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

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ABSTRACT

Checkpoint gene function prevents meiotic progression when recombination is blocked by mutations in the *recA* homologue *DMC1*. 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 synapsis 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 reductional 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 Petes 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 *SPO11*, which encodes the transesterase that forms DSBs at the initiation of recombination (Keeney *et al.* 1997). *spo11 dmc1* mutant cells progress through both meiotic divisions, but the resulting spores are all inviable due to failed reductional 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

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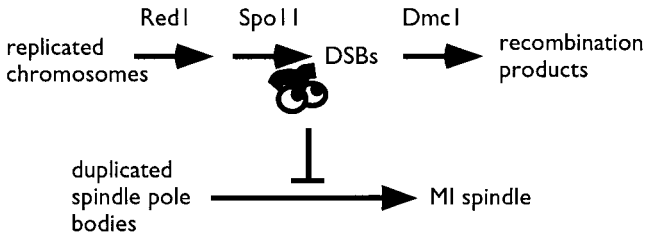


Figure 1.—Model for the meiotic recombination checkpoint. Regulatory surveillance is specific to a stage after the formation of Red1-dependent DSBs and before formation of recombination products.

pairs during prophase (Smith and Roeder 1997). In *red1* 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 *red1* mutant does not promote proper reductional segregation in meiosis I (Rockmill and Roeder 1990). Mutation of *red1* allows meiotic progression to occur in a *dmc1* mutant but, as with *spo11*, the spores produced are inviable. It has been proposed that the *red1* 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 *mec1/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 UV damage during mitotic S phase (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 polycomplex 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 TRAFO Method (Geitz and Scheistl 1995).

Checkpoint 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). *mec1-1 smlX* 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 colonies were screened by PCR to confirm the correct structure of *ndt80Δ::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). *ppx1::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^+ spores to determine the frequency of recombination have been described (Goldman and Lichten 1996). The percentage of Arg^+ recombinants was the mean of 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^+ 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:

$$\frac{(1 - 0.5R_{wt})\,E_{rad17}}{(1 - 0.5R_{rad17})E_{wt}}.$$

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 nucleoids was as described in Bishop (1994), using Rabbit anti-Zip1 (Symand Roeder 1995; gift of S. Roeder) at a 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 nucleoids 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 polycomplexes.

Southern blotting analysis: Preparation of DNA for meiotic time course analysis of reciprocal and ectopic recombinants was as described by Cao *et al.* (1990), except that purification of the DNA on Sephadex was replaced by ethanol precipitation as 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 *Pst*I (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 S&S Nytran Plus nylon membranes (Schleicher & Schuell, Keene, NH), and cross linked with 1200 J/m^2 UV light. Sequences were probed with the 677-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 dmc1*, *rad24 dmc1*, and *mec1-1 dmc1* double mutants (Lydall *et al.* 1996). To identify the product, *Pst*I 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 5′ 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). A *Bsi*WI 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 *leu2::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 dmc1*, *rad24 dmc1*, and *mec1-1 dmc1* mutants five- to sevenfold more frequently than in wild-type meiosis (Figure 2C). In a wild-type background, the EC1 and EC2 products occur with approximately equal frequency. However, in a *dmc1* background, the primary crossover product in this ectopic recombination

