Rad51 and Dmc1 have similar tolerance for mismatches in yeast meiosis

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Abstract

In many eukaryotes, including both budding yeast and mammals, repair of double-strand breaks (DSBs) is carried out by different apparatus in somatic and meiotic cells. In mitotic cells, Rad51 recombinase, acting with Rad54, facilitates the search for homology and DNA strand exchange. In meiosis, Rad51 is inhibited by Hed1 and plays only an effector role, while strand exchange is driven by Rad51’s homolog, Dmc1, acting with Rad54’s homolog, Rdh54/Tid1. To directly compare the activities of Rad51 and Dmc1 and especially their tolerance for recombination between divergent sequences, we created diploids in which a site-specific DSB was created by HO endonuclease, either under control of a galactose-inducible promoter or a meiosis-specific SPO13 promoter. Homologous recombination was measured by an ectopic break-induced replication (BIR) assay in which a 108-bp homologous sequence shared between the DSB end and the donor sequence could be easily modified. As previously shown for a haploid mitotic strain, BIR efficiency decreased with increasing divergence between donor and recipient, but repair occurred even when every 6th base pair was mismatched. There was little difference in the tolerance of mismatches in mitotic haploids or meiotic diploids; however, there were notable differences in meiotic diploids when recombination was facilitated by Dmc1 or when Rad51 took over from Dmc1 in both hed1Δ and dmc1Δ hed1Δ mutants. We found that Dmc1 and Rad51 are similarly tolerant of mismatches during meiotic recombination in budding yeast. Surveillance of mismatches by the Msh2 mismatch repair protein proved to be Dmc1-specific. In all cases, assimilation of mismatches into the BIR product was dependent on the 3’ to 5’ exonuclease activity of DNA polymerase δ.
**Author Summary**

In many eukaryotes, including both budding yeast and mammals, repair of double-strand breaks (DSBs) is carried out by different apparatus in somatic and meiotic cells. In mitotic cells, Rad51 recombinase, acting with Rad54, facilitates the search for homology and DNA strand exchange. In budding yeast meiosis, Rad51 is inhibited by Hed1 and plays only an effector role, while strand exchange is driven by Rad51’s homolog, Dmc1, acting with Rad54’s homolog, Rdh54/Tid1. To directly compare the activities of Rad51 and Dmc1 and especially their tolerance for recombination between divergent sequences, we created diploids in which a site-specific DSB was created by HO endonuclease. Homologous recombination was measured by an ectopic break-induced replication (BIR) assay in which recombination occurred between a 108-bp homologous sequence shared between the DSB end and the donor sequence. The donor sequence could be easily modified to introduce different arrangements of mismatches. BIR efficiency decreased with increasing divergence between donor and recipient, but repair occurred even when every 6\textsuperscript{th} base pair was mismatched. There was little difference in the tolerance of mismatches in mitotic or meiotic diploids; however, there were notable differences in meiotic diploids when recombination was facilitated by Dmc1 or when Dmc1 was deleted and Rad51 was activated. We found that Dmc1 and Rad51 are similarly tolerant of mismatches during meiotic recombination. Surveillance of mismatches by the Msh2 mismatch repair protein proved to be Dmc1-specific. As in mitotic cells, the assimilation of mismatches into the BIR product was dependent on the 3’ to 5’ exonuclease activity of DNA polymerase δ.
Introduction

In somatic cells, double-strand breaks (DSBs) are repaired in ways that have the least genetic consequence. DSBs may be repaired by recombining with a genetically identical sister chromatid or with a homologous chromosome or, non-allelically, with an ectopic homologous site, but in these instances, repair is most often accomplished without a crossing-over that could lead to loss of heterozygosity of more distal markers. Many of the recombination intermediates that could be resolved as crossovers are removed by a BLM/Sgs1- and Top3-dependent “dissolvase” that leads to a noncrossover outcome (1, 2). In contrast, in meiosis, recombination between homologs is strongly favored over sister chromatid events and is frequently accompanied by crossing-over, which provides the necessary anchoring to establish proper bivalent alignment on the meiotic spindle and generates genetic diversity among the haploid germ cells (3, 4).

Associated with these important differences in the outcomes of mitotic and meiotic recombination are differences in both the origins of DSBs and in the basic recombination machinery itself. Mitotic DSBs arise primarily from DNA replication errors that can be attributed to various impediments to replication fork progression or by the processing of altered nucleotides (5-7). In addition, some developmental events are based on the creation of specific DSBs, including Spo11-mediated meiotic recombination, AID cytidine deaminase-induced immunoglobulin rearrangements and - in yeast - mating-type gene switching (8-10). Budding yeast mating-type gene (MAT) switching is triggered by the site-specific HO endonuclease (11, 12). DSB ends are resected first by the Mre11-Rad50-Xrs2\(^{NBS1}\) (MRX) complex, but more extensive 5’ to 3’ resection depends on two other exonucleases, Exo1 or the Sgs1-
Rmi1-Top3-Dna2 complex, to create long single-stranded 3′-ended tails (13). These strands are first bound by the single-strand DNA binding complex, RPA, and then by the Rad51 recombinase that initiates the search for homology and the repair of the DSB by homologous recombination (14-16).

In meiosis, dozens of DSBs are created by the topoisomerase-like Spo11 complex whose association at meiotic hotspots requires the participation of many other proteins to create an accessible chromatin structure (10). Spo11 generates a DSB in which both 5′ ends are covalently linked to Spo11 itself. These protein-blocked ends are processed by the Mre1-Rad50-Xrs2NBS1 (MRX/MRN) complex, excising the Spo11 protein and a short oligonucleotide, yielding 3′ single-stranded ends that can be further enlarged by 5′ to 3′ resection, mostly by Exo1 (17).

