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Genetics

Simple Mendelian inheritance of the reiterated ribosomal DNA of yeast

(ribosomal RNA genes/tetrad analysis/meiotic recombination/mitotic recombination/restriction endonuclease *Eco*RI)

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ABSTRACT **A diploid strain of yeast (*Saccharomyces cerevisiae*) was found to be heterozygous for two forms of the highly repetitious ribosomal DNA. These forms could be distinguished by the pattern of fragments produced after digestion with the site-specific restriction endonuclease *Eco*RI. The mode of inheritance of ribosomal DNA was determined by tetrad analysis.**

Of 14 tetrads analyzed, 12 clearly showed the ribosomal DNA forms segregating as a single Mendelian unit. The simplest interpretation of this result is that all of the approximately 100 copies of the ribosomal DNA genes of the yeast cell are located on one chromosome and that meiotic recombination within these genes is suppressed. Two of the 14 tetrads showed the segregation patterns expected as the result of mitotic recombination within the ribosomal DNA.

The yeast *Saccharomyces cerevisiae*, in common with other eukaryotes, has many copies of the genes coding for ribosomal RNA. Each haploid cell has about 100 to 140 copies of the genes coding for the 25S, 18S, 5.8S, and 5S rRNA species (1, 2). Experiments of various types have been reported which suggest that the rRNA genes are organized into units, each containing a single copy of the genes coding for the four rRNA species (refs. 3, 4, and †; R. Davis, personal communication). There is also evidence indicating that the ribosomal units are organized into long tandem arrays (5, †). The number of such arrays and their chromosomal location, however, are less clear. Using saturation hybridization of rRNA to DNA from monosomic yeast strains (strains with $2n - 1$ chromosomes), several groups have obtained evidence suggesting that about 70% of the ribosomal DNA (rDNA) is located on chromosome I (6–8).

In this paper, we present evidence indicating that almost all (at least 90%) of the rDNA is located on a single chromosome. These results suggest that there might be a single long stretch of repeating rDNA units in the yeast genome. In addition, we have not observed meiotic recombination within the 100 copies of yeast rDNA, although mitotic recombination appears to occur. This indicates that the structure of the chromosomal region containing rDNA may be different from that of other comparably long segments of yeast chromosomes.

MATERIALS AND METHODS

Yeast Strains. The diploid +D4 was provided by L. H. Hartwell (University of Washington). The genotype of the strain is:

$$\frac{a \;+\; \textit{gal1}\ \textit{lys2}\ \textit{tyr1}\ \textit{his7}\ \textit{ura1}\ \textit{ade1} \;+\; +\; \textit{ade2}}{\alpha\ \textit{leu2}\ \textit{gal1} \;+\; +\; +\; \textit{ura1}\ \textit{ade1}\ \textit{his5}\ \textit{lys11} \;+}$$

Hartwell made the diploid by mating the haploid strains A364a (a *ade1 ura1 gal1 ade2 tyr1 his7 lys2*) and 2262 (α *ade1 ura1 gal1 his5 lys11 leu2*). A364a was provided by Hartwell and 2262 by C. McLaughlin (University of California, Irvine). Both A364a and 2262 are laboratory stocks which have been in use for many years; the degree of relatedness between them cannot reliably be estimated.

Genetic Analysis. The diploid was induced to sporulate by using the conditions described by Brandriss *et al.* (9). Other methods of tetrad dissection were standard. Genetic markers in the spores were checked either by growth on minimal plates lacking various amino acids or by mating to tester strains of known genotype, which were as follows: *ade2*, DBY222 (a *ade2*) and DBY 223 (α *ade2*); *his7*, DBY167 (α *ade2 his7*) and DBY170 (a *trp5 his7*); *lys2*, A35 (α *lys2*) and A36 (a *lys2*); *his5*, DBY128 (a *can1 his5*) and DBY173 (α *his5 ade2*); mating type, DBY3 (α *ade1 his1 trp1*). The only marker not checked in these experiments was *lys11*.

Isolation of Yeast rDNA. Yeast rDNA was isolated using CsCl density gradients that contained the fluorescent dye Hoechst 33258 (refs. 10 and 11; D. H. Williamson, personal communication). Control experiments indicate that this procedure provides a 2- to 3-fold enrichment of rDNA.

