Saccharomyces cerevisiae Checkpoint Genes MEC1, RAD17 and RAD24 Are Required for Normal Meiotic Recombination Partner Choice

Jeremy M. Grushcow,* Teresa M. Holzen,* Ken J. Park,* Ted Weinert,¹ Michael Lichten³ and Douglas K. Bishop*

*Departments of Radiation and Cellular Oncology and Molecular Genetics and Cell Biology, University of Chicago, Chicago, Illinois 60637, ¹Department of Molecular and Cellular Biology, University of Arizona, Tucson, Arizona 85721 and ³Laboratory of Biochemistry, Division of Cancer Biology, Diagnosis and Centers, National Cancer Institute, Bethesda, Maryland 20892

Manuscript received December 10, 1998
Accepted for publication June 8, 1999

ABSTRACT

Checkpoint gene function prevents meiotic progression when recombination is blocked by mutations in the recA homologue DM C1. Bypass of dmc1 arrest by mutation of the DNA damage checkpoint genes MEC1, RAD17, or RAD24 results in a dramatic loss of spore viability, suggesting that these genes play an important role in monitoring the progression of recombination. We show here that the role of mitotic checkpoint genes in meiosis is not limited to maintaining arrest in abnormal meioses; mec1-1, rad24, and rad17 single mutants have additional meiotic defects. All three mutants display Zip1 polycomplexes in two-to threefold more nuclei than observed in wild-type controls, suggesting that symmetry may be aberrant. Additionally, all three mutants exhibit elevated levels of ectopic recombination in a novel physical assay. rad17 mutants also alter the fraction of recombination events that are accompanied by an exchange of flanking markers. Crossovers are associated with up to 90% of recombination events for one pair of alleles in rad17, as compared with 65% in wild type. Meiotic progression is not required to allow ectopic recombination in rad17 mutants, as it still occurs at elevated levels in ndt80 mutants that arrest in prophase regardless of checkpoint signaling. These observations support the suggestion that MEC1, RAD17, and RAD24, in addition to their proposed monitoring function, act to promote normal meiotic recombination.

Meiosis involves a change in the pattern of chromosome segregation from that normally seen in mitosis. The first meiotic division is a reductional division, in which homologous chromosome pairs are separated from each other. Sister chromatids remain together until the second, equational division. Recombination is required in most organisms to create the physical connections between homologues that allow development of appropriate spindle tension during reduced segregation. In Saccharomyces cerevisiae, ~300 double-strand breaks (DSBs) are formed in prophase and serve to initiate the requisite homologous recombination. Repair of these breaks requires the recombination machinery to distinguish between three “donor” templates: one copy on the sister chromatid of the initiating strand and two copies on the homologous chromosome. Several meiosis-specific genes act to ensure that recombinational repair uses allelic sequences on homologues as preferred donors during meiosis (reviewed by Pet es and Pukkila 1995; Kleckner 1996; Roeder 1997). The choice of donor can be further complicated by the existence of duplicated copies of a sequence that may be dispersed through the genome. Meiotic recombination between homologous sequences at dispersed loci, termed “ectopic” recombination, occurs with frequencies that can be nearly as high as allelic recombination (Jinks-Robertson and Petes 1985; Lichten et al. 1987; Goldman and Lichten 1996).

One key component of the meiotic machinery is Dmc1, which is a homologue of the bacterial strand exchange protein RecA (Bishop et al. 1992). Dmc1 is required for strand exchange during meiotic recombination, as evidenced by the fact that dmc1 mutants arrest in prophase with hyperresected, unrepaired DSBs (Bishop et al. 1992). Arrest caused by dmc1 mutations can be avoided by upstream mutations that block normal initiation of recombination (Figure 1). For example, mutations in genes required for meiotic DSB formation eliminate the requirement for Dmc1 for execution of meiotic divisions (Bishop et al. 1992, 1999). One such gene is SP011, which encodes the transesterase that forms DSBs at the initiation of recombination (Kee ney et al. 1997). spo11 dmc1 mutant cells progress through both meiotic divisions, but the resulting spores are all inviable due to failed reduced segregation.

Mutations in RED1 create a second type of block to normal meiotic recombination (Schwacha and Kleckner 1997; Xu et al. 1997). Red1 is a meiosis-specific component of chromatin that is required for formation of the synaptonemal complex (SC), a proteinaceous structure that forms between homologous chromosome
pairs during prophase (Smith and Roeder 1997). In rad1 mutants, DSB formation is reduced (Mao-Draayer et al. 1996; Xu et al. 1997), and this reduction appears to correspond to a reduction in interhomologue recombination (Rockmill and Roeder 1990; Mao-Draayer et al. 1996; Schwacha and Kleckner 1997; Xu et al. 1997). Furthermore, the residual meiotic recombination events are abnormal: they do not require DMC1, and they exhibit reduced levels of interhomologue recombination intermediates but normal levels of corresponding intersister intermediates (Schwacha and Kleckner 1997). The residual crossover recombination that occurs in the rad1 mutant does not promote proper reductional segregation in meiosis I (Rockmill and Roeder 1990). Mutation of rad1 allows meiotic progression to occur in a dmc1 mutant but, as with spo11, the spores produced are inviable. It has been proposed that the rad1 mutation specifically eliminates a "highly-specified interhomologue-only" recombination pathway and that only this special pathway is monitored by cell-cycle regulators during meiosis (Schwacha and Kleckner 1997; Xu et al. 1997).

