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Correlation between suppressed meiotic recombination and the lack of DNA strand-breaks in the rRNA genes of *Saccharomyces cerevisiae*

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ABSTRACT.

We have examined wether the suppressed homologous meiotic recombination within the rDNA of S. cerevisiae is reflected by a lack of possibly recombination-initiating strand-breaks in this part of the genome. Our findings indicate that bulk DNA in the ds-break repair deficient mutant rad52/rad52 accumulates a limited number of both ss- and ds-breaks during meiosis as compared to a RAD^+/rad52 heterozygote. The rDNA-containing chromosome is however protected against these breaks, and thus this may be an explanation for the suppression of recombination in the rDNA. The fact that ds-breaks seem to be involved gives indirect support to the ds-break-repair model for recombination.

INTRODUCTION.

The rDNA in Saccharomyces cerevisiae is composed of about 100 copies of a 9 kb repeating unit, arranged in tandem as one cluster on the right arm of chromosome XII (1,2). This chromosome also contains other genes (3) and since the rDNA-region per se is about as large as an average chromosome (4-6), it means that chromosome XII is among the larger yeast chromosomes. From the overall recombination frequency in the yeast genome, one would expect 4-5 recombination incidents in the rDNA per meiosis (7). The general meiotic recombination within the rDNA is however strongly supressed (7-9). On the other hand mitotic recombination seems to occur (7), and unequal sister chromatid exchange within the rDNA has been shown to occur both in meiotic (9) and mitotic cells (10). Such mechanisms may explain the observed unequal segregation of the rDNA-containing γDNA-fraction (11).

In the present work we have tried to find a molecular basis for the suppression of reciprocal meiotic recombination within the rDNA. Meiosis in yeast can be induced by a shift from growth

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medium to a nitrogen and glucose depleted medium. In response, the cells go through a complex series of genetic and biochemical processes collectively referred to as sporulation (for review, see ref. 12). These processes include premeiotic DNA-synthesis, commitment to high levels of genetic recombination, meiotic chromosome segregation, and formation of haploid ascospores. The molecular mechanism of genetic recombination is not known, but, largely based on genetic data, several models have been proposed. In the model of Meselson and Radding (13) recombination is initiated by a single-stranded (ss) break in the DNA of one of the homologous chromosomes, while Szostak et al. (14) suggests that initiation is effected by a double-stranded (ds) break.

A suppressed meiotic recombination within the rDNA could then be reflected by the abscence of the initial event, either a ss- or a ds-break, in this region of the genome. We have therefore analyzed high molecular weight DNA before, during and after the premeiotic DNA-synthesis and estimated the amount of strand-breaks in the rDNA as compared to bulk DNA where recombination is known to occur. Since such breaks would probably be short-lived and therefore difficult to detect, we have used a mutant, rad52-1 which is defective in ds-break repair (15) and which is known to accumulate ss-breaks during meiosis (16). Originally the rad52 mutation was detected as conferring an x-ray sensitive phenotype to afflicted strains (17,18), but it has later been found to have highly pleiotropic effects. These include reduced sporulation and formation of inviable spores (18), reduced mitotic recombination (19) and defects in meiotic gene conversion and reciprocal recombination (19,20). In spite of these defects rad52 homozygotes go through a normal premeiotic DNA-synthesis (19,20). The RAD52 gene product is not however necessary for sister chromatid exchange (21,22).

Our findings show that a limited amount of both ds- and ss-breaks have been accumulated in the rad52 mutant at the end of premeiotic DNA-synthesis, but that the rDNA-containing chromosome is protected against these breaks. It thus seems to be a correlation between the abscence of strand-breaks in rDNA and the suppression of meiotic recombination in this region of the yeast genome. Indirectly the results give support to the double-strand break repair model for recombination of Szostak et al. (14).

MATERIALS AND METHODS.

Strains.

The strain g654 and the parents of strain AR-1 were kindly provided by dr. J. C. Game. Both strains are closely related to the strain SK-1 known to yield synchronous meiosis and a high level of sporulation (23). The strain g654 is a rad52/rad52 homozygote, while AR-1 is a RAD^+/rad52 heterozygote.

Media and culture conditions.

Presporulation medium (PSP2), sporulation medium (SPM) and culture conditions were as in Simchen et al. (24). The media were supplemented by 1-2 μCi [^{3}H]-uracil per ml to label DNA. To improve the uptake of label, the initial pH in SPM was adjusted to 5.0 (unpublished).