In all eukaryotes, DSB repair in mitotic cells is accomplished by the Rad51 recombinase (18-21). A set of mediator proteins (in budding yeast, Rad52, Rad55 and Rad57) enable Rad51 to assemble into a nucleoprotein filament on the single-stranded ends of a DSB that are created by 5′ to 3′ resection (15, 22-24). Rad51’s ability to promote DNA strand exchange also depends on the Rad54 chromatin remodeler, which binds to Rad51 and promotes strand invasion into homologous sequences and later branch migration steps in recombination (25-29). In contrast, meiotic recombination in many eukaryotes, including both budding yeast and mammals, recombination depends on Dmc1, a Rad51 paralog (19, 30). In meiosis, Rad51 is reduced to an auxiliary, effector role such that even a mutant that can only bind ssDNA, but is unable to carry out strand exchange, is able to accomplish its meiotic functions (31-33). Furthermore, Rad51 is restrained by the Hed1 protein that disrupts Rad51’s ability to interact with Rad54 (34-38).

Forming the Dmc1 nucleoprotein filament depends on a different set of auxiliary factors that
are either specifically expressed in meiosis (Sei3 and Mei5, Hop2 and Mnd1) or play only minor roles in mitotic DSB repair (most notably, Rad54’s homolog, Rdh54, also known as Tid1) (39-44).

In vitro, Rad51 and Dmc1, which are about 45% identical in amino acid sequence, have distinctive strand exchange behaviors (45-47). Moreover, in vivo, Dmc1-mediated recombination strongly favors recombination between homologous chromosomes rather than exchanges between sister chromatids (48, 49). Although dmc1Δ diploids are severely defective in meiotic recombination, spore viability is significantly restored in a dmc1Δ hed1Δ double mutant, where Rad51 can interact with Rad54 and carry out meiotic recombination; however, the outcomes are different, as the strong bias for interhomolog recombination is lost (34, 35, 37, 38, 48, 50).

Among the differences that may be important in the evolution of a meiosis-specific DSB repair system is the fact that the favored template for repair is a homologous chromosome which – unlike a sister chromatid – is likely to differ at many polymorphic sites. Hence, it may be the case that Dmc1 would be more tolerant of mismatches in the formation of a strand invasion intermediate. Such an increased tolerance has been suggested by in vitro studies of DNA strand exchange involving short oligonucleotides, in which Dmc1, but not Rad51, could “step over” a mismatch in the formation of a displacement loop (D-loop) intermediate (51-53). Structural differences between Rad51 and Dmc1 that might reflect a change in mismatch tolerance have also been reported (54, 55).

Mismatches in heteroduplex DNA created by strand invasion are also monitored by the mismatch repair (MMR) system including the Msh2-Msh6 and Pms1-Mlh1 heterodimers (56-59). Msh2-Msh6 plays two roles when confronted with mismatches during strand invasion.
Msh2-Msh6, independent of Mlh1-Pms1, detects heterologies and promotes dismantling of strand invasion intermediates (heteroduplex rejection) by the Sgs1-Rmi1-Top3 helicase complex (60-62). Meiotic recombination between divergent strains, and even between species, with significantly homeologous chromosomal sequences, can be enhanced by eliminating Msh2 (59, 63, 64).

In this study, we exploit the HO endonuclease to create site-specific DSBs to study repair by break-induced replication (BIR), in which only one end of the DSB shares homology with a donor sequence elsewhere in the genome and thus leads to a nonreciprocal translocation.

Here, we have compared the mismatch tolerance and repair efficiency of both Dmc1 and Rad51 during meiotic recombination in budding yeast, induced by the same site-specific DSB. We find that Rad51 and Dmc1 show similar tolerances for mismatches but the monitoring of mismatches by the Msh2-dependent mismatch repair system is significantly different between mitotic and meiotic cells. Consistent with our observations from mitotic BIR (65), the incorporation of mismatches in the heteroduplex DNA formed by strand invasion is not through the mismatch repair system but via the 3’ to 5’ exonuclease activity of DNA polymerase δ during meiotic BIR.

Results

Effect of sequence divergence on mitotic and meiotic BIR in diploids

Previously we have studied ectopic break-induced replication (BIR) in haploid strain yRA253 in which a site-specific DSB is created by the HO endonuclease expressed under the control of a galactose-inducible promoter (65) (Fig. 1A). An HO cleavage site is located in the
nonessential left end of chromosome V, distal to the 5' end of an inserted URA3 gene (UR)

oriented toward the telomere. Rad51-mediated BIR depends on a 108-bp sequence containing
one end of the HO cleavage site and the 5' end of an artificial intron, including its splice donor
(SD). On the opposite arm of chromosome V lies the 108-bp donor sequence and the 3' end of
the intron, with its splice acceptor (SA), followed by the 3' end of the URA3 gene (A3). Following
HO cleavage, BIR between the 108-bp segments generates a complete, intron-containing,
reconstituted URA3 gene. The sequence of the 108-bp donor can be easily modified without
altering expression of the BIR URA3 product. From these studies, we learned several important
facts. First, the efficiency of BIR decreased with the increasing density of evenly-spaced
mismatches across the 108-bp donor sequence, but recombination could still occur even when
every 6th base was mismatched. Second, assimilation of the mismatches in the donor sequence
into the BIR product was strongly polar from the 3' invading end and was heavily dependent on
the 3' to 5' proofreading exonuclease activity of DNA polymerase δ and largely independent of
Msh2 and Mlh1. Third, Msh2 promoted heteroduplex rejection and thus inhibited
recombination between divergent 108-bp sequences, but this rejection only when the 3' end of
the DSB contained a nonhomologous “tail” that would need to be clipped off before the 3' end
could be used to prime DNA repair synthesis. However, the constructs we used in current study
do not produce a nonhomologous tail.

