Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 7072–7076, September 1989
Genetics

Evidence for two pathways of meiotic intrachromosomal recombination in yeast

(*RAD50*/haploid meiosis/rDNA)

SHOSHANNA GOTTLIEB*, JOSEPH WAGSTAFF†‡, AND ROCHELLE EASTON ESPOSITO*†

*Committee on Genetics and †Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL 60637

Communicated by Gerald R. Fink, February 13, 1989

ABSTRACT **This study shows that *RAD50*, a yeast DNA repair gene required for meiotic interchromosomal exchange between homologs, also is required for meiotic intrachromosomal recombination. However, only intrachromosomal events in nonribosomal DNA are dependent on *RAD50*; those in ribosomal DNA (rRNA-encoding DNA) occur in the absence of this gene. Furthermore, nonribosomal DNA sequences retain their *RAD50*-dependence even when inserted into the ribosomal DNA array. We argue that these data provide evidence for at least two pathways of meiotic intrachromosomal recombination whose activity depends on the specific sequences involved or their structural context in the chromosome. In contrast to its role in meiosis, *RAD50* is not required for either inter- or intrachromosomal spontaneous mitotic recombination.**

Genetic exchange functions in several important ways in eukaryotic cells. In addition to contributing to genetic diversity during gametogenesis, it also is required for proper distribution of homologs at meiosis I (1, 2), maintenance of copy number (3–5) and sequence homogeneity (6–8) in repeated gene families, creation of new alleles (9, 10), and control of gene expression (11–13). Despite the importance of these various recombination (Rec) events, relatively little is known at present about eukaryotic recombination genes particularly in higher organisms.

In this study we used the yeast *Saccharomyces cerevisiae* as a model system to examine the mechanism of intrachromosomal recombination. Using a process of single-division meiosis in haploid and diploid cells that eliminates meiosis I segregation of homologs (2, 14), we have inquired whether intrachromosomal recombination and interchromosomal recombination utilize the same recombination functions and occur via a common recombination gene pathway(s).

In this system it was previously shown that (*i*) interchromosomal reciprocal exchange occurs at similar levels to standard two-division meiosis (14, 15); (*ii*) genetic map distances along the length of chromosome III are the same in recombination-proficient (Rec^+) diploids ($2n$) and disomic haploids ($n + 1$)—i.e., the presence or absence of pairing and exchange on other homologs does not affect recombination on this chromosome (15); (*iii*) intrachromosomal events between duplicated genes are elevated in the absence of a paired homolog (16); and (*iv*) the *SPO11* gene encodes a recombination function required in common for exchange between and within individual chromosomes (16).

Here we report that a second gene, *RAD50*, also is used in common; however, *RAD50*, unlike *SPO11*, is not essential for all intrachromosomal recombination. Based on this evidence we propose that there are at least two partially overlapping pathways of intrachromosomal exchange acting on different sequences in the genome. Recombination functions in both of these pathways are shared with those that operate in interchromosomal exchange.

MATERIALS AND METHODS

Media and Genetic Procedures. Growth and sporulation media are as described (17). The *rad50-1* mutation, which confers sensitivity to ionizing radiation (18) and methyl methanesulfonate, was scored on standard YPD medium (17) containing 0.01% methyl methanesulfonate added after autoclaving. Crosses, ascus dissection, mitotic recombination experiments, and other genetic procedures were done as described (16, 19).

Cloning *RAD50*. A *rad50-1 ura3* strain was transformed with a YRP17 yeast DNA library containing the *URA3* gene (provided by R. T. Elder), and Ura^+ transformants were screened for the ability to grow on methyl methanesulfonate-containing medium. A 10-kilobase (kb) fragment of yeast DNA was identified that complements the methyl methanesulfonate phenotype and integrates at the *RAD50* locus (data not shown); its restriction map overlaps that reported by Kupiec and Simchen (20).

Construction of Strains Containing *his3* Truncated Genes. Two plasmids, each containing the yeast *URA3* gene and truncated *his3* genes (21), were kindly provided by S. Roeder; 5′ flanking sequences of *HIS4* present in one plasmid (pR656) and a *Bgl* II fragment of rDNA in the other (pR658) were used to target the *his3* genes to the *HIS4* and rRNA-encoding DNA (rDNA) regions, respectively, in strains carrying a genomic deletion of *his3*. Transformants were selected on medium lacking uracil, and the site of integration was confirmed by genetic and physical tests.

