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Single-Strand Scissions of Chromosomal DNA During Commitment to Recombination at Meiosis

(*Saccharomyces cerevisiae*/sporulation/sedimentation velocity)

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ABSTRACT **Diploid cells of the yeast *Saccharomyces cerevisiae* induced to undergo meiosis accumulate single-strand scissions in both template and newly synthesized DNA during commitment to genetic recombination. No evidence for accumulation of double-strand breaks during meiosis was obtained. When commitment to recombination is at the full meiotic level there are approximately 70 to 200 single-strand scissions per meiotic cell in which approximately 150 recombination events have been reported to occur.**

Genetic analysis of meiotic recombination has illustrated that recombination occurs by both gene conversion and reciprocal exchange (1). Molecular models proposed to explain these phenomena invoke the introduction of single-strand nicks (breakage of one phosphodiester bond) or gaps into the DNA of homologous chromosomes to initiate interactions which result in both conversion and reciprocal events (2, 3). The purpose of this study was to determine the timing and extent of single-strand and double-strand scissions of chromosomal DNA as diploid cells stimulated to enter meiosis become committed to genetic recombination. Yeast diploids are favorable material for a study of this type. Intact chromosomal DNA duplexes can be obtained by lysis of spheroplasts prepared from yeast cells. The chromosomes of yeast are unineme and consist of DNA duplexes ranging in molecular weight from 5×10^7 to 1.4×10^9 (4–6). These molecules can be fractionated by sedimentation velocity in neutral sucrose gradients under appropriate conditions (4, 5) and can be examined for single-strand nicks or gaps by analysis of sedimentation patterns in alkaline sucrose gradient (7).

We have found that meiotic yeast cells do not accumulate double-strand breaks but do accumulate single-strand breaks after the onset of premeiotic DNA synthesis and commitment to genetic recombination. Nicking of both template and newly synthesized DNA was observed. When commitment to recombination is at the full meiotic level we estimate there are two to six single-strand scissions/10^9 daltons of DNA per 4C meiotic cell (4C indicates four copies of each locus). The relationship of these observations to related studies of meiotic DNA metabolism and pertinent yeast genetic data is discussed.

MATERIALS AND METHODS

Organism. A diploid strain of *Saccharomyces cerevisiae* (Z193) was employed in this study. Z193 is heteroallelic at the lysine 2 (*lys* 2-1/*lys* 2-2) and tryptophan 5 (*trp* 5-R/*trp* 5-20) loci.

Procedure for Sporulation. Diploid Z193 was grown in acetate nutrient medium (YPA) at 30°, harvested during the exponential phase of growth (at approximately 1×10^7 cells/ml), washed free of medium, and transferred to acetate sporulation medium at the same cell density. The compositions of YPA and sporulation medium have been described (8). The appearance of asci was determined by hemocytometer counts of samples fixed in 4% formaldehyde.

Dectection of Intragenic Recombination. Intragenic recombination at the *lys* 2 and *trp* 5 loci leading to the production of lysine and tryptophan prototrophs, respectively, was monitored by plating aliquots on synthetic complete medium to assay viability and on lysineless and tryptophanless media to detect prototrophic intragenic recombinants. Before plating samples were sonicated to separate daughter cells that complete cell division in sporulation medium (8). After sonication the percentage of budded versus unbudded cells was determined by hemocytometer counts. The composition of synthetic complete medium has been reported (9).

Labeling of DNA During Growth and Sporulation. Cells were grown in YPA containing 2 μCi/ml of [2-^{14}C]uracil (New England Nuclear) to label DNA during growth and were transferred to sporulation medium containing 20 μCi/ml of [6-^{3}H]-uracil (Amersham-Searle) to label DNA synthesized during sporulation. YPA was supplemented with 20 μg/ml of unlabeled uracil and sporulation medium was supplemented with 50 μg/ml of unlabeled uracil. Labeling of DNA was monitored by measuring incorporation of ^{3}H and ^{14}C into alkali-stable material as previously described (8).

Spheroplast Formation. Spheroplasts were prepared by Glusulase (Endo Laboratories, Inc.) treatment by the method of Goldring *et al.* (10).