Spheroplasting.

Cells were pelleted, washed twice in cold water, resuspended in ice-cold TG-buffer (0.5 M thioglycolate, 0.1 M tris, 0.03 M EDTA, pH 8.8) and left on ice for 30 minutes (25,26). The cells were then washed twice in cold KPE-buffer (0.05 M KH_2PO_4, 10 mM EDTA, pH 6.5), resuspended in KPE, and 1.25 ml cell suspension (containing about 1 x 10^8 cells) was transferred to polyallomer sentrifuge tubes (Kontron 25.4 x 89 mm) that had been boiled 10 min in 0.05 M EDTA (27). 0.25 ml 10% Nonidet P-40 and 0.40 ml zymolyase 5000 (Kirin Breweries, 10 mg/ml in KPE) were added, and the suspension was incubated at 37 °C for 10 minutes. Then 0.30 ml of a detergent solution (2% sarcosyl, 3% Na deoxycholat, 5% SDS, 0.02 M EDTA, 3% sorbitol) was added, and a sucrose gradient was pumped into the tube beneath the lysed suspension (5-20% sucrose, for neutral gradients: in 1.0 M NaCl, 0.03 M EDTA, 0.01 M tris, pH 8.0, for alkaline gradients: in 0.7 M NaCl, 0.3 M NaOH, 0.03 M EDTA) (16).

Sucrose gradient sedimentation.

The lysate was sentrifuged for 12 hours at 12 000 rpm in a Beckman SW 27 rotor at 20 °C. Freshly deproteinized (5% sarcosyl, 65 °C, 10 min) phage T4 (prepared by CsCl-centrifugation as described in ref. 28) [^{3}H]-DNA was run in parallell gradients as a molecular weight marker. DNA in an aliquot of each fraction was detected as alkali-stable trichloroacetic acid (TCA)-precipitable radioactivity (24). The number average molecular weight (M_n) was

calculated according to Green at al. (29). The molecular weight (MW_i) of DNA sedimented to the middle of each fraction (i) was estimated from the equations of Freifelder (30) and Levin and Hutchinson (31) for neutral and alkaline gradients respectively. For presentation we calculated the percent of total amount DNA-molecules in each fraction (P_i) using the following rationale: Prerequisite: Uniform labelling of the DNA. Approximation: All DNA-molecules in fraction i have the MW of MW_i.
Then $cpm_i = k \times MW_i \times X_i$, where cpm_i is cpm in fraction i, X_i is the number of molecules in this fraction, and k is a constant which depends on the specific activity and thus is the same for all fractions. Hence the fraction cpm_i/MW_i is proprotional to X_i, and accordingly $P_i = (100cpm_i/MW_i)/\Sigma(cpm_i/MW_i)$.

rRNA-DNA hybridization.

An aliquot of each fraction was mixed with an equal volume of 1,0 N NaOH and incubated overnight at 37 °C to hydrolyse RNA. After neutralization, DNA was fixed to nitrocellulose filters (Schleicher and Schuell BA 85) and hybridized to [^{32}P]-rRNA as described (32). [^{32}P]-rRNA was prepared as described by Rubin (33).

RESULTS.

Premeiotic DNA-synthesis and appearance of asci.

In order to study meiotic DNA-metabolism, it is necessary to use strains that exhibit a high degree of synchrony in premeiotic DNA-synthesis and sporulation. The time course of these processes is shown in Fig.1. Both strains go through a synchronous premeiotic DNA-synthesis, which is 2-3 hours delayed in the rad52 homozygote as compared to the RAD$^+$/rad52 heterozygote. The same was true concerning the appearance of asci. AR-1 reached the very high level of over 90% sporulation, while g654 reached only about 20%, which is a normal value in rad52 homozygotes (19,20). In the following experiments, 8 and 11 hours respectively was taken as the end of premeiotic DNA-synthesis in our strains, and chosen as the last time of harvesting because cells at later stages in meiosis become resistant to the spheroplasting enzymes.

Examination of the sedimentation pattern of nuclear DNA during meiosis.

