A Study of Crossing Over in *Saccharomyces cerevisiae*
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ABSTRACT

During meiosis DNA undergoes recombination through crossing over within homologous chromosomes to produce genetic variability. It has been previously shown that a crossover at any given point interferes (crossover interference) with the occurrence of crossovers at nearby locations on the same chromosome. How interference occurs is unknown. Crossover interference could be induced by a release of tension in the chromosome (“stress model”) or by some unknown system that counts a specific number of genetic distances away from the point of crossover before allowing another crossover (“counting model”). Studying the effects of a crossover between nonhomologous chromosomes will give light to the necessity for the participation of homologues in crossing over and interference. Preliminary data show that under conditions in which an ectopic recombination event is occurring, interference is nonexistent, indicating that both homologues of a chromosome must be present for crossing over and interference to occur, a necessity in proper segregation and propagation of genetic diversity.

INTRODUCTION

In meiosis, double strand breaks (DSBs) formed by topoisomerase-like SPO11 protein are believed to be responsible for the resolution of both crossovers and noncrossovers. Noncrossover recombinants are not effective in mediating the disjunction of homologs at meiosis 1. Therefore, the use of crossovers is essential for proper chromosome segregation and genetic diversity among progeny (Malkova et al., 2004; Storlazzi et al., 1995). Recombinational repair of double strand breaks (DSBs) results in gene conversion, the process by which information carried on DNA is transferred from one DNA helix which remains unchanged to another DNA helix whose sequence is
altered. These gene conversions are frequently associated with crossing over in meiosis. The number of DSBs compared to crossovers has been quantified in many organisms with some organisms showing greater than ten fold more DSBs than crossovers (Moens et al., 2002); in yeast about 35% of gene conversions become crossovers (Allers & Lichten, 2001). These DSBs that do not become crossovers are repaired and resolved as noncrossovers. There are contradicting data regarding the temporal formation of noncrossovers. Allers and Lichten (2001) found that noncrossovers do not form at the same time as crossovers. This, according to Allers and Lichten, is because crossovers and noncrossovers are under the control of different genetic pathways that form the products we see. However, according to Storlazzi et al., noncrossovers are found to be matured at the same time as crossovers, indicating the use of two separate pathways where the “decision” to enter a pathway is determined early in DSB repair (Storlazzi et al., 1995). This differentiation involves some sort of decision by which some precursors enter one pathway to be resolved as crossover, while all other DSBs enter some other unknown pathway to be resolved as noncrossovers. The mechanism by which one DSB product is chosen for a crossover fate over another is still not known. However, one hypothesis is that crossovers are resolved as a mechanism inherently linked to interference. This also suggested that all other breaks, regardless of how many, or how few, are resolved as noncrossovers (Martini et al., 2006).

