INVESTIGATION

Pch2 Modulates Chromatid Partner Choice During Meiotic Double-Strand Break Repair in Saccharomyces cerevisiae

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ABSTRACT In most organisms, the segregation of chromosomes during the first meiotic division is dependent upon at least one crossover (CO) between each pair of homologous chromosomes. COs can result from chromosome double-strand breaks (DSBs) that are induced and preferentially repaired using the homologous chromosome as a template. The PCH2 gene of budding yeast is required to establish proper meiotic chromosome axis structure and to regulate meiotic interhomolog DSB repair outcomes. These roles appear conserved in the mouse ortholog of PCH2, Trip13, which is also involved in meiotic chromosome axis organization and the regulation of DSB repair. Using a combination of genetic and physical assays to monitor meiotic DSB repair, we present data consistent with pch2 mutants showing defects in suppressing intersister DSB repair. These defects appear most pronounced in dmc1 mutants, which are defective for interhomolog repair, and explain the previously reported observation that pch2 dmc1 cells can complete meiosis. Results from genetic epistasis analyses involving spo13, rad54, and mek1/MEK1 alleles and an intersister recombination reporter assay are also consistent with Pch2 acting to limit intersister repair. We propose a model in which Pch2 is required to promote full Mek1 activity and thereby promotes interhomolog repair.

DNA double-strand breaks (DSBs) that occur during vegetative growth are preferentially repaired via homologous recombination in which the Rad51 recombinase and its partner Rad54 mediate strand exchange with the sister chromatid. This intersister repair occurs even in diploid cells where a homologous chromosome template is available and is thought to help prevent chromosome rearrangements (Kadyk and Hartwell 1992; M. Shinohara et al. 1997; Arbel et al. 1999; Krogh and Symington 2004). In meiosis, formation of programmed DSBs and their repair using the homologous chromosome as a template is essential for the production of viable gametes (Roeder 1997). Although Rad51 and Rad54 are still present, meiotic interhomolog strand exchange is accomplished by their respective orthologs, Dmc1 and Rdh54 (Dresser et al. 1997; Klein 1997; A. Shinohara et al. 1997; M. Shinohara 1997; Arbel et al. 1999; Hollingsworth 2010). Interhomolog DSB repair creates linkages provided by genetic exchanges, or crossovers (COs), between homologous chromosomes. In many organisms, these COs are required for reductional chromosome segregation at the meiosis I (MI) division, which lowers cell ploidy by one-half, allowing for the generation of haploid gametes (Roeder 1997). If any pair of homologous chromosomes fails to receive a CO, MI nondisjunction can occur and produce aneuploid gametes, which cause conditions such as Down syndrome or infertility in humans (Hasbold et al. 2007). During meiotic prophase I in budding yeast, ~140–170 DNA DSBs are introduced into the genome by a group of 10 proteins, of which Spo11 is the catalytic component (Blitzblau et al. 2007; Buhler et al. 2007; Mancera et al. 2008). Although COs are the only repair products known to promote MI disjunction, only ~50% of DSBs in yeast meiosis are repaired as interhomolog COs. Some DSBs are repaired using the homologous chromosome without producing a CO; this is known as a noncrossover (NCO). Obligate CO formation, CO interference, and CO homeostasis are manifestations of interhomolog DSB repair regulation acting to ensure each pair of homologous chromosomes disjoins at
MI (Bishop and Zickler 2004; Borner et al. 2004; Martini et al. 2006; Blitzblau et al. 2007; Buhler et al. 2007; Chen et al. 2008; Mancera et al. 2008; Berchowitz and Copenhaver 2010). The obligate CO refers to the observation that all homologous chromosome pairs receive at least one CO. CO interference describes the nonrandom, evenly spaced distribution of CO events, and CO homeostasis describes the finding that CO levels are maintained as DSB frequencies decrease (reviewed in Jones and Franklin 2006; Berchowitz and Copenhaver 2010). Little is known about the mechanisms or relatedness of the different aspects of CO control, although one mutant, pch2Δ, has decreased CO interference and may also be defective for CO homeostasis (Joshi et al. 2009; Zanders and Alani 2009).

