Decreased Meiotic Intergenic Recombination and Increased Meiosis I Nondisjunction in *exo1* Mutants of *Saccharomyces cerevisiae*

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Manuscript received July 23, 1999

Accepted for publication September 6, 2000

ABSTRACT

Exonuclease I was originally identified as a 5' → 3' deoxyribonuclease present in fractionated extracts of *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae*. Genetic analysis of *exo1* mutants of both yeasts revealed no major defect in meiosis, suggesting that exonuclease I is unlikely to be the primary activity that processes meiosis-specific double-strand breaks (DSBs). We report here that *exo1* mutants of *S. cerevisiae* exhibit subtle but complex defects in meiosis. Diploids containing a homozygous deletion of *EXO1* show decreased spore viability associated with an increase in meiosis I nondisjunction, while intergenic recombination is reduced about twofold. Exo1p functions in the same pathway as Msh5p for intergenic recombination. The length of heteroduplex tracts within the *HIS4* gene is unaffected by the *exo1* mutation. These results suggest that Exo1p is unlikely to play a major role in processing DSBs to form single-stranded tails at *HIS4*, but instead appears to promote crossing over to ensure disjunction of homologous chromosomes. In addition, our data indicate that exonuclease I may have a minor role in the correction of large DNA mismatches that occur in heteroduplex DNA during meiotic recombination at the *HIS4* locus.

IN *Saccharomyces cerevisiae*, meiotic recombination is initiated by double-strand breaks (DSBs) that are subsequently processed to form 3' single-stranded tails (CAO *et al.* 1990; SUN *et al.* 1991). Spo11p is an atypical type II topoisomerase that is thought to catalyze DSB formation (KEENEY *et al.* 1997), but the identity of the nuclease(s) that processes the breaks to form single-stranded tails remains unknown. The processing reaction is thought to require the activity of a 5' → 3' double-stranded exonuclease, a 5' → 3' single-stranded exonuclease in conjunction with a DNA helicase, or a single-stranded endonuclease, again working with a DNA helicase. In some proposed models, the Mre11/Rad50/Xrs2 complex participates in the removal of Spo11p from DSB sites in meiosis and might also participate in the removal of the 5' strand (FURUSE *et al.* 1998; USUI *et al.* 1998; MOREAU *et al.* 1999). However, the *in vitro* exonuclease activities of yeast Mre11p and the human Mre11 complex are of the opposite polarity to that predicted for the processing reaction (FURUSE *et al.* 1998; TRUJILLO *et al.* 1998; USUI *et al.* 1998). Although the endonuclease activity of Mre11p might be sufficient to remove Spo11p and the 5' strand, another possibility

is that the 5' strand is removed by the sequential action of Mre11p and a different nuclease.

The resulting 3' single-stranded tailed molecules participate in homologous pairing and strand invasion of a homologous duplex to form heteroduplex DNA. The length of heteroduplex DNA at the *ARG4* locus is dictated by the extent of degradation of the 5' strand (SUN *et al.* 1991). DNA mismatches present in heteroduplex DNA are repaired by the mismatch correction system giving rise to gene conversion (6:2 or 2:6 segregation of markers) or restoration to the parental markers (4:4 segregation of markers). Failure to repair results in post-meiotic segregation of markers to yield 5:3 or 3:5 segregations, manifested as sectorized spore colonies in *S. cerevisiae*. Although single base pair (except C/C) and small insertion/deletion mismatches are efficiently repaired by the mismatch repair system, palindromic insertions are refractory to repair (NAG *et al.* 1989). Hence, palindromic insertions can be used to monitor heteroduplex DNA formation without the complication of effects on meiotic recombination and sporulation caused by defects in the mismatch repair system. At the *HIS4* locus in *S. cerevisiae*, heteroduplex formation in meiosis is initiated at the 5' end of the gene and often extends to the 3' end, a distance of ~2.5 kb (PORTER *et al.* 1993).

Exonuclease I was originally characterized because the biochemical activity suggested that it might play a role in processing DSBs *in vivo* (SZANKASI and SMITH 1992; HUANG and SYMINGTON 1993; FIORENTINI *et al.* 1997). The *exo1* gene of *Schizosaccharomyces pombe* is transcriptionally induced during meiosis, suggesting a role in

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meiotic DNA metabolism (SZANKASI and SMITH 1995). The observations that *exo1* mutants are radiation resistant, proficient at mating-type switching, and have wild-type levels of sporulation argue against the possibility that exonuclease I is the primary DSB-processing activity (SZANKASI and SMITH 1995; FIORENTINI *et al.* 1997). Instead, the elevated rates of spontaneous mutation observed in *exo1* strains and the physical interaction between Exo1p and Msh2p are more consistent with a role for Exo1p in mismatch repair in mitotic cells. Tsubouchi and OGAWA (2000) isolated *EXO1* as a high-copy suppressor of the methyl methanesulfonate sensitivity caused by mutations in *MRE11*, *RAD50*, and *XRS2*. They also show that *EXO1* is induced in meiosis; processing of double-strand breaks is reduced in an *exo1 dmc1* strain; the level of crossing over, but not the frequency of gene conversion, is reduced by the *exo1* mutation; and Exo1p and Msh4p act in the same pathway for intergenic recombination. KHAZANEHDARI and BORTS (2000) also demonstrate that Exo1p and Msh4p act in the same pathway for crossing over but act in different pathways for spore viability. They show that the *exo1* mutation confers a high rate of meiosis I nondisjunction and that the level of gene conversion in the mutant is reduced at some but not all alleles examined.