Data have shown that the distribution of crossovers among chromosomes is not random. There are on average one to two crossovers per chromosome. While this number is very low, there are always crossovers; nonexchange chromosomes are very rare. The distribution of crossovers along a chromosome also appears to be nonrandom. A
crossover in one location makes it less likely to see another crossover in a nearby location. This phenomenon is known as crossover interference (Martini et al., 2006). Crossover interference occurs specifically when the proportion of closely spaced crossovers is lower than would be expected from a random distribution and can be measured by a lower than expected incidence of nonparental ditypes within a single interval (Papazian 1951; Roeder 1997; Fung et al., 2004; Malkova et al., 2004). It has been found that interference depends on the MSH4-MSH5-ZIP1 epistasis group. Mutant strains lacking one of the genes associated with this group show a decrease in the amount of interference seen. However, MUS81 and MMS4 have been shown to reduce interference without affecting crossovers. Therefore, it is believed that there are two pathways that lead to crossovers, one that influences interference and one that does not (Malkova et al., 2004; Stahl et al., 2004). It is believed that DSBs are repaired through a recombination pathway that involves a Holliday junction, a four strand complex through which crossovers and noncrossovers can be resolved. A Holliday junction must yield a crossover if the intermediate is stabilized. A noncrossover occurs when the Holliday junction is not stable or possibly does not even exist. However, the duality of data seen by both Malkova and Stahl addressing the presence of crossovers and the lack of interference in certain mutant strains is believed to occur through ligated Holliday junctions as opposed to the standard unligated junction. The resolution of crossovers and noncrossovers in a ligated Holliday junction has no such limitations to become a crossover or not. The ligation stabilizes the intermediate and abolishes the nick that would have directed the resolvase to one substrate. Because of this, any resolution of the Holliday junction is possible, resulting in the dimorphic results seen (Stahl et al., 2004).
Research has shown that a specific resolution of DSBs as crossovers leads to a decrease in the number of adjacent crossovers along that chromosome. How one recombination event at one location may influence the recombination at large distances away is not well understood. Various models have been proposed to explain interference. One hypothesis is that the interference is due to some unknown type of signal sent between the synaptonemal complex linking the homologous pairs of chromosomes preventing the crossing over for a certain distance downstream of the cross over. Double strand breaks (DSBs) form intermediates of recombination. One model suggests that a resolvase then “counts” some number of intermediate events that it resolves as noncrossovers between two events that are resolved as crossover. As shown by McPeek and Speed (1995), this fixed number of noncrossovers between adjacent crossovers was found to be four for Drosophila and two for Neurospora. According to Stahl et al. (2004) and Malkova et al. (2004), the fixed number of noncrossovers between adjacent crossovers is believed to be three in budding yeast. This theoretical prediction was correlated with experimental data from Hilliker and Chovnick (1981), Hilliker et al. (1991), and Perkins et al. (1993). The distance between the two events resolved as crossovers is the distance through which interference occurs (Stahl et al., 2004). It has been shown that within a defined chromosome segment, crossover density is higher and interference is weaker when that segment is embedded in a short chromosome than when it is in a long chromosome (Kaback et al., 1999). If Saccharomyces cerevisiae do indeed follow a “counting model” for resolving crossovers and noncrossovers, then there should be a fixed number of noncrossovers seen between each resolved crossover. However, as shown by Foss and Stahl in 1995, this was not the case. In 2004, Malkova et al.
suggested a third possibility for that could support the "counting model". Malkova suggested that if a third category of resolved DSB products existed, those that are resolved as crossovers but that do not exhibit interference, there would be a randomly distributed number of noninterfering resolved products between two resolved, interfering crossover products. The presence of noninterfering crossovers supports data found by Zhao et al. (1995) that shorter chromosomes are more susceptible to interference and that interference suppresses crossover density. Essentially it was suggested that regions of weaker interference reflects a relatively higher density of noninterfering crossovers while a region of stronger interference would signal a relatively higher concentration of interfering crossovers (Stahl et al., 2004). It is also shown that within a defined chromosome segment, crossover density is higher and interference is weaker when the segment is embedded in a short chromosome than when it is in a long chromosome. This data supports the notion that one kind of crossover occurs at a roughly fixed number per kilobase, while the other occurs at a roughly fixed number per chromosome. The latter would result in higher densities of crossing over on shorter chromosomes (Stahl et al., 2004). It is this clustering that strongly supports a "counting model". However there is not any evidence as to what this signal is or how it is propagated over such long distances. The counting model is refuted by Martini et al. (2006) who showed that the ratio of crossovers to noncrossovers changes as SPO11 activity is varied, thereby contradicting the fixed nature of the ratio that is implied in the counting model.

Another model proposes that a crossover is a mechanism by which two homologs can release built up stress, similar in mechanism to the stress released when a polymer coated piece of an elastic beam is quickly heated causing the beam to expand without the
polymer expanding. Release of this stress results in the local release of stress on either side of the affected site. Once the stress is released, another crossover will not occur close by because there is no stress point for a crossover to occur at. This model is based on the concept that the spatial patterning of the chromosome is crucial to the location at which crossing over and interference occurs (Kleckner et al., 2004; Borner et al., 2004). The signal sent from the release of stress could behave in a similar manner to the signals sent in the “counting model” although there is not much evidence for what this signal might be. This model is also supported by the idea that interference drops off as a function of distance (Martini et al., 2006).

