The \textit{pch2\,\textalpha} Mutation in Baker’s Yeast Alters Meiotic Crossover Levels and Confers a Defect in Crossover Interference

Sarah Zanders, Eric Alani*

Department of Molecular Biology and Genetics, Cornell University, Ithaca, New York, United States of America

Abstract

\textit{Pch2} is a widely conserved protein that is required in baker’s yeast for the organization of meiotic chromosome axes into specific domains. We provide four lines of evidence suggesting that it regulates the formation and distribution of crossover events required to promote chromosome segregation at Meiosis I. First, \textit{pch2\,\textalpha} mutants display wild-type crossover levels on a small (III) chromosome, but increased levels on larger (VII, VIII, XV) chromosomes. Second, \textit{pch2\,\textalpha} mutants show defects in crossover interference. Third, crossovers observed in \textit{pch2\,\textalpha} require both Msh4-Msh5 and Mms4-Mus81 functions. Lastly, the \textit{pch2\,\textalpha} mutation decreases spore viability and disrupts crossover interference in \textit{spo11} hypomorphic strains that have reduced levels of meiosis-induced double-strand breaks. Based on these and previous observations, we propose a model in which \textit{Pch2} functions at an early step in crossover control to ensure that every homolog pair receives an obligate crossover.

Introduction

Meiosis generates haploid gametes from diploid progenitor cells. The reduction in ploidy results from the segregation of homologous chromosome pairs in the first meiotic division (MI) [1]. Prior to MI, each chromosome is joined to its homolog at chiasmata, which serve to tether homologs to each other. This interaction promotes the tension between homologs needed to form a bipolar spindle that facilitates homolog segregation. Homologous chromosome pairs lacking chiasmata connections often fail to segregate properly at MI. Chromosome nondisjunction can also result if chiasmata are present, but not properly placed on chromosomes, or if sister chromatid cohesion is disrupted [2–5]. Regardless of the cause, chromosome missegregation produces aneuploid gametes that lead to infertility or conditions like Down syndrome in humans [6].

Chiasmata form at sites where programmed Spo11-catalyzed DNA double-stranded breaks (DSBs), induced early in meiotic prophase, are repaired to form crossovers [1]. In baker’s yeast, crossovers (COs) are formed via two main pathways. The first pathway, by which the majority of COs are made, involves Msh4-Msh5 and Mlh1-Mlh3 [7–15]. In this pathway, DSBs are processed and acted upon by strand exchange enzymes to form single-end invasion intermediates (SEIs) that are converted into double Holliday junctions (dHJs). The latter are resolved into crossovers which display interference; the COs are more uniformly spaced than if placed at random (see below; [16–22]). The COs formed via the second major pathway, which require Mms4-Mus81, are not subject to CO interference [10,11,23]. Little is known about the intermediates that form in this latter pathway.

The recombination steps that lead to CO formation occur in meiotic prophase. In leptotene, when meiotic DSB formation initiates recombination, an axial element containing Hop1 and Red1 proteins assembles along each pair of sister chromatids. In zygotene, when SEIs are detected, mature tripartite synaptonemal complex (SC) starts to form when the Zip1-containing central element connects the axial elements, which are now termed “lateral elements.” Mature SC initiation begins at centromeres and later at CO-designated sites. These SC initiation events then spread outward until synopsis is completed in pachytene [24,25]. Hop1/Red1 and Zip1 are enriched in separate domains on the mature SC. This organization is \textit{Pch2}-dependent because in \textit{pch2\,\textalpha} mutants, Zip1 and Hop1 appear to be more uniformly distributed along the chromosome axes [26,27]. At the end of pachytene, recombination intermediates are resolved [reviewed in [28]].

In yeast, ~40% of the ~140–170 meiotic DSBs are repaired to generate noncrossover (NCO) products [29,30]. These NCO products are thought to form by a synthesis-dependent strand annealing mechanism (SDSA, [31]), separate from the interfering CO mechanism, and do not result in MI disjunction-promoting chiasmata. Martini \textit{et al.} [32] found that when meiotic programmed DSBs are decreased in \textit{spo11} hypomorphic strains, COs are favored at the expense of NCOs [24]. This CO homeostasis phenomenon may be an additional manifestation of CO interference [32,33]. The above studies indicate that DSBs are subject to a CO vs. NCO decision step, which is regulated by interference. Interference regulates this decision by ensuring that CO designation for a given DSB inhibits nearby DSBs from receiving this designation, thereby relegating them to a NCO fate. It is not clear whether non-interfering COs are formed through
Author Summary

During meiosis, cells that ultimately become gametes (such as eggs or sperm) undergo a single round of DNA replication followed by two consecutive divisions. In most organisms, the segregation of chromosomes at the first meiotic division is dependent upon genetic exchange, or crossing over, at homologous sites along chromosomes. Crossing over must therefore be regulated to ensure that every pair of matched chromosomes receives at least one crossover. Matched chromosomes that do not receive a crossover frequently undergo missegregation at the first meiotic division, yielding gametes that do not contain the normal chromosome number. Such missegregation events have been linked to human infertility syndromes. We used a genetic approach to study meiotic crossover control in baker's yeast. Our work suggests that Pch2 is required in crossover control during meiosis; mutants lacking Pch2 display altered crossover levels and distribution. Furthermore, pch2 mutations cause enhanced gamete inviability in strains that are mildly defective in initiating recombination. Based on these observations, we hypothesize that Pch2 acts early in crossover control, in steps that occur prior to those proposed for previously characterized crossover-promoting factors.

such a decision process; these COs are thought to form through a parallel pathway [10,23]. For this paper, the CO vs. NCO decision refers solely to COs that are subject to interference.

The interference-regulated CO vs. NCO decision likely occurs very early in recombination, roughly at the time of SEI formation [3,17,18,20,21,34,35]. CO interference is strongest near a CO event and weakens with distance along the chromosome [17,18,20,21,34,35]. CO interference is strongest in CO interference likely reflect problems in CO formation and not in the early CO vs. NCO decision (reviewed in [28]). For example, mutants defective in either the SC central element protein Zip1 or the CO-promoting factor Msh4 have reduced COs in strains that are mildly defective in initiating recombination. Based on these observations, we hypothesize that Pch2 acts early in crossover control, in steps that occur prior to those proposed for previously characterized crossover-promoting factors.

In the EAY strain background, the total map distance across four intervals on chromosome XV was 152 cM in pch2Δ compared to 101 cM in wild-type (Figure 1; Table 1). Increased crossing over in pch2Δ was statistically significant in all four intervals (G-test where p<0.017 is considered significant due to Dunn-Sidak correction for multiple tests; see Table 1 for p values). Similar results were observed on the large (VII) and medium (VIII) chromosomes in the NHY background (Figure 2B; Table 1). Significantly more crossing over was observed in each of three intervals on chromosome VII, raising the map distance of the marked region from 69 cM in wild-type to 115 cM in pch2Δ (G-test where p<0.017 is considered significant due to Dunn-Sidak correction for multiple tests; see Table 1 for p values). For chromosome VIII, statistically significant increases in crossing over were observed in both genetic intervals, raising the map distance from 46 cM in wild-type to 72 cM in pch2Δ. The increases in crossing over observed in pch2Δ on chromosomes XV, VII, and VIII resulted from an increase in both tetranet and non-parental ditype tetrads (Table 1). These data argue against the increase being due to multiple COs resulting from a single initiating DSB [52].

The effect of pch2Δ on crossing over on the small chromosome III was similar to that reported by San Segundo and Roeder [26], who saw no effect of the pch2Δ mutation on crossing over. We observed a significant increase in crossing over in pch2Δ in one of the two genetic intervals (Figure 2B; Table 1). However, the overall map distance for the marked region in pch2Δ was 37 cM, which was not significantly different from wild-type (35 cM).

Results

Genetic analysis of recombination

A new phenotype for pch2Δ mutants: increased crossing over on large chromosomes. We analyzed the pch2Δ phenotype in two different strain backgrounds at 30°C. In the EAY11088/1112 (EAY) SK1 congenic strain background, one large chromosome (XV, 1095 kb) is marked, whereas large (VII, 1040 kb), medium (VIII, 582 kb), and small (III, 333 kb) chromosomes are marked in the SK1 isogenic NHY942/943 (NY) strain background ([10,11]; Figure 1A, Figure 2A; Table S1). Similar to previous studies, pch2Δ mutants show wild-type spore viability (~95%; [26,47,51]; Figure 3, Figure S1).

In the EAY strain background, the total map distance across four intervals on chromosome XV was 152 cM in pch2Δ compared to 101 cM in wild-type (Figure 1; Table 1). Increased crossing over in pch2Δ was statistically significant in all four intervals (G-test where p<0.017 is considered significant due to Dunn-Sidak correction for multiple tests; see Table 1 for p values). Similar results were observed on the large (VII) and medium (VIII) chromosomes in the NHY background (Figure 2B; Table 1). Significantly more crossing over was observed in each of three intervals on chromosome VII, raising the map distance of the marked region from 69 cM in wild-type to 115 cM in pch2Δ (G-test where p<0.017 is considered significant due to Dunn-Sidak correction for multiple tests; see Table 1 for p values). For chromosome VIII, statistically significant increases in crossing over were observed in both genetic intervals, raising the map distance from 46 cM in wild-type to 72 cM in pch2Δ. The increases in crossing over observed in pch2Δ on chromosomes XV, VII, and VIII resulted from an increase in both tetranet and non-parental ditype tetrads (Table 1). These data argue against the increase being due to multiple COs resulting from a single initiating DSB [52].