Plasmid Constructions. pSG(RAD50)1 was constructed by inserting a *Hin*dIII fragment complementing *RAD50* function into a yeast centromere plasmid containing the *TRP1* selectable marker. pSG(RAD50)2 was constructed by inserting the *Hin*dIII fragment into the integrating vector YIP5. Plasmids containing the mating type loci were constructed by inserting a *Hin*dIII fragment containing *MAT***a** or *MAT*α information (provided by I. Herskowitz) into a yeast centromere plasmid containing the *LEU2* selectable marker.

RESULTS

***RAD50* Is Required for Meiotic Recombination Between Homologs in Chromosome III Disomic Haploids.** The experimental system used in this analysis involves three essential components (16): (*i*) the presence of the *spo13-1* mutation, which eliminates reductional chromosome segregation at meiosis I and permits either diploid cells or haploid cells to complete a single meiosis II-like division and form two viable

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: SCE, sister chromatid exchange; rDNA, rRNA-encoding DNA.

‡Present address: Division of Hematology and Oncology, The Childrens Hospital, Boston, MA 02115.

Table 1. Effect of *rad50-1* on *CEN3-MAT* meiotic recombination in *MATa/MATα* disomic haploids

Genotype/ strain	Dyads, no.	Dyads with Mat phenotypes, no. N,N	a,α	a,N	α,N
RAD50					
K355-13A	128	69	27	17	15
JW165-19D	115	61	22	13	19
JW165-37D	127	70	33	14	10
JW167-171B	441	243	103	50	45
Total (%)	811	443 (55)	185 (23)	94 (12)	89 (11)
rad50-1					
JW168-23A	334	323	0	4	7
JW168-62B	398	371	0	9	18
Total (%)	732	694 (95)	0 (<0.2)	13 (2)	25 (3)

All strains contain a *MATa/MATα* chromosome III disome, *spo13-1*, *ura3*, and *URA3* in rDNA. N,N dyads contain two nonmating *MATa/MATα* spores and result from no *CEN3–MAT* exchange followed by equational division; a,α dyads contain *MATa/MATa* and *MATα/MATα* spores and occur half of the time when there is *CEN3–MAT* exchange and equational segregation and whenever there is no exchange and reductional segregation; a,N and α,N dyads result most frequently from aberrant segregation in which one chromatid separates from three chromatids—e.g., a,N = MATa, MATa/MATα/MATα (see ref. 15).

meiotic products (2, 14); (*ii*) repeated genes, either naturally occurring tandem arrays such as the rDNA or artificially constructed repeats, for monitoring exchange; and (*iii*) haploid yeast strains that can enter meiotic development because they express both alleles of the *MAT* locus.

The *rad50-1* amber mutation was previously shown to dramatically reduce meiotic gene conversion and reciprocal exchange between homologs in both *SPO13* and *spo13-1* diploids (22, 23). Its Rec⁻ phenotype was also seen during *spo13-1* haploid meiosis (Table 1). In *rad50-1 spo13-1* haploids disomic for chromosome III, the frequency of recombination in the *MAT–CEN3* interval [23 centimorgans (cM)] (24) was reduced >100-fold (<0.2%); no exchange (0 of 732 dyads) occurred in the *his4–MAT* interval (50cM) (24). Thus, *RAD50* is essential for intergenic recombination between homologs in *MATa/α* disomic haploids, as well as in diploids.

***RAD50* Is Required for Intrachromosomal Recombination Between Duplicated *his4* Genes.** To determine whether *RAD50* is also essential for meiotic recombination within single chromosomes, exchange was analyzed in haploid strains (n) containing duplicate copies of *his4* on chromosome III separated by pBR313 plasmid DNA (Fig. 1). These haploids contain a single chromosome III but are functionally *MATa/MATα* because of the presence of the *sir2* mutation, which causes derepression of the normally silent *HML* and *HMR* mating-type loci (25). The *his4* duplication contains noncomplementing mutations in the *his4A* and *his4C* regions (26). In Rec⁺ haploid meiosis, the frequency of detectable intrachromosomal recombination in the *his4* system was ≈30% (Table 2; see also ref. 16), compared with 1–2% previously reported in Rec⁺ diploid meiosis (27). The vast majority of events (80%) were evenly divided between reciprocal unequal sister chromatid exchanges (SCEs) and reciprocal intrachromatid exchanges (16). The high level of intrachromosomal exchange during haploid meiosis suggests that the presence of the other homolog in diploid cells suppresses recombination events within single chromosomes (16).

