Changes in DNA During Meiosis in a Repair-Deficient Mutant (*rad* 52) of Yeast

Abstract. *The kinetic patterns of DNA synthesis in wild-type* (RAD$^+$) *and* rad *52 mutants of yeast, which exhibit high levels of synchrony during meiosis, are comparable. However,* RAD *52 mutants accumulate single-strand breaks in parental DNA during the DNA synthesis period. Thus, the product of the* RAD *52 gene has a role in meiotic DNA metabolism, as well as in the repair of DNA damage during mitotic growth. The observed breaks may be unresolved recombination intermediates.*

Genetic evidence has established the importance of DNA repair mechanisms during meiosis (*1*). For example, several meiotic mutants of *Drosophila* are sensitive to mutagens (*2, 3*) and, conversely, various mutagen-sensitive mutants of fungi (*4, 5*) and *Drosophila* (*2*) affect meiosis. In some cases in mitotically growing cells, the genetic defects have been identified at the molecular level as repair defects (*6–8*). Although events of a repair type have been observed during normal meiosis (*9, 10*), the function of the resulting molecular changes has not been demonstrated; nor have repair functions identified in mitotically growing cells been shown to have a role in meiosis.

Mutants of the yeast *Saccharomyces cerevisiae*, deficient in some aspects of DNA repair during mitotic growth, are able to initiate meiosis, but do not undergo normal genetic recombination (*5, 11*), and for some mutants the meiotic products (ascospores) are inviable. In particular, strains carrying mutations of the *RAD* 52 gene do not undergo radiation-induced genetic recombination and lack the ability to repair DNA double-strand breaks produced by ionizing radiation (*8, 11, 12*). Since the repair of double-strand breaks in yeast is believed to involve recombination between homologous chromosomes or sister chromatids (*12*), the *rad* 52 mutant might exhibit changes in DNA during meiosis. Therefore, using recently developed techniques (*13*) for detecting small numbers of single- and double-strand breaks in high-molecular-weight DNA molecules, we examined the chromosomal DNA of yeast throughout meiosis in *rad* 52 and wild-type (*RAD*$^+$) strains. We have shown that a *rad* 52 mutant, unlike the *RAD*$^+$ strain, accumulates a limited number of single-strand breaks during meiosis.

For the detection of changes in DNA during meiosis we used strains and conditions that would provide a high degree of meiotic synchrony; this would improve the detection of small numbers of breaks that might occur transiently during the meiotic cycle. By using a strain (SK-1) known to yield synchronous meiosis (*14*) as a parent in a series of backcrosses with a *rad* 52 mutant (*15*), we obtained nearly isogenic *rad* 52 and *RAD*$^+$ derivatives that gave exceptional sporulation synchrony when they were grown in acetate medium and transferred to the sporulation medium.

To define synchrony during meiosis, we monitored (i) the uptake of radioactive label into DNA, (ii) the increase in DNA as measured by diphenylamine assays, and (iii) the appearance of ascospores (*16*). The *RAD*$^+$ strain began to incorporate label into DNA 2 hours after transfer to sporulation medium (Fig. 1). Increases in total DNA were first detected at 2 hours, and the increase continued linearly for approximately 3 hours. The total DNA in the culture doubled, as expected for one round of meiotic DNA synthesis. Sporulation began at 8 hours, and the frequency of sporulated cells (asci) increased rapidly, reaching 93 percent by 14 hours. Similar kinetics were observed in these three processes for the *rad* 52 mutant, except that the total frequency of sporulation was only 29 percent, and the spores were inviable [similar to results in (*5*)]. From these results with the *RAD*$^+$ and the *rad* 52 strains, we conclude that strains with the SK-1 background can yield a synchronous meiosis [for comparison, see (*5*)], and the *rad* 52 mutation does not affect the rate or extent of DNA synthesis or the rate of spore formation.

