HO endonuclease-induced recombination in yeast meiosis resembles Spo11-induced events

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In meiosis, gene conversions are accompanied by higher levels of crossing over than in mitotic cells. To determine whether the special properties of meiotic recombination can be attributed to the way in which Spo11p creates double-strand breaks (DSBs) at special hot spots in *Saccharomyces cerevisiae*, we expressed the site-specific HO endonuclease in meiotic cells. We could therefore compare HO-induced recombination in a well-defined region both in mitosis and meiosis, as well as compare Spo11p- and HO-induced meiotic events. HO-induced gene conversions in meiosis were accompanied by crossovers at the same high level (52%) as Spo11p-induced events. Moreover, HO-induced crossovers were reduced 3-fold by a *msh4*Δ mutation that similarly affects Spo11p-promoted events. In a *spo11*Δ diploid, where the only DSB is made by HO, crossing over was significantly higher (27%) than in mitotic cells (≤7%). This single meiotic DSB failed to induce the formation of a synaptonemal complex. We also show that HO-induced gene conversion tract lengths are shorter in meiotic than in mitotic cells. We conclude that a hallmark of meiotic recombination, the production of crossovers, is independent of the nature of Spo11p-generated DSBs at special hotspots, but some functions of Spo11p are required in *trans* to achieve maximum crossing over.

Meiotic recombination in *Saccharomyces cerevisiae* differs from mitotic recombination in several respects. Beyond the fact that meiotic gene conversions occur 100 to 10,000 times more often than equivalent spontaneous mitotic events, the individual events themselves have different outcomes. Most notably, meiotic gene conversions have a higher association with crossing over than is seen in mitotic cells (reviewed in refs. 1 and 2). In addition to generating genetic diversity, crossing over is critical in ensuring accurate chromosome segregation during meiosis (3, 4).

The origins of most spontaneous gene conversion events in mitosis are unknown, but they are certainly different from the way in which double strand breaks (DSBs) are induced by the meiosis-specific Spo11 protein (5, 6). DSBs created by Spo11p are generated at many nearby sites in meiotic hotspots (7, 8). These hotspots represent a very special chromosomal context influenced by perhaps 10 other genes, the deletion of which abolishes or severely reduces DSB formation (reviewed in refs. 1, 3, 4, 9, and 10). Deletion of genes encoding Mre11p, Rad50p, and Xrs2p causes changes in the chromatin structure of hotspots (11). Similarly, the absence of protein components such as Hop1p that comprise the axial elements lying between sister chromatids also reduces DSB formation (12).

Other mutations reduce the proportion of meiotic gene conversions accompanied by meiotic crossovers without affecting DSB formation. The *zip1*Δ or *zip2*Δ mutations, which eliminate formation of the central element of the synaptonemal complex (SC), have this effect (13, 14), providing support for the general view that crossover regulation depends on this structure (3, 4). Deletion of the mismatch repair-related *MSH4* and *MSH5* genes also reduces the proportion of gene conversions accompanied by crossing over without reducing the total number of events (15, 16).

In the absence of Spo11-induced DSBs, meiotic crossing over is abolished. X-irradiation of such cells can produce an increase in exchange events, which is probably due to the induction of DSBs (17); but that study could not determine whether repair of such breaks is "meiotic-like," that is, whether the frequency of crossing over associated with each repair event is higher than in mitotic cells.

The increase in crossover-associated gene conversion can be explained in several ways that are not mutually exclusive. For example, the specific DNA ends produced by Spo11p could predetermine the fate of these events. It might also depend on the creation of a special chromatin environment at hotspots by other proteins (e.g., ref. 11) that would subsequently influence the way recombination proceeds. Alternatively, it could depend on diffusible proteins that associate with DSB ends, for example, by a meiosis-specific DNA strand exchange protein such as Dmc1p (18), or that enhance stabilization of intermediate structures such as Holliday junctions that could be resolved by crossing over (reviewed in ref. 1).

To explore the differences between mitotic and meiotic recombination we have made use of the site-specific HO endonuclease to create identical DSBs in the two types of cells, at the same well-defined 2-kb region. HO-induced events in meiosis can also be compared directly with Spo11p-induced recombination in the same region. HO endonuclease creates 3′-ended 4-bp overhanging ends at a large, defined recognition site (19), whereas Spo11p appears to produce 5′-ended, 2-bp overhangs at many sites within hotspot regions (7, 8). Thus we can also evaluate how the DSB ends themselves influence recombination. We have previously shown that expression of HO from a meiosis-specific *SPO13* promoter (*SPO13*::*HO*) caused the appearance of DSBs at approximately the same time as those created by Spo11p and that the kinetics of appearance of recombinants by HO was similar to those of normal meiotic events (20). We report here that HO-induced recombination in meiotic cells has many of the characteristics of Spo11-induced events.

