Sgs1 and Mph1 Helicases Enforce the Recombination Execution Checkpoint During DNA Double-Strand Break Repair in *Saccharomyces cerevisiae*

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ABSTRACT We have previously shown that a recombination execution checkpoint (REC) regulates the choice of the homologous recombination pathway used to repair a given DNA double-strand break (DSB) based on the homology status of the DSB ends. If the two DSB ends are synapsed with closely-positioned and correctly-oriented homologous donors, repair proceeds rapidly by the gene conversion (GC) pathway. If, however, homology to only one of the ends is present, or if homologies to the two ends are situated far away from each other or in the wrong orientation, REC blocks the rapid initiation of new DNA synthesis from the synapsed end(s) and repair is carried out by the break-induced replication (BIR) machinery after a long pause. Here we report that the simultaneous deletion of two 3' → 5' helicases, *Sgs1* and *Mph1*, largely abolishes the REC-mediated lag normally observed during the repair of large gaps and BIR substrates, which now get repaired nearly as rapidly and efficiently as GC substrates. Deletion of *SGS1* and *MPH1* also produces a nearly additive increase in the efficiency of both BIR and long gap repair; this increase is epistatic to that seen upon *Rad51* overexpression. However, *Rad51* overexpression fails to mimic the acceleration in repair kinetics that is produced by *sgs1Δ mph1Δ* double deletion.

KEYWORDS recombination execution checkpoint (REC); *Sgs1*; *Mph1*; gene conversion (GC); break-induced replication (BIR)

DNA double-strand breaks (DSBs) are potentially lethal lesions that can be repaired either by nonhomologous end joining (NHEJ) or by homologous recombination (HR) (Krogh and Symington 2004; Haber 2008; Haber 2013). While NHEJ involves simple religation of the DSB ends with little or no homology, HR requires the presence of intact homologous sequences to serve as a template for repair. During HR-mediated repair, the DSB ends are resected to produce 3'-ended single-stranded DNA tails, which get coated with the *Rad51* recombinase protein to form *Rad51* nucleoprotein

filaments. These *Rad51* filaments then search for and strand invade homologous sequences to form a three-strand displacement loop (D-loop), which is followed by extension of the invading strand by new DNA synthesis using the paired homologous sequence as a template. When both DSB ends share homology with a sister chromatid, a homologous chromosome or an ectopically placed donor; repair occurs by gene conversion (GC), resulting in the synthesis of a short patch of new DNA at the recipient locus using the homologous donor as a template. New DNA synthesis is initiated within 30 min after strand invasion (White and Haber 1990; Sugawara *et al.* 2003; Hicks *et al.* 2011) and does not require components of the lagging strand DNA-synthesis machinery such as *Polα* and primase (Wang *et al.* 2004). In mitotic cells of budding yeast, GC proceeds primarily by the synthesis-dependent strand annealing (SDSA) mechanism, in which the newly-synthesized strands dissociate from the donor template and are returned to the recipient locus. GC is thus distinct from normal semiconservative

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replication in that all the newly-copied DNA is found in the recipient (Ira *et al.* 2006).

However, if there is homology to only one DSB end, repair occurs by another HR pathway called break-induced replication (BIR) (McEachern and Haber 2006; Llorente *et al.* 2008; Malkova and Ira 2013). Here, strand invasion by the homologous end results in the establishment of a migrating D-loop that can copy all sequences distal to the site of homology, resulting in a nonreciprocal translocation (Donnianni and Symington 2013; Saini *et al.* 2013; Wilson *et al.* 2013). Sequences on the other side of the break, lacking homology, are lost by degradation.