Below we report that Exo1p has several meiotic functions. Strains with the *exo1* mutation have decreased spore viability and intergenic recombination and have elevated levels of chromosome nondisjunction. Genetic data argue that Exo1p acts in the same pathway as Msh5p to generate intergenic recombinants, but in overlapping pathways for spore viability. The extent of heteroduplex formation is not affected by the *exo1* mutation, indicating that exonuclease I does not have an essential role in processing of double-strand breaks to generate long 3' single-stranded DNA molecules for strand invasion during meiotic recombination. In addition, the patterns of aberrant segregation observed in *exo1* strains suggest that Exo1p functions in the repair of the DNA mismatches that lead to gene conversion events at some loci. These results are consistent with Exo1p having complex roles in both meiotic intergenic recombination and mismatch repair.

MATERIALS AND METHODS

Media and genetic procedures: With the exceptions noted below, standard protocols and media were used (SHERMAN *et al.* 1986). Sporulation plates contained 1% potassium acetate, 0.1% yeast extract, 0.05% glucose, 6 µg/ml adenine, and 2% agar. For studies on the frequencies of crossovers and aberrant segregation, diploids were sporulated at 18° and dissected onto plates containing rich growth medium. After colonies formed, they were replica plated to various omission media to score segregation of heterozygous markers. Postmeiotic segregation events at the *HIS4* locus were detected as sectored His⁺/His⁻ colonies (DETLOFF *et al.* 1992). Nomenclature for aberrant segregation events follows the standard set for eight-spored fungi—for segregation of a heterozygous allele A/a, Men-

delian segregation is designated 4A:4a, while a gene conversion event is 6A:2a or 2A:6a and single postmeiotic segregation events (one sectored colony) are 5A:3a and 3A:5a.

Studies on spore viability were done in two different genetic backgrounds. Diploids in the AS4/AS13 genetic background (described below) were sporulated at 18°. Diploids in the W303 background were sporulated at 30°. Following matings of the haploids to generate diploids, these strains were grown for either 15 or 60 generations prior to sporulation. The mating phenotype of germinated spores was determined by replica plating dissection plates to lawns of mating-type tester strains MCY14 and SJR13, followed by selection for diploids on minimal medium. Spores that failed to mate to the two tester strains were considered to be nonmatters due to missegregation of chromosome III.

Strains: Diploid strains used in the analysis of recombination were constructed by mating haploid strains derived from AS4 (*MATα trp1-1 arg4-17 tyr7-1 ade6 ura3*) or AS13 (*MATα leu2-Bst ade6 ura3 rme1*) (STAPLETON and PETES 1991) or from published derivatives of these strains. The haploid DTK299 was made from AS4 by one-step gene replacement using a PCR-generated DNA fragment designed to replace the *EXO1* gene with the *URA3* gene. The PCR fragment was generated by using the primers (5' AAAGGAGCTCGAAAACTGAAAGGCGTAGAAAGGAATGGGTATCCAAGGTgattgactgagagtgacc and 5' CCTCCGATATGAAACGTGCAGTACTTAACTTTATTACCTTTATAAACAAATTGGGgggctgtgcggtattcacaccg) to amplify the *URA3* gene from the plasmid pRS306 (SIKORSKI and HIETER 1989). Capital letters represent *EXO1* sequences and are identical to those used previously to construct the *exo1::HIS3* allele (FIORENTINI *et al.* 1997); the small letters represent sequences flanking *URA3*. Other strains with the *exo1* mutation were constructed in the same way including: DTK300, derived from DNY24 [AS13 but *his4-lopd*, (NAG *et al.* 1989)]; LSY612, derived from DNY16 [AS13 but *his4-B2* (NAG *et al.* 1989)]; LSY613, derived from DNY25 [AS13 but *his4-lopc* (NAG *et al.* 1989)]; and LSY611, derived from PD98 [AS13 but *his4-3133* (DETLOFF *et al.* 1992)]. *MSH5* was disrupted in DNY24, creating DTK492, using a PCR-generated DNA fragment from the primers (5' CAACTCATTCAAATAACTTACTCATTCATATACTGCCACCAAATGGAATcgtagctgcaggtcgc and 5' TTATTAACCTAAATATGTTACAGGTGGGCGTTTTTTTATTCTTTGATATAatgatgaattcgagctcg) to amplify the KanMX4 cassette from pFA6-kanMX4 (WACH *et al.* 1994); transformants were selected by resistance to geneticin. Capital letters in the primers represent *MSH5* sequences, while small letters represent pFA6-kanMX4 sequences. DTK492 was crossed with AS4 and transformed by a one-step transformation using the *msh5::URA3* plasmid pNH190-11 (HOLLINGSWORTH *et al.* 1995), selecting for Ura⁺ geneticin-resistant transformants to create the diploid DTK498. *MSH5* was disrupted in a Ura⁻ derivative of DTK299 using pNH190-11, generating DTK502. *EXO1* was disrupted in DTK492 using the *exo1::URA3* primers described above, creating DTK504. Diploid strains were constructed by mating: DTK308 (DTK299 × DTK300), DTK332 (DTK299 × LSY612), DTK333 (DTK299 × LSY613), DTK335 (DTK299 × LSY611), and DTK505 (DTK502 × DTK504). Except for the *exo1* and/or *msh5* mutations, strains DTK332, DTK333, and DTK335 are isogenic with strains DNY16, DNY26, and PD98, while DTK308, DTK498, and DTK505 are isogenic with DNY27 (NAG *et al.* 1989; DETLOFF *et al.* 1992). The diploid strain DTK347 is also isogenic with the diploid DNY26 (NAG *et al.* 1989), except for a homozygous insertion of a 60-bp simple repetitive sequence (CCCGNN)₁₂ that replaces transcription factor binding sites located upstream of *HIS4* (KIRKPATRICK *et al.* 1999).