Materials and Methods

Strains used in this study are derivatives of those used previously (21). Diploid strain WYL237 has a genotype *MAT***a**/*MATα ura3/ura3 trp1/trp1 met13-4/met13-4 lys2C/lys2C ade1/ade1 CAN1/can1 CYH2/cyh2* and contains a well-defined *URA3-LEU2-ADE1* interval on chromosome III (Fig. 1*A*). A 2.2-kb *ADE1* gene was inserted at the *AseI* site of *LEU2* located 1076 bp centromere proximal to the *Asp*718 site in *LEU2* (19), whereas the opposite site of *LEU2* is bounded by a large *URA3*-marked deletion removing part of the *HIS4* gene and all of the intervening 17 kb to the *Xho*I site distal to *LEU2*

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Abbreviation: DSB, double strand break.

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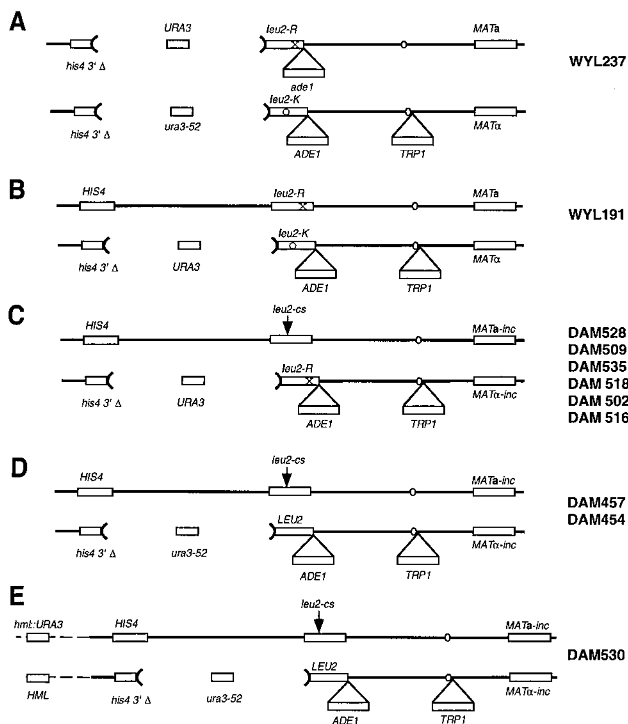

Fig. 1. (*A*) A well-defined interval on chromosome III in which a 1.8-kb *LEU2* gene is flanked closely by *URA3* and *ADE1* genes. The positions of *leu2-K* and *leu2-R* are shown by o and x, respectively. (*B*) Recombination in strain WYL 191 is largely confined to the 1.8-kb interval containing the 2-kb *LEU2* gene bounded between flanking heterologies, a 20-kb deletion (*URA3*) and a 2.2-kb insertion (*ADE1*). (*C*) A 117-bp HO cleavage site (*leu2-cs*, arrow) was inserted into the *LEU2* gene at Asp718. Sites of Spo11p cleavage are located approximately 400 bp centromere-distal to *leu2-cs*. The opposite homologue carries *leu2-R*. All strains expressing *HO* contain mutations at *MAT* that prevent HO cleavage. (*D*) DAM454 and DAM457 carry *leu2-cs* and a wild-type *LEU2* allele. (*E*) DAM530 has the same chromosome III structure as that shown in *D*, except that the *HML* locus on the *leu2-cs*-containing chromosome was disrupted by the insertion of *URA*.

(E. J. Louis and J.E.H., unpublished observation). The *leu2-R* allele contains a 4-bp fill-in of the *Eco*RI site of *LEU2* (22). The *leu2-K* allele is a 4-bp deletion of the *Kpn*I site, located 399 bp from *Eco*RI (22). A 1.7-kb *TRP1* marker was inserted next to *CEN3*. WYL191 is isogenic to WYL237, except that the *LEU2* gene in this strain is flanked by the large heterologies marked by *URA3* and *ADE1* (Fig. 1*B*). Diploid strain DAM454 has the genotype *MAT***a**-inc/*MAT*α-inc *ade1*/*ade1 ura3*/*ura3 lys2*::*SPO13*::*HO*/*lys2*::*SPO13*::*HO*. The *LEU2* gene is flanked by large heterologies (Fig. 1*D*). The *URA3* marker was replaced by *ura3-52*, by an interchromosomal gene conversion event, selected using 5-fluoroorotic acid (23). One copy of *LEU2* contains an HO endonuclease cut site (*leu2-cs*) at the Asp718 site (24), and another copy of *LEU2* is wild type.

