ANALYSIS OF MEIOSIS-DEFECTIVE MUTATIONS IN YEAST BY PHYSICAL MONITORING OF RECOMBINATION

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ABSTRACT

We have developed a method by which the extent of physical exchange of DNA molecules can be determined throughout meiosis in the yeast *Saccharomyces cerevisiae*. We have used this technique to analyze the effect of five meiosis-defective mutations (*rad6*, *rad50*, *rad52*, *rad57* and *spo11*) on the physical exchange of DNA molecules. In the same experiments, we have also measured other meiotic parameters, such as premeiotic DNA synthesis, commitment to intragenic recombination, haploidization, ascus formation, and viability. *rad50* and *spo11* diploids make an undetectable amount of physically recombined DNA and <1% of wild-type levels of viable intragenic recombinants. In contrast, diploids homozygous for *rad52*, *rad6* or *rad57* all yield significant amounts of novel restriction fragments which arise by recombination. *rad57* diploids make nearly wild-type levels of the recombined restriction fragments, although they produce <10% of the wild-type levels of viable intragenic recombinants. *rad52* strains are also capable of a significant (33%) amount of exchange of DNA molecules, but make <1% of wild-type levels of viable intragenic recombinants. *rad6* diploids are also capable of undergoing a high level of exchange, as measured by the appearance of the recombined restriction fragment. In addition, *rad6* diploids show an unusual allele- or locus-specific variability in the level of viable intragenic recombinants produced. Although *rad6* diploids produce no viable spores, they are able to complete a significant amount of haploidization upon return to vegetative growth conditions.

AN important approach to the analysis of the major events in meiosis has been to isolate meiosis-defective mutations. In *Saccharomyces cerevisiae*, attention has focused on two types of mutations, those that were isolated directly as sporulation-defective (*spo*) mutations (ESPOSITO *et al.* 1972; ESPOSITO and ESPOSITO 1974a) and those that were described initially as radiation-sensitive (*rad*) and were subsequently shown to prevent normal meiosis (GAME *et al.* 1980; PRAKASH *et al.* 1980).

One problem in characterizing these mutations has been the difficulty in establishing whether they directly affect meiotic recombination. In many cases,

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the mutations completely block the production of viable spores, so that haploid meiotic products cannot be analyzed and the nature of the mutational defect cannot be accurately defined. Meiotic reversal experiments, which demonstrate that meiotic levels of recombination can be attained in wild-type strains without subsequent haploidization (SHERMAN and ROMAN 1963; ESPOSITO and ESPOSITO 1974b), have been used to assess recombination in the absence of successful completion of meiosis. For example, the γ-ray-sensitive mutations *rad50*, *rad52* and *rad57*, the UV-sensitive *rad6* mutation and the sporulation-defective mutant, *spo11*, all show defects in the production of significant increases in the frequency of intragenic recombinants, even when cells are returned to vegetative conditions (GAME *et al.* 1980; PRAKASH *et al.* 1980; RESNICK *et al.* 1986; KLAPHOLZ, WADDELL and ESPOSITO 1985). However, diploids homozygous for these mutations do carry out meiotic DNA synthesis (GAME *et al.* 1980; PRAKASH *et al.* 1980; KLAPHOLZ, WADDELL and ESPOSITO 1985). These results have been interpreted as suggesting that such mutants enter meiosis, but are blocked before the time at which cells become committed to intragenic recombination. In these experiments, it is not possible to establish if the failure to recover intragenic recombinants is due to failure to undergo recombination, to the inability of the cells to return to mitotic growth or to a secondary effect of the mutation.

Further genetic analysis has suggested that some of these mutations do, in fact, affect different aspects of recombination. The *spo13* mutation has been used by bypass the first meiotic division (KLAPHOLZ and ESPOSITO 1980a,b; MALONE and ESPOSITO 1981; MALONE 1983; KLAPHOLZ, WADDELL and ESPOSITO 1985) and, thus, avoid spore inviability caused by extensive chromosome nondisjunction. Using *spo13* in combination with other meiosis-defective mutations, these workers have demonstrated that both *spo13 rad50* and *spo13 spo11* strains produced viable meiotic progeny which had not undergone intergenic or intragenic recombination, whereas *spo13 rad52* and *spo13 rad6* diploids did not yield viable progeny. *spo13 rad50 rad52* strains produced viable spores, indicating that *rad50* blocks meiosis before the defect of *rad52*. In contrast, a *rad6 rad50 spo13* diploid produced no viable meiotic progeny (MALONE 1983). MALONE (1983) has proposed that the time of *rad50* action may be after that of *rad6*. Alternatively, *rad6* may be on a different, independent pathway from that of *rad50*, *rad52* and *spo11* (MALONE 1983). A similar analysis has indicated that the *spo11* mutation, which is defective in both intragenic and intergenic recombination, is also epistatic to the *rad57* mutation (R. E. ESPOSITO, personal communication).

We have sought a more direct way to investigate the molecular defects associated with various meiosis-defective mutations. We have developed a method to monitor recombination by a Southern blot analysis of DNA extracted from cells undergoing meiosis (BORTS *et al.* 1984). Crossing over in a 11-kb interval flanked by pairs of restriction endonuclease recognition-site polymorphisms yields novel restriction fragments that can be distinguished from the two parental fragments (Figure 1). In wild-type strains, these recombined fragments appear approximately 1.8 hr after the time of commitment to intragenic recombination in the same interval. This molecular approach to