Restriction Analysis of Yeast rDNA. The isolated rDNA was treated with restriction endonuclease *Eco*RI (Miles Research Products) in a buffer containing 0.1 M Tris, 0.05 M NaCl, and 0.01 M $MgCl_2$ (pH 7.5). After 1 hr at 37°, the digestion products were electrophoresed on 1.4% agarose gels containing ethidium bromide at 0.5 μg/ml (12). Gels were photographed under ultraviolet illumination with Polaroid 55 or 57 film.

DNA·DNA Hybridization with Restriction Fragments from Gels. ^{32}P-Labeled DNA probe was hybridized to rDNA restriction fragments that had been transferred from a gel to a nitrocellulose filter (13). The DNA probe was prepared from an *Escherichia coli* strain (pY1rG12) that carried a recombinant plasmid (yeast rDNA inserted in the plasmid vector pMB9†). The plasmid contained several of the *Eco*RI fragments found in yeast rDNA (including B, C, E, G, and parts of D and F). Experiments involving this plasmid were conducted under P2 conditions, as described in the NIH recombinant DNA research guidelines. The plasmid was labeled to a specific activity of 5×10^7 cpm/μg by nick translation with DNA polymerase I of *E. coli* (Boehringer-Mannheim) (14). In control experiments, this technique easily detects single copy sequences (D. Botstein, unpublished data).

The transfer of fragments from the gel to the filters and the hybridization were done as described by Botchan *et al.* (15).

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Abbreviation: rDNA, DNA coding for ribosomal RNA.

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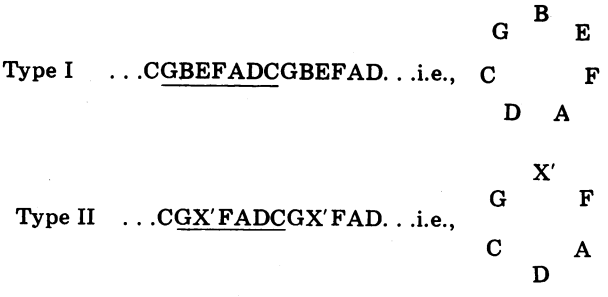
† T. D. Petes, L. M. Hereford, and K. G. Skryabin, unpublished data.

RESULTS

In the course of analyzing the structure of the rDNA repeating unit in yeast, we recently observed that the diploid strain +D4 contained two different repeating units of rDNA in approximately equal amounts.† The two alternatives were distinguished by patterns of fragments produced after digestion with the site-specific restriction endonuclease *Eco*RI: Type I units produce seven fragments, while Type II units produce six fragments. The restriction maps of these types of repeating units are as follows:



where the underlined region gives the repeating unit. The unit is also represented as a circular map, the topological equivalent of tandem repetition. Fragments X′, A, B, C, D, E, F, and G have, respectively, molecular lengths of 2.86, 2.71, 2.26, 1.87, 0.60, 0.55, 0.34, and 0.26 kilobase pairs (representing molecular weights of 1.87, 1.77, 1.48, 1.22, 0.39, 0.36, 0.22, and 0.17 × 10^6). The difference between the Type I and Type II units is the substitution in Type II units of the X′ fragment for fragments B and E of the Type I pattern. This conclusion is supported by the fact that fragment X′ has a molecular length approximately equal to the sum of the lengths of fragments B and E and by the fact that fragments B and E hybridize to fragment X′ (†; see also below).