In mitosis, broken chromosomes are monitored by "checkpoint" genes such as RAD17, RAD24, MEC1, and RAD9 that prevent cells from attempting to segregate damaged DNA (Weinert and Hartwell 1988). Dmc1 arrest in meiosis is similarly monitored: at least three of the same checkpoint genes (RAD17, RAD24, and MEC1) are required to maintain dmc1 arrest (Lydall et al. 1996). In dmc1 checkpoint double mutants, arrest is bypassed and meiosis progresses, but breaks remain unrepaired and <1% of spores are viable (Lydall et al. 1996). These properties of Rad17, Rad24, and Mec1 function in meiosis fit with the classical view of G2/M checkpoint activity (Hartwell and Weinert 1989). In meiosis, as in mitosis, the checkpoint seems to act to prevent cell division until recombination is complete and the genome is intact. One interesting difference between the meiotic and the mitotic checkpoint is that RAD9 is required only for the latter; dmc1 rad9 double mutants remain arrested in meiotic prophase (Lydall et al. 1996).

The classical definition of checkpoint function included the stipulation that checkpoint single mutants should have no phenotype when the process they monitored was unperturbed (Hartwell and Weinert 1989). For example, the original checkpoint mutant rad9 shows normal mitotic viability and growth in the absence of DNA damage. Contrary to this stipulation is our previous observation of reduced spore viability in rad17, rad24, and mec1-1 single mutants (Lydall et al. 1996). The reduced viability of these mutants could be accounted for by a model in which activation of checkpoint control was a normal feature of meiosis, restraining the first division until recombination was complete. Mutation of RAD17, RAD24, or MEC1 does appear to allow progression from prophase to metaphase I (MI) in the presence of a small amount of ongoing recombination (Lydall et al. 1996), indicating that the checkpoint proteins normally act to enforce a dependency relationship between completion of recombination and the prophase-metaphase transition (Figure 1).

However, a second possibility for the reduced meiotic viability of checkpoint mutants is that Rad17, Rad24, and Mec1 are required to effect normal prophase processes (Lydall et al. 1996). Some previous data using genetic assays have suggested that checkpoint proteins may participate in recombination and DNA repair. For example, immortalized human ataxia telangiectasia (AT) cell lines, which carry mutations in the MEC1 homologue ATM, show increased nonallelic recombination during mitotic growth (Meyn 1993). In Drosophila melanogaster, the frequency of meiotic recombination is reduced in mutants in the ATM homologue mei-41 (Carpenter 1979). Third, the original characterization of mei1/esr1 showed reduced levels of recombination when meiotic cells were returned to mitotic growth (Kato and Ogawa 1994). Fourth, Rad17 and Rad24 act in complex ways to influence the choice of pathway used to circumvent irreparable DNA damage during mitotic Sphase (Paulovich et al. 1998). However, because these assays all depend on continued growth to detect genetic recombinants, it has been difficult to determine whether the recombination defects are a primary effect of the checkpoint mutations or whether they are secondary effects of unregulated cell-cycle progression.

Here we report physical evidence that checkpoint gene functions are required for normal meiotic recombination. An ectopic recombination event that initiates at the HIS4::LEU2 hotspot is characterized and shown to occur with increased frequency in rad17, rad24, and mec1-1 mutants. Homologous recombination is somewhat reduced in these mutants. In addition, checkpoint mutants display increased frequencies of Zip1 polycistron formation, suggesting that aberrant synapsis may be related to the recombination defect. Importantly, we show that the defect in recombination is not an indirect consequence of inappropriate progression from prophase to MI in the checkpoint mutants, but instead...
defines a novel function for checkpoint proteins during meiotic prophase.

MATERIALS AND METHODS

Strain construction and plasmids: Transformations: All transformations were performed according to the LiAc TRAF0 Method (Geitz and Schiestl 1995).

Check mutant strains: rad24 strains were derived from transformations of diploid DKB1265 with the rad24::TRP1 plasmid pDL225 (Lydall and Weinert 1997), followed by sporulation and tetrad dissection of transformants. rad17 strains were similarly derived from DKB1265 using the rad17::LEU2 plasmid pDL183 (Lydall and Weinert 1997). mcl-1 smX strains were previously described (Lydall and Weinert 1996). ndt80 strains were constructed by transforming DKB973 with a PCR-amplified KAN-MX4 cassette (Wach et al. 1994) that had 40 nucleotides (nt) of terminal homology to NDT80 flanking sequences. Geneticin-resistant clones were screened by PCR to confirm the correct structure of ndt80::KAN-MX4 alleles (Table 1).

Strains for genetic determination of ectopic recombination efficiency: SK1-derived haploid strains containing the arg4-nsp or arg4-bgl heteroallelic inserts at put2 have been described previously (Goldman and Lichten 1996). px1::arg4 strains were a generous gift from A. Goldman. rad17 strains were generated by transforming these strains with a rad17::LEU2 plasmid pDL183 (Lydall and Weinert 1997). The RAd17 and rad17 haploids for each pair of inserts were then mated with one another to form diploids with arg4 heteroalleles in allelic or ectopic configuration (Table 1).

Measurement of efficiency of ectopic recombination: Preparation, plating, and counting of Arg R strains to determine the frequency of recombination have been described (Goldman and Lichten 1996). The percentage of Arg R recombinants was the mean observed recombination frequencies from at least three independent trials for each diploid strain.

Ectopic recombination efficiencies were adjusted to account for ectopic recombination events that produce inviable spores. At least 200 Arg R colonies were picked from each ectopic allele configuration. DNA from these colonies was separated by pulsed-field gel electrophoresis (PFGE), and Southern blotted using a probe to the chromosome VIII subtelomeric region (Goldman and Lichten 1996). The observed efficiencies of recombination for wild type and rad17 were normalized to account for lethal crossover-associated events and compared using the following formula (Goldman and Lichten 1996), where R is the frequency of crossing over and E is the efficiency of ectopic recombination:

\[
\frac{(1 - 0.5R_{wt}) \cdot E_{rad17}}{(1 - 0.5R_{rad17}) \cdot E_{wt}}.
\]

The efficiency of ectopic recombination (E) between two loci is the ratio of the total ectopic recombination frequency to the total allelic recombination frequency for two given loci (Equation 6 in Goldman and Lichten 1996).