In *rad50-1 sir2 spo13-1* haploid strains, the rate of intrachromosomal recombination per meiosis was reduced ≈100-fold (Table 2). Thus, RAD50 is required for exchange between duplicated *his4* genes within a single chromosome as well as for exchange between homologs.

A Cloned *RAD50* Gene Restores Intrachromosomal Exchange at *his4* in *rad50-1* Haploid Meiosis. Transformation of the *rad50-1* haploid G15-4 with plasmid pSG(RAD50)1 (see *Materials and Methods*) yielded a haploid that exhibited 27% recombination in the *his4* duplication, similar to other Rec⁺ haploids (Table 2). Southern hybridization analysis of 18 of the recombinant dyads indicated that 7 of 18 (39%) arose from reciprocal unequal SCE, 7 of 18 (39%) from reciprocal intrachromatid exchange, and 4 of 18 (22%) from intrachromosomal gene conversion, analogous to other Rec⁺ haploids (16). This result shows that the lack of intrachromosomal recombination in *rad50-1* haploids is due to the *rad50* mutation and not another gene(s) in the strain background.

Meiotic Intrachromosomal Recombination Within the Ribosomal DNA Occurs in the Absence of *RAD50*. Recombination

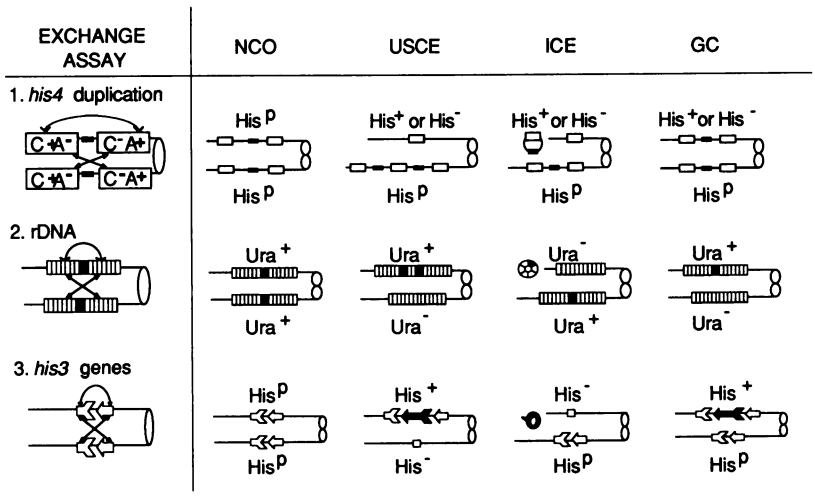

FIG. 1. Spores produced after either no exchange or meiotic intrachromosomal recombination in *spo13-1* single-division meiosis. In all cases, spore phenotypes are written above or below the representative chromatid. NCO, no crossover; USCE, reciprocal unequal sister chromatid exchange; ICE, reciprocal intrachromatid exchange; GC, gene conversion. Assay 1: *his4* duplication. Open rectangles represent the *his4* gene; the filled rectangles represent pBR313 sequences. The two copies of *his4* contain noncomplementing mutations in either *his4A* or *his4C*. Recombination produces one His^P spore (His⁻ with His⁺ papillae) and either one His⁺ or one His⁻ spore depending on the site of the exchange event between the duplicated genes. Assay 2: rDNA. Open boxes represent individual rDNA repeats; the filled boxes represent the yeast *URA3* gene. Recombination will lead to loss of the *URA3* insert on one chromatid. Assay 3: *his3* truncated genes. The 3′ end of the *his3* gene is represented by an arrowhead; the 5′ end is represented by an arrowtail. The darkened arrow represents an intact *his3* gene. The two sequences overlap by 300 base pairs.

