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Genetics

Meiotic disjunction of circular minichromosomes in yeast does not require DNA homology

(recombination/chromosome segregation/chromosome pairing)

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ABSTRACT **Circular plasmids containing an autonomously replicating sequence (ARS) and a centromeric DNA sequence (CEN) segregate as independent linkage groups during meiosis in *Saccharomyces cerevisiae*. If two genetically marked plasmids are present in the same diploid cell, their segregation during meiosis may be determined relative to each other. It has been observed that for centromere plasmids containing some DNA sequences in common, these plasmids tend to segregate away from each other at the first meiotic division [Clarke, L. & Carbon, J. (1980) *Nature (London)* 287, 504–509; Clarke, L., Fitzgerald-Hayes, M., Buhler, J.-M. & Carbon, J. (1981) *Stadler Genet. Symp.* 13, 9–23]. Here we show that nonhomologous plasmids, having no detectable DNA sequence cross-hybridization, also tend to disjoin from each other at the first meiotic division. Therefore, this nonrandom segregation to opposite poles can occur by mechanisms that do not involve DNA sequence homology. This process may be an active nonhomologous pairing system or it may reflect unknown physical restraints on the meiotic segregation of the two plasmids. In either case, this process cannot be used as a possible assay for homologous meiotic pairing.**

After premeiotic DNA replication, two meiotic divisions are required to segregate the replicated diploid chromosome set into four haploid products (1). Pairing and recombination between homologous chromosomes occurs in the first meiotic prophase. The first meiotic division is reductional. Pairs of sister chromatids, held together by their centromeres, are segregated to opposite poles. The second meiotic division is equational and resembles a mitotic division. The centromeres divide, allowing sister chromatids to be segregated from one another.

Yeast undergoes a typical meiotic process in which the segregation of genetic markers on different linkage groups may be examined by tetrad analysis. Circular plasmids containing sequences that allow autonomous replication (ARS) and segregation [centromeric (CEN) DNA sequences] have been followed through meiosis in yeast (2, 3). These plasmids segregate as independent linkage groups through meiosis. Tetrad analysis indicates that the centromere on the plasmid faithfully holds its sister chromatids together through the first meiotic division [precocious centromere separation would lead to tetratype segregation (4) for the plasmid marker relative to other centromere-linked markers, which is only rarely observed]. In a number of instances, CEN plasmids containing two different selectable markers and various amounts of homologous DNA sequence were introduced into the same diploid cell, and their segregation during meiosis was examined. In all cases, the plasmids tended to segregate away from each other at the first meiotic division. A determination of the dependence of this process on sequence homology can be made by examining the meiotic segregation of two totally nonhomologous plasmids in yeast.

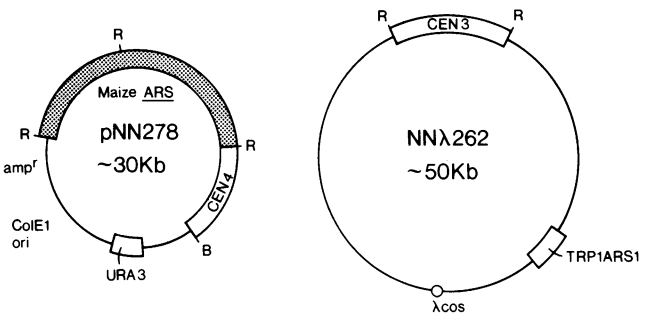

FIG. 1. Diagram of pNN278 and NNλ262. R, *Eco*RI; B, *Bam*HI.

MATERIALS AND METHODS

Two plasmids containing no DNA sequence homology (pNN278 and NNλ262; Fig. 1) were constructed and tested for their segregation during meiosis relative to one another. pNN278 was constructed by cloning genomic *Eco*RI fragments from the maize genome into the *Eco*RI site of the vector YIp5-Sc4137 (3). The maize DNA in this plasmid contains sequences that allow autonomous replication of this plasmid in yeast. The plasmid was made and generously provided by Roberta Berlani. NNλ262 was constructed by cloning a 5.2-kilobase (kb) *Eco*RI *CEN3* fragment into the vector λgt7 (5) and then performing a phage cross with λsep6TRP1 (3) and screening for recombinants that contained *CEN3*, *TRP1*, and *ARS1* sequences in *Escherichia coli*.