Sedimentation Velocity in Sucrose Gradients. Spheroplasts were layered directly onto a 0.10 ml layer of detergent-containing lysing buffer (4) on top of 10–25% (w/v) sucrose gradients at room temperature. Lysis was complete in 10 min. Neutral sucrose gradients (pH 8.1–8.2) consisted of 1 M NaCl, 1% Sarkosyl (Geigy Industrial Chemicals), and 0.01 M EDTA. Alkaline gradients (pH 12.1–12.5) consisted of 0.3 N NaOH, 0.7 M NaCl, 1% Sarkosyl, and 0.01 M EDTA. Gradients were prepared in nitrocellulose tubes previously boiled for 10 min or soaked overnight in 0.05 M EDTA to minimize adherence of DNA to the sides of tubes. Gradients were sedimented in Beckman SW 50.1 rotors at 10,000 rpm for 20 hr at 10°. Gradients were fractionated by puncturing the bottom of tubes and fractions were collected into 1 ml of 0.3 N NaOH containing 0.1 mg/ml of uracil. The fractions were hydrolyzed overnight at room temperature, refrigerated, neutralized with 0.5 ml of cold 0.6 N HCl and precipitated with 0.25 ml of cold

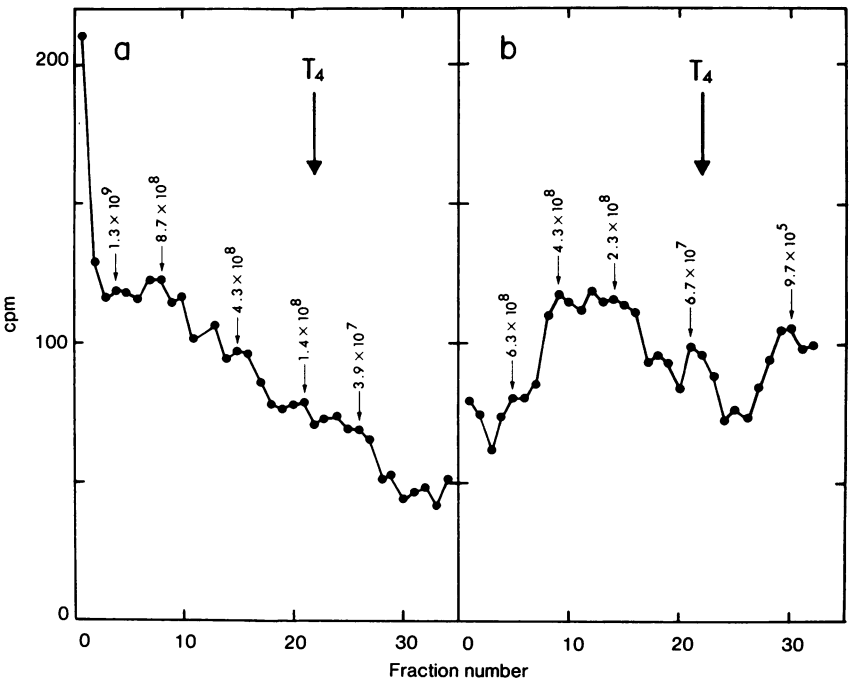

FIG. 1. Sedimentation of yeast whole cell DNA in neutral (a) and alkaline (b) sucrose gradients (10–25% w/v sucrose) centrifuged at 10,000 rpm, 20 hr. Spheroplasts prepared from [^{14}C]uracil-labeled cells were harvested during the exponential phase of growth (1×10^7 cells per ml) and lysed on the surface of gradients. Arrow denotes the position of T4 [^{3}H]DNA molecular weight 1.1×10^8 (neutral) and 5.5×10^7 (alkaline). The direction of sedimentation is from right to left.

50% trichloroacetic acid. Bovine serum albumin was added to a concentration of 0.1 mg/ml as carrier. The precipitate was collected on Whatman GF/A or Reeve-Angel glass fiber filters and prepared for scintillation counting in a toluene based scintillation fluid containing 4.0 g/liter of Omnifluor (New England Nuclear). Molecular weights were calculated using the equation of Burgi and Hershey (11) as modified by Levin and Hutchinson (12). T4 bacteriophage DNA was used as a molecular weight reference, taking 1.1×10^8 as the double-stranded molecular weight (12) and 5.5×10^7 as the single-stranded molecular weight.

