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The Role of *CSM3*, *MRC1*, and *TOF1* in Minisatellite Stability and Large Loop DNA Repair During Meiosis in Yeast

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Abstract

Double-stranded break (DSB) repair during meiotic recombination in yeast *Saccharomyces cerevisiae* leads to the formation of heteroduplex DNA, a hybrid DNA molecule composed of single strands from two homologous chromosomes. Differences in sequence between the strands within heteroduplex DNA generate mismatches or large unpaired loops that are substrates for repair. At least two pathways function to repair large loops that form within heteroduplex DNA: the *RAD1*-dependent large loop repair (LLR) pathway and another as yet uncharacterized *RAD1*-independent LLR pathway. Repair of large loops during meiotic recombination is especially important for the genomic stability of the repetitive DNA sequences known as minisatellites. Minisatellite DNA tracts are generally stable during mitotic cell divisions but frequently alter in length during meiosis. Using a yeast minisatellite system in which the human minisatellite associated with the *HRAS1* proto-oncogene has been inserted into the recombination hotspot region upstream of *HIS4* in *S. cerevisiae*, our lab previously showed that the *RAD1*-dependent LLR pathway controls minisatellite length expansions, but not contractions. Here we show that minisatellite length expansions are controlled by the products of the *CSM3* and *TOF1* genes, while contractions are controlled by *MRC1*. By examining meiotic segregation patterns in yeast strains heterozygous for the 26bp *his4-lopd* insert, we found that deleting *CSM3* caused a loss of LLR activity similar to that seen in a *RAD1* mutant. Double mutant analysis revealed that failure to repair loops is exacerbated upon deleting both *RAD1* and *CSM3* - specifically the type of repair that fills in loops, which would generate minisatellite length expansions. A model for minisatellite length alteration based on these results is presented.

Keywords

Meiosis; Recombination; DNA repair; Gene conversion

1. Introduction

Meiotic recombination is an elaborate process that functions to repair a programmed DNA double-stranded break (DSB) during meiosis, using the homologous chromosome as the template (Hunter, 2006; Roeder, 1997). During recombination, 3' single-stranded DNA from the broken chromosome invades the DNA duplex of the homologue, forming a

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heteroduplex molecule composed of DNA strands from each of the chromosomes. If a difference exists in the DNA sequences that make up the heteroduplex, a mismatch or a loop will form that is a substrate for repair (Figure 1) (Alani et al., 1994; Fogel et al., 1981; Jensen et al., 2005; Kearney et al., 2001; Kirkpatrick, 1999).

In yeast *Saccharomyces cerevisiae*, there are several possible fates for a loop-generating mismatch after formation. Depending on which strand initiates repair, repair of the loop can either restore normal Mendelian segregation or generate a gene conversion (GC) (Kirkpatrick, 1999). If the loop is not detected or repaired, one of the four spores will receive the duplex DNA molecule containing the mismatch. When this spore germinates and goes through the first round of DNA synthesis, the two strands comprising the mismatched region will each serve as a template, producing daughter DNA molecules with different sequences in the mismatched region. The result will be a spore colony with a sectored phenotype that can easily be detected if the mismatched region is within an auxotrophic marker; this type of segregation is known as a post-meiotic segregation (PMS) (Alani et al., 1994; Fogel and Hurst, 1967; Fogel et al., 1981; Kirkpatrick, 1999; Petes et al., 1991). The efficiency with which a mismatch is detected and repaired during meiotic recombination is reflected in the percent of GC and PMS tetrads (Alani et al., 1994; Fogel et al., 1981; Jensen et al., 2005; Kearney et al., 2001; Kirkpatrick, 1999; Kirkpatrick and Petes, 1997).

In the AS4/AS13 *Saccharomyces cerevisiae* strain background used in this study, the *HIS4* locus exhibits a very high level of recombination activity during meiosis (Detloff et al., 1992; Jauert et al., 2002; Kearney et al., 2001; Kirkpatrick and Petes, 1997; Kirkpatrick et al., 1999; Nag and Petes, 1993; Nag et al., 1989; White et al., 1993). This hotspot recombination activity leads to a high level of mismatch formation in diploids heterozygous for *HIS4* alleles. Research using strains that are heterozygous for *his4-lopd*, a 26 bp non-palindromic insertion in *HIS4*, has shown that at least two pathways function to repair large loop mismatches during meiosis. The first large loop repair (LLR) pathway, consisting of *RAD1*, *RAD10*, *MSH2*, and *MSH3*, can repair loops no less than 16 bp and up to 5.6 kb in size (Jensen et al., 2005; Kearney et al., 2001; Kirkpatrick and Petes, 1997). Although no genes have been identified, studies indicate that a second *RAD1*-independent large loop repair pathway exists, as significant large loop repair is still seen when the *RAD1*-dependent LLR pathway is eliminated (Kearney et al., 2001; Kirkpatrick and Petes, 1997).

One of the goals of our lab has been to identify other genes involved in meiotic large loop repair. This is especially important for characterizing the meiotic stability of repetitive DNA sequences such as minisatellite DNA. Minisatellites are composed of tandemly repeated units that are 15 to 100 bp in length. Our lab utilizes the minisatellite found in the 3' region of the human proto-oncogene *HRAS1*, which is composed of 28 bp units that are repeated in tandem (Capon et al., 1983; Cohen et al., 1987). Studies have shown that the *HRAS1* minisatellite is generally stable during mitosis but frequently alters in length during meiosis (Jauert et al., 2002; Jeffreys et al., 1998). Meiotic alterations generate new *HRAS1* minisatellite alleles that can enhance the expression of the *HRAS1* gene and predispose cells to becoming malignant (Krontiris, 1995; Krontiris et al., 1993; Krontiris et al., 1985; Spandidos and Holmes, 1987; Trepicchio and Krontiris, 1992). By inserting the *HRAS1* minisatellite into the yeast genome upstream of *HIS4*, our lab has been able to identify factors that influence meiotic minisatellite stability (Jauert et al., 2002; Jauert and Kirkpatrick, 2005; Jensen et al., 2005). Previously, our lab has shown that the *RAD1*-dependent LLR pathway controls the expansions, but not the contractions, of the common *HRAS1-A1* minisatellite allele, and thereby plays a direct role in the meiotic stability of the *HRAS1* minisatellite (Jauert et al., 2002).