TABLE 1

Yeast strains

DKB no.	Genotype
974	*lys2 ho::LYS2 ura3 leu2::hisG arg4-nsp his4B::LEU2* *lys2 ho::LYS2 ura3 leu2::hisG arg4-bgl his4X::LEU2-URA3*
977	*lys2 ho::LYS2 ura3 leu2::hisG arg4-bgl his4B::LEU2 dmcl*Δ*::ARG4* *lys2 ho::LYS2 ura3 leu2::hisG arg4-bgl his4X::LEU2-URA3 dmcl*Δ*::ARG4*
835	*lys2 ho::LYS2 ura3 leu2::hisG arg4-nsp his4B::LEU2 mecl-l sml-X* *lys2 ho::LYS2 ura3 leu2::hisG arg4-bgl his4X::LEU2-URA3 mecl-l sml-X*
839	*lys2 ho::LYS2 ura3 leu2::hisG arg4-nsp his4B::LEU2 dmcl*Δ*::ARG4 mecl-l sml-X* *lys2 ho::LYS2 ura3 leu2::hisG arg4-bgl his4X::LEU2-URA3 dmcl*Δ*::ARG4 mecl-l sml-X*
1361	*lys2 ho::LYS2 ura3 leu2::hisG arg4 TRP1 his4B::LEU2 rad17::LEU2* *lys2 ho::LYS2 ura3 leu2::hisG arg4 trp1 his4X::LEU2-URA3 rad17::LEU2*
1367	*lys2 ho::LYS2 ura3 leu2::hisG arg4 TRP1 his4B::LEU2 dmcl*Δ*::ARG4 rad17::LEU2* *lys2 ho::LYS2 ura3 leu2::hisG arg4 trp1 his4X::LEU2-URA3 dmcl*Δ*::ARG4 rad17::LEU2*
1435	*lys2 ho::LYS2 ura3 leu2::hisG arg4 trp1 his4B::LEU2 rad24::TRP1* *lys2 ho::LYS2 ura3 leu2::hisG arg4 trp1 his4X::LEU2-URA3 rad24::TRP1*
1439	*lys2 ho::LYS2 ura3 leu2::hisG arg4 trp1 his4B::LEU2 dmc1*Δ*::ARG4 rad24::TRP1* *lys2 ho::LYS2 ura3 leu2::hisG arg4 trp1 his4X::LEU2-URA3 dmc1*Δ*::ARG4 rad24::TRP1*
1557	*lys2 ho::LYS2 ura3 leu2::hisG arg4 trp1 his4B::LEU2* *lys2 ho::LYS2 ura3 leu2::hisG arg4 trp1 his4X::LEU2-URA3*
1478	*lys2 ho::LYS2 ura3 leu2::hisG arg4 trp1 his4B::LEU2 ndt80*Δ*::KAN-MX4* *lys2 ho::LYS2 ura3 leu2::hisG arg4 trp1 his4X::LEU2-URA3 ndt80*Δ*::KAN-MX4*
1482	*lys2 ho::LYS2 ura3 leu2::hisG arg4 trp1 his4B::LEU2 rad17::LEU2* *lys2 ho::LYS2 ura3 leu2::hisG arg4 trp1 his4X::LEU2-URA3 rad17::LEU2*
1486	*lys2 ho::LYS2 ura3 leu2::hisG arg4 trp1 his4B::LEU2 ndt80*Δ*::KAN-MX4 dmc1*Δ*::ARG4* *lys2 ho::LYS2 ura3 leu2::hisG arg4 trp1 his4X::LEU2-URA3 ndt80*Δ*::KAN-MX4 dmc1*Δ*::ARG4*
1490	*lys2 ho::LYS2 ura3 leu2::hisG arg4 trp1 his4B::LEU2 dmc1*Δ*::ARG4 rad17::LEU2* *lys2 ho::LYS2 ura3 leu2::hisG arg4 trp1 his4X::LEU2-URA3 dmc1*Δ*::ARG4 rad17::LEU2*
1494	*lys2 ho::LYS2 ura3 leu2::hisG arg4 trp1 his4B::LEU2 ndt80*Δ*::KAN-MX4 rad17::LEU2* *lys2 ho::LYS2 ura3 leu2::hisG arg4 trp1 his4X::LEU2-URA3 ndt80*Δ*::KAN-MX4 rad17::LEU2*
1498	*lys2 ho::LYS2 ura3 leu2::hisG arg4 trp1 his4B::LEU2 ndt80*Δ*::KAN-MX4 dmc1*Δ*::ARG4 rad17::LEU2* *lys2 ho::LYS2 ura3 leu2::hisG arg4 trp1 his4X::LEU2-URA3 ndt80*Δ*::KAN-MX4 dmc1*Δ*::ARG4 rad17::LEU2*
1390	*lys2 ho::LYS2 ura3 leu2::hisG arg4-nsp leu2-K put2::URA3-[arg4-nsp]* *lys2 ho::LYS2 ura3 leu2::hisG arg4-bgl leu2-R put2::URA3-[arg4-bgl]*
1391	*lys2 ho::LYS2 ura3 leu2::hisG arg4-nsp leu2-K put2::URA3-[arg4-nsp]* *lys2 ho::LYS2 ura3 leu2::hisG arg4-bgl leu2-R ppx1::URA3-[arg4-bgl]*
1393	*lys2 ho::LYS2 ura3 leu2::hisG arg4-nsp leu2-K ppx1::URA3-[arg4-nsp]* *lys2 ho::LYS2 ura3 leu2::hisG arg4-bgl leu2-R put2::URA3-[arg4-bgl]*
1394	*lys2 ho::LYS2 ura3 leu2::hisG arg4-nsp leu2-K ppx1::URA3-[arg4-nsp]* *lys2 ho::LYS2 ura3 leu2::hisG arg4-bgl leu2-R ppx1::URA3-[arg4-bgl]*
1399	*lys2 ho::LYS2 ura3 leu2::hisG arg4-nsp leu2-K put2::URA3-[arg4-nsp] rad17::LEU2* *lys2 ho::LYS2 ura3 leu2::hisG arg4-bgl leu2-R put2::URA3-[arg4-bgl] rad17::LEU2*
1400	*lys2 ho::LYS2 ura3 leu2::hisG arg4-nsp leu2-K put2::URA3-[arg4-nsp] rad17::LEU2* *lys2 ho::LYS2 ura3 leu2::hisG arg4-bgl leu2-R ppx1::URA3-[arg4-bgl] rad17::LEU2*
1402	*lys2 ho::LYS2 ura3 leu2::hisG arg4-nsp leu2-K ppx1::URA3-[arg4-nsp] rad17::LEU2* *lys2 ho::LYS2 ura3 leu2::hisG arg4-bgl leu2-R put2::URA3-[arg4-bgl] rad17::LEU2*
1403	*lys2 ho::LYS2 ura3 leu2::hisG arg4-nsp leu2-K ppx1::URA3-[arg4-nsp] rad17::LEU2* *lys2 ho::LYS2 ura3 leu2::hisG arg4-bgl leu2-R ppx1::URA3-[arg4-bgl] rad17::LEU2*

