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Meiotic Synapsis in the Absence of Recombination

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Although in *Saccharomyces cerevisiae* the initiation of meiotic recombination, as indicated by double-strand break formation, appears to be functionally linked to the initiation of synapsis, meiotic chromosome synapsis in *Drosophila* females occurs in the absence of meiotic exchange. Electron microscopy of oocytes from females homozygous for either of two meiotic mutants (*mei-W68* and *mei-P22*), which eliminate both meiotic crossing over and gene conversion, revealed normal synaptonemal complex formation. Thus, synapsis in *Drosophila* is independent of meiotic recombination, consistent with a model in which synapsis is required for the initiation of meiotic recombination. Furthermore, the basic processes of early meiosis may have different functional or temporal relations, or both, in yeast and *Drosophila*.

In the classical view of meiosis, homologous chromosome synapsis, as indicated by the formation of an elaborate ribbonlike structure called the synaptonemal complex (SC), was thought to be the first and primary event of meiotic prophase, essential for the initiation of meiotic recombination (*1*). Studies in *Saccharomyces cerevisiae*, however, have created a different view of the meiotic process in which the initiation of recombination, as evidenced by a double-strand break (DSB), precedes the initiation of synapsis (*2*, *3*). Three lines of evidence support this view of early meiotic prophase in yeast. First, the initiating event of meiotic recombination, the formation of a DSB, appears before SC formation (*4*). Second, meiotic mutants that either fail to create DSBs or to process DSBs to make single-stranded tails prevent the formation of a mature SC (*2*). Third, some mutants allow high levels of meiotic recombination but prevent the production of a mature SC (*5*). These data are consistent with a model in which single-stranded DNA generated by a DSB carries out a homology search required for synapsis and SC formation. In contrast, synapsis is not an absolute prerequisite for either the initiation (*6*) or completion of meiotic recombination (*7*).

To assess the relation between synapsis and the initiation of recombination in *Drosophila* oocytes, we examined both recombination and SC formation in oocytes homozygous for either of two null-recombination mutations. The *mei-W68* and *mei-P22* (*8*) mutants prevent the initiation of meiotic recombination as defined by four independent assays: (i) reduction or elimination of meiotic gene conversion; (ii) elimination of meiotic crossing over, as assayed by measuring either intragenic crossing over or the frequency of meiotic crossing over along entire chromosome arms; (iii) lack of double-strand DNA breaks that persist into metaphase or anaphase I; and (iv) failure to produce either early or late recombination nodules (RNs).

To assay the effects of the *mei-W68* and *mei-P22* mutations on meiotic crossing over, we examined intragenic recombination at the *rosy* locus (*9*). No gene conversion events or intragenic crossovers were observed among the progeny of *mei-W68* or *mei-P22* females (Table 1 and Fig. 1). Compared to controls, the frequency of both intragenic exchange and simple gene conversion was reduced by a factor of at least 30 to 40 and, most likely, was eliminated. A small reduction in gene conversion frequency was also observed in the *mei-W68*/+ females, suggesting a dosage effect.

The effect of these mutations on crossing over was also assayed by more conventional means. In both *mei-W68* and *mei-P22* mutant females, the frequency of crossing over along the entire X and second chromosomes was reduced to less than 0.5% of normal. Moreover, the few crossover events that were observed tended to be recovered in clusters of identical recombinants among the progeny of single females, suggesting that they resulted from mitotic and not meiotic recombination events (*10*). The failure to observe meiotic recombination events in progeny of *mei-W68* and *mei-P22* mutant