The effect of pch2Δ on crossing over on the small chromosome III was similar to that reported by San Segundo and Roeder [26], who saw no effect of the pch2Δ mutation on crossing over. We observed a significant increase in crossing over in pch2Δ in only one (LEU2-GE3) of three genetic intervals (Figure 2B; Table 1). However, the overall map distance for the marked region in pch2Δ was 37 cM, which was not significantly different from wild-type (35 cM).
Gene conversion is elevated in \textit{pch2D} mutants. The \textit{pch2D} mutation conferred an increase in gene conversion for 15 of the 17 markers that were examined (Table 2). Two markers with the most dramatic increases in gene conversion were \textit{met13} (2.4\% in wild-type, 11.0\% in \textit{pch2D}) and \textit{thr1} (5.1\% in wild-type, 11.9\% in \textit{pch2D}), both in the NHY strain background. Tetrads in which high levels of gene conversion were observed (\textit{THR1}, chromosome VIII, \textit{MET13}, chromosome VII) were analyzed for exchange of flanking markers (Table 3; see [32]). For example, tetrads containing \textit{MET13} gene conversions were scored in the CO class if \textit{LYS5} and \textit{CYH2} markers were non-parental ditype or tetratype, but were in the NCO class if those markers were parental ditype. A ratio of CO:NCO was then computed from these classes. At \textit{MET13}, the CO:NCO ratio was 1.8 in wild-type and 2.6 in \textit{pch2D}, but this difference was not statistically significant (G-test where \(p < 0.05\) is significant). At \textit{THR1} the ratio was 1.9 in wild-type and 9.4 in \textit{pch2D} (\(p < 0.0001\); Table 3). Assuming no change in DSB formation in \textit{pch2D} (see below), these data suggest that at least for the \textit{THR1} locus, the increase in crossing over observed in \textit{pch2D} was accompanied by a relative decrease in

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{\textit{pch2D} has increased levels of meiotic COs on the large chromosome XV. Recombination levels in four genetic intervals were analyzed on chromosome XV in the EAY1108/EAY1112 strain background (A). CO frequencies were calculated in cM from tetrads (B) and as recombination frequencies in spores (C). See Table 1 and Table S3 for raw data and statistical analyses. doi:10.1371/journal.pgen.1000571.g001}
\end{figure}
noncrossover events. However, more extensive genetic analyses at multiple loci, using markers that can eliminate incidental COs, will be required to solidify this observation (see Discussion).

Genetic analysis of meiotic CO control

**Pch2 is required for wild-type levels of CO interference.** The crossover phenotype of pch2Δ mutants, increased crossing over on large chromosomes, encouraged us to test a role for Pch2 in CO interference. As shown below, our data, and work by Joshi et al. [53], indicate that pch2Δ mutants are defective in CO interference. We employed three methods to measure CO interference. First, we measured the NPD ratio for those loci in which a significant number of NPD events were expected (>10; chromosome III data are thus excluded) using Stahl’s “Better Way” calculator. This method compares the observed number of each tetrad class (NPD, PD and TT), to the numbers expected if CO distribution was random [54–56]. In the absence of CO interference, the NPD ratio is expected to be one. Values significantly less than one indicate interference, with smaller numbers indicating stronger interference. We found pch2Δ mutants had a larger NPD ratio than wild-type in all genetic intervals in both strain backgrounds (Table 4). In the EAY strain, statistically significant levels of interference were seen in all genetic intervals in wild-type, pch2Δ/pch2Δ, spo11-HA/spo11-HA and pch2Δ spo11-HA/spo11-HA strains. See Table 1 for raw data and statistical analyses.

Second, we measured the coefficient of coincidence (COC). This method compares the observed number of times that a CO occurs in each of two adjacent genetic intervals to the number of such double COs expected due to chance. In the absence of interference, the COC value is expected to equal one. Values significantly less than one indicate interference, with smaller numbers indicating stronger interference. All intervals in the two strain backgrounds displayed COC values that were higher in pch2Δ than in wild-type (Table 5). For three intervals, interference could not be detected in either wild-type or pch2Δ. In one interval, interference was seen in wild-type, but not pch2Δ. For the remaining four intervals, interference was seen in both wild-type and pch2Δ, but was weaker in pch2Δ.

Lastly, we employed the method of Malkova et al. [37] to analyze CO interference. This method compares the map distance calculated for a given interval when a CO has occurred in the adjacent interval to the map distance calculated for the same given interval when a CO has not occurred in the adjacent interval. In the absence of interference, these map distances are expected to be the same and a ratio of the map distances is equal to one. However, in the presence of interference, a CO in one interval would make a nearby CO less likely. This would cause the map distance ratio to be less than one, with smaller ratios resulting from stronger interference [37]. In both strain backgrounds the map distance ratios were larger in pch2Δ than wild-type for all adjacent intervals, indicating that, as seen with the NPD ratio and COC tests, pch2Δ disrupted CO interference (Figure 4; Table S2). In the EAY strain background, G-tests indicated that interference was statistically detectable in wild-type between all three interval pairs, but was detectable between only two interval pairs in pch2Δ (Figure 4A; Table S2). In the NHY strain background, interference was statistically detectable in pch2Δ for two out of five interval pairs, although it was weaker than in wild-type. For
Figure 3. Pch2 promotes spore viability in spo11 hypomorphs. (A) Spore viability distributions of wild-type and mutant strains in the NHY942/NHY943 strain background are displayed. The X-axes represent the number of viable spores per tetrad and the Y-axes represent the percent of tetrads comprising each class. The total number of tetrads dissected (n) and the overall percent spore viability (% SV) are shown. (B) Bar graph showing spore viability in wild-type (gray) and pch2Δ (black) mutants containing the indicated spo11 mutations. The SPO11/SPO11, spo11-HA/spo11-HA, spo11-HA/spo11yf-HA, and spo11da-HA/spo11da-HA alleles confer 100, 80, 30 and 20% total DSB levels, respectively, in the PCH2 background [32]. doi:10.1371/journal.pgen.1000571.g003
Table 1. Genetic map distances calculated from four-spore viable tetrads.

| Chromosome | 4-spore viable tetrads | PD | TT | NPD | cM | SE | to wild-type | p values to pch2.1 | p values to msh5.1 |
|------------|------------------------|----|----|-----|----|----|-------------|-------------------|-------------------|
| XV         |                        |    |    |     |    |    |             |                   |                   |
| URA3-LEU2  | wild-type              | 1087 | 607 | 456 | 5  | 22.8 | 1.0         | <0.0001           |                   |
| pch2.1     |                        | 1015 | 563 | 423 | 18 | 26.4 | 1.4         |                   | <0.0001           |                   |
| msh5.1     |                        | 757  | 643 | 76  | 1  | 5.7  | 0.7         | <0.0001           | <0.0001           |                   |
| pch2.1 msh5.1 |                    | 94   | 79  | 14  | 0  | 7.5  | 1.9         | <0.0001           | <0.0001           | 0.3620            |
| LEU2-LYS2  | wild-type              | 1087 | 496 | 569 | 3  | 27.5 | 0.9         |                   | <0.0001           |                   |
| pch2.1     |                        | 1015 | 395 | 561 | 39 | 40.0 | 1.8         |                   | <0.0001           |                   |
| msh5.1     |                        | 757  | 659 | 61  | 0  | 4.2  | 0.5         | <0.0001           | <0.0001           | <0.0001           |
| pch2.1 msh5.1 |                    | 94   | 74  | 17  | 1  | 12.5 | 3.7         | <0.0001           | <0.0001           | 0.5576            |
| LYS2-ADE2  | wild-type              | 1087 | 803 | 263 | 2  | 12.9 | 0.8         |                   |                   |                   |
| pch2.1     |                        | 1015 | 649 | 344 | 7  | 19.3 | 1.1         | <0.0001           |                   |                   |
| msh5.1     |                        | 757  | 562 | 155 | 3  | 5.4  | 1.6         | <0.0001           | <0.0001           | 0.7296            |
| ADE2-HIS3  | wild-type              | 1087 | 343 | 709 | 16 | 37.7 | 1.2         |                   |                   |                   |
| pch2.1     |                        | 1015 | 243 | 638 | 115| 66.7 | 2.3         | <0.0001           |                   |                   |
| msh5.1     |                        | 757  | 496 | 215 | 9  | 18.7 | 1.5         | <0.0001           | <0.0001           |                   |
| pch2.1 msh5.1 |                    | 94   | 54  | 37  | 2  | 26.3 | 4.9         | <0.0001           | <0.0001           | 0.0845            |
| Chromosome III |                        |    |    |     |    |    |             |                   |                   |
| HIS4-LEU2  | wild-type              | 572  | 414 | 142 | 2  | 13.8 | 1.2         |                   |                   |                   |
| pch2.1     |                        | 611  | 427 | 150 | 3  | 14.5 | 1.3         | 0.8093            | 0.0001            |
| spo11-HA   |                        | 518  | 411 | 96  | 1  | 10.0 | 1.0         | 0.0016            |                   |                   |
| pch2.1 spo11-HA |                | 556  | 438 | 100 | 1  | 9.8  | 1.0         | 0.0005            | 0.9772            | 0.0001            |
| LEU2-CEN3  | wild-type              | 572  | 499 | 70  | 0  | 6.2  | 0.7         |                   |                   |                   |
| pch2.1     |                        | 611  | 503 | 100 | 0  | 8.3  | 0.8         | 0.0092            | 0.9995            |
| spo11-HA   |                        | 518  | 429 | 85  | 0  | 8.3  | 0.8         | 0.0200            |                   |                   |
| pch2.1 spo11-HA |                | 556  | 453 | 96  | 2  | 9.8  | 1.1         | 0.0004            | 0.1311            | 0.0939            |
| CEN3-MAT   | wild-type              | 572  | 405 | 164 | 1  | 14.9 | 1.1         |                   |                   |                   |
| pch2.1     |                        | 611  | 440 | 162 | 1  | 13.9 | 1.0         | 0.5796            | 0.0007            |
| spo11-HA   |                        | 518  | 398 | 112 | 5  | 13.8 | 1.5         | <0.0001           | <0.0001           | <0.0001           |
| pch2.1 spo11-HA |                | 556  | 487 | 68  | 0  | 6.1  | 0.7         | <0.0001           | <0.0001           | <0.0001           |
| Chromosome VII |                        |    |    |     |    |    |             |                   |                   |
| TRPS-CYH2  | wild-type              | 572  | 202 | 352 | 12 | 37.5 | 1.9         |                   |                   |                   |
| pch2.1     |                        | 611  | 153 | 371 | 67 | 65.4 | 3.6         | <0.0001           | <0.0001           |                   |
| spo11-HA   |                        | 518  | 155 | 336 | 22 | 45.6 | 2.6         | 0.0008            |                   |                   |
| pch2.1 spo11-HA |                | 556  | 173 | 335 | 43 | 53.8 | 3.2         | <0.0001           | 0.0006            | 0.0014            |
| CYH2-MET13 | wild-type              | 572  | 452 | 104 | 0  | 9.4  | 0.8         |                   |                   |                   |
| pch2.1     |                        | 611  | 372 | 156 | 5  | 17.5 | 1.5         | <0.0001           | <0.0001           |                   |
| spo11-HA   |                        | 518  | 378 | 102 | 0  | 10.6 | 0.9         | 0.3720            |                   |                   |
| pch2.1 spo11-HA |                | 556  | 433 | 82  | 1  | 8.5  | 1.0         | 0.0730            | 0.0036            | <0.0001           |
Table 1. cont.