By gentle lysis of a spheroplast suspension and subsequent

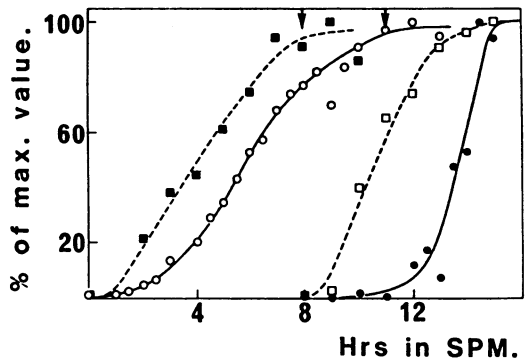

Fig.1. Time course of premeiotic DNA-synthesis and appearance of asci in the strains used. DNA-synthesis was measured as alkali-stable, TCA-precipitable material after labelling with [^{3}H]-uracil in the sporulation medium only. The maximum incorporation into the DNA of the RAD$^+$/rad52 and rad52/rad52 strains was 11 x 10^3 cpm and 26 x 10^3 cpm respectively per 10^7 cells. Per cent asci was determined by counting in a haemacytometer. (■----■) cpm in RAD$^+$/rad52 DNA (max. value 148 000 cpm). (○——○) cpm in rad52/rad52 DNA (max. value 29 000 cpm). (□- - -□) asci in RAD$^+$/rad52 (max. value 90%). (●——●) asci in rad52/rad52 (max. value 20%). Arrows indicate the times taken as the end of premeiotic DNA-synthesis.

sedimentation through a neutral sucrose gradient it is possible to fractionate intact yeast chromosomal DNA according to MW (4). If ds-breaks occur in DNA during sporulation it should be reflected by a reduction of the MW of the afflicted DNA-molecules, and consequently a change in the sedimentation profiles towards lower molecular weight. Fig.2 shows the sedimentation profiles of DNA from cultures harvested before and after premeiotic DNA-synthesis. The material at the top of the gradient represents mitochondrial DNA (4,5). Unbroken chromosomal DNA-molecules have molecular sizes in the range about 300-1500 kb (4-6), corresponding to relative sedimentation distances of 0.4 to 0.7 in our experiments. The number average molecular weights (M_n) of mitotic nuclear DNA (calculated from the fractions with sedimentation distances greater than 0.275) in these experiments were 2.9 x 10^8 for RAD$^+$/rad52 and 2.5 x 10^8 for rad52/rad52 (0 hours). This is comparable to the values published by other groups (5,15,34), and indicates that the sedimentation profiles of mitotic DNA represent largely intact chromosomal DNA. Recovery of DNA

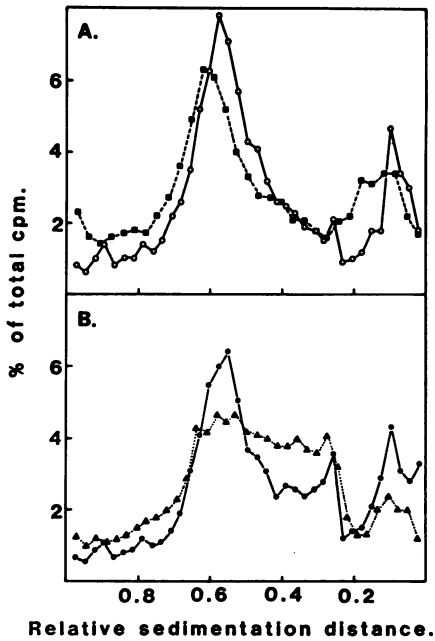

Fig.2. Sedimentation profiles of DNA from cells harvested before and after premeiotic DNA-synthesis. Cells were labelled both in PSP2 and SPM, and spheroplasted and sedimented in neutral gradients as described. Sedimentation was from right to left. DNA was detected as alkali-stable, TCA-precipitable material. A: $RAD^+/rad52$. (○——○) 0 h in SPM (total cpm: 26028). (■- - -■) 8 h in SPM (total cpm: 55086). B: rad52/rad52. (●——●) 0 h in SPM (total cpm: 71313). (▲·····▲) 11 h in SPM (total cpm: 114 622).

in the gradients was generally greater than 80%. It appears (Fig.2) that at the end of premeiotic DNA-synthesis the DNA of the rad52/rad52 mutant had accumulated a limited number of ds-breaks. The M_n values at this stage were 3.3×10^8 for $RAD^+/rad52$ and 2.1×10^8 for rad52/rad52.