Isopycnic CsCl Density Gradient Centrifugation. Dialyzed fractions from sucrose gradients as well as lysed spheroplasts were centrifuged to equilibrium in CsCl gradients to resolve chromosomal DNA (1.699 g/ml) from mitochondrial DNA (1.683 g/ml). Samples were mixed with CsCl in 0.01 M Tris·HCl, pH 8.0, and the final density was adjusted to 1.700 g/ml. Centrifugation was performed at 31,000 rpm at 18° for 60–70 hr. Fractions were collected from the bottom of the tubes and prepared for scintillation counting as for sucrose gradients.

RESULTS

Diploid cells of yeast can be stimulated to undergo meiosis and ascospore formation by transfer of exponential phase cells grown in acetate nutrient medium (YPA) to acetate sporulation medium (13). Shortly after the onset of premeiotic DNA synthesis cells become committed to genetic recombination (14). In the experiments described below we examined the sedimentation patterns of yeast chromosomal DNA in both neutral and alkaline sucrose gradients before and after exposure of cells to sporulation medium. A sporulation-proficient diploid (Z193) which has been the subject of extensive genetic analysis was employed in this study (15, 16).

Sedimentation of DNA from Acetate-Grown Cells. Previous workers have described the sedimentation profile of DNA released from spheroplasts prepared from mitotic cells grown in glucose nutrient medium (4–7). For this study it was important to determine whether cells growing in acetate medium exhibit a similar profile. The results are shown in Fig. 1. At low rotor speed (10,000 rpm) which minimizes rotor speed dependency effects, molecules of 5×10^7 to 1.4×10^9 daltons are observed in the neutral sucrose gradient (Fig. 1a). The distribution of molecular weights observed in the alkaline gradient (Fig. 1b) reflects the size range expected from the molecular weight distribution of duplex DNA molecules observed in neutral sucrose. The largest single-stranded molecules seen (Fig. 1b) have a molecular weight of approximately 7×10^8. These results indicate that the DNA of both glucose- and acetate-grown cells sediments in the same manner in neutral and alkaline sucrose gradients.

Contribution of Mitochondrial DNA to Sedimentation Patterns. In our studies the centrifugation time employed for neutral and alkaline sucrose gradients restricts mitochondrial DNA molecules mainly to the top one-third of gradients. In this manner the lower two-thirds of gradients could be examined for changes in the sedimentation profile of chromosomal DNA. The sedimentation pattern of mitochondrial DNA under the conditions we employed was determined by labeling the total cellular DNA of mitotic cells by growth in [^{14}C]uracil and then specifically labeling mitochondrial DNA with [^{3}H]uracil by the cycloheximide treatment regimen of Grossman *et al.* (17). Spheroplasts prepared from these cells were lysed on both neutral and alkaline sucrose gradients (Fig. 2a and b). Isopycnic CsCl gradient centrifugation of the cell lysate confirmed the incorporation of ^{3}H label into mitochondrial DNA (Fig. 2c).