Since diploids derived from AS4 and AS13 exhibit lower spore viability than most other laboratory strains, we also used

TABLE 1
Viability of spores from wild-type and *exo1* diploids (W303 genetic background)

Strain	<i>EXO1</i>	No. of generations ^b	No. of tetrads	% tetrads with indicated spore viability pattern (nos. of tetrads) ^a					% spore viability
				4:0	3:1	2:2	1:3	0:4	
W303	+/+	15	1012	82 (833)	14 (138)	3 (30)	0.5 (5)	0.5 (6)	94
LSY529a	-/-	15	492	72 (355)	13 (63)	9 (45)	1 (6)	5 (23)	87
LSY529b	-/-	15	513	74 (379)	11 (56)	9 (48)	2 (8)	4 (22)	87
LSY529 ^c (summed)	-/-	15	1005	73 (734)	12 (119)	9 (93)	1 (14)	5 (45)	87
W303	+/+	60	197	77 (152)	8 (16)	10 (19)	3 (6)	2 (4)	89
LSY529	-/-	60	213	66 (140)	9 (18)	13 (28)	2 (5)	10 (22)	79

^a Strains were sporulated and four-spored tetrads were dissected. The number of viable spores is shown, followed by the number of inviable spores. For example, the 4:0 class represents tetrads with four viable and no inviable spores.

^b Following construction of the diploid strains, the cells were grown for 15 or 60 generations prior to meiosis.

^c Summed data from LSY529a (*exo1::HIS3/exo1::HIS3*) and LSY529b (*exo1::URA3/exo1::HIS3*) diploids. No statistical difference was noted between LSY529a and LSY529b, but the heterozygous *exo1* insertion in LSY529b allows monitoring of nondisjunction events involving chromosome XV (see text).

W303 derivatives to measure spore viability. Strains W303-1A (*MATa leu2-3, 112 trp1-1 can1-100 ura3-1 ade2-1 his3-11, 15*) and isogenic derivatives W303-1B (*MATα*), LSY496-10D (*MATa exo1::HIS3*), and LSY496-20A (*MATα exo1::HIS3*) were described previously (FIORENTINI *et al.* 1997). LSY625 (*MATa exo1::URA3*) was constructed from W303-1A as described above for the AS4 and AS13 *exo1* derivatives. The wild-type diploid (W303) was constructed by crossing W303-1A with W303-1B while the *exo1* diploid derivatives were constructed by crossing LSY496-10D with LSY496-20A (to generate LSY529a) and LSY496-20A with LSY625 (to generate LSY529b). The *MSH5* gene was disrupted in the haploid W303-derived strains by the one-step replacement method using plasmid pNH190-11 (HOLLINGSWORTH *et al.* 1995). The resulting strains, LSY810 (*MATa msh5::URA3*), LSY811 (*MATα msh5::URA3*), LSY812 (*MATa msh5::URA3 exo1::HIS3*), and LSY813 (*MATα msh5::URA3 exo1::HIS3*), were crossed to make the diploids LSY899 (LSY810 × LSY811) and LSY900 (LSY812 × LSY813). The genotypes of the mating-type tester strains were: *MATa suc2-437 lys2-802* (MCY14) and *MATα lys2-802* (SJR13).

Statistical analysis: Statistical comparisons were done using the Instat 1.12 program for Macintosh (GraphPad Software). Chi-square test or Fisher's exact variant were used for comparisons. Results were considered statistically significant if $P < 0.05$.