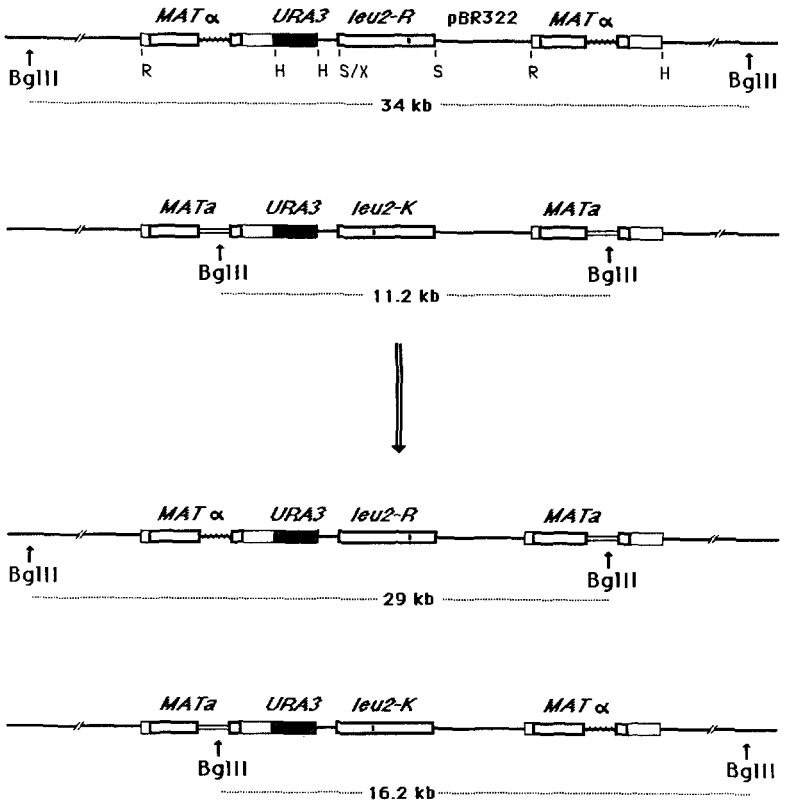

FIGURE 1.—Creation of two novel *Bgl*II restriction fragments by meiotic recombination. Reciprocal exchange in the [*MAT-URA3-leu2*-pBR322-*MAT*] interval produces two nonmating segregants. Since the two parental strains contain pairs of *Bgl*II recognition-site polymorphisms in this interval, the two nonmating recombinants are characterized by pBR322-containing restriction fragments that can be distinguished from the two parental fragments. In practice, the 29-kb recombined fragment is not clearly resolved from the parental 34-kb fragment on autoradiograms; consequently, the appearance of recombined DNA is monitored by the appearance of the 16.2-kb fragment, which is well resolved from both parental bands.

the study of recombination bypasses the need to recover viable meiotic products and, thus, allows a more direct measure of the extent of exchange of DNA molecules in meiosis-defective mutants.

We have applied physical monitoring of recombination to examine the effects of five mutations that prevent the recovery of viable, meiotic progeny. We have found that mutations with apparently similar effects on the recovery of viable, intragenic recombinants could be phenotypically distinguished by their ability to complete the physical exchange of DNA. We have shown that several mutations which severely block the recovery of intragenic recombinants are still competent to execute significant amounts of physical exchange of DNA molecules.

MATERIALS AND METHODS

Strains: Duplications of the yeast mating-type locus (*MAT*) flanking pBR322 and other yeast sequences were created by the integration at *MAT* of several different

derivatives of pBR322. All plasmids contained the 3.5-kb *Eco*RI-*Hin*dIII fragment of *MAT* (ASTELL *et al.* 1981) inserted between the *Eco*RI and *Hin*dIII sites of pBR322, and most contained the *Hin*dIII-*Hin*dIII fragment of *URA3* (ROSE, GRISAFI and BOTSTEIN 1983) inserted into the *Hin*dIII site of pBR322. Other plasmids (pJH12, pJH118 and pJH119) contained either wild-type or mutant copies of the 2.2-kb *LEU2 Xho*I-*Sal*I fragment (ANDREADIS and SCHIMMEL 1982) inserted at the *Sal*I site of pBR322. Strains DR210 and DR212 have the structures [*MAT***a**-*URA3*-pBR322-*MAT***a**] and [*MATα-URA3*-pBR322-*MATα*] and are described elsewhere (HABER and ROGERS 1982). MJH76 [*MATα-LEU2*-pBR322-*MATα*] was created by integrative transformation (ITO *et al.* 1983) of pJH12. MJH148 [*MATα-URA3-leu2R*-pBR322-*MATα*] and MJH149 [*MATα-URA3-leu2K*-pBR322-*MATα*] were created by the integration of plasmids pJH118 and pJH119, respectively. These plasmids contain *MATα*, *URA3* and the *Sal*I-*Xho*I *LEU2* fragment containing different mutations of *LEU2* (Figure 1). The *leu2K* and *leu2R* mutations are in the *LEU2* coding sequence and simultaneously eliminate both *LEU2* function and either the *Kpn*I or the *Eco*RI restriction site. Both mutations were generated by cutting the parental plasmid with the appropriate restriction enzyme and incubating the cut plasmid with the large fragment of *E. coli* DNA polymerase I and all four deoxyribonucleotide triphosphates. The blunt ends were then ligated with T4 DNA ligase (MANIATIS, FRITSH and SAMBROOK 1982). All enzymes were purchased from New England Biolabs.

All *MAT* duplication strains were backcrossed at least three times to strain Y55 to ensure synchronous and efficient sporulation. In the process of backcrossing, auxotrophic markers, drug-resistance markers and *met13* heteroalleles were introduced into each parental haploid. All genetic manipulations were as described previously (SHERMAN, FINK and HICKS 1982).

Strains containing *rad50-1*, *rad52-1*, *rad57-1*, *rad6-1* and *spo11-1* were backcrossed at least three times to Y55 and were then crossed to strains containing the structures [*MAT***a**-*URA3-leu2K*-pBR322-*MAT***a**] and [*MATα-URA3-leu2R*-pBR322-*MATα*] or [*MAT***a**-*URA3*-pBR322-*MAT***a**] and [*MATα-URA3*-pBR322-*MATα*]. The complete genotypes of all strains employed in this study are given in Table 1.

The radiation-sensitive phenotype of *rad50*, *rad57* and *rad52* was scored by the absence of growth after exposure to 4.45 kilorads of γ-radiation from a cobalt-60 source (Gamma cell 220, Atomic Energy of Canada, Ltd.). The radiation-sensitive phenotype of *rad57* is cold-sensitive; therefore, it was scored by differential growth at 18° and 30° after exposure to γ-radiation. *rad6* was scored following exposure to ultraviolet light ($1.2 \times 10^2 J/M^2$). *spo11* segregants were identified as those which failed to complement the original *spo11* allele (KLAPHOLZ and ESPOSITO 1982). Both *rad6* and *rad52* diploid strains were maintained on YEP-ethanol-glycerol (YEPG) plates to select against petites. All strains were stored in 25% glycerol at −70°. The *rad50-1* allele was the gift of J. GAME, and *rad6-1* was given to us by L. PRAKASH. *spo11-1* was obtained from R. E. ESPOSITO, and *rad52-1* and *rad57-1* were obtained from the Berkley Yeast Stock collection.

Media: YEPD, synthetic complete and sporulation media have been described previously (SHERMAN, FINK and HICKS 1982). YEPG plates are complete media (YEP) supplemented with 1% (w/v) succinic acid, 2.6% (v/v) glycerol and 2.6% (v/v) ethanol (J. MCCUSKER, personal communication).

Liquid sporulation was carried out in 2% potassium acetate (KAc) (w/v), pH 7.0, supplemented with all amino acids for which the strain was auxotrophic. Supplementation was at 0.006% leucine (w/v), 0.002% methionine (w/v) and 0.003% lysine (w/v). Glass distilled or commercial distilled water (Belmont Springs) was used for all sporulation solutions.