Using two parental strains containing heterozygous nutritional markers surrounding the met13 gene on chromosome 7, I studied the effects of different factors on crossing over and recombination frequencies. At the crux of this study is the understanding that the frequency of crossovers is directly related to the distance between genes in morgans and is defined as the average number of points of crossing over in the interval that separates the genes (Stahl et al., 2004). Using this standard and the knowledge of distances based on wildtype meioses, we can measure the affect different factors have on the crossover frequencies between given heterozygous markers. In 2004, Malkova et al., using strains containing heterozygous dominant markers surrounding an isogenic methionine locus, showed that interference occurs for tens of kilobases along the left arm of chromosome 7. In the same year, Hollingsworth published a paper studying the effects of chromosome size on interference. She found that chromosome size had little to no effect on interference; a finding that contradicted the findings of other published experiments. Very little is known about recombination and the factors that
influence it. Contradicting data make it probable that different segments of DNA, different sized chromosomes, or even different segments of chromosomes behave differently in respect to the rates of recombination. Because of this, the data collected by Malkova (2004), data only for chromosome VII, cannot be extrapolated. In my experiments, however, I used derivatives of the strains used by Malkova in 2004 to study the effects of different factors on the recombination frequencies and interference of chromosome VII.

One system to gain insight into the mechanism of crossing over interference is to study the effects of an ectopic nonhomologous crossover on the adjacent heterozygous markers. Results from this experiment should indicate if it is necessary for the homologs of a chromosome to both be involved in the resolution of a crossover for interference to be felt. By inducing an ectopic crossover and studying the effects on the surrounding nutritional markers we should get some indication of the strength of interference and the necessity of both homologs in the mechanism of crossing over.

Another factor that I studied is nonhomologous end joining. DNA end joining is a common mechanism of double-strand-break (DSB) DNA repair. The process is called nonhomologous, or illegitimate, recombination because it requires little or no DNA homology to join the ends of DNA (Moore & Haber, 1996). NEJl is thought to have an effect on nonhomologous end joining (NHEJ) by facilitating the movement of LIFl into the nucleus. Lack of NEJl reduced NHEJ by 100 fold and constitutive expression of NEJl restores NHEJ in mitotic cells (Valencia et al., 2001). It has been proposed that repression of the NHEJ pathway may promote homologous recombination and crossing over in diploid cells (Valencia et al., 2001). Because of this, we were interested in the
effects of NEJ1 in meiotic systems. Preliminary data indicated that over expression of NEJ1 had no effect on the level of crossing over in the intervals on chromosome VII (See Results). However, one explanation for this could be that NHEJ was not the most efficient repair process occurring during meiosis and there was a more efficient process whose actions repaired the damage. Because of this any effects that NHEJ would have had on crossing over would not have been detected in this system. Another way to study the effects of NHEJ is through the manipulation of another gene, RME1. RME1 is a zinc finger protein that blocks meiosis by preventing the expression of IME1. RME1 is expressed in a and α cells that are unable to enter into meiosis. It is repressed in a/α cells, which enter meiosis in response to starvation (Covitz et al., 1994). By using the rme1::KAN inserted strains, and deleting the α mating type to create an a/α strain, we can allow the cells to undergo meiosis in a system that would normally not allow it. In this strain, NEJ1 is not repressed because the system behaves as a haploid, mitotic system. Because of this, NHEJ can compete in DSB repair. In this system, we may be able to see a difference in crossover frequencies, and allow us to understand better the relationship between homologous recombination and crossovers.