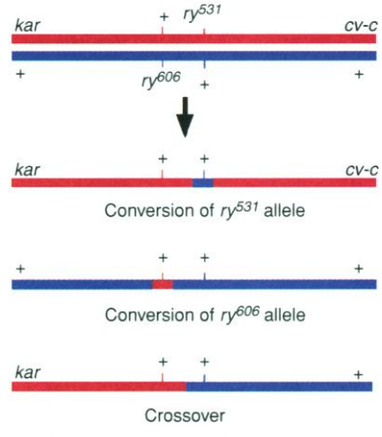

Fig. 1. Gene conversion at the *rosy* locus (genetic map position 52.0). A schematic of the parental chromosomes and the three products of intragenic recombination that yield *rosy*$^+$ recombinants. The distances between the loci are not drawn to scale. The recombinants were classified as convertants or crossovers on the basis of the flanking mutations. In the *mei-P22* experiment, the flanking markers were *kar*, an eye color mutant mapping 0.3 cM to the left, and *cv-c*, a wing vein mutant mapping 2.1 cM to the right of *ry*. In the *mei-W68* experiment, *Ace* (52.5) or *red* (53.6) replaced *cv-c* (54.1). The two *rosy* alleles used in this study, *ry*^{531} and *ry*^{606}, were chosen because they are at opposite ends of the *rosy* gene, 3780 nucleotides and 0.012 cM apart (*29*). The average length of conversion tracts in *Drosophila* is 885 base pairs (*32*), and therefore co-conversion events are expected to have a minimal effect on our experiments.

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Table 1. Intragenic recombination at the *rosy* locus in *mei-P22*, *mei-W68*, and control females.

Genotype	Crossover	Conversion of ry^{606}	Conversion of ry^{531}	Zygotes sampled	ry^+ frequency
	kar^+ ry^+ cv-c or kar^+ ry^+ Ace^{126}	kar^2 ry^+ cv-c^1 or kar^2 ry^+ Ace^{126}	kar^+ ry^+ cv-c^+ or kar^+ ry^+ Ace^+	($\times 10^5$)	($\times 10^{-5}$)
thr^1 kar^2 ry^{606} cv-c^1/ry^{531}	20	9	5	5.7	5.96
mei-W68/+; kar^2 ry^{606} Ace^{126}/ry^{531}	9	1	3	3.88	3.35
mei-W68; kar^2 ry^{606} Ace^{126} or red/ry^{531}*	0	0	0	3.82	<0.9
mei-P22 thr^1 kar^2 ry^{606} cv-c^1/mei-P22 ry^{531}	0	0	0	5.6	<0.6

*Two ry^{606} chromosomes were used, with the right marker either *Ace* or *red*. A recombinant chromosome was not recovered in either case, and the data were pooled. In each experiment, 1.91×10^5 progeny were screened.

oocytes is consistent with our failure to observe either early or late RNs in the oocytes of these females [(*11*) and see below].

Recombination events may be initiated in *mei-P22* and *mei-W68* oocytes, but then redirected into a pathway that results in sister-chromatid exchanges. We tested this possibility by examining the effects of these mutations on sister-chromatid exchange assayed by ring-chromosome loss. Sister-chromatid exchange within a ring chromosome results in the formation of a dicentric chromosome that is not transmissible. No excess of ring loss was observed in *mei-P22* females compared with +/+ or +/*mei-P22* sisters (*12*).

The above data demonstrate that meiotic recombination events involving either homologous chromosomes or sister chromatids do not occur in *mei-W68* and *mei-P22* mutant oocytes. The ablation of gene conversion further demonstrates that mature recombination intermediates are also not formed. Based on three lines of evidence we can also rule out the possibility that DSBs are created in *mei-W68* and *mei-P22* mutant oocytes but left unrepaired. First, cytological analysis of meiotic chromosomes during metaphase and anaphase I from both *mei-W68* and *mei-P22* mutant oocytes showed no evidence of chromosome fragmentation or of gapped chromosomes (*13*), as would be expected if the DSBs were not repaired (*14*). Second, there was no excess of *nullo*-X oocytes relative to diplo-X oocytes among the progeny of either type of mutant female, and thus chromosome loss is not common (*15*). Finally, these females were no less fertile than expected, on the basis of expected frequencies of zygotic death due to aneuploidy, suggesting that there was no excessive dominant lethality from broken chromosomes in the oocytes. Although we cannot rule out the possibility that DSBs are made but are rapidly repaired in a manner that leaves no genetic trace, all available data argue that recombination is not initiated in these mutants.