RESULTS AND DISCUSSION

Reduced spore viability in diploids homozygous for the *exo1* mutation: The diploid strains W303 (*EXO1/EXO1*) and LSY529 (*exo1/exo1*) are isogenic except for the *exo1* mutation. When these strains were sporulated after a limited number of generations (about 15) of vegetative growth following their construction, we found that the wild-type diploid had better spore viability than the homozygous *exo1* diploid (94 vs. 87%; Table 1); by a chi-square test this difference is highly significant ($P < 0.0001$). Since strains with the *exo1* mutation have elevated levels of spontaneous mutations (TISHKOFF *et al.* 1997), one interpretation of this result is that recessive

lethal mutations accumulate in the diploid *exo1* strain, leading to a loss of spore viability. By this interpretation, additional generations of vegetative growth of the diploid should lead to further reductions in spore viability. As shown in Table 1, although 60 generations of vegetative growth of LSY529 slightly reduced spore viability (from 87 to 79%), a reduction was also observed in the wild-type diploid (from 94 to 89%). We conclude, therefore, that the reduction in spore viability in the *exo1* diploid is unlikely to reflect the accumulation of recessive lethal mutations.

Another potential cause of spore inviability is chromosome nondisjunction. A single nondisjunction event during meiosis I would be expected to result in two disomic spores and two spores missing one chromosome. Nondisjunction events involving two different chromosomes during meiosis I would be expected to result in either two double disome spores and two spores missing two chromosomes or four spores that are disomic for one chromosome and lack another. Thus, in strains with a moderate level of nondisjunction in meiosis I, one would expect to detect an elevation in classes of tetrads with two viable spores or with no viable spores. In contrast, classes of tetrads with three viable spores or one viable spore would not be substantially elevated as a consequence of meiosis I nondisjunction.

Two arguments are consistent with the conclusion that the *exo1* mutations increase the rate of chromosome nondisjunction in meiosis I. First, when we compare the sum of the number of tetrads with two viable spores and no viable spores with the sum of the other classes of tetrads in *exo1* and wild-type strains, we find a significant elevation in the *exo1* strain ($P < 0.0001$). When we compare the sum of the number of tetrads with three viable spores and one viable spore with the sum of the other classes of tetrads, no significant elevation is ob-

served in the *exo1* strains ($P = 0.6$). The second argument is based on finding nonmating spores. If a nondisjunction event involves chromosome *III* (the location of the *MAT* locus), tetrads with two viable nonmating spores should be produced. We tested the mating phenotype of all of the spores derived from *exo1* and wild-type strains shown in Table 1. As expected, all except six of the tetrads with four viable spores showed 4:4 segregation for *MAT*. The exceptions were gene conversion events observed in both strains. Of the tetrads with two viable spores, 11 of 93 from the LSY529 *exo1* strains represented pairs of nonmating spores, indicative of chromosome *III* nondisjunction during meiosis I. No pairs of nonmating spores were observed for the wild-type strain. A second diploid version of LSY529 (LSY529b) was constructed in which one allele of *EXO1* was disrupted with *URA3 (exo1::URA3)* and one allele with *HIS3 (exo1::HIS3)*. As the *EXO1* locus resides on chromosome *XV*, nondisjunction events involving chromosome *XV* can be detected by monitoring the segregation of these two alleles. From the 48 2:2 spores (Table 1), there were 2 with *Ura*⁺ *His*⁺ spores, indicative of nondisjunction of chromosome *XV*. This number might be an underestimate, as the *Ura*⁺ *His*⁺ spores grow very poorly, presumably due to the extra copy of chromosome *XV*.

Chromosome nondisjunction during meiosis II would be expected to produce tetrads with three viable spores and one inviable spore. None of the tetrads with three viable spores contained a nonmating spore, indicating no detectable meiosis II nondisjunction in the wild-type or *exo1* strains. In addition, as described above, the fraction of tetrads with three viable spores was not significantly affected by the *exo1* mutation. In summary, we conclude that the spore inviability in *exo1* strains reflects an increase in chromosome nondisjunction during meiosis I.

A number of other yeast mutants have been identified that decreased spore viability associated with reduced intergenic recombination including *msh4*, *msh5*, *mlh1*, *mlh3*, *zip1*, *zip2*, and *tam1* (SYM *et al.* 1993; ROSS-MACDONALD and ROEDER 1994; HOLLINGSWORTH *et al.* 1995; CHUA and ROEDER 1997, 1998; WANG *et al.* 1999). The *MSH4* and *MSH5* genes are meiosis specific and encode homologues of bacterial MutS proteins (ROSS-MACDONALD and ROEDER 1994; HOLLINGSWORTH *et al.* 1995). Unlike other MutS homologues, *msh4* and *msh5* mutants show no apparent defect in mismatch correction during mitosis or meiosis. Instead, the proteins are proposed to form a heterodimer that binds to Holliday junctions and promotes resolution of recombination intermediates to form crossover products (POCHART *et al.* 1997). The meiotic phenotype of *exo1* mutants resembles *msh4* and *msh5* mutants, except that the defect in spore viability in *exo1* strains is less severe. *mlh1* mutants also show a decrease in intergenic recombination and spore viability and epistasis studies indicate that *MLH1* functions in