| MET13-LYS5 | 4-spore viable tetrads | PD | TT | NPD | cM | SE | to wild-type | p values to pch2-D | p values to msh5-D |
|------------|------------------------|----|----|-----|----|----|-------------|-----------------|-----------------|
| wild-type  | 572                    | 335| 209| 5   | 21.8| 1.5|             | <0.0001         | <0.0001         |
| pch2.1     | 611                    | 273| 243| 17  | 32.4| 2.4|             |                 |                 |
| spo11-HA   | 518                    | 277| 204| 1   | 21.8| 1.3|             | 0.0295          |                 |
| pch2.1 spo11-HA | 556          | 343| 161| 11  | 22.0| 2.1|             | 0.0005          | <0.0001         | <0.0001         |

Chromosome VIII

| CEN8-THR1 | 4-spore viable tetrads | PD | TT | NPD | cM | SE | to wild-type | p values to pch2-D | p values to msh5-D |
|-----------|------------------------|----|----|-----|----|----|-------------|-----------------|-----------------|
| wild-type | 572                    | 319| 221| 2   | 21.5| 1.3|             |                 |                 |
| pch2.1    | 611                    | 302| 228| 7   | 25.1| 1.7|             | 0.0133          | 0.0227          |
| spo11-HA  | 518                    | 309| 195| 3   | 21.0| 1.4|             | 0.4436          |                 |
| pch2.1 spo11-HA | 556          | 375| 161| 4   | 17.1| 1.4|             | <0.0001         | 0.0001          | <0.0001         |

| THR1-CUP1 | 4-spore viable tetrads | PD | TT | NPD | cM | SE | to wild-type | p values to pch2-D | p values to msh5-D |
|-----------|------------------------|----|----|-----|----|----|-------------|-----------------|-----------------|
| wild-type | 572                    | 278| 260| 1   | 24.7| 1.2|             |                 |                 |
| pch2.1    | 611                    | 189| 305| 31  | 46.8| 3.0|             | <0.0001         | <0.0001         |
| spo11-HA  | 518                    | 186| 312| 7   | 35.1| 1.8|             | <0.0001         |                 |
| pch2.1 spo11-HA | 556          | 227| 292| 20  | 38.2| 2.5|             | <0.0001         | <0.0001         | 0.0027          |

The map distances in cM between the indicated markers and the number of each tetrad type observed (as calculated by RANA software; Argueso et al. [11]) are shown. Chromosome XV data were obtained from EAY background strains; chromosomes III, VII and VIII data were obtained from NHY background strains. The Stahl lab online tools (http://www.molbio.uoregon.edu/~fstahl/) were used to calculate the genetic distances and standard errors (SE). p values for G-tests comparing the tetrad type distributions for all mutant combinations were calculated using the spreadsheet available from The Online Handbook of Biological Statistics (http://udel.edu/~mcdonald/statintro.html).

doi:10.1371/journal.pgen.1000571.t001

Table 2. pch2-D increases the frequency of aberrant marker segregation.

| Chromosome XV | Tetrads | TRP1 | URA3 | LEU2 | LYS2 | ADE2 | HIS3 | Total |
|---------------|---------|------|------|------|------|------|------|-------|
| wild-type     | 1087    | 0.0  | 0.0  | 0.2  | 0.6  | 0.1  | 0.8  | 1.7   |
| pch2.1        | 1015    | 0.3  | 0.2  | 1.1  | 0.4  | 0.4  | 1.5  | 3.9   |
| msh5.1        | 757     | 0.1  | 0.1  | 1.6  | 1.2  | 0.8  | 1.2  | 5.0   |
| pch2.1 msh5.1 | 94      | 0.0  | 0.0  | 1.1  | 1.1  | 1.1  | 0.0  | 3.3   |

| Chromosome III | Tetrads | HIS4 | LEU2 | ADE2 | MATa | Total |
|----------------|---------|------|------|------|------|-------|
| wild-type      | 572     | 2.1  | 0.3  | 0.2  | 0.2  | 2.8   |
| pch2.1         | 611     | 3.8  | 1.3  | 0.0  | 1.3  | 6.4   |
| spo11-HA       | 518     | 1.2  | 0.8  | 0.0  | 0.6  | 2.6   |
| pch2.1 spo11-HA| 556     | 2.2  | 0.9  | 0.0  | 0.2  | 3.3   |

| Chromosome VII | Tetrads | LYS5 | MET13 | CYH2 | TRPS | Total |
|----------------|---------|------|-------|------|------|-------|
| wild-type      | 572     | 1.6  | 2.4   | 0.3  | 0.7  | 5.0   |
| pch2.1         | 611     | 1.8  | 11.0  | 1.8  | 1.5  | 16.1  |
| spo11-HA       | 518     | 0.2  | 6.8   | 0.6  | 0.4  | 8.0   |
| pch2.1 spo11-HA| 556     | 0.4  | 7.0   | 0.2  | 0.7  | 8.3   |

| Chromosome VIII | Tetrads | URA3 | THR1 | CUP1 | Total |
|-----------------|---------|------|------|------|-------|
| wild-type       | 572     | 0.2  | 5.1  | 0.7  | 6.0   |
| pch2.1          | 611     | 0.2  | 11.9 | 2.1  | 14.2  |
| spo11-HA        | 518     | 0.0  | 2.1  | 0.4  | 2.5   |
| pch2.1 spo11-HA | 556     | 0.0  | 2.9  | 0.2  | 3.1   |

The percent of non 2:2 marker segregations were calculated for the indicated loci. Chromosome XV data were obtained from EAY background strains; chromosomes III, VII, and VIII data were obtained from NHY background strains. Most events were 3:1 or 1:3 gene conversions although one 4:0 event in the EAY background and two 4:0 events in the NHY background were observed in pch2-D mutants. In addition, one post-meiotic segregation event (5:3) was observed in the pch2-D spo11-HA mutant.

doi:10.1371/journal.pgen.1000571.t002
Table 3. CO:NCO ratio of markers flanking gene conversion events on chromosomes VII and VIII.

| 4-spore viable tetrads | MET13 conversions |  | NCO (LYS5-CYH2) | CO:NCO ratio | p value |
|------------------------|-------------------|---|------------------|--------------|---------|
| wild-type              | 572               | 14| 9                | 5            | 1.8     |
| pch2.D                 | 611               | 65| 47               | 18           | 2.6     | 0.169   |

| 4-spore viable tetrads | THR1 conversions |  | NCO (CEN8-CUP1) | CO:NCO ratio | p value |
|------------------------|------------------|---|------------------|--------------|---------|
| wild-type              | 572               | 29| 19               | 10           | 1.9     |
| pch2.D                 | 611               | 73| 66               | 7            | 9.4     | <0.0001 |

Tetrads containing a gene conversion at the MET13 or THR1 loci were analyzed in wild-type and pch2.D mutants in the NHY background. The markers flanking the gene conversion event were scored as CO (TT or NPD) or NCO (PD) and the CO: NCO ratio was calculated at each site. The wild-type and pch2.D CO and NCO numbers were compared using a G-test and the p values are shown.

doi:10.1371/journal.pgen.1000571.t003

Table 4. Interference calculations using NPD ratios.

| 4-spore viable tetrads | NPD obs. | NPD exp. | obs./exp. | p     | I     |
|------------------------|----------|----------|-----------|-------|-------|

Chromosome XV

| URA3-LEU2 |          |          |           |       |
|-----------|----------|----------|-----------|-------|-------|
| wild-type | 1087     | 5        | 27.7      | 0.18  | <0.0001 | YES   |
| pch2.D    | 1015     | 18       | 28.7      | 0.63  | 0.0212  | YES   |