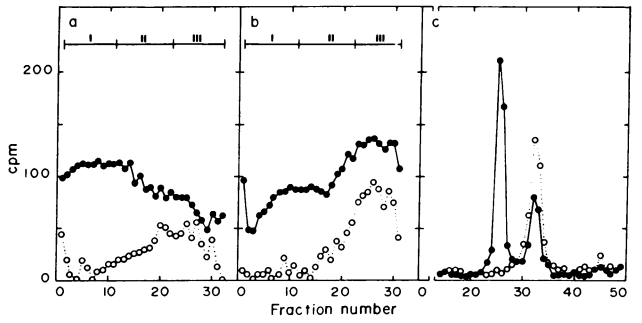

FIG. 2. Sedimentation of yeast mitochondrial DNA in neutral (a) and alkaline (b) sucrose gradients. Whole cell DNA was labeled by growth in the presence of [^{14}C]uracil (●) and mitochondrial DNA was specifically labeled with [^{3}H]uracil (○) by the method of Grossman *et al.* (17). Molecular weight distributions for neutral sucrose are: I, 1.6×10^9 to 5.6×10^8; II, 4.9×10^8 to 9.0×10^7; III, 7.0×10^7 to 1.6×10^5; and for alkaline sucrose: I, 7.7×10^8 to 2.9×10^8; II, 2.6×10^8 to 4.5×10^7; and III, 3.5×10^7 to 1.4×10^5. Sedimentation is from right to left. Isopycnic CsCl gradient centrifugation (c) confirmed the incorporation of ^{3}H label into mitochondrial DNA. Density increases from right to left.

Fig. 2 illustrates that ^{3}H-labeled mitochondrial DNA sediments in the molecular weight range of 10^8–10^6 in neutral sucrose gradients and 10^7–10^6 in alkaline sucrose. It can be seen from Fig. 2 that chromosomal DNA is essentially restricted to the bottom two-thirds of the sucrose gradients. Additional control experiments in which neutral sucrose gradients were divided into thirds, the fractions pooled, and the nuclear versus mitochondrial DNA content determined by isopycnic CsCl centrifugation, indicate that mitochondrial DNA accounts for 22% of the ^{14}C-labeled DNA in the upper third, 20% in the middle third and 0% in the lower third. The same analysis of alkaline sucrose gradients indicated that mitochondrial DNA accounts for 30% of the radioactivity in the upper third, 6% in the middle third, and 0% in the lower third. The molecular weight distribution of molecules in the top, middle, and lower third of sucrose gradients is summarized in Fig. 2. The molecular weights were calculated with reference to bacteriophage T4 marker DNA. The behavior of mitochondrial DNA in the sucrose gradients is consistent with estimates of 5×10^7 for the molecular weight of the mitochondrial genome (18) and reports of some higher-molecular-weight forms (4).

Sporulation of Diploid Z193. The sedimentation analysis of DNA during meiosis was performed in two experiments. In one experiment sedimentation in neutral sucrose gradients was monitored and in the other sedimentation in alkaline gradients was examined. In each experiment two sporulation cultures were prepared. Both cultures were established from the same starting inoculum. The culture employed for sedimentation analysis was grown in YPA medium containing [^{14}C]-uracil to label DNA and transferred to sporulation medium containing [^{3}H]uracil to label DNA made during sporulation. This culture was sampled at intervals to monitor the completion of mitotic cell division by budded cells, commitment to intragenic recombination at the *lys* 2 and *trp* 5 loci leading to prototrophy, and appearance of asci. The unlabeled culture was employed to monitor in greater detail the kinetics of completion of cell division, commitment to intragenic recombination, and ascus formation.