the same pathway as *MSH4* and *MSH5* (HUNTER and BORTS 1997). To determine whether *EXO1* functions in the same pathway as *MSH5*, double mutants were generated and examined for spore viability. In the W303 genetic background, a strain homozygous for the *msh5* mutation (LSY899) had substantially reduced spore viability (55% spore viability from 230 tetrads dissected). In the diploid strain LSY900, homozygous for both *msh5* and *exo1*, the spore viability was not significantly different from the *msh5* homozygote (53% from 637 tetrads dissected). A strain homozygous for *exo1* and *msh4* mutations showed similar spore viability (47% from 200 tetrads dissected).

We also examined the effects of the *exo1* and *msh5* mutations on spore viability in a different genetic background [crosses of AS4- and AS13-derived haploid strains (STAPLETON and PETES 1991)]. In the *EXO1/EXO1* diploid strain DTK347, we observed 85% spore viability. In the *exo1/exo1* strains DTK308, DTK332, DTK333, and DTK335 (differing in alleles at the *HIS4* locus), the average spore viability was 78%, a significant ($P < 0.0001$) reduction from that observed for the wild-type strain. An *msh5/msh5* strain (DTK498) had a spore viability of 80%, also a significant reduction ($P = 0.0001$) compared to wild type. The average spore viability in a strain homozygous for deletions in both *exo1* and *msh5* (DTK505) was 67%, a significant reduction compared to wild-type, *msh5*, and *exo1* strains ($P < 0.0001$), but above the level expected for independent pathways ($80\% \times 78\% = 62.4\%$). Thus, in two different genetic backgrounds, spore viability is reduced by mutations in *EXO1* and *MSH5*, and analysis of spore viability in the double mutant indicates that Exo1p and Msh5p function in the same or overlapping pathways for chromosome disjunction. This contrasts with the results obtained with *exo1 msh4* homozygous diploids in two other strain backgrounds, in which synergistic decreases in spore viability were observed (KHAZANEHDARI and BORTS 2000; TSUBOUCHI and OGAWA 2000).

Reduced spore viability associated with elevated levels of meiosis I chromosome nondisjunction is often a consequence of decreased meiotic crossing over (ROEDER 1997). Consequently, as described below, we examined the effects of the *exo1* mutation on the frequency of reciprocal and nonreciprocal meiotic recombination.

Reduced meiotic crossing over in strains homozygous for the *exo1* mutation: We examined meiotic recombination in four pairs of diploid wild type and *exo1* mutant strains (Figure 1). Each diploid was constructed from AS4- and AS13-derived haploid strains and was heterozygous for mutations at the *HIS4* locus (located on chromosome *III*), the linked (centromere-proximal) *LEU2* locus, and the centromere-linked *TRP1* locus. We monitored crossovers between *HIS4* and *LEU2* and between *LEU2* and *CEN3*, as well as the frequency of aberrant segregation at the *HIS4* locus. As will be discussed in more detail below, the four pairs of strains differed in

FIGURE 1.—Location of markers within the *his4* gene. Haploid strains LSY611, LSY613, DTK300, and LSY612 were made by one-step gene replacement of strains PD98, DNY25, DNY24, and DNY16, respectively, using a PCR-generated DNA fragment designed to disrupt the *EXO1* gene. The vertical arrow indicates the position of the recombination initiation site upstream of the *HIS4* gene; the horizontal arrows indicate the direction of transcription. The lollipops indicate the position of palindromic insertions within *his4*. The inverted triangle represents the nonpalindromic insertion within *his4*. Each haploid was crossed to the *His*⁺ strain DTK299 to create the diploids DTK308, DTK332, DTK333, and DTK335. The corresponding diploid strain number is given in parentheses.

the nature and/or position of the heterozygous *his4* mutations.

As shown in Table 2, for all four pairs of strains the map distances between *HIS4* and *LEU2* and between

LEU2 and the centromere were reduced by the *exo1* mutation. This reduction was statistically significant ($P < 0.03$) for both genetic intervals in every strain pair, except for the *HIS4-LEU2* interval in strains PD99 and DTK335 ($P = 0.2$). We conclude, therefore, that the *exo1* mutation reduces but does not eliminate crossing over. It is likely that this reduction is responsible for the elevation in chromosome nondisjunction and the reduced spore viability observed in the *exo1* strains.