LEU2-LYS2

| wild-type | 1087     | 3        | 43.2      | 0.07  | <0.0001 | YES   |
| pch2.D    | 1015     | 39       | 58.8      | 0.66  | 0.0011  | YES   |

Ade2-His3

| wild-type | 1087     | 16       | 74.8      | 0.21  | <0.0001 | YES   |
| pch2.D    | 1015     | 115      | 117.0     | 0.98  | 0.7522  | NO    |

Chromosome VII

| TRP5-CYH2 |          |          |           |       |
|-----------|----------|----------|-----------|-------|-------|
| wild-type | 572      | 12       | 36.0      | 0.33  | <0.0001 | YES   |
| pch2.D    | 611      | 67       | 66.4      | 1.00  | 0.9191  | NO    |
| spo11-HA  | 518      | 22       | 41.6      | 0.53  | <0.0001 | YES   |
| pch2.D spo11-HA | 556 | 43 | 47.9 | 0.90 | 0.3362 | NO |

MET13-LYS5

| wild-type | 572      | 5        | 11.8      | 0.42  | 0.0256  | YES   |
| pch2.D    | 611      | 17       | 20.0      | 0.85  | 0.4298  | NO    |
| spo11-HA  | 518      | 1        | 12.0      | 0.08  | 0.0003  | YES   |
| pch2.D spo11-HA | 556 | 11 | 8.7 | 1.26 | 0.3834 | NO |

Chromosome VIII

| CEN8-THR1 |          |          |           |       |
|-----------|----------|----------|-----------|-------|
| Wild-type | 572      | 2        | 12.6      | 0.16  | 0.0007  | YES   |
| pch2.D    | 611      | 7        | 14.9      | 0.47  | 0.0185  | YES   |
| spo11-HA  | 518      | 3        | 10.7      | 0.28  | 0.0077  | YES   |
| pch2.D spo11-HA | 556 | 4 | 7.0 | 0.57 | 0.2138 | NO |

| THR1-CUP1 |          |          |           |       |
|-----------|----------|----------|-----------|-------|
| wild-type | 572      | 1        | 17.5      | 0.06  | <0.0001 | YES   |
| pch2.D    | 611      | 31       | 37.4      | 0.83  | 0.1725  | NO    |
| spo11-HA  | 518      | 7        | 30.2      | 0.23  | <0.0001 | YES   |
| pch2.D spo11-HA | 556 | 20 | 29.1 | 0.69 | 0.0359 | YES |

The number of NPDs was compared to the expected number using the Stahl Online Laboratory “Better Way” calculator (http://www.molbio.oregon.edu/~fstahl/). Chromosome XV data were obtained from EAY background strains; chromosomes III, VII, and VIII data were obtained from NHY background strains. The total number of 4-spore viable tetrads used for analysis is shown. The number of PD, NPD and TT tetrads can be found in Table 1. p values were calculated from the chi-square values provided by the “Better Way” program using Vassernets (http://faculty.vassar.edu/lowry/VassarStats.html) chi-square to p calculator with one degree of freedom. “I” indicates if interference was statistically detectable.

doi:10.1371/journal.pgen.1000571.t004
one interval pair, interference was not detected in pch2A, whereas it was present in wild-type. For the remaining two intervals, interference was not detected in wild-type or pch2D (Figure 4B; Table S2).

We saw no evidence of chromatid interference in any strain analyzed in this study. We also found no evidence for negative interference between genetic intervals on different chromosomes or between widely spaced intervals on the same chromosome in pch2D, suggesting that the decreases in positive interference we observed did not result from variability in recombination between meioses (data not shown; [38,39]).

**Pch2 is required for maintaining spore viability in spo11 hypomorphs.** Previously, Martini et al. [32] observed that CO levels were maintained at the expense of NCOs when meiotic DSBs became limiting in spo11 hypomorphs showing reduced DSB levels (20–80%; [24,32,33,60]). This homeostasis mechanism is thought to ensure obligate CO formation between all homologous chromosome pairs and thereby promote spore viability. If

| Chromosome XV | 4-spore viable tetrads | DCO obs. | DCO exp. | COC | p    | I   |
|---------------|------------------------|----------|----------|-----|------|-----|
| URA3-LEU2-LYS2 |                        |          |          |     |      |     |
| wild-type     | 1087                   | 177      | 246.9    | 0.72| <0.0001| YES |
| pch2A         | 1015                   | 232      | 265.9    | 0.87| 0.017| YES |
| LEU2-LYS2-ADE2 |                        |          |          |     |      |     |
| wild-type     | 1087                   | 65       | 141.9    | 0.46| <0.0001| YES |
| pch2A         | 1015                   | 181      | 210.6    | 0.86| 0.024| YES |
| LYS2-ADE2-HIS3 |                        |          |          |     |      |     |
| wild-type     | 1087                   | 158      | 179.9    | 0.88| 0.080| NO  |
| pch2A         | 1015                   | 258      | 265.4    | 0.97| 0.624| NO  |

| Chromosome III | 4-spore viable tetrads | DCO obs. | DCO exp. | COC | p    | I   |
|----------------|------------------------|----------|----------|-----|------|-----|
| HIS3-LEU2-CEN3 |                        |          |          |     |      |     |
| wild-type     | 572                    | 5        | 17.7     | 0.28| 0.003| YES |
| pch2A         | 611                    | 14       | 25.4     | 0.55| 0.027| YES |
| spo11-HA      | 518                    | 8        | 16.0     | 0.50| 0.057| NO  |
| pch2A spo11-HA | 556                   | 11       | 18.0     | 0.61| 0.119| NO  |

| Chromosome VII | 4-spore viable tetrads | DCO obs. | DCO exp. | COC | p    | I   |
|---------------|------------------------|----------|----------|-----|------|-----|
| TRPS-CYH2-MET13 |                        |          |          |     |      |     |
| wild-type     | 572                    | 59       | 68.1     | 0.87| 0.267| NO  |
| pch2A         | 611                    | 122      | 132.3    | 0.92| 0.337| NO  |
| spo11-HA      | 518                    | 63       | 76.1     | 0.83| 0.119| NO  |
| pch2A spo11-HA | 556                   | 55       | 60.8     | 0.91| 0.472| NO  |

| Chromosome VIII | 4-spore viable tetrads | DCO obs. | DCO exp. | COC | p    | I   |
|----------------|------------------------|----------|----------|-----|------|-----|
| CEN8-THR1-CUP1 |                        |          |          |     |      |     |
| wild-type     | 572                    | 67       | 108.0    | 0.62| <0.0001| YES |
| pch2A         | 611                    | 125      | 150.4    | 0.83| 0.019| YES |
| spo11-HA      | 518                    | 85       | 125.1    | 0.68| <0.0001| YES |
| pch2A spo11-HA | 556                   | 76       | 95.5     | 0.80| 0.032| YES |

Chromosome XV data were obtained from EAY background strains; chromosomes III, VII and VIII data were obtained from NHY background strains. The number of double crossovers observed was compared to the expected number (as calculated by RANA software; [11]) for the EAY (A) and NHY (B) strain backgrounds. Two-tailed p values were calculated using the Vasserstats binomial properties calculator using a normal distribution. “I” indicates if interference was statistically detectable.

doi:10.1371/journal.pgen.1000571.t005
Interference and homeostasis result from a common mechanism, a mutation disrupting CO interference (e.g. pch2Δ) would severely compromise the spore viability of spo11 hypomorph strains [33].

We tested the effect of the pch2Δ mutation on the spore viability of spo11 hypomorph strains (NHY background). As shown in Figure 3, spore viability was similar in wild-type (91%) and the spo11-HA/spo11-HA hypomorph (91%), which displays 80% of the wild-type level of DSBs. These results confirm work by Martini et al. [32]. Interestingly, the pch2Δ/spo11-HA/spo11-HA mutant displayed significantly lower spore viability, 73%, despite having CO levels (165 cM total) that were similar to spo11-HA/spo11-HA (166 cM) and above wild-type levels (150 cM; Figure 2 and Figure 3). Spore viability in pch2Δ strains was compromised even further, relative to PCH2, in strains bearing more defective spo11 alleles (Figure 3; 86% spore viability in spo11-HA/spo11yf-HA vs. 16% in pch2Δ/spo11-HA/spo11yf-HA; 50% in spo11da-HA/spo11da-HA vs. 1% in pch2Δ/spo11da-HA/spo11da-HA). We also observed that the pch2-G319A mutation, which maps to the Walker A motif and is predicted to disrupt Pch2 ATP binding/hydrolysis activities [26,47], is unable to complement the pch2Δ mutation (S. Zanders, J. Olszewski, M. Dowicki, E. Alani, unpublished data).

The excess of tetrads with 4, 2, and 0 viable spores per tetrad observed in the pch2Δ spo11-HA hypomorph double mutants suggests that the spore death results from MI chromosome nondisjunction, although we are unable to rule out additional causes (see Discussion). In support of this, we observed that 68% (n = 130) of two-spore viable tetrads were sisters in the pch2Δ/spo11-HA/spo11-HA double mutant, as determined by the centromere-linked markers URA3 and ADE2. This was higher than what we observed in spo11-HA/spo11-HA and pch2Δ/spo11-HA where only 35% (n = 52) and 48% (n = 29), respectively, of the two-spore viable tetrads were sisters (G-test where p<0.025 is significant due to correction for multiple comparisons). We also observed significantly more (9/936) tetrads in which chromosome III had undergone MI nondisjunction, as determined by the ADE2 centromere-linked marker and an inability to mate, in the pch2Δ/spo11-HA/spo11-HA double mutant as compared to spo11-HA/spo11-HA (0/649) and pch2Δ/spo11-HA (1/707). Together
these observations are consistent with Pch2 regulating the distribution of CO events required to promote MI disjunction.