Compared to GC, BIR is less efficient; it is kinetically slower and new DNA synthesis does not initiate until ~3–4 hr after strand invasion (Malkova *et al.* 2005; Lydeard *et al.* 2007; Jain *et al.* 2009). BIR requires leading and lagging strand DNA synthesis and essentially all of the DNA replication factors including Pol α /primase, Cdc7, Cdt1, and the Cdc45-MCM-GINS helicase complex (Lydeard *et al.* 2007; Lydeard *et al.* 2010b); only the components specifically needed for origin-dependent DNA replication (Cdc6 and the ORC proteins) are dispensable for BIR. Additionally, while Pol δ and Pol ϵ are redundantly required for GC (Holmes and Haber 1999; Wang *et al.* 2004), Pol δ alone is required for the initiation of BIR whereas Pol ϵ becomes important for the completion of repair (Lydeard *et al.* 2007).

In *Saccharomyces cerevisiae*, when both ends of a DSB share homology to the donor, GC nearly always outcompetes BIR (Malkova *et al.* 2005). Although GC is the more commonly-used mode of DSB repair, BIR is required for the maintenance of telomeres in telomerase-deficient cells (Lundblad and Blackburn 1993; Le *et al.* 1999; Teng *et al.* 2000; McEachern and Haber 2006; Lydeard *et al.* 2007). A single homologous end can also arise when a DSB occurs in the vicinity of a dispersed, repeated sequence such as a transposable element that is present at other chromosome locations (Malkova *et al.* 2001; VanHulle *et al.* 2007).

Correct channeling of the DSBs is crucial to ensure that repair occurs with high fidelity. Independent initiation of BIR events from DSB ends that could repair by GC will produce nonreciprocal translocations if the ends are repaired by using two nonallelic, repeated donor sequences; or cause aneuploidy if one end is repaired using a sister chromatid while the other end used a homologous chromosome for repair. We previously showed evidence for the existence of a recombination execution checkpoint (REC) that acts prior to the actual initiation of the repair synthesis, apparently by sensing the presence and topology of the engaged ends (Jain *et al.* 2009). When DSB ends are engaged with closely-positioned and correctly-oriented homologous donors, a break is rapidly repaired by GC. This topological requirement to allow rapid repair is fulfilled even if the two ends are derived from two separate DSBs (Jain *et al.* 2016). However, if homology to only one end is present, or if the two ends engage far from each other, or if the homologies are arranged close together but in the wrong orientation; REC blocks the rapid initiation

of repair by GC and repair occurs by BIR after a long pause (Jain *et al.* 2009). The delayed initiation of BIR may provide more time for the DSB ends to dissociate from their initial site of synapsis and continue searching for homology in the vicinity of each other, so that repair can proceed via the more conservative GC pathway. We have previously estimated that even GC repair involves, on average, four encounters between the broken end and the donor before the synapsis is irreversibly converted to a repair event (Coic *et al.* 2011).

One indication of how REC monitors the location and orientation of the DSB ends came from the observation that Sgs1, a 3' $\rightarrow$ 5' helicase of the evolutionarily-conserved RecQ family, modulates the REC-mediated switch from GC to BIR (Jain *et al.* 2009). Whereas a 1.2-kb gap is rapidly repaired by GC, a 5-kb gap is repaired more slowly, apparently by Pol32-dependent BIR. However, deletion of Sgs1 accelerates the repair kinetics of the 5-kb gap to resemble repair of the 1.2-kb gap. This observation led us to postulate that capture of both DSB ends within a continuous D-loop may be required to signal repair by GC, and that Sgs1 might limit the enlargement of the D-loops formed upon strand invasion by the DSB ends, thereby preventing the GC machinery from repairing a 5-kb gap. However, *sgs1* Δ did not alter the repair kinetics of much larger gaps, e.g., one of 18 kb, although it increased the efficiency of BIR (Jain *et al.* 2009; Lydeard *et al.* 2010a).

Here we report that Mph1, another well-conserved 3' $\rightarrow$ 5' helicase, also regulates the REC-mediated choice between GC and BIR. Moreover, double deletion of *SGS1* and *MPH1* virtually eliminates the REC, resulting in a mechanistic shift in the repair of long gaps and BIR substrates, and much faster initiation of new DNA synthesis.