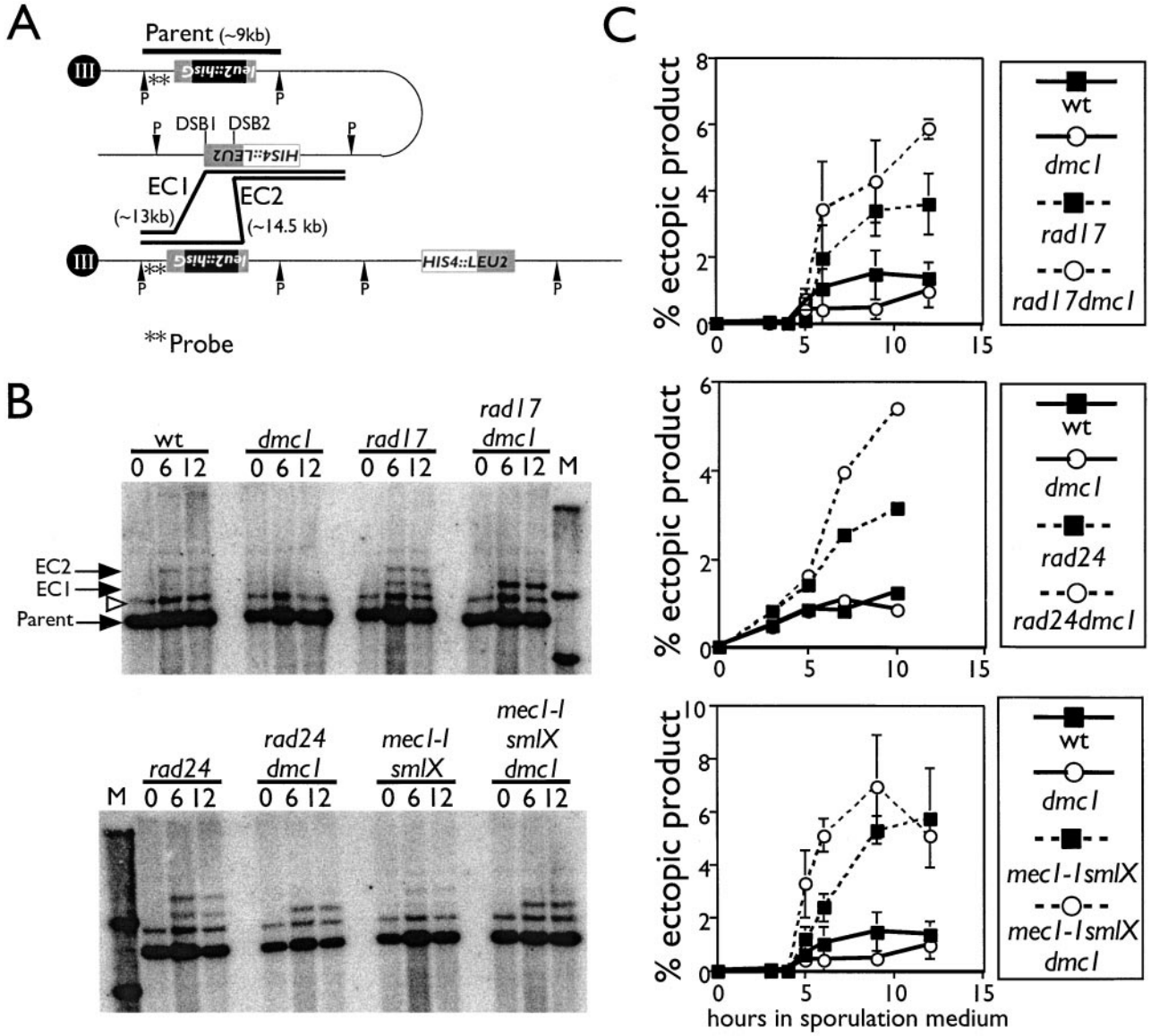


Figure 2.—Physical assay of ectopic recombination. (A) The ectopic recombination event observed between the *HIS4::LEU2* hotspot and the *leu2::hisG* allele. The configuration of the chromatids is meant only to emphasize the inverted configuration of the *leu2* sequences on chromosome III. The assay does not distinguish between intrachromatid, interhomologue or intersister ectopic recombinants. (B) *Pst*I digest of genomic DNA isolated at 0, 6, or 12 hr after synchronous start of meiosis and probed with the sequence adjacent to *leu2::hisG* indicated by the double asterisk in A. The open arrow indicates a nonspecific cross-reacting band intermediate in size between the parental fragment and the two ectopic recombinants. (C) *Pst*I blots were quantitated for the total amount of ectopic product as a percentage of parental DNA in wild-type, *dmc1*, *checkpoint*, and *dmc1 checkpoint* strains. Plots of *rad17* and *mec1-1* are averaged values of at least three experiments ±SD. Plot of *rad24* is a single experiment. Additional experiments were carried out with the *rad24* mutant and these gave essentially identical results, but different time points were taken (data not shown).

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 *Pst*I 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.*