The interference defect in pch2Δ is not dependent upon extra COs. Previous studies suggested that the CO interference mechanism is intact in msh1 and mec4 mutants but appears to be disrupted due to excess non-interfering COs [7,8,10,15,23,45]. We entertained such a mechanism to explain the interference defect in pch2Δ by examining interference in pch2Δ spo11Δ hypmorphs and pch2Δ mutants defective in the non-interfering (Mms4-Mus81) and interfering (Msh4-Msh5) CO pathways. As described below, our data do not support the excess non-interfering CO hypothesis. First, pch2Δ/pch2Δ spo11-1 HA/spo11-1 HA mutants showed interference defects similar to pch2Δ/pch2Δ (Figure 2, Figure 4B; Table 4, Table 5, Table S2). This defect was seen even though the total number of COs decreased from 224 cM in pch2Δ/pch2Δ to 165 cM in pch2Δ/pch2Δ spo11-1 HA/spo11-1 HA.

Second, we tested if the decreased interference in pch2Δ was due to additional COs formed through the Mms4-Mus81 or Msh4-Msh5 pathways. This was done by analyzing pch2Δ mms4Δ and pch2Δ msh5Δ tetrads in the EAY strain background. The pch2Δ mms4Δ mutant had considerably lower spore viability (16%) than the mms4Δ mutant (32%; Figure S1). Overall, the recombination frequency of pch2Δ mms4Δ spores was about 14% higher than mms4Δ spores, but still lower than pch2Δ (Figure 1C; Table S3). In three out of four genetic intervals, the recombination frequencies were significantly higher in pch2Δ mms4Δ than in mms4Δ spores (G-test where p < 0.025 is considered significant due to Dunn-Sidak correction for multiple comparisons; Table S3). These data suggest that the elevated crossing over seen in pch2Δ was not solely due to Mms4-Mus81-specific crossing over. The spore viability of the pch2Δ msh5Δ mutant was 26%, compared to 36% for the msh5Δ single mutant (Figure S1). Like the mms4Δ mutant, overall CO frequencies (in tetrads and spores) were higher in the pch2Δ msh5Δ double mutant than in msh5Δ (~60%; Figure 1B and 1C; Table 1, Table S3), but were much lower than in pch2Δ. When only data from complete tetrads were compared, there were no statistically significant differences between msh5Δ and pch2Δ msh5Δ (G-test where p < 0.025 is considered significant due to Dunn-Sidak correction for multiple tests).

However, when data from all surviving spores were analyzed, pch2Δ msh5Δ had significantly higher recombination frequencies than msh5Δ in two out of the four genetic intervals (G-test where p < 0.025 is considered significant due to Dunn-Sidak correction for multiple tests). A caveat to these analyses is that the low spore viabilities observed in both the pch2Δ mms4Δ and pch2Δ msh5Δ mutants constrained analysis to a selected minority of meiotic products. Together, these data are consistent with COs in pch2Δ requiring both Mms4-Mus81 and Msh4-Msh5 pathways and argue against the idea that pch2Δ mutants show decreased CO interference due to additional COs formed through a non-interfering CO pathway.

pch2Δ does not increase DSB formation at two sites

Previous work indicated that pch2Δ mutants show delays in meiotic DSB repair; thus, a time course comparison of DSB levels in meiotic prophase between pch2Δ and wild-type could be misleading [27,47,61]. Wu and Burgess [47] assayed DSB formation at the well-characterized HIS4LEU2 hotspot in wild-type and pch2Δ in a sae2Δ strain background where DSBs are formed but not resected or repaired. They reported that wild-type and pch2Δ strains displayed similar DSB levels. More recently, the Hochwagen group, using microarray analysis, observed increases in DSB formation in pch2Δ surrounding the rDNA on chromo-
some XII, but nowhere else in the genome (A. Hochwagen personal communication).

We assayed DSB formation in pch2Δ mutants at the YCR048W hotspot on chromosome III and near the centromere on chromosome XV [42,62,63]. These experiments were performed in a dnc1Δ background where DSBs are formed at wild-type levels and resected (eventually hyperresected), but not repaired [29,42,64]. This approach allowed us to assay total DSB at loci other than HIS4LEU2, where DSBs are thought to occur at saturating levels, and avoid the use of the sae2Δ background where maximal DSB levels may not be reached [29,32,42,65]. One concern with performing this analysis in the dnc1Δ background is that two reports [26,66] indicated that the checkpoint arrest seen in dnc1Δ mutants is bypassed in pch2Δ dnc1Δ strains; however, a more recent report [61] indicated that it is not. Our pch2Δ dnc1Δ mutants displayed a meiotic arrest as measured by a failure to form spores (< 0.6% spore formation for pch2Δ dnc1Δ vs. ~90% for wild-type at T = 24 hrs). However as shown below, we observed a significant bypass of the dnc1Δ arrest in pch2Δ spo11-1 HA dnc1Δ strains.

Quantification of DSB levels in the dnc1Δ background is difficult due to the extensive resection of the breaks. We therefore analyzed five independent cultures of dnc1Δ and pch2Δ dnc1Δ strains. Similar to previous work [47]; A. Hochwagen personal communication), we saw no difference in DSB levels (% of total DNA) between dnc1Δ and pch2Δ dnc1Δ strains at the YCR048W (5 and 6 kb DSB bands; 19±6% for dnc1Δ, 18±5% for pch2Δ dnc1Δ) and CEN15 (3 kb DSB band; 4.7±1.2% for dnc1Δ, 4.5±1.0% for pch2Δ dnc1Δ) hotspots (Figure 5A and 5B; T = 7 hrs in meiosis). It is important to note that Hochwagen et al. [61] reported that pch2Δ dnc1Δ mutants do not resect DSB ends as rapidly as dnc1Δ; however, such a difference in resection rate could only result in an overestimation of the level of DSBs in pch2Δ dnc1Δ. These data, together with previous work, suggest that the pch2Δ mutation does not disrupt DSB levels in a spo11Δ background.

As shown above, the pch2Δ mutation severely compromised the spore viability of spo11Δ hypmorph strains. Because some spo11Δ mutants confer semi-dominant and conditional phenotypes, as well as alter DSB patterns [60], we assayed DSB levels at YCR048W in spo11-1 HA dnc1Δ strains in the presence or absence of the pch2Δ mutation (Figure 5C). At T = 3.5 hrs in meiosis, similar DSB levels were observed in pch2Δ spo11-1 HA dnc1Δ (16%) and spo11-1 HA dnc1Δ (15%) strains. However, at T = 7 hrs, lower levels were observed in pch2Δ spo11-1 HA dnc1Δ (13±2.6%; seven independent cultures) compared to spo11-1 HA dnc1Δ (18±2.6%; seven independent cultures). In time courses performed side by side, pch2Δ spo11-1 HA dnc1Δ strains displayed 30 to 90% of the spo11-1 HA dnc1Δ levels at T = 7 hrs. Such variability was not observed in side-by-side experiments involving pch2Δ dnc1Δ and dnc1Δ strains. As shown below and analyzed in the Discussion, we attribute the variability in DSB levels to the bypass of the dnc1Δ arrest in pch2Δ spo11-1 HA dnc1Δ. This was determined by measuring the completion of the MI division in spo11-1 HA dnc1Δ and pch2Δ spo11-1 HA dnc1Δ strains. At T = 28 hrs in meiosis, only 1-2% of spo11-1 HA dnc1Δ strains completed MI; this indicates that the dnc1Δ arrest is maintained in these strains. For pch2Δ spo11-1 HA dnc1Δ, at T = 4.5 hrs, no cells (n > 200) had completed the MI division. However, at T = 6.5 hrs, 8 to 30% of the cells completed MI, and these values increased to 54 to 60% (with similar spore formation levels) at T = 28 hrs. As predicted for a dnc1Δ mutant, the spores produced by pch2Δ spo11-1 HA dnc1Δ were inviable.

Analysis of meiotic progression

The spo11-1 HA hypmorph suppresses the MI delay of pch2Δ. Wu and Burgess [47] showed that the pch2Δ MI delay is
suppressed by a null mutation in the mitotic and meiotic checkpoint gene RAD17. The delay is also suppressed by the spo11D mutation [47,67]. One interpretation of these and our data is that the greater than wild-type number of COs in pch2D, rather than a recombination-associated DNA aberration inherent to the mutant, triggers the Rad17-dependent checkpoint. If the additional time required to complete the additional COs causes the delay in pch2D, then reducing the number of recombination events by lowering the number of DSBs should suppress the delay. We assayed MI division timing in pch2D/pch2D spo11-HA/spo11-HA mutants displaying total CO levels (165 cM) that are somewhat similar to wild-type (150 cM) but significantly lower than pch2D/pch2D spo11-HA/spo11-HA strains progressing through meiosis with timing indistinguishable from spo11-HA/spo11-HA and wild-type (Figure 6). These data suggest there are no inherent recombination defects recognized by a Rad17-dependent checkpoint in pch2D mutants, unless the defect appears only when DSBs are at wild-type levels [32, but see 27]. We favor the idea that the MI delay in pch2D is caused by the prolonged recombination period needed to generate the additional COs observed in pch2A. Alternatively, the extra COs observed in pch2A could result from, rather than cause, the MI delay [68]. In this case, it is unclear what could be eliciting the delay in pch2A. Importantly, the fact that the pch2A spo11-HA double mutant has wild-type MI timing and disrupted CO interference (Figure 4, Figure 6; Table 4, Table 5, Table S2) demonstrates that the interference defects observed in pch2A are not simply the result of a prolonged CO designation period [68].