In this study we demonstrate that *CSM3* plays a role in the meiotic stability of the *HRAS1* minisatellite in yeast. *CSM3* (chromosome segregation in meiosis) was first identified in a screen for genes that are necessary for wild-type meiosis in *S. cerevisiae* (Rabitsch et al., 2001). The loss of *CSM3* resulted in a “mild chromosome missegregation” phenotype and a loss of spore viability, but the functional role of *CSM3* during meiosis remains poorly understood. Since then research has focused on the role of *CSM3* during S phase in mitotically dividing cells. Studies have shown that the products of the *CSM3*, *MRC1*, and *TOF1* genes form a heterotrimeric complex that interacts directly with the replisome, specifically the MCM helicase, during S phase (Bando et al., 2009; Calzada et al., 2005; Nedelcheva et al., 2005). The *CSM3/MRC1/TOF1* complex is not required for DNA synthesis, but instead serves as a mediator of replication fork progression. In the event of DNA damage or replicative stress, DNA synthesis pauses leading to the accumulation of RPA bound single-stranded DNA regions, the activation of the replication checkpoint, and phosphorylation of Mrc1 by Mec1. The Csm3/Mrc1/Tof1 complex stabilizes the MCM helicase and prevents further DNA unwinding, effectively keeping the replisome intact and stabilizing the replication fork (Bando et al., 2009; Calzada et al., 2005; Nedelcheva et al., 2005). Additionally, Mrc1 and Tof1, and presumably Csm3, have been shown to play a role in replication fork progression recovery (Tourriere et al., 2005).

In this study we analyzed *CSM3*, *MRC1*, and *TOF1* for a role in meiotic minisatellite stability. We further characterized the role of *CSM3* in large loop repair by analyzing the loss of *CSM3* in strains heterozygous for the *his4-lopd* allele and compared the results to a *RAD1* mutant. Finally, we analyzed *CSM3 RAD1* double mutants in a heterozygous *his4-lopd* diploid to determine if they are in the same LLR pathway, or if *CSM3* is the first gene to be identified that is part of the *RAD1*-independent LLR pathway. We present a model for the activities of the proteins based on their known enzymatic roles during mitotic DNA repair and our observations of the effects on meiotic recombination upon deletion of these genes.

2. Materials and Methods

2.1 Strain construction

All strains are derived from the haploid strains AS4 (*MATa trp1-1 tyr7-1 arg4-17 ade6 ura3*) and AS13 (*MATa leu2-3 ura3 ade6 rme1*) and are isogenic except for alterations introduced by transformation (Stapleton and Petes, 1992). The *his4-A1* minisatellite allele was inserted into PD63 and PD57 derived strains at the site of the deleted promoter sequences upstream of *HIS4* (Jauert et al., 2002), as shown in Figure 2. The *his4-H10* minisatellite allele was derived from the *his4-A1* allele as a naturally occurring tract reduction during meiosis (Jauert and Kirkpatrick, 2005). The *his4-lopc* and *his4-lopd* alleles, 26 bp insertions into the coding region of *HIS4*, were inserted into the AS13-derived haploids (Jauert et al., 2002; Jensen et al., 2005). Candidate gene deletions were constructed with one-step transformation by replacing the ORF of the respective gene with the *KANMX4* cassette encoding geneticin resistance (Jauert et al., 2005). *RAD1* deletions were previously constructed by two-step transformation (Kirkpatrick and Petes, 1997).

2.2 Strains generated

All haploid strains are listed in Table 1. Diploid strains were constructed by the following crosses: DNY27k (DNY24 × AS4), DTK314 (DTK288 × DTK305), DTK508 (DTK506 × DTK507), DTK751 (DTK608 × DTK750), DTK802 (DTK669 × DTK796), DTK1459 (DTK1451 × DTK1452), DTK1460 (DTK1454 × DTK1453), DTK1480 (DTK1470 × DTK1469), DTK1487 (DTK1462 × DTK1461), DTK1567 (DTK1566 × DTKDTK1565), DTK1667 (DTK1659 × DTK1660), DTK1684 (DTK1679 × DTK1680), DTK1685

(DTK1681 × DTK1682), DTK1724 (DTK1661 × DTK1662), DTK1731 (DTK221 × DTK225), DTK1785 (DTK1782 × DTK1783), DTK1887 (DTK1886 × DTK1885)

2.3 Media, meiotic protocols and minisatellite tract-length analysis

Standard media were used (Guthrie and Fink, 1991; Jauert et al., 2002). Diploid sporulation was performed using standard protocols (Jauert et al., 2002). All sporulating strains were incubated at 18°C for three days. Whole-cell PCR was performed on spore colonies to analyze the minisatellite tract length in four-spore tetrads. The following 50 µl reaction was used to amplify the *his4-H10* allele: 5 µl 10X PCR buffer (Sigma P-2317), 7.5 µl 25 mM MgCl₂ (Sigma M-8787), 7.5 µl of 1.25 mM dNTPs, 1.25 µl each of 10 mM Ras 3' and Ras 5' primers (Jauert et al., 2002), 2 µl *Taq* polymerase, and 19.5 µl of H₂O for a total of 44 µl, which was added to 6 µl of H₂O containing DNA. Reagents were identical but the following volumes were changed for the *his4-A1* allele: 3.75 µl 25 mM MgCl₂, 8.3 µl of 1.25 mM dNTPs, and 22.45 µl of H₂O. PCR was performed in a Hybaid PCR Express machine using the following parameters: 1 cycle of 94°C for 1 min; 30 cycles of 92°C for 1 min, 58°C for 45 s, and 72°C for either 1 min (for the *his4-H10* allele) or 2 min (for the *his4-A1* allele); 72°C for 10 min; and then held at 15°C. At least 200 tetrads were examined per strain.

2.4 *HIS4* marker segregation analysis

Marker segregation is described using the nomenclature of the eight-spored fungi - a tetrad exhibiting a Mendelian segregation event is described as 4 His⁺: 4 His⁻, while deviations from Mendelian segregation (aberrant segregation) events include gene conversions (6:2, 2:6, 0:8 and 8:0) and post-meiotic segregation (PMS) events (5:3, 3:5, aberrant 2:6, aberrant 6:2, deviant 3:5, deviant 5:3, deviant 4:4, 1:7 and 7:1). At least 300 tetrads were evaluated for segregation and crossover patterns. In order to distinguish meiotic events from mitotic alterations, only four viable-spore tetrads were analyzed.

2.5 Mathematical analyses

Aberrant segregation and minisatellite tract-length alteration comparisons were done with Instat 1.12 (GraphPad) for Macintosh, using the Fisher's Exact version of the chi-square test. Results are considered statistically significant if $p \leq 0.05$. Crossover frequencies were calculated using the Perkins equation (Perkins, 1949). To compare crossover frequencies, the Fisher's Exact test was performed using the number of nonrecombinant ($PD - NPD + \frac{1}{2}(T - 2NPD)$) and recombinant ($PD - NPD + \frac{1}{2}(T - 2NPD)$) tetrads (Chua and Roeder, 1997).