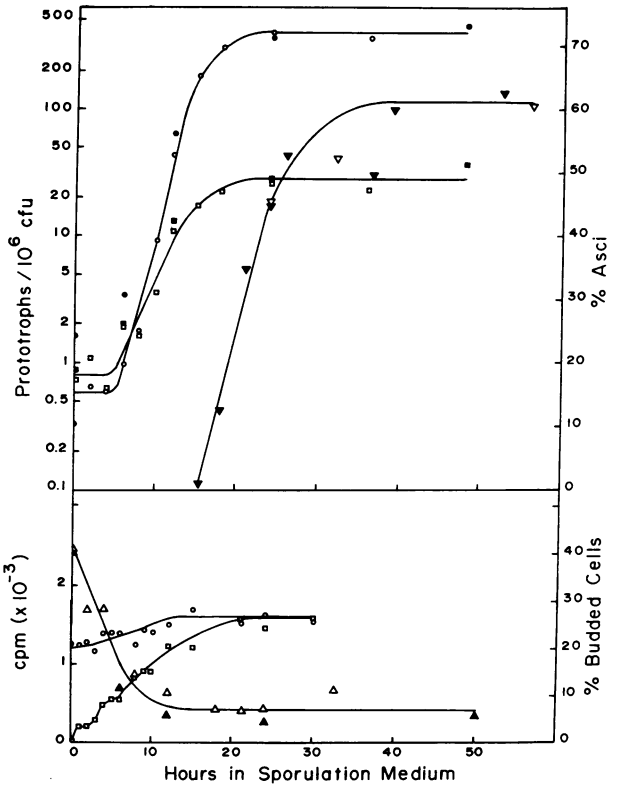

FIG. 3. Sporulation of diploid Z193. Upper panel: lysine prototrophs/10^6 colony-forming units (cfu) (○,●), tryptophan prototrophs/10^6 colony forming units (□, ■), percent asci (▽, ▼). Open symbols refer to the unlabeled culture and closed symbols refer to the culture labeled with [^{14}C]- and [^{3}H]uracil. Lower panel: cpm [^{3}H]DNA/0.5 ml of sample (□), cpm [^{14}C]-DNA per 0.5 ml of sample (○), percent budded cells in the unlabeled culture (△), percent budded cells in the labeled culture (▲).

Typical data for completion of cell division, premeiotic DNA synthesis, commitment to recombination at *lys* 2 and *trp* 5, and ascus formation are shown in Fig. 3. Premeiotic DNA synthesis in yeast draws extensively upon endogenous DNA precursor pools; therefore ^{14}C-labeled cells transferred to sporulation medium continue to incorporate ^{14}C label into DNA (19). Both the further incorporation of ^{14}C and appearance of ^{3}H label in DNA reflect net premeiotic DNA synthesis but the latter is less reliable as an indicator of net nuclear DNA synthesis due to preferential labeling of mitochondrial DNA during the first 3–4 hr of sporulation by precursors supplied in sporulation medium (20).

Sedimentation Velocity Analysis of DNA During Meiosis. Figs. 4 and 5 depict neutral and alkaline sedimentation patterns of DNA released from spheroplasts prepared from sporulation cultures. Three features of the sedimentation studies are evident:

(*1*) Incorporation of [^{3}H]uracil into DNA at early times is confined to regions of the gradient where mitochondrial DNA sediments. This result is expected, since previous studies have shown that exogenous label is preferentially incorporated into mitochondrial DNA during early sporulation (20). At later times the incorporation of ^{3}H label into chromosomal DNA is evident. The sedimentation patterns of ^{3}H-labeled DNA

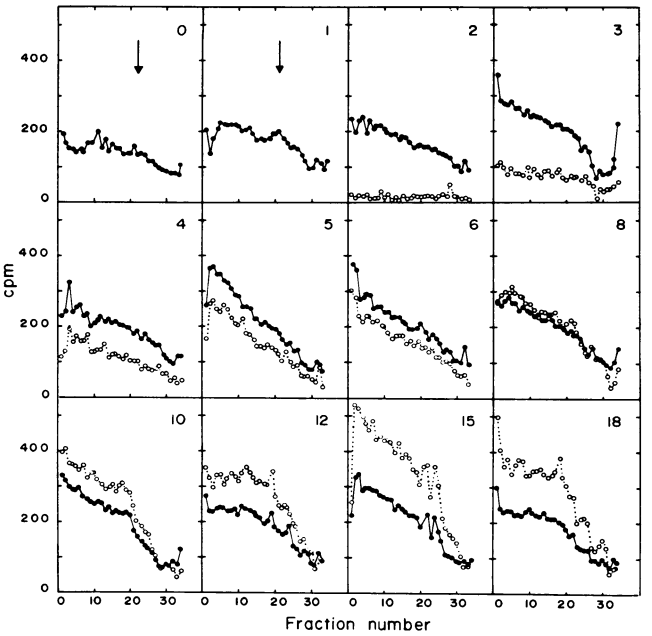

FIG. 4. Sedimentation profiles in neutral sucrose gradients of DNA released from spheroplasts prepared after exposure of Z193 to sporulation medium from 0 to 18 hr. Arrow denotes the position of T4 [^{3}H]DNA, molecular weight 1.1×10^8 [^{14}C]DNA (●), [^{3}H]DNA (○). Sedimentation is from right to left.