An obvious possible explanation for the two patterns found in the diploid +D4 is that this strain is heterozygous for the two types of rDNA repeating units. Because the haploid parents of this diploid were known, a comparison of the total rDNA from +D4 and its parents (strains A364a and 2262; L. M. Hartwell, personal communication) was carried out. Total rDNA was isolated from each strain by taking advantage of the fact that rDNA differs in its base composition (and therefore density in CsCl equilibrium gradients) from the nuclear and mitochondrial DNA of yeast (5, 16). Fig. 1*a* shows the results obtained when rDNA from the diploid +D4 is digested with *Eco*RI and analyzed by electrophoresis on agarose gels. Eight bands are observed, labeled X′, A, B, C, D, E, F, and G in order of decreasing size. The bands correspond to the fragments given in the map. The bands representing fragments X′, B, and E are less intense than the remaining bands because X′ is present only in Type II units and B and E are present only in Type I units, whereas all the other fragments are common to both types of repeating rDNA units. Fig. 1*b* shows that the strain A364a, one of the haploid parents of +D4, has Type I rDNA genes, containing the seven *Eco*RI fragments A, B, C, D, E, F, and G. Fig. 1*c* shows that strain 2262, the other haploid parent of +D4, has Type II rDNA genes; six fragments (X′, A, C, D, F, and G) are observed after digestion with *Eco*RI. These results, therefore, show that the diploid +D4 is heterozygous for the two types of rDNA: its haploid parents each have one of the two types of rDNA. Further, these results confirm that the X′ fragment is an alternative allele of fragments B and E, as expected from previous results.† It should be noted that the nonhomology between the two rDNA Types II is probably small, because all but one of the *Eco*RI sites and all *Hin*dIII and *Bgl*II sites are held in common (T. Petes, unpublished data).

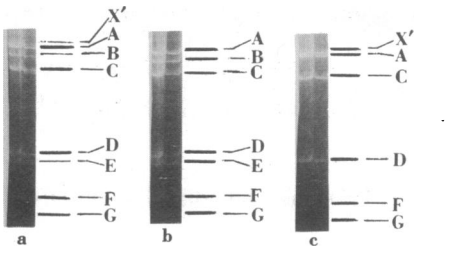


FIG. 1. Analysis of *Eco*RI fragments of ribosomal DNA from the diploid yeast strain +D4 and its haploid parents, strains A364a and 2262. Yeast rDNA was isolated by density gradient centrifugation (11), digested with *Eco*RI, and the fragments produced were separated by agarose gel electrophoresis in the presence of ethidium bromide. Next to each photograph, a schematic drawing of the bands is given, because the smallest bands are not well visualized. (*a*) *Eco*RI digest of rDNA from +D4. Thin lines in the schematic drawing for X′, B, and E indicate that the bands are present in smaller amounts than the other bands. (*b*) *Eco*RI digest of rDNA from the haploid parent A364a. (*c*) *Eco*RI digest of rDNA from the haploid parent 2262.

Inheritance of the rDNA Repeating Units. Having established that the existence of two types of rDNA repeating units in the diploid +D4 is the result of heterozygosity for the rDNA, it became possible to use the difference between Type I and Type II rDNA units as a genetic marker that can be followed through meiosis. If the rDNA is on a single chromosome, then one might expect (in the absence of recombination) to find simple Mendelian segregation of the Type I/Type II difference: i.e., 2 Type I:2 Type II spores in tetrads. Of approximately 30 tetrads dissected, 14 produced four viable spores. Of the remaining 16 tetrads, 14 produced three viable spores and 2 produced two viable spores. These spores were analyzed with respect to the meiotic segregation of various nutritional markers and of mating type. We then isolated rDNA from cultures grown from individual spores. The isolated rDNA was treated with *Eco*RI and examined by agarose gel electrophoresis.

The results of this analysis are shown in Table 1. In 12 of 14 tetrads, the Type I and Type II rDNA patterns segregated 2:2, two of the spores resembling one parent and two spores the other. This is the result expected for segregation of a single Mendelian factor. Likewise, each of the other genetic markers segregated 2:2 in at least $^{13}/_{14}$ tetrads. All the exceptional cases (rDNA in tetrads 1 and 10; *his*7 and *tyr*1 in tetrad 4; mating type in tetrad 6) are most easily explained if the particular diploid cells which gave rise to the tetrad had undergone a mitotic recombination event prior to meiosis. The exceptions are discussed in more detail below.

A photograph of the agarose gel analysis of *Eco*RI-digested rDNA from the four spores of a single tetrad (tetrad 4) is shown in Fig. 2. It is clear that two of the spores show the Type I pattern (seven fragments) and the other two show the Type II pattern (six fragments). This kind of analysis should detect as little as 10% contamination of one pattern by the other; reconstruction experiments (not shown) with deliberate mixtures of the two types confirm this.