Cytological analysis: Spread meiotic nuclei were prepared at 0, 3, 5, and 7 hr after the start of synchronous meiosis as described (Klein et al. 1992). Immunostaining of spread nuclei oids was as described in Bishop (1994), using Rabbit anti-Zip1 (Sym and Roeder 1995; gift of S. Roeder) at 1:1000 dilution, followed by secondary staining with either FITC-conjugated goat anti-rabbit IgG (1 μg/ml) or Alexa-488-conjugated goat anti-rabbit IgG (1 μg/ml), both from Molecular Probes (Eugene, OR). Stained nuclei oids were examined using an epifluorescence microscope as described previously (Bishop 1994). Pictures of 50 unselected nuclei were taken from each strain at each time point and scored for the presence of Zip1 foci.

Southern blotting analysis: Preparation of DNA for meiotic time course analysis of reciprocal and ectopic recombination events was as described by Cao et al. (1990), except that purification of the DNA on Sephadex was replaced by ethanol precipitation in Bishop et al. (1992). For assaying ectopic recombination, 5-20% of the DNA from each time point was digested in 30 μl final reaction volume using 60 units of PstI (New England Biolabs, Beverly, MA) overnight at 37°. Digests were run at 55 V for 20 hr in 0.8% agarose in Tris acetate buffer, transferred to SS/Nytran Plus nylon membranes (Schleicher & Schuell, Keene, NH), and cross linked with 1200 J/m2 UV light. Sequences were probed with the 67 nt fragment ChrIII 92973-93649. Homologous recombination was assayed as previously described (Cao et al. 1990). Blots were quantitated on a Molecular Dynamics (Sunnyvale, CA) STORM 860 Phosphorimager, using ImageQuant software (Molecular Dynamics).

RESULTS

Analysis of checkpoint mutants using a physical method to detect ectopic crossover recombination products: A putative ectopic recombination product was fortuitously observed during Southern blotting for DSB fragments at the HIS4::LEU2 hotspot in rad17, rad24, and mec1-1 single mutants. This product was also present in rad17 mec1, rad24 mec1, and mec1-1 mec1 double mutants (Lydall et al. 1996). To identify the product, PstI digests were designed (Figure 2A) using a map of the region obtained by analysis of DNA from the SK1 strain background, which lacks the Ty-2 transposable element S of LEU2 that is present in the Stanford Saccharomyces Genome Database sequence (http://genome-www. stanford.edu/ Saccharomyces; Baudat and Nicolas 1997). These digests were probed with unique sequences adjacent to the endogenous LEU2 allele, which confirmed that the product was an ectopic recombinant that contained sequences from the two dispersed copies of LEU2 (Figure 2A). The HsI digest was used to further confirm the identity of the ectopic recombinants (data not shown). These digests revealed that the ectopic recombination event occurred between the hotspot HIS4::LEU2 and the LEU2 locus, which, in the strains examined, harbors the disruption allele las2::HisG. The LEU2 locus is located 19 kb centromere-proximal and in inverted orientation relative to the HIS4::LEU2 hotspot on chromosome III (Figure 2B). In rad17, rad24, and mec1-1 single mutants, this ectopic recombination event occurs at frequencies two- to fivefold above those seen in wild type (Figure 2C), indicating that the checkpoint proteins have a role in ensuring allelic recombination partner choice. Ectopic recombination also occurred in rad17 mec1, rad24 mec1, and mec1-1 mec1 mutants five- to sevenfold more frequently than in wild-type mei6/6 (Figure 2C). In a wild-type background, the EC1 and EC2 products occur with approximately equal frequency. However, in a mec1 background, the primary crossover product in this ectopic recombination
<table>
<thead>
<tr>
<th>DKB no.</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>974</td>
<td>lys2 ho:LYS2 ura3 leu2::hisG arg4-nsp his4B::LEU2</td>
</tr>
<tr>
<td>977</td>
<td>lys2 ho:LYS2 ura3 leu2::hisG arg4-bgl his4X::LEU2-URA3</td>
</tr>
<tr>
<td></td>
<td>lys2 ho:LYS2 ura3 leu2::hisG arg4-bgl his4B::LEU2 dmclΔ::ARG4</td>
</tr>
<tr>
<td>835</td>
<td>lys2 ho:LYS2 ura3 leu2::hisG arg4-nsp his4B::LEU2 medI sml-X</td>
</tr>
<tr>
<td>839</td>
<td>lys2 ho:LYS2 ura3 leu2::hisG arg4-bgl his4X::LEU2-URA3 medI sml-X</td>
</tr>
<tr>
<td>1361</td>
<td>lys2 ho:LYS2 ura3 leu2::hisG arg4 TRP1 his4B::LEU2 rad17::LEU2</td>
</tr>
<tr>
<td>1367</td>
<td>lys2 ho:LYS2 ura3 leu2::hisG arg4 TRP1 his4X::LEU2-URA3 rad17::LEU2</td>
</tr>
<tr>
<td>1435</td>
<td>lys2 ho:LYS2 ura3 leu2::hisG arg4 trp1 his4B::LEU2 rad24·TRP1</td>
</tr>
<tr>
<td>1439</td>
<td>lys2 ho:LYS2 ura3 leu2::hisG arg4 trp1 his4X::LEU2-URA3 rad24·TRP1</td>
</tr>
<tr>
<td>1557</td>
<td>lys2 ho:LYS2 ura3 leu2::hisG arg4 trp1 his4B::LEU2</td>
</tr>
<tr>
<td>1478</td>
<td>lys2 ho:LYS2 ura3 leu2::hisG arg4 trp1 his4B::LEU2 ndt80Δ::KAN-MX4</td>
</tr>
<tr>
<td>1482</td>
<td>lys2 ho:LYS2 ura3 leu2::hisG arg4 trp1 his4X::LEU2-URA3 rad17::LEU2</td>
</tr>
<tr>
<td>1486</td>
<td>lys2 ho:LYS2 ura3 leu2::hisG arg4 trp1 his4B::LEU2 ndt80Δ::KAN-MX4 dmclΔ::ARG4</td>
</tr>
<tr>
<td>1490</td>
<td>lys2 ho:LYS2 ura3 leu2::hisG arg4 trp1 his4B::LEU2 dmclΔ::ARG4 rad17::LEU2</td>
</tr>
<tr>
<td>1494</td>
<td>lys2 ho:LYS2 ura3 leu2::hisG arg4 trp1 his4X::LEU2-URA3 rad17::LEU2</td>
</tr>
<tr>
<td>1498</td>
<td>lys2 ho:LYS2 ura3 leu2::hisG arg4 trp1 his4B::LEU2 ndt80Δ::KAN-MX4 dmclΔ::ARG4 rad17::LEU2</td>
</tr>
<tr>
<td>1390</td>
<td>lys2 ho:LYS2 ura3 leu2::hisG arg4-nsp leu2·K put2·URA3-[arg4-nsp]</td>
</tr>
<tr>
<td>1391</td>
<td>lys2 ho:LYS2 ura3 leu2::hisG arg4-bgl leu2-R put2·URA3-[arg4-bgl]</td>
</tr>
<tr>
<td>1393</td>
<td>lys2 ho:LYS2 ura3 leu2::hisG arg4-bgl leu2·K put2·URA3-[arg4-nsp]</td>
</tr>
<tr>
<td>1394</td>
<td>lys2 ho:LYS2 ura3 leu2::hisG arg4-bgl leu2·K pp1·URA3-[arg4-nsp]</td>
</tr>
<tr>
<td>1399</td>
<td>lys2 ho:LYS2 ura3 leu2::hisG arg4-nsp leu2·K put2·URA3-[arg4-nsp] rad17::LEU2</td>
</tr>
<tr>
<td>1400</td>
<td>lys2 ho:LYS2 ura3 leu2::hisG arg4-bgl leu2-R put2·URA3-[arg4-bgl] rad17::LEU2</td>
</tr>
<tr>
<td>1402</td>
<td>lys2 ho:LYS2 ura3 leu2::hisG arg4-nsp leu2·K pp1·URA3-[arg4-nsp] rad17::LEU2</td>
</tr>
<tr>
<td>1403</td>
<td>lys2 ho:LYS2 ura3 leu2::hisG arg4-bgl leu2-R pp1·URA3-[arg4-bgl] rad17::LEU2</td>
</tr>
</tbody>
</table>