The ~10–33% of meiotic DSBs estimated to not be repaired using a homologous chromosome are repaired by homologous recombination using the sister chromatid as a template (e.g., Goldfarb and Lichten 2010). The shift in DSB repair template preference from the sister chromatid in the mitotic cell cycle to the homologous chromosome in meiosis is referred to as “interhomolog bias” (Jackson and Fink 1985; Schwacha and Kleckner 1994, 1997; Wan et al. 2004; Webber et al. 2004; Niu et al. 2005, 2007, 2009; Goldfarb and Lichten 2010). Interhomolog bias is established shortly after DSB formation and requires components of the axial elements, which are linear structures that form along each pair of sister chromatids early in meiotic prophase (Hollingsworth 2010). An early step in instituting interhomolog bias is phosphorylation of Hop1 of the Hop1/Red1 axial element duo by the Mec1 and Tel1 kinases (Hollingsworth and Byers 1989; Hollingsworth et al. 1990; Rockmill and Roeder 1990; Carballo et al. 2008). Red1 and phosphorylated Hop1 are required for the activation of the effector kinase Mek1 (Niu et al. 2005, 2007; Carballo et al. 2008). Mek1 appears to directly promote interhomolog repair (Terentyev et al. 2010). In addition, Mek1 phosphorylates Rad54, which inhibits the interaction between Rad51 and Rad54 (Niu et al. 2009). Phosphorylation of Rad54 contributes to, but is not sufficient for, complete interhomolog bias (Niu et al. 2009). The meiosis-specific protein Hed1 also acts to prevent Rad51–Rad54 complex formation by competing with Rad54 for Rad51 binding, although the role of Hed1 in interhomolog bias is yet to be determined (Tsubouchi and Roeder 2006; Bussygina et al. 2008). Interhomolog bias is maintained in haploid meiosis and inhibits DSB repair, suggesting that interhomolog interactions are not required (Demassy et al. 1994; Callender and Hollingsworth 2010). At hemizygous DSB sites in diploid meiosis, intersister repair is constrained by a Mek1-dependent delay, although efficient intersister DSB repair does occur (Goldfarb and Lichten 2010).

The mechanisms promoting interhomolog bias are often studied in dmc1 null mutant backgrounds in which unrepaired DSBs trigger the meiotic recombination checkpoint to arrest cells at pachytene, the last stage of meiotic prophase before cells are committed to undergo the MI division (Hollingsworth 2010). There are two ways in which recombination checkpoint arrest can be overcome in a dmc1 mutant background. The first is to eliminate any of the essential recombination checkpoint genes such as MEC1, RAD17, or RAD24. In such cases, meiosis proceeds with unrepaired breaks to form inviable gametes (Lydall et al. 1996). The second is to eliminate (or reduce; see below) the checkpoint-eliciting DNA lesions either by preventing DSB formation or by allowing inappropriate Dmc1-independent DSB repair (Bishop et al. 1992, 1999; Schwacha and Kleckner 1994, 1997; Xu et al. 1997; Thompson and Stahl 1999). The latter can be accomplished by several mechanisms. Overexpressing RAD51 or RAD54 and/or mutating HED1 in a dmc1 background allows for meiotic progression and the production of moderate to wild-type levels of interhomolog COs and thus of viable spores (Bishop et al. 1999; Tsubouchi and Roeder 2003, 2006; Busygina et al. 2008). Alternatively, when RED1, HOP1, or MEK1 are mutated, interhomolog bias is lost and DSBs are rapidly repaired via Rad51–Rad54-dependent strand exchange using the sister chromatid as a template, and meiosis progresses to produce inviable spores (Bishop et al. 1999; Wan et al. 2004; Niu et al. 2005, 2007).

Pch2 (pachytene checkpoint) is a putative AAA ATPase that promotes the checkpoint arrest/delays observed in zip1, rad17, mms4, and sac2 recombination mutants. The pch2Δ mutation also suppressed the dmc1Δ checkpoint arrest in some but not all studies (San-Segundo and Roeder 1999; Zierhut et al. 2004; Hochwagen et al. 2005; Wu and Burgess 2006; Mitra and Roeder 2007; Zanders and Alani 2009). In budding yeast, Pch2 is also required for wild-type kinetics of meiotic progression, CO interference, and establishing proper organization of Hop1 and Zip1 on meiotic chromosomes (Sym and Roeder 1995; Borner et al. 2008; Joshi et al. 2009; Zanders and Alani 2009). Several of these roles appear conserved in the mouse PCH2 ortholog Trip13, which is required for wild-type levels of DSB repair, wild-type CO distribution, and proper organization of HORMADs (which share homology with Hop1) and the synaptonemal complex central element protein SYCP1 on meiotic axes (Li and Schimenti 2007; Wojtasz et al. 2009; Roig et al. 2010).