Diploid DAM518 is a *leu2-R* isogenic derivative of DAM454 created by gene replacement (21) (Fig. 1*C*). DAM 518 also contains a *spo13*Δ mutation introduced as previously described (20). *SPO11* was deleted by the method of Wach *et al.* (25) to produce DAM502. *msh4*Δ derivatives were created using a plasmid provided by N. Hollingsworth (State University of New York, Stony Brook, NY). DAM 516 was created by a crossing an isogenic *msh4*Δ derivative of DAM454 and a backcrossed *MAT*α-inc *msh4*Δ strain. Strain DAM509 is identical to DAM518 except that it carries a *GAL*::*HO* gene integrated at *ade3* (26) and is *SPO13*/*spo13*Δ. DAM528 is identical to DAM518 except that it lacks the *lys2*::*SPO13*::*HO* construct, is *SPO13*/*spo13*Δ and its *MAT***a**-inc parent is isogenic to a sibling of that used to construct DAM518. DAM535 is identical to DAM528 except it is homozygous for *spo13*Δ. DAM457 is identical to DAM454 except that it lacks the *lys2*::*SPO13*::*HO* construct and its *MAT*α-inc parent was a sibling of that used to construct DAM454. DAM530 is identical to DAM454, except that it is homozygous for *spo13*Δ and *spo11*Δ and it carries an insertion of *URA3* at *HML* (27) on the chromosome carrying *leu2-cs* (Fig. 1*E*).

Galactose inductions, yeast spreads and immunocytological analysis, and genetic and DNA methods have been described previously (20, 24, 28). The statistical significance of various comparisons was evaluated by using the G-test (29), with a program provided by Ed Louis (University of Leicester, Leicester, U.K.).

Results

HO-Induced Recombination in Meiosis Resembles *SPO11*-Mediated Events. We have examined recombination in a well-defined interval on the left arm of chromosome III, in which a 1.8-kb *LEU2* gene is flanked closely by *URA3* and *ADE1* genes that can be used to determine how often crossing over accompanies gene conversion in the *LEU2* locus (Fig. 1*A*). Spo11-mediated events were first analyzed by examining random spore segregants from a diploid carrying the *leu2-K* and *leu2-R* alleles, as previously described (21). Previous studies, using strains closely related to those used here, have shown that virtually all Leu$^+$ recombinants arise by gene conversion rather than by reciprocal exchange between the two (21). The strong bias in which crossover chromatid contains the Leu2$^+$ gene convertant is consistent with previous analyses of heteroallelic recombination, supporting the conclusion that crossover-associated gene conversions usually involve heteroduplex DNA that covers only one of the two heteroallelic sites (30, 31). Among Leu2$^+$ meiotic gene convertants in strain WYL237, 60% were accompanied by crossing over between the *URA3* and *ADE1* flanking markers (Table 1*A*).

To facilitate subsequent analysis of crossing over by Southern blot or PCR approaches, we then created strains in which the flanking *URA3* and *ADE1* regions were present as large heterologies, leaving the 1.8-kb *leu2* region surrounded by a 20-kb deletion and a 2.2-kb insertion (WYL191; Fig. 1*B*). The presence of these heterologies had no significant effect on the association of gene conversion and crossing over, as 63% (502/797) of the Leu$^+$ heteroallelic recombinants exhibited an exchange of flanking markers. Among Leu$^-$ spores, most of which had not undergone a recombination event in this interval, only 7% (56/766) exhibited crossing over of flanking markers.

To be able to compare Spo11*p*- and HO-mediated recombination, we further modified this region by replacing the *leu2-K* allele with the 117-bp cleavage site for HO endonuclease, yielding the *leu2-cs* allele (Fig. 1*C*). First, we examined random spores from diploids heterozygous for *leu2-cs* and *leu2*-R, which are separated by 399 bp (Fig. 1*C*). The rate of Leu2$^+$ recombinants in a *SPO11* strain DAM528 heteroallelic for *leu2-cs* and *leu2-R* (0.24 ± 0.1%) was similar to the rate of Leu2$^+$ recombinants obtained in isogenic strains with *leu2-K* and *leu2-R* (0.3 ± 0.1%). When the *leu2-cs*/*leu2-R* diploid expressed both *SPO11* and *SPO13*::*HO* (DAM509) the rate of Leu2$^+$ recombinants increased 10-fold to 2.5 ± 0.4%. In both of these diploids approximately half of the Leu2$^+$ random spores exhibited a crossover of the flanking heterologous markers (49% for DAM528 and 46% for DAM509) (Table 1*C*).

We then analyzed recombination in this interval by tetrad analysis, comparing strain DAM457 expressing only *SPO11* and DAM454 expressing both *SPO11* and *SPO13*::*HO*, both diploids carrying *leu2-cs* and *LEU2* (Fig. 1D and Table 2). In the absence of *SPO13*::*HO*, 3% of tetrads contained three *LEU2* and one *leu2-cs* and another 2% had the reverse 1:3 gene conversion pattern, as expected if either *leu2* segment could be cleaved by Spo11p. The majority of Spo11p-induced events in this region most likely to occur in the promoter region of *LEU2* gene, which is a prominent

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Table 1. The frequency of crossing over associated with recombination at the *LEU2* locus