In Fig.2 the cpm in each fraction depend on the size of the DNA-molecules. To get a quantitative picture of the distribution of breaks in the different size-classes of DNA, the relative number of DNA-molecules (expressed as P_i) in each fraction was calculated (Materials and methods). The results from different times during premeiotic DNA-synthesis are shown in Fig.3. It can be seen that a significant fraction of the chromosomal DNA-molecules are broken during sporulation in the rad52 homozygote.

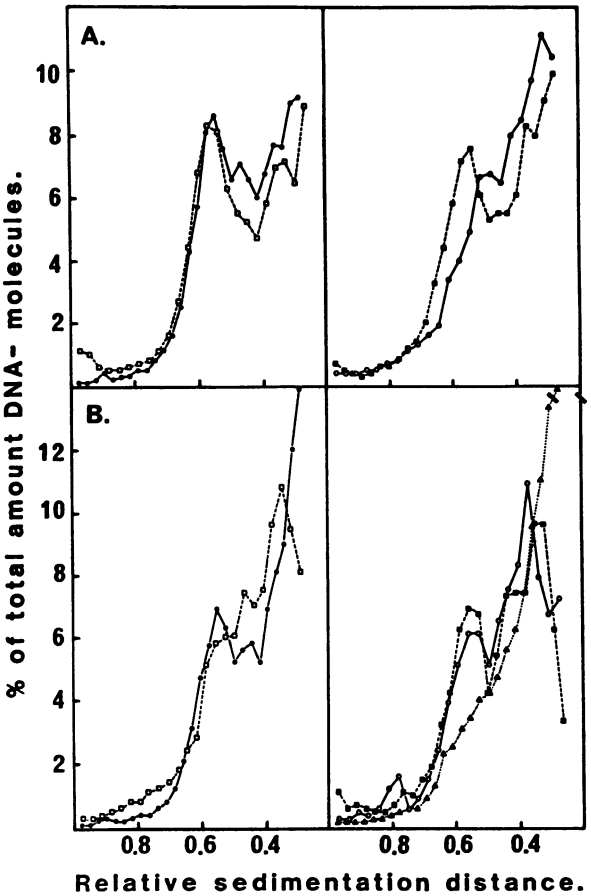

Fig.3. Size distribution of chromosomal DNA-molecules from cells harvested at different times during sporulation. Cells were treated, sedimented and DNA detected as in the legend of Fig.2. Per cent (P_i) of total amount DNA-molecules in each fraction (i) was calculated by the equation $P_i = (100cpm_i/MW_i)/\Sigma(cpm_i/MW_i)$, and plotted against the sedimentation distance. A: $RAD^+/rad52$. (●——●) 0 h. (□- - -□) 2 h. (○——○) 5 h. (■- - -■) 8 h. B: rad52/rad52. Symbols as in A, except (▲·····▲) 11 h (the upmost point represents 19.1%).

Most of the breaks seem to occur between 8 and 11 hours after transfer to SPM, which corresponds to the end of premeiotic DNA-synthesis (Fig.1). It can be estimated that 30-40% of the DNA-molecules originally in the main peak (size range 590-1900 kb, corresponding to sedimentation distances of 0.5-0.8 in the

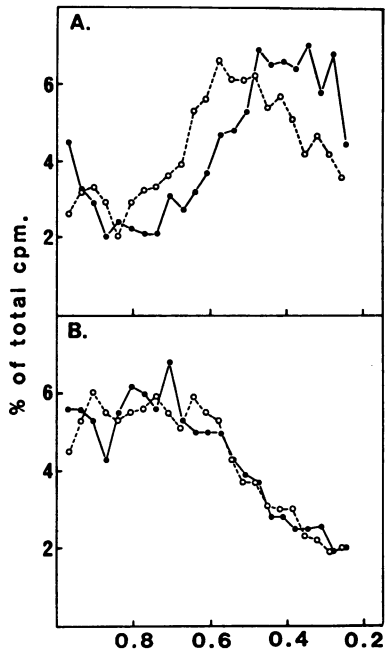

Fig.4. A: Sedimentation profiles of chromosomal ss-DNA from cells harvested after premeiotic DNA-synthesis. Cells were grown, labelled and spheroplasted as in the legend of Fig.2, and sedimented in alkaline sucrose gradients as described. DNA was detected as in Fig.2. (○- - -○) RAD^+/rad52. (●——●) rad52/rad52.
B: Sedimentation profiles of the rDNA-containing ss-molecules from cells harvested after premeiotic DNA-synthesis. Experimental conditions were as in A. rDNA in the fractions was detected by fixing an aliquot of DNA from each fraction to nitrocellulose filters and hybridizing with [^{32}P]-rRNA as described. Symbols as in A.

figures) have disappeared from this size range at 11 hours as compared to 0 hours.