**MATERIALS & METHODS:**

**Strains:** Haploid strains YPB1 and YPB2 were derived from the diploid MAG100. The diploid, MAG100 was created by a series of auxotrophic and drug-resistant mutations isolated in the homothallic Y55 strain background (McCusker and Haber, 1988b; Malkova et al., 2004). Haploids were isolated from MAG100 with the genotype *MATa ura3-Y1 ADE5 KAN lys5-Y2 NAT met13-Y1E cyh2-Y1 CRL3 TRP5 LEU1* and *MATa ura3-Y1 ade5-Y7 LYS5 MET13 HPH CYH2 crl3-2 trp5-Y1 leu1-Y1*, the
Figure 1: A) Map of chromosomes V and VII of YPB1. Transformation with plasmid containing wildtype copy of URA3 gene along with met13-5 allele created ura3 repeat on chromosome V of YPB1. B) Map of chromosomes V and VII or YPB2. Created by crossing Nat24 (Genotype: *MATα ura3-Y1 ADE5 KAN lys5-Y2 NAT met13-Y1E cyh2-Y1 CRL3 TRP5 LEU1) with Hyg1 (Genotype: *MATα ura3-Y1 ade5-Y7 lys5 MET13 HPH CYH2 crl3-2 trp5-Y1 leu1-Y1) and selecting for spores containing the met13-1 allele.

former was called Nat24 and the latter called Hyg1. Creation of YPB1 (Figure 1a) was done through insertion of *met13*-5 into a URA3 plasmid, and subsequent transformation into Nat24. YPB2 was created by selecting for *met*- colonies in a cross between Nat24 and Hyg1 (Figure 1b).

Using strains Nat24 and YPB2, transformations were done to create strains containing *NEJ1* constitutively expressed under the ADH promoter (transformation of pMV01) or *RME1* deleted by a KAN cassette (transformation of rme1::KAN PCR product). These strains are respectively called YPB3 and 4 (*Nej1::ADH transformed into both Nat24 and YPB2) and YPB5 and 6 (rme1::KAN transformed into both Nat24 and YPB2). YPB5 and 6 were crossed to create a diploid. This diploid was transformed with pJH526 to delete MATα and create an aΔ diploid called YPB7.

**Genetic Analysis:** Diploids were grown in yeast extract-peptone-dextrose (YEPD) medium and sporulated at 30°, as described by McCusker and Haber (1988b). Random spores were produced by digesting tetrads in 1X Glusulase overnight at 30° and subsequently sonicating. Cells were plated on nutritional drop out medium lacking
methionine. Tetrad dissection was done on YEPD plates and germinated at 30\(^\circ\). Spores were then isolated and analyzed. Auxotrophic markers were scored by replica plating on standard nutritional drop-out media lacking one amino acid. The temperature sensitive crl3-2 allele was scored by replica plating to YEPD plates subsequently incubated at 37\(^\circ\). Resistance to antibiotics was scored by replica plating to YEPD plates containing 10 mg/ml cycloheximide, 25 mg/ml nourseothricin, 300 \(\mu\)g/ml hygromycin B, or 300 \(\mu\)g/ml kanamycin.

Differentiation between conversion with or without associated crossovers at MET13 was determined through sensitivity or resistance to 5-Fluoroorotic Acid (5-FoA). If a gene conversion occurred without an associated crossover, the strains should be able to

![Diagram](image-url)

**Fig. 2**: a) Use of FoA plates can determine if ectopic conversion was associated with a crossover. When crossing over does not occur, the strain has a ura3 locus, and therefore is FoA sensitive. However, because the URA3 and ura3 loci are on the same chromosome, they can recombine on FoA plates to form a single ura3 locus with the met13 gene deleted. This will be FoA resistant. Because of this, when a conversion is not associated with crossing over, the colonies will papillate on 5-FoA plates. b) When crossing over does occur, the strain will be FoA sensitive because there is a copy of the wildtype URA3 gene.
papillate on 5-FoA plates as they contain a ura3-met13-URA3 repeat that can recombine to become 5-FoA resistant half the time and 5-FoA sensitive the other half (Figure 2a). However, if a conversion occurs with an associated crossover, the wildtype and mutated URA3 alleles move to different chromosomes and therefore cannot recombine to become 5-FoA resistant (Figure 2b). This can occur either in meiosis or mitosis. All colonies that papillate are classified as noncrossovers. Those colonies that are 5-FoA resistant are part of a set of data that are not applicable to this experiment. All colonies that are 5-FoA sensitive are classified as crossovers.