Table 2. Effect of *rad50-1* on meiotic intrachromosomal exchange in the *his4* duplication

Genotype/ strain	Dyads, no.	Dyads with His phenotypes, no.					% recombination
		p,p	p,−	p,+	+,−	−,−	
RAD50							
JW201-4B	157	108	43	5	0	1	30.8
JW211-15C	76	57	14	2	2	1	24.0
G15-4/ pSG(RAD50)1	370	269	85	15	0	1	27.1
Total	603	434	142	22	2	3	27.7
rad50-1							
G15-4	445	440	1	0	0	4	0.2

Strains JW201-4B (*MATa*), JW211-15C (*MATα*), and G15-4 (*MATa*) contain the *his4* duplication (*his4-39,260 pBR313 his4-1176,864*), *sir2*, and *spo13-1*. G15-4 also has *trp1-1*, *ura3*, and *URA3* in rDNA. Three spore phenotypes are observed: His^+, His^-, and His^p (His^- with His^+ papillae). In the absence of meiotic intrachromosomal recombination, the two spore clones in a dyad are His^p, designated above as p,p. The other phenotypes are generated by exchange during meiosis and mitosis [see Fig. 1 and ref. 16]. Percent recombination = [(p,−) + (p,+) + (+,−)]/[(p,p) + (p,−) + (p,+) + (+,−)]; the −,− dyads are assumed to be due to prior mitotic recombination and are omitted from this calculation.

was next examined between the repeated rDNA genes present in a tandem array of ≈150 copies on chromosome XII (28). In contrast to the near absence of meiotic intrachromosomal exchange between duplicated *his4* genes in *rad50-1* mutants, the level of recombination in the rDNA was unaffected by the absence of *RAD50* function (Table 3). Exchange was assayed in strains containing the *URA3* gene integrated in the rDNA array (Fig. 1) and were either MATa/MATα disomic haploids or haploids transformed with a *CEN* plasmid containing the opposite mating-type allele. The *URA3*-rDNA inserts were derived either from strain TP406pSS31(1) (29) or by independent transformations with the integrating plasmid pSS31(29). Intrachromosomal exchange between repeats flanking the *URA3* insert yields dyads containing 1 Ura^+ spore and 1 Ura^- spore. In *RAD50* and *rad50-1* strains, the frequency of these dyads was similar, 2.1% and 1.2%, respectively. In all dyads examined by Southern hybridization analysis, the Ura^- spore contained no *URA3* sequences integrated in the rDNA array. Therefore, these events are due to recombination between rDNA repeats leading to loss of the *URA3* gene rather than gene conversion between the *URA3* gene inserted in the array and the chromosomal *ura3* allele on chromosome V. Although there was some variability among strains, the observed frequencies indicate that the *RAD50* function is dispensable for intrachromosomal events between rDNA genes.

Recombination Between Truncated *his3* Genes, Inserted Either at the *HIS4* Locus or in the rDNA, Is *RAD50*-Dependent. To address whether *RAD50*-independent recombination between rDNA repeats is a property of the region containing the tandem array, a duplication of non-rDNA sequences was inserted into the rDNA, and exchange was measured between the non-rDNA repeats in wild-type and *rad50-1* backgrounds. The non-rDNA duplication contains 5′ and 3′ truncated *his3* sequences with ≈300 base pairs of internal sequence homology (Fig. 1; ref. 21). It was inserted at both the *HIS4* locus and into the rDNA in haploids containing a deletion of the *HIS3* locus. In such strains, the only way to produce a His^+ cell is by recombination between the homologous sequences present in the inserted duplication.

In Rec^+ strains, the frequency of exchange yielding His^+ prototrophs was similar at both *HIS4* and in the rDNA: $\approx 1 \times 10^{-3}$ events per meiosis (Table 4). Strikingly, the *rad50-1* mutation caused a nearly complete loss of meiotic exchange between the *his3* genes at both locations (Table 4). Therefore, unlike intrachromosomal recombination between rDNA repeats, which can occur in the absence of *RAD50*, exchange between unique sequences inserted in the rDNA is *RAD50*-dependent. One possible explanation for the *RAD50*-dependence of *his3* recombination in rDNA is that the duplication may have been inserted near the junction between rDNA and unique sequences enabling them to retain their original recombination properties. We consider this possibility unlikely because exchange between rDNA repeats leading to loss of the entire *his3* insert is *RAD50*-independent, providing an internal control (S.G., data not shown).