Sporulation: Diploid strains were grown in liquid YEPD at 30° to mid-stationary phase. Cell titer was measured by optical density (Beckman Model 25 spectrophotometer). When a 1:10 dilution of the culture reached an optical density (600 nm) of 1.2–1.4 (0.7–0.9 for *rad52* strains), the culture was shifted to room temperature and incu-

TABLE 1

Complete genotypes of strains used in this study

Strains	Genotype
RHB 105	*MAT***a**-*URA3*-pBR322-*MAT***a** *leu2-3,112* *lys2-a* *his6-1* + *cyh2* *met13-2* *can1* *ura3* / *MATα*-*URA3*-pBR322-*MATα* *leu2-3,112* *lys2-b* + *trp5* + *met13-4* + +
RHB 129	*MAT***a**-*LEU2*-pBR322-*MATα* *leu2-3,112* *met13-2* *lys2k* *ade1* *cyh2* + + + *ura3* / *MATα*-*URA3*-pBR322-*MAT***a** *leu2-3,112* *met13-2* *lys2a* + + *trp5* *his6* *can1* *ura3*
RHB 219	*rad57-1* / *rad57-1* *MAT***a**-*URA3*-pBR322-*MAT***a** *leu2-3,112* *lys2-a* *his6-1* *met13-2* + *cyh2* *can1* *ura3* / *MATα*-*URA3*-pBR322-*MATα* *leu2-3,112* *lys2-b* + *met13-4* *trp5* + + *ura3*
RHB 206	*rad50-1* / *rad50-1* *MAT***a**-*URA3*-pBR322-*MAT***a** *leu2-3,112* *lys-a* *his6-1* *met13-2* + *cyh2* *can1* *ura3* / *MATα*-*URA3*-pBR322-*MATα* *leu2-3,112* *lys2-b* + *met13-4* *trp5* + + *ura3*
RHB 220	*rad57-1* / + *MAT***a**-*URA3*-pBR322-*MAT***a** *leu2-3,112* *lys2-a* *his6-1* *met13-2* + *cyh2* *can1* *ura3* / *MATα*-*URA3*-pBR322-*MATα* *leu2-3,112* *lys2-b* + *met13-4* *trp5* + + *ura3*
RHB 226	*rad52-1* / *rad52-1* *MAT***a**-*URA3*-pBR322-*MAT***a** *leu2-3,112* *lys2-a* *his6-1* *met13-2* + *cyh2* *can1* *ura3* / *MATα*-*URA3*-pBR322-*MATα* *leu2-3,112* *lys2-b* + *met13-4* *trp5* + + *ura3*
RHB 229	*rad52-1* / + *MAT***a**-*URA3*-pBR322-*MAT***a** *leu2-3,112* *lys2-a* *his6-1* *met13-2* + *cyh2* *can1* *ura3* / *MATα*-*URA3*-pBR322-*MATα* *leu2-3,112* *lys2-b* + *met13-4* *trp5* + + *ura3*
RHB 300	*MAT***a**-*URA-leu2K*-pBR322-*MAT***a** *leu2-3,112* *lys2-c* *his6-1* *met13-2* + *cyh2* *can1* *ura3* / *MATα*-*URA-leu2R*-pBR322-*MATα* *leu2-3,112* *lys2-d* + *met13-4* *trp5* + + *ura3*
RHB 334	*rad6-1* / + *MAT***a**-*URA-leu2K*-pBR322-*MAT***a** *leu2-3,112* *lys2-c* *his6-1* *met13-2* + *cyh2* *can1* *ura3* / *MATα*-*URA-leu2R*-pBR322-*MATα* *leu2-3,112* *lys2-d* + *met13-4* *trp5* + + *ura3*
RHB 336	*rad6-1* / *rad6-1* *MAT***a**-*URA-leu2K*-pBR322-*MAT***a** *leu2-3,112* *lys2-c* *his6-1* *met13-2* + *cyh2* *can1* *ura3* / *MATα*-*URA-leu2R*-pBR322-*MATα* *leu2-3,112* *lys2-d* + *met13-4* *trp5* + + *ura3*
RHB 339	*spo11-1* / + *MAT***a**-*URA-leu2K*-pBR322-*MAT***a** *leu2-3,112* *lys2-c* *his6-1* *met13-2* + *cyh2* *can1* *ura3* / *MATα*-*URA-leu2R*-pBR322-*MATα* *leu2-3,112* *lys2-d* + *met13-4* *trp5* + + *ura3*
RHB 340	*spo11-1* / *spo11-1* *MAT***a**-*URA-leu2K*-pBR322-MA**T**a *leu2-3,112* *lys2-c* *his6-1* + *cyh2* *met13-2* *can1* *ura3* / *MATα*-*URA-leu2R*-pBR322-*MATα* *leu2-3,112* *lys2-d* + *trp5* + *met13-4* + *ura3*
RHB 357	*rad50-1* / *rad50-1* *MAT***a**-*URA-leu2K*-pBR322-*MAT***a** *leu2-3,112* *lys2-c* *his6-1* + *cyh2* *met13-2* *can1* *ura3* / *MATα*-*URA-leu2R*-pBR322-*MATα* *leu2-3,112* *lys2-d* + *trp5* + *met13-4* + *ura3*
RHB 359	*rad50-1* / + *MAT***a**-*URA-leu2K*-pBR322-*MAT***a** *leu2-3,112* *lys2-c* *his6-1* + *cyh2* *met13-2* *can1* *ura3* / *MATα*-*URA-leu2R*-pBR322-*MATα* *leu2-3,112* *lys2-d* + *trp5* + *met13-4* + *ura3*
RHB 361	*rad52-1* / *rad52-1* *MAT***a**-*URA-leu2K*-pBR322-MA**T**a *leu2-3,112* *lys2-c* *his6-1* + *cyh2* *met13-2* *can1* *ura3* / *MATα*-*URA-leu2R*-pBR322-*MATα* *leu2-3,112* *lys2-d* + *trp5* + *met13-4* + *ura3*
RHB 362	*rad52-1* / + *MAT***a**-*URA-leu2K*-pBR322-*MAT***a** *leu2-3,112* *lys2-c* *his6-1* + *cyh2* *met13-2* *can1* *ura3* / *MATα*-*URA-leu2R*-pBR322-*MATα* *leu2-3,112* *lys2-d* + *trp5* + *met13-4* + *ura3*

bated, without shaking, for 10–12 hr. The cells were removed from growth media by filtration and were washed once with 2% KAc. Cells were resuspended in supplemented 2% KAc, at a density of 1–3 × 10^7 cells/ml, and were aerated at 30° with a culture volume to flask volume ratio no greater than 1:5. Only acid-washed glassware or plasticware was used.

Samples were removed from the sporulating culture at various times throughout sporulation. At each time point, aliquots of cells were removed from the media by centrifugation and were frozen in 25% glycerol at −70°. These samples were used in subsequent analysis for physically recombined DNA, determination of DNA content (BURTON 1968) and DAPI staining. At each time, point cells were also diluted and plated onto YEPD, synthetic complete plates lacking methionine or leucine and canavanine-containing plates, to assess viability, prototroph formation and canavanine resistance. The frequency of prototrophic or canavanine-resistant colony forming units was determined and plotted as a percentage of the maximum value attained in that experiment.