We first determined if mitotic Rad51-mediated BIR in diploids was similar to that
observed in the haploid strain. Diploids were created by mating yRA353 with yAM153, a strain
that lacks an HO cleavage site at MATα-inc (Fig. 1B) (see Methods and Table 1). The frequency
of Ura+ BIR outcomes even for the fully-matched donor was about 4 times lower than for the
haploid strain (Fig. 2B). This reduction can be attributed to two alternative outcomes not available in the haploid system: (1) a competing interchromosomal gene conversion (GC) that replaced the entire UR-SD sequence and an adjacent NAT-resistance marker, producing NAT−Ura−diploids, and (2) complete loss of the broken chromosome, producing NAT−Ura−Trp− outcomes (Fig. 1B). Among the survivors of HO induction in a strain with no mismatches in the 108-donor template, 97.3% of the diploids lost the NAT marker adjacent to the HO cleavage site, of which 4.1% were Ura3+, the BIR outcome. Only 0.2% were Ura−NAT−TRP1−, indicating that loss of the broken chromosome was infrequent but repair by interchromosomal GC was favored over the ectopic event with only 108 bp homology. The remaining 2.7% of the cells retained the NAT marker and are likely to have arisen by nonhomologous end-joining, which is not repressed in mata/MATα diploids (66).

We then compared the viability and BIR efficiencies of strains carrying mismatches every 12th, every 9th, and every 6th base across the 108-bp donor region (Fig. 2A and 2B). Viability was equivalent in all three strains compared to no mismatches, as expected in diploids that could either repair the DSB or tolerate loss of the broken chromosome (Fig. 1B). As we saw for haploid strains, there was a pronounced decrease in Ura+ frequencies with increasing mismatch density. To compare the effect of mismatch density on BIR, we compared the ratio of Ura3+ outcomes for each set of mismatches relative to the fully homologous template (no mismatches) as a control (Fig. 3A). The somewhat lower ratios of BIR with mismatches compared to no mismatches between haploid and diploid strains were not statistically significant, but may reflect the mixed genetic background in the diploid compared to the
haploid strain. All further comparisons were made only among the set of isogenic diploid strains.

**Effect of sequence divergence on meiotic Dmc1-mediated BIR in diploids**

To examine the same HO-induced BIR events in meiotic cells we deleted the \textit{GAL::HO} sequences from the \textit{ade3} locus and introduced a \textit{SPO13::HO} construct (67, 68) at the \textit{lys2} locus. We had previously shown that \textit{SPO13::HO}-induced DSBs occurred in the same time frame as normal, Spo11-induced DSBs (67, 69). Moreover, \textit{SPO13::HO}-induced gene conversion events are distinctly meiotic-like, being strongly associated with crossovers and with much shorter co-conversion tract lengths than \textit{GAL::HO}-induced mitotic events (69). Because the haploid parent carrying the BIR assay sequences was deleted for \textit{MATa} and lacked the \textit{MATa1} gene function needed to promote meiotic recombination, the \textit{mat\alpha\Delta/MATa} diploids cannot enter meiosis. To enable meiosis and sporulation we created \textit{rme1\Delta} derivatives that bypass the need for mating-type control. Rme1 (Repressor of MEiosis 1) is a negative regulator of meiosis that repress transcription of \textit{IME1} (Inducer of Meiosis) and thereby inhibit cells from entering meiosis and form spores (70). We sporulated these \textit{rme1\Delta} diploids and then eliminated vegetative cells by glusulase treatment and derived random spores by sonication (See Methods). Because the efficiency of \textit{SPO13::HO}-induced cleavage is considerably less than the near-complete cleavage seen with \textit{GAL::HO} (68), we did not compare the absolute frequencies of Ura\textsuperscript{+} BIR products in mitotic and meiotic diploids, but instead compared the outcomes of a diploid with no mismatches to those with every 12\textsuperscript{th}, 9\textsuperscript{th} and 6\textsuperscript{th} base-pair mismatched. As shown in Fig. 2B, we found a similar decrease in recombination efficiency with increasing divergence as we had seen...
in the mitotic diploids. A comparison of the ratios of Ura* strains in the mismatched constructs versus no mismatches (Fig. 3A) showed that the tolerance of mismatches in meiosis, driven by Dmc1, was not statistically significantly different from the same diploid using Rad51 in mitotic cells. A better comparison of Rad51- and Dmc1-mediated meiotic events can be made if we analyze the same SPO13::HO-induced events, but in which recombination depended on Rad51 instead of Dmc1.

**Effect of mismatched substrates in **hed1Δ **and dmc1Δ hed1Δ** **diploids**

Previous studies have shown that Rad51 can substitute for Dmc1’s meiotic strand exchange activity by enhancing Rad51 activity in dmc1Δ diploids, either by deleting HED1, by overexpressing Rad51’s partner, RAD54, or by preventing Mek1-mediated phosphorylation of Rad54 (34, 35, 37, 38, 48, 71). We chose to delete Hed1, an inhibitor of Rad51’s interaction with Rad54. Though less efficient than wild type, both hed1Δ and dmc1Δ hed1Δ cells were able to successfully complete meiosis, as judged by percent sporulation (Fig. S3A). Dissection of 4- and 3-spored tetrads showed that the spore viability of dmc1Δ hed1Δ diploids (87.2%) was comparable to that of wild type diploids (88.9%).