In order to assess the degree of purity of the rDNA patterns in a more sensitive way, a second procedure was employed. Tetrad 4 (whose pattern is shown in Fig. 2) has spores (4a and 4b) that exhibit the Type I pattern, containing the *Eco*RI fragments A, B, C, D, E, F, and G. The other two spores (4c and 4d) show the Type II pattern, containing the *Eco*RI fragments X′, A, C, D, F, and G. The experimental procedure was designed to detect a small amount of the X′ fragment in spores 4a

Table 1. Segregation of markers in tetrads from the diploid +D4

	Markers on chromosome:							
	III		II			IX	XV	rDNA
Spores	m.t.	*leu2*	*lys2*	*tyr1*	*his7*	*his5*	*ade2*	pattern
1a	a	+	−	−	+	+	−	I ≫ II
1b	a	+	−	−	−	−	+	I ≫ II
1c	α	−	+	+	+	+	+	I
1d	α	−	+	+	−	−	−	I
2a	α	−	−	+	+	+	−	I
2b	a	+	+	−	−	+	+	II
2c	a	+	−	−	+	−	+	I
2d	α	−	+	+	−	−	−	II
3a	α	−	−	−	+	+	+	II
3b	a	+	−	−	+	−	−	I
3c	a	+	+	+	−	−	+	I
3d	α	−	+	+	−	+	−	II
4a	a	+	+	+	+	+	−	I
4b	α	−	−	+	+	−	−	I
4c	a	+	−	+	+	−	+	II
4d	α	−	+	+	+	+	+	II
5a	α	−	−	−	−	+	−	II
5b	α	−	+	+	+	+	−	II
5c	a	+	−	−	−	−	+	I
5d	a	+	+	+	+	−	+	I
6a	α	+	−	−	−	−	−	II
6b	α	−	+	+	+	+	−	II
6c	α	+	+	−	−	−	+	I
6d	α	−	−	+	+	+	+	I
7a	α	−	−	+	−	−	−	II
7b	a	+	+	+	+	+	−	I
7c	α	−	+	−	−	−	+	II
7d	a	+	−	−	+	+	+	I
8a	a	+	−	−	−	−	−	I
8b	α	−	−	−	−	+	+	II
8c	α	−	+	+	+	+	−	I
8d	a	+	+	+	+	−	+	II
9a	a	+	+	+	−	−	−	I
9b	α	−	−	−	+	−	−	II
9c	α	−	−	−	−	+	+	I
9d	a	+	+	+	+	+	+	II
10a	α	+	+	+	+	+	+	I ≫ II
10b	a	+	+	−	−	−	−	I ≫ II
10c	a	−	−	+	+	−	+	II ≫ I
10d	α	−	−	−	−	+	−	II ≫ I
11a	α	−	−	−	+	−	+	II
11b	a	+	−	+	−	+	−	I
11c	a	+	+	+	+	+	+	I
11d	α	−	+	−	−	−	−	II
12a	a	−	+	+	+	+	+	I
12b	a	+	−	−	−	−	−	II
12c	α	+	+	+	+	−	+	I
12d	α	−	−	−	−	+	−	II
13a	a	+	−	+	+	+	−	I
13b	a	+	−	−	−	−	+	II
13c	α	−	+	−	−	−	+	II
13d	α	−	+	+	+	+	−	I
14a	α	+	−	+	+	−	+	II
14b	α	−	+	−	−	−	+	I
14c	a	+	−	−	−	+	−	I
14d	a	−	+	+	+	+	−	II

Genotype of +D4 =

$$\frac{a\ +\ gal1\ lys2\ tyr1\ his7\ ura1\ ade1\ +\ +\ ade2}{\alpha\ leu2\ gal1\ +\ +\ +\ ura1\ ade1\ his5\ lys11\ +}.$$

I ≫ II means primarily Type I pattern with a minor amount (less than 25%) of Type II. m.t., mating type.

and 4b or a small amount of fragment B in spores 4c and 4d. The *Eco*RI fragments of the gel shown in Fig. 2 were transferred to nitrocellulose filter paper (13). ^{32}P-Labeled DNA from a recombinant plasmid that contains the yeast rDNA fragments B, C, E, and G was hybridized to the filter paper. The position of hybridization was found by comparing the gel photograph shown in Fig. 2 to the autoradiograph of the filter after hybridization reaction.