**Discussion**

In this study we show that pch2 mutants display elevated crossing over on medium and large chromosomes and are defective in CO interference. Based on this work, our initial studies suggesting an increased CO:NCO ratio in pch2A mutants (Table 3), and previous work [27,32,47], we hypothesize that the increase in COs in pch2A on the medium and large chromosomes results from a greater than normal proportion of DSBs being repaired as COs at the expense of non COs, due to the loss of CO interference, rather than an increase in initiating DSBs (Figure 5;

---

**Figure 5. pch2 does not appear to have increased levels of meiotic DSBs.** Southern blots were performed to measure meiotic DSBs in dmc1Δ, pch2Δ dmc1Δ, spo11-HA dmc1Δ, and pch2Δ spo11-HA dmc1Δ strains. For the YCR048w hotspot on chromosome III (A) DNA was digested with BglII and probed with a chromosome III fragment (SGD coordinates 215,422-216,703, [63]). For the CEN15 hotspot (B), DNA was digested with SphI and NheI and probed with a chromosome XV fragment (SGD coordinates 331,713-332,402, [42]). The parental bands are marked with asterisks and arrows show bands that form due to DSB formation. Approximate sizes for all bands are shown [42,63]. The lanes on the CEN15 blot have been reordered for easy comparison of the two strains. (C) Analysis of DSBs at the YCR048W hotspot at T = 3.5 and 7 hrs in spo11-HA dmc1Δ (EAY2562/EAY2563) and pch2Δ spo11-HA dmc1Δ (EAY2564/EAY2565) strains. A representative blot is shown. In side-by-side experiments the DSB levels at T = 7 hrs in pch2Δ spo11-HA dmc1Δ ranged from 30-90% (30, 61, 72, 76, 80, 89, and 90%) of the levels observed in spo11-HA dmc1Δ. doi:10.1371/journal.pgen.1000571.g005
HIS4LEU2 through recombination at increased at this site [27,69]. They also monitored progression and found that CO levels were decreased and NCO levels were increased at this site [27,69]. They also monitored progression and found that CO levels were decreased and NCO levels were increased at this site [27,69].

Specifically, the additional load of SEIs and dHJs that the chromosome axis seen in pch2Δ mutants results from, but is not limited to the overall number of COs. The same defect in pch2Δ that disrupts interference could lead to longer heteroduplex tracts, causing the increases in gene conversion frequencies observed in pch2Δ.

We favor a model in which Pch2 promotes wild-type levels of CO interference at the CO vs. NCO decision, which is though to occur in late leptotene, perhaps by acting in meiotic axis organization/assembly (Figure 7; [17,18,20]). In this model, CO designation at one site inhibits nearby DSBs from receiving CO designation; such a decision could then influence the Pch2-dependent domainal organization of Hop1 and Zip1 observed in pachytene [27; see below]. Two recent studies support the idea that Pch2 acts in early prophase. 1. Hochwagen et al. observed changes in DSB processing in pch2Δ mutants [61]. 2. Shinohara and colleagues (personal communication) found that meiotic depletion of CDC53 causes a defect in meiotic axis construction in leptotene, resulting in aberrant SC formation. A pch2 mutation fully suppresses the SC construction defect of CDC53 meiotic depletion, suggesting that Pch2 is a negative regulator of meiotic axis assembly.

Our proposal that Pch2 acts at the CO vs. NCO decision differs from interpretations presented by Borner et al. [27]. They examined NCO and CO formation at the HIS4LEU2 hotspot on chromosome III in pch2Δ mutants using Southern blot analysis and found that CO levels were decreased and NCO levels were increased at this site [27,69]. They also monitored progression through recombination at HIS4LEU2 and found that pch2Δ mutants were delayed after SEI formation and accumulate SEIs and dHJs. Given these results and the finding that CO and NCO formation were coordinately delayed, Borner et al. [27] proposed that the meiotic delay in pch2Δ is caused by a defect downstream of the CO vs. NCO decision. We did not observe an effect of the pch2Δ mutation on crossing over on chromosome III. One explanation for this difference is that the Borner et al. [27] performed their analysis at HIS4LEU2, which was shown previously to lack CO homeostasis [32]. Additionally, the delay that they saw in processing recombination intermediates in pch2Δ may be due to an upstream defect at the CO vs. NCO decision. Specifically, the additional load of SEIs and dHJs that the recombination machinery must process in a pch2Δ mutant could delay their turnover genome-wide. It is important to note that we do not have a clear explanation for why CO levels on chromosome III are not elevated in pch2Δ. However, smaller chromosomes in yeast, such as chromosome III, have higher map distances per kb compared to larger chromosomes, and CO interference appears weaker on smaller chromosomes compared to larger ones [38,39]. Thus, because interference is stronger on larger chromosomes, eliminating interference should have a more pronounced effect on CO levels on larger chromosomes, as was seen in our study.

Meiotic axis organization appears to be conserved in S. cerevisiae and C. elegans

Martinez-Perez [70] recently reported a link between meiotic axis protein organization and CO interference in C. elegans. They analyzed the distribution patterns of the central element protein SYP-1 and the axial element proteins HTP-1 and HTP-2, which, like Hop1, are HORMA domain proteins. Analogous to observations made for Hop1 and Zip1 in yeast, Martinez-Perez et al. [70] found that the HTP axial element and the SYP-1 central element proteins sort into reciprocal domains on late pachytene chromosome axes. Based on the above, the finding that HTP1/2 is depleted at COs, the fact that Spo11 and Msh5 are required for domain formation, and the correlation seen between HTP1/2 depletion sites and chiasmata, Martinez-Perez et al. [70] suggest that HTP/SYP-1 domain boundaries mark CO sites. This information suggests that Hop1/Zip1 boundaries indicate where the CO/NCO decision marks subsequent CO sites. Such a model takes into account the finding that C. elegans displays only one domain of each type whereas S. cerevisiae contains a large number of alternating Zip1/Hop1 domains. This pattern is consistent with the fact that each chromosome pair in C. elegans typically enjoys a single CO whereas chromosome pairs in S. cerevisiae enjoy multiple COs (~80–90 total COs in S. cerevisiae [30,35] vs. six in C. elegans [21,70]).
the cause of, the increase in COs. In this interpretation, the defect in CO control in pch2A mutants leads to additional COs, reflected by a greater number of domains, thus making the axis distribution of Hop1 and Zip1 appear more uniform. This model fits with respect to the known timing of the CO vs. NCO decision [17,19,20,21,34,35], and the finding that early Hop1 organization appears normal in pch2A mutants [27]. Testing such a model will require an examination of Hop1 and Zip1 localization patterns in strains (e.g., pch2A spo11 hypomorphs) containing decreased levels of DSBs; our model predicts that the Hop1 and Zip1 domains would become more distinct due to fewer COs, although not completely like wild-type due to defects in CO interference.

Why do pch2 mutants show wild-type spore viability?

The wild-type spore viability seen in pch2A mutants suggests that Pch2-mediated CO control is not required to maintain the viability of yeast grown in lab conditions. We offer two explanations for this finding: 1) COs are present in excess (~90-90 per cell) of the number needed for all homologs to receive an obligate CO (16 per cell). 2) The reduction in interference in pch2A is accompanied by, and likely causes, an increase in the overall number of COs. This increase in crossing over could compensate for distribution failures that jeopardized obligate CO formation [(30,33]; Figure 3, Figure 4; Table 4, Table 5, Table S2). Our results and those of Martini et al. [32] demonstrate a buffered system in baker’s yeast in which excess DSBs and COs lessen the need for interference to ensure obligate CO formation. Because of this buffer, obligate CO formation can be maintained if interference or DSBs are reduced, but not both (Figure 3; [32]). Such buffering may exist because the consequences of having too many COs are less severe than too few. For example, pch2A mutants have dramatic increases in CO levels, but show wild-type spore viability, whereas mutants that significantly decrease CO levels like mlh3Δ, have reduced spore viabilities due to MI nondisjunction [9,11]. Future searches for mutants that disrupt the CO vs. NCO decision must be broadened to include genes with high spore viability or synthetic phenotypes with spo11 hypomorphs.

Although the role of Pch2 in limiting CO levels, after the requisite number required for ensuring obligate CO formation is reached, is not required, it is likely to be advantageous. Too many COs, especially closely spaced ones, have been suggested to disrupt the sister chromatid cohesion required to create tension on the MI spindles and ensure proper homolog disjunction at MI [71,72]. In addition, our data suggests that the CO limiting role of Pch2 also promotes timely meiotic progression, which could also be advantageous to cells (Figure 6).

What causes the loss in spore viability seen in pch2A spo11 hypomorphs? pch2A/pch2A spo11-HA/spo11-HA strains displayed an excess of tetrads with 4, 2, and 0 viable spores, a high percentage of two-spore viable tetrads containing sisters, and an increased frequency of chromosome 3 nondisjunction. Our data are consistent with MI chromosome nondisjunction being a major component of the spore death phenotype, perhaps due to a failure to ensure obligate CO formation on all chromosomes. In such a model, when DSBs become limiting, the proper distribution of COs becomes even more critical to ensure obligate CO formation. Similar DSB levels were seen at YCRO48W at 3.5 hours in meiosis in spo11-HA dmc1A and pch2A spo11-HA dmc1A; however, by 7 hrs, fewer breaks were observed in the triple mutant (Figure 5C and 5D). Our DSB level measurements are not definitive due to the checkpoint bypass observed in the triple mutant. We provide two explanations for the triple mutant phenotype. In one scenario, early forming DSBs appear at wild-type levels while later-forming DSBs form at lower levels that are insufficient for sustained recombination checkpoint activation. In a second scenario, DSBs form normally, but undergo some level of Dmc1-independent, possibly interstitial, repair that permits a bypass of the checkpoint. Such repair would not lead to MI disjunction-promoting chiasmata. Both of these scenarios are sufficient to explain the spore inviability seen in pch2A spo11 hypomorphs (Figure 5). Future experiments to distinguish these hypotheses should include an analysis of meiotic Rad51 foci in spo11-HA dmc1A and pch2A spo11-HA dmc1A strains [73].