**TABLE 1**

*Yeast strains*
event switches to EC1 (Figure 2, A and B). The EC1 product results from crossing over probe-proximal to the hisG insertion, whereas the EC2 product results from crossing over probe-distal to hisG (Figure 2A). The nature of these products was confirmed by probing a PstI blot with hisG sequences, which hybridized with only EC2 and the parental band (data not shown). The reason for the change in EC1/EC2 ratio in the ectopic recombination products in dmc1 background is not understood but is likely to be a consequence of altered strand exchange complex function caused by the absence of Dmc1 (Bishop 1994; Shinohara et al. 1997).

The increase in ectopic recombination seen in rad17, rad24, and mec1-1 mutants could be accounted for either by an overall increase in meiotic recombination, by a redirection of events that would normally be allelic, or by redirection of events (such as sister-chromatid exchange) that would normally not be detected. The HIS4::LEU2 hotspot was originally designed for measuring interhomologue allelic recombination (Cao et al. 1993).
1990; Figure 3A). By performing both assays on the same DNA samples, it was possible to compare allelic and ectopic events at the same locus. No increase in allelic interhomologue recombination was observed by this assay (Figure 3B). In fact, the checkpoint single mutants show an approximately twofold decrease in interhomologue recombinants (Figure 3C). Therefore, the increased ectopic recombination in rad17, rad24, and mec1-1 single mutants does not result from an overall increase in the level of recombination at the H154 locus.

Analysis of the levels of interhomologue and ectopic recombination products indicates that DMC1 and the checkpoint genes make independent contributions to interhomologue partner choice during normal meiosis. The ratio of allelic interhomologue recombination to ectopic recombination can be used as a measure of interhomologue recombination partner choice “fidelity.” In an otherwise wild-type strain background, a checkpoint mutant reduces interhomologue recombination at HIS4 2-fold while increasing ectopic recombination roughly 2.5-fold, thereby decreasing the partner choice fidelity index 5-fold. In a dmc1 single-mutant background, interhomologue recombination events are reduced 5-fold (Bishop et al. 1992; Shinohara et al. 1997; data not shown) while ectopic recombination events are reduced only 1.7-fold, amounting to a 3-fold reduction in partner choice fidelity. Combining a checkpoint mutation with a dmc1 mutation increases ectopic recombination 7-fold but does not affect the level of interhomologue recombination relative to that observed in a dmc1 single mutant. Thus partner choice fidelity is reduced 21-fold in dmc1 checkpoint double mutants compared to wild type. This multiplicative effect of combining the two mutations suggests that the corresponding genes make distinct and possibly independent contributions to partner choice fidelity.