The pattern shown in Fig. 3 shows the hybridization of the

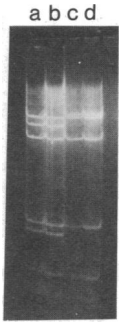


FIG. 2. Analysis of *Eco*RI fragments of rDNA from the four spores in tetrad 4 (Table 1). After dissection, each spore was grown into a culture from which rDNA was isolated and analyzed. Spores a and b show the Type I pattern (seven fragments) and spores c and d show the type II pattern (six fragments).

probe to the larger *Eco*RI fragments (X′, A, B, and C) of the spores. The labeled C fragment hybridized to rDNA from all the spores at the expected position. The B fragment contains sequences in common with the X′ fragment.† The pattern shown in Fig. 3, therefore, is the expected pattern; rDNA from spores 4a and 4b hybridized to the probe at the position of the B fragment and rDNA from 4c and 4d hybridized at the position of the X′ fragment. No contamination of Type I rDNA and Type II rDNA was observed in the spore hybridization patterns. This experiment should have detected as little as 1–2% contamination of rDNA patterns in the spores, because the technique detects single-copy yeast sequences of comparable length.

In 2 tetrads of the 14 analyzed, the Type I and Type II rDNA cistrons did not segregate 2:2. As indicated in Table 1, one tetrad (tetrad 1) had two spores that had only Type I rDNA and two spores that were predominantly Type I but also had a small amount of Type II rDNA (Fig. 4). The second tetrad (tetrad 10) had two spores that were predominantly the Type I pattern with a small amount of the Type II pattern and two spores of the reciprocal class. As will be discussed in more detail later, the segregation patterns of these two tetrads can be explained by a mitotic recombination event preceding meiosis.

DISCUSSION

The finding of two types of repeating ribosomal DNA units in the diploid yeast strain +D4 made possible a genetic analysis

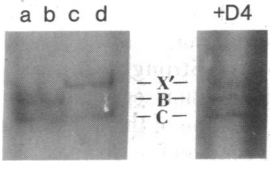


FIG. 3. Hybridization analysis of rDNA segregation in the four spores of tetrad +D4-4. The restriction fragments from the gel shown in Fig. 2 were transferred to a nitrocellulose filter (13). The filter was hybridized to ^{32}P-labeled DNA from a recombinant plasmid containing an insertion of yeast ribosomal DNA. The rDNA *Eco*RI fragments B, C, F, and G were present in the recombinant plasmid. Spores a and b show two positions of hybridization, corresponding to the positions of fragments B and C in the gel. Spores c and d show two positions of hybridization corresponding to the positions of C and X′. Because the B fragment contains sequences homologous to X′,† this is the expected result. The gel labeled +D4 represents rDNA isolated from the diploid +D4 that was hybridized to the same probe as the spore DNA. As expected, hybridization is observed at the positions of X′, B, and C. The bottom part of the gel showing hybridization to E, F, and G is not shown.

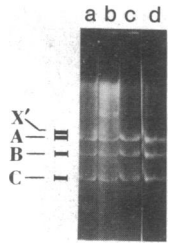


FIG. 4. Analysis of *Eco*RI fragments of rDNA from the four spores of tetrad 1 (Table 1), two spores of which contain both types of rDNA in unequal amounts. Spores a and b are seen to contain both fragment B (characteristic of Type I rDNA) and fragment X′ (characteristic of Type II rDNA) as well as the common fragments A and C. Spores c and d show only the Type I pattern. The lower molecular weight fragments (D, E, F, and G) are not shown.

of the inheritance of rDNA. The genetic results can be summarized simply as follows: the difference between Type I and Type II rDNA units behaves as a simple Mendelian trait. The parents of the diploid +D4 differ in their rDNA; the diploid itself is heterozygous for this difference; and the difference segregates 2:2 in tetrads.

By itself, the 2:2 segregation leads to several conclusions. First, most of the yeast rDNA (greater than 90%) is located on a single chromosome. Second, meiotic recombination within the tandemly arranged rDNA genes is rare. Third, yeast rDNA is not extra-chromosomal unless extra-chromosomal DNA is degraded before meiotic segregation. Each of these conclusions will be discussed in turn below.