Compared to wild type diploids, hed1Δ and dmc1Δ hed1Δ cells exhibited a significant increase in the fraction of viable spores that completed BIR and reconstituted Ura3* (Fig. 3B). Meiotic BIR outcomes in hed1Δ were 1.3-fold higher (4.1% in hed1Δ versus 3.1% in WT) and dmc1Δ hed1Δ were 1.8-fold higher (5.7% in dmc1Δ hed1Δ versus 3.1% in WT). The significant increase in BIR efficiency was also observed in the substrates with every 9th bp mismatched (Fig. 3B). In this comparison, we presume that the frequency of SPO13::HO-generated DSBs was
unchanged by deleting DMC1 or HED1, so the difference is most likely a reflection of a change in homologous partner choice and/or in the completion of successful BIR. This increase is consistent with previous studies showing that there were marked differences in the choice of recombination partner in dmc1Δ hed1Δ diploids versus wild type; specifically, the strong inter-homolog bias in forming joint molecules (double Holliday junctions) was eliminated and there was an increase in ectopic recombination (48). The increase in ectopic interactions can explain the increase in the fraction of Ura3+ recombinants; but intrinsic differences between Rad51 and Dmc1 could still have an effect.

We then compared how Dmc1 and Rad51 dealt with the same mismatched substrates under the same meiotic conditions (Fig. 3C). For DMC1, the ratio of BIR outcomes for every 9th mismatch versus no mismatches was 0.24, whereas for hed1Δ the ratio was 0.36 and dmc1Δ hed1Δ the ratio was 0.51 (Fig. 3C). These statistically significant differences suggests that Dmc1 itself is not intrinsically more tolerant of mismatches than Rad51 and that Rad51 is more tolerant. However, there could be other contributing factors, such as surveillance of the recombination intermediates by the mismatch repair system, that are different when Rad51 or Dmc1 promotes recombination (see below).

Assimilation of mismatches into BIR products in meiosis is highly dependent on DNA polymerase δ

We compared the assimilation of heterologies into BIR products in mitosis and meiosis. In the haploid BIR assay, once heteroduplex DNA is formed by strand invasion, mismatches are assimilated into the BIR product in a strongly polar fashion (65). Mismatches close to the 3’ end
of the invading strand are almost always replaced by the donor template sequence, with the
likelihood of incorporating a mismatch declining rapidly until about 40 bp from the 3’ end (65,
72). The assimilation of the template sequence almost completely depends on the 3’ to 5’
proofreading exonuclease activity of DNA polymerase δ, which chews back the 3’ end of the
invading strand before initiating new DNA synthesis (65, 72)(Fig. S1). Almost all mismatch
assimilation is lost in a pol3-01 proofreading-defective mutant. In fact, Pol3-dependent 3’ to 5’
resection could be demonstrated even if the first 26 nt of the 3’ invading end was identical to
the donor (72).

The mitotic diploid strains with mismatches every 12th, 9th, and 6th bp all showed the
same strong polarity as seen in the haploid system (65) (Fig. 4A), with mismatch incorporation
extending as far as 45 nt. A similar result was found for wild type (Dmc1+) meiotic diploids (Fig.
4A). When proofreading exonuclease activity of DNA polymerase δ was impaired, in a pol3-01
diploid, almost all mismatch incorporation in meiosis with every 9th bp mismatched was
eliminated (Fig. 4B). The pattern of mismatch incorporation into BIR products was similar in
wild type, hed1Δ and dmc1Δ hed1Δ meiotic diploids (Fig. 3D).

Mismatch surveillance by Msh2 is different in mitotic and meiotic cells

The mismatch repair (MMR) system is closely linked with recombination. In a variety of
assays in budding yeast, the bacterial MutS homolog, Msh2, reduces recombination between
divergent sequences both in mitotic and meiotic contexts (63, 73-76). However, in the specific
context of HO endonuclease-induced mitotic BIR, Msh2 does not inhibit break-induced
replication (BIR) unless the 3’ end of the DSB contains a nonhomologous segment that must be
cleaved before new DNA synthesis could be initiated (65). Specifically, deleting MSH2 had little
effect on the efficiency of haploid mitotic BIR even when the level of mismatches exceeded 10%.
In mitotic diploids there also was no significant difference in BIR recombination
frequency between wild type and msh2Δ, either for substrates with no mismatches or with
every 9th base mismatched (Fig. 5A); however, MSH2 proved to have an important role when
the same HO-induced BIR events occurred in meiosis. Consistent with previous studies (76),
deleting MSH2 suppressed the reduction in meiotic recombination between highly mismatched
substrates (Fig. 5A). Thus, in the absence of Msh2, Dmc1-mediated BIR appears to be
significantly more tolerant of every 9th base mismatched than Rad51 in mitotic cells. Again, a
better comparison is with the same meiotic break, comparing Dmc1 and Rad51-driven events.
Indeed, increased BIR recombination between the mismatched sequences was not seen was
not seen when Rad51 was the sole recombinase acting in meiosis (Fig. 6A). Together, these data
suggest that Msh2 plays a distinctive role in monitoring Dmc1-mediated recombination that is
not seen for the same SPO13::HO-induced recombination carried out by Rad51. When we
compare Dmc1 and Rad51, both acting in meiosis and in the absence of Msh2, there is no
significant difference in the tolerance of mismatches for the two recombinases (Fig. 6B).
The mismatch repair system plays two distinguishable roles in confronting mismatches
during strand invasion. First, it participates with the Sgs1 helicase complex in rejecting
heteroduplexes (62, 77). Second, it directs the repair of mismatches, favoring the correction of
the mismatched base that is present on the unbroken (donor) strand (65, 73, 74, 78). As with
mitotic haploids, in the absence of a nonhomologous 3’ end of the invading strand, there was
no significant Msh2-dependent rejection during strand invasion when Rad51 was the active
recombinase. However, there was a notable suppression of BIR recombination in wild type meiotic cells with every 9\textsuperscript{th} base mismatched (i.e. where Dmc1 is the recombinase), suggesting that Msh2 enforced heteroduplex rejection, even though there is no 3’ end nonhomology.