Materials and Methods

Strains and plasmids

The construction of the break-repair strain, YSJ119; gap-repair strains, YSJ130 (1.2-kb gap), YSJ133 (5-kb gap), YSJ135 (18-kb gap); and the BIR strain, YSJ131; and their *sgs1* Δ derivatives (YSJ190, YSJ191, YSJ192, YSJ230, and YSJ193, respectively) is described in (Jain *et al.* 2009). The *mph1* Δ and *mph1* Δ *sgs1* Δ strains were made by the standard PCR-based gene disruption method to obtain strains YSJ204 (YSJ119 *mph1* Δ), YSJ205 (YSJ130 *mph1* Δ), YSJ206 (YSJ133 *mph1* Δ), YSJ207 (YSJ131 *mph1* Δ), YSJ211 (YSJ135 *mph1* Δ), YSJ244 (YSJ191 *mph1* Δ), YSJ245 (YSJ193 *mph1* Δ), YSJ281 (YSJ192 *mph1* Δ), YSJ417 (YSJ190 *mph1* Δ), and YSJ418 (YSJ230 *mph1* Δ). Wild type (WT) and mutant strains were transformed with the Rad51 overexpressing plasmid pSJ5 (Lydeard *et al.* 2010a) to obtain strains YSJ178 (YSJ119 pSJ5), YSJ179 (YSJ130 pSJ5), YSJ180 (YSJ131 pSJ5), YSJ181 (YSJ133 pSJ5), YSJ182 (YSJ135 pSJ5), YSJ279 (YSJ245 pSJ5), YSJ282 (YSJ207 pSJ5), and YSJ283 (YSJ193 pSJ5). WT and mutant strains were transformed with the Pol30-89 expressing plasmid pTR1 [which was obtained by

moving the *pol30-89* allele from pBL248-89 (Eissenberg *et al.* 1997) into the pRS314 vector (Sikorski and Hieter 1989)], or just the empty pRS314 vector alone to obtain strains YAM007 (YSJ131 pTR1), YAM008 (YSJ207 pTR1), YAM009 (YSJ193 pTR1), YAM010 (YSJ245 pTR1), YAM011 (YSJ135 pTR1), YAM012 (YSJ418 pTR1), YAM013 (YSJ119 pTR1), YAM014 (YSJ417 pTR1), YAM015 (YSJ131 pRS314), YAM016 (YSJ207 pRS314), YAM017 (YSJ193 pRS314), YAM018 (YSJ245 pRS314), YAM019 (YSJ135 pRS314), YAM020 (YSJ418 pRS314), YAM021 (YSJ119 pRS314), and YAM022 (YSJ417 pRS314). Strains and plasmids are available upon request.

Viability measurements

Yeast cells were grown in YEP containing 2% raffinose to a density of $\sim 1 \times 10^7$ cells/ml. Equal volumes of appropriate dilutions were plated on YEP containing 2% galactose (YEP-Gal; to induce the HO break) and YEP containing 2% dextrose (YEPD; no DSB control). Viability was determined from the ratio of CFUs able to survive the break (number of colonies that grew on YEPGal) to the total number of CFUs plated (number of colonies that appeared on YEPD). For the Pol30-89 experiments (Figure 5), Trp drop-out medium was used instead of YEP to ensure plasmid retention. Data represent mean $\pm$ SD ($n \geq 5$), and *P*-values were calculated using the two-tailed, unpaired Student's *t*-test.

HO induction for kinetic analysis of DSB repair

Yeast cells were grown in YEP containing 2% raffinose to a density of $\sim 1 \times 10^7$ cells/ml and HO endonuclease was induced by adding galactose to a final concentration of 2%. Samples were collected for DNA analysis just prior to and at different time points following addition of galactose, as described before (Holmes and Haber 1999).

