1 and Srs2 helicases. *Nature genetics* 25: 192–194.
- LaRocque JR, Stark JM, Oh J, Bojilova E, Yusa K, et al. (2011) Interhomolog recombination and loss of heterozygosity in wild-type and Bloom syndrome helicase (BLM)-deficient mammalian cells. *Proceedings of the National Academy of Sciences of the United States of America* 108: 11971–11976.
- McVey M, Larocque JR, Adams MD, Sekelsky JJ (2004) Formation of deletions during double-strand break repair in *Drosophila* DmBlm mutants occurs after strand invasion. *Proceedings of the National Academy of Sciences of the United States of America* 101: 15694–15699.
- Wu L, Hickson ID (2003) The Bloom’s syndrome helicase suppresses crossing over during homologous recombination. *Nature* 426: 870–874.
- Plank JL, Wu J, Hsieh TS (2006) Topoisomerase IIIalpha and Bloom’s helicase can resolve a mobile double Holliday junction substrate through convergent branch migration. *Proceedings of the National Academy of Sciences of the United States of America* 103: 11118–11123.
- Dayani Y, Simchen G, Lichten M (2011) Meiotic recombination intermediates are resolved with minimal crossover formation during return-to-growth, an analogue of the mitotic cell cycle. *PLoS Genet* 7: e1002083. doi:10.1371/journal.pgen.1002083
- Liberi G, Maffioletti G, Lucca C, Chiolo I, Baryshnikova A, et al. (2005) Rad51-dependent DNA structures accumulate at damaged replication forks in *sgs1* mutants defective in the yeast ortholog of BLM RecQ helicase. *Genes & development* 19: 339–350.
- Adams MD, McVey M, Sekelsky JJ (2003) *Drosophila* BLM in double-strand break repair by synthesis-dependent strand annealing. *Science* 299: 265–267.
- McVey M, Andersen SL, Broze Y, Sekelsky J (2007) Multiple functions of *Drosophila* BLM helicase in maintenance of genome stability. *Genetics* 176: 1979–1992.
- van Brabant AJ, Ye T, Sanz M, German IJ, Ellis NA, et al. (2000) Binding and melting of D-loops by the Bloom syndrome helicase. *Biochemistry* 39: 14617–14625.

49. Bachrati CZ, Borts RH, Hickson ID (2006) Mobile D-loops are a preferred substrate for the Bloom's syndrome helicase. *Nucleic acids research* 34: 2269–2279.
50. Chen CF, Brill SJ (2010) An essential DNA strand-exchange activity is conserved in the divergent N-termini of BLM orthologs. *The EMBO journal* 29: 1713–1725.
51. De Muyt A, Jessop L, Kolar E, Sourirajan A, Chen J, et al. (2012) BLM helicase ortholog Sgs1 is a central regulator of meiotic recombination intermediate metabolism. *Molecular cell* 46: 43–53.
52. Zakharyevich K, Tang S, Ma Y, Hunter N (2012) Delineation of joint molecule resolution pathways in meiosis identifies a crossover-specific resolvase. *Cell* 149: 334–347.
53. Robert T, Dervins D, Fabre F, Gangloff S (2006) Mrc1 and Srs2 are major actors in the regulation of spontaneous crossover. *The EMBO journal* 25: 2837–2846.
54. Blanck S, Kobbe D, Hartung F, Fengler K, Focke M, et al. (2009) A SRS2 homolog from *Arabidopsis thaliana* disrupts recombinogenic DNA intermediates and facilitates single strand annealing. *Nucleic acids research* 37: 7163–7176.
55. Paques F, Leung WY, Haber JE (1998) Expansions and contractions in a tandem repeat induced by double-strand break repair. *Molecular and cellular biology* 18: 2045–2054.
56. Miura T, Yamana Y, Usui T, Ogawa HI, Yamamoto MT, et al. (2012) Homologous recombination via synthesis-dependent strand annealing in yeast requires the Irc20 and Srs2 DNA helicases. *Genetics* 191: 65–78.
57. Inbar O, Liefshitz B, Bitan G, Kupiec M (2000) The relationship between homology length and crossing over during the repair of a broken chromosome. *The Journal of biological chemistry* 275: 30833–30838.
58. Mazon G, Lam AF, Ho CK, Kupiec M, Symington LS (2012) The Rad1-Rad10 nuclease promotes chromosome translocations between dispersed repeats. *Nature structural & molecular biology* 19: 964–971.
59. Sikorski RS, Hieter P (1989) A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. *Genetics* 122: 19–27.