Here we investigated the mechanisms by which the pch2Δ mutation suppresses the meiotic arrest/delay phenotypes of dmc1Δ mutations. First, we found that lowering DSB levels in dmc1Δ mutants reduced the fraction of cells that arrest, indicating that the recombination checkpoint is sensitive to DSB levels. Second, we found that Pch2 inhibited some DSB repair in dmc1Δ cells that likely includes, but may not be limited to, intersister recombination. Third, we identified genetic interactions between PCH2 and RAD54 and PCH2 and MEK1 that support a role for Pch2 in limiting intersister repair. Finally, we present a genetic assay that demonstrates an increase in intersister repair at one locus in pch2Δ mutants. We synthesize our data with published results to propose a model in which Pch2 is required for full Mek1 activity.
Materials and Methods

Media and yeast strains

All yeast strains (Table 1) were grown at 30°C on yeast peptone dextrose (YPD) supplemented with complete amino acid mix, synthetic complete, or synthetic complete −histidine (Argueso et al. 2004). All strains were sporulated at 30°C. The sporulation media and sporulation conditions used to generate the data in Tables 2–4 were described previously (Zanders and Alani 2009). Differences in spore formation and viability were analyzed by an χ² test in which P-values <0.05 were considered statistically significant. Geneticin (Invitrogen), nourseothricin (Hans-Knoll Institute für Naturstoff-Forschung), and hygromycin B (Calbiochem) were added in standard concentrations to YPD media when required (Wach et al. 1994; Goldstein and McCusker 1999).

Strains described in Tables 2 and 3 are isogenic to the NHY943 or NHY942 SK1 strains described in de los Santos et al. (2003). The spo11 hypomorphic mutants and the NHY943 strains containing these alleles are described in Martini et al. (2006). As in Martini et al. (2006), we refer to spo11-HA3His6 as spo11-HA. The dmc1Δ and rad54Δ alleles used in this work were all complete open reading frame deletions. The pch2Δ allele contains a deletion of amino acids 17–587 (Zanders and Alani 2009). All deletion cassettes were made via PCR, and the deleted regions were replaced with HPHMX4, KANMX4, or NATMX4 as shown in Table 1. A BamHI fragment of pNKYS8 was integrated into the genome to create the spo13::hisG-URA3-hisG mutation, and a BglII to EcoRI fragment of pNKYS49 was used to replace RAD50 with rad50s::URA3 (Alani et al. 1990). All mutations were initially integrated into the genome using standard transformation techniques (Gietz et al. 1995). Standard genetic crosses were used to generate the various mutant combinations. Details on strain construction and primer sequences are available upon request.

The pch2Δ spo11-HA mekl-as and pch2Δ spo11-HA MEK1-GST strains presented in Table 4 were constructed as follows. The mekl-as strain was constructed by digesting the plasmid pJR2 with RsrII and then by transforming the mekl-Q241G::URA3 segment into the ura3 mekl1Δ SK1 diploid YTS1 (plasmid and strain provided by Nancy Hollingworth). The diploid was then tetrad-dissected, selecting for Ura+ haploid segregants. The homozygous MEK1-GST diploid SK1 strain SBY2901 (provided by Sean Burgess) was also tetrad-dissected to obtain haploid segregants. The mekl-as and MEK1-GST segregants described above were mated to EAY2581 (pch2Δ), EAY2263 (pch2Δ spo11-HA), and SKY635 (spo11-HA). The resulting diploids were sporulated and tetrad-dissected to obtain the haploids in Table 1 that were then mated to create diploids that were tetrad-dissected.