Most of the Yeast rDNA Is on a Single Chromosome. The argument that virtually all the rDNA of yeast is on a single chromosome can be illustrated as follows: if the rDNA were divided among two chromosomes, half the tetrads would still exhibit 2:2 segregation, but the other half would contain four spores, each of which contained a mixture of the two types of rDNA in the ratio of division of the rDNA between the two chromosomes. As the number of chromosomes containing rDNA increases, the probability that two spores of a tetrad will contain only Type I rDNA and two spores will contain only Type II rDNA gets smaller. Thus, the finding that at least $^{12}/_{14}$ tetrads contain two spores with Type I rDNA and two spores with Type II rDNA is not consistent with more than one independently segregating chromosome containing a substantial fraction of the rDNA. Clearly we cannot rule out a division of rDNA between two chromosomes in the ratio 100:1; however, it is extremely unlikely that as much as 10% of the rDNA could be on a second chromosome.

Yeast rDNA Is Not Strongly Centromere-Linked. The frequency of second-division segregation of a genetic marker during meiosis is a function of the recombinational distance of the marker from its centromere. In yeast, second-division segregation can be assessed by the frequency of tetratype asci using a known centromere-linked marker (17). *leu*2 is such a marker; Table 1 shows that $^{9}/_{14}$ asci are tetratype with respect to the rDNA pattern and *leu*2. Because *leu*2 itself shows 13% second-division segregation (17), a rough minimum estimate is that rDNA shows 51% second-division segregation. This value should be compared to 67% (no centromere linkage). Clearly the rDNA is not tightly linked to its centromere; in such a case we would have observed no second-division segregation beyond the 13% attributable to *leu*2. This result also implies that the rDNA units are on a single chromosome arm, because if they were on both arms of a chromosome, they could not be linked to each other without also being linked to their centromere.

Yeast rDNA Recombines Less Frequently during Meiosis than Nonribosomal Nuclear DNA. The segregation of the ribosomal DNA as a single unit in 12 of 14 tetrads indicates not only that most of the rDNA is on a single chromosome but also that the yeast rDNA repeating units do not recombine frequently during meiosis. It has been estimated (18) that each yeast cell undergoes at least 70 recombination events each meiosis. Because the ribosomal DNA represents 5% of the total cellular DNA (1), there should be at least 4 crossover events in rDNA in every meiosis. Because, as will be discussed later, the segregation of rDNA in tetrads 1 and 10 can best be explained by mitotic recombination, in none of the 14 tetrads analyzed was meiotic recombination of the rDNA observed. This suggests that meiotic recombination in yeast rDNA is at least 50-fold less frequent than recombination elsewhere in the nuclear genome. Because unequal crossover events within the tandemly arranged rDNA sequences could result in spores having large deletions or duplications of rDNA, it is possible that suppression of meiotic recombination has a selective advantage.

Several alternative explanations for the apparent lack of meiotic recombination of the rDNA should be considered. For example, it is possible that the 100 or more copies of the ribosomal repeating units found in mitotically dividing yeast cells do not represent the number of copies of rDNA present at meiosis. There must be at least two (possibly more) such copies because several of the spores contain both Type I and Type II rDNA in unequal amounts. The few copies present at meiosis could be amplified after spore germination and degraded prior to the next meiosis.

A second model is that although the number of physical copies of the rDNA repeating units is large, the number of informational copies is small. Meiotic recombination events could be obscured if the tandem arrays of rDNA units were made homogenous by a "master-slave" correction mechanism (19) after meiosis. The finding of spores with both Type I and Type II rDNA units places special restrictions on this model. The possibility of a structural explanation (i.e., inversion or translocation) accompanied by normal recombination is unlikely because spore viability, although not perfect in our cross, is much higher than expected for an average of four lethal recombinations per meiosis. On the other hand, chromosomal rearrangements that prevent pairing and subsequent crossover are not ruled out.

In our view, the most likely explanation is the relatively simple idea that the rDNA region has a structure not recognized efficiently by the meiotic recombination systems.

Mitotic Recombination of rDNA. In two tetrads (1 and 10), the Type I and Type II rDNA did not segregate 2:2. Tetrad 1 (Fig. 4) contained two spores with the Type I pattern and two spores that were predominantly Type I but also had Type II rDNA. Because unequal amounts of the two types of rDNA were recovered, such a tetrad cannot straightforwardly be explained by either independent segregation of two or more rDNA-containing chromosomes or a reciprocal meiotic recombination. A simple explanation for this segregation pattern is that mitotic recombination occurred in a diploid cell that was the precursor to tetrad 1 prior to meiosis (Fig. 5). As shown in the diagram, the products of a mitotic recombination can segregate in several ways. One of these ways yields a diploid cell that results, after meiosis, in the tetrad 1 pattern.