For the RAD^+/rad52 heterozygote there was no accumulation of ds-breaks at the end of premeiotic DNA-synthesis (8 hours in this strain, Fig.1), but a few breaks could be detected at 5 hours. These breaks were apparently repaired beetween 5 and 8 hours. Similar results were found when the DNA of the two strains was labelled with [^{3}H]-uracil and [^{14}C]-uracil respectively, the cells harvested after premeiotic DNA-synthesis, mixed, and spheroplasted and sedimented as described. Thus whatever causes

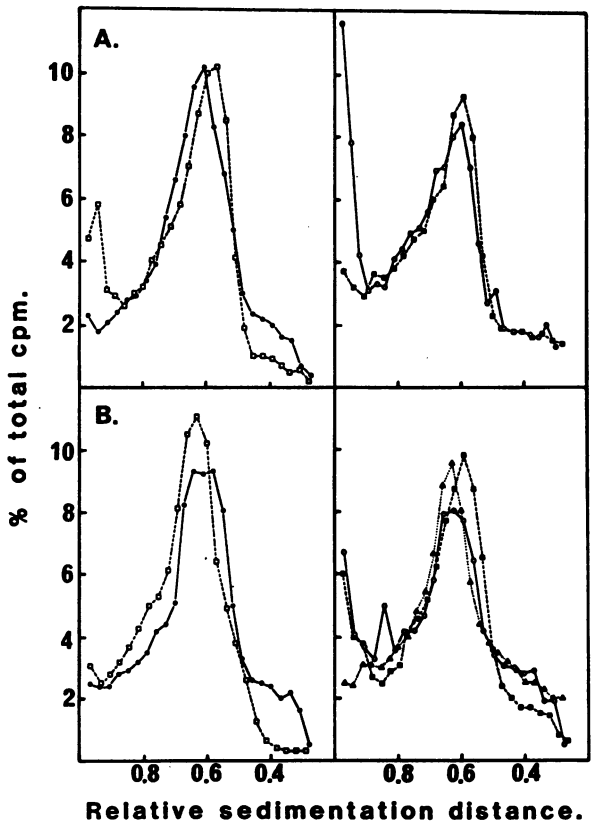

Fig.5. Sedimentation profiles of the rDNA-containing molecules from cells harvested at different times during sporulation. Cells were spheroplasted and sedimented as in the legend of Fig.2. An aliqout of the DNA in each fraction was fixed to nitrocellulose filters and hybridized to [^{32}P]-rRNA as described. A: RAD$^+$/rad52. (●——●) 0 h. (□- - -□) 2 h. (○——○) 5 h. (■- - -■) 8 h. B: rad52/rad52. Symbols as in A, except (▲.....▲) 11 h.

the breaks in the rad52 homozygote, it does not introduce breaks in the DNA of the RAD$^+$/rad52 heterozygote during the preparation procedure. Fig.4A shows the sedimentation profiles of ss-DNA from cells harvested after premeiotic DNA-synthesis, and indicates that the DNA from the rad52 homozygote is fragmented as compared to the RAD$^+$/rad52 heterozygote. At time zero the sedimentation profiles of ss-DNA from the two strains were almost completely overlapping (data not shown). This is in accordance with the report by Resnick et al. (16) who found accumulated ss-breaks at

the end of premeiotic DNA-synthesis in the rad52 homozygote strain g654. The amount of ss-breaks in the present study can be estimated (by the method of Green et al. (29)) to be about twice the amount needed to account for the observed ds-breaks.

Although alternative possibilities cannot be ruled out, our conclusion is that yeast DNA is subjected to a limited amount of both ss- and ds-breaks during premeiotic DNA-synthesis. The rad52 homozygote accumulates these breaks, while the RAD^+/rad52 heterozygote repairs the breaks before the end of premeiotic DNA-synthesis.

Sedimentation pattern of the rDNA-containing molecules.