3. Results

3.1 Loss of *CSM3*, *MRC1*, or *TOF1* affects minisatellite stability during meiosis

We took a candidate gene approach to identify genes that have a role in minisatellite stability during meiosis, focusing on genes whose products are known to play a role in some aspect of DNA repair and/or recombination during meiosis. *SLX1*, *SLX4*, and *MLH3* are involved in crossing over (Fekairi et al., 2009; Munoz et al., 2009; Santucci-Darmanin et al., 2002; Svendsen et al., 2009; Wang et al., 1999), *UBX7* has been implicated in minisatellite instability in stationary phase yeast cells (Alver and Kirkpatrick, unpublished results), and *CSM3*, *MRC1* and *TOF1* form a complex that acts at the replication fork (Bando et al., 2009; Calzada et al., 2005; Nedelcheva et al., 2005). We used a yeast minisatellite model system developed by our lab, in which an *HRAS1* minisatellite allele is inserted upstream of the *HIS4* gene on chromosome III (Jauert et al., 2002). The primary minisatellite allele used in this study is the *his4-H10* allele (Figure 2), which contains ten and a half repeats derived from the most common human *HRAS1* allele *A1* (Jauert and Kirkpatrick, 2005). In diploid

strains homozygous for the *his4-H10* insert and a deletion of the candidate gene of interest we used PCR to analyze the length of the minisatellite tracts in four-spore tetrads, to determine the total number of tetrads with an altered minisatellite allele after meiosis.

The wild-type *his4-H10* strain had a total meiotic alteration frequency of 22% (46 altered alleles in 205 tetrads - Table 2). The most common minisatellite alteration during meiosis was a minisatellite length contraction in a single spore (16%), followed by tetrads with a minisatellite length expansion in a single spore (5%), while tetrads with two, three, or four spores with an altered minisatellite were observed at 0–1% (Table 2).

Four of the candidate genes we analyzed (*SLX1*, *SLX4*, *UBX7*, and *MLH3*) had no statistically significant effect on the meiotic stability of the *his4-H10* minisatellite (Table 2). We included *SLX1*, *SLX4*, and *MLH3* in this study because they have each been previously determined to have a role in promoting meiotic crossing over in yeast and in humans (Fekairi et al., 2009; Munoz et al., 2009; Santucci-Darmanin et al., 2002; Svendsen et al., 2009; Wang et al., 1999), and in the case of *SLX1* and *SLX4* also have a role in maintaining the stability of the repetitive ribosomal DNA (rDNA) loci in *S. cerevisiae* and *S. pombe* (Coulon et al., 2004; Fricke and Brill, 2003; Mullen et al., 2001). While *UBX7* has no role in meiotic crossing over, it has been implicated in minisatellite stability during stationary phase in yeast (Alver and Kirkpatrick, unpublished). Loss of these candidate genes had no significant effect on the pattern or the total percent of minisatellite length alterations (Table 2).

Our lab has previously shown that *RAD1* plays a direct role in the meiotic stability of the larger *HRAS1-A1* allele (Jauert et al., 2002). To determine if the loss of *RAD1* also has an effect on the shorter *A1*-derived *H10* allele, we examined minisatellite length alteration in a *RAD1* mutant. We found that the total percent of tetrads with an altered minisatellite significantly decreased from 22% to 14% ($p = 0.021$, Table 2). Specifically, we observed a significant decrease in the percent of tetrads with a minisatellite length expansion (from 5% to 1%, $p = 0.021$), and an insignificant decrease in tetrads with a length contraction (from 16% to 11%, $p = 0.17$), while the percent of tetrads with minisatellite alterations in two, three, or four spores remained at 1% or less (Table 2). These data demonstrate that *RAD1* functions in the meiotic stability of the *his4-H10* allele, primarily in the type of repair that generates expansions, in agreement with our prior work with the *HRAS1-A1* allele.

As our lab recently identified *CSM3* in a forward screen for genes that have a significant effect on minisatellite stability during stationary phase in *S. cerevisiae* (B. Alver and D. Kirkpatrick, unpublished data), we examined *CSM3* mutants for a meiotic minisatellite stability phenotype. In a *CSM3* mutant, the total percent of tetrads with an altered minisatellite significantly decreased from 22% to 13% ($p = 0.008$, Table 2). Similar to the *RAD1* mutant, we observed a significant decrease in the percent of tetrads with a length expansion (to 1%, $p = 0.044$) and a small but not significant decrease in length contractions (to 11%, $p = 0.12$), while the percent of tetrads with two, three or four altered spores remained at 1% or less (Table 2).

This result prompted us to analyze *his4-H10* stability in strains bearing deletions in the *CSM3* interacting partners *MRC1* and *TOF1*. The *his4-H10 mrc1Δ* and *tof1Δ* strains also exhibited significant decreases in the total percent of minisatellite alterations, to 15% each ($p = 0.038$ and $p = 0.046$, respectively, Table 2). Similar to *CSM3*, a *TOF1* mutation decreased the percent of tetrads with a minisatellite length expansion (to 2%, $p = 0.19$) and moderately decreased length contractions (to 11%, $p = 0.15$), although neither of these changes was significant (Table 2). In contrast, the *MRC1* mutant exhibited a significant loss of minisatellite length contractions (to 8%, $p = 0.018$), about half that of a wild-type strain,

and very little change in length expansions (Table 2). These results demonstrate that *CSM3*, *MRC1*, and *TOF1* play roles in the meiotic stability of the *HRAS1* minisatellite in yeast; *CSM3* and *TOF1* mutants have a loss of LLR activity similar to a *RAD1* mutant, while *MRC1* mutants exhibit a different spectrum of meiotic alterations, possibly indicating a different LLR activity (Table 2).

3.2 Loss of *CSM3*, *MRC1*, or *TOF1* has no effect on *HIS4* segregation and crossovers

As part of our analysis on minisatellite stability during meiosis, we measured the level of meiotic recombination at the *HIS4* locus. Each diploid strain is heterozygous for the *HIS4* gene, containing one wild-type allele and a mutant *his4-lopc* allele that bears a twenty-six base pair palindromic insertion within the *HIS4* coding region that forms a stem-loop structure when incorporated into heteroduplex DNA (Nag et al., 1989). Utilizing an insert that is not easily repaired gives a better indication of the level of recombination events initiating at the *HIS4* locus because it prevents large loop repair that restores Mendelian segregation (Figure 1). By following the segregation pattern of the *HIS4/his4-lopc* alleles we can measure the level of recombination at the *HIS4* locus. A high frequency of aberrant segregation indicates a high level of recombination.

In a wild-type *his4-H10* strain, the majority of tetrads exhibited 4:4 Mendelian segregation (53%, Table 3). Of the tetrads with an aberrant segregation, most exhibited a 3:5 or 5:3 PMS event (14% and 16%, respectively), while a smaller percent exhibited a 2:6 or 6:2 GC event (4% and 5%, respectively - Table 3). Deleting *RAD1*, *CSM3*, *MRC1*, or *TOF1* in *his4-H10* strains heterozygous for *his4-lopc* did not cause a significant deviation from the wild-type pattern of segregation, or in the overall percent of aberrant segregation (from 47% to 52% (*rad1Δ*), 50% (*csm3Δ*), 47% (*mrc1Δ*), and 48% (*tof1Δ*), respectively, Table 3).

We measured the level of intergenic crossing over occurring in the *his4-H10* minisatellite-spanning interval by examining linkage between the *HIS4/his4-lopc* and *LEU2/leu2-3* alleles. A wild-type *his4-H10* strain had a map distance of 32 cM (Table 3). Deleting *RAD1*, *CSM3*, *MRC1*, or *TOF1* did not significantly affect the level of crossovers in the *HIS4-LEU2* interval (to 34, 29, 35, and 33 cM, respectively, Table 3).