PCR-based primer extension assay

Equal amounts of genomic DNA isolated from samples collected at different time points were amplified within linear range as described before (White and Haber 1990). A pair of primers 500-bp upstream of the *U2* donor and 800-bp downstream of *leu2::HOcs* was used to analyze the repair kinetics. The PCR reactions were run on agarose gels and the repair product was quantified using Bio-Rad (Hercules, CA) Quantity One software. For the break-repair strain, YSJ119, the primer pair used to study *U2* repair gives a band at 0 hr time points as well; however, that band is ~ 120 -bp bigger than the one obtained after repair [owing to the presence of the HO cleavage site (HOcs)], and was not taken into account in our analysis. PCR signal from an independent locus (*LDB16*) was used to normalize for input DNA. The ratio of test and reference signals obtained from the DNA of a repaired colony was set to 100%.

Colony PCR

A small quantity of cells taken from colonies growing on YEPGal (from the viability assay described above) were heated at 100° for 10 min and the lysates were amplified

with primers upstream of the *LE* donor to assay for the loss of the distal end of chromosome (Chr) 5.

Data availability

The authors state that all data necessary for confirming the conclusions presented in the article are represented fully within the article.

Results

Mph1 exerts an effect opposite to that of *Sgs1* during short gap repair

We had previously constructed a series of haploid yeast strains to study competition between GC and BIR. These strains harbor a HOcs within a *LEU2* gene (*leu2::HOcs*) inserted at the *can1* locus on Chr 5. Homologies to the *LE* and *U2* ends of the HO break (~ 700 -bp 5' and ~ 1 -kb 3' segments, respectively, of the *LEU2* gene) are present on the left arm of Chr 3. The strains differ from each other by the distance separating the *LE* and *U2* donors on Chr 3 (Jain *et al.* 2009; Figure 1A). A strain carrying the entire *LEU2* gene as donor serves as the break-repair (GC) control (YSJ119), and a strain carrying only the *U2* donor serves as the BIR control (YSJ131). In the gap-repair strains, repair can occur either by GC (resulting in copying of all the Chr 3 sequences between the *LE* and *U2* donors on to Chr 5) or by BIR (leading to duplication of Chr 3 sequences distal to the *U2* donor, and a concomitant loss of the nonessential distal left end of Chr 5). We showed previously that increasing the gap between the donors from 1.2 kb (YSJ130) to 5 kb (YSJ133) or 18 kb (YSJ135) caused the ends to lose sight of each other resulting in a shift from the quick and efficient GC to the slower and less efficient Pol32-dependent BIR pathway of repair (Figure 1; data from Jain *et al.* 2009).

As reported previously, deleting *Sgs1* increased the efficiency of BIR and accelerated the kinetics of 5-kb gap repair (Figure 2, A and D; data from Jain *et al.* 2009). We have now tested the *Mph1* helicase for a possible role in modulating gap repair. *Mph1* has been implicated in promoting HR-mediated, error-free bypass of DNA lesions (Scheller *et al.* 2000; Schürer *et al.* 2004; Panico *et al.* 2009). *Mph1*, as well as its *Schizosaccharomyces pombe* ortholog Fml1, have been shown to restrict crossover formation during GC by channeling repair into the SDSA pathway (Prakash *et al.* 2009; Lorenz *et al.* 2012). It has also been implicated in the generation of Rad51-dependent regressed fork intermediates (Chen *et al.* 2009; Mankouri *et al.* 2009). Its human ortholog FANCM has been shown to promote branch migration of Holliday junctions and replication forks *in vitro* (Gari *et al.* 2008). However, *Mph1* suppresses BIR (Luke-Glaser and Luke 2012; Stafa *et al.* 2014) and its overexpression increases gross chromosomal rearrangements, apparently by inhibiting HR (Banerjee *et al.* 2008). *Mph1* has also been shown to play a role in processing Okazaki fragments during replication (Kang *et al.* 2009). Consistent with previous studies examining the role of *Mph1* in ectopic GC (Prakash *et al.* 2009),