These results suggest that wild type meiotic cells, even acting at an HO-induced DSB not created by Spo11 and thus not necessarily in association with the entire apparatus of the meiotic recombination machinery (79) interacts differently with Msh2 than does Rad51.

Heteroduplex DNA formed at the initial step of BIR is transient and any defect of mismatch correction should be reflected in a reduction in the incorporation of mismatches into the BIR product rather than in the appearance of sectored colonies (Fig. S1) (78). Mismatch assimilation in \textit{dmc1\Delta hed1\Delta} meiotic cells was not affected by deleting \textit{MSH2}, and heterologies were still incorporated as far as 45 bp from the 3’ end of the DSB, as in mitotic haploids and diploids (Fig. 5C) (72). However, in Dmc1-driven (wild type) meiotic cells there was less assimilation of mismatches near the 3’ invading end and in a small fraction of cases, the extent of donor sequence incorporation was extended over nearly the length of homology (Fig. 5C).

These observations suggest that Msh2-dependent mismatch correction plays a more significant role in the incorporation of mismatches in Dmc1-promoted events than in meiotic or mitotic Rad51-promoted exchanges, but there is still a strong bias that focuses this activity on the 3’ invading end. Moreover, DNA sequencing of the BIR events in which there is a long conversion tract unexpected showed that about 25% of them showed the apparent presence of unrepaired heteroduplex DNA (Fig. 5D). However, because we selected BIR events by selecting Ura\textsuperscript{*} segregants from random spores, it is possible that these represent a small fraction of cases where there were two Ura\textsuperscript{*} spores in a tetrad whose spores were not completely separated.
Although *in vitro* strand exchange and structural studies have suggested that Dmc1 may be more tolerant to mismatched substrates than Rad51, we find that this difference is not evident when we analyze ectopic break-induced replication in budding yeast cells undergoing meiosis. When Msh2 is active, Rad51 (in *dmc1Δ hed1Δ* cells) appears to be more tolerant of highly mismatched substrates than Dmc1 (Fig. 3C). But this comparison is confounded by the apparently selective action of Msh2 on Dmc1-mediated events; so when this effect is taken into account, Rad51 and Dmc1 are not statistically distinguishable in their tolerance of substrates with every 9th base pair mismatched (Fig. 6B).

There are, of course, many differences between *in vitro* strand exchange assays, using purified proteins but lacking many of the auxiliary factors that may be important *in vivo*, compared to the BIR assay used here, which requires not only strand invasion but the completion of recombination. Our previous study of mitotic BIR also differed in notable ways from a Rad51-mediated strand exchange assay; notably – and as we also show here – recombination was possible even when every 6th base pair was mismatched, whereas *in vitro*, using short oligonucleotide substrates, Rad51-mediated strand exchange required 8 consecutive matched base pairs. However, in vitro studies using 90 nt substrates yielded results quite similar to those seen in our *in vivo* assay (M. Kong and E. Greene, personal communication).

Previous work had also suggested that Rad51 was less tolerant than Dmc1 in meiosis when the entire genome had a density of mismatches of about 1 per 200 bases (38). Whereas
wild type and dmc1Δ hed1Δ diploids had similar spore viability with diploids homozygous for one set of variants (Sk1 or S288c), there was a 5-fold drop in spore viability with a Sk1/S288c diploid. We note that our strains were also of mixed parentage, but we were focused only on the tolerance of the diploid for a specific HO-induced ectopic BIR event with very high levels of mismatch in a 108-bp target. It will be important to determine if allelic gene conversions display similar tolerances.

There are several notable differences between wild type diploids, using Dmc1, and dmc1Δ hed1Δ diploids, using Rad51. In between these two poles lie the results in hed1Δ, where both Dmc1 and Rad51 are apparently active. First, the level of ectopic BIR in strains with no mismatches between the 108-bp donor and recipient sequences is significantly higher in hed1Δ and dmc1Δ hed1Δ compared to wild type. This increase is consistent with previous analysis of joint molecule intermediates in meiotic recombination that found dmc1Δ hed1Δ diploids lacked the strong inter-homolog bias seen in wild type diploids and that ectopic interactions were increased (48, 80). The presence of Dmc1 is inhibitory to Rad51-mediated strand exchange and blocks Rad51 filaments from catalyzing strand exchange between homologs (48). Notably, hed1Δ in the dmc1Δ mutant relieves the inhibition of Rad51-mediated recombination and restores DSB repair efficiency, which is a similar effect of overexpression of Rad51 in dmc1Δ cells (34). Based on this observation, meiotic recombination events resulting from Rad51- and Dmc1-mediated BIR demonstrate different repair efficiency and potentially work differently as a recombinase when the repair become dependent on either one. A reduction in interchromosomal repair of the SPO13::HO-induced DSB would yield more ectopic BIR events, as we found.
Mechanistically, meiotic BIR events appear to obey very similar rules to those in mitotic cells, with a strong polarity of assimilating mismatches near the 3’ end of the invading strand. The incorporation of mismatches is almost entirely dependent on the 3’ to 5’ exonuclease proofreading activity of DNA polymerase δ, although there is a small fraction of cases where mismatch assimilation extends over most of the 108-bp region rather than stopping about halfway. These events still apparently do not involve Msh2-dependent mismatch correction (Fig. 5, and previous results (65, 72).