Another difference in the exchange properties of rDNA repeats and unique DNA has been reported recently. The *SIR2* gene, which represses transcription of the silent mating type loci (25), also suppresses recombination between rDNA repeats while having no effect on exchange between duplicated *HIS4* genes (30). Therefore, we inquired whether the property of enhanced recombination in rDNA characteristic of *sir2* strains is acquired by the *his3* duplication when it is embedded in the rDNA array. The experiments shown in Table 4 were performed in *sir2* strains. Comparable studies in *SIR2* strains gave similar frequencies of His^+ recombinants: 1.42×10^{-4} during mitosis and 8.92×10^{-4} during meiosis. Thus, recombination between duplicated non-rDNA se-

Table 3. Effect of *rad50-1* on intrachromosomal recombination in the rDNA array

Genotype/ strain	Dyads, no.	Ura phenotype, no.			% recombination
		+,+	+,−	−,−	
RAD50					
K355-13A	128	118	2	8	1.7
JW165-19D	115	99	7	9	6.6
JW165-37D	127	119	2	6	1.7
JW167-171B	441	426	8	7	1.8
JW230-5A	93	86	5	2	5.5
SG25-34C	232	231	1	0	0.4
SG25-36D	156	154	2	0	1.3
Total	1292	1233	27	32	2.1
rad50-1					
JW168-23A	334	280	5	49	1.8
JW168-62B	398	359	4	35	1.1
JW231-4D	102	90	2	10	2.2
SG25-12A	122	120	1	1	0.8
SG25-29C	218	205	1	12	0.5
Total	1174	1054	13	107	1.2

All strains are disomic ($n + 1$) for chromosome III (*MATa*/*MATα*), except for haploids (n) SG25-12A (*MATa*), SG25-29C (*MATα*), SG25-34C (*MATa*), and SG25-36D (*MATα*), each of which carries the opposite *MAT* allele on a *CEN* plasmid. All of the above strains also contain *spo13-1*, *ura3*, and *URA3* in rDNA. The absence or presence of meiotic intrachromosomal exchange is detected by Ura^+,Ura^+ and Ura^+,Ura^- dyads, respectively [see Fig. 1 and ref. 16). Percent recombination = (+,−)/[(+,−) + (+,+)]; Ura^-,Ura^- dyads are assumed to be due to prior events during mitotic growth and are not included in this calculation.

Table 4. Effect of *rad50-1* on intrachromosomal recombination in a *his3* duplication integrated at *HIS4* or in the rDNA array

Genotype/ strain	His$^+$ per 10^4 cfu, mean		
	Mitotic	Meiotic	Meiotic increase
his3 at *HIS4*			
RAD50			
SG9-36B	1.19	12.80	11.61
SG9-24B/ pSG(RAD50)1	0.50	10.47	9.97
rad50-1			
SG9-24B	1.06	1.50	0.44
his3 in rDNA			
RAD50			
SG9-36B	1.89	13.84	11.95
SG9-24B pSG(RAD50)1	4.13	15.86	11.73
rad50-1			
SG9-24B	2.98	2.89	−0.09

SG9-24B (*MATα*) and SG9-36B (*MATα*) are *his3Δ200 sir2 spo13-1 trp1-1 ura3* haploids and have plasmids pR656 and pR658 containing the *his3* duplication and *URA3* integrated at either *HIS4* or in the rDNA, respectively. His$^+$ recombinants retain the integrated plasmid and are therefore Ura$^+$. Mean frequencies of His$^+$ are an average of five (or six in the case of SG9-36B/pR658) independent cultures per strain and are calculated from His$^+$ colony-forming units (cfu)/ Ura$^+$ cfu to correct for loss of the plasmid. Sporulated cells were treated with Glusulase for 4 hr, and spores were plated on His$^-$ or Ura$^-$ selective media. The *rad50-1* and *RAD50 spo13-1* haploids both sporulated ≈40–50%. The meiotic increase was determined by subtracting the mitotic frequency of His$^+$ cfus from the meiotic frequency of His$^+$ cfus.

quences inserted in the rDNA is unaffected by the presence or absence of *SIR2*.