**Tetrad analysis and calculation of interference:** Dissected tetrad data were analyzed using MacTetrad, version 6.9 written by Jonathan Greene, Warren Voth, and David Stillman. Map distances between markers were calculated by the formula of Perkins (1949), where map distance (in centimorgans) = 100([6NPD + (TT)]/[2 X [PD + NPD + TT]], where PD, NPD, and TT stand for parental ditype, nonparental ditype, and tetratype, respectively. Map distances for random spores were calculated by dividing the number of recombinants by total sample size for each interval being studied. This was done for all intervals. Map distances were compared to those found in 2004 by Malkova et al. (Figure 3).

Interference for the random spores was calculated by comparing the random spore data from Malkova et al. (2004) to data obtained in these experiments using an

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**Table:**

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*Figure 3: Map distances as found by Malkova et al (2004)*
interactive chi-square ($\chi^2$) site at http://www.psych.ku.edu/preacher/chisq/chisq.htm.

RESULTS

Creation of the Nat24 parental strain

A PCR purified met13-5 product was transformed into a plasmid containing URA3 (YIP5). This plasmid containing met13-5 (MYIP5) (Figure 4) was then digested with several different enzymes to confirm that the met13-5 allele was inserted into the correct location. Figure 5 shows MYIP5 digested with EcoR1 (producing a 1.3 kb and 6.7 kb band), HindIII (producing a 6.1 kb and 1.8 kb band), and Stu1 (producing 1 band that is 7.9 kb). MYIP5 was then transformed into Nat24 to create the parental strain YPB1 (Figure 1a). This strain was tested by plating on 5-FoA plates and selecting colonies that form papillae (Figure 6). In addition, a southern was done to confirm the presence of the insertion (Figure 7).

Figure 4: Map of MYIP5 with Stu1, EcoR1, and HindIII digestion sites.

Figure 5: Digestion of YIP5 and MYIP5 with EcoR1 and HindIII. Lane 1 is 1kb ladder. Lane 2 is YIP5 digested with EcoR1. Lanes 3 & 4 are MYIP5 digested with EcoR1. Lane 5 is YIP5 digested with
Cross of Parental Strains

Five Nat24 parental transformants containing the met13-5 allele on chromosome V were crossed to YPB2 to create diploids. These diploids were randomly sporulated and plated on both methionine drop out plates and YEPD plates. Only Met\(^+\) spores grew on the methionine drop out plates. These spores were all the products of some ectopic conversion event, associated with or without a crossover. These spores were tested for all markers by replica plating. Three classes of spores were obtained (Figure 8). Of all markers, only KAN and MAT segregated in a 1:1 fashion. About sixty percent of the spores segregated as Hyg\(^+\). It is believed that because of this bias towards the Hyg\(^+\) spores, for whatever reason, about fifty-five percent of the spores segregated as nat\(^-\). Further along the chromosome, the markers directly adjacent to HYG and NAT, cyh2 and lys5 both segregated with a slight bias towards minus and plus respectively. Fifty-four percent of the spores were cyh\(^+\) and fifty-five percent of
the spores were $LYS^+$. Analysis of the 612 spores in the data set showed that 32 spores were $nat^-$ and $hyg^-$ indicating a crossover in the $NAT/HYG$ interval. 302 spores were $nat^+$ and $HYG^+$, indicative of a parental formation, but showing the bias towards the larger $HYG^+$ data set. 233 spores were $NAT^+$ and $hyg^-$. Finally, 45 spores in the data set were $NAT^-$ and $HYG^+$, another set of products formed by a crossover in the $NAT/HYG$ interval. If we look at the whole data set, without narrowing it, there are statistically insignificant changes in map distance ($\chi^2 P = 0.754$) (Table 1a). When looking at all of the data, the $KAN/LYS$ interval has a map distance of 22.0 cM compared to previous data which indicated that the map distance of this same interval is 21.4 cM. Similarly, the $LYS/NAT$ interval was found to be 18 cM when previously it was set at 20.9 cM. The distance in the $NAT/HYG$ interval increased by about 4 cM, going from 8.6 cM in previous studies, to 12.6 cM in this data set. Lastly, the $HYG/CYH$ interval went from 10.2 cM to 8.2 cM. All of these changes, while noticeable, are not statistically significant and would indicate no effect of ectopic events on interference.
The data set can be broken down to look at specific data sets. One such way is to look at just the \( URA^+ \) spores that would select for the ectopic events that involved the parental chromosome V that contained the \( met13-5 \) allele. If we look at this data set, the map distances are once again not statistically significant (\( \chi^2 P = 0.874 \)) (Table 1b). However, this data set can further be broken down into those colonies which form papillae on 5-FoA plates (conversions with no associated crossovers) and those colonies which do not grow on 5-FoA plates (conversions with associated crossovers). It is possible that this breakdown will show interference at a statistically significant level.