	Spores with no crossing over			Spores with crossing over				
A. Analysis of random spores from WYL237 bearing no heterologies around *LEU2* (Fig. 1*A*)								
Random spores	His$^-$Ura$^+$Ade$^-$	His$^-$Ura$^-$Ade$^+$	Total	His$^-$Ura$^+$Ade$^+$	His$^-$Ura$^-$Ade$^-$	Total	Total	% crossing over
Leu$^-$	84	99	183	28	23	31	214	14
Leu$^+$	17	81	98	127	14	141	239	60
B. Analysis of random spores from WYL191 containing heterologies flanking *LEU2* (Fig. 1*B*)								
Random spores	His$^+$Ura$^-$Ade$^-$	His$^-$Ura$^+$Ade$^+$	Total	His$^+$Ura$^-$Ade$^+$	His$^-$Ura$^+$Ade$^-$	Total	Total	% crossing over
Leu$^-$	379	331	710	33	23	56	766	7
Leu$^+$	50	245	295	457	45	502	797	63
C. Analysis of Leu$^+$ random spores from strains with and without *SPO13*::*HO* (Fig. 1*C*)								
Strain	His$^+$Ura$^-$Ade$^-$	His$^-$Ura$^+$Ade$^+$	Total	His$^+$Ura$^-$Ade$^+$	His$^-$Ura$^+$Ade$^-$	Total	Total	% crossing over
DAM528 *SPO11*	144	8	152	28	115	143	295	49
DAM509 *SPO11* *SPO13*::*HO*	145	4	149	7	119	126	275	46

hot spot for the formation of meiotic DSBs located 400 bp distal to the HO cleavage site (32). In the *SPO11 SPO13*::*HO* strain, 12% of tetrads were 3 *LEU2*:1 *leu2-cs*, whereas only 1% were 1:3 events. This is the expected result if *SPO13*::*HO*-induced DSBs in the *leu2-cs* allele are produced more frequently than SPO11-induced events in *LEU2*. We estimate that at least 70% of the 3 *LEU2*:1 *leu2* gene conversions in the *SPO13*::*HO* strain were induced by HO, in agreement with the random spore events discussed above. We will discuss later the 12% 4:0 events induced by HO, as they originate from DSBs of both sister chromatids and proved to have distinctly different behaviors.

In the case of 3 *LEU2*:1 *leu2-cs* tetrads in the *SPO11 SPO13*::*HO* diploid DAM454, 58% were tetratype tetrads with respect to the flanking *ADE1* and *HIS4* markers; that is, two of the four chromatids had undergone a crossing over in the *LEU2* interval. The four tetrads in which the *ADE1* marker was coconverted were not considered. In 34 of 38 tetratype asci, crossing over involved the Leu2$^+$-containing chromatid. After correcting for the four unassociated crossovers, we calculate that the proportion of gene conversions associated with crossovers was 52% (34/66), in good agreement with random spore measurements. These results show that HO-induced events in meiotic cells exhibit the same high level of crossing over as those created by *SPO11*.

About 6% (4/70) of the 3 *LEU2*:1 *leu2* gene conversion events in the *SPO11 SPO13*::*HO* strain DAM454 were coconverted for the adjacent *ADE1* marker, inserted 1076 bp centromere proximal to the HO cleavage site. This is not significantly different from the 2/10 coconversions found among 3 *LEU2*:1 *leu2 tetrads* in the *SPO11* strain DAM457, lacking *SPO13*::*HO*, where the presumed site of DSBs is located 400 bp distal to *leu2-cs*. Among tetrads without a *leu2* gene conversion *ADE1* conversion was

Table 2. Effect of *SPO13*::*HO* on meiotic recombination involving *leu2*::*cs*

Strain	Leu$^+$:Leu$^-$	Total number of tetrads	Tetrads with no crossover between *HIS4* and *ADE1*	Tetrads with crossover between *HIS4* and *ADE1**	% crossover	Tetrads with coconversion of *ADE1*
DAM 457	2:2	286	264	21	7	1
SPO11	3:1	10 (3%)	4	4	50	2
	4:0	0	—	—		—
	0:4	1	—	—		1
	1:3	5 (2%)	0	4		1
	Total	302				
DAM 454	2:2	457	421	36	8	0
SPO11	3:1	70 (12%)†	28	38‡	52§	4
SPO13::*HO*	4:0	74 (12%)†	28	18	23¶	28
	0:4	0				
	1:3	4 (1%)	0	4		
	Total	605				

*Parental ditype (PD), tetratype (TT), and nonparental ditype (NPD) asci were identified with respect to the flanking *ADE1* and *HIS4* markers (see Fig. 1*D*). Tetrads in which *ADE1* was coconverted were not considered. In (2:2), (3:1), and (1:3) cases, all crossover tetrads are TT. Among the 18 4:0 tetrads from DAM454, 15 were TT and 3 were NPD.

†Statistically significant difference ($P < 0.001$) from the absence of *SPO13*::*HO.*

‡In 34 of 38 cases a Leu$^+$ spore experiencing gene conversion contained a crossover product, whereas in 4 cases the crossover did not involve the *LEU2*-containing chromatid.