Our studies also reveal a role for Msh2 in Dmc1-mediated recombination that we do not see in dmc1Δ hed1Δ diploids. Previous investigations have identified Msh2 in several different aspects of double-strand break repair, beyond simply correcting mismatches. One process is heteroduplex rejection, in which mismatched substrates are unwound by the Sgs1BLM helicase complex, triggered by Msh2 and Msh6. However, at least in mitotic BIR, this rejection is only provoked when the strand invasion intermediate has a 3’ end that is not homologous to the donor template; such flaps are bound by Msh2, working with Msh3 (81). Msh2-Msh3, associated with the SLX4 scaffold, also stimulates cleavage of the 3’ flap by Rad1-Rad10XRCC1 endonuclease (82). When there is a 3’ nonhomologous tail, as in single-strand annealing (SSA), a separation-of-function mutant that allows tail clipping but not single base pair mismatch recognition allows homeologous strands to complete recombination (62). In the experiments described here, however, the 3’ end is perfectly matched to the donor. Nevertheless, in meiotic cells, using Dmc1, deletion of MSH2 significantly elevates the recovery of BIR events in substrates with every 9th base mismatched but did not change the outcomes with perfectly matched templates. A similar suppression does not occur when Rad51 becomes the active
recombinase. These data support the idea that the MMR system acts as a genetic barrier for meiotic recombination between divergent sequences and reduces exchange between homologs during meiosis (76). The basis of this apparently Dmc1-dependent inhibition of heteroduplex formation is not yet known.

However, Msh2 appears also to affect the assimilation of mismatches into BIR products, as there is a notable reduction in the proportion of BIR outcomes that incorporate the mismatches encoded in the donor in meiotic cells (Fig. 5C). These results are reminiscent of the role of the mismatch repair protein Pms1 in incorporating mismatches in HO-induced DSB repair at the MAT locus during mating-type switching (78). In that gene conversion system, we presumed that sequences differences near the invading strand were preferentially repaired to the genotype of the (intact) donor and that this bias was lost in the pms1Δ mutant. This may explain the much lower proportion of mismatch incorporation in msh2Δ in Fig. 5C.

We conclude that, in the context of the intact recombination machinery, Dmc1-mediated recombination is not intrinsically more tolerant of mismatches than that driven by Rad51, at least during ectopic BIR in meiosis, using a site-specific DSB. Although meiotic recombination outcomes of SPO13::HO-initiated events are distinctly meiosis-like (69), it is distinctly possible that some parts of the meiosis machinery are intrinsically linked to the elaborate protein assemblies associated with the creation of Spo11-mediated DSBs (79). The ability to create a specific Spo11 hotspot in a normally “cold” region of the genome (83) may help elucidate these questions.
Yeast strains

Haploid and diploid strains are listed in Table 1. Diploids were constructed by mating a series of yRA haploid strains carrying the components of the BIR assay (65) with derivatives of the MATα-inc strain yAM153 that carries SPO13::HO (69) (Table 1). MATα-inc cannot be cleaved by HO endonuclease. Strain yLX9 (Mismatched every 9th bp) and yLX11 (Perfect homology) lack GAL::HO, by replacing ade3::GAL::HO (84) by ade3::LEU2. All yRA haploid strains contain the recipient sequences composed of the 5’ sequences of URA3 gene (UR), an artificially inserted split-intron with the splice donor site (5’ SD) and the HO recognition site (HOcs), located at the CAN1 locus in a non-essential terminal region of chromosome V (65). Divergent donors with different densities of mismatches were assembled into pRS314-based (CEN4, TRP1) plasmid containing the 3’ splice-acceptor (3’ SA) of the intron, the 3’ sequence from the URA3 gene (A3) and the TRP1 marker (called bRA29 plasmid in this study) using in vivo recombination and plasmid rescue from a yeast host strain as described previously (65). The donor cassettes were PCR-amplified from plasmid bRA29 and integrated at the FAU1 locus, about 30 kb from the right end of on chromosome V. Because the yRA haploid strains used in these experiments lack a functional MATa1 gene, the diploids obtained by mating yRA strains with derivatives of yAM153 cannot initiate meiosis. To restore sporulation and meiotic recombination, the meiosis repressor gene RME1 was knocked out in both haploid parents. In addition, the ura3-1 single base-pair mutation in the MATα parent strain was replaced by a compete open reading frame deletion to prevent the possibility of this homologous sequence becoming an alternative ectopic repair template. msh2Δ and pol3-01 mutations were made via CRISPR-Cas9 with an
ssODN repair template. gRNAs were ligated into a BpII digested site in a backbone containing a constitutively active Cas9 in URA3 marker (pJH2972). Cas9 plasmid was verified by sanger sequencing via GENEWIZ from Azenta and transformed as described in a previous study (85). A list of plasmids is found in S2 Table. After the plasmid containing the gRNA and repair template was introduced via transformation, colonies are plated onto 5-fluoroorotic acid media (FOA), which counter-selects against URA3 (86). Spore viability was assessed by tetrad dissection using a micromanipulator.

Rad51-mediated BIR assay in mitotic diploids

Selected diploid strains with DSBs induced by GAL::HO were grown in selective plates at 30°C. Individual colonies from strains were picked and serially diluted for 100- and 1000-fold. Serial dilutions of cells were plated on YEP-Gal, to measure the recombination-dependent survivors after inducing HO endonuclease expression with galactose, and on YEPD, to obtain the total number of cells. Cells on YEP-Gal were then replica plated to plates lacking uracil to count colonies that survived the break via BIR and to nourseothricin (NAT) plates to count colonies which survived the break via NHEJ and retained the distal part of the left arm of chromosome V.