We can also look at the data as a subset of \( hyg^+ \) spores to eliminate false positives that might be contained within the data as was possible by the significant bias towards \( HYG^+ \) spores. However, this data set, once again, does not show any statistically significant difference (\( \chi^2 P = 0.899 \)) (Table 1c). The data can also be broken down by scoring of 5-FoA plates. By analyzing the data that are 5-FoA papillating for their map distances and comparing this to the spores that at 5-FoA nonpapillating, we can see if an

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ectopic conversion with associated crossover has an effect on interference when compared to an ectopic conversion without an associated crossover. Once again there is no statistical significance in the map distances ($\chi^2 P = 0.840$) (Table 1d). However, my entire data set contains the spores that would have come about through segregation with the parental chromosome V that does not contain the met13-5 allele. Because these would have arisen through homologous events, they can be used as a control group but not in studying the effects of ectopic conversions in crossing over. A last way to compare the data is by looking at the $hyg^r$ spores that either 5-FoA papillating or 5-FoA nonpapillating ($\chi^2 P = 0.424$) (Table 1e). This data set is once again not statistically significant but still has the same concerns as when looking at the previous data set. We cannot be entirely sure about the scoring of the "crossover" and "noncrossover" classes because both 5-FoA$^R$ and 5-FoA$^p$ spores were classified in the same way and cannot be differentiated. Therefore cannot make any conclusions about what we see. However, by replating all spores onto 5-FoA plates and rescoring, this problem can be easily fixed.

Analysis of Interference in Intervals surrounding met13

Interference in the intervals surrounding the met13 locus on chromosome VII, specifically the LYS/NAT interval and the HYG/CYH interval, can be studied to determine if some level of interference is detected. By dividing the data set into several categories, once again, we can compare this data to that of Malkova et al. (2004) to get a statistical distribution. Table 2 shows the intervals analyzed and the level of statistical significance present in the data. It becomes evident based on the $\chi^2 P$ values that very little interference is occurring in adjacent intervals, the same intervals in which interference was readily detected when recombination occurred between homologous chromosomes.
Table 2

<table>
<thead>
<tr>
<th>Analysis of interference based on random spores – URA+ Spores</th>
<th>Analysis of interference based on random spores – All Data</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Met+ spores</strong> that are <strong>NAT-hyg</strong> crossovers (P = 0.549; Yates P = 0.797)</td>
<td><strong>Met+ spores</strong> that are <strong>NAT-hyg</strong> crossovers (P = 0.125; Yates P = 0.301)</td>
</tr>
<tr>
<td><strong>Crossovers</strong></td>
<td><strong>Noncrossovers</strong></td>
</tr>
<tr>
<td>X2 P (interference)</td>
<td>0.717 (none)</td>
</tr>
<tr>
<td>Yates P</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>Met+ spores</strong> that are <strong>NAT-hyg</strong> noncrossovers (P = 0.896; Yates P = 0.697)</td>
<td><strong>Met+ spores</strong> that are <strong>NAT-hyg</strong> noncrossovers (P = 0.461; Yates P = 0.732)</td>
</tr>
<tr>
<td><strong>Crossovers</strong></td>
<td><strong>Noncrossovers</strong></td>
</tr>
<tr>
<td>X2 P (interference)</td>
<td>0.051 (none)</td>
</tr>
<tr>
<td>Yates P</td>
<td>N/A</td>
</tr>
</tbody>
</table>