We cannot rule out that other cellular defects contribute to the MI non-disjunction phenotype seen in pch2A spo11 mutants. For example, both pch2A and spo11 hypomorphs have SC defects, which could lead to CO control-independent synthetic phenotypes in the double mutants [24,27]. It is also possible that Pch2 promotes MI disjunction by regulating sister chromatid cohesion establishment and/or removal, or by preventing/resolving chromosome entanglements [2-4,68], or that some spore death in pch2A spo11 hypomorphs is independent of MI non-disjunction.

Additional factors are likely to act early in meiosis to establish CO interference

Interference mutants have been proposed to act downstream of the CO vs. NCO decision (e.g. zip1Δ, msh4Δ; Introduction; [22,33]), or display an apparent defect in interference due to an increase in non-interfering COs (aff1, con6) [15,33]. The only other yeast interference mutants that appear similar to pch2A are tid1Δ and dmc1A-Red54 [34; but see 30]. We will focus on tid1Δ, because its CO phenotype is better characterized. Tid1/Rdh54 is a member of the Swi2/Snf2 family, and thus may act in meiotic chromatin axis remodeling, though this has yet to be tested [74]. tid1Δ mutants display moderate levels of spore viability (58% 4-spore viable tetrads), and Tid1 has been shown to be involved in the strand exchange step of recombination [57]. Similar to pch2A, tid1Δ mutants display a defect in interference and increased gene conversion. Also, like pch2A, CO levels in tid1Δ appear similar to wild-type on a small chromosome (III). On a medium-sized chromosome (V), tid1Δ mutants displayed wild-type CO levels in two intervals, but a significant (2-4-fold) increase in a third [34]. These data suggest that tid1Δ and pch2A have similar CO patterns. We are eager to test this hypothesis in the strain sets used in this study. Furthermore, we are intrigued by the idea that strand exchange and meiotic chromatin axis components are both required/involved in interference-regulation of the CO vs. NCO decision.

Materials and Methods

Media and yeast strains

Yeast strains are listed in Table S1. All strains were grown at 30°C on standard YPD (yeast peptone dextrose; [75]). The sporulation media was described previously [11,68]. For tetrad genotyping, synthetic minimal selective media, synthetic complete media with 5 μM Cu, and YPD supplemented with complete amino acid mix and 3 mg/L cycloheximide were used [75]. When required, Geneticin (Invitrogen), nourseothricin (Hans-Knoll Institut für Naturstoff-Forschung), and hygromycin B (Callbiochem) were added to YPD media as described [76,77].

The EAY1108/EAY1112 SK1 congenic strain set is described in Argueso et al. [11], and the NYH942/NYH943 SK1 isogenic strain set is described in de los Santos et al. [10]. The spo11 hypomorphic mutants were described by Diaz et al. [60] and Henderson and Keene [24] although the NYH942/NYH943 strains containing these alleles, which are used in this work, are described in Martini et al. [32]. As in Martini et al. [32], we refer to spo11-HA3His6 as spo11-HA, spo11(D290Y)-HA3His6 as spo11Δa-HA, and spo11(T135F)-HA3His6 as spo11fΔ-HA. Strains EAY2562-
2565 are derivatives of a cross between EAY2260 and SKY633. The msh5Δ, mms4Δ, and dmc1Δ alleles used in this work were all complete open reading frame (ORF) deletions. The pch2Δ allele contains a deletion of amino acids 17–507 (in the 603 amino acid ORF). All deleted regions were replaced with HPMMX4, KANMX4, or NATMX4 as shown in Table S1 [76,77]. The deletion cassettes were made via PCR and integrated into the genome using standard techniques [78]. Details on strain construction and primer sequences are available on request.

Tetrad analysis

Diploids for tetrad analysis were all made using the zero growth mating protocol [79]. The haploid parental strains were patched together on YPD for 4 hours and then spread on sporulation plates. The plates were incubated at 30°C for 2 days, after which tetrads were dissected. Tetrads from the EAY1108/EAY1112 strain background were dissected on synthetic complete media, whereas tetrads from the NHY942/NHY943 strain background were dissected on YPD media supplemented with complete amino acids. All tetrads were incubated 3–4 days at 30°C and then replica-plated to various selective media. The replica plates were scored after one day of incubation at 30°C. In the EAY strain background, the data for wild-type, mms4Δ, and msh5Δ were originally published in Argueso et al. [11]. In the NHY strain background, a subset of the wild-type data was originally published in Wanat et al. [68]. The distributions of each tetrad type were calculated using RANA software [11].

Genetic map distances ± standard error were calculated using the Stahl Laboratory Online Tools [http://www.molbio.uoregon.edu/~fstahl/] which utilizes the formula of Perkins [54]. The G-test spreadsheet, available from The Online Handbook of Biological Statistics [http://udel.edu/~mcdonald/statintro.html], was used to compare tetrad distribution patterns between strains. The Dunn-Sidak correction (p value of 0.05/number of comparisons) was applied when multiple comparisons per data set were performed [80]. Recombination frequencies from spore data were calculated as described previously (RANA software; [11]), with p-values determined as above [http://udel.edu/~mcdonald/statintro.html].

Three different analyses were performed to measure interference. The NPD ratio (Table 4) was determined using the “Better Way” calculator [http://www.molbio.uoregon.edu/~fstahl/]. This method compares the number of each tetrad type observed to the numbers expected if CO distribution was random and calculates a chi square value, which was converted to a p value using VassarStats [http://faculty.vassar.edu/lowry/VassarStats.html]. Coefficients of coincidence (Table 5) were determined as described previously [11,68]. Tetrads were sorted using Mactetrad 6.9 software to calculate interference via the Malkova et al. method ([37, Figure 4; Table S2]).

Meiotic time courses and DSB Southern blotting

For all time courses, a saturated YPD overnight culture from each strain to be analyzed was diluted in 200 ml YPA (2% potassium acetate) and grown for 17 hours. The YPA culture was then spun down, washed once in 1% potassium acetate and resuspended in 100 ml 1% potassium acetate (similar to [81]). All strains were grown in the same batches of media and treated identically. DAPI staining to analyze progression past MI (MI + MII) was performed as described [81]. Cells were visualized using an Olympus BX60 microscope and at least 200 cells were counted for each time point. DNA was isolated from meiotic cultures as described [29]. Southern blotting was performed using standard techniques [82]. The percent of DSB formation for four to six independent time courses (% of hybridizing bands ± standard deviation, SD) was calculated using Image Quant software.

Supporting Information

Figure S1 Spore viability distributions from tetrads in the EAY1108/EAY1112 strain background. The X-axes indicate the number of viable spores per tetrad and the Y-axes indicate the percent of tetrads represented by each class. The number of tetrads dissected (n) is indicated as well as the overall percentage of viable spores (SV). Strains homozygous for the indicated genotypes were analyzed (Table S1).

Table S1 The strains used are listed with their genotypes and the papers in which the strains were originally used. EAY1108 and EAY1112 and their derivatives are SK1 congenic strains. NHY942 and NHY943 and their derivatives are SK1 isogenic strains.

Table S2 Chromosome XV data were obtained from EAY background strains; chromosomes III, VII, and VIII data were obtained from NHY background strains. All pair-wise comparisons between adjacent intervals are shown. The top genetic interval listed in each box is the reference interval. All tetrads were divided into two classes: those with (CO+; i.e. NPD or TT) and those without (CO−; i.e. PD) an observable CO event within the reference interval using Mactetrad 6.9. The genetic size and standard error (SE) of the adjacent genetic interval (the lower listing at the top of the box) was then calculated for each class (CO+ and CO−) using the Stahl Laboratory Online Tools [http://www.molbio.uoregon.edu/~fstahl/]. A ratio of the CO+/CO− class CM values was computed. Interference was considered significant if the CO+ and CO− classes were found to be significantly different via G-tests calculated using the spreadsheet available from The Online Handbook of Biological Statistics [http://udel.edu/~mcdonald/statintro.html].

Table S3 The recombination frequencies between the indicated markers and the number of parental and recombinant spores (as calculated by RANA software; Argueso et al. [11]) in the EAY strain background are shown. p values for G-tests comparing the recombinant and parental spore numbers for all mutant combinations were calculated using the spreadsheet available from The Online Handbook of Biological Statistics [http://udel.edu/~mcdonald/statintro.html].

Acknowledgments

We thank Valentin Borner, Andreas Hochwagen, and Akira Shinohara for sharing unpublished data; Scott Keeney for providing strains; Demelza Kool for DSB Southern blot advice; and Ken Kemphues and Tom Fox for sharing equipment. We are also grateful to Sean Burgess, Neil Hunter, Nancy Kleckner, Frank Stahl, and all Alani lab members for helpful discussions and the anonymous reviewers for their comments. We thank Megan Sonntag for constructing the msh5Δ pch2Δ strain.