High-molecular-weight DNA was recovered during meiosis by use of a sucrose gradient technique that minimizes the handling of samples (*13*) (Fig. 2). It relies on the removal of cell walls in the presence of a nonionic detergent to cause immediate lysis; all operations are performed in a centrifuge tube so that gradients can be formed under the lysis suspension. In this way we avoided artifacts that could result from handling spheroplasts during sucrose gradient analysis (these artifacts could arise during the concentration of spheroplasts by centrifugation) and during transfer to the

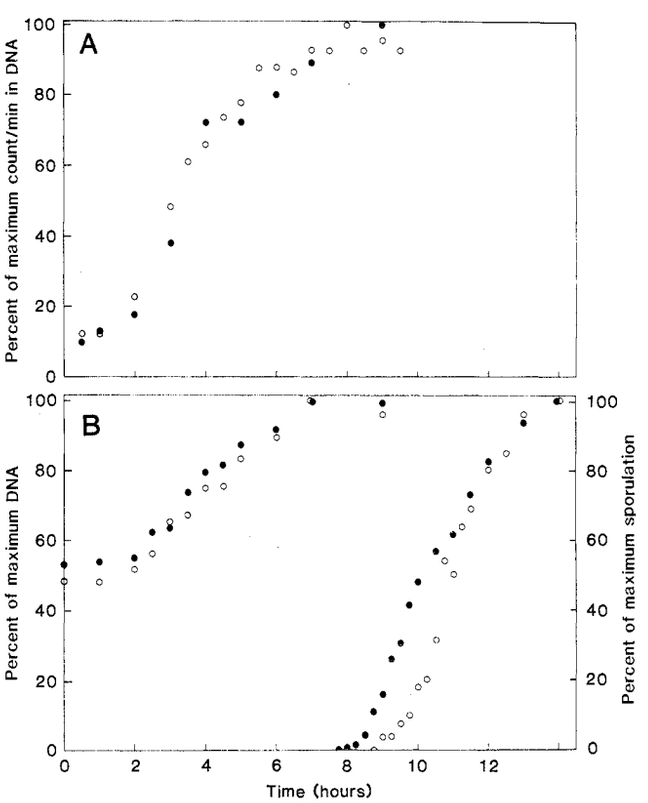

Fig. 1. DNA synthesis and sporulation during meiosis in (●) *RAD*$^+$ and (○) *rad* 52 strains. Cells were grown in presporulation medium and subsequently resuspended in sporulation medium (1 percent potassium acetate) at time 0 (*16, 21*). (A) At 30 minutes after resuspension of cells in the sporulation medium [5, 6-^{3}H]uracil (10 μCi/ml, 40 Ci/mmole) plus uracil (0.5 μg/ml) was added. The maximum incorporation into the DNA of *RAD*$^+$ and *rad* 52 strains was 1.5×10^3 and 1.9×10^3 count/min per 10^7 cells, respectively. (B) The left part of the figure corresponds to DNA as measured by diphenylamine assays (*16*); the maximum DNA for *RAD*$^+$ and *rad* 52 was 5.27 and 5.68 μg per 10^8 cells, respectively. The right portion of the figure corresponds to the appearance of asci; the maximum percentage of cells forming asci are 93 percent and 30 percent for *RAD*$^+$ and *rad* 52, respectively.

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sucrose gradients. As few as 10 to 20 breaks per haploid genome (about 10^{10} daltons), corresponding to about one break per chromosome, are detectable (*13*).

As a control, ^{14}C-labeled cells, which had been treated in the same manner as the ^{3}H-labeled cells up to the time of resuspension in sporulation medium, were included with the ^{3}H-labeled meiotic cells for gradient analysis. There do not appear to be significant changes in the DNA profiles of cells harvested at any stage of meiosis. We have detected small shifts, which are probably short-lived since they are not seen in successive 1-hour samples. The number average molecular weight (M_n) of the nuclear DNA in these experiments, 1.8×10^8, is comparable to that reported for mitotic cells (*8*, *13*) and indicates that the profiles correspond to full-length chromosomal DNA (*17*). Radioactive counts in the peak near the top of the gradient correspond to mitochondrial DNA as determined by CsCl density gradient analysis (*18*). We conclude that there is little, if any, accumulation of single- or double-strand breaks in parental DNA (data not shown) during meiosis in our *RAD*$^+$ strains of yeast. Breaks that do occur must be few or short-lived. Although reports of experiments on wild-type yeast (*19*) have indicated that long-lived single-strand breaks begin to appear during meiotic DNA synthesis, these reports lacked well-resolved profiles, and in some of the experiments the presence of large amounts of small-molecular-weight nuclear DNA was observed. In addition, many of the breaks may have occurred during the preparation of cells for sucrose gradient analysis. (We have found that during meiosis the spheroplasts recovered after removal of the cell wall are often fragile and lyse easily, so that breaks could inadvertently be introduced into the DNA.)