**Analysis of an Exceptional Class of Spores**

Of the set of 612 spores, 32 of them were nat and hyg and 45 of them were NAT⁺ and HYG⁺. Such high numbers of crossovers in the NAT/HYG interval were not expected because this would be a rare event because it would involve an ectopic event between chromosome V and VII and a homologous event between both chromosome VIIIs in a small genetic interval. These numbers indicated that perhaps a high level of nondisjunction was occurring, one of the few ways that NAT and HYG spores could have the same scoring product and arise from the same meiosis to yield a high number of NAT⁺/HYG⁺ spores. However, these spores, and the nat-hyg- spores could also come about by a double conversion event; one between chromosomes V and VII and one between both chromosome VIIIs. These spores were analyzed through polymerase chain reactions (PCR), a southern, and a CHEF gel to determine if there was a higher level of nondisjunction as had previously been seen in the literature.

The PCR primers amplified the region surrounding the open reading frame into
which the \textit{HYG} marker was inserted. In one parent the primers amplify the ORF which is 585 bp. In the other parent, the primers amplify the \textit{HYG} marker which is about 2.5 kb. If nondisjunction occurred in the spores, then both bands should be present as both copies of chromosome VII would be present, that which carries the \textit{HYG} marker and that which does not. Figure 9 shows the analysis of 5 \textit{NAT}/\textit{HYG} spores that were analyzed through PCR. It is clear that both bands are present, indicative of nondisjunction.

In addition to the PCR, a southern blot was done to confirm the presence of crossover products and nondisjunction. After digestion with Stu1, the gels were probed with \textit{met13} to select for all fragments that should be present if either a crossover product existed or if nondisjunction occurred (Figure 10). The southern blot was not informative for either case. While bands were present, the expected bands were not seen and extra bands were present. However, the banding pattern was similar to that of the southern done on the YPB1 colonies in Figure 7. The “crossover” products have the same banding patterns, as the “noncrossover” products. This is indicative of the fact that there may be
The expected shift of chromosome V at 600 kb and chromosome VII at 1100 kb to their crossover products at 950 kb and 750 kb were not decipherable (Figure 11a). However, a southern blot was subsequently done on this CHEF gel to probe for specific fragments of DNA. When probed with pBR322 (the DNA surrounding met13-5 on chromosome V), it was clear that out of eleven samples, only one was a clear crossover product (Figure 11b). Some information that we are missing about cut sites. Further analysis will have to be done to confirm this result.

A CHEF gel was also run to analyze this exceptional class of products. A CHEF gel runs whole chromosomes at their respective sizes such that any translocations can be identified through shifts in the gel. The CHEF gel was hard to decipher.
The other samples that were supposedly crossover products did not appear as such which brought up the question of whether we were scoring crossovers and noncrossovers correctly.

**Nonhomologous Endjoining (NHEJ)**

NEJ1 is one gene responsible for NHEJ. Two strains were created that would overexpress NEJ1 under the control of the ADH promoter (Valencia et al., 2001). These strains were YPB3 and YPB4. YPB3 contained the *met13-5* allele on chromosome VII while YPB4 contained the *met13-1* allele on chromosome VII. Surrounding these mutations were heterozygous nutritional markers including HYG, cyh2, NAT, and lys5. Around 200 tetrads were dissected and map distances between adjacent markers were determined. The data showed that NHEJ does not affect the genetic map distances of adjacent markers (Figure 12). This would suggest that NHEJ did not compete with normal meiotic homologous recombination. However, we were concerned that *NEJ1*, while under the ADH promoter, was not properly expressed in meiosis, although in mitotic cells this plasmid was capable of complementing *nej1Δ* (Valencia et al., 2001).