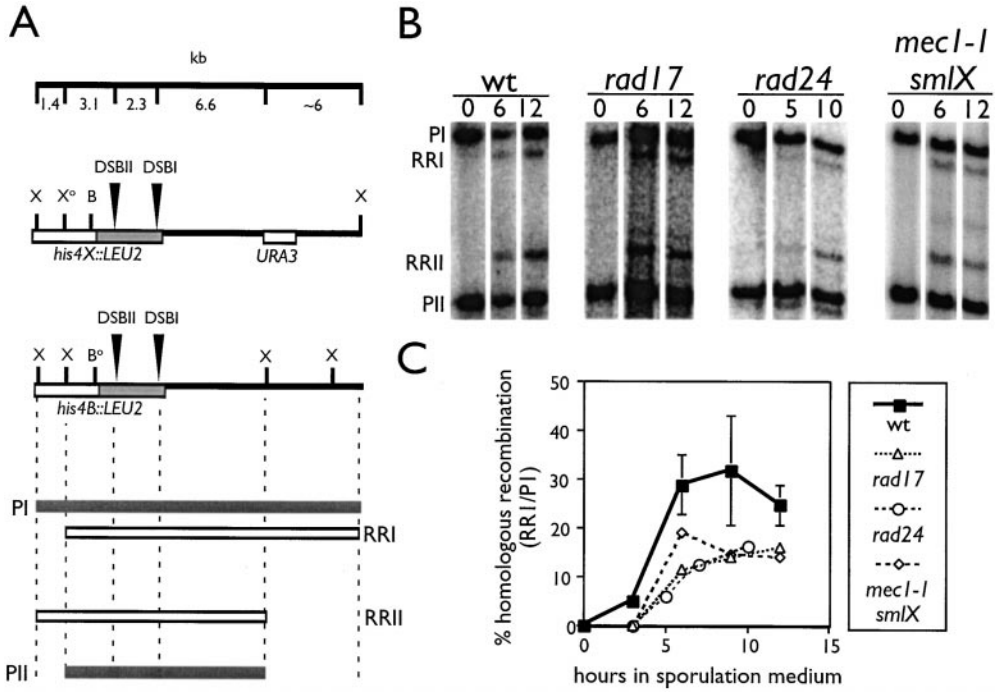
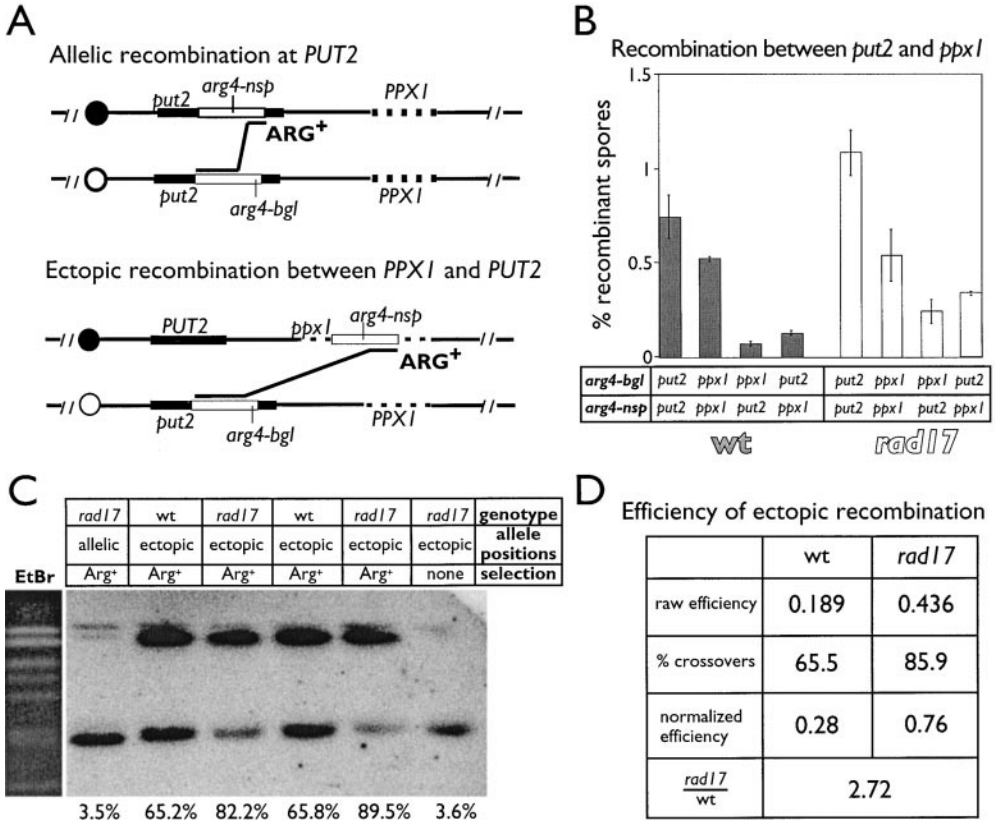


Figure 3.—Homologous recombination in *checkpoint* mutants. (A) The differentially marked *HIS4::LEU2* hotspot and the diagnostic interhomologue recombinants produced by *Xho*I digestion. (B) *Xho*I digests of meiotic DNA. (C) Quantitation of the amount of homologous recombination as expressed by the ratio of R1 recombinant product to P1 parental band. Wild-type plot is the mean of four experiments ±SD.

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 *HIS4* 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* heteroalleles 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-



	wt	*rad17*
raw efficiency	0.189	0.436
% crossovers	65.5	85.9
normalized efficiency	0.28	0.76
$\frac{rad17}{wt}$	2.72	

Figure 4.—Genetic assay of the efficiency of ectopic recombination. (A) The *ARG4* heteroalleles in allelic or ectopic configuration on chromosome VIII (Goldman and Lichten 1996). (B) Frequency of Arg^+ prototrophs among viable spores. Filled bars, recombination frequency in wild type; open bars, recombination frequency in *rad17* strains. All measurements are the mean of at least three experiments ±SD. (C) The frequency of crossing over in wild-type and *rad17* strains as determined by PFGE. Experiments were done in duplicate and samples from both are shown. (D) The fold increase in the efficiency of ectopic recombination due to the *rad17* mutation is expressed in the bottom row normalized for the level of crossing over (see materials and methods).