DNA extraction: From each time point, 1.5 × 10 cells were thawed on ice and were washed once with cold distilled water, washed once with cold 1 M sorbitol and transferred to a 1.5-ml microfuge tube. Cells were incubated in 0.3 ml of 1.2 M sorbitol, 200 mM Tris-HCl, 20 mM EDTA, 1.7% β-mercaptoethanol, pH 8.4, for 30 min at 0°. Cells were removed from this solution by centrifugation and were resuspended in 0.3 ml of 1.2 M sorbitol, 10 mM Tris-HCl, 10 mM $CaCl_2$, 1.7 mg/ml Zymolyase 5000 (Seikagaku Kogyo Co.), pH 7.5, and spheroplasted at 37° for 10–20 min. Spheroplasts were removed from zymolyase by centrifugation and were washed once in cold 1 M sorbitol. The spheroplast pellet was gently resuspended in 50 μl of 1 M sorbitol and lysed at 65° by addition of 0.4 ml of 50 mM Tris-HCl, 100 mM NaCl, 100 mM EDTA, 0.5% SDS, 0.5 mg/ml Proteinase K (Merck), pH 6.8, for 1 hr. Denatured protein was removed by two successive phenol/chloroform (1 v/1 v) extractions. DNA was precipitated by the addition of 50 μl of 3 M sodium acetate and 1 ml of cold 100% EtOH. The precipitated DNA was spooled by gentle inversion of the microfuge tube and washed once with cold 70% EtOH. The DNA pellet was resuspended in 0.5 ml TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) containing 0.02 mg/ml ribonuclease and incubated at 37° for 1 hr. RNase was removed by a phenol/chloroform extraction followed by an EtOH precipitation. The DNA was gently resuspended in 25–50 μl of TE, such that the final concentration of DNA was 0.1–0.2 mg/ml.

Southern analysis: Physically recombined DNA was detected in DNA from sporulating cultures by Southern analysis (SOUTHERN 1975). From each time point, 0.5–2.0 μg of DNA was digested with a 20-fold unit excess of *Bgl*II and was electrophoresed in a horizontal 0.5% agarose gel at 2.2 V/cm for 48 hr. Electrophoresis was carried out in 1× TBE (89 mM Tris, 89 mM boric acid, 2.5 mM EDTA, pH 8.3) buffer (DAVIS, BOTSTEIN and ROTH 1980). Depurination, denaturation and transfer of the gel to nitrocellulose (Schleicher & Schuell BA 85) was carried out as described in MANIATIS, FRITSH and SAMBROOK (1982). The filter was hybridized with 1 × 10^7 cpm of ^{32}P nick-translated pBR322 (DAVIS, BOTSTEIN and ROTH 1980) and exposed to film (Kodak XAR-5). For some exposures, enhancing screens were used.

Densitometry: The relative amount of DNA present as physically recombined DNA was determined by densitometric scanning (Joyce-Loebl Densitometer) of an autoradiogram of Southern blots. For each time point, the amount of pBR322-homologous DNA present as the recombined *Bgl*II fragment was normalized to the amount of pBR322-homologous DNA present in the [*MATα*-pBR322-*MATα*] parental *Bgl*II fragment. The exposure times of autoradiograms were varied to obtain recombinant and parental bands of similar intensity for scanning. An Apple II Graphics Tablet was used to determine the area beneath each peak obtained from the densitometric scans.

DAPI staining: Cells were fixed in 3:1 methanol:acetic acid (v/v) for 30 min, washed twice with 1% (w/v) NaCl and resuspended in 0.1 μg/ml DAPI (4′,6-diamidino-2-

phenylindole), 0.1% (w/v) *o*-phenylenediamine, 100 mM Tris-HCl (pH 7.4). The nuclei were visualized with a Zeiss fluorescence microscope.

RESULTS

Physical exchange of DNA in meiosis-defective mutants: Diploid strains with the structure

$$\frac{\textit{MAT}\mathbf{a}\text{-}\textit{URA3}\text{-}\textit{leu2K}\text{-pBR322-}\textit{MAT}\mathbf{a}}{\textit{MAT}\alpha\text{-}\textit{URA3}\text{-}\textit{leu2R}\text{-pBR322-}\textit{MAT}\alpha}$$

were created by the integration of plasmids containing *URA3, leu2* and *MAT* sequences at the *MAT* locus on chromosome *III*. Diploids with this structure exhibit a high level of crossing over within the 11.2-kb interval defined by the *MAT* loci, and 20.8% of all tetrads contain a reciprocal exchange of the flanking *MAT* genes. These crossovers can be detected both genetically and physically. Tetrads containing the products of reciprocal exchange in the *MAT-URA3-leu2*-pBR322-*MAT* interval contain two nonmating spores with the genotype [*MAT***a**-*URA3-leu2*-pBR322-*MAT*α] or [*MAT*α-*URA3-leu2*-pBR322-*MAT***a**] (Figure 1). Because of the *Bgl*II site found in *MAT***a** but not *MAT*α (ASTELL *et al.* 1981), the two haploid nonmating spores yield pBR322-containing *Bgl*II restriction fragments that are distinct from each other and from the *Bgl*II fragments produced by both parents. We have previously used this system to determine when physical exchange in meiosis occurs by measuring the appearance of the recombined *Bgl*II fragment relative to other meiotic events (BORTS *et al.* 1984).

In this paper we employ the appearance of the novel *Bgl*II fragment to evaluate the extent of crossing over in diploids homozygous for one of a number of meiosis-defective mutations (*rad50, rad52, rad57, rad6* or *spo11*). Although identified on the basis of other phenotypes (radiation sensitivity and diminished ascus formation), all of these mutations have been shown to be defective in the production of viable intragenic recombinants and fail to produce viable haploid progeny (GAME *et al.* 1980; PRAKASH *et al.* 1980; KLAPHOLZ, WADDELL and ESPOSITO 1985). For all mutations studied, we have measured levels of intragenic recombination, haploidization, increase in DNA content, and physically recombined DNA in at least two separate experiments. The results of these experiments are summarized in Table 2.

rad52: *rad52* strains undergo a limited increase in meiotic intragenic recombinants (Figure 2B; Table 2; M. A. RESNICK, personal communication). Although the increase over the mitotic level of prototrophs can be as high as tenfold, the final meiotic levels are still <1% of the wild-type levels for both *MET13* and *LEU2*. No haploidization occurs, as detected by an increase in canavanine-resistant colonies. Although no mature asci are formed, DAPI staining reveals a few tetranucleate cells (data not shown). Viability drops to about 40% of the initial population by 24 hr after the initiation of sporulation.

Although only a limited number of viable intragenic recombinants are produced in these strains (Figure 2B; Table 2), physical exchange of DNA molecules is not as severely affected. *rad52* strains are capable of approximately

TABLE 2

Effect of *rad* mutants on meiotic recombination and sporulation

Mutant	Increase in DNA synthesis	Percent increase relative to heterozygous control					
		MET13	*LEU2*	Recombined *Bgl*II fragment	Can^R	Spo[a]	Viability[b]
rad50	60	0		<20[c]	23	0	78
rad52	80	0.09	1	33	0	0	39
rad57[d]	80	1.4		109	3	0	51
rad57[e]	70	7		78	1.5	0	40
rad6	80	16	74	64	27	8.8	74
spo11	50	0.1	0	<20[c]	0.1	0	60

[a] Percent sporulation was measured after 24 hr for all strains, except that *rad57* at its restrictive temperatures (18° or 25°) was measured after 48 hr.