Taken together, these results indicate that sequences integrated in the rDNA do not acquire the properties of exchange characteristic of the rDNA, suggesting that the recombination behavior of the array is due either to specific rDNA sequences themselves or general properties of genes that are present in many multiple copies.

Mitotic Intrachromosomal Recombination in *rad50-1* Haploids Occurs at Normal or Increased Frequencies. The radiation sensitivity of *rad50* strains indicates that the *RAD50* gene product acts during mitosis. Malone and Esposito (23) noted that *rad50-1* diploids in some intervals show elevated levels of spontaneous mitotic recombination between homologs, although Saeki *et al.* (31) have reported reduced rates of γ-ray-induced mitotic gene conversion and reciprocal exchange in *rad50* diploids. To determine the effect of *rad50-1* on spontaneous mitotic intrachromosomal exchange in our strains, *spo13-1* haploids containing either the *his4* duplication or *URA3* integrated in the rDNA were examined. In contrast to meiotic cells, the lack of *RAD50* function in mitosis did not abolish exchange and therefore is not absolutely required for exchange in non-rDNA regions (Table 5). As in previous studies (23), the absence of *RAD50* appears to be associated with a hyper-Rec phenotype in some regions of the genome. Recombination in rDNA (monitored by the percentage of Ura$^+$:Ura$^-$ sectored colonies) and between *leu2* alleles is increased, whereas there is no enhancement of exchange between duplicated *his4* genes. The basis of this variable hyperrecombination behavior is presently unknown. It has been proposed that it may be due to DNA lesions that accumulate in the absence of *RAD50* that stimulate recombination (23).

Table 5. Spontaneous mitotic recombination in *RAD50* and *rad50-1* haploids

Genotype/ strain	Cultures, no.	Recombination event		
		leu2 alleles, Leu$^+$ per 10^6 cfu	*URA3* in rDNA, Ura$^+$:Ura$^-$ per 10^2 cfu	*HIS4* duplication, His$^+$ per 10^5 cfu
RAD50				
JW167-171B	5	9.2	0.1	
JW168-7B	3	7.8	0.1	
JW211-43B/ pSG(RAD50)2	5			10.5
rad50-1				
JW168-23A	5	NT*	1.3	
JW168-62B	3	36.7	0.8	
JW211-43B	6			9.7
rad50-1/RAD50†		4.3	14.0	0.9

JW211-43B is G15-4 (see Table 2) without the *URA3* gene in the rDNA. All other strains are disomic for chromosome III *MATa/ MATα*, are heteroallelic at the *LEU2* and *HIS4* loci (*leu2-27 his4A-25/leu2-1 his4B-331*), and contain *spo13-1*, *ura3*, and *URA3* in rDNA. All values are the means of the indicated number of cultures. Since Ura$^+$:Ura$^-$ are sectored colonies, they indicate the rate of these events per cell division.

*JW168-23A could not be tested since it is a *leu1* auxotroph in addition to being heteroallelic at *leu2*.

†Relative increase in *rad50-1* in all cultures tested.

DISCUSSION

The conclusion that reciprocal intrachromosomal meiotic recombination and interchromosomal meiotic recombination require common recombination functions and utilize the same recombination pathway, at least in non-rDNA, is supported by the following observations. In the presence of both homologs, reciprocal exchange between chromosomes occurs much more frequently (>10-fold) than intrachromosomal events involving the same gene duplication (ref. 27; R.E.E., unpublished observations). Moreover, when only one homolog is present, reciprocal intrachromosomal events increase (≈15-fold) (ref. 16; this study). Taken together these results suggest that reciprocal events between homologs can suppress events within chromosomes. One explanation for this behavior is that both types of recombination events may compete for common recombination functions and/or intermediates. The results presented here, and in a previous report (16), support this view: at least two meiotic recombination genes, *SPO11* and *RAD50*, have been shown to be required for both events. How might such functions be executed preferentially in interchromosomal events? One possibility is that the structure or conformation of paired homologs reduces the probability of forming appropriate recombination intermediates within a chromosome.