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 *clb1*, *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-

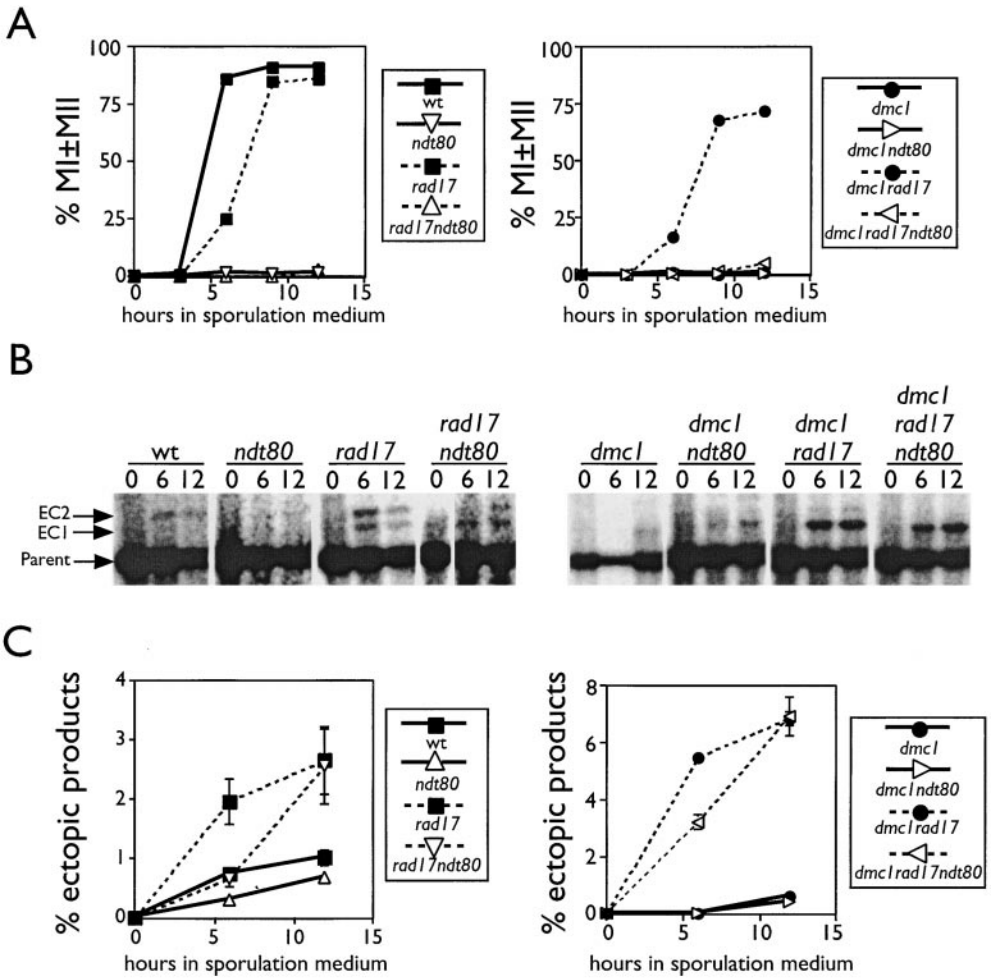


Figure 5.—The role of *NDT80* in progression and ectopic recombination. (A) Progression of meiotic divisions in *ndt80* mutants (open symbols) in wild-type or *dmc1* backgrounds was assessed by counting the number of staining bodies visible in DAPI-stained cells fixed at the indicated time after synchronous induction of meiosis. Plots represent the percentage of nuclei that have undergone at least one meiotic division and therefore contain two or more DAPI-staining bodies. (B) *Pst*I digests of genomic DNA fixed at the indicated time points from the same cultures analyzed in A and probed to detect ectopic recombinants as in Figure 2. (C) Quantitation of ectopic recombination in *ndt80* mutants. Average values of at least two experiments ±SD are shown, except for the *dmc1* single mutant (one experiment); averaged values of two experiments are plotted in A.

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 recombinants accumulate 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 interhomologue 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 ~3 hr, progresses to extended structures peaking at ~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 ~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