The relationship between *EXO1*-dependent crossing over and the previously defined pathway for crossing over that involves *MLH1*, *MLH3*, *MSH4*, and *MSH5* (described above) was determined by examining the level of intergenic recombination in *msh5* and *msh5 exo1* strains (Table 2). Deletion of *MSH5* reduced the map distance between *HIS4* and *LEU2* to the same degree as a deletion of *EXO1* [from 39 cM to 24 (*msh5*) and 22 cM (*exo1*), respectively]. The *HIS4-LEU2* map distance in the double mutant (19 cM) was not statistically different than the distances in the single mutants, indicating that the two gene products function in the same pathway for intergenic recombination.

Effect of the *exo1* mutation on aberrant segregation at the *HIS4* and *ARG4* loci: Three of the pairs of AS4 × AS13 strains used in this study are heterozygous for palindromic insertions; one member of each pair was wild type and one member was homozygous for *exo1*. The location of these insertions relative to the *HIS4* initiating codon are: −50 (*his4-B2*), +467 (*his4-lopc*), and +2327 (*his4-3133*) (Figure 1). The fourth pair of strains (DNY27 and DTK308) was heterozygous for a nonpalindromic insertion at the same position in *HIS4* as the *his4-lopc* insertion. Tetrads from each strain were

TABLE 2
Map distances between various loci in *EXO1* or *exo1* strains

Strain	<i>HIS4</i>	<i>EXO1</i>	<i>HIS4-LEU2</i> interval					<i>LEU2-CEN3</i> interval			
			PD	NPD	T	cM	% of WT	FDS	SDS	cM	% of WT
DNY18 ^a	<i>his4-B2</i> /+	+/+	155	10	124	32	100	280	75	11	100
DTK332	<i>his4-B2</i> /+	−/−	113	4	37	20	63	163	17	5	45
DNY26 ^b	<i>his4-lopc</i> /+	+/+	191	8	202	31	100	388	91	10	100
DTK333	<i>his4-lopc</i> /+	−/−	109	1	53	18	58	170	13	4	40
PD99 ^c	<i>his4-3133</i> /+	+/+	202	12	141	30	100	320	88	11	100
DTK335	<i>his4-3133</i> /+	−/−	112	2	67	22	73	187	13	3	27
DNY27 ^d	<i>his4-lopd</i> /+	+/+	130	15	108	39	100	289	65	9	100
DTK308	<i>his4-lopd</i> /+	−/−	156	4	79	22	56	261	21	4	44
DTK498	<i>his4-lopd</i> /+	+/+ <i>msh5</i> / <i>msh5</i>	143	5	82	24	61	249	32	6	67
DTK505	<i>his4-lopd</i> /+	−/− <i>msh5</i> / <i>msh5</i>	124	1	69	19	49	216	43	8	89

All strains are diploids that are isogenic except for the differing heterozygous *HIS4* allele and the presence or absence of *EXO1* or *MSH5*. The location of each of the *HIS4* alleles is depicted in Figure 1. PD, parental ditype; NPD, nonparental ditype; T, tetatype; FDS, first division segregation; SDS, second division segregation; cM, centimorgans; WT, wild type. The number of tetrads of each segregation class is listed in the appropriate column.

^a From NAG *et al.* (1989).

^b From FAN *et al.* (1995).

^c Rescored from original data (DETLOFF and PETES 1992).

^d From KIRKPATRICK and PETES (1997).

dissected and the level of aberrant segregation at the *HIS4* locus was monitored (Table 3). All of the strains are also heterozygous for a mutation in *ARG4*; the level of aberrant segregation of this marker was also determined.

The level of aberrant segregation for strains containing a palindromic insertion at *HIS4* is only slightly affected by the *exo1* mutation (Table 3); the effects on aberrant segregation are considerably smaller than on crossing over. The *exo1* mutation has its strongest effect on the level of aberrant segregation for the strain with the nonpalindromic insertion (compare DNY27 and DTK308); an interpretation of this result will be given below. The *exo1* mutation does not significantly reduce the frequency of aberrant segregation of the palindromic insertion near the 3' end of *HIS4* (comparison of PD98 and DTK335). Since most of the heteroduplexes located at the 3' end of *HIS4* reflect processing of a DSB located at the 5' end of *HIS4*, this result indicates that Exo1p is not required for producing the single-stranded DNA "tail" required for heteroduplex formation.

The effect of deletion of *exo1* on aberrant segregation of a heterozygous allele at the *ARG4* locus contrasts with the effect on aberrant segregation at *HIS4*. In the wild-type strain DNY27, 44 out of 359 tetrads exhibited aberrant segregation at *ARG4* (24 6:2 events and 20 2:6 events). In the *exo1* strains (DTK308, DTK332, DTK333, and DTK335) there were 37 tetrads of a total of 870 that exhibited aberrant segregation of the *ARG4* marker (23 6:2 events and 14 2:6 events). This decrease in the level of aberrant segregation, from 12% in the wild type to 4% in the *exo1* mutant strains, is highly significant ($P < 0.0001$). All events in both wild-type and *exo1* strains were gene conversions; no postmeiotic segregation (PMS) tetrads were detected. Removal of Exo1p affects the observed level of aberrant segregation differently at *HIS4* and *ARG4*. At *HIS4*, the data indicate that the level of aberrant segregation is not significantly affected by loss of Exo1p, whereas the level of aberrant segregation observed at the *ARG4* locus is reduced. Locus-specific effects resulting from the loss of Exo1p have been reported previously (KHAZANEHDARI and BORTS 2000). This study on the effects of a deletion of *EXO1* on the aberrant segregation rate of a number of two- or four-base insertions into *HIS4* and *LEU2* demonstrated that the *HIS4* alleles, but not the *LEU2* alleles, showed reduced aberrant segregation when *EXO1* was deleted (KHAZANEHDARI and BORTS 2000).