To create the strains used in the sister-chromatid exchange assays, the HIS3 gene was deleted from a haploid segregant of the SK1 diploid EAY28 to create EAY2908. A cross of EAY2908 by EAY2209 (pch2Δ in NHY943) (Zanders and Alani 2009) generated EAY2910 and EAY2913. The HIS3 sister-chromatid recombination reporter assay contained on plasmid pNN287 (provided by Mike Fasullo) was integrated into the genome near TRP1 in EAY2913, as described by Fasullo and Davis (1987), to create EAY2918. Correct integration of the sister-chromatid recombination assay in EAY2918 was confirmed using Southern blot analysis. EAY2918 was then crossed to EAY2910 to generate strains EAY2951 and EAY2952 (wild type) and EAY2955 and EAY2956 (pch2Δ) used in the sister-chromatid recombination experiments.

To measure sister-chromatid recombination, saturated YPD overnight cultures were diluted into 22 ml YPA and grown for 17 hr. A sample of each YPA culture (0.4–2 ml) was plated on synthetic complete −His plates to detect early mitotic sister-chromatid recombination events that would skew meiotic analyses. No such His+ jackpots were observed in cells plated from YPA cultures (generally, fewer than one His+ cell/ml plated was observed for all strains).

Correct integration of the sister-chromatid recombination assay in EAY2918 was confirmed using Southern blot analysis. EAY2918 was then crossed to EAY2910 to generate strains EAY2951 and EAY2952 (wild type) and EAY2955 and EAY2956 (pch2Δ) used in the sister-chromatid recombination experiments.

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After 17 hr, the YPA cultures were spun down, washed once in 1% potassium acetate, resuspended in 10 ml 1% potassium acetate, and then allowed to sporulate 24 hr. Undiluted sporulated cells were then plated on synthetic complete −His, and cell dilutions were plated on synthetic complete media. The frequency of His+ colony-forming units (cfu) (His+ prototrophy in sporulated cells in which spores in asci were not separated) was found by dividing the number of His+ cfu/ml by the total number of colony-forming units per millimeter. Experimental replicates in which <90% of cells sporulated were not included in the data presented.

Meiotic time courses and DSB Southern blotting

For the time courses to analyze meiotic DSB levels, 0.3 ml (for RAD54 strains) or 0.6 ml (for rad54Δ strains) of a saturated YPD overnight culture from each strain to be analyzed was diluted into 200 ml YPA (2% potassium acetate) plus complete amino acid mix and grown for 17 hr. The YPA culture was then spun down, washed once in 1% potassium acetate, and resuspended in 100 ml 1% potassium acetate (Zanders and Alani 2009). All strains were grown in the same batches of media and treated identically. DNA was isolated from meiotic cultures as in Buhler et al. (2007) for dmc1Δ strains and as in Goyon and Lichten (1993) for rad50S strains. The percentage of DSBs was calculated using Image Quant software. In this analysis, a lane profile was generated and used to calculate the total lane signal. Lane background was determined from the blot regions below DSB signals. Only the peaks above lane background were quantified as DSB-specific signals.

Results

pch2Δ suppresses dmc1Δ arrest by allowing DSB repair

We initiated this study in SK1 budding yeast to examine a role for Pch2 in ensuring a meiotic arrest/delay in the absence of Dmc1 (San-Segundo and Roeder 1999; Zierhut et al. 2004;
Hochwagen et al. 2005; Wu and Burgess 2006; Mitra and Roeder 2007; Zanders and Alani 2009). On the basis of recent work showing a role for Pch2 in regulating crossing over in meiosis, we and others hypothesized that Pch2 acts directly in DSB repair (Hochwagen et al. 2005; Wu and Burgess 2006; Borner et al. 2008; Joshi et al. 2009; Zanders...
and Alani 2009). In such a model, pch2Δ relieves the dmc1Δ arrest by allowing Dmc1-independent DSB repair. Below are physical and genetic studies that are consistent with Pch2 inhibiting DSB repair in dmc1Δ cells.

We analyzed visible meiotic DSBs at the YCR048W (chromosome III) and HIS2 (chromosome VI) hotspots in pch2Δ, dmc1Δ, spo11-HA, rad50S, and rad54Δ strain backgrounds. Six hours after meiotic induction, dmc1Δ mutants averaged 11.7 ± 3.0% (±SD; n = 4 independent cultures) DSBs at YCR048W. pch2Δ dmc1Δ (9.2 ± 1.0%, n = 2) and spo11-HA dmc1Δ (7.3 ± 3.0%, n = 2) mutants displayed slightly fewer DSBs at this hotspot. Consistent with previous results, the pch2Δ spo11-HA dmc1Δ triple mutant showed lower DSB levels (2.7 ± 0.7%, n = 4; Figure 1A; Zanders and Alani 2009). A similar pattern of DSBs was observed at the HIS2 DSB hotspot on chromosome VI (Figure 1B) (Bullard et al. 1996).