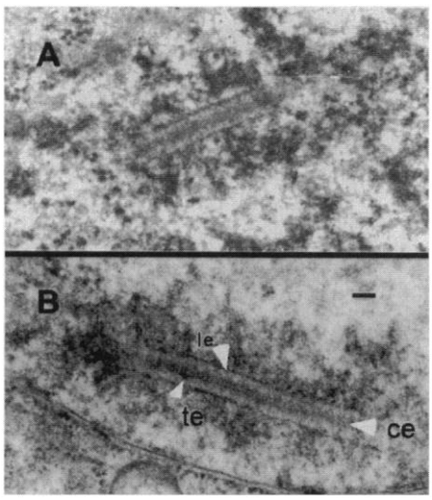

Fig. 2. Transmission electron microscopy of ovaries from females homozygous for *mei-W68* and *mei-P22*. (**A**) Section from a *mei-W68* female (*16*) and (**B**) a section from a *mei-P22* female (*33*). The complete SC is visible in either mutant, including lateral elements (le), transverse elements (te), and central elements (ce). Bar, 100 nm.

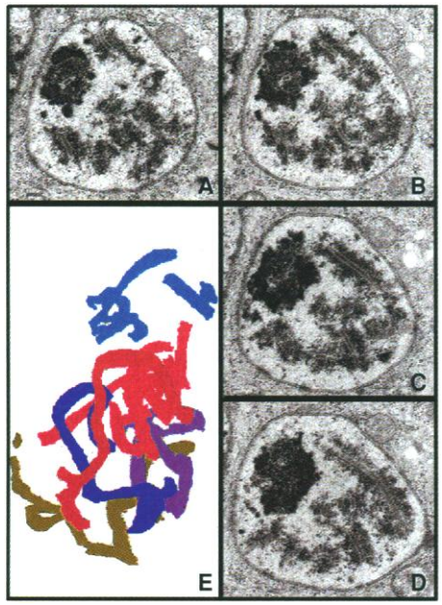

Fig. 3. (**A** to **D**) Consecutive sections from *mei-P22* females. (**E**) A tracing generated by superimposing all the sections from this nucleus, with each chromosome arm represented by a different color.

Saccharomyces cerevisiae mutants exhibiting recombination defects as severe as those observed here do not make SC. To compare the effects of such a recombination defect in *Drosophila* oocytes, we examined SC formation in *mei-W68* and *mei-P22* oocytes. SC formation was analyzed by the reconstruction of serial sections examined by transmission electron microscopy. All of the pachytene nuclei from a single *mei-W68* germarium plus several nuclei from two other germaria were reconstructed (Fig. 2A) (*16*). Three *mei-P22* pachytene nuclei, two from a single germarium and one from another germarium, were completely reconstructed and 10 nuclei from several germaria were thoroughly examined (Figs. 2B and 3). Formation of the central and lateral elements and transverse filaments of the synaptonemal complex was normal in both *mei-W68* and *mei-P22* females. The width of the central region in euchromatic SC for both *mei-W68* and *mei-P22* was equal to that of wild-type (109 ± 8 nm) (*17*). SC is continuous along each of the X, *2R*, *2L*, *3R*, *3L*, and *4th* chromosome arms (Fig. 3), and the age-adjusted euchromatic lengths of the SCs for the X and *2L* are equal to those of wild type. There was no evidence for failed or nonhomologous synapsis. Consistent with the recombination phenotype, however, we failed to observe either early or late RNs in the mutants (*11*).

Meiotic progression in these mutants also appeared to be normal. We observed the same progression of SC shortening and thickening as in wild type. Three *mei-W68* germaria were examined in detail (*16*), and in all respects the timing of developmental events was indistinguishable from that in wild type (*17*). In particular, there was no delay in reaching full pachytene. In *mei-W68* germaria there was an average of 1.5 ± 1.7 16-cell cysts in pre-pachytene (*16*), compared with 1.3 ± 0.9 and 2.0 ± 1.8 in two different wild-type samples (*18*).