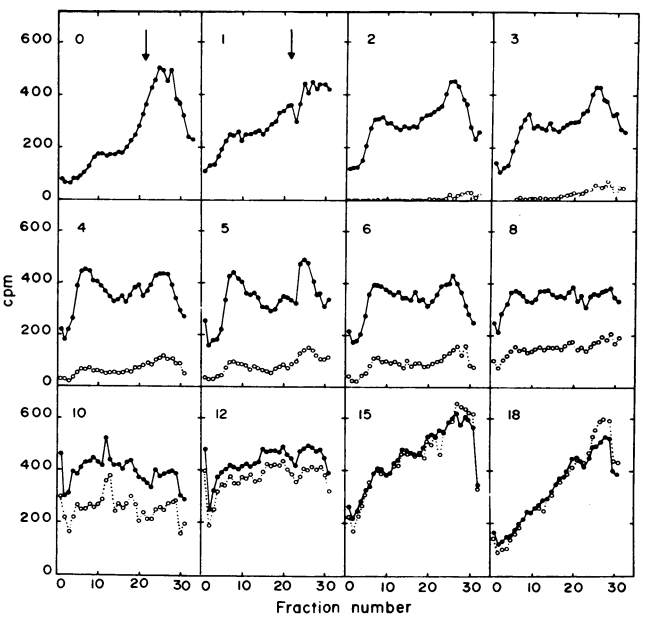

FIG. 5. Sedimentation profiles in alkaline sucrose gradients of DNA released from spheroplasts prepared after exposure of Z193 to sporulation medium from 0 to 18 hr. Arrow denotes the position of T4 [^{3}H]DNA, molecular weight 5.5×10^7 [^{14}C]DNA (●), [^{3}H]DNA (○). Sedimentation is from right to left.

(newly synthesized) and ^{14}C-labeled DNA (parental + newly synthesized) do not differ significantly from one another.

(*2*) There is no evidence for accumulation of double-strand breaks during the first 18 hr of sporulation. By this time 10% of the cells have formed asci.

(*3*) The sedimentation profiles in alkaline sucrose reveal large-scale changes in the banding pattern of single-stranded DNA. Brief exposure to sporulation medium for about 10 min apparently interferes with joining activity. Time 0 cultures which have been suspended in sporulation medium and quickly harvested exhibit more nicking of DNA than is detected in mitotic cells (Fig. 1b). From 1 to 4 hr the nicks present at 0 hr are sealed and the alkaline profile at 4 hr resembles that of mitotic cells (Fig. 1b). By 5 hr two discrete size classes of molecules are present. At 6 hr the population of molecules becomes more heterogeneous and remains so until 10 hr. After 10 hr a loss of fast-sedimenting DNA molecules is noticeable. This accumulation of single-strand nicks or gaps thus begins well after the onset of premeiotic DNA synthesis and both newly synthesized strands (^{3}H-labeled) and parental DNA strands are nicked to the same degree. Control experiments in which spheroplasts recovered beyond 10 hr were lysed in the presence of labeled phage T4 marker DNA indicated that the nicking observed at 12, 15, and 18 hr does not occur during lysis of the spheroplasts.

The changes in sedimentation profile in alkaline sucrose are not due to selective release of DNA from spheroplasts. The recovery of radioactivity from gradients was greater than 85%. At times after 10 hr approximately 15% of the radioactivity in DNA was recovered in the pellet fractions. These contained ascospores that are refractory to Glusulase treatment.

Commitment to Recombination and Accumulation of Single-Strand Scissions. During the interval from 10 to 18 hr the fraction of ^{14}C-labeled chromosomal DNA of molecular weight 7.7×10^8 to 2.9×10^8 (Fig. 2) sedimenting in the lower third of the alkaline gradients declines by 50% (i.e., from 36% at 10 hr to 19% at 18 hr). The extent of the decline was calculated by subtracting the contribution of mitochondrial [^{14}C]DNA to the total [^{14}C]DNA observed in gradients and expressing the total ^{14}C cpm in the bottom third of alkaline gradients as a percent of the total ^{14}C cpm in chromosomal DNA. The proportion of ^{14}C cpm in mitochondrial DNA does not change during sporulation (20) and the data shown in Fig. 2c were employed to obtain this value. From 10 to 18 hr the fraction of sporulated cells increased from 0% to 10% (Fig. 3). The frequency of recombination among unsporulated cells present at 18 hr is at 60% of the meiotic level for the *lys* 2 heteroalleles and 64% of the meiotic level for the *trp* 5 heteroalleles. These values for unsporulated cells at 18 hr were obtained by subtracting the contribution of the asci (in which recombinants are always present at the full meiotic level) from the frequencies of lysine and tryptophan prototrophs observed by direct plating at 18 hr (Fig. 3).