One explanation for the reduced level of breaks observed in pch2Δ spo11-HA dmc1Δ mutants is that fewer DSBs are formed in pch2Δ mutants. Previous genetic analyses, however, suggest that pch2Δ mutants do not form fewer DSBs; pch2Δ mutants have increased COs on large chromosomes and increased gene conversion frequencies on chromosomes of all sizes. The opposite effect would be expected from a mutant with reduced DSB frequencies (Zanders and Alani 2009). To formally test if pch2Δ spo11-HA dmc1Δ mutants affect DSB formation, we measured DSBs in the rad50S mutant background in which they persist (Alani et al. 1990). At the YCR048w hotspot, rad50S and pch2Δ rad50S mutants showed similar average DSB levels: 8.7% (same value in

![Figure 1 A](image-url) DSBinduced by dmc1Δ mutants averaged 11.7 ± 3.0% (±SD; n = 4 independent cultures) DSBs at YCR048W. pch2Δ dmc1Δ (9.2 ± 1.0%, n = 2) and spo11-HA dmc1Δ (7.3 ± 3.0%, n = 2) mutants displayed slightly fewer DSBs at this hotspot. Consistent with previous results, the pch2Δ spo11-HA dmc1Δ triple mutant showed lower DSB levels (2.7 ± 0.7%, n = 4; Figure 1A; Zanders and Alani 2009). A similar pattern of DSBs was observed at the HIS2 DSB hotspot on chromosome VI (Figure 1B) (Bullard et al. 1996).

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Table 2 Spore formation efficiency and viability in pch2Δ mutants

<table>
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<tr>
<th>Genotype</th>
<th>% sporulation</th>
<th>No. analyzed</th>
<th>% spore viability</th>
<th>Spores analyzed</th>
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<td>93.5</td>
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Sporulation efficiencies for the above strains were counted after 5 days on sporulation media at 30°. Tetrad strains (for SPO13 strains) or dyads (from spo13 strains) were dissected on YPD and scored for spore viability after 3 days. NA indicates that the percentage of spore viability is not applicable for strains that do not sporulate. ND indicates that spore viability was not assayed.

two independent experiments) and 7.8 ± 0.4%, respectively (n = 2; Figure 1C). The spo11-HA rad50S (5.8 ± 1.1%) and pch2Δ spo11-HA rad50S mutants (5.3 ± 1.6%) also showed similar levels of DSBs, although the levels were lower than rad50S alone, as expected because of the presence of spo11-HA (n = 2; Figure 1C). These results suggest that the decrease in DSBs observed in pch2Δ spo11-HA dmc1Δ was not due to a decrease in DSB formation.

Low levels of DSBs were observed in pch2Δ spo11-HA dmc1Δ. Interestingly, these strains showed a dramatic increase in meiotic completion—39% spore formation, compared to 0% in dmc1Δ strains, which showed high levels of DSBs (Bishop et al. 1999) (Table 2; P < 0.005; Figure 1). The pch2Δ and spo11-HA mutations contribute synergistically to the triple-mutant phenotype because only a small percentage of pch2Δ dmc1Δ (4.6%) and spo11-HA dmc1Δ (0.4%) cells formed spores. One explanation for the phenotype is that the recombination checkpoint is sensitive to the level of unrepaired breaks (see Discussion), such that fewer breaks elicit a less robust checkpoint arrest. Consistent with this interpretation, dmc1Δ/dmc1Δ spo11-HA/spo11yf-HA (30% of wild-type DSB levels) have increased meiotic progression [dmc1Δ, 0% spore formation; spo11-HA/spo11yf-HA dmc1Δ/dmc1Δ, 5% spore formation (Table 2; P < 0.005)].