§The frequency of crossing over associated with conversion to Leu$^+$ was determined after omitting four tetrads in which the adjacent *ADE1* marker was coconverted and four tetrads in which crossing over did not involve the gene-converted chromatid.

¶For the 4:0 tetrads, the frequency of crossing over was calculated on the basis of two gene conversion events per tetrad. Thus there were 15 TTs, with one crossover out of a possible two and three NPDs with two crossovers, of a total of 92 possible exchange events in 46 tetrads.

rare (Table 2), suggesting that most conversions of *ADE1* were dependent on the DSBs that converted *leu2*.

***SPO13*::*HO*-Induced Meiotic Events Are Different from *GAL*::*HO*-Induced Mitotic Recombination.** For this same *leu2* interval, we examined HO-induced recombination in mitotic cells, using a galactose-inducible HO gene that was also present in DAM509. The results were different from those induced in meiosis by *SPO13*::*HO*. We selected DSB-initiated repair events arising in the G_2 stage of the mitotic cell cycle (equivalent to the time when recombination happens in meiosis), by plating cells on galactose-containing medium. We considered colonies that were sectored Leu^+/Leu^-, where only one of the HO-cut chromatids had converted to *LEU2* whereas the second *leu2-cs* allele was uncut or coconverted to *leu2-R*. The majority of these events apparently occurred at the G_2 stage, although some of them could have occurred by repairing the HO-induced DSB in subsequent cell cycles. These colonies were analyzed by Southern blots to identify crossovers. Gene conversions coming from the G_2 phase of the mitotic cell cycle are rarely ($\leq$7%) associated with crossing over (3/46 cases).

The gene conversion tract lengths of HO-induced events in mitotic cells were longer than those in meiotic cells, in agreement with previous studies of conversion tract lengths (1). Forty-eight percent (26/54) of all HO-induced mitotic gene conversions examined in G_1 cells resulted in coconversion of the 2.2-kb *ADE1* marker, compared with only 6% (4/70) of HO-induced gene conversion in meiosis (Table 2).

We also examined spontaneous mitotic recombination between *leu2-K* and *leu2-R* in strain WYL191 with the arrangement of markers shown in Fig. 1*B*, to compare it to HO-induced recombination. Only 4 of 107 independent Leu^+ colonies selected in mitosis were homozygous for the distal *his4* marker, indicative of a crossover event in G_2 cells. Correcting for the fact that only half of the crossover events should be recovered as $His4^-$ because of the random segregation of chromatids in mitosis (31), we estimated that 7% of gene conversions were accompanied by crossing over. Thus HO-induced and spontaneous mitotic recombination events are similar to each other, and both are statistically significantly different from what occurs for both Spo11*p*- and HO-induced gene conversions in meiosis in the same interval.

Analysis of *SPO13*::*HO*-Induced Recombination in the Absence of the *MSH4*. We then asked whether the high level of crossing over of HO-induced events in meiosis depended on the *MSH4* gene function, as do *SPO11*-induced events. Msh4 and Msh5 proteins are involved in regulating the proportion of gene conversions that are associated with crossovers, and mutations in these genes reduce crossing over about 2- to 3-fold without affecting the total number of gene conversions (15, 16). To carry out these experiments, we used diploids homozygous for *spo13*Δ, which rescues spore viability in recombinationless mutants like *spo11*Δ (33, 34) and for mutations such as *msh4*Δ that significantly reduce crossing over (15, 16). *spo13*Δ causes meiotic cells to undergo only a single meiotic division and to produce two diploid spores.

Before analyzing the effects of *msh4*Δ on HO-induced meiotic events, we confirmed that *spo13*Δ did not affect HO-promoted recombination. We dissected the dyads produced by strain DAM518 (*spo13*Δ *SPO11 SPO13*::*HO*) and determined the frequency of dyads in which one spore had undergone a gene conversion resulting in the formation of a Leu^+ spore, whereas the other spore was still heteroallelic for *leu2-cs* and *leu2-R* (confirmed by the ability of such diploids to give rise to Leu^+ papillae). These dyads have had a single gene conversion and are therefore the equivalent of a 3:1 tetrad. In DAM518 the frequency of such dyads was 6% (13/223), whereas strain DAM535, carrying *SPO11* without *SPO13*::*HO*, gave only 1% (2/209) dyads with one $Leu2^+$ spore ($P < 0.01$) (Table 3*A*). Thus, as with the *SPO13* tetrads analyzed above, approximately 80% of the events in DAM518 are likely to have been induced by *SPO13*::*HO*.