DISCUSSION

The studies presented in this paper provide evidence for the accumulation of single-strand scissions in chromosomal DNA of meiotic yeast cells. Similar results have been obtained in studies of meiotic cells of *Lilium* (21). As in the case of *Lilium*, accumulation of single-strand breaks does not result in the accumulation of double-strand breaks (21, 22). We have observed the accumulation of single-strand breaks in both template and newly synthesized DNA after the onset of premeiotic DNA synthesis and during commitment of cells to recombination at the *lys* 2 and *trp* 5 loci. The accumulation of single-strand nicks may serve to explain certain features of recombination during meiosis of yeast. Yeast cells exposed to sporulation medium become committed to both intragenic and

intergenic recombination before they become committed to meiotic chromosomal segregation (15, 23). Cells selected for recombination in one region of the genome exhibit a gradual increase in the frequency of exchange in other unselected intervals (15, 16). The frequencies of recombination in unselected intervals among cells uncommitted to meiotic chromosomal segregation eventually achieve the values typical of the haploid products of meiosis (16). If the number of single-strand nicks per 4C nucleus contributes to the probability of exchange, their accumulation could account for the progressive increase in recombination.

We have estimated the number of single-strand nicks per 10^9 daltons of DNA when yeast meiotic cells achieve full commitment to recombination by comparing the rate of single-strand nick accumulation with the rate at which cells become committed to recombination. In the interval from 10 to 18 hr unsporulated cells become committed to recombination (at the *lys* 2 and *trp* 5 loci) at approximately 60% of the meiotic level. The fraction of single-stranded DNA molecules in the size range 2.9 to 7.7 $\times$ 10^8 daltons sedimenting in the lower third of alkaline sucrose gradients is halved. The longest molecules in this section of the gradient would require approximately three nicks to remove them from the lower third of alkaline gradients. The average molecule (5 $\times$ 10^8 daltons) would require a minimum of one nick. If this rate of nicking in relation to commitment to recombination is maintained, then we estimate that there are two to six nicks per 10^9 daltons of DNA when cells are fully committed to recombination.

Genetic data indicate that there are approximately 150 gene conversion events per meiosis in yeast and that 75 of these are accompanied by reciprocal exchange between homologs (24). If one single-strand nick is sufficient to initiate recombination, then commitment to recombination at the full meiotic level would require 150 single-strand nicks per 4C cell or approximately four nicks per 10^9 daltons of DNA, assuming a haploid genome size of 9 $\times$ 10^9 daltons (25). Thus, the frequency of single-strand scissions we have observed is sufficient to account for meiotic levels of recombination. Our data also indicate that accumulated nicks are utilized very efficiently in recombinational events by meiotic yeast cells.

We would expect single-strand scissions of yeast DNA to be repaired after recombination, as is found in *Lilium* (21). In our studies we were not able to determine whether this repair takes place during sporulation, since ascospores and some ascogenous cells are resistant to spheroplast formation. Examination of this question would perhaps be most feasible with sporulation mutants that complete meiosis but do not form ascospore walls (26). Recent data of Roth (27) suggest that recombination is complete in only a few percent of cells when commitment to recombination is at 50–60% of the full meiotic level. The sedimentation profiles of DNA in alkaline sucrose gradients which we have observed support this view.

Certain sporulation mutants proficient in premeiotic DNA synthesis begin to acquire but subsequently lose commitment to recombination (15). The state of chromosomal DNA with respect to single-strand scissions in these mutants may provide further information with respect to the quantitative relationships of single-strand scissions and genetic exchange during meiosis.

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