3.3 Effect of the *CSM3* mutation on *his4-A1* minisatellite allele

Our lab has previously shown that *RAD1* plays a direct role in the meiotic stability of the *his4-A1* allele (Jauert et al., 2002). Because we identified *CSM3* as having a role in the meiotic stability of the shorter *his4-H10* allele, we analyzed the effect of deleting *CSM3* on *his4-A1* stability. A wild-type *his4-A1* strain (Figure 2) exhibited minisatellite alterations in 45% of all tetrads (Table 2), with 19% of all tetrads exhibiting a minisatellite length decrease and 9% exhibiting a length increase (Table 2). These data demonstrate that the longer *his4-A1* allele is significantly more unstable than the shorter *his4-H10* derivative. (45% alteration versus 22%)

The *his4-A1 rad1Δ* strain showed a significant drop in the percent of tetrads that exhibited a minisatellite length expansion in a single spore, although the frequency of tetrads with a minisatellite alteration did not significantly differ from the wild-type strain (Jauert et al., 2002). In contrast, the *his4-A1 csm3Δ* strain did not show any deviation in the pattern of minisatellite alterations and the overall frequency of tract alterations was the same as in the wild-type strain (Table 2). Thus, loss of *CSM3* appears to specifically affect the shorter *his4-H10* tract, unlike *RAD1* loss which affects both tracts. The reason behind this tract length difference for *CSM3* is not known; it is possible that longer tract lengths adopt structures during alteration that are not Csm3 substrates. Neither the *CSM3* nor the *RAD1*

mutation altered the level of *his4-lop*c aberrant segregation or the level of intergenic recombination between *HIS4* and *LEU2* in the *his4-A1* strain (Table 3).

3.4 *CSM3* and *RAD1* mutations affect *his4-lop*d repair

The experiments above focused on minisatellite stability evaluation. To characterize the role of *CSM3* in meiotic large loop repair (LLR) and compare it to the LLR activity of *RAD1*, we examined the meiotic segregation pattern of diploid strains heterozygous for the *his4-lop*d insertion. The *his4-lop*d insert is a 26 bp non-palindromic insertion within the *HIS4* coding region that has been used extensively in examining large loop repair pathways during meiotic recombination (Jauert et al., 2002; Jensen et al., 2005; Kearney et al., 2001; Kirkpatrick and Petes, 1997). When incorporated into heteroduplex DNA, the *his4-lop*d allele forms a large 26 bp loop that is frequently repaired.

A wild-type strain exhibited *his4-lop*d aberrant segregation in 28% of tetrads, comprised of 21% GC events and 7% PMS events (Table 4). Within the class of tetrads with aberrant segregation of *HIS4* alleles, 24% exhibited PMS (Table 4). These data indicate that the 26 bp loop formed by the *his4-lop*d insert is efficiently recognized and repaired when incorporated into heteroduplex DNA. Deleting either *CSM3* or *RAD1* did not significantly alter the total percent of *his4-lop*d aberrant segregation (27%; $p = 0.75$ and 30%; $p = 0.5$, respectively), however both mutations decreased the percent of GC events (from 21% to 15 and 17% respectively; Table 4) and nearly doubled the percent of PMS events (from 7% to 12 and 14%; Table 4). This shift from GC to PMS events reflects a loss of repair of the 26 bp loop formed by the *his4-lop*d insert when incorporated into heteroduplex DNA (Figure 1). Both mutations increased the percent of 3:5 and 5:3 tetrads and reduced the percent of 2:6 tetrads, while the percent of 6:2 tetrads did not significantly change (Table 4). These data suggest that *CSM3* and *RAD1* are used primarily in the type of repair that fills-in loops when either the wild-type or *his4-lop*d strand initiates recombination (Figure 1), in agreement with previous data on meiotic large loop repair in *RAD1* mutants (Jauert et al., 2002; Kearney et al., 2001; Kirkpatrick and Petes, 1997).

We also examined the effect of *MRC1* loss on meiotic repair of the *his4-lop*d mismatch. Unlike the *rad1Δ* and *csm3Δ* strains, the *mrc1Δ* strain exhibited significantly lower levels of aberrant segregation ($p = 0.03$), with reductions in both classes of gene conversion tetrads (to 15% from 21% GC in the WT strain), although post-meiotic segregation tetrads were not affected (Table 4). This decrease in GC led to an apparent increase in the PMS/Ab seg ratio (Table 4), but for different reasons than the increases seen in the *rad1Δ* and *csm3Δ* strains. In addition, the *mrc1Δ* strain had a significantly lower level of spore viability (74%) compared to the other strains (i.e. *csm3Δ* = 82%, $p < 0.0001$). The *mrc1Δ* strain also showed the largest drop in spore viability in the *his4-H10 his4-lop*c strain set (Table 5). Finally, the individual isolates of *mrc1Δ* had a large amount of variability in sporulation frequency, viability and recombination frequency from plate to plate; these phenotypes were not seen with the other mutants we examined. The spore viability pattern indicated random spore death, rather than defects in meiosis I or II chromosome segregation (Table 5). These data suggest that *MRC1* is required in at least a subset of gene conversion events, and lack of *MRC1* function leads to a loss of those spores.

3.5 *CSM3 RAD1* double mutant analysis

Components of the *RAD1*-dependent LLR pathway were previously identified in the *his4-lop*d background; these studies indicated the existence of at least one other meiotic LLR pathway that is independent of *RAD1* (Kearney et al., 2001; Kirkpatrick and Petes, 1997). We analyzed *HIS4* segregation in a *csm3Δ rad1Δ* strain heterozygous for *his4-lop*d to determine if both genes function in the same meiotic LLR pathway.

A *csn3Δ rad1Δ* strain exhibited *his4-lop* aberrant segregation in 34% of tetrads, which is slightly but not significantly increased compared to wild-type ($p = 0.09$) and single mutant strains ($p = 0.06$ and $p = 0.35$, Table 4). Compared to a wild-type strain, the percent of GC events decreased by almost half and the percent of PMS events nearly tripled (Table 4). These data reveal that the *CSM3 RAD1* double mutant has an even greater loss of large loop repair activity than either single mutant. Specifically, the change in the percent of 2:6, 6:2, and 3:5 tetrads is similar to either single mutant; the greatest difference is the increased percent of 5:3 tetrads, which is twice that of either single mutant and quadruple that observed in a wild-type strain (Table 4). This is reflected in the increased percent of aberrant segregation tetrads that exhibited a post-meiotic segregation (64%; Table 4), which is significantly higher than either single mutant. However, the increase seen in the double mutant is not a fully additive effect, as might be expected if *CSM3* and *RAD1* were in two completely separable LLR pathways. These results suggest that *CSM3* and *RAD1* play distinct but overlapping roles in the type of LLR activity that fills-in loops.