Spores produced by pch2Δ dmc1Δ and spo11-HA pch2Δ dmc1Δ were mostly inviable (<3% spore viability for each), suggesting that interhomolog recombination was not restored in these mutants (Table 2). We tested whether other types of repair occurred in the spo13 mutant background in which a mixed chromosome division occurs: some chromosomes undergo an equalizational division whereas others segregate reductionally (Klapholz and Esposito 1980; Hugerat and Simchen 1980). spo13 mutants can produce viable meiotic progeny in the absence of meiotic DSBs or if DSB repair does not yield COs (e.g., Malone and Esposito 1981). Because of this, spore viability analyses in the spo13 mutant background can detect DSB repair that does not facilitate proper MI chromosome segregation (Bishop et al. 1999).

Similar to previous work in SK1 strains, spo13 dmc1Δ showed low levels of sporulation (10%) and spore viability
(7%) compared to spo13 (63% sporulation, 47% viability; Table 2; P < 0.005 for both sporulation and spore viability) (Bishop et al. 1999). Introducing the spo11-HA allele to dmc1Δ spo13 increased sporation formation in the resulting triple mutant to 17% and spore viability to 16% (Table 2; P < 0.005 for both sporulation and spore viability). This result is expected because fewer DSBs are produced in spo11-HA strains (Martini et al. 2006; Johnson et al. 2007). Deleting PCH2 in dmc1Δ spo13 had a strong effect; sporulation in this triple mutant increased to 43% and spore viability increased to 16% (Table 2; P < 0.005 for both sporulation and spore viability). The spo11-HA pch2Δ spo13 dmc1Δ quadruple mutant showed even greater sporulation (58%) and spore viability (26%; Table 2; P < 0.005 for both sporulation and spore viability). These results are consistent with some Dmc1-independent repair occurring in pch2Δ dmc1Δ mutants.

**pch2Δ mutants have an increased dependence on Rad54-mediated repair**

Sporulation of pch2Δ spo11-HA dmc1Δ mutants and spore viability of the pch2Δ spo11-HA dmc1Δ spo13 mutants were Rad54-dependent, suggesting that Rad54 is repairing breaks in these mutants (Table 2). During vegetative growth, DSBs are preferentially repaired by homologous recombination involving sister chromatids in steps that are mediated by the Rad51 recombinase and its partner Rad54 (Kadyk and Hartwell 1992; M. Shinohara et al. 1997; Arbel et al. 1999; Krogh and Symington 2004). Consistent with Rad54-dependent recombination in pch2Δ mutants, we observed a reduction in sporulation (46%) and spore viability (47%) in the pch2Δ rad54Δ double mutant compared to pch2Δ (81% sporulation and 95% spore viability) and rad54Δ single mutants (58% sporulation and 66% spore viability; Table 2; P < 0.005 for all comparisons). Interestingly, CO levels were not reduced in pch2Δ rad54Δ compared to pch2Δ, suggesting the Rad54-dependent recombination in pch2Δ mutants is intersister (Table 3).

An important prediction of the above genetic analyses is that the rad54Δ mutation should result in a restoration of observed DSBs in the pch2Δ spo11-HA dmc1Δ mutant. This prediction was not met; 4.6 ± 1.4% DSBs (n = 3) were observed at YCRO48W in pch2Δ spo11-HA dmc1Δ rad54Δ, compared to 2.7 ± 0.7% (n = 4) in pch2Δ spo11-HA dmc1Δ and 10.3 ± 1.5% (n = 3) in spo11-HA dmc1Δ rad54Δ 6 hr after meiotic induction (Figure 1). It is possible that both Dmc1- and Rad54-independent repair can occur in the absence of these factors in a pch2Δ background. Another possibility is that hyper-resected DSBs form in pch2Δ spo11-HA dmc1Δ rad54Δ that cannot be detected by Southern blot. More experimentation is needed to understand this phenotype.

**pch2Δ spo11-HA phenotype is modulated by Mek1 activity**

Previously, we showed (Zanders and Alani 2009) that pch2Δ spo11-HA strains have a spore viability defect and hypothesized that the decreased spore viability was due to defects in CO interference and partner choice. If a compromised interhomolog bias contributes to the pch2Δ spo11-HA phenotype, then further undermining interhomolog bias should enhance the phenotype. Alternatively, reinforcing interhomolog bias should suppress the pch2Δ spo11-HA phenotype. To test this hypothesis, we utilized two MEK1 alleles: mek1-as (mek1-Q241G hypomorph) (Callender and Hollingsworth 2010) and MEK1-GST (hypermorph) (Wu et al. 2010). mek1-as strains complete meiosis efficiently in the absence of a 1-Na-PP1 inhibitor and display nearly wild-type spore viability; however, Mek1-as has reduced affinity for ATP in vitro, and in one dmc1Δ strain background, the mek1-as
mutation conferred phenotypes consistent with defects in interhomolog bias (Wan et al. 2004; Niu et al. 2009; Callender and Hollingsworth 2010). Wu et al. (2010) recently showed that MEK1-GST is a semidominant allele that shows increased interhomolog recombination events, primarily noncrossovers, and fewer intersister events, with no change in DSB levels.