BIR assay in meiotic diploids

Diploid strains were streaked on YEPD and replica plated to KAc plates (2% potassium acetate) to induce sporulation of diploids at 30°C. Cells were plated for ~5 days until they form 30% of tetrads. Cells were harvested from KAc plates and treated with 2X glusulase (diluted from 10X glusulase stock) and incubated at 30°C for 12h. Then spores were vortexed for 5 min and
sonicated using a Qsonica Q70 sonicator (power 80 Amps, duration 20 min) until more than 50% of the spores were singles. Spores were counted and plated on YEPD and subsequently replica plated to uracil dropout plates for 3-4 days at 30°C. The number of colonies were counted to determine the mean and standard deviation of Ura3+ cells.

**DNA sequence analysis of the break-repair junctions**

Ura+ colonies were confirmed to have completed BIR by using PCR primers amplifying the region at the start and end of the URA3 gene using primers DG31 (GGAACGTGCTGCTACTCATC) and DG32 (TTGCTGGCCGCATCTTCTCA). PCR products were initially checked by gel electrophoresis and sent to GENEWIZ for Sanger sequencing. Individual PCR sequences were aligned with corresponding 108-bp donor templates and analyzed by DNA analyses software Geneious Prime software.

**Statistical Analysis**

GraphPad Prism 9 software was used to calculate statistical significance of data.

**Figure Legends**

**Figure 1. Studying repair outcomes and break-induced replication (BIR) in meiotic diploids in budding yeast**

A. BIR-dependent formation of a functional Ura3+ recombinant. The recipient sequence shares the 108-bp region of homology and contain the 5’ sequences from the URA3 gene (UR), the splice donor site (5’ SD) of an artificial intron and the HO cut site. 108-bp donor sequences...
containing different mismatch distributions were assembled into a plasmid containing the 3’
sequences from the \textit{URA3} gene (A3), the 3’ splice-acceptor (3’ SA) of the intron and the \textit{TRP1}
auxotrophic maker. A DSB was created using the galactose-inducible HO endonuclease. This
break is repaired by BIR using the donor sequences that share 108-bp of homology located on
the opposite arm of chromosome V. Once BIR is complete, a functional intron is formed, and
yeast become Ura3\textsuperscript{+} recombinants. \textbf{B.} Schematic diagram of possible recombination outcomes
in the diploid system: (1) intrachromosomal break-induced replication (BIR) by using the donor
sequence sharing 108-bp homology located on the opposite arm of Chromosome V to produce
NAT\textsuperscript{-} Ura\textsuperscript{+} outcomes; (2) interchromosomal gene conversion (GC) replaces the entire insertion
in the left arm of the chromosome, including the beginning of the \textit{URA3} gene (UR) and the
splice donor site (5’S\textsubscript{D}) of an artificial intron and an adjacent NAT resistance marker , to
produce NAT\textsuperscript{-} Ura\textsuperscript{-} outcomes; (3) complete loss of the broken chromosome to produce NAT\textsuperscript{-}
Ura\textsuperscript{-} Trp\textsuperscript{-} outcomes.

\textbf{Figure 2. The effect of sequence divergence on mitotic and meiotic diploids in Dmc1-mediated
break-induced replication (BIR)}

\textbf{A.} Percent viability for haploid mitotic and diploid mitotic and meiotic cells and % sporulation
for diploid meiotic cells carrying mismatches every 12th, every 9th, and every 6th bp across the
108-bp donor template. \textbf{B.} Percent BIR efficiency of haploid and diploid mitotic and meiotic
cells carrying mismatches every 12th, every 9th, and every 6th bp across the 108-bp donor
template. Significance determined using an unpaired t-test with ordinary one-way ANOVA
comparing every 12th, every 9th, and every 6th arrangement to perfect homology (no mismatch). Error bars show the standard deviation.

**Figure 3. Rad51 is more tolerant of mismatches than Dmc1 in diploid meiotic cells.**

**A.** The ratios of Ura+ BIR events in the templates containing mismatches (every 12th, every 9th, and every 6th) versus no mismatches in haploid mitotic, diploid mitotic and diploid meiotic. **B.** Percent BIR efficiency of WT, hed1Δ, and dmc1Δ hed1Δ in the templates containing mismatches every 9th and no mismatches (WT). **C.** The ratios of Ura+ BIR events in the templates containing mismatches every 9th versus no mismatches in diploid meiotic for WT, hed1Δ, and dmc1Δ hed1Δ. Significance determined using ordinary one-way ANOVA multiple comparisons. Error bars refer to the standard deviation. **D.** Percent mismatch incorporation in the donor template into BIR products for donor containing mismatches every 9th in WT, hed1Δ, and dmc1Δ hed1Δ mutants. A minimum of 40 DNA samples were sequenced.

**Figure 4. Mismatch assimilation in meiosis depend on DNA polymerase δ**

**A.** Percent assimilation of mismatches in the donor template into BIR products, for donors containing mismatches every 12th, every 9th, and every 6th. A minimum of 40 samples were DNA sequenced **B.** Percent mismatch incorporation of diploid meiotic cells containing mismatches every 9th and no mismatches with proofreading-defective DNA Polymerase δ mutant (pol3-01)
Figure 5. Msh2 act as a negative modulator on diploid meiotic cells containing heterologous template.

A. Percent BIR efficiency of WT and $msh2^\Delta$ versions in the templates containing mismatches every 9th versus no mismatches in haploid mitotic, diploid mitotic, and diploid meiotic. B. The ratio of BIR efficiency in $msh2^\Delta$ in the template containing mismatches every 9th base. C. Percent mismatch incorporation of WT versus $msh2^\Delta$ in haploid mitotic, diploid mitotic, and diploid meiotic. D. Percent of incorporated mismatches versus of heteroduplex formation in $msh2^\Delta$ in haploid mitotic, diploid mitotic, and diploid meiotic. Significance determined using an unpaired t-test. Error bars refer to the standard deviation.