Figure 1 *Mph1* modulates the efficiency and kinetics of gap repair. (A) Schematic representation of the strains used to study competition between GC and BIR (Jain *et al.* 2009). *leu2::HOcs* is inserted at the *can1* locus on Chr 5. Homology to right end of the break, the *U2* end, is present upstream of the *SPS22* locus ~41 kb from the left end of Chr 3. Homology to left end of the break, the *LE* end, is present at indicated distances from the *U2* donor. The endogenous *MAT*, *HML*, *HMR*, and *LEU2* sequences are deleted. (B) Viability of the indicated WT (black) and *mph1Δ* (red) strains. Data represent mean $\pm$ SD ($n \geq 5$). * denotes that viabilities of WT and *mph1Δ* strains are significantly different ($P \leq 0.005$). (C and D) Kinetics of repair measured from the *U2* end in the indicated WT and *mph1Δ* strains as determined by a quantitative

PCR-based primer extension assay using primers shown schematically in (A). The amount of PCR product obtained from a repaired colony was set to 100%. Data represent mean of three PCR reactions from each of two or three independent time courses $\pm$ SD. The WT data has been published in Jain *et al.* (2009).

deleting *Mph1* did not affect the efficiency of break repair (when the donors are not separated by a gap) (Figure 1B). However, it significantly reduced the efficiency of 1.2-kb gap repair from ~56 to ~38% (Figure 1B). Intriguingly, *mph1Δ* did not affect the repair efficiency of a 5-kb gap but increased the efficiency of repairing an 18-kb gap, as well as a BIR substrate. Deletion of *MPH1* resulted in a small shift in the kinetics of break repair, and it significantly slowed down the initiation of new DNA synthesis during 1.2-kb gap repair, as determined by a PCR-based primer extension assay (Figure 1C). However, the kinetics of 5-kb and 18-kb gap repair as well as BIR remained largely unaltered (Figure 1D). Although we observed an apparent small shift in the kinetics of 18-kb gap repair and BIR in the absence of *Mph1*, this difference disappeared when the primer extension data were normalized to the amount of product obtained 15 hr after HO induction (Figure 2F). Therefore, the apparent shift in the kinetics of 18-kb gap repair (as well as BIR) could be attributed solely to the increased efficiency of repair in an *mph1Δ* background.

As postulated above, if coordination between the DSB ends occurs by strand invasion/annealing within the same D-loop, then these data suggest that *Mph1* could potentially facilitate DSB end coordination by promoting the extension of the D-loops formed upon strand invasion by the broken ends. Therefore, in the absence of *Mph1*, even ends that are synapsed close together (only 1.2 kb apart) may behave as though they are separated by a larger distance. If *Mph1* indeed promotes D-loop extension, it may act only over short distances, because *mph1Δ* did not compromise the already slow and inefficient repair of 5-kb or 18-kb gaps (Figure 1, B and D).

In contrast to *Mph1*, *Sgs1* seems to restrict the enlargement of the D-loops (Jain *et al.* 2009).

Double deletion of *SGS1* and *MPH1* eliminates *REC*-mediated delay in the initiation of long gap repair and BIR

Since *Sgs1* and *Mph1* appeared to play opposing roles in the regulation of 1.2-kb and 5-kb gap repair, we made double deletions to examine their functional interactions. Deleting *Sgs1* suppressed the *mph1Δ* defect of break repair as well as the 1.2-kb gap repair, as both the efficiency (1.2-kb gap repair; Figure 2A) and kinetics of repair (break repair and 1.2-kb gap repair; Figure 2, B and C) were restored to WT levels. Conversely, *mph1Δ* did not suppress the *sgs1Δ*-dependent acceleration of repair kinetics in the 5-kb gap-repair strain (Figure 2D). Strikingly, while neither of the single mutations affected the kinetics of the 18-kb gap repair, *sgs1Δ mph1Δ* double deletion resulted in a marked acceleration in the appearance of the primer-extension product (Figure 2, E and F).