If the observed strand-breaks were a part of the recombination process, one could expect that the rDNA-region is not subjected to these breaks. In that case the rDNA-containing molecules should not, or if breaks occur outside rDNA, only slightly, be fragmented during sporulation. To investigate this, we localized rDNA-containing molecules in the gradients by hybridization with [^{32}P]-rRNA. Fig.5 shows that for both strains there were only minor differences in the sedimentation profiles of these molecules in neutral gradients when different times of harvesting were compared. Hence, the rDNA must be protected against ds-breaks during sporulation. The same is true concerning ss-breaks (Fig. 4B). If the rDNA-containing chromosome during premeiotic DNA-synthesis was subjected to as many ss-breaks per DNA-length as bulk DNA, one would expect the sedimentation profile of rDNA from the rad52 homozygote to be overlapping the profile of bulk DNA from this strain after premeiotic DNA-synthesis. Fig.4 shows that this is clearly not the case. Fig.3, 4 and 5 also show that the rDNA-containing molecules from RAD^+/rad52 and from mitotic rad52/rad52 cells sediment faster than the main peak of chromosomal DNA. This is consistent with the fact that the rDNA-containing chromosome must be among the largest of the yeast chromosomes. Taken together these findings suggest that the suppression of recombination in the rDNA-region might be explained by a lack of recombination-initiating strand-breaks in these genes. The fact that a significant fraction of the breaks are ds-breaks also give indirect support to the double-strand break repair model for meiotic recombination proposed by Szostak et al. (14).

DISCUSSION.

Significance of DNA strand-breaks during sporulation in yeast.

The results presented in this work indicate that a limited number of ds-breaks accumulate in the DNA of a rad52 homozygote at the end of premeiotic DNA-synthesis. This observation may be consistent with the study by Resnick et al. (16) who mentioned the appearance of a few ds-breaks late in the sporulation process in a rad52 homozygote. These authors (16) and other workers (25,27), claim that ds-breaks cannot be detected during meiosis in wildtype strains, while in the present study we have found a few breaks in the rad52/RAD$^+$ heterozygote after 5 hours in SPM. These breaks are apparently repaired before the end of premeiotic DNA-synthesis. The reason for this discrepancy may be that our reference strain was a RAD$^+$/rad52 heterozygote. If the RAD52 gene product is the limiting factor in ds-break repair, ds-breaks could be more slowly repaired in a RAD$^+$/rad52 heterozygote than in RAD$^+$ homozygotes. Thus breaks that are too short-lived to be detected in RAD$^+$ homozygotes might be discovered in RAD$^+$/rad52 heterozygotes. The fact that the ds-breaks are not a result of the preparation procedure and are not introduced at random, since the rDNA is not afflicted, suggests that they are created by specific in vivo incidents. The relation between the observed ds-breaks and the ss-breaks found by us and by other workers (16,25,27) is unclear. The ss-breaks detected in a rad52 homozygote by Resnick et al. (16) seem to occur at an earlier stage in meiosis than the ds-breaks found in the present work. This indicates that the ds-breaks are not created mechanically from ss-breaks during the preparation procedure. One possibility may be that the initial DNA-scission is a ss-break, which later in meiosis is converted to a ds-break or -gap, for example by means of a ss-specific endonuclease. Howell and Stern (35) have found a nicking endonuclease that appear during meiosis in lily, and a similar enzyme might exist in yeast. Chow and Resnick (36) have found a nuclase-activity which is controlled by the RAD52 gene and seem to appear during meiosis in yeast.

Possible explanations for the suppressed recombination in the rDNA region.

The suppression of meiotic recombination within the rDNA

(7,9) is reflected by a deficiency in the possibly recombination-initiating strand-breaks. The basis of this lack of strand-breaks is unknown, but one might speculate along two lines. Firstly the rDNA might lack sites for initiation of recombination. There are data supporting the view that recombination is initiated from special sites, possibly DNA-sequences, in the genome (37-39). Such sites might be recognition sites for nucleases, either directly, or indirectly for example by means of proteins or RNA-molecules (40). Sequence specific endonucleases have been found in mitotic yeast cells (41-44), and such a nuclease probably plays a role in the recombinational event of mating type switching (43,44). To our knowledge no study has yet been made on site-specific endonucleases in meiotic cells.

The protection of rDNA against strand-breaks might also be a consequence of a chromatin structure that renders the rDNA region insusceptible to nuclease attack as compared to the rest of the genome. Such a mechanism is a possible reason for the unidirectionality of mating type switching in yeast (45) and may also explain certain features of recombination in Drosophila (46). In an attempt to test out these possibilities work is in progress to search for endonucleases in meiotic yeast cells, and to study the chromatin structure of meiotic rDNA by means of nuclease sensitivity.