As shown in Table 4, we constructed mek1-as pch2Δ spo11-HA and MEK1-GST pch2Δ spo11-HA strains and examined their spore formation efficiency and viability (mek1-as strains were analyzed in the absence of a 1-Na-PP1 inhibitor). We found that the mek1-as mutation reduced the spore viability of pch2Δ spo11-HA strains from 57 to 41% (P < 0.005). The mek1-as mutation did not significantly affect the spore viability of either single mutant alone (Table 4). The MEK1-GST allele increased spore viability in pch2Δ spo11-HA strains from 57 to 89% (P < 0.005); however, as described previously (Wu et al. 2010), there was a general defect in spore formation due to MEK1-GST. MEK1-GST did not increase the spore viability in either single mutant (Table 4). These data are consistent with our hypothesis that excess intersister repair contributes to spore inviability of pch2Δ spo11-HA.

**Evidence that Pch2 promotes interhomolog DSB repair**

To more directly test if there is an increase in intersister repair in pch2Δ, we adapted an assay developed by Fasullo and Davis (1987) to measure sister-chromatid exchange in meiosis. This assay utilizes a HIS3 reporter gene in which cells become His+ if a sister-chromatid recombination event (either a CO or gene conversion) occurs between two his3 truncations to produce full-length HIS3. The mean frequency of His+ colonies was 1.6 × 10^{-6} (n = 22 independent cultures) in wild type and 5.3 × 10^{-6} in cells lacking Pch2 (n = 24). pch2Δ values were significantly higher than wild type (P = 0.008, Mann–Whitney U-test).

**Discussion**

We provide several independent lines of evidence consistent with a role for Pch2 in inhibiting Dmc1-independent intersister DSB repair in meiosis. First, pch2Δ contributes to a reduction of unrepairable DSBs visible on Southern blots in spo11-HA dmc1Δ mutants (Figure 1). This reduction in detectable DSBs appears to be due to DSB repair because the levels of DSBs formed, as measured in a rad50Δ background, are not affected by pch2Δ and the spore inviability seen in pch2Δ dmc1Δ and pch2Δ spo11-HA dmc1Δ mutants is suppressed by spo13Δ (Table 2). We hypothesize that the DSB repair occurring in pch2Δ spo11-HA dmc1Δ is intersister because it does not facilitate proper MI chromosome segregation. Our epistasis analysis of pch2Δ rad54Δ suggests that pch2Δ mutants are more dependent on Rad54-dependent repair, but that Rad54 does not appear to contribute to intersister DSB repair in pch2Δ. These data further support the idea that pch2Δ mutants have increased intersister repair. In addition, we demonstrated that a mek1 hypomorph enhanced the spore death phenotype of pch2Δ spo11-HA whereas a MEK1 gain-of-function allele suppressed the phenotype. These experiments are consistent with excess intersister DSB repair contributing to spore inviability of pch2Δ spo11-HA. Finally, our genetic reporter assay demonstrated an increase in intersister DSB repair at one locus.

Our data and those of other groups are consistent with recombination checkpoint signaling being sensitive to unrepairable DSB levels, such that more DSBs trigger checkpoint arrest in a greater proportion of the cell population (Malkova et al. 1996; Bhalla and Dernburg 2005; MacQueen et al. 2005; Johnson et al. 2007; Callender and Hollingsworth 2010; Goldfarb and Lichten 2010). In wild-type cells, DSBs are quickly repaired and the Mek1-mediated checkpoint delay is transient. In dmc1Δ, DSBs are not repaired and Mek1 elicits checkpoint arrest. When DSBs are reduced in dmc1Δ strains containing spo11 hypomorphic alleles, the checkpoint response is less robust and fewer cells arrest. We hypothesize that the spo11-HA and pch2Δ mutations independently contribute to reducing the level of unrepairable DSBs available to trigger the recombination checkpoint in dmc1Δ mutants: spo11-HA forms fewer DSBs and pch2Δ acts by allowing Dmc1-independent repair. In this model, the combination of spo11-HA and pch2Δ in a dmc1Δ background synergistically contribute to DSB repair due to a positive feedback loop wherein fewer DSBs elicit less checkpoint activation, which allows even more DSB repair and meiotic progression. At present we do not have a good sense of the number of DSBs that would be needed to elicit checkpoint activation; however, work from Malkova et al. (1996) showed that a single unrepairable DSB does not arrest meiosis.