A larger number of Leu^+ recombinants arising from a single HO cleavage were then obtained by plating complete dyads without microdissection and analyzing Leu^+/Leu^- sectored colonies where the Leu^- sector was still heterozygous for *leu2-cs* and *leu-R* (Table 3*B*). Southern blot and genetic analysis showed that 52% of the predominantly *SPO13*::*HO*-induced events in strain DAM518 were associated with crossing over (Table 3*B*), similar to the 52% we observed in tetrad analysis performed in $Spo13^+$ diploids (Table 2). Again, we observed that HO-induced gene conversions are associated with crossing over in meiosis almost 8 times more frequently than in mitosis, again suggesting that the high level of crossing over is not a special feature of *SPO11*-induced events.

Having shown that *spo13*Δ diploids behaved similarly to wild-type diploids in the way HO-induced events were repaired, we analyzed Leu^+-containing dyads obtained from meiosis of *spo13*Δ *msh4*Δ diploids carrying *leu2-cs* and l*eu2-R*. As shown in Table 3*B*, a *msh4*Δ mutation reduces *SPO13*::*HO*-induced crossovers by about 3-fold (from 52% to 18%), similar to the reductions that have been observed for *SPO11*-induced events in previous studies (15, 16). The overall frequency of Leu^+-containing dyads was not significantly different between *msh4*Δ mutants (4.4 ± 1.7%) and wild-type controls (6.6 ± 2.7%).

***SPO13*::*HO* Induces Gene Conversions with a High Proportion of Crossovers, Even in the Absence of SPO11.** After showing that Spo11p is not required at the site of the DSB (i.e., in *cis*) to ensure high levels of crossing over, we asked whether some function of Spo11p is nevertheless needed to ensure the full level of crossing over associated with gene conversion (i.e., that Spo11p plays a role in *trans*). We used the *spo13*Δ *spo11*Δ *SPO13*::*HO* diploid DAM502, heterozygous for *leu2-cs* and *leu2-R*. Here, the only DSBs in meiosis come from HO cleavage. Crossing over in those dyads with one $Leu2^+$ spore was reduced from 52% to 27% (Table 3*B*). Thus in the absence of Spo11p, crossing over still occurs at a level 4 times higher than in mitotic cells, but 2-fold lower than in a *SPO11* strain. This 2-fold reduction could be due to a modulating effect of other nearby recombination events that would be created by Spo11p and/or to the fact that *spo11*Δ mutants fail to form SC. Our result is analogous to previous studies showing that *zip1*Δ or *zip2*Δ mutations, which prevent formation of SC in *SPO11* diploids, reduce the proportion of gene conversions accompanied by crossing over by about 2-fold (13, 14).

The absence of Spo11p also affects the lengths of gene conversion tracts of HO-induced meiotic gene conversions. In the *spo13*Δ *spo11*Δ *SPO13*::*HO* diploid DAM502, 9% (30/344) of the dyads had a single gene conversion of *leu2-cs* (Table 3*C*), compared with 13% (28/223) in the $Spo11^+$ diploid, but there was a significant reduction in the percentage of $Leu2^+$ segregants among single gene conversion events (13% in *spo11*Δ (4/30) instead of 42% (13/28) in *SPO11*). Most of the reduction in $Leu2^+$ recombinants was due to enhanced coconversion of *leu2-cs* to *leu2-R* in the *spo11*Δ diploid compared with the *SPO11* strain, so that the gene convertants were $Leu2^-$. In addition to the increased coconversion of the *leu2-R* marker 400 bp away from the HO cleavage site, the *spo11*Δ *SPO13*::*HO* strain also showed a significant ($P < 0.01$) increase in the frequency of coconversions of the adjacent 20-kb heterology extending to *HIS4* (40% versus 11% in *SPO11 SPO13*::*HO*). This same trend was found when we analyzed a subset of events for coconversions of the adjacent *ADE1* insertion by Southern blots (Table 3*C*).

The long conversion tracts extending 20 kb distal to the *leu2* region could in fact be much longer, extending all the way to the end of the chromosome. To examine how long those conversion tracts arising in the *spo11*Δ *spo13*Δ *SPO13*::*HO* strain could become, we used a diploid in which the *leu2-cs*-containing chromosome carried *URA3*, inserted at the *HML* locus, 12 kb

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Table 3. Analysis of Leu$^+$-containing dyads from *spo13*Δ diploids

A. Analysis of unselected dyads from diploids with and without *SPO13*::*HO*

		Leu$^+$ spore-containing dyads			
Strain	Relevant genotype	Dyads with a single gene conversion[†]	Others[‡]	Dyads containing only Leu$^-$ spores	Total
DAM518	*SPO11 SPO13*::*HO* *spo11*Δ *SPO13*::*HO*	13 (6%)*	24	186	223
DAM535	*SPO11* *spo11*Δ	2 (1%)	3	204	209

B. Analysis of Leu$^+$/Leu$^-$ dyads obtained from asci plated on growth medium

		Analysis of *ADE1–HIS4* crossing over among Leu$^+$-containing dyads[§]				
Strain	Relevant genotype	No crossover	Crossover	% crossover	Coconversion of *HIS4*	Coconversion of ADE1
DAM518	*SPO11* *spo11*Δ	12	13	52[¶]	1	0
DAM502	*spo11*Δ *spo11*Δ	30	11	27	5	3
DAM516	*SPO11 msh4*Δ *spo11*Δ *msh4*Δ	37	8	18	1	3