The relation between DNA ds-breaks and recombination.

The present results suggest a connection between ds-breaks and meiotic recombination, but the amount of ds-breaks accumulated in the rad52 homozygote is probably not high enough to account for all the recombination incidents that occur in RAD^+-strains during meiosis. A possible explanation for this may be that rad52 homozygotes are rendered inviable during sporulation, and that a large fraction of the cells therefore are not able to reach their full level of initiation of recombination. It is known that yeast is commited to recombination during premeiotic DNA-synthesis (47) but it is not known if this commitment involves the actual strand-break incident postulated in several recombination models (for exampel 13,14). Some data indicate that the actual exchange event takes place after the end of premeiotic DNA-synthesis (48,49). Another possibility is that there are more than one mechanism for meiotic recombination in yeast and that

only a fraction of the recombination events involve ds-breaks. Studies of chromosomal integration of plasmids (50) and recombination between plasmids in cellfree extracts (51) indicate that this may be the case, at least in mitotic cells.

Resnick (52) suggested a model for repair of ds-breaks which involved recombination. Since then other observations have indicated that ds-breaks may play a role in the molecular mechanism for normal recombination and gene conversion (for review, see ref. 14). Among these are the fact that the ds-break repair deficient mutation rad52 also conferres defects in recombination (19,20), that ds-breaks in plasmids are recombinogenic in mitotic cells (50,53), and the finding that a specific ds-break probably is involved in the recombinational event of mating type switching in yeast (54,55) which also requires the RAD52 gene product (54,56). Our findings of ds-breaks during sporulation and the correlation between suppressed recombination and the lack of such breaks give further indications that ds-breaks may play a role in the process of meiotic recombination.

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REFERENCES.

1. Petes,T.D. (1979) Proc. Natl. Acad. Sci. 76, 410-414.
2. Kaback,D.B. and Davidson,N. (1980) J. Mol. Biol. 138, 745-754.
3. Mortimer,R.K. and Schild,D. (1980) Microbiol. Rev. 44, 519-571.
4. Blamire,J., Cryer,D.R., Finkelstein,D.B. and Marmur,J. (1972) J. Mol. Biol. 67, 11-24.
5. Petes,T.D. and Fangman,W.L. (1972) Proc. Natl. Acad. Sci. 69, 1188-1191.
6. Petes,T.D., Byers,B. and Fangman,W.L. (1973) Proc. Natl. Acad. Sci. 70, 3072-3076.
7. Petes,T.D. and Botstein,D. (1977) Proc. Natl. Acad. Sci. 74, 5091-5095.
8. Petes,T.D. (1979) J. Bacteriol. 138, 185-192.
9. Petes,T.D. (1980) Cell 19, 765-774.
10. Szostak,J.W. and Wu,R. (1980) Nature 284, 426-430.