We conclude that, in *Drosophila melanogaster* oocytes, SC can form in the absence of detectable recombination events. Moreover,

classical cytogenetic studies argue that in *Drosophila* oocytes it is synapsis that is required to initiate recombination, and not vice versa. For example, in *D. melanogaster*, *Caenorhabditis elegans*, maize and a variety of other organisms, heterozygosity for a translocation breakpoint substantially reduces crossing over for large regions surrounding that breakpoint (*19*). In yeast, however, similar rearrangements have little or no effect on the frequency of meiotic recombination in the vicinity of the breakpoint (*20*). Similarly, although small regions of ectopic DNA undergo recombination with their normally located homologous regions in yeast (*7*), the same is not true in *Drosophila* (*21*). Even large translocated regions only very rarely recombine or synapse (or both) with homologous intervals in *Drosophila* (*22*), worms (*23*), and mice (*24*).

We interpret these data to mean that large-scale synapsis is required to initiate meiotic recombination in *Drosophila*. Meiotic chromosome synapsis in yeast, which appears to require only a homology search done at a "gene-by-gene" level (*25*), may be representative of organisms with relatively small and noncomplex genomes. Indeed, such a strategy might well be disastrous in organisms with large amounts of dispersed repetitive DNA or with large dispersed gene families. We imagine that in higher organisms synapsis is required before the initiation of exchange both to prevent recombination events between homologous DNA sequences on nonhomologous chromosomes and to facilitate the nonrandom positioning of exchanges along the arms of meiotic chromosomes.