Pch2 acts in meiotic CO control to limit CO formation on large chromosomes and promote CO interference (Joshi et al. 2009; Zanders and Alani 2009). This work suggests a broader role for Pch2 in DSB repair that includes inhibiting

### Table 4 Spore formation efficiency and viability in mek1-as and MEK1-GST mutants

<table>
<thead>
<tr>
<th>Genotype</th>
<th>% sporulation</th>
<th>No. analyzed</th>
<th>% spore viability</th>
<th>Spores analyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>79.1</td>
<td>436</td>
<td>93.5</td>
<td>400</td>
</tr>
<tr>
<td>pch2Δ</td>
<td>80.9</td>
<td>429</td>
<td>95.3</td>
<td>400</td>
</tr>
<tr>
<td>spo11-HA</td>
<td>81.1</td>
<td>434</td>
<td>92.5</td>
<td>400</td>
</tr>
<tr>
<td>mek1-as</td>
<td>84.7</td>
<td>163</td>
<td>98.8</td>
<td>80</td>
</tr>
<tr>
<td>MEK1-GST</td>
<td>66.9</td>
<td>178</td>
<td>87.5</td>
<td>160</td>
</tr>
<tr>
<td>pch2Δ spo11-HA</td>
<td>74.9</td>
<td>453</td>
<td>56.8</td>
<td>400</td>
</tr>
<tr>
<td>mek1-as spo11-HA</td>
<td>87.8</td>
<td>164</td>
<td>92.3</td>
<td>400</td>
</tr>
<tr>
<td>mek1-as pch2Δ</td>
<td>88.5</td>
<td>191</td>
<td>92.0</td>
<td>400</td>
</tr>
<tr>
<td>MEK1-GST spo11-HA</td>
<td>69.8</td>
<td>162</td>
<td>85.4</td>
<td>546</td>
</tr>
<tr>
<td>MEK1-GST pch2Δ</td>
<td>67.5</td>
<td>155</td>
<td>86.5</td>
<td>408</td>
</tr>
<tr>
<td>pch2Δ spo11-HA</td>
<td>85.8</td>
<td>183</td>
<td>40.8</td>
<td>400</td>
</tr>
<tr>
<td>mek1-as</td>
<td>63.5</td>
<td>143</td>
<td>89.0</td>
<td>552</td>
</tr>
</tbody>
</table>

Sporulation efficiencies for the above strains were counted after 5 days on sporulation media at 30°C. Tetrads were dissected on YPD and scored for spore viability after 3 days.
intersister DSB repair. Although the mechanism of Pch2 function in DSB repair is unknown, there are several avenues worthy of investigation. RTEL-1, the Caenorhabditis elegans homolog of the yeast Srs2 helicase has been shown to be defective in CO interference and CO homeostasis (Youds et al. 2010). Thus one possibility is that Pch2 facilitates access of a helicase to remove inappropriate strand invasion events. Borner et al. (2008) first posited another attractive (and not mutually exclusive) hypothesis that Pch2 somehow promotes Mec1 regulatory action. Indeed, at least one Mek1 effector (a Mec1 target) that promotes interhomolog bias is still unknown (Niu et al. 2009; Hollingsworth 2010). This effector could be Pch2 acting to augment or promote Mek1 activity. Under this model, Mek1 signaling is attenuated in pch2Δ mutants, allowing excess intersister repair. Such an idea is consistent with work from Wu et al. (2010), who showed that an activated MEK1 allele (MEK1-GST) promoted an increase in interhomolog events that were primarily repaired as NCOs and fewer intersister recombination hotspots near centromeres and telomeres. Curr. Biol. 17: 2003–2012.


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Communicating editor: J. Engebrecht