The behavior of rDNA is intriguing in this regard. In wild-type cells, rDNA reciprocal exchange between homologs occurs at <2% the rate predicted by physical distance, while intrachromosomal events are relatively more frequent (3, 32). These findings are also compatible with the notion that intra- and interchromosomal events are competitive. In this case however, intrachromosomal events predominate and occur at similar levels in diploid and haploid meiosis, while interchromosomal events are highly suppressed (3, 16, 32). These observations suggest that the properties of either the rDNA sequences themselves and/or the physical arrangement of a large number of tandem repeats favors intrachromosomal events and utilizes a different recombination function(s) and/or intermediate(s). Some evidence for this proposal comes from the discovery that rDNA exchange in meiosis does not depend on the presence of *RAD50*, which is required for non-rDNA exchange.

Below we present two explanations for why *RAD50* does not appear to be required for meiotic intrachromosomal recombination events in the rDNA. The first is that *RAD50* either regulates or promotes meiotic pairing and that it does

so only in non-rDNA regions, so that its absence has no consequences for rDNA exchange. This proposal is based on the findings that (*i*) *rad50* diploids (ref. 33 and B. Byers, personal communication) and *rad50 spo13-1* haploids (S.G., J.W., L. Jensen, and R.E.E., unpublished data), lack synaptonemal complexes (SCs), structures thought to mediate homolog pairing during meiotic prophase (34); (*ii*) SCs cannot be visualized in the rDNA-containing nucleolus in yeast (35); and (*iii*) genetic and molecular tests suggest that the *RAD50* gene functions early in the recombination process (23, 36, 37). These results suggest that *rad50* is defective in a pairing mechanism involving SC formation, although the absence of SCs in *rad50* mutants and in the nucleolus could be due to other reasons. However, if this model is correct then one may infer that SCs can initiate between relatively short stretches of homology, as suggested by Carpenter (38). This is based on the striking finding that intrachromosomal recombination between *his3* truncated genes with only 300 bases of homology still requires the *RAD50* function, even when inserted in the rDNA. Furthermore, the fact that recombination between the *his3* sequences occurs at approximately the same frequency at both *HIS4* and in the rDNA in wild-type cells suggests that embedding sequences within the array does not affect their accessibility as recombination substrates.

The second explanation is that *RAD50* is required for meiotic rDNA exchange, but the events observed during *rad50-1* meiosis occur by an independent recombination pathway operating in the absence of *RAD50*. This possibility is considered in the light of our data on mitotic recombination in the rDNA in *rad50-1* mutants, where the rate of mitotic recombination in the rDNA is seen to increase 14-fold in *rad50-1* mutants (Table 5). Since the induction of meiotic rDNA exchange in wild-type strains is only ≈20-fold, the recombination events observed in *rad50-1* mutants during meiosis could occur by the same pathway as the enhanced rDNA recombination events in *rad50-1* mitosis. Evidence against this view is that a correlation between the rates of mitotic and meiotic rDNA recombination has not been observed—e.g., strains with both high (see strain JW194-64B in 15) or low (SG25-12A in Table 3) levels of mitotic exchange in rDNA exhibit similar frequencies (≈2%) of meiotic events. We emphasize that the second explanation requires the existence of a substitute function for *RAD50* that restores wild-type levels of recombination in rDNA. Thus, in considering either hypothesis, one must conclude that there are at least two pathways by which intrachromosomal exchange occurs.

In summary, our data show that *RAD50*, like *SPO11*, is required for meiotic intrachromosomal recombination between non-rDNA repeated DNA sequences. However, unlike *SPO11*, *RAD50* is dispensable for meiotic intrachromosomal recombination in rDNA, indicating the operation of an alternate pathway of recombination in the rDNA.

We thank C. Atcheson, S. Honigberg, S. Klapholz, and F. Stahl for helpful comments on the manuscript and M. Ruzinski for technical assistance. This work was supported by Public Health Service Grants GM29182 and HD19252 (to R.E.E.) and National Research Service Award Predoctoral Training Grant GM07197 (to J.W. and S.G.) from the National Institutes of Health.