**Genetic detection of ectopic recombinants:** To confirm and extend our observations at the HIS4::LEU2 hotspot, we utilized a genetic assay for ectopic recombination (Goldman and Lichten 1996) to compare the efficiency of ectopic recombination between wild-type and rad17 strains (Figure 4). This assay differs from the physical assay in several respects. First, the configuration of recombinogenic sequences is different. In the case of the genetic assay, each of the two interacting ARG4 alleles is present in single copy and the two heteroalleles are present on homologous chromosomes (the position of the alleles relative to one another is varied). Only when recombination occurs between the two homologous chromatids is a detectable product generated (sister-chromatid and intrachromatid interactions are not detected). In the physical assay, the alleles that interact are present at two locations: one allelic and one ectopic. Recombination products are detected when recombination occurs between one copy of LEU2 at the normal locus and a second copy of LEU2 at the ectopic site. Recombination products are detected regardless of whether sisters or homologues contributed the two recombination partners. The genetic assay also differs from the physical assay in that it depends on the viability of the resulting meiotic products. It is therefore necessary to normalize the raw frequency of observed Arg+ spores to account for those spores that have undergone recombination associated with a lethal deletion of sequences between the Arg heteroalleles. Viable duplications and lethal deletions of the region between the Arg heteroalleles should occur at equal levels. The duplica-
tions and deletions associated with the Arg\(^{+}\) recombinants could arise via a reciprocal mechanism such as crossing over, which covalently joins the initiating chromatid to the donor chromatid, or via a nonreciprocal mechanism such as break-induced replication (BIR), which initiates DNA replication at the site of invasion and copies all distal sequences to the initiating chromatid, leaving the donor chromatid intact. In either case, the Arg\(^{+}\) chromatid that results from the duplication events can be observed by PFGE and quantitated by Southern blotting as a fraction of the Arginine prototrophs (Goldman and Lichten 1996).

We constructed isogenic wild-type and rad17 diploids with arg4 heteroalleles inserted on chromosome VIII at PUT2 or PPX1 in allelic or ectopic configuration (Figure 4A). The frequency of recombination was measured for Arg heteroalleles in allelic and ectopic configurations as the fraction of Arg\(^{+}\) prototrophs among viable spores (Figure 4B). These figures were normalized to account for the lethal deletion events discussed above. The frequency of exchange resulting in duplication of the region between PUT2 and PPX1 was increased in the rad17 mutants. In fact, up to 90% of the Arg\(^{+}\) spores in rad17 mutants were associated with crossovers as compared to 65% in wild-type cells (Figure 4C). Using these values, we calculated that the efficiency of ectopic recombination by this assay is 2.7-fold higher in rad17 strains than in their isogenic wild-type controls (Figure 4D).

Because the physical assay measured only ectopic crossing over, we considered the possibility that the increase in ectopic recombination observed in that assay could be a secondary effect of increased crossing over rather than a primary effect on partner choice. However, while the high level of duplications in rad17 mutants is dramatic, it is only 1.3-fold higher than in wild-type cells. This increase is not sufficient to account for the 3- to 5-fold increase in ectopic crossovers observed in rad17 mutants by the physical assay. Therefore, mutations in rad17 seem to affect both partner choice and the ratio of crossing over genome-wide.

**Separation of Rad17 functions:** Because checkpoints are classically believed to function by allowing inappropriate cell-cycle progression, we attempted to determine if the recombination defects we observed were a secondary effect of allowing cell-cycle progression from prophase to MI before recombination is complete, or if Rad17, Rad24, and Mec1 proteins might exert their effects on recombination independently of exit from prophase. To distinguish between these possibilities, exit from prophase was blocked by combining the rad17 mutation with mutations in NDT80, a meiosis-specific gene required for exit from prophase (Xu et al. 1995). Ndt80 is a transcription factor required for expression of middle sporulation genes (Chu and Herskowitz 1998; Chu et al. 1998; Clancy 1998; Hepworth et al. 1998), including the B-type cyclins clb\(1\), 3, 4, and 5 that promote the first meiotic division (Epstein and Cross 1992; Grandin and Reed 1993; Dahmann and Futcher 1995). Although Ndt80 is produced in dmc1 mutants, Rad17 prevents Ndt80 from functioning, and the requi-
site cyclins are not transcribed. In a dmc1 rad17 mutant, the checkpoint is inactive, and Ndt80 promotes meiosis I (Chu and Herskowitz 1998; Hepworth et al. 1998).

Based on these results, we reasoned that the ndt80 mutations would be epistatic to rad17 mutations with respect to meiotic progression. The dmc1 rad17 ndt80 mutant was constructed and confirmed this prediction—the triple mutant did not progress through meiotic divisions nor did a rad17 ndt80 double mutant (Figure 5A).

Ectopic recombination was assayed in the rad17 ndt80 double mutant and in the dmc1 rad17 ndt80 triple mutant to determine whether progression into metaphase was required for formation of ectopic recombination products (Figure 5B). In both cases, ectopic recombination occurred at similar frequencies as in the NDT80 background (Figure 5C). Ectopic recombination accumulated to similar levels in rad17 ndt80 mutants as in rad17 mutants, although there was a delay in accumulation in the double mutant. Similarly, ectopic recombinants accumulated to the same level in the dmc1 rad17 ndt80 triple mutant as in the dmc1 rad17 double mutant. This high level of ectopic recombination observed in rad17 ndt80 and dmc1 rad17 ndt80 strains is not due to an independent effect of ndt80 on recombination, because neither ndt80 single mutants nor dmc1 ndt80 double mutants show an increase in ectopic recombination over their NDT80 counterparts. Furthermore, ndt80 mutants do not exhibit increased allelic crossing over (Xu et al. 1995), so a general hyperrecombinant phenotype also cannot account for the elevated ectopic recombination in the ndt80-arrested strains. These results indicate that progression through meiosis I is not required for the high level of ectopic recombination observed in the checkpoint mutants.

Zip1 defects in checkpoint mutants suggest that synapsis may be aberrant: Components of the synaptonemal complex are known to play a role in establishing the correct structural context that ensures proper interhomolog recombination (Engebrecht et al. 1990; Rockmill and Roeder 1990; Sym and Roeder 1995; Storlazzi et al. 1996; Schwacha and Kleckner 1997). We therefore attempted to determine whether rad17, rad24, or mec1-1 mutants might display a synaptic defect. Immunolocalization of Zip1, an SC component, was performed on spread meiotic nuclei as an indication of whether synapsis occurred normally in rad17, rad24, and mec1-1 mutants. In wild-type cells, Zip1 shows a punctate...
pattern at its first appearance at \( \sim 3 \) hr, progresses to extended structures peaking at \( \sim 5 \) hr, and then disappears before the cells undergo the first meiotic division. Disruption of the SC can result in the appearance of large structures called “polycomplexes,” which stain brightly with anti-Zip1 antibody (Sym and Roeder 1995). Polycomplexes appear in addition to or instead of the extended structures typical of normal meiosis (Figure 6A), especially in mutants with defects in synapsis (Sym and Roeder 1995).