Effect of the *exo1* mutation on patterns of aberrant segregation at the *HIS4* locus: In strains heterozygous for a single marker, most tetrads segregate 4:4 (nomenclature used for eight-spored fungi). As discussed in the Introduction, the most common aberrant segregation types are of two classes, gene conversion events (6:2 or 2:6 segregations) or postmeiotic segregation events (5:3 or 3:5 segregation). PMS tetrads reflect asymmetric het-

eroduplex formation between wild-type and mutant alleles and failure to correct the resulting DNA mismatch. The efficiency of repair of a mismatch is reflected by the fraction of the aberrant segregation events that are PMS. In *S. cerevisiae*, single base mismatches (excluding C/C) and mismatches resulting from nonpalindromic insertions are efficiently repaired (low level of PMS compared to gene conversion), whereas C/C mismatches and mismatches resulting from small palindromic insertions are inefficiently repaired (PETES *et al.* 1991).

In the AS4 $\times$ AS13 genetic background, the *HIS4* locus has a very high level of aberrant segregation (NAG *et al.* 1989). Most aberrant segregation events at the *HIS4* locus reflect an initiating double-strand DNA break near the 5' end of the gene (FAN *et al.* 1995), followed by heteroduplex formation that often extends from the break to the 3' end of *HIS4* (PORTER *et al.* 1993). One argument supporting the conclusion that heteroduplex formation usually involves the entire *HIS4* coding region is that the level of aberrant segregation for markers leading to inefficiently repaired DNA mismatches at the 5' and 3' ends of the *HIS4* gene is similar (DETLOFF *et al.* 1992). For most of the *exo1* strains, there was a significant increase in the number of PMS events at the *HIS4* locus compared with the wild-type diploids, confirming the role of Exo1p in mismatch repair (Table 3). Both the *his4-B2* and *his4-lopc* strains showed a significant ($P = 0.04$ and 0.03 , respectively) increase in the number of PMS tetrads when *EXO1* was deleted. The greatest increase ($P = 0.007$) in such tetrads was seen in the strain DTK308 containing the well-repaired *his4-lopd* allele, which generates a 26-base loop when contained in a heteroduplex tract. We previously demonstrated (KIRKPATRICK and PETES 1997) that correction of this loop was also dependent on the products of the *RAD1* and *MSH2* genes, although deletion of either of these two genes has a greater effect on correction of *his4-lopd* mismatches than a deletion of *EXO1*. In all pairs of strains, the *exo1* member of the pair had a reduced frequency of gene conversion relative to the wild-type member (Table 3). For example, in strains heterozygous for the nonpalindromic *his4-lopd* insertion, the gene conversion frequency was reduced from 26 to 14% by the *exo1* mutation, and the PMS frequency was elevated from 4.3 to 7%. These results are the first demonstration of a role for Exo1p in DNA mismatch repair during meiosis; other studies did not show an increase in the frequency of PMS of heteroalleles when *EXO1* was deleted (KHAZANEHDARI and BORTS 2000; TSUBOUCHI and OGAWA 2000). Also, in our study no increase in PMS was detected at the *ARG4* locus, as described above. The alleles that show no increase in PMS are those that will generate a small mismatch (one to four bases) when present in heteroduplex, while the alleles that have a significant increase in PMS are those that will generate a large mismatch (26 to 36 bases) in heteroduplex DNA.