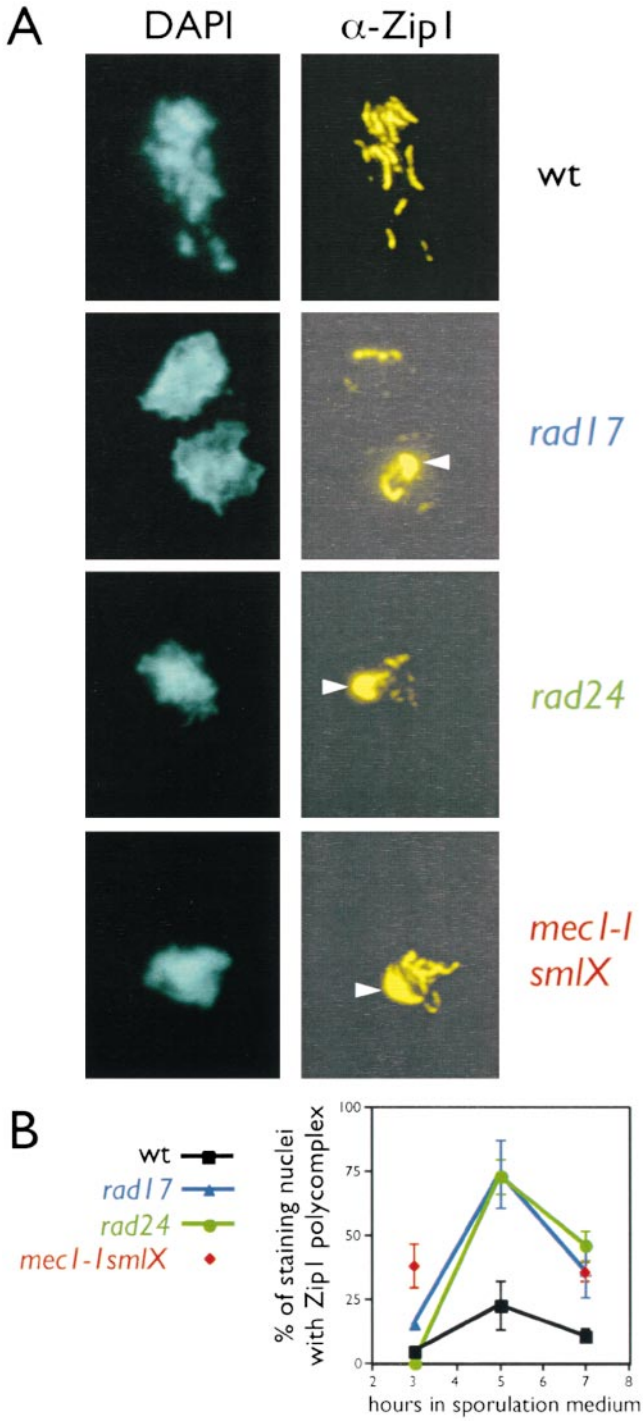


Figure 6.—Zip1 polycomplex formation in *checkpoint* mutants. (A) Spread meiotic nuclei were immunostained with rabbit anti-Zip1, and structures were visualized by secondary staining with fluorochrome-conjugated anti-rabbit antibodies. Open arrowheads identify polycomplexes typical of aberrant synapsis. (B) The frequency of defective synapsis is approximated by recording the fraction of staining nuclei that contains polycomplex structures. Two groups of 25 unselected nuclei are photographed at each time point and scored for the presence of Zip1 staining and for the presence of polycomplex (see text). Data plotted are the mean of the two groups at each time point ±SD.

physical assays used to detect recombinants at *HIS4::LEU2*.

Recently, alleles of *RAD24*, *RED1*, *MEK1*, 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 MI. 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 MI 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 MI. 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 MI 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 unrepaired 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-

portant features that distinguish *checkpoint* mutants from *red1* 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 *red1* 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 Weinert 1995). Additionally, certain mutations in the *Schizosaccharomyces pombe* homologue of *RAD24* (sp*RAD17*) 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 Murnane 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.

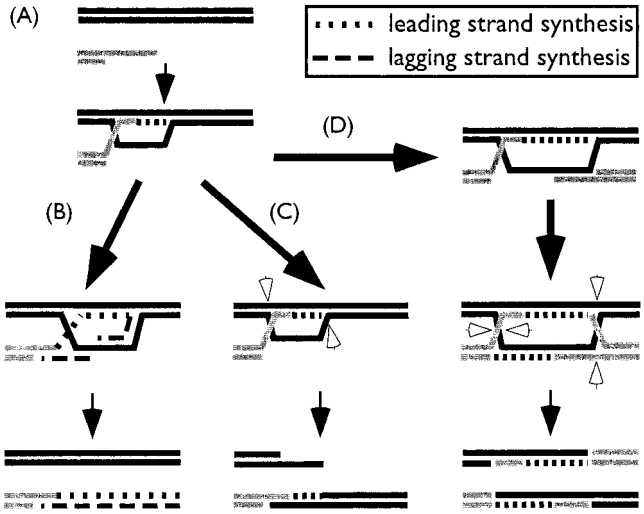


Figure 7.—Model for the role of checkpoint proteins in coordinating the two ends of double strand breaks. Invasion of homologous sequences by one end of a DSB (A) can proceed in three ways. (B) The invasion event promotes the formation of a full replication fork, which replicates to the end of the chromosome. (C) Cleavage of the one-sided intermediate by an endonuclease or a topoisomerase yields a half-crossover product. (D) Through the recruitment of the second end of the DSB, a typical double Holliday junction intermediate is formed, yielding two noncrossover or two reciprocal crossover products, as in Szostak *et al.* (1983).

The fraction of meiotic recombination events that results 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 *mec1* 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.

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LITERATURE CITED

Baker, B. S., and A. T. Carpenter, 1972 Genetic analysis of sex chromosomal meiotic mutants in *Drosophilia melanogaster*. Genetics **71:** 255–286.

Barlow, C., M. Liyanage, P. B. Moens, C. X. Deng, T. Ried *et al.*, 1997 Partial rescue of the prophase I defects of Atm-deficient mice by p53 and p21 null alleles. Nat. Genet. **17:** 462–466.

Barlow, C., M. Liyanage, P. B. Moens, M. Tarsounas, K. Nagashima *et al.*, 1998 *Atm* deficiency results in severe meiotic disruption as early as leptonema of prophase I. Development **125:** 4007–4017.

Baudat, F., and A. Nicolas, 1997 Clustering of meiotic double-strand breaks on yeast chromosome III. Proc. Natl. Acad. Sci. USA **94:** 5213–5218.