Spread nuclei of wild-type as well as rad17, rad24, and mec1-1 cells undergoing synchronous meiosis were scored for the presence of Zip1 polycomplex structures. Most often, polycomplexes appeared as very bright oval-shaped structures toward one side of the spread nuclei. However, their appearance was somewhat heterogeneous, varying from round to more extended structures in all mutants (Figure 6A). Polycomplexes were therefore defined quantitatively as structures that stained at least twice as brightly as any other structure in the nucleus. This allowed objective scoring of their presence. Polycomplexes appeared in \( \sim 75\% \) of nuclei from rad17, rad24, and mec1-1 mutants, and appeared in \(< 25\% \) of nuclei from wild-type cells (Figure 6B). As was seen previously, polycomplexes also appeared at high frequency in nuclei from dmc1 cells (Bishop et al. 1992), but no additive effect on the frequency polycomplex formation was observed in rad17 dmc1, rad24 dmc1, or mec1 dmc1 mutants (data not shown).

**DISCUSSION**

Partner choice defects in rad17, rad24, and mec1-1 single mutants suggest a role in controlling recombination pathways: We characterized an ectopic crossover recombination product that forms between the hotspot construct at HIS4::LEU2 and the endogenous LEU2 locus. The ability to detect ectopic recombination by a physical assay provides a novel and direct measure of aberrant recombination partner choice. Using this assay, it was observed that levels of ectopic recombination are increased from 1% in wild type to 3–5% in rad17, rad24, or mec1-1 single mutants. Interhomologue recombination at HIS4 is also reduced approximately twofold in these mutants, from 25 to 30% in wild type to 15% in rad17, rad24, and mec1-1. These numbers indicate that the increase in ectopic recombination does not quantitatively account for the decrease in interhomologue allelic recombination. Because the level of DSB formation is unaffected by mutations in RAD17, RAD24, or MEC1 (Lydall et al. 1996), the fate of most remaining DNA that suffers a DSB at HIS4::LEU2 can be explained by either of two mechanisms. First, some broken DNA may be resolved by allelic sister chromatid recombination. Second, some breaks may be repaired by ectopic gene conversion without associated crossing over. Both of these events would be invisible to the

![Figure 6. Zip1 polycomplex formation in checkpoint mutants.](image)
physical assays used to detect recombinants at HIS4::LEU2.

Recently, alleles of RAD24, RED1, MEC1, and MEC3 were isolated in a screen for mutants with increased unequal sister chromatid exchange (SCE) in meiosis (D. Thompson and F. Stahl, unpublished results). This result could reflect a role for these genes in the normal meiotic preference for interhomologue recombination over intersister recombination. Alternatively, it could reflect a general role in suppressing ectopic interaction that is not specific in terms of homologue vs. sister. Overall, rad17, rad24, and mec1-1 mutations likely result in a general redirection of interhomologue events into pathways that favor ectopic and possibly intersister recombination.

Possible functions of checkpoint proteins in meiotic prophase: There are three possible models for the role of checkpoint proteins in meiotic prophase. First, it is possible that checkpoint proteins act to ensure that recombination is complete before meiosis I division begins in a manner analogous to the monitoring of DNA damage in mitotic G2 and that increased ectopic recombination in checkpoint mutants is a secondary effect of inappropriate progression through M1. Second, it is possible that checkpoint proteins form a structure required to make DSB intermediates "monitorable" in a manner analogous to that proposed for Red1 (Xu et al. 1997). In this case, checkpoint proteins act to form a structure required for both proper recombination partner choice and regulation of meiotic progression. Third, it is possible that checkpoint proteins have separable effects during prophase, one that influences partner choice and a second that regulates progression.

Rad17 function in promoting proper recombination partner choice does not depend on a classical G2/M checkpoint delay: In mitosis, the G2/M checkpoint serves to sense DNA damage and prevent metaphase when damage is present. In rad9 mutants, inappropriate progression in the presence of broken chromosomes decreases intersister recombinational repair, because the sister chromatids are separated from each other by the mitotic division (Fasullo et al. 1998). If RAD17, RAD24, and MEC1 had a similar role in meiosis, the increased ectopic recombination in checkpoint mutants could be a secondary effect of inappropriate progression—premature separation of homologous chromosomes could decrease interhomologue recombination and increase the frequency of intersister and ectopic events. This model is consistent with the previous observation that Rad51 foci, which are associated with ongoing recombination events (Bishop 1994; Shinohara et al. 1997; Gasior et al. 1998), persist in rad17, rad24, and mec1-1 cells even after the M1 spindle is formed (Lydall et al. 1996). It seemed possible that ectopic recombinants could form as these final recombination events attempted to find any available recombination donor sequences during or after M1. If this was the case, then formation of ectopic recombinants would depend on progression into metaphase I.

By blocking progression using ndt80 mutants, it was possible to test this hypothesis directly. Ectopic recombination levels are elevated in checkpoint mutants even in the absence of progression. Therefore, the recombination phenotypes of rad17, rad24, and mec1-1 mutants are not dependent on progression to M1 and are not accounted for by a classical G2/M model of checkpoint function.