We next examined the effect of *sgs1Δ mph1Δ* double deletion on BIR. Consistent with the previous reports (Jain *et al.* 2009; Lydeard *et al.* 2010a; Luke-Glaser and Luke 2012; Stafa *et al.* 2014), single deletion of either *Sgs1* or *Mph1* increased the efficiency of BIR by ~twofold (Figure 3A). In addition, we found that the simultaneous deletion of both *Sgs1* and *Mph1* increased the efficiency of BIR by nearly fourfold (Figure 3A). Thus, akin to their additive/synergistic roles in suppression of heteroallelic recombination and crossover formation in mitotic cells (Prakash *et al.* 2009; Sebesta *et al.* 2011; Mitchel *et al.* 2013), *Mph1* and *Sgs1* seem to act in

Figure 2 Deletion of Mph1 and Sgs1 eliminates the REC-mediated delay in the initiation of long gap repair. (A) Viability of the indicated strains. Data represent mean $\pm$ SD ($n \geq 5$). * denotes that viabilities of the *mph1Δ sgs1Δ* double-mutant strains are significantly different from the *mph1Δ* single mutants ($P \leq 0.005$). (B–E) Repair kinetics of the indicated break-repair, 1.2-kb, 5-kb, and 18-kb gap-repair strains, respectively, as determined by a quantitative PCR-based primer extension assay using primers shown schematically in Figure 1A. The amount of PCR product obtained from a repaired colony was set to 100%. (F) Data from (E) plotted after normalizing the amount of product obtained at 15 hr to 100% for each strain. In all cases, the kinetic data represent mean of three PCR reactions from each of two or three independent time courses $\pm$ SD. Data from WT and *sgs1Δ* single mutants have been published in Jain *et al.* (2009).

parallel pathways to inhibit BIR. The *sgs1Δ mph1Δ* double deletion also resulted in a dramatic acceleration in the initiation of repair of the BIR substrate (Figure 3, B and C). Thus, the simultaneous deletion of both *Sgs1* and *Mph1* eliminates the REC-mediated kinetic differences between the different gap-repair and BIR strains such that the repair kinetics in all cases is comparable to the repair of a 1.2-kb gap in the WT background (Figure 3, B and C).

It is possible that the improved efficiency of BIR in the absence of *Sgs1* and *Mph1* could be due to the increased stability of the D-loop intermediates. In this regard, both *Sgs1* and *Mph1* have been shown to disrupt the strand-invasion intermediates (Bachrati *et al.* 2006; Prakash *et al.* 2009). The human ortholog of *Sgs1* has also been shown to disrupt *Rad51* filament formation *in vitro* (Bugreev *et al.* 2007). *Sgs1* might additionally affect the stability of the *Rad51* filament near the DSB by stimulating 5' $\rightarrow$ 3' resection to generate more single-stranded DNA substrate for *Rad51* binding further away from the break (Mimitou and Symington 2009; Lydeard *et al.* 2010a). Consistent with the role of *Sgs1* and *Mph1* in modulating D-loop formation, we found that *Rad51* overexpression also improved the efficiency of BIR as well as long gap repair (Figure 3D; also Lydeard *et al.* 2010a). There was no further increase in the efficiency of BIR when *Rad51* was overexpressed with *sgs1Δ* and *mph1Δ* single or double deletions (Figure 3A). These data suggest that *Sgs1* and *Mph1* act in parallel pathways to suppress BIR, presumably by destabilizing the *Rad51* filament and/or the strand-invasion intermediates.

We next examined the effect of overexpressing *Rad51* on the kinetics of repair. Interestingly, while overexpression increased the efficiency of gap repair and BIR (Figure 3D; also Lydeard *et al.* 2010a), it did not accelerate the kinetics of either the 18-kb gap repair (Figure 2, E and F) or BIR (Lydeard *et al.* 2010a). This is in stark contrast to the *sgs1Δ mph1Δ* double deletion, which increased the efficiency as well as the kinetics of BIR and 18-kb gap repair (Figure 2, A and F, and Figure 3C). These data argue that in addition to regulating the stability/topology of the D-loops, *Sgs1* and *Mph1* must play a more direct role in imposing the REC, either by slowing down BIR and/or by preventing the GC machinery from loading inappropriately at single-end invasion intermediates.