[b] Percentage of initial population remaining viable at 24 hr. For *rad57* at the restrictive temperatures (18° and 25°), percent viability was determined at 48 hr.

[c] Ten to twenty percent of the wild-type recombined *Bgl*II fragment is the minimum amount of fragment detectable in these experiments.

[d] *rad57* cells were sporulated at 18° or 25°, and the data were averaged.

[e] *rad57* cells were sporulated at 30° or 34°, and the data were averaged.

30% of the wild-type levels of exchange of DNA molecules (Table 2). In the time course experiment shown in Figure 3A, the 16.2-kb recombined *Bgl*II fragment can be first detected in DNA samples made from *rad52* cells 10 hr after the initiation of sporulation; by 12 hr after, recombined fragment has attained its maximum value of 21% of the heterozygous control (RHB362, Figure 3B). Since this level of recombined *Bgl*II fragment is near the limits of detection of these experiments, accurate determination of the kinetics of accumulation in *rad52* cells is not possible.

We have previously reported experiments with *rad52* strains that showed no detectable recombined *Bgl*II fragment 12 hr after the initiation of sporulation (BORTS *et al.* 1984). In those experiments we used strain RHB226 (Table 1), which contains the structure [*MAT-URA3*-pBR322-*MAT*]. In wild-type strains, this structure yields 25% less crossing over in the [*MAT*-pBR322-*MAT*] interval than does the *MAT-URA3-leu2*-pBR322-*MAT* structure (BORTS *et al.* 1984). Further experiments with RHB226, in which a 24-hr time point was analyzed, showed detectable, but not quantifiable, amounts of recombined *Bgl*II fragment (data not shown). These data suggest that the accumulation of recombined *Bgl*II may be slower in *rad52* strains than in wild type.

Three independent experiments with *rad52* strains gave a mean value of the total pBR322-homologous DNA present as recombined *Bgl*II fragment as 33% (range 16–51%) of the maximum level attained in the heterozygous controls. This value is substantially greater than the amount of *LEU2* intragenic recombination (1% relative to the heterozygous control) measured in the same region or intragenic recombination at another genomic location (*MET13*) (Table 2; Figure 2A and B).

rad50: In contrast to *rad52, rad50* strains (RHB206 and RHB357, Table 1)

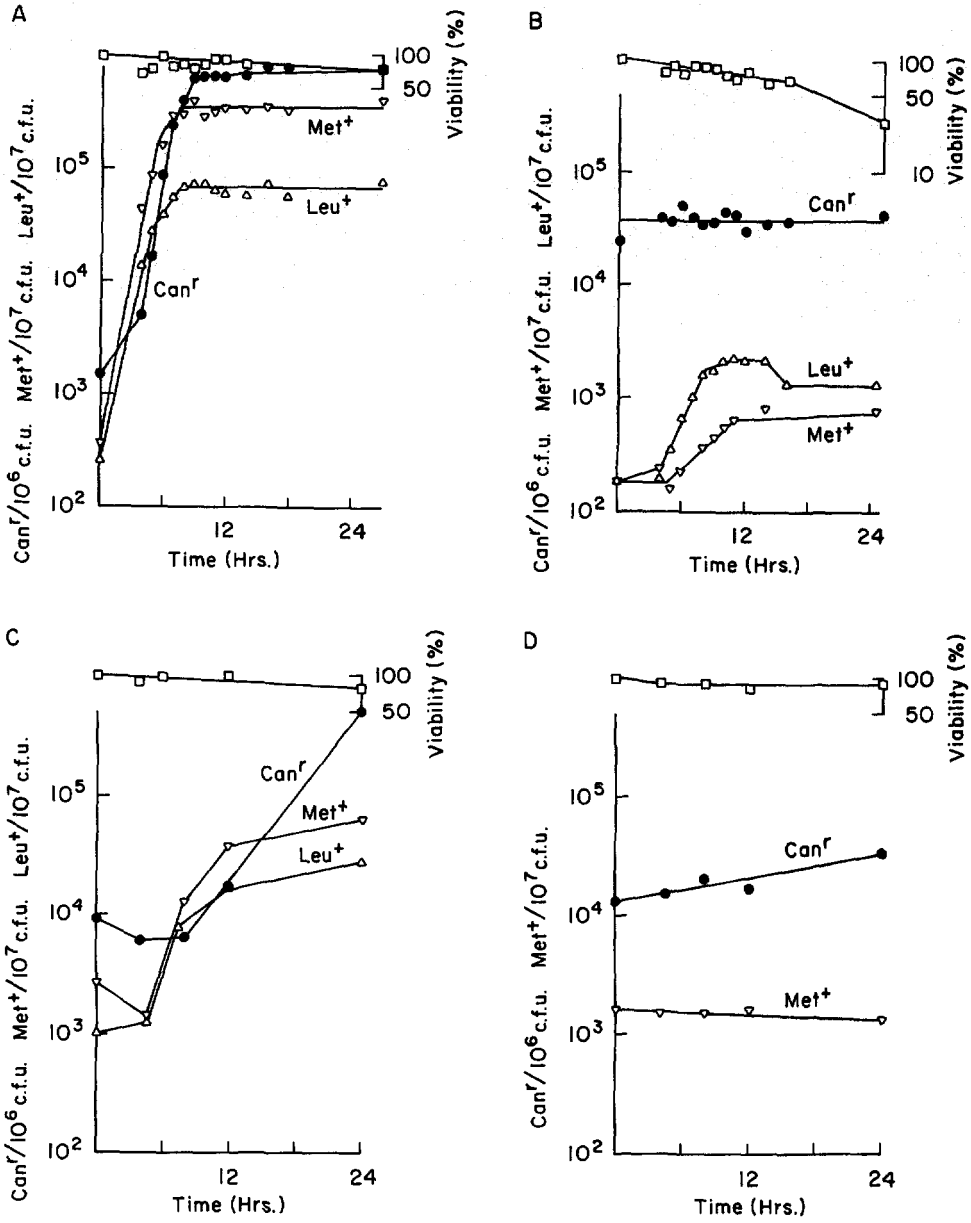

FIGURE 2.—Intragenic recombination, haploidization and viability during sporulation in meiosis-defective mutants. Strains (complete genotypes are listed in Table 1) were removed from growth media and were placed in sporulation conditions as described in MATERIALS AND METHODS. Cells were removed from sporulation media at the indicated times and were assayed for viable cells (□), *MET13* prototrophs (▽), *LEU2* prototrophs (△) and canavanine-resistant colonies (●). A, *RAD52/rad52* (strain RHB362); B, *rad52/rad52* (strain RHB361); C, *rad6/rad6* (strain RHB336); D, *rad50/rad50* (RHB206). The data for each mutant strain is the average of at least two independent experiments.