The role of Rad17, Rad24, and Mec1 in progression may be similar to the role of Red1 in creating or enforcing upstream constraints: Red1 is physically present on synapsed chromosomes and is thought to be a physical component of the SC (Roeder 1997). Red1 mutants show defects in recombination and synapsis that are thought to be primary effects of losing or altering the SC (Rockmill and Roeder 1990). Mutation of red1 creates an upstream block in a specific recombination pathway termed the "interhomologue" pathway. This pathway accounts for 75% of the total recombination events that occur at the HIS4::LEU2 hot spot. In the absence of Red1 function, DSBs are reduced fourfold with a corresponding loss of interhomologue recombination products. If the prophase checkpoint was specifically sensitized to observe these interhomologue recombinants, then the absence of these recombinants in red1 mutants would account for their ability to bypass dmc1 arrest. The observation that rad17, rad24, and mec1-1 mutants show defects in Zip1 assembly suggested that the ability of checkpoint proteins to bypass dmc1 arrest might be analogous to the role of Red1. Specifically, checkpoint proteins might block recombination before formation of monitorable recombination intermediates, possibly also acting through the SC to establish appropriate constraints on the DSBs. However, Rad17, Rad24, and Mec1 arrest both mitotic and meiotic progression when unrepair'd DSBs are present. It seems likely, therefore, that the role of the checkpoint genes in promoting meiotic arrest involves a DSB-associated structural feature common to both mitotic and meiotic checkpoint control pathways, rather than acting through the SC, which is a structural feature specific to meiosis. The possibility remains that the Zip1 assembly defect we observe and the recombination defects we observe are both divergent consequences of a single role for checkpoint proteins in determining aspects of chromosome structure common to mitosis and meiosis. In other words, despite the common perception that MEC1, RAD17, and RAD24 act in a signaling pathway activated by damage-induced DSBs in mitosis, it remains formally possible that these genes act to promote chromosomal structures needed to make unrepaired DSBs detectable by the signal transducers that control cell-cycle progression (e.g., RAD53, CHK2, PDS1, etc.).

Even if they act in similar ways (or along the same pathway) to control cell-cycle progression, there are im-
important features that distinguish checkpoint mutants from rad1 mutants in terms of their effects on meiotic recombination. First, DSB formation is partially blocked in red1 mutants (Mao-Draayer et al. 1996; Schwacha and Kleckner 1997; Xu et al. 1997), whereas DSBs accumulate to wild-type levels in rad17, rad24, and mec1-1 mutants (Lydall et al. 1996). Second, the residual DSBs that form in red1 dmc1 double mutants are efficiently repaired (Schwacha and Kleckner 1997; Xu et al. 1997; data not shown). In contrast, breaks persist at least until 10 hr in dmc1 checkpoint double mutants (Lydall et al. 1996; data not shown). These differences indicate that red1 and the checkpoint mutants each have unique effects on recombination. Nevertheless, some underlying similarity between checkpoint mutants and rad1 mutants was suggested by the observation that red1 mutants also show elevated levels of ectopic recombination in our physical assay (data not shown; M. Shinohara, unpublished results).

Checkpoint proteins have a role in promoting proper recombination partner choice and repair distinct from their role in progression: Previous work has shown that it is possible to separate checkpoint functions that block inappropriate progression from functions that promote proper repair. For example, mutation of RAD24 can alter the processing of cdc13-induced damage intermediates, even when cell-cycle progression is blocked by a downstream mutation in cdc15 (Lydall and Weiner 1995). Additionally, certain mutations in the Schizosaccharomyces pombe homologue of RAD24 (spRAD17) can continue to show radiation sensitivity despite a normal checkpoint delay (Griffiths et al. 1995), indicating that distinct regions of the protein can mediate different functions. Atm-defective cell lines also display sensitivity to DNA damage that is separable from defects in cell-cycle checkpoints (reviewed by Murane and Schwartz 1993; Jeggo et al. 1998).

Our results do not distinguish between two alternative explanations of Rad17 function. Rad17 may have only one function during prophase, with loss of recombination checkpoint control and loss of normal recombination partner choice being divergent and therefore separable consequences of a single initial event possibly related to some aspect of chromosome or DSB-associated structure. Alternatively, Rad17 may act twice during prophase: once to influence recombination partner choice and a second time as part of a recombination checkpoint control mechanism. Nevertheless, by examining the phenotype of rad17 mutants in wild-type and ndt80 backgrounds, we can rule out the possibility that defective recombination partner choice is merely a secondary effect of inappropriate progression to MI.

Model for the role of checkpoint proteins in controlling recombination partner choice: We propose that Rad17, Rad24, and Mec1 act to create or maintain the normal axial association of recombination events. A dissociation of recombination events from the SC could result in an increase in ectopic recombination, because the SC helps in the pairing of homologues, which in turn promotes allelic over ectopic partner choice in meiotic recombination (A. Goldman and M. Lichten, unpublished results). The dissociation of recombination events from chromosome axes could also account for the increased frequency of crossovers observed in rad17 mutants in the genetic assay for Arg+ recombinants. While 65% of Arg+ recombinants in wild-type cells were associated with duplicated chromatids, up to 90% of Arg+ recombinants in rad17 mutants were associated with this type of exchange.

The fraction of meiotic recombination events that result in exchange of flanking markers in wild-type strains fluctuates around 50% and is therefore usually attributed to random resolution of double Holliday junction (DHJ) intermediates (Szostak et al. 1983; Stahl 1994; Schwacha and Kleckner 1995). It is possible that the high level of duplications in rad17 mutants results from radically biased resolution of the same symmetrical DHJ intermediate. However, it seems much more likely that a large fraction of the ectopic recombination events we observe do not involve DHJs but are formed through an alternate mechanism involving one-ended invasions (Figure 7A). Invasion of only one DSB end could prime DNA synthesis over long distances (Figure 7B), as shown in telomere capture and BIR experiments (see Pâques and Haber 1999), or could promote the formation of half crossovers (Figure 7C).
Either way, dissociation of DSB ends from the SC and from each other could help explain the increase in duplications associated with Arg recombinants in checkpoint mutants. It is even possible that the recruitment of the second end of the DSB to one-ended joint molecules is a separate intermediate step in some allelic meiotic recombination (Figure 7D).