Because of this, we developed another system to study this phenomenon to ensure that NHEJ does not, in fact, have any effect on the map distances between markers. NHEJ is repressed in meiosis, perhaps for protection against random recombination events. *RME1*
is a gene responsible for repressing meiosis in haploid mitotic cells or in diploids homozygous for one mating-type. Using strains YPB5 and YPB6 which have \( rme1 \) deleted by a KAN cassette, we can allow meiotic processes to occur in diploids that are not MATa/MATα. Then, by deleting MATα we can create a diploid system which can undergo meiosis. In this system, \( NEJ1 \) is not repressed and NHEJ becomes a competing process in DSB repair. Studying the effects of this system on adjacent markers should give insight into the role of NHEJ or homologous recombination in meiosis. After dissecting around 100 tetrads, it became very apparent that the viability in this system dropped drastically. Only about 10 whole tetrads survived. The remaining 90 tetrads had 1-3 lethal spores per tetrad. This observation can be confirmed by repressing \( NEJ1 \) in the \( rme1 \) deleted strains to see if viability is being restored. This experiment is currently being done.

**DISCUSSION**

Recombination and repair play significant roles in meiosis. However, many questions still exist concerning the exact role of repair in meiotic systems. This paper sought to answer a few of those compelling questions.

**Does interference occur when an ectopic crossover is induced?**

The answer at this time appears to be no. Based on all the analysis, it appears that an ectopic conversion event plays no role in interference. However, this conclusion is confounded by two very pressing issues that will be addressed. One, the spores that were obtained were scored incorrectly for crossovers or noncrossovers. Because of this, the data reflect all events, including those in which an ectopic event did not occur. This can readily be fixed by rescoring all the spores on 5-FoA plates. Secondly, the data set was so
small, any statistical significance was negated. In 2004, Malkova et al. found that only
gene conversions with associated crossovers exhibited interference. At this time, we
cannot differentiate between gene conversion with or without associated crossovers. In
addition, by looking at URA4+ spores that are 5-FoA resistant and are hyg+, the data set
becomes statistically inconsequential. By getting a larger data set and rescoring the
spores, we can analyze the data with more confidence.

However, if after fixing these two problems, the findings are the same, then the
conclusion that can be extrapolated is a very interesting one. Further analysis could
indicate that both homologs of a chromosome need to be present for interference to
occur. In some sense, the cell does not believe that a crossover is occurring unless both
homologs are involved in the crossover. If an ectopic event occurs in a cell, interference
would be eliminated and the control over genetic variability would be negated.

The lack of interference in this ectopic system indicates that the cell is somehow
able to communicate the use of both homologues in crossing over prior to segregation
during meiosis 1. This communication, be it by a series or protein cascades, an electrical
signal, or some other unknown method of propagating information, lends support to the
idea of a stress model.

**Does an ectopic event lead to nondisjunction?**

Preliminary data would indicate that an ectopic event, whether a gene conversion
or a crossover, does lead to a significantly higher number of nondisjunction events. This
finding was previously seen by Jinks-Robertson et al. in 1997. Dr. Jinks-Robertson found
that nondisjunction occurred in about 15% of the ectopic events (Jinks-Robertson et al.,
1997). In addition, Lichten et al. (1986) found in their ectopic crossing over studies a
similar finding of nondisjunction associated with ectopic crossing over. While more clarification is required of my data to separate those events that were crossovers from those noncrossover events, it can be extrapolated that there is a higher than expected number of nondisjunction events when looking at all ectopic conversion events, whether associated with crossing over or not.

The significance of this, with the previous finding of the necessity of the use of both homologues, indicate some form of communication by the cell down the length of the homologous chromosome. If this "signal" is not propagated, the cell does not register the ectopic cross over as a legitimate one and as such does not allow for interference or proper segregation of the chromosomes at a higher frequency.

What is the role of homologous recombination in meiosis?

Nonhomologous recombination is a way in which the cell repairs DNA breaks. However, during meiosis it is shut off. When forced to turn on, in the \textit{rme1} system, viability of the cell was severely affected. Viability of a meiotic diploid in which nonhomologous recombination could occur is significantly lower than a normal meiotic cell. To confirm these results, \textit{NEJ1} is being deleted from the \textit{rme1} deleted, A/Δ strains to see if viability is restored. If viability is restored, as hypothesized, then we can extrapolate that the randomness and variability involved in homologous recombination is detrimental to the cell and is therefore shut off in meiotic systems in order to protect the highly regulated systems of replication and chromosome segregation.


**Literature Cited**