1. Baker, B. S., Carpenter, A. T. C., Esposito, M. S., Esposito, R. E. & Sandler, L. (1976) *Annu. Rev. Genet.* **10,** 53–134.
2. Klapholz, S., Waddell, C. S. & Esposito, R. E. (1985) *Genetics* **110,** 187–216.
3. Petes, T. D. (1980) *Cell* **19,** 765–774.
4. Szostak, J. W. & Wu, R. (1980) *Nature (London)* **284,** 426–430.
5. Endow, S. A., Komma, D. J. & Atwood, K. C. (1984) *Genetics* **108,** 969–983.
6. Slightom, J. L., Blechl, A. E. & Smithies, O. (1980) *Cell* **21,** 627–638.
7. Liebhaber, S. A., Goosens, M. & Kan, Y. W. (1981) *Nature (London)* **290,** 26–29.
8. Nagylaki, T. & Petes, T. D. (1982) *Genetics* **100,** 315–337.
9. Weiss, E. H., Mellor, A., Golden, L., Fahrner, K., Simpson, E., Hurst, J. & Flavell, R. A. (1983) *Nature (London)* **301,** 671–674.
10. Krawinkel, V., Zoebelein, G., Bruggemann, M., Radbruch, A. & Rajewsky, K. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 4997–5001.
11. Haber, J. E. (1983) in *Mobile Genetic Elements*, ed. Shapiro, J. A. (Academic, New York), pp. 539–619.
12. Borst, P. (1983) in *Mobile Genetic Elements*, ed. Shapiro, J. A. (Academic, New York), pp. 621–659.
13. Tonegawa, S. (1983) *Nature (London)* **302,** 575–581.
14. Klapholz, S. & Esposito, R. E. (1980) *Genetics* **96,** 567–588.
15. Wagstaff, J. E., Klapholz, S. & Esposito, R. E. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 2986–2990.
16. Wagstaff, J. E., Klapholz, S., Waddell, C. S., Jensen, L. & Esposito, R. E. (1985) *Mol. Cell Biol.* **5,** 3532–3544.
17. Klapholz, S. & Esposito, R. E. (1982) *Genetics* **100,** 387–412.
18. Game, J. C. & Mortimer, R. K. (1974) *Mutat. Res.* **24,** 281–292.
19. Mortimer, R. K. & Hawthorne, D. C. (1969) in *The Yeasts*, eds. Rose, A. M. & Harrison, J. S. (Academic, New York), Vol. 1, pp. 385–460.
20. Kupiec, M. & Simchen, G. (1984) *Mol. Gen. Genet.* **193,** 525–531.
21. Fasullo, M. & Davis, R. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 6215–6219.
22. Game, J. C., Zamb, T. J., Braun, R. J., Resnick, M. & Roth, R. M. (1980) *Genetics* **94,** 51–68.
23. Malone, R. E. & Esposito, R. E. (1981) *Mol. Cell Biol.* **1,** 891–901.
24. Mortimer, R. & Schild, D. (1980) *Microbiol. Rev.* **44,** 519–571.
25. Klar, A. J. S., Fogel, S. & Macleod, K. (1979) *Genetics* **93,** 37–50.
26. Jackson, J. A. & Fink, G. R. (1981) *Nature (London)* **292,** 306–311.
27. Jackson, J. A. & Fink, G. R. (1985) *Genetics* **109,** 303–332.
28. Petes, T. D. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 410–414.
29. Zamb, T. J. & Petes, T. D. (1981) *Curr. Genet.* **3,** 125–132.
30. Gottlieb, S. & Esposito, R. E. (1989) *Cell* **56,** 771–776.
31. Saeki, T., Machida, I. & Nakai, S. (1980) *Mutat. Res.* **73,** 251–265.
32. Petes, T. D. & Botstein, D. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5091–5095.
33. Farnet, C., Padmore, R., Cao, L., Raymond, W., Alani, E. & Kleckner, N. (1988) in *Mechanisms and Consequences of DNA Damage Processing*, UCLA Symposia on Molecular Biology, eds. Friedberg, E. & Hanawalt, P. (Liss, New York), Vol. 83.
34. von Wettstein, D., Rasmussen, S. W. & Holm, P. B. (1984) *Annu. Rev. Genet.* **18,** 331–413.
35. Byers, B. & Goetsch, L. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 5056–5060.
36. Malone, R. E. (1983) *Mol. Gen. Genet.* **189,** 405–412.
37. Borts, R. H., Lichten, M. & Haber, J. E. (1986) *Genetics* **113,** 551–567.
38. Carpenter, A. T. C. (1987) *BioEssays* **6,** 232–236.