Consistent with our proposal that ectopic recombination is elevated in the checkpoint mutants by loss of axial association is the recent observation that mouse Atm-/- spermatocytes show a dissociation of Rad51 (Barlow et al. 1997, 1998) and Dmc1 (Barlow et al. 1998) foci from chromosomal axes. Atm-/- spermatocytes also exhibit extremely defective synapsis (Keegan et al. 1996; Xu et al. 1996; Barlow et al. 1998). However, interpretation of the meiotic synapsis defect in the single mutant is complicated by the fact that the Atm mutation also causes the induction of apoptosis during meiotic prophase. Mutation of p53 or p21 partially alleviates both the Atm synaptic defect and induction of apoptosis (Barlow et al. 1997). It is possible, therefore, that most or all of the synaptic defects observed in the single mutant are indirect consequences of induction of apoptotic mechanisms. On the other hand, disruption of Rad51-axis association is observed even in Atm-/-p53-/- and Atm-/-p21-/- double mutant nuclei with relatively normal SC (Barlow et al. 1997). This observation is reminiscent of the effects of mutations in Mei-41, a Drosophila MEC1 homologue, which result in the aberrant localization of recombination nodules away from chromosome cores (Carpenter 1979). It seems plausible that the more subtle phenotype observed when apoptosis is suppressed in the Atm-/-p53-/- or Atm-/-p21-/- double mutant is more analogous to the meiotic phenotype of mei-41 flies and mec-1 yeast than the dramatic SC defects observed in the Atm-/- single mutant.

While it is possible that checkpoint proteins act directly to tether recombination events to the SC, we currently favor the possibility that the checkpoint proteins play an indirect role. If they acted directly as a bridge, one would predict that checkpoint proteins would colocalize with recombination proteins on the SC. However, although the mouse homologue of Rad17 does localize to meiotic chromosome cores, it does not colocalize with Dmc1 (Freire et al. 1998). Furthermore, because the checkpoint proteins can influence SCE in mitosis (Paulovich et al. 1998; see below), we believe that they act to generate or maintain a feature of chromosome structure that is common to mitosis and meiosis rather than exerting their effects directly on (or through) a meiosis-specific structure.

A possible role for checkpoint proteins in influencing chromosome structure is suggested by the observation that mei-41 mutants show reduced chromosome compaction (Carpenter 1979). We speculate that Rad17, Rad24, and Mec1 may be playing similar roles in yeast meiosis, possibly linking condensing chromatin to axial cores without specifically interacting with recombination proteins. Loss of such a function in checkpoint mutants would reduce interhomologue interaction, making intersister and ectopic interactions more likely, and could also interfere with the proper elaboration of the SC.

Control of the association of condensing chromatin with axial structure was previously proposed to play a role in regulation of the distribution of reciprocal crossover events during meiosis (Kleckner 1996), a process manifested as crossover interference (reviewed by Foss et al. 1993). Crossover interference is defective in mei-41 mutants (Baker and Carpenter 1972). If MEC1 and MEI-41 have similar functions, in yeast and flies respectively, it is possible that crossover interference and suppression of ectopic recombination are different manifestations of the same underlying molecular mechanism.

A previous study of Drosophila ribosomal DNA (rDNA) rearrangement may be relevant to the observations presented here. The frequency of meiotic translocation events involving the rDNA locus was shown to be increased in male mei-41 mutants (Hawley and Tartof 1983). However, important differences between the previous study and this one make the relationship of the two studies unclear. The rDNA is normally excluded from meiotic recombination in yeast, and male Drosophila do not undergo meiotic recombination. Furthermore, there is no evidence that the translocation events observed in the Drosophila study were homology mediated. Nonetheless, it is possible that the effect of mei-41 mutations on rDNA rearrangement is mechanistically similar to the effect of yeast checkpoint mutations on ectopic recombination reported here.

**Relationship of mitotic vs. meiotic homologous recombination functions of checkpoint genes:** The role of checkpoint genes in tolerance of UV-induced damage in mitosis may also be related to their role in meiosis. UV-induced lesions can block the progress of DNA polymerase, thereby resulting in formation of single-stranded DNA gaps in daughter DNA strands (reviewed in Friedberg et al. 1995). Cells possess multiple mechanisms for allowing the completion of replication when polymerase-blocking lesions are present. One of these tolerance mechanisms promotes SCE. RAD17 and RAD24 appear to suppress UV-induced SCE. The rad17 rad24 double mutant shows a very high level of SCE compared to wild type or the two corresponding single mutants (Paulovich et al. 1998). While there are many differences between meiotic recombination and postreplicative repair of UV damage, it is interesting to consider the possibility that the two types of suppression also reflect common underlying features. If so, the suppression of SCE during mitotic S phase is not caused by a reduction in recombination activity, because clearly no such reduction occurs in meiosis. Instead, DNA configuration may be altered by checkpoint proteins in such a way as to promote...
interhomologue recombination in meiosis and nonrecombinogenic damage tolerance pathways in mitosis.

We thank David Lydall for strains and Stephen Gasior for instruction on cytological methods. We are very grateful to Alistair Goldman for providing strains for genetic detection of ectopic recombination. Special thanks to Dawn Thompson and Franklin Stahl for sharing unpublished results and for many productive discussions. Thanks also to members of the Bishop lab for technical assistance and helpful conversation. This work was supported by National Institutes of Health grant GM 50936 to D. K. B.

LITERATURE CITED


Paulovich, A. G., C. D. A. K. Bishop, and H. Hartwell, 1998 The Saccharomyces cerevisiae RAD9, RAD17, RAD24 and MEC3 genes
are required for tolerating irreparable, ultraviolet-induced DNA damage. Genetics 150: 75-93.


Shinohara, A., S. Gasior, T. Ogawa, N. Kleckner and D. K. Bishop, 1997 Saccharomyces cerevisiae RAD51 and DMC1 have both distinct and overlapping roles in meiotic recombination. Genes Cells 2: 615-629.