4. Discussion

4.1 *RAD1*, *CSM3*, *TOF1*, and *MRC1* control minisatellite stability during meiosis

At least two pathways exist that function to repair large loops that form within heteroduplex DNA during meiotic recombination: the *RAD1*-dependent large loop repair (LLR) pathway and an uncharacterized *RAD1*-independent LLR pathway (Jauert et al., 2002; Kearney et al., 2001; Kirkpatrick and Petes, 1997). Our lab has previously shown that the *RAD1*-dependent LLR pathway controls the expansion, but not the contraction, of the *HRAS1-A1* allele when inserted upstream of the *HIS4* gene in yeast, and that these alterations are specific to meiosis (Jauert et al., 2002). In this study we identified three more factors that control meiotic minisatellite stability: *CSM3*, *MRC1*, and *TOF1*. Deleting each of these genes significantly decreases the frequency of meiotic minisatellite alterations. *RAD1*, *CSM3*, and *TOF1* deletions significantly reduced expansion of the *HRAS1* minisatellite tract length, while deletion of *MRC1* significantly reduced the frequency of length contractions (Table 2). In Figure 3a, the frequency of alterations is expressed as a percentage of the total number of alterations seen in the strain. Loss of *RAD1*, *CSM3* or *TOF1* leads to a significant drop in observed expansions, while contractions are slightly increased. The *RAD1* results match previous research on its role in minisatellite stability (Jauert et al., 2002; Kearney et al., 2001; Kirkpatrick and Petes, 1997), and suggests that *CSM3* and *TOF1* have a similar function in minisatellite stability. In contrast, *MRC1* appears to act in a different manner, influencing tract contraction rather than expansion. Loss of *MRC1* function decreases contractions from 70% of events to 57%, while expansions are mildly increased – a reversal of the *rad1Δ/csn3Δ/tof1Δ* pattern. *MRC1* is the first gene we have identified that influences this aspect of minisatellite stability/

4.2 An expanded model for minisatellite alterations during meiosis

We previously proposed a model for meiotic minisatellite alterations that involved misalignment of repeat units during strand invasion (Jauert et al., 2002). In this model, a DSB forms adjacent to the minisatellite, and strand resection generates 3' single-stranded DNA which then invades the homologue, creating a region of heteroduplex DNA that includes the minisatellite. The repetitive minisatellite DNA can misalign, causing one or more of the repeat units to loop out of the heteroduplex region. Each repeat unit of the *HRAS1* minisatellite is 28bp in length, so a one-repeat misalignment would generate a 28bp loop. The size of this loop is well within the functional range of the *RAD1*-dependent LLR pathway (Jensen et al., 2005).

The *RAD1*-dependent LLR pathway involves the *RAD1*, *RAD10*, *MSH2*, and *MSH3* genes (Kearney et al., 2001; Kirkpatrick and Petes, 1997). We previously proposed a model, based on our research and the known mitotic activities of each of these gene products, in which the *RAD1* and *RAD10* endonucleases generate a nick in the strand opposite of the loop, with the *MSH2* and *MSH3* proteins serving as loop-recognition factors (Jauert et al., 2002; Jensen et al., 2005). The cleaved DNA strand is filled in by DNA synthesis, generating a minisatellite length expansion (Jauert et al., 2002; Jensen et al., 2005).

This model can be expanded to incorporate *CSM3*, *MRC1*, and *TOF1*. During mitotic growth, Csm3, Mrc1 and Tof1 form a heterotrimeric complex that interacts directly with the MCM helicase of the replisome, serving as a mediator of replication fork progression and DNA repair (Bando et al., 2009; Calzada et al., 2005; Nedelcheva et al., 2005). During meiotic recombination, DNA synthesis is necessary to repair the DSB using the homologue as template, and utilizes DNA replication factors such as *POL30* and *POL3* (Maloisel et al., 2004; Stone et al., 2008). *CSM3*, *MRC1*, and *TOF1* genetically interact with *POL30* and *POL3* (Collins et al., 2007; Tong et al., 2004), with the MRX complex (*MRE11/RAD50/XRS2*) required for formation and processing of meiotic DSBs (Borde, 2007), and with *RAD51*, a strand exchange factor (Collins et al., 2007; Hunter, 2006; Pan et al., 2006; Tong et al., 2004). These data along with our results suggest that the *CSM3/MRC1/TOF1* complex forms part of the DNA synthesis machinery involved in meiotic recombination, and is likely recruited to the heteroduplex intermediate.

We propose that the *CSM3/MRC1/TOF1* complex localizes to heteroduplex DNA, and when a minisatellite length misalignment generates a large loop, the complex functions as a central player that mediates how the loop is repaired. Our data argue that *CSM3* and *TOF1* are involved in the type of repair that fills in loops (generating minisatellite length expansions), possibly by directly interacting with a helicase that opens the DNA duplex up around the region of the loop, providing easier access for the Rad1/10 endonuclease when it cleaves the strand opposite the loop. Candidate helicases include *RAD3* and *RAD5*; *CSM3* and *TOF1* (but not *MRC1*) have been shown to genetically interact with both (Moriel-Carretero and Aguilera, 2010; Pan et al., 2006; Tong et al., 2004). Csm3 and Tof1 co-depend on each other for proper localization (Bando et al., 2009); this is likely why both mutants exhibit similar LLR phenotypes.

Intriguingly, our minisatellite data suggest that Mrc1 plays a role in the type of repair that removes the loop to generate minisatellite length contractions. In the event of replication stress during mitotic growth, Mrc1 (but not Csm3 or Tof1) is phosphorylated and recruits the sensor kinase Rad53 (Tanaka and Russell, 2001). It is possible that Mrc1 is also phosphorylated in the event of minisatellite misalignment and large loop formation, and transmits a signal to recruit an endonuclease to cleave the loop and generate a minisatellite length contraction (Figure 4). A candidate endonuclease is *MUS81*, which interacts genetically with *MRC1* but not *CSM3* or *TOF1* (Collins et al., 2007). Unfortunately, *MUS81* mutants do not sporulate in our AS4/AS13 strain background, preventing us from determining the role of *MUS81* in minisatellite stability (unpublished data).

While several studies have shown that *MRC1*, *TOF1*, and *CSM3* have overlapping roles in DNA replication checkpoint activation (Bando et al., 2009; Calzada et al., 2005; Nedelcheva et al., 2005), our research is not the first to have observed a difference in function between *MRC1* and *CSM3/TOF1*. Studies have shown that Mrc1 depends on Csm3-Tof1 for proper localization but the reverse is not true (Bando et al., 2009), and that Csm3-Tof1 but not Mrc1 is required for replication fork pausing at protein barriers (Calzada et al., 2005; Hodgson et al., 2007). Our data suggest a central role for the Csm3/Tof1/Mrc1 complex in discriminating between differing modes of repair during minisatellite tract alterations.