Bishop, D. K., 1994 RecA homologues Dmc1 and Rad51 interact to form multiple nuclear complexes prior to meiotic chromosome synapsis. Cell **79:** 1081–1092.

Bishop, D. K., D. Park, L. Xu and N. Kleckner, 1992 *DMC1*: a meiosis-specific yeast homologue of E. coli *recA* required for recombination, synaptonemal complex formation, and cell cycle progression. Cell **69:** 439–456.

Bishop, D. K., Y. Nikolski, J. Oshiro, J. Chon, M. Shinohara *et al.*, 1999 High copy number suppression of the meiotic arrest caused by a dmc1 mutation: *REC114* imposes an early recombination block and *RAD54* promotes a *DMC1*-independent DSB repair pathway. Genes Cells (in press).

Cao, L., E. Alani and N. Kleckner, 1990 A pathway for generation and processing of double-strand breaks during meiotic recombination in *S. cerevisiae*. Cell **61:** 1089–1101.

Carpenter, A. T., 1979 Recombination nodules and synaptonemal complex in recombination- defective females of *Drosophila melanogaster*. Chromosoma **75:** 259–292.

Chu, S., and I. Herskowitz, 1998 Gametogenesis in yeast is regulated by a transcriptional cascade dependent on Ndt80. Mol. Cell **1:** 685–696.

Chu, S., J. DeRisi, M. Eisen, J. Mulholland, D. Botstein *et al.*, 1998 The transcriptional program of sporulation in budding yeast. Science **282:** 699–705.

Clancy, M. J., 1998 Meiosis: step-by-step through sporulation. Curr. Biol. **8:** R461–463.

Dahmann, C., and B. Futcher, 1995 Specialization of B-type cyclins for mitosis or meiosis in *S. cerevisiae*. Genetics **140:** 957–963.

Engebrecht, J., J. Hirsch and G. S. Roeder, 1990 Meiotic gene conversion and crossing over: their relationship to each other and to chromosome synapsis and segregation. Cell **62:** 927–937.

Epstein, C. B., and F. R. Cross, 1992 CLB5: a novel B cyclin from budding yeast with a role in S phase. Genes Dev. **6:** 1695–1706.

Fasullo, M., T. Bennett, P. AhChing and J. Koudelik, 1998 The *Saccharomyces cerevisiae RAD9* checkpoint reduces the DNA damage-associated stimulation of directed translocations. Mol. Cell. Biol. **18:** 1190–1200.

Foss, E., R. Lande, F. W. Stahl and C. M. Steinberg, 1993 Chiasma interference as a function of genetic distance. Genetics **133:** 681–691.

Freire, R., J. R. Murguia, M. Tarsounas, N. F. Lowndes, P. B. Moens *et al.*, 1998 Human and mouse homologs of *Schizosaccharomyces pombe rad1*(+) and *Saccharomyces cerevisiae RAD17*: linkage to checkpoint control and mammalian meiosis. Genes Dev. **12:** 2560–2573.

Friedberg, E. C., G. C. Walker and W. Siede, 1995 *DNA Repair and Mutagenesis*. ASM Press, Washington, DC.

Gasior, S. L., A. K. Wong, Y. Kora, A. Shinohara and D. K. Bishop, 1998 Rad52 associates with RPA and functions with Rad55 and Rad57 to assemble meiotic recombination complexes. Genes Dev. **12:** 2208–2221.

Geitz, R. D., and R. H. Scheistl, 1995 Transforming yeast with DNA. Methods Mol. Cell. Biol. **5:** 255–269.

Goldman, A. S., and M. Lichten, 1996 The efficiency of meiotic recombination between dispersed sequences in *Saccharomyces cerevisiae* depends upon their chromosomal location. Genetics **144:** 43–55.

Grandin, N., and S. I. Reed, 1993 Differential function and expression of *Saccharomyces cerevisiae* B-type cyclins in mitosis and meiosis. Mol. Cell. Biol. **13:** 2113–2125.

Griffiths, D. J., N. C. Barbet, S. McCready, A. R. Lehmann and A. M. Carr, 1995 Fission yeast *rad17*: a homologue of budding yeast *RAD24* that shares regions of sequence similarity with DNA polymerase accessory proteins. EMBO J. **14:** 5812–5823.

Hartwell, L. H., and T. A. Weinert, 1989 Checkpoints: controls that ensure the order of cell cycle events. Science **246:** 629–634.

Hawley, R. S., and K. D. Tartof, 1983 The effect of *mei-41* on rDNA redundancy in *Drosophila melanogaster*. Genetics **104:** 63–80.

Hepworth, S. R., H. Friesen and J. Segall, 1998 *NDT80* and the meiotic recombination checkpoint regulate expression of middle sporulation-specific genes in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **18:** 5750–5761.

Jeggo, P. A., A. M. Carr and A. R. Lehmann, 1998 Splitting the ATM: distinct repair and checkpoint defects in ataxia-telangiectasia. Trends Genet. **14:** 312–316.

Jinks-Robertson, S., and T. D. Petes, 1985 High-frequency meiotic gene conversion between repeated genes on nonhomologous chromosomes in yeast. Proc. Natl. Acad. Sci. USA **82:** 3350–3354.

Kato, R., and H. Ogawa, 1994 An essential gene, *ESR1*, is required for mitotic cell growth, DNA repair and meiotic recombination in *Saccharomyces cerevisiae*. Nucleic Acids Res. **22:** 3104–3112.