C. Analysis of unselected dyads containing a single gene conversion event

			Gene conversion of one spore in the dyad			
Strain	Genotype	Total number of dyads analyzed	Single gene conversion, with Leu$^+$ spore	Single gene conversion, coconversion of *leu2-R*	Among gene conversions of one spore, coconversion of *HIS4*[‖]	Among gene conversions of one spore, coconversion of *ADE1*[††]
DAM518	*SPO11* *spo11*Δ	223	13/28 (42%)	15/28 (58%)	3/28 (11%)	1/9 (11%)
DAM502	*spo11*Δ *spo11*Δ	344	4/30 (13%)**	26/30 (87%)**	12/30 (40%)**	3/9 (33%)

[†]Dyads in which one spore had undergone a gene conversion resulting in Leu$^+$ phenotype while the other spore was still heterozygote for *leu2-cs* and *leu2-R* (confirmed by the ability of such diploids to give rise to Leu$^+$ papillae). * indicates a statistically significant difference from the absence of *SPO13*::*HO* ($P < 0.01$).
[‡]Dyads in which both spores undergone gene conversion events (analogous to 4:0 tetrads) or dyads in which a Leu$^-$ spore did not give rise to Leu$^+$ papillae because of reductional chromosomal division, chromosome missegregation, or crossing over in the interval between the *LEU2* gene and the centromere.
[§]Sectored colonies arising from the germination of two spores in an ascus were identified after germination on YEPD medium by replica plating them to medium lacking leucine. Leu$^+$/Leu$^-$ sectored colonies were then analyzed to show that the Leu$^-$ half still retained the *leu2-cs* allele; hence there was a single gene conversion event in this meiosis.
[¶]Statistically significant difference from the *spo11*Δ strain ($P < 0.05$) and the *msh4*Δ strain ($P < 0.01$).
[‖]One of three His$^-$ segregants obtained from DAM518 was Leu$^+$. The other two His$^-$ segregants obtained from DAM518 and all 12 His$^-$ segregants obtained from DAM502 were Leu$^-$.
**Indicates a statistically significant difference from the Spo11$^+$ strain ($P < 0.01$).
[††]Only a subset of events were analyzed to determine if *ADE1* was heterozygous or homozygous.

from the chromosome end (Fig. 1*E*). Of 110 dyads analyzed, 13 were identified as having one His$^-$ and one His$^+$ spore. Of these, two were the result of chromosome loss, as they had also lost *MAT***a** on the opposite side of the centromere. By Southern blot analysis two others were shown to be reciprocal crossovers, but nine were the result of a nonreciprocal event that is consistent with a break-induced replication mechanism (35, 36) that apparently copied the His$^-$ chromosome all the way to the end. This demonstrates that break-induced replication can occur in meiotic cells. These data reveal that HO-induced meiotic recombination in the absence of *SPO11* is significantly different from what is seen in a *SPO11 spo13*Δ *SPO13*::*HO* strain: the proportion of crossovers accompanying gene conversion is reduced, the length of gene conversion tracts is greatly increased, and many breaks are apparently repaired by nonreciprocal break-induced replication rather than by gene conversion.

***SPO13*::*HO spo11*Δ Mutants Do Not Form Visible SC.** *spo11*Δ cells fail to form the tripartite synaptonemal complex (SC) that has been implicated in the control of crossing over (37). Hence it was of great interest to know whether a single DSB induced by SPO13::HO in a *spo11*Δ strain would be sufficient to promote SC formation. We examined two independent *spo13*Δ *spo11*Δ *SPO13*::*HO* diploids and their isogenic *spo11*Δ and *SPO11* controls lacking *SPO13*::*HO* by immunostaining of chromosome spreads (28), using the synapsis-specific anti-Zip1 antibody (38). Whereas *SPO11* diploids showed normal levels of partial and complete SCs at 5, 6, and 7.3 h after induction (30–65%), SC-like structures were found exceedingly rarely in the *spo11*Δ strains (0.2–1%), regardless of the presence of the *SPO13*::*HO* construct. More than 1000 nuclei were inspected per time point. Many of the spread *spo11*Δ nuclei contained a Zip1 positive polycomplex (PC) and occasionally rigid linear structures, which, because they did not correspond to DAPI-labeled chromatin, were interpreted as fragments of the PC. Thus, the induction of a single DSB by *SPO13*::*HO* was not sufficient to induce formation of extensive SCs. This result raises the possibility that lack of synaptonemal complex by itself is responsible for the very unusual long gene conversion tracts seen in *spo11*Δ *SPO13*::*HO* cells, as well as for crossover levels that are 2-fold lower than maximum.