4.3 Analysis of *CSM3* LLR activity in *his4-lop*d strains

CSM3 or *RAD1* deletion in strains heterozygous for the *his4-lop*d insert lead to a nearly identical shift from GC to PMS tetrads in both strains (Table 4). Both mutants exhibited an increase in 3:5 and 5:3 tetrads and a decrease in 2:6 tetrads GC events (Figure 3b), while the total percent of *HIS4* aberrant segregation remained unchanged (Table 4). These data can be explained by a loss of a specific type of large loop repair that fills in the loop (which would be accomplished by nicking the strand opposite the loop and filling in the gap with DNA synthesis), rather than repair that initiates with loop removal. This type of loop repair is consistent with the effect on minisatellite alterations observed in these mutants (Table 2). As shown in Figure 1, loss of fill-in loop repair would shift some of the 2:6 GC tetrads to 3:5 PMS tetrads when the DSB forms on the WT strand, and would shift the restored 4:4 tetrads to 5:3 tetrads when the DSB forms on the mutant strand. The expected result would be an increase in 3:5 and 5:3 tetrads, a loss of 2:6 tetrads, and no effect on 6:2 tetrads, which is what was observed in the *RAD1* and *CSM3* mutants (Table 4).

A double mutant analysis revealed that deleting both *CSM3* and *RAD1* exacerbated the phenotypes observed in either single mutant; the percent of PMS tetrads is significantly increased compared to either single mutant (Table 4, Figure 3b). This suggests that the *CSM3* and *RAD1* gene products may not have completely overlapping functions in LLR loop fill-in reactions.

4.4 Broader implications on minisatellite stability and human disease

This research has identified more factors that function in meiotic large loop repair. *CSM3* appears to play a role in the type of large loop repair that fills-in loops, similar to the already characterized *RAD1*-dependent LLR pathway (Jensen et al., 2005; Kearney et al., 2001; Kirkpatrick and Petes, 1997). In contrast, *MRC1* appears to be the first factor identified to function in the type of large loop repair that remove loops. The identification of large loop repair components is important for our understanding of factors that play a role in minisatellite stability. Minisatellite alterations during meiosis generate novel minisatellite alleles that are often associated with various types of human disease, as demonstrated by the correlation between human *HRAS1* minisatellite alleles and cancer (Krontiris, 1995; Krontiris et al., 1993; Krontiris et al., 1985; Tamimi et al., 2003; Trepicchio and Krontiris, 1992). Furthering our understanding of how large loops are recognized and repaired during meiotic recombination will allow us to better understand the predisposition and initiation of human diseases associated with minisatellite DNA.

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Highlights

- We find that Csm3, Mrc1 and Tof1 affect minisatellite stability during yeast meiosis
- Minisatellite expansions are influenced by Csm3 and Tof1 and contractions by Mrc1
- Csm3 also affects large loop repair during gene conversion recombination
- Csm3 acts in an overlapping pathway with Rad1/10 during gene conversion
- We propose that Csm3/Mrc1/Tof1 mediates direction of repair during recombination

Figure 1.

DNA Repair events during meiotic recombination. Two chromosomes containing the *HIS4* locus are shown following DNA replication in early meiosis. One homolog is in blue, the other in red, and both strands of each sister chromatid are depicted as thin lines. The solid black boxes on the red chromosome indicate a region of inserted DNA. Double-strand breaks (DSBs) initiate recombination on either the wild-type (WT) chromosome (left side) or the mutant chromosome (right side). The breaks are processed to generate single-stranded ends (B and B'), which invade the homolog to form heteroduplex DNA (C and C'). Mismatches that occur in the heteroduplex DNA are shown as loops. Repair synthesis is indicated by dashed lines. The mismatched loops in heteroduplex DNA can be resolved by either removing the loop or nicking the strand opposite the loop and filling it in via repair synthesis, leading to either a gene conversion event or a restoration of normal Mendelian segregation. Failure to recognize or repair the mismatched loop will generate a post-meiotic segregation event.

Figure 2.

Diagram of the *HIS4* locus of chromosome III in diploid strains. A) The *HIS4-BIK1* locus, shown in a diploid strain bearing wildtype *HIS4* promoter sequences is heterozygous for either the *his4-lopd* or *his4-lopc* insert (dark vertical bar) that disrupts the *HIS4* coding sequence. B) A diploid strain isogenic to (A) but bearing the *his4-H10* minisatellite insert (white boxes) upstream of *HIS4* replacing the endogenous promoter sequence. The TATA box (white circle) is left intact. Small grey arrows represent the primers used in PCR reactions to amplify the repetitive DNA tract. C) A diploid strain bearing the *his4-A1* minisatellite insert (white boxes) upstream of *HIS4*, replacing the same endogenous promoter sequences as in (B). D) A representative gel showing minisatellite tract alterations in spore colonies from DTK751. Three complete tetrads are shown, with each spore colony labeled a – d. PCR was conducted as described in Materials and Methods, using the primers indicated in (B) above. The first tetrad had no spores that contained an altered *his4-H10* tract. The second tetrad had a spore (b) that lost a repeat unit, while the third tetrad had a spore (a) that gained two repeat units.

Figure 3.

A) Frequency of Meiotic Minisatellite Tract Alterations. Each bar represents the percentage of tetrads exhibiting a tract expansion or a contraction in a single spore for that particular mutant strain. Deletion of *RAD1*, *CSN3* or *TOF1* leads to a loss of expansions, while loss of *MRC1* leads to a loss of contractions. B) Frequency of Meiotic *his4-lopd* Recombination Events. The left bar graph shows the frequency of unrepaired recombination events (PMS) to total aberrant segregation events as a percentage, demonstrating that deletion of *RAD1* or *CSN3* has equivalent effect on repair, while a double mutant exhibits significantly poorer repair. The right graph shows the frequencies of 2:6 and 6:2 gene conversion events in the mutant strains. 2:6 GC events are reduced in the mutants, indicating a loss of loop fill-in events (see Figure 1). Both graphs use data from Table 4.

Figure 4.

A model for minisatellite length alterations during meiosis. An overview is shown at the top, with a detailed model for alterations at the bottom. During strand invasion and heteroduplex formation, a misalignment can occur within the repetitive minisatellite DNA (orange boxes). Extrusion of one of the repeat units will form a large 28bp loop; other misalignments are possible but not shown. Detection and repair of the loop can generate a minisatellite length expansion (left) or a contraction (right). Our data suggest that *RAD1*, *RAD10*, *CSM3*, and *TOF1* function in large loop repair that generates length expansions. Our model depicts Rad1/10, directed by Csm3/Tof1, nicking the strand opposite the loop (red strand). The resulting gap will be filled in by DNA repair synthesis. Mrc1 functions in large loop removal (blue strand), which will generate a length contraction by recruiting an unknown endonuclease (possibly Mus81/Mms4) in response to the phosphorylation state of Mrc1.