Keegan, K. S., D. A. Holtzman, A. W. Plug, E. R. Christenson, E. E. Brainerd *et al.*, 1996 The Atr and Atm protein kinases associate with different sites along meiotically pairing chromosomes. Genes Dev. **10:** 2423–2437.

Keeney, S., C. N. Giroux and N. Kleckner, 1997 Meiosis-specific DNA double-strand breaks are catalyzed by Spo11, a member of a widely conserved protein family. Cell **88:** 375–384.

Kleckner, N., 1996 Meiosis: how could it work? Proc. Natl. Acad. Sci. USA **93:** 8167–8174.

Klein, F., T. Laroche, M. E. Cardenas, J. F. Hoffman, D. Schweizer *et al.*, 1992 Localization of RAP1 and topoisomerase II in nuclei and meiotic chromosomes of yeast. J. Cell Biol. **117:** 935–948.

Lichten, M., R. H. Borts and J. E. Haber, 1987 Meiotic gene conversion and crossing over between dispersed homologous sequences occurs frequently in *Saccharomyces cerevisiae*. Genetics **115:** 233–246.

Lydall, D., and T. Weinert, 1995 Yeast checkpoint genes in DNA damage processing: implications for repair and arrest. Science **270:** 1488–1491.

Lydall, D., and T. Weinert, 1996 From DNA damage to cell cycle arrest and suicide: a budding yeast perspective. Curr. Opin. Genet. Dev. **6:** 4–11.

Lydall, D., and T. Weinert, 1997 G2/M checkpoint genes of *Saccharomyces cerevisiae*: further evidence for roles in DNA replication and/or repair. Mol. Gen. Genet. **256:** 638–651.

Lydall, D., Y. Nikolsky, D. K. Bishop and T. Weinert, 1996 A meiotic recombination checkpoint controlled by mitotic checkpoint genes. Nature **383:** 840–843.

Mao-Draayer, Y., A. M. Galbraith, D. L. Pittman, M. Cool and R. E. Malone, 1996 Analysis of meiotic recombination pathways in the yeast *Saccharomyces cerevisiae*. Genetics **144:** 71–86.

Meyn, M. S., 1993 High spontaneous intrachromosomal recombination rates in ataxia-telangiectasia. Science **260:** 1327–1330.

Murnane, J. P., and J. L. Schwartz, 1993 Cell checkpoint and radiosensitivity. Nature **365:** 22.

Pâques, F., and J. E. Haber, 1999 Multiple pathways of double-strand break-induced recombination in *Saccharomyces cerevisiae*. Microbiol. Mol. Biol. Rev. **63:** 349–404.

Paulovich, A. G., C. D. Armour and L. 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.

Petes, T. D., and P. J. Pukkila, 1995 Meiotic sister chromatid recombination. Adv. Genet. **33:** 41–62.

Rockmill, B., and G. S. Roeder, 1990 Meiosis in asynaptic yeast. Genetics **126:** 563–574.

Roeder, G. S., 1997 Meiotic chromosomes: it takes two to tango. Genes Dev. **11:** 2600–2621.

Schwacha, A., and N. Kleckner, 1995 Identification of double Holliday junctions as intermediates in meiotic recombination. Cell **83:** 783–791.

Schwacha, A., and N. Kleckner, 1997 Interhomolog bias during meiotic recombination: meiotic functions promote a highly differentiated interhomolog-only pathway. Cell **90:** 1123–1135.

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

Smith, A. V., and G. S. Roeder, 1997 The yeast Red1 protein localizes to the cores of meiotic chromosomes. J. Cell Biol. **136:** 957–967.

Stahl, F. W., 1994 The Holliday junction on its thirtieth anniversary. Genetics **138:** 241–246.

Storlazzi, A., L. Xu, A. Schwacha and N. Kleckner, 1996 Synaptonemal complex (SC) component Zip1 plays a role in meiotic recombination independent of SC polymerization along the chromosomes. Proc. Natl. Acad. Sci. USA **93:** 9043–9048.

Sym, M., and S. Roeder, 1995 Zip1-induced changes in synaptonemal complex structure and polycomplex assembly. J. Cell Biol. **128:** 455–466.

Szostak, J. W., T. L. Orr-Weaver, R. J. Rothstein and F. W. Stahl, 1983 The double-strand-break repair model for recombination. Cell **33:** 25–35.

Wach, A., A. Brachat, R. Pöhlmann and P. Philippsen, 1994 New heterologous modules for classical or PCR-based gene disruptions in *Saccharomyces cerevisiae.* Yeast **10:** 1793–1808.

Weinert, T. A., and L. H. Hartwell, 1988 The *RAD9* gene controls the cell cycle response to DNA damage in *Saccharomyces cerevisiae*. Science **241:** 317–322.

Xu, L., M. Ajimura, R. Padmore, C. Klein and N. Kleckner, 1995 *NDT80*, a meiosis-specific gene required for exit from pachytene in *Saccharomyces cerevisiae.* Mol. Cell. Biol. **15:** 6572–6581.

Xu, Y., T. Ashley, E. E. Brainerd, R. T. Bronson, M. S. Meyn *et al.*, 1996 Targeted disruption of *ATM* leads to growth retardation, chromosomal fragmentation during meiosis, immune defects, and thymic lymphoma. Genes Dev. **10:** 2411–2422.

Xu, L., B. M. Weiner and N. Kleckner, 1997 Meiotic cells monitor the status of the interhomolog recombination complex. Genes Dev. **11:** 106–118.

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