To test whether any sequence homology exists between pNN278 and NNλ262, 1 μg each of pNN278 and NNλ262 DNA was digested together with the restriction enzyme *Eco*RI. The digest was split into three equal aliquots and loaded in three separate lanes of a 0.7% agarose gel. After electrophoresis, the DNA was transferred to nitrocellulose sheets (5). Each lane was cut and removed for hybridization with a ^{32}P-labeled nick-translated probe (Fig. 2). The hybridization was done in probe excess with a probe average length of ≈250 base pairs (bp). Hybridization was performed in 50% (vol/vol) formamide/5× SSPE (0.9 M NaCl) (5) at 37°C for 24 hr. The filters were then washed in 5× SSPE at 23°C. We estimate that a perfectly homologous sequence of 30 bp would provide at least a moderate hybridization signal under these conditions. This must be a crude estimate, as we do not know the base composition of the maize DNA fragments. The estimate is based on the equation $t_m = 79.9 - 498/n$ determined by Hayes *et al.* (in which t_m is the melting temperature of DNA) (6) for oligomers of DNA of 50% G+C base content in 0.02 M NaCl. The addition of formamide to

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Abbreviations: ARS, autonomously replicating sequence; CEN, centromeric DNA sequence; kb, kilobase(s); bp, base pair(s).

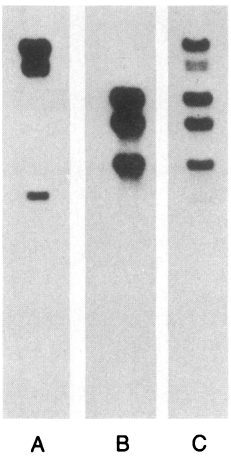

FIG. 2. Test for sequence homology of pNN278 and NNλ262 by hybridization analysis. Plasmid pNN278 and NNλ262 DNAs were mixed, digested with *Eco*RI, and separated on a 1% agarose gel. A DNA blot was prepared and cut into three strips. The strips were hybridized with the following probes: lane A, ^{32}P-labeled NNλ262 DNA; lane B, ^{32}P-labeled pNN278 DNA; lane C, a mixture of the two probes. Only the expected DNA bands hybridized, indicating no sequence homology between the two plasmids (see *Materials and Methods*).

50% decreases the t_m of DNA in 0.9 M NaCl to approximately the level that occurs in 0.02 M NaCl (7). Therefore, this equation should be approximately correct for our hybridization conditions. We expect duplexes to be efficiently formed and stable if hybridization occurs at 15°C below the t_m of the resulting duplex. Since we hybridize at 37°C, duplexes with t_m = 52°C should be formed. Substituting t_m = 52°C in the above equation yields n = 16 bp. Thus, 30 bp is a conservative estimate of the maximum amount of homology the plasmids may share.

Genomic Southern analysis verifying the structural integrity of pNN278 and NNλ262 plasmids in YNN289 yeast cells was performed as follows: rapid lysate DNA was prepared from spheroplasted YNN289 [(**a**/α *trp1Δ/trp1Δ his3Δ200/ his3Δ200 ura3-52/ura3-52 lys2-801/+ ade2-101/+ leu1/+*)/ pNN278 + NNλ262] yeast cells as described (8). The DNA was digested with *Eco*RI, electrophoresed on a 0.7% agarose gel along with plasmid markers and then transferred to nitrocellulose. The nitrocellulose filter was hybridized with a

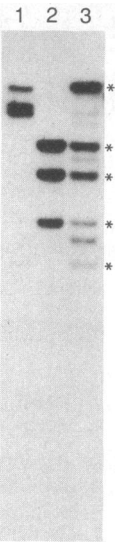

FIG. 3. Genomic Southern analysis of YNN289 containing the plasmids NNλ262 and pNN278. Lane 1, purified NNλ262 marker DNA (1 ng) cleaved with *Eco*RI; lane 2, purified pNN278 marker DNA (1 ng) cleaved with *Eco*RI; lane 3, YNN289 DNA (5 μg) digested with *Eco*RI. A gel blot of these DNAs was probed with a mixture of ^{32}P-labeled NNλ262 and pNN278 DNA. The bands marked by an asterisk indicate those anticipated for unrearranged plasmid DNA. The other bands in lane 3 derive from the genomic *Eco*RI fragments that hybridize with the yeast *URA3*, *CEN3*, and *CEN4* DNA in the nick-translated probe. The doublet of bands in lane 1 represent the left and right arms of the phage λ DNA that are annealed and ligated at the λ cos site upon circularization in yeast to give a single higher molecular weight band that comigrates with the largest band in lane 1.

^{32}P-labeled nick-translated probe made from a mixture of pNN278 and NNλ262 DNA (Fig. 3).