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8. The isolation and initial characterization of the second chromosome recessive mutation, *mei-W68*, was performed by B. S. Baker (*26*). The allele studied here is the more severe of the two existing alleles. The third chromosomal recessive mutant, *mei-P22*, was isolated in a large scale P-element mutagenesis screen for mutants with a high frequency of X-chromosome nondisjunction in the female germline (*27*). Subsequent analysis has shown that this mutant is caused by the insertion of a P-element (*28*).
9. Because of the low frequency of meiotic recombination in small intervals, gene conversion events in *D. melanogaster* are rare. The *rosy* system facilitates the isolation of intragenic recombinants, including gene conversions, because of a selection system for wild-type recombinants (*29*). Females with the genotypes shown in Table 1 were crossed to either *TM2, ry/MKRS, ry kar* males as virgins, or as nonvirgins to their phenotypically *rosy* brothers. These crosses were done in bottles with 30 females and 30 males for the controls, or 50 females and 50 males for the meiotic mutants. The flies were transferred to new bottles every 3 days at which time 0.75 ml of 0.2% purine (Sigma) was added to the media. A total of five broods were set. To estimate the total number of progeny scored in each experiment, all the progeny were scored from the ~5% of the bottles that were not given the purine treatment.
10. An exact estimate of the frequency of meiotic exchange in *mei-W68* and *mei-P22* mutants was complicated by the presence of premeiotic-exchange events. This problem is illustrated by an example with *mei-P22*. In our analysis of X-chromosome crossing over in females homozygous for *mei-P22*, 25 crossovers were observed among 1935 regular progeny. Twenty-three of the crossovers occurred in clusters (one cluster of 18 identical recombinants and one cluster of 5 identical recombinants). Similar results were obtained on the left arm of chromosome *2* and in two sets of data regarding the effect of the *mei-W68* mutation on second chromosome crossing over. Those recombinants arising in clusters can be ascribed to premeiotic (that is mitotic) recombination events, and thus the frequency of meiotic exchange in *mei*-P22 and *mei-W68* females is either zero or very close to zero. In support of this conclusion, in males, which are normally achiasmatic, the *mei-W68* mutation caused an increase in the spontaneous crossover frequency to an amount similar to that in *mei-W68* females (*30*). Although the clusters of recombinants from *mei-P22* females were larger than from *mei-W68*, the similarity of the *mei-P22* and *mei-W68* meiotic phenotypes (effects on gene conversion, and reductions in crossing over combined with increases in clustered events and their effects on SC formation) suggests that any differences between *mei-P22* and *mei-W68* in the production of crossover progeny are probably the result of differences in their effects on mitotic recombination.
11. Of the nine *mei-W68* nuclei that were in the right developmental stage to have late RNs (between the onset of organelle passage through the ring canals and overt oocyte determination), no RNs were observed (*16*), despite the fact that with an average number of late nodules of 3.37 per nucleus (*18*), about 30 were expected. Similarly, no RNs were observed in three *mei-P22* oocytes examined (*16*), although 10 were expected. No early RNs were observed in the four *mei-W68* nuclei at the stage expected to display early RNs (that is, just before and just after the onset of organellar passage), when 11 would have been expected. The absence of RNs in *mei-W68* and *mei-P22* oocytes is concordant with the null-recombination phenotype of these mutants and is consistent with the hypothesis that neither mutant initiates recombination events.
12. In this experiment ring loss was measured by crossing $R(1)w^{vc}/FM7$ females to $y\ w/y^{+}Y$ males, where $R(1)w^{vc}$ is a ring X chromosome, *FM7* is a multiply inverted X chromosome balancer chromosome that strongly suppresses X-chromosomal exchange, and $y^{+}Y$ is a marked Y chromosome. Ring recovery was assayed by comparing the frequency of $R(1)w^{vc}/y\ w$ female progeny with that of the corresponding *FM7/y w* sisters. In the case of control females the Ring/Rod(*FM7*) ratio was 0.854 (n = 2731), whereas in *mei-P22* females the Ring/Rod ratio was increased to 1.079 (n = 659). Thus, rather than reducing recovery of the ring chromosome, as might be expected if *mei-P22* oocytes exhibited an increased frequency of sister-chromatid exchange, the *mei-P22* mutation appears to increase the frequency of ring recovery. A very similar set of results was obtained with a second null-recombination mutant $c(3)G^{17}$ in a similar experiment. Using a different ring-X chromosome [*R(1)2*], Hall observed a Ring/Rod ratio of 0.755 (n = 7552) in controls and an elevated ratio of 0.894 (n = 5355) in *c(3)G* females (*31*). One reasonable explanation for these observations is that the reduced ring recovery observed in the two control experiments reflects the background frequency of meiotic sister-chromatid exchange, and that *mei-P22* and *c(3)G* actually inhibit these sister-chromatid exchange events, as they do interhomolog events, and in doing so increase the transmissibility of the ring-X chromosome.
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15. Among a total of 709 progeny from *mei-P22* homozygous females, 11.6% were nullo-X exceptions and 18.3% diplo-X exceptions. Among a total of 1024 progeny from *mei-W68* homozygous females, 20.7% were nullo-X exceptions and 18.0% diplo-X exceptions.
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33. Ovaries from females homozygous for *mei-P22* were removed in a 0.7% NaCl solution as described by Carpenter (*18*) with minor modifications; 4% glutaraldehyde in cacodylate buffer (pH 7.0) was used instead of Karnovsky's fixative, and instead of storing overnight in buffer, the ovaries were rinsed and immediately placed in 2% osmium tetroxide in cacodylate buffer (pH 7.0) for 1.5 hours. Ovaries were thoroughly rinsed with distilled H_2O, dehydrated with ethanol to 70%, and en bloc stained with 2% uranyl acetate dissolved in 70% ethanol overnight. Ovaries were embedded in Spurr's resin instead of Epon-Araldite. Whole ovaries were flat-embedded with the ovarioles positioned so that the long axis of the germarium was parallel to the cutting plane. The germarium end of the block was serial sectioned with a thickness of 100 nm per section. The sections were collected on formvar, carbon-coated copper single-slot grids (1 mm by 2 mm). The sections were then poststained with uranyl acetate (in 50% ethanol) for 20 min and concentrated lead citrate for 30 s, with thorough rinsing after each staining. The sections were examined by electron microscopy (Philips EM-410) and photographed at low magnification. When the cells with four ring canals were located, they were photographed at a magnification of 14,000.

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