Mechanistic shift in the repair of long gap repair and BIR substrates in the absence of both *Sgs1* and *Mph1*

The accelerated repair kinetics of *sgs1Δ mph1Δ* gap-repair and BIR strains could either be attributed to a more rapid initiation of the BIR pathway itself or it could indicate a mechanistic shift from the slower BIR to the quicker GC-like mode of repair. To distinguish between these possibilities, we carried out several tests, including an analysis of repair products and the effect of impairing BIR-specific repair with a *pol30-89* mutation. First, we examined ~ 100 repaired colonies from WT and *sgs1Δ mph1Δ* strains for the retention (indicative of GC) or loss (indicative of BIR) of the distal nonessential end of Chr 5. We have previously shown that increasing the gap between the donors results in an increase in the proportion of colonies that repair the break by BIR

Figure 3 Mph1 and Sgs1 act independently to suppress BIR. (A and D) Viabilities of the indicated strains. Data represent mean $\pm$ SD ($n \geq 4$). * in (A) denotes that the viabilities of *mph1Δ* and *sgs1Δ* single-mutant strains are significantly different from the WT strain ($P \leq 0.005$). ** denotes that the viabilities of the indicated strains are significantly different from WT as well as *mph1Δ* and *sgs1Δ* single-mutant strains ($P \leq 0.005$). * in (D) denotes that the viabilities of the indicated Rad51 over-expressing strains are significantly different from the parent strains ($P \leq 0.005$). (B) Kinetics of the indicated *mph1Δ* and *sgs1Δ* double-mutant strains as determined by a quantitative PCR-based primer extension assay using primers shown schematically in Figure 1A. Kinetics of WT break-repair and BIR strains (dotted lines) are shown for comparison. (C) Kinetic data of *mph1Δ* and *sgs1Δ* double-

mutant strains from (B) plotted after normalizing the amount of product obtained at 15 hr to 100% in each strain. Kinetic data represent mean of three PCR reactions from each of two or three independent time courses $\pm$ SD. Data from WT and *sgs1Δ* single mutants have been published in Jain *et al.* (2009).

(Jain *et al.* 2009). We note that during long gap repair, independently initiated BIR events from the widely-separated *LE* and *U2* ends may resolve to yield GC-like outcomes, potentially resulting in a gross underestimation of the actual frequency of BIR. Nevertheless, if double deletion of *SGS1* and *MPH1* merely accelerates the kinetics of BIR, the proportion of colonies that lose the distal end of Chr 5 should not change relative to the WT background. Conversely, if there is a switch from the BIR to GC mode of repair, the proportion of colonies that lose the distal end might be expected to decrease; however an increase in the proportion of GC events that are accompanied by crossing over (CO) could also result in the loss of the distal end of Chr 5 (Figure 4A). If repair occurred in the G_2 phase of the cell cycle such that one of the chromatids repaired by GC leading to a CO while the other repaired by GC without a CO, the chromatids could segregate in a manner that would give rise to one daughter cell which has lost the distal end of Chr 5, and another one, which lacks the left end of Chr 3 (Figure 4A, panel ii). Since the left end of Chr 3 is essential for cell survival, only the daughter that has lost the distal nonessential end of Chr 5 would be viable and grow into a BIR-like colony. In the absence of *SGS1* and *MPH1* we observed a significant increase in the proportion of repaired colonies that had lost the distal end of Chr 5; even for break repair, this proportion increased from $\sim 1\%$ in WT to $\sim 25\%$ in the *sgs1Δ mph1Δ* background (Figure 4B). Since both *Sgs1* and *Mph1* are known to suppress mitotic crossovers (Ira *et al.* 2003; Prakash *et al.* 2009), the increase in the proportion of colonies lacking the distal