RESULTS AND DISCUSSION

The two nonhomologous plasmids used in these experiments, pNN278 and NNλ262, are shown in Fig. 1. pNN278 contains yeast *URA3* and *CEN4* sequences, bacterial pBR322 sequences, and maize DNA providing *ARS* function. NNλ262 contains yeast *TRP1*, *ARS1*, and *CEN3* sequences along with phage λ DNA. These sequences were expected to be non-

Table 1. Meiotic segregation of nonhomologous plasmids in yeast

Plasmid	4:0	3:1	2:2	1:3	0:4	Tetratypes
			A. YNN289 transformant 1: 63 tetrads			
NNλ262 (*TRP1*)	0	0	57 (90%)	1 (2%)	5 (8%)	2
pNN278 (*URA3*)	0	3 (5%)	53 (84%)	1 (2%)	6 (10%)	4
			B. YNN289 transformant 2: 43 tetrads			
NNλ262 (*TRP1*)	1 (2%)	0	38 (88%)	2 (5%)	2 (5%)	3
pNN278 (*URA3*)	0	0	35 (81%)	1 (2%)	7 (16%)	7
			C. Total of A + B = 106 tetrads			
NNλ262 (*TRP1*)	1 (1%)	0	95 (90%)	3 (3%)	7 (7%)	5
pNN278 (*URA3*)	0	3 (3%)	88 (83%)	2 (2%)	13 (12%)	11

Frequency with which both plasmids went 2:2 parental or nonparental ditype (PD or NPD) = 71/106 (67%).

Opposite pole	Same pole
60/71 (85%)	11/71 (15%)

Tetrad analysis of YNN289 (**a**/α *trp1Δ/trp1Δ his3Δ200/his3Δ200 lys2-801/+ ade2-101/+ leu1/+*) containing plasmids NNλ262 plus pNN278. The centromere-linked marker *leu1* was used to score the frequency of tetratypes for the plasmid markers.

homologous, and Fig. 2 shows that by standard nucleic acid hybridization they share no detectable sequence homology. These two plasmids were introduced into a diploid yeast strain by simultaneous DNA transformation (9), selecting for the markers *TRP1* and *URA3*.

Two independently transformed diploids were selected and shown to contain both plasmids in an unrearranged form. The Southern blot analysis for one of these diploids is presented in Fig. 3. These diploids were sporulated and tetrads were dissected. Table 1 gives the data for segregation of *TRP1* and *URA3* marking the two plasmids. Of 106 tetrads examined from the two diploids, 71 (67%) had both plasmids segregating $2^+:2^0$ with both markers segregating in parental or nonparental configuration, indicating absence of recombination and that sister chromatids were held together until the second meiotic division. Of these 71 tetrads, 60/71 (85%) had the two plasmids segregating to opposite poles at the first meiotic division yielding two Trp^+ Ura^- spores and two Trp^- Ura^+ spores. If segregation of the plasmids was occurring randomly at the first meiotic division, equal numbers of tetrads should have had both plasmids going to the same pole as to opposite poles. Spore viability for these tetrads was 95%, eliminating the possibility that the class of tetrads with plasmids segregating to the same pole might have been underrepresented due to a lethality associated with this class. We conclude that these two nonhomologous plasmids are nonrandomly segregating away from each other at the first meiotic division.

The 85% frequency with which nonhomologous plasmids segregate from each other in this experiment is similar to the 75–90% frequency with which plasmids containing up to 7 kb of homologous DNA disjoin from each other at meiosis I (refs. 2 and 10; unpublished data). Thus, this meiosis I disjunction can occur by a mechanism that does not require DNA sequence homology. Moreover, cytogenetic analysis in higher eukaryotes indicates that homologous meiotic pairing occurs in meiosis I prophase and that, in the absence of meiotic recombination, homologous chromosomes tend to undergo precocious separation before the metaphase of meiosis I. This can lead to nondisjunction in the absence of a pairing mechanism for nonexchange chromosomes (11). Thus, there is no reason to believe that, in the absence of recombination, homologous pairing would tend to promote disjunction of two yeast plasmids during the first meiotic division. The observed meiosis I disjunction of yeast plasmids may be due to an active nonhomologous or nonexchange pairing system as exists in *Drosophila melanogaster* (11, 12). Alternatively, this mechanism may arise in part by the tendency to segregate equal numbers of chromosomes in meiosis I, possibly because of a limited number of spindle attachment sites. Examination of the meiotic segregation in yeast of three or more plasmids with different extents of homologous DNA may allow further properties of this segregation mechanism to be uncovered.

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