Table 1

Haploid Strains

Strain	<i>HIS4</i> promoter	<i>HIS4</i> coding sequence	Relevant Mutation	Parental Strain	Source
<i>Matα</i> :					
AS4	WT	WT	WT		Stapleton and Petes 1991
PD63	<i>his4-Δ52</i>	WT	WT	AS4	Detloff, White and Petes 1992
DTK225	WT	WT	<i>rad1Δ</i>	AS4	Kirkpatrick and Petes 1997
DTK288	<i>his4-A1</i>	WT	WT	PD63	Jauert et al. 2002
DTK506	<i>his4-A1</i>	WT	<i>rad1Δ</i>	DTK288	Jauert et al. 2002
DTK750	<i>his4-H10</i>	WT	WT	PD63	Jauert and Kirkpatrick 2005
DTK796	<i>his4-H10</i>	WT	<i>rad1Δ</i>	DTK750	Jauert and Kirkpatrick 2005
DTK1452	<i>his4-H10</i>	WT	<i>csm3Δ</i>	DTK750	this study
DTK1454	<i>his4-H10</i>	WT	<i>slx1Δ</i>	DTK750	this study
DTK1462	<i>his4-H10</i>	WT	<i>slx4Δ</i>	DTK750	this study
DTK1470	<i>his4-H10</i>	WT	<i>ubx7Δ</i>	DTK750	this study
DTK1566	<i>his4-H10</i>	WT	<i>mlh3Δ</i>	DTK750	this study
DTK1659	WT	WT	<i>csm3Δ</i>	AS4	this study
DTK1662	<i>his4-A1</i>	WT	<i>csm3Δ</i>	DTK288	this study
DTK1680	<i>his4-H10</i>	WT	<i>mre1Δ</i>	DTK750	this study
DTK1682	<i>his4-H10</i>	WT	<i>tof1Δ</i>	DTK750	this study
DTK1783	WT	WT	<i>rad1Δ csm3Δ</i>	DTK225	this study
DTK1885	WT	WT	<i>mre1Δ</i>	AS4	this study
<i>Matα</i> :					
AS13	WT	WT	WT		Stapleton and Petes 1991
DNY24	WT	<i>his4-lopΔ</i>	WT	AS13	Nag, White and Petes 1989
PD57	<i>his4-Δ52</i>	WT	WT	AS13	Detloff, White and Petes 1992
PD80	<i>his4-Δ52</i>	<i>his4-lopΔ</i>	WT	PD57	Detloff, White and Petes 1992
DTK221	WT	<i>his4-lopΔ</i>	<i>rad1Δ</i>	DNY24	Kirkpatrick and Petes 1997
DTK294	<i>his4-A1</i>	WT	WT	PD57	Jauert et al. 2002
DTK305	<i>his4-A1</i>	<i>his4-lopΔ</i>	WT	DTK294	Jauert et al. 2002

Strain	<i>HIS4</i> promoter	<i>HIS4</i> coding sequence	Relevant Mutation	Parental Strain	Source
DTK507	<i>his4-A1</i>	<i>his4-lope</i>	<i>rad1Δ</i>	DTK305	Jauert et al. 2002
DTK608	<i>his4-H10</i>	<i>his4-lope</i>	WT	PD80	Jauert and Kirkpatrick 2005
DTK669	<i>his4-H10</i>	<i>his4-lope</i>	<i>rad1Δ</i>	DTK608	Jauert and Kirkpatrick 2005
DTK1451	<i>his4-H10</i>	<i>his4-lope</i>	<i>csn3Δ</i>	DTK608	this study
DTK1453	<i>his4-H10</i>	<i>his4-lope</i>	<i>slx1Δ</i>	DTK608	this study
DTK1461	<i>his4-H10</i>	<i>his4-lope</i>	<i>slx4Δ</i>	DTK608	this study
DTK1469	<i>his4-H10</i>	<i>his4-lope</i>	<i>ubx7Δ</i>	DTK608	this study
DTK1565	<i>his4-H10</i>	<i>his4-lope</i>	<i>mlh3Δ</i>	DTK608	this study
DTK1660	WT	<i>his4-lopd</i>	<i>csn3Δ</i>	DNY24	this study
DTK1661	<i>his4-A1</i>	<i>his4-lope</i>	<i>csn3Δ</i>	DTK305	this study
DTK1679	<i>his4-H10</i>	<i>his4-lope</i>	<i>mrc1Δ</i>	DTK608	this study
DTK1681	<i>his4-H10</i>	<i>his4-lope</i>	<i>tof1Δ</i>	DTK608	this study
DTK1782	WT	<i>his4-lopd</i>	<i>rad1Δ csm3Δ</i>	DTK221	this study
DTK1886	WT	<i>his4-lopd</i>	<i>mrc1Δ</i>	DNY24	this study

Table 2

tion of minisatellite length alterations in various strain backgrounds

	Relevant mutation	<i>HIS4</i> promoter	Total tetrads	% (No.) of tetrads with alteration						Total alterations
				No alteration	Single spore expansion	Single spore contraction	Two spore alterations	Three spore alterations	Four spore alterations	
1	+	<i>his4-H10</i>	205	78 (159)	5 (10)	16 (32)	1 (2)	1 (2)	0 (0)	22 (46)
2	<i>rad1Δ</i>	<i>his4-H10</i>	263	86 (226)	1 (3)*	11 (29)	1 (3)	0.4 (1)	0.4 (1)	14 (37)*
39	<i>esm3Δ</i>	<i>his4-H10</i>	237	87 (207)	1 (3)*	11 (25)	0.4 (1)	0.4 (1)	0 (0)	13 (30)*
44	<i>mrc1Δ</i>	<i>his4-H10</i>	255	85 (218)	4 (11)	8 (21)*	0.4 (1)	1 (2)	1 (2)	15 (37)*
45	<i>tof1Δ</i>	<i>his4-H10</i>	224	85 (191)	2 (5)	11 (24)	1 (3)	0.4 (1)	0 (0)	15 (33)*
50	<i>slx1Δ</i>	<i>his4-H10</i>	204	78 (159)	2 (4)	15 (31)	2 (4)	3 (6)	0 (0)	22 (45)
57	<i>slx4Δ</i>	<i>his4-H10</i>	222	83 (184)	2 (5)	13 (28)	1 (2)	1 (2)	0.5 (1)	17 (38)
80	<i>ubx7Δ</i>	<i>his4-H10</i>	222	84 (186)	2 (4)	11 (25)	2 (4)	1 (2)	0.5 (1)	16 (36)
57	<i>mlh3Δ</i>	<i>his4-H10</i>	220	77 (169)	2 (5)	17 (38)	2 (5)	1 (3)	0 (0)	23 (51)
44	+	<i>his4-A1</i>	213	55 (117)	9 (19)	19 (41)	10 (22)	6 (13)	0.5 (1)	45 (95)
34	<i>rad1Δ</i>	<i>his4-A1</i>	275	52 (142)	3 (9)	24 (67)	16 (44)	4 (11)	1 (2)	48 (133)
44	<i>esm3Δ</i>	<i>his4-A1</i>	206	57 (117)	8 (17)	22 (45)	8 (16)	4 (9)	1 (2)	43 (89)

Indicates statistically significant difference compared to wild-type strain: DTK751 vs: DTK802 p = 0.0207; DTK1459 p = 0.0078; DTK1684 p = 0.0377; DTK1685 p = 0.0460.

al. 2002.