Figure 6. The effect of MSH2 deletion in Rad51-mediated ($dmc1^\Deltahed1^\Delta$) meiotic recombination.

A. Percent BIR efficiency of WT, $msh2^\Delta$, $dmc1^\Delta hed1^\Delta$, and $dmc1^\Delta hed1^\Delta msh2^\Delta$ in diploid meiotic cells. B. The ratio of BIR efficiency in $msh2^\Delta$ and $dmc1^\Delta hed1^\Delta msh2^\Delta$ in the template containing mismatches every 9th. C. Percent mismatch incorporation of WT and mutants for the template containing mismatches every 9th in diploid meiotic. Significance determined using an unpaired t-test. Error bars refer to the standard deviation.

Supplement Figure 1. Mechanism of mismatch incorporation of heteroduplex DNA formation during BIR

Once a DSB is created, a broken end of DSB will be resected by 5’$\rightarrow$3’ exonuclease to generate 3’ single-stranded DNA (ssDNA) which interact with several recombination proteins to carry out
homology search and strand invasion. Since both donor and recipient sequence share a 108-bp homology, the broken and resected end of recipient sequence (indicated in red) will find this homology and initiate strand invasion into donor sequence (indicated in blue). Mismatches are created in the heteroduplex region during strand invasion and polymerase δ get recruited to perform its proofreading 3’→5’ exonuclease activity. DNA Polymerase δ “backs up” into the heteroduplex region and resynthesizes the region in reference to donor templates to incorporate mismatches. BIR completes and results in BIR products with mismatches close to the 3’ end frequently being incorporated.

**Supplement Figure 2.** A. Percent viability of diploid mitotic cells in the template containing mismatches every 9th and no mismatches. The right upper graph with blue bars show % viability for all three possible repair outcomes in templates with no mismatches in our system; interchromosomal break-induced replication (BIR), non-homologous end joining (NHEJ), and intrachromosomal gene conversion (GC). The right bottom graph with red bars shows templates with mismatches every 9th. B. Percent viability of msh2Δ of no mismatch and every 9th bp mismatched templates in haploid and diploid mitotic. Percent sporulation of msh2Δ of no mismatch and every 9th bp mismatched templates in diploid meiotic.

**Supplement Figure 3.** A. Percent sporulation of WT and dmc1Δ hed1Δ for no mismatch versus every 9th bp mismatched. B. Percent sporulation of no mismatch versus every 9th bp mismatched in mutant strains. Significance determined using an unpaired t-test. Error bars
refer to the standard deviation. C. Percent BIR of WT and pol3-01 for no mismatch versus every 9th mismatch.

Supplement Figure 4. A model for Dmc1-mediated BIR versus Rad51-mediated BIR during meiotic recombination

In wild type cells during meiosis, Hed1 inhibits the activity of Rad51 by preventing the assembly of Rad51-Rad54 complex required for Rad51-mediated recombination. This allows both Dmc1 and Rad51 to cooperate to mediate efficient repair during meiotic recombination. Deletion of Hed1 and Dmc1 restores the activity of Rad51 and allows efficient Rad51-mediated recombination during meiosis.

Reference


Figure 1

A. Functional intron-containing URA3 gene

B. Intrachromosomal BIR (Ura⁺ NAT Trp⁺) Interchromosomal GC (Ura⁺ NAT Trp⁺) Chromosome loss (Ura⁺ NAT Trp⁺)
Figure 3

A.

Every 12th

Every 9th

Every 6th

B. Diploid Meiotic: No MM

Diploid Meiotic: Every 9th

C. Diploid Meiotic: Every 9th

D. Diploid Meiotic: Every 9th

% BIR

WT
hed1Δ

% MM Incorporation

100 80 60 40 20 0

108 99 90 81 72 63 54 45 36 27 18 9 0

Mismatch every 9th

WT
hed1Δ
dmc1Δ hed1Δ
Figure 4

A. Haploid Mitotic

B. Diploid Mitotic

C. Diploid Meiotic

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Figure 5

A. Haploid Mitotic

No MM  |  Every 9th

% BIR

WT  |  mshΔ |  WT  |  mshΔ

No MM  |  Every 9th

% BIR

ns

B. Every 9th: mshΔ

M. No MM ratio

MM: No MM ratio

C. Haploid Mitotic

100  |  % MM Incorporation

10  |  Mismatched every 9th

WT  |  mshΔ

D. Haploid Mitotic

100  |  % Incorporation

M. No MM ratio

MM: No MM ratio

100  |  % Asymmetric

M. No MM ratio

MM: No MM ratio
Figure 6

A. Diploid Meiotic: No MM

B. Every 9th

C. % MM Incorporation

Mismatched every 9th
Supplement Figure 1.

The 3' end of recipient strand invade

Mismatch occurs between recipient and donor

3'→5' exonuclease activity of DNA Pol δ resects invading end

New DNA synthesis

BIR products
Supplement Figure 2.

A. Mitotic Diploid % Viability

B. Haploid Mitotic msh2Δ, Diploid Mitotic msh2Δ, Diploid Meiotic msh2Δ
Supplement Figure 3.

A. % Sporulation

B. 

- dmc1Δ hed1Δ
- dmc1Δ hed1Δ msh2Δ
- pol3-01

C. 

- Diploid Meiotic

% BIR

- No MM
- Every 9th
Supplement Figure 4.

Dmc1-Mediated Recombination

Rad51 and Rad51 cooperate to mediate efficient repair

Rad51-Mediated Recombination

Rad51 mediates efficient repair