The DNA profile of the *rad* 52 is similar to that of the *RAD*$^+$ strain during mitotic growth (Fig. 2B), indicating that DNA breaks do not occur under normal growth conditions. When *rad* 52 cells are incubated in sporulation medium, single-strand breaks begin to appear during the period of DNA synthesis (Fig. 2, C and D); none are observed before this time. By 6 hours, the number of breaks is approximately one per average-sized molecule (Table 1); the DNA is larger than the mitochondrial DNA, and no breaks are observed in the mitochondrial DNA under these conditions. These observations did not differ in several experiments. We did not observe any repair of these breaks during the course of individual experiments, although we have not excluded the possibility of an equilibrium after the establishment of breaks between break appearance and repair. The breaks do not appear to be the consequence of double-strand breakage, as indicated by analysis of chromosomal DNA with neutral sucrose gradients (data not shown). Although some double-strand breaks can be detected in *rad* 52, they occur later than DNA synthesis and account for only a small number of the single-strand breaks (*20*). Since the counts in DNA increase slightly during meiotic DNA synthesis [presumably due to incorporation from RNA (*21*)] and then remain constant, the breaks that occur during meiosis cannot be attributed to any generalized DNA degradation (see Table 1).

We conclude that the absence of the

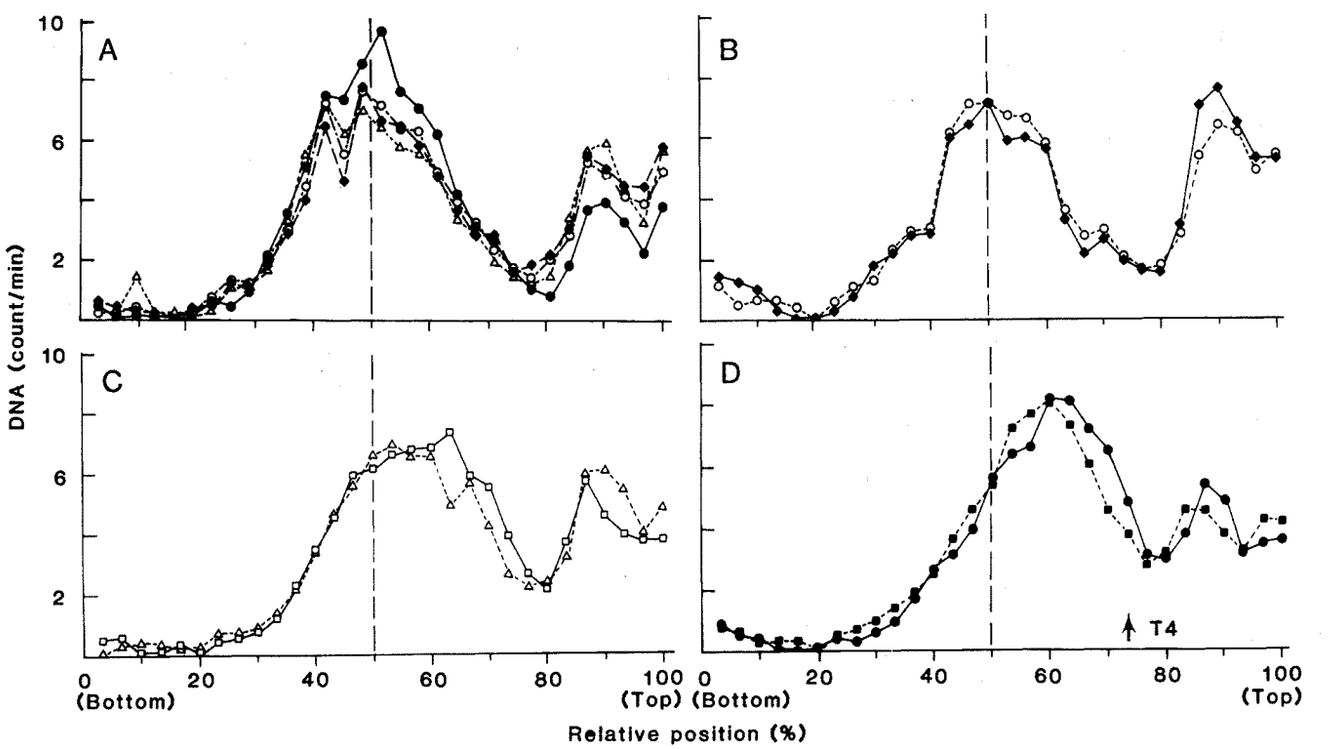

Fig. 2. Alkaline sucrose gradient analysis of the DNA from *RAD*$^+$ and *rad* 52 cells during meiosis. Cells were grown in presporulation medium containing [5, 6-^{3}H]uracil (10 μCi/ml, 40 Ci/mole), plus uracil (0.5 μg/ml) or [^{14}C]uracil (7.5 μCi/ml, 57 Ci/mole) plus uracil (0.5 μg/ml). To follow changes during meiosis, the cells were resuspended at time 0 in sporulation medium lacking radioactive label. The processing of samples is described in (*13*). Sedimentation is from right to left. Each profile is the result obtained from a gradient that contained ^{14}C-labeled mitotic DNA and the ^{3}H-labeled DNA from meiotic cells. The profiles of the control ^{14}C-labeled DNA did not vary significantly between gradients. Therefore, the profiles of ^{3}H-labeled DNA from separate gradients were compiled in single figures. (A) *RAD*$^+$: (♦) ^{14}C-labeled mitotic cells; ^{3}H-labeled cells after (○) 1 hour, (△) 3.5 hours, and (●) 6 hours of meiosis. (B to D) *rad* 52: (♦) ^{14}C-labeled mitotic cells; ^{3}H-labeled cells after (○) 1 hour, (△) 3 hours, (□) 4 hours, (●) 6 hours, and (■) 7 hours of meiosis. Included in (D) is the position to which T4 bacteriophage DNA sediments under these conditions.

RAD 52 function results in the accumulation of a limited number of single-strand breaks beginning in the DNA synthesis period of meiosis. Although these single-strand breaks are prevented by hydroxyurea (*18*), an inhibitor of meiotic DNA synthesis (*22*), it is not clear that the breaks are directly associated with DNA synthesis. This report shows that a mutation affecting meiosis leads to specific DNA changes during meiosis. (While apurinic or apyrimidinic sites would yield similar alkaline sucrose gradient results, it is unlikely that they would significantly affect spore viability or recombination.) These breaks were correlated with lethality and a lack of recombination in *rad* 52 mutants during meiosis [see also (*5*)].

Since the ^3H label in these experiments is primarily located in the parental strands (after switching from growth medium to sporulation medium, there is less than a 25 percent increase in counts in DNA; Table 1), the measured breaks in these experiments are primarily located in parental strands. These breaks are probably different from single-strand breaks induced by ionizing radiation, since the latter are repaired in *rad* 52 mutants (*23*). Presumably they occur after DNA synthesis because breaks or gaps in parental DNA would inhibit synthesis and would yield double-strand breaks during replication. We propose that they are associated with the recombination process and that in RAD^+ strains the recombination intermediates are rapidly resolved so that breaks or gaps are not detectable. The number of breaks per cell that can be estimated from these results is in the expected range for the number of recombinational events per cell (*24*). If this hypothesis is correct, then the first appearance of breaks would correspond to the beginning of recombination in yeast. Based on the results of Olson and Zimmerman (*25*), recombination can begin during the meiotic DNA synthesis period, before the formation of the synaptonemal complex (*25*).