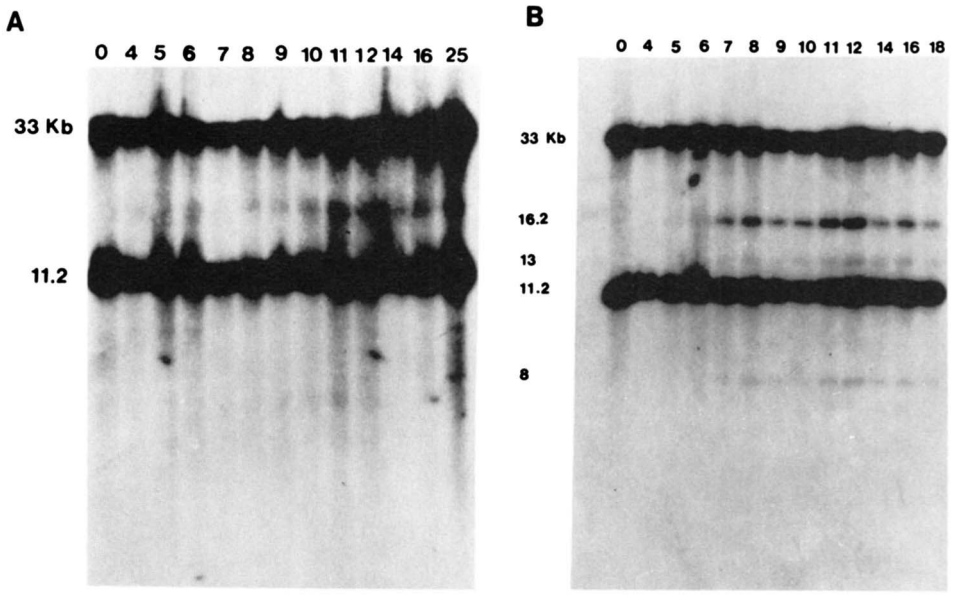

FIGURE 3.—Time course of appearance of the recombined *Bgl*II fragment in *rad52/rad52* and *rad52/RAD52* strains. Diploids were placed in sporulation media as described in MATERIALS AND METHODS and DNA made from samples taken at the indicated times. Autoradiograms of a Southern blot of this DNA, digested with *Bgl*II and probed with ^{32}P-labeled pBR322, revealed the 16.2 recombined *Bgl*II fragment 10 hr after the initiation of sporulation in *rad52/rad52* (panel A) and 6 hr after the initiation of sporulation in *rad52/RAD52* (panel B).

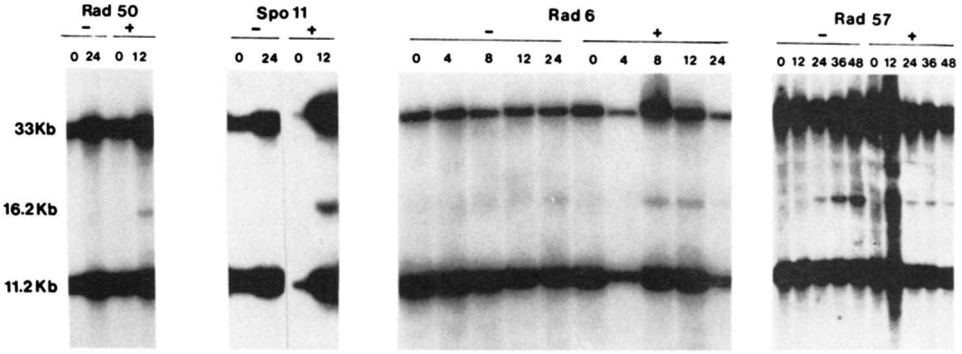

FIGURE 4.—Physical monitoring of recombination in meiosis-defective mutants. DNA was prepared from sporulating cultures at the times indicated, digested with *Bgl*II and the fragments resolved by electrophoresis. Southern blots of each gel were probed with ^{32}P-labeled pBR322. For each mutant strain, a congenic strain heterozygous for the mutation is shown as a control.

show virtually no increase in *MET13* (Figure 2D; Table 2) or *LEU2* prototrophs (data not shown). These strains undergo normal meiotic DNA synthesis (1.6-fold increase in DNA content) and remain viable (78%) over the course of the experiment (Figure 2D; Table 2). Although no mature asci are produced, DAPI staining revealed a few tetranucleate cells (data not shown). No recombined *Bgl*II fragment can be detected in these strains (Figure 4). In experi-

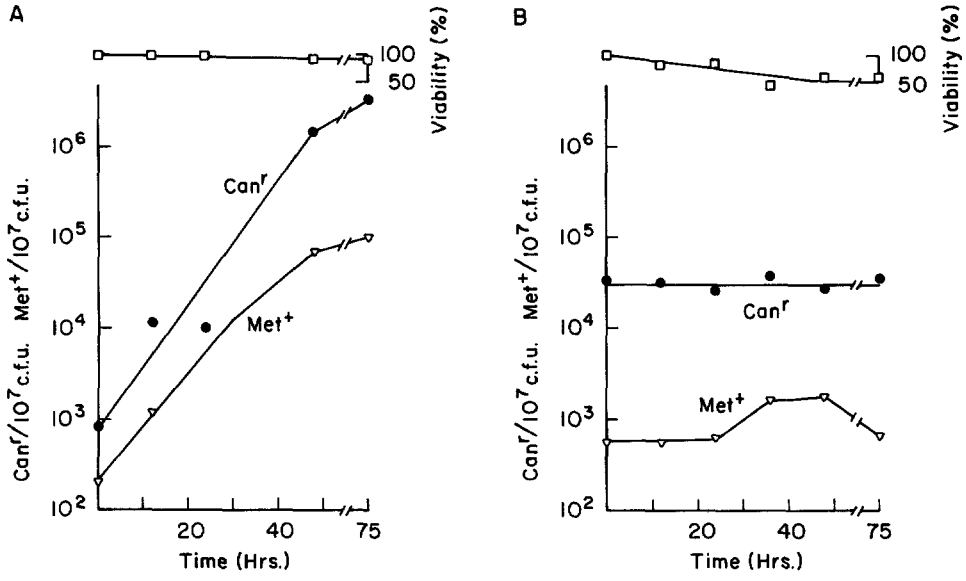

FIGURE 5.—Intragenic recombination, haploidization and viability in *rad57/rad57* diploids. Cells were placed in sporulation medium, and samples were assayed at the indicated times for *MET13* prototrophs (▽), canavanine-resistant colonies (●) and viability (□) as described previously (BORTS *et al.* 1984). A, *RAD57/rad57* (RHB220). B, *rad57/rad57* (RHB219). Sporulation was at 22°. The data from two independent sporulation experiments were pooled, and the average value at each time point was plotted.