Table 3
Meiotic segregation patterns and crossover frequencies of diploid strains heterozygous for *his4-lopc*

Strain	Relevant mutation	<i>HIS4</i> promoter	Total tetrads	% Ab seg ^b	Percent (No.) of tetrads with <i>HIS4</i> segregation pattern ^a							<i>HIS4-LEU2</i> distance (cM) ^d
					4:4	2:6	6:2	3:5	5:3	Ab 4:4	Other ^d	
DTK751	WT	<i>his4-H10</i>	866	47	53 (455)	4 (38)	5 (47)	14 (121)	16 (137)	3 (30)	5 (46)	32
DTK802	<i>rad1Δ</i>	<i>his4-H10</i>	311	52	48 (150)	2 (6)	7 (22)	14 (43)	18 (56)	5 (16)	6 (18)	34
DTK1459	<i>csn3Δ</i>	<i>his4-H10</i>	318	50	50 (158)	5 (15)	3 (9)	16 (52)	19 (60)	3 (9)	5 (15)	29
DTK1684	<i>mrc1Δ</i>	<i>his4-H10</i>	361	47	53 (190)	4 (14)	6 (21)	11 (41)	15 (55)	1 (5)	10 (35)	35
DTK1685	<i>tof1Δ</i>	<i>his4-H10</i>	345	48	52 (178)	2 (8)	7 (25)	15 (51)	16 (55)	2 (8)	6 (20)	33
DTK1460	<i>slx1Δ</i>	<i>his4-H10</i>	389	55	45 (175)	3 (13)	4 (16)	19 (75)	19 (74)	4 (15)	5 (21)	34
DTK1487	<i>slx4Δ</i>	<i>his4-H10</i>	345	50	50 (173)	2 (8)	6 (20)	17 (59)	16 (54)	4 (13)	5 (18)	28
DTK1480	<i>ubx7Δ</i>	<i>his4-H10</i>	381	50	50 (189)	3 (10)	6 (21)	13 (50)	21 (79)	4 (17)	4 (15)	32
DTK1567	<i>mlh3Δ</i>	<i>his4-H10</i>	357	55	45 (161)	4 (14)	8 (27)	14 (51)	18 (64)	5 (18)	6 (22)	35
DTK314 ^c	WT	<i>his4-A1</i>	733	47	53 (392)	2 (17)	9 (64)	15 (109)	15 (108)	2 (16)	4 (27)	30
DTK508 ^c	<i>rad1Δ</i>	<i>his4-A1</i>	487	43	57 (279)	5 (24)	6 (28)	13 (66)	12 (56)	5 (22)	2 (12)	25
DTK1724	<i>csn3Δ</i>	<i>his4-A1</i>	323	37	63 (205)	5 (15)	8 (26)	7 (24)	11 (37)	3 (10)	2 (6)	33

^aSee Materials and Methods.

^bAb seg, aberrant segregation.

^cJauert et al. 2002.

^dIncludes: aberrant 2:6, ab 6:2, deviant 3:5, dev 5:3, dev 4:4, 1:7, 0:8, and 8:0. WT = wild-type

Table 4

Meiotic segregation patterns and crossover frequencies of WT promoter strains heterozygous for *his4-lop*^d

Strain	Relevant mutation	Total tetrads	Percent (No.) of tetrads with <i>HIS4</i> segregation pattern ^a							% Ab Seg	% PMS	% PMS/Ab Seg	<i>HIS4-LEU2</i> distance (cM) ^d
			4:4	2:6	6:2	3:5	5:3	Ab 4:4	Other ^b				
DNY27	WT	407	72 (293)	11 (45)	9 (35)	1 (6)	4 (17)	0.5 (2)	2 (9)	28	7	24	28
DTK1667	<i>csn3Δ</i>	357	73 (261)	6 (22)	8 (27)	3 (12)	8 (29)	0 (0)	2 (6)	27	12	44	30
DTK1731	<i>rad1Δ</i>	326	70 (227)	4 (14)	12 (38)	5 (15)	8 (26)	0.6 (2)	1 (4)	30	14	46	29
DTK1785	<i>csn3Δ rad1Δ</i>	320	66 (211)	3 (10)	9 (28)	3 (10)	16 (52)	2 (5)	1 (4)	34	22	64	23
DTK1887	<i>mrc1Δ</i>	568	78 (444)	7 (38)	7 (42)	2 (12)	4 (25)	0.2 (1)	1 (6)	22	7	33	27

^aSee Materials and Methods.

^bIncludes: aberrant 2:6, ab 6:2, deviant 3:5, dev 5:3, dev 4:4, 1:7, 7:1, 0:8, and 8:0. WT = wild-type.

Table 5

Meiotic spore viability patterns

Strain	Relevant mutation	HIS4 promoter	HIS4 mutation	Total spore viability %	Spore viability pattern ^a				
					4:0	3:1	2:2	1:3	0:4
DTK751	WT	<i>his4-H10</i>	<i>his4-topc</i>	90	866	188	101	18	9
DTK802	<i>rad1Δ</i>	<i>his4-H10</i>	<i>his4-topc</i>	87	311	119	49	9	4
DTK1459	<i>csn3Δ</i>	<i>his4-H10</i>	<i>his4-topc</i>	80	318	148	81	26	26
DTK1684	<i>mrc1Δ</i>	<i>his4-H10</i>	<i>his4-topc</i>	67	361	253	231	108	76
DTK1685	<i>tof1Δ</i>	<i>his4-H10</i>	<i>his4-topc</i>	73	345	214	192	80	25
DTK1460	<i>slx1Δ</i>	<i>his4-H10</i>	<i>his4-topc</i>	86	389	86	36	9	33
DTK1487	<i>slx4Δ</i>	<i>his4-H10</i>	<i>his4-topc</i>	85	345	110	58	12	15
DTK1480	<i>ubx7Δ</i>	<i>his4-H10</i>	<i>his4-topc</i>	92	381	55	25	8	4
DTK1567	<i>mlh3Δ</i>	<i>his4-H10</i>	<i>his4-topc</i>	80	357	129	84	35	27
DTK314	WT	<i>his4-A1</i>	<i>his4-topc</i>	80	733	369	238	62	31
DTK508	<i>rad1Δ</i>	<i>his4-A1</i>	<i>his4-topc</i>	77	487	357	172	77	29
DTK1724	<i>csn3Δ</i>	<i>his4-A1</i>	<i>his4-topc</i>	62	323	366	350	192	87
DNY27	WT	WT	<i>his4-topd</i>	90	407	89	41	9	5
DTK1667	<i>csn3Δ</i>	WT	<i>his4-topd</i>	82	357	181	94	25	10
DTK1731	<i>rad1Δ</i>	WT	<i>his4-topd</i>	83	326	186	92	17	4
DTK1785	<i>csn3Δ rad1Δ</i>	WT	<i>his4-topd</i>	79	320	233	110	36	13
DTK1887	<i>mrc1Δ</i>	WT	<i>his4-topd</i>	74	568	411	291	94	48

^alive spore:dead spore. WT = wild-type