Cleavage of Both Sister Chromatids Causes a Disruption of Meiotic Recombination. In normal meiosis, there are few, if any, tetrads in which both sister chromatids are cleaved simultaneously at the same hot spot, to produce a 4:0 tetrad by two independent gene conversion events. This is also evident from the fact that Spo11-mediated DSBs created in a haploid strain undergoing meiosis can be repaired by homologous recombination, using their sister chromatids as templates (39); such a repair would be

impossible if both chromatids were cut, in the absence of a homologous chromosome. Therefore the consequences of cleaving both sister chromatids of one homologue at the same site have not previously been assessed.

In the *SPO13*::*HO* diploids about 12% of the tetrads had four *LEU2* spores (Table 2), apparently the consequence of two gene conversion events resulting from the cleavage of both sister chromatids. Although some events might have been the consequence of HO-induced recombination before meiosis, we could show that most of them most likely arose after premeiotic DNA replication, as 38% of them had one crossover and one noncrossover event and another 12% showed different coconversions of the flanking *ADE1* or *HIS4* regions. The recombination events in these cases appeared to be different from what was obtained in tetrads with a single *SPO13*::*HO*-induced event. First, although 52% of tetrads with 3 *LEU2*:1 *leu2-cs* were accompanied by crossing over, only 23% were crossover-associated in the 4 *LEU2*:0 *leu2-cs* cases. This value was calculated on the basis that there are two conversion events per tetrad, including 15 with one crossover and 3 tetrads with 2 crossovers, of a total of 92 events in 46 tetrads. The calculated crossover rate of 23% is significantly different ($P < 0.001$) from the rate of 52% among the 3:1 tetrads. It is possible that this reduced level of crossing over accompanying gene conversions could be a reflection of the longer apparent gene conversion tracts among the 4:0 tetrads than the 3:1 tetrads, although no other data support this possibility.

The proportion of observed four-strand double crossovers in the 4:0 tetrads (6.5%) corresponds to the expected frequency, based on the calculated crossover rate of 0.23 among these 4:0 events and assuming that only four-strand double crossovers can result when the two sister chromatids are cleaved at the same site. This conclusion suggests that there is no interference in double crossovers in the interval containing *leu2*. We note that apparent interference in crossing over was seen previously by Kolodkin *et al.* (40), who studied *GAL*::*HO*-induced 4:0 events at the *MAT* locus in meiotic cells; but in their case, only 4:0 tetrads were recovered, and it was not clear whether many of these events were initiated before premeiotic DNA replication.

Discussion

We have compared the outcomes of recombination events in the same chromosomal location, initiated by DSBs created by the same HO endonuclease, in mitotic and meiotic cells. This study examines the recombination induced by the same DSB under these two very different situations. We find that the fraction of gene conversions accompanied by crossing over is 8 times greater in meiosis than in mitotic cells and is comparable to Spo11p-generated events in the same well-defined genetic interval. We conclude therefore that two of the special properties of meiotic recombination, the frequent crossovers accompanying gene conversion and the shorter conversion tract lengths compared with mitotic events, are not dependent on initiation by Spo11p. Thus, neither the different overhanging ends of Spo11p- and HO-generated DSBs nor the specific sequences in which DSBs are made account for the difference between mitotic and meiotic gene conversions. Moreover, although the ability of Spo11p to create DSBs depends on more than 10 proteins, some of which have been shown to bind to DNA and in some cases alter the chromatin structure of a hotspot (11, 41), this special chromatin context does not appear to be required to achieve the characteristic meiotic outcome.

By creating an HO-induced DSB in the absence of any other meiotic DSBs, we also demonstrated that a single HO-induced gene conversion had about half the level of crossing over (27%) as was seen when Spo11p was also active (52%). Apparently a single HO-induced DSB is insufficient to create a visible extent of SC. One explanation for the reduced level of crossing over in the *spo11*Δ *SPO13*::*HO* strain is that it lacks the synaptonemal complex that is somehow responsible for increasing the proportion of gene conversions accompanied by crossing over. The reduced level of crossing over we obtained in a *spo11*Δ *SPO13*::*HO* strain is analogous to what has been seen previously in *SPO11* strains lacking the SC proteins Zip1p and Zip2p (13, 14).

Our results suggest that the special features of meiotic recombination can be attributed to processes occurring after the DSB is generated. This assumption focuses our attention on meiosis-specific recombination proteins such as Dmc1p and/or meiosis-specific chromosome structures, including the synaptonemal complex, meiosis-specific cohesins (42), or other possible chromatin modifications. Further support for this conclusion is that the absence of Msh4p reduces crossing over of HO-induced gene conversions to the same extent that others have reported for Spo11p-mediated events. Thus we are now in a position to use several revealing assays, developed in mitotic cells using HO endonuclease (24, 43), to compare in detail the molecular mechanisms of meiotic and mitotic recombination. Further experiments should also establish whether HO-induced recombination is sufficient to drive reductional division, another key characteristic of meiosis.

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