ments with wild-type strains we have determined that the minimum detectable level of recombined *Bgl*II fragment was 0.5–1.0% of total pBR322 homologous DNA (BORTS *et al.* 1984). Since the maximum amount of *Bgl*II fragment produced in wild-type strains is 5% of the total pBR322 homologous DNA, this allows us to place an upper limit on the amount of physical exchange in the [*MAT*-pBR322-*MAT*] interval producing recombined *Bgl*II fragment as <20% of that found in wild-type strains.

spo11: As with *rad50* strains, *spo11* strains accumulate no detectable recombined fragment (Figure 4). *spo11* strains produce essentially no viable haploid progeny which have undergone meiotic intragenic recombination at *MET13* and *LEU2* (Table 2). Premeiotic DNA synthesis is normal in these strains, and no haploidization, as determined by an increase in canavanine-resistant colonies, is detected. Although no mature asci are formed in liquid sporulation, DAPI staining indicates that most cells are tetranucleate by 24 hr after initiation of sporulation (data not shown).

rad57: *rad57-1* is a mutation causing sensitivity to γ-rays at 18°. GAME *et al.* (1980) have also shown *rad57-1* to be cold-sensitive (18–25°) for sporulation and the production of viable intragenic recombinants. In our experiments, *rad57-1* strains show drastically reduced prototroph formation at both the restrictive temperature (18° or 25°) and the permissive temperature (30–34°) (Figure 5; Table 2), although they retain the cold-sensitive γ-radiation-sensitive phenotype. Premeiotic DNA synthesis is normal, but no asci are formed at

either temperature. At neither temperature could haploidization be detected by an increase in canavanine-resistant colonies. The reasons for these differences are unclear, but may be due to differences in strain background or to the presence of modifying mutations.

Unlike *rad50*, *spo11* and *rad52*, diploids homozygous for *rad57* make nearly wild-type levels of recombined *Bgl*II fragment, even at the temperature restrictive for γ-ray sensitivity (Figure 4; Table 2). The kinetics of appearance of the physically recombined fragment are normal (data not shown). Thus, although *rad57* diploids are severely deficient in the production of viable recombinant progeny, exchange of DNA molecules appears to be occurring at nearly normal levels.

rad6: *rad6* has previously been reported as undergoing limited prototroph formation, no haploidization, no ascus formation and very little cell death after initiation of sporulation (GAME *et al.* 1980). These results have been interpreted to suggest that *rad6* strains are blocked shortly after completion of premeiotic DNA synthesis. Here, we report experimental results that lead to a different interpretation of the role of *rad6* in recombination and meiosis. *rad6* appears to have little or no effect on the physical exchange of DNA molecules, as *rad6* strains produce nearly wild-type levels of recombined *Bgl*II fragment (Figure 4). The average maximum value for three independent experiments with *rad6* strains was about 65% of the heterozygous control; however, unlike *rad57*, the appearance of the fragment is delayed relative to heterozygous controls. In three separate experiments, at a time when the heterozygous control had produced the maximum amount of the 16.2-kb *Bgl*II fragment, *rad6* strains had only accumulated approximately 50% of the total recombined *Bgl*II fragment that was seen at later times.

Approximately 9% of the cells make visible, but immature, deformed asci. The increase in canavanine-resistant colonies is consistent with 16% haploidization. Both binucleate and tetranucleate cells are visible by DAPI staining. Although *rad6* is deficient in the completion of meiotic events, *rad6* strains are unusual in that they show a dramatic allele- or locus-specific effect on intragenic recombination. *MET13* prototrophs increase only fivefold and accumulate to approximately 16% of the heterozygous control level by 24 hr. This result is similar to that reported by GAME *et al.* (1980) for *HIS1* prototrophs. In contrast, *LEU2* prototrophs accumulate to 74% of the level of prototrophs found in the heterozygous controls (Figure 2C; Table 2).

In spite of the observation that *rad6* strains are severely defective in ascus formation, they undergo a significant amount of haploidization upon return to vegetative growth. We examined 169 *LEU2* prototrophs from four independent experiments to determine the proportion that expressed the recessive allele at four heterozygous loci. About 30% expressed a recessive marker at one or more of the four loci (28% *ade1*, 32% *trp5*, 29% *his6* and 33% *cyh2*). Since *ade1*, *trp5* and *his6* are all centromere-linked, these results indicate that about 60% of the Leu^+ population has undergone haploidization or chromosome nondisjunction. As a further measure of the proportion of Leu^+ prototrophs that were haploid, we determined what proportion of *LEU2* prototrophs con-

tained only one copy of chromosome *III*. Of 47 Leu$^+$ analyzed, 23 contained only one copy of chromosome *III*. Seven of these expressed an **a-** or α-mating phenotype as determined by genetic analysis. Sixteen contained a single copy of the structure [*MAT***a**-*URA3-leu2-MAT*α] or [*MAT*α-*URA3-leu2-MAT***a**] as determined from Southern blots. These 23 segregants are apparently haploid: all recessive markers show 1:1 segregation (12 of 23 Ade$^-$, 15 of 23 Trp$^-$, 9 of 23 His$^-$, 12 of 23 Cyh^r), as would be expected from a population that had completed haploidization. Of the remaining 24 segregants, all of which contain two copies of chromosome *III*, there is only one Ade$^-$ segregant and are no His$^-$, Trp$^-$ or Cyh^r segregants, indicating that these recombinant segregants are still diploid.

DISCUSSION

We have used physical detection of recombined DNA to expand the criteria by which meiosis- and recombination-defective mutants can be phenotypically distinguished. The utility of such a method is demonstrated by the distinctions it has allowed us to make between the group of γ-ray-sensitive mutations, *rad50*, *rad52* and *rad57*. Although diploids homozygous for any of these mutations fail to yield viable haploid spores and permit only very small increases in intragenic recombinants, the actual level of physical exchange of DNA molecules in the *MAT-URA3-leu2*-pBR322-*MAT* interval is quite different for each mutant. *rad50* diploids yield no detectable recombined restriction fragment, whereas both *rad52* and *rad57* strains accumulate significant levels of recombined DNA. In this regard, it is important to note viable spores are also not recovered from *spo13 rad52* or *spo13 rad57* diploids (MALONE and ESPOSITO 1981; R. E. ESPOSITO, personal communication). This result has been interpreted to mean that *rad57* and *rad52* fail to complete meiotic recombination events and, thus, generate many lethal lesions. The fact that *rad57* cells make wild-type levels of recombined fragment raises the question of the source of lethality in these strains, as the ability to complete exchange of DNA molecules (as measured in one chromosomal interval) did not ensure the production of viable haploid progeny.