Author Contributions

Conceived and designed the experiments: SZ EA. Performed the experiments: SZ. Analyzed the data: SZ EA. Contributed reagents/materials/analysis tools: EA. Wrote the paper: SZ EA.
References

1. Roeder GS (1997) Meiotic chromosomes: it takes two to tango. Genes Dev 11: 2600–2621.

2. Buonomo SB, Glyne RK, Piriou J, Loidl J, Uhlmann F, Nasmuth K (2000) Disruption of homologous chromosomes in meiosis I depends on proteolytic cleavage of the meiotic cohesin Rec8 by separin. Cell 103: 387–398.

3. Bickel SE, Orr-Weaver TL, Balicky EM (2002) The sister-chromatid cohesion protein ORC is required for chiasma maintenance in Drosophila oocytes. Curr Biol 12: 952–960.

4. Hodges CA, Revenkova E, Jessberger R, Hassold TJ, Hunt PA (2005) SMC1/2 beta-deficient female mice provide evidence that cohesins are a missing link in age-related nondisjunction. Nat Genet 37: 1531–1535.

5. Nishant KT, Plys AJ, Alani E (2008) A mutation in the putative MLH3 endonuclease domain confers a defect in both mismatch repair and meiosis in Saccharomyces cerevisiae. Genetics 174: 1745–1754.

6. Hassold T, Hall H, Hunt P (2007) The origin of human aneuploidy: where we have been, where we are going. Hum Mol Genet 16: R203–208.

7. Ross-Macdonald P, Roeder GS (1994) Mutation of a meiosis-specific MutS homolog decreases crossing over but not mismatch correction. Cell 79: 1069–1080.

8. Hollingsworth NM, Ponte L, Halsey C (1995) ABF3, a novel MutS homolog, facilitates meiotic recombination between homologs in Saccharomyces cerevisiae but not mismatch repair. Genes Dev 9: 1728–1739.

9. Wang TF, Kleckner N, Hunter N (1999) Functional specificity of MutS homologs in yeast: evidence for three MutS1-based heterocomplexes with distinct roles during meiosis in recombination and mismatch correction. Proc Natl Acad Sci USA 96: 13914–13919.

10. de los Santos T, Loidl J, Larkin B, Loidl J, Hollingsworth NM (2003) The Mus81/Mms4 endonuclease acts independently of double-Holliday junction resolution to promote a distinct subset of crossovers during meiosis in budding yeast. Genetics 164: 81–94.

11. Argueso JL, Wanat J, Gemici Z, Alani E (2004) Competing crossover pathways act during meiosis in Saccharomyces cerevisiae. Genetics 168: 1805–1816.

12. Getz TJ, Higgin JD, Sanchez-Moran E, Armstrong SJ, Osman KE, et al. (2006) Control of meiotic recombination in budding yeast: the role of the MutS and MutL homologues. Biochem Soc Trans 34: 542–544.

13. de Boer E, Stam P, Dietrich AJ, Pastink A, Heyting C (2006) Control of meiotic recombination in Saccharomyces cerevisiae requires a TID1/RMD4+ and DMC1-dependent pathway. Genetics 163: 1273–1296.

14. Fung JC, Rockmill B, Odell M, Roeder GS (2004) Impression of crossover interference through the nonrandom distribution of synopsis initiation complexes. Cell 116: 795–802.

15. Lawrie NM, Tease C, Hulton MA (1995) Chromias, frequency, distribution and interference maps of mouse autosomes. Chromosoma 104: 308–314.

16. Malkova A, Swanson J, German M, Mcusker JH, Houssow PA, et al. (2004) Evidence and deletion of breakpoints along the 405-kb left arm of Saccharomyces cerevisiae chromosome VII. Genetics 168: 49–63.

17. Kaback DB, Guacci V, Barber D, Mahon JW (1992) Chromosome size-dependent control of meiotic recombination. Science 256: 228–232.

18. Kaback DB, Barber D, Mahon J, Lamb J, You J (1999) Chromosome size-dependent control of meiotic reciprocal recombination in Saccharomyces cerevisiae: the role of crossover interference. Genetics 152: 1475–1486.

19. Stahl FW, Foss HM, Young LS, Borts RH, Abdullah MF, Copenhagen GP (2004) Does crossover interference count in Saccharomyces cerevisiae? Genetics 168: 35–48.

20. Turney D, de los Santos T, Hollingsworth NM (2004) Does chromosome size affect map distance and genetic interference in budding yeast? Genetics 168: 2421–2424.

21. Blizthea HG, Bell GW, Rodriguez J, Bell SP, Hochwagen A (2007) Mapping of meiotic single-stranded DNA reveals double-stranded-break hotspots near centromeres and telomeres. Curr Biol 17: 2003–2012.

22. McMahill MS, Sham CW, Bishop DK (2007) Synthesis-dependent strand invasion by the meiotic cohesin Rec8 by separin. Cell 103: 387–398.

23. Buhler CV, Borde V, Lichten M (2007) Mapping meiotic single-strand DNA reveals a new landscape of DNA double-strand breaks in Saccharomyces cerevisiae. PLoS Biol 5: e324. doi:10.1371/journal.pbio.0050324.
61. Hochwagen A, Tham WH, Bear GA, Amon A (2005) The FK506 binding protein Fpr3 counteracts protein phosphatase 1 to maintain meiotic recombination checkpoint activity. Cell 122: 861–873.
62. Liu J, Wu TC, Lichten M (1995) The location and structure of double-strand DNA breaks during yeast meiosis: evidence for a covalently linked DNA-protein intermediate. EMBO J 14: 4599–4608.
63. Yamashita K, Shinohara M, Shinohara A (2004) Rad6-Bre1-mediated histone H2B ubiquitylation modulates the formation of double-strand breaks during meiosis. Proc Natl Acad Sci USA 101: 11380–11385.
64. Bishop DK, Park D, Xu L, Kleckner N (1992) \textit{DMC1}: a meiosis-specific yeast homolog of \textit{E. coli} \textit{recA} required for recombination, synaptonemal complex formation, and cell cycle progression. Cell 69: 439–456.
65. Alani E, Padmore R, Kleckner N (1990) Analysis of wild-type and \textit{rad50} mutants of yeast suggests an intimate relationship between meiotic chromosome synapsis and recombination. Cell 61: 419–436.
66. Zierhut C, Berlinger M, Rupp C, Shinohara A, Klein F (2004) Mnd1 is required for meiotic interhomolog repair. Curr Biol 14: 752–762.
67. Hochwagen A, Amon A (2006) Checking your breaks: surveillance mechanisms of meiotic recombination. Curr Biol 16: R217–28.
68. Wanat JJ, Kim KP, Kosmol R, Zanders S, Weiner B, Kleckner N, Alani E (2008) Csm4, in collaboration with Ndj1, mediates telomere-led chromosome dynamics and recombination during yeast meiosis. PLoS Genet 4: e1000188. doi:10.1371/journal.pgen.1000188.
69. Cao L, Alani E, Kleckner N (1990) A pathway for generation and processing of double-strand breaks during meiotic recombination in \textit{S. cerevisiae}. Cell 61: 1089–1101.
70. Martinez-Perez E, Schvarzstein M, Barroso C, Lightfoot J, Dernburg AF, Villeneuve AM (2008) Crossovers trigger a remodeling of meiotic chromosome axis composition that is linked to two-step loss of sister chromatid cohesion. Genes Dev 22: 2886–2901.
71. Nilsson NO, Sall T (1995) A model of chiasma reduction of closely formed crossovers. J Theor Biol 173: 93–98.
72. van Veen JE, Hawley RS (2003) Meiosis: when even two is a crowd. Curr Biol 13: R531–R533.
73. Lydall D, Nikolovsky Y, Bishop DK, Weinert T (1996) A meiotic recombination checkpoint controlled by mitotic checkpoint genes. Nature 383: 840–843.
74. Petushkova G, Sung P, Klein H (2000) Promotion of Rad51-dependent D-loop formation by yeast recombination factor Rdh54/Tid1. Genes Dev 14: 2206–2215.
75. Rose MD, Winston F, Hieter P (1990) Methods in yeast genetics: A laboratory course manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
76. Wach A, Brachat A, Pohlmann R, Philippsen P (1994) New heterologous modules for classical or PCR-based gene disruptions in \textit{Saccharomyces cerevisiae}. Yeast 10: 1793–1808.
77. Goldstein AL, McComber JH (1999) Three new dominant drug resistance cassettes for gene disruption in \textit{Saccharomyces cerevisiae}. Yeast 15: 1541–1553.
78. Gietz RD, Schiestl RH, Willems AR, Woods RA (1995) Studies on the transformation of intact yeast cells by the LzAc/SS-DNA/PEG procedure. Yeast 11: 355–360.
79. Angues JL, Kijas AW, Sarin S, Heck J, Waase M, Alani E (2003) Systematic mutagenesis of the \textit{Saccharomyces cerevisiae} \textit{MLH1} gene reveals distinct roles for Mlh1p in meiotic crossing over and in vegetative and meiotic mismatch repair. Mol Cell Biol 23: 873–886.
80. Hoffmann ER, Schurbockova PV, Kunkel TA, Borts RH (2003) \textit{MLHI} mutations differentially affect meiotic functions in \textit{Saccharomyces cerevisiae}. Genetics 163: 515–526.
81. Galbraith AM, Bullard SA, Jiao K, Nau JJ, Malone RE (1997) Recombination and the progression of meiosis in \textit{Saccharomyces cerevisiae}. Genetics 146: 481–489.
82. Lyndaker AM, Goldfarb T, Alani E (2008) Mutants defective in Rad1-Rad10-SxI exhibit a unique pattern of viability during mating-type switching in \textit{Saccharomyces cerevisiae}. Genetics 179: 1007–1021.