TABLE 3
Aberrant segregation of *HIS4* alleles in *EXO1* or *exo1* strains

Strain	<i>HIS4</i>	<i>EXO1</i>	Total tetrads	% of tetrads with indicated segregation patterns								Ab. Seg. (%)	PMS/ Ab. Seg. (%)
				4:4	6:2	2:6	5:3	3:5	Ab 4:4	Other Ab. Seg.			
DNY18 ^a	<i>his4-B2/+</i>	+/+	383	57 (218)	9 (36)	5 (20)	13 (49)	11 (41)	3 (10)	2 (9)	43	62	
DTK332	<i>his4-B2/+</i>	-/-	182	59 (108)	7 (13)	3 (5)	15 (28)	9 (17)	4 (8)	2 (3)	41	76	
DNY26 ^b	<i>his4-lop/+</i>	+/+	494	50 (248)	6 (30)	3 (16)	18 (89)	15 (72)	3 (15)	5 (24)	50	79	
DTK333	<i>his4-lop/+</i>	-/-	191	60 (114)	5 (10)	1 (2)	13 (25)	15 (29)	4 (8)	2 (3)	40	84	
PD98 ^c	<i>his4-3133/+</i>	+/+	441	63 (276)	5 (23)	5 (23)	12 (51)	11 (49)	2 (10)	2 (9)	37	72	
DTK335	<i>his4-3133/+</i>	-/-	205	62 (128)	4 (8)	3 (6)	15 (30)	12 (25)	2 (5)	1 (3)	38	81	
DNY27 ^d	<i>his4-lop/+</i>	+/+	359	70 (252)	15 (54)	11 (38)	3 (11)	0.3 (1)	0 (0)	1 (3)	30	12	
DTK308	<i>his4-lop/+</i>	-/-	292	79 (232)	10 (30)	4 (11)	4 (11)	2 (6)	0 (0)	1 (2)	21	29	
DTK498	<i>his4-lop/+</i>	+/+ <i>msh5/msh5</i>	288	76 (220)	8 (22)	9 (26)	3 (9)	1 (4)	0 (0)	2 (7)	24	19	
DTK505	<i>his4-lop/+</i>	-/- <i>msh5/msh5</i>	267	71 (190)	10 (27)	10 (27)	2 (5)	4 (10)	0.4 (1)	3 (7)	29	24	

For all segregation patterns, the first number is the percentage, while the number in parentheses is the number of tetrads observed with that segregation pattern. In the heading of each column of segregation pattern data, the first number represents the wild-type allele and the second, the mutant allele. The segregation patterns include: 4:4 (normal Mendelian segregation), 6:2 and 2:6 (gene conversion), 5:3 and 3:5 (tetrads with a single PMS event), Ab 4:4 (aberrant 4:4; one wild-type, one mutant, and two sector colonies). The Other Ab. Seg. class includes aberrant 6:2 and 2:6 tetrads as well as tetrads with three or four PMS or gene conversion events. The percentage of aberrant segregation tetrads that are not repaired is indicated in the PMS/Ab. Seg. column.

^a From NAG *et al.* (1989).

^b From FAN *et al.* (1995).

^c Rescued from original data (DETLOFF and PETES 1992).

^d From KIRKPATRICK and PETES (1997).

We propose that Exo1p may have a role specifically in the repair of large mismatches.

Conclusions: In conclusion, we find that Exo1p promotes meiotic crossing over, ensuring proper disjunction of chromosomes during meiosis I. This function involves a previously identified pathway involving the *MSH4*, *MSH5*, *MLH1*, and *MLH3* gene products (ROSS-MACDONALD and ROEDER 1994; HOLLINGSWORTH *et al.* 1995; HUNTER and BORTS 1997; WANG *et al.* 1999), as the level of crossovers in strains lacking both Exo1p and Msh5p is higher than expected if the two proteins work in distinct pathways. Loss of Exo1p also leads to a decrease in spore viability. This decrease is not due to the accumulation of mutations during mitotic growth and is partially overlapping with the decrease in spore viability seen in strains lacking Msh5p. Furthermore, the length of the heteroduplex tract at the *HIS4* locus does not appear to be reduced in *exo1* mutant strains, indicating that the Exo1p deoxyribonuclease does not participate in generating long 3' single-stranded tailed molecules following double-strand break generation during meiotic recombination at *HIS4*. It is possible that the nuclease function of Exo1p acts postsynaptically to direct resolution of recombination intermediates instead of the predicted presynaptic function. Alternatively, the *EXO1* protein could participate in generating a small amount of single-stranded DNA, after which another protein or protein complex continues the process of generating the long 3' single-stranded tailed molecules present at *HIS4*. In addition, we find that the *exo1* mutation affects the frequency of aberrant segregation in a manner that suggests that Exo1p has a minor role in the repair of DNA mismatches at *HIS4*. Exo1p is involved in the repair of a 26-bp loop mismatch in heteroduplex DNA, although to a lesser extent than several other proteins (Rad1p and Msh2p) known to act on DNA loops of this size (KIRKPATRICK and PETES 1997). Finally, there are locus-specific effects to the loss of Exo1p, as the level of aberrant segregation at *ARG4* is significantly lowered by loss of Exo1p, unlike the situation at *HIS4*. Also in contrast to the data from the *HIS4* locus, repair of mismatches at *ARG4* is not affected by the *exo1* mutation, indicating that at *ARG4* initiation, rather than repair, is primarily affected by the *exo1* mutation.

This work was supported by Public Health Service grants from the National Institutes of Health GM41784 (L.S.S.), 2 T32 CA09503 (J.R.F.), and GM24110 (T.D.P.). D.T.K. is a Special Fellow of the Leukemia and Lymphoma Society.

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Communicating editor: M. LICHTEN