Our results are consistent with the concept that the product of the *RAD* 52 gene plays a central role for recombination in yeast. It mediates ultraviolet-induced and ionizing radiation–induced mitotic recombination, spontaneous mitotic and meiotic recombination, and the events (presumably recombinational) involved in mating-type switching (*26*). The DNA changes detected in the present experiments presumably correspond to intermediates in recombination, and further studies with *rad* 52 and other DNA repair mutants in this genetic pathway (*27*) will contribute to the elucidation of the molecular mechanism of recombination during meiosis as well as the importance of mitotically defined DNA repair mechanisms.

Table 1. Changes in the molecular weight (M_n) of nuclear DNA and the amount of ^3H in RNA and DNA during meiosis of cells grown in the presence of [^3H]uracil.

Time* (hours)	^3H RNA ($\times 10^5$ count/min)†	^3H DNA ($\times 10^3$ count/min)†	M_n‡ ($\times 10^8$ daltons)
	RAD^+		
0	2.2	4.8	1.9
1	2.3	5.2	1.8
2.5	2.2	5.3	1.9
3.5	1.9	6.2	1.7
6	1.5	6.0	1.9
7	1.3	6.1	2.0
	rad *52*		
0	3.0	6.6	1.8
1	3.0	8.3	1.8
3	2.7	7.7	1.3
4	2.3	7.2	1.2
6	2.1	7.4	0.9
7	1.9	8.3	1.1

*Time after introduction of cells into sporulation medium. †Samples from the sucrose gradient experiments of Fig. 2 were processed as described in (*16*, *21*); these values correspond to counts per minute per 10^7 cells. ‡The M_n for the nuclear DNA of gradients in Fig. 2 was determined according to the method of Lehmann (*17*).

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13. M. A. Resnick, J. Boyce, B. Cox, *J. Bacteriol.*, in press. Cells for sucrose gradient analysis are suspended in 0.1*M* tris sulfate, 0.01*M* EDTA (*p*H 9.3) containing beta-mercaptoethanol (2 percent) and incubated for 10 minutes at 30°C. They are then resuspended in 0.05*M* KH_2PO_4, 10 m*M* EDTA (*p*H 6.5) at 10^8 cells per milliliter, and 2×10^7 cells are placed in a cellulose nitrate centrifuge tube (treated with calf thymus DNA). To this are added 0.02 ml of 10 percent Nonidet (Bethesda Research Laboratory, Rockville, Md.) and 0.04 ml of Zymolyase (10 mg/ml; Kirin Breweries, Japan), and the mixture is incubated at 37°C for 10 minutes. A sucrose gradient is pumped into the tube beneath the lysed suspension (15 to 30 percent sucrose, 0.3*N* NaOH, 0.03*M* EDTA, and 0.7*M* NaCl); the gradients are topped with 0.05 ml of a solution containing 2 percent sarkosyl, 3 percent sodium deoxycholate, 5 percent sodium dodecyl sulfate, 0.02*M* EDTA, 0.01*M* tris (*p*H 8.0), and 18 percent sorbitol. Centrifugation is for 12 hours at 12,000 rev/min in a Beckman SW50.1 rotor at 23°C. The gradients are fractionated 8 to 10 hours later to ensure digestion of the RNA in the gradient by the alkali; they are fractionated onto Whatman No. 17 filter strips and the strips are placed in 5 percent trichloroacetic acid at room temperature for approximately 5 hours and then in 95 percent ethanol. The individual fractions are counted in a Beckman scintillation counter.
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$$M_n = \left(\sum_a^x c_i / \sum_a^x c_i/M_i \right) - M_x$$
where c_i are the counts in the ith fraction, M_i corresponds to the molecular weight of DNA in this fraction, a is the next to bottom fraction, and x is the last fraction of nuclear DNA before the "trough" between mitochondrial and nuclear DNA in Fig. 2 (that is, fractions 9 or 10 from the top).
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