end of Chr 5 could be a result of the much higher frequency of CO-associated GC events in the absence of these helicases. To test this, we analyzed the repaired colonies by Southern blot. As expected, all of the WT colonies analyzed gave products consistent with repair by GC without an associated CO (Figure 4C). Conversely, 3 out of 12 (25%) colonies derived from the *sgs1Δ mph1Δ* break-repair strain harbored products of a CO as well as a noncrossover (NCO) event, and a similar proportion of colonies yielded a BIR-like outcome (Figure 4D). It is likely that these two populations represent the alternate segregation outcomes of GC-mediated repair outlined in Figure 4A, in which case the colonies lacking the distal end of Chr 5 would not have arisen from a true BIR event but from the enhanced level of CO associated with GC. Thus, it is difficult to assess whether the elevated repair seen in *sgs1Δ mph1Δ* arose from increased BIR or GC.

As an alternate approach to distinguish between the GC and BIR modes of repair, we employed the *pol30-89* allele of *PCNA*, which has a dominant-negative effect on BIR but does not affect GC (Lydeard *et al.* 2010b). We reasoned that if the *sgs1Δ mph1Δ* double deletion merely accelerates the kinetics of BIR, then *pol30-89* would compromise the repair efficiency of the gap-repair and BIR strains. However, if the cells switched from BIR to GC in the absence of both *Sgs1* and *Mph1*, the efficiency of repair would remain unaffected upon addition of *pol30-89*. We note that even though *pol32Δ* has a much more profound effect on BIR (> 20 -fold reduction) (Lydeard *et al.* 2007; Jain *et al.* 2009) compared to the three- to fourfold reduction imparted by the *pol30-89* allele (Lydeard

donors in a GC-compatible configuration could generate a positive “GO” signal to rapidly recruit the GC machinery to carry out repair. In the second scenario, the inability of both the DSB ends to pair in a GC-compatible configuration could send a negative “STOP” signal to prevent the recruitment of the GC machinery to a single engaged end (or distantly engaged ends). The failure to initiate new DNA synthesis in the absence of a GO signal or due to the generation of a STOP signal presumably leads to repeated cycles of Rad51 filament dissociation and reinvasion in the attempt to find homologies to both DSB ends in the vicinity of each other so that repair can proceed via the more conservative GC pathway. However, after several failed attempts to find homologies in a GC-compatible configuration, the cells would resort to BIR to initiate repair from a single engaged end. According to the first scenario, if the REC is disrupted, even the ends that can be repaired by GC would fail to generate/perceive the GO signal, and therefore be inappropriately channeled into the slow and inefficient BIR pathway. Conversely, disruption of REC in the second scenario would result in a failure to generate the STOP signal if only one of the DSB ends is paired with its homologous donor, thereby channeling even the BIR substrates into the GC pathway. Our results support the creation of a STOP signal, since disruption of the REC by *Sgs1* and *Mph1* double deletion results in rapid, Pol30-89-independent repair of the BIR and gap-repair substrates. We propose that in the absence of REC, the repair machinery is able to initiate new DNA synthesis soon after a DSB end strand invades its homologous donor, irrespective of whether homology to the other end has been found. Although there is a dramatic acceleration of BIR and gap repair in the absence of *Sgs1* and *Mph1*, their repair kinetics are still slower than the kinetics of break repair (Figure 3, B and C). The reason for this is not clear, although it is plausible that when both DSB ends are juxtaposed right next to each other, they might act cooperatively to modify the chromatin structure and load the repair synthesis machinery or prevent the D-loop from collapsing.

It will be worthwhile to test whether any other factors (besides Pol30-89) that are normally required for BIR, are also dispensable for repair in the absence of REC. It also remains to be determined what really triggers the REC and how *Sgs1* and *Mph1* act as sensors or mediators/effectors of this pathway. Since mutations in the human orthologs of these helicases have been known to cause Bloom’s syndrome and Fanconi Anemia, both of which are characterized by genomic instability and cancer predisposition, a deeper understanding of the REC could potentially provide useful insights into the etiology of these debilitating disorders in the future.

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