The segregation pattern observed for the tetrad 10 was two spores containing predominantly Type I rDNA with a small amount of Type II and the other two spores with mainly Type II rDNA and a small amount of Type I rDNA. This pattern can also readily be explained by mitotic recombination as shown

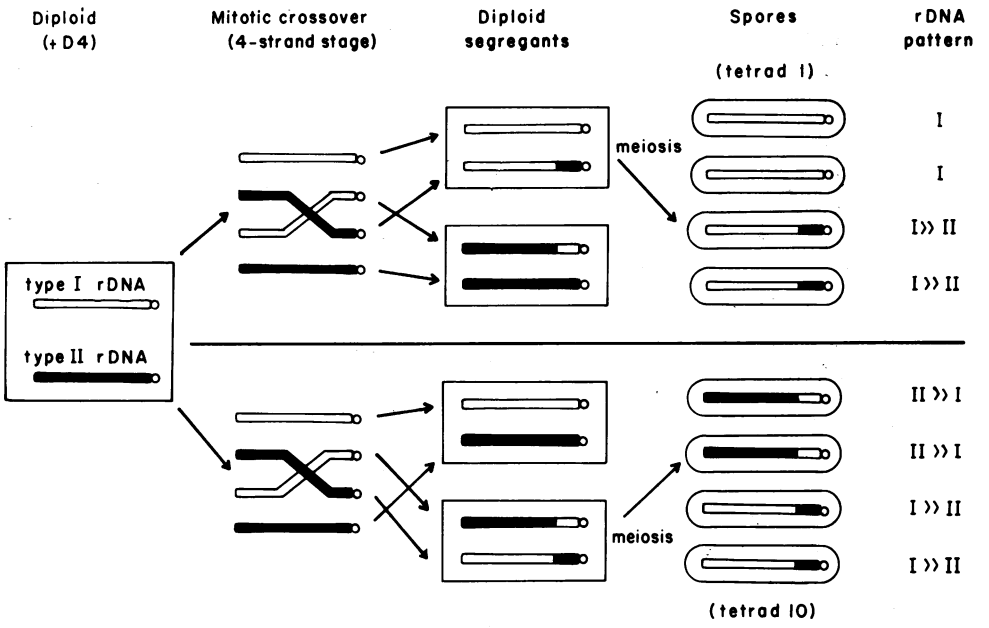


FIG. 5. Schematic diagram showing how mitotic recombination within the rDNA prior to meiosis can account for the distribution of rDNA patterns in tetrads 1 and 10 of Table 1. For convenience, rDNA is shown as comprising an entire chromosome, although this is not known to be the case. I ≫ II indicates that the rDNA is largely Type I with a minor amount of Type II.

in the bottom panel of Fig. 5. It should be noted that tetrads 1 and 10 must have been produced by independent mitotic events.

The segregation of rDNA in tetrad 10 could also be explained by a double crossover during meiosis or by independent segregation of two chromosomes carrying unequal amounts of rDNA. However, double crossovers should be much less frequent than single crossovers; no single meiotic crossovers were observed in our 14 tetrads. Similarly, rDNA on a second chromosome is unlikely for the reasons given above.

It should also be pointed out that several of the nonribosomal genetic markers showed segregation patterns indicating two separate mitotic recombination events (Table 1). For example, in the diploid that gave rise to tetrad 4, a mitotic crossover clearly occurred between *lys*2 and *tyr*1 on chromosome II, because both *tyr*1 and *his*7 (a third marker further away from the centromere on the same arm of chromosome II) segregated 4:0. The overall frequency of mitotic recombination observed in our experiments is somewhat higher than expected from previous studies (20). This might reflect strain differences or environmental effects.

In conclusion, it should be pointed out that our results indicate that there must be some differences in specificity between the enzyme systems responsible for mitotic and meiotic recombination, because the chromosomal region containing the reiterated rDNA seems to be recognized differently by these two recombination systems. Furthermore, our results indicate that very long stretches of yeast chromosomes can successfully undergo meiosis without accompanying crossover events.

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