The production of physically exchanged DNA molecules in the absence of virtually any viable haploid or diploid intragenic recombinants can be explained in several ways. It is possible that, in *rad52* and *rad57* strains, exchange events in some regions of the genome (of which [*MAT-URA3*-pBR322-*MAT*] might be one) might be more readily completed than in others. Examination of other regions of the genome would test this possibility. Alternatively, the apparently wild-type level of crossing over seen in *rad57* strains (or the one-third of wild-type level in *rad52* diploids) does not preclude the possible accumulation of a significant number of unrepaired lesions in other regions of the genome. In *S. cerevisiae* there are approximately 100 exchange events per cell per meiosis; consequently, the failure to repair even a few percent of the DNA breaks would lead to aneuploid, and hence inviable, progeny. Further, it is possible that the novel *Bgl*II fragments we monitor need not always reflect a completed exchange event. The presence of an unrepaired nick, a small gap

or even a short single-stranded tail would not affect the electrophoretic mobility of the *Bgl*II fragment sufficiently for us to detect such an alteration. An analysis of denatured DNA from *rad52* and *rad57* cells will be useful for testing this hypothesis.

It is also possible that the types of recombination events detected in *rad52* and *rad57* are not the same as those observed in wild-type strains, where more than 95% of the recombined fragments are the products of an exchange of DNA molecules, rather than of gene conversion of one of the flanking *Bgl*II polymorphisms (BORTS *et al.* 1984). In mutant strains, gene conversion events of the flanking *MAT* regions might contribute a substantial proportion of the recombined *Bgl*II fragment (although conversion frequencies of *MAT* would have to be elevated one to two orders of magnitude to account for the levels of recombined *Bgl*II fragment observed). A further possibility is that the recombined DNA is generated by a *RAD52*- or *RAD57*-independent mechanism that may be intrinsically nonreciprocal. For example, mitotic recombinants in *rad52* strains result from gene-conversion associated exchange events in which the reciprocal recombined chromosome is not recovered (HABER and HEARN 1985). Thus, although it is clear that by physical monitoring techniques we can detect recombination events not indicated by a genetic analysis of viable meiotic products, further study will be necessary to determine if the type of exchange events are inherently different from wild-type events.

Our analysis of *rad50* and *spo11* substantiates previous results obtained using *spo13* to bypass the first meiotic division (ESPOSITO and KLAPHOLTZ 1981; MALONE 1983; KLAPHOLZ, WADDELL and ESPOSITO 1985). Those genetic studies suggested that both *rad50* and *spo11* abolished meiotic crossing over and were meiotic-lethal mutations because of massive nondisjunction caused by the absence of proper chromosome pairing and/or crossing over. In our hands, neither *rad50* nor *spo11* strains yielded recombined DNA.

In addition to the γ-sensitive *rad* mutations, we have studied the UV-sensitive mutation *rad6*. Previous workers have suggested that *rad6* is blocked very early in meiosis. The results of GAME *et al.* (1980) and of MALONE (1983) suggested that *RAD6* acted before commitment to intragenic recombination or chromosome pairing, or else was on a pathway independent of the recombination pathway involving *RAD50* (and, therefore, *RAD52* and *RAD57*). We have found *rad6* strains to be capable of a limited amount of abnormal sporulation (although no dissectable asci are formed) and a range of intragenic recombination from 16 to 74% of wild-type levels. It is possible that our observation of some abnormal ascospores and a higher level of canavanine-resistant colonies than was observed by GAME *et al.* (1980) represents a difference in genetic background. We do not believe that we have acquired a partial suppressor of *rad6,* since all meiotic Leu^+ segregants analyzed retained the UV-sensitive phenotype. Despite these apparent differences, both our analysis of *LEU2* prototrophs and the examination of *HIS1* prototrophs (GAME *et al.* 1980) showed that a significant fraction of the prototrophic colonies had undergone haploidization.

The fact that physical exchange in the [*MAT-URA3-leu2*-pBR322-*MAT*] in-

terval occurred in *rad6* strains at levels that were >60% of wild type indicates that the *rad6* mutation does not preclude crossing over. It is possible that a primary lesion in *rad6* may be in mismatch repair processes, not in the physical exchange of DNA molecules. This is also consistent with its known role in the repair of damage due to ultraviolet irradiation and chemical mutagens in mitotic cells (PRAKASH 1974; KERN and ZIMMERMAN 1978). Although not on the same recombination pathway as *rad50*, *spo11*, *rad52* and *rad57*, the completion of *RAD6* function would still be required for ascus formation and viable spore production, as well as for normal levels of intragenic recombination. The possibility that *rad6* strains accumulate intermediates of recombination, such as heteroduplex DNA, can be investigated by an extension of the physical techniques we have described.

Alternatively, it is possible that exchange events in the [*MAT-URA3-leu2*-pBR322-*MAT*] interval, in contrast to some other chromosomal regions, occur in a predominantly *RAD6*-independent manner. This might explain why the level of *LEU2* prototrophs arising in the same interval were only slightly reduced (74% of wild type), whereas recovery of intragenic recombinants in another region, *MET13*, was more significantly affected (16% of wild-type levels).

It seems clear that each of the *rad* mutations that we have examined affects recombination in fundamentally different ways. The object of our work and that of other laboratories is to establish the molecular role that each of these genes plays in chromosome pairing, recombination and chromosome segregation. A complete understanding of these functions must also account for the fact that mutations in each gene have different effects in meiotic *vs.* mitotic recombination, both singly and in combination. Although *rad50*, *rad52* and *rad57* are all γ-ray-sensitive, this does not necessarily mean that they are all involved in double-strand break repair, *per se*. For example, *rad52* is lethal to cells undergoing the apparent double-strand break repair associated with mating-type gene switching (MALONE and ESPOSITO 1980; WEIFFENBACH and HABER 1981), whereas neither *rad50* nor *rad57* affect this process (MALONE and ESPOSITO 1980; J. E. HABER and R. H. BORTS, unpublished observations; J. GAME, PERSONAL COMMUNICATION). Furthermore, *rad52*, but not *rad50* or *rad57*, reduces mitotic intragenic recombination to 10% of wild-type levels and also significantly alters the types of recombinants that are recovered (MALONE and ESPOSITO 1980; JACKSON and FINK 1981; HABER and HEARN 1985). In mitotic cells, *rad50* fails to rescue *rad52* homothallic strains or restore normal mitotic recombination and is therefore not epistatic to these *rad52* phenotypes, although *rad50* does rescue *rad52* meiotic cells (MALONE 1983).

In meiotic cells, these three *rad* mutants also display distinct differences. DNA isolated from sporulating cells has revealed that *rad52* and *rad57* strains accumulate single-strand interruptions during meiosis (RESNICK *et al.* 1981, 1984a,b; HØGSTET and ØYEN 1984; J. L. NITISS and M. A. RESNICK, personal communication), whereas *rad50* strains accumulate neither single- or double-strand lesions (RESNICK *et al.* 1984a,b). Despite the accumulation of single-strand breaks, *rad57* is capable of apparently completing physical exchange of

DNA molecules at wild-type levels, and *rad52* yields recombined DNA at approximately 30% of its heterozygous control. A more complete understanding of the role of the gene functions will require further characterization of the ways in which meiotic and mitotic recombination events are initiated and resolved.

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