11. Øyen,T.B., Saelid,G. and Skuladottir,G.V. (1978) Biochim. Biophys. Acta 520, 88-102.
12. Esposito,R.E. and Klapholz,S. (1981) in The Molecular Biology of the Yeast Saccharomyces, Life Cycle and Inheritance, Strathern,J.N., Jones, E.W. and Broach,J.R. Eds., pp. 211-287, Cold Spring Harbor Laboratory, New York.
13. Meselson,M.S. and Radding,C.M. (1975) Proc. Natl. Acad. Sci. 72, 358-361.
14. Szostak,J.W., Orr-Weaver,T.L., Rothstein,R.J. and Stahl,F.W. (1983) Cell 33, 25-35.
15. Resnick,M.A. and Martin,P. (1976) Molec. Gen. Genet. 143, 119-129.
16. Resnick,M.A., Kasimos,J.N., Game,J.C., Braun,R.J. and Roth,R.M. (1981) Science 212, 543-545.
17. Resnick,M.A. (1969) Genetics 62, 519-531.
18. Game,J.C. and Mortimer,R.K. (1974) Mutat. Res. 24, 281-292.
19. Prakash,S., Prakash,L., Burke,W. and Montelone,B.A. (1980) Genetics 94, 31-50.
20. Game,J.C., Zamb,T.J., Braun,R.J., Resnick,M. and Roth,R.M. (1980) Genetics 94, 51-68.
21. Zamb,T.J. and Petes,T.D. (1981) Curr. Genet. 3, 125-132.
22. Prakash,L. and Taillon-Miller,P. (1981) Curr. Genet. 3, 247-250.
23. Kane,S.M. and Roth,R. (1974) J. Bacteriol. 118, 8-14.
24. Simchen,G., Pinon,R. and Salts,Y. (1972) Exp. Cell Res. 75, 207-218.
25. Kassir,Y. and Simchen,G. (1980) Curr. Genet. 2, 79-85.
26. Pinon,R. and Salts,Y. (1977) Proc. Natl. Acad. Sci. 74, 2850-2854.
27. Jacobson,G.K., Pinon,R., Esposito,R.E. and Esposito M.S. (1975) Proc. Natl. Acad. Sci. 72, 1887-1891.
28. Thomas,C.A.jr. and Abelson,J. (1966) in Procedures in Nucleic Acid Research, Cantoni,G.L. and Davies,D.R. Eds., pp. 553-561, Harper and Row, New York.
29. Green,M.H.L., Medcalf,A.S.C., Arlett,C.F., Harcourt,S.A. and Lehman,A.R. (1974) Mutat. Res. 24, 365-378.
30. Freifelder,D. (1970) J. Mol. Biol. 54, 567-577.
31. Levin,D. and Hutchinson,F. (1973) J. Mol. Biol. 75, 495-502.
32. Karijord,Ø. and Øyen,T.B. (1975) Biochim. Biophys. Acta 383, 255-265.
33. Rubin,G.M. (1975) in Methods in Cell Biology, Prescott,D.M. Ed., vol XII, pp. 45-64, Academic Press, New York.
34. Forte,M.A. and Fangman,W.L. (1976) Cell 8, 425-431.
35. Howell,S.H. and Stern,H. (1971) J. Mol. Biol. 55, 357-378.
36. Chow,T.Y.K. and Resnick,M.A. (1983) in Cellular Responses to DNA Damage, Friedberg,E.C. and Bridges,B.A. Eds., pp. 447-455. Alan R. Liss, New York.
37. Stahl,F.W. (1979) Ann. Rev. Genet. 13, 7-24.
38. Fogel,S., Mortimer,R.K. and Lusnak,K. (1981) in The Molecular Biology of the Yeast Saccharomyces, Life Cycle and Inheritance, Strathern,J.N., Jones,E.W. and Broach,J.R. Eds., pp. 289-339, Cold Spring Harbor Laboratory, New York.
39. Friedman,B.E., Bouchard,R.A. and Stern,H. (1982) Chromosoma 87, 409-424.
40. Hotta,Y, and Stern,H. (1981) Cell, 309-319.
41. Watabe,H-o., Shibata,T. and Ando,T. (1981) J. Biochem. 90, 1623-1632.

42. Watabe,H-o, Iino,T., Kaneko,T., Shibata,T. and Ando,T. (1983) J. Biol. Chem. 258. 4663-4665.
43. Nasmyth, K. (1983) Nature 302, 670-676.
44. Kostriken,R., Strathern,J.N., Klar,A.J.S., Hicks,J.B. and Heffron,F. (1983) Cell 35, 167-174.
45. Nasmyth,K.A. (1982) Cell 30, 567-578.
46. Szauter,P. (1984) Genetics 106, 45-71.
47. Sherman,F. and Roman,H. (1963) Genetics 48, 255-261.
48. Silva-Lopez,E., Zamb,T.J. and Roth,R. (1975) Nature 253, 212-214.
49. Davidow,L.S. and Byers,B. (1984) Genetics 106,165-183.
50. Orr-Weaver,T.L., Szostak,J.W. and Rothstein,R.J. (1981) Proc. Natl. Acad. Sci. 78, 6354-6358.
51. Symington,L.S., Fogarty, L.M. and Kolodner,R. (1983) Cell 35, 805-813.
52. Resnick,M.A. (1976) J. Theor. Biol. 59, 97-106.
53. Orr-Weaver,T.L. and Szostak,J.W. (1983) Proc. Natl. Acad. Sci. 80, 4417-4421.
54. Weiffenbach,B. and Haber,J.E. (1981) Mol. Cell. Biol. 1, 522-534.
55. Strathern,J.N., Klar,A.J.S., Hicks,J.B., Abraham,J.A., Ivy,J.M., Nasmyth,K.A. and McGill,C. (1982) Cell 31, 183-192.
56. Malone,R.E. and Esposito,R.E. (1980) Proc. Natl. Acad. Sci. 77, 503-507.